ABSTRACT

WHERRY, BRYAN MICHAEL. Use of Acid Whey as an Ingredient in Yogurt and Measurement of Furfuryl Alcohol. (Under the direction of Dr. MaryAnne Drake).

Whey protein ingredients are widely used in a variety of products and are added primarily for functionality or nutritional benefits. Acid whey, a relatively low-pH whey, resulting from the production of soft cheeses, is a disposal problem for the dairy industry. Disposal of acid whey is not only a financial burden of many dairies, but can have a negative impact on the surrounding environment. Understanding the potential of the protein in acid whey is essential to moving forward with recycling this byproduct.

In the first study, recovery of whey protein from cottage cheese acid whey was explored for use in yogurt applications. Cottage cheese acid whey and Cheddar cheese whey were produced from standard cottage cheese and Cheddar cheese make procedures, respectively. Skim milk was concentrated to 6% total protein to make skim milk concentrate. Nonfat, unflavored set-style yogurt made with acid whey protein (AWP) was compared to yogurt made with additional sweet whey protein (SWP) and yogurt made from skim milk concentrate (SM). The yogurts were measured over a period of 8 weeks for pH, titratable acidity, whey separation, color, and gel strength. Trained panel profiling was conducted on days 0, 14, 28, and 56. Yogurt made with AWP had similar color and titratable acidity to those made with SWP ($p>0.05$). Yogurt with AWP had higher values of syneresis, lower gel strength, higher sour taste and lower firmness and viscosity compared to yogurts made with SWP ($p<0.05$). Both yogurts with AWP and SWP were distinct in sensory characteristics from the control yogurt made from SM and from each other. These results indicate that AWP can be used as an ingredient in yogurt.

The secondary study had two objectives, the first of which was to determine the most accurate and reliable technique for furfuryl alcohol extraction and detection in dairy products.

The second objective was to report concentrations of furfuryl alcohol in fluid milk, Cheddar cheese, mozzarella cheese, cottage cheese, sour cream, yogurt, milk protein powders (MPI and MPC), whey protein powders (WPI and WPC) and skim milk powders (SMP). Solvent extraction (SAFE), head space solid phase microextraction with gas chromatography mass spectrometry (HS-SMPE-GC/MS), stir bar sorptive extraction with gas chromatography mass spectrometry (SBSE-GC/MS), and head space solid phase microextraction with gas chromatography triple quadrupole mass spectrometry (HS-SPME-GC/MS/MS) were compared for recovery of FA. Internal standards for the quantitation of furfuryl alcohol (2-methyl-3-heptanone, deuterated furfuryl alcohol, 2,5 dimethyl phenol, 5-methyl 2-furfuryl alcohol, and 5-methyl furfural) were also compared. HS-SPME-GC/MS/MS was the most precise method of extraction and quantification of FA, with more precise peaks and higher signal-to-noise ratios than other methods. Deuterated furfuryl alcohol was the best internal standard due to its high signal to noise ratio and distinct retention time. UP milks had higher levels of FA than HTST milks ($p<0.05$) (54.0 ppb vs. 4.90 ppb). FA concentrations ranged from 0.63 to 56.2 ppb in WPI and WPC, and 8.31 to 122 ppb in MPI and MPC, and concentrations increased with powder storage ($p<0.05$). High heat SMP had higher concentrations of FA than low heat SMP (11.8 vs. 1.36 ppb, $p<0.05$) and concentrations increased with storage time ($p<0.05$). Concentrations of FA in Cheddar and mozzarella cheese ranged from 2.36 to 110 ppb and were higher than FA concentrations in cottage cheese or sour cream (0.33 to 1.01 ppb). Concentrations in yogurt ranged from 4.96 to 135 ppb. Fat content had no impact on FA concentration ($p>0.05$).

Use of Acid Whey as an Ingredient in Yogurt Manufacture and Measurement of Furfuryl Alcohol

by
Bryan Michael Wherry

A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the degree of
Master of Science

Food Science

Raleigh, North Carolina

2018

APPROVED BY:

_____	_____
Dr. Maryanne Drake Committee Chair	Dr. K. P. Sandeep
_____	
Dr. Dana Hanson	

DEDICATION

I would like to dedicate this thesis to my grandfather, Keith Wherry. Over the past two and a half years, I feel that we have become very close and our relationship has grown very strong. I enjoy our weekly phone calls and the many three hour drives I made to come visit you for the weekend. I have learned many lessons from you, from various aspects of life, that have helped mold me into the person I am. These lessons will help me be a successful person throughout my life, and I have great appreciation for you. Thank you.

BIOGRAPHY

Bryan Wherry was born in Buffalo, New York to Bryan and Maryfrances Wherry, and is the oldest of three children. Growing up, he enjoyed playing sports and had an interest in math and science. Bryan graduated from Niagara University in 2013 with a B.S. in Biochemistry. During his time as an undergraduate, Bryan played for the University lacrosse team and was a part of the chemistry club. Upon graduation, he accepted a position working for Upstate Niagara Cooperative as a Research and Development Technician. Bryan moved to North Carolina in May 2016 to pursue his M.S. in Food Science at North Carolina State University under Dr. Maryanne Drake.

ACKNOWLEDGMENTS

I would like to thank my parents first of all, for their constant support and always urging me to chase my goals. I hope I have made you proud. I would also like to acknowledge all of my lab family. Without all of you and you're help in the pilot plant making yogurt and taking measurements on the GCs, none of this would have been possible. Last, but certainly not least, thank you to Dr. MaryAnne Drake. Thank you for this opportunity and your constant support of my goals and always pushing me to do my best. Your mentorship has taught me many very valuable lessons that I will carry with me throughout my life.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1:

LITERATURE REVIEW. ACID WHEY PROCESSING AND APPLICATIONS, AND A REVIEW OF YOGURT PROCESSING AND FORMULATION.

Introduction

Bovine milk has been consumed by humans, in different forms, for many centuries. Although it is intended for nutrition and growth of a young calf, it contains many nutritionally valuable molecules that can benefit humans (de Wit, 1998). Dairy products are highly consumed in today's population because of their high nutritional value, and dairy ingredients are widely used in the food industry. Cheese whey is the result of precipitation and removal of casein from milk in the cheese making process. On average, 9 kilograms of cheese whey are produced as the byproduct of 1 kilogram of cheese produced (Remon et al., 2016). Worldwide, $40.7x10^6$ tons of cheese whey are produced each year; half of which is produced in the USA (Prazeres et al., 2012). Lactose and protein are the major solid components in whey and account for approximately 75 and 10% of the solids, respectively.

Whey protein is extracted from cheese whey by ultrafiltration. The remaining solution is whey permeate which consists mainly of lactose, minerals, and water (Parashar et al., 2015). Two main whey varieties are produced, acid whey (pH <5) and sweet whey (pH 6-7) (Siso, 1996). Whey protein products are widely used as food ingredients due to their excellent functional and nutritional properties (Wang et al., 2003; Whetstine et al., 2005; Kontopidis et al., 2002; 2004; Bals et al., 2003; Kersten et al., 2005; Leman et al., 2005). Many whey protein powders are produced by the dairy industry today. Sweet whey powder (SWP) is spray dried liquid whey from cheese manufacture. Whey powders also can be concentrated. Whey protein concentrates (34-90% protein) are commonly used as skim milk powder replacers (Sodini and Tong, 2013). Using ultrafiltration, protein content can be concentrated to 35-80% and then spray dried to make a whey protein concentrate (WPC). Often, manufacturers will diafiltrate and/or apply microfiltration to increase protein content up to 90% which makes whey protein isolate

(WPI) (Tunick, 2008). Products that use whey powders as ingredients include ready-to-mix protein beverages, yogurt, ice cream, ready-to-drink protein beverages, protein bars, flavored milks, cereals, and baby formula (Smithers, 2015).

Acid whey, a relatively low-pH whey (4.2-4.6), resulting from the production of soft cheeses, is a disposal problem for the dairy industry. Sweet whey is currently fully utilized and manufactured into a variety of dairy ingredients. Acid whey, in contrast, because of its low pH, is often disposed. This is not only a large economical issue, but also an ecological issue. Acid whey has a high biological oxygen demand and chemical oxygen demand, which, when introduced into a biological system like a river or steam, will pull oxygen away from organisms already existing in that environment (Parashar et al., 2015). Not only does disposal of acid whey hurt the environment, but it also throws away a chance to recycle something with a high nutritional potential. Few uses have been found for acid whey because of its high ash content, low pH, and high organic content, which requires costly waste treatment (Silva and Yang, 1995). Additionally, disposal of acid whey results in losses of over 18 million pounds of whey protein (USDA, 2015).

Acid Whey Review

Composition of Milk

Bovine milk is described as "The lacteal secretion, practically free from colostrum, obtained by the complete milking of one or more healthy cows. Milk that is in final package form for beverage use shall have been pasteurized or ultra-pasteurized, and shall contain not less than 8.25% milk solids not fat and not less than 3.25% milkfat," by the code of federal regulations under Title 21: Subchapter B: Part 131: Subpart B: Section 131.110. Milk may have vitamins added. Vitamin A can be added so that milk contains not less than 2000 IU per quart,

and vitamin D can be added such that milk contains not less than 400 IU per quart (FDA, Grade A Pasteurized Milk Ordinance, 2015).

Milk contains many nutrients that humans can utilize on a daily basis. The composition of milk can vary based on which breed of cow it comes from and the different seasons it is sampled from (Lindmark-Mansson et al., 2003). The main components of milk include fat, casein and serum proteins, and lactose. Milk is comprised mostly of water with lactose (4.6-4.9% w/w), fat (3.5-4.0% w/w), protein (2.9- 3.4% w/w), and ash (<1% w/w) (Varnam and Sutherland, 2001; Weimer, 2001).

The milk proteins, casein and serum or whey protein, can be further separated into sub-protein groups. Casein proteins mainly exist in micelle form, which are a combination of α_{s1}-Casein, α_{s2}-Casein, β-casein and κ-casein, and have an average diameter of 150nm (Haug et al., 2007) (Table 1.1). The casein micelles carry phosphate and calcium, which are the main structural components of the micelle. The α_{s1}-Casein, α_{s2}-Casein, and β-casein all have calcium sensitivities, meaning they can be precipitated by calcium, whereas κ-casein is not. K-casein has a hydrophilic tail, and these proteins surround the micelle and protect the other casein proteins from precipitation (Horne, 2006). The remaining proteins in milk come from the serum or whey proteins, which are globular shaped and more water soluble than casein. The main whey proteins are β-lactoglobulin (β-Lg), α-lactalbumin (α-La), immunoglobulins (Ig), and bovine serum albumin (BSA) (Prazeres et al., 2012).

Where does whey come from?

Whey is a coproduct of cheese and yogurt manufacture and accounts for about 85-95% of total milk used in the process (Parashar et al., 2015). There are two main types of whey produced: acid whey (pH <5) and sweet whey (pH 6-7), according to the procedure for

precipitation of casein. Acid whey has a higher ash and lower protein content than sweet whey and is limited by its acidity and higher salt content (Siso, 1996).

Sweet whey comes from the manufacture of hard cheeses such as Cheddar or mozzarella. The cheese make process includes pasteurizing whole milk at legal temperatures and then cooling to about 33°C. Milk is then inoculated with cheese culture and allowed to ferment until the pH reaches approximately 6.4. Then, rennet is added to start the coagulation process. Rennet is a type of milk protease that refers to calf or recombinant chymosin. There are other types of rennet (*Mucor meihei* protease, *Mucor pusillus* protease, *Cryophonectria prasitica* protease or fermentation derived chymosins) which are product specific. These enzymes typically work by cleaving the κ-casein from the casein micelles which destabilizes the casein micelles and facilitates coagulation (Farkye, 2004). Once the cheese is coagulated, it is cut and allowed to heal. Then the cooking process to expel whey from the curds is started. After cooking, the whey is drained from the curd and the curd is used to make cheese (Prazeres et al., 2012). The whey, now at pH of 6-7 is collected and pasteurized, which results in sweet whey (Tsakali et al., 2007).

Acid whey can come from multiple sources. Cottage cheese and other soft cheeses are the main products that result in acid whey. Cottage cheese is made by pasteurizing skim milk and cooling to about 33°C. It is then either inoculated with cheese culture and allowed to ferment until the pH drops to about 4.6, which can take up to 8 hours (Covacevich and Kosikowski, 1978), or direct acid set with a food grade acid, usually lactic or citric (Mohamed and Bassette, 1979). The cheese is then cut and the curd is allowed to heal. The curd is then cooked to expel more whey. Once the cooking process is finished, the whey is drained and the curd continues in the cottage cheese make process. The resulting acid whey has a pH similar to the cheese at cutting (pH 4.6) and is usually drained into a tank and disposed of accordingly (Barrantes and

Morr, 1997).

Greek-style yogurt is another cultured dairy product that produces acid whey. It has become very popular in the USA over the past few years and is estimated to be responsible for about 25% of the total yogurt market. Greek-style yogurt typically has higher solids content than regular yogurt and is commonly claimed to have twice the protein of traditional yogurt. Greek-style yogurt can be manufactured with skim, low-fat, or whole milk, but it is typically made using skim (Erickson, 2017).

Greek-style yogurt can be manufactured in multiple ways. The traditional method is to strain the finished yogurt through cloth bags to allow the acid whey to drain. This process is started by pasteurizing the milk. Once pasteurized, the milk is cooled to inoculation temperature and transferred to a fermentation tank. Here the milk is inoculated with yogurt culture and allowed to incubate until the final pH (usually 4.6) is reached. After the pH is reached, the yogurt is blended and transferred into cloth bags where it is either hung in a cooler or onto ice with applied pressure to squeeze all of the acid whey out. This process usually takes 24-48 hours to complete. This process can work for small manufacturers, but for larger production it is not an ideal method (Kilara and Chandan, 2013).

More commonly, centrifugation is used for larger manufacturers. The pasteurization and inoculation process is the same as for strained Greek-style yogurt, however, once the final pH is reached, the yogurt is blended and then pumped to a separator where the acid whey is removed from the white mass. A schematic of a separator is shown in figure 1.1. The yogurt is pumped into the top (1) where it enters and goes to the bottom of the bowl. The disk stack (5) spins at a high speed which allows the yogurt to rise through rising channels (4) and separates the acid whey from the white mass. The resulting white mass exits (3) to a collection tank. The acid whey

exits (2) to a waste tank.

Commonly, acid whey, is not used by the companies producing the products. In the past acid whey was dumped and exited into streams and other bodies of water. However, this process depletes the water of oxygen which can destroy the marine environment. Because of this, many states have passed environmental laws that prohibit the dumping of acid whey into or near bodies of water (Pilarska et al., 2016). The New York State Department of Environmental Conservation has been regulating land application of food processed waste for the past 20 years. New York generates 70% of the $6 billion Greek yogurt business nationwide. From a 4oz volume of milk, Greek yogurt produces about 3oz of acid whey and 1oz of yogurt (NYS Department of Environmental Conservation, 2017).

Composition of Whey/Acid whey

With only about 6% solids, whey is a significantly dilute product. During the cooking of cheese, most of the water is expelled from the casein curd, which makes up the majority of whey. Other content includes protein, fat, lactose and ash (Tsakali et al., 2007). Percentages of each of these components are listed in Table 1.2. Cheese whey also contains amounts of lactic acid, citric acid and B group vitamins (Pilarska et al., 2016). Depending on the cheese-type, most of the milkfat will be retained in the curd, and therefore, fat levels in whey are low. Although most of the fat is retained by the curd, fat separation can be applied to whey to meet product specifications (Mattews, 1984). Protein makes up <1.0% of the total solids in whey.

β-Lactoglobulin is one of the most widely studied proteins and is the most abundant whey protein. It is a large factor in forming whey protein gels (van Vliet et al., 2004). It has a molecular mass of about 18.3 kDa (Brisson and Singh, 2013). pH has an effect on the structure and functionality of β-Lg. Hambling et al. (1992) noted that in physiological conditions, β-Lg

exists as a dimer, but begins to dissociate at pH 3.5. The secondary structure of the β–Lg is stabilized by hydrophobic, ionic, and hydrogen bond interactions along with two disulfide bridges (de Wit, 2009). The β-Lg denaturation and aggregation process very closely resembles a polymerization process unlike α-La which has no free thiol groups. β-Lg can form disulfide bridges with α_{s2}-Casein and κ-casein (Vasbinder and de Kruif, 2003).

α-Lactalbumin is the second most abundant whey protein, comprising about 20% of all whey proteins (Raikos, 2010). α-La is a metallo-protein that binds a calcium ion to its core and is much more thermally stable than other whey proteins (Brisson and Singh, 2013). It has a molecular mass of 14.2 kDa and an isoelectric point of 4.2 (Lam and Nickerson, 2015). Increasing temperature alters the structure of α-La, which increases hydrophobicity. Both influence the aggregation at changing pH by altering surface charge (Lam and Nickerson, 2015).

Immunoglobulin proteins are antibodies that protect against pathogenic microorganisms. Igs in bovine milk are divided into three classes: IgA (20-25%), IgG (60-70%), and IgM (10-15%); and make up about 10% of total whey proteins (Brisson and Singh, 2013). IgG is composed of two light chains and two heavy chains of 23 kDa and 53 kDa respectively. These combine through disulfide bonds to create a polymer of 150 kDa. IgM is a pentamer that weighs about 900 kDa and IgA exists as a monomer or dimer of 160 kDa or 380 kDa (Edwards et al., 2009). Igs are essential in providing calves with immune protection, and have also been studied for treatment of microbial diseases in humans (Korhonen et al., 2000). Igs have shown antibody effects against rotavirus gastroenteritis, *Shigella flexneri*, *E. coli*, and *Streptococcus mutans* (Brussow et al., 1987; Tacket et al., 1992,1988; Michalek et al., 1987).

Bovine serum albumin (BSA) is a plasma protein that is relatively large at 66.4 kDa and accounts for about 5% of whey proteins (Brisson and Singh, 2013). BSA has been studied

extensively and because of its low concentrations in milk, has little effect on physiological conditions and has no known biological function for milk (Fox, 2009). In blood, BSA is used as a carrier of molecules to cells throughout the body (Hu et al., 2004). BSA may affect flavor of milk by controlling levels of oxygen through various redox reactions that it can facilitate (Edwards et al., 2009). BSA has specific binding sites for metal ligands and long chain fatty acids that endow it with high thermal stability (Relkin and Mulvihill, 1996).

Benefits of Whey Proteins

Whey proteins have many positive effects on the human body. While casein proteins coagulate in the stomach and are slowly digested, whey proteins do not coagulate under acidic conditions and are considered fast proteins, and thus, peak amino acid concentrations in blood from whey proteins is achieved faster than casein proteins. Once in the small intestine, hydrolysis of whey is slower than casein, which allows for more absorption (Marshall, 2004). When combined with resistance exercise, they can help improve and increase skeletal muscle (Tipton et al., 2004). Whey proteins have also been found to stimulate the synthesis of glutathione, support biosynthesis of lactose, bind free fatty acids in blood, and act as potential modulators of various regulatory processes (de Wit, 1998). Arnal et al. (1999) demonstrated that pulse feeding protein led to better absorption of protein and more available nitrogen in older women.

Whey protein improves yogurt texture (Isleten and Karagul-Yuceer, 2006), increases water holding capacity (Sodini et al., 2005), increase density (Puvanenthrian et al., 2002), and reduces lumpiness (Laiho et al., 2017). Hudson et al. (2000) demostrated that whey protein isolate powders are capable of forming cold-set weak gel structures suitable for thickening over a wide range of temperature and pH food systems. Whey protein coupled with locust bean gum

can be used to make a mesoscopic protein particle suspension which can make a dough similar to a wheat dough and used to make gluten free bread (van Riemsdijk et al., 2011).

Acid Whey Processing and Applications

Current Uses of Acid Whey

With increasing cheese manufacture, there is an increasing amount of whey. With few ways to utilize the whey, it became customary to dispose of whey in streams, waterways or sewage drains (Tsakali et al., 2007; Kosikowski, 1979; Siso, 1996). When governmental agencies discovered that whey had negative effects on the environment and a hefty biological oxygen demand, appropriate discard regulations were placed on the dairy industry (Kosikowski, 1979; Tsakali et al., 2007). Farmers began to use whey as a part of animal feed and spread it over fields, but were still limited by the regulations. Sweet whey can be concentrated and dried to use as an ingredient in other dairy products, but acid whey is not typically utilized because of the low pH and characteristic off flavors (Sodini and Tong, 2013). Low pH is attributed to high lactic acid content which makes drying acid whey troublesome. Lactic acid agglomerates during spray drying, which causes lumping and caking of particles (Dec and Chojnowski, 2006). Greek yogurt acid whey is even harder to find a use for than cottage cheese acid whey because of the higher mineral content, higher acidity and lower protein levels (Bell, 2013).

How is Acid Whey Processed?

Currently, many companies in the dairy industry are attempting to discover new implementations for the acid whey produced from cottage cheese and Greek yogurt manufacture. Erickson (2017) recently outlined acid whey and industrial standards for disposal. It was noted that a few companies have invested in anaerobic digesters that result in methane production. The methane is used to fuel generators to produce electricity that is used to power the plant. Excess

electricity gets sold back to the power companies. Other companies use filtration units such as Reverse Osmosis (RO) to concentrate the acid whey. This process uses pressure to separate water from the whey, resulting in a concentrated whey. This process allows companies to transport the whey using fewer trucks to local farms. Some companies use acid whey to direct set milk for cheese production. General Mills has a patent for an enzyme-based process that converts sugars in the whey to soluble fiber (Gonzalez and Smith, 2014). Others have explored extracting the lactic acid from the whey for use in other areas. Using nanofiltration, acid whey can be demineralized and concentrated (Roman et al., 2009). By adjusting pH prior to nanofiltration, 60% lactic acid removal can be achieved without major fouling of the nanofiltration membranes (Chandrapala et al., 2017). Monodisperse superparamagnetic polyglycidyl methacrylate particles have been used to isolate lactoferrin from acid whey because of its high affinity for the molecule (Chen et al., 2007). These modern approaches to dealing with the acid whey produced from Greek yogurt and cottage cheese are huge steps forward to recycling and re-using this byproduct.

Products that use Acid Whey as an ingredient

Many products in the food industry use whey protein powders; such as WPC and WPI; as an ingredient in formulation because of their high protein content and ability to replace nonfat dry milk (Smithers, 2008). Most commonly, whey protein powders are used as a supplement in protein beverages and snacks (Prazeres et al., 2012). WPC and WPI have been used in a wide range of products, from dairy products such as ice cream and yogurt to meat sauces and soups, infant formula beverages, baked goods, and salad dressings (Tunick, 2009). Acid whey is harder to incorporate into such foods because of its high acidity and off flavors, but has been incorporated into some products. Acid whey powder enhances flavor and improves crust color in baked goods such as bread, biscuits and crackers (Tunick, 2009). Acid whey powder can be used

as a base for edible coatings of meats (Haque et al., 2009). Fruit-flavored beverages have been manufactured using acid whey protein, because of the solubility at high acid levels (Morr, 1992).

Potential uses for Acid Whey

Ideally, acid whey ingredients would have the potential to be used in any and all circumstances that sweet whey ingredients are used. Sweet whey protein powders, most popularly used for protein supplements, are often used in many food products as a protein source, thickener or stabilizer (Isleten and Karagul-Yuceer, 2006). Acid whey protein has not been considered for these applications because of its low pH. Other than pH, acid whey and sweet whey have very few differences, most being slight nutritional differences. By neutralizing the acid whey, it essentially is equal to sweet whey. By concentrating this product, we can make WPC 34, 80 or WPI. Considering that extra processing needs to occur, possibly involving neutralization, a cost to benefit analysis would need to be evaluated. Assuming that these costs would be minimal compared to the amount of usable whey protein that could be produced by a single cottage cheese plant, it is reasonable to suggest that a dairy making these products could produce their own whey ingredients for other products.

Yogurt Manufacture

Basic Principles of Milk Processing

Pasteurization of fluid milk allows safe consumption and extends shelf life. In order for milk to be considered a Grade A product, it must have a standard of identity in accordance to 21 CFR 131.110 and is required to follow the Pasteurized Milk Ordinance (PMO), to be sold in the United States. According to the PMO: “The terms ‘pasteurization’, ‘pasteurized’ and similar terms shall mean the process of heating every particle of milk or milk product, in properly designed and operated equipment, to one of the temperatures given in Table 1.3, and held

continuously at or above that temperature for at least the corresponding specified time". It was also noted that if the fat content of milk is 10% or greater, or the total solids are 18% or greater, the temperatures for pasteurization would be increased by 3°C (5°F). Heat treatment of milk is necessary because it is an excellent growing medium for microorganisms. Milk processing usually follows a standard procedure: clarification to remove dirt and body cells, separating of cream and skim milk by centrifugation, standardization of fat content, pasteurization, homogenization, and then packaging (Goff and Griffiths, 2006).

The dairy industry has developed many methods of pasteurizing products over the past few decades. Milk plants commonly use high temperature short time (HTST) pasteurization for yogurt. This process requires that raw milk be heated to 71.7°C (161°F) and held at this temperature for 15s. Yogurt mix is made with raw milk and other ingredients such as dairy protein powders and stabilizers. According to the PMO, if fat content of milk is ten percent or higher, or a total solids of 18% or greater, or if it contains added sweeteners, the specified temperature would be increased by 3°C (5°F).

Although homogenization isn't required by the FDA or USDA, it is a standard practice in most dairy facilities. Homogenization is a technique that uses high pressure (10-20 MPa) at temperatures of 55-70°C to force milk through small areas that reduces particle size. This is mostly performed to reduce the size of fat particles to prevent separation from the milk during storage (Hongyu et al., 2001). Once the milk has been thermally processed according to FDA standards, it is cooled and packaged for transport.

