

ABSTRACT

PARK, CURTIS WAYNE. The Influence of Processing Variables on the Flavor of Dried Dairy Ingredients. (Under the direction of Dr. MaryAnne Drake).

Production of dried dairy ingredients continues to increase due to their unique functional and nutritional properties. The most important factor that determines their successful use in a variety of ingredient applications is flavor. Understanding how unit operations affect the flavor of these ingredients is critical to producing products that will succeed in a variety of applications with an increased shelf-life. The objective of this dissertation was to investigate how unit operations during ingredient manufacture influence the flavor of various dried dairy ingredients. Five different studies were conducted to investigate: the effects of liquid storage and bleaching on the flavor of whey protein concentrate (WPC) 80%, the effects of condensed milk storage and evaporation on the flavor of nonfat dry milk (NFDM), the effect of spray drying parameters on the flavor of NFDM and milk protein concentrate (MPC) 70%, the effect of homogenization pressure on the flavor and flavor stability of whole milk powder (WMP), and the effect of facility run time on the flavor of skim milk powder (SMP).

In the first study, WPC80 was produced with or without bleaching and liquid whey or liquid WPC80 storage. When liquid whey was bleached, off-flavors increased with liquid WPC80 storage and when whey was not bleached, off-flavors increased with liquid whey storage.

This study demonstrates that bleaching and liquid composition during storage play a significant role in the flavor of WPC80.

In the second study, NFDM was produced by concentrating milk first to 30% solids by reverse osmosis (RO) or evaporation (E) and then 24 h storage or no storage of condensed milk prior to E to 50% solids and spray drying. A follow-up experiment was designed to explore the mechanism behind the observed results. Evaporation and condensed milk storage

increased cardboard flavor in the spray dried product. The follow-up experiment indicated that the heat and vacuum applied to the milk during E removed many key flavor compounds, resulting in off-flavors that were more pronounced.

In the third study, the effects of inlet temperature and feed solids concentration on the flavor of NFDM and MPC70 were investigated. For both NFDM and MPC70 higher inlet temperature and feed solids concentration decreased cardboard off-flavor and increased sweet aromatic flavor, particle size, and furosine, a Maillard reaction product. These results indicated that flavor is improved at higher inlet temperatures and solids concentrations despite increased heat load to the product.

The fourth study evaluated how homogenization pressure of condensed whole milk affects the flavor of WMP throughout shelf-life. Condensed whole milk was homogenized at four different pressures and spray dried. Fat globule size in the condensed whole milk decreased with increasing homogenization pressure. Higher homogenization pressure decreased cardboard, grassy, and painty flavors and lipid oxidation products throughout shelf-life. Free fat increased and encapsulated fat decreased in WMP produced at lower homogenization pressures. These results indicate that free fat plays a large role in the shelf-life of WMP and that free fat is altered by homogenization pressure.

In the fifth study, the effect of facility run time on the flavor and functionality of SMP was investigated. Low heat (LH) and medium heat (MH) condensed skim milk (CSM) and spray dried SMP samples across production runs (0, 12, 24, 36 h) were collected from four commercial facilities and evaluated for sensory and volatile compound analyses as well as various functional analyses. Functionality of milk powders was unaffected by facility run time. Cardboard flavor intensity and lipid oxidation products increased in both CSM and

SMP after 24 h run time. These results indicate that SMP quality decreases as the production run time increases past 24 h.

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The Influence of Processing Variables on the Flavor of Dried Dairy Ingredients

by
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DEDICATION

I would like to dedicate this work to my wife Chelsea who has always been there to love and support me. We did it!

BIOGRAPHY

Curtis Wayne Park was born in Champagne, Illinois on December 27, 1986 to Wayne and Nancy Park. He has 2 sisters, Renae and Corinne. Curtis spent the majority of his childhood growing up in Kennewick, WA where he graduated from Southridge High School in 2005. He attended Brigham Young University for his undergraduate studies and received a B.S. degree in Food Science with a minor in Chemistry. During his undergraduate education he took two years off to serve as a missionary for the Church of Jesus Christ of Latter-day Saints in the Tuxtla Gutierrez Mexico mission. Also during his time at BYU he met his wife Chelsea and they were married in August 2009. Immediately after graduation in 2011 he came to NC State University to begin his Master's degree in Food Science under Dr. MaryAnne Drake. He finished the Master's degree in 2013 and decided to continue under Dr. Drake to pursue a PhD in Food Science. Curtis is very proud of the family members who have paved the way for him to receive his education. He is a third generation PhD as his grandfather William Park and father Wayne Park both received doctorate degrees in agricultural economics.

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**CHAPTER 1: LITERATURE REVIEW: THE DISTRIBUTION OF FAT
IN DRIED DAIRY PARTICLES DETERMINES FLAVOR RELEASE
AND FLAVOR STABILITY**

The distribution of fat in dried dairy particles determines flavor release and
flavor stability

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Practical Application

Dried dairy ingredients are utilized in various food and beverage applications for their nutritional, functional, and sensory properties. Lipid oxidation is the main mechanism for off-flavor development in dried dairy ingredients, and the distribution of fat may play a critical role in flavor and flavor stability. Some hypotheses for the role of surface free fat on the flavor of dried dairy ingredients are presented along with proposed mechanisms.

Abstract

Dried dairy ingredients are utilized in various food and beverage applications for their nutritional, functional, and sensory properties. Dried dairy ingredients include milk powders of varying fat content and heat treatment and buttermilk powder, along with both milk and whey proteins of varying protein contents. The flavor of these ingredients is the most important characteristic that determines consumer acceptance of the ingredient applications. Lipid oxidation is the main mechanism for off-flavor development in dried dairy ingredients. The effects of various unit operations on the flavor of dried dairy ingredients have been investigated. Recent research documented that increased surface free fat in spray dried WPC80 was associated with increased lipid oxidation and off-flavors. Surface free fat in spray dried products is fat on the surface of the powder that is not emulsified. The most common emulsifiers present in dried dairy ingredients are proteins and phospholipids. Currently only an association between surface free fat and lipid oxidation has been presented. The link between surface free fat in dried dairy ingredients and flavor and flavor stability has not been investigated. In this review, some hypotheses for the role of free fat on the flavor of dried dairy ingredients are presented along with proposed mechanisms.

Keywords: flavor, dairy powders, free fat, spray drying, lipid oxidation

Introduction

Bovine milk contains 88% water along with the macronutrients fat, protein, and carbohydrates. Due to the high water content and available macronutrients for microbial growth the shelf-life of milk is relatively short. Dehydration is used to extend the shelf-life of milk and milk-derived products. Milk and milk derived products are generally dehydrated in two ways, by spray drying or by roller drying. The resulting product is a powder with very low moisture content. Spray drying is the most common method used in the dairy industry due to the intense heat treatment involved in roller drying. These dried dairy ingredients extend the shelf-life and provide functional benefits and convenience.

Dried dairy ingredients are generally classified by their physical and compositional properties and can be divided into two general groups, milk powders and protein powders. All milk powders are defined as having less than 5% moisture (USDEC 2005). Non-fat dry milk (NFDM) and skim milk powder (SMP) are very similar in that both are produced from pasteurized skim milk and have less than 1.5% fat by weight (USDEC 2005). SMP must have at least 34% protein by weight, which can be regulated by the addition of milk permeate, whereas NFDM does not have a legal definition in regards to protein (USDEC 2005). The cumulative thermal treatments for low-heat, medium-heat, and high-heat SMP and NFDM are 70°C for 2 min, 70-78°C for 20 min, and 88°C for 30 min respectively (USDEC 2005). Whole milk powder (WMP) is produced from pasteurized whole milk and has between 26% and 40% fat by weight (USDEC 2005). Buttermilk powder is produced from buttermilk during butter manufacture and contains greater than 4.5% milkfat (USDEC

2005). In 2012, over 1 million metric tons of dry milk products were produced in the United States with the majority as NFDM (USDA 2013).

Concentrated protein ingredients are available in the dairy industry due to advances in membrane filtration as well as ion exchange chromatography. The membrane filtration processes commonly used are microfiltration (MF), ultrafiltration (UF), nanofiltration, and reverse osmosis. Milk protein can be concentrated by UF of skim milk to remove lactose and minerals and produce milk protein concentrates (MPC) with protein concentrations of 40-90% of the total solids. Using a combination of MF and UF, whey proteins can be concentrated to produce whey protein concentrate (WPC) with 25-89% protein or whey protein isolate (WPI) with greater than 90% protein of the total solids. Whey proteins that are removed prior to cheesemaking are called serum proteins and can be further concentrated to make serum protein concentrate (Nelson and Barbano 2005; Evans and others 2009, 2010). Production of WPC and WPI in the United States reached 230,000 metric tons in 2012 while production of dry milk protein concentrate reached 46,000 metric tons (USDA 2013). Other dried dairy protein ingredients include caseins (rennet or acid) and caseinates.

Dried dairy ingredients are used in numerous applications due to their nutritional and functional properties (Foegeding and others 2002; Davis and Foegeding 2007; Raikos 2010; Anema and others 2006; Kenny and others 2000) but the most important factor in consumer acceptance of dried dairy ingredient applications is flavor (Drake 2006; Childs and others 2007; Caudle and others 2005). In order to characterize the flavor of dried dairy ingredients, flavor lexicons have been developed (Drake and others 2003; Carunchia Whetstine and others 2005; Drake and others 2009). Off-flavors resulting from lipid oxidation in dried dairy ingredients result in decreased consumer acceptance of dried dairy ingredient

applications (Caudle and others 2005; Evans and others 2010; Lloyd and others 2009b). Raw milk quality has a substantial impact on the off-flavors in milk powders and is affected by animal feed, season, or microbiological quality (Coulon and Priolo 2002, Croissant and others 2007; Celestino and others). Stapelfeldt and others (1997) reported that both water activity and storage temperature were important in reducing off-flavors in whole milk powder and Lloyd and others (2009) confirmed that temperature and oxygen levels were crucial to minimize lipid oxidation. Lipid oxidation is also a primary contributor to loss of shelf life in SMP although shelf stability is substantially longer than WMP (Drake and others 2006). Sources of off-flavors in protein ingredients have been attributed to various unit operations such as starter culture, storage, bleaching, agglomeration, and instantization (Campbell and others 2011a; Campbell and others 2011b; Croissant and others 2009; Wright and others 2009; White and others 2013).

The physical properties of dairy powders impact various functional characteristics and may also impact the sensory properties. Because lipid oxidation is the source of many off-flavors in dried dairy ingredients, an understanding of the effect that distribution and physical characteristics of lipids in dried dairy ingredients have on flavor and flavor stability is of great importance to the dairy industry. The goal of this manuscript is to investigate the influence of the distribution of fat on the flavor of dried dairy ingredients. A link between fat distribution in dried dairy ingredients and flavor and flavor stability has yet to be investigated.

Flavor Deterioration in Dairy Products

It is generally recognized that the main reactions that deteriorate the flavor of dried dairy ingredients are lipid oxidation and Maillard browning (Farkye 2006; Karagul-Yuceer and others 2001). Generally, lipids alone do not contribute to the flavor of foods due to their low volatility but products formed during the decomposition of lipids can impact the flavor significantly (McClements and Decker 2008). Hydrolytic rancidity and autoxidation are the two main types of decomposition of lipids. Hydrolytic rancidity refers to the liberation of free fatty acids from the glycerol backbone whereas autoxidation involves a complex sequence of chemical changes due to the interaction of unsaturated lipids with oxygen (Frankel 1998a; McClements and Decker 2008). Hydrolytic rancidity in milk is mostly attributed to endogenous lipoprotein lipase enzymes (Deeth 2006). Lipid autoxidation decomposes fatty acids into volatile compounds that are generally aldehydes, ketones, carbonyls, alcohols, and acids (Frankel 1998b) and these are the primary source of off-flavors in dried dairy ingredients.

Lipid oxidation in milk powders has been studied extensively. Lloyd and others (2009a) demonstrated that common off-flavors in WMP produced in the United States were grassy and painty. These off-flavors increased with storage time and were correlated to an increase in various lipid oxidation products. Aldehydes and ketones are among the main volatile compounds responsible for off-flavors in WMP and SMP (Shiratsuchi and others 1994; Lloyd and others 2009a, 2009b; Carunchia Whetstine and others 2007; Karagul-Yuceer and others 2001; Karagul-Yuceer and others 2002). Lipid oxidation and flavor of both SMP and WMP can be influenced by many factors including light exposure, antioxidant addition, pre-heating treatment, storage temperature, nitrogen flushing, moisture content, and

relative humidity (Hardas and others 2002; McCluskey and others 1997; Hall and Lignert 1984; Stapelfeldt and others 1997; Lloyd and others 2009b). The concentration of unsaturated fatty acids plays a role in the oxidation stability of milk powders (Romeu-Nadal and others 2007). The physical distribution of fat in the powders also affects lipid oxidation in milk powders and will be discussed in subsequent sections of this manuscript.

Lipid oxidation is the primary contributor to a decrease in shelf-life and an increase in off-flavors in WPC34, WPC80, and WPI (Carunchia Whetstine and others 2005; Evans and others 2009, 2010; Wright and others 2009). In sweet whey powder a combination of lipid oxidation and Maillard reactions contribute to off-flavors (Mahajan and others 2004; Sithole and others 2005). Off-flavors in dried whey proteins are often associated with different processing steps. The use of starter culture increases lipid oxidation in liquid whey due to their lipolytic enzymatic activity which can then carry through into WPC or WPI (Campbell and others 2011a; Tomaino and others 2004). Mesophilic starter cultures impact the oxidative stability of WPC more than thermophilic starter cultures (Liaw and others 2011). Because the orange colorant annatto used in Cheddar cheese manufacture is also found in liquid Cheddar whey, it must be bleached to obtain a colorless powder. Bleaching of Cheddar whey increases off-flavors and lipid oxidation (Croissant and others 2009; Kang and others 2012; Listiyani and others 2011; Jervis and others 2012). Other unit operations that increase lipid oxidation include storage of liquid whey or retentate, agglomeration, and instantization (Whitson and others 2011; Campbell and others 2011b; Wright and others 2009; White and others 2013). Because lipid oxidation is responsible for off-flavors and loss of shelf life in both milk powders and dried protein powders, increased lipid oxidation due to

increased surface free fat in the powders could be detrimental to the flavor and flavor stability.

Flavor Binding

In order for flavor perception to occur, flavor compounds must be volatile in the food system as well as in the mouth. Interactions between flavor compounds and constituents in dried dairy ingredients can impact flavor release. Thus, flavor quality of dried dairy ingredients could be improved if volatile compounds responsible for off-flavors were less volatile, therefore increasing the sensory detection threshold of the volatile compounds. Milk proteins that contain non-polar amino acids are of interest in flavor binding due to the non-polar nature of flavor compounds. The measurement of flavor binding of proteins is generally done either by headspace analysis or by equilibrium dialysis (O'Neill 1996).

Roberts and Pollien (2000) investigated the influence of milk components on the volatility of different flavor compounds. The main factor in flavor retention in milk was the milk fat content with milk fat concentrations up to 1.5% in their experimental design. This was due to the lipophilic nature of many flavor compounds. The volatility of some compounds (diacetyl, 2,3-pentanedione, guaiacol) were not affected by the concentrations of the different milk components and some (3-methyl butanal, 2-methylpropanal, 4-ethylguaiacol) decreased with decreased milk fat content. Volatility of other compounds (β -damascenone and 1-octen-3-one) decreased with decreasing milk solids-not-fat suggesting that there was also binding with protein. In another study, the effects of lipid type and solid fat content on volatile compound release were investigated. Lipid type did not have a significant effect but an increase in solid fat content increased the volatile compound release

in milk based emulsions (Roberts and others 2003). These studies demonstrated that fat in milk products influences volatile compound release whether it is due to concentration or physical state. Given the large impact that fat has on volatile compound release it is probable that the distribution and emulsification of fat in dried dairy powders has a strong influence on the volatile compound release and overall flavor.

Hansen and Booker (1996) investigated the influence of casein and whey protein on the binding of flavor compounds used in ice cream mixes. Their work demonstrated that whey proteins reduced flavor compound intensities more than casein. It was hypothesized that the high thermal treatment denatured more of the whey proteins than caseins which exposed more non-polar regions to bind the flavor compounds (Hansen and Book 1996; O'Neill 1996). However, other studies have observed a decrease in flavor binding by β -lactoglobulin and WPI upon heating above denaturation temperatures (O'Neill and Kinsella 1988; McNeill and Schmidt 1993). This has been suggested to be due to structural changes and aggregation that occurred during the heat treatment (Kuhn and others 2006). Sodium caseinate also decreased vanillin concentrations in dairy protein beverages (McNeill and Schmidt 1993; Li and others 2000) but WPC decreased vanillin flavor intensity more than sodium caseinate (Hansen and Heinis 1991). Extensive research has been done on the flavor binding properties of various milk proteins and has been reviewed by Kuhn and others (2006). In WPI, the major protein responsible for binding flavor compounds was β -lactoglobulin (Kuhn and others 2007). Collectively, these studies provide strong evidence for the binding of flavors by milk derived proteins. The encapsulation of fat in dried dairy powders by proteins capable of binding flavors could increase the sensory quality by reducing the volatility of the compounds responsible for off-flavors.

Surface Free Fat

The term free fat is defined as fat that is no longer emulsified (Palanuwech and others 2003). In dried dairy ingredients, proteins and phospholipids are the most common emulsifiers. Free fat can be an indicator of damage to the milk fat globule membrane (Kim and others 2002). A more complete definition of free fat in dairy powders is fat that is not entirely coated by amphiphilic molecules or protected by a matrix of carbohydrates and proteins during drying (Vignolles and others 2007). When free fat is on the surface of the powder particles it is referred to as surface free fat. The surface free fat in milk powders can alter important properties of the dried milk powder such as: oxidative stability, wettability, dispersability, solubility, flowability, and ability to be used in chocolate processing applications (Vignolles and others 2007).

Free fat is most commonly extracted using an organic solvent such as hexane or petroleum ether (Vignolles and others 2007). The use of polar solvents are avoided because they can lead to the extraction of total fat (Buma 1971). During a free fat extraction, a fixed amount of organic solvent is added to a fixed amount of powder and swirled gently for a given amount of time. The solvent is then filtered and the fat is measured gravimetrically after evaporation of the solvent. Because of this, free fat can also be referred to extractable fat. Increasing extraction time and temperature increased the amount of free fat that was extracted (Buma 1971; Kim and others 2002).

Because it can take more time to extract the free fat from the interior of the particle there is the ability to extract different fractions of free fat, whether from the surface or the interior (Kim and others 2005b). Kim and others (2005b) were able to separate three different fractions of fat: surface free fat, inner free fat, and encapsulated fat. Only minor

changes in fatty acid composition were found in the fat extracted from different fat fractions with the high melting saturated fatty acids being slightly more represented in the surface free fat than the interior free fat or the encapsulated fat. In both the surface free fat and the inner free fat, the oleic, linoleic, and linolenic acid composition accounted for approximately 22% of the total fatty acid profile. These unsaturated fatty acids are highly susceptible to lipid oxidation (Frankel 1998a). These results demonstrate that the surface free fat is rich in unsaturated fatty acids and suggest that surface free fat could be susceptible to lipid oxidation. Truyen and Orsi (1977) observed greater concentrations of unsaturated fatty acids in surface free fat in milk powders and increased concentrations of polar lipids in emulsified fat, fat not solvent extractable. In the manuscript it was not specified whether the free fat was surface or inner free fat but it is assumed to be surface free fat due to the extraction being very similar to that of surface free fat done by Kim and others (2005b). The extraction time was 10 min whereas the extraction time for inner free fat by Kim and others (2005b) was 48 h.

High levels of unsaturated fatty acids such as oleic, linoleic, and linolenic acid make lipid oxidation a concern in milk and milk derived products because they are among the most common unsaturated fatty acids to undergo lipid oxidation (Frankel 1998a). Surface free fat may be more susceptible to oxidation than emulsified fat because emulsified fat is encapsulated with proteins and phospholipids which have antioxidant properties and will be discussed further.

Surface free fat in dairy powders can be highly influenced by processing and storage. In general, parameters that can be manipulated include inlet air temperature, outlet air temperature, feed solids concentration, and atomizing conditions. Elevated inlet air

temperatures increase the particle size because the crust on the particle surface is formed more quickly, leaving less time for the particle to shrink (Birchal and others 2005; Nijdam and Langrish 2006). Larger particles can encapsulate more fat, thus decreasing the surface free fat content (Buma 1971; Beristain and others 2001). Increased inlet air temperatures decreased surface free fat in whole milk powder whereas increasing outlet air temperatures increased free fat (De Vilder and others 1976; Kelly and others 2002). Increased inlet temperatures and increased feed solids concentration increased the surface free fat in spray dried WPC80 (Park and others 2013). Lactose crystallization increased general free fat by damaging the MFGM and proteins that encapsulate the fat droplets (Aguilar and Ziegler 1994).

Surface Composition of Dairy Powders

The way that dairy powders are produced can greatly influence the composition of the surface of the dried particles. During spray drying, the concentrate feed is sprayed into small droplets which are mixed with hot air to evaporate the water, leaving a dry particle with a low moisture content (<5.0%). The drying of particles in the spray dryer can be classified into two different periods. The first period is when the bulk of the water is evaporated. During this period the water can move freely to the surface of the droplet and thus keeps the surface saturated with water. The temperature of the droplet during this time is prevented by rising above the wet bulb temperature due to the cooling that occurs when water evaporates (Fellows 2009). The wet bulb temperature during drying is generally no greater than 60 °C (Schuck 2013). During the second period, enough moisture has been removed from the droplet that the surface is no longer saturated with water. At this point a crust made of solid

particles forms and the amount of water that is evaporated decreases. Because the water evaporation rate decreases, the temperature of the dried particles increases (Birchal and others 2006; Kim and others 2009b).

The solids composition of dried dairy ingredients is primarily made up of fat, protein, and carbohydrate (lactose). The distribution of these components on the surface of dried powder particles can affect different functional properties. Kim and others (2009a) demonstrated that the surface composition of SMP, WMP, and instantized WMP was determined solely by the spray drying process and not by subsequent fluidized bed drying. During spray drying, the fat, protein, and lactose reorient themselves where the fat and protein migrate to the surface due to their hydrophobicity and the lactose migrates to the center due to its hydrophilic nature. This makes the surface composition of the powder different from the composition of the entire powder including the interior, or bulk composition. In order to analyze the surface composition of dairy powders scanning electron microscopy and a technique called electron spectroscopy for chemical analysis (ESCA) (Kim and others 2009a). In ESCA, the milk powder is assumed to be made of protein, fat, and lactose. By analyzing the elemental composition of the surface, mainly carbon, oxygen, and nitrogen, the relative percentages of fat, protein, and lactose on the surface can be calculated (Kim and others 2009). A more in depth explanation of this technique has been described by Faldt and others (1993).

Kim and others (2002) investigated the bulk and surface compositions of commercially produced SMP, WMP, cream powder, and WPC. In SMP, the bulk composition of lactose, protein, and fat was 58%, 41%, and 1% respectively and the surface composition was 36%, 46%, and 18% respectively. In WPC, the bulk composition of

lactose, protein, and fat was 8%, 86%, and 6%, respectively, with the surface composition much different, 6%, 41%, and 53%, respectively. In WMP and cream powder, fat represented 98% and 99% of the surface composition, respectively. Gaiani and others (2007a) observed that a native caseinate powder with 0.4% lipid had a surface lipid content of 6%. It was hypothesized that the high spray drying outlet temperature (90 °C) was above the melting temperature of the powder lipids. As a consequence, the lipids were in the melted state and had increased mobility throughout the particle (Kim and others 2005; Nijdam and Langrish 2006). The fact that fat is overrepresented at the surface of dairy powders can have implications for different functional properties such as flowability, particle stickiness and solubility (Kim and others 2005; Nijdam and Langrish 2006). Free fat on the surface of dairy powders could be more susceptible to lipid oxidation due to greater access to oxygen. Thus, decreased surface free fat could reduce off-flavors and increase flavor stability.

The presence of fat on the surface decreased the wettability of spray dried emulsions stabilized by both whey and milk proteins due to the hydrophobic nature of the fat (Faldt and Bergenstahl 1996; Millqvist and others 2001). Increasing concentrations of lactose on the particle surface increased the wettability. When stored in a humid environment, the fat was redistributed to the surface at the expense of lactose (Faldt and Bergenstahl 1996, Faldt and Bergenstahl 1995). This observation was confirmed by Shrestha and others (2007) who observed in SMP that fat and protein were more likely to migrate to the powder particle surface than lactose. Kim and others (2005a) reported that surface fat inhibited the flowability of dairy powders. SMP with low surface fat was observed to flow better than powders with high surface fat coverage (WMP, cream powder, WPC). Higher surface fat

has been correlated to increased oxidation in dairy powders (Granelli and others 1996). In contrast, Lloyd and others (2009) did not observe any correlations with free fat and flavor stability in WMP produced in the United States. The range of surface free fat in the U.S. WMP was 1.1-7.7% and international WMP ranged from 2.8-6.7%. A possible reason for the lack of correlation was that the WMP were made at four different manufacturing facilities, confounding the effect that surface free fat alone would have on flavor stability in WMP.

Kim and others (2009a) reported that spray drying was the most important manufacturing process in determining the surface composition of spray dried milk powders. Fluidized bed drying had no significant effect on the surface composition of the milk powders. As the particle dries, the Peclet number and the initial saturation of the concentrate to be dried influence the particle formation (Vehring and others 2007). The Peclet number is defined as the ratio between the diffusion coefficient of the solute and the evaporation rate. As the particle dries, the shape, size, and surface composition are determined by the ability of the components to reposition themselves due to the droplet viscosity or the presence of precipitates (Vehring and others 2007). This was observed by Nijdam and Langrish (2006) with the drying of milk powder. Increasing fat content in milk powders increased the surface fat coverage with the most dramatic increase seen in powders ranging from 0 to 5% fat. Spray drying at increased inlet temperatures favored the accumulation of lactose on the surface rather than protein. The theory proposed was that higher temperatures lead to accelerated formation of the surface crust, leaving less time for larger molecules such as proteins to reach the surface. The increased viscosity of the droplets would also reduce the amount of fat able to migrate to the surface. An increase in concentrate viscosity during

spray drying was observed to decrease solubility of SMP (Baldwin and others 1980). Kim and others (2002) observed that of the milk components, fat migrated to the surface more than lactose or protein. Their results also showed a dramatic increase in surface free fat in dairy powders with less than 6% fat with a WPC of only 6% fat bulk composition had 53% of the surface covered in free fat. These results along with those of Nijdam and Langrish (2006) suggest that the bulk fat content of the powder has a significant effect on surface free fat when the bulk fat content is low. As the percentage of bulk fat in the powder increases its effect on surface free fat diminishes significantly.

Particle size distribution of dairy powders is also of importance. Dairy powders are often agglomerated to increase the average particle size and porosity. Larger sized particles are more soluble because they are more porous and thus allow for an increase in wetting ability. Nijdam and Langrish (2006) observed that regardless of the fat content, milk powders spray dried at elevated inlet temperatures resulted in an increased average particle size. Increased fat content in the milk powders decreased the particle size and increased surface free fat. Elevated inlet temperatures also increased the particle size of spray dried WPC80 but increased feed solids concentration increased the particle size to a greater extent (Park and others 2013). In the spray dried WPC80 a decrease in surface free fat was observed in WPC80 that had a larger particle size. Fitzpatrick and others (2004) documented the particle sizes of 26% fat milk powders with varying free fat contents. While not a major focus of the study it is of interest to note that for the powders with the lowest free fat content increased particle size reduced the free fat content. These results suggest that particle size also plays a large role in the surface free fat content in dried dairy ingredients. As the

particle size decreases the surface area per unit mass increases, leaving more particle surface to be covered by free fat and less to be encapsulated (Buma 1971).

Free Fat and Lipid Oxidation

The two main classes of compounds responsible for encapsulating fat in dried dairy ingredients are phospholipids and proteins. Due to their close proximity to fat during emulsification both are of interest in regards to their ability to promote or inhibit lipid oxidation. Also, due to their hydrophilic and hydrophobic properties they may be able to slow the migration of fat to the surface by interacting with both the fat and the hydrophilic lactose interior. Phospholipids are amphiphilic and contain two hydrophobic acyl chains and a hydrophilic portion (Rombaut and others 2006). Phospholipids contain two fatty acids esterified on the glycerol backbone at the sn-1 and sn-2 positions with a phosphoric acid on the sn-3 position through a phosphate ester bond (Rombaut and Dewettinck 2006).

Phospholipids account for about 1% of the total bovine milk lipids and about 60% of these come from the milk fat globule membrane (MFGM) (Gallier and others 2010). The phospholipid contents of various dairy products derived from milk are shown in Table 1. During processing steps (heating, agitation, homogenization, and aeration) the MFGM is ruptured and the phospholipids enter into the aqueous phase (Rombaut and Dewettinck 2006). This is why phospholipids can be found in products that are not fat rich. In many cases, the proportion of phospholipids to total fat is higher in products with little fat.

It is unclear whether phospholipids native to dried dairy products are pro- or anti-oxidants. It appears that their effect on lipid oxidation is product specific. Phospholipids can negatively affect the flavor of dairy products through oxidation of lipids due to their high

levels of unsaturated fatty acids (Sessa 1985; Sattar and deMan 1975). Phospholipids also act as pro-oxidants by lowering the surface tension of the lipid, allowing oxygen from the headspace to diffuse to the oil and thus increase lipid oxidation (Choe and Min 2006). In WPC phospholipids are concentrated along with the protein. In WPC75, phospholipids have been documented to be as much as 23% of the total lipid content (Vaghela and Kilara 1995). This renders proteins as an important part in the encapsulation of fat in dried dairy ingredients. The concentrated phospholipids associated with the MFGM increase the potential for lipid oxidation to occur in dried whey ingredients. Wright and others (2009) observed that WPI that was instantized with lecithin, a phospholipid, had decreased shelf life due to increases in lipid oxidation products and off-flavors.

Phospholipids have also been reported to have anti-oxidant activity which can impact the flavor of foods (Chen and Nawar 1991). The mechanism for the antioxidative effects of phospholipids is still not clear. The phospholipids with polar groups containing nitrogen are effective antioxidants under most conditions (Choe and Min 2006). Phospholipids also chelate metals which decrease lipid oxidation. If the concentration of the phospholipids is too high then the phospholipids act as pro-oxidants. Yoon and Min (1987) reported that phospholipids were only antioxidants when Fe^{2+} was present and chelated. Sources of Fe^{2+} in dried dairy ingredients include metalloproteins such as lactoferrin, serum transferrin, and ovotransferrin and are found in many dried dairy ingredients (Jervis and Drake 2012). It is possible that in dried dairy ingredients with increased fat emulsified by native phospholipids that decreased off-flavors would result due to anti-oxidant properties of the native phospholipids. Phospholipids also could reduce lipid oxidation by encapsulating the fat and

preventing it from migrating to the surface due to their hydrophilic properties and the hydrophilic nature of the lactose rich interior of the spray dried particles.

Milk proteins are the other main class of compounds involved in the encapsulation of fat in dried dairy ingredients. Antioxidant properties of native milk proteins that encapsulate and reduce surface free fat may also in turn reduce lipid oxidation and off-flavors in dried dairy products. The ability to unfold at the oil/water interface and expose the hydrophilic and hydrophobic amino acids allows proteins to encapsulate fat globules. Casein has been demonstrated to have greater antioxidant effects than whey proteins (Allen and Wrieden 1982; Hu and others 2003). It was hypothesized that differences in antioxidant properties among the proteins was due to interfacial thickness, chelating properties, and free radical scavenging amino acids. The antioxidant activity can come from the electrical charge proteins impart on the fat droplets, repelling pro-oxidant metals away from fat droplets (Donnelly and others 1998). Others have stated that the effectiveness of casein to prevent lipid oxidation is due to its ability to bind copper and other pro-oxidant metals and to unfold and surround the fat globule membrane (Frankel 1998b). β -lactoglobulin was observed to be a mild antioxidant and a loss in antioxidant activity was attributed to structural changes during heating (Liu and others 2007). The antioxidant or pro-oxidant properties of proteins are highly dependent on the food system and unexpected activity can arise from different interactions between food components.

Milk proteins may also decrease lipid oxidation in dried dairy ingredients by encapsulating milk fat and preventing it to reach the surface of the powders. Kim and others (2002) observed that fat that was encapsulated by protein was preferentially located beneath the layer of surface free fat. They also demonstrated that oxygen uptake for powders with

higher levels of surface free fat was greater than in powders with lower levels of surface free fat. Because oxygen is key to lipid oxidation and an increase in oxygen content correlates to greater lipid oxidation this demonstrated that powders with higher surface free fat were more susceptible to lipid oxidation and potentially off-flavor development. De Vilder and others (1977) observed a positive correlation between surface free fat and particle porosity.

