Sources and Strategies for Improving the Isolation of Oligosaccharides from Milk and Dairy Streams

By

SIERRA DIANE DURHAM
DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Food Science

in the

OFFICE OF GRADUATE STUDIES

of the

UNIVERSITY OF CALIFORNIA

DAVIS

Approved:

Daniela Barile, Chair

Danielle Lemay

J. Bruce German

Committee in Charge

2022

ACKNOWLEDGEMENTS

I would like to acknowledge and offer my sincerest gratitude to everyone who has helped me complete my educational journey and contributed to the success of this research.

To my advisor, Professor Daniela Barile, who has provided unwavering support and encouragement since the moment we met during my graduate student recruitment. I could not have asked for a better PI, mentor, or cheerleader.

To Dr. Randall Robinson, who has been an amazing mentor, lab mate, and friend since the very beginning of my graduate school experience. His example of patience and attention to detail has helped make me a better researcher and better person.

To all of the members of the Barile lab, past and present, who laid the foundations of my research and have been an incredible support system during my tenure in the lab. You all have become my second family, and I look forward to when our paths will cross again in the future.

To Dr. Danielle Lemay, who provided incredibly valuable feedback and statistical advice while serving on my dissertation committee, and who has been a role model for me in more ways than she knows.

To Professor Bruce German, who served on my dissertation committee and has continually invited me to see the bigger picture and to frame my research in the context of the wider world.

To all of the collaborators who have generously shared their expertise. This research would not have been possible without their input.

To my friends and fellow graduate students, who made my graduate school experience about more than just the research and reminded me to take breaks and live life outside the lab too. I look forward to seeing where all of our bright futures lead us.

To the faculty and staff of my undergraduate program at Cal Poly San Luis Obispo for fostering my love of chemistry, particularly my former PI, Dr. Scott Eagon, who helped me build my scientific research skills and enthusiastically encouraged all of his students to set our goals high and continue pursuing our scientific interests.

And finally, to my parents, Kim and Scott Durham who have always nurtured my curiosity and surrounded me with science; and to my extended family who have offered their love and support throughout my educational journey, even when they didn't fully understand what I was studying.

ABSTRACT

Milk oligosaccharides are a class of carbohydrates composed of three to twenty monosaccharides, which are found in mammalian milk and other dairy products. Bioactivities, including prebiotic, anti-pathogenic, and immunomodulatory activities, as well as roles in cognition, have been ascribed to milk oligosaccharides featuring particular structural motifs. The most prevalent source of milk oligosaccharides for humans is breast milk, but a comparable source of these beneficial compounds for formula-fed infants or individuals at other life stages is not currently available. As a result, milk oligosaccharides are recent targets for addition to infant formulas and nutraceuticals. Harnessing the bioactive potential of naturally occurring milk oligosaccharides, however, is challenged by low commercial availability of human breast milk and low concentrations of similarly structured milk oligosaccharides in traditional bovine dairy streams. This dissertation presents a look into the abundances of milk oligosaccharides and their potential sources form several non-traditional angles and proposes potential alternative sources for their isolation.

Chapter I introduces bovine milk oligosaccharides, highlights the current sources for bovine milk oligosaccharide isolation and their associated challenges, and proposes the more concentrated dairy stream, delactosed permeate, as a potential new source for bovine milk oligosaccharide isolation.

Chapter II focuses on the ‘gold standard’ human milk oligosaccharides and how the concentrations of key oligosaccharides in human milk vary with lactation stage and maternal gene expression.

Building off of the foundation established in Chapters I and II, Chapters III through VI delve more in-depth into how non-human milk oligosaccharide abundances are impacted by specific factors, and how harnessing these elements may allow for improved milk oligosaccharide isolation by increasing their source concentrations.

Chapter III examines the impact of maternal diet on bovine milk oligosaccharide abundances. This study was the first to successfully demonstrate significant differences in bovine milk oligosaccharide yields with changes in dietary fiber levels.

Chapter IV takes a more in-depth look at the composition of delactosed permeate, and is the first study to quantify bovine milk oligosaccharides in this promising concentrated dairy waste stream.

Chapter V expands beyond traditional western sources of commercial milk to investigate the milk oligosaccharide profiles of all mammalian species through the compilation and analysis of five decades of published milk oligosaccharide research. A comprehensive review of milk oligosaccharide literature at this magnitude has never before been undertaken. The analysis of the compiled data revealed overarching influences of phylogeny and evolution on milk oligosaccharide profiles and allowed for the identification of non-bovine milks that feature oligosaccharide profiles with key similarities to human breastmilk that are promising potential sources for milk oligosaccharide isolation.

Finally, Chapter VI summarizes the main conclusions of the dissertation, provides perspective on the current challenges relating to milk oligosaccharide analysis, and proposes future directions for research in this field.

RELEVANT PUBLICATIONS

Durham, S.D.; Cohen, J.L.; Bunyatratchata, A.; Fukagawa, N.; Barile, D.; "Oligosaccharides," Encyclopedia of Dairy Science, 3rd Ed., Vol. 5, McSweeney, P.L.H.; McNamara, J.P. eds. Elsevier, Amsterdam, The Netherlands. **2022**. 141-153.

Durham, S.D.; Robinson, R.C.; Olga, L.; Ong, K.O.; Chichlowski, M.; Dunger, D.B.; Barile, D.; "A one-year study of human milk oligosaccharide profiles in the milk of healthy UK mothers and their relationship to maternal FUT2 genotype," *Glycobiology*. **2021**. *31*, 1254-1267.

Durham, S.D.; Lemay, D.G.; Wei, Z.; Kalsceur, K.K.; Finley, J.W.; Fukagawa, N.K.; Barile, D.; "Dietary fiber to starch ratio affects bovine milk oligosaccharide profiles," *Curr. Dev. Nutr*. **2022**. (*in press*).

Durham, S.D.; Huang, Y-P.; Tian, T.; Liu, Y.; Barile D.; "Delactosed permeate as a source for extracting oligosaccharides: Compositional variation and processing strategies," (*manuscript in preparation*).

Durham, S.D.; Wei, Z.; Lange, M.; Laborie, E.; German, J.B.; Lemay, D.G.; Barile D.; "Fifty years of research on milk oligosaccharides: Querying the body of literature for humans and other mammals," (*manuscript in preparation*).

ABBREVIATIONS

ADF, acid detergent fiber

APTS, 8-aminopyrene-1,3,6-trisulfonic acid

BMO, bovine milk oligosaccharide

DLP, delactosed permeate

DP, degree of polymerization

DSL, disialyllactose

EPEC, enteropathogenic *Escherichia coli*

ETEC, enterotoxigenic *Escherichia coli*

FOS, fructooligosaccharides

Fuc, fucose

FUT2, fucosyltrasferase 2

FUT3, fucosyltransferase 3

Gal, galactose

GalNAc, *N*-acetylgalactosamine

Glc, glucose

GlcNAc, *N*-acetylgalactosamine

GOS, galactooligosaccharides

Hex, hexose

HexNAc, *N*-acetylhexosamine

HMO, human milk oligosaccharide

HPAEC-PAD, high-performance anion-exchange chromatography with pulsed amperometric detection

HPLC, high-performance liquid chromatography

HSLF, high starch low fiber

LC, liquid chromatography

LC-MS/MS, liquid chromatography tandem mass spectrometry

LNFP I, lacto-*N*-fucopentaose I

LNnT, lacto-*N*-neotetraose

LNT, lacto-*N*-tetraose

LOD, limit of detection

LOQ, limit of quantification

LSHF, low starch high fiber

MS, mass spectrometry

NASH, non-alcoholic steatohepatitis

NDF, neutral detergent fiber

Neu5Ac, *N*-acetylneruaminic acid

Neu5Gc, *N*-glycolylneruaminic acid

NMR, nuclear magnetic resonance

Q-ToF, quadrupole time-of-flight

SNP, single-nucleotide polymorphism

SPE, solid phase extraction

TMR, total mixed ration

TMT, tandem mass tag

USDA-ARS, United States Department of Agriculture Agricultural Research Service

UV, ultraviolet

WHO, World Health Organization

2'-FL, 2'-fucosyllactose

3-FL, 3-fucosyllactose

3'-SL, 3'-sialyllactose

6'-SL, 6'-sialyllactose

TABLE OF CONTENTS

CHAPTER I:

Obtaining milk oligosaccharides from milk and other dairy streams: Potential sources and considerations on increasing oligosaccharide concentrations

Published as:

Durham, S.D.; Cohen, J.L.; Bunyatratchata, A.; Fukagawa, N.; Barile, D.; "Oligosaccharides" in Encyclopedia of Dairy Science, 3rd Ed., Vol. 5, McSweeney, P.L.H.; McNamara, J.P. eds. Elsevier, Amsterdam, The Netherlands. **2022**. 141-153.

ABSTRACT

Bovine milk oligosaccharides (BMOs) have demonstrated and hypothesized benefits for infants, including protecting against pathogens and promoting cognitive development, making them promising ingredients for infant formulas and nutraceuticals. Isolation of BMOs from traditional dairy streams is challenged by low BMO concentrations compared to non-bioactive, simpler sugars like lactose. Delactosed permeate presents a promising alternative dairy stream for sourcing BMOs, yet improving oligosaccharide concentrations in the starting milk, possibly through modifications to cows' diets, may be needed. Understanding how dietary components influence milk composition and selecting ideal source(s) will be vital to meet the growing demand for milk oligosaccharides as ingredients.

BACKGROUND

Breast milk is widely considered to be the ideal source of nutrition for infants, and the World Health Organization (WHO) recommends that mothers exclusively breastfeed their newborns for at least the first six months of life and continue breastfeeding with the addition of complementary foods for up to two years. (WHO, 2009) In addition, many recent studies have shown associations between breastfeeding and a reduction of the risk of diseases such as obesity, (Owen, et al., 2005) asthma, (Kull et al., 2009) and necrotizing enterocolitis (Meinzen-Derr et al., 2009) as well as a reduction in infant mortality. (Meinzen-Derr, et al., 2009; Vennemann et al., 2009) However, breastfeeding is not always a practical nor attainable option for all mothers. When mothers are unable to breastfeed or cannot provide sufficient milk for their babies, infant formula, which attempts to mimic human milk composition, is often used as a substitute. (Martin et al., 2016)

Because infant formula is composed primarily of bovine milk-derived ingredients, which inherently contain much lower levels of oligosaccharides than human milk, (Dong et al., 2016; Fong, et al., 2011; Martin-Sosa, et al., 2003) infants consuming formula instead of breastmilk receive only trace amounts of the bioactive molecules responsible for many of the benefits attributed to breastfeeding. (Salli, et al., 2019) In attempts to counteract this, several infant formulas include non-milk oligosaccharide supplements such as galactooligosaccharides (GOS), inulin, fructooligosaccharides (FOS), or polydextrose. (Akkerman et al., 2019; Ma, et al., 2018; Nijman, et al., 2018; Fanaro, et al., 2005) GOS consists of a combination of galactose dimers and compounds with a degree of polymerization (DP) between 3 and 15, composed of multiple galactose units and a terminal glucose. (Tzortzis & Vulevic, 2009) Most commercial GOS products contain primarily DP 2 to 5 oligosaccharides with the constituent linkages (β1-2, 3, 4, or 6) depending on the enzyme used for GOS synthesis. FOS and inulin are fructans containing almost exclusively β2,1-linked fructose monomers, with or without a terminal glucose. The use of the terms inulin and FOS is inconsistent; however, they are most commonly distinguished as inulin having a DP of 10 to 60 and short-chain FOS as having a DP of less than 10. (Roberfroid, 2007) Polydextrose is a highly branched glucose polymer of DP 2-120 (average DP=12) that contains primarily α or β1-6 glycosidic linkages, but may also include α or β 1-2, 2-3, and 2-4 linkages. (Do Carrmo et al., 2016; Cho et al., 1999) Because of their homooligomeric composition, these alternative oligosaccharides, do not contain the wide array of structural characteristics featured by human milk oligosaccharides (HMOs) that are key for many of their beneficial biological effects. (Bode et al., 2016; Barile & Rastall, 2013)

More recently, a few infant formulas have been supplemented with a small number of synthetically produced HMOs; however, with only a few compounds included at low concentrations, these formulas still lack the unique structural diversity exhibited by HMOs in breastmilk. Thus, other alternative sources that more closely mimic the structural complexity and diversity of HMOs seen in breast milk are needed.

Bovine milk oligosaccharides (BMOs) are a class of carbohydrates that are indigestible to mammals, yet they have the potential to play a significant role in human health. BMOs are a promising alternative to help fill this void due to their structural similarity to many HMOs as well as their demonstrated safety and tolerability. Another advantage of applying BMOs as a supplement to infant formula is their wide availability in dairy processing side streams and waste streams. BMOs are composed of between 3 and 11 monosaccharides connected through a variety of glycosidic linkages. BMO constituent monosaccharides may include glucose (Glc), galactose (Gal), *N*-acetylglucosamine (GlcNAc), *N*-acetylgalactosamine (GalNAc), fucose (Fuc), *N*-acetylneuraminic acid (Neu5Ac) and *N*-glycolylneuraminic acid (Neu5Gc). BMOs are based on one of two core structures at their reducing end: lactose (Gal(β1-4)Glc) or lactosamine (Gal(β1-4)GlcNAc). These core structures can be further expanded through the addition of β1-3-, β1-4- or β1-6-linked Glc, Gal, GlcNAc or GalNAc units, and the resulting backbones may be decorated with α2-3- or α2-6-linked sialic acid (Neu5Ac or Neu5Gc) or, more rarely, α1-2- or α1-3-linked fucose. (Tao et al., 2008; Aldredge et al., 2013)

BMOs are classified based on their monosaccharide compositions, with those that contain one or more sialic acid monomers categorized as acidic, while BMOs without any Neu5Ac or Neu5Gc are classified as neutral. Neutral BMOs can be further subcategorized as fucosylated and unfucosylated based on the presence or absence of fucose in their structures. Unlike HMOs, which are highly fucosylated, the majority of BMOs are acidic, and only six neutral fucosylated structures have been identified so far. (Aldredge et al., 2013; Robinson et al., 2018; Albrecht et al., 2014; Mehra et al., 2014) No oligosaccharides featuring both sialylation and fucosylation have been found in cows' milk. Table 1.1 summarizes the classes of BMOs identified in prior studies. Although they make up a smaller percentage of the BMO fraction, neutral BMOs show a similar level of structural diversity as acidic BMOs. The wide range in the numbers of BMOs reported by the studies in Table 1.1 may be due to variation in the BMO profiles of the milk samples analyzed as well as differences in the techniques employed for analysis. Because the full structures of many larger BMOs have not yet been fully elucidated, they are often referenced by their composition via a five-digit code delineating the number of each monosaccharide included in the structure in the format: Hex_HexNAc_Fuc_Neu5Ac_Neu5Gc, where Hex is the number of hexose monomers (Glc and Gal) and HexNAc is the number of *N*-acetylhexosamine monomers (GlcNAc and GalNAc).

Table 1.1. Distributions of bovine milk oligosaccharides previously studies reporting more than ten BMO compounds

	Number of unique structures identified			
Publication	Total	Sialylated	Neutral Unfucosylated	Neutral Fucosylated
Remoroza et al., 2020	36	16	18	2
Liu et al., 2019	12	7	5	--
Robinson et al., 2019	15	3	8	4
Liu et al., 2017	14	8	6	--
Schwendel et al., 2017	11	6	5	--
Sischo et al., 2017	29	7	20	2
Albrecht et al., 2014	34	21	10	3
Liu et al., 2014	13	8	5	--
Aldredge et al., 2013	25	8	11	6
Sundekilde et al., 2012	50	13	32	5
Marino et al., 2011	34	22	10	2
Tao et al., 2009	24	17	7	--
Tao et al., 2008	24	17	7	--

Like HMOs, BMOs are of interest for their wide array of demonstrated and hypothesized bioactivities. Although their bioactivities have not yet been investigated as thoroughly as HMOs, BMOs have numerous proven properties that would be beneficial in human nutrition, particularly for infants. BMOs have shown antiadhesive and pathogen decoy activities against a number of pathogens *in vitro* including the enteric pathogens *Campylobacter jejuni* (Lane et al., 2012) and enterotoxigenic *Escherichia coli* (ETEC) (Martín-Sosa et al., 2002) which have been recognized as a leading cause of enteritis in humans worldwide. In addition, bovine colostrum, as well as its ultrafiltration and nanofiltration permeates, have demonstrated antiadhesive effects *in vitro* against the enteric pathogens *Salmonella enterica* serotype Typhimurium, enteropathogenic *E.*

coli (EPEC), and *Cronobacter sakazakii*. (Maldonado-Gomez et al., 2015) This adherence inhibition can be at least partially attributed to the BMOs present in the dairy fractions; however, since BMOs were not the exclusive ingredient in the products tested, peptides and glycopeptides may contribute to the activity as well. Purified BMOs have been shown to also act as immunomodulators by decreasing gut permeability and reducing inflammation in animal studies, as well as contributing to gains in lean body mass in animal models of infant undernutrition (Boudry et al., 2017; Charbonneau et al., 2016) In addition, the two most abundant acidic. BMOs, 3’-sialyllactose (3’-SL) and 6’-sialyllactose (6’-SL), have exhibited a role in improving neonatal cognitive development in animal models. (Obelitz-Ryom et al., 2019; Oliveros et al., 2018)

The prebiotic activity of oligosaccharides derived from bovine milk specifically have been minimally investigated; however, because of their structural homology with HMOs, BMOs are hypothesized to have similar prebiotic effects. This hypothesis is supported by emerging *in vitro* studies of the effects of BMOs on beneficial bacteria. *In vitro* supplementation with a BMO isolate has been shown to improve the growth of the beneficial infant gut microbes *Bifidobacterium longum* ssp. *longum* and *Parabacteroides distasonis,* as well as the probiotic *B. animalis* ssp. *lactis*. (Jakobsen et al., 2019; Marsaux et al., 2020) In addition, 3’-SL and 6’-SL have been demonstrated to promote the *in vitro* growth of select strains of *B. breve,* a prevalent gut microbe in infants. (Ruiz-Moyano et al., 2013) BMO supplementation has also been shown to increase the relative abundance of bifidobacteria among *in vitro* infant fecal-derived microbial cultures, including increased average relative abundances of operational taxonomic units (OTUs) for *B. longum, B. bifidum, B. adolescentis,* and *B. breve*. (Marsaux et al., 2020) Purified BMOs were also used in an animal model of cancer-prone non-alcoholic steatohepatitis (NASH) mouse, alone and in combination with *B. longum* ssp. *infantis.* Protective effects were observed for both *B. infantis* and BMOs in terms of reduced hepatic and ileal inflammation, which could be correlated with increased short chain fatty acid production and reduced hydrogen sulfide and methane in the gut. Improved outcomes were also shown for the combination of the BMOs and *B. infantis*. Importantly, this study was the first to demonstrate that BMO supplementation alone increased the abundance of butyrate-generating bacteria (which have proven useful to prevent NASH) in addition to other direct benefits to the host, independent of the beneficial outcomes attributable to support of the growth of *B. infantis*. (Jena et al., 2018)

EXISTING SOURCE: WHEY PERMEATE

One dairy side stream that has been investigated as a source for BMO isolation is whey permeate, a byproduct of cheesemaking and whey protein isolation (process flowchart in Figure 1.1, dotted outline). In 2018, more than 217.5 billion pounds of cow milk were produced in the US, about 1.1 billion pounds of which became cheese whey permeate. (American Dairy Products Institute, 2018; USDA NASS, 2020; USDA NASS, 2019) The ultrafiltration process to isolate whey proteins often involves the addition of some water in diafiltration mode to increase protein purity by enhancing the removal of salts and lactose from the whey protein retentate. A side effect of this process is the dilution of the obtained permeate, generally resulting in total solids as low as 3 to 5% in the final whey permeate. Of that solids content, the vast majority is lactose, and the remaining balance is composed of nitrogenous materials, residual lipids, salts, and other components including BMOs (Table 1.2, Figure 1.2). (Barile et al., 2009; Tetra Pak, 2020; Smith et al., 2016; Frankowski et al., 2014) It should be noted that this composition, while generally representative, will vary substantially between batches or producers depending on the cheese type and applied processing techniques.

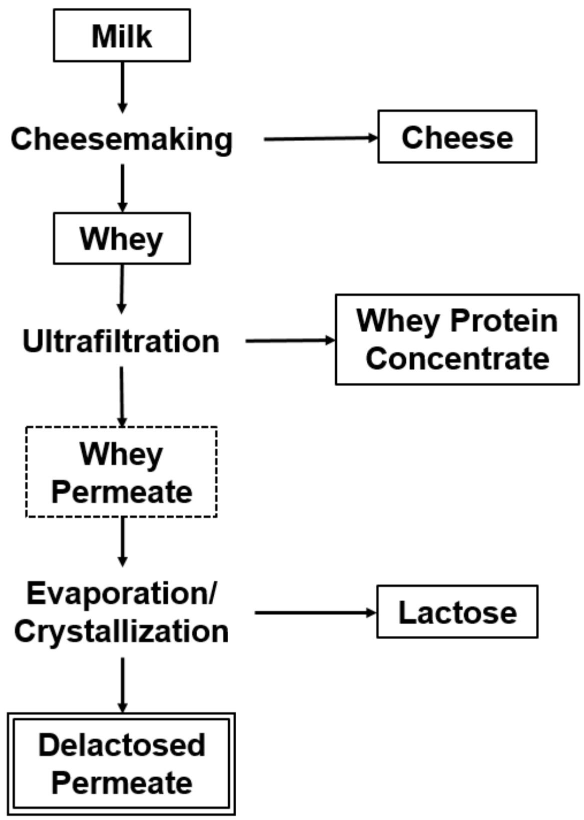


Figure 1.1. Generation of coproducts from cheesemaking and whey processing

Table 1.2. Dry basis composition of whey permeate from multiple sources, along with average composition. Values for components other than pH reported in percent (gram per 100 grams dry matter)

	Barile 2009	**Smith 2016**		**Tetrapak 2020**	**Frankowski 2014**	**Average (range)**
Solids	4.87 ±0.02	5-6	5-6	5.38	11.1	**6.47** (4.87-11.1)
pH	6.50 ±0.02	--	--	--	6.3	**6.4** (6.3-6.52)
Lactose	--	82*	81*	87.17	85	**83.8** (81-87.17)
Proteins	3.49 ±1.03	0.003 ±0.003	0.003 ±0.003	0.19	0	**0.74** (0-4.52)
Non-protein N	--	2.50 ±0.04	2.50 ±0.04	3.16	3.36	**2.88** (2.46-3.36)
Lipids	2.05 ±0.82	--	--	Trace	--	**2.05**
Salts/ash	--	--	--	9.48	8.26	**8.87** (8.26-9.48)
Na	--	0.66	0.65	--	0.98	**0.76** (0.65-0.98)
K	--	2.51	2.43	--	2.13	**2.36** (2.13-2.51)
Ca	--	0.48	0.50	--	0.54	**0.51** (0.48-0.54)
Mg	--	0.13	0.13	--	0.12	**0.13** (0.12-0.13)
Cl	--	--	--	--	0.21	**0.21**

*****Values from graph reported in %weight/weight

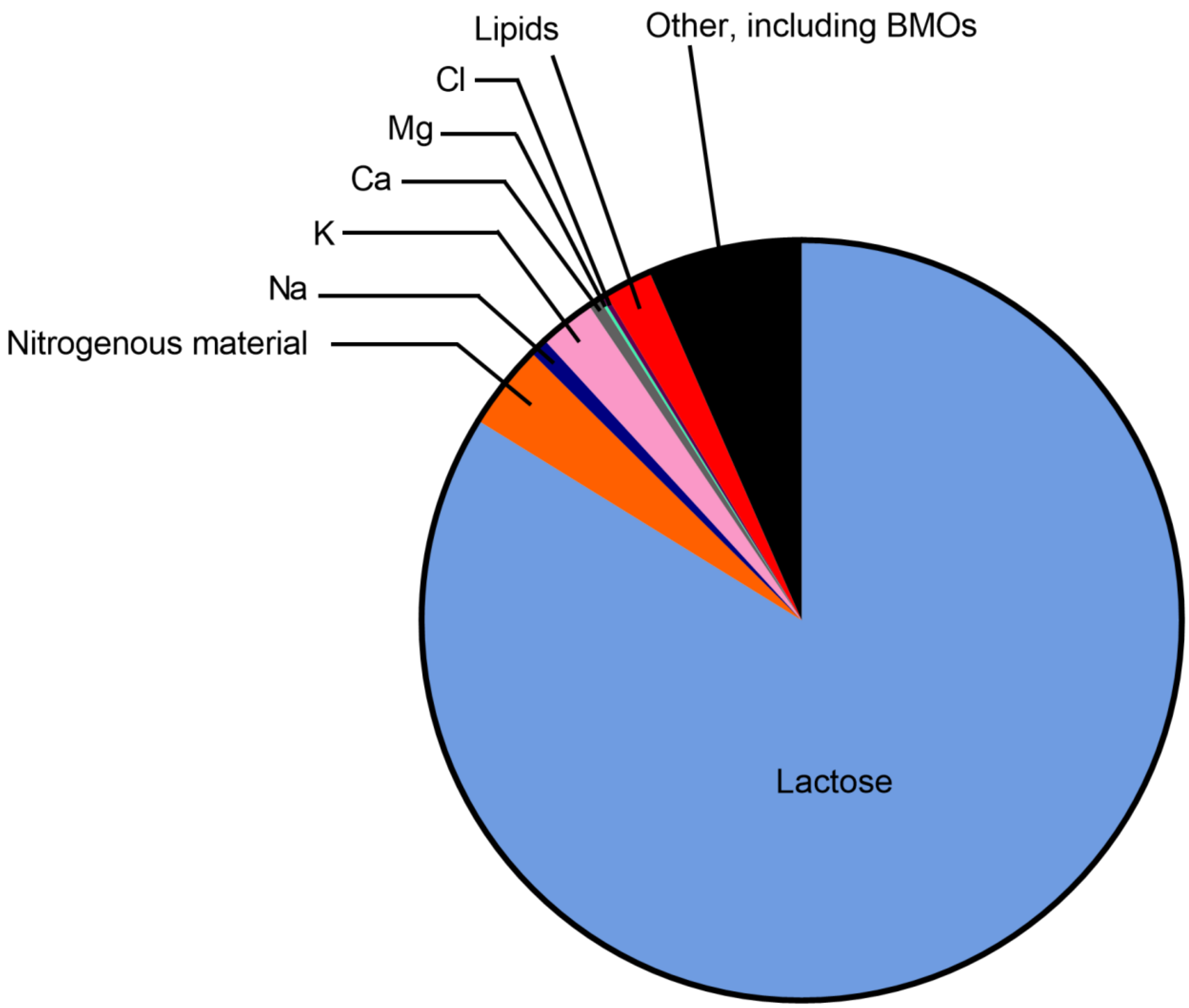


Figure 1.2. Average composition of major components of whey permeate

Because of its high lactose concentration, whey permeate has a high biochemical oxygen demand, making it a difficult waste stream to dispose of for dairy processors, demonstrating an urgency for valorization avenues for this dairy stream. (Jelen et al., 2011) Applications of whey permeate as a food ingredient, (Milkner et al., 2020; Beucler et al., 2006; Bradley & Rexroat, 1988; Hargrove et al., 1976) livestock feed, (Kim et al., 2012; Naranjo et al., 2010) and feedstock for fermentative production of biosurfactants, (Daverey & Pakshirajan, 2010) biopolymers, (Koller et al., 2005; Ahn et al., 2001; Ahn et al., 2000) biogas, (Lee et al., 2009) biohydrogen, (Yang et al., 2007) ethanol (Pasotti et al., 2017; Gabardo et al., 2014; Koushki et al., 2012; Silveira et al., 2005; Domingues et al., 2001) and other chemicals (Dornburg et al., 2008; Ennis & Maddox, 1985; Qureshi & Maddox, 1985) have all been investigated but none of these uses has yet proven to be widely commercially viable.

Several groups have developed and optimized techniques for the isolation of BMOs from whey permeate at different scales using various combinations of pH adjustment, enzymatic hydrolysis, microbial fermentation, and membrane filtration, with many nanofiltration processes exhibiting BMO recovery yields greater than 90% (Table 1.3), making the isolation of BMOs from whey permeate an encouraging potential valorization of this dairy processing stream. (de Moura Bell et al., 2019; Cohen et al., 2017; Altmann et al., 2016; Altmann et al., 2015) Despite the promise of these membrane filtration techniques, however, their high operating costs and capital investment limit their availability primarily to large commercial producers, and because the shipping of diluted material is not practical, consistent sources of large volumes of permeate near such producers are also required.

Table 1.3. Recovery of major bovine milk oligosaccharides from whey permeate after nanofiltration

Publication	Starting Material	Scale	% Recovery			
			3'-SL	6'-SL	6'-SLN	2_1_0_0_0
Bell et al. 2018	Colostrum whey permeate	Pilot	94.3	93.7	93.7	--
Cohen et al. 2017	Colostrum whey permeate	Pilot	91.8-100	92.0-100	92.7-100	--
Altmann et al. 2015	Milk ultrafiltration permeate	Lab	49.8±6.0	84.0±11.4	--	58.7±7.9
		Pilot	77.5±9.3	--	--	51.6±18.7
		Industrial	99.3±13.7	97.4±14.2	--	70.4±17.7

Monosaccharide compositions reported as the numbers of Hex_HexNAc_Fuc_Neu5Ac_Neu5Gc

The main challenge with using whey permeate as the starting material for BMO isolation is its extremely dilute nature. Cow milk, which contains around 80 to 100 mg/L BMOs, is often inadvertently further diluted during the ultrafiltration process. (Fischer-Tlustos et al., 2020; Fong et al., 2011; Gopal et al., 2000) Additionally, the disproportionately high lactose content of whey permeate relative to BMOs, and the structural similarity of lactose and many small BMOs further complicates the isolation of target BMOs at high purity. To overcome such challenges and speed up the process for BMO isolation, a more concentrated source of BMOs would be helpful. Such a source might be achieved either through the use of a more concentrated dairy processing stream or by improving the concentration of BMOs naturally present in the starting milk.

ALTERNATIVE SOURCE: DELACTOSED PERMEATE

A potential source of more concentrated BMOs may be found in the form of delactosed permeate (DLP). Many large cheesemakers and dairy processing co-ops worldwide concentrate and subsequently crystallize the substantial quantity of lactose present in whey permeate for food, and less commonly, pharmaceutical applications. To isolate lactose, whey permeate is pooled and concentrated from a range of 4 to 6% solids up to 60 to 65% total solids using some combination of membrane filtration and evaporation, yielding a wet basis lactose concentration ranging from 40 to 55%. The supersaturated solution is cooled and seeded with crystalline lactose for nucleation. The mother liquor from this crystallization process is decanted and the lactose crystals are washed to improve purity. The decanted mother liquor, known as DLP (Figure 1.1, doubled outline), is typically concentrated to approximately 20 to 30% total solids in an evaporator. (Wong & Hartel, 2014)

Lactose production in the United States has more than doubled over the past 15 years, yet suitable outlets for its co-product, DLP, are lacking. (USDA NASS, 2020) As a result, DLP is widely viewed as a problematic co-product of lactose manufacture, with many processors considering it valueless. Currently, most DLP is given to animals or treated as wastewater by dairy processors.

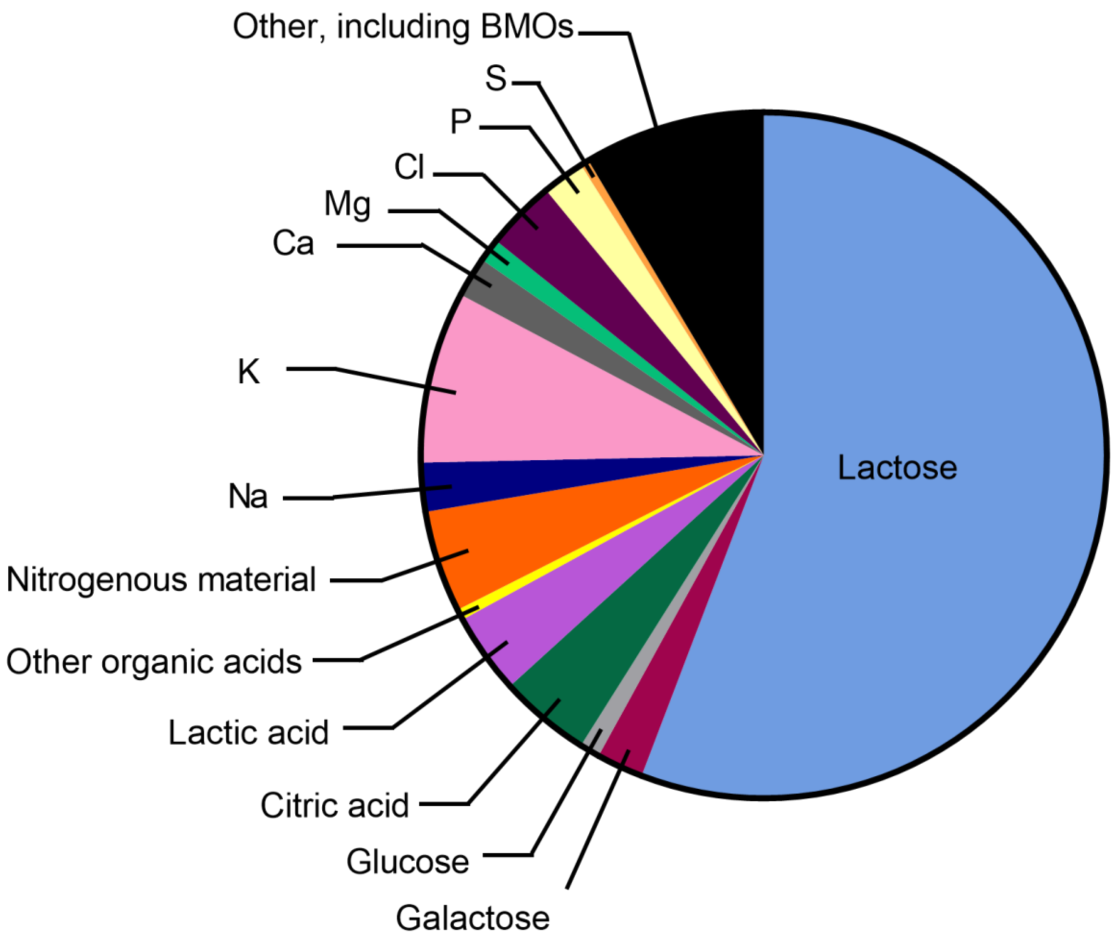


Figure 1.3. Average composition of major components of delactosed permeate

Table 4: Dry basis composition of delactosed permeate from multiple sources, along with average composition. Values for components other than pH reported in percent (gram per 100 grams dry matter)

Reference	Wagner 2014	Liang 2009			Smith 2016	Burrington 2014	Friend 2004	Frankowski 2014	Levin 2016	**Average** (range)
Solids	30	33.8	25.9	35.6	37.3±0.14	--	35.2±2.9	34.5	--	**33.2** (25.9-37.4)
pH	--	5.6	5.2	5.2	--	--	--	5.5	--	**5.34** (5.2-5.6)
Lactose	60±6.7	64.2	55.21	41.29	60	59.6	55.8±3.1	46	59.87	**55.8** (41.3-66.7)
Galactose	--	2.66	0	3.93	--	--	--	--	--	**2.2** (0-3.9)
Glucose	--	1.18	0	1.69	--	--	--	--	--	**0.95** (0-1.69)
Citric acid	--	5.74	3.46	4.92	--	--	6.3±0.8	0.864	--	**4.3** (0.86-7.1)
Lactic acid	--	2.47	5.71	7.28	--	--	2.4±1.1	1.38	--	**3.8** (1.4-7.3)
Orotic acid	--	--	--	--	--	--	--	0.28	--	**0.28**
Uric acid	--	--	--	--	--	--	--	0.221	--	**0.22**
Hippuric acid	--	--	--	--	--	--	--	0.007	--	**0.01**
Proteins	10±3.3	1.36	2.14	2.37	0.027	7.32	3.7±0.6	0.54	0.66	**4.8** (0.66-13)*
Non-protein N	--	--	--	--	6.7	--	--	8.29	--	
Salts/ash	53.3	--	--	--	--	12.29	22.9±3.6	21.8	26.61	**27.4** (12.3-53.3)
Na	3.48	1.23	2.33	2.3	2.25	2	2.7±0.4	2.39	--	**2.3** (1.2-3.5)
K	12.78	3.93	6.87	8.37	20.21	0.24	6.2±1.9	6.04	--	**8.1** (0.24-20.2)
Ca	0.48	0.86	0.69	1.12	4.96	3.76	2.0±0.2	1.51	--	**1.9** (0.5-5.0)
Mg	0.733	0.21	0.23	0.3	0.72	6.29	0.4±0.1	0.25	--	**1.1** (0.2-6.3)
Cl	--	1.31	5.25	5.45	--	--	--	1.03	--	**3.3** (1.0-5.5)
P	--	1.74	1.63	2.32	--	--	2.4±2	--	--	**2.0** (1.6-2.4)
S	--	--	--	--	--	--	0.4±0.2	--	--	**0.4**

*Proteins and Non-protein nitrogen combined in average value

We compiled compositional information of DLP from multiple sources (Table 1.4). Although various sources reported different components, we determined that the sum of the average composition of all reported components to be approximately 91%. The most abundant components in DLP on a dry basis were lactose (56%), minerals (19%), organic acids (8.6%), and nitrogen-containing compounds (4.8%) (Figure 1.3). The organic acid fraction, particularly lactic acid content, can vary depending upon the type of cheese, its manufacturing process and whey treatment, as well as the degree of conversion of lactose to lactic acid during storage of DLP. Based on moisture sorption isotherms, the water activity at a typical value of 33% w/w solids ranges from 0.92 to 0.96, depending upon composition. (Liang *et al*., 2009)

DLP also contains a substantial quantity of the bovine milk oligosaccharides present in the original milk. On a mass basis, the lactose-to-BMO ratio for whey permeate is approximately 400:1, while in DLP it is 100:1 based on measurements conducted in our laboratory (Table 1.5). The observed decrease in lactose relative to BMO in DLP as compared with whey permeate would likely facilitate BMO purification efforts starting from DLP. Lactose and mineral removal will further facilitate BMO enrichment, which would yield a prebiotic product with proper attention to mineral content in the final product.

Table 1.5. Concentrations of 6 bovine milk oligosaccharides and lactose measured as-is in delactosed permeate using high-performance anion-exchange chromatography with pulsed amperometric detection

Oligosaccharide	**Concentration (mg L^{-1})**
3'-Sialyllactose	650
6'-Sialyllactose	250
Lacto-*N*-hexaose	49
3 Hex	78
Lacto-*N*-neotetraose	35
2 Hex 1 HexNAc	450
Total quantified BMOs	1.512 g L^{-1}
Lactose	150 g L^{-1}

Ion exchange with cation-exchange resins, electrodialysis and nanofiltration have all been applied to the desalination of DLP to good effect. (Wagner et al., 2014; Holst et al., 2007; Mikhaylin & Bazinet, 2006; Vembu & Rathinam, 1997; Mahmoud & Kosikowski, 1982; Pratt et al., 1952) Electrodialysis has been the most effective at removing monovalent ions, particularly potassium ions, and to a lesser extent, sodium ions. Although electrodialysis was effective for removing monovalent ions, it was unable to remove more than 25% of divalent ions, especially calcium ions, and thus ion exchange or precipitation have been suggested as alternative desalination methods. (Mikhaylin and Bazinet, 2006) A process implementing phosphate addition, pH adjustment, and heating of DLP to precipitate divalent cations has also been developed. Monovalent cations are by far the most abundant minerals in DLP; thus, a combination of nanofiltration and precipitation may be most appropriate to remove both monovalent and divalent ions. Additional technoeconomic evaluation and understanding of the advantages and disadvantages of the involved unit operations required to optimally demineralize

DLP and purify BMO will be needed to make such processes feasible and efficient on a commercial scale.

Preliminary analysis by our lab of one commercially produced DLP sample displayed a BMO concentration of approximately 1.5 g/L on an as-is basis with 150 g/L lactose (Table 1.5). Interestingly, 1.5 g/L BMO is higher than the highest typical reported concentration of BMO in bovine colostrum (1 g/L) and is substantially higher than the 50 to 100 mg/L found in whey permeate. Beyond the six BMOs listed in Table 1.5, our lab has shown that high molecular weight, fucosylated oligosaccharides are also present in dairy co-products. (Mehra et al., 2014) Although these are low in abundance, enriching these compounds in particular will lend even more specific and potent biological functionality due to their higher degree of similarity to human milk oligosaccharides.

Assuming this BMO composition is representative of most DLP in the United States, we can estimate an amount of BMO in DLP produced in the U.S. With 1.2 billion pounds of lactose produced in 2019, and assuming a 60% recovery of lactose from the whey permeate and an average composition of DLP reported in Table 4, we can calculate that at least 1.69 liters of DLP of that composition is produced per pound of lactose. (USDA NASS, 2020) This yields an overall amount of 2.02 billion liters of DLP in 2019, which potentially contain a total of 3100 metric tons of BMOs in that DLP.

Despite its promise for BMO isolation, we want to acknowledge that DLP presents a number of challenges as a source. Drying DLP as-is to a stable powder is problematic due to its hygroscopic and syrupy nature deriving from the high mineral, organic acid, and residual lactose content. (Bund & Hartel, 2010; Liang et al., 2009) These properties make incorporation into final food products, storage, and general powder stability and flow characteristics difficult. In general, residual lactose concentrations remain high because lactose crystallization yields rarely surpass 65% due to minerals and other components in whey permeate. (Paterson, 2009) The high lactose concentration (~15%) in DLP makes it an attractive feedstock for industrial biotechnology. Unfortunately, high mineral content along with high lactose concentrations lead to a high osmolarity and subsequent slow or limited growth of ethanol- or oil-producing microorganisms. In addition, the relatively low pH of DLP (pH 5.3), resulting from the presence of organic acids may further inhibit or slow the growth of desirable fermentative organisms to aid in lactose removal, especially bacteria. (Frankowski et al., 2014; Liang & Hartel, 2009) One potential avenue for utilizing the lactose in DLP and in doing so, facilitating further BMO purification, is to apply a fermentation step with a yeast to consume most of the lactose. Such a process has already been developed for the isolation of BMO from bovine colostrum, however, the higher lactose and salt concentration of DLP would not be suitable for conventionally used *Saccharomyces cerevisiae* strains. Alternative yeast such as *Kluyveromyces marxianus* have already been examined in many biotechnological roles, including the production of ethanol and single cell protein from dairy streams. This species is particularly well suited for DLP as it can ferment at high temperatures, is salt tolerant, and readily and rapidly assimilates lactose.

The use of DLP as a BMO source is further complicated by the inconsistency in its composition. Because DLP is the resultant stream from production processes involving many steps to capture valuable co-products like whey protein and lactose, the variability in each cheesemaking, protein isolation, and lactose crystallization step must be accounted for, in addition to the variation in the composition of the starting milk itself. At present, there is no standard of identity for DLP, complicating potential efforts to standardize DLP processing and BMO isolation methods.

BMO VARIATION

Even when using more concentrated dairy streams as starting points for BMO isolation, the starting concentrations of BMOs in the initial milk are a limiting factor. An alternative approach to improving commercial BMO isolation, which could be applied in-tandem with isolation techniques tailored for dairy streams like mother liquor is to improve the BMO isolate by modifying the concentration of BMOs produced in the original milk. Such a modification also presents the opportunity to modify BMO profiles in addition to total BMO concentrations, potentially increasing the abundances of larger, more structurally complex, and fucosylated BMOs, which would allow BMO compositions to more closely mirror those of HMOs. Factors that influence BMO profiles and concentrations may include lactation timepoint, breed, parity, season, farming system, and diet; however not all of these factors are realistically modifiable in existing dairy herds. In addition, because commercial dairy streams are the result of pooling milk from a wide range of cows and farms, it is important to consider the widespread applicability of any potential modification.

Lactation Time Point

The abundance of BMOs in milk varies depending on the individual mother and state of lactation. Colostrum is the thick yellowish fluid rich in immunological components that is produced leading up to and immediately following parturition. (McGrath et al., 2016) The term ‘bovine colostrum’ is often used to described milk produced in the first few days postpartum; however, following the definition set by the USDA, colostrum is the first milking harvested after calving. (USDA NASS, 2007) A more appropriate descriptor of the milk produced between colostrum (the first milking) and mature milk is transitional milk. The composition of transitional milk may vary substantially between consecutive days as milk composition approaches that of mature, or saleable, milk. Total BMO concentrations range from about 1 g/L in colostrum to between 80 and 100 mg/L in mature milk (Table 1.6). Increases in BMO concentrations in very late lactation milk may be due to the concentrating effect of lower milk yields just before cows dry off. (Martin et al., 2001)

In addition to the potential sources of variation described in the following sections, the differences exhibited in Table 1.6 between BMO concentrations reported by different studies for the same milking or day of lactation may be due, in part, to differences in sample preparation and BMO analysis techniques. Although multi-step BMO extraction is often key for analysis, each additional sample preparation step increases the risk of BMO losses. It is important to find a balance that reduces matrix effects that hinder analysis while not sacrificing BMO recoveries. Van Leeuwen (2019) recently reviewed the pros and cons of a wide range of sample preparation techniques for HMO analysis, and while there is minimal parallel research on the influence of sample preparation on BMO analysis, it would be reasonable to expect similar pitfalls and benefits for BMO extraction. In addition, while derivatization of extracted BMOs prior to analysis can be useful for increasing detector sensitivity for some analytical techniques, such measures may be subject to uneven or incomplete derivatization and require additional sample clean-up steps, which can introduce further variation to the analysis.

Table 1.6. Concentrations of the most abundant bovine milk oligosaccharides at varying lactation timepoints

Lactation Timepoint		BMO concentration (mg L^{-1}) 3'-SL	6'-SL	6'-SLN	DSL	Publication
Days 0-2	Prepartum	717 ± 27	64 ± 6	100 ± 7	--	Nakamura 2003
Days 3-6	Prepartum	557 ± 175	52 ± 10	75 ± 17	--	Nakamura 2003
Days 7-10	Prepartum	262 ± 76	40 ± 5	74 ± 4	--	Nakamura 2003
Days 11-14	Prepartum	135 ± 73	18 ± 10	64 ± 22	--	Nakamura 2003
1st Milking	Postpartum	681-867	136-243	220-239	201-283	McJarrow 2004*
1st Milking	Postpartum	590	100	140	225	Fischer-Tlustos 2020‡
2nd Milking	Postpartum	1245 ± 82	85 ± 6	119 ± 7	126 ± 8	Fong 2011
2nd Milking	Postpartum	310	80	75	100	Fischer-Tlustos 2020‡
Day 1	Postpartum	280	60	60	--	Nakamura 2003‡
3rd Milking	Postpartum	170	75	40	50	Fischer-Tlustos 2020‡
4th Milking	Postpartum	739 ± 53	73 ± 2	117 ± 10	80 ± 7	Fong 2011
4th Milking	Postpartum	100	50	20	25	Fischer-Tlustos 2020‡
Day 2	Postpartum	190	70	45	--	Nakamura 2003‡
5th Milking	Postpartum	80	45	10	20	Fischer-Tlustos 2020‡
6th Milking	Postpartum	50	40	5	20	Fischer-Tlustos 2020‡
Day 3	Postpartum	100	40	25	--	Nakamura 2003‡
8th Milking	Postpartum	45	35	3	15	Fischer-Tlustos 2020‡
Day 5	Postpartum	75	20	15	--	Nakamura 2003‡

14th Milking	Postpartum	40	25	2	10	Fischer-Tlustos 2020‡
Day 7	Postpartum	30	25	12	--	Nakamura 2003‡

Data reported as mean ± standard error, when available. *Concentrations reported from more than one breed. ‡Data derived from their figure expressed as mg/L

Breed

Among cows of similar lactation stages, notable differences in BMO profiles have been documented between different breeds. Comparisons between Danish Jersey and Holstein-Friesian BMO profiles have revealed higher abundances of neutral fucosylated compounds including 4_5_1_0_0 and 3_6_1_0_0 as well as acidic BMOs such as 3'-SL, 6'-SL, and disialyllactose (DSL) in Jersey milk, as well as greater diversity of BMO abundances between Jersey cows compared to Holstein-Friesians. (Robinson et al., 2019; Sundekilde et al., 2012) In contrast, McJarrow and van Amelsfort-Schoonbeek (2004) observed higher concentrations of 6'-SL in the colostrums of New Zealand Friesian dairy cattle than Jerseys, and no significant differences between the breeds for 3'-SL and DSL. In addition, Angus and Angus Hybrid beef cows have been noted to express milk with higher abundances of 3_1_0_0_0, 2_2_0_1_0, and 4_1_0_1_0 compared to Holstein dairy cows. (Sischo et al., 2017) A variety of Nordic dairy breeds including Doela and Telemark cattle from Norway, Swedish Mountain cattle, Danish Red anno 1970, Icelandic cattle, Native Black cattle and Native White cattle from Lithuania, Western Fincattle and Eastern Fincattle were also recently compared by Sunds et al. (2021). Though not normalized for other variables like days in milk or farming practices, they found that all of the breeds included in the study featured an array of the same 19 BMOs, but in varying proportions. Western Fincattle were found to have significantly higher total BMO abundances, while Telemark cattle had significantly lower total BMO abundances than the other breeds. In addition, Western Fincattle, Doela cattle, and Icelandic cattle had higher abundances of the large fucosylated BMO, 3_6_1_0_0. Sischo et al. (2017) and Sunds et al. (2021) have speculated that the variations in BMO profiles and higher BMO concentrations found in the milk of non-commercial dairy breeds may be the result of their milk compositions favoring the needs of their calves rather than higher milk yield.

Parity

Differences in BMO abundances have also been shown between cows of different parities in both Jersey and Holstein-Friesian cows over the first three parities, with the highest BMO abundances in the 2^{nd} parity. Robinson et al. (2019) have proposed that this phenomenon may be the result of incomplete mammary gland maturity at the time of the first lactation. Although not divided by individual parities, Fisher-Tlustos et al. (2020) also observed higher concentrations of 3’-SL, 6’-SL, and 6’-sialyllactosamine (6’-SLN) in multiparous Holstein dairy cows compared to their primiparous herd-mates.

Though their impacts are well-documented in the literature, lactation timepoint, breed and parity are all difficult, if not impossible, to modify in an existing dairy herd. Other factors that have been investigated for their influence on BMO profiles include season, farming system and cow diet composition; however, the complex nature of such studies has led to challenges in interpreting the results due to the presence of potential confounding factors.

Season

Liu et al. (2017) noted substantial variation in BMO profiles for monthly samples collected from New Zealand Holstein-Friesian dairy cattle, with most BMO’s reaching peak abundance in late autumn (May). Because the cows were pasture fed with varying supplementation of cereal grains or pelleted concentrates as needed, however, the influence of the cows' diets likely played a role in the observed variation. New Zealand grazing pasture quality and composition is known to vary seasonally, (Waghorn & Clark, 2004; Litherland et al., 2002) which, paired with inconsistent supplementation of non-pasture feedstocks, likely caused variation in cow nutrient intake over the course of the lactation period. McJarrow and van Amelsfort-Schoonbeek (2004) observed a similar pattern in BMO concentrations over the lactation season in bulk milk samples from a New Zealand herd of mixed Jerseys and Friesians, but specific dietary intake information was not reported for the study.

Farming System

Schwendel *et al*. (2017) analyzed BMO profiles from bulk milk samples collected from two organic and two conventional dairy farms with pasture-fed cows. They found that BMO concentrations significantly differed ($p < 0.05$) between farming systems, with higher average abundances of 2_1_0_1_0, 3_0_0_0_0, 3_0_0_1_0, 3_2_0_0_0 and 4_1_0_0_0 in milk samples from the organic farms. In addition to the difference in farming systems, the groups also had different breed compositions, with fewer Jersey, more Holstein-Friesian and no Ayrshire cows in the conventional compared to the organic farm groups, which likely had an influence on the BMO profiles of the bulked milk.

Diet

The influence of a diet composed of alfalfa and corn silage, earlage, and grain compared to an exclusively grass diet on BMO profiles in colostrum and early lactation milk was investigated by

Vicaretti et al. (2018). Milk samples were collected from 3 cows of varying breeds on each of 2 farms, with dietary groups segregated by farm. No significant differences in BMO profiles or monosaccharide composition of BMOs between the two cow groups were observed; however, the small sample size likely caused this study to be too underpowered to observe meaningful differences between the groups. Additionally, there is the potential for the differences in breed, location, and farm management practices between the two dietary groups to have confounding effects on the data.

The impact on BMO profiles of supplementing cows' diets by adding either almond hulls or citrus peels to a base total mixed ration of corn grain, canola meal, and alfalfa cubes was investigated by Liu et al. (2014). This study looked at 13 BMOs in the milk of 32 mid-lactation Holstein-Friesian dairy cows after 28 days of dietary treatment. The identified BMOs were found to have greater inter-cow variation within dietary treatment groups than inter-group variation, preventing any conclusions from being made about the influence of the diets on BMO production.

Although a clear effect of cow diet on BMO profiles has not yet been shown, dietary composition is well documented to influence yield, (Sanchez-Duarte et al., 2019; Ranathunga et al., 2013; Cant et al., 1991; Thomson et al.; 1985) lipid profiles, (Xue et al., 2019; Ranathunga et al., 2013; Miron et al., 2007, 2003; Carroll, et al., 2006; Jahreis et al., 1997; Cant et al., 1991; Spain et al., 1990) nitrogen content, (Sanchez-Duarte et al., 2019; Cant et al., 1991; Miron et al., 2007; Carroll et al., 2006; Spain et al., 1990) and monosaccharide composition (Asakuma et al.,

2010) of cows' milk. As an easily modifiable factor with the potential to influence BMO profiles, further controlled studies on the impact of cow diet on BMO production are warranted.

Considerations for Future Dietary Studies

The question that follows from this is what aspect of cow diet is likely to have the greatest impact on the resulting BMO profiles. Many previous studies investigating the effects of diet on other aspects of milk composition and yield have centered around supplementing or exchanging specific feed ingredients in the diet. While this approach makes study design and diet formulation straight-forward, long-term or wide-spread application of findings may pose challenges depending on the seasonal and regional availability and nutrient composition of the target feed ingredients. Additionally, comparing diets composed entirely of single feed ingredients may be unfeasible because of the need to meet overall nutritional requirements to maintain good cow health. (Thomson et al, 1985)

The alternative dietary modification approach would be to alter the ratios of feed ingredients to change the compositional characteristics of the diet (i.e. starch, fiber, lipid, protein content) while maintaining the same ingredients in the total mixed ration (TMR). This approach is beneficial in that it gets more to the foundation of what biochemical elements in the feed are driving the metabolic changes in the cow that lead to modified milk compositions. Additionally, if a specific compositional component or combination of components of the feed is identified as having a beneficial effect on the resulting BMO profile without negatively impacting other compositional, physiochemical, or sensorial properties of the milk, there may be greater potential for widespread translation of such a finding with TMRs composed of different feed ingredients based on regional availability or seasonal variation in crop quality, but formulated to replicate the identified biochemical composition.

The digestive system of a cow consists of six major components: the rumen, reticulum, omasum, abomasum, small intestine, and large intestine, as shown in Figure 1.4. Of these, the rumen, abomasum, and small intestine have the greatest influence on the breakdown and absorption of nutrients by the cow. The behavior of the rumen in particular is especially susceptible to being influenced by the feed consumed.

The majority of previous studies on the effect of cow diet on milk composition have focused on the impact on milk yield and lipid profiles because of their economic relevance both to dairy farmers and the cheesemaking industry. Additionally, most studies have found milk lipid profiles to be more easily manipulated through dietary changes, compared to milk protein content or lactose concentration. However, other compositional aspects of a cow's diet that bear consideration for potential influence on BMO profiles include the ratios and amounts of fiber and non-fiber carbohydrates, as well as degradable and metabolizable protein.

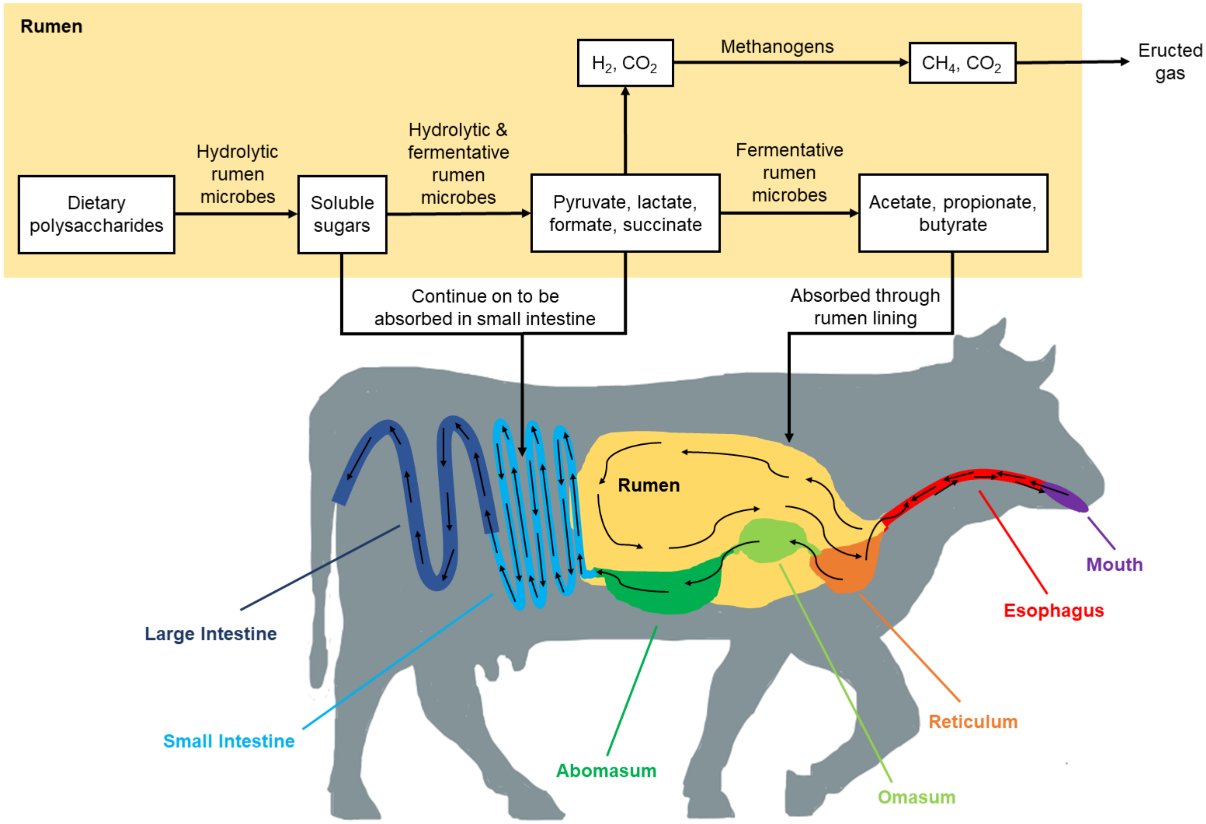


Figure 1.4. Simplified graphical representation of the bovine digestive system and its functions

The carbohydrate portion of feed is generally classified as either fiber – including neutral detergent fiber (NDF; cellulose + hemicellulose + lignin) or acid detergent fiber (ADF; cellulose + lignin) – or non-fiber carbohydrates – including starch and simple sugars. The balance of fiber and non-fiber carbohydrates in feed affects ruminal buffering capacity, with levels of non-fiber carbohydrates greater than 42% dry matter or levels of fiber less than 14 to 16% dry matter often causing ruminal acidosis and the loss of ruminal buffering capacity as fermentable carbohydrates are rapidly broken down by rumen microbes and converted to volatile fatty acids. (Eastridge & Firkins 2011; Ranathunga et al., 2010; Kennelly et al., 1999; Spain et al., 1990) Because fibrolytic rumen microbes are generally pH sensitive, such fluctuations in ruminal pH are likely to lead to decreased fiber breakdown. The fiber content of the diet also stimulates chewing and influences the digesta passage rate, which determines the balance between the breakdown of components in the rumen and absorption of breakdown products in the rumen and small intestine (Figure 1.4). (Ranathunga et al., 2019) The composition and absorption location of these breakdown products may influence how they are utilized by the cow, including as potential precursors for BMO synthesis. Increasing the ratio of NDF to starch has been shown to lead to increased milk yield as well as higher total milk lipids and lactose. Additional considerations for many feeds are what the ratios of cellulose, hemicellulose, and lignin are in the fiber fraction and how the ratio of fiber and non-fiber carbohydrates is impacted by treatment and storage practices, including ensiling. (Miron et al., 2007; Keady et al., 1998; Kelly et al., 1998)

Similarly, the balance of degradable protein, which can be utilized by rumen microbes, and metabolizable protein, which is not broken down by rumen microbes and is available to be digested and absorbed by the cow, influence both rumen and overall cow health. The amino acid composition of metabolizable protein is also an important consideration for cow health and milk production. The limiting amino acids for dairy cattle are generally lysine and histidine, however, methionine may also be limiting for cows fed high-forage or soy hull-based diets. The amino acid composition of feed may also be impacted by feed treatment and storage practices, especially in the case of lysine which is particularly heat sensitive. (Schwab, 2011) Studies examining the impact of changes in protein source on milk composition have found changes in lipid, protein, and lactose concentrations. (Spain et al., 1990; Bernard, 1997; Ørskov et al., 1981) (Bernard, 1997; Ørskov et al., 1981) In addition, diets with a higher ratio of metabolizable to degradable protein have been found to result in increased levels of milk fat and protein. (Ørskov et al., 1981)

In addition to other well-established effects of dietary lipids on milk yield and total milk fat, (van Knegsel et al., 2007; Petit et al., 2001; Cant et al., 1991) modifications to the lipid profile of feed, particularly the ratio of saturated and unsaturated fatty acids, may also impact digestion, nutrient absorption, and milk production. Cant *et al*. (1991) documented decreased fiber digestion with increased levels of yellow grease supplementation and hypothesized that it may have been the result of changes in membrane composition of rumen microbes as the result of incorporating unsaturated fatty acids from the supplemented feed. Such changes in microbial membrane composition and fluidity, if taken to an extreme, could lead to the loss of function of some rumen microbes, vastly impacting the required time for rumination, degree of feed digestion, and nutrient absorption.

Additionally, changing feed compositions or ingredients may also lead to changes in feeding behavior, including the frequency and duration of feeding, (Su et al., 2017; Miron et al., 2007) feed sorting, (Su et al., 2017) and overall levels of dry matter intake. (Su et al., 2017; Ranathunga et al., 2013, 2010) Such changes in behavior may alter the expected quantities and ratios of feed components ingested from the expected values. Maintaining a dietary composition that leads to adequate levels of feed consumption to support lactation and meets all the nutritional needs of the cow is also a necessary consideration. Although the impact of cow health on BMO production is unknown, BMO synthesis is an energy-intensive process, so it is expected that healthier cows would have the capacity to produce BMOs with more complex structures and/or higher concentrations of BMOs.

Another important consideration for any study implementing a dietary modification variable is the duration of the treatment period. Elgersma et al. (2004) found that 4 to 14 days was sufficient treatment length to see a leveling out of changes in total milk fat and milk fatty acid composition due to dietary modification transitioning from fresh grass to ensiled forage. In contrast, Thomson et al. (1985) didn’t see any leveling-off of changes in milk yield, or total milk protein during a 16-week study period looking at the effects of perennial ryegrass versus white clover grazing on milk production and composition. No comparable study has yet been carried out on milk oligosaccharides, but existing studies on the influence of diet on BMO profiles have featured treatment periods of 7 to 28 days. (Liu et al., 2014; Vicaretti et al., 2018) Ensuring that treatment periods are sufficiently long to reveal the full results of dietary alterations will be essential for future dietary treatment studies.

Regardless of the source of the change in BMO profile, it is crucial to consider what effect, if any, increasing BMO content has on other milk components and properties. The Danish-Swedish Milk Genomics Initiative offers a uniquely large dataset including information on a wide range of milk components from cows of several breeds and parities, although the same subsets of samples were not used for all analyses. A study from this initiative reported higher abundances of some BMOs in Danish Jersey compared to Danish Holstein cows, as well as higher abundances of some BMOs in the milk of second parity cows, compared to those in their first and third parities. (Robinson et al., 2019) Other studies have shown that compared to Danish

Holsteins, the milk of Danish Jerseys has increased percent fat, percent protein, and percent casein, particularly κ-casein, which likely contributes to the better coagulative properties of Jersey milk. (Gustavsson et al., 2014; Poulsen et al., 2013, 2012) Though differences in milk composition between breeds is likely more influenced by genetic than environmental factors, these observations suggest that increased BMO abundances and more favorable BMO profiles do not necessarily come at the expense of reduced concentrations of other more traditionally valuable milk components.

CONCLUSIONS

Bovine milk oligosaccharides are a promising ingredient for infant formulas and nutraceuticals due to their numerous demonstrated and hypothesized bioactivities and their potential for large-scale isolation from dairy processing side and waste streams. The low concentrations of BMOs in milk and traditional dairy processing streams present a challenge to isolation. This may be overcome by using more concentrated dairy streams like delactosed permeate and modifying the naturally occurring BMO composition of the starting milk through changes in cows' diets to increase BMO concentrations while also potentially modifying BMO profiles to be more similar to those of human breast milk.

REFERENCES

Ahn WS, Park SJ, Lee SY. 2001. Production of poly(3-hydroxybutyrate) from whey by cell recycle fed-batch culture of recombinant *Escherichia coli*. *Biotechnol. Lett.* 23:235–240.

Ahn WS, Park SJ, Lee SY. 2000. Production of poly(3-hydroxybutyrate) by fed-batch culture of recombinant *Escherichia coli* with a highly concentrated whey solution. *Appl. Environ. Microbiol.* 66:3624–3627.

Akkerman R, Faas MM, de Vos P. 2019. Non-digestible carbohydrates in infant formula as substitution for human milk oligosaccharide functions: Effects on microbiota and gut maturation. *Crit. Rev. Food Sci. Nutr.* 59:1486–1497.

Albrecht S, Lane JA, Mariño K, Al Busadah KA, Carrington SD, Hickey RM, Rudd PM. 2014. A comparative study of free oligosaccharides in the milk of domestic animals. *Br. J. Nutr*. 111:1313–1328.

Aldredge DL, Geronimo MR, Hua S, Nwosu CC, Lebrilla CB, Barile D. 2013. Annotation and structural elucidation of bovine milk oligosaccharides and determination of novel fucosylated structures. *Glycobiology* 23:664–676.

Altmann K, Clawin-Rädecker I, Hoffmann W, Lorenzen P. 2016. Nanofiltration enrichment of milk oligosaccharides (MOS) in relation to process parameters. *Food Bioprocess Technol.* 9:1924–1936.

Altmann K, Wutkowski A, Kämpfer S, Klempt M, Lorenzen PC, Clawin-Radecker I. 2015. Comparison of the efficiency of different NF membranes for the enrichment of milk oligosaccharides from bovine milk. *Eur. Food Res. Technol.* 241:803–815.

American Dairy Products Institute. 2018. Dairy Products Utilization and Production Trends.

Asakuma S, Ueda Y, Akiyama F, Uemura Y, Miyaji M, Nakamura M, Murai M, Urashima T. 2010. Effect of grazing on the concentrations of total sialic acid and hexose in bovine milk. *J. Dairy Sci.* 93:4850–4854.

Barile D, Tao N, Lebrilla CB, Coisson J-D, Arlorio M, German JB. 2009. Permeate from cheese whey ultrafiltration is a source of milk oligosaccharides. *Int. Dairy J*. 19:524–530.

Barile D, Rastall RA. 2013. Human milk and related oligosaccharides as prebiotics. *Curr. Opin. Biotechnol.* 24:214–219.

Bernard JK. 1997. Milk Production and composition responses to the source of protein supplements in diets containing wheat middlings. *J. Dairy Sci.* 80:938–942.

Beucler J, Drake M, Foegeding EA. 2006. Design of a Beverage from Whey Permeate. *J. Food Sci.* 70:S277–S285.

Bode L, Contractor N, Barile D, Pohl N, Prudden AR, Boons G-J, Jin Y-S, Jennewein S. 2016. Overcoming the limited availability of human milk oligosaccharides: challenges and opportunities for research and application. *Nutr. Rev.* 74:635–644.

Boudry G, Hamilton MK, Chichlowski M, Wickramasinghe S, Barile D, Kalanetra KM, Mills DA, Raybould HE. 2017. Bovine milk oligosaccharides decrease gut permeability and improve inflammation and microbial dysbiosis in diet-induced obese mice. *J. Dairy Sci.* 100:2471–2481.

Bradley RL, Rexroat TM. 1988. Acceptance and stability of chocolate and strawberry fountain syrups made with concentrate with concentrated, decolorized, deionized hydrolyzed whey permeate. *J. Food Process. Preserv.* 12:163–170.

Bund RK, Hartel RW. 2010. Effect of protein on sorption characteristics of delactosed permeate. *J. Food Eng.* 96:388–393.

Burrington K, Schoenfuss T, Patel S. 2014. Coproducts of milk and whey processing.

Cant JP, DePeters EJ, Baldwin RL, 1991. Effect of dietary fat and postruminal casein administration on milk composition of lactating dairy cows. *J. Dairy Sci.* 74:211–219.

Carroll SM, DePeters EJ, Taylor SJ, Rosenberg M, Perez-Monti H, Capps VA. 2006. Milk composition of Holstein, Jersey, and Brown Swiss cows in response to increasing levels of dietary fat. *Anim. Feed Sci. Technol.* 131:451–473.

Charbonneau MR, O'Donnell D, Blanton LV, Totten SM, Davis JCC, Barratt MJ, Cheng J, Guruge J, Talcott M, Bain JR, Muehlbauer MJ, Ilkayeva O, Wu C, Struckmeyer T, Barile D, Mangani C, Jorgensen J, Fan Y-M, Maleta K, Dewey KG, Ashorn P, Newgard GB, Lebrilla C, Mills DA, Gordon JI. 2016. Sialylated milk oligosaccharides promote microbiota-dependent growth in models of infant undernutrition. *Cell* 164:859–871.

Cho S, Prosky L, Dreher ML. 1999. *Complex Carbohydrates in Foods*. vol. 93. Marcel Dekker, New York, New York, USA.

Cohen JL, Barile D, Liu Y, de Moura Bell JMLN. 2017. Role of pH in the recovery of bovine milk oligosaccharides from colostrum whey permeate by nanofiltration. *Int. Dairy J.* 66:68–75.

Daverey A, Pakshirajan K. 2010. Sophorolipids from *Candida bombicola* using mixed hydrophilic substrates: Production, purification and characterization. *Colloids Surf. B Biointerfaces* 79:246–253.

de Moura Bell JMLN, Cohen JL, de Aquino LFMC, Lee H, de Melo Silva VL, Liu Y, Domizio P, Barile D. 2018. An integrated bioprocess to recover bovine milk oligosaccharides from colostrum whey permeate. *J. Food Eng.* 216:27–35.

Do Carmo MMR, Walker JCL, Novello D, Caselato VM, Sgarbieri VC, Ouwehand AC, Andreollo NA, Hiane PA, dos Santos EF. 2016. Polydextrose: Physiological Function, and Effects on Health. *Nutrients* 8.

Domingues L, Lima N, Teixeira JA. 2001. Alcohol production from cheese whey permeate using genetically modified flocculent yeast cells. *Biotechnol. Bioeng.* 72:8.

Dong X, Zhou S, Mechref Y. 2016. LC-MS/MS analysis of permethylated free oligosaccharides and N-glycans derived from human, bovine, and goat milk samples: Liquid Phase Separations. *Electrophoresis* 37:1532–1548.

Dornburg V, Hermann BG, Patel MK. 2008. Scenario projections for future market potentials of biobased bulk chemicals. *Environ. Sci. Technol.* 42:2261–2267.

Eastridge ML, Firkins JL. 2011. Feed concentrates: Cereal grains, in: Encyclopedia of Dairy Science, 2rd Ed., Vol. 5, Fuquay JW, ed. Elsevier, Amsterdam, The Netherlands. 335–341.

Elgersma A, Ellen G, van der Horst H, Boer H, Dekker PR, Tamminga S. 2004. Quick changes in milk fat composition from cows after transition from fresh grass to a silage diet. *Anim. Feed Sci. Technol.* 117:13–27.

Ennis BM, Maddox IS. 1985. Use of *Clostridium acetobutylicum* P262 for production of solvents from whey permeate. *Biotechnol. Lett.* 7:601–606.

Fanaro S, Boehm G, Garssen J, Knol J, Mosca F, Stahl B, Vigi V. 2005. Galacto-oligosaccharides and long-chain fructo-oligosaccharides as prebiotics in infant formulas: A review: GOS and FOS in infant formulas. *Acta Paediatr.* 94:22–26.

Fischer-Tlustos AJ, Hertogs K, van Niekerk JK, Nagorske M, Haines DM, Steele MA. 2020. Oligosaccharide concentrations in colostrum, transition milk, and mature milk of primi- and multiparous Holstein cows during the first week of lactation. *J. Dairy Sci.* 103:3683–3695.

Fong B, Ma K, McJarrow P. 2011. Quantification of bovine milk oligosaccharides using liquid chromatography–selected reaction monitoring–mass spectrometry. *J. Agric. Food Chem.* 59:9788–9795.

Frankowski KM, Miracle RE, Drake MA. 2014. The role of sodium in the salty taste of permeate. *J. Dairy Sci*. 97:5356–5370.

Friend MA, Kaiser AG, Piltz JW, Sillence MN, Jolliffe SK. 2004. Use of delactose whey permeate as a supplement for cattle on a cereal straw based diet. *Aust. J. Exp. Agric.* 44:833.

Gabardo S, Rech R, Rosa CA, Ayub MAZ. 2014. Dynamics of ethanol production from whey and whey permeate by immobilized strains of *Kluyveromyces marxianus* in batch and continuous bioreactors. *Renew. Energy* 69:89–96.

Gopal PK, Gill HS. 2000. Oligosaccharides and glycoconjugates in bovine milk and colostrum. *Br. J. Nutr.* 84:69–74.

Gustavsson F, Buitenhuis AJ, Johansson M, Bertelsen HP, Glantz M, Poulsen NA, Mansson HL, Stalhammar H, Larsen LB, Bendixen C, Paulsson M, Andren A. 2014. Effects of breed and casein genetic variants on protein profile in milk from Swedish Red, Danish Holstein, and Danish Jersey cows. *J. Dairy Sci.* 97:3866–3877.

Hargrove RE, McDonough FE, LaCroix DE, Alford JA. 1976. Production and properties of deproteinized whey powders. *J. Dairy Sci.* 59:25–33.

Holst HH, Gunther WS, Mogensen MT, Jorgensen AS. 2007. Concentrate derived from a milk product enriched in naturally occuring sialyllactose and a process for preparation thereof. United States Patent 20070104843A1.

Jahreis G, Fritsche J, Steinhart H. 1997. Conjugated linoleic acid in milk fat: High variation depending on production system. *Nutr. Res.* 17:1479–1484.

Jakobsen LMA, Sundekilde UK, Andersen HJ, Nielsen DS, Bertram HC. 2019. Lactose and bovine milk oligosaccharides synergistically stimulate *B. longum* subsp. *longum* growth in a simplified model of the infant gut microbiome. *J. Proteome Res.* 18:3086–3098.

Jelen P. 2011. Whey processing: Utilization and products, in: Encyclopedia of Dairy Science, 2nd Ed., Vol. 5, Fuquay JW, ed. Elsevier, Amsterdam, The Netherlands. 731–737.

Jena PK, Sheng L, Nagar N, Wu C, Barile D, Mills DA, Wan Y-JY. 2018. Synbiotics *Bifidobacterium infantis* and milk oligosaccharides are effective in reversing cancer-prone nonalcoholic steatohepatitis using western diet-fed FXR knockout mouse models. *J. Nutr. Biochem.* 57:246–254.

Jenkins TC, McGuire MA. 2006. Major advances in nutrition: Impact on milk composition. *J. Dairy Sci.* 89:1302–1310.

Keady TWJ, Mayne CS, Marsden M. 1998. The effects of concentrate energy source on silage intake and animal performance with lactating dairy cows offered a range of grass silages. *Anim. Sci.* 66:21–33.

Kelly ML, Kolver ES, Bauman DE, Amburgh MEV, Muller LD. 1998. Effect of intake of pasture on concentrations of conjugated linoleic acid in milk of lactating cows. *J. Dairy Sci.* 80:1630–1636.

Kennelly JJ, Robinson B, Khorasani GR. 1999. Influence of carbohydrate source and buffer on rumen fermentation characteristics, milk yield, and milk composition in early-lactation Holstein cows. *J. Dairy Sci.* 82:2486–2496.

Kim BG, Lee JW, Stein HH. 2012. Energy concentration and phosphorus digestibility in whey powder, whey permeate, and low-ash whey permeate fed to weanling pigs. *J. Anim. Sci.* 90:289–295.

Koller M, Bona R, Braunegg G, Hermann C, Horvat P, Kroutil M, Martinz J, Neto J, Pereira L, Varila P. 2005. Production of polyhydroxyalkanoates from agricultural waste and surplus materials. *Biomacromolecules* 6:561–565.

Koushki M, Jafari M, Azizi M. 2012. Comparison of ethanol production from cheese whey permeate by two yeast strains. *J. Food Sci. Technol.* 49:614–619.

Kull I, Almqvist C, Lilja G, Pershagen G, Wickman M. 2004. Breast-feeding reduces the risk of asthma during the first 4 years of life. *J. Allergy Clin. Immunol.* 114:755–760.

Lane JA, Mariño K, Naughton J, Kavanaugh D, Clyne M, Carrington SD, Hickey RM. 2012. Anti-infective bovine colostrum oligosaccharides: *Campylobacter jejuni* as a case study. *Int. J. Food Microbiol.* 157:182–188.

Lee C, Kim J, Hwang K, O'Flaherty V, Hwang S. 2009. Quantitative analysis of methanogenic community dynamics in three anaerobic batch digesters treating different wastewaters. *Water Res.* 43:157–165.

Levin MA, Burrington KJ, Hartel RW. 2016. Whey protein phospholipid concentrate and delactosed permeate: Applications in caramel, ice cream, and cake. *J. Dairy Sci.* 99:6948–6960.

Liang B, Bund RK, Hartel RW. 2009. Effect of composition on moisture sorption of delactosed permeate. *Int. Dairy J.* 19:630–636.

Litherland AJ. Woodward SJR, Stevens DR, McDougal DB, Boom CJ, Knight TL, Lambert MG. 2002. Seasonal variations in pasture quality on New Zealand sheep and beef farms. *Proc. N. Z. Soc. Anim. Prod.* **62**:138–142.

Liu Z, Wang T, Pryce JE, MacLeod IM, Hayes BJ, Chamberlain AJ, Jagt CV, Reich CM, Mason BA, Rochfort S, Cocks BG. 2019. Fine-mapping sequence mutations with a major effect on oligosaccharide content in bovine milk. *Sci. Rep.* 9:2137.

Liu Z, Auldist M, Wright M, Cocks B, Rochfort S. 2017. Bovine milk oligosaccharide contents show remarkable seasonal variation and intercow variation. *J. Agric. Food Chem.* 65:1307–1313.

Liu Z, Moate P, Cocks B, Rochfort S. 2014. Simple liquid chromatography–mass spectrometry method for quantification of major free oligosaccharides in bovine milk. *J. Agric. Food Chem.* 62:11568–11574.

Ma L, McJarrow P, Mohamed HJBJ, Liu X, Welman A, Fong BY. 2018. Lactational changes in the human milk oligosaccharide concentration in Chinese and Malaysian mothers' milk. *Int. Dairy J.* 87:1–10.

Mahmoud MM, Kosikowski FV. 1982. Alcohol and single cell protein production by *Kluyveromyces* in concentrated whey permeates with reduced ash. *J. Dairy Sci.* 65:2082–2087.

Maldonado-Gomez MX, Lee H, Barile D, Lu M, Hutkins RW. 2015. Adherence inhibition of enteric pathogens to epithelial cells by bovine colostrum fractions. *Int. Dairy J.* 40:24–32.

Marino K, Lane JA, Abrahams JL, Struwe WB, Harvey DJ, Marotta M, Hickey RM, Rudd PM. 2011. Method for milk oligosaccharide profiling by 2-aminobenzamide labeling and hydrophilic interaction chromatography. *Glycobiology* 21:1317–1330.

Marsaux B, Van den Abbeele P, Ghyselinck J, Prioult G, Marzorati M, Bogicevic B. 2020. Synbiotic effect of *Bifidobacterium lactis* CNCM I-3446 and bovine milk-derived oligosaccharides on infant gut microbiota. *Nutrients* 12:2268.

Martin C, Ling P-R, Blackburn G. 2016. Review of infant feeding: Key features of breast milk and infant formula. *Nutrients* 8:279.

Martín M-J, Martín-Sosa S, García-Pardo L-A, Hueso P. 2001. Distribution of bovine milk sialoglycoconjugates during lactation. *J. Dairy Sci.* 84:995–1000.

Martín-Sosa S, Martín M-J, Hueso P. 2002. The sialylated fraction of milk oligosaccharides is partially responsible for binding to enterotoxigenic and uropathogenic *Escherichia coli* human strains. *J. Nutr.* 132:3067–3072.

McGrath BA, Fox PF, McSweeney PLH, Kelly AL. 2016. Composition and properties of bovine colostrum: a review. *Dairy Sci. Technol.* 96:133–158.

McJarrow P, van Amelsfort-Schoonbeek J. 2004. Bovine sialyl oligosaccharides: seasonal variations in their concentrations in milk, and a comparison of the colostrums of Jersey and Friesian cows. *Int. Dairy J.* 14:571–579.

Mehra R, Barile D, Marotta M, Lebrilla CB, Chu C, German JB. 2014. Novel high-molecular weight fucosylated milk oligosaccharides identified in dairy streams. *PLoS ONE* 9:e96040.

Meinzen-Derr J, Poindexter B, Wrage L, Morrow AL, Stoll B, Donovan EF. 2009. Role of human milk in extremely low birth weight infants' risk of necrotizing enterocolitis or death. *J. Perinatol.* 29:57–62.

Mikhaylin S, Bazinet L, 2006. Electrodialysis applications in the food industry, in: Advances in Food and Nutrition Research. Academic Press. 265–360.

Milner L, Kerry JP, O'Sullivan MG, Gallagher E. 2020. Physical, textural and sensory characteristics of reduced sucrose cakes, incorporated with clean-label sugar-replacing alternative ingredients. *Innov. Food Sci. Emerg. Technol.* 59:102235.

Miron J, Zuckerman E, Adin G, Solomon R, Shoshani E, Nikbachat M, Yosef E, Zenou A, Weinberg ZG, Chen Y, Halachmi I, Ben-Ghedalia D. 2007. Comparison of two forage sorghum varieties with corn and the effect of feeding their silages on eating behavior and lactation performance of dairy cows. *Anim. Feed Sci. Technol.* 139:23–39.

Miron J, Yosef E, Maltz E, Halachmi I. 2003. Soybean hulls as a replacement of forage neutral detergent fiber in total mixed rations of lactating cows. *Anim. Feed Sci. Technol.* 106:20–28.

Nakamura T, Kawase H, Kimura K, Watanabe Y, Ohtani M, Arai I, Urashima T. 2003. Concentrations of sialyloligosaccharides in bovine colostrum and milk during the prepartum and early lactation. *J. Dairy Sci.* 86:1315–1320.

Naranjo VD, Bidner TD, Southern LL. 2010. Comparison of dried whey permeate and a carbohydrate product in diets for nursery pigs. *J. Anim. Sci.* 88:1868–1879.

Nijman RM, Liu Y Bunyatratchata A, Smilowitz JT, Stahl B, Barile D. 2018. Characterization and quantification of oligosaccharides in human milk and infant formula. *J. Agric. Food Chem.* 66:6851–6859.

Obelitz-Ryom K, Bering SB, Overgaard SH, Eskildsen SF, Ringgaard S, Olesen JL, Skovgaard K, Pankratova S, Wang B, Brunse A, Heckmann AB, Rydal MP, Sangild PT, Thymann T. 2019. Bovine milk oligosaccharides with sialyllactose improves cognition in preterm pigs. *Nutrients* 11:1335.

Oliveros E, Vazquez E, Barranco A, Ramirez M, Gruart A, Delgado-Garcia JM, Buck R, Rueda R, Martin MJ. 2018. Sialic acid and sialylated oligosaccharide supplementation during lactation improves learning and memory in rats. *Nutrients* 10:1519.

Ørskov ER, Reid GW, McDonald I. 1981. The effects of protein degradability and food intake on milk yield and composition in cows in early lactation. *Br. J. Nutr.* 45:547–555.

Owen CG. 2005. Effect of infant feeding on the risk of obesity across the life course: A quantitative review of published evidence. *Pediatrics* 115:1367–1377.

Pasotti L. Zucca S, Casanova M, Micoli G, de Angelis MGC, Magni P. 2017. Fermentation of lactose to ethanol in cheese whey permeate and concentrated permeate by engineered *Escherichia coli*. *BMC Biotechnol.* 17:48.

Paterson AHJ. 2009. Production and Uses of Lactose. in *Advanced Dairy Chemistry* vol. 3. Springer. 105–120.

Petit HV, Dewhurst RJ, Proulx JG, Khalid M, Haresign W, Twagiramungu. 2001. Milk production, milk composition, and reproductive function of dairy cows fed different fats. *Can. J. Anim. Sci.* 81:263–271.

Poulsen NA, Bertelsen HP, Jensen HB, Gustavsson F, Glantz M, Mansson HL, Andren A, Paulsson M, Bendixen C, Buitenhuis AJ, Larsen LB. 2013. The occurrence of noncoagulating milk and the association of bovine milk coagulation properties with genetic variants of the caseins in 3 Scandinavian dairy breeds. *J. Dairy Sci.* 96:4830–4842.

Poulsen NA, Gustavsson F, Glantz M, Paulsson M, Larsen LB, Larsen MK. 2012. The influence of feed and herd on fatty acid composition in 3 dairy breeds (Danish Holstein, Danish Jersey, and Swedish Red). *J. Dairy Sci.* 95:6362–6371.

Pratt WJ, Seibert HF, Stribley RC. 1952. Recovery of lactose from mother liquor. United States Patent 2584158A.

Qureshi N, Maddox IS. 2005. Reduction in Butanol Inhibition by Perstraction. *Food Bioprod. Process.* 83:43–52.

Ranathunga SD, Kalscheur KF, Herrick KJ. 2019. Ruminal fermentation, kinetics, and total-tract digestibility of lactating dairy cows fed distillers dried grains with solubles in low- and high-forage diets. *J. Dairy Sci.* 102:7980–7996.

Ranathunga SD, Abdelqader MM, Kalscheur KF. 2013. Nutrient digestion by dairy cows fed diets replacing starch with non- forage fiber. in Energy and protein metabolism and nutrition in sustainable animal production. eds. Oltjen JW, Kebreab E, Lapierre H. Wageningen Academic Publishers. 61–63.

Ranathunga SD, Kalscheur KF, Hippen AR, Schingoethe DJ. 2010. Replacement of starch from corn with nonforage fiber from distillers grains and soyhulls in diets of lactating dairy cows. *J. Dairy Sci.* 93:1086–1097.

Remoroza CA, Liang Y, Mak TD, Mirokhin Y, Sheetlin SL, Yang X, San Andres JV, Power ML, Stein SE. 2020. Increasing the coverage of a mass spectral library of milk oligosaccharides using a hybrid-search-based bootstrapping method and milks from a wide variety of mammals. *Anal. Chem.* 92:10316–10326.

Roberfroid MB. 2007. Inulin-type fructans: Functional food ingredients. *J. Nutr.* 137:2493S-2502S.

Robinson RC, Poulsen NA, Colet E, Duchene C, Larsen LB, Barile D. 2019. Profiling of aminoxyTMT-labeled bovine milk oligosaccharides reveals substantial variation in oligosaccharide abundance between dairy cattle breeds. *Sci. Rep.* 9:5465.

Ruiz-Moyano S, Totten SM, Garrido DA, Smilowitz JT, German JB, Lebrilla CB, Mills DA. 2013. Variation in consumption of human milk oligosaccharides by infant gut-associated srains of *Bifidobacterium breve*. *Appl. Environ. Microbiol.* 79:6040–6049.

Salli K, Anglenius H, Hirvonen J, Hibberd AA, Ahonen I, Saarinen MT, Tiihonen K, Maukonen J, Ouwehand AC. 2019. The effect of 2′-fucosyllactose on simulated infant gut microbiome and metabolites; a pilot study in comparison to GOS and lactose. *Sci. Rep.* 9:13232.

Sánchez-Duarte JI, Kalscheur KF, Casper DP, García AD. 2019. Performance of dairy cows fed diets formulated at 2 starch concentrations with either canola meal or soybean meal as the protein supplement. *J. Dairy Sci.* 102:7970–7979.

Schwab CG. 2011. Feed supplements: Ruminally protected amino acids, in: Encyclopedia of Dairy Science, 2nd Ed., Vol. 5, Fuquay JW, ed. Elsevier, Amsterdam, The Netherlands. 991–997.

Schwendel BH, Wester TJ, Morel PCH, Fong B, Tavendale MH, Deadman C, Shadbolt NM, Otter DE. 2017. Pasture feeding conventional cows removes differences between organic and conventionally produced milk. *Food Chem.* 229:805–813.

Silveira WB, Passos FJV, Mantovani HC, Passos FML. 2005. Ethanol production from cheese whey permeate by *Kluyveromyces marxianus* UFV-3: A flux analysis of oxido-reductive metabolism as a function of lactose concentration and oxygen levels. *Enzyme Microb. Technol.* 36:930–936.

Sischo WM, Short DM, Geissler M, Bunyatratchata A, Barile D. 2017. Comparative composition, diversity, and abundance of oligosaccharides in early lactation milk from commercial dairy and beef cows. *J. Dairy Sci.* 100:3883–3892.

Smith ST, Metzger L, Drake MA. 2016. Evaluation of whey, milk, and delactosed permeates as salt substitutes. *J. Dairy Sci.* 99:8687–8698.

Spain JN, Alvarado MD, Polan CE, Miller CN, McGilliard ML. 1990. Effect of protein source and energy on milk composition in midlactation dairy cows. *J. Dairy Sci.* 73:445–452.

Su H, Akins MS, Esser NM, Ogden R, Coblentz WK, Kalscheur KF, Hatfield R. 2017. Effects of feeding alfalfa stemlage or wheat straw for dietary energy dilution on nutrient intake and digestibility, growth performance, and feeding behavior of Holstein dairy heifers. *J. Dairy Sci.* 100:7106–7115.

Sundekilde UK, Barile D, Meyrand M, Poulsen NA, Larsen LB, Lebrilla CB, German JB, Bertram HC. 2012. Natural variability in bovine milk oligosaccharides from Danish Jersey and Holstein-Friesian breeds. *J. Agric. Food Chem.* 60:6188–6196.

Sunds AV, Bunyatratchata A, Robinson R, Glantz M, Paulsson M, Leskauskaite D, Pihlanto A, Inglingstad R, Devold TG, Vegarud GE, Birgisdottir BE, Gudjonsdottir M, Barile D, Larsen LB, Poulsen NA. 2021. Comparison of bovine milk oligosaccharides in native North European cattle breeds. *Int. Dairy J.* 114.

Tao N, DePeters EJ, Freeman S, German JB, Grim R, Lebrilla CB. 2008. Bovine milk glycome. *J. Dairy Sci.* 91:3768–3778.

Tao N, DePeters EJ, German JB, Grimm R, Lebrilla CB. 2009. Variations in bovine milk oligosaccharides during early and middle lactation stages analyzed by high-performance liquid chromatography-chip/mass spectrometry. *J. Dairy Sci.* 92:2991–3001.

Tetra Pak. 2020. Whey Processing, in: Dairy Processing Handbook.

Thomson DJ, Beever DE, Haines MJ, Cammell SB, Evans RT, Dhanoa MS, Austin AR. 1985. Yield and composition of milk from Friesian cows grazing either perennial ryegrass or white clover in early lactation. *J. Dairy Res.* 52:17–31.

Tzortzis G, Vulevic J. 2009. Galacto-oligosaccharide prebiotics. in Prebiotics and Probiotics Science and Technology. Springer. 207–244.

United States Department of Agriculture National Agricultural Statistics Service. 2020. Dairy Products 2019 Summary.

United States Department of Agriculture National Agricultural Statistics Service. 2019. Dairy Products.

United States Department of Agriculture National Agricultural Statistics Service. 2007. Dairy 2007 Heifer Calf Health and Management Practices on U.S. Dairy Operations.

van Knegsel ATM, van den Brand H, Dijkstra J, wan Straalen WM, Heetkamp MJW, Tamminga S, Kemp B. 2007. Dietary energy source in dairy cows in early lactation: Energy partitioning and milk composition. *J. Dairy Sci.* 90:1467–1476.

van Leeuwen SS. 2019. Challenges and pitfalls in human milk oligosaccharide analysis. *Nutrients* 11:2684.

Vembu R, Rathinam V. 1997. Separation of minerals from whey permeate. United States Patent 5639501A.

Vennemann MM, Bajanowski T, Brinkmann B, Jorch G, Yucesan K, Sauerland C, Mitchell EA. 2009. Does breastfeeding reduce the risk of sudden infant death syndrome? *Pediatrics* 123:e406–e410.

Vicaretti SD, Mohtarudin NA, Garner AM, Zandberg WF. 2018. Capillary electrophoresis analysis of bovine milk oligosaccharides permits an assessment of the influence of diet and the discovery of nine abundant sulfated analogues. *J. Agric. Food Chem.* 66:8574–8583.

Waghorn G, Clark D. 2004. Feeding value of pastures for ruminants. *N. Z. Vet. J.* 52:320–331.

Wagner C, Benecke C, Buchholz H, Beutel S. 2014. Enhancing bioethanol production from delactosed whey permeate by upstream desalination techniques. *Eng. Life Sci.* 14:520–529.

Wong SY, Hartel RW. 2014. Crystallization in lactose refining-A review. *J. Food Sci.* 79:R257–R272.

World Health Organization. 2009. Infant and Young Child Feeding: Model chapter for textbooks for medical students and allied health professionals.

Xue F, Sun F, Jiang L, Hua D, Wang Y, Nan X, Zhao Y, Xiong B. 2019. Effects of partial replacement of dietary forage using kelp powder (*Thallus laminariae*) on ruminal fermentation and lactation performances of dairy cows. *Animals* 9:852.

Yang P, Zhang R, Mcgarvey J, Benemann J. 2007. Biohydrogen production from cheese processing wastewater by anaerobic fermentation using mixed microbial communities. *Int. J. Hydrog. Energy* 32:4761–4771.

CHAPTER II:

A one-year study of human milk oligosaccharide profiles in the milk of healthy UK mothers and their relationship to maternal FUT2 genotype

Published as:

Durham, S.D., Robinson, R.C., Olga, L., Ong, K.K., Chichlowski, M., Dunger, D.B., Barile, D. A one-year study of human milk oligosaccharide profiles in the milk of healthy UK mothers and their relationship to maternal FUT2 genotype. *Glycobiology*. **2021**, *31(10)*, 1254-1267.

ABSTRACT

Human milk oligosaccharides (HMOs) are indigestible carbohydrates with prebiotic, pathogen decoy, and immunomodulatory activities that are theorized to substantially impact infant health. The objective of this study was to monitor HMO concentrations over one year to develop a long-term longitudinal dataset. HMO concentrations in the breast milk of healthy lactating mothers of the Cambridge Baby Growth and Breastfeeding Study (CBGS-BF) were measured at birth, 2 weeks, 6 weeks, 3 months, 6 months and 12 months postpartum. HMO quantification was conducted by high-performance anion-exchange chromatography with pulsed amperometric detection using a newly validated "dilute-and-shoot" method. This technique minimizes sample losses and expedites throughput, making it particularly suitable for the analysis of large sample sets. Varying patterns of individual HMO concentrations were observed with changes in lactation time point and maternal secretor status, with the most prominent temporal changes occurring during the first 3 months. This data provides valuable information for the development of human milk banks in view of targeted distribution of donor milk based on infant age. Maternal FUT2 genotype was determined based on identification at single-nucleotide polymorphism rs516246 and compared with the genotype expected based on phenotypic markers in the HMO profile. Surprisingly, two mothers genotyped as secretors produced milk that displayed very low levels of 2'-fucosylated moieties. This unexpected discrepancy between genotype and phenotype suggests that differential enzyme expression may cause substantial variation in HMO profiles between genotypically similar mothers, and current genotypic methods of secretor status determination may require validation with HMO markers from milk analysis.

INTRODUCTION

Human milk oligosaccharides (HMOs) are a class of bioactive carbohydrates that are one of the most abundant components of breast milk, with total estimated concentrations in the range of 5 – 25 g/L. (Gabrielli et al., 2011; Huang et al., 2019; Ma et al., 2018) These carbohydrates are composed of between 3 and 20 monosaccharide units and generally feature a lactose core at the reducing end. The backbones of HMOs are extended from the lactose core through the addition of galactose and *N*-acetylglucosamine units and may be further decorated with fucose or *N*-acetylneuraminic acid. More than 200 unique HMOs have been reported to date, with at least 164 structures fully elucidated. (Urashima et al., 2018; Chen et al., 2015; Kobata et al., 2010; Ninonuevo et al., 2006; Wu et al., 2011, 2010) HMOs have garnered substantial recent interest because, despite being assembled at considerable energetic cost to the mother, they are mostly undigested by the neonate. (Gnoth et al., 2000; Leoz et al., 2013; Rudloff et al., 1996) A small portion is absorbed, entering the infant's circulatory system, (Goehring et al., 2014) while the majority reach the colon largely intact. (Chaturvedi et al., 2001; Engfer et al., 2000; Gnoth et al., 2000) Much HMO-related research has therefore focused on identifying the functional purpose of these molecules.

HMOs are prebiotic, selectively promoting the growth of beneficial bacteria in the infant gut. (Bai et al., 2018; Marcobal et al., 2010; Pacheco et al., 2015; Underwood et al., 2015; Ward et al., 2007; Yu et al., 2013) These beneficial bacteria bind to the intestinal epithelium, reducing the opportunities for pathogens to colonize, (Chichlowski et al., 2012) as well as producing short chain fatty acids which lower the pH of the gut, (Langhendries et al., 1995; Midtvedt and Midtvedt, 1992; Scott et al., 2014) making the environment unfavorable for pathogen colonization. The produced short chain fatty acids further benefit human health by serving as substrates for host processes such as colonocyte metabolism and gluconeogenesis. (Wong et al., 2006) In addition, studies suggest that HMOs have other structure-specific functions including acting as receptor decoys to which pathogens may bind in place of host epithelial cells, (Coppa et al., 2006; Manthey et al., 2014; Ruiz-Palacios et al., 2003) strengthening gut-barrier function, (Boudry et al., 2017) and reducing gut inflammation by limiting the binding of lymphocytes, monocytes, and neutrophils to epithelial cells. (Bode et al., 2004; Terrazas et al., 2001) Consumption of sialylated HMOs has also contributed to brain development in experiments with piglet models. (Jacobi et al., 2016)

HMO profiles vary between mothers based on gestational age at birth, (Austin et al., 2019; Gabrielli et al., 2011; Spevacek et al., 2015) and maternal secretor and Lewis status, (Azad et al., 2018; Cabrera-Rubio et al., 2019; Chaturvedi et al., 2001; Erney et al., 2000; Sprenger et al., 2017; Thurl et al., 2010; van Leeuwen et al., 2018; Xu et al., 2017) as well as between milk samples from the same mother based on lactation stage. (Austin et al., 2016; Ma et al., 2018; McJarrow et al., 2019; Samuel et al., 2019; Sprenger et al., 2017; Thurl et al., 2010) Secretor status is linked with the expression of the secretor gene which codes for the fucosyltransferase 2 (FUT2) enzyme. Secretor positive mothers are characterized by the presence of α1,2-fucosylated HMOs in their milk, while secretor negative mothers produce milk with little to no α1,2-fucosylated HMOs. Similarly, Lewis status is based on the expression of the Lewis gene which encodes fucosyltransferase 3 (FUT3). Lewis positive mothers produce milk with substantial levels of α1,3- and α1,4-fucosylated HMOs, while the milk of Lewis negative mothers contains lower levels of α1,3-fucosylated and little or no α1,4-fucosylated HMOs. (Bode, 2015; Newburg et al., 2004) It is especially important to determine the mother's secretor status in clinical studies because there is strong evidence that maternal secretor status and the concentration of α1,2-fucosylated HMOs in breast milk influence the infant gut microbiota composition, (Bai et al., 2018; Borewicz et al., 2020; Cabrera-Rubio et al., 2019; Lewis et al., 2015; Moossavi et al., 2019; Underwood et al., 2015) which has been associated with differential health outcomes for the infant. (Davis et al., 2017; Morrow et al., 2004)

From the perspective of promoting healthy infant development, understanding the changes that occur in milk's HMO content over time could be particularly informative. The World Health Organization recommends exclusive breastfeeding for the first six months of life, followed by complementary feeding in which breastfeeding is continued up to two years of age. (World Health Organization, 2018) Longitudinal studies that track HMO concentrations and infant health over this time span could identify important trends in, and relationships between, these variables. When mother's own milk is not available, infant formula is commonly used as an alternative source of infant nutrition. Several companies have successfully produced HMOs, a couple of which are added in low amounts to some infant formulas. Identifying changes over time in milk composition could also ensure that biologically appropriate amounts of HMOs are added to infant formulas according to infant age. Similarly, data on how milk composition changes over time could be applied in human milk banks to develop batches segregated based on appropriate corresponding infant age. Several studies have measured HMO concentrations in populations of mothers across multiple time points, primarily within the range of 0 to 6 months postpartum. (Chaturvedi et al., 2001; Coppa et al., 1999; Kunz et al., 2017; Ma et al., 2018; Perrin et al., 2017; Samuel et al., 2019; Sprenger et al., 2017; Thurl et al., 2010) Furthermore, as

HMO discovery-based studies lead to translational applications such as infant formula production and milk bank development to target specific infant subgroups based on age and developmental stage, it will be particularly important to reconcile variations among HMO datasets arising from differences in sampling procedures, genetics, geographic location, HMO extraction, and analytical methodology. In this study, absolute quantities of HMOs were measured in a one-year longitudinal sampling of hind milk from mothers residing in the United Kingdom who gave birth to a healthy term infant, with the objectives of identifying significant variations in HMO concentrations based on sampling time, maternal genetics, and infant growth. Lastly, we have specifically investigated the relationship between maternal genotype and the concentration α1-2-linked fucose in human milk. HMO measurements were performed using a novel analytical approach that minimizes sample handling and extraction steps, reducing opportunities for losses during sample preparation and increasing throughput.

RESULTS

HPAEC-PAD "Dilute-and-Shoot" Method Validation

HMOs were quantified on a ThermoFisher Scientific Dionex ICS 5000+ high-performance anion-exchange chromatography system with pulsed amperometric detection (HPAEC-PAD) with a Dionex IonPac NG1 column that served as a trap column for on-line removal of hydrophobic sample components. To verify that HMOs were not retained during this on-line sample clean-up, measurements of recovery and repeatability were evaluated for each HMO. Recovery values varied from 89.1 – 106.6% (Table 2.1), indicating minimal losses and reasonably high measurement accuracy. Repeated injections of a breast milk sample showed reproducible results for all quantified HMOs with coefficients of variation less than 3% for all HMOs except 3-fucosyllactose (3-FL), which had a coefficient of variation of 8.5% due to challenges with peak integrations caused by a closely eluting peak in some samples (Table 2.2). Additionally, sample replicates injected several hundred injections apart produced very consistent results without significant rise in the baseline or loss of signal.

Table 2.1. HMO recovery measurements. Values are expressed as the mean ± standard deviation of triplicate measurements. Spiking levels 1 – 5 signify the addition of 1, 5, 9, 13, and 17 mg/L, respectively, for 3-fucosyllactose and 2'-fucosyllactose. For the remaining oligosaccharides, spiking levels 1 – 5 signify the addition of 4, 8, 12, 16, and 20 mg/L, respectively

	Spiking level				
Oligosaccharide	**1**	**2**	**3**	**4**	**5**
3-Fucosyllactose	91.9% ± 2.4%	94.0% ± 0.7%	93.4% ± 0.5%	93.9% ± 0.4%	96.2% ± 0.1%
2'-Fucosyllactose	106.2% ± 5.9%	99.4% ± 6.6%	96.8% ± 2.1%	99.1% ± 0.7%	95.8% ± 0.7%
Lacto-*N*-fucopentaose I	90.3% ± 1.0%	106.6% ± 3.5%	103.1% ± 1.7%	103.9% ± 0.6%	101.6% ± 1.2%
Lacto-*N*-*neo*tetraose	99.5% ± 0.3%	100.5% ± 2.6%	99.5% ± 1.2%	100.7% ± 1.0%	98.8% ± 0.5%
Lacto-*N*-tetraose	93.7% ± 1.3%	100.6% ± 5.1%	98.7% ± 2.4%	100.5% ± 0.2%	97.7% ± 0.2%
6'-Sialyllactose	89.1% ± 3.1%	97.9% ± 2.7%	94.8% ± 1.6%	98.4% ± 1.0%	98.3% ± 1.5%
3'-Sialyllactose	93.4% ± 1.2%	98.1% ± 0.5%	97.4% ± 2.1%	97.8% ± 1.6%	98.8% ± 0.9%

Table 2.2. HMO repeatability measurements. Values represent the average of five replicate injections of a 6-week postpartum human milk sample

Oligosaccharide	Average (g/L)	Standard deviation (g/L)	Coefficient of variation
3-Fucosyllactose	0.394	0.033	8.5%
2'-Fucosyllactose	2.065	0.015	0.7%
Lacto-*N*-fucopentaose I	0.621	0.008	1.3%
Lacto-*N*-*neo*tetraose	0.069	0.002	2.6%
Lacto-*N*-tetraose	2.527	0.033	1.3%
6'-Sialyllactose	0.193	0.003	1.5%
3'-Sialyllactose	0.071	0.002	2.8%

An external calibration for each HMO was constructed to cover a wide range of natural variations in HMO concentrations. All seven HMOs showed good linearity in response over the given concentration range (Table 2.3, R^2 = 0.9998-1.0000). The limits of quantification (LOQs) were set at a signal-to-noise ratio of 6 to 1, and the limits of detection (LODs) were set at a signal to noise ratio of 3 to 1. For most of the HMOs examined, these ratios translated into LODs ≤1 ng and LOQs ≤3 ng. LOQ and LOD values for individual quantified HMOs are displayed in Table 2.3.

Table 2.3. HMO limits of detection, limits of quantification and linear dynamic ranges. Limits of detection and quantification were established at signal-to-noise ratios of 3 to 1 and 6 to 1, respectively

Oligosaccharide	LOD (ng)	LOQ (ng)	Linear Dynamic Range
3-Fucosyllactose	0.1	0.2	0.3 – 20 mg/L
2'-Fucosyllactose	0.3	0.6	0.3 – 20 mg/L
Lacto-*N*-fucopentaose I	0.3	0.5	0.3 – 30 mg/L
Lacto-*N*-*neo*tetraose	0.2	0.3	0.3 – 30 mg/L
Lacto-*N*-tetraose	1.0	3.0	0.6 – 30 mg/L
6'-Sialyllactose	1.5	3.0	0.6 – 30 mg/L
3'-Sialyllactose	0.45	0.7	1.0 – 30 mg/L

HMO Trends over Lactation

A total of 167 milk samples were analyzed from 71 term mothers. Average concentrations for most quantified HMOs decreased over the course of lactation for both secretor and non-secretor mothers, as shown in Figure 2.1. The exceptions to this trend were 3-FL and 3'-sialyllactose (3'-SL), which were lower in early lactation and increased in concentration over time. Since concentrations of many HMOs vary depending on maternal secretor status, statistical tests were applied to secretor and non-secretor data separately to identify differences in HMO concentrations by time point. Participation rates were the highest at two and six weeks postpartum, and our data shows that the most significant changes occur during this early phase of lactation among the HMOs that decrease over time, both in secretors and non-secretors. Increases in 3-FL and 3'-SL over time were statistically significant, except for 3-FL in non-secretors (Figure 2.1, right panel).

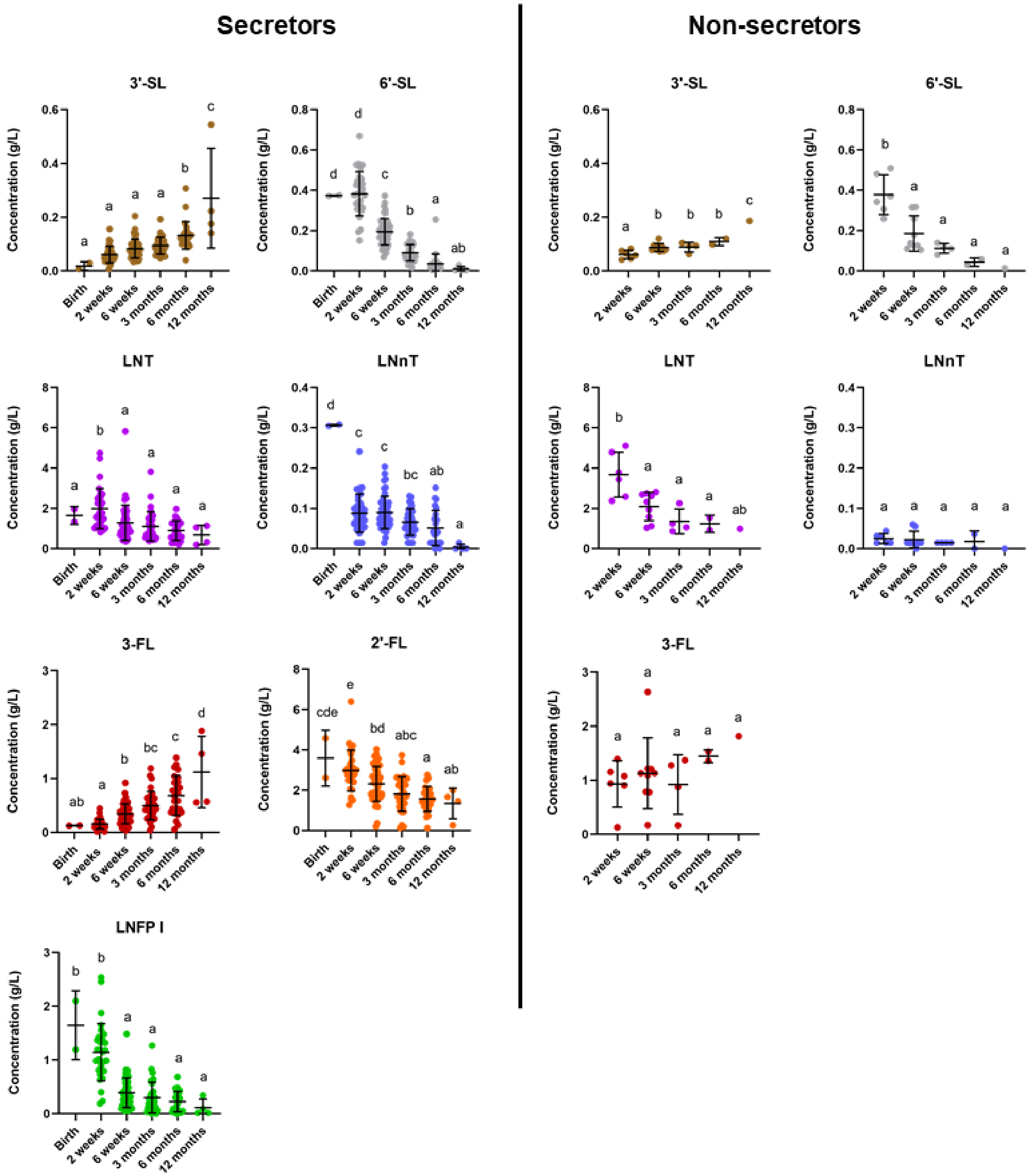


Figure 2.1. Human milk oligosaccharide concentrations at birth, 2 weeks, 6 weeks, 3 months, 6 months, and 12 months postpartum in secretor (left) and non-secretor (right) mothers. For each oligosaccharide, different letters indicate significant differences (α=0.05) as identified by single factor ANOVA and Tukey pairwise comparisons.

3-FL and lacto-*N*-tetraose (LNT) concentrations were notably higher among non-secretor mothers compared to secretor mothers, with a significant difference (p<0.05) in concentrations for the two groups at 2 weeks and 6 weeks postpartum for both 3-FL and LNT, as well as at 6 months postpartum for 3-FL (Figure 2.2).

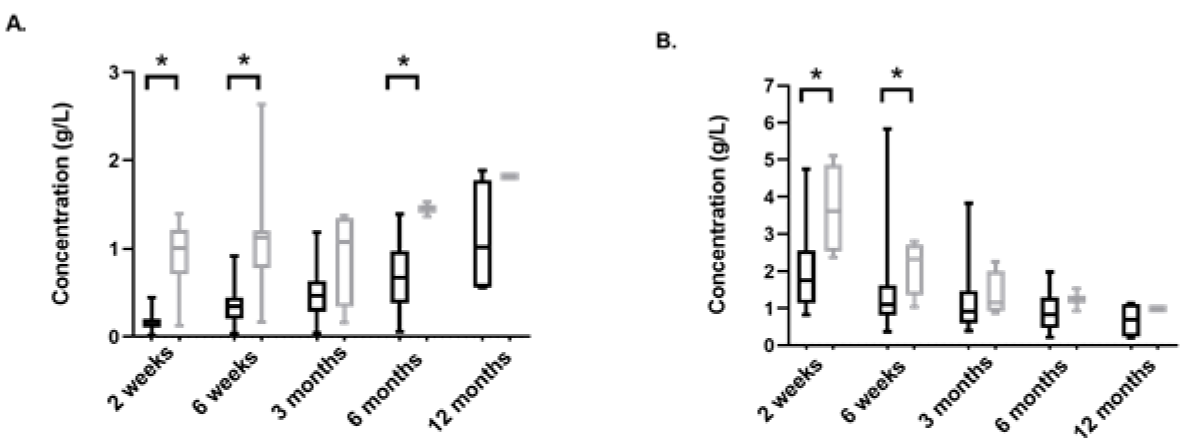


Figure 2.2. Concentrations of (**A**) 3-fucosyllactose and (**B**) lacto-*N*-tetraose in the breast milk of secretor (black) and non-secretor (grey) mothers across lactation. Boxes represent the interquartile range (25 to 75%) and the interior line indicates the mean. An asterisk indicates significant difference ($P < 0.05$) in HMO concentration between secretor and non-secretor mothers at the bracketed time point.

Both secretors and non-secretors had opposite trends in average 3’-SL versus 6’-sialyllactose (6’-SL) concentrations over the course of lactation, in which the more abundant acidic oligosaccharide reversed at around 3 months of lactation, as shown in Figure 2.3. Although the average 3’-SL concentrations were low in early lactation, their increase over time resulted in an average 3’-SL concentration at 1 year postpartum that was comparable to early-lactation concentrations of 6’-SL. Intrigued by this finding, we re-analyzed existing longitudinal quantitative HMO data in the published literature to validate our finding and assess whether the switch in the concentration of acidic HMOs does indeed occur at around 3 months of lactation in a consistent manner across longitudinal clinical studies. We found our observation to be consistent with patterns in 3'-SL and 6'-SL concentration changes in previous reports from numerous cohorts across Europe (Austin et al., 2019; Coppa et al., 1999; Gabrielli et al., 2011; Samuel et al., 2019; Thurl et al., 2010) and eastern Asia (Austin et al., 2016; Ma et al., 2018; Sprenger et al., 2017; Sumiyoshi et al., 2003) (Supplementary Figures 2.2 & 2.3).

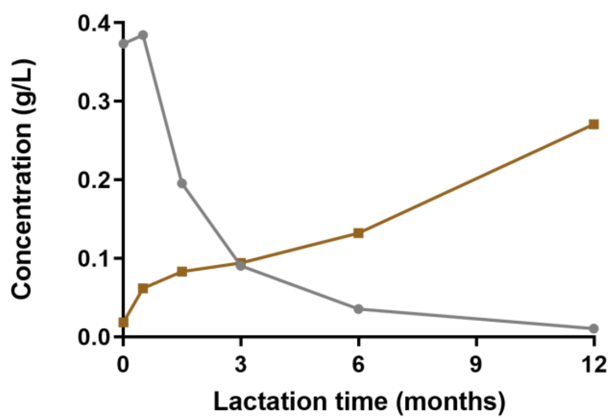


Figure 2.3. Intersecting trends in average 3'-sialyllactose (brown squares) and 6'-sialyllactose (grey circles) concentrations over the first 12 months of lactation.

Secretor Status Determination

In this study, the secretor status of a subset of the mothers was determined through FUT2 genotyping based on the single-nucleotide polymorphism (SNP) at rs516246, with mothers possessing G/G and A/G alleles being secretors, and those with A/A alleles being non-secretors. A large variation was observed for 2'-FL levels in breastmilk, with nearly all genotypic secretor mothers expressing 2'-FL at concentrations between 0.12 and 6.4 g/L, while genotypic non-secretors always produced 2'-FL concentrations below 0.1 g/L, suggesting that 0.1 g/L 2'-FL could be used as a threshold to phenotypically distinguish secretor from non-secretor mothers (Figure 2.4). All mothers with 2'-FL levels below 0.1 g/L also produced milk with low levels of lacto-*N*-fucopentaose I (LNFP I), the other quantified α1,2-fucosylated HMO.

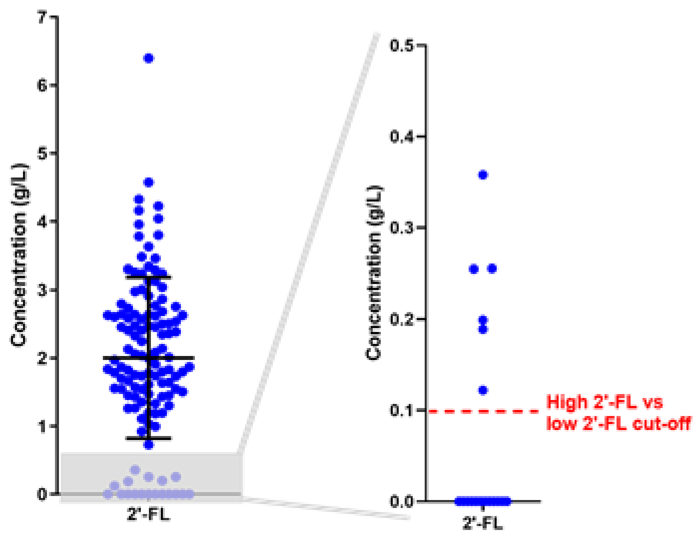


Figure 2.4. 2'-Fucosyllactose concentration cut-off used for the designation of phenotypic secretors and non-secretors from breast milk samples across the first year of lactation.

Two mothers, despite being genotyped as secretors, produced milk that featured exceptionally low concentrations of α1,2-fucosylated HMOs throughout lactation. For these mothers, the genotyping procedure was repeated to ensure that the secretor genotype had not been assigned in error, and the results of the re-test confirmed the FUT2 positive genotypes. In addition, the phenotypic analyses for these mothers were repeated and the presence of very low levels of α1,2-fucosylated HMOs was confirmed by monitoring LNFP I on a capillary electrophoresis system featuring a lower LOD than the HPAEC-PAD method used for HMO quantification. The milk of both mothers with discrepancies between secretor status genotype and phenotype was found to have a consistently lower relative abundances of LNFP I than levels typical of other FUT2 positive mothers in the dataset, across lactation (Supplementary Figure 1). All other mothers with secretor genotype produced breastmilk with 2'-FL concentrations above the 0.1 g/L cut-off at all measured lactation time points.

DISCUSSION

The trends in concentration over the course of lactation for all quantified HMOs were consistent with the results of previously published HMO profiles for longitudinal cohorts, (Coppa et al., 1999; Ma et al., 2018; Samuel et al., 2019; Sprenger et al., 2017; Sumiyoshi et al., 2003; Thurl et al., 2010) although very few other studies span points across a full year of lactation. With the exception of 3-FL and 3'-SL, average HMO concentrations declined over the first year of lactation, with these changes occurring rapidly over the first three months (Figure 2.1). In both secretors and non-secretors, 3-FL and 3'-SL increased steadily throughout the study, and 3-FL was consistently more concentrated in non-secretors at each time point. The lack of statistical significance in the increase of 3-FL over time in non-secretors is likely due to the smaller proportion of non-secretor milk samples available to the study (Figure 2.1, right panel).

Neutral HMOs

The higher 3-FL and LNT concentrations in non-secretor mothers were further explored, with 3-FL concentrations found to be significantly higher ($p<0.05$) in the milk of non-secretor mothers compared to secretor mothers at 2 weeks, 6 weeks, and 6 months post-partum and LNT concentrations found to be significantly higher ($p<0.05$) for non-secretor mothers at 2- and 6-weeks post-partum (Figure 2.2). The increased synthesis of these compounds in the mammary gland is likely the result of decreased competition for sugar nucleotide substrates in the absence of active FUT2 enzymes. This absence of FUT2 activity increases the effective GDP-fucose substrate availability and allows for greater rates of fucosylation by other fucosyltransferase enzymes, such as the FUT3 enzyme that produces 3-FL. If not all of the additional available substrate undergoes alternative fucosylation, increased concentrations of remaining unfucosylated precursor oligosaccharides would remain, as with LNT. This is consistent with previous observations reporting that the concentration of LNT in milk was higher for non-secretor mothers compared to secretor mothers, and among non-secretors, was higher in Lewis negative mothers than Lewis positive mothers. (Samuel et al., 2019)

Unlike some previous studies, (Austin et al., 2016; Erney et al., 2000; Nakhla et al., 1999; Prieto, 2012) we did not observe any mothers with exceptionally low levels of 3-FL at any point during lactation, which suggests that none of the mothers in the present study were both Lewis negative and missing the function of the secondary α1,3-fucosyltransferase. (van Leeuwen et al., 2018) The absence of graphitized carbon solid phase extraction in our sample preparation method is key for ascertaining 3-FL levels with confidence, as 3-FL has been demonstrated to have poor retention on graphitized carbon, (Xu et al., 2017) and the application of graphitized carbon solid phase extraction for HMO isolation prior to analysis may lead to substantial under-quantification of this compound.

Acidic HMOs

Because the inverted trends in average 3’-SL and 6’-SL concentrations had not been noted in previous studies, we further investigated our own results (Figure 2.3) and compared them with information we extracted from the published literature (Supplementary Figures 2.2 & 2.3). The initially high but decreasing average 6'-SL concentrations paired with the initially low but increasing average 3'-SL concentrations result in a relatively steady average concentration of the quantified acidic HMOs over the first year of lactation. Although the same degree of increase in 3'-SL concentration was not observed for all of the previous longitudinal HMO studies, the decline in 6'-SL concentrations is clear across the existing longitudinal HMO literature. Similar observations of substantially increased 3'-SL concentrations in later lactation may have been hindered by shorter lactation durations sampled for previous cohorts. This switch in 6'-SL and 3'-SL abundances will be an important factor for consideration as manufacturers consider which sialyllactose isomer(s) to include in infant formula for targeted age groups to best align its composition with breast milk.

Very few studies have investigated both 3'-SL and 6'-SL in a side-by-side comparison for antiadhesive or other antipathogenic effects against common infant gastrointestinal pathogens, and in those that have, there is a lack of reporting whether any significant differences exist between the effects of the two isomers. Among *in vitro* studies, there have been reports of greater inhibition of adhesion of *Salmonella enterica* ssp. *enterica* ser. fyris by 6'-SL compared to 3'-SL, (Coppa et al., 2006) strain dependence in the degree of inhibition of hemagglutination of enterotoxigenic *Escherichia coli* (ETEC) and enteropathogenic *E. coli* (EPEC) by both 6'-SL and 3'-SL, (Coppa et al., 2006; Martín-Sosa et al., 2002) and greater inhibition of both hemagglutination and adhesion of S. fimbriated *E. coli* by 3'-sialylated oligosaccharides including 3'-SL compared to their 6'-sialyllated analogues. (Parkkinen et al., 1986)

In relation to prebiotic activity, the sialidases of *Bifidobacterium longum* ssp. *infantis*, a prevalent beneficial gut microbe in infants, have shown a greater affinity for α2,6-linked than α2,3-linked Neu5Ac.(Sela et al., 2011) In addition, several strains of *B. breve* have demonstrated a greater percent consumption of sialyllacto-*N*-tetraose b (LSTb, Gal(β1,3)[Neu5Ac(α2,6)]GlcNAc(β1,3)Gal(β1,4)Glc) compared to its α2,3-linked Neu5Ac-containing counterpart, sialyllacto-*N*-tetraose a (LSTa, Neu5Ac(α2,6)Gal(β1,3)GlcNAc(β1,3)Gal(β1,4)Glc), in an *in vitro* study. (Ruiz-Moyano et al., 2013) In a pre-clinical piglet model, microbiome differences were observed in the proximal and distal colons of piglets fed control compared with 6'-SL-enriched formulas, but no significant differences were observed between control and 3'-SL-enriched formulas. (Jacobi et al., 2016) In another study, both 3'-SL and 6'-SL were shown to support normal microbial communities and behavioral responses in piglets during stressor exposure, potentially through effects on the gut microbiota–brain axis. (Tarr et al., 2015)

Both 3'-SL and 6'-SL have been linked with sialylation of brain gangliosides and improved learning outcomes compared to non-sialyllactose-supplemented controls. (Jacobi et al., 2016; Oliveros et al., 2018; Sakai et al., 2006) In a preclinical model, increasing doses of 3'-SL increased enrichment of ganglioside-bound sialic acid in the cerebellum of neonatal pigs. In addition, total sialic acid was also increased in the corpus callosum of pigs fed the lower doses of both 3'-SL and 6'-SL. (Jacobi et al., 2016)

Secretor Status Determination

The most commonly differentiated maternal phenotype impacting HMO profiles is secretor status. In most previously published HMO studies, maternal secretor status has been assigned based on the degree to which α1,2-fucosylated moieties are present in a given mother's milk as measured by NMR (Smilowitz et al., 2013; Spevacek et al., 2015, van Leeuwen 2018, van Leeuwen 2014) and a variety of chromatographic methods, including HPAEC-PAD (Erney et al., 2000; Gabrielli et al., 2011; Sprenger et al., 2017) as well as HPLC coupled with fluorescence-, (Alderete et al., 2015; Austin et al., 2019; Azad et al., 2018; Ferreira et al., 2020; Larsson et al., 2019; Saben et al., 2020; Samuel et al., 2019) UV-, (Ma et al., 2018; McGuire et al., 2017) or mass spectrometry-based detectors. (Goehring et al., 2014; Tonon et al., 2019a; Tonon et al., 2019b) Those with little to no α1,2-fucosylated HMOs are categorized as non-secretors and assumed to be homozygous for the recessive FUT2 allele (*se/se*), while all other mothers are characterized as secretors and assumed to be either heterozygous or homozygous for the dominant FUT2 allele (*Se/se* or *Se/Se*). Although some studies have observed a complete absence of α1,2-fucosylated HMOs in the milk of the mothers they categorized as non-secretors, (Borewicz et al., 2019; van Leeuwen et al., 2018; van Leeuwen et al., 2014) other studies, including the present analysis, have observed very low concentrations of α1,2-fucosylated HMOs in phenotypic non-secretors. In the latter situation, however, the threshold separating designated secretors and non-secretors varies substantially between studies, often being set at either the LOD or LOQ for 2'-FL or LNFP I – so that it varies between analytical methods – or where there is a natural break in concentrations of 2'-FL and/or LNFP I – so that the cut-off varies between cohorts.

In this study, we had a unique opportunity to compare FUT2 genotyping results for a subset of the mothers in the cohort with their corresponding HMO profiles to determine whether a concentration threshold for a specific HMO can indeed be used to differentiate secretor genotypes. After examining each dataset, we were able to distinguish secretor mothers from non-secretors by selecting a 2'-FL concentration cut-off of 0.1 g/L, with genotypic non-secretors always producing 2'-FL concentrations below the cut-off (Figure 2.4). This value successfully distinguished the FUT2 status of all mothers with the potential exception of two subjects for which the maternal genotype and phenotype were consistently not in alignment. These mothers were secretors by genotype but had 2'-FL concentrations below the LOD (6 mg/L in the milk) for all time points. However, LNFP I concentrations for both mothers were frequently in between the LOD and LOQ, indicating a low level of α1-2 fucosyltransferase activity. This is a novel observation that has not been noted in previously published HMO studies. We propose that these individuals may have an alternate mutation to the FUT2 gene that limits the activity of the FUT2 enzyme.

The nonsense mutation exchanging adenine for guanine 428 in the FUT2 gene is the most commonly reported in Caucasian populations and was thus selected as the target SNP for the present study, which features a cohort of predominantly Caucasian mothers (91.5% Caucasian, 3.2% Asian, 1.0% Black, 4.3% multiple racial or ethnic identities); however, several other SNPs in the FUT2 gene have also been documented to result in limited enzyme function after translation. Among these are the nonsense mutations at nucleotides 571 (C→T), 357 (C→T) and 628 (C→T), and missense mutations at nucleotides 302 (C→T) and 385 (A→T). (Chang et al., 1999; Guo et al., 2017; Henry et al., 1996; Koda et al., 1996; Liu et al., 1998; Pang et al., 2001, 2000; Park et al., 2010; Yip et al., 2007) The missense mutation substituting adenine 385 in the FUT2 gene to thymine causes the transcription of phenylalanine instead of isoleucine at amino acid position 129 in the resulting protein. This mutant FUT2 enzyme has a similar substrate binding affinity to the wild type, but only 20% of the enzyme activity. (Henry et al., 1996) This mutation, frequently referred to as the weak secretor (Se^{385} or Se^{W}) allele, is well documented in Asian populations, but much more rarely identified among Caucasians. All of the other listed mutations result in FUT2 expression levels characteristic of non-secretors.

Although alternative methods of identifying secretor status have been applied in previous HMO studies, most techniques, including saliva hemagglutinin inhibition, blood typing, and thresholds based on concentrations or ratios of specific HMOs rely on phenotypic rather than genotypic markers, and therefore may not reflect true maternal genotype. Additionally, apparent secretor or Lewis phenotype in some body fluids or tissues may change with disease, organ transplants, or pregnancy. (Henry et al., 1996) Genotypic identification of secretors, however, is fraught with its own challenges due to the large number of potential SNPs in the FUT2 gene and the wide variation of SNPs between regional and ethnic populations, even between groups of similar phenotypic proportions of secretors and non-secretors. (Ferrer-Admetlla et al., 2009)

The subset of the study population that was FUT2 genotyped at SNP rs516246 (50 mothers) was 88% secretors. Based on phenotypic expression of α1,2-fucosylated HMOs in all 69 mothers in the cohort, the study population is 84% secretors. Both of these distributions are consistent with those reported in previous HMO studies for geographically similar populations. (Erney et al., 2000; Samuel et al., 2019; Thurl et al., 2010)

Variation of HMO Concentrations in the Published Literature

Even among studies with populations of similar reported secretor status distributions, concentrations of HMOs at comparable lactation time points vary substantially (Supplementary Table 2.1). The source of this variation has been attributed to a number of factors including geographical location, (Azad et al., 2018; Chaturvedi et al., 2001; Erney et al., 2000; McGuire et al., 2017; Samuel et al., 2019) maternal pre-pregnancy BMI, (Azad et al., 2018; Ferreira et al., 2020; McGuire et al., 2017; Samuel et al., 2019; Wang et al., 2020) gestation duration, (Austin et al., 2019; Gabrielli et al., 2011; Spevacek et al., 2015; Sundekilde et al., 2016; Wang et al., 2020) parity, (Azad et al., 2018; Ferreira et al., 2020; Samuel et al., 2019; Wang et al., 2020) mode of delivery, (Samuel et al., 2019; Wang et al., 2020) maternal age, (Azad et al., 2018; McGuire et al., 2017) infant sex, (Tonon et al., 2019a; Wang et al., 2020) and maternal disease status. (Bode et al., 2012; Olivares et al., 2015; Tonon et al., 2019a; Van Niekerk et al., 2014) Interestingly, many of these variables have also been found not to influence HMO profiles in other cohorts. (Austin et al., 2016; Azad et al., 2018; Nakhla et al., 1999) Additional studies will be needed to determine whether these are biologically driving factors of HMO composition or have previously correlated with HMO abundances for other reasons.

Samuel et al. (2019) recently suggested that the variation in α1,2-fucosylated HMO concentrations among secretor individuals may be partially explained by whether the mother is homozygous or heterozygous for the dominant FUT2 allele (*G*/*G* versus *A*/*G*, as assessed at the rs516246 SNP). As depicted in Figure 2.5, for the present cohort, no significant difference ($p = 0.1349$) was found in 2'-FL concentrations between *G/G* and *A/G* mothers, based on FUT2 genotyping at SNP rs516246. A significant difference was observed, however, in LNFP I concentrations ($p = 0.01$) between the milk of heterozygous and homozygous FUT2 positive mothers. It is possible that the homozygous versus heterozygous status of secretor mothers does play a role in the degree of α1,2-fucosylated HMO expression; however, based on our results it seems unlikely that this is the sole cause of the wide distribution of 2'-FL and LNFP I concentrations among secretor mothers at similar stages of lactation. Further studies comparing maternal FUT2 status and α1,2-fucosylated HMO concentrations are needed to draw a more concrete conclusion.

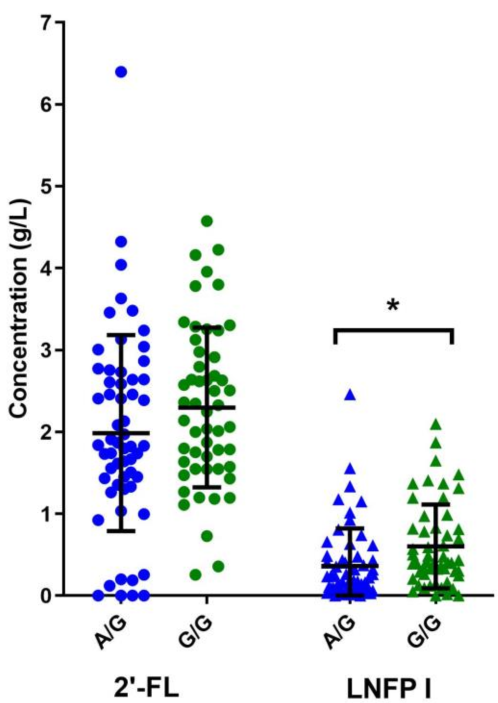


Figure 2.5. 2'-Fucosyllactose and lacto-*N*-fucopentaose concentrations in breast milk samples across the first year of lactation for mothers genotyped as heterozygous (A/G, blue) and homozygous (G/G, green) for the dominant FUT2 allele based on SNP rs516246. An asterisk indicates significant difference ($P < 0.05$) in HMO concentration between heterozygous and homozygous mothers for the given HMO.

Comparison of HMO Analytical Methods

The wide variation in reported HMO concentrations between mothers of similar lactation stages also demonstrates the importance of considering the analytical method used for HMO studies. Despite their acceptability in the early years of the field and citation in several seminal HMO publications, (Coppa et al., 1993; Montreuil and Mullet, 1960; Viverge et al., 1985) the application of rudimentary techniques like paper chromatography, thin layer chromatography, (Mernie et al., 2019; Srivastava et al., 2014; Stepans et al., 2006) liquid chromatography methods that elute all HMOs as only a few unresolved peaks, and the recent approach of subtracting simple sugar concentrations from spectrophotometrically determined total carbohydrate content, (Gridneva et al., 2019) have been surpassed and replaced by modern techniques with considerably improved precision and accuracy. Chromatographic techniques, including HPLC coupled with fluorescence-, UV-, or mass spectrometry-based detectors, as well as capillary electrophoresis and HPAEC-PAD are the most common modern techniques for isomer-specific HMO quantification or profiling. In most cases, it is essential that these quantification methods are validated for accuracy and precision, and that peak identities are validated by mass spectrometry (MS), nuclear magnetic resonance (NMR), or enzymatic breakdown.

The results of all of these analyses, however, have the potential to be distorted by sample preparation procedures. The roles, benefits, and possible pitfalls of a wide variety of sample preparation techniques for HMO analysis have been recently reviewed by van Leeuwen. (2019) The application of graphitized carbon solid phase extractions is of particular concern in the realm of HMO analysis, due to its potential to alter the ratios and/or concentrations of specific HMOs from those present in the original milk. Graphitized carbon solid phase extraction has been previously demonstrated to yield poor recovery of 3-FL, (Xu et al., 2017) and poorer recoveries for 6'-SL than 3'-SL have been reported in bovine milk as well. (Robinson et al., 2018) Previous work from our lab on the quantification oligosaccharides in infant formula and human milk provides a unique comparison of relative HMO quantifications for the same set of milk samples with and without the use of graphitized carbon solid phase extraction. The results for samples prepared with the use of graphitized carbon and analyzed by nano-chip LC quadrupole time-of-flight MS show a higher abundance of 3'-SL compared to 6'-SL, while the same samples prepared without graphitized carbon solid phase extraction and analyzed by HPAEC-PAD show a higher abundance of 6'-SL than 3'-SL. (Nijman et al., 2018) Solid phase extraction recoveries are an especially important consideration for studies measuring absolute HMO concentrations, as the potential for loss during the extraction can lead to substantial under-quantification. Unfortunately, very few studies have measured milk oligosaccharide recoveries with graphitized carbon. In our past work we have found that bovine milk oligosaccharide recovery from the extraction sorbent is substantially reduced by the presence of lactose in the sample matrix, (Robinson et al., 2018) and we therefore decided to conduct sample preparation without graphitized carbon extractions in this study.

It is possible that other sample preparation techniques may result in comparably skewed recoveries of particular HMOs. Protein precipitation with acetonitrile results in losses when applied to bovine milk oligosaccharide purification, (Liu et al., 2014) but a similar method evaluation has not yet been published for HMOs. Although derivatization methods may be incorporated to increase detection sensitivity for UV or fluorescence detection, these measures are subject to potential uneven or incomplete derivatization and require sample clean-up, which may introduce substantial additional variation to the analysis. Verification of high reaction efficiencies and recoveries are therefore quite important here as well. Likewise, the influence of matrix effects or the presence of coelution may cause reported HMO concentrations to be disproportionately high. While HPAEC-PAD is limited by the varying response factors for each HMO and therefore requires individual external HMO calibration standards, as well necessitating multiple sample dilutions to quantify both high- and low-abundance HMOs due to a linear detector range of approximately two orders of magnitude (Table 2.3), the present study's "dilute-and-shoot" method within-line sample clean-up, paired with distinct gradients optimized for the separation of either neutral or acidic HMOs substantially reduces the risk of the potential pitfalls surrounding target compound loss and matrix effects by minimizing sample preparation and ensuring an absence of coelution for the targeted HMO peaks. Accordingly, our results call into question HMO quantification efforts in previously published studies that report individual HMO concentrations 3 to 11 times higher than those in the present study after substantially more sample preparation, in populations with similar demographics and proportions of secretor mothers. (Elwakiel et al., 2018; Goehring et al., 2014; Larsson et al., 2019; McGuire et al., 2017; Thurl et al., 2010).

Reaching a field-wide consensus on reasonable ranges for HMO concentrations at time points across lactation and on standardized method(s) for determining secretor status will help guide supplementation of HMOs in food for infants. Current HMO supplementation of infant formulas is limited primarily to 2'-FL, present at concentrations of around 0.2 g/L (BJ Marriage, personal communication) – a concentration which in human milk would classify the mother as a non-secretor under the phenotypic secretor status criteria set by many studies, and which falls far below total HMO concentrations in mother's milk, even in very late lactation.

Although establishing a single universal method for HMO analysis is unlikely to occur in the foreseeable future, ensuring that both new and existing techniques have been validated will be an important step as the field moves forward. Measures of repeatability, recovery, accuracy, and precision as well as the levels of detection and quantification for individual HMOs will be critical to include as new or under-validated analytical methods are applied in future publications. In addition, maternal demographics, infant health and milk collection parameters including the time since the last feeding, milk expression method, time of milk collection and whether the sample is fore milk, hind milk or full breast expression should be reported, as these factors may also influence HMO profiles. (Choi et al., 2015; Viverge et al., 1986)

CONCLUSION

The present study focuses on HMO concentrations in the breast milk of healthy lactating mothers collected over 12 months postpartum. Our study also offers the validation and application of a new "dilute-and-shoot" analytical technique on samples from a large clinical study achieving quantification of HMOs by HPAEC-PAD with minimized sample preparation to limit potential HMO losses. Maternal genetic data generally agreed with α1-2-linked fucosyloligosaccharide expression, with the exception of two potential "weak secretor" mothers identified in this cohort, based on FUT2 positive genotype but extremely low expression of α1,2-fucosylated HMOs. Because data on human milk is frequently used as the basis for determining optimum practices for infant formula and weaning food compositions and supplementation, it is important to determine reasonable concentration ranges for individual HMOs at time points across lactation, as well as to identify how milk collection, oligosaccharide extraction, and analytical techniques may influence the reported HMO concentrations. Cross-lab validation studies are needed to reach a consensus on expected HMO concentrations across lactation and on the analytical methods best suited for HMO analysis and determining maternal genetics.

MATERIALS AND METHODS

Study Design

This study is part of the Cambridge Baby Growth and Breastfeeding Study (CBGS-BF), a UK-based prospective observational infant cohort which recruited mother-infant pairs at birth from a single maternity unit in Cambridge, England. The CBGS-BF is the continuation of the original Cambridge Baby Growth Study, (Prentice et al., 2016a) an ongoing birth cohort since 2001, aiming to investigate ante- and postnatal determinants of infant growth and body composition. All infants recruited to this cohort were singletons and vaginally born at term from healthy mothers with normal pre-pregnancy BMI and without any significant comorbidities. All infants received exclusive breastfeeding for at least 6 weeks. The study was approved by the Cambridge Local Research Ethics Committee and all mothers gave written informed consent.

To collect breast milk samples, mothers were asked to hand express milk liquid after feeding their infants, as described previously.(Prentice et al., 2016b, 2019) Expression was done from the breast last used to feed their infants. Samples were kept frozen until processed at a single time point. At the time of assay, breastmilk samples were thoroughly mixed.

HPAEC-PAD

167 milk samples from 71 mothers were analyzed in duplicate via HPAEC-PAD using a "dilute-and-shoot" sample preparation method. Breast milk samples were diluted between 15 and 400 times, passed through a 0.2 µm polyethersulfone syringe filter (Pall Life Sciences, Port Washington, NY USA) and directly injected. Analysis was carried out on a Dionex ICS-5000+ ion chromatography system outfitted with dual pumps and a detector consisting of an electrochemical cell with a disposable gold working electrode and a pH-Ag/AgCl reference electrode (ThermoFisher Scientific, Waltham, MA). Chromatographic eluents consisted of 18.2 MΩ-cm (Milli-Q) water (A), 200 mM sodium hydroxide (B) and 100 mM sodium acetate with 100 mM sodium hydroxide (C). The instrument and column configurations were based partially on ThermoFisher Customer Application Note 119. (Tan et al., 2015) Diluted samples were injected (5 µL injection volume) and passed through an IonPac NG1 column (4 x 35 mm, ThermoFisher Scientific) to eliminate hydrophobic milk components. The NG1 column was operated continuously at 0.5 mL/min and 100% A using pump 1. HMOs were eluted from the NG1 column onto a 500 µL sample loop, then passed onto a CarboPac PA20 guard column (3 x 30 mm, ThermoFisher Scientific) and CarboPac PA20 analytical column (3 x 150 mm, ThermoFisher Scientific) for chromatographic separation. For neutral HMO separation, pump 2 had a flow rate of 0.5 mL/min with a 34-minute gradient that was isocratic at 15% B for 10 min, followed by an increase from 15 to 70% B from 10 to 20 min, an isocratic period at 70% B from 20 to 29 min, an increase from 0 to 20% C from 29 to 29.1 min, and a final isocratic period at 70% B and 20% C from 29.1 to 34 min. For acidic HMO separation pump 2 had a flow rate of 0.5 mL/min with a 35-minute gradient that was isocratic at 60% B and 20% C for 30 min, followed by a simultaneous decrease to 55% B and increase to 30% C from 30 to 30.1 min, and a

final isocratic period from 30.1 to 35 min at 55% B and 30% C. Column temperatures were set to 15 °C, and the detector temperature was 20 °C. The instrument configuration is depicted in Figure 2.6.

