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Exploring consumer motivations for fermented food consumption and the impact of small-scale production on safety and quality of fermented foods

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Exploring consumer motivations for fermented food consumption and the impact of small-scale production on safety and quality of fermented foods

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Melanie Hanlon

Abstract

Food fermentations are an ancient practice, integral to many global cultural and culinary practices. Traditional fermented foods (FF) have developed from access to unique raw materials, with distinctive properties of aroma, texture, appearance, and ingredients. FFs have long been relied on for their ability to transform raw substrates into a safe, nutritious, and flavorful final product. While documentation of FF dates back to ancient civilizations, our understanding of these foods was greatly expanded in the seventeenth century when early microbiologists determined this transformation process to be controlled by microorganisms. This understanding led to the commercial production of FFs, such as sauerkraut and pickled cucumbers, in large quantities, thus establishing them as a dietary staple. The prevalence of more highly processed and convenience foods in the diet of Western countries led to decreased interest in and consumption of FFs. However, recent consumer demand for FFs has led to a revival of such products which has coincided with a growing movement towards artisanal and locally sourced food production. This research aims to improve understanding of this recent trend within the U.S. with the main objectives: 1) to evaluate consumer familiarity with consumption patterns, and perceptions of FF; 2) determine frequency and type of fermentations conducted in U.S. households; and 3) determine replicability of a novel cabbage-based fermentation.

To evaluate consumer perceptions and consumption patterns of FFs, an online public survey was developed. This survey assessed participants' recognition of different types of FFs, the most frequently consumed categories, and gained insight into motivations for consumption.

A total of 751 participants completed this survey in 2022; survey questions are listed in Appendix 1, with responses for general questions and demographic questions in Appendix 2 and Appendix 4, respectively. From a list of provided FFs, only 7% (n=55) of participants correctly identified all as fermented with yogurt the most frequently identified by all participants. The least frequently identified FF was escabeche, by 15.71% (n=118) of participants. Of the provided categories, participants reported consuming fermented cereal grains (46.17%; n=307), fruits and vegetables (42.26%; n=281), dairy (39.70%; n=264), meat (30.68%; n=204), and rice or soybean products (37.60%; n=250). Approximately half (50.68%; n=337) of participants reported taste of FFs as the primary motivator for consumption while health benefits and cultural reasons were selected by 35.54% (n=235) and 12.03% (n=80) of participants, respectively. The most frequently associated health benefits of fermented foods were “improved gut microbiome” (77.14%; n=513), “digestive benefits” (76.39%; n=508), and “probiotic” (68.87%; n=458). Responses to matched questions about both non-alcoholic fermented foods and fermented fruits and vegetables did not vary, suggesting consumers do not differentiate expectations and motivations for different categories of FFs.

A second portion of this survey assessed if people in the U.S. ferment fruits and vegetables at home. All survey questions are listed in Appendix 1 with participant responses of each question in Appendix 3. Of the 751 total participants, 49.20% (n=367) reported fermenting fruits and vegetables at home. When asked what fruits or vegetables they ferment at home, most frequently reported from free responses were cabbage (53.30%; n=202), cucumbers (23.22%; n=88), alliums (14.25%; n=54), carrots (13.72%; n=52), and peppers (12.66%; n=48). Participants reported using recipe style books (46.32%; n=170) most frequently as a source of information on fermenting fruits or vegetables. Participants expressed interest in learning more about the fermentation of cucumbers (37.60%; n=138), berries (29.16%; n=107), peppers (28.61%; n=105), olives (28.33%; n=104), and cabbage (26.43%; n=97).

Due to this interest in home fermentations, a recipe for a small-batch fermentation was developed containing red cabbage, carrots, and ginger. Two salt concentrations, 2% and 3% NaCl, were evaluated at 22°C in a laboratory environment. To assess environmental influence on the fermentation repeatability, the 3% NaCl recipe was replicated in five household kitchens near Davis, CA. Brine pH and salinity measurements were collected across a 21-day fermentation period for laboratory ferments. Microbial growth was measured through culturing of fungal and lactic acid bacteria, evaluated in combination with V4-16S rRNA gene sequencing. By day 2 of the fermentation, all treatments achieved a pH of below 4.6. While observed pH reductions were consistent across fermentations, salinity had an effect on microbial population dynamics. In the 3% NaCl lab fermentation, statistically higher counts of LAB were detected on days 1, 2, 3, 4, 5 and 7. Alpha diversity of fermentations decreased during fermentation for all treatments yet was higher for the 2% NaCl fermentation compared to those with 3% NaCl, with a greater abundance of Enterobacterales and lower abundance of Lactobacillales across all measured time points. *Leuconostoc* was the most abundant genus by day 2 of the laboratory-based fermentations. However, all fermentations, including those conducted in households, had differing *Leuconostoc* amplicon sequencing variants (ASV) represented. These results indicate that while differences in salinity and fermentation environment influenced the final fermentation microbial population, all fermentation treatments reached the key critical control point of a pH decline to below 4.6.

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Table of Contents

Chapter 1: Relevance and background of fermented foods

1.1 Overview of food fermentation

Fermentation is a food preservation technique that has been utilized for thousands of years. Communities across the globe have developed their unique fermented products based on access to raw materials, environment to influence fermentation, and establishment of social food networks. Table 1 includes traditional fermented foods (FFs) from across the globe highlighting how different cultures have utilized indigenous food substrates to create a value-added product. For example, traditional FFs in Mexico include products made from native species of maize, agave, or cacti fruits while Scandinavian cultures have a history of fermenting fish dating back to early Holocene settlements (c. 9701 BCE) (Boethius, Kjällquist, Kielman-Schmitt, Ahlström, & Larsson, 2021; Ojeda-Linares et al., 2021). Origins of vegetable fermentations can be traced back to 960 CE China, and around the same time fermentation of olives was documented in ancient Rome (Jyoti Prakash Tamang et al., 2020). Fermented cereals and breads originated as early as 10,000 BCE in ancient Egypt and other cultures have prepared compositionally similar foods with “dosa,” a mixture of rice and black gram, made in India and “puto,” also made from rice, produced in the Philippines (Jyoti Prakash Tamang et al., 2020). Fermentation of milk into cheese is traced to West Asia about 8,000 years ago but now is produced globally with approximately 130 countries and regions producing different styles of cheese (X. Zheng, Shi, & Wang, 2021).

The long-standing importance of these foods has been complemented by our understanding of microorganisms in the fermentation process. In the seventeenth century, Pasteur’s work established a framework through identifying the biological role microorganisms have in fermentation, understanding the significance in starter cultures, and characterizing differences in kinetics, chemical features, and conditions involved in unique fermentations (Buchholz & Collins, 2013). These discoveries led to an inclusion of industrialization in fermentations with Pasteur patenting a closed vessel for brewing to mitigate quality issues from failed ferments.

According to the recent consensus statement from the International Scientific Association for Probiotic and Prebiotics (ISAPP) “fermented foods are defined as foods made through desired microbial growth and enzymatic conversions of food components” (Marco et al., 2021). Based on this definition, there are many foods that are considered fermented and are generated from a variety of starting ingredients. Table 1 includes select FFs and the types of microorganisms generally involved in the fermentation process for each product. Lactic acid bacteria (LAB), acetic acid bacteria (AAB), bacilli or other bacteria, or fungi contribute to the fermentation of various products (Caplice & Fitzgerald, 1999; Hansen & Miller, 1896; Hutkins, 2007; Marco et al., 2021; Ray & Bhunia, 2008). Among the numerous groups of microorganisms involved in food fermentations, the LAB are the most predominant and are involved in the fermentation of dairy, meat, cereal, and vegetable products (Di Cagno, Coda, De Angelis, & Gobbetti, 2013; M. G. Gänzle, 2015; Hutkins, 2007; Marco et al., 2021; Petrova & Petrov, 2020; Ray & Bhunia, 2008; Van Reckem et al., 2019). AAB are important for the production of vinegar, imparting its characteristic flavor through production of acetic acid during fermentation (De Roos & De Vuyst, 2018; Hutkins, 2007; Lynch, Zannini, Wilkinson, Daenen, & Arendt, 2019). *Saccharomyces cerevisiae*, an ethanol producing yeast, is responsible for the fermentation of beer, wine, and bread (Amerine & Kunker, 1968; Cavalieri, McGovern, Hartl, Mortimer, & Polsinelli, 2003; M. Gänzle & Ripari, 2016; Stewart, 2003). Other bacteria and fungi are important for the fermentation of dairy, meat, and soy products (Boyaci Gunduz, Agirman, & Erten, 2022; Hutkins, 2007; K. Ito & Matsuyama, 2021).

Though FFs have been produced for thousands of years, recent consumer demand for fermented products in the US has led to a surge in the FF product market (Domonoske & Schneider, 2020; Everett, 2019; Manskar & Raskin, 2020; Nielson-Stowell, 2017, 2020; Prince, 2018; Ramos, Louvau, Kim, Marco, & Dicaprio, 2022). Overwhelmingly, as consumers are interested in functional foods, they turning to FFs to fulfill this need (Unilever, 2022). The term functional food is generally used to describe foods that have value beyond basic nutrition, including physiological benefits, reductions of chronic disease risk, or improved well-being (Henry, 2010). Japan was the first country to establish a regulatory system for approving products as functional foods in 1991 and this led to the adoption of nutritional claims by the Codex Alimentarius in 1997 (Shimizu, 2003). Currently in the U.S., a few label claims are regulated by the FDA. Structure function claims, such as "calcium builds strong bones," are used for products that contain a nutrient or dietary ingredient with an intended effect on normal function or structure of the human body (FDA, 2022). Nutrient content claims describe the level of a given nutrient either on a scale of low to high or comparative to levels in another product, such as a “low fat” food (FDA & CFSAN, 2013). Health claims are applied to products containing a substance of which the level or presence in a food is connected to a disease or health related condition (FDA & CFSAN, 2013). A common health claim is made of products containing phytosterols which are marketed to lower blood cholesterol and reduce the risk of chronic heart conditions (Weiner & Will, 2015). The presence of health promoting antimicrobial compounds, antioxidants, and peptides qualifies certain FFs as functional. FFs such as milk products and kimchi have been found to contain antioxidant properties, serving as functional through the scavenging of free radicals which accumulation of has been connected to increased risk of some diseases (Abubakr, Hassan, Imdakim, & Sharifah, 2012; Najgebauer-Lejko & Sady, 2015; J. M. Park et al., 2011). Bioactive peptides are commonly found in FFs, especially those containing proteolytic microorganisms such as *Bacillus* species (Jyoti P. Tamang, Shin, Jung, & Chae, 2016). Most frequently these peptides are angiotensin-I-converting enzyme (ACE) inhibitors, antioxidants, or cholesterol lowering (Chaudhary, Bhalla, Patiyal, Raghava, & Sahni, 2021; Daskaya-Dikmen, Yucetepe, Karbancioglu-Guler, Daskaya, & Ozcelik, 2017). Probiotic is an additional common functional claim used with respect to FFs, however most FFs do not meet the definition to be classified as a probiotic product.

Probiotics are defined as “live microorganisms that, when administered in adequate amounts confer a health benefit on the host” (Hill et al. 2014). FFs contain a diversity of microbes most of which do not have defined host health benefits and, therefore, most commercially produced FFs are not considered probiotic (Marco et al., 2021). An exception to this rule would be if a defined probiotic strain of bacteria, such as *Lactobacillus acidophilus* NCFM, is added at sufficient levels to confer a health benefit to a FF product, such as fermented dairy products labeled as probiotic, and the levels of viable probiotics are maintained for the duration of the product’s shelf life (Gao et al., 2022; Kariyawasam, Lee, & Paik, 2021; Mukherjee, Gómez-Sala, O’Connor, Kenny, & Cotter, 2022; Sanders & Marco, 2010). Unpasteurized FFs may contain live microorganisms that are involved in fermentation, but should not be labeled “probiotic”, but rather “contains live and active cultures” (Hill et al., 2014; Marco et al., 2021).

FFs may also act as a vehicle for prebiotics, defined as “a substrate that is selectively utilized by host microorganisms conferring a health benefit” (Gibson et al., 2017). Presently, prebiotics are understood to be carbohydrate-based, with studies to substantiate the benefit of fructans and galactans to human health as they are fermented to short chain fatty acids by microorganisms in the intestinal microbiota (Kolida, Tuohy, & Gibson, 2002; Marco et al., 2017). Fermented cereal grains using such substrates as oat, wheat, maize, or barley, contain prebiotics although processing with heat treatment, as with bread making, can alter the composition of these (Salmerón, 2017). Beer and wine have also been considered as prebiotic carriers for containing oligosaccharides and polyphenols, respectively (Dueñas et al., 2015; Kanyer, Bornhorst, Marco, & Bamforth, 2017). Homopolysaccharides and heteropolysaccharides produced by species of LAB have been evaluated for their potential as prebiotics yet need further investigation of in-vivo effects (Salazar, Gueimonde, de los Reyes-Gavilán, & Ruas-Madiedo, 2016).

Postbiotics were recently defined as a “preparation of inanimate microorganisms and/or their components that confers a health benefit on the host” (Salminen et al., 2021). Although further research is needed, FFs provide potential as sources of postbiotics as they typically contain mixed cultures of LAB producing health promoting metabolites of bioactive peptides and short chain fatty acids (Salminen et al., 2021).

1.2. Fermentation of fruits and vegetables

While fermented fruits and vegetables have been consumed worldwide for centuries, often as side dishes, a microbiological understanding of these foods emerged in the early 1900s (Linda J. Harris, 1998). Since then the industrialization of food processing has allowed this market to grow tremendously with some producers in the U.S. currently housing 40 million liters of pickles at one location (Fred Breidt, McFeeters, Perez-Diaz, & Lee, 2012).

1.2.1. Introduction to fruit and vegetable fermentation

Fermented fruits and vegetables are consumed ubiquitously across the globe with olives, pickles, and sauerkraut largely popular in Western countries although many cultures have unique fermented products with native fruits and vegetables (Hutkins, 2019; Ray & Bhunia, 2008; Jyoti Prakash Tamang et al., 2020). Fermentation of vegetables is typically initiated by dry-salting or brining vegetables followed by storage at a cool ambient temperature until noticeable changes in texture, aroma, and taste occur (Hutkins, 2019). Table olives are a salty and acidic fermented product quintessential to the Mediterranean diet, but also a staple to many households in the U.S. (Hutkins, 2019; Rocha, Borges, & Pinho, 2020). Olives must undergo processing before consumption, due to their bitterness caused by the presence of oleuropein; this can be achieved either through an alkaline treatment or salt brining (Rocha et al., 2020). During the process of fermentation in this salt brine (8-10% w/v NaCl), oleuropein diffuses from the fruit into the brine after which acid hydrolysis of the compound occurs (Charoenprasert & Mitchell, 2012). Olive fermentations without lye treatment are typically Greek-style or Sicilian (Hutkins, 2019). Lye treated olives are typically harvested earlier when the fruit is green or yellow and are treated with 0.5-3.5% NaOH for four to 12 h during which oleuropein is hydrolyzed (Hutkins, 2019; Perpetuini, Prete, Garcia-Gonzalez, Alam, & Corsetti, 2020). Typical fermentations last about 120 days, and the final product is dominated by *Lactoplantibacillus. plantarum, L. mesenteroides, L. brevis,* and *Lactococcus* spp. and some yeast species. Many fermentations occur spontaneously which allows for variation in the microbial populations and safety risks if the brine remains above or near a pH of 4.5 (Poiana & Romeo, 2006; Randazzo et al., 2017).

Pickling is a process of preserving fruit or vegetables in salt and/or acid, which is commonly associated with cucumbers but can be applied to other fruits or vegetables (Hutkins, 2019). Not all cucumbers sold as pickles are fermented however an estimated one-third of the cucumber pickles produced in the U.S. are fermented by lactic acid bacteria (Hutkins, 2019). Fresh cucumbers have LAB endogenously present at low levels, below 0.4% of the total bacterial community, and Gram-negative bacterial species are dominant which may result in quality defects or a slowed fermentation if improper conditions are not established (Zhongjing Lu, Pérez-Díaz, Hayes, & Breidt, 2020; Mundt, 1970). Cucumbers are typically fermented in a 5-8% w/v NaCl brine with a target terminal pH of 3.2 (Fred Breidt et al., 2012; S. D. Johanningsmeier, Franco, Perez-Diaz, & McFeeters, 2012). Pickle fermentation is usually dominated by *L. mesenteroides*, *L. plantarum*, *L. brevis* and *P. pentosaceus* which tolerate the low pH, high salt environment well; presence of yeast or non-lactic acid bacteria species can cause defects such as softening or internal cavities from CO_2 accumulation (Fred Breidt et al., 2012; Hutkins, 2019).

Cabbage is fermented under different names throughout many cultures: as sauerkraut in the U.S., Europe, and Australia, kimchi in Korea and East Asia, suan cai in China, and curtido in El Salvador (Fred Breidt et al., 2012; Louis-Jean et al., 2021; Jyoti Prakash Tamang et al., 2020). White cabbage is traditionally used in sauerkraut for its sweet, mild flavor and composition of fermentable sugars, glucose (2.5%) and fructose (4 to 5%) primarily with lower levels of sucrose (2%) (Fred Breidt et al., 2012; Hutkins, 2019). Sauerkraut will be discussed at length later in this text, however cabbage ferments generally are high in vitamin C, lightly acidic (final acidity 0.4–0.6%), and have a terminal pH near 4.0 (Fred Breidt et al., 2012; Pederson & Albury, 1969a; Jyoti Prakash Tamang et al., 2020).

The commonality of these fruit and vegetable fermentations is their reliance on LAB for the fermentation process. LAB are abundant in the environment, found on plants, as commensal organisms with animals, insects, and humans, and others are pathogenic organisms (W. H.

Holzapfel & Wood, 2014). In 2020, a taxonomic reorganization of LAB occurred with a reclassification of 300 species into one family, *Lactobacillaceae,* containing 31 genera along with 23 new genera encompassing the former classification of *Lactobacillus* species (J. Zheng et al., 2020). This reclassification accommodates for the increased awareness of LAB diversity of ecological and metabolic traits that emerged from the relatively recent surge in 16S rRNA and whole genome sequencing (J. Zheng et al., 2020). Presently, LAB refers to a group of Gram-positive, non-spore forming, and aerotolerant bacteria that produce lactic acid as a byproduct of a type of heterotrophic metabolism called fermentation (W. H. Holzapfel & Wood, 2014; Orla-Jensen, 1910; Qiao et al., 2022). LAB are highly valuable in food fermentations for their ability to grow in either aerobic or anaerobic conditions. The presence or lack of oxygen in addition to composition of carbohydrates in the environment for LAB dictates the metabolic pathways through which fermentation will proceed (M. G. Gänzle, 2015; Orla-Jensen, 1910). Homofermentative metabolism of hexoses proceeds through Emden-Meyerhoff pathway while homofermentation of pentoses occurs through the pentose phosphate pathway; heterofermentative metabolism of both hexoses and pentoses occurs through the phosphoketolase pathway (M. G. Gänzle, 2015). These metabolic pathways and end products of fermentation are detailed in Figure 1. In meat and dairy fermentations, only homofermentative are usually present. However, in fruit, vegetable and cereal fermentations, a mixture of homo and heterofermentative LAB are found (M. G. Gänzle, 2015; Pedersen et al., 2008). Homofermentative species such as *Lactococcus lactis* in food fermentations usually will metabolize glucose through lactate dehydrogenase into lactate either aerobically or anaerobically (M. G. Gänzle, 2015). However, in conditions of carbohydrate limitation or anaerobic growth, alternative metabolites produced by LAB may be ethanol, acetate, lactate, or acetoin (Bacteriol, Hols Philippe Goffin, Lorquet, &

Kleerebezem, 2004; M. G. Gänzle, 2015; Gaudu, Lamberet, Poncet, & Gruss, 2003). Heterofermentative species such as *Leuconostoc* spp. and *Oenococcus oeni* typically convert pentoses to lactate and hexoses to pyruvate with alternative end products to pyruvate (N. Wagner, Tran, Richter, Selzer, & Unden, 2005). Heterofermentative LAB are present in a variety of FFs, sourdough, wine, sauerkraut, and kimchi, attributed to their versatility in using alternative electron acceptors to oxygen and fructose (M. G. Gänzle, Vermeulen, & Vogel, 2007; Lin & Gänzle, 2014). Table 2 details common genera of LAB in food fermentations and their respective fermentation type (Kandler, 1983).

When in high acid environments of FFs, LAB can become limited in carbohydrate metabolism and shift to amino acid utilization (M. G. Gänzle, 2015). This metabolic change can produce desirable compounds such as γ-aminobutyrate (GABA), from decarboxylation of LAB in dairy, meat, and cereal fermentations (Li & Cao, 2010). However, decarboxylation of amino acids other than glutamate can allow biogenic amine accumulation, which will be discussed at length further in this text (Jin, Lee, Park, Lee, & Mah, 2019). While carbohydrate metabolism is essential to the success of LAB in food fermentations, especially of fruits and vegetables, additional physiological responses allow LAB to succeed in harsh environments that may add potential value or risk to food products.

1.2.2. By-products of LAB fermentation of fruits and vegetables that influence food safety

Very few foodborne outbreaks have been attributed to fermented fruits and vegetables. In one example, kimchi caused an outbreak of Enterotoxigenic *E. coli* in South Korea. This outbreak was caused by kimchi produced with poor sanitation and employee hygiene practices (Shin et al., 2016). Properly fermented fruits and vegetables manufactured in a manner that limits the risk of the production environment or food handlers introducing pathogens generally are considered low risk foods. The safety of fermented fruits and vegetables is achieved through the generation of multiple by-products produced by the microbes involved in fermentation.

1.2.2.A Organic Acid Production

All LAB produce lactic acid as a by-product of carbohydrate metabolism. The safety of fermented fruits and vegetables is largely attributed to production of organic acids by LAB, with the most notable being lactic acid. The production of these organic acids, in combination with production of bacteriocins and competitive exclusion, as LAB proliferate, contribute to the long history of safety associated with fermented fruits and vegetables. Organic acids (lactic acid, acetic acid, citric acid, etc.) are weak acids. Where a strong acid is one that completely dissociates into a proton (hydrogen ion) and a negative hydroxyl ion in water, a weak acid only partially dissociates in water. Therefore, a proportion of the weak acid will be in the ionized form and a proportion un-ionized form of the weak acid in water. The pH where the dissociated and undissociated forms of the weak acid are equal is called the pKa. The antimicrobial effect of the weak (organic) acids is achieved when the acid is in the un-ionized (undissociated) form. This allows for the movement of the weak acid across the lipid membrane of microbial cells. Once inside the cell, the weak acid dissociates and the ionized form alters the pH of the cytoplasm and effects other cellular functions. Generally, if a food is one pH unit less than the pKa of the weak acid, 90% of the acid is in the non-dissociated or un-ionized form.

LAB are either homofermentative, producing only lactic acid as the major product of glycolysis or heterofermentative producing lactic acid, carbon dioxide, and acetate or ethanol (Basso et al., 2014; Zúñiga, Pardo, & Ferrer, 1993). Homolactic fermenters include lactobacilli and species of *Lactococcus* while heterolactic fermenters include *Leuconostoc* and *Oenococcus*

(D. Wang et al., 2021). The acidification of the fermentation matrix challenges spoilage as well as pathogenic microorganisms while preventing unregulated growth of lactic acid bacteria. In many spontaneous vegetable and fruit fermentations the growth kinetics are largely correlated with organic acid production. Using sauerkraut as an example, within the first 72 h of fermentation, the pH drops below 4, viable counts of LAB peak, and the fermentation is stabilized allowing further degradation of plant tissue and production of secondary fermentation metabolites such as flavor and aroma compounds. (Plengvidhya, Breidt, Lu, & Fleming, 2007). This rapid acidification process results is important for inhibiting the growth of bacterial pathogens (Snyder, Fred Breidt, Andress, & Ingham, 2020).

Common vegetative pathogens present on fresh produce include *Escherichia coli, Listeria monocytogenes,* and *Salmonella* (Snyder et al., 2020). In vegetable products containing acetic acid, studied at a pH of 3.3, *E. coli* O157:H7 is generally the most acid tolerant with a required holding time of 5.75 days to reach a 5-log reduction at 10°C (Fred Breidt, Hayes, & Mcfeeters, 2007; Frederick Breidt & Caldwell, 2011). Under these same acidity and temperature parameters, *Listeria* and *Salmonella* require 11.2 and 51.3 h, respectively to reach a 5-log reduction (Fred Breidt et al., 2007). When exposed to 0.5% lactic acid (LA), 5-log reductions of *Salmonella* and *E. coli* are achieved within 1 h; *Listeria* within 2 h (C. Wang, Chang, Yang, & Cui, 2015). Many foodborne pathogens such as *L. monocytogenes* and *E. coli* are known to have acid tolerance responses (ATR), with an induced protection to highly acidic conditions (pH 3.0) after exposure to mild acidic conditions (pH 5.0) (Kroll & Patchett, 1992).

1.2.2.B Production of bacteriocins by LAB during the fermentation of fruits and vegetables

In addition to inhibiting non-acid tolerant organisms through production of organic acids, many LAB also produce antimicrobial compounds (Choi, Patra, Kim, & Kang, 2018). The production of bacteriocins by LAB allows for a competitive advantage in the fermentation process by the inhibition of the growth and survival of other bacteria through distinct cellular mechanisms. Bacteriocins are a type of antimicrobial peptide (AMPs) produced by bacteria, typically ranging from 30 to 60 amino acids in length (Mokoena, 2017). Bacteriocins are ribosomally synthesized and may be post-translationally modified with enzymes before being transported outside the cell membrane. Bacteriocins may be either bacteriostatic or bactericidal with broad inhibitory activity or targeted against specific bacteria typically of a different genus (Agriopoulou, Stamatelopoulou, Sachadyn-Król, & Varzakas, 2020). Generally, bacteriocins are grouped into three classes: lantibiotics, small heat stable non-lantibiotics, and large heat labile proteins (Nes et al., 1996). The majority of LAB synthesized bacteriocins are in classes I and II. A well-studied class I lantibiotic is nisin A, which is produced by *L. lactis* and has broad antibacterial activity against such species as *L. monocytogenes*, *Staphylococcus aureus*, and *Clostridium botulinum* (Badaoui Najjar, Chikindas, & Montville, 2009; Linda J Harris, Fleming, & Klaenhammer, 1992). Bacteriocins are frequently used as a food preservative due to their stability during thermal processing and across a wide pH range (Darbandi et al., 2022). The peptides are inactivated by human proteolytic enzymes in-vivo which prevents action against the native gut microbiota (Perez, Zendo, & Sonomoto, 2014). Bacteriocins produced by various LAB can be found widely in fermented products (Table 3). which demonstrates potential for strategic selection of species that produce these peptides for starter cultures. Whereas the use of other types of preservatives or processing methods can compromise taste and flavor, these bacteriocin producing starter cultures can contribute to the fermentation process and contribute to the control of growth of spoilage and pathogenic microbes beyond that achieved simply by organic acid production and carbohydrate depletion within the system.

LAB-produced bacteriocins were once thought to be effective only against Gram positive bacteria. However, the diversity of bacteriocins that may be produced during fermentation with a heterogeneous population of LAB have been shown to be broadly effective against a range of Gram-positive and Gram-negative bacteria (Soltani et al., 2021). This diversity of bacteriocins and spectrum of efficacy can vary widely among spontaneous FFs produced from similar or the same ingredients, based on the microbes involved. For example, a crude extract of total bacteriocins was obtained from 29 individual fermented cucumber samples and while 15 showed bactericidal impacts on both Gram negative and Gram positive bacteria, the remainder were effective against one or the other (Yi, Qi, Hong, Deng, & Zeng, 2020).

While LAB are well-established producers of bacteriocins, not all species produce these AMPs. LAB strains isolated from fermented cucumber pickles and dry-cured meat were sequenced to identify the presence of bacteriocin-encoding genes. Of the 219 LAB strains sequenced, 38 strains (17%) were found to harbor known bacteriocin encoding genes (Yi et al., 2020). Beyond the limitation of not having bacteriocin-producing isolates within the LAB fermentative community in a product, bacteriocin production is also influenced by the fermentation environment. The addition of salt to FFs is an important control process that selectively promotes the growth of LAB and maintains a pleasant texture of vegetable and fruit pieces by satisfying osmotic balance between the brine and fermented substrates. However, this presence of salt can also modify the rate of bacteriocin production, and some work has shown that concentrations above 4% w/v halts bacteriocin synthesis, while lower (1-2% w/v) concentrations increase production (Kumar, Sheoran, Gupta, Yadav, & Tiwari, 2016; Leroy & De Vuyst, 1999).

Vegetative organisms known to have ATR also have been shown to confer resistance to bacteriocins (Guan & Liu, 2020; Yap, MatRahim, AbuBakar, & Lee, 2021). A study of ATR *L. monocytogenes* found that wild type cells with an induced ATR also demonstrated resistance to nisin (Badaoui Najjar et al., 2009). These findings suggest that bacteriocin-producing organisms may be strategically used for targeting foodborne pathogens while also considering their acid adaptive mechanisms. Moreover, there is consistent evidence of bacteriocins found in FFs such as pickles, sauerkraut, and kimchi (Table 3). Yet these peptides are often isolated and studied for their applications in biopreservation and improved product stability. A better understanding of how these AMPs contribute to fermented vegetable and fruit safety would allow for improved use of strains in starter cultures to minimize the risk of pathogenic and spoilage growth.

1.2.2.C. Production of biogenic amines by LAB during fruit and vegetable fermentation

Although there are several metabolic processes associated with positive attributes of fermented fruits and vegetables, LAB also produce compounds that can be hazardous when consumed in high concentrations. Biogenic amines are a by-product of amino acid metabolism to maintain intracellular pH through decarboxylation reactions (Ordonez, Ibanez, Torre, & Barcina, 1997). Common biogenic amines are histamine and tryptamine which in low quantities are digestible and show no adverse effect on health, but at a high dose can result in headaches, nausea, or rashes (Yu, Yu, & Xu, 2021). Biogenic amines have been detected in fermented fruits and vegetables (Paramithiotis, Das, Shin, & Patra, 2022). Fermented cabbage products produced in China contained putrescine, cadaverine, tryptamine, histamine, and β-Phenethylamine (Yu et al., 2021). There is no regulation pertaining to the level of biogenic amines in fermented fruit and vegetable products. The US FDA does have a histamine limit for seafood which is 35 ppm, however the survey of biogenic amine levels in fermented cabbage referenced above had histamine levels of 45.44 ppm (Food and Drug Administration, 2021; Yu et al., 2021).

Alteration of ingredients in FFs can have an impact on the production of biogenic amines. In kimchi for example, the inclusion of fish sauce or other seafood ingredients has been shown to increase the formation of biogenic amines in the product. *Levilactobacillus brevis* has been found responsible for production of tyramine in kimchi and inclusion of seafood ingredients was responsible for histamine and cadaverine production (Jin et al., 2019). In kimchi fermentations with varying amounts of fish sauce, higher amounts of biogenic amines were associated with higher proportions of fish sauce in the formulation (Kim, Dang, & Ha, 2022).

1.2.3 Summary

There is long history of safe production of fermented fruits and vegetables. The production of organic acids (antimicrobial agents), pH reduction, reduction in carbohydrate sources, and bacteriocin production during the fermentation process all contribute to enhancing the safety and shelf life of these products. Safe production is also influenced by minimizing introduction of foodborne pathogens through the implementation of Good Manufacturing Practices (GMPs) before and during fermentation. High-quality ingredients that are free from obvious signs of spoilage and that have been produced in a manner to minimize risk of pathogen cross contamination should be used in all fermentations. Cleaning and sanitizing of equipment and utensils, as well as employee hygiene practices, are also critical to minimize cross contamination.

1.3. Production of sauerkraut

1.3.1. Overview of commercial production practices

The Food Safety and Modernization Act (FSMA) Preventive Controls for Human Food Rule, requires facilities commercially producing FFs to conduct a hazard analysis, establish preventive controls, and implement a food safety plan to mitigate food safety risks (Snyder et al., 2020). Retail food establishments, which are likely to be subject to the FDA Food Code, are required to develop a Hazard Analysis and Critical Control Points (HACCP) plan and will likely have to establish a variance for production of FFs (Ceylan et al., 2021; Snyder et al., 2020). The process of conducting a hazard analysis, developing a validated process, and implementing a food safety plan can be more cumbersome for small food service facilities without access to adequate training and resources on this process. For the production of fermented fruits and vegetables, guidance for small-scale processors includes the rapid and sustained reduction in pH as a critical control point (Snyder et al., 2020). Other critical factors include the formulation of the product with a specific focus on the salt concentration, monitoring and maintaining appropriate temperature during fermentation, and implementing cleaning and sanitation procedures. The monitoring of the critical control points along with critical factors also influence the final quality of the fermented product. Moreover, as many fermented fruit and vegetable products are produced by “spontaneous fermentation”, meaning they are conducted without the use of starter cultures, the final product can be significantly influenced by the quality of the starting material, the production environment, and the salt concentration used.

Some commercial sauerkraut producers may use starter cultures to account for the inherent variability in fermentation to help ensure that final products are reproducible in taste, texture, and appearance. Considerations for the selection of appropriate strains for use as a starter culture include: tolerance for a high acid environment, ability to metabolize the carbohydrates found in cabbage, the fermentation pathway, rate of growth and lactic acid production, and production of secondary compounds such as bacteriocins (W. Holzapfel, Schillinger, & Buckenhüskes, 2003). Starter cultures of *L. mesenteroides*, *L. plantarum*, or *L. lactis* have been found to successfully initiate the sauerkraut fermentation process and in some cases allow for a reduction in the sodium concentration used (Linda J. Harris, Fleming, & Klaenhammer, 1992; S. Johanningsmeier, McFeeters, Fleming, & Thompson, 2007; Pederson & Albury, 1969a). "Back slopping" or adding brine from a completed fermentation to a fresh batch, is also used as a low cost alternative to the use of starter cultures (Wiander & Ryhänen, 2005). This process produces more variable results, as the LAB present in the back-slopped brine are not typically those involved in the initial stages of fermentation.

Sauerkraut is sold packaged, pasteurized and shelf stable or packaged and refrigerated (Pederson & Albury, 1969a; Peñas, Martinez-Villaluenga, & Frias, 2017). A generalized schematic for sauerkraut production is detailed in Figure 4. Sauerkraut production begins with selection of quality cabbage heads, devoid of spoiled leaves or pest damage, after which washing of the head and removal of outer leaves occurs (W. Holzapfel et al., 2003) The inner core should be removed as this primarily contains sucrose which can be metabolized to dextran by the organism *L. mesenteroides*, creating a slimy texture (Fred Breidt et al., 2012). Cabbage is shredded to 0.7 to 2 mm wide strips, which helps release sugars from the cabbage cells (Ray & Bhunia, 2008). Cabbage is then salted in a range of 0.7 to 3% NaCl using canning salt, free of any preservatives or anti-caking agents, to inhibit growth of spoilage organisms and prevent pectinolytic enzyme activity from softening the cabbage (Center for Food Safety and Applied Nutrition Office of Regulatory Affairs, 2005; W. Holzapfel et al., 2003; Pederson & Albury, 1969a). Once the salt and cabbage has been thoroughly mixed, initiating release of juices from the vegetable substrate, the homogenate is packed tightly into fermentation vessels with the self-made brine covering the solids to prevent aeration (Fred Breidt et al., 2012; W. Holzapfel et al., 2003; Pederson & Albury, 1969a). From this point the cabbage can be fermented from 1 week to several months, as long as anaerobic conditions are maintained (W. Holzapfel et al., 2003).