Definition of Yogurt

According to the Code of Federal Regulations, under Title 21: Subchapter B: Part 131: Subpart B: Section 131.200, Yogurt is the food produced by culturing cream, milk, partially

skimmed milk, or skim milk, used alone or in combination, with a bacteria culture that contains lactic acid-producing bacteria *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Yogurt, before addition of culture should have no less than 3.25% milkfat, 8.25% milk solids not fat, and a titratable acidity of no less than 0.9%" (FDA, 2016).

Since 1960, in the United States, yogurt production has increased from 43.98 million pounds to 4.7 billion pounds in 2013 with an annual per capita consumption of 14.92 pounds (IDFA, 2017). The yogurt business in the United States was worth $3.9 billion dollars in 2010 and continues to grow. Blended and fruit on the bottom style yogurts are the main sellers, contributing up to 40% of yogurt, Greek style has moved into 30% of sales, and specialty yogurts and kids yogurts make up the rest of yogurt sales (IDFA, 2017).

Yogurt Processing

Heat Treatment

Yogurt, like milk, requires a specific pasteurization process to get rid of any competitive bacteria in the mix that would prevent desirable bacteria from growing. This process also creates reducing conditions, produces protein-cleaved nitrogenous compounds and reduces oxygen. All of these effects enhance the nutritional status of the medium for optimal bacterial growth (Kilara, 2013). Not only does the heat treatment provide a method to kill potentially dangerous bacteria in the yogurt, it also provides the backbone to the gel set for the yogurt. Denaturing whey proteins with heat causes the viscosity of the yogurt to increase. This is because whey protein denaturation enhances water-absorption capacity and creates a smooth consistency and stability from whey separation. Denatured whey proteins also interact with casein proteins to form a gel structure. *B*-Lactoglobulin, specifically, interacts with *k*-Casein on the surface of casein micelles and crosslinks the two proteins (Lee and Lucey, 2004).

Heat treatment of raw yogurt mix is higher than milk pasteurization temperatures. Heat treatment of yogurt can be completed in multiple ways. The more traditional way to pasteurize is in a jacketed processing tank known as vat pasteurization. In this method, the raw mix is brought up to the yogurt pasteurization temperature (~75C) and held at that temperature for about 30 minutes. The most common pasteurization method is by using a plate heat exchanger or HTST processing. The mix is pumped through the plate and brought up to pasteurization temperature (90-95C). Yogurt pasteurization hold times range from 30 seconds to 7 minutes (Kilara, 2013).

Homogenization

Homogenization is a process that reduces fat particle size to less than 1 um in diameter and uniformly distributes the milk fat in the yogurt. This is done to prevent a "creamy layer" from forming on top of the yogurt. This also prevents whey separation in yogurt by increasing the stability of the yogurt. Homogenization temperatures are usually from 55-80C with pressures being between 1500-3000 psi. In general, the homogenizer is placed between the pre-heater and the final heater in the system. This is done because the homogenization is most efficient when fat is in the liquid stage (Kilara, 2013). Another reason the yogurt is homogenized between the two heat treatments is that some ingredients, particularly stabilizers, can lose effectiveness from being exposed to high pressures such as homogenization pressures at pasteurization temperatures. Homogenizers can have one valve or two valves, also known as "single-stage" or "two-stage" homogenization. The first stage is always responsible for the homogenizing, but the second stage also has important roles. It provides constant back pressure on the first stage, and prevents clumping of fat particles after passage through the first stage. Homogenization can also help improve the consistency and viscosity of yogurt, and increase stability (Kilara, 2013).

Coagulation Process

Once the yogurt mix has been processed, it is inoculated with a starter culture and incubated between 43-45°C (Vedamuthu, 2013). The cultures are thermophilic starter bacteria that are a mixture of *Streptococcus* thermophiles and *Lactobacillus bulgaricus* (Lee and Lucey, 2004). The general function of the bacteria is to lower the pH of the mix to the isoelectric point of casein, which is about 4.6 on the pH scale. This is accomplished by generating lactic acid by the fermentation of lactose; the major sugar in milk; which is what the bacteria use as a food source. As more lactose is fermented to lactic acid, the pH of the mix will progressively drop. There is increased casein-casein attraction as the pH decreases during the fermentation, which results in gelation as casein approaches the isoelectric point. Acidification also leads to a slightly weaker gel network due to the solubilization of colloidal calcium phosphate (Lucey, 2002).

Lee and Lucey (2004) studied the structure and physical properties of yogurt gels by comparing the inoculation rate and the incubation temperature of heated milk. Using a commercial yogurt culture, heated skim milk was inoculated at 0.5, 1, 2, 3, or 4% (wt/wt) and incubated at either 40 or 45.7°C until the milk reached a pH oh 4.6. The study found that average time to gelation for 0.5, 1, 2, 3, and 4% inoculation were 180, 166, 142, 124, and 119 minutes respectively. The pH at gelation was not different between samples. The time to pH of 4.6 was reported for 0.5, 1, 2, 3, and 4% inoculation was 392, 348, 321, 305, and 260 minutes respectively. The difference between the samples shows that there was a significant difference between the percent of culture used and time until gelation except for 3 and 4% inoculation. All of the samples were significantly different for time to pH 4.6. Comparing the incubation temperatures, average time to gelation was 160 minutes for the samples at 40°C and 132 minutes for the samples at 45.7°C. Yogurt incubated at 40°C had a lower pH at gelation than the yogurt

at 45.7°C, 5.41 and 5.45 respectively. Not significantly different were the time to pH 4.6, where both samples took about 330 minutes. The study concluded that inoculation rate and incubation temperature were significant processing parameters that affected the physical properties and micro structure of yogurt gels.

Packaging

Packaging materials are important in all food categories. They provide a protective barrier from environmental conditions that may affect the shelf-life or safety of foods, as well as a means to market the features of foods (Routray and Mishra, 2011). Packaging is very important to preserve quality during storage of physiochemical and sensory aspects of yogurt. Effects of yogurt packaging on sensory and textural characteristics have been documented (Linssen et al., 1991; Salvador and Fiszman, 2004; Saint-Eve et al., 2008).

Yogurt flavor is affected by packaging material. Saint-Eve et al. (2008) compared yogurts with 0 and 4% fat stored in glass, polypropylene and polystyrene containers over a shelf life of 28 days. Packaging type had a greater impact on lower fat yogurts than on full fat yogurts, for both sensory and physiochemical characteristics. Type of package also had an effect on yogurt flavor. Yogurts in glass containers had the least amount of aroma decrease over shelf life. Polystyrene was preferred for preventing loss of fruity notes and limiting odor defects. Linssen et al. (1991) also confirmed that polyethylene containers absorbed volatile compounds from yogurt drinks. These studies outlined the importance of packaging with respect to yogurt manufacture and distribution.

Cooling

The objectives of cooling the yogurt are to restrict the growth of the yogurt bacterial culture and restrict their enzyme activity, and to maintain desired body, texture and pH.

Generally, cooling takes place once the yogurt has reached a pH of 4.6 (Kilara, 2013). Often, yogurt products are cooled by blasting cold air in refrigerated storage to prevent further acid production. Set yogurts are usually sent right to cold storage after fermentation is completed and blended yogurts are usually cooled in a jacketed vat or through a cooling press (Lee and Lucey, 2010). Zhong et al. (2007) studied the rheology and microstructure of yogurts as they were cooled at varying cooling rates. Yogurts with differing protein levels (15%, 16%, 17% and 18%) and pH (5.8, 6.5, 7.2 and 12) were compared. Storage moduli were always highest at the fastest rate of cooling (0.5°C/min). Yogurts with higher protein levels also exhibited larger storage moduli. Higher storage moduli have been reported as ideal for yogurt structure (Lee and Lucey, 2010). At a molecular level, Haque et al. (2001) noted that as cooling of yogurt occured, hydrophobic contacts become weaker but were replaced by other interactions such as hydrogen bonding, however the overall gel structure remained the same. This suggests that gel structure is more affected by fermentation temperature and time than by cooling. Cooling slows fermentation of yogurt rather than strengthening the gel.

Ingredient Contributions to Yogurt Physical and Sensory Properties

Typical Yogurt Ingredients

The main ingredient in most yogurt formulas is milk, whether it is skimmed or whole or somewhere in between. Low-fat and fat free yogurts have gained popularity among people who seek healthy options across product categories. Production of these types of yogurts requires very careful control of processing conditions and formulation to perfect the textural and flavor characteristics of the yogurt (Isleten and Karagul-Yuceer, 2006). Defects in such characteristics could result in poor gel strength or surface whey separation (Lucey, 2002). Many yogurts utilize plant hydrocolloids or animal proteins to impact the stability, thickness and gelling properties

(Akalin et al., 2012; Barrantes et al., 1994; Schmidt et al., 2000). These ingredients can lead to off-flavors in yogurts, even at low usage levels (Sodini et al., 2004). With more demand for "all natural" product, combined with the potential off-flavor issue, there is a trend to use milk-based proteins and milk solids to fortify yogurt (Modler et al., 1983). Commonly, skim milk powder is used to increase the solids and fortify yogurt, but other dried dairy ingredients such as calcium caseinate, sodium caseinate, whey protein concentrate, and whey protein isolate have also been used as a viable way to increase total solids in low or nonfat yogurts (Isleten and Karagul-Yuceer, 2006).

Peng et al. (2009) conducted a study comparing different milk protein powders in yogurt. Yogurts were made by reconstituting skim milk powder to yield skim milk with 2.5% protein. Additions of skim milk powder, milk protein isolate, micellar casein, or sodium caseinate brought the total protein to 3.5%. They found that different types of milk proteins had significant effects on rheological proterties of yogurt. Sodium caseinate was the most effective in improving texture while milk protein isolate and micellar casein were not as effective but still improved texture over yogurts made with skim milk powder (Peng et al., 2009). Isleten and Karagul-Yuceer (2006) had similar results with sodium caseinate when compared to control yogurt made with skim milk powder. Sodium caseinate yogurts had improved texture and less syneresis than the control. Sodium caseinate yogurts also had better sensory properties and were preferred by consumers over the control (Isleten and Karagul-Yuceer, 2006).

How whey protein powders affect yogurt

Whey protein powders are commonly used as ingredients in yogurt formulations for textural reasons as cost effective replacements to other dairy ingredients such as nonfat dry milk. Sodini et al. (2005) examined the physical and rheological effects of fortifying yogurt with whey

protein concentrates as opposed to skim milk powder. Whey proteins were provided from various sources (Cheddar, mozzarella, and other cheeses). They found that yogurts made with whey protein had higher water holding capacities than those made with skim milk powder. These results were attributed to the possibility of higher cross-linkage networks. The data also showed strong variations in rheological properties such as firmness and viscosity. These traits were related to higher levels of non-protein nitrogen and denatured whey protein, which could affect the functionality of the whey proteins (Sodini et al., 2005). Puvanenthrian et al. (2002) studied the effect of changing the casein to whey protein ratio in yogurt mixes. Yogurts were made using skim milk powder and whey protein concentrates of differing protein contents (34-63%). By decreasing the casein to whey ratio, the gel networks within the yogurts became finer and denser with smaller aggregates. This was believed to be related to the lower whey drainage in yogurts with lower casein to whey ratios and higher gel strength attributes (Puvanenthrian et al., 2002). Zhao et al. (2015) reported similar results. Viscoelastic and gelation properties of yogurts were improved by decreasing the casein to whey protein ratio (Zhao et al., 2015). Barrantes et al. (1994) determined that using whey protein powders as a fat substitute increased apparent viscosity but decreased firmness over storage time and increased serum separation compared to control yogurt made with fat. More recently, Aziznia et al. (2008) determined that using whey protein concetrate to replace fat can benefit yogurt. They reported that reduction of fat reduced firmness due to interactions between fat globules and the gel network. However, as they increased fortification with whey protein concentrate, firmness increased and syneresis decreased (Aziznia et al., 2008). Similar results were found by Akalin et al. (2012), Isleten and Karagul-Yuceer (2006), Jorgensen et al. (2015), and Laiho et al. (2017).

Sensory evaluations of whey protein addition to yogurts have also been documented.

Isleten and Karagul-Yuceer (2006) studied the effects of whey protein fortification on sensory properties of yogurt. They found that yogurts fortified with skim milk powder had higher free whey and lower thickness than yogurts fortified with whey protein. Lumpiness was described as large protein aggregates, for which whey protein yogurts scored higher than control yogurts. Significant differences were found in flavor attributes. Creaminess, whey flavor, and astringency were all higher in whey protein fortified yogurts compared to control yogurts. Surprisingly, cardboard flavor, which is a known flavor defect of whey protein addition to foods (Whetstine et al. 2005), was not different among yogurts fortified with skim milk powder and whey protein isolate (Isleten and Karagul-Yuceer, 2006). Laiho et al. (2017) found similar results. Comparing the differences in casein to whey protein ratios in yogurts, sensory perceptions were altered as whey protein increased. They found that although some characteristics were decreased (sheen, smoothness, grain), thickness and gelation were increased. They concluded that increasing whey protein in yogurts increased whey protein interactions but may have negative effects on sensory properties (Laiho et al., 2017).

How other ingredients affect yogurt

Many ingredients have been added to yogurt formulations in the past to improve textural and sensory properties. Sugars have a water binding effect on yogurts which inherently increases viscosity (Sodini et al., 2004). Fernandez-Garcia et al. (1998) compared the differences of sugar usage in yogurt. They used differing concentrations of sucrose and fructose added to reconstituted skim milk powder and whole milk. Yogurts with fructose added had longer fermentation times. Both sucrose and fructose increased the viscosity of yogurts by 5-25%. Sugar also had an effect on sensory properties. Yogurts supplemented with sucrose or fructose had higher apparent viscosities than the control, decreased acetaldehyde flavor, increased

sweetness, and had better texture attributes than unsweetened yogurts (Fernandez-Garcia et al., 1998). McGregor and White (1986) found similar results with comparing different sweeteners in plain and fruited yogurts. Yogurts with sweeteners added had higher flavor and liking scores in consumer testing than unsweetened yogurt, regardless of sweetener used.

Often, stabilizers such as gelatin or polysaccharides are used as ingredients in yogurt bases. These ingredients can have multiple purposes such as increasing stability, desirable texture, helping to gel or simply thicken yogurt (Sodini et al., 2004). Stabilizers function through their ability to form gel structures in water, and can form complexes with casein to increase yogurt body and reduce syneresis. An ideal yogurt stabilizer will not have any off-flavor characteristics, be effective at lower pH ranges, have good water holding capacity, is easily soluble and promotes stable foam formation (in whipped yogurt) (Sodini and Tong, 2013). Gelatin, a common stabilizer has been tested in milk systems for use in yogurt applications. Salvador and Fiszman (1998) compared different levels of gelatin in both milk and water. Results showed that increasing gelatin concentrations increased gel strength, breaking force, and hardness of the gel, without affecting flavor. It has also been reported that gelatin adds increased water holding capacity, increased viscosity, and gel networks to yogurts without affecting flavor (Fiszman et al., 1999; Pang et al., 2014; Nguyen et al., 2017; Teles and Flores, 2007).

Starches are common stabilizers used in yogurt applications as well. They may be modified to better suit the product needs or in the native form of the starch (Sodini and Tong, 2013). An ideal starch stabilizer would have cross-linked bonds which can provide a resistance to acid over long periods of storage. This is key for a product at high acid levels such as yogurt (Schmidt et al., 2000). While native starches are essentially natural and unmodified, they can have limitations both in product and processing areas. Modified starches are designed to

withstand processing conditions such as high heat and shear, while also having the ability to function in high-acid environments such as in yogurt. In general, modified starches have better functionality and stability than native starches (Sodini and Tong, 2013). Starches can come from many different sources. Common types of starches are potato, corn, wheat, and tapioca (Schmidt et al., 2000). Tapioca starches have been found to have very little flavor carry-over into dairy products (LaBell, 2000). Schmidt et al. (2000) compared the effects of adding different starches to yogurt bases in place of gelatin. They found that native starches increased firmness in yogurts while modified starches were outperformed by gelatin. Yogurts made with gelatin had lower values of titratable acidity than yogurts made with starches. They concluded that native starches could replace gelatin in set style yogurts and modified starches may be considered for stirred yogurts. Hess et al. (1997) found similar results with combinations of modified food starch with pectin and modified food starch with gelatin. Compared to control yogurts, stabilized yogurts had higher apparent viscosity. These results demonstrate that by altering stabilizers or their concentrations, different yogurt texture properties can be achieved.

Conclusion

As cheese and other fermented dairy products have gained popularity, there has been an increased need to recycle and reuse the byproduct acid whey. Many companies and agencies have taken great strides forward to incorporate acid whey into other acidic products or to process the whey into other products such as lactose or lactic acid. Although these newly developed methods have aided in dealing with the growing acid whey problem, the issue of having too much acid whey still exists. Yogurt is a healthy and affordable food product that people have been consuming more of over time. Often, yogurt products use whey protein as a supplement to increase the milk solids non-fat, which can attribute to better creaminess, viscosity and texture of

the finished product. This thesis will explore processing of neutralized acid whey to whey protein concentrate (80% protein) and its incorporation into yogurt.

References

Akalin, A. S., G. Unal, N. Dinkci, and A. A. Hayaloglu. 2012. Microstructural, textural, and sensory characteristics of probiotic yogurts fortified with sodium calcium caseinate or whey protein concentrate. J. Dairy Sci. 95: 3617-3628.

Arnal, M. A., L. Mosoni, Y. Boirie, M. L. Houlier, L. Morin, E. Verdier, P. Ritz, J. M. Antoine, J. Prugnaud, B. Beaufrère, and P. P. Mirand. 1999. Protein pulse feeding improves protein retention in elderly women. Am. J. for Clin. Nutr. 69: 1202-1208.

Aziznia, S., A. Khosrowshahi, A. Madadlou, and J. Rahimi. 2008. Whey protein concentrate and gum tragacanth as fat replacers in nonfat yogurt: chemical, physical, and microstructural properties. J. Dairy Sci. 91: 2545-2552.

Bals, A., and U. Kulozik. 2003. Effect of preheating on the foaming properties of whey protein isolate using a membrane foaming apparatus. Int. Dairy J. 13: 903-908.

Barrantes, E., A. Y. Tamime, D. D. Muir, and A. M. Sword. 1994. The effect of substitution of fat by microparticulate whey protein on the quality of set-type, natural yogurt. J. Dairy Sci. 47: 61-68.

Barrantes, L. D., and C. V. Morr. 1997. Partial Deacidification and Demineralization of Cottage Cheese Whey by Nanofiltration. J. Food Sci. 62: 338-341.

Bell, L. I. 2013. Nutritious beverage formed from fluid acid whey and a method of forming a nutritious beverage by combining fluid acid whey and a juice. Bell, L. I. US Pat. No. 20140335226A1.

Bilge, G., B. Sezer, K. E. Eseller, H. Berberoglu, A. Topcu, and I. H. Boyaci. 2016. Determination of whey adulteration in milk powder by using laser induced breakdown spectroscopy. J. Food Chem. 212: 183-188.

Brisson, G., and H. Singh. 2013. Milk composition, physical and processing characteristics. Pages 21-48 in Manufacturing yogurt and fermented milks, Vol 1. Chandan, R. C. and A. Kilara, ed. Wiley-Blackwell, West Sussex, UK.

Brody, A. L. 2013. Fermented dairy packaging materials. Pages 149-176 in Manufacturing yogurt and fermented milks, Vol. 1. Chandan, R. C. and A. Kilara, ed. Wiley-Blackwell, West ussex, UK.

Brussow, H., H. Hilpert, I. Walther, J. Sidoti, C. Mietens, and P. Bachmann. 1987. Bovine milk immunoglobulins for passive immunity to infantile rotavirus gastroenteritis. J. Clin. Microbiol. 25: 982-986.

Chandrapala, J., M. C. Duke, S. R. Gray, M. Weeks, M. Palmer, and T. Vasiljevic. 2017 Strategies for maximizing removal of lactic acid from acid whey – addressing the un-processability issue. Sep. Purif. Technol. 172: 489-497.

Chen, L., C. Guo, Y. Guan, and H. Liu. 2007. Isolation of lactoferrin from acid whey by

magnetic affinity separation. Sep. Purif. Technol. 56: 168-174.

Covacevich, H. R., and F. V. Kosikowski. 1978. Cottage Cheese by Ultrafiltration. J. Dairy Sci. 61: 529-535.

de Wit, J. N. 1998. Nutritional and Functional characteristics of whey proteins in food products. J. Dairy Sci. 81: 597-608.

de Wit, J. N. 2009. Thermal behavior of bovine β-lactoglobulin at temperatures up to 150°C: a review. Trends Food Sci. Technol. 20: 27-34.

Dec, B., and W. Chojnowski. 2006. Characteristics of acid whey powder partially demineralised by nanofiltration. Pol. J. Food Nutr. Sci. 15: 87-90.

Edwards, P. B., L. K. Creamer, and G. B. Jameson. 2009. Structure and stability of whey proteins. Pages 163-205 in Milk proteins from expression to food, Vol. 1. Thompson, A., M. Boland, and H. Singh, ed. Acedemic Press, San Diego, CA.

Erickson, B. E. 2017. Acid whey: is the waste product an untapped goldmine?. Accessed Feb. 6, 2017. http://cen.acs.org/articles/95/i6/Acid-whey-waste-product-untapped.html.

Farkye, N. Y. 2004. Cheese Technology. Int. J. Dairy Technol. 57: 91-98.

FDA. 2016. 21 CFR 1240.61. Code of Federal Regulations. Accessed May 15, 2017. http://ecfr.gpoaccess.gov/cgi/t/text/text-idx?c=ecfr&sid=d66e31091e21e93f7cba1d2d0d5fc570&rgn=div8&view=text&node=21:8.0.1.5.48.4.1.2&idno=21

FDA. 2016. 21 CFR 131.200. Code of Federal Regulations. Accessed Feb. 20, 2017. http://www.accessdata.fda.gov/SCRIPTs/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=131.200

FDA. 2016. 21 CFR 131.3. Code of Federal Regulations. Accessed Jun. 19, 2017. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=131.3

FDA. 2015. Grade A Pasteurized Milk Ordinance. Accessed Feb. 3, 2017. https://www.fda.gov/downloads/food/guidanceregulation/guidancedocumentsregulatoryinformation/milk/ucm513508.pdf

Fernandez-Garcia, E., J. U. McGregor, and S. Traylor. 1998. The addition of oat fiber and natural alternative sweeteners in the manufacture of plain yogurt. J. Dairy Sci. 81: 655-663.

Fiszman, S. M., M. A. Lluch, and A. Salvador. 1999. Effect of addition of gelatin on microstructure of acidic milk gels and yoghurt and on their rheological properties. Int. Dairy J. 9: 895-901.

Fox, P. F. 2009. Milk: and overview. Pages 1-54 in Milk proteins: from expression to food, Vol. 1. Thompson, A., M. Boland, abd H. Singh, ed. Acedemic Press, San Diego, CA.

Goff, H. D., and M. W. Griffiths. 2006. Major advances in fresh milk and milk products: fluid

milk. J. Dairy Sci. 89: 1163-1173.

Gonzalez, T., and E. Smith. 2014. Soluble fiber from yogurt whey. Assignee, General Mills Inc. US Patent 20140348979.

Hambling, S. G., A. S. McAlpine, and L. Sawyer. 1992. β-Lactoglobulin. Pages 141-190 in Advanced dairy chemistry, proteins, Vol. 1. P.F. Fox, ed. Elsevier Applied Science, London, UK.

Haque, A., R. K. Richardson, and E. R. Morris. 2001. Effect of temperature on the reology of set and stirred yogurt. Food Hydro. 15: 593-602.

Haque, Z. U., J. Shon, and J. B. Williams. 2009. Efficacy of sour whey as a shelf-life enhancer: use in antioxidative edible coatings of beef steak. J. Food Qual. 32: 381-397.

Haug, A, A. T. Hostmark, and O. M. Harstad. 2007. Bovine milk in human nutrition - A review. Lipids Health Dis. 6: 1-25.

Hess, S. J., R. F. Roberts, and G. R. Ziegler. 1997. Rheological properties of nonfat yogurt stabilized using lactobacillus delbrueckii ssp. bulgaricus producing exopolysaccharide or using commercial stabilizer systems. J. Dairy Sci. 80: 252-263.

Hiraoka, Y., T. Segawa, and K. Kuwajima. 1980. α-Lactalbumin: A calcium metalloprotein. Biochem. Biophys. Res. Commun. 95: 1098-1104.

Hongyu, W., G. J. Hulbert, and J. R. Mount. 2001. Effects of ultrasound on milk homogenization and fermentation with yogurt starter. Innov. Food Sci. Emerg. Technol. 1: 211-218.

Horne, DS. 2006. Casein micelle structure: models and muddles. Curr. Opin. Colloid Interface Sci. 11: 148-153.

Hu, Y. J., Y. Liu, J. B. Wang, X. H. Xiao, and S. S. Qu. 2004. Study of the interaction between monoammonium glycyrrhizinate and bovine serum albumin. J pharm biomed anal. 36: 915-919.

Hudson, H. M., C. R. Daubert, and E. A. Foegeding. 2000. Rheological and physical properties of derivitized whey protein isolate powders. J. Agric. Food Chem. 48: 3112-3119.

IDFA. 2017. Cultured products. Accessed May 17, 2017. https://www.idfa.org/resource-center/industry-facts/cultured-products

Isleten, M, and K. Karagul-Yuceer. 2006. Effects of dried dairy ingredients on physical and sensory properties of nonfat yogurt. J. Dairy Sci. 89: 2865-2872.

Jalen, P. 1979. Industrial whey processing technology: An overview. J. Ag. Food Chem. 4: 658-661.