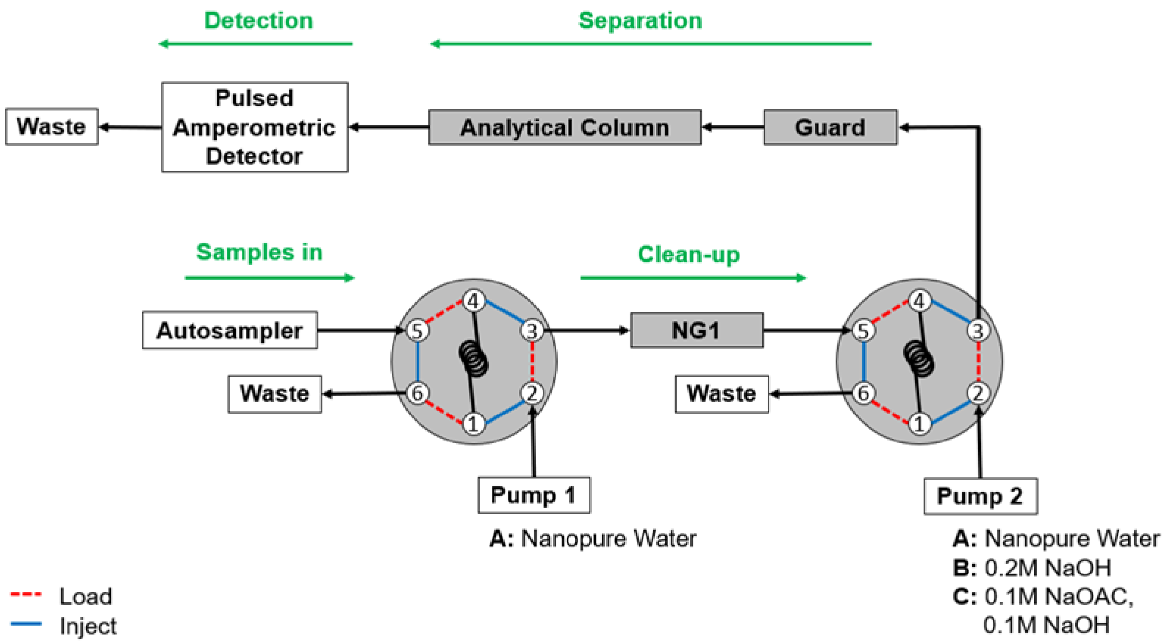


Figure 2.6. Pump and column configuration for the newly validated HPAEC-PAD "dilute-and-shoot" HMO analysis.

HPAEC-PAD Method Validation: Recovery, Repeatability, Limits of Detection, and Limits of Quantification

To evaluate recovery, five concentrations of each HMO standard (3-FL, 2'-FL, LNFP I, Lacto-*N*-neotetraose (LNnT), LNT, 3'-SL and 6'-SL) were spiked into a 6-week milk sample during sample dilution. Spiked samples were analyzed as described above, and recoveries were expressed as the ratio of the measured spiked quantity to the theoretical spiked quantity. Repeatability was evaluated by injecting the same 6-week sample five times and calculating the coefficient of variation among the repeated measurements. The limit of detection of each HMO was defined as the concentration that produced a signal-to-noise ratio of 3:1. This was determined empirically by sequential injection of low concentrations of each analytical standard. The limit of quantification was defined as the concentration of each analytical standard that produced a signal-to-noise ratio of 6:1.

Capillary Electrophoresis

Additional analysis to confirm the presence or absence of α1,2-fucosylated HMOs was performed on a Gly-Q capillary electrophoresis system (Prozyme, now part of Agilent, Santa Clara, CA, USA). Milk samples were centrifuged at 4000 xg at 4 °C for 30 minutes and 10 µL of the aqueous portion were transferred to a new tube and dried by centrifugal evaporation (Eppendorf Vacufuge plus; Eppendorf, Hamburg, Germany). Dried samples were reduced and labeled with 8-aminopyrene-1,3,6-trisulfonic acid (APTS) using a GlykoPrep Rapid-Reductive Amination APTS Labeling kit, following the manufacturer's instructions (Prozyme), as described previously. (Bonen et al., 2018) Briefly, dried samples were combined with the reducing agent, catalyst, and APTS labeling solution and incubated at 65 °C for 1 hour. Samples were allowed to cool to room temperature, open, in a fume hood, before undergoing solid phase extraction with GlykoPrep CU cartridges (Prozyme) to remove excess APTS label. Collected eluate, containing APTS-labeled HMOs, was diluted ten to 100 times and injected on the Gly-Q capillary electrophoresis system. Separation was achieved on a Gly-Q Cartridge with Gly-Q Separation Buffer as the mobile phase (Prozyme). The applied potential gradient consisted of a 10 s High voltage purge at 4.00 V, followed by a 2 s migration standard injection at 2.00 V, a 2 s sample injection at 2.00 V and 120 s of separation and detection at 10.00 V. Detector sensitivity was set to medium. Sample alignment was achieved with an external Instant-Q-labeled maltodextrin ladder reference with degrees of polymerization (DP) between 2 and 15 and co-injected migration standards of Instant-Q-labeled maltose and maltopentadecaose.

Presence of α1,2-fucosylated HMOs was determined based on observation of a peak corresponding to LNFP I. Peak identification was based on migration time relative to the co-injected sample brackets, using a commercial LNFP I reference standard.

FUT2 Genotyping

Saliva samples were collected from the mothers using the Oragene.DNA kit OG-500 (DNA Genotek, Ottawa, Canada). Manual DNA purification from the samples was done using prepIT.L2P kit following the manufacturer's instructions (DNA Genotek, Ottawa, Canada). Following that, DNA was fragmented by applying a restriction enzyme (restriction fragment length polymorphism or RFLP).

Maternal FUT2 genotype was then determined via sodium dodecyl sulfate-polyacrylamide gel electrophoresis based on the identification of single nucleotide polymorphisms at rs516246 (A allele producing 202-base pairs and G allele producing 125 and 77 base pairs).

Statistics

The concentration at each time point of each HMO was compared using ANOVA followed by Tukey pairwise comparisons, which were adjusted to an overall α of 0.05. Comparisons between concentrations of individual HMOs at each time point were carried out using 2-sided independent t-tests (α=0.05). All statistical analysis was done in R, version 3.6.0.

ACKNOWLEDGEMENTS

The authors wish to acknowledge Dr. Gabriele Gross and Dr. Janna van Diepen for their support and expertise. Special thanks are also given to the mothers who participated in this study and provided breast milk samples. The authors acknowledge Prozyme (now part of Agilent, Santa Clara, CA, USA) for the use of a Gly-Q capillary electrophoresis system and supplies.

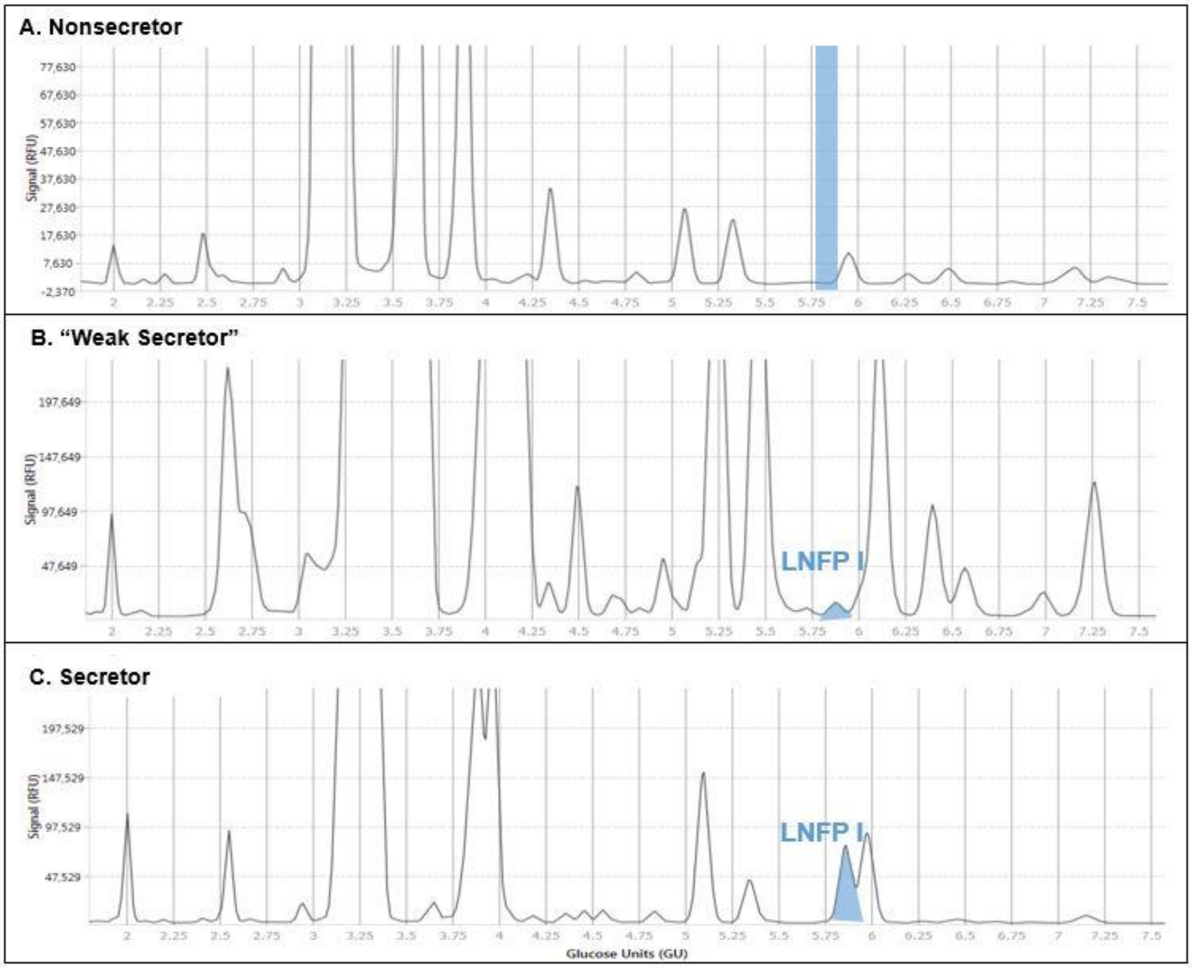
Funding

This work was supported by Reckitt Benckiser (former Mead Johnson Nutrition) and USDA NIFA project CA-D-FST-2187-H. The CBGS-BF also received additional support from MRC Epidemiology Unit; NIHR Clinical Research Network; Newlife; Mothercare; and NIHR Cambridge Biomedical Research Centre.

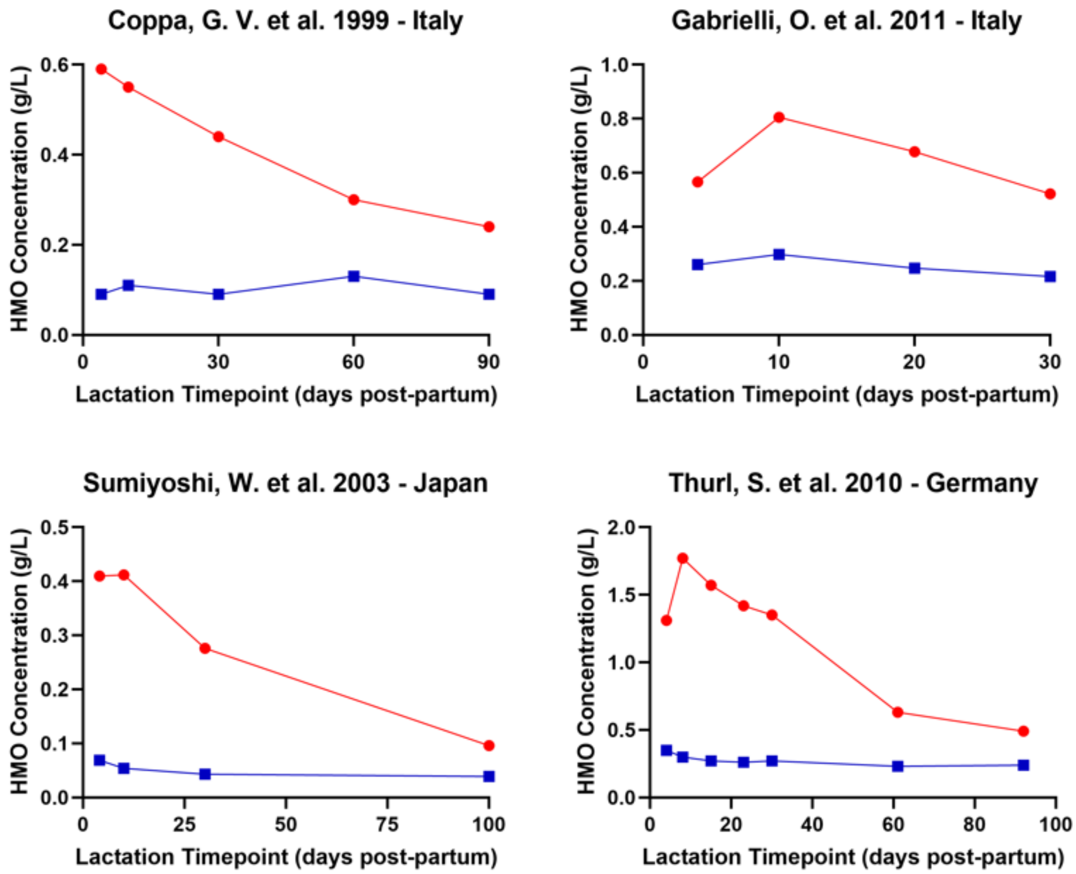
Author Disclosures

M.C. is an employee of Reckitt Benckiser. D.B. is a cofounder of Evolve Biosystems, a company focused on diet-based manipulation of the gut microbiota. Evolve Biosystems played no role in the design, execution, interpretation, or publication of this work.

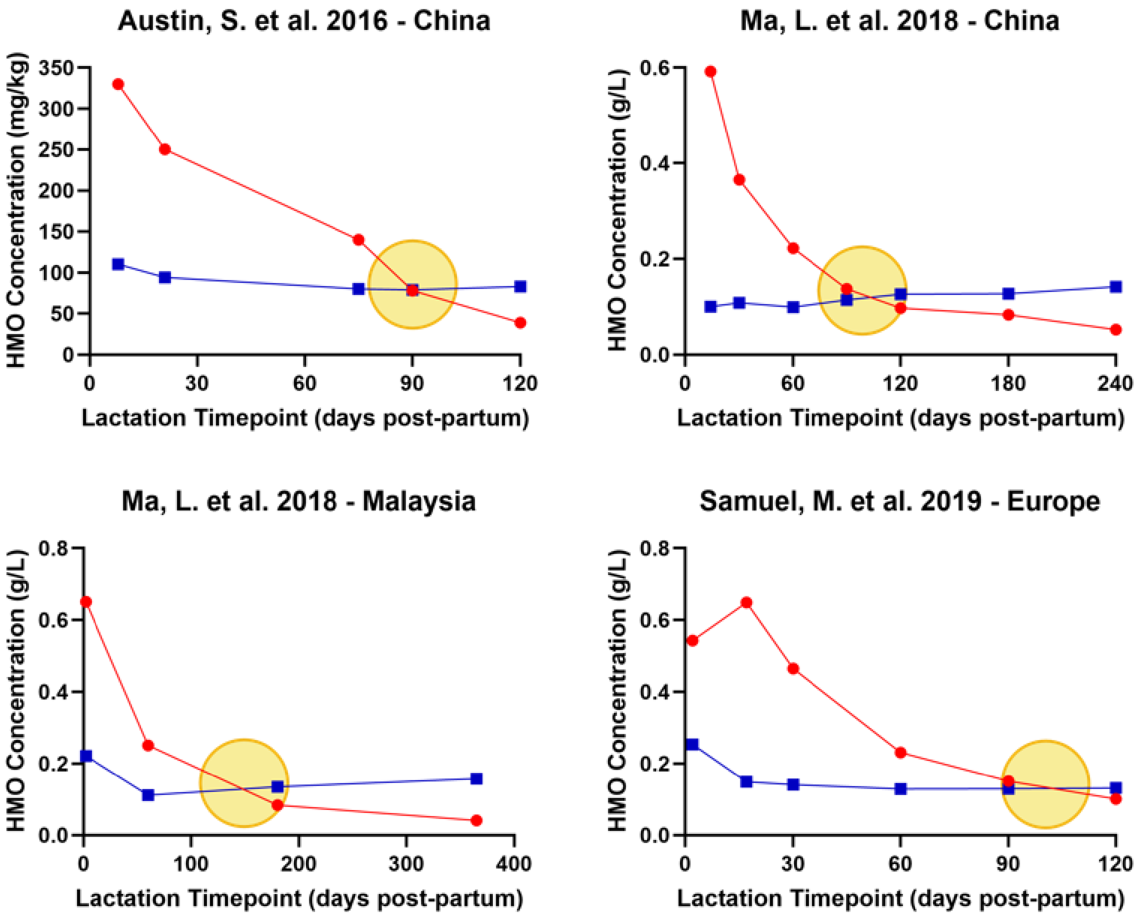
SUPPLEMENTARY MATERIAL



Supplementary Figure 2.1. Electropherograms from capillary electrophoresis analysis of breast milk samples from (**A**) non-secretor, (**B**) "weak secretor," and (**C**) secretor mothers with representative lacto-N-fucopentaose I abundances.



Supplementary Figure 2.2. Concentrations of 6'-SL (red circles) and 3'-SL (blue squares) during the first 3 months of lactation in previously published longitudinal HMO studies.



Supplementary Figure 2.3. Concentrations of 6'-SL (red circles) and 3'-SL (blue squares) during the first 3 to 12 months of lactation in previously published longitudinal HMO studies.

Supplementary Table 2.1. Summary of longitudinal human milk oligosaccharide studies

First Author	Year	Country	Lactation Stage	# Mothers	Preterm?	Quantification Method	% Secretors
Present study	2020	UK	Birth 2 weeks 6 weeks 3 months 6 months 12 months	71	N	HPAEC-PAD	85
Alderete	2015	USA	1 month 6 months	25	N	HPLC-Fluoresecne	72
Asakuma	2007	Japan	1 day 2 days 3 days	20	N	HPLC-UV	Unspecified
Austin	2019	Switzerland	1 week 2 weeks 3 weeks 4 weeks 5 weeks 6 weeks 7 weeks 8 weeks	34	N	HPLC-Fluorescence	79
Austin	2019	Switzerland	1 week 2 weeks 3 weeks 4 weeks 5 weeks 6 weeks 7 weeks 8 weeks 10 weeks 12 weeks 14 weeks 16 weeks	27	Y	HPLC-Fluorescence	80
Azad	2018	Canada	(3-4 months)	427	N	HPLC-Fluorescence	72
Bao	2013	USA	colostrum mature milk	4	N	LC-MS	Unspecified
Bao	2007	USA	(2-4 days) (12-67 days)	8	N	CE-UV	Unspecified
Bode	2012	Zambia	1 month	36	N	HPLC-Fluorescence	69
Borewicz	2019	The Netherlands	1 month	121	N	UHPLC-MS	Unspecified
Borewicz	2020	The Netherlands	2 weeks 6 weeks 12 weeks	24	N	UHPLC-MS, HPAEC-PAD	79
Coppa	2011	Italy	1 month	39	N	HPAEC-PAD	41 (biased selection)
Coppa	1999	Italy	4 days 10 days 1 month 2 months 3 months	18	N	HPAEC-PAD	100 (biased selection)
Erney	2000	Latin America	(3-10 days) (11-30 days) (31-452 days)	200	N	HPAEC-PAD	96
Erney	2000	Asia	(3-10 days) (11-30 days) (>31 days)	54	N	HPAEC-PAD	65
Erney	2000	Europe	(3-10 days) (11-30 days) (>31 days)	76	N	HPAEC-PAD	88
Erney	2000	USA	(3-10 days) (11-30 days) (>31 days)	79	N	HPAEC-PAD	68
Ferriera	2020	Brazil	(2-8 days) (28-50 days) (88-119 days)	75	N	HPLC-Fluorescece	89

First Author	2'-FL	3-FL	LNFP I	LNFP II	LNFP III	LNFP V	LDFT	LNT	LNnT	LNH	LNnH
Present study	3.560	0.124	1.647					1.651	0.306		
	2.495	0.280	0.959					2.264	0.076		
	1.965	0.463	0.331					1.416	0.078		
	1.623	0.542	0.266					1.136	0.057		
	1.453	0.740	0.209					0.900	0.045		
	1.074	1.257	0.086					0.743	0.000		
Alderete	2.750	0.163	0.971	1.340	0.071			1.423	0.039		
	2.430	0.516	0.438	1.616	0.161			1.227	0.105		
Asakuma											
Austin	3.157	0.346	1.743	0.415	0.421	0.059	0.340	0.993	0.333		
	2.207	0.456	1.317	0.523	0.371	0.075	0.255	1.334	0.227		
	2.139	0.494	1.214	0.507	0.305	0.081	0.258	1.286	0.182		
	2.007	0.558	1.061	0.531	0.267	0.068	0.202	1.216	0.166		
	1.938	0.622	0.822	0.456	0.302	0.065	0.256	1.056	0.153		
	1.801	0.683	0.681	0.438	0.323	0.064	0.299	0.939	0.144		
	1.664	0.721	0.642	0.423	0.332	0.058	0.272	0.877	0.138		
	1.621	0.726	0.562	0.403	0.319	0.053	0.248	0.804	0.133		
	2.504	0.461	1.337	0.542	0.346	0.076	0.346	1.179	0.274		
	1.672	0.479	0.982	0.616	0.317	0.085	0.202	1.522	0.223		
	1.582	0.566	0.876	0.626	0.303	0.091	0.267	1.448	0.190		
	1.651	0.629	0.762	0.620	0.321	0.090	0.303	1.319	0.178		
	1.595	0.730	0.704	0.556	0.328	0.084	0.294	1.162	0.158		
	1.578	0.690	0.625	0.603	0.337	0.092	0.286	1.158	0.169		
	1.533	0.758	0.568	0.618	0.357	0.089	0.292	1.120	0.163		
	1.556	0.831	0.529	0.563	0.350	0.079	0.354	0.996	0.159		
	1.499	0.869	0.466	0.475	0.389	0.061	0.341	0.797	0.155		
	1.306	1.074	0.358	0.564	0.389	0.072	0.455	0.755	0.122		
	1.294	1.205	0.362	0.503	0.369	0.067	0.296	0.669	0.125		
	1.389	1.037	0.334	0.516	0.343	0.069	0.419	0.660	0.126		
Azad	2.255	0.267	0.788	1.852	0.092		0.313	1.047	0.284	0.072	
Bao	1.118	0.131	1.420	0.042	0.173	0.003		0.230	0.231		
	1.079	0.231	1.549	0.148	0.169	0.014		0.194	0.629		
Bao											
Bode	0.399	0.033	0.139								
Borewicz	0.327	0.248	0.467	0.339	0.270	0.041	0.040			0.105	0.072
Borewicz	1.007	0.522	0.761	0.448	0.357	0.050	0.081			0.064	0.042
	0.840	0.780	0.447	0.431	0.379	0.054	0.201			0.043	0.040
	0.702	1.003	0.296	0.382	0.407	0.049	0.072			0.017	0.024
Coppa	1.066	0.375	0.495	0.184							
Coppa	3.930	0.340	1.360	0.290				0.840	2.040	0.070	0.180
	3.020	0.220	1.360	0.480				0.730	1.830	0.050	0.100
	2.780	0.280	0.990	0.430				0.710	1.400	0.060	0.090
	1.840	0.710	0.970	0.290				1.560	0.950	0.090	0.130
	2.460	0.530	1.350	0.330				1.780	1.370	0.170	0.280
Erney	2.790	0.660	1.730	0.290	0.420	0.680	0.190		0.410		
	2.610	0.750	1.390	0.320	0.420	0.480	0.150		0.310		
	1.910	0.880	0.620	0.350	0.440	0.440	0.120		0.190		
	2.260	1.380	1.810	0.730	0.400	0.350	0.400		0.360		
	2.360	1.760	1.200	0.660	0.380	0.230	0.460		0.230		
	1.500	2.150	0.490	0.570	0.380	0.120	0.460		0.100		
	2.690	0.970	1.560	0.490	0.740	0.190	0.700		0.550		
	2.380	0.630	1.060	0.450	0.710	0.180	0.570		0.290		
	2.360	1.360	0.610	0.470	0.770	0.190	0.580		0.200		
	2.780	1.030	1.570	0.670	0.580	0.270	0.450		0.360		
	2.560	1.480	1.010	0.890	0.810	0.270	0.540		0.200		
	1.640	2.590	0.510	0.670	0.720	0.140	0.410		0.190		
Ferriera	2.460	0.132	2.270	0.657	0.068		0.159	0.990	0.410	0.064	
	2.460	0.176	1.331	0.956	0.060		0.235	1.026	0.198	0.086	
	2.080	0.918	0.649	1.442	0.034		0.279	0.962	0.233	0.075	

First Author	LNDFH I	LNDFH II	6'-SL	3'-SL	LST a	LST b	LST c	DSLNT
Present study			0.374	0.015				
			0.382	0.061				
			0.193	0.084				
			0.092	0.094				
			0.036	0.130				
			0.008	0.254				
Alderete				0.756		0.096	0.206	0.247
				1.125		0.149	0.077	0.184
Asakuma			0.342	0.362	0.107	0.068	0.659	0.480
			0.371	0.269	0.155	0.064	0.707	0.447
			0.396	0.258	0.162	0.062	0.693	0.459
Austin	0.943		0.498	0.223		0.082	0.578	0.361
	0.987		0.646	0.147		0.076	0.481	0.360
	1.025		0.567	0.135		0.083	0.306	0.363
	0.913		0.477	0.134		0.080	0.216	0.305
	0.841		0.358	0.129		0.077	0.158	0.251
	0.826		0.306	0.131		0.074	0.132	0.231
	0.793		0.258	0.124		0.077	0.108	0.203
	0.669		0.220	0.120		0.066	0.090	0.173
	0.976		0.492	0.239		0.100	0.433	0.423
	0.808		0.506	0.198		0.111	0.275	0.466
	0.869		0.455	0.197		0.118	0.208	0.468
	0.871		0.396	0.193		0.113	0.178	0.416
	0.908		0.320	0.194		0.116	0.128	0.378
	0.786		0.291	0.186		0.111	0.126	0.323
	0.757		0.248	0.183		0.107	0.106	0.306
	0.798		0.226	0.185		0.102	0.096	0.272
	0.761		0.174	0.172		0.086	0.076	0.233
	0.699		0.144	0.182		0.095	0.055	0.210
	0.636		0.112	0.164		0.084	0.040	0.185
	0.647		0.100	0.176		0.078	0.037	0.185
Azad			0.161	0.360		0.118	0.043	0.315
Bao	0.544	0.001						
	0.802	0.015						
Bao			0.276	0.082				0.777
			0.306	0.063				0.660
Bode				0.114				
Borewicz	0.475		0.111	0.091	0.028	0.256	0.116	
Borewicz	0.736		0.362	0.175	0.041	0.194	0.275	
	1.311		0.451	0.209	0.038	0.489	0.199	
	0.489		0.074	0.164	0.011	0.147	0.034	
Coppa		0.163						
Coppa	0.790	0.570	0.590	0.090	0.180	0.170	1.050	0.800
	0.790	0.320	0.550	0.110	0.120	0.100	0.470	0.740
	0.430	0.120	0.440	0.090	0.110	0.230	0.210	0.670
	1.180	1.020	0.300	0.130	0.000	0.250	0.210	0.640
	0.920	0.740	0.240	0.090	0.000	0.200	0.120	0.630
Erney								
Ferriera			0.234	0.203		0.090	0.769	0.748
			0.399	0.241		0.110	0.270	0.387
			0.310	0.342		0.070	0.100	0.232

First Author	Year	Country	Lactation Stage	# Mothers	Preterm?	Quantification Method	% Secretors
Gabrielli	2011	Italy	4 days	63	Y	HPAEC-PAD	67
			10 days				
			20 days				
			1 month				
Galeotti	2014	Italy	1 month	9	N	CE	56
Goehring	2014	USA	2 weeks	17	N	LC-MS	76
Hong	2014	USA	35 days	20	N	LC-MS	50 (biased selection)
Kunz	1999	Germany	(2-28 days)	4	N	HPAEC-PAD	50
Larsson	2019	Denmark	(5-6.5 months)	30	N	HPLC-Fluorescence	77
			9 months				
Leo	2010	Samoa	(5-10 days)	16	N	HPLC-UV	Unspecified
			(22-155 days)				
Ma	2018	China	2 weeks	20	N	HPLC-UV	63
			1 month				
			2 months				
			3 months				
			4 months				
			6 months				
			8 months				
		Malaysia	2 days	26			88
			2 months				
			6 months				
			12 months				
McGuire	2017	Ethiopia (Rural)	(2 weeks-5 months)	40	N	HPLC-UV	65
		Ethiopia (Urban)	(2 weeks-5 months)	40			78
		Gambia (Rural)	(2 weeks-5 months)	40			65
		Gambia (Urban)	(2 weeks-5 months)	40			85
		Ghana	(2 weeks-5 months)	40			68
		Kenya	(2 weeks-5 months)	42			81
		Peru	(2 weeks-5 months)	43			98
		Spain	(2 weeks-5 months)	41			76
		Sweeden	(2 weeks-5 months)	24			79
		Washington	(2 weeks-5 months)	41			68
		California (Hispanic)	(2 weeks-5 months)	19			95
McJarrow	2019	United Arab Emerates	(5-15 days)	41	N	LC-MS	74
			6 monts				
Morrow	2004	Mexico	(1-5 weeks)	93	N	HPLC-UV	100
Musumeci	2006	Burkina Faso	1 day	53	N	HPAEC-PAD	Unspecified
			2 days				
			3 days				
		Italy	1 day	50	N	HPAEC-PAD	Unspecified
			2 days				
			3 days				
Nakhla	1999	USA	(1-128)	15	Y	HPAEC-PAD	92
Newburg	2004	Mexico	1-5 weeks	93	N	HPLC-UV	100
Nijman	2018	USA	day 3	10	N	HPAEC-PAD	Unspecified
			day 42				
Olivares	2015	The Netherlands	1 month	12	N	CE-Fluorescence	58
Saben	2020	USA	2 monts	136	N	HPLC-Fluorescence	74
Samuel	2019	Europe	2 days	290	N	HPLC-Fluorescence	83
			17 days				
			30 days				
			60 days				
			90 days				
			120 days				
Sjogren	2007	Sweeden	(2-4 days)	20	N	HPLC-UV	95
Smilowitz	2013	USA	90 days	52	N	NMR	77
Spevacek	2015	USA	(0-5 days)	15	N	NMR	71
			2 weeks				
			28 days				
			(0-5 days)	13	Y		76
			2 weeks				
			28 days				

First Author	2'-FL	3-FL	LNFP I	LNFP II	LNFP III	LNFP V	LDFT	LNT	LNnT	LNH	LNnH
Gabrielli	4.834	0.782	1.351	0.587	0.552		0.710	1.561	1.699	0.073	0.085
	3.721	0.997	1.432	0.853	0.452		0.508	1.956	1.699	0.070	0.085
	3.270	0.755	1.208	0.853	0.493		0.414	1.882	1.623	0.065	0.085
	3.100	1.001	1.041	0.771	0.448		0.503	1.675	1.462	0.047	0.078
Galeotti	3.400	3.820	1.940		0.778			3.700	2.744	0.089	
Goehring	2.192										
Hong	1.490		0.255					0.975		0.047	
Kunz	0.450	0.070	1.260					1.090			
Larsson	2.633	0.231	0.702	1.544	0.069		0.525	0.877	0.509	0.100	
	3.142	0.336	0.597	1.230	0.077		0.684	0.468	0.429	0.046	
Leo	0.220	1.670	0.280				0.070	3.900	0.460		0.160
	0.690	2.350	0.350				0.140	1.310	0.200		0.050
Ma	1.281	0.543						1.979	1.033		
	1.371	0.894						1.225	0.708		
	1.176	1.158						0.851	0.569		
	0.984	1.366						0.947	0.513		
	0.866	1.427						0.866	0.525		
	0.704	1.476						0.785	0.446		
	0.709	1.588						0.823	0.448		
	2.249	0.429						2.393	1.420		
	1.286	0.762						1.217	0.609		
	1.003	1.146						0.867	0.571		
	0.741	1.138						1.156	0.642		
McGuire	1.105	0.092	0.771	1.381	0.038		0.114	0.922	0.593	0.073	
	1.393	0.090	1.089	1.462	0.020		0.184	0.996	0.656	0.092	
	1.440	0.050	0.984	1.643	0.034		0.214	1.602	1.006	0.120	
	2.060	0.079	1.146	1.323	0.026		0.225	1.115	0.552	0.099	
	0.702	0.094	1.102	0.967	0.040		0.249	1.296	0.612	0.117	
	1.650	0.095	0.786	1.422	0.039		0.214	1.154	0.759	0.099	
	3.187	0.151	0.952	0.951	0.045		0.298	0.674	0.377	0.115	
	1.907	0.101	0.901	1.707	0.027		0.195	1.110	0.388	0.062	
	2.764	0.231	1.190	1.615	0.230		0.174	1.508	0.598	0.121	
	2.030	0.060	0.725	1.813	0.021		0.171	0.803	0.549	0.100	
	3.438	0.189	1.167	1.058	0.065		0.237	1.017	0.561	0.042	
McJarrow	2.021	0.581	1.932					1.429	0.765		
	0.997	1.194	0.650					0.504	0.250		
Morrow	1.879		2.739				0.444				
Musumeci	1.800		0.800								
	4.500		1.100								
	8.400		4.400								
	1.000		1.500								
	2.100		2.500								
	4.200		5.000								
Nakhla	1.134	0.432	0.234	0.048	0.060	0.017	0.166	0.225	0.081		
Newburg	1.879	0.283	2.739				0.444	0.898	0.297		
Nijman	3.750		1.810				0.360	0.480		0.080	
	2.480		0.580				0.240	0.510		0.160	
Olivares				0.429			0.065	1.880			
Saben	0.617	1.801	0.734	1.548	0.037		0.204	0.988	0.072	0.181	
Samuel	3.691	0.422	1.928	0.422	0.445	0.108	0.607	0.912	0.307		
	2.627	0.594	1.431	0.595	0.320	0.124	0.349	1.213	0.177		
	2.450	0.720	1.071	0.549	0.311	0.112	0.277	1.009	0.153		
	2.075	0.970	0.611	0.474	0.358	0.091	0.280	0.700	0.128		
	1.819	1.140	0.469	0.433	0.353	0.085	0.273	0.594	0.108		
	1.625	1.209	0.384	0.394	0.339	0.076	0.269	0.526	0.098		
Sjogren	3.177	0.087	1.420				0.202	0.679	0.330		
Smilowitz	1.220	1.025	0.161	0.179	0.199		0.169	0.358	0.086		
Spevacek	2.651	0.444	1.408	0.401	0.358		0.159	1.054	0.255		
	2.060	0.581	0.862	0.358	0.247		0.178	0.870	0.149		
	1.753	0.766	0.546	0.367	0.222		0.140	0.750	0.113		
	2.421	0.483	0.922	0.341	0.239		0.330	0.679	0.163		
	1.660	0.591	0.683	0.444	0.205		0.203	0.969	0.120		
	1.133	0.967	0.256	0.674	0.230		0.197	1.280	0.141		

First Author	LNDFH I	LNDFH II	6'-SL	3'-SL	LST a	LST b	LST c	DSLNT
Gabrielli		0.181	0.566	0.260	0.282	0.114	1.207	1.230
		0.252	0.805	0.298	0.352	0.158	1.100	1.284
		0.237	0.678	0.247	0.366	0.184	0.594	1.205
		0.248	0.522	0.216	0.382	0.151	0.399	1.050
Galeotti		0.067		0.367	0.133	0.000	0.000	2.500
Goehring			0.718					
Hong			0.305	0.175			0.053	
Kunz			0.380	0.270	0.140		0.170	
Larsson			0.094	0.525		0.132	0.032	0.381
			0.082	0.704		0.472	0.140	0.519
Leo	0.750	0.860	0.343	0.163	0.078	0.084	0.620	0.638
	1.220	0.700	0.189	0.133	0.044	0.193	0.201	0.317
Ma			0.592	0.100			0.941	
			0.365	0.108			0.159	
			0.222	0.099			0.152	
			0.137	0.114			0.085	
			0.097	0.126			0.056	
			0.083	0.127			0.047	
			0.052	0.142			0.042	
			0.651	0.222			1.326	
			0.251	0.112			0.130	
			0.084	0.135			0.145	
			0.041	0.158			0.054	
McGuire			0.237	0.262		0.086	0.101	0.400
			0.345	0.333		0.079	0.169	0.713
			0.293	0.294		0.132	0.159	1.122
			0.370	0.320		0.096	0.146	0.615
			0.564	0.391		0.115	0.245	0.723
			0.275	0.334		0.086	0.158	0.573
			0.403	0.334		0.041	0.182	0.353
			0.319	0.384		0.105	0.072	0.460
			0.127	0.296		0.140	0.092	0.279
			0.255	0.356		0.082	0.112	0.571
			0.155	0.300		0.079	0.103	0.355
McJarrow			0.621	0.226			0.488	
			0.091	0.134			0.011	
Morrow	1.259							
Musumeci								
Nakhla		0.007						
Newburg								
Nijman	2.100		0.340	0.110				
	1.930		0.250	0.120				
Olivares	0.415							
Saben			0.451	0.271		0.107	0.131	0.417
Samuel	1.232		0.543	0.254		0.079	0.497	0.405
	1.275		0.649	0.149		0.080	0.258	0.385
	1.105		0.465	0.141		0.077	0.148	0.290
	0.842		0.231	0.129		0.064	0.070	0.169
	0.719		0.151	0.130		0.057	0.044	0.136
	0.619		0.101	0.132		0.050	0.029	0.121
Sjogren	1.117							
Smilowitz			0.075	0.091				
Spevacek			0.519	0.228				
			0.557	0.165				
			0.367	0.146				
			0.545	0.228				
			0.722	0.184				
			0.659	0.177				

First Author	Year	Country	Lactation Stage	# Mothers	Preterm?	Quantification Method	% Secretors
Sprenger	2017	Singapore	1 month	50	N	HPAEC-PAD	68
			2 months				
			4 months				
Stepans	2006	USA	2 weeks	49	N	HPTLC-Optical Density	Unspecified
			6 weeks				
			12 weeks				
			24 weeks				
Sumiyoshi	2003	Japan	4 days	20	N	HPLC-UV	Unspecified
			10 days				
			30 days				
			100 days				
Thurl	2010	Germany	(2-5 days)	21	N	HPAEC-PAD	83
			(6-9 days)				
			(13-18 days)				
			(20-26 days)				
			(28-33 days)				
			(57-65 days)				
			(88-96 days)				
Thurl	1996	Not specified	Not specified	1		HPAEC-PAD	100
Tonon	2019	Brazil	(17-45 days)	41		CE-ESI-MS	87
Tonon	2019	Brazil	(17-76 days)	78		LC-MS	87
Zhang	2019	China	(2-6 months)	61		LC-MS	Unspecified

First Author	2'-FL	3-FL	LNFP I	LNFP II	LNFP III	LNFP V	LDFT	LNT	LNnT	LNH	LNnH
Sprenger	1.484							1.138	0.239		
	1.206							0.741	0.148		
	0.949							0.500	0.095		
Stepans				0.008							
				0.008							
				0.009							
				0.008							
Sumiyoshi											
Thurl	4.130	0.240	2.000	0.140	0.340		0.490	0.780	0.490	0.060	
	3.370	0.260	2.050	0.230	0.340		0.330	1.550	0.480	0.170	
	3.040	0.380	1.640	0.290	0.370		0.480	1.520	0.280	0.140	
	3.020	0.440	1.720	0.300	0.370		0.360	1.590	0.320	0.130	
	2.960	0.420	1.480	0.240	0.370		0.370	1.410	0.230	0.140	
	2.820	0.560	1.060	0.180	0.400		0.380	1.000	0.230	0.080	
	2.590	0.670	0.940	0.170	0.440		0.400	0.860	0.200	0.060	
Thurl	1.840	0.460	0.670	0.200	0.280	0.000	0.170	0.860	0.110		
Tonon	3.233	0.687	1.310							0.013	0.003
Tonon	2.080	0.632	0.797							0.185	0.035
Zhang	0.410	0.590	0.158		0.219						0.002

First Author	LNDFH I	LNDFH II	6'-SL	3'-SL	LST a	LST b	LST c	DSLNT
Sprenger			0.540	0.230				
			0.275	0.210				
			0.129	0.205				
Stepans								
Sumiyoshi			0.410	0.069	0.104	0.056	0.294	0.199
			0.412	0.054	0.083	0.054	0.145	0.176
			0.276	0.043	0.052	0.044	0.074	0.111
			0.096	0.039	0.037	0.029	0.040	0.056
Thurl	1.120	0.100	1.310	0.350	0.060	0.050	0.480	0.290
	1.300	0.170	1.770	0.300	0.090	0.060	0.530	0.380
	1.460	0.230	1.570	0.270	0.050	0.070	0.310	0.440
	1.550	0.260	1.420	0.260	0.030	0.090	0.250	0.410
	1.360	0.240	1.350	0.270	0.030	0.100	0.240	0.410
	1.020	0.190	0.630	0.230	0.010	0.080	0.110	0.230
	1.050	0.170	0.490	0.240	0.010	0.080	0.090	0.210
Thurl		0.250						
Tonon	1.257	0.189	0.433	0.174	0.008	0.082	0.198	
Tonon	0.935	0.063	0.377	0.179	0.072	0.072	0.154	
Zhang		0.497	0.739	0.196	0.098	0.155	0.139	4.443

REFERENCES

Alderete TL, Autran C, Brekke BE, Knight R, Bode L, Goran MI, Fields DA. 2015. Associations between human milk oligosaccharides and infant body composition in the first 6 mo of life. *Am. J. Clin. Nutr.* 102:1381–1388.

Asakuma, S. Akahori M, Kimura K, Watanabe Y, Nakamura T, Tsunemi M, Arai I, Sanai Y, Urashima T. 2007. Sialyl oligosaccharides of human colostrum: Changes in concentration during the first three days of lactation. *Biosci. Biotechnol. Biochem.* 71:1447–1451.

Austin S, De Castro C, Bénet T, Hou Y, Sun H, Thakkar S, Vinyes-Pares G, Zhang Y, Wang P. 2016. Temporal change of the content of 10 oligosaccharides in the milk of Chinese urban mothers. *Nutrients*. 8:346.

Austin S, De Castro CA, Sprenger N, Binia A, Affolter M, Garcia-Rodenas CL, Beauport L, Tolsa J-F, Fumeaux CJF. 2019. Human milk oligosaccharides in the milk of mothers delivering term versus preterm infants. *Nutrients*. 11:1282.

Azad MB, Robertson B, Atakora F, Becker AB, Subbarao P, Moraes TJ, Mandhane PJ, Turvey SE, Lefebvre DL, Sears MR, Bode L. 2018. Human milk oligosaccharide concentrations are associated with multiple fixed and modifiable maternal characteristics, environmental factors, and feeding practices. *J. Nutr*. 148:1733–1742.

Bai Y, Tao J, Zhou J, Fan Q, Liu M, Hu Y, Xu Y, Zhang L, Yuan J, Li W, Ze X, Malard P, Guo Z, Yan J, Li M. 2018. Fucosylated human milk oligosaccharides and N-glycans in the milk of Chinese mothers regulate the gut microbiome of their breast-fed infants during different lactation stages. *mSystems*. 3:e00206-18.

Bao Y, Chen C, Newburg DS. 2013. Quantification of neutral human milk oligosaccharides by graphitic carbon high-performance liquid chromatography with tandem mass spectrometry. *Anal. Biochem.* 433:28–35.

Bao Y, Zhu L, Newburg DS. 2007. Simultaneous quantification of sialyloligosaccharides from human milk by capillary electrophoresis. *Anal. Biochem.* 370:206–214.

Bode L. 2015. The functional biology of human milk oligosaccharides. *Early Hum. Dev*. 91:619–622.

Bode L, Kuhn L, Kim H-Y, Hsiao L, Nissan C, Sinkala M, Kankasa C, Mwiya M, Thea DM, Aldrovandi GM. 2012. Human milk oligosaccharide concentration and risk of postnatal transmission of HIV through breastfeeding. *Am. J. Clin. Nutr*. 96:831–839.

Bode L, Kunz C, Muhly-Reinholz M, Mayer K, Seeger W, Rudloff S. 2004. Inhibition of monocyte, lymphocyte, and neutrophil adhesion to endothelial cells by human milk oligosaccharides. *Thromb. Haemost*. 92:1402–1410.

Bonen H, Guerrero A, Brownell EA, Smith KC, Barile D, Hagadorn JI, Lebrilla CB, Lussier MM, Matson AP, Sela DA. 2018. Human milk oligosaccharide analysis of large sample cohorts using a novel capillary electrophoresis platform. *Glycobiology*. 28:1027–1028.

Borewicz K, Gu F, Saccenti E, Hechler C, Beijers R, de Weerth C, van Leeuwen SS, Schols HA, Smidt H. 2020. The association between breastmilk oligosaccharides and faecal microbiota in healthy breastfed infants at two, six, and twelve weeks of age. *Sci. Rep*. 10:4270.

Borewicz K, Gu F, Saccenti E, Arts ICW, Penders J, Thijs C, van Leeuwen SS, Lindner C, Nauta A, van Leusen E, Schols HA, Smidt H. 2019. Correlating infant fecal microbiota composition and human milk oligosaccharide consumption by microbiota of 1-month-old breastfed infants. *Mol. Nutr. Food Res.* 63:1801214.

Boudry G, Hamilton MK, Chichlowski M, Wickramasinghe S, Barile D, Kalanetra KM, Mills DA, Raybould HE. 2017. Bovine milk oligosaccharides decrease gut permeability and improve inflammation and microbial dysbiosis in diet-induced obese mice. *J. Dairy Sci*. 100:2471–2481.

Cabrera-Rubio R, Kunz C, Rudloff S, García-Mantrana I, Crehuá-Gaudiza E, Martínez-Costa C, Collado MC. 2019. Association of maternal secretor status and human milk oligosaccharides with milk microbiota: An observational pilot study. *J. Pediatr. Gastroenterol. Nutr*. 68:256–263.

Chang JG, Yang TY, Liu TC, Lin TP, Hu CJ, Kao MC, Wang NM, Tsai FJ, Peng CT, Tsai CH. 1999. Molecular analysis of secretor type alpha(1,2)-fucosyltransferase gene mutations in the Chinese and Thai populations. *Transfusion (Paris)* 39:1013–1017.

Chaturvedi P, Warren CD, Altaye M, Morrow AL, Ruiz-Palacios G, Pickering LK, Newburg DS. 2001. Fucosylated human milk oligosaccharides vary between individuals and over the course of lactation. *Glycobiology*. 11:365–372.

Chen X. 2015. Human Milk Oligosaccharides (HMOS), in: Advances in Carbohydrate Chemistry and Biochemistry. Elsevier. p. 113–190.

Chichlowski M, De Lartigue G, German JB, Raybould HE, Mills DA. 2012. Bifidobacteria isolated from infants and cultured on human milk oligosaccharides affect intestinal epithelial function. *J. Pediatr. Gastroenterol. Nutr*. 55:321–327.

Choi A, Fusch G, Rochow N, Sheikh N, Fusch C. 2015. Establishment of micromethods for macronutrient contents analysis in breast milk: Micromethodology for measuring macronutrient contents in breast milk. *Matern. Child Nutr*. 11:761–772.

Coppa GV, Gabrielli O, Zampini L, Galeazzi T, Ficcadenti A, Padella L, Santoro L, Soldi S, Carlucci A, Bertino E, Morelli L. 2011. Oligosaccharides in 4 different milk groups, Bifidobacteria, and Ruminococcus obeum. *J. Pediatr. Gastroenterol. Nutr.* 53:80–87.

Coppa GV, Zampini L, Galeazzi T, Facinelli B, Ferrante L, Capretti R, Orazio G. 2006. Human milk oligosaccharides inhibit the adhesion to Caco-2 cells of diarrheal pathogens: *Escherichia coli*, *Vibrio cholerae*, and *Salmonella fyris*. *Pediatr. Res*. 59:377–382.

Coppa G, Pierani P, Zampini L, Carloni I, Carlucci A, Gabrielli O. 1999. Oligosaccharides in human milk during different phases of lactation. *Acta Paediatr*. 88:89–94.

Coppa GV, Gabrielli O, Pierani P, Catassi C, Carlucci A, Giorgi PL. 1993. Changes in carbohydrate composition in human milk over 4 months of lactation. *Pediatrics*. 91:637–641.

Davis JCC, Lewis ZT, Krishnan S, Bernstein RM, Moore SE, Prentice AM, Mills DA, Lebrilla CB, Zivkovic AM. 2017. Growth and morbidity of Gambian infants are influenced by maternal milk oligosaccharides and infant gut microbiota. *Sci. Rep*. 7:40466.

Elwakiel M, Hageman JA, Wang W, Szeto IM, van Goudoever JB, Hettinga KA, Schols HA. 2018. Human milk oligosaccharides in colostrum and mature milk of Chinese mothers: Lewis positive secretor subgroups. *J. Agric. Food Chem*. 66:7036–7043.

Engfer MB, Stahl B, Finke B, Sawatzki G, Daniel H. 2000. Human milk oligosaccharides are resistant to enzymatic hydrolysis in the upper gastrointestinal tract. *Am. J. Clin. Nutr*. 71:1589–1596.

Erney RM, Malone WT, Skelding MB, Marcon AA, Kleman-Leyer KM, O'Ryan ML, Ruiz-Palacios G, Hility MD, Pickering LK, Prieto PA. 2000. Variability of human milk neutral oligosaccharides in a diverse population. *J. Pediatr. Gastroenterol. Nutr*. 30:181–192.

Ferreira AL, Alves R, Figueiredo A, Alves-Santos N, Freitas-Costa N, Batalha M, Yonemitsu C, Manivong N, Furst A, Bode L, Kac G. 2020. Human milk oligosaccharide profile variation throughout postpartum in healthy women in a Brazilian cohort. *Nutrients*. 12:790–810.

Ferrer-Admetlla A, Sikora M, Laayouni H, Esteve A, Roubinet F, Blancher A, Calafell F, Bertranpetit J, Casals F. 2009. A natural history of FUT2 polymorphism in humans. *Mol. Biol. Evol*. 26:1993–2003.

Gabrielli O, Zampini L, Galeazzi T, Padella L, Santoro L, Peila C, Giuliani F, Bertino E, Fabris C, Coppa GV. 2011. Preterm milk oligosaccharides during the first month of lactation. *Pediatrics*. 128:e1520–e1531.

Galeotti F, Coppa GV, Zampini L, Maccari F, Galeazzi T, Padella L, Santoro L, Gabrielli O, Volpi N. 2014. Capillary electrophoresis separation of human milk neutral and acidic oligosaccharides derivatized with 2-aminoacridone: CE and CEC. *Electrophoresis.* 35:811–818.

Gnoth MJ, Kunz C, Kinne-Saffran E, Rudloff S. 2000. Human milk oligosaccharides are minimally digested *in vitro*. *J. Nutr*. 130:3014–3020.

Goehring KC, Kennedy AD, Prieto PA, Buck RH. 2014. Direct evidence for the presence of human milk oligosaccharides in the circulation of breastfed infants. *PLoS ONE*. 9:e101692.

Gridneva Z, Rea A, Tie WJ, Lai CT, Kugananthan S, Ward LC, Murray K, Hartmann PE, Geddes DT. 2019. Carbohydrates in human milk and body composition of term infants during the first 12 months of lactation. *Nutrients*. 11:1472.

Guo M, Luo G, Lu R, Shi W, Cheng H, Lu Y, Jin K, Yang C, Wang Z, Long J, Xu J, Ni Q, Liu C, Yu X. 2017. Distribution of *Lewis* and *Secretor* polymorphisms and corresponding CA19-9 antigen expression in a Chinese population. *FEBS Open Bio.* 7:1660–1671.

Henry S, Mollicone R, Fernandez P, Samuelsson B, Oriol R, Larson G. 1996. Molecular Basis for Erythrocyte Le(a+b+) and salivary ABH partial-secretor phenotypes: Expression of a FUT2 secretor allele with an A→T mutation at nucleotide 385 correlates with reduced α(1,2)fucosyltransferase activity. *Glycoconj. J*. 13:985–993.

Hong Q, Ruhaak LR, Totten SM, Smilowitz JT, German JB, Lebrilla CB. 2014. Label-free absolute quantitation of oligosaccharides using multiple reaction monitoring. *Anal. Chem.* 86:2640–2647.

Huang X, Zhu B, Jiang T, Yang C, Qiao W, Hou J, Han Y, Xiao H, Chen L. 2019. Improved Simple Sample Pretreatment Method for Quantitation of Major Human Milk Oligosaccharides Using Ultrahigh Pressure Liquid Chromatography with Fluorescence Detection. *J. Agric. Food Chem*. 67. 12237–12244.

Jacobi SK, Yatsunenko T, Li D, Dasgupta S, Robert KY, Berg BM, Chichlowski M, Odle J. 2016. Dietary isomers of sialyllactose increase ganglioside sialic acid concentrations in the corpus callosum and cerebellum and modulate the colonic microbiota of formula-fed piglets. *J. Nutr*. 146:200–208.

Kobata A. 2010. Structures and application of oligosaccharides in human milk. *Proc. Jpn. Acad. Ser. B*. 86:731–747.

Koda Y, Soejima M, Liu Y, Kimura H. 1996. Molecular basis for Secretor type a(1,2)-fucosyltransferase gene deficiency in a Japanese population: A fusion gene generated by unequal crossover responsible for the enzyme deficiency. *Am. J. Hum. Genet*. 59:343–350.

Kunz C, Meyer C, Collado MC, Geiger L, García-Mantrana I, Bertua-Ríos B, Martínez-Costa C, Borsch C, Rudloff S. 2017. Influence of gestational age, secretor, and Lewis blood group status on the oligosaccharide content of human milk. *J. Pediatr. Gastroenterol. Nutr*. 64:789–798.

Kunz C, Rudloff S, Schad W, Braun D. 1999. Lactose-derived oligosaccharides in the milk of elephants: comparison with human milk. *Br. J. Nutr.* 82:391–399.

Langhendries JP, Detry J, Van Hees J, Lamboray JM, Darimont J, Mozin MJ, Secretin MC, Senterre J. 1995. Effect of a fermented infant formula containing viable Bifidobacteria on the fecal flora composition and pH of healthy full-term infants. *J. Pediatr. Gastroenterol. Nutr*. 21:177–181.

Larsson MW, Lind MV, Laursen RP, Yonemitsu C, Larnkjær A, Mølgaard C, Michaelsen KF, Bode L. 2019. Human milk oligosaccharide composition is associated with excessive weight gain during exclusive breastfeeding—An explorative study. *Front. Pediatr*. 7:297.

Leo F, Asakuma S, Fukuda K, Senda A, Urashima T. 2010. Determination of sialyl and neutral oligosaccharide levels in transition and mature milks of Samoan women, using anthranilic derivatization followed by reverse phase high performance liquid chromatography. *Biosci. Biotechnol. Biochem.* 74:298–303.

Leoz MLAD, Wu S, Strum JS, Niñonuevo MR, Gaerlan SC, Mirmiran M, German JB, Mills DA, Lebrilla CB, Underwood MA. 2013. A quantitative and comprehensive method to analyze human milk oligosaccharide structures in the urine and feces of infants. *Anal. Bioanal. Chem*. 405:4089–4105.

Lewis ZT, Totten SM, Smilowitz JT, Popovic M, Parker E, Lemay DG, Van Tassell ML, Miller MJ, Jin Y-S, German JB, Lebrilla CB, Mills DA. 2015. Maternal fucosyltransferase 2 status affects the gut bifidobacterial communities of breastfed infants. *Microbiome*. 3:13.

Liu Y, Koda Y, Soejima M, Pang H, Schlaphoff T, du Toit ED, Kimura H. 1998. Extensive polymorphism of the FUT2 gene in an African (Xhosa) population of South Africa. *Hum. Genet*. 103:204–210.

Liu Z, Moate P, Cocks B, Rochfort S. 2014. Simple liquid chromatography–mass spectrometry method for quantification of major free oligosaccharides in bovine milk. *J. Agric. Food Chem*. 62:11568–11574.

Ma L, McJarrow P, Jan Mohamed HJB, Liu X, Welman A, Fong BY. 2018. Lactational changes in the human milk oligosaccharide concentration in Chinese and Malaysian mothers' milk. *Int. Dairy J*. 87:1–10.

Manthey CF, Autran CA, Eckmann L, Bode L. 2014. Human milk oligosaccharides protect against Enteropathogenic *Escherichia coli* attachment *in vitro* and EPEC colonization in suckling mice. *J. Pediatr. Gastroenterol. Nutr*. 58:167–170.

Marcobal A, Barboza M, Froehlich JW, Block DE, German JB, Lebrilla CB, Mills DA. 2010. Consumption of human milk oligosaccharides by gut-related microbes. *J. Agric. Food Chem*. 58:5334–5340.

Martín-Sosa S, Martín M-J, Hueso P. 2002. The sialylated fraction of milk oligosaccharides is partially responsible for binding to enterotoxigenic and uropathogenic *Escherichia coli* human strains. *J. Nutr*. 132:3067–3072.

McGuire MK, Meehan CL, McGuire MA, Williams JE, Foster J, Sellen DW, Kamau-Mbuthia EW, Kamundia EW, Mbugua S, Moore SE, Prentice AM, Kvist LJ, Otoo GE, Brooker SL, Price WJ, Shafii B, Placek C, Lackey KA, Robertson B, Manzano S, Ruíz L, Rodríguez JM, Pareja RG, Bode L. 2017. What's normal? Oligosaccharide concentrations and profiles in milk produced by healthy women vary geographically. *Am. J. Clin. Nutr*. 105:1086–1100.

McJarrow P, Radwan H, Ma L, MacGibbon AKH, Hashim M, Hasan H, Obaid RS, Naja F, Mohamed HJJ, Al Ghazal H, Fong BY. 2019. Human milk oligosaccharide, phospholipid, and ganglioside concentrations in breast milk from United Arab Emirates mothers: Results from the MISC cohort. *Nutrients*. 11:2400.

Mernie EG, Tolesa LD, Lee M-J, Tseng M-C, Chen Y-J. 2019. Direct oligosaccharide profiling using thin-layer chromatography coupled with ionic liquid-stabilized nanomatrix-assisted laser desorption-ionization mass spectrometry. *Anal. Chem*. 91:11544–11552.

Midtvedt A-C, Midtvedt T. 1992. Production of short chain fatty acids by the intestinal microflora during the first 2 years of human life. *J. Pediatr. Gastroenterol. Nutr*. 15:395–403.

Montreuil J, Mullet S. 1960. Etude des variations des constituants glucidiques du lait de femme au cours de la lactation. *Bull. Soc. Chim. Biol. (Paris)*. 42:365–378.

Moossavi S, Atakora F, Miliku K, Sepehri S, Robertson B, Duan QL, Becker AB, Mandhane PJ, Turvey SE, Moraes TJ, Lefebvre DL, Sears MR, Subbarao P, Field CJ, Bode L, Khafipour E, Azad MB. 2019. Integrated analysis of human milk microbiota with oligosaccharides and fatty acids in the CHILD cohort. *Front. Nutr*. 6:58.

Morrow AL, Ruiz-Palacios GM, Altaye M, Jiang X, Lourdes Guerrero M, Meinzen-Derr JK, Farkas T, Chaturvedi P, Pickering LK, Newburg DS. 2004. Human milk oligosaccharides are associated with protection against diarrhea in breast-fed infants. *J. Pediatr*. 145:297–303.

Musumeci M, Simpore J, D'Agata A, Sotgiu S, Musumeci S. 2006. Oligosaccharides in colostrum of Italian and Burkinabe women. *J. Pediatr. Gastroenterol. Nutr.* 43:372–378.

Nakhla T, Fu D, Zopf D, Brodsky NL, Hurt H. 1999. Neutral oligosaccharide content of preterm human milk. *Br. J. Nutr*. 82:361–367.

Newburg DS, Ruiz-Palacios GM, Altaye M, Chaturvedi P, Meinzen-Derr J, Guerrero M de L, Morrow AL. 2004. Innate protection conferred by fucosylated oligosaccharides of human milk against diarrhea in breastfed infants. *Glycobiology*. 14:253–263.

Nijman RM, Liu Y, Bunyatratchata A, Smilowitz JT, Stahl B, Barile D. 2018. Characterization and quantification of oligosaccharides in human milk and infant formula. *J. Agric. Food Chem*. 66:6851–6859.

Ninonuevo MR, Park Y, Yin H, Zhang J, Ward RE, Clowers BH, German JB, Freeman SL, Killeen K, Grimm R, Lebrilla CB. 2006. A strategy for annotating the human milk glycome. *J. Agric. Food Chem*. 54:7471–7480.

Olivares M, Albrecht S, De Palma G, Ferrer MD, Castillejo G, Schols HA, Sanz Y. 2015. Human milk composition differs in healthy mothers and mothers with celiac disease. *Eur. J. Nutr*. 54:119–128.

Oliveros E, Vázquez E, Barranco A, Ramírez M, Gruart A, Delgado-García J, Buck R, Rueda R, Martín M. 2018. Sialic acid and sialylated oligosaccharide supplementation during lactation improves learning and memory in rats. *Nutrients*. 10:1519.

Pacheco AR, Barile D, Underwood MA, Mills DA. 2015. The impact of the milk glycobiome on the neonate gut microbiota. *Annu. Rev. Anim. Biosci*. 3:419–445.

Pang H, Koda Y, Soejima M, Fujitani N, Ogaki T, Saito A, Kawasaki T, Kimura H. 2001. Polymorphism of the human ABO-Secretor locus (FUT2) in four populations in Asia: Indication of distinct Asian subpopulations. *Ann. Hum. Genet*. 65:429–437.

Pang H, Fujitani N, Soejima M, Koda Y, Islam MN, Islam MS, Kimura H. 2000. Two distinct alu-mediated deletions of the human ABO-secretor (FUT2) locus in Samoan and Bangladeshi populations. *Human Mutation*.

Park H-D, Park KU, Song J, Ki C-S, Han KS, Kim JQ. 2010. The relationship between *Lewis/Secretor* genotypes and serum carbohydrate Antigen 19-9 levels in a Korean population. *Korean J. Lab. Med*. 30:51–57.

Parkkinen J, Rogers GN, Korhonen T, Dahr W, Finne J. 1986. Identification of the O-linked sialyloligosaccharides of glycophorin A as the erythrocyte receptors for S-fimbriated *Escherichia coli*. *Infect. Immun*. 54:37–42.

Perrin MT, Fogleman AD, Newburg DS, Allen JC. 2017. A longitudinal study of human milk composition in the second year postpartum: implications for human milk banking. *Matern. Child Nutr*. 13.

Prentice P, Acerini CL, Eleftheriou A, Hughes IA, Ong KK, Dunger DB. 2016a. Cohort profile: the Cambridge Baby Growth Study (CBGS). *Int. J. Epidemiol*. 45.

Prentice P, Ong KK, Schoemaker MH, van Tol EAF, Vervoort J, Hughes IA, Acerini CL, Dunger DB. 2016b. Breast milk nutrient content and infancy growth. *Acta Paediatr*. 105:641–647.

Prentice PM, Schoemaker MH, Vervoort J, Hettinga K, Lambers TT, van Tol EAF, Acerini CL, Olga L, Petry CJ, Hughes IA, Koulman A, Ong KK, Dunger DB. 2019. Human milk short-chain fatty acid composition is associated with adiposity outcomes in infants. *J. Nutr*. 149:716–722.

Prieto PA. 2012. Profiles of human milk oligosaccharides and production of some human milk oligosaccharides in transgenic animals. *Adv. Nutr*. 3:456S-464S.

Robinson RC, Colet E, Tian T, Poulsen NA, Barile D. 2018. An improved method for the purification of milk oligosaccharides by graphitised carbon-solid phase extraction. *Int. Dairy J*. 80:62–68.

Rudloff S, Pohlentz G, Diekmann L, Egge H, Kunz C. 1996. Urinary excretion of lactose and oligosaccharides in preterm infants fed human milk or infant formula. *Acta Paediatr*. 85:598–603.

Ruiz-Moyano S, Totten SM, Garrido DA, Smilowitz JT, German JB, Lebrilla CB, Mills DA. 2013. Variation in consumption of human milk oligosaccharides by infant gut-associated strains of *Bifidobacterium breve*. *Appl. Environ. Microbiol*. 79:6040–6049.

Ruiz-Palacios GM, Cervantes LE, Ramos P, Chavez-Munguia B, Newburg DS. 2003. *Campylobacter jejuni* binds intestinal H(O) antigen (Fucα1, 2Galβ1, 4GlcNAc), and fucosyloligosaccharides of human milk inhibit its binding and infection. *J. Biol. Chem*. 278:14112–14120.

Saben JL, Abraham A, Bode L, Sims CR, Andres A. 2020. Third-trimester glucose homeostasis in healthy women is differentially associated with human milk oligosaccharide composition at 2 months postpartum by secretor phenotype. *Nutrients*. 12:2209.

Sakai F, Ikeuchi Y, Urashima T, Fujihara M, Ohtsuki K, Yanahira S. 2006. Effects of feeding sialyllactose and galactosylated *N*-acetylneuraminic acid on swimming learning ability and brain lipid composition in adult rats. *J. Appl. Glycosci*. 53:249–254.

Samuel TM, Binia A, de Castro CA, Thakkar SK, Billeaud C, Agosti M, Al-Jashi I, Costeira MJ, Marchini G, Martínez-Costa C, Picaud J-C, Stiris T, Stoicescu S-M, Vanpeé M, Domellöf M, Austin S, Sprenger N. 2019. Impact of maternal characteristics on human milk oligosaccharide composition over the first 4 months of lactation in a cohort of healthy European mothers. *Sci. Rep*. 9:11767.

Scott KP, Martin JC, Duncan SH, Flint HJ. 2014. Prebiotic stimulation of human colonic butyrate-producing bacteria and bifidobacteria, *in vitro*. *FEMS Microbiol. Ecol*. 87:30–40.

Sela DA, Li Y, Lerno L, Wu S, Marcobal AM, German JB, Chen X, Lebrilla CB, Mills DA. 2011. An infant-associated bacterial commensal utilizes breast milk sialyloligosaccharides. *J. Biol. Chem*. 286:11909–11918.

Sjögren YM, Duchén K, Lindh F, Björkstén B, Sverremark-Ekström E. 2007. Neutral oligosaccharides in colostrum in relation to maternal allergy and allergy development in children up to 18 months of age: Breast milk, gut flora and allergy. *Pediatr*. *Allergy Immunol.* 18:20–26.

Smilowitz JT, O'Sullivan A, Barile D, German JB, Lönnerdal B, Slupsky CM. 2013. The human milk metabolome reveals diverse oligosaccharide profiles. *J. Nutr*. 143:1709–1718.

Spevacek AR, Smilowitz JT, Chin EL, Underwood MA, German JB, Slupsky CM. 2015. Infant maturity at birth reveals minor differences in the maternal milk metabolome in the first month of lactation. *J. Nutr*. 145:1698–1708.

Sprenger N, Lee LY, de Castro CA, Steenhout P, Thakkar SK. 2017. Longitudinal change of selected human milk oligosaccharides and association to infants' growth, an observatory, single center, longitudinal cohort study. *PLoS ONE*. 12:e0171814.

Srivastava, A., Tripathi, R., Verma, S., Srivastava, N., Rawat, A.K.S., Deepak, D., 2014. A novel method for quantification of lactose in mammalian milk through HPTLC and determination by a mass spectrometric technique. *Anal. Methods.* 6, 7268–7276.

Stepans MBF, Wilhelm SL, Hertzog M, Rodehorst TKC, Blaney S, Clemens B, Polak JJ, Newburg DS. 2006. Early consumption of human milk oligosaccharides is inversely related to subsequent risk of respiratory and enteric disease in infants. *Breastfeed. Med*. 1:207–215.

Sumiyoshi W, Urashima T, Nakamura T, Arai I, Nagasawa T, Saito T, Tsumura N, Wang B, Brand-Miller J, Watanabe Y, Kimura K. 2003. Sialyl oligosaccharides in the milk of Japanese women: Changes in concentration during the course of lactation. *J. Appl. Glycosci*. 50:461–467.

Sundekilde U, Downey E, O'Mahony J, O'Shea C-A, Ryan C, Kelly A, Bertram H. 2016. The effect of gestational and lactational age on the human milk metabolome. *Nutrients*. 8:304–318.

Tan J, Chai F, Zhang C. 2015. Determination of human milk oligosaccharides in human breast milk by HPAE-PAD with on-line sample cleanup. ThermoFisher Customer Application Note 119.

Tarr AJ, Galley JD, Fisher SE, Chichlowski M, Berg BM, Bailey MT. 2015. The prebiotics 3′-sialyllactose and 6′-sialyllactose diminish stressor-induced anxiety-like behavior and colonic microbiota alterations: Evidence for effects on the gut–brain axis. *Brain Behav. Immun*. 50:166–177.

Terrazas LI, Walsh KL, Piskorska D, McGuire E, Harn DA. 2001. The Schistosome oligosaccharide lacto- *N* -neotetraose expands Gr1 $^{+}$ cells that secrete anti-inflammatory cytokines and inhibit proliferation of naive CD4 $^{+}$ cells: A potential mechanism for immune polarization in Helminth Infections. *J. Immunol*. 167:5294–5303.

Thurl S, Munzert M, Henker J, Boehm G, Müller-Werner B, Jelinek J, Stahl B. 2010. Variation of human milk oligosaccharides in relation to milk groups and lactational periods. *Br. J. Nutr*. 104:1261–1271.

Thurl S, Müller-Werner B, Sawatzki G. 1996. Quantification of individual oligosaccharide compounds from human milk using high-pH anion-exchange chromatography. *Anal. Biochem.* 235:202–206.

Tonon KM, de Morais, MB, Abrão ACFV, Miranda A, Morais TB. 2019a. Maternal and infant factors associated with human milk oligosaccharides concentrations according to secretor and Lewis phenotypes. *Nutrients*. 11:1358.

Tonon KM, Miranda A, Abrão ACFV, de Morais MB, Morais TB. 2019b. Validation and application of a method for the simultaneous absolute quantification of 16 neutral and acidic human milk oligosaccharides by graphitized carbon liquid chromatography – electrospray ionization – mass spectrometry. *Food Chem*. 274:691–697.

Underwood MA, Gaerlan S, de Leoz MLA, Dimapasoc L, Kalanetra KM, Lemay DG, German JB, Mills DA, Lebrilla CB. 2015. Human milk oligosaccharides in premature infants: absorption, excretion, and influence on the intestinal microbiota. *Pediatr. Res*. 78:670–677.

Urashima T, Hirabayashi J, Sato S, Kobata A. 2018. Human milk oligosaccharides as essential tools for basic and application studies on galectins. *Trends Gycosc. Glycotech.* 30:SE51-SE65.

van Leeuwen SS. 2019. Challenges and pitfalls in human milk oligosaccharide analysis. *Nutrients*. 11:2684.

van Leeuwen SS, Stoutjesdijk E, ten Kate GA, Schaafsma A, Dijck-Brouwer J, Muskiet FAJ, Dijkhuizen L. 2018. Regional variations in human milk oligosaccharides in Vietnam suggest FucTx activity besides FucT2 and FucT3. *Sci. Rep*. 8:16790.

van Leeuwen SS, Schoemaker RJW, Gerwig GJ, van Leusen-van Kan EJM, Dijkhuizen L, Kamerling JP. 2014. Rapid milk group classification by ^{1}HNMR analysis of Le and H epitopes in human milk oligosaccharide donor samples. *Glycobiology*. 24:728-739.

Van Niekerk E, Autran CA, Nel DG, Kirsten GF, Blaauw R, Bode L. 2014. Human milk oligosaccharides differ between HIV-infected and HIV-uninfected mothers and are related to necrotizing enterocolitis incidence in their preterm very-low-birth-weight infants. *J. Nutr*. 144:1227–1233.

Viverge D, Grimmonprez L, Cassanas G, Bardet L, Bonnet H, Solere M. 1985. Variations of lactose and oligosaccharides in milk from women of blood types Secretor A or H, Secretor Lewis, and Secretor H/Nonsecretor Lewis during the course of lactation. *Ann. Nutr. Metab*. 29:1–11.

Viverge D, Grimmonprez L, Cassanas G, Bardet L, Solere M. 1986. Diurnal variations and within the feed in lactose and oligosaccharides of human milk. *Ann. Nutr. Metab*. 30:196–209.

Wang M, Zhao Z, Zhao A, Zhang J, Wu W, Ren Z, Wang P, Zhang Y. 2020. Neutral human milk oligosaccharides are associated with multiple fixed and modifiable maternal and infant characteristics. *Nutrients*. 12:826.

Ward RE, Niñonuevo M, Mills DA, Lebrilla CB, German JB. 2007. *In vitro* fermentability of human milk oligosaccharides by several strains of bifidobacteria. *Mol. Nutr. Food Res*. 51:1398–1405.

Wong JMW, de Souza R, Kendall CWC, Emam A, Jenkins DJ. 2006. Colonic health: Fermentation and short chain fatty acids. *J. Clin. Gastroenterol*. 40:235–243.

World Health Organization Health Topics - Breastfeeding. 2018. www.who.int/health-topics/breastfeeding.

Wu S, Grimm R, German JB, Lebrilla CB. 2011. Annotation and structural analysis of sialylated human milk oligosaccharides. *J. Proteome Res*. 10:856–868.

Wu S, Tao N, German JB, Grimm R, Lebrilla CB. 2010. Development of an annotated library of neutral human milk oligosaccharides. *J. Proteome Res*. 9:4138–4151.

Xu G, Davis JC, Goonatilleke E, Smilowitz JT, German JB, Lebrilla CB. 2017. Absolute quantitation of human milk oligosaccharides reveals phenotypic variations during lactation. *J. Nutr*. 147:117–124.

Yip SP, Lai SK, Wong ML. 2007. Systematic sequence analysis of the human fucosyltransferase 2 (FUT2) gene identifies novel sequence variations and alleles. *Transfusion (Paris)*. 47:1369–1380.

Yu Z-T, Chen C, Kling DE, Liu B, McCoy JM, Merighi M, Heidtman M, Newburg DS. 2013. The principal fucosylated oligosaccharides of human milk exhibit prebiotic properties on cultured infant microbiota. *Glycobiology*. 23:169–177.

Zhang W, Wang T, Chen X, Pang X, Zhang S, Ujiroghe OJ, Shilong J, Lu J, Lv J. 2019. Absolute quantification of twelve oligosaccharides in human milk using a targeted mass spectrometry-based approach. *Carbohydr. Polym.* 219:328–333.

CHAPTER III:

Dietary fiber to starch ratios affect bovine milk oligosaccharide profiles

Published as:

Durham, S.D., Lemay, D.G.; Wei, Z.; Kalcheur, K.F.; Finley, J.W.; Fukagawa, N.; Barile, D. Dietary fiber to starch ratios affect bovine milk oligosaccharide profiles. *Current Developments in Nutrition*. **2022**.

ABSTRACT

Bovine milk oligosaccharides (BMOs) have several demonstrated and hypothesized benefits including roles in cognitive development and anti-pathogenic activities, making them promising ingredients for infant formulas and nutraceutical applications. BMO extraction from bovine milk is challenged by low concentrations relative to non-bioactive simple sugars like lactose. BMO abundances are known to vary with a cow's lactation stage, breed, and parity, but these characteristics are difficult to modify in existing dairy herds. In contrast, diet modification is an accessible target, and is already known to influence milk yield, lipid content, protein levels, and monosaccharide compositions. The objective of this study was to determine the impact of a low starch high fiber versus a high starch low fiber diet on overall BMO profiles and individual BMO abundances in Holstein dairy cattle. Milk samples were collected from 59 mid-lactation Holsteins in a crossover study featuring dietary modification with either a low starch high fiber or high starch low fiber feed. BMO profiles were evaluated by nano-liquid chromatography quadrupole time-of-flight tandem mass spectrometry, and differences in BMO abundances between diets were evaluated using linear mixed-effects modeling. 19 BMOs were identified across the sample set, including four large fucosylated compounds. 7 BMOs were found to have significantly more positive percent changes in yield-adjusted abundance from the pre-experiment baseline period for milk samples collected during feeding with the low starch high fiber diet compared to the high starch low fiber diet. Consuming the low starch high fiber diet promoted greater overall BMO production than the high starch low fiber diet in a population of mid-lactation Holsteins. Additionally, this study afforded the opportunity to investigate the impact of other factors potentially influencing BMO abundances, furthering understanding of how dairy

herd management practices can positively impact milk composition and support the potential use of BMOs as functional ingredients.

INTRODUCTION

Bovine milk oligosaccharides (BMOs) are a class of carbohydrates found in cows' milk composed of between 3 and 11 monosaccharide subunits connected by glycosidic linkages. The core of a BMO structure is either a lactose (galactose(β1-4)glucose) or lactosamine (galactose(β1-4)*N*-acetylglucosamine) reducing end. These core structures may then be expanded through the addition of further galactose (Gal), *N*-acetylglucosamine (GlcNAc), or *N*-acetylgalactosamine (GalNAc) units and decorated with α2-3- or α2-6-linked *N*-acetylneuraminic acid (Neu5Ac) or *N*-glycolylneuraminic acid (Neu5Gc) or, less commonly, α1-2- or α1-3-linked fucose (Fuc) (1). BMOs may be classified as either acidic or neutral based on the presence of absence of sialic acid (Neu5Ac or Neu5Gc) in their structures. Neutral BMOs can be further designated as either neutral fucosylated or neutral unfucosylated based on whether or not they contain fucose monomers. BMOs discussed herein are referred to by their monosaccharide composition as the number of Hex_HexNAc_Fuc_Neu5Ac_Neu5Gc, followed by an isomer designation when applicable. Following this nomenclature, acidic BMOs can be identified by the presence of a non-zero number in either the fourth or fifth positon of the 5-digit numerical code, while neutral fucosylated BMOs can be distinguished by the presence of a non-zero number as the third digit of the compositional code, as shown in Supplemental Table 1 and Supplemental Figure 1.

BMOs have numerous demonstrated health and development benefits which are particularly relevant for human infants. BMOs exhibit anti-adhesive and anti-pathogen activity against major enteric pathogens including *Campylobacter jejuni* (2) and enterotoxigenic *Escherichia coli* (ETEC) (3). The two most abundant acidic BMOs, 3'-sialyllactose (3'-SL) and 6'-sialyllactose (6'-SL) have also been shown to exhibit anti-pathogenic effects against enteropathogenic *E. coli* (EPEC) (4), S fimbriated *E. coli* (5), *Salmonella enterica* ssp. *enterica* ser. Fyris (4), and *Pseudomonas aeruginosa* (6). In addition, BMOs have demonstrated improved gut barrier function *in vitro* (7), as well as decreased gut permeability, increased lean body mass, and healthy organ growth in animal models of infant undernutrition (8-9). Sialylated milk oligosaccharides including 3'-SL and 6'-SL have also been linked with increased sialylation of cerebellum gangliosides, upregulated genes for myelination and ganglioside synthesis in the hippocampus, and improved learning outcomes in animal models (10-12).

Due to their structural similarities with human milk oligosaccharides, BMOs are also hypothesized to have prebiotic activity. Recent *in vitro* studies featuring BMOs support this hypothesis. Isolated BMOs or sialyllactose have been shown to promote the *in vitro* growth of the beneficial infant gut microbes *Parabacteroides distasonis*, *Bifidobacterium breve*, and *B. longum* ssp. *longum* (13) as well as the probiotic *B. animalis* ssp. *lactis* (14). In addition, BMOs have been shown to promote the colonization of *B. longum* ssp. *infantis* when co-administered in mouse models (15).

Despite the clear benefits, isolating BMOs for use in products like infant formulas and nutraceuticals is challenging due to their low concentrations both in milk and dairy processing streams like whey permeate. Unlike human milk oligosaccharides which are present in concentrations of around 12-16 g/L in colostrum and 5 to 11 g/L in milk (16-19), BMOs are only found at around 1 g/L in bovine colostrum and fall to 80 to 100 mg/L in mature milk (20-21). Increasing the concentrations of BMOs in milk would facilitate their isolation. In addition, modifying BMO profiles to be more similar to human milk oligosaccharides with greater abundances of larger and more fucosylated structures would improve the bioactivity of the resultant BMO isolate.

BMO abundances have been previously shown to vary with lactation time point (20-21), cow breed (22-26), and parity (20, 23). However, these factors are difficult to modify in existing dairy herds. Cow diet has been well documented to influence the yield (27-30), lipid profiles (28, 29, 31-36), nitrogen content (27, 29, 32-34, 36), and monosaccharide composition (37) of cows' milk. Dietary supplementation with chitooligosaccharides in sows has also been previously linked with increased abundances of some pig milk oligosaccharides (38). Although a connection between diet and milk oligosaccharides has not yet been shown in ruminants, cow diet is an easily modified factor that has the potential to favorably impact BMO profiles and concentrations.

The impact of the ratio of dietary fiber to starch on BMO content is of particular interest because the balance of these components in feed influences both the ruminal buffering capacity and the digesta passage rate in the cow. These factors, in turn, affect the balance between the breakdown of feed components in the rumen and the absorption of breakdown products in the rumen and small intestine. Although the biochemical pathways for BMO synthesis and the precursors involved have not yet been fully elucidated, the absorption of more energetically favorable building blocks, as influenced by the composition of digestion breakdown products absorbed in the small intestine, may favor BMO production. In this study BMO profiles were evaluated in a herd of Holstein dairy cattle across a 3-period cross-over study design with the objectives of identifying significant variations in BMO profiles and abundances based on dietary fiber to starch ratio, cow parity, and lactation time point.

MATERIALS AND METHODS

Study Design

Milk samples were collected from 76 mid-lactation Holstein dairy cattle in a crossover study design that included sampling during a 4-week pre-experimental baseline period and two subsequent 70-day treatment periods in which cows were fed either a low starch high fiber diet (LSHF; 37% neutral detergent fiber (NDF), 13% starch) or a high starch low fiber diet (HSLF; 29% NDF, 27% starch). At the end of each period, cows were assigned to the opposite diet, as shown in Figure 1, such that each cow acted as its own control. There was an 11-day transition period between each period. Milk samples were collected across the three dietary periods, with a sample collected from each cow during two consecutive morning milkings in the final week of the pre-experimental baseline period and week 5 of each experimental period (39).

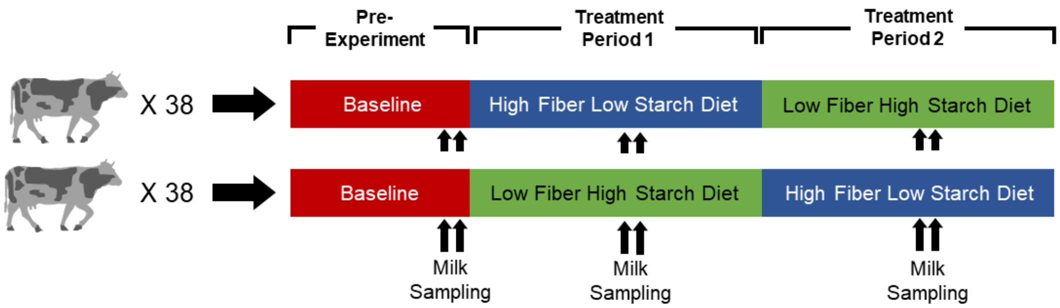


Figure 3.1. Crossover design of this study featuring a baseline period followed by two 70-day treatment periods, with milk samples collected for oligosaccharide profiling on two consecutive days in the final week of the baseline period and during the fifth week of each dietary period.

Both treatment diets and the baseline diet were composed of a combination of beet pulp, alfalfa silage, corn silage, canola meal, high moisture corn, corn distillers' grains, roasted soybeans and soy hulls, mixed at different proportions such that the diets differed in fiber and starch levels but were balanced for protein availability and other key nutrients (Supplemental Table 2). The baseline diet fed in the pre-experiment period was formulated to have starch and fiber contents halfway between those of the LSHF and HSLF diets. Cows were assigned to the two groups in a balanced manner based on evaluation of their parity, dry matter intake, milk production, and body weight during the pre-experimental baseline period. Cows were housed in indoor tie-stalls throughout the duration of the study. Feed was provided *ad libitum* once a day, with feed amounts adjusted daily to allow a maximum of 10% refusals individually, determined based on the refusals measured 2 days prior. Cows were milked three times per day (0400 h, 1030 h, and 1800 h). All milk for BMO analysis was collected during the first morning milking after teats were stripped (3 streams of milk), treated and disinfected with Gladiator Barrier (BouMatic, Wisconsin, USA) and towel dried. Raw milk was collected for BMO analysis on two consecutive days after five weeks of consumption of each experimental diet in portions of approximately 48 mL each. Aliquots were stored at -10 °C immediately after collection and shipped on dry ice to the USDA-ARS Western Human Nutrition Research Center. Here the samples were thawed, portioned into smaller 2 mL aliquots and stored at -20 °C until later analysis.

All feeding and milk collection portions of this study were conducted at the USDA-ARS Dairy Forage Research Center Dairy Farm (Prairie du Sac, WI) under protocols approved by the University of Wisconsin-Madison Institutional Animal Care and Use Committee (Protocol #A005945).

Sample Subset Selection

From the full set of 456 available milk samples, 338 (from 59 cows) were selected for BMO analysis. 38 total samples from 9 cows were excluded because the cows received antibiotic treatment during the corresponding or prior study period. 4 samples were removed because at least one sample was missing in a given period or collected outside of the morning milking. 4 samples were removed because they were collected after 300 days of lactation and had particularly low milk yields. 54 total samples from 9 cows were removed due to technical issues related to accurately estimating their feed intake. In addition, 18 total samples from 5 cows were removed because they were outliers for a given period for either the lactose concentration or BMO abundances, as evaluated by the standard error with a cut-off of 3 (39).

Oligosaccharide Extraction and Multiplexing

Oligosaccharides were extracted, labeled, and analyzed from milk samples as described previously (40-41) with some modifications. Samples were skimmed to remove lipids, and for each cow, skimmed milk samples collected on consecutive days within the same period were pooled to minimize the influence of day-to-day variations in milk composition. Pooled samples then underwent ethanol precipitation to remove proteins, followed by C18 microplate solid phase extraction (SPE) to remove peptides, and graphitized carbon microplate SPE to remove lactose and salts. A 4% acetonitrile/0.1% trifluoroacetic acid solution was used for solid phase equilibration and sample washing during graphitized carbon microplate SPE to maximize lactose removal while minimizing BMO loss.

Extracted oligosaccharides from samples were then isobarically labeled with aminoxy tandem mass tags (TMTs) with reporter ions of 127, 128, 129, 130, or 131 Da, and a previously characterized bovine milk oligosaccharide mixture (42) labeled with the aminoxy TMT 126 Da reporter ion was used for as an internal standard. Labeled samples were multiplexed such that each set of six aminoxy TMTs contained the labeled internal standard and five unique samples, each labeled with a different one of the five remaining TMTs. Multiplexed samples underwent an additional SPE clean-up employing Oasis Hydrophilic-Lipophilic Balance cartridges to remove excess labeling reagents prior to LC-MS analysis.

LC-MS/MS Analysis

Glycoprofiling of oligosaccharides in the collected samples was conducted using nano-liquid chromatography chip quadrupole time-of-flight mass spectrometry (nano-LC-chip-Q-ToF MS) using our previously published LC-MS method (41) with slight modifications. Briefly, samples were dissolved in 3% acetonitrile, and passed through 0.2 μm polyethersulfone filters, and loaded onto the nano-LC chip with a 40 nL enrichment column and a 75 μm x 43 mm analytical column, packed with 5 μm particles of 250Å pore size. Flow rates were operated at 4 μL/min (enrichment column) and 0.3 μL/min (analytical column). Mobile phase solvents were 3% acetonitrile/0.1% formic acid (A), and 89.9% acetonitrile/0.1% formic acid (B). After equilibrating both the analytical and enrichment columns with 100% A, and a 65-minute gradient was used for chromatographic separation. The gradient was ramped from 4 to 20.6% B from 0 to 23 min, 20.6 to 50% B from 23 to 30 min, 50 to 100% B from 30 to 35 min, held at 100% B from 35 to 50 min, then lowered from 100 to 0% B from 50 to 50.1 min.

Mass spectra were collected in positive mode over a scan range of 400 to 2500 m/z at a rate of two spectra/s for MS scans and 100 to 2500 m/z at a rate of one spectra/s for MS/MS. The drying gas was held at 350 °C with a flow of 5 L/min. An in-house library of BMO masses assembled from the literature (1, 23, 43-45) was entered in the acquisition software as a list for targeted fragmentation. The five most abundant precursors in each MS scan matching to the targeted list were fragmented, with a quadrupole isolation window of ~4 m/z. A minimum precursor threshold of 5,000 ion counts/spectrum was set to ensure substantial reporter ion abundance in the MS/MS scans. Capillary voltage was varied from 1900 to 1975 V as needed to maintain a stable spray. In-run mass calibration was performed with infused calibrant ions of m/z 922.009798 and 1221.990637.

BMOs were identified using a customized bioinformatics library of bovine milk oligosaccharide compounds assembled from prior publications (1, 23, 43-45) and their identities were confirmed by the examination of MS/MS spectra using Agilent MassHunter B.07.00 (Agilent Technologies, Santa Clara, CA). For relative quantification, raw data was exported in .mzData format with

MassHunter and then imported into SimGlycan Enterprise Edition 5.61 (PREMIER Biosoft, Palo Alto, CA) (46). BMOs with confirmed identities were added to a custom library on the SimGlycan server, which was used by the software to identify those BMOs in the data files through matching retention time and precursor mass using the "High Throughput Search and Score" feature. Precursor ion and reporter ion m/z tolerances were set to 10 ppm and 0.025 Da, respectively. For each BMO, the reporter ion abundances from all the MS/MS spectra were summed, and the ratios of these sums were calculated. The reporter ion abundances for each sample were normalized to the signal for the TMT 126-labeled BMO internal standard to give the BMO relative abundances (Supplemental Table 3).

Statistical Analysis

Glycoprofiling relative abundances were log transformed to improve normality as evaluated by the Shapiro-Wilks test prior to comparative statistical analysis, with the exception of the results for the BMOs with compositions 2_1_0_0_0 isomer 2 and 4_4_1_0_0, which were transformed via a cube root, and 3_6_1_0_0 and 5_4_1_0_0, which did not require a transformation to achieve a normal distribution (Shapiro-Wilks test, $p > 0.05$). Relative abundances were also multiplied by the average morning milk weight (lbs) for the corresponding period to give yield-adjusted relative abundances.

Yield-adjusted relative abundance results were log transformed to improve normality as evaluated by the Shapiro-Wilks test prior to comparative statistical analysis, with the exception of BMO with composition 3_6_1_0_0, which did not require a transformation to achieve a normal distribution (Shapiro-Wilks test, $p > 0.05$). Transformed relative abundances and yield-adjusted relative abundances were evaluated with 2-sided student's t-tests to compare the two post-diet arms and 1-way ANOVA with post hoc evaluation using Tukey's Test to compare the three diet time points. Linear mixed effects modeling was used to determine the significance ($\alpha = 0.05$) of the effects of diet, cow ID, treatment period, dietary sequence, parity, milk yield, and lactation timepoint on the oligosaccharide profiles. In addition, the percent change in transformed relative abundances and percent change in transformed yield-adjusted relative abundances were calculated as

$$\%\ change = \frac{transformed\ relative\ abundance_X\ - transformed\ relative\ abundance_{pre-exp}}{transformed\ relative\ abundance_{pre-exp}}$$

where X is a dietary treatment period. Percent change in relative abundances from the pre-experimental baseline period were evaluated similarly using 2-sided Student's t-test, 1-way ANOVA with post-hoc evaluation using Tukey's Test, and linear mixed effects modeling. Calculation of Pearson's correlations were conducted on transformed data, with all Pearson correlation figures and their significances were generated using the R package corrplot (47). Principal component analysis was conducted on untransformed data. All statistical analyses were conducted using R version 4.0.2.

RESULTS

Identification of Bovine Milk Oligosaccharides and their Abundance in Milk

Milk samples from all of the cows in the study showed a high degree of similarity in BMO composition. Abundances of 19 major BMOs were measured in all samples, including 5 acidic structures and 4 neutral fucosylated compounds. Identified BMOs ranged in size from degrees of polymerization of 3 to 10.

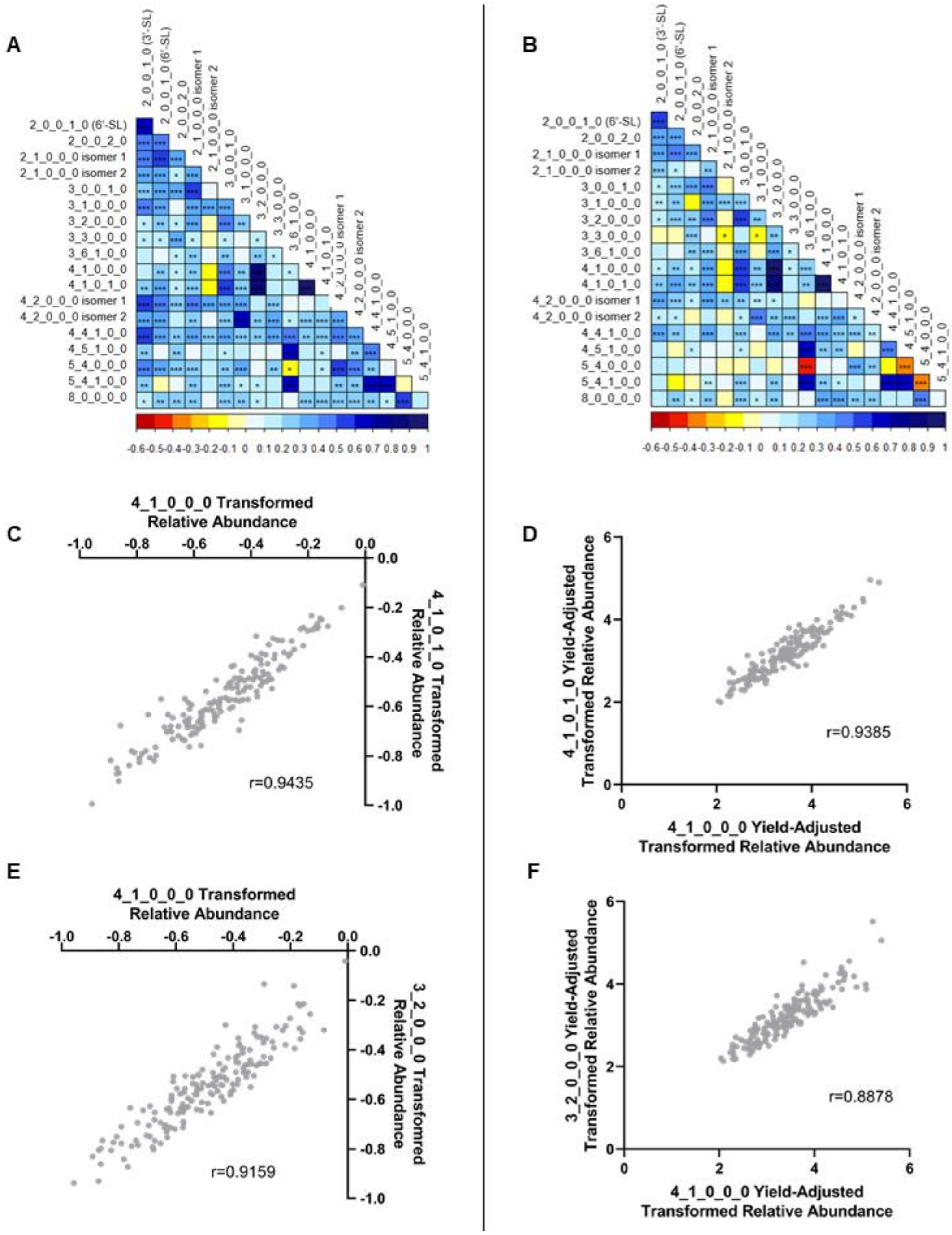


Figure 3.2. Pearson's correlations among oligosaccharide pairs for non-yield corrected (left) and yield-corrected (right) relative abundance data organized as heat maps for all oligosaccharide pairs (A & B), as well as individual plots for the strongest correlations between 4_1_0_0_0 and 4_1_0_1_0 (C, r=0.9504 & D, r=0.9400), and between 4_1_0_0_0 and 3_2_0_0_0 (E, r=0.8651 & F, r=0.8553). BMOs are described by their monosaccharide compositions as the number of Hex_HexNAc_Fuc_Neu5Ac_Neu5Gc, followed by the isomer number, as appropriate. * $0.01 < p \leq 0.05$, ** $0.001 < p \leq 0.01$, *** $p < 0.001$

Diet effects on BMO Profiles

Correlations in abundance between BMOs were identified for transformed data, both without and with adjustment for milk yield. In both cases the strongest correlations were observed between the BMOs 4_1_0_0_0 and 4_1_0_1_0 (Figure 2C & D) and between 4_1_0_0_0 and 3_2_0_0_0 (Figure 2E & F). Significant positive correlations were also observed among the four identified fucosylated BMOs, as well as between the two sialyllactose isomers (Figure 2A & B).

Sources of variation in BMO profiles

A wide spread of BMO abundances was observed both within and across treatment groups. Principal component analysis was conducted to evaluate which, if any, of the main recorded study variables contributed to the observed variation. Although some clustering was present (Figure 3B), very little separation based on cow diet, dietary treatment period, or diet sequence was observed (Figure 3C, D & E). Some separation did occur based on parity, particularly between parity 1 and parities 5 and 6 along the second principal component (Figure 3F). Thus, the largest source of variance in BMO profiles remains as one or more unrecorded factors.
The percent change in the transformed relative abundance from the pre-experiment baseline diet differed significantly ($p < 0.001$) between the LSHF and HSLF dietary treatments for four BMOs (with compositions 3_0_0_1_0, 3_2_0_0_0, 4_1_0_0_0, and 4_1_0_1_0) based on initial t-test comparisons (Figure 1A). For all 4 of these oligosaccharides the abundance was significantly higher ($p < 0.05$) in samples from cows fed the LSHF diet compared to both the HSLF diet and the pre-experimental diet (Supplemental Figure 2).

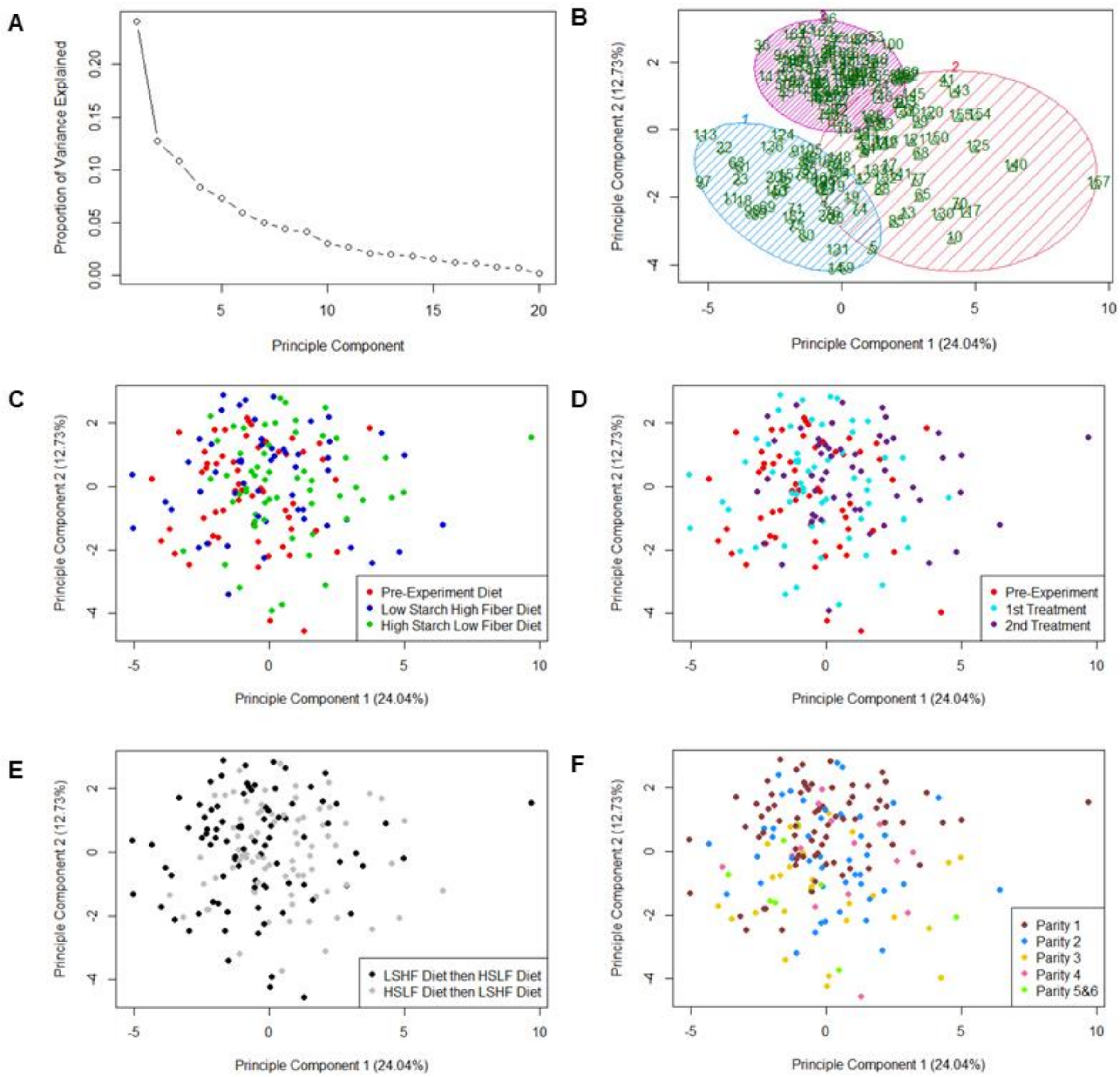


Figure 3.3. (A) Scree plot, (B) cluster plot, and principal component analysis of BMO relative abundance data organized by (C) diet, (D) study period, (E) diet sequence, and (F) parity.

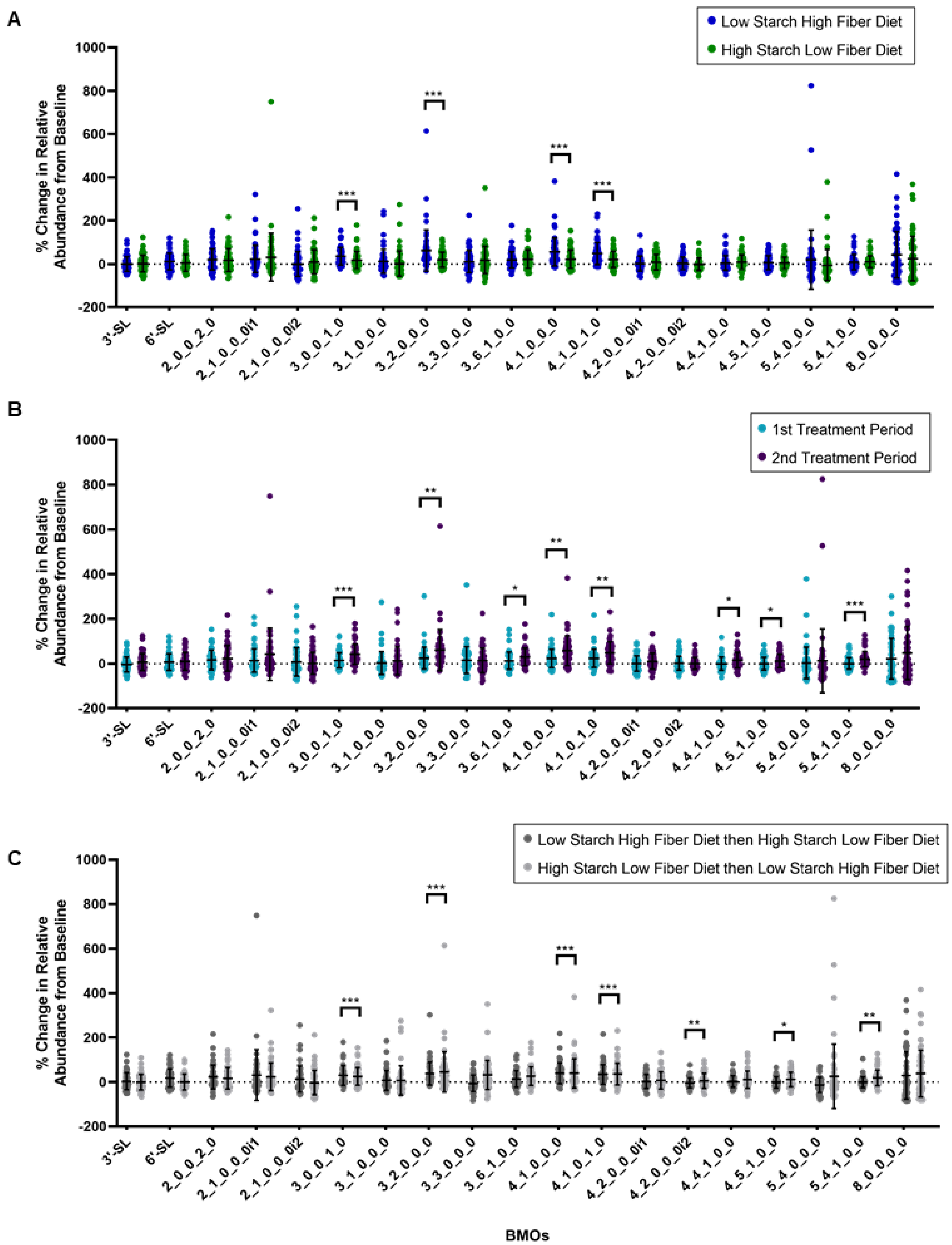


Figure 3.4. BMO % change in relative abundance data organized by (A) diet, (B) study period, and (C) diet sequence with BMOs described by monosaccharide composition as the number of Hex_HexNAc_Fuc_Neu5Ac_Neu5Gc, followed by the isomer number, as appropriate. Statistics are from parametric analyses of transformed data, while graphs present untransformed data. * $0.01 < p \leq 0.05$, ** $0.001 < p \leq 0.01$, *** $p < 0.001$

Dietary treatment period significantly influenced ($p < 0.05$) the percent change in relative abundance from the pre-experiment baseline period for 8 BMOs (Figure 4B). Similarly, t-test comparisons showed that the percent change in the transformed relative abundances from the pre-experimental baseline period significantly differed ($p < 0.05$) for 7 BMOs based on the sequence of dietary treatments (Figure 4C). All four of the BMOs for which the percent change in their transformed relative abundances from the pre-experiment baseline period differed significantly by diet also differed significantly by treatment period and dietary treatment sequence. An interaction between diet and period was observed for 3_1_0_0_0, 3_2_0_0_0, 4_1_0_0_0, and 4_1_0_1_0 with more negative percent changes in transformed relative abundances (corresponding with smaller percent changes from the pre-experiment baseline period in the untransformed abundance data) for both the second dietary treatment period and the LSHF diet for all 4 BMOs, as shown in Supplemental Figure 3.

Linear mixed effects modeling was conducted to determine whether the effects of diet on the percent change in the transformed relative abundances from the pre-experiment baseline diet remained significant after adjustment for other study parameters including treatment period, diet sequence, parity, days in milk, cow ID, and milk yield. The influence of diet was not significant ($p > 0.05$) for nearly all linear mixed effects models constructed for 3_0_0_1_0 and 4_1_0_1_0; however, the effect of diet remained significant ($p < 0.05$) across all models for 3_2_0_0_0 and 4_1_0_0_0. In addition, the effect of diet emerged as significant for 3_1_0_0_0 in all linear mixed effect models including cow ID as a variable. In summary, the LSHF diet increased the abundance of 3_2_0_0_0, 4_1_0_0_0, and possibly also 3_1_0_0_0. No BMOs were decreased in abundance on the LSHF diet relative to the HSLF diet.

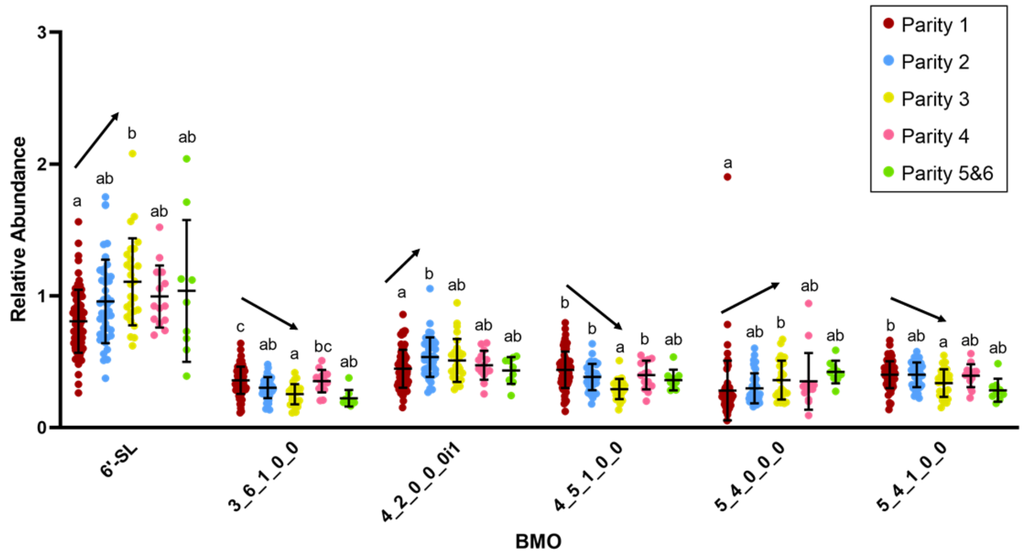


FIGURE 3.5. BMO relative abundance data organized by parity with BMOs described by monosaccharide composition as the number of Hex_HexNAc_Fuc_Neu5Ac_Neu5Gc, followed by the isomer number, as appropriate. Statistics are from parametric analyses of transformed data, while graph present untransformed data. Parities that share a letter are not significantly different ($\alpha = 0.05$). Arrows indicate the direction of average relative abundance changes across the first three parties.

Parity Affects BMO Profiles

Significant differences ($p < 0.05$) were observed between milk samples from cows of different parities for 6 BMOs, with most significant differences in BMO abundances being observed between parities 1 and 3 (Figure 5). 6'-SL, 4_2_0_0_0 isomer 1, and 5_4_0_0_0 increase with increasing parity while 3_6_1_0_0, 4_5_1_0_0, and 5_4_1_0_0 decrease with increasing parity.

Differences in BMO Abundances are Not Merely Due to Changes in Yield

BMO abundances were also adjusted for milk yield by multiplying BMO abundance by the average milk weight collected during the morning milkings on the days of each sample collection during the corresponding study period.

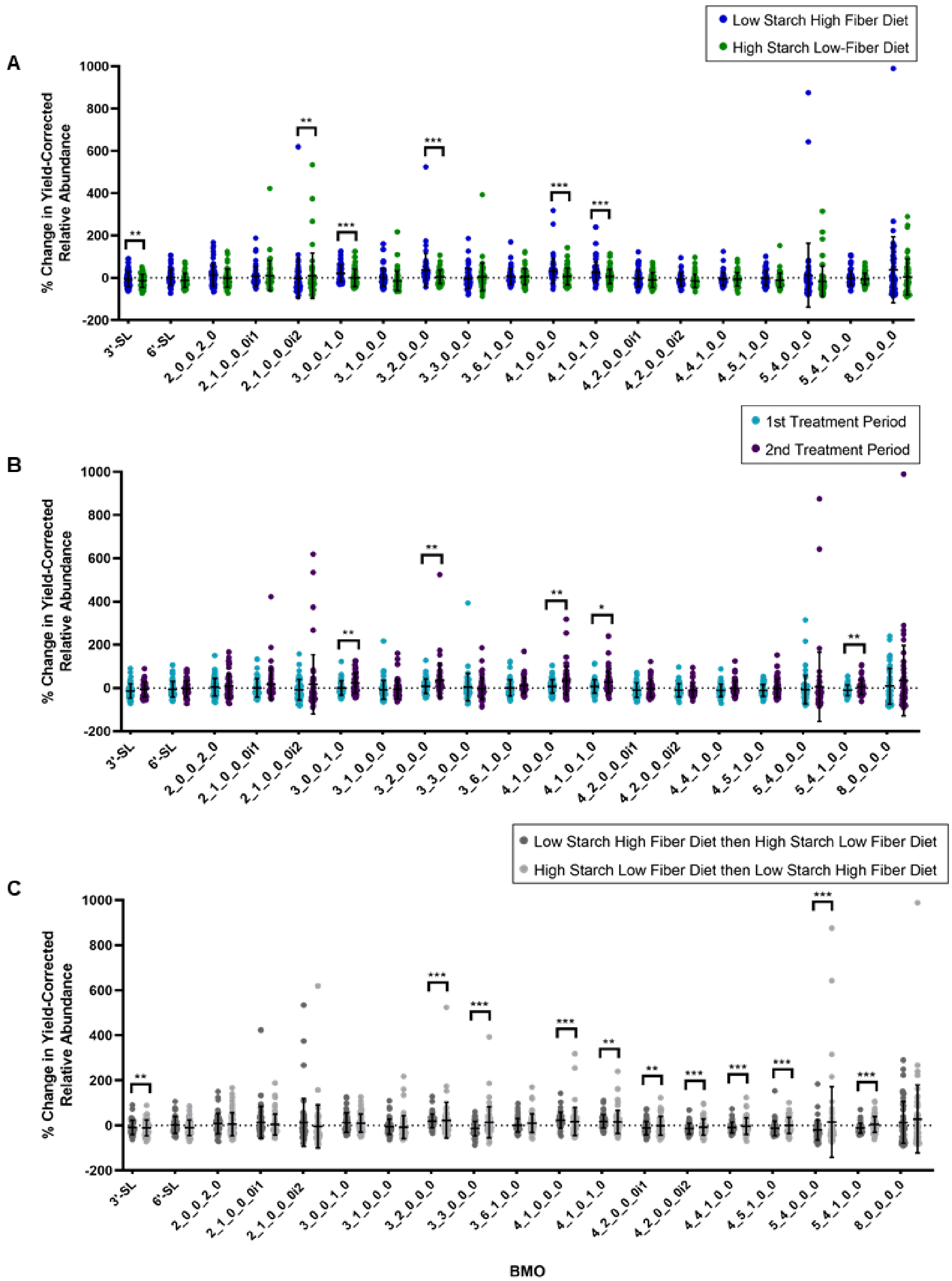


Figure 3.6. BMO % change in yield-adjusted relative abundance data organized by (A) diet, (B) study period, and (C) diet sequence with BMOs described by monosaccharide composition as the number of Hex_HexNAc_Fuc_Neu5Ac_Neu5Gc, followed by the isomer number, as appropriate. Statistics are from parametric analyses of transformed data, while graphs present untransformed data. * $0.01 < p \leq 0.05$, **$0.001 < p \leq 0.01$, *** $p < 0.001$

Similar to the non-yield-adjusted data, the yield-adjusted relative abundances of 3_0_0_1_0, 3_2_0_0_0, 4_1_0_0_0, and 4_1_0_1_0 were all highest with the LSHF diet (Supplemental Figure 4A). Adjusting for yield, however, also revealed differences in BMO abundances between dietary treatments for the additional BMOs 3'-SL and 2_1_0_0_0 isomer 2, which had significantly lower abundances ($p < 0.05$) with the HSLF diet compared to the pre-experiment baseline diet (Supplemental Figure 4A).

As with the non-yield-adjusted data, t-test comparisons showed that the percent change in the transformed yield-adjusted relative abundances from the pre-experimental baseline period significantly differed ($p < 0.05$) based on study period and/or the sequence of dietary treatments for most of the same BMOs that showed significant differences between diets (Figure 6B & C).

Linear mixed effects modeling was conducted to determine whether the effects of diet on the percent change in the transformed yield-adjusted relative abundances from the pre-experiment baseline diet remained significant when other study parameters including treatment period, diet sequence, parity, days in milk, and cow ID were also accounted for. The influence of diet became non-significant ($p > 0.05$) for all linear mixed effects models constructed for 3'-SL and 2_1_0_0_0 isomer 2; however, the effect of diet remained significant ($p < 0.05$) across all models for 3_0_0_1_0, 3_2_0_0_0, 4_1_0_0_0, and 4_1_0_1_0. In addition, the effect of diet emerged as significant for 3_1_0_0_0, 4_2_0_0_0 isomer 2, and 5_4_0_0_0 in all linear mixed effect models including cow ID as a variable. In summary, when the influence of differences in milk yield are accounted for, no BMOs decreased in abundance with the LSHF diet compared to the HSLF diet. In addition, the LSHF diet increased the abundance of 3_0_0_1_0, 3_2_0_0_0, 4_1_0_0_0, and 4_1_0_1_0 and may have also increased the abundance of 3_1_0_0_0, 4_2_0_0_0 isomer 2, and 5_4_0_0_0.

DISCUSSION

Diet

Two acidic BMOs (3_0_0_1_0 and 4_1_0_1_0) and two neutral unfucosylated BMOs (3_2_0_0_0 and 4_1_0_0_0) exhibited significantly more positive percent changes of the transformed abundances from the pre-experiment baseline diet for the LSHF diet compared to the HSLF diet, based on initial t-test comparisons ($p < 0.001$).

Interestingly, the relative abundances of 3_2_0_0_0 and 4_1_0_0_0 were found to correlate with each other in the present study (Figure 2), as well as in a recent analysis of milk from 634 Danish Jerseys and Holstein-Friesians (23). Given these correlations across differences in both breed and feeding, the changes in abundance of these compounds with diet in the present study suggest that dietary fiber levels may impact a key enzyme or reaction involved in the synthesis of both of these oligosaccharides. Further, the fact that BMO abundances only increase with the LSHF diet suggests that substrate increases the shared synthesis of these correlated BMOs.

Two previous studies have also investigated the influence of diet on BMO profiles. Vicaretti *et al*. compared milk samples from cows that were either exclusively grass fed or consumed a feed composed of alfalfa and corn silage, earlage, and grain (48). No significant differences in BMO profiles were observed between cows in the two dietary groups; however, only six cows were included per dietary group, likely causing the study to be too underpowered to observe any meaningful differences between diets, if such a difference existed. In addition, differences in breed composition and farm between the two dietary groups may have had a confounding influence on the data.

Liu *et al*. compared BMO profiles between 32 Holstein-Friesian dairy cows with diets supplemented with either almond hulls or citrus peels to a base total mixed ration of corn grain, canola meal, and alfalfa cubes (49). As a result of BMO measurements only being taken at one time point during the study, the identified BMOs were found to have greater inter-cow variation within dietary treatment groups than inter-group variation, preventing any conclusions from being made about the influence of the diets on BMO production.

Although the present study also showed minimal effects of diet on non-yield-adjusted BMO profiles, our results are more meaningful and conclusive as a result of the greater study power and the use of a cross-over study design, which accounted for both the inherent cow-to-cow variation through the inclusion of pre-experimental baseline BMO profiling as well as many potential confounding factors that may have impacted the results of prior studies. The design of the present study is also advantageous in the inclusion of cows from a single breed, all located on the same farm, and all without access to an alternative feed source (i.e. pasture) outside of the study diets. In addition, in the present study, cows in the two groups were balanced by parity and pre-experimental average milk yields.

Beyond the influence of diet, this study also affords the opportunity to investigate the impact of other BMO-influencing factors in a large set of milk samples from mid-lactation dairy cattle.

Parity

Similar to the differences in BMO abundances between cows of different parities observed in previous studies (20, 23), primiparous cows were found to have significantly lower abundances of 6’-SL, 4_2_0_0_0 isomer 1, and 5_4_0_0_0 in their milk compared to cows in either their second or third parity (Figure 5). Unlike prior studies however, cows in the present study were also shown to have significantly higher abundances of the large neutral fucosylated BMOs 3_6_1_0_0, 4_5_1_0_0, and 5_4_1_0_0 in the first parity compared to those in the third parity (Figure 5), a direct contrast to the previously observed trend. This pattern of some BMOs increasing in abundance with increasing parity while other BMOs decrease, suggests that trade-offs may occur in BMO synthesis pathways as the mammary gland is remodeled with each lactation cycle through epigenetics (50-51). The higher abundances of larger fucosylated oligosaccharides, which have greater demonstrated bioactivities (52), in earlier parities may also be evidence of the corresponding fucosylation genes being naturally activated prior to the first lactation and silenced during subsequent lactations.

Lactation Time Point

Nearly all previous BMO studies with samples collected at more than one time point have focused on detecting BMO in early lactation, with samples collected only through the end of second week postpartum (20-21, 48, 53). However, most milk used for commercial purposes is collected outside of this timeframe, and very little is known about if and how BMO profiles change over time in mature cows' milk. McJarrow and van Amelsfort-Schoonbeek followed the concentrations of 5 BMOs in bulk milk samples across a milking season in grass-fed New Zealand Jersey and Friesian dairy cattle and noted a seasonal variation (26); however, no parallel study has been conducted with non-grass-fed cows or cows from other breeds or regions to determine whether similar patterns in BMO variations occur.

The number of days in milk at the time of milk sample collection significantly influenced BMO abundances for several BMOs, including 2_1_0_0_0 isomer 1, 3_0_0_1_0, 3_2_0_0_0, 3_6_1_0_0, 4_1_0_0_0, 4_1_0_1_0, 4_4_1_0_0, and 5_4_1_0_0. Although the correlation coefficients for the abundances of these oligosaccharides over time are not particularly strong - likely due in part to the wide degree of natural variation in BMO abundances between cows - general increasing trends can be observed for these 6 BMOs across lactation (Figure 7). This trend disappears when looking at the yield-adjusted relative abundance data, suggesting that the apparent increase in abundances for these BMOs in later lactation may be due, at least in part, to a concentrating effect caused by similar levels of total BMO production despite decreasing total milk volumes. Although this concentrating effect has been previously hypothesized (54), this is the first report, to our knowledge, of yield-adjusted BMO concentrations across the lactation cycle.

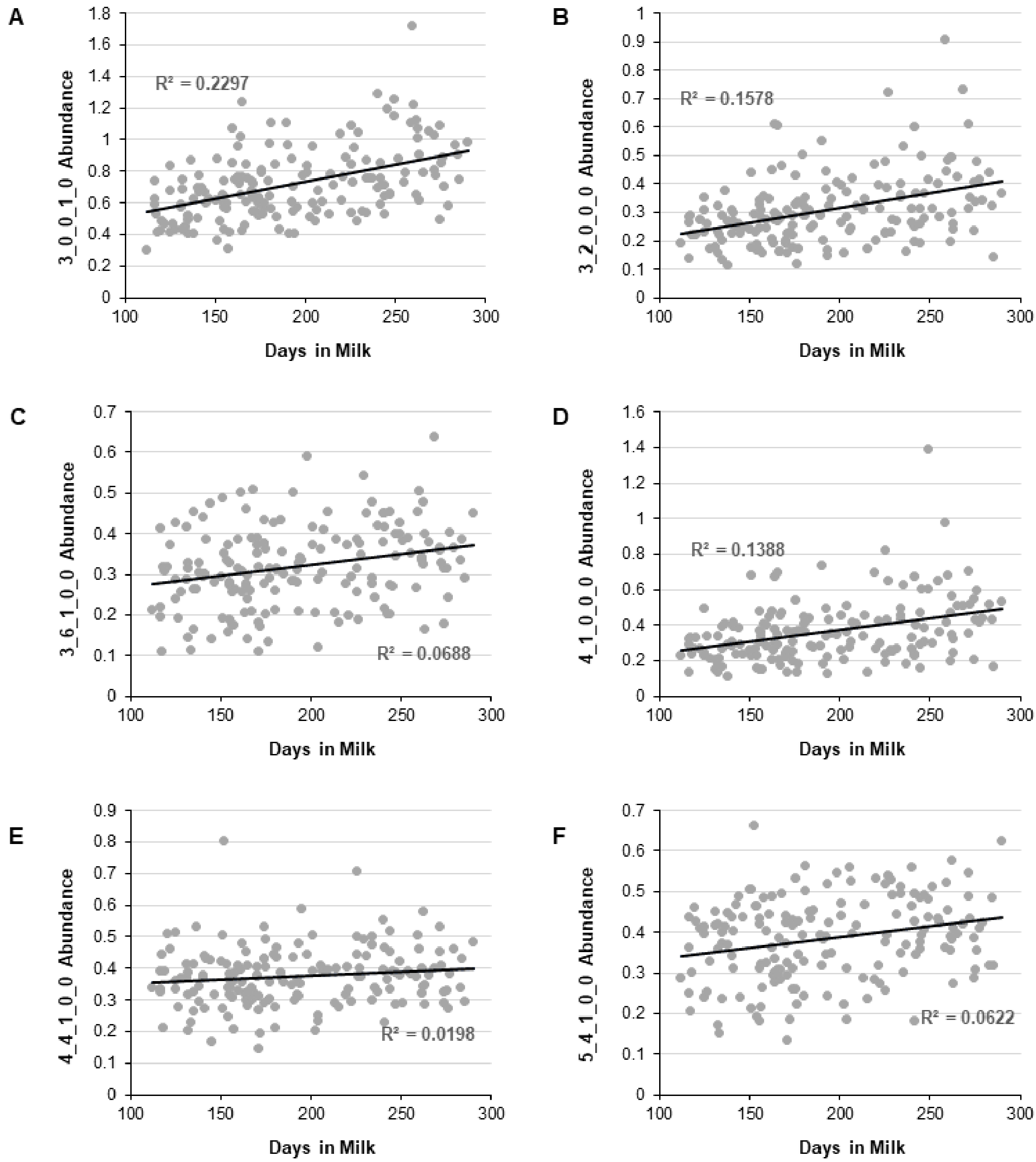


Figure 3.7. Increasing trends of BMO relative abundance across lactation for (A) 3_0_0_1_0, (B) 3_2_0_0_0, (C) 3_6_1_0_0, (D) 4_1_0_0_0, (E) 4_4_1_0_0, and (F) 5_4_1_0_0. BMOs are described by their monosaccharide composition as the number of Hex_HexNAc_Fuc_Neu5Ac_Neu5Gc.

Unmeasured Factors

The principal component analyses suggest that the largest source of variance in BMO abundance is due to one or more factors that were not measured in the study. Prior work demonstrating differences in BMO concentration between breeds (23, 55) suggests that genetics is an important determinant of BMO production and is therefore likely to be at least one source of variance. Future studies involving both breed and feeding will be needed to increase BMO production.

Correlations in BMO Abundances

Significant correlations in abundance between several BMOs were identified both without and with adjustment for milk yield (Figure 2), providing insight into their co-occurrences in milk from a milk consumption and milk synthesis perspectives, respectively. The strongest correlations were observed between the BMOs 4_1_0_0_0 and 4_1_0_1_0 (non-yield-adjusted r=0.94, yield-adjusted r=0.94) and between 4_1_0_0_0 and 3_2_0_0_0 (non-yield-adjusted r=0.92, yield-adjusted r=0.89), suggesting that these three BMOs may share a common core structure or key glycosyltransferase enzyme. Significant positive correlations were also observed among the four identified fucosylated BMOs, which may indicate a shared fucosyltransferase enzyme utilized in their synthesis. In addition, the negative correlation of 5_4_0_0_0 with 5_4_1_0_0 may suggest that 5_4_0_0_0 is a precursor structure for its larger, fucosylated BMO, causing its abundance to decrease as it is used to create its fucosylated counterpart. Overall, the correlations among BMO abundances provide tantalizing clues regarding BMO synthesis that should be investigated in future studies.

Yield-adjusted BMO Abundances

Although most previous BMO studies have focused more on a consumer perspective and therefore have not accounted for milk yield in their analyses, adjusting for milk yield is important for understanding whether milk oligosaccharide production is truly increasing, decreasing, or remaining unchanged from a biological and mechanistic perspective on milk production.

Analysis of the transformed yield-adjusted percent change in relative abundance from the pre-experiment baseline diet in the present data with linear mixed effects modeling including cow ID as a variable revealed 7 BMOs that differed significantly by diet, with all 7 BMOs featuring a more positive percent change from the pre-experiment baseline diet for the LSHF diet compared to the HSLF diet.

The observed significant changes in transformed yield-adjusted relative abundances from the baseline period for 7 out of the 19 measured BMOs suggests that there is indeed a relation between cows' dietary fiber and starch intake levels and their production of milk oligosaccharides. Among these 7 BMOs are 2 acidic (3_0_0_1_0 and 4_1_0_1_0) and 5 neutral unfucosylated compounds (3_1_0_0_0, 3_2_0_0_0, 4_1_0_0_0, 4_2_0_0_0 isomer 2, and 5_4_0_0_0). The significant impact of dietary fiber levels on the yield-adjusted abundances of these BMOs but not the 4 identified neutral fucosylated BMOs may indicate that these fucosylated compounds do not share the same core structures as the 7 impacted unfucosylated BMOs or that the availability of fucose or the occurrence of the fucosylation reaction is a limiting factor in the synthesis of these fucosylated BMOs under the conditions of the present study.

Additional investigation into the biological mechanisms of milk oligosaccharide synthesis and the absorption of carbohydrates and potential carbohydrate precursors from the digestive track in cows are needed to better understand the observed relationship between bovine dietary fiber intake and yield-adjusted BMO abundances. The inclusion of more detailed analysis of the dietary fiber consumed by the cows (i.e. monosaccharide compositions and linkage analysis) as well as linkage analysis of the produced BMOs in future studies will aid in the further investigation of the observed link between cow dietary fiber to starch intake ratio and BMO production.

Conclusions

In this study we have implemented a three-period cross-over design paired with high-throughput nano-LC-chip-Q-ToF MS analysis to evaluate the impact of dietary fiber and starch ratios on BMO abundances. 19 BMOs were identified across 338 samples from 59 cows, including 7 BMOs with a more positive percent change in yield-adjusted abundance from the pre-experimental baseline period with a LSHF diet compared to a HSLF diet. In addition, significant differences were observed for six BMOs based on parity, including three for which abundances were greater in primiparous cows compared to their secundiparous or triparous herd mates. While parity had a mixed effect on BMO abundances with some increasing and others decreasing with increasing parity, the LSHF diet only increased BMOs, suggesting the utility of this diet regardless of other cow-specific factors.

ACKNOWLEDGEMENTS

The authors would like to extend special thanks to Dr. Randall Robinson for his advisement during the tandem mass tagging and mass spectrometry optimization processes. We would like to thank Shan Betzold and Kurt Pickar for animal care, and Amélie Fischer, Alicia Pelletier and Diane Amundson for data and sample collection.

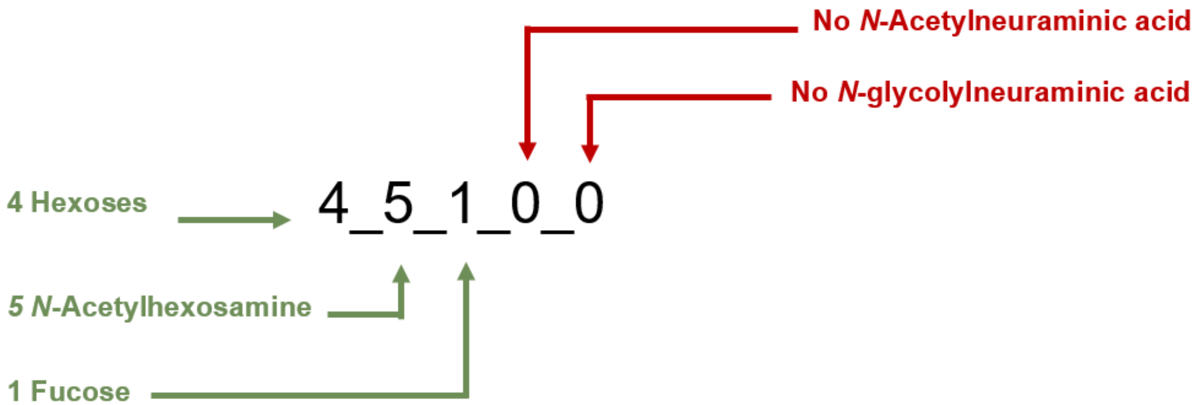
Funding

This work was supported by U.S. Department of Agriculture Agricultural Research Service (USDA-ARS) (NACA #58-8040-8-013), project CA-D-FST-2187-H, USDA-ARS Project # 5090-31000-025-00D, USDA-ARS Project # 2032-51530-026-000-D, and by the USDA-ARS Grand Challenge Synergy project "Dairy Agriculture for People and Planet".

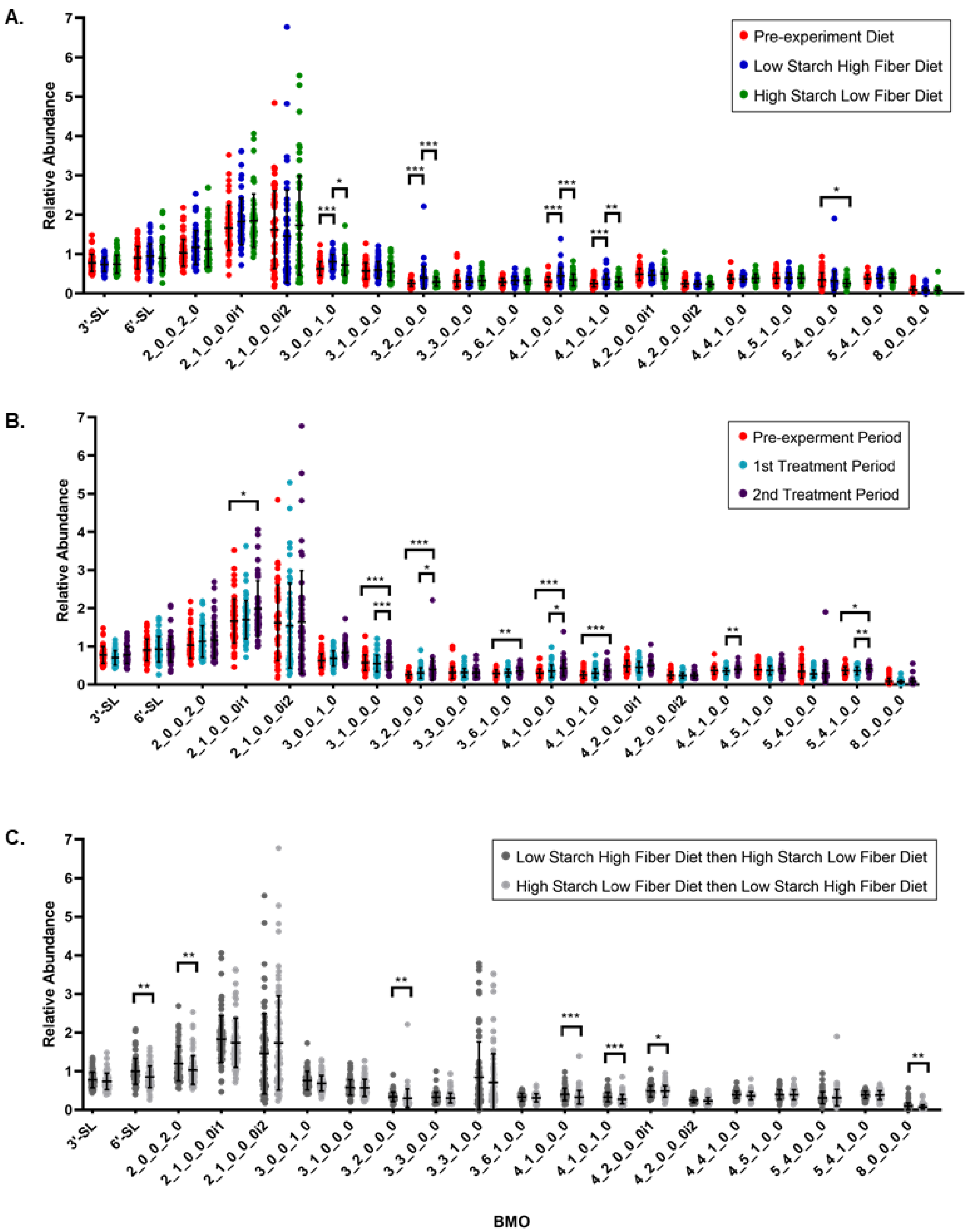
Author Disclosures

John Finley is an Editor on Current Developments of Nutrition and played no role in the Journal's evaluation of the manuscript. D.G.L., K.F.K., J.W.F., and N.K.F. are employees of the U.S. Department of Agriculture. The USDA is an Equal Opportunity Employer. D.B. is a cofounder of Evolve Biosystems, a company focused on diet-based manipulation of the gut microbiota. Evolve Biosystems played no role in the design, execution, interpretation, or publication of this work.

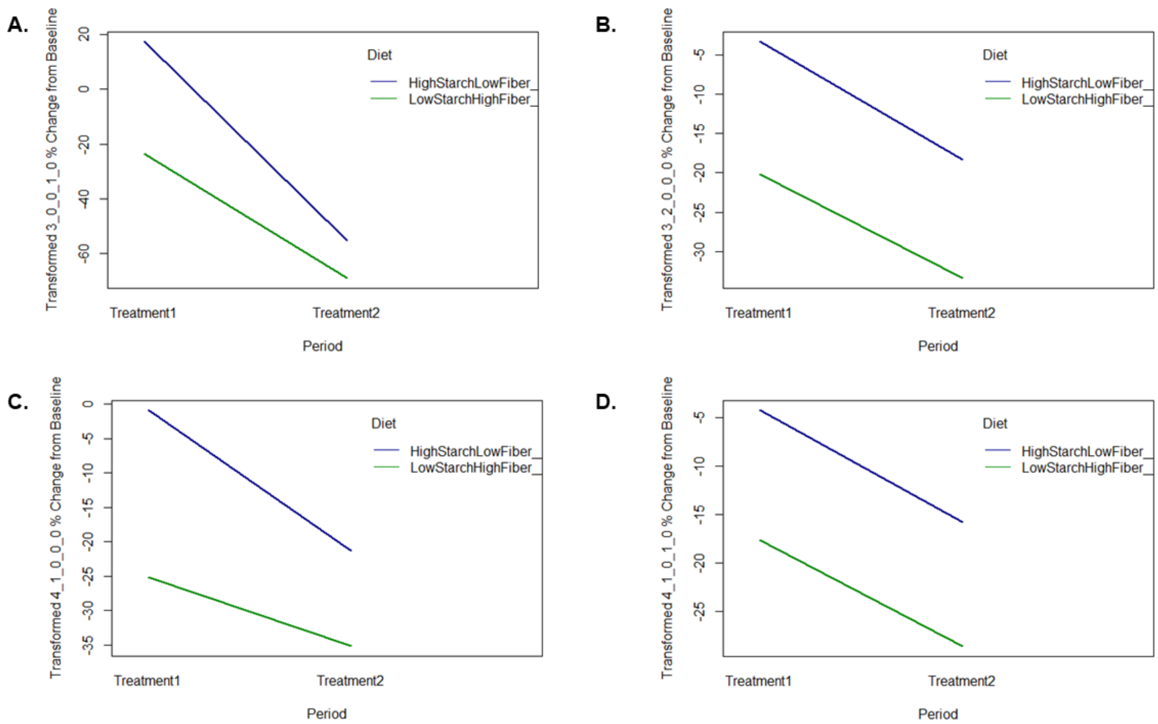
SUPPLEMENTARY MATERIAL



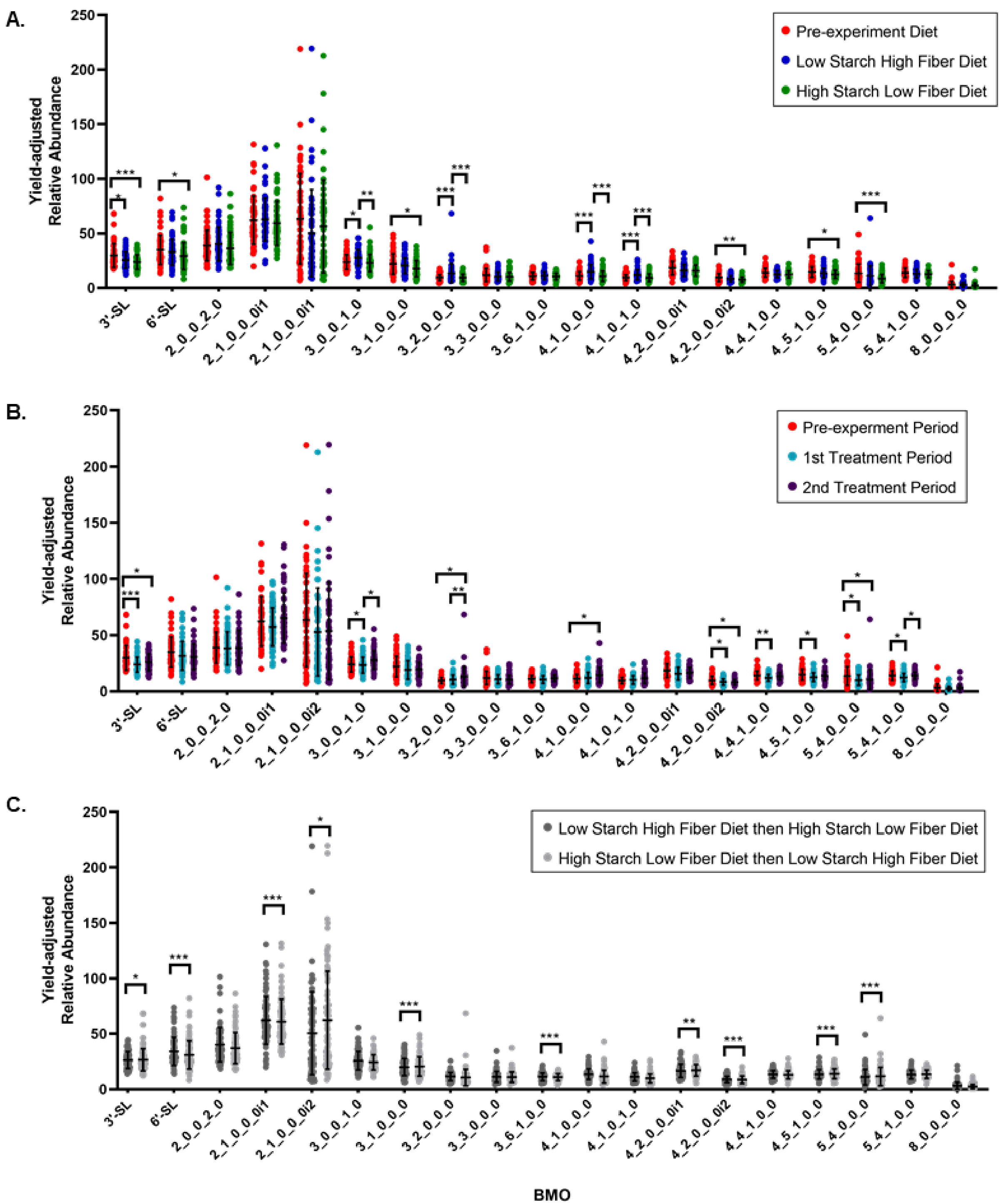
Supplementary Figure 3.1. Example interpretation of the 5-digit numerical code for milk oligosaccharide identification.



Supplementary Figure 3.2. BMO relative abundance data organized by (A) diet, (B) study period, and (C) diet sequence with BMOs described by monosaccharide composition as the number of Hex_HexNAc_Fuc_Neu5Ac_Neu5Gc, followed by the isomer number, as appropriate. Statistics are from parametric analyses of transformed data, while graphs present untransformed data. * $0.01 < p \leq 0.05$, **$0.001 < p \leq 0.01$, *** $p < 0.001$



Supplementary Figure 3.33 Interaction plots of BMO percent change in from the baseline period data showing the interaction between dietary treatment period and diet for (A) 3_0_0_1_0, (B) 3_2_0_0_0, (C) 4_1_0_0_0, (D) 4_1_0_1_0. BMOs are described by monosaccharide composition as the number of Hex_HexNAc_Fuc_Neu5Ac_Neu5Gc.



Supplementary Figure 3.4. BMO yield-adjusted relative abundance (yield-adjusted signal intensity) data organized by (A) diet, (B) study period, and (C) diet sequence with BMOs described by monosaccharide composition as the number of Hex_HexNAc_Fuc_Neu5Ac_Neu5Gc, followed by the isomer number, as appropriate. Statistics are from parametric analyses of transformed data, while graphs present untransformed data. * $0.01 < p \leq 0.05$, **$0.001 < p \leq 0.01$, *** $p < 0.001$

Supplementary Table 3.1. Identified BMOs and their classifications

BMO	Constituent Monosaccharides					Classification	DP
	Hex	HexNAc	Fuc	Neu5Ac	Neu5Gc		
3'-SL	2	0	0	1	0	Acidic	3
6'-SL	2	0	0	1	0		3
2_0_0_2_0	2	0	0	2	0		4
3_0_0_1_0	3	0	0	1	0		4
4_1_0_1_0	4	1	0	1	0		6
2_1_0_0_0 isomer 1	2	1	0	0	0	Neutral unfucosylated	3
2_1_0_0_0 isomer 2	2	1	0	0	0		3
3_1_0_0_0	3	1	0	0	0		4
3_2_0_0_0	3	2	0	0	0		5
3_3_0_0_0	3	3	0	0	0		6
4_1_0_0_0	4	1	0	0	0		5
4_2_0_0_0 isomer 1	4	2	0	0	0		6
4_2_0_0_0 isomer 2	4	2	0	0	0		6
5_4_0_0_0	5	4	0	0	0		9
8_0_0_0_0	8	0	0	0	0		8
3_6_1_0_0	3	6	1	0	0	Neutral fucosylated	10
4_4_1_0_0	4	4	1	0	0		9
4_5_1_0_0	4	5	1	0	0		10
5_4_1_0_0	5	4	1	0	0		10

Hex = hexose; HexNAc = *N*-acetylhexosamine; Fuc = fucose; Neu5Ac = *N*-acetylneuraminic acid; Neu5Gc = *N*-glycolylneuraminic acid; DP = degree of polymerization; 3'-SL = 3'-sialyllactose; 6'-SL = 6'-sialyllactose

Supplementary Table 3.2. Ingredient and chemical composition of experimental diets

	LSHF[1]	HSLF
Ingredients (% of diet DM)		
Alfalfa silage	33.9	24.2
Corn silage	32.4	23.2
High moisture corn	0	24.5
Beet pulp pelleted	8.9	2.8
Canola meal	2.7	9.7
Corn distillers' grain	9.2	2.7
Roasted soybean	4.1	4.1
Soybean hulls	6.1	6.1
Mineral and vitamin mix[2]	2.7	2.7
Chemical composition (% of diet DM)		
Dry matter (DM), % of diet	45.9	50.1
Crude protein	16.5	16.2
Neutral detergent fiber (NDF)	36.9	29.0
Forage NDF	24.9	17.7
Acid detergent fiber (ADF)	27.4	21.1
Lignin	3.9	3.0
Ether extract	5.1	4.9
Ash	7.4	5.7
Starch	13.0	26.7

[1]LSHF = low starch high fiber diet; HSLF = high starch low fiber diet.

