Authentication of Organic Bovine Milk by Analysis of Fatty Acid Profiles

By

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Abstract

Organic bovine milk in the U.S. is considered and labeled differently from conventional bovine milk because of the regulations and certification by the USDA. These regulations by the USDA for organic bovine milk in the U.S. are claimed to change the fatty acid profile of organic milk and increase levels of n-3 fatty acids. This study investigated differences in fatty acid profiles between 12 organic and 12 conventional retail bovine milk brands in California to test the hypothesis that organic milk will contain more n-3 fatty acids. The milk samples were collected from U.S. retail stores in California, once each in the early late Winter of March in 2020 and late Summer of August in 2020 to account for seasonal variations of the fatty acid profiles between organic and conventional milk. Milk samples from dairy farms in California were selected to consider regional variations. Milk fatty acids were identified and their levels in each sample measured through a GC-FID. Two Sample T-Test showed that levels of caprylic, myristic, myristoleic, and α-linolenic acid (ALA) were significantly ($P<0.05$) greater in organic retail milk (OM) than conventional retail milk (CM) and levels of linoleic (LA) and stearic acid were significantly greater in CM than OM in both Winter and Summer. ANOVA Single Factor with Tukey HSD Test resulted in levels of myristic, myristoleic, and ALA to be significantly greater in OM than CM and levels of LA to be significantly greater in CM than OM. OM demonstrated no significant differences ($P>0.05$) in levels of any of the 13 identified fatty acids between Summer and Winter samples through T-Test and ANOVA Single Factor with Tukey HSD Tests. CM demonstrated significant differences, through T-Tests, in levels of fatty acids capric, lauric, myristic, oleic/elaidic, and LA between Winter and Summer samples. ANOVA Single Factor with Tukey HSD Test on CM resulted in no significant differences in levels of any of the fatty acids between Winter and Summer samples. Through performing linear discriminant

analysis, we were able to accurately authenticate organic and conventional retail milk samples using their fatty acid profiles with an accuracy of 95.83% without considering seasons, Winter and Summer. The accuracy of the linear discriminant analysis dropped to 83.33% when having to also authenticate organic and conventional retail milk samples in their respective Winter and Summer sample groups. Overall, this thesis demonstrates significantly greater ALA in organic retail milk than conventional retail milk in California.

Introduction

Organic and conventional dairy milk are differentiated by the way their farms are managed. In the U.S., organic dairy farms must follow the special regulations enforced by the USDA to be certified an organic dairy farm.[2] Dairy farms are considered conventional or nonorganic if they are not first organically certified by the USDA.[2] The *USDA* prohibits organic dairy farms from using antibiotics, pesticides, herbicides, growth hormones, and chemical fertilizers.[2] Dairy cows on organic farms must also be out on pastures for the entire grazing season and no less than 120 days in the year.[2] Organic dairy cows must also receive at least 30 percent of their feed or dry matter intake (DMI) from pastures on average over the course of the grazing season.[2] In most of Europe, the European Union certifies and regulates organic certification through organic control bodies such as Ecocert and Soil Association.[3,4] Regulations for EU organic certification are similar to those in the U.S. in that pesticides, herbicides, growth hormones, antibiotics, and chemical fertilizers are generally restricted. However, organic livestock are to have adequate and permanent access to pastures unless specific circumstances arise, such as weather conditions or the health of the animal is at risk.[3,5] Conventional dairy farms are not required to follow any of these regulations, but can do so without receiving a certified organic approval by the USDA or European organic control bodies.[2,3] Dairy cows on conventional farms are traditionally fed non-organically certified grains and concentrates, but most of these management decisions are up to individual farms.[2,6] The regulations required for organic dairy farms in the U.S. and in other parts of the world are claimed to produce desirable fatty acid profiles containing significantly more n-3 polyunsaturated fatty acids than fatty acid profiles from conventional dairy farms for consumers.[6,7] However, there are currently no regulations or standards for fatty acid profiles in organic milk in anywhere in the world.

In several studies and a meta-analysis of over 170 published articles, fatty acid profiles were compared between organic and conventional milk to test their significance. Studies in the U.S., U.K. (North East (NE) England), and meta-analysis by Średnicka-Tober et al. found negligible differences on average in total saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs) between organic and conventional milk, as seen in **Table 1.**[6–8] However, another study in the U.K. MUFAs on average to be significantly greater in conventional milk and a study in South Korea found both SFAs and MUFAs to be significantly greater in conventional milk, on average.[9,10] When looking at total polyunsaturated fatty acids (PUFAs), on average, between organic and conventional milk, studies in the U.K., South Korea, and the meta-analysis by Średnicka-Tober et al. found significantly greater PUFAs in organic milk, as seen in **Table 1**.[8–10] However, the study conducted by Benbrook et al. in the U.S. found conventional milk on average to contain significantly greater concentrations of PUFAs.[6] Total n-3 fatty acids on average, in studies conducted in the U.S., U.K. (NE England), U.K., South Korea, and the metaanalysis by Średnicka-Tober et al., were found to have significantly greater concentrations in organic milk.[6–10] The study in the U.S. found significantly greater concentrations of total n-6 fatty acids in conventional milk on average than organic milk, while studies conducted in U.K.

(NE England) and South Korea found total n-6 fatty acids to be significantly greater in organic milk on average.[6,8,9] However, another study conducted in the U.K. and the meta-analysis by Średnicka-Tober et al. found that differences in total n-6 fatty acids to be negligible between organic and conventional milk on average.[7,10] Overall, linoleic acid (LA) was found to be significantly greater in organic milk than conventional milk in studies conducted in the U.K. (NE England) and South Korea, while the study conducted in the U.S. found LA to be significantly greater in conventional milk than organic milk on average.[6,8,9] The meta-analysis by ŚrednickaTober et al. determined LA to have no significance between organic and conventional milk.[7] αlinolenic acid (ALA) in the studies conducted in the U.S., U.K. (NE England), South Korea, Germany, and the meta-analysis by Średnicka-Tober et al. was found to be significantly greater in organic milk than conventional milk on average, as seen in **Table 1.**[6–9]

Table 1: Results of Fatty Acid Concentrations and Levels from Referenced Studies

	Organic								**Conventional**						
Studies	**SFAs**	**MUFAs**	**PUFAs**	**n-3**	**n-6**	**ALA**	**LA**	**Studies**	**SFAs**	**MUFAs**	**PUFAs**	**n-3**	**n-6**	**ALA**	**LA**
U.S. (g/100g) (n=143)	2.116	0.741	0.1037*	0.0321*	0.0711*	0.0255*	0.0639*	**U.S. (g/100g) (n=107/108)**	2.043	0.7944	0.1147*	0.0198*	0.0948*	0.0159*	0.0856*
U.K. (g/kg) (n=40)	699	261	39.4*	8.8*	23.2*	6.9*	20.1*	**U.K. (g/kg) (n=48)**	707	262	31.8*	5.5*	20.7*	4.4*	17.5*
U.K. (% of total FA) (n=17)	68.13	26.19*	3.89*	1.11*	1.68	n/a	n/a	**U.K. (% of total FA) (n=19)**	67.25	27.63*	3.33*	0.66*	1.68	n/a	n/a
South Korea (g/kg) (n=60)	665.4*	289.6*	44.99*	4.84*	39.61*	4.51*	35.49*	**South Korea (g/kg) (n=60)**	693.9*	272.5*	33.56*	2.46*	30.59*	2.24*	26.88*
Germany (g/100g) (n=122)	n/a	n/a	n/a	n/a	n/a	0.69*	n/a	**Germany (g/100g) (n=124)**	n/a	n/a	n/a	n/a	n/a	0.39*	n/a

"*" indicates significance between the same fatty acid in organic and conventional milk on average ($P<0.05$) n = number of samples, SFAs = saturated fatty acids, MUFAs = monounsaturated fatty acids, PUFAs = polyunsaturated fatty acids, n-3 = total n-3 fatty acids, n-6 = total n-6 fatty acids, ALA = α-linolenic acid, and LA = linoleic acid. U.S.[6], U.K.[8], U.K.[10], South Korea[9], and Germany.[11]

During each study, challenges were faced in authenticating organic milk from conventional milk due to variations in fatty acid profiles that overlap between the two systems due to factors such as, regional and seasonal variations as seen from the studies in the U.S., U.K. (NE England), U.K., New Zealand, and Germany.[6,8,10–12] The study conducted in the U.S. found, on average, California conventional milk, specifically from dairy farms in Humboldt County, had fatty acid profiles similar to nationwide organic milk fatty acid profiles, with unusually low concentrations of LA and high concentrations in ALA, about 0.060 and 0.025 g/100g, respectively.[6] The study explained that the similarities could be because both organic and conventional dairy farms in Humboldt County commonly grazed their cows for more than 250 days per year on pastures, due to the type of environment allowed in the region.[6] Additionally, a study in Germany found that, although organic milk always had greater levels of ALA on all single sampling days compared to conventional milk, when considering all year variations (18 months of biweekly milk collection from organic and conventional retail milk), ALA levels between organic and conventional milk did overlap to some degree.[11] The highest ALA levels in a particular conventional milk brand compared to the lowest organic milk brand was 0.67% and 0.52%, respectively.[11] The study determined the cause was possibly because the conventional milk brand had dairy farms that were located in a region in southern Germany where there observed more increased pasture feeding compared to other conventional dairy farms in Germany.[11] Furthermore, when looking at the resulting values of ALA or LA in **Table 1,** we can see how different countries produce different concentrations of the same fatty acid in organic and conventional milk. Thus, it would be important to consider regional variations when trying to discriminate organic milk fatty acid profiles from conventional milk fatty acid profiles from different regions.

Seasonality as a factor also creates difficulties to authenticate organic and conventional milk due to the significant fluctuations in concentration or levels in fatty acid profiles in certain times of the year, as seen in the studies in Germany, U.K., U.S., and South Korea.[6,8,10,11] In the study conducted by Molkentin in Germany, one of the conventional retail milk analyzed and almost all organic retail milk showed elevated levels of ALA, with the max level of ALA in conventional retail milk to be 0.67% in the Summer.[11] Although all the organic retail milk analyzed still displayed ALA levels greater than 0.67% in the Summer, this level of concentration of 0.67% would be surpassed by one particular conventional milk, exceeding some ALA concentrations of organic retail milk in other times of the year.[11] The study then selected a threshold for ALA levels to authenticate organic milk based on the all year variations, which resulted in the retail conventional milk with ALA levels of 0.67% in the summer to be labeled an organic milk.[11] Thus, it appears important to collect milk samples of both organic and conventional milk in the same season or in the same time frame in order to determine the correct level of ALA to be used as a threshold to authenticate organic milk. In contrast, for the U.S. study by Benbrook et al., both ALA and LA concentrations showed little seasonal variations from January 2011 to May 2012.[6] Furthermore, ALA and LA concentrations did not have any overlap and remained significantly different by the Mann-Whitney test ($P<0.02$) between organic and conventional retail milk from January 2011 to May 2012.[6] The study in South Korea also had concurring data, with n-3 and n-6 fatty acids significantly greater in organic milk than conventional milk throughout the four seasons performed in the study, as seen in **Table 1.**[9] However, in the study conducted by K.A. Ellis et al. in the U.K., all samples decreased significantly in SFAs over the Spring and early Summer, but increased significantly in the Fall and Winter.[10] Furthermore, in the Spring and early Summer, in all samples, PUFAs and n-3 fatty acids increased significantly, as shown in the study's Figure 1.[10] The study indicated that the significant variations in fatty acids in different seasons throughout the year could likely be due to increased pasture grazing seasons in the U.K. that takes place in the Summer. While in the Fall and Winter, with less grazing available, the use of total mixed rations, corn silage, and whole crop was found to significantly increase SFA and decrease PUFAs.[10] The study conducted by Butler et al., also in the U.K., found concurring results to Ellis et al., SFAs decreasing significantly in all samples in the Summer and increasing significantly in the Winter, 682 g/kg SFAs in the Summer and 725 g/kg SFAs in the Winter. PUFAs and n-3 fatty acids were also significantly greater in the Summer and significantly lower in the Winter, 37.6 g/kg PUFAs in the Summer vs. 32.8 g/kg PUFAs in the Winter and 8.1 g/kg n-3 fatty acids in the Summer vs. 5.9 g/kg n-3 fatty acids in the Winter.[8]

Other factors, such as climate change, stage of lactation, and breed of dairy cows can significantly affect the fatty acid composition of milk and can create challenges to authenticating organic milk. A surprising result occurred in a 2-year study by Butler et al. in the U.K., specifically northeast England, where fatty acid profiles in the first year were found to be significantly different than the second year.[8] Milk collected in year one had greater concentrations of PUFAs (37.5 vs 32.9 g/kg), ALA (6.0 vs 5.1 g/kg), and LA (19.9 vs 17.5 g/kg) than year two.[8] Milk collected in year one also had lower concentrations of myristic (110 vs 118 g/kg) and palmitic acid (332 vs 357 g/kg) than year two.[8] The study suggested the reason for the differences between fatty acid profiles in different years was due to differences in climate conditions between sampling years.[8] Between 2006-2007 and 2007-2008, the Summer of 2007 recorded 30% higher rainfall and 12% lower air temperatures than the recorded data in 2006.[8]