Jorgensen, C. E., R. K. Abrahamsen, E. O. Rukke, A. G. Johansen, R. B. Schuller, and S. B. Skeie. 2015. Improving the structure and rheology of high protein, low fat yoghurt with indenatured whey proteins. Int. Dairy J. 47: 6-18.

Kersten, S., B. S. Murray, and E. Dickinson. 2005. Confocal microscopy of heat-induced aggregation and gelation of b-lactoglobulin in presence of non-ionic surfactant. Food Hydro. 19: 625-633.

Kilara, A. 2013. Principles of dairy processing. Pages 95-114 in Manufacturing yogurts and fermented dairy products, Vol. 1. Chandan R. C., and A. Kilara, ed. Wiley-Blackwell, West Sussex, UK.

Kilara, A., and R. C. Chandan. 2013. Greek-style yogurt and related products. Pages 297-318 in Manufacturing yogurt and fermented milks, Vol. 1. Chandan R. C., and A. Kilara, ed. Wiley-Blackwell, West Sussex, UK.

Kontopidis, G., C. Holt, and L. Sawyer. 2002. The ligand-binding site of bovine b-lactoglobulin: Evidence for a function?. J. Mol. Biol. 318: 1043-1055.

Korhonen, H., P. Marnila, and H. S. Gill. 2000. Milk immunoglobulins and complement factors. Br. J. Nutr. 84: S75-S80.

Kosikowski, F. V. 1979. Whey utilization and whey products. J. Dairy Sci. 62: 1149-1160.

LaBell, F. 2000 Modified tapioca starches provide smooth textures. Prep. Foods 169: 63.

Laiho, S., R. P. W. Williams, A. Poelman, I. Appelqvist, and A. Logan. 2017. Effect of whey protein phase volume on the tribology, rheology and sensory protperties of fat-free yoghurts. Food Hydro. 67: 166-177.

Lam, R. S. H., and M. T. Nickerson. 2015. The effect oh pH and temperature pre-treatments on the structure, surface characteristics and emulsifying properties of alpha-lactalbumin. J. Food Chem. 173: 163-170.

Lee, W. J., and J. A. Lucey. 2010. Formation and physical properties of yogurt. Asian-Australas J. Anim. Sci. 23: 1127-1136.

Lee, W. J., and J. A. Lucey. 2004. Structure and physical properties of yogurt gels: effect of inoculation rate and incubation temperature. J. Dairy. Sci. 87: 3153-3164.

Leman, J., T. Dolgan, M. Smoczynski, and Z. Dziuba. 2005. Fractal characteristics of microstructure of beta-lactoglobulin preparations and their emulsifying properties. Elec. J. Pol. Ag. Univ. 8:3.

Liao, S. Y., and M. E. Mangino. 1987. Characterization of the composition, physicochemical and functional properties of acid whey protein concentrates. J. Food Sci. 52: 1033-1037.

Lindmark-Mansson, H., R. Fonden, and H. E. Pettersson. 2003. Composition of swedish dairy milk. Int. Dairy J. 13: 409-425.

Linssen, J. P. H., A. Verheul, and J. P. Roozen. 1991. Absorption of flavour compounds by packaging material: drink yoghurts in polyehtylene bottles. Int. Dairy J. 1: 33-40.

Lucey, J. A. 2002. Formation and physical properties of milk protein gels. J. Dairy Sci. 85: 281-

294.

Marshall, K. 2004. Therapeutic applications of whey proteins. Alt. Med. Review. 9: 136-156.

Mattews, M. E. 1984. Whey protein recovery processes and products. J. Dairy Sci. 67: 2680-2692.

McGregor, J. U., and C. H. White. 1986. Effect of sweeteners on the quality and acceptability of yogurt. J. Dairy Sci. 69: 698-703.

Michalek, S. M., R. L. Gregory, C. C. Harmon, J. Katz, G. J. Richardson, T. Hilton, S. J. Filler, and J. R. Mcghee. 1987. Protection of gnotobiotic rats against dental caries by passive immunization with bovine milk antibodies to Streptococcus mutans. Infect. Immun. 55: 2341-2347.

Modler, H. W., M. E. Larmond, C. S. Lin, D. Froehlich, and D. B. Emmons. 1983. Physical and sensory properties of yogurt stabilized with milk proteins. J. Dairy Sci. 66: 422-429.

Mohamed, F. O., and R. Bassette. 1979. Quality and yield of cottage cheese influenced by psychrotrophic microorganisms in milk. J. Dairy Sci. 62: 222-226.

Morr, C. V. 1992. Whey utilization. Pages 133-153 in Whey and lactose processing, Vol. 1. J. G. Zadow, ed. Springer, Dordrecht, Netherlands.

Nguyen, P. T. M., O. Kravchuk, B. Bhandari, and S. Prakash. 2017. Effect of different hydrocolloids on texture, rheology, tribology and sensory perception of texture and mouthfeel of low-fat pot-set yoghurt. Food Hydro. 72: 90-104.

NYS DOEC. 2017. Whey management for agriculture. Accessed Jan. 13, 2017. https://www.dec.ny.gov/chemical/94164.html

Pang, Z., H. Deeth, P. Sopade, R. Sharma, and N. Bansal. 2014. Rheology, texture and microstructure of gelatin gels with and without milk proteins. Food Hydro. 35: 484-493.

Parashar, A., Y. Jin, B. Mason, M. Chae, and D. Bressler. 2015. Incorporation of whey permeate, a dairy effluent, in ethanol fermentaion to provide a zero waste solution for the dairy industry. J. Dairy Sci. 99: 1859-1867.

Peng, Y., M. Serra, D. S. Horne, and J. A. Lucey. 2009. Effect of fortification with various types of milk proteins on the rheological properties and permeability of nonfat set yogurt. J. Food Sci. 74: 666-673.

Pilarska, A. A., K. Pilarski, K. Witaszek, H. Waliszewska, M. Zborowska, B. Waliszewska, M. Kolasiński and K. Szwarc-Rzepka. 2016. Treatment of dairy waste by anaerobic co-digestion with sewage sludge. Ecol. Chem. Eng. 23: 99-115.

Prazeres, A. R., F. Carvalho, and J. Rivas. 2012. Cheese whey management: A review. J. Environ. Manage. 110: 48-68.

Puvanenthrian, A., R. P. W. Williams, and M. A. Augustin. 2002. Structure and visco-elastic

properties of set yoghurt with altered casein to whey protein ratios. Int. Dairy J. 12: 383-391.

Raikos, V. 2010. Effect of heat treatment on milk protein functionality at emulsion interfaces: A review. Food Hydro. 24: 259-265.

Relkin, P., and D. M. Mulvihill. 1996. Thermal unfolding of β-lactoglobulin, α-lactalbumin, and bovine serum albumin: A thermodynamic approach. Crit. Rev. Food Sci. Nutr. 36: 565-601.

Remon, J., J. Ruiz, M. Olivia, L. Garcia, and J. Arauzo. 2016. Cheese Whey Valorisation: Production of valuable gaseous and liquid chemicals from lactose by aqueous phase reforming. Energy Convers. Manag. 124: 453-469.

Roman, A., J. Wang, J. Csanadi, C. Hodur, and G. Vatai. 2009. Partial demineralization and concentration of acid whey by nanofiltration combined with diafiltration. Desalination. 241: 288-295.

Routray, W., and H. N. Mishra. 2011. Scientific and technical aspects of yogurt aroma and taste: A review. Compr. Rev. Food Sci. Food Saf. 10: 208-220.

Saint-Eve, A., C. Levy, M. Le Moigne, V. Ducruet, and I. Souchon. 2004. Quality changes in yogurt during storage in different packaging materials. Food Chem. 110: 285-293.

Salvador, A., and S. M. Fiszman. 2004. Textural and sensory characteristics of whole and skimmed flavored set-type yogurt during long storage. J. Dairy Sci. 87: 4033-4041.

Schmidt, K. A, T. J. Herald, and K. A. Khatib. 2000. Modified wheat starches used as stabilizers in set-style yogurt. Food Qual. 24: 421-434.

Silva, E. M., and S. T. Yang. 1995. Kinetics and stability of a fibrous-bed bioreactor for continuous production of lactic acid from unsupplemented acid whey. Biotech. 41: 59-70.

Siso, M.I. Gonzalez. 1996. The biotechnological utilization of cheese whey: A review. Bioresour. Technol. 57: 1-11.

Smithers, G. W. 2008. Whey and whey proteins: from 'gutter to gold'. Int. Dairy J. 18: 695-704.

Smithers, G. W. 2015. Whey-ing up the options: Yesterday, today and tomorrow. Int. Dairy J. 48: 2-14.

Sodini, I., J. Montella, and P. S. Tong. 2005. Physical properties of yogurt fortified with various commercial whey protein concentrates. J. Sci. Food Ag. 85: 853-859.

Sodini, I., and P. S. Tong. 2013. Milk and milk based ingredients. Pages 177-192 in Manufacturing of Yogurt and Fermented Milks, Vol. 1. Chandan, R. C. and A. Kilara, ed. Wiley-Blackwell, West Sussex, UK.

Sodini, I., F. Remeuf, S. Haddad, and G. Corrieu. 2004. The relative effect of milk base, starter, and process on yogurt texture: A review. J. Food Sci. Nutr. 44: 113-137.

Tacket, C. O., S. B. Binion, E. Bostwick, G. Losonsky, M. J. Roy, and R. Edelman. 1992. Efficacy of bovine milk immunoglobulin concentrate in preventing illness after shigella flexneri challenge. Am. J. Trop. Med. Hygen. 47: 276-283.

Teles, C. D., and S. H. Flores. 2007. The influence of additives on the rheological and sensory. Int. J. Dairy Tech. 60: 270-276.

Tipton, K, T. Elliott, M. Cree, S. Wolf, A. Sanford, and R. Wolfe. 2004. Ingestion of casein and whey proteins results in muscle anabolism after resistance exercise. Med. Sci. Sports Exerc. 36: 2073-2081.

Tsakali, E., K. Petrotos, A. D'Allessandro, and P. Goulas. 2007. A review on whey composition and the methods used for its utilization for food and pharmaceutical products. 6th International conference on simulation and modelling in the food and bio-industry (FoodSim 2010). 195-201.

Tunick, M. H. 2009. Whey protein production and utilization: a breif history. Pages 1-13 in Whey processing, functionality and health benefits, Vol. 1. Onwulata, C. I. and P. J. Huth, ed. John Wiley and Sons, Inc, Ames, IA.

USDA. 2015. Dairy products 2015 summary: USDA. Accessed Feb. 6, 2017. http://usda.mannlib.cornell.edu/usda/nass/DairProdSu//2010s/2015/DairProdSu-04-29-2015.pdf

van Reimsdijk, L. E., A. J. van der Goot, R. J. Hamer, and R. M. Boom. 2011. Preparation of gluten-free bread using a meso-structured whey protein particle system. J. Cer. Sci. 53: 355-361.

van Vliet, T., C. M. M. Lakemond, and R. W. Visschers. 2004. Rheology and structure of milk protein gels. Curr. Opin. Colloid Interface Sci. 9: 298-304.

Varnam, A. H., and J. P. Sutherland. 2001. Introduction. Pages 1-42 in Milk and Milk Products, Vol. 2. Aspen Publishers, Gaithersburg, Maryland.

Vasbinder, A. J., and C. G. de Kruif. 2003. Casein-whey protein interactions in heated milk: the influence of pH. Int. Dairy J. 13: 669-677.

Vedamuthu, E. R. 2013. Starter cultures for yogurt and fermented milks. Pages 115-148 in Manufacturing yogurts and fermented milks, Vol. 1. Chandan, R. C. and A. Kilara, ed. Wiley-Blackwell, West Sussex, UK.

Wang, T., and J. A. Lucey. 2003. Use of multi-angle laser light scattering and size-exclusion chromatography to characterize the molecular weight and types of aggregates present in commercial whey protein product. J. Dairy Sci. 86: 3090-3101.

Weimer, P. J. 2001. Microbiology of the Dairy Animal. Pages 1-58 in Applied Dairy Microbiology, Vol. 2. Marcel Dekker Inc, New York, NY.

Whetstine, M. E., K. R. Cadwallader, and M. A. Drake. 2005. Characterization of aroma

compounds responsible for rosy/floral flavor in cheddar cheese. J. Ag. Food Chem. 53: 3126-3132.

Zhao, L. L., X. L. Wang, Q. Tian, and X. Y. Mao. 2015. Effect of casein to whey protein ratios on the protein interactions and coagulation properties of low-fat yogurt. J. Dairy Sci 99: 7768-7775.

Table 1.1 Concentrations of proteins in milk (Barrantes and Morr, 1997; Edwards et al., 2009; Brisson and Singh, 2013).

Protein	***Relative Abundance (mg/mL)***
α-Casein	16.16
β-casein	8.43
κ-casein	2.38
β-lactoglobulin	4.51
α-lactalbumin	1.47
Bovine Serum Albumin	0.46
Immunoglobulins	0.49

Table 1.2 Composition comparison between Cheddar whey and acid whey (Tunick, 2003; (Barrantes and Morr, 1997).

Parameter	***Cheddar Whey***	***Acid Whey***
Total Solids	6.30%	5.50%
Lactose	4.60%	4.41%
Lactic Acid	0.05%	0.58%
Protein	0.80%	0.52%
pH	6.2	4.6
Ash	0.50%	0.80%
Fat	1.00%	0.32%

Table 1.3 Pasteurization temperature-time requirements for milk (FDA, 2015).

Pasteurization	***Temperature***	***Time***
Vat Pasteurization	63°C (145°F)	30 min
HTST	72°C (161°F)	15 s
HHST	100°C (212°F)	0.01 s
Ultra-pasteurized	138°C (280°F)	2 s
UHT Sterilized	140°C (284°F)	4 s

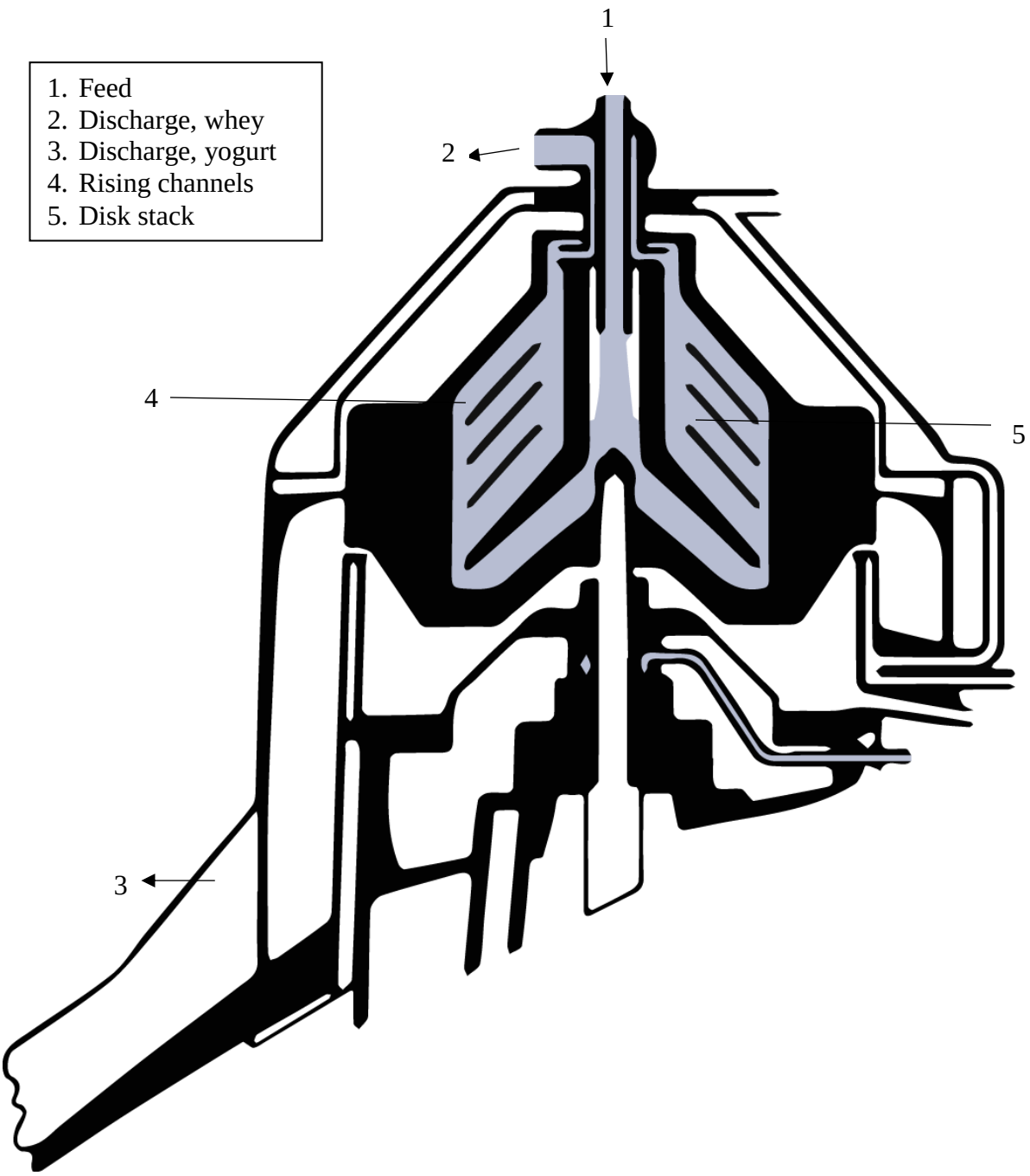


Figure 1.1 Schematic of a separator used for Greek-style yogurt. (Kilara and Chandan, 2013)

CHAPTER 2:

USE OF ACID WHEY PROTEIN AS AN INGREDIENT IN NONFAT SET-STYLE YOGURT.

Use of acid whey protein as an ingredient in nonfat set-style yogurt.

B.M. Wherry[1], D.M. Barbano[2], M.A. Drake[1]

[1]Southeast Dairy Foods Research Center, North Carolina State University, Raleigh, NC 27695

[2]Northeast Dairy Research Center, Cornell University, Ithaca, NY 14850

The contents of this chapter have been submitted to:

Journal of Dairy Science

ABSTRACT

Acid whey resulting from the production of soft cheeses is a disposal problem for the dairy industry. Few uses have been found for acid whey because of its high ash content, low pH, and high organic content. The objective of this study was to explore the potential of recovery of whey protein from cottage cheese acid whey for use in yogurt. Cottage cheese acid whey and Cheddar cheese whey were produced from standard cottage cheese and Cheddar cheese make procedures, respectively. The whey was separated and pasteurized by high temperature short time (HTST) pasteurization and stored at 4°C. Food grade ammonium hydroxide was used to neutralize the acid whey to a pH of 6.4. The whey was heated to 50°C and concentrated using an ultrafiltration system equipped with 11 polyethersulfone cartridge membrane filters (10,000 kDa cutoff) to 25% total solids, 80% protein. Skim milk was concentrated to 6% total protein. Nonfat, unflavored set-style yogurts (6.0±0.1% protein, 15±1.0% solids) were made from skim milk with added acid whey protein (AWP), skim milk with added sweet whey protein (SWP) or skim milk concentrate (SMC). Yogurt mixes were standardized to protein, lactose, and fat of 6.00%, 6.50% and 0.10% respectively. Yogurt was fermented at 43°C to pH 4.6 and stored at 4°C. The experiment was replicated in triplicate. Titratable acidity, pH, whey separation, color, and gel strength were measured weekly in yogurts through 8 weeks. Trained panel profiling was conducted on days 0, 14, 28, and 56. Yogurt made with AWP had similar color and titratable acidity to those made with SWP ($p>0.05$). Yogurts with AWP and SWP were distinct in sensory characteristics from the control yogurt made from SMC. Yogurt with AWP had higher values of syneresis, lower gel strength, higher sour taste and lower firmness and viscosity compared to yogurts made with SWP ($p<0.05$). Consumer ($n=100$) liking scores indicated that texture and thickness of AWP yogurt were less liked than SMC or SWP ($P < 0.05$). Reformulation of

yogurts with increased fat and hydrocolloids resulted in trained panel texture attribute intensities and consumer liking scores for AWP yogurts that were similar to SMC yogurts ($P < 0.05$). These results indicate that AWP can be used as an ingredient in yogurt.

Introduction

Whey protein, which originates from milk, is usually collected following the coagulation or precipitation of casein for cheese production. After removal from cheese vats, liquid whey is centrifuged to reduce fat content and pasteurized to prevent further lactic acid production. The whey protein is then concentrated through ultrafiltration (UF) and/or microfiltration (MF) (Modler et al., 1983; de Wit, 1998) and dried to produce whey protein concentrate (WPC) (protein content 30-90%) or whey protein isolate (WPI) (protein content > 90%) (Lopes et al., 2006).

Commonly, whey protein is used in a wide variety of food applications. Whey protein supplementation in foods includes flavored bars, infant formula and various other foods to increase protein density (Onwulata et al., 2001). Enriching foods with protein via WPC or WPI has become a popular trend in food manufacturing because of the economic, physical, and nutritional benefits (Tunick, 2009). Whey protein has excellent binding (Kontopidis et al., 2002; 2004), foaming (Bals et al., 2003), gelling (Kersten et al., 2005) and emulsifying (Leman et al., 2005) properties.

Due to increased cheese production, dairy products such as ice cream, yogurt, and flavored beverages have used whey protein as a replacement to nonfat dry milk (Morr et al., 1993). Whey protein addition to ice cream can be an effective fat replacer (Yilsay et al., 2006). Whey proteins have been added to protein beverages for increased nutritional density (Wagoner et al., 2015). Whey protein powders have been added to milk for yogurt manufacture to increase

totals solids and protein load, which increases firmness and viscosity and reduces syneresis (Lucey et al., 1999; Lopes et al., 2006). Since 1960, in the United States, yogurt production has increased from 43.98 million pounds to 4.7 billion pounds in 2013 with an annual per capita consumption of 14.92 pounds (IDFA 2017). The yogurt community in the United States was worth $3.9 billion dollars in 2010 and continues to grow. Blended and single style yogurts are the main sellers, contributing up to 40% of yogurt, Greek style has moved into 30% of sales, and specialty yogurts and kid's yogurts make up the majority of the rest of yogurt sales (Kilara and Chandan, 2013). Whey protein concentrate provides a cost effective and functionally sound ingredient as an alternative to nonfat dry milk (NFDM) in yogurt formulations (Sodini et al., 2005).

Most whey protein powders come from sweet whey from hard cheeses such as Cheddar or mozzarella (Smithers, 2015). Another type of whey stream is acid whey, which comes from the production of soft cheeses (pH ~4.5) and is a disposal issue for the dairy industry. In 2015, about 400 million pounds of cottage cheese curd was produced in the United States, which resulted in acid whey disposal of over 18 million pounds of whey protein annually (USDA, 2015). Due to its high Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD), acid whey can have negative effects on the environment and ecosystems where it may be disposed (Prazeres et al., 2012).

The low pH of acid whey makes it difficult to implement into foods. Drying acid whey is troublesome due to its high lactic acid content. Lactic acid agglomerates during spray drying, which causes lumping and caking of particles (Dec and Chojnowski, 2006). There are two main sources of acid whey. That which comes from Greek yogurt, and that which comes from soft cheeses, such as cottage cheese. Acid whey from Greek yogurt contains less available protein

than acid whey from soft cheese production (Barrantes et al., 1997; Smithers, 2015). Some larger Greek-yogurt producers have invested in methods of transforming Greek acid whey into a useable substance such as lactose or biofuel or the application of nanofiltration to demineralize the acid whey and separate it into lactose and lactic acid (Chandrapala et al., 2015; Erickson, 2017). In contrast, the protein from cottage cheese acid whey has not been evaluated as a possible protein source. The goal of this investigation was to evaluate the potential of acid whey protein from cottage cheese as a whey protein source for addition to yogurt in substitution for sweet whey protein. Using neutralization and filtration technology, this study outlines a potential use for protein from cottage cheese acid whey.

Materials and Methods

Protein Manufacture and Experimental Overview

Acid whey protein, sweet whey protein and skim milk concentrate were used to manufacture 6.0% protein nonfat yogurts. All whey proteins and ultrafiltered skim milk were manufactured at North Carolina State University dairy research pilot plant (Raleigh, NC). Fat free yogurts were then manufactured from skim milk and each protein source. The entire experiment was repeated three times.

Acid Whey Production. Cottage cheese was made using a standard cottage cheese make procedure. Approximately 800 Kg of skim milk (0.2% fat, 3.3% protein) was obtained from the North Carolina State University Research and Education System. Milk was pasteurized using high temperature short time (HTST) at 72°C for 15 seconds (model T4 RGS-16/2, SPX Flow Technology, Greensboro, NC), cooled to 31°C and transferred to a 1500 liter cheese vat (Kusel Equipment, Watertown, WI). The warm pasteurized milk was inoculated with R-604 cheese culture containing *Lactococcus lactis ssp.* (CHR Hansen, Milwaukee, WI) and allowed to

ferment to a pH of 4.6 at 31°C. Once fermentation was complete, the set cheese was cut with 1 cm cheese knives and allowed to heal for 10 min. The curd was then stirred and the temperature was raised gradually to 39°C over a period of 30 min. Once the curd was fully cooked, the whey was drained and the unseparated acid whey was HTST pasteurized (72^{o}C for 15 s), separated using an inline hot bowl centrifugal separator (Modle J5-OSCP-1, JSC PLAVA, Savery, Orlando, FL, U.S.A.) at 50^{o}C, cooled to 4^{o}C, weighed and placed in a jacketed tank for overnight storage at 4^{o}C. Approximately 450 kg of pasteurized separated cottage cheese whey was generated from each batch.

