ABSTRACT

SMITH, TUCKER JAMES. The Effect of Processing and Environmental Parameters on the Sensory and Functional Characteristics of Dried Dairy Protein Ingredients. (Under the direction of Dr. MaryAnne Drake.)

The importance of dairy protein ingredients to the domestic and global dairy industry is increasing due to the abundance of functional and nutritional properties they possess. Flavor is a critical factor in the success of any food product. The dairy protein ingredient category includes a wide variety of products, including dried milk, milk protein concentrates, isolates, caseinates, and whey and serum protein concentrates, isolates, and hydrolysates. The objectives of this dissertation were to evaluate the effect of processing and environmental parameters on the sensory and functionality characteristics of a variety of dried dairy ingredients. Four different studies were conducted to investigate the effect of bleaching and whey source on the sensory and functionality characteristics of whey protein isolates (WPI), to determine the effects of benzoyl peroxide bleaching temperature and time on bleaching efficiency and sensory properties of whey protein concentrate, and to characterize the flavor, flavor chemistry, and shelf life of milk proteins.

In the first study, sensory analysis and gas chromatography-mass spectrometry (GC-MS) were conducted to determine flavor differences between bleached and unbleached WPI. Functionality differences were evaluated by measurement of foam stability, protein solubility, and heat stability. Bleaching caused higher off-flavors and increased lipid oxidation but also increased soluble protein at pH 4.6 and heat stability of WPI. These results demonstrated that bleaching had flavor effects as well as effects on many of the functionality characteristics of whey proteins. In the second study, three factors (temperature, bleach type, bleach concentration) were evaluated for norbixin destruction
using a response surface model – central composite design (RSM–CCD) in liquid whey. Subsequently, norbixin concentration, residual benzoic acid, and flavor differences were explored in WPC80 from whey bleached by two benzoyl peroxides (soluble and insoluble). Soluble BP bleached more norbixin than insoluble BP. The WPC80 bleached at 50°C had less norbixin, benzoic acid, cardboard flavor, and aldehydes than WPC80 bleached at 4°C. These results suggested that soluble BP can be used to reduce benzoic acid and improve flavor in WPC80.

In the third study, WPI from Cheddar, Mozzarella, and Cottage cheese, and rennet casein whey were evaluated by sensory analysis and GC-MS. Foam stability, heat stability, and protein solubility were measured. The WPI from Cheddar and Cottage cheese wheys had the highest cardboard flavor and aldehyde concentrations. Cottage cheese WPI foams had a lower overrun and air phase fraction (p<0.05). Cottage cheese WPI was less soluble at pH 4.6 and exhibited higher turbidity loss at pH 3-7 compared to other WPI (p<0.05). These results indicated that WPI from different sources could be used in targeted applications due to functional and flavor differences.

In the fourth study, flavor, flavor chemistry and shelf stability of spray dried milk protein ingredients (MPC, MPI, caseins, MCC) were evaluated. Milk proteins were evaluated by sensory analysis, GC-MS, and gas chromatography olfactometry (GCO). Pilot plant manufactured MPC were stored at 3, 25, and 40°C and solubility, furosine, sensory properties, and GC-MS were performed over 1 y. Milk proteins and caseins were diverse in flavor and exhibited sweet aromatic and cooked/milky flavors as well as cardboard, brothy, tortilla, soapy, and fatty flavors. Key aroma active compounds in milk proteins and caseins included many lipid oxidation and heat-induced volatile compounds. Solubility of MPC
decreased and furosine concentration increased with storage time and temperature. Storage temperature had the greatest effect on MPC solubility.
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Dedication

To Lauren. Your unconditional support and love can never adequately be repaid, but I promise to try.
Biography

Tucker James Smith was born in Corpus Christi, Texas on March 30th, 1984 to Brad and Lois Smith. He has three brothers and one sister; Shad, Scott, Sam, and Barbi. Tucker’s childhood was spent growing up in Wilmington, NC, where he graduated from John T. Hoggard high school in 2002. After graduation, Tucker spent one semester at Brigham Young University – Idaho in Rexburg, ID, after which he served a two year mission for the Church of Jesus Christ of Latter-Day Saints in the Samara, Russia mission. After his mission, Tucker transferred to Brigham Young University – Provo, where he majored in Food Science. In 2007, Tucker met and married his beautiful wife, Lauren. Tucker graduated with his Bachelors of Science in Food Science in 2009 and began his graduate studies under Dr. MaryAnne Drake in 2010. In 2011, Lauren and Tucker had their first child, Nettie. He finished his Master’s degree in 2012 and immediately began work towards his PhD. In 2013 Lauren and Tucker were surprised to find out that they were expecting identical twin boys. Nolan and Dean were born in November of that year. Since then Tucker has been as focused (as he can be) on finishing his PhD.
Acknowledgements

I would like to acknowledge everyone who has carried me to this point in my life, but unfortunately I couldn’t possibly mention so many. My family has been my foundation, a source of never-ending love and support, the center of my universe. My professors and advisors, who have so patiently pushed me along this path, every bit of success or distinction I achieve will be in large part an accomplishment I share with them. Dr. MaryAnne Drake, who kept me going, dragged me kicking and screaming towards our mutual goal of finishing this dissertation, I am truly in awe of the work you do.
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CHAPTER 1: LITERATURE REVIEW. SENSORY PROPERTIES OF MILK PROTEIN INGREDIENTS

Sensory Properties of Milk Protein Ingredients

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Introduction

Dairy Proteins provide significant value to the food industry and have an abundance of functional and nutritional properties (Foegeding et al., 2002; Miller, 2005; O’Connell and Flynn, 2007). The term “dairy protein” encompasses an increasingly large variety of ingredients, including whey, serum, and milk concentrated and unconcentrated products. Included in this category also are caseins and caseinates. Dairy proteins have many valuable functional properties, such as gelation, thermal stability, foam formation and emulsification properties (Foegeding et al., 2002). Consumption of whey proteins, specifically, provides many health benefits, including exercise recovery, weight management, cardiovascular health, anti-cancer effects, anti-infection activity, wound repair and infant nutrition (Smithers, 2008). Due to their ability to enhance functional characteristics of foods, dairy proteins are included in many different types of foods. Foods which use dairy proteins for their functional properties include fish products (used as a binding agent), cheese and yogurt
(to improve yields), cakes (as a replacement for egg whites), minced meats, coffee whiteners, salad dressings, frozen desserts (as an emulsifier) and many others (Jayaprakasha and Brueckner, 1999).

The varied useful functional characteristics of dairy proteins would not benefit the food industry, however, if their flavor profile was such that use as an ingredient detrimentally affected the purchase habits of consumers. Flavor plays a large role in acceptance and product success in all foods, and dried ingredients impact the flavor of the final product in which they are included (Caudle et al., 2005; Wright et al., 2009). As such, the sensory profiles of dairy proteins and ingredients which contain them cannot be overlooked. Due to their primary use as functional or nutritional enhancers, dairy proteins typically have a bland and mild flavor.

**Sensory Analysis**

Sensory science has been around for less than 70 years, making it a fairly young discipline. Sensory science analyzes all properties of food or other materials which are perceived by the human senses including, but not limited to, sight, taste, touch, and smell. Sensory properties are critical for a product to enjoy success in the marketplace. There are two main types of sensory testing: analytical tests and affective tests (Drake, 2007). Several types of different tests exist for each of these two main categories giving scientists a variety of tests from which to select.

Analytical sensory tests use trained or screened panelists and are objective in nature. Some examples of these tests include descriptive analysis, discrimination tests and threshold
Affective sensory tests use consumers, who are untrained, and are subjective in nature. These tests often measure degree of liking using a 9-point hedonic scale or measure preference among products (Meilgaard et al., 2007). Selection of the appropriate sensory test before data collection is imperative in order to fulfill the specific objective. Should an inappropriate test be selected, or if sensory testing is left as an afterthought, meaningful results and conclusions cannot be made (Drake, 2007a).

Dried dairy ingredients should ideally be bland or very mild in flavor so that ingredient flavor does not carry through into the finished product and influence consumer liking. Dairy foods can possess two types of off-flavors: those that are already present in the milk and those that develop later as a consequence of actions, such as processing or storage (Bodyfelt et al., 1988). Since off flavors are designated by consumers and not by trained experts, flavors are more appropriately classified as dairy and nondairy flavors (Drake, 2004). A sensory language has been developed for whey and dried whey ingredients, milk powders, and milk proteins, which includes both dairy and nondairy flavors (Tables 1 and 2).

Undesirable flavors that carry through to the finished product limit the utilization of dairy ingredients. In general, dairy products display a wholesome and flavorful image making them well accepted with consumers. In order for dairy products to continue a positive perception by consumers, understanding flavor, flavor variability, sources of off-flavors and consumer perception is imperative (Drake et al., 2009).

**Off Flavor Formation in Milk Products**

One would expect dairy proteins to inherit flavors primarily from the milk, but secondary flavors are introduced by processing. Figure 1 elucidates some of the common
processes through which dairy proteins and ingredients are produced, any of which may introduce flavors.

While unprocessed dairy ingredients tend to have a very bland flavor, once processed and stored, dried dairy protein ingredients can develop undesirable flavors, such as cardboard, cabbage, animal, tortilla, fatty, brothy and soapy (Carunchia Whetstine et al., 2005; Drake et al., 2009a; Wright et al., 2009). Undesirable ingredient flavors are often detectable in finished products and are a limiting factor in foods which lack flavor strong enough to overpower or mask the flavor of the dried dairy protein ingredient (Caudle et al., 2005; Drake, 2006; Drake et al., 2009; Wright et al., 2009; Evans et al., 2010). Lipid oxidation and Maillard reactions have a significant effect on off flavor formation.

**Lipid Oxidation**

Dairy protein ingredients contain varying amounts of fat, but flavor volatiles caused by lipid oxidation play a major role in the flavor of all dried dairy ingredients. The two types of lipid degradation reactions in dairy protein ingredients are hydrolytic rancidity and autoxidation. Hydrolytic rancidity is the release of fatty acids from the glycerol backbone of a glyceride molecule (McClements and Decker, 2008). Flavors associated with free fatty acids are the result of hydrolytic rancidity. In the case of certain cheeses, this reaction is enzyme mediated (lipase) and is a source of desirable flavor. In dried dairy ingredients, free fatty acid flavors (rancid, butyric, sweaty, soapy flavors) are not desirable. Autoxidation refers to the production of radicals caused by the reaction of lipids with oxygen and is a much more frequent source of off-flavors in dried dairy ingredients (Frankel, 2005). Lipid oxidation occurs in the presence of initiators (oxidizers) when unsaturated lipids lose a
hydrogen radical and form lipid free radicals. The alkyl radical of the unsaturated lipids containing a labile hydrogen reacts with molecular oxygen to form peroxy radicals. This is followed by a hydrogen transfer reaction with unsaturated lipids to form hydroperoxides (Frankel, 2005). Once the peroxy radicals accumulate, they begin to interact with each other to form non-radical products, terminating the reaction. Lipid oxidation begins in the earliest stages of dairy protein ingredient processing and can decrease sensory quality. Oleic, linoleic, and linolenic acids are unsaturated fatty acids commonly found in milk which, when auto-oxidized cause volatile flavor compound formation. Specifically, auto oxidation of oleic acid produces octanal, nonanal, decanal, 2-decenal, 2-undecenal, while auto-oxidation of linoleic acid produces hexanal, 2-octenal, 3-nonenal, and 2,4-decadienal, and auto-oxidation of linolenic acid produces propanal, 2-hexenal, 2,4-heptadienal, 3,6-nonadienal, and 2,4,7-decatrienal (Cadwallader and Singh, 2009). These compounds, at the appropriate concentrations, cause a host of undesirable flavors including cardboard, painty, fatty and cucumber (Drake et al., 2009; Whitson et al., 2010; Lloyd et al., 2009b).

Maillard Reactions

Maillard reaction products are also a major component of off flavors in milk and milk protein ingredients. Maillard reactions typically occur during processing and storage (Sikorski et al., 2008). Maillard reactions require a reducing sugar and an amine-containing compound such as a protein, peptide, or amino acid. Lactose is a reducing sugar and is a component of virtually all dairy protein ingredients, making the Maillard reaction a predominant interaction in dairy protein ingredients. Maillard reactions have three main stages as follows:
1. Reaction of an amine with a reducing sugar to form a glycosyl amine, which is then followed by the Amadori rearrangement;  
2. Dehydration of intermediate products, fragmentation of the saccharidic moiety, and Strecker degradation of the products;  
3. Reactions of intermediate products to produce heterocyclic flavor compounds (Sikorski et al., 2008)

Figure 2 is a representation of many of the off flavor compounds produced during different steps of the Maillard reaction.

The moisture content of a dry dairy protein ingredient must fall below 3% before Maillard reactions cease, which is below the target moisture content for most dried dairy ingredients (Sienkiewicz et al., 1990). As such, it is rare for Maillard reactions not to affect the flavor of a dairy protein ingredient. Maillard reactions are sourced to sweet aromatic and caramelized flavors in freshly manufactured milk and whey ingredients and brothy, vitamin and stale flavors in stored milk and whey ingredients (Kargul Yucer et al., 2001, 2002; Drake, 2006; Drake, 2007b; Wright et al., 2009; Drake et al., 2009b).

**Fluid Milk**

As the precursor of all dairy protein ingredients, fluid milk flavor is an important factor. Fluid milk is a complicated system and many compositional factors affect flavor formation. Calvo and de la Hoz (1992) stated that the origins of flavor-contributing compounds are typically due to the feed, microbial metabolism before dairy processing, or are generated by heat treatment or caused by storage of the fluid milk. Fat content, fatty acid composition, vitamin and mineral content, lactose, and salts all have an effect on the flavor of
fluid milk (Clark et al., 2009). Seasonal variability, primarily of milk fat levels, is seen due to differences in feed and stage of lactation (Varnam and Sutherland, 1994). The age and genetics of the cow, and the feed also have an effect on milk composition and flavor (Varnam and Sutherland, 1994; Croissant et al., 2007) as does processing. (z)-4 Heptenal (doughy/fatty), 1-octen-3-one (metallic/mushroom), hexanal (grassy), 12:2 lactone (baby powder) and dimethyl disulfide (onion) are significant flavor components of fluid milk (Chandan, 1997). Bendall (2001) identified 66 different aroma-active compounds in fluid milk, and classified them as nitrogen heterocycles, linolenic acid oxidation products, γ-lactones, phenolics, phytol derivatives, fatty acids, strecker esters, sulfur compounds, δ-lactones, terpenes, diacetyl and related compounds, and Strecker degradation products.

Croissant et al. (2007) studied the sensory attributes of fluid milk from both Jersey and Holstein cows on a total mixed ration (TMR) and a pasture based (PB) diet. Sensory profiles of the milks from different feeding methods were different, with pasture-fed cows producing milk with distinct grassy, mothball flavors, and salty taste profiles, while TMR fed cows produced milk characterized by a sweet feed/malty flavor (Table 3).

Figure 3 is a principal component biplot (PCA) of these descriptive analysis results and shows that the variation in flavor of fluid milks was due to feed (pasture versus TMR) more so than breed. Dimick et al. (1969) also found that pasture-fed cows had fewer lactones in their milk than silage-fed cows. Badings (1980) and Bendall (2001) reported that pasture feeding of cows caused an increase in indole, skatole, sulfides, mercaptans, nitriles, and thiocyanates, compounds that cause common off-flavors and potential sources of grassy and mothball flavors.
It is also well established that processing has an effect on fluid milk flavor. To ensure microbial safety, the vast majority of commercial milk is thermally processed. High-temperature short-time (HTST, typically 72°C for 15 s) ultra-pasteurized (UP, typically 138°C for 2 s), and ultra high-temperature (UHT, 135-150°C for 3-5s) processing are typical thermal treatments for fluid milk. Heat treatment increases lipid oxidation products and causes thermal degradation of milk proteins (Scanlan et al., 1968; Badings and Neeter, 1980; Moio et al., 1994). Impactful sulfur compounds, methyl ketones and lactones are all formed during heat treatment and as severity of heat treatment increases, levels of volatile flavor compounds increase (Scanlan et al., 1968; Badings and Neeter 1980; de la Hoz, 1992) and cooked, sulfurous, cabbage, and caramelized flavors also are increased with heat treatment.

**Milk Powders**

Milk powders are another widely used raw material in the formulation of many different foods. They have many uses including recombination back into milk, in the baking industry to increase volume of bread and improve water binding capacity, as a substitute for eggs in baked goods, as the milk solids in milk chocolate, in sausages and ready-cooked meals, baby food, ice cream, and as animal feed (USDEC, 2013).

Milk powders in the US are separated into three categories: dry whole milk/whole milk powder (WMP), nonfat dry milk (NFDM) and skim milk powder (SMP). WMP in the US is 24.5-27% protein, 26-40% fat, 2-4.5% moisture, 36-38.5% lactose, and 5.5-6.5% ash while NFDM and SMP are 34-37% protein, 49.5-52% lactose, 0.6-1.25% fat, 8.2-8.6% ash, and 3-4.5% moisture (USDEC, 2013). WMP is usually obtained by removing water from whole milk although it may also be a blending of fluid, condensed or dry SMP with liquid or
dry cream or with fluid, condensed or dry milk. NFDM and SMP are very similar to each other and are both made by removing water from pasteurized skim milk. The difference between the two powders is that SMP has a minimum milk protein content of 34% while NFDM has no standardized protein level (USDEC, 2013). The protein content of SMP can also be adjusted by adding either ultrafiltered milk retentate or permeate while NFDM cannot (ADPI, 2013).

**Skimmed Milk Powder/Non-fat Dry Milk**

SMP and NFDM can be designated low, medium or high heat based on heat treatment of milk prior to spray drying and subsequent whey protein nitrogen index (WPNI). WPNI is a method for the determination of soluble whey proteins in NFDM and gives a good indication of the heat treatment used on the milk before spray drying (USDEC, 2013b). Low heat NFDM has a WPNI of over 6.00 mg soluble whey protein per g powder, while medium heat NFDM has a WPNI between 1.51 and 5.99 mg/g, and high heat has WPNI under 1.50 mg/g (USDEC, 2013b). Sensory profiles of SMP/NFDM vary with heat treatment, age, and country of origin.

Freshly-manufactured low heat skim milk powders are characterized by cooked/milky and sweet aromatics. Like fluid milk from pasture feeding, skim milk powders from countries where pasture-feeding is common, may have grassy/hay flavors. These sweet fresh milk flavors decrease with storage and nondairy flavors (cardboard, animal, fatty) increase in intensity (Caudle *et al*., 2005; Drake *et al*., 2007b). Karagul-Yuceer *et al*. (2001) also conducted a study on NFDM with low, medium and high heat treatments which differed in their sensory characteristics (Table 5).
By using direct solvent extraction, gas chromatography-olfactometry and gas chromatography-mass spectrometry, Karagul-Yuceer et al. (2001) found that free fatty acids, lactones and browning/Maillard reaction products including maltol, Furaneol and aldehydes were the primary contributors to the aroma of NFDM while ketones and skatole played a smaller, but significant part. Heat-induced compounds such as Furaneol, maltol, sotolon, vanillin, butanoic acid, o-aminoacetophenone, (e)-4,5-epoxy-(e)-2-decenal, nonanal, and 1-octen-3-one were detected at higher levels in high-heat-treated NFDM.

**Whole Milk Powder**

Studies have also evaluated the flavor of WMP (Biolatto et al., 2007; Lloyd et al., 2009a,b). WMP has a flavor distinct from SMP and undergoes seasonal shifts in dimethyl sulfide, n-pentanal, n-hexanal and butyric acid concentrations (Biolatto et al., 2007). An increase in n-hexanal, n-pentanal and dimethyl sulfide concentration occurs in the summer season due to changes in the feed availability (Biolatto et al., 2007). Lloyd et al. (2009a) performed a study on a range of WMP produced in the US which had been stored for up to a year. Figure 4 is a PCA biplot of the initial descriptive analysis of US WMP from 4 different facilities.