Nitrogen was able to penetrate the milk powder particles with increased surface free fat. This suggests that decreased surface free fat could also decrease lipid oxidation by limiting oxygen exposure to the interior of the powder particles. Hardas and others (2000) observed increased oxidation in surface free fat compared to encapsulated under the surface in emulsions made with milk fat. In their study surface free fat had a greater increase in peroxide value and hexanal over time and a greater decrease in linoleic and linolenic acid contents compared to the encapsulated fat. These studies suggest that efforts should be made to reduce the amount of free fat on the surface of dried dairy ingredients to improve flavor and flavor stability.

Implications for Flavor and Flavor Stability

The protein binding properties, surface composition, and surface free fat are all important characteristics that influence the flavor of dried dairy ingredients. Because spray drying and storage of dried dairy ingredients influence these properties, an emphasis should be placed on parameters during these unit operations to improve the flavor and flavor stability. A reduction in surface free fat could reduce off-flavors in dried dairy ingredients due to the properties of the emulsifying native proteins and phospholipids. Emulsifying proteins in dried dairy ingredients can bind more off-flavors, decrease oxygen permeability,

and decrease lipid oxidation and native phospholipids potentially have anti-oxidant effects. Park and others (2013) observed this effect directly in WPC80 where decreased surface free fat corresponded to decreased off-flavor intensity and associated lipid oxidation compounds. Lower levels of surface free fat were observed in WPC80 spray dried at increased feed solids concentration. Higher feed solids concentration during spray drying in WPI was demonstrated to increase whey protein denaturation (Anandharamakrishnan and others 2007). The denatured whey proteins would have a greater ability to encapsulate the fat due to their exposed hydrophobic regions. This is of importance because pre-heat treatment is an important unit operation in the manufacture of dried dairy ingredients. While it is possible that pre-heat treatment could in theory reduce surface free fat due to greater milk fat encapsulation, its effect on flavor would be more difficult to predict since many thermally induced flavor compounds would result and confound any benefits of reduced surface free fat. Keogh and O’Kennedy (1999) observed higher levels of fat oxidation on the surface of spray dried whey protein/milk fat emulsions. A summary of research related to the influence of process parameters on characteristics of dairy powders related to flavor and surface free fat is shown in Table 2.

Future Work

Future experiments should be conducted to investigate the effect of decreased free fat content on the flavor and oxidative stability of various dried dairy ingredients, both milk powders and protein ingredients of differing fat contents. While free fat has been studied extensively in WMP, a link between free fat and flavor has only been investigated recently in WPC80. In particular, spray drying parameters and processing steps prior to spray drying

should be optimized for the sensory properties of the dried powders. These parameters should include homogenization pressures, concentrate solids concentration, inlet and outlet air temperatures, and atomization conditions. The effect of surface free fat in various dried dairy ingredients on flavor stability over time would be very useful to the dairy industry as a whole.

Conclusion

Dried dairy ingredients continue to be a focus for the dairy industry due to their reduction in shipping costs, versatility, and extended shelf-life. Because flavor is the limiting factor in consumer liking of dried dairy ingredient applications, improved flavor and flavor stability by altering powder characteristics is of great importance. Advances in spray drying technology and research have resulted in discoveries regarding the influence that spray drying parameters have on protein binding, particle surface composition, and free fat. Understanding how these powder characteristics relate to the flavor and flavor stability of dried dairy ingredients will help the dairy industry produce products with improved flavor and increase their implementation in the food industry.

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Table 1 Polar lipid content of various dairy products.

^aAdapted from Boyd and others (1999)

^bAdapted from Rombaut and Dewettink (2006) whole milk was calculated from 100 g of product using 4.0% total fat content.

^cAdapted from Vaghela and Kilara (1995)

^dAdapted from Gaiani and others (2007a)

^eAdapted from Morr and Foegeding (1990)

Dairy Product	g polar lipid/100g total lipid
Whey powder ^a	21.1-41.7
Cream ^b	0.35-0.86
Butter ^b	0.20-0.27
Whole milk ^b	0.36
Skim milk ^b	19.06
Buttermilk ^b	21.7-33.1
Cheddar cheese ^b	0.47
Cheddar whey ^b	5.32
Cottage cheese ^b	5.30
WPC34 ^c	17.53
WPC75 ^c	23.6
Native phosphocaseinate ^d	67.7
WPC ^e	10.8-45

Table 2 The influence of various process parameters investigated in relation to the flavor and surface free fat of dairy powders.

Powder	Process Parameters Investigated	Effect	Author
WMP	Spray dryer nozzle size, outlet air temperature, feed solids concentration	Increased nozzle size and outlet air temperature increased surface free fat and increased feed solids concentration decreased surface free fat	Kelly and others (2002)
WMP	One stage and two stage drying and homogenization	The use of a two stage drying process involving spray drying and fluidized bed drying decreased surface free fat. Homogenization also decreased surface free fat	de Vilder (1980)
WMP	Lactose crystallization by high shear and elevated temperature in a mixer	Increasing lactose crystallization with high shear and elevated temperature increased the amount of free fat in WMP to almost 80% compared to low shear and decreased temperature	Koc and others (2003)
WMP	Preheat treatment prior to spray drying	WMP that was classified as low-heat WMP was consistently higher in lipid oxidation throughout extended storage than medium or high-heat WMP	Stapelfeldt and others (1997)
WPC80	Spray dryer inlet temperature, feed solids concentration	Increased inlet temperature and feed solids concentration decreased off-flavors along with surface free fat while increasing particle size	Park and others (2013)
WPC34 and SPC34	Freeze drying and spray drying	The heat used during the spray drying process had little effect on the flavor of WPC34 and SPC34 because no consistent differences in flavor were observed between freeze drying and spray drying	Evans and others (2009)
WPC/WPI Emulsion	Homogenization conditions and composition of the feed	Increasing the lactose : WPC concentration reduced free fat but not surface fat. The higher levels of fat on the surface of powder particles increased the level of oxidation during storage	Keogh and O'Kennedy (1999)
WPI	Spray dryer outlet air temperature, feed solids concentration	Increased outlet air temperature and feed solids concentration increased the amount of whey protein denaturation	Anandharamakrishnan and others (2007)

CHAPTER 2: SHORT COMMUNICATION: THE EFFECT OF LIQUID STORAGE ON THE FLAVOR OF WHEY PROTEIN CONCENTRATE

**Short Communication: The Effect of Liquid Storage on the Flavor of Whey
Protein Concentrate**

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* Use of names, names of ingredients, and identification of specific models of equipment is for scientific clarity and does not constitute any endorsement of product by authors, North Carolina State University, or the Southeast Dairy Foods Research Center.

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Abstract

Unit operations in dried dairy ingredient manufacture significantly influence sensory properties and, consequently, their use and consumer acceptance in a variety of ingredient applications. In whey protein concentrate (WPC) manufacture, liquid can be stored as whey or WPC prior to spray drying. The objective of this study was to determine the effect of storage, composition, and bleaching on the flavor of spray dried WPC80. Liquid whey was manufactured and subjected to the following treatments: bleached or unbleached and liquid whey or liquid WPC storage. The experiment was replicated three times and included no storage controls. All liquid storage was performed at 4°C for 24 h. Flavor of the final spray dried WPC80 was evaluated by a trained panel and volatile compound analyses. Storage of liquids increased cardboard flavor and decreased sweet aromatic flavor and resulted in increased volatile lipid oxidation products ($p < 0.05$). Bleaching altered the effect of liquid storage. Storage of unbleached liquid whey decreased sweet aromatic and increased cardboard flavor and volatile lipid oxidation products compared to liquid WPC80 and no storage ($p < 0.05$). In contrast, storage of liquid bleached WPC decreased sweet aromatic flavor and increased cardboard flavor and associated volatile lipid oxidation products compared to bleached liquid whey or no storage ($p < 0.05$). These results confirm that liquid storage increases off flavors in spray dried protein but to variable degrees depending on whether bleaching has been applied. If liquid storage is necessary, to mitigate off-flavors, bleached WPC80 should be stored as liquid whey and unbleached WPC80 should be stored as liquid WPC.

Key words: Whey protein, flavor, bleaching, storage

Short Communication

Due to increased consumer demand for foods with increased protein content, whey proteins have become much more than a by-product of cheese manufacture. In 2014 over 244,000 metric tons of whey protein concentrate (34-89% protein; WPC) were produced in the United States which constituted an 8.1% increase from 2013 (USDA, 2015). Whey proteins are commonly used due to their unique nutritional and functional properties. The successful use of whey protein ingredients requires that they do not negatively affect the flavor of their ingredient applications. Due to delicate flavors in whey protein ingredients, off-flavors can become prominent and negatively affect consumer acceptance of food applications (Childs et al., 2007; Wright et al., 2009; Evans et al., 2010; Oltman et al., 2015).

Many of the off-flavors in WPC arise from the various unit operations applied to fluid whey to concentrate proteins. In the United States, annatto (norbixin) is an approved color additive in Cheddar cheese with usage consistent with good manufacturing practice (US FDA, 2015). The majority of the colorant is retained in the cheese but approximately 10% of annatto remains in the whey (Smith et al., 2014). This colorant is then concentrated along with the protein during WPC or WPI manufacture and therefore must be removed by bleaching. Unit operations such as cheese manufacture, bleaching, spray drying, agglomeration, and instantization have all been demonstrated to influence the flavor of WPC or whey protein isolate (WPI; Campbell et al., 2011; Smith et al., 2015; Park et al., 2014a; Park et al., 2014b; Wright et al., 2009; White et al., 2013).

During the manufacture of WPC or WPI, liquid product may be stored for extended periods of time. This can be due to shipping product from one facility to another or due to spray drying capacities not meeting production capacities. Whitson et al. (2011) investigated

how storage of liquid whey protein isolate prior to spray drying impacted the flavor of WPI and WPC80. They observed that lipid oxidation products and off flavors increased in both spray dried Mozzarella WPC80 and Cheddar WPI with increased storage of liquid protein product (liquid WPC80 or WPI). Liaw et al. (2010) demonstrated that lipid oxidation increased with storage of fluid whey but did not investigate how or if fluid whey storage impacted the flavor of the resulting WPC. To our knowledge, the role of bleaching and fluid composition during storage on the flavor of WPC80 has not been investigated. The objective of this study was to determine the effect of bleaching and fluid composition across liquid storage on the flavor of spray dried WPC80.

Approximately 235 kg of raw whole milk was obtained from the North Carolina State University Dairy Education Unit (Raleigh, NC). The milk was pasteurized at 72°C for 16 s with a plate heat exchanger (model T4 RGS-16/2, SPX Flow Technology, Greensboro, NC). Liquid Cheddar whey was manufactured as described by Park et al. (2014b). The whey was defatted with a centrifugal separator (Model SI600E, Agri-Lac, Miami, FL) and pasteurized at 72°C for 15 s. The following treatments were then applied to the manufacture of spray dried WPC80: hydrogen peroxide bleach or no bleach, 24 h fluid whey storage or no storage. Half of the pasteurized whey was bleached with 250 ppm hydrogen peroxide (35% w/v, VWR International, Westchester, PA) for 1 h at 50°C after which 20 ppm catalase was added (FoodPro CAT, Danisco, New Century, NJ). The other half of the whey was held at 50°C for 1 h prior to ultrafiltration (UF). Then, bleached or unbleached whey was concentrated and spray dried or concentrated and held for 24 h prior to spray drying. A total of six treatments (WPC80) were manufactured. A summary of the experimental design is shown in Figure 1.

Wheys (47 kg each) were concentrated to WPC80 by UF/diafiltration (DF) with a UF system containing 10 polyethersulfone membrane cartridges (model P2B010V05, nominal cutoff = 10 kDa, surface area = 0.5 m², Millipore Inc., Billerica, MA) and a variable-speed peristaltic pump (model P2B010V05, Cole Parmer, Vernon Hills, IL) which was used to circulate the product in a batch process. Prior to UF, the membrane cartridges were cleaned as described by Park et al. (2014a). A concentration factor of 3x was achieved and then DF water was added to equal the original weight followed by concentration to a solids content of 15% (w/w) (12% protein, w/w) to produce liquid WPC80. Total solids were measured by a rapid moisture analyzer (SMART Trac II, CEM Corp., Matthews, NC) and protein was measured with a Sprint Rapid Protein Analyzer (CEM Corp.). Total time for each batch of UF/DF was approximately 1 h.

The liquid WPC80 produced from the no storage controls and liquid whey storage treatments were spray dried immediately whereas the liquid WPC80 storage treatments were spray dried after 24 h at 4°C (Figure 1). Spray drying was performed with a pilot scale spray drier (model Lab 1, Anhydro Inc., Soeberg, Denmark) with an inlet temperature of 200°C and an outlet temperature of 90°C. WPC80 powders were stored in Mylar bags (TF-4000, Impak Corp., Central City, SD) at -80°C until analyses were performed. The entire experiment was replicated 3 times with 3 different lots of milk.

Proximate analysis was performed on the WPC80 powders. Percent moisture was measured by vacuum oven drying (AOAC International, 2000; method 990.20; 33.2.44), fat content was measured by Mojonnier ether extraction (AOAC International, 2000; method 932.06; 33.5.08), and protein was measured using the Kjeldahl method (AOAC International, 2000; method 991.20; 33.2.11) with a nitrogen conversion factor of 6.38.

Descriptive sensory analysis was performed in compliance with the North Carolina State University Institutional Review Board for Human Subjects guidelines. The WPC80 powders were rehydrated to 10% solids (w/v) in deionized water. The solutions were dispensed into 3-digit coded 60 ml soufflé cups (Solo Cup, Highland Park, IL), lidded, and tempered to 21°C. Flavor attributes were evaluated by a trained panel (n=8) using an established dried dairy ingredient lexicon and scored using a 0 to 15 Spectrum™ intensity scale (Wright et al., 2009). Panelists were between the ages of 22 and 47 y with >150 h of experience of descriptive analysis of dried dairy ingredients. Each sample was evaluated in duplicate by each panelist and data was collected with Compusense Five version 5.6 (Compusense, Guelph, ON, Canada).

Extraction of volatile compounds was performed with headspace-solid phase microextraction-gas chromatography-mass spectrometry (HS-SPME-GC-MS) as described by Park et al. (2014a). WPC80 powders were rehydrated to 10% solids (w/v) in HPLC grade water along with 10% (w/v) sodium chloride (VWR International) and 10µl of internal standard solution (2-methyl-3-heptanone in water, 81 mg/kg; Sigma-Aldrich, St. Louis, MO). Five ml of WPC80 solution were added to 20 ml amber autosampler vials and sealed with a Teflon-sided silicon septum with screw cap (MicroLiter Analytical Supplies Inc., Suwanee, FL). A Combi PAL autosampler (CTC Analytics AG, Zwingen, Switzerland) was used to inject samples in an Agilent 7820A gas chromatograph with 5975 inert mass selective detector (Agilent Technologies Inc., Santa Clara, CA) and a ZB-5 MS column (30 m x 0.25 mm i.d. x 0.25 µm; Phenomenex, Torrance, CA). The SPME fiber (1-cm divinylbenzene/carboxen/polydimethylsiloxane; Supelco, Bellefonte, PA) was exposed to each sample as described by Park et al. (2014a). GC oven conditions were also as described

by Park et al. (2014a). Compounds were identified using the NIST 2014 library of spectra and comparison of spectra and retention time under identical conditions (NIST, 2014). Compounds of interest were quantified in selective ion monitoring mode by relative abundance using the internal standard.

Statistical analysis was performed with XL STAT 2015.1 (Addinsoft, New York, NY). Sensory and volatile data were analyzed by 2-way ANOVA (bleach * storage composition) with Fisher's least significant difference (LSD) test to determine differences among sample means.

The percent moisture, fat, and protein in the WPC80 powders were $3.83 \pm 0.76\%$, $4.12 \pm 0.54\%$, and $81.5 \pm 0.89\%$ respectively, and no significant differences between treatments were observed ($p > 0.05$). The trained panel profiles are shown in Table 1. Interaction effects between bleaching and storage composition were significant ($p < 0.05$). Consistent with previous research (Croissant et al., 2009; Kang et al., 2012; Qiu et al., 2015) bleaching increased overall aroma intensity, cardboard, and cabbage flavors and decreased sweet aromatic flavor ($p < 0.05$). The effect of liquid composition during storage on the flavor of WPC80 was different between bleached and unbleached treatments. For unbleached treatments, storage of liquid whey increased cardboard and decreased sweet aromatic flavors compared to the no storage control ($p < 0.05$) whereas liquid WPC80 storage was not different in flavor from the no storage control ($p > 0.05$). In contrast to the unbleached treatments, storage of liquid whey was not different in cardboard flavor intensity than the no storage control ($p > 0.05$). Storage of bleached liquid WPC80 increased cardboard flavor compared to the no storage control and the liquid whey storage treatments ($p < 0.05$).

Volatile compound results were consistent with sensory analysis (Table 2, Figure 2). Interaction effects were significant between bleaching and storage composition for all volatile compounds ($p < 0.05$). In agreement with the descriptive sensory analysis results, in general, volatile lipid oxidation products were increased in the bleached WPC80 treatments. In unbleached WPC80, storage of liquid whey increased lipid oxidation observed by increases in pentanal, hexanal, heptanal, octanal, nonanal, decanal, 3-methyl butanal, 1-octen-3-one, and 2-pentyl furan concentrations compared to the no storage control and liquid WPC80 storage treatments ($p < 0.05$). In bleached WPC80, storage of liquid product increased hexanal, nonanal, 2-methyl butanal, DMTS, 1-octen-3-one, and 2-pentyl furan concentrations compared to the no storage control ($p < 0.05$). Storage of bleached liquid whey increased heptanal, DMDS concentrations compared to the no storage control and increased octanal concentration compared to both no storage and liquid WPC80 storage ($p < 0.05$). Storage of liquid WPC80 increased pentanal, decanal, and DMTS concentrations compared to no storage and liquid whey storage ($p < 0.05$).

It is important to understand how unit operations affect cardboard flavor and lipid oxidation because they are the main contributors to decreased quality and shelf-life (Wright et al., 2009; Whitson et al. 2010, 2011). In bleached WPC80, we observed increases of pentanal and DMTS which have been demonstrated to contribute to cardboard flavor in whey protein ingredients (Whitson et al., 2010). Similar to our results, Whitson et al. (2011) observed an increase in cardboard flavor and lipid oxidation in Mozzarella WPC80 with increased liquid WPC80 storage but to a greater extent in liquid Cheddar WPI. This could be due to the chemical bleaching step that was applied to Cheddar whey to remove residual annatto colorant. Common bleaching agents, hydrogen peroxide and benzoyl peroxide,

increase lipid oxidation in whey protein ingredients (Croissant et al., 2009; Smith et al., 2015). In our experiment, we observed similar results where storage of bleached liquid WPC80 increased cardboard flavor and lipid oxidation in the spray dried WPC80 but in WPC80 from unbleached liquid WPC80, storage was not significantly higher than the no storage control. Bleached liquid WPC80 was higher in cardboard flavor than its no storage control whereas the unbleached liquid WPC80 was not different from its respective control. Another source of difference in flavor stability between liquid Mozzarella and Cheddar whey protein products is due to the starter culture. Campbell et al. (2011) observed that mesophilic starter cultures used in Cheddar cheese manufacture increased lipid oxidation in liquid whey compared to thermophilic starter culture used in Mozzarella cheese manufacture.

Whether or not the liquid whey was bleached had a large impact on the effect of storage composition. In unbleached WPC80, liquid whey storage increased lipid oxidation and cardboard flavor more than liquid WPC80 storage. This could be due to the pro-oxidant mineral compositional differences between liquid whey and liquid WPC80. The endogenous pro-oxidant transition metals copper and iron are documented throughout the literature to significantly increase lipid oxidation in milk fat (O'Connor and O'Brien, 2006). The copper and iron content in liquid sweet whey have been reported as 3.5 and 89 $\mu\text{g}/100\text{ g}$ respectively (Wong et al., 1978). Wong et al. (1978) reported that concentrations of copper and iron in fluid sweet whey increased with increased volume reduction with ultrafiltration membranes. There may be two reasons they did not see a decrease in iron and copper concentrations. First, they did not use diafiltration to produce WPC80 which would have increased the removal of soluble minerals. Second, with increasing protein concentration the trace minerals that are bound to protein would also be concentrated. Thus, the pro-oxidant trace

minerals would mainly be bound to protein and therefore less able to catalyze lipid oxidation (Sugiarto et al., 2010; Ueno et al., 2012). In the current study, diafiltration was applied and therefore unbound minerals would be removed in the permeate. As such, liquid WPC80 would have decreased concentrations of unbound trace minerals compared to fluid whey. Unbleached liquid whey storage could also have increased cardboard flavor and lipid oxidation more than liquid WPC80 storage because the higher protein content in liquid WPC80 is an effective anti-oxidant. Tong et al. (2000) reported that the antioxidant activity of whey proteins increased with concentration. This was attributed to their sulfhydryl groups, their ability to chelate iron and free radicals scavenging.

In bleached WPC80, lipid oxidation and cardboard flavor increased with liquid WPC80 storage compared to liquid whey storage and the no storage control. This could be due to the increased lipid concentration in the fluid during storage. The reason that this difference was not observed in unbleached product could be that the antioxidant activity of the system in the bleached product was not high enough to slow lipid oxidation due to free radicals formed during bleaching. Fox et al. (2013) observed increased lipid oxidation products when bleaching was performed on liquid WPC80 compared to liquid whey suggesting decreased oxidative stability of liquid WPC80. Bleaching by both hydrogen peroxide and benzoyl peroxide generates free radicals which greatly accelerate lipid oxidation (Jervis et al., 2012; Jervis and Drake 2013). The lipid concentration in liquid WPC80 is roughly 10 times higher than in fluid whey. The increased lipid concentration combined with the free radicals generated from bleaching could explain why spray dried bleached WPC80 that included liquid WPC80 storage had increased lipid oxidation and cardboard flavor whereas this was not observed in unbleached WPC80.

These results confirm previous studies that in order to mitigate off-flavors, WPC manufacturers should avoid liquid storage if possible. However, this study establishes that if liquid storage is necessary specific steps can be taken to mitigate off flavors. An understanding of fluid composition and pre-treatment during storage is critical in reducing lipid oxidation and off-flavor development in WPC. If liquid storage is necessary, manufacturers of bleached and unbleached product should store liquid whey and liquid WPC80 respectively.

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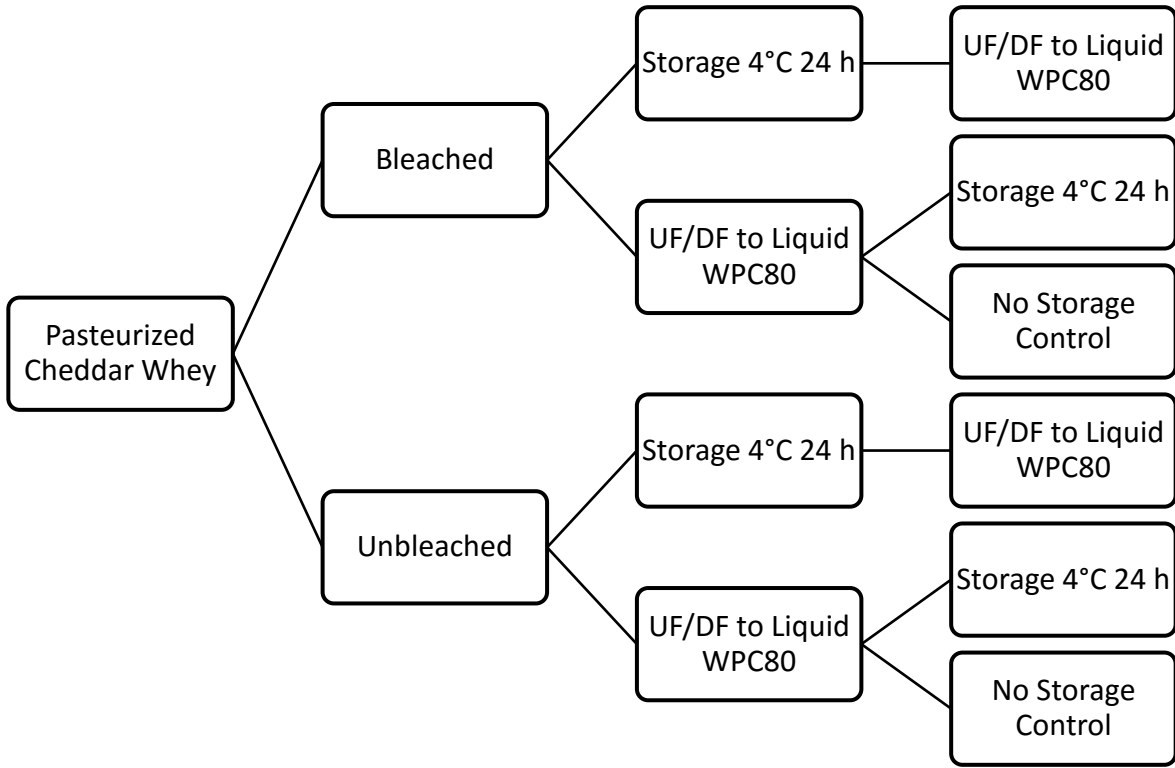


Figure 1 Diagram of experimental design. All samples were spray dried under identical conditions. This experiment was replicated three times.

Table 1 Trained panel profiles of rehydrated WPC80 powders. Means in the same column followed by a different letter indicate significant differences ($p < 0.05$). ¹ND—Not Detected.

Treatment	Aroma Intensity	Sweet Aromatic	Cardboard	Cabbage
Unbleached Control	2.5 ab	1.9 a	0.5 d	ND ¹
Unbleached Liquid Whey Storage	2.1 b	1.0 b	1.2 c	ND
Unbleached Liquid WPC80 Storage	2.3 ab	1.4 ab	0.9 cd	ND
Bleached Control	2.8a	ND	2.3b	1.1a
Bleached Liquid Whey Storage	2.8a	ND	2.2b	1.2a
Bleached Liquid WPC80 Storage	2.6a	ND	2.9a	1.1a

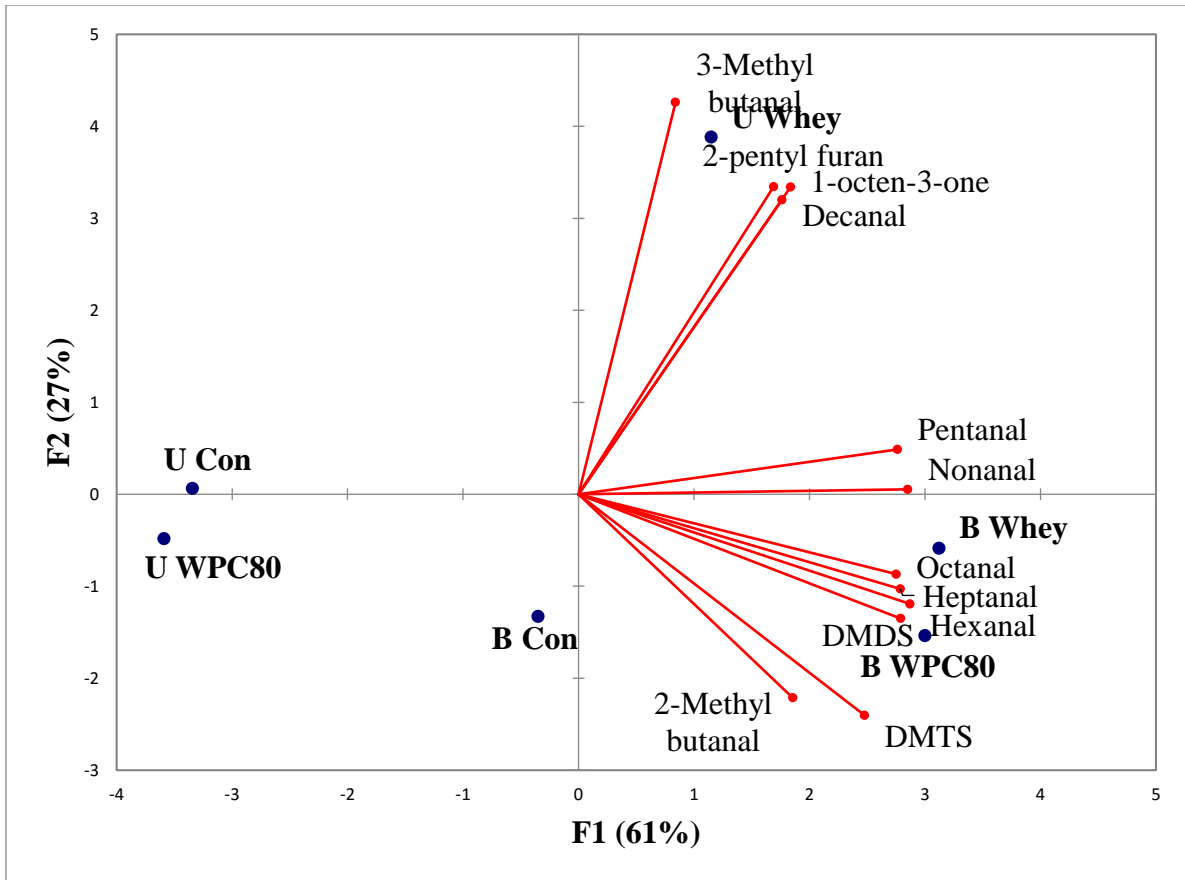


Figure 2 Principal component analysis (PCA) biplot of volatile compound relative abundance ($\mu\text{g}/\text{kg}$) in rehydrated WPC80 treatments. B—Bleached, U—Unbleached, Con—Control.

Table 2 Relative abundance ($\mu\text{g}/\text{kg}$) of selected volatile compounds in rehydrated WPC80.

Means in the same row followed by a different letter indicate a significant difference

($p < 0.05$). ¹ND—Not detected.

Volatile Compound	Bleached Control	Bleached Liquid Whey	Bleached Liquid WPC80	Unbleached Control	Unbleached Liquid Whey	Unbleached Liquid WPC80
Pentanal	1.17 c	1.55 bc	2.33 a	0.606 d	1.81 b	0.504 d
Hexanal	54.7 b	73.2 a	76.2 a	14.3 c	44.7 b	20.3 c
Heptanal	2.49 bc	3.22 a	2.69 ab	0.719 d	1.85 c	0.636 d
Octanal	0.513 bc	0.877 a	0.586 b	0.143 d	0.400 c	0.069 d
Nonanal	2.95 c	4.19 a	3.69 ab	2.25 d	3.26 bc	1.23 e
Decanal	0.041 cd	0.052 c	0.090 b	0.037 cd	0.144 a	0.015 d
2-Methyl butanal	0.002 c	0.018 b	0.033 a	0.002 c	0.002 c	0.010 bc
3-Methyl butanal	0.211 b	0.308 b	0.220 b	0.286 b	0.555 a	0.186 b
DMDS	0.172 bc	0.379 a	0.296 ab	ND ¹	0.123 c	0.029 c
DMTS	0.017 c	0.024 b	0.035 a	0.002 d	0.005 d	0.002 d
1-octen-3-one	0.077 cd	0.170 b	0.159 b	0.070 d	0.271 a	0.104 c
2-pentyl furan	1.73 d	8.16 b	3.42 c	1.91 d	10.5 a	2.85 c

**CHAPTER 3: CONDENSED MILK STORAGE AND EVAPORATION
AFFECT THE FLAVOR OF NONFAT DRY MILK**

**Condensed milk storage and evaporation affect the flavor of nonfat dry
milk**

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* Use of names, names of ingredients, and identification of specific models of equipment is for scientific clarity and does not constitute any endorsement of product by authors, North Carolina State University, or the Southeast Dairy Foods Research Center.

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ABSTRACT

Unit operations in nonfat dry milk (NFDM) manufacture influence sensory properties, and consequently, its use and acceptance in ingredient applications. Condensed skim milk may be stored at refrigeration temperatures for extended periods prior to spray drying due to shipping or lack of drying capacity. Currently, NFDM processors have two options for milk concentration up to 30% solids: evaporation (E) or reverse osmosis (RO). The objective of this study was to determine the effect of condensed milk storage and milk concentration method (E vs RO) on the flavor of NFDM and investigate mechanisms behind flavor differences. For experiment 1, skim milk was pasteurized and concentrated to 30% solids by E or RO and then either stored for 24 h at 4°C or concentrated to 50% solids by E and spray dried immediately. To investigate mechanisms behind the results from experiment 1, experiment 2 was constructed. In experiment 2, pasteurized skim milk was subjected to one of four treatments: control (no E), heated in the evaporator without vacuum, E concentration to 30% solids, E concentration to 40% solids. The milks were then diluted to the same solids content and evaluated. Volatile compounds were also measured during concentration in the vapor separator of the evaporator. Sensory properties were evaluated by descriptive sensory analysis and instrumental volatile compound analysis was conducted to evaluate volatile compounds. Interaction effects between storage and method of concentration were investigated. In experiment 1, E decreased sweet aromatic flavor and many characteristic milk flavor compounds and increased cardboard and cooked flavors in NFDM compared to RO. Liquid storage increased cardboard flavor and hexanal and octanal and decreased sweet

aromatic flavors and vanillin concentration. Results from experiment 2 indicated that the characteristic milk flavors and their associated volatile compounds were removed by the vapor separator in the evaporator due to the heat and vacuum applied during concentration. These results demonstrate that off-flavors are significantly reduced when RO is used in place of E and storage of condensed milk is avoided.