The next day, the cooled acid whey was neutralized and the protein was concentrated by ultrafiltration. Acid whey pH was measured at 4^{o}C using a pH meter calibrated at 4°C and continuously monitored throughout the neutralization process. Ammonium hydroxide (Food grade - USP, 30% w/v, VWR Analytical, Radnor, PA) was used to neutralize the acid whey. The ammonium hydroxide was pumped into the whey using a peristaltic pump at 20 mL doses. This process was to ensure that there was no localized neutralization. Ammonium hydroxide was added every minute and pH was measured 30 sec after each addition. Neutralization was continued until the pH of the whey reached 6.4 (approximately 800 mL). Temperature was monitored throughout the neutralization process. Titratable acidity measurements were taken before and after neutralization. Temperature of the whey was increased to 50^{o}C after neutralization and before filtration. The pH of the neutralized whey at 50^{o}C was confirmed with a pH probe and meter calibrated to appropriate reference pH for 50^{o}C.

The neutralized whey was ultrafiltered using a pilot scale ultrafiltration unit (Model Lab 46, Filtration Engineering, Champlin, MN). Four spiral wound UF membranes were used (Synder Filtration, Vacaville, CA; nominal cutoff: 10,000 Da, surface area 5.0 m^{2}). The

temperature for UF was 50°C. The flux was recorded every 15 min. Deionized water was added as 40% (w/w) of the original weight of whey for diafiltration. The run time was approximately 2.5 h. A Lactoscope FTIR (Delta Instruments, Drachten, Netherlands) was used to measure protein, fat and lactose at each flux measurement. Final whey protein percent and solids of the liquid protein were 16.5% (w/w) and 20.5% (w/w) respectively.

Sweet Whey Production. Cheddar cheese was manufactured using a standard Cheddar cheese make procedure. Approximately 200 Kg of raw whole milk (3.7% fat, 3.0% protein) was obtained from the North Carolina State University Research and Education System (Raleigh, NC). Milk was pasteurized using high temperature short time (HTST) at 72°C for 15 seconds (model T4 RGS-16/2, SPX Flow Technology), cooled to 31°C and transferred to a 1500 liter cheese vat (Kusel Equipment). The warm pasteurized milk was inoculated with R-604 cheese culture containing *Lactococcus lactis ssp.* (CHR Hansen) and allowed to ferment to a pH of 6.2 at 31°C. Next, the milk was coagulated with double strength recombinant rennet (Dairy Connection Inc.) for 30 min at a rate of 0.09 mL/kg of milk diluted 80 times in deionized water. The set cheese was cut with 1 cm cheese knives and allowed to heal for 10 min. The curd was then stirred and the temperature was raised gradually to 39°C over a period of 30 min. Once the curd was fully cooked, the unseparated whey was drained and HTST pasteurized (72^{o}C for 15 s), separated using an inline hot bowl centrifugal separator (Modle J5-OSCP-1, JSC PLAVA, Savery) at 50^{o}C, weighed and placed in a 300 liter tank. Approximately 150 kg of pasteurized separated Cheddar cheese whey at 50^{o}C was collected.

The whey was ultrafiltered using a pilot scale ultrafiltration unit (Model Lab 46, Filtration Engineering). Four spiral wound UF membranes were used (Synder Filtration). The temperature for UF was 50°C. The flux was recorded every 15 min. Diafiltration water was

added as 40% of the original weight of whey. The run time was approximately 2.5 h. A Lactoscope FTIR (Delta Instruments) was used to measure protein, fat and lactose at each flux measurement. Final whey protein percent and solids of the liquid protein were 16.9% (w/w) and 20.4% (w/w) respectively.

Skim Milk Concentrate. Approximately 70 kg of raw skim milk (0.2% fat, 3.3% protein) was obtained from the North Carolina State University Research and Education System (Raleigh, NC). Raw skim milk was subjected to ultrafiltration to concentrate the protein to 6.0%. Prior to UF, the membrane cartridges were cleaned with a 0.1N sodium hydroxide solution (VWR Analytical, Radnor, PA) followed by a rinsing of deionized water. After the rinse step, each batch of raw skim milk was concentrated using a UF system (model Pellicon 2, Millipore Inc., Billerica, MA) using 11 cartridges of polyethersulfone membrane filters (model P2B010V05, nominal separation cutoff = 10,000 Da, surface area = 0.5 m^2). The pump used to circulate the product was a variable speed peristaltic pump (model 77410–10, Cole Palmer, Vernon Hills, Il) equipped with model 77601–00 pump heads with silicone tubing (model 96440–73, Cole Palmer). A Lactoscope FTIR (Delta Instruments) was used to measure protein, fat and lactose at each flux measurement. Final protein, fat, lactose and solids of the skim milk concentrate were 6.13% (w/w), 0.15% (w/w), 4.41% (w/w) and 12.4% (w/w) respectively.

Yogurt Production. Fat free yogurt mixes were made based on calculations to produce mix with 6% protein, 6.5% lactose, and less than 0.2% fat. Three yogurts were manufactured: a control made from the skim milk concentrate (SMC), a second control made by adding the WPC 80 from Cheddar whey to raw skim milk (fat – 0.10%, protein – 3.35%, lactose – 4.72%) (SWP) and an experimental yogurt made by adding the WPC 80 from acid whey to raw skim milk (AWP). Formulations for each type of yogurt were calculated prior to the processing day using a

Lactoscope FTIR (Delta Instruments) for protein, fat and lactose concentrations. Yogurts were processed on a Microthermics EHVH pasteurization unit (Microthermics, Raleigh, NC) with a two-stage homogenizer (GEA Niro Soavi, Parma, Italy). The processing conditions were as follows: pump speed – 2.0 L/minute, preheat – 60°C, homogenizer – 20.7 MPa (17.2 MPa first stage, 3.5 MPa second stage), final heat – 88°C, 7 min hold time, cool to 43°C for inoculation. Approximately 22 kg of yogurt mix was collected in a sanitized milk can. The mix was weighed and inoculated at 0.03% (w/w) with Yo-Fast 20 yogurt culture (CHR Hansen, Milwaukee, WI). The cultured solution was mixed for 2 min and then poured into sanitized containers (177 mL, Choice-Pac, San Francisco, CA) and placed in an incubator at 43^{o}C. The pH was monitored every half hour until a pH of 4.8 was reached, then was measured every 10 min until pH 4.65 (about 5.5 h). Yogurt was then placed in a cooler at 4^{o}C to cool. Yogurts were cooled to < 10°C within 8 h.

Color. Hunter L, and a, and CIE b* values were measured on the raw and pasteurized mixes using an Ultra Scan Pro Spectrophotometer (Hunter Associates Laboratory, Inc., Reston, VA) with L being Luminosity (the degree of lightness from dark to light), a being the degree of redness or greenness, and b* being the degree of yellowness or blueness (Quinones et al., 1997). Hunter values were computed from the reflectance data in the range of 360-750 nm at 5 nm intervals, Illuminant A with a 10 degree observer angle. Measurements were taken on liquid mixes tempered at 4°C.

Analytical Techniques.

Yogurts were evaluated starting on Day 1, with Day 0 being the day the yogurt was produced. Each yogurt was tested on the same day each week for 8 weeks. Each test was performed in triplicate.

pH Determination. pH was determined by calibrated pH meter measurements (Orion Model 0290, Thermo Scientific, Waltham, MA) at 4°C. Each week the pH meter was calibrated in the cooler at 4°C, and yogurt pH was measured at 4°C.

Titratable Acidity. Titratable acidity was measured using the standard methods for examination of dairy products: Method 15.021 (Hooi et al., 2004a). Yogurt was mixed and 9 g of sample was added to a beaker using a pipet. Then, 18 g of DI water was added to the beaker and mixed thoroughly. Phenolphthalein was added at 0.5mL. The solution was titrated with 0.1N NaOH to the first permanent color change. Percent acidity was calculated as follows:

$$\%\ acidity = \frac{(mL\ NaOH)\ x\ (Normality\ of\ NaOH)\ x\ 9}{Sample\ Weight\ in\ Grams\ Titrated}$$

Mojonnier. Fat content of yogurts was measured using the Mojonnier method, Method 15.086 (Hooi et al., 2004b).

Syneresis. Syneresis (whey separation) was measured using a modified method from Lucey et al. (1998). At each time point, 3 cups of each yogurt treatment were examined for syneresis. Cups were weighed to the nearest mg. Any visible liquid on top of the yogurt was suctioned off with a pipet and the cup was reweighed. The initial weight minus the final weight represented the weight of the free whey. Whey separation was expressed as percent of total yogurt weight.

Gel Strength. Gel strength was measured using a modified method from Schmidt et al. (2000), Pang et al. (2016), and Houze et al. (2005). Gel strength attributes were measured using an Instron 5542 rheometer equipped with a 1.27 cm diameter spherical stainless steel probe (Instron, Norwood, MA). The limits of texture parameters were set up using a 0.7 kg load cell with 0.8 mm/s pretest speed, 1.0 mm/s test speed, 1.0 mm/s post-test speed and a 0.001 N trigger force. Measurements of firmness, compression, adhesion and cohesion were analyzed using BlueHill 2.0 software (Instron, Norwood, MA). Firmness was measured by the max force of

compression. Compression was measured as the area under the curve for compression. Adhesion was measured as the max force of retraction. Cohesion was measured by the area under the curve for the retraction.

Sensory Testing

All sensory testing was performed in compliance with the North Carolina State University Institutional Review Board for Human Subjects. The yogurts were dispensed into 3-digit coded soufflé cups (Solo Cup, Highland Park, Il), lidded, and tempered to 15°C. Aromatics and basic taste intensities were evaluated in duplicate by trained panelists (n = 8) using an established sensory language for yogurts (Desai et al., 2013) and a 0 to 15 point universal Spectrum^{TM} intensity scale on days 0, 14, 28, and 56. Panelists were between the ages of 23 and 55 y and each had over 150 h of experience with descriptive analysis of yogurts. Panelists expectorated samples and were provided with room temperature DI water for palate cleansing. Texture attributes were evaluated in separate sessions on individual coded cups of yogurt to evaluate yogurt before and after stirring (Desai et al., 2013). Conditions for texture evaluation were similar to those described for flavor. For all sensory evaluations, each panelist evaluated each yogurt in duplicate. Data were collected using Compusense Cloud (Guelph, Canada).

Consumer Testing

A consumer acceptance test was conducted after 14 days of storage on replicate 3 following analysis of trained panel and instrumental measurements to ensure that analytical properties were consistent with previous trials. Yogurts were mixed with a strawberry fruit base at 20% (Fruit Gel, Fruit Crown, Farmingdale, NY) (w/w). Consumers (n=100) were self-reported yogurt consumers. Yogurts were dispensed into lidded 60 mL soufflé cups labeled with random 3 digit codes. Consumers evaluated samples monadically, in a randomized balanced block design

and data was collected using Compusense Cloud (Guelph, Canada). A 120 sec rest was enforced between samples, during which panelists were instructed to clean their palates with DI water and unsalted crackers. Consumers answered questions about overall liking, sweetness, texture, thickness, and aftertaste. Liking was scored on a 9-pt hedonic scale with 1=dislike extremely and 9=like extremely. Aftertaste liking was scored only when aftertaste was indicated.

Data Analysis

All analyses were performed at 95% confidence ($P < 0.05$). Statistical analyses were conducted with XLSTAT version 2017.19.5 (Addinsoft, Paris, France) and SAS (Version 9.4, Cary, NC). Two way analysis of variance (ANOVA) (protein source x time; with time as a continuous variable) was performed on the analytical data (pH, gel strength, syneresis, color and titratable acidity) and trained panel sensory data with means separation performed using least square means (SAS). Proximate analysis and consumer hedonic scores were evaluated by one-way analysis of variance with Fisher's LSD for means separation. Just About Right (JAR) questions were analyzed with penalty analysis and means separation via chi square analysis (XLSTAT).

Results

Protein Source Compositions

There was no difference in the whey protein content of liquid WPC 80 made from acid or Cheddar whey ($P > 0.05$). There was a difference in true protein content of liquid WPC 80 made from acid and Cheddar whey ($P < 0.05$) (Table 2.1). There was a large difference in fat content between the two ingredients ($P < 0.05$) (Table 2.1) which was expected because of the milk source; acid whey was produced from skim milk and Cheddar whey was produced from whole milk. This was not a concern because the final fat content of yogurts were standardized. Another

difference between the two ingredients was the ammonia content. Concentrated acid whey had higher ammonia (17.04 mg/100g) than concentrated Cheddar whey (1.462 mg/100g). Again, this result was expected due to the use of ammonium hydroxide to neutralize the acid whey. Ammonium hydroxide was chosen as a neutralizing agent instead of sodium hydroxide or potassium hydroxide to avoid salty or bitter off tastes (Neta et al., 2009).

Yogurt Compositions

Proximate analysis was measured on yogurt mixes after pasteurization. There was no difference between AWP and SWP in total solids ($P > 0.05$), however, SMC had lower total solids ($P < 0.05$) (Table 2.2). True protein concentrations in AWP and SWP were similar ($P > 0.05$), while SMC had lower true protein ($P < 0.05$), however all values were within ranges that were targeted. Whey protein concentrations in AWP and SWP were similar ($P > 0.05$), but lower in SMC ($P < 0.05$), which was expected as there was no added whey protein ingredient used in the SMC formulation. Conversely, casein protein was higher in SMC compared to AWP and SWP ($P < 0.05$), which is due to UF skim milk as the base ingredient.

Fermentation and pH

Fermentation time (min to reach pH 4.7) varied between yogurts ($P < 0.05$) (Figure 2.1). AWP took approximately 90 min longer to ferment to final pH than SMC and SWP ($P < 0.05$). pH was affected both by time and protein source ($P < 0.05$). There was a strong linear and moderate quadratic effect of time of fermentation on mix pH and an interaction effect of protein source with both the linear and quadratic term for time with the linear effect being the strongest. The interaction effect with protein source with time of fermentation indicates the rate of change with time was different ($P < 0.05$) among the different protein sources. pH was measured on a weekly basis in triplicate, starting the day after fermentation, for 8 weeks. During the 8 week

shelf life, the pH of SMC, AWP and SWP yogurts decreased on average by 0.26, 0.21, and 0.25 respectively, and AWP yogurt had a higher pH at all time points than SMC and SWP ($P < 0.05$) (Figure 2.2). pH was affected by both time of storage and protein source ($P < 0.05$) (Table 2.3). For interactions between pH and time, all protein sources had quadratic relationships.

Titratable Acidity

Titratable acidity (TA) was affected by time ($P < 0.05$) but not protein source ($P > 0.05$) (Figure 2.2) (Table 2.3). TA was measured on a weekly basis in triplicate, starting the day after fermentation, for 8 weeks. During the 8 week shelf life, the TA of SMC, AWP and SWP yogurts increased on average by 0.23%, 0.25%, and 0.25%, respectively. There was a strong quadratic and moderate linear effect of storage time on TA. There was no interaction (linear or quadratic) of protein source on TA, indicating the rate of acid production with storage time was not different ($P > 0.05$).

Syneresis

Syneresis was affected by both time and protein source ($P < 0.05$) (Table 2.3). AWP yogurt (2.90%) had higher syneresis than SMC (0.30%) or SWP (0.40%) yogurts at all time points during shelf life ($P < 0.05$) (Figure 2.3). AWP yogurt experienced an increase in syneresis over 8 weeks ($P < 0.05$), while SMC and SWP yogurts had no significant change ($P > 0.05$). There was a strong quadratic and moderate linear effect of time of storage on syneresis and an interaction effect of protein source with both the linear and quadratic term with the quadratic term being the strongest. The interaction effect of protein source with storage time indicates that the change during storage time was different for the different protein sources ($P < 0.05$).

Gel Strength

Gel strength attributes (firmness, compression, adhesion and cohesion) were affected by

both protein source and time of storage ($P < 0.05$) (Table 2.3). All gel strength attributes, for all protein sources, increased with time ($P < 0.05$) (Figures 2.4, 2.5). AWP had lower gel strength attributes than other yogurts at all time points ($P < 0.05$). For all gel strength attributes, SMC was higher than SWP which was higher than AWP ($P < 0.05$). There was a strong quadratic and moderate linear effect of storage time on firmness and compression, and an interaction effect of protein source on firmness and compression with both the linear and quadratic term for time with quadratic being the strongest. There was also a strong quadratic and moderate linear effect of storage time on adhesion and cohesion, and an interaction effect of protein source on adhesion and cohesion with both the linear and quadratic term for time with quadratic being the strongest.

Color Attributes

Color values of raw and pasteurized mixes were measured using Hunter L (whiteness), a (green-redness) and CIE b* (yellow-blueness), on yogurt mixes at day 0. The final color of yogurt was affected by pasteurization and whey protein addition ($P < 0.05$). Raw mixes of AWP and SWP had higher values of yellowness and redness than SMC ($P < 0.05$) (Table 2.4). After pasteurization, AWP and SWP increased in lightness, redness and yellowness ($P < 0.05$), but SMC did not change in yellowness ($P > 0.05$) and decreased in redness ($P < 0.05$), indicating that whey protein addition and pasteurization had an effect on final color.

Descriptive Sensory Analysis

Descriptive analysis confirmed that protein source had an effect on sensory properties of yogurts. Flavor of yogurts was distinct ($P < 0.05$) (Table 2.5). Yogurts differed in aroma intensities, cooked, dairy sour, cardboard, and acetaldehyde flavors and sour and umami tastes and aftertaste intensity on day 1 of tasting. Aroma intensity was higher in AWP yogurt than SWP and SMC yogurts ($P < 0.05$). There was a strong quadratic effect of time of storage on aroma

intensity, but no interaction of protein source with time. Cooked flavor had a strong quadratic effect of time, but there was no protein source interaction. There was a strong quadratic effect of time of storage on dairy sour flavor, but no interaction of protein source with time. Beefy flavor was only detected in AWP and SWP and decreased over time ($P < 0.05$). This flavor has been previously documented in sour creams (Shepard et al., 2013) and fortified Greek yogurts (Desai et al., 2013). Cardboard flavor was also only detected in AWP and SWP yogurts. Cardboard flavor has been documented in whey protein and foods with added whey protein, including Greek yogurt (Whitson et al., 2010; Whetstine et al., 2005; Leksrisompong et al., 2010; Desai et al., 2013). Cardboard flavor decreased over time. Soapy flavor was higher in SWP than AWP ($P < 0.05$), but not detected in SMC. This flavor had a small but significant change over time across all protein sources. Soapy flavor has been documented in nonfat Greek yogurts (Desai et al., 2013) and has also been documented in whey proteins and whey protein beverages (Whetstine et al., 2005; Oltman et al., 2015). Acetaldehyde flavor was lower in AWP yogurt compared to SMC and SWP yogurts ($P < 0.05$) and had a small but significant change over time. There was no significant difference in sweet taste between the protein sources ($P > 0.05$). Sour taste was higher in AWP yogurt compared to SMC and SWP yogurts ($P < 0.05$), and there was a quadratic effect of time with sour taste. There was no significant difference in astringency between protein sources ($P > 0.05$), and there was increase over time for all protein sources for this attribute. Umami taste had a slight increase with time in AWP yogurt ($P < 0.05$) and was not detected in yogurts with other protein sources. There was a quadratic effect of time of storage on aftertaste intensity and an interaction of protein source with time of storage. Aftertaste intensity was higher in SWP than AWP and SMC ($P < 0.05$).

Texture properties between yogurts were also distinct ($P < 0.05$) (Table 2.6). There was

no significant effect of time of storage on surface shine, spoon indent or mouth coating ($P > 0.05$), or protein with time interactions. Initially, there were no significant differences among yogurts for the texture attributes of denseness and cohesiveness. There was a strong quadratic effect of time of storage on denseness and cohesiveness and an interaction with protein source and time of storage. AWP yogurt decreased in denseness and cohesiveness over shelf life ($P < 0.05$), but SMC and SWP yogurts stayed the same ($P > 0.05$). There was a quadratic effect of time of storage on graininess and an interaction effect of protein source with the quadratic term for time. Graininess was higher in SMC and AWP yogurts than in SWP yogurt ($P < 0.05$). After 28 days, AWP yogurt increased in graininess and was higher than both SMC and SWP yogurts ($P < 0.05$). There was a moderate quadratic effect of time of storage on both viscosity and firmness. There was also an interaction effect of protein source with the quadratic term for time for firmness but not viscosity. Viscosity and firmness were higher in SMC and SWP yogurts than AWP yogurt ($P < 0.05$). There was a slight decrease in viscosity for all protein sources with time. Firmness increased with time in SMC and SWP yogurts but not AWP yogurt.

Consumer Testing

Yogurts were evaluated by consumers after 14 days of storage. One hundred self-reported yogurt consumers participated. Males made up 40.0% of the consumers, and females 60.0%. All consumers were between the ages of 18-64 y, with the majority between 18-34 y (68.5%). The majority of consumers reported consuming yogurt at least once a week (73.3%).

SMC yogurts had higher liking scores than AWP yogurts in all categories ($P < 0.05$) (Table 2.7). On the basis of appearance, consumers preferred SMC yogurt ($P < 0.05$), and AWP and SWP yogurts were at parity ($P > 0.05$). Flavor liking was significantly higher for SWP and SMC yogurts than AWP yogurt ($P < 0.05$). Consumers indicated that they liked the tartness and

sweetness of SMC and SWP yogurts more than AWP yogurt ($P < 0.05$). Thickness and texture liking scores were significantly lower in AWP than SMC and SWP ($P < 0.05$). Consumers preferred SMC, then SWP, then AWP yogurts ($P < 0.05$). AWP yogurts had significant penalties in overall liking for having "too little" thickness and "too little" texture ($P < 0.05$) (results not shown). These results suggested that texture was the primary reason for the lower overall liking score of AWP yogurt compared to SMC yogurt.

Reformulation. The objective of this study was to develop a yogurt that used whey protein from acid whey and was acceptable to consumers. Upon completion of consumer testing, improvements to AWP yogurts were necessary to increase consumer liking scores. Consumer scores indicated that texture was the primary reason yogurts were not liked. Trained panel and instrumental data were consistent with consumer panel scores. All yogurts were reformulated with increased total fat (0.2% to 2.0%), decreased total protein (6.0% to 5.0%), and increased sugar (6.5% to 8.0%). Janiaski et al. (2016) documented that increasing fat content in yogurts improved sensory texture attributes viscosity and smoothness. Modified food starch (Ingredion, Westchester, IL) was also added to all yogurts at 1.0% (w/w). Modified food starch has been documented to increase thickness of yogurt (Nguyen et al., 2017; Schmidt et al., 2000). AWP yogurt also had added gelatin (0.20%) (Geliko, New York, NY) (w/w) to increase thickness and gellan gum (0.02%) (Tic Gums, Bellcamp, MD) (w/w) to reduce graininess (Fiszman et al., 1999). Gelatin reduces syneresis and improves sensory perception of yogurt texture (Nguyen et al., 2017; Pang et al., 2017). Yogurts were processed and fermented in duplicate as previously described. Yogurts were stored for 14 days and trained panel profiling and consumer acceptance testing (replicate 2 only) were conducted as previously described. Yogurts for consumer testing were mixed with a strawberry fruit base at 20% (w/w).

Descriptive analysis results of reformulated yogurts showed expected changes in AWP yogurt for texture and flavor attributes such that the flavor and texture profiles were more similar to SMC yogurts (Table 2.7). Milkfat flavor and sweet taste were higher in the reformulated yogurts compared to the initial formulations, which was expected because of the added sugar and increase in fat content. Cardboard and soapy notes were no longer detected in the reformulated AWP yogurt also consistent with previous work with fat free versus fat containing yogurts (Desai et al., 2013). Firmness and viscosity were increased in the reformulated AWP yogurt ($P < 0.05$), compared to SWP and SMC reformulated yogurts (Table 2.8).

Consumer liking scores for reformulated yogurts are shown in Table 2.10. Consumer demographics were similar to the previous consumer test. The reformulated AWP yogurt had increased liking scores for all categories tested ($P < 0.05$). AWP was favored or at parity with SMC and SWP ($P < 0.05$). Consumers preferred thickness of AWP over SMC and SWP ($P < 0.05$). Consumers also preferred texture of AWP and SWP over SMC ($P < 0.05$).

Discussion

Fermentation time and pH differed between AWP yogurt and SWP/SMC yogurts. The mean initial pH of the AWP and SWP fortified yogurt mixes were lower ($P < 0.05$) 6.49 and 6.52, than for the SMC protein source (6.65). All yogurt mixes were cultured under the same conditions. The fermentation time to reach a finished pH of 4.7 was longer for both AWP and SWP (390 and 300 min, respectively) than for SMC (270 min) (Figure 2.1). The higher resistance to pH decrease by the AWP fortified yogurt mix may have been due to the residual buffering capacity in the acid whey ingredient due to the presence of the aqueous ammonia neutralizer in the AWP versus the SMC and SWP. The level of measured ammonia in the AWP was higher ($P < 0.05$) than the SWP (17.6 mg/100g versus 1.46 mg/100 g, respectively).

Yogurt texture is affected by pH. Martin et al. (1999) and Martens (1972) reported that yogurts at pH 4.2-4.4 had higher thickness than yogurts at pH 4.7-4.8. Ronnegard and Dejkmek (1993) observed higher viscosities in yogurts as pH was decreased from 4.5 to 4.25. AWP yogurts had higher pH values than SMC and SWP yogurts at all time points, and never reached below a pH of 4.52. Increase of gel strength in yogurts could be explained by the effect of lower pH on the electrical charge on casein (Harwalker and Kalab, 1983), which causes a more rigid gel structure. The higher pH in AWP yogurts led to lower instrumental gel strength attributes and less idea sensory texture attributes compared to SMC and SWP yogurts without reformulation. Water holding capacity is also affected by pH. Aguilera and Kessler (1989) showed that acidified gels had higher water holding capacity at pH 3.7 than at pH 5.35. AWP yogurt had higher values of syneresis than SMC and SWP yogurts at all time points. This is likely due to the pH difference among the yogurts. Yogurt texture descriptive analysis results were consistent with analytical measurements. There was also a distinct umami taste in the AWP yogurt. Smith et al. (2016b) documented higher intensities of umami taste in acid and cottage whey than in Cheddar whey. Monosodium glutamate and 5'-nucleotides are recognized as the components that provide umami taste (Reineccius, 2006) as well as organic acids (Drake et al., 2007; Rubico and McDaniel, 1992). Differences in processing conditions among Cheddar and cottage cheese whey allow more bacteria to grow in cottage cheese whey as fermentation takes longer for a lower final pH. Increased amounts of bacteria and organic acids are likely the source of the umami taste in the AWP yogurt.