Flavor variability was evident amongst freshly manufactured WMP. Lactones play a large role in flavor of WMP and contribute a distinct caramelized flavor (Carunchia Whetstine and Drake, 2007a). Flavor changes with storage, and lipid oxidation flavors (fatty, grassy and painty) were quickly evident due to the high fat content of WMP (Table 6).
**Milk Protein Concentrates**

Milk protein concentrates (MPCs) and isolates (MPIs) are a relatively new category of dairy ingredients with USDA statistics of US production only going back to 2008 (USDA, 2013). Production of MPC is on the rise in the US with an increase from 34,000 tonnes in 2008 to almost 47,000 tonnes in 2012 (USDA, 2013). Milk protein concentrates are manufactured by removing most of the lactose and minerals from skim milk using ultrafiltration (O’Donnell and Butler, 1996).

Like whey proteins, MPC are separated by protein content into different categories and have different sensory properties. MPC with lower protein levels have fluid milk-type flavors including cooked/milky, sweet aromatic, cereal, and sweet taste (Drake *et al.*, 2009a). As protein content increases the flavor profile changes. MPC (70 and 80% protein) and MPI (<90% protein) are characterized by tortilla, brothy, cardboard, and animal flavors in addition to higher astringency, and a decrease in sweet aromatic and milky flavors. Sensory profiles of MPC at 45 and 85% protein directly after production (0 month), and after 1 and 3 months of storage at 3, 25, and 40°C demonstrate the impact of protein, storage temperature and storage time on the sensory characteristics (Tables 7,8). Overall, higher protein MPC had lower cooked/milky and sweet aromatic flavors. The MPC85 developed higher cardboard flavor during storage while sweet aromatic flavor decreased. Tortilla flavor was only detected in MPC85 and increased with storage time and storage temperature. Both time and temperature variables decreased cooked milky and sweet aromatic flavors of low and high protein MPC (Table 7). High protein MPC were characterized by tortilla flavors with some exhibiting potato brothy and soapy characteristics.
Caseins

The primary protein fraction in bovine milk is casein. Caseins are extremely heat stable and have been isolated from milk and produced commercially for over 80 years (Mulvihill and Ennis, 2003). Originally caseins were used as glues or synthetic fibers and caseins did not gain popularity as a functional food ingredient until about 50 years ago (Mulvihill and Ennis, 2003). To remove caseins from milk, the milk is first skimmed to remove fat and then the caseins are destabilized so that the casein proteins will precipitate. Destabilization can be achieved by lowering the pH to the isoelectric point (pH 4.6) and achieving isoelectric precipitation (acid casein) or by enzymatic destabilization (rennet casein) (Rollema and Muir, 2009).

Acid Casein

Changing the pH of milk from about 6.7 (its natural pH) to 4.6 causes caseins to precipitate. This decrease in pH can be achieved two ways: mineral acids (HCl) or through fermentation of the lactose in the skim milk which produces lactic acid (O’Kennedy, 2011). The functionality of acid casein is severely limited due to its insolubility and thus is often pH neutralized through alkali addition to form a caseinate (O’Kennedy, 2009). Some alkalis commonly used include sodium hydroxide, calcium hydroxide, or sodium carbonate (O’Kennedy, 2009, 2011; Drake et al., 2009b).

Caseins and caseinates have distinct and generally non-dairy-like flavors and as such, are generally selected/used for their functionality rather than pleasant or dairy-like flavor (Table 9).
Both acid and rennet caseins are high in aroma intensity and tortilla flavor. Acid and rennet casein are also characterized by a dirty brothy/animal flavor which has been documented in the literature (Karagul-Yuceer et al., 2003; Isleten and Karagul-Yuceer, 2006). Since caseins are functional proteins and can be used to alter viscosity and other texture attributes, they have been studied recently in yogurt (Gonzalez et al., 2000; Isleten and Karagul-Yuceer, 2006; Guzman and Saint-Eve et al., 2006; Damin et al., 2009; Routray and Mishra, 2011; Akalin et al., 2012). To increase total solids in low and non-fat yogurt, dried dairy ingredients such as sodium caseinate are added to prevent textural defects such as poor gel firmness and syneresis (Isleten and Karagul-Yuceer, 2006; Routray and Mishra, 2011).

Istelen and Karagul-Yuceer (2006) compared physical and sensory properties of fat-free yogurts made with SMP, whey protein isolate (WPI), sodium caseinate, or a yogurt texture improver, during storage. Yogurts which used sodium caseinate displayed a distinct animal-like off flavor which had been previously identified as a key sensory attribute in dried rennet caseins (Karagul-Yuceer et al., 2003; Isleten and Karagul-Yuceer, 2006). Cardboard flavor and astringency were also detected in yogurts which used dried dairy ingredients but were highest in those fortified with sodium caseinate. Although these off-flavors were present, yogurts enriched with sodium caseinate had higher viscosities and less syneresis than the control yogurt. In addition, consumers preferred products fortified with sodium caseinate to all other treatments in this study (Istelen and Karagul-Yuceer, 2006).

Saint-Eve et al. (2006) investigated the role of protein composition at a constant protein level on 4% fat, strawberry flavored, stirred yogurt. Yogurts enriched with caseinates had a higher viscosity than those enriched with whey proteins. In addition, release of major
aroma compounds were lower in caseinate-enriched yogurts leading to lower flavor intensity and fruity notes which was in agreement with physicochemical measurements as yogurts with a high caseinate level had higher retention of aroma compounds suggesting protein/flavor binding (Hansen and Booker, 1996; Saint-Eve et al., 2006). The inclusion of dirty brothy/animal and tortilla flavors present in acid caseins and caseinates must be carefully weighed against their many functional benefits.

**Rennet Casein**

The production of rennet casein is nearly identical to the production of cheese curd and depends on the enzyme (rennet) cleaving the crucial peptide bond (Phe105-Met106) in K-casein (O’Kennedy, 2009). Like acid casein, in order to render rennet casein soluble, polyphosphates or citrates must be used to produce caseinates (Wallington, 1997). Rennet casein is used in the food industry not nearly as much as caseinates due to its insolubility (Wallington, 1997). Rennet caseins are used in cheese analog production and to make non-food items such as plastics (Wallington, 1997). Like caseinates, rennet caseins exhibit a distinct odor described as that of animal or wet dog. Figure 5 documents the aroma profiles of two rehydrated rennet caseins.

Karagul-Yuceer et al. (2003) identified 20 neutral/basic and 14 acidic odorants from rennet caseins. While O-aminoacetophenone was detected at the highest flavor dilution factor, sensory studies of model mixtures demonstrated that this compound may play only a minor role in the flavor of rennet casein with hexanoic acid, indole, guiacol, and P-cresol being the major contributors to the previously described animal/wet dog-like odor (Karagul-Yuceer et al., 2003).
A relatively recent review of cheese analogs detailed the texture properties of various proteins, such as caseinates and rennet casein, but only mentioned one study which incorporated sensory properties (Bachmann, 2001).

**Whey Proteins**

The United States is the largest cheese producer in the world, at almost 4.9 million tonnes in 2012 (USDA, 2012). Whey proteins are the byproduct of cheesemaking and as such, the US is the largest whey producer in the world. The US has seen growth in both whey protein concentrate (WPC, 34-89% protein) and whey protein isolate (WPI, >90% protein) production over the past 10 years. WPC production has increased from 142,000 tonnes in 2002 to 200,000 tonnes in 2012. WPI production in 2003 was about 10,000 tonnes while 2012 production was almost 30,000 tonnes (USDA, 2013). Whey protein concentrates are typically membrane processed and concentrated to reach the desired protein levels. Different whey products are used for a variety of food products. Whey concentrates and protein concentrates are used in breads and other baked goods, pre-cooked meats, confections, dry beverage mixes, beverages, seafood products, ice cream mixes, mayonnaise-type dressings, dairy products such as yogurt and processed cheese, snacks, animal feed, baby food, diet foods, and soups (USDEC, 2013d).

To understand better the introduction of flavor and off-flavor development, it is important to understand the processing steps that whey proteins are subject to before a final product is reached. During Cheddar cheese manufacture, for instance, the milk is received, pretreated, standardized, and heat treated/pasteurized. Calcium chloride and color (typically annatto) are also often added. The cheese milk is acidified through the addition of a lactic
acid-producing starter culture or by direct addition of lactic acid and then a coagulant enzyme (rennet) is added. After curd formation and initial whey drainage, the curds are cut and cooked to help expel the whey from the casein coagulant (curd). These steps influence the flavor and flavor chemistry of the fluid whey.

**Liquid Whey**

Liquid whey is the unconcentrated byproduct of Cheddar, Swiss, Mozzarella, Monterey Jack and similar cheeses (Mahajan *et al.*, 2004). The dried powder is typically composed of about 70% lactose, 1.5% fat, 12% protein, 4% moisture and 8.5% other solids (USDEC, 2013). Many studies have evaluated the flavor attributes of liquid whey (Carunchia-Whetstine *et al.*, 2003; Karagul-Yuceer *et al.*, 2003; Tomaino *et al.*, 2004; Drake *et al.*, 2009; Wright *et al.*, 2009; Liaw *et al.*, 2010, 2011; Campbell *et al.*, 2011b). Starter culture and type of set (rennet or acid) both have significant effects on the flavor of the liquid whey. Gallardo-Escamilla *et al.* (2005) reported that whey from rennet-set cheese was bland, sweet, and milky, while acid casein wheys were described as bitter, stale, rancid and chemical.

Campbell *et al.* (2011a,b) also reported bland, milky flavor and lower oxidation products in rennet-set whey without the addition of starter culture compared to that from rennet-set whey with starter culture. The type of cheese is also a source of variability (Tomaino *et al.*, 2004; Gallardo Escamilla *et al.*, 2005; Drake *et al.*, 2009; Campbell *et al.*, 2011b). Liaw *et al.* (2011) studied the differences between liquid wheys from Mozzarella and Cheddar manufacture. Mozzarella and Cheddar wheys were distinct in flavor profile following 3 days of storage at 3°C. Lipid oxidation continues in fluid whey despite
pasteurization, fat separation and cold storage (Tomaino et al., 2004; Liaw et al., 2011; Campbell et al., 2011a,b). This increase in lipid oxidation products results in increases in cardboard and serummy flavors in liquid wheys (Liaw et al., 2011; Campbell et al., 2011a,2011b).

Cheddar whey is more prone to lipid oxidation than Mozzarella whey, and further differences exist within different strains of starter cultures (Carunchia Whetstine et al., 2003; Campbell et al., 2011a; Liaw et al., 2011).

**Sweet Whey Powder**

Sweet whey powder (SWP) is the spray dried product of liquid whey manufactured from rennet coagulation of casein in milk. SWP goes through several steps before drying, including separations, pasteurization, concentration and crystallization (USDEC, 2013c; Tunick, 2008). SWPs are characterized by cooked, carmelized, cardboard, oxidized, and barny flavors (Sithole et al., 2005, 2006a,b). Many factors affect the stability of SWPs including variability in liquid whey, raw milk composition and processing (Sithole et al., 2006a). Different suppliers of SWP produce distinct products with unique flavor profiles (Table 11).

Mahajan et al. (2004) found that the important aroma volatiles in sweet whey powder were the short-chain fatty acids, aldehydes and ketones, lactones, sulfur compounds, phenols, indoles, pyrazines, furans and pyrroles and that autooxidation of lipids, caramelization of sugar, and Maillard reactions could explain the generation of many aroma compounds. Sithole et al. (2005) concluded that the shelf life of SWP is longer than 12 months; however, the deterioration rates of SWP from different commercial suppliers vary and should be taken
SWP contains a high concentration of lactose and is susceptible to Maillard reactions, which play a role in the rate of deterioration of flavor profiles of whey powders.

**Whey Protein Concentrates**

Processing variables have perhaps the most significant effect on the flavor of whey protein ingredients. A typical whey protein concentrate process includes holding, clarification/fat separation, pasteurization, ultrafiltration, diafiltration, evaporation and spray drying (Varnam and Sutherland, 1994; Huffman, 1996). Additional steps are necessary depending on the specific whey protein product. Production of some lower protein whey concentrates do not require diafiltration, while whey protein isolates (WPI) often require an extra fat removal step, such as microfiltration, in order to reach desired protein levels.

Tremendous variability in sensory profile is documented among WPC80 (80% protein) from different suppliers (Carunchia-Whetstine *et al.*, 2005; Wright *et al*., 2009; Evans *et al*., 2010). Differences are due to the source of whey as well as differences in processing procedures. Differences in fluid whey from different cheese manufacture translate to differences in whey protein flavor. WPC80 produced from Mozzarella whey had higher sweet aromatic and cereal flavors compared to WPC80 from Cheddar whey (Wright *et al*., 2009; Drake *et al*., 2009b). WPC66 (66% protein) from rennet-set whey with no starter culture was characterized by milky flavor and low cardboard flavor (Campbell *et al*., 2011a).

Storage of fluid product is an important factor in whey protein ingredient flavor. Figures 6 and 7 show a comparison of cardboard flavor intensity to aldehyde relative abundance (µg/kg) in WPC spray dried from Mozzarella whey and Cheddar WPI produced from liquid retentates stored for 0, 6, 12, 24, and 48 h before processing. Similar increases in
lipid oxidation and cardboard flavor were demonstrated by Liaw et al. (2011) in WPC produced from fresh and stored fluid Cheddar and Mozzarella wheys. Fluid storage of whey or retentate should be minimized (< 6 h) to minimize lipid oxidation and associated off-flavors.

Bleaching is another common liquid whey processing step for colored Cheddar whey which increases off flavor formation. Annatto, a pigment extract from the *Bixa orellana* shrub, is often added to milk intended for Cheddar cheese manufacture to provide desired orange color many consumers expect. Chemical bleaching is the most common method applied to remove the color despite the oxidative load this introduces to the system. Numerous publications have connected the bleaching of liquid whey with off-flavor formation in dried whey protein products (Croissant et al., 2009; Listiyani et al., 2011; Campbell et al., 2011a, 2012; Kang et al., 2012; Jervis et al., 2012). Jervis et al. (2012) studied volatile components produced by lipid oxidation and sensory attributes of WPC80 made from fluid whey bleached by benzoyl peroxide (BP) or hydrogen peroxide (HP). Figure 6 is a principal component analysis biplot of sensory attributes and lipid oxidation volatile components in those WPC80 ingredients. The HP-bleached WPC80 were characterized by higher concentrations of hexanal, heptanal, octanal, nonanal, decanal, dimethyl disulfide and 1-octen-3-one when compared with unbleached and BP-bleached WPC80. HP-bleached WPC80 were also characterized by higher intensities of cardboard and fatty flavors.

Hydrogen peroxide and benzoyl peroxide are the two chemical bleaching agents currently allowed for liquid whey, but the effect of alternative bleaching agents has also been
studied (Kang et al., 2012; Campbell et al., 2012). Alternative bleaching agents, including ultraviolet light, acid-activated bentonite, ozone, and lactoperoxidase compared to hydrogen peroxide and benzoyl peroxide, have variable effects on cardboard flavor intensity and total aldehyde levels in WPC80 (Figure 9). Every bleaching agent increased aldehyde levels and cardboard flavor intensity when compared to an unbleached control (Campbell et al., 2012; Jervis et al., 2012; Kang et al., 2012). Of the bleaching agents evaluated, only bentonite did not use oxidation as the primary method of norbixin destruction, which explains the low aldehyde increase compared to other bleaching agents studied. Bleaching of colored whey is a major factor in off-flavor formation.

Storage and agglomeration of the dried powder also have significant effects on flavor. Agglomeration is a process which produces small clumps of several whey powder particles, and is a common process because it increases dispersability and decreases dispersion time due to an increase in the particle size and porosity of the powder (Pietsch 2005; Turchiuli et al., 2005). A wetting agent may also be applied to increase solubility further, and soy lecithin is the most commonly used agent. Wright et al. (2009) studied the effect of agglomeration and storage on the flavor of WPC80. Both agglomeration and instantization decreased storage stability of WPC80 and WPI (Figures 10 and 11). The addition of lecithin also can contribute grassy and cucumber flavors and bitter taste to WPC80 and WPI.

**Whey Protein Isolates**

Many of the processing steps for WPI are the same as those used for high protein content WPC (>80% protein) production, although a considerable reduction in fat content is necessary to reach >90% protein levels. As such, the flavor profiles for the two are similar,
although differences do exist. Table 13 displays sensory profiles of rehydrated unbleached and hydrogen peroxide and bleached with benzoyl peroxide WPI. As with WPC80, chemical bleaching alters flavor profiles of WPI. WPIs from bleached whey have lower sweet aromatic flavor and higher cardboard flavor than WPI from unbleached whey. Flavor variability within commercial WPI has been studied, especially the occurrence of cabbage off-flavor. Wright et al. (2009) studied the volatile origin of this off-flavor. WPI with cabbage off-flavor had higher concentrations of dimethyl trisulfide, a sulfurous compound with a threshold in water of 0.07 parts per trillion (Wright et al., 2006). Dimethyltrisulfide, along with dimethyldisulfide and dimethyl sulfide, are all caused by degradation of methionine which can occur from oxidative processes during whey processing or storage.

**Whey Protein Hydrolysates**

Whey protein hydrolysates (WPH) are another category within whey protein concentrates and isolates that are commonly used as food ingredients. Whey proteins are first ultrafiltered/diafiltered to desired protein levels. Enzymatic hydrolysis using a variety of proteolytic enzymes is used to reach the desired level of proteolysis after which they are heated to inactivate the added enzymes (Havea et al., 2009). Whey protein hydrolysates are often touted for their bioactive properties stemming from specific protein fragments that may improve health (Nnanna and Wu, 2007). WPH have been suggested to have a protective effect against cardiovascular disease in addition to ion binding, antioxidant, immunomodulatory, satiety, and antiallergenicity effects (Nnanna and Wu, 2007).
Despite the health effects of WPHs, their general use is hindered due to strong, and often objectionable, sensory attributes (Table 14). WPHs differ from whey protein concentrates and isolates in enzymatic treatments, additional thermal treatments, and clarification steps. Reducing peptide chain length through enzymatic hydrolysis can also significantly increase bitter taste, further differentiating WPH from typical WPC and WPI (Spellman et al., 2009). Bitter taste of whey protein hydrolysates is variable, and dependent on the specific enzyme used for hydrolysis, the degree to which the protein is hydrolyzed, and processing (Spellman et al., 2009; Drake et al., 2009). Lekrisompong et al. (2010) reported that high bitter taste was correlated with low concentrations of large and medium chain peptides (2 to >10 kDa) and higher concentrations of low molecular weight peptides (<0.5 to 1 kDa). These findings are consistent with previous studies linking low molecular weight peptides with bitter taste (Cheison et al., 2007; Spellman et al., 2009). Lekrisompong et al. (2012) conducted work on masking the bitter taste inherent in whey protein hydrolysates, and suggested that fructose, sucrose, sucralose, monosodium glutamate, sodium acetate, sodium gluconate, 5’adenosine monophosphate (AMP), and 5’AMP disodium were all effective to some degree, with sweeteners showing the most promise.

Bitter taste is not the only sensory challenge of WPH. Many of the flavors in WPH have been documented in WPC and WPI, but the bitterness can range anywhere from 2.4 to 13.0 on a 15 point universal scale, far higher than what is detected in WPC or WPI. Other flavors documented in WPH were cooked/sulfur, potato/brothy, cheesy, tortilla/animal, and malty flavors. It is likely that the unique flavor profile of WPH is due to protein degradation compounds in addition to lipid oxidation products found in WPI and WPC. Lekrisompong et
al. (2010) reported that 2-methyl butanal (malty/chocolate) and methional (potato brothy), protein degradation products, were key volatile compounds in all WPH. Protein degradation products were key volatile compounds in all WPH. Sulfur compounds commonly found in WPH were dimethyl sulfide, 2-methyl-3-furanthiol, methyl 2-methyl-3furyl disulfide, dimethyl trisulfide, and dimethyl tetrasulfide. Other Strecker degradation compounds detected in some of the WPH samples were phenylacetaldehyde, 2-phenethanal, P-cresol, guaiacol, 2-acetyl pyrroline and sotolon. Lipid oxidation compounds were also evident.