1.3.2. Microbiology of sauerkraut

When sauerkraut is spontaneously fermented, the fermentation process initiates immediately after the cabbage mixture is packed into vessels. The endogenous organisms on cabbage prior to fermentation are primarily aerobic spoilage bacteria, including *Enterobacter* and *Pseudomonas*, at counts of approximately 10^4-10^6 CFU/g (Fred Breidt et al., 2012; Peñas, Frias, Sidro, & Vidal-Valverde, 2010; Peñas et al., 2017). Also present are native species of yeasts and molds, at counts of~ 10^1-10^4 CFU/g (Fred & Peterson, 1921; Satora, Skotniczny, Strnad, & Ženišová, 2020). The endogenous LAB population on the raw cabbage is lower, around 10^2-10^3 CFU/g (Fred Breidt et al., 2012; Peñas, Frias, Sidro, et al., 2010). The inclusion of NaCl and exclusion of oxygen in fermentation vessels favors the LAB population over aerobic, non-salt tolerant organisms. The heterofermentative organism *L. mesenteroides* initiates the fermentation, due to its fast growth rate, ability to grow well in microaerophilic conditions, and low acid tolerance (S. Johanningsmeier et al., 2007; Z. Lu, Breidt, Plengvidhya, & Fleming, 2003; Peñas et al., 2017). This first stage of the fermentation allows for lactic and acetic acid to accumulate, lowering the pH below 4.6, and CO_2 is produced contributing to the creation of an anaerobic environment. Once the pH has dropped, more acid tolerant species, including homofermentative *L. plantarum* and *L. brevis*, takeover the fermentation producing almost exclusively lactic acid leading to a further decrease in the pH to 3.4 to 3.7 (Peñas et al., 2017; Plengvidhya et al., 2007). By the end of this fermentation process, there is approximately 1% acetic and 2% lactic acid in the sauerkraut (Fred Breidt et al., 2012; Pederson & Albury, 1969a).

1.3.3. Sensory profile of sauerkraut

Traditionally sauerkraut has been produced with white cabbage, thus extensive chemical profiling of varietals is available. Between different varieties, growth regions, and time of harvest there is variation in the cabbage chemical composition and therefore differences in the resulting sauerkraut (Adelanwa & Medugu, 2015). Sugar content, for example, varies within varieties of white cabbage with Ambrosia and Avak producing kraut with greater acidity and Cabton, Jaguar, and Kamienna Glowa having greater residual sugars (Table 1.4) (Satora, Skotniczny, Strnad, & Piechowicz, 2021). Total carbohydrate levels in red cabbage (*Brassica oleracea* var. *capitata* f. *alba*), may be higher than white, with comparable levels of glucose, fructose, and sucrose although further research is needed on the application of red cabbage in sauerkraut (Table 1.4) (Adelanwa & Medugu, 2015; Volden et al., 2008). Understanding the difference in chemical composition among different varieties allows for strategic selection to produce a desirable sauerkraut given such considerations as fermentation temperature, duration, and sodium concentration.

Sauerkraut is known for a crunchy, effervescent, and sour sensory experience (Satora et al., 2021). Direct acidification of shredded cabbage with acid will not produce or replicate the unique flavor and aroma of sauerkraut (Lonergan & Lindsay, 1979). Individual consumer preference can influence the desired sensory profile of sauerkraut as manipulations of the cabbage variety, salt concentration, temperature during fermentation, and duration of fermentation can change the sensory characteristics of the final product. Conversion of alcohols and acids into esters are important to the development of characteristic sauerkraut flavors. Off-flavor development in sauerkraut may range from cheese like flavors to putrid (Hang & Woodams, 1993; Pederson & Albury, 1969b). The cheesy off flavor can development as a result of n- butyric, n-valeric, and n-caproic acids production from high levels of yeast growth on the surface of the ferment (Vorbeck, Mattick, Lee, & Pederson, 1961).

The sulfur aroma associated with cabbage products is attributed to glucosinolates (Higdon, Drake, Delage, & Traka, 2017). This sulfur aroma is often characteristic of sauerkraut and has been found to increase as salt concentration increases (S. Johanningsmeier et al., 2007). Sulfur aromas are more frequently associated with shelf-stable canned sauerkraut (thermally processed) compared to untreated refrigerated sauerkraut(S. Johanningsmeier et al., 2007).

1.3.4. Nutritional and health benefits associated with sauerkraut consumption

Sauerkraut is known to contain high levels of glucosinolates coming from the cabbage, which contains high levels of this antioxidant along with other cruciferous vegetables such as broccoli, kohlrabi, and Brussels sprouts. The most studied glucosinolate from cruciferous vegetables is glucobrassicin (GB) (A. E. Wagner & Rimbach, 2009). Due to tissue disruption, GB is converted by myrosinases to ascorbigen (ABG) and this conversion is promoted under acidic conditions (Higdon et al., 2017; McFeeters, 2004; A. E. Wagner & Rimbach, 2009). Thus, the fermentation of cabbage into sauerkraut promotes the generation of ABG. ABG has been shown in animal studies to have an anticarcinogenic effect (Ciska & Pathak, 2004; A. E. Wagner & Rimbach, 2009). Beyond ABG, cabbage also contains high in vitamins A and C, as well as being a good source of dietary fiber. The FDA has specific requirement for health claims that can be made for foods, and these health claims must have scientific evidence supporting consumption the food and the association with a specific health outcome. Recently, the FDA published several health claims that “meet the significant scientific agreement standard” and can be used for labeling foods with defined health benefits (CFSAN, 2009). Under the FDA for health claims for fruits and vegetables “Low fat diets rich in fruits and vegetables (foods that are low in fat may contain dietary fiber, vitamin A, and vitamin C) may reduce the risk of some types of cancer, a disease associated with many factors. Cabbage is high in vitamins A and C, and it is a good source of dietary fiber” (21 C.F.R. Part 101). Levels of both ABG and vitamin C can decrease during storage time and may be reduced by pasteurization.

The FDA has also established nutrient content claims related to sodium labeling. A food can be labeled as “no sodium” if it contains less than 5 mg/serving, “very low sodium” if it contains less than 35 mg/serving, and “low sodium” if it contains less than 140 mg/serving (21 C.F.R. Part 110, n.d.). Sauerkraut cannot exceed 3% salt concentration by weight per the FDA definition. If we assume a serving size of 100 g, a portion of 3% salt sauerkraut would contain 3000 mg (3 g) sodium. There has been some work to reduce the level of NaCl added to sauerkraut by substituting with KCl (Jones, 1986; Nayara Ribeiro, Sergio Monteiro, De Paiva Cun Ha, De Oliveira, & Cristina Silva, 2020; Wiander & Ryhänen, 2005). The ionic potential of KCl is lower compared to NaCl, which generally allows for a more diverse microbial population to grow during fermentation (Jones, 1986). Moreover, using KCl alone led to a decrease in consumer acceptance due to “metallic” flavor imparted to the sauerkraut (Jones, 1986). However, by substituting a portion of the total salt content with KCl (approximately 20% of the total salt content) fermentation dynamics were not negatively impacted and sensory attributes were less affected (Nayara Ribeiro et al., 2020; Wiander & Ryhänen, 2005). With a growing interest in reduced sodium fermentations, there also is interest in mineral supplementation to increase the functionality of sauerkraut. One study found that the addition of selenium in sauerkraut fermentation can selectively promote growth of LAB, allowing for reductions in NaCl, enhancement of GB degradation into ascorbigen, and can have potential anti-inflammatory effects through downregulation of proinflammatory gene expression (Peñas et al., 2012).

1.4. Consumer interest in fermented foods

1.4.1. Preferences and purchasing practices

With the globalization of food products, FFs from different continents have become more accessible and increasingly industrialized (Jyoti Prakash Tamang et al., 2020). Consumers within the U.S. are reportedly interested in incorporating FFs in their diet to add unique flavors and incorporate more dishes from other cultures (Everett, 2019; Prince, 2018). Sales of FFs, such as kombucha, sauerkraut, and kimchi reportedly increased since early 2020, coinciding with the onset the global COVID-19 pandemic (Domonoske & Schneider, 2020; Manskar & Raskin, 2020). News coverage at the time headlined FFs as health promoting functional foods based on research of a potential correlation between sauerkraut consumption and decreased COVID-19 severity (Bousquet et al., 2021; Fonseca et al., 2020).

In westernized countries like the U.S. this interest in FFs has followed a period of declining consumption, attributed in part to an interest in more sustainable, artisan, and health promoting foods (El Bilali, 2019; Flachs & Orkin, 2021). However in less urban areas of the world with reduced access to ready to eat foods, FFs are a way of preserving crops and consuming nutritive food while also a central part of the social food network (Jyoti Prakash Tamang et al., 2020). Through a qualitative interview of 11 individuals, reporting fermenting their own food at home, core themes emerged for why the ferment: enjoying the creative process, feeling a connection to their food, and managing a more balanced microbiota (Flachs & Orkin, 2021). A study investigating purchasing intent of labneh (strained yogurt) found that fat levels and sensory properties were equally important considerations, supporting that consumers are seeking FFs in part, for their taste (Haddad et al., 2007). Further investigation is needed into consumer preferences to better understand motivation for producing or consuming FFs, and for where knowledge may be missing about the appeal.

1.4.2. Consumer awareness of the nutritional and health benefits of fermented foods

Overwhelmingly as consumers are interested in functional foods, they turn to FFs for their numerous health benefits (Unilever, 2022). Of all FFs, the most research has been conducted on health benefits of consuming fermented dairy products. Consistent consumption of yogurt has been associated with weight maintenance based on human trials (Mozaffarian, Hao, Rimm, Willett, & Hu, 2011). Consumption of whole fat yogurt has also been associated with decreased risk for type 2 diabetes in some populations (Ibsen et al., 2017). Assessing the benefits of other FFs is more complex; with increased consumption of dietary fiber from fruits, vegetables, and grains already supporting the recommended dietary shift for improved gastrointestinal function, insulin sensitivity, and immune function (Anderson et al., 2009). Fruit and vegetable consumption has also been associated with affective benefits, specifically reduced depressive symptoms and improved mood as compared to cooked or canned produce (Brookie, Best, & Conner, 2018). Fermentation of fruits and vegetables can increase the potential health benefits through increased nutrient density as naturally occurring sugars are metabolized by LAB or through modification of phenolic compounds (Septembre-Malaterre, Remize, & Poucheret, 2018).

While it is evident that consumer demand for FFs has increased recently, there is still a lack of understanding about what quantity of these products should be included in a balanced diet and what level of live microorganisms is needed to confer benefits. A study aiming to create a classification system for microbes consumed in the U.S. diet found that consumption of foods with live microorganisms (>10^7 CFU/g) had increased over an 18 year period from 2001 to 2018 (Marco et al., 2022). The final product can vary in level of live microorganisms especially throughout the shelf life, especially for small scale produced FFs, but most FFs are estimated to contain 10^5-10^7 CFU/g LAB (Rezac, Kok, Heermann, & Hutkins, 2018). Fermented dairy products tend to contain higher levels of LAB, up to 10^9 CFU/g (Rezac et al., 2018). In the case of probiotics the format of delivery, mechanism of action, and processing steps all affect the final concentration of live microorganism available to the consumer (Sanders et al., 2014). The ability for microorganisms to confer health benefits can also vary with individual host factors of diet, genetics, age, presence of chronic disease, and geographic location (Lozupone, Stombaugh, Gordon, Jansson, & Knight, 2012). Further research is needed to understand both the live microbial loads in FFs and the impact consumption has on individuals with diverse diet, age, race, and health (Marco, 2021).

While few studies have explored consumer perceptions of FFs, a survey of North-eastern Slovenians found participants were most aware of benefits to intestinal health (Šikić-Pogačar, Turk, & Fijan, 2022). Most were familiar of benefits to digestion, the immune system, and gut microbiota while affective benefits, such as reduction of depressive symptoms, were least recognized (Šikić-Pogačar et al., 2022). Results of this study also found participants with a chronic disease were more likely to prepare FFs at home (Šikić-Pogačar et al., 2022). A similar study on fermented plant beverages in Thailand found participants expect consumption to alleviate or improve symptoms of exhaustion, musculoskeletal pain, constipation and insomnia (Chaiyasut, Sivamaruthi, Makhamrueang, Peerajan, & Kesika, 2018).

1.4.3. Consumer food preservation practices

Home food processing and preservation practices are prevalent in U.S. households. Online communities as well as the number of in-person classes being taught around the country evidence the high level of consumer interest and practice. The COVID-19 pandemic has also increased consumer interest in home preservation. Shortages of canning supplies, notably jars and lids, were reported early in the pandemic (Gray, 2020; Rysdall, 2020). In home fermentation has also gained traction during the COVID-19 pandemic, a notable example being the popularity of bread making (Holmes, 2020).

Limited data exists on the motivations that drive individuals to preserve foods in the home. One study which utilized a consumer survey and interviews found that the primary source of food preserved in the home was from home gardens with the primary motivation for preserving being “to know and control what’s in my food” and “it tastes better” (Click & Ridberg, 2010). Among the motivation for “to know and control what’s in my food” common themes emerged such as controlling sensory attributes, avoiding additives or preservatives, and to avoid unsafe food (Click & Ridberg, 2010). Participants generally expressed a distrust in the global agri-food industry and a motivation to store locally and sustainably produced foods (Click & Ridberg, 2010).

There is no shortage of information available online related to production methods (recipes) and health benefits for FFs. The authors of most of these materials are not trained food safety specialists or microbiologists and do not provide any data to support the nutritional and bioactivity claims of the final product. A recent study evaluating the knowledge related to the safety and nutritional indicated a lack of knowledge of the health benefits associated with FFs, the distinction between FFs and probiotics, and the biological safety hazards that can be associated with FF products among participants in a FF educational webinar (Ramos et al., 2022).

1.4.4. Summary

Despite the presence of FFs in cultures across the world for centuries, there has been a recent increase in the US in FFs, as more households and local producers in the U.S. are beginning fermentation practices. However, this comes without centralized guidance and resources for the small-scale production of FFs and vegetables, along with an understanding of the variability that may be introduced by preparation practices (Ramos et al., 2022). As the FFs industry is anticipated to continue growing in the US, it is important to understand the preferences of consumers for various types of FFs and the drivers for what motivates them to consume them.

1.5. Tables and figures

Table 1.1. FFs from across the world with their substrate and isolated microorganisms.

Fermented Food	Substrate	Country/ Region	Dominant Organisms	Source
Chorizo	Pork	Spain	*L. sake, L. curvatus, L. plantarum*	(García Fontán, Lorenzo, Parada, Franco, & Carballo, 2007)
Gari	Cassava	West and Central Africa	*Leuconostoc* spp., *Streptococcus* spp. *Corynebacterium mannihot*	(Anukam & Reid, 2009)
Gulbi	Shell-fish	Korea	*Bacillus licheniformis*, *Staphylococcus* sp., *Aspergillus* sp., *Candida* sp.	(Jyoti P. Tamang, Watanabe, & Holzapfel, 2016)
Gundruk	Leafy vegetables	India, Nepal, Bhutan	*L. fermentum, L. plantarum, L. casei, P. pentosaceus*	(Jyoti P. Tamang et al., 2005)
Kefir	Milk (goat, sheep, cow)	Russia	*Lactiplantibacillus, Acetobacter, Streptococcus, Saccharomyces*	(Bourrie, Willing, & Cotter, 2016)
Mesu	Bamboo shoots	India, Nepal, Bhutan	*L. plantarum, L. brevis, L. curvatus, L. citreum, P. pentosaceus*	(B. Tamang et al., 2008)

Pao cai	Cabbage	China	*L. pentosus, L. plantarum, L. brevis, L. lactis, L. fermentum, L. mesenteroides, P. pentosaceus*	(Yan, Xue, Tan, Zhang, & Chang, 2008)
Pulque	Agave sap	Mexico	*Lactiplantibacillus* sp., *Z. mobilis*	(Escalante et al., 2004; Ojeda-Linares et al., 2021)
Puto	Rice	Philippines	*L. mesenteroides, E. faecalis, P. pentosaceaus*, yeasts	(Jyoti P. Tamang, Watanabe, et al., 2016)
Sauerkraut	Cabbage	Europe, USA, Canada, Australia	*L. mesenteroides, P. pentosacaeus, L. brevis, L. plantarum, L sakei*	(S. Johanningsmeier et al., 2007)
Sayur asin	Mustard leaves, cabbage, coconut	Indonesia	*L. mesenteroides, L. plantarum, L. brevis, L confuses, P. pentosaceus*	(Puspito & Fleet, 1985)
Sua chua	Dried skim milk	Vietnam	*L. bulgaricus, S. thermophilus*	(Jyoti P. Tamang, Watanabe, et al., 2016)
Surströmming	Herring	Sweden	*Haloanaerobium praevalens*	(Kobayashi, Kimura, & Fujii, 2000)
Tarhana	Milk (sheep), wheat	Cyprus, Greece, Turkey	*L. bulgaricus, S. thermophilus*, yeasts	(Sengun, Nielsen, Karapinar, & Jakobsen, 2009)

Tejuino	Maize	Mexico	*S. cerevisiae, Pichia* sp., *Lactiplantibacillus* sp.	(Ojeda-Linares et al., 2021; Rubio-Castillo et al., 2021)
Thua Nao	Soybean	Thailand	*B. subtilis*	(Inatsu et al., 2006)

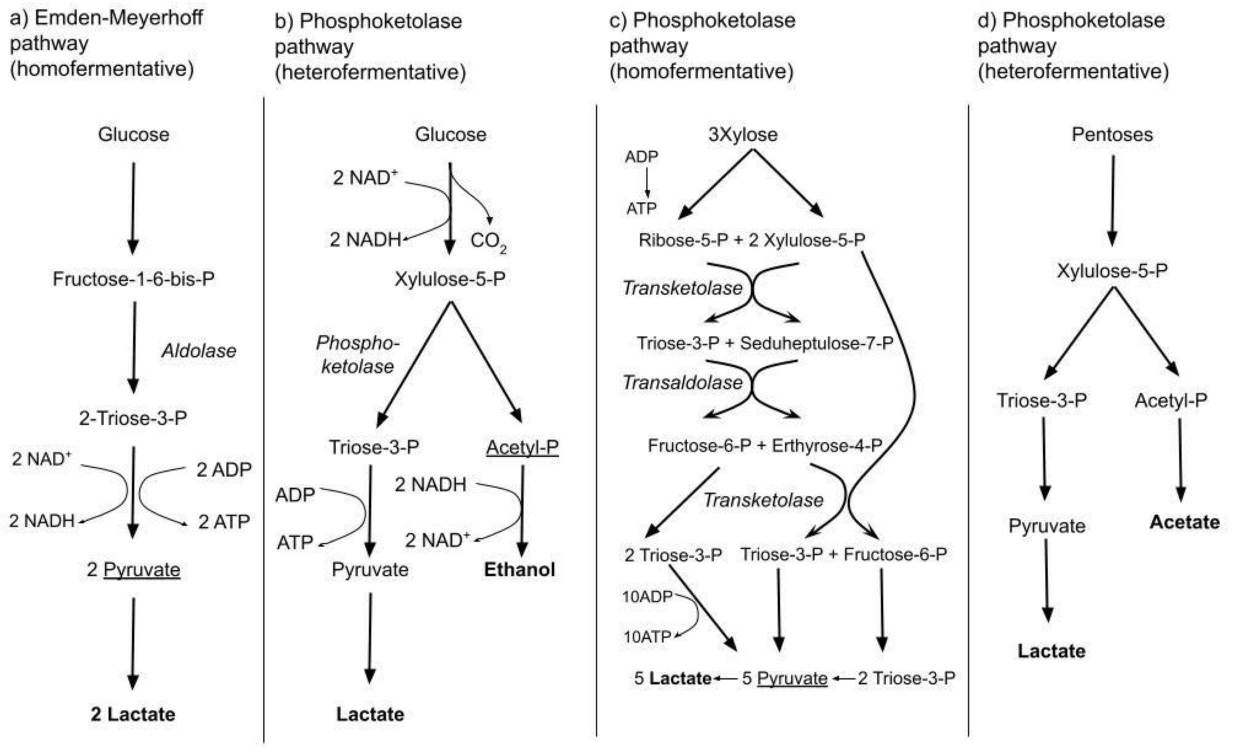

Figure 1.1. **Metabolic pathways for carbohydrate metabolism of lactic acid bacteria.** Terms in bold are the major end products of metabolism for their respective pathway. a) Homofermentative metabolism of hexoses in the Emden Meyerhoff pathway. b) Heterofermentative metabolism of hexoses in the phosphoketolase pathway. c) Homofermentative metabolism of pentoses in the pentose phosphate pathway. d) Heterofermentative metabolism of pentoses through the phosphoketolase pathway. Adapted from (M. G. Gänzle, 2015).

Table 1.2. Selected genera of LAB common in food fermentations and their fermentation type and products. Adapted from (Kandler, 1983).

Genus	**Fermentation type**	**Main product (molar ratio)**
Streptococcus	Homofermentative	Lactate
Pediococcus	Homofermentative	Lactate
Lactobacillus	Homofermentative	Lactate
Leuconostoc	Heterofermentative	Lactate:acetate:CO_2 (1:1:1)

Table 1.3. Isolated and characterized bacteriocins from FF products. The associated producing organism is listed at the species level and presumed class is identified.

Product	**Bacteriocin**	**Producing Organism**	**Class**	**Reference**
Chinese pickle	Pentocin DZ1	*Lactiplantibacillus pentosus*	Class II C	(Yi et al., 2020)
Chinese pickle	Pentocin DZ2	*Lactiplantibacillus pentosus*	Class II A	(Yi et al., 2020)
Chinese Sauerkraut	Paracin 1.7	*Lacticaseibacillus paracasei*	Class II D	(Ge, Sun, Xin, Wang, & Ping, 2016)
Japanese Pickles	Leucocyclicin Q	*Leuconostoc mesenteroides*	Class II C	(Masuda et al., 2011)
Kimchi	kimchicin GJ7	*Leuconostoc citreum*	Class I	(Chang, Lee, & Chang, 2007)
Sauerkraut	Nisin	*Lactoccus lactis* subsp. *lactis*	Class I	(Harris et al. 1992)

Table 1.4. Levels of glucose and fructose in cabbage varieties. Adapted from (Satora et al., 2021; Volden et al., 2008).

Variety of cabbage	**Glucose (g/kg)**	**Fructose (g/kg)**
Ambrosia	25.7	15.3
Avak	20.8	19.4
Cabton	20.8	20.0
Galaxy	14.3	9.6
Jaguar	16.2	12.3
Kamienna Glowa	18.6	11.8
Manama	25.8	21.7
Ramco	11.1	8.6
Capitata	15.8	13.2

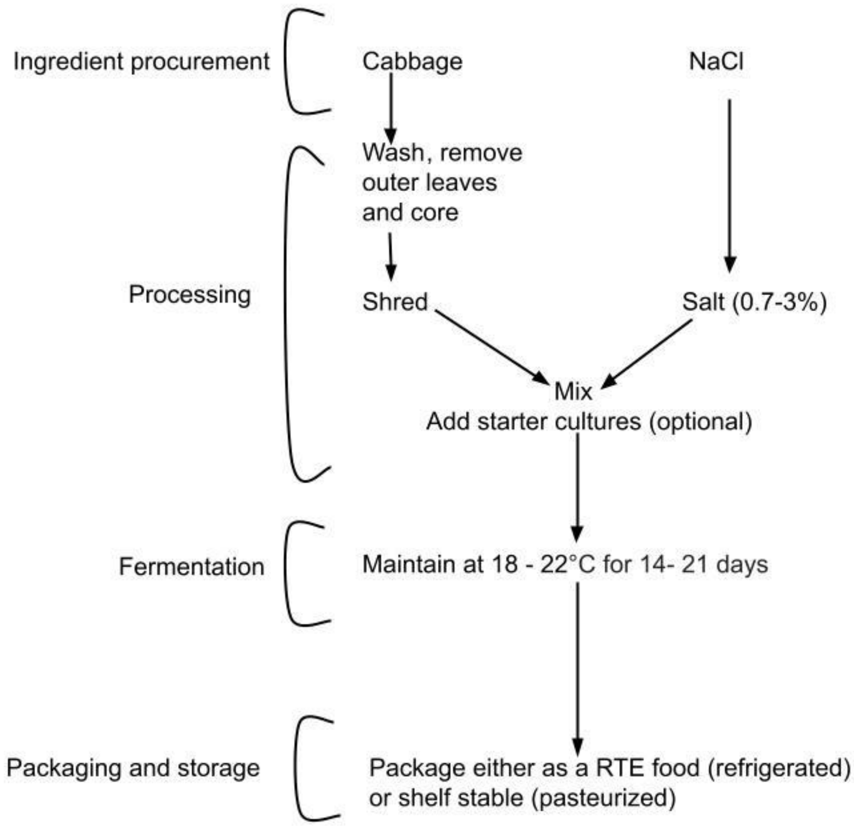

Figure 1.2. Production flow diagram for sauerkraut. Adapted from (Peñas et al., 2017).

Chapter 2: Consumer understanding of and motivations for consumption of fermented foods

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2.1. Abstract

Fermented foods (FFs) are a popular food group with consumers, however limited studies exist evaluating the motivations for consuming FFs and the frequency of consumption. In this work, an online survey was developed to assess participant familiarity with different types of FFs, determine consumption frequency, and gain insight into the motivation for consumption. A total of 751 participants completed the survey between March and September of 2022. Only 7% of participants correctly identified all FFs, with yogurt as the most frequently (87.62%) identified FF by participants. These findings suggest that participants may not be aware of the diversity of FFs or of all the different types of FFs they consume on a regular basis. Participants reported consuming fermented cereal grains (46.17% of respondents), fruits and vegetables (42.26%), dairy products (39.70%), soy/rice products (37.60%) and fermented meats (30.68%). The daily frequency of consumption was highest for categories of fermented cereal and dairy products, compared to the other categories which typically were consumed on a weekly or monthly basis.

The primary motivator for consumption was taste (50.68%) compared to health benefits (35.34%) and cultural reasons (12.03%). The most highly selected health benefits associated with FF consumption were “improved gut microbiome” (77.14%), “digestive benefits” (76.39%), and “probiotic” (68.87%). Participants associated health benefits with yogurt consumption including improved gut microbiome, digestive benefits, and probiotics with all FFs in the survey. Therefore, consumers may assume that all FF products confer the same health benefits. The motivations for consumption (sensory attributes, health benefits, cultural reasons) did not vary when participants were asked to respond for FFs as a broad category versus specifically for fermented fruits and vegetables. This suggests that consumers view FFs similarly regardless of the starting ingredients and fermentative process involved. Findings of this study, in the U.S., indicate a need for more education on individual types of FFs and the health benefits that may be associated specific FF consumption.

2.2. Introduction

Fermentation is a well-established practice of enhancing flavor, increasing safety, and preserving food reliant upon growth of desired microbial species and communities. While fermentation has been a long-standing tradition across the globe, there has also been a resurgence of these foods within the last decade topping the “What’s Trending in Nutrition” survey curated by Pollock Communications and Today’s Dietitian for the fifth year in a row in 2021 (The Fermentation Association, 2022). In the 2022-2027 period, the fermented food (FF) and beverages market is expected to grow at a CAGR of 6.35% (Mordor Intelligence, 2022). This increased interest and demand for FFs has become notable since the beginning of the COVID 19 pandemic in 2020 but continued to surge in popularity with consumers seeking the complex global flavor profiles of FFs (Thiel, 2022). During the pandemic, consumer preference increased for immunity boosting foods and this, in combination with broader implementation of plant-based food diets, has directed more people towards eating FFs (Mordor Intelligence, 2022).

The fermentation of dairy products relies on species of *Lactobacillus, Lactococcus,* and *Streptococcus thermophilus*, typically added as a starter or adjunct culture, which are important for lactose metabolism in addition to flavor development through production of volatile flavor compounds such as acetic acid, acetaldehyde, and diacetyl (Johnson & Steele, 2012). The production of many fermented fruits and vegetables such as sauerkraut or kimchi occurs through spontaneous fermentation, through the growth of endogenous microorganisms present on the raw food. Fruits and vegetables are mostly fermented by species of lactic acid bacteria, specifically *Leuconostoc mesenteroides*, *Weissella,* and *Lactiplantibacillus plantarum* with addition of a salt brine (Fred Breidt et al., 2012). These organisms, collectively classified as lactic acid bacteria (LAB) produce lactic acid, carbon dioxide, acetic acid, or ethanol as products of glycolysis. In addition, LAB produce volatile compounds that contribute to the taste of many FFs and antimicrobial peptides that inhibit growth of other bacterial species (Chikindas, Weeks, Drider, Chistyakov, & Dicks, 2018; S. D. Johanningsmeier, McFeeters, & Drake, 2005). Citral, hexanol, and 2-phenylethanol are examples of volatile compounds produced by *L. plantarum* (Lorn et al., 2021).

FFs are well established in our global history, with recipes and knowledge being passed down in various regions through indigenous peoples. While these traditional preparation methods remain important globally, the onset of accessible internet has allowed for rapid sharing of and access to personal recipes, advice, or guidelines which may include less safe food preparation practices or inaccurate information related to health benefits. Probiotic fermented dairy products were one of the first probiotic foods and continue to be a growing portion of the functional foods market (Yilmaz-Ersan, Ozcan, & Akpinar-Bayizit, 2020). In 2017, the global market value of probiotic foods was $40.09 billion U. S. and is expected to increase to $65.87 billion U. S. by 2024, with dairy products accounting for 69% of the product market (Yilmaz-Ersan et al., 2020).

There have been several surveys assessing consumer understanding of FFs and probiotics in FFs. Focus groups conducted in California in 1998, found that a wide range of consumer awareness and perception of probiotics in dairy products, ranging from unaware to having some level of understanding of the health benefits associated with probiotic consumption (Bruhn et al., 2006). A study conducted in Brazil in 2007 found that 29.05% and 21.67% of individuals interviewed were able to correctly define and identify a probiotic food, respectively (Viana, Da Cruz, Zoellner, Silva, & Batista, 2008). A 2018 study conducted in Turkey found 43.95% of participants were familiar with probiotic dairy products, while 56.05% had never heard of probiotic dairy products (Yilmaz-Ersan et al., 2020). Based on the available studies, there is a need for consumer education related to probiotic dairy products. However, data on the general knowledge of consumers related to FFs broadly, consumption habits, as well as perceived sensory and health benefits is not available. This study aims to determine awareness of different types of FFs and motivating factors for FF consumption.

2.3. Methods

2.3.1 Development of questionnaire

Globally, the information the consumer survey aimed to collect a) what types of FFs are people consuming, b) what motivates people to consume FFs, and c) are individuals producing their own FFs. The key research questions were, 1) do consumers recognize and consume FFs?;

2) what are the primary motivators for purchasing and consuming FFs?; 3) what health benefits do consumers feel they receive if consuming FFs?; 4) are there differences in these motivators or perceived health benefits when the primary ingredients in a FF are fruits or vegetables?; 5) are consumers interested in fresh fermented fruits and vegetables?; 6) what are the motivators for purchasing fresh fermented fruits and vegetables?; 7) are consumers fermenting their own fruits and vegetables?; and 8) what resources are consumers relying for home fermentation guidance? Based on these questions, 18 questions were developed. To accompany the questions, pertinent background information defining FFs and fresh fermented fruits and vegetables were embedded within the questionnaire to provide background information to participants. The complete survey questions and logic can be found in Appendix 1, Tables 1-5.

The first block of questions focused on the general category of FFs. Question (Q) 1 asked participants to identify the FFs from a provided list, all of which were FF products. Q2 asked participants to self-report whether they consumed FFs other than fermented alcoholic beverages. In Q3, participants were asked to indicate whether they consumed FFs based on category (cereal grain, fruits and vegetables, dairy, soybean or rice, meats, or other) and provide the frequency of consumption of each category (daily, weekly, monthly, or a few times a year). In Q4, participants were asked to provide their primary motivation for consuming FFs as either taste, health benefits, cultural reasons, or other (free response). The following questions (Q5, Q6, Q7) asked participants to provide the sensory attributes, health benefits, and cultural relevance related to consuming FFs.

Fermented fruits and vegetables were the focus of the second and third block of questions in this survey. After providing background information on fermented fruits and vegetables, including delineating between pasteurized and fresh (refrigerated and non-pasteurized) fermented fruits and vegetables, participants were asked in Q8 if they consumed fermented fruits and vegetables. Participants were asked if the fermented fruits and vegetables they consumed required refrigeration in Q9. The next series of questions (Q10-Q14), focused solely on fresh fermented fruits and vegetables. Q10 asked participants to indicate the frequency of consumption of fresh fermented fruits and vegetables as either daily, weekly, monthly, a few times a year, or never. The same questions were asked for motivations for consumption as above for FFs in general including, the primary motivation for consuming fresh fermented fruits and vegetables, flavors associated with these foods, perceived health benefits associated with consumption, and cultural reasons for consuming fermented fruits and vegetables (Q11-Q14).

The final block of questions focused on the in-home production of fermented fruits and vegetables. Participants were first asked if they ferment fruits or vegetables at home (Q15) and if "yes" to list the types of fruits and vegetables they ferment (Q16). The next question (Q17) asked them to identify resources they use for information on how to ferment fruits and vegetables at home including university extension, Unites States Department of Agriculture (USDA), textbooks, other books, websites, blog posts, other, or none. The final question (Q18) in the block asked participants to indicate what type of fruit and vegetable they are most interested in fermenting at home that they currently are not fermenting. Demographic questions were also included in the survey (Q19-26) and asked participants to self-identify gender, age, ethnicity, race, level of education, country of residence, state of residence, and annual family income.

The developed questionnaire went through multiple internal reviews for clarity and ability to deliver desired research outcomes. The survey research questions, Qualtrics survey, and survey logic were validated by four external reviewers, one fruit and vegetable fermentation expert (government affiliated), two food safety extension specialists (university affiliated), and one evaluation specialist (university affiliated). Based on the feedback from external review, the survey tool was updated and then piloted with a group of 30 University of California Division of Agriculture and Natural Resources (UC ANR) Master Food Preserver (MFP) volunteers. The pilot participants were asked to provide feedback on question clarity, functionality in Qualtrics, and estimated duration to completion. The survey was reviewed by the UC Davis Institutional Review Board (IRB) Administration (Project Title: [1657403-1] Survey of consumer consumption habits; Principal Investigator: Erin DiCaprio, PhD) and was deemed Exempt. All survey participants needed to provide consent to complete the survey.

2.3.2 Data collection

The developed Qualtrics survey was open from April 2022 to September 2022. The survey was shared through multiple mechanisms. First, the survey was distributed to a listserv of ~500 contacts curated through outreach activities associated with the project team (EATLAC project). The survey was shared via social media associated with the project team (EATLAC Instagram, EATLAC Facebook, UCD FST Facebook). The email was also shared through home preservation and food safety extension networks. Finally, the survey was included in The Fermentation Association (TFA) newsletters. A total of 853 responses were collected, with 851 respondents consenting to participate in the survey.