Color is an influential attribute of appearance and quality. Lightness increase in milk has been reported with pasteurization (Schamberger and Labuza, 2006). This is a result of the denaturation of soluble milk proteins that coagulated and reflected light. Lightness in yogurt

mixes was increased after pasteurization compared to raw mixes. Cheng et al. (2018) reported increased b* values with increased levels of whey protein as a percentage of total protein in milks. Similar trends were found for a values in milks (Cheng et al., 2018). This data was consistent with increased b* values and a values in AWP and SWP yogurt mixes because of the added whey protein ingredient. Since consumer perception is a large driver for yogurt purchase, it was important that all yogurts had similar appearance. There were some instrumental color differences between the samples that had added whey protein (AWP and SWP) and SMC yogurts, however, consumer liking scores did not differ.

Conclusions

Yogurt with acid whey protein had lower gel strength attributes which translated to differences in trained panel texture attributes and consumer liking. pH was the main contributor to texture differences as higher pH in AWP yogurts restricted gel structure formation. Although there were some instrumental color differences, consumers had no appearance preference. Reformulation to address texture differences resulted in AWP yogurts that performed at consumer parity with control SMC yogurts. These results indicate that acid whey protein can be used as a protein source in yogurt.

Acknowledgements

Funding in part provided by the New York State Milk Promotion Board and the National Dairy Council.

References

Aguilera, J. M. and H. G. Kessler. 1989. Properties mixed and filled-type dairy gels. J. Food Sci. 54: 1213-1217.

Bals, A., and U. Kulozik. 2003. Effect of preheating on the foaming properties of whey protein isolate using a membrane foaming apparatus. Int Dairy J 13: 903-908.

Barrantes, L. D., and C. V. Morr. 1997. Partial Deacidification and Demineralization of Cottage Cheese Whey by Nanofiltration. J Food Sci. 62: 338-341.

Chandrapala, J., M. C. Duke, S. R. Gray, B. Zisu, M. Weeks, M. Palmer, T. Vasiljevic. 2015. Properties of acid whey as a function of pH and temperature. J Dairy Sci. 98: 4352-4363.

Cheng, N., D. M. Barbano and M. A. Drake. 2018. Hunter verses CIE color measurement systems for analysis of milk-based beverages. J. Dairy Sci. 101: 4891-4905.

Drake, S. L., M. E. Carunchia Whetstine, M. A. Drake, P. Courtney, K. Fligner, J. Jenkins, and C. Pruitt. 2007. Sources of umami taste in cheddar and swiss cheeses. J. Food Sci. 72: S360-S366.

de Wit, J. N. 1998. Nutritional and functional characteristics of whey proteins in food products. J Dairy Sci. 81: 597-608.

Dec, B. and Chojnowski, W. 2006. Characteristics of acid whey powder partially demineralised by nanofiltration. Pol J Food Nutr Sci. 15: 87-90.

Desai, N. T., L. Shepard, and M. A. Drake. 2013. Sensory properties and drivers of liking for greek yogurts. J. Dairy Sci. 96: 7454-7466.

Erickson, Britt E. 2017. Acid whey: is the waste product an untapped goldmine?. Accessed Feb. 6, 2017. http://cen.acs.org/articles/95/i6/Acid-whey-waste-product-untapped.html.

Fiszman, S. M., M. A. Lluch, and A. Salvador. 1999. Effect of addition of gelatin on microstructure of acidic milk gels and yoghurt and on their rheological properties. Int. Dairy J. 9: 895-901.

Harwalker, V. R. and M. Kalab. 1983. Susceptibility of yogurt to syneresis. Comparison of centrifugation and drainage methods. Milchwissenschaft. 38: 517-522.

Hooi, R., D. M. Barbano, R. L. Bradley, D. Budde, M. Bulthaus, M. Chettiar, J. Lynch, and R. Reddy. 2004a. Acidity, titratable - phenolphthalein indicator. Pages 427-434 in: Standard Methods for the Examination of Dairy Products, Vol. 17. H. M. Wehr & J. F. Frank, ed. American Public Health Association, Washington, DC.

Hooi, R., D. M. Barbano, R. L. Bradley, D. Budde, M. Bulthaus, M. Chettiar, J. Lynch, and R. Reddy. 2004b. Mojonnier Method, Milk and Cream; Other products. Pages 364-366 in: Standard Methods for the Examination of Dairy Products, Vol. 17. H. M. Wehr & J. F.

Frank, ed. American Public Health Association, Washington, DC.

Houze, G., Cases, E., Colas, B. and Cayot, P. 2005. Viscoelastic properties of acid milk gel as affected by fat nature at low level. Int Dairy J. 1006-1016.

IDFA. 2017. Cultured Products. Accessed May 17, 2017. https://www.idfa.org/resource-center/industry-facts/cultured-products

Janiaski, D. R., T. C. Pimentel, A. G. Cruz and H. Prudencio. 2016. Strawberry-flavored yogurts and whey beverages: whay is the sensory profile of the ideal product?. J. Dairy Sci. 99: 5273-5283.

Kersten, S., B. S. Murray, and E. Dickinson. 2005. Confocal microscopy of heat-induced aggregation and gelation of b-lactoglobulin in presence of non-ionic surfactant. Food Hydro. 19: 625-633.

Kilara, A., and R. C. Chandan. 2013. Greek-style yogurt and related products. Pages 297-318 in Manufacturing yogurt and fermented milks, Vol. 1. Chandan R. C., and A. Kilara, ed. Wiley-Blackwell, West Sussex, UK.

Kontopidis, G., C. Holt, and L. Sawyer. 2002. The ligand-binding site of bovine b-lactoglobulin: Evidence for a function?. J Mol Biol. 318: 1043-1055.

Kontopidis, G., C. Holt, and L. Sawyer. 2004. Invited review: b-lactoglobulin: binding properties, structure, and function. J Dairy Sci. 87: 785-796.

Leksrisompong, P. P., R. E. Miracle and M. A. Drake. 2010. Characterization of flavor of whey protein hydrolysates. J. Agric. Food Chem. 58: 6318-6327.

Leman, J., T. Dolgan, M. Smoczynski, and Z. Dziuba. 2005. Fractal characteristics of microstructure of beta-lactoglobulin preparations and their emulsifying properties. Elec J Pol Ag Univ. 8:3.

Lopes, G. K., D. S. Alviano, D. Torres, M. P. Goncalves, C. T. Andrade. 2006. Gelation of whey protein concentrate in the presence of partially hydrolyzed waxy maize starch and urea at pH 7.5. J Coll Poly Sci. 285: 203-210.

Lucey, J. A., P. A. Munro, and H. Singh. 1998. Whey seperation in acid skim milk gels made with glucono-delta-lactone: effects of heat treatment and gelation temperature. J Text Stud. 29: 413-426.

Lucey, J. A., P. A. Munro, and H. Singh. 1999. Effects of heat treatment and whey protein addition on the rheological properties and structure of acid skim milk gels. Int Dairy J. 9: 275-279.

Martens, R. 1972. Influence of some factors on the consistency and the taste of stirred yogurt. Rev. Agric. 25: 461-480.

Martin, N. C., J. Skokanova, E. Latrille, C. Beal and G. Corrieu. 1998. Influence of fermentation and storage conditions on the sensory properties of plain lowfat stirred yogurts. J. Sen

Stud. 14: 139-160.

Modler, H. W., M. E. Larmond, C. S. Lin, D. Froehlich, and D. B. Emmons. 1983. Physical and sensory properties of yogurt stabilized with milk proteins. J Dairy Sci. 66: 422-429.

Morr, C. V., and E. Y. W. Ha. 1993. Whey protein concentrates and isolates: processing and functional properties. Crit rev food sci nut. 33: 431-476.

Neta, E. R. D., S. D. Johanningsmeier, M. A. Drake, and R. F. McFeeters. 2009. Effects of pH adjustment and sodium ions on sour taste intensity of organic acids. J. Food Sci. 74: S165-S169.

Nguyen, P. T. M., O. Kravchuk, B. Bhandari, and S. Prakash. 2017. Effect of different hydrocolloids on texture, rheology, tribology and sensory perception of texture and mouthfeel of low-fat pot-set yoghurt. Food Hydro. 72: 90-104.

Oltman, A. E., K. Lopetcharat, E. Bastian, and M. A. Drake. 2015. Identifying key attributes for protein beverages. J. Food Sci. 80: S1383-S1390.

Onwulata, C. I., Smith, P. W., Konstance, R. P. & Holsinger, V. H., 2001. Incorporation of whey products in extruded corn, potato or rice snacks. Food Res Int, 34: 679-687.

Pang, Z., H. Deeth, H. Yang, S. Prakash, N. Bansal. 2016. Evaluation of tilapia skin gelatin as a mammalian gelatin replacer in acid milk gels and low-fat stirred yogurt. J Dairy Sci. 100: 1-12.

Prazeres, Ana R., Fatima Carvalho, and Javier Rivas. 2012. Cheese whey management: A review. J Environ Manage 110: 48-68.

Quinones, H. J., D. M. Barbano, and L. G. Phillips. 1997. Influence of protein standardization by ultrafiltration on the viscosity, color, and sensory properties of skim, and 1% milk. J Dairy Sci. 80: 3142-3151.

Reineccius, G. 2006. Flavor analysis. Pages 33-72 in Flavor chemistry and technology, Vol. 2. Taylor and Francis Group, Boca Raton, FL.

Ronnegard, E. and P. Dejmek. 1993. Development and breakdown of structure in yoghurt studied by oscillatory rheological measurements. Lait. 73: 371-379.

Rubico, S. M., and M. R. McDaniel. 1992. Sensory evaluation of acids by free-choice profiling. Chem. Senses. 17: 273-289.

Schamberger, G. P., and T. P. Labuza. 2006. Evaluation of front-face fluorescence for assessing thermal processing of milk. J. Food Sci. 71: C69-C74.

Schmidt, K A, T J Herald, and K A Khatib. 2000. Modified wheat starches used as stabilizers in set-style yogurt. Food Qual. 24: 421-434.

Shepard, L., R. E. Miracle, P. Leksrisompong, and M. A. Drake. 2013. Relating sensory and chemical properties of sour cream to consumer acceptance. J. Dairy Sci. 96: 5435-5454.

Smith, S., T. J. Smith, and M. A. Drake. 2016. Short communication: flavor and flavor stability of cheese, rennet, and acid wheys. J. Dairy Sci. 99: 3434-3444.

Smithers, Geoffrey W. 2015. Whey-ing up the options: Yesterday, today and tomorrow. Int Dairy J. 48: 2-14.

Sodini, I., J. Montella, and P. S. Tong. 2005. Physical properties of yogurt fortified with various commercial whey protein concentrates. J Sci Food Ag. 85: 853-859.

Tunick, Michael H. 2009. Whey protein production and utilization: a breif history. Pages 1-13 in Whey processing, functionality and health benefits, Vol. 1. Charles I. Onwulata and Peter J. Huth, ed. John Wiley and Sons, Inc, Ames, IA.

USDA. 2015. Dairy Products 2015 Summary: USDA. Accessed Feb. 6, 2017. http://usda.mannlib.cornell.edu/usda/nass/DairProdSu//2010s/2015/DairProdSu-04-29-2015.pdf

Wagoner, T. B., L. Ward, and E. A. Foegeding. 2015. Using state diagrams for predicting colloidal stability of whey protein beverages. J Ag Food Chem. 63: 4335-4344.

Whetstine, M. E., K. R. Cadwallader, and M. A. Drake. 2005. Characterization of aroma compounds responsible for rosy/floral flavor in cheddar cheese. J. Ag. Food Chem. 53: 3126-3132.

Whitson, M. E., R. E. Miracle and M. A. Drake. 2010. Sensory characterization of chemical components responsible for cardboard flavor in whey protein. J. Sen. Stud. 25: 616-636.

Yilsay, T. O., L. Yilmaz, and A. A. Bayizit. 2006. The effect of using a whey protein fat replacer on textural and sensory characteristics of low-fat vanilla ice cream. Eur Food Res Tech. 222: 171-175.

Table 2.1. Proximate analysis of liquid acid or Cheddar whey proteins.

	Neutralized Concentrated Acid Whey	***Concentrated Cheddar Whey***
Fat Content (g/100g)	0.24b	1.03a
Total Solids (g/100g)	27.1a	22.0b
True Protein (g/100g)	19.5a	17.6b
Casein Protein (g/100g)	2.99a	0.70b
Whey Protein (g/100g)	16.5a	16.9a
Ammonia (mg/100g)	17.0a	1.46b

Different lowercase letters in rows following means indicate a significant difference ($P < 0.05$).

Table 2.2. Proximate analysis of yogurts expressed as a percentage.

	Protein Source		
	SMC[1]	***AWP[1]***	***SWP[1]***
Fat Content	0.16b	0.17ab	0.21a
Total Solids	13.9b	14.7a	14.5a
True Protein	5.91b	6.04a	6.00a
Casein Protein	4.76a	3.79b	3.65b
Whey Protein	1.15b	2.25a	2.35a
Whey Protein from WPC	N/A	1.28b	1.46a

Different lowercase letters in rows following means indicate a significant difference ($P < 0.05$).
[1]SMC – skim milk concentrate yogurt mix; AWP – acid whey protein yogurt mix; SWP – sweet whey protein yogurt mix; N/A – not applicable.

Table 2.3. Least squares mean instrumental pH, TA, and texture metrics of fat free yogurt using 3 different protein sources measured of at 0, 7, 14, 21, 28, 42, 49, and 56 days of 4°C storage.

Parameter	*Protein Source Least Square Mean*			*LSD*	*Time of Storage[2]*	*Time of Storage[2]*	*R-squared*
	SMC[1]	*AWP[1]*	*SWP[1]*		*Linear*	*Quadratic*	
pH	4.33b	4.61a	4.34b	0.264	Sig	Sig	0.954
TA[3]	1.17a	1.22a	1.19a	0.063	Sig	Sig	0.780
Syneresis[4]	0.30b	2.90a	0.40b	0.081	Sig	Sig	0.895
Firmness[5]	0.70a	0.26b	0.67ab	0.411	Sig	Sig	0.979
Compression[6]	4.72a	1.64b	4.17ab	2.77	Sig	Sig	0.967
Adhesion[5]	0.11a	0.03b	0.08ab	0.070	Sig	Sig	0.880
Cohesion[6]	0.36a	0.11b	0.30ab	0.232	Sig	Sig	0.706

Means followed by a different lowercase letter within a row indicate a significant difference among protein sources. LSD = least significant difference ($P < 0.05$)
[1] Protein Sources: SMC – skim milk concentrate yogurt; AWP – acid whey protein yogurt; SWP – sweet whey protein yogurt. ND - not detected.
[2] Time of storage: NS = not significant ($P > 0.05$); Sig = Significant ($P < 0.05$).
[3] Expressed in percent acid
[4] Expressed in percent weight of white mass
[5] Expressed in N
[6] Expressed in mJ

Table 2.4. Color values of raw and pasteurized yogurt mixes using Hunter L (whiteness), a (green-redness) and CIE b* (yellow-blueness).

Color property		***Protein Source***		
		SMC[1]	***AWP[1]***	***SWP[1]***
L	*Raw*	80.88d (0.020)	80.50d (0.090)	79.64e (0.020)
	Pasteurized	82.37c (0.097)	84.34a (0.077)	83.87b (0.088)
a	*Raw*	-2.335d (0.005)	-1.550c (0.040)	-1.255b (0.005)
	Pasteurized	-3.082e (0.041)	-0.822a (0.035)	-0.792a (0.041)
b*	*Raw*	5.815e (0.005)	7.335d (0.025)	7.705c (0.035)
	Pasteurized	5.664e (0.094)	8.400b (0.077)	8.940a (0.028)

Measurements were taken at 4°C.

Means are compared across rows for L, a, and b* measurements. Raw and pasteurized mixes are compared to each other for significance. Standard error values are in parentheses below mean values. Different lowercase letters in color property rows following means indicate a significant difference ($P < 0.05$).

SMC – skim milk concentrate yogurt mix; AWP – acid whey protein yogurt mix; SWP – sweet whey protein yogurt mix.

Table 2.5 Trained panel flavor profiles of skim milk yogurts evaluated at 1, 14, 28 and 56 days of 4°C storage. Attributes were scored on a 0 to 15 point universal intensity scale (Meilgaard et al., 2007; Desai et al., 2013).

	Protein Source[1] Least Square Means			***LSD***	***Time of Storage[2]***	***R-squared***
Parameter	***SMC[1]***	***AWP[1]***	***SWP[1]***			
Aroma Intensity	2.27a	2.53a	2.54a	NS	sig	0.469
Cooked	3.69a	3.67a	3.62a	0.09	sig	0.779
Dairy Sour	1.67a	1.69a	1.75a	0.10	sig	0.612
Beefy/Brothy	ND	0.48a	0.54a	0.31	sig	0.679
Cardboard	ND	1.05ab	1.70a	1.51	sig	0.896
Soapy	ND	0.85ab	1.20a	0.99	sig	0.789
Acetaldehyde	2.21a	1.41a	2.29a	1.03	sig	0.752
Sweet	1.38a	1.41a	1.35a	NS	sig	0.557
Sour	2.39a	2.40a	2.68a	0.33	sig	0.700
Aftertaste	1.08b	1.23ab	1.41a	0.31	sig	0.546
Umami	ND	1.38a	ND	1.16	sig	0.901
Astringency	2.75a	2.86a	2.84a	0.12	sig	0.599

Means followed by a different lowercase letter within a row indicate a significant difference among protein sources ($P < 0.05$).

[1] Protein Sources: SMC – skim milk concentrate yogurt; AWP – acid whey protein yogurt; SWP – sweet whey protein yogurt. ND - not detected.

[2] Time of storage: NS = not significant ($P > 0.05$); Sig = Significant ($P < 0.05$)

Table 2.6. Trained panel texture profiles of skim milk yogurts evaluated at 1, 14, 28 and 56 days of 4°C storage. Attributes were scored on a 0 to 15 point product specific intensity scale (Meilgaard et al., 2007; Desai et al., 2013).

	Protein Source[1] Least Square Means			***LSD***	***Time of Storage[2]***	***R-squared***
Parameter	***SMC[1]***	***AWP[1]***	***SWP[1]***			
Surface Shine	14.5a	13.8a	14.3a	0.848	NS	0.388
Surface Grain	0.7b	3.8a	0.8b	2.76	Sig	0.863
Spoon Indent	14.5ab	12.3b	14.7a	2.40	NS	0.600
Viscosity	9.6a	7.7a	9.7a	2.72	Sig	0.666
Firmness	3.4a	2.2b	3.3ab	1.15	Sig	0.736
Denseness	6.8a	6.1a	6.9a	0.83	Sig	0.659
Cohesiveness	3.1a	2.5a	3.0a	0.74	Sig	0.537
Graininess	4.5ab	5.6a	4.3b	1.25	Sig	0.627
Meltaway	8.4a	9.5a	8.5a	1.33	Sig	0.531
Mouth Coating	6.7a	7.0a	7.0a	NS	NS	0.335
Spoon Ropey	0.6a	ND	ND	NS	Sig	0.643
Spoon Grainy	3.8a	4.0a	3.1a	1.11	Sig	0.474
Jiggle	3.6b	5.7a	4.4ab	2.06	Sig	0.696
No Stir Firmness	4.2b	2.4b	4.4ab	1.95	Sig	0.457
No Stir Denseness	7.1a	5.9a	7.1a	1.81	Sig	0.767
Ropey	ND	1.2a	ND	0.94	Sig	0.783
Slurp Viscosity	12.3a	9.7b	11.5ab	2.60	Sig	0.746

Means followed by a different lowercase letter within a row indicate a significant difference among protein sources ($P < 0.05$).

[1] Protein Sources: SMC – skim milk concentrate yogurt; AWP – acid whey protein yogurt; SWP – sweet whey protein yogurt. ND - not detected.

[2] Time of storage: NS = not significant ($P > 0.05$); Sig = Significant ($P < 0.05$)

Table 2.7. Consumer (n=100) liking scores for yogurts mixed with strawberry fruit prep base at 20% (w/w).

	Protein Source		
	SMC[1]	***AWP[1]***	***SWP[1]***
Overall	7.0a (0.075)	5.0c (0.122)	6.0b (0.108)
Appearance	6.8a (0.070)	6.0b (0.081)	6.0b (0.102)
Flavor	6.9a (0.080)	5.6c (0.109)	6.4b (0.090)
Tartness	6.6a (0.079)	5.6b (0.098)	6.3a (0.089)
Sweetness	6.6a (0.087)	5.7b (0.105)	6.5a (0.085)
Thickness	6.9a (0.085)	4.6c (0.106)	5.9b (0.112)
Texture	6.7a (0.089)	4.2c (0.118)	5.3b (0.122)
Aftertaste	6.0a (0.089)	4.9b (0.096)	5.9a (0.078)

Different lowercase letters in rows following means indicate a significant difference within a day ($P < 0.05$). Standard error values are in parentheses below mean values.

[1] Protein Sources: SMC – skim milk concentrate yogurt; AWP – acid whey protein yogurt; SWP – sweet whey protein yogurt. ND - not detected.

Liking was scored on a 9-point hedonic scale where 1=dislike extremely and 9=like extremely.

Table 2.8. Trained panel flavor profiles of reformulated yogurts on day 14.

Flavor Attribute	*Protein Source*		
	SMC[1]	***AWP[1]***	***SWP[1]***
Aroma Intensity	2.4a (0.060)	1.8b (0.099)	1.8b (0.084)
Cooked	4.0b (0.017)	3.9b (0.030)	4.1a (0.063)
Dairy Sour	2.0a (0.032)	1.6c (0.037)	1.7b (0.039)
Milkfat	1.0b (0.104)	1.4a (0.067)	1.2b (0.064)
Cardboard	ND	ND	0.9a (0.063)
Beefy/Brothy	ND	ND	ND
Soapy	ND	ND	ND
Acetaldehyde	2.7a (0.067)	1.9b (0.050)	1.7c (0.070)
Sweet	5.4a (0.066)	5.5a (0.066)	4.7b (0.113)
Sour	2.0c (0.031)	2.3b (0.050)	2.6a (0.043)
Aftertaste	1.1a (0.079)	1.0a (0.067)	1.0a (0.059)
Umami	ND	0.7a (0.061)	ND
Astringency	2.7b (0.044)	2.9a (0.075)	2.8ab (0.082)

Different lowercase letters in rows following means indicate a significant difference within a day ($P < 0.05$). Standard error values are in parentheses below mean values.

[1] Protein Sources: SMC – skim milk concentrate yogurt; AWP – acid whey protein yogurt; SWP – sweet whey protein yogurt. ND - not detected. NA – not available.

Attributes were scored on a 0 to 15 point product specific scale (Meilgaard et al., 2007).

Table 2.9. Trained panel texture profiles of reformulated yogurts on day 14.

Texture Attribute	*Protein Source*		
	SMC[1]	***AWP[1]***	***SWP[1]***
Surface Shine	10.5a (0.081)	9.9b (0.114)	10.4a (0.128)
Surface Grain	3.4b (0.097)	5.7a (0.138)	3.8ab (0.180)
Spoon Indent	10.2a (0.311)	11.2a (0.144)	10.2a (0.237)
Viscosity	8.4b (0.101)	9.1a (0.125)	8.4b (0.160)
Firmness	2.4b (0.073)	2.9a (0.093)	2.5b (0.079)
Denseness	6.5b (0.097)	7.1a (0.090)	6.6b (0.180)
Cohesiveness	2.2b (0.059)	2.6a (0.089)	2.4b (0.110)
Graininess	3.0a (0.089)	2.4b (0.084)	2.7b (0.115)
Meltaway	11.1a (0.081)	10.9a (0.141)	11.1a (0.129)
Mouth Coating	6.6b (0.143)	7.1a (0.170)	7.0ab (0.125)

Different lowercase letters in rows following means indicate a significant difference within a day ($P < 0.05$). Standard error values are in parentheses below mean values.

[1] Protein Sources: SMC – skim milk concentrate yogurt; AWP – acid whey protein yogurt; SWP – sweet whey protein yogurt. ND - not detected.

Attributes were scored on a 0 to 15 point product specific intensity scale (Meilgaard et al., 2007).

Table 2.10. Consumer (n=100) liking scores for yogurts after reformulation, mixed with strawberry fruit prep base at 20% (w/w).

	Protein Source		
	SMC[1]	***AWP[1]***	***SWP[1]***
Overall	6.9a (0.141)	7.2a (0.131)	7.2a (0.126)
Appearance	6.9b (0.140)	7.3a (0.076)	7.3a (0.115)
Flavor	7.0a (0.131)	7.2a (0.131)	7.2a (0.124)
Tartness	6.8a (0.139)	6.8a (0.153)	6.9a (0.127)
Sweetness	6.7a (0.154)	6.9a (0.161)	6.8a (0.165)
Thickness	6.9b (0.148)	7.3a (0.117)	7.1ab (0.133)
Texture	6.6b (0.177)	7.4a (0.120)	7.0a (0.130)
Aftertaste	6.0a (0.142)	6.7a (0.157)	6.2a (0.170)

Different lowercase letters in rows following means indicate a significant difference within a day ($P < 0.05$). Standard error values are in parentheses below mean values.