Serum Protein

Whey proteins that have been removed prior to the cheese making process have been referred to as “native” whey proteins or milk serum proteins (Drake et al., 2009; Evans et al., 2009, 2010). Milk serum proteins are a valuable milk fraction and are not exposed to cheese making, thus leaving them free of any functional or sensory effects from this process. It may be advantageous to remove the milk serum proteins prior to cheese making for several reasons, particularly to produce more mild-tasting and consistent value-added whey protein products. When milk serum proteins are removed before the manufacture of cheese, the subsequent composition of the cheese is the same because the majority of these proteins are not retained in the cheese (Nelson and Barbano, 2005a). Serum proteins are prepared by microfiltering and diafiltering skimmed milk (Nelson and Barbano, 2005a).

Lipid oxidation is initiated during the cheese making process (Campbell et al., 2011b) and subsequent steps in whey processing increase levels of volatile oxidation products (Croissant et al., 2009; Campbell et al., 2011a; Whitson et al., 2011). Serum protein concentrates are lower in fat (0.53% vs 4.67% on a dry weight basis) than WPC (Evans et al.,
2009, 2010) because the fat remaining in skim milk is retained in the retentate of the MF process, while the serum proteins pass into the permeate. During production of WPC, the fat not removed from whey is concentrated by UF and DF in the WPC retentate. The high content of fat in WPC has a negative effect on flavor (and functionality) and provides a source for increased levels of lipid oxidation compounds, such as aldehydes, compared to SPC (Figure 9) (Liaw et al., 2010; Evans et al., 2010; Jervis et al., 2012; Campbell et al., 2013). Serum proteins contain lower amounts of volatile compounds and thus lower intensities of flavors commonly found in WPC due not only to the decreased fat content, but also to the absence of starter culture and cheese making residuals. In addition, WPC often has to be bleached as some of the annatto added to the cheese milk to impart desired color, remains in the fluid whey. Previous studies have demonstrated that bleaching significantly impacts flavor in both WPC and SPC but by removing proteins prior to cheese making, bleaching can be avoided (Campbell et al., 2013; Jervis et al., 2012). Bleaching using hydrogen peroxide, the most commonly used bleaching agent in US industry, increases total aldehydes (hexanal, heptanal, octanal, nonanal, decanal) nearly 20-fold in WPC and SPC (Figure 12).

Evans et al. (2009) compared the flavor profile of serum protein concentrates at 34% protein to whey protein concentrations at 34% protein. Spray dried SPC lacked both diacetyl and cardboard flavors (Table 15). Evans et al. (2010) subsequently compared the flavor profile of SPC80 (80% protein) to that of WPC80 (80% protein) as well as to several commercial WPC80s. The WPC80 had higher overall aroma intensity and also had cardboard and diacetyl flavors that were not present in the SPC80 (Tables 15 and 16).
Conclusions

Milk protein ingredient products and applications continue to increase in number as technology and processing methodologies continue to adapt to the demand for functional and nutritious ingredients. An understanding of the sensory properties of milk proteins and milk protein ingredients is essential to ensure the healthy growth and development of the dairy industry. While the nutritional and functional characteristics of milk protein ingredients are a marketing boon, disregarding the sensory aspects of these products would most certainly lead to missed opportunities and underutilization. A significant amount of information on the flavor characterization of different milk protein products exists in literature; however, continued research must be done on flavor reduction and improvement to realize fully the potential of milk protein products.
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Table 1: Language and preparation of reference materials for dried skim and whole milk powders and milk proteins (Adapted from Drake et al., 2003; Lloyd et al., 2009a, 2009b; Drake et al., 2009a)

<table>
<thead>
<tr>
<th>Descriptor</th>
<th>Reference</th>
<th>Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooked/sulfurous</td>
<td>Heated Milk</td>
<td>Heat pasteurized skim milk to 85°C for 45 min</td>
</tr>
<tr>
<td>Caramelized/butterscotch</td>
<td>Autoclaved milk</td>
<td>Autoclave whole milk at 121°C for 30 min</td>
</tr>
<tr>
<td>Caramel syrup</td>
<td></td>
<td>Dilute a tablespoon of caramel syrup in 400 mL skim milk</td>
</tr>
<tr>
<td>Sweet aromatic/cake mix</td>
<td>Pillsbury-White cake mix</td>
<td>Dilute 5 mg of vanillin in skim milk</td>
</tr>
<tr>
<td>Cereal/grass-like</td>
<td>Breakfast cereals (corn flakes, oat and wheaties)</td>
<td>Soak one cup cereal into three cups milk for 30 min and filter to remove cereals</td>
</tr>
<tr>
<td>Grassy/Hay</td>
<td>Timothy Hay</td>
<td></td>
</tr>
<tr>
<td>Barny</td>
<td>p-Cresol</td>
<td>20 ppm in skim milk</td>
</tr>
<tr>
<td>Brothy/potato-like</td>
<td>Canned white potato slices</td>
<td>Remove the sliced potatoes from the broth</td>
</tr>
<tr>
<td>Animal/gelatin-like/wet dog</td>
<td>Knox-unflavored gelatin</td>
<td>Dissolve one bag of gelatin (28 g) in two cups of distilled water</td>
</tr>
<tr>
<td>Milkfat/lactone</td>
<td>Heavy cream</td>
<td></td>
</tr>
<tr>
<td>Fried fatty/fryer oil/painty</td>
<td>(E,E)-2,4-decadienal or nonanal</td>
<td>2 ppb on filter paper in sniff jar</td>
</tr>
<tr>
<td>Fishy</td>
<td>Fresh fish with skin</td>
<td></td>
</tr>
<tr>
<td>Mushroom/metallic</td>
<td>Fresh mushroom</td>
<td>Slice fresh mushroom in skim milk for 30 min and filter to remove mushroom slices</td>
</tr>
<tr>
<td>Papery/cardboard</td>
<td>Cardboard paper</td>
<td>Soak pieces of cardboard paper in skim milk or water overnight</td>
</tr>
<tr>
<td>Tortilla</td>
<td>Packaged corn tortilla, or o-aminoaceto phenone</td>
<td>Packaged corn tortillas or 100 ppm o-aminoaceto phenone on filter paper in sniff jar</td>
</tr>
<tr>
<td>Musty/earthy</td>
<td>Aroma of damp basement or potting soil</td>
<td>1 part per trillion trichloroanisole on filter paper in sniff jar, potting soil</td>
</tr>
<tr>
<td>Burnt feathers/glue</td>
<td>Stale, degraded proteinaceous aroma</td>
<td>Elmers glue stored at 21°C for more than 12 months</td>
</tr>
<tr>
<td>Burnt/charcoal</td>
<td>Over toasted bread slice</td>
<td></td>
</tr>
<tr>
<td>Vitamin/rubber</td>
<td>Enfamil Liquid polyvisol vitamins</td>
<td></td>
</tr>
<tr>
<td>Diacetyl/buttery</td>
<td>Diacetyl</td>
<td>Diacetyl, 20 ppm on filter paper</td>
</tr>
<tr>
<td>Sweet taste</td>
<td>Sucrose</td>
<td>5% sucrose solution</td>
</tr>
<tr>
<td>Salty taste</td>
<td>NaCl</td>
<td>2% NaCl solution</td>
</tr>
<tr>
<td>Sour taste</td>
<td>Citric acid</td>
<td>1% citric acid solution</td>
</tr>
<tr>
<td>Bitter taste</td>
<td>Caffeine</td>
<td>0.5% caffeine solution</td>
</tr>
<tr>
<td>Umami</td>
<td>Monosodium glutamate</td>
<td>1% monosodium glutamate in water</td>
</tr>
<tr>
<td>Astringent</td>
<td>Tea</td>
<td>Soak 6 tea bags in water for 10 min</td>
</tr>
</tbody>
</table>
Table 2: Sensory language for descriptive analysis of fluid whey and whey proteins. (Adapted from Russell et al., 2006; Carunchia Whetstine et al., 2003; Liaw et al., 2011; Drake et al., 2009; Wright et al., 2009)

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
<th>Reference</th>
<th>Example/Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall aroma intensity</td>
<td>The overall orthonasal aroma impact</td>
<td></td>
<td>Evaluated as the lid is removed from the cupped sample</td>
</tr>
<tr>
<td>Sweet aromatic</td>
<td>Sweet aromatics associated with dairy products</td>
<td></td>
<td>Vanilla cake mix or 20 ppm vanillin in milk</td>
</tr>
<tr>
<td>Cooked/milky</td>
<td>Aromatics associated with cooked milk</td>
<td>Cooked milk</td>
<td>Heating skim milk to 85°C for 30 min</td>
</tr>
<tr>
<td>Doughy</td>
<td>Aromatics associated with canned biscuit dough</td>
<td>(z) - 4- heptenal</td>
<td>1 ppm (z)-4-heptenal in water from canned biscuit dough, or cooked pasta water</td>
</tr>
<tr>
<td>Fatty/frying oil</td>
<td>Aromatics associated with old frying oil and lipid oxidation products</td>
<td>2,4 - Decadienal</td>
<td>Old (stored) vegetable oil</td>
</tr>
<tr>
<td>Metallic/meat serum</td>
<td>Aromatic associated with metals or with juices of raw or rare beef</td>
<td>Aroma of fresh raw beef steak</td>
<td></td>
</tr>
<tr>
<td>Cucumber</td>
<td>Aromatics associated with freshly sliced cucumber</td>
<td>(e)-2-nonenal</td>
<td>1 ppm (e)-2-nonenal or freshly sliced cucumbers</td>
</tr>
<tr>
<td>Grassy/hay</td>
<td>Aromatics associated with dried grasses</td>
<td>Alfalfa or grass hay</td>
<td></td>
</tr>
<tr>
<td>Cardboard/wet paper</td>
<td>Aromatics associated with wet cardboard and brown paper</td>
<td>Cardboard paper</td>
<td>Brown cardboard or brown paper bag cut into strips and soaked in water</td>
</tr>
<tr>
<td>Potato brothy</td>
<td>Aromatics associated with boiled potatoes</td>
<td>Boiled potatoes</td>
<td>Drained broth from canned potatoes</td>
</tr>
<tr>
<td>Cabbage brothy</td>
<td>Aromatics associated with boiled cabbage</td>
<td>Boiled cabbage</td>
<td>Cabbage leaf boiled in 500 mL water for 5 min</td>
</tr>
<tr>
<td>Raisin/Spicy</td>
<td>Aromatic associated with stewed raisins</td>
<td>Boiled raisins</td>
<td>Boil 50 g dark raisins in 500 mL water</td>
</tr>
<tr>
<td>Animal/wet dog</td>
<td>Aromatics associated with wet dog hair</td>
<td>Knox gelatin</td>
<td>One bag of gelatin (28 g) dissolved in two cups of distilled water</td>
</tr>
<tr>
<td>Pasta water/cereal</td>
<td>Aromatics associated with water after pasta has been boiled in it or oatmeal</td>
<td>Boiled pasta or plain boiled oats</td>
<td>Pasta boiled in water for 30 min</td>
</tr>
<tr>
<td>Soapy</td>
<td>Aromatics associated with soap</td>
<td>Lauric acid</td>
<td>1 ppm lauric acid or shaved bar soap</td>
</tr>
<tr>
<td>Bitter</td>
<td>Basic taste associated with bitterness</td>
<td>Caffeine</td>
<td>Caffeine, 0.5% in water</td>
</tr>
<tr>
<td>Astringency</td>
<td>Chemical feeling factor characterized by a drying or puckering of the oral tissues</td>
<td>Alum</td>
<td>Alum, 1% in water</td>
</tr>
</tbody>
</table>
Table 3: Sensory profiles of 1.5% pasteurized milk from pasture-based and total mixed ration feeding system for each treatment and breed group.

<table>
<thead>
<tr>
<th>Sensory Attribute</th>
<th>Jersey TMR</th>
<th>Jersey Pasture</th>
<th>Holstein TMR</th>
<th>Holstein Pasture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aroma Intensity</td>
<td>2.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sweet Aromatic</td>
<td>2.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cooked</td>
<td>2.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Milk fat</td>
<td>2.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Grassy</td>
<td>ND</td>
<td>1.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>1.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mothball</td>
<td>ND</td>
<td>1.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>1.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sweet feed/malty</td>
<td>1.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>1.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
</tr>
<tr>
<td>Sweet</td>
<td>2.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Salty</td>
<td>ND</td>
<td>0.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>1.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Astringency</td>
<td>1.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a-c</sup> Means within rows with different superscripts are different (P<0.05).

Scores based on a universal 0-15 point intensity scale.

Means are from duplicate analyses by 10 trained panelists.

ND = not detected.

Adapted from Croissant <i>et al.</i> (2007).
Table 4: Descriptive analysis profiles of various SMP (Adapted in part from Caudle et al., 2005).

<table>
<thead>
<tr>
<th>Attribute</th>
<th>SMP1</th>
<th>SMP2</th>
<th>SMP3</th>
<th>SMP4</th>
<th>SMP5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooked</td>
<td>3.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sweet Aromatic</td>
<td>3.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.0&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Grassy</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>1.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Animal</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>2.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
</tr>
<tr>
<td>Fatty/fryer Oil</td>
<td>ND</td>
<td>ND</td>
<td>2.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Sweet Taste</td>
<td>2.5&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Astringency</td>
<td>1.5&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.5&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a-c</sup> Means within rows with different superscripts are different (P<0.05).
Scores based on a universal 0-15 point intensity scale.
Means are from duplicate analyses by 10 trained panelists.
ND = not detected.
Table 5: Descriptive analysis profiles of nonfat dry milk treated with low, medium or high heat

<table>
<thead>
<tr>
<th>Attributes</th>
<th>Low</th>
<th>Medium</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooked/sulfurous</td>
<td>3.0c</td>
<td>3.6b</td>
<td>4.3a</td>
</tr>
<tr>
<td>Sweet aromatic/cake mix</td>
<td>1.0a</td>
<td>0.5b</td>
<td>ND</td>
</tr>
<tr>
<td>Cardboard</td>
<td>1.0b</td>
<td>1.5a</td>
<td>1.5a</td>
</tr>
<tr>
<td>Sweet Taste</td>
<td>1.9a</td>
<td>1.5b</td>
<td>1.0c</td>
</tr>
<tr>
<td>Astringent</td>
<td>0.5b</td>
<td>0.7b</td>
<td>1.2a</td>
</tr>
</tbody>
</table>

*a-c Means within rows with different superscripts are different (P<0.05).
Scores based on a universal 0-15 point intensity scale.
Means are from duplicate analyses by 10 trained panelists.
Adapted from Karagul-Yuceer *et al.* (2001).
ND = not detected.
Table 6: Mean flavor attributes in US whole milk powders through 12 mo storage. (Adapted from Lloyd *et al.*, 2009a.)

<table>
<thead>
<tr>
<th>Storage time (mo)</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aroma Intensity</strong></td>
<td>2.0b</td>
<td>2.0b</td>
<td>2.0b</td>
<td>2.1b</td>
<td>2.2b</td>
<td>2.4a</td>
<td>2.5a</td>
</tr>
<tr>
<td><strong>Cooked/sulfurous</strong></td>
<td>2.5a</td>
<td>2.3b</td>
<td>2.2c</td>
<td>2.1cd</td>
<td>2.0d</td>
<td>2.0d</td>
<td>2.0d</td>
</tr>
<tr>
<td><strong>Milk fat</strong></td>
<td>2.6a</td>
<td>2.4b</td>
<td>2.2c</td>
<td>2.1d</td>
<td>1.9d</td>
<td>2.0d</td>
<td>1.9d</td>
</tr>
<tr>
<td><strong>Sweet aromatic/caramelized</strong></td>
<td>2.5a</td>
<td>2.2b</td>
<td>2.1c</td>
<td>2.0cd</td>
<td>1.8d</td>
<td>1.8d</td>
<td>1.8d</td>
</tr>
<tr>
<td><strong>Grassy/hay</strong></td>
<td>ND</td>
<td>1.1d</td>
<td>1.4c</td>
<td>1.6bc</td>
<td>1.8b</td>
<td>1.9b</td>
<td>2.4a</td>
</tr>
<tr>
<td><strong>Fatty/fryer oil/painty</strong></td>
<td>ND</td>
<td>ND</td>
<td>0.5e</td>
<td>1.0d</td>
<td>1.4c</td>
<td>1.6b</td>
<td>2.0a</td>
</tr>
<tr>
<td><strong>Sweet taste</strong></td>
<td>2.1a</td>
<td>2.1a</td>
<td>2.1a</td>
<td>2.0a</td>
<td>2.0a</td>
<td>1.9a</td>
<td>1.9a</td>
</tr>
<tr>
<td><strong>Astringency</strong></td>
<td>1.3b</td>
<td>1.3b</td>
<td>1.3b</td>
<td>1.4b</td>
<td>1.4b</td>
<td>1.5ab</td>
<td>1.7a</td>
</tr>
</tbody>
</table>

Values represent pooled means from duplicate panel measurements from 5 shipments from 4 facilities. Different letter with a row indicate significant difference (P <0.05). Intensities were scored on a 0-15 point universal Spectrum™ intensity scale where 0 = absence of the attribute and 15 = very high intensity of the attribute. ND – not detected.
Table 7: Sensory profiles of milk protein concentrates containing 45% and 85% protein (MPC 45 and MPC 85 samples stored for 0, 1, and 3 months at 3, 25 or 40°C).

<table>
<thead>
<tr>
<th></th>
<th>45 0 mo</th>
<th>85 0 mo</th>
<th>45 3°C 1 mo</th>
<th>45 3°C 3 mo</th>
<th>45 25°C 1 mo</th>
<th>45 25°C 3 mo</th>
<th>45 40°C 1 mo</th>
<th>45 40°C 3 mo</th>
<th>85 3°C 1 mo</th>
<th>85 3°C 3 mo</th>
<th>85 25°C 1 mo</th>
<th>85 25°C 3 mo</th>
<th>85 40°C 1 mo</th>
<th>85 40°C 3 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aroma Intensity</td>
<td>2.5</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
<td>1.5</td>
</tr>
<tr>
<td>Cooked/Milky</td>
<td>3.5</td>
<td>1.8</td>
<td>3.1</td>
<td>3.0</td>
<td>3.0</td>
<td>2.8</td>
<td>2.4</td>
<td>2.3</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
<td>1.0</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Sweet aromatic</td>
<td>3.0</td>
<td>1.0</td>
<td>2.2</td>
<td>2.0</td>
<td>2.5</td>
<td>2.3</td>
<td>2.0</td>
<td>2.0</td>
<td>1.0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Cardboard</td>
<td>1.5</td>
<td>1.5</td>
<td>1.0</td>
<td>1.3</td>
<td>1.5</td>
<td>1.5</td>
<td>1.3</td>
<td>1.5</td>
<td>1.5</td>
<td>2.0</td>
<td>1.5</td>
<td>2.2</td>
<td>1.5</td>
<td>2.7</td>
</tr>
<tr>
<td>Cereal</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Tortilla</td>
<td>ND</td>
<td>1.5</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Sweet taste</td>
<td>1.8</td>
<td>ND</td>
<td>1.8</td>
<td>2.0</td>
<td>1.7</td>
<td>1.7</td>
<td>1.4</td>
<td>1.3</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Astringency</td>
<td>1.8</td>
<td>2.5</td>
<td>2.5</td>
<td>2.0</td>
<td>2.5</td>
<td>2.5</td>
<td>2.8</td>
<td>2.8</td>
<td>3.0</td>
<td>3.0</td>
<td>3.5</td>
<td>3.7</td>
<td>3.5</td>
<td>3.5</td>
</tr>
</tbody>
</table>

Values represent pooled means from duplicate panel measurements.