2.3.3. Data processing and analysis

Participants with missing data were removed, for a total of 751 responses included in analysis. Missing data was defined as missing responses for Q19 through Q24. Entries with identical responses for both closed ended questions and free responses were deleted.

Frequency data was used for categorical variables and descriptive statistics. Open ended questions were included intermittently in the survey to ensure closed questions were being properly addressed by respondents and to inquire for additional information when necessary (Singer & Couper, 2017). Income values were reported as an open response so values were changed to numeric manually and any value range listed was changed to the respective average. Open ended questions were approached as recommended by O'Cathain and Thomas: responses were previewed, a coding frame was developed to encompass responses, and codes were assigned to each comment manually (O'Cathain & Thomas, 2004). In the present work, 15 questions are used for analysis excluding demographic information. The remaining questions are discussed separately.

For further analysis, specific answers to questions were grouped by unique values and compared against total respondents of a second question from which proportions were calculated for each respective category in a question, as used by Šikić-Pogačar and others (Šikić-Pogačar et al., 2022). This method was used to draw comparisons between Q3 and Q6, Q4 and Q11, Q4 and Q22, Q1 and Q22, Q1 and Q20, Q1 and Q23, and Q1 and Q21. A two-way analysis of variance (ANOVA) with interaction effect was used to analyze differences in responses between Q3 and Q6 (King & Eckersley, 2019). A paired t-test was used to compare the mean responses for each matched question between the question block about non-alcoholic FFs (Q4, Q6, Q7) and the block related to fresh fermented fruits and vegetables (Q11, Q13, Q14) (Alasuutari, Bickman, & Brannen, 2008). A P-value below 0.05 was considered significant. Data processing and analysis was performed using R studio and Python in Jupyter Notebook (Kluyver et al., 2016; R Core Team, 2022).

Due to the significant amount of data collected in this survey, this chapter includes the data from question blocks 1, 2, 3, and 5 only. This allows for comparisons between data on FFs generally and fermented fruits and vegetables with survey demographics. Question block 4, focused on home fermentation practices, was removed from this current manuscript for brevity.

2.3.4. Limitations

As with all survey tools, there are inherent limitations to this study. While the participant pool was sufficiently large, the demographic data indicates a high proportion of respondents were female, aged 18-39, white, and had achieved a level of education above a high school diploma. The distribution methods for the survey, including university affiliated emails and social media posts, likely influenced this skewing of the sample population to a younger and educated population. Moreover, previous studies using non-specifically targeted survey tools have found that survey participants that are assigned female at birth are more inclined to participate (Becker 2022). Also, women are also the majority gender demographic currently enrolled in higher education (Parker, 2021). Although this survey was available to and advertised to individuals outside of the U.S., 87% (n=656) resided within the US with other participants from primarily Canada, Argentina, Brazil, and the UK. While the sample size is of sufficient power for analysis, correlations should not necessarily be extrapolated to demographic populations with low representation in the study.

2.4. Results

2.4.1. Demographics of survey respondents

A total of 751participants were included in analysis after incomplete and duplicate entries were removed. As shown in Table 1, 463 respondents (61.65%) identified as female, 258 respondents (34.35%) identified as male, 14 (1.86%) identified as non-binary, 2 (0.27%) identified as transmale, 1 (0.13%) identified as transfemale, and 13 participants (1.73%) selected "prefer not to respond." The highest number of respondents were in the 18-29 age range (n=302, 40.21%), followed by 30-39 (n=232, 30.89%), 40-49 (n=106; 14.11%), 50-59 (n=49; 6.52%), 60-69 (n=45; 5.99%) and 70 and above (n=17; 2.26%). Participants were also asked to report their highest level of education: 2.53% (n=19) of participants had received no formal degree, 7.99% received a high school degree (n=60), 11.98% received an associate degree (n=90), 41.41% received a bachelor's degree (n=311), 24.63% received a master's degree (n=185), 10.12% received a doctoral or professional degree (n=76), and 1.20% preferred not to respond (n=9). A total of 158 (21.04%) of participants identified as Hispanic or Latino(a) and 593 (78.96%) identified as not Hispanic or Latino(a). The majority of respondents were White (n=532; 70.84%), followed by Asian (n=95; 12.65%), two or more races (n=35, 4.66%), Black/African American (n=21; 2.80%), other/not listed (n=18; 2.40%), Native Hawaiian/other Pacific Islander (n=15, 2.00%), and American Indian/Alaska Native (n=7; 0.93%) and a total of 26 (3.46%) preferred not to respond. The United States was the primary country of residence of respondents (n=656; 87.35%) with only 85 respondents residing outside of the US and residing in 29 countries. All 50 states had at least one participant, with the majority from California (n=314, 41.81%). Most international respondents were from Canada (n=11) and Argentina (n=10).

2.4.2. Participant identification of fermented foods

Prior to the first question in the survey, participants were provided a basic definition of FFs (Appendix 1, Table 1). The first block of questions focused on the general category of FFs. In Q1, a list of FFs was provided (yogurt, sour cream, kefir, cheese, wine, beer, distilled spirits, kalamata olives, bread, coffee, chocolate, kombucha, salami, vinegar, kimchi, sauerkraut, curtido, escabeche, soy sauce, or none of these) and participants were asked to identify which foods were produced through fermentation (Figure 1). All foods provided on the list were fermented and 7% (n=55) of respondents correctly identified all foods as fermented. Yogurt was the most frequently identified FF by participants (n=658;87.62%). Other fermented dairy products, such as cheese, kefir, and sour cream, were identified by 67.51% (n=507), 64.98% (n=488), and 62.98% (n=473) of respondents, respectively. Alcoholic beverages (wine, beer, distilled spirits) were the second most identified type of FFs with 627 (83.49%) participants correctly identifying these beverages as fermented. Vinegar was identified by 323 (43.01%) participants as fermented. Beverages such kombucha and coffee were identified by 467 (62.18%) and 222 (29.56%) participants, respectively. The only fermented fruit included in the list was kalamata olives and 267 (35.56%) participants identified this food as fermented. The only fermented soy product, soy sauce, was correctly identified as fermented by 349 (46.47%) participants. Fermented vegetables included in the list included kimchi, sauerkraut, curtido, and escabeche. Kimchi and sauerkraut were the third and fourth most frequently identified FF, with 549 (73.10%) respondents identifying kimchi and fermented and 536 (71.37%) respondents identifying sauerkraut as fermented. Curtido and escabeche were least frequently recognized FF, with 141 (18.77%) participants identifying curtido as fermented and 118 (15.71%) identifying escabeche as fermented.

2.4.3. Participant reported consumption of different categories of non-alcoholic fermented foods

Participants were asked in Q2 if they consumed non-alcoholic FFs and beverages, excluding beer, wine, and distilled spirits, to which 85.22% (n=640) responded “Yes,” 10.79% (n=81) responded “no,” and 3.33% (n=25) were uncertain (Appendix 2, Table 2). Participants were asked to indicate which categories of non-alcoholic FFs and beverages (cereal grains, fruits and vegetables, dairy, soybean or rice, meats, and other) they consumed and to indicate the frequency of consumption of each category as daily, weekly, monthly, or a few times a year (Figure 2). For this question, participants were allowed to select all that applied, meaning they could select a frequency for consuming without selecting the option for consuming the food. Results are reported below without adjustment, and the inconsistencies in this question are considered as a limitation of the survey design. Percentages stated are calculated from the responses “yes” and “uncertain” in the previous question. A total of 46.17% (n=307) reported consuming fermented cereal grains, which was the category of FFs with the highest reported frequency of consumption. Of the participants that reported consuming fermented cereal foods, 51.58% (n=343) reported eating these foods daily, 28.72% (n=191) consumed weekly, 4.67% (n=31) consumed the food monthly and 3.31% (n=22) reported consuming these foods a few times a year. A total of 42.26% (n=281) participants indicated consuming fermented fruits and vegetables, 39.70% (n=264) consumed fermented dairy products, 30.68% (n=204) reported consuming fermented meats, and 37.60% (n=250) reported consuming fermented rice or soybean products (Appendix 2 Table 3). Participants indicated consuming fermented fruits and vegetables and fermented soybean or rice products most frequently on a weekly basis, as compared to daily for fermented dairy, and monthly for meats. A total of 8.87% (n=59) respondents selected "other" and were asked to describe; open responses included chili sauce, vinegar, kombucha, non-dairy yogurt, and fish.

2.4.4. Motivators for consuming non-alcoholic fermented foods including sensory attributes, health benefits, and cultural reasons

Participants were asked to select one answer for the primary motivator for consuming FFs as either 1) taste, 2) health benefits, 3) cultural reasons, or 4) other, in Q4. Most respondents indicated that "taste" was the primary reason for consuming FFs (n=337, 50.68%) (Figure 3 Panel A). "Health benefits" was the second highest frequency response (n=235; 35.34%), followed by "cultural reasons" (n=80, 12.03%). A total of 1.95% (n=13) respondents selected "other" with responses including "enjoying the fermentation process" and "food waste prevention". Regardless of the primary motivator selected in Q4, all participants were asked to complete Q5, Q6, and Q7 related to sensory attributes, health benefits, and cultural motivators related to or associated with the consumption of non-alcoholic FFs.

In Q5, participants were asked to select all the flavors and/or aromas associated with FFs from a list of 12 (sourness, umami, sweet, acid, sharp, spicy, bitter, salty, fizzy or bubbly, bland, pungent, rancid, other, none). Sourness (n=540; 81.20%) and acid (n=401; 60.30%) were the most frequent responses, while bland (n=17; 2.56%) and rancid (n=38; 5.71%) were least frequently chosen (Figure 4 Panel A). Participants selecting "other" (n=10; 1.50%) could also provide additional aromas or flavors and responses included: yeasty, fruity, tangy, or earthy.

In Q6, participants were asked to indicate which (if any) health benefits they associated with the consumption of FFs (digestive benefits, improved gut microbiome, probiotic, prebiotic, improved nutritional value, immune system benefits, other, none). The most highly selected health benefits associated with FF consumption were “improved gut microbiome” (n=513; 77.14%), “digestive benefits” (n=508; 76.39%), and “probiotic” (n=458; 68.87%) (Figure 5 Panel A). Participants also indicated associating “improved nutritional value” (n=330; 49.62%), “immune system benefits” (n=273;41.05%), and “prebiotic” (n=243; 36.54%) with the consumption of FFs. A total of 2.11% (n=14) participants selected “none.” If participants selected other (n=21; 3.16%), they were asked to provide a free response and responses included “mood”, “mental health”, “sleep”, and “memory”.

Participants were then asked to provide any cultural reasons for consuming non-alcoholic FFs in Q7. The most selected cultural reason for consuming FFs was “part of a culture I enjoy experiencing” (n=280; 42.11%) (Figure 6 Panel A). Participants also selected “part of the normal diet in my part of the world” (n=248; 37.29%), “part of a culture in an area I have lived or visited” (n=181; 27.22%), “popular with my ethnic group” (n=189; 28.42%), and “heritage recipe or practice” (n=178; 26.77%). A total of 16.99% (n=113) participants selected none. If participants selected “other” (n=16; 2.41%) they were asked to provide a free response to which responses included “part of a community affiliation” or having a career as a “chef” or “nutritionist.”

2.4.5. Consumer purchasing habits of fermented fruits and vegetables

The next question (Q8) addressed whether consumers were purchasing fermented fruits or vegetables. A total of 398 respondents (53.00%) indicated they purchased fermented fruits and vegetables. Of those responding yes, some provided the type of fermented fruits and vegetables they purchased with coded free responses including “kimchi,” “sauerkraut,” “olives,” “hot sauce,” “umeboshi plums,” “citrus,” and “pickles”. Others listed non-fruit or vegetable fermented products including “yogurt,” “natto,” and “sourdough.” Participants also listed responses of not traditionally fermented products including “corned beef,” “pickled cucumbers,” and “banana peppers.” To determine whether the purchased fermented fruits and vegetables were non-pasteurized participants were asked to indicate if the products they purchased always required refrigeration. Respondents were asked if they purchase fresh fermented fruits and vegetables that require refrigeration to which 58.87% (n=282) responded yes, 12.94% (n=62) responded no, and 28.18% (n=135) purchase both refrigerated and shelf-stable FFs.

2.4.6 Consumption frequency and motivators related to fresh fermented fruits and vegetables

In Q10 participants were provided a definition of fresh fermented fruits and vegetables and then asked answer how often they consumed these foods as either: daily, weekly, monthly, a few times a year, or never. A total of 7.19% (n=54) respondents indicated they never eat these foods while 92.41% (n=694) responded that they do consume these foods (Appendix 2 Table 10). Of those that did consume fresh fermented fruits and vegetables 21.76% (n=151) ate the products daily, 36.74% (n=255) ate the products weekly, 23.20% (n=161) ate the products monthly, and 18.01% (n=125) consumed these products a few times a year. The participants were asked to select one primary motivator for consuming fresh fermented fruits and vegetables (Q11), 50.14% (n=348), selected taste, 36.17% (n=251) reported health benefits, 10.23% (n=71) indicated cultural reasons and 3.17% (n=22) selected “other” (Figure 3 Panel B). Responses from those that selected “other” included “reduced cost,” “food preservation,” and “enjoying fermentation." In Q12, participants were asked what flavors and/or aromas they associate with fresh fermented fruits or vegetables from a list of 11 (sourness, umami, sweet, acid, sharp, bitter, salty, fizzy or bubbly, bland, pungent, rancid, other, none). Sourness (n=517; 74.50%) and acid (n=378;54.47%) were most frequently selected while bland (n=11;1.59%) and rancid (n=29;4.18%) were selected least frequently (Figure 4 Panel B). A total 0.43% (n=3) of participants selected "none" and 1.59% (n=11) selected "other." Open responses to "other" included "spicy," "fruity," "tangy," "earthy," and "crunchy." In Q13, participants were asked what health benefits they associate with the consumption of fresh fermented fruits and vegetables. Health benefits most associated with fresh fermented fruits and vegetables was "improved gut microbiome" (n=488; 70.32%), "digestive benefits" (n=472; 68.01%), "probiotic" (n=384;55.33%), and "improved nutritional value" (n=332; 47.84%) (Figure 5 Panel B). Participants also associated "immune system benefits" (n=263;37.90%) and "prebiotic" (n=246; 35.45%) with fresh fermented fruits and vegetables. In total, 4.61% (n=32) of participants selected "none" and 1.73% (n=12) selected "other." Free responses to this option included "mental health" and "mood" benefits. In Q14, participants were asked if there are cultural reasons for which they consume fermented fruits or vegetables. The most selected cultural reasons were "part of a culture I enjoy experiencing" (n=272;39.19%), "part of the normal diet in my area of the world" (n=203;29.25%), and "popular within my ethnic group" (n=180;25.94%) (Figure 6 B). Participants also selected "part of a culture in an area I have lived or visited" (n=178;25.65%) and "none" (n=139;20.03%). In total, 1.44% (n=10) selected "other" of which free responses included "community affiliations," "working as a chef," or "revival of cultural practices."

2.4.7 Comparisons of demographics with survey responses

We compared demographic data to correctly identifying all FFs listed in Q1. There were correlations identified associated with age and education status of participants. Of all age groups, the largest percentage of respondents that correctly identified all foods as fermented were between 60 to 69 (n=9; 20.93%) (Figure 7 Panel A). The age group that least frequently identified all FFs correctly was in the range of 50 to 59 (n=1; 2.04%). Of all reported education levels, the largest proportion of respondents to correctly identify all FFs correctly were participants with a doctoral or professional degree (n=8; 10.53%), except for those who chose "prefer not to answer" (n=1;12.50%) (Figure 7 Panel B).

Curtido and escabeche are traditionally Hispanic fermented products, so we identified which participants selected curtido and escabeche, not exclusively, for Q1 and compared with responses for ethnicity, Q21 (Appendix 2 Table 17). In total, 14.01% (n=22) of Hispanic or Latino(a) participants identified escabeche as fermented while 19.11 % (n=30) of Hispanic or Latino(a) participants identified curtido as fermented. Of not Hispanic or Latino participants, 16.3% (n=96) identified escabeche as fermented and 18.85% (n=111) identified curtido as fermented.

Similarly, kimchi is a traditional Asian FF, so we identified which participants selected kimchi in Q1 and compared with responses for race, Q22. Participants that identified as being two or more races had the largest proportion to correctly identify kimchi (n=31; 88.57%) (Appendix 2 Table 18). In total, 86.17% (n=81) of participants that identified themselves as Asian correctly identified kimchi as fermented. The lowest proportion of participants that selected kimchi as fermented identify as Native Hawaiian/ Other Pacific Islander (n=8; 53.33%).

Both Q4 and Q11 asked participants what their main reason for consuming non-alcoholic FFs and fresh fermented fruits and vegetables, respectively. We isolated the participants that selected “cultural reasons” and compared to their response to Q22, to assess if race had an association with this response. The largest proportion of participants that selected “cultural reasons” for consuming FFs differed for each question. For Q4, 15% (n=3) of Black/African American participants selected cultural reasons for consuming non-alcoholic foods whereas for Q11, 14.29% (n=1) of American Indian/ Alaska Natives selected cultural reasons for consuming fresh fermented fruits and vegetables (Appendix 2 Table 19).

2.4.8. Comparisons between health benefits and fermented foods with defined health benefits

Responses to Q3 and Q6 were compared, isolating FF consumption frequency data for participants that selected “digestive benefits,” “improved gut microbiome,” or “probiotic” as the primary health benefits associated with non-alcoholic FFs and beverages (Appendix 2 Table 20). Participants were able to select all that apply for health benefits expected from FFs. Results of a two-way ANOVA with interaction effect for the reason (ie., probiotic) and category (i.e., dairy) against response frequency found no significant differences ($p = 0.73$). For example, consumption habit responses of individuals that selected probiotic as a health benefit were not significantly different between fermented dairy and fruits and vegetables from an ad-hoc Tukey comparison test ($p=0.2$).

2.4.9. Comparisons between matched questions in blocks 1 and 3

Question block 1 asked about non-alcoholic FFs and question block 3 asked participants about exclusively fresh fermented fruits and vegetables. Three questions were identically repeated in each section and a t-test was used to determine differences in the mean response. Percentages were used in this analysis to account for the different number of responses in each section, as all participants that consume FFs were asked question block 1 however only those that consume fresh fermented fruits and vegetables were asked in question block 3. Between Q4 and Q11, asking the main reason for eating FFs, no significant difference was found in responses ($p=0.92$). Between Q6 and Q13, asking about associated health benefits, no significant difference was found ($p=0.05$). Between Q7 and Q14, asking about cultural reasons, no significant difference was found ($p=0.11$).

2.5. Discussion

Some consumers are expressing renewed interest in nutritional value, methods of food preparation, and functionality of their diet (Sloan, 2019). This has correlated with a recent increase in consumer demand for fermented products in the US which has allowed for the expansion of the market with more people seeking out FFs at dining establishments, in grocery stores, and fermenting in their households (Nielson-Stowell, 2017; Prince, 2018; Ramos et al., 2022). This growth in the FF market was exacerbated at the beginning of the COVID-19 pandemic as media coverage promoted FFs, specifically sauerkraut, for its potential to reduce severity of infection (Domonoske & Schneider, 2020; Manskar & Raskin, 2020). FFs are often appealing for their actual or perceived functional properties, e.g., through delivery of probiotic microorganisms, containing live microbes, synthesis of new nutrients, or through the nutrients present in the starting ingredients (Jyoti P. Tamang, Shin, et al., 2016). The survey in the present study was designed to assess consumer familiarity with FFs and expectations of FFs to better understand the basis for this trend.

The most studied FF category in terms of health promoting benefits is fermented dairy. Randomized control trials (RCTs) show evidence of favorable effects on gastrointestinal (GI) health and disease, cardiovascular health, weight, cancer, and diabetes (Savaiano & Hutkins, 2021). Fermented dairy products supplemented with probiotics and prebiotics have been found to modulate the host GI microbiota and positively regulate immunity (Bogovič Matijašic et al., 2016; S. Wang et al., 2012). Yogurt, sour cream, kefir, and cheese were among the most frequently identified as FFs by participants in this survey. Participants identified health benefits associated with yogurt consumption including improved gut microbiome, digestive benefits, and probiotic with all FFs in the survey, not just yogurt. While this study did not evaluate participants' underlying reasoning or understanding of this, yogurt was the most correctly identified FF in this survey and as participants are most familiar with these products, they are also likely familiar with the associated health benefits.

Participants were provided with a definition of FFs, foods made through "desired microbial growth and enzymatic conversions of food components" (Marco et al., 2021) with additional details, yet only 7% (n=55) of respondents correctly identified all the listed foods as fermented. Kimchi, curtido, and sauerkraut are all FFs made through the fermentation of cabbage (with addition of differing minor ingredients) by species of LAB such as *Pediococcus, Leuconostoc,* and *Lactobacillus* (S. W. Hong, Choi, Lee, Yang, & Lee, 2016; Zabat, Sano, Wurster, Cabral, & Belenky, 2018). Curtido is made from cabbage, onions, and carrots and is traced back to El Salvador; kimchi is produced from cabbage, spices, and often fish products and originated in South Korea; sauerkraut is traditionally made from green cabbage and originated in Germany (Chilton, Burton, Reid, & Reid, 2015). Despite these similarities, kimchi was most frequently identified by 79.49% (n=597), sauerkraut second most identified by 77.50% (n=582) of participants, and curtido by just 20.91% (n=157). Soy sauce was identified by less than half of participants as fermented which could be attributed to confusion about the product processing. Soy sauce is traditionally made from fermentation of soybeans and referred to as shoyu, but can also be produced through acid hydrolysis which yields a less flavorful product (K. S. Liu, 2008).

Over two-thirds of respondents reported associating probiotic health benefits with non-alcoholic FFs and beverages. Separating the renewed cultural interest of FFs from interest in probiotics is challenging, due to the rapidly expanding market for probiotics which lacks extensive regulation for safety, quality, and efficacy (de Simone, 2019). LAB are important to many food fermentations and some specific strains, such as *Lactobacillus rhamnosus* GG and *Lactobacillus acidophilus* NCFM, having been characterized as probiotics (Doron, Snydman, & Gorbach, 2005; Gao et al., 2022). Probiotic strains of LAB are generally recognized as beneficial to host health through regulating cytokine expression, interacting with immune cells, and strengthening the epithelial barrier (Chabot et al., 2001; Corcoran, Stanton, Fitzgerald, & Ross, 2005; Lebeer, Vanderleyden, Keersmaecker, & De Keersmaecker, 2008). The definition of probiotics is “live microorganisms that, when administered in adequate amounts confer a health benefit on the host” (FAO & WHO, 2001; Hill et al., 2014). Yogurt is a FF that is often enriched with *Lactobacillus acidophilus* and other bacteria at sufficient levels to confer a health benefit and levels of viable organism are maintained for the duration of the product’s shelf life (Gao et al., 2022; Kariyawasam et al., 2021; Sanders & Marco, 2010). However, the microbial contents of most FFs are largely unclassified microorganisms and while some probiotic strains may indeed be present, they would not be at sufficient levels to confer a health benefit. To determine if participants that identified “probiotic” as a health benefit were associating that benefit with fermented dairy products, we compared responses of “probiotic” in Q6 to responses for different

FF categories consumed (dairy, cereal, fruits and vegetables, meat, soy/rice) in Q3. Based on our data, probiotic associated as a health benefit was not more frequent for those that consume fermented dairy products compared to other FFs. Undefined microorganisms present in fermentations may impact the host through mechanisms similar to probiotics, however these products based on the current definition of probiotics should not be marketed as such and rather include a label statement "contains live cultures" (Marco et al., 2017).

FFs can contain other health-promoting components besides microorganisms. Prebiotics are "substrate[s] that [are] selectively utilized by host microorganisms conferring a health benefit" and these compounds can be present in FFs from initial ingredients or in-situ synthesis from microorganisms (Gibson et al., 2017; Marco et al., 2021). While further research and regulation is necessary to develop label claims for prebiotics in FFs, fermented cereal grains, beer, wine, and some LAB fermented products may contain health-benefiting fructans and galactans (Dueñas et al., 2015; Kanyer et al., 2017; Kolida et al., 2002; Salazar et al., 2016; Salmerón, 2017). While 68.87% of participants stated associating probiotics with FFs, only 36.54% selected prebiotic. Probiotics have been marketed for some time to consumers for their health promoting benefits and associated with FFs, yogurt in particular (Gibson et al., 2017). This data suggests consumers are not as familiar with prebiotics as probiotics and may not understand other health benefiting properties of consuming FFs beyond live microbes. A study among Romanian consumers in 2018 found that while 74% of respondents were familiar with the term prebiotics, the definition was not well understood (Precup, Pocol, Teleky, & Vodnar, 2022). Improved nutritional value, which was selected by half of respondents, is also an understood benefit of FFs with increased bioavailability of amino acids and nutrients. Increased content of free amino acids in FFs are attributed to LAB conversion of peptides present in raw materials (Christensen, Dudley, Pederson, & Steele, 1999; Moore, DuVivier, & Johanningsmeier, 2022).

When asked what their primary reason for consuming FFs was, nearly half of participants (n=355) selected taste. Currently, there has been limited development of standardized methods for assessing FF palatability (Fushiki & Nakano, 2019). Taste within single fermentations can vary greatly due to the length of fermentation, characteristics of starting ingredients, and temperature of the surrounding environment (S. M. Lee, Kim, & Kim, 2019; Peñas, Frias, Sidro, et al., 2010; Peng, Li, Cui, & Guo, 2015). Our finding that taste is the primary motivator related to consumption, is supported by other work finding that yogurt purchasing preferences is primarily driven by taste as sensory characteristics are more likely to influence purchase of a product compared to health claims (Haddad et al., 2007). In our study, 81.20% of participants selected sourness and 60.30% selected acid as the most frequently associated organoleptic properties of FFs. Sour and acid tastes are imparted by the production of organic acids characteristic of many fermented fruits and vegetables (S. D. Johanningsmeier et al., 2005). In our question block focused only on fermented fruits and vegetables, the sensory attributes remained largely the same. In the present study, 46.62% of participants selected umami as a flavor associated with FFs. The umami flavor in FFs is attributed to the presence of glutamate above 1mM and is typically found in fermented cheese, soy sauce, meat, bread, or fish (C. J. Zhao, Schieber, & Gänzle, 2016). The lower frequency of umami compared to sourness and acid may be related to the fact most respondents were most familiar with fermented dairy compared to other categories of FFs.

Our survey also examined the cultural reasons for which people consume FFs. Participants indicated that FFs are a “part of a culture that the participant enjoys experiencing”

(42.11%) and that FFs are a “normal part of the diet in the participants part of the world” (37.29%). A review of FF consumption in Africa from 1985 to 2008 found decreased consumption in urban areas compared to rural areas and this was attributed to increased westernization of diets in urban areas and loss of generational knowledge related to FF nutritional and health benefits (Anukam & Reid, 2009). This highlights a potential risk that comes with upscaling FFs popular in the western diet without supporting small-scale and artisanal FFs. The increased availability of FFs such as yogurt and kimchi, the most frequently identified FFs by consumers in this survey, may lead to a reduced diversity of lesser known, regional ferments (Materia, Linnemann, Smid, & Schoustra, 2021). A study of lesser-known FFs in Eastern Europe found that knowledge of FF ingredients and processes has decreased, and thus more traditional fermentations have become marginalized (Sõukand et al., 2015). Small-scale fermenters offer the potential to invest in more artisanal FFs, with potential for greater diversity supporting a more heterogenous landscape of FFs (Flachs & Orkin, 2021).

2.6. Conclusion

Results from this survey suggests that while consumers have strong interest in FFs, a lack of understanding on the fermentation process, health benefits, and variety of FFs is present. Even with a provided definition of what qualifies a food as fermented, less than one tenth of participants correctly identified FFs. When asked if participants consume fermented fruits and vegetables, 10.79% stated they were uncertain. In free responses, multiple participants listed foods that are traditionally not produced by fermentation as a type of FF. These data highlight that more education is needed to help consumers understand FF from non-fermented products. A notable limitation of this study is the cultural and individual differences in preparation methods of such foods. Creating an all-encompassing definition of foods that individuals may prepare differently, such as with curtido which may be fermented or quick pickled by mixing cabbage with vinegar, is challenging. Therefore, more studies are necessary to examine the ethnobiology of FFs to better understand the microbial and social implications of food fermentations. Despite these knowledge gaps, there is an overwhelming interest in the practice of fermentation attributed to preservation of fruits and vegetables, convenience, health potential, and general enjoyment of learning more.

2.7. Figures and tables

Table 2.1. Demographic responses of study participants. Total response size n=751.

Characteristics	N=751	%
Gender		
Male	258	34.35
Female	463	61.65
TransMale	2	0.27
TransFemale	1	0.13
Non-binary	14	1.86
Prefer not to respond	13	1.73
Age		
18-29	302	40.21
30-39	232	30.89
40-49	106	14.11
50-59	49	6.52
60-69	45	5.99
70 and above	17	2.26
Ethnicity		
Hispanic or Latino(a)	158	21.04
Not Hispanic or Latino(a)	593	78.96
Race		
American Indian/ Alaska Native	7	0.93
Asian	95	12.65
Black/African American	21	2.80
Native Hawaiian/ Other Pacific Islander	15	2.00
White	532	70.84
Two or more races	35	4.66
Other/not listed	18	2.40
Prefer not to respond	26	3.46
Highest Education Level		
No formal degree	19	2.53
High school degree or diploma	60	7.99
Associates degree	90	11.98
Bachelor's degree	311	41.41
Master's degree	185	24.63
Doctoral or professional degree	76	10.12
Prefer not to respond	9	1.20
Country of residence		
United States	656	87.35
Outside of United States	85	11.32
Prefer not to respond	10	1.33

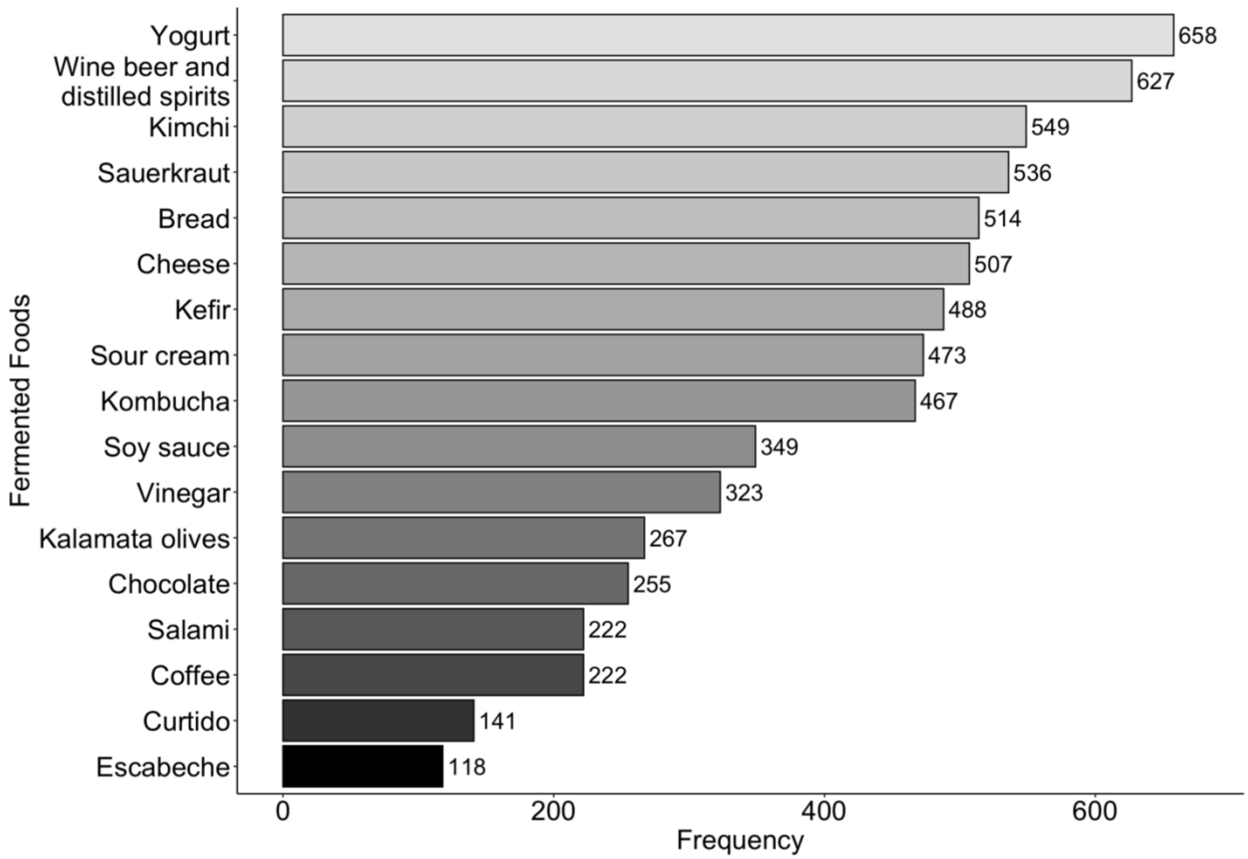

Figure 2.1. Response frequency for question 1: "Based on this explanation and your experience, please indicate below which of the following foods can be made by fermentation or are examples of FFs and beverages. Please check all response options that apply." Numbers listed along y axis are total response frequency for that FF. (n=751)

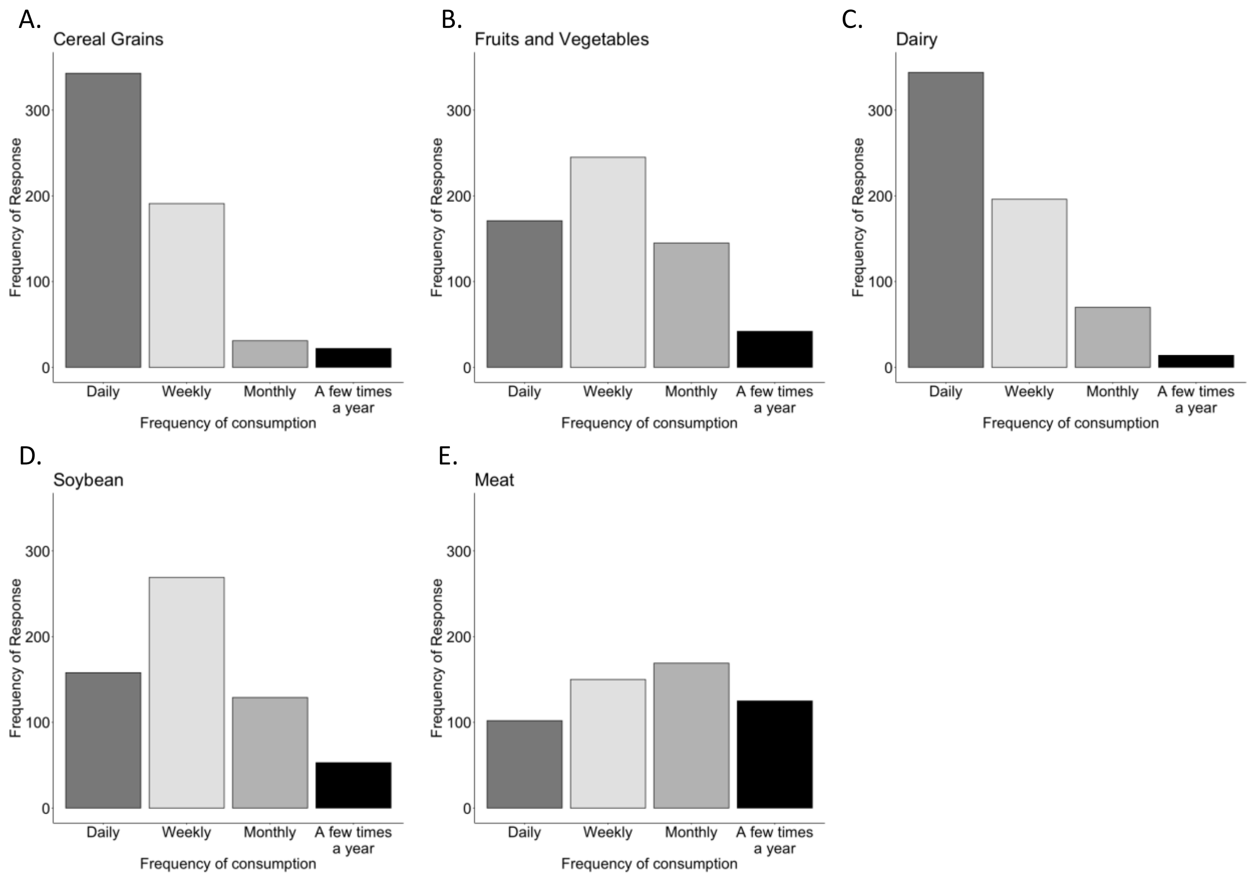

Figure 2.2. Responses for Q3: "Which types of FFs do you eat (excluding alcoholic beverages)? Please indicate the frequency of consuming that food by checking the box next to: daily, weekly, monthly, or a few times a year." Total responses for Q3 n=665.