The study explained that these conditions could have affected the cow's behavior in grazing by reduced grazing intake and lowering milk yields.[8] Thus, due to lower milk yields, farmers would tend to supplement with concentrates or conserved forage in order to maintain efficient milk yields.[8] Due to the increase in concentrates or conserved forage and decreased fresh pasture intake, we would expect lower concentrations of PUFAs and greater concentrations of SFAs.[13] The study in New Zealand also found that sampling of milk in different times of the day resulted in different fatty acid profiles due to different grazing behaviors.[12] The study in New Zealand also investigated if different breeds produced different fatty acid profiles, specifically between Holstein Friesian and Jersey cows.[12] However, due to lack of purebred lines in their study, as purebreds between Holstein and Jersey cows showed some significant differences in fatty acids, differences in fatty acid profiles between crossbreeds disappeared and the study determined that breed did not significantly affect fatty acid profiles in their study.[12] Stage of lactation also proved to be significant in the study conducted in New Zealand where the first 4 weeks of lactation showed lower concentrations of butyric, capric, lauric, and palmitic acid, but greater concentrations of the same fatty acids in the mid lactation, around 17-21 weeks.[12] No significant differences in concentrations of ALA, LA, CLA, and vaccenic acid were observed in the mid and late lactations.[12]

Factors that can significantly affect fatty acid profiles in milk such as regional, seasonal, climate change, breed, stage of lactation, and management of diet at individual farms bring about challenges to authenticate organic milk and therefore it becomes important to consider these factors in our results. However, for most of the studies conducted in the U.S., U.K., Germany, South Korea, and the meta-analysis by Średnicka-Tober et al., n-3 fatty acids, especially PUFAs and ALA, demonstrated significantly greater concentrations in organic milk than conventional milk.[6–10] This result is likely due to organic farms being regulated to allow dairy cows to feed on fresh pastures regularly which is known to contain high concentrations of ALA, about 0.5-0.75.[13]

Studies by Benbrook et al. in the U.S. and Ellis et al. in the U.K. have suggested that organic milk generally have LA/ALA ratios of 2.3 or 1.1, respectively, which is associated for being healthier for consumers, rather than LA/ALA ratios greater than 10:1, which are typically found in conventional milk.[6,10] Studies further suggests that these differences between organic and conventional milk fatty acid profiles result because of different diets of forage throughout the year.[1,7] The diet of dairy cows in both organic and conventional dairy farms is a clear factor that affects the milk fatty acid profiles, increasing desirable fatty acids such as PUFAs and n-3 fatty acids like ALA.[13–15] Fresh grass on pastures contain a high proportion of ALA, LA, and palmitic acid, about 95%.[13] Plants are the primary sources of n-3 fatty acids and grasses found on pastures contain a proportion of 0.50-0.75 of total FA content as ALA.[13,16] Phenolic compounds present in fresh forage have been found to inhibit biohydrogenation, relating to phenolic toxicity to microbial species in the rumen involved in fatty acid biohydrogenation and have seen a decrease in biohydrogenation activity, leading to an increased concentration of desirable PUFAs in the fatty acid composition of milk.[17,18] Also, the reduced intake of SFAs, usually found in grains, rations, and silage has been found to reduce the *de novo* activity in the rumen of the cow and could be a reason for an increase in concentrations of PUFAs as well.[14,15] However, when fresh grass from pastures are no longer abundantly available due to the change in season or weather, the use of ensiling to create silage as feed is common.[16] However, these other types of feed for dairy cows other than fresh pastures in general contain significantly less total fatty acids due to the oxidative loss during the wilting and ensiling process.[16] This type of feed also has many varieties made from different ingredients such as red clover silage, white clover silage, maize silage, and grass silage, which can contain significantly varying amounts of ALAs and LAs, with clover silage being able to recover a higher concentration of ALA than grass silage, from 0.04-0.05 to about 0.08-0.10 in clover silage.[16] These are also the same feeds commonly available to dairy cows on conventional farms.[19] Another option for feeding dairy cows is hay.[16] Through the process of hay making, the grass and forage dried to make hay can lose over 50% of all total fatty acids by the oxidative loss during wilting.[16,20] Total mixed rations (TMR) are another type of feed used to feed dairy cattle, but contain 30% less total fatty acids and with a reduction of about 40% less ALA and contain more SFAs.[7,13] Thus, we would expect dairy cows who feed mainly on fresh forage throughout the year to generally have a greater concentration of PUFAs, especially ALA in their milk fat, when compared to dairy cows who only feed on rations, grains, silage, and hay.[6,8,13]

Dairy cows typically do not synthesize ALA. Most ingested unsaturated fatty acids like ALA or LA, derived from fresh forages, are biohydrogenated in the rumen, 85%-95% and 75%90%, respectively.[13,14] Long-chain unsaturated fatty acids that do get biohydrogenated are mostly transformed into intermediates such as stearic acid.[21,22] Unsaturated fatty acids that do avoid biohydrogenation are absorbed and incorporated into the body fat, which can then later be mobilized and added into milk fat by the mammary glands by the same process of the preformed long-chain fatty acids.[15] A possible reason for an increased concentration of polyunsaturated fatty acids in milk fat could be due to the reduction of *de novo* activity because of reduced intake of saturated fatty acids, usually found in grains, rations, and silage.[15,23,24]

The composition of bovine milk fat is made up of more than 400 different fatty acids in trace quantities with only 15 of the fatty acids at 1% level or higher.[15,25] The fatty acids in organic and conventional bovine milk fat that can be detected through gas-chromatography are shown in **Table 2** and was conducted by O'Donnel et al.[15] Milk fat in bovine milk originates mainly from 2 sources, *de novo* synthesis in the mammary gland and the mammary uptake of preformed long-chain fatty acids.[15,25]

During *de novo* synthesis, the mammary glands produce fatty acids with even numbered carbons of 4-16 in length, which accounts for approximately 60% and 45% of the total milk fatty acids on a molar and weight basis, respectively.[15] Acetate and β-hydroxybutyrate are utilized in the mammary cells during *de novo* synthesis, which are produced from fermentation of carbohydrates in the rumen.[15] Butyric acid is produced from the fermentation in the rumen, which is then converted into β-hydroxybutyrate by the rumen wall and liver. In the mammary cells, acetate and β-hydroxybutyrate are activated to a coenzyme A derivative which allows acetyl-CoA carboxylase 1 to catalyze the formation of malonyl-CoA from acetyl-CoA. From here, malonyl-CoA is condensed with acetyl-CoA by fatty acid synthase (FASN) to produce the first 4-carbon acyl chain.[15] Condensing more malonyl-CoAs would produce a larger acyl chain to create longer-chain fatty acids. FASN creates a range of fatty acids 4-16 in length and the enzyme that terminates FASN's function is acylthioesterase, which cleaves the fatty acids of varying lengths from the FASN complex and then synthesized into triglycerides (TAGs).[15]

Preformed long-chain fatty acids, such as saturated, unsaturated, and trans fatty acids, making up the 16 and greater carbon length fatty acids, originate from lipids absorbed from the cow's diet and lipids lipolyzed from body fat reserves. Since lipids are not soluble in water, they are packaged in lipoproteins within the blood called very low-density lipoproteins (VLDLs). VLDLs containing lipids reach the mammary glands where lipoprotein lipases cleave the VLDL triacylglycerols into glycerol and non-esterified fatty acids (NEFAs), such as saturated or unsaturated fatty acids.[15] Also, plasma NEFAs, originating from the body adipose triglycerides, are taken up by the mammary gland. The NEFAs are then taken up into the mammary cells by fatty acid transport proteins and fatty acid binding proteins where they are activated by CoA esters and synthesized with glycerol-phosphate, converted from glycerol, into TAGs. Plasma triglycerides and NEFAs contribute about 55% and 40% of total milk fat on a weight and molar basis, respectively.[15] After the formation of TAGs in the mammary gland is complete, TAGs come together on the luminal side, moving through the epithelial cell, where a it is pinched off with a part of membrane into the lumen.[15] The membrane now surrounding the fat droplets is referred to as the milk fat globular membrane present in milk.[15] **Table 2** displays the concentrations of fatty acids in both organic and conventional milk in the U.S. and referenced from O'Donnell et al.[1]

Table 2: Fatty acid composition (% of total fatty acids) of conventional, rbST-free, and organic retail milk referenced from O'Donnel et al.[1]

	Treatment		
Fatty Acids	**Conventional**	**rbST-free**	**Organic**
C4:0	4.17 ± 0.03	4.22 ± 0.03	4.36 ± 0.03
C6:0	2.12 ± 0.01	2.11 ± 0.01	2.30 ± 0.01
C8:0	1.15 ± 0.01	1.13 ± 0.01	1.26 ± 0.01
C10:0	2.53 ± 0.02	2.49 ± 0.02	2.81 ± 0.02
C12:0	2.89 ± 0.03	2.83 ± 0.02	3.24 ± 0.03
C14:0	9.63 ± 0.06	9.42 ± 0.05	10.62 ± 0.06
C14:1, *cis*-9	0.89 ± 0.01	0.87 ± 0.01	0.98 ± 0.01
C15:0	0.90 ± 0.01	0.87 ± 0.01	1.07 ± 0.01
C16:0	27.99 ± 0.13	27.78 ± 0.09	29.27 ± 0.17
C16:1, *cis*-9	1.55 ± 0.02	1.53 ± 0.01	1.47 ± 0.02
C17:0	0.50 ± <0.01	0.50 ± <0.01	0.55 ± <0.01
C18:0	10.88 ± 0.10	11.04 ± 0.08	10.21 ± 0.12
C18:1, *trans*-6-8	0.29 ± 0.01	0.30 ± <0.01	0.22 ± <0.01
C18:1, *trans*-9	0.28 ± <0.01	0.29 ± <0.01	0.21 ± <0.01

C18:1, *trans*-10	0.52 ± 0.02	0.55 ± 0.01	0.28 ± 0.01
C18:1, trans-11	1.45 ± 0.02	1.47 ± 0.02	1.71 ± 0.05
C18:1, trans-12	0.50 ± 0.01	0.52 ± 0.01	0.38 ± 0.01
C18:1, cis-9	23.90 ± 0.14	24.38 ± 0.09	21.44 ± 0.11
C18:2, n-6	3.50 ± 0.06	3.43 ± 0.04	2.55 ± 0.08
C20:0	0.09 ± <0.01	0.09 ± <0.01	0.10 ± <0.01
C18:3, n-3	0.41 ± 0.01	0.40 ± 0.01	0.65 ± 0.01
C18:2, cis-9, trans-11	0.57 ± 0.01	0.57 ± 0.01	0.70 ± 0.02
20:4, n-6	0.14 ± <0.01	0.14 ± <0.01	0.11 ± <0.01
20:5, n-3	0.03 ± <0.01	0.03 ± <0.01	0.06 ± <0.01
22:5, n-3	0.06 ± <0.01	0.06 ± <0.01	0.11 ± <0.01
22:6, n-3	ND	ND	ND
Other	2.95 ± 0.04	2.94 ± 0.03	3.17 ± 0.03

Values represent least square means ± SE for retail milk (n =292).
ND = not detected (<0.01% of total fatty acids)

There are several unknowns that have not been addressed by the literature. First, there are not many retail milk fat studies between organic and conventional milk conducted in the U.S. Second, there are no specific retail milk fat studies conducted in California. Another unknown in the literature is that there is no data on the fatty acids that are significantly different between organic and conventional retail milk in California, such as ALA with regards to seasonality.

Thus, we hypothesize that organic retail milk contains significantly greater levels of n-3 fatty acids than conventional retail milk in California. The alternate hypothesis is there is no significant difference in levels of n-3 fatty acids between organic and conventional retail milk in California.

The objectives of this study are therefore to: (i) compare the fatty acid profiles of organic and conventional milk, with consideration to seasonality and region in California; (ii) investigate if levels of n-3 fatty acids are significantly greater in organic retail milk than conventional retail milk in California; (iii) test if authentication of organic retail milk is possible with the collected samples.

Materials and Methods

Chemicals and Reagents

Distilled water/Type I water/Deionized water (Sourced from UC Davis Robert Mondavi Institute Laboratories, Davis, California), Hexanes (Fisher Scientific, Hexanes (Optima^{TM}), Fisher Chemical, Massachusetts, U.S.), 37% Hydrochloric acid (Sigma-Aldrich, 37% HCl (ACS reagent), St. Louis, Missouri), Toluene (Fisher Scientific, Toluene (Optima^{TM}), Fisher Chemical, Massachusetts, U.S.), Methanol (Fisher Scientific, Methanol (Optima^{TM}), Fisher Chemical, Massachusetts, U.S.), Chloroform (Fisher Scientific, Chloroform (HPLC), Fisher Chemical, Massachusetts, U.S.), Solution of 8% HCl in methanol (mixture created from reagents referenced above), Fatty Acid Standards (Nuchek, Prep Inc., TG-17:0, Minnesota, USA).