[1] Protein Sources: SMC – skim milk concentrate yogurt; AWP – acid whey protein yogurt; SWP – sweet whey protein yogurt. ND - not detected.

Liking was scored on a 9-point hedonic scale where 1=dislike extremely and 9=like extremely.

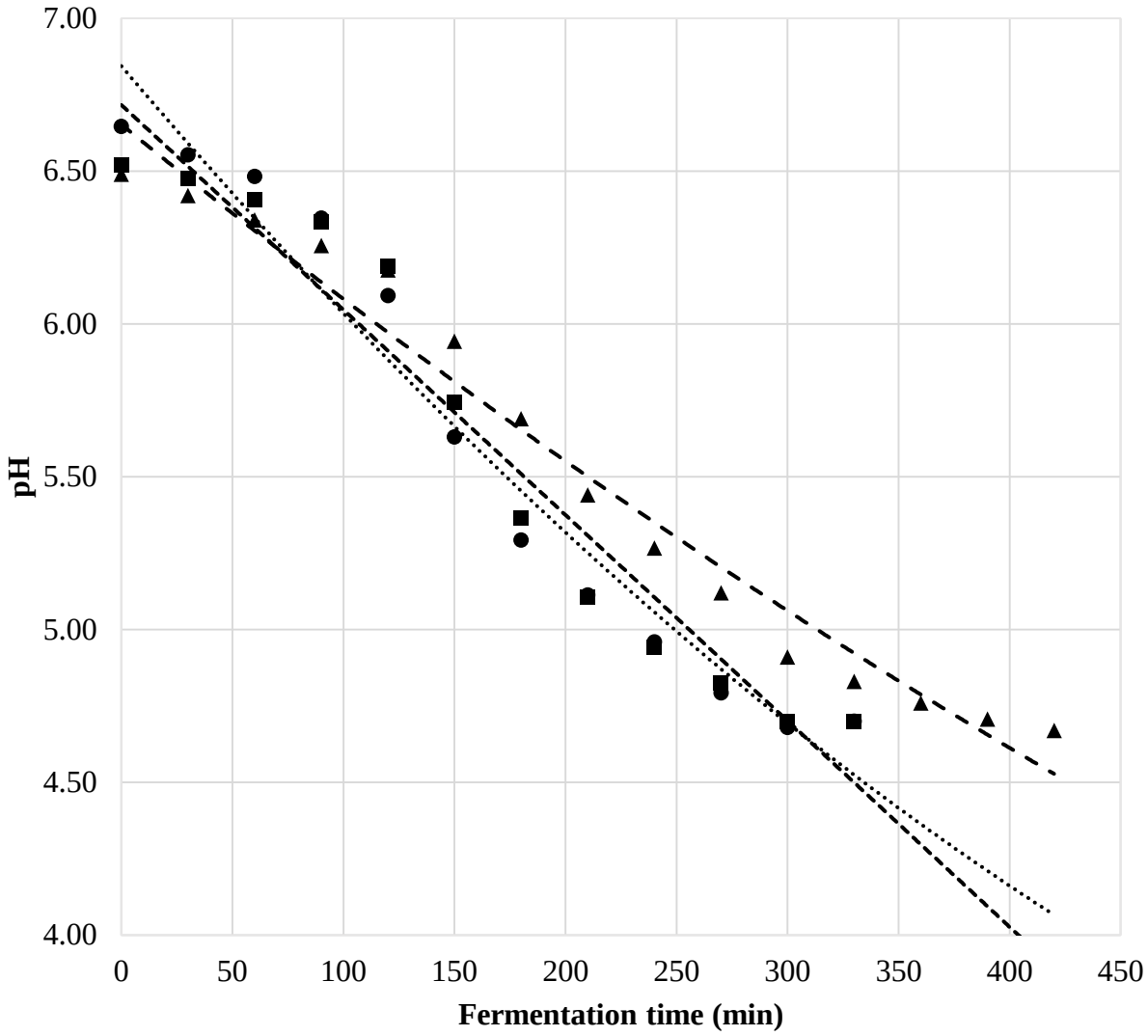


Figure 2.1. Time-course of pH change during fermentation of SMC (skim milk concentrate yogurt) (●) and quadratic regression line (···), AWP (acid whey protein yogurt) (▲) and quadratic regression line (—), and SWP (sweet whey protein yogurt) (■) and quadratic regression line (---).
Yogurts were inoculated with 0.03% (w/w) yogurt culture and incubated at 43°C.

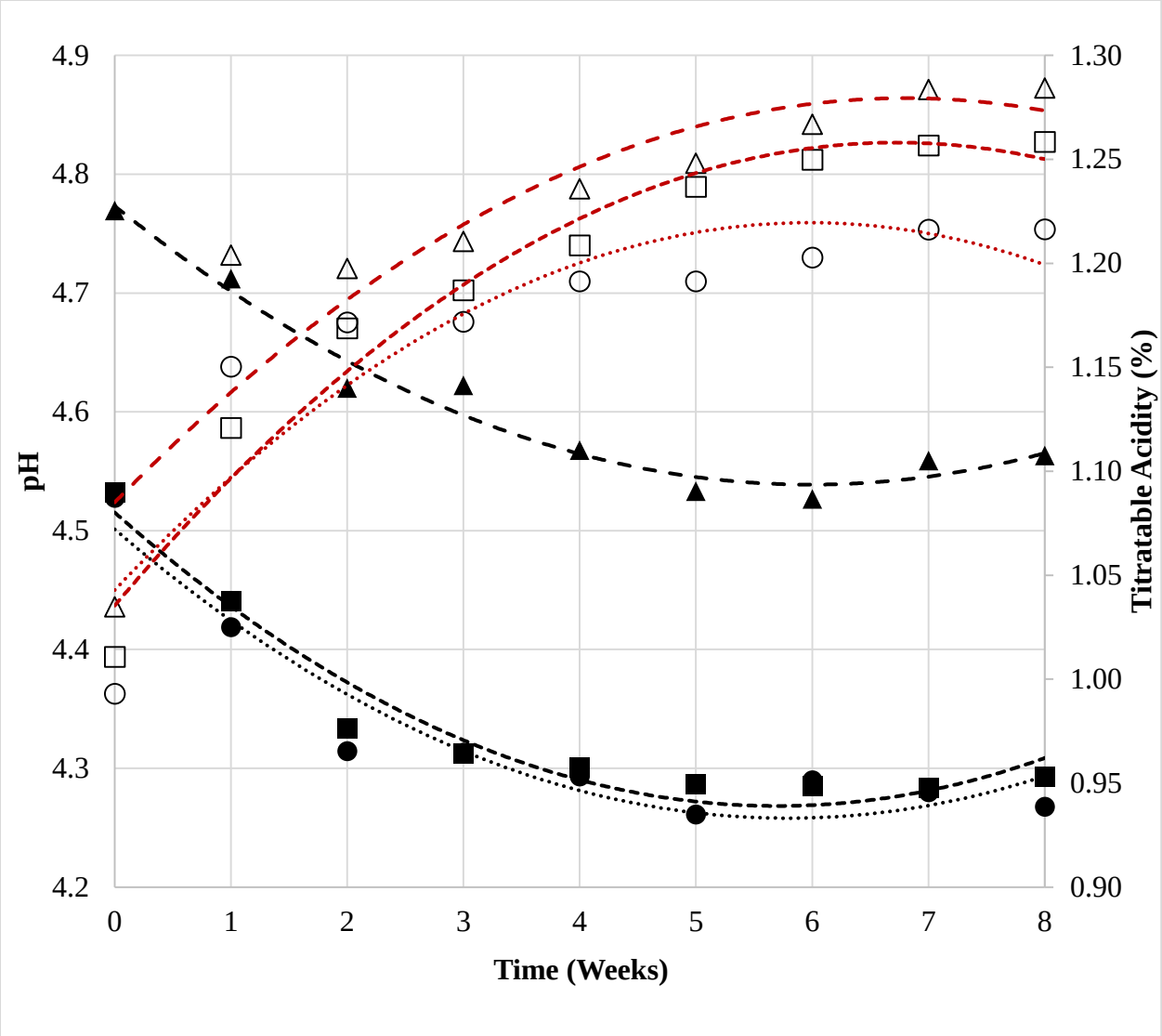


Figure 2.2. Time-course of pH and titratable acidity after cooling of yogurts during an 8 week shelf life.

pH is represented by SMC (skim milk concentrate yogurt) (●) and quadratic regression line (···), AWP (acid whey protein yogurt) (▲) and quadratic regression line (—), and SWP (sweet whey protein yogurt) (■) and quadratic regression line (---).

Titratable acidity is represented by SMC (○) and quadratic regression line (···), AWP (Δ) and quadratic regression line (—), and SWP (□) and quadratic regression line (---).

Both properties were measured at 4°C.

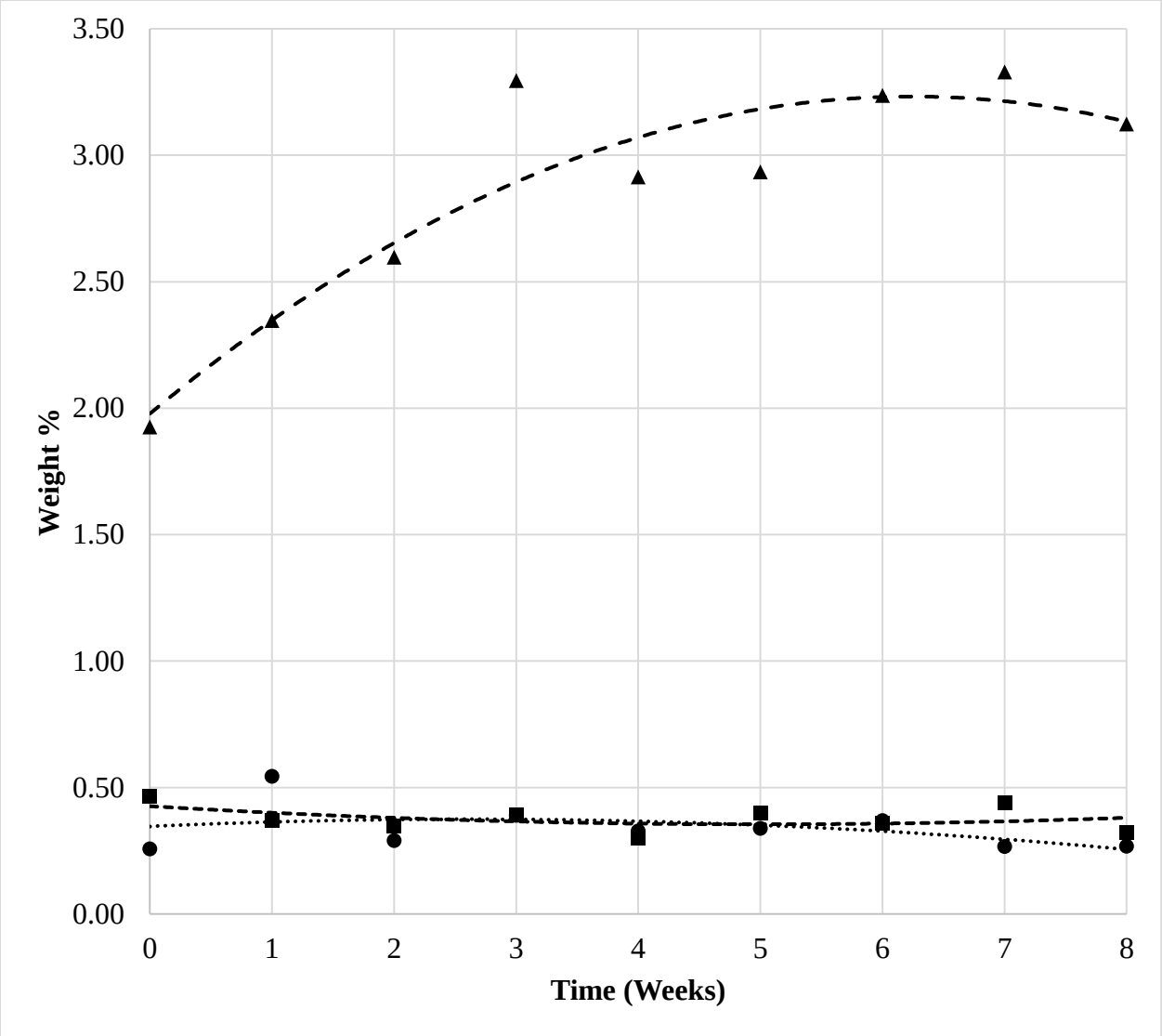


Figure 2.3. Time-course of syneresis amounts expressed in percent weight for SMC (skim milk concentrate yogurt) (●), AWP (acid whey protein yogurt) (▲), and SWP (sweet whey protein yogurt) (■), over an 8 week shelf life.
Measurements were taken from untested yogurts each week, at 4°C.

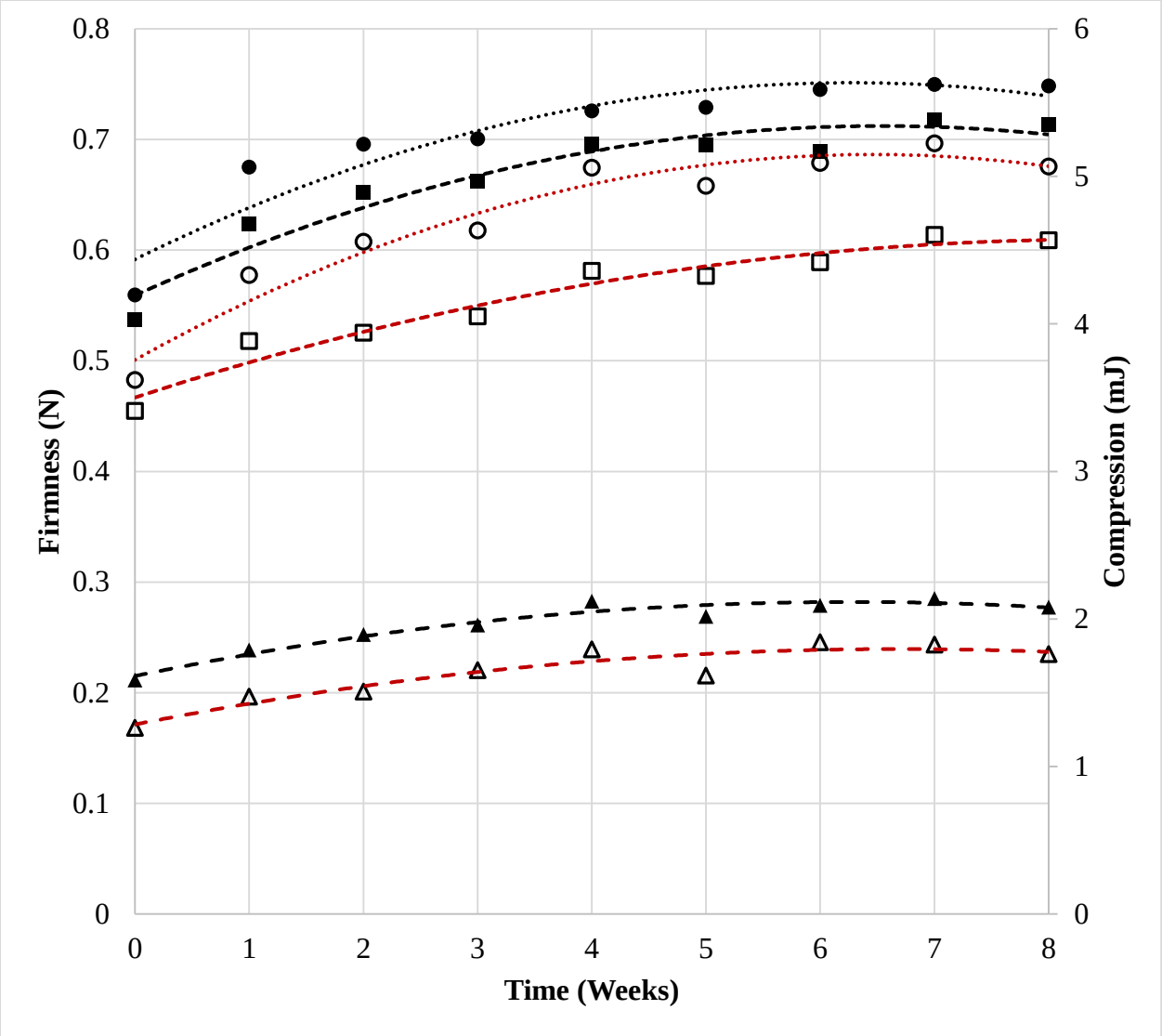


Figure 2.4. Time-course of gel strength attributes for all yogurts over an 8 week shelf life. Firmness is represented by SMC (skim milk concentrate yogurt) (●) and quadratic regression line (···), AWP (acid whey protein yogurt) (▲) and quadratic regression line (—), and SWP (sweet whey protein yogurt) (■) and quadratic regression line (---).
Compression is represented by SMC (○) and quadratic regression line (···), AWP (Δ) and quadratic regression line (—), and SWP (□) and quadratic regression line (---).
Gel strength attributes were measured at 4°C.

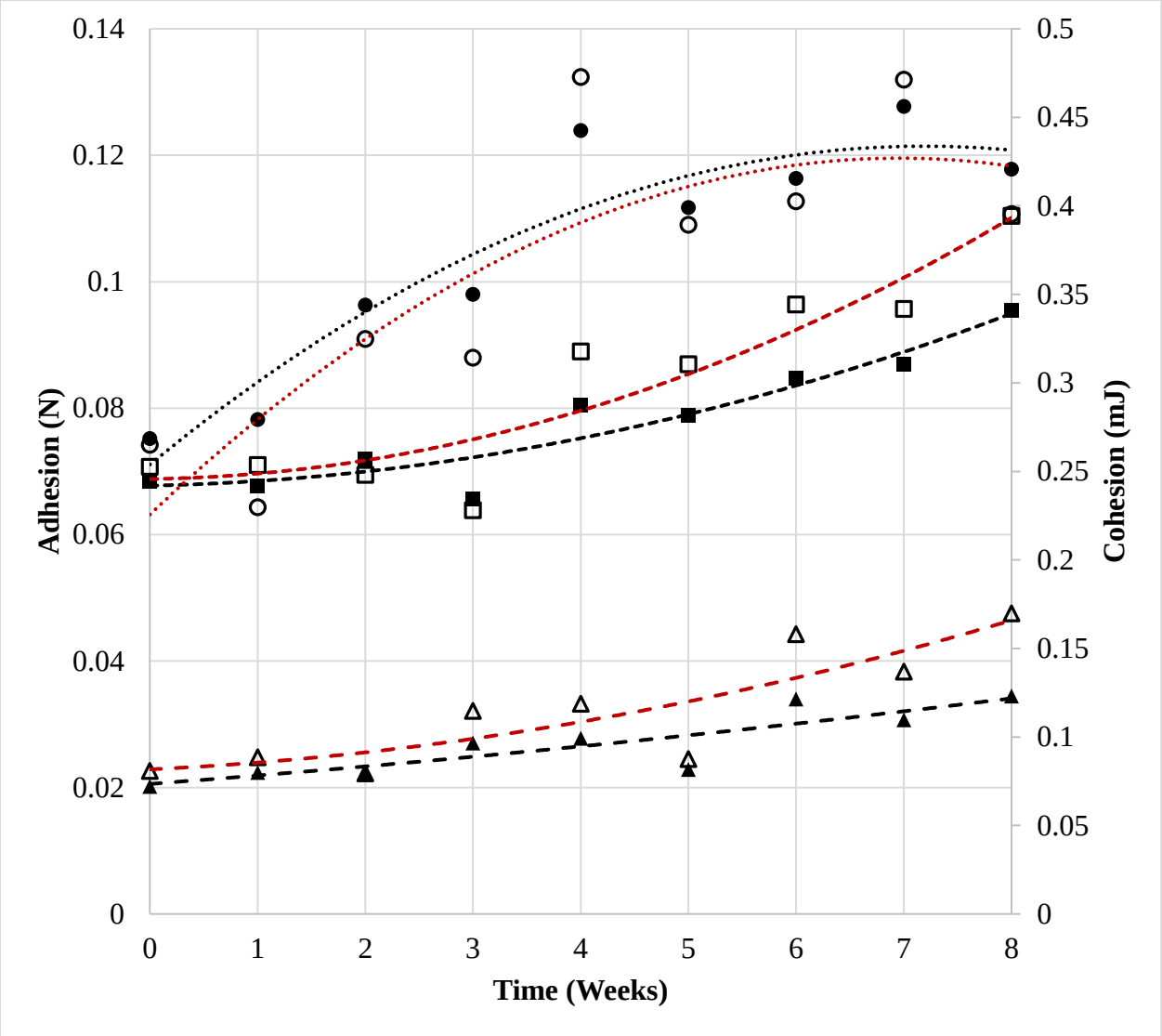


Figure 2.5. Time-course of gel strength attributes for all yogurts over an 8 week shelf life.
Adhesion is represented by SMC (skim milk concentrate yogurt) (●) and quadratic regression line (···), AWP (acid whey protein yogurt) (▲) and quadratic regression line (—), and SWP (sweet whey protein yogurt) (■) and quadratic regression line (---).
Cohesion is represented by SMC (○) and quadratic regression line (···), AWP (Δ) and quadratic regression line (—), and SWP (□) and quadratic regression line (---).
Gel strength attributes were measured at 4°C.

CHAPTER 3:

FURFURYL ALCOHOL CONCENTRATIONS IN FLUID MILK AND CULTURED AND DRIED DAIRY PRODUCTS

Furfuryl alcohol concentrations in fluid milk and cultured and dried dairy products

B.M. Wherry, Y. Jo, M.A. Drake

Southeast Dairy Foods Research Center, North Carolina State University, Raleigh, NC 27695

The contents of this chapter have been submitted to:

Journal of Dairy Science

Abstract

Maillard browning occurs in dairy products during heat treatment. Furfuryl alcohol (FA) may be found in dairy products as a result of Maillard browning. The recent posting in California proposition 65 indicates that FA may be carcinogenic, and for this reason it is crucial to accurately measure FA concentrations in dairy products. The objective of this study was to identify an extraction and quantitation method for FA from dairy products and to determine FA concentrations in milk, dairy powders and fermented dairy products. Solvent assisted flavor extraction (SAFE), solid phase microextraction (SPME), stir bar sorptive extraction (SBSE) with gas chromatography mass spectrometry (GC-MS), and triple quadrupole mass spectrometry (GC-MS/MS) were compared for recovery of FA. Internal standards for the quantitation of FA (2-methyl-3-heptanone, D_5-furfuryl alcohol, 2,5-dimethylphenol, 5-methyl-2-furfuryl alcohol, and 5-methyl furfural) were also compared. Subsequently, fluid milk (high temperature short time (HTST) and ultrapasteurized (UP)), whey protein isolates (WPI) (3 mo - 4 y), whey protein concentrates (WPC) (3 mo - 4 y), high and low heat skim milk powders (SMP) (0 - 8 y), milk protein isolates (MPI) (3 mo - 3 y), milk protein concentrates (MPC) (3 mo - 3 y), Cheddar cheese (mild, medium, sharp and extra sharp), mozzarella cheese (whole and part skim), cottage cheese (nonfat, low fat, and full fat), sour cream (full and low fat) and yogurts (traditional and Greek-style) (n=139 products total) were evaluated. FA was extracted from products by HS-SPME followed by gas GC-MS/MS using a ZB-5ms column (30m x 0.25mm ID x 0.25µm). D_5-furfuryl alcohol was used as an internal standard. Each food was extracted in triplicate. UP milks had higher levels of FA than HTST milks ($P < 0.05$) (7.350 vs. 122.3 ppb). FA concentrations ranged from 0.634 to 26.55 ppb in WPI, 2.251 to 56.19 ppb in WPC, 11.99 to 121.9 ppb in MPI, and 8.312 to 49.71 ppb in MPC and concentrations increased with powder storage ($P < 0.05$).

High heat SMP had higher concentrations of FA than low heat SMP (11.8 vs. 1.36 ppb, $P < 0.05$) and concentrations increased with storage time ($P < 0.05$). Concentrations of FA in Cheddar and mozzarella cheese ranged from 2.361 to 110.5 ppb and were higher ($P < 0.05$) than FA concentrations in cottage cheese or sour cream (0.049 to 1.017 ppb). These results indicate that FA is present at higher levels in dairy products that have been subjected to higher temperatures or have been stored longer. Fermented dairy products, such as sour cream or cottage cheese, had lower levels of FA. Compared to other food products with detected levels of FA, such as coffee (200-400 ppm), dairy products have very low levels of FA.

Introduction

Proposition 65 was enacted in 1986 to help consumers in California make informed decisions about protecting themselves from chemicals known to cause cancer, birth defects, or other reproductive harm (American Cancer Society, 2015). There are over 800 chemicals on the proposition 65 list that are known to the state of California to cause cancer or birth defects. These chemicals may be naturally occurring or synthetic. They can be additives or ingredients in pesticides, household products, food, drugs, dyes or solvents (California Environmental Protection Agency, 2018a). If any food, drug or household product has the potential to contain one of the listed chemicals, then the product must be labeled appropriately. Several compounds in foods are on the proposition 65 list including acrylamide, which is formed in certain plant-based foods during the cooking process at high temperatures; methyl mercury, which is bioaccumulated in fish over time and is often found in canned tuna; and lead, which can be found in chocolate because of natural uptake by the cocoa plant (California Environmental Protection Agency, 2018b).