ND – not detected.

Intensities were scored on a 0-15 point universal Spectrum™ intensity scale where 0 = absence of the attribute and 15 = very high intensity of the attribute.
Table 8: Sensory profiles of pilot plant and commercial milk protein concentrates 45, 70, 80, 85 and milk protein isolate within 4 weeks of manufacture.

<table>
<thead>
<tr>
<th></th>
<th>MPC45 (PP)</th>
<th>MPC85 (PP)</th>
<th>MPC70 (A)</th>
<th>MPC85(A)</th>
<th>MPI90(A)</th>
<th>MPC80(B)</th>
<th>MPC80(C)</th>
<th>MPC85(C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aroma Intensity</td>
<td>2.5</td>
<td>1.5</td>
<td>2.0</td>
<td>2.5</td>
<td>2.5</td>
<td>1.0</td>
<td>2.0</td>
<td>1.2</td>
</tr>
<tr>
<td>Sweet aromatic</td>
<td>3.2</td>
<td>1.2</td>
<td>1.8</td>
<td>1.5</td>
<td>ND</td>
<td>1.0</td>
<td>0.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Cardboard</td>
<td>1.4</td>
<td>2.0</td>
<td>1.6</td>
<td>2.3</td>
<td>2.5</td>
<td>ND</td>
<td>1.5</td>
<td>2.0</td>
</tr>
<tr>
<td>Potato brothy</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>1.5</td>
<td>1.5</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Sweet taste</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>1.0</td>
<td>2.2</td>
<td>1.8</td>
<td>2.2</td>
<td>1.0</td>
</tr>
<tr>
<td>Fatty</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>2.0</td>
<td>ND</td>
<td>1.0</td>
<td>ND</td>
</tr>
<tr>
<td>Astringency</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Values represent pooled means from duplicate panel measurements.
ND – not detected.
PP = pilot plant
A,B, and C denote products from three different commercial suppliers.
Intensities were scored on a 0-15 point universal Spectrum™ intensity scale where 0 = absence of the attribute and 15 = very high intensity of the attribute.
Table 9: Sensory profile of commercial milk protein concentrates containing 80 or 85% protein (C80 and 85, respectively), acid casein (AC), and rennet casein (RC).

<table>
<thead>
<tr>
<th></th>
<th>C80</th>
<th>C85</th>
<th>AC</th>
<th>RC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aroma Intensity</strong></td>
<td>2.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sweet aromatic</td>
<td>0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Sour aromatic</td>
<td>ND</td>
<td>ND</td>
<td>1.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
</tr>
<tr>
<td>Cardboard</td>
<td>1.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ND</td>
<td>2.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dirty brothy/animal</td>
<td>ND</td>
<td>ND</td>
<td>3.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tortilla</td>
<td>2.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Soapy</td>
<td>1.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Fatty</td>
<td>1.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Sour taste</td>
<td>ND</td>
<td>ND</td>
<td>1.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
</tr>
<tr>
<td>Astringency</td>
<td>2.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Values represent pooled means from duplicate panel measurements.

ND – not detected.

Intensities were scored on a 0-15 point universal Spectrum™ intensity scale where 0 = absence of the attribute and 15 = very high intensity of the attribute.

Means in a row followed by different letters are different (p<0.05)

C80 = MPC80, C85 = MPC85, AC = Acid Casein, RC = Rennet Casein
Table 10: Sensory flavor attributes of liquid wheys from Cheddar and Mozzarella manufacture initially and after 3 d at 3°C.

<table>
<thead>
<tr>
<th>Sensory attribute</th>
<th>Cheddar No fat separation</th>
<th>Cheddar Fat Separation</th>
<th>Mozzarella No fat separation</th>
<th>Mozzarella Fat Separation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 d</td>
<td>3 d</td>
<td>0 d</td>
<td>3 d</td>
</tr>
<tr>
<td>Aroma intensity</td>
<td>2.4&lt;sup&gt;a&lt;/sup&gt; 2.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.4&lt;sup&gt;a&lt;/sup&gt; 2.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.2&lt;sup&gt;ab&lt;/sup&gt; 2.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.0&lt;sup&gt;bc&lt;/sup&gt; 1.6&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sweet aromatic</td>
<td>2.0&lt;sup&gt;a&lt;/sup&gt; 1.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.0&lt;sup&gt;a&lt;/sup&gt; 1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.1&lt;sup&gt;b&lt;/sup&gt; 0.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.1&lt;sup&gt;b&lt;/sup&gt; 0.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sour aromatic</td>
<td>ND 1.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND 0.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND 1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ND 0.5&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cardboard</td>
<td>3.2&lt;sup&gt;ab&lt;/sup&gt; 2.7&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>3.2&lt;sup&gt;a&lt;/sup&gt; 2.8&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>3.2&lt;sup&gt;ab&lt;/sup&gt; 2.4&lt;sup&gt;de&lt;/sup&gt;</td>
<td>2.9&lt;sup&gt;abc&lt;/sup&gt; 2.2&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cooked/milky</td>
<td>2.0&lt;sup&gt;a&lt;/sup&gt; 2.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.0&lt;sup&gt;a&lt;/sup&gt; 2.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.7&lt;sup&gt;b&lt;/sup&gt; 1.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.6&lt;sup&gt;b&lt;/sup&gt; 1.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Astringent</td>
<td>1.5&lt;sup&gt;a&lt;/sup&gt; 1.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.5&lt;sup&gt;a&lt;/sup&gt; 1.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.5&lt;sup&gt;a&lt;/sup&gt; 1.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.5&lt;sup&gt;a&lt;/sup&gt; 1.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means in a row followed by different superscript letters signify differences (P<0.05). ND = not detected. Attributes were scored using a 0 to 15 point universal Spectrum™ scale where 0 = absence of the attribute and 15 = extremely high intensity of attribute. Used with permission from Liaw et al. (2011).
Table 11: Sensory profiles of commercial sweet whey powders.

<table>
<thead>
<tr>
<th></th>
<th>SW1</th>
<th>SW2</th>
<th>SW3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sweet aromatic</strong></td>
<td>1.4</td>
<td>1.2</td>
<td>1.1</td>
</tr>
<tr>
<td><strong>Caramelized</strong></td>
<td>2.4</td>
<td>2.7</td>
<td>2.9</td>
</tr>
<tr>
<td><strong>Cooked</strong></td>
<td>3.9</td>
<td>3.8</td>
<td>4.0</td>
</tr>
<tr>
<td><strong>Cardboard</strong></td>
<td>1.8</td>
<td>1.9</td>
<td>2.0</td>
</tr>
<tr>
<td><strong>Oxidized</strong></td>
<td>1.2</td>
<td>1.7</td>
<td>1.6</td>
</tr>
<tr>
<td><strong>Barny</strong></td>
<td>0.8</td>
<td>1.6</td>
<td>1.6</td>
</tr>
<tr>
<td><strong>Sweet taste</strong></td>
<td>4.1</td>
<td>4.8</td>
<td>3.5</td>
</tr>
<tr>
<td><strong>Sour taste</strong></td>
<td>1.6</td>
<td>1.2</td>
<td>1.4</td>
</tr>
<tr>
<td><strong>Salty taste</strong></td>
<td>2.6</td>
<td>2.2</td>
<td>2.6</td>
</tr>
</tbody>
</table>

Rating scale was a 15-point scale, where 3 = slight, and 7 = moderate
Adapted from Sithole *et al.* (2005).
Table 12: Descriptive sensory analysis of whey protein concentrates (WPC80; 80% protein) from different manufacturers.

<table>
<thead>
<tr>
<th></th>
<th>WPC 1</th>
<th>WPC 2</th>
<th>WPC 3</th>
<th>WPC 4</th>
<th>WPC 5</th>
<th>WPC 6</th>
<th>WPC 7</th>
<th>WPC 8</th>
<th>WPC 9</th>
<th>WPC 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aroma intensity</td>
<td>1.6c</td>
<td>3.0a</td>
<td>2.0b</td>
<td>3.0a</td>
<td>1.8b</td>
<td>3.5a</td>
<td>3.5a</td>
<td>3.3a</td>
<td>2.0b</td>
<td>1.4c</td>
</tr>
<tr>
<td>Sweet aromatic</td>
<td>0.8c</td>
<td>ND</td>
<td>ND</td>
<td>2.9a</td>
<td>2.0b</td>
<td>1.5c</td>
<td>ND</td>
<td>ND</td>
<td>1.3c</td>
<td>2.1b</td>
</tr>
<tr>
<td>Cooked milky</td>
<td>1.1b</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>2.0a</td>
<td>1.5ab</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Cardboard</td>
<td>1.3e</td>
<td>3.3a</td>
<td>2.0b</td>
<td>ND</td>
<td>1.5b</td>
<td>ND</td>
<td>ND</td>
<td>3.3a</td>
<td>1.6b</td>
<td>0.6c</td>
</tr>
<tr>
<td>Pasta water</td>
<td>ND</td>
<td>ND</td>
<td>1.5d</td>
<td>1.5d</td>
<td>2.5c</td>
<td>3.3b</td>
<td>4.0a</td>
<td>2.5c</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Brothy</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>1.5</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Doughy fatty</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>1.5</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>Cereal</td>
<td>1.9</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Astringent</td>
<td>2.3b</td>
<td>3.4a</td>
<td>3.0ab</td>
<td>2.8b</td>
<td>2.5b</td>
<td>3.0ab</td>
<td>2.5b</td>
<td>2.4b</td>
<td>2.3b</td>
<td>2.3b</td>
</tr>
</tbody>
</table>

*a-c* Means in a row followed by different letters are different (p<0.05).

WPC from different manufacturing sites
Intensities were scored on a 0 to 15-point universal scale where 0 = none and 15 = very high (Meilgaard et al., 1999). ND = Not detected
Adapted from Carunchia-Whetstine et al. (2005), Wright et al. (2009), and Evans et al. (2010).
Table 13: Descriptive sensory profiles of unbleached WPI and WPI bleached using 250ppm hydrogen peroxide (HP) or 50 ppm benzoyl peroxide (BP)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>HP</th>
<th>BP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aroma intensity</strong></td>
<td>2.0b</td>
<td>2.5a</td>
<td>2.0b</td>
</tr>
<tr>
<td><strong>Sweet aromatic</strong></td>
<td>2.0a</td>
<td>ND</td>
<td>0.5b</td>
</tr>
<tr>
<td><strong>Cardboard</strong></td>
<td>1.5c</td>
<td>2.5a</td>
<td>2.0b</td>
</tr>
<tr>
<td><strong>Cabbage</strong></td>
<td>ND</td>
<td>1.0</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Fatty</strong></td>
<td>ND</td>
<td>ND</td>
<td>1.2</td>
</tr>
<tr>
<td><strong>Bitter</strong></td>
<td>ND</td>
<td>1.1a</td>
<td>ND</td>
</tr>
</tbody>
</table>

Means in a row followed by different lowercase letters signify differences (P<0.05). ND = not detected. Attributes were scored using a 0 to 15 point universal Spectrum™ scale where 0 = absence of the attribute and 15 = extremely high intensity of attribute.
Table 14: Descriptive sensory profiles of selected whey protein hydrolysates.

<table>
<thead>
<tr>
<th>Aroma Intensity</th>
<th>Cooked/ milky</th>
<th>Cooked/ sulfur</th>
<th>Malty</th>
<th>Cardboard</th>
<th>Potato broth</th>
<th>Cheesy broth</th>
<th>Grassy/ herbal</th>
<th>Tortilla/ animal</th>
<th>Cucumber</th>
<th>Scorched</th>
<th>Sour</th>
<th>Bitter</th>
<th>Astringency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 2.9cde</td>
<td>ND</td>
<td>1.7ab</td>
<td>ND</td>
<td>0.8a</td>
<td>1.1c</td>
<td>1.3a</td>
<td>1.0a</td>
<td>ND</td>
<td>ND</td>
<td>2.0b</td>
<td>2.3a</td>
<td>12.4ab</td>
<td>3.0bc</td>
</tr>
<tr>
<td>2 2.9cde</td>
<td>2.0a</td>
<td>1.0ab</td>
<td>ND</td>
<td>ND</td>
<td>0.5d</td>
<td>ND</td>
<td>0.5b</td>
<td>ND</td>
<td>ND</td>
<td>2.5a</td>
<td>10.1c</td>
<td>3.1bc</td>
<td></td>
</tr>
<tr>
<td>3 2.1ef</td>
<td>1.1ab</td>
<td>1.4ab</td>
<td>ND</td>
<td>ND</td>
<td>1.5c</td>
<td>0.5b</td>
<td>1.5a</td>
<td>ND</td>
<td>ND</td>
<td>0.9c</td>
<td>ND</td>
<td>2.4h</td>
<td>2.5cde</td>
</tr>
<tr>
<td>4 1.9f</td>
<td>ND</td>
<td>0.9bc</td>
<td>ND</td>
<td>ND</td>
<td>1.3c</td>
<td>1.0a</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.8c</td>
<td>ND</td>
<td>5.6e</td>
<td>2.1e</td>
</tr>
<tr>
<td>5 3.5bc</td>
<td>ND</td>
<td>0.9ab</td>
<td>3.0a</td>
<td>1.0a</td>
<td>2.7b</td>
<td>1.4a</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.9c</td>
<td>ND</td>
<td>3.6f</td>
<td>2.4de</td>
</tr>
<tr>
<td>6 2.6def</td>
<td>ND</td>
<td>ND</td>
<td>0.6bc</td>
<td>0.5b</td>
<td>1.6c</td>
<td>0.9ab</td>
<td>ND</td>
<td>ND</td>
<td>0.6d</td>
<td>3.1a</td>
<td>2.6a</td>
<td>11.7b</td>
<td>2.3de</td>
</tr>
<tr>
<td>7 2.5def</td>
<td>ND</td>
<td>ND</td>
<td>0.5bc</td>
<td>1.0a</td>
<td>1.6c</td>
<td>1.5a</td>
<td>ND</td>
<td>ND</td>
<td>2.6b</td>
<td>ND</td>
<td>0.8b</td>
<td>13.0a</td>
<td>3.8ab</td>
</tr>
<tr>
<td>8 3.7b</td>
<td>ND</td>
<td>2.0ab</td>
<td>ND</td>
<td>ND</td>
<td>2.3b</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>2.4b</td>
<td>0.9b</td>
<td>ND</td>
<td>2.8g</td>
<td>3.0bc</td>
</tr>
<tr>
<td>9 3.7b</td>
<td>ND</td>
<td>1.2ab</td>
<td>ND</td>
<td>ND</td>
<td>1.5c</td>
<td>ND</td>
<td>1.3a</td>
<td>ND</td>
<td>2.3b</td>
<td>ND</td>
<td>ND</td>
<td>4.0f</td>
<td>3.1bc</td>
</tr>
<tr>
<td>10 4.6a</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>3.6a</td>
<td>ND</td>
<td>ND</td>
<td>4.4a</td>
<td>ND</td>
<td>2.8a</td>
<td>2.8a</td>
<td>9.2cd</td>
<td>4.1a</td>
</tr>
<tr>
<td>11 2.7b</td>
<td>ND</td>
<td>3.4a</td>
<td>1.8ab</td>
<td>ND</td>
<td>1.5c</td>
<td>ND</td>
<td>2.0b</td>
<td>ND</td>
<td>2.4b</td>
<td>2.4a</td>
<td>ND</td>
<td>4.7ef</td>
<td>2.4de</td>
</tr>
</tbody>
</table>

*a-c* Means in a row followed by different lowercase letters signify differences (P<0.05). ND = not detected. Attributes were scored using a 0 to 15 point universal Spectrum™ scale where 0 = absence of the attribute and 15 = extremely high intensity of attribute.

Used with permission (Lekrisompong et al., 2010).
Table 15: Sensory attributes of spray-dried (SD) serum protein concentrate (SPC) and whey protein concentrate (WPC).

<table>
<thead>
<tr>
<th>Product</th>
<th>Aroma Intensity</th>
<th>Sweet aromatic</th>
<th>Diacetyl</th>
<th>Cardboard</th>
<th>Cereal</th>
<th>Cooked/milky</th>
<th>Sweet taste</th>
<th>Astringent</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD WPC</td>
<td>1.8^b</td>
<td>1.1^b</td>
<td>0.5^a</td>
<td>0.6^a</td>
<td>0.7^b</td>
<td>1.4^a</td>
<td>2.0^a</td>
<td>1.7^a</td>
</tr>
<tr>
<td>SD SPC</td>
<td>1.7^bc</td>
<td>0.7^c</td>
<td>ND</td>
<td>ND</td>
<td>1.0^a</td>
<td>1.5^a</td>
<td>2.0^a</td>
<td>1.7^a</td>
</tr>
</tbody>
</table>

^a-cMeans in the same column not sharing a common superscript are different (p<0.05).

Intensities were scored on a 0 to 15-point universal scale where 0 = none and 15 = very high intensity (Meilgaard et al., 1999)

ND = not detected.

Adapted from Evans et al. (2009).
Table 16: Mean sensory attributes of the experimental 80% serum protein concentrate (SPC) and whey protein concentrate (WPC) and 5 commercial 80% WPC products.

<table>
<thead>
<tr>
<th>Source</th>
<th>Product</th>
<th>Aroma Intensity</th>
<th>Sweet aromatic</th>
<th>Diacetyl</th>
<th>Cardboard</th>
<th>Cereal</th>
<th>Cooked/milky</th>
<th>Potato</th>
<th>Astringent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial WPC</td>
<td>1</td>
<td>1.8&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>1.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>2.3&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ND</td>
<td>1.1&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>2.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.4&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>1.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>1.0&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>1.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>2.3&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>2.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.1</td>
<td>1.3&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>0.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>2.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Experimental</td>
<td>SPC</td>
<td>1.2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>0.8&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>1.6&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Experimental</td>
<td>WPC</td>
<td>1.6&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ND</td>
<td>1.3&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>1.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>1.9&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a-c</sup>Means in the same column not sharing a common superscript are different (p<0.05).

Intensities were scored on a 0 to 15-point universal scale where 0 = none and 15 = very high intensity (Meilgaard <i>et al.</i>, 1999)

ND = not detected.

Adapted from Evans <i>et al.</i> (2010).
Figure 1: Various typical steps in a milk processing application. (Adapted from Cadwallader and Singh, 2009) UHT: Ultra-high temperature processing; UF: ultrafiltration; MF: microfiltration.
Figure 2: Formation of aroma compounds via Maillard reaction/non-enzymatic browning (Cadwallader and Singh, 2009; used with permission).
Figure 3: Principal component analysis biplot of sensory analysis of fluid milk derived from TMR (total mixed ration) and PB (pasture-based) Jersey and Holstein cows. Croissant et al., 2007. Used with permission.
Figure 4: Principal component analysis biplot of initial descriptive analysis of whole milk powder from 4 facilities. Numbers represent shipments (1 to 5) and letters represent facilities (A to D). Each point represents duplicate analyses from 2 bags produced on different days. Reproduced with permission from Lloyd et al. (2009a).
Figure 5: Sensory profiles of rehydrated rennet casein (adapted from Karagul-Yuceer et al., 2003).
Figure 6: Comparison of cardboard flavor intensity to total aldehyde relative abundance (µg/kg) in Mozzarella whey protein concentrate from liquid retentate stored for 0, 6, 12, 24, or 48 h at 3°C.