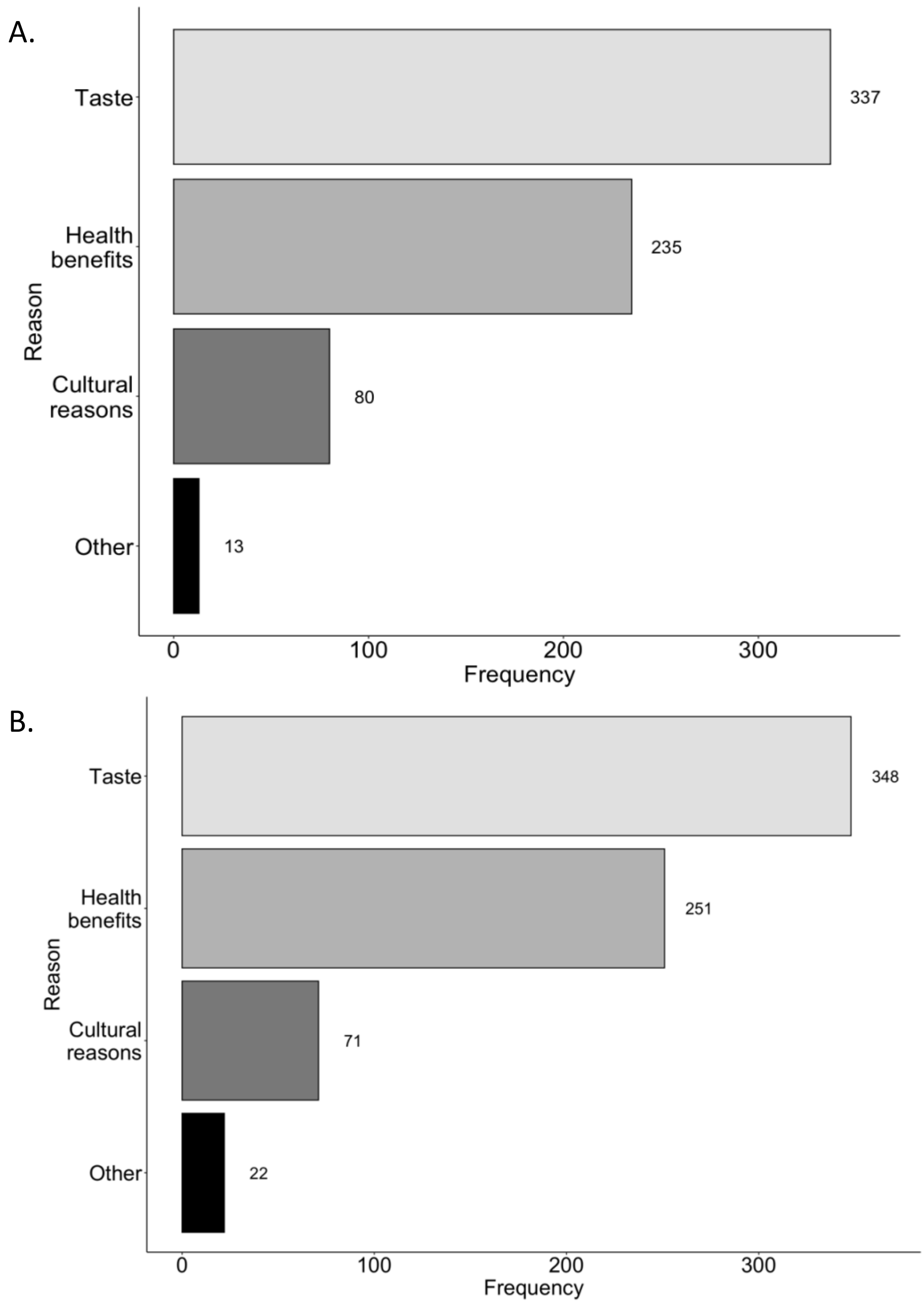

Figure 2.3. Responses for Q4(A) and Q11(B): “what is your main reason for eating [non-alcoholic FFs]/[fresh fermented fruits or vegetables]?” Single response allowed. Total response for Q4 n=665 and for Q11 n=694.

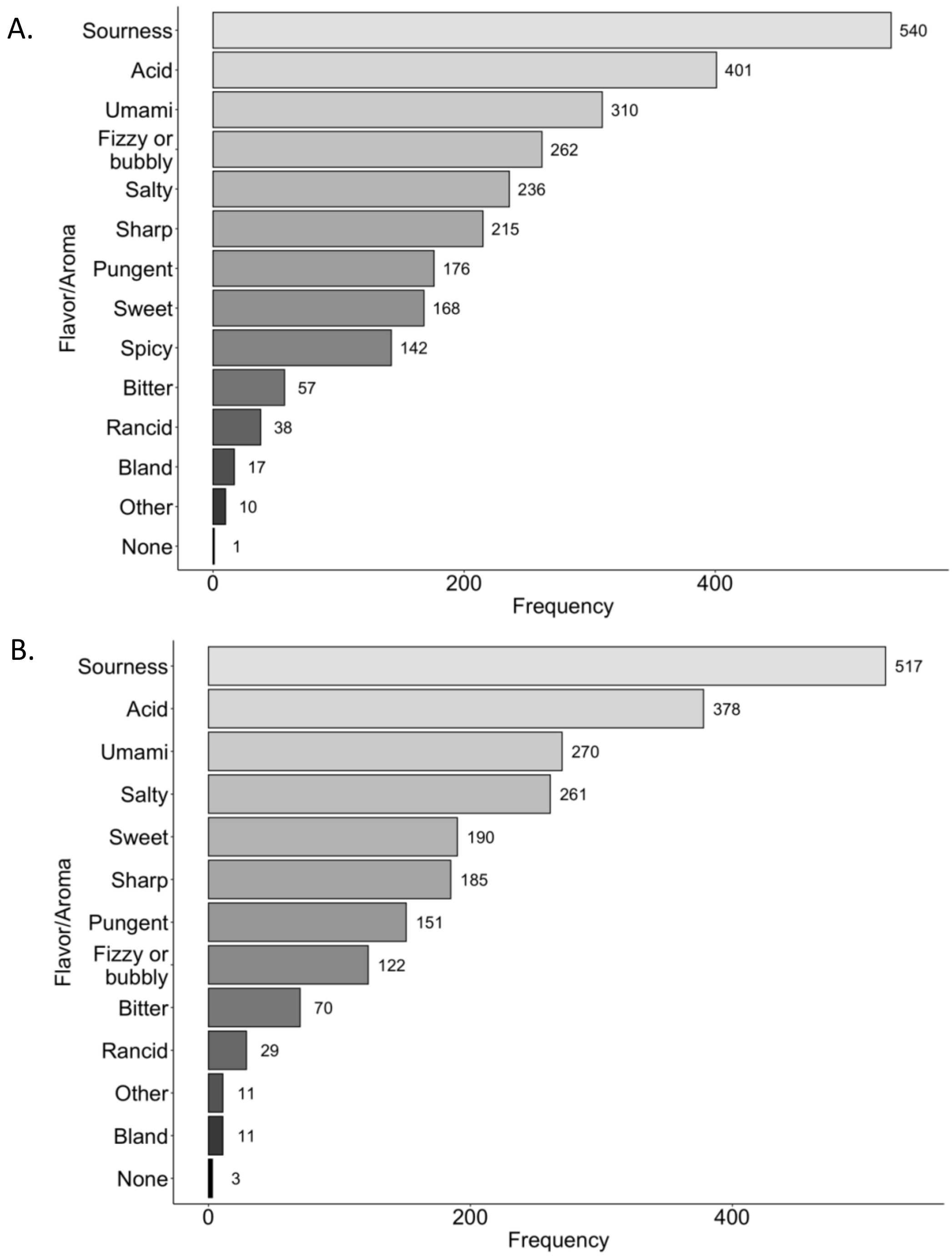

Figure 2.4. Responses for Q5(A) and Q12(B): "What flavors or aromas do you associate with [FFs] / [fermented fruits or vegetables]? Select all that apply." Total responses for Q5 n=665 and for Q12 n=694.

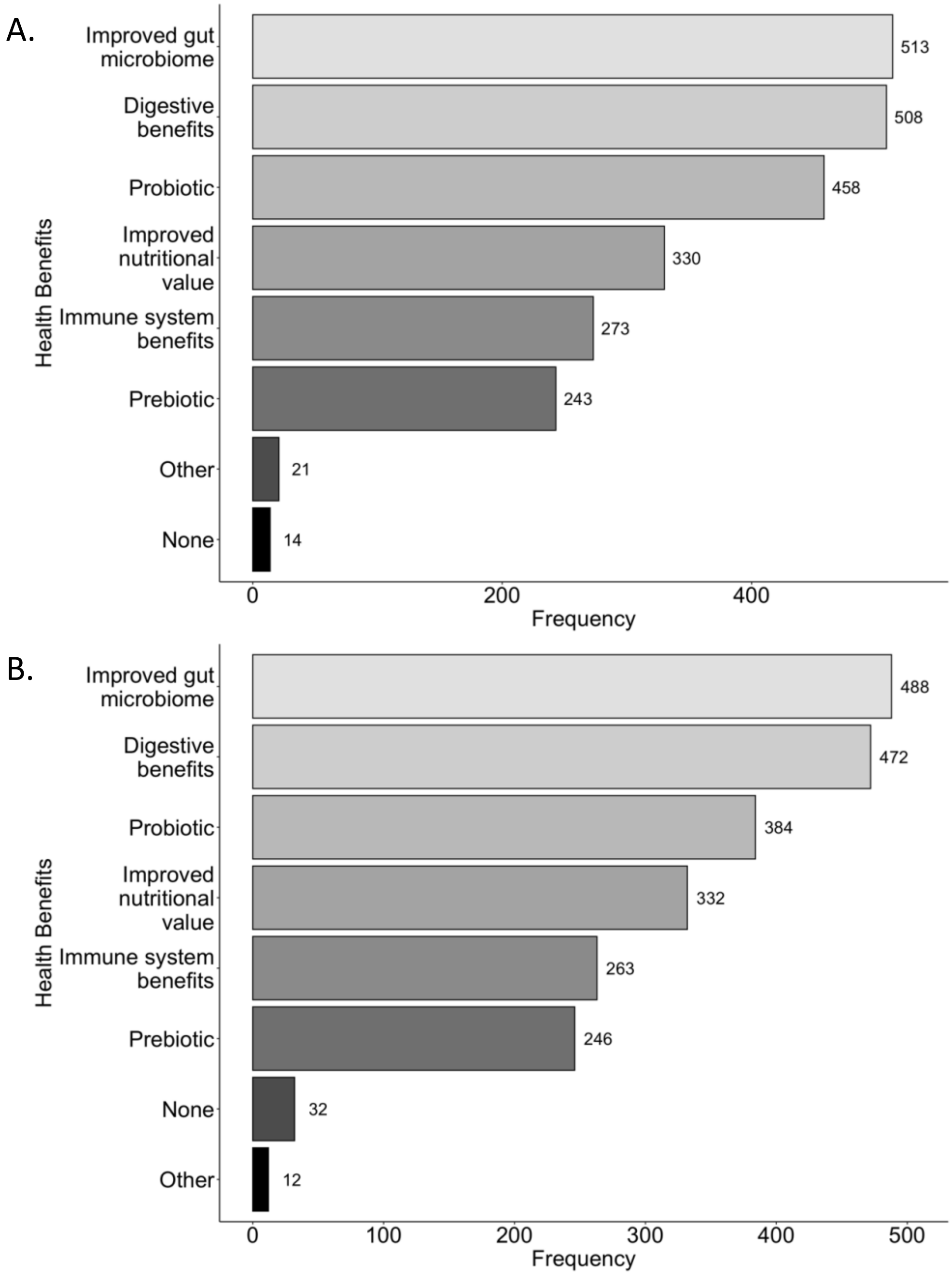

Figure 2.5. Responses for Q6(A) and Q13(B): "What, if any, health benefits do you associate with the consumption of [FFs] / [fresh fermented fruits and vegetables]? Select all that apply." Total responses for Q6 n=665 and for Q13 n=694.

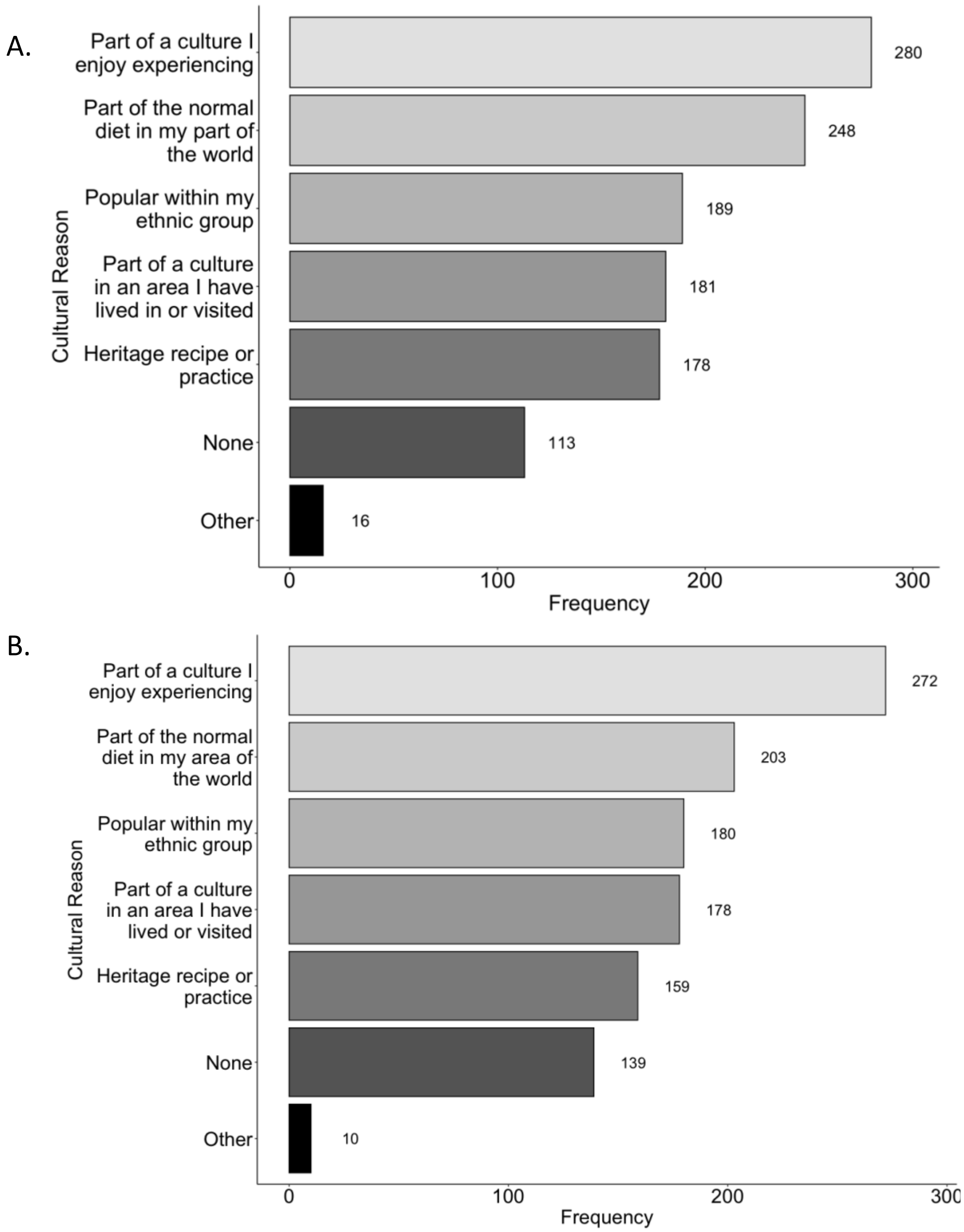

Figure 2.6. Responses for Q7(A) and Q14(B): Are there any cultural reasons you consume [non-alcoholic FFs and beverages] / [fresh fermented fruits and vegetables]? Select all that apply." Total responses for Q6 n=665 and for Q13 n=694.

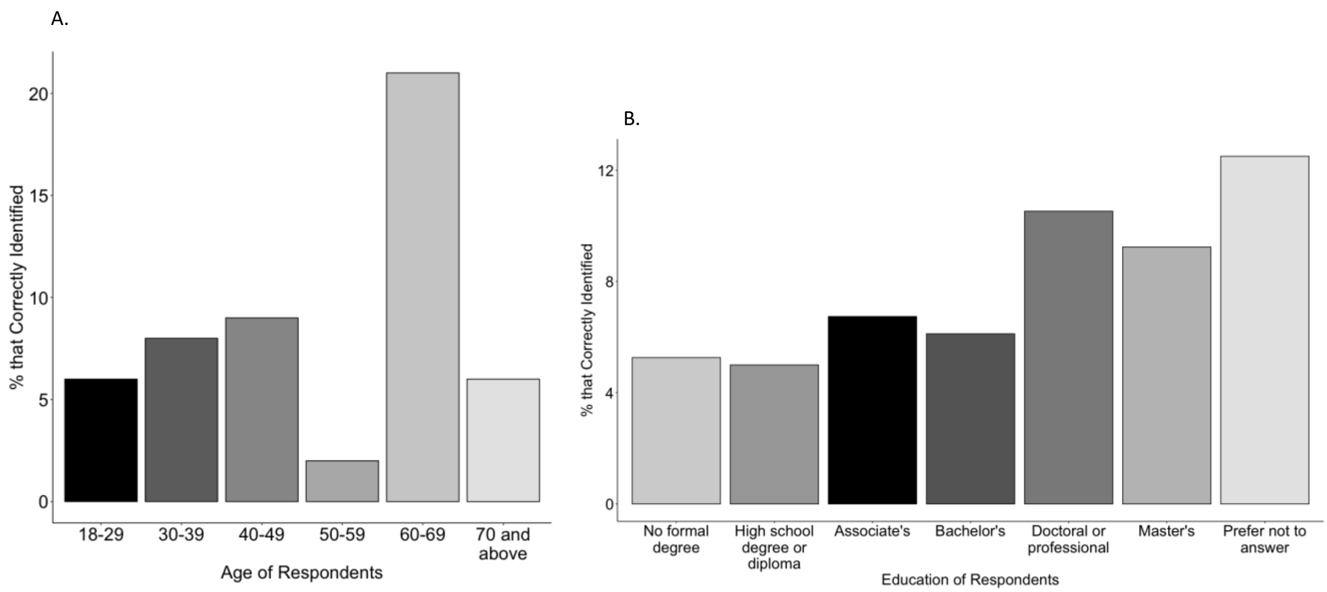

Figure 7. Percentages of total responses to Q20 (A) and Q23 (B) calculated from respondents that selected all foods as fermented in Q1 (n=55).

Chapter 3: Determining trends of in-home fermentation as a needs assessment for the development of new extension programming related to fermentation

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Author contribution statement: MH, HL, ZM, PF, AT, MM, TMR, WB, LW, MLM, and ED, contributed to the study conception and design. Funding was acquired by MLM and ED. Material preparation, data collection and analysis were performed by MH. The first draft of the manuscript was written by MH and ED.

3.1. Abstract

Small-scale fermentations are important dietary stables, relying on principally autochthonous microorganisms, that allow for a unique engagement with the food system while extending fruit and vegetable shelf life and developing novel organoleptic properties. The purpose of this study was to understand the prevalence of in-home fermentation of fruits and vegetables, types of foods that are fermented at home, resources that are used as reference material, and what types of fruits and vegetables individuals have interest in fermenting in the future. These data were collected as part of a larger survey on fermented food (FF) consumption and preferences with a total of 751 participants. Fermenting fruits and vegetables at home were reported by 49.20% of participants; 50.80% did not ferment fruits and vegetables. The 367 individuals that did ferment fruits and vegetables were asked to respond to the questions related specifically to home fermentation. Respondents indicated which types of fruits and vegetables were currently fermented at home and the most frequent responses were cabbage, cucumbers,

alliums, carrots, peppers, and citrus. Respondents also indicated a broad interest in expanding in home fermentation to other specialty crops. Participants were asked what resources they use as reference material for fermenting fruits and vegetables; books from authors popular in the FF community were most frequently used by participants (46.32%, n=170) followed by content on YouTube (38.69%; n=142). Approximately 25% of respondents indicated that they used the United States Department of Agriculture Complete Guide to Home Canning or other university extension publications. These data suggest a broad general interest in fermenting a wide variety of fruits and vegetables. While extension resources are used by some respondents, nearly twice as many respondents used books or YouTube. Given that in-home fermentation (namely fermented cabbage, olives, cucumbers) extension resources are limited, there is a unique opportunity to develop new resources for products of high interest including berries, peppers, and tomatoes. Using streaming content (e.g., YouTube) in extension programming, could enhance the impact of FF education and lead to increases in preparation.

3.2. Introduction

Home food processing and preservation practices remain prevalent in U.S. households. Limited data exists on the motivations that drive individuals to preserve foods in the home. A consumer survey and interviews found that the primary source of food preserved was from home gardens with the primary motivations for preserving being “to know and control what’s in my food” and “it tastes better” (Click and Ridberg 2010). Among the motivation for “to know and control what’s in my food” common themes emerged such as controlling sensory attributes, avoiding additives or preservatives, and to avoid unsafe food (Click and Ridberg 2010). Participants generally expressed a distrust in the global agro-food industry and a motivation to store locally and sustainably produced foods (Click and Ridberg 2010). Coupled with a lack of information on motivations for home preservation, there is limited information on prevalence of different types of preservation being utilized to extend the shelf-life and quality of food products by consumers.

U.S. consumers are interested in functional foods, they turn to fermented foods (FFs) for their numerous health benefits (Unilever, 2022). Consumers within the U.S. are reportedly interested in incorporating FFs in their diet to add unique flavors and incorporate more dishes from other cultures (Prince 2018; Everett 2019). FFs have remained vital to cultures across the globe for their preservation of perishable crops, improved digestibility, and ethnobiological tradition (Quave & Pieroni, 2018). As such, there has been a movement to catalog indigenous FFs that use native plant species and autochthonous microorganisms (L. Hong et al., 2015; Quave & Pieroni, 2018; Sõukand et al., 2015). As fermented fruits and vegetables can be produced through spontaneous fermentation (i.e., without the use of starter cultures) they are generally a product that is easy to prepare at home.

As the volume of FF production has shifted away from small-scale to large scale commercialized operations, the consistency needed for fermentations has increased. While fermentations are by nature highly variable and dynamic, scaled up production of FFs puts greater emphasis on uniformity of the product where a deviation in pH could result in a significant financial loss (Hutkins, 2019). Extensive development of starter cultures for fermentations has enabled this shift in production style while also allowing for improved quality, safety, and sensory attributes such as decreased nitrite synthesis, or developing off-flavors (Caplice & Fitzgerald, 1999; Han et al., 2014; Johansen, 2018). However, for small scale producers of FFs there is relatively limited guidance on best production practices. A recent study evaluating the knowledge related to the safety and nutritional properties of FFs indicated a lack of knowledge of the health benefits associated with FFs, the distinction between FFs and probiotics, and the biological safety hazards that can be associated with FF products among participants in a FF educational webinar (Ramos et al., 2022).

Understanding where individuals fermenting products in the home are obtaining information on production practices can help inform where gaps in information exist and what mechanisms of delivery may be of greatest value for increasing knowledge on best production practices. In this study, we survey individuals producing fermented fruits and vegetables in their home to indicate the types of commodities fermented, resources used for fermenting, and what types of commodities they have interest in fermenting in the future.

3.3. Methods

3.3.1 Questionnaire Development

Globally, the information the consumer survey aimed to collect were a) what types of FFs are people consuming, b) what motivates people to consume FFs, and c) are individuals producing their own FFs. The key research questions were, 1) do consumers recognize and consume FFs?; 2) what are the primary motivators for purchasing and consuming FFs?; 3) what health benefits do consumers feel they receive if consuming FFs?; 4) are there differences in these motivators or perceived health benefits when the primary ingredients in a FF are fruits or vegetables?; 5) are consumers interested in fresh fermented fruits and vegetables?; 6) what are the motivators for purchasing fresh fermented fruits and vegetables?; 7) are consumers fermenting their own fruits and vegetables?; and 8) what resources are consumers relying for home fermentation guidance? A team of microbiologists and food scientists developed a series of 18 questions based on the above research interests. To accompany the questions, pertinent background information defining FFs and fresh fermented fruits and vegetables were embedded within the questionnaire to provide background information to participants. The complete survey questions and logic can be found in Appendix 1, Tables 1-5.

The first block of questions focused on the general category of FFs. Fermented fruits and vegetables were the focus of the second and third block of questions developed in this survey. The final block of questions focused on the in-home production of fermented fruits and vegetables and that data is the focus of the analysis presented here. Participants were first asked if they ferment fruits or vegetables at home (Q15) and if "yes" to list the types of fruits and vegetables they ferment (Q16). The next question (Q17) asked them to identify resources they use for information on how to ferment fruits and vegetables at home including university extension, U.S. Department of Agriculture (USDA), textbooks, other books, websites, blog posts, other, or none. The final question (Q18) in the block asked participants to indicate what type of fruit and vegetable they are most interested in fermenting at home that they currently are not fermenting. Demographic questions were also included in the survey (Q19-26) and asked participants to self-identify gender, age, ethnicity, race, level of education, country of residence, state of residence, and annual family income.

The developed questionnaire went through multiple internal reviews for clarity and ability to deliver desired research outcomes. The survey research questions, Qualtrics survey, and survey logic were validated by four external reviewers, one fruit and vegetable fermentation expert (government affiliated), two food safety extension specialists (University affiliated), and one evaluation specialist (University affiliated). Based on the feedback from external review, the survey tool was updated and then piloted with a group of 30 University of California Division of

Agriculture and Natural Resources (UC ANR) Master Food Preserver (MFP) volunteers. The pilot participants were asked to provide feedback on question clarity, functionality in Qualtrics, and estimated duration to completion. The survey was reviewed by the UC Davis Institutional Review Board Administration (Project Title: [1657403-1] Survey of consumer consumption habits; Principal Investigator: Erin DiCaprio, PhD) and was deemed Exempt. All survey participants needed to provide consent to complete the survey.

3.3.2. Data collection

The developed Qualtrics survey was open from April 2022 to September 2022. The survey was shared through multiple mechanisms. First, the survey was distributed to a listserv of ~500 contacts curated through outreach activities associated with the project team (EATLAC project). The survey was shared via social media associated with the project team (EATLAC Instagram, EATLAC Facebook, UCD FST Facebook). The email was also shared through home preservation and food safety extension networks. Finally, the survey was included in The Fermentation Association (TFA) newsletters. A total of 853 responses were collected, with 851 respondents consenting to participate in the survey.

3.3.3. Data processing and analysis

Participants with missing data were removed, for a total of 751 responses included in analysis. Missing data was defined as missing responses for Q19 through Q24. Entries with identical responses for both closed ended and free response questions were deleted.

Frequency data was used for categorical variables and descriptive statistics. Open ended questions were included intermittently in the survey to ensure closed questions were being properly addressed by respondents and to inquire for additional information when necessary (Singer & Couper, 2017). Income values were reported as an open response so values were changed to numeric manually and any value range listed was changed to the respective average. Open ended questions were manually coded into defined groups based on similarity of meaning. This process was done as recommended by O'Cathain and Thomas: responses were briefly read, a coding frame was developed to represent the thematic content, and codes were assigned to all comments manually (O'Cathain & Thomas, 2004). In the present work, questions are used for analysis excluding demographic information. The remaining 15 questions are discussed separately.

For further analysis, specific answers to questions were grouped by unique values and compared against total respondents of a second question from which proportions were calculated for each respective category in a question, as used by Šikić-Pogačar and others (Šikić-Pogačar et al., 2022).This method was used to draw comparisons between Q15 and Q20 and Q15 and Q23. Data processing and analysis was performed using R studio and Python in Jupyter Notebook (Kluyver et al., 2016; R Core Team, 2022).

Due to the significant amount of data collected in this survey, this chapter includes the data from question blocks 4 and 5 only. This allows for comparisons between data on home fermentations with participant demographics.

3.3.4 Limitations

As with all survey tools, there are inherent limitations to this study. While the participant pool was sufficiently large, the demographic data indicates a high proportion of respondents were female, aged 18-39, white, and had achieved a level of education above a high school diploma.

The distribution methods for the survey, including university affiliated emails and social media posts, likely influenced this skewing of the sample population to a younger and educated population. Moreover, previous studies using non-specifically targeted survey tools have found generally in survey participation, participants that are assigned female at birth are more inclined to participate (Becker 2022). Also, women are also the majority gender demographic currently enrolled in higher education (Parker, 2021). Although this survey was available to and advertised to individuals outside of the U.S., 87% (n=656) resided within the US with other participants from primarily Canada, Argentina, Brazil, and the UK. While the sample size provides sufficient power for analysis, it should be noted that correlations should not necessarily be extrapolated to demographic populations with low representation in the study.

3.4.Results

3.4.1. Demographics of survey respondents:

A total of 751participants were included in analysis after incomplete and duplicate entries were removed. As shown in Table 1, 463 respondents (61.65%) identified as female, 258 respondents (34.35%) identified as male, 14 (1.86%) identified as non-binary, 2 (0.27%) identified as transmale, 1 (0.13%) identified as transfemale, and 13 participants (1.73%) selected "prefer not to respond." The highest number of respondents were in the 18-29 age range (n=302, 40.21%), followed by 30-39 (n=232, 30.89%), 40-49 (n=106; 14.11%), 50-59 (n=49; 6.52%), 60-69 (n=45; 5.99%) and 70 and above (n=17; 2.26%). Participants were also asked to report their highest level of education: 2.53% (n=19) of participants had received no formal degree, 7.99% received a high school degree (n=60), 11.98% received an associate degree (n=90), 41.41% received a bachelor's degree (n=311), 24.63% received a master's degree (n=185),10.12% received a doctoral or professional degree (n=76), and 1.20% preferred not to respond (n=9). A total of 158 (21.04%) of participants identified as Hispanic or Latino(a) and 593 (78.96%) identified as not Hispanic or Latino(a). The majority of respondents were White (n=532; 70.84%), followed by Asian (n=95; 12.65%), two or more races (n=35, 4.66%), Black/African American (n=21; 2.80%), other/not listed (n=18; 2.40%), Native Hawaiian/other Pacific Islander (n=15, 2.00%), and American Indian/Alaska Native (n=7; 0.93%) and a total of 26 (3.46%) preferred not to respond. The US was the primary country of residence of respondents (n=656; 87.35%) with only 85 respondents residing outside of the US and residing in 29 countries. Most international respondents were from Canada (n=11) and Argentina (n=10). All 50 states had at least one participant, with the majority from California (n=314, 41.81%).

3.4.2. Types of fermented fruits and vegetables produced by survey participants

Participants were asked in Q15 if they currently ferment fruits and vegetables at home to which 49.20% (n=367) responded “Yes” and 50.80% (n=379) responded “No” (Appendix 3 Table 2). Of the 751 total respondents, 5 (0.67%) did not respond to this question. If participants selected “yes” they were directed to Q16 which asked for an open response list of fruits and vegetables fermented at home. At the top of this list were cabbage (n=202; 53.30%), cucumber (n=88; 23.22%), alliums (n=54; 14.25%), carrot (n=52; 13.72%), peppers (n=48; 12;66%), and citrus (n=45;11.87%) (Appendix 3 Table 2). A total of 38 coded types of fruits and vegetables were reported for this question with the least responses of interest in fermenting kvass (n=2; 0.52%), eggplant (n=2; 0.52%), bamboo shoot (n=2; 0.52%), avocado (n=2; 0.52%), salsa (n=3; 0.79%), potatoes (n=3; 0.79%), pear (n=3; 0.79%), passion fruit (n=3; 0.79%), and beans (n=3; 0.79%). Responses also included radish (40; 10.55%), apple(n=22; 5.80%); leafy greens (n=22;

5.80%), tomatoes (n=18; 4.75%), beets (n=17; 4.49%), pineapple (n=16; 4.22%), berries (n=15; 3.96%), stone fruit (n=15; 3.96%), grape (n=13; 3.43%), assorted vegetables (n=11; 2.90%), assorted fruits (n=9; 2.37%), olives (n=9; 2.37%), turnip (n=8; 2.11%), asparagus (n=6; 1.58%), cauliflower (n=6; 1.58%), ginger (n=6; 1.58%), green beans (n=5; 1.23%), vinegar (n=5; 1.23%), kiwi (n=4; 1.06%), and mango (n=4; 1.06%). Some reported making fermented products that do not have fruit and vegetables as the major fermentable substrate but may contain fruit in secondary fermentations such as: kombucha (n=11; 2.90%) and water kefir (n=2; 0.53%). FFs not made from fruits and vegetables, such as bread, yogurt, and cheese, were excluded from the coding of these results.

3.4.3. Resources utilized as reference material for procedures for in-home fermentations

In Q17 participants were asked what resources they use as a source of information for the ingredients and processes for fermenting fruits and vegetables at home. The largest number of participants (46.32%, n=170) selected the resource category of "books" as the source of fermentation guidance including resources referenced in our survey such as *Farmhouse Culture Guide to Fermentation, NOMA Guide to Fermentation, the Art of Fermentation, Fermented Vegetables*, and *Wild Fermentation* (Appendix 3 Table 3). A total of 115 (31.34%) participants selected textbooks, 91 (24.80%) reported using the USDA Complete Guide to Home Canning, and 89 (24.25%) selected university extension publications. A total of 51 (23.90%) participants reported using websites as a resource, 21 (5.72%) reported using blog posts, and 50 (13.62%) reported using other sources as resources. Coded free responses to "other" included personal knowledge (n=7), community knowledge (n=28), and social media accounts (n=2). A total of 12

(3.27%) of participants reported using none of the listed resources for fermenting fruits and vegetables at home (Figure 1).