[2]The mineral and vitamin mix contained (on a DM basis): 16.0% Ca, 5.85% Mg, 0.54% K, 14.8% Na, 6.67% Cl, 0.73% S, 42.5 mg of Co/kg, 519 mg of Cu/kg, 60.2 mg of I/kg, 778 mg of Fe/kg, 2,601 mg of Mn/kg,14.6 mg of Se/kg, 2,808 mg of Zn/kg, 292 kIU of vitamin A/kg, 58.5 kIU of vitamin D/kg, 1.36 kIU of vitamin E/kg, and 0.494 g of monensin/kg (Vita Plus Corporation, Madison, WI).

Supplementary Table 3.3. Abundances of bovine milk oligosaccharides normalized to the multiplexed internal standard

Cow ID	Period	Diet	Sequence	Parity	Days in milk at start of study	Days in milk at sample collection	Milk Weight at sample collection (lbs, average of 2 pooled samples)
4221	First Treatment period	High Starch Low Fiber	HSLF then LSHF	6	91	155	37.0
4403	First Treatment period	Low Starch High Fiber	LSHF then HSLF	6	110	174	36.9
4668	First Treatment period	Low Starch High Fiber	LSHF then HSLF	5	87	151	49.9
4889	First Treatment period	Low Starch High Fiber	LSHF then HSLF	4	106	170	36.7
5002	First Treatment period	Low Starch High Fiber	LSHF then HSLF	4	138	202	38.4
5007	First Treatment period	High Starch Low Fiber	HSLF then LSHF	4	143	207	31.1
5034	First Treatment period	Low Starch High Fiber	LSHF then HSLF	4	197	261	26.9
5046	First Treatment period	High Starch Low Fiber	HSLF then LSHF	4	145	209	47.4
5249	First Treatment period	Low Starch High Fiber	LSHF then HSLF	3	110	174	39.4
5282	First Treatment period	Low Starch High Fiber	LSHF then HSLF	3	137	201	27.5
5298	First Treatment period	Low Starch High Fiber	LSHF then HSLF	3	108	172	41.6
5409	First Treatment period	High Starch Low Fiber	HSLF then LSHF	3	194	258	21.3
5417	First Treatment period	Low Starch High Fiber	LSHF then HSLF	3	92	156	44.7
5439	First Treatment period	High Starch Low Fiber	HSLF then LSHF	3	100	164	29.9
5455	First Treatment period	High Starch Low Fiber	HSLF then LSHF	3	113	177	38.8
5472	First Treatment period	High Starch Low Fiber	HSLF then LSHF	3	111	175	39.1
5473	First Treatment period	Low Starch High Fiber	LSHF then HSLF	3	129	193	40.2
5658	First Treatment period	Low Starch High Fiber	LSHF then HSLF	2	149	213	30.3
5663	First Treatment period	High Starch Low Fiber	HSLF then LSHF	2	101	165	42.1
5676	First Treatment period	Low Starch High Fiber	LSHF then HSLF	2	121	185	31.0
5694	First Treatment period	Low Starch High Fiber	LSHF then HSLF	2	120	184	35.0
5696	First Treatment period	High Starch Low Fiber	HSLF then LSHF	2	151	215	37.3
5808	First Treatment period	High Starch Low Fiber	HSLF then LSHF	2	150	214	31.4
5823	First Treatment period	Low Starch High Fiber	LSHF then HSLF	2	131	195	25.7
5828	First Treatment period	High Starch Low Fiber	HSLF then LSHF	2	94	158	25.4
5834	First Treatment period	High Starch Low Fiber	HSLF then LSHF	2	125	189	41.4
5838	First Treatment period	High Starch Low Fiber	HSLF then LSHF	2	140	204	33.5
5840	First Treatment period	High Starch Low Fiber	HSLF then LSHF	2	137	201	49.3
5844	First Treatment period	High Starch Low Fiber	HSLF then LSHF	2	128	192	32.6
5849	First Treatment period	High Starch Low Fiber	HSLF then LSHF	2	128	192	41.2
5858	First Treatment period	Low Starch High Fiber	LSHF then HSLF	2	107	171	30.4
5862	First Treatment period	Low Starch High Fiber	LSHF then HSLF	2	91	155	36.6
6058	First Treatment period	Low Starch High Fiber	LSHF then HSLF	1	129	193	31.5
6076	First Treatment period	High Starch Low Fiber	HSLF then LSHF	1	130	194	35.2
6090	First Treatment period	Low Starch High Fiber	LSHF then HSLF	1	142	206	33.2
6091	First Treatment period	Low Starch High Fiber	LSHF then HSLF	1	113	177	34.9
6098	First Treatment period	Low Starch High Fiber	LSHF then HSLF	1	156	220	27.1
6201	First Treatment period	Low Starch High Fiber	LSHF then HSLF	1	141	205	36.8
6205	First Treatment period	Low Starch High Fiber	LSHF then HSLF	1	107	171	30.4
6213	First Treatment period	High Starch Low Fiber	HSLF then LSHF	1	140	204	41.7
6218	First Treatment period	High Starch Low Fiber	HSLF then LSHF	1	126	190	31.6
6219	First Treatment period	Low Starch High Fiber	LSHF then HSLF	1	134	198	29.1
6221	First Treatment period	High Starch Low Fiber	HSLF then LSHF	1	91	155	44.9
6222	First Treatment period	Low Starch High Fiber	LSHF then HSLF	1	115	179	27.5
6226	First Treatment period	Low Starch High Fiber	LSHF then HSLF	1	117	181	29.8
6230	First Treatment period	High Starch Low Fiber	HSLF then LSHF	1	116	180	28.1
6231	First Treatment period	High Starch Low Fiber	HSLF then LSHF	1	107	171	29.3
6232	First Treatment period	High Starch Low Fiber	HSLF then LSHF	1	116	180	34.2
6234	First Treatment period	Low Starch High Fiber	LSHF then HSLF	1	110	174	34.9
6235	First Treatment period	Low Starch High Fiber	LSHF then HSLF	1	103	167	29.5
6236	First Treatment period	High Starch Low Fiber	HSLF then LSHF	1	119	183	22.7
6238	First Treatment period	High Starch Low Fiber	HSLF then LSHF	1	97	161	28.1
6239	First Treatment period	Low Starch High Fiber	LSHF then HSLF	1	127	191	27.1
6240	First Treatment period	High Starch Low Fiber	HSLF then LSHF	1	100	164	31.2
6242	First Treatment period	Low Starch High Fiber	LSHF then HSLF	1	93	157	35.1
6243	First Treatment period	High Starch Low Fiber	HSLF then LSHF	1	100	164	32.6
6245	First Treatment period	Low Starch High Fiber	LSHF then HSLF	1	106	170	32.2
6247	First Treatment period	High Starch Low Fiber	HSLF then LSHF	1	95	159	33.85

Cow ID	Period	Diet	3'-sialyllactose	6'-sialyllactose	2_0_0_2_0	2_1_0_0_0 isomer 1	2_1_0_0_0 isomer 2
4221	First Treatment period	High Starch Low Fiber	0.959958824	1.710594875	1.173786399	1.561376774	1.908355204
4403	First Treatment period	Low Starch High Fiber	0.460627549	0.729920662	0.861055146	1.222486853	1.789280492
4668	First Treatment period	Low Starch High Fiber	0.449781147	0.675613191	1.159706206	1.049765667	1.388565186
4889	First Treatment period	Low Starch High Fiber	0.628001666	0.790024571	0.761030709	1.422258671	1.501301639
5002	First Treatment period	Low Starch High Fiber	0.483805607	0.700752206	0.856537302	1.531757115	0.536784943
5007	First Treatment period	High Starch Low Fiber	0.586706268	0.921825052	1.547461285	2.435393693	1.143728432
5034	First Treatment period	Low Starch High Fiber	0.960204359	1.178840817	1.817736101	3.630343472	3.714058911
5046	First Treatment period	High Starch Low Fiber	0.540475707	0.736093572	0.732023832	1.725918407	1.749012654
5249	First Treatment period	Low Starch High Fiber	0.585624277	0.809935631	1.211971076	1.895898294	2.170131791
5282	First Treatment period	Low Starch High Fiber	0.961526092	1.357378887	0.881927719	2.36960341	1.97247368
5298	First Treatment period	Low Starch High Fiber	0.627117919	0.994366969	1.564466507	1.703210968	0.808505051
5409	First Treatment period	High Starch Low Fiber	0.68338527	1.356835701	1.496285981	2.716331695	1.906613602
5417	First Treatment period	Low Starch High Fiber	0.567768941	1.158280321	0.664337853	1.655599815	0.66830255
5439	First Treatment period	High Starch Low Fiber	0.662759094	1.214983018	0.76758723	1.269475321	0.530710651
5455	First Treatment period	High Starch Low Fiber	0.504114617	0.89340527	1.352166155	1.336543038	1.870531375
5472	First Treatment period	High Starch Low Fiber	0.720002523	1.312801442	1.265975223	1.944177632	1.860922317
5473	First Treatment period	Low Starch High Fiber	0.850364418	1.408408803	1.072331207	1.968781037	5.292493254
5658	First Treatment period	Low Starch High Fiber	0.850467428	1.110720639	1.331057819	2.878214522	2.823150666
5663	First Treatment period	High Starch Low Fiber	0.918956193	1.146134047	2.190971537	1.423934653	0.460422436
5676	First Treatment period	Low Starch High Fiber	0.898953936	1.295581949	0.789975096	1.980795903	2.247134824
5694	First Treatment period	Low Starch High Fiber	0.519066002	0.509464103	1.10127499	1.409648996	0.643755722
5696	First Treatment period	High Starch Low Fiber	1.06042576	1.75003883	0.97189552	1.895046556	0.251810961
5808	First Treatment period	High Starch Low Fiber	0.687759485	0.850900197	0.968091947	0.719944295	0.933470571
5823	First Treatment period	Low Starch High Fiber	1.007632455	1.200897747	0.871155525	2.134569771	2.771779411
5828	First Treatment period	High Starch Low Fiber	0.960545854	1.101244503	2.109219565	2.017528259	2.66843535
5834	First Treatment period	High Starch Low Fiber	0.732535053	0.984202079	1.43486929	1.78495096	2.397878402
5838	First Treatment period	High Starch Low Fiber	0.949470602	1.193948827	1.180357091	1.959746404	0.81130263
5840	First Treatment period	High Starch Low Fiber	0.494171322	0.970307586	0.716457458	1.933988607	0.641472132
5844	First Treatment period	High Starch Low Fiber	0.441645595	0.516014285	0.876399711	1.421289582	0.381138119
5849	First Treatment period	High Starch Low Fiber	1.077201927	1.686704558	1.15311079	1.781939226	2.808967843
5858	First Treatment period	Low Starch High Fiber	0.657067617	0.591598939	1.608555793	1.13544843	0.662454612
5862	First Treatment period	Low Starch High Fiber	0.569858007	0.741452246	0.801576338	1.282912855	2.974663001
6058	First Treatment period	Low Starch High Fiber	0.627717328	0.262390601	0.587086293	1.306831038	4.620556713
6076	First Treatment period	High Starch Low Fiber	0.799877718	0.782792964	0.703344693	1.608082975	0.601977843
6090	First Treatment period	Low Starch High Fiber	0.600793191	0.521475492	0.741650344	1.214065763	0.420737559
6091	First Treatment period	Low Starch High Fiber	0.556913439	0.678296853	0.72065582	1.450522677	2.065287053
6098	First Treatment period	Low Starch High Fiber	0.852430853	0.875718296	0.98671743	1.452614771	0.708287876
6201	First Treatment period	Low Starch High Fiber	0.627874308	0.728776332	0.639162863	0.924937448	1.06844275
6205	First Treatment period	Low Starch High Fiber	0.447761939	0.509504725	0.603396005	1.009394792	0.746803289
6213	First Treatment period	High Starch Low Fiber	0.455206817	0.589204398	0.691148036	1.326297598	1.498247809
6218	First Treatment period	High Starch Low Fiber	0.805690892	1.267020856	1.06725826	1.680782825	1.578900861
6219	First Treatment period	Low Starch High Fiber	0.79634293	0.727105252	0.926671112	1.883166549	0.481299186
6221	First Treatment period	High Starch Low Fiber	0.710514289	0.580899015	1.129428652	1.879522608	1.520515576
6222	First Treatment period	Low Starch High Fiber	0.710431438	0.653272775	0.942331543	1.799675354	1.028168481
6226	First Treatment period	Low Starch High Fiber	1.176101976	0.698924094	1.020176143	1.582459718	0.849401482
6230	First Treatment period	High Starch Low Fiber	0.773193732	1.561251288	1.640674326	1.540463329	0.436074733
6231	First Treatment period	High Starch Low Fiber	0.777477754	1.056720518	1.382812203	1.934312934	3.403174233
6232	First Treatment period	High Starch Low Fiber	0.744987248	0.87062435	1.583510685	2.346134769	0.640218931
6234	First Treatment period	Low Starch High Fiber	0.812090147	0.737541301	2.135260938	2.255765011	3.585101281
6235	First Treatment period	Low Starch High Fiber	0.421717076	0.632296056	1.013668201	1.557847427	1.513043161
6236	First Treatment period	High Starch Low Fiber	0.727812633	0.99490976	1.081125386	1.112295633	0.622211939
6238	First Treatment period	High Starch Low Fiber	0.600761098	0.88885915	2.153494428	1.703091431	0.306765702
6239	First Treatment period	Low Starch High Fiber	0.527788632	0.480271988	0.926035257	1.320644041	1.227063255
6240	First Treatment period	High Starch Low Fiber	0.597671996	0.785764292	0.884567292	1.496884394	1.087272376
6242	First Treatment period	Low Starch High Fiber	0.453690306	0.6379557	0.667843015	1.214402215	2.106771779
6243	First Treatment period	High Starch Low Fiber	0.735502513	0.875515548	1.125849734	1.775112082	1.488655805
6245	First Treatment period	Low Starch High Fiber	0.81108759	1.021218591	1.709082348	2.063946103	0.368612475
6247	First Treatment period	High Starch Low Fiber	0.698007365	0.863322647	1.221881385	1.862667798	1.363590334

Cow ID	Period	Diet	3_0_0_1_0	3_1_0_0_0	3_2_0_0_0	3_3_0_0_0	3_6_1_0_0
4221	First Treatment period	High Starch Low Fiber	0.643538985	0.574147662	0.344346067	0.146800945	0.189808688
4403	First Treatment period	Low Starch High Fiber	0.519958409	0.772113869	0.257880617	0.516553394	0.377560743
4668	First Treatment period	Low Starch High Fiber	0.361819842	0.476397467	0.176434921	0.200070955	0.191855383
4889	First Treatment period	Low Starch High Fiber	0.666399377	0.754890916	0.193393208	0.350636792	0.391380062
5002	First Treatment period	Low Starch High Fiber	0.511352235	0.415738664	0.157702114	0.259209587	0.205421867
5007	First Treatment period	High Starch Low Fiber	0.677887982	0.618516158	0.420010921	0.509062041	0.412143912
5034	First Treatment period	Low Starch High Fiber	1.129541894	0.512857831	0.492933748	0.24892714	0.341079799
5046	First Treatment period	High Starch Low Fiber	0.608352766	0.521564257	0.323730063	0.279231032	0.45419542
5249	First Treatment period	Low Starch High Fiber	0.639138208	0.513253105	0.179772835	0.209617159	0.375262327
5282	First Treatment period	Low Starch High Fiber	0.836783167	1.054840389	0.39869925	0.440046692	0.41668278
5298	First Treatment period	Low Starch High Fiber	0.552235269	0.556006179	0.231602144	0.402894807	0.172291748
5409	First Treatment period	High Starch Low Fiber	1.111164654	0.645899082	0.909773678	0.259483119	0.266471532
5417	First Treatment period	Low Starch High Fiber	0.563360213	0.772276102	0.309356865	0.397084391	0.175191258
5439	First Treatment period	High Starch Low Fiber	0.649862923	0.501412898	0.30153662	0.263190617	0.278803426
5455	First Treatment period	High Starch Low Fiber	0.56898314	0.67246982	0.337206859	0.530278395	0.29058578
5472	First Treatment period	High Starch Low Fiber	0.797937873	0.551565886	0.429407872	0.24219537	0.204335906
5473	First Treatment period	Low Starch High Fiber	0.549071045	0.785189872	0.147592534	0.165201992	0.210757645
5658	First Treatment period	Low Starch High Fiber	0.531026537	0.58803673	0.309567431	0.32108738	0.207080897
5663	First Treatment period	High Starch Low Fiber	0.767068536	0.630171341	0.608218631	0.413821855	0.297243628
5676	First Treatment period	Low Starch High Fiber	0.435362788	0.750013949	0.254049602	0.257530159	0.300682709
5694	First Treatment period	Low Starch High Fiber	0.513545949	0.217959241	0.247778719	0.306853938	0.355791233
5696	First Treatment period	High Starch Low Fiber	0.711573779	0.53951731	0.171538672	0.172475565	0.183483731
5808	First Treatment period	High Starch Low Fiber	0.785189038	0.412877173	0.350578116	0.335821075	0.387707795
5823	First Treatment period	Low Starch High Fiber	0.574803197	0.713396474	0.349831695	0.206152999	0.335580519
5828	First Treatment period	High Starch Low Fiber	0.415162905	0.47352203	0.356147621	0.342534216	0.265307625
5834	First Treatment period	High Starch Low Fiber	1.104988915	0.729698191	0.291308024	0.221212706	0.283474852
5838	First Treatment period	High Starch Low Fiber	0.956016522	1.207357851	0.373621393	0.435475009	0.287451826
5840	First Treatment period	High Starch Low Fiber	0.794755139	0.473844497	0.264785904	0.220016617	0.372777969
5844	First Treatment period	High Starch Low Fiber	0.607564331	0.249552122	0.447803787	0.262638146	0.31188058
5849	First Treatment period	High Starch Low Fiber	0.879660319	0.924575912	0.382611647	0.230343673	0.28141791
5858	First Treatment period	Low Starch High Fiber	0.725767561	0.480698152	0.309294376	0.314466764	0.181629548
5862	First Treatment period	Low Starch High Fiber	0.577921911	0.694816205	0.168480166	0.301973367	0.282591306
6058	First Treatment period	Low Starch High Fiber	0.709612694	0.926781686	0.166736166	0.268086615	0.330064899
6076	First Treatment period	High Starch Low Fiber	0.410040621	0.406320883	0.322884224	0.222447225	0.343479304
6090	First Treatment period	Low Starch High Fiber	0.55469701	0.334867738	0.244635918	0.352566366	0.304810073
6091	First Treatment period	Low Starch High Fiber	0.598643343	0.343234961	0.171369032	0.280113974	0.315612942
6098	First Treatment period	Low Starch High Fiber	0.619731608	0.434678791	0.204433358	0.350695421	0.372683954
6201	First Treatment period	Low Starch High Fiber	0.486492517	0.255819084	0.234541997	0.290897773	0.362090463
6205	First Treatment period	Low Starch High Fiber	0.545272926	0.275099212	0.159350851	0.353442879	0.112034703
6213	First Treatment period	High Starch Low Fiber	0.605067757	0.631906541	0.285978699	0.383020561	0.122639585
6218	First Treatment period	High Starch Low Fiber	0.965191433	0.660424915	0.554760122	0.247696187	0.50373523
6219	First Treatment period	Low Starch High Fiber	0.779824517	0.231602947	0.408503462	0.335656698	0.591646216
6221	First Treatment period	High Starch Low Fiber	0.744862844	0.445560293	0.331173328	0.429712702	0.273496715
6222	First Treatment period	Low Starch High Fiber	0.737124552	0.340882348	0.501998128	0.714522513	0.429133609
6226	First Treatment period	Low Starch High Fiber	1.111482949	0.423838575	0.353132311	0.299893934	0.312170605
6230	First Treatment period	High Starch Low Fiber	0.975070084	0.390219045	0.289576832	0.298622326	0.261468198
6231	First Treatment period	High Starch Low Fiber	0.633132703	0.345160222	0.228380616	0.353677153	0.319138525
6232	First Treatment period	High Starch Low Fiber	0.648128569	0.454278387	0.324036505	0.318258404	0.213660109
6234	First Treatment period	Low Starch High Fiber	0.600734628	0.413529244	0.220730326	0.212359881	0.435442103
6235	First Treatment period	Low Starch High Fiber	0.607182049	0.450982519	0.16275033	0.427792608	0.392076008
6236	First Treatment period	High Starch Low Fiber	0.847645584	0.781733658	0.309497818	0.275533715	0.435345722
6238	First Treatment period	High Starch Low Fiber	0.752399231	0.424808203	0.347969862	0.370925318	0.504109197
6239	First Treatment period	Low Starch High Fiber	0.409434623	0.407553323	0.202225752	0.324684016	0.283894516
6240	First Treatment period	High Starch Low Fiber	1.018028	0.744793104	0.611253813	0.220022887	0.2724892
6242	First Treatment period	Low Starch High Fiber	0.309436829	0.312794947	0.156391206	0.140319219	0.176882097
6243	First Treatment period	High Starch Low Fiber	0.745728379	0.593174215	0.275328306	0.262137464	0.461154882
6245	First Treatment period	Low Starch High Fiber	0.744589089	0.292914958	0.202658811	0.611380354	0.371903829
6247	First Treatment period	High Starch Low Fiber	1.077604331	1.038764004	0.274044216	0.222913302	0.316708846

Cow ID	Period	Diet	4_1_0_0_0	4_1_0_1_0	4_2_0_0_0 isomer 1	4_2_0_0_0 isomer 2	4_4_1_0_0
4221	First Treatment period	High Starch Low Fiber	0.479673248	0.392555327	0.530612375	0.212654122	0.341595726
4403	First Treatment period	Low Starch High Fiber	0.361564127	0.264402844	0.345661081	0.399252981	0.290355723
4668	First Treatment period	Low Starch High Fiber	0.233923653	0.218900808	0.243585886	0.214672468	0.27602544
4889	First Treatment period	Low Starch High Fiber	0.240006434	0.216802768	0.424468204	0.33389311	0.381192567
5002	First Treatment period	Low Starch High Fiber	0.209282578	0.198737207	0.254607563	0.205579345	0.204799138
5007	First Treatment period	High Starch Low Fiber	0.418397565	0.275715297	0.472395012	0.301034628	0.386186686
5034	First Treatment period	Low Starch High Fiber	0.475186642	0.473619395	0.633790377	0.190540819	0.322030261
5046	First Treatment period	High Starch Low Fiber	0.406311266	0.326228685	0.334613752	0.289245094	0.395301851
5249	First Treatment period	Low Starch High Fiber	0.198070888	0.207357458	0.462978934	0.207613724	0.431105538
5282	First Treatment period	Low Starch High Fiber	0.454286517	0.374323162	0.569576734	0.405594409	0.506181249
5298	First Treatment period	Low Starch High Fiber	0.236664874	0.214029359	0.446248933	0.161137282	0.196590745
5409	First Treatment period	High Starch Low Fiber	0.980566574	0.778994369	0.359496194	0.19953528	0.376685556
5417	First Treatment period	Low Starch High Fiber	0.36156181	0.326279634	0.399239071	0.267406285	0.307591866
5439	First Treatment period	High Starch Low Fiber	0.377567035	0.319974384	0.287848731	0.226181287	0.300894806
5455	First Treatment period	High Starch Low Fiber	0.371993571	0.267409584	0.381026853	0.32058526	0.304665724
5472	First Treatment period	High Starch Low Fiber	0.545244637	0.410650869	0.554394993	0.260858679	0.404099744
5473	First Treatment period	Low Starch High Fiber	0.128295465	0.15203119	0.661698933	0.183472491	0.315867267
5658	First Treatment period	Low Starch High Fiber	0.340730174	0.298559054	0.629943676	0.204086029	0.293639044
5663	First Treatment period	High Starch Low Fiber	0.695382966	0.57082704	0.633952174	0.277416049	0.409245363
5676	First Treatment period	Low Starch High Fiber	0.276334166	0.244474366	0.790120978	0.255488613	0.355494056
5694	First Treatment period	Low Starch High Fiber	0.284105746	0.258077176	0.454046355	0.191173556	0.369877003
5696	First Treatment period	High Starch Low Fiber	0.138907053	0.210349291	0.61374587	0.246432214	0.277219381
5808	First Treatment period	High Starch Low Fiber	0.458653762	0.447092632	0.288058586	0.249114222	0.403471828
5823	First Treatment period	Low Starch High Fiber	0.355762582	0.300253674	0.627042295	0.277997898	0.588854664
5828	First Treatment period	High Starch Low Fiber	0.40712627	0.346282051	0.682126566	0.265678299	0.389783276
5834	First Treatment period	High Starch Low Fiber	0.287197473	0.258364707	0.385556936	0.163235668	0.347326847
5838	First Treatment period	High Starch Low Fiber	0.508356249	0.363289881	0.618629247	0.462304586	0.253449485
5840	First Treatment period	High Starch Low Fiber	0.259974196	0.217610287	0.552900802	0.223245806	0.393658643
5844	First Treatment period	High Starch Low Fiber	0.497687652	0.354128755	0.394468533	0.153978089	0.317539768
5849	First Treatment period	High Starch Low Fiber	0.339608654	0.364565704	0.542296841	0.295934055	0.366049171
5858	First Treatment period	Low Starch High Fiber	0.36966641	0.349113544	0.528705146	0.214697407	0.270105134
5862	First Treatment period	Low Starch High Fiber	0.151612538	0.166025401	0.409035905	0.218803847	0.318401531
6058	First Treatment period	Low Starch High Fiber	0.221699389	0.191054072	0.529113932	0.210075797	0.478380911
6076	First Treatment period	High Starch Low Fiber	0.43689132	0.314914641	0.303812427	0.248554029	0.351386348
6090	First Treatment period	Low Starch High Fiber	0.30899058	0.262680374	0.274651303	0.219433211	0.392867044
6091	First Treatment period	Low Starch High Fiber	0.181900254	0.151470488	0.325541659	0.155068579	0.357153895
6098	First Treatment period	Low Starch High Fiber	0.265840758	0.214560782	0.527648572	0.13571815	0.404611869
6201	First Treatment period	Low Starch High Fiber	0.276674761	0.289020134	0.534894565	0.278833509	0.408633491
6205	First Treatment period	Low Starch High Fiber	0.137038065	0.135353007	0.473594111	0.226089845	0.146638549
6213	First Treatment period	High Starch Low Fiber	0.361169406	0.201702791	0.417175333	0.224834642	0.234573219
6218	First Treatment period	High Starch Low Fiber	0.739388944	0.530159215	0.328990803	0.28786029	0.435524817
6219	First Treatment period	Low Starch High Fiber	0.34902591	0.327564783	0.858514073	0.294993805	0.446077545
6221	First Treatment period	High Starch Low Fiber	0.310685381	0.27003862	0.710774537	0.21071598	0.38594958
6222	First Treatment period	Low Starch High Fiber	0.374055968	0.291313496	0.260606118	0.134493832	0.446050785
6226	First Treatment period	Low Starch High Fiber	0.414542033	0.48324036	0.549680242	0.329308083	0.434223458
6230	First Treatment period	High Starch Low Fiber	0.412002842	0.349018036	0.365663541	0.161131759	0.278559098
6231	First Treatment period	High Starch Low Fiber	0.265611238	0.227303153	0.357673973	0.188696794	0.312125514
6232	First Treatment period	High Starch Low Fiber	0.448065357	0.4068317	0.241773249	0.16774514	0.211317443
6234	First Treatment period	Low Starch High Fiber	0.254735125	0.19557541	0.424720915	0.173056139	0.432616507
6235	First Treatment period	Low Starch High Fiber	0.175781308	0.156320245	0.427914485	0.231230465	0.341835377
6236	First Treatment period	High Starch Low Fiber	0.442134191	0.338637922	0.393811082	0.228938102	0.388961328
6238	First Treatment period	High Starch Low Fiber	0.449849848	0.316936344	0.299681666	0.33845356	0.399092403
6239	First Treatment period	Low Starch High Fiber	0.184085045	0.171784975	0.571292582	0.158361271	0.298538406
6240	First Treatment period	High Starch Low Fiber	0.671113454	0.527819773	0.406055735	0.266291193	0.45562014
6242	First Treatment period	Low Starch High Fiber	0.148979322	0.145173082	0.151096882	0.111038681	0.206434917
6243	First Treatment period	High Starch Low Fiber	0.297133529	0.227225697	0.355318148	0.278330882	0.320404341
6245	First Treatment period	Low Starch High Fiber	0.221392684	0.208744522	0.530659607	0.294641929	0.403771586
6247	First Treatment period	High Starch Low Fiber	0.398282512	0.404713601	0.600571513	0.315269181	0.411044033

Cow ID	Period	Diet	4_5_1_0_0	5_4_0_0_0	5_4_1_0_0	8_0_0_0_0
4221	First Treatment period	High Starch Low Fiber	0.282530145	0.587108246	0.182751003	0.29464224
4403	First Treatment period	Low Starch High Fiber	0.534666684	0.275495066	0.310436869	0.032916499
4668	First Treatment period	Low Starch High Fiber	0.285152572	0.427819226	0.212837145	0.118832847
4889	First Treatment period	Low Starch High Fiber	0.348477467	0.326315915	0.374963084	0.104380516
5002	First Treatment period	Low Starch High Fiber	0.200294495	0.264647462	0.224467277	0.040459314
5007	First Treatment period	High Starch Low Fiber	0.348620725	0.313264075	0.398283959	0.111603466
5034	First Treatment period	Low Starch High Fiber	0.292997857	0.333044529	0.35542857	0.073438709
5046	First Treatment period	High Starch Low Fiber	0.508115031	0.196653816	0.418260158	0.061116667
5249	First Treatment period	Low Starch High Fiber	0.339405901	0.188819046	0.429926359	0.054441277
5282	First Treatment period	Low Starch High Fiber	0.283499206	0.229820186	0.442468868	0.066123939
5298	First Treatment period	Low Starch High Fiber	0.198595359	0.363554407	0.187024578	0.040175675
5409	First Treatment period	High Starch Low Fiber	0.284195454	0.187677373	0.364750718	0.055732655
5417	First Treatment period	Low Starch High Fiber	0.228054931	0.415400546	0.215935577	0.027001681
5439	First Treatment period	High Starch Low Fiber	0.245715785	0.262662832	0.27739275	0.138565258
5455	First Treatment period	High Starch Low Fiber	0.319590167	0.242938053	0.344668702	0.077825876
5472	First Treatment period	High Starch Low Fiber	0.32352279	0.27899902	0.26201432	0.05548439
5473	First Treatment period	Low Starch High Fiber	0.281202596	0.400758946	0.244288586	0.042953017
5658	First Treatment period	Low Starch High Fiber	0.284469482	0.320013173	0.288231195	0.057794106
5663	First Treatment period	High Starch Low Fiber	0.367531307	0.364986133	0.309108616	0.109176758
5676	First Treatment period	Low Starch High Fiber	0.336262909	0.223482671	0.454770867	0.020995809
5694	First Treatment period	Low Starch High Fiber	0.393881031	0.19634855	0.433613116	0.0327664
5696	First Treatment period	High Starch Low Fiber	0.324400801	0.55012438	0.238508327	0.047406587
5808	First Treatment period	High Starch Low Fiber	0.636392841	0.203780595	0.469114741	0.049948391
5823	First Treatment period	Low Starch High Fiber	0.413026105	0.326394914	0.349167207	0.085394633
5828	First Treatment period	High Starch Low Fiber	0.561569468	0.310408111	0.486781442	0.113862843
5834	First Treatment period	High Starch Low Fiber	0.262154835	0.249615268	0.394782921	0.033659863
5838	First Treatment period	High Starch Low Fiber	0.318028733	0.498923798	0.311902795	0.180778968
5840	First Treatment period	High Starch Low Fiber	0.297761983	0.34477996	0.471882735	0.103218655
5844	First Treatment period	High Starch Low Fiber	0.390381844	0.233310616	0.390991644	0.034825406
5849	First Treatment period	High Starch Low Fiber	0.333186431	0.245728325	0.344715703	0.032918588
5858	First Treatment period	Low Starch High Fiber	0.345561706	0.202163513	0.325394125	0.081601997
5862	First Treatment period	Low Starch High Fiber	0.477390055	0.25720418	0.420510983	0.103722959
6058	First Treatment period	Low Starch High Fiber	0.355022086	0.195685109	0.521038514	0.045542683
6076	First Treatment period	High Starch Low Fiber	0.341020963	0.234880409	0.422820687	0.061538174
6090	First Treatment period	Low Starch High Fiber	0.415032997	0.188028267	0.527727534	0.021034233
6091	First Treatment period	Low Starch High Fiber	0.392731451	0.203954375	0.428155165	0.018218388
6098	First Treatment period	Low Starch High Fiber	0.598417563	0.190367835	0.534743107	0.028142703
6201	First Treatment period	Low Starch High Fiber	0.663722349	0.253793112	0.55971597	0.05475476
6205	First Treatment period	Low Starch High Fiber	0.181215493	0.296189588	0.135649687	0.034098045
6213	First Treatment period	High Starch Low Fiber	0.122488902	0.515450164	0.187411708	0.062823149
6218	First Treatment period	High Starch Low Fiber	0.458351971	0.145433597	0.318095613	0.055438091
6219	First Treatment period	Low Starch High Fiber	0.443648688	0.274441144	0.546245568	0.051304538
6221	First Treatment period	High Starch Low Fiber	0.237955425	0.469498596	0.312372267	0.150403963
6222	First Treatment period	Low Starch High Fiber	0.47078926	0.144128059	0.395250306	0.039620749
6226	First Treatment period	Low Starch High Fiber	0.516675764	0.308738709	0.563370434	0.080191239
6230	First Treatment period	High Starch Low Fiber	0.37487423	0.182192279	0.330417976	0.035880101
6231	First Treatment period	High Starch Low Fiber	0.397672453	0.263151994	0.29644882	0.047534738
6232	First Treatment period	High Starch Low Fiber	0.298295207	0.246036235	0.245572148	0.031794474
6234	First Treatment period	Low Starch High Fiber	0.4729482	0.337429801	0.417852378	0.056181092
6235	First Treatment period	Low Starch High Fiber	0.491882198	0.197733699	0.28426084	0.022436145
6236	First Treatment period	High Starch Low Fiber	0.643708533	0.234456745	0.452426326	0.123271408
6238	First Treatment period	High Starch Low Fiber	0.41976646	0.204600281	0.444697268	0.031682152
6239	First Treatment period	Low Starch High Fiber	0.330456934	0.218391736	0.383957205	0.02516471
6240	First Treatment period	High Starch Low Fiber	0.343336546	0.23631968	0.335526256	0.052926011
6242	First Treatment period	Low Starch High Fiber	0.213163042	0.176144116	0.263703007	0.036483322
6243	First Treatment period	High Starch Low Fiber	0.463308535	0.263215603	0.30153857	0.07092279
6245	First Treatment period	Low Starch High Fiber	0.548275661	0.266386242	0.442819889	0.133241544
6247	First Treatment period	High Starch Low Fiber	0.441880993	0.279074249	0.403348846	0.076484005

Cow ID	Period	Diet	Sequence	Parity	Days in milk at start of study	sample collection (first day of 2	Milk Weight at sample collection (lbs, average of 2 pooled samples)
4221	Pre-experiment baseline	Pre-experiment baseline	HSLF then LSHF	6	91	116	39
4403	Pre-experiment baseline	Pre-experiment baseline	LSHF then HSLF	6	110	135	38
4668	Pre-experiment baseline	Pre-experiment baseline	LSHF then HSLF	5	87	112	59
4889	Pre-experiment baseline	Pre-experiment baseline	LSHF then HSLF	4	106	131	47
5002	Pre-experiment baseline	Pre-experiment baseline	LSHF then HSLF	4	138	163	46
5007	Pre-experiment baseline	Pre-experiment baseline	HSLF then LSHF	4	143	168	38
5034	Pre-experiment baseline	Pre-experiment baseline	LSHF then HSLF	3	197	222	38
5046	Pre-experiment baseline	Pre-experiment baseline	HSLF then LSHF	4	145	170	52
5249	Pre-experiment baseline	Pre-experiment baseline	LSHF then HSLF	3	110	135	43
5282	Pre-experiment baseline	Pre-experiment baseline	LSHF then HSLF	3	137	162	26
5298	Pre-experiment baseline	Pre-experiment baseline	LSHF then HSLF	3	108	133	46
5405	Pre-experiment baseline	Pre-experiment baseline	HSLF then LSHF	3	115	140	
5409	Pre-experiment baseline	Pre-experiment baseline	HSLF then LSHF	3	194	219	25
5417	Pre-experiment baseline	Pre-experiment baseline	LSHF then HSLF	3	92	117	51
5439	Pre-experiment baseline	Pre-experiment baseline	HSLF then LSHF	3	100	125	37
5455	Pre-experiment baseline	Pre-experiment baseline	HSLF then LSHF	3	113	138	43
5472	Pre-experiment baseline	Pre-experiment baseline	HSLF then LSHF	3	111	136	39
5473	Pre-experiment baseline	Pre-experiment baseline	LSHF then HSLF	3	129	154	48
5658	Pre-experiment baseline	Pre-experiment baseline	LSHF then HSLF	2	149	174	40
5663	Pre-experiment baseline	Pre-experiment baseline	HSLF then LSHF	2	101	126	43
5676	Pre-experiment baseline	Pre-experiment baseline	LSHF then HSLF	2	121	146	38
5694	Pre-experiment baseline	Pre-experiment baseline	LSHF then HSLF	2	120	145	41
5696	Pre-experiment baseline	Pre-experiment baseline	HSLF then LSHF	2	151	176	40
5808	Pre-experiment baseline	Pre-experiment baseline	HSLF then LSHF	2	150	175	37
5823	Pre-experiment baseline	Pre-experiment baseline	LSHF then HSLF	2	131	156	31
5828	Pre-experiment baseline	Pre-experiment baseline	HSLF then LSHF	2	94	119	35
5834	Pre-experiment baseline	Pre-experiment baseline	HSLF then LSHF	2	125	150	45
5838	Pre-experiment baseline	Pre-experiment baseline	HSLF then LSHF	2	140	165	34
5840	Pre-experiment baseline	Pre-experiment baseline	HSLF then LSHF	2	137	162	52
5844	Pre-experiment baseline	Pre-experiment baseline	HSLF then LSHF	2	128	153	43
5858	Pre-experiment baseline	Pre-experiment baseline	LSHF then HSLF	2	107	132	44
5862	Pre-experiment baseline	Pre-experiment baseline	LSHF then HSLF	2	91	116	38
6058	Pre-experiment baseline	Pre-experiment baseline	LSHF then HSLF	1	129	154	35
6076	Pre-experiment baseline	Pre-experiment baseline	HSLF then LSHF	1	130	155	37
6090	Pre-experiment baseline	Pre-experiment baseline	LSHF then HSLF	1	142	167	42
6091	Pre-experiment baseline	Pre-experiment baseline	LSHF then HSLF	1	113	138	39
6098	Pre-experiment baseline	Pre-experiment baseline	LSHF then HSLF	1	156	181	35
6201	Pre-experiment baseline	Pre-experiment baseline	LSHF then HSLF	1	141	166	42
6205	Pre-experiment baseline	Pre-experiment baseline	LSHF then HSLF	1	107	132	45
6213	Pre-experiment baseline	Pre-experiment baseline	HSLF then LSHF	1	140	165	39
6218	Pre-experiment baseline	Pre-experiment baseline	HSLF then LSHF	1	126	151	35
6219	Pre-experiment baseline	Pre-experiment baseline	LSHF then HSLF	1	134	159	32
6221	Pre-experiment baseline	Pre-experiment baseline	HSLF then LSHF	1	91	116	41
6222	Pre-experiment baseline	Pre-experiment baseline	LSHF then HSLF	1	115	140	25
6226	Pre-experiment baseline	Pre-experiment baseline	LSHF then HSLF	1	117	142	30
6230	Pre-experiment baseline	Pre-experiment baseline	HSLF then LSHF	1	116	141	30
6231	Pre-experiment baseline	Pre-experiment baseline	HSLF then LSHF	1	107	132	32
6232	Pre-experiment baseline	Pre-experiment baseline	HSLF then LSHF	1	116	141	35
6234	Pre-experiment baseline	Pre-experiment baseline	LSHF then HSLF	1	110	135	38
6235	Pre-experiment baseline	Pre-experiment baseline	LSHF then HSLF	1	103	128	36
6236	Pre-experiment baseline	Pre-experiment baseline	HSLF then LSHF	1	119	144	24
6238	Pre-experiment baseline	Pre-experiment baseline	HSLF then LSHF	1	97	122	29
6239	Pre-experiment baseline	Pre-experiment baseline	LSHF then HSLF	1	127	152	35
6240	Pre-experiment baseline	Pre-experiment baseline	HSLF then LSHF	1	100	125	34
6242	Pre-experiment baseline	Pre-experiment baseline	LSHF then HSLF	1	93	118	44
6243	Pre-experiment baseline	Pre-experiment baseline	HSLF then LSHF	1	100	125	32
6245	Pre-experiment baseline	Pre-experiment baseline	LSHF then HSLF	1	106	131	36
6247	Pre-experiment baseline	Pre-experiment baseline	HSLF then LSHF	1	95	120	34

Cow ID	Period	Diet	3'-sialyllactose	6'-sialyllactose	2_0_0_2_0	2_1_0_0_0 isomer 1	2_1_0_0_0 isomer 2
4221	Pre-experiment baseline	Pre-experiment baseline	0.818979909	1.11994389	1.380036731	1.896715985	2.464938384
4403	Pre-experiment baseline	Pre-experiment baseline	0.897305613	0.589733161	0.86289814	1.231892987	2.816360471
4668	Pre-experiment baseline	Pre-experiment baseline	0.990755043	1.128233552	0.783659972	1.179386826	2.192187036
4889	Pre-experiment baseline	Pre-experiment baseline	0.566583139	0.905997618	0.699944894	1.488721871	1.874182745
5002	Pre-experiment baseline	Pre-experiment baseline	1.482747034	1.520533565	1.33010084	2.87387155	1.847558125
5007	Pre-experiment baseline	Pre-experiment baseline	0.783031671	1.179434151	0.939479342	2.20135191	1.539063112
5034	Pre-experiment baseline	Pre-experiment baseline	0.917578809	1.288976984	0.883907059	1.750976863	2.76229686
5046	Pre-experiment baseline	Pre-experiment baseline	0.826497696	0.823241067	1.941452606	2.042320411	1.551740039
5249	Pre-experiment baseline	Pre-experiment baseline	1.076781184	1.087997412	0.798204233	1.67034732	2.779018166
5282	Pre-experiment baseline	Pre-experiment baseline	0.752259501	0.984809656	1.20905388	3.036632439	2.309488002
5298	Pre-experiment baseline	Pre-experiment baseline	0.526496614	0.872457343	0.802979806	0.797240421	0.478600331
5405	Pre-experiment baseline	Pre-experiment baseline	0.933642199	0.916555137	1.702275708	2.611080832	0.53654998
5409	Pre-experiment baseline	Pre-experiment baseline	0.757247207	1.564546102	1.12863333	1.937911811	1.609011722
5417	Pre-experiment baseline	Pre-experiment baseline	1.334808963	1.60162147	0.910911709	1.776187344	0.729685836
5439	Pre-experiment baseline	Pre-experiment baseline	0.73232553	1.234035066	0.89719616	1.755809594	0.657648927
5455	Pre-experiment baseline	Pre-experiment baseline	0.564902888	0.687996183	1.218882747	0.743097089	1.255927841
5472	Pre-experiment baseline	Pre-experiment baseline	0.787062984	1.111647502	1.438302599	2.056397593	1.80400203
5473	Pre-experiment baseline	Pre-experiment baseline	0.575856999	0.93774529	1.392055624	0.713226585	3.154417796
5658	Pre-experiment baseline	Pre-experiment baseline	1.006061195	1.39749772	1.14546141	2.304381239	3.044406843
5663	Pre-experiment baseline	Pre-experiment baseline	0.477030684	0.664593404	0.865564926	1.310656876	0.3839689
5676	Pre-experiment baseline	Pre-experiment baseline	0.491015812	0.642805459	0.821478684	1.475946885	0.720976913
5694	Pre-experiment baseline	Pre-experiment baseline	0.462199845	0.373953281	0.65461957	0.828981521	0.848754646
5696	Pre-experiment baseline	Pre-experiment baseline	0.762396865	0.790513528	0.727851706	1.321971435	0.171838317
5808	Pre-experiment baseline	Pre-experiment baseline	0.829363811	0.754094028	1.106461332	1.306820817	0.381082951
5823	Pre-experiment baseline	Pre-experiment baseline	0.88519151	1.236730651	1.048722615	1.878711735	2.397034824
5828	Pre-experiment baseline	Pre-experiment baseline	0.884472821	0.824238977	1.061463339	1.101261163	1.884535752
5834	Pre-experiment baseline	Pre-experiment baseline	0.695678679	1.039422981	0.851775997	2.038239901	4.841679552
5838	Pre-experiment baseline	Pre-experiment baseline	0.792350983	0.86751121	1.20361626	1.566545307	0.609282806
5840	Pre-experiment baseline	Pre-experiment baseline	0.673843697	1.178184635	0.728128282	2.181032702	0.820377387
5844	Pre-experiment baseline	Pre-experiment baseline	0.898354456	0.559083295	0.983573853	0.462876875	0.809823297
5858	Pre-experiment baseline	Pre-experiment baseline	1.045468554	0.945366023	1.106007428	1.811724779	2.670611949
5862	Pre-experiment baseline	Pre-experiment baseline	0.634273654	0.682813285	1.012432318	1.591024045	2.523556429
6058	Pre-experiment baseline	Pre-experiment baseline	0.49491139	0.526491699	0.52738838	1.440073527	3.054875108
6076	Pre-experiment baseline	Pre-experiment baseline	0.578861345	0.569597559	0.563186453	1.531843708	0.75145373
6090	Pre-experiment baseline	Pre-experiment baseline	0.577231708	0.644691987	1.15452709	1.331297283	1.656203231
6091	Pre-experiment baseline	Pre-experiment baseline	0.608406698	0.553694862	0.815270255	1.734610725	1.210191681
6098	Pre-experiment baseline	Pre-experiment baseline	0.625987467	0.805220352	0.791164406	1.466616053	0.779506513
6201	Pre-experiment baseline	Pre-experiment baseline	0.830616847	0.681770116	0.903596851	1.025491246	1.677681668
6205	Pre-experiment baseline	Pre-experiment baseline	0.507665571	0.843695614	1.084222365	1.569949621	1.192901265
6213	Pre-experiment baseline	Pre-experiment baseline	0.714500172	0.524110711	0.548417738	1.218960839	1.586011523
6218	Pre-experiment baseline	Pre-experiment baseline	0.750280133	1.116730538	0.831452766	1.993585984	2.263080733
6219	Pre-experiment baseline	Pre-experiment baseline	0.941800015	0.890464861	0.979876752	2.216688151	0.403985198
6221	Pre-experiment baseline	Pre-experiment baseline	0.446246672	0.880500921	0.770199778	1.774119108	1.045608959
6222	Pre-experiment baseline	Pre-experiment baseline	0.754090275	0.827321429	0.72944677	1.871423741	1.193804582
6226	Pre-experiment baseline	Pre-experiment baseline	0.850757693	0.926992335	1.622847319	2.73324278	2.233361498
6230	Pre-experiment baseline	Pre-experiment baseline	0.66343321	1.041482953	1.259120018	1.69570037	0.828020707
6231	Pre-experiment baseline	Pre-experiment baseline	0.91689858	1.303996356	1.090980985	1.288722777	2.76887311
6232	Pre-experiment baseline	Pre-experiment baseline	0.781187565	0.902329409	0.920547382	1.459701625	0.698961676
6234	Pre-experiment baseline	Pre-experiment baseline	0.801227161	0.709548893	1.046003064	1.250020108	1.812520122
6235	Pre-experiment baseline	Pre-experiment baseline	0.542445825	0.691174545	0.76960047	0.983464063	1.127263425
6236	Pre-experiment baseline	Pre-experiment baseline	1.090218459	1.398113219	1.238517504	1.828536178	1.467072431
6238	Pre-experiment baseline	Pre-experiment baseline	0.68957418	0.641051748	1.87306807	1.926571436	0.370850484
6239	Pre-experiment baseline	Pre-experiment baseline	0.79108321	0.756057076	0.642621988	1.669298116	3.206810452
6240	Pre-experiment baseline	Pre-experiment baseline	0.575147669	0.400665872	0.74437902	2.128688097	0.676201472
6242	Pre-experiment baseline	Pre-experiment baseline	0.654108119	0.730104831	0.984245096	1.185223999	2.309920533
6243	Pre-experiment baseline	Pre-experiment baseline	1.069569774	0.968566599	2.18247041	3.521589383	3.177019852
6245	Pre-experiment baseline	Pre-experiment baseline	0.705520029	0.871567021	1.328176956	1.256469341	0.231075737
6247	Pre-experiment baseline	Pre-experiment baseline	1.309174752	1.047828296	0.88076909	1.63515484	1.780337473

Cow ID	Period	Diet	3_0_0_1_0	3_1_0_0_0	3_2_0_0_0	3_3_0_0_0	3_6_1_0_0
4221	Pre-experiment baseline	Pre-experiment baseline	0.626319102	0.3992317	0.285436438	0.427605948	0.197890699
4403	Pre-experiment baseline	Pre-experiment baseline	0.494417267	0.670036565	0.201349243	0.249982369	0.164329683
4668	Pre-experiment baseline	Pre-experiment baseline	0.301015619	0.780415546	0.194034583	0.187240532	0.214707073
4889	Pre-experiment baseline	Pre-experiment baseline	0.592335791	0.868098751	0.177852562	0.39411741	0.287067021
5002	Pre-experiment baseline	Pre-experiment baseline	0.722928196	0.843434435	0.193411444	0.2400151	0.207302734
5007	Pre-experiment baseline	Pre-experiment baseline	0.758486968	0.616164412	0.466184664	0.357045318	0.508585653
5034	Pre-experiment baseline	Pre-experiment baseline	0.891106874	0.444261484	0.31661081	0.152124935	0.296562339
5046	Pre-experiment baseline	Pre-experiment baseline	0.67899476	0.576978678	0.190832831	0.386818129	0.353808474
5249	Pre-experiment baseline	Pre-experiment baseline	0.404282137	0.600802313	0.134230128	0.139740338	0.264497973
5282	Pre-experiment baseline	Pre-experiment baseline	0.961499875	1.271516368	0.450222017	0.486127707	0.309373082
5298	Pre-experiment baseline	Pre-experiment baseline	0.453776433	0.522034006	0.222620858	0.215369496	0.116237374
5405	Pre-experiment baseline	Pre-experiment baseline	0.506671045	0.745936523	0.2265981	0.345311521	0.295564723
5409	Pre-experiment baseline	Pre-experiment baseline	1.042398463	0.534530094	0.468525635	0.24562982	0.277250518
5417	Pre-experiment baseline	Pre-experiment baseline	0.529468553	0.956464965	0.289953918	0.271086524	0.111453201
5439	Pre-experiment baseline	Pre-experiment baseline	0.41744404	0.393352701	0.229330212	0.248361558	0.24183235
5455	Pre-experiment baseline	Pre-experiment baseline	0.625269286	0.782411598	0.27111436	0.392666973	0.305350356
5472	Pre-experiment baseline	Pre-experiment baseline	0.869643168	0.641318403	0.377565776	0.293401585	0.263431091
5473	Pre-experiment baseline	Pre-experiment baseline	0.608430793	0.209613002	0.166606591	0.363350506	0.157297657
5658	Pre-experiment baseline	Pre-experiment baseline	0.62066175	0.28499774	0.292398689	0.93483426	0.276375828
5663	Pre-experiment baseline	Pre-experiment baseline	0.468792999	0.571177347	0.263338338	0.325296878	0.19181634
5676	Pre-experiment baseline	Pre-experiment baseline	0.624467458	0.516093162	0.246717728	0.283568645	0.279863323
5694	Pre-experiment baseline	Pre-experiment baseline	0.603426197	0.320214522	0.228811153	0.224266999	0.140999452
5696	Pre-experiment baseline	Pre-experiment baseline	0.535080196	0.452536255	0.117506675	0.243377333	0.135404028
5808	Pre-experiment baseline	Pre-experiment baseline	0.8113419	0.385879939	0.329103136	0.374189124	0.364883571
5823	Pre-experiment baseline	Pre-experiment baseline	0.602729776	0.7467258	0.283221912	0.193821266	0.257298654
5828	Pre-experiment baseline	Pre-experiment baseline	0.429026254	0.201282895	0.223106187	1.001375899	0.310141366
5834	Pre-experiment baseline	Pre-experiment baseline	0.61913893	0.811498567	0.240345086	0.259854121	0.294090808
5838	Pre-experiment baseline	Pre-experiment baseline	1.23766599	0.651360721	0.27151817	0.37638184	0.142196885
5840	Pre-experiment baseline	Pre-experiment baseline	0.763774937	0.590480782	0.20812291	0.310228598	0.256961529
5844	Pre-experiment baseline	Pre-experiment baseline	0.627085738	0.348269934	0.229901643	0.333039779	0.337443448
5858	Pre-experiment baseline	Pre-experiment baseline	0.690789851	0.777592714	0.322234839	0.276040792	0.315739976
5862	Pre-experiment baseline	Pre-experiment baseline	0.739933279	0.536772163	0.137748236	0.192437779	0.219378482
6058	Pre-experiment baseline	Pre-experiment baseline	0.64969114	0.934480153	0.186053984	0.287476469	0.372550367
6076	Pre-experiment baseline	Pre-experiment baseline	0.439020833	0.38148524	0.247538107	0.272124251	0.330201541
6090	Pre-experiment baseline	Pre-experiment baseline	0.850739731	0.347796766	0.28155262	0.200783877	0.211497391
6091	Pre-experiment baseline	Pre-experiment baseline	0.407765528	0.369697697	0.115353763	0.260463571	0.32875244
6098	Pre-experiment baseline	Pre-experiment baseline	0.566485456	0.450698868	0.288215781	0.247314888	0.314789196
6201	Pre-experiment baseline	Pre-experiment baseline	0.464917873	0.612290639	0.161362516	0.204495819	0.261627725
6205	Pre-experiment baseline	Pre-experiment baseline	0.75016803	0.474751665	0.15671667	0.25981484	0.146593095
6213	Pre-experiment baseline	Pre-experiment baseline	0.429680676	0.81458713	0.287919338	0.290986484	0.239507055
6218	Pre-experiment baseline	Pre-experiment baseline	0.884849814	0.738730471	0.440663336	0.321019915	0.489550072
6219	Pre-experiment baseline	Pre-experiment baseline	0.853697174	0.548528211	0.24821976	0.219394988	0.387101736
6221	Pre-experiment baseline	Pre-experiment baseline	0.590240347	0.501611569	0.263752698	0.467002242	0.41567684
6222	Pre-experiment baseline	Pre-experiment baseline	0.701143173	0.432010278	0.310063223	0.158553316	0.442572694
6226	Pre-experiment baseline	Pre-experiment baseline	0.696963598	0.395528786	0.250172832	0.330765117	0.387332634
6230	Pre-experiment baseline	Pre-experiment baseline	0.777445776	0.347751294	0.301838473	0.246410049	0.301294573
6231	Pre-experiment baseline	Pre-experiment baseline	0.432555826	0.43012898	0.232149747	0.226955229	0.327655659
6232	Pre-experiment baseline	Pre-experiment baseline	0.674658455	0.569364986	0.269250039	0.222297737	0.301505553
6234	Pre-experiment baseline	Pre-experiment baseline	0.521827829	0.514020954	0.289446783	0.202949947	0.455397789
6235	Pre-experiment baseline	Pre-experiment baseline	0.435178639	0.457094668	0.170974097	0.24495718	0.259201966
6236	Pre-experiment baseline	Pre-experiment baseline	0.674951888	0.71249045	0.262584674	0.259896922	0.475245437
6238	Pre-experiment baseline	Pre-experiment baseline	0.46734916	0.376568588	0.277482226	0.558072088	0.375028003
6239	Pre-experiment baseline	Pre-experiment baseline	0.485797226	0.548644666	0.29677086	0.227937512	0.352542685
6240	Pre-experiment baseline	Pre-experiment baseline	0.679779893	0.854521422	0.352138723	0.39239452	0.288092697
6242	Pre-experiment baseline	Pre-experiment baseline	0.420290553	0.593364581	0.222705692	0.184834754	0.319156669
6243	Pre-experiment baseline	Pre-experiment baseline	0.833797752	0.466655325	0.310822921	0.509563888	0.428160742
6245	Pre-experiment baseline	Pre-experiment baseline	0.571874103	0.5781436	0.178117444	0.425048974	0.416529746
6247	Pre-experiment baseline	Pre-experiment baseline	0.483554102	0.87803057	0.221542274	0.227981686	0.317609776

Cow ID	Period	Diet	4_1_0_0_0	4_1_0_1_0	4_2_0_0_0 isomer 1	4_2_0_0_0 isomer 2	4_4_1_0_0
4221	Pre-experiment baseline	Pre-experiment baseline	0.325580335	0.299350808	0.360743193	0.180747894	0.392510954
4403	Pre-experiment baseline	Pre-experiment baseline	0.266517808	0.21792769	0.50585822	0.399884765	0.328927661
4668	Pre-experiment baseline	Pre-experiment baseline	0.22890836	0.185140338	0.402820582	0.269273282	0.339183861
4889	Pre-experiment baseline	Pre-experiment baseline	0.155778083	0.163529463	0.399693828	0.417519016	0.368768338
5002	Pre-experiment baseline	Pre-experiment baseline	0.265172165	0.237867943	0.587382348	0.328136276	0.323135598
5007	Pre-experiment baseline	Pre-experiment baseline	0.471634513	0.297029543	0.516226216	0.375668336	0.327615776
5034	Pre-experiment baseline	Pre-experiment baseline	0.30073272	0.321235151	0.543286374	0.226669576	0.297810784
5046	Pre-experiment baseline	Pre-experiment baseline	0.248703608	0.195896966	0.512105256	0.232117405	0.374495455
5249	Pre-experiment baseline	Pre-experiment baseline	0.17022392	0.16605657	0.427428272	0.192408809	0.413494781
5282	Pre-experiment baseline	Pre-experiment baseline	0.481726211	0.407379749	0.947027139	0.512335381	0.478595332
5298	Pre-experiment baseline	Pre-experiment baseline	0.211573726	0.22587869	0.413529341	0.211062809	0.230894113
5405	Pre-experiment baseline	Pre-experiment baseline	0.307494495	0.246646279	0.791511045	0.16732705	0.379911892
5409	Pre-experiment baseline	Pre-experiment baseline	0.694892437	0.56745429	0.550648181	0.284720003	0.320259973
5417	Pre-experiment baseline	Pre-experiment baseline	0.335384304	0.246034374	0.51083628	0.394004995	0.445182457
5439	Pre-experiment baseline	Pre-experiment baseline	0.233290093	0.270028489	0.312387386	0.236783357	0.333684158
5455	Pre-experiment baseline	Pre-experiment baseline	0.296601705	0.219373527	0.313803797	0.357745206	0.343975591
5472	Pre-experiment baseline	Pre-experiment baseline	0.412124132	0.353887895	0.603690353	0.274112083	0.532307942
5473	Pre-experiment baseline	Pre-experiment baseline	0.161687033	0.155299849	0.567402284	0.170985376	0.248005296
5658	Pre-experiment baseline	Pre-experiment baseline	0.31146328	0.239757824	0.664359985	0.180231062	0.533315803
5663	Pre-experiment baseline	Pre-experiment baseline	0.336432687	0.267623591	0.593499235	0.245277236	0.278946993
5676	Pre-experiment baseline	Pre-experiment baseline	0.242012155	0.172454724	0.512021356	0.228115359	0.319461825
5694	Pre-experiment baseline	Pre-experiment baseline	0.296411078	0.264072119	0.293916939	0.124493309	0.170191126
5696	Pre-experiment baseline	Pre-experiment baseline	0.134660406	0.134093068	0.442797424	0.175731392	0.300368665
5808	Pre-experiment baseline	Pre-experiment baseline	0.3658797	0.322553709	0.657063165	0.448334706	0.495211689
5823	Pre-experiment baseline	Pre-experiment baseline	0.342968317	0.249506116	0.642179526	0.17278802	0.35853627
5828	Pre-experiment baseline	Pre-experiment baseline	0.265828819	0.174609193	0.719444975	0.150035547	0.392722502
5834	Pre-experiment baseline	Pre-experiment baseline	0.265063124	0.22094344	0.461856823	0.19379217	0.42153231
5838	Pre-experiment baseline	Pre-experiment baseline	0.351105273	0.266925386	0.532049889	0.381443071	0.328436716
5840	Pre-experiment baseline	Pre-experiment baseline	0.211133825	0.18481462	0.653271935	0.206756935	0.358519084
5844	Pre-experiment baseline	Pre-experiment baseline	0.26824939	0.228803406	0.343537363	0.190202147	0.41306797
5858	Pre-experiment baseline	Pre-experiment baseline	0.337918295	0.314978339	0.673612475	0.258590945	0.388594271
5862	Pre-experiment baseline	Pre-experiment baseline	0.136970857	0.125394919	0.362659997	0.139907292	0.325677611
6058	Pre-experiment baseline	Pre-experiment baseline	0.232745767	0.183539437	0.339510414	0.221403248	0.339601481
6076	Pre-experiment baseline	Pre-experiment baseline	0.368854521	0.221039207	0.335859016	0.194189196	0.367382147
6090	Pre-experiment baseline	Pre-experiment baseline	0.457724772	0.339249192	0.433492304	0.171917551	0.302594806
6091	Pre-experiment baseline	Pre-experiment baseline	0.110323957	0.101425836	0.327807797	0.107641198	0.294289944
6098	Pre-experiment baseline	Pre-experiment baseline	0.359619613	0.285126419	0.702526322	0.201514219	0.468592989
6201	Pre-experiment baseline	Pre-experiment baseline	0.20829139	0.209126448	0.290636162	0.196227468	0.313571741
6205	Pre-experiment baseline	Pre-experiment baseline	0.134692438	0.141398306	0.631405167	0.246012096	0.206058876
6213	Pre-experiment baseline	Pre-experiment baseline	0.295968296	0.234526338	0.596211044	0.281967394	0.281945377
6218	Pre-experiment baseline	Pre-experiment baseline	0.682858659	0.527317272	0.334961409	0.410407204	0.505146195
6219	Pre-experiment baseline	Pre-experiment baseline	0.284470163	0.243125364	0.574047677	0.19677255	0.330492554
6221	Pre-experiment baseline	Pre-experiment baseline	0.291773748	0.242171592	0.451507704	0.261729042	0.337433636
6222	Pre-experiment baseline	Pre-experiment baseline	0.28782491	0.257291216	0.201654284	0.152154528	0.370064535
6226	Pre-experiment baseline	Pre-experiment baseline	0.241611163	0.224895405	0.408175519	0.16718163	0.432956011
6230	Pre-experiment baseline	Pre-experiment baseline	0.413681629	0.332624013	0.615074547	0.233324359	0.442288798
6231	Pre-experiment baseline	Pre-experiment baseline	0.173713969	0.232462089	0.471892742	0.213836741	0.316631944
6232	Pre-experiment baseline	Pre-experiment baseline	0.390442488	0.326305315	0.270525223	0.220697455	0.276286043
6234	Pre-experiment baseline	Pre-experiment baseline	0.395840337	0.301529051	0.320523445	0.229643978	0.266142809
6235	Pre-experiment baseline	Pre-experiment baseline	0.21173137	0.185623918	0.519053646	0.267762956	0.371448679
6236	Pre-experiment baseline	Pre-experiment baseline	0.388551046	0.268046285	0.381281241	0.242306099	0.426551144
6238	Pre-experiment baseline	Pre-experiment baseline	0.269163655	0.240436739	0.459553751	0.299227545	0.461035574
6239	Pre-experiment baseline	Pre-experiment baseline	0.245211037	0.192384123	0.733532472	0.19872124	0.802215366
6240	Pre-experiment baseline	Pre-experiment baseline	0.493519832	0.465905136	0.406087915	0.307253239	0.363613041
6242	Pre-experiment baseline	Pre-experiment baseline	0.239647829	0.260486615	0.276478146	0.256540131	0.212140403
6243	Pre-experiment baseline	Pre-experiment baseline	0.246113122	0.255148376	0.484114198	0.245704864	0.514780209
6245	Pre-experiment baseline	Pre-experiment baseline	0.203692346	0.199104628	0.275371349	0.296664125	0.415830537
6247	Pre-experiment baseline	Pre-experiment baseline	0.332957744	0.31183602	0.452943376	0.339064086	0.510340296

Cow ID	Period	Diet	4_5_1_0_0	5_4_0_0_0	5_4_1_0_0	8_0_0_0_0
4221	Pre-experiment baseline	Pre-experiment baseline	0.394254486	0.486390024	0.251491463	0.114413111
4403	Pre-experiment baseline	Pre-experiment baseline	0.391697502	0.402158007	0.238567632	0.02335661
4668	Pre-experiment baseline	Pre-experiment baseline	0.372247719	0.374666657	0.287814718	0.029684625
4889	Pre-experiment baseline	Pre-experiment baseline	0.410908256	0.293707125	0.418246994	0.054970065
5002	Pre-experiment baseline	Pre-experiment baseline	0.316736572	0.698004139	0.294832884	0.202005752
5007	Pre-experiment baseline	Pre-experiment baseline	0.515925477	0.321654285	0.497711136	0.107279941
5034	Pre-experiment baseline	Pre-experiment baseline	0.267607566	0.327901697	0.275303529	0.027975602
5046	Pre-experiment baseline	Pre-experiment baseline	0.548833166	0.941534678	0.425198303	0.409875573
5249	Pre-experiment baseline	Pre-experiment baseline	0.353590561	0.259943843	0.372604347	0.025724449
5282	Pre-experiment baseline	Pre-experiment baseline	0.304858341	0.669325436	0.363714634	0.371760402
5298	Pre-experiment baseline	Pre-experiment baseline	0.212101952	0.396580362	0.151764402	0.036903301
5405	Pre-experiment baseline	Pre-experiment baseline	0.369703504	0.466901376	0.341514874	0.334186672
5409	Pre-experiment baseline	Pre-experiment baseline	0.336879461	0.486266023	0.285261227	0.207131133
5417	Pre-experiment baseline	Pre-experiment baseline	0.180975509	0.51905064	0.206928343	0.027016442
5439	Pre-experiment baseline	Pre-experiment baseline	0.247574436	0.245075397	0.24185632	0.119019007
5455	Pre-experiment baseline	Pre-experiment baseline	0.349284503	0.292885421	0.447818535	0.034780075
5472	Pre-experiment baseline	Pre-experiment baseline	0.365372834	0.639405914	0.413202856	0.122600822
5473	Pre-experiment baseline	Pre-experiment baseline	0.204875436	0.544954465	0.191586877	0.052410635
5658	Pre-experiment baseline	Pre-experiment baseline	0.178844459	0.368088948	0.261716483	0.051176593
5663	Pre-experiment baseline	Pre-experiment baseline	0.258158426	0.390855718	0.255099816	0.057508546
5676	Pre-experiment baseline	Pre-experiment baseline	0.361215668	0.31365065	0.436648841	0.056349165
5694	Pre-experiment baseline	Pre-experiment baseline	0.256500806	0.214457924	0.240904801	0.018925641
5696	Pre-experiment baseline	Pre-experiment baseline	0.261743149	0.457697579	0.222682966	0.017877161
5808	Pre-experiment baseline	Pre-experiment baseline	0.503770732	0.443697877	0.534329246	0.0509671
5823	Pre-experiment baseline	Pre-experiment baseline	0.226174112	0.51240181	0.322711581	0.099341341
5828	Pre-experiment baseline	Pre-experiment baseline	0.492488826	0.21054654	0.462037573	0.043410018
5834	Pre-experiment baseline	Pre-experiment baseline	0.37917007	0.237422496	0.507613731	0.051299816
5838	Pre-experiment baseline	Pre-experiment baseline	0.24901789	0.496163664	0.299337049	0.073026472
5840	Pre-experiment baseline	Pre-experiment baseline	0.357497531	0.283429235	0.489346347	0.039146783
5844	Pre-experiment baseline	Pre-experiment baseline	0.394632605	0.20770317	0.464251757	0.102844841
5858	Pre-experiment baseline	Pre-experiment baseline	0.368034597	0.262825113	0.421901314	0.045938904
5862	Pre-experiment baseline	Pre-experiment baseline	0.454035778	0.220105324	0.436515738	0.043101435
6058	Pre-experiment baseline	Pre-experiment baseline	0.371077064	0.23938959	0.467004208	0.033057522
6076	Pre-experiment baseline	Pre-experiment baseline	0.376827049	0.148139788	0.433814687	0.072974632
6090	Pre-experiment baseline	Pre-experiment baseline	0.424027622	0.62750207	0.310023171	0.094249968
6091	Pre-experiment baseline	Pre-experiment baseline	0.485044385	0.167303247	0.371685755	0.026825311
6098	Pre-experiment baseline	Pre-experiment baseline	0.592483905	0.303655563	0.503071311	0.066124178
6201	Pre-experiment baseline	Pre-experiment baseline	0.497958369	0.052940327	0.3853751	0.032306127
6205	Pre-experiment baseline	Pre-experiment baseline	0.268009212	0.498344572	0.173893957	0.121404636
6213	Pre-experiment baseline	Pre-experiment baseline	0.192681267	0.562952694	0.285578377	0.066840922
6218	Pre-experiment baseline	Pre-experiment baseline	0.705833506	0.23022263	0.505139438	0.133352026
6219	Pre-experiment baseline	Pre-experiment baseline	0.345661161	0.122014608	0.326780936	0.037763245
6221	Pre-experiment baseline	Pre-experiment baseline	0.289404463	0.277746518	0.362830201	0.094755329
6222	Pre-experiment baseline	Pre-experiment baseline	0.432483534	0.14738516	0.439050406	0.066298305
6226	Pre-experiment baseline	Pre-experiment baseline	0.397469093	0.097642835	0.469289462	0.058257707
6230	Pre-experiment baseline	Pre-experiment baseline	0.642446219	0.233718057	0.404605684	0.070164386
6231	Pre-experiment baseline	Pre-experiment baseline	0.613201705	0.30269445	0.360384492	0.109811913
6232	Pre-experiment baseline	Pre-experiment baseline	0.301961726	0.279790513	0.310482027	0.110368083
6234	Pre-experiment baseline	Pre-experiment baseline	0.625518364	0.18902003	0.400678332	0.076358731
6235	Pre-experiment baseline	Pre-experiment baseline	0.464835222	0.298887628	0.330395442	0.084377392
6236	Pre-experiment baseline	Pre-experiment baseline	0.750075489	0.216135702	0.488107884	0.125727606
6238	Pre-experiment baseline	Pre-experiment baseline	0.302886206	0.168225043	0.409735817	0.030068025
6239	Pre-experiment baseline	Pre-experiment baseline	0.444864503	0.302710599	0.661929479	0.096569739
6240	Pre-experiment baseline	Pre-experiment baseline	0.466748947	0.782521535	0.397877467	0.273704736
6242	Pre-experiment baseline	Pre-experiment baseline	0.437039238	0.24805683	0.302702853	0.069797622
6243	Pre-experiment baseline	Pre-experiment baseline	0.481099802	0.234095281	0.414463957	0.051067775
6245	Pre-experiment baseline	Pre-experiment baseline	0.534345396	0.219615981	0.451350454	0.064242198
6247	Pre-experiment baseline	Pre-experiment baseline	0.328676198	0.233404025	0.429465611	0.023623023

Cow ID	Period	Diet	Sequence	Parity	Days in milk at start of study	sample collection (first day of 2	Milk Weight at sample collection (lbs, average of 2 pooled samples)
4221	Second Treatment period	Low Starch High Fiber	HSLF then LSHF	6	91	225	31.4
4403	Second Treatment period	High Starch Low Fiber	LSHF then HSLF	6	110	244	37.9
4668	Second Treatment period	High Starch Low Fiber	LSHF then HSLF	5	87	221	52.2
4889	Second Treatment period	High Starch Low Fiber	LSHF then HSLF	4	106	240	35.4
5002	Second Treatment period	High Starch Low Fiber	LSHF then HSLF	4	138	272	31.2
5007	Second Treatment period	Low Starch High Fiber	HSLF then LSHF	4	143	277	27.8
5046	Second Treatment period	Low Starch High Fiber	HSLF then LSHF	4	145	279	37.3
5249	Second Treatment period	High Starch Low Fiber	LSHF then HSLF	3	110	244	46.8
5282	Second Treatment period	High Starch Low Fiber	LSHF then HSLF	3	137	271	24.7
5298	Second Treatment period	High Starch Low Fiber	LSHF then HSLF	3	108	242	42.3
5417	Second Treatment period	High Starch Low Fiber	LSHF then HSLF	3	92	226	36.8
5439	Second Treatment period	Low Starch High Fiber	HSLF then LSHF	3	100	234	31.1
5455	Second Treatment period	Low Starch High Fiber	HSLF then LSHF	3	113	247	33.5
5472	Second Treatment period	Low Starch High Fiber	HSLF then LSHF	3	111	245	35.4
5473	Second Treatment period	High Starch Low Fiber	LSHF then HSLF	3	129	263	32.4
5658	Second Treatment period	High Starch Low Fiber	LSHF then HSLF	2	149	283	31.8
5663	Second Treatment period	Low Starch High Fiber	HSLF then LSHF	2	101	235	34.1
5676	Second Treatment period	High Starch Low Fiber	LSHF then HSLF	2	121	255	30.5
5694	Second Treatment period	High Starch Low Fiber	LSHF then HSLF	2	120	254	39.5
5696	Second Treatment period	Low Starch High Fiber	HSLF then LSHF	2	151	285	33.4
5808	Second Treatment period	Low Starch High Fiber	HSLF then LSHF	2	150	284	28.6
5823	Second Treatment period	High Starch Low Fiber	LSHF then HSLF	2	131	265	29.4
5828	Second Treatment period	Low Starch High Fiber	HSLF then LSHF	2	94	228	25.6
5834	Second Treatment period	Low Starch High Fiber	HSLF then LSHF	2	125	259	32.2
5838	Second Treatment period	Low Starch High Fiber	HSLF then LSHF	2	140	274	22.3
5840	Second Treatment period	Low Starch High Fiber	HSLF then LSHF	2	137	271	33.6
5844	Second Treatment period	Low Starch High Fiber	HSLF then LSHF	2	128	262	26.5
5849	Second Treatment period	Low Starch High Fiber	HSLF then LSHF	2	128	262	34.0
5858	Second Treatment period	High Starch Low Fiber	LSHF then HSLF	2	107	241	30.3
5862	Second Treatment period	High Starch Low Fiber	LSHF then HSLF	2	91	225	36.4
6058	Second Treatment period	High Starch Low Fiber	LSHF then HSLF	1	129	263	31.9
6090	Second Treatment period	High Starch Low Fiber	LSHF then HSLF	1	142	276	34.2
6091	Second Treatment period	High Starch Low Fiber	LSHF then HSLF	1	113	247	37.3
6098	Second Treatment period	High Starch Low Fiber	LSHF then HSLF	1	156	290	33.7
6201	Second Treatment period	High Starch Low Fiber	LSHF then HSLF	1	141	275	38.2
6205	Second Treatment period	High Starch Low Fiber	LSHF then HSLF	1	107	241	34.4
6213	Second Treatment period	Low Starch High Fiber	HSLF then LSHF	1	140	274	36.9
6218	Second Treatment period	Low Starch High Fiber	HSLF then LSHF	1	126	260	30.9
6219	Second Treatment period	High Starch Low Fiber	LSHF then HSLF	1	134	268	27.9
6221	Second Treatment period	Low Starch High Fiber	HSLF then LSHF	1	91	225	36.4
6222	Second Treatment period	High Starch Low Fiber	LSHF then HSLF	1	115	249	30.9
6226	Second Treatment period	High Starch Low Fiber	LSHF then HSLF	1	117	251	29.9
6231	Second Treatment period	Low Starch High Fiber	HSLF then LSHF	1	107	241	26.8
6234	Second Treatment period	High Starch Low Fiber	LSHF then HSLF	1	110	244	34.1
6235	Second Treatment period	High Starch Low Fiber	LSHF then HSLF	1	103	237	37.4
6236	Second Treatment period	Low Starch High Fiber	HSLF then LSHF	1	115	249	30.9
6238	Second Treatment period	Low Starch High Fiber	HSLF then LSHF	1	97	231	26.2
6239	Second Treatment period	High Starch Low Fiber	LSHF then HSLF	1	127	261	33.8
6240	Second Treatment period	Low Starch High Fiber	HSLF then LSHF	1	100	234	32.0
6242	Second Treatment period	High Starch Low Fiber	LSHF then HSLF	1	93	227	42.2
6243	Second Treatment period	Low Starch High Fiber	HSLF then LSHF	1	100	234	30.1
6245	Second Treatment period	High Starch Low Fiber	LSHF then HSLF	1	106	240	34.1
6247	Second Treatment period	Low Starch High Fiber	HSLF then LSHF	1	95	229	31.0

Cow ID	Period	Diet	3'-sialyllactose	6'-sialyllactose	2_0_0_2_0	2_1_0_0_0 isomer 1	2_1_0_0_0 isomer 2
4221	Second Treatment period	Low Starch High Fiber	1.272229344	2.039089253	1.62869797	1.838176097	2.201544317
4403	Second Treatment period	High Starch Low Fiber	0.677251807	0.391161377	0.663207809	0.99475302	2.126177469
4668	Second Treatment period	High Starch Low Fiber	0.801715982	0.950445048	1.340938525	1.30242117	1.158450552
4889	Second Treatment period	High Starch Low Fiber	0.793014763	1.055997238	1.181419917	3.616042524	3.386855258
5002	Second Treatment period	High Starch Low Fiber	1.040185305	1.092396147	1.095900726	3.267914887	1.403929175
5007	Second Treatment period	Low Starch High Fiber	0.549749783	0.814833168	0.820705657	1.480684146	1.154656898
5046	Second Treatment period	Low Starch High Fiber	0.747262911	0.922445367	0.757498248	1.514404988	2.092043707
5249	Second Treatment period	High Starch Low Fiber	0.741810858	0.840140136	1.191029542	1.745481399	1.250846496
5282	Second Treatment period	High Starch Low Fiber	0.797332272	1.227314716	0.732167002	2.915144008	1.37452081
5298	Second Treatment period	High Starch Low Fiber	0.73538448	0.884132873	0.85778858	1.964693847	0.464714433
5417	Second Treatment period	High Starch Low Fiber	0.828773961	0.620865722	0.702798985	1.667938787	0.591345957
5439	Second Treatment period	Low Starch High Fiber	0.525890053	0.770939717	0.594832134	1.932657867	0.68947233
5455	Second Treatment period	Low Starch High Fiber	0.698162817	0.681702614	1.284574493	1.68339732	1.860855777
5472	Second Treatment period	Low Starch High Fiber	1.038649552	2.080011217	1.309870772	2.82889995	1.977098599
5473	Second Treatment period	High Starch Low Fiber	0.729564777	1.275482476	1.090865817	3.008015328	6.775036045
5658	Second Treatment period	High Starch Low Fiber	0.811837379	1.390088154	1.0033066	2.127933749	2.285283722
5663	Second Treatment period	Low Starch High Fiber	0.715212392	0.935957409	1.542050926	1.841336388	0.348799607
5676	Second Treatment period	High Starch Low Fiber	0.397072832	0.868200027	0.913449174	1.597797684	0.928156309
5694	Second Treatment period	High Starch Low Fiber	0.527126615	0.700352007	1.308491116	1.390351431	0.401748942
5696	Second Treatment period	Low Starch High Fiber	0.595401846	0.825326524	0.92948791	1.426571714	0.300945026
5808	Second Treatment period	Low Starch High Fiber	1.225851965	1.116750806	1.146311839	1.841400376	0.275806595
5823	Second Treatment period	High Starch Low Fiber	0.439365571	0.696422857	0.658624033	1.750492311	1.050226557
5828	Second Treatment period	Low Starch High Fiber	1.349449843	1.145498669	1.535499987	1.827209474	3.773333701
5834	Second Treatment period	Low Starch High Fiber	1.15387625	1.691746054	2.688897638	4.062995451	5.543165612
5838	Second Treatment period	Low Starch High Fiber	0.620531252	0.707189489	1.133337073	2.277543631	0.52748315
5840	Second Treatment period	Low Starch High Fiber	0.518098449	0.845727552	0.726107032	1.983600909	0.709901524
5844	Second Treatment period	Low Starch High Fiber	0.907311989	0.961056091	1.796668193	3.929616165	0.982444216
5849	Second Treatment period	Low Starch High Fiber	0.686798104	0.881946045	1.076829901	1.845725101	2.288856262
5858	Second Treatment period	High Starch Low Fiber	0.637208064	0.754482579	0.564600037	1.959221206	1.234457582
5862	Second Treatment period	High Starch Low Fiber	1.030173181	0.976929053	1.062488643	1.764235688	3.475841727
6058	Second Treatment period	High Starch Low Fiber	1.037317894	0.518352318	0.763989049	1.600843187	4.822192558
6090	Second Treatment period	High Starch Low Fiber	0.560360887	0.600479036	0.929571779	1.515777393	1.43140677
6091	Second Treatment period	High Starch Low Fiber	0.794807828	1.08749303	0.872985203	1.7701177	1.51399248
6098	Second Treatment period	High Starch Low Fiber	0.774285421	0.732465	1.253310804	2.236517241	0.685172187
6201	Second Treatment period	High Starch Low Fiber	0.85920392	0.935839534	1.7424761	1.114732781	0.359176396
6205	Second Treatment period	High Starch Low Fiber	0.653279449	0.821178976	0.999397479	1.490072066	1.32446544
6213	Second Treatment period	Low Starch High Fiber	0.822819537	0.680623517	0.664837634	1.568982475	1.964391134
6218	Second Treatment period	Low Starch High Fiber	0.960615545	1.173741828	1.49015595	2.932078009	1.500430388
6219	Second Treatment period	High Starch Low Fiber	1.070247282	1.079471408	2.1933949	2.275890054	0.522732905
6221	Second Treatment period	Low Starch High Fiber	0.998187845	1.023137138	0.751229118	2.014006065	1.675443116
6222	Second Treatment period	High Starch Low Fiber	0.878571189	1.018567051	1.222257947	3.611688226	1.378986836
6226	Second Treatment period	High Starch Low Fiber	0.633450444	0.628719879	1.38337874	1.315098361	0.758146505
6231	Second Treatment period	Low Starch High Fiber	0.910192655	1.015477321	2.018907041	1.020851085	3.385036275
6234	Second Treatment period	High Starch Low Fiber	1.019433852	1.045265266	2.533844132	1.713535622	1.331178074
6235	Second Treatment period	High Starch Low Fiber	0.41990666	0.328133893	1.013826435	1.152207553	0.800317049
6236	Second Treatment period	Low Starch High Fiber	0.529105539	0.826235958	1.261766628	1.627762439	0.918650929
6238	Second Treatment period	Low Starch High Fiber	0.656221507	0.570345247	1.361460127	1.573766549	0.277855274
6239	Second Treatment period	High Starch Low Fiber	0.652675633	0.73169066	0.994782441	1.375811274	2.248703387
6240	Second Treatment period	Low Starch High Fiber	0.718116477	0.819819778	0.817825855	1.648999069	1.792151433
6242	Second Treatment period	High Starch Low Fiber	0.639264536	0.605717089	0.584928336	1.441013187	0.638724547
6243	Second Treatment period	Low Starch High Fiber	0.606676181	0.929434877	0.72088911	2.015661296	2.674354572
6245	Second Treatment period	High Starch Low Fiber	0.61029118	0.975944494	1.060218781	2.182236199	0.293895279
6247	Second Treatment period	Low Starch High Fiber	0.913493777	0.779729295	1.958394599	2.867614193	1.725794919

Cow ID	Period	Diet	3_0_0_1_0	3_1_0_0_0	3_2_0_0_0	3_3_0_0_0	3_6_1_0_0
4221	Second Treatment period	Low Starch High Fiber	0.847041099	0.420919864	0.47913249	0.770962893	0.258603984
4403	Second Treatment period	High Starch Low Fiber	0.528405649	0.565299814	0.262038426	0.333922222	0.204358937
4668	Second Treatment period	High Starch Low Fiber	0.765381889	0.487304544	0.39777386	0.365478883	0.199636258
4889	Second Treatment period	High Starch Low Fiber	1.294193867	0.840905089	0.31268156	0.406443508	0.379833903
5002	Second Treatment period	High Starch Low Fiber	0.772720153	0.735506956	0.351105972	0.294093376	0.328623489
5007	Second Treatment period	Low Starch High Fiber	0.70960912	0.400820442	0.414996245	0.376369426	0.404710345
5046	Second Treatment period	Low Starch High Fiber	0.580866841	0.538939392	0.345326995	0.216020663	0.366500589
5249	Second Treatment period	High Starch Low Fiber	0.710946913	0.273197016	0.194793219	0.288946573	0.273271647
5282	Second Treatment period	High Starch Low Fiber	1.028541638	1.094564885	0.611498213	0.494518093	0.366054708
5298	Second Treatment period	High Starch Low Fiber	0.716145604	0.38463865	0.370174325	0.309533169	0.203502655
5417	Second Treatment period	High Starch Low Fiber	0.735940668	0.680738206	0.38694084	0.404387008	0.190320396
5439	Second Treatment period	Low Starch High Fiber	0.874939492	0.515422385	0.362172159	0.290580064	0.277109208
5455	Second Treatment period	Low Starch High Fiber	0.650316558	0.561642605	0.369738457	0.317303661	0.346245179
5472	Second Treatment period	Low Starch High Fiber	1.193707938	0.543519302	0.497416808	0.327891284	0.272052185
5473	Second Treatment period	High Starch Low Fiber	0.905934707	0.720036182	0.260427733	0.272013	0.16579632
5658	Second Treatment period	High Starch Low Fiber	0.966980003	0.93361335	0.442916194	0.221356103	0.334840026
5663	Second Treatment period	Low Starch High Fiber	0.758496619	0.420278861	0.533760362	0.40257198	0.2448776
5676	Second Treatment period	High Starch Low Fiber	0.795793773	0.715015806	0.286862216	0.230122275	0.352190688
5694	Second Treatment period	High Starch Low Fiber	0.731226445	0.432099188	0.403370112	0.214025076	0.390273466
5696	Second Treatment period	Low Starch High Fiber	0.753865915	0.4498106	0.144031146	0.239103927	0.290099735
5808	Second Treatment period	Low Starch High Fiber	0.908127868	0.696685993	0.323507362	0.278849249	0.386873926
5823	Second Treatment period	High Starch Low Fiber	0.798052027	0.695695608	0.42528048	0.204614035	0.38052694
5828	Second Treatment period	Low Starch High Fiber	0.487386698	0.571378085	0.230970398	0.162205038	0.349059996
5834	Second Treatment period	Low Starch High Fiber	1.726463768	0.695879011	0.444099246	0.301088701	0.454288713
5838	Second Treatment period	Low Starch High Fiber	1.091818334	1.072538794	0.405807787	0.345175845	0.316148726
5840	Second Treatment period	Low Starch High Fiber	0.808294363	0.505371407	0.312602458	0.235605778	0.359716728
5844	Second Treatment period	Low Starch High Fiber	1.078239779	0.548177553	0.493245938	0.221874559	0.479234634
5849	Second Treatment period	Low Starch High Fiber	1.015381416	1.125815106	0.300705257	0.325406087	0.326161803
5858	Second Treatment period	High Starch Low Fiber	0.758429761	0.708757943	0.600135721	0.300812872	0.418035086
5862	Second Treatment period	High Starch Low Fiber	1.0890149	0.803149499	0.227015089	0.200396145	0.386482417
6058	Second Treatment period	High Starch Low Fiber	0.617681917	0.981253273	0.241788175	0.18371152	0.40170487
6090	Second Treatment period	High Starch Low Fiber	0.892439375	0.366889542	0.477542433	0.248067332	0.242349541
6091	Second Treatment period	High Starch Low Fiber	0.662360586	0.504463385	0.278464857	0.169378173	0.401196178
6098	Second Treatment period	High Starch Low Fiber	0.982277525	0.468738333	0.368396469	0.218179541	0.451804404
6201	Second Treatment period	High Starch Low Fiber	0.853003398	0.399385157	0.336437712	0.373258773	0.31401684
6205	Second Treatment period	High Starch Low Fiber	0.858969582	0.617867565	0.198260693	0.270939621	0.216603303
6213	Second Treatment period	Low Starch High Fiber	0.495709192	0.653708569	0.236150593	0.253873818	0.177654584
6218	Second Treatment period	Low Starch High Fiber	1.223809546	0.824079818	0.48630732	0.437000728	0.504686806
6219	Second Treatment period	High Starch Low Fiber	1.057769987	0.604420835	0.734776924	0.391715675	0.638592249
6221	Second Treatment period	Low Starch High Fiber	0.539601605	0.449336481	0.229614979	0.155297595	0.315281324
6222	Second Treatment period	High Starch Low Fiber	1.26150309	0.886091342	2.213954322	0.281908432	0.427713737
6226	Second Treatment period	High Starch Low Fiber	0.88024027	0.514865889	0.314324166	0.236966492	0.384273785
6231	Second Treatment period	Low Starch High Fiber	0.592768669	0.22540033	0.252424376	0.339758317	0.450479985
6234	Second Treatment period	High Starch Low Fiber	0.642195509	0.261638797	0.312810014	0.657363175	0.4553827
6235	Second Treatment period	High Starch Low Fiber	0.759569293	0.446418393	0.164464508	0.383442749	0.421719603
6236	Second Treatment period	Low Starch High Fiber	1.155876076	0.738246511	0.41732153	0.257048268	0.401191941
6238	Second Treatment period	Low Starch High Fiber	0.546028311	0.239264668	0.268569724	0.378361068	0.450072677
6239	Second Treatment period	High Starch Low Fiber	0.650223768	0.368379519	0.197038635	0.297889469	0.337430005
6240	Second Treatment period	Low Starch High Fiber	0.759411655	0.522862946	0.404053457	0.257746663	0.477570882
6242	Second Treatment period	High Starch Low Fiber	0.947813909	0.625597502	0.722694785	0.383484705	0.375728872
6243	Second Treatment period	Low Starch High Fiber	0.540541061	0.7444894	0.350995625	0.246403384	0.291089315
6245	Second Treatment period	High Starch Low Fiber	0.697958949	0.444164494	0.266731674	0.354839271	0.45084563
6247	Second Treatment period	Low Starch High Fiber	1.043600054	0.452281449	0.27775192	0.423405182	0.544386861

Cow ID	Period	Diet	4_1_0_0_0	4_1_0_1_0	4_2_0_0_0 isomer 1	4_2_0_0_0 isomer 2	4_4_1_0_0
4221	Second Treatment period	Low Starch High Fiber	0.825235708	0.629878991	0.542108705	0.222225189	0.709140234
4403	Second Treatment period	High Starch Low Fiber	0.296255666	0.261164689	0.508194716	0.287139433	0.455642776
4668	Second Treatment period	High Starch Low Fiber	0.428620351	0.327105672	0.458906538	0.291225859	0.336569679
4889	Second Treatment period	High Starch Low Fiber	0.270701437	0.209030269	0.641140374	0.317522855	0.555535311
5002	Second Treatment period	High Starch Low Fiber	0.519045565	0.415336443	0.431173468	0.326055174	0.408547122
5007	Second Treatment period	Low Starch High Fiber	0.416036553	0.28839927	0.445645099	0.285638344	0.382809699
5046	Second Treatment period	Low Starch High Fiber	0.43749471	0.347477314	0.423978409	0.236981948	0.461053126
5249	Second Treatment period	High Starch Low Fiber	0.161738176	0.149317074	0.366311288	0.151544174	0.337507544
5282	Second Treatment period	High Starch Low Fiber	0.701998031	0.56397549	0.556160708	0.477970131	0.453059838
5298	Second Treatment period	High Starch Low Fiber	0.404650948	0.321882473	0.538640008	0.184187132	0.365582891
5417	Second Treatment period	High Starch Low Fiber	0.429244403	0.389130384	0.464153687	0.220292352	0.373751137
5439	Second Treatment period	Low Starch High Fiber	0.456558066	0.320814254	0.346900295	0.205258952	0.503695511
5455	Second Treatment period	Low Starch High Fiber	0.382576701	0.301147825	0.426880617	0.296745734	0.389217555
5472	Second Treatment period	Low Starch High Fiber	0.606331011	0.524220292	0.770262778	0.198471095	0.393630944
5473	Second Treatment period	High Starch Low Fiber	0.24234839	0.208146687	0.726284749	0.173924456	0.34899105
5658	Second Treatment period	High Starch Low Fiber	0.519027066	0.359827356	0.505719271	0.238854854	0.341704533
5663	Second Treatment period	Low Starch High Fiber	0.628417624	0.507693144	0.551966462	0.262368553	0.34037381
5676	Second Treatment period	High Starch Low Fiber	0.364867543	0.327006813	0.497597337	0.236198323	0.385674572
5694	Second Treatment period	High Starch Low Fiber	0.652645466	0.513646372	0.266712721	0.197833414	0.391638505
5696	Second Treatment period	Low Starch High Fiber	0.165083879	0.161856605	0.398721248	0.149276128	0.294976912
5808	Second Treatment period	Low Starch High Fiber	0.431938896	0.411669215	0.439051156	0.325494428	0.433273976
5823	Second Treatment period	High Starch Low Fiber	0.507714188	0.365716981	0.395804751	0.194796954	0.378675183
5828	Second Treatment period	Low Starch High Fiber	0.260891475	0.203768383	1.054434519	0.169129739	0.374140524
5834	Second Treatment period	Low Starch High Fiber	0.414771638	0.395433775	0.7234933	0.177850333	0.506163229
5838	Second Treatment period	Low Starch High Fiber	0.551460441	0.462520837	0.54087064	0.385573096	0.293143716
5840	Second Treatment period	Low Starch High Fiber	0.355743939	0.309826328	0.594166821	0.254662883	0.531425418
5844	Second Treatment period	Low Starch High Fiber	0.563310987	0.427348766	0.409149452	0.163730294	0.581104085
5849	Second Treatment period	Low Starch High Fiber	0.346520754	0.368060915	0.462116011	0.244566647	0.345588464
5858	Second Treatment period	High Starch Low Fiber	0.67799986	0.542832545	0.535191275	0.283687554	0.465282004
5862	Second Treatment period	High Starch Low Fiber	0.192511081	0.268780048	0.44996386	0.221414588	0.44317384
6058	Second Treatment period	High Starch Low Fiber	0.324196034	0.272462522	0.483579422	0.254865196	0.287405676
6090	Second Treatment period	High Starch Low Fiber	0.592903889	0.51599074	0.453738554	0.184847384	0.272959436
6091	Second Treatment period	High Starch Low Fiber	0.307679632	0.253780858	0.514605907	0.162539819	0.287366341
6098	Second Treatment period	High Starch Low Fiber	0.5381682	0.40197792	0.632882906	0.154310737	0.485842076
6201	Second Treatment period	High Starch Low Fiber	0.431391575	0.371575565	0.349824625	0.3591953	0.33228562
6205	Second Treatment period	High Starch Low Fiber	0.230476966	0.232196959	0.565128519	0.364029448	0.229426757
6213	Second Treatment period	Low Starch High Fiber	0.203873537	0.232944576	0.478557083	0.264714959	0.324874542
6218	Second Treatment period	Low Starch High Fiber	0.678679227	0.519920161	0.427131607	0.344618666	0.460862928
6219	Second Treatment period	High Starch Low Fiber	0.510140253	0.464011994	0.72560814	0.212568145	0.401870943
6221	Second Treatment period	Low Starch High Fiber	0.238242538	0.206743715	0.73272698	0.290562473	0.501789803
6222	Second Treatment period	High Starch Low Fiber	1.389805641	0.851983017	0.469627457	0.219703818	0.380050767
6226	Second Treatment period	High Starch Low Fiber	0.301514983	0.229831947	0.377385593	0.159434021	0.292321648
6231	Second Treatment period	Low Starch High Fiber	0.242188791	0.24500309	0.320731182	0.198916667	0.393471847
6234	Second Treatment period	High Starch Low Fiber	0.469439093	0.324012365	0.5017254	0.176219242	0.519145862
6235	Second Treatment period	High Starch Low Fiber	0.184758911	0.15920204	0.537704775	0.175296331	0.324716528
6236	Second Treatment period	Low Starch High Fiber	0.605526544	0.442403054	0.460742637	0.197963092	0.421502883
6238	Second Treatment period	Low Starch High Fiber	0.267985641	0.21783671	0.343033872	0.237017179	0.441827021
6239	Second Treatment period	High Starch Low Fiber	0.201594336	0.198050889	0.284857447	0.186514987	0.39919475
6240	Second Treatment period	Low Starch High Fiber	0.430571043	0.304938516	0.70681793	0.214838681	0.473596864
6242	Second Treatment period	High Starch Low Fiber	0.647756229	0.584550302	0.387673179	0.208726117	0.402215146
6243	Second Treatment period	Low Starch High Fiber	0.497361524	0.355073532	0.398001854	0.234120239	0.300791814
6245	Second Treatment period	High Starch Low Fiber	0.331799672	0.26598876	0.265970088	0.349725215	0.396483699
6247	Second Treatment period	Low Starch High Fiber	0.252649437	0.207853711	0.561095698	0.299631341	0.492807343

Cow ID	Period	Diet	4_5_1_0_0	5_4_0_0_0	5_4_1_0_0	8_0_0_0_0
4221	Second Treatment period	Low Starch High Fiber	0.341923194	0.458565075	0.257400615	0.140404039
4403	Second Treatment period	High Starch Low Fiber	0.302594802	0.36910862	0.485316825	0.052091805
4668	Second Treatment period	High Starch Low Fiber	0.342893888	0.417916536	0.320381442	0.107692032
4889	Second Treatment period	High Starch Low Fiber	0.417037373	0.09224607	0.561714124	0.078987258
5002	Second Treatment period	High Starch Low Fiber	0.52489022	0.291637967	0.434212886	0.098345762
5007	Second Treatment period	Low Starch High Fiber	0.396683245	0.212011393	0.411202892	0.025691766
5046	Second Treatment period	Low Starch High Fiber	0.488232246	0.29214405	0.423452533	0.054369035
5249	Second Treatment period	High Starch Low Fiber	0.26891163	0.260798683	0.376058964	0.043533187
5282	Second Treatment period	High Starch Low Fiber	0.507493032	0.276345218	0.547854988	0.106073166
5298	Second Treatment period	High Starch Low Fiber	0.332450783	0.533718507	0.345552221	0.106484514
5417	Second Treatment period	High Starch Low Fiber	0.134026013	0.481350805	0.394490342	0.052710199
5439	Second Treatment period	Low Starch High Fiber	0.323846278	0.191670481	0.495170457	0.557451233
5455	Second Treatment period	Low Starch High Fiber	0.375020405	0.184188662	0.491825249	0.030020045
5472	Second Treatment period	Low Starch High Fiber	0.293344763	0.207200578	0.393355325	0.037758409
5473	Second Treatment period	High Starch Low Fiber	0.22375955	0.514573975	0.275447191	0.070230218
5658	Second Treatment period	High Starch Low Fiber	0.337109685	0.254479147	0.319836044	0.097521071
5663	Second Treatment period	Low Starch High Fiber	0.273645238	0.235396299	0.30070585	0.159070127
5676	Second Treatment period	High Starch Low Fiber	0.46268474	0.261608069	0.423909126	0.060929878
5694	Second Treatment period	High Starch Low Fiber	0.41252052	0.157965329	0.434486185	0.036906475
5696	Second Treatment period	Low Starch High Fiber	0.332008127	0.480119831	0.319025078	0.074976705
5808	Second Treatment period	Low Starch High Fiber	0.498237185	0.2034186	0.48420446	0.051246239
5823	Second Treatment period	High Starch Low Fiber	0.401516461	0.201433506	0.39709814	0.058038026
5828	Second Treatment period	Low Starch High Fiber	0.486441065	0.187279696	0.531725337	0.074887169
5834	Second Treatment period	Low Starch High Fiber	0.488087575	0.172835425	0.523510085	0.061969237
5838	Second Treatment period	Low Starch High Fiber	0.411145404	0.602989944	0.385748758	0.138921509
5840	Second Treatment period	Low Starch High Fiber	0.422255749	0.223220578	0.488022443	0.101894966
5844	Second Treatment period	Low Starch High Fiber	0.409000152	0.209389342	0.576313089	0.040175587
5849	Second Treatment period	Low Starch High Fiber	0.41170025	0.17828987	0.382702183	0.039694701
5858	Second Treatment period	High Starch Low Fiber	0.502462664	0.272233397	0.495922526	0.07069597
5862	Second Treatment period	High Starch Low Fiber	0.564726642	0.313473328	0.519694409	0.092515191
6058	Second Treatment period	High Starch Low Fiber	0.533404422	0.223617652	0.410360296	0.024636106
6090	Second Treatment period	High Starch Low Fiber	0.367709838	0.247086997	0.35672561	0.095581393
6091	Second Treatment period	High Starch Low Fiber	0.45668479	0.262198156	0.408295074	0.108965849
6098	Second Treatment period	High Starch Low Fiber	0.796129215	1.90333224	0.626011641	0.340746021
6201	Second Treatment period	High Starch Low Fiber	0.374540177	0.489321066	0.309962238	0.082593525
6205	Second Treatment period	High Starch Low Fiber	0.27279738	0.485750618	0.182048875	0.146634823
6213	Second Treatment period	Low Starch High Fiber	0.202459554	0.445082822	0.288831946	0.05407427
6218	Second Treatment period	Low Starch High Fiber	0.621924615	0.232910551	0.454912361	0.069004016
6219	Second Treatment period	High Starch Low Fiber	0.444429563	0.261973173	0.422105071	0.056085583
6221	Second Treatment period	Low Starch High Fiber	0.296434198	0.302570395	0.376513191	0.027249832
6222	Second Treatment period	High Starch Low Fiber	0.593908908	0.231703736	0.482741387	0.063774275
6226	Second Treatment period	High Starch Low Fiber	0.35790464	0.157653139	0.512357593	0.036346561
6231	Second Treatment period	Low Starch High Fiber	0.465163813	0.251709983	0.42851473	0.108607253
6234	Second Treatment period	High Starch Low Fiber	0.627717511	0.249019043	0.361462282	0.053601545
6235	Second Treatment period	High Starch Low Fiber	0.50356087	0.151707302	0.369708739	0.03639788
6236	Second Treatment period	Low Starch High Fiber	0.673253699	0.052247605	0.43920913	0.118734624
6238	Second Treatment period	Low Starch High Fiber	0.368315245	0.123269916	0.445631342	0.021279872
6239	Second Treatment period	High Starch Low Fiber	0.369156418	0.146250007	0.391401912	0.018041819
6240	Second Treatment period	Low Starch High Fiber	0.382434736	0.297849174	0.511983086	0.112187387
6242	Second Treatment period	High Starch Low Fiber	0.484352649	0.196845998	0.540338047	0.049142504
6243	Second Treatment period	Low Starch High Fiber	0.324046272	0.242452442	0.350482304	0.049330188
6245	Second Treatment period	High Starch Low Fiber	0.46130429	0.176382618	0.465572582	0.055207676
6247	Second Treatment period	Low Starch High Fiber	0.466668344	0.21335394	0.492822721	0.040195845

REFERENCES

1. Aldredge DL, Geronimo MR, Hua S, Nwosu CC, Lebrilla CB, Barile D. Annotation and structural elucidation of bovine milk oligosaccharides and determination of novel fucosylated structures. Glycobiology 2013;23:664–676.

2. Lane JA, Mariño K, Naughton J, Kavanaugh D, Clyne M, Carrington SD, Hickey RM. Anti-infective bovine colostrum oligosaccharides: *Campylobacter jejuni* as a case study. Int J Food Microbiol 2010;157:182–188.

3. Martín-Sosa S, Martín J-J, Hueso P. The sialylated fraction of milk oligosaccharides is partially responsible for binding to Enterotoxigenic and Uropathogenic *Escherichia coli* human strains. J Nutr 2002;32:3067–3072.

4. Coppa GV, Zampini L, Galeazzi T, Facinelli B, Ferrante L, Capretti R, Orazio G. Human milk oligosaccharides inhibit the adhesion to Caco-2 cells of diarrheal pathogens: *Escherichia coli*, *Vibrio cholerae*, and *Salmonella fyris*. Ped Res 2006;59:377–382.

5. Parkkinen J, Rogers GN, Korhonen T, Dahr W, Finne J. Identification of the O-linked sialyloligosaccharides of glycophorin A as the erythrocyte receptors for S-fimbriated *Escherichia coli*. Infect Immun 1986;54:37–42.

6. Marotta M, Ryan JT, Hickey RM. The predominant milk oligosaccharide 6′-sialyllactose reduces the internalisation of *Pseudomonas aeruginosa* in human pneumocytes. J Funct Foods 2014;6:367–373.

7. Perdijk O, van Baarlen P, Fernandez-Gutierrez MM, van den Brink E, Schuren FHJ, Brugman S, Savelkoul HFJ, Kleerebezem M, van Neerven RJJ. Sialyllactose and galactooligosaccharides promote epithelial barrier functioning and distinctly modulate microbiota composition and short chain fatty acid production *in vitro*. Front Immunol 2019;10:94.

8. Boudry G, Hamilton MK, Chichlowski M, Wickramasinghe S, Barile D, Kalanetra KM, Mills DA, Raybould HE. Bovine milk oligosaccharides decrease gut permeability and improve inflammation and microbial dysbiosis in diet-induced obese mice. J Dairy Sci 2017;100: 2471–2481.

9. Charbonneau MR, O’Donnell D, Blanton LV, Totten SM, Davis JCC, Barratt MJ, Cheng J, Guruge J, Talcott M, Bain JR, et al. Sialylated milk oligosaccharides promote microbiota-dependent growth in models of infant undernutrition. Cell 2016;164:859–871.

10. Obelitz-Ryom K, Bering SB, Overgaard SH, Eskildsen SF, Ringgaard S, Olesen JL, Skovgaard K, Pankratova S, Wang B, Brunse A, et al. Bovine milk oligosaccharides with sialyllactose improves cognition in preterm pigs. Nutrients 2019;11:1335.

11. Oliveros E, Vázquez E, Barranco A, Ramírez M, Gruart A, Delgado-García J, Buck R, Rueda R, Martín M. Sialic acid and sialylated oligosaccharide supplementation during lactation improves learning and memory in rats. Nutrients 2018;10:1519.

12. Jacobi SK, Yatsunenko T, Li D, Dasgupta S, Yu RK, Berg BM, Chichlowski M, Odle J. Dietary isomers of sialyllactose increase ganglioside sialic acid concentrations in the corpus callosum and cerebellum and modulate the colonic microbiota of formula-fed piglets. J Nutr 2016;146:200–208.

13. Jakobsen LMA, Sundekilde UK, Andersen HJ, Nielsen DS, Bertram HC. Lactose and bovine milk oligosaccharides synergistically stimulate *B. longum subsp. longum* growth in a simplified model of the infant gut microbiome. J Prot Res 2019;18:3086–3098.

14. Marsaux B, Van den Abbeele P, Ghyselinck J, Prioult G, Marzorati M, Bogićević B. Synbiotic effect of *Bifidobacterium lactis* CNCM I-3446 and bovine milk-derived oligosaccharides on infant gut microbiota. Nutrients 2020;12:2268.

15. Jena PK, Sheng L, Nagar N, Wu C, Barile D, Mills DA, Wan Y-JY. Synbiotics Bifidobacterium infantis and milk oligosaccharides are effective in reversing cancer-prone nonalcoholic steatohepatitis using western diet-fed FXR knockout mouse models. J Nutr Biochem 2018;57:246–254.

16. Austin S, De Castro CA, Sprenger N, Binia A, Affolter M, Garcia-Rodenas CL, Beauport L, Tolsa J-F, Fumeaux CJF. Human milk oligosaccharides in the milk of mothers delivering term versus preterm infants. Nutrients 2019;11:1282.

17. Samuel TM, Binia A, de Castro CA, Thakkar SK, Billeaud C, Agosti M, Al-Jashi I, Costeira MJ, Marchini G, Martínez-Costa C, et al. Impact of maternal characteristics on human milk oligosaccharide composition over the first 4 months of lactation in a cohort of healthy European mothers. Sci Rep 2019;9,:11767.

18. Gabrielli O, Zampini L, Galeazzi T, Padella L, Santoro L, Peila C, Giuliani F, Bertino E, Fabris C, Coppa GV. Preterm milk oligosaccharides during the first month of lactation. Pediatrics 2011;128:e1520–e1531.

19. Thurl S, Munzert M, Henker J, Boehm G, Müller-Werner B, Jelinek J, Stahl B. Variation of human milk oligosaccharides in relation to milk groups and lactational periods. Br J Nutr 2010;104:1261–1271.

20. Fischer-Tlustos AJ, Hertogs K, van Niekerk JK, Nagorske M, Haines DM, Steele MA. Oligosaccharide concentrations in colostrum, transition milk, and mature milk of primi- and multiparous Holstein cows during the first week of lactation. J Dairy Sci 2020:103:3683–3695.

21. Nakamura T, Kawase H, Kimura K, Watanabe Y, Ohtani M, Arai I, Urashima T. Concentrations of sialyloligosaccharides in bovine colostrum and milk during the prepartum and early lactation. J Dairy Sci 2003;86:1315–1320.

22. Sunds AV, Bunyatratchata A, Robinson R, Glantz M, Paulsson M, Leskauskaite D, Pihlanto A, Inglingstad R, Devold TG, Vegarud, et al. Comparison of bovine milk oligosaccharides in native North European cattle breeds. Int Dairy J 2021;114:104917.

23. Robinson RC, Poulsen NA, Colet E, Duchene C, Larsen LB, Barile D. Profiling of aminoxyTMT-labeled bovine milk oligosaccharides reveals substantial variation in oligosaccharide abundance between dairy cattle breeds. Scientific Reports 2019;9:5465.

24. Sischo WM, Short DM, Geissler M, Bunyatratchata A, Barile D. Comparative composition, diversity, and abundance of oligosaccharides in early lactation milk from commercial dairy and beef cows. J Dairy Sci 2017;100:3883–3892.

25. Sundekilde U, Downey E, O'Mahony J, O'Shea C-A, Ryan C, Kelly A, Bertram H. The effect of gestational and lactational age on the human milk metabolome. Nutrients 2016;8:304.

26. McJarrow P, van Amelsfort-Schoonbeek J. Bovine sialyl oligosaccharides: seasonal variations in their concentrations in milk, and a comparison of the colostrums of Jersey and Friesian cows. Int Dairy J 2004;14:571–579.

27. Sánchez-Duarte JI, Kalscheur KF, Casper DP, García AD. Performance of dairy cows fed diets formulated at 2 starch concentrations with either canola meal or soybean meal as the protein supplement. J Dairy Sci 2019;102:7970–7979.

28. Ranathunga SD, Abdelqader MM, Kalscheur KF. Nutrient digestion by dairy cows fed diets replacing starch with non- forage fiber. In: Oltjen, J.W., Kebreab, E., & Lapierre, H. (Eds.), Energy and Protein Metabolism and Nutrition in Sustainable Animal Production. (pp. 61-63). Wageningen, NL: Wageningen Academic Publishers, 2013.

29. Cant JP, DePeters EJ, Baldwin RL. Effect of dietary fat and postruminal casein administration on milk composition of lactating dairy cows. J Dairy Sci 1991;74:211–219.

30. Thomson DJ, Beever DE, Haines MJ, Cammell SB, Evans RT, Dhanoa MS, Austin AR. Yield and composition of milk from Friesian cows grazing either perennial ryegrass or white clover in early lactation. J Dairy Res 1985;52:17–31.

31. Xue F, Sun F, Jiang L, Hua D, Wang Y, Nan X, Zhao Y, Xiong B. Effects of partial replacment of dietary forage using kelp powder (*Thallus laminariae*) on ruminal fermentation and lactation performances of dairy cows. *Animals* 2019;9:852.

32. Miron J, Zuckerman E, Adin G, Solomon R, Shoshani E, Nikbachat M, Yosef E, Zenou A, Weinberg ZG, Chen Y, et al. Comparison of two forage sorghum varieties with corn and the effect of feeding their silages on eating behavior and lactation performance of dairy cows. Anim Feed Sci Technol 2007;139:23–39.

33. Carroll SM, DePeters EJ, Taylor SJ, Rosenberg M, Perez-Monti H, Capps VA. Milk composition of Holstein, Jersey, and Brown Swiss cows in response to increasing levels of dietary fat. Anim Feed Sci Tech 2009;131z:451–473.

34. Miron J, Yosef E, Maltz E, Halachmi I. Soybean hulls as a replacement of forage neutral detergent fiber in total mixed rations of lactating cows. Anim Feed Sci Technol 2003;106:20–28.

35. Jahreis G, Fritsche J, Steinhart H. Conjugated linoleic acid in milk fat: high variation depending on production system. Nutr Res 1997;17:1479–1484.

36. Spain JN, Alvarado MD, Polan CE, Miller CN, McGilliard ML. Effect of protein source and energy on milk composition in midlactation dairy cows. J Dairy Sci 1990;73:445–452.

37. Asakuma S, Ueda Y, Akiyama F, Uemura Y, Miyaji M, Nakamura M, Murai M, Urashima T. Effect of grazing on the concentrations of total sialic acid and hexose in bovine milk. J of Dairy Sci 2010;93:4850–4854.

38. Cheng LK, Wang LX, Xu QS, Huang LJ, Zhou DS, Li Z, Li SG, Du YG, Yin H. Chitooligosaccharide supplementation improves the reproductive performance and milk composition of sows. Livestock Sci 2015;174:74–81.

39. Aguinis H, Gottfredson RK, Joo H. Best-practice recommendations for defining, identifying, and handling outliers. Organ Res Methods 2013;16:270-301.

40. Robinson RC, Colet E, Tian T, Poulsen NA, Barile D. An improved method for the purification of milk oligosaccharides by graphitised carbon-solid phase extraction. Int Dairy J 2018;80, 62–68.

41. Robinson RC, Poulsen NA, Barile D. Multiplexed bovine milk oligosaccharide analysis with aminoxy tandem mass tags. PLoS One 2018;13:e0196513.

42. Mehra R, Barile D, Marotta M, Lebrilla CB, Chu C, German JB. Novel high-molecular weight Fucosylated milk oligosaccharides identified in dairy streams. PLoS One 2014;9:e96040.

43. Barile D, Marotta M, Chu C, Mehra R, Grimm R, Lebrilla CB, German JB. Neutral and acidic oligosaccharides in Holstein-Friesian colostrum during the first 3 days of lactation measured by high performance liquid chromatography on a microfluidic chip and time-of-flight mass spectrometry. J Dairy Sci 2010;93:3940–3949.

44. Tao N, DePeters EJ, German JB, Grimm R, Lebrilla CB. Variations in bovine milk oligosaccharides during early and middle lactation stages analyzed by high-performance liquid chromatography-chip/mass spectrometry. J Dairy Sci 2009;92:2991–3001.

45. Tao N, DePeters EJ, Freeman S, German JB, Grimm R, Lebrilla CB. Bovine milk glycome. J Dairy Sci 2008;91:3768–3778.

46. Meitei NS, Apte A, Snovida SI, Rogers JC, Saba J. Automating mass spectrometry-based quantitative glycomics using aminoxy tandem mass tag reagents with SimGlycan. J Proteomics 2015;127:211–222.

47. Wei T, Simko V, Levy M, Xie Y, Jin Y, Zemla J, Freidank M, Cai J, Protivinsky T. Corrplot: Visualization of a correlation matrix. R Package Version 0.90. 2021.

48. Vicaretti SD, Mohtarudin NA, Garner AM, Zandberg WF. Capillary electrophoresis analysis of bovine milk oligosaccharides permits an assessment of the influence of diet and the discovery of nine abundant sulfated analogues. J Agric Food Chem 2018;66:8574–8583.

49. Liu Z, Moate P, Cocks B, Rochfort S. Simple liquid chromatography–mass spectrometry method for quantification of major free oligosaccharides in bovine milk. J Agric Food Chem 2014;62:11568–11574.

50. Lemay DG, Pollard KS, Martin WF, Freeman-Zadrowski C, Hernandez J, Korf I, German JB, Rijnkels M. From genes to milk: Genomic organization and epigenetic regulation of the mammary transcriptome. PLoS One 2013;8:e75030.

51. Rijnkels M, Freeman-Zadrowski C, Hernandez J, Potluri V, Wang L, Li W, Lemay DG. Epigenetic modifications unlock the milk protein gene loci during mouse mammary gland development and differentiation. PLoS One 2013;8:e53270.

52. Weinborn V, Li Y, Shah IM, Yu H, Dallas DC, German JB, Mills DA, Chen X, Barile D. Production of functional mimics of human milk oligosaccharides by enzymatic glycosylation of bovine milk oligosaccharides. Int Dairy J 2020;102:104583.

53. Fong B, Ma K, McJarrow P. Quantification of bovine milk oligosaccharides using liquid chromatography–selected reaction monitoring–mass spectrometry. J Agric Food Chem 2010;59:9788–9795.

54. Martin M-J, Martin-Sosa S, Garcia-Pardo L-A, Hueso P. Distribution of bovine milk sialoglycoconjgates during lactation. J Dairy Sci 2001;84:995-1000.

55. Poulsen NA, Robinson RC, Barile D, Larson LB, Buitenhuis B. A genome-wide association study reveals specific transferases as candidate loci for bovine milk oligosaccharide synthesis. BMC Genomics 2019;20:404.

CHAPTER IV:

Delactosed Permeate as a Source for Extracting Oligosaccharides: Compositional Variation and Processing Strategies

ABSTRACT

Bovine milk contains an array of naturally-occurring bioactive compounds including oligosaccharides, peptides, and organic acids; however, isolation of these bioactive compounds from traditional dairy streams is challenged by their low concentrations. Delactosed permeate, the dairy side stream resulting from protein isolation by ultrafiltration and lactose crystallization of the ultrafiltration permeate, is a promising, more concentrated, alternative source of these bioactive peptides, oligosaccharides, and organic acids. Although these bioactive compounds are known to be present in delactosed permeate, the specific details of their individual compound identities and abundances remain largely unknown. In addition, the lack of a standard of composition for delactosed permeate and the many potential variations in the processing steps leading to its formation cause the potential for wide variations in the make-up of this stream. In this study, the composition of 10 commercial delactosed permeate batches from two production facilities were analyzed and their macronutrient, mineral, B vitamin, and organic acid contents were determined, in addition to peptidomic and glycomic profiling. Significant variations were found between delactosed permeates from the two production sites, as well as substantial variation between batches within a production facility. These findings highlight the extensive compositional variation resulting from differences in delactosed permeate starting materials and the cumulative effects of differences in the preceding processing steps, and they underscore the need for further research into the bioactive potential of delactosed permeate.

INTRODUCTION

Delactosed permeate is the coproduct of lactose production for food and pharmaceutical applications. Milk permeate or whey permeate remaining from cheese making and whey protein isolation is concentrated using a combination of evaporation and membrane filtration to produce a supersaturated solution with a wet basis total solids content of 60 to 65% and lactose concentration of 40 to 55%. This solution is then seeded with crystalline lactose to initiate lactose crystallization. (Wong and Hartel, 2014; Bund and Hartel, 2013) The mother liquor remaining after recovery of the newly crystallized lactose is known as delactosed permeate.

Lactose production in the United States doubled from 614 million pounds to 1.23 billion pounds between 2005 and 2019, (USDA, 2019) resulting in a parallel increase in delactosed permeate production. However, feasible productive applications for delactosed permeate are still lacking. The main current outlets for delactosed permeate are as animal feed or wastewater, but because of the high mineral content and biological oxygen demand of delactosed permeate, additional pre-treatment is still often required. (Slavov, 2017) While simple and reliable outlets for delactosed permeate are important in the short term, the inherent biological value of this stream deserves more creative solutions to harness the health benefits of the underutilized bioactive compounds it contains.

As the final coproduct of multiple product manufacturing ad isolation processes, delactosed permeate has a highly variable composition. It’s final make-up is dependent on factors including the type of cheese produced, how the whey was treated, the manufacturing processes implemented for whey protein and lactose isolation, and the extent of lactose conversion to lactic acid during storage. Due to its variable nature, there is not yet a standard of identity for delactosed permeate. While several major delactosed permeate components, including crude protein, lactose, lactic acid, citric acid, and major minerals have been fairly well documented in

the literature, (Burrington et al., 2014; Frankowski et al., 2014; Friend et al., 2004; Levin et al., 2016; Liang et al., 2009; Smith et al., 2016; Wagner et al., 2014) other components like peptides, additional organic acids, and bovine milk oligosaccharides (BMOs), which are known to be present in delactosed permeate, have received minimal compositional investigation.
Although most of the protein content from the starting milk or whey is removed during ultrafiltration, low molecular weight compounds like peptides remain in delactosed permeate. (Dallas et al., 2014b) Hundreds of naturally occurring peptides have been identified in bovine milk, (Dallas et al., 2014a; Guerrero et al., 2015) and additional peptide structures resulting from protein or larger peptide degradation may also be created during the processing treatments leading up to the production of delactosed permeate. These peptides are generally composed of 2 to 50 amino acid residues, and many are homologous to known anti-hypertensive (Cicero et al., 2011; Jauhiainen et al., 2012) immunomodulatory, (Gill et al., 2000) antioxidant, (El- Nawawy et al., 2012; Sah et al., 2018) and antimicrobial (Sah et al., 2018) peptides. These bioactive peptides are of interest for potential applications based on their antimicrobial-, immunomodulatory-, gastrointestinal-, and cardiovascular-related activities. (Auestad and Layman, 2021; Miralles et al., 2018; Nongonierma and FitzGerald, 2015)

Lactic acid and citric acid have been routinely identified in delactosed permeate, but other organic acids are relatively uncharacterized in this stream. Uric acid is of particular interest because of its antioxidant activity, which is believed to promote the oxidative stability of dairy products, helping prevent peroxidase-induced oxidation of peptides and other oxidation-sensitive bioactives. (Østdal et al., 2000)

Delactosed permeate also contains a substantial quantity of the BMOs originally present in the starting milk. BMOs are a class of carbohydrates composed of between 3 and 11 monosaccharide subunits connected by glycosidic linkages, which are present in cows' milk. BMO structures are based off of either a lactose (galactose(β1-4)glucose) or lactosamine (galactose(β1-4)*N*-acetylglucosamine) reducing end, which is expanded through the addition of further galactose (Gal), *N*-acetylglucosamine (GlcNAc), or *N*-acetylgalactosamine (GalNAc) units and decorated with α2-3- or α2-6-linked *N*-acetylneuraminic acid (Neu5Ac) or *N*-glycolylneuraminic acid (Neu5Gc) or, less commonly, α1-2- or α1-3-linked fucose (Fuc). (Aldredge et al., 2013) BMOs are of increasing recent interest due to their numerous demonstrated health and development benefits that bear particular relevance for human infants. BMOs have been shown to contribute to improved gut barrier function *in vitro* (Perdijk et al., 2019), as well as decreased gut permeability, increased lean body mass, and healthy organ growth in animal models of infant undernutrition. (Boudry et al., 2017; Charbonneau et al., 2016) BMOs also exhibit anti-adhesive and anti-pathogen activity against major enteric pathogens including enterotoxigenic *Escherichia coli* (Martín-Sosa et al., 2002) and *Campylobacter jejuni*. (Lane et al., 2012) Of particular interest are 3'-sialyllactose (3'-SL) and 6'-sialyllactose (6'-SL), two of the most abundant oligosaccharides in bovine milk, which have also been shown to exhibit anti-pathogenic effects against enteropathogenic and S fimbriated *E. coli*, (Coppa et al., 2006; Parkkinen et al., 1986) *Salmonella enterica* ssp. *enterica* ser. fyris, (Coppa et al., 2006) and *Pseudomonas aeruginosa*. (Marotta et al., 2014) Sialic acid and sialylated milk oligosaccharides including 3'-SL and 6'-SL have also been linked with upregulated genes for myelination and ganglioside synthesis in the hippocampus, increased sialylation of cerebellum gangliosides, and improved learning outcomes in animal models. (Jacobi et al., 2016; Obelitz-Ryom et al., 2019; Oliveros et al., 2018) 25

BMOs, including six high molecular weight, fucosylated compounds have been identified in delactosed permeate, (Mehra et al., 2014) but little is known about their concentrations and whether BMO profiles vary with delactosed permeate production processes.

One of the main challenges limiting the harnessing of bioactive compounds in delactosed permeate is the high mineral content of this stream, with delactosed permeate often featuring ash levels of 12 to 26% on a dry basis. (Burrington et al., 2014; Frankowski et al., 2014; Friend et al., 2004; Levin et al., 2016) Before delactosed permeate can be applied in *in vitro* or *in vivo* studies testing its bioactivity, salt levels must be reduced to prevent the impact of the bioactive compounds from being overshadowed by the exceptionally high salt concentrations. Applications of delactosed permeate that require it to be dried are also complicated by the high mineral content which interferes with drying delactosed permeate as-is to a stable, free-flowing powder, due to its hygroscopic and syrupy nature, (Bund and Hartel, 2010; Liang et al., 2009) creating a further need for its demineralization.

In this study, detailed compositional analysis of delactosed permeate samples from multiple production lots was conducted, including peptidomic, organic acid, and BMO profiling, to gain insight into the full composition of this stream and to determine the degree of variation in these components across multiple production sites and batches. In addition, a pilot batch of delactosed permeate was demineralized to determine the extent of potential mineral removal and the impact of this additional processing on key bioactive compounds, including BMOs.

MATERIALS AND METHODS

Delactosed Permeate Sourcing

Delactosed permeate samples were collected from production batches across two manufacturing sites (5 samples per location) belonging to Milk Specialties Global. All samples were stored at -20°C until the time of analysis. Thawed samples were heated to 40°C and inverted to redissolve precipitated solids prior to all extractions. All extractions and analyses were conducted in duplicate.

Proximate Analyses

Proximate analyses were conducted by Milk Specialties Global (Eden Prairie, MN). Protein content of the delactosed permeates was evaluated using IDF 185:2002 (LECO). Total lipids were measured through the Mojonnier method (AOAC 989.05). The concentrations of simple sugars including lactose, glucose, and galactose were determined using AOAC 977.20 and AOAC 979.06. Ash content was measured with AOAC 930.30.

Protein content was additionally analyzed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Each gel lane was normalized to 25µg of protein and combined with 0.5 equivalent volumes of 4x Laemmli sample buffer and 0.5 equivalent volumes of 0.2M dithiothreitol. Sample mixtures were incubated at 95ºC for 5 minutes, and then loaded onto a 4-15% acrylamide gel. The gel was run at 116V for 40 minutes. Precision Plus Protein Standard (Bio-Rad, Hercules, CA) was used as a positive control.

Organic Acids

Major organic acids were analyzed by Eurofins (Food Integrity Innovation – Madison, WI). Citric, lactic, and acetic acid concentrations were determined using AOAC method 986.13.

In addition, uric acid content was determined by ThermoFisher Scientific (Sunnyvale, CA) using high-performance anion-exchange chromatography with UV detection (HPAEC-UV) at 295nm on a Dionex ICS 5000+ HPAEC system. Chromatographic separation was achieved using 50mM KOH with a flow rate of 0.3mL/min with an IonPac AS11-HC4µm column (3 x 250mm, ThermoFischer Scientific, Sunnyvale, CA) at 30ºC.

Micronutrients

Vitamins

B vitamin analysis was conducted by Medallion Labs (Minneapolis, MN). Riboflavin (B2) was quantified using AOAC methods 942.23, 970.65, and 981.15. Pantothenic acid (B5) analysis was conducted following AOAC methods 945.74, 960.46, and 992.07. Cobalamin (B12) quantification was carried out through AOAC methods 952.20 and 986.23.

Minerals

The mineral content of the delactosed permeates was analyzed by Milk Specialties Global (Eden Prairie, MN). Calcium, magnesium, and phosphorous contents were measured using AOAC 985.01. Sodium analysis was conducted using AOAC 985.02.

Bovine Milk Oligosaccharides

Oligosaccharide Profiling

BMOs in the delactosed permeate samples were purified by microplate C18 solid phase extraction (SPE; Glygen, Columbia, MD) and microplate graphitized carbon SPE (Glygen) prior to analysis by mass spectrometry. Each C18 SPE well was activated with acetonitrile and equilibrated with nanopure water. Samples were loaded, and each C18 well was washed with 3 column volumes (600µL total) nanopure water. All eluate from the sample loading and washing steps was collected and purified by graphitized carbon SPE. Graphitized carbon SPE wells were activated with 80% acetonitrile/0.1% trifluoroacetic acid (TFA) and equilibrated with water. The samples were loaded, and each well was washed with 6 column volumes (1.2mL total) nanopure water. BMOs were eluted with 3 column volumes (600µL total) 40% acetonitrile/0.1% TFA. The samples were dried and re-dissolved in nanopure water prior to mass spectrometry analysis. Samples were analyzed on an Agilent 6520 nano-liquid chromatography chip quadrupole time-of-flight mass spectrometry (nano-LC-chip-Q-ToF MS) system (Agilent Technologies, Santa Clara, CA). Chromatographic separation was performed on a porous graphitized carbon nano-LC chip, consisting of a 40nL enrichment column and a 75µm x 43mm analytical column with 5µm particles (Agilent Technologies). Instrumental parameters for chromatographic separation and BMO analysis by MS have been described previously. (Sunds et al., 2021)

The monosaccharide composition of each BMO was determined by examination of the MS/MS spectra. Relative abundances of a selection of these BMOs were calculated with Profinder B.08.00 software (Agilent Technologies). Precursor ions of the BMOs were identified from the MS-level data with an error tolerance of 15 ppm, and the chromatographic area of each BMO was calculated.

Oligosaccharide Quantification

BMOs in the delactosed permeate samples were purified by microplate C18 SPE, as described above, prior to analysis by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD).

3'-SL and 6'-SL were quantified on a Dionex ICS 5000+ HPAEC-PAD system outfitted with dual pumps and a detector consisting of an electrochemical cell with a disposable gold working electrode and a pH-Ag/AgCl reference electrode (ThermoFischer Scientific, Sunnyvale, CA). Chromatographic eluents consisted of nanopure water (A), 200mM sodium hydroxide (B), and 100mM sodium acetate in 100mM sodium hydroxide. Chromatographic separation was carried out with a CarboPac PA200 guard column (3 x 50mm, ThermoFisher Scientific) and CarboPac PA200 analytical column (3 x 250mm, ThermoFisher Scientific) with a pump flow rate of 0.5mL/min. The gradient was held constant at 8% B and 9% C for 60min. Column and detector temperatures were set to 25°C.

Naturally Occurring Peptides

Peptidomic Profiling

Delactosed permeate samples were acidified by mixing 1:1 with 0.2% TFA. Peptides were purified by column C18 SPE prior to analysis by mass spectrometry. Each C18 SPE cartridge (Supelco, Bellefonte, PA) was activated with acetonitrile and equilibrated with 0.1% TFA. Samples were loaded, and each C18 cartridge was washed with 3 column volumes (6mL total)

0.1% TFA. Peptides were eluted with 3 column volumes (6mL total) 80% acetonitrile/0.1% TFA. The samples were dried and re-dissolved in 2% acetonitrile for mass spectrometry analysis. Peptidomics samples were analyzed by nano-LC-chip-Q-ToF MS. Chromatographic separation was performed on a C18 nano-LC chip, consisting of a 40 nL enrichment column and a 75 µm x 150 mm analytical column, each with 5 µm Zorbax C18 particles (Agilent Technologies, Santa Clara, CA). Mobile phase solvents consisted of 3% acetonitrile/0.1% formic acid (A) and 89.9% acetonitrile/0.1% formic acid (B). Samples were loaded onto the enrichment column by a capillary pump operating at a flow rate of 4.0µL/min at 100% A. Separation was performed on the analytical column by a nanopump operating at 0.3µL/min. The gradient was ramped from 0 to 30% B from 0 to 40min, 30 to 45% B from 40 to 45min, 45 to 100% from 45 to 45.1min, then held at 100% B from 45.1 to 50min, and re-equilibrated at 100% A from 50.01 to 65min. Upon eluting from the column, peptides were analyzed in positive ionization mode with scan ranges of m/z 130-1400 (MS) and 50-1700 (MS/MS), collected at a rate of 8 spectrum/s. Drying gas flow was 5L/min at 350°C. Capillary voltage was 1875V. In each MS scan, the eight most abundant ions were selected for MS/MS fragmentation, with a dynamic exclusion of 0.30min subsequently applied to each fragmented ion. Collision energies were specified with the linear equation *collision energy = ((m/z)/100)*slope + offset*, with slope and offset values of 3 and 2, respectively. In-run calibration was performed with infused calibrant ions of m/z 322.048121 and 922.009798. Data was stored in centroid mode.

PEAKS Studio X Pro (Bioinformatics Solutions Inc., Waterloo, ON, Canada) was used for analyzing LC-MS/MS data for peptide identification. Peptides containing at least five amino acid residues were identified through database search using *Bos taurus* (bovine) protein sequences in the Swiss-Prot database. (https://www.uniprot.org/, Accessed 12/19/2020) Enzyme and digestion mode were set as “none” and “unspecific,” respectively. Variable modifications allowed included deamidation (+0.98; N and Q), phosphorylation (+79.97; S, T, and Y), and oxidation (+15.99; M). Mass error tolerance was set at 20 ppm and 0.02 Da for precursors and fragments, respectively. Peptide-spectrum matches were filtered at 1% false-discovery rate (FDR) to get the final peptide identifications.

Peptide sequences found in the samples were searched against Milk Bioactive Peptide Database (http://mbpdb.nws.oregonstate.edu/, Accessed 12/19/2020) after removing modifications for annotating potential bioactivities. Peptide sequences with 100% match with the bioactive peptides in the database were reported.

Peptide Measurement

Proteins were removed from diluted samples via ethanol precipitation. Two equivalents of cold ethanol were added to each sample. Samples were held at -30°C for 1 hour and then centrifuged at 4000*xg* for 30min. The resulting supernatants were dried and reconstituted in nanopure water. The approximate peptide content of each sample was determined using a Qubit Fluorometer (ThermoFisher Scientific, Waltham, MA). Reconstituted supernatants from ethanol precipitation were mixed with a buffered solution of Qubit fluorescent dye, incubated, and analyzed according to the manufacturer’s instructions.

Demineralization

A 16.65L pilot batch of delactosed permeate produced at plant 1, separate from the 5 un-demineralized delactosed permeate batches analyzed from this site, was demineralized using electrodialysis by Ameridia (Napa, CA) in a series of 9 test demineralization batches. The electrodialysis stack employed ten cationic and anionic membranes with an average production rate of 1.03L/h at 26ºC and a potassium nitrate diluate maintained at approximately 20mS/cm via the addition of demineralized water.

Statistical Analysis

Within versus between group variation was assessed using 1-way ANOVA with post hoc evaluation using Tukey's Test. All statistical analyses were conducted using R version 4.0.2.

RESULTS AND DISCUSSION

Proximate Analyses

Delactosed permeate composition was variable between production batches and processing plants but comparable to previously reported values. (Burrington et al., 2014; Frankowski et al., 2014; Friend et al., 2004; Liang et al., 2009; Smith et al., 2016; Wagner et al., 2014) All samples contained less than 0.5% fat and 5.2% protein (Table 4.1). The significantly higher ($p<0.01$) levels of protein in samples from production plant 2 are likely the result of greater incorporation of milk permeate in the starting material at this site compared to the entirely whey permeate starting material used at production site 1, and higher protein content in the permeate starting material due to a protein leak in the permeate supplier's ultrafiltration process for production plant 2. The significantly higher ($p<0.001$) total solids content in the delactosed permeate samples from production plant 2 is likely due to a combination of this less precise ultrafiltration and a lower lactose recovery during crystallization.

Table 4.1. Proximate analyses of delactosed permeate batches from both production plants. Values are expressed as the mean ± standard deviation. Significant differences within a row are indicated as * $0.01 < p \leq 0.05$, **$0.001 < p \leq 0.01$, *** $p < 0.001$

	Production Plant 1	**Production Plant 2**	
Total Solids (g/100g)	25.93 ± 2.04	40.76 ± 0.48	***
Ash (g/100g)	4.82 ± 0.19	10.21 ± 0.17	***
Protein (g/100g)	2.04 ± 0.12	4.96 ± 0.12	***
Fat (g/100g)	0.17 ± 0.04	0.33 ± 0.11	*

Proteins

Protein profiles of the delactosed permeates, with the gel loadings normalized to 25μg of protein each, also differed between the two production plants, as shown in Figure 4.1. Delactosed permeates from both plants contained proteins with masses corresponding to β-lactoglobulin (18.4kDa) and caseins (19-32kDa). (Zhang et al., 2022) Although the delactosed permeates from production plant 1 have a lower total protein content, they also appear to have a higher relative content of proteins with molecular weights greater than 25kDa. Other proteins commonly found in milk, including α-lactalbumin (14kDa), bovine serum albumin (66.4kDa), and lactoferrin (77-80kDa) do not appear to contribute substantially to the protein profiles of any of the delactosed permeates. The darkened portion of the gel below the 10kDa mark for lanes 11 and 12, as seen in Figure 4.1, is the result of residual stain that was not fully removed during the destaining process, not very low-mass protein or high-mass peptide content, as confirmed by additional SDS-PAGE analyses of the two corresponding delactosed permeate samples.

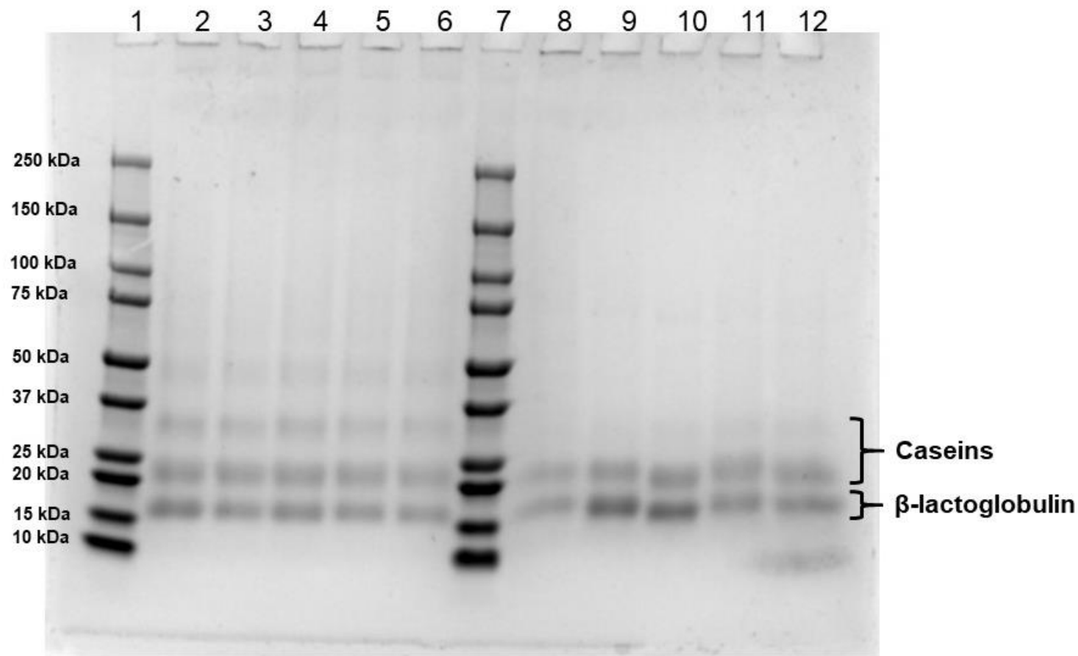


Figure 4.1. SDS-PAGE analysis of delactosed permeates. Lanes: (1) protein standards, (2) production plant 1 batch A, (3) production plant 2 batch B, (4) production plant 1 batch C, (5) production plant 1 batch D, (6) production plant 1 batch E, (7) protein standards, (8) production plant 2 batch A, (9) production plant 2 batch B, (10) production plant 2 batch C, (11) production plant 2 batch D, (12) production plant 2 batch E. Each gel lane was normalized to 25μg of protein.

Vitamins and Minerals

Three B vitamins, riboflavin (B2), pantothenic acid (B5), and cobalamin (B12) were measured in the delactosed permeates. Vitamins B2 and B5 were present at significantly higher ($p<0.001$) concentrations in delactosed permeate batches from production plant 2 (Figure 4.2). There was no significant difference ($p>0.05$) in the concentration of vitamin B12 between production plants, but there was substantially more variation in vitamin B12 concentration between batches from plant 1 compared to plant 2, as shown in Figure 4.2.

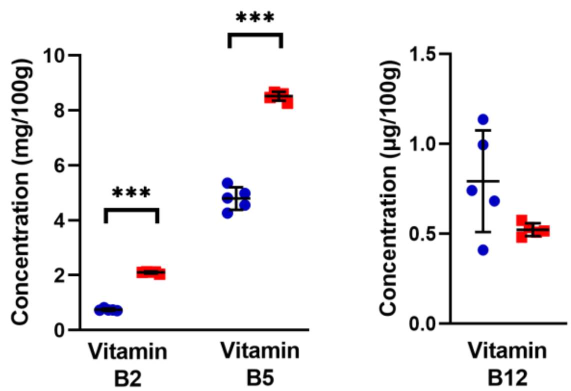


Figure 4.2. B vitamin concentrations in delactosed permeate batches produced at production plant 1 (●) and production plant 2 (■). * 0.01 < p ≤ 0.05, **0.001 < p ≤ 0.01, *** p < 0.001

Sodium was the most abundant mineral, followed by potassium (Figure 4.3). All measured minerals were present at significantly higher concentrations (p<0.001) in the delactosed permeate produced at plant 2, as a result of more substantial pH adjustments carried out on the starting material at this facility.

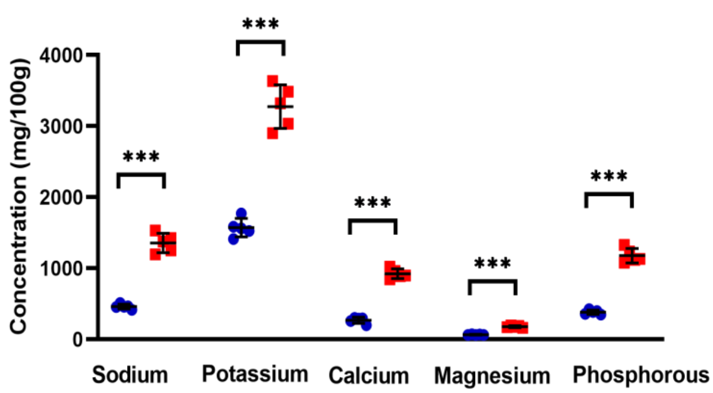


Figure 4.3. Mineral concentrations in delactosed permeate batches produced at production plant 1 (●) and production plant 2 (■). * 0.01 < p ≤ 0.05, **0.001 < p ≤ 0.01, *** p < 0.001

Organic Acids

Citric acid was the most abundant organic acid delactosed permeate samples, particularly for delactosed permeates produced at plant 2 (Figure 4.4), which is consistent with previous reports of delactosed permeated organic acid content. (Frankowski et al., 2014; Friend et al., 2004; Liang et al., 2009) Acetic acid was only present in trace amounts (<400ppm) across all ten delactosed permeate batches. Lactic acid was one of the only measured compounds that did not differ significantly (p>0.05) in concentration between production plants (Figure 4.4). The comparable levels of lactic acid across all analyzed delactosed permeate batches suggest minimal variability in the degree of microbial degradation of lactose into lactic acid during prior production processes as well as during storage of the milk and whey permeate starting materials and delactosed permeate batches during processing.

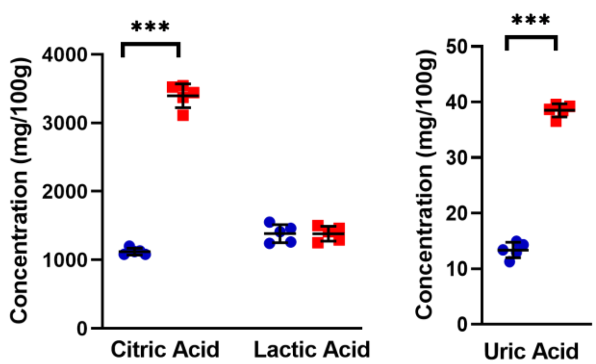


Figure 4.4. Organic acid concentrations in delactosed permeate batches produced at production plant 1 (●) and production plant 2 (■). * $0.01 < p \leq 0.05$, **$0.001 < p \leq 0.01$, *** $p < 0.001$

Carbohydrates

Simple Sugars

The delactosed permeates from production plant 2 had significantly higher (p<0.05) concentrations of lactose compared to those from production plant 1 (Figure 4.5), which aligns with the lower lactose crystallization recovery reported by production plant 2. In general, the yield of lactose crystallization rarely surpasses 65% because of interferences from minerals and other components. (Paterson, 2009) Following this pattern, the reduced lactose crystallization at production plant 2 was likely caused by higher levels of protein, minerals, and other non-lactose compounds in the concentrated permeate at this site.