Means followed by different superscripts indicate differences (p < 0.05) among hold times. Used with permission from Whitson et al. (2011).
Figure 7: Comparison of cardboard flavor intensity to total aldehyde relative abundance (µg/kg) in Cheddar whey protein isolate from liquid retentate stored for 0, 6, 12, 24, or 48 h. Means followed by different superscripts indicate differences (p < 0.05) among hold times. Used with permission from Whitson et al. (2011).
Figure 8: Principal component analysis biplot of sensory attributes and selected lipid oxidation volatile components of WPC80 with and without annatto color added to the milk followed by no bleaching, bleaching with benzoyl peroxide (BP; 50 mg/kg), or bleaching with hydrogen peroxide (HP; 500 mg/kg). Used with permission (Jervis et al., 2012).
Figure 9: Comparison of descriptive analysis cardboard flavor intensity to percent total aldehyde increase in Cheddar whey protein concentrates bleached with 250 ppm hydrogen peroxide (HP250), 50 ppm benzoyl peroxide (BP50), acid activated bentonite (BT), ultraviolet light (UV), ozone (OZ), and the lactoperoxidase system using 20 ppm hydrogen peroxide (LP).

Adapted from Kang et al. (2012), Jervis et al. (2012), and Campbell et al. (2012).
Figure 10: Principal component analysis biplot of sensory changes in nonagglomerated and agglomerated WPC80 from Plant 1 over time. Numbers indicate treatment type and storage time. The letter “C” represents the nonagglomerated control. “S” represents the steam-agglomerated samples. “L” represents the lecithin-agglomerated samples. The number next to each letter represents the storage in months at the time of analysis. Used with permission (Wright et al., 2009).
Figure 11: Principal component analysis biplot of sensory changes in nonagglomerated and agglomerated whey protein concentrate (WPC80) from Plant 2 over time. Numbers indicate treatment type and storage time. The letter “C” represents the nonagglomerated control. “S” represents the steam-agglomerated samples. The number next to each letter represents the storage in months at the time of analysis. Used with permission (Wright et al., 2009).
Figure 12. Total aldehydes (hexanal, heptanal, octanal, nonanal, decanal) (µg/kg) in bleached (500 mg/kg hydrogen peroxide) and unbleached whey protein concentrate (WPC) and serum protein concentrate (SPC).
Adapted from Jervis et al. (2012) and Campbell et al. (2013).
Sensory and Functionality Differences of Whey Protein Isolate Bleached by Hydrogen or Benzoyl Peroxide

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Abstract:
Whey protein is a highly functional food ingredient used in a wide variety of applications. A large portion of fluid whey produced in the United States is derived from Cheddar cheese manufacture and contains annatto (norbixin), and therefore must be bleached. The objective of this study was to compare sensory and functionality differences between whey protein isolate (WPI) bleached by benzoyl peroxide (BP) or hydrogen peroxide (HP). HP and BP bleached WPI and unbleached controls were manufactured in triplicate. Descriptive sensory analysis and gas chromatography-mass spectrometry were conducted to determine flavor differences between treatments. Functionality differences were evaluated by measurement of foam stability, protein solubility, SDS-PAGE, and effect of NaCl concentration on gelation relative to an unbleached control. HP bleached WPI had higher concentrations of lipid oxidation and sulfur containing volatile compounds than both BP and unbleached WPI (p<0.05). HP bleached WPI was characterized by high aroma intensity, cardboard, cabbage, and fatty flavors, while BP bleached WPI was differentiated by low bitter taste. Overrun and yield stress were not different among WPI (p<0.05). Soluble protein loss at pH 4.6 of WPI decreased by bleaching with either hydrogen peroxide or benzoyl peroxide (p<0.05), and the heat stability of WPI was also distinct among WPI (p<0.05). SDS PAGE results suggested that bleaching of whey with either BP or HP resulted in protein degradation, which likely contributed to functionality differences. These results demonstrate that bleaching has flavor effects as well as effects on many of the functionality characteristics of whey proteins.

Keywords Whey protein isolate, bleaching, functionality, sensory
Practical Applications

WPI is often used for its functional properties, but the effect of oxidative bleaching chemicals on the functional properties of WPI is not known. This study identifies the effects of hydrogen peroxide and benzoyl peroxide on functional and flavor characteristics of WPI bleached by hydrogen and benzoyl peroxide and provides insights for the product applications which may benefit from bleaching.

Introduction

Whey protein concentrate (WPC, 34-89% protein) and isolate (WPI, >90% protein) production in the United States has steadily increased over the last decade. United States manufacture of cheese reached almost 4.9 million metric tons in 2012, making it the largest producer of cheese, and subsequently whey, in the world (USDA 2012). WPC production has increased from 142,000 metric tons in 2002 to 200,000 metric tons in 2012, while WPI production has tripled in the last 10 years, from 10,000 metric tons in 2003 to 30,000 metric tons in 2012 (USDA 2013a). A large portion of dried whey protein produced in the US is the byproduct of Cheddar cheese manufacture colored by annatto (Scotter 2009). Annatto is a coloring agent extracted from the seed of the Bixa orellana shrub. The primary carotenoid responsible for the yellow color in annatto added to Cheddar cheese is norbixin (Kang and others 2010). Approximately 10% of the norbixin added to cheese milk partitions into the liquid whey and results in a yellow color in whey protein ingredients if it is not removed (Smith and others 2014; Kang and others 2010). Currently, benzoyl peroxide (BP) and hydrogen peroxide (HP) are the only two chemical bleaching agents approved by the FDA.
for bleaching of whey (US FDA 2013a,b), although enzymatic alternatives to chemical bleaching also exist (Campbell and others 2012, 2014b).

Cheese and fluid whey processing variables have significant effects on the flavor of whey protein ingredients. A typical WPI process includes fluid storage, clarification/fat separation, pasteurization, bleaching, microfiltration or ion exchange, ultrafiltration, diafiltration, and spray drying (Varnam and Sutherland 1994; Drake and others 2009). Bleaching, in particular, has been extensively studied for its detrimental effects on the flavor of whey protein products (Kang and others 2012; Campbell and others 2012; Croissant and others 2009; Listiyani and others 2011; Jervis and others 2012). Studies have consistently demonstrated that bleaching with BP results in greater norbixin destruction and less lipid oxidation (and off flavors) compared to HP (Croissant and others 2009; Listiyani and others 2012; Fox and others 2013). Residual benzoic acid (BA) residues remain a concern due to restrictions in international markets (Listiyani and others 2011), but a previous study with WPC34 suggested that some BA was removed with ultrafiltration (UF) and diafiltration (DF) (Listiyani and others 2011). Jervis and others (2015) reduced residual benzoic acid levels in sweet whey powders by almost 4x by bleaching liquid whey after concentrating rather than before concentrating. Further investigation into reduction of benzoic acid could make bleaching with BP an increasingly attractive option.

Chemical and enzymatic bleaching agents bleach by production of free radicals that oxidize the norbixin isoprenoid structure, rendering it colorless. This non-specific oxidation process also results in lipid oxidation (Jervis and others 2012; Campbell and others 2012; Campbell and Drake 2013). Lipid oxidation is the primary source of off-flavors in WPC 80 and WPI,
despite the relatively low fat content of WPI (Wright and others 2009). General flavor attributes of WPC80 and WPI have been studied (Wright and others 2009; Wright and others 2006; Carunchia Whetstine and others 2005), but the effect of bleaching on WPI specifically has not yet been studied.

WPI is typically used for dairy, bakery, meat, snack, and confectionery products due to its high-quality protein for supplementation, and good emulsifying, fat binding, water binding/thickening, gelling, and whipping properties (USDEC 2013b). Functionality of WPI lends itself to a variety of food applications and it would be advantageous to understand differences caused by processing parameters. Jervis and others (2012) and Campbell and others (2013) reported that bleaching fluid whey with HP may improve the heat stability of rehydrated WPC80 and serum protein concentrate (SPC, 80% protein) respectively, but, again, the effect of bleaching agent on WPI functionality has not been studied. The objectives of this study were to determine the functionality and sensory effects of bleaching WPI with HP or BP.

**Methods and Materials:**

**Whey production and bleaching**

WPI manufacture was conducted across three days. Each bleaching treatment (control [no bleach], benzoyl peroxide at 50 mg/kg [BP50], and hydrogen peroxide at 250 mg/kg [HP250]) was manufactured on separate days, but from the same lot of pasteurized milk. Bleaching treatment order was randomized across days. Raw whole milk, 720 kg, was obtained from the North Carolina State University Dairy Research and Education Farm.
Milk was HTST pasteurized (720 kg/h) with a plate heat exchanger (model T4 RGS-16/2, SPX Flow Technology, Greensboro, NC) at 72°C with a hold time of 16 sec. The milk was then cooled to 31°C and transferred to a cheese vat (Kusel Equipment, Watertown, WI) or chilled to 10°C and refrigerated at 4°C for subsequent cheesemakes. A standard colored Cheddar cheese-make procedure was then followed as described by Park and others (2013) and colored liquid whey obtained. The liquid whey was HTST pasteurized under the same parameters as the milk, and fat-separated. The liquid whey was then bleached with hydrogen peroxide (250 mg/kg, HP250, 35% w/v, VWR international, Westchester, PA), benzoyl peroxide (50 mg/kg, BP50, Oxylite Type XX Benzoyl Peroxide 32% wt/wt, Nelson Jameson, Marshfield, WI), or left unbleached (Con) for 1 h at 50°C. Following 1h, catalase (20 mg/kg, FoodPro CAT, Danisco, New Century, NJ) was added to the HP250 treatment to deactivate the bleaching agent.

Treated whey was microfiltered and ultrafiltered using a pilot scale membrane system (Model Lab 46, Filtration Engineering, Champlin, MN). Fat was removed from the whey by two 800,000 Da spiral wound microfiltration (MF) membranes (Synder, Vacavile, CA; nominal cutoff: 800,000 Da, surface area 7.15 m²) running at 0.052 Mpa transmembrane pressure. Temperature for MF was 50°C. Once the whey was 5x concentrated, diafiltration (DF) commenced. 30% w/w deionized (DI) water was added during DF. MF run time was approximately 1 h. MF permeate was collected and used for ultrafiltration (UF). MF permeate was concentrated via UF and DF (40% w/w) using a UF membrane (Synder filtration, Vacavile, Ca; nominal cutoff: 10,000 Da, surface area 5.0 m²) running at 0.086
Mpa transmembrane pressure to a target composition of 18% solids (w/v) and 90% protein (w/w). UF run time was approximately 2 h.

Once target solids (18%) and protein (90%) levels were achieved, the WPI retentate was spray dried (model Lab 1, Anhydro Inc., Soeberg, Denmark) at an inlet temperature of 200°C and outlet temperature of 95°C. All samples were collected in mylar bags (TF-4000, Impak Corp., Central City, SD) and stored at -80°C until analysis. Spray drying of each WPI took approximately 4 h. WPI were manufactured in triplicate.

**Composition analysis**

The WPI powders were analyzed for total solids (TS), fat, crude protein, and minerals. TS were determined by air oven drying (AOAC 2000; method number 990.20; 33.2.44). Fat was quantified by ether extraction (AOAC 2000; method number 989.05; 33.2.26). Protein was determined using the Kjeldahl method (AOAC 2000; method number 991.20; 33.2.11). Analysis of phosphorus, calcium, magnesium, potassium, sulfur, sodium and iron was performed by the North Carolina State University analytical services laboratory (Raleigh, NC) using a standard dry ash method with inductively coupled plasma optical emission spectroscopy (Lloyd and others 2009). All samples were measured in duplicate.

**Benzoic Acid Extraction and Measurement**

Benzoic acid in the WPI powders was determined by HPLC-PDA as described by Listiyani and others (2011) with modifications. 2.5 g of WPC80 was measured into a 50 ml volumetric flask with 5 ml of 1 M zinc acetate (Mallinckrodt Baker Inc., Phillipsburg, NJ) solution, and 5 ml of 0.25 M potassium hexacyanoferrate(II) trihydrate (Alfa Aesar, Ward...
hill, MA) and filled to volume with HPLC water (EMD Chemicals). WPI samples were shaken for 1 min and placed into centrifuge tubes (VWR International) and centrifuged for 10 min at 10,000 x g. The supernatant was filtered with a 0.45 um nylon filter (VWR International) after which 750 µl of filtrate and 750 µl of mobile phase were added to HPLC vials (Phenomenex). Quantitation was done by HPLC (Breeze HPLC, Waters, Milford, MA) using standard curves ranging from 0.1 to 50 mg/kg benzoic acid. HPLC analysis was performed using reversed phase separation (Kinetex C18, 2.6 um, 100 x 4.6 mm; Phenomenex, Torrance, CA). The PDA detection was set at 230 µm. The mobile phase used was 4% methanol (Sigma Aldrich, Milwaukee, Wis., USA) and 96% 0.02M ammonium acetate (Sigma Aldrich, Milwaukee, Wis., USA) buffer with a flow rate of 1 ml/min pumped through a binary pump (Waters 1525, Waters, Milford, MA). 20µL of sample was injected (Waters 2707 autosampler) onto the column.

**Color Analysis**

Hunter L (lightness), a (red-green), and b (yellow-blue) values for WPI powders and reconstituted liquids (10% solids, wt/vol) were determined using a MacBeth Color-Eye spectrophotometer (model 2020, Kollmorgen Instruments Corp., Newburgh, NY) using methods described by Jervis and others (2012). Values were determined in triplicate.

**Norbixin extraction**

Norbixin extraction from WPI powder was performed following the procedure described by Campbell and others (2014a). Briefly, 1g of powder was placed into a 10mL volumetric flask and HPLC grade water (EMD Chemicals) was added to volume. The sample was the
mixed for 10 min after which 2 g of whey solution was placed into another 10 mL volumetric flask and filled to volume with dilution solution (80% acetonitrile, 20% HPLC water, and 0.1% w/v formic acid, EMD Chemicals). The solution was again mixed for 10 min, after which 2 mL of sample was placed into an Eppendorf tube (VWR International) and centrifuged at 14,000 x g for 5 min. The clear supernatant was removed and placed into a vial (Phenomenex) for injection. Norbixin was quantified by HPLC (Breeze HPLC, Waters, Milford, MA) using an isocratic mobile phase (70% acetonitrile/30% water with 0.1% formic acid, EMD Chemicals) at a flow rate of 1 mL/min pumped through a binary pump (Waters 1525, Waters, Milford, MA). Fifty µL of sample was injected (Waters 2707 autosampler) onto the column (Phenomenex Kinetex 2.6µm particle size, 10 cm length, 4.6 mm inner diameter, 100 Å pore size) at 40ºC. Sample was analyzed using a photodiode array detector (Waters 2998) with a maxima of 482 nm. Sample run time was 3 min. All WPI were extracted and evaluated in triplicate. Norbixin destruction was determined by calculating the percent reduction of norbixin concentration in the bleached WPI compared to the norbixin concentration of the unbleached control WPI.

**Gas chromatography/mass spectrometry**

Selected volatile compounds were extracted and analyzed by headspace solid-phase microextraction following the method used by Campbell and others (2011) with modification. Each WPI was rehydrated to 10% solids (w/v) using HPLC water (EMD Chemicals Inc., Gibbstown, NJ), and 5 mL were added to 20 mL SPME vials (MicroLiter Analytical Supplies, Inc., Suwanee, GA) along with 10% (w/v) salt. Sample was placed into 20mL autosampler vials with steel screw tops containing silicone septa faced in Teflon.
(Microliter analytical, Suwannee, FL). Ten µL of 81 mg/kg 2-methyl-3-heptanone in methanol (Sigma Aldrich., Milwaukee, WI) was added as an internal standard. Samples were injected by a CombiPal autosampler (CTC Analytics, Zwingen, Switzerland) into an Agilent 6890N GC with 5973 inert MSD (Agilent Technologies Inc., Santa Clara, CA). Samples were held at 10ºC before fiber exposure and brought up to 40ºC for 25 min before 30 min fiber exposure of a 1 cm DVB/CAR/PDMS (divinylbenzene/carboxen/polydimethylsiloxane) fiber at 31 mm with 4 sec pulsed agitation at 250 rpm. The fibers were injected for 5 min at a depth of 50 mm. The GC method used a start temperature of 40ºC for 3 min with a ramp rate of 10ºC/min until 250ºC was reached. The sample was then held for 5 min. The column used was a Zb-5ms(Zb-5ms, 30 m length x 0.25 mm inner diameter x 0.25 µm film thickness, Phenomenex) with a constant flow rate of 1 mL/min in SIM and SCAN mode. Purge time was 1 min. Compounds were identified using the National Institute of Standards and Technology (2002) (Gaithersburg, MD) mass spectral database and comparison of mass spectra of authentic standards. Retention indexes (RI) were based on an alkane series (Sigma Aldrich). Internal standard concentration was used to calculate the relative abundance of each compound.

Descriptive Sensory Analysis

Descriptive sensory analysis (DA) was conducted in compliance with North Carolina State University Institutional Review Board for Human Subjects guidelines. Samples were rehydrated at 10% solids (w/v) and dispensed into 59 mL cups with lids (Solo Cup Co., Champaign, Ill) labeled with 3 digit codes. Eight panelists (23-45y, 6 females, 2 males), each with more than 100 h of experience with descriptive analysis of dried dairy ingredients
and the Spectrum™ 0 to 15 point universal scale (Meilgaard and others 2007) evaluated the samples using an established sensory language (Wright and others 2009). Each sample was evaluated by each panelist in duplicate. Compusense five version 5.6 (Compusense, Guelph, Canada) was used for data collection.

**Foam Generation**

An Artisan KitchenAid Mixer (KitchenAid, St. Joseph, MI) with a 4.5-quart stationary bowl and a rotating wire beater was used to generate WPI foams. Solutions (10% wt/vol) of each WPI were rehydrated for 6 h at room temperature (20-24ºC) on a stir plate and refrigerated overnight at 4ºC. Before foaming, the temperature was brought back up to 25ºC and pH adjusted to 7 using 1 N NaOH (VWR International). 200 mL samples of the solution were whipped at speed 10 (beater rpm of 752) for 19 min and 36 s (Davis and Foegeding 2007). Foams were all prepared in triplicates.

**Yield Stress**

A Brookfield 25xLVTDV-CIP viscometer (Brookfield Engineering Laboratories Inc., Middleboro, MA) was used to test yield stress of foams using vane rheometry (Pernell and others 2000; Davis and Foegeding 2007). Tests were conducted at a speed of 0.3 rpm with a vane diameter of 10 mm and 40 mm length. Maximum torque response (M_o) was determined in each sample in triplicate. M_o was converted to yield stress using the following formula (Dzuy and Boger 1983, 1985; Steffe 1996):

\[ T_o = M_o / [(h/d) + (1/6)][(\pi d^3)/2] \]
\( \tau_o \) is yield stress and \( h \) and \( d \) are the height and diameter of the vane respectively. Torque measurements were taken in triplicate.

**Overrun**

Overrun of the foams was determined after yield stress of the foams was determined. Foam was removed from the mixing bowl with a rubber spatula and placed into a 100 mL weighing dish in triplicate. Overrun and air phase fraction were determined using the following formulas:

\[
\text{Overrun} = (\text{wt of 100 mL solution}) - (\text{wt of 100 mL of foam}) / \text{wt of 100 mL of foam}
\]

\[
\text{Air phase fraction (\( \Phi \))} = \% \text{ overrun}/(\% \text{ overrun} + 100)
\]

Treatments were measured in triplicate (Dickinson 1999; Wilde 2000; Davis and Foegeding 2007).

**Foam Stability**

Foam stability was determined by measuring the time necessary for half of the prefoam mass to drain through a hole in the whipping bowl (Phillips and others 1990; Luck and others 2001). Starting time for the measurements began immediately after foam formation (Davis and Foegeding 2007).