3.4.4. Commodities of interest for in-home fermentation by survey respondents

In Q18, participants were asked the types of fruits and vegetables are of interest for fermenting but are currently not types of fruits and vegetables they ferment at home. From a list of 14 fruits and vegetables (apple, berries, pears, citrus, olives, cranberries, cucumbers, peppers, tomatoes, cabbage, carrots, onions, leafy greens, mixed vegetables, other), cucumbers (n=138, 37.60%), berries (n=107, 29.16%), peppers (n=105, 28.61%), olives (n=104, 28.33%), and cabbage (n=97, 26.43%) were the most frequently selected by participants (Figure 2). Tomatoes (n=95; 25.89%), citrus (n=89; 24.25%), apple (n=88; 23.98%) carrots (n=86; 23.43%), and mixed vegetables (n=75; 20.44%) were also identified as commodities of interest for fermenting. The least frequently selected fruits and vegetables by participants were pears (n=69; 18.80%), leafy greens (n=68; 18.53%), onions (n=62; 16.89%), and cranberries (n=54; 14.71%). A total of 22 (5.99%) participants selected "other" and the free responses included plant-based cheese (n=3), stone-fruit (n=2), miso (n=2), and eggplant (n=1) (Appendix 3, Table 4).

3.4.5. Comparisons of demographics with survey responses

Demographic information, of Q20 and Q23, was compared to responses to whether the respondent currently fermented fruits at vegetables at home (Q15). In the age demographic, those 70 and above (total respondents n=17; respondents fermenting n=12; 75.00%) were the age demographic with the highest reported level of home preparation of FFs followed by 60 to 69 years old (total respondents n=45; respondents fermenting n=31; 72.09%) (Figure 3, panel A).

The age group of 18 to 29 years reported fermenting fruits and vegetables at home less frequently (total respondents n=302; respondents fermenting n=91; 30.13%).

This process was replicated between Q15 and Q23, reported participant highest level of education. The largest proportion of participants fermenting at home were those with “no formal degree” (total respondents n=19; respondents fermenting n=13; 68.42%), (Figure 3, panel B.). The smallest proportion of participants that ferment at home were those with a “Master’s degree” (total respondents n=185, respondents fermenting n=87; 47.28%) and high school degree or diploma (total respondents n=60; respondents fermenting=29; 48.33%). Of participants with a “Doctoral or Professional degree” 48.68% (total respondents n=76; respondents fermenting n=37) ferment fruits and vegetables at home.

3.5. Discussion

FFs provide a convenient and safe way to consume fruits and vegetables through extending their shelf life and the potential for enhanced nutrition or health benefits (Marco et al., 2017). The recent popularity of FFs in the US has led to increased availability of many products in grocery stores, supermarkets, and dining establishments, with sauerkraut, kombucha, olives, and kimchi experiencing notable growth in this sector (Domonoske & Schneider, 2020; Nielson-Stowell, 2017; Ramos et al., 2022). Here, we found that approximately 50% of individuals that participated in our survey were actively fermenting fruits and vegetables in the home. Respondents were invited to participate in a survey related to consumption and preparation habits for FFs. As such, the participant pool likely screwed more toward a group that had a higher propensity to ferment their own foods because they have a general interest in FFs. Regardless, the participant pool that did ferment food (>300) provided important information on current FF production trends, resources utilized, and interest in creating other FFs that can be beneficial for conducting future research and developing extension education programs.

The results of this survey reveal that in-home fermentation practices are most prevalent for commodities that are also fermented on a commercial scale, cabbage and olives for example. While we did not determine the motivation for fermenting products in-home as part of this survey, in our larger survey we found a primary motivation of consumers for consumption of FFs was taste. Consumers are likely driven to produce FFs with familiar sensory attributes, which may explain the correlation of similarities between food fermented in-home and those available at the market. Studies have speculated that cultural shifts towards a Western lifestyle and a lack of accurate education has decreased collective knowledge and understanding of FFs' value and preparation practices (Šikić-Pogačar et al., 2022; Watson, Ngesa, Onyang'o, Alnwick, & Tomkins, 1996). Historically on a global scale, household and non-commercial fermentations contributed to the production of a diverse range of fermented products (Sõukand et al., 2015). With industrialization many of these regional and traditional fermentations fell out of popularity, leading to a loss of generational knowledge on production practices and nutritional importance of FFs (Šikić-Pogačar et al., 2022; Sõukand et al., 2015). The demographic information collected in this survey indicates respondents lack parity in ethnic and racial diversity. With most respondents selecting "white" and "non-Hispanic" this also likely contributed significantly to the most commonly in-home fermented products being those most familiar in the Western diet.

To gain insights into the resources utilized for in-home ferment production participants were asked to select which types of reference materials were utilized for fermentation. The most frequently selected references were lay press books such as *Farmhouse Culture Guide to Fermenting* and *The Noma Guide to Fermentation* (Lukas & Petersen, 2019; Redzepi & Zilber, 2018). The authors of these works have extensive experience in fermentation, though they are not affiliated with academic institutions. With increased popularity of and demand for FFs, more information is readily available online to individuals however this content is not necessarily vetted for accuracy and safety (Ramos et al., 2022). The results of this survey suggest a high proportion of individuals obtain information from YouTube. The USDA Complete Guide to Home Canning and extension publications from various land-grant institutions on the production of fermented sauerkraut, cucumbers, and olives are available online for downloading. However, there is a lack of information available on YouTube from these institutions. There is an opportunity to develop new extension resources on fermented fruits and vegetables and adaptation of this new, as well as existing content, into YouTube videos and online media has the potential to increase accessibility (Stroud & Christian, 2021; USDA, 2009).

Estimates of food loss in the US occurring at the retail and consumer level topped $165 billion in 2008. The top three types of food wasted based on value are meat, poultry, and fish (41%), vegetables (17%), and dairy products (14%) (Buzby & Hyman, 2012). In our survey, nearly half of respondents reported fermenting fruits and vegetables at home. Cabbage was overwhelmingly the most common fruit or vegetable to be fermented at home by survey respondents and is one vegetable that has Extension and USDA resources for production in home. However, there was interest in fermentation of a wide variety of commodities including cucumbers, berries, peppers, leafy greens, and many other commodities that are considered highly perishable. There is potential to expand in home fermentation as a mechanism of preservation for other commodities that often-become food waste if resources can be dedicated to understanding the key parameters (i.e., salt concentration, other ingredient ratios, temperature and time, sensory attributes) to ensure safety and quality. While our study did not evaluate in-home fermentation practices related to meat, poultry, fish, or dairy there is potential to develop best practices for fermenting these types of commodities as well to help reduce food waste at the consumer level.

Reducing food waste is important not only because of economic and environmental impacts, but because increasing numbers of the population are food insecure. A 2010 survey found that 49 million people lived in food insecure households in the US out of a population of 304 million (Coleman-Jensen, Nord, Andrews, & Carlson, 2012). This correlates to 14% of U.S. households struggling with access to food (Buzby & Hyman, 2012; Coleman-Jensen et al., 2012). Since 2011, levels of food insecurity have declined slightly to now 10.2% of households experiencing some level of food insecurity in 2021 (USDA Economic Research Service, 2022). It is well established that food insecurity disproportionally impacts minority populations and single-parent families (USDA Economic Research Service, 2022). In home food preservation is often included in the discussion of how to reduce national levels of food insecurity. Equipment required for home canning (water bath canners, pressure canners, jar and lid systems) may not be accessible by low-income households. Preservation via fermentation would have a lower cost barrier compared to other preservation methods, as the major costs would be a fermentation vessel and ingredients. Resources for recipes and best practices would need to be low-cost, include a diversity of ingredients, have cultural relevance, and be accessible in multiple languages and formats, to have impact. As previously discussed, our survey did not include many minority participants and therefore follow-up studies should aim to assess which fermented products would have the most relevance based on cultural food practices.

3.6. Conclusion

In a group of home fermenters of fruits and vegetables, the most fermented commodity was cabbage. There was a general interest among respondents to ferment additional fruits and vegetables beyond their current practices. The most popular resources for in-home fermentation were books and YouTube. There is an opportunity for Cooperative Extension to provide more information to consumers on the production of fermented fruits and vegetables and to deliver the content via multiple channels, increasing accessibility and awareness.

3.7 Figures and Tables

Table 1. Survey participant demographics from Q19 through Q24. Total survey size n=751.

Characteristics	**N=751**	**%**
Gender		
Male	258	34.35
Female	463	61.65
TransMale	2	0.27
TransFemale	1	0.13
Non-binary	14	1.86
Prefer not to respond	13	1.73
Age		
18-29	302	40.21
30-39	232	30.89
40-49	106	14.11
50-59	49	6.52
60-69	45	5.99
70 and above	17	2.26
Ethnicity		
Hispanic or Latino(a)	158	21.04
Not Hispanic or Latino(a)	593	78.96
Race		
American Indian/ Alaska Native	7	0.93
Asian	95	12.65
Black/African American	21	2.80
Native Hawaiian/ Other Pacific Islander	15	2.00
White	532	70.84
Two or more races	35	4.66
Other/not listed	18	2.40
Prefer not to respond	26	3.46
Highest Education Level		
No formal degree	19	2.53
High school degree or diploma	60	7.99
Associates degree	90	11.98
Bachelor's degree	311	41.41
Master's degree	185	24.63
Doctoral or professional degree	76	10.12
Prefer not to respond	9	1.20
Country of residence		
United States	656	87.35
Outside of United States	85	11.32
Prefer not to respond	10	1.33

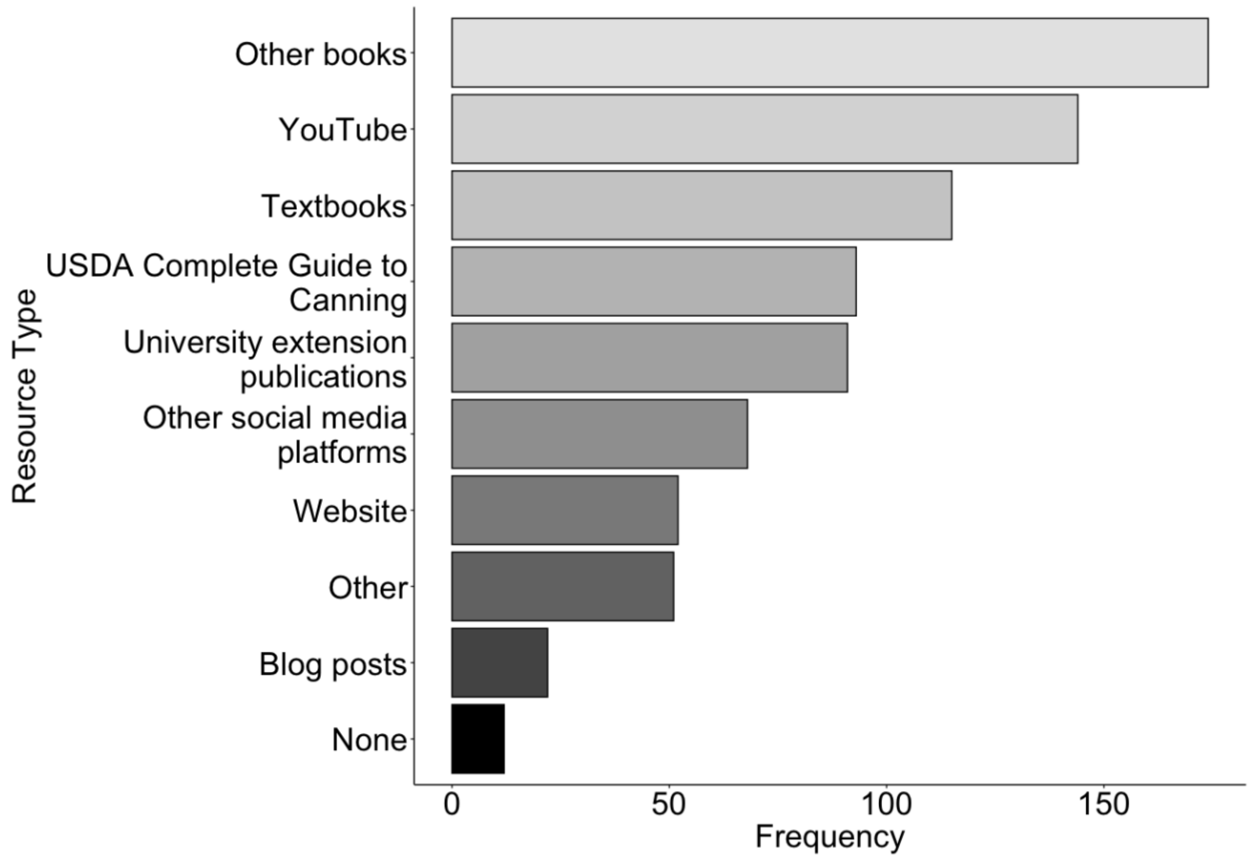

Figure 1. Frequency of resources that participants use as a source of information when fermenting fruits and vegetables at home. Participants could select all that applied. (n=367)

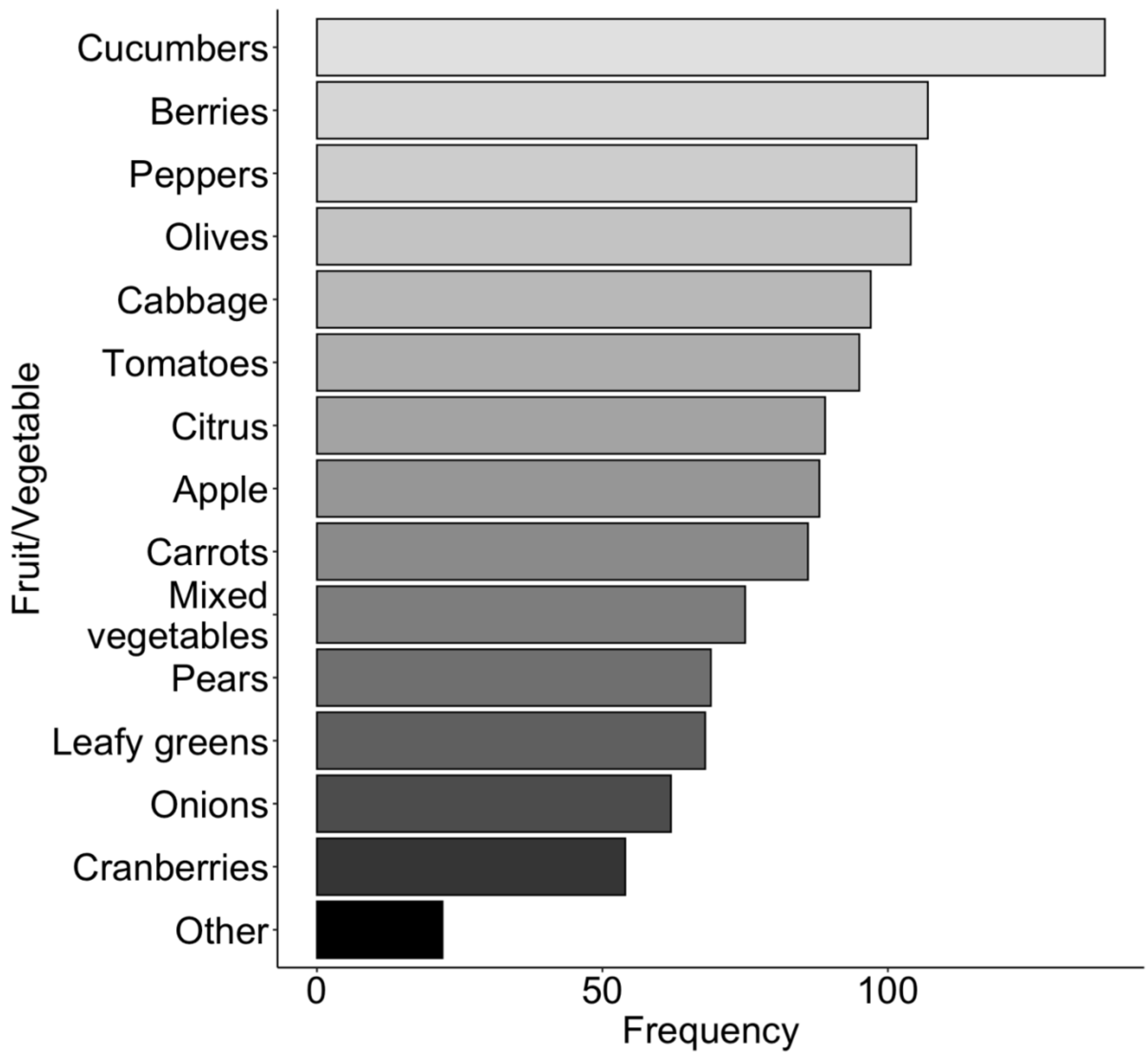

Figure 2. Fruits and vegetables that participants are most interested in fermenting at home, that they are not already making. Participants could select all that applied. (n=367)

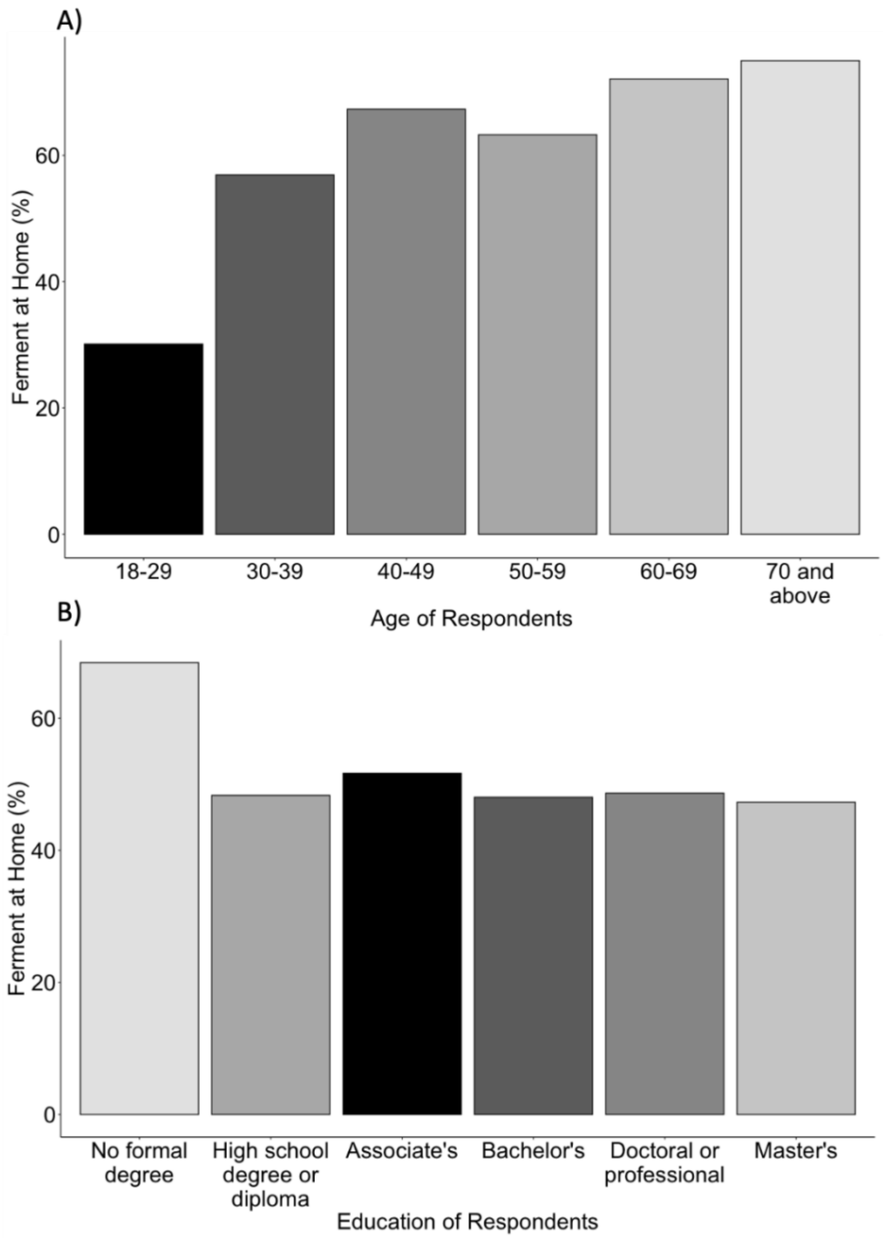

Figure 3. Percentage of participants based on A) age and B) education that ferment fruits and vegetables at home. (n=751)

Chapter 4: Comparative analysis of fermentation dynamics and microbial ecology of cabbage-based fermentations

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Author contribution statement: MH, WVB, HL, ZM, PF, MM, TMR, LW, MLM, and ED, contributed to the study conception and design. Funding was acquired by MLM and ED. Material preparation and data collection of fermentations were performed by MH, HL, ZM, PF, MM, MLM, and ED. Data analysis and first draft preparation was done by MH, WVB, and ED.

4.1. Abstract

Although most fermented fruits and vegetables are made in small batches at home, there is currently a lack of information on the microbial ecology and characteristics of fermented foods (FFs) made at home. The present study aimed to determine the variation in fermentation dynamics introduced by modulating salt concentrations and production environment of small-batch, cabbage-based ferment including red cabbage, carrots, and ginger. Two salt concentrations (2% and 3% NaCl w/w) were evaluated and, to assess the influence of environment on the fermentation process and assess recipe repeatability, the 3% w/w NaCl recipe was replicated in five household kitchens. The brine pH and salinity were measured across a 21-day fermentation period. Fungal and lactic acid bacteria (LAB) growth was monitored in combination with microbiota analysis targeting the V4 region of the 16S rRNA genes. Across all fermentations, the pH decreased to below 4.6 by day 2. Although the pH reductions were similar across all ferments, the microbial population dynamics differed based on salt concentration and

the production environment. Significantly higher counts of LAB were detected in the 3% NaCl compared to 2% NaCl laboratory fermentations over the first week. There were also differences in the dominant microbial community between the 2% and 3% NaCl lab fermentations. *Leuconostoc* were the most abundant genus by day 2 for both fermentations. However, there were differences in composition with five different *Leuconostoc* amplicon sequencing variants (ASV) represented. Fermentations conducted in household kitchens also had differences in *Leuconostoc* ASVs. Alpha diversity decreased with time across all fermentations but was higher for 2% NaCl lab fermentations which had greater abundance of Enterobacterales and lower abundance of Lactobacillales compared to fermentations with 3% NaCl across all measured time points. These results indicate that while slight differences exist in the microbial population dynamics over time among fermentations made in different environments and with different salt concentration, the pH decline and maintenance of the low pH was similar under all conditions tested.

4.2. Introduction

Fermentation is a well-established preservation method to enhance to shelf life of many types of highly perishable foods. Although the practice has been utilized for thousands of years, recent consumer demand for fermented products in the U.S. has led to a surge in the fermented food (FF) product market (Domonoske & Schneider, 2020; Everett, 2019; Manskar & Raskin, 2020; Nielson-Stowell, 2017, 2020; Prince, 2018; Ramos et al., 2022). The COVID-19 pandemic further amplified this popularity. Sales of cabbage-based fermented products, such as sauerkraut, increased by 960% in March 2020 compared to the previous year and news coverage of the potential for sauerkraut consumption to decrease the symptom severity associated with SARS-

CoV-2 infection likely bolstered these sales (Bousquet et al., 2021; Fonseca et al., 2020; Manskar & Raskin, 2020; Nielson-Stowell, 2020; Ramos et al., 2022). Due to the popularity and relative simplicity of production, cabbage-based fermented products are frequently produced by small-scale processors entering the food industry or by consumers in their home (Holmes, 2020; Prince, 2018; Ramos et al., 2022; Snyder et al., 2020). Little information exists comparing the influence of ingredient variations or the production environment on the chemical and microbial dynamics of cabbage-based fermentations produced on a small-scale.

During spontaneous fermentation of cabbage, the native LAB microbiota in raw ingredients is relied on produce sauerkraut. However, the microbiota of fresh vegetables is diverse. Fungi (example genera: *Candida, Saccharomyces, Hansenula*, *Pichia*, *Rhodotorula* and aerobic molds), aerobic bacteria (example genera: *Pseudomonas*, *Flavobacterium*, *Bacillus*), facultative anaerobic bacteria (example genera: *Enterobacter*, *Escherichia*, *Klebsiella*), aerotolerant anaerobes (example genera: *Leuconostoc*, *Lactiplantibacillus*, *Pediococcus*, *Weissella*), and sporeforming anerobes (example genera: *Clostridium*) are common endophytes in vegetables (Hutkins, 2007). Traditional sauerkraut is produced by the addition of salt to shredded cabbage and subsequent spontaneous fermentation by lactic acid bacteria (LAB), a group of bacteria characterized by having lactic acid as a main product of their carbohydrate metabolism. LAB metabolism can be divided in two distinct groups: heterofermentative and homofermentative. Heterofermentative LAB metabolize hexoses and pentoses through the phosphoketolase pathway to produce lactate, acetate, ethanol or acetoin (M. G. Gänzle, 2015; Gaudu et al., 2003). Homofermentative LAB metabolize hexoses through the Emden-Meyerhoff pathway and pentoses through the pentose phosphate pathway to produce primarily lactate (M. G. Gänzle, 2015). The fermentation of sauerkraut has two distinct successive stages, a heterofermentive stage followed by a homofermentative stage induced by the changing ecological conditions during the process (Fred Breidt et al., 2012; Peñas et al., 2017).

Historically sauerkraut fermentation has been associated primarily with five genera of LAB, *Leuconostoc* spp. *(*mainly *L. mesenteriodes), Lactococcus* spp., *Pediococcus* spp., and *Lactiplantibacillus* spp. *(*mainly *L. plantarum*) and *Levilactobacillus* (mainly *L. brevis*), although *L. mesenteroides* and *L. plantarum* are reported to dominate commercial sauerkraut fermentations (Fred Breidt et al., 2012; Z. Liu et al., 2021; Ray & Bhunia, 2008; Xiaozhe Yang et al., 2020; Zabat et al., 2018). During the heterofermentative stage (initial 1-2 days of fermentation at 18°C) a combination of *Leuconostoc* species (*L. mesenteroides* typically predominates) produce carbon dioxide gas as well as lactic and acetic acid (Fred Breidt et al., 2012; Pederson & Albury, 1969a; Peñas et al., 2017). *L. mesenteroides* is less acid tolerate, microaerophilic, metabolizes hexoses and pentoses and has a shorter generation time compared to the other LAB involved in sauerkraut fermentation (Fred Breidt et al., 2012; M. G. Gänzle, 2015; Linda J. Harris et al., 1992; Z. Lu et al., 2003; Peñas et al., 2017). When the level of organic acids produced by *Leuconostoc* spp. reaches approximately 0.7-1.0% and the pH is reduced below 4.5, approximately three days after the start of the fermentation, homofermentative LAB dominate (McDonald, Fleming, & Hassan, 1990). *L. plantarum* metabolizes remaining carbohydrates and produces lactic acid as main end-product, further reducing the pH (Linda J. Harris et al., 1992; McDonald et al., 1990; Plengvidhya et al., 2007). *L. plantarum* is largely responsible for lactic acid production, yielding three to four times as much acid as *Leuconostoc* spp.. (Hughes & Lindsay, 1985).

In spontaneous fermentations, the growth of each species relies on the initial level present in raw ingredients, carbohydrate sources present in cabbage, concentration of salt, and temperature of the fermentation. Assessment of LAB levels in Napa cabbage grown under field conditions demonstrated a low abundance of LAB in cabbage compared to other bacterial species using both culture-based methods (0.51%-1.09% relative LAB abundance when compared to total aerobic plate counts) and 16S rRNA sequence analysis (0.30%-1.68% relative abundance of *Lactobacillales* compared to total number of bacterial reads) (Miller et al., 2019). Genera of LAB important for vegetables fermentation including *Lactobacillus*, *Fructobacillus*, *Leuconostoc*, *Lactococcus*, and *Streptococcus* were detected in cabbage evaluated in the same study (Miller et al., 2019). Fermentation of these field-grown cabbages with 2% NaCl (w/w) revealed low levels of LAB present were sufficient to lead to fermentation and subsequent pH lowering effects, however the microbial population varied depending on the growing site for the cabbage (Miller et al., 2019).

The carbohydrate composition of the cabbage utilized for sauerkraut production can influence the abundance of LAB species in the fermentation (Satora et al., 2021). In white cabbage (*Brassica oleracea* var. *capitata* f. *alba*), the monosaccharides glucose and fructose are the predominant carbohydrates and in some varieties relatively low amounts of sucrose present (Rosa, David, & Gomes, 2001; Tlais et al., 2022). *L. mesenteroides* rapidly initiates the fermentation of vegetables by reducing fructose and sucrose to mannitol and dextran, respectively, which allows other species to subsequently utilize products for growth in addition to residual glucose and fructose (S. Johanningsmeier et al., 2007; Z. Lu et al., 2003; Pederson & Albury, 1969a). Comparisons of sauerkraut fermentations of different varieties of white cabbage revealed that a major factor influencing LAB levels and lactic acid production was the amount of fermentable sugars present and that eight varieties of cabbage had significantly different initial levels of glucose and fructose (Satora et al., 2021). Red cabbage (*Brassica oleracea* var. *capitata*

f. *rubra*) is typically similar in chemical composition to white cabbage but has been less extensively studied in the application of sauerkraut (Adelanwa & Medugu, 2015; Hunaefi, Akumo, & Smetanska, 2013). However, red cabbage has been found to have a higher total anthocyanin content with levels of increased polyphenol and antioxidants, including higher Vitamin C content compared to white cabbage (Ashfaq, Butt, Bilal, Tehseen, & Suleria, 2020). Based on the higher level of carbohydrates, polyphenols, and antioxidants, red cabbage has the potential to serve as a fermentable substrate with enhanced nutritional benefits compared to white cabbage.

Salt is an important component in successful sauerkraut fermentations and the United States Food and Drug Administration (US FDA) standards require that sauerkraut contains “not less than 2% nor more than 3% salt by weight” (Center for Food Safety and Applied Nutrition Office of Regulatory Affairs, 2005). The LAB involved in cabbage fermentation are more salt tolerant compared to many other endogenous microorganisms present in the raw cabbage, such as members of the *Enterobacteriaceae* family (Fred Breidt et al., 2012; S. Johanningsmeier et al., 2007; Peñas et al., 2017). In the spontaneous fermentation of sauerkraut a salt concentration above 1% NaCl inhibits the growth of many spoilage bacteria and fungi, but salt concentrations above 3.5% NaCl may also restrict growth of LAB (Pederson & Albury, 1969a; Xiong et al., 2016). Inclusion of less than 2% salt by weight can result in more growth of undesirable microbes (spoilage microbes and potentially pathogens) and failed fermentations due to competition for carbohydrate sources (He, Li, Wang, Wu, & Yang, 2021). Salt inclusion below 2% may also result in more textural degradation of cabbage and greater variability in the product of fermentation due to less competition from LAB species and growth of yeasts and/or molds (S. Johanningsmeier et al., 2007; Pederson & Albury, 1969a).

The use of LAB starter cultures, such as *L. mesenteroides, L. lactis,* or *L. plantarum,* can permit the use of reduced salt concentrations of 0.5% to 2% (Linda J. Harris et al., 1992; S. Johanningsmeier et al., 2007; Lopez, Shwartz, Pratt, & Powers, 1954; Pederson & Albury, 1969a). The starter cultures will rapidly produce organic acids and possibly bacteriocins that can lead to competitive inhibition of other native non-LAB species preventing them from proliferating to high levels during the early stages of cabbage fermentation. However, starter cultures are not routinely used in industrial production of sauerkraut (Peñas et al., 2017). In some commercial operations "back-slopping," or adding brine from a previous batch of a FF, is used to prime the new ferment with microbes but may lead to an inconsistent quality product (Wiander & Ryhänen, 2005). By the completion of fermentation, the *Leuconostoc* spp. involved in the initial stages of fermentation may no longer be viable in brine and depending on the volume added, native *Leuconostoc* on the cabbage may be inhibited by levels of organic acids and pH in the new batch, as growth of this organism is suppressed when a pH of 5.5 to 5.7 is reached (McDonald et al., 1990). Whether the range of salt concentrations (2-3% salt w/w) used for traditional sauerkraut is appropriate for the small-scale production of cabbage-based spontaneous fermentations including additional ingredients other than cabbage has not been investigated.

The optimal temperature for commercially produced sauerkraut is 18°C, but sauerkraut can be produced in a range of temperatures from 16-24°C (Pederson & Albury, 1969a; Ray & Bhunia, 2008). Temperatures above this optimal range but still within the growth temperature of LAB, between 10-25°C, can result in rapid progression of fermentation (< 3 weeks) with all available sugars being depleted (Drašković Berger et al., 2021; Pederson & Albury, 1969a). Elevated temperatures can lead to more competition at the initiation of fermentation and *Leuconostoc* spp. may not produce sufficient lactic acid, acetic acid and carbon dioxide to inhibit growth of halotolerant spoilage microorganisms, such as yeast (Hughes & Lindsay, 1985; McDonald et al., 1990). This effect would be further exacerbated by reduction in salt levels, allowing a greater variety of spoilage microorganisms to proliferate in a low salt environment (Pederson & Albury, 1969a). At temperatures below the optimal range, the typical LAB involved in cabbage fermentation may be unable to rapidly proliferate leading to failed fermentation or more diverse microbial populations as compared to optimal temperature ranges during fermentation (Marten, Peterson, & Fred, 1929; Pederson & Albury, 1969a; Tlais et al., 2022). For small-scale or home production of fermented cabbage products, the temperature during fermentation cannot be easily controlled. The impact of the fluctuations in temperature during non-temperature controlled spontaneous fermentation cabbage, mimicking small-scale production, has not been investigated for impact on final product quality and safety.

Aerobic organisms dominate in fresh cabbage with populations between 10^4-10^6 CFU/g while the native LAB present only represent a small fraction of the total microflora with levels from 10^2-10^3 CFU/g (Hutkins 2007; Peñas et al. 2010, 2017; Breidt et al. 2012). However, the impact of the microbes in the production environment cannot be ruled out for their contribution to the process, notably for their potential contribution to microbial diversity and abundance during fermentation. Microbial analysis of a commercial vegetable fermentation facility showed that environmental samples contain similar LAB as samples from the fermenting vegetables (Einson et al., 2018). In a home setting, more variability will be introduced via the lack of commercial sanitization practices and equipment for controlling temperature. Different levels of LAB, associated with the production location were noted in small-scale Chinese sauerkraut (Z. Liu et al., 2021). However, the evaluation in microbial community dynamics based on variations introduced by the in-home production environment has not been explored.

Given the popularity of FFs and the interest in producing them on a small-scale local level there is a need to gain an understanding of the influence of environment on the quality and safety of fermentation. In this study, we utilized a variation on traditional sauerkraut, a ferment including red cabbage and carrots, as a model system to evaluate the impact of salt concentration and the production environment on the fermentation process. We assessed the key safety parameter (pH reduction) during fermentation and the microbial dynamics using standard culture-based methods coupled with 16S analysis to gain insight into whether these variables influence the bacterial populations.