Key Words: NFDM, unit operations, flavor

INTRODUCTION

Dried milk powders are widely used in the food industry. In 2014, approximately 970,000 metric tons of non-fat dry milk (NFDM) and skim milk powder (SMP) were produced in the United States (USDA, 2015). Both SMP and NFDM must have <1.5% Fat, <5.0% moisture, and >34% protein (ADPI, 2015). SMP differs from NFDM in that milk permeate, milk retentate, or lactose can be added to adjust the protein content in SMP. As such, all NFDM can be called SMP but not all SMP are NFDM. SMP and NFDM are expected to taste like fluid skim milk. NFDM and SMP are used in the food industry to alter texture and flavor of ingredient applications. Flavor is a critical characteristic of milk powders for consumer acceptance of their ingredient applications (Caudle et al., 2005; Isleten and Karagul-Yuceer, 2006; Lloyd et al., 2009a; Lloyd et al. 2009b).

Skimmed milk powders are expected to have flavor similar to fluid skim milk but often off-flavors are present. These off-flavors can be due to lipid oxidation, Maillard browning, enzymatic-induced changes, or microbial growth. Many studies have documented off-flavors that develop due to unit operations during the manufacture and storage of whole milk powder (WMP) and NFDM (Karagul-Yuceer et al., 2001; Karagul-Yuceer et al., 2002;

Drake et al., 2003; Drake et al., 2007; Lloyd et al., 2009a). Milk-like flavors in NFDM include sweet aromatic and cooked, and off-flavors can include cardboard, potato/brothy, cereal, animal/wet dog (Drake et al., 2003). The sweet aromatic flavor has been attributed to lactones, vanillin, and free fatty acids (Karagul-Yuceer et al., 2001; Karagul-Yuceer et al., 2002; Shiratsuchi et al., 1995). Off-flavors in NFDM were caused by a variety of aldehydes, ketones, alcohols, and free fatty acids, primarily lipid oxidation compounds (Karagul-Yuceer et al., 2002; Drake et al., 2007).

Typical unit operations in NFDM manufacture include heat treatment, vacuum evaporation (E), and spray drying. E is performed with a falling film evaporator where water is boiled under vacuum to increase the solids content of the skim milk up to 50% total solids before spray drying (Drake et al., 2007; Oldfield et al., 2005). Stapelfeldt et al. (1997) demonstrated that heat treatment significantly affected oxidation rate during storage of WMP. Higher heat treatments decreased the amount of free radicals during storage due to exposed sulfhydryl groups on the whey proteins allowing them to scavenge the free radicals. In addition, heat treatment temperature during pasteurization significantly affected whey protein denaturation while evaporation and spray drying temperatures had little effect (Oldfield et al., 2005). Heat treatment also influenced the functional properties of NFDM in a set yogurt (Augustin et al., 1999). Spray drying temperatures did alter the surface composition of SMP and whole milk powder and their functional properties (Kim et al., 2009; Nijdam and Langrish, 2006). Evaporation and spray drying significantly alter the flavor of SMP (Drake et al., 2007).

The use of reverse osmosis (RO) membranes can also remove water from skim milk up to 30% total solids, replacing a portion of the E. Because the RO membranes do not

require heating like E they can provide a significant reduction in energy costs (Stabile, 1983). Condensed skim milk (<36% solids) is often stored for extended periods of time due to shipping from one facility to another or inadequate spray drying capacities of the manufacturing facility. Condensed skim milk is also widely used as an ingredient in various dairy products. Drake et al. (2007) observed that when evaporated skim milk (50% solids) was diluted to 9% solids nonfat that cooked and sweet aromatic flavor intensities were decreased compared to rehydrated spray dried NFDM. They did not investigate the cause of the loss of flavor and how evaporation affects the resulting milk powder flavor. In order to do so two different methods of concentration of skim milk need to be investigated. The objective of this study was to determine the effect of condensed milk storage and method of concentration (RO vs E) on the flavor of the resulting NFDM and investigate a possible mechanism for the observed effects.

MATERIALS AND METHODS

NFDM Production

Experiment 1

Raw bovine skim milk (390 kg) was obtained from the North Carolina State University Dairy Research and Education Unit. The skim milk was HTST pasteurized at 73°C for 16 s with a plate heat exchanger (model T4 RGS-16/2, SPX Flow Technology, Greensboro, NC). The milk was cooled to 4°C and stored in a bulk tank. Half of the skim milk was concentrated to 30% solids (wt/wt) by RO and the other half by E. Condensed milks were further split in two treatments, 4°C storage for 24 h or no storage control. Prior to spray drying, the condensed milks were further concentrated to 50% solids by E. A diagram of the experimental design is shown in Figure 1.

E was carried out using a single effect pilot scale falling film evaporator. For concentration of skim milk to 30% solids, the milk was pre-heated to 50°C prior to entering the calandria. The calandria temperature was 71°C with a vacuum of 74.5 kPa. Upon exiting the evaporator, the condensed milk was cooled through a tubular heat exchanger to 10°C. The condensed milk was then cooled to 4°C and either placed in storage for 24 h or collected for further concentration. When evaporating the condensed milk (30% solids) to 50% solids, the calandria temperature was 60°C and the vacuum was 81 kPa. The 50% solids condensed milk was 50°C upon exiting the evaporator and was immediately spray dried.

RO was performed using two spiral wound RO membrane elements (TRO 3838; Toray Membrane USA Inc., Poway, CA). The skim milk was recirculated in batch mode at 15°C with a pressure of 3.79 MPa and a flow rate of 55 L/min. Total solids were measured by a rapid moisture analyzer (SMART Trac II, CEM Corp., Matthews, NC). All spray drying was performed with a pilot scale spray dryer (model Lab 1, Anhydro Inc., Soeberg, Denmark) with an inlet temperature of 200°C and an outlet temperature of 90°C. The entire experiment was replicated 3 separate times.

Experiment 2

In order to further investigate the effect of evaporation on the retention of volatile flavor compounds, a second experiment was performed. Raw skim milk was obtained from the North Carolina State University Dairy Research and Education Unit. The skim milk was pasteurized as described previously. A portion of the pasteurized skim milk was saved as a control while the rest was subjected to one of the following treatments: A) heating (63°C) in the evaporator with no vacuum, B) condensed to 30% solids (w/w) with vacuum, and C) condensed to 40% solids (w/w) with vacuum. Calandria temperature was 71°C and vacuum

was 74.5 kPa for treatments B and C. The temperature for treatment A was 63°C because that was the temperature of the condensed milk exiting the calandria and the temperature at which the milk spends the majority of the time. All milks were subsequently cooled to 4°C and sampled. The condensed milks were diluted to the original percent solids as the pasteurized skim milk with deionized water. Descriptive and volatile compound analyses on the milks were performed as described below. In addition, 3 solid phase micro-extraction fibers (SPME; 2 cm divinylbenzene/Carboxen/polydimethylsiloxane fiber, Supelco, Bellefonte, PA) were exposed for 15 min through a silicone septa (Fisher Scientific, Waltham, MA) in the vapor separator of the evaporator during manufacture of treatments A, B, and C. The pH of the undiluted condensed milks and original milk was measured at 63°C using a pH meter (VWR International, Radnor, PA) calibrated with buffers at 63°C. This entire experiment was replicated 3 times.

Proximate Analysis

Moisture in NFDM was measured using vacuum oven drying (AOAC International, 2000; method 990.20; 33.2.44). Total fat in NFDM was determined by ether extraction (AOAC International, 2000; method 923.06; 33.5.08). Total protein was measured using the Kjeldahl method and multiplying total nitrogen by a conversion factor of 6.38 (AOAC International, 2000; method 991.20; 33.2.11). WPNI was measured as described by ADPI (2002).

Particle Size, Surface Free Fat

Particle size of the NFDM in experiment 1 was measured using a Mastersizer 3000 (Malvern, Malvern, UK) with an Aero S dry powder dispenser. Surface free fat was measured by a petroleum ether extraction (GEA Niro Method No. 10a; GEA, 2005).

Descriptive Sensory Analysis

All descriptive sensory analysis was done in compliance with the North Carolina State University Institutional Review Board for Human Subjects guidelines. NFDM was rehydrated to 10% solids (wt/vol) in deionized water with a handheld blender until fully dissolved. NFDM solutions were dispensed into 60 ml soufflé cups (Solo Cup, Highland Park, IL). They were then lidded and tempered to 21°C. Flavor attributes were evaluated using the 0 to 15 Spectrum™ intensity scale with a trained panel (n=8). Panelists were between the ages of 22 and 47 y with > 150 h of previous experience with descriptive analysis of dried dairy ingredients using an established lexicon (Drake et al., 2003). Data was collected using Compusense Cloud (Compusense, Guelph, ON, Canada).

Volatile Compound Analysis

Experiment 1

Volatile compounds in experiment 1 were extracted using sorptive stir bar extraction (SSBE) (Prieto et al., 2010). Each NFDM manufacture replicate was extracted in triplicate. Prior to analysis, the stir-bars and thermal desorption unit (TDU) tubes were conditioned for 1 h at 300°C. First, 0.5 g of NFDM was placed into a 10 ml amber screw top vial (Gerstel, Inc., Linthicum, MD) along with 5 ml of HPLC grade water. Next, 10 µl of internal standard was added (0.81 mg/kg 2-methyl-3-heptanone in water; Sigma Aldrich, St. Louis, MO). Sequential stir bar extraction (SBSE) was used due because the adsorption of compounds onto the PDMS layer is affected by the addition of salt. Polar compounds are extracted more effectively with salt while hydrophobic compounds are extracted more efficiently without salt (Prieto et al., 2010). SBSE allows for the extraction of both classes of compounds. Preliminary experiments determined that the extraction of volatile compounds of interest

increased with SBSE using 20% salt in the second extraction compared to one stir bar alone or two stir bars simultaneously (data not shown). One PDMS coated stir bar (10 mm x 0.5 mm thickness, Gerstel, Inc.) was placed in the vial, sealed, and stirred for one hour at 1000 rpm. After 1 h, the stir bar was briefly rinsed in HPLC grade water, dried, and placed in an autosampler tube (Gerstel, Inc.) and 1 g of NaCl was added along with another PDMS stir bar into the sample vial. This stir bar was stirred for 1 h at 1000 rpm, rinsed, dried, and placed in the same TDU tube previously mentioned. Stir bars were injected using an autosampler (MPS Autosampler, Gerstel, Inc.). The stir bars were desorbed at 250°C for 10 min (TDU, Gerstel, Inc.) and the volatile compounds were cryogenically trapped at -120°C (CIS 4, Gerstel, Inc.).

Volatile compounds were analyzed by gas chromatography mass spectrometry (GCMS). An Agilent 7890B GC (Agilent Technologies Inc., Santa Clara, CA) with an inert mass selective detector (model 5970A, Agilent) with a ZB-5MS column (30 m x 0.25 mm x 0.25 μ m) (Phenomenex, Torrance, CA) was used to identify and quantify volatile compounds of interest (Karagul-Yuceer et al., 2001; Karagul-Yuceer et al., 2002; Drake et al., 2007). Initial GC oven conditions were 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. Purge time was set to 1.2 min using helium as the carrier gas at a constant flow rate of 1 ml/min. Compounds were identified by comparison with the 2014 NIST mass spectral library (NIST, 2014), retention index, and retention time of authentic standards injected under identical conditions. Relative abundance of selected compounds was calculated using recovery of the internal standard.

Experiment 2

Volatile compounds of the milks diluted to the original percent solids (approximately 9% w/w) were analyzed as described for experiment 1. Volatile compounds that were extracted in the vapor separator of the evaporator were identified qualitatively by GC-olfactometry (GC-O) as well as by GC-MS. GC-O analysis was performed with a gas chromatograph with a flame ionization detector and olfactometry port (model 6850, Agilent Technologies Inc.) and the same non-polar column in experiment 1. Two highly trained sniffers (>50 h experience each) sniffed each sample once. Oven temperature program was as follows: 40°C held for 3 min, 10°C/min ramp to 150°C, and 30°C/min ramp to 200°C held for 10 min. GC-MS analysis was performed on an Agilent 7820A GC (Agilent Technologies Inc.) with an inert mass selective detector (model 5975 MSD, Agilent Technologies Inc.). Column and oven temperature program were the same as in experiment 1. All fibers were exposed into the column inlet at 250°C. Helium carrier gas was used on both GC-O and GC-MS at a rate of 1 ml/min with a purge time of 1.5 min.

Furosine Analysis

Furosine (FUR) was measured in NFDM as described by Resmini et al. (1990) with minor modifications. Between 110 and 140 mg of NFDM was accurately weighed into a screw-cap Pyrex tube with a PTFE-lined septa along with 8 ml of 10.6M HCl (Sigma Aldrich). Nitrogen gas was bubbled through the samples for 2 min and then capped and heated to 110°C for 23 h. After cooling to room temperature, the hydrolysate was filtered and 0.5 ml of filtrate was loaded onto a solid phase extraction column (500 mg Discovery DSC-18, Supelco) previously conditioned with 5 ml of methanol followed by 10 ml of water. FUR was eluted with 3 ml of 3M HCl. Detection was performed with a Waters HPLC system (Milford, MA). A furosine dedicated column was used (Altima C8, 250 x 4.6 mm,

5 μ m, Alltech-Grace, Columbia, MD) with isocratic conditions. The mobile phase consisted of 0.4% acetic acid (Sigma Aldrich) and the column temperature was 35°C. UV detection was performed at 280 nm. A 5 point external standard curve ranging from 50 to 400 ng was constructed for quantification.

Statistical Analysis

The data was analyzed by two-way analysis of variance with means separation (SAS version 9.3, SAS, Cary, NC). Interaction effects between storage time and method of concentration to 30% solids were investigated. Differences between sample means were analyzed using Fisher's least significant difference test.

RESULTS

Experiment 1

The fat content in the NFDM was $0.743 \pm 0.11\%$ (wt/wt) and was not different among treatments and replications ($p>0.05$). Moisture in the NFDM was $3.01 \pm 0.23\%$ and was not different among treatments and replications ($p>0.05$). Protein and WPNI were not different among treatments and replications and were $36.1 \pm 0.25\%$ (wt/wt) and 7.76 ± 0.27 mg/g respectively ($p>0.05$).

Descriptive analysis results indicated that the flavor profiles of the different NFDM treatments were distinct (Table 1). Interaction effects between method of concentration (RO vs. E) and storage time were not significant ($p>0.05$). The use of RO in place of E for concentration to 30% solids (wt/wt) increased sweet aromatic flavor intensity (2.8 vs 2.2, $p<0.05$) and decreased cardboard (ND vs. 1.1) and cooked (2.9 vs. 3.6, $p<0.05$) flavor intensities. Storage of condensed milk for 24 h increased cardboard flavor intensity (1.1 vs. ND) and decreased sweet aromatic flavor intensity (2.2 vs. 2.8, $p<0.05$).

The volatile compound profiles of the different spray dried NFDM treatments were also distinct (Figure 2, Table 2) and consistent with sensory results. In general, the RO treatments had increased volatile compound concentrations than the E treatments ($p < 0.05$). Use of RO to concentrate to 30% (wt/wt) increased concentrations of furfural, hexanal, vanillin, δ -nonalactone, δ -decalactone, 2,4-nonadienal, butyric acid, hexanoic acid, octanoic acid, decanoic acid, dodecanoic acid, tridecanoic acid, 1-octen-3-one, and benzaldehyde and decreased the concentration of maltol ($p < 0.05$). Many of these compounds are attributed to sweet aromatic flavor in milk powders (Karagul Yuceer et al., 2001; Drake et al., 2007) and explain the higher sweet aromatic intensity. Storage time also impacted the volatile compound profile of the NFDM ($p < 0.05$). Storage of 30% solids (wt/wt) condensed milk decreased the concentration of vanillin, and increased the concentrations of hexanal, octanal, and 2,4-nonadienal ($p < 0.05$). These aldehyde compounds are attributed to cardboard flavor in dried ingredients (Whitson et al., 2010; Drake et al., 2007).

Furosine (FUR) is a product of early Maillard reactions and is used as an indicator of heat treatment. FUR was only affected by the method of concentration to 30% solids (wt/wt) (Figure 3). RO decreased FUR concentration compared to E (30.9 vs. 41.6 mg FUR/100 g protein, $p < 0.05$). This is most likely due to the fact that the milk during RO was $< 15^{\circ}\text{C}$ compared to elevated temperatures during E ($> 63^{\circ}\text{C}$). During heating of milk, lysine residues of proteins react with lactose forming lactulosyl-lysine (Mehta and Deeth, 2015). Because other reducing sugars can react with lysine forming, for example fructosyl-lysine or glucosyl-lysine, all of the sugar-lysine products are converted to FUR by acid hydrolysis to then be quantified (Mehta and Deeth, 2015; Resmini et al., 1990). The decreased water

content in the condensed milks compared to the original skim milk could have also increased the Maillard reaction when heated during E.

The particle size distribution of the NFDM were distinct (Table 3). Surface free fat (SFF) was not different among treatments ($p>0.05$) and averaged 1.28 g SFF/100g total fat. Interaction effects between storage time and method of concentration were significant ($p<0.05$). Storage of liquid E condensed skim milk increased the average particle size (D[4,3]) and the particle size distribution of the NFDM compared to all other treatments ($p<0.05$). In contrast, storage of RO condensed skim milk decreased the D[4,3] and the size distribution of NFDM compared to all other treatments ($p<0.05$). E increased the D[4,3] (63.9 vs 48.1 μm) and size distribution compared to RO when condensed skim milk was stored for 24 h ($p<0.05$) but when no storage was applied, E decreased the D[4,3] (58.0 vs 51.0 μm) and size distribution compared to RO ($p<0.05$).

Experiment 2

The descriptive sensory means of the different treatments in experiment 2 are shown in Table 4. Heating the milk in the evaporator with no vacuum (treatment A) decreased overall aroma and sweet aromatic flavors and increased astringency compared to the unheated control ($P<0.05$). Condensing milk to 30 or 40% solids (treatments B and C respectively) removed much of the aroma and characteristic milk flavors. These flavor effects were observed with a decrease in overall aroma and sweet aromatic flavor and an increase in cardboard flavor compared to the control and treatment A ($p<0.05$). Evaporation to 30% or 40% increased astringency compared to the control, and evaporation to 40% also increased astringency compared to treatment A ($p<0.05$).

The volatile compound profiles of the evaporator treatments in experiment 2 were in agreement with the descriptive sensory results (Table 5, Figure 4). In general, concentration of the milk by evaporation decreased the flavor compound concentrations. This was observed with decreases in furfural, hexanal, maltol, furaneol, vanillin, δ -nonalactone, δ -decalactone, 2,4-nonadienal, 2,4-decadienal, butyric acid, hexanoic acid, octanoic acid, decanoic acid, and 2-acetyl-thiazole compared to the control ($p < 0.05$). Many of these compounds were also identified in the vapor separator of the evaporator (Table 6).

The skim milk, 30% condensed milk, and 40% condensed milk pH values at 63°C were 6.57 ± 0.02 , 6.25 ± 0.01 , and 6.17 ± 0.01 respectively. The pH was measured at 63°C because that was the temperature of the condensed milk in the vapor separator. The pH of the condensed milk in the vapor separator could play a large role in the solubility of the flavor compounds and therefore influence their partitioning out of the milk and into the headspace air going to the vacuum pump. In general, acidic volatile compounds decrease in solubility with decreasing pH and therefore would be more easily removed by vacuum evaporation.

DISCUSSION

The flavor of dried dairy ingredients can be grouped into one of two groups: dairy and non-dairy flavors (Carunchia Whetstine et al., 2005). During the storage of SMP/NFDM, sweet aromatic flavor decreases while non-dairy flavors such as cardboard and potato/brothy develop (Caudle et al., 2005; Karagul-Yuceer et al., 2002; Drake et al., 2007). To increase the shelf-life of NFDM it is then important to preserve fresh milk flavors such as sweet aromatic flavor. We observed that there was a decrease in dairy flavors in NFDM and

an increase in non-dairy flavors when solely E was used and when liquid condensed milk was stored for even just 24 h. These unit operations could have a detrimental impact on the shelf-life and consumer acceptance of ingredient applications that use NFDM (Caudle et al., 2005).

Karagul-Yuceer et al. (2001) and Karagul-Yuceer et al. (2004) identified and confirmed volatile compounds in NFDM with high odor activity and flavor impact. These compounds were identified as furaneol, butyric acid, methional, o-aminoacetophenone, δ -decalactone, vanillin, hexanoic acid, 2-acetyl-1-pyrroline, octanoic acid, γ -dodecalactone, 2,4-decadienal, and maltol. Drake et al. (2007) also reported many of these compounds in fresh SMP/NFDM and documented that they decreased with storage at 21°C while lipid oxidation compounds increased in relative abundance. In experiment 1 we observed decreases in furaneol, vanillin, δ -decalactone, butyric acid, hexanoic acid, and octanoic acid when E was used to concentrate to 30% solids instead of RO ($p < 0.05$). Experiment 2 further demonstrated that these compounds were being removed with the water vapor as they decreased in concentration with treatments B and C and were identified in the vapor separator. This supports the descriptive sensory results that observed a decrease in sweet aromatic, a characteristic dairy flavor. E increased cooked flavor in the NFDM compared to RO which can be explained by the increased heating during the unit operation of evaporation and was confirmed with increased maltol and furosine concentrations ($p < 0.05$). Maltol is a product of the Maillard reaction and was demonstrated to be in higher concentrations in milk powders that were exposed to more heat (Pischetsrieder et al., 1999; Karagul-Yuceer et al., 2001). In general, lipid oxidation compounds did not increase with E compared to RO. Cardboard flavor did increase in E NFDM and cardboard flavor has been linked to volatile

lipid oxidation products (Whitson et al., 2010). We hypothesize that the higher cardboard flavor in E NFDM was attributed to the lower sweet aromatic flavor. In the NFDM produced with only E, the sweet aromatic decreased in intensity and therefore cardboard flavor was perceived more readily by the trained panel. Cardboard flavor was not detected in RO NFDM even though lipid oxidation products were detected. This is most likely due to the increased sweet aromatic flavor intensity masking cardboard flavor perception. Park et al. (2014) demonstrated that spray drying parameters significantly affected the flavor of WPC80 with certain conditions increasing cardboard flavor intensity. Because experiment 2 demonstrated that a wide range of volatile compounds were removed during E, the effect of spray drying on the flavor of NFDM could be magnified. This is because variations in spray drying parameters influence flavor and this flavor variability could be more pronounced because the flavor intensity in the condensed milk is significantly decreased, thus small changes in cardboard flavor could be perceived when they are not perceived when sweet aromatic flavor intensity is higher.

The effect of liquid storage was similar to that of the method of concentration to 30% solids. Storage of liquid condensed milk decreased sweet aromatic flavor and increased cardboard flavor. Decreases in the same characteristic milk flavor compounds were also observed as stated with the effect of E versus RO. In contrast to the effect of the method of concentration, storage of condensed milk increased the concentrations of the lipid oxidation compounds hexanal and octanal, indicating that lipid oxidation did occur during 24 h storage. The combined effect of a decrease in the potential suppression of sweet aromatic flavor on cardboard flavor and the increase in lipid oxidation compounds is most likely the cause of the increase in cardboard flavor intensity due to liquid storage. Liquid storage also affects the

flavor of liquid whey and whey protein ingredients. Storage of liquid whey (sweet or acid, cultured and uncultured), liquid WPC80 and liquid WPI increased cardboard flavor and lipid oxidation compounds in spray dried WPC80 and WPI (Park et al., 2016; Smith et al., 2016; Whitson et al., 2012).

The FUR results demonstrate that E does heat the product more than RO and that Maillard reaction products were increased. In our study the FUR values of RO NFDM decreased by 28.7% (41.6 vs 30.9 mg FUR/100 g protein) compared to E NFDM. Because RO did not have any heat applied to it, the FUR from the RO NFDM must have come from either pasteurization, E concentration from 30 to 50% solids, and/or spray drying. It can be concluded, however, that in our case approximately 30% of the total FUR from the E NFDM was produced during E to 30% solids. Future work should investigate the role of spray drying parameters on FUR concentrations in NFDM. FUR is an indicator of the Maillard reaction and can be used to measure blocked lysine leading to decreased digestibility of the protein in the milk powder (Erbersdobler and Somoza, 2007; Mehta and Deeth, 2015). Using the formula given by Mehta and Deeth (2015) the percentage of blocked lysine at 41 mg FUR/100 g protein is 1.39%. Lysine blockage in low heat NFDM that we produced is therefore very minimal but in medium and high heat powders it could be much more significant. Van Renterghem and De Block (1996) reported values for freshly prepared low heat NFDM between 100 and 120 mg FUR/100 g protein. These differences could be due to temperatures and hold times used during pasteurization, preheating, E, and spray drying.

Previous studies have investigated how spray drying parameters affect the particle size of milk powders and whey protein concentrate (Park et al., 2014; Nijdam and Langrish 2006; Kim et al., 2009). In general, increased feed solids concentration and inlet temperature

increase the particle size of dried dairy ingredients. To our knowledge, the effect of condensed milk storage and method of concentration on particle size in NFDM has not been investigated prior to our study. Bienvenue et al. (2003) demonstrated that 4 h of storage of condensed skim milk (45% solids) at 50°C significantly increased the viscosity. There could be a slight increase in viscosity with 24 h storage of E condensed milk due to protein aggregation that occurred during evaporation due to the heat applied. Jinapong et al. (2008) observed a positive correlation between feed viscosity entering the spray dryer and particle size of instant soymilk powders. In addition, larger casein micelles during spray drying could lead to larger particle size even if viscosity is similar. Bienvenue et al. (2003) observed that the particle size of condensed skim milk (45% solids) increased dramatically during 8 h of storage at 50°C. This increase was irreversible. They hypothesized that the casein micelles either fused or aggregated together, forming much larger particles. In our experiment, casein micelle aggregation could have initiated in the condensed milk with E due to the heat involved and increased during storage. A decrease in pH with increased temperature has been observed in skim milk (Chaplin and Lyster, 1988; Anema, 2009). The pH values of 30% solids condensed milk at 63°C (E processing temperature) and 18°C (RO processing temperature) were 6.25 and 6.48 respectively. Because the RO condensed milk was not heated during concentration aggregation of casein micelles would have been hindered and the effect of storage on particle size of the NFDM would not be the same as we observed.

The increase in D[4,3] from E 0 h and E 24 h constituted a 25% increase whereas the decrease in D[4,3] from RO 0 h and RO 24 h constituted a 20% decrease. Park et al. (2014) observed a decrease in cardboard flavor with an increase in particle size in whey protein

concentrate 80%. They hypothesized that the reason was due to decreased surface free fat with increased particle size which decreased lipid oxidation. We did not observe differences in surface free fat even though particle size was different and that may be attributed to the fact that the total fat content in the NFDM was very low (0.743%). It could also possibly be due to the fact that the greatest difference in D[4,3] among the treatments they observed was approximately 100% whereas our greatest difference was only about 20%. We hypothesize that we did not see this same trend due to the smaller difference in particle size, the lower fat content, and other confounding variables such as unit operation differences and liquid storage.

While we did not observe differences in SFF along with the particle size in NFDM they could be used as indicators of drying conditions. Future work should attempt to link spray drying conditions to the flavor and shelf life of NFDM. Also, because our work demonstrates that particle size of NFDM is affected by unit operations, future work should investigate how these unit operations influence the functional properties of NFDM and other dried dairy ingredients.

CONCLUSION

Unit operations during the manufacture of NFDM affect NFDM flavor. Use of RO to concentrate to 30% solids decreased cardboard off-flavor and increased sweet aromatic flavor of NFDM compared to E concentration to 30% solids. In order to mitigate off-flavors, storage of condensed milk should be avoided as it increased cardboard off-flavor and decreased sweet aromatic flavor. We demonstrated that the cause of off-flavors in NFDM was a combination of heat and vacuum during E due to the removed characteristic milk

flavor compounds. These results demonstrate to milk powder manufacturers that RO should be used as much as possible during milk concentration and liquid storage should be avoided where possible.

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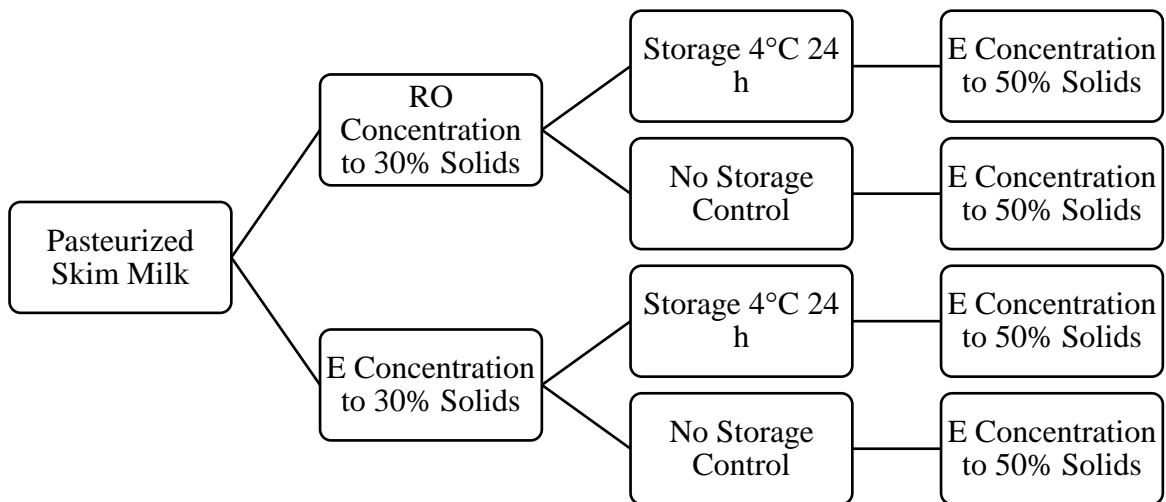


Figure 2. Flow diagram of NFDM Production (Experiment 1). The entire experiment was replicated three times.

Table 1 Descriptive sensory means of rehydrated nonfat dried milk powders with or without 24 h of liquid storage and processed by reverse osmosis (RO) or evaporation (E). Means in the same column and color not sharing a common superscript are different ($P < 0.05$).

Attribute intensities were scored on a 0 to 15 point universal intensity scale (Meilgaard et al., 2009). Most dried ingredient flavors fall between 0 and 4 on this scale (Drake et al., 2003).

Interaction effects were not significant ($p > 0.05$). ND – not detected

	Variable	Overall Aroma	Sweet Aromatic	Cooked	Cardboard
Storage Time	0 h	2.5 a	2.8 a	3.2 a	ND
	24 h	2.4 a	2.2 b	3.3 a	1.1 a
Method of Concentration	RO	2.4 a	2.8 a	2.9 b	ND
	E	2.5 a	2.2 b	3.6 a	1.1 a

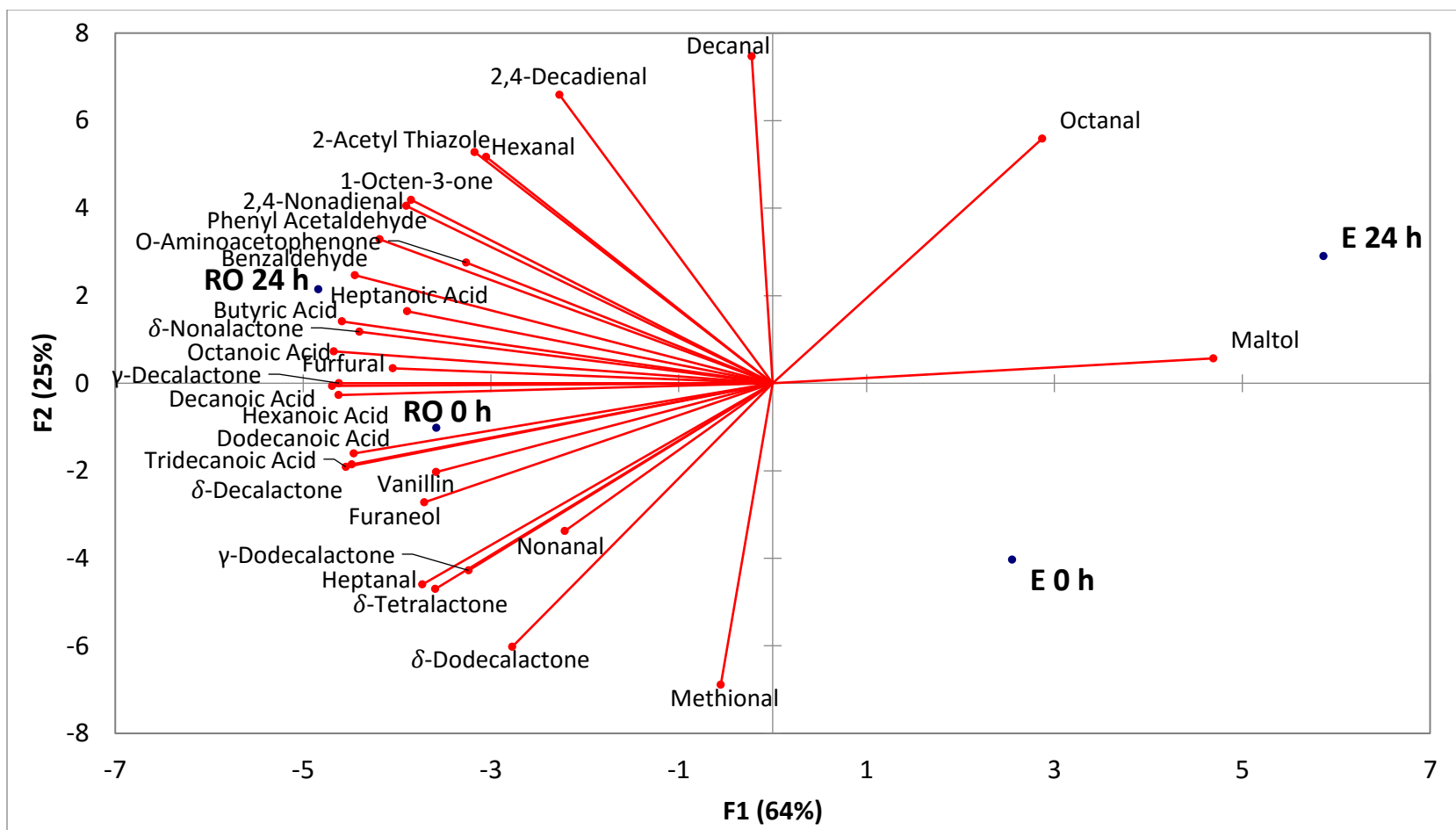


Figure 3 PCA biplot of volatile compound profiles of NFDM. E— Evaporation, RO—Reverse Osmosis, 0 h—No storage, 24 h—24 h storage.