Materials

50mL Centrifuge tubes with blue screw caps (Thermo Fisher Scientific, Waltham, MA), 8 mL borosilicate glass tubes (Corning Life Sciences, Pyrex 13X100mm, North Carolina, U.S.), Corning Cap, Phenolic, Rubber Liner (Corning Life Sciences, Corning Cap, North Carolina, U.S.), 1.5 mL Microcentrifuge Graduated Tubes (Fisher Scientific, 1.5 mL MCT graduated natural, Fisherbrand, Massachusetts, U.S.), Dry Heat Block (Thermolyne, Type 17600 Dri-Bath, Barnstead/Thermolyne, Iowa, U.S.), Dry Heat Block (Dupont, Qualicon BAX System, Boekel Scientific, Pennsylvania, U.S.), Vortex Mixer (Fisher Scientific, Vortex Mixer, Massachusetts, U.S.), Centrifuge (Eppendorf, Centrifuge 5424 R, Eppendorf AG, Hamburg, Germany), Gas Chromatography – Flame Ionization Detector (Perkin Elmer Clarus 500 GC with FID and

AutoSampler, PerkinElmer, Connecticut, U.S.) with DB-FFAP column ID: 0.25mm; length: 30m; Film: 0.25 micrometers (Agilent Technologies, DB-FFAP, California, U.S.), High Pressure Compressed Specialty Grade Helium Gas (Central Storehouse, UHP Helium 99.999%, CGA 580, California, U.S.), Pipettors and Pipette tips (Fisher Scientific, Massachusetts, U.S.), Pasteur pipettes (Fisher Scientific, Massachusetts, U.S.), GC vials and Cap (Phenomenex Verex vial kit, 2mL Amber 51, 9mm screw top, and PTFE/Silicone Cap, California, U.S.).

Milk Sample Collection

Organic milk (OM) (n=12, ~1quart) and conventional milk (CM) (n=12, ~1quart) brands were each collected in the Winter and Summer from grocery stores in Davis and Sacramento, CA (**Details are listened in Table 3).** Milk samples were collected in early March 2020 and late August 2020. The reference raw organic milk samples were supplied from Crescent City, California in early March 2020 and late August 2020. Each of the collected retail milk brands were transferred to centrifuge tubes and stored on racks and frozen at -30℃ on March 2020 and August 2020. Any excess samples were stored and frozen in a -30℃ freezer room. A total of 192 milk samples were frozen. The direct transesterification method used in this thesis was modified and referenced to the methods reported by Dias et al.[26] No internal standard was added because we were interested in the % composition of the milk fatty acids.

Table 3: List of Retail Milk with Origin of Milk and Collection Month in 2020

Brand	Sample No.	Milk Type	Location	Season Collected	Month Collected
A	1 & 13	O	Crescent City, CA	Winter/Summer	February/September
B	2 & 14	O	Crescent City, CA	Winter/Summer	February/September
C	3 & 15	O	San Leandro, CA	Winter/Summer	March/August
D	4 & 16	O	Fairfield, CA	Winter/Summer	March/August
E	5 & 17	O	Petaluma, CA	Winter/Summer	March/August
F	6 & 18	O	Crescent City, CA	Winter/Summer	March/August
G	7 & 19	O	Petaluma,CA	Winter/Summer	March/August
H	8 & 20	O	City of Industry, CA	Winter/Summer	March/August
I	9 & 21	O	Fortuna, CA	Winter/Summer	March/August
J	10 & 22	O	Fresno, CA	Winter/Summer	March/August
K	11 & 23	O	Sonoma, CA	Winter/Summer	March/August
L	12 & 24	O	Fresno, CA	Winter/Summer	March/August
M	25 & 37	C	San Leandro, CA	Winter/Summer	March/August
N	26 & 38	C	San Leandro, CA	Winter/Summer	March/August
O	27 & 39	C	San Leandro, CA	Winter/Summer	March/August
P	28 & 40	C	Hanford, CA	Winter/Summer	March/August
Q	29 & 41	C	Fairfield, CA	Winter/Summer	March/August
R	30 & 42	C	Modesto, CA	Winter/Summer	March/August
S	31 & 43	C	Fairfield,CA	Winter/Summer	March/August
T	32 & 44	C	Petaluma, CA	Winter/Summer	March/August
U	33 & 45	C	Modesto, CA	Winter/Summer	March/August
V	34 & 46	C	Panoche, CA	Winter/Summer	March/August
W	35 & 47	C	Panoche, CA	Winter/Summer	March/August
X	36 & 48	C	Petaluma, CA	Winter/Summer	March/August

O = Organic, C = Conventional

Direct Transesterification of Fatty Acids from Bovine Milk

8mL borosilicate glass tubes used for derivatization were rinsed with 2mL of chloroform prior to analysis to remove any potential trace of lipids and other contaminations. Two extra sample tubes were rinsed with 2mL of chloroform as well to be used as blanks prior to analysis. Dry heat blocks were turned on and set to 90°C ahead of time to prepare for heating. After the tubes were completely dried, 400 µL of toluene, 3 mL of methanol, and 600 µL of HCl solution (8% in methanol prepared ahead of time) were added to every tube, respectively. Milk samples were removed from the -30°C freezer and defrosted ahead of time and 200 µL of each milk sample brand were added to 8mL tubes, capped, and thoroughly vortexed.

Samples were placed in a heating block and heated at 90°C for 1 hour. Samples were removed from the heat block and cooled for approximately 5 minutes at room temperature. 1 mL of hexane was added to each sample and the mixtures were thoroughly vortexed. Then, 1 mL of distilled water was added to each sample and the mixtures were vortexed. 10 minutes later, 900 µL of the hexane upper layer from every sample were transferred to a labeled 1.5 mL microcentrifuge tube containing 450 µL of distilled water. The mixtures were thoroughly vortexed and centrifuged for 2 minutes at 15,871 x g at room temperature. Hexane upper layer of each sample were transferred to newly labeled 1.5 mL Eppendorf tubes. Bottom layer from each sample were discarded. Hexane upper layer of each sample were dried under nitrogen and samples were reconstituted with 200 µL of hexane. 100 µL of hexane supernatant from each sample were transferred to labeled GC vials and the fatty acid methyl esters were analyzed by GC-FID. The injector and detector temperatures were set to 240℃ and 300℃, respectively. Flow rate of carrier gas, helium was at 1.3 mL/min. Oven temperatures were maintained at 80℃ for 2 min, increased to 180℃ at 10 ℃/min, increased to 240℃ at 5 ℃/min, and held at

240℃ for 13 minutes. Conditions were referenced from the study conducted by Dias et al.[26] Fatty acids were identified by comparing the retention time to that of authentic fatty acid standards. All milk samples were extracted and analyzed in triplicates and the results were expressed as the mean of the normalized peak areas (%).

Statistical Analysis

Two Tailed T-Test: Two Sample T-Test assuming Equal or Unequal Variances were performed between the fatty acids in organic and conventional milk collected from the Winter and Summer to test for their significance ($P<0.05$) in Microsoft Excel Version 2203.

ANOVA Single Factor was performed for each fatty acid in organic and conventional milk samples collected from the Winter and Summer to test for their significance ($P < 0.05$). For each fatty acid, significance was tested between the four groups, Organic/Winter, Organic/Summer, Conventional/Winter, and Conventional/Summer using Tukey HSD Post Hoc Tests in Microsoft Excel Version 2203.

Principal Component Analysis (PCA) was performed to investigate the significance of the similarities and differences between our milk fatty acid profiles between organic and conventional milk samples collected in the Winter and Summer. Principal Component Analysis was conducted in RStudio Version 1.4.1717.

Linear Discriminant Analysis (LDA) was performed to investigate if the milk fatty acid profiles of organic and conventional milk could be used to correctly discriminate and classify retail milk samples in their correct milk type and/or season. Linear Discriminant Analysis was conducted in RStudio Version 1.4.1717.

Results

In total, 13 fatty acids were consistently found in almost all milk samples except sample 47, which had 1 run in the triplicate run that did not produce a peak for ALA and 2 runs that did not produce peaks for margaric acid. The drop out of some peaks could be because ALA values in these samples were close to the limits of detection. The fatty acids were averaged over the three runs and calculated using % of the total fatty acids as seen in **Table 4A, 5A, and 6.** ANOVA Single Factor with Tukey HSD Test was performed between fatty acids in organic and conventional milk from both Winter and Summer samples and found that, on average, 4 out of 13 fatty acids were found to be significantly ($P<0.05$) different in % of total fatty acids between organic and conventional retail milk: myristic, myristoleic, linoleic, and α-linolenic acid, as displayed in **Table 8**. Levels of palmitoleic acid was the only fatty acid found not to be significantly different ($P>0.05$) for any of the milk sample groups or Winter and Summer groups in both T-Test and ANOVA Single Factor with Tukey HSD Tests, displayed in **Table 7** and **Table 8.**

Levels of myristic, myristoleic, and ALA were found to be significantly greater in our organic than conventional milk samples in both Winter and Summer samples, as displayed in **Table 8**. Levels of LA were found to be significantly greater in our conventional than organic milk samples in both Winter and Summer samples, as displayed in **Table 8**. The fatty acid with the greatest level of significant ($P<0.05$) difference between our organic and conventional milk samples on average was ALA with 0.870% and 0.476%, respectively, as displayed in **Table 4A**. Our organic milk samples overall had 82% greater levels of ALA than conventional milk samples. The next fatty acid with the greatest level of significant ($P<0.05$) difference on average between our organic and conventional milk samples was LA with 2.871% and 4.062%, respectively, as displayed in **Table 4A**. Our conventional milk samples on average had 41% greater levels of LA than our organic milk samples.

Table 4A: Average % of All Detected Fatty Acids of Retail Organic and Conventional Milk in Summer and Winter

Total Fatty Acids	Organic (n=24)				Conventional (n=24)			
	Mean	SD	CV%	SE	Mean	SD	CV%	SE
Caprylic Acid	1.272	0.165	12.980	0.034	1.122	0.093	8.281	0.019
Capric Acid	3.441	0.533	15.487	0.109	2.938	0.297	10.116	0.061
Lauric Acid	4.257	0.679	15.957	0.139	3.545	0.370	10.425	0.075
Myristic Acid	13.401	0.958	7.152	0.196	11.564	0.730	6.312	0.149
Myristoleic Acid	1.076	0.118	10.989	0.024	0.881	0.076	8.606	0.015
Pentadecylic Acid	1.439	0.183	12.685	0.037	1.220	0.170	13.906	0.035
Palmitic Acid	36.600	2.603	7.113	0.531	35.275	0.954	2.705	0.195
Palmitoleic Acid	1.524	0.152	9.983	0.031	1.461	0.122	8.382	0.025
Margaric Acid	0.713	0.084	11.724	0.017	0.620	0.095	15.368	0.019
Stearic Acid	11.948	1.556	13.023	0.318	14.158	1.058	7.475	0.216
Oleic/Elaidic Acid	20.587	2.361	11.470	0.482	22.706	1.411	6.216	0.288
Linoleic Acid	2.871	0.756	26.344	0.154	4.06	0.447	10.999	0.091
α-Linolenic Acid	0.870	0.186	21.361	0.038	0.476	0.199	41.813	0.041

Table 4B: Total Sum of Grouped Fatty Acids of Retail Organic and Conventional Milk

Total Fatty Acids	Organic (n=24)	Conventional (n=24)
	Grouped Means	Grouped Means
Total SFAs	73.072	70.424
Total MUFAs	23.187	25.049
Total PUFAs	3.741	4.527
LA/ALA Ratio	3.668	9.442

SD – Standard Deviation, CV% - Percent Coefficient of Variation, and SE – Standard Error for the total organic and conventional milk samples. Mean is expressed as % of total detected fatty acid. n = 48 total samples, 24 samples from organic and 24 samples from conventional milk brands ran in triplicates. SFAs: Saturated fatty acids, MUFAs: Monounsaturated fatty acids, PUFAs: Polyunsaturated fatty acids, LA/ALA Ratio: Linoleic / α-Linolenic Acid ratio

Table 5A: Average % of Total Fatty Acid of Retail Organic and Conventional Milk in the Winter and Summer

Total Fatty Acids	Organic/Winter (n=12)				Organic/Summer (n=12)				Conventional/Winter (n=12)				Conventional/Summer (n=12)			
	Mean	SD	CV%	SE	Mean	SD	CV%	SE	Mean	SD	CV%	SE	Mean	SD	CV%	SE
Caprylic Acid	1.295	0.210	16.233	0.061	1.248	0.108	8.627	0.031	1.158	0.061	5.301	0.018	1.087	0.108	9.896	0.031
Capric Acid	3.492	0.681	19.491	0.196	3.390	0.353	10.420	0.102	3.096	0.176	5.687	0.051	2.780	0.315	11.325	0.091
Lauric Acid	4.285	0.862	20.115	0.249	4.229	0.469	11.096	0.135	3.743	0.229	6.122	0.066	3.348	0.384	11.482	0.111
Myristic Acid	13.222	1.185	8.965	0.342	13.580	0.668	4.920	0.193	11.876	0.355	2.991	0.103	11.251	0.880	7.821	0.254
Myristoleic Acid	1.033	0.136	13.129	0.039	1.120	0.082	7.346	0.024	0.906	0.205	22.593	0.059	0.856	0.093	10.808	0.027
Pentadecylic Acid	1.399	0.226	16.149	0.065	1.480	0.123	8.312	0.036	1.271	0.205	16.112	0.059	1.169	0.113	9.639	0.033
Palmitic Acid	35.888	2.569	7.157	0.742	37.312	2.543	6.816	0.734	35.178	0.814	2.313	0.235	35.373	1.105	3.123	0.319
Palmitoleic Acid	1.491	0.144	9.678	0.042	1.556	0.159	10.218	0.046	1.472	0.104	7.094	0.030	1.451	0.142	9.803	0.041
Margaric Acid	0.701	0.098	14.052	0.028	0.726	0.068	9.320	0.020	0.627	0.135	21.563	0.039	0.613	0.025	4.030	0.007
Stearic Acid	12.384	1.774	14.324	0.512	11.513	1.226	10.646	0.354	13.770	0.740	5.375	0.214	14.546	1.211	8.324	0.350
Oleic/Elaidic Acid	21.024	2.529	12.030	0.730	20.151	2.202	10.925	0.636	22.125	0.821	3.709	0.237	23.287	1.660	7.129	0.479
Linoleic Acid	2.912	0.806	27.679	0.233	2.830	0.737	26.033	0.213	4.260	0.428	10.054	0.124	3.865	0.386	9.979	0.111
α-Linolenic Acid	0.873	0.202	23.137	0.058	0.866	0.177	20.431	0.051	0.518	0.259	49.866	0.075	0.433	0.109	25.137	0.031