4.3. Materials and methods

4.3.1. Preparation of cabbage-based ferment in the laboratory. to mimic small-scale production practices

Purple cabbage, carrots, ginger, canning salt, and distilled water were purchased from a local market in Davis, CA in the fall of 2021. In order to mimic small scale production practices, all utensils, tools, jars, and lids were sterilized by submerging in boiling water for 10 min (USDA, 2009). All ingredient preparation occurred on the open bench top. The purple cabbage was prepared by removing the outer leaves followed by rinsing the cabbage head under deionized (DI) water to remove dirt or debris. The core of the cabbage was removed using a sterile knife and then cabbage was shredded using a sterilized cheese grater on the panel with the largest diameter hole. Carrots were prepared by rinsing in water followed by removal of the exterior layer with a sterilized vegetable peeler. Carrots were shredded on a sterilized cheese grater using the same panel used for shredding the cabbage. Next, 454 g (1 lb.) of finely grated carrots and 907 g (2 lbs.) of grated purple cabbage were weighed and combined along with 10 g of ginger in a large mixing bowl. The cabbage and carrots were mixed at a ratio of 2:1 w/w (907 g cabbage:454 g carrots) with addition of a small amount of grated ginger for flavor (10 g). Two salt concentrations were evaluated to determine the impact on fermentation. Non-iodized NaCl containing no anti-caking agents (i.e. canning salt) was added to achieve a salt concentration of either 2% w/w or 3% w/w in two independent fermentations. Salt was distributed by hand mixing and squeezing vegetables for 10 min until visible liquid accumulated in the bottom of the mixing bowl.

As brine samples were to be collected throughout the fermentation process, a supplemental brine was prepared to ensure that the cabbage and carrots remained completely submerged for the duration of fermentation. The supplemental brine was prepared by combining distilled water with 2% or 3% w/v canning salt for the respective fermentations, boiling until salt was dissolved, and then cooling to room temperature. The prepared salted cabbage and carrot mixture was divided into three portions and 454 g was packed into sterilized glass quart jars with a continuous thread closure. Supplemental brine (956 mL) was added to each of the three jars and a plastic zip-top bag filled with canning salt was used as a weight to keep the cabbage and carrot mixture submerged in the brine. Jars were sealed using a canning ring from a two-piece lid system for a continuous thread closure glass jar. A plastic zip top bag was used to cover the opening prior to apply the canning ring to create a seal and allow for CO_2 expansions during fermentation. The glass jars were placed in a Heratherm microbiological incubator (Thermo Fischer Scientific, Waltham, MA) held at a constant temperature of 22°C. Although 18°C is more optimal for sauerkraut fermentation, 22°C more closely mimicked the temperature of fermentations done in households in this study. Samples were fermented for 21 days.

4.3.2. In home preparation of cabbage-based ferments

To assess the variability associated with production of the cabbage-based ferment in the home, the same formulation was used to produce the fermented product at five individual residences in and surrounding Davis, CA. All utensils, tools, jars, and lids were sterilized by submerging in boiling water for 10 min. Purple cabbage, carrots, ginger, canning salt, and distilled water from the same lot were purchased from a local market in Davis, CA in the spring of 2021. All ingredients and materials were collected and then distributed to households, where preparation of the fermentation was initiated. The in-home ferments included the same ingredient ratios as laboratory-based ferment (907 g cabbage, 454 g carrots, 10 g ginger) and 3% salt (w/w). All individuals fermented in quart glass jars and divided cabbage/carrot mixture equally (454 g/jar) into three jars. Supplemental 3% salt brine was prepared as described in section 2.1 and 956 mL was added to each jar. Each fermentation vessel was weighted and sealed as described in Section 2.1. Temperature indicating strips (Propogate Pro, Silverdale, WA) were affixed to the exterior of each of the three fermentation jars and the vegetable mixture was allowed to ferment at room temperature for 21 days.

On days 0 (day fermentation started), 1, 2, 3, 7, 14, and 21 samples of brine and vegetables were collected for analysis of salinity, pH, temperature, and bacterial community analysis. A fork was sterilized by boiling in water for 10 minutes and then used to collect vegetable mixture from each container which was transferred to a 7 oz Whirl-pak bag. A single use plastic bulb end pipette was used to collect 1 mL of brine which was added to the vegetable mixture in the bag. One sample of this type was collected on all sample days and frozen in the -20°C freezer in each home, with the exception of day 0 and day 21 where two samples were collected. The additional samples on day 0 and day 21 were transported directly to the laboratory on ice for plating on

MRS agar and RBCA as described in Section 2.4. The samples stored at -20°C were transported to the laboratory on ice at the conclusion of fermentation and subsequently stored at -80°C until 16S rRNA gene amplicon DNA sequencing analysis.

Brine was utilized to measure temperature, pH and salinity. At each timepoint, brine temperature and salinity were measured first, immediately after sample collection. A calibrated Traceable salinity/temperature pen (Sper scientific, Scottsdale, AZ) was utilized to measure both temperature (°C) and salinity (ppt). Next, the brine pH was measured using a calibrated Fisherbrand pH pen (Thermo Fisher Scientific, Waltham, MA) with accuracy ±0.1. The temperature, pH, and salinity for three jars was determined and results are reported as the mean ± 1 standard deviation.

4.3.3. Sample collection for cabbage-based ferments conducted under laboratory conditions

For each cabbage-based ferment (2% and 3% salt) three separate jars were fermented and samples were collected for analysis from each individual jar. On days 0 (day fermentation started), 1, 2, 3, 4, 5, 7, 14, and 21 samples of brine and vegetables were collected for analysis of salinity, pH, temperature, lactic acid bacterial counts, yeast counts, and bacterial community analysis. After sampling 2% NaCl on day 21, samples were packaged into small glass jars with a plastic screw lid and stored at 4°C until day 80, when an additional time point of microbial counts, pH, and salinity was measured to assess shelf life of the finished product. Sterile forceps were used to collect a 10 g sample of vegetables which was transferred to a 7 oz Whirl-pak bag. A 1 mL sample of brine was collected using serological pipette and was added to the bag with the vegetable mixture. Two samples were collected in this manner at each time point, one was used for plating and the other was frozen in a -80°C freezer for bacterial 16S rRNA gene sequence analysis. At each time point, 8 mL of brine was collected and transferred to a 15 mL conical tube to measure pH, salinity, and temperature.

4.3.4. Measurement of brine pH and salinity

Brine was utilized to measure pH and salinity, rather than cabbage-ferment homogenate, as recommended for traditional fermented vegetable fermentation (Snyder et al., 2020). At each timepoint, brine temperature and salinity were measured first, immediately after sample collection. A calibrated Traceable salinity/ temperature pen (Sper scientific, Scottsdale, AZ) was utilized to measure both temperature (°C) and salinity (ppt). Next, the brine pH was measured using a calibrated Mettler Toledo Five Easy pH meter (Mettler Toledo, Columbus, OH) with accuracy ± 0.01. The temperature, pH, and salinity for three jars was determined and results are reported as the mean ± 1 standard deviation.

4.3.5. Microbial counts

One of the samples collected in Whirl-pak bags at each timepoint was homogenized by stomaching in a Corning S Blender (Corning, Corning, NY) for 5 min at 650 strokes/min. After stomaching, 100 μL of homogenate was collected using a sterile serological pipette and was transferred to 900 μL phosphate buffered saline (PBS) to achieve a 1:10 dilution. De Man, Rogosa and Sharpe (MRS) agar (Thermo Fisher Scientific, Waltham, MA) was utilized to determine level of LAB. MRS was supplemented with 25 mg/L natamycin to prevent fungal growth. Rose-Bengal chloramphenicol agar (RBCA) (Neogen, Lansing, MI) supplemented with an additional 100mg/L chloramphenicol was utilized to enumerate the levels of fungi in samples. Samples were serially diluted in PBS and plated in triplicate. MRS plates were incubated at 37°C for 24-48 h. RBCA plates were incubated at 37°C for 48-72 h. The limit of detection (LOD) for both RBCA and MRS media was 0.52 log CFU/mL, calculated from 1 CFU present across three replicates for each jar with a total of 300 µL plated. This was determined as a threshold for enumeration. Colonies were counted after incubation and the results are reported as the mean log CFU/mL of the three jars ± 1 standard deviation. One limitation of this method is that the LOD relies on a small plating volume and establishes a low detection threshold which may result in false negative detections when measuring low CFU counts of fungi or LAB.

4.3.6. DNA extraction, library preparation, and sequencing

The second sample collected in a Whirl-pak bag was frozen at -80°C until the conclusion of the fermentation. After all samples at all time points were collected, samples were thawed on ice for DNA extraction and subsequent 16S rRNA gene amplicon DNA sequence analysis. Total DNA was extracted using the QIAGEN DNeasy PowerSoil Pro Kit (QIAGEN, Hilden, Germany) and FastPrep-24 5G bead beating grinder and lysis system (MP Biomedicals, Irvine, CA). The extraction procedure followed manufacturer's instructions, with custom bead beating procedure consisting of two repetitions of 60 seconds bead beating at 6 m/s to enhance lysis of Gram-positive cells. DNA was eluted in 100 µl elution buffer and stored at -20°C for further batch processing. A barcoded PCR was performed amplifying the V4 region of the 16S rRNA gene using custom barcoded primers (Bokulich & Mills, 2012) consisting of 806R reverse primer (GGACTAC**N**VGGGTWTCTAAT), and 515F forward primer (GTG**Y**CAGCMGCCGCGGTAA) with the following PCR conditions: denaturation at 94°C for 3 min, 94°C for 45 s, annealing at 55°C for 1 min and elongation at 72°C for 30 s. Gel electrophoresis at 135V for 35 min was performed to assess success of PCR and used for equimolar pooling of DNA samples (i.e. bright DNA band samples were pooled at 5 µL and samples with a faded band were pooled at 10 µL). After pooling, for the DNA was purified for size selection and removal of primer dimers using wizard® SV Gel and PCR clean-up system (Promega, Madison,Wi) according to the manufacturer's protocol. Ion torrent fragment plus library preparation kit (Thermo Fischer Scientific, Waltham, MA) was used for library preparation following the manufacturers protocol. A final library of 30 pM was achieved and transferred to Ion Chef for templating using the Ion Chef 530 kit (Thermo Fischer Scientific, Waltham, MA). Samples were loaded on 530 Ion torrent chip (Thermo Fischer Scientific, Waltham, MA) and sequenced on the Ion S5 sequencing system (Thermo Fischer Scientific, Waltham, MA) for 850 flows (one initialization per chip).

Raw sequencing data was analyzed using Ion reporter and qiime2 (version 2020.8), in short sequences were demultiplexed using Ionreporter software with default settings. After demultiplexing, raw data was imported into qiime2 and denoised using DADA2's denoise-single version (Callahan et al., 2016). Reads were classified up to ASV level using VSEARCH consensus classifier on the silva SSU database (138.1 update release) (Rognes, Flouri, Nichols, Quince, & Mahé, 2016). Afterwards data was exported and further analyzed in the R environment (version 4.1.2) using tidyamplicons package (version 0.2.2) (https://github.com/SWittouck/tidyamplicons) for visualization and data handling.

4.3.7. Statistical Analysis

All experiments were performed in triplicate. Data was analyzed with R Studio and statistical analysis for enumeration, pH, and salinity data was performed using multifactor variance analysis (ANOVA) with ad-hoc Tukey honest significant differences. P values <0.05 were considered significant.

4.4. Results

4.4.1. Brine pH, temperature, and salinity for laboratory and home-based cabbage fermentations

Environmental measurements were collected at nine sampling points for laboratory produced cabbage ferments (days 0, 1, 2, 3, 4, 5, 7, 14, 21) and on seven sampling points for home ferments (days 0, 1, 2, 3, 7, 14, 21). The temperature of laboratory-based fermentations was maintained at a constant 22°C. The overall average temperature for the measured timepoints during home fermentation was 23.7°C (Figure 4.7). The lowest temperature, averaged for each day, across the five home fermentations was 21.9°C and the highest average temperature was 25.2°C.

The average brine pH across all fermentations (n=7) was 5.8 ± 0.04 with a range from 5.9 ± 0.06 (3% NaCl) to 5.8 ± 0.07 (3% NaCl home) (Figure 4.1). After one day incubation, the 3% NaCl home fermentations reached a pH of 5.0 ± 0.5. This was significantly ($p<0.01$) lower than the comparable lab-based 3% NaCl fermentation that reached a pH of 5.5 ± 0.03. The 2% NaCl fermentation (5.3 ± 0.07) was not significantly different from the other fermentations on day 1. On day 2, the pH of the 3% NaCl home fermentation was lower (pH3.8 ± 0.04) compared to 2% NaCl fermentation (pH 4.0 ± 0.02) and 3% NaCl (pH 4.0 ± 0.03). The pH of both 3% NaCl fermentations continued to decrease from day 2 to day 21. The final pH of 2% NaCl, 3% NaCl, and 3% NaCl home fermentations were 3.9 ± 0.07, 3.6 ± 0.04, and 3.4 ± 0.2 respectively. On day 80, the 2% NaCl fermentation pH was 3.9 ± 0.22.

Salinity measures were in the unit parts per thousand, meaning a 3% NaCl brine would be expected to have a salinity measurement around 30 ppt, and 2% NaCl near 20 ppt. The salinity on day 0 of 3% NaCl home and 3% NaCl lab brines was 35.8 ± 1.88 ppt and 32.4±0.21 ppt, respectively (Figure 4.2). At day 21 of fermentation, the brine salinity values decreased to 26.7 ± 1.53 for 3% NaCl home and 28.2 ± 0.56 for 3% NaCl lab. The 2% lab fermentation brine began at a value of 10.1 ± 0.15 ppt at day 0 and increased to a value of 19.3 ± 0.92 by the end of fermentation at day 21.

4.4.2. Counts of yeast present in laboratory and home-based 2% NaCl and 3% NaCl cabbage-based ferments

Counts of yeast colonies were determined at each time point for laboratory-based ferments. For the sauerkraut made with 2% NaCl, yeast were only detected on day 0, 1, and 2 and were below the detection limit for all other study days (Figure 4.6 Panel A). Counts were highest on day 1 of fermentation reaching a level of 6.88 ± 0.24 log CFU/mL, which was significantly higher compared to day 0 (3.09 ± 0.72 log CFU/mL) ($p<0.01$) and day 2 (3.68 ± 0.35 log CFU/mL) ($p<0.05$). Yeast were detected in the 3% NaCl fermentation on days 0, 1, 2, 14, and 21. Colony counts peaked on day 1 of fermentation to a level of 6.08 ± 0.63 log CFU/mL across three replicates and was significantly higher compared to growth in replicate 'B' on day 0 (0.66 log CFU/mL) and replicates 'A' and 'B' on day 2 (3.50 log CFU/mL) ($p<0.01$). On day 14 only replicate 'C' of the 3% NaCl ferment had detectable counts (1.43±1.25 log CFU/mL) and on day 21 replicates 'A' and 'B' had detectable counts (0.67±1.15 and 0.76±1.33 log CFU/mL). On days 0, 1, and 2 there were no statistically significant difference in yeast counts between 2% NaCl and 3% NaCl fermentations. On day 80 of the sauerkraut made with 2% NaCl, no yeast were detected. For home fermentations (3% NaCl), yeast levels were measured only for days 0 and 21 in one replicate for each location (Figure 4.6 Panel B). On day 0, all 5 locations had yeast present in the ferment at cell counts of 4.84 ± 0.92 log CFU/mL. On day 21 the, only three samples were positive for yeast with an average level of 4.36 ± 1.98 log CFU/mL. Samples from two home fermentations had no detectable fungal counts.

4.4.3. Counts of LAB in 2% and 3% NaCl lab and home-based ferments

In the 2% NaCl lab fermentation, the average LAB counts on day 0 were 2.76 ± 0.15 log CFU/mL. On day 1, the count of LAB in 2% NaCl increased to 5.96 ± 0.16 log CFU/mL and continued to increase through day 21 to a maximum count of 8.61 ± 0.09 log CFU/mL. On day 0, only two of the three replicates of the 3% NaCl lab fermentation had detectable counts of LAB with a combined average of 2.10 ± 0.17 log CFU/mL. On day 1, 3% NaCl had a LAB count of 7.01 ± 0.09 log CFU/mL. The 3% NaCl had a maximum count detected on day 2 (9.82 ± 0.05 log CFU/mL) with a gradual decline from days 3 to 21 with a final count on day 21 of 7.53 ± 0.09 log CFU/mL. The 3% NaCl ferment had significantly higher LAB counts on days 1, 2, 3, 4, 5, and 7 compared to 2% NaCl, while on day 21 LAB counts were significantly higher for 2% NaCl compared to 3% NaCl ($p<0.01$) (Figure 4.3). On day 80 of the 2% lab fermentation, one replicate (B) had no detectable counts of LAB while replicate A had counts of 6.91 ± 0.07 log CFU/mL and replicate C 5.95 ± 0.05 log CFU/mL (Figure 4.8).

LAB were detected in only two of the five 3% NaCl home fermentations on day 0 with an average count of 2.25 log CFU/mL in positive samples. The average LAB count for 3% NaCl home (n=5) on day 21 was 7.69 ± 0.38 log CFU/mL, which was similar to laboratory 3% NaCl counts but significantly lower than 2% NaCl counts at the same timepoint ($p<0.01$) (Figure 4.5).

4.4.4. Alpha diversity shows distinct patterns between household fermentations and is influenced by salinity

Alpha diversity at the genus level was calculated based on Inverse Simpson (accounts for both community evenness and richness). Alpha diversity decreased as fermentation progressed. For the home ferments, alpha diversity decreased from 2.32 ± 0.48 at day 1 to 1.39 ± 0.26 at day 21. A significant difference could be observed among the different households regarding the alpha diversity dynamics. A significantly faster decrease in alpha diversity was observed for Households C and D, as indicated by a significant lower alpha diversity on days 2, 3, 7. On days 14 and 21, no significant difference among households could be observed. For the laboratory conducted ferments, the alpha diversity decreased from 2.80 ±1.01 at day 1 to 1.31 ± 0.14 at day 21. Salinity had a significant impact on the alpha diversity with 3% NaCl fermentations having a significantly lower alpha diversity at days 3, 4, 7, 14 and 21 (Figure 4.12 Panel B).

4.4.5. Sauerkraut fermentations are dominated by LAB

Bacterial community composition was assessed by 16S rRNA gene amplicon DNA sequencing. The initial day of the fermentation, for both home and laboratory ferments, was characterized by the presence of bacterial genera native to the raw produce and members of the Enterobacterales order (e.g. *Pantoea, Erwinia, Enterobacter).* As fermentation progressed, LAB initiated the fermentation with *Leuconostoc* reaching relative abundance of 79% ± 4% relative abundance and 84% ± 9% relative abundance after two days of fermentation for 2% and 3% NaCl laboratory fermentations, respectively (Figure 4.9 Panel B). The relative abundance of

Leuconostoc remained constant throughout the fermentation for both 2% and 3% NaCl laboratory fermentations (Figure 4.9 Panel B).

Community composition at the genus level was comparable between both laboratory fermentations (both were dominated by *Leuconostoc* after one day). However, the 3% laboratory NaCl fermentations had a significantly higher relative abundance of Lactobacillales compared to the 2% laboratory NaCl fermentation and a significantly lower relative abundance of Enterobacterales(Figure 4.10). These differences were also observed at the level of amplicon sequence variants (most specific taxonomic unit possible with this approach). The 2% and 3% NaCl laboratory fermentations differed in ASV composition. *Leuconostoc* ASV1 dominated 2% NaCl fermentations whereas four different *Leuconostoc* ASVs (1, 2, 3, 4) existed together within the 3% NaCl fermentations (Figure 4.9 Panel A).

For the home fermentations, bacterial community dynamics were similar between the four households. There was an increase of *Leuconostoc* after 2 days incubation (81 ± 7% relative abundance). *Leuconostoc* ASV1 dominated the bacterial community of all four households but several different LAB ASVs were also found (Figure 4.9, panel A). *Leuconostoc* ASV2 had a higher relative abundance in household C compared to the other four households and *Leuconostoc* ASV3 had higher relative abundance in household D. *Leuconostoc* ASV4 had a higher relative abundance in households A and B. These differences could reflect the impact of household on the microbial community throughout the fermentation.

Beta diversity was calculated using the Bray-Curtis metric. All four households had similar bacterial communities, but small clusters could be observed for household A and B, whereas C and D overlapped to a greater extent according to PCoA (Figure 4.11 Panel E). These results suggest a possible effect of the household environment on the bacterial community and the presence of different LAB ASVs.

4.5. Discussion

In this study we compared the chemical and microbial dynamics of a cabbage and carrot fermentation utilizing the minimum and maximum salt concentration appropriate to meet the standard definition of sauerkraut (2 and 3% salt by weight). Additionally, we compared environmental variability introduced by preparing the cabbage-based ferment in home kitchens and controlled laboratory conditions.

Traditional, spontaneous ferments of sauerkraut typically begin in the pH range of 5.7 to 6.0 and decrease within 2 to 3 days to a range of 3.2 to 3.9 (S. D. Johanningsmeier, Fleming, & Breidt, 2004; Peñas, Frias, Sidro, et al., 2010; Plengvidhya et al., 2007). The safety and ultimate quality of fermented vegetables relies in large part to the rapid and sustained drop in the pH of the brine. A pH reduction to below 4.6 and sustaining a pH below 4.6 has been suggested as a critical control point (CCP) for small-scale manufacture of fermented vegetables (Snyder et al., 2020). The low oxygen environment achieved during the fermentation process has the potential to allow for *Clostridium botulinum* spore germination, growth, and toxin production should sufficient acid not be produced during fermentation reducing the pH to below 4.6 (Snyder et al., 2020)(K. A. Ito, Chen, Lerke, Seeger, & Unverferth, 1976). Other vegetative foodborne bacteria (Shiga-toxin producing *Escherichia coli* (STEC), *Salmonella* spp., *Listeria monocytogenes*) must also be controlled during fermentation and previous work has shown STEC to be the most acid tolerant of these organisms (reference). Work using fermented cucumbers as a model system, determined the pH and time needed to reach a 5-log reduction of STEC to provide guidance for naturally acidified foods sold without thermal processing (Frederick Breidt & Caldwell, 2011). At a pH of 4.0, 12 days of subsequent fermentation is needed to reach this 5-log reduction of *E. coli* (Frederick Breidt & Caldwell, 2011).

At the beginning of the fermentation, for all treatments, the brine pH was approximately 5.8.. In this study, the pH of all cabbage-based fermentations, regardless of salt concentration or environment, was below pH 4.0 after two days and that pH was maintained for the duration of the study period.

In both the laboratory and home 3% NaCl fermentations the brine salinity decreased slightly from days 0 to 3 and this correlates to other studies showing the sauerkraut brines have decreases in salinity during the progression of fermentation (Major et al., 2022). The decrease is salinity has been attributed to osmotic release of water from cabbage pieces (K. W. Lee et al., 2018). The observed increase in salinity of the 2% NaCl fermentation is likely a result of insufficient mixing of salt with vegetable ingredients on day 0, as the expected value would be near 20 ppt.

The 2% w/w NaCl sauerkraut had higher yeast counts on day 0 than the laboratory 3% NaCl fermentation (below LOD). The starting ingredients were the same, however the cabbage and carrots were from different lots. This indicates that the vegetable microflora varies between lots of products. This has been demonstrated for the bacterial community of Napa cabbage, with cabbage grown in different fields having variation in the bacterial population (Miller et al., 2019). Yeast counts at day 21 of all 2% ferment replicates were below the LOD. These findings are consistent with other cabbage-based fermentations, where yeast growth decreases from a peak at the onset of fermentation to be undetectable by day 10 or later (Viander, Mäki, & Palva, 2003).

Interestingly, yeast were detected in later stages of 3% laboratory fermentations and a subset of 3% home fermentations. Yeast were measured on days 0 and 21 in 3% home fermentations and fungi was detected at both time points. High salt concentration, typically above 3%, can support growth of yeast in the later stage of fermentation and can lead to development of pink color in traditional sauerkraut (Fred & Peterson, 1921). This fungal growth is attributed to the lack of carbon dioxide production from homofermentative LAB that are favored at higher salt concentrations, as CO_2 present provides a flushing effect and replaces environmental oxygen preferred by fermentative LAB (Pederson & Albury, 1969a).

Sampling 2% NaCl ferment at day 80 was to assess the viable levels of LAB that persist in packaged product after long-term refrigeration, as may occur with small scale producers or home fermentations. The large variability seen in counts of LAB highlighted an error in the experimental set up, where jars of cabbage mixture had been exposed to oxygen and environmental conditions at nine sampling times previously. Possibly due to this environmental exposure and gradual brine removal, visible mold was evident on multiple of the packaged jars that had been reserved for shelf-life testing and were disposed of after day 80. Previous work has shown that growth of spoilage microorganisms, in a fermented product, during extended periods of storage leads to a degradation of lactic acid and out competition of LAB through competitive exclusion. (S. D. Johanningsmeier et al., 2012).

The plating done to enumerate LAB colonies provides a quantifiable estimate of culturable LAB present in the fermentations, however there are limitations with this approach. MRS was selected for this study as most LAB will be able to grow with the available nutrients thus the counts obtained can generally represent the total LAB population. Comparison of culturable LAB isolates from kefir grains and beverage with results of culture independent methods found that the dominant species, *Lactobacillus kefiranofaciens,* could not be cultured on MRS (Kesmen & Kacmaz, 2011). This can occur from the media lacking necessary supplementation to support colony growth, or damage of the species in the sampling and culturing process. Thus, the combination of culture-dependent and culture-independent methods are applied in the present study to holistically consider the microbiota of non-traditional cabbage-based ferments.

The present study compared the impact of initial salt concentration (2% NaCl vs. 3% NaCl) on the bacterial community dynamics in a cabbage-based fermentation produced in a controlled laboratory environment (constant temperature of 22°C). In the day 0 and 1 samples, *Enterobacteriaceae* had the highest relative abundance in 2% NaCl ferments and 3% NaCl ferments. This family of bacteria is commonly found within the microbial community of fresh produce (Leff & Fierer, 2013). The microbial community of spontaneous vegetable fermentations is characterized by this group of bacteria within the first days of fermentation (E. J. Park et al., 2012; Wouters, Grosu-Tudor, Zamfir, & De Vuyst, 2013; Wuyts et al., 2018). After 2 days, *Leuconostoc*, a heterofermentative member of lactic acid bacteria, dominated the fermentation resulting in a rapid decline of pH through production of organic acids. Similar dynamics, with an initial dominance of heterofermentative LAB, has been documented in sauerkraut (Gaudioso et al., 2022; Plengvidhya et al., 2007; Xiaozhe Yang et al., 2020), other fermented vegetables such as carrots (Raghuvanshi et al., 2019; Wuyts et al., 2018), and kimchi (Jung et al., 2011).

At the genus level, both 2% and 3% NaCl lab ferments had similar microbial communities, however the 3% NaCl ferment had higher relative abundance of Lactobacillales and significantly lower relative abundance of Enterobacterales than the 2% NaCl fermentation.

These findings are similar with fermentations of Chinese sauerkraut, where growth of LAB was higher in a 2.5% NaCl ferment than fermentations at or below 2.0% NaCl and above 3% NaCl (X. Yang et al., 2020). On an ASV level a difference between LAB ASVs dominating the fermentation could be observed. *Leuconostoc* 1 dominated the 2% NaCl fermentation whereas the 3% NaCl lab fermentation had co-occurrence of five different *Leuconostoc* ASVs. The 2% NaCl fermentation had greater abundance of the ASV *Weissella* 1 through day 4 of fermentation. In sauerkraut fermentations, growth of *Lactiplantibacillus* typically dominates the second, homolactic, stage of fermentation around day 7 of fermentation (Pederson & Albury, 1969a; Zabat et al., 2018). In the bacterial DNA isolation step of this study, an additional length of bead beating incorporated was to account for presence of complex carbohydrates, microbial metabolites, and salt requiring a more rigorous extraction method to break the cell wall of LAB (N. Zhao et al., 2017). However *Lactiplantibacillus* species can be notoriously difficult to detect with 16S rRNA gene DNA sequencing, so there is a possibility that the detection accuracy affected results (Church et al., 2020)**.** This recipe contained raw ginger, which has been show to exert antimicrobial effects against primarily gram positive bacteria, and may have inhibited their growth (Geremew, Kebede, & Andualem, 2015). The higher salinity in the 3% fermentation reduces oxygen solubility in the liquid brine, limiting the growth of aerobic microorganisms present and allowing more salt tolerant species of LAB to gain dominance with production of antimicrobial compounds and competitive inhibition by organic acid production (Choi et al., 2018). These differences in the microbial community suggest an effect of salt concentration, however both fermentations were prepared as asynchronous batches so the effect of raw produce variability should not be overlooked.

The bacterial community between the cabbage-based ferment with 3% NaCl prepared under laboratory conditions to ferments prepared in five different home kitchens was also compared. On the genus level both household fermentations and lab fermentations were dominated by *Leuconostoc* for the duration of the fermentation period (starting from day 2). Household specific ASVs of *Leuconostoc* could be observed with *Leuconostoc* 2 having a higher relative abundance in household C compared to the other four households and *Leuconostoc* 3 having higher relative abundance in household D. *Leuconostoc* 4 had higher relative abundance in households A and B. These differences suggest an effect of the household environment on the microbiota present in the final ferment. A study of Chinese sauerkraut from three different households similarly found differences in the abundance of *Clostridium, Enterobacter,* and *Lactobacillus* supporting that the environment of fermentations may influence the final microbial composition (Xiaozhe Yang et al., 2020). In sampling of sauerkraut produced in two different artisanal production facilities, different abundances of *Leuconostoc* and *Lactiplantibacillus* were detected in final products (Gaudioso et al., 2022). Environmental sampling of a vegetable processing facility found that bacterial communities of fermentation rooms were similar to the community on raw vegetable surfaces, suggesting that bacterial community of fermentations may become widely distributed in a production setting (Einson et al., 2018). Further studies are needed to assess influence of production and manufacturing environment on the final community, and how differences in the microbiota can affect the metabolome of sauerkraut.

In all fermentations, alpha diversity decreased from day 1 to day 21, which is in agreement with results of other cabbage-based fermentations as LAB outcompete and inhibit growth of other organisms (Fred Breidt et al., 2012; Müller et al., 2018). Between the laboratory-based fermentations, 3% NaCl had significantly lower alpha diversity on days 3, 4, 7, 14, and 21 which can be attributed to the higher salt content inhibiting growth of non-LAB organisms and is supported from higher total LAB abundance in the 3% ferment. These results are consistent with microbial dynamics studied in other sauerkraut productions, of a diverse bacterial community on fresh vegetables being rapidly transformed and controlled by LAB (S. D. Johanningsmeier et al., 2012; Wolkers-Rooijackers, Thomas, & Nout, 2013; Zabat et al., 2018). However, the two laboratory produced fermentations were not synchronous; the 3% NaCl ferment was produced in April, while the 2% NaCl ferment was produced in February. Fermentations done in households were prepared in April of the prior year. Thus, initial ingredient variability prevents conclusions from these comparisons as seasonal and inherent fluctuations in the raw cabbage and carrot microbiota hinder fermentation repeatability (Peñas, Frias, Sidro, et al., 2010; Rosa et al., 2001).

Presently, few studies have examined the application of red cabbage in a cabbage-based fermentation or the fermentation of sauerkraut on a small-scale with environmental variation. Here we evaluated the use of the red cabbage for the production of cabbage based ferment using the low (2%) and high (3%) range of salt required to meet the sauerkraut standard of identity (Center for Food Safety and Applied Nutrition Office of Regulatory Affairs, 2005). The pH of these fermentations was reduced to below the 4.6 after 48 h and low pH was maintained for the duration of fermentation. The fermentations were dominated by the genus *Leuconostoc* with differences in ASVs dependent on salt concentration and environment of fermentation. Alpha diversity decreased across all fermentations after day 1, though the Alpha diversity for 2% NaCl was higher compared to 3% NaCl fermentations at multiple time points. Use of 3% salt allowed for LAB to establish dominance early in the fermentation whereas use of 2% salt allowed more competition for LAB amongst endogenous species and created more overall bacterial diversity. Although working to establish the safety of ferments is critically important, the evaluation of flavor, texture, and general consumer acceptability should be considered in future work. There is also a need for more validated recipes of other fermented fruits and vegetables beyond cabbage.

4.6. Figures and tables

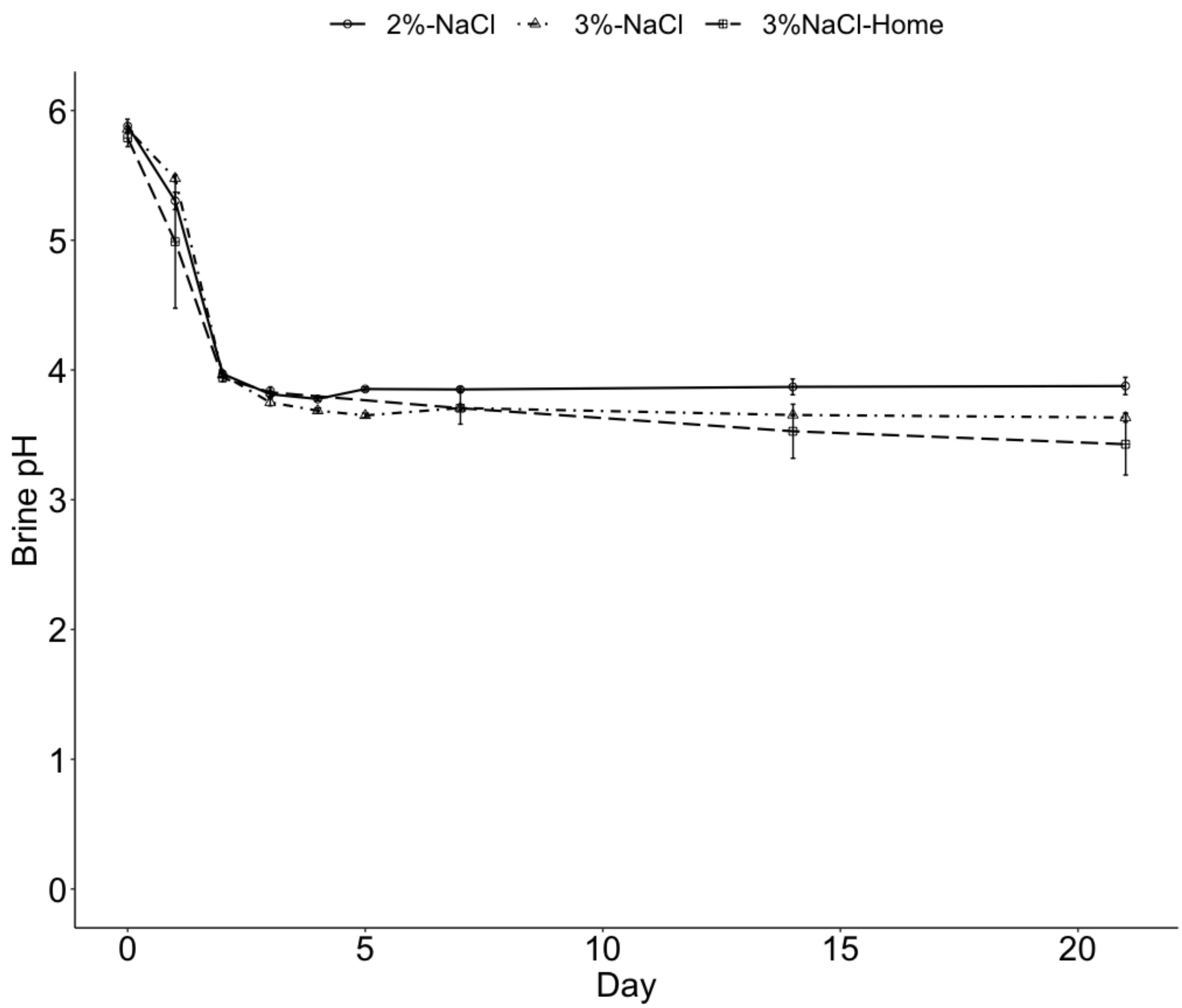


Figure 4.1. pH of 2% NaCl, 3% NaCl fermentations conducted in laboratory at 22°C and 3% NaCl fermentations conducted at five individual homes. All fermentations were performed in triplicate. Samples were collected on days 0, 1, 2, 3, 4, 5, 7, 14, 21.