**Protein Solubility**

Protein solubility assays were performed as described by Jervis and others (2012). Solutions (10% wt/vol) of each WPI were made by rehydrating the WPI to 80% of the desired volume for 6 h at room temperature (20-24°C) on a stir plate set to 200 rpm (Campbell and others 2011). Solutions were then held overnight at 4°C. After bringing the temperature to 25°C
the next day, the solutions were adjusted to a pH of 3, 4, 5, 6, or 7 using 1 N HCl or 1 N NaOH, after which each solution was brought to a total volume of 100 mL with deionized water. The solutions were then 10% (wt/vol) protein. Turbidity and solubility using the micro bicinchoninic acid (micro-BCA) assay were performed on the solutions, after which they were centrifuged at 16,500 × g for 10 min (model RC5B centrifuge; Thermo Scientific) and the turbidity and solubility were performed again on the supernatants. Micro-BCA was performed using a kit from Thermo Fisher Scientific/Pierce (Rockfor, IL). Protein solutions were diluted 1:100 in deionized water and added at a ratio of 1:8 to a working reagent and pipetted into a 96-well plastic plate. Micro-BCA was performed in triplicate. The plate was placed on a shaker for 30 s and incubated at 37ºC for 30 min. The plate was allowed to return to room temperature and read on a Tecan Safire plate reader spectrophotometer at wavelength 562nm (Tecan, Durham, NC). Solubility was calculated using the following equation (Abs = absorbance):

\[
\text{Protein solubility} = 100 - \frac{(\text{Abs}_{\text{before}} - \text{Abs}_{\text{after}})}{\text{Abs}_{\text{before}}} \times 100
\]

Analyses were performed in quadruplicate. Turbidity was measured using a Hach 2100AN Turbidimeter (Loveland, CO). A tungsten filament lamp was used as a light source focused by a lens and measured transmitted light, back scattering, forward scattering, and 90° angle scattering light. Centrifuged and uncentrifuged samples were pipetted into glass cuvettes and measured in quadruplicate. Turbidity was calculated using the following equation:

\[
\text{Turbidity} = \frac{(\text{Turbidity}_{\text{before}} - \text{Turbidity}_{\text{after}})}{\text{Turbidity}_{\text{before}}} \times 100
\]
Heat Stability

Heat stability was measured using the methods described by Jervis and others (2012). Solutions were prepared at 10% (wt/vol) protein and then brought to pH 7 using 1 N NaOH. The solutions were then heated in a water bath at 90°C for 0, 10, and 20 min and then placed in an ice bath until reaching an internal temperature of 25°C. Turbidity and micro-BCA measurements were then taken before and after centrifugation as described above. Solubility and turbidity calculations were then made as described above. The supernatant was then collected and brought to a pH of 4.6 using 1 N HCl. Turbidity and solubility were then measured as previously described. All measurements were measured in triplicate.

SDS PAGE

A NuPAGE Bis-Tris electrophoresis system (Invitrogen, Carlsbad, CA) was utilized to perform SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) on all samples. The protein concentrations were standardized to the same protein concentration prior to loading on to the gel (1 mg/ml). Fifty µl of sample was dissolved in NuPAGE LDS sample buffer (25 µl) with NuPAGE reducing agent (10 µl). Final protein concentrations were 2.0 mg/ml. Samples were then heated at 70°C for 10 min and a 20 µL aliquot of each sample was loaded per well. Proteins were separated on precast NuPAGE 4-12% Bis-Tris gels (Invitrogen) using NuPAGE MES SDS running buffer (Invitrogen) at a constant 200 volts for 50 min. The gel was then removed from the cast and soaked in 50% methanol (EMD Chemicals Inc., Gibbstown, NJ) and 10% acetic acid (99.5%; J.T. Baker Chemicals, Phillipsburg, NJ) solution for 10 min after which it was transferred and stained with colloidal blue staining solution (Invitrogen). After 3 h the gel was destained by adding 100 mL DI
water to gel and letting it sit overnight. A Novex sharp protein standard (Invitrogen) was used to identify components in the samples. A gel-dry drying solution (Invitrogen) was used to preserve the gel. Each experimental replication was evaluated by SDS-PAGE in duplicate.

**Statistical Analysis**

Data was analyzed by analysis of variance with means separation (ANOVA) (XLSTAT, version 2013.5.03, Addinsoft, New York, NY). Differences were analyzed by Tukey’s honestly significant difference (HSD).

**Results**

**Composition and Benzoic Acid Analysis**

There were no differences in moisture, protein, or fat of WPI (p>0.05). Moisture was 3.56 ± 0.12%, fat (dry basis) was 0.39% ± 0.05%, and protein (dry basis) was 90.9% ± 0.45%. No differences (p>0.05) were found in calcium, potassium, magnesium, and sodium levels among WPI. HP bleached WPI had lower levels of phosphorus and iron (p<0.05) than the unbleached control while BP bleached samples had lower sulfur (p<0.05) than the unbleached WPI (Table 1). Lower iron levels in HP bleached WPI were consistent with the lower iron levels reported in HP bleached WPC80, SPC80 and sweet whey powder reported by Campbell and others (2013), Jervis and others (2012), and Jervis and others (2015) respectively. Benzoic acid (BA) concentrations were higher in BP bleached WPI than unbleached or HP bleached WPI (p<0.05) at 42.2 mg/kg, 2.76 mg/kg, and 3.42 mg/kg respectively. BA concentrations in BP bleached WPI were lower than WPC34 (272 mg/kg) and SWP (approximately 130 mg/kg or 420 mg/kg) bleached by 50 mg/kg BP, determined by
Listiyani and others (2011) and Jervis and others (2015) respectively. BA concentrations in BP bleached WPI were within the range of BA concentrations found in WPC80 (30.6 mg/kg and 60.0 mg/kg) bleached by 50 mg/kg BP, determined by Listiyani and others (2011). These results confirm the finding by Listiyani and others (2011) that diafiltration decreases BA concentration in concentrated liquid retentate and powders.

**Color and norbixin destruction**

Both bleached powders were visibly whiter than the unbleached WPI, but the BP bleached WPI was also visibly whiter than the HP bleached WPI. Both HP and BP bleached powders had increased whiteness (L value) compared to the unbleached WPI (Table 2). HP WPI L values were higher than BP WPI (p<0.05). As expected, the yellowness (b value) was reduced by both HP and BP bleaching, but BP bleached WPI had much lower yellowness in both the powder and liquid compared to HP bleached WPI (p<0.05). This data is consistent with the findings of both Campbell and others (2013) and Jervis and others (2012) in SPC80 and WPC80. Norbixin destruction results showed a 12% decrease in norbixin due between the HP bleached WPI and the unbleached WPI, while the BP bleached WPI showed a 95% decrease in norbixin compared to the unbleached WPI.

**Volatile compounds**

Volatile compound profiles were distinct among WPI (Table 3). Campbell and others (2013) and Jervis and others (2012) reported that HP bleached WPC80 were higher in lipid oxidation compounds than unbleached and BP bleached treatments. Both bleached WPI were higher (p<0.05) in hexanal, heptanal, octanal, nonanal, decanal and 2 pentyl furan than
unbleached WPI, all lipid oxidation compounds (Table 3). HP WPI was higher (p<0.05) in hexanal, dimethyl disulfide, dimethyl trisulfide, z-4-heptenal, methional, 1-octen-3-one, and nonanal than the BP bleached WPI, all volatile compounds which have been previously reported in whey products (Wright and others 2006; Croissant and others 2009; Evans and others 2010; Whitson and others 2010; Listiyani and others 2011; Jervis and others 2012; Campbell and others 2013). BP bleached WPI was only higher than HP in 2-pentylfuran and 3-methylbutanal. Jervis and others (2012) also reported higher 2-pentylfuran in BP bleached WPC80 than HP bleached WPC80. Lipid oxidation products, such as the aldehydes found in increased concentrations in the bleached WPI are the source of cardboard flavor (Whitson and others 2010).

**Descriptive Analysis**

Sensory differences between liquid whey, sweet whey powder, WPC 34, and WPC 80 bleached by different bleaching agents have been reported in several studies (Croissant and others 2009; Campbell and others 2011; Listyani and others 2011; Jervis and others 2012; Jervis and others 2015). Both hydrogen peroxide (250 mg/kg) and benzoyl treatments had significant effects (p<0.05) on flavor profiles of WPI (Table 4). Hydrogen peroxide bleaching increased aroma intensity and cardboard flavor and decreased sweet aromatic flavor of rehydrated WPI (p<0.05). Benzoyl peroxide treatment decreased sweet aromatic flavor and caused a bitter taste. The control WPC80 was lower in cardboard and aroma intensity compared to HP bleached WPI and higher in sweet aromatic compared to both BP and HP bleached WPI (p<0.05). These results are consistent with HP and BP effects on other
dried whey ingredients (Listiyani and others 2011; Jervis and others 2012; Campbell and others 2013; Jervis and others 2015).

**Functionality**

All samples of WPI foamed, but no differences were found between the percent overrun or air phase fraction of the WPI (p>0.05, Table 5). Foams made using either bleached WPI were more stable than the control WPI (p<0.05, Table 5). Yield stress of the control foams was different from the bleached WPI (p<0.05, Table 5). Both HP and BP bleached WPI had a lower soluble protein loss than the unbleached control, but again were not different from each other (p<0.05, Figure 1). Solubility of WPI at different pH values without heat treatment was not different among treatments within a specific pH value (p>0.05; results not shown). Solubility of BP and HP bleached WPI at pH 4.6 and 7 was not different from the solubility of unbleached WPI (Figure 1, p>0.05). Solubility at pH 4.6 and 7 without heating represents inherent degrees of denaturation/aggregation in the powders. Aggregates formed during processing could remain soluble at pH 7 but would be more prone to undergo secondary aggregation and precipitate at pH 4.6. The slightly lower solubility at pH 4.6 for all treatments indicates a mild degree of aggregation produced during processing. Heating causes denaturation/aggregation and aggregates that are large enough to be removed by centrifugation at 16,500 x g for 10 min account for the loss of solubility. The HP bleached WPI were more stable to aggregation after heating and, even at pH 4.6, 80% of the protein remained dispersed (Figure 1) (p<0.05). Turbidity is determined by several factors including aggregate size and amount of aggregates. Note that 40 to 60% of the turbidity was lost in unheated solutions at pH 7 while this accounted for a minor loss of solubility (< 10%,
see Figures 1 and 2). The HP treatment after heating had 100% turbidity loss at pH 4.6 but this only accounted for a 20% loss in protein (Figures 1 and 2). **SDS PAGE**

SDS PAGE showed only small differences between the unbleached WPI and BP bleached WPI. HP bleached WPI had lighter bands of bovine serum albumin (67kDa) and smearing of the bands around 20kDa suggesting a disruption and breakdown of BSA into smaller peptides (results not shown). Protein breakdown is consistent with the descriptive analysis and volatile compound results for HP bleached WPI. HP bleached WPI had higher concentrations of DMDS and DMTS, both protein degradation products, than unbleached and BP bleached WPI.

**Discussion**

The composition of powders and benzoic acid concentrations were largely consistent with previous studies (Sieber and others, 1995; Qi and others, 2009; Listiyani and others, 2011; Jervis and others, 2015). In contrast, norbixin results were not. Hydrogen peroxide bleaching of liquid whey reduced the norbixin concentration of WPI by 12% while BP bleaching of liquid whey reduced the norbixin concentration of WPI by 95%. Previous studies with hydrogen peroxide bleaching of fluid whey, liquid whey protein retentate, and WPC80 have shown hydrogen peroxide (250-500 mg/kg) caused a norbixin reduction of 20-44% (Fox and others 2013; Kang and others 2012; Jervis and others 2012). A result of 12% norbixin destruction appears inconsistent with past studies, but previous studies have not addressed WPI. A large portion of the norbixin retained in the whey during the cheese make process is removed by microfiltration and discarded with the MF retentate (Qiu and others 2015). While the unbleached control was not chemically bleached, a study performed by Qiu
and others (2015) demonstrated that microfiltration reduced norbixin levels by approximately 40% in WPI. The liquid whey was bleached before MF, which reduced norbixin concentration in both the serum and fat phase of the liquid whey. This effectively reduced the perceived norbixin destruction caused by HP bleaching. This would have the same or similar effect on the effectiveness of BP bleaching, but due to the much greater degree of norbixin destruction when bleaching with BP compared to HP, the effect is not as pronounced and the BP bleaching levels are consistent with past findings (Jervis and others 2012).

The useful functional characteristics of WPI would ultimately be wasted if the flavor profile was such that it adds an undesirable flavor to the end product. Off-flavor formation is often unavoidable. A typical whey protein concentrate or isolate goes through many process steps which have the potential to introduce flavor variability or off-flavors. Whey source (Wright and others 2009; Drake and others 2009a) starter culture (Campbell and others 2011), fluid storage (Liaw and others 2011; Whitson and others 2011), spray drying (Park and others 2013), dry powder storage and instantization (Wright and others 2009), and bleaching (Croissant and others 2009; Listiyani and others 2011; Campbell and others 2012; Kang and others 2012; Jervis and others 2012; Fox and others 2013) all cause variability and off-flavor formation in fluid whey and dried ingredients. Flavor of dried ingredients, such as WPI, does impact the flavor of the final product in which they are included and even seemingly small differences in flavor can decrease the acceptability of the final product (Evans and others 2010; Caudle and others, 2005; Wright and others 2009). Sensory characteristics and flavor
volatile compound concentrations from HP and BP bleached WPI were distinct from unbleached WPI confirming the effects of bleaching across the spectrum of dried whey ingredients.

Previous studies have also documented the effects of bleaching agent on flavor and volatile compound profile. These studies include sweet whey powder (Jervis and others 2015), WPC34 (Listiyani and others 2011), WPC66 (Croissant and others 2009), WPC80 (Campbell and others 2012; Kang and others 2012; Jervis and others 2012; Fox and others 2013), and SPC80 (Campbell and others 2013). Combined with the present study, these studies collectively demonstrate increased levels of lipid oxidation and protein degradation products in HP bleached whey products compared to BP bleached whey products.

Consistent with results in WPC80 (Jervis and others 2012) and SPC80 (Campbell and others 2013), WPI bleached with HP were higher than unbleached and BP bleached WPI in fatty and cardboard flavors. HP bleached WPI was higher than unbleached or BP bleached WPI in nonanal, 1 octen-3-one, and DMTS, explaining the higher intensity of cardboard flavor by sensory analysis. DMTS has also been connected to cabbage off-flavor in WPI (Wright and others 2006). As expected, HP bleached WPI was unique among the WPI in that it had a distinct cabbage flavor, and also had higher DMTS concentrations than unbleached or BP bleached WPI (p<0.05).

Oxidation of various protein products and its effect on functional properties has been well studied, including oxidation of various proteins by reactive oxygen species generated via lipid oxidation, metal, and enzyme-catalyzed oxidative reactions, (Wolf and others 1986;
Butterfield and Stadtman 1997) and iron catalyzed Fenton reactions and enzymatic oxidation through the lactoperoxidase system, especially in iron-rich proteins such as lactoferrin (Jervis and Drake 2013). Oxidation of muscle proteins has been shown to affect functional properties, including gelation, emulsification, viscosity, solubility, and hydration, of muscle foods made with the oxidized proteins (Xiong and others 2000). Whey protein isolate is typically used for dairy, bakery, meat, snack, and confectionery products due to its high-quality protein for supplementation, and ability to form and stabilize foams, emulsions and gels (USDEC 2013b). Depending on the final product, specific functional properties may be desirable, and the influence of bleaching on functional properties of WPI has not been addressed.

As expected, the WPI readily formed foams. The WPC80 manufactured by Jervis and others (2012) did not foam while the SPC manufactured by Campbell and others (2013) did, which supports the supposition by Campbell and others (2013) that fat content was an important factor in foaming properties of whey protein concentrates and isolates, a fact well established in past literature (Peter and Bell, 1930; El-Rafey and Richardson, 1943). Hydrophobic particles are antifoaming agents (Aveyard and others, 1992; Wang and others, 1999) and the plausible reason for removal of foaming properties. Due to the low fat level, one would expect SPC80 and WPI to have more similar functional characteristics than WPC80 and WPI. Foam stability and yield stress of the WPI foams were increased by bleaching. This is likely due to oxidative effects on whey proteins which has been reported to improve foam stability, depending on the level of oxidation (Hansen and Black 1972; Schmidt 1984; Campbell and others 2013).
Solubility of WPI was not affected by the HP and BP bleaching (Figure 1; p>0.05), however HP bleached WPI was more heat stable than BP bleached and unbleached WPI. This result was consistent with the WPC80 results of Jervis and others (2012). It should be noted that heat stability in this situation is the colloidal stability of the protein sol after heating. Aggregation produces large particles that are removed by centrifugation and counted as a loss of solubility. At pH 4.6 and after heating for 10 min at 90°C, both the BP bleached and unbleached WPI gelled, while the HP bleached WPI did not, and retained 81.2% solubility. This is surprisingly high and could be due to an artifact created that produced falsely high micro bicinchoninic acid results or caused a reduction in the degree of protein aggregation. At pH 7 and after heating for 10 min at 90°C, both the BP bleached and unbleached WPI gelled, while the HP bleached WPI did not, and retained 94.3% solubility. These results are also consistent with those of the heat stability of SPC80 and WPC80 reported by Campbell and others (2013) and Jervis and others (2012). It is likely that the HP damaged the protein to a greater degree than the BP, or that it broke apart aggregates that had already formed due to heat or other parameters during processing. Forming large aggregates or a gel network are due to whey proteins denaturing and having elements of the denatured structure favor intermolecular interactions. It would appear that HP bleached WPI would not form gels but this also makes it more functional in applications in beverages where aggregation is undesirable. It is possible that a combination of BP and HP treatment could produce a WPI with acceptable color and functional properties.
Conclusions
Bleaching of fluid whey results in changes in the sensory and functionality characteristics of WPI. BP was a much more effective bleaching agent for WPI than HP. Sensory characteristics of the bleached and unbleached treatments were all distinct (p<0.05). BP bleached WPI had lower levels of most lipid oxidation volatiles than HP bleached WPI, despite much more effective bleaching. Both HP and BP increased foam stability and protein solubility of WPI. HP bleaching modified heat-induced aggregation more than unbleached or BP bleached WPI. These results, when compared to the results of bleaching SPC80 and WPC80 that protein oxidation has a large effect on the protein solubility and heat stability of WPI. Variability among WPI functional and sensory characteristics demands that specific WPI parameters are targeted for specific applications, depending on the desired functional properties. Oxidation through bleaching needs to be taken into account when producing value-added protein ingredients containing WPI.

Acknowledgements
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References


USDEC. 2013b. WPI: Typical Applications.

http://www.usdec.org/Products/content.cfm?ItemNumber=82508&navItemNumber=8258


Table 1. Mean (n=3) mineral composition (mg/kg, calculated on a dry basis) of spray-dried whey protein isolate (WPI) bleached with benzoyl peroxide (BP, 50 mg/kg), bleached with hydrogen peroxide (HP, 250 mg/kg), or unbleached (Con)\(^1\).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>P</th>
<th>Ca</th>
<th>K</th>
<th>Mg</th>
<th>S</th>
<th>Na</th>
<th>Fe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con</td>
<td>0.416(^a)</td>
<td>0.542(^a)</td>
<td>0.682(^a)</td>
<td>0.068(^a)</td>
<td>1.13(^ab)</td>
<td>1789(^a)</td>
<td>8.82(^a)</td>
</tr>
<tr>
<td>BP50</td>
<td>0.398(^{ab})</td>
<td>0.556(^a)</td>
<td>0.685(^a)</td>
<td>0.068(^a)</td>
<td>1.10(^b)</td>
<td>1756(^a)</td>
<td>8.91(^a)</td>
</tr>
<tr>
<td>HP250</td>
<td>0.378(^b)</td>
<td>0.541(^a)</td>
<td>0.699(^a)</td>
<td>0.067(^a)</td>
<td>1.15(^a)</td>
<td>1801(^a)</td>
<td>6.12(^b)</td>
</tr>
</tbody>
</table>

\(^{a-b}\) Means in the same column not sharing a common superscript are different (p<0.05)
Table 2. Mean (n=3) color (L, a, and b values) of liquid (10% wt/vol powders) and spray-dried whey protein isolate (WPI) bleached with hydrogen peroxide (HP 250; 250 mg/kg), benzoyl peroxide (BP 50; 50 mg/kg), or unbleached (Con).