Table 5B: Total Sum of Grouped Fatty Acids of Retail Organic and Conventional Milk in the Winter and Summer

Total Fatty Acids	Organic/Winter (n=12)	Organic/Summer (n=12)	Conventional/Winter (n=12)	Conventional/Summer (n=12)
	Grouped Mean	Grouped Mean	Grouped Mean	Grouped Mean
Total SFAs	72.667	73.477	70.718	70.167
Total MUFAs	23.548	22.826	24.503	25.594
Total PUFAs	3.785	3.697	4.778	4.297
LA/ALA Ratio	3.868	3.469	9.443	9.440

SD: Standard deviation, %CV: Percent coefficient of variation, SE: Standard Error. Mean is expressed as % of total fatty acid. n = 12 samples for each retail organic or conventional milk in the Summer and Winter.
SFAs: Saturated fatty acids, MUFAs: Monounsaturated fatty acids, PUFAs: Polyunsaturated fatty acids, LA/ALA Ratio: Linoleic / α-Linolenic Acid ratio

Table 6: Fatty Acids and their levels in Retail Organic and Conventional Milk collected over Winter and Summer of 2020 (% of total fatty acid)

Samples	MilkType/ Season	Caprylic Acid	Capric Acid	Lauric Acid	Myristic Acid	Myristoleic Acid	Pentadecylic Acid	Palmitic Acid	Palmitoleic Acid	Margaric Acid	Stearic Acid	Oleic/ Elaidic Acid	Linoleic Acid	α-Linolenic Acid
1	OW	1.505	3.916	4.726	13.903	1.224	1.353	34.723	1.734	0.595	12.684	20.754	2.138	0.744
2		1.131	2.988	3.726	13.550	1.076	1.636	39.459	1.835	0.884	10.553	20.614	1.511	1.037
3		1.017	2.680	3.353	12.054	0.912	1.535	41.310	1.491	0.853	9.376	21.556	2.779	1.082
4		1.167	3.100	3.812	12.850	0.985	1.270	34.007	1.357	0.679	14.164	22.768	3.138	0.703
5		1.243	3.231	3.852	12.638	0.965	1.290	32.756	1.405	0.687	13.242	24.268	3.498	0.928
6		1.169	3.133	3.844	12.661	0.992	1.336	33.022	1.431	0.711	13.104	24.288	3.371	0.940
7		1.161	3.164	3.987	13.150	0.981	1.221	35.298	1.499	0.685	13.627	22.049	2.355	0.823
8		1.403	3.739	4.503	13.664	1.073	1.429	33.961	1.467	0.664	12.721	21.403	2.938	1.036
9		1.109	2.859	3.383	10.698	0.774	1.024	37.252	1.413	0.524	14.308	21.801	4.500	0.353
10		1.601	4.367	5.338	14.587	1.151	1.444	36.183	1.425	0.658	11.606	18.247	2.532	0.861
11		1.669	4.996	6.292	15.245	1.265	1.921	37.077	1.450	0.757	9.315	15.279	3.771	0.962
12		1.366	3.733	4.609	13.668	0.996	1.333	35.605	1.390	0.712	13.905	19.261	2.415	1.008
13	OS	1.332	3.689	4.699	14.296	1.229	1.471	38.547	1.788	0.634	10.153	18.993	2.306	0.863
14		1.030	2.634	3.281	12.492	0.942	1.436	38.488	1.754	0.802	11.284	22.734	1.844	1.280
15		1.248	3.392	4.242	13.524	1.101	1.389	35.908	1.444	0.669	13.045	20.368	3.026	0.645
16		1.375	3.602	4.263	13.595	1.143	1.393	35.630	1.483	0.703	12.003	20.830	3.174	0.807
17		1.277	3.432	4.197	13.412	1.088	1.403	35.536	1.485	0.672	11.818	21.452	3.373	0.854
18		1.252	3.406	4.311	13.543	1.154	1.398	37.604	1.846	0.729	11.365	20.269	2.266	0.857
19		1.346	3.637	4.480	14.234	1.187	1.581	39.926	1.584	0.788	9.722	17.825	2.729	0.961
20		1.068	2.835	3.521	12.429	1.004	1.332	34.796	1.434	0.687	13.719	22.909	3.543	0.721
21		1.184	3.284	4.237	14.027	1.196	1.757	40.766	1.542	0.847	10.674	17.213	2.179	1.094
22		1.287	3.760	4.851	13.802	1.133	1.574	33.659	1.325	0.691	12.461	20.462	4.293	0.702
23		1.339	3.750	4.734	14.537	1.172	1.603	41.539	1.512	0.808	10.055	16.204	1.990	0.757

24		1.245	3.257	3.933	13.066	1.086	1.421	35.338	1.474	0.685	11.852	22.550	3.242	0.853
25		1.117	2.984	3.591	11.681	0.826	1.202	34.583	1.384	0.606	14.972	21.976	4.635	0.441
26		1.164	3.072	3.671	11.584	0.915	1.148	36.559	1.526	0.528	13.684	21.369	4.369	0.410
27		1.207	3.150	3.736	11.625	0.920	1.158	36.005	1.560	0.559	13.466	21.676	4.520	0.417
28		1.240	3.506	4.300	12.269	0.948	1.558	34.607	1.545	0.665	12.545	21.662	4.691	0.464
29		1.106	2.944	3.593	11.848	0.911	1.165	36.227	1.466	0.604	14.073	21.333	4.282	0.448
30	CW	1.082	3.082	3.836	12.010	0.975	1.508	34.239	1.642	0.670	13.595	22.846	4.036	0.478
31		1.160	3.090	3.773	12.153	0.949	1.209	35.867	1.460	0.588	13.463	21.835	4.014	0.439
32		1.085	2.836	3.439	11.452	0.865	1.117	34.908	1.325	0.558	14.240	23.398	4.305	0.471
33		1.123	3.045	3.707	11.866	0.874	1.209	35.584	1.432	0.591	14.610	20.997	4.548	0.415
34		1.248	3.227	3.719	11.822	0.915	1.124	34.467	1.354	0.549	13.669	23.099	4.309	0.498
35		1.237	3.260	4.012	12.680	0.934	1.723	34.508	1.606	1.034	12.557	22.041	3.074	1.334
36		1.122	2.957	3.541	11.526	0.839	1.124	34.575	1.362	0.573	14.367	23.273	4.336	0.406
37		1.000	2.555	3.072	10.459	0.670	1.003	33.733	1.250	0.612	17.418	23.530	4.313	0.385
38		1.009	2.616	3.201	10.921	0.869	1.157	36.501	1.645	0.613	14.219	22.539	4.338	0.373
39		1.061	2.699	3.224	10.812	0.895	1.209	35.701	1.566	0.577	13.042	24.688	4.145	0.381
40		1.129	2.994	3.637	11.641	0.873	1.235	34.855	1.427	0.609	13.336	23.787	4.077	0.399
41		1.023	2.586	3.147	10.973	0.833	1.140	36.371	1.510	0.629	15.194	22.431	3.725	0.438
42	CS	1.127	2.958	3.579	11.706	0.974	1.390	33.651	1.574	0.627	13.379	24.785	3.868	0.383
43		1.032	2.604	3.139	10.765	0.829	1.119	35.045	1.479	0.612	14.360	25.070	3.578	0.368
44		1.185	3.007	3.557	11.487	0.854	1.125	36.055	1.370	0.596	15.128	21.387	3.791	0.458
45		1.018	2.501	2.962	10.570	0.773	1.061	33.991	1.348	0.594	15.457	25.070	4.237	0.418
46		1.110	2.866	3.433	11.231	0.828	1.105	35.546	1.344	0.588	14.971	22.797	3.730	0.450
47		1.364	3.551	4.300	13.724	1.041	1.356	36.518	1.245	0.669	13.618	19.542	3.009	0.763
48		0.984	2.422	2.917	10.721	0.837	1.134	36.515	1.653	0.636	14.424	23.818	3.563	0.376

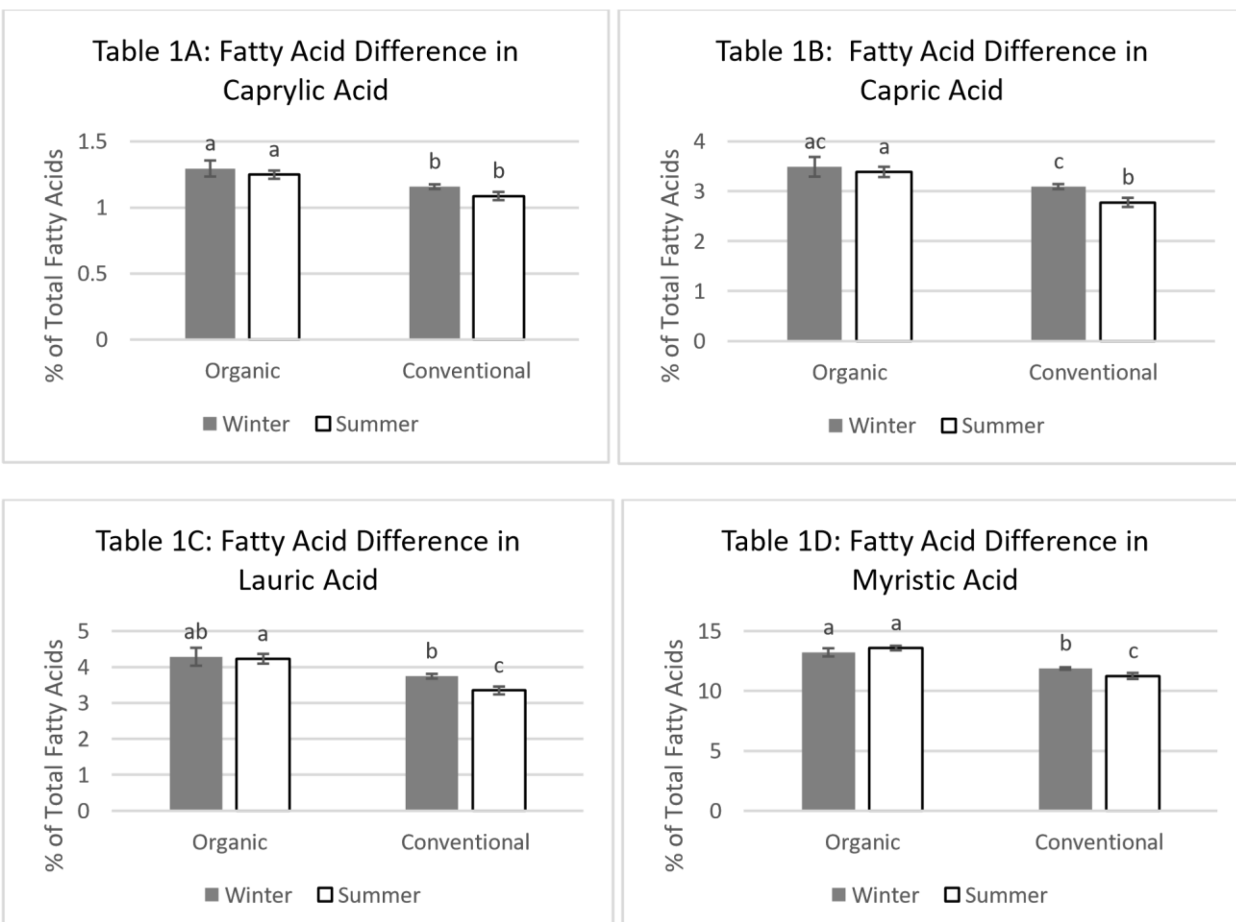
% Fatty acids were calculated by averaging the three runs ran for each sample. Sample 47, on the second run had peaks from margaric and ALAdrop out. Sample 47, on the third run had peaks from ALAdrop out. Margaric acid's % of total fatty acid was from the first run of Sample 47. α-linolenic acid's % of total fatty acid was calculated from the mean of the first and third run of Sample 47.
OW: Organic/Winter, OS: Organic/Summer, CW: Conventional/Winter, CS: Conventional/Summer.