Table 2. Relative abundance ($\mu\text{g}/\text{kg}$) of selected volatile compounds of NFDM with or without 24 h of liquid storage and processed by reverse osmosis (RO) or evaporation (E). Interaction effects between method of concentration and storage time were not significant ($p>0.05$). Means shaded in the same color followed by different letters indicate significant differences ($p<0.05$).

Compound	0 h	24 h	p value	RO	E	p value
Furfural	0.122 a	0.115 a	0.109	0.132 a	0.105 b	0.023
Hexanal	0.680 b	0.826 a	<0.001	0.836 a	0.670 b	0.001
Heptanal	0.240 a	0.177 a	0.556	0.239 a	0.178 a	0.568
Octanal	0.483 b	0.764 a	0.018	0.542 a	0.705 a	0.205
Nonanal	4.51 a	4.37 a	0.070	4.53 a	4.36 a	0.103
Decanal	0.758 a	0.855 a	0.676	0.818 a	0.794 a	0.781
Maltol	0.083 a	0.090 a	0.567	0.065 b	0.108 a	0.002
Furaneol	0.031 a	0.017 b	0.122	0.031 a	0.017 b	0.003
Vanillin	0.118 a	0.081 b	0.019	0.126 a	0.073 b	0.002
δ -Nonalactone	0.195 a	0.188 a	0.757	0.230 a	0.152 b	0.001
δ -Decalactone	1.92 a	1.88 a	0.713	2.14 a	1.67 b	0.260
δ -Dodecalactone	2.88 a	2.54 a	0.448	2.80 a	2.62 a	0.706
δ -Tetralactone	1.89 a	1.71 a	0.568	1.89 a	1.72 a	0.586
γ -Decalactone	0.533 a	0.478 a	0.831	0.646 a	0.365 a	0.277
γ -Dodecalactone	0.488 a	0.449 a	0.673	0.489 a	0.448 a	0.660
2,4-Nonadienal	0.025 b	0.029 a	0.023	0.033 a	0.021 b	<0.001
2,4-Decadienal	0.066 a	0.078 a	0.126	0.077 a	0.067 a	0.199
Butyric Acid	1.64 a	1.60 a	0.857	1.78 a	1.46 b	0.133
Hexanoic Acid	2.29 a	2.24 a	0.827	2.57 a	1.97 b	0.012
Heptanoic Acid	0.312 a	0.342 a	0.488	0.363 a	0.291 a	0.104
Octanoic Acid	6.64 a	6.50 a	0.608	7.71 a	5.43 b	<0.001
Decanoic Acid	15.0 a	14.8 a	0.537	15.6 a	14.2 b	0.001
Dodecanoic Acid	17.0 a	15.9 a	0.148	17.7 a	15.2 b	0.002
Tridecanoic Acid	14.1 a	13.0 a	0.342	14.8 a	12.3 b	0.030
1-Octen-3-one	0.065 a	0.095 a	0.065	0.107 a	0.052 b	0.001
Methional	0.013 a	0.012 a	0.589	0.013 a	0.012 a	0.675
2-Acetyl Thiazole	0.031 a	0.038 a	0.587	0.041 a	0.028 a	0.294
O-Aminoacetophenone	0.014 a	0.018 a	0.179	0.018 a	0.013 a	0.079
Phenyl Acetaldehyde	0.088 a	0.094 a	0.886	0.101 a	0.081 a	0.578
Benzaldehyde	0.784 a	0.839 a	0.356	0.937 a	0.687 b	0.002

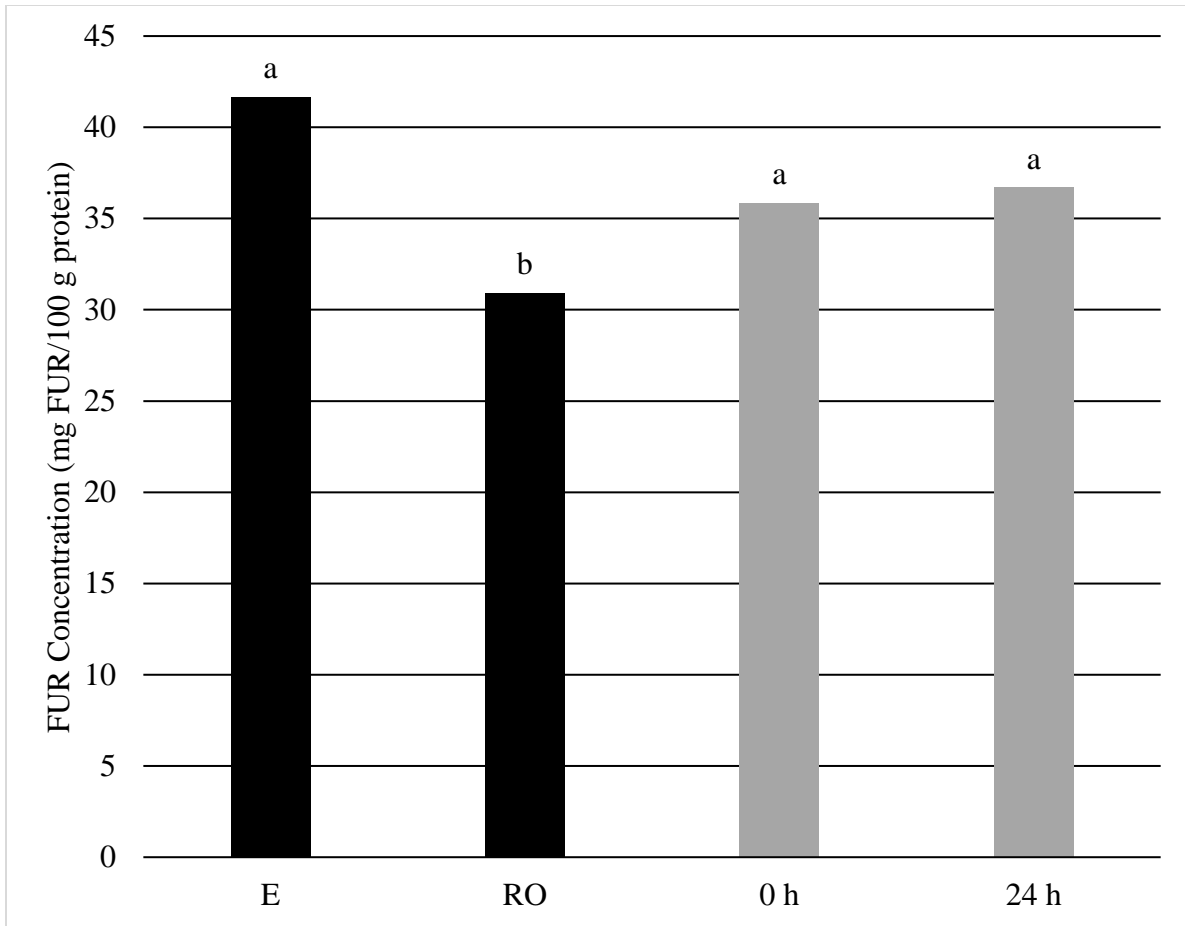


Figure 4. FUR content in NFDM (mg FUR/ 100g protein) with or without 24 h of liquid storage and processed by reverse osmosis (RO) or evaporation (E). Bars with the same color and different letters indicate significant differences ($p < 0.05$). Interaction effects between storage time and method of concentration were not significant ($p > 0.05$).

Table 3. Particle size of NFDM powders with or without 24 h of liquid storage and processed by reverse osmosis (RO) or evaporation (E). Interaction effects between storage time and method of concentration were significant ($p < 0.05$). $D_x(10)$ – 10% of particles below that value, $D_x(50)$ – 50% of particles below that value, $D_x(90)$ – 90% of particles below that value. $D[4,3]$ - Volumetric mean. Numbers in the same column followed by a different letter are significantly different ($p < 0.05$).

Treatment	$D_x(10)$	$D_x(50)$	$D_x(90)$	$D[4,3]$
E 24 h	0.858 a	55.8 a	129 a	63.9 a
RO 0 h	0.609 c	48.0 b	114 b	58.0 b
E 0 h	0.679 b	46.0 c	106 c	51.0 c
RO 24 h	0.599 c	42.6 d	96.5 d	48.1 d

Table 4. Descriptive sensory means for skim milks diluted to 9% solids from experiment 2. Treatment A— Heated in evaporator without vacuum, treatment B— Evaporated to 30% solids (w/w), treatment C— Evaporated to 40% solids (w/w). Means in the same column and color not sharing a common superscript are different ($P < 0.05$). Attribute intensities were scored on a 0 to 15 point universal intensity scale (Meilgaard et al., 2009). Most dried ingredient flavors fall between 0 and 4 on this scale (Drake et al., 2003). Interaction effects were not significant ($p > 0.05$). ND – not detected.

Treatment	Overall Aroma	Sweet Aromatic	Cooked	Cardboard	Sweet	Astringency
Control	1.9 a	1.8 a	3.2 a	ND	2.3 a	2.0 c
A	1.5 b	1.3 b	3.4 a	ND	2.3 a	2.6 b
B	0.5 c	0.6 c	3.2 a	0.6 a	2.3 a	2.8 ab
C	0.5 c	0.5 c	3.4 a	0.7 a	2.2 a	3.0 a

Table 5. Relative abundance ($\mu\text{g}/\text{kg}$) of selected volatile compounds in skim milks from experiment 2. Means in the same row followed by different letters indicate significant differences ($p < 0.05$).

Volatile Compound	Treatment			
	Control	A	B	C
Furfural	0.203 a	0.192 ab	0.171 ab	0.151 b
Hexanal	1.08 a	1.07 a	0.490 b	0.490 b
Heptanal	0.009 a	0.009 a	0.010 a	0.008 a
Octanal	0.175 c	0.203 bc	0.324 a	0.238 b
Nonanal	2.60 b	2.83 ab	3.14 a	3.16 a
Decanal	0.528 c	0.507 c	0.985 a	0.718 b
Maltol	0.089 a	0.023 b	0.017 b	0.018 b
Furaneol	0.037 a	0.034 a	0.019 b	0.022 b
Vanillin	0.016 a	0.015 a	0.008 b	0.008 b
δ -Nonalactone	0.136 a	0.144 a	0.052 b	0.060 b
δ -Decalactone	0.480 a	0.503 a	0.250 b	0.249 b
δ -Dodecalactone	0.058 a	0.050 a	0.061 a	0.047 a
δ -Tetralactone	0.063 a	0.039 a	0.044 a	0.048 a
γ -Decalactone	0.123 a	0.147 a	0.096 a	0.090 a
γ -Dodecalactone	0.042 a	0.037 a	0.038 a	0.035 a
2,4-Nonadienal	0.009 a	0.008 a	0.003 b	0.003 b
2,4-Decadienal	0.022 a	0.025 a	0.014 b	0.014 b
Butyric Acid	0.747 a	0.659 ab	0.571 b	0.595 b
Hexanoic Acid	1.51 a	1.43 ab	1.04 c	1.26 bc
Heptanoic Acid	0.141 a	0.116 ab	0.106 ab	0.097 b
Octanoic Acid	4.48 a	4.73 a	2.69 b	2.84 b
Decanoic Acid	2.22 a	2.27 a	1.59 b	1.56 b
Dodecanoic Acid	1.51 a	1.60 a	1.17 a	1.18 a
1-Octen-3-one	0.019 ab	0.023 a	0.019 ab	0.014 b
2-Acetyl Thiazole	0.003 a	0.002 ab	0.002 ab	0.001 b
O-Aminoacetophenone	0.004 a	0.004 a	0.003 a	0.004 a
Phenyl Acetaldehyde	0.009 b	0.009 b	0.012 a	0.009 b
DMDS	0.695 a	0.646 a	0.669 a	0.692 a
Benzaldehyde	0.245 b	0.332 a	0.390 a	0.333 a

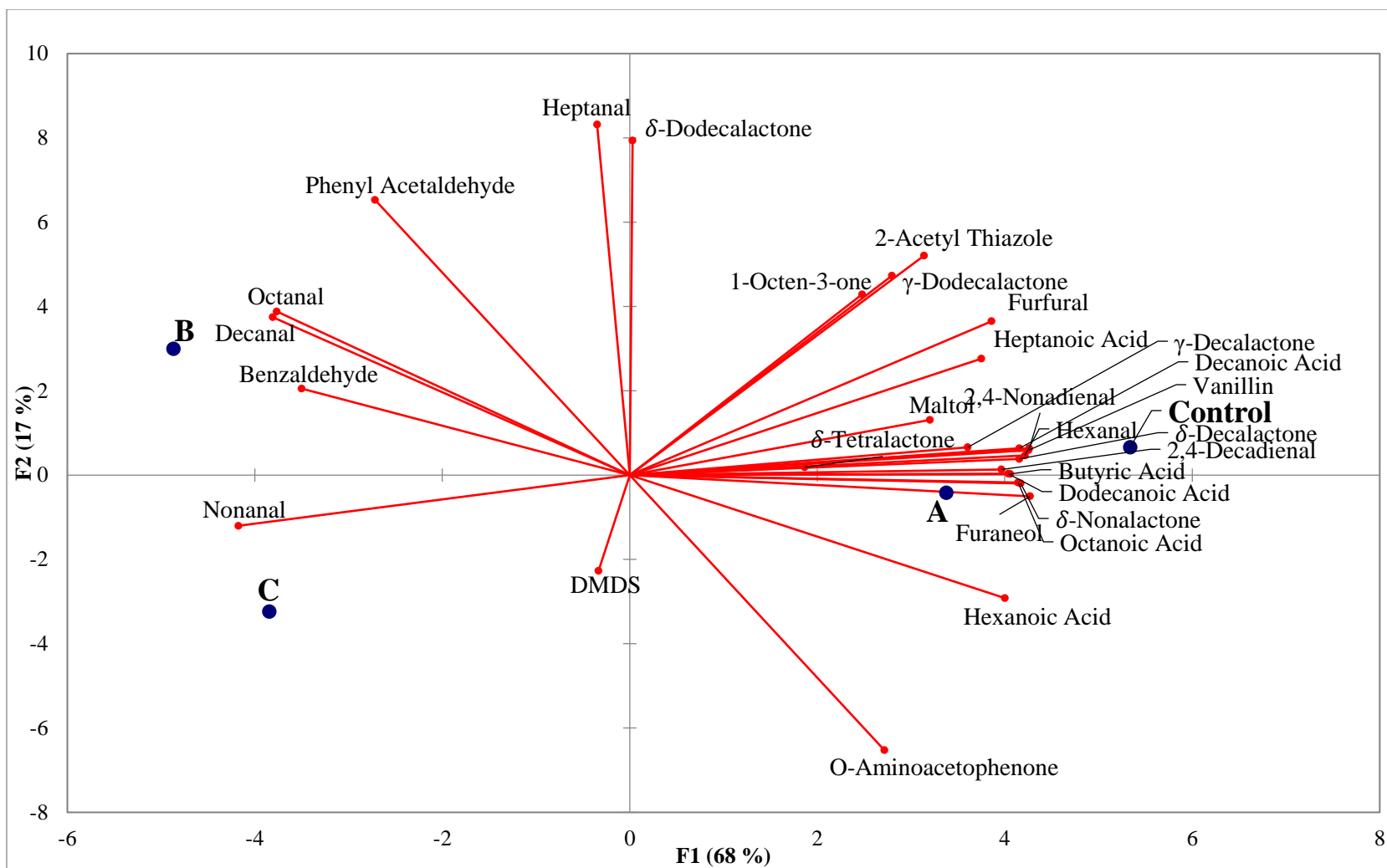


Figure 4. PCA Biplot of volatile compound profiles of skim milks from experiment 2. Treatment A— Heated in evaporator without vacuum, treatment B— Evaporated to 30% solids (w/w), treatment C— Evaporated to 40% solids (w/w).

Table 6. Volatile compounds identified in the vapor separator of the evaporator during production of 30% and 40% solids (w/w) condensed skim milk (Experiment 2). RI—Retention index, O—Odor, MS—Mass spectra. ND—odor not detected (not aroma active).

Compound	Method of ID	Odor	RI
Diacetyl	RI, MS	ND	<600
Acetic Acid	RI, MS	ND	<600
Dimethyl sulfide	RI, O	Sulfur, garbage	<600
Butyric Acid	RI, O, MS	Cheesy	801
Hexanal	RI, O, MS	Grassy	803
Furfuryl alcohol	RI, O, MS	Rubber	861
2-Acetylpyrroline	RI, O	Cooked, roasted	878
Methional	RI, O, MS	Cooked potato	896
Heptanal	RI, MS	ND	901
1-Octen-3-one	RI, O	Mushroom	986
Hexanoic Acid	RI, MS	ND	993
Octanal	RI, MS	ND	1007
2-Acetyl-2-thiazoline	RI, O	Toasted grain	1044
Furaneol	RI, O, MS	Sweet, caramel	1056
Heptanoic Acid	RI, MS	ND	1091
Nonanal	RI, MS	ND	1104
Maltol	RI, O, MS	Cooked, sweet	1109
Octanoic Acid	RI, MS	ND	1184
Decanal	RI, O, MS	Soapy	1206
o-Aminoacetophenone	RI, O, MS	Tortilla, corn chip	1318
Decanoic Acid	RI, MS	ND	1381
Vanillin	RI, MS	ND	1393
δ -Decalactone	RI, O, MS	Sweet	1491
Dodecanoic Acid	RI, MS	ND	1566
δ -Dodecalactone	RI, MS	ND	1702

**CHAPTER 4: THE EFFECT OF SPRAY DRYING PARAMETERS ON
THE FLAVOR OF NONFAT DRY MILK AND MILK PROTEIN
CONCENTRATE 70%**

**The Effect of Spray Drying Parameters on the Flavor of Nonfat Dry Milk
and Milk Protein Concentrate 70%**

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* Use of names, names of ingredients, and identification of specific models of equipment is for scientific clarity and does not constitute any endorsement of product by authors, North Carolina State University, or the Southeast Dairy Foods Research Center.

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ABSTRACT

Unit operations during production influence the sensory properties of nonfat dry milk (NFDM) and milk protein concentrate (MPC). Off-flavors in dried dairy ingredients decrease consumer acceptance of ingredient applications. Previous work has shown that spray drying parameters affect physical and sensory properties of whole milk powder and whey protein concentrate. The objective of this study was to determine the effect of inlet temperature and feed solids concentration on the flavor of NFDM and MPC70. Condensed skim milk (50% solids) and condensed liquid MPC70 (32% solids) were produced using pilot scale dairy processing equipment. The condensed products were then spray dried at either 160°C, 210°C, or 260°C inlet temperature and 30%, 40%, or 50% total solids for NFDM and 12%, 22%, or 32% for MPC70 in a randomized order. The entire experiment was replicated three times. Flavor of the NFDM and MPC70 were evaluated by sensory and instrumental volatile compound analyses. Surface free fat, particle size, and furosine were also analyzed. Both main effects (30%, 40%, 50% solids and 160°C, 210°C, and 260°C inlet temperature) and interactions between solids concentration and inlet temperature were investigated. Interactions were not significant ($p>0.05$). In general, results were consistent for NFDM and MPC70. Increasing inlet temperature and feed solids concentration increased sweet aromatic flavor and decreased cardboard flavor and associated lipid oxidation products. Increases in furosine ($p<0.05$) with increased inlet temperature and solids concentration indicated increased Maillard reactions during drying. Particle size increased and surface free fat decreased with increasing inlet temperature and solids concentration ($p<0.05$). These results demonstrate that increasing inlet temperatures and solids concentration during spray drying

decrease off-flavor intensities in NFDM and MPC70 even though the heat treatment is greater compared to low temperature and low solids.

INTRODUCTION

Dairy powders have developed over the years as a way to extend the shelf-life of milk through various drying techniques. Milk powders made from skim milk contain less than 1.5% fat (wt/wt) are classified as either non-fat dry milk (NFDM) or skim milk powder (SMP) (ADPI, 2015a). Milk retentate, milk permeate, and lactose can be used to adjust protein content to 34% in SMP but not in NFDM (ADPI, 2015a). Over 1 million metric tons of SMP and NFDM are used annually as both an ingredient in food applications as well as for direct consumption (USDA, 2015). Milk protein concentrate (MPC) is any concentrated milk product with protein ranging from 40-90% (ADPI, 2015b). The flavor of NFDM and MPC is important because off-flavors negatively affect the consumer acceptance of ingredient applications (Caudle et al., 2005). Flavor variability has been documented in fresh NFDM from different manufacturers (Caudle et al., 2005; Drake et al., 2006) as well as MPC (Drake et al., 2009, Smith et al. 2016a) but currently there are no published studies documenting the role of unit operations on the flavor of MPC. Current available literature on the flavor of NFDM is limited to flavor properties throughout storage, key aroma compounds, and the effects of off-flavors in ingredient applications (Karagul-Yuceer et al., 2001, Karagul-Yuceer et al., 2002; Isleten and Karagul-Yuceer, 2006; Caudle et al., 2005; Drake et al., 2003). Lipid oxidation compounds are the primary source of off flavors in NFDM and MPC stored at 21°C (Drake et al., 2006; Smith et al., 2016a).

Typical manufacture of NFDM includes fat separation, pasteurization, evaporation, and spray drying. For MPC, ultrafiltration is performed before concentration to remove the majority of lactose and soluble minerals. Concentration of MPC can be done either by nanofiltration (NF) or evaporation. The benefits of using a NF process include reduced product heating and improved solubility (Cao et al., 2015). Typical solids concentration for condensed skim milk during drying is 50% whereas for condensed MPC it is lower (around 30%). Manufacturers are limited on how much solids can be increased due to the high viscosity of condensed skim milk and condensed MPC (Velez-Ruiz and Barbosa-Canovas, 1998; Bienvenue et al., 2003). Spray drying involves atomization of milk into fine particles which are mixed with hot air to remove the majority of the water. During drying, the temperature of the individual particles does not generally reach above 60°C due to evaporative cooling (Schuck, 2013). The spray drying process not only affects the water content but the powder structural and physiochemical characteristics as well (Vignolles et al., 2007).

Surface free fat (SFF), an important characteristic in dairy powders, is described as fat that is not entirely coated by amphiphilic molecules or protected by a matrix of carbohydrates and proteins during drying (Vignolles et al., 2007). The SFF in whole milk powders can alter important properties of the dried milk powder such as: oxidative stability, wettability, dispersability, solubility, flowability, shelf life, and ability to use in chocolate processing applications (Vignolles et al., 2007). Although NFDM and MPC have a low fat content, lipid oxidation plays a critical role in flavor and flavor stability of NFDM and MPC (Karagul-Yuceer et al., 2002; Caudle et al., 2006; Smith et al., 2016a). Control of SFF through changing processing parameters could potentially affect the flavor and flavor

stability of NFDM and MPC (Park and Drake, 2014). Park et al. (2014) recently demonstrated that SFF influenced flavor stability of dried whey proteins and that SFF was impacted by spray drying parameters. Vignolles et al. (2010) also observed that SFF was affected by spray drying temperatures, with decreasing SFF with increasing inlet temperatures.

Spray drying parameters impact the flavor of whey protein concentrate (WPC) (Park et al., 2014). Decreased off-flavor intensities and corresponding lipid oxidation products were observed with increased inlet temperatures and feed solids concentrations. Increased inlet temperatures may increase the heat treatment given to the dairy powder and decrease nutritional quality. To our knowledge, Maillard reaction products, such as furosine, have not been investigated in dried dairy ingredients spray dried under varying conditions. Given differences in composition and unit operations between WPC, MPC, and NFDM, it is important to investigate the influence of spray drying parameters on the flavor of NFDM. The objective of this study was to determine the influence of feed solids concentration and inlet temperature on the flavor of NFDM and MPC70.

MATERIALS AND METHODS

NFDM Production

Raw bovine skim milk (313 kg) was obtained from the North Carolina State University Dairy Research and Education Unit. The milk was HTST pasteurized at 73°C for 16 s with a plate heat exchanger (model T4 RGS-16/2, SPX Flow Technology, Greensboro, NC) and subsequently cooled to 4°C and stored until evaporation (<6 h). The pasteurized skim milk was evaporated to 50% solids (wt/wt) in a single stage falling film evaporator.

The milk was pre-heated to 54°C and introduced to the evaporator operating at 71°C with 75

kPa vacuum. The condensed milk was then standardized with warm deionized water to either 30, 40, or 50% solids (wt/wt) and was approximately 50°C for <30 min prior to drying. Drying was performed with a spray dryer (model Lab 1, Anhydro Inc., Soeberg, Denmark) with a two fluid nozzle with compressed air, operated at an inlet temperature of either 160, 210, or 260°C with a constant outlet temperature of 90°C. Outlet temperature was controlled with the feed flow rate into the spray dryer in order to keep a constant percent moisture in the resulting powders. The total solids content in the condensed milk and the spray dried NFDM was measured using the Smart Turbo moisture/solids analyzer (CEM, Matthews, NC). The order of treatments was completely randomized within one day of production. The entire experiment was replicated three times.

MPC70 Production

Raw bovine skim milk was obtained from the NC State University Dairy Research and Education Unit. The milk was pasteurized as described previously for NFDM production. The pasteurized skim milk was then subjected to ultrafiltration (UF) using a pilot scale unit (Model Lab 46, Filtration Engineering, Champlin, MN). Two spiral wound membranes were used (Microdyn, Raleigh, NC; nominal cutoff 10kDa, surface area 6.5 m² per element) with a recirculation rate of 132 L/min. The skim milk was heated with a plate heat exchanger to 50°C prior to introduction into the UF unit. UF was performed in two stages with concentration factors of 3x and 4x in stages 1 and 2 respectively. After stage 1, diafiltration was performed by adding warm deionized water in an amount equaling 30% of the original weight of starting skim milk. Solids content of the liquid MPC70 was measured using the Smart Turbo moisture/solids analyzer (CEM). The protein content was confirmed using a Sprint Protein Analyzer (CEM) and using an infrared milk analyzer (LactoScope

FTIR Advanced, Delta Instruments, Drachten, the Netherlands). The liquid MPC70 was 12% solids (w/w) and 8.4% protein (w/w/).

Next, the liquid MPC70 was evaporated to 32% solids (wt/wt) in the falling film evaporator. The liquid MPC70 was pre-heated to 54°C and introduced to the evaporator operating at 63°C with 81 kPa vacuum. The liquid MPC70 was then standardized with warm deionized water to either 12, 22, or 32% solids (w/w) and was approximately 50°C for <30 min prior to drying. Spray drying was performed as described previously in NFDMM manufacture.

Proximate Analysis

Percent moisture was measured using a vacuum oven (AOAC International 2000; method 990.20) and fat was measured by ether extraction (AOAC International 2000; method 932.06).

Descriptive Sensory Analysis

Sensory testing was done in compliance with the North Carolina State University Institutional Review Board for Human Subjects. Spray dried NFDMM and MPC70 were rehydrated to 10% solids (w/v) in deodorized deionized water. Rehydrated powders were dispensed into soufflé cups (Solo Cup, Highland Park, IL, USA), lidded, and tempered to 21°C. Rehydrated powders were profiled by a highly trained sensory panel (n=8) each with >150 h of training using the Spectrum™ method with an established dried dairy ingredient lexicon (Drake et al., 2003; Smith et al., 2016b). Panelists expectorated the samples and were given deionized water to cleanse their palates. Compusense Cloud (Compusense, Guelph, Canada) was used for data collection. Each treatment replication was evaluated in duplicate.

Volatile Compound Analysis

Volatile compounds were extracted using sorptive stir bar extraction (SSBE) (Prieto et al., 2010). Each NFDM and MPC70 manufacture replicate was extracted in triplicate. SSBE was used in place of headspace solid phase micro extraction (SPME) due to the very low concentrations of the volatile compounds in the powders. SSBE has a significantly larger surface area of stationary phase to absorb the volatile compounds than SPME. SSBE also has the ability to extract compounds of higher boiling point because the stir bars are immersed in the sample rather than in the headspace. Thermal desorption unit (TDU) tubes were conditioned for 1 h at 300°C prior to analysis. First, 0.5 g of powder was dissolved in 5 ml of HPLC grade water into a 10 ml amber screw top vial (Gerstel, Inc., Linthicum, MD). Next, 10 µl of internal standard was added (0.81 mg/kg 2-methyl-3-heptanone in water; Sigma Aldrich, St. Louis, MO). Sequential stir bar extraction (SSBE) was used with salt addition in the second extraction to more effectively extract compounds of various classes. Extraction of polar compounds is improved with salt whereas extraction of hydrophobic compounds is decreased with salt (Prieto et al., 2010). One PDMS coated stir bar (10 mm x 0.5 mm thickness, Gerstel, Inc.) was placed in the vial, sealed, and stirred for one hour at 800 rpm. After 1 h, the stir bar was briefly rinsed in HPLC grade water, dried, and placed in a TDU autosampler tube (Gerstel, Inc.). Next, 1 g of NaCl was added to the sample along with another PDMS stir bar. This stir bar was stirred for 1 h at 800 rpm, rinsed, dried, and placed in the same TDU tube previously mentioned. Compounds were desorbed on to the GC TDU inlet using an autosampler (MPS Autosampler, Gerstel, Inc.). The stir bars were desorbed at 250°C for 10 min (TDU, Gerstel, Inc.) and the volatile compounds were cryogenically trapped at -120°C (CIS 4, Gerstel, Inc.).

Volatile compounds were analyzed by gas chromatography mass spectrometry (GCMS). An Agilent 7890B GC (Agilent Technologies Inc., Santa Clara, CA) with an inert mass selective detector (model 5970A, Agilent) with a ZB-5MS column (30 m x 0.25 mm x 0.25 μ m) (Phenomenex, Torrance, CA) was used to identify and quantify volatile compounds of interest (Karagul-Yuceer et al., 2001; Karagul-Yuceer et al., 2002; Drake et al., 2007;). Initial GC oven conditions were 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. Purge time was set to 1.2 min using helium as the carrier gas at a constant flow rate of 1 ml/min. Compounds were identified by comparison with the 2014 NIST mass spectral library (NIST, 2014), retention index, and retention time of authentic standards injected under identical conditions. Relative abundance of selected compounds was calculated using recovery of the internal standard.

Surface Free Fat and Particle Size

Surface free fat was measured by solvent extraction with petroleum ether (GEA Niro Method No. A 10a; GEA, 2005) and was expressed as g free fat/100 g total fat. Particle size of the NFDM and MPC70 was measured by laser diffraction (Mastersizer 3000, Malvern, UK). All measurements were performed in duplicate.

Furosine

The method of Resmini et al. (1990) was followed to analyze furosine with minor modifications. An amount of NFDM/MPC70 corresponding to 40-50 mg of protein was placed in a screw-cap Pyrex tube with a PTFE-lined septa along with 8 ml of 10.6M HCl (Sigma Aldrich). Nitrogen was bubbled through the samples for 2 min and then capped and heated to 110°C for 23 h. After cooling, the hydrolysate was filtered and 0.5 ml was loaded

onto a 500 mg reversed phase solid phase extraction column (Discovery DSC-18, Supelco) previously conditioned with 5 ml of methanol and 10 ml of water. Furosine was eluted with 3 ml of 3M HCl. Furosine was separated and detected with a Waters HPLC system (Milford, MA). A furosine dedicated column was used (Altima C8, 250 x 4.6 mm, 5 μ m, Alltech-Grace, Columbia, MD) with isocratic conditions. The mobile phase consisted of 0.4% acetic acid (Sigma Aldrich) and the column temperature was 35°C. UV detection was performed at 280 nm. A 5 point external standard curve ranging from 50 to 400 ng was constructed for quantification of both NFDM and MPC70.