<table>
<thead>
<tr>
<th></th>
<th>Powder</th>
<th>Liquid</th>
<th>Powder</th>
<th>Liquid</th>
<th>Powder</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con</td>
<td>88.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HP250</td>
<td>90.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.35&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>BP50</td>
<td>90.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.23&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.15&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.14&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a-c</sup>Means in a row not sharing a common superscript are different (p<0.05)

<sup>1ND</sup> = not detected.
Table 3. Relative abundance table of selected volatile compounds by treatment. Mean (n=3) concentrations of selected volatile compounds (µg/L) of spray-dried WPI bleached with hydrogen peroxide (HP 250; 250 mg/kg), benzoyl peroxide (BP 50; 50 mg/kg), or unbleached (Con).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Con</th>
<th>HP 250</th>
<th>BP 50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diacetyl</td>
<td>1.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.72&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.91&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>0.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.37&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pentanal</td>
<td>0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.21&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hexanal</td>
<td>0.53&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.74&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.19&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Heptanal</td>
<td>0.46&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.58&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Octanal</td>
<td>ND</td>
<td>0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.07&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Nonanal</td>
<td>0.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.42&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Decanal</td>
<td>0.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.49&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>DMDS</td>
<td>0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.09&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>DMTS</td>
<td>0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.02&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>2-Pentyl furan</td>
<td>15.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>119&lt;sup&gt;b&lt;/sup&gt;</td>
<td>272&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Z-4-Heptenal</td>
<td>0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Methional</td>
<td>0.98&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.94&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>3 methyl butanal</td>
<td>13.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2 methyl butanal</td>
<td>2.98&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.31&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>1-octen-3-one</td>
<td>0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.14&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Benzaldehyde</td>
<td>0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.18&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a-c</sup>Means in a row not sharing a common superscript are different (p<0.05)  
ND = not detected.
Table 4: Sensory profiles of rehydrated WPI. Mean (n = 3 replicates with 8 panelists) sensory attributes\(^1\) of spray-dried WPI bleached with hydrogen peroxide (HP 250; 250 mg/kg), benzoyl peroxide (BP 50; 50 mg/kg), or unbleached (Con).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Aroma</th>
<th>Sweet aromatic</th>
<th>Cardboard</th>
<th>Cabbage</th>
<th>Fatty</th>
<th>Bitter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con</td>
<td>2.0(^b)</td>
<td>2.0(^a)</td>
<td>1.5(^c)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>HP250</td>
<td>2.5(^a)</td>
<td>ND</td>
<td>2.5(^a)</td>
<td>1.0(^a)</td>
<td>1.1</td>
<td>ND</td>
</tr>
<tr>
<td>BP50</td>
<td>2.0(^b)</td>
<td>0.5(^b)</td>
<td>2.0(^b)</td>
<td>ND</td>
<td>ND</td>
<td>0.8</td>
</tr>
</tbody>
</table>

\(^{a-b}\)Means in the same column not sharing a common superscript are different (p<0.05).

\(^1\)Intensities were scored on a 0-15 universal scale, where 0 = none detected and 15 = very high intensity (Meilgaard and others 1999). Dried whey ingredient intensities usually fall between 0 and 4 on this scale (Drake and others 2003; Wright and others 2009).
Table 5. Mean (n=3) overrun, air phase fraction, foam stability, and yield stress of foams from whey protein isolate (WPI) bleached with benzoyl peroxide (BP, 50 mg/kg), bleached with hydrogen peroxide (HP, 250 mg/kg), or unbleached (Con)\(^1\)

<table>
<thead>
<tr>
<th>Item</th>
<th>Control</th>
<th>BP50</th>
<th>HP250</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overrun (%)</td>
<td>1302(^a)</td>
<td>1208(^a)</td>
<td>1333(^a)</td>
</tr>
<tr>
<td>Air Phase Fraction (%)</td>
<td>92.9(^a)</td>
<td>92.3(^a)</td>
<td>92.9(^a)</td>
</tr>
<tr>
<td>Foam Stability (min)</td>
<td>15.9(^b)</td>
<td>19.6(^a)</td>
<td>18.2(^a)</td>
</tr>
<tr>
<td>Yield Stress (Pa)</td>
<td>32.6(^b)</td>
<td>40.1(^a)</td>
<td>35.4(^b)</td>
</tr>
</tbody>
</table>

\(^{a-b}\) Means in the same row not sharing a common superscript are different (p<0.05)
Figure 1. Mean percentage solubility of whey protein isolate (WPI) bleached with hydrogen peroxide (HP 250; 250 mg/kg), benzoyl peroxide (BP 50; 50 mg/kg), or unbleached (Con) at pH 4.6 and 10% (wt/vol) heated for 0 and 10 min at 90°C.
Figure 2. Mean percentage turbidity loss of whey protein isolate (WPI) bleached with hydrogen peroxide (HP 250; 250 mg/kg), benzoyl peroxide (BP 50; 50 mg/kg), or unbleached (Con) at pH 4.6 and 10% (wt/vol) heated for 0, 10, and 20 min at 90ºC.
Impact of Temperature and Concentration on Benzoyl Peroxide Bleaching Efficacy and Benzoic Acid Levels in Whey Protein Concentrate

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Abstract

Much of the fluid whey produced in the US is a byproduct of Cheddar cheese manufacture and must be bleached. Benzoyl peroxide (BP) is currently one of only two legal chemical bleaching agents for fluid whey in the US, but benzoic acid (BA) is an unavoidable byproduct of BP bleaching. Benzoyl peroxide is typically a powder, but new liquid BP dispersions are available. A greater understanding of the bleaching characteristics of BP is necessary. The objective of the study was to compare norbixin destruction, residual BA, and flavor differences between liquid whey and 80% whey protein concentrates bleached at different temperatures with two different benzoyl peroxides (soluble and insoluble). Two experiments were conducted in this study. For experiment 1, three factors (temperature, bleach type, bleach concentration) were evaluated for norbixin destruction using a response surface model – central composite design (RSM–CCD) in liquid whey. For experiment 2, norbixin concentration, residual benzoic acid, and flavor differences were explored in WPC80 from whey bleached by the two commercially available BP (soluble and insoluble) at 5 mg/kg. In liquid whey, soluble BP bleached more norbixin than insoluble BP, especially at lower concentrations (5 and 10 mg/kg) at both cold (4°C) and hot (50°C) temperatures. The WPC80 from liquid whey bleached with BP at 50°C had lower norbixin concentration, benzoic acid levels, cardboard flavor, and aldehyde levels than WPC80 from liquid whey bleached with BP at 4°C. Regardless of temperature, soluble BP destroyed more norbixin at lower concentrations than insoluble BP (p<0.05). The WPC80 from soluble BP bleached wheys had lower cardboard flavor and lower aldehyde levels than WPC80 from insoluble BP bleached whey. This study suggests that new, soluble (liquid) BP can be used at lower
concentrations than insoluble BP to achieve equivalent bleaching and that less residual benzoic acid remains in WPC80 powder from liquid whey bleached hot (50°C) than cold (4°C), which may provide opportunities to reduce benzoic acid residues in dried whey ingredients, expanding their marketability.

Keywords: Benzoyl peroxide, benzoic acid, whey protein concentrate, bleaching

Introduction

Dried whey ingredients provide significant value to the dairy industry. They possess many useful functional properties, such as gelation, thermal stability, foam formation, and emulsification properties, and provide many health benefits, including exercise recovery, weight management, cardiovascular health, anti-cancer effects, anti-infection activity, wound repair and infant nutrition (Foegeding et al., 2002; Miller, 2005; O’Connell and Flynn, 2007; Smithers, 2008). Much of the whey produced in the US is a byproduct of Cheddar cheese manufacture. Cheddar cheese in the US is typically colored with annatto (norbixin), a carotenoid extracted from the seed of the *Bixa Orellana* shrub (Scotter, 2009). Approximately 10% of annatto partitions into the liquid whey during Cheddar cheese manufacture (Smith et al., 2014).

To provide a colorless whey ingredient, colored liquid whey must be bleached; however, the use of chemical bleaching agents has detrimental effects, including off-flavor development, chemical residues, and protein functional changes (Listiyani et al., 2011; Jervis et al., 2012). There are currently two chemical bleaching agents approved for use in liquid whey in the US, hydrogen peroxide (HP) and benzoyl peroxide (BP) (21CFR184.1366 and 21CFR184.1157, respectively). Bleaching of liquid whey has been extensively studied and it
has been consistently demonstrated that BP results in greater norbixin destruction and less lipid oxidation and subsequent off-flavors than bleaching with HP (Listiyani et al., 2012; Jervis et al., 2012; Fox et al., 2013; Jervis et al., 2015). Benzoyl peroxide reacts with oxidizable compounds in the liquid whey and is converted into water-soluble benzoic acid. Benzoic acid residues are a concern due to restrictions in international markets, especially China. Due to the breakdown of BP into BA, BP is not an approved bleaching agent for whey ingredients in China or Japan (USDA 2013). In Canada, BP is allowed to be used as a bleaching agent in fluid whey not to be used in infant formula, at a maximum concentration of 100 mg/kg BP (CFIA, 2008).

The safety of BA has been extensively studied (Sharratt et al., 1964; Nair, 2001; Qi et al., 2009). Benzoic acid and its salt are used in the food industry as preservatives with antibacterial, yeast, and fungal properties (Chipley et al., 1993). Sieber et al. (1995) found native benzoic acid in dairy products, especially fermented dairy products, at concentrations as high as 50 mg/kg (Sieber et al., 1995) while Qi et al. (2009) reported benzoic acid concentrations in Chinese infant formula as high as 85 mg/kg and in milk powder as high as 110 mg/kg. Listiyani et al. (2011) reported concentrations in BP bleached WPC34 of 272 mg/kg and 634 mg/kg, depending on BP concentration used to bleach liquid whey at 60°C (50 mg/kg and 100 mg/kg respectively). In commercial WPC80 from BP bleached whey, 30-60 mg/kg BA were reported (Listiyani et al., 2011) but BP concentrations and conditions were not identified. Jervis et al. (2015) evaluated the impact of solids concentration at bleaching on residual BA. Benzoic acid concentrations in sweet whey powder from BP bleached (50 mg/kg BP) whey of 130 mg/kg or 420 mg/kg were reported, depending on total
solids concentration when bleached (6.7% solids and 14% solids respectively). These studies suggest that parameters in addition to BP concentration impact residual BA in dried whey ingredients.

The Joint Expert Committee for Food Additives (JECFA) has set the acceptable daily intake (ADI) for benzoic acid and its salts (benzoate calcium, potassium, and sodium), benzaldehyde, benzyl acetate, and benzyl alcohol at 0-5 mg/kg of body weight (WHO, 1996). The JEFCA has also stated that no safety concern exists for BP in bleaching of whey when used at up to 100 mg/kg (JEFCA, 2004). Benzoic acid is generally recognized as safe (GRAS), but exposure to benzoic acid has been linked to eye, skin, and respiratory tract irritation (Qi et al., 2009), skin sensitization (Filho et al., 2004), and non-immunological contact reactions such as asthma, urticarial, metabolic acidosis, and convulsions (Tfouni and Toledo, 2002; WHO, 2000). Due to health concerns and international restrictions of benzoyl peroxide use due to benzoic acid residues, it is important to investigate possible methods of reducing benzoic acid residues in dried whey protein ingredients. Benzoyl peroxide suspended in liquid medium, rather than powder, has recently come to the market and is commercially available. The objectives of this study were to compare the bleaching characteristics of commercially available soluble (liquid) and insoluble (powder) benzoyl peroxides at different concentrations (5, 10, 25, 50 mg/kg BP) and temperatures (4 and 50°C), and to determine their effect on benzoic acid residues in WPC80 bleached at 4 and 50°C.
Methods

Experimental Overview

Two experiments (experiments 1 and 2) were included in this study. The purpose of experiment 1 was to determine the effects of benzoyl peroxide concentration and temperature on norbixin destruction in liquid whey using soluble or insoluble benzoyl peroxide, and to generate a response surface and prediction equation for each temperature/benzoyl peroxide treatment. Liquid Cheddar whey was manufactured and bleached at 2 temperatures (4°C and 50°C), using 2 commercial forms of BP at 4 concentrations (5, 10, 25, and 50 mg/kg). Samples were taken at 9 (50°C treatment - 1,5,10,15,20,25, 30, 45, and 60) or 5 (4°C treatment - 1, 2, 4, 6, and 16 h) time points for norbixin analysis. Experiment 2 determined the effect of temperature and benzoyl peroxide type (soluble or insoluble) on residual benzoic acid and flavor properties of WPC80. Conditions for experiment 2 were based on the results of experiment 1. Bleaching liquid whey with 5 mg/kg BP was selected for experiment 2. The prediction equations generated in experiment 1 also predicted a norbixin destruction level of 90% using 5 mg/kg soluble BP at 50°C. A norbixin destruction level of 90% approximates the bleaching efficacy of BP treatments reported previously in literature (Jervis et al., 2012; Jervis et al., 2015; Listiyani et al., 2011). The prediction equations generated in experiment 1 also predicted that approximately 5 mg/kg soluble BP at 4°C would destroy 40% of norbixin in 1 h, which is equivalent to or greater than HP bleaching in previous literature (Campbell et al., 2013; Jervis et al., 2012; Jervis et al., 2015). The WPC80 powders were manufactured from liquid whey at 2 temperatures (4°C and 50°C), with 5 mg/kg of each of the 2 commercial forms of benzoyl peroxides.
Composition analysis

The liquid whey and WPC80 powders were analyzed for total solids (TS), fat, and crude protein. True solids were determined by vacuum oven drying (AOAC, 2000; method number 990.20; 33.2.44), fat by ether extraction (AOAC, 2000; method number 989.05; 33.2.26), and protein by the Kjeldahl method (AOAC, 2000; method number 991.20; 33.2.11). All samples were measured in duplicate.

Whey production and bleaching

Bleaching of liquid whey

Raw whole milk, 195 kg, was obtained from the North Carolina State University Dairy Research and Education Farm. Milk was pasteurized (720 kg/h) with a plate heat exchanger (model T4 RGS-16/2, SPX Flow Technology, Greensboro, NC) at 72ºC with a hold time of 16 seconds. The milk was then cooled to 31ºC and transferred to a cheese vat (Kusel Equipment, Watertown, WI). A standard colored Cheddar cheese-make procedure was then followed as described by Park et al. (2014) and colored liquid whey obtained. The liquid whey was HTST pasteurized under the same parameters as the milk, and fat-separated. Pasteurized liquid whey was then placed into autoclaved 1L amber glass jars (VWR International) and held in a water bath at either 4ºC or 50ºC until desired temperature was reached. Once temperature was reached, benzoyl peroxide (soluble, (liquid) Arkema, Prince of Prussia, PA or insoluble, Oxylite Type XX, (powder) Nelson Jameson, Marshfield, WI) at the appropriate concentration (5, 10, 25, or 50 mg/kg), was added to each whey. Whey samples (0.5mL) were taken (1, 5, 10, 15, 20, 25, 30, 45, and 60 min for 50ºC; 1h, 2h, 4h, 6h,
and 16h for 4°C) and immediately added to 2 mL dilution solution (80% acetonitrile, 20% HPLC water, and 0.1% w/v formic acid, EMD Chemicals) to suspend the bleaching reaction. Samples were then stored at -20°C until norbixin extraction. Unbleached controls were also evaluated and sampled. This experiment was replicated 3 times.

**WPC80 bleaching and production**

Liquid whey was produced as described above. After fat separation and pasteurization, liquid whey was separated into 6 batches (30 kg) using sanitized 38 L milk cans, covered, and placed into water baths at desired temperatures (4°C, 50°C). Once desired temperature was reached, bleaching (control no bleach 4°C, 50°C, 5 mg/kg Insoluble BP 4°C, 50°C, 5 mg/kg Soluble BP 4°C, or 50°C) commenced. All treatments were held or bleached for 1 h at their respective temperatures. After bleaching, the whey underwent ultrafiltration (UF) and diafiltration (DF) to concentrate the protein to 80% on a dry weight basis (18% solids) as described by Park et al. (2014). Briefly, the UF system (model Pellicon 2, Millipore Inc., Billerica, MA) using 5 cartridges of polyethersulfone membrane filters (model P2B010V05, nominal separation cutoff = 10,000 kDa, surface area = 0.5 m²) was cleaned before use with a 0.1 N sodium hydroxide solution (VWR international) followed by a rinse of deionized water. Total deionized water addition during DF was 50% of the original weight of the liquid whey. Protein concentration was confirmed using a Sprint Rapid Protein Analyzer (CEM Corp., Matthews, NC). Percent solids were analyzed using Smart System 5 moisture/solids analyzer (CEM Corp.). The total time for UF was approximately 3 h. The WPC80 retentates were then dried in a spray drier (model Lab 1, Anhydro Inc., Soeberg, Denmark) with an inlet temperature of 200°C and outlet temperature of 90°C. The powders
were collected in Mylar bags (TF-4000, Impak Corp., Central city, SD) and stored at -80°C until analysis. The experiment was replicated 3 times.

**Color Analysis**

Hunter L* (lightness), a* (red-green), and b* (yellow-blue) values for WPC80 powders and reconstituted WPC80 liquids (10% solids, wt/vol) were determined using Minolta chroma meter (CR-410, Ramsey, N.J., U.S.A.) using methods described by Fox et al. (2013). Measurements were taken in triplicate.

**Norbixin extraction**

Norbixin extraction from liquid whey and WPC80 powder was performed following the procedure described by Campbell et al. (2014), slightly modified for liquid whey. For WPC80 powder, 1 g of powder was placed into a 10 mL volumetric flask and HPLC grade water (EMD Chemicals) was added to volume. The sample was then mixed for 10 min after which 1 g of WPC80 solution was placed into a 5 mL volumetric flask and filled to volume with dilution solution (80% acetonitrile, 20% HPLC water, and 0.1% w/v formic acid, EMD Chemicals). The solution was again mixed for 10 min, after which 2 mL of sample was placed into an Eppendorf tube (VWR International) and centrifuged at 14,000 x g for 5 min. The clear supernatant was removed and placed into a vial (Phenomenex) for injection. For liquid whey, 0.5 mL of bleached or unbleached whey was added to 2 mL of dilution solution (80% acetonitrile, 20% HPLC water, and 0.1% w/v formic acid) at each time point. Two milliliters of sample were placed in an Eppendorf tube (VWR International) and
centrifuged at 14,000 x g for 5 min. The clear supernatant was removed and placed into a vial (Phenomenex) for injection.

Norbixin was quantified by HPLC (Breeze HPLC, Waters, Milford, MA) using an isocratic mobile phase (70% acetonitrile/30% water with 0.1% formic acid, EMD Chemicals) at a flow rate of 1 mL/min pumped through a binary pump (Waters 1525, Waters, Milford, MA). Fifty microliters of sample was injected (Waters 2707 autosampler) onto the column (Phenomenex Kinetex 2.6µm particle size, 10 cm length, 4.6 mm inner diameter, 100 Å pore size) at 40ºC. Sample was analyzed using a photodiode array detector (Waters 2998) with a maxima of 482 nm. Sample run time was 3 min. All WPC80 and liquid wheys were extracted and evaluated in triplicate. Norbixin destruction was determined by calculating the percent reduction of norbixin concentration in the bleached WPC80 or liquid whey compared to the norbixin concentration of the unbleached control WPC80 or liquid whey.