Seasonality

Differences in % of total fatty acids of all 13 fatty acids between Winter and Summer samples in organic retail milk were found to be not significant (P>0.05) after performing T-Test and ANOVA Single Factor with Tukey HSD Test, as displayed in **Figure 7** and **Table 8.** However, seasonal variations were identified within our conventional retail milk between Winter and Summer samples and displayed significant (P<0.05) differences in levels of capric, lauric, myristic, oleic/elaidic, and LA, (see **Figure 1** and **Table 7**)**;** after performing a T-Test**.** Capric, lauric, myristic, and LA in conventional milk were found to be significantly greater in the Winter than in the Summer samples, (see **Figure 1** and **Table 7**). In contrast, through ANOVA Single Factor and Tukey HSD Test, when comparing between Organic/Winter, Organic/Summer, Conventional/Winter, and Conventional/Summer, no significant differences were identified in the levels of all 13 fatty acids when looking at organic and conventional retail milk samples in the Winter and Summer, as displayed in **Table 8**. A possible reason for the different results concluded for the seasonal variations in organic and conventional milk in both Winter and Summer could likely be due to different comparisons that are being made between T-Test and ANOVA Single Factor with Tukey HSD Test. With the T-Test, we are only testing between two means and standard deviations, which considers variability from only two groups. However, ANOVA Single Factor tests between all four groups of our sample and takes into consideration the variability of all four groups as well. Thus, due to the consideration of more variability in ANOVA Single Factor, it is possible ANOVA Single Factor would not be able to discern small but possibly significant differences in concentrations of fatty acids, such as the % fatty acid differences between Winter and Summer in conventional milk.

Through T-Test, levels of caprylic, myristic, myristoleic, and ALA had significantly greater levels in organic than conventional retail milk in both Winter and Summer samples, displayed in **Figure 1** and **Table 7.** Levels of stearic and LA had significantly ($P<0.05$) greater levels in conventional milk than organic milk in both Winter and Summer, **Figure 1** and **Table 7**. Levels of oleic/elaidic acid in conventional milk were found to be significantly greater in the Summer than Winter, displayed in **Figure 1** and **Table 7.**

Figure 1A-1M: T-Test Results on % of Total Fatty Acid Differences of Organic and Conventional Milk Samples in Winter and Summer displayed as Bar Graphs (n=12 for each bar plot)



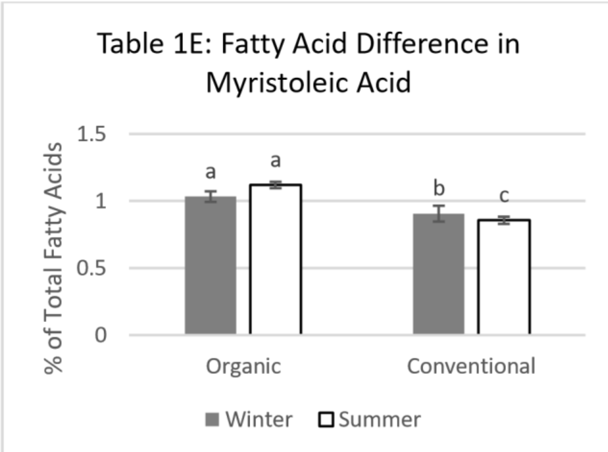


Table 1E: Fatty Acid Difference in Myristoleic Acid
% of Total Fatty Acids
1.5
1
0.5
0
a
a
b
c
Organic
Conventional
Winter
Summer

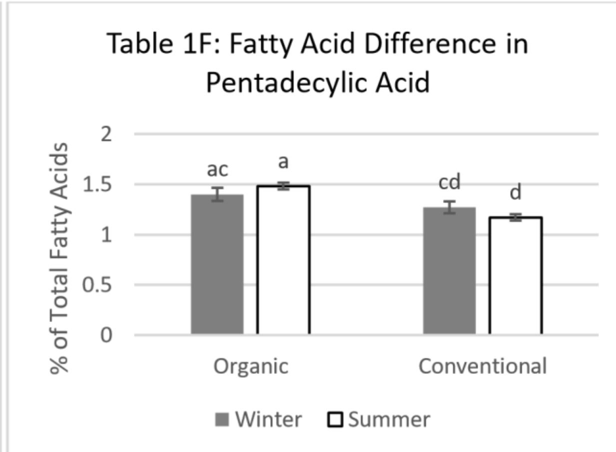


Table 1F: Fatty Acid Difference in Pentadecylic Acid
% of Total Fatty Acids
2
1.5
1
0.5
0
ac
a
cd
d
Organic
Conventional
Winter
Summer

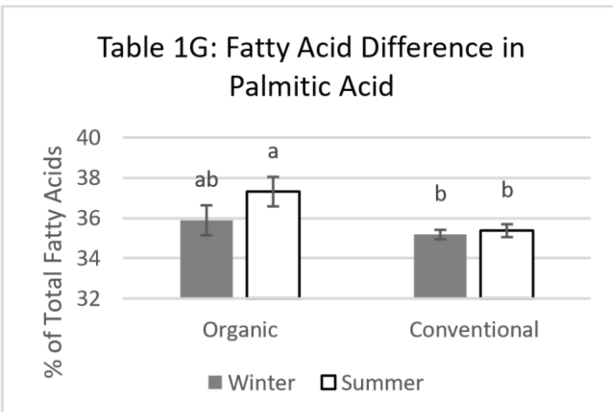


Table 1G: Fatty Acid Difference in Palmitic Acid
% of Total Fatty Acids
40
38
36
34
32
ab
a
b
b
Organic
Conventional
Winter
Summer

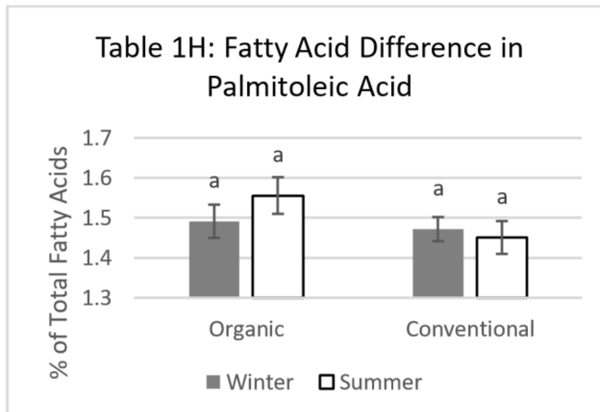


Table 1H: Fatty Acid Difference in Palmitoleic Acid
% of Total Fatty Acids
1.7
1.6
1.5
1.4
1.3
a
a
a
a
Organic
Conventional
Winter
Summer

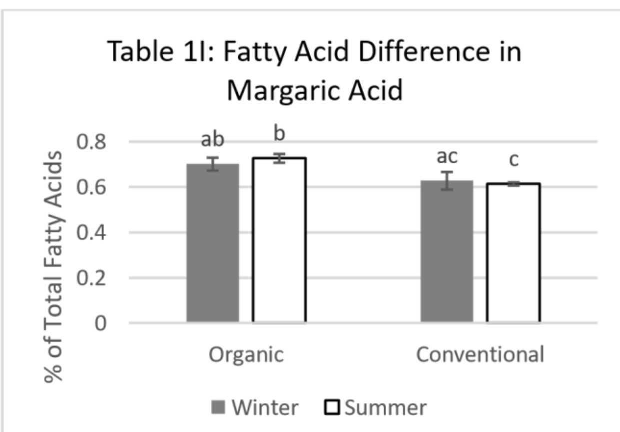


Table 1I: Fatty Acid Difference in Margaric Acid
% of Total Fatty Acids
0.8
0.6
0.4
0.2
0
ab
b
ac
c
Organic
Conventional
Winter
Summer

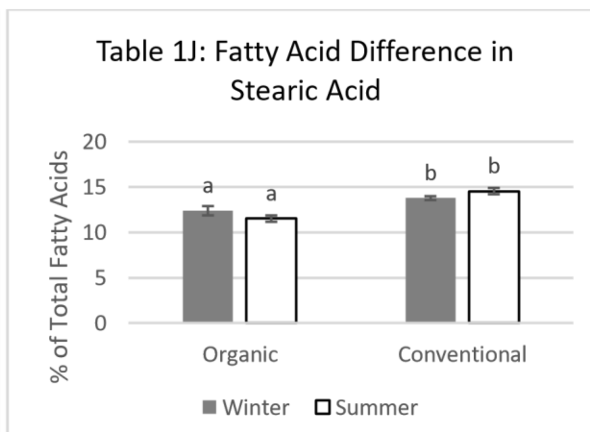
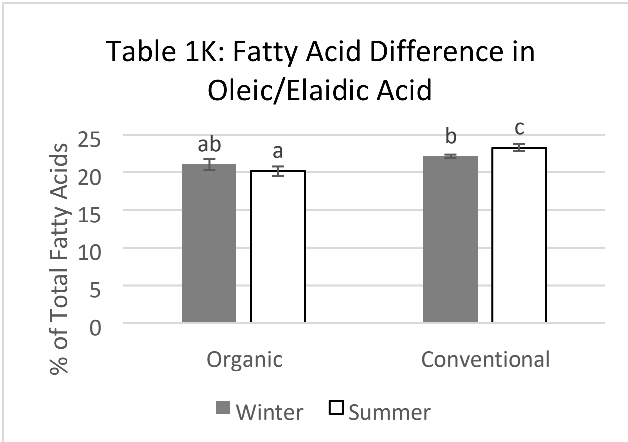
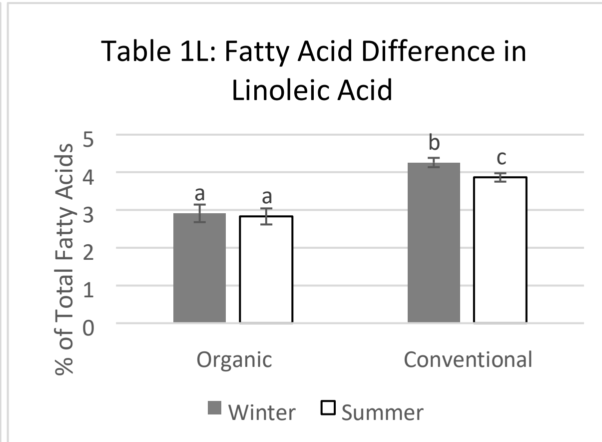
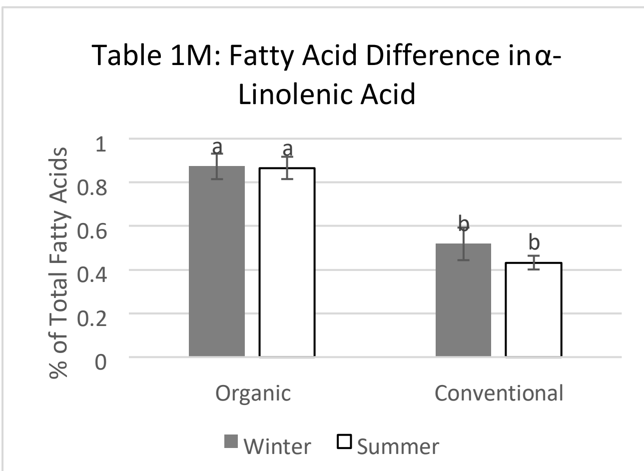


Table 1J: Fatty Acid Difference in Stearic Acid
% of Total Fatty Acids
20
15
10
5
0
a
a
b
b
Organic
Conventional
Winter
Summer







Calculated mean % of total fatty acid for each fatty acid for Organic and Conventional milk in the Winter and Summer. Error bars represent standard error of mean. Two Tailed T-Test: Two Sample T-Test assuming Equal or Unequal Variances was performed. Mean % FA with different letters, indicated by a, b, c, and/or d are significantly different (P<0.05).

Table 7: T-Test Results on Fatty Acid Differences of Organic and Conventional Milk Samples in the Winter and Summer displayed as % Mean of each Fatty Acid

Total Fatty Acids	Organic/Winter	Organic/Summer	Conventional/Winter	Conventional/Summer
	Mean (n=12)	Mean (n=12)	Mean (n=12)	Mean (n=12)
Caprylic Acid	1.295[a]	1.248[a]	1.158[b]	1.087[b]
Capric Acid	3.492[ac]	3.390[a]	3.096[c]	2.780[b]
Lauric Acid	4.285[ab]	4.229[a]	3.743[b]	3.348[c]
Myristic Acid	13.222[a]	13.580[a]	11.876[b]	11.251[c]
Myristoleic Acid	1.033[a]	1.120[a]	0.906[b]	0.856[c]
Pentadecylic Acid	1.399[ac]	1.480[a]	1.271[cd]	1.169[d]
Palmitic Acid	35.888[ab]	37.312[a]	35.178[b]	35.373[b]
Palmitoleic Acid	1.491[a]	1.556[a]	1.472[a]	1.451[a]
Margaric Acid	0.701[ab]	0.726[b]	0.627[ac]	0.613[c]
Stearic Acid	12.384[a]	11.513[a]	13.770[b]	14.546[b]
Oleic/Elaidic Acid	21.024[ab]	20.151[a]	22.125[b]	23.287[c]
Linoleic Acid	2.912[a]	2.830[a]	4.260[b]	3.865[c]
α-linolenic acid	0.873[a]	0.866[a]	0.518[b]	0.433[b]

Two Tailed T-Test: Two Sample T-Test assuming Equal or Unequal Variances was performed on all four groups of organic and conventional milk samples in the Winter and Summer. Mean % FA with different letters, indicated by a, b, c, and/or d are significantly different ($P<0.05$).