Statistical Analysis

Two-way analysis of variance (ANOVA) with means separation with the Fisher's least significant difference post-hoc test was used to analyze the data (XLStat, version 2015.1, Addinsoft, France). Interaction effects between feed solids concentration and inlet temperature were investigated.

RESULTS

Moisture and fat were not significant between treatments ($p > 0.05$) and were $3.12 \pm 0.35\%$ and $0.651 \pm 0.17\%$ respectively for NFDM and $3.98 \pm 0.41\%$ and $1.38 \pm 0.08\%$ for MPC70 respectively.

The NFDM treatments had distinct flavor profiles (Table 1). Interaction effects between inlet temperature and feed solids concentration for descriptive sensory analysis were not significant ($p > 0.05$). Sweet taste was not affected by feed solids concentration or inlet temperature. A feed solids concentration of 30% decreased overall aroma intensity compared to 50% ($p < 0.05$; 1.7 vs. 1.9). A 30% feed solids concentration decreased sweet aromatic (1.6 vs. 2.0 vs. 2.1) and cooked flavors (3.1 vs. 3.3 vs. 3.3) compared to 40% and

50% solids concentrations respectively ($p < 0.05$). Decreasing feed solids concentration from 50% to 30% increased cardboard flavor intensity ($p < 0.05$; ND vs. 0.5 vs 1.1). An inlet temperature of 160°C decreased overall aroma (1.7 vs. 1.9 vs. 1.9) and sweet aromatic (1.4 vs. 2.1 vs. 2.2) compared to 210°C and 260°C respectively ($p < 0.05$). Increasing inlet temperature increased cooked flavor intensity (3.1 vs. 3.3 vs. 3.4). Cardboard flavor was only detected with an inlet temperature of 160°C.

The volatile compound profiles of the NFDM treatments were in congruence with the descriptive sensory profiles (Figure 1, Table 2). In general, lipid oxidation products increased with decreasing inlet temperature and feed solids concentration. This is consistent with what has been previously reported in WPC80 (Park et al., 2014). Interaction effects between feed solids concentration and inlet temperature were not significant for the following compounds: furfural, nonanal, furaneol, vanillin, delta-decalactone, delta-dodecalactone, gamma-dodecalactone, butyric acid, hexanoic acid, heptanoic acid, octanoic acid, decanoic acid, dodecanoic acid, methional, 2-acetyl thiazole, phenyl acetaldehyde, and benzaldehyde ($p > 0.05$).

Both feed solids concentration and inlet temperature significantly affected the volatile compound profile of the rehydrated NFDM. Spray drying at a feed solids concentration of 30% increased concentrations of furfural and nonanal and decreased delta-decalactone and benzaldehyde compared to 40 or 50% ($p < 0.05$). When spray dried at 160°C, a feed solids concentration of 30% increased hexanal, 2,4-nonadienal, 2,4-decadienal, 1-octen-3-one, and o-aminoacetophenone compared to 40 or 50% and heptanal compared to 50% ($p < 0.05$). At an inlet temperature of 210°C, spray drying at 30% solids increased concentrations of hexanal, heptanal, maltol, 2,4-nonadienal, 1-octen-3-one, and o-aminoacetophenone

compared to 40 or 50% and 2,4-decadienal compared to 50% ($p < 0.05$). At an inlet temperature of 210°C a solids concentration of 50% increased delta-nonalactone ($p < 0.05$). When spray dried at 260°C, a solids concentration of 30% increased maltol, 2,4-nonadienal, 2,4-decadienal, and o-aminoacetophenone concentrations compared to 40 or 50% solids ($p < 0.05$).

An inlet temperature of 260°C increased the concentration of furfural compared to 160 or 210°C and delta-dodecalactone and heptanoic acid compared to 160°C ($p < 0.05$). An inlet temperature of 210°C decreased nonanal concentration compared to 160°C ($p < 0.05$). Spray drying at 160°C resulted in decreased butyric acid concentrations compared to 210 or 260°C ($p < 0.05$). When dried at 30% solids, increasing inlet temperature decreased hexanal, 2,4-nonadienal, and 1-octen-3-one concentrations ($p < 0.05$). An inlet temperature of 160°C increased decanal, gamma-decalactone, and 2,4-decadienal compared to 210 or 260°C and 260°C decreased heptanal concentration when NFDM was dried at 30% solids ($p < 0.05$). When dried at 40% solids, an inlet temperature of 160°C increased hexanal, heptanal, decanal, 2,4-nonadienal, and 1-octen-3-one compared to 210 or 260°C and 2,4-decadienal compared to 260°C ($p < 0.05$). Also, when dried at 40% solids, maltol concentration increased with a temperature of 260°C compared to 160 or 210°C ($p < 0.05$). When dried at 50% solids, an inlet temperature of 160°C increased hexanal and 1-octen-3-one concentrations compared to 210 or 260°C and heptanal and octanal compared to 210°C ($p < 0.05$). At 50% solids, 210°C increased delta-nonalactone and decreased octanal concentrations compared to 160 or 260°C and 260°C increased maltol concentration compared to 160 or 210°C ($p < 0.05$).

As observed in NFDM, inlet temperature and feed solids concentration also affected the sensory and volatile compound profiles of MPC70 ($p < 0.05$). The descriptive sensory profiles are displayed in Table 3. Interaction effects between solids concentration and inlet temperature were not significant ($p > 0.05$). Aroma intensity and cooked flavor were not affected by solids concentration or inlet temperature ($p > 0.05$). A solids concentration of 12% decreased sweet aromatic flavor compared to 22 or 32% and increased cardboard flavor compared to 32% ($p < 0.05$). Fatty flavor was only detected in MPC70 spray dried at 12% solids or 160°C. Increasing inlet temperature increased sweet aromatic flavor ($p < 0.05$). Cardboard flavor intensity increased when MPC70 was spray dried at 160°C compared to 210 or 260°C ($p < 0.05$).

Volatile compound profiles of rehydrated MPC70 followed similar trends as in NFDM. In general, lipid oxidation products increased with decreasing inlet temperature and feed solids concentration. Interaction effects were not significant for furfuryl alcohol, furfural, maltol, furaneol, delta-nonolactone, delta-decalactone, delta-dodecalactone, dodecanoic acid, phenyl acetaldehyde, and DMDS ($p > 0.05$). An inlet temperature of 260°C increased furfuryl alcohol and maltol concentrations compared to 160°C ($p < 0.05$). A solids concentration of 32% during spray drying increased furfuryl alcohol, delta-dodecalactone, and dodecanoic acid concentrations compared to 12% ($p < 0.05$).

When MPC70 was spray dried at 160°C, a solids concentration of 12% increased hexanal, decanal, 2,4-nonadienal, and 2,4-decadienal compared to 22 or 32% and gamma-decalactone compared to 22% ($p < 0.05$). At 160°C, 32% solids increased butyric acid, heptanoic acid, decanoic acid, 2-acetyl thiazole, and benzaldehyde compared to 12 or 22% solids and heptanal, octanal, nonanal, and hexanoic acid compared to 22% solids ($p < 0.05$).

At an inlet temperature of 210°C, increasing solids decreased hexanal and octanal concentrations ($p < 0.05$). At 210°C, 12% solids increased heptanal, nonanal, and o-aminoacetophenone and decreased delta tetralactone compared to 22 or 32% solids ($p < 0.05$). When dried at 210°C, a solids concentration of 32% increased vanillin, gamma-dodecalactone, butyric acid, hexanoic acid, heptanoic acid, and 2-acetyl thiazole compared to 12 or 22% solids ($p < 0.05$). When dried at 260°C, increasing solids concentration decreased hexanal concentration ($p < 0.05$). In addition, at 260°C, 12% solids increased heptanal, 1-octen-3-one, and o-aminoacetophenone compared to 22 or 32% solids and octanal and 2,4-nonadienal compared to 22% solids ($p < 0.05$). At an inlet temperature of 260°C, 32% solids increased vanillin, octanoic acid, and 2-acetyl thiazole compared to 12 or 22% solids and decreased nonanal compared to 12% solids ($p < 0.05$). When MPC70 was dried at 12% solids and an inlet temperature of 160°C increased hexanal, heptanal, nonanal, decanal, 2,4-decadienal, and o-aminoacetophenone concentrations were observed compared to 210 or 260°C ($p < 0.05$). At 22% solids, drying at 160°C increased hexanal, heptanal, octanal, and nonanal compared to 210 or 260°C and drying at 260°C increased butyric acid, heptanoic acid, and 2-acetyl thiazole compared to 160 or 210°C ($p < 0.05$). When spray dried at 32% solids, an inlet temperature of 160°C increased hexanal, heptanal, octanal, and nonanal compared to 210 or 260°C and an inlet temperature of 260°C increased gamma-decalactone and 2-acetyl thiazole compared to 160°C ($p < 0.05$).

Furosine (FUR) concentration was also affected by spray drying parameters in both NFDM and MPC70 (Figure 3, Figure 4). Furosine is an indicator of early Maillard reactions and has been used to measure nutritional quality of dairy proteins as it measures the amount of blocked lysine (Mehta and Deeth, 2015). Interaction effects between inlet temperature

and feed solids concentration were not significant for furosine content in both NFDM and MPC70 ($p>0.05$). In NFDM, an inlet temperature of 260°C increased FUR concentration compared to 160 or 210°C (20.3 vs 13.9 vs 15.2 mg FUR/100 g protein) ($p<0.05$). Also, a feed solids concentration of 30% decreased FUR compared to 40 or 50% (12.3 vs 17.9 vs 19.3 mg FUR/100 g protein) ($p<0.05$). Similarly, in MPC70 an inlet temperature of 260°C increased FUR concentrations compared to 160 or 210°C (15.9 vs 9.5 vs 13.4 mg FUR/100 g protein) ($p<0.05$). Solids concentration did not affect FUR concentration in MPC70 ($p>0.05$).

Particle size and surface free fat (SFF) were also different between treatments (Table 5, Figure 5). Interaction effects between temperature and solids concentration were not significant for either particle size or SFF in NFDM and MPC70 ($p>0.05$). In NFDM, both inlet temperature and solids concentration affected particle size ($p<0.05$) but neither affected SFF ($p>0.05$). Increasing inlet temperature increased both the $D_x(90)$ (77.6 vs 97.8 vs 105 μm) and the $D[4,3]$ (35.1 vs 45.8 vs 62.5 μm) ($p<0.05$). Similarly, increasing solids concentration increased the $D_x(90)$ (77.4 vs 85.8 vs 118 μm) and the $D[4,3]$ (35.3 vs 40.1 vs 68.0 μm). In MPC70, increasing inlet temperature increased the $D[4,3]$ (31.4 vs 43.5 vs 49.2 μm) and 160°C decreased the $D_x(90)$ compared to 210 or 260°C (72.7 vs 93.3 vs 99.5 μm) ($p<0.05$). The specific surface area increased in MPC70 with decreasing inlet temperature (1850 vs 2180 vs 3080 m^2/kg) ($p<0.05$) which is consistent with the smaller particle size at lower inlet temperatures. Particle size of MPC70 spray dried at 12% decreased compared to 22 or 32% as observed in the $D_x(90)$ (76.9 vs 96.9 vs 91.7 μm), $D[4,3]$ (34.2 vs 46.8 vs 43.1 μm), and specific surface area (2600 vs 2140 vs 2300 m^2/kg) ($p<0.05$). SFF increased in MPC70 spray dried at 160°C compared to 210 or 260°C (0.036 vs 0.027 vs 0.022 g SFF/100

g MPC70) and at 12% compared to 22 or 32% (0.046 vs 0.020 vs 0.020 g SFF/100g MPC70) ($p < 0.05$). The increase in SFF is most likely due to the increase in surface area due to the smaller particle size.

DISCUSSION

The flavor of NFDM and MPC70 are distinct even though many of the same volatile compounds are responsible for their flavor (Karagul-Yuceer et al., 2001, Karagul-Yuceer et al., 2004, Smith et al., 2016a), likely due to their distinct composition. MPC70 contains more protein and fat. However, lipid oxidation is the main source of off-flavors in dried dairy ingredients stored at 21°C across a wide range of protein and fat and must be controlled in order to improve flavor and shelf-life (Karagul-Yuceer et al., 2002; Park et al., 2014; Wright et al., 2009; Lloyd et al. 2009; Smith et al., 2016a, 2016b).

Volatile compounds with high odor activity in nonfat dry milk (NFDM) and MPC have been identified and confirmed previously (Karagul-Yuceer et al., 2001; Smith et al., 2016a). In NFDM these compounds include furaneol, butyric acid, methional, o-aminoacetophenone, delta-decalactone, vanillin, hexanoic acid, 2-acetyl-1-pyrroline, octanoic acid, gamma-dodecalactone, 2,4-decadienal, and maltol (Karagul-Yuceer et al., 2001). Many of the same compounds were identified with a high odor activity in milk proteins. Those with the highest odor activity were: o-aminoacetophenone, nonanal, 1-octen-3-one, dimethyl trisulfide, 2-acetyl-1-pyrroline, heptanal, methional, 1-hexen-3-one, hexanal, dimethyl disulfide, butyric acid, and acetic acid (Smith et al., 2016a).

We identified and quantified many of these compounds in both NFDM and MPC70. The studies mentioned previously used solvent assisted flavor evaporation to extract the

flavor compounds due to the ability to extract compounds of many different classes. We were able to extract many of the same compounds using SSBE which reduces labor, variability, solvent waste, and increases sample throughput tremendously. Increases in lipid oxidation compounds such as aldehydes, 1-octen-3-one, 2,4-nonadienal, and 2,4-decadienal were observed with increasing cardboard flavor. Smith et al. (2016a) observed increases in these compounds during storage of MPC and they were also correlated with increased cardboard flavor. We also observed that increasing inlet temperature and solids concentration increased sweet aromatic flavor intensity and some associated compounds associated such as vanillin and 2-acetyl thiazole (Karagul-Yuceer et al., 2001). These increases could be due to generation with the higher heat treatment or a reduction of these compounds by oxidation in samples with increased lipid oxidation.

The fact that increased temperature during spray drying improved flavor may be counter intuitive but because water is constantly evaporating, the particle temperature does not reach the inlet temperature (Kim et al., 2009). However, we did observe increases in FUR, a marker for Maillard reactions, with elevated inlet temperatures. To our knowledge, FUR has not been reported in dried dairy ingredients that were spray dried under varying conditions. In NFDM, an inlet temperature of 260°C increased FUR compared to 160 or 210°C and in MPC70 210 and 260°C increased FUR compared to 160°C. This suggests that the heat treatment during spray drying was higher with elevated inlet temperatures. Our results demonstrate that although heat treatment during drying increased with higher inlet temperatures, off-flavors are minimized as observed in the reduction of cardboard flavor by sensory profiling.

Decreased solubility of MPC during storage has been attributed to protein cross-linking with Maillard reaction products (Le et al., 2011a; Le et al., 2011b). Increases in FUR during storage of MPC have been correlated with decreases in solubility consistent with increased Maillard reaction products (Le et al., 2011a; Smith et al., 2016a). Our results suggest that spray drying parameters may also play a role in MPC70 solubility through shelf-life due to differences in initial FUR content.

Park et al. (2014) observed that lipid oxidation in WPC80 decreased with increasing inlet temperature and feed solids concentration which is consistent with what we observed for NFDM and MPC70. They hypothesized that it was due to the rate at which the particles dried that influenced flavor. Larger particles are formed when the droplets dry quicker which is the case when elevated inlet temperatures and feed solids concentration (Kim et al., 2009). With increased particle size there is a decrease in SFF due to a decrease in surface area which also leads to a decrease in lipid oxidation. In NFDM we observed differences in particle size with no difference in SFF. This could be because the fat content in NFDM was too low to be able to detect differences in SFF by the method employed. Differences in SFF were detected in MPC70 which had a fat content of more than double that of NFDM (0.651% vs 1.38%). Decreases in free fat have been correlated with decreased lipid oxidation and increased shelf-life (Park et al., 2014; Keogh et al., 2001, Vega and Roos, 2006; Vignolles et al., 2007).

CONCLUSION

Spray drying parameters significantly affect the flavor of dried dairy ingredients. Strong consideration should be taken when spray drying parameters are chosen by ingredient manufacturers in order to improve the flavor and shelf-life of either NFDM or MPC70. Our

results also suggest that functionality may be impacted and that future work should investigate the relationship between spray drying parameters and functionality of dried dairy ingredients. Both NFDM and MPC70 were affected by inlet temperature and feed solids concentration during drying. Increasing inlet temperatures and solids concentrations increased sweet aromatic and decreased cardboard flavor and associated volatile lipid oxidation products. We also demonstrated that although the particles do receive a greater heat treatment during drying, flavor quality is improved at elevated inlet temperatures.

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Table 1 Sensory profiles of rehydrated NFDM spray dried at different inlet temperatures and feed solids concentrations. Interaction effects between solids concentration and inlet temperature were not significant ($p>0.05$). Means in the same column and shading followed by a different letter are different ($p<0.05$). ND—not detected.

Treatment	Overall Aroma	Sweet Aromatic	Cooked	Cardboard	Sweet Taste
30%	1.7b	1.6b	3.1b	1.1a	2.1a
40%	1.8ab	2.0a	3.3a	0.5b	2.1a
50%	1.9a	2.1a	3.3a	ND	2.0a
160°C	1.7b	1.4b	3.1c	1.2a	2.0a
210°C	1.9a	2.1a	3.3b	ND	2.1a
260°C	1.9a	2.2a	3.4a	ND	2.1a

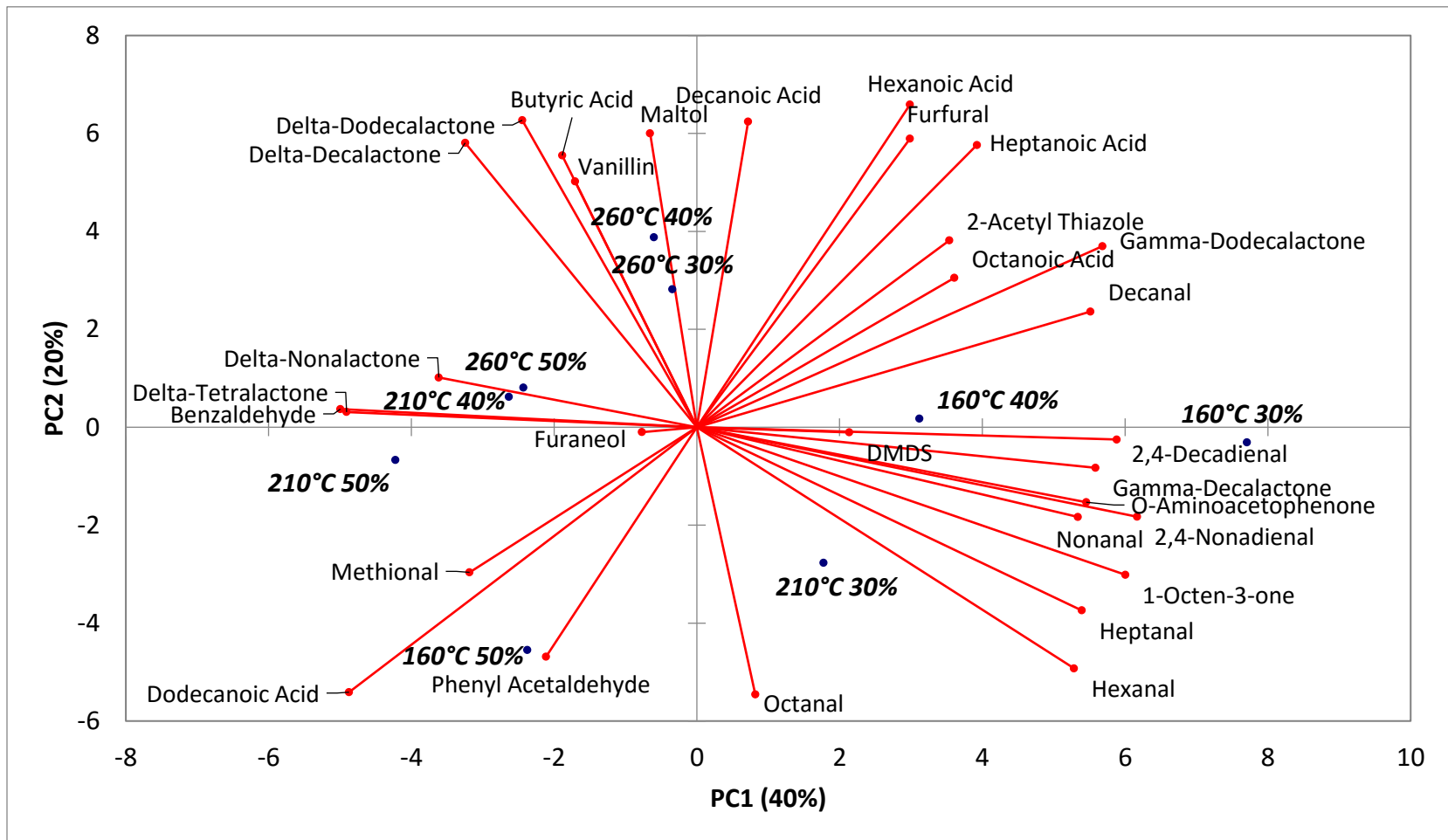


Figure 1 Principal component biplot of volatile compound analysis of rehydrated NFDM spray dried at different temperatures and feed solids concentrations.

Table 2 Volatile compound analysis of rehydrated NFDM ($\mu\text{g}/\text{kg}$). Means in the same column followed by a different letter indicate significant differences ($p < 0.05$). Interaction effects between solids concentration and inlet temperature were not significant for compounds highlighted in grey ($p > 0.05$).

Compound	160°C 30%	160°C 40%	160°C 50%	210°C 30%	210°C 40%	210°C 50%	260°C 30%	260°C 40%	260°C 50%
Furfural	0.214 a	0.164 abcd	0.137 d	0.185 abcd	0.148 bcd	0.145 cd	0.207 abc	0.222 a	0.208 ab
Hexanal	1.41 a	1.07 b	1.050 b	1.03 b	0.654 c	0.655 c	0.593 c	0.674 c	0.654 c
Heptanal	0.654 ab	0.674 a	0.551 bc	0.644 ab	0.418 cd	0.389 d	0.470 cd	0.458 cd	0.435 cd
Octanal	0.396 abc	0.371 bc	0.465 a	0.393 abc	0.319 c	0.324 c	0.332 c	0.350 c	0.443 ab
Nonanal	4.86 a	3.81 cd	3.73 cde	4.50 ab	3.40 de	3.14 e	4.13 bc	3.43 de	3.85 cd
Decanal	1.06 a	0.927 ab	0.576 cde	0.625 cde	0.517 de	0.446 e	0.765 bc	0.707 cd	0.788 bc
Maltol	0.181 d	0.168 d	0.114 d	0.289 c	0.164 d	0.178 d	0.614 a	0.382 b	0.424 b
Furaneol	0.018 a	0.017 a	0.017 a	0.022 a	0.016 a	0.024 a	0.025 a	0.016 a	0.017 a
Vanillin	0.019 b	0.017 b	0.013 b	0.018 b	0.033 a	0.019 b	0.022 ab	0.022 ab	0.023 ab
Delta-Nonalactone	0.113 c	0.135 b	0.122 bc	0.131 bc	0.129 bc	0.181 a	0.131 b	0.139 b	0.130 bc
Delta-Decalactone	1.05 bc	1.21 ab	1.17 abc	0.954 c	1.21 ab	1.20 ab	1.21 ab	1.33 a	1.18 abc
Delta-Dodecalactone	0.292 bc	0.287 bc	0.260 c	0.259 c	0.280 bc	0.371 a	0.339 ab	0.354 ab	0.341 ab
Delta-Tetralactone	0.096 de	0.091 e	0.148 ab	0.117 cde	0.132 bc	0.133 bc	0.120 cd	0.144 bc	0.173 a
Gamma-Decalactone	0.213 a	0.165 b	0.122 de	0.152 bc	0.130 cde	0.148 bcd	0.144 bcde	0.121 e	0.128 cde
Gamma-Dodecalactone	0.061 a	0.053 a	0.044 a	0.049 a	0.048 a	0.042 a	0.050 a	0.054 a	0.049 a
2,4-Nonadienal	0.034 a	0.024 b	0.017 cd	0.024 b	0.016 cd	0.014 d	0.020 c	0.014 d	0.015 d
2,4-Decadienal	0.076 a	0.051 b	0.041 c	0.051 b	0.045 bc	0.041 c	0.052 b	0.041 c	0.037 c
Butyric Acid	0.613 cd	0.608 cd	0.537 d	0.923 b	1.01 ab	0.769 bc	1.17 a	0.932 b	0.796 bc
Hexanoic Acid	1.70 ab	1.66 ab	1.24 c	1.36 bc	1.50 abc	1.54 abc	1.78 a	1.61 abc	1.37 bc
Heptanoic Acid	0.200 ab	0.187 abc	0.157 cd	0.173 bcd	0.172 bcd	0.148 d	0.178 abcd	0.209 a	0.185 abc
Octanoic Acid	3.34 ab	3.81 a	2.83 d	2.83 cd	2.92 bcd	2.95 bcd	2.89 bcd	3.30 bc	3.08 bcd
Decanoic Acid	2.16 bc	2.55 ab	1.91 c	1.89 c	2.036 c	1.93 c	2.29 abc	2.54 ab	2.711 a
Dodecanoic Acid	1.30 b	1.50 ab	1.81 a	1.64 ab	1.65 ab	1.77 a	1.38 b	1.52 ab	1.60 ab
1-Octen-3-one	0.150 a	0.106 b	0.067 d	0.087 c	0.044 e	0.033 e	0.040 e	0.041 e	0.032 e
Methional	0.003 b	0.003 ab	0.005 a	0.003 ab	0.003 ab	0.004 ab	0.003 ab	0.003 b	0.006 a
2-Acetyl Thiazole	0.004 abc	0.004 abc	0.002 d	0.004 a	0.004 ab	0.003 cd	0.003 bcd	0.004 a	0.003 abc
O-Aminoacetophenone	0.015 a	0.012 b	0.006 def	0.016 a	0.008 cd	0.005 ef	0.007 de	0.009 bc	0.004 f

Table 2 continued.

Phenyl Acetaldehyde	0.119 a	0.135 a	0.163 a	0.229 a	0.159 a	0.181 a	0.173 a	0.091 a	0.186 a
DMDS	0.235 a	0.216 ab	0.212 ab	0.217 ab	0.190 b	0.229 a	0.195 b	0.233 a	0.222 ab
Benzaldehyde	0.700 b	0.856 ab	0.872 ab	0.760 b	0.966 a	0.876 ab	0.789 b	0.845 ab	0.854 ab

Table 3 Sensory profiles of rehydrated MPC70 spray dried at different inlet temperatures and feed solids concentrations. Interaction effects between solids concentration and inlet temperature were not significant ($p>0.05$). Means in the same column and shading followed by a different letter are different ($p<0.05$). ND—not detected.

Treatment	Aroma Intensity	Sweet Aromatic	Cooked	Cardboard	Fatty
160°C	1.6a	0.9c	2.0a	1.8a	1.1a
210°C	1.6a	1.1b	2.0a	1.5b	ND
260°C	1.5a	1.4a	2.0a	1.3b	ND
12%	1.5a	0.8b	2.0a	1.7a	1.4a
22%	1.6a	1.2a	2.0a	1.5ab	ND
32%	1.6a	1.4a	2.1a	1.3b	ND

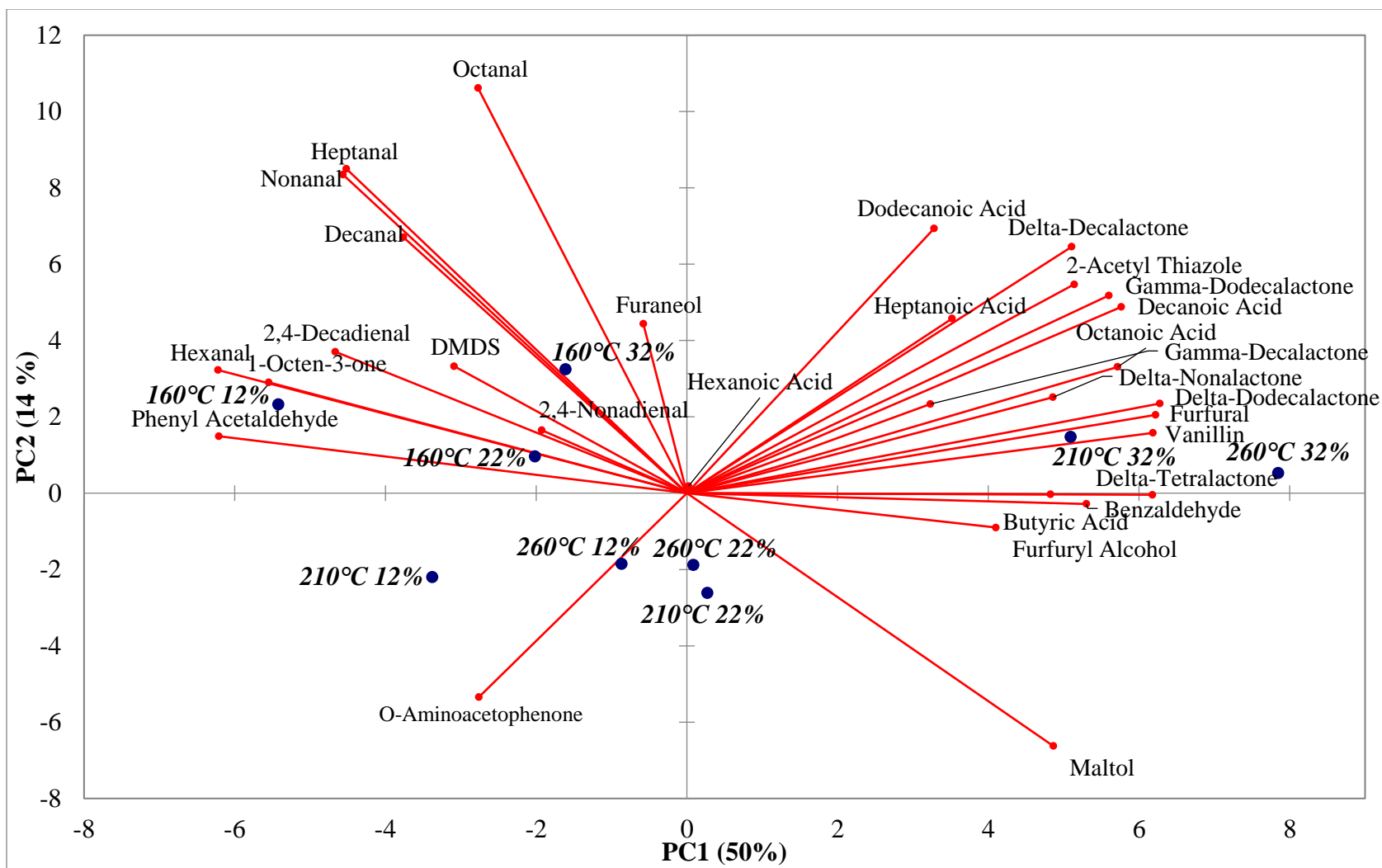


Figure 2 Principal component biplot of volatile compound analysis of rehydrated MPC70 spray dried under various conditions.

Table 4 Volatile compound analysis of rehydrated MPC70 ($\mu\text{g}/\text{kg}$). Means in the same column followed by a different letter indicate significant differences ($p < 0.05$). Interaction effects between solids concentration and inlet temperature were not significant for compounds highlighted in grey ($p > 0.05$).

Compound	160°C 12%	160°C 22%	160°C 32%	210°C 12%	210°C 22%	210°C 32%	260°C 12%	260°C 22%	260°C 32%
Furfuryl Alcohol	0.382 b	0.332 b	0.461 b	0.435 b	0.393 b	0.464 ab	0.507 ab	0.441 b	0.680 a
Furfural	0.430 abcd	0.317 cd	0.377 abcd	0.288 d	0.343 bcd	0.559 a	0.468 abc	0.370 abcd	0.545 ab
Hexanal	2.56 a	2.25 b	2.21 b	2.20 b	1.41 c	0.867 d	2.08 b	1.47 c	0.941 d
Heptanal	1.77 ab	1.58 bc	1.88 a	1.37 cd	0.895 ef	1.05 e	1.26 d	0.823 ef	0.806 f
Octanal	0.811 ab	0.748 b	0.889 a	0.731 bc	0.562 d	0.750 b	0.785 b	0.644 cd	0.717 bc
Nonanal	13.1 a	9.35 c	11.4 b	8.28 cd	6.03 f	6.03 f	7.26 de	6.16 ef	5.41 f
Decanal	1.21 a	0.611 b	0.619 b	0.583 b	0.411 b	0.609 b	0.532 b	0.584 b	0.625 b
Maltol	0.088 ab	0.036 b	0.052 ab	0.072 ab	0.063 ab	0.106 ab	0.094 ab	0.120 a	0.115 a
Furaneol	0.021 b	0.021 b	0.052 a	0.024 b	0.023 b	0.023 b	0.035 ab	0.029 b	0.023 b
Vanillin	0.013 c	0.011 c	0.012 c	0.011 c	0.014 c	0.025 b	0.013 c	0.015 c	0.032 a
Delta-Nonalactone	0.110 ab	0.092 b	0.113 ab	0.098 b	0.097 b	0.111 ab	0.106 b	0.116 ab	0.177 a
Delta-Decalactone	0.500 a	0.438 a	0.518 a	0.428 a	0.458 a	0.542 a	0.468 a	0.508 a	0.589 a
Delta-Dodecalactone	0.456 c	0.523 bc	0.599 abc	0.465 c	0.555 abc	0.735 ab	0.608 abc	0.606 abc	0.782 a
Delta-Tetralactone	0.365 d	0.486 cd	0.498 cd	0.381 d	0.571 abc	0.672 ab	0.600 abc	0.521 bcd	0.707 a
Gamma-Decalactone	0.096 abc	0.060 d	0.066 cd	0.082 bcd	0.061 d	0.093 abc	0.103 ab	0.058 d	0.115 a

Table 4 continued.