Furfuryl alcohol (FA) was recently added to the list of chemicals in proposition 65 as a

carcinogen (California Environmental Protection Agency, 2018c). FA has been the subject of safety research and studies have documented that FA can become a DNA-reactive intermediate that has a mutagenic effect (Glatt and Sommer, 2006). Monien et al. (2011) detected DNA adducts in *S. typhimurium* that was exposed to FA and Hoie et al. (2015) reported enhanced adduct levels in colon and liver cells of mice that had been exposed to FA. The safety of FA poses an issue to the food industry because FA is formed during heat treatment of food products due to Maillard browning or by sugar degradation due to high processing temperatures (Patton, 1950; Yaylayan and Keyhani, 2000; Swasti and Murlovic, 2012).

FA is responsible for burnt (Lee et al., 2006; Wang and Kays, 2000), cooked-sugar (Bonvehi, 2005), and rubber-like odors in foods (Karagul-Yuceer et al., 2002), and has been documented in many roasted and heated food sources. Albouchi and Murkovic (2018) documented amounts of FA in soybeans (25-100 ppm), coffee (200-400 ppm), beans (10-120 ppm), rice (5-20 ppm), wheat (25-90 ppm), corn (10-20 ppm), chickpeas (20-130 ppm) and sesame seeds (10-20 ppm). This study concluded that FA concentrations were increased in foods that received higher heat treatments during roasting.

Dairy foods have desirable flavors that are formed by microorganisms, enzymes, thermal treatments, storage conditions and chemical reactions. Safety concerns surrounding FA pose an issue to the dairy industry because the compound has been detected in dairy products. Previous studies have noted the presence of FA in milk powders (Karagul-Yuceer et al., 2002), Cheddar cheese and parmesan cheese (Whetstine et al., 2005; Qian and Reineccius, 2002), and sweet whey powder (Mahajan et al., 2004). However, FA amounts in a representative array of dairy products have not been documented. There were two objectives for this paper. The first, was to find the most accurate and reliable technique for FA extraction and detection in dairy products.

The second objective was to report concentrations of FA in fluid milk, Cheddar cheese, mozzarella cheese, cottage cheese, sour cream, yogurt, milk and whey protein powders and skim milk powders.

Materials and Methods

Experimental Overview

Two experiments were conducted as part of this study. Experiment 1 included the selection of an appropriate extraction procedure and an appropriate internal standard for the quantitation of FA in dairy products. Four methods were tested for the extraction and detection of FA: Head Space-Solid Phase Microextraction-Gas Chromatography-Triple Quadrupole Mass Spectrometry (HS-SPME-GC-MS/MS), Head Space-Solid Phase Microextraction-Gas Chromatography-Mass Spectrometry (HS-SPME-GC-MS), Stir-Bar Sorptive Extraction-Thermal Desorption-Gas Chromatography-Mass Spectrometry (SBSE-TDU-GC-MS), and Direct Solvent Extraction-Solvent Assisted Flavor Evaporation (DSE-SAFE). Skim milk was used as the test medium (high temperature short time (HTST) pasteurized, purchased locally) and 2-methyl-3-heptanone (Sigma Aldrich, St. Louis, MO) was used as the internal standard. Subsequently, five internal standards were evaluated with the selected extraction method. Once an extraction method was optimized, FA concentrations were documented in 139 dairy products (experiment 2).

Experiment 1: Comparison of extraction and detection methods for FA

Sample Preparation. For HS-SPME-GC-MS and HS-SPME-GC-MS/MS, 5 mL of milk were placed in a 20 mL amber SPME vial with steel screw tops containing silicone septa faced in Teflon (Microliter Analytical, Suwanee, GA). Twenty (20) μL of internal standard (2-methyl-3-heptanone, 81 ppm) were added to each sample for quantitation. For SBSE-GC-MS, magnetic

stir bars coated with polydimethylsiloxane (PDMS) (10 x 0.5mm, Gerstel, Linthicum, MD) were added to 10 mL milk along with 20 µL of internal standard in a 10 mL amber SPME vial (Microliter Analytical) and rotated on a magnetic stir plate at 900 rpm for 60 min at 25ºC. After sample extraction, stir bars were rinsed with HPLC grade water (Fisher Scientific, Hampton, NH) and dried briefly. For DSE-SAFE, milks were extracted by ether extraction and distilled by SAFE glassware (Evans et al., 2009). Samples were separated into acidic and basic fractions and injected separately on GC-MS.

HS-SPME-GC-MS/MS analysis. GC-triple quadrupole-MS was performed using an Agilent 7890B GC applied to Agilent 7000C triple quadrupole MS (Agilent Technologies Inc., Santa Clara, CA). The sample preparation and SPME-GC-MS/MS method were modified from the method used by Jo et al. (2018). Separations were performed on a ZB-5ms column (30 m length x 0.25 mm i.d. x 0.25 µm film thickness; Phenomenex Inc, Torrance, CA). Helium was used as a carrier gas at constant flow of 1 mL/min. Vials were equilibrated for 25 min at 35°C with 4 sec pulsed 250 rpm agitation. A three phase 50/30 µm DVB/CAR/PDMS (Supelco, Bellefonte, PA) 1 cm fiber was used for analysis. The SPME fiber was exposed to the samples for 40 min at a depth 31 mm. The fiber was retracted and injected at 50 mm in the GC inlet for 5 min. The GC oven was initially held at 35°C for 3 min with a ramp rate of 10°C/min to 150°C held for 1 min then raised at a rate of 20°C/min to 250°C and maintained for 5 min. The MS transfer line was maintained at 250°C with the quad at 150°C and source at 250°C. The flow rate of helium quench gas and nitrogen collision gas was 1.0 ml/min and 2.5 ml/min, respectively. The MS/MS was operated by previously optimized multiple reaction monitoring (MRM) (Table 1) for FA. Triplicate analyses were performed on each sample. On the basis of MS results, relative abundance was calculated using 2-methyl-3-heptanone as the internal standard.

SBSE-GC-MS analysis. SBSE-GC-MS analyses were performed using an Agilent 7890B series GC and Agilent inert 5977A MSD (Agilent Technologies Inc.) equipped with a ZB-5ms column (30 m length x 0.25 mm i.d. x 0.25 µm film thickness; Phenomenex Inc.). The analysis parameters were modified from the method used by Park and Drake (2016). PDMS stir bars (10 x 0.5mm, Gerstel) and thermal desorption unit (TDU) tubes (Gerstel) were conditioned prior to analysis for 1 h at 300ºC. Stir bars were injected using an autosampler (MPS Autosampler, Gerstel, Inc., Linthicum, MD) and desorbed on a TDU in combination with a CIS-4 PTV injector (Gerstel). Volatile compounds from the stir bars were thermally desorbed at 280°C assisted by cryofocusing at -100°C for 5 min. Initial GC oven conditions were 40°C for 3 min with ramp rates of 10°C/min to 90°C, and 5°C/min to 200°C held for 10 min, then 20°C/min to 250°C held for 10 min. Purge time was set to 1.2 min using helium as the carrier gas at a constant flow rate of 1 ml/min. A 3 min solvent delay was included in the MS acquisition parameters. The MS transfer line was maintained at 280°C with the quad at 150°C and source at 230°C. A combination of scanning from 40 to 200 *m/z* and selective ion mode for ions 98 (furfuryl alcohol) and 128 (2-methyl-3-heptanone) was performed to identify compounds of interest. On the basis of MS results, relative abundance was calculated 2-methyl-3-heptanone as the internal standard.

HS-SPME-GC-MS analysis. Volatile compounds were extracted using SPME followed by Agilent 7820 GC with 5975 MSD (Agilent Technologies Inc.) equipped with ZB-5ms column (30 m length x 0.25 mm i.d. x 0.25 µm film thickness; Phenomenex Inc.). The sample preparation and SPME-GC-MS method were modified from the method used by White et al. (2013). Sample introduction was accomplished using a CTC Analytics CombiPal Autosampler (Zwingen, Switzerland). Vials were equilibrated for 25 min at 35°C with 4 sec pulsed 250 rpm agitation. A three phase 50/30 µm DVB/CAR/PDMS (Supelco) was used for all analysis. The

SPME fiber was exposed to the samples for 40 min at depth 3.1 cm. The fiber was retracted and injected at 5.0 cm in the GC inlet for 5 minutes. Samples were held at 5°C before fiber exposure. The GC oven temperature was 40°C for 3 min with ramp rates of 10°C/min to 90°C, 5°C/min to 200°C held for 10 min, and 20°C/min to 250°C held for 5 min. Inlet temperature was 250°C and set to splitless mode. A constant flow rate of 1 ml/min (helium) was maintained throughout. The MS transfer line was maintained at 250°C with the quad at 150°C and source at 250°C. Selective ion mode for ions 98 (furfuryl alcohol) and 128 (2-methyl-3-heptanone) was performed to identify compounds of interest. FA was quantified by relative abundance using 2-methyl-3-heptanone as an internal standard 2-methyl-3-heptanone as the internal standard.

DSE-SAFE analysis. Milks were extracted according to the methods of Milo and Reineccius (1997), with some modifications. Two aliquots of 80 mL of milk were placed into two Teflon bottles (capacity of 250 mL) with Tefzel closures (160 mL total for each milk) (Nalgene, Rochester, NY). Ethyl ether (50 mL; Fisher Scientific, Hampton, NH) and 20 μL of 2-methyl-3-heptanone (81 ppm in methanol; Sigma Aldrich) were added to each bottle. The mixture was shaken for 30 min on a Rotomix type 50800 (Thermolyne, Dubuque, IA) at high speed. The bottles were then centrifuged at 735 g for 10 min to separate the solvent phase from the mixture, which was subsequently collected into a glass jar. The procedure was repeated twice with 50 mL of ethyl ether. The extract was concentrated to 120 mL under a constant stream of nitrogen gas.

Solvent extracts were distilled by SAFE (Ace Glassware, Vineland, NJ) with an assembly similar to those described by Evans et al. (2009). The glass SAFE head, with the 2 L round-bottom flask attached, was connected to 2 glass traps. The bases of the primary trap (for receiving the final distilled solvent extract) and the secondary trap (for waste) were both submerged in liquid nitrogen by suspension over nitrogen-filled insulated Nalgene buckets. The

liquid nitrogen was replaced throughout the extraction so that the liquid nitrogen level remained constant. The temperature of the round bottom flask maintained at 40°C by submerging the base of the flask in a water bath. Vacuum was reached in the glassware using a rough pump/turbo pump combination. The solvent extract was introduced drop-wise into the vacuum system. Distillation was carried out for 45 min under vacuum (10^5 torr). After distillation, the distillate was collected in an amber glass jar, filtered through anhydrous sodium thiosulfate (VWR International, Radnor, PA) to remove residual water, and concentrated under a stream of nitrogen gas to 20 mL. The concentrated distillate was transferred to a 50 mL screw-top glass tube for phase separation.

To separate the distillate into neutral/basic and acid fractions, the concentrated distillate was first washed twice with 3 mL of sodium bicarbonate (0.5 M; Fisher Scientific) and mixed vigorously. After washing, the aqueous phase was placed into another glass tube. This washing and aqueous layer removal was repeated twice. The resulting ether layer, which will now be referred to as the neutral/basic fraction, was then filtered through anhydrous sodium thiosulfate to remove any residual water and concentrated to 0.5 mL under a stream of nitrogen gas. The aqueous phase, which will now be referred to as the acidic fraction, was acidified through the addition hydrochloric acid (18 % wt/vol; Sigma Aldrich) until a pH of 2 to 2.5 was reached. Five mL of diethyl ether was added to the acidic fraction and mixed vigorously. The ether phase was removed and collected in a separate glass tube. This process was repeated twice. The acidic fraction was then filtered through anhydrous sodium thiosulfate to remove residual water and concentrated to 0.5 mL under a stream of nitrogen gas.

One (1) μL of each solvent extract fraction (neutral/basic and acidic) was injected in triplicate on an Agilent 7820 GC with 5975 MS (Agilent Technologies Inc.) with a ZB-5ms

column (30 m length x 0.25 mm i.d. x 0.25 µm film thickness; Phenomenex Inc.). Injections were performed in the pulsed splitless mode. A 3.00 min solvent delay was included in the MS acquisition parameters. The GC oven was initially held at 40°C for 3 min with ramp rates of 10°C/min to 90°C, 5°C/min to 200°C held for 10 min, and 20°C/min to 250°C held for 5 min. Selective ion mode for ions 98 (furfuryl alcohol) and 128 (2-methyl-3-heptanone) was performed to identify compounds of interest. FA was quantified by relative abundance using 2-methyl-3-heptanone as an internal standard. There was no quantifiable amount of FA in the basic fraction, and so only the acid fraction was considered in calculations.

Method Selection. The optimal method was chosen by precision. Precision was determined by relative standard deviation percentage (RSD %), and sensitivity, limit of detection (LOD; signal-to-noise (S/N) of 3:1) and quantitation (LOQ; S/N of 10:1), as well as economical and temporal limitations. The LOD and LOQ determinations for each extraction method were generated from FA standards spiked with raw skim milk to account for background interference to be calculated into these limits.

Internal Standard Identification. Once the preferred method of extraction was selected, different internal standards were evaluated to determine the best internal standard for quantifying FA. Five different compounds were selected for testing: 2-methyl-3-heptanone (Sigma Aldrich), 5-methyl furfuryl alcohol (BOC Sciences, Shirley, NY), 5-methyl furfural (Sigma Aldrich), 2,5-dimethylphenol (Sigma Aldrich), and D_5-furfuryl alcohol (CDN Isotopes, Quebec, Canada). Each standards (20 µL of 1 ppm in methanol v/v) were spiked into skim milk and evaluated by HS-SPME-GC-MS/MS and injected with the conditions described previously for this extraction method. Internal standard peaks were analyzed using Agilent MassHunter quantitative and qualitative software (Agilent Technologies Inc.). The base peak (the most intensive/unique ion

peak) was selected as precursor ions for each compound on the basis of the full scan single mass spectrometry (MS 1 Scan mode) of authentic standards. Following the selection of these precursor ions for compounds of interest, their product ions were optimized using different collision energies (CE) of 3, 5, 10, 15, 20, 25, or 30 V with nitrogen gas (product ion scan mode). MRM transition was developed with chosen CE that gave the most intense/unique product ion to each compound. The most intense product ion was chosen for the quantifier ion, and the second or third most intense product ions were selected for qualitative ions (Table 1). Dwell times were set to ensure 3~3.1 cycles over a peak. S/N ratio was determined from measuring the height of the peak of interest divided by the height of the noise in the chromatogram. The internal standard for FA quantitation was selected based on S/N ratio and molecular similarity to FA.

Experiment 2: FA concentrations in dairy foods

After determining the best method of extraction and internal standard for quantitation (HS-SPME with GC-MS/MS with D_5-furfuryl alcohol), concentrations of FA in fluid milk, and fermented and dried dairy products were determined.

Food samples. Fluid skim milks (n=12) (3 different lots, processed internally, both HTST and Direct steam injection-ultrapasteurization (DSI-UP); 3 locally purchased HTST; 3 locally purchased UP), commercial dried dairy ingredients; whey protein isolate (WPI), whey protein concentration (WPC), milk protein isolate (MPI), milk protein concentrate (MPC), whole milk powder (WMP), and skim milk powder (SMP) (n=59); and commercial fermented dairy products; cottage cheese, Cheddar cheese, mozzarella cheese, sour cream, yogurt and Greek yogurt (n=68); were evaluated. The three lots of raw fluid milks (0.1 % fat, 3.3 % protein) were processed in the North Carolina State University dairy processing pilot plant and stored at 4°C

until analysis within 24 h of processing. Skim milks were processed on a Microthermics EHVH pasteurization unit (Microthermics, Raleigh, NC) with a two-stage homogenizer (GEA Niro Soavi, Parma, Italy) as described by Lee et al. (2017). For HTST treatment, raw skim milks were preheated to 60°C, homogenized and pasteurized at 75°C, for 15 s and cooled to 4°C. For the DSI-UP milk, raw milks were also preheated to 90°C, heated to 140°C for 2.3s by direct steam injection, then cooled to 85°C by vacuum cooling to remove both heat and added water. The DSI milk was then homogenized and cooled to 4°C. All milks were homogenized at 20.7 MPa total pressure with 3.4 MPa on the second stage. Dried dairy ingredients were sourced from commercial facilities. Fermented dairy products were purchased locally (Raleigh, NC). A minimum of two lots from each product were evaluated. A total of 139 dairy products were evaluated.

HS-SPME-GC-MS/MS. Milk (5 mL) was dispensed into 20 mL amber vials. Cheddar cheese and mozzarella cheese were prepared by first grating the sample and then placing 2.0 g into a 20 mL amber vial. Sour cream and yogurt were first stirred and then 2.0 g of each were placed into a 20 mL amber vial. Cottage cheese was first pureed to create a homogenous sample and then 2.0 g were placed into a 20 mL amber vial. For dried dairy ingredients, a 10 % solution (w/v) was made using MS grade deionized water (Fisher Chemical), and 5 mL of solution were placed in a 20 mL amber vial. All samples had 20 μL of D_5-furfuryl alcohol (10 ppm in methanol v/v) as an internal standard added. All samples were prepared in triplicate and analyzed using the method identified for extraction and quantitation. GC-MS/MS analysis condition was identical as described above.

Data Analysis

All data was analyzed using XLSTAT software (version 2018.1, Addinsoft, New York,

NY). Differences among sample means was determined by analysis of variance followed by Tukey's honestly significant difference post-hoc test at 95 % confidence.

Results

Method Selection

FA was detected by SPME-GC-MS/MS, SBSE-GC-MS, and SAFE-GC-MS, but was not detected by SPME-GC-MS in fluid skim milk (Table 2). FA was detected by SBSE-GC-MS but results were not quantifiable. Method selection between SPME-GC-MS/MS and SAFE-GC-MS was determined based on precision, LOD and LOQ. Precision was defined as the RSD %. SPME-GC-MS/MS had a lower RSD than SAFE-GC-MS, suggesting that results were more precise over a series of samples. LOD and LOQ for SPME-GC-MS/MS were 0.001 and 0.005 ppb, respectively, compared to SAFE-GC-MS which were 0.894 and 5.271 ppb, respectively.

Internal Standard Selection

For the generation of reliable quantitative data, the use of a proper internal standard is required for analysis. We selected internal standards with similar molecular structure and mass to FA. Internal standards were analyzed for retention time and signal to noise ratio to determine the best compound for quantitation. Of the five compounds tested, D_5-furfuryl alcohol had the highest S/N ration (2553, Table 1) compared to the other four compounds. 5-methyl furfural had the second highest S/N ratio (2001). Based on these results, SPME-GC-MS/MS with D_5-furfuryl alcohol as the internal standard was selected for FA evaluation in dairy products.

FA Concentration in Dairy Foods

Fluid Milk. FA content in fluid milks ranged from 0.505 - 122.3 ppb (Table 3). FA content in commercial milks were similar in content to milks processed in the pilot plant after one week of storage ($P > 0.05$). Milks processed in the pilot plant were tested over a 4 week shelf life to

determine FA concentrations over time (Figure 1). At each time point, UP pasteurized milks had higher levels of FA than HTST pasteurized milks ($P < 0.05$) with a large decrease in FA content of UP milk in the first 7 days. During the 4 week shelf life period, FA content decreased in both HTST and UP pasteurized milks ($P < 0.05$).

Dried Dairy Ingredients. SMP is made by condensing skim milk through evaporation and then spray drying the condensed milk (solids content ~50 %) into a powder (solids content ~95 %). Higher heat SMP are defined by a higher heat treatment prior to the spray dryer than lower heat SMP. In SMP, FA was detected in the range of 0.771 – 80.58 ppb (Table 3). High heat and low heat SMP were tested, and ranged from 0 to 8 y in age. For all ages tested, high heat SMP had higher levels of FA than low heat SMP ($P < 0.05$) (Figure 2). FA content increased with longer storage times ($P < 0.05$).

Milk protein ingredients are any concentrated milk with protein content greater than 40 % on a dry basis. Milk is concentrated by ultrafiltration and/or microfiltration and then spray dried to a powder. In MPC and MPI, FA was detected in the range of 8.312 – 49.71 ppb and 11.99 – 121.9 ppb respectively (Table 3). MPC powders were tested in a range of 0 - 6 y of storage (Figure 3) and MPI powders were tested in the range of 0 - 4 y of storage. Both MPC and MPI had increased levels of FA with longer storage times ($P < 0.05$).

Whey protein ingredients come from concentrated whey streams after cheese make. After whey is drained from cheese curd, it is pasteurized and concentrated through ultrafiltration and/or microfiltration to a target protein content. The solution is then spray dried to a powder (solids content ~95 %). Whey protein powders range in protein content from 34 % to greater than 90 % protein on a solids basis. In WPC (80 % protein) (w/w) and WPI (90 % protein) (w/w), FA was detected in the range of 2.251 – 56.19 ppb and 0.634 – 26.55 respectively (Table

3). WPC and WPI powders were tested in a range of 0 - 6 y of storage (Figure 3). Whey protein powders showed no change in FA levels with storage time ($P > 0.05$).

Cultured Dairy Products. FA content in Cheddar cheese ranged from 2.797 – 73.82 ppb (Table 3). Mild Cheddar cheese had the highest levels of FA ($P < 0.05$), and FA decreased with longer ageing of cheese ($P < 0.05$). In cottage cheeses, FA ranged from 0.231 – 1.017 ppb (Table 3). Full fat (n=5), low fat (n=4) and fat free (n=2) cottage cheeses were tested. Fat content had no impact on FA ($P < 0.05$). Sour cream is, as the name suggests, a high fat fermented dairy product. Full fat sour cream must have a fat level of 18 % (w/w) and low fat sour cream must have a fat level of 9% (w/w). In sour creams (n=11), FA concentrations ranged from 0.049 – 0.988 ppb (Table 3). Full fat (n=7), low fat (n=2) and fat free (n=2) sour cream samples were tested. Fat content had no impact on FA ($P < 0.05$). Yogurt is made by fermenting pasteurized milk to a pH of 4.6 or lower. Greek-style yogurt is further processed by straining the whey from the white mass after fermentation. Three (3) Swiss-style yogurts and six (6) Greek-style yogurts were tested for FA content. In Swiss-style yogurts, FA content ranged from 10.88 – 18.01 ppb and 4.963 – 144.6 ppb in Greek-style yogurts (Table 3).

Discussion

Formation of FA in dairy products has been hypothesized and tested since the 1940's. Patton and Josephson (1949) isolated FA from heated skim milk by ether extraction. They theorized that FA was produced from the reduction of furfural by sulfhydryl groups in milk. Furfural is a Maillard reaction product from the reaction between lactose and lysine (Berg, 1993). FA was found in concentrated skim milk and weakly alkaline lactose solutions (Patton, 1950), suggesting that pH plays a role in FA formation. Hydroxymethyl-furfural (HMF) was formed in lactose solutions with lower pH and FA was not detected. FA formed in the same

solutions at higher pH, suggesting a relationship between HMF and FA. However, HMF was not a precursor to FA because addition of HMF to skim milk before heating had no effect on the amount of FA (Patton, 1950). Patton (1950) predicted that FA was formed from lactose. Berg (1993) reported that FA was formed from degradation of lactulose, which is a product of lactose degradation and heat. Furfural and FA were also formed in stored casein (Ramshaw and Dunstone, 1969), likely a result of a Maillard reaction from lysine residues and lactose. Like Patton (1950) and Flipse (1957), Albouchi and Murkovic (2018) presented a mechanism for FA formation from glucose degradation, but in a coffee model system. Future studies are required to elucidate FA formation in different types of dairy foods.

Comparing fluid milk content of FA, milks that were subjected to higher heat treatment formed more FA than milks with lower heat treatment. This was also reported by Albouchi and Murkovic (2018) in coffee model systems. Previous studies extracted FA from milks that had been heated at high temperatures (above 140°C/30 min or longer) as well (Patton and Josephson, 1949; Patton, 1950; Patton and Flipse, 1957; Berg, 1993). This result demonstrates that higher heat treatment favors formation of FA. This trend was also expressed in higher heat skim milk powders compared with lower heat skim milk powders.

FA was significantly higher in milk protein powders than in whey protein powders. One hypothesis is that the difference in processing of the two powders affected final FA content. Whey protein powders are generally made by concentrating whey from cheese production. The cheese making process uses lactic starter cultures that convert lactose into lactic acid to drive down pH. With lower levels of starting lactose in the whey, FA content is lower in whey protein powders compared to milk protein powders. Milk protein powders and skim milk powders had increased levels of FA with longer storage time. Maillard reaction products continue to form in

milk powders during storage due to the close proximity of reactive molecules (Kelly and Fox, 2016). Furfuryl alcohol, being a product of Maillard reaction, is likely continuing to form in these powders as they are stored.

Cottage cheese and sour cream had the lowest levels of FA. This result coincides with previous studies finding no FA in lower pH lactose solutions after heating (Patton, 1950). It is also possible that FA is degraded at lower pH. Yogurt samples also had low levels of FA, with the exception of one Greek-style yogurt, further suggesting low pH does not favor FA formation in dairy products. The Greek-style yogurt sample with higher FA content was made by fortifying with milk protein powder, and according to the findings of this paper, milk protein powders can contain up to 121 ppb of FA which would contribute to the FA in that sample of Greek-style yogurt. Cheddar cheese had more interesting results with mild Cheddars having higher concentrations than more sharp Cheddars. Spillman et al. (1998) reported microflora converting FA into FA ethers in wine. It is possible that cheese cultures have a similar effect on FA in cheese during the ageing process. Longer aged cheeses would experience this effect over a longer period of time and have lower levels of FA.

Conclusion

Furfuryl alcohol was present in higher levels in dairy products that have been subjected to higher heat treatments or have been stored for longer periods of time. Dairy products with lower pH, such as sour cream or cottage cheese, had lower levels of FA, suggesting that pH plays a role in formation or degradation of FA. More importantly, dairy products have lower levels of FA compared to other food products with documented concentrations of FA. Based on these results from over 100 dairy products, FA concentrations in dairy foods are up 1000 fold lower than other heated or processed foods.