Table 8: ANOVA Single Factor and Tukey HSD Test Results on % of Total Fatty Acid Differences of Organic and Conventional Milk Samples in Winter and Summer displayed as % Mean of each Fatty Acid

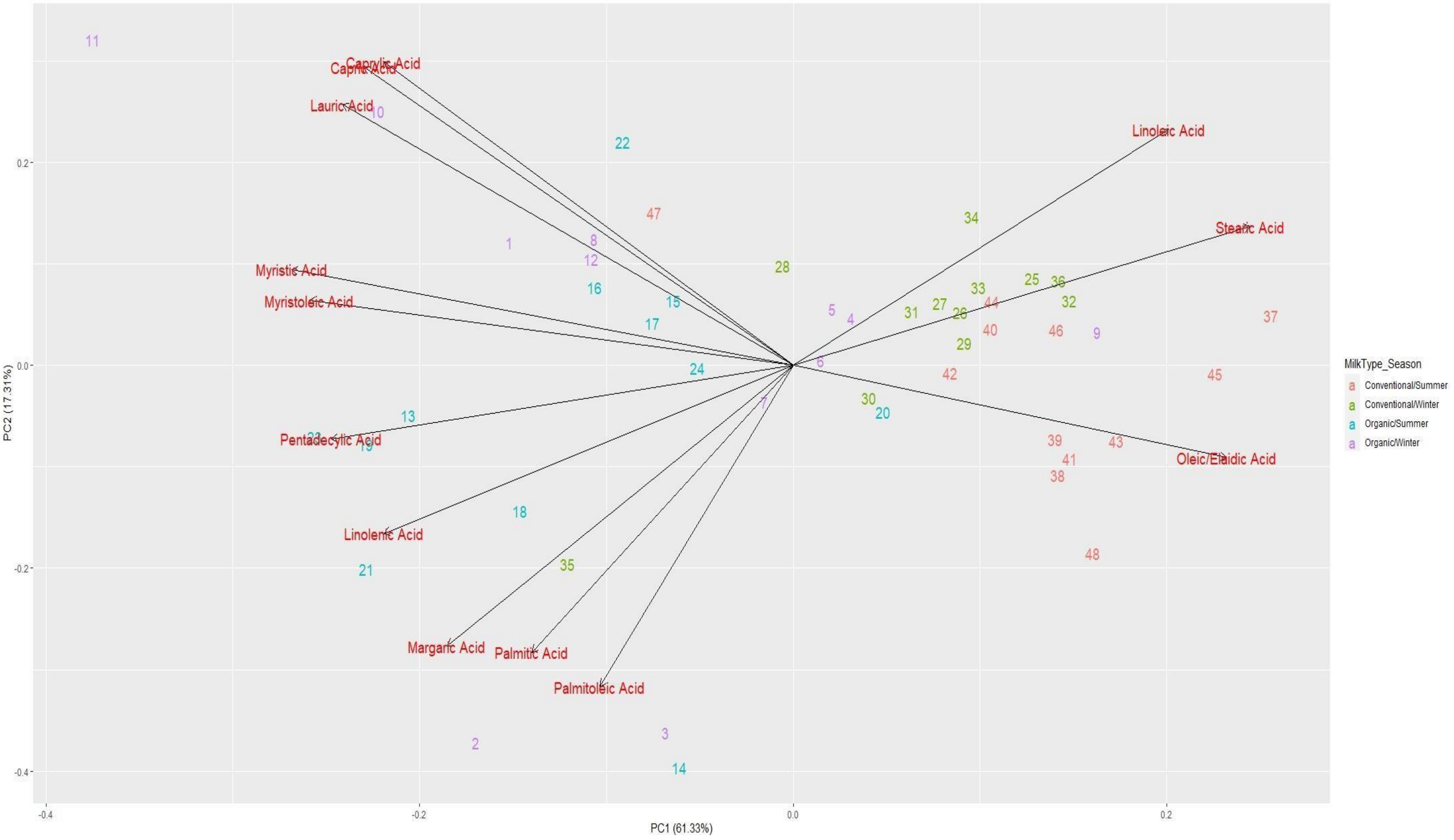
Total Fatty Acids	Organic/Winter	Organic/Summer	Conventional/Winter	Conventional/Summer
	Mean (n=12)	Mean (n=12)	Mean (n=12)	Mean (n=12)
Caprylic Acid	1.295^{AC}	1.248^{AC}	1.158^{BC}	1.087^{B}
Capric Acid	3.492^{AC}	3.390^{AC}	3.096^{BC}	2.780^{B}
Lauric Acid	4.285^{AC}	4.229^{AC}	3.743^{BC}	3.348^{B}
Myristic Acid	13.222^{A}	13.580^{A}	11.876^{B}	11.251^{B}
Myristoleic Acid	1.033^{A}	1.120^{A}	0.906^{B}	0.856^{B}
Pentadecylic Acid	1.399^{AB}	1.480^{B}	1.271^{AC}	1.169^{C}
Palmitic Acid	35.888^{AB}	37.312^{A}	35.178^{B}	35.373^{AB}
Palmitoleic Acid	1.491^{A}	1.556^{A}	1.472^{A}	1.451^{A}
Margaric Acid	0.701^{AB}	0.726^{A}	0.627^{AB}	0.613^{B}
Stearic Acid	12.384^{AB}	11.513^{B}	13.770^{AC}	14.546^{C}
Oleic/Elaidic Acid	21.024^{AC}	20.151^{AC}	22.125^{BC}	23.287^{B}
Linoleic Acid	2.912^{A}	2.830^{A}	4.260^{B}	3.865^{B}
α-linolenic acid	0.873^{A}	0.866^{A}	0.518^{B}	0.433^{B}

ANOVA Single Factor and Tukey HSD Test tested on averages of all four groups of organic and conventional milk samples in the Winter and Summer. Significance between groups of organic and conventional milk in the Winter and Summer were determined using Tukey HSD Post Hoc Tests. Mean values in the same row of their corresponding fatty acid with different letter, indicated by A, B, and/or C, superscripts are significantly different.

ANOVA Single Factor and Tukey HSD Test results were considered significant with a $P<0.05$.

Principal component analysis (PCA) was performed to investigate and illustrate the significance of separation between the fatty acid profiles of organic and conventional milk, displayed in **Figure 2.** As shown in **Figure 2**, we observed a separation between organic and conventional samples, regardless of seasons, by 10 fatty acids associated with organic milk and 3 fatty acids associated with conventional milk. Conventional milk samples seemed to show a tighter grouping when compared to organic milk samples, which is observed to be more spread apart. This indicates that conventional milk samples displayed more similar fatty acid profiles that organic milk samples. A slight separation between Winter and Summer conventional samples can be observed in **Figure 2,** with Winter conventional samples plotted near LA and stearic acid and Summer conventional samples plotted towards oleic/elaidic acid. There is also a small observed grouping of conventional samples associated near caprylic, capric, and lauric acid, displayed in **Figure 2.** In the PCA plot, we found five of our retail organic milk samples to display similar fatty acid profiles to most of our conventional milk samples, as seen in **Figure 2**, four of which were collected in the Winter of March 2020 and one in the Summer of August 2020. Also, one retail conventional milk brand W (Sample 35 and 47) collected in Winter of 2020 and Summer of 2020 were found to have similar fatty acid profiles to most of our organic milk samples according to **Figure 2**. We also observed sample 28 in **Figure 2** to be in between organic and conventional milk samples, indicating a fatty acid profile that is not similar to organic or conventional milk fatty acid profiles overall.

Figure 2: Organic and Conventional Milk Fatty Acids in the Winter and Summer PCA Plot



PCA Plot of Organic and Conventional retail milk samples from the Winter and Summer of the 13 identified fatty acids.

Linear discriminant analysis (LDA) was performed to demonstrate the ability to classify organic and conventional samples collected in the Winter and Summer based on their fatty acid profiles. Three 3 cross-validations were performed with our samples and 2 samples from each milk type and season were randomly chosen through a random number generating program for each test to achieve around an 80/20 training and testing split. **Figures 3A**, **3B**, and **3C** represent the data used for our training split for all three tests. The LDA plots in **Figure 3A-3C** display better separation than observed in our PCA plot. The LDA plot is also able to separate the seasons, Winter and Summer, in our organic and conventional milk samples. After running 3 cross validations using our identified fatty acids, the percentage accuracy of classifying our samples into the correct milk type and season were, 87.5%, 62.5%, and 100%, resulting in 83.33% accuracy of classification on average. The percentage accuracy of classifying our samples in just the correct milk type were, 100%, 87.5%, and 100%, resulting in 95.83% accuracy of classification on average. Accuracy on classifying our test milk samples into the correct milk type and season was expected to be lower, since our ANOVA Single Factor tests indicated that there were not any fatty acids affected significantly by seasonal variations in our organic and conventional milk samples. T-Tests also only resulted in some significant ($P>0.05$) seasonal variations in conventional milk samples. However, we did expect somewhat good level of accuracy in classifying our test milk samples into the correct milk type because our T-Test and ANOVA did display significant differences in almost all fatty acids between organic and conventional milk samples.