Gamma-Dodecalactone	0.136 bcd	0.138 bcd	0.153 abc	0.120 d	0.132 cd	0.169 ab	0.147 bcd	0.158 abc	0.182 a
2,4-Nonadienal	0.038 a	0.019 c	0.021 c	0.027 bc	0.018 c	0.021 c	0.037 ab	0.018 c	0.029 abc
2,4-Decadienal	0.054 a	0.034 b	0.038 b	0.039 b	0.033 b	0.036 b	0.041 b	0.036 b	0.030 b
Butyric Acid	0.950 cd	0.820 d	1.32 ab	0.873 d	0.864 d	1.55 a	0.976 cd	1.40 ab	1.22 bc
Hexanoic Acid	0.359 abcd	0.283 cd	0.468 a	0.263 d	0.284 bcd	0.479 a	0.268 cd	0.432 ab	0.398 abc
Heptanoic Acid	0.069 bc	0.057 cd	0.093 a	0.051 cd	0.060 c	0.100 a	0.041 d	0.091 a	0.087 ab
Octanoic Acid	0.262 b	0.217 b	0.285 b	0.203 b	0.230 b	0.268 b	0.216 b	0.330 b	0.640 a
Decanoic Acid	0.856 cd	0.871 bcd	1.10 a	0.805 d	0.780 d	1.02 abcd	0.877 bcd	1.09 ab	1.08 abc
Dodecanoic Acid	1.01 bc	1.21 abc	1.41 a	0.949 c	1.24 abc	1.34 ab	1.35 ab	1.35 ab	1.30 abc
1-Octen-3-one	0.116 ab	0.097 bc	0.094 bc	0.087 bc	0.066 c	0.060 c	0.136 a	0.061 c	0.059 c
2-Acetyl Thiazole	0.005 cd	0.002 e	0.010 b	0.003 de	0.003 de	0.011 ab	0.004 cde	0.007 bc	0.014 a
O-Aminoacetophenone	0.012 b	0.010 b	0.011 b	0.021 a	0.009 b	0.010 b	0.023 a	0.011 b	0.009 b
Phenyl Acetaldehyde	0.259 a	0.153 a	0.183 a	0.167 a	0.147 a	0.157 a	0.194 a	0.155 a	0.130 a
DMDS	0.098 a	0.085 a	0.097 a	0.080 a	0.087 a	0.084 a	0.080 a	0.098 a	0.074 a
Benzaldehyde	1.75 e	1.87 de	2.63 abc	2.43 bcde	1.94 cde	3.18 ab	3.30 a	2.59 bcd	2.63 abc

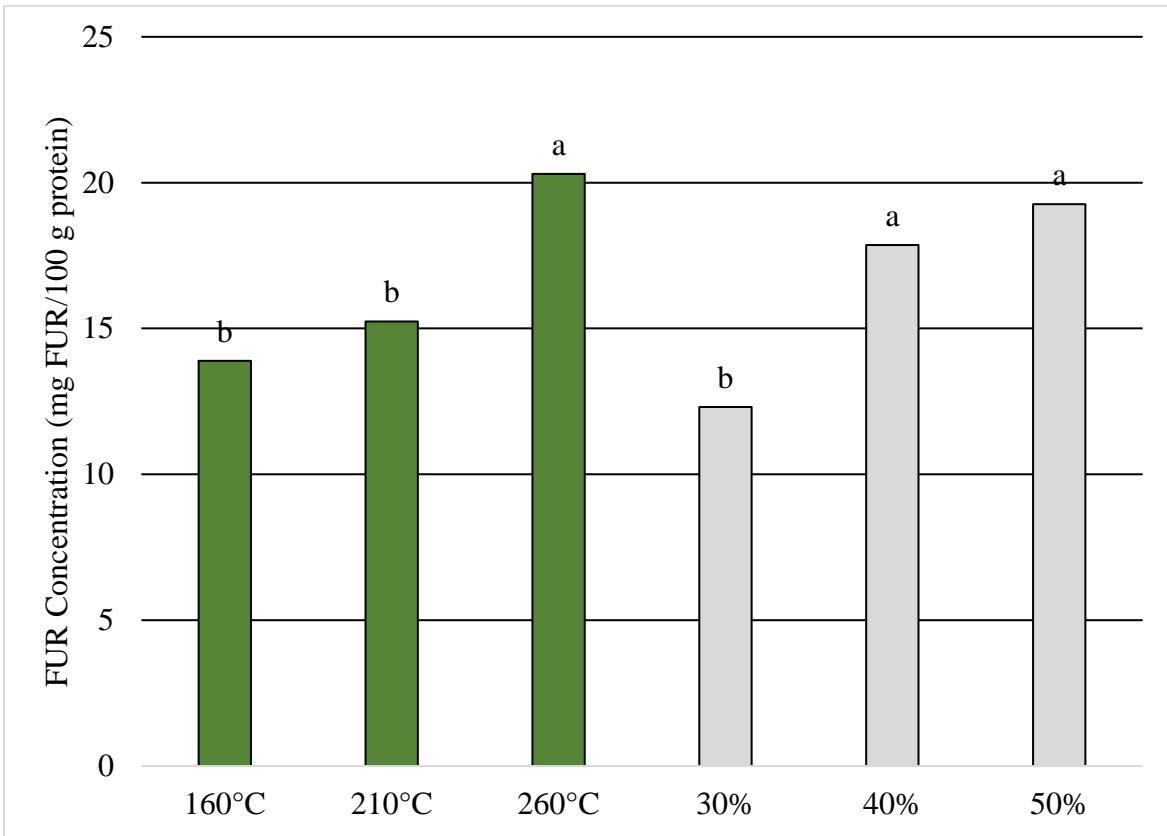


Figure 3 Furosine concentration (mg/100 g protein) in spray dried NFDm. Bars with the same shading and different lettering indicates a significant difference ($p > 0.05$). Interaction effects between solids concentration and inlet temperature were not significant ($p > 0.05$).

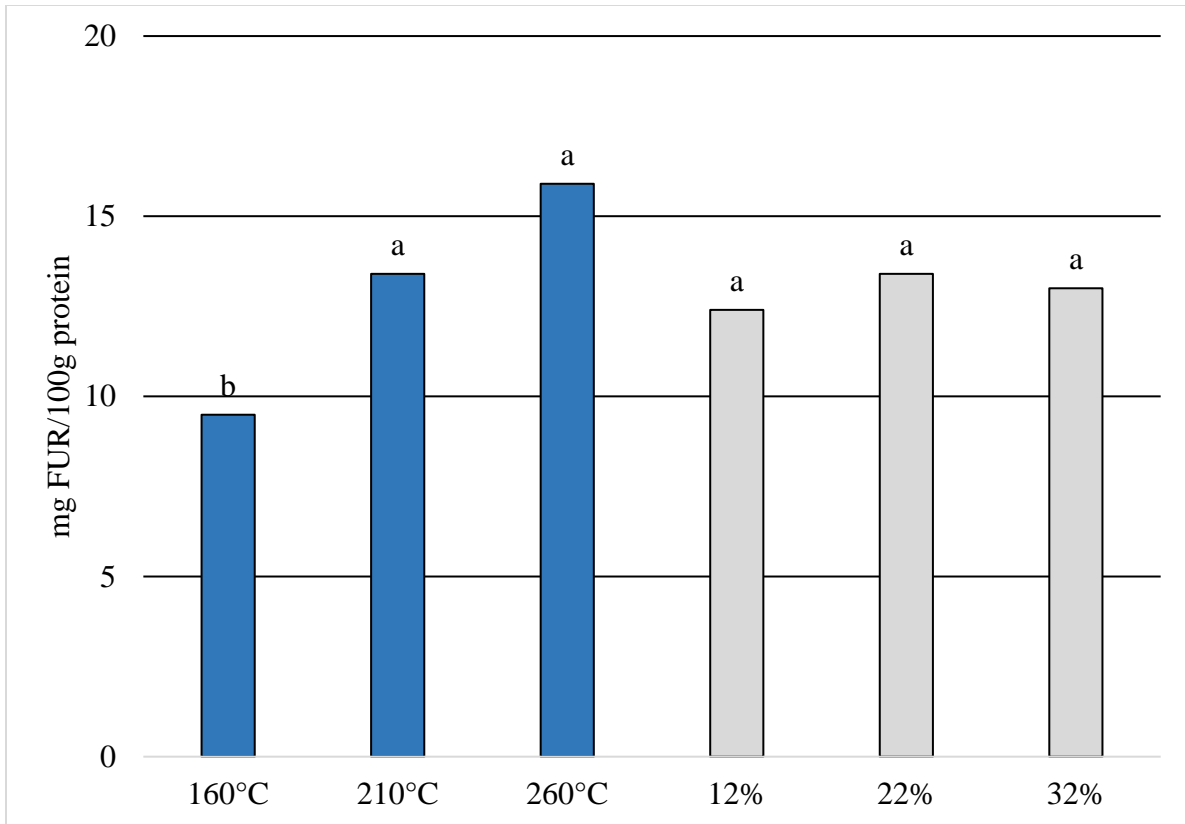


Figure 4 Furosine concentration (mg/100 g protein) in spray dried MPC70. Bars with the same shading and different lettering indicates a significant difference ($p > 0.05$). Interaction effects between solids concentration and inlet temperature were not significant ($p > 0.05$).

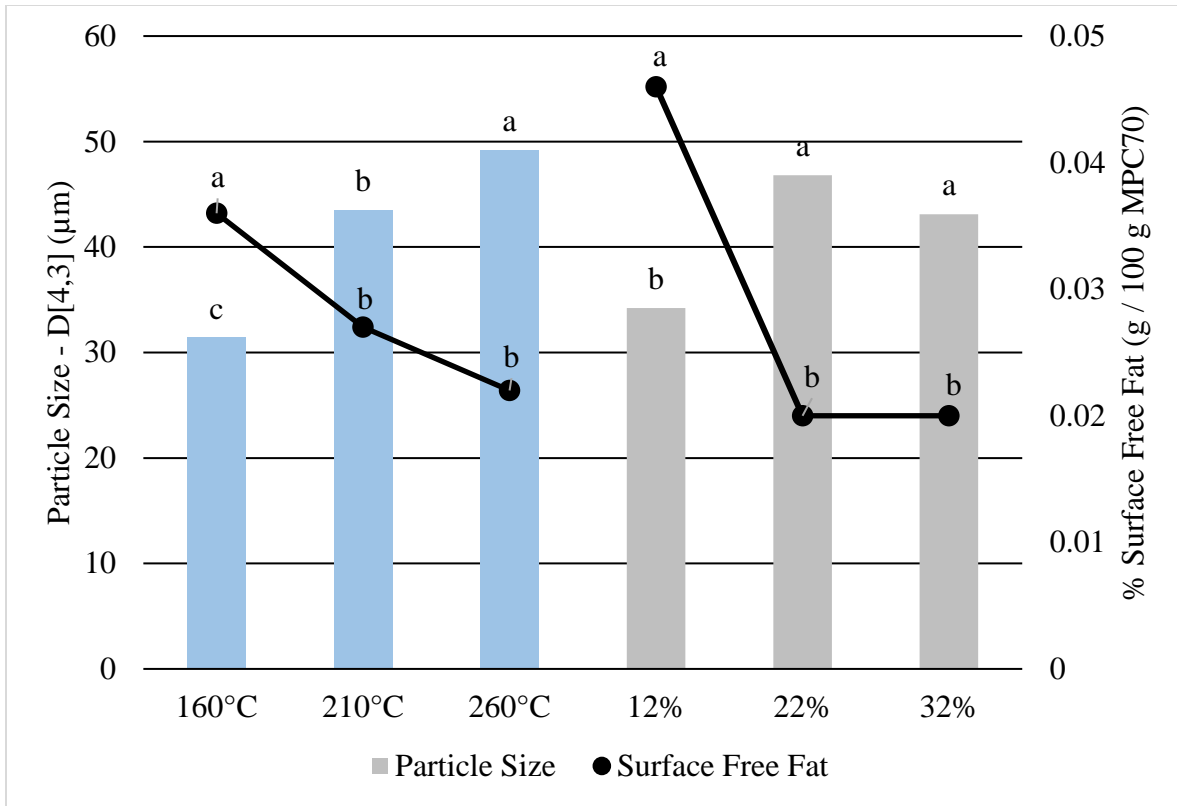


Figure 5 Particle size (D[4,3]) and surface free fat (g / 100 g MPC70) of spray dried MPC70. Bars with the same shading and different lettering indicates a significant difference ($p > 0.05$). Interaction effects between solids concentration and inlet temperature were not significant ($p > 0.05$).

Table 5 Surface free fat, particle size, and specific surface area of NFDM and MPC70 powders. Means in the same column followed by a different letter indicates a significant difference ($p < 0.05$). Interaction effects between solids concentration and inlet temperature were not significant ($p > 0.05$).

Treatment		NFDM		Treatment		MPC70		
Inlet Temperature	Surface Free Fat (g/100 g)	Dx (90) (μm)	D[4,3] (μm)	Inlet Temperature	Surface Free Fat (g/100 g)	Dx (90) (μm)	D[4,3] (μm)	Specific Surface Area (m^2/kg)
160°C	0.011a	77.6 c	35.1 c	160°C	0.036a	72.7 b	31.4 c	3080 a
210°C	0.011a	97.8 b	45.8 b	210°C	0.027b	93.3 a	43.5 b	2180 b
260°C	0.011a	105 a	62.5 a	260°C	0.022b	99.5 a	49.2 a	1850 c
Feed Solids Concentration				Feed Solids Concentration				
30%	0.011a	77.4 c	35.3 c	12%	0.046a	76.9 b	34.2 b	2660 a
40%	0.011a	85.8 b	40.1 b	22%	0.020b	96.9 a	46.8 a	2140 b
50%	0.011a	118 a	68.0 a	32%	0.020b	91.7 a	43.1 a	2300 b

**CHAPTER 5: THE EFFECT OF HOMOGENIZATION PRESSURE ON
THE FLAVOR AND FLAVOR STABILITY OF WHOLE MILK
POWDER**

The effect of homogenization pressure on the flavor and flavor stability of
whole milk powder

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* Use of names, names of ingredients, and identification of specific models of equipment is for scientific clarity and does not constitute any endorsement of product by authors, North Carolina State University, or the Southeast Dairy Foods Research Center.

ABSTRACT

Flavor is a limiting factor in the application and shelf life of dried dairy ingredients. Many off-flavors are caused during ingredient manufacture which carry through into ingredient applications and decrease consumer acceptance. The objective of this research was to investigate the effect of homogenization pressure on the flavor and flavor stability of whole milk powder (WMP). WMP was produced from standardized pasteurized whole milk that was evaporated to 50% solids (w/w), homogenized in two stages with varying pressures (0/0, 5.5/1.4, 11.0/2.8, or 16.5/4.3 MPa), and spray dried. WMP was evaluated at 0, 3, and 6 mo storage at 21°C. Sensory properties were evaluated by descriptive analysis. Volatile compound analyses were analyzed by sorptive stir bar extraction (SBSE) with gas chromatography mass spectrometry (GC-MS). Fat globule size in condensed whole milk and particle size of powders were measured by laser diffraction. Surface free fat, inner free fat, and encapsulated fat of WMP were measured by solvent extractions. Phospholipid content was measured by UPLC-ELS. Furosine in WMP was analyzed by UPLC-MS. Increased homogenization pressure decreased cardboard and painty flavors, volatile lipid oxidation compound concentrations, fat globule size in condensed milk, surface free fat, and inner free fat in WMP. Encapsulated fat increased and phospholipid to encapsulated fat ratio decreased with higher homogenization pressure. Surface free fat in powders increased cardboard flavor and lipid oxidation. These results indicate that off-flavors are decreased throughout shelf-life with increased homogenization pressures in WMP due to the decrease in free fat. To

decrease off-flavor intensities in WMP, manufacturers should carefully evaluate these parameters during ingredient manufacture.

Key Words: homogenization, flavor, whole milk powder

INTRODUCTION

Whole milk powder (WMP) is produced using the following unit operations: fat standardization, pasteurization, evaporation, homogenization, and spray drying. The resulting powder must be between 26 and 40% fat and <5% moisture (USDEC, 2005). The shelf-life of WMP is generally 6 to 9 mo when stored <27°C and <65% relative humidity (USDEC, 2005). Flavor of WMP is critical because it is the number one factor influencing consumer acceptance of WMP applications (Lloyd et al., 2009b; Hough et al., 2002).

Typical flavors of fresh WMP include milk fat, cooked, caramelized, and sweet aromatic while off-flavors such as grassy, painty, and cardboard develop during storage due to lipid oxidation (Lloyd et al., 2009a; Lloyd et al., 2009b). Because lipid oxidation is the primary source of off-flavors and reduced shelf-life in WMP, it has been studied extensively. Lipid oxidation in WMP occurs more readily than nonfat dry milk due to its higher fat content. The flavor of WMP produced in the United States is highly variable with lipid oxidation being the main source of off-flavors (Lloyd et al., 2009a). Sources of flavor variability among manufacturers of WMP are likely differences in how they are processed. Factors that affect lipid oxidation and shelf-life of WMP include animal feed quality, raw milk storage, heat treatment, storage conditions, water activity, and packaging conditions (Lloyd et al., 2009b; Stapelfeldt et al., 1997; Hall and Lingnert, 1984; McCluskey et al., 1997).

During spray drying, fat migrates to the surface at the expense of protein and lactose due to its hydrophobic nature (Kim et al., 2009b). Free fat is defined as fat that is not entirely coated/stabilized by amphiphilic molecules (i.e. phospholipids or protein) or protected by amorphous carbohydrates or proteins during spray drying (Vignolles et al., 2007). Spray drying parameters significantly influence the amount of free fat on the surface of the whey protein, milk protein, and milk powders (Park et al., 2014; Vignolles et al., 2010; Kim et al., 2009a). Keogh and O’Kennedy (1999) observed that higher lactose:protein ratios decreased the aggregation of fat globules and free fat in spray dried whey protein stabilized milk fat emulsions. Crystallization of lactose during storage increases free fat by increasing solvent accessibility to the fat (Saito, 1985). During WMP manufacture, homogenization is performed to decrease the fat globule size. Because homogenization of condensed whole milk prior to spray drying decreases fat globule size it decreases the migration of fat to the surface of WMP (Vignolles et al., 2007). During homogenization, the milk fat globule membrane ruptures and milk proteins and phospholipids interact with the newly formed smaller fat droplets (Ye et al., 2004; Vignolles et al., 2007; Ye et al., 2008). Increased homogenization pressures have been reported to increase the amount of protein on the surface of the milk fat droplets in condensed whole milk (Ye et al., 2008). The increased protein on the surface of the fat droplets along with the smaller size due to homogenization decreases the amount of free fat in the spray dried WMP (Tamsma et al., 1959). Spray drying conditions also cause a change in the fat droplet size as well as amount of adsorbed proteins on the surface (Vignolles et al., 2010; Ye et al., 2007).

Increased free fat has been associated with increased lipid oxidation in dried dairy ingredients (Park et al., 2014; Keogh and O’Kennedy, 1999). Because off-flavors in dried

dairy ingredients are directly related to lipid oxidation, it is hypothesized that off-flavors increase with increased free fat of dairy powders (Park and Drake, 2014; Park et al., 2014). Keogh and O’Kennedy (1999) related free fat and fat globule size to flavor in spray dried whey protein stabilized dairy emulsions with decreased free fat in general resulting in decreased off-flavor levels. To our knowledge, there is a lack of published work determining the effect that homogenization conditions have on the flavor and flavor stability of WMP. The objective of this work was to determine the effect of homogenization pressure of condensed whole milk (50% solids) on the flavor of WMP throughout shelf-life.

MATERIALS AND METHODS

WMP Manufacture

Raw bovine whole milk (310 kg) and raw skim milk (80 kg) were obtained from the North Carolina State University Dairy Research and Education Unit. The whole milk was standardized to a milk solids nonfat to fat ratio of 2.48 using skim milk. The percent fat and solids were measured using the Smart Turbo moisture/solids analyzer and Smart Trac II (CEM, Matthews, NC) with the percent solids nonfat being calculated as total solids - fat. The standardized milk was pasteurized at 73°C for 16 s using a plate heat exchanger (model T4 RGS-16/2, SPX Flow Technology, Greensboro, NC). The milk was subsequently cooled to 4°C and stored in a bulk tank with constant stirring until introduction into the evaporator.

Evaporation was performed on a single effect pilot scale falling film evaporator. The milk was pre-heated to 50°C upon introduction into the evaporator. The calandria temperature was 71°C with a vacuum of 74.5 kPa. The condensed whole milk exiting the evaporator was 50% solids and at a temperature of 60°C. The solids content was confirmed using the Smart Turbo moisture/solids analyzer (CEM). The condensed whole milk was then

homogenized with a two stage homogenizer (model NS2006H, GEA Niro Soavi, Parma, Italy) at one of the following pressures: 0/0, 5.51/1.38, 11.0/2.76, or 16.5/4.14 MPa at 60°C. The order of treatments was completely randomized. The condensed whole milk was then spray dried with a pilot scale spray dryer (model Lab 1, Anhydro Inc., Soeborg, Denmark) with an inlet temperature of 200°C and an outlet temperature of 95°C. The entire experiment was replicated 3 times.

Storage and Sampling

The fresh WMP was placed in Mylar bags (TF-4000, Impak Corp., Central City, S.Dak., U.S.A.) and heat sealed until analysis. Storage conditions were 21°C and 50% relative humidity. WMP was sampled at 0, 3, and 6 mo post manufacture. A new bag was used for each sampling time point.

Proximate Analysis

Percent fat and moisture of the fresh WMP were measured using ether extraction (AOAC International 2000; method 932.06) and a vacuum oven (AOAC International 2000; method 990.20) respectively.

Descriptive Sensory Analysis

Flavor of the WMP at all timepoints was evaluated using descriptive sensory analysis in compliance with the North Carolina State University Institutional Review Board for Human Subjects guidelines. The WMP was rehydrated to 10% solids nonfat with deionized water using a handheld blender as described by Lloyd et al. (2009b). WMP solutions were dispensed into 60 ml soufflé cups (Solo Cup, Highland Park, IL), lidded, and tempered to 21°C prior to evaluation. A trained panel (n=8; 6 females, 2 males, ages 22-47 y) with > 200 h of experience with descriptive analysis of dried dairy ingredients evaluated the samples

using an established lexicon (Drake et al., 2003; Lloyd et al., 2009a). Each panelist evaluated each rehydrated WMP in duplicate at each timepoint. Compusense Cloud (Compusense, Guelph, ON, Canada) was used to collect the data.

Volatile Compound Analysis

WMP was sampled at each timepoint for volatile compound analysis. Volatile flavor compounds were extracted by sorptive stir bar extraction (SBSE; Park et al., 2016). Prior to analysis, stir bars (PDMS, 10 x 0.5 mm; Gerstel Inc., Linthicum, MD) were cleaned by soaking 10 stir bars in 40 ml of a 1:1 mixture of methanol and methylene chloride for 4 h, air drying in a fume hood for 2 h, and conditioning at 280°C for 1 h with 75 ml/min nitrogen gas. WMP was rehydrated to 10% solids nonfat with HPLC grade water as performed for sensory analysis. Selected volatile compounds (Lloyd et al., 2009b; Karagul-Yuceer et al., 2001) were extracted as described by Park et al. (2016). Next, 5 ml of rehydrated WMP was placed in a 10 ml amber screw top vial with a Teflon lined lid (Gerstel Inc.) along with 10 µl of internal standard (0.81 mg/L 2-methyl-3-heptanone in water; Sigma Aldrich, St. Louis, MO). In order to more efficiently extract multiple classes of compounds, sequential stir bar extraction was employed. The first stir bar was placed into the vial, lidded, and then stirred for 1 h at 800 rpm at room temperature. The stir bar was removed, briefly rinsed with water, and placed in a thermal desorption unit (TDU) tube. Next, 1 g of NaCl was added to the vial and another stir bar was added and then stirred again for 1 h at 800 rpm at room temperature. After stirring, the second stir bar was removed, rinsed briefly with water, and placed in the same TDU tube as the first stir bar.

The TDU tubes with stir bars were injected using an autosampler (MPS Autosampler, Gerstel, Inc.). The tubes were heated to 250°C for 10 min in a TDU (Gerstel Inc.) with

cryogenic trapping of the compounds at -120°C (CIS 4, Gerstel Inc.). The volatile compounds were injected onto an Agilent 7890B GC (Agilent Technologies, Santa Clara, CA) and separated using a non-polar column (ZB-5MS, 30 m x 0.25 mm x 0.25µm; Phenomenex, Torrence, CA). Oven conditions were as described by Park et al. (2016). Helium was used as the carrier gas with a column flow rate of 1 ml/min and a purge time of 1.2 min. Compounds were detected with an inert mass selective detector (model 5970A, Agilent) using selective ion monitoring mode. Compounds were identified using the 2014 NIST mass spectral library (NIST, 2014), retention index, and retention time of authentic standards injected under identical conditions.

Fat Globule Size, and Particle Size

Particle size of the dry WMP at time 0 only was measured using a Mastersizer 3000 Particle Size Analyzer (Malvern, Malvern, UK) with the Aero S dry powder dispenser. Fat globule size in the condensed whole milks (50% solids) prior to spray drying was measured with the Mastersizer 3000 with the Hydro EV attachment with water (40°C; refractive index 1.33) as the dispersant. The refractive index used for milk fat was 1.46.

Milk Fat Fractions and Phospholipids

Surface free fat (SFF) was measured in WMP at every timepoint following the method described by GEA Niro (2005; method No. 10a). Inner free fat (IFF) and encapsulated fat (EF) were measured as described by Kim et al. (2009b) with minor modifications. After extracting the surface free fat, 1 g of WMP was weighed and mixed with 40 ml of hexane. The solutions were shaken frequently for 48 h, filtered, and dried. The inner free fat was measured gravimetrically. The powder from the filter was dried in a fume hood and approximately 0.5 g were mixed for 15 min with 22.5 ml of 3:1

hexane:isopropanol (v/v). Afterwards, they were centrifuged and the clear upper layer was extracted. This was repeated one more time and the extracts were pooled together. The encapsulated fat was measured gravimetrically.

To quantify phospholipids in the different fat fractions, the extracted fats were re-dissolved in 1 ml in a mixture of chloroform and methanol (2:1 v/v). Neutral lipids were removed by solid phase extraction as described by Donato et al. (2011). The final polar lipid residue was dissolved in chloroform/methanol (2:1, v/v). The polar lipid extracts were filtered with a 0.2 μm PTFE filter into amber HPLC vials. The polar lipids were separated by UPLC using an evaporative light scattering (ELS) detector with a drift tube temperature of 85°C and nitrogen gas flow rate of 1.75 L/min (25 psi) (Acquity H-Class, Waters Corporation, Milford, MA). A HILIC column was used (BEH HILIC 1.7 μm 150 x 2.1 mm; Waters Corporation) with a column temperature of 30°C. Compounds of interest were quantified using standard curves ranging from 2 $\mu\text{g}/\text{ml}$ to 5 mg/ml. Gradient elution was used with mobile phase A as 100% acetonitrile, B as 100% water, and C as 200mM ammonium acetate adjusted to pH 5.5 with acetic acid. The gradient conditions are shown in Table 1.

Furosine Analysis

Furosine was measured as described by Resmini et al. (1990) with minor modifications. Approximately 230 mg of WMP was dissolved in 2 ml of HPLC grade water and placed in a glass tube with a Teflon lined lid. Next, 6 ml of 10.6N HCl was added and nitrogen gas was bubbled through the sample for 1 min. Tubes were lidded and heated to 110°C for 23 h. Upon cooling, the samples were diluted 1:10 with HPLC grade water, centrifuged at 14,000 rpm for 10 min, and placed into HPLC autosampler vials

(Phenomenex). Furosine was detected using UPLC-MS. A C18 column was used (HSS T3, 2.1 x 100 mm, 1.7 μ m; Waters Corporation, Milford, MA) with a mobile phase of 0.1% formic acid in water run at 40°C at a flow rate of 0.5 ml/min. Furosine was detected using a single quadrupole mass spectrometer (SQ Detector 2, Waters) in ES+ mode using a mass of 255.03. Capillary and cone voltages were 2.5 kV and 25V respectively. A standard curve was constructed in raw milk that was determined to have no detectable furosine.

Concentrations ranged from 0 to 500 mg furosine/100 g protein.

Statistical Analysis

Two-way analysis of variance with means separation was used to analyze the data (XL Stat, Addinsoft, New York, NY). Interaction effects between homogenization pressure and storage time were investigated. Fisher's least significant difference test was used to determine differences among samples.

RESULTS

The percent moisture and fat in the WMP was $3.28 \pm 0.18\%$ and $27.3 \pm 0.33\%$ respectively and were not different among treatments ($p > 0.05$). The varying homogenization treatments affected the flavor and flavor stability of the WMP. Interaction effects were significant between homogenization pressure and storage time ($p < 0.05$). The flavor profiles of the various homogenization treatments were distinct (Table 2; Figure 1). In fresh WMP, 0 MPa homogenization decreased aroma intensity and caramelized flavor decreased in the order 20.8 MPa and 13.8 MPa > 6.9 MPa > 0 MPa ($p < 0.05$). Cardboard flavor was only detected in the 0 MPa treatment in fresh WMP. Sweet aromatic was only detected in the 13.8 and 20.8 MPa treatments in fresh WMP. At 3 mo storage, 0 MPa homogenization decreased cooked flavor and increased cardboard and grassy flavors compared to all other

treatments ($p < 0.05$). The 13.8 MPa treatment decreased cardboard and grassy flavors compared to all other treatments ($p < 0.05$). Grassy flavor was detected at 3 mo storage but was not detected in any of the treatments at 0 or 6 mo storage. At 6 mo storage, painty flavor was detected in the 0 and 6.9 MPa treatments with an increase observed in 0 MPa WMP ($p < 0.05$). Cardboard flavor intensity increased in the following order: 13.8 and 20.8 MPa < 6.9 MPa < 0 MPa ($p < 0.05$). Caramelized and cooked flavors decreased at each timepoint across 6 mo storage for all treatments except caramelized flavor for the 0 MPa treatment ($p < 0.05$). These sensory changes are consistent with those reported by Lloyd et al. (2009a; 2009b) for commercial U.S. WMP. U.S. WMP are characterized by milkfat, cooked, and caramelized flavors initially followed by development of grassy and then painty flavors as the WMP is stored and lipid oxidation occurs (Lloyd et al., 2009a; 2009b).

Volatile compound profiles were also distinct and were consistent with sensory profiles (Table 2; Figure 2). Interaction effects between homogenization pressure and storage time were significant ($p < 0.05$). Initially, there were few differences in volatile compound concentrations. In fresh WMP, 0 MPa homogenization increased maltol and furaneol concentrations compared to all other treatments, decanal compared to 13.8 or 20.8 MPa, and phenyl acetaldehyde compared to 6.9 MPa ($p < 0.05$). After 3 mo, 0 MPa homogenization increased hexanal and heptanal compared to all other treatments, phenyl acetaldehyde compared to 13.8 MPa, and decanal compared to 13.8 or 20.8 MPa ($p < 0.05$). Vanillin concentration decreased at 3 mo storage when WMP was homogenized at 0 or 6.9 MPa compared to 13.8 or 20.8 MPa ($p < 0.05$). At 6 mo storage, 0 MPa homogenization increased concentrations of octanal, nonanal, decanal, 2,4-nonadienal, E2-nonenal, E2-decenal, 1-octen-3-one, 3-octen-2-one, o-aminoacetophenone, gamma-decalactone, and

gamma-dodecalactone compared to all other treatments ($p < 0.05$). Homogenization at 138 or 20.8 MPa decreased concentrations of 2-heptanone compared to 0 or 6.9 MPa and delta-tetralactone compared to 0 MPa ($p < 0.05$). The concentrations of hexanal, heptanal, and 2,4-decadienal were in the order 0 MPa > 6.9 MPa > 138 and 20.8 MPa ($p < 0.05$).