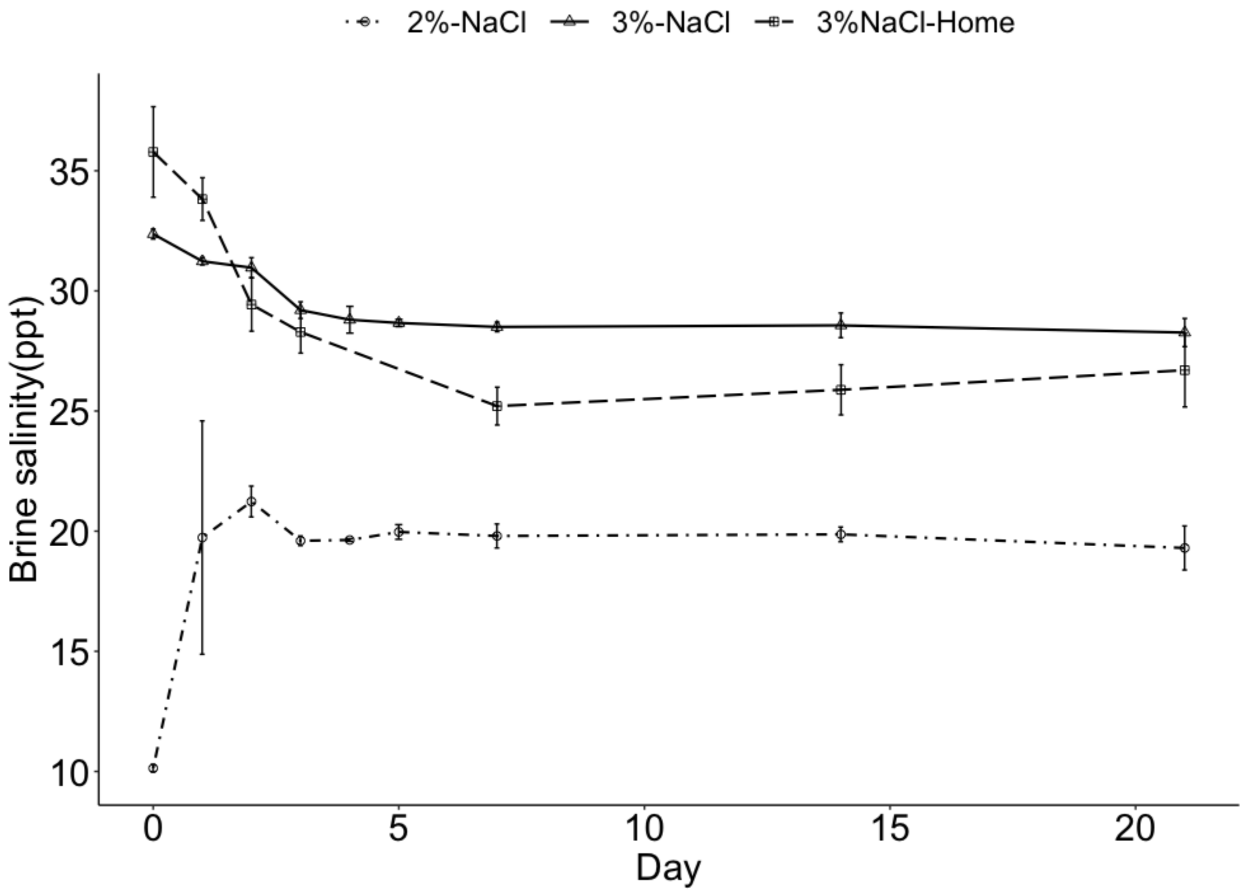


Figure 4.2. Salinity of 2% NaCl, 3% NaCl fermentations conducted in laboratory at 22°C and 3% NaCl fermentations conducted at five individual homes. All fermentations were performed in triplicate. Samples were collected on days 0, 1, 2, 3, 4, 5, 7, 14, 21.

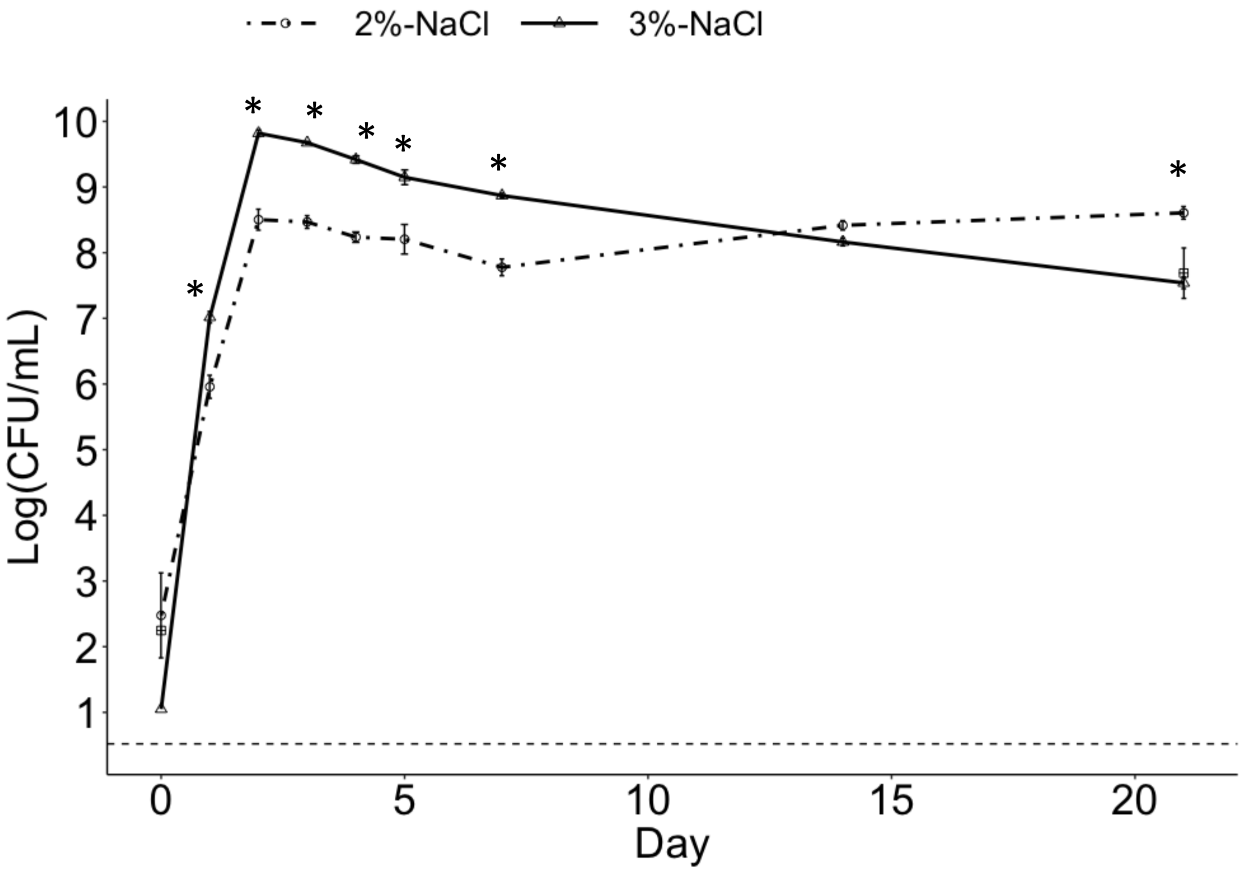


Figure 4.3. Dynamics of LAB growth in cabbage-based fermentation produced under laboratory conditions. All fermentations were performed in triplicate. Samples were collected on days 0, 1, 2, 3, 4, 5, 7, 14, 21. *: p<0.01.

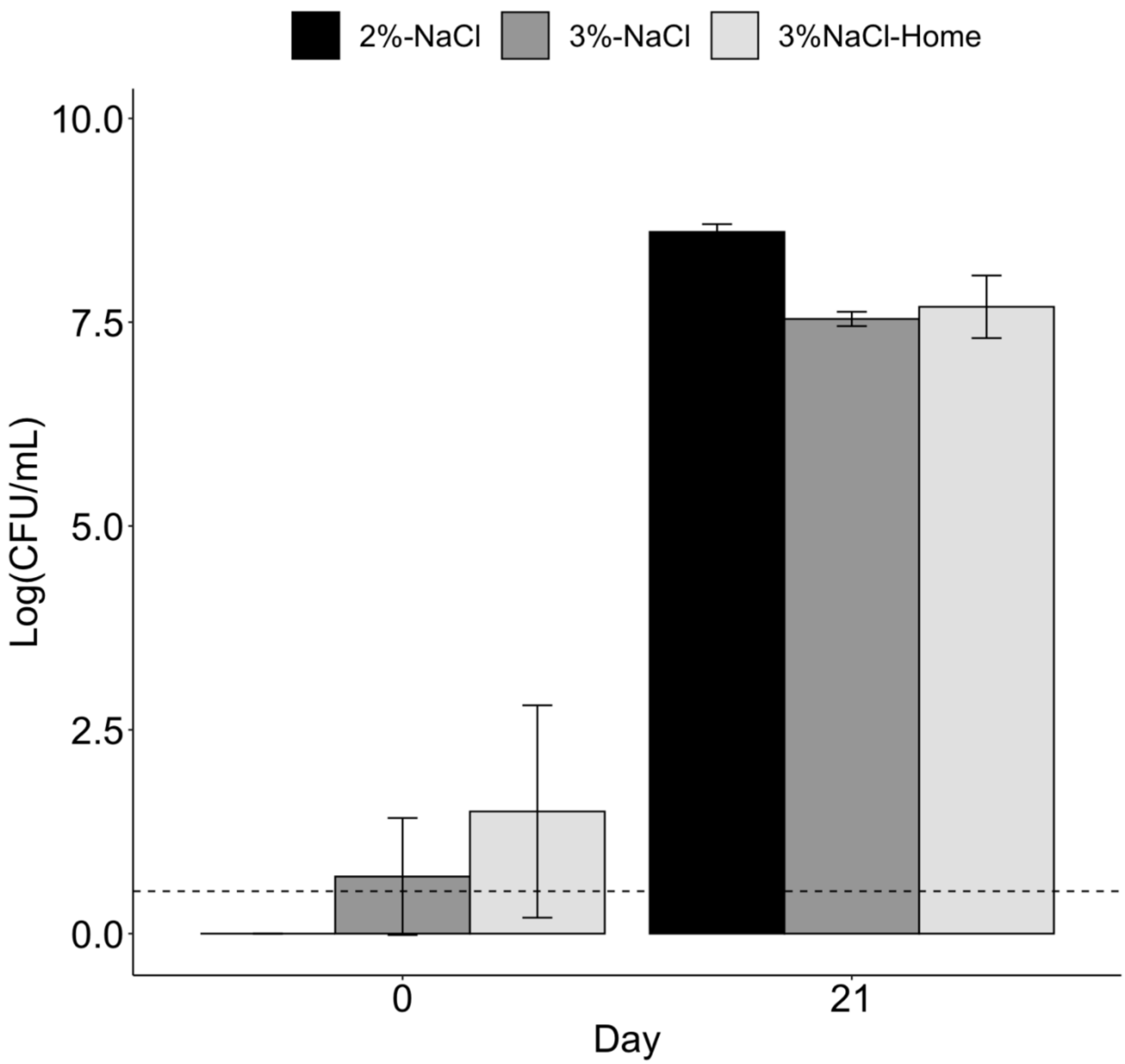
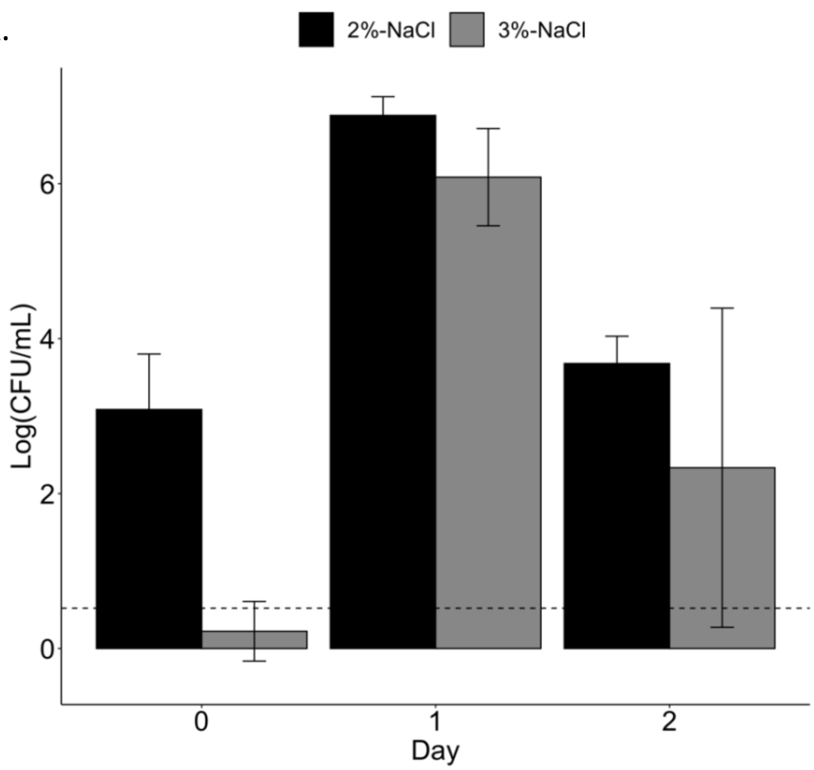


Figure 4.4. Comparison of total LAB colony counts on day 0 and day 21 of LAB in 2%, 3% fermentations conducted under laboratory conditions and 3% home based fermentation. All fermentations were performed in triplicate.

A.



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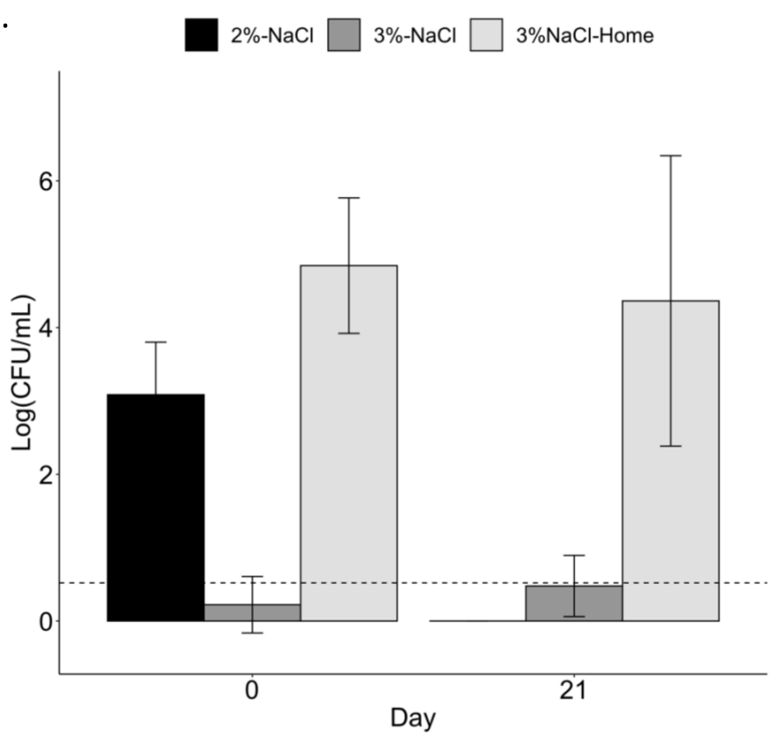


Figure 4.5. Fungal counts in 2%, 3% laboratory, and household fermentations. All fermentations were performed in triplicate.

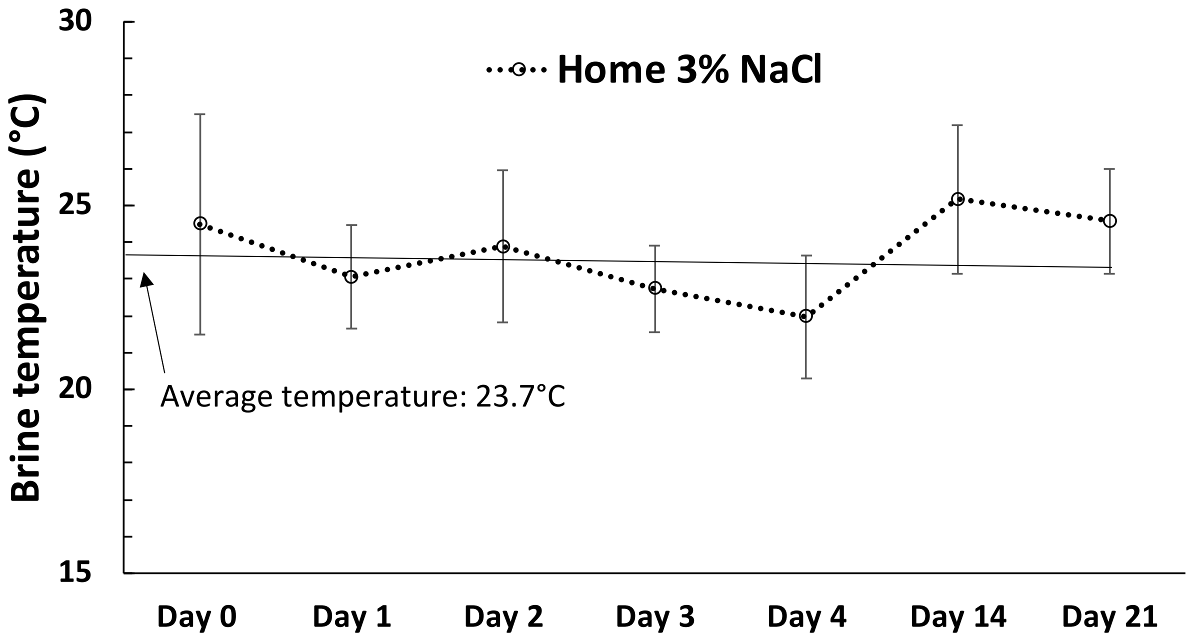


Figure 4.7. Temperature during home fermentations. Fermentations were conducted at five individual homes. All fermentations were performed in triplicate.

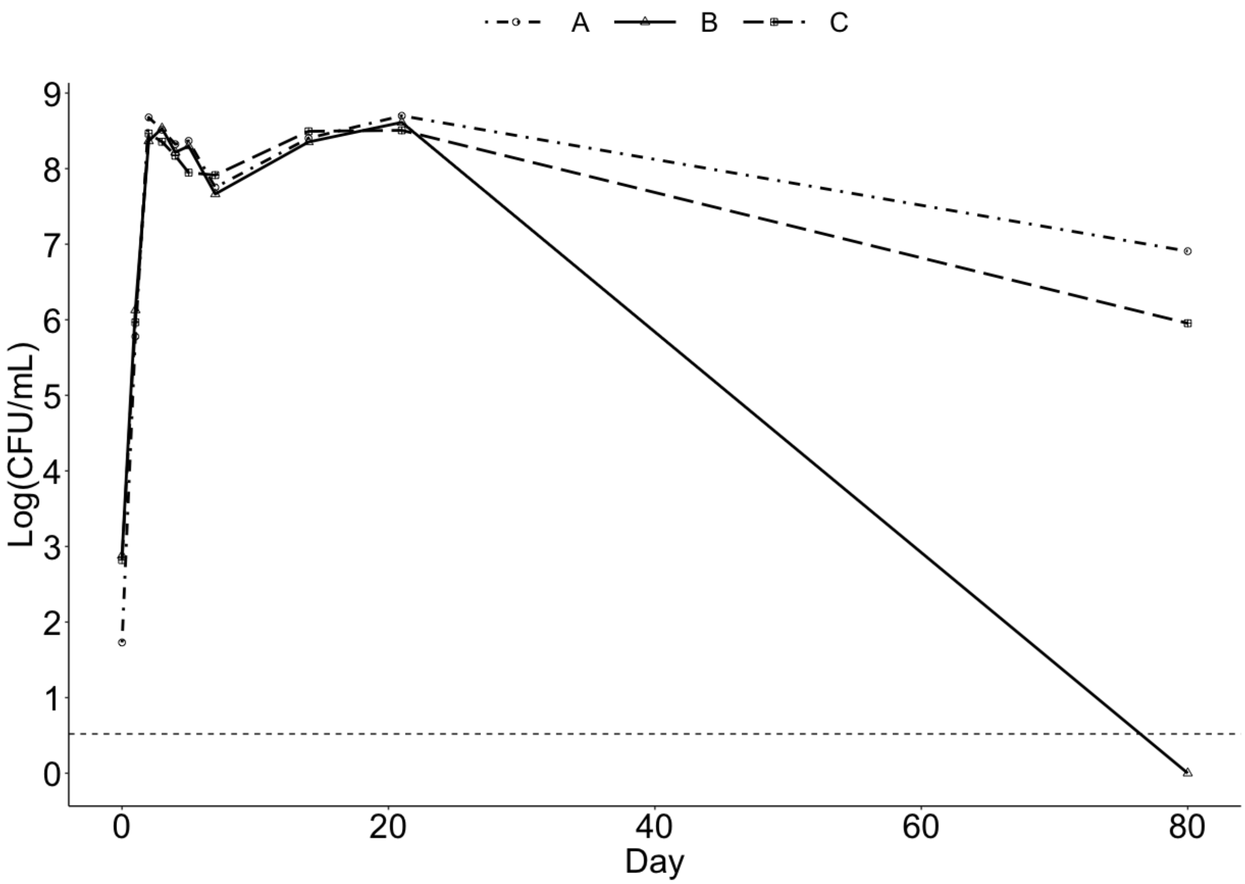


Figure 4.8. LAB counts of 2% NaCl fermentation through day 80. All fermentations were performed in triplicate. Samples were collected on days 0, 1, 2, 3, 4, 5, 7, 14, 21, and 80.

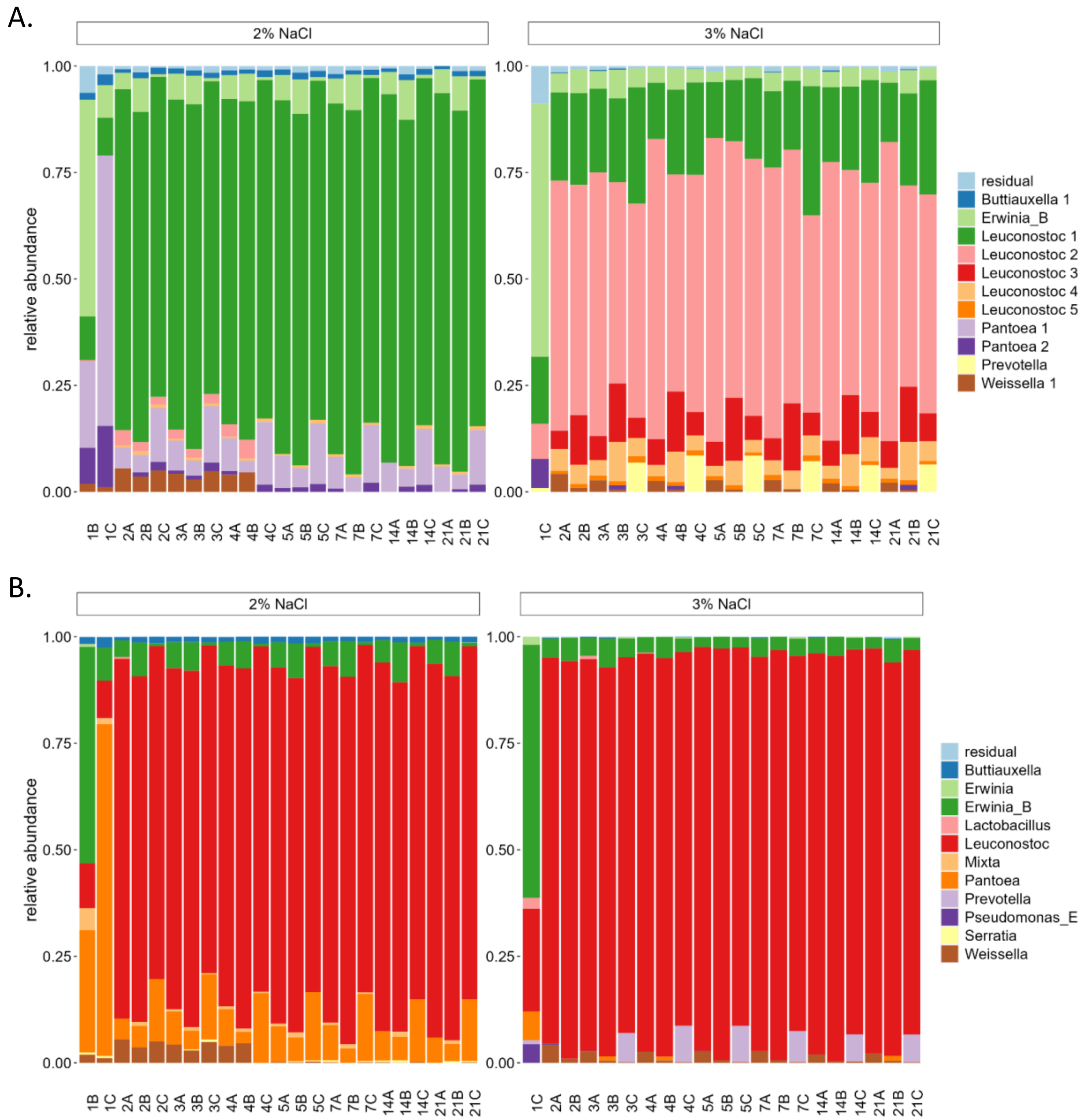


Figure 4.9: Bacterial community composition on ASV level (A) and genus level (B). Top 11 most abundant taxa are visualized in a stacked bar graph. Fermentations were performed in the lab at 22°C, in triplicate and followed for 21 days.

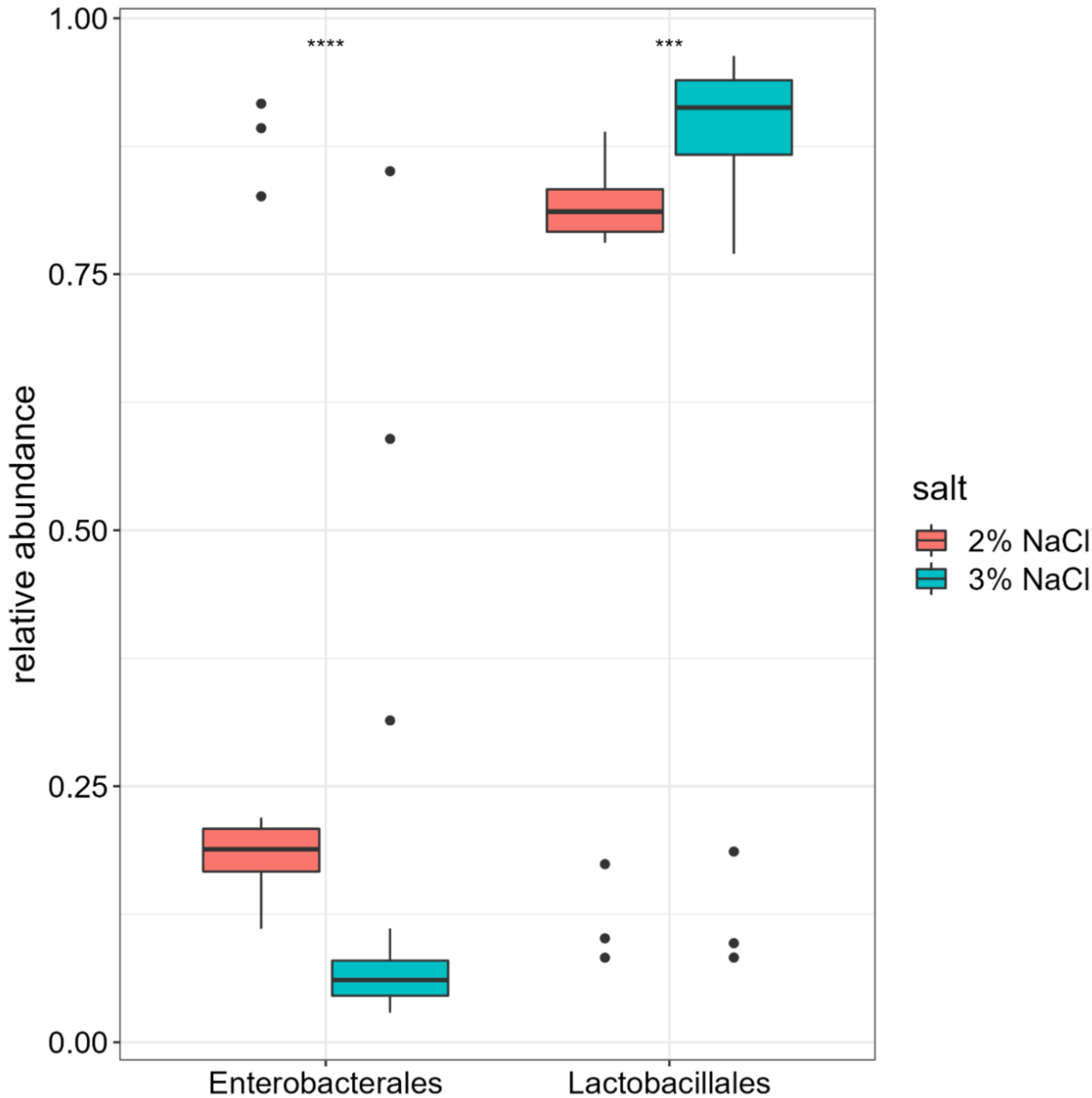
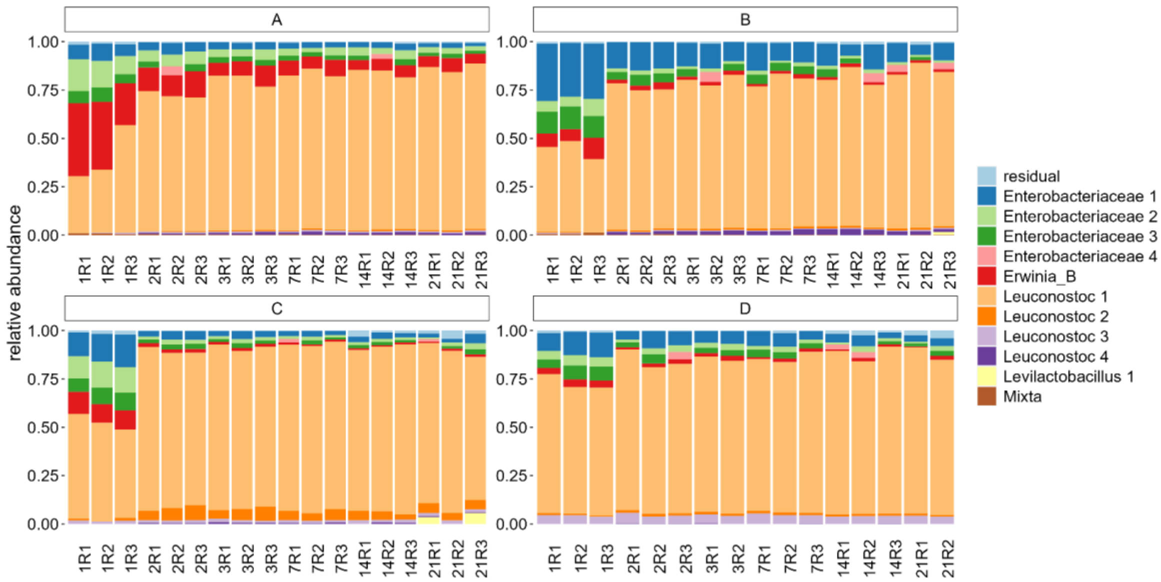


Figure 4.10. Relative abundance of orders Enterobacterales and Lactobacillales within the two lab fermentations (2% and 3% NaCl). Fermentations were set up in triplicate at 22°C. Relative abundance is calculated across all time points. *** : $p < 0.001$, ****: $p < 0.0001$



E.

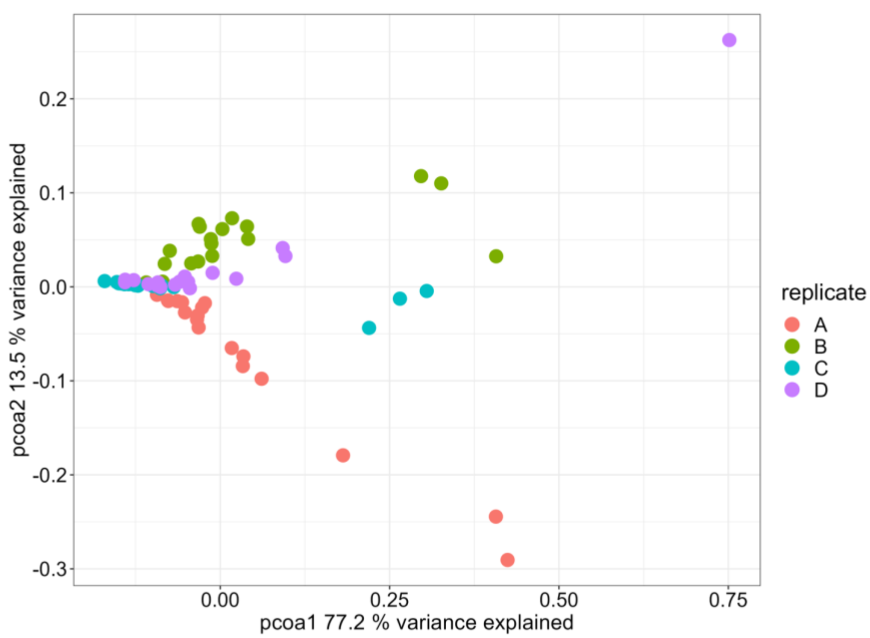


Figure 4.11: Bacterial community composition on ASV level (A). Top 11 most abundant taxa are visualized in a Stacked bar graph. Fermentations (3% NaCl) were performed in four different households (A, B, C, D) in triplicates and followed for 21 days. Beta diversity analysis using Bray-Curtis dissimilarity index (E). Principle coordinate analysis is used to visualize the samples in a 2D figure. PcoA 1 explains variation in diversity among samples from different households.

A.

B.