Glucose had the greatest variation across all batches, but there was no significant difference (p>0.05) in glucose concentrations between production plants.

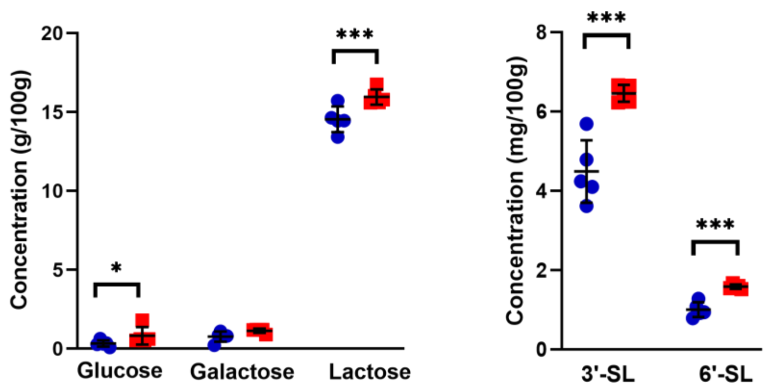


Figure 4.5. Simple sugar and sialyllactose concentrations in delactosed permeate batches produced at production plant 1 (●) and production plant 2 (■). * $0.01 < p \leq 0.05$, **$0.001 < p \leq 0.01$, *** $p < 0.001$

Bovine Milk Oligosaccharides

21 BMOs, including 12 unique monosaccharide compositions and 9 additional isomers, were identified across all of the delactosed permeate samples. Three of the identified BMOs were acidic, including two of the most abundant structures in bovine milk, 3’-SL and 6’-SL. No fucosylated BMOs were identified in the delactosed permeate samples.

Significantly higher (p<0.001) concentrations of 3’-SL and 6’-SL were measured in the delactosed permeate batches from production plant 2 (Figure 4.5). This trend may be the result of more extensive ultrafiltration carried out to maximize protein removal at production plant 1, which may lead to additional, unintentional retention of oligosaccharides, as an unintended side effect. Acidic oligosaccharides like 3'-SL and 6'-SL, which maintain a negative charge under the mildly acidic conditions of milk and whey permeates, may be more susceptible to this effect because of their greater interactions with the charged filtration membranes. (Cheryan, 1998; Cohen et al., 2017; Luo and Wan, 2013) No significant difference in relative abundance ($p>0.05$) was observed between the two production plants for any of the other BMOs, but substantially greater variation in BMO abundances between delactosed permeate batches was observed for production plant 1 (Table 4.1). Relative abundance data for all BMOs is reported in Supplementary Table 4.1.

Table 4.1. Relative abundances of bovine milk oligosaccharides in delactosed permeate from two production plants. Abundances are expressed as the mean relative abundance per gram of delactosed permeate ± standard deviation.

Bovine Milk Oligosaccharide	Neutral Mass (Da)	Production Plant 1 Relative Abundance	Production Plant 2 Relative Abundance
2_0_0_1_0 (3'-SL)	633.2116	1617.8490 ± 1134.444	1252.085 ± 548.541
2_0_0_1_0 (6'-SL)	633.2116	192.549 ± 85.535	224.875 ± 70.827
2_1_0_0_0 isomer 1	545.1956	1128.319 ± 389.905	1421.359 ± 566.052
2_1_0_0_0 isomer 2	545.1956	16.315 ± 6.854	12.938 ± 2.611
2_1_0_0_0 isomer 3	545.1956	20.092 ± 12.074	11.268 ± 4.523
2_2_0_0_0	748.2750	13.663 ± 8.771	9.460 ± 3.236
3_0_0_0_0 isomer 1	504.1690	1011.583 ± 706.352	449.193 ± 161.156
3_0_0_0_0 isomer 2	504.1690	166.481 ± 105.960	109.556 ± 31.637
3_0_0_1_0	795.2645	138.671 ± 76.261	79.571 ± 16.035
3_1_0_0_0 isomer 1	707.2484	18.385 ± 9.367	17.637 ± 6.836
3_1_0_0_0 isomer 2	707.2484	20.973 ± 13.174	16.259 ± 3.350
3_1_0_0_0 isomer 3	707.2484	62.416 ± 41.641	38.453 ± 7.728
3_2_0_0_0	910.3278	27.024 ± 17.630	20.560 ± 4.013
3_3_0_0_0	1113.4072	14.132 ± 8.710	12.288 ± 2.685
4_0_0_0_0 isomer 1	666.2219	44.752 ± 43.261	31.532 ± 8.950
4_0_0_0_0 isomer 2	666.2219	13.840 ± 11.006	7.533 ± 2.667
4_0_0_0_0 isomer 3	666.2219	29.177 ± 23.432	17.584 ± 4.180
4_1_0_0_0	869.3012	84.502 ± 50.725	57.370 ± 9.758
4_2_0_0_0	1072.3806	13.761 ± 7.509	12.229 ± 2.921
6_0_0_0_0 isomer 1	990.3275	78.833 ± 62.487	27.583 ± 9.641
6_0_0_0_0 isomer 2	990.3275	16.731 ± 15.341	12.946 ± 3.552

Bovine milk oligosaccharides are listed based on their monosaccharide compositions as the number of Hex_HexNAc_Fuc_Neu5Ac_Neu5Gc.

Naturally Occurring Peptides

A total of 77 to 577 unique peptide sequences were identified in the delactosed permeate samples, and a comparison across different samples is shown in Table 4.3. Major parent proteins for the peptides identified in the delactosed permeates included β-casein, α-S1-casein, glycosylation-dependent cell adhesion molecule 1, α-S2-casein, κ-casein, polymeric immunoglobulin receptor, and β-lactoglobulin, with the greatest number of sequences originating from β-casein and α-S1-casein. In addition, several bioactive peptide sequences were identified by matching with the Milk Bioactive Peptide Database (Figure 4.6). (http://mbpdb.nws.oregonstate.edu/, Accessed 12/19/2020) Prominent bioactivities of the identified peptides include Angiotensin-converting enzyme (ACE)-inhibitory, antimicrobial, antioxidant, anti-inflammatory, and immunomodulatory activities. The complete lists of naturally occurring peptide sequences identified in all the delactosed permeate batches can be found in Appendix 1, and the corresponding activity annotations are listed in Supplementary Tables 4.2 through 4.11.

Table 4.3. Number of unique peptide sequences and estimated peptide concentrations in delactosed permeates. Concentrations are expressed as the mean ± standard deviation.

	Production Plant 1		**Production Plant 2**	
	Number of Peptide Sequences	**Peptide Concentration (mg/g delactosed permeate)**	**Number of Peptide Sequences**	**Peptide Concentration (mg/g delactosed permeate)**
Batch A	399	14.69 ± 5.18	159	1.82 ± 0.08
Batch B	326	14.05 ± 1.11	130	2.12 ± 0.97
Batch C	577	14.58 ± 3.77	77	1.61 ± 0.48
Batch D	264	14.87 ± 0.41	131	2.07 ± 0.62
Batch E	457	13.60 ± 0.66	77	1.35 ± 0.04

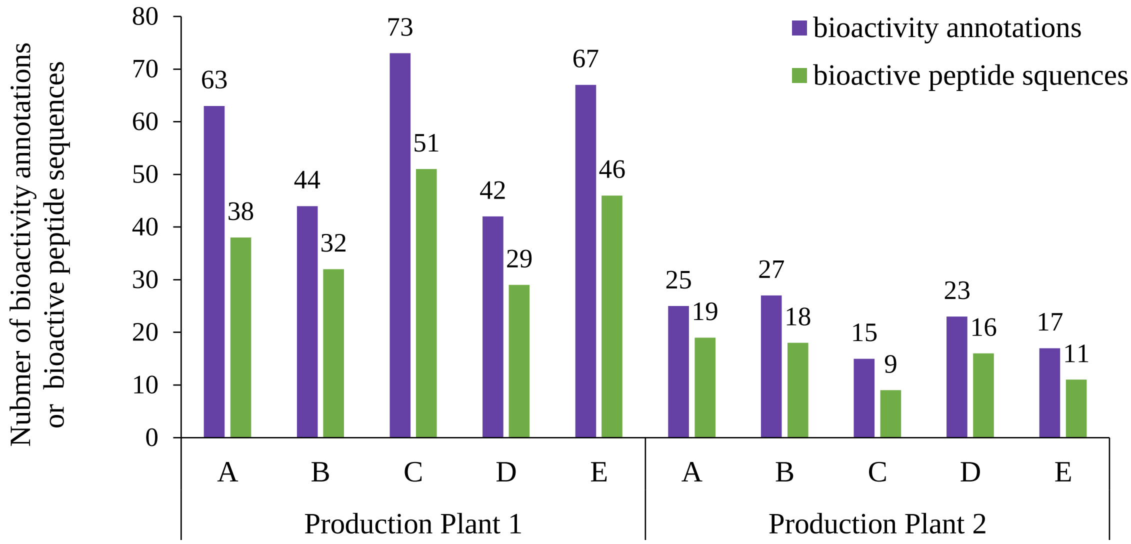


Figure 4.6. Numbers of bioactivity annotations and unique bioactive peptide sequences in delactosed permeate batches (A-E) from the two production plants.

In addition, peptide concentrations were estimated for all delactosed permeate samples using a fluorometric assay (Table 4.3). Peptide contents measured for delactosed permeates from production plant 1 were significantly higher ($p<0.001$) than those from production plant 2, consistent with the higher number of peptide sequences identified in the delactosed permeates from plant 1. This phenomenon is likely the result of differing composition of the delactosed permeate starting material between the two plants, with lower incorporation of whey permeate and greater levels of milk permeate, which has a lower natural peptide content than cheesemaking byproducts like whey permeate, in the starting material at production plant 2. Additional influence on the delactosed permeate peptide content may also have arisen from the higher mineral content in the ultrafiltration starting material at production plant 2, which could lead to greater formation of salt bridges between peptides and the ultrafiltration membrane, increasing peptide retention. (Cheryan, 1998)

Because the fluorometric assay employed to estimate peptide concentrations was designed for protein quantification and we cannot confirm that all peptides react in the same manner, the provided peptide concentrations are only estimates. Similarly, it is possible that other, as yet unidentified components of the delactosed permeates may react with the fluorescent agent in the assay, contributing to the measured peptide content. These effects may also be contributing to the differences in peptide content measured between the two production plants.

Demineralization

As a pilot study, 16.65L of delactosed permeate from production plant 1 underwent demineralization through electrodialysis. An average of 95.8% reduction in conductivity was achieved after demineralization, with approximately 85% of the reduction in conductivity occurring within the first hour of electrodialysis. This drop in conductivity corresponds to substantial reduction in mineral content, including more than 95% removal of sodium and potassium, and more than 80% removal of calcium and magnesium (Table 4.4).

Table 4.4. Percent reduction in major solid components of delactosed permeate with demineralization. Values are expressed as the mean ± standard deviation from three demineralization trials.

Component	**Reduction in Concentration (%)**
Total Solids	16.88 ± 6.65
Ash	89.69 ± 0.04
Sodium	96.19 ± 0.21
Potassium	97.99 ± 0.21
Calcium	83.71 ± 6.22
Magnesium	85.09 ± 5.15
Phosphorous	71.20 ± 2.67
Protein	25.51 ± 1.99
Lactose	2.20 ± 0.07

The concentrations of the two most abundant charged BMOs, 3’-SL and 6’-SL, were also measured in both the diluent and final delactosed permeate product after demineralization (Table 4.5). Because of their charged nature and comparatively low molecular weight, 3’-SL and 6’-SL were determined to hold the greatest risk of loss during the electrodialytic demineralization, and thus made good markers for any potential removal of other BMOs from the delactosed permeate during this process. No loss of sialyllactose to the diluent was detected (limit of detection of 0.1 mg/100g), demonstrating good recovery of BMOs after demineralization.

Table 4.5. Concentration of sialyllactose in delactosed permeate demineralization fractions. Values are expressed as the mean ± standard deviation from two replicates of the final pooled delactosed permeate fractions.

Fraction	**3'-Sialyllactose** (mg/100g)	**6'-Sialyllactose** (mg/100g)
Demineralized delactosed permeate	16.59 ± 1.91	5.44 ± 0.45
Diluate	None detected	None detected

In addition to significantly reducing the mineral content without a detectable loss of BMOs, demineralization of the delactosed permeate pilot batch also allowed the material to be easily spray dried to a free-flowing powder. This demonstrates that previously reported challenges with drying this stream (Bund and Hartel, 2010; Liang et al., 2009) can also be overcome through demineralization.

Conclusions

This study offers an in-depth compositional analysis of delactosed permeate, illustrates its substantial variance in composition between production sites and between production batches, and demonstrates the ability of this stream to be successfully desalinated without loss of key bioactive compounds. In addition, the present study provides the first comparative analysis of the peptide and bovine milk oligosaccharide profiles of delactosed permeate. The findings from this study indicate the strong potential for delactosed permeate to be harnessed as a source of bioactive oligosaccharides, peptides, and organic acids. Further research on this dairy stream will be needed to determine which variations of the dairy processing procedures leading up to delactosed permeate production are optimal for bioactive compound isolation.

ACKNOWLEDGEMENTS

The author would like to extend special thanks to Yu-Ping Huang for her peptidomic analysis, Dr. Tian Tian for her measurement of the uric acid concentrations, Dr. Aidong Wang for her SDS-PAGE protein analysis, and Dr. Lindsey Ormond from Milk Specialties Global for providing the samples and some of the compositional parameters of the delactosed permeates.

SUPPLEMENTAL DATA

Batch	Replicate	Mass of original sample (mg)	Mass of original sample per injection	3_1_0_0_0 isomer 1	3_1_0_0_0 isomer 2	3_1_0_0_0 isomer 3	3_2_0_0_0	3_3_0_0_0	4_0_0_0_0 isomer 1	4_0_0_0_0 isomer 2	4_0_0_0_0 isomer 3	4_1_0_0_0	4_2_0_0_0	6_0_0_0_0 isomer 1	6_0_0_0_0 isomer 2

Supplementary Table 4.1. Raw abundances of bovine milk oligosaccharides identified in delactosed permeates

	Batch	Replicate	Mass of original sample (mg)	Mass of original sample per injection	xylosylcellobiose (internal standard)	1_0_0_1_0 (3'-SL fragment)	2_0_0_1_0 (3'-SL)	2_0_0_1_0 (6'-SL)	2_1_0_0_0 isomer 1	2_1_0_0_0 isomer 2	2_1_0_0_0 isomer 3	2_2_0_0_0	3_0_0_0_0 isomer 1	3_0_0_0_0 isomer 2	3_0_0_1_0
Production Plant 1	A	1	315.6	0.0252	2474266.6	25742312.8	11998213.8	6608868.5	49289152.0	580016.7	743219.7	413065.3	23759463.4	4850012.7	3754707.2
		2	320.5	0.0256	1866023.1	34689068.5	15375614.2	7653682.2	52090457.1	525465.5	715555.6	456210.4	25466740.3	5311169.2	3962222.9
	B	1	325.5	0.0260	1164124.6	41822954.3	17953371.9	6454837.7	36094998.9	477944.8	504742.9	374538.0	38647621.0	5649457.6	5207999.5
		2	326.6	0.0261	1298158.7	38382769.3	16675959.6	7275372.9	34320520.3	526788.8	609567.7	420067.5	37380776.6	5430635.7	4970252.9
	C	1	330.6	0.0264	643428.7	49991598.6	21220119.0	6483603.7	34367166.3	545518.6	798818.7	584602.6	44488057.4	7082289.1	5394660.7
		2	331.4	0.0265	911150.8	48205201.4	21287883.8	6944065.1	37760092.7	546953.1	849180.7	589374.4	42725441.4	6808793.5	4934897.8
	D	1	323.5	0.0259	1897277.8	38047936.2	17147836.8	6172453.6	48018819.1	553212.6	484471.4	440792.4	29641780.2	5286936.4	5316700.7
		2	322.4	0.0258	1767592.3	40958523.9	17722311.4	7123889.2	47860742.2	553855.3	540935.1	423489.1	28068832.1	5468309.8	4705486.4
	E	1	319.6	0.0256	2258035.1	29251771.7	13409909.5	7273922.7	44449726.6	818730.9	682318.6	501450.2	36008522.1	5621778.6	5706364.6
		2	322.6	0.0258	2152818.6	27691362.4	12374188.5	8660460.6	45699369.3	1071721.9	1322343.9	557651.6	33345923.0	5925561.9	5212528.8
Production Plant 2	A	1	161.7	0.0078	3602897.5	16037058.5	7135359.7	5467761.4	26798921.2	347530.3	266646.8	213338.1	10967887.5	2511556.6	1903087.9
		2	177.7	0.0085	2711934.2	25619501.6	11310215.0	6921730.5	37526993.0	335754.1	305289.6	277597.2	15348256.9	2854912.2	2185757.7
	B	1	191.9	0.0092	2208892.7	25848053.2	11486381.0	5816975.1	45677046.8	338216.4	383298.1	282444.3	12027108.4	3118502.7	2074049.2
		2	243.4	0.0117	1783166.5	32111442.3	13857239.7	6068763.1	43625842.7	316877.7	383546.2	296079.2	12990404.7	3456231.0	2013284.5
	C	1	150.8	0.0072	2779748.9	23486911.6	10397839.8	6786214.5	43776619.1	308026.0	241860.2	234999.5	12503752.5	2531725.7	1922602.9
		2	147.4	0.0071	4487604.4	13938485.0	6463182.9	5339515.1	25970778.6	252227.5	153745.2	182220.5	7717710.6	2329242.7	1877714.7
	D	1	173.6	0.0083	3799958.9	13924410.1	6175310.8	4514975.9	26438255.2	383294.8	238877.3	171213.0	7935285.4	2392066.6	1989429.0
		2	173.6	0.0083	3519928.1	18915818.1	8580993.4	5245950.4	32045224.9	295945.8	255986.1	216910.3	9880012.4	2728645.2	1961024.3
	E	1	176.8	0.0085	3366060.3	17689106.7	8127812.9	4391664.2	34925258.3	369540.6	310965.7	227507.5	10205275.7	2641187.0	2217320.5
		2	184.2	0.0088	2938364.8	22520478.5	9996526.4	5096528.5	29881078.5	317472.2	225379.3	225891.2	10712293.2	2674396.4	1885817.9

Supplementary Table 4.2. Bioactive peptide sequences identified in delactosed permeate from production plant 1, batch A

Search peptide	Protein	Peptide	Protein description	Species	Intervals	Function	DOI
ALNEINQFYQK	P02663	ALNEINQFYQK	Alpha-S2-casein	Bos taurus	96-106	ACE-inhibitory	10.1016/S0014-5793(02)03576-
DAQSAPLRVY	P02754	DAQSAPLRVY	Beta-lactoglobulin	Bos taurus	49-58	ACE-inhibitory	10.1016/j.peptides.2011.02.005, 10.1016/j.foodchem.2011.09.052
ENLLRF	P18626	ENLLRF	Alpha-S1-casein	Capra hircu:	33-38	ACE-inhibitory	0302(05)73032-0
EPVLGPVRGPFP	P02666	EPVLGPVRGPFP	Beta-casein	Bos taurus	210-221	ACE-inhibitory	10.1128/AEM.00096-07
FPEVFGK	P02662	FPEVFGK	Alpha-S1-casein	Bos taurus	43-49	ACE-inhibitory	10.1080/00021369.1987.108682
FVAPFPEVFG	P02662	FVAPFPEVFG	Alpha-S1-casein	Bos taurus	39-48	ACE-inhibitory	10.1021/jf049510t
INNQFLPYPYYAKPA	P02668	INNQFLPYPYYAKPA	Kappa-casein	Bos taurus	72-86	Antioxidant	10.3390/antiox9020117
IPIQY	P02668	IPIQY	Kappa-casein	Bos taurus	47-51	DPP-IV Inhibitory	10.1016/j.foodchem.2013.08.097 , 10.1039/c6fo01411a
KVLPVPQ	P02666	KVLPVPQ	Beta-casein	Bos taurus	184-190	ACE-inhibitory	10.3168/jds.S0022-0302(96)76487-1,
KVLPVPQ	P02666	KVLPVPQ	Beta-casein	Bos taurus	184-190	Anti-inflammatory	10.3168/jds.2019-17976
LLYQEPVLGPVRGPF	P02666	LLYQEPVLGPVRGPF	Beta-casein	Bos taurus	206-224	ACE-inhibitory	10.3168/jds.S0022-
MKPWIQPK	P02663	MKPWIQPK	Alpha-S2-casein	Bos taurus	205-212	ACE-inhibitory	0302(96)76487-1
NIPPLTQTPV	P02666	NIPPLTQTPV	Beta-casein	Bos taurus	88-97	ACE-inhibitory	10.1128/AEM.66.9.3898-
NVPGEIVESL	P02666	NVPGEIVESL	Beta-casein	Bos taurus	22-31	Antioxidant	10.3390/antiox9020117
PFPEVFGK	P02662	PFPEVFGK	Alpha-S1-casein	Bos taurus	42-49	ACE-inhibitory	10.3168/jds.2015-10437
PVVVPPFLQPE	P33048	PVVVPPFLQPE	Beta-casein	Capra hircu:	96-106	Antimicrobial	10.1017/S0007114511001085
QEPVLGPVRGPFPIIV	P02666	QEPVLGPVRGPFPIIV	Beta-casein	Bos taurus	209-224	ACE-inhibitory	10.3168/jds.2015-9569
RDMPIQAF	P02666	RDMPIQAF	Beta-casein	Bos taurus	198-205	ACE-inhibitory	0302(94)77026-0
SDIPNPIGSENSEK	P02662	SDIPNPIGSENSEK	Alpha-S1-casein	Bos taurus	195-208	Antimicrobial	10.1128/AEM.72.3.2260-
SKVLPVPQ	P02666	SKVLPVPQ	Beta-casein	Bos taurus	183-190	ACE-inhibitory	10.3168/jds.S0022-
SQSKVLPVPQ	P02666	SQSKVLPVPQ	Beta-casein	Bos taurus	181-190	ACE-inhibitory	10.1128/AEM.00096-07
SQSKVLPVPQKAVP\	P02666	SQSKVLPVPQKAVP\	Beta-casein	Bos taurus	181-197	Antioxidant	10.3390/antiox9020117
TQTPVVVPPFLQPE	P02666	TQTPVVVPPFLQPE	Beta-casein	Bos taurus	93-106	Antioxidant	10.1016/j.foodchem.2010.05.029
VAGTWY	P02754	VAGTWY	Beta-lactoglobulin	Bos taurus	31-36	stimulates proliferation	10.1016/j.idairyj.2010.02.013
VAGTWY	P02754	VAGTWY	Beta-lactoglobulin	Bos taurus	31-36	DPP-IV Inhibitory	10.1016/j.jff.2014.04.002
VAGTWY	P02754	VAGTWY	Beta-lactoglobulin	Bos taurus	31-36	Antioxidant	10.1016/j.jff.2014.04.002
VAGTWY	P02754	VAGTWY	Beta-lactoglobulin	Bos taurus	31-36	Antimicrobial	10.1016/S0304-4165(01)00116-7
VAGTWY	P02754	VAGTWY	Beta-lactoglobulin	Bos taurus	31-36	ACE-inhibitory	10.1017/S0022029999003982
VAPFPE	P02662	VAPFPE	Alpha-S1-casein	Bos taurus	40-45	Inhibition of cholesterol solubility	10.3168/jds.2019-17586
VLNENLLR	P02662	VLNENLLR	Alpha-S1-casein	Bos taurus	30-37	Antimicrobial	10.1128/AEM.72.3.2260-2264.2006, 10.1111/j.1472-765X.2012.03271.x,
VLPVPQ	P02666	VLPVPQ	Beta-casein	Bos taurus	185-190	Inhibition of cholesterol solubility	10.3168/jds.2019-17586
VLPVPQK	P02666	VLPVPQK	Beta-casein	Bos taurus	185-191	Antioxidant	10.1016/j.idairyj.2014.11.001, 10.1016/j.lwt.2019.108816
VLPVPQK	P02666	VLPVPQK	Beta-casein	Bos taurus	185-191	Antimicrobial	10.1016/j.lwt.2015.12.019
VLPVPQK	P02666	VLPVPQK	Beta-casein	Bos taurus	185-191	ACE-inhibitory	10.1016/j.foodchem.2015.05.121
VLPVPQK	P02666	VLPVPQK	Beta-casein	Bos taurus	185-191	Wound healing	10.1002/jcb.28246
VLPVPQK	P02666	VLPVPQK	Beta-casein	Bos taurus	185-191	Osteoanabolic	10.1007/s00394-016-1346-2, 10.1021/acs.jafc.0c03385
VLPVPQK	P02666	VLPVPQK	Beta-casein	Bos taurus	185-191	anti-apoptotic effect	10.1016/j.fbio.2020.100566
VLPVPQKAVPYPQR	P02666	VLPVPQKAVPYPQR	Beta-casein	Bos taurus	185-198	Antimicrobial	10.1016/j.lwt.2015.12.019
VPGEIVE	P02666	VPGEIVE	Beta-casein	Bos taurus	23-29	DPP-IV Inhibitory	10.1016/j.peptides.2016.03.005
VQVTSTAV	P02668	VQVTSTAV	Kappa-casein	Bos taurus	183-190	Antimicrobial	N/A
VYPFPGPIPN	P02666	VYPFPGPIPN	Beta-casein	Bos taurus	74-83	Antioxidant	10.1007/s00217-012-1894-5, 10.3390/antiox9020117
VYPFPGPIPN	P02666	VYPFPGPIPN	Beta-casein	Bos taurus	74-83	ACE-inhibitory	10.1007/s00217-012-1894-5
YLGYLE	P02662	YLGYLE	Alpha-S1-casein	Bos taurus	106-111	Opioid	10.1021/jf301279k, 10.1021/bi00288a034
YLGYLE	P02662	YLGYLE	Alpha-S1-casein	Bos taurus	106-111	Antioxidant	10.3390/foods9080991
YLGYLE	P02662	YLGYLE	Alpha-S1-casein	Bos taurus	106-111	ACE-inhibitory	10.3390/foods9080991
YLGYLEQ	P02662	YLGYLEQ	Alpha-S1-casein	Bos taurus	106-112	Anxiolytic	10.1021/jf202890e, 10.1021/jf104089c
YPVEPF	P02666	YPVEPF	Beta-casein	Bos taurus	129-134	Opioid	10.1016/S0196-9781(99)00088-1, 10.1017/S0022029914000533

Search peptide	Protein	Peptide	Protein description	Species	Intervals	Function	DOI
YPVEPF	P02666	YPVEPF	Beta-casein	Bos taurus	129-134	Increase MUC4 expression	10.1017/S0022029914000533
YPVEPF	P02666	YPVEPF	Beta-casein	Bos taurus	129-134	DPP-IV Inhibitory	10.1016/j.peptides.2016.03.005, 10.1016/j.foodchem.2017.10.033
YPVEPF	P02666	YPVEPF	Beta-casein	Bos taurus	129-134	Antioxidant	10.3390/foods9080991
YPVEPF	P02666	YPVEPF	Beta-casein	Bos taurus	129-134	Antimicrobial	10.3389/fmicb.2018.01148
YQEPVL	P02666	YQEPVL	Beta-casein	Bos taurus	208-213	ACE-inhibitory	10.1016/S0958-6946(98)00048-X, 10.1016/j.idairyj.2007.02.009
YQEPVLGPVR	P02666	YQEPVLGPVR	Beta-casein	Bos taurus	208-217	ACE-inhibitory	10.3168/jds.2015-9569
YQEPVLGPVR	P02666	YQEPVLGPVR	Beta-casein	Bos taurus	208-217	Immunomodulatory	10.1016/0014-5793(96)00207-4
YQEPVLGPVR	P02666	YQEPVLGPVR	Beta-casein	Bos taurus	208-217	Antithrombotic	10.1039/c8fo02235f
YQEPVLGPVR	P02666	YQEPVLGPVR	Beta-casein	Bos taurus	208-217	Antioxidant	10.1007/s10989-018-9708-7
YQEPVLGPVR	P02666	YQEPVLGPVR	Beta-casein	Bos taurus	208-217	Anti-inflammatory	10.1007/s10989-018-9708-7
YQEPVLGPVRGPFPI	P33048	YQEPVLGPVRGPFPI	Beta-casein	Capra hircu:	206-220	Antimicrobial	10.1017/S0007114511001085, 10.1111/j.1365-
YQEPVLGPVRGPFPII	P02666	YQEPVLGPVRGPFPII	Beta-casein	Bos taurus	208-224	immunomodulatory	N/A
YQEPVLGPVRGPFPII	P02666	YQEPVLGPVRGPFPII	Beta-casein	Bos taurus	208-224	antithrombin	10.1016/j.idairyj.2012.05.002
YQEPVLGPVRGPFPII	P02666	YQEPVLGPVRGPFPII	Beta-casein	Bos taurus	208-224	Antimicrobial	10.1111/j.1365-
YQEPVLGPVRGPFPII	P02666	YQEPVLGPVRGPFPII	Beta-casein	Bos taurus	208-224	ACE-inhibitory	10.3168/jds.S0022-
YQEPVLGPVRGPFPII	P02666	YQEPVLGPVRGPFPII	Beta-casein	Bos taurus	208-224	Immunomodulatory	N/A

Supplementary Table 4.3. Bioactive peptide sequences identified in delactosed permeate from production plant 1, batch B

Search peptide	Protein I	Peptide	Protein description	Species	Intervals	Function	DOI
ALNEINQFYQK	P02663	ALNEINQFYQK	Alpha-S2-casein	Bos taurus	96-106	ACE-inhibitory	10.1016/S0014-5793(02)03576-7
AYFYPE	P02662	AYFYPE	Alpha-S1-casein	Bos taurus	158-163	Antioxidant	10.1016/j.idairyj.2013.05.008, 10.3390/foods9080991
AYFYPE	P02662	AYFYPE	Alpha-S1-casein	Bos taurus	158-163	ACE-inhibitory	10.1016/j.idairyj.2013.05.008, 10.3168/jds.S0022-0302(94)77026-0,
DAQSAPLRVY	P02754	DAQSAPLRVY	Beta-lactoglobulin	Bos taurus	49-58	ACE-inhibitory	10.1016/j.peptides.2011.02.005, 10.1016/j.foodchem.2011.09.052
EPVLGPVRGPFP	P02666	EPVLGPVRGPFP	Beta-casein	Bos taurus	210-221	ACE-inhibitory	10.1128/AEM.00096-07
FALPQY	P02663	FALPQY	Alpha-S2-casein	Bos taurus	189-194	ACE-inhibitory	10.1016/S0014-5793(02)03576-7
FFVAPFPEVFGK	P02662	FFVAPFPEVFGK	Alpha-S1-casein	Bos taurus	38-49	ACE-inhibitory	10.1016/j.foodchem.2014.09.098, 10.1080/00021369.1982.10865255, 10.1016/S0014-
FPEVFGK	P02662	FPEVFGK	Alpha-S1-casein	Bos taurus	43-49	ACE-inhibitory	10.1080/00021369.1987.1086824
FVAPFPEVFG	P02662	FVAPFPEVFG	Alpha-S1-casein	Bos taurus	39-48	ACE-inhibitory	10.1021/jf049510t
IHPFAQTQ	P02666	IHPFAQTQ	Beta-casein	Bos taurus	64-71	prolyl endopeptidase-inhibitory	10.1007/BF02019390
IHPFAQTQ	P02666	IHPFAQTQ	Beta-casein	Bos taurus	64-71	PEP-inhibitory	10.1271/bbb.56.976
IPIQY	P02668	IPIQY	Kappa-casein	Bos taurus	47-51	DPP-IV Inhibitory	10.1016/j.foodchem.2013.08.097, 10.1039/c6fo01411a
KVLPVPQ	P02666	KVLPVPQ	Beta-casein	Bos taurus	184-190	ACE-inhibitory	10.3168/jds.S0022-0302(96)76487-1,
KVLPVPQ	P02666	KVLPVPQ	Beta-casein	Bos taurus	184-190	Anti-inflammatory	10.3168/jds.2019-17976
LLYQEPVLGPVRGPF	P02666	LLYQEPVLGPVRGPF	Beta-casein	Bos taurus	206-224	ACE-inhibitory	10.3168/jds.S0022-
LYQEPVLGPVRGPFF	P02666	LYQEPVLGPVRGPFF	Beta-casein	Bos taurus	207-224	Immunomodulatory	10.1016/0165-2478(92)90091-2
NIPPLTQTPV	P02666	NIPPLTQTPV	Beta-casein	Bos taurus	88-97	ACE-inhibitory	10.1128/AEM.66.9.3898-
NVPGEIVESL	P02666	NVPGEIVESL	Beta-casein	Bos taurus	22-31	Antioxidant	10.3390/antiox9020117
QEPVLGPVRGPFPIIV	P02666	QEPVLGPVRGPFPIIV	Beta-casein	Bos taurus	209-224	ACE-inhibitory	10.3168/jds.2015-9569
RDMPIQAF	P02666	RDMPIQAF	Beta-casein	Bos taurus	198-205	ACE-inhibitory	10.3168/jds.S0022-
SDIPNPIGSENSEK	P02662	SDIPNPIGSENSEK	Alpha-S1-casein	Bos taurus	195-208	Antimicrobial	10.1128/AEM.72.3.2260-
SKVLPVPQ	P02666	SKVLPVPQ	Beta-casein	Bos taurus	183-190	ACE-inhibitory	10.3168/jds.S0022-
SQSKVLPVPQ	P02666	SQSKVLPVPQ	Beta-casein	Bos taurus	181-190	ACE-inhibitory	10.1128/AEM.00096-07
SQSKVLPVPQKAVPY	P02666	SQSKVLPVPQKAVPY	Beta-casein	Bos taurus	181-197	Antioxidant	10.3390/antiox9020117
VAPFPE	P02662	VAPFPE	Alpha-S1-casein	Bos taurus	40-45	Inhibition of cholesterol solubility	10.3168/jds.2019-17586
VLNENLLR	P02662	VLNENLLR	Alpha-S1-casein	Bos taurus	30-37	Antimicrobial	10.1128/AEM.72.3.2260-2264.2006, 10.1111/j.1472-765X.2012.03271.x,
VLPVPQ	P02666	VLPVPQ	Beta-casein	Bos taurus	185-190	Inhibition of cholesterol solubility	10.3168/jds.2019-17586
VQVTSTAV	P02668	VQVTSTAV	Kappa-casein	Bos taurus	183-190	Antimicrobial	N/A
VRGPFPIIV	P02666	VRGPFPIIV	Beta-casein	Bos taurus	216-224	ACE-inhibitory	10.1016/j.idairyj.2005.12.011, 10.3168/jds.S0022-
VYPFPGPIPN	P02666	VYPFPGPIPN	Beta-casein	Bos taurus	74-83	Antioxidant	10.1007/s00217-012-1894-5, 10.3390/antiox9020117
VYPFPGPIPN	P02666	VYPFPGPIPN	Beta-casein	Bos taurus	74-83	ACE-inhibitory	10.1007/s00217-012-1894-5
YQEPVL	P02666	YQEPVL	Beta-casein	Bos taurus	208-213	ACE-inhibitory	10.1016/S0958-6946(98)00048-X, 10.1016/j.idairyj.2007.02.009
YQEPVLGPVR	P02666	YQEPVLGPVR	Beta-casein	Bos taurus	208-217	ACE-inhibitory	10.3168/jds.2015-9569
YQEPVLGPVR	P02666	YQEPVLGPVR	Beta-casein	Bos taurus	208-217	Immunomodulatory	10.1016/0014-5793(96)00207-4
YQEPVLGPVR	P02666	YQEPVLGPVR	Beta-casein	Bos taurus	208-217	Antithrombotic	10.1039/c8fo02235f
YQEPVLGPVR	P02666	YQEPVLGPVR	Beta-casein	Bos taurus	208-217	Antioxidant	10.1007/s10989-018-9708-7
YQEPVLGPVR	P02666	YQEPVLGPVR	Beta-casein	Bos taurus	208-217	Anti-inflammatory	10.1007/s10989-018-9708-7
YQEPVLGPVRGPFPI	P33048	YQEPVLGPVRGPFPI	Beta-casein	Capra hircu:	206-220	Antimicrobial	10.1017/S0007114511001085, 10.1111/j.1365-
YQEPVLGPVRGPFPII	P02666	YQEPVLGPVRGPFPII	Beta-casein	Bos taurus	208-224	immunomodulatory	N/A
YQEPVLGPVRGPFPII	P02666	YQEPVLGPVRGPFPII	Beta-casein	Bos taurus	208-224	antithrombin	10.1016/j.idairyj.2012.05.002
YQEPVLGPVRGPFPII	P02666	YQEPVLGPVRGPFPII	Beta-casein	Bos taurus	208-224	Antimicrobial	10.1111/j.1365-
YQEPVLGPVRGPFPII	P02666	YQEPVLGPVRGPFPII	Beta-casein	Bos taurus	208-224	ACE-inhibitory	10.3168/jds.S0022-
YQEPVLGPVRGPFPII	P02666	YQEPVLGPVRGPFPII	Beta-casein	Bos taurus	208-224	Immunomodulatory	N/A
YQKFPQYLQY	P02663	YQKFPQYLQY	Alpha-S2-casein	Bos taurus	104-113	ACE-inhibitory	10.1016/j.peptides.2017.09.021

Supplementary Table 4.4. Bioactive peptide sequences identified in delactosed permeate from production plant 1, batch C

Search peptide	Protein	Peptide	Protein description	Species	Intervals	Function	DOI
AMKPWIQPK	P02663	AMKPWIQPK	Alpha-S2-casein	Bos taurus	204-212	ACE-inhibitory	10.3168/jds.S0022-0302(96)76487-1
APSFSDIPNPIGSENSI	P02662	APSFSDIPNPIGSENSI	Alpha-S1-casein	Bos taurus	191-207	Antioxidant	10.3390/antiox9020117
DAQSAPLRVY	P02754	DAQSAPLRVY	Beta-lactoglobulin	Bos taurus	49-58	ACE-inhibitory	10.1016/j.peptides.2011.02.005, 10.1016/j.foodchem.2011.09.052
ENLLRF	P18626	ENLLRF	Alpha-S1-casein	Capra hircus	33-38	ACE-inhibitory	0302(05)73032-0
EPVLGPVRGPFP	P02666	EPVLGPVRGPFP	Beta-casein	Bos taurus	210-221	ACE-inhibitory	10.1128/AEM.00096-07
FFVAP	P02662	FFVAP	Alpha-S1-casein	Bos taurus	38-42	ACE-inhibitory	10.1080/00021369.1985.10866901, 10.3168/jds.2019-17976
FPEVFGK	P02662	FPEVFGK	Alpha-S1-casein	Bos taurus	43-49	ACE-inhibitory	10.1080/00021369.1987.1086824
FPKYPVEPF	P02666	FPKYPVEPF	Beta-casein	Bos taurus	126-134	Antioxidant	10.3390/antiox9020117
FVAPFPEVFG	P02662	FVAPFPEVFG	Alpha-S1-casein	Bos taurus	39-48	ACE-inhibitory	10.1021/jf049510t
IHPFAQTQ	P02666	IHPFAQTQ	Beta-casein	Bos taurus	64-71	prolyl endopeptidase-inhibitory	10.1007/BF02019390
IHPFAQTQ	P02666	IHPFAQTQ	Beta-casein	Bos taurus	64-71	PEP-inhibitory	10.1271/bbb.56.976
IPIQY	P02668	IPIQY	Kappa-casein	Bos taurus	47-51	DPP-IV Inhibitory	10.1016/j.foodchem.2013.08.097, 10.1039/c6fo01411a
KHQGLPQEVLNENLL	P02662	KHQGLPQEVLNENLL	Alpha-S1-casein	Bos taurus	22-36	Antioxidant	10.3390/antiox9020117
KVLPVPQ	P02666	KVLPVPQ	Beta-casein	Bos taurus	184-190	ACE-inhibitory	10.3168/jds.S0022-0302(96)76487-1,
KVLPVPQ	P02666	KVLPVPQ	Beta-casein	Bos taurus	184-190	Anti-inflammatory	10.3168/jds.2019-17976
LHLPLPL	P02666	LHLPLPL	Beta-casein	Bos taurus	148-154	ACE-inhibitory	10.1016/j.idairyj.2005.12.011
LLYQEPVLGPVRGPF	P02666	LLYQEPVLGPVRGPF	Beta-casein	Bos taurus	206-224	ACE-inhibitory	0302(94)77026-0
LPLPL	P02666	LPLPL	Beta-casein	Bos taurus	150-154	DPP-IV Inhibitory	10.1016/j.foodchem.2013.08.097, 10.1016/j.peptides.2016.03.005
LPLPLL	P02666	LPLPLL	Beta-casein	Bos taurus	150-155	DPP-IV Inhibitory	10.1016/j.peptides.2016.03.005
LPLPLL	P02666	LPLPLL	Beta-casein	Bos taurus	150-155	ACE-inhibitory	10.1016/j.jff.2017.03.008
LPVPQ	P02666	LPVPQ	Beta-casein	Bos taurus	186-190	DPP-IV Inhibitory	10.1016/j.peptides.2016.03.005
LPYPY	P02668	LPYPY	Kappa-casein	Bos taurus	77-81	DPP-IV Inhibitory	10.1016/j.foodchem.2013.08.097, 10.1039/c6fo01411a
LPYPY	P02668	LPYPY	Kappa-casein	Bos taurus	77-81	ACE-inhibitory	10.1002/elps.200700324, 10.3168/jds.2018-15901
LVYPFPGPIPNSLPQ	P02666	LVYPFPGPIPNSLPQ	Beta-casein	Bos taurus	73-87	ACE-inhibitory	10.1016/S0141-0229(97)00261-5
LYQEPVLGPVRGPFF	P02666	LYQEPVLGPVRGPFF	Beta-casein	Bos taurus	207-224	Immunomodulatory	10.1016/0165-2478(92)90091-2
NIPPLTQTPV	P02666	NIPPLTQTPV	Beta-casein	Bos taurus	88-97	ACE-inhibitory	10.1128/AEM.66.9.3898-
NLHLPLP	P05814	NLHLPLP	Beta-casein	Homo sapiens	138-144	ACE-inhibitory	10.1080/00021369.1989.1086962
NLHLPLPLL	P02666	NLHLPLPLL	Beta-casein	Bos taurus	147-155	ACE-inhibitory	10.1021/jf049510t
NVPGEIVESL	P02666	NVPGEIVESL	Beta-casein	Bos taurus	22-31	Antioxidant	10.3390/antiox9020117
PFPEVFGK	P02662	PFPEVFGK	Alpha-S1-casein	Bos taurus	42-49	ACE-inhibitory	10.3168/jds.2015-10437
PVVVPPFLQPE	P33048	PVVVPPFLQPE	Beta-casein	Capra hircus	96-106	Antimicrobial	10.1017/S0007114511001085
QEPVLGPVRGPFPIIV	P02666	QEPVLGPVRGPFPIIV	Beta-casein	Bos taurus	209-224	ACE-inhibitory	10.3168/jds.2015-9569
RDMPIQAF	P02666	RDMPIQAF	Beta-casein	Bos taurus	198-205	ACE-inhibitory	10.3168/jds.S0022-
RDMPIQAF	P02666	RDMPIQAF	Beta-casein	Bos taurus	198-205	ACE-inhibitory	0302(94)77026-0
SDIPNPIGSENSEK	P02662	SDIPNPIGSENSEK	Alpha-S1-casein	Bos taurus	195-208	Antimicrobial	10.1128/AEM.72.3.2260-
SKVLPVPQ	P02666	SKVLPVPQ	Beta-casein	Bos taurus	183-190	ACE-inhibitory	10.3168/jds.S0022-
SQSKVLPVPQ	P02666	SQSKVLPVPQ	Beta-casein	Bos taurus	181-190	ACE-inhibitory	10.1128/AEM.00096-07
SQSKVLPVPQKAVPY	P02666	SQSKVLPVPQKAVPY	Beta-casein	Bos taurus	181-197	Antioxidant	10.3390/antiox9020117
TEDELQDKIHPF	P33048	TEDELQDKIHPF	Beta-casein	Capra hircus	56-67	Antimicrobial	10.1017/S0007114511001085
TQTPVVVPPFLQPE	P02666	TQTPVVVPPFLQPE	Beta-casein	Bos taurus	93-106	Antioxidant	10.1016/j.foodchem.2010.05.029
VAPFPE	P02662	VAPFPE	Alpha-S1-casein	Bos taurus	40-45	Inhibition of cholesterol solubility	10.3168/jds.2019-17586
VLGPVRGPFP	P02666	VLGPVRGPFP	Beta-casein	Bos taurus	212-221	ACE-inhibitory	10.3168/jds.S0022-0302(06)72372-4
VLNENLLR	P02662	VLNENLLR	Alpha-S1-casein	Bos taurus	30-37	Antimicrobial	10.1128/AEM.72.3.2260-2264.2006, 10.1111/j.1472-765X.2012.03271.x,
VLPVPQ	P02666	VLPVPQ	Beta-casein	Bos taurus	185-190	Inhibition of cholesterol solubility	10.3168/jds.2019-17586
VLPVPQK	P02666	VLPVPQK	Beta-casein	Bos taurus	185-191	Antioxidant	10.1016/j.idairyj.2014.11.001, 10.1016/j.lwt.2019.108816
VLPVPQK	P02666	VLPVPQK	Beta-casein	Bos taurus	185-191	Antimicrobial	10.1016/j.lwt.2015.12.019
VLPVPQK	P02666	VLPVPQK	Beta-casein	Bos taurus	185-191	ACE-inhibitory	10.1016/j.foodchem.2015.05.121

Search peptide	Protein	Peptide	Protein description	Species	Intervals	Function	DOI
VLPVPQK	P02666	VLPVPQK	Beta-casein	Bos taurus	185-191	Wound healing	10.1002/jcb.28246
VLPVPQK	P02666	VLPVPQK	Beta-casein	Bos taurus	185-191	Osteoanabolic	10.1007/s00394-016-1346-2, 10.1021/acs.jafc.0c03385
VLPVPQK	P02666	VLPVPQK	Beta-casein	Bos taurus	185-191	anti-apoptotic effect	10.1016/j.fbio.2020.100566
VLPVPQKAVPYPQR	P02666	VLPVPQKAVPYPQR	Beta-casein	Bos taurus	185-198	Antimicrobial	10.1016/j.lwt.2015.12.019
VPGEIVE	P02666	VPGEIVE	Beta-casein	Bos taurus	23-29	DPP-IV Inhibitory	10.1016/j.peptides.2016.03.005
VQVTSTAV	P02668	VQVTSTAV	Kappa-casein	Bos taurus	183-190	Antimicrobial	N/A
VRGPFPIIV	P02666	VRGPFPIIV	Beta-casein	Bos taurus	216-224	ACE-inhibitory	10.3168/jds.S0022-0302(06)72372-4
VYPFPGPIPN	P02666	VYPFPGPIPN	Beta-casein	Bos taurus	74-83	Antioxidant	10.1007/s00217-012-1894-5, 10.3390/antiox9020117
VYPFPGPIPN	P02666	VYPFPGPIPN	Beta-casein	Bos taurus	74-83	ACE-inhibitory	10.1007/s00217-012-1894-5
YPFPGPIPN	P02666	YPFPGPIPN	Beta-casein	Bos taurus	75-83	DPP-IV Inhibitory	10.1016/j.idairyj.2011.08.002
YPFPGPIPN	P02666	YPFPGPIPN	Beta-casein	Bos taurus	75-83	ACE-inhibitory	10.3168/jds.S0022-0302(00)75013-2,
YPFPGPIPN	P02666	YPFPGPIPN	Beta-casein	Bos taurus	75-83	Antioxidant	10.3390/foods9080991
YQEPVL	P02666	YQEPVL	Beta-casein	Bos taurus	208-213	ACE-inhibitory	10.1016/S0958-6946(98)00048-X, 10.1016/j.idairyj.2007.02.009
YQEPVLGPVR	P02666	YQEPVLGPVR	Beta-casein	Bos taurus	208-217	ACE-inhibitory	10.3168/jds.2015-9569
YQEPVLGPVR	P02666	YQEPVLGPVR	Beta-casein	Bos taurus	208-217	Immunomodulatory	10.1016/0014-5793(96)00207-4
YQEPVLGPVR	P02666	YQEPVLGPVR	Beta-casein	Bos taurus	208-217	Antithrombotic	10.1039/c8fo02235f
YQEPVLGPVR	P02666	YQEPVLGPVR	Beta-casein	Bos taurus	208-217	Antioxidant	10.1007/s10989-018-9708-7
YQEPVLGPVR	P02666	YQEPVLGPVR	Beta-casein	Bos taurus	208-217	Anti-inflammatory	10.1007/s10989-018-9708-7
YQEPVLGPVRGPFPI	P33048	YQEPVLGPVRGPFPI	Beta-casein	Capra hircus	206-220	Antimicrobial	10.1017/S0007114511001085, 10.1111/j.1365-
YQEPVLGPVRGPFPII	P02666	YQEPVLGPVRGPFPII	Beta-casein	Bos taurus	208-224	immunomodulatory	N/A
YQEPVLGPVRGPFPII	P02666	YQEPVLGPVRGPFPII	Beta-casein	Bos taurus	208-224	antithrombin	10.1016/j.idairyj.2012.05.002
YQEPVLGPVRGPFPII	P02666	YQEPVLGPVRGPFPII	Beta-casein	Bos taurus	208-224	Antimicrobial	10.1111/j.1365-
YQEPVLGPVRGPFPII	P02666	YQEPVLGPVRGPFPII	Beta-casein	Bos taurus	208-224	ACE-inhibitory	10.3168/jds.S0022-
YQEPVLGPVRGPFPII	P02666	YQEPVLGPVRGPFPII	Beta-casein	Bos taurus	208-224	Immunomodulatory	N/A
YQKFPQY	P33049	YQKFPQY	Alpha-S2-casein	Capra hircus	105-111	Antioxidant	10.3168/jds.S0022-0302(06)72370-0,
YQKFPQY	P33049	YQKFPQY	Alpha-S2-casein	Capra hircus	105-111	ACE-inhibitory	10.3168/jds.S0022-0302(06)72370-0,

Supplementary Table 4.5. Bioactive peptide sequences identified in delactosed permeate from production plant 1, batch D

Search peptide	Protein I	Peptide	Protein description	Species	Intervals	Function	DOI
AMKPWIQPK	P02663	AMKPWIQPK	Alpha-S2-casein	Bos taurus	204-212	ACE-inhibitory	10.3168/jds.S0022-
DAQSAPLRVY	P02754	DAQSAPLRVY	Beta-lactoglobulin	Bos taurus	49-58	ACE-inhibitory	10.1016/j.peptides.2011.02.005, 10.1016/j.foodchem.2011.09.052
EPVLGPVRGPFP	P02666	EPVLGPVRGPFP	Beta-casein	Bos taurus	210-221	ACE-inhibitory	10.1128/AEM.00096-07
FPEVFGK	P02662	FPEVFGK	Alpha-S1-casein	Bos taurus	43-49	ACE-inhibitory	10.1080/00021369.1987.1086824
FPKYPVEPF	P02666	FPKYPVEPF	Beta-casein	Bos taurus	126-134	Antioxidant	10.3390/antiox9020117
FVAPFPEVFG	P02662	FVAPFPEVFG	Alpha-S1-casein	Bos taurus	39-48	ACE-inhibitory	10.1021/jf049510t
IHPFAQTQ	P02666	IHPFAQTQ	Beta-casein	Bos taurus	64-71	prolyl endopeptidase-inhibitory	10.1007/BF02019390
IHPFAQTQ	P02666	IHPFAQTQ	Beta-casein	Bos taurus	64-71	PEP-inhibitory	10.1271/bbb.56.976
IPIQY	P02668	IPIQY	Kappa-casein	Bos taurus	47-51	DPP-IV Inhibitory	10.1016/j.foodchem.2013.08.097, 10.1039/c6fo01411a
KVLPVPQ	P02666	KVLPVPQ	Beta-casein	Bos taurus	184-190	ACE-inhibitory	10.3168/jds.S0022-0302(96)76487-1,
KVLPVPQ	P02666	KVLPVPQ	Beta-casein	Bos taurus	184-190	Anti-inflammatory	10.3168/jds.2019-17976
LIVTQTMK	P02754	LIVTQTMK	Beta-lactoglobulin	Bos taurus	17-24	Cytotoxic	10.1016/j.idairyj.2010.02.013
LLYQEPVLGPVRGPF	P02666	LLYQEPVLGPVRGPF	Beta-casein	Bos taurus	206-224	ACE-inhibitory	10.3168/jds.S0022-
LYQEPVLGPVRGPFF	P02666	LYQEPVLGPVRGPFF	Beta-casein	Bos taurus	207-224	Immunomodulatory	10.1016/0165-2478(92)90091-2
NIPPLTQTPV	P02666	NIPPLTQTPV	Beta-casein	Bos taurus	88-97	ACE-inhibitory	10.1128/AEM.66.9.3898-
NVPGEIVESL	P02666	NVPGEIVESL	Beta-casein	Bos taurus	22-31	Antioxidant	10.3390/antiox9020117
PFPEVFGK	P02662	PFPEVFGK	Alpha-S1-casein	Bos taurus	42-49	ACE-inhibitory	10.3168/jds.2015-10437
QEPVLGPVRGPFPIIV	P02666	QEPVLGPVRGPFPIIV	Beta-casein	Bos taurus	209-224	ACE-inhibitory	10.3168/jds.2015-9569
RDMPIQAF	P02666	RDMPIQAF	Beta-casein	Bos taurus	198-205	ACE-inhibitory	10.3168/jds.S0022-
SKVLPVPQ	P02666	SKVLPVPQ	Beta-casein	Bos taurus	183-190	ACE-inhibitory	10.3168/jds.S0022-
SQSKVLPVPQ	P02666	SQSKVLPVPQ	Beta-casein	Bos taurus	181-190	ACE-inhibitory	10.1128/AEM.00096-07
VAPFPE	P02662	VAPFPE	Alpha-S1-casein	Bos taurus	40-45	Inhibition of cholesterol solubility	10.3168/jds.2019-17586
VLNENLLR	P02662	VLNENLLR	Alpha-S1-casein	Bos taurus	30-37	Antimicrobial	10.1128/AEM.72.3.2260-2264.2006, 10.1111/j.1472-765X.2012.03271.x,
VLPVPQ	P02666	VLPVPQ	Beta-casein	Bos taurus	185-190	Inhibition of cholesterol solubility	10.3168/jds.2019-17586
VQVTSTAV	P02668	VQVTSTAV	Kappa-casein	Bos taurus	183-190	Antimicrobial	N/A
VYPFPGPIPN	P02666	VYPFPGPIPN	Beta-casein	Bos taurus	74-83	Antioxidant	10.1007/s00217-012-1894-5, 10.3390/antiox9020117
VYPFPGPIPN	P02666	VYPFPGPIPN	Beta-casein	Bos taurus	74-83	ACE-inhibitory	10.1007/s00217-012-1894-5
YPFPGPIPN	P02666	YPFPGPIPN	Beta-casein	Bos taurus	75-83	DPP-IV Inhibitory	10.1016/j.idairyj.2011.08.002
YPFPGPIPN	P02666	YPFPGPIPN	Beta-casein	Bos taurus	75-83	ACE-inhibitory	10.3168/jds.S0022-0302(00)75013-2,
YPFPGPIPN	P02666	YPFPGPIPN	Beta-casein	Bos taurus	75-83	Antioxidant	10.3390/foods9080991
YQEPVL	P02666	YQEPVL	Beta-casein	Bos taurus	208-213	ACE-inhibitory	10.1016/S0958-6946(98)00048-X, 10.1016/j.idairyj.2007.02.009
YQEPVLGPVR	P02666	YQEPVLGPVR	Beta-casein	Bos taurus	208-217	ACE-inhibitory	10.3168/jds.2015-9569
YQEPVLGPVR	P02666	YQEPVLGPVR	Beta-casein	Bos taurus	208-217	Immunomodulatory	10.1016/0014-5793(96)00207-4
YQEPVLGPVR	P02666	YQEPVLGPVR	Beta-casein	Bos taurus	208-217	Antithrombotic	10.1039/c8fo02235f
YQEPVLGPVR	P02666	YQEPVLGPVR	Beta-casein	Bos taurus	208-217	Antioxidant	10.1007/s10989-018-9708-7
YQEPVLGPVR	P02666	YQEPVLGPVR	Beta-casein	Bos taurus	208-217	Anti-inflammatory	10.1007/s10989-018-9708-7
YQEPVLGPVRGPFPI	P33048	YQEPVLGPVRGPFPI	Beta-casein	Capra hircu:	206-220	Antimicrobial	10.1017/S0007114511001085, 10.1111/j.1365-
YQEPVLGPVRGPFPII	P02666	YQEPVLGPVRGPFPII	Beta-casein	Bos taurus	208-224	immunomodulatory	N/A
YQEPVLGPVRGPFPII	P02666	YQEPVLGPVRGPFPII	Beta-casein	Bos taurus	208-224	antithrombin	10.1016/j.idairyj.2012.05.002
YQEPVLGPVRGPFPII	P02666	YQEPVLGPVRGPFPII	Beta-casein	Bos taurus	208-224	Antimicrobial	10.1111/j.1365-
YQEPVLGPVRGPFPII	P02666	YQEPVLGPVRGPFPII	Beta-casein	Bos taurus	208-224	ACE-inhibitory	10.3168/jds.S0022-
YQEPVLGPVRGPFPII	P02666	YQEPVLGPVRGPFPII	Beta-casein	Bos taurus	208-224	Immunomodulatory	N/A

Supplementary Table 4.6. Bioactive peptide sequences identified in delactosed permeate from production plant 1, batch E

Search peptide	Protein I	Peptide	Protein description	Species	Intervals	Function	DOI
AMKPWIQPK	P02663	AMKPWIQPK	Alpha-S2-casein	Bos taurus	204-212	ACE-inhibitory	10.3168/jds.S0022-
DAQSAPLRVY	P02754	DAQSAPLRVY	Beta-lactoglobulin	Bos taurus	49-58	ACE-inhibitory	10.1016/j.peptides.2011.02.005, 10.1016/j.foodchem.2011.09.052
ENLLRF	P18626	ENLLRF	Alpha-S1-casein	Capra hircu:	33-38	ACE-inhibitory	10.3168/jds.S0022-
EPVLGPVRGPFP	P02666	EPVLGPVRGPFP	Beta-casein	Bos taurus	210-221	ACE-inhibitory	10.1128/AEM.00096-07
FPEVFGK	P02662	FPEVFGK	Alpha-S1-casein	Bos taurus	43-49	ACE-inhibitory	10.1080/00021369.1987.1086824
FPKYPVEPF	P02666	FPKYPVEPF	Beta-casein	Bos taurus	126-134	Antioxidant	10.3390/antiox9020117
FVAPFPEVFG	P02662	FVAPFPEVFG	Alpha-S1-casein	Bos taurus	39-48	ACE-inhibitory	10.1021/jf049510t
HKEMPFPKYPVEPFTESQ	P02666	HKEMPFPKYPVEPFTESQ	Beta-casein	Bos taurus	121-138	Antioxidant	10.3390/antiox9020117
HPHPHLSF	P02669	HPHPHLSF	Kappa-casein	Ovis aries	119-126	ACE-inhibitory	10.1002/mnfr.200900453, 10.1002/elps.200700324
HPHPHLSF	P02669	HPHPHLSF	Kappa-casein	Ovis aries	119-126	Osteoanabolic	10.1007/s00394-016-1346-2
IHPFAQTQ	P02666	IHPFAQTQ	Beta-casein	Bos taurus	64-71	prolyl endopeptidase-inhibitory	10.1007/BF02019390
IHPFAQTQ	P02666	IHPFAQTQ	Beta-casein	Bos taurus	64-71	PEP-inhibitory	10.1271/bbb.56.976
INNQFLPYPYYAKPA	P02668	INNQFLPYPYYAKPA	Kappa-casein	Bos taurus	72-86	Antioxidant	10.3390/antiox9020117
IPIQY	P02668	IPIQY	Kappa-casein	Bos taurus	47-51	DPP-IV Inhibitory	10.1016/j.foodchem.2013.08.097, 10.1039/c6fo01411a
KHQGLPQEVLNENLL	P02662	KHQGLPQEVLNENLL	Alpha-S1-casein	Bos taurus	22-36	Antioxidant	10.3390/antiox9020117
KVLPVPQ	P02666	KVLPVPQ	Beta-casein	Bos taurus	184-190	ACE-inhibitory	10.3168/jds.S0022-0302(96)76487-1,
KVLPVPQ	P02666	KVLPVPQ	Beta-casein	Bos taurus	184-190	Anti-inflammatory	10.3168/jds.2019-17976
LIVTQTMK	P02754	LIVTQTMK	Beta-lactoglobulin	Bos taurus	17-24	Cytotoxic	10.1016/j.idairyj.2010.02.013
LLYQEPVLGPVRGPFPIIV	P02666	LLYQEPVLGPVRGPFPIIV	Beta-casein	Bos taurus	206-224	ACE-inhibitory	10.3168/jds.S0022-
LVYPFPGPIPNSLPQ	P02666	LVYPFPGPIPNSLPQ	Beta-casein	Bos taurus	73-87	ACE-inhibitory	10.1016/S0141-0229(97)00261-5
LYQEPVLGPVRGPFPIIV	P02666	LYQEPVLGPVRGPFPIIV	Beta-casein	Bos taurus	207-224	Immunomodulatory	10.1016/0165-2478(92)90091-2
NIPPLTQTPV	P02666	NIPPLTQTPV	Beta-casein	Bos taurus	88-97	ACE-inhibitory	10.1128/AEM.66.9.3898-
NVPGEIVESL	P02666	NVPGEIVESL	Beta-casein	Bos taurus	22-31	Antioxidant	10.3390/antiox9020117
PFPEVFGK	P02662	PFPEVFGK	Alpha-S1-casein	Bos taurus	42-49	ACE-inhibitory	10.3168/jds.2015-10437
QEPVLGPVRGPFPIIV	P02666	QEPVLGPVRGPFPIIV	Beta-casein	Bos taurus	209-224	ACE-inhibitory	10.3168/jds.2015-9569
RDMPIQAF	P02666	RDMPIQAF	Beta-casein	Bos taurus	198-205	ACE-inhibitory	10.3168/jds.S0022-
RPKHPIKHQGLPQEVLNENL	P02662	RPKHPIKHQGLPQEVLNENL	Alpha-S1-casein	Bos taurus	16-38	Antimicrobial	10.1016/0278-6915(95)00097-6, 10.1111/j.1365-
RPKHPIKHQGLPQEVLNENL	P02662	RPKHPIKHQGLPQEVLNENL	Alpha-S1-casein	Bos taurus	16-38	Immunomodulatory	10.1016/0278-6915(95)00097-6
SDIPNPIGSENSEK	P02662	SDIPNPIGSENSEK	Alpha-S1-casein	Bos taurus	195-208	Antimicrobial	10.1128/AEM.72.3.2260-
SKVLPVPQ	P02666	SKVLPVPQ	Beta-casein	Bos taurus	183-190	ACE-inhibitory	10.3168/jds.S0022-
SQSKVLPVPQ	P02666	SQSKVLPVPQ	Beta-casein	Bos taurus	181-190	ACE-inhibitory	10.1128/AEM.00096-07
SQSKVLPVPQKAVPYPQ	P02666	SQSKVLPVPQKAVPYPQ	Beta-casein	Bos taurus	181-197	Antioxidant	10.3390/antiox9020117
TEDELQDKIHPF	P33048	TEDELQDKIHPF	Beta-casein	Capra hircu:	56-67	Antimicrobial	10.1017/S0007114511001085
TKVIPYVRYL	P02663	TKVIPYVRYL	Alpha-S2-casein	Bos taurus	213-222	Antimicrobial	10.1128/AEM.01394-13
TQTPVVVPPFLQPE	P02666	TQTPVVVPPFLQPE	Beta-casein	Bos taurus	93-106	Antioxidant	10.1016/j.foodchem.2010.05.029
VAPFPE	P02662	VAPFPE	Alpha-S1-casein	Bos taurus	40-45	cholesterol solubility	10.3168/jds.2019-17586
VLGPVRGPFP	P02666	VLGPVRGPFP	Beta-casein	Bos taurus	212-221	ACE-inhibitory	10.1016/j.idairyj.2005.12.011, 10.3168/jds.S0022-
VLNENLLR	P02662	VLNENLLR	Alpha-S1-casein	Bos taurus	30-37	Antimicrobial	10.1128/AEM.72.3.2260-2264.2006, 10.1111/j.1472-765X.2012.03271.x,
VLPVPQ	P02666	VLPVPQ	Beta-casein	Bos taurus	185-190	cholesterol solubility	10.3168/jds.2019-17586
VLPVPQK	P02666	VLPVPQK	Beta-casein	Bos taurus	185-191	Antioxidant	10.1016/j.idairyj.2014.11.001, 10.1016/j.lwt.2019.108816
VLPVPQK	P02666	VLPVPQK	Beta-casein	Bos taurus	185-191	Antimicrobial	10.1016/j.lwt.2015.12.019
VLPVPQK	P02666	VLPVPQK	Beta-casein	Bos taurus	185-191	ACE-inhibitory	10.1016/j.foodchem.2015.05.121
VLPVPQK	P02666	VLPVPQK	Beta-casein	Bos taurus	185-191	Wound healing	10.1002/jcb.28246
VLPVPQK	P02666	VLPVPQK	Beta-casein	Bos taurus	185-191	Osteoanabolic	10.1007/s00394-016-1346-2, 10.1021/acs.jafc.0c03385
VLPVPQK	P02666	VLPVPQK	Beta-casein	Bos taurus	185-191	anti-apoptotic	10.1016/j.fbio.2020.100566
VPSERYL	P04653	VPSERYL	Alpha-S1-casein	Ovis aries	101-107	ACE-inhibitory	10.1016/j.idairyj.2004.04.007
VQVTSTAV	P02668	VQVTSTAV	Kappa-casein	Bos taurus	183-190	Antimicrobial	N/A
VRGPFPIIV	P02666	VRGPFPIIV	Beta-casein	Bos taurus	216-224	ACE-inhibitory	10.1016/j.idairyj.2005.12.011, 10.3168/jds.S0022-

Search peptide	Protein I	Peptide	Protein description	Species	Intervals	Function	DOI
VYPFPGPIPN	P02666	VYPFPGPIPN	Beta-casein	Bos taurus	74-83	Antioxidant	10.1007/s00217-012-1894-5, 10.3390/antiox9020117
VYPFPGPIPN	P02666	VYPFPGPIPN	Beta-casein	Bos taurus	74-83	ACE-inhibitory	10.1007/s00217-012-1894-5
YPFPGPIPN	P02666	YPFPGPIPN	Beta-casein	Bos taurus	75-83	DPP-IV Inhibitory	10.1016/j.idairyj.2011.08.002
YPFPGPIPN	P02666	YPFPGPIPN	Beta-casein	Bos taurus	75-83	ACE-inhibitory	10.3168/jds.S0022-0302(00)75013-2,
YPFPGPIPN	P02666	YPFPGPIPN	Beta-casein	Bos taurus	75-83	Antioxidant	10.3390/foods9080991
YQEPVL	P02666	YQEPVL	Beta-casein	Bos taurus	208-213	ACE-inhibitory	10.1016/S0958-6946(98)00048-X, 10.1016/j.idairyj.2007.02.009
YQEPVLGPVR	P02666	YQEPVLGPVR	Beta-casein	Bos taurus	208-217	ACE-inhibitory	10.3168/jds.2015-9569
YQEPVLGPVR	P02666	YQEPVLGPVR	Beta-casein	Bos taurus	208-217	Immunomodulatory	10.1016/0014-5793(96)00207-4
YQEPVLGPVR	P02666	YQEPVLGPVR	Beta-casein	Bos taurus	208-217	Antithrombotic	10.1039/c8fo02235f
YQEPVLGPVR	P02666	YQEPVLGPVR	Beta-casein	Bos taurus	208-217	Antioxidant	10.1007/s10989-018-9708-7
YQEPVLGPVR	P02666	YQEPVLGPVR	Beta-casein	Bos taurus	208-217	Anti-inflammatory	10.1007/s10989-018-9708-7
YQEPVLGPVRGPFPI	P33048	YQEPVLGPVRGPFPI	Beta-casein	Capra hircu:	206-220	Antimicrobial	10.1017/S0007114511001085, 10.1111/j.1365-
YQEPVLGPVRGPFPIIV	P02666	YQEPVLGPVRGPFPIIV	Beta-casein	Bos taurus	208-224	immunomodulatory	N/A
YQEPVLGPVRGPFPIIV	P02666	YQEPVLGPVRGPFPIIV	Beta-casein	Bos taurus	208-224	antithrombin	10.1016/j.idairyj.2012.05.002
YQEPVLGPVRGPFPIIV	P02666	YQEPVLGPVRGPFPIIV	Beta-casein	Bos taurus	208-224	Antimicrobial	10.1111/j.1365-
YQEPVLGPVRGPFPIIV	P02666	YQEPVLGPVRGPFPIIV	Beta-casein	Bos taurus	208-224	ACE-inhibitory	10.3168/jds.S0022-
YQEPVLGPVRGPFPIIV	P02666	YQEPVLGPVRGPFPIIV	Beta-casein	Bos taurus	208-224	Immunomodulatory	N/A
YQKFPQY	P33049	YQKFPQY	Alpha-S2-casein	Capra hircu:	105-111	Antioxidant	10.3168/jds.S0022-0302(06)72370-0,
YQKFPQY	P33049	YQKFPQY	Alpha-S2-casein	Capra hircu:	105-111	ACE-inhibitory	10.3168/jds.S0022-0302(06)72370-0,

Supplementary Table 4.7. Bioactive peptide sequences identified in delactosed permeate from production plant 2, batch A

Search peptide	Protein I	Peptide	Protein description	Species	Intervals	Function	DOI
DKIHPF	P02666	DKIHPF	Beta-casein	Bos taurus	62-67	ACE-inhibitory	10.1128/AEM.66.9.3898-
EPVLGPVRGPFP	P02666	EPVLGPVRGPFP	Beta-casein	Bos taurus	210-221	ACE-inhibitory	10.1128/AEM.00096-07
FVAPFPEVFG	P02662	FVAPFPEVFG	Alpha-S1-casein	Bos taurus	39-48	ACE-inhibitory	10.1021/jf049510t
IHPFAQTQ	P02666	IHPFAQTQ	Beta-casein	Bos taurus	64-71	prolyl endopeptidase-inhibitory	10.1007/BF02019390
IHPFAQTQ	P02666	IHPFAQTQ	Beta-casein	Bos taurus	64-71	PEP-inhibitory	10.1271/bbb.56.976
IPIQY	P02668	IPIQY	Kappa-casein	Bos taurus	47-51	DPP-IV Inhibitory	10.1016/j.foodchem.2013.08.097, 10.1039/c6fo01411a
KVLPVPQ	P02666	KVLPVPQ	Beta-casein	Bos taurus	184-190	ACE-inhibitory	10.3168/jds.S0022-0302(96)76487-1,
KVLPVPQ	P02666	KVLPVPQ	Beta-casein	Bos taurus	184-190	Anti-inflammatory	10.3168/jds.2019-17976
LLYQEPVLGPVRGPF	P02666	LLYQEPVLGPVRGPF	Beta-casein	Bos taurus	206-224	ACE-inhibitory	10.3168/jds.S0022-
LYQEPVLGPVRGPFF	P02666	LYQEPVLGPVRGPFF	Beta-casein	Bos taurus	207-224	Immunomodulatory	10.1016/0165-2478(92)90091-2
NIPPLTQTPV	P02666	NIPPLTQTPV	Beta-casein	Bos taurus	88-97	ACE-inhibitory	10.1128/AEM.66.9.3898-
NVPGEIVESL	P02666	NVPGEIVESL	Beta-casein	Bos taurus	22-31	Antioxidant	10.3390/antiox9020117
QEPVLGPVRGPFPIIV	P02666	QEPVLGPVRGPFPIIV	Beta-casein	Bos taurus	209-224	ACE-inhibitory	10.3168/jds.2015-9569
SQSKVLPVPQ	P02666	SQSKVLPVPQ	Beta-casein	Bos taurus	181-190	ACE-inhibitory	10.1128/AEM.00096-07
TQTPVVVPPFLQPE	P02666	TQTPVVVPPFLQPE	Beta-casein	Bos taurus	93-106	Antioxidant	10.1016/j.foodchem.2010.05.029
VAPFPE	P02662	VAPFPE	Alpha-S1-casein	Bos taurus	40-45	Inhibition of cholesterol	10.3168/jds.2019-17586
VPSERYL	P04653	VPSERYL	Alpha-S1-casein	Ovis aries	101-107	ACE-inhibitory	10.1016/j.idairyj.2004.04.007
VQVTSTAV	P02668	VQVTSTAV	Kappa-casein	Bos taurus	183-190	Antimicrobial	N/A
YQEPVL	P02666	YQEPVL	Beta-casein	Bos taurus	208-213	ACE-inhibitory	10.1016/S0958-6946(98)00048-X, 10.1016/j.idairyj.2007.02.009
YQEPVLGPVRGPFPI	P33048	YQEPVLGPVRGPFPI	Beta-casein	Capra hircu:	206-220	Antimicrobial	10.1017/S0007114511001085, 10.1111/j.1365-
YQEPVLGPVRGPFPII	P02666	YQEPVLGPVRGPFPII	Beta-casein	Bos taurus	208-224	immunomodulatory	N/A
YQEPVLGPVRGPFPII	P02666	YQEPVLGPVRGPFPII	Beta-casein	Bos taurus	208-224	antithrombin	10.1016/j.idairyj.2012.05.002
YQEPVLGPVRGPFPII	P02666	YQEPVLGPVRGPFPII	Beta-casein	Bos taurus	208-224	Antimicrobial	10.1111/j.1365-
YQEPVLGPVRGPFPII	P02666	YQEPVLGPVRGPFPII	Beta-casein	Bos taurus	208-224	ACE-inhibitory	0302(94)77026-0
YQEPVLGPVRGPFPII	P02666	YQEPVLGPVRGPFPII	Beta-casein	Bos taurus	208-224	Immunomodulatory	N/A

Supplementary Table 4.8. Bioactive peptide sequences identified in delactosed permeate from production plant 2, batch B

Search peptide	Protein I	Peptide	Protein description	Species	Intervals	Function	DOI
AVPYPQR	P02666	AVPYPQR	Beta-casein	Bos taurus	192-198	Antioxidant	10.1021/jf0003911,
AVPYPQR	P02666	AVPYPQR	Beta-casein	Bos taurus	192-198	Antimicrobial	10.1002/biof.1023,
AVPYPQR	P02666	AVPYPQR	Beta-casein	Bos taurus	192-198	ACE-inhibitory	10.1080/00021369.1985.10866901, 10.1016/S0958-6946(98)00048-X
FFVAPFPEVFGK	P02662	FFVAPFPEVFGK	Alpha-S1-casein	Bos taurus	38-49	ACE-inhibitory	10.1016/j.foodchem.2014.09.098, 10.1080/00021369.1982.10865255, 10.1016/S0014-5793(02)03576-7
FVAPFPEVFG	P02662	FVAPFPEVFG	Alpha-S1-casein	Bos taurus	39-48	ACE-inhibitory	10.1021/jf049510t
IHPFAQTQ	P02666	IHPFAQTQ	Beta-casein	Bos taurus	64-71	prolyl endopeptidase-	10.1007/BF02019390
IHPFAQTQ	P02666	IHPFAQTQ	Beta-casein	Bos taurus	64-71	PEP-inhibitory	10.1271/bbb.56.976
KVLPVPQ	P02666	KVLPVPQ	Beta-casein	Bos taurus	184-190	ACE-inhibitory	10.3168/jds.S0022-0302(96)76487-1, 10.3168/jds.2019-17976
KVLPVPQ	P02666	KVLPVPQ	Beta-casein	Bos taurus	184-190	Anti-inflammatory	10.3168/jds.2019-17976
LLYQEPVLGPVRGPF	P02666	LLYQEPVLGPVRGPF	Beta-casein	Bos taurus	206-224	ACE-inhibitory	10.3168/jds.S0022-0302(94)77026-0
LYQEPVLGPVRGPFF	P02666	LYQEPVLGPVRGPFF	Beta-casein	Bos taurus	207-224	Immunomodulatory	10.1016/0165-2478(92)90091-2
NIPPLTQTPV	P02666	NIPPLTQTPV	Beta-casein	Bos taurus	88-97	ACE-inhibitory	10.1128/AEM.66.9.3898-3904.2000
NVPGEIVESL	P02666	NVPGEIVESL	Beta-casein	Bos taurus	22-31	Antioxidant	10.3390/antiox9020117
PVVVPPFLQPE	P33048	PVVVPPFLQPE	Beta-casein	Capra hircu:	96-106	Antimicrobial	10.1017/S0007114511001085
QEPVLGPVRGPFPIIV	P02666	QEPVLGPVRGPFPIIV	Beta-casein	Bos taurus	209-224	ACE-inhibitory	10.3168/jds.2015-9569
SKVLPVPQ	P02666	SKVLPVPQ	Beta-casein	Bos taurus	183-190	ACE-inhibitory	10.3168/jds.S0022-0302(94)77026-0
SQSKVLPVPQ	P02666	SQSKVLPVPQ	Beta-casein	Bos taurus	181-190	ACE-inhibitory	10.1128/AEM.00096-07
VESTVATL	P02668	VESTVATL	Kappa-casein	Bos taurus	160-167	Antimicrobial	N/A
VQVTSTAV	P02668	VQVTSTAV	Kappa-casein	Bos taurus	183-190	Antimicrobial	N/A
VYPFPGPIPN	P02666	VYPFPGPIPN	Beta-casein	Bos taurus	74-83	Antioxidant	10.1007/s00217-012-1894-5, 10.3390/antiox9020117
VYPFPGPIPN	P02666	VYPFPGPIPN	Beta-casein	Bos taurus	74-83	ACE-inhibitory	10.1007/s00217-012-1894-5
YQEPVLGPVRGPFPI	P33048	YQEPVLGPVRGPFPI	Beta-casein	Capra hircu:	206-220	Antimicrobial	10.1017/S0007114511001085, 10.1111/j.1365-2672.2008.03996.x
YQEPVLGPVRGPFPII	P02666	YQEPVLGPVRGPFPII	Beta-casein	Bos taurus	208-224	immunomodulatory	N/A
YQEPVLGPVRGPFPII	P02666	YQEPVLGPVRGPFPII	Beta-casein	Bos taurus	208-224	antithrombin	10.1016/j.idairyj.2012.05.002
YQEPVLGPVRGPFPII	P02666	YQEPVLGPVRGPFPII	Beta-casein	Bos taurus	208-224	Antimicrobial	10.1111/j.1365-2672.2008.03996.x
YQEPVLGPVRGPFPII	P02666	YQEPVLGPVRGPFPII	Beta-casein	Bos taurus	208-224	ACE-inhibitory	10.3168/jds.S0022-0302(94)77026-0
YQEPVLGPVRGPFPII	P02666	YQEPVLGPVRGPFPII	Beta-casein	Bos taurus	208-224	Immunomodulatory	N/A

Supplementary Table 4.9. Bioactive peptide sequences identified in delactosed permeate from production plant 2, batch C

Search peptide	Protein	Peptide	Protein description	Species	Intervals	Function	DOI
KVLPVPQ	P02666	KVLPVPQ	Beta-casein	Bos taurus	184-190	ACE-inhibitory	10.3168/jds.S0022-0302(96)76487-1,
KVLPVPQ	P02666	KVLPVPQ	Beta-casein	Bos taurus	184-190	Anti-inflammatory	10.3168/jds.2019-17976
LLYQEPVLGPVRGPF	P02666	LLYQEPVLGPVRGPF	Beta-casein	Bos taurus	206-224	ACE-inhibitory	0302(94)77026-0
LYQEPVLGPVRGPFF	P02666	LYQEPVLGPVRGPFF	Beta-casein	Bos taurus	207-224	Immunomodulatory	10.1016/0165-2478(92)90091-2
NIPPLTQTPV	P02666	NIPPLTQTPV	Beta-casein	Bos taurus	88-97	ACE-inhibitory	10.1128/AEM.66.9.3898-
VQVTSTAV	P02668	VQVTSTAV	Kappa-casein	Bos taurus	183-190	Antimicrobial	N/A
VYPFPGPIPN	P02666	VYPFPGPIPN	Beta-casein	Bos taurus	74-83	Antioxidant	10.1007/s00217-012-1894-5, 10.3390/antiox9020117
VYPFPGPIPN	P02666	VYPFPGPIPN	Beta-casein	Bos taurus	74-83	ACE-inhibitory	10.1007/s00217-012-1894-5
YQEPVL	P02666	YQEPVL	Beta-casein	Bos taurus	208-213	ACE-inhibitory	10.1016/S0958-6946(98)00048-X, 10.1016/j.idairyj.2007.02.009
YQEPVLGPVRGPFPI	P33048	YQEPVLGPVRGPFPI	Beta-casein	Capra hircu:	206-220	Antimicrobial	10.1017/S0007114511001085, 10.1111/j.1365-
YQEPVLGPVRGPFPII	P02666	YQEPVLGPVRGPFPII	Beta-casein	Bos taurus	208-224	immunomodulatory	N/A
YQEPVLGPVRGPFPII	P02666	YQEPVLGPVRGPFPII	Beta-casein	Bos taurus	208-224	antithrombin	10.1016/j.idairyj.2012.05.002
YQEPVLGPVRGPFPII	P02666	YQEPVLGPVRGPFPII	Beta-casein	Bos taurus	208-224	Antimicrobial	10.1111/j.1365-
YQEPVLGPVRGPFPII	P02666	YQEPVLGPVRGPFPII	Beta-casein	Bos taurus	208-224	ACE-inhibitory	0302(94)77026-0
YQEPVLGPVRGPFPII	P02666	YQEPVLGPVRGPFPII	Beta-casein	Bos taurus	208-224	Immunomodulatory	N/A

Supplementary Table 4.20. Bioactive peptide sequences identified in delactosed permeate from production plant 2, batch D

Search peptide	Protein I	Peptide	Protein description	Species	Intervals	Function	DOI
FVAPFPEVFG	P02662	FVAPFPEVFG	Alpha-S1-casein	Bos taurus	39-48	ACE-inhibitory	10.1021/jf049510t
IHPFAQTQ	P02666	IHPFAQTQ	Beta-casein	Bos taurus	64-71	prolyl endopeptidase-	10.1007/BF02019390
IHPFAQTQ	P02666	IHPFAQTQ	Beta-casein	Bos taurus	64-71	PEP-inhibitory	10.1271/bbb.56.976
IPIQY	P02668	IPIQY	Kappa-casein	Bos taurus	47-51	DPP-IV Inhibitory	10.1016/j.foodchem.2013.08.097, 10.1039/c6fo01411a
KVLPVPQ	P02666	KVLPVPQ	Beta-casein	Bos taurus	184-190	ACE-inhibitory	10.3168/jds.S0022-0302(96)76487-1,
KVLPVPQ	P02666	KVLPVPQ	Beta-casein	Bos taurus	184-190	Anti-inflammatory	10.3168/jds.2019-17976
LLYQEPVLGPVRGPF	P02666	LLYQEPVLGPVRGPF	Beta-casein	Bos taurus	206-224	ACE-inhibitory	10.3168/jds.S0022-
LYQEPVLGPVRGPFF	P02666	LYQEPVLGPVRGPFF	Beta-casein	Bos taurus	207-224	Immunomodulatory	10.1016/0165-2478(92)90091-2
NIPPLTQTPV	P02666	NIPPLTQTPV	Beta-casein	Bos taurus	88-97	ACE-inhibitory	10.1128/AEM.66.9.3898-
QEPVLGPVRGPFPIIV	P02666	QEPVLGPVRGPFPIIV	Beta-casein	Bos taurus	209-224	ACE-inhibitory	10.3168/jds.2015-9569
SQSKVLPVPQ	P02666	SQSKVLPVPQ	Beta-casein	Bos taurus	181-190	ACE-inhibitory	10.1128/AEM.00096-07
TQTPVVVPPFLQPE	P02666	TQTPVVVPPFLQPE	Beta-casein	Bos taurus	93-106	Antioxidant	10.1016/j.foodchem.2010.05.029
VAPFPE	P02662	VAPFPE	Alpha-S1-casein	Bos taurus	40-45	Inhibition of cholesterol solubility	10.3168/jds.2019-17586
VQVTSTAV	P02668	VQVTSTAV	Kappa-casein	Bos taurus	183-190	Antimicrobial	N/A
VYPFPGPIPN	P02666	VYPFPGPIPN	Beta-casein	Bos taurus	74-83	Antioxidant	10.1007/s00217-012-1894-5, 10.3390/antiox9020117
VYPFPGPIPN	P02666	VYPFPGPIPN	Beta-casein	Bos taurus	74-83	ACE-inhibitory	10.1007/s00217-012-1894-5
YQEPVL	P02666	YQEPVL	Beta-casein	Bos taurus	208-213	ACE-inhibitory	10.1016/S0958-6946(98)00048-X, 10.1016/j.idairyj.2007.02.009
YQEPVLGPVRGPFPI	P33048	YQEPVLGPVRGPFPI	Beta-casein	Capra hircu:	206-220	Antimicrobial	10.1017/S0007114511001085, 10.1111/j.1365-
YQEPVLGPVRGPFPII	P02666	YQEPVLGPVRGPFPII	Beta-casein	Bos taurus	208-224	immunomodulatory	N/A
YQEPVLGPVRGPFPII	P02666	YQEPVLGPVRGPFPII	Beta-casein	Bos taurus	208-224	antithrombin	10.1016/j.idairyj.2012.05.002
YQEPVLGPVRGPFPII	P02666	YQEPVLGPVRGPFPII	Beta-casein	Bos taurus	208-224	Antimicrobial	10.1111/j.1365-
YQEPVLGPVRGPFPII	P02666	YQEPVLGPVRGPFPII	Beta-casein	Bos taurus	208-224	ACE-inhibitory	10.3168/jds.S0022-
YQEPVLGPVRGPFPII	P02666	YQEPVLGPVRGPFPII	Beta-casein	Bos taurus	208-224	Immunomodulatory	N/A

Supplementary Table 4.21. Bioactive peptide sequences identified in delactosed permeate from production plant 2, batch E

Search peptide	Protein I	Peptide	Protein description	Species	Intervals	Function	DOI
FVAPFPEVFG	P02662	FVAPFPEVFG	Alpha-S1-casein	Bos taurus	39-48	ACE-inhibitory	10.1021/jf049510t
LLYQEPVLGPVRGPF	P02666	LLYQEPVLGPVRGPF	Beta-casein	Bos taurus	206-224	ACE-inhibitory	10.3168/jds.S0022-
LYQEPVLGPVRGPFF	P02666	LYQEPVLGPVRGPFF	Beta-casein	Bos taurus	207-224	Immunomodulatory	10.1016/0165-2478(92)90091-2
NIPPLTQTPV	P02666	NIPPLTQTPV	Beta-casein	Bos taurus	88-97	ACE-inhibitory	10.1128/AEM.66.9.3898-
QEPVLGPVRGPFPIIV	P02666	QEPVLGPVRGPFPIIV	Beta-casein	Bos taurus	209-224	ACE-inhibitory	10.3168/jds.2015-9569
TQTPVVVPPFLQPE	P02666	TQTPVVVPPFLQPE	Beta-casein	Bos taurus	93-106	Antioxidant	10.1016/j.foodchem.2010.05.029
VQVTSTAV	P02668	VQVTSTAV	Kappa-casein	Bos taurus	183-190	Antimicrobial	N/A
YQEPVLGPVRGPFPI	P33048	YQEPVLGPVRGPFPI	Beta-casein	Capra hircu	206-220	Antimicrobial	10.1017/S0007114511001085, 10.1111/j.1365-2672.2008.03996.x

REFERENCES

Aldredge DL, Geronimo MR, Hua S, Nwosu CC, Lebrilla CB, Barile D. 2013. Annotation and structural elucidation of bovine milk oligosaccharides and determination of novel fucosylated structures. *Glycobiology* 23:664–676.

Auestad N, Layman DK. 2021. Dairy bioactive proteins and peptides: a narrative review. *Nutr. Rev.* 79:36–47.

Boudry G, Hamilton MK, Chichlowski M, Wickramasinghe S, Barile D, Kalanetra KM, Mills DA, Raybould HE. 2017. Bovine milk oligosaccharides decrease gut permeability and improve inflammation and microbial dysbiosis in diet-induced obese mice. *J. Dairy Sci.* 100:2471–2481.

Bund RK, Hartel RW. 2013. Blends of delactosed permeate and pro-cream in ice cream: Effects on physical, textural and sensory attributes. *Int Dairy J.* 31:132-138.

Bund RK, Hartel RW. 2010. Effect of protein on sorption characteristics of delactosed permeate. *J. Food Eng.* 96:388–393.

Burrington K, Schoenfuss T, Patel S. 2014. Coproducts of milk and whey processing.

Charbonneau MR, O'Donnell D, Blanton LV, Totten SM, Davis JCC, Barratt MJ, Cheng J, Guruge J, Talcott M, Bain JR, Muehlbauer MJ, Ilkayeva O, Wu C, Struckmeyer T, Barile D, Mangani C, Jorgensen J, Fan Y, Maleta K, Dewey KG, Ashorn P, Newgard CB, Lebrilla C, Mills DA, Gordon JI. 2016. Sialylated milk oligosaccharides promote microbiota-dependent growth in models of infant undernutrition. *Cell* 164:859–871.

Cheryan M. 1998. Ultrafiltration and Microfiltration Handbook. Taylor & Francis Routledge.

Cicero AFG, Gerocarni B, Laghi L, Borghi C. 2011. Blood pressure lowering effect of lactotripeptides assumed as functional foods: a meta-analysis of current available clinical trials. *J. Hum. Hypertens.* 25:425–436.

Cohen JL, Barile D, Liu Y, de Moura Bell JMLN. 2017. Role of pH in the recovery of bovine milk oligosaccharides from colostrum whey permeate by nanofiltration. *Int. Dairy J.* 66:68–75.

Coppa GV, Zampini L, Galeazzi T, Facinelli B, Ferrante L, Capretti R, Orazio G. 2006. Human milk oligosaccharides inhibit the adhesion to Caco-2 cells of diarrheal pathogens: *Escherichia coli*, *Vibrio cholerae*, and *Salmonella* fyris. *Pediatr. Res.* 59:377–382.

Dallas DC, Guerrero A, Parker EA, Garay LA, Bhandari A, Lebrilla CB, Barile D, German JB. 2014a. Peptidomic profile of milk of Holstein cows at peak lactation. *J. Agric. Food Chem.* 62:58–65.

Dallas DC, Weinborn V, de Moura Bell JMLN, Wang M, Parker EA, Guerrero A, Hettinga KA, Lebrilla CB, German JB, Barile D. 2014b. Comprehensive peptidomic and glycomic evaluation reveals that sweet whey permeate from colostrum is a source of milk protein-derived peptides and oligosaccharides. *Food Res. Int.* 63:203–209.

El-Nawawy M, Radwan U, El-Behairy S, Hassan M. 2012. Derived peptides from milk permeate as a health ingredient: Properties and applicable aspects. *J. Food Dairy Sci.* 3:447–460.

Frankowski KM, Miracle RE, Drake MA. 2014. The role of sodium in the salty taste of permeate. *J. Dairy Sci.* 97:5356–5370.

Friend MA, Kaiser AG, Piltz JW, Sillence MN, Jolliffe SK. 2004. Use of delactose whey permeate as a supplement for cattle on a cereal straw based diet. *Aust. J. Exp. Agric.* 44:833.

Gill HS, Doull F, Rutherfurd KJ, Cross ML. 2000. Immunoregulatory peptides in bovine milk. *Br. J. Nutr.* 84:111–117.

Guerrero A, Dallas DC, Contreras S, Bhandari A, Cánovas A, Islas-Trejo A, Medrano JF, Parker EA, Wang M, Hettinga K, Chee S, German JB, Barile D, Lebrilla CB. 2015. Peptidomic analysis of healthy and subclinically mastitic bovine milk. *Int. Dairy J.* 46:46–52.

Jacobi SK, Yatsunenko T, Li D, Dasgupta S, Yu RK, Berg BM, Chichlowski M, Odle J. 2016. Dietary isomers of sialyllactose increase ganglioside sialic acid concentrations in the corpus callosum and cerebellum and modulate the colonic microbiota of formula-fed piglets. *J. Nutr.* 146:200–208.

Jauhiainen T, Niittynen L, Orešič M, Järvenpää S, Hiltunen TP, Rönnback M, Vapaatalo H, Korpela R. 2012. Effects of long-term intake of lactotripeptides on cardiovascular risk factors in hypertensive subjects. *Eur. J. Clin. Nutr.* 66:843–849.

Lane JA, Mariño K, Naughton J, Kavanaugh D, Clyne M, Carrington SD, Hickey RM. 2012. Anti-infective bovine colostrum oligosaccharides: *Campylobacter jejuni* as a case study. *Int. J. Food Microbiol.* 157:182–188.

Levin MA, Burrington KJ, Hartel RW. 2016. Whey protein phospholipid concentrate and delactosed permeate: Applications in caramel, ice cream, and cake. *J. Dairy Sci.* 99:6948–6960.

Liang B, Bund RK, Hartel RW. 2009. Effect of composition on moisture sorption of delactosed permeate. *Int. Dairy J.* 19:630–636.

Luo J, Wan Y. 2013. Effects of pH and salt on nanofiltration - a critical review. *J. Membr. Sci.* 438:18–28.

Marotta M, Ryan JT, Hickey RM. 2014. The predominant milk oligosaccharide 6′-sialyllactose reduces the internalisation of *Pseudomonas aeruginosa* in human pneumocytes. *J. Funct. Foods* 6:367–373.

Martín-Sosa S, Martín M-J, Hueso P. 2002. The sialylated fraction of milk oligosaccharides is partially responsible for binding to Enterotoxigenic and Uropathogenic *Escherichia coli* human strains. *J. Nutr.* 132:3067–3072.

Mehra R, Barile D, Marotta M, Lebrilla CB, Chu C, German JB. 2014. Novel high-molecular weight fucosylated milk oligosaccharides identified in dairy streams. *PLoS ONE* 9:e96040.

Milk Bioactive Peptide Database, http://mbpdb.nws.oregonstate.edu/, Accessed 12/19/2020.

Miralles B, Hernández-Ledesma B, Fernández-Tomé S, Amigo L, Recio I. 2018. Health-related functional value of dairy proteins and peptides, in: Proteins in Food Processing. Elsevier, pp. 523–568.

Nongonierma AB, FitzGerald RJ. 2015. The scientific evidence for the role of milk protein-derived bioactive peptides in humans: A review. *J. Funct. Foods* 17:640–656.

Obelitz-Ryom K, Bering SB, Overgaard SH, Eskildsen SF, Ringgaard S, Olesen JL, Skovgaard K, Pankratova S, Wang B, Brunse A, Heckmann AB, Rydal MP, Sangild PT, Thymann T. 2019. Bovine milk oligosaccharides with sialyllactose improves cognition in preterm pigs. *Nutrients* 11:1335.

Oliveros E, Vázquez E, Barranco A, Ramírez M, Gruart A, Delgado-García J, Buck R, Rueda R, Martín M. 2018. Sialic acid and sialylated oligosaccharide supplementation during lactation improves learning and memory in rats. *Nutrients* 10:1519.

Østdal H, Andersen HJ, Nielsen JH. 2000. Antioxidative activity of urate in bovine milk. *J. Agric. Food Chem.* 48:5588–5592.

Parkkinen J, Rogers GN, Korhonen T, Dahr W, Finne J. 1986. Identification of the O-linked sialyloligosaccharides of glycophorin A as the erythrocyte receptors for S-fimbriated *Escherichia coli*. *Infect. Immun.* 54:37–42.

Paterson AHJ. 2009. Production and Uses of Lactose, in: McSweeney, P.L.H., Fox, P., F. (Eds.), Advanced Dairy Chemistry. Springer Science+Business Media, New York, NY, USA, pp. 105–120.

Perdijk O, van Baarlen P, Fernandez-Gutierrez MM, van den Brink E, Schuren FHJ, Brugman S, Savelkoul HFJ, Kleerebezem M, van Neerven RJJ. 2019. Sialyllactose and galactooligosaccharides promote epithelial barrier functioning and distinctly modulate microbiota composition and short chain fatty acid production *in vitro*. *Front. Immunol.* 10:94.

Sah BNP, Vasiljevic T, McKechnie S, Donkor ON. 2018. Antioxidative and antibacterial peptides derived from bovine milk proteins. *Crit. Rev. Food Sci. Nutr.* 58:726–740.

Slavov AK. 2017. Dairy wastewaters – General characteristics and treatment possibilities – a review. *Food Technol. Biotechnol.* 55:14-28.

Smith ST, Metzger L, Drake MA. 2016. Evaluation of whey, milk, and delactosed permeates as salt substitutes. *J. Dairy Sci.* 99:8687–8698.

Sunds AV, Bunyatratchata A, Robinson R, Glantz M, Paulsson M, Leskauskaite D, Pihlanto A, Inglingstad R, Devold TG, Vegarud GE, Birgisdottir BE, Gudjonsdottir M, Barile D, Larsen LB, Poulsen NA. 2021. Comparison of bovine milk oligosaccharides in native North European cattle breeds. *Int. Dairy J.* 114:104917.

Swiss-Prot Database, https://www.uniprot.org/, Accessed 12/19/2020.

United States Department of Agriculture National Agricultural Statistics Service. 2019. Dairy Products Summary.

Wagner C, Benecke C, Buchholz H, Beutel S. 2014. Enhancing bioethanol production from delactosed whey permeate by upstream desalination techniques. *Eng. Life Sci.* 14:520–529.

Wong SY, Hartel RW. 2014. Crystallization in lactose refining – A review. *J. Food Sci.* 79:257-272.

Zhang M, Sun X, Cheng J, Guo M. 2022. Analysis and “Comparison of nutrition profiles of canine milk with bovine and caprine milk. *Foods*. 11:472.

CHAPTER V:

Fifty years of research on milk oligosaccharides: Querying the body of literature for humans and other mammals

ABSTRACT

The carbohydrate fraction of most mammalian milks contains a variety of oligosaccharides that encompass a range of structures and monosaccharide compositions. Human milk oligosaccharides have received considerable recent attention due to their biological roles contributing to the establishment and maintenance of beneficial gut microbiota, prevention of pathogen binding to the intestinal epithelium, immunomodulation, and brain development in the neonate. Non-human mammals have varying milk oligosaccharide profiles that are adapted to their gestational systems and the needs of their offspring. Parity, genotype, breed, and lactation time point may also contribute to observed variation in milk oligosaccharide profiles. Despite this, many species have considerable overlap with the oligosaccharides found in human milk. The milk oligosaccharides of some non-human mammals may also have the potential for commercial isolation and supplementation in human infant formula and other products for human health.

In the present study a database was created to compile the existing milk oligosaccharide profile data across all mammalian species. This database facilitates the comparison of milk oligosaccharide profiles across species by compiling milk oligosaccharide data across more than fifty years of publications and translating the often disparate methods for reporting milk oligosaccharide profiles into a single standardized identification format. Through the consolidation of all existing milk oligosaccharide profiles, this queryable database promotes further analysis of the existing milk oligosaccharide literature, revealing patterns and trends not apparent from the examination of individual publications.

INTRODUCTION

Mammals are characterized as homeothermic vertebrates with mammary glands. Beings within the class *Mammalia* can be divided into placental mammals, marsupials and monotremes, based on how their young are gestated and born. Placental mammals belong to the clade *Eutheria* and are characterized by fetuses which remain in the uterus of the mother and are nourished by the placenta until a comparatively late stage of neonatal development. In contrast, marsupial offspring undergo a brief uterine gestation followed by a period of further development in the mother's pouch, where they begin nursing. Diverging even farther, the young of monotremes are laid in eggs and then undergo further development in their mother's pouch after hatching. While all types of mammalian mothers produce milk to nourish their young after birth, the composition of this milk varies between species.[1,2]

In addition to protein and lipids, carbohydrates are one of the main components of mammalian milk, with oligosaccharides often featuring as the third or fourth most abundant milk component, depending on the species and lactation time point. Milk oligosaccharides are composed of three to twenty monosaccharides. Constituent monosaccharides may include D-glucose (Glc), D-galactose (Gal), D-*N*-acetylglucosamine (GlcNAc), D-*N*-acetylgalactosamine (GalNAc), L-Fucose (Fuc), D-*N*-acetylneuramic acid (Neu5Ac), or D-*N*-glycolylneuraminic acid (Neu5Gc). Milk oligosaccharides feature either a lactose or, less commonly, a lactosamine unit at the reducing end, and their structures may be extended through the addition of Gal, GlcNAc, or GalNAc monomers. Milk oligosaccharides composed of more than three monosaccharides are divided into two basic categories based on their core structures as either type I or type II. Type I cores feature the structure of lacto-*N*-tetraose (LNT, Gal(β1-3)GlcNAc(β1-3)Gal(β1-4)Glc), while type II cores are based off of lacto-*N*-neotetraose (LNnT, Gal(β1-4)GlcNAc(β1-3)Gal(β1-4)Glc), Core structures may also be decorated with Fuc, Neu5Ac, or Neu5Gc. Neu5Ac and Neu5Gc are two forms of sialic acid, and oligosaccharides containing either of these monosaccharides are classified as acidic, while those without any sialic acid are categorized as neutral.

Milk oligosaccharides are of particular interest because, although they are assembled at considerable energetic cost to the mother, they are largely undigested by the neonate. Human milk oligosaccharides have been demonstrated to have prebiotic activity, selectively promoting the growth of beneficial bacteria in the infant gut.[3–8] These probiotics then occupy space on the intestinal epithelium, consume human milk oligosaccharides and produce short chain fatty acids, which lower the pH of the gut, making it difficult for pathogens to colonize the infant gut. In addition, the structural homology of milk oligosaccharides to cell surface glycans of the intestinal epithelium allows them to act as receptor decoys to which pathogens may bind in place of host epithelial cells, resulting in the flushing of pathogens from the gut.[9] Human milk oligosaccharides also have anti-inflammatory and immunomodulatory activities and have been shown to decrease gut permeability associated with obesity.[10–14] In addition, the sialic acid found in milk oligosaccharides has been linked to neonatal brain development and learning.[15–17]
The functions of milk oligosaccharides demonstrated to date are dependent upon their structural motifs. As such, oligosaccharides that share monosaccharide compositions may have distinctly different activities depending on their unique isomer structures. Despite the benefits of human milk oligosaccharides, no equally diverse source of bioactive carbohydrates is currently available outside of mother's milk. Some infant formulas are beginning to be supplemented with prebiotic

oligosaccharides, but in most cases the added compounds are not equivalent to those in human breast milk. Despite their demonstrated prebiotic activity, homooligomers like galactooligosaccharies (GOS) and fructooligosaccharides (FOS) lack the structural complexity and compositional diversity of human milk oligosaccharides.[18] The human milk oligosaccharides commercially produced in quantities sufficient for supplementation to infant formula are relatively small, simple structures as more complex human milk oligosaccharide structures have proven to be difficult and expensive to produce through enzymatic synthesis or in genetically modified microbes.[19]

However, many oligosaccharide structures have been identified in the milk or colostrum of non-human mammals with varying degrees of similarity to human milk oligosaccharides. Some non-human mammalian milks are potential sources of oligosaccharides for commercial isolation for supplementation in human infant formulas and functional foods, while others represent possible biomedical models for developing a further understanding of the roles of human milk oligosaccharides. The biological significance of variations in milk oligosaccharide profiles among mammalian species is not yet fully understood.

The main challenge in building further understanding of milk oligosaccharides from the existing literature lies in the scattering of the relevant data across decades of publications in dozens of academic books and journals. Any cross-publication analysis is additionally hindered by the vast inconsistencies in how milk oligosaccharides have been historically reported, ranging from figures depicting oligosaccharide structures to tables of monosaccharide constituents, to full linkage descriptions in the text. These disparate data reporting methods make it prohibitively difficult to make direct comparisons between oligosaccharide profiles reported using different descriptive methods.

The present study overcomes these challenges through the creation of a database that reconciles all existing milk oligosaccharide profiles through the use of a standardized form of representing milk oligosaccharide structures. This database facilitates the comparison of oligosaccharides between individual species and across groups of species. In addition, the database holds the potential to contribute to answering questions about the biological significance of specific oligosaccharide structural variations across mammalian milks. When combined with queries and visualizations, it will also serve as a generator of hypotheses able to be investigated in future milk oligosaccharide studies.

METHODS

Literature Selection

To enable comparisons between milk oligosaccharide profiles of different species, a database was constructed, containing compilations of the existing published milk oligosaccharide profiles for each species discussed herein. Any studies reporting milk oligosaccharide structures published in a peer reviewed journal or book between January 1970 and January 2022 were considered for inclusion in the database. Publications were excluded from consideration if they had not undergone peer review, were not full articles (i.e. abstract-only publications), did not report original results (i.e. reviews, meta-analyses, secondary analyses of existing published milk oligosaccharides data), did not describe the method through which oligosaccharide analysis was conducted, did not adequately describe the species from which milk was obtained, or were published prior to January 1970 or after January 2022. The number of subjects, milk sample collection method, lactation time point at milk collection, and pooling of milk samples were not used as selection criteria. In cases where the milk oligosaccharides of a species were reported in numerous publications meeting the specified criteria, such as with human and cow milk, papers were selected so as to build an oligosaccharide profile covering the full scope of identified milk oligosaccharides for the species with minimal redundancy. 210 publications covering the milk oligosaccharide profiles of 75 species were included in the database (Supplementary Table 5.2).

Database Construction

Oligosaccharide isomers were distinguished in the database based on the compositional information available in the corresponding literature, with varying degrees of identification based on the analytical technique applied in the study. When available, the sequence of monosaccharides, branching, and monosaccharide linkages were specified in the isomer designation. While this strategy allows for the greatest extent of comparison between milk oligosaccharide profiles presented in different studies, there are likely some remaining isomer redundancies. In particular, this may result when comparing data from NMR, enzymatic, or standard-based chromatographic isomer identifications that contain complete structural information with less detailed identifications made by mass spectral or chromatographic techniques. In total, entries for 672 oligosaccharide isomers were included in the database (Supplementary Table 5.1).

All oligosaccharides are represented by a unique six-digit alphanumeric code where the first five digits sequentially represent the numbers of hexose_*N*-acetylhexosamine_fucose_*N*-

acetylneuraminic acid_*N*-glycolylneuraminic acid (Hex_HexNAc_Fuc_Neu5Ac_Neu5Gc) monomers contained in the oligosaccharide structure and the final letter designates the isomer. For example, 4_2_1_1_0b is composed of 4 hexoses, 2 *N*-acetylhexosamines, 1 fucose, 1 *N*-acetylneuraminic acid, and no *N*-glycolylneuraminic acids, and has been assigned to the specific oligosaccharide Neu5Ac(α2-3)Gal(β1-3)GlcNAc(β1-3)[Gal(β1-4)[Fuc(α1-3)]GlcNAc(β1-6)]Gal(β1-4)Glc. The full list of oligosaccharide isomers and their respective alphanumeric codes is provided in Supplementary Table 5.1.

Analysis of Database Queries

The database was queried to compare oligosaccharide profiles for a variety of groups of species, and the ensuing data was transformed into concept maps using Cmap Tools to visualize the results.

The resulting concept maps can be read from left to right by following the arrows connecting the species names, linking phrases, and oligosaccharides, as exemplified in Figure 5.1. Oligosaccharides color-coded as black, with arrows connecting them to multiple species have been reported in the milk of each species to which they share a connecting arrow. Oligosaccharides that are unique to the milk of a single species in a given concept map are color-coded to match that species and bear only a single connecting arrow.

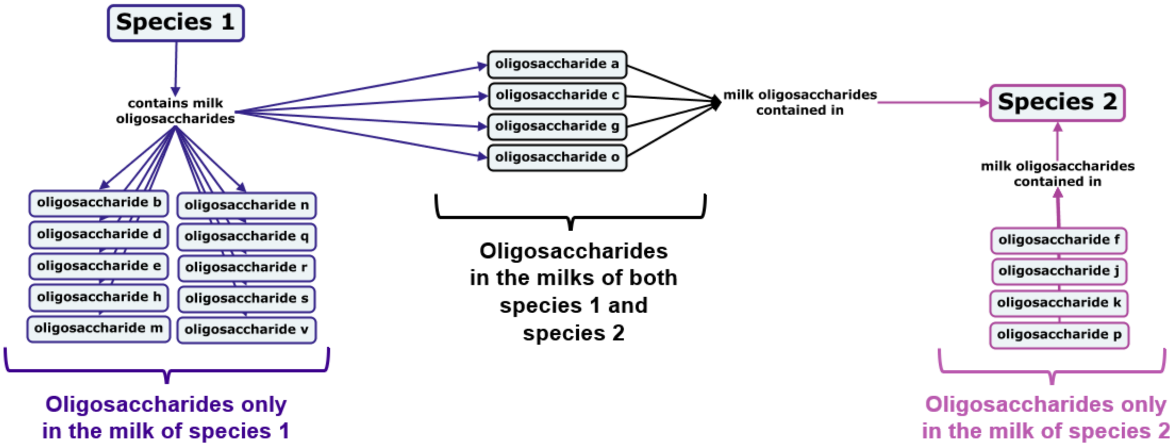


Figure 5.1. Sample concept map depicting the shared and unshared oligosaccharides for two species.

RESULTS AND DISCUSSION

Milk Oligosaccharides of Placental Mammals

Humans

Human milk oligosaccharides are by far the most studied set of milk oligosaccharides of any mammalian species. They feature five constituent monosaccharides: Glc, Gal, Fuc, GlcNAc and Neu5Ac, with twelve possible linkages.[19] To date, more than 200 HMO structures have been identified through the use of various analytical techniques, of which 215 unique human milk oligosaccharide structures have been fully elucidated.[19–53]Variation in human milk oligosaccharide profiles and concentrations due to the secretor and Lewis status of the mother is well documented.[54–62] The secretor gene codes for α1-2-fucosyltransferase, FUT2, and the Lewis gene codes for the α1-3/4-fucosyltransferase, FUT3. Mothers who are positive for the secretor gene, known as secretors, express the FUT2 gene and produce milk containing an abundance of α1-2-linked fucose moieties, while non-secretor mothers produce little to no α1-2-linked fucose-containing human milk oligosaccharides. Individuals who are Lewis positive express the FUT3 gene and produce milk containing oligosaccharides with α1-3- and α1-4-linked fucoses.[63] The milk of Lewis negative mothers does not contain α1-4-linked fucose moieties but may have human milk oligosaccharides with α1-3-linked fucose units due to the activity of a secretor- and Lewis-independent fucosyltransferase.[56] Secretor status is known to vary between regional, racial, or ethnic groups, as shown in Figure 5.2, which contributes to variations in human milk oligosaccharide profiles between cohorts around the world.[54,56,60,61,64–77]

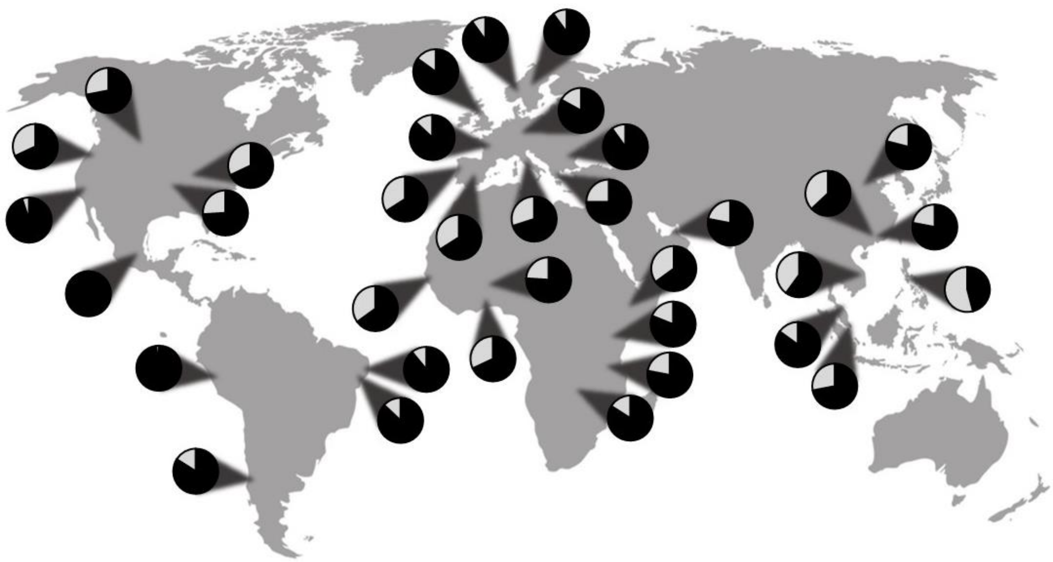


Figure 5.2. Distribution of secretor status in human mothers around the world based on the abundance of α1-2-linked fucose in breast milk, where the black sections of the pie charts represent the percent of mothers in the population who are secretors and the light grey represents the percentage of non-secretors.

In addition, human milk oligosaccharide concentrations have been shown to vary over the course of lactation, with typical oligosaccharide concentrations in human colostrum as high as 20 g/L but falling to as low as 5 g/L in mature milk.[58,59,61,78,79] The concentration of lactose in human milk is comparatively steady across lactation, at around 60 g/L.[80] The oligosaccharide profile of human milk is unique in that it does not contain the Neu5Gc form of sialic acid and contains almost no structures with α1-3-linked galactose. Both Neu5Gc and α1-3-linked galactose may be recognized as allergens in many people.[81,82] For most lactating individuals, neutral fucosylated milk oligosaccharides predominate. In addition, the majority of human milk oligosaccharides contain type I, core structures, a unique feature compared to the predominantly type II oligosaccharides found in most non-human mammalian milks.

Non-human Primates

As the closest relatives to humans, data on the milk oligosaccharides of non-human primates can aid the understanding of human milk oligosaccharides and their roles. The milk oligosaccharides of a number of non-human primates have been investigated, including those of apes (*Pongidae* and *Hylobatidae*), old world monkeys (*Cercopithecidae*), new world monkeys (*Cebidae, Callitrichidae*, and *Atelidae*), and strepsirrhine primates. Of the primate groups, the great apes, including chimpanzees, bonobos, gorillas, and orangutans, are the closest phylogenetic relatives to humans. Chimpanzee and bonobo milks have oligosaccharide profiles that are about 50% fucosylated with both type I and II cores and a 1 to 4 or 1 to 5 ratio of oligosaccharides to lactose, making them the closest in terms of free carbohydrate composition to human milk. Unlike human milk however, chimpanzee and bonobo milk oligosaccharides contain Neu5Gc and have more LNnT- than LNT type core structures (Figure 5.3).[83–85] 2’-FL has been shown to decrease in concentration in bonobo milk over the course of lactation while 3-FL increases in concentration, a trend also observed in human milk. [74,85] In contrast, only α1-2-linked fucose has been identified in gorilla milk, which also contains oligosaccharides with Neu5Gc monomers and both LNT- and LNnT-type cores structures.[83,84] Orangutans have milk with a substantially higher ratio of oligosaccharides to lactose (1 to 0.8) than the other great apes, and their milk oligosaccharide profile contains structures with Neu5Gc and predominantly type II cores (Figure 5.3).[84,85]

The only lesser ape for which milk oligosaccharides have been analyzed is the siamang. Although siamang milk's 1 to 3 ratio of oligosaccharides to lactose is similar to those of the great apes, siamang milk oligosaccharides are the most sialylated of any primate, with only trace amounts of fucosylation (Figure 5.4).[83,84]

Three species of old world monkeys, hamadryas baboon, toque macaque and rhesus macaque, all have milk oligosaccharides with α1-3-linked fucose moieties, but no α1-2-linked fucose-containing oligosaccharides have been identified.[86] Type I core and Neu5Gc-containing oligosaccharides have both been identified in milk of the rhesus macaque, but not in toque macaque or hamadryas baboon milk (Figure 5.4).[83,86]

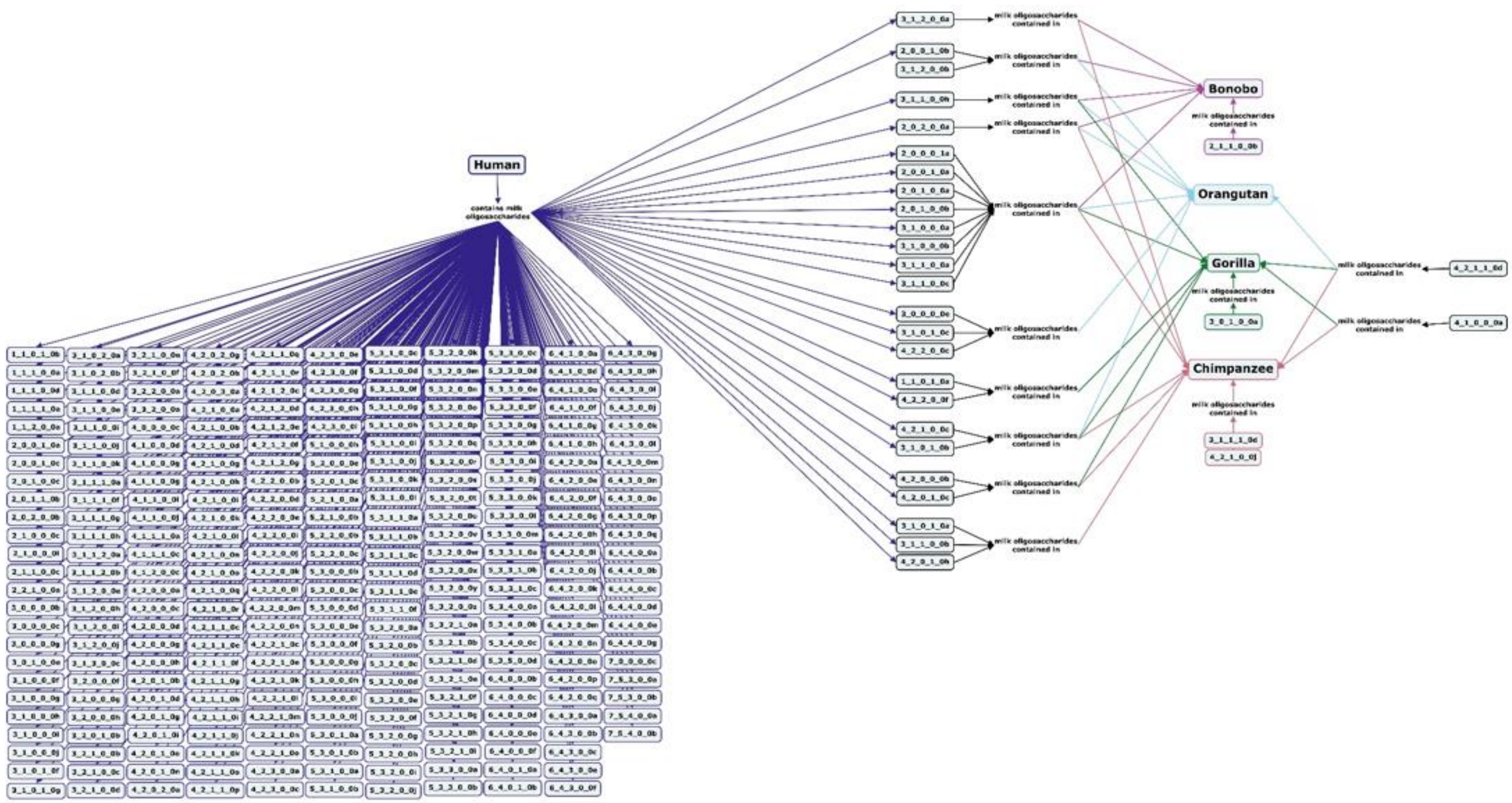


Figure 5.3. Concept map comparing the milk oligosaccharide profiles of four great ape species, bonobos, orangutans, gorillas, and chimpanzees, with human milk oligosaccharides. Oligosaccharides are given as the number of hexose_*N*-acetylhexosamine_fucose_*N*-acetylneuraminic acid_*N*-glycolylneuraminic acid monomers contained in the structure, followed by the isomer designation. The full list of oligosaccharide isomers and their respective alphanumeric codes is provided in Supplementary Table 5.1.

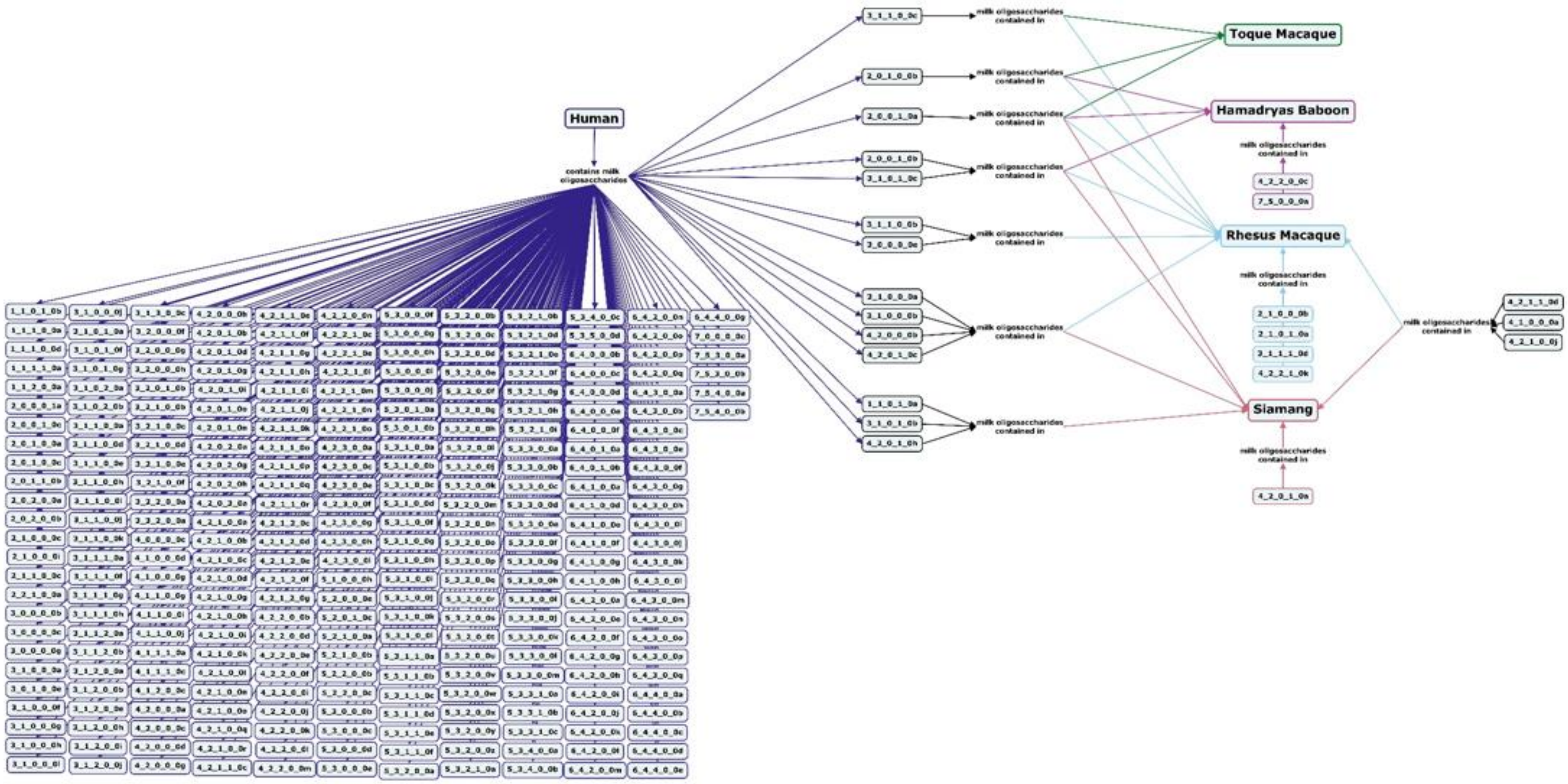


Figure 5.4. Concept map comparing the milk oligosaccharide profiles of four primate species, toque macaque, Hamadryas baboon, rhesus macaque, and siamang, with human milk oligosaccharides. Oligosaccharides are given as the number of hexose_*N*-acetylhexosamine_fucose_*N*-acetylneuraminic acid_*N*-glycolylneuraminic acid monomers contained in the structure, followed by the isomer designation. The full list of oligosaccharide isomers and their respective alphanumeric codes is provided in Supplementary Table 5.1.

Milk oligosaccharides from three of the five families of new world monkeys have been profiled, including samples of mantled howler, brown capuchin, Bolivian squirrel monkey, golden lion tamarin, and common marmoset milk. With the exception of the common marmoset, which has the greatest proportion of fucosylated milk oligosaccharides of all non-human primates, the milk of new world monkeys appears to contain little to no fucosylated or type I oligosaccharides (Figure 5.5). [83,86,87]

Strepsirrhine primates split off from the lineage of other monkeys and apes an estimated 76 to 87 million years ago. Milk oligosaccharides from four species in this suborder have been analyzed to date, including the greater galago, aye-aye, mongoose lemur, and Coquerel’s sifaka. The milk of these species has a similar ratio of lactose and free oligosaccharides as humans and great apes, but LNT-type core structures have only been identified in aye-aye milk (Figure 5.6).[88]

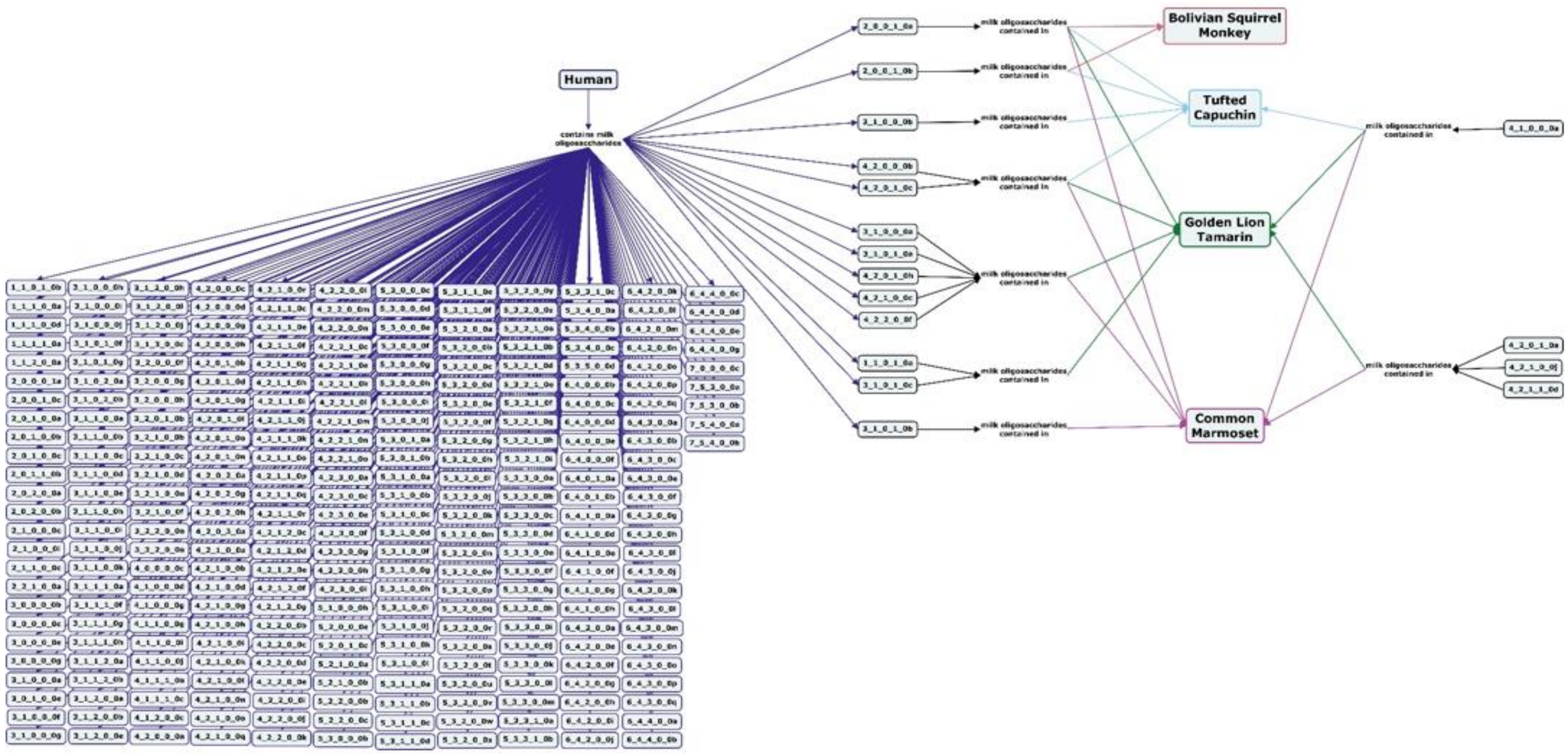


Figure 5.5. Concept map comparing the milk oligosaccharide profiles of four new world monkey species, Bolivian squirrel monkey, tufted capuchin, golden lion tamarin, and common marmoset, with human milk oligosaccharides. Oligosaccharides are given as the number of hexose_*N*-acetylhexosamine_fucose_*N*-acetylneuraminic acid_*N*-glycolylneuraminic acid monomers contained in the structure, followed by the isomer designation. The full list of oligosaccharide isomers and their respective alphanumeric codes is provided in Supplementary Table 5.1.

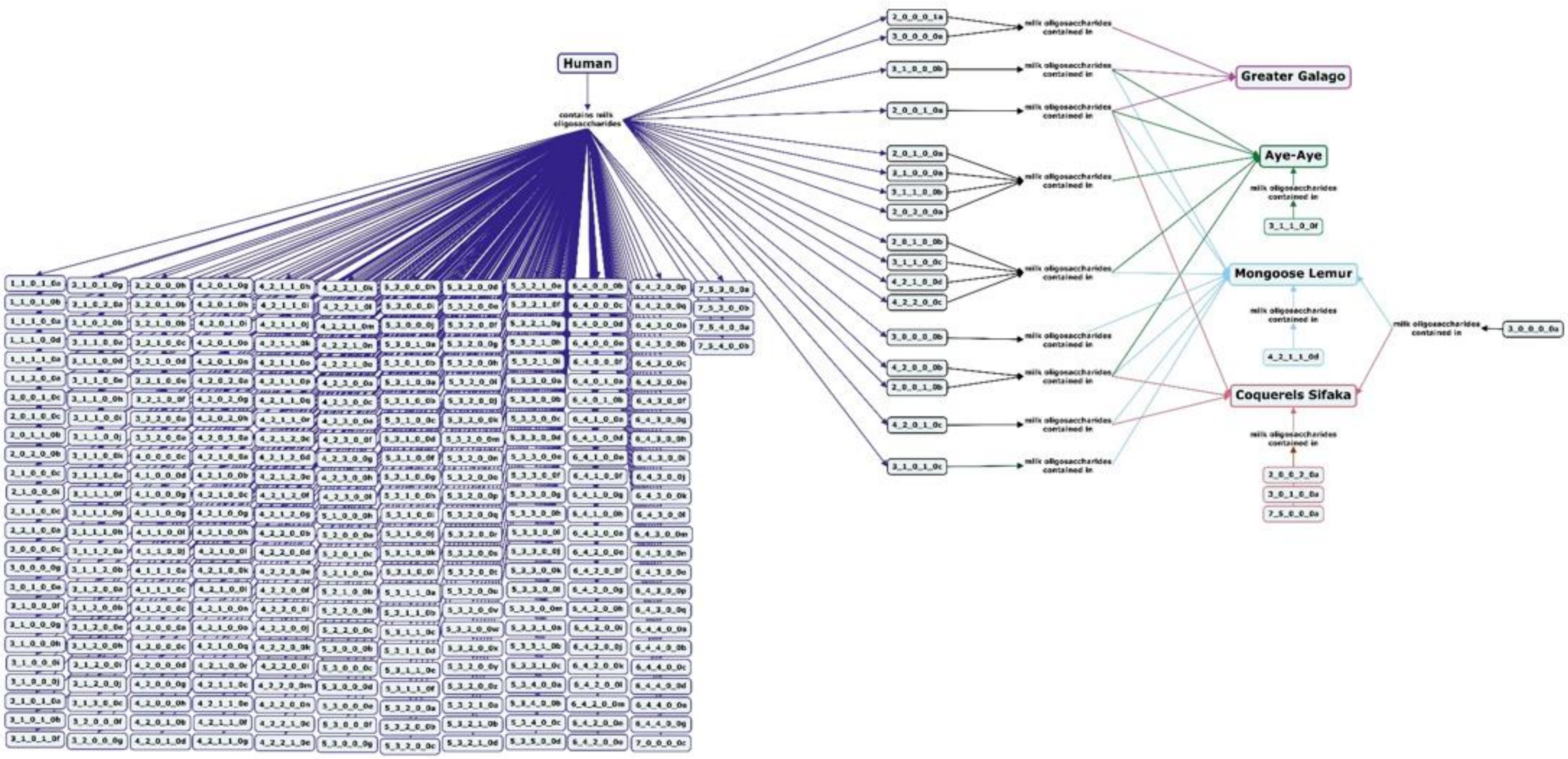


Figure 5.6. Concept map comparing the milk oligosaccharide profiles of four Strepsirrhine primate species, greater galago, aye-aye, mongoose lemur, and coquerels sifaka, with human milk oligosaccharides. Oligosaccharides are given as the number of hexose_*N*-acetylhexosamine_fucose_*N*-acetylneuraminic acid_*N*-glycolylneuraminic acid monomers contained in the structure, followed by the isomer designation. The full list of oligosaccharide isomers and their respective alphanumeric codes is provided in Supplementary Table 5.1.

Overall, primate milk oligosaccharide profiles are more diverse than those of bovine, caprine, or porcine milks and contain similar types of structures as human milk oligosaccharides, but in different proportions (Figures 5.3-5.6).[85] With an average degree of polymerization (DP) of 4 to 6, milk oligosaccharide structures of non-human primates tend to be smaller than human milk oligosaccharides (average DP of 7 to 9).[83] Current research shows only minimal evidence of correlation between milk oligosaccharide profiles of non-human primates and their phylogenetic relations or social structures.[83,88]

Terrestrial Carnivores

The species within the order *Carnivora* can be divided into two suborders, *Feloidea* and *Canoidea*. A handful of species within *Feloidea* have been the subject of milk oligosaccharide investigations. Primarily small neutral oligosaccharides have been identified in the milk of cheetahs, spotted hyenas, and clouded leopards,[89–91] but larger structures, including a variety of fucosylated oligosaccharides have been identified in the milk of house cats and African lions (Figure 5.7).[90,92,93] Only two acidic oligosaccharides have been identified in *Feloidea* milk, with 6'-sialyllactose (6'-SL) identified in the milk of house cats and α2-3-Neu5Gc-lactose found in all profiled milks except cheetah (Figure 5.7).[89,90,92,93] Lions, leopards, and cheetahs all have a milk oligosaccharide to lactose ratio of 1:1 to 1:2, although lion milk has considerably less lactose (about 27 g/kg) compared to cheetah milk (40.2 g/kg).[90,91,94]

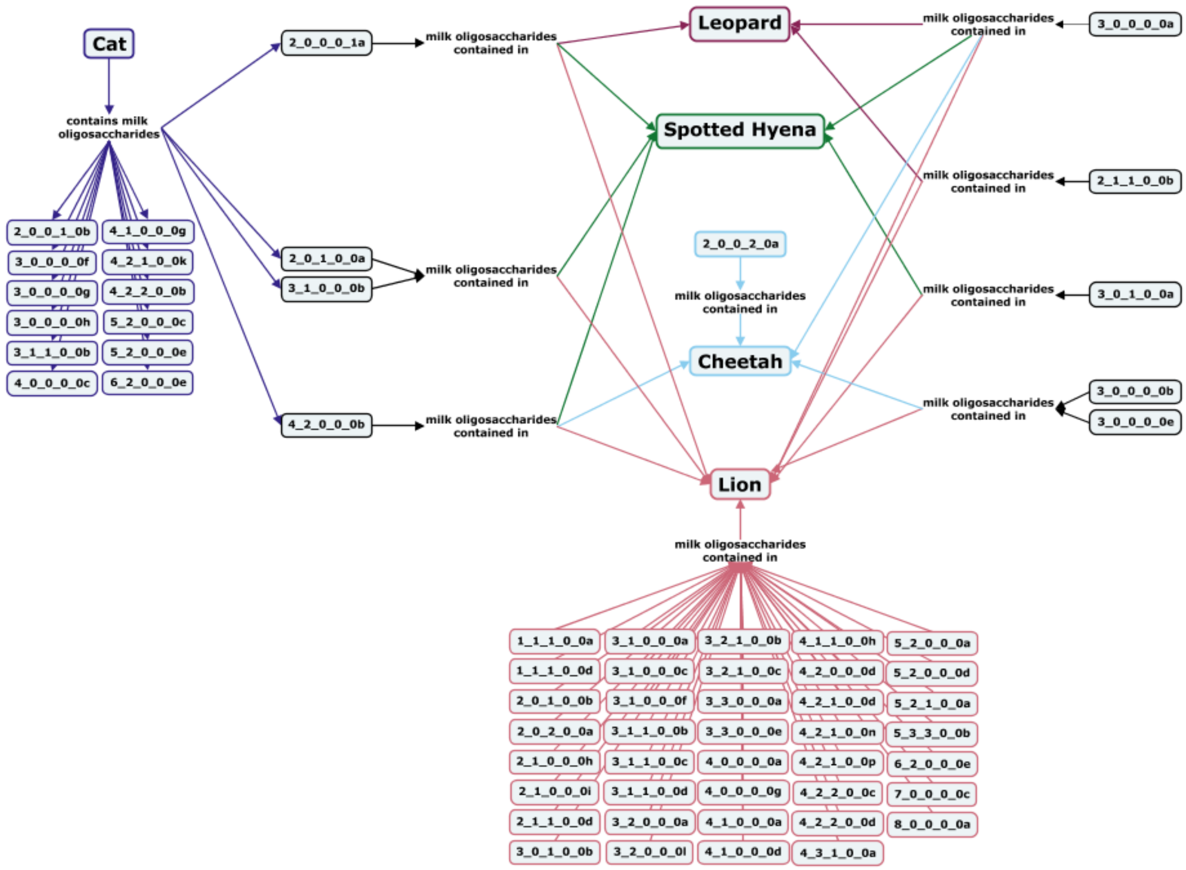


Figure 5.7. Concept map comparing the milk oligosaccharide profiles of four *Feloidea* species, domestic cats, spotted hyenas, cheetahs, and lions. Oligosaccharides are given as the number of hexose_*N*-acetylhexosamine_fucose_*N*-acetylneuraminic acid_*N*-glycolylneuraminic acid monomers contained in the structure, followed by the isomer designation. The full list of oligosaccharide isomers and their respective alphanumeric codes is provided in Supplementary Table 5.1.

Substantially more investigations into the milk oligosaccharide profiles of species within the *Canoidea* suborder of *Carnivora* have been conducted. The milk oligosaccharide profiles of several species of bears have been studied, including those of the American black bear, Japanese black bear, Ezo brown bear, grizzly bear, polar bear, and giant panda. Both American and Japanese black bear milk contains large α1-2- and α1-3-linked fucosylated oligosaccharides, although only type II core structures were identified in Japanese black bear milk, while both LNT- and LNnT-type core milk oligosaccharides have been identified for the American black bear.[85,91,95] No acidic oligosaccharides are present in American black bear milk, but Japanese black bears were shown to produce several α2-3- and α2-6-linked Neu5Ac-containing oligosaccharides.[96] Among the brown bears, milk of the Ezo brown bear is dominated by trisaccharides, especially 2'-FL, while grizzly bear milk contains more DP 4 and 5 fucosylated oligosaccharides with both LNT- and LNnT-type core structures (Figure 5.8).[85,97] Although the total carbohydrate concentration of polar bear milk remains relatively constant, the oligosaccharide profile varies over the course of lactation, with a high 3'-sialyllactose (3'-SL) concentration in colostrum but an abundance of isoglobotriose in mid to late lactation milk. [98,99] In contrast, the carbohydrate fraction of giant panda milk increases over the course of lactation, with isoglobotriose as the main oligosaccharide throughout.[100,101] Lactose concentrations in bear milk are low at around 1 to 4 g/kg, which makes them a notable exception to the typically high lactose concentrations in the milk of placental mammals. This low lactose content serves to protect the hibernating mother during lactation both because lipid content is a more efficient method of energy transfer from mother to nursing offspring and because lower lactose concentrations lead to less osmolytic pressure on the milk, lessening the risk of maternal dehydration. [91,100,102]

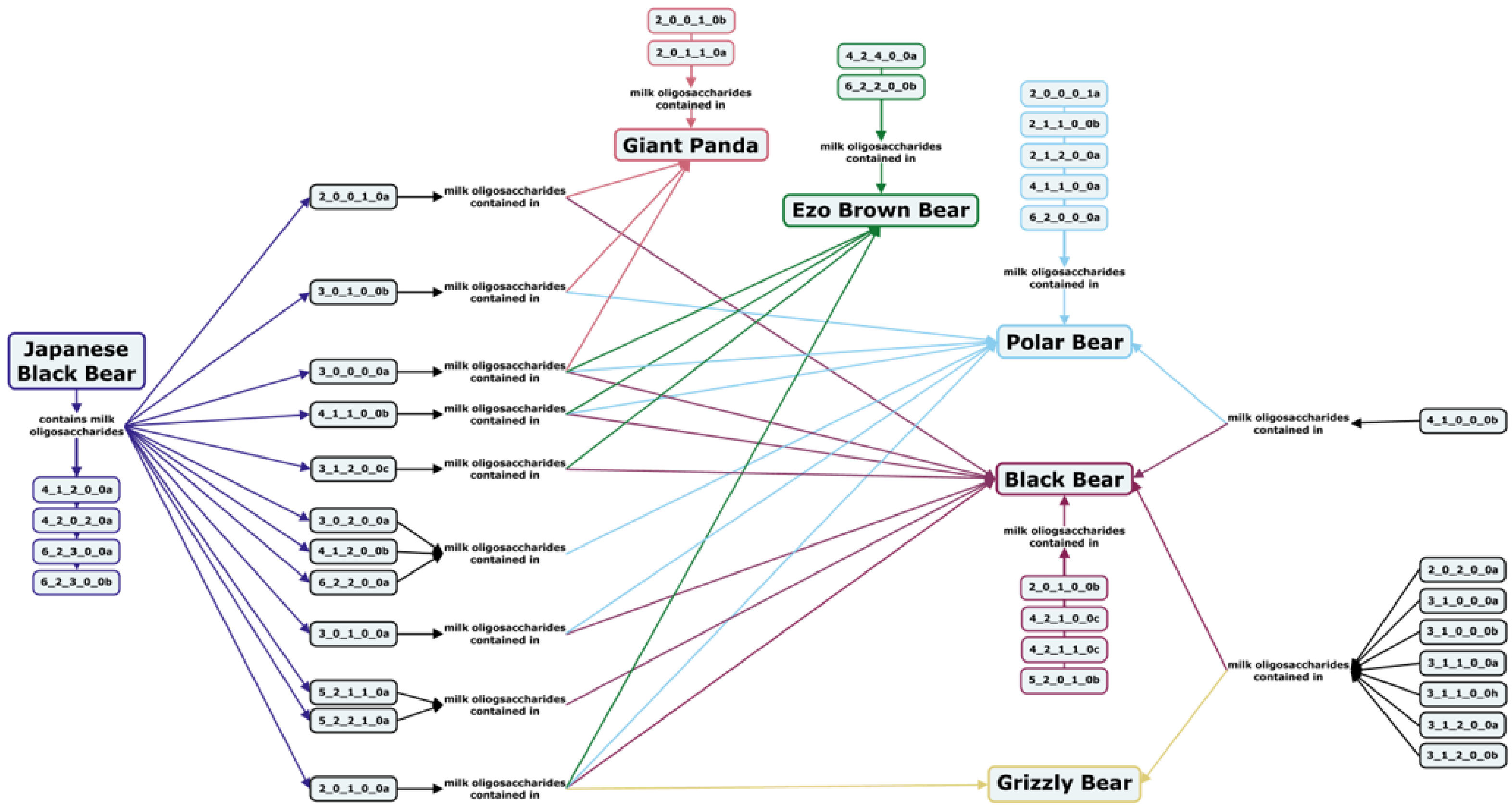


Figure 5.8. Concept map comparing the milk oligosaccharide profiles of five bear species, including Japanese black bears, giant pandas, ezo brown bears, polar bears, black bears, and grizzly bears. Oligosaccharides are given as the number of hexose_*N*-acetylhexosamine_fucose_*N*-acetylneuraminic acid_*N*-glycolylneuraminic acid monomers contained in the structure, followed by the isomer designation. The full list of oligosaccharide isomers and their respective alphanumeric codes is provided in Supplementary Table 5.1.