Figure 3A: Linear Discriminant Analysis Plot of Training Sample 1

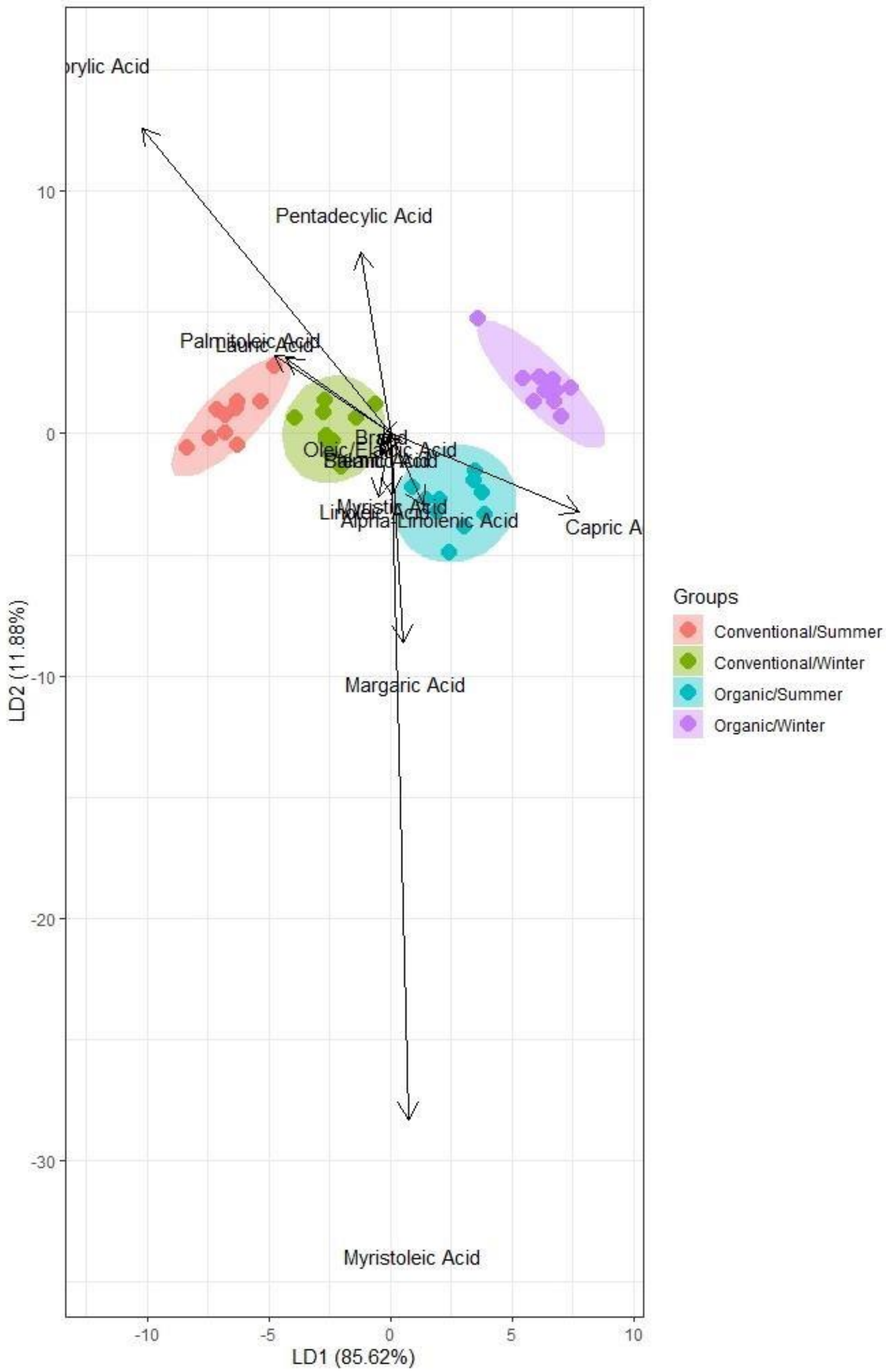


Figure 3B: Linear Discriminant Analysis Plot of Training Sample 2

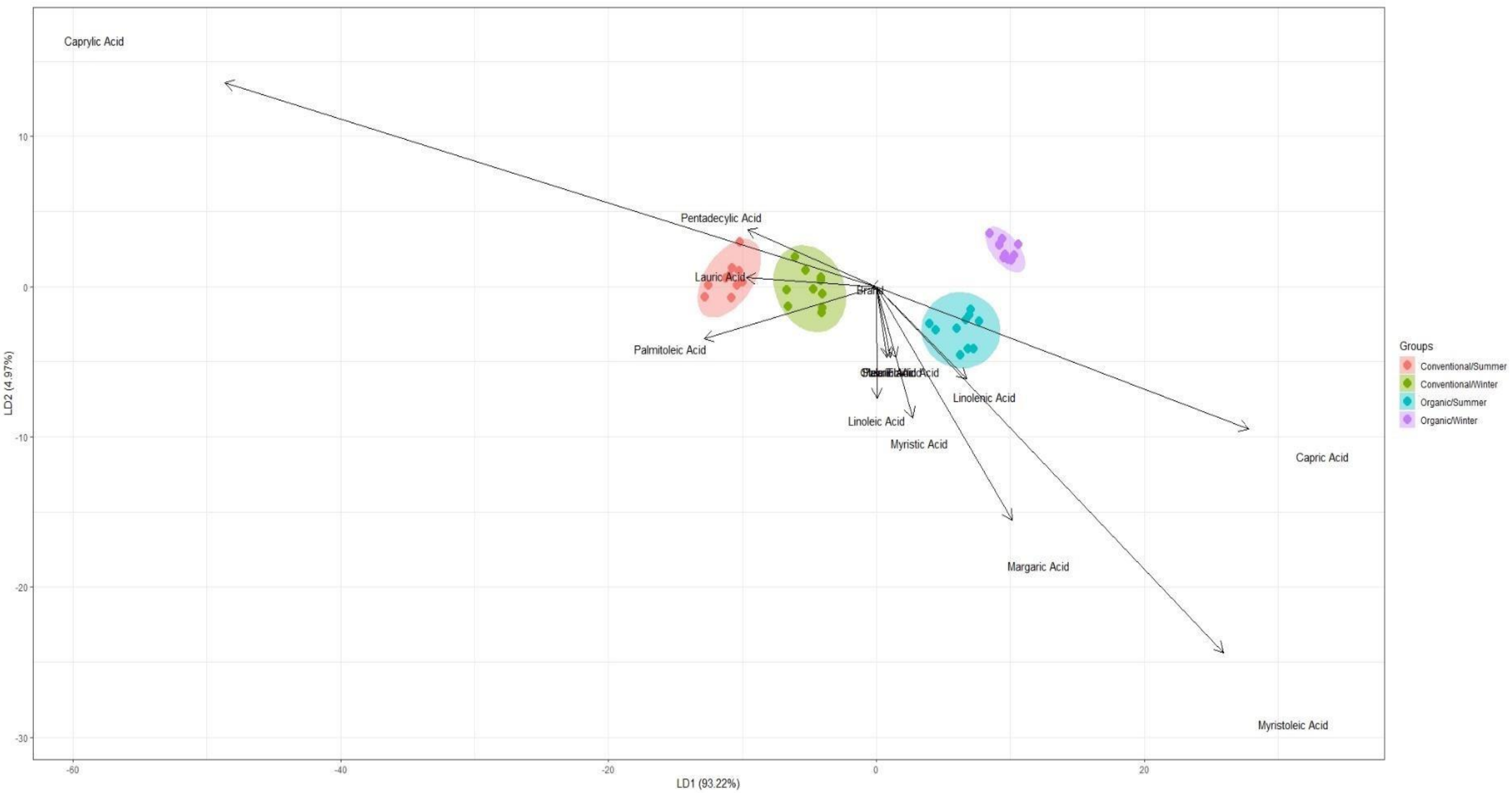
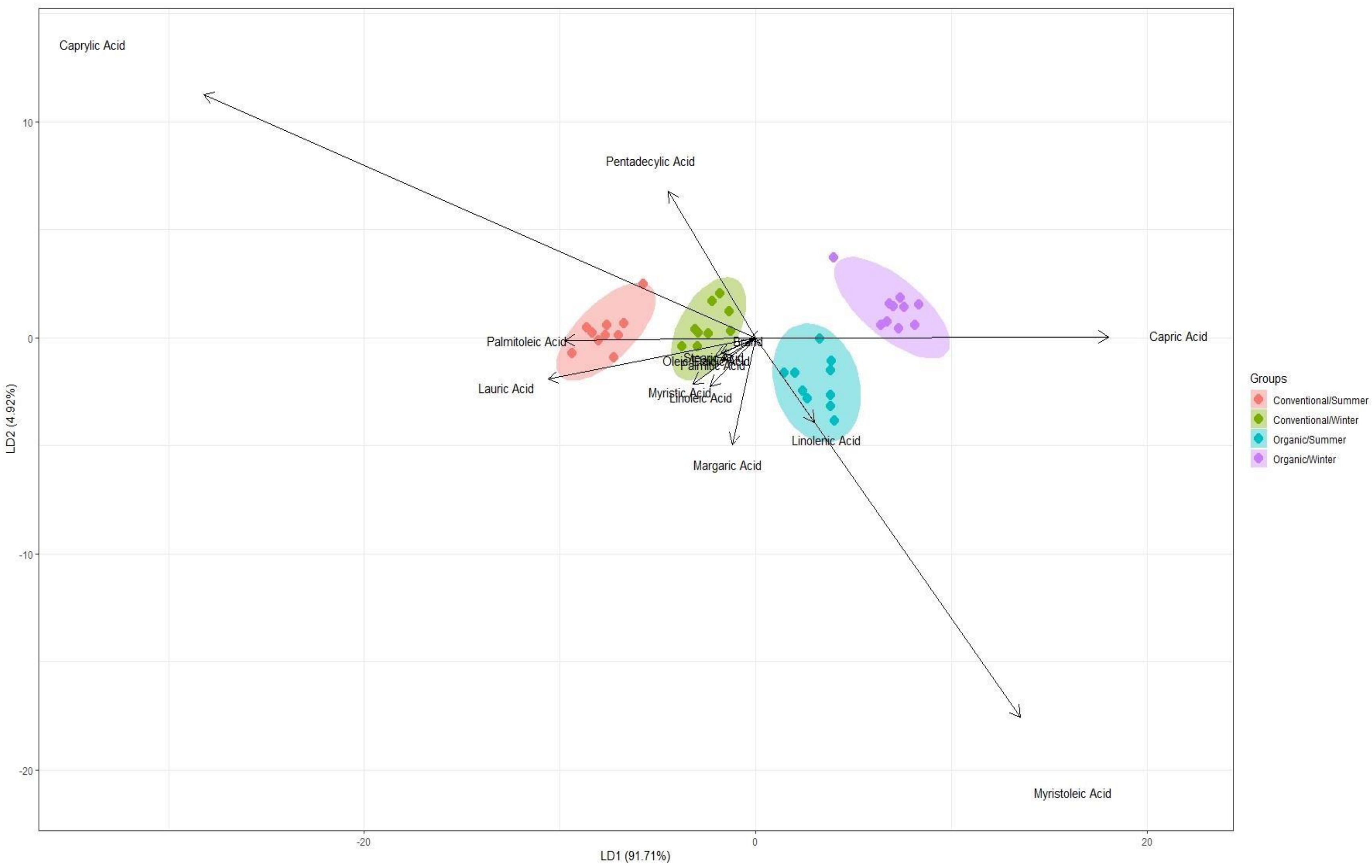


Figure 3C: Linear Discriminant Analysis Plot of Training Sample 3



Discussion

Overall, our organic milk samples had significantly greater levels of myristic, myristoleic, and ALA than our conventional milk samples according to ANOVA Single Factor with Tukey HSD Test. Previous studies from the U.S. and South Korea agreed with our findings in general, but the study conducted in South Korea also found concentrations of LA to be significantly greater in organic milk overall.[6,9] The study conducted in the U.K. by Butler et al. also found significantly greater concentrations of LA in organic milk than conventional milk.[8] ALA levels found to be significantly ($P<0.05$) greater in concentration or levels in organic milk on average agrees with the studies conducted in Germany, the U.K., South Korea, the U.S., and in the meta-analyses conducted by Średnicka-Tober et al, as seen in **Table 1**.[6,8–11] LA levels found to be significantly greater in conventional milk agrees with the study conducted in the U.S. by Benbrook et al., but did not agree with the studies conducted in the U.K., South Korea, and in the meta-analyses, as seen in **Table 1**.[6–9] The study conducted in the U.K. and South Korea found LA concentrations overall to be significantly greater in organic milk, however, the metaanalyses by Średnicka-Tober et al. found differences in LA concentrations to be negligible ($P>0.05$) on average between organic and conventional milk.[7–9] Total PUFAs and LA/ALA ratios were also cited as important markers for distinguishing between organic and conventional milk, as previously discussed.[6–10,27] Total PUFAs were greater in our conventional milk than organic milk samples overall, 4.527% and 3.741%, respectively, displayed in **Table 4.** This result agrees with the study conducted by Benbrook et al. in the U.S., but contrasts with studies conducted in the U.K., South Korea, and the meta-analyses by Średnicka-Tober et al. where PUFAs were found to be significantly ($P<0.05$) greater in organic milk**.**[6–10] LA/ALA ratios between organic and conventional milk on average were, 3.668 and 9.442, respectively, which is consistent with the findings by Benbrook et al., 2.568 in organic milk and 6.272 in conventional milk overall, where organic milk had a smaller ratio closer to 1:1 and conventional milk had a much greater ratio closer the 10:1.[6,10]

As previously discussed, the fatty acid profile of milk from dairy cows is significantly affected by diet and other factors such as region, season, stage of lactation, and breed.[8,12,15] The result of significantly greater levels of ALA in organic milk than conventional can be contributed to the greater intake of fresh forage and green pastures generally found on organic dairy farms which are made up abundantly of n-3 fatty acids, such as ALA.[13,16] However, the LA concentrations were found to be greater in organic than conventional milk from the studies conducted in the U.K and South Korea.[8,9] Furthermore, LA concentrations were found to be negligible between organic and conventional milk.[7] Generally, TMRs, silage, and grains have been found to increase SFAs, n-6 fatty acids, and LA in milk.[7,24] As for the different conclusions reached for total PUFAs from the studies conducted in the U.S., U.K., New Zealand, South Korea, and the meta-analyses, this could be due to the variations of how many PUFAs were identified within each study, 6 PUFAs, 10 PUFAs, 13 PUFAs, 6 PUFAs, and 8 PUFAs, respectively.[6–9] Our study was only able to identify two PUFAs, ALA and LA. The possible drop out of other PUFAs could be due to samples reaching close to our limits of detection. Another issue could be that our column could not differentiate other PUFAs.

Our results of levels of ALA remaining significantly ($P<0.05$) greater in organic milk and levels of LA remaining significantly greater in conventional milk in both Winter and Summer agrees with the study conducted in the U.S. by Benbrook et al, displayed in **Table 1.**[6] The study conducted in the U.K. found ALA concentrations to significantly ($P<0.05$) decrease from the Summer to Winter in both organic and conventional milk.[8] However, in the same transition of seasons the year after, ALA concentrations significantly decreased from Summer to Winter in only organic milk, not conventional milk.[8] Concentration of total n-6 fatty acids in the first sampling year of 2006 displayed negligible ($P>0.05$) differences between organic and conventional milk in both Summer and Winter.[8] However, in the next sampling year of 2007, concentration of total n-6 fatty acids in organic milk in the Summer was significantly ($P<0.05$) less, but still negligible to conventional milk.[8] In the same year of 2007, concentration of total n-6 fatty acids in the Winter was significantly greater in organic milk than conventional milk.[8] The study contributed the sampling year variations between 2006 and 2007 in ALA and LA to changes in weather that significantly affected the forage, quality, and intake of that sampling year.[8] The study conducted in South Korea found ALA and LA to have significantly ($P<0.05$) greater concentrations in organic milk than conventional milk throughout the 4 seasons.[9] Furthermore, organic milk contained significantly greater concentrations of n-3 and n-6 fatty acids in each of the individual four seasons.[9] These differences in seasonal variations have been attributed to many factors such as feeding regime, fresh pasture availability throughout the year, and weather changes from year to year that can affect the availability of fresh forage available.[6–9] As previously discussed, the increased reliance of fresh pastures in the diet of dairy cows has been associated with increasing ALA concentrations and levels in milk fatty acid profiles.[7,13,15] Thus, perhaps organic dairy farms, with the capability to continually feed fresh pastures, forages, or feed with a high content of ALA throughout the year could explain how seasonality would have a negligible effect on our organic milk fatty acid profiles overall.[7,13] However, when fresh pastures aren't available during certain times of the season, such as in the Winter, farms have to rely on rations, hays, grains, and silage, which are known to contain high concentrations of

SFAs, n-6 FAs, and LA.[7,13] This would lead to a decrease in ALA and nutritionally favorable PUFAs in milk fatty acids, while also increasing SFAs, n-6 FAs, and LA in milk fatty acid profiles.[7,13]

From our PCA plot in **Figure 2**, samples 4, 5, 6, 9, and 20 were grouped together with conventional milk samples. Samples 4, 5, 6, and 9 were organic retail milk collected in the Winter and Sample 20 was an organic retail milk collected in the Summer. A possible reason for this overlap could be due to pastures being insufficient or unavailable during the Winter and having to supplement with hays, rations, silage, and grains, which are known to contain elevated concentrations of n-6 FAs such as LA.[7,13,15,16]

One retail conventional milk brand W (Sample 35 and 47) collected in the Winter and Summer of 2020 was found to have similar fatty acid profiles to most of our organic milk samples according to **Figure 2**. Milk brand W is retailed as raw milk and manufactured from a farm in Panoche, California. At this conventional farm, their website states that their dairy cows are fed a diet of pastures, organic hay, and non-GMO grain-based dairy feed and have access to pastures during the natural growing season in that region in the Spring and Winter.[28] As previously discussed, a reliance on fresh pastures has been associated with increased n-3 FAs and ALA in milk.[7,13,16] Since dairy cows at this farm are fed a pasture based diet in the Winter and fed organic hay and non-GMO grain-based dairy feed in the Summer, this may be the reason for the similarities in fatty acid profiles to that of our organic milk samples in **Figure 2**.