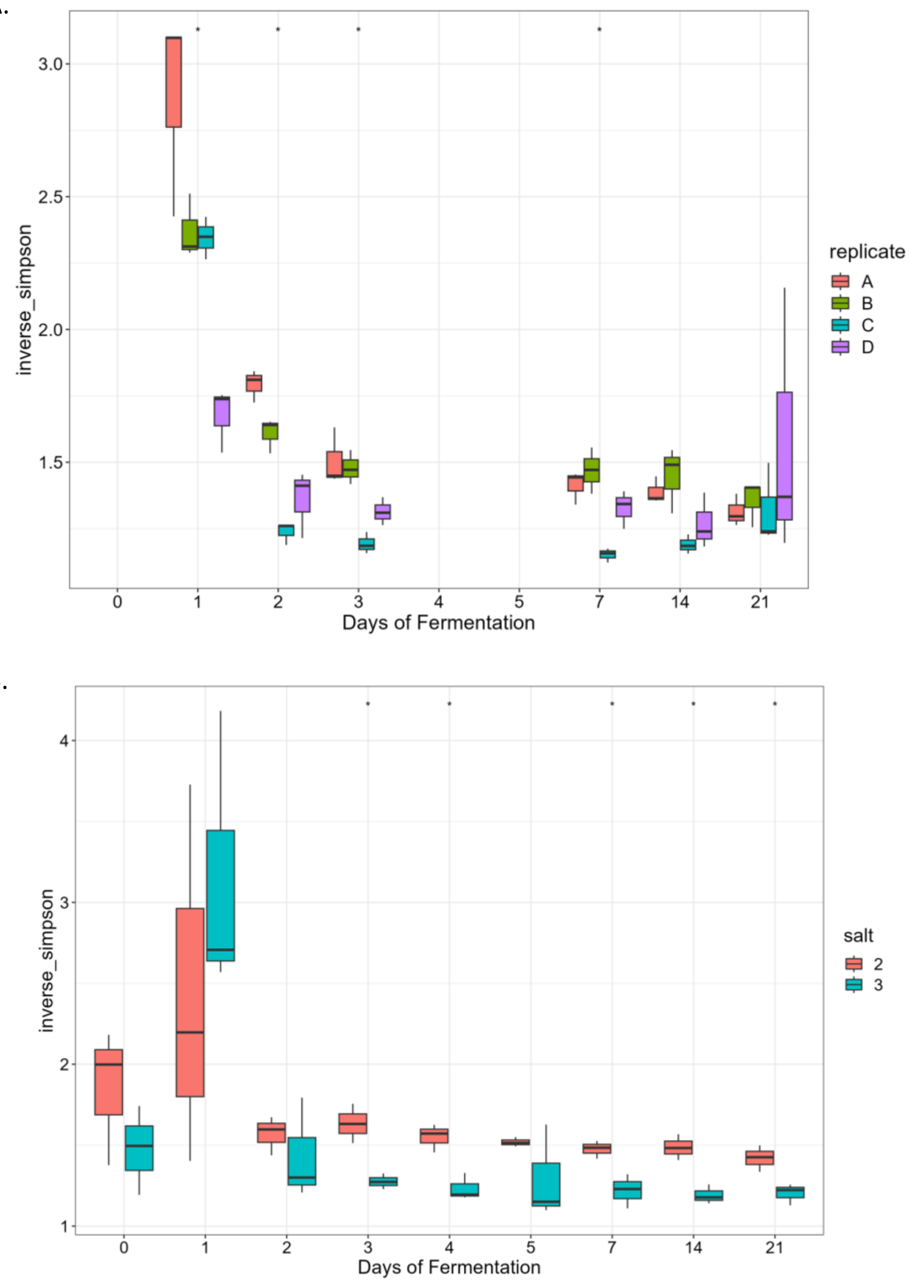


Figure 4.12: Alpha diversity decreases as the fermentation progresses in all household fermentations (A) and the lab fermentations (B). Alpha diversity was calculated using the inverse Simpson index that accounts for both evenness and richness. All fermentations were performed in triplicates. *: $p < 0.05$

Supplementary Material

Appendix 1

Appendix 1, Table 1. Survey question block related to all FFs.

Question block related to all fermented foods	
Background information (prior to Q1)	FFs and beverages are popular foods and could provide beneficial health effects. Making any type of FF requires a waiting step during preparation where the food or beverage is held for a period of time before getting the finished fermented product. Frequently that step is performed at room (or similar) or warmer temperatures (for example, letting dough rise). FFs and beverages are made through desired microbial growth and enzymatic conversions of food components. These foods and beverages are not the same as those that are preserved by adding acid such as vinegar, and the food is immediately canned or baked after making.
Question (Q) number and type of response allowed	**Question text included in survey**
Q1 (multiple selections allowed)	Based on this explanation and your experience, please indicate below which of the following foods can be made by fermentation or are examples of FFs and beverages. Please check all response options that apply. ▪ Yogurt ▪ Sour cream ▪ Kefir ▪ Cheese ▪ Wine, beer, distilled spirits ▪ Kalamata olives ▪ Bread ▪ Coffee ▪ Chocolate ▪ Kombucha ▪ Salami ▪ Vinegar ▪ Kimchi ▪ Sauerkraut ▪ Curtido ▪ Escabeche ▪ Soy sauce ▪ None of these
Q2 (single response allowed)	Besides alcoholic beverages (example: beer, wine, spirits), there are many other types of FFs and beverages. Do you currently consume non-alcoholic FFs and beverages? ▪ Yes ▪ No (if No, then skip to Q8) ▪ Uncertain
Q3	Which types of FFs and beverages do you eat (excluding alcoholic beverages)? Please check the box next to the category of FFs and beverages you eat and

(multiple responses allowed and free response for "other")	indicate the frequency of consuming that food by checking box next to: daily, weekly, monthly, or a few times a year ▪ Cereal grain (such as bread) ▪ Fruits and vegetables (such as sauerkraut, fermented pickles, kimchi) ▪ Dairy (such as yogurt, cheese, kefir) ▪ Soybean or rice (such as miso or soy sauce) ▪ Meats (such as salami) ▪ Other (please describe)
Q4 (single response allowed and free response for "other")	What is your main reason for eating non-alcoholic FF and beverages? • Taste • Health benefits • Cultural reasons • Other (please specify)
Q5 (multiple responses allowed and free response for "other")	What flavors or aromas do you associate with FFs? Select all that apply. ▪ Sourness ▪ Umami ▪ Sweet ▪ Acid ▪ Sharp ▪ Spicy ▪ Bitter ▪ Salty ▪ Fizzy or bubbly ▪ Bland ▪ Pungent ▪ Rancid ▪ Other (please specify) ▪ None
Q6 (multiple responses allowed and free response for "other")	What, if any, health benefits do you associate with non-alcoholic FFs and beverages? Select all that apply. ▪ Digestive benefits ▪ Improved gut microbiome ▪ Probiotic ▪ Prebiotic ▪ Improved nutritional value ▪ Immune system benefits ▪ Other (please specify) ▪ None
Q7 (multiple responses allowed and free response for "other")	Are there any cultural reasons you consume non-alcoholic FFs and beverages? Select all that apply. ▪ Heritage recipe or practice (passed down through the generations of my family) ▪ Popular with my ethnic group ▪ Part of the normal diet in my part of the world ▪ Part of a culture in an area I have lived or visited ▪ Part of a culture I enjoy experiencing ▪ Other (please specify) ▪ None

Appendix 1, Table 2. Survey question block related to fermented fruits and vegetables foods.

Question block related to all fermented fruits and vegetables	
Background information (prior to Q8)	The remainder of the survey is now focused specifically on fermented fruits and vegetables. Examples of fermented fruits include olives (such as those found in olive bars, not cans) and pickles (fermented cucumbers), as well as preserved citrus, fermented berries, etc. Examples of fermented vegetables include sauerkraut and kimchi (fermented cabbage), and curtido (fermented cabbage relish). This part of the survey does not include fermented alcoholic beverages like wine or beer, fermented dairy, grains, legumes, or meats. You will also be asked questions about ready-to-eat fresh (non-thermally processed) fermented fruits and vegetables. Fresh (non-thermally processed) fermented fruits and vegetables are consumed directly after fermentation or after storage in the refrigerator. Fresh (non-thermally processed) fermented fruits and vegetables have not gone through a process to make them shelf stable by thermal processing. You can find a fresh fermented fruit or vegetables in the refrigerated section of the grocery store or sold chilled at Farmers' Markets. Many FFs found on the shelves at room temperature in grocery stores or markets are heated-treated after fermentation. This heating process extends the shelf life of the final products while at the same time keeps the food from spoiling, but also inactivates the live microbes found in FFs. In our survey, we want to know if you prepare and/or consume FFs that contain the live organisms responsible for fermentation.
Question (Q) number and type of response allowed	**Question text included in survey**
Q8 (single response allowed and free response for "yes")	Do you currently buy fermented fruits or vegetables? ▪ Yes (please specify the product type and brand if you know) ▪ No (skip to Q10) ▪ Uncertain
Q9 (single response allowed)	If you buy fermented fruits and vegetable foods, do they always require refrigeration? ▪ Yes ▪ No ▪ I purchase both refrigerated and shelf-stable FFs

Appendix 1, Table 3. Survey question block related fresh fermented fruits and vegetables.

Question block related to fresh fermented fruits and vegetables	
Question (Q) number and type of response allowed	**Question text included in survey**
Q10 (single response allowed)	How often do you consume **fresh** (non-thermally processed) **fermented** fruits or vegetables? **Fresh fermented** fruits and vegetables are consumed directly after fermentation or after storage in the refrigerator. Fresh fermented fruits and vegetables have not gone through a process to make them shelf stable (thermal processing). You can find a fresh fermented fruit or vegetable in the refrigerated section of the grocery store or sold chilled at Farmers' Markets. ▪ Daily ▪ Weekly ▪ Monthly ▪ A few times a year ▪ Never (skip to Q15)
Q11 (single response allowed and free response for "other")	What is your main reason for eating fresh fermented fruits or vegetables? ▪ Taste ▪ Health benefits ▪ Cultural reasons ▪ Other (please specify)
Q12 (multiple responses allowed and free response for "other")	What flavors or aromas do you associate with fermented fruits or vegetables? Select all that apply. ▪ Sourness ▪ Umami ▪ Sweet ▪ Acid ▪ Sharp ▪ Bitter ▪ Salty ▪ Fizzy or bubbly ▪ Bland ▪ Pungent ▪ Rancid ▪ Other (please specify) ▪ None
Q13 (multiple responses allowed and free response for "other")	What, if any, health benefits do you associate with the consumption of fresh fermented fruits and vegetables? ▪ Digestive benefits ▪ Improved gut microbiome ▪ Probiotic ▪ Prebiotic ▪ Improved nutritional value ▪ Immune system benefits ▪ Other (please specify) ▪ None

Q14 (multiple responses allowed and free response for "other")	Are there any cultural reasons you consume non-alcoholic FFs and beverages? Select all that apply. ▪ Heritage recipe or practice (passed down through the generations of my family) ▪ Popular with my ethnic group ▪ Part of the normal diet in my part of the world ▪ Part of a culture in an area I have lived or visited ▪ Part of a culture I enjoy experiencing ▪ Other (please specify) ▪ None

Appendix 1, Table 4. Survey question block related to making fermented fruits and vegetables at home.

Question block related to making fermented fruits and vegetables at home	
Question (Q) number and type of response allowed	**Question text included in survey**
Q15 (single response allowed)	This part of the survey is on making fermented fruits and vegetables at home. Do you currently ferment fruits or vegetables at home? Yes No (skip to demographic questions)
Q16 (free response)	What type of fruits and vegetables do you ferment at home? (Please list)
Q17 (multiple responses allowed and free response for "website", "blog posts", and "other")	What type of resources do you use as a source of information on how to ferment fruits and vegetables at home? ▪ University Extension publications (examples: National Center for Home Food Preservation, University of California Cooperative Extension Resources, Colorado State Extension, etc.) ▪ United States Department of Agriculture (USDA) Complete Guide to Home Canning ▪ Textbooks (examples: Microbiology and Technology of FFs, Fermentation Microbiology and Biotechnology, Principles of Fermentation Technology, etc.) ▪ Other Books (examples: Farmhouse Culture Guide to Fermentation, NOMA guide to Fermentation, the Art of Fermentation, Fermented Vegetables, Wild Fermentation, etc.) ▪ Website (please specify) ▪ Blog posts (please specify) ▪ Other (please specify) ▪ None
Q18 (multiple responses allowed and free response for "other")	What types of fruits and vegetables would you be most interested in fermenting at home that you are not making already? ▪ Apple ▪ Berries (strawberry, blueberry, raspberry, etc.) ▪ Pears ▪ Citrus ▪ Olives ▪ Cranberries ▪ Cucumbers ▪ Peppers (including hot sauces) ▪ Tomatoes (including tomato-based products like salsa) ▪ Cabbage ▪ Carrots ▪ Onions ▪ Leafy greens ▪ Mixed vegetables ▪ Other (please specify)

Appendix 1, Table 5. Survey demographic question block

Question block on demographic information	
Question (Q) number and type of response allowed	**Question text included in survey**
Q19 (single response allowed)	What is your gender? ▪ Male ▪ Female ▪ Transmale ▪ Transfemale ▪ Non-binary/non-conforming ▪ Prefer not to respond
Q20 (single response allowed)	Please identify your age range. ▪ 18-29 ▪ 30-39 ▪ 40-49 ▪ 50-59 ▪ 60-69 ▪ 70 and above
Q21 (single response allowed)	Please identify your ethnicity. ▪ Hispanic or Latino(a) ▪ Not Hispanic or Latino(a)
Q22 (single response allowed)	Please identify your race. ▪ American Indian/Alaska Native ▪ Asian ▪ Black/African American ▪ Native Hawaiian/Other Pacific Islander ▪ White ▪ Two or more races ▪ Other/not listed ▪ Prefer not to state
Q23 (single response allowed)	Indicate your highest level of education. ▪ No formal degree ▪ High school degree or diploma ▪ Associate's degree ▪ Bachelor's degree ▪ Master's degree ▪ Doctoral or professional degree ▪ Prefer not to answer
Q24 (single response allowed and free response for "outside the United States")	Please share the country in which you reside. ▪ United States ▪ Outside the United States (please write the country name) (skip to Q26) ▪ Prefer not to answer
Q25	Please share the state in which you reside. ▪ Alabama

- Alaska
- Arizona
- Arkansas
- California
- Colorado
- Connecticut
- Delaware
- Florida
- Georgia
- Hawaii
- Idaho
- Illinois
- Indiana
- Iowa
- Kansas
- Kentucky
- Louisiana
- Maine
- Maryland
- Massachusetts
- Michigan
- Minnesota
- Mississippi
- Missouri
- Montana
- Nebraska
- Nevada
- New Hampshire
- New Jersey
- New Mexico
- New York
- North Carolina
- North Dakota
- Ohio
- Oklahoma
- Oregon
- Pennsylvania
- Rhode Island
- South Carolina
- South Dakota
- Tennessee
- Texas
- Utah
- Vermont
- Utah
- Vermont
- Virginia
- Washington
- West Virginia
- Wisconsin
- Wyoming

Q26 (free response)	What is your estimated annual average family income?

Appendix 2

Appendix 2, Table 1. Frequency of responses for Q1: "Based on this explanation and your experience, please indicate below which of the following foods can be made by fermentation or are examples of FFs and beverages. Please check all response options that apply". Total respondents for Q1 (n=751).

Fermented food	Response Frequency (n=751)	Percentage (%)
Yogurt	658	87.62
Sour cream	473	62.98
Kefir	488	64.98
Cheese	507	67.51
Wine, beer, distilled spirits	627	83.49
Kalamata olives	267	35.55
Bread	514	68.44
Coffee	222	29.56
Chocolate	255	34.09
Kombucha	467	62.18
Salami	222	29.56
Vinegar	323	43.01
Kimchi	549	73.10
Sauerkraut	536	71.37
Curtido	141	18.77
Escabeche	118	15.71
Soy sauce	349	46.47
None of these	0	0

Appendix 2, Table 2. Frequency of responses for Q2: "Besides alcoholic beverages (example: beer, wine, spirits), there are many other types of FFs and beverages. Do you currently consume non-alcoholic FFs and beverages? Total respondents for Q2 n=751.

Consumption of non-alcoholic fermented foods	**Response (n=751)**	**Percentage (%)**
Yes	640	85.22
No	81	10.79
Uncertain	25	3.33

Appendix 2, Table 3. Frequency of responses for Q3: "Which types of FFs and beverages do you eat (excluding alcoholic beverages)? Please check the box next to the category of FFs and beverages you eat and indicate the frequency of consuming that food by checking box next to: daily, weekly, monthly, or a few times a year".

Fermented food category	Consume the food	Reported frequency of consumption			
		Daily	Weekly	Monthly	A few times a year
Cereal grain	307	343	191	31	22
Fruits and vegetables	281	42	171	145	245
Dairy	264	344	196	70	14
Soybean or rice	250	158	269	129	53
Meats	204	102	150	169	125
Other*	59	45	45	24	11

*Other response frequencies: Kombucha (49), chili sauce (17), vinegar (6), temeph (5), water kefir (6), chocolate (4), natto (4), fish (3), coffee (3), kvass (3), non-dairy yogurt (2), tofu (2), amazake (1), pulque (1), millet (1).

Appendix 2, Table 4. Frequency of responses for Q4: "What is your main reason for eating non-alcoholic fermented beverages?" Total respondents for Q4 n=665.

Reason for eating	**Frequency (n=665)**	**Percent** (%)
Taste	337	50.68
Health Benefits	235	35.34
Cultural Reasons	80	12.03
Other (please specify)	13	1.95

Appendix 2, Table 5. Frequency of responses for Q5: "What flavors or aromas do you associate with FFs? Select all that apply" Total respondents for Q4 n=665.

Flavor/ Aroma	**Frequency** (n=665)	**Percent** (%)
Sourness	540	81.20
Umami	310	46.62
Sweet	168	25.26
Acid	401	60.30
Sharp	215	32.33
Spicy	142	21.35
Bitter	57	8.57
Salty	236	35.49
Fizzy or bubbly	262	39.40
Bland	17	2.56
Pungent	176	26.47
Rancid	38	5.71
Other (please specify)	10	1.50
None	1	0.15

Appendix 2, Table 6. Frequency of responses for Q6: "What, if any, health benefits do you associate with non-alcoholic FFs and beverages? Select all that apply" Total respondents for Q6 n=665.

Health Benefit	**Frequency (n=665)**	**Percent (%)**
Digestive benefits	508	76.39
Improved gut microbiome	513	77.14
Probiotic	458	68.87
Prebiotic	243	36.54
Improved nutritional value	330	49.62
Immune system benefits	273	41.05
Other (please specify)	21	3.16
None	14	2.11

Appendix 2, Table 7. Frequency of responses for Q7: "Are there any cultural reasons you consume non-alcoholic FFs and beverages? Select all that apply." Total respondents for Q7 n=665.

Cultural Reasons	**Frequency (n=665)**	**Percent (%)**
Heritage recipe or practice (passed down through the generations)	178	26.77
Popular with my ethnic group	189	28.42
Part of the normal diet in my part of the world	248	37.29
Part of a culture in an area I have lived or visited	181	27.22
Part of a culture I enjoy experiencing	280	42.11
Other (please specify)	16	2.41
None	113	16.99

Appendix 2, Table 8. Frequency of responses for Q8: "Do you currently buy fermented fruits or vegetables?" Total respondents for Q8 n=751.

Response	**Frequency** (n=751)	**Percent** (%)
Yes (please specify the product type and brand if you know) *	398	53.00
No	267	35.55
Uncertain	81	10.79

*Frequency of responses to "Yes":

Fermented food	**Frequency**
Kimchi	129
Pickles (cucumber)	110
Sauerkraut	97
Olives	52
Assorted vegetables	21
Miso	8
Kombucha	7
Yogurt	5
Kefir	4
Vinegar	4
Hot sauce	4
Radish	3
Citrus	4
Banana peppers	3
Garlic	3
Beans	3
Natto	3
Fermented berries	2

Apple	2
Dairy products	2
Umeboshi plums	2
Assorted fruits	2
Soy sauce	2
Green Tomatoes	1
Weinkraut	1
Bread	1
Salami	1
Sakura	1
Coffee	1
Chocolate	1
Corned beef	1
Gochujang	1

Appendix 2, Table 9. Frequency of responses for Q9: "If you buy fermented fruit and vegetable foods, do they always require refrigeration?" Total respondents for Q9 n=479.

Response	**Frequency** (n=479)	**Percent** (%)
Yes	282	58.87
No	62	12.94
I purchase both refrigerated and shelf-stable FFs	135	28.18

Appendix 2, Table 10. Frequency of responses for Q10: "How often do you consume fresh (non-thermally processed) fermented fruits or vegetables?" Total respondents for Q10 n=751.

Response	**Frequency** (n=751)	**Percent** (%)
Daily	151	20.11
Weekly	255	33.95
Monthly	161	21.44
A few times a year	125	16.64
Never	54	7.19

Appendix 2, Table 11. Frequency of responses for Q11: "What is your main reason for eating fresh fermented fruits or vegetables?" Total respondents for Q11 n=694.

Response	**Frequency** (n=694)	**Percent** (%)
Taste	348	50.14
Health benefits	251	36.17
Cultural Reasons	71	10.23
Other (please specify)	22	3.17

Appendix 2, Table 12. Frequency of responses for Q12: "What flavors or aromas do you associate with fermented fruits or vegetables? Select all that apply." Total respondents for Q12 n=694.

Response	**Frequency** (n=694)	**Percent** (%)
Sourness	517	74.50
Umami	270	39.05
Sweet	190	27.38
Acid	378	54.47
Sharp	185	26.66
Bitter	70	10.09
Salty	261	37.61
Fizzy or bubbly	122	17.58
Bland	11	1.59
Pungent	151	21.76
Rancid	29	4.18
Other (please specify)	11	1.59
None	3	0.43

Appendix 2, Table 13. Frequency of responses for Q13: "What, if any, health benefits do you associate with the consumption of fresh fermented fruits and vegetables?" Total respondents for Q13 n=694.

Response	**Frequency** (n=694)	**Percent** (%)
Digestive benefits	472	68.01
Improved gut microbiome	488	70.32
Probiotic	384	55.33
Prebiotic	246	35.45
Improved nutritional value	332	47.84
Immune system benefits	263	37.90
Other (please specify)	12	1.73
None	32	4.61

Appendix 2, Table 14. Frequency of responses for Q14: "Are there any cultural reasons you consume non-alcoholic FFs and beverages? Select all that apply." Total respondents for Q14 n=694.

Response	**Frequency** (n=694)	**Percent** (%)
Heritage recipe or practice (passed down through the generations)	159	22.91
Popular within my ethnic group	180	25.94
Part of the normal diet in my area of the world	203	29.25
Part of a culture in an area I have lived or visited	178	25.65
Part of a culture I enjoy experiencing	272	39.19
Other (please specify)	10	1.44
None	139	20.03

Appendix 2, Table 15. Frequency and percentages for association between respondents that correctly identified all FFs in Q1 and responses for Q20.

Age Group	Frequency	Percent
18-29	17	5.63
30-39	18	7.76
40-49	9	8.65
50-59	1	2.04
60-69	9	20.93
70 and above	1	6.25

Appendix 2, Table 16. Frequency and percentages for association between respondents that correctly identified all FFs in Q1 and responses for Q23.

Degree	Frequency	Percent
No formal degree	1	5.26
High school degree or diploma	3	5.00
Associate's degree	6	6.74
Bachelor's degree	19	6.13
Master's degree	17	9.24
Doctoral or professional degree	8	10.53
Prefer not to answer	1	12.5

Appendix 2, Table 17. Frequency and percentages for association between respondents that identified curtido and escabeche as fermented in Q1 and their response for Q20.

Response	"escabeche"	"escabeche" %	"curtido"	"curtido" %
Hispanic or Latino(a)	22	14.01	30	19.11
Not Hispanic or Latino(a)	96	16.3	111	18.85

Appendix 2, Table 18. Frequency and percentages for association between respondents that identified kimchi as fermented in Q1 and their responses for Q22.

Response	Frequency	Percentage
American Indian/Alaska Native	5	71.43
Asian	81	86.17
Black/ African American	14	70.00
Native Hawaiian/ Other Pacific Islander	8	53.33
Other/ not listed	12	66.67
Prefer not to state	20	80.00
Two or more races	31	88.57
White	376	70.94

Appendix 2, Table 19. Frequency and percentages for association between respondents that selected "cultural reasons" as their main reason for eating non-alcoholic and fresh fermented fruits and vegetables in Q4 and Q11, respectively, and responses for Q22.

Response	**Frequency (Q4)**	**Percentage (Q4)**	**Frequency (Q11)**	**Percentage (Q11)**
American Indian/Alaska Native	0	0.00	1	14.29
Asian	9	9.57	7	7.45
Black/ African American	3	15.00	2	10.00
Native Hawaiian/ Other Pacific Islander	2	13.33	2	13.33
Other/ not listed	1	5.56	0	0.00
Prefer not to state	0	0.00	0	0.00
Two or more races	3	8.57	4	11.43
White	62	11.70	55	10.38

Appendix 2 Table 20. Comparison between responses to Q6 with responses to Q3 for the three most selected health benefits associated with FFs: probiotic, improved gut microbiome, and digestive benefits.

Reason (Q6)	**Category (Q3)**	**Consumption (Q3)**	**Frequency (Q3)**
probiotic	cereal grain	A few times a year	19
probiotic	cereal grain	Daily	224
probiotic	cereal grain	I eat this food	43
probiotic	cereal grain	Monthly	21
probiotic	cereal grain	Weekly	138
probiotic	fruits and vegetables	A few times a year	33
probiotic	fruits and vegetables	Daily	117
probiotic	fruits and vegetables	I eat this food	27
probiotic	fruits and vegetables	Monthly	99
probiotic	fruits and vegetables	Weekly	167
probiotic	dairy	A few times a year	10
probiotic	dairy	Daily	226
probiotic	dairy	I eat this food	20
probiotic	dairy	Monthly	44
probiotic	dairy	Weekly	142
probiotic	soybean	A few times a year	39
probiotic	soybean	Daily	99
probiotic	soybean	I eat this food	20
probiotic	soybean	Monthly	92
probiotic	soybean	Weekly	189
probiotic	meats	A few times a year	95
probiotic	meats	Daily	57
probiotic	meats	I eat this food	16
probiotic	meats	Monthly	121
probiotic	meats	Weekly	102
probiotic	other	A few times a year	4
probiotic	other	Daily	33
probiotic	other	I eat this food	3
probiotic	other	Monthly	19
probiotic	other	Weekly	31
improved gut microbiome	cereal grain	A few times a year	18
improved gut microbiome	cereal grain	Daily	247
improved gut microbiome	cereal grain	I eat this food	43

improved gut microbiome	cereal grain	Monthly	23
improved gut microbiome	cereal grain	Weekly	168
improved gut microbiome	fruits and vegetables	A few times a year	38
improved gut microbiome	fruits and vegetables	Daily	131
improved gut microbiome	fruits and vegetables	I eat this food	37
improved gut microbiome	fruits and vegetables	Monthly	103
improved gut microbiome	fruits and vegetables	Weekly	190
improved gut microbiome	dairy	A few times a year	14
improved gut microbiome	dairy	Daily	271
improved gut microbiome	dairy	I eat this food	17
improved gut microbiome	dairy	Monthly	49
improved gut microbiome	dairy	Weekly	146
improved gut microbiome	soybean	A few times a year	44
improved gut microbiome	soybean	Daily	108
improved gut microbiome	soybean	I eat this food	25
improved gut microbiome	soybean	Monthly	96
improved gut microbiome	soybean	Weekly	219
improved gut microbiome	meats	A few times a year	109
improved gut microbiome	meats	Daily	61
improved gut microbiome	meats	I eat this food	22
improved gut microbiome	meats	Monthly	122
improved gut microbiome	meats	Weekly	114
improved gut microbiome	other	A few times a year	10
improved gut microbiome	other	Daily	36
improved gut microbiome	other	I eat this food	6
improved gut microbiome	other	Monthly	18
improved gut microbiome	other	Weekly	41
digestive benefits	cereal grain	A few times a year	21

digestive benefits	cereal grain	Daily	257
digestive benefits	cereal grain	I eat this food	33
digestive benefits	cereal grain	Monthly	27
digestive benefits	cereal grain	Weekly	156
digestive benefits	fruits and vegetables	A few times a year	38
digestive benefits	fruits and vegetables	Daily	123
digestive benefits	fruits and vegetables	I eat this food	33
digestive benefits	fruits and vegetables	Monthly	119
digestive benefits	fruits and vegetables	Weekly	181
digestive benefits	dairy	A few times a year	12
digestive benefits	dairy	Daily	253
digestive benefits	dairy	I eat this food	11
digestive benefits	dairy	Monthly	54
digestive benefits	dairy	Weekly	164
digestive benefits	soybean	A few times a year	41
digestive benefits	soybean	Daily	113
digestive benefits	soybean	I eat this food	20
digestive benefits	soybean	Monthly	99
digestive benefits	soybean	Weekly	216
digestive benefits	meats	A few times a year	110
digestive benefits	meats	Daily	57
digestive benefits	meats	I eat this food	18
digestive benefits	meats	Monthly	130
digestive benefits	meats	Weekly	114
digestive benefits	other	A few times a year	8
digestive benefits	other	Daily	40
digestive benefits	other	I eat this food	6
digestive benefits	other	Monthly	16
digestive benefits	other	Weekly	39

Appendix 3

Appendix 3, Table 1. Frequency of responses for Q15: "This part of the survey is on making fermented fruits and vegetables at home. Do you currently ferment fruits or vegetables at home?" Total respondents for Q15 n=746.

Ferment fruits or vegetables at home	**Response** (n=746)	**Percentage (%)**
Yes	367	49.20
No	379	50.80

Appendix 3, Table 2. Frequency of responses for Q16: "What type of fruits and vegetables do you ferment at home? (Please list)" Total respondents for Q16 n=367.

Fruit or vegetable fermented at home	**Response Frequency** (n=367)
Cabbage	202
Cucumber	88
Alliums	54
Carrot	52
Peppers	48
Citrus	45
Radish	40
Apple	22
Leafy greens	22
Tomatoes	18
Beets	17
Pineapple	16
Berries	15
Stone fruit	15
Grape	13
Kombucha	11
Assorted vegetables	11
Assorted fruit	9
Olives	9
Turnip	8
Asparagus	6
Bread	6
Cauliflower	6
Ginger	6

Green beans	5
Vinegar	5
Kiwi	4
Mango	4
Beans	3
Passion fruit	3
Pear	3
Potatoes	3
Salsa	3
Avocado	2
Bamboo shoot	2
Eggplant	2
Kvass	2
Water kefir	2

Appendix 3, Table 3. Frequency of responses for Q17: "What type of resources do you use as a source of information on how to ferment fruits and vegetables at home?" Total respondents for Q15 n=367.

Resource type	**Response** (n=367)	**Percentage (%)**
University Extension publications (examples: National Center for Home Food Preservation, University of California Cooperative Extension Resources, Colorado State Extension, etc.)	89	24.25
United States Department of Agriculture (USDA) Complete Guide to Home Canning	91	24.80
Textbooks (examples: Microbiology and Technology of FFs, Fermentation Microbiology and Biotechnology, Principles of Fermentation Technology, etc.)	115	31.34
Other Books (examples: Farmhouse Culture Guide to Fermentation, NOMA guide to Fermentation, the Art of Fermentation, Fermented Vegetables, Wild Fermentation, etc.)	170	46.32
Website (please specify)	51	23.90
YouTube	142	38.69
Blog posts (please specify)	21	5.72
Other (specified below)	50	13.62
Community knowledge	28	
Personal knowledge	7	
Social media	2	
None	12	3.27

Appendix 3, Table 4. Frequency of responses for Q18: "What types of fruits and vegetables would you be most interested in fermenting at home that you are not making already?" Total respondents for Q18 n=367.

Ferment fruits or vegetables at home	Response (n=367)	Percentage (%)
Apple	88	23.98
Berries (strawberry, blueberry, raspberry, etc.)	107	29.16
Pears	69	18.80
Citrus	89	24.25
Olives	104	28.33
Cranberries	54	14.71
Cucumbers	138	37.60
Peppers (including hot sauces)	105	28.61
Tomatoes (including tomato-based products like salsa)	95	25.89
Cabbage	97	26.43
Carrots	86	23.43
Onions	62	16.89
Leafy greens	68	18.53
Mixed vegetables	75	20.44
Other (specified below)	22	5.99
Plant-based cheese	3	
Stone fruit	2	
Miso	2	
Eggplant	1	
Squash	1	
Radish	1	
Yuzu	1	
Edible flowers	1	
Beans	1	

Beets	1
Mushrooms	1

Appendix 3 Table 5. Comparison between "Yes" responses to Q15 ("Do you currently ferment fruits or vegetables at home?") and Q23 ("Indicate your highest level of education").

Education Level	**Frequency**	**Percentage**
No formal degree	13	68.42
High school degree or diploma	29	48.33
Associate's degree	46	51.69
Bachelor's degree	149	48.07
Master's degree	87	47.28
Doctoral or professional degree	37	48.68
Prefer not to answer	6	75.00

Appendix 3 Table 6. Comparison between "Yes" responses to Q15 ("Do you currently ferment fruits or vegetables at home?") and Q20("Please identify your age range").

Age	Frequency	Percentage
18-29	91	30.13
30-39	132	56.90
40-49	70	67.31
50-59	31	63.27
60-69	31	72.09
70 and above	12	75.00

Appendix 4

Appendix 4. Table 1. Frequency of free responses for Q24: “Please share the country in which you reside.” Total respondents for Q24 n=751.

Country Outside the United States	Response (n=751)
Canada	11
Argentina	10
Argentina	8
United Kingdom	8
Italy	7
Peru	3
Switzerland	3
Brazil	3
India	3
Korea	2
Germany	2
Mexico	2
Japan	2
India	2
Chile	1
Qatar	1
France	1
Ireland	1
Taiwan	1
Ireland	1
Netherlands	1
China	1
Philippines	1

Colombia	1
Australia	1
Czechia	1
Belize	1
Latvia	1
Estonia	1

Appendix 4, Table 2. Frequency of responses for Q25: “Please share the state in which you reside.” Total respondents for Q25 n=751.

State	Frequency (n=751)	Percentage (%)
Alabama	13	1.73
Alaska	20	2.66
Arizona	16	2.13
Arkansas	11	1.46
California	314	41.81
Colorado	13	1.73
Connecticut	10	1.33
Delaware	7	0.93
Florida	22	2.93
Georgia	11	1.46
Hawaii	9	1.20
Idaho	7	0.93
Illinois	9	1.20
Indiana	5	0.67
Iowa	7	0.93
Kansas	15	2.00
Kentucky	5	0.67
Louisiana	3	0.40
Maine	2	0.27
Maryland	3	0.40
Massachusetts	4	0.53
Michigan	11	1.46
Minnesota	7	0.93
Mississippi	2	0.27
Missouri	3	0.40

Montana	1	0.13
Nebraska	5	0.67
Nevada	3	0.40
New Hampshire	1	0.13
New Jersey	2	0.27
New Mexico	1	0.13
New York	18	2.40
North Carolina	12	1.60
North Dakota	1	0.13
Ohio	4	0.53
Oklahoma	3	0.40
Oregon	11	1.46
Pennsylvania	6	0.80
Rhode Island	2	0.27
South Carolina	2	0.27
South Dakota	1	0.13
Tennessee	7	0.93
Texas	14	1.86
Utah	4	0.53
Vermont	2	0.27
Virginia	5	0.67
Washington	15	2.00
West Virginia	9	1.20
Wisconsin	2	0.27
Wyoming	1	0.13

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