Like the larger members of *Canoidea*, milk oligosaccharides are dominated by α1,3-linked galactose-containing cores and Neu5Gc-containing structures are absent from raccoon, striped skunk, mink, dog, and white-nosed coati milk (Figure 5.9).[85,92,103–108] No acidic oligosaccharides have been reported in mink or white-nosed coati milk, and no LNT-type core structures or α1-3-linked fucose-containing oligosaccharides have been found in the milk of any of the smaller terrestrial carnivores. Unlike most other *Canoidea*, the oligosaccharides identified in raccoon milk include very large structures (DP 13 to 18) in addition to the smaller neutral fucosylated oligosaccharides (Figure 5.9).[103]

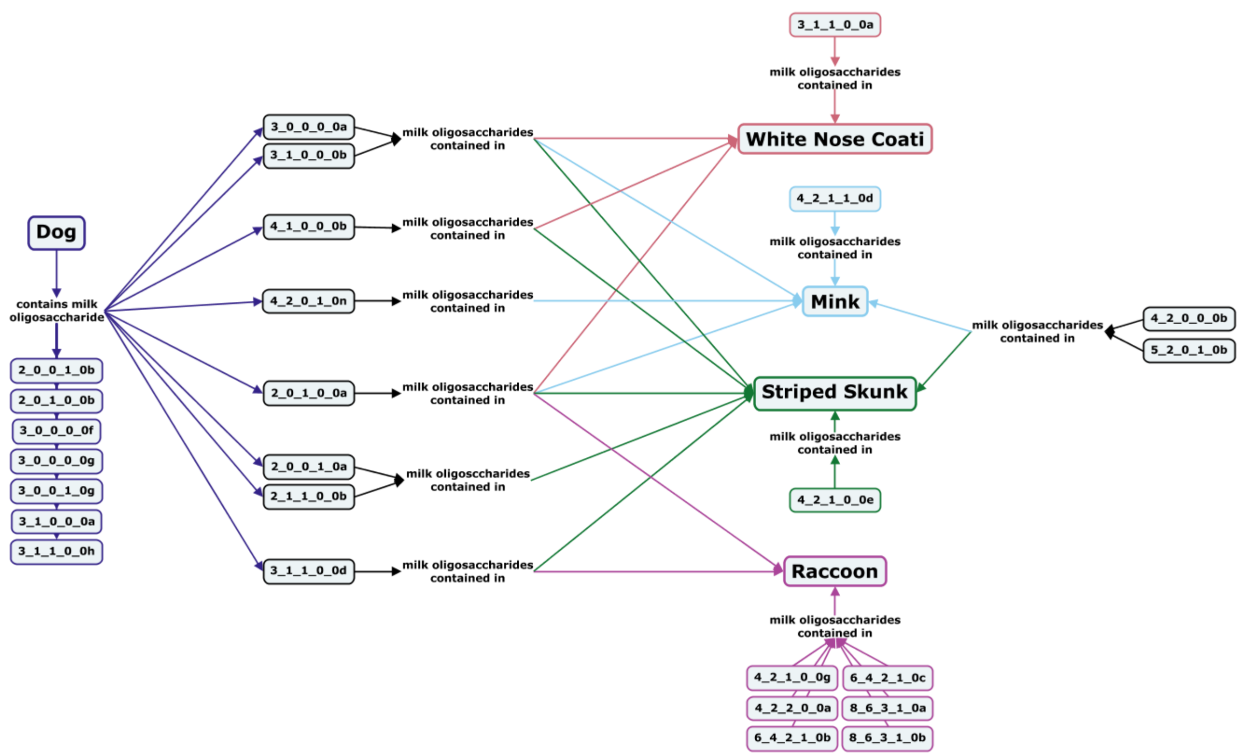


Figure 5.9. Concept map comparing the milk oligosaccharide profiles of five small *Canoidea* carnivore species, dogs, white nose coatis, minks, striped skunks, and raccoons. Oligosaccharides are given as the number of hexose_*N*-acetylhexosamine_fucose_*N*-acetylneuraminic acid_*N*-glycolylneuraminic acid monomers contained in the structure, followed by the isomer designation. The full list of oligosaccharide isomers and their respective alphanumeric codes is provided in Supplementary Table 5.1.

Even-toed Ungulates

Milks from many species within the *Artiodactyla* order have been analyzed for their oligosaccharide content. These species include ruminants such as cows, goats, sheep, buffalo, antelope, and deer, as well as non-ruminants like pigs.

Milk and dairy products from cows, goats and sheep are commonly consumed across much of the world. Milk oligosaccharides are present in concentrations of around 1.57 g/L in cow colostrum but fall to between 200 and 300 mg/L in mature cow and goat milk or 2 to 3 mg/L in mature sheep milk. [109–115] Milk oligosaccharides in these species are much less concentrated than lactose, which is expressed at levels of 49 g/L for cows, 43 g/L for goats, and 48 g/L for sheep.[102] The oligosaccharide profiles for all three species are dominated by acidic structures, but while cow milk features predominantly Neu5Ac-containing oligosaccharides, acidic goat and sheep milk oligosaccharides are largely Neu5Gc-containing compounds (Figure 5.10).[109–112,115–122] Neutral fucosylated oligosaccharides and LNT-type core structures have been observed in cow and goat milk, but at lower abundances – especially for cow milk – than in the milk of humans and other primates.[121,123–128] In contrast, most neutral sheep milk oligosaccharides are small, unfucosylated compounds with no type I core structures reported.[110,121,122,129] The oligosaccharide profiles of cows and goats have been shown to vary over the course of lactation[117,130,131] and between animals of different breeds or parities,[111,132–134] in addition to seasonal variation of cow milk oligosaccharides.[114,116] As in humans, genotype may influence the oligosaccharide profiles in goats and cows with changes in goat milk oligosaccharide profiles observed based on the α_{s1}-casein production gene *CSN1S1*,[135] and two recent genome-wide association studies strongly correlating changes in milk oligosaccharide expression to several genes in cows.[136,137]

Yak milk is consumed as a food source in regions of China, India, Mongolia, Nepal, and Tibet. Yak milk contains similar levels of lactose and oligosaccharides as dairy cattle.[102,138] Several neutral oligosaccharides have been identified in yak milk, including both an α1,3- and an α1,2-fucosylated structure (Figure 5.10).[138–140] The yak milk oligosaccharide profile also includes 3'-SL and 6'-SL, with substantially more 3'-SL than 6'-SL, similar to the milk of commercial dairy cows.[138]

The oligosaccharide content of buffalo milk has been investigated in several different studies, although not all studies specify what type of buffalo the milk was collected from. The carbohydrate composition of buffalo milk varies significantly between species, with a 1 to 5 ratio of milk oligosaccharides to lactose in water buffalo[141] but a lactose concentration 500 times higher than the oligosaccharide concentration in African buffalo milk.[142] Water buffalo have predominantly small neutral and acidic oligosaccharide structures (Figure 5.10), and oligosaccharide profiles that vary over the course of lactation.[110,141,143,144]

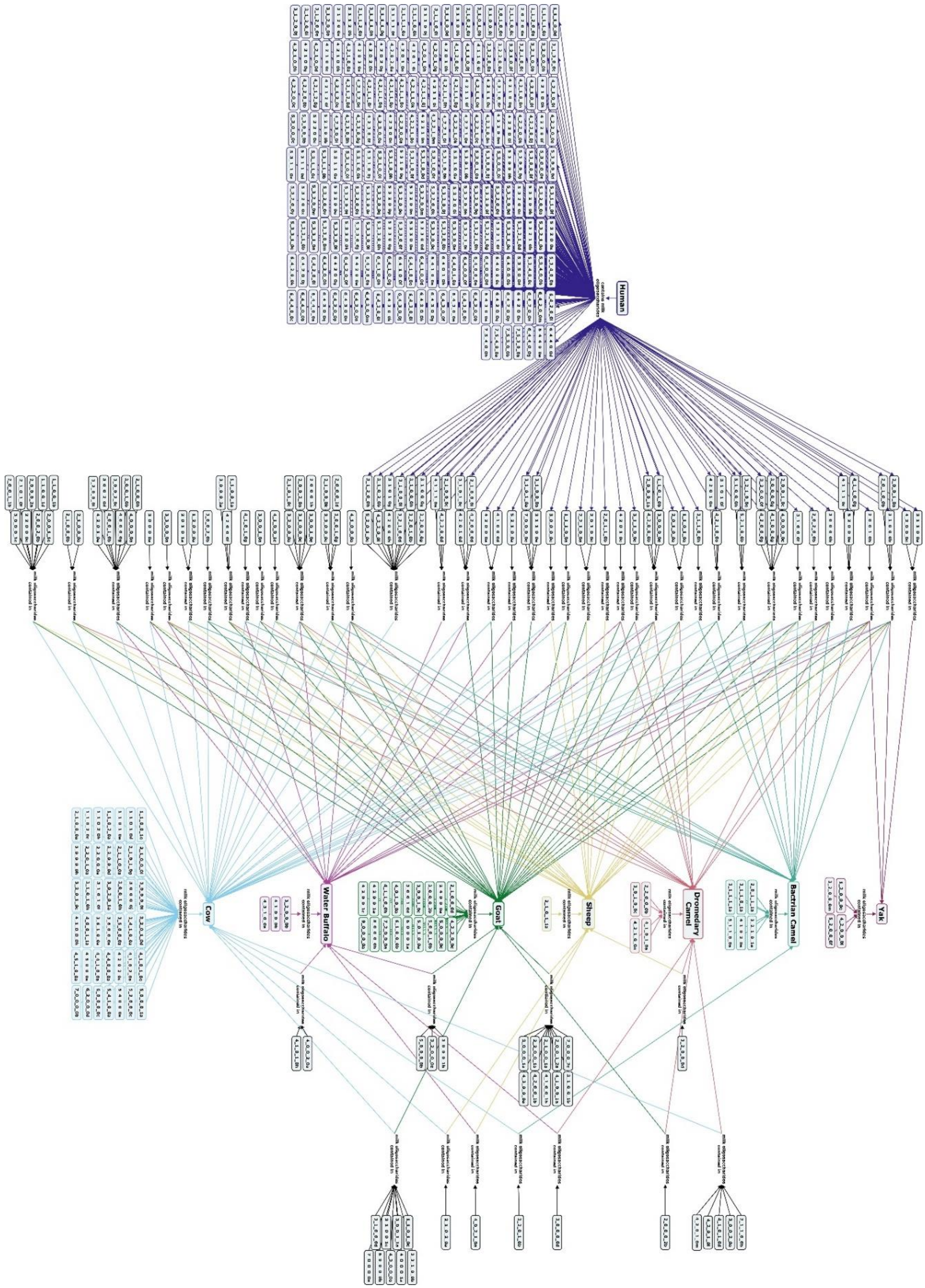

Human
Cow
Water Buffalo
Goat
Sheep
Dromedary Camel
Bactrian Camel
Yak

Figure 5.10. Concept map comparing the milk oligosaccharide profiles of seven routinely milked *Artiodactyla* species, cows, goats, water buffalo, sheep, dromedary camels, Bactrian camels, and yaks, with human milk oligosaccharides. Oligosaccharides are given as the number of hexose_*N*-acetylhexosamine_fucose_*N*-acetylneuraminic acid_*N*-glycolylneuraminic acid monomers contained in the structure, followed by the isomer designation. The full list of oligosaccharide isomers and their respective alphanumeric codes is provided in Supplementary Table 5.1.

Camel milk is frequently consumed in eastern Europe, north-eastern Africa, and parts of Asia. The majority of camels are dromedary, but Bactrian camels may also be milked as a food source. Compared to other commercially milked mammals, very little research has been done on the oligosaccharide content of camel milk. Dromedary camel milk has low levels of fucose- and Neu5Gc-containing oligosaccharides and no LNT-type cores (Figure 5.10).[121,145] In both species, acidic oligosaccharides are more abundant than neutral oligosaccharides, but in Bactrian camel milk, acidic oligosaccharide do not contain Neu5Gc and decrease over the course of lactation.[122,145,146]

Although milk from okapi as well as a number of antelope and deer species has been analyzed, the individual milk oligosaccharides of most species have not been profiled. Oligosaccharides were characterized in Addax milk and found to contain similar concentrations of Neu5Ac and Neu5Gc, with more α2-3-linked than α2-6-linked sialic acid.[147] A few small neutral and fucosylated oligosaccharides have been identified in giraffe milk, with no type II structures reported.[85,141] Several neutral and acidic oligosaccharides have been identified in reindeer milk too, which was found to be unique in both its lack of Neu5Gc- and α2-6-linked Neu5Ac-containing oligosaccharides and the predominance of phosphorylated oligosaccharides over α2-3-linked Neu5Ac-containing structures.[14] The milk of antelope species contains about 40 to 50 g/kg lactose, while deer milk has lower lactose concentrations of around 26 to 28 g/kg. [100] Many deer and antelope milk samples were collected after hunting-related deaths of the animals, but the effects of post-mortem milk sampling on oligosaccharide concentrations is unknown.

The milk oligosaccharide profiles of several breeds of pigs have been analyzed, and while minimal variation has been reported between breeds, differences have been observed between pigs of different parities, as with cows and goats.[149] Pig milk contains very low levels of NeuGc-containing oligosaccharides, making it more similar to human milk than other domesticated large mammals.[120,150,151] Unlike human milk oligosaccharides however, pig milk oligosaccharides are primarily acidic, with 3'-SL as the most abundant oligosaccharides, and less than 4% of pig milk oligosaccharide structures are fucosylated.[150,152,153]

Odd-toed Ungulates

Within the order *Perissodactyla,* only black rhinoceros, donkey and horse milks have been analyzed for their oligosaccharide profiles. Black rhinoceros milk oligosaccharides are predominantly small, neutral fucosylated structures with both α1-2- and α1-3-linked fucose moieties (Figure 5.11).[85] Donkey milk oligosaccharides are primarily small, Neu5Ac-containing structures. [152–154] In horses, the typical milk oligosaccharide concentration in colostrum is 0.217 to 4.63 g/L but falls to 0.0798 g/L in mature milk, with variation in oligosaccharide profiles between breeds and over the course of lactation.[157,158] The majority of horse milk oligosaccharide are small neutral or acidic structures, with lower levels of Neu5Gc-containing compounds and lactose than cows or goats.[121,155–161]

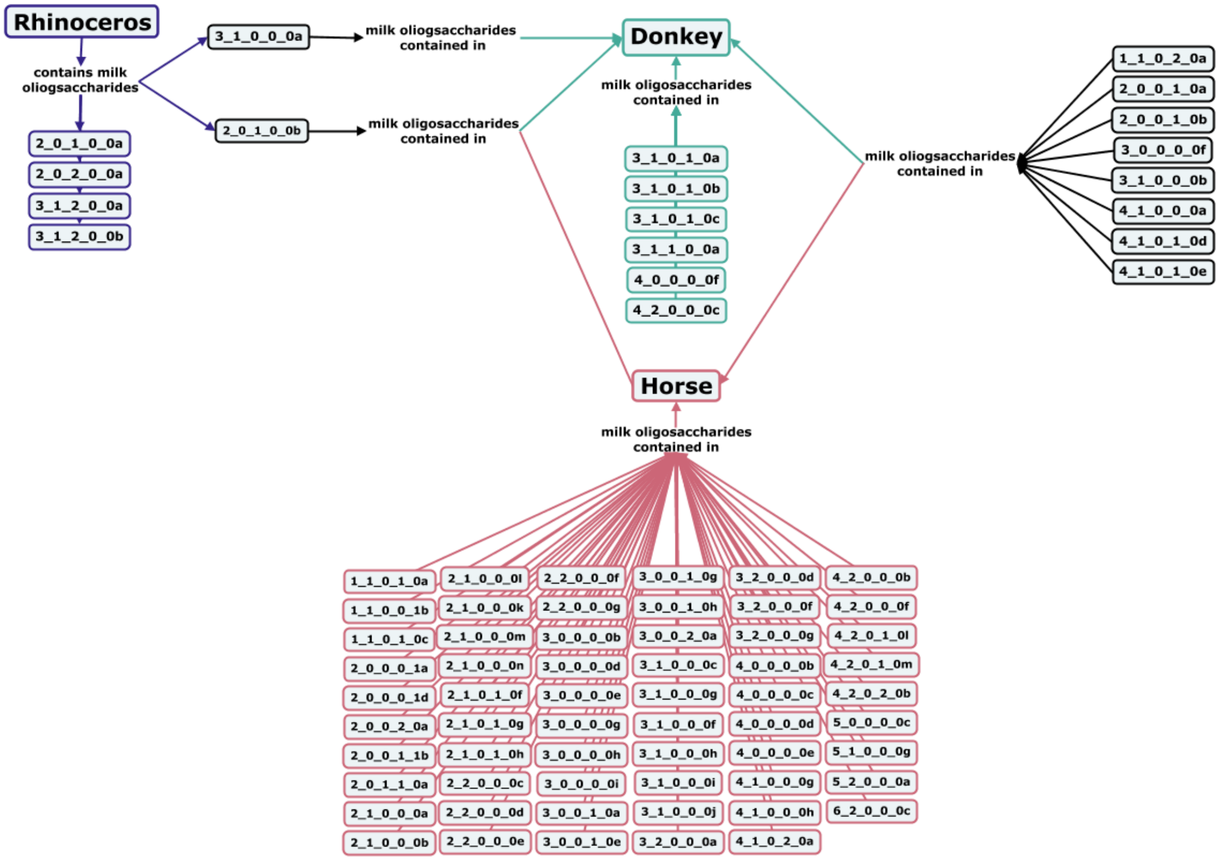


Figure 5.11. Concept map comparing the milk oligosaccharide profiles of three *Perissodactyla* species, black rhinoceroses, horses, and donkeys. Oligosaccharides are given as the number of hexose_*N*-acetylhexosamine_fucose_*N*-acetylneuraminic acid_*N*-glycolylneuraminic acid monomers contained in the structure, followed by the isomer designation. The full list of oligosaccharide isomers and their respective alphanumeric codes is provided in Supplementary Table 5.1.

Other Terrestrial Placental Mammals

From the order *Proboscidea*, both Asian and African elephants have undergone milk oligosaccharide analysis. The concentration of milk oligosaccharide changes over the course of lactation in both species, decreasing from 53.7 to around 20 g/L from early to middle lactation in Asian elephants and increasing from 8 to 21.5 g/kg from mid to late lactation in African elephants.[162–165] Isoglobotriose was found to be the most abundant oligosaccharide in the milk of both species, although a range of fucosylated and Neu5Ac sialylated oligosaccharides, as well as

structures with type I and II cores, have also been reported in Asian elephant milk.[162,163,165] African elephant milk contains about 5 times more lactose than oligosaccharides, while Asian elephant milk only contains about twice as much lactose as oligosaccharides.[162,164]
In the order *Pilosa*, milk oligosaccharides have only been analyzed for one species, the giant anteater. Giant anteater milk has a 3.4 to 1 ratio of lactose to oligosaccharides. No fucosylated or α2-3-linked Neu5Ac-containing oligosaccharides have been reported in giant anteater milk, but α2-6 sialylated structures were detected.[166]

The only species from the order *Chiroptera* for which milk oligosaccharides have been profiled is the island flying fox, a bat whose milk was found to lack LNT-type core, fucosylated, and Neu5Ac-containing oligosaccharides, but does feature milk oligosaccharides with Neu5Gc and α1-3-linked galactose, making the oligosaccharide profile of island flying fox milk very dissimilar to that of human milk.[167]

Aquatic Placental Mammals

The order *Cetacea* is divided into marine mammals with and without teeth. Of the toothed cetaceans, milk of a beluga whale and bottlenose dolphins have been analyzed. 3'-SL was the only free carbohydrate identified with certainty in beluga milk; however, because the milk sample was collected at one year postpartum, lactose and additional oligosaccharides may be present in earlier lactation milk.[168] Reports on the oligosaccharide profile of bottlenose dolphin milk vary, with some studies reporting no milk oligosaccharides,[169] and others reporting up to 9 g/L of oligosaccharides.[170] In most baleen whales, lactose has been reported as the most abundant free carbohydrate. Only Neu5Ac-containing oligosaccharides were detected in Bryde's whale and Sei whale milk,[171] whereas fucosylated, unfucosylated neutral, and Neu5Ac-containing oligosaccharides were detected in Minke whale milk.[168] All baleen whale milk analyzed in these studies was collected in late lactation, and it is unknown if milk collection post-mortem impacted some oligosaccharide profiles.[168,171]

Within the order *Pinnipedia*, no milk oligosaccharides or lactose have been detected in species within the *Otariidae* family but, a number of oligosaccharides have been identified in the milk of *Phocidae* family seals[172,173] In crabeater seal milk, sialylated and fucosylated oligosaccharides, including 2'-FL have been detected.[174,175] In bearded seal, hooded seal, and arctic harbor seal milk, only type II core structures, α1-2-linked fucosylation, and α2-6-linked Neu5Ac sialylation of oligosaccharides were detected (Figure 5.12).[173,176–178] Milk composition in Weddell seals has been shown to vary over the course of lactation, especially around two weeks postpartum when the mothers stop fasting and the total carbohydrate concentration of their milk drops. In early lactation, the carbohydrate fraction of Weddell seal milk is around 90% free oligosaccharides, which is substantially higher than that of terrestrial carnivores. Similar to bears, the low lactose concentration in pinniped milk is likely the result of evolutionary pressure toward rapid nutrient transfer from mother to offspring to more quickly prepare the pup for cold ocean temperatures and increase the size of offspring to hinder predators, a feat more easily achieved with high milk fat rather than lactose content.

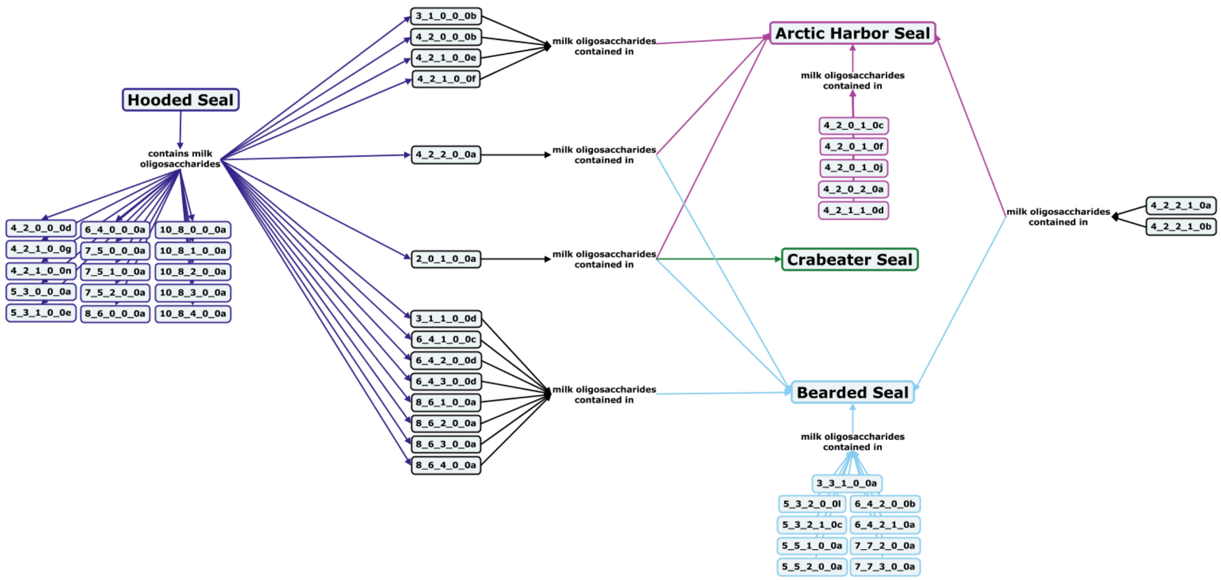


Figure 5.12. Concept map comparing the milk oligosaccharide profiles of four pinniped species, including hooded seals, arctic harbor seals, crabeater seals, and bearded seals. Oligosaccharides are given as the number of hexose_*N*-acetylhexosamine_fucose_*N*-acetylneuraminic acid_*N*-glycolylneuraminic acid monomers contained in the structure, followed by the isomer designation. The full list of oligosaccharide isomers and their respective alphanumeric codes is provided in Supplementary Table 5.1.

The only species for which milk oligosaccharides have been analyzed in the order *Sirenia* is the Florida manatee, whose milk contains little to no lactose and low concentrations of oligosaccharides, are consistent with the milk compositions of other aquatic mammals. The milk oligosaccharides that are present in Florida manatee milk are largely neutral structures containing *N*-acetylglucosamine or fucose residues.[85,169]

Milk Oligosaccharides of Marsupials

Unlike most placental mammals, the milk of many marsupials contains little to no lactose, because they lack intestinal brush border lactase, making lactose largely indigestible as a nutrient. In addition, marsupial milk does not contain oligosaccharides with Neu5Gc or LNnT-type core structures.[179] Koalas, wombats, and common brushtail possums all have predominantly linear oligosaccharide structures, including acidic milk oligosaccharides, although no α2,6-linked Neu5Ac has been reported in Wombat milk.[180–182] Koalas are one of the only marsupials investigated to date that has milk containing fucosylated oligosaccharides (Figure 5.13).[181] Among macropods, small and medium neutral unfucosylated oligosaccharides have been routinely identified, and acidic oligosaccharides in a range of sizes have been reported in red kangaroo and tammar wallaby milk.[183–189] In contrast to their plant-eating relatives, the carnivorous tiger quoll and eastern quoll have more branched than linear oligosaccharide structures with DPs of 3 to 11 (Figure 5.13).[190,191] The carbohydrate content of tammar wallaby, eastern quoll, and common brushtail possum have all been shown to change over the course of lactation, with tammar wallaby milk showing a distinct shift in composition between milk for pouch-bound offspring and more independent, plant-eating joeys that have begun to develop a more ruminant-like digestive system.[192–195] Many marsupial milk oligosaccharide samples were subjected to long-term freezer storage (25 to 35 years) prior to analysis, but the impact of such storage on milk oligosaccharide profiles is unknown.

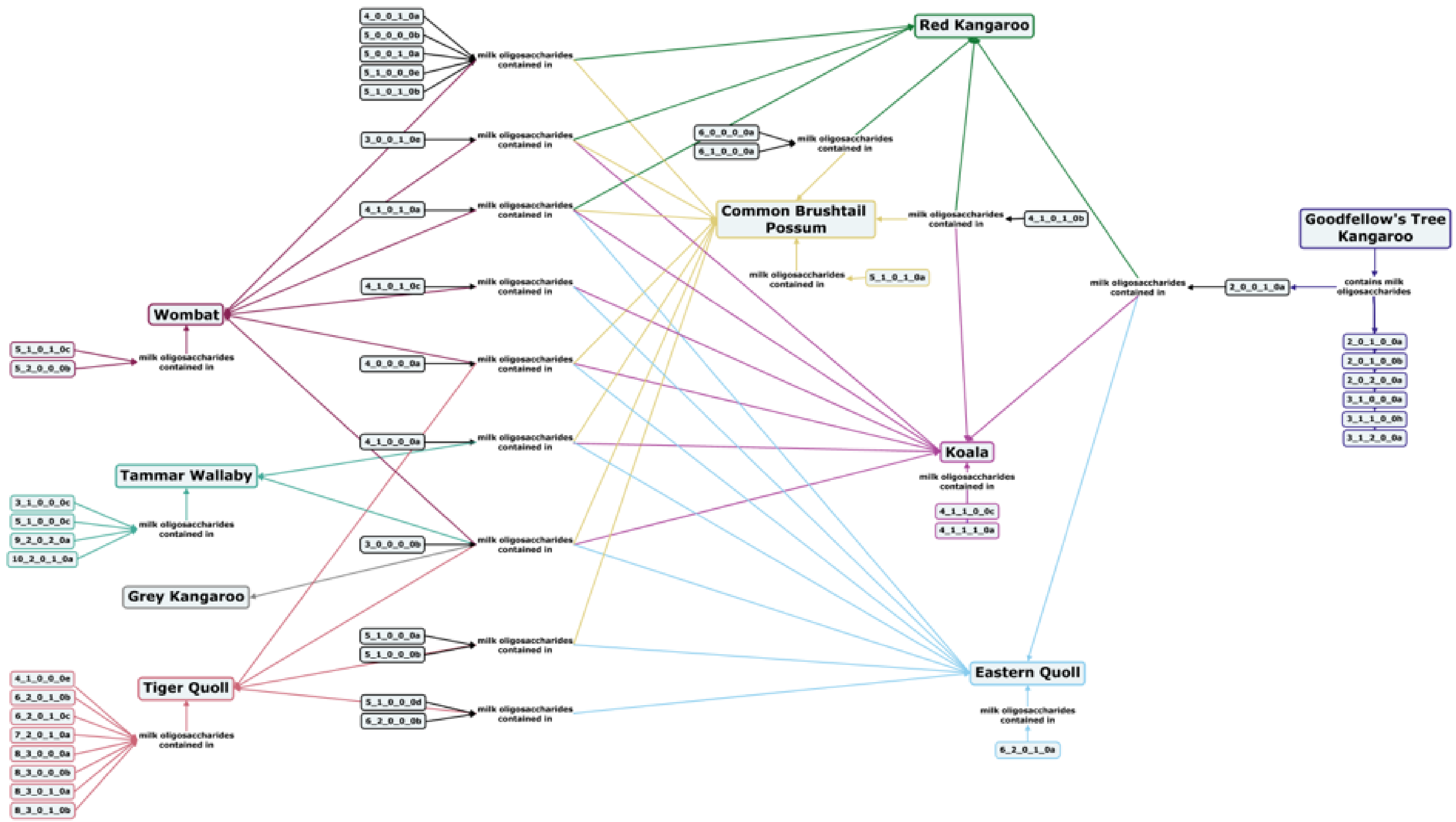


Figure 5.13. Concept map comparing the milk oligosaccharide profiles of nine marsupial species, including Goodfellow's tree kangaroo, red kangaroo, grey kangaroo, tammar wallaby, common brushtail possum, wombat, koala, eastern quoll, and tiger quoll. Oligosaccharides are given as the number of hexose_*N*-acetylhexosamine_fucose_*N*-acetylneuraminic acid_*N*-glycolylneuraminic acid monomers contained in the structure, followed by the isomer designation. The full list of oligosaccharide isomers and their respective alphanumeric codes is provided in Supplementary Table 5.1.

Milk Oligosaccharides of Monotremes

Monotremes diverged evolutionarily from the ancestors of eutherians and marsupials an estimated 200 million years ago. Although monotremes don't have nipples, they still secrete milk to nourish their young.[196] Both platypus and echidna milks have levels of sialic acid similar to those of marsupials, but nearly all monotreme milk sialic acid is diacetylated Neu4,5Ac.[197,198] Platypus milk features oligosaccharides with α1,2- and α1,3-linked fucosylation as well as LNnT-type core structures, with primarily di- and tri-fucosylated compounds (Figure 14). [197,199–201] In contrast, echidna milk oligosaccharides are primarily small, simple, mono-fucosylated or mono-sialylated structures (Figure 5.14).[200,202,203]

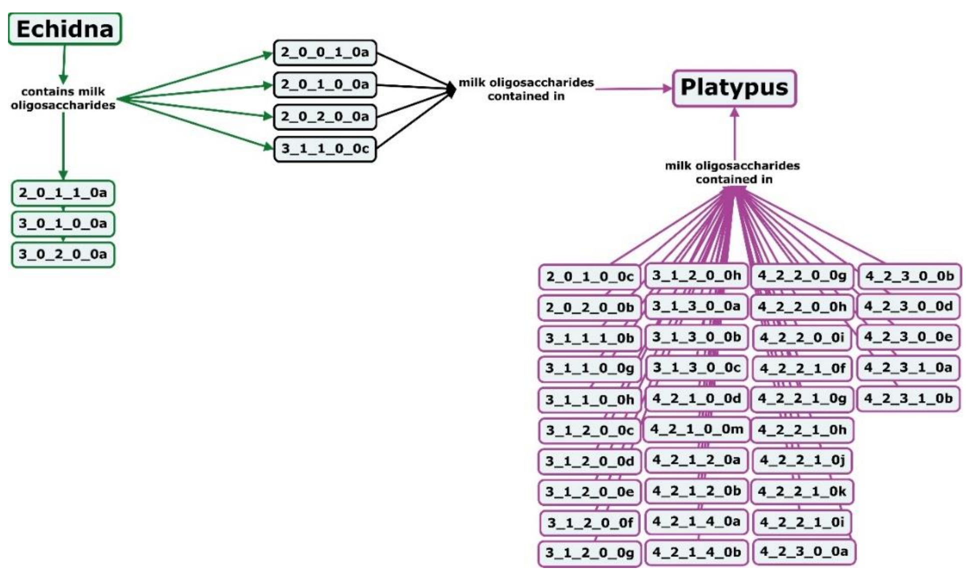


Figure 5.14. Concept map comparing the milk oligosaccharide profiles of two monotreme species, echidna and platypus, with human milk oligosaccharides. Oligosaccharides are given as the number of hexose_*N*-acetylhexosamine_fucose_*N*-acetylneuraminic acid_*N*-glycolylneuraminic acid monomers contained in the structure, followed by the isomer designation. The full list of oligosaccharide isomers and their respective alphanumeric codes is provided in Supplementary Table 5.1.

Inter-species Milk Oligosaccharide Comparisons

The unique oligosaccharide profiles of different species are likely the result of evolutionary pressures adapting milk compositions to the needs of both the mother and the neonate.[204–206] Species in which the mothers fast during all or part of lactation appear to produce milk in which oligosaccharides are more concentrated than other carbohydrates including lactose. This pattern has been observed in bears,[95–99] *Phocidae* seals,[172,173] and baleen whales.[168,171] In these species, oligosaccharides are likely the main free carbohydrates in milk because energy is transferred from mother to offspring mainly in the form of lipids, not carbohydrates. In some cases, this may be due to the need for rapid offspring growth to increase mobility and avoid predations or the need to increase in neonatal body fat to ensure survival under conditions of extreme cold. In other cases, the lack of mono- and disaccharides in the mother's milk may instead be the result of evolutionary pressures selecting for the preservation for the mother who, with limited energy stores, must transfer nutrients to her offspring in the manner that results in the least energy and water loss.

In placental mammal species with less-developed neonates at birth, including bears,[95–97,100,101] dogs,[104] minks,[107] raccoons,[103] skunks,[106] and primates[83,84,88] including humans[3,20,53,76,79] the milk oligosaccharide profiles feature more fucosylated structures than those of species with more precocial offspring. Because the neonates of these species have less-developed immune systems at birth, they are likely more dependent on prebiotic and immunomodulatory compounds, including fucosylated oligosaccharides, delivered by their mother's milk.

Other species, like elephants and primates including humans, which are phylogenetically distant but developmentally similar in terms of nervous and immune system maturation, show similar trends in oligosaccharide composition over lactation.[165] This may be related to the long, slow growth and long lactation periods in these species. Although dolphin and toothed whale milk oligosaccharide profiles have not been monitored over the course of lactation, it is possible that similar trends would be observed in these species, given their similarly prolonged lactation.

Sources of Milk Oligosaccharide Variation within Species

In addition to the variation in oligosaccharide profiles that occurs between species, intra-species variations have also been observed. These differences in reported oligosaccharide profiles or concentrations may be due to a number of natural causes. Variation in oligosaccharide profiles between different breeds has been observed in cows,[132,134,206] goats,[111] pigs,[149,208] horses,[157] and dogs.[104] Even within a breed, differences in oligosaccharide abundances have been observed in cows,[132] pigs,[149] goats,[111] and humans[54] based on parity and, in humans, based on whether a birth is full- or preterm.[76,78,207,210] Genotypes have also been shown to influence oligosaccharide profiles, specifically those associated with α_{s1}-casein production in goats[135] and secretor and Lewis status in humans.[54,58,62,72,78,211] In humans, variations in oligosaccharide profiles have also been associated with the presence of immune diseases, including HIV[75] and celiac disease.[212] The mother's diet may also impact the oligosaccharide profile, with a distinct shift observed in Weddell seal milk when mothers stop fasting[172] and changes observed in the milk of cows fed different diets.[213–215]

Oligosaccharide profiles are known to vary over the course of lactation too as the needs of the neonate change and they shift away from consuming mother's milk as their sole food source. Variation in milk oligosaccharides over the course of lactation has been well documented in cows,[117,119,130] pigs,[149,150,153,208] and humans.[57–59,66,74,216–220] Variation in milk oligosaccharide profiles or concentrations of some milk oligosaccharides at multiple lactation points have also been noted in elephants,[165] bonobos,[82] dogs,[102] polar bears,[96] and tammar wallabies.[193] This variation in milk carbohydrate profile of tammar wallaby milk is especially notable because this species can co-express milk of different compositions from different teats simultaneously if nursing both a latched, pouch-bound joey and mobile joey at the same time. With such widespread variation in milk oligosaccharide profiles over the course of lactation, it is exceedingly important that future studies report the lactation time point from which milk is being analyzed. Without this crucial information, studies on the milk oligosaccharides of the same species may seem to present conflicting data, when in fact they may simply be from disparate lactation time points.

Approximating Human Milk Oligosaccharides

Despite the wide sources of variation, several mammalian species have milk oligosaccharide profiles with characteristics quite similar to human milk oligosaccharides, as shown in Figure 5.15. Camels, pigs, and terrestrial carnivores express milk with low levels of Neu5Gc-containing oligosaccharides. Chimpanzee and common marmoset milks contain relatively high concentrations of an array of neutral fucosylated oligosaccharides. The milk of giraffes and most primates has low levels of α1-3-linked galactose and type II core structures. To most closely mirror human milk oligosaccharides however, a milk oligosaccharide profile should have low levels of Neu5Gc- and α1,3-linked galactose-containing OS, high concentrations of a diverse array of fucosylated oligosaccharides, and substantially more type I structures than type II oligosaccharides. Based on the currently available research, Asian elephant milk presents the best balance of all three of these features. Despite their promising similarities to humans in terms of milk oligosaccharide content, not all of these species are reasonable sources for milk oligosaccharide isolation. Successful milk oligosaccharide isolation at the pilot scale has been demonstrated for both cow and goat milk, and similar techniques could be applied to harness the oligosaccharide available in the milk or dairy streams originating from other commercially milked mammals.[110,221–226] Though not at the same scale as cows, the milk or dairy side streams from producing butter and cheese from horses, Bactrian camels and goat breeds with relatively high concentrations of fucosylated oligosaccharides and low abundances of Neu5Gc-containing oligosaccharides, provide promising dairy streams for isolating milk oligosaccharides that could be used to create supplements for human infant nutrition or for use as a food ingredient in other products for human consumption. In addition, other camelid species like llamas and alpacas, which have the potential to be commercially milked, pose further possibilities for species whose milk oligosaccharide profiles warrant investigation for these purposes. More studies of the milk of these species detailing their full milk oligosaccharide profiles and oligosaccharide concentrations are still needed.

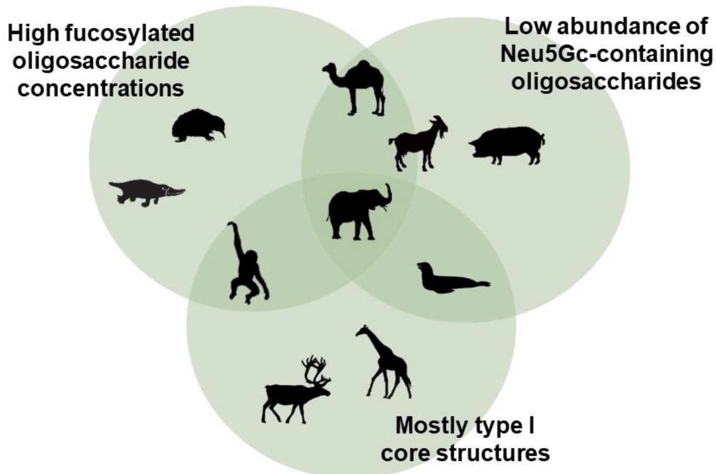


Figure 5.15. Venn diagram comparing the milk oligosaccharide profiles of non-human mammals to the 3 key features of human milk oligosaccharides.

Database Advantages and Limitations

The comparisons drawn in this study are inherently limited by the depth and scope of the published studies reviewed herein. In many cases, the published results are only an indication of what the overall profile of the milk oligosaccharides for a given species may look like. A number of studies have been limited by small sample size availability, occasionally with as little as one individual chosen to represent an entire breed or species. Such sweeping assumptions come with the known risk that milk oligosaccharide profiles vary, sometimes widely, between individuals within a group. Factors such as parity, season, location, genotype, captivity status and days in milk may have inherent influences on milk oligosaccharide profiles. Additional variation in reported results between studies may be due to the application of a wide range milk collection methods, sample storage conditions and analytical techniques. The work reviewed here spans more than five decades, over which time methodology, instrumentation, and commercially available standards for milk oligosaccharide analysis have improved greatly.

Additionally, because the concentrations of individual oligosaccharides were not reported in most of the reviewed literature, no comparisons of the abundance of particular oligosaccharide classes or structures was made during this analysis. All descriptions of milk oligosaccharide profiles have “more” or “less” of a specific category of oligosaccharides are based on the number of reported structures of that type. As such, the analysis of any milks potentially containing a large number of very low abundant compounds with a given structural feature, or a high concentration of a single oligosaccharide may be skewed by this analysis.

Despite these limitations, this database and the concept maps derived from it facilitate a cumulative analysis of all existing published milk oligosaccharide profile data that has not been previously undertaken at this magnitude. Reconciling the oligosaccharide data from existing publications into a common format allows for cross-species and cross-publication comparisons that would otherwise be hindered by the unstandardized multitude of textual, tabular, and visual formats in which oligosaccharide profiles are reported. In particular, the queryable nature of the database and visual format of its output facilitate observations of trends, particularly within and between phylogenetic groups that would not otherwise be readily apparent by examining the publications individually. In addition, the concept map format reveals areas that have been comparatively underinvestigated or in which there are substantial gaps or inconsistencies in the existing literature. At its heart, this platform is not only a way to compile data, but also an avenue to generate new data-driven hypotheses for future research.

Conclusions

All mammals produce milk from mammary glands to suckle their young; however, the OS content of their milk can differ greatly. Although it is unlikely that the milk oligosaccharides of all mammalian species will be profiled in the near future, targeted investigations of the milk oligosaccharides of particular mammals could advance the field on several fronts. Minimal to no research has been done on the milk oligosaccharides of species from nearly half of the 19 orders within the class *Mammalia*. Profiling milk oligosaccharides from species in theses relatively untouched orders, including *Dermoptera*, *Insectivora*, and *Lagomorpha* would provide improved understanding of how and why milk oligosaccharides developed from an evolutionary perspective. Further investigation into domestic species that are more commonly milked in non-western countries, such as yaks, camels, water buffalo, llamas, and alpacas would aid in the identification of potential dairy streams from which oligosaccharides could be isolated for supplementation in infant formulas and other nutraceutical products. Additional investigation into the influence of the impact of milk collection conditions, including the impact of oxytocin administration to induce milk let-down, collection of milk post-mortem, and milk oligosaccharide profiles from captive versus wild animals would also provide further context for the interpretation of existing milk oligosaccharide data.

ACKNOWLEDGEMENTS

The author would like to thank to Ettiene Laborie, Anne-Laure Pacaud, Emeline Colet, and Chloe Duchenne for their work on preliminary versions of the milk oligosaccharide database.

SUPPLEMENTARY MATERIAL

Supplementary Table 5.1. Alphanumeric oligosaccharide codes and corresponding structural composition for all milk oligosaccharides

Oligosaccharide Isomer Designation	Full Oligosaccharide Structural Information
0_2_0_0_0a	GalNAc(b1-4)GlcNAc
0_2_0_1_0a	Neu5Ac(a2-6)GalNAc(b1-4)GlcNAc
1_0_0_1_0a	Hex+Neu5Ac
1_0_0_1_0b	Hex+Neu5Ac
1_1_0_0_0a	Gal(b1-4)HexNAc
1_1_0_0_0b	Gal(b1-4)GlcNAc (LacNAc)
1_1_0_0_0c	GalNAc(b1-4)Glc
1_1_0_0_0d	Hex+HexNAc
1_1_0_0_1a	Neu5Gc(a2-3)Gal(b1-4)GlcNAc (3'-GLN)
1_1_0_0_1b	Neu5Gc(a2-6)Gal(b1-4)GlcNAc (6'-GLN)
1_1_0_0_1c	Neu5Gc-Gal(b1-4)GlcNAc
1_1_0_0_1d	Hex+HexNAc+Neu5Gc
1_1_0_0_1e	Hex+HexNAc+Neu5Gc
1_1_0_1_0a	Neu5Ac(a2-3)Gal(b1-4)GlcNAc (3'-SLN)
1_1_0_1_0b	Neu5Ac(a2-6)Gal(b1-4)GlcNAc (6'-SLN)
1_1_0_1_0c	Hex+HexNAc+Neu5Ac
1_1_0_1_0d	Hex+HexNAc+Neu5Ac
1_1_0_1_0e	Hex+HexNAc+Neu5Ac
1_1_0_2_0a	Neu5Ac(a2-8)Neu5Ac(a2-3)Gal(b1-4)GlcNAc (DSLN)
1_1_0_2_0b	Hex+HexNAc+2Neu5Ac
1_1_0_2_0c	Hex+HexNAc+2Neu5Ac
1_1_1_0_0a	Gal(b1-4)[Fuc(a1-3)]GlcNAc
1_1_1_0_0b	Hex+HexNAc+Fuc
1_1_1_0_0c	Hex+HexNAc+Fuc
1_1_1_0_0d	Fuc(a1-2)Gal(b1-4)GlcNAc
1_1_2_0_0a	Hex+HexNAc+Fuc
1_1_1_1_0a	Neu5Ac(a2-6)Gal(b1-3)[Fuc(a1-4)]GlcNAc (3'SIe)
1_1_1_1_0b	Hex+HexNAc+Fuc+Neu5Ac
1_1_2_0_0a	Hex+HexNAc+2Fuc
1_2_0_0_0a	Hex+2HexNAc
1_2_0_0_0b	Hex+2HexNAc
1_2_0_0_0c	Hex+2HexNAc
1_2_0_0_0d	Hex+2HexNAc
1_2_0_0_0e	GlcNAc(1-6)GalNAc(1-4)Glc (bosiose)
1_2_0_1_0a	Hex+2HexNAc+Neu5Ac
1_2_0_1_0b	Hex+2HexNAc+Neu5Ac
1_2_0_1_0c	Hex+2HexNAc+Neu5Ac
1_3_0_0_0a	HexNAc-HexNAc-Gal(b1-4)GlcNAc
2_0_0_0_1a	Neu5Gc(a2-3)Gal(b1-4)Glc (3'-NGc-SL/ 3'-GL)
2_0_0_0_1b	Neu5Gc(a2-6)Gal(b1-4)Glc (6'-GL)
2_0_0_0_1c	Neu5Gc-Gal(b1-4)Glc
2_0_0_0_1d	2Hex+Neu5Gc
2_0_0_0_2a	Neu5Gc(a2-8)Neu5Gc(a2-3)Gal(b1-4)Glc (DGL)
2_0_0_0_2b	Neu5Gc-Neu5Gc-Gal(b1-4)Glc
2_0_0_0_2c	2Hex+2Neu5Gc
2_0_0_1_0a	Neu5Ac(a2-3)Gal(b1-4)Glc (3'-SL)
2_0_0_1_0b	Neu5Ac(a2-6)Gal(b1-4)Glc (6'-SL)
2_0_0_1_0c	2Hex+Neu5Ac
2_0_0_1_1a	Neu5Ac(a2-8)Neu5Gc(a2-3)Gal(b1-4)Glc
2_0_0_1_1b	Neu5Gc(a2-8)Neu5Ac(a2-3)Gal(b1-4)Glc (GSL)
2_0_0_1_1c	2Hex+Neu5Ac+Neu5Gc
2_0_0_1_1d	Neu5Ac(a2-8)Neu5Gc(a2-6)Gal(b1-4)Glc
2_0_0_1_2a	2Hex+Neu5Ac+2Neu5Gc
2_0_0_2_0a	Neu5Ac(a2-8)Neu5Ac(a2-3)Gal(b1-4)Glc (DSL)
2_0_0_2_0b	2Hex+2Neu5Ac
2_0_0_2_0c	2Hex+2Neu5Ac
2_0_1_0_0a	Fuc(a1-2)Gal(b1-4)Glc (2'-FL)
2_0_1_0_0b	Gal(b1-4) [Fuc(a1-3)]Glc (3-FL)
2_0_1_0_0c	2Hex+Fuc
2_0_1_0_1a	Hex2+Fuc+Neu5Gc
2_0_1_1_0a	Neu5Ac(a2-3)Gal(b1-4)[Fuc(a1-3)]Glc
2_0_1_1_0b	2Hex+Fuc+Neu5Ac
2_0_1_1_1a	2Hex+Fuc+Neu5Ac+Neu5Gc
2_0_2_0_0a	Fuc(a1-2)Gal(b1-4)GlcFuc(a1-3) (DFL)
2_0_2_0_0b	2Hex+2Fuc

Oligosaccharide Isomer Designation	Full Oligosaccharide Structural Information
2_1_0_0_0a	GalNAc(a1-3)Gal(b1-4)Glc (a 3'-GalNAcL)
2_1_0_0_0b	GalNAc(b1-3)Gal(b1-4)Glc (b 3'-GalNAcL)
2_1_0_0_0c	GlcNAc(b1-3)Gal(b1-4)Glc
2_1_0_0_0d	GlcNAc(b1-6)Gal(b1-4)Glc (6'-GlcNAcL)
2_1_0_0_0e	Gal-Gal(b1-4)Glc
2_1_0_0_0f	Gal-Gal(b1-4)Glc
2_1_0_0_0g	Gal-Gal(b1-4)GlcNAc
2_1_0_0_0h	HexNAc-Gal(b1-4)Glc
2_1_0_0_0i	HexNAc-Gal(b1-4)Glc
2_1_0_0_0j	HexNAc-Gal(b1-4)Glc
2_1_0_0_0k	2Hex+HexNAc
2_1_0_0_0l	2Hex+HexNAc
2_1_0_0_0m	2Hex+HexNAc
2_1_0_0_0n	2Hex+HexNAc
2_1_0_0_0o	2Hex+HexNAc
2_1_0_0_0p	2Hex+HexNAc
2_1_0_0_1a	2Hex+HexNAc+Neu5Gc
2_1_0_1_0a	GalNAc(b1-4)[Neu5Ac(a2-3)]Gal(b1-4)Glc (GM2 tetrasaccharide/GM2 tetra)
2_1_0_1_0b	Neu5Ac(a2-3) + HexNAc-Gal(b1-3)Glc
2_1_0_1_0c	Neu5Ac(a2-3)GlcNAc(b1-3)Gal(b1-4)Glc
2_1_0_1_0d	Neu5Ac(a2-6)GlcNAc(b1-6)Gal(b1-4)Glc
2_1_0_1_0e	Neu5Ac(a2-6)GlcNAc(b1-3)Gal(b1-4)Glc
2_1_0_1_0f	2Hex+HexNAc+Neu5Ac
2_1_0_1_0g	2Hex+HexNAc+Neu5Ac
2_1_0_1_0h	2Hex+HexNAc+Neu5Ac
2_1_0_1_1a	2Hex+HexNAc+Neu5Ac+Neu5Gc
2_1_0_2_0a	2Hex+HexNAc+2Neu5Ac
2_1_1_0_0a	Fuc(a1-2)Gal(b1-4)[GalNAc(a1-3)]Glc
2_1_1_0_0b	GalNAc(a1-3)[Fuc(a1-2)]Gal(b1-4)Glc (A-tetrasaccharide)
2_1_1_0_0c	2Hex+HexNAc+Fuc
2_1_1_0_0d	Fuc(a1-4)GlcNAc(b1-3)Gal(b1-4)Glc
2_1_2_0_0a	GalNAc(a1-3)[Fuc(a1-2)]Gal(b1-4)[Fuc(a1-3)]Glc (A-pentasaccharide)
2_2_0_0_0a	Gal-HexNAc-Gal(b1-4)GlcNAc
2_2_0_0_0b	Gal(b1-4)GlcNAc-Gal(b1-4)GlcNAc
2_2_0_0_0c	2Hex+2HexNAc
2_2_0_0_0d	2Hex+2HexNAc
2_2_0_0_0e	2Hex+2HexNAc
2_2_0_0_0f	2Hex+2HexNAc
2_2_0_0_0g	2Hex+2HexNAc
2_2_0_0_1a	2Hex+2HexNAc+Neu5Gc
2_2_0_1_0a	2Hex+2HexNAc+Neu5Ac
2_2_1_0_0a	2Hex+2HexNAc+Fuc
2_2_1_1_0a	2Hex+2HexNAc+Fuc+Neu5Ac
2_4_0_0_0a	2Hex+4HexNAc
3_0_0_0_0a	Gal(a1-3)Gal(b1-4)Glc (isoglobotriose)/ (a 3'-GL)
3_0_0_0_0b	Gal(b1-3)Gal(b1-4)Glc (b 3'-GL)
3_0_0_0_0c	Gal(b1-4)Gal(b1-4)Glc (4'-GL)
3_0_0_0_0d	Gal(a1-4)Gal(b1-4)Glc (globotriose)
3_0_0_0_0e	Gal(b1-6)Gal(b1-4)Glc (6'-GL)
3_0_0_0_0f	3Hex
3_0_0_0_0g	3Hex
3_0_0_0_0h	3Hex
3_0_0_0_0i	3Hex
3_0_0_0_0j	3Hex
3_0_0_0_0k	3Hex
3_0_0_0_1a	Gal(b1-3)[Neu5Gc(a2-6)]Gal(b1-4)Glc
3_0_0_0_1b	Neu5Gc(a2-3)Gal(b1-3)Gal(b1-4)Glc
3_0_0_0_1c	3Hex+Neu5Gc
3_0_0_0_2a	Neu5Gc(a2-3)Gal(b1-3)[Neu5Gc(a2-6)]Gal(b1-4)Glc
3_0_0_0_2b	3Hex+2Neu5Gc
3_0_0_1_0a	Gal(b1-3)[Neu5Ac(a2-6)]Gal(b1-4)Glc
3_0_0_1_0b	Gal(b1-6)[Neu5Ac(a2-3)]Gal(b1-4)Glc
3_0_0_1_0c	Gal(b1-6)[Neu5Ac(a2-6)]Gal(b1-4)Glc
3_0_0_1_0d	Gal(b1-6)[Neu5Ac(a2-3)]Gal(b1-4)Glc
3_0_0_1_0e	Neu5Ac(a2-3)Gal(b1-3)Gal(b1-4)Glc (sialyl 3'-galactosyllactose)
3_0_0_1_0f	Neu5Ac(a2-3) + Gal-Gal(b1-3)Glc
3_0_0_1_0g	3Hex+Neu5Ac
3_0_0_1_0h	3Hex+Neu5Ac
3_0_0_1_0i	3Hex+Neu5Ac

Oligosaccharide Isomer Designation	Full Oligosaccharide Structural Information
3_0_0_1_1a	3Hex+Neu5Ac+Neu5Gc
3_0_0_2_0a	Neu5Ac(a2-3)Gal(b1-3)[Neu5Ac(a2-6)]Gal(b1-4)Glc
3_0_0_2_0b	3Hex+2Neu5Ac
3_0_0_2_0c	Neu5Ac(a2-3)Gal(b1-6)[Neu5Ac(a2-3)]Gal(b1-4)Glc
3_0_0_2_0d	Neu5Ac(a2-8)Neu5Ac(a2-3)[Gal(b1-6)]Gal(b1-4)Glc
3_0_1_0_0a	Gal(a1-3)[Fuc(a1-2)]Gal(b1-4)Glc (B-tetrasaccharide)
3_0_1_0_0b	Gal(a1-3)Gal(b1-4)[Fuc(a1-3)]Glc (fucosyl isoglobotriose)
3_0_1_0_0c	Fuc + Gal-Gal(b1-4)Glc
3_0_1_0_0d	Fuc + Gal-Gal(b1-4)Glc
3_0_1_0_0e	3Hex+Fuc
3_0_2_0_0a	Gal(a1-3)[Fuc(a1-2)]Gal(b1-4)[Fuc(a1-3)]Glc (B-pentasaccharide)
3_0_2_0_1a	3Hex+2Fuc+Neu5Gc
3_1_0_0_0a	Gal(b1-3)GlcNAc(b1-3)Gal(b1-4)Glc (LNT)
3_1_0_0_0b	Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc (LNnT)
3_1_0_0_0c	Gal(b1-4)(GlcNAc(b1-6))Gal(b1-4)Glc (iso-LNnT)
3_1_0_0_0d	Gal(b1-3)[GlcNAc](b1-6)Gal(b1-4)Glc
3_1_0_0_0e	Gal(b1-6)[GlcNAc(b1-3)]Gal(b1-4)Glc
3_1_0_0_0f	3Hex+1HexNAc
3_1_0_0_0g	3Hex+1HexNAc
3_1_0_0_0h	3Hex+1HexNAc
3_1_0_0_0i	3Hex+1HexNAc
3_1_0_0_0j	3Hex+HexNAc
3_1_0_0_1a	Neu5Gc(a2-6)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc (a2-6N-glycolylneuraminyl lacto-N-neotetraose)
3_1_0_0_1b	Neu5Gc + Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc (GLNnT)
3_1_0_0_1c	3Hex+HexNAc+Neu5Gc
3_1_0_1_0a	Neu5Ac(a2-3)Gal(b1-3)GlcNAc(b1-3)Gal(b1-4)Glc (LST a)
3_1_0_1_0b	Gal(b1-3)[Neu5Ac(a2-6)]GlcNAc(b1-3)Gal(b1-4)Glc (LST b)
3_1_0_1_0c	Neu5Ac(a2-6)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc (LST c/ 6"-SLNnT)
3_1_0_1_0d	Neu5Ac(a2-3)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc (3'-SLNnT)
3_1_0_1_0e	Neu5Ac(a2-3)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc
3_1_0_1_0f	3Hex+HexNAc+Neu5Ac
3_1_0_1_0g	3Hex+HexNAc+Neu5Ac
3_1_0_1_0h	3Hex+HexNAc+Neu5Ac
3_1_0_1_0i	3Hex+HexNAc+Neu5Ac
3_1_0_1_0j	3Hex+HexNAc+Neu5Ac
3_1_0_1_0k	3Hex+HexNAc+Neu5Ac
3_1_0_1_0l	3Hex+HexNAc+Neu5Ac
3_1_0_2_0a	Neu5Ac(a2-3)Gal(b1-3)[Neu5Ac(a2-6)]GlcNAc(b1-3)Gal(b1-4)Glc (DSLNT)
3_1_0_2_0b	3Hex+HexNAc+2Neu5Ac
3_1_0_2_0c	3Hex+HexNAc+2Neu5Ac
3_1_1_0_0a	Fuc(a1-2)Gal(b1-3)GlcNAc(b1-3)Gal(b1-4)Glc (LNFP I)
3_1_1_0_0b	Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)Gal(b1-4)Glc (LNFP II)
3_1_1_0_0c	Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)Gal(b1-4)Glc (LNFP III)
3_1_1_0_0d	Fuc(a1-2)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc (LNFP IV)
3_1_1_0_0e	Gal(b1-3)GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]Glc (LNFP V)
3_1_1_0_0f	Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]Glc (LNFP VI or LNnFP V)
3_1_1_0_0g	Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)Gal(b1-4)Glc
3_1_1_0_0h	3Hex+HexNAc+Fuc
3_1_1_0_0i	3Hex+HexNAc+Fuc
3_1_1_0_0j	3Hex+HexNAc+Fuc
3_1_1_0_0k	3Hex+HexNAc+Fuc
3_1_1_0_1a	3Hex+HexNAc+Fuc+Neu5Gc
3_1_1_1_0a	Neu5Ac(a2-6)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]Glc (F-LSTc)
3_1_1_1_0b	Neu5Ac(a2-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)Gal(b1-4)Glc
3_1_1_1_0c	Neu5Ac(a2-6)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]Glc
3_1_1_1_0d	Neu5Ac(a2-6)Gal(b1-4)GlcNAc(b1-3)Gal(b2-4)[Fuc(a1-3)]Glc (SFLNnT)
3_1_1_1_0e	Neu5Ac+Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)Gal(b1-4)Glc (S LNFP II)
3_1_1_1_0f	3Hex+HexNAc+Fuc+Neu5Ac
3_1_1_1_0g	Neu5Ac(a2-3)Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)Gal(b1-4)Glc (F-LSTa)
3_1_1_1_0h	Fuc(a1-2)Gal(b1-3)[Neu5Ac(a2-6)]GlcNAc(b1-3)Gal(b1-4)Glc (F-LSTb)
3_1_1_1_1a	3Hex+HexNAc+Fuc+Neu5Ac+Neu5Gc
3_1_1_2_0a	Neu5Ac(a2-3)Gal(b1-3)[Fuc(a1-4)][Neu5Ac(a2-6)]GlcNAc(b1-3)Gal(b1-4)Glc (DS-LNF II or FDS-LNT I)
3_1_1_2_0b	Neu5Ac(a2-3)Gal(b1-3)[Neu5Ac(a2-6)]GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]Glc (DS-LNF V or FFDS-LNT II)
3_1_2_0_0a	Fuc(a1-2)Gal(b1-3)GlcNAc(b1-3)[Fuc(a1-4)]Gal(b1-4)Glc(LNDFH I)
3_1_2_0_0b	Gal(b1-3)GlcNAc[Fuc(a1-4)](b1-3)Gal(b1-4)[Fuc(a1-3)]Glc (LNDFH II)
3_1_2_0_0c	Fuc(a1-2)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)Gal(b1-4)Glc
3_1_2_0_0d	Fuc(a1-2)Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)Gal(b1-4)Glc
3_1_2_0_0e	Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]Glc
3_1_2_0_0f	Fuc(a1-2)Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)Gal(b1-4)Glc
3_1_2_0_0g	Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]Glc

Oligosaccharide Isomer Designation	Full Oligosaccharide Structural Information
3_1_2_0_0h	3Hex+HexNAc+2Fuc
3_1_2_0_0i	3Hex+HexNAc+2Fuc
3_1_2_0_0j	3Hex+HexNAc+2Fuc
3_1_3_0_0a	Fuc(a1-2)Gal(b1-4)GlcNAc[Fuc(a1-3)](b1-3)Gal(b1-4)[Fuc(a1-3)]Glc
3_1_3_0_0b	Fuc(a1-2)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-?)Gal(b1-4)[Fuc(a1-3)]Glc
3_1_3_0_0c	3Hex+HexNAc+3Fuc
3_2_0_0_0a	Gal(b1-4)GlcNAc(b1-6)[GlcNAc(b1-3)]Gal(b1-4)Glc
3_2_0_0_0b	GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc
3_2_0_0_0c	Gal-HexNAc-Gal-Gal(b1-4)GlcNAc
3_2_0_0_0d	GlcNAc + Gal(b1-4)GlcNAc-Gal(b1-4)Glc
3_2_0_0_0e	HexNAc-HexNAc-Gal-Gal(b1-4)Glc
3_2_0_0_0f	3Hex+2HexNAc
3_2_0_0_0g	3Hex+2HexNAc
3_2_0_0_0h	3Hex+2HexNAc
3_2_0_0_0i	3Hex+2HexNAc
3_2_0_0_0j	3Hex+2HexNAc
3_2_0_0_0k	3Hex+2HexNAc
3_2_0_0_0l	Gal(b1-4)GlcNAc(b1-3)[GlcNAc(b1-6)]Gal(b1-4)Glc
3_2_0_0_0m	Gal(a1-3)GlcNAc(b1-6)Gal(b1-4)Glc(a1-3)GalNAc (grunniose)
3_2_0_1_0a	Neu5Ac(a2-3)Gal(b1-4)GlcNAc(b1-6)[GlcNAc(b1-3)]Gal(b1-4)Glc
3_2_0_1_0b	3Hex+2HexNAc+Neu5Ac
3_2_0_1_0c	3Hex+2HexNAc+Neu5Ac
3_2_0_1_0d	3Hex+2HexNAc+Neu5Ac
3_2_0_2_0a	3Hex+2HexNAc+2Neu5Ac
3_2_0_2_0b	3Hex+2HexNAc+2Neu5Ac
3_2_1_0_0a	HexNAc-HexNAc[Fuc]-Gal[Gal]-Glc
3_2_1_0_0b	Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)[GlcNAc(b1-6)]Gal(b1-4)Glc
3_2_1_0_0c	Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[GlcNAc(b1-3)]Gal(b1-4)Glc
3_2_1_0_0d	Fuc + Gal(b1-4)GlcNAc(b1-3)[GlcNAc(b1-6)]Gal(b1-4)Glc
3_2_1_0_0e	3Hex+2HexNAc+Fuc
3_2_1_0_0f	3Hex+2HexNAc+Fuc
3_2_1_0_1a	3Hex+2HexNAc+Fuc+Neu5Gc
3_2_2_0_0a	GlcNAc(b1-3)[Fuc(a1-2)]Gal(b1-3)GlcNAc[Fuc(a1-4)](b1-3)Gal(b1-4)Glc
3_3_0_0_0a	Gal(b1-4)GlcNAc + Gal-GlcNAc-Gal(b1-4)GlcNAc
3_3_0_0_0b	Gal(b1-4)HexNAc-HexNAc-Gal-Gal(b1-4)Glc
3_3_0_0_0c	3Hex+3HexNAc
3_3_0_0_0d	3Hex+3HexNAc
3_3_0_0_0e	Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc
3_3_1_0_0a	Fuc+Gal(b1-4)GlcNAc(b1-6)[Gal(b1-4)GlcNAc(b1-3)]Gal(b1-4)GlcNAc
3_3_1_0_0b	3Hex+3HexNAc+Fuc
3_3_2_0_0a	3Hex+3Hex+2Fuc
3_4_1_0_0a	3Hex+4HexNAc+Fuc
3_5_0_0_0a	3Hex+5HexNAc
3_6_0_0_0a	3Hex+6HexNAc
3_6_1_0_0a	3Hex+6HexNAc+Fuc
4_0_0_0_0a	Gal(b1-3)Gal(b1-3)Gal(b1-4)Glc (3",3'-digalactosyllactose)
4_0_0_0_0b	Gal-Gal-Gal(b1-4)Glc
4_0_0_0_0c	4Hex
4_0_0_0_0d	4Hex
4_0_0_0_0e	4Hex
4_0_0_0_0f	4Hex
4_0_0_0_0g	Gal(b1-3)[Gal(b1-6)]Gal(b1-4)Glc
4_0_0_0_1a	4Hex+Neu5Gc
4_0_0_1_0a	Neu5Ac(a2-3)Gal(b1-3)Gal(b1-3)Gal(b1-4)Glc
4_0_0_1_0b	4Hex+Neu5Ac
4_0_0_1_1a	4Hex+Neu5Ac+Neu5Gc
4_0_0_2_0a	4Hex+2Neu5Ac
4_0_1_0_0a	4Hex+Fuc
4_0_2_1_0a	Neu5Ac+Gal(b1-4)GlcNAc(b1-6)[Gal(b1-4)GlcNAc(b1-3)]Gal(b1-4)Glc
4_1_0_0_0a	Gal(b1-4)GlcNAc(b1-6)[Gal(b1-3)]Gal(b1-4)Glc (LNnP I)
4_1_0_0_0b	Gal(a1-3)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc (Galili pentasaccharide)
4_1_0_0_0c	Gal(b1-3)[Gal(b1-6)GlcNAc(b1-6)]Gal(b1-4)Glc
4_1_0_0_0d	Gal(b1-4)[Gal(b1-3)]GlcNAc(b1-6)Gal(b1-4)Glc
4_1_0_0_0e	Gal(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc
4_1_0_0_0f	Gal + Gal(b1-4)GlcNAc-Gal(b1-4)Glc
4_1_0_0_0g	4Hex+HexNAc
4_1_0_0_0h	4Hex+HexNAc
4_1_0_0_0i	4Hex+HexNAc
4_1_0_0_1a	Neu5Gc(a2-3) + Gal(b1-4)GlcNAc(b1-6)(Gal(b1-3))Gal(b1-4)Glc (3GLNnP I)
4_1_0_0_1b	Neu5Gc(a2-6) + Gal(b1-4)GlcNAc(b1-6)(Gal(b1-3))Gal(b1-4)Glc (6GLNnP I)
4_1_0_0_1c	4Hex+HexNAc+Neu5Gc

Oligosaccharide Isomer Designation	Full Oligosaccharide Structural Information
4_1_0_1_0a	Neu5Ac(a2-3)Gal(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc (SLNnP a)
4_1_0_1_0b	Gal(b1-3)[Neu5Ac(a2-6)Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc (SLNnP b)
4_1_0_1_0c	Gal(b1-3)[Neu5Ac(a2-3)Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc (SLNnP c)
4_1_0_1_0d	Neu5Ac(a2-3) + Gal(b1-4)GlcNAc(b1-6)[Gal(b1-3)]Gal(b1-4)Glc (3-SLNnP I)
4_1_0_1_0e	Neu5Ac(a2-6) + Gal(b1-4)GlcNAc(b1-6)[Gal(b1-3)]Gal(b1-4)Glc (6-SLNnP I)
4_1_0_1_0f	Neu5Ac(a2-6)Gal(b1-4)GlcNAc(b1-6)-Gal(b1-4)[Gal(b1-3)]Glc
4_1_0_1_0g	4Hex+HexNAc+Neu5Ac
4_1_0_1_0h	4Hex+HexNAc+Neu5Ac
4_1_0_1_0i	4Hex+HexNAc+Neu5Ac
4_1_0_2_0a	2Neu5Ac + Gal(b1-4)GlcNAc(b1-6)(Gal(b1-3))Gal(b1-4)Glc (DSLNnP I)
4_1_0_2_0a	4Hex+HexNAc+2Neu5Ac
4_1_1_0_0a	Gal(a1-3)[Fuc(a1-2)]Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc
4_1_1_0_0b	Gal(a1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)Gal(b1-4)Glc (galactosyl LNFP III)
4_1_1_0_0c	Gal(b1-3)[Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)]Gal(b1-4)Glc (F LNnP I)
4_1_1_0_0d	Gal-HexNAc[Fuc]-Gal-Gal-Glc
4_1_1_0_0e	Fuc+Gal(b1-4)GlcNAc(b1-6)[Gal(b1-3)]Gal(b1-4)Glc
4_1_1_0_0f	Gal-[Fuc-]HexNAc-Gal-Gal-Glc
4_1_1_0_0g	4Hex+HexNAc+Fuc
4_1_1_0_0h	Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Gal(b1-3)]Gal(b1-4)Glc
4_1_1_0_0i	4Hex+HexNAc+Fuc
4_1_1_0_0j	4Hex+HexNAc+Fuc
4_1_1_1_0a	Neu5Ac(a2-3)Gal(b1-3)[Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)]Gal(b1-4)Glc (FS LNnP a)
4_1_1_1_0b	Fuc+Neu5Ac(a2-6)Gal(b1-4)GlcNAc(b1-6)[Gal(b1-3)]Gal(b1-4)Glc
4_1_1_1_0c	4Hex+HexNAc+Fuc+Neu5Ac
4_1_2_0_0a	Gal(a1-3)[Fuc(a1-2)]Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)Gal(b1-4)Glc
4_1_2_0_0b	Gal(a1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]Glc
4_1_2_0_0c	4Hex+HexNAc+2Fuc
4_2_0_0_0a	Gal(b1-3)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc (LNH)
4_2_0_0_0b	Gal(b1-4)GlcNAc(b1-6)[Gal(b1-4)GlcNAc(b1-3)]Gal(b1-4)Glc (LNnH)
4_2_0_0_0c	Gal(b1-3)GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc (p-LNH)
4_2_0_0_0d	Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc (para LNnH)
4_2_0_0_0e	Gal(b1-4)HexNAc-HexNAc-Gal-Gal(b1-4)Glc
4_2_0_0_0f	Gal(b1-4)GlcNAc + Gal(b1-4)GlcNAc-Gal(b1-4)Glc
4_2_0_0_0g	4Hex+2HexNAc
4_2_0_0_0h	4Hex+2HexNAc
4_2_0_0_0i	Gal(b-13)GlcNAc(b1-6)Gal(b1-3)GlcNAc(b1-3)Gal(b1-4)Glc (bovisose)
4_2_0_0_1a	Neu5Gc(a2-3) + Gal(b1-4)GlcNAc(b1-6)(Gal(b1-4)GlcNAc(b1-3))Gal(b1-4)Glc (3-GLNnH)
4_2_0_0_1b	Neu5Gc(a2-6) + Gal(b1-4)GlcNAc(b1-6)(Gal(b1-4)GlcNAc(b1-3))Gal(b1-4)Glc (6GLNnH)
4_2_0_0_1c	4Hex+2HexNAc+Neu5Gc
4_2_0_1_0a	Neu5Ac(a2-6)Gal(b1-4)GlcNAc(b1-6)[Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)Glc
4_2_0_1_0b	Neu5Ac(a2-6)Gal(b1-4)GlcNAc(b1-6)[Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)Glc (S-LNH)
4_2_0_1_0c	Neu5Ac(a2-6)Gal(b1-4)GlcNAc(b1-6)[Gal(b1-4)GlcNAc(b1-3)]Gal(b1-4)Glc (S-LNnH)
4_2_0_1_0d	Neu5Ac(a2-6)Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc (S-LNnH II)
4_2_0_1_0e	Neu5Ac(a2-3)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc (S para LNnH)
4_2_0_1_0f	Gal(b1-4)[Neu5Ac(a2-6)]GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc
4_2_0_1_0g	Gal(b1-3)[Neu5Ac(a2-6)]GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc (S-LNH II)
4_2_0_1_0h	Neu5Ac(a2-6)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc
4_2_0_1_0i	Neu5Ac(a2-6)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc
4_2_0_1_0j	Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)[Neu5Ac(a2-6)]GlcNAc(b1-6)]Gal(b1-4)Glc
4_2_0_1_0k	Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)][Neu5Ac-]Gal(b1-4)Glc
4_2_0_1_0l	Neu5Ac(a2-3) + Gal(b1-4)GlcNAc(b1-6)[Gal(b1-4)GlcNAc(b1-3)]Gal(b1-4)Glc (3-SLNnH)
4_2_0_1_0m	Neu5Ac(a2-6) + Gal(b1-4)GlcNAc(b1-6)[Gal(b1-4)GlcNAc(b1-3)]Gal(b1-4)Glc (6-SLNnH)
4_2_0_1_0n	4Hex+2HexNAc+Neu5Ac
4_2_0_1_0o	4Hex+2HexNAc+Neu5Ac
4_2_0_2_0a	Neu5Ac(a2-6)Gal(b1-4)GlcNAc(b1-3)[Neu5Ac(a2-6)Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc (DS LNnH I)
4_2_0_2_0b	2Neu5Ac+Gal(b1-4)GlcNAc(b1-6)[Gal(b1-4)GlcNAc(b1-3)]Gal(b1-4)Glc
4_2_0_2_0c	2Neu5Ac+Gal(b1-4)GlcNAc(b1-6)[Gal(b1-4)GlcNAc(b1-3)]Gal(b1-4)Glc
4_2_0_2_0d	Neu5Ac+Gal(b1-4)GlcNAc(b1-6)[Neu5Ac-Gal(b1-4)GlcNAc(b1-3)]Gal(b1-4)Glc
4_2_0_2_0e	4Hex+2HexNAc+2Neu5Ac
4_2_0_2_0f	4Hex+2HexNAc+2Neu5Ac
4_2_0_2_0g	Neu5Ac(a2-6)Gal(b1-4)GlcNAc(b1-6)[Neu5Ac(a2-3)Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)Glc (DS LNH I)
4_2_0_2_0h	Gal(b1-4)GlcNAc(b1-6)[Neu5Ac(a2-3)Gal(b1-3)[Neu5Ac(2-6)]GlcNAc(b1-3)]Gal(b1-4)Glc (DS LNH II)
4_2_0_3_0a	Neu5Ac(a2-6)Gal(b1-4)GlcNAc(b1-6)[Neu5Ac(a2-3)Gal(b1-3)[Neu5Ac(a2-6)]GlcNAc(b1-3)]Gal(b1-4)Glc (TS-LNH)
4_2_1_0_0a	Fuc(a1-2)Gal(b1-3)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc (MFLNH I)
4_2_1_0_0b	Gal(b1-3)GlcNAc(b1-3)[Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)]Gal(b1-4)Glc (MFLNH III)
4_2_1_0_0c	Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)Glc (MFLNH II)
4_2_1_0_0d	Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)]Gal(b1-4)Glc (FLNnH a)
4_2_1_0_0e	Fuc(a1-2)Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc (MFLNnH a)
4_2_1_0_0f	Gal(b1-4)GlcNAc(b1-3)[Fuc(a1-2)Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc (MFLNnH b)
4_2_1_0_0g	Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc (MF para LNH II)
4_2_1_0_0h	Fuc(a1-2)Gal(b1-3)GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc (MF para LNH I)

Oligosaccharide Isomer Designation	Full Oligosaccharide Structural Information
4_2_1_0_0i	Fuc(a1-2)Gal(b1-3)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc
4_2_1_0_0j	Gal(b1-3)GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-3)[Fuc(a1-3)]Gal(b1-4)Glc (MFpLNH V)
4_2_1_0_0k	Gal(b1-3)GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)Gal(b1-4)Glc (MFpLNH IV)
4_2_1_0_0l	Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc
4_2_1_0_0m	Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc
4_2_1_0_0n	Fuc+Gal(b1-4)GlcNAc(b1-6)[Gal(b1-4)GlcNAc(b1-3)]Gal(b1-4)Glc
4_2_1_0_0o	4Hex+2HexNAc+Fuc
4_2_1_0_0p	Gal(b1-4)GlcNAc(b1-4)Gal(b1-6)[Fuc(a1-3)]GlcNAc(b1-3)Gal(b1-4)Glc
4_2_1_0_0q	Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)Gal(b1-4)Glc (F-para LNnH)
4_2_1_0_0r	4Hex+2HexNAc+Fuc
4_2_1_1_0a	Fuc(a1-2)Gal(b1-4)[Neu5Ac(a2-6)]GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc
4_2_1_1_0b	Fuc(a1-2)Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)[Neu5Ac(a2-6)]GlcNAc(b1-6)]Gal(b1-4)Glc
4_2_1_1_0c	Neu5Ac(a2-3)Gal(b1-3)GlcNAc(b1-3)[Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)]Gal(b1-4)Glc (FS-LNH II)
4_2_1_1_0d	Neu5Ac(a2-6)Gal(b1-4)GlcNAc(b1-3)[Fuc(a1-2)Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc
4_2_1_1_0e	Neu5Ac(a2-6)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)Gal(b1-4)Glc
4_2_1_1_0f	Neu5Ac(a2-6)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)Gal(b1-4)Glc (FS-LNnH I)
4_2_1_1_0g	Neu5Ac(a2-6)Gal(b1-4)GlcNAc(b1-6)[Fuc(a1-3)Gal(b1-4)GlcNAc(b1-3)]Gal(b1-4)Glc
4_2_1_1_0h	Neu5Ac(a2-6)Gal(b1-3)GlcNAc(b1-3)[Gal(b1-4)[Fuc(a1-2)]GlcNAc(b1-6)]Gal(b1-4)Glc
4_2_1_1_0i	Neu5Ac(a2-6)Gal(b1-4)GlcNAc(b1-6)[Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)Glc (FS-LNH III)
4_2_1_1_0j	Neu5Ac(a2-6)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc (FS-para LNnH I)
4_2_1_1_0k	Gal(b1-3)[Neu5Ac(a2-6)]GlcNAc(b1-3)[Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)]Gal(b1-4)Glc (FS-LNH I)
4_2_1_1_0l	Neu5Ac(a2-6)Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)]Gal(b1-4)Glc
4_2_1_1_0m	Fuc+Neu5Ac(a2-6)Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc
4_2_1_1_0n	4Hex+2HexNAc+Fuc+Neu5Ac
4_2_1_1_0o	Neu5Ac(a2-6)Gal(b1-4)GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)Glc (FS-LNH)
4_2_1_1_0p	Gal(b1-4)GlcNAc(b1-6)[Neu5Ac(a2-3)Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)Glc (FS-LNH IV)
4_2_1_1_0q	Fuc+Neu5Ac(a2-6)Gal(b1-4)GlcNAc(b1-6)[Gal(b1-4)GlcNAc(b1-3)]Gal(b1-4)Glc (FS-LNnH II)
4_2_1_1_0r	4Hex+2HexNAc+Fuc+Neu5Ac
4_2_1_2_0a	Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)[Neu4,5Ac2(a2-6)Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc
4_2_1_2_0b	Neu5Ac(a2-6)Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)]Gal(b1-4)Glc
4_2_1_2_0c	Fuc(a1-2)Gal(b1-4)GlcNAc(b1-6)[Neu5Ac(a2-3)Gal(b1-3)[Neu5Ac(a2-6)]GlNAc(b1-3)]Gal(b1-4)Glc (FDS-LNH I)
4_2_1_2_0d	Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Neu5Ac(a2-3)Gal(b1-3)[Neu5Ac(a2-6)]GlcNAc(b1-3)]Gal(b1-4)Glc (FDS-LNH II)
4_2_1_2_0e	Neu5Ac(a2-6)Gal(b1-4)GlcNAc(a1-6)[Neu5Ac(a2-3)Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)Glc (FDS-LNH III)
4_2_1_2_0f	Neu5Ac(a2-6)Gal(b1-4)GlNAc(b1-6)[Neu5Ac(a2-6)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)]Gal(b1-4)Glc
4_2_1_2_0g	Neu5Ac(a2-3)Gal(b1-4)GlNAc(b1-6)[Neu5Ac(a2-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)]Gal(b1-4)Glc
4_2_1_4_0a	Neu5Ac(a2-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)[Neu4,5Ac2(a2-3)Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc
4_2_1_4_0b	Neu5Ac(a2-3)Gal(b1-4)GlcNAc(b1-3)[Neu4,5Ac2(a2-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)]Gal(b1-4)Glc
4_2_2_0_0a	Fuc(a1-2)Gal(b1-4)GlcNAc(b1-3)[Fuc(a1-2)Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc (DFLNnH)
4_2_2_0_0b	Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)[Gal(b1-3)GlcNAc[Fuc(a1-3)](b1-6)]Gal(b1-4)Glc (DFLNH II)
4_2_2_0_0c	Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)[Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)]Gal(b1-4)Glc (DFLNnH)
4_2_2_0_0d	Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)Gal(b1-4)Glc (DF-paraLNnH)
4_2_2_0_0e	Fuc(a1-2)Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc (DFLNHc)
4_2_2_0_0f	Fuc(a1-3)Gal(b1-4)GlcNAc(b1-6)Fuc(a1-2)Gal(b1-3)GlcNAc(b1-3)Gal(b1-4)Glc (DFLNHa/2,3-DF-LNH)
4_2_2_0_0g	Gal(b1-3)[Fuc(a1-3)]GlcNAc(b1-3)[Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)]Gal(b1-4)Glc
4_2_2_0_0h	Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)]Gal(b1-4)Glc
4_2_2_0_0i	4Hex+2HexNAc+2Fuc
4_2_2_0_0j	Fuc(a1-2)Gal(b1-3)GlcNAc(b1-3)[Fuc(a1-3)[Gal(b1-4)]GlcNAc(b1-6)]Gal(b1-4)Glc (DFLNH I)
4_2_2_0_0k	Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)Gal(b1-4)Glc (DF-para LNH I)
4_2_2_0_0l	Fuc(a1-2)Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc (DF-para LNH II)
4_2_2_0_0m	Fuc(a1-2)Gal(b1-3)GlcNAc(b1-3)Gal(b1-4)[jFuc(a1-3)]GlcNAc(b1-3)Gal(b1-4)Glc (DF-para LNH III)
4_2_2_0_0n	4Hex+2HexNAc+2Fuc
4_2_2_1_0a	Fuc(a1-2)Gal(b1-4)[Neu5Ac(a2-6)]GlcNAc(b1-3)[Fuc(a1-2)Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc
4_2_2_1_0b	Fuc(a1-2)Gal(b1-4)GlcNAc(b1-3)[Fuc(a1-2)Gal(b1-4)[Neu5Ac(a2-6)]GlcNAc(b1-6)]Gal(b1-4)Glc
4_2_2_1_0c	Fuc(a1-2)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Neu5Ac(a2-3)Gal(b1-4)GlcNAc(b1-3)]Gal(b1-4)Glc
4_2_2_1_0d	Neu5Ac(a2-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)Gal(b1-4)Glc
4_2_2_1_0e	Neu5Ac(a2-6)Gal(b1-4)GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-3)[Fuca1-4)]GlcNAc(b1-3)]Gal(b1-4)Glc (DFS-LNH I)
4_2_2_1_0f	Fuc(a1-2)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)[Neu5Ac(a2-3)Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc
4_2_2_1_0g	Fuc(a1-2)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)[Neu5Ac(a2-6)Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc
4_2_2_1_0h	Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)[Neu5Ac(a2-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)]Gal(b1-4)Glc
4_2_2_1_0i	Neu5Ac(a2-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)GlcNAc(b1-6)]Gal(b1-4)Glc
4_2_2_1_0j	Neu5Ac(a2-3)Gal(b1-4)GlcNAc(b1-3)[Fuc(a1-2)Gal(b1-4)[Fuc(a1-3)GlcNAc(b1-6)]Gal(b1-4)Glc
4_2_2_1_0k	Neu5Ac(a2-6)Gal(b1-4)GlcNAc(b1-3)[Fuc(a1-2)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)]Gal(b1-4)Glc (DFS-LNnH)
4_2_2_1_0l	Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Neu5Ac(a2-3)Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)Glc (DFS-LNH II)
4_2_2_1_0m	Neu5Ac(a2-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)Glc (DFS-LNH III)
4_2_2_1_0n	Neu5Ac(a2-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)Glc (DFS-LNH IV)
4_2_2_1_0o	4Hex+2HexNAc+2Fuc+1Neu5Ac
4_2_3_0_0a	Fuc(a1-2)Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)[Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)]Gal(b1-4)Glc (TFLNH I)
4_2_3_0_0b	Fuc(a1-2)Gal(b1-4)GlcNAc(b1-6)[Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)]Gal(b1-4)Glc
4_2_3_0_0c	Fuc(a1-2)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Gal(b1-3)[Fuc(a1-4)GlcNAc(b1-3)]Gal(b1-4)Glc (TFLNH II)

Oligosaccharide Isomer Designation	Full Oligosaccharide Structural Information
4_2_3_0_0d	Fuc(a1-2)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)[Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)]Gal(b1-4)Glc
4_2_3_0_0e	4Hex+2HexNAc+3Fuc
4_2_3_0_0f	Fuc(a1-2)Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)Gal(b1-4)Glc (TF-para LNH I)
4_2_3_0_0g	Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]Glc (TF-para LNH II)
4_2_3_0_0h	Gal(b1-4)[Fuc(1-3)]GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]Glc (TF-para LNnH)
4_2_3_0_0i	4Hex+2HexNAc+3Fuc
4_2_3_1_0a	Fuc(a1-2)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)[Neu5Ac(a2-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)]Gal(b1-4)Glc
4_2_3_1_0b	Neu5Ac(a2-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-)[Fuc(a1-2)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)]Gal(b1-4)Glc
4_2_4_0_0a	Fuc(a1-2)Gal(b1-4)[Fuc(a1-3)]FlcNAc(b1-6)[Fuc(a1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)]Gal(b1-4)Glc
4_3_0_0_0a	4Hex+3HexNAc
4_2_1_0_0a	Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[GlcNAc(b1-3)]Gal(b1-4)Glc
4_3_0_0_0a	4Hex+3HexNAc
4_3_2_0_0a	Fuc(a1-2)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)]Gal(b1-4)Glc
4_3_2_0_0b	Fuc(a1-2)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)[Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)]Gal(b1-4)Glc
4_4_0_0_0a	4Hex+4HexNAc
4_4_1_0_0a	4Hex+4HexNAc+Fuc
4_5_0_0_0a	4Hex+5HexNAc
4_5_1_0_0a	4Hex+5HexNAc+Fuc
5_0_0_0_0b	Gal(b1-3)Gal(b1-3)Gal(b1-3)Gal(b1-4)Glc
5_0_0_0_0c	5Hex
5_0_0_0_1a	5Hex+Neu5Gc
5_0_0_1_0a	Neu5Ac(a2-3)Gal(b1-3)Gal(b1-3)Gal(b1-3)Gal(b1-4)Glc
5_0_0_1_0b	5Hex+1Neu5Ac
5_0_1_0_0a	5Hex+Fuc
5_1_0_0_0a	Gal(b1-3)Gal(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc (galactosyl LNnP I)
5_1_0_0_0b	Gal(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-3)Gal(b1-4)Glc (galactosyl LNnP II)
5_1_0_0_0c	Gal(b1-4)GlcNAc(b1-6)[Gal(b1-4)Gal(b1-3)]Gal(b1-4)Glc
5_1_0_0_0d	Gal(b1-3)[Gal(b1-3)Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc
5_1_0_0_0e	Gal(b1-3)Gal(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc
5_1_0_0_0f	Gal(b1-4)GlcNAc(b-16)[Gal(b1-4)Gal(b1-3)]Gal(b1-4)Glc
5_1_0_0_0g	Gal(a1-3) + Gal(b1-4)GlcNAc(b1-6)[Gal(b1-3)]Gal(b1-4)Glc
5_1_0_0_0h	5Hex+HexNAc
5_1_0_1_0a	Gal(b1-3)Gal(b1-3)[Neu5Ac(a2-6)Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc (galactosyl sialyl LNnP b)
5_1_0_1_0b	Neu5Ac(a2-3)Gal(b1-3)Gal(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc
5_1_0_1_0c	Gal(b1-3)Gal(b1-3)[Neu5Ac(a2-3)Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc
5_1_1_0_0a	5Hex+HexNAc+Fuc
5_2_0_0_0a	Gal(a1-3) + Gal(b1-4)GlcNAc(b1-6)(Gal(b1-4)GlcNAc(b1-3))Gal(b1-4)Glc
5_2_0_0_0b	Gal(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc
5_2_0_0_0c	5Hex+2HexNAc
5_2_0_0_0d	Gal(b1-4)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc
5_2_0_0_0e	5Hex+2HexNAc
5_2_0_0_0f	Glc(a1-3)Gal(b1-3)GlcNAc(b1-6)Gal(b1-3)GlcNAc(b1-3)Gal(b1-4)Glc (vakose)
5_2_0_1_0a	Neu5Ac + Gal(a1-3) + Gal(b1-4)GlcNAc(b1-6)(Gal(b1-4)GlcNAc(b1-3))Gal(b1-4)Glc
5_2_0_1_0b	Neu5Ac(a2-6)Gal(b1-4)GlcNAc(b1-3)[Gal(a1-3)Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc (MS monogalactosyl LNnH)
5_2_0_1_0c	5Hex+2HexNAc+Neu5Ac
5_2_1_0_0a	Gal(b1-4)Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)]Gal(b1-4)Glc
5_2_1_0_0b	5Hex+2HexNAc+Fuc
5_2_1_1_0a	Neu5Ac(a2-6)Gal(b1-4)GlcNAc(b1-3)[Gal(a1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)]Gal(b1-4)Glc (MSMF monogalactosyl LNnH)
5_2_2_0_0a	Gal(a1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)Gal(b1-4)Glc
5_2_2_0_0b	5Hex+2HexNAc+2Fuc
5_2_2_0_0c	5Hex+2HexNAc+2Fuc
5_2_2_1_0a	Neu5Ac(a2-6)Gal(b1-4)GlcNAc(b1-3)[Gal(a1-3)[Fuc(a1-2)]Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)]Gal(b1-4)Glc
5_3_0_0_0a	Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(1-4)Glc
5_3_0_0_0b	5Hex+3HexNAc
5_3_0_0_0c	Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-6)[Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)Glc (LNO)
5_3_0_0_0d	Gal(b1-3)GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-6)[Gal(b1-4)GlcNAc(b1-3)]Gal(b1-4)Glc (LNnO)
5_3_0_0_0e	Gal(b1-3)GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-6)[Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)Glc (iso LNO)
5_3_0_0_0f	Gal(b1-3)GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc (para LNO)
5_3_0_0_0g	Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc (para LNnO)
5_3_0_0_0h	Gal(b1-4)GlcNAc(b1-6)Gal(b1-4)GlcNAc(b1-6)[Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)Glc
5_3_0_0_0i	Gal(b1-4)GlcNAc(b1-6)[Gal(b1-4)GlcNAc(b1-3)]Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc (nova-LNnO)
5_3_0_0_0j	5Hex+3HexNAc
5_3_0_1_0a	Neu5Ac(a2-6)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)GlcNc(b1-6)[Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)Glc (S-LNO)
5_3_0_1_0b	5Hex+3HexNAc+1Neu5Ac
5_3_1_0_0a	Gal(b1-3)GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Gal(b1-4)GlcNAc(b1-3)]Gal(b1-4)Glc (FLNnO I)
5_3_1_0_0b	Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)Glc (FLNO III)
5_3_1_0_0c	Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-6)[Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)Glc (FLNO II)
5_3_1_0_0d	Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)Glc (FLNO I)

Oligosaccharide Isomer Designation	Full Oligosaccharide Structural Information
5_3_1_0_0e	Fuc+Gal(b1-4)GlcNAc(b-13)Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(1-4)Glc
5_3_1_0_0f	Fuc(a1-2)Gal(b1-3)GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-6)[Gal(b1-4)GlcNAc(b1-3)]Gal(b1-4)Glc (FLNnO II)
5_3_1_0_0g	Gal(b1-3)GlcNAc(b1-3)Gal(b1-4)[uc(a1-3)]GlcNAc(b1-6)[Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)Glc (F-iso-LNO)
5_3_1_0_0h	Gal(b1-4)GlcNAc(b1-3)Gal(b-14)[Fuc(a1-3)]GlcNAc(b1-6)[Gal(b1-4)GlcNAc(b1-3)]Gal(b1-4)Glc (F-iso-LNnO I)
5_3_1_0_0i	Gal(b1-4)GlcNAc(b1-6)[Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)]Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc (F-nova-LLNnO)
5_3_1_0_0j	Gal(b1-3)GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)Glc (F-para-LNO)
5_3_1_0_0k	5Hex+3HexNAc+1Fuc
5_3_1_0_0l	5Hex+3HexNAc+1Fuc
5_3_1_1_0a	Neu5Ac(a2-3)Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc (FS-LNO
5_3_1_1_0b	Neu5Ac(a2-6)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)Glc (FS-LNO II)
5_3_1_1_0c	Gal(b1-3)GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-6)[Neu5aC(a2-3)Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)Glc (FS-iso LNO)
5_3_1_1_0d	Gal(b1-4)GlcNAc(b1-6)Gal(b1-4)GlcNAc(b1-6)[Neu5Ac(a2-3)Gal(b1-3)[Fuc(a1-4)[]GlcNac(b1-3)Gal(b1-4)Glc
5_3_1_1_0e	5Hex+3HexNAc+1Fuc+1Neu5Ac
5_3_1_1_0f	5Hex+3HexNAc+1Fuc+1Neu5Ac
5_3_2_0_0a	Fuc(a1-2)Gal(b1-3)GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-6)[Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)]Gal(b1-4)Glc (DFLNnO III)
5_3_2_0_0b	Fuc(a1-2)Gal(b1-3)GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)Glc (DF-iso LNO VII)
5_3_2_0_0c	Gal(b1-3)GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)]Gal(b1-4)Glc (DFLNnO II)
5_3_2_0_0d	Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)Glc (DFLNO I)
5_3_2_0_0e	Gal(b1-4)GlcNAc(b1-6)Gal(b1-4)GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-4)[Fuc(a1-3)]GlcNAc(a1-3)]Gal(b1-4)Glc
5_3_2_0_0f	Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)Gal(b1-4)GlcNAc(b1-6)[Fuc(a1-2)Gal(1-3)GlcNAc(b1-3)]Gal(b1-4)Glc
5_3_2_0_0g	Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)GlcNAc(b1-6)Gal(b1-4)Glc
5_3_2_0_0h	Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)GlcNAc(b1-6)Gal(b1-4)Glc
5_3_2_0_0i	Gal(b1-4)GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)GlcNAc(b1-6)Gal(b1-4)Glc
5_3_2_0_0j	Gal(b1-4)GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)]Gal(b1-4)GlcNAc(b1-6)Gal(b1-4)Glc
5_3_2_0_0k	Gal(b1-4)GlcNAc(b1-6)Gal(b1-4)GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-3)[Fuc(a1-4)]GlcNAc(a1-3)]Gal(b1-4)Glc
5_3_2_0_0l	2Fuc(a1-2)+Gal(b1-4)GlcNAc(b1-3)+Gal(b1-4)GlcNAc(b1-6)[Gal(b1-4)GlcNAc(b1-3)]Gal(b1-4)Glc
5_3_2_0_0m	Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)Glc (DF-LNO II)
5_3_2_0_0n	Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Gal(b1-4)GlcNAc(b1-3)]Gal(b1-4)Glc (DF-LNnO I)
5_3_2_0_0o	Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)Glc (DF-iso LNO I)
5_3_2_0_0p	Gal(b1-3)GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]GlcNac(b1-6)[Fuc(a1-2)Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)Glc (DF-iso LNO II)
5_3_2_0_0q	Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]GlcNac(b1-6)[Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)]Gal(b1-4)Glc (DF-iso LNnO)
5_3_2_0_0r	Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)Glc (DFLNO III)
5_3_2_0_0s	Gal(b1-3)GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)Glc (DF-iso LNO III)
5_3_2_0_0t	Gal(b1-3)GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)Glc (DF-iso LNO IV)
5_3_2_0_0u	Fuc(a1-2)Gal(b1-3)GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)Glc (DF-iso LNO V)
5_3_2_0_0v	Fuc(a1-2)Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)Gal(b1-4)GlcNaC(b1-6)[Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)Glc (DF-iso LNO VI)
5_3_2_0_0w	Gal(b-14)GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAC(b1-3)Gal(b1-4)[Guc(b1-3)]GlcNAc(b1-3)Gal(b1-4)Glc (DF-para LNnO)
5_3_2_0_0x	5Hex+3HexNAc+2Fuc
5_3_2_0_0y	5Hex+3HexNAc+2Fuc
5_3_2_0_0z	5Hex+3HexNAc+2Fuc
5_3_2_1_0a	Neu5Ac(a2-6)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)Glc (DFS-LNO III)
5_3_2_1_0b	Neu5Ac(a2-6)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)Glc (DFS-LNO II)
5_3_2_1_0c	2Fuc(a1-2)+Gal(b1-4)Glc(b1-3)+Neu5Ac(a2-6)+Gal(b1-4)GlcNAc(b1-6)[Gal(b1-4)GlcNAc(b1-3)]Gal(b1-4)Glc
5_3_2_1_0d	Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-6)[Neu5Ac(a2-3)Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)Glc (DFS-LNO I)
5_3_2_1_0e	Gal(b1-3)GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Neu5Ac(a2-3)Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)Glc (DFS-iso LNO I)
5_3_2_1_0f	Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-6)[Neu5Ac(a2-3)Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)Glc (DFS-iso LNO II)
5_3_2_1_0g	Fuc(a1-2)Gal(b1-4)GlcNAc(b1-6)Gal(b1-4)GlcNAc(b1-6)[Neu5Ac(a2-3)Gal(b1-4)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)Glc
5_3_2_1_0h	5Hex+3HexNAc+2Fuc+1Neu5Ac
5_3_2_1_0i	5Hex+3HexNAc+2Fuc+1Neu5Ac
5_3_3_0_0a	Fuc(a1-2)Gal(b1-3)GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)Glc (TF-iso LNO I)
5_3_3_0_0b	Fuc(a1-2)Gal(b1-3)GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)Glc
5_3_3_0_0c	Fuc(a1-2)Gal(b1-3)GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)Glc (Tetra-iso-LNO)

Oligosaccharide Isomer Designation	Full Oligosaccharide Structural Information
5_3_3_0_0c	Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)Glc (TFLNO I)
5_3_3_0_0d	Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)]Gal(b1-4)Glc (TFLNnO I)
5_3_3_0_0e	Fuc(a1-2)Gal(b1-3)GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-4)GlcNAc(b1-3)]Gal(b1-4)Glc (TFLNnO II)
5_3_3_0_0f	Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-4)]GlcNAc(b1-6)[Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)Glc (TF-iso LNO II)
5_3_3_0_0g	Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-3)[Fuc(a1-4)]GlcNaC(b1-3)]Gal(b1-4)Glc (TFLNO II)
5_3_3_0_0h	Gal(b1-3)[Fuc(a1-4)]GlcNAC(b1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)Glc (TF-iso LNO II)
5_3_3_0_0i	Gal(b1-3)GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)Glc (TF-iso LNO III)
5_3_3_0_0j	Fuc(a1-2)Gal(b1-3)GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)Glc (TF-iso LNO IV)
5_3_3_0_0k	Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]GlcNac(b1-6)[Gal(b1-4)[Fuc(a1-3)]GlcNAc(a1-3)]Gal(b1-4)Glc (TF-iso LNnO)
5_3_3_0_0l	5Hex+3HexNAc+3Fuc
5_3_3_0_0m	5Hex+3HexNAc+3Fuc
5_3_3_1_0a	Neu5Ac(a2-6)Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)Glc (TFS-LNO)
5_3_3_1_0b	Fuc(a1-32)Gal(b1-3)GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Neu5Ac(a2-3)Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)Glc (TFS-iso LNO)
5_3_3_1_0c	5Hex+3HexNAc+3Fuc+1Neu5Ac
5_3_4_0_0a	Fuc(a1-2)Gal(b1-3)GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)Glc (TetraF-iso LNO)
5_3_4_0_0b	Fuc(a1-2)Gal(b1-3)GlcNAc(b1-3)[Fuc(a1-4)]Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)Gal(b1-4)Glc (TetraF-para LNO)
5_3_4_0_0c	5Hex+3HexNAc+4Fuc
5_3_5_0_0a	Fuc(a1-2)Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)Glc (PentaF-iso LNO)
5_4_0_0_0a	5Hex+4HexNAc
5_4_1_0_0a	5Hex+4HexNAc+Fuc
5_5_1_0_0a	Fuc+Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc
5_5_2_0_0a	2Fuc+Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc
6_0_0_0_0a	Gal(b1-3)Gal(b1-3)Gal(b1-3)Gal(b1-3)Gal(b1-4)Glc
6_0_0_0_0b	6Hex
6_0_0_0_0c	6Hex
6_0_0_0_1a	6Hex+Neu5Gc
6_1_0_0_0a	Gal(b1-3)Gal(b1-3)Gal(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc
6_1_0_0_0b	6Hex+HexNAc
6_2_0_0_0a	Gal(a1-3)Gal(b1-4)GlcNAc(b1-3)[Gal(a1-3)Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc
6_2_0_0_0b	Gal(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc
6_2_0_0_0c	2Gal(a1-3) + Gal(b1-4)GlcNAc(b1-6)(Gal(b1-4)GlcNAc(b1-3))Gal(b1-4)Glc
6_2_0_0_0d	6Hex+2HexNAc
6_2_0_0_0e	Gal(b1-4)Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc
6_2_0_1_0a	Neu5Ac-Gal(b1-4)GlcNAc(b1-6)+Gal(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc
6_2_0_1_0b	Neu5Ac(a2-3)Gal(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc
6_2_0_1_0c	Neu5Ac(a2-3)+Gal(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc)
6_2_2_0_0a	Gal(a1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)[Gal(a1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)]Gal(b1-4)Glc
6_2_2_0_0b	Gal(a1-3)[Fuc(a1-3)]Gal(b1-4)GlcNAc(b1-6)[Gal(a1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)]Gal(b1-4)Glc
6_2_3_0_0a	Gal(a1-3)[Fuc(a1-2)]Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)[Gal(a1-3)Gal(b1-4)[Fuc(a1-3)]-GlcNAc(b1-6)]Gal(b1-4)Glc
6_2_3_0_0b	Gal(a1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Gal(a1-3)[Fuc(a1-2)]Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)]Gal(b1-4)Glc
6_4_0_0_0a	Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc
6_4_0_0_0b	Gal(b1-4)GlcNAc(b1-6)[Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)GlcNAc(b1-6)[Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)Glc (LND)
6_4_0_0_0c	Gal(b1-4)GlcNAc(b1-6)[Gal(b1-4)GlcNAc(b1-3)]Gal(b1-4)GlcNAc(b1-6)[Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)Glc (LNnD)
6_4_0_0_0d	Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-6)[Gal(b1-3)GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-3)]Gal(b1-4)Glc
6_4_0_0_0e	Gal(b-14)GlcNAc(b1-6)[Gal(b1-4)GlcNAc(b1-3)]Gal(b1-4)GlcNAC(b1-6)[Gal(b1-4)GlcNAc(b1-3)]Gal(b1-4)Glc (iso-LND)
6_4_0_0_0f	6Hex+4HexNAc
6_4_0_1_0a	Neu5Ac(a2-6)Gal(b1-4)GlcNAc(b1-6)[Gal(b1-4)GlcNAc(b1-3)]Gal(b1-4)GlcNAc(b1-6)[Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)Glc (SLNnD)
6_4_0_1_0b	6Hex+4HexNAc+1Neu5Ac
6_4_1_0_0a	Gal(b1-3)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-3)]Gal(b1-4)GlcNAc(b1-6)[Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)Glc
6_4_1_0_0b	Gal(b1-3)GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)]Gal(b1-4)Glc
6_4_1_0_0c	Fuc+Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc
6_4_1_0_0d	Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)GlcNAc(b1-6)[Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)Glc (FLND I)

Oligosaccharide Isomer Designation	Full Oligosaccharide Structural Information
6_4_1_0_0e	Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Gal(b1-4)GlcNAc(b1-3)]Gal(b1-4)GlcNAc(b1-6)[Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)Glc (FLNnD I)
6_4_1_0_0f	Gal(b1-4)GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)GlcNAc(b1-6)[Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)Glc
6_4_1_0_0g	Gal(b1-4)GlcNAc(b1-6)[Gal(b1-4)GlcNAc(b1-3)]Gal(b1-4)GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)Glc (FLNnD II)
6_4_1_0_0h	Gal(b1-4)GlcNAc(b1-6)[Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)GlcNAc(b1-6)[Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)Glc (FLND II)
6_4_1_0_0i	6Hex+4HexNAc+1Fuc
6_4_2_0_0a	Gal(b1-3)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-3)]Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)Glc
6_4_2_0_0b	2Fuc(a1-2)+2Gal(b1-4)Glc(b1-3)+Gal(b1-4)GlcNAc(b1-6)[Gal(b1-4)GlcNAc(b1-3)]Gal(b1-4)Glc
6_4_2_0_0c	Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Gal(b1-3)GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)]Gal(b1-4)Glc
6_4_2_0_0d	2Fuc+Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc
6_4_2_0_0e	Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)Glc (DFLND I)
6_4_2_0_0f	Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Gal(b1-4)GlcNAc(b1-3)]Gal(b1-4)GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)Glc (DFLNnD)
6_4_2_0_0g	Gal(b1-4)GlcNAc(b1-6)[Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)GlcNAc(b1-6)[Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)Glc (DFLND II)
6_4_2_0_0h	Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)GlcNAc(b1-6)[Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)Glc (DFLND III)
6_4_2_0_0i	Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)GlcNAc(b1-6)[Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)Glc (DFLND IV)
6_4_2_0_0j	Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)GlcNAc(b1-6)[Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)Glc (DFLND V)
6_4_2_0_0k	Gal(b1-4)GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)GlcNAc(b1-6)[Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)Glc
6_4_2_0_0l	Gal(b1-4)GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)]Gal(b1-4)GlcNAc(b1-6)[Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)Glc
6_4_2_0_0m	Gal(b1-4)GlcNAc(b1-6)[Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)Glc (DFLND VI)
6_4_2_0_0n	Gal(b1-4)GlcNAc(b1-6)[Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)GlNAc(b1-6)[Fuc(a1-2)Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)Glc
6_4_2_0_0o	Gal(b1-3)GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Gal(b1-3)GlcNAc(b1-3)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)Gal(b1-4)Glc (DF-novo LND)
6_4_2_0_0p	6Hex+4HexNAc+2Fuc
6_4_2_0_0q	6Hex+4HexNAc+2Fuc
6_4_2_1_0a	2Fuc(a1-2)+2Gal(b1-4)Glc(b1-3)+Neu5Ac(a2-6)+Gal(b1-4)GlcNAc(b1-6)[Gal(b1-4)GlcNAc(b1-3)]Gal(b1-4)Glc
6_4_2_1_0b	2Fuc(a1-2)+Neu5Ac(a2-3)+Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc
6_4_2_1_0c	2Fuc(a1-2)+Neu5Ac(a2-6)+Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc
6_4_3_0_0a	Fuc(a1-2)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)Glc (TriF-LND VII)
6_4_3_0_0b	Gal(b1-3)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)Glc
6_4_3_0_0c	Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)Glc (TriF-LND VI)
6_4_3_0_0d	3Fuc+Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc
6_4_3_0_0e	Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)GlcNAc(b1-6)[Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)Glc (TriF-LND I)
6_4_3_0_0f	Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)Glc (TriF-LND II)
6_4_3_0_0g	Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)GlcNAc(b1-6)[Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)Glc (TriF-LND III)
6_4_3_0_0h	Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)Glc (TriF-LND IV)
6_4_3_0_0i	Gal(b1-4)GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)Glc
6_4_3_0_0j	Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)]Gal(b1-4)GlcNAc(b1-6)[Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)Glc
6_4_3_0_0k	Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)GlcNAc(b1-6)[Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)Glc
6_4_3_0_0l	Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)Glc
6_4_3_0_0m	Gal(b1-4)GlcNAc(b1-6)[Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)Glc (TriF-LND V)
6_4_3_0_0n	Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-6)[Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)GlcNAc(b1-3)]Gal(b1-4)Glc

Oligosaccharide Isomer Designation	Full Oligosaccharide Structural Information
6_4_3_0_0o	6Hex+4HexNAc+3Fuc
6_4_3_0_0p	6Hex+4HexNAc+3Fuc
6_4_3_0_0q	6Hex+4HexNAc+3Fuc
6_4_4_0_0a	Fuc(a1-2)Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)[Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)Glc
6_4_4_0_0b	Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)Glc (TetraF-LND I)
6_4_4_0_0c	Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)Glc (TetraF-LND II)
6_4_4_0_0d	Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-3)GlcNAc(b1-3)]Gal(b1-4)Glc (TetraF-LND III)
6_4_4_0_0e	Gal(b1-4)GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)Glc
6_4_4_0_0f	Gal(b1-4)GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-3)[Fuc(a1-4)]GlcNAc(b1-3)]Gal(b1-4)GlcNAc(b1-6)[Fuc(a1-2)Gal(b1-4)[Fuc(a1-3)]GlcNAc(b1-3)]Gal(b1-4)Glc
6_4_4_0_0g	6Hex+4HexNAc+4Fuc
7_0_0_0_0a	7Hex
7_0_0_0_0b	7Hex
7_0_0_0_0c	Gal(b1-4)Gal(1-4)Gal(b1-4)Gal(b1-4)Gal(b1-4)Gal(b1-4)Glc
7_2_0_0_0a	7Hex+2HexNAc
7_2_0_1_0a	Neu5Ac(a2-3)Gal(b1-3)+Gal(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc
7_5_0_0_0a	Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc
7_5_1_0_0a	Fuc+Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc
7_5_2_0_0a	2Fuc+Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-3)Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc
7_5_3_0_0a	7Hex+5HexNAc+3Fuc
7_5_3_0_0b	7Hex+5HexNAc+3Fuc
7_5_4_0_0a	7Hex+5HexNAc+4Fuc
7_5_4_0_0b	7Hex+5HexNAc+4Fuc
7_7_2_0_0a	2Fuc+Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc
7_7_3_0_0a	3Fuc+Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc
8_0_0_0_0a	Gal(b1-4)Gal(1-4)Gal(b1-4)Gal(b1-4)Gal(b1-4)Gal(b1-4)Gal(b1-4)Glc
8_0_0_0_0b	8Hex
8_3_0_0_0a	Gal(b1-3)[Gal(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-6)]Gal(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc
8_3_0_0_0b	Gal(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-3)[Gal(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc
8_3_0_1_0a	Neu5Ac(a2-3)+Gal(b1-3)[Gal(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-6)]Gal(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc
8_3_0_1_0b	Neu5Ac(a2-3)+Gal(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-3)[Gal(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc
8_6_0_0_0a	Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc
8_6_1_0_0a	Fuc+Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc
8_6_2_0_0a	2Fuc+Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc
8_6_3_0_0a	3Fuc+Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc
8_6_3_1_0a	3Fuc(a1-2)+Neu5Ac(a2-3)+Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc
8_6_3_1_0b	3Fuc(a1-2)+Neu5Ac(a2-6)+Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc
8_6_4_0_0a	4Fuc+Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc
9_0_0_0_0a	9Hex
9_2_0_2_0a	2[Neu5Ac-Gal(b1-4)GlcNAc(b1-6)]+Gal(b1-3)Gal(b-13)Gal(b1-3)Gal(b1-3)Gal(b1-3)Gal(b1-4)Glc
10_2_0_1_0a	Neu5Ac-Gal(b1-4)GlcNAc(b1-6)+Gal(b1-4)GlcNAC(b1-6)+Gal(b1-3)Gal(b1-3)Gal(b1-3)Gal(b1-3)Gal(b1-3)Gal(b1-3)Gal(b1-4)Glc
10_8_0_0_0a	Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc
10_8_1_0_0a	Fuc+Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc
10_8_2_0_0a	2Fuc+Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc
10_8_3_0_0a	3Fuc+Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc
10_8_4_0_0a	4Fuc+Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-3)[Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)GlcNAc(b1-6)]Gal(b1-4)Glc

Supplementary Table 5.2. Non-human milk oligosaccharide publications included in the database

Genus	Species	Common Name	Number of Donors	Total Number of Samples	Number of Oligosaccharides Identified	Analytical Method	Publication	
Non-human primates								
Pan	troglodytes	Chimpanzee	1	1	>100	LC-MS	Tao	2011
Pan	troglodytes	Chimpanzee	2	2	7	NMR	Urashima	2009
Pan	troglodytes	Chimpanzee	1	1	8	HPLC	Warren	2001
Pan	paniscus	Bonobo	1	1	11	NMR	Urashima	2009
Pan	paniscus	Bonobo	1	4	10	HPLC	Warren	2001
Gorilla	gorilla	Gorilla	1	1	52	LC-MS	Tao	2011
Gorilla	gorilla	Gorilla	2	1	5	NMR	Urashima	2009
Gorilla	gorilla	Gorilla	1	1	5	HPLC	Warren	2001
Pongo	pygmaeus	Orangutan	1	1	12	NMR	Urashima	2009
Papio	hamadryas	Hamadryas Baboon	3	3	6	NMR	Goto	2010
Macaca	sinica	Toque Macaque	2	2	3	NMR	Goto	2010
Macaca	mulatta	Rhesus Macaque	9	9	9	NMR	Goto	2010
Macaca	mulatta	Rhesus Macaque	1	1	69	LC-MS	Tao	2011

Genus	Species	Common Name	Number of Donors	Total Number of Samples	Number of Oligosaccharides Identified	Analytical Method	Publication	
				Non-human primates (continued)				
Alouatta	palliata	Mantled Howler	3	3	2	NMR	Goto	2010
Sapajus	apella	Brown Capuchin	3	3	6	NMR	Goto	2010
Sapajus	apella	Brown Capuchin	1	2	6	NMR	Urashima	1999
Saimiri	boliviensis	Bolivian Squirrel Monkey	3	3	2	NMR	Goto	2010
Leontopithecus	rosalia	Golden Lion Tamarin	1	1	66	LC-MS	Tao	2011
Callithrix	jacchus	Common Marmoset	1	4	>100	LC-MS	Tao	2011
Symphalangus	syndactylus	Siamang	1	1	69	LC-MS	Tao	2011
Symphalangus	syndactylus	Siamang	1	1	6	NMR	Urashima	2009
Otolemur	crassicaudatus	Greater Galago	4	pooled	4	NMR	Taufik	2012
Daubentonia	madagascariensis	Aye-aye	4	pooled	12	NMR	Taufik	2012
Propithecus	coquereli	Coquerel's Sifaka	4	pooled	8	NMR	Taufik	2012

Genus	Species	Common Name	Number of Donors	Total Number of Samples	Number of Oligosaccharides Identified	Analytical Method	Publication	
Non-human primates (continued)								
Eulemur	mongoz	Mongoose Lemur	3	pooled	13	NMR	Taufik	2012
Feloidea Carnivores								
Felis	catus	Domestic Cat	6	139	33	LC-MS	Wrigglesworth	2020
Panthera	leo	African Lion	1	1	3	NMR	Senda	2010
Panthera	leo	African Lion	unknown	unknown	63	LC-MS	Remoroza	2020
Neofelis	nebulosa	Clouded Leopard	1	1	3	NMR	Senda	2010
Acinonyx	jubatus	Cheetah	3	3	6	NMR, MALDI-MS	Urashima	2020
Crocuta	crocuta	Hyena	1	1	4	NMR	Uemura	2009
Canoidea Carnivores								
Canis	lupus	Domestic Dog	23	230	53	LC-MS	Wrigglesworth	2020
Canis	lupus	Domestic Dog	4	4	6	HPLC-Fluorescence	Rostami	2014
Canis	lupus	Domestic Dog	1	1	4	HPLC	Warren	2001

Genus	Species	Common Name	Number of Donors	Total Number of Samples	Number of Oligosaccharides Identified	Analytical Method	Publication	
Canoidea Carnivores (continued)								
Canis	lupus	Domestic Dog	1	1	2	NMR, MS	Bubb	1999
Procyonidae	lotor	Racoon	5	5	6	NMR, MALDI-MS	Urashima	2018
Nasua	nasua	Coati	1	1	5	NMR	Urashima	1999
Neovison	vison	Mink	1	1	9	NMR	Urashima	2005
Mephitis	mephitis	Striped Skunk	5	7	6	NMR	Taufik	2013
Ursus	americanus	Black Bear	5	5	12	NMR, MALDI-MS	Urashima	2020
Ursus	americanus	Black Bear	1	1	9	HPLC	Warren	2001
Ursus	thibetanus	Japanese Black Bear	2	3	4	NMR	Urashima	2004
Ursus	thibetanus	Japanese Black Bear	4	12	11	NMR, FAB-MS, MALDI-MS	Urashima	1999
Ursus	arctos	Ezo Brown Bear	1	1	6	NMR	Urashima	1997

Genus	Species	Common Name	Number of Donors	Total Number of Samples	Number of Oligosaccharides Identified	Analytical Method	Publication	
Canoidea Carnivores (continued)								
Ursus	arctos	Grizzly Bear	1	1	8	HPLC	Warren	2001
Ursus	maritimus	Polar Bear	2	2	8	NMR	Urashima	2003
Ursus	maritimus	Polar Bear	7	7	10	NMR	Urashima	2000
Ailuropoda	melanoleuca	Panda	1	1	4	NMR	Nakamura	2003
Even-toed Ungulates								
Bos	Tarus	Cow	20	pooled	29	LC-MS	Shi	2021
Bos	Tarus	Cow	20	200	4	LC-MS	Fischer	2020
Bos	Tarus	Cow	18	108	11	HPAEC-PAD	Quinn	2020
Bos	Tarus	Cow	unknown	unknown	35	LC-MS	Remoroza	2020
Bos	Tarus	Cow	634	634	15	LC-MS	Robinson	2019
Bos	Tarus	Cow	6	18	34	LC-MS, CE-LIF	Vicaretti	2018
Bos	Tarus	Cow	unknown (bulk milk)	160	11	LC-MS	Schwendel	2017
Bos	Tarus	Cow	unknown	pooled	33	LC-MS	Albrecht	2014

Genus	Species	Common Name	Number of Donors	Total Number of Samples	Number of Oligosaccharides Identified	Analytical Method	Publication	
Even-toed Ungulates (continued)								
Bos	Tarus	Cow	6	pooled	50	LC-MS, MALDI-MS, FT-ICR MS	Aldredge	2013
Bos	Tarus	Cow	892	892	52	LC-MS, MALDI-MS	Sundekilde	2012
Bos	Tarus	Cow	2	10	5	HPAEC-PAD	McJarrow	2004
Bos	grunniens	Yak	5	5	6	HPAEC-PAD	Wang	2020
Bos	grunniens	Yak	1	1	2	NMR	Singh, AK	2016
Bos	grunniens	Yak	1	1	2	NMR	Singh, M	2016
Bubalus	bubalis	Water Buffalo	unknown	unknown	49	LC-MS	Remoroza	2020
Bubalus	bubalis	Water Buffalo	1	1	3	NMR, MALDI-MS	Mineguchi	2017
Ovis	aires	Sheep	20	pooled	32	LC-MS	Shi	2021
Ovis	aires	Sheep	unknown	pooled	35	LC-MS	Albrecht	2014
Ovis	aires	Sheep	unknown	pooled	3	NMR	Nakamura	1998
Ovis	aires	Sheep	5	5	3	NMR	Urashima	1989

Genus	Species	Common Name	Number of Donors	Total Number of Samples	Number of Oligosaccharides Identified	Analytical Method	Publication	
			Even-toed Ungulates (continued)					
Capra	aegagrus	Goat	20	pooled	43	LC-MS	Shi	2021
Capra	aegagrus	Goat	unknown	unknown	54	LC-MS	Remoroza	2020
Capra	aegagrus	Goat	6	6	9	HPAEC-PAD	Wang	2020
Capra	aegagrus	Goat	unknown	pooled	7	HPAEC-PAD	Acquino	2017
Capra	aegagrus	Goat			78	LC-MS	Martin-Ortiz	2016
Capra	aegagrus	Goat	unknown	pooled	40	LC-MS	Albrecht	2014
Capra	aegagrus	Goat	20	100	3	HPAEC-PAD	Claps	2016
Capra	aegagrus	Goat	16	32	29	LC-MS	Meyrand	2013
Capra	aegagrus	Goat	10	10	20	HPAEC-PAD, FAB-MS	Martinez-Ferez	2006
Capra	aegagrus	Goat	unknown	pooled	4	NMR	Urashima	1997
Capra	aegagrus	Goat	unknown	unknown	2	HPAEC-PAD, GC, FAB-MS, NMR	Viverge	1997
Capra	aegagrus	Goat	unknown	pooled	4	NMR	Urashima	1994

Genus	Species	Common Name	Number of Donors	Total Number of Samples	Number of Oligosaccharides Identified	Analytical Method	Publication	
Even-toed Ungulates (continued)								
Capra	aegagrus	Goat	unknown (bulk milk)	pooled	3	HPLC, NMR	Chaturvedi	1990
Capra	aegagrus	Goat	unknown (bulk milk)	pooled	3	HPLC, NMR	Chaturvedi	1988
Addax	nasomaculatus	Addax	1	1	9	NMR, MALDI-MS	Ganzorig	2018
Cervus	nippon	Yezo Sika Deer	unknown	unknown	5	NMR, MALDI-MS	Mineguchi	2017
Tragelaphus	spekii	Sitatunga	1	1	4	NMR, MALDI-MS	Mineguchi	2018
Rangifer	tarandus	Reindeer	1	1	4	NMR	Taufik	2014
Giraffa	camelopardalis	Giraffe	1	pooled	2	NMR, MALDI-MS	Mineguchi	2018
Giraffa	camelopardalis	Giraffe	1	1	6	HPLC	Warren	2001
Camelus	dromedarius	Dromedary Camel	unknown	pooled	33	LC-MS	Albrecht	2014

Genus	Species	Common Name	Number of Donors	Total Number of Samples	Number of Oligosaccharides Identified	Analytical Method	Publication	
Even-toed Ungulates (continued)								
Camelus	dromedarius	Dromedary Camel	unknown	pooled	12	NMR	Alhaj	2013
Camelus	bactrianus	Bactrian Camel	20	pooled	34	LC-MS	Shi	2021
Camelus	bactrianus	Bactrian Camel	2	4	14	NMR	Fukuda	2010
Sus	scrofa	Pig	17	51	55	LC-MS	Wei	2018
Sus	scrofa	Pig	14	28	61	LC-MS	Winkel	2018
Sus	scrofa	Pig	unknown	unknown	41	LC-MS	Cheng	2016
Sus	scrofa	Pig	6	8	35	CE-FID-MS	Difilippo	2016
Sus	scrofa	Pig	7	14	60	HPAEC-PAD, LC-MS	Mudd	2016
Sus	scrofa	Pig	3	12	33	LC-MS	Salcedo	2016
Sus	scrofa	Pig	unknown	pooled	39	LC-MS	Albrecht	2014
Sus	scrofa	Pig	3	12	29	LC-MS	Tao	2010

Genus	Species	Common Name	Number of Donors	Total Number of Samples	Number of Oligosaccharides Identified	Analytical Method	Publication	
				Proboscidea				
Elephas	maximus	Asian Elephant	3	3	10	HPAEC-PAD, FAB-MS, NMR	Kunz	1999
Elephas	maximus	Asian Elephant	1	1	8	HNMR	Uemura	2006
Loxodonta	africana	African Elephant	1	1	15	NMR	Uemura	2008
Loxodonta	africana	African Elephant	3	3	1	NMR, HPLC-RI	Osthoff	2007
				Pilosa				
Mymecophaga	tridactyla	Giant Anteater	1	1	5	NMR	Urashima	2008
				Muridae				
Ratus		Rat	2	pooled	15	LC-MS	Li	2021
Mus		Mouse	unknown	pooled	15	LC-MS	LI	2021
				Chiroptera				
Pteropus	hypomelanus	Island Flying Fox	7	21	4	NMR	Senda	2011

Genus	Species	Common Name	Number of Donors	Total Number of Samples	Number of Oligosaccharides Identified	Analytical Method	Publication	
				Cetacea				
Delphinapterus	leucas	Beluga	1	1	1	NMR	Urashima	2002
Tursiops	truncatus	Bottlenose Dolphin	1	1	4	NMR	Uemura	2005
Tursiops	truncatus	Bottlenose Dolphin	1	1	4	HPLC	Warren	2001
Balaenoptera	acutorostrata	Minke Whale	2	2	7	NMR	Urashima	2002
Balaenoptera	brydei	Bryde's Whale	1	1	3	NMR	Urashima	2007
Balaenoptera	borealis	Sei Whale	1	1	3	NMR	Urashima	2007
				Pinnipeds				
Arctocephalus	pusillus	Australian Fur Seal	unknown	pooled	9	NMR	Urashima	2001
Lobodon	carcinophagus	Crabeater Seal	unknown	3	1	NMR	Urashima	1997
Phoca	vitulina	Arctic Harbor Seal	1	1	9	NMR	Urashima	2003
Erignathus	barbatus	Bearded Seal	1	1	10	NMR	Urashima	2004
				Sirenia				
Trichechus	manatus	Florida Manatee	1	1	3	HPLC	Warren	2001

Genus	Species	Common Name	Number of Donors	Total Number of Samples	Number of Oligosaccharides Identified	Analytical Method	Publication	
Marsupials								
Macropus	rufus	Red Kangaroo	1	pooled	12	NMR, MALDI-MS	Anraku	2012
Macropus	giganteus	Grey Kangaroo	2	2	1	NMR	Messer	1980
Dendrolagus	goodfellowi	Goodfellows Tree Kangaroo	1	1	6	HPLC	Warren	2001
Macropus	eugenii	Tammar Wallaby	unknown	pooled	2	GC, NMR	Urashima	1994
Macropus	eugenii	Tammar Wallaby	unknown	unknown	2	NMR	Bradbury	1983
Macropus	eugenii	Tammar Wallaby	unknown	unknown	1	NMR	Messer	1982
Macropus	eugenii	Tammar Wallaby	unknown	unknown	4	NMR	Collins	1981
Macropus	eugenii	Tammar Wallaby	6	6	1	NMR	Messer	1980
Vombatus	ursinus	Wombat	1	2	12	NMR	Hirayama	2016
Trichosurus	vulpecula	Brushtail Possum	1	pooled	21	NMR, MALDI-MS	Urashima	2014

Genus	Species	Common Name	Number of Donors	Total Number of Samples	Number of Oligosaccharides Identified	Analytical Method	Publication	
Marsupials (continued)								
Phascolarctos	cinereus	Koala	6	pooled	10	NMR	Urashima	2013
Dasyurus	maculatus	Tiger Quoll	1	pooled	13	NMR, MALDI-MS	Urashima	2016
Dasyurus	viverrinus	Eastern Quoll	unknown	pooled	12	NMR	Urashima	2015
Monotremes								
Ornithorhynchus	anatinus	Platypus	12	pooled	10	NMR, MALDI-MS	Urashima	2015
Ornithorhynchus	anatinus	Platypus	unknown	unknown	8	Enzymatic	Amano	1985
Ornithorhynchus	anatinus	Platypus	2	1	1	NMR	Jenkins	1984
Ornithorhynchus	anatinus	Platypus	12	12	6	Enzymatic	Messer	1983
Ornithorhynchus	anatinus	Platypus	1	1	2	Enzymatic	Messer	1973
Tachyglossus	aculeatus	Echidna	unknown	unknown	1	NMR	Jenkins	1984
Tachyglossus	aculeatus	Echidna	unknown	unknown	3	NMR, GLC-MS	Kamerling	1982
Tachyglossus	aculeatus	Echidna	unknown	unknown	2	Enzymatic	Messer	1974
Tachyglossus	aculeatus	Echidna	2	3	3	Enzymatic	Messer	1973

REFERENCES

(1) Skibiel, A. L.; Downing, L. M.; Orr, T. J.; Hood, W. R. The evolution of the nutrient composition of mammalian milks. *J. Anim. Ecol.* **2013**, *82*, 1254–1264.

(2) Urashima, T.; Saito, T.; Nakamura, T.; Messer, M. Oligosaccharides of milk and colostrum in non-human mammals. *Glycoconj. J.* **2001**, *18*, 357–371.

(3) Bai, Y.; Tao, J.; Zhou, J.; Fan, Q.; Liu, M.; Hu, Y.; Xu, Y.; Zhang, L.; Yuan, J.; Li, W.; Ze, X.; Malard, P.; Guo, Z.; Yan, J.; Li, M. Fucosylated Human Milk Oligosaccharides and N-Glycans in the Milk of Chinese Mothers Regulate the Gut Microbiome of Their Breast-Fed Infants during Different Lactation Stages. *mSystems* **2018**, *3*.

(4) Pacheco, A. R.; Barile, D.; Underwood, M. A.; Mills, D. A. The impact of the milk glycobiome on the neonate gut microbiota. *Annu. Rev. Anim. Biosci.* **2015**, *3*, 419–445.

(5) Underwood, M. A.; Gaerlan, S.; De Leoz, M. L. A.; Dimapasoc, L.; Kalanetra, K. M.; Lemay, D. G.; German, J. B.; Mills, D. A.; Lebrilla, C. B. Human milk oligosaccharides in premature infants: absorption, excretion, and influence on the intestinal microbiota. *Pediatr. Res.* **2015**, *78*, 670–677.

(6) Yu, Z.-T.; Chen, C.; Newburg, D. S. Utilization of major fucosylated and sialylated human milk oligosaccharides by isolated human gut microbes. *Glycobiology* **2013**, *23*, 1281–1292.

(7) Marcobal, A.; Barboza, M.; Froehlich, J. W.; Block, D. E.; German, J. B.; Lebrilla, C. B.; Mills, D. A. Consumption of human milk oligosaccharides by gut-related microbes. *J. Agric. Food Chem.* **2010**, *58*, 5334–5340.

(8) Ward, R. E.; Niñonuevo, M.; Mills, D. A.; Lebrilla, C. B.; German, J. B. *In vitro* fermentability of human milk oligosaccharides by several strains of bifidobacteria. *Mol. Nutr. Food Res.* **2007**, *51*, 1398–1405.

(9) Hickey, R. M. The role of oligosaccharides from human milk and other sources in prevention of pathogen adhesion. *Int. Dairy J.* **2012**, *22*, 141–146.

(10) Boudry, G.; Hamilton, M. K.; Chichlowski, M.; Wickramasinghe, S.; Barile, D.; Kalanetra, K. M.; Mills, D. A.; Raybould, H. E. Bovine milk oligosaccharides decrease gut permeability and improve inflammation and microbial dysbiosis in diet-induced obese mice. *J. Dairy Sci.* **2017**, *100*, 2471–2481.

(11) Bode, L.; Kunz, C.; Muhly-Reinholz, M.; Mayer, K.; Seeger, W.; Rudloff, S. Inhibition of monocyte, lymphocyte, and neutrophil adhesion to endothelial cells by human milk oligosaccharides. *Thromb. Haemost.* **2004**, *92*, 1402–1410.

(12) Daddaoua, A.; Puerta, V.; Requena, P.; Martinez-Ferez, A.; Guadix, E.; Sanchez de Medina, F.; Zarzuelo, A.; Suarez, M. D.; Boza, J. J.; Martinez-Augustin, O. Goat milk oligosaccharides are anti-inflammatory in rats with hapten-induced colitis. *J. Nutr.* **2006**, *136*, 672–676.

(13) Lara-Villoslada, F.; Debras, E.; Nieto, A.; Concha, A.; Gálvez, J.; López-Huertas, E.; Boza, J.; Obled, C.; Xaus, J. Oligosaccharides isolated from goat milk reduce intestinal inflammation in a rat model of dextran sodium sulfate-induced colitis. *Clin. Nutr.* **2006**, *25*, 477–488.

(14) Li, M.; Monaco, M. H.; Wang, M.; Comstock, S. S.; Kuhlenschmidt, T. B.; Fahey, G. C.; Miller, M. J.; Kuhlenschmidt, M. S.; Donovan, S. M. Human milk oligosaccharides shorten rotavirus-induced diarrhea and modulate piglet mucosal immunity and colonic microbiota. *ISME J.* **2014**, *8*, 1609–1620.

(15) Sakai, F.; Ikeuchi, Y.; Urashima, T.; Fujihara, M.; Ohtsuki, K.; Yanahira, S. Effects of Feeding Sialyllactose and Galactosylated N-Acetylneuraminic Acid on Swimming Learning Ability and Brain Lipid Composition in Adult Rats. *Journal of Applied Glycoscience* **2006**, *53*, 249–254.

(16) Scholtz, S. A.; Gottipati, B. S.; Gajewski, B. J.; Carlson, S. E. Dietary sialic acid and cholesterol influence cortical composition in developing rats. *J. Nutr.* **2013**, *143*, 132–135.

(17) Wang, B.; Yu, B.; Karim, M.; Hu, H.; Sun, Y.; McGreevy, P.; Petocz, P.; Held, S.; Brand-Miller, J. Dietary sialic acid supplementation improves learning and memory in piglets. *Am. J. Clin. Nutr.* **2007**, *85*, 561–569.

(18) Fanaro, S.; Boehm, G.; Garssen, J.; Knol, J.; Mosca, F.; Stahl, B.; Vigi, V. Galacto-oligosaccharides and long-chain fructo-oligosaccharides as prebiotics in infant formulas: A review: GOS and FOS in infant formulas. *Acta Paediatr.* **2005**, *94*, 22–26.

(19) Chen, X. Human Milk Oligosaccharides (HMOS). In *Advances in Carbohydrate Chemistry and Biochemistry*; Elsevier, 2015; Vol. 72, pp. 113–190.

(20) Amano, J.; Osanai, M.; Orita, T.; Sugahara, D.; Osumi, K. Structural determination by negative-ion MALDI-QIT-TOF MSn after pyrene derivatization of variously fucosylated oligosaccharides with branched decaose cores from human milk. *Glycobiology* **2009**, *19*, 601–614.

(21) Kobata, A.; Ginsburg, V. Oligosaccharides of Human Milk: Isolation and characterization of a new hexasaccharide, lacto-N-hexaose. *J. Biol. Chem.* **1972**, *247*, 1525–1529.

(22) Yamashita, K.; Tachibana, Y.; Kobata, A. Oligosaccharides of human milk: Isolation and characterization of three new disialylfucosyl hexasaccharides. *Arch. Biochem. Biophys.* **1976**, *174*, 582–591.

(23) Yamashita, K.; Tachibana, Y.; Kobata, A. Oligosaccharides of human milk: isolation and characterization of two new nonasaccharides, monofucosyllacto-N-octaose and monofucosyllacto-N-neooctaose. *Biochemistry* **1976**, *15*, 3950–3955.

(24) Yamashita, K.; Tachibana, Y.; Kobata, A. Oligosaccharides of human milk: structures of three lacto-N-hexaose derivatives with H-haptenic structure. *Arch. Biochem. Biophys.* **1977**, *182*, 546–555.

(25) Tachibana, Y.; Yamashita, K.; Kobata, A. Oligosaccharides of human milk: structural studies of di-and trifucosyl derivatives of lacto-N-octaose and lacto-N-neoctaose. *Arch. Biochem. Biophys.* **1978**, *188*, 83–89.

(26) Dua, V. K.; Goso, K.; Dube, V. E.; Bush, C. A. Characterization of lacto-N-hexaose and two fucosylated derivatives from human milk by high-performance liquid chromatography and proton NMR spectroscopy. *J. Chromatogr.* **1985**, *328*, 259–269.

(27) Ashline, D. J.; Yu, Y.; Lasanajak, Y.; Song, X.; Hu, L.; Ramani, S.; Prasad, V.; Estes, M. K.; Cummings, R. D.; Smith, D. F.; Reinhold, V. N. Structural characterization by multistage mass spectrometry (MSn) of human milk glycans recognized by human rotaviruses. *Mol. Cell Proteomics* **2014**, *13*, 2961–2974.

(28) Sabharwal, H.; Nilsson, B.; Grönberg, G.; Chester, M. A.; Dakour, J.; Sjöblad, S.; Lundblad, A. Oligosaccharides from feces of preterm infants fed on breast milk. *Arch. Biochem. Biophys.* **1988**, *265*, 390–406.

(29) Haeuw-Fievre, S.; Wieruszeski, J.-M.; Plancke, Y.; Michalski, J.-C.; Montreuil, J.; Strecker, G. Primary structure of human milk octa-, dodeca- and tridecasaccharides determined by a combination of' ' ’H-NMR spectroscopy and fast-atom-bombardment mass spectrometry. *Eur. J. Biochem.* **1993**, *215*, 361–371.

(30) Blank, D.; Geyer, H.; Maass, K.; Yamashita, K.; Geyer, R. Elucidation of a novel lacto-N-decaose core structure in human milk using nonlinear analytical technique combinations. *Anal. Biochem.* **2012**, *421*, 680–690.

(31) Strecker, G.; Fièvre, S.; Wieruszeski, J. M.; Michalski, J. C.; Montreuil, J. Primary structure of four human milk octa-, nona-, and undeca-saccharides established by 1H- and 13C-nuclear magnetic resonance spectroscopy. *Carbohydr. Res.* **1992**, *226*, 1–14.

(32) Guérardel, Y.; Morelle, W.; Plancke, Y.; Lemoine, J.; Strecker, G. Structural analysis of three sulfated oligosaccharides isolated from human milk. *Carbohydr. Res.* **1999**, *320*, 230–238.

(33) Strecker, G.; Wieruszeski, J. M.; Michalski, J. C.; Montreuil, J. Primary structure of human milk nona- and deca- saccharides determined by a combination of fast atom bombardment mass spectrometry and 1H-/13C-nuclear magnetic resonance spectroscopy: Evidence for a new core structure, iso-lacto-N-octaose. *Glycoconj. J.* **1989**, *6*, 169–182.

(34) Wieruszeski, J. M.; Chekkor, A.; Bouquelet, S.; Montreuil, J.; Strecker, G.; Peter-Katalinic, J.; Egge, H. Structure of two new oligosaccharides isolated from human milk: Sialylated lacto-N-fucopentaoses I and II. *Carbohydr. Res.* **1985**, *137*, 127–138.

(35) Strecker, G.; Wieruszeski, J. M.; Michalski, J. C.; Montreuil, J. Structure of a new nonasaccharide isolated from human milk: Vl2Fuc,V4Fuc, lll3Fuc-p-Lacto-N - hexaose. *Glycoconj. J.* **1988**, *5*, 385–396.

(36) Chai, W.; Piskarev, V. E.; Zhang, Y.; Lawson, A. M.; Kogelberg, H. Structural determination of novel lacto-N-decaose and its monofucosylated analogue from human milk by electrospray tandem mass spectrometry and 1H NMR spectroscopy. *Arch. Biochem. Biophys.* **2005**, *434*, 116–127.

(37) Grönberg, G.; Lipniunas, P.; Lundgren, T.; Lindh, F.; Nilsson, B. Structural analysis of five new monosialylated oligosaccharides from human milk. *Arch. Biochem. Biophys.* **1992**, *296*, 597–610.

(38) Grönberg, G.; Lipniunas, P.; Lundgren, T.; Lindh, F.; Nilsson, B. Isolation and structural analysis of three new disialylated oligosaccharides from human milk. *Arch. Biochem. Biophys.* **1990**, *278*, 297–311.

(39) Gronberg, G.; Lipniunas, P.; Lundgren, T.; Erlansson, K.; Lindh, F.; Nilsson, B. Isolation of monosialylated oligosaccharides from human milk and structural analysis of three new compounds. *Carbohydr. Res.* **1989**, *191*, 261–278.

(40) Wang, W.; Lundgren, T.; Lindh, F.; Nilsson, B.; Gronberg, G.; Brown, J. P.; Mentzer-Dibert, H.; Zopf, D. Isolation of two novel sialyl-Lewis X-active oligosaccharides by high-performance liquid affinity chromatography using monoclonal antibody Onc-M26. *Arch. Biochem. Biophys.* **1992**, *292*, 433–441.

(41) Kogelberg, H.; Piskarev, V. E.; Zhang, Y.; Lawson, A. M.; Chai, W. Determination by electrospray mass spectrometry and 1H-NMR spectroscopy of primary structures of variously fucosylated neutral oligosaccharides based on the iso-lacto-N-octaose core. *Eur. J. Biochem.* **2004**, *271*, 1172–1186.

(42) Tarrago, M. T.; Tucker, K. H.; Van Halbeek, H.; Smith, D. F. A novel sialylhexasaccharide from human milk: Purification by affinity chromatography on immobilized wheat germ agglutinin. *Arch. Biochem. Biophys.* **1988**, *267*, 353–362.

(43) Smith, D. F.; Prieto, P. A.; McCrumb, D. K.; Wang, W. C. A novel sialylfucopentaose in human milk. Presence of this oligosaccharide is not dependent on expression of the secretor or Lewis fucosyltransferases. *J. Biol. Chem.* **1987**, *262*, 12040–12047.

(44) Kitagawa, H.; Nakada, H.; Fukui, S.; Funakoshi, I.; Kawasaki, T.; Yamashina, I.; Tate, S.; Inagaki, F. Novel oligosaccharides with the sialyl-Lea structure in human milk. *Biochemistry* **1991**, *30*, 2869–2876.

(45) Kitagawa, H.; Takaoka, M.; Nakada, H.; Fukui, S.; Funakoshi, I.; Kawasaki, T.; Tate, S.; Inagaki, F.; Yamashina, I. Isolation and Structural Studies of Human Milk Oligosaccharides That Are Reactive with a Monoclonal Antibody MSW 1131. *The Journal of Biochemistry* **1991**, *110*, 598–604.

(46) Kitagawa, H.; Nakada, H.; Fukui, S.; Funakoshi, I.; Kawasaki, T.; Yamashina, I.; Tate, S.; Inagaki, F. Novel Oligosaccharides with the Sialyl-Lea Structure in Human Milk1. *The Journal of Biochemistry* **1993**, *114*, 504–508.

(47) Kitagawa, H.; Nakada, H.; Kurosaka, A.; Hiraiwa, N.; Numata, Y.; Fukui, S.; Funakoshi, I.; Kawasaki, T.; Yamashina, I. Three novel oligosaccharides with the sialyl-Lea structure in human milk: Isolation by immunoaffinity chromatography. *Biochemistry* **1989**, *28*, 8891–8896.

(48) Fievre, S.; Wieruszeski, J.-M.; Michalski, J.-C. Primary structure of a trisialylated oligosaccharide from human milk. *Biochem. Biophys. Res. Commun.* **1991**, *177*, 720–725.

(49) Wu, S.; Tao, N.; German, J. B.; Grimm, R.; Lebrilla, C. B. Development of an annotated library of neutral human milk oligosaccharides. *J. Proteome Res.* **2010**, *9*, 4138–4151.

(50) Wu, S.; Grimm, R.; German, J. B.; Lebrilla, C. B. Annotation and structural analysis of sialylated human milk oligosaccharides. *J. Proteome Res.* **2011**, *10*, 856–868.

(51) Urashima, T.; Hirabayashi, J.; Sato, S.; Kobata, A. Human milk oligosaccharides as essential tools for basic and application studies on galectins. *Trends Glycosci Glycotechnol* **2018**, *30*, SE51–SE65.

(52) Kobata, A. Structures and application of oligosaccharides in human milk. *Proc. Jpn. Acad., Ser. B* **2010**, *86*, 731–747.

(53) Ninonuevo, M. R.; Park, Y.; Yin, H.; Zhang, J.; Ward, R. E.; Clowers, B. H.; German, J. B.; Freeman, S. L.; Killeen, K.; Grimm, R.; Lebrilla, C. B. A strategy for annotating the human milk glycome. *J. Agric. Food Chem.* **2006**, *54*, 7471–7480.

(54) Azad, M. B.; Robertson, B.; Atakora, F.; Becker, A. B.; Subbarao, P.; Moraes, T. J.; Mandhane, P. J.; Turvey, S. E.; Lefebvre, D. L.; Sears, M. R.; Bode, L. Human Milk

Oligosaccharide Concentrations Are Associated with Multiple Fixed and Modifiable Maternal Characteristics, Environmental Factors, and Feeding Practices. *J. Nutr.* **2018**, *148*, 1733–1742.