Acknowledgments

Funding provided in part by the National Dairy Council (Rosemont, IL). The use of tradenames does not imply endorsement not lack of endorsement by those not mentioned.

References

Albouchi, A., and M. Murkovic. 2018. Formation kinetics of furfuryl alcohol in a coffee model system. J. Food Chem. 243:91-95.

American Cancer Society. 2015. Cancer warning labels based on California's proposition 65. Accessed Jul. 23, 2018. https://www.cancer.org/cancer/cancer-causes/general-info/cancer-warning-labels-based-on-californias-proposition-65.html

Berg, H. E. 1993. Reactions of lactose during heat treatment of milk: a quantitative study. Ph.D. thesis, Agricultural University, Wageningen.

Bonvehi, J. S. 2005. Investigation of aromatic compounds in roasted cocoa powder. Eur. Food Res. Technol. 221:19-29.

California Environmental Protection Agency. 2018a. About proposition 65. Accessed Jul. 23, 2018. https://oehha.ca.gov/proposition-65/about-proposition-65

California Environmental Protection Agency. 2018b. Foods. Accessed Jul. 30, 2018. https://www.p65warnings.ca.gov/fact-sheets/foods

California Environmental Protection Agency. 2018c. Furfuryl alcohol. Accessed Jul. 23, 2018. https://oehha.ca.gov/proposition-65/chemicals/furfuryl-alcohol

Evans, J., J. Zulewska, M. Newbold, M. A. Drake, and D. M. Barbano. 2009. Comparison of composition, sensory, and volatile components of thirty-four percent whey protein and milk serum protein concentrates. J. Dairy Sci. 92:4773-4791.

Glatt, H., and Y. Sommer. 2006. Health risks of 5-hydroxymethylfurfural (HMF) and related compounds. Pages 328-357 in Acrylamide and other hazardous compounds in heat-treated foods, Vol. 1. Skog, K., and J. Alexander, eds. Woodhead Publishing, Cambridge, UK.

Hoie, A. H., B. H. Monien, A. K. Sakhi, H. Glatt. H. Hjertholm, and T. Husey. 2015. Formation of DNA adducts in wild-type and transgenic mice expressing human sulfotransferases 1A1 and 1A2 after oral exposure to furfuryl alcohol. Mutagenesis. 30:643-649.

Jo, Y., D. M. Benoist, D. M. Barbano, and M. A. Drake. 2018. Flavor and flavor chemistry differences among milks processed by high temperature short time or ultra-pasteurization. J. Dairy Sci. 101:3812-3828.

Karagul-Yuceer, Y., K. R. Cadwallader, and M. A. Drake. 2002. Volatile flavor compounds of stored nonfat dry milk. J. Ag. Food Chem. 50:305-312.

Kelly, A. L., and P. F. Fox. 2016. Manufacture and properties of dairy powders. Pages 1-33 in Advanced Dairy Chemistry Vol.1. Part B. McSweeney P. L. H. and O. Mahoney, ed. Springer Science, New York, NY.

Lee, A. P., D. M. Barbano, and M. A. Drake. 2017. The influence of ultrapasteurization by indirect and direct steam injection processing on sensory perception of skim and 2% fat milks. J. Dairy Sci. 100:1688-1701.

Lee, S. M., B. C. Seo, and Y. S. Kim. 2006. Volatile compounds in fermented and acid-hydrolyzed soy sauces. J. Food Sci. 71:146-156.

Mahajan, S. S., L. Goddik, and M. C. Qian. 2004. Aroma compounds in sweet whey powder. J. Dairy Sci. 87:4057-4063.

Milo, C., and G. A. Reineccius. 1997. Identification and quantification of potent odorants in regular-fat and low-fat mild cheddar cheese. J. Agric. Food Chem. 45:3590-3594.

Monien, B. H., K. Herrmann, S. Florian, and H. Glatt. 2011. Metabolic activation of furfuryl alcohol: formation of 2-methylfuranyl DNA adducts in Salmonella typhimurium strains expressing human sulfotransferase 1A1 and in FVB/N mice. Carcinogenesis. 32:1533-1539.

Park, C. W., and M. A. Drake. 2016. Condensed milk storage and evaporation affect the flavor of nonfat dry milk. J. Dairy Sci. 99:9586-9597.

Patton, S. 1950. Studies of heated milk III: mode of formation of certain furan compounds. J. Dairy Sci. 33:904-910.

Patton, S. and R. J. Flipse. 1957. Carbon-14 activity of some heat-degradation products of milk containing lactose-1-C^{14}. Science. 125:1087-1088.

Patton, S. and D. V. Josephson. 1949. The isolation of furfuryl alcohol from heated skim milk. J. Dairy Sci. 32:222-227.

Ramshaw, E. H. and E. A. Dunstone. 1969. Volatile compounds associated with the off-flavor in stored casein. J. Dairy Res. 36:215-223.

Qian, M., and G. Reineccius. 2002. Identification of aroma compounds in parmigiana-reggiano cheese by gas chromatography/olfactometry. J. Dairy Sci. 85:1362-1369.

Spillman, P. J., A. P. Pollnitz, D. Liacopoulos, K. H. Pardon, and M. A. Sefton. 1998. Formation and degradation of furfuryl alcohol, 5-methylfurfuryl alcohol, vanillyl alcohol, and their ethyl ethers in barrel-aged wines. J. Agric. Food Chem. 46:657-663.

Swasti, Y. R. and M. Murkovic. 2012. Characterization of the polymerization of furfuryl alcohol during roasting of coffee. Food Funct. 3:965-969.

Wang, Y., and S. J. Kays. 2000. Contribution of volatile compounds to the characteristic aroma of baked jewel sweetpotatoes. J. Amer. Soc. Hort. Sci. 125:638-643.

Whetstine, M. E. C., K. R. Cadwallader, and M. A. Drake. 2005. Characterization of aroma compounds responsible for the rosy/floral flavor in cheddar cheese. J. Agric. Food Chem.

53:3126-3132.

White, S. S., K. M. Fox, S. M. Jervis, and M. A. Drake. 2013. Influence of heating and acidification on the flavor of whey protein isolate. J. Dairy Sci. 96:1366-1379.

Yaylayan, V. A., and A. Keyhani. 2000. Origin of carbohydrate degradation products in L-alanine/D-[13C] glucose model systems. J. Agric. Food Chem. 48:2415-2419.

Table 3.1 Multiple reaction monitoring (MRM) transition of furfuryl alcohol and internal standards for GC-MS/MS. Properties of furfuryl alcohol (FA) and internal standards evaluated for use in quantifying FA.

Compound Name	*RT (min)*	*Precursor Ion (m/z)*	*Product Ion*						*S/N Ratio*
			Quantifier (m/z)	CE (V)	Qualifier 1 (m/z)	CE (V)	Qualifier 2 (m/z)	CE (V)	
Furfuryl Alcohol	10.7	98	70	3	69	5	81	5	-
2-Methyl-3-Heptanone	10.9	128	85	3	86	3	71	10	33.97
5-Methyl Furfuryl Alcohol	11.5	112	96	5	69	15	-	-	249.1
5-Methyl Furfural	11.3	110	54	15	80	5	-	-	2001
2,5-Dimethylphenol	14.0	122	107	15	78	20	-	-	94.27
D_5-Furfuryl Alcohol	8.70	103	75	10	60	15	-	-	2553

CE = Collison energy
S/N = Signal to noise
S/N was determined from measuring the height of the peak of interest divided by the height of the noise in the chromatogram

Table 3.2 Concentrations of furfuryl alcohol detected in commercial HTST skim milk using each method specified.

	SPME GC MS/MS	*SBSE GCMS*	*SPME GCMS*	*SAFE GCMS*
Pasteurized milk (ppb)	11.26a	NQ	ND	9.671a
Repeatability (RSD %)	0.338b	NA	NA	12.021a
LOD (ppb)	0.001b	NQ	NA	0.894a
LOQ (ppb)	0.005b	NQ	NA	5.271a

LOD and LOQ are expressed in ppb.
Means in a row not sharing the same letters are different ($P < 0.05$).
ND = Not detected. NQ = Not quantifiable. NA = Not applicable.

Table 3.3 Ranges of furfuryl alcohol content in dairy products.

Category	Product	FA Content (ppb)
Fluid Skim Milks	HTST (n=6)	0.505-7.350
	DSI-UP (n=6)	1.059-122.4
Cultured Products	Mild Cheddar (Aged 3 mo) (n=10)	9.688-73.82
	Medium Cheddar (Aged 3-6 mo) (n=5)	2.361-67.51
	Sharp Cheddar (Aged 6 mo) (n=9)	15.31-24.91
	Extra Sharp Cheddar (Aged 12+ mo) (n=5)	2.797-16.17
	Sour Cream (n=5)	0.166-0.988
	Low Fat Sour Cream (n=4)	0.261-0.811
	Organic Sour Cream (n=2)	0.049-0.226
	4% Cottage Cheese (n=5)	0.284-0.716
	Lowfat Cottage Cheese (n=4)	0.405-1.017
	Nonfat Cottage Cheese (n=2)	0.231-0.371
	Mozzarella (n=6)	19.28-42.82
	2% Mozzarella (n=3)	28.35-89.76
	Organic Mozzarella (n=2)	59.93-110.5
	Swiss-style Yogurt (n=3)	10.88-18.00
	Greek-style Yogurt (n=6)	4.964-144.6
Powder Products	WPC (n=13)	2.251-56.19
	WPI (n=14)	0.634-26.55
	High Heat SMP (n=7)	11.81-80.58
	Low Heat SMP (n=8)	0.771-37.44
	MPC (n=8)	8.312-49.71
	MPI (n=3)	11.99-121.9
	WMP (n=3)	49.14-112.6

Ranges represent the mean range from triplicate extractions from the number of samples listed in parentheses.

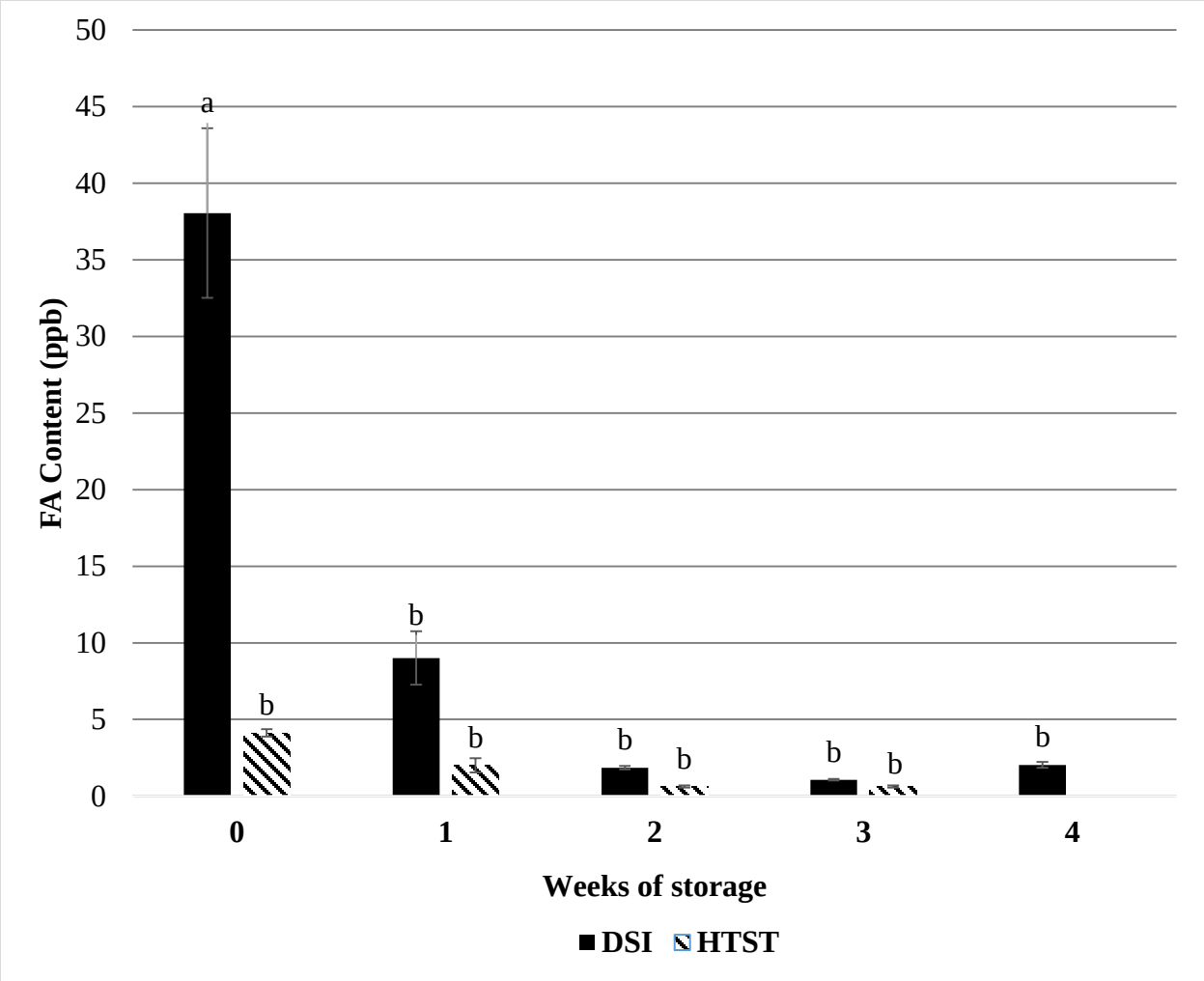


Figure 3.1 Furfuryl alcohol concentrations in pilot plant processed fluid skim milk across four weeks at 3°C. Means not sharing common letters (a to c) are different ($P < 0.05$).
DSI – Direct steam injected ultrapasteurized milk. HTST – High temperature short time pasteurized milk.
Each bar represents the mean of triplicate analyses from three different lots of processed milk at each time point.

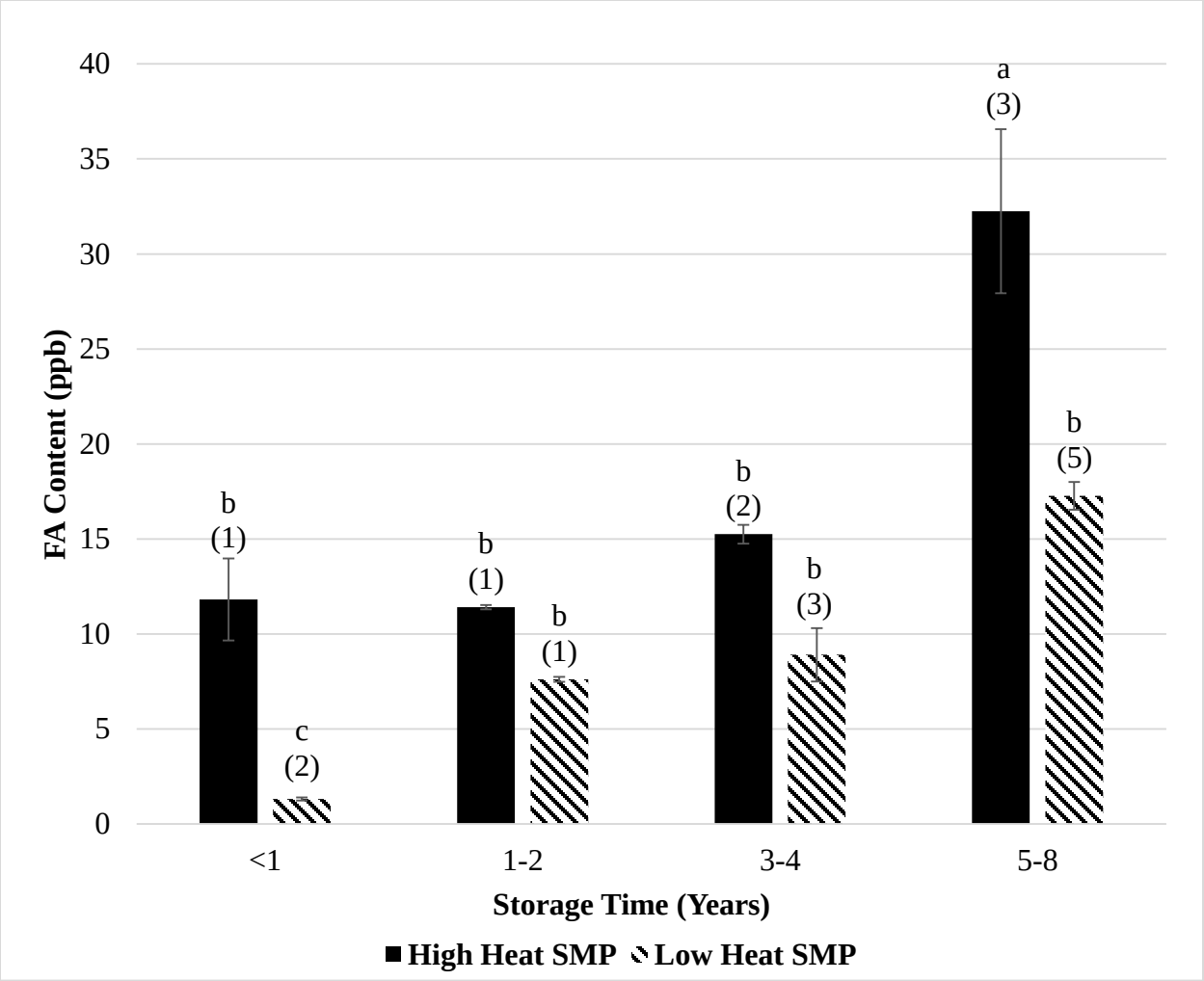


Figure 3.2 Furfuryl alcohol content in skim milk powders of various ages. Means not sharing common letters (a to e) are different ($P < 0.05$). Numbers in parentheses above bars represent the number of samples tested.

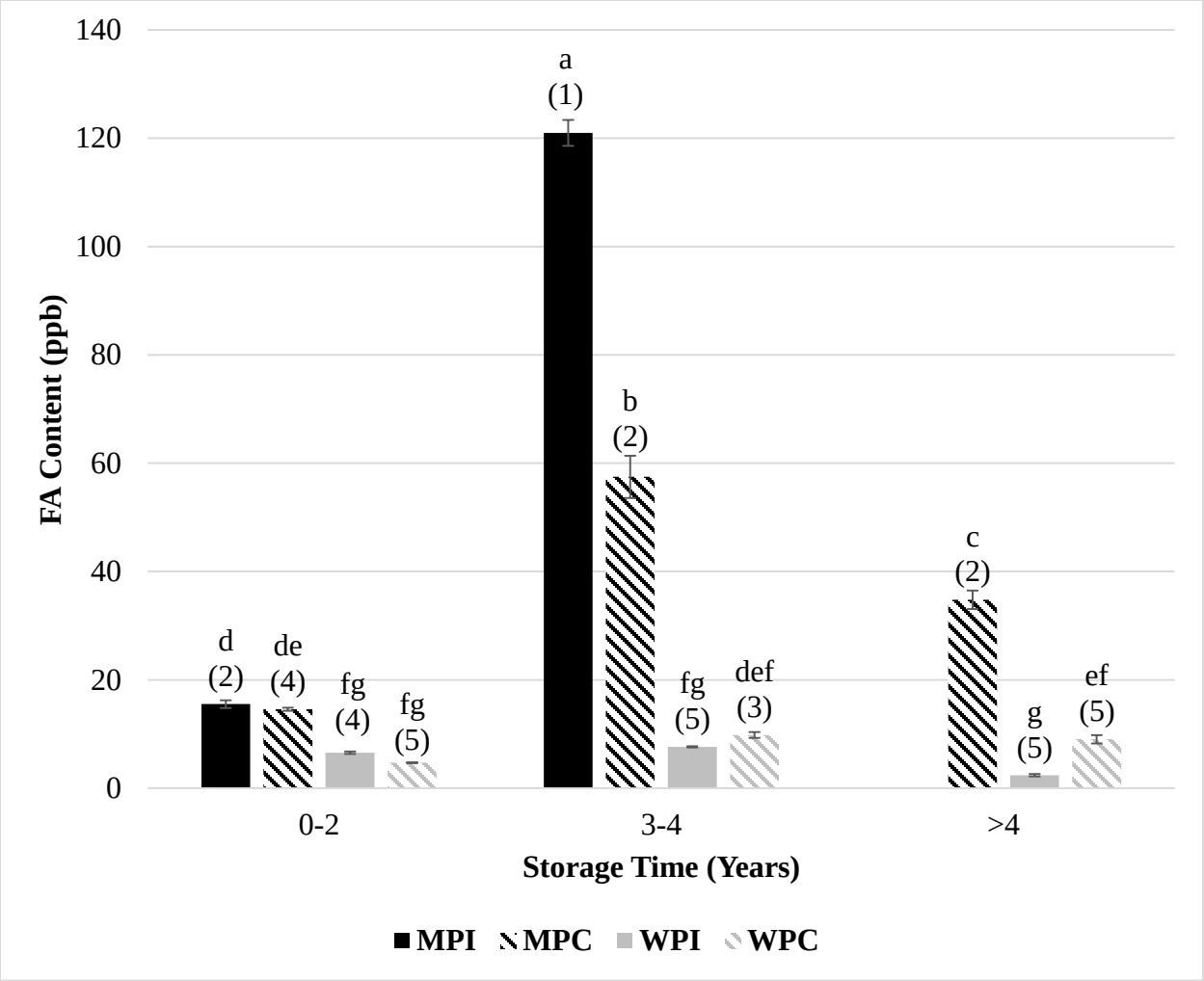
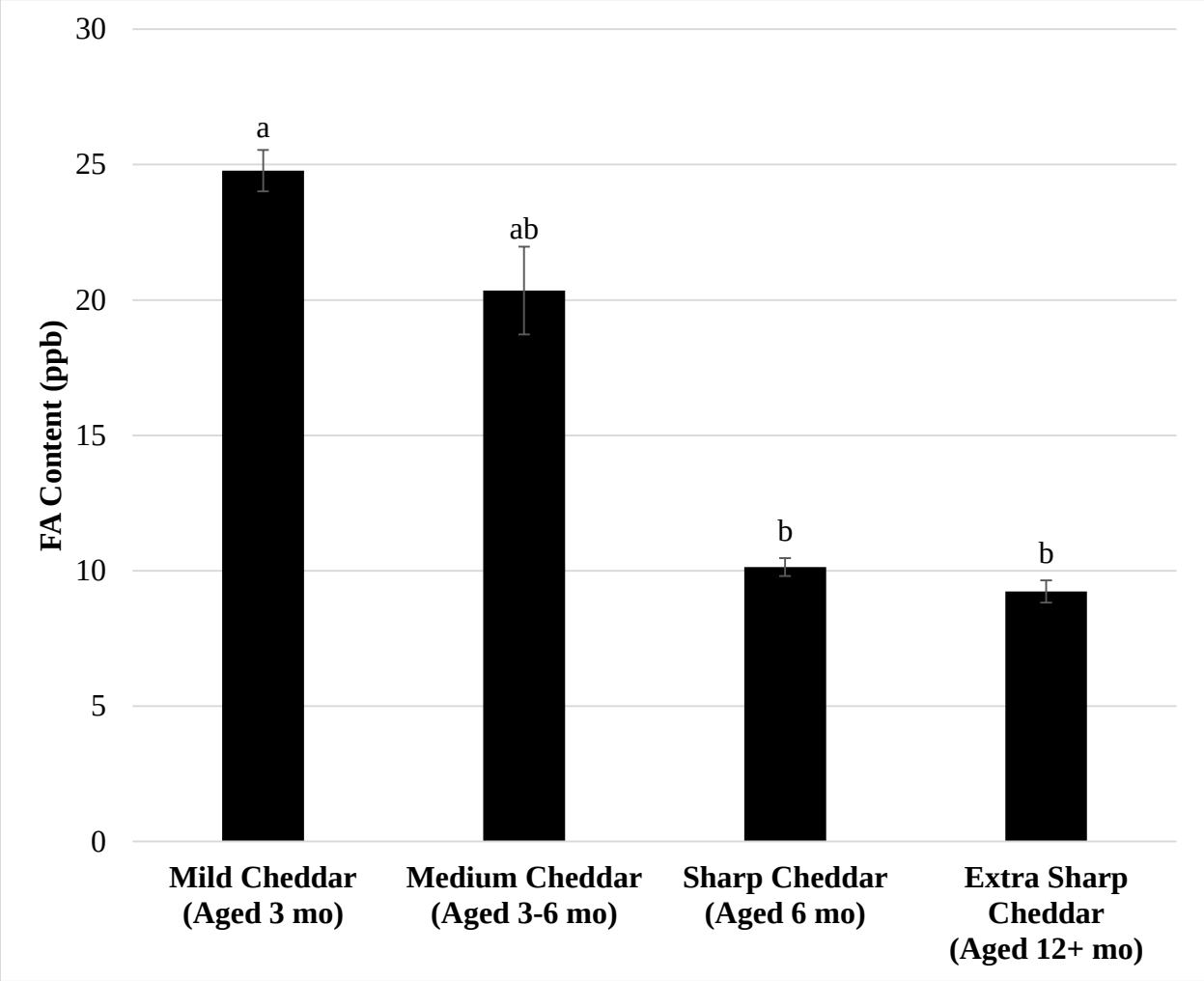


Figure 3.3 Furfuryl alcohol content in milk and whey protein powders at storage times ranging from 0-6 years. Means not sharing common letters (a to e) are different ($P < 0.05$). Numbers in parentheses above bars represent the number of samples tested.

APPENDICES

Appendix A. Furfuryl alcohol in Cheddar cheeses with varying levels of sharpness. Means not sharing common letters (a to c) are different ($P < 0.05$).



Appendix B. Furfuryl alcohol content in cottage cheese and sour cream with varying levels of fat. Means not sharing common letters (a to c) are different ($P < 0.05$).