Homogenization of WMP at 13.8 MPa decreased E,Z-3,5-octadien-2-one and E,E-3,5-octadien-2-one compared to 0 or 6.9 MPa, and homogenization at 20.8 MPa decreased E,Z-3,5-octadien-2-one and E,E-3,5-octadien-2-one compared to 0 MPa ($p < 0.05$). Lloyd et al. (2009a) reported that increased 3-methylbutanal, 2-methylbutanal, hexanal, octanal, and 3-octen-2-one concentrations were predictors of painty flavor development in WMP. We observed that the 0 MPa treatment, the sample with the highest painty flavor intensity at 6 mo storage, also had the highest concentrations of hexanal and octanal after 3 mo storage and the highest 3-octen-2-one concentration at 6 mo storage. With SSBE we were able to extract more volatile compounds than Lloyd et al. (2009a), including heavier molecular weight compounds, because the stir bars were immersed into the liquid. With SPME, compounds must be in the headspace of the sample vial and therefore heavier molecular weight compounds are extracted less efficiently. Examples of compounds we measured throughout storage that Lloyd et al. (2009b) did not, include lactones, vanillin, and o-aminoacetophenone.

Storage, as expected, affected the volatile compound profile of each treatment. In general, lipid oxidation products increased with increasing storage time, similar to what was observed by Lloyd et al. (2009b) who used SPME. Specifically, hexanal, heptanal, octanal, o-aminoacetophenone, phenyl acetaldehyde, and lactones increased in all treatments at each subsequent time point ($p < 0.05$). Also, E,Z-3,5-octadien-2-one, E,E-3,5-octadien-2-one, 3-

octen-2-one, and 2-heptanone were only detected after 6 mo storage. In the 0 MPa WMP, decanal, 2,4-nonadienal, 2,4-decadienal, E2-nonenal and E2-decenal increased at every time point and nonanal and 1-octen-3-one increased after 6 mo storage ($p < 0.05$). In the 6.9 MPa WMP, nonanal, 2,4-nonadienal, and 1-octen-3-one increased with increasing storage and 2,4-decadienal, E2-nonenal, and E2-decenal increased and vanillin decreased after 6 months storage ($p < 0.05$). When homogenized at either 13.8 or 20.8 MPa, 6 mo storage increased nonanal, decanal, 2,4-nonadienal, 2,4-decadienal, E2-nonenal, E2-decenal, and 1-octen-3-one concentrations in WMP compared to 0 or 3 months storage ($p < 0.05$).

Furosine (FUR) is an indicator of Maillard reactions in dairy products and can be used to measure heat treatment (Mehta and Deeth, 2015). When dairy products are heated, lysine residues from proteins react with lactose, a reducing sugar, to form lactulosyl-lysine which is converted to FUR by acid hydrolysis (Mehta and Deeth, 2015). Both homogenization and storage time affected the FUR content in WMP (Table 3) and interaction effects between homogenization and storage time were not significant ($p > 0.05$). No homogenization (0 MPa) increased FUR compared to 6.9 or 20.8 MPa (51.2 vs 46.7 vs 47.3 mg FUR/100g protein; $p < 0.05$). As expected, FUR increased with 6 mo storage compared to 0 or 3 mo (55.2 vs 44.7 vs 45.8 mg FUR/100g protein; $p < 0.05$). FUR has been reported to increase during storage of dried dairy ingredients, even at ambient temperatures (Le et al., 2011; Smith et al., 2016).

Particle size analysis indicated differences among treatments before and after spray drying (Table 4, Table 5). As expected, fat globule size decreased with increased homogenization pressure in the order 0 MPa > 6.9, 13.8 MPa > 20.8 MPa ($p < 0.05$) as observed in the D(90) (4.56 vs 2.10 vs 1.94 vs 1.71 μm) and D[4,3] (1.71 vs 0.85 vs 0.83 vs

0.77 μm). In the spray dried WMP, the D(90) particle size decreased in the 6.9 MPa treatment compared to 0, 13.8, and 20.8 MPa (118 vs 130 vs 130 vs 128 μm ; $p < 0.05$).

Varying the homogenization pressure also affected the distribution of milk fat in the spray dried WMP (Table 6). Interaction effects between homogenization pressure and storage time were not significant and storage time did not affect milk fat distribution ($p > 0.05$). SFF decreased with increasing homogenization pressure in the order 0 MPa > 6.9 MPa > 13.8 MPa, 20.8 MPa (5.16 vs 3.32 vs 2.22 vs 1.85 g SFF/100g WMP; $p < 0.05$). IFF decreased when WMP was homogenized at 13.8 or 20.8 MPa compared to 0 or 6.9 MPa (1.79 vs 1.67 vs 3.62 vs 3.12 g IFF/100g WMP; $p < 0.05$). Milk fat encapsulation increased with increasing homogenization pressure in the order 13.8, 20.8 MPa > 6.9 MPa > 0 MPa (23.5 vs 24.1 vs 20.2 vs 17.1 g EF/100g WMP; $p < 0.05$). Phospholipids were not detected in the SFF or IFF fractions. In the EF fraction, g phospholipids/100 g EF increased with decreasing homogenization pressure in the order 20.8, 13.8 MPa < 6.9 MPa < 0 MPa (0.77 vs 0.88 vs 1.25 vs 1.75; $p < 0.05$).

DISCUSSION

Previous work has demonstrated that lipid oxidation is the primary factor in increased off-flavors in WMP (Lloyd et al., 2009a; Lloyd et al., 2009b; Hall and Lingnert et al., 1984). Increases in lipid oxidation during storage of WMP, like we observed, have also been documented extensively (Lloyd et al., 2009b; Stapelfeldt et al., 1997; Ulberth and Roubicek, 1995; Celestino et al., 1997). The off-flavors associated with lipid oxidation include cardboard, grassy, and painty (Lloyd et al., 2009a; Whitson et al., 2010). Lipid oxidation is slowed in WMP by a number of steps. First, storage of raw milk should be of high quality by

using high quality animal feed (McCluskey et al., 1997) and extended storage of raw milk should be avoided (Celestino et al., 1997). Increased heat treatment during the manufacture of WMP decreases lipid oxidation (Stapelfeldt et al., 1997; McCluskey et al., 1997). Calligaris et al. (2004) demonstrated that the anti-oxidant activity in milk decreased with short heat treatments and increased with more prolonged heat treatments. The increase in anti-oxidant activity with longer heat treatments is due to the unfolding of proteins and the exposure of sulfhydryl groups (Calligaris et al., 2004; Tong et al., 2000). Elevated temperature and water activity also increase lipid oxidation throughout storage (Stapelfeldt et al., 1997). Storage conditions also play a large role in the flavor stability of WMP. Lloyd et al. (2009b) demonstrated that flushing the WMP packaging with nitrogen significantly improved the shelf-life and reduced lipid oxidation throughout 1 y of storage. The WMP in this study was given a mild heat treatment during pasteurization (73°C 16 s) and thus limited the potential anti-oxidant activity. The pasteurization temperature that we used is similar to temperatures used by U.S. WMP manufacturers. We observed a 6 mo shelf-life which is consistent with the shelf-life of U.S. WMP as reported by Lloyd et al. (2009a). Sensory and volatile compound profiles initially and across shelf-life for the 13.8 MPa treatment were consistent with commercial U.S. WMP (Lloyd et al., 2009a).

It has been hypothesized that the distribution of milk fat influences flavor and lipid oxidation in dried dairy particles (Park and Drake 2014; Park et al., 2014). Similar to what we observed in this study, Vignolles et al. (2010) observed that homogenization of milk emulsions decreased the free fat content in the resulting spray dried powders regardless of the total fat content. Also, similar to our study, they observed decreased fat globule size with 20 MPa homogenization, but did not test multiple homogenization pressures as we did. The

decreases in free fat that we observed with higher homogenization pressure can be attributed to the decrease in fat globule size and the encapsulation of the fat with proteins and phospholipid, as observed in the increased amount of encapsulated fat. The smaller fat globule size also decreased the surface free fat because it reduces the creaming velocity of the fat in the atomized droplets during spray drying.

The increase in milk fat encapsulation observed in higher homogenization pressures most likely decreased lipid oxidation due to the anti-oxidant activity of the proteins adsorbed onto the surface of the fat droplets (Lethuaut et al., 2002). Park et al. (2014) reported that in spray dried WPC80, lipid oxidation products increased in powders with increased surface free fat. Lipid oxidation in polyunsaturated fatty acids is reduced when free fat content is reduced by means of microencapsulation (Gharsallaoui et al., 2007). Homogenization of condensed whole milk, as performed in this study, could be seen as a microencapsulation step because increasing amounts of proteins are adsorbed onto the surface of the milk fat globules (Ye et al., 2004). We observed, similar to Park et al. (2014) in WPC80, that surface free fat increased lipid oxidation and cardboard flavor in WMP. Park et al. (2014) did not evaluate the spray dried WPC80 throughout shelf-life. We have demonstrated that not only is lipid oxidation elevated initially with increased surface free fat but that it also increases lipid oxidation throughout shelf-life.

Decreased particle size has been reported to increase surface free fat (Park et al., 2014). While we observed decreased particle size in the 6.9 MPa treatment, it did not increase surface free fat compared to the 0 MPa treatment. This is most likely due to the homogenization that occurred and the relatively small difference in particle size (118 vs 130

µm). These results indicate that spray drying parameters influence the particle size of the powders more than homogenization (Park et al., 2014; Kim et al., 2009b).

Native phospholipids in dairy products have been reported to have both pro- and anti-oxidant activity. The anti-oxidant activity is mainly due to their ability to chelate pro-oxidant metals (Chen and Nawar, 1987). The pro-oxidant activity of phospholipids is twofold. First, there is a high degree of unsaturation in the fatty acids associated with phospholipids (Sessa, 1985). Second, phospholipids reduce the surface tension of the fat droplets which allows for greater oxygen uptake and therefore increased lipid oxidation (Choe and Minn, 2006). While the total phospholipids was not different among treatments, the ratio of phospholipid to encapsulated fat increased in the 0 and 6.9 MPa treatments due to decreased total encapsulated fat. The increased phospholipids around the encapsulated fat could partially explain the increased lipid oxidation and off-flavors in WMP homogenized at 0 or 6.9 MPa compared to 13.8 or 20.8 MPa. The increase in encapsulated fat in the 13.8 and 20.8 MPa treatments could have also decreased lipid oxidation because of the proteins adsorbed on the surface of the fat globules. Milk proteins have been reported a high antioxidant activity (Cervato et al., 1999; Tong et al., 2000).

FUR is an indicator of early Maillard reactions and can be used as an indicator of heat treatment in dairy products. In this study we observed that homogenization affected the FUR content in the spray dried WMP very little. While 0 MPa was statistically higher than 6.9 and 20.8 MPa ($p < 0.05$), the greatest difference was only 4.5 mg FUR/100g protein. As expected, storage increased FUR levels in WMP, which has been reported in other dried dairy ingredients (Le et al., 2011; Smith et al., 2016). While increased Maillard reaction products have been reported as having anti-oxidant activity, they are brown melanoidins

which are late Maillard products as opposed to FUR which is an indicator of early Maillard reactions (Calligaris et al., 2004). As such, the increased Maillard reactions observed in the 0 MPa treatment and after 6 mo storage for all treatments would not be expected to impact the antioxidant activity, and therefore flavor stability of the resulting WMP.

CONCLUSION

Homogenization pressure significantly affects the flavor and flavor stability of WMP. Our results indicate that WMP flavor stability was improved with increased homogenization pressure and decreased fat globule size in the condensed whole milk. This led to decreased free fat and an increase in fat encapsulation. Particle size of the resulting WMP was not affected by homogenization. Homogenization had little effect on Maillard reactions as observed in furosine levels in the WMP. Manufacturers of WMP should continually evaluate the effectiveness of their homogenizer to improve the flavor and shelf-life of their product with the goal of smaller fat globule size in their condensed whole milk.

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Table 1 Gradient conditions for UPLC analysis of phospholipids. Mobile phases: A—acetonitrile, B—water, C—200 mM ammonium acetate in water pH 5.5.

Time (min)	Flow (ml/min)	%A	%B	%C
0	0.6	95	0	5
2	0.6	95	0	5
12	0.6	73	22	5
13	0.6	95	0	5

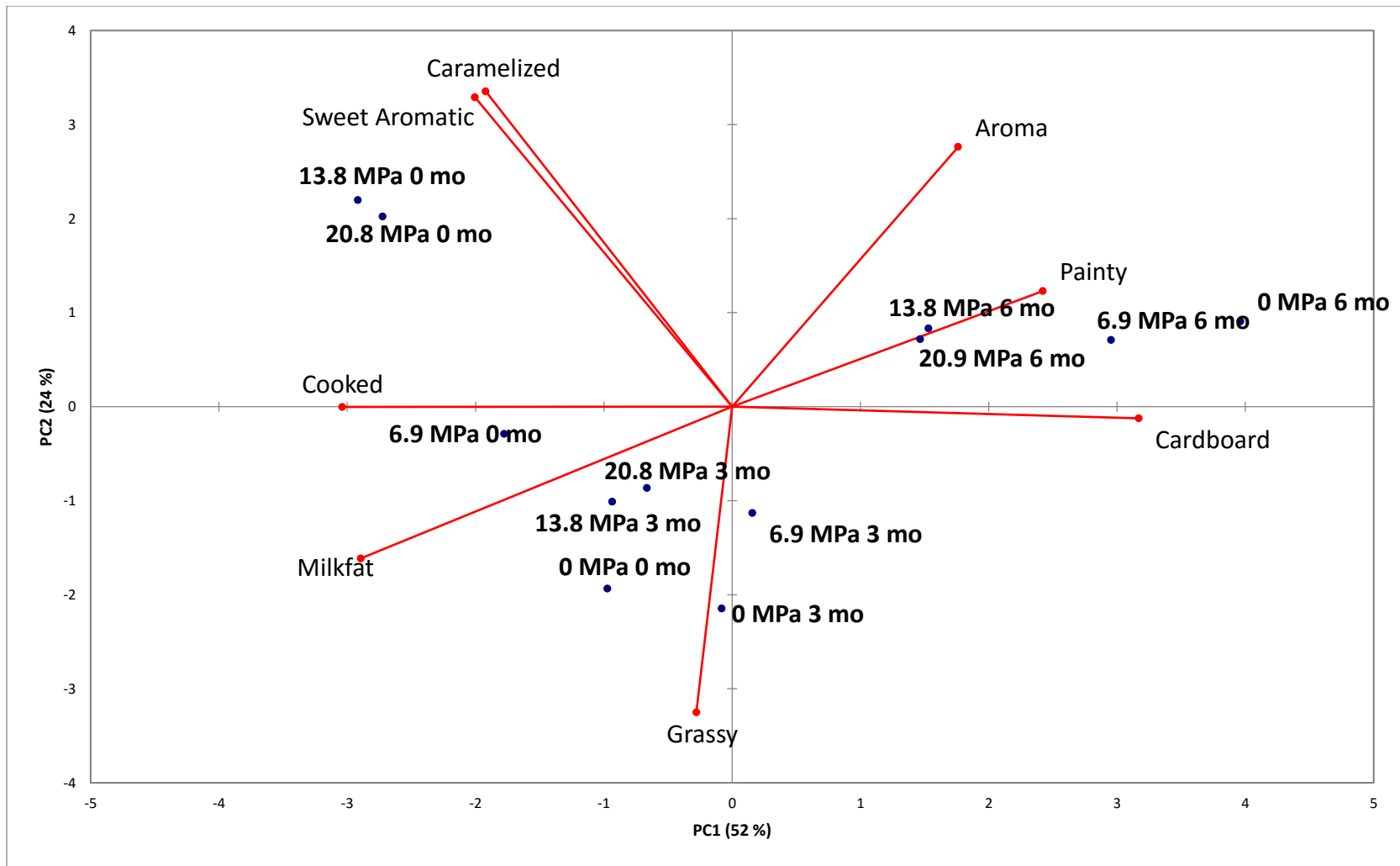


Figure 1 Principal component biplot of descriptive sensory analysis means of rehydrated WMP across 6 mo storage.

Table 2 Descriptive sensory means of rehydrated WMP across 6 mo storage. Means in the same column followed by a different letter indicates a significant difference ($p < 0.05$). ND—Not detected.

Treatment	Time (mo)	Aroma	Cooked	Caramelized	Cardboard	Sweet Aromatic	Grassy	Milkfat	Painty
0 MPa	0	1.6 e	3.4 a	1.0 e	0.9 d	ND	ND	2.4 c	ND
6.9 MPa	0	1.8 d	3.4 a	2.0 b	ND	ND	ND	2.5 a	ND
13.8 MPa	0	1.9 abcd	3.5 a	2.7 a	ND	1.6 a	ND	2.5 a	ND
20.8 MPa	0	1.9 abcd	3.4 a	2.7 a	ND	1.4 a	ND	2.5 a	ND
0 MPa	3	1.8 cd	2.5 c	1.6 bcd	1.6 c	ND	1.4 a	2.5 a	ND
6.9 MPa	3	2.0 a	2.8 b	1.4 de	1.0 d	ND	1.0 b	2.4 bc	ND
13.8 MPa	3	1.8 bcd	2.8 b	1.8 bcd	0.5 e	ND	0.5 c	2.5 ab	ND
20.8 MPa	3	1.9 abc	2.8 b	2.0 b	0.8 de	ND	0.9 b	2.5 a	ND
0 MPa	6	2.0 ab	2.0 e	1.4 cde	3.0 a	ND	ND	2.0 d	1.0 a
6.9 MPa	6	2.0 a	2.1 e	1.5 cd	2.5 b	ND	ND	2.0 d	0.5 b
13.8 MPa	6	2.0 a	2.3 cd	1.9 bc	1.7 c	ND	ND	2.0 d	ND
20.8 MPa	6	2.0 a	2.2 de	1.9 bc	1.8 c	ND	ND	2.1 d	ND

Table 3 Relative abundance ($\mu\text{g}/\text{kg}$) of selected volatile compounds of WMP throughout 6 mo storage. Means in the same row followed by a different letter indicate a significant difference ($p < 0.05$).

Compound	0 Month				3 Month				6 Month			
	0 MPa	6.9 MPa	13.8 MPa	20.8 MPa	0 MPa	6.9 MPa	13.8 MPa	20.8 MPa	0 MPa	6.9 MPa	13.8 MPa	20.8 MPa
Hexanal	1.03 f	0.983 f	0.910 f	0.895 f	10.3 d	6.70 e	5.89 e	6.10 e	53.6 a	20.3 b	15.4 c	15.3 c
Heptanal	0.978 f	0.907 f	0.734 f	0.749 f	4.64 d	3.17 e	3.04 e	2.93 e	21.9 a	9.23 b	7.09 c	6.90 c
Octanal	0.638 d	0.595 d	0.596 d	0.599 d	1.83 bc	1.44 c	1.29 c	1.30 c	7.52 a	2.42 b	2.19 b	2.14 b
Nonanal	4.10 cde	3.10 e	3.57 de	3.17 e	5.48 c	4.96 cd	4.66 cde	3.99 cde	14.5 a	7.46 b	7.52 b	7.24 b
Decanal	0.410 de	0.305 efg	0.241 fg	0.236 g	0.546 bc	0.458 cd	0.371 def	0.347 defg	0.990 a	0.584 bc	0.610 b	0.601 b
2,4-Nonadienal	0.036 e	0.025 e	0.017 e	0.021 e	0.421 cd	0.312 d	0.244 de	0.252 de	1.99 a	0.922 b	0.658 bc	0.688 b
2,4-Decadienal	0.068 e	0.046 e	0.038 e	0.037 e	0.957 cd	0.558 de	0.490 de	0.468 de	4.16 a	2.29 b	1.47 c	1.47 c
E2-Nonenal	0.125 d	0.088 d	0.062 d	0.074 d	0.584 c	0.339 cd	0.326 cd	0.341 cd	4.41 a	1.15 b	0.967 b	1.04 b
E2-Decenal	0.073 e	0.043 e	0.031 e	0.037 e	0.277 d	0.156 de	0.160 de	0.165 de	2.86 a	0.819 bc	0.756 c	0.980 b
1-Octen-3-one	0.114 cd	0.062 d	0.021 d	0.027 d	0.267 c	0.231 c	0.171 cd	0.152 cd	3.65 a	0.549 b	0.480 b	0.519 b
EZ-3,5-Octadien-2-one	ND	ND	ND	ND	ND	ND	ND	ND	24.3 a	21.9 ab	15.1 c	19.3 bc
EE-3,5-Octadien-2-one	ND	ND	ND	ND	ND	ND	ND	ND	16.9 a	13.2 b	8.95 c	11.9 bc
3-Octen-2-one	ND	ND	ND	ND	ND	ND	ND	ND	15.1 a	5.70 b	4.60 b	5.91 b
2-Heptanone	ND	ND	ND	ND	ND	ND	ND	ND	31.3 a	31.2 a	20.4 b	20.4 b
O-Aminoacetophenone	0.003 d	0.002 d	0.002 d	0.002 d	0.008 c	0.007 c	0.007 c	0.008 c	0.023 a	0.016 b	0.013 b	0.017 b

Table 3 continued.

Phenyl Acetaldehyde	0.206 de	0.158 f	0.172 ef	0.172 ef	0.253 bc	0.234 bcd	0.210 de	0.218 cd	0.316 a	0.317 a	0.278 ab	0.276 ab
Maltol	0.561 a	0.118 b	0.116 b	0.105 b	0.174 b	0.165 b	0.123 b	0.120 b	0.248 b	0.155 b	0.174 b	0.143 b
Furaneol	0.052 ab	0.021 ef	0.019 f	0.017 f	0.046 abc	0.044 abcd	0.037 bcd	0.032 de	0.053 a	0.033 cde	0.050 ab	0.040 abcd
Vanillin	0.014 bcd	0.012 cd	0.013 bcd	0.014 abc	0.011 def	0.012 cde	0.016 a	0.016 ab	0.011 cdef	0.007 g	0.009 efg	0.008 fg
Delta-Nonalactone	0.674 d	0.567 d	0.621 d	0.647 d	5.12 c	5.62 c	5.74 bc	5.59 c	7.07 a	6.01 bc	5.57 c	6.65 ab
Delta-Decalactone	9.10 d	7.48 d	8.87 d	9.14 d	62.3 c	65.7 c	64.8 c	66.5 c	93.3 a	81.2 b	80.8 b	88.0 ab
Delta-Dodecalactone	8.19 d	6.83 d	7.76 d	7.82 d	65.2 c	68.8 c	67.1 c	69.1 c	99.3 a	87.6 b	88.5 b	92.5 ab
Delta-Tetralactone	3.05 d	2.11 d	2.25 d	2.23 d	29.6 c	27.6 c	27.6 c	26.2 c	42.4 a	38.1 ab	36.5 b	36.8 b
Gamma-Decalactone	0.132 d	0.150 d	0.130 d	0.124 d	0.901 c	0.895 c	1.12 c	1.03 c	3.68 a	1.91 b	1.94 b	1.95 b
Gamma-Dodecalactone	0.923 d	0.598 d	0.651 d	0.674 d	11.8 c	10.4 c	11.8 c	11.1 c	21.3 a	17.8 b	17.8 b	19.0 ab

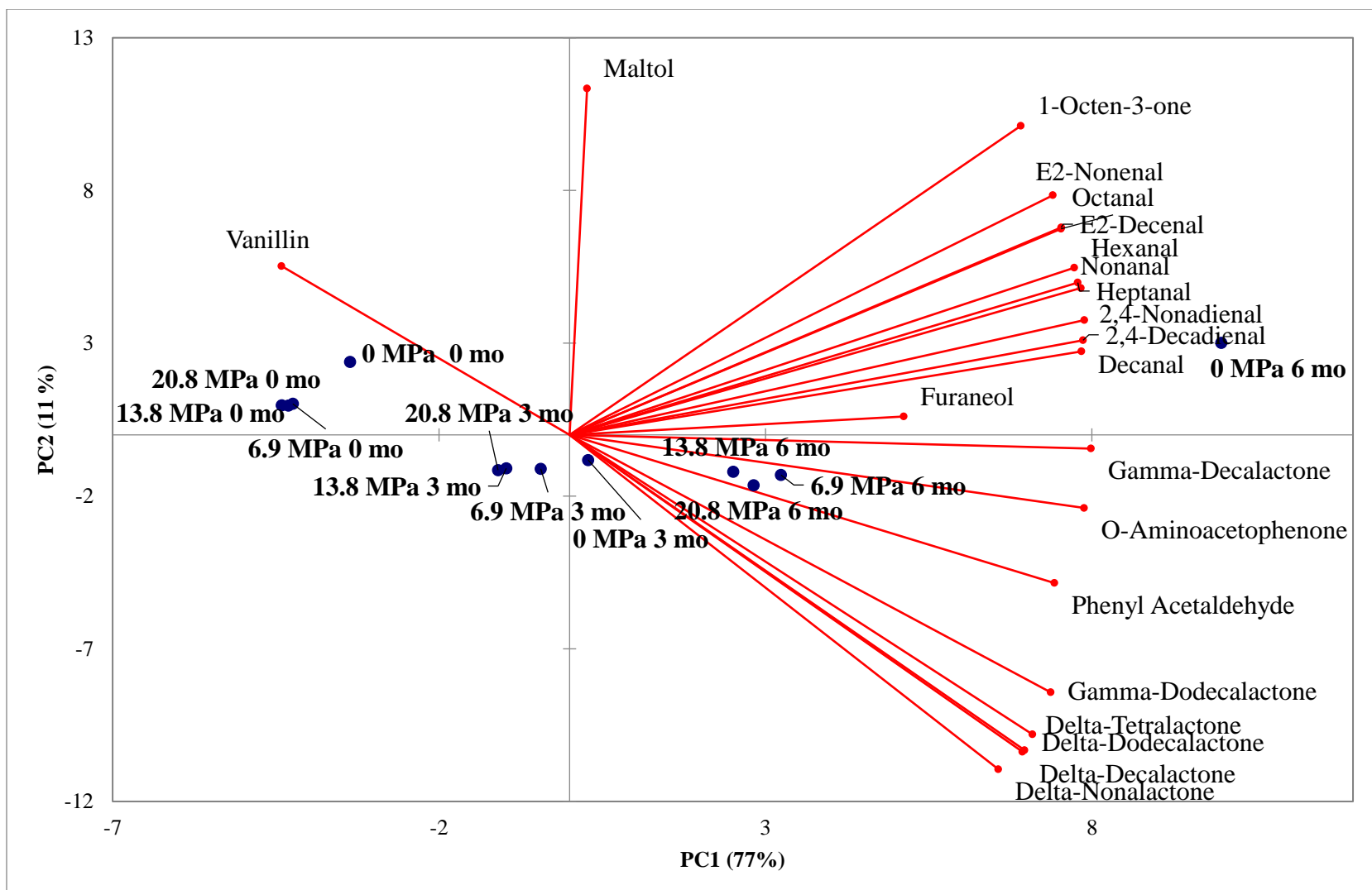


Figure 2 Principal component biplot of volatile compound analysis of WMP through 6 mo storage at 21°C.

Table 4 Furosine content (mg furosine/100 g protein) in WMP throughout storage. Means followed by a different letter indicate significant differences ($p < 0.05$). Interaction effects between homogenization pressure and storage time were not significant ($p > 0.05$).

Pressure (MPa)	mg FUR/100 g protein
0	51.2 a
6.9	46.7 b
13.8	49.0 ab
20.8	47.3 b
Storage	
0 mo	44.7 b
3 mo	45.8 b
6 mo	55.2 a

Table 5 Particle size of fresh WMP homogenized at varying pressures. Means in the same column followed by different letters indicate significant differences ($p < 0.05$). $D_x(90)$ – 90% of particles below that value. $D[4,3]$ - Volumetric mean.

Treatment	$D_x(90)$	$D[4,3]$
0 MPa	130 a	65.5 a
13.8 MPa	130 a	66.6 a
20.8 MPa	128 a	65.6 a
6.9 MPa	118 b	60.0 b

Table 6 Milk fat globule size in condensed whole milks (50% solids) prior to spray drying.

Means in the same column followed by a different letter indicate significant differences

($p < 0.05$). $D_x(90)$ – 90% of particles below that value. $D[4,3]$ - Volumetric mean.

Treatment	$D(90)$ (μm)	$D[4,3]$ (μm)
0 MPa	4.56 a	1.71 a
6.9 MPa	2.10 b	0.85 b
13.8 MPa	1.94 b	0.83 b
20.8 MPa	1.71 c	0.77 c

Table 7 Fat fractions of WMP treatments. Storage time had no effect on fat fractions ($p>0.05$). Means in the same column followed by a different letter indicate significant differences ($p<0.05$). SFF—Surface free fat, IFF—Inner free fat, EF—Encapsulated fat, PL—Phospholipids.

Treatment	g SFF/100g WMP	g IFF/100g WMP	g EF/100g WMP	g PL/100 g EF
0 MPa	5.16a	3.62a	17.1c	1.75a
6.9 MPa	3.32b	3.12a	20.2b	1.25b
13.8 MPa	2.22c	1.79b	23.5a	0.90c
20.8 MPa	1.85c	1.67b	24.1a	0.93c

**CHAPTER 6: SHORT COMMUNICATION: THE EFFECT OF
FACILITY RUN TIME ON THE FLAVOR OF SKIM MILK POWDER**

**Short communication: The effect of facility run time on the flavor of skim
milk powder**

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* Use of names, names of ingredients, and identification of specific models of equipment is for scientific clarity and does not constitute any endorsement of product by authors, North Carolina State University, or the Southeast Dairy Foods Research Center.

ABSTRACT

The sensory and functional qualities of skim milk powder (SMP) influence the use and consumer acceptance of powdered milk in various applications. Previous research demonstrated that flavor variability of low heat SMP influenced consumer acceptance in SMP applications. Previous research has also demonstrated that microbiological quality of SMP decreases the longer a facility manufactures milk powder before cleaning. The objective of this study was to investigate the impact of production run time on sensory and functional properties of SMP. Four different domestic spray drying facilities were selected for sampling of low and medium heat SMP. Samples taken were condensed skim milk (CSM) from the last evaporator effect (50% solids) and the spray dried powder at 0, 12, 24, and 36 h throughout production. Flavor was evaluated by descriptive sensory analysis and volatile compound analysis on both the CSM and SMP. Aerobic thermophilic sporeformers were enumerated. Particle size, solubility, and WPNI were performed on the SMP, and furosine was quantified in both the CSM and SMP. Increasing production run time up to 36 h increased cardboard and decreased sweet aromatic flavor intensities in both LH and MH products ($p < 0.05$). Cardboard and beefy flavors increased in SMP compared to CSM in LH and MH products respectively ($p < 0.05$), implicating spray drying as a source of off-flavors. Volatile compound analysis results were in agreement with sensory results and indicated lipid oxidation as the primary cause of off-flavors. Solubility, WPNI, and furosine were not affected by production run time ($p < 0.05$). Furosine was higher in MH CSM and SMP compared to LH CSM and SMP indicating increased Maillard reactions ($p < 0.05$). Particle size was not affected by run time in MH SMP, but increasing run time decreased the particle size in LH SMP. This study demonstrates that as the production run time increases, the

sensory quality of SMP deteriorates. Identifying production run time as a source of SMP quality gives SMP manufacturers needed information to improve SMP quality for use in various applications.

Key Words: milk powder, flavor, run time

Milk powders have become an important ingredient used globally in the food industry because of their nutritional and functional properties as well as their ability to extend shelf life. Dried skim milk is classified into skim milk powder (SMP) and non-fat dry milk (NFDM). SMP and NFDM both contain less than 5% moisture and 1.5% fat (ADPI, 2015). SMP contains a minimum of 34% protein whereas NFDM has no standardized protein content. In addition, milk retentate, milk permeate, and lactose can be used to adjust the protein content of SMP but not NFDM. SMP and NFDM are further classified by heat treatment for use in various applications. These classifications are low heat (LH), medium heat (MH), and high heat (HH) and are defined by their undenatured whey protein nitrogen index with values of >6.00, 1.51-5.99, and <1.50 mg/g respectively (USDEC, 2005). In the U.S., LH NFDM is the most common with MH SMP increasing due to its increased heat stability for various applications. Globally, MH SMP is the primary type used (Varnum and Sutherland, 1994). Skim milk powders are expected to taste like fluid skim milk but off-flavors can develop during processing which decrease consumer acceptance of the reconstituted powders as well as ingredient applications (Caudle et al., 2005; Isleten and Karagul-Yuceer, 2006; Karagul-Yuceer et al., 2002).

Unit operations during SMP manufacture include fat separation, heat treatment, evaporation to approximately 50% solids, and spray drying. Depending on the capacity and design of the processing facility, SMP manufacturers may run product for up to 40 h prior to

shutting down for cleaning and sanitation. Plant sanitation must be high in order to run a facility for up to 40 h. A great deal of research has demonstrated that the longer a facility produces product prior to cleaning, the greater the number of thermophilic spores in the resulting SMP (Scott et al., 2007; Watterson et al., 2014; Murphy et al., 1999). Thermophilic spores in SMP can lead to defects in recombined or UHT products (Marchand et al., 2012). Heat exchangers and evaporators are considered the main source of spores in milk powders due to the elevated temperatures. Biofilms can develop and spores are sloughed off into the product during the processing run (Scott et al., 2007). Spores are of importance because they survive heat treatments and spray drying and germinate when conditions are favorable. Because of this, SMP destined for infant formula has strict specifications for how many spores can be present (Watterson et al., 2014).