(55) Cabrera-Rubio, R.; Kunz, C.; Rudloff, S.; García-Mantrana, I.; Crehuá-Gaudiza, E.; Martínez-Costa, C.; Collado, M. C. Association of maternal secretor status and human milk oligosaccharides with milk microbiota: an observational pilot study. *J. Pediatr. Gastroenterol. Nutr.* **2019**, *68*, 256–263.

(56) van Leeuwen, S. S.; Stoutjesdijk, E.; Ten Kate, G. A.; Schaafsma, A.; Dijck-Brouwer, J.; Muskiet, F. A. J.; Dijkhuizen, L. Regional variations in human milk oligosaccharides in Vietnam suggest FucTx activity besides FucT2 and FucT3. *Sci. Rep.* **2018**, *8*, 16790.

(57) Sprenger, N.; Lee, L. Y.; De Castro, C. A.; Steenhout, P.; Thakkar, S. K. Longitudinal change of selected human milk oligosaccharides and association to infants' growth, an observatory, single center, longitudinal cohort study. *PLoS One* **2017**, *12*, e0171814.

(58) Thurl, S.; Munzert, M.; Henker, J.; Boehm, G.; Müller-Werner, B.; Jelinek, J.; Stahl, B. Variation of human milk oligosaccharides in relation to milk groups and lactational periods. *Br. J. Nutr.* **2010**, *104*, 1261–1271.

(59) Chaturvedi, P.; Warren, C.; Altaye, M.; Morrow, A.; Palacios, G.; Pickering, L.; Newburg, D. Fucosylated human milk oligosaccharides vary between individuals and over the course of lactation. *Glycobiology* **2001**, *11*, 365–372.

(60) Xu, G.; Davis, J. C.; Goonatilleke, E.; Smilowitz, J. T.; German, J. B.; Lebrilla, C. B. Absolute quantitation of human milk oligosaccharides reveals phenotypic variations during lactation. *J. Nutr.* **2017**, *147*, 117–124.

(61) Erney, R. M.; Malone, W. T.; Skelding, M. B.; Marcon, A. A.; Kleman-Leyer, K. M.; O'Ryan, M. L.; Ruiz-Palacios, G.; Hilty, M. D.; Pickering, L. K.; Prieto, P. A. Variability of human milk neutral oligosaccharides in a diverse population. *J. Pediatr. Gastroenterol. Nutr.* **2000**, *30*, 181–192.

(62) Thurl, S.; Henker, J.; Siegel, M.; Tovar, K.; Sawatzki, G. Detection of four human milk groups with respect to Lewis blood group dependent oligosaccharides. *Glycoconj. J.* **1997**, *14*, 795–799.

(63) Bode, L. The functional biology of human milk oligosaccharides. *Early Hum. Dev.* **2015**, *91*, 619–622.

(64) Durham, S. D.; Robinson, R. C.; Olga, L.; Ong, K. K.; Chichlowski, M.; Dunger, D. B.; Barile, D. A one-year study of human milk oligosaccharide profiles in the milk of healthy UK mothers and their relationship to maternal FUT2 genotype. *Glycobiology* **2021**, *31*, 1254–1267.

(65) Saben, J. L.; Sims, C. R.; Abraham, A.; Bode, L.; Andres, A. Human milk oligosaccharide concentrations and infant intakes are associated with maternal overweight and obesity and predict infant growth. *Nutrients* **2021**, *13*.

(66) Ferreira, A. L.; Alves, R.; Figueiredo, A.; Alves-Santos, N.; Freitas-Costa, N.; Batalha, M.; Yonemitsu, C.; Manivong, N.; Furst, A.; Bode, L.; Kac, G. Human milk oligosaccharide profile variation throughout postpartum in healthy women in a Brazilian cohort. *Nutrients* **2020**, *12*.

(67) Moubareck, C. A.; Lootah, M.; Tahlak, M.; Venema, K. Profiles of human milk oligosaccharides and their relations to the milk microbiota of breastfeeding mothers in Dubai. *Nutrients* **2020**, *12*.

(68) McJarrow, P.; Radwan, H.; Ma, L.; MacGibbon, A. K. H.; Hashim, M.; Hasan, H.; Obaid, R. S.; Naja, F.; Mohamed, H. J. J.; Al Ghazal, H.; Fong, B. Y. Human milk oligosaccharide, phospholipid, and ganglioside concentrations in breast milk from United Arab Emirates mothers: Results from the MISC Cohort. *Nutrients* **2019**, *11*.

(69) M Tonon, K.; B de Morais, M.; F V Abrão, A. C.; Miranda, A.; B Morais, T. Maternal and Infant Factors Associated with Human Milk Oligosaccharides Concentrations According to Secretor and Lewis Phenotypes. *Nutrients* **2019**, *11*.

(70) Samuel, T. M.; Binia, A.; de Castro, C. A.; Thakkar, S. K.; Billeaud, C.; Agosti, M.; Al-Jashi, I.; Costeira, M. J.; Marchini, G.; Martínez-Costa, C.; Picaud, J.-C.; Stiris, T.; Stoicescu, S.-M.; Vanpeé, M.; Domellöf, M.; Austin, S.; Sprenger, N. Impact of maternal characteristics on human milk oligosaccharide composition over the first 4 months of lactation in a cohort of healthy European mothers. *Sci. Rep.* **2019**, *9*, 11767.

(71) Ma, L.; McJarrow, P.; Jan Mohamed, H. J. B.; Liu, X.; Welman, A.; Fong, B. Y. Lactational changes in the human milk oligosaccharide concentration in Chinese and Malaysian mothers' milk. *International Dairy Journal* **2018**, *87*, 1–10.

(72) Dessì, A.; Briana, D.; Corbu, S.; Gavrili, S.; Cesare Marincola, F.; Georgantzi, S.; Pintus, R.; Fanos, V.; Malamitsi-Puchner, A. Metabolomics of breast milk: The importance of phenotypes. *Metabolites* **2018**, *8*.

(73) McGuire, M. K.; Meehan, C. L.; McGuire, M. A.; Williams, J. E.; Foster, J.; Sellen, D. W.; Kamau-Mbuthia, E. W.; Kamundia, E. W.; Mbugua, S.; Moore, S. E.; Prentice, A. M.; Kvist, L. J.; Otoo, G. E.; Brooker, S. L.; Price, W. J.; Shafii, B.; Placek, C.; Lackey, K. A.; Robertson, B.; Manzano, S.; Ruíz, L.; Rodríguez, J. M.; Pareja, R. G.; Bode, L. What's normal? Oligosaccharide concentrations and profiles in milk produced by healthy women vary geographically. *Am. J. Clin. Nutr.* **2017**, *105*, 1086–1100.

(74) Austin, S.; De Castro, C.; Bénet, T.; Hou, Y.; Sun, H.; Thakkar, S.; Vinyes-Pares, G.; Zhang, Y.; Wang, P. Temporal change of the content of 10 oligosaccharides in the milk of Chinese urban mothers. *Nutrients* **2016**, *8*, 346.

(75) Bode, L.; Kuhn, L.; Kim, H.-Y.; Hsiao, L.; Nissan, C.; Sinkala, M.; Kankasa, C.; Mwiya, M.; Thea, D. M.; Aldrovandi, G. M. Human milk oligosaccharide concentration and risk of postnatal transmission of HIV through breastfeeding. *Am. J. Clin. Nutr.* **2012**, *96*, 831–839.

(76) De Leoz, M. L. A.; Gaerlan, S. C.; Strum, J. S.; Dimapasoc, L. M.; Mirmiran, M.; Tancredi, D. J.; Smilowitz, J. T.; Kalanetra, K. M.; Mills, D. A.; German, J. B.; Lebrilla, C. B.; Underwood, M. A. Lacto-N-tetraose, fucosylation, and secretor status are highly variable in human milk oligosaccharides from women delivering preterm. *J. Proteome Res.* **2012**, *11*, 4662–4672.

(77) Musumeci, M.; Simpore, J.; D'Agata, A.; Sotgiu, S.; Musumeci, S. Oligosaccharides in colostrum of Italian and Burkinabe women. *J. Pediatr. Gastroenterol. Nutr.* **2006**, *43*, 372–378.

(78) Gabrielli, O.; Zampini, L.; Galeazzi, T.; Padella, L.; Santoro, L.; Peila, C.; Giuliani, F.; Bertino, E.; Fabris, C.; Coppa, G. V. Preterm milk oligosaccharides during the first month of lactation. *Pediatrics* **2011**, *128*, e1520–31.

(79) Hong, Q.; Ruhaak, L. R.; Totten, S. M.; Smilowitz, J. T.; German, J. B.; Lebrilla, C. B. Label-free absolute quantitation of oligosaccharides using multiple reaction monitoring. *Anal. Chem.* **2014**, *86*, 2640–2647.

(80) Fischer Fumeaux, C. J.; Garcia-Rodenas, C. L.; De Castro, C. A.; Courtet-Compondu, M.-C.; Thakkar, S. K.; Beauport, L.; Tolsa, J.-F.; Affolter, M. Longitudinal analysis of macronutrient composition in preterm and term human milk: A prospective cohort study. *Nutrients* **2019**, *11*.

(81) Nguyen, D. H.; Tangvoranuntakul, P.; Varki, A. Effects of natural human antibodies against a nonhuman sialic acid that metabolically incorporates into activated and malignant immune cells. *J. Immunol.* **2005**, *175*, 228-236.

(82) Wilson, J. M.; Schuyler, A. J.; Schroeder, N.; Platts-Mills, T. A. Galactose-a-1,3-galactose: Atypical food allergen or model IgE hypersensitivey. *Curr. Allergy Asthma Rep.* **2017**, *17*, 8.

(83) Tao, N.; Wu, S.; Kim, J.; An, H. J.; Hinde, K.; Power, M. L.; Gagneux, P.; German, J. B.; Lebrilla, C. B. Evolutionary glycomics: characterization of milk oligosaccharides in primates. *J. Proteome Res.* **2011**, *10*, 1548–1557.

(84) Urashima, T.; Odaka, G.; Asakuma, S.; Uemura, Y.; Goto, K.; Senda, A.; Saito, T.; Fukuda, K.; Messer, M.; Oftedal, O. T. Chemical characterization of oligosaccharides in chimpanzee, bonobo, gorilla, orangutan, and siamang milk or colostrum. *Glycobiology* **2009**, *19*, 499–508.

(85) Warren, C. D.; Chaturvedi, P.; Newburg, A. R.; Oftedal, O. T.; Tilden, C. D.; Newburg, D. S. Comparison of oligosaccharides in milk specimens from humans and twelve other species. In *Bioactive Components of Human Milk*; Newburg, D. S., Ed.; Plenum Publishers: New York, NY, USA, 2001; pp. 325–332.

(86) Goto, K.; Fukuda, K.; Senda, A.; Saito, T.; Kimura, K.; Glander, K. E.; Hinde, K.; Dittus, W.; Milligan, L. A.; Power, M. L.; Oftedal, O. T.; Urashima, T. Chemical characterization of oligosaccharides in the milk of six species of New and Old World monkeys. *Glycoconj. J.* **2010**, *27*, 703–715.

(87) Urashima, T.; Kawai, Y.; Nakamura, T.; Arai, I.; Saito, T.; Namiki, M.; Yamaoka, K.; Kawahawa, K.; Messer, M. Chemical characterisation of six oligosaccharides in a sample of colostrum of the brown capuchin, *Cebus apella* (Cebidae: Primates). *Comp. Biochem. Physiol. C* **1999**, *124*, 295–300.

(88) Taufik, E.; Fukuda, K.; Senda, A.; Saito, T.; Williams, C.; Tilden, C.; Eisert, R.; Oftedal, O.; Urashima, T. Structural characterization of neutral and acidic oligosaccharides in the milks of strepsirrhine primates: greater galago, aye-aye, Coquerel's sifaka and mongoose lemur. *Glycoconj. J.* **2012**, *29*, 119–134.

(89) Uemura, Y.; Takahashi, S.; Senda, A.; Fukuda, K.; Saito, T.; Oftedal, O. T.; Urashima, T. Chemical characterization of milk oligosaccharides of a spotted hyena (*Crocuta crocuta*). *Comp. Biochem. Physiol. B.* **2009**, *152*, 158–161.

(90) Senda, A.; Hatakeyama, E.; Kobayashi, R.; Fukuda, K.; Uemura, Y.; Saito, T.; Packer, C.; Oftedal, O. T.; Urashima, T. Chemical characterization of milk oligosaccharides of an African lion (*Panthera leo*) and a clouded leopard (*Neofelis nebulosa*). *Anim. Sci. J.* **2010**, *81*, 687–693.

(91) Urashima, T.; Umewaki, M.; Taufik, E.; Ohshima, T.; Fukuda, K.; Saito, T.; Whitehouse-Tedd, K.; Budd, J. A.; Oftedal, O. T. Chemical structures of oligosaccharides in milks of the American black bear (*Ursus americanus americanus*) and cheetah (*Acinonyx jubatus*). *Glycoconj. J.* **2020**, *37*, 57–76.

(92) Wrigglesworth, D. J.; Goonatilleke, E.; Haydock, R.; Hughes, K. R.; Lebrilla, C. B.; Swanson, K. S.; Jones, P.; Watson, P. High-throughput glycomic analyses reveal unique oligosaccharide profiles of canine and feline milk samples. *PLoS One* **2020**, *15*, e0243323.

(93) Remoroza, C. A.; Liang, Y.; Mak, T. D.; Mirokhin, Y.; Sheetlin, S. L.; Yang, X.; San Andres, J. V.; Power, M. L.; Stein, S. E. Increasing the coverage of a mass spectral library of milk oligosaccharides using a hybrid-search-based bootstrapping method and milks from a wide variety of mammals. *Anal. Chem.* **2020**, *92*, 10316–10326.

(94) Osthoff, G.; Hugo, A.; de Wit, M. The composition of cheetah (*Acinonyx jubatus*) milk. *Comp. Biochem. Physiol. B.* **2006**, *145*, 265–269.

(95) Urashima, T.; Sumiyoshi, W.; Nakamura, T.; Arai, I.; Saito, T.; Komatsu, T.; Tsubota, T. Chemical characterization of milk oligosaccharides of the Japanese black bear, *Ursus thibetanus japonicus*. *Biochim. Biophys. Acta* **1999**, *1472*, 290–306.

(96) Urashima, T.; Nakamura, T.; Teramoto, K.; Arai, I.; Saito, T.; Komatsu, T.; Tsubota, T. Chemical characterization of sialyl oligosaccharides in milk of the Japanese black bear, *Ursus thibetanus japonicus*. *Comp. Biochem. Physiol. B.* **2004**, *139*, 587–595.

(97) Urashima, T.; Kusaka, Y.; Nakamura, T.; Saito, T.; Maeda, N.; Messer, M. Chemical characterization of milk oligosaccharides of the brown bear, *Ursus arctos yesoensis*. *Biochim. Biophys. Acta* **1997**, *1334*, 247–255.

(98) Urashima, T.; Nagata, H.; Nakamura, T.; Arai, I.; Saito, T.; Imazu, K.; Hayashi, T.; Derocher, A. E.; Wiig, O. Differences in oligosaccharide pattern of a sample of polar bear colostrum and mid-lactation milk. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* **2003**, *136*, 887–896.

(99) Urashima, T.; Yamashita, T.; Nakamura, T.; Arai, I.; Saito, T.; Derocher, A. E.; Wiig, O. Chemical characterization of milk oligosaccharides of the polar bear, *Ursus maritimus*. *Biochim. Biophys. Acta* **2000**, *1475*, 395–408.

(100) Zhang, Z.; Hou, R.; Lan, J.; Wang, H.; Kurokawa, H.; Takatsu, Z.; Kobayashi, T.; Koie, H.; Kamata, H.; Kanayama, K.; Watanabe, T. Analysis of the breast milk of giant pandas (*Ailuropoda melanoleuca*) and the preparation of substitutes. *J. Vet. Med. Sci.* **2016**, *78*, 747–754.

(101) Nakamura, T.; Urashima, T.; Mizukami, T.; Fukushima, M.; Arai, I.; Senshu, T.; Imazu, K.; Nakao, T.; Saito, T.; Ye, Z.; Zuo, H.; Wu, K. Composition and oligosaccharides of a milk sample of the giant panda, *Ailuropoda melanoleuca*. *Comp. Biochem. Physiol. B.* **2003**, *135*, 439–448.

(102) Rezaei, R.; Wu, Z.; Hou, Y.; Bazer, F. W.; Wu, G. Amino acids and mammary gland development: nutritional implications for milk production and neonatal growth. *J Anim Sci Biotechnol* **2016**, *7*, 20.

(103) Urashima, T.; Yamaguchi, E.; Ohshima, T.; Fukuda, K.; Saito, T. Chemical structures of oligosaccharides in milk of the raccoon (*Procyon lotor*). *Glycoconj. J.* **2018**, *35*, 275–286.

(104) Rostami, S. M.; Bénet, T.; Spears, J.; Reynolds, A.; Satyaraj, E.; Sprenger, N.; Austin, S. Milk oligosaccharides over time of lactation from different dog breeds. *PLoS One* **2014**, *9*, e99824.

(105) Bubb, W. A.; Urashima, T.; Kohso, K.; Nakamura, T.; Arai, I.; Saito, T. Occurrence of an unusual lactose sulfate in dog milk. *Carbohydr. Res.* **1999**, *318*, 123–128.

(106) Taufik, E.; Sekii, N.; Senda, A.; Fukuda, K.; Saito, T.; Eisert, R.; Oftedal, O. T.; Urashima, T. Neutral and acidic milk oligosaccharides of the striped skunk (Mephitidae: *Mephitis mephitis*). *Anim. Sci. J.* **2013**, *84*, 569–578.

(107) Urashima, T.; Nakamura, T.; Ikeda, A.; Asakuma, S.; Arai, I.; Saito, T.; Oftedal, O. T. Characterization of oligosaccharides in milk of a mink, *Mustela vison*. *Comp. Biochem. Physiol. A* **2005**, *142*, 461–471.

(108) Urashima, T.; Yamamoto, M.; Nakamura, T.; Arai, I.; Saito, T.; Namiki, M.; Yamaoka, K.; Kawahara, K. Chemical characterisation of the oligosaccharides in a sample of milk of a white-nosed coati, *Nasua narica* (Procyonidae: Carnivora). *Comp. Biochem. Physiol. A* **1999**, *123*, 187–193.

(109) Martín-Ortiz, A.; Salcedo, J.; Barile, D.; Bunyatratchata, A.; Moreno, F. J.; Martin-García, I.; Clemente, A.; Sanz, M. L.; Ruiz-Matute, A. I. Characterization of goat colostrum oligosaccharides by nano-liquid chromatography on chip quadrupole time-of-flight mass spectrometry and hydrophilic interaction liquid chromatography-quadrupole mass spectrometry. *J. Chromatogr. A* **2016**, *1428*, 143–153.

(110) Lee, H.; Cuthbertson, D. J.; Otter, D. E.; Barile, D. Rapid screening of bovine milk oligosaccharides in a whey permeate product and domestic animal milks by accurate mass database and tandem mass spectral library. *J. Agric. Food Chem.* **2016**, *64*, 6364–6374.

(111) Claps, S.; Di Napoli, M. A.; Sepe, L.; Caputo, A. R.; Rufrano, D.; Di Trana, A.; Annicchiarico, G.; Fedele, V. Sialyloligosaccharides content in colostrum and milk of two goat breeds. *Small Ruminant Res.* **2014**, *121*, 116–119.

(112) Hernández-Ledesma, B.; Ramos, M.; Gómez-Ruiz, J. Á. Bioactive components of ovine and caprine cheese whey. *Small Ruminant Res.* **2011**, *101*, 196–204.

(113) Fong, B.; Ma, K.; McJarrow, P. Quantification of bovine milk oligosaccharides using liquid chromatography-selected reaction monitoring-mass spectrometry. *J. Agric. Food Chem.* **2011**, *59*, 9788–9795.

(114) McJarrow, P.; van Amelsfort-Schoonbeek, J. Bovine sialyl oligosaccharides: Seasonal variations in their concentrations in milk, and a comparison of the colostrums of Jersey and Friesian cows. *Int. Dairy J.* **2004**, *14*, 571–579.

(115) Nakamura, T.; Urashima, T.; Nakagawa, M.; Saito, T. Sialyllactose occurs as free lactones in ovine colostrum. *Biochim. Biophys. Acta* **1998**, *1381*, 286–292.

(116) Liu, Z.; Moate, P.; Cocks, B.; Rochfort, S. Simple liquid chromatography-mass spectrometry method for quantification of major free oligosaccharides in bovine milk. *J. Agric. Food Chem.* **2014**, *62*, 11568–11574.

(117) Barile, D.; Marotta, M.; Chu, C.; Mehra, R.; Grimm, R.; Lebrilla, C. B.; German, J. B. Neutral and acidic oligosaccharides in Holstein-Friesian colostrum during the first 3 days of lactation measured by high performance liquid chromatography on a microfluidic chip and time-of-flight mass spectrometry. *J. Dairy Sci.* **2010**, *93*, 3940–3949.

(118) Tao, N.; DePeters, E. J.; Freeman, S.; German, J. B.; Grimm, R.; Lebrilla, C. B. Bovine milk glycome. *J. Dairy Sci.* **2008**, *91*, 3768–3778.

(119) Nakamura, T.; Kawase, H.; Kimura, K.; Watanabe, Y.; Ohtani, M.; Arai, I.; Urashima, T. Concentrations of sialyloligosaccharides in bovine colostrum and milk during the prepartum and early lactation. *J. Dairy Sci.* **2003**, *86*, 1315–1320.

(120) Urashima, T.; Murata, S.; Nakamura, T. Structural determination of monosialyl trisaccharides obtained from caprine colostrum. *Comp. Biochem. Physiol. B* **1997**, *116*, 431–435.

(121) Albrecht, S.; Lane, J. A.; Mariño, K.; Al Busadah, K. A.; Carrington, S. D.; Hickey, R. M.; Rudd, P. M. A comparative study of free oligosaccharides in the milk of domestic animals. *Br. J. Nutr.* **2014**, *111*, 1313–1328.

(122) Shi, Y.; Han, B.; Zhang, L.; Zhou, P. Comprehensive identification and absolute quantification of milk oligosaccharides in different species. *J. Agric. Food Chem.* **2021**, *69*, 15585–15597.

(123) Dong, X.; Zhou, S.; Mechref, Y. LC-MS/MS analysis of permethylated free oligosaccharides and N-glycans derived from human, bovine, and goat milk samples: Liquid Phase Separations. *Electrophoresis* **2016**, *37*, 1532–1548.

(124) Mehra, R.; Barile, D.; Marotta, M.; Lebrilla, C. B.; Chu, C.; German, J. B. Novel high-molecular weight fucosylated milk oligosaccharides identified in dairy streams. *PLoS One* **2014**, *9*, e96040.

(125) Aldredge, D. L.; Geronimo, M. R.; Hua, S.; Nwosu, C. C.; Lebrilla, C. B.; Barile, D. Annotation and structural elucidation of bovine milk oligosaccharides and determination of novel fucosylated structures. *Glycobiology* **2013**, *23*, 664–676.

(126) Urashima, T.; Bubb, W. A.; Messer, M.; Tsuji, Y.; Taneda, Y. Studies of the neutral trisaccharides of goat (*Capra hircus*) colostrum and of the one- and two-dimensional ^{1}H and ^{13}C NMR spectra of 6'-*N*-acetylglucosaminyllactose. *Carbohydr. Res.* **1994**, *262*, 173–184.

(127) Chaturvedi, P.; Sharma, C. B. Purification, by high-performance liquid chromatography, and characterization, by high-field ^{1}H NMR spectroscopy, of two fucose-containing pentasaccharides of goat's milk. *Carbohydr. Res.* **1990**, *203*, 91–101.

(128) Chaturvedi, P.; Sharma, C. B. Goat milk oligosaccharides: Purification and characterization by HPLC and high-field ^{1}H-NMR spectroscopy. *Biochim. Biophys. Acta* **1988**, *967*, 115–121.

(129) Urashima, T.; Saito, T.; Nishimura, J.; Ariga, H. New galactosyllactose containing α-glycosidic linkage isolated from ovine (*Booroola dorset*) colostrum. *Biochim. Biophys. Acta* **1989**, *992*, 375–378.

(130) Tao, N.; DePeters, E. J.; German, J. B.; Grimm, R.; Lebrilla, C. B. Variations in bovine milk oligosaccharides during early and middle lactation stages analyzed by high-performance liquid chromatography-chip/mass spectrometry. *J. Dairy Sci.* **2009**, *92*, 2991–3001.

(131) Martín-Ortiz, A.; Barile, D.; Salcedo, J.; Moreno, F. J.; Clemente, A.; Ruiz-Matute, A. I.; Sanz, M. L. Changes in caprine milk oligosaccharides at different lactation stages analyzed by high performance liquid chromatography coupled to mass spectrometry. *J. Agric. Food Chem.* **2017**, *65*, 3523–3531.

(132) Robinson, R. C.; Poulsen, N. A.; Colet, E.; Duchene, C.; Larsen, L. B.; Barile, D. Profiling of aminoxyTMT-labeled bovine milk oligosaccharides reveals substantial variation in oligosaccharide abundance between dairy cattle breeds. *Sci. Rep.* **2019**, *9*, 5465.

(133) Sischo, W. M.; Short, D. M.; Geissler, M.; Bunyatratchata, A.; Barile, D. Comparative composition, diversity, and abundance of oligosaccharides in early lactation milk from commercial dairy and beef cows. *J. Dairy Sci.* **2017**, *100*, 3883–3892.

(134) Sundekilde, U. K.; Barile, D.; Meyrand, M.; Poulsen, N. A.; Larsen, L. B.; Lebrilla, C. B.; German, J. B.; Bertram, H. C. Natural variability in bovine milk oligosaccharides from Danish Jersey and Holstein-Friesian breeds. *J. Agric. Food Chem.* **2012**, *60*, 6188–6196.

(135) Meyrand, M.; Dallas, D. C.; Caillat, H.; Bouvier, F.; Martin, P.; Barile, D. Comparison of milk oligosaccharides between goats with and without the genetic ability to synthesize αs_1-casein. *Small Ruminant Res.* **2013**, *113*, 411–420.

(136) Liu, Z.; Wang, T.; Pryce, J. E.; MacLeod, I. M.; Hayes, B. J.; Chamberlain, A. J.; Jagt, C. V.; Reich, C. M.; Mason, B. A.; Rochfort, S.; Cocks, B. G. Fine-mapping sequence mutations with a major effect on oligosaccharide content in bovine milk. *Sci. Rep.* **2019**, *9*, 2137.

(137) Poulsen, N. A.; Robinson, R. C.; Barile, D.; Larsen, L. B.; Buitenhuis, B. A genome-wide association study reveals specific transferases as candidate loci for bovine milk oligosaccharides synthesis. *BMC Genomics* **2019**, *20*, 404.

(138) Wang, Y.; Zhou, X.; Gong, P.; Chen, Y.; Feng, Z.; Liu, P.; Zhang, P.; Wang, X.; Zhang, L.; Song, L. Comparative major oligosaccharides and lactose between Chinese human and animal milk. *International Dairy Journal* **2020**, 104727.

(139) Singh, M.; Kumar, A.; Srivastava, G.; Deepak, D.; Singh, M. P. V. V. Isolation, structure elucidation and DFT study on two novel oligosaccharides from yak milk. *J. Mol. Struct* **2016**, *1117*, 69–78.

(140) Singh, A. K.; Ranjan, A. K.; Srivastava, G.; Deepak, D. Structure elucidation of two novel yak milk oligosaccharides and their DFT studies. *J. Mol. Struct* **2016**, *1108*, 87–91.

(141) Mineguchi, Y.; Miyoshi, M.; Taufik, E.; Kawamura, A.; Asakawa, T.; Suzuki, I.; Souma, K.; Okubo, M.; Saito, T.; Fukuda, K.; Asakuma, S.; Urashima, T. Chemical characterization of the milk oligosaccharides of some Artiodactyla species including giraffe (*Giraffa camelopardalis*), sitatunga (*Tragelaphus spekii*), deer (*Cervus nippon yesoensis*) and water buffalo (*Bubalus bubalis*). *Glycoconj. J.* **2018**, *35*, 561–574.

(142) Osthoff, G.; Hugo, A.; de Wit, M.; Mai Nguyen, T. P. The Chemical Composition of Milk from Free-Ranging African Buffalo (*Syncerus caffer*). *S. Afr. J. Wildl. Res.* **2009**, *39*, 97–102.

(143) Saksena, R.; Deepak, D.; Khare, A.; Sahai, R.; Tripathi, L. M.; Srivastava, V. M. L. A novel pentasaccharide from immunostimulant oligosaccharide fraction of buffalo milk. *Biochim. Biophys. Acta* **1999**, *1428*, 433–445.

(144) Aparna, H. S.; Salimath, P. V. Disialyl lactose from buffalo colostrum: Isolation and characterization. *Carbohydr. Res.* **1995**, *268*, 313–318.

(145) Alhaj, O. A.; Taufik, E.; Handa, Y.; Fukuda, K.; Saito, T.; Urashima, T. Chemical characterisation of oligosaccharides in commercially pasteurised dromedary camel (*Camelus dromedarius*) milk. *International Dairy Journal* **2013**, *28*, 70–75.

(146) Fukuda, K.; Yamamoto, A.; Ganzorig, K.; Khuukhenbaatar, J.; Senda, A.; Saito, T.; Urashima, T. Chemical characterization of the oligosaccharides in Bactrian camel (*Camelus bactrianus*) milk and colostrum. *J. Dairy Sci.* **2010**, *93*, 5572–5587.

(147) Ganzorig, K.; Asakawa, T.; Sasaki, M.; Saito, T.; Suzuki, I.; Fukuda, K.; Urashima, T. Identification of sialyl oligosaccharides including an oligosaccharide nucleotide in colostrum of an addax (*Addax nasomaculatus*) (Subfamily Antelopinae). *Anim. Sci. J.* **2018**, *89*, 167–175.

(148) Taufik, E.; Ganzorig, K.; Nansalmaa, M.; Fukuda, R.; Fukuda, K.; Saito, T.; Urashima, T. Chemical characterisation of saccharides in the milk of a reindeer (*Rangifer tarandus tarandus*). *Int. Dairy J.* **2014**, *34*, 104–108.

(149) Wei, J.; Wang, Z. A.; Wang, B.; Jahan, M.; Wang, Z.; Wynn, P. C.; Du, Y. Characterization of porcine milk oligosaccharides over lactation between primiparous and multiparous female pigs. *Sci. Rep.* **2018**, *8*, 4688.

(150) Mudd, A. T.; Salcedo, J.; Alexander, L. S.; Johnson, S. K.; Getty, C. M.; Chichlowski, M.; Berg, B. M.; Barile, D.; Dilger, R. N. Porcine milk oligosaccharides and sialic acid concentrations vary throughout lactation. *Front. Nutr.* **2016**, *3*, 39.

(151) Salcedo, J.; Frese, S. A.; Mills, D. A.; Barile, D. Characterization of porcine milk oligosaccharides during early lactation and their relation to the fecal microbiome. *J. Dairy Sci.* **2016**, *99*, 7733–7743.

(152) Difilippo, E.; Pan, F.; Logtenberg, M.; Willems, R. H. A. M.; Braber, S.; Fink-Gremmels, J.; Schols, H. A.; Gruppen, H. Milk oligosaccharide variation in sow milk and milk oligosaccharide fermentation in piglet intestine. *J. Agric. Food Chem.* **2016**, *64*, 2087–2093.

(153) Tao, N.; Ochonicky, K. L.; German, J. B.; Donovan, S. M.; Lebrilla, C. B. Structural determination and daily variations of porcine milk oligosaccharides. *J. Agric. Food Chem.* **2010**, *58*, 4653–4659.

(154) Licitra, R.; Li, J.; Liang, X.; Altomonte, I.; Salari, F.; Yan, J.; Martini, M. Profile and content of sialylated oligosaccharides in donkey milk at early lactation. *LWT* **2019**, *115*, 108437.

(155) Orlandi, M.; Curadi, M. C.; Monti, L.; Cattaneo, T. M. P. Sialylated oligosaccharides in mare and ass milk: Preliminary results. *Prog. Nutr.* **2016**, *18*, 283–287.

(156) Monti, L.; Cattaneo, T. M. P.; Orlandi, M.; Curadi, M. C. Capillary electrophoresis of sialylated oligosaccharides in milk from different species. *J. Chromatogr. A* **2015**, *1409*, 288–291.

(157) Difilippo, E.; Willems, H. A.; Vendrig, J. C.; Fink-Gremmels, J.; Gruppen, H.; Schols, H. A. Comparison of milk oligosaccharides pattern in colostrum of different horse breeds. *J. Agric. Food Chem.* **2015**, *63*, 4805–4814.

(158) Karav, S.; Salcedo, J.; Frese, S. A.; Barile, D. Thoroughbred mare's milk exhibits a unique and diverse free oligosaccharide profile. *FEBS Open Bio* **2018**, *8*, 1219–1229.

(159) Nakamura, T.; Amikawa, S.; Harada, T.; Saito, T.; Arai, I.; Urashima, T. Occurrence of an unusual phosphorylated *N*-acetyllactosamine in horse colostrum. *Biochim. Biophys. Acta* **2001**, *1525*, 13–18.

(160) Urashima, T.; Saito, T.; Kimura, T. Chemical structures of three neutral oligosaccharides obtained from horse (thoroughbred) colostrum. *Comp. Biochem. Physiol. B* **1991**, *100*, 177–183.

(161) Urashima, T.; Sakamoto, T.; Ariga, H. Structure determination of three neutral oligosaccharides obtained from horse colostrum. *Carbohydr. Res.* **1989**, *194*, 280–287.

(162) Uemura, Y.; Asakuma, S.; Yon, L.; Saito, T.; Fukuda, K.; Arai, I.; Urashima, T. Structural determination of the oligosaccharides in the milk of an Asian elephant (*Elephas maximus*). *Comp. Biochem. Physiol. A* **2006**, *145*, 468–478.

(163) Osthoff, G.; de Wit, M.; Hugo, A.; Kamara, B. I. Milk composition of three free-ranging African elephant (*Loxodonta africana africana*) cows during mid lactation. *Comp. Biochem. Physiol. B* **2007**, *148*, 1–5.

(164) Osthoff, G.; De Waal, H. O.; Hugo, A.; de Wit, M.; Botes, P. Milk composition of a free-ranging African elephant (*Loxodonta africana*) cow during early lactation. *Comp. Biochem. Physiol. A* **2005**, *141*, 223–229.

(165) Kunz, C.; Rudloff, S.; Schad, W.; Braun, D. Lactose-derived oligosaccharides in the milk of elephants: comparison with human milk. *Br. J. Nutr.* **1999**, *82*, 391–399.

(166) Urashima, T.; Komoda, M.; Asakuma, S.; Uemura, Y.; Fukuda, K.; Saito, T.; Oftedal, O. T. Structural determination of the oligosaccharides in the milk of a giant anteater (*Myrmecophaga tridatyla*). *Anim. Sci. J.* **2008**, *79*, 699–709.

(167) Senda, A.; Kobayashi, R.; Fukuda, K.; Saito, T.; Hood, W. R.; Kunz, T. H.; Oftedal, O. T.; Urashima, T. Chemical characterization of milk oligosaccharides of the island flying fox (*Pteropus hypomelanus*) (Chiroptera: Pteropodidae). *Anim. Sci. J.* **2011**, *82*, 782–786.

(168) Urashima, T.; Sato, H.; Munakata, J.; Nakamura, T.; Arai, I.; Saito, T.; Tetsuka, M.; Fukui, Y.; Ishikawa, H.; Lydersen, C.; Kovacs, K.-. . Chemical characterization of the

oligosaccharides in beluga (*Delphinapterus leucas*) and Minke whale (*Balaenoptera acutorostrata*) milk. *Comp. Biochem. Physiol. B* **2002**, *132*, 611–624.

(169) Fervaiz, S.; Brew, K. Composition of the milks of the bottlenose dolphin (*Tursiops truncatus*) and the Florida manatee (*Trichechus manatus latirostris*). *Comp Biochem. Physiol. A* **1986**, *84*, 357–360.

(170) Uemura, Y.; Asakuma, S.; Nakamura, T.; Arai, I.; Taki, M.; Urashima, T. Occurrence of a unique sialyl tetrasaccharide in colostrum of a bottlenose dolphin (*Tursiops truncatus*). *Biochim. Biophys. Acta* **2005**, *1725*, 290–297.

(171) Urashima, T.; Kobayashi, M.; Asakuma, S.; Uemura, Y.; Arai, I.; Fukuda, K.; Saito, T.; Mogoe, T.; Ishikawa, H.; Fukui, Y. Chemical characterization of the oligosaccharides in Bryde's whale (*Balaenoptera edeni*) and Sei whale (*Balaenoptera borealis lesson*) milk. *Comp. Biochem. Physiol. B.* **2007**, *146*, 153–159.

(172) Eisert, R.; Oftedal, O. T.; Barrell, G. K. Milk composition in the weddell seal *Leptonychotes weddellii*: evidence for a functional role of milk carbohydrates in pinnipeds. *Physiol. Biochem. Zool.* **2013**, *86*, 159–175.

(173) Urashima, T.; Arita, M.; Yoshida, M.; Nakamura, T.; Arai, I.; Saito, T.; Arnould, J. P. Y.; Kovacs, K. M. Chemical characterisation of the oligosaccharides in hooded seal (*Cystophora cristata*) and Australian fur seal (*Arctocephalus pusillus doriferus*) milk. *Comp. Biochem. Physiol. B* **2001**, *128*, 307–323.

(174) Urashima, T.; Hiramatsu, Y.; Murata, S.; Nakamura, T.; Messer, M. Identification of 2′-fucosyllactose in milk of the crabeater seal (*Lobodon carcinophagus*). *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* **1997**, *116*, 311–314.

(175) Messer, M.; Crisp, E. A.; Newgrain, K. Studies on the carbohydrate content of milk of the crabeater seal (*Lobodon carcinophagus*). *Comp. Biochem. Physiol. B* **1988**, *90*, 367–370.

(176) Urashima, T.; Nakamura, T.; Nakagawa, D.; Noda, M.; Arai, I.; Saito, T.; Lydersen, C.; Kovacs, K. M. Characterization of oligosaccharides in milk of bearded seal (*Erignathus barbatus*). *Comp. Biochem. Physiol. B* **2004**, *138*, 1–18.

(177) Urashima, T.; Nakamura, T.; Yamaguchi, K.; Munakata, J.; Arai, I.; Saito, T.; Lydersen, C.; Kovacs, K. M. Chemical characterization of the oligosaccharides in milk of high Arctic harbour seal (*Phoca vitulina vitulina*). *Comp. Biochem. Physiol. A* **2003**, *135*, 549–563.

(178) Kinoshita, M.; Ohta, H.; Higaki, K.; Kojima, Y.; Urashima, T.; Nakajima, K.; Suzuki, M.; Kovacs, K. M.; Lydersen, C.; Hayakawa, T.; Kakehi, K. Structural characterization

of multibranched oligosaccharides from seal milk by a combination of off-line high-performance liquid chromatography-matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry and sequential exoglycosidase digestion. *Anal. Biochem.* **2009**, *388*, 242–253.

(179) Sturman, J. A.; Lin, Y. Y.; Higuchi, T.; Fellman, J. H. *N*-acetylneuramin lactose sulfate: a newly identified nutrient in milk. *Pediatr. Res.* **1985**, *19*, 216–219.

(180) Hirayama, K.; Taufik, E.; Kikuchi, M.; Nakamura, T.; Fukuda, K.; Saito, T.; Newgrain, K.; Green, B.; Messer, M.; Urashima, T. Chemical characterization of milk oligosaccharides of the common wombat (*Vombatus ursinus*). *Anim. Sci. J.* **2016**, *87*, 1167–1177.

(181) Urashima, T.; Taufik, E.; Fukuda, R.; Nakamura, T.; Fukuda, K.; Saito, T.; Messer, M. Chemical characterization of milk oligosaccharides of the koala (*Phascolarctos cinereus*). *Glycoconj. J.* **2013**, *30*, 801–811.

(182) Urashima, T.; Fujita, S.; Fukuda, K.; Nakamura, T.; Saito, T.; Cowan, P.; Messer, M. Chemical characterization of milk oligosaccharides of the common brushtail possum (*Trichosurus vulpecula*). *Glycoconj. J.* **2014**, *31*, 387–399.

(183) Anraku, T.; Fukuda, K.; Saito, T.; Messer, M.; Urashima, T. Chemical characterization of acidic oligosaccharides in milk of the red kangaroo (*Macropus rufus*). *Glycoconj. J.* **2012**, *29*, 147–156.

(184) Urashima, T.; Saito, T.; Tsuji, Y.; Taneda, Y.; Takasawa, T.; Messer, M. Chemical characterization of sialyl oligosaccharides isolated from tammar wallaby (*Macropus eugenii*) milk. *Biochim. Biophys. Acta* **1994**, *1200*, 64–72.

(185) Bradbury, J. H.; Collins, J. G.; Jenkins, G. A.; Trifonoff, E.; Messer, M. ^{13}C-NMR study of the structures of two branched oligosaccharides from marsupial milk. *Carbohydr. Res.* **1983**, *122*, 327–331.

(186) Messer, M.; Trifonoff, E.; Collins, J. G.; Bradbury, J. H. Structure of a branched tetrasaccharide from marsupial milk. *Carbohydr. Res.* **1982**, *102*, 316–320.

(187) Collins, J. G.; Bradbury, J. H.; Trifonoff, E.; Messer, M. Structures of four new oligosaccharides from marsupial milk, determined mainly by ^{13}C NMR spectroscopy. *Carbohydr. Res.* **1981**, *92*, 136–140.

(188) Messer, M.; Trifonoff, E. Structure of a marsupial milk trisaccharide. *Carbohydr. Res.* **1980**, *83*, 327–334.

(189) Messer, M.; Mossop, G. S. Milk carbohydrates of marsupials I. Partial separation and characterization of neutral milk oligosaccharides of the eastern grey kangaroo. *Aust. J. Biol. Sci.* **1977**, *30*, 379–388.

(190) Urashima, T.; Yamamoto, T.; Hirayama, K.; Fukuda, K.; Nakamura, T.; Saito, T.; Newgrain, K.; Merchant, J.; Green, B.; Messer, M. Chemical characterization of milk oligosaccharides of the tiger quoll (*Dasyurus maculatus*), a marsupial. *Glycoconj. J.* **2016**, *33*, 797–807.

(191) Urashima, T.; Sun, Y.; Fukuda, K.; Hirayama, K.; Taufik, E.; Nakamura, T.; Saito, T.; Merchant, J.; Green, B.; Messer, M. Chemical characterization of milk oligosaccharides of the eastern quoll (*Dasyurus viverrinus*). *Glycoconj. J.* **2015**, *32*, 361–370.

(192) Messer, M.; Fitzgerald, P. A.; Merchant, J. C.; Green, B. Changes in milk carbohydrates during lactation in the eastern quoll, *Dasyurus viverrinus* (marsupialia). *Comp. Biochem. Physiol. B.* **1987**, *88*, 1083–1086.

(193) Messer, M.; Green, B. Milk carbohydrates of marsupials II. Quantitative and qualitative changes in milk carbohydrates during lactation in the tammar wallaby (*Macropus eugenii*). *Aust. J. Biol. Sci.* **1979**, *32*, 519–531.

(194) Cowan, P. E. Changes in milk composition during lactation in the common brushtail possum, *Trichosurus vulpecula* (*Marsupialia: Phalangeridae*). *Reprod Fertil Dev* **1989**, *1*, 325–335.

(195) Crisp, E. A.; Cowan, P. E.; Messer, M. Changes in milk carbohydrates during lactation in the common brushtail possum, *Trichosurus vulpecula* (*Marsupialia: Phalangeridae*). *Reprod Fertil Dev* **1989**, *1*, 309–314.

(196) Bininda-Emonds, O. R. P.; Cardillo, M.; Jones, K. E.; MacPhee, R. D. E.; Beck, R. M. D.; Grenyer, R.; Price, S. A.; Vos, R. A.; Gittleman, J. L.; Purvis, A. The delayed rise of present-day mammals. *Nature* **2007**, *446*, 507–512.

(197) Urashima, T.; Inamori, H.; Fukuda, K.; Saito, T.; Messer, M.; Oftedal, O. T. 4-*O*-Acetyl-sialic acid (Neu4,5Ac2) in acidic milk oligosaccharides of the platypus (*Ornithorhynchus anatinus*) and its evolutionary significance. *Glycobiology* **2015**, *25*, 683–697.

(198) Messer, M.; Kerry, K. R. Milk carbohydrates of the echidna and the platypus. *Science* **1973**, *180*, 201–203.

(199) Amano, J.; Messer, M.; Kobata, A. Structures of the oligosaccharides isolated from milk of the platypus. *Glycoconj. J.* **1985**, *2*, 121–135.

(200) Jenkins, G. A.; Bradbury, J. H.; Messer, M.; Trifonoff, E. Determination of the structures of fucosyl-lactose and difucosyl-lactose from the milk of monotremes, using ^{13}C NMR spectroscopy. *Carbohydr. Res.* **1984**, *126*, 157–161.

(201) Messer, M.; Gadiel, P.; Ralston, G.; Griffiths, M. Carbohydrates of the milk of the platypus. *Aust. J. Bio. Sci.* **1983**, *36*, 129.

(202) Kamerling, J. P.; Dorland, L.; vanHalbeek, H.; Vliegent-Hart, J. F. G. Structural studies of 4-*O*-acetyl-a-*N*-acetylneuraminyl-(2->3)-lactose, the main oligosaccharide in echidna milk. *Carbohydr. Res.* **1982**, *100*, 331–340.

(203) Messer, M. Identification of *N* -acetyl-4–*O*-acetylneuraminyl-lactose in echidna milk. *Biochem. J.* **1974**, *139*, 415–420.

(204) Urashima, T.; Fukuda, K.; Messer, M. Evolution of milk oligosaccharides and lactose: a hypothesis. *Animal* **2012**, *6*, 369–374.

(205) Varki, A.; Altheide, T. K. Comparing the human and chimpanzee genomes: searching for needles in a haystack. *Genome Res.* **2005**, *15*, 1746–1758.

(206) Messer, M.; Urashima, T. Evolution of milk oligosacharides and lactose. *Trends in Glycoscience and Glycotechnology* **2002**, *14*, 153–176.

(207) Sunds, A. V.; Bunyatratchata, A.; Robinson, R.; Glantz, M.; Paulsson, M.; Leskauskaite, D.; Pihlanto, A.; Inglingstad, R.; Devold, T. G.; Vegarud, G. E.; Birgisdottir, B. E.; Gudjonsdottir, M.; Barile, D.; Larsen, L. B.; Poulsen, N. A. Comparison of bovine milk oligosaccharides in native North European cattle breeds. *Int. Dairy J.* **2021**, *114*.

(208) Cheng, L.; Xu, Q.; Yang, K.; He, J.; Chen, D.; Du, Y.; Yin, H. Annotation of porcine milk oligosaccharides throughout lactation by hydrophilic interaction chromatography coupled with quadruple time of flight tandem mass spectrometry. *Electrophoresis* **2016**, *37*, 1525–1531.

(209) Spevacek, A. R.; Smilowitz, J. T.; Chin, E. L.; Underwood, M. A.; German, J. B.; Slupsky, C. M. Infant maturity at birth reveals minor differences in the maternal milk metabolome in the first month of lactation. *J. Nutr.* **2015**, *145*, 1698–1708.

(210) Nakhla, T.; Fu, D.; Zopf, D.; Brodsky, N. L.; Hurt, H. Neutral oligosaccharide content of preterm human milk. *Br. J. Nutr.* **1999**, *82*, 361–367.

(211) Totten, S. M.; Zivkovic, A. M.; Wu, S.; Ngyuen, U.; Freeman, S. L.; Ruhaak, L. R.; Darboe, M. K.; German, J. B.; Prentice, A. M.; Lebrilla, C. B. Comprehensive profiles of human milk oligosaccharides yield highly sensitive and specific markers for determining secretor status in lactating mothers. *J. Proteome Res.* **2012**, *11*, 6124–6133.

(212) Olivares, M.; Albrecht, S.; De Palma, G.; Ferrer, M. D.; Castillejo, G.; Schols, H. A.; Sanz, Y. Human milk composition differs in healthy mothers and mothers with Celiac disease. *Eur J Nutr* **2015**, *54*, 119–128.

(213) Durham, S. D.; Lemay, D. G.; Wei, Z.; Kalscheur, K. F.; Finley, J. W.; Fukagawa, N. K.; Barile, D. Dietary fiber to starch ratio affects bovine milk oligosaccharide profiles. *Curr. Dev. Nutr.* **2022**.

(214) Vicaretti, S. D.; Mohtarudin, N. A.; Garner, A. M.; Zandberg, W. F. Capillary electrophoresis analysis of bovine milk oligosaccharides permits an assessment of the influence of diet and the discovery of nine abundant sulfated analogues. *J. Agric. Food Chem.* **2018**, *66*, 8574–8583.

(215) Schwendel, B. H.; Wester, T. J.; Morel, P. C. H.; Fong, B.; Tavendale, M. H.; Deadman, C.; Shadbolt, N. M.; Otter, D. E. Pasture feeding conventional cows removes differences between organic and conventionally produced milk. *Food Chem.* **2017**, *229*, 805–813.

(216) Asakuma, S.; Akahori, M.; Kimura, K.; Watanabe, Y.; Nakamura, T.; Tsunemi, M.; Arai, I.; Sanai, Y.; Urashima, T. Sialyl oligosaccharides of human colostrum: changes in concentration during the first three days of lactation. *Biosci. Biotechnol. Biochem.* **2007**, *71*, 1447–1451.

(217) Coppa, G. V.; Pierani, P.; Zampini, L.; Carloni, I.; Carlucci, A.; Gabrielli, O. Oligosaccharides in human milk during different phases of lactation. *Acta Paediatr Suppl* **1999**, *88*, 89–94.

(218) Sumiyoshi, W.; Urashima, T.; Nakamura, T.; Arai, I.; Nagasawa, T.; Saito, T.; Tsumura, N.; Wang, B.; Brand-Miller, J.; Watanabe, Y.; Kimura, K. Sialyl oligosaccharides in the milk of Japanese women: Changes in concentration during the course of lactation. *J. Appl. Glycosci.* **2003**, *50*, 461–467.

(219) Asakuma, S.; Urashima, T.; Akahori, M.; Obayashi, H.; Nakamura, T.; Kimura, K.; Watanabe, Y.; Arai, I.; Sanai, Y. Variation of major neutral oligosaccharides levels in human colostrum. *Eur. J. Clin. Nutr.* **2008**, *62*, 488–494.

(220) Plows, J. F.; Berger, P. K.; Jones, R. B.; Alderete, T. L.; Yonemitsu, C.; Najera, J. A.; Khwajazada, S.; Bode, L.; Goran, M. I. Longitudinal changes in human milk oligosaccharides (HMOs) over the course of 24 months of lactation. *J. Nutr.* **2021**, *151*, 876–882.

(221) de Moura Bell, J.; Cohen, J. L.; de Aquino, L.; Lee, H.; de Melo Silva, V. L.; Liu, Y.; Domizio, P.; Barile, D. An integrated bioprocess to recover bovine milk oligosaccharides from colostrum whey permeate. *J. Food Eng.* **2018**, *216*, 27–35.

(222) Aquino, L.; de Moura Bell, J.; Cohen, J. L.; Liu, Y.; Lee, H.; de Melo Silva, V. L.; Domizio, P.; Conte, C. A.; Barile, D. Purification of caprine oligosaccharides at pilot-scale. *J. Food Eng.* **2017**, *214*, 226–235.

(223) Cohen, J. L.; Barile, D.; Liu, Y.; de Moura Bell, J. Role of pH in the recovery of bovine milk oligosaccharides from colostrum whey permeate by nanofiltration. *Int. Dairy J.* **2017**, *66*, 68–75.

(224) Thum, C.; Cookson, A.; McNabb, W. C.; Roy, N. C.; Otter, D. Composition and enrichment of caprine milk oligosaccharides from New Zealand Saanen goat cheese whey. *Journal of Food Composition and Analysis* **2015**, *42*, 30–37.

(225) Barile, D.; Tao, N.; Lebrilla, C. B.; Coisson, J. D.; Arlorio, M.; German, J. B. Permeate from cheese whey ultrafiltration is a source of milk oligosaccharides. *Int. Dairy J.* **2009**, *19*, 524–530.

(226) Martinez-Ferez, A.; Rudloff, S.; Guadix, A.; Henkel, C. A.; Pohlentz, G.; Boza, J. J.; Guadix, E. M.; Kunz, C. Goats' milk as a natural source of lactose-derived oligosaccharides: Isolation by membrane technology. *Int. Dairy J.* **2006**, *16*, 173–181.

CHAPTER VI:

Conclusions, Current Limitations, and Future Directions

CONCLUSIONS

The preceding chapters have delineated strategies for sourcing, isolating, and analyzing milk oligosaccharides. This work contributes to the field's knowledge of naturally occurring free milk oligosaccharide profiles and concentrations as well as how they are impacted by an array of inherent and external factors. In particular, the discovery of additional effects of parity on milk oligosaccharide abundances and the novel demonstration of the impact of dietary fiber levels on milk oligosaccharide yields in cows, and the challenge to the previously established link between maternal secretor genotype and levels of α1,2-fucosylated oligosaccharides in human breast milk are substantial new contributions to the field.

This dissertation also introduces and applies two recently developed methods for milk oligosaccharide analysis, with focuses on either in-depth milk oligosaccharide profiling with improved detection of large, low-abundance compounds and multiplexed samples for greater throughput in the tandem mass tag-labeled nano-chip liquid chromatography quadrupole time-of-flight tandem mass spectrometry (nano-chip LC Q-ToF MS) method (Durham et al., 2022; Robinson et al., 2018) applied in Chapter III, or accurate milk oligosaccharide quantification with minimized sample preparation to eliminate the loss of milk oligosaccharides prior to analysis in the "dilute-and-shoot" high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) method (Durham et al, 2021; Tan et al., 2015) applied in Chapter II. These techniques for milk oligosaccharide analysis will both be particularly useful for future milk oligosaccharide research, depending on the analytical priorities of forthcoming studies.

In addition, the meta-analysis of all milk oligosaccharide profiling research from the past 5 decades accomplishes a cumulative review of milk oligosaccharide literature that has never before been undertaken and which reveals several previously unnoted phylogenetic trends in oligosaccharide profiles that provide insight into the evolutionary development of milk oligosaccharide synthesis. In addition, this analysis highlights gaps in the existing milk oligosaccharide profiling literature and underscores the importance of viewing milk oligosaccharide data in the greater context of the field.

Although the preceding chapters have addressed separate strategies for improving oligosaccharide recovery in a milk oligosaccharide isolate, their true application almost certainly lies in a combined approach. Achieving a milk oligosaccharide functional ingredient that most closely mirrors the human milk oligosaccharide target will likely require the combined efforts of optimizing husbandry practices (including but not limited to dietary modifications to naturally increase oligosaccharide concentrations in milk), as well as employing alternative, non-bovine dairy sources, and utilizing concentrated dairy streams.

CURRENT LIMITATIONS

The milk oligosaccharide data referenced herein originates across a timespan of over 50 years. Sample extraction practices and analytical technologies have evolved substantially over this period and rudimentary methods of analysis like paper chromatography, thin layer chromatography, subtractive derivations from spectrophotometrically determined total carbohydrate content and liquid chromatography approaches without sufficient chromatographic separation of oligosaccharides have been eclipsed by modern techniques with greater precision and accuracy. Despite this, much of our knowledge of milk oligosaccharides produced in species beyond humans and cows hinges on single analyses that are decades old, and the occurrence of updated, more in-depth studies continue to be limited by the ongoing need for standardized techniques that allow for routine, cost-effective identification of milk oligosaccharides with full compositional and linkage information. In addition, data on the concentrations of milk oligosaccharides from non-human mammals is sorely needed to assess potential alternative sources of milk oligosaccharides for isolation. While the milks of domesticated, routinely milked species like goats and camels identified in Chapter IV appear promising based on their oligosaccharide profiles, little is known about the concentrations or ratios of these compounds or how they vary with lactation time, feeding, or herd management systems.

In viewing milk oligosaccharides from a more evolutionary or basic research-oriented perspective additional knowledge gaps in the field warrant future research attention. The influence of the timing and strategies employed for milk collection and storage on the composition and oligosaccharide profiles of the milk, including the effect of oxytocin administration, diurnal variation, the health of nursing offspring, milk collection post-mortem, multiple freeze-thaw cycles, and prolonged milk sample storage warrant investigation, and future studies would benefit from documenting such methodological details.

FUTURE DIRECTIONS

Moving forward, the field of milk oligosaccharide research as a whole will benefit from additional research in several key areas.

First, the continued optimization of membrane filtration and demineralization techniques for milk oligosaccharide isolation, particularly as they apply to non-traditional dairy streams, like delactosed permeate, and non-bovine milk sources will be imperative for the successful commercial-scale application of the research discussed in the preceding chapters. Without these techniques, the large-scale production of milk oligosaccharide isolates for applications as nutraceuticals and supplements for infant formulas will be severely hindered.

As evidenced by the high degree of variation in delactosed permeate composition between batches and production sites in Chapter IV, investigation into the factors driving compositional variation in concentrated dairy streams will also be an important step toward product standardization and, consequently, the development of appropriate isolation protocols. Key considerations will be in assessing the impact of different cheese-making processes (i.e. mozzarella versus Hispanic-style cheeses) on the composition of the resulting whey and determining the effects of different ultrafiltration parameters on ultrafiltration permeate compositions, because the ultrafiltration permeates from milk and cheese whey become the starting materials from which concentrated dairy streams like delactosed permeates are produced.

In addition, development of a method for the enzymatic modification of existing oligosaccharides through the addition of fucose would help boost the bioactive potential of less-decorated milk oligosaccharide isolates. A small-scale *in-vitro* study of externally fucosylated bovine milk oligosaccharides demonstrated increased prebiotic activity of the newly fucosylated oligosaccharides compared to their unmodified precursors. (Weinborn et al., 2020) Identifying a large-scale source of fucose and developing a method for applying this technique at commercial scale could allow for the fucosylation of less-bioactive milk oligosaccharide streams, like those originating form bovine milk, to increase their structural similarity to human milk oligosaccharides and create a milk oligosaccharide isolate with improved bioactivity.

Finally, further investigation into the milk oligosaccharide profiles and concentrations of mammalian species milked for human consumption outside of North America and Western Europe are needed to identify better sources of milk oligosaccharides for isolation. Based on current research, camels and some breeds of goats appear to have milk oligosaccharide profiles with promising similarities to human milk oligosaccharides, (Shi et al., 2021; Lu et al., 2020; Remoroza et al., 2020; Albrecht et al., 2014; Alhaj et al., 2013; Meyrand et al., 2013; Fukuda et al., 2010) but additional research will be needed to confirm these findings and look into the milk oligosaccharide profiles of other camelid species, including llamas and alpacas. Investigations of how these milk oligosaccharide profiles are impacted by lactational and environmental factors, including lactation time point, parity, diet, and herd management style will also been needed to fully understand the potential of milks from these species as oligosaccharide sources.

Through the combination of strategic sourcing of non-bovine and non-traditional milk and dairy streams as well as applying techniques to increase milk oligosaccharide concentrations and their resemblance to human milk oligosaccharide profiles, this milk oligosaccharide profiling, isolation, and bioactivity research can enable the creation of an extremely beneficial value-added product from existing dairy waste streams, in the form of a human-like milk oligosaccharide isolate with applications in infant formula and nutraceuticals.

REFERENCES

Albrecht S, Lane JA, Marino K, Al Busadah KA, Carrington SD, Hickey RM, Rudd PM. 2014. A comparative study of free oligosaccharides in the milk of domestic animals. *Br. J. Nutr*. 111:1313-1328.

Alhaj OA, Taufik E, Handa Y, Fukuda K, Saito T, Urashima T. 2013. Chemical characterization of oligosaccharides in commercially pasteurized dromedary camel (*Camelus dromedarius*) milk. *Int. Dairy J.* 28:70-75

Durham SD, Lemay DG, Wei Z, Kalcheur KF, Fnley JW, Fukagawa N, Barile D. 2022. Dietary fiber to starch ratios affect bovine milk oligosaccharide profiles. *Curr. Dev. Nutr.*

Durham SD, Robinson RC, Olga L, Ong KK, Chichlowski M, Dunger DB, Barile D. 2021. A one-year study of human milk oligosaccharide profiles in the milk of healthy UK mothers and their relationship to maternal FUT2 genotype. *Glycobiology*. 31:1254-1267.

Fukuda K, Yamamoto A, Ganzorig K, Khuukhenbaatar J, Senda A, Saito T, Urashima T. 2010. Chemical characterization of the oligosaccahrides in Bactrian camel (Camelus bactrianus) milk and colostrum. *J. Dairy Sci.* 93:5572-5587.

Lu J, Zhang Y, Song B, Zhang S, Pang X, Sari RN, Liu L, Wang J. 2020. Comparative analysis of oligosaccharides in Guanzhong and Saanen goat milk using LC-MS/MS. *Carbohydr. Polym.* 15965.

Meyrand M, Dallas DC, Caillat H, Bouvier F, Martin P, Barile D. 2013. Comparison of mlk oligosaccharides between goats with and without the genetic ability to synthesize αs1-casein. *Small Rumin. Res.* 113:411-420.

Remoroza CA, Liang Y, Mak TD, Mirokhin Y, Sheetlin SL, Yang X, San Andres JV, Power ML, Stein SE. 2020. Increasing the coverage of a mass spectral library of milk oligosaccharides using a hybrid-search based bootstrapping method and milks from a wide variety of mammals. *Anal. Chem.* 92:10316-10326.

Robinson RC, Poulsen NA, Barile D. 2018. Multiplexed bovine milk oligosaccharide analysis with aminoxy tandem mass tags. *PLoS One* 13:e0196513.

Shi Y, Han b, Zhang L, Zhou P. 2021. Comprehensive identification and absolute quantification of milk oligosaccharides in different species. *J. Agric. Food Chem.* 69:15585-15597.

Tan J, Chai F, Zhang C. 2015. Determination of human milk oligosaccharides in human breast milk by HPAE-PAD with on-line sample cleanup. ThermoFisher Customer Application Note 119.

Weinborn V, Li Y, Shah IM, Yu H, Dallas D, German JB, Mills DA, Chen X, Barile D. 2020. Production of functional mimics of human milk oligosaccharides by enzymatic glycosylation of bovine milk oligosaccharides. *Int. Dairy J.* 102:104583.

APPENDIX I:

Additional Tables

Table A1.1. Peptide sequences identified in delactosed permeate from production plant 1, batch A (Chapter IV)

Peptide	-10lg	Mass	Length	ppm	m/z	RT	Area	Accession	PTM
YQEPVLGPVRGPFPIIV	78.4	1880.1	17	4.2	941.04	44.2	5.43E+05	P02666|CASB_BOVIN	
YQEPVLGPVRGPFPI	66.6	1667.9	15	-4.5	834.96	39	4.15E+04	P02666|CASB_BOVIN	
YQEPVLGPVRGPFPII	66.6	1781	16	-1.4	891.5	42.6	7.09E+04	P02666|CASB_BOVIN	
EPVLGPVRGPFPIIV	65.7	1588.9	15	2.8	795.48	43.2	1.97E+05	P02666|CASB_BOVIN	
QEPVLGPVRGPFPIIV	64.6	1717	16	1.6	859.51	43.1	4.67E+04	P02666|CASB_BOVIN	
LLYQEPVLGPVRGPFPIIV	61.6	2106.2	19	0.7	1054.1	46.4	2.66E+03	P02666|CASB_BOVIN	
KVLPVPQ	60	779.49	7	3.1	390.75	17.6	1.23E+04	P02666|CASB_BOVIN	
VAPFPEVFGKEK	59.3	1346.7	12	8.1	449.92	27.9	1.42E+05	P02662|CASA1_BOVI	
AQPTDASAQFIR	59	1303.7	12	-2.6	435.56	19.6	4.39E+04	P80195|GLCM1_BOVI	
YQEPVLGPVRGPFP	58.3	1554.8	14	-3.2	778.42	33.5	1.04E+05	P02666|CASB_BOVIN	
DAAGGPGAPADPGRPT	57.2	1405.7	16	-2.9	703.84	12.4	2.72E+04	P81265|PIGR_BOVIN: P81265-	
EPVLGPVRGPFPII	57.2	1489.9	14	-3.2	745.94	41.8	3.24E+04	P02666|CASB_BOVIN	
TVQVTSTAV	56.8	904.49	9	-0.6	905.5	16.7	2.60E+05	P02668|CASK_BOVIN	
LPQEVLNENLLR	56.6	1436.8	12	-0.1	719.41	30.6	4.02E+03	P02662|CASA1_BOVI	
APFPEVFGKEK	56.2	1247.7	11	0.6	416.89	25	7.31E+04	P02662|CASA1_BOVI	
GLPQEVLNENLLR	55.5	1493.8	13	-3.9	747.92	33.8	9.98E+04	P02662|CASA1_BOVI	
SSSEESITRIN	54.3	1221.6	11	0	611.8	15	2.17E+04	P02666|CASB_BOVIN	
TPVVVPPFLQPEVM(+15.99)	54.2	1567.8	14	3.9	784.93	39.8	1.05E+04	P02666|CASB_BOVIN	Oxidation (M)
DTIAQAASTTTISDAVSK	54	1778.9	18	-2.2	890.45	24.6	5.37E+03	P80025|PERL_BOVIN	
PVLGPVRGPFPIIV	53.4	1459.9	14	-3.9	730.95	43.2	8.84E+03	P02666|CASB_BOVIN	
TIASGEPTSTPTTE	53	1390.6	14	-1.5	696.33	14.7	8.43E+03	P02668|CASK_BOVIN	
FVAPFPEVFGKEK	52.8	1493.8	13	6.4	498.94	34.1	5.79E+04	P02662|CASA1_BOVI	
EVIESPPEINTVQVTSTAV	52.3	2012	19	1.2	1007	31.3	2.81E+04	P02668|CASK_BOVIN	
ESRNPDEEGLFTVR	52.3	1647.8	14	3.3	550.27	23.3	1.13E+04	P18892|BT1A1_BOVIN	
AAGGPGAPADPGRPT	51.9	1290.6	15	-1.3	646.32	11.9	1.79E+04	P81265|PIGR_BOVIN: P81265-	
GLPQEVLNENLL	51.9	1337.7	12	3.5	669.87	36.8	1.46E+05	P02662|CASA1_BOVI	
GPIVLNPWDQVK	51.7	1364.7	12	-1.7	683.38	32.5	3.73E+03	P02663|CASA2_BOVI	
SSRQPQSQNPKLPL	51.6	1578.8	14	4.6	527.29	20.3	1.30E+05	P80195|GLCM1_BOVI	
NIPPLTQTPV	51.2	1078.6	10	-5.9	1079.6	27	7.13E+04	P02666|CASB_BOVIN	
SLVYPFPGPIPN	51.1	1299.7	12	-2.2	650.85	36.8	8.73E+03	P02666|CASB_BOVIN	
SQNPKLPLSIL	50.9	1208.7	11	-2.8	605.36	35.8	9.41E+04	P80195|GLCM1_BOVI	
APFPEVFGK	50.9	990.52	9	4.6	496.27	28.6	1.02E+05	P02662|CASA1_BOVI	
ILNKPEDETHLEAQPTDASAQFIR	50.9	2722.4	24	2.7	681.6	25.1	4.04E+04	P80195|GLCM1_BOVI	
LVYPFPGPIPN	50.6	1212.7	11	2.9	607.34	35.8	1.05E+04	P02666|CASB_BOVIN	
DASAQFIRNL	50.3	1133.6	10	-3.9	567.8	28.4	1.54E+04	P80195|GLCM1_BOVI	
EELNVPGEIVESL	50	1426.7	13	-7.2	714.36	38.7	1.32E+04	P02666|CASB_BOVIN	
LPQEVLNENLLRF	50	1583.9	13	-6.2	528.96	39.3	1.01E+04	P02662|CASA1_BOVI	
VAPFPEVFGK	49.8	1089.6	10	1.3	545.8	31.3	2.50E+05	P02662|CASA1_BOVI	
FVAPFPEVFGK	49.8	1236.7	11	3.6	619.34	37.5	1.77E+05	P02662|CASA1_BOVI	
GLPQEVLNENLLRF	49.5	1640.9	14	-1.4	821.45	41.7	3.87E+04	P02662|CASA1_BOVI	
DAQSAPLRVY	49.1	1118.6	10	-1.5	560.29	20.4	8.38E+03	P02754|LACB_BOVIN	
ILNKPEDETHLE	49	1436.7	12	2.7	479.92	16.2	6.48E+05	P80195|GLCM1_BOVI	
AQPTDASAQF	48.9	1034.5	10	-0.9	1035.5	16	1.38E+05	P80195|GLCM1_BOVI	
VEDHIAEGSVAVR	48.5	1380.7	13	10.8	461.25	15.2	1.40E+04	P18892|BT1A1_BOVIN	
VAPFPEVFG	48.5	961.49	9	-2.5	962.5	35.7	6.51E+04	P02662|CASA1_BOVI	
AQPTDASAQFIRNL	48.2	1530.8	14	-2.4	766.4	30.8	1.04E+04	P80195|GLCM1_BOVI	
VAPFPEVFGKE	47.9	1218.6	11	-6.6	610.32	31.7	2.65E+04	P02662|CASA1_BOVI	
DLISKEQIVIR	47.9	1312.8	11	8.1	438.6	25.1	2.22E+04	P80195|GLCM1_BOVI	
TKVIPYVRY	47.7	1137.7	9	4.5	380.23	22.7	3.83E+03	P02663|CASA2_BOVI	
SDIPNPIGSENSE	47.7	1357.6	13	-4.3	679.81	22.1	1.69E+04	P02662|CASA1_BOVI	
VLPVPQKAVPYPQ	47.6	1434.8	13	3	718.42	25.7	3.63E+04	P02666|CASB_BOVIN	
GLPQEVLNENL	47.5	1224.6	11	6.3	613.33	31.2	2.25E+04	P02662|CASA1_BOVI	
VYPFPGPIPN	47.5	1099.6	10	-2.5	550.79	32	6.89E+03	P02666|CASB_BOVIN	
SQNPKLPL	47.4	895.51	8	-5.1	448.76	21.8	1.14E+05	P80195|GLCM1_BOVI	
SHAFEVVKT	47.4	1016.5	9	6.2	339.85	15.8	4.54E+04	P80195|GLCM1_BOVI	
SSEESITRIN	47.3	1134.6	10	1.7	568.29	14.8	6.01E+03	P02666|CASB_BOVIN	
FVAPFPEVFG	47.3	1108.6	10	0.3	1109.6	41.8	1.61E+04	P02662|CASA1_BOVI	
HQGLPQEVLNENLLR	47	1758.9	15	2.7	587.32	30.9	0	P02662|CASA1_BOVI	
VDMESTEVFTK	46.9	1284.6	11	0.4	643.31	22.4	8.47E+03	P02663|CASA2_BOVI	
APFPEVFGKEKV	46.8	1346.7	12	1.6	449.92	28.7	6.29E+04	P02662|CASA1_BOVI	
ILNKPEDETHLEAQPTDASAQFIRNL	46.8	2949.5	26	4.1	738.38	32.4	3.34E+04	P80195|GLCM1_BOVI	
VPPFLQPEVM(+15.99)	46.8	1171.6	10	2.1	586.81	30.5	1.21E+05	P02666|CASB_BOVIN	Oxidation (M)
SQNPKLPLS	46.7	982.54	9	-2.8	492.28	19.4	2.13E+04	P80195|GLCM1_BOVI	
YKVPQLEIVPN	46.6	1298.7	11	-3.5	650.37	30.5	2.59E+04	P02662|CASA1_BOVI	
ILNKPEDETHL	46.5	1307.7	11	2.9	436.9	17.2	2.74E+05	P80195|GLCM1_BOVI	
EPVLGPVRGPFP	46.5	1263.7	12	5.1	632.86	32	7.59E+04	P02666|CASB_BOVIN	
NAVPITPTLN	46.4	1038.6	10	4.7	520.3	24.3	6.34E+03	P02663|CASA2_BOVI	
SQSKVLPVPQK	46.4	1209.7	11	8.4	404.25	15.1	2.45E+04	P02666|CASB_BOVIN	
GPVRGPFPIIV	46.3	1150.7	11	3.9	576.35	37.1	3.37E+05	P02666|CASB_BOVIN	
VQVTSTAV	46.2	803.44	8	-7.7	804.44	15.3	8.60E+03	P02668|CASK_BOVIN	
TKVIPYVRYL	46.1	1250.7	10	0.3	417.92	29.7	2.31E+03	P02663|CASA2_BOVI	
EVLNENLLRF	45.9	1245.7	10	5.9	623.85	35.3	3.08E+04	P02662|CASA1_BOVI	
TVDM(+15.99)ESTEVFTK	45.8	1401.6	12	-1.4	701.83	18	1.38E+04	P02663|CASA2_BOVI	Oxidation (M)
SVLSLSQS	45.8	819.43	8	-8.7	820.44	20.6	1.28E+04	P02666|CASB_BOVIN	
APFPEVFG	45.7	862.42	8	-0.8	863.43	33.2	2.19E+04	P02662|CASA1_BOVI	

Peptide	-10lg	Mass	Length	ppm	m/z	RT	Area	Accession	PTM
APPPPPPPP	45.7	865.47	9	-3.9	433.74	15	1.01E+04	A2VDK6|WASF2_BOVIN:Q32LP2|RADI_BOVI	
QEPVLGPVRGPFPII	45.6	1617.9	15	3.7	809.97	41.6	6.00E+03	P02666|CASB_BOVIN	
VVPPFLQPEVM(+15.99)	45.6	1270.7	11	0	636.34	33.4	9.85E+04	P02666|CASB_BOVIN	Oxidation (M)
HQGLPQEVL	45.5	1019.5	9	3.2	510.78	23.2	3.03E+04	P02662|CASA1_BOVI	
GLPQEVLNEN	45.4	1111.6	10	-0.8	556.78	23.4	1.54E+04	P02662|CASA1_BOVI	
LNKPEDETHLE	45.1	1323.6	11	10	442.22	13.8	1.05E+05	P80195|GLCM1_BOVI	
LIVTQTMKGL	45.1	1102.6	10	-3.7	552.33	27.8	6.66E+03	P02754|LACB_BOVIN	
SLVYPFPGPIPNSLPQ	45	1724.9	16	1.8	863.47	39.9	1.13E+04	P02666|CASB_BOVIN	
VDMES(+79.97)TEVFTK	45	1364.6	11	6.6	683.29	22.7	8.31E+03	P02663|CASA2_BOVIN	Phosphorylation (STY)
HIQKEDVPSERYL	44.8	1612.8	13	2.6	538.62	18.7	1.23E+05	P02662|CASA1_BOVI	
VAPFPEVFGKEKV	44.7	1445.8	13	0.4	482.94	31	5.10E+04	P02662|CASA1_BOVI	
PEVIESPPEINTVQVTSTAV	44.7	2109.1	20	-2.6	1055.6	32.3	2.86E+03	P02668|CASK_BOVIN	
LPVPQKAVPYPQ	44.6	1335.8	12	-0.4	668.89	23.6	2.60E+04	P02666|CASB_BOVIN	
SQNPKLPLSILK	44.6	1336.8	12	-5.4	446.61	29.6	7.63E+03	P80195|GLCM1_BOVI	
TQTPVVVPPFLQPEVM(+15.99)	44.5	1796.9	16	1.6	899.48	40	6.55E+04	P02666|CASB_BOVIN	Oxidation (M)
VPQLEIVPNSAEERLH	44.5	1830	16	-0.7	611	28	5.65E+03	P02662|CASA1_BOVI	
GLDIQKVAGTW	44.3	1186.6	11	-3	594.32	31.5	1.23E+03	P02754|LACB_BOVIN	
PPPPPPPPP	44.1	891.49	9	1.4	446.75	15.6	5.24E+04	A2VDK6|WASF2_BOVIN:A5PKL7|LZTS2_BO	
FPEVFGKEK	44.1	1079.6	9	9.7	360.87	21.9	9.24E+03	P02662|CASA1_BOVI	
ILNKPEDETHLEAQPTDASAQF	44	2453.2	22	-0.3	818.73	23.9	1.41E+05	P80195|GLCM1_BOVI	
VIESPPEINTVQ	43.9	1324.7	12	-0.1	663.35	23.2	1.52E+04	P02668|CASK_BOVIN	
IESPPEIN	43.9	897.44	8	-4.9	898.45	17.8	1.17E+03	P02668|CASK_BOVIN	
AAGGPGAPADPGRPTGY	43.8	1510.7	17	-10.6	756.36	15.6	5.50E+03	P81265|PIGR_BOVIN:P81265-	
SRQPQSQNPKLPL	43.7	1491.8	13	-2.6	498.28	20	1.76E+04	P80195|GLCM1_BOVI	
NAVPITPTL	43.2	924.53	9	-6.8	925.53	27.9	0	P02663|CASA2_BOVI	
VPPFLQPEVM	43.1	1155.6	10	-0.4	578.81	35.8	2.50E+04	P02666|CASB_BOVIN	
VDM(+15.99)ES(+79.97)TEVFTK	43.1	1380.6	11	3.7	691.29	16.4	3.04E+04	P02663|CASA2_BOVIN	Oxidation (M); Phosphorylation (STY)
LNKPEDETHL	43	1194.6	10	3.3	399.21	14.4	6.18E+04	P80195|GLCM1_BOVI	
SRNPDEEGLFTVR	43	1518.7	13	-1.9	507.26	23	6.09E+03	P18892|BT1A1_BOVIN	
VPPFLQPEVM(+15.99)GV	43	1327.7	12	2.2	664.85	34.5	1.05E+04	P02666|CASB_BOVIN	Oxidation (M)
FSHAFEVVKT	42.8	1163.6	10	1.1	388.87	21.2	1.33E+04	P80195|GLCM1_BOVI	
FVAPFPEVFGKEKV	42.7	1592.9	14	1.2	531.96	36.3	4.77E+04	P02662|CASA1_BOVI	
VAPFPEVF	42.6	904.47	8	-3.7	905.48	36.6	7.15E+04	P02662|CASA1_BOVI	
SLSQSKVLPVPQ	42.6	1281.7	12	-2.1	641.87	23.3	1.88E+04	P02666|CASB_BOVIN	
PPPPPPPP	42.6	794.43	8	-5	795.44	14.3	4.35E+04	A2VDK6|WASF2_BOVIN:A5PKL7|LZTS2_BOVIN:Q32LP2|RADI_BOVIN:Q2KJE5|G3PT_B	
SQSKVLPVPQ	42.5	1081.6	10	4.2	541.82	19.1	8.40E+04	P02666|CASB_BOVIN	
HIQKEDVPSERY	42.5	1499.7	12	1.8	500.92	13.3	6.98E+03	P02662|CASA1_BOVI	
AASTTTISDAVSK	42.5	1250.6	13	-8.8	626.32	15.5	0	P80025|PERL_BOVIN	
RELEELNVPGEIVE	42.4	1624.8	14	2.1	813.43	29.7	8.55E+04	P02666|CASB_BOVIN	
VDM(+15.99)ESTEVFTK	42.3	1300.6	11	0.3	651.3	16.8	1.74E+04	P02663|CASA2_BOVI	Oxidation (M)
SKVLPVPQ	42.2	866.52	8	-1.6	434.27	18.6	3.50E+04	P02666|CASB_BOVIN	
VSREGQEQEGEEMAEYR	42	2025.9	17	10.4	676.31	15.5	1.81E+03	P18892|BT1A1_BOVIN	
FVAPFPEVF	41.9	1051.5	9	1.9	526.78	42.5	2.38E+04	P02662|CASA1_BOVI	
NAVPITPT	41.9	811.44	8	-0.2	812.45	17.4	3.93E+04	P02663|CASA2_BOVI	
KVLPVPQKAVPYPQ	41.8	1562.9	14	-1.2	521.98	23.1	1.04E+04	P02666|CASB_BOVIN	
VIESPPEIN	41.8	996.51	9	-0.8	997.52	20	8.06E+04	P02668|CASK_BOVIN	
HIQKEDVPSER	41.8	1336.7	11	-5.3	446.56	9.63	1.39E+02	P02662|CASA1_BOVI	
FVAPFPEVFGKE	41.4	1365.7	12	-1.5	683.86	37.7	1.22E+04	P02662|CASA1_BOVI	
KEDVPSERYL	41.4	1234.6	10	9.6	412.55	18.4	2.04E+04	P02662|CASA1_BOVI	
LYQGPIVLNPWDQVKR	41.4	1925.1	16	0.3	642.69	34.2	1.45E+04	P02663|CASA2_BOVI	
GLPQEVLN	41.4	868.47	8	-3.2	869.47	22.5	2.12E+05	P02662|CASA1_BOVI	
ALLDPSFFAKESVKDAAGGPGAPA	41.3	2938.5	30	4.5	735.63	33.2	2.18E+04	P81265|PIGR_BOVIN:P81265-	
VLPVPQKAVPYPQRDMPIQAF	41.3	2393.3	21	-7.5	798.77	34.6	6.08E+03	P02666|CASB_BOVIN	
RELEELNVPGEIVESL	41.3	1824.9	16	0.5	913.48	39.9	6.02E+03	P02666|CASB_BOVIN	
PPPAPPPPP	41.3	865.47	9	1.6	433.74	15	4.76E+03	A2VDK6|WASF2_BOVIN:A6QR00|ZN526_BO	
NLHLPLPLLQ	41.2	1156.7	10	3.1	579.36	42.3	1.21E+04	P02666|CASB_BOVIN	
SSS(+79.97)EESITRIN	41.2	1301.6	11	-0.8	651.78	15.9	9.69E+03	P02666|CASB_BOVIN	Phosphorylation (STY)
ELEELNVPGE	41.1	1127.5	10	1.6	564.78	24.5	2.83E+03	P02666|CASB_BOVIN	
KHQGLPQEVLNENLLRF	40.8	2034.1	17	6.1	509.54	36.2	2.61E+03	P02662|CASA1_BOVI	
SLSQSKVLPVPQK	40.7	1409.8	13	4.1	470.95	19.5	1.40E+04	P02666|CASB_BOVIN	
SPPPPPPPP	40.7	881.46	9	0.7	441.74	14.6	2.18E+03	A5PKL7|LZTS2_BOVI	

Peptide	-10lg	Mass	Length	ppm	m/z	RT	Area	Accession	PTM
GYLEQLLR	40.3	990.55	8	5.9	496.29	31.4	4.22E+03	P02662|CASA1_BOVI	
VVPPFLQPEVM	40.3	1254.7	11	0.9	628.34	38.5	1.93E+04	P02666|CASB_BOVIN	
PPPPPPPPPP	40.3	988.54	10	-3.7	495.28	16.8	1.61E+04	A2VDK6|WASF2_BOVIN:A5PKL7|LZTS2_BO	
HQGLPQEVLNENLL	40.3	1602.8	14	-1.7	802.43	33.9	1.26E+04	P02662|CASA1_BOVI	
FLPYPYYAKPA	40.3	1328.7	11	5.8	665.35	28.5	1.86E+03	P02668|CASK_BOVIN	
FVAPFPE	40.2	805.4	7	-3.7	806.41	28.9	3.73E+04	P02662|CASA1_BOVI	
TKVIPYVR	40.2	974.59	8	4.9	325.87	17.7	3.35E+03	P02663|CASA2_BOVI	
DM(+15.99)ESTEVFTK	40.2	1201.5	10	4.2	601.77	15.1	3.63E+03	P02663|CASA2_BOVI	Oxidation (M)
FPEVFGK	40	822.43	7	1.7	412.22	24.7	2.31E+04	P02662|CASA1_BOVI	
LYQEPVLGPVRGPFPIIV	40	1993.1	18	-3.2	997.58	45.4	2.49E+03	P02666|CASB_BOVIN	
DKTEIPTIN	39.9	1029.5	9	-0.1	515.78	19.5	3.10E+04	P02668|CASK_BOVIN	
SSRQPQSQNPKLPLSILK	39.8	2020.1	18	-0.6	506.04	27.6	1.44E+04	P80195|GLCM1_BOVI	
VDM(+15.99)EST(+79.97)EVFTK	39.7	1380.6	11	2.2	691.29	16.9	3.04E+04	P02663|CASA2_BOVIN	Oxidation (M); Phosphorylation (STY)
SQSKVLPVPQKAVPYPQ	39.7	1865	17	3.8	622.69	24	2.74E+04	P02666|CASB_BOVIN	
QEPVLGPVRGPFP	39.6	1391.8	13	1.3	696.89	31.9	4.11E+03	P02666|CASB_BOVIN	
QEPVLGPVRGPFPI	39.5	1504.8	14	-2.3	753.43	37.6	2.29E+03	P02666|CASB_BOVIN	
APFPEVF	39.4	805.4	7	-2.4	806.41	34.2	2.03E+04	P02662|CASA1_BOVI	
SDIPNPIGSE	39.4	1027.5	10	-9.1	514.75	22.8	5.47E+03	P02662|CASA1_BOVI	
SQNPKLPLSILKEK	39.4	1593.9	14	13.6	399.5	26.3	3.11E+03	P80195|GLCM1_BOVI	
SLPQNIPPLTQTPV	39.3	1503.8	14	-0.5	752.92	32.1	2.00E+04	P02666|CASB_BOVIN	
ALLDPSFFAK	39.3	1107.6	10	1	554.81	34.2	1.87E+03	P81265|PIGR_BOVIN:P81265-	
TDVENLHLPLPLLQ	39.2	1600.9	14	4.6	801.45	43.2	4.72E+03	P02666|CASB_BOVIN	
DHIAEGSVAVR	39.2	1152.6	11	6.1	385.21	13.6	2.03E+03	P18892|BT1A1_BOVIN	
VLGPVRGPFP	39.1	1037.6	10	1	519.81	28.5	3.62E+03	P02666|CASB_BOVIN	
YLEQLLRL	39.1	1046.6	8	5.3	524.32	37.5	2.94E+03	P02662|CASA1_BOVI	
LIVTQTM(+15.99)KGL	39.1	1118.6	10	6.2	560.33	21.1	2.72E+04	P02754|LACB_BOVIN	Oxidation (M)
TVDM(+15.99)EST(+79.97)EVFTK	38.9	1481.6	12	-4.2	741.81	17.5	1.66E+04	P02663|CASA2_BOVIN	Oxidation (M); Phosphorylation (STY)
NVPGEIVESL	38.8	1055.5	10	-2.5	1056.6	31.1	1.56E+05	P02666|CASB_BOVIN	
GLPQEVL	38.8	754.42	7	3.5	755.43	25.6	3.19E+05	P02662|CASA1_BOVI	
SLPQNIPPLTQTPVVVPPFLQPEVM	38.8	2756.5	25	4.8	919.84	45.7	1.50E+04	P02666|CASB_BOVIN	Oxidation (M)
YQKFPQY	38.8	972.47	7	2	487.25	18.9	6.09E+03	P02663|CASA2_BOVI	
SDIPNPIGSENSEK	38.6	1485.7	14	2.7	743.86	20.2	1.56E+04	P02662|CASA1_BOVI	
GYLEQLLRLK	38.6	1231.7	10	7	411.59	35.8	1.05E+03	P02662|CASA1_BOVI	
AAGGPGAPADPGRPTGYS	38.6	1597.7	18	-4.2	799.88	14.7	2.76E+03	P81265|PIGR_BOVIN:P81265-	
RDMPIQAFLL	38.5	1202.6	10	1.4	602.33	40.6	8.57E+03	P02666|CASB_BOVIN	
DM(+15.99)PIQAF	38.5	836.37	7	-0.8	837.38	22.3	8.96E+03	P02666|CASB_BOVIN	Oxidation (M)
LPYPYYAKPA	38.4	1181.6	10	-2.3	591.81	23	1.80E+04	P02668|CASK_BOVIN	
IGVNQEL	38.4	771.41	7	-4.7	772.42	20	1.02E+03	P02662|CASA1_BOVI	
DKIHPFAQTQ	38.3	1183.6	10	2.8	592.81	14.8	4.67E+03	P02666|CASB_BOVIN	
YQEPVL	38.3	747.38	6	0.4	748.39	21.6	3.23E+04	P02666|CASB_BOVIN	
DASAQFIR	38.2	906.46	8	3.7	454.24	16.3	3.03E+04	P80195|GLCM1_BOVI	
GPVRGPFP	38.2	825.45	8	0.4	413.73	20.7	3.54E+04	P02666|CASB_BOVIN	
GPFPIIV	38	741.44	7	-0.4	742.45	37.9	7.61E+03	P02666|CASB_BOVIN	
IPPLTQTPV	37.9	964.56	9	-6.8	965.56	26.2	4.31E+04	P02666|CASB_BOVIN	
AVPYPQ	37.8	673.34	6	-0.4	674.35	14.4	8.49E+03	P02666|CASB_BOVIN	
VVVPPFLQPEVM(+15.99)	37.8	1369.7	12	1.1	685.88	37.7	2.27E+03	P02666|CASB_BOVIN	Oxidation (M)
TQTPVVVPPFLQPEVM	37.6	1780.9	16	0.1	891.48	43.6	1.25E+04	P02666|CASB_BOVIN	
NENLLRF	37.6	904.48	7	4.7	453.25	27.6	1.82E+05	P02662|CASA1_BOVI	
ASAQFIRNL	37.6	1018.6	9	-4.5	510.28	25	3.35E+03	P80195|GLCM1_BOVI	
HAFEVVKT	37.5	929.5	8	7.6	465.76	15.2	3.08E+03	P80195|GLCM1_BOVI	
NVPGEIVE	37.4	855.43	8	-0.8	856.44	18.6	8.56E+04	P02666|CASB_BOVIN	
EMPFPKYPVEPF	37.3	1479.7	12	3.4	740.87	36.8	1.49E+04	P02666|CASB_BOVIN	
EVLNENLLR	37.3	1098.6	9	1.1	550.31	24.4	4.81E+04	P02662|CASA1_BOVI	
HQGLPQEVLN	37.3	1133.6	10	-3.4	567.8	20.3	2.95E+04	P02662|CASA1_BOVI	
TLTDVENL	37.3	903.45	8	-3	904.46	24	1.18E+04	P02666|CASB_BOVIN	
TTLSSEAPTTQ	37.2	1134.5	11	2.5	568.28	13.7	2.60E+03	P80025|PERL_BOVIN	
PPAPPPPPP	37.1	865.47	9	-3.9	433.74	15	1.01E+04	A2VDK6|WASF2_BOV	
DMPIQA	37	673.31	6	3.4	674.32	17.9	5.04E+03	P02666|CASB_BOVIN	
AGEIQNKALLD	37	1170.6	11	-1.4	586.32	18.5	1.66E+04	P81265|PIGR_BOVIN:P81265-	
ELEELNVPGEIVE	37	1468.7	13	3.4	735.38	31.8	1.56E+04	P02666|CASB_BOVIN	
TVDM(+15.99)ES(+79.97)TEVFTK	37	1481.6	12	-4.2	741.81	17.5	1.66E+04	P02663|CASA2_BOVIN	Oxidation (M); Phosphorylation (STY)
VLNENLLR	36.9	969.56	8	-4.5	485.79	22	6.37E+03	P02662|CASA1_BOVI	
YQGPIVLNPWDQVKR	36.9	1812	15	9.9	605	32	1.18E+04	P02663|CASA2_BOVI	
EAQPTDASAQF	36.9	1163.5	11	-0.3	582.76	17	6.32E+04	P80195|GLCM1_BOVI	
DMPIQAF	36.9	820.38	7	-6.1	821.38	29.2	6.01E+03	P02666|CASB_BOVIN	

Peptide	-10lg	Mass	Length	ppm	m/z	RT	Area	Accession	PTM
FPEVFGKE	36.8	951.47	8	5.5	476.75	25.1	1.02E+03	P02662|CASA1_BOVI	
PVEPFTESQ	36.8	1032.5	9	5.3	517.25	18.9	9.30E+03	P02666|CASB_BOVIN	
SLSQSKVLPVPQKAVPYPQ	36.5	2065.2	19	4.2	689.4	26.5	4.61E+03	P02666|CASB_BOVIN	
FPKYPVEPF	36.5	1122.6	9	4.8	562.3	31.3	1.19E+04	P02666|CASB_BOVIN	
VSREGQEQEGEEM(+15.99)AEYR	36.4	2041.9	17	-1.4	681.63	11.7	1.79E+03	P18892|BT1A1_BOVIN	Oxidation (M)
MAIPPKKNQ	36.4	1025.6	9	0.4	342.86	11.3	1.30E+04	P02668|CASK_BOVIN	
PFPEVFGK	36.3	919.48	8	0.6	460.75	31.3	5.69E+03	P02662|CASA1_BOVI	
APFPEVFGKE	36.3	1119.6	10	0.6	560.79	29.2	1.09E+04	P02662|CASA1_BOVI	
AVPITPT	36.3	697.4	7	-4.1	698.41	16	4.16E+03	P02663|CASA2_BOVI	
GHLKALINN	36.2	978.56	9	-13.7	490.28	21.1	3.65E+03	Q9TTK4|LYST_BOVIN	
GQVWEESLK	36.1	1074.5	9	2.5	538.28	21.4	0	P80025|PERL_BOVIN	
VLGPVRGPFPIIV	36	1362.8	13	4.4	682.43	41.9	2.32E+04	P02666|CASB_BOVIN	
SVLSLSQSK	36	947.53	9	-1.2	474.77	17.6	9.20E+03	P02666|CASB_BOVIN	
VDMEST(+79.97)EVFTK	35.9	1364.6	11	6.6	683.29	22.7	8.31E+03	P02663|CASA2_BOVI N	Phosphorylation (STY)
LPQEVLNENLL	35.9	1280.7	11	4.7	641.36	33.9	2.52E+04	P02662|CASA1_BOVI	
STLVPLA	35.8	699.42	7	-5.3	700.42	26.4	9.96E+03	P81265|PIGR_BOVIN: P81265-	
IHPFAQTQ	35.8	940.48	8	1.2	471.25	14.9	6.50E+03	P02666|CASB_BOVIN	
NQFLPYPYYAKPA	35.7	1570.8	13	1.3	786.4	30.7	2.91E+03	P02668|CASK_BOVIN	
GPVRGPFPII	35.7	1051.6	10	1.4	526.82	34.6	3.41E+04	P02666|CASB_BOVIN	
DMPIQAFLL	35.7	1046.5	9	-3	524.28	44.9	2.90E+03	P02666|CASB_BOVIN	
SVLSLSQ	35.6	732.4	7	-13.5	733.4	20.9	1.11E+03	P02666|CASB_BOVIN	
SDIPNPIGSENSEKTTM(+15.99)PLW	35.6	2231	20	0.8	1116.5	32.6	2.20E+04	P02662|CASA1_BOVI	Oxidation (M)
IPYVRYL	35.5	922.53	7	-0.9	462.27	29.3	3.75E+03	P02663|CASA2_BOVI	
ASTTTISDAVSK	35.5	1179.6	12	-1.3	590.81	14.8	1.24E+03	P80025|PERL_BOVIN	
MAIPPKKN	35.5	897.51	8	8.2	300.18	11.4	7.17E+04	P02668|CASK_BOVIN	
ALLDPSFFAKE	35.5	1236.6	11	3	619.33	34.6	5.23E+03	P81265|PIGR_BOVIN: P81265-	
EIVPNSAEERLH	35.5	1392.7	12	-0.6	465.24	17.6	4.36E+03	P02662|CASA1_BOVI	
GLPQEVLNE	35.4	997.51	9	5.8	499.77	24.4	6.31E+04	P02662|CASA1_BOVI	
VVPPFLQPE	35.4	1024.6	9	-2.6	513.29	31.4	1.24E+04	P02666|CASB_BOVIN	
RPKHPIKHQGLPQEVL	35.2	1876.1	16	13.5	376.23	16.6	8.47E+03	P02662|CASA1_BOVI	
GYLEQL	35.1	721.36	6	-2.4	722.37	24.7	7.07E+03	P02662|CASA1_BOVI	
LVYPFPGPIPNSLPQ	35.1	1637.9	15	0.9	819.95	39.2	4.81E+03	P02666|CASB_BOVIN	
TKLTEEEKNRL	35.1	1359.7	11	0.9	454.25	13.5	7.97E+03	P02663|CASA2_BOVI	
RDMPIQAF	35	976.48	8	-5.3	489.25	25	6.98E+03	P02666|CASB_BOVIN	
AFEVVKT	35	792.44	7	-0.6	397.23	17.7	6.94E+03	P80195|GLCM1_BOVI	
EAQPTDASAQFIR	35	1432.7	13	-2.5	717.36	20.2	4.09E+03	P80195|GLCM1_BOVI	
VPGEIVESL	34.9	941.51	9	3.7	471.76	27.8	2.27E+03	P02666|CASB_BOVIN	
EPVLGPVR	34.9	865.5	8	4	433.76	17.9	1.06E+04	P02666|CASB_BOVIN	
ARHPHPHLSF	34.8	1197.6	10	7.8	300.41	14.7	1.78E+03	P02668|CASK_BOVIN	
LPQYLKT	34.5	861.5	7	-6	431.75	20.2	4.38E+03	P02663|CASA2_BOVI	
NENLLRFF	34.5	1051.5	8	1.7	526.78	38.1	2.09E+04	P02662|CASA1_BOVI	
KHQGLPQEVLN	34.3	1261.7	11	0.3	631.85	18.2	3.68E+04	P02662|CASA1_BOVI	
APFPEV	34.2	658.33	6	-3	659.34	24.9	8.15E+03	P02662|CASA1_BOVI	
FSDKIAKY	34.2	970.51	8	6.8	324.51	15.8	1.47E+03	P02668|CASK_BOVIN	
VAPFPE	33.8	658.33	6	0.5	659.34	20	2.58E+04	P02662|CASA1_BOVI	
KVPQLEIVPN	33.7	1135.7	10	5.1	568.84	26.4	3.72E+04	P02662|CASA1_BOVI	
SLPQNIPPLT	33.6	1078.6	10	7.8	540.31	28.6	6.12E+03	P02666|CASB_BOVIN	
LPQYL	33.6	632.35	5	-3.2	633.36	24.4	2.53E+03	P02663|CASA2_BOVI	
IPQYI	33.6	632.35	5	-3.2	633.36	24.4	2.53E+03		
LPQEVL	33.6	697.4	6	2.7	698.41	22.7	7.66E+03	P02662|CASA1_BOVI N:Q9TTK4|LYST_BOV	
IPQEVL	33.6	697.4	6	2.7	698.41	22.7	7.66E+03		
VLPVPQ	33.6	651.4	6	-1	652.4	19	2.17E+04	P02666|CASB_BOVIN	
HLPLPLLQ	33.5	929.57	8	5	465.8	35	8.24E+03	P02666|CASB_BOVIN	
SPPEINTVQ	33.5	983.49	9	7	492.76	16	0	P02668|CASK_BOVIN	
TQTPVVVPPFLQPE	33.4	1550.8	14	-3.3	776.42	38.8	1.09E+04	P02666|CASB_BOVIN	
VPQLEIVPNSAEER	33.4	1579.8	14	-8.9	790.91	26.2	2.38E+03	P02662|CASA1_BOVI	
ALPQYLK	33.4	831.49	7	-0.2	416.75	20.1	3.80E+04	P02663|CASA2_BOVI	
MAIPPKKNQD	33.3	1140.6	10	-9.3	381.2	9.8	1.34E+04	P02668|CASK_BOVIN	
EM(+15.99)PFPKYPVEPF	33.2	1495.7	12	-0.9	748.86	32.9	2.09E+04	P02666|CASB_BOVIN	Oxidation (M)
HQGLPQEVLN(+.98)ENLLR	33.1	1759.9	15	14	587.66	30.9	6.65E+03	P02662|CASA1_BOVI N	Deamidation (NQ)
ALNEINQF	33	947.47	8	3	474.75	24.7	3.09E+03	P02663|CASA2_BOVI	
NIPPLTQTPVVVPPFLQPEVM(+15.9	32.9	2331.3	21	-0.6	1166.6	45.3	1.30E+04	P02666|CASB_BOVIN	Oxidation (M)
VPQLEIVPN	32.9	1007.6	9	-6.2	1008.6	28	5.18E+04	P02662|CASA1_BOVI	
ELNVPGEIVESL	32.9	1297.7	12	-4.4	649.84	38.3	1.20E+03	P02666|CASB_BOVIN	
PVEPF	32.8	587.3	5	-3.7	588.3	20.2	5.44E+03	P02666|CASB_BOVIN	
HIQKEDVPSERYLG	32.8	1669.8	14	3.2	557.62	17.5	2.48E+03	P02662|CASA1_BOVI	
ALLDPSFFAKES	32.8	1323.7	12	4.1	662.85	34.1	4.57E+03	P81265|PIGR_BOVIN: P81265-	
S(+79.97)PEVIESPPEINTVQVTSTA'	32.8	2276.1	21	-3.2	1139	32.3	4.14E+03	P02668|CASK_BOVIN	Phosphorylation (STY)
NLHLPLPL	32.7	915.55	8	9.8	458.79	39.4	2.38E+03	P02666|CASB_BOVIN	

Peptide	-10lg	Mass	Length	ppm	m/z	RT	Area	Accession	PTM
YPFPGPIPN	32.7	1000.5	9	2	1001.5	29.8	4.99E+03	P02666|CASB_BOVIN	
TVDMESTEVFTK	32.6	1385.6	12	0.6	693.83	23.3	4.19E+03	P02663|CASA2_BOVI	
DKIHPFAQTQS	32.6	1270.6	11	3.1	424.55	14.7	3.36E+03	P02666|CASB_BOVIN	
AVESTVATL	32.5	889.48	9	6.4	445.75	21	7.33E+03	P02668|CASK_BOVIN	
SKVLPVPQKAVPYPQ	32.5	1650	15	1.7	550.99	23.7	7.77E+03	P02666|CASB_BOVIN	
LPQYLK	32.5	760.45	6	2.2	381.23	18.3	1.57E+04	P02663|CASA2_BOVI	
DM(+15.99)PIQAFLL	32.4	1062.5	9	2.8	1063.6	38.9	7.09E+03	P02666|CASB_BOVIN	Oxidation (M)
IGVNQELAY	32.3	1005.5	9	0.5	1006.5	23.9	1.50E+04	P02662|CASA1_BOVI	
DVPSERYL	32.2	977.48	8	5	489.75	20	3.78E+04	P02662|CASA1_BOVI	
RPKHPIKHQGLPQEVLN	32.2	1990.1	17	5.9	498.54	15.3	5.59E+04	P02662|CASA1_BOVI	
ALLDPSF	32.1	761.4	7	-7.3	762.4	30.4	4.64E+03	P81265|PIGR_BOVIN: P81265-	
M(+15.99)PFPKYPVEPF	32.1	1366.7	11	3.7	684.34	32.4	1.14E+04	P02666|CASB_BOVIN	Oxidation (M)
VPPFL	32.1	571.34	5	-2.5	572.34	30	0	P02666|CASB_BOVIN	
MPFPKYPVEPF	32	1350.7	11	0.6	676.34	36	1.30E+04	P02666|CASB_BOVIN	
M(+15.99)ES(+79.97)TEVFTK	32	1166.5	9	1.6	584.24	14.7	9.13E+03	P02663|CASA2_BOVIN	Oxidation (M); Phosphorylation (STY)
SSRQPQSQNPKLPLS	32	1665.9	15	-11.5	556.3	18.4	3.39E+03	P80195|GLCM1_BOVI	
HQGLPQEVLNENLLRF	32	1906	16	-2.7	636.34	38.6	1.99E+03	P02662|CASA1_BOVI	
TVDMES(+79.97)TEVFTK	31.9	1465.6	12	4.7	733.82	23	1.44E+03	P02663|CASA2_BOVIN	Phosphorylation (STY)
LEQLLRLK	31.9	1011.6	8	-2.3	338.22	24.1	1.91E+03	P02662|CASA1_BOVI	
AM(+15.99)KPWIQPK	31.8	1113.6	9	1.3	372.21	15.2	9.82E+03	P02663|CASA2_BOVI	Oxidation (M)
ALPQYLKT	31.8	932.53	8	-2.1	467.27	22.1	6.53E+03	P02663|CASA2_BOVI	
DLIS(+79.97)KEQIVIR	31.7	1392.7	11	5	465.26	28.1	2.73E+04	P80195|GLCM1_BOVIN	Phosphorylation (STY)
NAVPITPTLNRE	31.7	1323.7	12	-10.1	662.86	22.2	4.62E+03	P02663|CASA2_BOVI	
SEESITRIN	31.7	1047.5	9	-4.6	524.77	14.5	1.36E+03	P02666|CASB_BOVIN	
LPQEVLN	31.7	811.44	7	-1.3	812.45	18.8	2.98E+03	P02662|CASA1_BOVI	
IEKFQS(+79.97)EEQQ	31.7	1344.6	10	-4.2	673.29	12.6	3.12E+03	P02666|CASB_BOVIN	Phosphorylation (STY)
IPIQY	31.6	632.35	5	-1.2	633.36	21.1	7.05E+03	P02668|CASK_BOVIN	
LPLQY	31.6	632.35	5	-1.2	633.36	21.1	7.05E+03		
IPLQY	31.6	632.35	5	-1.2	633.36	21.1	7.05E+03		
ENTVKETIKY	31.6	1223.6	10	-7.6	612.82	15.7	2.76E+03	P80195|GLCM1_BOVI	
S(+79.97)SSEESITRIN	31.6	1301.6	11	-1.7	651.78	15.9	4.66E+03	P02666|CASB_BOVIN	Phosphorylation (STY)
PGRPTGYSGSSKAL	31.5	1376.7	14	2.1	459.91	13.5	8.68E+03	P81265|PIGR_BOVIN: P81265-	
AVPYPQRDMPIQA	31.5	1484.7	13	-13.4	743.37	24	2.15E+03	P02666|CASB_BOVIN	
ALLDPSFFAKESVKD	31.5	1665.9	15	4	556.3	33.1	5.84E+03	P81265|PIGR_BOVIN: P81265-	
IHPFAQTQSL	31.4	1140.6	10	-11.2	571.3	22.9	4.64E+03	P02666|CASB_BOVIN	
LPLSILKEK	31.3	1039.7	9	-0.1	347.56	25.1	4.27E+02	P80195|GLCM1_BOVI	
SPPEINTVQVTSTAV	31.3	1541.8	15	-0.7	771.91	26.4	1.07E+04	P02668|CASK_BOVIN	
VIPYVRYL	31.2	1021.6	8	-3.2	511.81	32.6	2.08E+03	P02663|CASA2_BOVI	
RPKHPIKHQGLPQEVLNENLLRF	31.2	2762.5	23	8.7	553.52	32.2	3.65E+03	P02662|CASA1_BOVI	
EIPTINTIAS	31.2	1057.6	10	-2.8	529.79	26.8	2.88E+03	P02668|CASK_BOVIN	
VPQLEIVPNS(+79.97)AEER	31.2	1659.8	14	13.2	554.28	26.4	1.36E+03	P02662|CASA1_BOVIN	Phosphorylation (STY)
LVYPFPGPIPNSLPQNIPPLT	31.1	2273.2	21	9.4	758.77	45	3.69E+03	P02666|CASB_BOVIN	
LEQLLRL	31	883.55	7	0.7	442.78	31.3	4.94E+03	P02662|CASA1_BOVI	
VLNENLL	31	813.46	7	-4.1	814.47	26.3	2.74E+03	P02662|CASA1_BOVI	
PVEPFTESQSL	31	1232.6	11	1.8	617.31	26.8	8.82E+03	P02666|CASB_BOVIN	
QFLPYPYYAKPA	31	1456.7	12	-6.8	729.37	30.7	1.70E+03	P02668|CASK_BOVIN	
TESQSLTL	30.9	877.44	8	-0.1	878.45	20.5	4.34E+03	P02666|CASB_BOVIN	
DVPSERYLGYL	30.9	1310.7	11	-0.5	656.33	31.6	2.86E+03	P02662|CASA1_BOVI	
ALPQYL	30.8	703.39	6	1.8	352.7	26.2	7.92E+03	P02663|CASA2_BOVI	
LHLPLPLLQ	30.7	1042.7	9	0	522.34	43.2	4.03E+04	P02666|CASB_BOVIN	
HKEMPFPKYPVEPFTESQ	30.7	2190	18	4.6	731.03	29.5	2.71E+03	P02666|CASB_BOVIN	
IHPFAQTQS	30.6	1027.5	9	0.6	514.76	14.8	3.90E+03	P02666|CASB_BOVIN	
LPQEVLNENL	30.6	1167.6	10	-8.9	584.81	27.7	2.24E+03	P02662|CASA1_BOVI	
FVAPFPEVFGKEKVN	30.6	1706.9	15	3.3	569.98	34.9	4.14E+03	P02662|CASA1_BOVI	
GLPQEV	30.3	641.34	6	-6.5	642.34	17.8	8.37E+03	P02662|CASA1_BOVI	
VIESPPEIN(+.98)	30.3	997.5	9	3.5	499.76	21.1	2.32E+03	P02668|CASK_BOVIN	Deamidation (NQ)
ENLLRFF	30.3	937.5	7	6.8	469.76	38	1.85E+03	P02662|CASA1_BOVI	
DVENLHLPLPLLQ	30.3	1499.8	13	-1.9	750.93	43.7	2.92E+03	P02666|CASB_BOVIN	
APFPE	30.2	559.26	5	-0.8	560.27	16.1	2.54E+03	P02662|CASA1_BOVI	
NVPGEIVES	30.2	942.47	9	-0.9	472.24	18.1	4.40E+03	P02666|CASB_BOVIN	
EVLNENLL	30.1	942.5	8	0.5	943.51	28.6	4.33E+04	P02662|CASA1_BOVI	
SSRQPQSQNPKLPLSILKEK	30	2277.3	20	6.4	456.47	24.8	3.71E+03	P80195|GLCM1_BOVI	

Peptide	-10lg	Mass	Length	ppm	m/z	RT	Area	Accession	PTM
EDVPSERYL	30	1106.5	9	-5	554.27	20.8	5.33E+03	P02662|CASA1_BOVI	
INNQFLPYPYYAKPA	30	1797.9	15	0.2	899.96	32	2.06E+03	P02668|CASK_BOVIN	
N(+.98)AVPITPT	30	812.43	8	-5.6	813.43	18.5	7.10E+03	P02663|CASA2_BOVI N	Deamidation (NQ)
HQGLPQ(+.98)EVLNENLLR	30	1759.9	15	14	587.66	30.9	3.70E+03	P02662|CASA1_BOVI N	Deamidation (NQ)
FPEVF	30	637.31	5	-2.6	638.32	30.7	9.01E+03	P02662|CASA1_BOVI	
TKVIPYV	29.9	818.49	7	-3.8	410.25	24	1.31E+03	P02663|CASA2_BOVI	
SKVLPVPQK	29.9	994.62	9	-2.7	332.55	14.7	1.35E+03	P02666|CASB_BOVIN	
IVTQTMKGL	29.9	989.56	9	-1.8	495.79	20.5	3.17E+03	P02754|LACB_BOVIN	
HKEM(+15.99)PFPKYPVEPF	29.8	1760.9	14	4	587.96	28.1	1.55E+04	P02666|CASB_BOVIN	Oxidation (M)
ILNKPEDETHLEAQPT	29.8	1833.9	16	5.3	612.32	17.8	8.43E+04	P80195|GLCM1_BOVI	
INTVQVTSTAV	29.8	1131.6	11	-5.3	566.81	21.7	3.37E+03	P02668|CASK_BOVIN	
RDM(+15.99)PIQAFLL	29.8	1218.6	10	-2.2	610.33	35.9	1.15E+04	P02666|CASB_BOVIN	Oxidation (M)
LENTVKETIKY	29.7	1336.7	11	2.8	446.58	19	4.16E+03	P80195|GLCM1_BOVI	
SPEVIESPPEINTVQVTSTAV	29.7	2196.1	21	2	733.05	32.3	6.11E+03	P02668|CASK_BOVIN	
LVSTLVPLA	29.7	911.57	9	-6.8	456.79	33.5	1.35E+03	P81265|PIGR_BOVIN: P81265-	
KVPQLEIVPNSAEER	29.7	1707.9	15	8.6	570.32	24.4	2.03E+03	P02662|CASA1_BOVI	
VSTLVPLA	29.7	798.49	8	-4.2	799.49	28.9	6.58E+03	P81265|PIGR_BOVIN: P81265-	
KTEIPTIN	29.7	914.51	8	-2.5	458.26	18.9	1.61E+04	P02668|CASK_BOVIN	
EIVESL	29.5	688.36	6	-7.9	689.37	21.3	4.53E+03	P02666|CASB_BOVIN	
SDIPNPIGSENSEKTTMPLW	29.4	2215	20	-3.4	1108.5	36.4	4.05E+03	P02662|CASA1_BOVI	
LIVTQTMK	29.3	932.54	8	-9.2	467.27	17.6	1.50E+03	P02754|LACB_BOVIN	
DVENLHLPLPL	29.3	1258.7	11	1.3	630.36	41.6	1.58E+03	P02666|CASB_BOVIN	
NNQFLPYPYYAKPA	29.3	1684.8	14	-7	843.42	30.5	2.70E+03	P02668|CASK_BOVIN	
VAPFP	29.3	529.29	5	0.6	530.3	21.1	0	P02662|CASA1_BOVI	
SSRQPQSQNPKLPLSIL	29.2	1892	17	-1.9	631.69	33.5	4.50E+04	P80195|GLCM1_BOVI	
SVLSL	29.1	517.31	5	-6.5	518.32	25.2	1.44E+03	P02666|CASB_BOVIN	
SVLSI	29.1	517.31	5	-6.5	518.32	25.2	1.44E+03		
SVISL	29.1	517.31	5	-6.5	518.32	25.2	1.44E+03		
SVISI	29.1	517.31	5	-6.5	518.32	25.2	1.44E+03		
EPVLGPVRGPFPI	29.1	1376.8	13	7.5	689.41	37.9	0	P02666|CASB_BOVIN	
VPYPQRDMPIQA	29.1	1413.7	12	-6.9	707.86	23.7	2.04E+03	P02666|CASB_BOVIN	
KHQGLPQEVLNENLL	28.9	1730.9	15	2.2	577.99	31.9	1.28E+04	P02662|CASA1_BOVI	
GLDIQKVA	28.9	842.49	8	4.6	422.25	20.2	3.49E+03	P02754|LACB_BOVIN	
SLPQNIPPL	28.9	977.55	9	3.8	489.79	30.6	4.77E+03	P02666|CASB_BOVIN	
LPLSILK	28.9	782.53	7	-5.2	392.27	28.7	1.14E+03	P80195|GLCM1_BOVI	
LPLSLLK	28.9	782.53	7	-5.2	392.27	28.7	1.14E+03		
VLPVPQK	28.8	779.49	7	3.2	390.75	15.1	9.50E+02	P02666|CASB_BOVIN	
KEPM(+15.99)IGVNQEL	28.8	1272.6	11	-8.4	637.32	22.3	6.20E+03	P02662|CASA1_BOVI	Oxidation (M)
YLEQL	28.8	664.34	5	-1.5	665.35	21	2.39E+03	P02662|CASA1_BOVI N:P61635|STAT3_BO	
FPEVFGKEKV	28.7	1178.6	10	2.8	393.89	25.1	7.89E+03	P02662|CASA1_BOVI	
IQKEDVPSERYL	28.7	1475.8	12	3.2	492.93	20.4	8.14E+03	P02662|CASA1_BOVI	
GYLEQLL	28.7	834.45	7	13.6	418.24	34.5	5.71E+03	P02662|CASA1_BOVI	
TVDMEST(+79.97)EVFTK	28.7	1465.6	12	13.7	733.82	22.9	0	P02663|CASA2_BOVI N	Phosphorylation (STY)
LGPVRGPFPIIV	28.6	1263.8	12	-3.5	632.89	40.8	2.65E+03	P02666|CASB_BOVIN	
KEEVPPPPP	28.5	988.52	9	9.6	495.27	16.8	1.61E+04	Q2KJE5|G3PT_BOVIN	
LEIVPN	28.5	683.39	6	-3.7	684.39	21.1	3.32E+03	P02662|CASA1_BOVI	
VYPFPGPIPNSLPQ	28.4	1524.8	14	3.6	763.41	36.3	4.29E+03	P02666|CASB_BOVIN	
VPSERYL	28.4	862.45	7	3	432.24	17.4	1.36E+04	P02662|CASA1_BOVI	
PPPPVI	28.4	618.37	6	14.3	619.39	30	1.67E+04	Q32LP2|RADI_BOVIN	
PPPPVL	28.4	618.37	6	14.3	619.39	30	1.67E+04		
LPLSIL	28.3	654.43	6	-1.1	655.44	36	5.01E+03	P80195|GLCM1_BOVI	
IPISLL	28.3	654.43	6	-1.1	655.44	36	5.01E+03		
LPLSLL	28.3	654.43	6	-1.1	655.44	36	5.01E+03		
LPISIL	28.3	654.43	6	-1.1	655.44	36	5.01E+03		
LPLSII	28.3	654.43	6	-1.1	655.44	36	5.01E+03		
IPLSII	28.3	654.43	6	-1.1	655.44	36	5.01E+03		
IPLSLL	28.3	654.43	6	-1.1	655.44	36	5.01E+03		
VSTLVPL	28.3	727.45	7	-7.1	728.45	30.3	4.76E+03	P81265|PIGR_BOVIN: P81265-	
IPIQYVL	28.3	844.51	7	0.6	423.26	34.1	2.62E+03	P02668|CASK_BOVIN	
VRGPFPIIV	28.3	996.61	9	14.2	499.32	36.3	3.73E+03	P02666|CASB_BOVIN	
PPLPPV	28.2	618.37	6	8.5	619.39	31	3.47E+03	A6QR00|ZN526_BOVI	
TEDELQDKIHPF	28.2	1470.7	12	7.9	491.25	25.4	1.46E+04	P02666|CASB_BOVIN	
FALPQ	28.2	574.31	5	8.8	575.33	25.4	1.67E+04	P02663|CASA2_BOVI	
ELEEL	28.1	631.31	5	0.3	632.32	18.4	5.17E+04	P02666|CASB_BOVIN	
EIEEL	28.1	631.31	5	0.3	632.32	18.4	5.17E+04		
EIEEI	28.1	631.31	5	0.3	632.32	18.4	5.17E+04		
ELEEI	28.1	631.31	5	0.3	632.32	18.4	5.17E+04		
KVPPLPA	28.1	720.45	7	-16	721.45	34.1	1.22E+04	F1MUG2|CEP41_BOVI	

Peptide	-10lg	Mass	Length	ppm	m/z	RT	Area	Accession	PTM
KTTLSS(+79.97)EAPTTQ	28	1342.6	12	-0.5	672.31	13.9	5.88E+03	P80025|PERL_BOVIN	Phosphorylation (STY)
AARTP	28	514.29	5	15	515.3	28.4	2.00E+04	Q05927|5NTD_BOVIN:P42891|ECE1_BOVIN:Q58D72|ATLA1_BOVIN:Q29RK0|ZN574_BOVIN:Q148I5|KTI12_BOV	
ALLDPSFFAKESVKDAAGGPGAPA	27.9	2430.2	25	0	811.08	34.3	1.04E+04	P81265|PIGR_BOVIN:P81265-	
EVIESPPEINTVQ	27.8	1453.7	13	-8.7	727.87	24.7	2.22E+03	P02668|CASK_BOVIN	
AAGRI	27.8	486.29	5	18.4	487.31	26.2	5.18E+03	Q8WN55|PTBP1_BOVI	
AAGRL	27.8	486.29	5	18.4	487.31	26.2	5.18E+03	N:Q3SYZ9|MED4_BOVIN:Q3T0B2|PSMD6_BOVIN:E1B9W9|UBP42_BOVIN:P79331|ATS2_BOVIN:A6QM06|SCAP_BOVIN:Q2KIS6|CP071_BOVIN	
FVAPFPEVFGKEKVNEL	27.8	1949	17	7.8	650.69	38.6	5.51E+03	P02662|CASA1_BOVI	
SVLSLS	27.7	604.34	6	-12.4	605.34	21.4	0	P02666|CASB_BOVIN	
RGPFPIIV	27.7	897.54	8	3.3	449.78	34.4	2.77E+03	P02666|CASB_BOVIN	
LIS(+79.97)KEQIVIR	27.7	1277.7	10	0.5	426.91	24.4	0	P80195|GLCM1_BOVIN	Phosphorylation (STY)
TLVPLA	27.6	612.38	6	-1.1	613.39	25.6	3.17E+03	P81265|PIGR_BOVIN:P81265-	
RDMPIQA	27.6	829.41	7	0.5	415.71	15.2	2.77E+03	P02666|CASB_BOVIN	
YYQQKPVAL	27.6	1108.6	9	-2.3	555.3	19.5	2.02E+03	P02668|CASK_BOVIN	
EVLNENL	27.5	829.42	7	-2.4	830.43	20.9	1.67E+03	P02662|CASA1_BOVI	
TLPAT	27.5	501.28	5	5.1	502.29	25.1	4.30E+03	O18964|SYNJ1_BOVIN:Q9GKZ4|TRAM1_BOVIN:Q17QT2|MTUS1_BOVIN:Q32PH0|TPPC9_	
TIPAT	27.5	501.28	5	5.1	502.29	25.1	4.30E+03	Q5J316|GTR12_BOVIN	
TAACK	27.4	492.24	5	-1.3	493.24	13.7	1.43E+03	P08169|MPRI_BOVIN	
MES(+79.97)TEVFTK	27.4	1150.5	9	6.6	576.24	17.4	2.49E+03	P02663|CASA2_BOVIN	Phosphorylation (STY)
RELEELNVPGE	27.4	1283.6	11	-9.1	642.82	22.8	7.78E+03	P02666|CASB_BOVIN	
HLPLPLLQS	27.3	1016.6	9	-0.8	509.31	34.1	1.68E+03	P02666|CASB_BOVIN	
ENLLRF	27.2	790.43	6	1	396.23	27.4	1.74E+05	P02662|CASA1_BOVI	
KEDVPSERYLG	27.2	1291.6	11	14.6	431.56	16.9	9.35E+02	P02662|CASA1_BOVI	
YPFPGPIPNSLPQ	27.1	1425.7	13	-0.4	713.87	34.6	6.85E+03	P02666|CASB_BOVIN	
VKDAAGGPGAPADPGRPT	27.1	1632.8	18	3	545.28	12.3	3.78E+03	P81265|PIGR_BOVIN:P81265-	
GTWYSL	27	725.34	6	-7.1	726.34	29.2	3.42E+03	P02754|LACB_BOVIN	
LTEEEKNRLNFLK	27	1632.9	13	-0.1	409.23	22	4.85E+03	P02663|CASA2_BOVI	
EDHIAEGSVAVR	27	1281.6	12	-0.8	428.22	14.4	2.91E+03	P18892|BT1A1_BOVIN	
GYSGSSKALVSTLVPLA	27	1648.9	17	4.4	825.47	36.2	4.01E+03	P81265|PIGR_BOVIN:P81265-	
IEKFQS(+79.97)EEQQQ	27	1472.6	11	-4.1	737.32	12.8	3.14E+03	P02666|CASB_BOVIN	Phosphorylation (STY)
LPQNIPPLTQTPV	26.8	1416.8	13	5.4	709.41	31.2	3.75E+03	P02666|CASB_BOVIN	
KHQGLPQEVL	26.8	1147.6	10	2.4	383.55	20.7	7.86E+03	P02662|CASA1_BOVI	
ALPQY	26.7	590.31	5	-3.5	591.31	16.8	3.99E+03	P02663|CASA2_BOVI	
AIPQY	26.7	590.31	5	-3.5	591.31	16.8	3.99E+03		
TRPGA	26.6	500.27	5	13.8	501.29	24.9	0	32L59|TMC5B_BOVIN:P52505|ACPM_BOVIN:P56965|DDAH1_BOVIN:A7YWL5|INSY1_BOVIN:O02751|CFDP2_BOVIN:Q2KIS1|RENBP_BOVIN:Q3ZBA6|DJB11_BOVIN	
IVPNSAEERLH	26.6	1263.7	11	2.1	422.23	14.5	1.94E+03	P02662|CASA1_BOVI	
HCPVL	26.6	567.28	5	10.7	568.3	21.5	8.45E+03	Q3MHJ7|EEPD1_BOVIN:Q2T9W1|SNX20_BO	
ISKIF	26.6	606.37	5	0.9	304.2	23.6	5.56E+03	P62285|ASPM_BOVIN:Q0P5E6|SNTA1_BOVIN:P48818|ACADV_BOVIN:A6QPB3|COHA1_	

Peptide	-10lg	Mass	Length	ppm	m/z	RT	Area	Accession	PTM
LSKIF	26.6	606.37	5	0.9	304.2	23.6	5.56E+03	A4IF87|GNPAT_BOVIN:P02687|MBP_BOVI	
LSKLF	26.6	606.37	5	0.9	304.2	23.6	5.56E+03	P48617|EPO_BOVIN:Q56JZ5|EIF3H_BOVIN	
ISKLF	26.6	606.37	5	0.9	304.2	23.6	5.56E+03	A5D785|XPO2_BOVIN	
HKEMPFPKYPVEPF	26.6	1744.9	14	-0.3	582.63	30.9	7.97E+03	P02666|CASB_BOVIN	
TIASGEPTSTPT	26.6	1160.6	12	1	581.29	13.9	3.47E+03	P02668|CASK_BOVIN	
HPHPHLSF	26.6	970.48	8	4.2	324.5	15.9	1.06E+03	P02668|CASK_BOVIN	
VPQLEIVPNS(+79.97)AEERLH	26.6	1909.9	16	0.3	637.65	28.9	7.41E+03	P02662|CASA1_BOVIN	Phosphorylation (STY)
KAVPYPQRDMPIQAF	26.5	1759.9	15	-2	587.64	28.9	2.06E+03	P02666|CASB_BOVIN	
EELNVPGEIVE	26.5	1226.6	11	2.5	614.31	27.1	3.87E+03	P02666|CASB_BOVIN	
YKVPQLEIVPNSAEERLH	26.5	2121.1	18	-1	531.29	29.4	2.04E+03	P02662|CASA1_BOVI	
GRPSV	26.5	514.29	5	15	515.3	28.4	2.00E+04	P23709|NDUS3_BOVIN:A1A4M4|TATD3_BOVIN	
YQEPVLGPVR	26.5	1156.6	10	-8.4	579.32	22	3.28E+03	P02666|CASB_BOVIN	
ALVSTLVPLA	26.5	982.61	10	-4.1	492.31	36.8	1.48E+03	P81265|PIGR_BOVIN:P81265-	
SSEESIISQETY	26.5	1371.6	12	3.5	686.81	22.9	1.21E+03	P02663|CASA2_BOVI	
PQEVL	26.4	584.32	5	-4.3	585.32	25.6	3.79E+03	P02662|CASA1_BOVIN:Q9TTK4|LYST_BOV	
PQEVI	26.4	584.32	5	-4.3	585.32	25.6	3.79E+03		
IQKEDVPSERY	26.4	1362.7	11	-3.5	455.23	14.3	2.26E+03	P02662|CASA1_BOVI	
RDM(+15.99)PIQAFL	26.4	1105.6	9	-0.1	553.79	29.8	2.60E+03	P02666|CASB_BOVIN	Oxidation (M)
PHQKK	26.4	636.37	5	-1.7	637.38	46.8	5.71E+03	Q8SQB8|ITB6_BOVIN	
EPGNLAG	26.3	656.31	7	-2.8	657.32	16.3	4.75E+03	Q3MHY6|NUBP2_BOV	

m/z = mass to charge ratio; RT = retention time; PTM = post-translational modification

Table A1.2. Peptide sequences identified in delactosed permeate from production plant 1, batch B (Chapter IV)

Peptide	-10lgP	Mass	Length	ppm	m/z	RT	Area	Accession	PTM
YQEPVLGPVRGPFPIIV	73.52	1880.06	17	0	941.0383	44.02	5.20E+05	P02666|CASB_BOVIN	
PVLGPVRGPFPIIV	63.16	1459.89	14	1.1	730.9562	42.95	9.86E+03	P02666|CASB_BOVIN	
QEPVLGPVRGPFPIIV	62.09	1716.99	16	1.6	859.5076	42.79	9.25E+04	P02666|CASB_BOVIN	
YQEPVLGPVRGPFPII	60.23	1780.99	16	-7	891.4976	42.33	6.63E+04	P02666|CASB_BOVIN	
HQGLPQEVLNENLLR	58.56	1758.94	15	3.4	587.3237	30.82	2.18E+04	P02662|CASA1_BOVIN	
KVLPVPQ	57.72	779.491	7	2.3	390.7547	17.57	7.57E+03	P02666|CASB_BOVIN	
LLYQEPVLGPVRGPFPIIV	56.61	2106.22	19	-3.4	1054.1191	46.31	2.71E+04	P02666|CASB_BOVIN	
GLPQEVLNENLLR	54.97	1493.82	13	4.3	747.923	33.63	7.51E+04	P02662|CASA1_BOVIN	
YQEPVLGPVRGPFPI	54.66	1667.9	15	0.3	834.962	38.84	4.68E+04	P02666|CASB_BOVIN	
FVAPFPEVFGKEK	54.22	1493.79	13	4.2	498.9416	33.95	7.99E+04	P02662|CASA1_BOVIN	
LPQEVLNENLLRF	53.32	1583.87	13	7	528.9683	39.02	1.27E+04	P02662|CASA1_BOVIN	
LYQEPVLGPVRGPFPIIV	53.26	1993.14	18	-2.5	997.578	45.28	2.70E+03	P02666|CASB_BOVIN	
VLPVPQKAVPYPQ	52.76	1434.82	13	2.5	718.4231	25.47	2.35E+04	P02666|CASB_BOVIN	
DAAGGPGAPADPGRPT	52.75	1405.66	16	0.8	703.8394	12.48	1.64E+04	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
AAGGPGAPADPGRPT	52.42	1290.63	15	1.1	646.3259	11.99	6.45E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
VSREGQEQEGEEMAEYR	52.38	2025.87	17	8.7	676.3052	15.43	1.80E+03	P18892|BT1A1_BOVIN	
ILNKPEDETHLEAQPTDASAQFIR	52.2	2722.36	24	9.2	681.6048	24.96	3.73E+04	P80195|GLCM1_BOVIN	
VAPFPEVFGK	52.06	1089.59	10	-1.1	545.8013	31.32	2.06E+05	P02662|CASA1_BOVIN	
AQPTDASAQFIR	51.37	1303.65	12	4.1	435.5611	19.49	3.33E+04	P80195|GLCM1_BOVIN	
AQPTDASAQF	51.33	1034.47	10	-0.5	1035.4769	16.03	1.48E+05	P80195|GLCM1_BOVIN	
QEPVLGPVRGPFPII	50.81	1617.92	15	2.5	809.974	41.26	1.41E+04	P02666|CASB_BOVIN	
SLVYPFPGPIPN	50.68	1299.69	12	-4.1	650.8499	36.71	7.97E+03	P02666|CASB_BOVIN	
SQSKVLPVPQK	50.61	1209.71	11	2.7	404.2457	15.11	7.91E+03	P02666|CASB_BOVIN	
EPVLGPVRGPFPII	50.59	1489.87	14	2.1	745.9441	41.55	2.88E+04	P02666|CASB_BOVIN	
FVAPFPEVFGK	50.15	1236.65	11	0.5	619.3367	37.3	1.40E+05	P02662|CASA1_BOVIN	
EPVLGPVRGPFPIIV	50.14	1588.93	15	-0.1	795.4768	43	1.25E+05	P02666|CASB_BOVIN	
APFPEVFGK	49.75	990.517	9	0.8	496.2679	28.45	8.71E+04	P02662|CASA1_BOVIN	
SQNPKLPLSIL	49.72	1208.71	11	1.5	605.3666	35.59	7.94E+04	P80195|GLCM1_BOVIN	
SSRQPQSQNPKLPL	48.49	1578.85	14	6.5	527.295	20.27	8.16E+04	P80195|GLCM1_BOVIN	
VIESPPEIN	48.46	996.513	9	-5.7	997.5175	19.99	5.53E+04	P02668|CASK_BOVIN	
ILNKPEDETHL	48.42	1307.67	11	4	436.9011	17.2	1.83E+05	P80195|GLCM1_BOVIN	
VIESPPEINTVQ	47.82	1324.69	12	-1.5	663.3521	23.1	1.22E+04	P02668|CASK_BOVIN	
SSEESITRIN	47.74	1134.55	10	0.3	568.2851	14.91	3.71E+03	P02666|CASB_BOVIN	
SQNPKLPLS	47.73	982.545	9	-7.2	492.2777	19.3	3.70E+03	P80195|GLCM1_BOVIN	
PPPPPPPPP	47.4	891.485	9	-5.9	446.7488	15.6	4.28E+04	A2VDK6|WASF2_BOVI	
HQGLPQEVLNENLLRF	47.34	1906.01	16	-9.6	636.3386	38.46	6.93E+03	P02662|CASA1_BOVIN	
DLISKEQIVIR	46.99	1312.77	11	2.3	438.6002	25.03	1.55E+04	P80195|GLCM1_BOVIN	
GLPQEVLNENLLRF	46.7	1640.89	14	-3.1	547.9702	41.4	5.73E+04	P02662|CASA1_BOVIN	
APFPEVFG	46.69	862.423	8	3.7	863.4357	33.02	1.79E+04	P02662|CASA1_BOVIN	
YQEPVLGPVRGPFP	46.52	1554.82	14	2.4	778.4213	33.36	5.50E+04	P02666|CASB_BOVIN	
EAQPTDASAQF	46.46	1163.51	11	-1	1164.5193	16.98	5.36E+04	P80195|GLCM1_BOVIN	
DASAQFIRNL	46.22	1133.58	10	-5.4	567.7975	28.3	1.53E+04	P80195|GLCM1_BOVIN	
SHAFEVVKT	45.59	1016.53	9	-3.3	509.2718	15.74	2.84E+04	P80195|GLCM1_BOVIN	
EVLNENLLRF	45.55	1245.67	10	-3.8	623.8428	35.12	2.58E+04	P02662|CASA1_BOVIN	
TVQVTSTAV	45.53	904.487	9	-3.6	905.4935	16.75	1.70E+05	P02668|CASK_BOVIN	
SSSEESITRIN	45.43	1221.58	11	0	611.8011	15.06	1.06E+04	P02666|CASB_BOVIN	
ILNKPEDETHLE	45.26	1436.71	12	2.8	479.9151	16.23	4.60E+05	P80195|GLCM1_BOVIN	
EPVLGPVRGPFP	45.23	1263.7	12	6.5	632.8622	31.82	1.69E+04	P02666|CASB_BOVIN	
LPVPQKAVPYPQ	45.1	1335.76	12	-7.4	668.882	23.44	1.70E+04	P02666|CASB_BOVIN	
VPQLEIVPN	45.04	1007.57	9	-0.2	1008.5755	27.96	3.74E+04	P02662|CASA1_BOVIN	
FVAPFPEVFG	44.98	1108.56	10	-4.7	1109.5649	41.65	9.84E+03	P02662|CASA1_BOVIN	
FVAPFPEVFGKE	44.86	1365.7	12	-8	683.8524	37.6	6.72E+03	P02662|CASA1_BOVIN	
FPEVFGK	44.65	822.428	7	3.3	412.2238	24.62	1.48E+04	P02662|CASA1_BOVIN	
VAPFPEVFG	44.55	961.491	9	-2.8	962.4986	35.6	5.69E+04	P02662|CASA1_BOVIN	
VLGPVRGPFPIIV	44.55	1362.84	13	-2.9	682.4268	41.55	1.87E+04	P02666|CASB_BOVIN	
NENLLRFF	44.54	1051.55	8	-2.4	526.7802	37.91	2.17E+04	P02662|CASA1_BOVIN	
VPPFLQPEVM(+15.99)	44.27	1171.59	10	5.5	586.8098	30.35	8.69E+04	P02666|CASB_BOVIN	Oxidation (M)
NVPGEIVE	44.22	855.434	8	2.7	856.4461	18.59	3.77E+04	P02666|CASB_BOVIN	
SQNPKLPLSILK	44.22	1336.81	12	9.6	446.6156	29.16	2.25E+03	P80195|GLCM1_BOVIN	
VDMESTEVFTK	44.14	1284.59	11	1	643.3053	22.26	8.24E+03	P02663|CASA2_BOVIN	
DAQSAPLRVY	44.05	1118.57	10	-4.9	560.2924	20.39	6.29E+03	P02754|LACB_BOVIN	
VQVTSTAV	44	803.439	8	-2.2	804.447	15.3	8.31E+03	P02668|CASK_BOVIN	
AQPTDASAQFIRNL	43.99	1530.78	14	3.3	511.2703	30.74	1.11E+04	P80195|GLCM1_BOVIN	
SQNPKLPL	43.77	895.513	8	3.9	448.7668	21.71	5.28E+04	P80195|GLCM1_BOVIN	
SRQPQSQNPKLPL	43.65	1491.82	13	-1.5	498.28	20.1	7.24E+03	P80195|GLCM1_BOVIN	
TVDM(+15.99)ESTEVFTK	43.6	1401.63	12	3.9	701.8289	18.03	1.07E+04	P02663|CASA2_BOVIN	Oxidation (M)

Peptide	-10lgP	Mass	Length	ppm	m/z	RT	Area	Accession	PTM
AAGGPGAPADPGRPTGYS	43.44	1597.75	18	0.2	799.8842	14.74	1.10E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
SVLSLSQS	43.39	819.434	8	-2.8	820.4414	20.55	3.49E+03	P02666|CASB_BOVIN	
FVAPFPEVF	43.39	1051.54	9	3.7	1052.5524	42.3	2.45E+04	P02662|CASA1_BOVIN	
LIVTQTMKGL	43.35	1102.64	10	-2.4	552.3287	27.56	3.59E+03	P02754|LACB_BOVIN	
SRNPDEEGLFTVR	43.19	1518.74	13	3.2	507.2581	22.87	2.80E+03	P18892|BT1A1_BOVIN	
NIPPLTQTPV	42.94	1078.6	10	-3.9	1079.6088	26.82	5.31E+04	P02666|CASB_BOVIN	
APPPPPPPP	42.89	865.47	9	3.3	433.745	15	6.76E+03	A2VDK6|WASF2_BOVIN:Q32LP2|RADI_BOVI	
KHQGLPQEVLNENLLRF	42.67	2034.1	17	4	509.5362	36.01	2.11E+03	P02662|CASA1_BOVIN	
PVEPFTESQSL	42.66	1232.59	11	-4.5	617.3027	26.65	6.91E+03	P02666|CASB_BOVIN	
HQGLPQEVLNENLL	42.66	1602.84	14	-3.1	802.4257	33.75	1.30E+04	P02662|CASA1_BOVIN	
APFPEVFGKEK	42.37	1247.65	11	4.3	416.8954	24.99	2.60E+04	P02662|CASA1_BOVIN	
VYPFPGPIPN	42.36	1099.57	10	-2.2	550.793	31.91	5.75E+03	P02666|CASB_BOVIN	
YKVPQLEIVPN	42.33	1298.72	11	-2.1	650.3697	30.4	1.94E+04	P02662|CASA1_BOVIN	
NAVPITPTL	42.26	924.528	9	-6	925.5328	27.85	4.59E+03	P02663|CASA2_BOVIN	
YQGPIVLNPWDQVKR	42.22	1811.97	15	-0.6	604.9983	31.9	5.20E+03	P02663|CASA2_BOVIN	
VAPFPEVF	42.12	904.469	8	-3.9	905.4761	36.48	6.96E+04	P02662|CASA1_BOVIN	
LPQEVLNENLL	41.89	1280.7	11	0.7	641.3586	33.75	2.32E+04	P02662|CASA1_BOVIN	
GLPQEVLNENLL	41.53	1337.72	12	1.7	669.8701	36.74	1.02E+05	P02662|CASA1_BOVIN	
SQSKVLPVPQ	41.48	1081.61	10	1.2	541.8163	19.05	4.33E+04	P02666|CASB_BOVIN	
ILNKPEDETHLEAQPTDASAQFIRNL	41.44	2949.48	26	3.9	738.3833	32.31	2.62E+04	P80195|GLCM1_BOVIN	
SKVLPVPQ	41.43	866.523	8	2.6	434.2711	18.57	2.03E+04	P02666|CASB_BOVIN	
YQEPVLGPVR	41.43	1156.62	10	-6.6	579.3173	21.94	3.53E+03	P02666|CASB_BOVIN	
VVPPFLQPEVM(+15.99)	41.38	1270.66	11	3.4	636.343	33.33	5.00E+04	P02666|CASB_BOVIN	Oxidation (M)
NLHLPLPLLQ	41.36	1156.7	10	-0.8	579.3571	42.02	1.42E+04	P02666|CASB_BOVIN	
VAPFPEVFGKEK	41.3	1346.72	12	3.1	449.9179	27.8	7.01E+04	P02662|CASA1_BOVIN	
MPFPKYPVEPF	41.07	1350.67	11	0.8	676.3441	35.78	4.21E+03	P02666|CASB_BOVIN	
RELEELNVPGEIVE	40.78	1624.83	14	-8.9	542.6144	29.42	3.92E+03	P02666|CASB_BOVIN	
VPQLEIVPNSAEERLH	40.75	1829.96	16	1.9	610.9982	27.88	2.57E+03	P02662|CASA1_BOVIN	
LPYPYYAKPA	40.73	1181.61	10	-2	591.814	22.91	1.28E+04	P02668|CASK_BOVIN	
ENTVKETIKY	40.59	1223.64	10	-1.7	408.8878	15.6	5.64E+03	P80195|GLCM1_BOVIN	
APFPEVFGKE	40.58	1119.56	10	-4.1	560.7868	28.91	5.59E+03	P02662|CASA1_BOVIN	
HQGLPQEVL	40.15	1019.54	9	1.8	510.7798	23.11	2.29E+04	P02662|CASA1_BOVIN	
NAVPITPT	40.12	811.444	8	-0.9	812.4532	17.35	3.67E+04	P02663|CASA2_BOVIN	
VVPPFLQPEVM	40.08	1254.67	11	4.4	628.3462	38.4	1.24E+04	P02666|CASB_BOVIN	
AFEVVKT	40.03	792.438	7	-1.3	397.2271	17.63	6.34E+03	P80195|GLCM1_BOVIN	
DMPIQAF	39.61	820.379	7	3.8	821.392	29.09	8.91E+03	P02666|CASB_BOVIN	
FPKYPVEPF	39.55	1122.58	9	5.3	562.2995	31.32	2.86E+03	P02666|CASB_BOVIN	
DASAQFIR	39.52	906.456	8	3.1	454.2381	16.32	2.59E+04	P80195|GLCM1_BOVIN	
GPIVLNPWDQVK	39.47	1364.75	12	-16.4	683.3709	32.34	1.27E+03	P02663|CASA2_BOVIN	
VDM(+15.99)ESTEVFTK	39.45	1300.59	11	2.9	651.3041	16.67	1.17E+04	P02663|CASA2_BOVIN	Oxidation (M)
DM(+15.99)PIQAFLL	39.4	1062.54	9	1.3	1063.5541	38.82	4.23E+03	P02666|CASB_BOVIN	Oxidation (M)
ESRNPDEEGLFTVR	39.39	1647.79	14	6.8	550.2745	23.27	6.74E+03	P18892|BT1A1_BOVIN	
GLPQEVLNE	39.26	997.508	9	1.6	499.7637	24.34	1.85E+04	P02662|CASA1_BOVIN	
SLSQSKVLPVPQ	39.25	1281.73	12	1.3	641.8748	23.28	3.57E+03	P02666|CASB_BOVIN	
QEPVLGPVRGPFPI	39.12	1504.84	14	-0.4	753.4294	37.47	7.83E+03	P02666|CASB_BOVIN	
PPPPPPPPPP	39.03	988.538	10	-3.6	495.2762	16.71	1.27E+04	A2VDK6|WASF2_BOVI	
VPPFLQPEVM	38.93	1155.6	10	-1.7	578.808	35.64	2.35E+04	P02666|CASB_BOVIN	
VPPFLQPEVM(+15.99)GV	38.93	1327.68	12	1.3	664.8526	34.36	9.53E+03	P02666|CASB_BOVIN	Oxidation (M)
TKVIPYVR	38.88	974.591	8	-6	325.8701	17.7	1.35E+03	P02663|CASA2_BOVIN	
APFPEVF	38.8	805.401	7	-2.7	806.4087	34.11	2.58E+04	P02662|CASA1_BOVIN	
ASAQFIRNL	38.79	1018.56	9	-2.3	510.2857	24.96	1.75E+03	P80195|GLCM1_BOVIN	
PPAPPPPPP	38.62	865.47	9	3.3	433.745	15	6.76E+03	A2VDK6|WASF2_BOVI	
TLTDVENL	38.5	903.455	8	-6.8	904.4589	23.98	7.29E+03	P02666|CASB_BOVIN	
GPFPIIV	38.47	741.443	7	-6.9	742.4471	37.8	9.08E+03	P02666|CASB_BOVIN	
SDIPNPIGSENSE	38.36	1357.6	13	-0.6	679.8089	22.07	5.29E+03	P02662|CASA1_BOVIN	
APFPEVFGKEKV	38.31	1346.72	12	2.5	449.9177	28.59	1.65E+04	P02662|CASA1_BOVIN	
HIQKEDVPSERYL	38.25	1612.82	13	8.6	538.6206	18.59	5.49E+04	P02662|CASA1_BOVIN	
GLPQEVLN	38.14	868.465	8	4.3	869.4792	22.35	3.99E+04	P02662|CASA1_BOVIN	
DM(+15.99)PIQAF	38.11	836.374	7	-1.2	837.3828	22.23	9.88E+03	P02666|CASB_BOVIN	Oxidation (M)
SPEVIESPPEINTVQVTSTAV	38.11	2196.12	21	-1.3	1099.0675	32.21	2.14E+03	P02668|CASK_BOVIN	
PPPPPPPP	38.06	794.433	8	-15.9	795.4299	14.3	2.95E+04	A2VDK6|WASF2_BOVIN:Q32LP2|RADI_BOVI	
SSRQPQSQNPKLPLSIL	38.03	1892.05	17	1.4	631.6928	33.33	2.62E+04	P80195|GLCM1_BOVIN	
SLSQSKVLPVPQK	37.74	1409.82	13	2.4	470.9514	19.46	2.81E+03	P02666|CASB_BOVIN	
HQGLPQEVLN	37.68	1133.58	10	2.4	567.8019	20.28	2.58E+04	P02662|CASA1_BOVIN	
KVPQLEIVPN	37.64	1135.66	10	-2.7	568.8376	26.23	2.43E+04	P02662|CASA1_BOVIN	
FVAPFPEVFGKEKV	37.64	1592.86	14	6.9	531.9661	36.2	1.11E+04	P02662|CASA1_BOVIN	