Our reference organic milk samples were directly collected from two physically different farms under the same brand (Brand A and B) from Crescent City. One farm (Brand A) was mostly pasture based with some grains and the other farm (Brand B) was all grass-fed per the farm's information. Their retail milk (Brand F) is a mixture of these farms.[29] For both reference organic milk samples (Brand A and B) in the Winter and Summer, both samples had fatty acid profiles that were grouped together with most other organic milk samples, as expected, from our study, as observed in **Figure 2**. However, the Winter sample of their retail milk, Brand F (Sample 6) was observed to have a fatty acid profile similar to our conventional milk samples. A reason for this case is unclear as we would expect a fatty acid profile similar to a mixture of our reference samples. In the Summer where pasture feeding is generally abundant, only one organic milk sample (Sample 20) collected in the Summer overlapped with conventional milk samples. However, organic dairy farms can change the diets of their dairy cows throughout the year as seasons change, since pasture feeding isn't guaranteed year-round depending on region and farm diet management.[7,13]

Conclusion

Our study investigated and compared the fatty acid profiles between organic and conventional retail milk in California and found 4 of 13 fatty acids identified had significantly ($P<0.05$) different % of total fatty acid overall between organic and conventional retail milk samples in the Winter and Summer through ANOVA Single Factor with Tukey HSD Test: myristic, myristoleic, LA, and ALA. Levels of caprylic, myristic, myristoleic, and ALA were significantly greater in organic than conventional retail milk in the Winter and Summer and levels of stearic and LA were significantly greater in conventional than organic retail milk in the Winter and Summer. In ANOVA, levels of myristic, myristoleic, and ALA remained significantly greater in organic than conventional retail milk in the Winter and Summer and levels of LA remained significantly greater in conventional than organic retail milk in the Winter and Summer. Seasonal differences in levels of fatty acids in the Winter and Summer were negligible ($P>0.05$) in organic and conventional retail milk through ANOVA. However, T-Test resulted in significant seasonal variations in the Summer and Winter in levels of capric, lauric, myristic, oleic/elaidic, and LA in conventional retail milk. The different results concluded in TTest and ANOVA Single Factor with Tukey HSD Test should be expected, as both tests consider different levels of variability. Two Sample T-Test considered the variability of two groups, while ANOVA Single Factor considers the variability of 4 groups: organic and conventional milk in the Winter and Summer. Thus, the conclusion is that significance in ANOVA Single Factor would be more difficult to reach when compared to the Two Sample T-Test. Fatty acids that did not demonstrate any significance in seasonal variations, such as myristic, myristoleic, LA, and ALA, but were significantly different between organic and conventional retail milk, regardless of season, in T-Test and ANOVA should be studied further as potential markers for authenticating organic milk in California. The PCA plot shown in **Figure 2** also agrees and shows myristic, myristoleic, LA, and ALA to be strong eigenvectors that correlated with the majority of organic and conventional samples. Through LDA, authentication of organic milk using fatty acid profiles is possible with an average accurate prediction of 95.83%. However, if we were to also include accurately determining the season as well with milk type, our accuracy drops to 83.33%, which is still good. From our results, we can conclude that there are significant differences between the fatty acid profiles of organic and conventional retail milk. Thus, authentication is possible and relatively accurate when predicting between organic and conventional retail milk in California when considering seasonal variations in the Winter and

Summer.

References

(1) O'Donnell, A. M.; Spatny, K. P.; Vicini, J. L.; Bauman, D. E. Survey of the Fatty Acid Composition of Retail Milk Differing in Label Claims Based on Production Management Practices1. *Journal of Dairy Science* **2010**, *93* (5), 1918–1925. https://doi.org/10.3168/jds.20092799.

(2) *7 CFR Part 205 Subpart C -- Organic Production and Handling Requirements*. https://www.ecfr.gov/current/title-7/subtitle-B/chapter-I/subchapter-M/part-205/subpart-C (accessed 2022-03-26).

(3) *Regulation (EU) 2018/848 of the European Parliament and of the Council of 30 May 2018 on Organic Production and Labelling of Organic Products and Repealing Council Regulation (EC) No 834/2007*; 2018; Vol. 150.

(4) *Explore Three Top Organic Certification Bodies in Europe | foodcircle*. https://www.foodcircle.com/magazine/european-organic-certification-ecocert-soil-associationabcert (accessed 2021-06-20).

(5) *Farming & Growing Standards | Soil Association.org*. https://www.soilassociation.org/ourstandards/read-our-organic-standards/farming-growing-standards/ (accessed 2021-06-19).

(6) Benbrook, C. M.; Butler, G.; Latif, M. A.; Leifert, C.; Davis, D. R. Organic Production Enhances Milk Nutritional Quality by Shifting Fatty Acid Composition: A United States–Wide, 18-Month Study. *PLoS One* **2013**, *8* (12). https://doi.org/10.1371/journal.pone.0082429.

(7) Średnicka-Tober, D.; Barański, M.; Seal, C. J.; Sanderson, R.; Benbrook, C.; Steinshamn, H.; Gromadzka-Ostrowska, J.; Rembiałkowska, E.; Skwarło-Sońta, K.; Eyre, M.; Cozzi, G.; Larsen, M. K.; Jordon, T.; Niggli, U.; Sakowski, T.; Calder, P. C.; Burdge, G. C.; Sotiraki, S.; Stefanakis, A.; Stergiadis, S.; Yolcu, H.; Chatzidimitriou, E.; Butler, G.; Stewart, G.; Leifert, C. Higher PUFA and N-3 PUFA, Conjugated Linoleic Acid, α-Tocopherol and Iron, but Lower Iodine and Selenium Concentrations in Organic Milk: A Systematic Literature Review and Meta- and Redundancy Analyses. *Br J Nutr* **2016**, *115* (6), 1043–1060. https://doi.org/10.1017/S0007114516000349.

(8) Butler, G.; Stergiadis, S.; Seal, C.; Eyre, M.; Leifert, C. Fat Composition of Organic and Conventional Retail Milk in Northeast England. *Journal of Dairy Science* **2011**, *94* (1), 24–36. https://doi.org/10.3168/jds.2010-3331.

(9) Chung, I.-M.; Kim, J.-K.; Lee, K.-J.; Son, N.-Y.; An, M.-J.; Lee, J.-H.; An, Y.-J.; Kim, S.-H. Discrimination of Organic Milk by Stable Isotope Ratio, Vitamin E, and Fatty Acid Profiling Combined with Multivariate Analysis: A Case Study of Monthly and Seasonal Variation in Korea for 2016–2017. *Food Chemistry* **2018**, *261*, 112–123. https://doi.org/10.1016/j.foodchem.2018.04.017.

(10) Ellis, K. A.; Innocent, G.; Grove-White, D.; Cripps, P.; McLean, W. G.; Howard, C. V.; Mihm, M. Comparing the Fatty Acid Composition of Organic and Conventional Milk. *Journal of Dairy Science* **2006**, *89* (6), 1938–1950. https://doi.org/10.3168/jds.S0022-0302(06)72261-5.

(11) Molkentin, J. Authentication of Organic Milk Using $\delta^{13}C$ and the α-Linolenic Acid Content of Milk Fat. *Journal of Agricultural and Food Chemistry* **2009**, *57* (3), 785–790. https://doi.org/10.1021/jf8022029.

(12) Schwendel, B. H.; Morel, P. C. H.; Wester, T. J.; Tavendale, M. H.; Deadman, C.; Fong, B.; Shadbolt, N. M.; Thatcher, A.; Otter, D. E. Fatty Acid Profile Differs between Organic and Conventionally Produced Cow Milk Independent of Season or Milking Time. *Journal of Dairy Science* **2015**, *98* (3), 1411–1425. https://doi.org/10.3168/jds.2014-8322.

(13) Elgersma, A.; Tamminga, S.; Ellen, G. Modifying Milk Composition through Forage. *Animal Feed Science and Technology* **2006**, *131* (3–4), 207–225.

https://doi.org/10.1016/j.anifeedsci.2006.06.012.
(14) Lock, A. L.; Bauman, D. E. Modifying Milk Fat Composition of Dairy Cows to Enhance Fatty Acids Beneficial to Human Health. *Lipids* **2004**, *39* (12), 1197–1206. https://doi.org/10.1007/s11745-004-1348-6.
(15) Harvatine, K. J.; Bauman, D. E.; McGuire, M. A. Mammary Gland, Milk Biosynthesis and Secretion: Milk Fat. *Encyclopedia of Dairy Sciences (Third Edition)*; McSweeney, P. L. H., McNamara, J. P., Eds.; Academic Press, 2022; pp 190–197. https://doi.org/10.1016/B978-0-12818766-1.00049-0.
(16) Dewhurst, R. J.; Shingfield, K. J.; Lee, M. R. F.; Scollan, N. D. Increasing the Concentrations of Beneficial Polyunsaturated Fatty Acids in Milk Produced by Dairy Cows in High-Forage Systems. *Animal Feed Science and Technology* **2006**, *131* (3–4), 168–206. https://doi.org/10.1016/j.anifeedsci.2006.04.016.
(17) Vasta, V.; Yáñez-Ruiz, D. R.; Mele, M.; Serra, A.; Luciano, G.; Lanza, M.; Biondi, L.; Priolo, A. Bacterial and Protozoal Communities and Fatty Acid Profile in the Rumen of Sheep Fed a Diet Containing Added Tannins. *Appl Environ Microbiol* **2010**, *76* (8), 2549–2555. https://doi.org/10.1128/AEM.02583-09.
(18) Khiaosa-Ard, R.; Bryner, S. F.; Scheeder, M. R. L.; Wettstein, H.-R.; Leiber, F.; Kreuzer, M.; Soliva, C. R. Evidence for the Inhibition of the Terminal Step of Ruminal α-Linolenic Acid Biohydrogenation by Condensed Tannins. *Journal of Dairy Science* **2009**, *92* (1), 177–188. https://doi.org/10.3168/jds.2008-1117.
(19) Somerville, S.; Sumner, D. A.; Fadel, J.; Fu, Z.; Hart, J. D.; Heguy, J. By-Product Use in California Dairy Feed Has Vital Sustainability Implications. 4.
(20) Doreau, M.; Poncet, C. Ruminal Biohydrogenation of Fatty Acids Originating from Fresh or Preserved Grass. *Reprod. Nutr. Dev* **2000**, *40*, 201.
(21) Bauman, D. E.; McGuire, M. A.; Harvatine, K. J. MAMMARY GLAND, MILK BIOSYNTHESIS AND SECRETION | Milk Fat. In *Encyclopedia of Dairy Sciences*; Elsevier, 2011; pp 352–358. https://doi.org/10.1016/B978-0-12-374407-4.00292-2.
(22) Jenkins, T. C.; Wallace, R. J.; Moate, P. J.; Mosley, E. E. BOARD-INVITED REVIEW: Recent Advances in Biohydrogenation of Unsaturated Fatty Acids within the Rumen Microbial Ecosystem1. *Journal of Animal Science* **2008**, *86* (2), 397–412. https://doi.org/10.2527/jas.20070588.
(23) Morales-Almaráz, E.; Soldado, A.; González, A.; Martínez-Fernández, A.; Domínguez-Vara, I.; de la Roza-Delgado, B.; Vicente, F. Improving the Fatty Acid Profile of Dairy Cow Milk by Combining Grazing with Feeding of Total Mixed Ration. *Journal of Dairy Research* **2010**, *77* (2), 225–230. https://doi.org/10.1017/S002202991000004X.
(24) Elgersma, A.; Ellen, G.; van der Horst, H.; Muuse, B. G.; Boer, H.; Tamminga, S. Comparison of the Fatty Acid Composition of Fresh and Ensiled Perennial Ryegrass (Lolium Perenne L.), Affected by Cultivar and Regrowth Interval. *Animal Feed Science and Technology* **2003**, *108* (1–4), 191–205. https://doi.org/10.1016/S0377-8401(03)00134-2.
(25) Månsson, H. L. Fatty Acids in Bovine Milk Fat. *Food Nutr Res* **2008**, *52*. https://doi.org/10.3402/fnr.v52i0.1821.
(26) Dias, F. F. G.; Augusto-Obara, T. R.; Hennebelle, M.; Chantieng, S.; Ozturk, G.; Taha, A. Y.; Vieira, T. M. F. de S.; Leite Nobrega de Moura Bell, J. M. Effects of Industrial Heat Treatments on Bovine Milk Oxylipins and Conventional Markers of Lipid Oxidation. *Prostaglandins, Leukotrienes and Essential Fatty Acids* **2020**, *152*, 102040. https://doi.org/10.1016/j.plefa.2019.102040.
(27) Calder, P. C. Very Long-Chain n-3 Fatty Acids and Human Health: Fact, Fiction and the Future. *Proceedings of the Nutrition Society* **2018**, *77* (1), 52–72.

https://doi.org/10.1017/S0029665117003950.
(28) *About Claravale Farm*. Claravale Farm. https://claravalefarm.com/pages/about-us (accessed 202110-24).
(29) *Home Page - Alexandre Family Farm*. https://alexandrefamilyfarm.com/ (accessed 2021-11-02).