Also of concern during extended production runs is heat exchanger fouling. In heat exchangers and evaporators, the deposits can build up on stainless steel tubing and increase with longer run times (de Jong, 1997; Bansal and Chen, 2006). The main foulants in dairy products are β -lactoglobulin and calcium phosphate (Bansal and Chen, 2006). As the β -lactoglobulin denatures, larger protein aggregates are formed which foul the heat exchange surface and the heating utilities must increase in order to compensate for the loss in heat conductivity and ultimately shorten the length that a manufacturing facility can operate before shutting down to clean and re-sanitize (de Jong, 1997). This represents a large portion of the costs (up to 80%) associated with manufacturing dairy products (van Asselt et al., 2005).

Drake et al. (2006) observed that the sensory profile of the milk changed throughout the SMP manufacturing process. They observed increases in cooked and sweet aromatic

flavors with heating steps such as pasteurization, evaporation, and spray drying. Drake et al. (2006) also reported that volatile compound profiles of SMP from the beginning (0 h), middle (20 h), and end (38 h) of the run changed. However, they did not report the sensory profiles of SMP from beginning, middle, and end of the run nor did they quantify the volatile compounds or functional properties. The objective of this study was to determine the effect of facility run time on the flavor and functionality of SMP.

Two LH SMP facilities and two MH SMP manufacturing facilities on the east coast and west coast of the United States were sampled throughout the production run time. Samples were collected 0, 12, 24, and 36 h from the beginning of the production run. The samples collected were condensed skim milk (CSM; ~50% solids) from the balance tank of the spray dryer and the resulting SMP. One L of CSM was collected in Teflon bottles and 1 kg of SMP was collected in Whirl-pak bags (Sigma-Aldrich, St. Louis, MO). Samples were frozen immediately and shipped overnight on blue ice packs to North Carolina State University. All sampling was replicated three times on three separate occasions.

Flavor of condensed milks and SMP was evaluated by descriptive sensory analysis in compliance with the North Carolina State University Institutional Review Board for Human Subjects guidelines. SMP and CSM were rehydrated to 10% solids (w/v) with a handheld blender until they were fully dissolved. The solutions were dispensed into 60 ml soufflé cups (Solo Cup, Highland Park, IL). Samples were lidded and tempered at 21°C for at least one hour prior to evaluation. Flavor and basic taste attributes were evaluated using the Spectrum™ scale with a highly trained panel (n=8; >150 h experience) using an established dried dairy ingredient lexicon (Drake et al., 2003). Each treatment replication was evaluated

by each panelist in duplicate. Data was compiled using Compusense Cloud (Compusense, Guelph, Canada).

Volatile flavor compounds were analyzed using sorptive stirbar extraction as described by Park and Drake (2016). SMP and CSM were rehydrated to 10% solids (w/v) with HPLC grade water. Next, 5 ml of solution was placed in a 10 ml amber vial with a Teflon lined screw top lid (Gerstel Inc., Linthicum, MD) along with 10 μ l of internal standard (0.78 mg/L 2-methyl-3-heptanone; Sigma Aldrich, St. Louis, MO). Two PDMS stirbars (10 x 0.5 mm; Gerstel Inc.) were used sequentially to extract volatile compounds. The first extraction was done at 800 rpm for 1 h at room temperature. For the second extraction, 1 g of salt was added to the vial and the second stirbar was added and stirred at 800 rpm for 1 h. The two stirbars were added together to the same thermal desorption tube to be injected at the same time. Compounds were injected, separated and detected as described by Park and Drake (2016) with the same equipment. Compounds were identified by comparing with the NIST Mass Spectral Library (NIST, 2014), retention indices, and retention times of authentic standards. Relative abundances (μ g/kg) were calculated using the internal standard.

Furosine, an indicator of Maillard reactions in dairy products, was measured as described by Resmini et al. (1990) with minor modifications. First, 2 ml of rehydrated SMP or CSM (10% solids w/v) was placed in a glass tube. Next, 6 ml of 10.6 N HCl was added and nitrogen gas was bubbled through the sample for 1 min. The tube was then sealed with a Teflon lined lid and stored in an oven at 110°C for 23 h. After 24 h, the hydrolysate was cooled to room temperature, diluted 1:10 with HPLC grade water, and centrifuged at 14,000 rpm for 10 min. The supernatant was placed in an HPLC autosampler vial (Waters

Corporation, Milford, MA). Separation and detection were performed using UPLC-MS. A reversed phase C18 column was used (HSS T3, 2.1 x 100 mm, 1.7 μ m; Waters Corporation, Medford, MA). The mobile phase consisted of 0.1% formic acid in water at 40°C with a flow rate of 0.5 ml/min. A single quadrupole mass spectrometer (SQ Detector 2, Waters) in ES+ mode was used to detect and quantify furosine using a mass of 255.03. Capillary and cone voltages were 2.5 kV and 25V respectively. A standard curve was constructed in raw milk free of furosine. Standard curve concentrations ranged from 0 to 500 mg furosine/100 g protein.

Other tests performed on the SMP only included particle size, solubility index, whey protein nitrogen index (WPNI), surface free fat, and thermophilic aerobic spores. Particle size was performed as described by Park and Drake (2016). Solubility index and WPNI were determined as described by ADPI (2002). Surface free fat was determined by ether extraction as described by GEA (2005). Thermophilic aerobic spores were determined by heating rehydrated SMP to 100°C for 30 min as described by Scott et al. (2007) and pour plated using tryptic soy agar (Sigma Aldrich, St. Louis, MO).

Statistical analysis was performed using XL Stat (version 2015.1; Addinsoft, New York, NY). Two-way analysis of variance with means separation with Fisher's least squared difference test was used to determine statistical differences.

Descriptive sensory analysis indicated that run time significantly affected the flavor of both the CSM and resulting SMP (Table 1; Table 2). Interaction effects between product (CSM vs SMP) and run time were not significant for LH nor MH products ($p > 0.05$).

Cardboard flavor was detected in LH SMP whereas it was not detected in the LH CSM. In LH products, as the run time increased, sweet aromatic flavor decreased and cardboard flavor

increased in the order 0 h < 12, 24 h < 36 h ($p < 0.05$). MH SMP had increased beefy and cooked flavors and decreased astringency compared to MH CSM ($p < 0.05$). As observed in LH products, sweet aromatic intensity decreased with run time in the order 0 h < 12 h < 24, 36 h ($p < 0.05$). Also, cardboard flavor was not detected in 0 or 12 h samples but was detected in 24 and 36 h samples.

Volatile compound analysis indicated differences in flavor were mainly due to lipid oxidation in both LH and MH products (Table 3, Table 4, Figure 1, Figure 2). Interaction effects between product (CSM vs SMP) and run time were significant ($p < 0.05$). In LH CSM, 36 h run time increased hexanal concentration compared to 0 or 12 h and octanal, 2,4-nonadienal, and 1-octen-3-one concentrations compared to 0 h ($p < 0.05$). Also, 36 h run time increased heptanal and nonanal concentrations compared to 0 or 24 h and decreased vanillin concentration compared to 0 h ($p < 0.05$). In LH SMP, 36 h run time increased hexanal, and 2,4-nonadienal concentrations and 24 h increased heptanal concentration compared to 0 or 12 h ($p < 0.05$). Increased run times of 24 or 36 h increased octanal, decanal, 2,4-decadienal, E2-decenal, and *o*-aminoacetophenone compared to 0 or 12 h ($p < 0.05$).

In MH CSM, 36 h run time increased furfural, 2,4-nonadienal, and 2,4-decadienal concentrations compared to all other time points, E2-decenal and heptanal compared to 0 h, and hexanal and nonanal compared to 0 or 12 h ($p < 0.05$). Delta-nonalactone in 36 h samples increased compared to 0 or 12 h and delta-decalactone increased in 24 or 36 h samples compared to 0 or 12 h ($p < 0.05$). In MH SMP 36 h run time increased concentrations of hexanal, heptanal, octanal, nonanal, 2,4-decadienal, and 1-octen-3-one compared to 0, 12, or 24 h ($p < 0.05$). After 24 and 36 h run time, decanal and E2-decenal increased and 2-acetyl

thiazole decreased compared to 0 or 12 h run time ($p < 0.05$). Vanillin concentration increased in 0 h MH SMP compared to 24 h ($p < 0.05$).

Solubility index, WPNI, surface free fat, and thermophilic aerobic spores were not different in SMP from beginning to end of production ($p > 0.05$). Solubility index of the LH and MH SMP was < 0.1 ml. WPNI for LH and MH SMP was 7.36 ± 1.23 and 3.94 ± 0.90 mg/g. Facility run time did not affect the particle size of MH SMP ($p > 0.05$) and the $D_x(90)$ and $D[4,3]$ were 211 ± 42.1 μm and 104 ± 20.6 μm respectively. In LH SMP particle size increased as the production run time increased in the order 0 h > 12 h > 24, 36 h ($p < 0.05$) observed in the D_x90 (178 vs 163 vs 151 vs 148 μm) and the $D[4,3]$ (96.7 vs 87.8 vs 78.2 vs 79.5).

Thermophilic aerobic spores averaged $3.69 \times 10^3 \pm 1.40$ CFU/g. As described in the introduction, previous literature has reported that thermophilic spores increase during the production of SMP due to unit operations containing heating zones such as heat exchangers. We did not observe differences in spore counts most likely due to the high standard deviation between replicates from each facility as well as among facilities. Kent et al. (2016) reported that the methodology used to enumerate spores also plays a large role in the results.

Furosine (FUR) indicated differences in Maillard reactions between LH and MH products (Table 6). Interaction effects were not significant between heat treatment and run time ($p > 0.05$). LH SMP had lower concentrations of FUR than MH SMP (76.1 vs 109 mg FUR/100 g protein) and the same effect was observed in CSM (53.1 vs 95.1 mg FUR/100 g protein). While average FUR concentrations were higher for both LH and MH products initially compared to 12, 24, or 36 h they were not statistically different ($p > 0.05$). This was due to a larger standard deviation among all reps and facilities. Unit operations such as

membrane filtration, evaporation, and spray drying during NFDM manufacture have been demonstrated to influence FUR concentrations (Park et al., 2016; Park and Drake, 2016). As such, differences in parameters of these unit operations among facilities would be expected to contribute to the large standard deviation observed.

We observed that lipid oxidation was the primary cause of off-flavors in SMP which is consistent with previous research (Karagul-Yuceer et al., 2002; Drake et al., 2006). Recently, Park and Drake (2016) and Park et al. (2016) have demonstrated that SMP flavor is significantly affected by unit operations, in particular evaporation and spray drying. We observed increased cardboard and beefy flavors along with lipid oxidation products in SMP compared to CSM which indicates that spray drying was one source of the flavor variability we observed. It is typical for industrial spray dryers to be shut down and cleaned once every 30 days due to the difficulty of cleaning them. The long time between cleanings could enhance the effect that spray drying has on off-flavors in SMP. In LH SMP, particle size decreased with increasing run time. In NFDM, milk protein concentrate, and whey protein concentrate, decreased particle size has been observed in spray dried powders with increased off-flavors (Park et al., 2016, Park et al., 2014). Evaporation uses a combination of heat and vacuum to remove water to concentrate skim milk prior to spray drying. In addition, many desirable flavor compounds are removed which make off-flavors more pronounced (Park and Drake, 2016). As the run time increases, fouling of the calandria in the evaporator can occur. This foulant layer reduces heat transfer and thus the heating medium has to be increased to compensate. This foulant layer can slough off into the product and possibly cause undesirable flavors.

As the run time increases it may be necessary to store condensed milk. This could be due to shipping product from one facility to another, storing in a bulk tank prior to spray drying because of limited spray drying capacity, or problems that arise during production. Storage of condensed milk has been demonstrated to increase lipid oxidation in the resulting SMP (Park and Drake, 2016).

These results identify production run time as a source of deteriorating flavor quality in SMP. In both LH and MH SMP, run times <24 h resulted in powders with decreased off-flavors and lipid oxidation products. Evaluation of both CSM and SMP also indicated that spray drying is a major source of off-flavors. Functional properties of SMP were unaffected by production run time. SMP manufacturers should consider the length of production runs in order to product a higher quality product to be used successfully in a variety of applications.

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Table 1. Sensory profiles of LH SMP across facility run time. Means in the same column followed by different letters indicate significant differences ($p < 0.05$). ND—Not detected.

Product	Overall Aroma	Sweet Aromatic	Cooked	Cardboard	Sweet Taste	Astringent
SMP	1.9 a	1.1 a	3.1 a	1.6 a	2.1 b	1.8 a
CSM	1.9 a	1.0 a	3.1 a	ND	2.6 a	2.0 a
Time Point	Overall Aroma	Sweet Aromatic	Cooked	Cardboard	Sweet Taste	Astringent
0	1.8 a	1.4 a	3.0 b	0.5 c	2.3 a	1.9 a
12	1.8 a	1.2 b	3.0 b	0.9 b	2.3 a	1.9 a
24	1.9 a	1.1 b	3.0 b	1.1 b	2.3 a	1.9 a
36	2.0 a	0.5 c	3.3 a	1.4 a	2.3 a	1.9 a

Table 2. Sensory profiles of MH SMP across facility run time. Means in the same column followed by different letters indicate significant differences ($p < 0.05$). ND—Not detected.

Product	Overall Aroma	Sweet Aromatic	Sulfur	Cooked	Beefy	Cardboard	Sweet Taste	Astringent
SMP	3.4 a	0.5 a	1.1 a	4.1 a	1.3 a	0.5 a	2.2 a	2.1 a
CSM	3.3 a	0.7 a	1.4 a	3.7 b	0.5 b	0.6 a	2.1 a	1.6 b
Time Point	Overall Aroma	Sweet Aromatic	Sulfur	Cooked	Beefy	Cardboard	Sweet Taste	Astringent
0	3.4 a	1.0 a	1.4 a	3.8 a	ND	ND	2.2 a	1.9 a
12	3.5 a	0.7 b	1.4 a	3.9 a	0.8 a	ND	2.2 a	1.9 a
24	3.4 a	ND	1.2 a	4.0 a	1.0 a	0.5 a	2.2 a	1.8 a
36	3.1 a	ND	0.9 a	3.8 a	1.5 a	0.9 a	2.3 a	1.7 a

Table 3. Relative abundance ($\mu\text{g}/\text{kg}$) of selected volatile compounds in LH SMP and CSM.

Means in the same row and shading followed by different letters indicate significant differences ($p < 0.05$).

Compound	LH CSM				LH SMP			
	0	12	24	36	0	12	24	36
Hexanal	0.393 c	0.576 b	0.640 ab	0.747 a	0.475 b	0.478 b	0.537 ab	0.684 a
Heptanal	0.151 c	0.231 ab	0.223 b	0.302 a	0.272 b	0.251 b	0.465 a	0.404 ab
Octanal	0.226 b	0.276 ab	0.277 ab	0.322 a	0.247 b	0.296 b	0.408 a	0.412 a
Nonanal	1.28 c	1.80 ab	1.48 bc	2.16 a	2.38 b	2.35 b	2.60 b	3.74 a
Decanal	0.291 a	0.371 a	0.323 a	0.332 a	0.213 b	0.210 b	0.294 a	0.304 a
Maltol	0.138 a	0.098 a	0.082 a	0.067 a	0.691 a	0.433 a	0.529 a	1.053 a
Furaneol	0.024 a	0.032 a	0.029 a	0.026 a	0.014 a	0.016 a	0.022 a	0.025 a
Vanillin	0.004 a	0.003 ab	0.003 ab	0.001 b	0.007 a	0.006 a	0.005 a	0.007 a
Delta-Nonalactone	0.044 a	0.039 a	0.048 a	0.042 a	0.023 a	0.023 a	0.036 a	0.022 a
Delta-Decalactone	0.274 a	0.260 a	0.234 a	0.262 a	0.266 a	0.305 a	0.350 a	0.378 a
Delta-Dodecalactone	0.067 a	0.056 ab	0.050 b	0.051 b	0.083 b	0.096 ab	0.100 ab	0.129 a
Delta-Tetralactone	0.150 a	0.121 a	0.108 a	0.094 a	0.135 a	0.143 a	0.114 a	0.143 a
Gamma-Decalactone	0.057 a	0.050 ab	0.040 b	0.047 ab	0.017 b	0.020 b	0.023 ab	0.032 a
2,4-Nonadienal	0.027 b	0.035 ab	0.042 a	0.039 a	0.010 c	0.011 bc	0.014 ab	0.018 a
2,4-Decadienal	0.024 a	0.028 a	0.035 a	0.030 a	0.023 b	0.025 b	0.037 a	0.049 a
Butyric Acid	0.446 a	0.454 a	0.421 a	0.389 a	0.706 a	0.719 a	0.555 a	0.798 a
Hexanoic Acid	0.923 a	1.003 a	0.964 a	1.116 a	1.09 a	1.01 a	1.41 a	1.32 a
Heptanoic Acid	0.071 a	0.076 a	0.060 a	0.047 a	0.118 a	0.114 a	0.100 a	0.137 a
Octanoic Acid	1.69 a	1.73 a	1.58 a	2.03 a	1.35 a	1.68 a	2.04 a	2.27 a
Decanoic Acid	1.23 a	1.08 a	1.00 a	1.10 a	0.497 b	0.696 a	0.743 a	0.634 ab
Dodecanoic Acid	0.832 a	0.816 a	0.781 a	0.900 a	0.341 b	0.441 a	0.476 a	0.459 a
E2-Decenal	0.035 a	0.044 a	0.040 a	0.033 a	0.012 b	0.013 b	0.025 a	0.029 a
1-Octen-3-one	0.021 b	0.027 ab	0.031 ab	0.033 a	0.023 b	0.030 a	0.080 a	0.082 a
2-Acetyl Thiazole	0.007 a	0.007 a	0.003 a	0.002 a	0.001 a	0.001 a	0.001 a	0.001 a
O-Aminoacetophenone	ND	ND	ND	ND	0.002 b	0.002 b	0.005 a	0.008 a

Table 4. Relative abundances ($\mu\text{g}/\text{kg}$) of selected volatile compounds in MH CSM and SMP.

Means in the same row and shading followed by different letters indicate significant differences ($p < 0.05$).

Compound	MH CSM				MH SMP			
	0	12	24	36	0	12	24	36
Furfural	0.035 b	0.034 b	0.032 b	0.047 a	0.037 a	0.032 a	0.036 a	0.036 a
Hexanal	0.220 c	0.278 bc	0.358 ab	0.415 a	0.338 b	0.377 b	0.400 b	0.645 a
Heptanal	0.154 b	0.227 ab	0.245 ab	0.271 a	0.221 b	0.238 b	0.246 b	0.419 a
Octanal	0.310 a	0.338 a	0.335 a	0.402 a	0.229 b	0.256 b	0.297 b	0.522 a
Nonanal	1.54 c	1.69 bc	2.20 ab	2.81 a	1.91 b	1.82 b	2.00 b	3.04 a
Decanal	0.321 a	0.373 a	0.338 a	0.421 a	0.233 b	0.241 b	0.303 a	0.315 a
Maltol	0.071 a	0.045 a	0.036 a	0.092 a	0.242 a	0.137 b	0.098 b	0.154 ab
Furaneol	0.011 a	0.008 a	0.008 a	0.015 a	0.014 a	0.010 b	0.010 ab	0.013 ab
Vanillin	0.008 a	0.007 a	0.002 a	0.004 a	0.006 a	0.004 ab	0.003 b	0.004 ab
Delta-Nonalactone	0.046 bc	0.031 c	0.056 ab	0.078 a	0.031 a	0.036 a	0.028 a	0.038 a
Delta-Decalactone	0.283 b	0.249 b	0.360 a	0.407 a	0.420 ab	0.366 b	0.374 b	0.540 a
Delta-Dodecalactone	0.121 a	0.082 a	0.106 a	0.140 a	0.151 ab	0.119 b	0.132 ab	0.174 a
Delta-Tetralactone	0.227 a	0.128 a	0.096 a	0.290 a	0.167 a	0.170 a	0.188 a	0.150 a
Gamma-Decalactone	0.049 a	0.043 a	0.056 a	0.056 a	0.038 a	0.035 a	0.031 a	0.044 a
2,4-Nonadienal	0.013 b	0.014 b	0.015 b	0.032 a	0.010 c	0.011 c	0.015 b	0.019 a
2,4-Decadienal	0.015 b	0.019 b	0.018 b	0.034 a	0.017 b	0.019 b	0.020 b	0.029 a
Butyric Acid	0.488 a	0.421 a	0.367 a	0.399 a	0.540 a	0.630 a	0.506 a	0.557 a
Hexanoic Acid	1.06 a	0.853 a	1.13 a	1.32 a	1.10 a	1.24 a	0.992 a	1.31 a
Heptanoic Acid	0.085 a	0.078 a	0.068 a	0.054 a	0.059 ab	0.061 ab	0.055 b	0.088 a
Octanoic Acid	1.53 a	1.54 a	2.03 a	2.08 a	1.80 a	2.10 a	1.91 a	2.59 a
Decanoic Acid	0.610 b	0.751 b	1.18 a	1.53 a	0.846 b	0.890 b	0.877 b	1.16 a
Dodecanoic Acid	1.08 a	0.540 a	0.712 a	1.02 a	0.617 a	0.818 a	0.605 a	0.882 a
E2-Decenal	0.017 b	0.026 ab	0.027 ab	0.035 a	0.011 b	0.011 b	0.016 a	0.018 a
1-Octen-3-one	0.012 a	0.018 a	0.010 a	0.016 a	0.018 b	0.017 b	0.016 b	0.029 a
2-Acetyl Thiazole	0.001 a	0.002 a	0.002 a	0.001 a	0.002 a	0.002 a	0.001 b	0.001 b

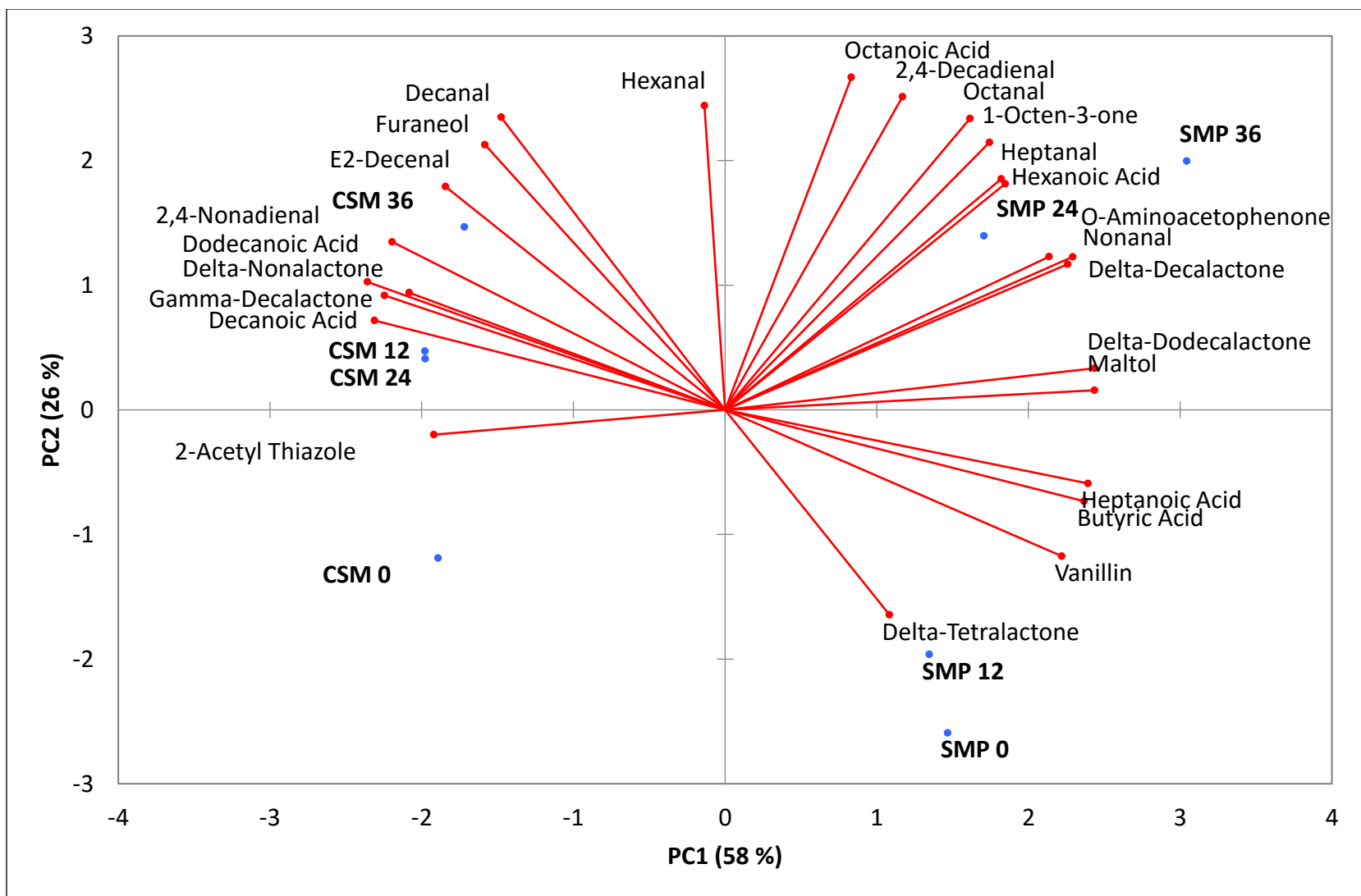


Figure 1. Principal component biplot of selected volatile compounds in LH CSM and SMP across facility run time.

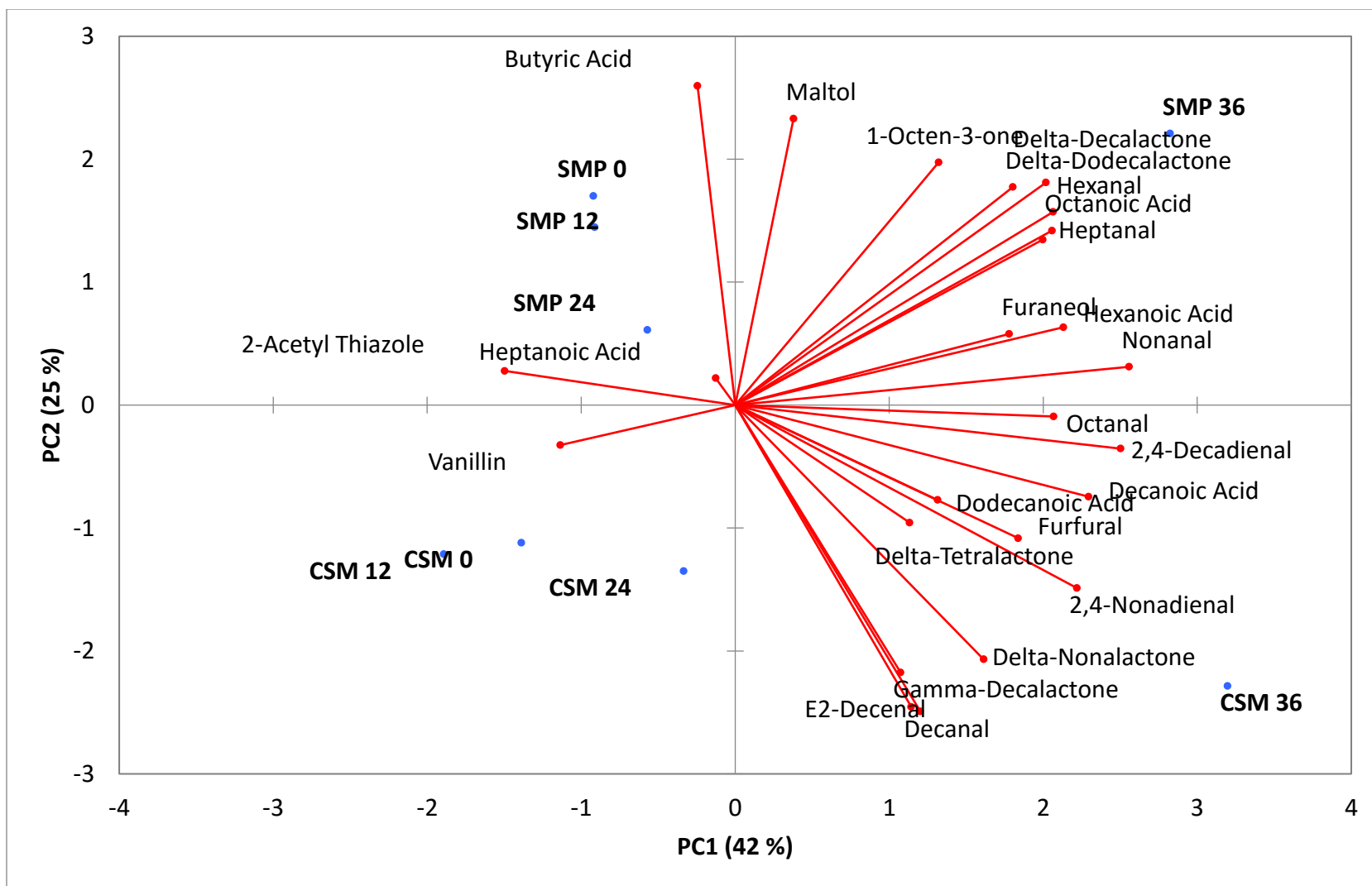


Figure 2. Principal component biplot of selected volatile compounds of MH CSM and SMP across facility run time.

Table 5. Particle size (μm) of LH and MH SMP. Means in the same column and SMP type (LH or MH) followed by different letters indicate significant differences ($p < 0.05$). $D_x(90)$ – 90% of particles below that value. $D[4,3]$ - Volumetric mean.

LH	$D_x(90)$	$D[4,3]$
0	178 a	96.7 a
12	163 b	87.8 b
24	151 c	78.2 c
36	148 c	79.5 c
MH	$D_x(90)$	$D[4,3]$
0	211 a	109 a
12	204 a	99.9 a
24	228 a	111 a
36	201 a	96.7 a

Table 6. Furosine (FUR) content in LH and MH SMP and CSM. Means in the same column followed by a different letter indicate significant differences ($p < 0.05$). Interaction effects between heat treatment and run time were not significant ($p > 0.05$).

		mg FUR/100 g Protein	
Heat Treatment		SMP	CSM
LH		76.1 b	53.1 b
MH		109 a	95.1 a
Time			
	0	100 a	88.1 a
	12	98.5 a	68.6 a
	24	78.4 a	76.9 a
	36	85.6 a	62.8 a

Interpretive Summary

Previous studies have established that lipid oxidation was the primary source of off-flavors in the ingredients tested in my studies: WPC80, MPC70, SMP/NFDM, and WMP. Previous studies have also established the specific volatile compounds that cause these off-flavors and their specific sources. My dissertation has applied these fundamental observations to practical application in dairy foods processing. I accomplished the goal of identifying processing variables that influence the flavor of dried dairy ingredients. I have demonstrated that lipid oxidation is influenced by processing steps during ingredient manufacture. This work is of importance to dairy manufacturers because it provides guidance on how to manufacture these ingredients with minimal off-flavors. Because these studies used existing technologies that are already common in the dairy industry, manufacturers should be able to use these guidelines with minimal investment in existing manufacturing plants. Also, when new manufacturing plants are built, this research should be consulted to produce products with minimal off-flavors.

Manufacturers of WPC80 should avoid liquid storage prior to spray drying, as off-flavors and lipid oxidation increased with liquid storage. If they must store liquid product, manufacturers of bleached WPC80 and unbleached WPC80 should store liquid whey and liquid WPC80 respectively to reduce off-flavors and lipid oxidation. Storage of condensed milk (30% solids) also increased off-flavors in NFDM. Manufacturers of NFDM should use reverse osmosis for partial water removal up to 30% in place of evaporation to produce spray dried NFDM with decreased off-flavors and lipid oxidation. Off-flavors in NFDM also increase when the production run time is longer than 24 hours. Lastly, homogenization pressure influences the flavor throughout shelf-life of WMP. Shelf-life of WMP was

extended with higher homogenization pressures of condensed whole milk due to decreased lipid oxidation products and decreased off flavors. Surface free fat was established as a key player in lipid oxidation of dried dairy ingredients and as such, a critical parameter for flavor and flavor stability of dried dairy ingredients. Surface free fat should be minimized by ingredient manufacturers for improved flavor.

Lastly, a new analytical tool for extracting flavor compounds from NFDM and WMP was developed. I developed a sorptive stir bar extraction method to extract a wide range of volatile compounds that are not extracted by headspace SPME. This method is simple, reproducible, and a time-saving alternative to solvent extraction.

This interpretive summary is intended to provide an overall comment on the research I conducted: however, because communication of scientific based processing information is critical to the dairy processing industry, I think it is important to point out that these findings have already been communicated to the industry at scientific and industry meetings and through the existing interface overlap of our laboratory with the dairy processing industry.