Peptide	-10lgP	Mass	Length	ppm	m/z	RT	Area	Accession	PTM
PGRPTGYSGSSKAL	37.59	1376.7	14	-0.7	459.91	13.56	4.15E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
DTIAQAASTTTISDAVSK	37.59	1778.89	18	-6.6	890.4492	24.53	5.92E+03	P80025|PERL_BOVIN	
GLPQEVLNEN	37.35	1111.55	10	2.3	556.7858	23.32	9.02E+03	P02662|CASA1_BOVIN	
LVYPFPGPIPN	37.26	1212.65	11	2.5	607.3379	35.75	3.66E+03	P02666|CASB_BOVIN	
TQTPVVVPPFLQPEVM(+15.99)	37.1	1796.94	16	-4.7	899.475	39.83	2.61E+04	P02666|CASB_BOVIN	Oxidation (M)
GLPQEVL	36.93	754.423	7	-2.3	755.4304	25.54	2.12E+05	P02662|CASA1_BOVIN	
AVPYPQRDMPIQAF	36.89	1631.81	14	-5.2	816.9121	31.65	1.34E+04	P02666|CASB_BOVIN	
QEPVLGPVRGPFP	36.87	1391.76	13	4.9	696.891	31.83	5.45E+03	P02666|CASB_BOVIN	
NENLLRF	36.86	904.477	7	1.9	453.2479	27.45	2.03E+05	P02662|CASA1_BOVIN	
YQEPVL	36.7	747.38	6	-6.2	748.3853	21.52	1.44E+04	P02666|CASB_BOVIN	
RELEELNVPG	36.68	1154.59	10	-6.3	578.3021	22.16	3.20E+03	P02666|CASB_BOVIN	
S(+79.97)PEVIESPPEINTVQVTSTA'	36.68	2276.08	21	-1	759.7031	32.29	1.20E+04	P02668|CASK_BOVIN	Phosphorylation (STY)
NVPGEIVESL	36.66	1055.55	10	-2.8	528.7824	31.04	1.21E+05	P02666|CASB_BOVIN	
GLDIQKVA	36.64	842.486	8	1	422.2521	20.17	2.25E+03	P02754|LACB_BOVIN	
KEDVPSERYL	36.57	1234.62	10	0.4	412.5485	18.36	6.82E+03	P02662|CASA1_BOVIN	
MAIPPKKNQ	36.56	1025.57	9	-1.3	342.8643	9.36	3.17E+03	P02668|CASK_BOVIN	
EVLNENLLR	36.56	1098.6	9	-3.3	550.3089	24.35	3.57E+04	P02662|CASA1_BOVIN	
MAIPPKKN	36.38	897.511	8	8.9	300.1811	11.36	2.28E+04	P02668|CASK_BOVIN	
ILNKPEDETHLEAQPTDASAQF	36.38	2453.17	22	4.8	818.7375	23.86	1.34E+05	P80195|GLCM1_BOVIN	
STLVPLA	36.25	699.417	7	-6.6	700.4216	26.29	8.00E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
FVAPFPE	36.25	805.401	7	-0.6	806.4104	29.01	2.04E+04	P02662|CASA1_BOVIN	
EPVLGPVR	36.24	865.502	8	-0.5	433.7595	17.85	6.30E+03	P02666|CASB_BOVIN	
PVEPFTESQ	36.22	1032.48	9	5.3	517.2498	18.86	8.41E+03	P02666|CASB_BOVIN	
GYLEQLLR	36.14	990.55	8	0.3	496.2839	31.52	2.20E+03	P02662|CASA1_BOVIN	
DMPIQAFLL	36.05	1046.55	9	3.3	524.2842	44.81	3.12E+03	P02666|CASB_BOVIN	
MHQPHQPLPPT	35.75	1281.63	11	2.4	428.2193	14.51	3.02E+03	P02666|CASB_BOVIN	
TVDMESTEVFTK	35.75	1385.64	12	3.3	693.8311	23.22	6.11E+03	P02663|CASA2_BOVIN	
DMPIQA	35.37	673.311	6	3.4	674.3223	17.85	4.79E+03	P02666|CASB_BOVIN	
QKFPQYLQY	35.24	1213.61	9	-2	607.8146	26.5	1.64E+03	P02663|CASA2_BOVIN	
YPFPGPIPN	35.18	1000.5	9	-14.6	1001.4977	29.58	5.10E+03	P02666|CASB_BOVIN	
LPQEVLN	35.17	811.444	7	-0.6	812.4534	18.81	3.00E+03	P02662|CASA1_BOVIN	
LYQGPIVLNPWDQVKR	35.01	1925.05	16	5.3	642.6968	33.99	1.02E+04	P02663|CASA2_BOVIN	
AVPITPT	34.93	697.401	7	-2.1	698.4091	15.96	4.50E+03	P02663|CASA2_BOVIN	
GPVRGPFPII	34.92	1051.62	10	1.8	526.8188	34.45	2.55E+04	P02666|CASB_BOVIN	
GPVRGPFP	34.8	825.45	8	-0.5	413.7332	20.57	1.90E+04	P02666|CASB_BOVIN	
RDMPIQAF	34.7	976.48	8	1.6	489.2496	24.93	8.75E+03	P02666|CASB_BOVIN	
LPQEVLNENLLR	34.62	1436.8	12	4.6	719.4123	30.54	4.86E+03	P02662|CASA1_BOVIN	
VLNENLL	34.59	813.46	7	0.8	814.4702	26.17	5.98E+03	P02662|CASA1_BOVIN	
LNKPEDETHLE	34.55	1323.63	11	2.4	442.22	13.89	4.61E+04	P80195|GLCM1_BOVIN	
LPQYL	34.46	632.353	5	-4.3	633.3599	24.31	4.03E+03	P02663|CASA2_BOVIN	
IPQYI	34.46	632.353	5	-4.3	633.3599	24.31	4.03E+03		
APFPEV	34.42	658.333	6	-3.9	659.3394	24.8	9.44E+03	P02662|CASA1_BOVIN	
VIESPPEINTV	34.42	1196.63	11	-10	599.3176	25.18	7.70E+02	P02668|CASK_BOVIN	
EM(+15.99)PFPKYPVEPF	34.33	1495.71	12	-2.5	748.8607	32.78	9.39E+03	P02666|CASB_BOVIN	Oxidation (M)
AVPYPQ	34.19	673.344	6	-4.5	674.3499	14.41	3.54E+03	P02666|CASB_BOVIN	
SRYPSYGLN	34.13	1055.5	9	4.1	528.7629	17.6	2.82E+03	P02668|CASK_BOVIN	
YKVPQLE	33.98	875.475	7	-4.3	438.7444	20.96	0	P02662|CASA1_BOVIN	
TVDM(+15.99)EST(+79.97)EVFTK	33.89	1481.6	12	-6	741.8051	17.49	4.12E+03	P02663|CASA2_BOVIN	Oxidation (M); Phosphorylation (STY)
ALLDPSF	33.78	761.396	7	-8.2	762.3994	30.19	4.08E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
VAGTWYSL	33.78	895.444	8	1.7	896.4556	30.56	7.01E+03	P02754|LACB_BOVIN	
M(+15.99)ES(+79.97)TEVFTK	33.77	1166.46	9	2.7	584.2391	14.74	1.14E+03	P02663|CASA2_BOVIN	Oxidation (M); Phosphorylation (STY)
VAPFPE	33.69	658.333	6	-1.3	659.3411	19.94	2.72E+04	P02662|CASA1_BOVIN	
IHPFAQTQSL	33.68	1140.59	10	-1.1	571.3049	22.89	4.81E+03	P02666|CASB_BOVIN	
IVTQTM(+15.99)KGLDIQ	33.61	1361.72	12	15.7	681.8814	19.3	1.31E+03	P02754|LACB_BOVIN	Oxidation (M)
NAVPITPTLN	33.59	1038.57	10	2.1	520.2955	24.17	1.85E+03	P02663|CASA2_BOVIN	
VLNENLLR	33.46	969.561	8	2.4	485.7904	21.89	2.82E+03	P02662|CASA1_BOVIN	
GLPQEVLNENL	33.41	1224.64	11	3	613.3286	31.13	1.75E+04	P02662|CASA1_BOVIN	
FSHAFEVVKT	33.38	1163.6	10	8.2	388.8775	21.05	4.66E+03	P80195|GLCM1_BOVIN	
YLEQLLRL	33.36	1046.61	8	7.7	524.3192	37.33	3.98E+03	P02662|CASA1_BOVIN	
YKVPQLEIVPNS(+79.97)AEERLH	33.35	2201.09	18	-1.3	551.2804	30.19	3.07E+03	P02662|CASA1_BOVIN	Phosphorylation (STY)
VLPVPQ	33.2	651.396	6	-3.3	652.4027	18.97	1.69E+04	P02666|CASB_BOVIN	

Peptide	-10lgP	Mass	Length	ppm	m/z	RT	Area	Accession	PTM
GTWYSL	32.97	725.338	6	-3	726.3458	29.06	4.56E+03	P02754|LACB_BOVIN	
GYLEQL	32.77	721.365	6	-1.9	722.3729	24.58	8.25E+03	P02662|CASA1_BOVIN	
IPIQYVL	32.71	844.506	7	-2.6	423.2604	34.01	3.04E+03	P02668|CASK_BOVIN	
VAPFPEVFGKE	32.66	1218.63	11	8.5	610.3287	31.69	1.44E+04	P02662|CASA1_BOVIN	
RELEELNVPGEIVESL	32.65	1824.95	16	-1.7	913.4821	39.77	1.39E+04	P02666|CASB_BOVIN	
VAPFPEV	32.57	757.401	7	-4.9	758.407	27.69	3.73E+03	P02662|CASA1_BOVIN	
N(+.98)AVPITPT	32.57	812.428	8	-4	813.4346	18.5	7.45E+03	P02663|CASA2_BOVIN	Deamidation (NQ)
GPFPII	32.56	642.374	6	-2.5	643.3818	34.84	1.80E+03	P02666|CASB_BOVIN	
TIASGEPTSTPT	32.48	1160.56	12	1.2	581.2878	13.96	1.13E+03	P02668|CASK_BOVIN	
TDVENLHLPLPLLQ	32.46	1600.88	14	-6.9	801.4456	42.95	1.74E+03	P02666|CASB_BOVIN	
EELNVPGEIVESL	32.36	1426.72	13	-0.1	714.3691	38.6	7.27E+03	P02666|CASB_BOVIN	
HIQKEDVPSERY	32.33	1499.74	12	5.4	500.9239	13.36	4.12E+03	P02662|CASA1_BOVIN	
GPVRGPFPIIV	32.2	1150.69	11	-3.3	576.3503	37.06	8.03E+04	P02666|CASB_BOVIN	
AGEIQNKALLD	32.08	1170.62	11	2.9	586.3231	18.44	5.08E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
TTLSSEAPTTQ	32	1134.54	11	-5.7	568.2761	13.81	0	P80025|PERL_BOVIN	
IHPFAQTQ	31.93	940.477	8	4.6	471.2493	14.97	2.10E+03	P02666|CASB_BOVIN	
AMKPWIQPK	31.87	1097.61	9	4.9	366.8788	19.18	2.78E+03	P02663|CASA2_BOVIN	
FPEVF	31.82	637.311	5	-4.3	638.3177	30.51	1.19E+04	P02662|CASA1_BOVIN	
PPPPVI	31.82	618.374	6	14	619.392	28.96	3.19E+04	Q32LP2|RADI_BOVIN	
PPPPVL	31.82	618.374	6	14	619.392	28.96	3.19E+04		
IPIQY	31.73	632.353	5	0.6	633.363	20.97	7.05E+03	P02668|CASK_BOVIN	
LPLQY	31.73	632.353	5	0.6	633.363	20.97	7.05E+03		
IPLQY	31.73	632.353	5	0.6	633.363	20.97	7.05E+03		
AAGGPGAPADPGRPTGYSGS	31.66	1741.8	20	0.9	871.9118	14.51	1.58E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
SLTLTDVEN	31.64	990.487	9	0.1	496.2524	22.24	1.90E+03	P02666|CASB_BOVIN	
QKVLPLLK	31.55	937.632	8	-9.2	469.8207	40.31	2.77E+04	A1A4J7-2|SMG8_BOVIN:A1A4J7|SMG8_BOVIN	
LPQEVL	31.45	697.401	6	-0.1	698.4105	22.61	8.44E+03	P02662|CASA1_BOVIN	
IPQEVL	31.45	697.401	6	-0.1	698.4105	22.61	8.44E+03		
AVESTVATL	31.41	889.476	9	-2.3	890.4838	20.91	2.17E+03	P02668|CASK_BOVIN	
VDMEST(+79.97)EVFTK	31.4	1364.56	11	3.4	683.2903	22.64	3.75E+03	P02663|CASA2_BOVIN	Phosphorylation (STY)
TQTMKGLDIQ	31.39	1133.58	10	4.9	567.7994	20.29	1.23E+04	P02754|LACB_BOVIN	
IPPLTQTPV	31.26	964.559	9	-4.2	483.2865	26.11	1.57E+04	P02666|CASB_BOVIN	
EIVESL	31.25	688.364	6	-10	689.3669	21.2	0	P02666|CASB_BOVIN	
EVIESPPEINTVQVTSTAV	31.24	2012.03	19	4.6	1007.0308	31.21	1.66E+04	P02668|CASK_BOVIN	
VGPMP	31.2	499.247	5	10.5	500.2606	24.49	0	P22600|HEMH_BOVIN:Q3ZBM6|GPAM1_BOVI	
LIVTQTMK	31.19	932.537	8	-4.4	467.275	17.58	1.49E+03	P02754|LACB_BOVIN	
GPVRGPFPI	31.18	938.534	9	5.1	470.2781	28.62	4.09E+03	P02666|CASB_BOVIN	
PVEPF	31.17	587.296	5	-5.8	588.3012	20.21	3.84E+03	P02666|CASB_BOVIN	
FPEVFG	31.06	694.333	6	-3	695.34	28.84	0	P02662|CASA1_BOVIN	
SSS(+79.97)EESITRIN	31.04	1301.55	11	0.1	651.7845	15.89	6.67E+03	P02666|CASB_BOVIN	Phosphorylation (STY)
YLEQLL	30.99	777.427	6	-0.9	778.4363	30.5	1.42E+03	P02662|CASA1_BOVIN	
YLEQLI	30.99	777.427	6	-0.9	778.4363	30.5	1.42E+03		
SLPQNIPPLTQTPVVVPPFLQPEVM	30.94	2756.48	25	2	919.8395	45.6	1.90E+04	P02666|CASB_BOVIN	Oxidation (M)
VSTLVPLA	30.93	798.485	8	-6.2	799.49	28.85	1.46E+04	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
NAVPITPTLNRE	30.84	1323.71	12	0.2	662.8668	22.12	2.98E+03	P02663|CASA2_BOVIN	
DIQKVAGTWYSL	30.76	1379.71	12	4.7	690.867	32.76	3.14E+03	P02754|LACB_BOVIN	
VSREGQEQEGEEM(+15.99)AEYR	30.72	2041.86	17	3.5	681.6334	11.82	1.36E+03	P18892|BT1A1_BOVIN	Oxidation (M)
EVLNENL	30.64	829.418	7	-7.2	830.4221	20.74	1.39E+03	P02662|CASA1_BOVIN	
LNKPEDETHL	30.63	1194.59	10	-2.6	598.3017	14.52	2.18E+03	P80195|GLCM1_BOVIN	
GLPQEV	30.62	641.338	6	1	642.3484	17.77	3.15E+03	P02662|CASA1_BOVIN	
LPQYLK	30.62	760.448	6	-0.7	381.2324	18.21	1.91E+04	P02663|CASA2_BOVIN	
LEQLLRL	30.38	883.549	7	-4.3	442.7813	30.9	3.80E+03	P02662|CASA1_BOVIN	
NAVPITPTLNREQL	30.33	1564.86	14	-0.8	783.4378	27.67	3.45E+03	P02663|CASA2_BOVIN	

Peptide	-10lgP	Mass	Length	ppm	m/z	RT	Area	Accession	PTM
AAHGV	30.32	453.234	5	-4.5	454.2403	16.51	0	Q1RMS6|INT7_BOVIN:P17694|NDUS2_BOVIN	
LPLPLLQ	30.3	792.511	7	-18.9	793.5057	36.91	9.86E+02	P02666|CASB_BOVIN	
VAPFP	30.28	529.29	5	5.1	530.3016	21.02	0	P02662|CASA1_BOVIN	
PFPEVFGK	30.2	919.48	8	-4	460.747	31.25	2.60E+03	P02662|CASA1_BOVIN	
LPKLVHA	30.19	776.491	7	13.6	777.5112	45.83	6.69E+03	7|NRX1A_BOVIN:Q28146-6|NRX1A_BOVIN:Q28146-5|NRX1A_BOVIN:Q28146-4|NRX1A_BOVIN:Q28146-3|NRX1A_BOVIN:Q28146-2|NRX1A_BOVIN:Q28146-9|NRX1A_BOVIN:Q2814	
NDATAQAF	30.18	836.367	8	8.4	419.1954	22.24	6.33E+03	Q0VBZ0|CSK_BOVIN	
YQEPVLGPV	30.15	1000.52	9	15.4	501.278	26.67	0	P02666|CASB_BOVIN	
VAPFPEVFGKEKV	30.15	1445.79	13	5.6	482.9421	30.93	1.31E+04	P02662|CASA1_BOVIN	
TLVPLA	30.13	612.385	6	-10.3	613.3876	25.46	1.52E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
SPPEINTVQ	30.03	983.492	9	-4.1	492.7531	16.08	4.57E+03	P02668|CASK_BOVIN	
VPQLEIVPNS(+79.97)AEERLH	30.02	1909.93	16	1.7	637.6537	28.76	5.58E+03	P02662|CASA1_BOVIN	Phosphorylation (STY)
DKTEIPTIN	30.01	1029.53	9	7.8	515.78	19.52	9.77E+03	P02668|CASK_BOVIN	
ALPQY	29.95	590.306	5	-1	591.315	16.77	5.21E+03	P02663|CASA2_BOVIN	
AIPQY	29.95	590.306	5	-1	591.315	16.77	5.21E+03		
SLPQNIPPLTQTPV	29.91	1503.83	14	-5.3	752.9206	31.99	6.56E+03	P02666|CASB_BOVIN	
AAGGPGAPADPGRPTGY	29.9	1510.72	17	-1.6	756.3667	15.57	1.54E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
EIPTINTIA	29.84	970.534	9	0.6	971.5445	28.11	1.74E+03	P02668|CASK_BOVIN	
EDVPSERY	29.62	993.44	8	-5.7	497.7262	13.56	2.65E+03	P02662|CASA1_BOVIN	

m/z = mass to charge ratio; RT = retention time; PTM = post-translational modification

Table A1.3. Peptide sequences identified in delactosed permeate from production plant 1, batch C (Chapter IV)

Peptide	-10lgP	Mass	Length	ppm	m/z	RT	Area	Accession	PTM
QEPVLGPVRGPFPIIV	63.26	1717	16	5.2	859.51	42.92	2.92E+05	P02666|CASB_BOVIN	
YQEPVLGPVRGPFPIIV	61.77	1880.1	17	0.5	941.04	44.03	7.98E+05	P02666|CASB_BOVIN	
YQEPVLGPVRGPFPII	54.16	1781	16	-4.1	891.5	42.55	1.68E+05	P02666|CASB_BOVIN	
YQEPVLGPVRGPFPI	53.91	1667.9	15	0.6	834.96	38.92	8.37E+04	P02666|CASB_BOVIN	
VLPVPQKAVPYPQ	53.05	1434.8	13	1.8	718.42	25.49	4.89E+04	P02666|CASB_BOVIN	
PVLGPVRGPFPIIV	50.95	1459.9	14	-5.2	730.95	43.01	4.56E+03	P02666|CASB_BOVIN	
EPVLGPVRGPFPIIV	50.64	1588.9	15	2.2	795.48	43.21	2.34E+05	P02666|CASB_BOVIN	
YQEPVLGPVRGPFP	49.78	1554.8	14	1.3	778.42	33.43	9.23E+04	P02666|CASB_BOVIN	
AQPTDASAQFIRNL	49.25	1530.8	14	1.7	766.4	30.72	4.14E+04	P80195|GLCM1_BOVI	
KVLPVPQ	48.83	779.49	7	2.1	390.75	17.34	2.51E+04	P02666|CASB_BOVIN	
LLYQEPVLGPVRGPFPIIV	48.7	2106.2	19	-2.4	1054.1	46.38	2.98E+04	P02666|CASB_BOVIN	
ILNKPEDETHLEAQPTDASAQFIRNL	47.83	2949.5	26	2.3	738.38	32.36	6.39E+04	P80195|GLCM1_BOVI	
GLPQEVLNENLLR	47.7	1493.8	13	1.4	747.92	33.68	1.22E+05	P02662|CASA1_BOVI	
QEPVLGPVRGPFPII	47.26	1617.9	15	-2.9	809.97	41.45	4.72E+04	P02666|CASB_BOVIN	
ILNKPEDETHLEAQPTDASAQFIR	46.55	2722.4	24	8.1	681.6	24.91	5.56E+04	P80195|GLCM1_BOVI	
TQTPVVVPPFLQPEVM(+15.99)	46.18	1796.9	16	0.2	899.48	39.94	4.47E+04	P02666|CASB_BOVIN	Oxidation (M)
LPLPLLQSW	46.05	1065.6	9	2	533.82	45.64	1.16E+04	P02666|CASB_BOVIN	
DAAGGPGAPADPGRPT	45.66	1405.7	16	-0.4	703.84	12.31	3.39E+04	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
SLVYPFPGPIPN	45.53	1299.7	12	0.9	650.85	36.82	1.78E+04	P02666|CASB_BOVIN	
FVAPFPEVFGKEK	45.3	1493.8	13	4.1	498.94	34.05	1.25E+05	P02662|CASA1_BOVI	
LPQEVLNENLLR	45.21	1436.8	12	-3.7	719.41	30.52	1.39E+04	P02662|CASA1_BOVI	
DASAQFIRNL	45.15	1133.6	10	1	567.8	28.31	3.78E+04	P80195|GLCM1_BOVI	
APFPEVFGKEK	44.49	1247.7	11	6.7	416.9	24.92	8.75E+04	P02662|CASA1_BOVI	
GPIVLNPWDQVK	44.49	1364.7	12	2.3	683.38	32.42	4.55E+03	P02663|CASA2_BOVI	
VDM(+15.99)ESTEVFTK	44.28	1300.6	11	-2.7	651.3	16.61	1.36E+04	P02663|CASA2_BOVI	Oxidation (M)
GLPQEVLNENLLRF	44.09	1640.9	14	-4.6	821.45	41.58	6.39E+04	P02662|CASA1_BOVI	
AQPTDASAQFIR	44.07	1303.7	12	7.7	435.56	19.47	5.91E+04	P80195|GLCM1_BOVI	
HQGLPQEVLNENLLRF	44.02	1906	16	-2.9	636.34	38.55	7.53E+03	P02662|CASA1_BOVI	
ILNKPEDETHLE	43.8	1436.7	12	1.5	719.37	15.92	1.12E+06	P80195|GLCM1_BOVI	
DAQSAPLRVY	43.79	1118.6	10	-4.7	560.29	20.34	8.40E+03	P02754|LACB_BOVIN	
LNKPEDETHLE	43.77	1323.6	11	2	442.22	13.67	1.90E+05	P80195|GLCM1_BOVI	
AAGGPGAPADPGRPT	43.72	1290.6	15	1.6	646.33	11.8	1.71E+04	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
FVAPFPEVFGK	43.6	1236.7	11	-1.2	619.34	37.44	1.20E+05	P02662|CASA1_BOVI	
LPVPQKAVPYPQ	43.48	1335.8	12	-1.8	668.89	23.42	5.65E+04	P02666|CASB_BOVIN	
TVQVTSTAV	43.44	904.49	9	-1.5	905.5	16.67	9.48E+04	P02668|CASK_BOVIN	
VDMESTEVFTK	43.34	1284.6	11	0.2	643.3	22.25	1.37E+04	P02663|CASA2_BOVI	
QEPVLGPVRGPFPI	43.28	1504.8	14	-5.4	753.43	37.58	2.30E+04	P02666|CASB_BOVIN	
YQGPIVLNPWDQVKR	43.06	1812	15	7	605	31.96	2.87E+04	P02663|CASA2_BOVI	
APFPEVFG	42.98	862.42	8	5.2	863.44	33.12	3.35E+04	P02662|CASA1_BOVI	
KVLPVPQKAVPYPQ	42.97	1562.9	14	3.9	521.98	23.02	4.29E+04	P02666|CASB_BOVIN	
LPQEVLNENLLRF	42.95	1583.9	13	-5.8	792.94	39.18	3.09E+04	P02662|CASA1_BOVI	
EAQPTDASAQF	42.82	1163.5	11	1.2	582.76	16.9	1.34E+05	P80195|GLCM1_BOVI	
SQNPKLPLSIL	42.77	1208.7	11	1.2	605.37	35.66	1.76E+05	P80195|GLCM1_BOVI	
NAVPITPTLN	42.61	1038.6	10	-7.9	520.29	24.16	1.02E+04	P02663|CASA2_BOVI	
SSEESITRIN	42.6	1134.6	10	-0.3	568.28	14.71	6.56E+03	P02666|CASB_BOVIN	
VVPPFLQPEVM	42.56	1254.7	11	-2.7	628.34	38.44	1.40E+04	P02666|CASB_BOVIN	
AQPTDASAQF	42.46	1034.5	10	2.6	1035.5	15.86	3.54E+05	P80195|GLCM1_BOVI	
VAPFPEVFGKEK	42.12	1346.7	12	8.8	449.92	27.76	1.53E+05	P02662|CASA1_BOVI	
SSSEESITRIN	42.09	1221.6	11	-2.8	611.8	14.97	2.15E+04	P02666|CASB_BOVIN	
TPVVVPPFLQPEVM	42.08	1551.8	14	-0.5	776.93	43.48	3.20E+03	P02666|CASB_BOVIN	
VDM(+15.99)ES(+79.97)TEVFTK	41.97	1380.6	11	6	691.29	16.4	2.84E+04	P02663|CASA2_BOVIN	Oxidation (M); Phosphorylation
SHAFEVVKT	41.89	1016.5	9	6.1	339.85	15.55	4.45E+04	P80195|GLCM1_BOVI	
VAPFPEVFGK	41.81	1089.6	10	5.4	545.8	31.31	2.65E+05	P02662|CASA1_BOVI	
APFPEVFGK	41.78	990.52	9	0.4	496.27	28.44	1.93E+05	P02662|CASA1_BOVI	
TPVVVPPFLQPEVM(+15.99)	41.77	1567.8	14	0.1	784.93	39.78	6.96E+03	P02666|CASB_BOVIN	Oxidation (M)
VPPFLQPEVM(+15.99)	41.67	1171.6	10	3.6	586.81	30.36	1.42E+05	P02666|CASB_BOVIN	Oxidation (M)
DVENLHLPL	41.41	1048.6	9	2.1	525.29	33.07	1.16E+03	P02666|CASB_BOVIN	
SRQPQSQNPKLPL	41.33	1491.8	13	3.8	498.28	20.04	2.48E+04	P80195|GLCM1_BOVI	

Peptide	-10lgP	Mass	Length	ppm	m/z	RT	Area	Accession	PTM
NIPPLTQTPV	41.28	1078.6	10	5.3	1079.6	26.95	7.47E+04	P02666|CASB_BOVIN	
APFPEVFGKE	41.19	1119.6	10	0.6	560.79	29	1.62E+04	P02662|CASA1_BOVI	
GLPQEVLNENLL	41.17	1337.7	12	4.6	669.87	36.74	1.42E+05	P02662|CASA1_BOVI	
DLISKEQIVIR	41.06	1312.8	11	-3	438.6	24.96	2.99E+04	P80195|GLCM1_BOVI	
VAPFPEVFG	40.9	961.49	9	-0.7	962.5	35.62	5.30E+04	P02662|CASA1_BOVI	
VDMES(+79.97)TEVFTK	40.74	1364.6	11	4.5	683.29	22.63	1.83E+04	P02663|CASA2_BOVI	Phosphorylation
AGEIQNKALLDPSF	40.74	1501.8	14	-4.8	751.9	29.4	3.70E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
SSRQPQSQNPKLPL	40.63	1578.8	14	-0.3	527.29	20.14	1.33E+05	P80195|GLCM1_BOVI	
SRNPDEEGLFTVR	40.6	1518.7	13	3.7	507.26	22.77	9.76E+03	P18892|BT1A1_BOVIN	
KHQGLPQEVLNENLLRF	40.49	2034.1	17	5.1	509.54	36.07	8.36E+03	P02662|CASA1_BOVI	
VIESPPEINTVQVTSTAV	40.48	1883	18	1.2	942.51	30.39	1.49E+04	P02668|CASK_BOVIN	
DETHLEAQPTDASAQFIR	40.34	2028	18	6.1	677	23.68	9.34E+03	P80195|GLCM1_BOVI	
ESRNPDEEGLFTVR	40.31	1647.8	14	8.7	550.28	23.16	1.62E+04	P18892|BT1A1_BOVIN	
VYPFPGPIPN	40.29	1099.6	10	4	550.8	31.91	2.03E+04	P02666|CASB_BOVIN	
DMPIQAF	40.25	820.38	7	0.8	821.39	29.09	3.31E+04	P02666|CASB_BOVIN	
SQNPKLPLS	40.17	982.54	9	-4.6	492.28	19.22	2.89E+04	P80195|GLCM1_BOVI	
RELEELNVPGEIVESL	40.07	1824.9	16	0.9	913.48	39.88	1.31E+04	P02666|CASB_BOVIN	
GLDIQKVAGTW	40.02	1186.6	11	-10	594.32	31.44	3.71E+03	P02754|LACB_BOVIN	
EPVLGPVRGPFP	40.02	1263.7	12	2.9	632.86	31.83	7.28E+04	P02666|CASB_BOVIN	
LVYPFPGPIPN	39.91	1212.7	11	0.8	1213.7	35.78	1.57E+04	P02666|CASB_BOVIN	
ILNKPEDETHL	39.78	1307.7	11	9.5	436.9	16.93	4.17E+05	P80195|GLCM1_BOVI	
VIESPPEIN	39.76	996.51	9	-5.5	997.52	19.94	7.74E+04	P02668|CASK_BOVIN	
EPVLGPVRGPFPII	39.65	1489.9	14	-5.2	745.94	41.61	6.95E+04	P02666|CASB_BOVIN	
HQGLPQEVLNENLLR	39.56	1758.9	15	-5.3	587.32	30.83	1.45E+04	P02662|CASA1_BOVI	
DHIAEGSVAVR	39.41	1152.6	11	4	385.21	13.44	3.24E+03	P18892|BT1A1_BOVIN	
SQSKVLPVPQK	39.22	1209.7	11	5.3	404.25	14.94	1.23E+04	P02666|CASB_BOVIN	
FVAPFPEVFGKEKV	39.04	1592.9	14	1	531.96	36.27	4.92E+04	P02662|CASA1_BOVI	
NENLLRFF	39.02	1051.5	8	-0.2	526.78	38.06	2.35E+04	P02662|CASA1_BOVI	
DPSFFAKE	38.87	939.43	8	-0.5	470.73	21.13	5.01E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
SLPQNIPPLTQTPVVVPPFLQPE	38.77	2510.4	23	-1.4	837.8	45.47	2.13E+04	P02666|CASB_BOVIN	
SQSKVLPVPQ	38.48	1081.6	10	0	541.82	19.02	6.93E+04	P02666|CASB_BOVIN	
PVEPFTESQSL	38.46	1232.6	11	-1.8	617.3	26.63	2.28E+04	P02666|CASB_BOVIN	
NVPGEIVESLSSSEESITRIN	38.46	2259.1	21	-8.4	754.04	38.48	5.13E+03	P02666|CASB_BOVIN	
LPQYLKT	38.43	861.5	7	-0.2	431.76	20.09	1.09E+04	P02663|CASA2_BOVI	
PPPPPPPPPP	38.37	988.54	10	-4.8	495.28	16.72	1.29E+04	A2VDK6|WASF2_BOV	
VLGPVRGPFPIIV	38.29	1362.8	13	3.4	682.43	41.73	3.12E+04	P02666|CASB_BOVIN	
ALLDPSFFAKESVK	38.29	1550.8	14	-3.2	517.95	33.09	6.10E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
SQNPKLPL	38.24	895.51	8	-0.9	448.76	21.71	1.05E+05	P80195|GLCM1_BOVI	
EELNVPGEIVESL	38.2	1426.7	13	-2.2	714.37	38.66	3.28E+04	P02666|CASB_BOVIN	
GPFPIIV	38.11	741.44	7	0.7	742.45	37.87	1.18E+04	P02666|CASB_BOVIN	
GPIVLNPWDQVKR	38.07	1520.8	13	4.6	507.96	29.62	4.45E+03	P02663|CASA2_BOVI	
VAPFPEVF	38.06	904.47	8	-2.6	905.48	36.59	9.53E+04	P02662|CASA1_BOVI	
NAVPITPTL	37.98	924.53	9	-1.4	925.54	27.82	8.65E+03	P02663|CASA2_BOVI	
RELEELNVPGEIVE	37.92	1624.8	14	-4.2	813.42	29.56	6.28E+04	P02666|CASB_BOVIN	
EVLNENLLRF	37.91	1245.7	10	1.1	623.85	35.19	2.05E+04	P02662|CASA1_BOVI	
PPPPPPPPP	37.89	891.49	9	-2.2	446.75	15.37	4.29E+04	A2VDK6|WASF2_BOVIN:A7XYH9|SOBP_BO	
DPSFFAKESVK	37.68	1253.6	11	3.8	418.89	20.98	1.60E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
FVAPFPE	37.67	805.4	7	-4	806.41	28.85	5.19E+04	P02662|CASA1_BOVI	
MAIPPKKNQ	37.54	1025.6	9	7.3	342.87	10.96	4.06E+04	P02668|CASK_BOVIN	
FVAPFPEVF	37.47	1051.5	9	-4	1052.5	42.47	1.35E+04	P02662|CASA1_BOVI	
KEDVPSERYL	37.45	1234.6	10	3.8	412.55	18.31	7.71E+04	P02662|CASA1_BOVI	
NAVPITPT	37.44	811.44	8	-1.5	812.45	17.19	7.94E+04	P02663|CASA2_BOVI	
LIVTQTMKGL	37.43	1102.6	10	3.1	552.33	27.58	4.49E+03	P02754|LACB_BOVIN	
DAAGGPGAPADPGRPTGYS	37.41	1712.8	19	-4.6	857.39	15.11	1.94E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
GLPQEVLN	37.37	868.47	8	2.2	435.24	22.31	2.56E+05	P02662|CASA1_BOVI	
DETHLEAQPTDASAQFIRNL	37.37	2255.1	20	2.2	752.71	32.78	1.20E+04	P80195|GLCM1_BOVI	
QPTDASAQFIR	37.31	1232.6	11	-1.5	617.32	18.14	6.34E+03	P80195|GLCM1_BOVI	

Peptide	-10lgP	Mass	Length	ppm	m/z	RT	Area	Accession	PTM
MAIPPKKN	37.29	897.51	8	5.6	300.18	11.06	1.00E+05	P02668|CASK_BOVIN	
GRVSLVEDHIAEGSVAVR	37.28	1893	18	10.6	474.27	22.95	1.08E+03	P18892|BT1A1_BOVIN	
ELNVPGEIVESL	37.26	1297.7	12	-7.3	649.84	38.27	5.49E+03	P02666|CASB_BOVIN	
VPPFLQPEVM	37.25	1155.6	10	-1.8	578.81	35.75	5.62E+04	P02666|CASB_BOVIN	
EAQPTDASAQFIRNL	37.25	1659.8	15	-1.7	830.92	31.16	1.85E+03	P80195|GLCM1_BOVI	
DETHLEAQPTDASAQF	37.18	1758.8	16	-4	880.39	21.96	1.60E+04	P80195|GLCM1_BOVI	
SDIPNPIGSENSE	37.11	1357.6	13	-1	679.81	22.06	1.82E+04	P02662|CASA1_BOVI	
NVPGEIVESL	37.1	1055.5	10	6.3	528.79	31	2.51E+05	P02666|CASB_BOVIN	
SDIPNPIGSE	37.05	1027.5	10	5.1	514.75	22.77	4.43E+03	P02662|CASA1_BOVI	
HQGLPQEVL	37.01	1019.5	9	0.8	510.78	23.07	6.04E+04	P02662|CASA1_BOVI	
NLHLPLPLL	36.96	1028.6	9	3.8	515.33	44.29	1.30E+04	P02666|CASB_BOVIN	
HLPLPLLQS	36.95	1016.6	9	-6.9	509.31	34.05	3.76E+03	P02666|CASB_BOVIN	
VSREGQEQEGEEM(+15.99)AEYR	36.87	2041.9	17	11.5	681.64	11.6	3.76E+03	P18892|BT1A1_BOVIN	Oxidation (M)
LPYPYYAKPA	36.85	1181.6	10	-1.7	591.81	22.89	3.26E+04	P02668|CASK_BOVIN	
VLGPVRGPFPI	36.84	1150.7	11	5.4	576.36	35.01	1.88E+03	P02666|CASB_BOVIN	
QEPVLGPVRGPFP	36.83	1391.8	13	8	696.89	31.81	1.88E+04	P02666|CASB_BOVIN	
IVTQTMKGL	36.81	989.56	9	4.6	495.79	20.46	1.05E+04	P02754|LACB_BOVIN	
SQNPKLPLSILKEK	36.81	1593.9	14	2.8	532.33	26.1	2.08E+04	P80195|GLCM1_BOVI	
AGEIQNKALLD	36.7	1170.6	11	0.9	586.32	18.38	1.17E+04	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
HIQKEDVPSERY	36.67	1499.7	12	-1.1	500.92	13.22	7.35E+03	P02662|CASA1_BOVI	
PPPPPPPP	36.6	794.43	8	-4.4	795.44	14.22	3.18E+04	A2VDK6|WASF2_BOVIN:A7XYH9|SOBP_BOVIN:Q32LP2|RADI_BO	
SDIPNPIGSENSEK	36.59	1485.7	14	-2.8	743.85	20.14	1.73E+04	P02662|CASA1_BOVI	
MPIQAF	36.57	705.35	6	-0.2	706.36	26.23	6.39E+03	P02666|CASB_BOVIN	
LDPSFFAKESVK	36.57	1366.7	12	8.4	456.58	25.83	8.66E+02	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
FPEVFGK	36.5	822.43	7	-1.8	412.22	24.58	3.45E+04	P02662|CASA1_BOVI	
GYLEQLLR	36.45	990.55	8	-0.6	496.28	31.5	4.17E+03	P02662|CASA1_BOVI	
PVEPFTESQ	36.38	1032.5	9	-1.8	517.25	18.78	8.56E+03	P02666|CASB_BOVIN	
VPYPQRDMPIQAF	36.38	1560.8	13	0.9	781.4	30.97	1.54E+04	P02666|CASB_BOVIN	
PVVVPPFLQPE	36.28	1220.7	11	0.4	611.35	38.06	3.86E+03	P02666|CASB_BOVIN	
MAIPPKKNQD	36.24	1140.6	10	5.5	381.21	10.89	3.56E+04	P02668|CASK_BOVIN	
LNKPEDETHL	36.24	1194.6	10	8	399.21	14.29	1.01E+05	P80195|GLCM1_BOVI	
APPPPPPPP	36.2	865.47	9	1.2	433.74	14.93	7.74E+03	A2VDK6|WASF2_BOVIN:Q32LP2|RADI_BOV	
NLHLPLPLLQS	36.16	1243.7	11	-8	622.87	41.48	3.83E+03	P02666|CASB_BOVIN	
GLPQEVLNENL	36.13	1224.6	11	2.9	613.33	31.16	3.02E+04	P02662|CASA1_BOVI	
VPYPQRDMPIQA	36.13	1413.7	12	-12.2	707.85	23.52	3.90E+03	P02666|CASB_BOVIN	
VPPFLQPEVM(+15.99)GV	36.03	1327.7	12	-0.9	664.85	34.43	1.09E+04	P02666|CASB_BOVIN	Oxidation (M)
VPQLEIVPN	35.74	1007.6	9	-3.2	1008.6	27.95	5.98E+04	P02662|CASA1_BOVI	
DM(+15.99)PIQAFLL	35.61	1062.5	9	-4.3	1063.5	38.92	1.23E+04	P02666|CASB_BOVIN	Oxidation (M)
SKVLPVPQKAVPYPQ	35.61	1650	15	-1.2	550.99	23.58	1.30E+04	P02666|CASB_BOVIN	
HQGLPQEVLN	35.59	1133.6	10	0.3	567.8	20.27	6.03E+04	P02662|CASA1_BOVI	
IPPLTQTPV	35.58	964.56	9	-4.3	965.57	26.07	4.27E+04	P02666|CASB_BOVIN	
QGLPQEVLN	35.49	996.52	9	0.8	499.27	22.64	1.51E+04	P02662|CASA1_BOVI	
VSREGQEQEGEEMAEYR	35.42	2025.9	17	-1.5	676.3	15.26	3.82E+03	P18892|BT1A1_BOVIN	
GLDIQKVA	35.34	842.49	8	1.1	422.25	20.07	1.34E+04	P02754|LACB_BOVIN	
RNAVPITPT	35.33	967.55	9	1.3	484.78	15.53	1.18E+03	P02663|CASA2_BOVI	
SPPEINTVQVTSTAV	35.31	1541.8	15	5.6	771.91	26.36	7.37E+03	P02668|CASK_BOVIN	
APFPEVF	35.27	805.4	7	-1.4	806.41	34.14	4.09E+04	P02662|CASA1_BOVI	
DASAQFIR	35.27	906.46	8	0.5	454.24	16.01	3.83E+04	P80195|GLCM1_BOVI	
QNPKLPLSIL	35.21	1121.7	10	0.8	561.85	35.95	1.63E+04	P80195|GLCM1_BOVI	
VLNENLLRF	35.19	1116.6	9	7.4	559.33	33.41	1.19E+04	P02662|CASA1_BOVI	
LPQEVLNENLL	35.19	1280.7	11	1.8	641.36	33.8	5.06E+04	P02662|CASA1_BOVI	
SKVLPVPQ	35.18	866.52	8	2.3	434.27	18.46	3.84E+04	P02666|CASB_BOVIN	
VLPVPQKAVPYPQR	35.17	1590.9	14	-5.6	531.31	23.09	4.18E+03	P02666|CASB_BOVIN	
NVPGEIVE	35.02	855.43	8	-1.9	856.44	18.54	1.20E+05	P02666|CASB_BOVIN	
VPQLEIVPNSAEERLH	35.01	1830	16	-5.9	610.99	27.87	5.48E+03	P02662|CASA1_BOVI	
HQGLPQEVLNENLL	34.99	1602.8	14	4.9	802.43	33.88	2.47E+04	P02662|CASA1_BOVI	
ILNKPEDETHLEAQPTDASAQFIRN	34.98	2836.4	25	0.1	710.11	24.3	9.16E+03	P80195|GLCM1_BOVI	
LGPVRGPFPIIV	34.96	1263.8	12	-2.4	632.89	40.71	7.24E+03	P02666|CASB_BOVIN	
ILNKPEDETHLEAQPTDASAQF	34.96	2453.2	22	5.9	818.74	23.81	2.19E+05	P80195|GLCM1_BOVI	
LLDPSFF	34.93	837.43	7	3.3	838.44	36.2	3.04E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
VPPFLQPEVMGV	34.92	1311.7	12	1.7	656.86	39.71	4.48E+03	P02666|CASB_BOVIN	

Peptide	-10lgP	Mass	Length	ppm	m/z	RT	Area	Accession	PTM
PPPAPPPPP	34.91	865.47	9	-3.4	433.74	14.82	3.93E+03	A2VDK6|WASF2_BOV	
VLGPVRGPFPII	34.91	1263.8	12	-2.3	632.89	39.68	6.13E+03	P02666|CASB_BOVIN	
TQTPVVVPPFLQPEVM	34.84	1780.9	16	4.8	891.49	43.59	1.63E+04	P02666|CASB_BOVIN	
YKVPQLE	34.76	875.48	7	-18.7	438.74	21.07	8.61E+03	P02662|CASA1_BOVI	
VIESPPEINTVQ	34.66	1324.7	12	-3.2	663.35	23.12	1.72E+04	P02668|CASK_BOVIN	
YQGPIVLNPWDQVK	34.65	1655.9	14	0	828.94	34.63	5.37E+03	P02663|CASA2_BOVI	
FPPQSVL	34.63	786.43	7	-6.3	787.43	27.07	5.18E+04	P02666|CASB_BOVIN	
SLTLTDVENL	34.58	1103.6	10	-4.3	552.79	33.16	3.74E+03	P02666|CASB_BOVIN	
VQVTSTAV	34.56	803.44	8	-4.8	804.45	15.13	1.67E+04	P02668|CASK_BOVIN	
TLTDVENL	34.55	903.45	8	-5	904.46	23.92	1.76E+04	P02666|CASB_BOVIN	
LYQEPVLGPVRGPFPIIV	34.53	1993.1	18	3.9	997.58	45.35	3.73E+03	P02666|CASB_BOVIN	
NLHLPLPLLQ	34.45	1156.7	10	-8	579.35	42.3	1.63E+04	P02666|CASB_BOVIN	
LIVTQTM(+15.99)KGL	34.29	1118.6	10	4.6	560.33	20.98	1.51E+04	P02754|LACB_BOVIN	Oxidation (M)
DMPIQAFL	34.24	933.46	8	-2.3	467.74	39.95	3.75E+03	P02666|CASB_BOVIN	
NLHLPLPLLQSW	34.24	1429.8	12	-0.8	715.91	46.91	1.21E+03	P02666|CASB_BOVIN	
EVLNENLLR	34.21	1098.6	9	4.7	550.31	24.3	4.57E+04	P02662|CASA1_BOVI	
DM(+15.99)ESTEVFTK	34.1	1201.5	10	-2.6	601.77	14.99	3.95E+03	P02663|CASA2_BOVI	Oxidation (M)
YQEPVLGPVR	34.09	1156.6	10	-9	579.32	21.82	4.36E+03	P02666|CASB_BOVIN	
AVPYPQRDMPIQAF	34.04	1631.8	14	1.2	816.92	31.67	1.26E+04	P02666|CASB_BOVIN	
GLPQEVLNE	33.97	997.51	9	15.6	499.77	23.68	4.47E+04	P02662|CASA1_BOVI	
TEIPTIN	33.93	786.41	7	-14.4	787.41	20.77	0	P02668|CASK_BOVIN	
HAFEVVKT	33.91	929.5	8	-1.5	465.76	15.08	8.38E+03	P80195|GLCM1_BOVI	
APFPEVFGKEKV	33.9	1346.7	12	4.8	449.92	28.56	4.86E+04	P02662|CASA1_BOVI	
GPVRGPFPII	33.88	1051.6	10	-1.9	526.82	34.52	4.15E+04	P02666|CASB_BOVIN	
ELNVPGEIVE	33.87	1097.6	10	-1.1	549.79	26.59	1.10E+04	P02666|CASB_BOVIN	
GHLKALINN	33.85	978.56	9	-8.8	490.29	21	4.13E+03	Q9TTK4|LYST_BOVIN	
TVDMES(+79.97)TEVFTK	33.85	1465.6	12	9.6	733.82	22.95	0	P02663|CASA2_BOVI	Phosphorylation
EELNVPGEIVE	33.84	1226.6	11	-2.5	1227.6	27.12	2.98E+04	P02666|CASB_BOVIN	
SQNPKLPLSILK	33.82	1336.8	12	6.1	446.61	29.25	5.25E+03	P80195|GLCM1_BOVI	
AAGGPGAPADPGRPTGYS	33.81	1597.7	18	3.3	799.89	14.57	3.37E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
VVPPFLQPEVM(+15.99)	33.74	1270.7	11	1.4	636.34	33.41	4.42E+04	P02666|CASB_BOVIN	Oxidation (M)
AFEVVKT	33.68	792.44	7	1.1	397.23	17.42	1.50E+04	P80195|GLCM1_BOVI	
MPFPKYPVEPF	33.63	1350.7	11	4.6	676.35	35.95	2.78E+04	P02666|CASB_BOVIN	
KEDVPSERYLG	33.6	1291.6	11	4.3	431.56	16.74	3.54E+03	P02662|CASA1_BOVI	
YQGPIVLNPWDQVKRN	33.58	1926	16	0.9	643.01	31.73	1.03E+04	P02663|CASA2_BOVI	
VAGTWYSL	33.5	895.44	8	0.8	896.45	30.63	1.02E+04	P02754|LACB_BOVIN	
SPPEINTVQ	33.48	983.49	9	2.7	492.76	15.92	6.48E+03	P02668|CASK_BOVIN	
TPVVVPPFLQPE	33.48	1321.7	12	-1.3	661.87	38.43	2.11E+03	P02666|CASB_BOVIN	
NIPPLTQTPVVVPPFLQPEVM(+15.9	33.48	2331.3	21	-5.3	778.09	45.26	1.83E+04	P02666|CASB_BOVIN	Oxidation (M)
DIQKVAGTW	33.47	1016.5	9	-3.4	509.27	22.06	4.56E+03	P02754|LACB_BOVIN	
FVAPFPEVFG	33.42	1108.6	10	-5.6	555.29	41.77	5.62E+03	P02662|CASA1_BOVI	
YQKFPQY	33.4	972.47	7	5.7	487.25	18.86	2.87E+03	P02663|CASA2_BOVI	
AVPYPQRDM(+15.99)PIQAF	33.38	1647.8	14	-2.4	824.91	26.56	5.09E+03	P02666|CASB_BOVIN	Oxidation (M)
LPQEVLNENL	33.37	1167.6	10	3.7	584.82	27.6	8.00E+03	P02662|CASA1_BOVI	
PPAPPPPPP	33.34	865.47	9	-3.4	433.74	14.82	3.93E+03	A2VDK6|WASF2_BOV	
TQTMKGLDIQ	33.33	1133.6	10	7.2	567.8	20.27	6.03E+04	P02754|LACB_BOVIN	
RDMPIQAF	33.29	976.48	8	-2.4	489.25	24.89	1.85E+04	P02666|CASB_BOVIN	
FVAPFPEVFGKEKVN	33.27	1706.9	15	3.4	569.98	34.76	6.30E+03	P02662|CASA1_BOVI	
PVEPFTE	33.21	817.39	7	-3.2	818.39	19.88	5.63E+03	P02666|CASB_BOVIN	
TVDM(+15.99)ES(+79.97)TEVFTK	33.17	1481.6	12	-1.7	741.81	17.42	1.30E+04	P02663|CASA2_BOVIN	Oxidation (M); Phosphorylation
TKLTEEEKNRL	33.16	1359.7	11	3.4	340.94	13.37	2.89E+03	P02663|CASA2_BOVI	
YQEPVL	33.14	747.38	6	-0.8	748.39	21.49	3.14E+04	P02666|CASB_BOVIN	
EMPFPKYPVEPF	33.14	1479.7	12	6.5	740.87	36.74	2.12E+04	P02666|CASB_BOVIN	
SLPQNIPPLTQTPV	33.14	1503.8	14	-1.2	752.92	32.01	2.11E+04	P02666|CASB_BOVIN	
YKVPQLEIVPNS(+79.97)AEERLH	33.14	2201.1	18	3.2	551.28	30.19	1.70E+04	P02662|CASA1_BOVI	Phosphorylation
STLVPLA	33.13	699.42	7	-4.5	700.42	26.29	1.66E+04	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
VSTLVPLA	33.05	798.49	8	-0.6	799.49	28.83	1.92E+04	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
DKTEIPTIN	33.02	1029.5	9	3.5	515.78	19.47	5.27E+04	P02668|CASK_BOVIN	
LDPSFFAKE	32.98	1052.5	9	5.3	527.27	26.24	1.59E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
DM(+15.99)PIQAF	32.95	836.37	7	0.6	837.38	22.17	3.30E+04	P02666|CASB_BOVIN	Oxidation (M)
QGPIVLNPWDQVK	32.9	1492.8	13	-7.8	747.41	32.6	2.30E+03	P02663|CASA2_BOVI	
NENLLRF	32.86	904.48	7	3.1	453.25	27.36	3.95E+05	P02662|CASA1_BOVI	
NLHLPLPL	32.78	915.55	8	1.1	458.79	39.32	1.03E+04	P02666|CASB_BOVIN	
AVPYPQRDMPIQA	32.78	1484.7	13	-6.8	743.38	24.5	1.05E+04	P02666|CASB_BOVIN	

Peptide	-10lgP	Mass	Length	ppm	m/z	RT	Area	Accession	PTM
GLPQEVL	32.67	754.42	7	2.8	755.43	25.49	3.29E+05	P02662|CASA1_BOVI	
DMPIQA	32.66	673.31	6	-0.1	674.32	17.74	6.93E+03	P02666|CASB_BOVIN	
TTLSSEAPTTQ	32.61	1134.5	11	2.8	568.28	13.66	0	P80025|PERL_BOVIN	
EPVLGPVR	32.59	865.5	8	-1.2	433.76	17.65	8.63E+03	P02666|CASB_BOVIN	
AVPYPQ	32.53	673.34	6	-5.4	674.35	14.28	1.02E+04	P02666|CASB_BOVIN	
ALLDPSF	32.51	761.4	7	0.1	762.41	30.22	5.99E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
EVLNENLL	32.46	942.5	8	0	943.51	28.57	3.35E+04	P02662|CASA1_BOVI	
VLPVPQKAVPYPQRDMPIQAF	32.44	2393.3	21	4.7	798.78	34.56	2.27E+04	P02666|CASB_BOVIN	
GYLEQL	32.39	721.36	6	-5.6	722.37	24.58	1.22E+04	P02662|CASA1_BOVI	
ALLDPSFFAKESVKD	32.35	1665.9	15	5.6	556.3	32.96	4.36E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
VPQKAVPYPQ	32.34	1125.6	10	-5.2	563.82	15.49	3.35E+03	P02666|CASB_BOVIN	
KVPQLEIVPN	32.34	1135.7	10	-1.1	568.84	26.24	4.66E+04	P02662|CASA1_BOVI	
SLVYPFPGPIPNSLPQ	32.3	1724.9	16	-6.9	863.46	39.9	1.24E+04	P02666|CASB_BOVIN	
KHQGLPQEVL	32.22	1147.6	10	0.3	574.83	20.51	2.98E+04	P02662|CASA1_BOVI	
GLPQEVLNEN	32.21	1111.6	10	7.6	556.79	23.28	2.42E+04	P02662|CASA1_BOVI	
HIQKEDVPSERYL	32.2	1612.8	13	5.2	538.62	18.56	2.96E+04	P02662|CASA1_BOVI	
LNENLLRF	32.18	1017.6	8	0.9	509.79	31.56	3.98E+03	P02662|CASA1_BOVI	
TQTPVVVPPFLQPE	32.16	1550.8	14	3.4	776.43	38.7	1.09E+04	P02666|CASB_BOVIN	
VAPFPEV	32.03	757.4	7	2.6	379.71	27.76	7.56E+03	P02662|CASA1_BOVI	
LPLSILKEK	32.01	1039.7	9	2.9	347.56	24.96	5.18E+02	P80195|GLCM1_BOVI	
YKVPQLEIVPN	31.97	1298.7	11	-2.6	650.37	30.39	3.20E+04	P02662|CASA1_BOVI	
LPQNIPPLTQTPV	31.93	1416.8	13	-8	709.4	31.08	3.52E+03	P02666|CASB_BOVIN	
GTWYSL	31.86	725.34	6	1.6	726.35	29.02	2.42E+04	P02754|LACB_BOVIN	
YQEPVLGPV	31.86	1000.5	9	2.1	501.27	26.62	4.00E+03	P02666|CASB_BOVIN	
PVVVPPFLQPEVM(+15.99)	31.85	1466.8	13	1.8	734.4	39.44	1.19E+03	P02666|CASB_BOVIN	Oxidation (M)
QKEDVPSERYL	31.82	1362.7	11	9.3	455.24	18.43	2.86E+03	P02662|CASA1_BOVI	
SLTLTDVEN	31.73	990.49	9	-2.5	496.25	22.11	2.71E+03	P02666|CASB_BOVIN	
AVPITPT	31.7	697.4	7	4.3	698.41	15.79	8.25E+03	P02663|CASA2_BOVI	
ELEELNVPGEIVE	31.68	1468.7	13	3	735.38	31.77	1.79E+04	P02666|CASB_BOVIN	
IVPNSAEERLH	31.63	1263.7	11	-6	422.23	14.38	6.79E+03	P02662|CASA1_BOVI	
VPQLEIVPNSAEER	31.55	1579.8	14	2.1	790.92	26.07	1.93E+03	P02662|CASA1_BOVI	
LPLLQSW	31.51	855.49	7	2.3	428.75	36.99	4.15E+03	P02666|CASB_BOVIN	
AGTWYSL	31.49	796.38	7	-3.2	797.38	29.27	2.84E+03	P02754|LACB_BOVIN	
DKIHPFAQTQ	31.47	1183.6	10	9.3	395.54	14.73	5.45E+03	P02666|CASB_BOVIN	
IGVNQEL	31.44	771.41	7	-1.1	772.42	19.84	4.36E+03	P02662|CASA1_BOVI	
DPSFF	31.41	611.26	5	-2.6	612.27	26.04	5.86E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN:Q9TU23|CE290_BOVIN	
VAPFPEVFGKEKVNEL	31.4	1802	16	3.9	601.67	33.95	1.05E+04	P02662|CASA1_BOVI	
VAPFPE	31.38	658.33	6	2.1	659.34	19.95	4.81E+04	P02662|CASA1_BOVI	
EVIESPPEIN	31.36	1125.6	10	7.9	563.79	21.93	2.10E+03	P02668|CASK_BOVIN	
LTDVENL	31.35	802.41	7	-1.2	803.42	20.98	4.97E+03	P02666|CASB_BOVIN	
EDVPSERY	31.33	993.44	8	4.8	497.73	13.46	3.20E+03	P02662|CASA1_BOVI	
AMKPWIQPK	31.32	1097.6	9	7.9	366.88	19.1	9.12E+03	P02663|CASA2_BOVI	
RDMPIQAFL	31.3	1089.6	9	2	545.79	35.01	1.55E+03	P02666|CASB_BOVIN	
VAPFPEVFGKEKV	31.28	1445.8	13	0.6	482.94	30.91	5.85E+04	P02662|CASA1_BOVI	
NVPGEIVESLS	31.25	1142.6	11	17	572.31	29.65	7.85E+03	P02666|CASB_BOVIN	
VPGEIVESL	31.23	941.51	9	-2.6	942.52	27.7	1.11E+04	P02666|CASB_BOVIN	
SSS(+79.97)EESITRIN	31.23	1301.6	11	-1.4	651.78	15.69	1.12E+04	P02666|CASB_BOVIN	Phosphorylation
KHQGLPQEVLN	31.16	1261.7	11	2.7	421.57	17.98	4.28E+04	P02662|CASA1_BOVI	
YPFPGPIPN	31.14	1000.5	9	-3.9	1001.5	29.67	1.04E+04	P02666|CASB_BOVIN	
GPFPII	31.13	642.37	6	-4.1	643.38	34.87	3.64E+03	P02666|CASB_BOVIN	
NIPPLTQTPVVVPPFLQPEVMGVSK	31	2686.5	25	0.3	896.5	45.82	2.56E+03	P02666|CASB_BOVIN	
VLGPVRGPFP	30.99	1037.6	10	-3.4	519.81	28.32	6.73E+03	P02666|CASB_BOVIN	
TIASGEPTSTPTTE	30.98	1390.6	14	5.7	696.34	14.57	7.02E+03	P02668|CASK_BOVIN	
KVPQLEIVPNSAEER	30.96	1707.9	15	-6.6	570.31	24.28	2.21E+03	P02662|CASA1_BOVI	
TVDM(+15.99)ESTEVFTK	30.89	1401.6	12	11.7	701.83	17.37	1.17E+04	P02663|CASA2_BOVI	Oxidation (M)
DVPSERYL	30.88	977.48	8	4.2	489.75	19.97	7.36E+04	P02662|CASA1_BOVI	
LPQYL	30.79	632.35	5	-5.2	633.36	24.28	4.62E+03	P02663|CASA2_BOVI	
IPQYI	30.79	632.35	5	-5.2	633.36	24.28	4.62E+03		
KTEIPTIN	30.76	914.51	8	1.2	458.26	18.74	2.36E+04	P02668|CASK_BOVIN	
QGLPQEVL	30.75	882.48	8	-7.7	883.48	25.72	2.72E+03	P02662|CASA1_BOVI	
HQGLPQ(+.98)EVLNENLLR	30.69	1759.9	15	6.8	587.65	30.8	6.84E+03	P02662|CASA1_BOVI	Deamidation (NQ)
M(+15.99)ES(+79.97)TEVFTK	30.68	1166.5	9	2.5	584.24	14.63	3.26E+03	P02663|CASA2_BOVIN	Oxidation (M); Phosphorylation
QPTDASAQF	30.67	963.43	9	-12.5	964.43	14.73	1.06E+04	P80195|GLCM1_BOVI	

Peptide	-10lgP	Mass	Length	ppm	m/z	RT	Area	Accession	PTM
IPIQYVL	30.63	844.51	7	-1.2	423.26	34.02	2.49E+03	P02668|CASK_BOVIN	
KEMPFPKYPVEPF	30.58	1607.8	13	9.4	536.95	32.72	2.07E+03	P02666|CASB_BOVIN	
EMPFPKYPVEPFTESQ	30.58	1924.9	16	-7.9	963.45	34.4	4.01E+03	P02666|CASB_BOVIN	
FVAPFPEVFGKE	30.57	1365.7	12	1.3	683.86	37.73	8.76E+03	P02662|CASA1_BOVI	
PVEPF	30.55	587.3	5	0.6	588.31	20.07	1.84E+04	P02666|CASB_BOVIN	
FFVAP	30.51	579.31	5	1.8	580.32	36.84	1.96E+03	P02662|CASA1_BOVI	
RPKHPIKHQGLPQEVLN	30.5	1990.1	17	5.9	498.54	15.16	8.10E+04	P02662|CASA1_BOVI	
SVKDAAGGPGAPADPGRPT	30.35	1719.9	19	-3.9	574.29	13.02	1.03E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
LHLPLPLLQ	30.32	1042.7	9	-3.9	522.33	43.31	4.86E+04	P02666|CASB_BOVIN	
VLPVPQ	30.3	651.4	6	-2.4	652.4	18.91	3.16E+04	P02666|CASB_BOVIN	
VPQLEIVPNS(+79.97)AEERLH	30.25	1909.9	16	-1.3	637.65	28.76	9.27E+03	P02662|CASA1_BOVI	Phosphorylation
FPEVFGKEK	30.24	1079.6	9	6.7	360.87	21.77	9.22E+03	P02662|CASA1_BOVI	
EAQPTDASAQFIR	30.09	1432.7	13	-9.8	717.35	20.19	9.93E+03	P80195|GLCM1_BOVI	
HLPLPLLQ	30.04	929.57	8	-0.9	465.79	34.9	6.11E+03	P02666|CASB_BOVIN	
FPEVF	30.03	637.31	5	2.2	638.32	30.52	1.60E+04	P02662|CASA1_BOVI	
EVLNENL	30.03	829.42	7	-4.6	830.42	20.76	4.93E+03	P02662|CASA1_BOVI	
GPVRGPFP	30.03	825.45	8	11.4	413.74	20.54	2.90E+04	P02666|CASB_BOVIN	
N(+.98)ENLLRFF	30.01	1052.5	8	-2.8	527.27	40.41	6.02E+02	P02662|CASA1_BOVI	Deamidation (NQ)
VAPFPEVFGKEKVNE	29.96	1688.9	15	5.9	563.97	29	1.48E+03	P02662|CASA1_BOVI	
YQQKPVAL	29.95	945.53	8	-4	473.77	16.35	2.27E+03	P02668|CASK_BOVIN	
APFPEV	29.91	658.33	6	-3.2	659.34	24.79	8.04E+03	P02662|CASA1_BOVI	
AAPAGAAIQSRAGEIQNK	29.87	1751.9	18	-3	584.98	16.16	1.64E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
VLNENLL	29.86	813.46	7	-8.5	814.46	26.15	1.55E+04	P02662|CASA1_BOVI	
DAVSKVKIQVN	29.85	1199.7	11	-3.3	600.85	17.6	1.36E+03	P80025|PERL_BOVIN	
EM(+15.99)PFPKYPVEPFTESQ	29.83	1940.9	16	-3.7	971.45	30.8	9.30E+03	P02666|CASB_BOVIN	Oxidation (M)
YLEQLL	29.8	777.43	6	-5.8	778.43	30.54	4.81E+03	P02662|CASA1_BOVI	
YLEQLI	29.8	777.43	6	-5.8	778.43	30.54	4.81E+03		
EVIESPPEINTVQVTSTAV	29.8	2012	19	-1.1	1007	31.29	1.73E+04	P02668|CASK_BOVIN	
GPVRGPFPIIV	29.75	1150.7	11	-6.8	576.35	37.12	2.46E+05	P02666|CASB_BOVIN	
IHPFAQTQ	29.74	940.48	8	-2.1	471.25	14.88	7.28E+03	P02666|CASB_BOVIN	
LYQGPIVLNPWDQVKR	29.73	1925.1	16	-1.3	642.69	34.14	1.30E+04	P02663|CASA2_BOVI	
ENLLRFF	29.72	937.5	7	-2.1	469.76	37.94	1.35E+03	P02662|CASA1_BOVI	
FPKYPVEPF	29.72	1122.6	9	6.1	562.3	31.32	1.76E+04	P02666|CASB_BOVIN	
HKEMPFPKYPVEPF	29.72	1744.9	14	-4.3	582.63	30.8	3.41E+04	P02666|CASB_BOVIN	
LLDPSF	29.7	690.36	6	-8.1	691.36	26.58	8.19E+02	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
NIPPLTQTPVVVPPFLQPE	29.7	2085.2	19	4.3	1043.6	44.87	1.31E+04	P02666|CASB_BOVIN	
FPEVFGKE	29.66	951.47	8	-1.4	476.74	24.92	1.17E+03	P02662|CASA1_BOVI	
ALLDPSFFAKESVKDAAGGPGAPA	29.63	2938.5	30	-1.6	735.63	33.11	4.06E+04	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
TKVIPYVR	29.62	974.59	8	1.9	325.87	17.47	1.68E+03	P02663|CASA2_BOVI	
LLDPSFFAKE	29.62	1165.6	10	-3.6	583.81	31.61	2.03E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
HKEM(+15.99)PFPKYPVEPF	29.57	1760.9	14	-2.1	587.96	27.9	1.64E+04	P02666|CASB_BOVIN	Oxidation (M)
MPIQAFLL	29.55	931.52	8	-3.3	466.77	42.6	2.01E+03	P02666|CASB_BOVIN	
MES(+79.97)TEVFTK	29.55	1150.5	9	8.4	576.24	17.31	7.85E+03	P02663|CASA2_BOVI	Phosphorylation
FSHAFEVVKT	29.52	1163.6	10	-0.5	388.87	21.05	6.96E+03	P80195|GLCM1_BOVI	
VSREGQEQEGEEM(+15.99)AEYRGI	29.52	2255	19	4.5	564.76	11.16	8.42E+02	P18892|BT1A1_BOVIN	Oxidation (M)
VRSPAQILQW	29.46	1196.7	10	1.2	599.34	33.2	1.75E+03	P02668|CASK_BOVIN	
VVPPFLQPE	29.45	1024.6	9	-6.3	513.29	31.44	1.09E+04	P02666|CASB_BOVIN	
LEQLLRL	29.44	883.55	7	2.9	442.78	31.2	4.39E+03	P02662|CASA1_BOVI	
QSKVLPVPQ	29.42	994.58	9	-0.5	498.3	18.8	2.41E+04	P02666|CASB_BOVIN	
KHQGLPQEVLNENLL	29.34	1730.9	15	6.6	577.99	31.74	1.76E+04	P02662|CASA1_BOVI	
N(+.98)AVPITPT	29.31	812.43	8	-1.4	813.44	18.47	1.19E+04	P02663|CASA2_BOVI	Deamidation (NQ)
KEPMIGVNQEL	29.29	1256.6	11	-13.9	629.32	24.53	3.13E+03	P02662|CASA1_BOVI	
LPLPL	29.26	551.37	5	-3.8	552.38	32.98	3.61E+03	P02666|CASB_BOVIN:P42916|CL43_BOVIN	
LPIPL	29.26	551.37	5	-3.8	552.38	32.98	3.61E+03		
IPIPI	29.26	551.37	5	-3.8	552.38	32.98	3.61E+03	A7XYH9|SOBP_BOVI	
LPLPI	29.26	551.37	5	-3.8	552.38	32.98	3.61E+03		
IPLPL	29.26	551.37	5	-3.8	552.38	32.98	3.61E+03		
IPLPI	29.26	551.37	5	-3.8	552.38	32.98	3.61E+03		
LPIPI	29.26	551.37	5	-3.8	552.38	32.98	3.61E+03		

Peptide	-10lgP	Mass	Length	ppm	m/z	RT	Area	Accession	PTM
LPQYLK	29.26	760.45	6	1.2	381.23	17.98	3.87E+04	P02663|CASA2_BOVI	
MES(+79.97)TEVFTKK	29.26	1278.6	10	0.8	427.19	15.33	1.47E+03	P02663|CASA2_BOVI	Phosphorylation
VPQLEIVPNSAEERLHS(+79.97)MK	29.24	2256.1	19	4.2	565.04	29.58	3.91E+03	P02662|CASA1_BOVI	Phosphorylation
IPIQY	29.23	632.35	5	-5.4	633.36	20.95	4.37E+03	P02668|CASK_BOVIN	
IPLQY	29.23	632.35	5	-5.4	633.36	20.95	4.37E+03		
LPLQY	29.23	632.35	5	-5.4	633.36	20.95	4.37E+03		
FPIIV	29.2	587.37	5	-7.8	588.37	33.82	2.00E+03	P02666|CASB_BOVIN	
FPLIV	29.2	587.37	5	-7.8	588.37	33.82	2.00E+03		
FPLLV	29.2	587.37	5	-7.8	588.37	33.82	2.00E+03		
NAVPITPTLNRE	29.2	1323.7	12	-2.5	662.87	21.98	6.68E+03	P02663|CASA2_BOVI	
ALLDPSFFAKE	29.19	1236.6	11	2.4	619.33	34.59	2.46E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
EM(+15.99)PFPKYPVEPF	29.19	1495.7	12	5	748.87	32.81	3.21E+04	P02666|CASB_BOVIN	Oxidation (M)
VPQLEIVPNS(+79.97)AEER	29.19	1659.8	14	1.2	830.9	26.2	6.14E+03	P02662|CASA1_BOVI	Phosphorylation
YLEQLLRL	29.11	1046.6	8	-2.9	524.31	37.49	5.44E+02	P02662|CASA1_BOVI	
EIVESL	29.06	688.36	6	-4.5	689.37	21.21	7.42E+03	P02666|CASB_BOVIN	
SRYPSYGLN	29.06	1055.5	9	6.1	528.76	17.51	3.28E+03	P02668|CASK_BOVIN	
DM(+15.99)PIQAFL	29.01	949.46	8	5.9	475.74	32.62	2.79E+03	P02666|CASB_BOVIN	Oxidation (M)
PFPEVFGK	29.01	919.48	8	-1.4	460.75	31.38	3.15E+03	P02662|CASA1_BOVI	
IGVNQELAY	29	1005.5	9	-2.2	1006.5	23.83	1.29E+04	P02662|CASA1_BOVI	
EDVPSERYL	28.98	1106.5	9	0.6	554.27	20.7	2.30E+04	P02662|CASA1_BOVI	
LPQEVLN	28.96	811.44	7	-8.5	812.45	18.78	8.68E+03	P02662|CASA1_BOVI	
FSDKIAKY	28.93	970.51	8	0.8	324.51	15.39	1.62E+03	P02668|CASK_BOVIN	
SSS(+79.97)EESITRINK	28.92	1429.6	12	4.8	477.56	14.23	5.60E+03	P02666|CASB_BOVIN	Phosphorylation
KEPM(+15.99)IGVNQELAY	28.86	1506.7	13	-5.7	754.38	24.87	7.49E+03	P02662|CASA1_BOVI	Oxidation (M)
KTTLS(+79.97)SEAPTTQ	28.78	1342.6	12	0.1	672.31	13.89	5.12E+03	P80025|PERL_BOVIN	Phosphorylation
IESPPEIN	28.76	897.44	8	2.9	449.73	17.78	6.96E+03	P02668|CASK_BOVIN	
LPQEVL	28.73	697.4	6	-1.7	698.41	22.53	1.40E+04	P02662|CASA1_BOVIN:Q9TTK4|LYST_BOV	
IPQEVL	28.73	697.4	6	-1.7	698.41	22.53	1.40E+04		
LPLPLLQ	28.67	792.51	7	-13.2	793.51	37.01	1.42E+04	P02666|CASB_BOVIN	
SDIPNPIGSENSEKTTM(+15.99)PLW	28.65	2231	20	-5.5	1116.5	32.53	2.37E+04	P02662|CASA1_BOVI	Oxidation (M)
IQKEDVPSERYL	28.63	1475.8	12	2.2	492.93	20.35	5.37E+03	P02662|CASA1_BOVI	
TEDELQDKIHPF	28.57	1470.7	12	4.3	491.24	25.2	1.02E+04	P02666|CASB_BOVIN	
LNENLLR	28.56	870.49	7	-1.7	436.25	18.85	1.40E+03	P02662|CASA1_BOVI	
VAPFP	28.49	529.29	5	-5.9	530.3	21.03	0	P02662|CASA1_BOVI	
DKIHPFAQTQS	28.43	1270.6	11	-5.1	636.32	14.67	1.05E+04	P02666|CASB_BOVIN	
KAVPYPQRDMPIQAF	28.41	1759.9	15	0.6	587.65	28.66	5.86E+03	P02666|CASB_BOVIN	
PEVIESPPEINTVQVTSTAV	28.38	2109.1	20	-4.3	704.03	32.25	3.27E+03	P02668|CASK_BOVIN	
GLPQEV	28.32	641.34	6	-6.8	642.34	17.64	1.16E+04	P02662|CASA1_BOVI	
EIPTINTIA	28.23	970.53	9	-2	971.54	28.04	8.28E+03	P02668|CASK_BOVIN	
EIVPNSAEERLH	28.21	1392.7	12	-4.3	465.24	17.47	2.19E+03	P02662|CASA1_BOVI	
PQVEAVLN	28.19	868.47	8	-2.2	869.47	22.31	2.56E+05	A6QL48|IL34_BOVIN	
SSRQPQSQNPKLPLSILK	28.17	2020.1	18	1.6	506.05	27.36	5.07E+03	P80195|GLCM1_BOVI	
APFPE	28.16	559.26	5	-2.1	560.27	15.8	5.24E+03	P02662|CASA1_BOVI	
SQSKVLPVPQKAVPYPQ	28.05	1865	17	-1.1	622.69	23.85	7.83E+03	P02666|CASB_BOVIN	
EIVPNSAEER	28.04	1142.6	10	-3.7	572.29	13.62	2.76E+03	P02662|CASA1_BOVI	
TLVPL	27.97	541.35	5	-1.2	542.36	26.68	1.03E+04	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN:C7EXK4|AT8A2_BOVIN:Q29449|AT8A1_BOVIN	
TLVPI	27.97	541.35	5	-1.2	542.36	26.68	1.03E+04		
TIVPI	27.97	541.35	5	-1.2	542.36	26.68	1.03E+04		
TIVPL	27.97	541.35	5	-1.2	542.36	26.68	1.03E+04		
MESTEVFTK	27.95	1070.5	9	-5	536.25	16.67	8.99E+03	P02663|CASA2_BOVI	
YLEQL	27.86	664.34	5	-2.9	665.35	20.9	7.50E+03	P02662|CASA1_BOVI	
SSRQPQSQNPKLPLS	27.86	1665.9	15	-4	556.3	18.24	1.06E+04	P80195|GLCM1_BOVI	
SDIPNPIGSENSEKTTMPLW	27.84	2215	20	-0.4	1108.5	36.4	8.27E+03	P02662|CASA1_BOVI	
LTLTDVENL	27.75	1016.5	9	4.8	509.28	32.3	2.89E+03	P02666|CASB_BOVIN	
DVPSERYLG	27.62	1034.5	9	3.3	518.26	18.11	5.75E+03	P02662|CASA1_BOVI	
VAPFPEVFGKE	27.62	1218.6	11	7.9	610.33	31.68	2.29E+04	P02662|CASA1_BOVI	
LEIVPNS(+79.97)AEERLH	27.58	1585.8	13	2.3	529.59	24.28	3.18E+03	P02662|CASA1_BOVI	Phosphorylation
APSFSDIPNPIGSENSE	27.58	1759.8	17	13.3	880.92	31.03	2.06E+03	P02662|CASA1_BOVI	
TLVPLA	27.54	612.38	6	-8.9	613.39	25.46	1.00E+04	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
VLPVPQK	27.53	779.49	7	3	390.75	15.11	1.18E+03	P02666|CASB_BOVIN	

Peptide	-10lgP	Mass	Length	ppm	m/z	RT	Area	Accession	PTM
LVSTLVPLA	27.52	911.57	9	-2.8	456.79	33.45	1.20E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
HQGLPQEVLNENL	27.44	1489.8	13	-0.3	745.89	28.5	3.82E+03	P02662|CASA1_BOVI	
YPYYAKPA	27.41	971.48	8	0.8	486.75	15.35	3.55E+03	P02668|CASK_BOVIN	
KEPMIGVNQELAY	27.41	1490.7	13	-6.7	746.38	27.07	4.81E+03	P02662|CASA1_BOVI	
ALPQY	27.35	590.31	5	-3.2	591.31	16.67	1.09E+04	P02663|CASA2_BOVI	
AIPQY	27.35	590.31	5	-3.2	591.31	16.67	1.09E+04		
LPLSIL	27.35	654.43	6	-2.8	655.44	35.92	2.62E+03	P80195|GLCM1_BOVIN:P42916|CL43_BOVI	
IPLSLL	27.35	654.43	6	-2.8	655.44	35.92	2.62E+03		
LPISIL	27.35	654.43	6	-2.8	655.44	35.92	2.62E+03		
IPISLL	27.35	654.43	6	-2.8	655.44	35.92	2.62E+03	C7EXK4|AT8A2_BOVIN:Q29449|AT8A1_BO	
LPLSLL	27.35	654.43	6	-2.8	655.44	35.92	2.62E+03	Q2KI51|PR15A_BOVIN	
IPLSII	27.35	654.43	6	-2.8	655.44	35.92	2.62E+03		
LPLSII	27.35	654.43	6	-2.8	655.44	35.92	2.62E+03		
PLLLPLQ	27.33	792.51	7	-2.4	793.52	37.06	1.07E+04	G3MZC5|AP5B1_BOVI	
VEDHIAEGSVAVR	27.28	1380.7	13	4.8	461.24	15.55	2.67E+03	P18892|BT1A1_BOVIN	
DTIAQAASTT	27.25	977.47	10	-9.5	978.47	13.83	6.18E+03	P80025|PERL_BOVIN	
EM(+15.99)PFPKY	27.19	926.42	7	3.3	464.22	19.09	1.88E+03	P02666|CASB_BOVIN	Oxidation (M)
LGYLEQL	27.18	834.45	7	-2.6	835.46	29.77	0	P02662|CASA1_BOVI	
TVDMESTEVFTK	27.18	1385.6	12	2.4	693.83	23.19	3.60E+03	P02663|CASA2_BOVI	
QSKVLPVPQKAVPYPQ	27.18	1778	16	-7.4	593.67	23.75	9.06E+03	P02666|CASB_BOVIN	
SPAQIL	27.17	627.36	6	-2.6	628.37	19.83	0	P02668|CASK_BOVIN	
SPAQLL	27.17	627.36	6	-2.6	628.37	19.83	0		
EIPTINTIAS	27.17	1057.6	10	-4.3	1058.6	26.69	1.43E+03	P02668|CASK_BOVIN	
HLPLPLL	27.12	801.51	7	-0.5	401.76	37.29	8.90E+02	P02666|CASB_BOVIN	
SVQAIN	27.1	630.33	6	1.7	631.34	20.84	6.91E+03	Q9TTK4|LYST_BOVIN	
IEKFQS(+79.97)EEQQ	27.08	1344.6	10	0.2	673.29	12.46	6.77E+03	P02666|CASB_BOVIN	Phosphorylation
EIPTINT	27.05	786.41	7	4.3	394.22	20.21	1.39E+03	P02668|CASK_BOVIN	
SSRQPQSQNPKLPLSILKEK	27.05	2277.3	20	1.5	456.47	24.63	6.72E+03	P80195|GLCM1_BOVI	
TDVENLHLPLPLLQ	27.03	1600.9	14	1.6	801.45	43.14	1.74E+03	P02666|CASB_BOVIN	
LVYPFPGPIPNSLPQ	27.01	1637.9	15	-0.5	819.95	39.22	6.52E+03	P02666|CASB_BOVIN	
ILNKPEDETHLEAQPT	26.96	1833.9	16	5.3	612.32	17.7	1.12E+05	P80195|GLCM1_BOVI	
LPLPLL	26.95	664.45	6	-4.7	665.46	40.56	1.45E+03	P02666|CASB_BOVIN	
IPLPLI	26.95	664.45	6	-4.7	665.46	40.56	1.45E+03		
LPIPLI	26.95	664.45	6	-4.7	665.46	40.56	1.45E+03		
LPIPII	26.95	664.45	6	-4.7	665.46	40.56	1.45E+03		
NDATAQAF	26.9	836.37	8	9.6	419.2	22.17	1.28E+04	Q0VBZ0|CSK_BOVIN	
LHLPLPL	26.88	801.51	7	-6.6	401.76	38.44	7.89E+02	P02666|CASB_BOVIN	
KVLPVPQKAVPYPQRDM(+15.99)P	26.88	2537.4	22	0.6	635.36	28.66	3.91E+03	P02666|CASB_BOVIN	Oxidation (M)
VLNENLLR	26.87	969.56	8	0.7	485.79	21.84	4.51E+03	P02662|CASA1_BOVI	
VYPFPGPIPNSLPQ	26.86	1524.8	14	3.7	763.41	36.29	4.27E+03	P02666|CASB_BOVIN	
KGLDIQKVA	26.81	970.58	9	-4	486.3	17.31	8.75E+02	P02754|LACB_BOVIN	
VFTKKT	26.79	722.43	6	-0.6	362.22	27.4	6.92E+03	P02663|CASA2_BOVI	
GYLEQLL	26.78	834.45	7	-5.2	835.45	34.49	1.10E+03	P02662|CASA1_BOVI	
CIKPLCDLLTVMDS	26.78	1549.8	14	11.8	775.9	29.72	1.23E+04	A2VE08|IMA5_BOVIN:Q0V7M0|IMA7_BOVIN	
LPYPY	26.77	651.33	5	1.1	652.34	22.17	0	P02668|CASK_BOVIN	
IPYPY	26.77	651.33	5	1.1	652.34	22.17	0		
ENLLRF	26.73	790.43	6	1.7	396.23	26.65	2.24E+05	P02662|CASA1_BOVI	
ALPQYLK	26.73	831.49	7	1.9	416.75	20.04	1.74E+04	P02663|CASA2_BOVI	
NAVPITPTLNREQL	26.72	1564.9	14	-7.1	783.43	27.62	5.61E+03	P02663|CASA2_BOVI	
GQPAPLPLSLL	26.64	1104.7	11	-6.4	1105.7	39.6	2.10E+03	Q2KI51|PR15A_BOVIN	
MHQPHQPLPPT	26.53	1281.6	11	2	428.22	14.37	2.05E+04	P02666|CASB_BOVIN	
SSRQPQSQNPKLPLSIL	26.53	1892	17	5.2	631.7	33.41	7.29E+04	P80195|GLCM1_BOVI	
RDM(+15.99)PIQAF	26.48	992.47	8	4.2	497.25	19.77	1.38E+04	P02666|CASB_BOVIN	Oxidation (M)
LEIVPN	26.47	683.39	6	-3.2	684.39	21.05	4.80E+03	P02662|CASA1_BOVI	
STLVPL	26.39	628.38	6	-3.8	629.39	27.02	9.12E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
VPYPQRDM(+15.99)PIQA	26.39	1429.7	12	3.1	715.86	18.84	3.05E+03	P02666|CASB_BOVIN	Oxidation (M)
ALLDPSFFAKES	26.38	1323.7	12	3.9	662.85	34.03	2.09E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	

Peptide	-10lgP	Mass	Length	ppm	m/z	RT	Area	Accession	PTM
PQEVL	26.36	584.32	5	-5.3	585.32	25.45	4.49E+03	P02662|CASA1_BOVI N:Q9TTK4|LYST_BOV	
PQEVI	26.36	584.32	5	-5.3	585.32	25.45	4.49E+03		
LPVPQ	26.35	552.33	5	-7.1	553.33	15.49	4.13E+03	P02666|CASB_BOVIN :A7YWD2|IPO13_BOVI	
TDASAQFIR	26.31	1007.5	9	-1.3	504.76	16.45	2.16E+03	P80195|GLCM1_BOVI	
GPVRGPFPI	26.31	938.53	9	2.9	470.28	28.57	1.45E+04	P02666|CASB_BOVIN	
TKLTEEEKNRLNFLK	26.3	1862	15	7	466.52	21.88	1.00E+04	P02663|CASA2_BOVI	
LPLLQ	26.29	582.37	5	-3	583.38	25.1	0	P02666|CASB_BOVIN	
LPIIQ	26.29	582.37	5	-3	583.38	25.1	0	Q95KV1|IKKA_BOVIN	
IPILQ	26.29	582.37	5	-3	583.38	25.1	0		
IPLIQ	26.29	582.37	5	-3	583.38	25.1	0		
LPILQ	26.29	582.37	5	-3	583.38	25.1	0		
LPLIQ	26.29	582.37	5	-3	583.38	25.1	0		
IPIIQ	26.29	582.37	5	-3	583.38	25.1	0		
IPLLQ	26.29	582.37	5	-3	583.38	25.1	0		
TMKGLDIQ	26.29	904.47	8	6.8	453.25	18.76	3.49E+03	P02754|LACB_BOVIN	
YKVPQLEIVPNSAEERLH	26.25	2121.1	18	9.1	531.29	29.24	4.92E+03	P02662|CASA1_BOVI	
VKDAAGGPGAPADPGRPT	26.24	1632.8	18	-2.5	545.28	12.31	2.78E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
ASAQFIRNL	26.23	1018.6	9	-4.9	510.28	24.94	6.85E+02	P80195|GLCM1_BOVI	
DLIS(+79.97)KEQIVIR	26.23	1392.7	11	5.6	465.26	27.96	4.26E+04	P80195|GLCM1_BOVI	Phosphorylation
RDMPIQA	26.19	829.41	7	4.3	415.72	15.21	7.47E+03	P02666|CASB_BOVIN	
EDLSKEPSISRE	26.19	1388.7	12	1.1	463.9	14.76	1.74E+03	P80195|GLCM1_BOVI	
TESQSLTL	26.18	877.44	8	4.8	878.45	20.38	2.74E+03	P02666|CASB_BOVIN	
RDMPIQAFLL	26.17	1202.6	10	-5.6	602.33	40.53	2.44E+03	P02666|CASB_BOVIN	
PPVIPP	26.15	618.37	6	12.3	619.39	30.42	3.13E+03	Q32LP2|RADI_BOVIN	
PPVLPP	26.15	618.37	6	12.3	619.39	30.42	3.13E+03		
VPPFLQPE	26.13	925.49	8	12.7	463.76	27.76	8.79E+03	P02666|CASB_BOVIN	
IEKFQS(+79.97)EEQQQ	26.12	1472.6	11	0.9	737.32	12.62	7.78E+03	P02666|CASB_BOVIN	Phosphorylation
LTEEEKNRLNFL	26.1	1504.8	12	0.3	502.61	26.29	5.02E+03	P02663|CASA2_BOVI	
S(+79.97)SEESITRINK	26.08	1342.6	11	-6.3	672.31	13.79	4.92E+03	P02666|CASB_BOVIN	Phosphorylation
DVPSERYLGYL	26.07	1310.7	11	-0.4	656.33	31.56	2.35E+03	P02662|CASA1_BOVI	
QGLPQEVLNENLL	26.02	1465.8	13	-4.2	733.9	36.45	3.52E+03	P02662|CASA1_BOVI	
VPGEIVE	26	741.39	7	-4.3	742.4	16.63	4.92E+03	P02666|CASB_BOVIN	
S(+79.97)AEERLHSM	25.99	1138.4	9	5.9	570.24	13.43	6.00E+03	P02662|CASA1_BOVI	Phosphorylation
DASAQFIRN	25.97	1020.5	9	-12.5	511.25	15.21	2.77E+03	P80195|GLCM1_BOVI	
PWIQPKTKVIPY	25.94	1468.8	12	6.6	490.63	27.98	2.65E+03	P02663|CASA2_BOVI	
GQVWEESLK	25.91	1074.5	9	-6.7	538.27	21.28	7.08E+03	P80025|PERL_BOVIN	
WEESLKRL	25.9	1059.6	8	-1.3	354.2	21.25	3.96E+03	P80025|PERL_BOVIN	
NLHLPLP	25.89	802.47	7	3.7	402.24	32.27	2.72E+03	P02666|CASB_BOVIN	
KVPQLEIVPNS(+79.97)AEERLH	25.84	2038	17	8.9	510.52	27.27	3.34E+03	P02662|CASA1_BOVI	Phosphorylation
KAVPYPQRDM(+15.99)PIQAFLL	25.81	2002.1	17	-1.8	668.36	36.01	1.86E+03	P02666|CASB_BOVIN	Oxidation (M)
GQVWEESLKRL	25.77	1343.7	11	1.6	448.92	28.33	2.28E+03	P80025|PERL_BOVIN	
ELEEL	25.76	631.31	5	1.4	632.32	18.16	1.56E+05	P02666|CASB_BOVIN :Q9TU23|CE290_BOVI	
EIEEL	25.76	631.31	5	1.4	632.32	18.16	1.56E+05	P41541|USO1_BOVIN	
ELEEI	25.76	631.31	5	1.4	632.32	18.16	1.56E+05		
EIEEI	25.76	631.31	5	1.4	632.32	18.16	1.56E+05		
M(+15.99)PFPKYPVEPF	25.75	1366.7	11	-3.6	684.34	32.28	1.21E+04	P02666|CASB_BOVIN	Oxidation (M)
QSKVLPVPQK	25.68	1122.7	10	2.5	375.23	14.8	3.85E+03	P02666|CASB_BOVIN	
KT(+79.97)TLSSEAPTTQ	25.66	1342.6	12	2.1	672.31	13.79	4.92E+03	P80025|PERL_BOVIN	Phosphorylation
VPPFLQPEV	25.63	1024.6	9	2.2	513.29	32.83	2.40E+03	P02666|CASB_BOVIN	
ALLDPS	25.62	614.33	6	-6.3	615.33	16.69	5.94E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
ALIDPS	25.62	614.33	6	-6.3	615.33	16.69	5.94E+03		
SSRQPQSQNPKLPLSILKEKHL	25.61	2527.4	22	2.1	506.49	26.17	5.71E+03	P80195|GLCM1_BOVI	
AVPITPTLNRE	25.6	1209.7	11	-1.3	605.84	21.46	2.29E+03	P02663|CASA2_BOVI	
RPKHPIKHQGLPQEVL	25.6	1876.1	16	4.2	376.23	16.39	5.71E+03	P02662|CASA1_BOVI	
VAPFPEVFGKEKVN	25.49	1559.8	14	5.8	520.96	28.96	3.36E+03	P02662|CASA1_BOVI	
FVAPFPEVFGKEKVNEL	25.39	1949	17	-9.4	650.68	38.51	8.17E+03	P02662|CASA1_BOVI	
LPLPLLQS	25.38	879.54	8	18.3	440.79	36.14	4.36E+03	P02666|CASB_BOVIN	
AQFIRNL	25.34	860.49	7	-12.9	431.25	22.94	1.23E+03	P80195|GLCM1_BOVI	
KVFIFR	25.31	808.5	6	17	405.26	36.92	1.42E+04	E1BGH8|MMS22_BOVI	
N(+.98)ENLLRF	25.31	905.46	7	-10.5	453.73	29.24	9.44E+03	N	Deamidation (NQ)
ASDISLL	25.27	717.39	7	-12.1	718.39	26.85	1.72E+03	P02754|LACB_BOVIN	
SIIS(+79.97)QETYK	25.24	1147.5	9	-1.7	574.77	19.72	2.90E+03	N	(STY)
VPPFL	25.15	571.34	5	-7.5	572.34	29.47	0	P02666|CASB_BOVIN	
ILNKPEDETHLEAQPTDASAQ	25.15	2306.1	21	0.4	769.71	18.16	5.50E+03	N	

Peptide	-10lgP	Mass	Length	ppm	m/z	RT	Area	Accession	PTM
DM(+15.99)ES(+79.97)TEVFTKK	25.13	1409.6	11	10.7	470.87	13.89	1.78E+03	P02663|CASA2_BOVIN	Oxidation (M); Phosphorylation
LGYLE	25.09	593.31	5	9	594.32	19.12	0	N	
IGYLE	25.09	593.31	5	9	594.32	19.12	0	Q95KV1|IKKA_BOVIN	
ALNEINQF	25.08	947.47	8	-4	474.74	24.58	2.23E+03	P02663|CASA2_BOVI	
PVRGPFPIIV	25.04	1093.7	10	-4.6	547.84	37.06	1.64E+03	P02666|CASB_BOVIN	
PKYPVEPFTESQ	25.02	1420.7	12	-7	711.35	23.42	2.77E+03	P02666|CASB_BOVIN	
VSTLVPL	25.01	727.45	7	-0.8	728.46	30.16	7.72E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
FPEVFGKEKV	25.01	1178.6	10	-0.3	393.89	24.97	4.44E+03	P02662|CASA1_BOVI	
DTIAQAASTTTISDAVSK	24.99	1778.9	18	-7.5	890.45	24.52	4.64E+03	P80025|PERL_BOVIN	
ERYLGYLEQL	24.97	1282.7	10	-8.9	642.33	32.71	4.25E+03	N	
APSFSDIPNPIGSENSEKTTM(+15.99	24.93	2633.2	24	7.7	878.76	37.96	5.83E+03	N	Oxidation (M)
VDMEST(+79.97)EVFTK	24.92	1364.6	11	4.5	683.29	22.63	8.83E+03	P02663|CASA2_BOVI	Phosphorylation
LEQLL	24.91	614.36	5	-6.3	615.37	24.2	5.30E+03	P02662|CASA1_BOVIN:A7YWD2|IPO13_BO	
LEQIL	24.91	614.36	5	-6.3	615.37	24.2	5.30E+03		
LEQLI	24.91	614.36	5	-6.3	615.37	24.2	5.30E+03		
IEQLI	24.91	614.36	5	-6.3	615.37	24.2	5.30E+03		
IEQLL	24.91	614.36	5	-6.3	615.37	24.2	5.30E+03		
DMPIQAFLL	24.88	1046.5	9	4.3	524.28	44.89	3.08E+03	P02666|CASB_BOVIN	
LVPLA	24.82	511.34	5	1.2	512.35	20.32	3.63E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
IVPLA	24.82	511.34	5	1.2	512.35	20.32	3.63E+03		
LVPIA	24.82	511.34	5	1.2	512.35	20.32	3.63E+03		
SKVLPVPQK	24.82	994.62	9	7.9	332.55	14.6	9.63E+02	P02666|CASB_BOVIN	
IPGMG	24.8	473.23	5	4.4	474.24	18.48	0	N	
RGPFPIIV	24.8	897.54	8	-1.4	449.78	34.27	2.40E+03	P02666|CASB_BOVIN	
GSSKALVSTLVPLA	24.78	1341.8	14	0.4	671.9	34.73	7.24E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
QPTDASAQFIRNL	24.61	1459.7	13	-1.7	730.88	29.73	3.89E+03	N	
YPFPGPIPNSLPQ	24.61	1425.7	13	-2.7	713.87	34.59	6.85E+03	P02666|CASB_BOVIN	
SLPQNIPPLTQTPVVVPPFLQPEVM	24.58	2756.5	25	-2.9	919.84	45.72	1.16E+04	P02666|CASB_BOVIN	Oxidation (M)
VKLLTSLLKQ	24.54	1141.7	10	7.7	571.88	46.7	2.93E+04	P41541|USO1_BOVIN	
EESSSLL	24.48	763.36	7	1.7	764.37	18.21	5.31E+03	Q29466-2|VPP1_BOVIN:Q29466|VPP1_BOVIN	
NPKLPLSIL	24.46	993.62	9	2.2	497.82	36.14	2.12E+03	N	
VRGPFPIIV	24.45	996.61	9	5.9	499.32	36.15	5.34E+03	P02666|CASB_BOVIN	
VDM(+15.99)EST(+79.97)EVFTK	24.43	1380.6	11	1.2	691.29	16.32	6.13E+03	P02663|CASA2_BOVIN	Oxidation (M); Phosphorylation
HPFAQTQSL	24.42	1027.5	9	-4.5	514.76	17.48	3.47E+03	P02666|CASB_BOVIN	

m/z = mass to charge ratio; RT = retention time; PTM = post-translational modification

Table A1.4. Peptide sequences identified in delactosed permeate from production plant 1, batch D (Chapter IV)

Peptide	-10lg	Mass	Length	ppm	m/z	RT	Area	Accession	PTM
YQEPVLGPVRGPFPIIV	75.1	1880.06	17	5.1	941.04	44.04	6.53E+05	P02666|CASB_BOVIN	
YQEPVLGPVRGPFPII	71.4	1780.99	16	4.7	891.505	42.43	1.61E+05	P02666|CASB_BOVIN	
EPVLGPVRGPFPIIV	64.6	1588.93	15	3	795.479	42.89	1.19E+05	P02666|CASB_BOVIN	
YQEPVLGPVRGPFPI	63.4	1667.9	15	-3.2	834.956	38.79	1.06E+05	P02666|CASB_BOVIN	
QEPVLGPVRGPFPIIV	62	1716.99	16	2.8	859.509	42.82	6.89E+04	P02666|CASB_BOVIN	
YQEPVLGPVRGPFP	60.7	1554.82	14	-0.2	778.417	33.3	9.75E+04	P02666|CASB_BOVIN	
KVLPVPQ	60.2	779.491	7	2.4	390.755	17.42	7.29E+03	P02666|CASB_BOVIN	
HQGLPQEVLNENLLR	59	1758.94	15	-2.8	587.318	30.85	1.26E+04	P02662|CASA1_BOVIN	
VLPVPQKAVPYPQ	58.6	1434.82	13	3	718.421	25.41	3.36E+04	P02666|CASB_BOVIN	
LLYQEPVLGPVRGPFPIIV	58.4	2106.22	19	3.4	1054.12	46.32	3.10E+04	P02666|CASB_BOVIN	
DAAGGPGAPADPGRPT	58.1	1405.66	16	-3.3	703.834	12.33	1.92E+04	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
AQPTDASAQFIR	58	1303.65	12	8.7	435.562	19.38	3.95E+04	P80195|GLCM1_BOVIN	
QEPVLGPVRGPFPII	57.5	1617.92	15	4.2	809.973	41.33	1.67E+04	P02666|CASB_BOVIN	
GLPQEVLNENLLR	56.5	1493.82	13	-1.5	747.919	33.45	1.52E+05	P02662|CASA1_BOVIN	
FVAPFPEVFGKEK	55.1	1493.79	13	-0.3	498.939	33.78	1.14E+05	P02662|CASA1_BOVIN	
GLPQEVLNENLLRF	54.5	1640.89	14	0	821.452	41.46	6.32E+04	P02662|CASA1_BOVIN	
SDIPNPIGSENSE	54	1357.6	13	0.4	679.81	21.94	1.45E+04	P02662|CASA1_BOVIN	
SQNPKLPLSIL	53.3	1208.71	11	9.4	605.369	35.53	1.43E+05	P80195|GLCM1_BOVIN	
EAQPTDASAQF	52.5	1163.51	11	1.4	1164.52	16.86	6.71E+04	P80195|GLCM1_BOVIN	
AQPTDASAQF	52.1	1034.47	10	-2.2	1035.48	15.8	2.10E+05	P80195|GLCM1_BOVIN	
LLYQEPVLGPVRGPFP	51.8	1780.99	16	0.9	891.502	38.36	6.98E+03	P02666|CASB_BOVIN	
VAPFPEVFGKEK	51.8	1346.72	12	6.1	449.918	27.55	8.25E+04	P02662|CASA1_BOVIN	
LYQGPIVLNPWDQVKR	51.7	1925.05	16	6	642.697	33.83	2.78E+04	P02663|CASA2_BOVIN	
LLYQEPVLGPVRGPFPI	51.6	1894.07	17	-2.9	948.044	42.66	9.60E+03	P02666|CASB_BOVIN	
VAPFPEVFGK	51.2	1089.59	10	1.9	545.803	31.1	2.49E+05	P02662|CASA1_BOVIN	
GPIVLNPWDQVK	50.8	1364.75	12	-5.7	683.378	32.26	2.87E+03	P02663|CASA2_BOVIN	
FVAPFPEVFG	50.5	1108.56	10	3.7	555.289	41.59	1.54E+04	P02662|CASA1_BOVIN	
PVLGPVRGPFPIIV	50.5	1459.89	14	-0.7	730.955	42.86	1.43E+04	P02666|CASB_BOVIN	
TVQVTSTAV	50.1	904.487	9	-2	905.492	16.59	2.60E+05	P02668|CASK_BOVIN	
FVAPFPEVFGK	50.1	1236.65	11	1.9	619.336	37.33	2.10E+05	P02662|CASA1_BOVIN	
APFPEVFGK	49.3	990.517	9	-0.1	496.266	28.35	7.13E+04	P02662|CASA1_BOVIN	
DASAQFIRNL	49.3	1133.58	10	2.8	567.8	28.17	2.30E+04	P80195|GLCM1_BOVIN	
ILNKPEDETHL	49.1	1307.67	11	8.9	436.902	17.02	2.62E+05	P80195|GLCM1_BOVIN	
LPVPQKAVPYPQ	48.9	1335.76	12	-8.4	668.882	23.3	1.39E+04	P02666|CASB_BOVIN	
LPQEVLNENLLRF	48.9	1583.87	13	14.1	528.97	39.1	1.29E+04	P02662|CASA1_BOVIN	
SQNPKLPLSI	48.7	1095.63	10	-3.3	548.822	29.03	1.26E+04	P80195|GLCM1_BOVIN	
VQVTSTAV	48.6	803.439	8	-1.2	804.445	15.19	9.85E+03	P02668|CASK_BOVIN	
TQTPVVVPPFLQPEVM(+15.99)	48.6	1796.94	16	1.8	899.478	39.91	3.36E+04	P02666|CASB_BOVIN	Oxidation (M)
VDMESTEVFTK	48.6	1284.59	11	0	643.303	22.1	7.97E+03	P02663|CASA2_BOVIN	
LLYQEPVLGPVRGPFPII	48.6	2007.16	18	0.2	1004.59	45.54	3.07E+03	P02666|CASB_BOVIN	
DMPIQAF	48.4	820.379	7	2.7	821.388	28.98	1.45E+04	P02666|CASB_BOVIN	
SSSEESITRIN	48.4	1221.58	11	1	611.802	14.95	9.85E+03	P02666|CASB_BOVIN	
LYQEPVLGPVRGPFPIIV	48.2	1993.14	18	4.3	997.582	45.31	4.59E+03	P02666|CASB_BOVIN	
RELEELNVPGEIVESL	48.2	1824.95	16	1.9	913.482	39.7	3.47E+04	P02666|CASB_BOVIN	
SHAFEVVKT	47.8	1016.53	9	2.2	509.273	15.59	1.58E+04	P80195|GLCM1_BOVIN	
SLSQSKVLPVPQK	47.8	1409.82	13	5.6	470.951	19.35	1.24E+04	P02666|CASB_BOVIN	
SSEESITRIN	47.7	1134.55	10	7.7	568.288	14.75	2.74E+03	P02666|CASB_BOVIN	
VVPPFLQPEVM(+15.99)	47.6	1270.66	11	4.2	1271.68	33.35	1.06E+05	P02666|CASB_BOVIN	Oxidation (M)
SVLSLSQS	47.5	819.434	8	-6.2	820.439	20.42	6.56E+03	P02666|CASB_BOVIN	
VLNENLLRF	47.4	1116.63	9	15.4	559.33	33.17	9.34E+03	P02662|CASA1_BOVIN	
SLVYPFPGPIPN	47.3	1299.69	12	1.4	650.854	36.71	1.13E+04	P02666|CASB_BOVIN	
ILNKPEDETHLE	47.3	1436.71	12	0.6	719.368	15.89	6.09E+05	P80195|GLCM1_BOVIN	
VPPFLQPEVM	47.3	1155.6	10	-1.1	578.807	35.67	3.10E+04	P02666|CASB_BOVIN	
PPPPPPPPPP	47.1	988.538	10	3.7	495.28	16.68	1.32E+04	A2VDK6|WASF2_BOVIN	
SSRQPQSQNPKLPL	47	1578.85	14	0.1	527.292	20.02	7.49E+04	P80195|GLCM1_BOVIN	
NVPGEIVESL	46.9	1055.55	10	1.2	1056.56	30.89	1.79E+05	P02666|CASB_BOVIN	
APFPEVFGKEK	46.8	1247.65	11	3.6	416.895	24.74	1.13E+04	P02662|CASA1_BOVIN	
ILNKPEDETHLEAQPTDASAQFIRNL	46.8	2949.48	26	7.5	738.386	32.12	3.72E+04	P80195|GLCM1_BOVIN	
PPPPPPPPP	46.6	891.485	9	-2.5	446.749	15.47	4.40E+04	A2VDK6|WASF2_BOVIN	
EPVLGPVRGPFPII	46.4	1489.87	14	-1.9	745.941	41.45	4.21E+04	P02666|CASB_BOVIN	
FVAPFPEVF	46.2	1051.54	9	1.4	1052.55	42.37	3.70E+04	P02662|CASA1_BOVIN	

Peptide	-10lg	Mass	Length	ppm	m/z	RT	Area	Accession	PTM
HLPLPLLQSW	46.1	1202.68	10	12.6	602.358	42.79	3.44E+03	P02666|CASB_BOVIN	
ILNKPEDETHLEAQPTDASAQFIR	46.1	2722.36	24	4.4	681.602	24.76	4.13E+04	P80195|GLCM1_BOVIN	
DLISKEQIVIR	46	1312.77	11	4	438.6	24.84	2.30E+04	P80195|GLCM1_BOVIN	
FPEVFGK	45.5	822.428	7	7.2	412.224	24.44	1.75E+04	P02662|CASA1_BOVIN	
VAPFPEVFG	45.5	961.491	9	4.5	962.502	35.53	4.32E+04	P02662|CASA1_BOVIN	
ILNKPEDETHLEAQPTDASAQF	45.4	2453.17	22	2.1	818.736	23.67	9.65E+04	P80195|GLCM1_BOVIN	
RELEELNVPGEIVE	45.2	1624.83	14	-0.3	813.425	29.3	1.34E+05	P02666|CASB_BOVIN	
NAVPITPTLN	45.1	1038.57	10	-8.4	520.288	24.1	1.61E+03	P02663|CASA2_BOVIN	
VPPFLQPEVM(+15.99)	45	1171.59	10	5.4	586.81	30.21	1.10E+05	P02666|CASB_BOVIN	Oxidation (M)
ASAQFIRNL	44.9	1018.56	9	-2.4	510.284	24.76	5.68E+03	P80195|GLCM1_BOVIN	
NENLLRFF	44.8	1051.55	8	-4	526.78	37.8	1.00E+04	P02662|CASA1_BOVIN	
ALLDPSFF	44.8	908.464	8	-3	909.469	39.1	3.31E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
APPPPPPPP	44.6	865.47	9	5.7	433.746	14.97	7.20E+03	A2VDK6|WASF2_BOVIN	
VDMES(+79.97)TEVFTK	44.4	1364.56	11	6.2	683.29	22.53	9.37E+03	P02663|CASA2_BOVIN	Phosphorylation (STY)
ALLDPSFFAK	44.4	1107.6	10	1.9	554.807	33.95	9.75E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
EVLNENLLRF	44.3	1245.67	10	-2.4	623.844	34.93	4.97E+04	P02662|CASA1_BOVIN	
APFPEVFG	44.2	862.423	8	-0.6	863.429	32.96	1.65E+04	P02662|CASA1_BOVIN	
GLPQEVLNENLL	44.2	1337.72	12	-3	669.865	36.69	1.23E+05	P02662|CASA1_BOVIN	
QEPVLGPVRGPFPI	43.9	1504.84	14	0.4	753.428	37.43	9.48E+03	P02666|CASB_BOVIN	
AAGGPGAPADPGRPT	43.9	1290.63	15	0.6	646.324	11.81	5.17E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
SQNPKLPLS	43.8	982.545	9	-11.1	492.274	19.11	2.66E+04	P80195|GLCM1_BOVIN	
LLYQEPVL	43.7	973.548	8	-9.4	974.547	30.82	4.23E+03	P02666|CASB_BOVIN	
AQPTDASAQFIRNL	43.7	1530.78	14	4.1	511.269	30.61	1.35E+04	P80195|GLCM1_BOVIN	
DTIAQAASTTTISDAVSK	43.6	1778.89	18	-3.7	890.452	24.37	8.33E+03	P80025|PERL_BOVIN	
VAPFPEVF	43.5	904.469	8	-4.3	905.476	36.43	7.17E+04	P02662|CASA1_BOVIN	
VIESPPEIN	43.4	996.513	9	-1.3	997.519	19.94	6.39E+04	P02668|CASK_BOVIN	
GPVRGPFPII	43.4	1051.62	10	3.2	526.818	34.3	4.12E+04	P02666|CASB_BOVIN	
VDM(+15.99)ES(+79.97)TEVFTK	43.2	1380.55	11	5.9	691.287	16.32	1.29E+04	P02663|CASA2_BOVIN	Oxidation (M); Phosphorylation (STY)
APFPEVFGKE	43.1	1119.56	10	-2.2	560.788	28.72	3.09E+03	P02662|CASA1_BOVIN	
DIQKVAGTWYSL	43.1	1379.71	12	-0.1	690.862	32.59	6.24E+03	P02754|LACB_BOVIN	
DAQSAPLRVY	42.8	1118.57	10	1.7	560.294	20.26	3.56E+03	P02754|LACB_BOVIN	
VLSRYPSYGLN	42.8	1267.66	11	2	634.837	21.59	4.41E+03	P02668|CASK_BOVIN	
NVPGEIVE	42.7	855.434	8	-6.2	856.439	18.45	2.02E+04	P02666|CASB_BOVIN	
VDM(+15.99)ESTEVFTK	42.7	1300.59	11	3.2	651.304	16.45	1.59E+04	P02663|CASA2_BOVIN	Oxidation (M)
HIQKEDVPSERYL	42.7	1612.82	13	6.4	538.618	18.4	7.42E+04	P02662|CASA1_BOVIN	
PVVVPPFLQPEVM(+15.99)	42.4	1466.78	13	13.3	734.409	39.39	1.67E+03	P02666|CASB_BOVIN	Oxidation (M)
VSREGQEQEGEEM(+15.99)AEYR	42.4	2041.86	17	5.8	681.633	11.52	2.92E+03	P18892|BT1A1_BOVIN	Oxidation (M)
SKVLPVPQ	42.4	866.523	8	-3.6	434.269	18.45	2.39E+04	P02666|CASB_BOVIN	
VPQLEIVPNSAEER	42.3	1579.82	14	17	790.931	25.99	0	P02662|CASA1_BOVIN	
SQSKVLPVPQ	42.3	1081.61	10	2.3	541.815	18.93	5.22E+04	P02666|CASB_BOVIN	
PVLGPVRGPFPII	42.2	1360.82	13	0.3	681.419	41.36	3.16E+03	P02666|CASB_BOVIN	
NAVPITPT	41.9	811.444	8	-2.3	812.452	17.24	2.36E+04	P02663|CASA2_BOVIN	
IVTQTMKGL	41.8	989.558	9	5	495.789	20.32	6.35E+03	P02754|LACB_BOVIN	
SQNPKLPL	41.8	895.513	8	7.1	448.767	21.59	5.19E+04	P80195|GLCM1_BOVIN	
NAVPITPTL	41.7	924.528	9	4.4	463.275	27.78	3.75E+03	P02663|CASA2_BOVIN	
NLHLPLPLLQ	41.5	1156.7	10	3.7	579.358	42.07	1.41E+04	P02666|CASB_BOVIN	
VVPPFLQPEVM	41.1	1254.67	11	0.2	628.344	38.33	3.14E+04	P02666|CASB_BOVIN	
APFPEVFGKEKV	41	1346.72	12	7.1	449.918	28.29	1.57E+04	P02662|CASA1_BOVIN	
SLSQSKVLPVPQKAVPYPQ	40.8	2065.16	19	0.4	689.393	26.26	7.74E+03	P02666|CASB_BOVIN	
DASAQFIR	40.7	906.456	8	1	454.236	16.07	1.37E+04	P80195|GLCM1_BOVIN	
YQEPVLGPVR	40.7	1156.62	10	-11.6	579.313	21.83	3.54E+03	P02666|CASB_BOVIN	
VPQLEIVPN	40.7	1007.57	9	-0.1	1008.58	27.87	3.98E+04	P02662|CASA1_BOVIN	
GLPQEVLN	40.6	868.465	8	2.6	869.478	22.23	8.92E+04	P02662|CASA1_BOVIN	
TVDM(+15.99)ESTEVFTK	40.6	1401.63	12	-3	701.824	17.83	1.54E+04	P02663|CASA2_BOVIN	Oxidation (M)
EM(+15.99)PFPKYPVEPF	40.5	1495.71	12	2.7	748.862	32.61	1.18E+04	P02666|CASB_BOVIN	Oxidation (M)
ALLDPSFFAKESVK	40.5	1550.83	14	6.3	517.957	32.82	4.92E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
HQGLPQEVL	40.4	1019.54	9	-12.2	510.771	22.02	2.48E+04	P02662|CASA1_BOVIN	
PPLLLLSA	40.2	822.522	8	-5.4	412.266	38.55	2.52E+04	A7MB27|RHG36_BOVIN	

Peptide	-10lg	Mass	Length	ppm	m/z	RT	Area	Accession	PTM
EAQPTDASAQFIR	40.2	1432.69	13	1	717.355	20.04	7.45E+03	P80195|GLCM1_BOVIN	
EPVLGPVRGPFPI	40.1	1376.78	13	-6.2	689.396	37.46	1.53E+04	P02666|CASB_BOVIN	
YLEQLLRL	40.1	1046.61	8	9	524.318	37.27	5.56E+03	P02662|CASA1_BOVIN	
TVDMESTEVFTK	40	1385.64	12	-11.9	693.821	23.03	3.18E+03	P02663|CASA2_BOVIN	
PPAPPPPPP	40	865.47	9	5.7	433.746	14.97	3.53E+03	A2VDK6|WASF2_BOVIN	
GYLEQLLRLK	39.6	1231.73	10	14	411.589	35.61	2.63E+03	P02662|CASA1_BOVIN	
LIVTQTM(+15.99)KGL	39.6	1118.64	10	6.8	560.332	20.82	4.70E+04	P02754|LACB_BOVIN	Oxidation (M)
PVEPFTESQ	39.5	1032.48	9	3.3	517.249	18.63	5.80E+03	P02666|CASB_BOVIN	
GYLEQLL	39.5	834.449	7	-5.1	835.455	34.25	1.73E+03	P02662|CASA1_BOVIN	
GPFPIIV	39.5	741.443	7	-7.2	742.447	37.75	7.86E+03	P02666|CASB_BOVIN	
LIVTQTMKGLDIQ	39.4	1458.81	13	0.3	730.413	31.23	6.44E+03	P02754|LACB_BOVIN	
PPPPPPPP	39.4	794.433	8	1	398.225	14.22	2.65E+04	A2VDK6|WASF2_BOVIN	
VPPFLQPEV	39.3	1024.56	9	5.1	513.29	32.7	1.46E+03	P02666|CASB_BOVIN	
LIVTQTMKGL	39.2	1102.64	10	1.5	552.331	27.4	1.98E+04	P02754|LACB_BOVIN	
SQNPKLPLSILK	39.1	1336.81	12	1.8	446.612	28.92	6.56E+03	P80195|GLCM1_BOVIN	
ELEELNVPGEIVE	39.1	1468.73	13	8.9	735.379	31.68	9.00E+03	P02666|CASB_BOVIN	
VAGTWYSL	39.1	895.444	8	-3.6	896.451	30.35	1.36E+04	P02754|LACB_BOVIN	
LPQEVLNENLL	38.9	1280.7	11	3	641.36	33.68	2.16E+04	P02662|CASA1_BOVIN	
LPLPLLQSW	38.9	1065.62	9	-0.6	533.82	45.57	3.97E+03	P02666|CASB_BOVIN	
NIPPLTQTPV	38.9	1078.6	10	7.3	540.314	26.8	3.83E+04	P02666|CASB_BOVIN	
VPPFLQPEVM(+15.99)GV	38.8	1327.68	12	1.5	664.851	34.35	1.05E+04	P02666|CASB_BOVIN	Oxidation (M)
FVAPFPEVFGKEKV	38.7	1592.86	14	10.1	531.966	36.18	2.47E+04	P02662|CASA1_BOVIN	
TLTDVENL	38.7	903.455	8	0.3	904.466	23.76	8.99E+03	P02666|CASB_BOVIN	
VYPFPGPIPN	38.6	1099.57	10	5.9	1100.58	31.87	5.48E+03	P02666|CASB_BOVIN	
EELNVPGEIVESL	38.6	1426.72	13	2.8	714.369	38.57	6.19E+03	P02666|CASB_BOVIN	
LNENLLRF	38.4	1017.56	8	-4.1	509.787	31.39	3.80E+03	P02662|CASA1_BOVIN	
YKVPQLEIVPN	38.4	1298.72	11	0.6	650.372	30.19	2.58E+04	P02662|CASA1_BOVIN	
SLSQSKVLPVPQ	38.3	1281.73	12	-3.7	641.872	23.12	9.45E+03	P02666|CASB_BOVIN	
DM(+15.99)PIQAF	38.2	836.374	7	-0.3	837.384	22.08	1.41E+04	P02666|CASB_BOVIN	Oxidation (M)
SSS(+79.97)EESITRIN	38	1301.55	11	-1.8	651.783	15.7	3.77E+03	P02666|CASB_BOVIN	Phosphorylation (STY)
FVAPFPE	38	805.401	7	-16.6	806.398	28.07	5.03E+04	P02662|CASA1_BOVIN	
TKVIPYVRY	38	1137.65	9	5.3	380.227	22.23	2.07E+03	P02663|CASA2_BOVIN	
SDIPNPIGSENSEK	37.9	1485.69	14	1.4	743.858	19.94	9.15E+03	P02662|CASA1_BOVIN	
APFPEVF	37.8	805.401	7	6.5	806.416	33.18	2.63E+04	P02662|CASA1_BOVIN	
SLPQNIPPLTQTPV	37.7	1503.83	14	-4.8	752.921	31.95	1.56E+04	P02666|CASB_BOVIN	
SKALVSTLVPLA	37.6	1197.73	12	4.2	599.876	34.25	3.29E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
YQGPIVLNPWDQVK	37.5	1655.87	14	1	828.945	34.41	2.74E+03	P02663|CASA2_BOVIN	
LPLALLPK	37.5	863.584	8	-11.1	432.796	41.43	2.11E+04	P50227|ST1A1_BOVIN	
HLPLPLLQS	37.1	1016.6	9	-5	509.307	33.84	2.43E+03	P02666|CASB_BOVIN	
PVEPFTE	37.1	817.386	7	-5.2	818.392	19.73	3.99E+03	P02666|CASB_BOVIN	
EPVLGPVRGPFP	37	1263.7	12	4.2	632.859	31.8	4.02E+04	P02666|CASB_BOVIN	
GQPAPLPLSLL	36.8	1104.65	11	1.4	1105.66	39.54	2.28E+03	Q2KI51|PR15A_BOVIN	
FPEVFGKEK	36.8	1079.57	9	-0.6	360.863	21.57	4.78E+03	P02662|CASA1_BOVIN	
AFEVVKT	36.8	792.438	7	3.4	397.229	17.39	5.97E+03	P80195|GLCM1_BOVIN	
SRQPQSQNPKLPLSIL	36.7	1805.02	16	-1.6	602.678	33.21	2.75E+03	P80195|GLCM1_BOVIN	
GLPQEVLNEN	36.6	1111.55	10	-0.1	556.783	23.21	1.09E+04	P02662|CASA1_BOVIN	
DMPIQA	36.6	673.311	6	-1.8	674.319	17.69	8.85E+03	P02666|CASB_BOVIN	
GYLEQL	36.5	721.365	6	-2.2	722.37	24.39	9.87E+03	P02662|CASA1_BOVIN	
SRQPQSQNPKLPL	36.5	1491.82	13	2.7	498.282	19.92	6.79E+03	P80195|GLCM1_BOVIN	
AAGGPGAPADPGRPTGYS	36.5	1597.75	18	-7.1	799.879	14.62	1.42E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
LVYPFPGPIPN	36.5	1212.65	11	7	607.339	35.73	4.74E+03	P02666|CASB_BOVIN	
NENLLRF	36.5	904.477	7	3.6	453.249	27.07	2.34E+05	P02662|CASA1_BOVIN	
EVLNENLLR	36.4	1098.6	9	-4.6	550.308	24.12	4.84E+04	P02662|CASA1_BOVIN	
VIESPPEINTVQ	36.3	1324.69	12	-5.7	663.35	23	1.84E+04	P02668|CASK_BOVIN	
SRNPDEEGLFTVR	36.3	1518.74	13	10.6	507.262	22.57	4.80E+03	P18892|BT1A1_BOVIN	
GLPQEVLNE	36.1	997.508	9	0.7	499.763	24.15	3.87E+04	P02662|CASA1_BOVIN	
GLPQEVLNENL	36	1224.64	11	3	613.327	31.12	1.29E+04	P02662|CASA1_BOVIN	
ALLDPSF	35.9	761.396	7	-2.3	762.402	30.05	7.59E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
LIVTQTM(+15.99)KGLDIQ	35.8	1474.81	13	3.3	738.416	25.1	1.15E+04	P02754|LACB_BOVIN	Oxidation (M)
DM(+15.99)PIQAFLL	35.8	1062.54	9	0.6	1063.55	38.76	9.86E+03	P02666|CASB_BOVIN	Oxidation (M)
GYLEQLLRL	35.8	1103.63	9	4.5	552.829	41.49	1.36E+03	P02662|CASA1_BOVIN	
GPVRGPFPIIV	35.8	1150.69	11	-2.8	576.351	36.9	1.91E+05	P02666|CASB_BOVIN	

Peptide	-10lg	Mass	Length	ppm	m/z	RT	Area	Accession	PTM
GPVRGPFP	35.7	825.45	8	5.9	413.736	20.36	1.86E+04	P02666|CASB_BOVIN	
DVENLHLPLPL	35.7	1258.69	11	9.6	630.359	41.44	2.26E+03	P02666|CASB_BOVIN	
TTLSSEAPTTQ	35.7	1134.54	11	2.6	568.279	13.67	0	P80025|PERL_BOVIN	
GLDIQKVAGTW	35.6	1186.63	11	-15.1	594.318	31.33	1.10E+03	P02754|LACB_BOVIN	
STLVPLA	35.5	699.417	7	-4.2	700.424	26.13	1.13E+04	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
HLPLPLLQ	35.5	929.57	8	10.6	465.797	34.76	1.10E+04	P02666|CASB_BOVIN	
EPVLGPVR	35.4	865.502	8	-0.5	433.76	17.68	5.81E+03	P02666|CASB_BOVIN	
IGVNQEL	35.3	771.413	7	-2.4	772.421	19.73	1.42E+03	P02662|CASA1_BOVIN	
GPVRGPFPI	35.3	938.534	9	4.4	470.276	28.47	9.87E+03	P02666|CASB_BOVIN	
IGVNQELAY	35.2	1005.51	9	-3.3	1006.52	23.65	9.86E+03	P02662|CASA1_BOVIN	
VAPFPEV	35.1	757.401	7	-4.6	758.407	27.59	4.19E+03	P02662|CASA1_BOVIN	
EDVPSERY	35.1	993.44	8	-4.4	497.725	13.44	1.55E+03	P02662|CASA1_BOVIN	
PEVIESPPEINTVQVTSTAV	35	2109.08	20	-1.1	704.037	32.18	1.89E+04	P02668|CASK_BOVIN	
MHQPHQPLPPT	34.9	1281.63	11	6.9	428.22	14.38	3.42E+03	P02666|CASB_BOVIN	
SVKDAAGGPGAPADPGRPT	34.9	1719.85	19	0.9	574.292	13.02	4.49E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
YQEPVL	34.8	747.38	6	0.1	748.388	21.41	2.16E+04	P02666|CASB_BOVIN	
GRVSLVEDHIAEGSVAVR	34.8	1893.01	18	-0.8	474.259	22.8	8.34E+02	P18892|BT1A1_BOVIN	
VLGPVRGPFPIIV	34.7	1362.84	13	1.3	682.43	41.55	2.26E+04	P02666|CASB_BOVIN	
VLPVPQKAVPYPQRDM(+15.99)PIQ	34.6	2409.29	21	-3.9	804.1	31.11	1.13E+04	P02666|CASB_BOVIN	Oxidation (M)
IHPFAQTQSL	34.6	1140.59	10	-2.1	571.303	22.68	7.25E+03	P02666|CASB_BOVIN	
DVPSERYL	34.6	977.482	8	2.8	489.75	19.87	2.73E+04	P02662|CASA1_BOVIN	
FVAPFPEVFGKE	34.5	1365.7	12	1	683.859	37.49	9.83E+03	P02662|CASA1_BOVIN	
IHPFAQTQ	34.5	940.477	8	7.1	471.249	14.84	2.16E+03	P02666|CASB_BOVIN	
LPQYL	34.5	632.353	5	-12.5	633.355	24.06	1.02E+03	P02663|CASA2_BOVIN	
IPQYI	34.5	632.353	5	-12.5	633.355	24.06	1.02E+03		
SVLSLSQSK	34.5	947.529	9	-1.2	474.771	17.43	4.99E+03	P02666|CASB_BOVIN	
YQGPIVLNPWDQVKR	34.4	1811.97	15	1	604.997	31.83	1.05E+04	P02663|CASA2_BOVIN	
GLPQEVL	34.4	754.423	7	1.4	755.433	25.32	1.48E+05	P02662|CASA1_BOVIN	
SDIPNPIGSE	34.3	1027.48	10	-4.2	514.746	22.69	2.11E+03	P02662|CASA1_BOVIN	
FALPQY	34.3	737.375	6	4.6	738.386	28.15	1.61E+03	P02663|CASA2_BOVIN	
ALNEINQFYQK	34.3	1366.69	11	-7.3	684.349	24.04	1.99E+03	P02663|CASA2_BOVIN	
SQSKVLPVPQK	34.2	1209.71	11	6.1	404.247	15.04	2.70E+03	P02666|CASB_BOVIN	
EVLNENLL	34.2	942.502	8	1.7	943.511	28.46	4.73E+04	P02662|CASA1_BOVIN	
VPQLEIVPNS(+79.97)AEERLH	34.2	1909.93	16	1.9	637.654	28.52	1.01E+04	P02662|CASA1_BOVIN	Phosphorylation (STY)
LGYLEQLLR	34.1	1103.63	9	1.4	552.825	35.15	1.07E+03	P02662|CASA1_BOVIN	
LNKPEDETHLE	34.1	1323.63	11	1.5	442.22	13.75	2.16E+04	P80195|GLCM1_BOVIN	
VAGTWYS	34.1	782.36	7	-3.6	783.367	18.59	7.34E+03	P02754|LACB_BOVIN	
EIPTINTIAS	34.1	1057.57	10	-0.7	529.79	26.63	3.02E+03	P02668|CASK_BOVIN	
KHQGLPQEVLNENLLRF	33.9	2034.1	17	-2.4	509.531	35.95	2.45E+03	P02662|CASA1_BOVIN	
AVPYPQ	33.9	673.344	6	-5.5	674.347	14.29	1.25E+03	P02666|CASB_BOVIN	
IPIQYVL	33.8	844.506	7	3.9	423.262	33.9	2.25E+03	P02668|CASK_BOVIN	
APFPEV	33.8	658.333	6	1.1	659.341	24.67	7.54E+03	P02662|CASA1_BOVIN	
ASTTTISDAVSK	33.8	1179.6	12	3.2	590.808	14.71	1.11E+03	P80025|PERL_BOVIN	
FPEVFG	33.7	694.333	6	-3	695.34	28.67	0	P02662|CASA1_BOVIN	
ESRNPDEEGLFTVR	33.7	1647.79	14	11.4	550.275	23.01	9.56E+03	P18892|BT1A1_BOVIN	
EVLNENL	33.7	829.418	7	-2.2	830.427	20.57	1.95E+03	P02662|CASA1_BOVIN	
HQGLPQEVLNENLL	33.7	1602.84	14	-2.7	802.423	33.72	5.58E+03	P02662|CASA1_BOVIN	
SLPQNIPPLTQTPVVVPPFLQPEVM	33.6	2756.48	25	0.8	919.835	45.71	1.35E+04	P02666|CASB_BOVIN	Oxidation (M)
DLIS(+79.97)KEQIVIR	33.5	1392.74	11	14.5	465.26	27.85	2.12E+04	P80195|GLCM1_BOVIN	Phosphorylation (STY)
VAPFPE	33.5	658.333	6	3.6	659.342	19.84	3.19E+04	P02662|CASA1_BOVIN	
LPQEVLNENLLR	33.5	1436.8	12	3.7	719.409	30.46	2.13E+03	P02662|CASA1_BOVIN	
VAPFPEVFGKE	33.5	1218.63	11	9.1	610.327	31.62	7.53E+03	P02662|CASA1_BOVIN	
ALPQYL	33.4	703.39	6	-3.6	704.398	25.77	8.33E+03	P02663|CASA2_BOVIN	
VLPVPQ	33.2	651.396	6	-0.8	652.402	18.9	2.48E+04	P02666|CASB_BOVIN	
PVEPF	33.1	587.296	5	2.2	588.306	19.97	9.51E+03	P02666|CASB_BOVIN	
MPFPKYPVEPF	33.1	1350.67	11	0.1	676.341	35.8	8.47E+03	P02666|CASB_BOVIN	
PEVIESPPEIN	33.1	1222.61	11	-18.5	612.302	23.54	1.79E+03	P02668|CASK_BOVIN	
GTWYSL	33	725.338	6	0.9	726.349	28.79	1.06E+04	P02754|LACB_BOVIN	
AGEIQNKALLD	33	1170.62	11	0.8	586.32	18.31	6.41E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
HQGLPQEVLN	33	1133.58	10	-13.2	567.793	20.08	1.24E+04	P02662|CASA1_BOVIN	
ALNEINQF	32.9	947.471	8	-7.7	948.471	24.42	4.92E+03	P02663|CASA2_BOVIN	
VIPYVRY	32.9	908.512	7	-6.8	455.26	23.93	1.91E+03	P02663|CASA2_BOVIN	
VLGPVRGPFPII	32.9	1263.77	12	2.6	632.894	39.54	3.31E+03	P02666|CASB_BOVIN	

Peptide	-10lg	Mass	Length	ppm	m/z	RT	Area	Accession	PTM
EVIESPPEINTVQVTSTAV	32.9	2012.03	19	2.6	1007.03	31.23	4.73E+04	P02668|CASK_BOVIN	
YQKFPQYLQY	32.9	1376.68	10	1	689.346	28	5.18E+03	P02663|CASA2_BOVIN	
FVAPFPEV	32.8	904.469	8	3	905.479	35	2.28E+03	P02662|CASA1_BOVIN	
ALLDPSFFAKES	32.8	1323.67	12	-0.7	662.845	33.79	2.83E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
ALLDPSFFAKESVKD	32.8	1665.86	15	11.2	556.303	32.69	4.60E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
IVTQTM(+15.99)KGLDIQ	32.8	1361.72	12	8.2	681.874	19.18	4.48E+03	P02754|LACB_BOVIN	Oxidation (M)
VIESPPEINTVQVTSTAV	32.7	1882.99	18	5.6	942.507	30.4	3.23E+03	P02668|CASK_BOVIN	
VAPFPEVFGKEKV	32.7	1445.79	13	2.6	482.941	30.72	2.95E+04	P02662|CASA1_BOVIN	
RDMPIQAF	32.6	976.48	8	1.9	489.25	24.71	1.45E+04	P02666|CASB_BOVIN	
SVLSLSQ	32.4	732.402	7	-4.9	733.406	20.68	3.41E+03	P02666|CASB_BOVIN	
VLNENLL	32.4	813.46	7	-3	814.467	25.94	2.39E+03	P02662|CASA1_BOVIN	
TLVPLA	32.3	612.385	6	1.5	613.393	25.37	1.96E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
FGKKRK	32.2	762.486	6	9.1	763.503	44.29	5.98E+03	Q1JQD9|LMBL2_BOVIN	
GLDIQKVAG	32.2	899.508	9	3.1	450.764	19.77	1.42E+03	P02754|LACB_BOVIN	
KVPQLEIVPN	32.1	1135.66	10	1.1	568.84	26.05	2.73E+04	P02662|CASA1_BOVIN	
FSHAFEVVKT	32.1	1163.6	10	8.2	388.876	20.91	5.33E+03	P80195|GLCM1_BOVIN	
FPEVF	32.1	637.311	5	-3.2	638.319	30.27	1.34E+04	P02662|CASA1_BOVIN	
IPIQY	32.1	632.353	5	-0.9	633.36	20.87	7.49E+03	P02668|CASK_BOVIN	
LPLQY	32.1	632.353	5	-0.9	633.36	20.87	7.49E+03		
IPLQY	32.1	632.353	5	-0.9	633.36	20.87	7.49E+03		
IPYVRYL	32	922.528	7	3.7	462.274	28.81	1.39E+03	P02663|CASA2_BOVIN	
ALPQYLK	31.9	831.485	7	8	416.753	19.92	3.28E+04	P02663|CASA2_BOVIN	
AVPITPT	31.8	697.401	7	-5.9	698.407	15.84	4.20E+03	P02663|CASA2_BOVIN	
DKTEIPTIN	31.8	1029.53	9	-1.2	515.776	19.35	1.34E+04	P02668|CASK_BOVIN	
DMPIQAFLL	31.8	1046.55	9	7.7	524.287	44.77	4.81E+03	P02666|CASB_BOVIN	
VSTLVPLA	31.8	798.485	8	-1.1	400.249	28.82	3.16E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
SLVYPFPGPIPNSLPQ	31.6	1724.91	16	1.9	863.466	39.87	5.64E+03	P02666|CASB_BOVIN	
VLNENLLR	31.6	969.561	8	4.8	485.79	21.73	2.62E+03	P02662|CASA1_BOVIN	
VPQLEIVPNS(+79.97)AEERLHSM(+	31.5	2144	18	-2.9	715.674	28.33	1.61E+04	P02662|CASA1_BOVIN	Phosphorylation (STY); Oxidation (M)
FFVAPFPEVFGK	31.5	1383.72	12	13.7	692.878	42.5	2.09E+03	P02662|CASA1_BOVIN	
EDVPSERYL	31.4	1106.52	9	2	554.271	20.54	5.03E+03	P02662|CASA1_BOVIN	
ALLDPSFFAKE	31.4	1236.64	11	3.2	619.329	34.42	7.74E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
FVAPFPEVFGKEKVN	31.4	1706.9	15	-5.5	569.974	34.47	3.33E+03	P02662|CASA1_BOVIN	
N(+.98)AVPITPT	31.4	812.428	8	-7.8	813.432	18.45	9.15E+03	P02663|CASA2_BOVIN	Deamidation (NQ)
LPQEVL	31.4	697.401	6	-3.3	698.406	22.49	9.22E+03	P02662|CASA1_BOVIN	
IPQEVL	31.4	697.401	6	-3.3	698.406	22.49	9.22E+03		
M(+15.99)EST(+79.97)EVFTK	31.2	1166.46	9	18.7	584.247	14.62	2.56E+03	P02663|CASA2_BOVIN	Oxidation (M); Phosphorylation (STY)
VPQLEIVPNS(+79.97)AEERLHSMK	31.2	2256.1	19	3.7	565.036	29.48	2.93E+03	P02662|CASA1_BOVIN	Phosphorylation (STY)
NAVPITPTLNREQL	31.1	1564.86	14	-1	783.435	27.54	4.62E+03	P02663|CASA2_BOVIN	
VRGPFPIIV	31.1	996.612	9	1.4	499.314	35.97	2.61E+03	P02666|CASB_BOVIN	
LVSTLVPLA	31	911.569	9	7.3	456.795	33.39	1.82E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
SSRQPQSQNPKLPLS	31	1665.88	15	5.4	556.304	18.18	1.02E+04	P80195|GLCM1_BOVIN	
PVRGPFPIIV	31	1093.66	10	-6.6	547.836	36.88	3.31E+03	P02666|CASB_BOVIN	
LGPVRGPFPIIV	30.9	1263.77	12	-0.6	632.894	40.56	3.51E+03	P02666|CASB_BOVIN	
LYQEPVLGPVRGPFP	30.9	1667.9	15	6.5	834.964	35.15	2.23E+03	P02666|CASB_BOVIN	
SQNPKLPLSILKEK	30.8	1593.95	14	2.9	399.496	25.85	1.80E+03	P80195|GLCM1_BOVIN	
TVDM(+15.99)EST(+79.97)EVFTK	30.8	1481.6	12	1.6	741.811	17.37	6.46E+03	P02663|CASA2_BOVIN	Oxidation (M); Phosphorylation (STY)

Peptide	-10lg	Mass	Length	ppm	m/z	RT	Area	Accession	PTM
HIQKEDVPSERY	30.7	1499.74	12	10.5	500.925	13.27	3.67E+03	P02662|CASA1_BOVIN	
FPEVFGKEKV	30.7	1178.63	10	-4.2	393.884	24.76	1.80E+03	P02662|CASA1_BOVIN	
KHQGLPQEVLN	30.7	1261.68	11	5.7	421.569	18.05	1.72E+04	P02662|CASA1_BOVIN	
LEQLLRL	30.6	883.549	7	0.2	442.783	30.72	3.28E+03	P02662|CASA1_BOVIN	
PGRPTGYSGSSKAL	30.6	1376.7	14	-1	459.908	13.44	4.51E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
KAVPYPQRDMPIQAF	30.6	1759.91	15	1.4	587.646	28.4	3.24E+03	P02666|CASB_BOVIN	
SLTLTDVE	30.5	876.444	8	-5	877.447	23.96	1.23E+03	P02666|CASB_BOVIN	
TKVIPYV	30.5	818.49	7	10	410.258	23.68	0	P02663|CASA2_BOVIN	
AVESTVATL	30.4	889.476	9	0.3	890.483	20.82	2.75E+03	P02668|CASK_BOVIN	
RDMPIQAFL	30.4	1089.56	9	-0.7	545.791	34.7	3.14E+03	P02666|CASB_BOVIN	
VDM(+15.99)EST(+79.97)EVFTK	30.4	1380.55	11	0.7	691.286	16.27	3.82E+03	P02663|CASA2_BOVIN	Oxidation (M); Phosphorylation (STY)
EIVESL	30.4	688.364	6	-6.1	689.367	21.11	2.52E+03	P02666|CASB_BOVIN	
DMPIQAFL	30.3	933.463	8	-4.3	467.737	39.82	1.40E+03	P02666|CASB_BOVIN	
LPLSIL	30.3	654.432	6	-1.1	655.438	35.79	3.82E+03	P80195|GLCM1_BOVIN	
IPISLL	30.3	654.432	6	-1.1	655.438	35.79	3.82E+03		
LPLSLL	30.3	654.432	6	-1.1	655.438	35.79	3.82E+03	Q2KI51|PR15A_BOVIN	
IPLSII	30.3	654.432	6	-1.1	655.438	35.79	3.82E+03		
IPLSLL	30.3	654.432	6	-1.1	655.438	35.79	3.82E+03		
LPLSII	30.3	654.432	6	-1.1	655.438	35.79	3.82E+03		
LPISIL	30.3	654.432	6	-1.1	655.438	35.79	3.82E+03		
AVPYPQRDM(+15.99)PIQA	30.2	1500.74	13	-2.8	751.378	19.86	6.03E+03	P02666|CASB_BOVIN	Oxidation (M)
LPQEVLN	30.1	811.444	7	-4.7	812.45	18.73	0	P02662|CASA1_BOVIN	
ALPQY	30	590.306	5	1.5	591.317	16.52	5.60E+03	P02663|CASA2_BOVIN	
AIPQY	30	590.306	5	1.5	591.317	16.52	5.60E+03		
HKEMPFPKYPVEPF	30	1744.86	14	-0.2	582.629	30.78	5.22E+03	P02666|CASB_BOVIN	
AKLKSTRGRALRIL	29.9	1582.02	14	2.8	528.347	48.57	0	Q58CW4|NMUR2_BOVIN	
HIQKEDVPSER	29.8	1336.67	11	-11.4	446.56	9.51	1.11E+02	P02662|CASA1_BOVIN	
LGYLEQL	29.8	834.449	7	-2.7	835.457	29.45	0	P02662|CASA1_BOVIN	
LVEDHIAEGSVAVR	29.8	1493.78	14	3.5	498.939	19.3	4.68E+03	P18892|BT1A1_BOVIN	
IEKFQS(+79.97)EEQQQ	29.7	1472.62	11	-0.5	737.319	12.58	1.81E+03	P02666|CASB_BOVIN	Phosphorylation (STY)
MAIPPKKNQD	29.6	1140.6	10	-3.1	381.206	11.4	1.38E+03	P02668|CASK_BOVIN	
SQSKVLPVPQKAVPYPQ	29.5	1865.04	17	-2.6	622.686	23.79	9.13E+03	P02666|CASB_BOVIN	
AYFYPE	29.4	788.338	6	7.9	395.179	22.21	6.69E+02	P02662|CASA1_BOVIN	
FFVAPFPE	29.4	952.469	8	16.2	477.25	36.13	0	P02662|CASA1_BOVIN	
VAPFP	29.4	529.29	5	8.2	530.302	20.93	3.37E+03	P02662|CASA1_BOVIN	

m/z = mass to charge ratio; RT = retention time; PTM = post-translational modification

Table A1.5. Peptide sequences identified in delactosed permeate from production plant 1, batch E (Chapter IV)

Peptide	-10lgP	Mass	Length	ppm	m/z	RT	Area	Accession	PTM
YQEPVLGPVRGPFPI	74.15	1667.9	15	1.2	834.96	38.77	1.79E+05	P02666|CASB_BOVIN	
YQEPVLGPVRGPFPII	69.05	1781	16	2.2	891.51	41.78	1.61E+05	P02666|CASB_BOVIN	
QEPVLGPVRGPFPIIV	66.94	1717	16	1.6	859.51	43.35	5.18E+04	P02666|CASB_BOVIN	
SHAFEVVKT	61.75	1016.5	9	8.9	339.85	15.76	1.53E+04	P80195|GLCM1_BOVIN	
VLPVPQKAVPYPQ	60.54	1434.8	13	-0.5	718.42	25.61	4.10E+04	P02666|CASB_BOVIN	
YQEPVLGPVRGPFPIIV	60.54	1880.1	17	-2.3	941.04	44.26	1.61E+05	P02666|CASB_BOVIN	
EPVLGPVRGPFPI	60.39	1376.8	13	0.3	689.4	37.63	6.61E+04	P02666|CASB_BOVIN	
KVLPVPQ	60.02	779.49	7	-5	390.75	17.59	2.20E+03	P02666|CASB_BOVIN	
DAAGGPGAPADPGRPT	59.08	1405.7	16	-1.6	703.84	12.43	1.53E+04	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
PVLGPVRGPFPIIV	59	1459.9	14	-3.7	730.95	43.42	6.89E+03	P02666|CASB_BOVIN	
PVVVPPFLQPEVM(+15.99)	58.08	1466.8	13	2	734.4	39.52	5.99E+03	P02666|CASB_BOVIN	Oxidation (M)
PVLGPVRGPFPII	58.03	1360.8	13	1.3	681.42	41.35	4.69E+03	P02666|CASB_BOVIN	
YQEPVLGPVRGPFP	56.97	1554.8	14	1.4	778.42	33.42	8.06E+04	P02666|CASB_BOVIN	
AQPTDASAQFIR	56.81	1303.7	12	3.7	435.56	19.55	2.89E+04	P80195|GLCM1_BOVIN	
DTIAQAASTTTISDAVSK	56.69	1778.9	18	-1.5	890.45	24.63	1.25E+04	P80025|PERL_BOVIN	
GLPQEVLNENLLRF	56.32	1640.9	14	-8.9	547.97	41.65	2.07E+04	P02662|CASA1_BOVIN	
FVAPFPEVFGKEK	56.07	1493.8	13	2.8	498.94	34.05	7.64E+04	P02662|CASA1_BOVIN	
SQNPKLPLSIL	55.89	1208.7	11	-2.5	605.36	35.85	5.43E+04	P80195|GLCM1_BOVIN	
GLPQEVLNENLLR	55.62	1493.8	13	-2.3	747.92	33.62	5.98E+04	P02662|CASA1_BOVIN	
APFPEVFGKEK	54.66	1247.7	11	4.8	416.9	25.06	2.00E+04	P02662|CASA1_BOVIN	
EPVLGPVRGPFPIIV	54.63	1588.9	15	-4	795.47	43.31	6.96E+04	P02666|CASB_BOVIN	
SQSKVLPVPQK	54.52	1209.7	11	7	404.25	15.07	5.98E+03	P02666|CASB_BOVIN	
SSSEESITRIN	53.66	1221.6	11	6.6	611.8	15.03	7.13E+03	P02666|CASB_BOVIN	
EVIESPPEINTVQVTSTAV	53.35	2012	19	-3.2	1007	31.3	3.86E+04	P02668|CASK_BOVIN	
FVAPFPEVFG	53.31	1108.6	10	7.1	555.29	41.76	3.42E+03	P02662|CASA1_BOVIN	
SSRQPQSQNPKLPL	53.01	1578.8	14	-2.2	527.29	20.34	6.67E+04	P80195|GLCM1_BOVIN	
QEPVLGPVRGPFPI	52.51	1504.8	14	-2.6	753.43	37.42	3.93E+04	P02666|CASB_BOVIN	
EPVLGPVRGPFPII	52.25	1489.9	14	2.1	745.94	41.7	5.67E+04	P02666|CASB_BOVIN	
APFPEVFGK	51.93	990.52	9	5	496.27	28.61	9.25E+04	P02662|CASA1_BOVIN	
VQVTSTAV	51.71	803.44	8	-7.1	804.44	15.3	6.28E+04	P02668|CASK_BOVIN	
DASAQFIRNL	51.57	1133.6	10	0.3	567.8	28.42	1.04E+04	P80195|GLCM1_BOVIN	
LPQEVLNENLLRF	51.46	1583.9	13	-5.1	792.94	39.12	1.34E+04	P02662|CASA1_BOVIN	
SLSQSKVLPVPQK	51.12	1409.8	13	7.4	470.95	19.52	4.57E+03	P02666|CASB_BOVIN	
AQPTDASAQF	50.98	1034.5	10	-1.9	1035.5	15.98	1.27E+05	P80195|GLCM1_BOVIN	
VAPFPEVFGK	50.42	1089.6	10	2	545.8	31.41	1.62E+05	P02662|CASA1_BOVIN	
TVQVTSTAV	50.27	904.49	9	1.8	905.5	16.74	1.71E+05	P02668|CASK_BOVIN	
VAPFPEVFGKEK	50.24	1346.7	12	9.9	449.92	27.97	5.72E+04	P02662|CASA1_BOVIN	
QEPVLGPVRGPFP	50.23	1391.8	13	-3.7	696.88	31.82	1.05E+04	P02666|CASB_BOVIN	
LPQEVLNENLLR	49.93	1436.8	12	7.3	479.94	30.63	7.59E+03	P02662|CASA1_BOVIN	
TVDMESTEVFTK	49.02	1385.6	12	4.9	693.83	23.21	4.96E+03	P02663|CASA2_BOVIN	
SLVYPFPGPIPN	48.86	1299.7	12	-5.2	650.85	36.89	5.99E+03	P02666|CASB_BOVIN	
LPYPYYAKPA	48.68	1181.6	10	-5.3	591.81	22.94	8.35E+03	P02668|CASK_BOVIN	
ELEELNVPGEIVE	48.62	1468.7	13	1.3	735.37	31.79	3.39E+04	P02666|CASB_BOVIN	
SSEESITRIN	48.42	1134.6	10	-1.7	568.28	14.87	2.08E+03	P02666|CASB_BOVIN	
FVAPFPEVFGK	48.38	1236.7	11	4.1	619.34	37.48	1.30E+05	P02662|CASA1_BOVIN	
VIESPPEIN	48.24	996.51	9	2.3	499.27	20.05	4.88E+04	P02668|CASK_BOVIN	
ASAQFIRNL	48.17	1018.6	9	-0.2	510.29	24.96	3.46E+03	P80195|GLCM1_BOVIN	
QEPVLGPVRGPFPII	48.15	1617.9	15	0	809.97	41.32	4.06E+04	P02666|CASB_BOVIN	
SQNPKLPLSI	48.03	1095.6	10	-3.4	548.82	29.41	1.49E+04	P80195|GLCM1_BOVIN	
DIQKVAGTWYSL	47.93	1379.7	12	1	690.86	32.8	2.02E+03	P02754|LACB_BOVIN	
VPPFLQPEVM(+15.99)	47.87	1171.6	10	4.1	586.81	30.54	1.21E+05	P02666|CASB_BOVIN	Oxidation (M)
NIPPLTQTPV	47.2	1078.6	10	-2.4	1079.6	27.01	2.55E+04	P02666|CASB_BOVIN	
ILNKPEDETHLEAQPTDASAQFIR	47.14	2722.4	24	3.8	681.6	25.01	4.79E+04	P80195|GLCM1_BOVIN	
SVLSLSQS	46.72	819.43	8	-9.5	820.44	20.58	7.69E+03	P02666|CASB_BOVIN	
ILNKPEDETHLE	46.57	1436.7	12	4.1	479.92	16.21	3.33E+05	P80195|GLCM1_BOVIN	
PVVVPPFLQPEVM(+15.99)GVS	46.56	1709.9	16	1	855.96	40.64	6.30E+03	P02666|CASB_BOVIN	Oxidation (M)

Peptide	-10lgP	Mass	Length	ppm	m/z	RT	Area	Accession	PTM
SQNPKLPL	46.47	895.51	8	4.2	448.77	21.8	4.09E+04	P80195|GLCM1_BOVIN	
ILNKPEDETHL	46.46	1307.7	11	2.4	654.85	17.17	1.42E+05	P80195|GLCM1_BOVIN	
SKVLPVPQ	46.39	866.52	8	2	434.27	18.62	1.48E+04	P02666|CASB_BOVIN	
APFPEVFG	46.05	862.42	8	-5.1	863.43	33.13	2.66E+04	P02662|CASA1_BOVIN	
LGYLEQLLR	45.8	1103.6	9	-7.1	552.82	35.5	1.48E+03	P02662|CASA1_BOVIN	
GYLEQLLR	45.58	990.55	8	1.7	496.28	31.53	3.51E+03	P02662|CASA1_BOVIN	
YQGPIVLNPWDQVKR	45.42	1812	15	3.2	605	31.96	9.44E+03	P02663|CASA2_BOVIN	
DAQSAPLRVY	45.13	1118.6	10	-1.2	560.29	20.39	5.44E+03	P02754|LACB_BOVIN	
VAPFPEVFG	44.99	961.49	9	-3.8	962.5	35.73	3.33E+04	P02662|CASA1_BOVIN	
EVLNENLLRF	44.88	1245.7	10	-3.6	623.84	35.25	2.42E+04	P02662|CASA1_BOVIN	
FVAPFPE	44.75	805.4	7	-4	806.41	28.8	9.37E+03	P02662|CASA1_BOVIN	
SPEVIESPPEINTVQVTSTAV	44.74	2196.1	21	-3.3	1099.1	32.32	6.87E+03	P02668|CASK_BOVIN	
PPPPPPPPPP	44.66	988.54	10	3.1	495.28	16.73	1.12E+04	A2VDK6|WASF2_BOVI	
SQNPKLPLS	44.3	982.54	9	-3.8	492.28	19.34	1.44E+04	P80195|GLCM1_BOVIN	
TIASGEPTSTPTTE	44.26	1390.6	14	-6.2	696.33	14.65	4.03E+03	P02668|CASK_BOVIN	
VPPFLQPEVM	44.25	1155.6	10	-0.8	578.81	35.81	2.06E+04	P02666|CASB_BOVIN	
TQTPVVVPPFLQPEVM	44.25	1780.9	16	-3.4	891.48	43.65	1.89E+03	P02666|CASB_BOVIN	
EPVLGPVRGPFP	44.23	1263.7	12	-1.8	632.86	31.82	3.47E+04	P02666|CASB_BOVIN	
AQPTDASAQFIRNL	44.16	1530.8	14	-3.8	766.4	30.84	9.39E+03	P80195|GLCM1_BOVIN	
APPPPPPPP	44.07	865.47	9	7.7	433.75	15.03	5.96E+03	A2VDK6|WASF2_BOVI	
LNKPEDETHLE	43.98	1323.6	11	1.6	442.22	13.84	3.38E+04	P80195|GLCM1_BOVIN	
DMPIQAF	43.78	820.38	7	-8.2	821.38	29.17	7.42E+03	P02666|CASB_BOVIN	
SQNPKLPLSILK	43.53	1336.8	12	2.5	446.61	29.34	1.82E+03	P80195|GLCM1_BOVIN	
VSREQQEQEGEEM(+15.99)AEYR	43.49	2041.9	17	-0.7	681.63	11.64	8.23E+02	P18892|BT1A1_BOVIN	Oxidation (M)
PVLGPVRGPFPI	43.46	1247.7	12	-3.8	624.88	37.3	9.38E+03	P02666|CASB_BOVIN	
NVPGEIVESL	43.33	1055.5	10	-3.3	1056.6	31.12	8.31E+04	P02666|CASB_BOVIN	
PPPPPPPPP	43.25	891.49	9	0.8	446.75	15.59	4.02E+04	A2VDK6|WASF2_BOVIN:A7XYH9|SOBP_BOVI	
HIQKEDVPSERYL	43.22	1612.8	13	2.2	538.62	18.57	2.87E+04	P02662|CASA1_BOVIN	
DM(+15.99)PIQAF	43.06	836.37	7	14.3	837.4	21.3	1.78E+04	P02666|CASB_BOVIN	Oxidation (M)
GPFPIIV	43	741.44	7	-4.5	742.45	37.96	8.14E+03	P02666|CASB_BOVIN	
VAPFPEVF	42.85	904.47	8	-4.9	905.47	36.67	2.24E+04	P02662|CASA1_BOVIN	
SLPQNIPPLTQTPVVVPPFLQPEVM	42.79	2756.5	25	4.2	919.84	45.7	7.44E+03	P02666|CASB_BOVIN	Oxidation (M)
GLPQEVLN	42.53	868.47	8	-0.3	869.47	22.37	9.37E+04	P02662|CASA1_BOVIN	
EAQPTDASAQF	42.49	1163.5	11	-1.9	1164.5	16.96	2.97E+04	P80195|GLCM1_BOVIN	
NAVPITPTLN	42.4	1038.6	10	3.6	520.3	24.27	1.38E+03	P02663|CASA2_BOVIN	
HIQKEDVPSERY	42.26	1499.7	12	1.5	500.92	13.21	7.70E+03	P02662|CASA1_BOVIN	
SQSKVLPVPQKAVPYPQ	42.22	1865	17	4.7	622.69	23.98	1.24E+04	P02666|CASB_BOVIN	
LPVPQKAVPYPQ	42.06	1335.8	12	-2.4	668.88	23.55	1.19E+04	P02666|CASB_BOVIN	
HQGLPQEVL	42.02	1019.5	9	3.3	510.78	23.14	1.99E+04	P02662|CASA1_BOVIN	
FPEVFGK	41.96	822.43	7	1.7	412.22	24.62	1.86E+04	P02662|CASA1_BOVIN	
VYPFPGPIPN	41.96	1099.6	10	-5.2	550.79	31.96	4.74E+03	P02666|CASB_BOVIN	
IVTQTMKGLDIQ	41.76	1345.7	12	1.3	673.87	24.73	8.40E+03	P02754|LACB_BOVIN	
FVAPFPEVFGKE	41.68	1365.7	12	-1.3	683.86	37.66	2.07E+03	P02662|CASA1_BOVIN	
MPIQAF	41.55	705.35	6	-0.4	706.36	26.35	3.92E+03	P02666|CASB_BOVIN	
ILNKPEDETHLEAQPTDASAQFIRNL	41.39	2949.5	26	4.3	738.38	32.44	1.83E+04	P80195|GLCM1_BOVIN	
VDM(+15.99)ES(+79.97)TEVFTK	41.33	1380.6	11	2.3	691.29	16.85	3.07E+03	P02663|CASA2_BOVIN	Oxidation (M); Phosphorylation (STY)
DASAQFIR	41.32	906.46	8	3.8	454.24	15.84	8.55E+03	P80195|GLCM1_BOVIN	
NAVPITPT	41.3	811.44	8	0.7	812.45	17.35	2.37E+04	P02663|CASA2_BOVIN	
YLGYLEQL	41.28	997.51	8	-5.9	998.52	33.6	1.73E+03	P02662|CASA1_BOVIN	
VVPPFLQPEVM(+15.99)	41.28	1270.7	11	4.9	636.34	33.47	1.86E+04	P02666|CASB_BOVIN	Oxidation (M)
DASAQFIRN	41.18	1020.5	9	0.9	511.26	15.39	4.21E+03	P80195|GLCM1_BOVIN	
GLPQEVLNENLL	41.15	1337.7	12	-2.3	669.87	36.74	5.43E+04	P02662|CASA1_BOVIN	
SRQPQSQNPKLPL	41.15	1491.8	13	1.9	498.28	20.07	5.38E+03	P80195|GLCM1_BOVIN	
IVTQTMKGL	40.9	989.56	9	2.2	495.79	20.49	1.08E+04	P02754|LACB_BOVIN	
GLPQEVLNEN	40.86	1111.6	10	1.2	556.78	23.34	7.95E+03	P02662|CASA1_BOVIN	
VLPVPQKAVPYPQR	40.8	1590.9	14	0.3	531.32	23.14	1.84E+03	P02666|CASB_BOVIN	
HQGLPQEVLN	40.66	1133.6	10	-4.5	567.8	20.34	1.95E+04	P02662|CASA1_BOVIN	
VAPFPEV	40.57	757.4	7	-1.7	758.41	27.79	1.30E+04	P02662|CASA1_BOVIN	
SLPQNIPPLTQTPV	40.55	1503.8	14	-6.3	752.92	32.02	1.19E+04	P02666|CASB_BOVIN	
VLGPVRGPFPII	40.43	1263.8	12	-10.7	632.89	39.59	7.49E+03	P02666|CASB_BOVIN	
RELEELNVPGEIVESL	40.3	1824.9	16	2.4	913.49	39.77	5.20E+03	P02666|CASB_BOVIN	
VDMESTEVFTK	40.26	1284.6	11	-2.5	643.3	22.27	1.29E+03	P02663|CASA2_BOVIN	
HQGLPQEVLNENLL	40.22	1602.8	14	3.6	802.43	33.8	5.21E+03	P02662|CASA1_BOVIN	
APFPEVF	40.12	805.4	7	-1.3	806.41	34.1	2.50E+04	P02662|CASA1_BOVIN	
GLPQEVL	40.1	754.42	7	1.4	755.43	25.65	1.14E+05	P02662|CASA1_BOVIN	
LPQEVLNENLL	40	1280.7	11	-1.1	641.36	33.86	2.71E+04	P02662|CASA1_BOVIN	
YLEQLLRL	39.79	1046.6	8	0.1	524.31	37.46	1.22E+03	P02662|CASA1_BOVIN	
PVEPFTESQSL	39.79	1232.6	11	0.8	617.31	26.75	6.36E+03	P02666|CASB_BOVIN	
INTVQVTSTAV	39.73	1131.6	11	4.5	566.82	21.71	1.40E+03	P02668|CASK_BOVIN	

Peptide	-10lgP	Mass	Length	ppm	m/z	RT	Area	Accession	PTM
SLSQSKVLPVPQKAVPYPQ	39.72	2065.2	19	6	689.4	26.47	1.10E+04	P02666|CASB_BOVIN	
SSRQPQSQNPKLPLS	39.54	1665.9	15	-4.9	556.3	18.31	1.20E+04	P80195|GLCM1_BOVIN	
VPPFLQPEVM(+15.99)GV	39.5	1327.7	12	-2.6	664.85	34.42	9.87E+03	P02666|CASB_BOVIN	Oxidation (M)
HLPLPLLQS	39.44	1016.6	9	12.1	509.32	34.12	1.84E+03	P02666|CASB_BOVIN	
AAGGPGAPADPGRPT	39.38	1290.6	15	-3.5	646.32	11.97	3.28E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
YKVPQLEIVPN	39.17	1298.7	11	-2.8	650.37	30.6	1.18E+04	P02662|CASA1_BOVIN	
LPQEVLN	39.12	811.44	7	1.2	812.45	18.83	1.84E+03	P02662|CASA1_BOVIN	
TLTDVENL	39.01	903.45	8	-9.9	904.46	24.02	1.47E+04	P02666|CASB_BOVIN	
HQGLPQEVLNENLLR	38.76	1758.9	15	3.3	587.32	30.96	0	P02662|CASA1_BOVIN	
ALLDPSFFAK	38.49	1107.6	10	-2.1	554.81	34.1	8.28E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
SQSKVLPVPQ	38.47	1081.6	10	3.4	541.82	19.07	3.27E+04	P02666|CASB_BOVIN	
ALLDPSF	38.45	761.4	7	-4.3	762.4	30.42	5.73E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
SLTLTDVENL	38.34	1103.6	10	-4.3	552.79	33.26	0	P02666|CASB_BOVIN	
STLVPLA	38.33	699.42	7	-2.4	700.42	26.43	6.08E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
TKVIPYVR	38.28	974.59	8	1.2	325.87	17.65	1.44E+03	P02663|CASA2_BOVIN	
PEVIESPPEINTVQVTSTAV	38.05	2109.1	20	-7.9	1055.5	32.29	1.82E+04	P02668|CASK_BOVIN	
AASTTTISDAVSK	38.03	1250.6	13	1.7	626.33	15.45	0	P80025|PERL_BOVIN	
NENLLRF	37.86	904.48	7	8.2	453.25	27.59	1.55E+05	P02662|CASA1_BOVIN	
GPVRGPFPII	37.84	1051.6	10	-0.7	526.82	34.56	3.47E+04	P02666|CASB_BOVIN	
SSS(+79.97)EESITRIN	37.8	1301.6	11	4.4	651.79	15.87	3.81E+03	P02666|CASB_BOVIN	Phosphorylation (STY)
APFPEVFGKE	37.66	1119.6	10	-6	560.79	29	8.33E+03	P02662|CASA1_BOVIN	
PVLGPVRGPFP	37.65	1134.7	11	2.3	568.34	31.19	2.59E+03	P02666|CASB_BOVIN	
VIESPPEINTVQ	37.5	1324.7	12	3.2	663.35	23.18	6.29E+03	P02668|CASK_BOVIN	
YLGYLEQ	37.46	884.43	7	-15	885.42	23.27	4.80E+03	P02662|CASA1_BOVIN	
SDIPNPIGSENSEK	37.32	1485.7	14	-0.1	743.86	20.14	2.27E+04	P02662|CASA1_BOVIN	
GPFPII	37.27	642.37	6	-7.1	643.38	34.86	8.71E+03	P02666|CASB_BOVIN	
EVLNENLLR	37.26	1098.6	9	-4.1	550.31	24.36	4.37E+04	P02662|CASA1_BOVIN	
FSHAFEVVKT	37.18	1163.6	10	1.1	388.87	21.11	3.52E+03	P80195|GLCM1_BOVIN	
SDIPNPIGSENSE	37.12	1357.6	13	0.9	679.81	22.12	9.71E+03	P02662|CASA1_BOVIN	
YQEPVL	37.1	747.38	6	-3.6	748.39	21.56	1.31E+04	P02666|CASB_BOVIN	
VDM(+15.99)ESTEVFTK	37.05	1300.6	11	1.2	651.3	16.65	1.02E+04	P02663|CASA2_BOVIN	Oxidation (M)
DMPIQA	37.03	673.31	6	0.8	674.32	17.91	6.73E+03	P02666|CASB_BOVIN	
APFPEVFGKEKV	36.96	1346.7	12	4.1	449.92	28.61	7.55E+03	P02662|CASA1_BOVIN	
SSRQPQSQNPKLPLSILKEK	36.86	2277.3	20	1.6	456.47	24.75	4.90E+03	P80195|GLCM1_BOVIN	
VPQLEIVPN	36.69	1007.6	9	3.2	1008.6	28.08	4.08E+04	P02662|CASA1_BOVIN	
NAVPITPTL	36.64	924.53	9	-3	925.53	27.89	3.88E+03	P02663|CASA2_BOVIN	
VPGEIVESL	36.49	941.51	9	-1.9	942.51	27.86	5.86E+03	P02666|CASB_BOVIN	
GPVRGPFPI	36.48	938.53	9	2.2	470.28	28.67	4.29E+04	P02666|CASB_BOVIN	
VAPFPE	36.42	658.33	6	2.7	659.34	20.02	2.84E+04	P02662|CASA1_BOVIN	
MPFPKYPVEPF	36.37	1350.7	11	0.9	676.34	35.88	8.63E+03	P02666|CASB_BOVIN	
SLSQSKVLPVPQ	36.24	1281.7	12	-2.8	641.87	23.34	1.46E+04	P02666|CASB_BOVIN	
PVVVPPFLQPE	36.18	1220.7	11	1.9	611.35	38.11	1.30E+03	P02666|CASB_BOVIN	
SSRQPQSQNPKLPLSIL	36.14	1892	17	-0.9	631.69	33.37	1.48E+04	P80195|GLCM1_BOVIN	
PFPEVFGK	36	919.48	8	-4.7	460.75	31.38	2.68E+03	P02662|CASA1_BOVIN	
PPPPPPPP	35.94	794.43	8	-4.1	398.22	14.23	2.66E+04	A2VDK6|WASF2_BOVIN:A7XYH9|SOBP_BOVI	
TVDM(+15.99)ESTEVFTK	35.6	1401.6	12	-0.5	701.83	18.04	1.13E+04	P02663|CASA2_BOVIN	Oxidation (M)
GLPQEVLNENL	35.52	1224.6	11	4.8	613.33	31.19	2.01E+04	P02662|CASA1_BOVIN	
SLVYPFPGPIPNSLPQ	35.23	1724.9	16	-0.7	863.47	39.98	3.39E+03	P02666|CASB_BOVIN	
YLGYLE	35.22	756.37	6	-3.1	757.38	24.51	0	P02662|CASA1_BOVIN	
IQKEDVPSERYL	35.2	1475.8	12	1.1	492.93	20.37	4.50E+03	P02662|CASA1_BOVIN	
VLGPVRGPFPI	35.16	1150.7	11	0	576.35	35	8.44E+03	P02666|CASB_BOVIN	
TIASGEPTSTPT	35.06	1160.6	12	-1.9	581.29	13.89	4.07E+03	P02668|CASK_BOVIN	
GPVRGPFP	35.03	825.45	8	2.1	413.73	20.65	1.94E+04	P02666|CASB_BOVIN	
HPFAQTQSL	35.03	1027.5	9	6	514.77	17.62	4.04E+03	P02666|CASB_BOVIN	
GYLEQL	34.85	721.36	6	-5.5	722.37	24.63	1.08E+04	P02662|CASA1_BOVIN	
FVAPFPEV	34.57	904.47	8	-7.2	905.47	35.22	5.89E+03	P02662|CASA1_BOVIN	
PDGNFRLI	34.49	930.49	8	-1.7	466.25	31.3	2.64E+03	Q24K11|AP3M1_BOVIN	
ALPQYL	34.46	703.39	6	-7.6	704.39	26.06	6.91E+03	P02663|CASA2_BOVIN	
IPIQYV	34.33	731.42	6	1	366.72	25.15	1.00E+03	P02668|CASK_BOVIN	

Peptide	-10lgP	Mass	Length	ppm	m/z	RT	Area	Accession	PTM
VLNENLL	34.29	813.46	7	-1.8	814.47	26.18	1.01E+04	P02662|CASA1_BOVIN	
LPQYL	34.21	632.35	5	-15.8	633.35	24.33	2.51E+03	P02663|CASA2_BOVIN	
IPQYI	34.21	632.35	5	-15.8	633.35	24.33	2.51E+03		
QKEDVPSERYL	34.17	1362.7	11	7.3	455.24	18.46	3.76E+03	P02662|CASA1_BOVIN	
DM(+15.99)PIQAFL	34.11	949.46	8	-3.8	950.46	32.63	3.71E+03	P02666|CASB_BOVIN	Oxidation (M)
ETHLEAQPTDASAQF	34.09	1643.7	15	2.7	822.88	21.03	1.70E+03	P80195|GLCM1_BOVIN	
QLEIVPN	34.05	811.44	7	-1.3	812.45	22.46	3.78E+03	P02662|CASA1_BOVIN	
RDMPIQAF	33.91	976.48	8	8.1	489.25	24.96	3.17E+03	P02666|CASB_BOVIN	
VLPVPQ	33.84	651.4	6	-0.6	652.4	18.99	3.06E+04	P02666|CASB_BOVIN	
IGVNQELAY	33.8	1005.5	9	-5.7	1006.5	23.86	3.42E+03	P02662|CASA1_BOVIN	
EVLNENLL	33.77	942.5	8	1.6	943.51	28.58	2.98E+04	P02662|CASA1_BOVIN	
AFEVVKT	33.73	792.44	7	2.3	397.23	17.64	4.92E+03	P80195|GLCM1_BOVIN	
AVPITPT	33.59	697.4	7	-0.1	698.41	15.98	3.92E+03	P02663|CASA2_BOVIN	
IGVNQEL	33.57	771.41	7	1.3	772.42	19.89	9.96E+02	P02662|CASA1_BOVIN	
VPQLEIVPNS(+79.97)AEERLHSM(+	33.52	2144	18	-0.8	715.67	28.66	7.08E+03	P02662|CASA1_BOVIN	Phosphorylation (STY); Oxidation (M)
VPPFLQPE	33.48	925.49	8	5.8	463.76	27.86	6.77E+03	P02666|CASB_BOVIN	
IASGEPTSTPTTE	33.45	1289.6	13	5.4	645.81	12.98	8.50E+02	P02668|CASK_BOVIN	
TQTPVVVPPFLQPEVM(+15.99)	33.43	1796.9	16	4.2	899.48	40	1.96E+04	P02666|CASB_BOVIN	Oxidation (M)
DLISKEQIVIR	33.39	1312.8	11	-5.5	438.6	25.11	3.56E+03	P80195|GLCM1_BOVIN	
M(+15.99)PFPKYPVEPF	33.36	1366.7	11	-1.5	684.34	32.29	7.84E+03	P02666|CASB_BOVIN	Oxidation (M)
TVDM(+15.99)ES(+79.97)TEVFTK	33.34	1481.6	12	4.8	741.81	17.5	4.63E+03	P02663|CASA2_BOVIN	Oxidation (M); Phosphorylation (STY)
TTMPL	33.2	561.28	5	-4.5	562.29	19.26	2.34E+03	P02662|CASA1_BOVIN	
IPPLTQTPV	33.14	964.56	9	3.9	483.29	26.15	5.05E+04	P02666|CASB_BOVIN	
FPEVF	33.03	637.31	5	-1.7	638.32	30.53	1.55E+04	P02662|CASA1_BOVIN	
LPQEVL	33.03	697.4	6	-2.7	698.41	22.69	1.25E+04	P02662|CASA1_BOVIN:Q9TTK4|LYST_BOVIN	
IPQEVL	33.03	697.4	6	-2.7	698.41	22.69	1.25E+04		
ALNEINQF	33.03	947.47	8	-9.3	948.47	24.56	7.04E+03	P02663|CASA2_BOVIN	
LLLLA	32.98	541.38	5	-14	542.39	31.33	7.43E+03	P02465|CO1A2_BOVIN:P00396|COX1_BOVIN:Q7SIB2|CO4A1_BOVIN:P19111|PPBL_BOVIN:Q3ZC80|LPAR5_BOVIN:Q08DE1|CCG1_BOVIN:Q3MHZ1|NAT14_BOVIN:P11151|LIPL_BOVIN:A6QQ85|UPK3L_BOVIN:Q3Y5Z3|ADIPO_BOVIN:Q3SYY9|LMBD1_BOVIN:P19238|CD5_BOVIN:A7YWM1|GGT6_BOVIN:Q95J56|DJC14_BOVIN:P07589|FINC_BOVIN:Q3SZD5|DCA15_BOVIN:P32592|ITB2_BOVIN:P80746|ITAV_BOVIN:P53710|ITA2_BOVIN:Q2UVX4|CO3_BOVIN:A6QR40|ELMO3_BOVIN:A7YY57|RHG29_BOVIN:A5D7M7|TMM88_BOVIN:A5D7K8|PD2R_BOVIN:Q08E36|TM198_BOVIN:Q05204|L	
LLLIA	32.98	541.38	5	-14	542.39	31.33	7.43E+03	O97583|NDST2_BOVIN:P35376|FSHR_BOVIN:Q32KP1|TSN31_BOVIN:P61625|ITAL_BOVIN:Q2TA14|PCP_BOVIN	
LIILA	32.98	541.38	5	-14	542.39	31.33	7.43E+03	A3KMY4|CTL4_BOVIN:Q3T181|LT4R1_BOVIN	
LILLA	32.98	541.38	5	-14	542.39	31.33	7.43E+03	Q1RMQ4|LYNX1_BOVIN:P01131|LDLR_BOVIN:Q08537|UPK2_BOVIN:Q86423|S22A6_BOVIN	

Peptide	-10lgP	Mass	Length	ppm	m/z	RT	Area	Accession	PTM
LLILA	32.98	541.38	5	-14	542.39	31.33	7.43E+03	Q2YDD4|S39AB_BOVIN:Q3SWX7|ANXA3_BOVIN:P00157|CYB_BOVIN:Q3MHM6|CTNA1_BO	
IILIA	32.98	541.38	5	-14	542.39	31.33	7.43E+03	Q1JPA3|TMM47_BOVIN	
IILLA	32.98	541.38	5	-14	542.39	31.33	7.43E+03	A7EZY6|MROH1_BOVIN:Q8HXQ5|MRP1_BOVIN	
IIILA	32.98	541.38	5	-14	542.39	31.33	7.43E+03	Q27977|ITA5_BOVIN:Q1LZE6|CHPT1_BOVIN	
LILIA	32.98	541.38	5	-14	542.39	31.33	7.43E+03	P68530|COX2_BOVIN:Q9BGI0|GABT_BOVIN	
IIIIA	32.98	541.38	5	-14	542.39	31.33	7.43E+03	Q2HJ88|RTCA_BOVIN:Q32LN6|F205C_BOVIN	
ILLLA	32.98	541.38	5	-14	542.39	31.33	7.43E+03		
ILLIA	32.98	541.38	5	-14	542.39	31.33	7.43E+03		
LIIIA	32.98	541.38	5	-14	542.39	31.33	7.43E+03		
ILILA	32.98	541.38	5	-14	542.39	31.33	7.43E+03		
LLDPSFFAK	32.93	1036.6	9	-2.3	519.29	31.25	2.34E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
ALLDPSFFAKE	32.9	1236.6	11	6.7	619.33	34.63	1.82E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
PGRPTGYSGSSKAL	32.8	1376.7	14	6.1	459.91	13.54	6.71E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
VLGPVRGPFPIIV	32.77	1362.8	13	2.4	682.43	41.78	8.73E+03	P02666|CASB_BOVIN	
SSRQPQSQNPKLPLSI	32.75	1779	16	7.9	594	27.3	1.31E+04	P80195|GLCM1_BOVIN	
GPVRGPFPIIV	32.68	1150.7	11	-9.3	576.35	37.2	5.00E+04	P02666|CASB_BOVIN	
PVEPF	32.65	587.3	5	-8.1	588.3	20.17	6.22E+03	P02666|CASB_BOVIN	
IPIQY	32.65	632.35	5	4.3	633.36	20.99	9.58E+03	P02668|CASK_BOVIN	
LPLQY	32.65	632.35	5	4.3	633.36	20.99	9.58E+03		
IPLQY	32.65	632.35	5	4.3	633.36	20.99	9.58E+03		
VPGEIVE	32.58	741.39	7	-3.2	742.4	16.72	2.13E+03	P02666|CASB_BOVIN	
YQGPIVLNPWDQVK	32.58	1655.9	14	1.7	828.94	34.71	2.12E+03	P02663|CASA2_BOVIN	
IPNPIGSENSEK	32.52	1283.6	12	-2.7	642.83	17.73	5.28E+03	P02662|CASA1_BOVIN	
NVPGEIVE	32.38	855.43	8	-1.4	856.44	18.63	3.06E+04	P02666|CASB_BOVIN	
FVAPFPEVFGKEKV	32.36	1592.9	14	-0.2	531.96	36.38	2.67E+03	P02662|CASA1_BOVIN	
PVVVPPFLQPEVM(+15.99)GVSK	32.36	1838	17	-4.6	613.67	38.07	4.86E+03	P02666|CASB_BOVIN	Oxidation (M)
ILNKPEDETHLEAQPTDASAQF	32.33	2453.2	22	7.4	818.74	23.89	5.24E+04	P80195|GLCM1_BOVIN	
TQTPVVVPPFLQPE	32.24	1550.8	14	7.3	776.43	38.74	4.59E+03	P02666|CASB_BOVIN	
ALPQYLKT	32.05	932.53	8	-5.1	467.27	22.01	2.89E+03	P02663|CASA2_BOVIN	
LPQEVLNENL	32.02	1167.6	10	-2.6	584.81	27.66	5.71E+03	P02662|CASA1_BOVIN	
SPPEINTVQVTSTAV	31.92	1541.8	15	7.1	771.91	26.47	7.20E+03	P02668|CASK_BOVIN	
GLPQEV	31.91	641.34	6	-2.2	642.35	17.91	0	P02662|CASA1_BOVIN	
LLDPSF	31.89	690.36	6	-2.8	691.37	26.65	1.64E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
DKTEIPTIN	31.85	1029.5	9	-4.4	515.77	19.5	9.18E+03	P02668|CASK_BOVIN	
NLHLPLPLLQ	31.75	1156.7	10	2.3	579.36	42.26	3.86E+03	P02666|CASB_BOVIN	
VAGTWY	31.57	695.33	6	-4.1	696.33	21.3	1.36E+04	P02754|LACB_BOVIN	
VLPVPQK	31.55	779.49	7	0.5	390.75	15.17	1.39E+03	P02666|CASB_BOVIN	
LGYLEQL	31.55	834.45	7	4.2	835.46	29.89	0	P02662|CASA1_BOVIN	
VPPFLQPEV	31.55	1024.6	9	3.5	513.29	32.8	1.23E+04	P02666|CASB_BOVIN	
M(+15.99)PIQAF	31.52	721.35	6	17.5	722.37	20.58	0	P02666|CASB_BOVIN	Oxidation (M)
VAPFP	31.49	529.29	5	9.3	530.3	21.09	0	P02662|CASA1_BOVIN	
APFPEV	31.48	658.33	6	-3.7	659.34	24.8	1.23E+04	P02662|CASA1_BOVIN	
HLPLPLLQ	31.45	929.57	8	-4.2	465.79	34.91	2.90E+03	P02666|CASB_BOVIN	
TLVPLA	31.36	612.38	6	0	613.39	25.59	1.14E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
AVPYPQRDMPIQA	31.34	1484.7	13	-3.6	743.38	24.63	6.24E+03	P02666|CASB_BOVIN	
EVLNENL	31.19	829.42	7	-0.1	830.43	20.75	5.38E+03	P02662|CASA1_BOVIN	
ALPQY	31.18	590.31	5	-2.8	591.31	16.67	0	P02663|CASA2_BOVIN	
AIPQY	31.18	590.31	5	-2.8	591.31	16.67	0		
NIPPLTQTPVVVPPFLQPEVM(+15.9	31.18	2331.3	21	0.2	778.09	45.27	4.24E+03	P02666|CASB_BOVIN	Oxidation (M)

Peptide	-10lgP	Mass	Length	ppm	m/z	RT	Area	Accession	PTM
VSTLVPLA	31.13	798.49	8	-7.2	799.49	28.92	4.64E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
IPIQYVL	31.12	844.51	7	5.4	423.26	34.09	1.51E+03	P02668|CASK_BOVIN	
KVPQLEIVPN	31.07	1135.7	10	2.8	568.84	26.4	2.13E+04	P02662|CASA1_BOVIN	
TIIIL	31.02	571.39	5	-17.1	572.39	29.48	9.30E+03		
TIILI	31.02	571.39	5	-17.1	572.39	29.48	9.30E+03		
TILIL	31.02	571.39	5	-17.1	572.39	29.48	9.30E+03		
TLIIL	31.02	571.39	5	-17.1	572.39	29.48	9.30E+03		
TLLLL	31.02	571.39	5	-17.1	572.39	29.48	9.30E+03	Q9XT96|PSN2_BOVIN:P02465|CO1A2_BOVIN:Q5E9P3|S1PR1_BOVIN	
TILII	31.02	571.39	5	-17.1	572.39	29.48	9.30E+03	E1BNG3|ASCC3_BOVIN	
TLLIL	31.02	571.39	5	-17.1	572.39	29.48	9.30E+03		
TLLLI	31.02	571.39	5	-17.1	572.39	29.48	9.30E+03		
TILLI	31.02	571.39	5	-17.1	572.39	29.48	9.30E+03		
TIILL	31.02	571.39	5	-17.1	572.39	29.48	9.30E+03		
TILLL	31.02	571.39	5	-17.1	572.39	29.48	9.30E+03		
TLILL	31.02	571.39	5	-17.1	572.39	29.48	9.30E+03		
TIIII	31.02	571.39	5	-17.1	572.39	29.48	9.30E+03		
TLLII	31.02	571.39	5	-17.1	572.39	29.48	9.30E+03		
TLIII	31.02	571.39	5	-17.1	572.39	29.48	9.30E+03		
TLILI	31.02	571.39	5	-17.1	572.39	29.48	9.30E+03		
INNQFLPYPYYAKPA	30.84	1797.9	15	-1.5	899.96	31.94	2.19E+03	P02668|CASK_BOVIN	
AVESTVATL	30.74	889.48	9	-4	445.74	20.97	4.86E+03	P02668|CASK_BOVIN	
PVEPFTESQ	30.71	1032.5	9	8.8	517.25	18.82	3.94E+03	P02666|CASB_BOVIN	
KTEIPTIN	30.62	914.51	8	0.2	458.26	18.88	4.14E+03	P02668|CASK_BOVIN	
VLNENLLR	30.62	969.56	8	0.3	485.79	21.96	4.60E+03	P02662|CASA1_BOVIN	
PVRGPFPIIV	30.47	1093.7	10	-0.9	547.84	37.08	1.66E+03	P02666|CASB_BOVIN	
ALNEINQFYQK	30.47	1366.7	11	-5.7	684.35	24.36	1.84E+03	P02663|CASA2_BOVIN	
TTLSSEAPTTQ	30.37	1134.5	11	2.1	568.28	13.71	0	P80025|PERL_BOVIN	
YQEPVLGPVR	30.28	1156.6	10	-3.5	579.32	22.05	8.17E+03	P02666|CASB_BOVIN	
ALLDPSFFAKESVK	30.25	1550.8	14	0.6	517.95	33.16	3.68E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
ALPQYLK	30.22	831.49	7	1.9	416.75	20.07	1.80E+04	P02663|CASA2_BOVIN	
TLVPL	30.2	541.35	5	-9.2	542.35	26.84	4.85E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN:F1MKX4|PSME4_BOVIN:C7EXK4|AT8A2_BOVIN:Q29449|AT8A1_BOVIN	
TIVPL	30.2	541.35	5	-9.2	542.35	26.84	4.85E+03		
TIVPI	30.2	541.35	5	-9.2	542.35	26.84	4.85E+03		
TLVPI	30.2	541.35	5	-9.2	542.35	26.84	4.85E+03		
VAPFPEVFGKE	30.17	1218.6	11	3.5	610.33	31.73	4.53E+03	P02662|CASA1_BOVIN	
VDM(+15.99)EST(+79.97)EVFTK	30.15	1380.6	11	9.1	691.29	16.82	3.36E+03	P02663|CASA2_BOVIN	Oxidation (M); Phosphorylation (STY)
NAVPITPTLNRE	30.15	1323.7	12	-4.2	662.86	22.2	2.36E+03	P02663|CASA2_BOVIN	
RELEELNVPGEIVE	30.13	1624.8	14	-1.7	813.42	29.71	1.95E+04	P02666|CASB_BOVIN	
VIESPPEINTVQVTSTAV	30.13	1883	18	-4	942.5	30.48	6.65E+03	P02668|CASK_BOVIN	
SDIPNPIGSENSEKTTM(+15.99)PL	29.87	2045	19	3.7	1023.5	26.36	4.11E+03	P02662|CASA1_BOVIN	Oxidation (M)
SVLSLSQSK	29.77	947.53	9	1.4	474.77	17.62	3.96E+03	P02666|CASB_BOVIN	
FPEVFG	29.73	694.33	6	-9.4	695.34	29.02	0	P02662|CASA1_BOVIN	
LEQLLRL	29.58	883.55	7	6.9	442.79	31.36	1.15E+03	P02662|CASA1_BOVIN	
N(+.98)AVPITPT	29.54	812.43	8	-4.5	813.43	18.52	4.20E+03	P02663|CASA2_BOVIN	Deamidation (NQ)
LPQYLK	29.53	760.45	6	4.9	381.23	18.23	8.04E+03	P02663|CASA2_BOVIN	
LEPPS	29.5	541.27	5	-3.9	542.28	17.15	5.05E+03	Q2T9U8|NMB_BOVIN:A6QQ70|MAP1S_BOVIN:Q29RT6|KI20A_BOVIN:Q0VBZ8|PRR14_BOVIN:Q2KIP2|HASP_BOVIN	
IEPPS	29.5	541.27	5	-3.9	542.28	17.15	5.05E+03	A6QNM2|RRF2M_BOVI	
HQGLPQEVLNENL	29.37	1489.8	13	-6	745.88	28.7	2.07E+03	P02662|CASA1_BOVIN	
ESVKDAAGGPGAPAD	29.34	1340.6	15	-0.9	671.32	12.05	5.39E+02	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	

Peptide	-10lgP	Mass	Length	ppm	m/z	RT	Area	Accession	PTM
RAGSP	29.3	486.26	5	17.2	487.27	23.1	2.63E+03	A7XYH9|SOBP_BOVIN:Q7SIB2|CO4A1_BOVIN:A6QLT2|MTMR2_BOVI	
ENLLRF	29.27	790.43	6	-1.5	396.22	26.86	6.56E+04	P02662|CASA1_BOVIN	
EIVESL	29.25	688.36	6	-12	689.37	21.23	1.74E+03	P02666|CASB_BOVIN	
VLNEN(+.98)LLRF	29.24	1117.6	9	14.5	559.82	33.44	2.19E+03	P02662|CASA1_BOVIN	Deamidation (NQ)
ATRIL	29.16	572.36	5	7.1	573.38	29.52	1.52E+03	P19111|PPBL_BOVIN:Q6TNJ1|KRIT1_BOVIN:A4FUB0|CE034_BOVIN:P53620|COPG1_BOVIN:Q3SYT7|PSMD8_BOVIN	
ATRLL	29.16	572.36	5	7.1	573.38	29.52	1.52E+03		
ATRII	29.16	572.36	5	7.1	573.38	29.52	1.52E+03		
ATRLI	29.16	572.36	5	7.1	573.38	29.52	1.52E+03		
ALVSTLVPLA	29.13	982.61	10	-0.1	492.31	36.79	2.91E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
SVLSLS	29.11	604.34	6	-10.2	605.35	21.51	1.53E+03	P02666|CASB_BOVIN	
GLPQEVLNE	29.09	997.51	9	-3.2	998.51	24.38	1.48E+04	P02662|CASA1_BOVIN	
FVAPFPEVF	28.98	1051.5	9	3.7	526.78	42.49	1.50E+03	P02662|CASA1_BOVIN	
IVTQTM(+15.99)KGLDIQ	28.9	1361.7	12	2.4	681.87	19.34	6.70E+03	P02754|LACB_BOVIN	Oxidation (M)
LLLSL	28.86	557.38	5	-1	558.39	26.31	1.06E+04	Q9TTK4|LYST_BOVIN:P00396|COX1_BOVIN:A6QQP7|DYSF_BOVIN:P41541|USO1_BOVIN:P69678|CUTA_BOVIN:Q0VCP2|PX11A_BOVIN:O02811|PI4KA_BOVIN:A3FPG8|GPAT4_BOVIN:Q2YDG0|GPC5C_BOVIN:Q3ZC98|NUP85_BOVIN:Q3MHH2|NOL11_BOVI	
LLLSI	28.86	557.38	5	-1	558.39	26.31	1.06E+04	Q5E9P3|S1PR1_BOVIN	
LLISI	28.86	557.38	5	-1	558.39	26.31	1.06E+04		
ILISI	28.86	557.38	5	-1	558.39	26.31	1.06E+04		
ILLSI	28.86	557.38	5	-1	558.39	26.31	1.06E+04	P80457|XDH_BOVIN:P55156|MTP_BOVIN	
ILLSL	28.86	557.38	5	-1	558.39	26.31	1.06E+04		
LLISL	28.86	557.38	5	-1	558.39	26.31	1.06E+04	Q6TNJ1|KRIT1_BOVIN:A6QP74|CALRL_BOVIN:Q92176|COR1A_BOVIN	
IIISI	28.86	557.38	5	-1	558.39	26.31	1.06E+04		
IILSI	28.86	557.38	5	-1	558.39	26.31	1.06E+04		
LIISL	28.86	557.38	5	-1	558.39	26.31	1.06E+04	A5PK14|LSME1_BOVIN:Q5J316|GTR12_BOVIN	
ILISL	28.86	557.38	5	-1	558.39	26.31	1.06E+04	Q9GJS9|GLRB_BOVIN:P27922|SC6A3_BOVIN	
LIISI	28.86	557.38	5	-1	558.39	26.31	1.06E+04		
IILSL	28.86	557.38	5	-1	558.39	26.31	1.06E+04	Q95L46|IF4G2_BOVIN	
LILSI	28.86	557.38	5	-1	558.39	26.31	1.06E+04		
LILSL	28.86	557.38	5	-1	558.39	26.31	1.06E+04		
IIISL	28.86	557.38	5	-1	558.39	26.31	1.06E+04		
KSKGR	28.86	574.36	5	5.3	575.37	28.68	2.69E+04	O97583|NDST2_BOVIN	
STLVPL	28.83	628.38	6	-6.7	629.38	27.2	8.11E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN:F1MKX4|PSME4_BOVIN	
VLPVPQKAVPYPQRDM(+15.99)PIC	28.83	2409.3	21	-2	804.1	31.19	4.37E+03	P02666|CASB_BOVIN	Oxidation (M)
PSHPPP	28.81	630.31	6	15.8	631.33	20.68	7.02E+03	A2VDK6|WASF2_BOVIN:A6QQP7|DYSF_BOVI	
ELEEL	28.8	631.31	5	1.2	632.32	18.41	3.48E+04	P02666|CASB_BOVIN	
EIEEI	28.8	631.31	5	1.2	632.32	18.41	3.48E+04		
ELEEI	28.8	631.31	5	1.2	632.32	18.41	3.48E+04		
EIEEL	28.8	631.31	5	1.2	632.32	18.41	3.48E+04	E1BNG3|ASCC3_BOVIN:P41541|USO1_BOVIN	
SPPEINTVQ	28.77	983.49	9	-2.2	492.75	16.06	3.61E+03	P02668|CASK_BOVIN	
IVTQTMKGLDIQK	28.74	1473.8	13	5.2	492.29	21.65	1.76E+03	P02754|LACB_BOVIN	
YLEQL	28.72	664.34	5	-6.8	665.35	20.87	5.82E+03	P02662|CASA1_BOVIN	

Peptide	-10lgP	Mass	Length	ppm	m/z	RT	Area	Accession	PTM
LPLSI	28.63	541.35	5	-7.2	542.35	28.69	3.97E+03	P80195|GLCM1_BOVIN:P42916|CL43_BOVIN:Q0IIL2|CLD12_BOVIN	
LPISI	28.63	541.35	5	-7.2	542.35	28.69	3.97E+03	Q9XT96|PSN2_BOVIN	
LPLSL	28.63	541.35	5	-7.2	542.35	28.69	3.97E+03	Q2KI51|PR15A_BOVIN	
IPISL	28.63	541.35	5	-7.2	542.35	28.69	3.97E+03	C7EXK4|AT8A2_BOVIN:Q29449|AT8A1_BOVIN	
LPISL	28.63	541.35	5	-7.2	542.35	28.69	3.97E+03		
IPLSL	28.63	541.35	5	-7.2	542.35	28.69	3.97E+03		
IPLSI	28.63	541.35	5	-7.2	542.35	28.69	3.97E+03		
IQKEDVPSERY	28.54	1362.7	11	-2.8	455.23	14.32	2.50E+03	P02662|CASA1_BOVIN	
GHLKALINN	28.53	978.56	9	-12.7	490.28	21.08	1.66E+03	Q9TTK4|LYST_BOVIN	
LPLSIL	28.5	654.43	6	0.8	655.44	36.04	1.75E+03	P80195|GLCM1_BOVIN:P42916|CL43_BOVIN:Q0IIL2|CLD12_BOVIN	
LPLSII	28.5	654.43	6	0.8	655.44	36.04	1.75E+03		
LPLSLL	28.5	654.43	6	0.8	655.44	36.04	1.75E+03	Q2KI51|PR15A_BOVIN	
IPISLL	28.5	654.43	6	0.8	655.44	36.04	1.75E+03	C7EXK4|AT8A2_BOVIN:Q29449|AT8A1_BOVIN	
IPLSII	28.5	654.43	6	0.8	655.44	36.04	1.75E+03		
IPLSLL	28.5	654.43	6	0.8	655.44	36.04	1.75E+03		
LPISIL	28.5	654.43	6	0.8	655.44	36.04	1.75E+03		
YPVEPF	28.47	750.36	6	9.7	751.38	25.73	1.60E+03	P02666|CASB_BOVIN	
SQNPKLPLSILKEK	28.47	1593.9	14	0.6	399.49	26.15	1.96E+03	P80195|GLCM1_BOVIN	
DVPSERYLG	28.45	1034.5	9	-2.2	518.26	18.22	3.67E+03	P02662|CASA1_BOVIN	
MKPWIQPK	28.43	1026.6	8	3.3	343.2	18.63	1.51E+03	P02663|CASA2_BOVIN	
FPGPK	28.4	544.3	5	8.5	545.31	26.8	1.64E+04	P02465|CO1A2_BOVIN	
LSLLL	28.39	557.38	5	-5.2	558.38	26.28	1.13E+04	F1MKX4|PSME4_BOVIN:G3MZC5|AP5B1_BOVIN:Q1JPG1|RS10B_BOVI	
ISILL	28.39	557.38	5	-5.2	558.38	26.28	1.13E+04		
LSILL	28.39	557.38	5	-5.2	558.38	26.28	1.13E+04	P42916|CL43_BOVIN	
ISILI	28.39	557.38	5	-5.2	558.38	26.28	1.13E+04		
ISLIL	28.39	557.38	5	-5.2	558.38	26.28	1.13E+04	Q5E9P3|S1PR1_BOVIN	
LSILI	28.39	557.38	5	-5.2	558.38	26.28	1.13E+04	Q0IIL2|CLD12_BOVIN	
LSLLI	28.39	557.38	5	-5.2	558.38	26.28	1.13E+04	P00396|COX1_BOVIN:Q2KI51|PR15A_BOVIN	
LSLII	28.39	557.38	5	-5.2	558.38	26.28	1.13E+04	Q1LZA0|PIGB_BOVIN	
LSLIL	28.39	557.38	5	-5.2	558.38	26.28	1.13E+04		
ISLLL	28.39	557.38	5	-5.2	558.38	26.28	1.13E+04		
ISIIL	28.39	557.38	5	-5.2	558.38	26.28	1.13E+04		
ISIII	28.39	557.38	5	-5.2	558.38	26.28	1.13E+04		
LSIIL	28.39	557.38	5	-5.2	558.38	26.28	1.13E+04	P79102|CP3AS_BOVIN	
ISLII	28.39	557.38	5	-5.2	558.38	26.28	1.13E+04		
IEKFQS(+79.97)EEQQQ	28.35	1472.6	11	1.7	737.32	12.64	1.34E+03	P02666|CASB_BOVIN	Phosphorylation (STY)
ELEELNVPGE	28.34	1127.5	10	7.6	564.78	24.5	4.00E+03	P02666|CASB_BOVIN	
MAIPPKKN	28.28	897.51	8	17.4	300.18	9.68	0	P02668|CASK_BOVIN	
VLN(+.98)ENLLRF	28.23	1117.6	9	14.5	559.82	33.44	2.19E+03	P02662|CASA1_BOVIN	Deamidation (NQ)
TKVIPYVRY	28.19	1137.7	9	1.3	380.23	22.67	1.54E+03	P02663|CASA2_BOVIN	
ALVSTLVPL	28.16	911.57	9	-4.8	912.57	37.72	1.83E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
PPVVLILK	28.09	877.6	8	-6.1	439.81	44.11	1.52E+04	Q32PJ3|ORC3_BOVIN	
LIVTQTM(+15.99)KGL	28.08	1118.6	10	0.9	560.33	21.03	4.78E+03	P02754|LACB_BOVIN	Oxidation (M)
LLYQEPVLGPVRGPFPIIV	28.05	2106.2	19	0.7	703.08	46.29	2.86E+03	P02666|CASB_BOVIN	
LGYLE	28.03	593.31	5	-6.1	594.31	19.24	0	P02662|CASA1_BOVIN	
IGYLE	28.03	593.31	5	-6.1	594.31	19.24	0		

m/z = mass to charge ratio; RT = retention time; PTM = post-translational modification

Table A1.6. Peptide sequences identified in delactosed permeate from production plant 2, batch A (Chapter IV)

Peptide	-10lgP	Mass	Length	ppm	m/z	RT	Area	Accession	PTM
YQEPVLGPVRGPFPIIV	71.01	1880.1	17	0.8	941.04	44.27	2.29E+05	P02666|CASB_BOVIN	
YQEPVLGPVRGPFPII	62.19	1781	16	5.5	891.51	42.66	2.06E+04	P02666|CASB_BOVIN	
KVLPVPQ	61.28	779.49	7	1.2	390.75	18	3.60E+03	P02666|CASB_BOVIN	
AQPTDASAQF	56.55	1034.5	10	5.2	1035.5	16.3	4.88E+04	P80195|GLCM1_BOVI	
EPVLGPVRGPFPIIV	56.05	1588.9	15	4.8	795.48	43.24	1.55E+04	P02666|CASB_BOVIN	
LLYQEPVLGPVRGPFPIIV	55.71	2106.2	19	-5.5	1054.1	46.38	5.47E+03	P02666|CASB_BOVIN	
DAAGGPGAPADPGRPT	54.69	1405.7	16	8.1	703.84	12.37	5.50E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
ILNKPEDETHLEAQPTDASAQFIR	54.3	2722.4	24	8.9	681.6	25.43	7.01E+03	P80195|GLCM1_BOVI	
YQEPVLGPVRGPFP	52.36	1554.8	14	-1.2	778.42	33.71	6.56E+03	P02666|CASB_BOVIN	
AQTQSLVYPFPGPIPN	52.12	1727.9	16	4.9	864.96	36.23	1.26E+04	P02666|CASB_BOVIN	
AAGGPGAPADPGRPT	52.11	1290.6	15	0.3	646.32	11.82	4.71E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
VIESPPEIN	51.99	996.51	9	-4.9	997.52	20.24	7.97E+04	P02668|CASK_BOVIN	
PYVPVHFDASV	51.63	1229.6	11	6	615.82	29.98	0	P61823|RNAS1_BOVI	
LYQEPVLGPVRGPFPIIV	51.38	1993.1	18	3.2	997.58	45.35	2.70E+03	P02666|CASB_BOVIN	
VVPPFLQPEVM	51.3	1254.7	11	12.8	628.35	38.57	6.99E+03	P02666|CASB_BOVIN	
YYQQKPVALINN	51.12	1449.8	12	-0.9	725.89	21.6	5.44E+03	P02668|CASK_BOVIN	
SLVYPFPGPIPN	50.34	1299.7	12	8.4	650.86	36.93	1.17E+04	P02666|CASB_BOVIN	
SHAFEVVKT	49.02	1016.5	9	9.6	339.85	16.27	8.86E+03	P80195|GLCM1_BOVI	
YYQQKPVAL	48.69	1108.6	9	5.8	555.31	19.89	2.38E+03	P02668|CASK_BOVIN	
PPPPPPPPP	48.55	891.49	9	4.6	446.75	15.91	1.88E+04	A2VDK6|WASF2_BOV	
VQVTSTAV	48.19	803.44	8	0.7	804.45	15.54	7.81E+03	P02668|CASK_BOVIN	
GLPQEVLNENLLR	48.13	1493.8	13	6.5	747.92	33.91	5.89E+03	P02662|CASA1_BOVI	
VAPFPEVFGKE	47.49	1218.6	11	-4.1	610.32	31.93	1.95E+03	P02662|CASA1_BOVI	
NAVPITPTLN	47.39	1038.6	10	2.9	520.29	24.59	2.17E+03	P02663|CASA2_BOVI	
VIESPPEINTVQ	47.16	1324.7	12	5.3	663.35	23.39	1.08E+04	P02668|CASK_BOVIN	
NIPPLTQTPV	46.68	1078.6	10	9.3	540.31	27.32	4.55E+04	P02666|CASB_BOVIN	
VAPFPEVFG	46.51	961.49	9	-0.4	962.5	35.83	7.48E+03	P02662|CASA1_BOVI	
TQTPVVVPPFLQPEVM(+15.99)	46.45	1796.9	16	2.4	899.48	40.02	2.82E+04	P02666|CASB_BOVIN	Oxidation (M)
SLSQSKVLPVPQ	46.17	1281.7	12	1.3	641.87	23.72	7.95E+03	P02666|CASB_BOVIN	
TVQVTSTAV	46.08	904.49	9	2.6	905.5	16.97	9.36E+04	P02668|CASK_BOVIN	
IESPPEIN	45.85	897.44	8	-1.6	898.45	18.02	4.18E+03	P02668|CASK_BOVIN	
GLPQEVLNENL	45.46	1224.6	11	4.1	613.33	31.27	2.98E+03	P02662|CASA1_BOVI	
PEVIESPPEINTVQVTSTAV	45.37	2109.1	20	8.8	1055.6	32.39	2.13E+04	P02668|CASK_BOVIN	
NAVPITPT	45.3	811.44	8	-0.1	812.45	17.6	3.36E+03	P02663|CASA2_BOVI	
QEPVLGPVRGPFPIIV	44.56	1717	16	3.6	859.51	43.24	2.46E+03	P02666|CASB_BOVIN	
VAPFPEVF	44.36	904.47	8	-3	905.47	36.76	1.50E+04	P02662|CASA1_BOVI	
SPPEINTVQVTSTAV	44.08	1541.8	15	5.6	771.91	26.63	1.36E+04	P02668|CASK_BOVIN	
LVYPFPGPIPN	43.62	1212.7	11	-4	607.33	35.92	4.31E+03	P02666|CASB_BOVIN	
SQNPKLPL	43.56	895.51	8	8.3	448.77	22.23	5.07E+03	P80195|GLCM1_BOVI	
NENLLRFF	43.42	1051.5	8	9.5	526.78	38.22	1.27E+04	P02662|CASA1_BOVI	
LHLPLPLLQ	43.05	1042.7	9	4.3	522.34	43.41	3.51E+04	P02666|CASB_BOVIN	
PVLGPVRGPFPIIV	42.32	1459.9	14	0.4	730.95	43.3	3.62E+03	P02666|CASB_BOVIN	
TQTPVVVPPFLQPE	42.31	1550.8	14	8.6	776.43	38.81	2.72E+03	P02666|CASB_BOVIN	
TIASGEPTSTPTTE	42.16	1390.6	14	0.9	696.33	14.85	6.09E+03	P02668|CASK_BOVIN	
FVAPFPEVF	41.92	1051.5	9	5.2	526.78	42.57	2.27E+03	P02662|CASA1_BOVI	
INTVQVTSTAV	41.91	1131.6	11	1.6	566.81	21.94	1.09E+04	P02668|CASK_BOVIN	
APFPEVF	41.81	805.4	7	-5.5	806.4	34.39	5.27E+03	P02662|CASA1_BOVI	
APFPEVFG	41.33	862.42	8	-7	863.42	33.26	5.08E+03	P02662|CASA1_BOVI	
GLPQEVLNENLL	41.2	1337.7	12	4.2	669.87	36.86	2.50E+04	P02662|CASA1_BOVI	
VVPPFLQPEVM(+15.99)	40.91	1270.7	11	-2.6	636.34	33.64	2.75E+04	P02666|CASB_BOVIN	Oxidation (M)
SSRQPQSQNPKLPL	40.87	1578.8	14	3.2	527.29	20.87	1.14E+04	P80195|GLCM1_BOVI	

Peptide	-10lgP	Mass	Length	ppm	m/z	RT	Area	Accession	PTM
HIQKEDVPSERYL	40.82	1612.8	13	9.1	538.62	19.05	1.36E+04	P02662|CASA1_BOVI	
YQEPVLGPVRGPFPI	40.82	1667.9	15	-0.4	834.96	39.06	5.09E+03	P02666|CASB_BOVIN	
SLPQNIPPLTQTPVVVPPFLQPEVM	40.8	2756.5	25	-0.3	919.83	45.73	2.12E+04	P02666|CASB_BOVIN	Oxidation (M)
PPPPPPPP	40.47	794.43	8	4.9	398.23	14.36	1.44E+04	A2VDK6|WASF2_BOVIN:Q2KJE5|G3PT_BOVIN:Q32LP2|RADI_BO	
PPEINTVQVTSTAV	40.23	1454.8	14	2.3	728.39	26.31	1.40E+03	P02668|CASK_BOVIN	
EVLNENLLR	40.14	1098.6	9	0.3	550.31	24.85	1.14E+04	P02662|CASA1_BOVI	
DVPSERYL	39.93	977.48	8	1.2	489.75	20.34	3.54E+03	P02662|CASA1_BOVI	
FVAPFPEVFGK	39.74	1236.7	11	-2.2	619.33	37.67	2.22E+04	P02662|CASA1_BOVI	
SDIPNPIGSENSE	39.69	1357.6	13	7.4	679.81	22.3	5.68E+03	P02662|CASA1_BOVI	
EVLNENLLRF	39.66	1245.7	10	-3.2	623.84	35.43	1.05E+04	P02662|CASA1_BOVI	
EVIESPPEINTVQVTSTAV	39.44	2012	19	0.8	1007	31.42	2.07E+04	P02668|CASK_BOVIN	
GLPQEVL	39.02	754.42	7	5.5	755.43	26.02	3.09E+04	P02662|CASA1_BOVI	
GLPQEVLNENLLRF	38.9	1640.9	14	3	547.97	41.74	8.79E+03	P02662|CASA1_BOVI	
VLSRYPSYGLN	38.7	1267.7	11	-2.1	634.83	22.16	8.37E+02	P02668|CASK_BOVIN	
RELEELNVPGEIVE	38.38	1624.8	14	2.2	813.42	29.84	1.99E+04	P02666|CASB_BOVIN	
TQTPVVVPPFLQPEVM	38.19	1780.9	16	3.2	891.48	43.61	3.82E+03	P02666|CASB_BOVIN	
FVAPFPEVFGKEK	38.05	1493.8	13	6.3	498.94	34.47	7.69E+03	P02662|CASA1_BOVI	
FVAPFPEVFGKEKV	38.02	1592.9	14	3.7	531.96	36.51	2.59E+03	P02662|CASA1_BOVI	
SLPQNIPPLTQTPV	37.85	1503.8	14	0.5	752.92	32.23	7.39E+03	P02666|CASB_BOVIN	
AVRSPAQILQWQVL	37.81	1607.9	14	7.1	804.97	40.62	1.43E+03	P02668|CASK_BOVIN	
AQPTDASAQFIRN	37.47	1417.7	13	-1.2	709.85	18.92	0	P80195|GLCM1_BOVI	
SVLSLSQS	37.25	819.43	8	0.9	410.72	20.81	9.16E+02	P02666|CASB_BOVIN	
SQNPKLPLSIL	37.19	1208.7	11	4.9	605.37	36.07	2.88E+03	P80195|GLCM1_BOVI	
NLHLPLPLLQ	37.15	1156.7	10	5.5	579.36	42.31	1.69E+03	P02666|CASB_BOVIN	
VPQLEIVPN	37.04	1007.6	9	1.7	504.79	28.28	6.64E+03	P02662|CASA1_BOVI	
VPPFLQPEVM(+15.99)	36.97	1171.6	10	1.1	586.81	30.56	1.21E+04	P02666|CASB_BOVIN	Oxidation (M)
VAPFPEVFGKEK	36.94	1346.7	12	6.1	449.92	28.44	5.96E+03	P02662|CASA1_BOVI	
VAPFPEVFGK	36.92	1089.6	10	2.6	545.8	31.57	2.71E+04	P02662|CASA1_BOVI	
NVPGEIVE	36.79	855.43	8	2.4	856.44	18.85	2.98E+04	P02666|CASB_BOVIN	
DKIHPFAQTQ	36.72	1183.6	10	-4.1	592.8	14.98	8.40E+02	P02666|CASB_BOVIN	
SLPQNIPPL	36.56	977.55	9	8.8	489.79	30.7	5.54E+03	P02666|CASB_BOVIN	
AVPYPQ	36.46	673.34	6	5.8	674.35	14.44	3.18E+03	P02666|CASB_BOVIN	
RDMPIQAFLL	36.41	1202.6	10	5.7	602.33	40.55	1.79E+03	P02666|CASB_BOVIN	
KAVPYPQ	36.34	801.44	7	-5.1	401.72	12.63	6.45E+02	P02666|CASB_BOVIN	
PVRGPFPIIV	35.99	1093.7	10	1.4	547.84	37.42	2.22E+03	P02666|CASB_BOVIN	
NENLLRF	35.95	904.48	7	2.6	453.25	28.13	3.90E+04	P02662|CASA1_BOVI	
APFPEVFGKEKV	35.8	1346.7	12	11.6	449.92	29.1	1.10E+03	P02662|CASA1_BOVI	
SQSKVLPVPQ	35.74	1081.6	10	6.2	541.82	19.54	4.81E+03	P02666|CASB_BOVIN	
NVPGEIVESL	35.7	1055.5	10	7.3	528.79	31.18	1.22E+04	P02666|CASB_BOVIN	
AVRSPAQIL	35.67	953.57	9	8.9	477.79	23.81	4.12E+03	P02668|CASK_BOVIN	
IPPLTQTPV	35.56	964.56	9	3.5	483.29	26.48	8.38E+03	P02666|CASB_BOVIN	
IPYVRYL	35.49	922.53	7	4.7	462.27	29.67	9.20E+02	P02663|CASA2_BOVI	
FVAPFPEVFG	35.42	1108.6	10	4.5	555.29	41.76	3.53E+03	P02662|CASA1_BOVI	
YQEPVL	35.28	747.38	6	-7.6	748.38	21.81	3.17E+03	P02666|CASB_BOVIN	
AVPITPT	35.12	697.4	7	0.9	698.41	16.33	2.94E+03	P02663|CASA2_BOVI	
VPPFLQPEVM	35.09	1155.6	10	-0.5	578.81	35.93	3.67E+03	P02666|CASB_BOVIN	
VLPVPQKAVPYPQ	35.07	1434.8	13	1.7	718.42	25.98	0	P02666|CASB_BOVIN	
GHLKALINN	34.86	978.56	9	-8.1	490.28	21.27	5.45E+03	Q9TTK4|LYST_BOVIN	
SILKEKHL	34.74	966.59	8	6.5	323.2	13.55	2.94E+02	P80195|GLCM1_BOVI	
VIESPPEINTVQVTSTAV	34.72	1883	18	-5.5	942.5	30.54	1.44E+03	P02668|CASK_BOVIN	
IHPFAQTQ	34.7	940.48	8	4.8	471.25	15.1	1.25E+03	P02666|CASB_BOVIN	
YAKPAAVRSPA	34.46	1129.6	11	6.1	377.55	12.63	5.76E+02	P02668|CASK_BOVIN	
ALLDPSF	34.39	761.4	7	-4.4	762.4	30.53	1.47E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
VAPFPE	34.37	658.33	6	-1.5	659.34	20.31	4.35E+03	P02662|CASA1_BOVI	
IPIQY	34.36	632.35	5	-8.5	633.36	21.28	2.86E+03	P02668|CASK_BOVIN	
LPLQY	34.36	632.35	5	-8.5	633.36	21.28	2.86E+03		
IPLQY	34.36	632.35	5	-8.5	633.36	21.28	2.86E+03		
PASTGA	34.16	502.24	6	-10.3	503.24	14.47	1.93E+03	Q2KJE5|G3PT_BOVIN	
APFPEV	33.83	658.33	6	6.6	659.34	25.19	3.27E+03	P02662|CASA1_BOVI	
EVLNENLL	33.83	942.5	8	4.1	943.51	28.87	3.65E+03	P02662|CASA1_BOVI	
VAPFPEVFGKEKV	33.72	1445.8	13	9.6	482.94	31.18	2.79E+03	P02662|CASA1_BOVI	
AVESTVATL	33.7	889.48	9	-4.7	890.48	21.19	5.69E+03	P02668|CASK_BOVIN	
GLPQEVLN	33.62	868.47	8	-2.1	869.47	22.74	4.90E+03	P02662|CASA1_BOVI	
ILNKPEDETHL	33.32	1307.7	11	5.5	436.9	17.56	9.90E+03	P80195|GLCM1_BOVI	
SSSEESITRIN	33.31	1221.6	11	-1.1	611.8	15.27	6.26E+02	P02666|CASB_BOVIN	
EPVLGPVRGPFP	33.13	1263.7	12	5.8	632.86	32.17	0	P02666|CASB_BOVIN	
HIQKEDVPSER	32.91	1336.7	11	16.8	446.57	9.48	1.44E+02	P02662|CASA1_BOVI	
PPPPPPPPPP	32.88	988.54	10	5.7	495.28	16.95	2.83E+03	A2VDK6|WASF2_BOV	

Peptide	-10lgP	Mass	Length	ppm	m/z	RT	Area	Accession	PTM
RELEELNVPGE	32.77	1283.6	11	4.9	642.83	23.1	3.81E+03	P02666|CASB_BOVIN	
GPVRGPFPIIV	32.71	1150.7	11	-2.9	576.35	37.53	2.21E+04	P02666|CASB_BOVIN	
PPPAPP	32.7	574.31	6	14.8	575.33	25.35	2.27E+03	A2VDK6|WASF2_BOV	
TVPAK	32.59	514.31	5	9.8	515.32	40.43	0	P02668|CASK_BOVIN :Q08DA0|RABL6_BO	
FPEVF	32.5	637.31	5	-3.4	638.32	30.84	1.76E+03	P02662|CASA1_BOVI	
DLISKEQIVIR	32.2	1312.8	11	6.9	438.6	25.58	8.97E+02	P80195|GLCM1_BOVI	
YYQQKPVALIN	32.15	1335.7	11	-4.6	668.86	22.62	1.38E+03	P02668|CASK_BOVIN	
AFEVVKT	32.09	792.44	7	4.1	397.23	18.08	8.80E+02	P80195|GLCM1_BOVI	
SVKDAAGGPGAPADPGRPT	32.08	1719.9	19	-4.2	574.29	13.09	8.65E+02	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
ILNKPEDETHLEAQPTDASAQFIRNL	32.06	2949.5	26	4.9	738.38	32.67	1.98E+03	P80195|GLCM1_BOVI	
DKIHPF	31.91	755.4	6	5.2	378.71	15.53	7.91E+02	P02666|CASB_BOVIN	
APFPEVFGK	31.74	990.52	9	6	496.27	29.05	3.40E+03	P02662|CASA1_BOVI	
KLKPGKVLFI	31.73	1141.8	10	3.2	571.89	46.73	3.34E+04	Q0VC74|TMLH_BOVI	
RDMPIQAFL	31.57	1089.6	9	-4.6	545.79	35.28	1.10E+03	P02666|CASB_BOVIN	
NAVPITPTLNRE	31.5	1323.7	12	-8.4	662.86	22.62	1.44E+03	P02663|CASA2_BOVI	
ARHPHPHLSF	31.37	1197.6	10	9.7	300.41	14.86	1.12E+03	P02668|CASK_BOVIN	
VGLLL	31.3	513.35	5	-6.2	514.36	25.33	0	P25930|CXCR4_BOVIN:Q08E36|TM198_BOVIN:P01017|ANGT_BOVIN:Q6B411|LYSM_BOVIN:E1B9E5|CTSRD_BOVIN:A1A4M2|EMC10_BOVIN:P0C6R2|ARMC2_BOVIN:Q6VE48-3|MCP_BOVIN:Q17QU7|DHR13_BOVIN:Q9BH13|CD166_BOVIN:Q6VE48|MCP_BOVIN:Q28042|OVGP1_BOVIN:Q32KP1|TSN31_BOVIN:Q0P583|KCNV1_BO	
VGILL	31.3	513.35	5	-6.2	514.36	25.33	0	Q2NKY8|DHX30_BOVIN:Q9BEG8|S26A2_BOVIN:O97827-3|AGRL3_BOVIN:O97827-2|AGRL3_BOVIN:O97827-5|AGRL3_BOVIN:O97827-6|AGRL3_BOVIN:O97827-7|AGRL3_BOVIN:O97827|AGRL3_BOVIN:O97827-4|AGRL3_BOVIN:O97827-8|AGRL3_BOVIN:O97827-11|AGRL3_BOVIN:O97827-12|AGRL3_BOVIN:O97827-9|AGRL3_BOVIN:O97827-10|AGRL3_BOVIN:Q1RMU3|P4HA1_BOVIN:Q06154|PMEL_BOVIN:Q	
VGIII	31.3	513.35	5	-6.2	514.36	25.33	0	Q5BIM9-2|GPHR_BOVIN:Q3T067|SCPDL_BOVIN:Q5BIM9|GPHR_BOVIN:Q32KQ5|TM225_BOVIN	

Peptide	-10lgP	Mass	Length	ppm	m/z	RT	Area	Accession	PTM
VGIIL	31.3	513.35	5	-6.2	514.36	25.33	0	P35071|CFTR_BOVIN:Q5E9E8|YIPF5_BOVIN	
VGLII	31.3	513.35	5	-6.2	514.36	25.33	0	Q28041|ACVR1_BOVIN:A5PK14|LSME1_BOVIN:P27595|HXK1_BO	
VGLLI	31.3	513.35	5	-6.2	514.36	25.33	0	P46411|EAA1_BOVIN:Q3ZBG6|UNC50_BOVIN:Q3ZBG6-2|UNC50_BOVIN:P79119|EPYC_BOVIN:Q3T0X5|PSA1_BOVIN	
VGILI	31.3	513.35	5	-6.2	514.36	25.33	0	P27674|GTR1_BOVIN:Q3SYU7|TNPO1_BOVIN:Q0P5M8|MPPA_BO	
VGLIL	31.3	513.35	5	-6.2	514.36	25.33	0	Q2HJ22|CLD5_BOVIN:Q1LZB0|DDRGK_BOVIN:A2VDP2|PCMD1_BOVIN:Q58CZ2|PCMD	
AVRSPAQILQ	31.1	1081.6	10	0.2	541.82	22.4	5.50E+03	P02668|CASK_BOVIN	
PPPPPPP	31.04	697.38	7	5.8	349.7	12.36	4.69E+02	A2VDK6|WASF2_BOVIN:Q2KJE5|G3PT_BOVIN:Q32LP2|RADI_BO	
PPPPVI	30.86	618.37	6	14	619.39	31.06	1.13E+04	Q32LP2|RADI_BOVIN	
PPPPVL	30.86	618.37	6	14	619.39	31.06	1.13E+04		
LPQEVL	30.83	697.4	6	0.4	698.41	23	2.76E+03	P02662|CASA1_BOVIN:Q9TTK4|LYST_BOV	
IPQEVL	30.83	697.4	6	0.4	698.41	23	2.76E+03		
LPQEVLNENLL	30.72	1280.7	11	0.3	641.36	33.99	2.56E+03	P02662|CASA1_BOVI	
AVPITPTLNRE	30.64	1209.7	11	3.7	605.85	21.94	9.25E+02	P02663|CASA2_BOVI	
AVPITPTLN	30.63	924.53	9	4.7	463.27	23.96	6.14E+02	P02663|CASA2_BOVI	
ALPQY	30.54	590.31	5	4.4	591.32	17	0	P02663|CASA2_BOVI	
AIPQY	30.54	590.31	5	4.4	591.32	17	0		
ILNKPEDETHLEAQPT	30.47	1833.9	16	6.5	612.31	18.06	3.99E+03	P80195|GLCM1_BOVI	
VPQLEIVPNS(+79.97)AEERLHSM(+	30.46	2144	18	2.8	715.68	28.77	3.39E+03	P02662|CASA1_BOVIN	Phosphorylation (STY); Oxidation
SLITIFSA	30.3	850.48	8	-1.8	426.25	29.29	1.59E+03	P48452-2|PP2BA_BOVIN:P48452|PP2BA_BOVIN	
SPPEINTVQ	30.23	983.49	9	5.1	492.76	16.41	2.46E+03	P02668|CASK_BOVIN	
VPSERYL	30	862.45	7	4.4	432.24	17.74	1.96E+03	P02662|CASA1_BOVI	

m/z = mass to charge ratio; RT = retention time; PTM = post-translational modification

Table A1.7. Peptide sequences identified in delactosed permeate from production plant 2, batch B (Chapter IV)

Peptide	-10lgP	Mass	Length	ppm	m/z	RT	Area	Accession	PTM
YQEPVLGPVRGPFPIIV	78.8	1880.1	17	3.5	941.04	44.38	1.56E+05	P02666|CASB_BOVIN	
LLYQEPVLGPVRGPFPIIV	62.29	2106.2	19	3.1	1054.1	46.28	1.23E+04	P02666|CASB_BOVIN	
KVLPVPQ	57.59	779.49	7	4.2	390.75	18.03	2.23E+03	P02666|CASB_BOVIN	
PEVIESPPEINTVQVTSTAV	57.12	2109.1	20	4.1	1055.6	32.31	2.99E+04	P02668|CASK_BOVIN	
YQEPVLGPVRGPFPI	56.38	1667.9	15	7	834.96	38.99	6.94E+03	P02666|CASB_BOVIN	
YQEPVLGPVRGPFPII	56.19	1781	16	4.9	891.51	42.59	2.84E+04	P02666|CASB_BOVIN	
TVQVTSTAV	53.88	904.49	9	4	905.5	17.02	6.51E+04	P02668|CASK_BOVIN	
SLVYPFPGPIPN	53.49	1299.7	12	3.5	650.85	36.94	7.35E+03	P02666|CASB_BOVIN	
ILNKPEDETHLEAQPTDASAQFIRNL	52.31	2949.5	26	-2.4	738.38	32.69	4.78E+03	P80195|GLCM1_BOVIN	
VAPFPEVFGK	51.62	1089.6	10	0	545.8	31.54	2.59E+04	P02662|CASA1_BOVI	
EPVLGPVRGPFPIIV	51.56	1588.9	15	7.4	795.48	43.19	1.11E+04	P02666|CASB_BOVIN	
DAAGGPGAPADPGRPT	51.51	1405.7	16	1.6	703.84	12.43	2.91E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
GLPQEVLNENLLRF	51.1	1640.9	14	8.1	547.97	41.76	1.19E+04	P02662|CASA1_BOVI	
SLSQSKVLPVPQ	50.98	1281.7	12	0.3	641.87	23.68	5.39E+03	P02666|CASB_BOVIN	
VQVTSTAV	50.69	803.44	8	0.8	804.45	15.57	2.97E+04	P02668|CASK_BOVIN	
VIESPPEIN	50.6	996.51	9	-1.4	997.52	20.23	4.66E+04	P02668|CASK_BOVIN	
ILNKPEDETHLEAQPTDASAQFIRN	50.25	2836.4	25	17.5	710.12	24.76	8.49E+03	P80195|GLCM1_BOVIN	
YQEPVLGPVRGPFP	49.96	1554.8	14	-1.5	778.42	33.73	5.60E+03	P02666|CASB_BOVIN	
VAPFPEVFG	49.5	961.49	9	-0.3	962.5	35.79	1.16E+04	P02662|CASA1_BOVI	
PPPPPPPPPP	49.19	988.54	10	8.5	495.28	16.97	5.41E+03	A2VDK6|WASF2_BOVI	
TQTPVVVPPFLQPEVM(+15.99)	49.15	1796.9	16	4.5	899.48	40.04	1.86E+04	P02666|CASB_BOVIN	Oxidation (M)
AQPTDASAQF	48.84	1034.5	10	-1.3	1035.5	16.38	1.30E+04	P80195|GLCM1_BOVIN	
FVAPFPEVFGKEK	48.71	1493.8	13	3.4	498.94	34.42	6.31E+03	P02662|CASA1_BOVI	
PPPPPPPPP	48.25	891.49	9	4.4	446.75	15.95	1.97E+04	A2VDK6|WASF2_BOVI	
SPPEINTVQVTSTAV	48.07	1541.8	15	0.1	771.9	26.68	5.17E+03	P02668|CASK_BOVIN	
LVYPFPGPIPN	48.03	1212.7	11	1.4	607.34	35.89	3.49E+03	P02666|CASB_BOVIN	
GLPQEVLNENLLR	47.69	1493.8	13	-3.2	747.92	33.94	7.24E+03	P02662|CASA1_BOVI	
APFPEVFG	47.15	862.42	8	-0.4	863.43	33.25	4.98E+03	P02662|CASA1_BOVI	
APFPEVFGK	47.13	990.52	9	10.8	496.27	29.07	2.60E+03	P02662|CASA1_BOVI	
LPQEVLNENLL	46.49	1280.7	11	-3.1	641.35	34.01	6.35E+03	P02662|CASA1_BOVI	
FVAPFPEVFG	45.66	1108.6	10	-1.1	555.29	41.76	2.13E+03	P02662|CASA1_BOVI	
PEVIESPPEINT	45.61	1323.7	12	-8.6	662.83	25.02	0	P02668|CASK_BOVIN	
VVPPFLQPEVM	45.32	1254.7	11	6.7	628.35	38.54	6.77E+03	P02666|CASB_BOVIN	
QEPVLGPVRGPFPIIV	45.26	1717	16	5	859.51	43.32	8.29E+02	P02666|CASB_BOVIN	
SLVYPFPGPIPNSLPQ	45.2	1724.9	16	8.1	863.47	39.98	9.59E+02	P02666|CASB_BOVIN	
PPPAPPPPP	45.08	865.47	9	4	433.74	15.23	7.45E+02	A2VDK6|WASF2_BOVIN:A6QR00|ZN526_BO	
LYQEPVLGPVRGPFPIIV	44.88	1993.1	18	-1.1	997.58	45.37	4.18E+03	P02666|CASB_BOVIN	
NENLLRFF	44.81	1051.5	8	0	526.78	38.19	1.13E+04	P02662|CASA1_BOVI	
SLPQNIPPLTQTPV	44.77	1503.8	14	5.9	752.93	32.23	4.58E+03	P02666|CASB_BOVIN	
IASGEPTSTPTTEA	44.61	1360.6	14	7.7	681.33	14.3	1.15E+04	P02668|CASK_BOVIN	
GLPQEVLNENLL	44.46	1337.7	12	1.3	669.87	36.91	2.65E+04	P02662|CASA1_BOVI	
FVAPFPEVFGK	44.08	1236.7	11	5.7	619.34	37.69	2.07E+04	P02662|CASA1_BOVI	
YYQQKPVAL	43.82	1108.6	9	-17.9	555.29	19.83	1.66E+03	P02668|CASK_BOVIN	
VAPFPEVF	43.7	904.47	8	-7.3	905.47	36.78	3.98E+03	P02662|CASA1_BOVI	
AAGGPGAPADPGRPT	43.18	1290.6	15	-1.1	646.32	11.87	3.05E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
VVPPFLQPEVM(+15.99)	43.11	1270.7	11	3.7	636.34	33.59	2.16E+04	P02666|CASB_BOVIN	Oxidation (M)
EVLNENLLRF	43.01	1245.7	10	1.5	623.84	35.44	1.22E+04	P02662|CASA1_BOVI	
PEINTVQVTSTAV	42.99	1357.7	13	5.5	679.87	24.91	7.99E+02	P02668|CASK_BOVIN	
SHAFEVVKT	42.83	1016.5	9	5.9	509.27	16.41	4.77E+03	P80195|GLCM1_BOVIN	
IASGEPTSTPTTE	42.66	1289.6	13	1.4	645.81	13.04	1.76E+03	P02668|CASK_BOVIN	
PPPPPPPP	42.34	794.43	8	3.7	398.23	14.37	1.45E+04	A2VDK6|WASF2_BOVI	
NAVPITPT	42.33	811.44	8	1.8	812.45	17.63	2.42E+03	P02663|CASA2_BOVI	
TEIPTINT	42.28	887.46	8	0.4	888.47	22.56	4.23E+04	P02668|CASK_BOVIN	
VESTVATL	42.27	818.44	8	1.2	819.45	19.31	1.39E+04	P02668|CASK_BOVIN	
APFPEVF	42.07	805.4	7	0.2	806.41	34.41	5.49E+03	P02662|CASA1_BOVI	
INTVQVTSTAV	41.78	1131.6	11	1.6	566.81	21.89	6.56E+03	P02668|CASK_BOVIN	
ILNKPEDETHLEAQPTDASAQFIR	41.72	2722.4	24	13	681.61	25.47	5.37E+03	P80195|GLCM1_BOVIN	
AVPYPQ	41.57	673.34	6	0.4	674.35	14.45	2.02E+03	P02666|CASB_BOVIN	
FSHAFEVVKT	41.56	1163.6	10	7.5	388.88	21.47	1.56E+03	P80195|GLCM1_BOVIN	
ILNKPEDETHLEAQPTDASAQF	40.96	2453.2	22	9.6	818.74	24.3	1.84E+04	P80195|GLCM1_BOVIN	

Peptide	-10lgP	Mass	Length	ppm	m/z	RT	Area	Accession	PTM
FVAPFPEVF	40.84	1051.5	9	8.2	526.78	42.5	4.25E+03	P02662|CASA1_BOVI	
EVIESPPEINTVQVTSTAV	40.76	2012	19	-3.4	1007	31.34	1.84E+04	P02668|CASK_BOVIN	
AQTQSLVYPFPGPIPN	40.67	1727.9	16	3.9	864.95	36.15	1.02E+04	P02666|CASB_BOVIN	
NIPPLTQTPV	40.57	1078.6	10	8.6	1079.6	27.34	1.68E+04	P02666|CASB_BOVIN	
GPFPIIV	40.43	741.44	7	-3.9	742.45	38.1	7.91E+02	P02666|CASB_BOVIN	
VPPFLQPEVM(+15.99)	40.38	1171.6	10	-0.1	586.8	30.55	3.62E+03	P02666|CASB_BOVIN	Oxidation (M)
VIESPPEINT	39.99	1097.6	10	6.2	549.79	21.6	8.76E+03	P02668|CASK_BOVIN	
PPPPPPP	39.31	697.38	7	4.8	349.7	12.41	6.82E+02	A2VDK6|WASF2_BOVIN:E1BB52|CDK13_BOVIN:Q0P5J8|STRP1_BO	
NAVPITPTLN	39.12	1038.6	10	-7	520.29	24.59	1.04E+03	P02663|CASA2_BOVI	
HIQKEDVPSERYL	38.84	1612.8	13	-3.4	538.61	19.06	4.87E+03	P02662|CASA1_BOVI	
GLPQEVLNENL	38.76	1224.6	11	-4.5	613.32	31.31	1.42E+03	P02662|CASA1_BOVI	
PYVPVHFDASV	38.71	1229.6	11	-6.4	615.81	29.92	0	P61823|RNAS1_BOVIN	
NVPGEIVESL	38.38	1055.5	10	2.4	528.78	31.16	1.25E+04	P02666|CASB_BOVIN	
SQNPKLPL	38.26	895.51	8	-1.3	448.76	22.2	4.50E+03	P80195|GLCM1_BOVIN	
KAVPYPQ	38.25	801.44	7	4.5	401.73	12.68	8.48E+02	P02666|CASB_BOVIN	
RDMPIQAFLL	38.19	1202.6	10	5.2	602.33	40.64	3.87E+03	P02666|CASB_BOVIN	
LPQEVLNENLLR	37.86	1436.8	12	12.3	719.42	30.66	4.51E+02	P02662|CASA1_BOVI	
IASGEPTSTPTT	37.58	1160.6	12	-0.2	581.29	12.85	7.22E+03	P02668|CASK_BOVIN	
VPPFLQPEVM(+15.99)GV	37.3	1327.7	12	3.5	664.85	34.54	1.69E+03	P02666|CASB_BOVIN	Oxidation (M)
VIESPPEINTVQVTSTAV	37.15	1883	18	1.5	942.5	30.51	1.35E+03	P02668|CASK_BOVIN	
VIESPPEINTVQ	37.1	1324.7	12	-4.6	663.35	23.35	3.63E+03	P02668|CASK_BOVIN	
GLPQEVL	36.62	754.42	7	6.6	755.43	26.05	7.88E+03	P02662|CASA1_BOVI	
VLNENLL	36.44	813.46	7	-12.4	814.46	26.57	6.85E+02	P02662|CASA1_BOVI	
FFVAPFPEVFGK	36.4	1383.7	12	4.1	692.87	42.78	9.64E+02	P02662|CASA1_BOVI	
LHLPLPLLQ	36.27	1042.7	9	3.1	522.34	42.46	2.05E+04	P02666|CASB_BOVIN	
YAKPAAVRSPA	36.13	1129.6	11	18.9	377.56	12.7	2.78E+02	P02668|CASK_BOVIN	
FVAPFPE	36.12	805.4	7	-2.4	403.71	29.21	9.33E+02	P02662|CASA1_BOVI	
ILNKPEDETHL	36.02	1307.7	11	3.6	436.9	17.61	7.73E+03	P80195|GLCM1_BOVIN	
NENLLRF	35.88	904.48	7	2.1	453.25	28.21	2.95E+04	P02662|CASA1_BOVI	
IASGEPTSTPT	35.84	1059.5	11	6	1060.5	12.06	6.74E+02	P02668|CASK_BOVIN	
AVPYPQR	35.77	829.44	7	6.2	415.73	12.73	5.21E+02	P02666|CASB_BOVIN	
VPPFLQPEVM	35.64	1155.6	10	-7.4	578.8	35.89	2.22E+03	P02666|CASB_BOVIN	
NVPGEIVE	35.59	855.43	8	2.6	856.44	18.82	1.50E+04	P02666|CASB_BOVIN	
S(+79.97)PEVIESPPEINTVQVTSTAV	35.25	2276.1	21	-4.9	1139	32.26	4.84E+03	P02668|CASK_BOVIN	Phosphorylation (STY)
GLPQEVLN	35.18	868.47	8	0.5	869.47	22.7	1.74E+03	P02662|CASA1_BOVI	
APFPEV	35.1	658.33	6	0.4	659.34	25.3	2.75E+03	P02662|CASA1_BOVI	
PVLGPVRGPFPIIV	35	1459.9	14	1.2	730.95	43.4	1.35E+03	P02666|CASB_BOVIN	
ALPQYLKT	34.75	932.53	8	8.7	467.28	22.34	5.09E+02	P02663|CASA2_BOVI	
FVAPFPEVFGKEKV	34.56	1592.9	14	7.8	531.96	36.55	4.24E+03	P02662|CASA1_BOVI	
SKVLPVPQ	34.48	866.52	8	4	434.27	19.03	1.10E+03	P02666|CASB_BOVIN	
SSRQPQSQNPKLPL	34.48	1578.8	14	1.5	527.29	20.8	4.87E+03	P80195|GLCM1_BOVIN	
VPQLEIVPN	34.2	1007.6	9	-0.6	504.79	28.27	6.77E+03	P02662|CASA1_BOVI	
VLSRYPSYGLN	34.14	1267.7	11	-3.3	634.83	22.12	6.91E+02	P02668|CASK_BOVIN	
EVLNENLLR	34.11	1098.6	9	0.6	550.31	24.89	1.18E+04	P02662|CASA1_BOVI	
PVRGPFPIIV	34.07	1093.7	10	1.2	547.84	37.45	3.90E+03	P02666|CASB_BOVIN	
RDMPIQAFL	34.06	1089.6	9	-2.4	545.79	35.24	9.01E+02	P02666|CASB_BOVIN	
YYQQKPVALINN	34.04	1449.8	12	-3	725.89	21.58	1.52E+03	P02668|CASK_BOVIN	
GHLKALINN	33.96	978.56	9	-6.6	490.28	21.27	3.29E+03	Q9TTK4|LYST_BOVIN	
ARHPHPHLSF	33.73	1197.6	10	9	300.41	15.03	7.54E+02	P02668|CASK_BOVIN	
PVVVPPFLQPE	33.71	1220.7	11	-5.7	611.34	38.21	1.13E+03	P02666|CASB_BOVIN	
ILNKPEDETHLE	33.42	1436.7	12	4.4	479.91	16.69	2.73E+04	P80195|GLCM1_BOVIN	
S(+79.97)PEVIESPPEINT	33.37	1490.7	13	-0.8	746.33	24.97	2.95E+03	P02668|CASK_BOVIN	Phosphorylation (STY)
IESPPEIN	33.28	897.44	8	12.4	449.74	18.07	1.09E+03	P02668|CASK_BOVIN	
IASGEPTSTPTTEAVE	33.25	1588.7	16	0.4	795.38	17.47	1.86E+03	P02668|CASK_BOVIN	
VYPFPGPIPN	33.05	1099.6	10	-3.5	550.79	32.06	1.72E+03	P02666|CASB_BOVIN	
RELEELNVPGEIVE	32.97	1624.8	14	1.6	813.42	29.8	9.59E+03	P02666|CASB_BOVIN	
PGLLLLLAVLSLGTA	32.97	1449.9	15	18.7	725.98	48.63	4.17E+04	P07589|FINC_BOVIN	
IPNSLPQNIPPLTQTPVVVPPFLQPEVM	32.9	3080.7	28	-18.8	1027.9	45.11	1.48E+04	P02666|CASB_BOVIN	Oxidation (M)
SQSKVLPVPQ	32.81	1081.6	10	8.2	541.82	19.5	1.47E+03	P02666|CASB_BOVIN	
GPVRGPFPIIV	32.51	1150.7	11	1.7	576.35	37.58	7.12E+03	P02666|CASB_BOVIN	
AVESTVATL	32.31	889.48	9	-2.7	890.48	21.15	1.35E+03	P02668|CASK_BOVIN	
SDIPNPIGSENSE	32.21	1357.6	13	4.7	679.81	22.26	1.85E+03	P02662|CASA1_BOVI	
TIASGEPTSTPTTE	32.15	1390.6	14	0	696.33	14.85	1.68E+03	P02668|CASK_BOVIN	
AVPITPT	32.01	697.4	7	2.9	698.41	16.41	8.39E+02	P02663|CASA2_BOVI	
PQVEAVLN	31.95	868.47	8	-2.8	869.47	22.68	1.98E+03	A6QL48|IL34_BOVIN	
SRYPSYGLN	31.65	1055.5	9	-6.4	528.76	17.99	0	P02668|CASK_BOVIN	
IHPFAQTQ	31.64	940.48	8	-0.1	471.25	15.15	1.43E+03	P02666|CASB_BOVIN	
ILNKPEDETHLEAQPTDASAQ	31.64	2306.1	21	2.2	769.71	18.49	1.21E+03	P80195|GLCM1_BOVIN	
FPEVF	31.58	637.31	5	4.2	638.32	30.84	1.04E+03	P02662|CASA1_BOVI	
APFPE	31.54	559.26	5	1.6	560.27	16.61	2.00E+03	P02662|CASA1_BOVI	

m/z = mass to charge ratio; RT = retention time; PTM = post-translational modification

Table A1.8. Peptide sequences identified in delactosed permeate from production plant 2, batch C (Chapter IV)

Peptide	-10lgF	Mass	Length	ppm	m/z	RT	Area	Accession	PTM
YQEPVLGPVRGPFPIIV	67.35	1880.06	17	2.8	941.038	44.15	1.25E+05	P02666|CASB_BOVIN	
YQEPVLGPVRGPFPII	63.31	1780.99	16	3.8	891.504	42.54	1.86E+04	P02666|CASB_BOVIN	
LLYQEPVLGPVRGPFPIIV	56.4	2106.22	19	3.2	1054.12	46.27	6.63E+03	P02666|CASB_BOVIN	
SHAFEVVKT	55.04	1016.53	9	8.5	339.854	16.3	6.84E+03	P80195|GLCM1_BOVIN	
AAGGPGAPADPGRPT	53.97	1290.63	15	-0.8	646.324	11.88	4.17E+03	P81265|PIGR_BOVIN:P81265-2|PIGR_BOVIN	
KVLPVPQ	53.58	779.491	7	8.1	390.757	17.98	3.12E+03	P02666|CASB_BOVIN	
YQEPVLGPVRGPFPI	53.25	1667.9	15	-0.6	834.96	39.03	4.80E+03	P02666|CASB_BOVIN	
SLVYPFPGPIPN	52.83	1299.69	12	0.8	650.851	36.88	9.85E+03	P02666|CASB_BOVIN	
GLPQEVLNENLLRF	51.9	1640.89	14	4.4	821.457	41.71	8.70E+03	P02662|CASA1_BOVIN	
AQPTDASAQF	51.63	1034.47	10	-2	1035.47	16.33	1.82E+04	P80195|GLCM1_BOVIN	
YYQQKPVALINN	50.41	1449.76	12	-3.1	725.886	21.6	2.04E+03	P02668|CASK_BOVIN	
APFPEVFG	49.82	862.423	8	-2.8	863.429	33.29	2.44E+03	P02662|CASA1_BOVIN	
VIESPPEIN	49.76	996.513	9	-1.7	997.521	20.24	6.68E+04	P02668|CASK_BOVIN	
EVIESPPEINTVQVTSTAV	49.48	2012.03	19	-0.9	1007.02	31.36	9.81E+03	P02668|CASK_BOVIN	
DAAGGPGAPADPGRPT	49.42	1405.66	16	-2.9	703.836	12.35	3.84E+03	P81265|PIGR_BOVIN:P81265-2|PIGR_BOVIN	
TQTPVVVPPFLQPEVM(+1!	48.61	1796.94	16	9.5	899.485	39.9	1.13E+04	P02666|CASB_BOVIN	Oxidation (M)
VAPFPEVFGK	48.32	1089.59	10	1.9	545.802	31.57	2.50E+04	P02662|CASA1_BOVIN	
EPVLGPVRGPFPIIV	48.07	1588.93	15	4.3	795.48	43.27	1.25E+04	P02666|CASB_BOVIN	
SLSQSKVLPVPQ	47.87	1281.73	12	-1.9	641.872	23.68	3.17E+03	P02666|CASB_BOVIN	
TIASGEPTSTPTTE	47.63	1390.65	14	3.6	696.335	14.83	2.19E+03	P02668|CASK_BOVIN	
VVPPFLQPEVM	47.4	1254.67	11	8.1	628.346	38.46	4.06E+03	P02666|CASB_BOVIN	
VQVTSTAV	46.62	803.439	8	-5	804.442	15.54	3.50E+03	P02668|CASK_BOVIN	
PVLGPVRGPFPIIV	45.73	1459.89	14	-3.2	730.952	43.24	3.67E+03	P02666|CASB_BOVIN	
NENLLRFF	45.68	1051.55	8	-1.5	526.78	38.22	1.17E+04	P02662|CASA1_BOVIN	
VPPFLQPEVM(+15.99)	45.5	1171.59	10	6.6	586.809	30.54	1.09E+04	P02666|CASB_BOVIN	Oxidation (M)
VAPFPEVFG	45.02	961.491	9	-3.2	962.495	35.76	3.84E+03	P02662|CASA1_BOVIN	
VIESPPEINTVQ	44.99	1324.69	12	2.7	663.354	23.35	9.30E+03	P02668|CASK_BOVIN	
TVQVTSTAV	44.8	904.487	9	-2.7	905.494	16.98	5.04E+04	P02668|CASK_BOVIN	
SDIPNPIGSENSE	44.45	1357.6	13	1	679.808	22.27	4.91E+03	P02662|CASA1_BOVIN	
ILNKPEDETHLEAQPTDAS/	44.42	2722.36	24	-4.5	681.595	25.41	6.21E+03	P80195|GLCM1_BOVIN	
SSRQPQSQNPKLPL	44.19	1578.85	14	9.8	527.295	20.86	4.71E+03	P80195|GLCM1_BOVIN	
VVPPFLQPEVM(+15.99)	43.98	1270.66	11	-1.8	636.339	33.58	2.50E+04	P02666|CASB_BOVIN	Oxidation (M)
APFPEVFGKEKV	43.68	1346.72	12	18.2	449.924	29.09	9.77E+02	P02662|CASA1_BOVIN	
SPPEINTVQVTSTAV	43.06	1541.79	15	1.1	771.907	26.63	5.73E+03	P02668|CASK_BOVIN	
EVLNENLLRF	42.53	1245.67	10	3.3	623.847	35.39	4.69E+03	P02662|CASA1_BOVIN	
FVAPFPEVFGK	42.17	1236.65	11	0.2	619.336	37.6	1.04E+04	P02662|CASA1_BOVIN	
LYQEPVLGPVRGPFPIIV	41.57	1993.14	18	2.6	997.582	45.31	2.05E+03	P02666|CASB_BOVIN	
PEVIESPPEINTVQVTSTAV	41.53	2109.08	20	2	1055.55	32.34	2.18E+04	P02668|CASK_BOVIN	
YQEPVLGPVRGPFP	41.05	1554.82	14	-1	778.418	33.74	5.87E+03	P02666|CASB_BOVIN	
LVYPFPGPIPN	40.92	1212.65	11	2.2	607.337	35.9	3.99E+03	P02666|CASB_BOVIN	
NVPGEIVE	40.68	855.434	8	1.8	856.445	18.86	3.37E+04	P02666|CASB_BOVIN	
AQPTDASAQFIR	40.29	1303.65	12	1.3	652.836	19.91	1.85E+03	P80195|GLCM1_BOVIN	
PPPPPPPPPP	40.03	988.538	10	-4.2	495.275	16.98	3.13E+03	7Z063|WASH1_BOVIN:Q95107|WASL_BOVIN:P55106|GDF6_BOVIN:A2VDK6|WASF2_BOVIN:Q08DG5|HXB4_BOVIN	
PPPPPPPP	39.88	794.433	8	1.5	398.224	14.33	7.29E+03	7Z063|WASH1_BOVIN:Q95107|WASL_BOVIN:P55106|GDF6_BOVIN:A2VDK6|WASF2_BOVIN:Q08DG5|HXB4_BOVIN	
AQTQSLVYPFPGPIPN	39.79	1727.89	16	-4.5	864.949	36.18	5.48E+03	P02666|CASB_BOVIN	
NAVPITPTLN	39.61	1038.57	10	7.2	520.298	24.56	1.11E+03	P02663|CASA2_BOVIN	
SLPQNIPPLTQTPV	39.1	1503.83	14	-0.6	752.922	32.14	6.35E+03	P02666|CASB_BOVIN	
FVAPFPEVF	38.95	1051.54	9	-0.4	526.776	42.45	2.20E+03	P02662|CASA1_BOVIN	
GLPQEVLNENLL	38.84	1337.72	12	1.4	669.868	36.79	1.34E+04	P02662|CASA1_BOVIN	
HIQKEDVPSERYL	38.65	1612.82	13	5.6	538.617	19.05	1.11E+04	P02662|CASA1_BOVIN	
GLPQEVLN	38.54	868.465	8	0	869.475	22.71	2.22E+03	P02662|CASA1_BOVIN	
PPPPPPPPP	38.39	891.485	9	2.2	446.751	15.9	1.99E+04	7Z063|WASH1_BOVIN:Q95107|WASL_BOVIN:P55106|GDF6_BOVIN:A2VDK6|WASF2_BOVIN:Q08DG5|HXB4_BOVIN	

<table>
<tr><th>Peptide</th><th>-10lgF</th><th>Mass</th><th>Length</th><th>ppm</th><th>m/z</th><th>RT</th><th>Area</th><th>Accession</th><th>PTM</th></tr>
<tr><td>VAPFPEVF</td><td>38.36</td><td>904.469</td><td>8</td><td>-1.3</td><td>905.476</td><td>36.69</td><td>9.90E+03</td><td>P02662|CASA1_BOVIN</td><td></td></tr>
<tr><td>SQNPKLPL</td><td>38.21</td><td>895.513</td><td>8</td><td>-4.5</td><td>448.762</td><td>22.16</td><td>1.93E+03</td><td>P80195|GLCM1_BOVIN</td><td></td></tr>
<tr><td>ILNKPEDETHL</td><td>37.86</td><td>1307.67</td><td>11</td><td>7</td><td>436.901</td><td>17.52</td><td>8.31E+03</td><td>P80195|GLCM1_BOVIN</td><td></td></tr>
<tr><td>NENLLRF</td><td>37.85</td><td>904.477</td><td>7</td><td>-0.3</td><td>453.247</td><td>28.15</td><td>1.53E+04</td><td>P02662|CASA1_BOVIN</td><td></td></tr>
<tr><td>APFPEVF</td><td>37.75</td><td>805.401</td><td>7</td><td>2.6</td><td>806.412</td><td>34.37</td><td>2.82E+03</td><td>P02662|CASA1_BOVIN</td><td></td></tr>
<tr><td>APFPEVFGK</td><td>37.59</td><td>990.517</td><td>9</td><td>6.6</td><td>496.269</td><td>28.95</td><td>1.84E+03</td><td>P02662|CASA1_BOVIN</td><td></td></tr>
<tr><td>RELEELNVPGEIVESL</td><td>37.23</td><td>1824.95</td><td>16</td><td>-0.7</td><td>913.482</td><td>39.89</td><td>1.97E+03</td><td>P02666|CASB_BOVIN</td><td></td></tr>
<tr><td>YQEPVL</td><td>37.22</td><td>747.38</td><td>6</td><td>-3.5</td><td>748.387</td><td>21.79</td><td>1.35E+03</td><td>P02666|CASB_BOVIN</td><td></td></tr>
<tr><td>VYPFPGPIPN</td><td>37.08</td><td>1099.57</td><td>10</td><td>-6.8</td><td>550.79</td><td>32.08</td><td>1.72E+03</td><td>P02666|CASB_BOVIN</td><td></td></tr>
<tr><td>GPVRGPFPIIV</td><td>37.03</td><td>1150.69</td><td>11</td><td>-2</td><td>576.351</td><td>37.48</td><td>1.00E+04</td><td>P02666|CASB_BOVIN</td><td></td></tr>
<tr><td>NIPPLTQTPV</td><td>36.81</td><td>1078.6</td><td>10</td><td>4.4</td><td>1079.62</td><td>27.3</td><td>1.81E+04</td><td>P02666|CASB_BOVIN</td><td></td></tr>
<tr><td>NAVPITPT</td><td>36.75</td><td>811.444</td><td>8</td><td>6.1</td><td>406.733</td><td>17.62</td><td>1.28E+03</td><td>P02663|CASA2_BOVIN</td><td></td></tr>
<tr><td>VPQLEIVPN</td><td>36.39</td><td>1007.57</td><td>9</td><td>3.4</td><td>504.793</td><td>28.29</td><td>3.30E+03</td><td>P02662|CASA1_BOVIN</td><td></td></tr>
<tr><td>AVESTVATL</td><td>35.9</td><td>889.476</td><td>9</td><td>-1.6</td><td>890.484</td><td>21.19</td><td>2.23E+03</td><td>P02668|CASK_BOVIN</td><td></td></tr>
<tr><td>VPPFLQPEVM</td><td>35.82</td><td>1155.6</td><td>10</td><td>-0.4</td><td>578.808</td><td>35.9</td><td>1.43E+03</td><td>P02666|CASB_BOVIN</td><td></td></tr>
<tr><td>GVILL</td><td>35.66</td><td>513.353</td><td>5</td><td>-6.8</td><td>514.358</td><td>25.13</td><td>7.91E+03</td><td>Q32KU6|TSN6_BOVIN:P00157|CYB_BOVIN:Q95L46|IF4G2_BOVIN:P85521|C163A_BOVIN</td><td></td></tr>
<tr><td>GVLLL</td><td>35.66</td><td>513.353</td><td>5</td><td>-6.8</td><td>514.358</td><td>25.13</td><td>7.91E+03</td><td>Q3T166|MPTX_BOVIN:Q2HJA4|PAR2_BOVIN:F1N5C8|ENPP6_BOVIN:P31783|CD8A_BOVIN:Q5E9J5|DHCR7_BOVIN:P41541|USO1_BOVIN:A3KMY4|CTL4_BOVIN:A4IF98|RMI1_BOVIN:Q32PB3|SACA4_BOVIN:Q28178|TSP1_BOVIN:A6QL94|IZUM3_BOVIN:Q9TT94|ACOD_BOVIN:A6QPF4|M4A18_BOVIN:</td><td></td></tr>
<tr><td>GVLIL</td><td>35.66</td><td>513.353</td><td>5</td><td>-6.8</td><td>514.358</td><td>25.13</td><td>7.91E+03</td><td>Q29RV1|PDIA4_BOVIN:P13752|HA1A_BOVIN:Q08DM8|TSN_BOVIN:Q3ZBE0|DQX1_BOVIN</td><td></td></tr>
<tr><td>GVIII</td><td>35.66</td><td>513.353</td><td>5</td><td>-6.8</td><td>514.358</td><td>25.13</td><td>7.91E+03</td><td>Q29RR6|VSIG1_BOVIN</td><td></td></tr>
<tr><td>GVLLI</td><td>35.66</td><td>513.353</td><td>5</td><td>-6.8</td><td>514.358</td><td>25.13</td><td>7.91E+03</td><td>P20004|ACON_BOVIN:Q9TTK4|LYST_BOVIN</td><td></td></tr>
<tr><td>GVIIL</td><td>35.66</td><td>513.353</td><td>5</td><td>-6.8</td><td>514.358</td><td>25.13</td><td>7.91E+03</td><td>Q17QT7|CYRIA_BOVIN:Q2KJI3|CYRIB_BOVIN</td><td></td></tr>
<tr><td>GVILI</td><td>35.66</td><td>513.353</td><td>5</td><td>-6.8</td><td>514.358</td><td>25.13</td><td>7.91E+03</td><td>Q08DV9|T131L_BOVIN:P79136-2|CAPZB_BOVIN:P79136|CAPZB_BOVIN</td><td></td></tr>
<tr><td>GVLII</td><td>35.66</td><td>513.353</td><td>5</td><td>-6.8</td><td>514.358</td><td>25.13</td><td>7.91E+03</td><td>Q2KI42|PSD11_BOVIN</td><td></td></tr>
<tr><td>AVPYPQ</td><td>35.61</td><td>673.344</td><td>6</td><td>-5.5</td><td>674.349</td><td>14.42</td><td>1.03E+03</td><td>P02666|CASB_BOVIN</td><td></td></tr>
<tr><td>PYVPVHFDASV</td><td>35.56</td><td>1229.61</td><td>11</td><td>3.2</td><td>615.815</td><td>30.04</td><td>0</td><td>P61823|RNAS1_BOVIN</td><td></td></tr>
</table>

m/z = mass to charge ratio; RT = retention time; PTM = post-translational modification

Table A1.9. Peptide sequences identified in delactosed permeate from production plant 2, batch D (Chapter IV)

Peptide	-10lgP	Mass	Length	ppm	m/z	RT	Area	Accession	PTM
YQEPVLGPVRGPFPIIV	76.04	1880.1	17	-1.4	941.04	43.99	2.05E+05	P02666|CASB_BOVIN	
YQEPVLGPVRGPFPII	58.85	1781	16	2.3	891.51	42.39	2.79E+04	P02666|CASB_BOVIN	
LLYQEPVLGPVRGPFPIIV	57.09	2106.2	19	1.5	1054.1	46.19	2.26E+04	P02666|CASB_BOVIN	
PVLGPVRGPFPIIV	55.84	1459.9	14	-4.9	730.95	42.86	4.47E+03	P02666|CASB_BOVIN	
VVPPFLQPEVM	53.74	1254.7	11	5.1	628.35	38.45	1.03E+04	P02666|CASB_BOVIN	
KVLPVPQ	52.61	779.49	7	-1	390.75	17.93	2.98E+03	P02666|CASB_BOVIN	
LVYPFPGPIPN	52.59	1212.7	11	2.7	607.34	35.82	4.68E+03	P02666|CASB_BOVIN	
SLVYPFPGPIPN	51.56	1299.7	12	5.8	650.86	36.82	1.06E+04	P02666|CASB_BOVIN	
QEPVLGPVRGPFPIIV	51.22	1717	16	-0.3	859.51	42.83	3.06E+03	P02666|CASB_BOVIN	
YQEPVLGPVRGPFP	50.26	1554.8	14	-0.5	778.42	33.57	6.61E+03	P02666|CASB_BOVIN	
LYQEPVLGPVRGPFPIIV	49.7	1993.1	18	1.3	997.58	45.13	5.88E+03	P02666|CASB_BOVIN	
NAVPITPTLN	49.61	1038.6	10	0.4	520.29	24.53	1.89E+03	P02663|CASA2_BOVIN	
YQEPVLGPVRGPFPI	49.43	1667.9	15	-0.4	834.96	38.86	3.10E+03	P02666|CASB_BOVIN	
VIESPPEIN	49.24	996.51	9	-2.6	997.52	20.21	7.04E+04	P02668|CASK_BOVIN	
SHAFEVVKT	47.88	1016.5	9	3	339.85	16.27	4.48E+03	P80195|GLCM1_BOVIN	
ILNKPEDETHLEAQPTDASAQFIR	47.82	2722.4	24	2.1	681.6	25.25	7.57E+03	P80195|GLCM1_BOVIN	
VQVTSTAV	47.72	803.44	8	-5.5	804.44	15.56	3.22E+03	P02668|CASK_BOVIN	
AQPTDASAQF	47.64	1034.5	10	-4.3	1035.5	16.34	3.32E+04	P80195|GLCM1_BOVIN	
GLPQEVLNENLLRF	47.03	1640.9	14	0.7	821.45	41.47	1.14E+04	P02662|CASA1_BOVIN	
FVAPFPEVFG	46.34	1108.6	10	8.4	555.29	41.62	4.81E+03	P02662|CASA1_BOVIN	
TVQVTSTAV	46.08	904.49	9	-0.2	905.5	16.93	7.97E+04	P02668|CASK_BOVIN	
VIESPPEINTVQ	45.81	1324.7	12	0	663.35	23.27	9.75E+03	P02668|CASK_BOVIN	
AAGGPGAPADPGRPT	45.81	1290.6	15	2.7	646.33	11.91	3.25E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
APFPEVFG	45.47	862.42	8	-1	863.43	33.2	7.50E+03	P02662|CASA1_BOVIN	
FVAPFPEVFGK	45.47	1236.7	11	1.4	619.34	37.51	2.36E+04	P02662|CASA1_BOVIN	
VLSRYPSYGLN	45.37	1267.7	11	-12.1	634.83	22.12	9.89E+02	P02668|CASK_BOVIN	
PEVIESPPEINTVQVTSTAV	45.27	2109.1	20	0.2	704.04	32.27	3.96E+04	P02668|CASK_BOVIN	
VAPFPEVFGKEK	45.19	1346.7	12	0	449.92	28.16	6.73E+03	P02662|CASA1_BOVIN	
TIASGEPTSTPTTE	45.16	1390.6	14	3.8	696.34	14.85	5.09E+03	P02668|CASK_BOVIN	
EPVLGPVRGPFPIIV	44.96	1588.9	15	-3.7	795.47	42.88	1.41E+04	P02666|CASB_BOVIN	
VAPFPEVF	44.8	904.47	8	-0.8	905.48	36.66	1.56E+04	P02662|CASA1_BOVIN	
INTVQVTSTAV	44.78	1131.6	11	2.9	566.82	21.9	9.11E+03	P02668|CASK_BOVIN	
DAAGGPGAPADPGRPT	44.77	1405.7	16	-1.3	703.84	12.43	4.52E+03	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
PPPPPPPPP	44.56	891.49	9	2.4	446.75	15.94	2.35E+04	A2VDK6|WASF2_BOVIN:O02755|CEBPB_BO	
PPAPPPPPP	44.52	865.47	9	-1.2	433.74	15.15	1.10E+03	A2VDK6|WASF2_BOVI	
GLPQEVLNENLLR	44.09	1493.8	13	3.5	747.92	33.82	6.87E+03	P02662|CASA1_BOVIN	
KVPQLEIVPN	44	1135.7	10	6.2	568.84	26.69	1.01E+03	P02662|CASA1_BOVIN	
NIPPLTQTPV	43.97	1078.6	10	-3.2	1079.6	27.23	3.79E+04	P02666|CASB_BOVIN	
VYPFPGPIPN	43.66	1099.6	10	9	550.8	32.03	1.75E+03	P02666|CASB_BOVIN	
APFPEVFGK	43.39	990.52	9	10.5	496.27	28.79	4.74E+03	P02662|CASA1_BOVIN	
VAPFPEVFGK	43.37	1089.6	10	3.5	545.8	31.58	2.76E+04	P02662|CASA1_BOVIN	
YYQQKPVAL	43.15	1108.6	9	-12.7	555.3	19.78	2.28E+03	P02668|CASK_BOVIN	
TQTPVVVPPFLQPEVM(+15.99)	42.95	1796.9	16	4	899.48	39.83	2.72E+04	P02666|CASB_BOVIN	Oxidation (M)
VVPPFLQPEVM(+15.99)	42.91	1270.7	11	-1.7	636.34	33.55	2.63E+04	P02666|CASB_BOVIN	Oxidation (M)
VIESPPEINTVQVTSTAV	42.87	1883	18	2.7	942.51	30.44	1.42E+03	P02668|CASK_BOVIN	
PYVPVHFDASV	42.55	1229.6	11	0.2	615.81	29.98	0	P61823|RNAS1_BOVIN	
NAVPITPT	41.8	811.44	8	-6.6	812.45	17.58	3.88E+03	P02663|CASA2_BOVIN	
APFPEVF	41.57	805.4	7	-0.7	806.41	34.24	6.51E+03	P02662|CASA1_BOVIN	
AAGGPGAPADPGRPTGY	41.4	1510.7	17	-8.7	756.36	15.97	8.90E+02	P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN	
GLPQEVLNENLL	41.08	1337.7	12	-7.9	669.86	36.71	3.27E+04	P02662|CASA1_BOVIN	
SLSQSKVLPVPQ	40.92	1281.7	12	-0.7	641.87	23.58	6.11E+03	P02666|CASB_BOVIN	
EVLNENLLRF	40.9	1245.7	10	1.7	623.85	35.3	1.18E+04	P02662|CASA1_BOVIN	
TQTPVVVPPFLQPEVM	40.9	1780.9	16	-0.3	891.48	43.47	5.43E+03	P02666|CASB_BOVIN	
VPPFLQPEVM	40.67	1155.6	10	1.6	578.81	35.79	2.05E+03	P02666|CASB_BOVIN	
VAPFPEVFG	40.59	961.49	9	-4	962.5	35.72	9.39E+03	P02662|CASA1_BOVIN	
SSRQPQSQNPKLPL	40.51	1578.8	14	3.5	527.29	20.82	1.08E+04	P80195|GLCM1_BOVIN	

Peptide	-10lgP	Mass	Length	ppm	m/z	RT	Area	Accession	PTM
PPPPPPPP	40.47	794.43	8	-3.1	795.44	14.4	1.68E+04	A2VDK6|WASF2_BOVIN:O02755|CEBPB_BOVIN:Q32LP2|RADL_BOV	
ILNKPEDETHLEAQPTDASAQFIRNL	40.41	2949.5	26	9.4	738.39	32.56	1.84E+03	P80195|GLCM1_BOVIN	
SQNPKLPL	39.54	895.51	8	-4.3	448.76	22.14	4.33E+03	P80195|GLCM1_BOVIN	
ILNKPEDETHLEAQPTDASAQF	39.15	2453.2	22	1	818.73	24.13	1.82E+04	P80195|GLCM1_BOVIN	
NENLLRFF	39.03	1051.5	8	5.3	526.78	38.03	7.55E+03	P02662|CASA1_BOVIN	
NVPGEIVE	38.34	855.43	8	0.9	856.44	16.82	3.31E+04	P02666|CASB_BOVIN	
AVPYPQ	37.94	673.34	6	-3.9	674.35	14.44	1.22E+03	P02666|CASB_BOVIN	
ARHPHPHLSF	37.71	1197.6	10	12.7	300.42	14.87	1.18E+03	P02668|CASK_BOVIN	
EVLNENLLR	37.29	1098.6	9	0	550.31	24.7	9.78E+03	P02662|CASA1_BOVIN	
VPPFLQPEVM(+15.99)	37.09	1171.6	10	3	586.81	30.51	8.27E+03	P02666|CASB_BOVIN	Oxidation (M)
SPPEINTVQVTSTAV	36.97	1541.8	15	-6.4	771.9	26.53	6.22E+03	P02668|CASK_BOVIN	
HIQKEDVPSERYL	36.73	1612.8	13	2.3	538.62	19.03	1.16E+04	P02662|CASA1_BOVIN	
IHPFAQTQSL	36.65	1140.6	10	-3.1	571.3	23.23	7.87E+02	P02666|CASB_BOVIN	
YYQQKPVALINN	36.53	1449.8	12	-0.9	725.89	21.52	4.15E+03	P02668|CASK_BOVIN	
YQEPVL	36.46	747.38	6	1.4	748.39	21.79	1.35E+03	P02666|CASB_BOVIN	
AVPITPTLN	36.31	924.53	9	-4.1	463.27	23.89	5.40E+02	P02663|CASA2_BOVIN	
GLPQEVLN	36.26	868.47	8	-4.6	869.47	22.65	1.18E+04	P02662|CASA1_BOVIN	
FVAPFPEVFGKEKV	36.25	1592.9	14	-4.1	531.96	36.33	2.55E+03	P02662|CASA1_BOVIN	
PPPPPPPPPP	36.18	988.54	10	3.3	495.28	16.95	6.92E+03	A2VDK6|WASF2_BOVI	
IPIQYVL	36.17	844.51	7	-3.9	423.26	34.19	2.23E+03	P02668|CASK_BOVIN	
VPQLEIVPN	35.75	1007.6	9	5.6	504.79	28.17	4.41E+03	P02662|CASA1_BOVIN	
NENLLRF	35.68	904.48	7	3.6	453.25	28.03	3.40E+04	P02662|CASA1_BOVIN	
PEINTVQVTSTAV	35.56	1357.7	13	-18.1	679.85	24.85	9.10E+02	P02668|CASK_BOVIN	
IPPLTQTPV	35.5	964.56	9	-4.4	483.29	26.24	6.77E+03	P02666|CASB_BOVIN	
VLNENLL	35.39	813.46	7	0.6	814.47	26.46	9.63E+02	P02662|CASA1_BOVIN	
SLPQNIPPLTQTPV	35.37	1503.8	14	1.2	752.92	32.15	1.41E+04	P02666|CASB_BOVIN	
VLPVPQKAVPYPQ	35.21	1434.8	13	1	718.42	25.82	2.01E+03	P02666|CASB_BOVIN	
RELEELNVPGEIVE	34.85	1624.8	14	2.8	813.43	29.78	2.63E+04	P02666|CASB_BOVIN	
VAPFPEVFGKE	34.48	1218.6	11	5.7	610.33	31.9	1.83E+03	P02662|CASA1_BOVIN	
FVAPFPEVFGKEK	34.39	1493.8	13	6.2	498.94	34.24	7.23E+03	P02662|CASA1_BOVIN	
ILNKPEDETHL	34.27	1307.7	11	-0.8	436.9	17.49	8.70E+03	P80195|GLCM1_BOVIN	
LPQEVL	34.22	697.4	6	-4.4	698.41	22.93	1.58E+03	P02662|CASA1_BOVIN:Q9TTK4|LYST_BOVIN	
IPQEVL	34.22	697.4	6	-4.4	698.41	22.93	1.58E+03		
YAKPAAVRSPA	34.21	1129.6	11	5.3	377.55	12.69	3.64E+02	P02668|CASK_BOVIN	
AVPITPT	34.16	697.4	7	-9.9	698.4	16.34	8.68E+02	P02663|CASA2_BOVIN	
IHPFAQTQ	34.12	940.48	8	0.6	471.25	15.15	1.23E+03	P02666|CASB_BOVIN	
IIQAKKRKTA	34.09	1155.7	10	14.8	578.89	48.68	4.99E+04	Q5E9K7|CCNE2_BOVIN	
LPQEVLNENLL	33.92	1280.7	11	3.4	641.36	33.92	4.12E+03	P02662|CASA1_BOVIN	
EVLNENLL	33.87	942.5	8	-1.6	943.51	28.7	2.73E+03	P02662|CASA1_BOVIN	
NAVPITPTLNRE	33.81	1323.7	12	-17.1	662.86	22.56	1.18E+03	P02663|CASA2_BOVIN	
DVPSERYL	33.74	977.48	8	2.7	489.75	20.34	6.60E+03	P02662|CASA1_BOVIN	
FVAPFPE	33.7	805.4	7	-1.4	806.41	29.05	0	P02662|CASA1_BOVIN	
GLPQEVL	33.63	754.42	7	-0.8	755.43	25.9	7.72E+03	P02662|CASA1_BOVIN	
LHLPLPLLQ	33.58	1042.7	9	-5.3	522.33	43.29	6.67E+04	P02666|CASB_BOVIN	
TQTPVVVPPFLQPE	33.46	1550.8	14	2.3	776.43	38.68	2.72E+03	P02666|CASB_BOVIN	
EVIESPPEINTVQVTSTAV	33.31	2012	19	-1.3	1007	31.31	2.70E+04	P02668|CASK_BOVIN	
AVESTVATL	33.23	889.48	9	-8.2	890.48	21.15	1.76E+03	P02668|CASK_BOVIN	
SRYPSYGLN	33.22	1055.5	9	0	528.76	17.88	1.55E+03	P02668|CASK_BOVIN	
PVRGPFPIIV	33.19	1093.7	10	-1.6	547.84	37.15	2.64E+03	P02666|CASB_BOVIN	
SLPQNIPPLTQTPVVVPPFLQPEVM	33.14	2756.5	25	5.2	919.84	45.58	1.05E+04	P02666|CASB_BOVIN	Oxidation (M)
GPVRGPFPIIV	32.99	1150.7	11	0.4	576.35	37.35	2.12E+04	P02666|CASB_BOVIN	
APFPEV	32.89	658.33	6	-6	659.34	25.1	2.11E+03	P02662|CASA1_BOVIN	
AQTQSLVYPFPGPIPN	32.48	1727.9	16	0.8	864.95	36.09	8.71E+03	P02666|CASB_BOVIN	
IPIQY	32.28	632.35	5	-15.3	633.35	21.27	3.01E+03	P02668|CASK_BOVIN	
LPLQY	32.28	632.35	5	-15.3	633.35	21.27	3.01E+03		
IPLQY	32.28	632.35	5	-15.3	633.35	21.27	3.01E+03		
AQPTDASAQFIR	32.12	1303.7	12	2.9	652.84	19.8	1.94E+03	P80195|GLCM1_BOVIN	
RELEELNVPGEIVESL	32.12	1824.9	16	-2.3	913.48	39.71	3.34E+03	P02666|CASB_BOVIN	
SQSKVLPVPQ	31.88	1081.6	10	8.7	541.82	19.45	1.89E+03	P02666|CASB_BOVIN	

<table>
<tr><th>Peptide</th><th>-10lgP</th><th>Mass</th><th>Length</th><th>ppm</th><th>m/z</th><th>RT</th><th>Area</th><th>Accession</th><th>PTM</th></tr>
<tr><td>FVAPFPEVF</td><td>31.71</td><td>1051.5</td><td>9</td><td>4.6</td><td>526.78</td><td>42.34</td><td>2.77E+03</td><td>P02662|CASA1_BOVIN</td><td></td></tr>
<tr><td>ILNKPEDETHLE</td><td>31.07</td><td>1436.7</td><td>12</td><td>5.1</td><td>479.92</td><td>16.59</td><td>1.79E+04</td><td>P80195|GLCM1_BOVIN</td><td></td></tr>
<tr><td>GHLKALINN</td><td>31</td><td>978.56</td><td>9</td><td>-18.7</td><td>490.28</td><td>21.27</td><td>2.31E+03</td><td>Q9TTK4|LYST_BOVIN</td><td></td></tr>
<tr><td>PPEINTVQVTSTAV</td><td>30.74</td><td>1454.8</td><td>14</td><td>-0.3</td><td>728.39</td><td>26.25</td><td>1.41E+03</td><td>P02668|CASK_BOVIN</td><td></td></tr>
<tr><td>PPAFK</td><td>30.7</td><td>558.32</td><td>5</td><td>12.8</td><td>559.33</td><td>29.85</td><td>2.79E+04</td><td>O02755|CEBPB_BOVIN</td><td></td></tr>
<tr><td>PPPPVI</td><td>30.59</td><td>618.37</td><td>6</td><td>10.9</td><td>619.39</td><td>29.97</td><td>2.99E+04</td><td>Q32LP2|RADI_BOVIN</td><td></td></tr>
<tr><td>PPPPVL</td><td>30.59</td><td>618.37</td><td>6</td><td>10.9</td><td>619.39</td><td>29.97</td><td>2.99E+04</td><td></td><td></td></tr>
<tr><td>ALLDPSF</td><td>30.57</td><td>761.4</td><td>7</td><td>-12.8</td><td>762.4</td><td>30.44</td><td>1.47E+03</td><td>P81265-2|PIGR_BOVIN:P81265|PIGR_BOVIN</td><td></td></tr>
<tr><td>EKKKK</td><td>30.39</td><td>659.43</td><td>5</td><td>16</td><td>660.45</td><td>32.3</td><td>8.88E+03</td><td>Q2NL08|DDX55_BOVIN:Q58DU0|MMTA2_BOVIN:Q08DA0|RABL6_BOVIN:Q32S26|BRD2_BOVIN:Q56JZ7|ZCRB1_BOVIN:Q00194|CNGA1_BOVIN:Q32LP0|URP2_BOVIN:Q58DT1|RL7_BOVIN:Q58CQ0|CSTOS_BOVIN:Q3B7L9|KRR1_BOVIN:P62866|RS30_BOVIN:Q1RMR5|TILB_BOVIN:Q24K12|GPTC1_BOVIN:P13789-2|TNNT2_BOVIN:P13789|TNNT2_BOVIN:A7Z019|SMCA4_BOVIN:Q3ZBE5|SLU7_BOVIN:Q58DQ3|RL6_BOVIN:Q9GL77|S4A4_BOVIN:D3K0R6-2|AT2B4_BOVIN:D3K0R6|AT2B4_BOVIN:E1B7L7|UBN2_BOVIN:A5D7J3|KNOP1_BOVIN:A7E3C4|F187A_BOVIN:Q0IIL1|MICU1_BOVIN:Q865S1</td><td></td></tr>
<tr><td>VAPFPE</td><td>30.35</td><td>658.33</td><td>6</td><td>-8.2</td><td>659.34</td><td>20.24</td><td>2.20E+03</td><td>P02662|CASA1_BOVIN</td><td></td></tr>
<tr><td>VFTKKTK</td><td>30.27</td><td>850.53</td><td>7</td><td>18.9</td><td>426.28</td><td>43.72</td><td>5.08E+04</td><td>P02663|CASA2_BOVIN</td><td></td></tr>
<tr><td>LLRAA</td><td>30.17</td><td>542.35</td><td>5</td><td>8.3</td><td>543.37</td><td>37.65</td><td>0</td><td>Q9TTK4|LYST_BOVIN:Q0VCK0|PUR9_BOVIN:A6QQ94|DMTA2_BOVIN:P46198|IF2M_BOVIN:Q5E9L7|VPS16_BOVIN:O46677|GROB_BOVIN:O46676|GROA_BOVIN:O46675|GROG_BOVIN:Q3ZBT2|FBX9_BOVIN:Q2KIF8|SYCM_BOVIN:</td><td></td></tr>
<tr><td>IIRAA</td><td>30.17</td><td>542.35</td><td>5</td><td>8.3</td><td>543.37</td><td>37.65</td><td>0</td><td></td><td></td></tr>
<tr><td>LIRAA</td><td>30.17</td><td>542.35</td><td>5</td><td>8.3</td><td>543.37</td><td>37.65</td><td>0</td><td></td><td></td></tr>
<tr><td>ILRAA</td><td>30.17</td><td>542.35</td><td>5</td><td>8.3</td><td>543.37</td><td>37.65</td><td>0</td><td></td><td></td></tr>
<tr><td>IESPPEIN</td><td>30.09</td><td>897.44</td><td>8</td><td>2.5</td><td>449.73</td><td>18.04</td><td>1.77E+03</td><td>P02668|CASK_BOVIN</td><td></td></tr>
</table>

m/z = mass to charge ratio; RT = retention time; PTM = post-translational modification

Table A1.10. Peptide sequences identified in delactosed permeate from production plant 2, batch E (Chapter IV)

Peptide	-10lgP	Mass	Length	ppm	m/z	RT	Area	Accession	PTM
YQEPVLGPVRGPFPIIV	72.42	1880.1	17	5.7	941.04	44.28	7.08E+04	P02666|CASB_BOVI	
YQEPVLGPVRGPFPII	60.7	1781	16	3.8	891.5	42.66	9.26E+03	P02666|CASB_BOVI	
KVLPVPQ	58.32	779.49	7	6.9	390.76	17.98	2.73E+03	P02666|CASB_BOVI	
YQEPVLGPVRGPFPI	56.5	1667.9	15	0	834.96	39.04	2.74E+03	P02666|CASB_BOVI	
LYQEPVLGPVRGPFPIIV	54.1	1993.1	18	7.1	997.58	45.32	2.23E+03	P02666|CASB_BOVI	
LVYPFPGPIPN	53.2	1212.7	11	4.7	607.34	35.9	2.43E+03	P02666|CASB_BOVI	
SLSQSKVLPVPQ	51.85	1281.7	12	4.4	641.87	23.68	3.47E+03	P02666|CASB_BOVI	
VQVTSTAV	51.72	803.44	8	-8.1	804.44	15.56	4.29E+03	P02668|CASK_BOVI	
AAGGPGAPADPGRPT	51.61	1290.6	15	-8.2	646.32	11.92	4.12E+03	P81265|PIGR_BOVIN :P81265-	
AQPTDASAQF	50.5	1034.5	10	0.3	1035.5	16.35	1.38E+04	P80195|GLCM1_BOVI	
YQEPVLGPVRGPFP	49.57	1554.8	14	1.7	778.42	33.77	3.18E+03	P02666|CASB_BOVI	
AQTQSLVYPFPGPIPN	49.27	1727.9	16	3.1	864.95	36.18	6.95E+03	P02666|CASB_BOVI	
PEVIESPPEINTVQVTSTAV	48.95	2109.1	20	2.4	1055.6	32.34	2.12E+04	P02668|CASK_BOVI	
SHAFEVVKT	48.39	1016.5	9	10.5	339.85	16.3	3.78E+03	P80195|GLCM1_BOVI	
DAAGGPGAPADPGRPT	47.74	1405.7	16	2	703.84	12.39	3.38E+03	P81265|PIGR_BOVIN :P81265-	
NIPPLTQTPV	47.73	1078.6	10	-5.1	540.31	27.53	2.18E+04	P02666|CASB_BOVI	
YYQQKPVALINN	47.71	1449.8	12	0.6	725.89	21.62	2.40E+03	P02668|CASK_BOVI	
GLPQEVLNENLLR	47.71	1493.8	13	-1.8	747.92	33.93	4.00E+03	P02662|CASA1_BOV	
VIESPPEIN	47.64	996.51	9	7	499.27	20.22	4.71E+04	P02668|CASK_BOVI	
QEPVLGPVRGPFPIIV	47.19	1717	16	0.2	859.5	43.15	1.21E+03	P02666|CASB_BOVI	
APFPEVFGK	47.04	990.52	9	1.6	496.27	29.02	1.75E+03	P02662|CASA1_BOV	
TVQVTSTAV	46.36	904.49	9	-4.5	905.49	17.13	5.23E+04	P02668|CASK_BOVI	
KAVPYPQ	45.3	801.44	7	4.7	401.73	12.69	9.78E+02	P02666|CASB_BOVI	
SPPEINTVQVTSTAV	44.96	1541.8	15	0	771.9	26.81	7.95E+03	P02668|CASK_BOVI	
GLPQEVLNENLLRF	44.62	1640.9	14	5.1	821.46	41.73	2.38E+03	P02662|CASA1_BOV	
EPVLGPVRGPFPIIV	44.33	1588.9	15	4.4	795.48	43.24	5.56E+03	P02666|CASB_BOVI	
RELEELNVPGEIVE	44.29	1624.8	14	3.1	813.43	29.83	1.21E+04	P02666|CASB_BOVI	
LLYQEPVLGPVRGPFPIIV	44.12	2106.2	19	-1.9	1054.1	46.34	7.75E+03	P02666|CASB_BOVI	
INTVQVTSTAV	44.08	1131.6	11	-4.4	1132.6	21.93	6.85E+03	P02668|CASK_BOVI	
VPPFLQPEVM	43.98	1155.6	10	0.9	578.81	35.94	2.03E+03	P02666|CASB_BOVI	
NENLLRFF	43.9	1051.5	8	6.7	526.78	38.28	5.98E+03	P02662|CASA1_BOV	
TQTPVVVPPFLQPEVM(+1!	43.53	1796.9	16	3	899.48	39.98	1.61E+04	P02666|CASB_BOVI	Oxidation (M)
PPPPPPPPP	43.43	891.49	9	8.5	446.75	15.91	1.73E+04	A7Z063|WASH1_BO VIN:Q08DG5|HXB4_ BOVIN:P55106|GDF6 _BOVIN:A5PKL7|LZT S2_BOVIN:Q95107|W ASL_BOVIN:A2VDK6	
SLVYPFPGPIPN	42.71	1299.7	12	8.3	650.86	36.93	5.65E+03	P02666|CASB_BOVI	
VAPFPEVFG	42.57	961.49	9	-5.4	962.49	35.86	2.62E+03	P02662|CASA1_BOV	
EVLNENLLRF	42.5	1245.7	10	1.5	623.84	35.42	5.38E+03	P02662|CASA1_BOV	
NAVPITPT	42.46	811.44	8	3.2	812.45	17.74	4.60E+03	P02663|CASA2_BO	
EVLNENLLR	42.09	1098.6	9	2.6	550.31	24.82	6.37E+03	P02662|CASA1_BOV	
APFPEVFG	41.95	862.42	8	0.1	863.43	33.32	3.29E+03	P02662|CASA1_BOV	
AVPYPQ	41.9	673.34	6	-3.9	674.35	14.54	2.28E+03	P02666|CASB_BOVI	
FVAPFPEVFGK	41.69	1236.7	11	3.9	619.34	37.65	1.20E+04	P02662|CASA1_BOV	
VYPFPGPIPN	41.29	1099.6	10	5.6	550.8	32.1	2.03E+03	P02666|CASB_BOVI	
FVAPFPEVF	41.14	1051.5	9	8	526.78	42.55	2.42E+03	P02662|CASA1_BOV	
TIASGEPTSTPTTE	41.08	1390.6	14	-5.5	696.33	14.9	2.61E+04	P02668|CASK_BOVI	
LHLPLPLLQ	40.85	1042.7	9	-4.8	522.33	41.59	0	P02666|CASB_BOVI	
PPPPPPPP	40.62	794.43	8	4.7	398.23	14.37	1.52E+04	VIN:Q08DG5|HXB4_ BOVIN:P55106|GDF6 _BOVIN:A5PKL7|LZT S2_BOVIN:Q95107|W ASL_BOVIN:A2VDK6 |WASF2_BOVIN	
VVPPFLQPEVM(+15.99)	40.45	1270.7	11	8.6	636.34	33.64	1.55E+04	P02666|CASB_BOVI	Oxidation (M)
HIQKEDVPSERYL	40.43	1612.8	13	11	538.62	19.46	1.17E+03	P02662|CASA1_BOV	

Peptide	-10lgP	Mass	Length	ppm	m/z	RT	Area	Accession	PTM
VAPFPEVF	40.27	904.47	8	-1.6	905.48	36.76	3.13E+03	P02662|CASA1_BOV	
NVPGEIVE	40.23	855.43	8	0.8	856.44	19.02	6.42E+03	P02666|CASB_BOVI	
FVAPFPE	40.06	805.4	7	-2.8	806.41	29.08	0	P02662|CASA1_BOV	
APFPEVF	40.04	805.4	7	1.7	806.41	34.4	3.51E+03	P02662|CASA1_BOV	
SDIPNPIGSENSE	39.96	1357.6	13	14.5	679.82	22.29	2.55E+03	P02662|CASA1_BOV	
PPPPPPPPPP	39.78	988.54	10	-6.3	495.27	16.98	3.65E+03	VIN:Q08DG5|HXB4_ BOVIN:P55106|GDF6 _BOVIN:A5PKL7|LZT S2_BOVIN:Q95107|W ASL_BOVIN:A2VDK6 |WASF2_BOVIN	
HIQKEDVPSER	39.51	1336.7	11	10.1	446.57	9.64	2.32E+02	P02662|CASA1_BOV	
NAVPITPTLN	39.39	1038.6	10	9.4	520.3	24.53	9.97E+02	P02663|CASA2_BO	
PVLGPVRGPFPIIV	39.12	1459.9	14	0.2	730.95	43.21	1.74E+03	P02666|CASB_BOVI	
VAPFPEVFGK	39.04	1089.6	10	3.6	545.8	31.59	1.55E+04	P02662|CASA1_BOV	
EVIESPPEINTVQVTSTAV	38.66	2012	19	1.2	1007	31.56	1.71E+03	P02668|CASK_BOVI	
AQPTDASAQFIR	38.52	1303.7	12	1.6	652.83	19.9	2.34E+03	P80195|GLCM1_BOVI	
TQTPVVVPPFLQPEVM	38.14	1780.9	16	-5.4	891.47	43.53	4.51E+03	P02666|CASB_BOVI	
FVAPFPEVFG	37.87	1108.6	10	-1	555.29	41.79	1.96E+03	P02662|CASA1_BOV	
SQNPKLPL	37.44	895.51	8	-6	448.76	22.2	2.61E+03	P80195|GLCM1_BOVI	
ILNKPEDETHLEAQPTDAS/	36.96	2722.4	24	12.9	681.61	25.69	4.63E+03	P80195|GLCM1_BOVI	
VAPFPEVFGKEK	36.84	1346.7	12	8.8	449.92	28.37	3.64E+03	P02662|CASA1_BOV	
VVPPFLQPEVM	36.55	1254.7	11	2.2	628.34	38.55	4.95E+03	P02666|CASB_BOVI	
LPQEVLNENLLRF	36.43	1583.9	13	3.4	792.94	39.27	1.06E+03	P02662|CASA1_BOV	
NENLLRF	36.01	904.48	7	3.1	453.25	28.15	1.85E+04	P02662|CASA1_BOV	
VAPFPEVFGKEKV	36	1445.8	13	4.1	482.94	31.24	2.88E+03	P02662|CASA1_BOV	
GLPQEVLN	35.5	868.47	8	-1.2	869.47	22.73	2.62E+03	P02662|CASA1_BOV	
AVPITPT	35.17	697.4	7	-4.6	698.41	16.47	1.43E+03	P02663|CASA2_BO	
SSRQPQSQNPKLPL	34.84	1578.8	14	3.5	527.29	20.88	6.05E+03	P80195|GLCM1_BOVI	
TQTPVVVPPFLQPE	34.56	1550.8	14	8.9	776.43	38.76	2.55E+03	P02666|CASB_BOVI	
AVESTVATL	34.39	889.48	9	4.3	445.75	21.21	6.38E+03	P02668|CASK_BOVI	
FVAPFPEVFGKEKV	34.33	1592.9	14	0.4	531.96	36.51	2.25E+03	P02662|CASA1_BOV	
VIESPPEINTVQ	34.26	1324.7	12	-1.6	663.35	23.35	6.25E+03	P02668|CASK_BOVI	
VLNENLL	34.22	813.46	7	-2.9	814.46	26.55	1.01E+03	P02662|CASA1_BOV	

m/z = mass to charge ratio; RT = retention time; PTM = post-translational modification