**Gas chromatography/mass spectrometry**

Selected volatile compounds were extracted from WPC80 powder by headspace solid-phase microextraction (SPME) followed by gas chromatography mass spectrometry (GCMS) using the method reported by Campbell et al. (2011) with modification. Each WPC80 was rehydrated to 10% solids (w/v) using HPLC water (EMD Chemicals Inc., Gibbstown, NJ), and 5 mL were added to 20 mL autosampler vials with steel screw tops containing silicone Teflon faced septa (Microliter analytical, Suwannee, FL) along with 10% (w/v) salt. Ten microliters of 81 mg/kg 2-methyl-3-heptanone in methanol (Sigma Aldrich., Milwaukee, WI) was added as an internal standard. Samples were injected by a CombiPal autosampler (CTC Analytics, Zwingen, Switzerland) into an Agilent 6890N GC with 5973
inert MSD (Agilent Technologies Inc., Santa Clara, CA). Samples were held at 10°C before fiber exposure and brought up to 40°C for 25 min before 30 min fiber exposure of a 1 cm DVB/CAR/PDMS (divinylbenzene/carboxen/polydimethylsiloxane) fiber at 31 mm with 4 sec pulsed agitation at 250 rpm. The fibers were injected for 5 min at a depth of 50 mm. The GC method used a start temperature of 40°C for 3 min with a ramp rate of 10°C/min until 250°C was reached. The sample was then held for 5 min. A Zb-5ms(Zb-5ms, 30 m length x 0.25 mm inner diameter x 0.25 µm film thickness, Phenomenex) column was used with a constant flow rate of 1 mL/min in SIM and SCAN mode. Purge time was 1 min. Compounds were identified using the National Institute of Standards and Technology (2002) (Gaithersburg, MD) mass spectral database and comparison of retention indices and mass spectra of authentic standards. Retention indexes (RI) were based on an alkane series (Sigma Aldrich). Internal standard concentration was used to calculate the relative abundance of each compound. The analyses were conducted in triplicate.

**Descriptive Sensory Analysis**

Descriptive sensory analysis (DA) was conducted on WPC80 manufactured in experiment 2 in compliance with North Carolina State University Institutional Review Board for Human Subjects guidelines. Samples were rehydrated to 10% solids (w/v) and dispensed into 59 mL cups with lids (Solo Cup Co., Champaign, Ill) labeled with random 3 digit codes. Eight panelists (23-45y, 6 females, 2 males), each with more than 100 h of experience with descriptive analysis of dried dairy ingredients and the Spectrum™ 0 to 15 point universal scale (Meilgaard et al. 2007) evaluated the samples using an established sensory language
(Wright et al., 2009). Each rehydrated sample was evaluated by each panelist in duplicate. Compusense five version 5.6 was used for data collection (Compusense, Guelph, Canada).

**Benzoic Acid Extraction and Measurement**

Benzoic acid in the WPC80 powders was determined by HPLC-PDA as described by Listiyani et al. (2011). The WPC80 (2.5 g) was measured into a 50 ml volumetric flask and 5 ml of 1 M zinc acetate (Mallinckrodt Baker Inc., Phillipsburg, NJ) solution, and 5 ml of 0.25 M potassium hexacyanoferrate(II) trihydrate (Alfa Aesar, Ward hill, MA) and filled to volume with HPLC water (EMD Chemicals). The samples were manually shaken for 1 min and transferred to centrifuge tubes (VWR International) and centrifuged for 10 min at 10,000 x g. The supernatant was filtered with a 0.45 um nylon filter (VWR International) and 750 µl of filtrate and 750 µl of mobile phase were added to HPLC vials (Phenomenex). Standard curves ranging from 0.1 to 50 mg/kg benzoic acid (BA; VWR International) were used to quantify BA in WPC80. High performance liquid chromatography analysis (Breeze HPLC, Waters, Milford, MA) was performed using reversed phase separation (Kinetex C18, 2.6 um, 100 x 4.6 mm; Phenomenex, Torrance, CA). The PDA detector was set at 230 nm. The mobile phase consisted of 4% methanol (VWR International) and 96% 0.02M ammonium acetate (VWR International) buffer with a flow rate of 1 ml/min pumped through a binary pump (Waters 1525, Waters, Milford, MA). Twenty microliters of sample was injected (Waters 2707 autosampler) onto the column.
Statistical analysis

Response surface methodology was performed on whey from experiment 1 using PROC RSREG (SAS version 9.2; SAS Institute Inc., Cary, NC) to create a predictive equation to predict log norbixin destruction as a function of benzoyl peroxide concentration and time for a given temperature and BP form for fluid whey. A second order response surface was fit to create a second order polynomial function of concentration and time for each temperature and BP form utilized in the experiment. Data from experiment 2 was analyzed by analysis of variance with means separation (ANOVA) (XLSTAT, version 2013.5.03, Addinsoft, New York, NY). Differences were analyzed by Tukey’s honestly significant difference (HSD).

Results and Discussion

Experiment 1

Norbixin Destruction

Benzoyl peroxide (soluble or insoluble), temperature, and benzoyl peroxide concentration all influenced norbixin destruction (p<0.05). At 4°C, and 5, 10, and 25 mg/kg concentration, soluble BP destroyed more norbixin than the insoluble BP (Figure 1). The soluble BP at 50 mg/kg bleached more norbixin than the insoluble BP until 16 h at which point norbixin was no longer detected (Figure 1). The insoluble BP, at 50 mg/kg, reduced norbixin 97.8% compared to the unbleached control by 16 h at 4°C (Figure 1). At 4°C, soluble BP was a more effective bleaching agent, especially at lower concentrations, than the insoluble BP.
At 50°C, and 5 and 10 mg/kg concentration, the soluble BP bleached more norbixin than the insoluble BP at all time points (Figure 2). At 25 and 50 mg/kg BP, the soluble BP bleached more norbixin than the insoluble BP until 45 min, at which point norbixin was no longer detected (Figure 2). The insoluble BP reduced norbixin 97.7% and 99.0% respectively at 25 and 50 mg/kg compared to the unbleached control after 45 min at 50°C (Figure 2). Like bleaching at 4°C, soluble BP was more effective at bleaching liquid whey at 50°C than insoluble, although at higher concentrations (25 and 50 mg/kg BP) virtually all norbixin was destroyed by both soluble and insoluble BP.

It is interesting to note that as BP concentration increased, the difference in norbixin destruction between the 2 different forms of BP decreased at both temperatures evaluated. This result suggests that at high concentrations of BP, little difference is seen between soluble and insoluble benzoyl peroxides in relation to bleaching efficiency, however, the soluble benzoyl peroxide was more effective than the insoluble benzoyl peroxide as concentration decreased. This observation is important considering the need to reduce benzoic acid residue in the final product. The lowest concentration of BP to reduce norbixin is desired.

At 4°C, the soluble BP at 5 mg/kg reduced norbixin concentration by 83.8% after 16 h (Figure 1). At 50°C, the soluble BP at 5 mg/kg reduced norbixin by 90.4% in 1 h (Figure 2). Recent studies evaluating hydrogen peroxide bleaching of fluid whey demonstrated that hydrogen peroxide reduced norbixin concentration by 20-44% at 250-500 mg/kg concentrations at temperatures ranging from 50-60°C (Fox et al., 2013; Kang et al., 2012; Jervis et al., 2012). Since HP is frequently used as a whey bleaching agent, it is likely that...
acceptable norbixin reduction in liquid whey is well below 90%. Listiyani et al. (2011) bleached liquid whey at both 50 and 100 mg/kg (insoluble) BP and demonstrated that there was a direct relationship between BP addition and subsequent BA levels in the final powder demonstrating a need to minimize the BP concentration used to bleach whey. Using the prediction equations from this study, at 4°C (Figures 3 and 4), it would take approximately 6 mg/kg of the soluble BP, and 10.5 mg/kg of insoluble BP to reduce norbixin by 45% in an hour (bleaching comparable to HP). At 50°C it would take approximately 0.5 mg/kg soluble BP, and 6.5 mg/kg insoluble BP to reduce norbixin by 45% in 40 min (Figures 5 and 6). It is unlikely that a concentration as high as the JECFA determined maximum BP concentration in liquid whey of 100 mg/kg would ever be necessary to reduce norbixin levels to desired levels. To reduce benzoic acid residues in whey protein ingredients, it is important to know exactly how much benzoyl peroxide is necessary to reduce norbixin to acceptable levels. Using soluble benzoyl peroxide allows for a significantly lower concentration to be used compared to benzoyl peroxide in an insoluble form with subsequently less residual benzoic acid.

**Experiment 2**

*Compositional analysis of WPC80*

No differences were detected (p>0.05) in the mean values for solids, fat, or protein among the WPC80 (Unbleached, 5 mg/kg soluble BP, 5 mg/kg insoluble BP, 4 and 50°C). Moisture was 3.32 ± 0.11%, fat (dry basis) was 4.29% ± 0.06%, and protein (dry basis) was
80.7% ± 0.45%. No differences (p>0.05) were found in mineral content (calcium, potassium, magnesium, sodium, iron, phosphorus) (results not shown).

Color and Norbixin Destruction

All bleached powders were visibly whiter (higher L* value) than the unbleached WPC80 (Table 1). The soluble BP 50°C (5 mg/kg) treatment was whiter (L* value) than the insoluble 4°C treatment. As expected, the yellowness (b* value) was reduced by both soluble and insoluble BP bleaching at 4 and 50°C, but soluble BP had much lower yellowness than the insoluble BP WPC80 at both 4 and 50°C (p<0.05, Table 1). These results are consistent with norbixin destruction results. Norbixin destruction in WPC80 from 5 mg/kg insoluble BP was 30 and 70% (4°C and 50°C, respectively) compared to the unbleached WPC80, while the norbixin concentration of WPC80 from 5 mg/kg soluble BP was 55 and 93% (4°C and 50°C, respectively; Figure 7). These results are consistent with those performed in liquid whey from experiment 1 and with the predicted norbixin destruction values from the generated equations. These results are also consistent with norbixin destruction from insoluble BP reported by Jervis et al. (2012) (92.3% norbixin destruction, 50 mg/kg BP, 50°C) in WPC80 and Campbell et al. (2013) (91.6% norbixin reduction, 50 mg/kg BP, 50°C) in SPC80. It is important to note that a direct comparison cannot be made due to the differences in commercial BP and that the WPC80 in this study was bleached at 5 mg/kg rather than 50 mg/kg. The results of this study, combined with those of Jervis et al. (2012) and Campbell et al. (2013) suggest that 5 mg/kg of soluble BP is as effective at reducing norbixin in the final product as 50 mg/kg insoluble BP.
Instrumental Volatile and Descriptive Analysis

Bleaching temperature and benzoyl peroxide (soluble or insoluble) impacted lipid oxidation and protein degradation compounds. Soluble and Insoluble BP bleached WPC80 had higher levels (p>0.05) of 2 methylbutanal, hexanal, 2-pentyl furan, and nonanal than unbleached WPC80 (results not shown), volatile compounds which have been previously reported in dried whey products (Wright et al., 2006; Croissant et al., 2009; Evans et al., 2010; Whitson et al., 2010; Listiyani et al., 2011; Jervis et al., 2012; Campbell et al., 2013). The WPC80 bleached by insoluble BP at 4°C had higher total aldehyde levels and higher levels of hexanal, 2-pentyl furan, and octanal than WPC80 bleached by soluble BP at 4 or 50°C and WPC80 bleached by insoluble BP at 50°C (Figure 8). Listiyani et al. (2012) and Fox et al. (2013) reported no differences among volatile compound levels between liquid whey bleached cold or hot (4°C or 68°C, 5°C or 50°C, respectively) with insoluble BP, however, 50 mg/kg and 25 mg/kg insoluble BP were the lowest concentrations evaluated and dried whey products were not evaluated. Campbell and Drake (2014) reported higher aldehyde levels in liquid whey bleached with various bleaching agents (HP, lactoperoxidase (LP), exogenous peroxidase (EP)) warm (35°C) compared to cold (4°C). Discrepancies between the findings of Fox et al. (2013), Listiyani et al. (2012), and Campbell and Drake (2014) may be due to differences in processing parameters and bleaching agents. Listiyani et al. (2012) bleached liquid whey with insoluble benzoyl peroxide at 68°C, which could cause more lipid oxidation compared to the “hot” bleaching temperature (50°C) used in the current study. Both Listiyani et al. (2012) and Fox et al. (2013) also bleached liquid whey using a higher concentration of benzoyl peroxide (50 mg/kg and 25 mg/kg respectively) and
evaluated liquid whey which had not been spray dried, which could also cause differences in lipid oxidation levels (Park et al., 2014). Campbell and Drake (2014) reported higher levels of aldehydes at 35°C compared to 4°C in WPC80 from liquid whey bleached with HP, LP or EP rather than BP.

Descriptive analysis results were consistent with volatile compounds. The WPC80 bleached at 4°C with insoluble BP was higher in cardboard flavor than the unbleached WPC80 and 50°C bleached WPC80 (p<0.05; Figure 8). The WPC80 bleached at 4°C by soluble BP was also higher in cardboard flavor than the unbleached control (p<0.05). Lipid oxidation products, such as the aldehydes found in elevated concentrations in the WPC80 bleached by insoluble BP at 4°C are the source of cardboard flavor (Whitson et al., 2010). Sweet aromatic flavor was lower in all BP bleached WPC80 compared to the unbleached control, and WPC80 bleached at 4°C by insoluble BP had lower sweet aromatic flavor than WPC80 bleached by soluble BP at 4 and 50°C.

**Benzoic Acid**

At equivalent concentration, bleaching temperature, but not BP form (soluble or insoluble) had a significant effect on benzoic acid levels in WPC80 (p<0.05; Figure 9). The WPC80 bleached at 4°C had higher benzoic acid residues than the WPC80 bleached at 50°C. Benzoic acid levels in UF permeate from WPC retentate bleached at 4°C were lower than BA residual levels in permeate from WPC retentate bleached at 50°C. This result may be due to benzoyl peroxide remaining unreacted during processing at cold temperatures. Unreacted BP could be concentrated during UF and subsequently spray dried with the WPC80 powder. It
would then be free to break down over time into benzoic acid, resulting in higher levels of BA in WPC80 bleached at 4°C than at 50°C.

Listiyani et al. (2011) suggested that not all BP was converted into benzoic acid during processing due to residual BA levels in WPC34 and UF permeate being too low to account for the amount of BP added to liquid whey. Jervis et al. (2015) demonstrated higher levels of lipid oxidation, residual BA, and BA recovery efficiency in liquid whey that had been BP bleached (insoluble, 50 mg/kg) at native solids levels (6.7%) compared to liquid whey bleached with BP at increased solids (14%). Bleaching efficacy for liquid wheys bleached at either solids level was the same (approximately 94% norbixin destruction), but as the current study shows, 50 mg/kg BP is a higher than necessary BP concentration to destroy virtually all norbixin in liquid whey. It is possible that at lower solids concentration, less BP degrades into BA during processing due to the decreased concentration of organics available for oxidation. Jervis et al. (2015) suggested that BA may bind to protein or solubilize in fat during the bleaching process. Moriguchi et al. (1968) and Wedzicha and Ahmed (1993) demonstrated that BA does bind to milk proteins. If BA binding to proteins takes place at a higher rate in liquid whey than it would as a dry powder (due to lack of BA mobility and subsequent protein contact and availability for reaction in the powder), then BA binding to whey proteins would also explain why BA recovery efficiency was much lower in SWP from liquid whey bleached at 14% solids compared to 6.7% solids (Jervis et al., 2015). The hypothesis that residual BP remains in the spray dried powder and continues to cause lipid oxidation is supported by the increased concentration of BA in the dried SWP powder (Jervis et al., 2015). During BP bleaching of liquid whey at higher solids (14%), more BP breaks
down to BA and more protein is available to bind with BP due to higher solids, while less BP breaks down into BA or binds to protein when bleaching occurs at native solids (6.7%). Residual BP in SWP from liquid whey bleached at native solids (6.7%) is free to continue to oxidize lipids in the dried powders. This result also explains the higher concentration of lipid oxidation products in SWP from fluid whey bleached at native solids. Residual BA in the powder is also unable to bind to protein in the dried powder, which would also explain the higher extraction efficiency and a higher concentration of residual BA.

In the current study, at 4°C, BP bleached WPC80 had higher levels of lipid oxidation than WPC80 bleached by BP at 50°C. A lower concentration of benzoic acid in 4°C bleached WPC80 UF permeate was present compared to 50°C bleached WPC80 UF permeate, which supports the hypothesis that benzoyl peroxide at 4°C was not completely decomposed and remained in the UF retentate (Figure 9). Benzoyl peroxide is fat soluble, and if unreacted BP was associated with the fat phase it would be concentrated during UF processing rather than being expelled into the permeate. It is possible that at lower temperatures, given only 1 h to bleach, residual insoluble benzoyl peroxide remained in the liquid retentate and subsequent WPC80 powder, while at higher temperatures (50°C), a higher proportion of the benzoyl peroxide was broken down into benzoic acid during bleaching. If this was the case, the increased lipid oxidation products associated with the 4°C insoluble BP bleached WPC80 are due to residual benzoyl peroxide continuing to cause lipid oxidation in the dried WPC80 powder. Future studies addressing BA as well as unreacted BP in dried powders would be necessary to confirm this hypothesis.
Sieber et al. (1995) and Qi et al. (2009) reported native benzoic acid in dairy products at concentrations as high as 110 mg/kg. A previous study performed by Listiyani et al. (2011) reported WPC34 powders from fluid whey bleached with 50 mg/kg benzoyl peroxide had a benzoic acid concentration of 272 mg/kg, and WPC34 powders from fluid whey bleached with 100 mg/kg benzoyl peroxide had a benzoic acid concentration of 634 mg/kg, while WPC80 from fluid whey bleached with 50 mg/kg BP had BA concentrations of 30-60 mg/kg. Listiyani et al. (2011) demonstrated that BA was removed with ultrafiltration (UF) and diafiltration (DF) during WPC production. Water soluble benzoic acid was washed out by DF and was detected in the UF permeate. Liquid permeate from the current study also contained BA. Ultrafiltered permeate from liquid whey bleached hot (50°C) had approximately 6x higher BA concentration than UF permeate from liquid whey bleached cold (4°C).

Based on the findings of the current study and Listiyani et al. (2011), it is possible that high protein whey concentrates (80-89%) and isolates (>90% protein wt/wt) could drastically reduce benzoic acid by UF and DF. Results from the current study suggest that approximately 5 mg/kg soluble BP would provide sufficient norbixin destruction under cold (4°C, 55% norbixin destruction) or hot bleaching (50°C, 90% norbixin destruction) conditions within 1 h and time could be increased to increase bleaching efficacy or BP decomposition. This result suggests possible avenues for reducing benzoic acid residues in WPC powders. To decrease benzoic acid residues in WPC powders, the lowest possible effective concentration of BP should be used. Diafiltration during processing also decreases BA, however, bleaching temperature, and ultimately benzoyl peroxide breakdown need to
also be accounted for during processing. Listiyani et al. (2012) demonstrated that bleaching before or after fat separation had no effect on volatile compound concentration or norbixin destruction in liquid whey. If BP is concentrated with the fat phase of the WPC80 retentate during UF, as the current study results suggest, it could be possible to remove remaining BP by bleaching before fat separation, without affecting the volatile composition in the final product. Reducing residual BP in the fat phase of liquid before UF and DF could further reduce BA residues in the WPC powder. As stated earlier, hydrogen peroxide bleaching of fluid whey reduces norbixin concentration by 20-44% at 250-500 mg/kg concentrations at temperatures ranging from 50-60°C (Fox et al., 2013; Kang et al., 2012; Jervis et al., 2012). The prediction equation from the current study predicts that as little as 0.5 mg/kg soluble BP at 50°C would be necessary to bleach 40% of norbixin in liquid whey in 1 h, which would further reduce benzoic acid levels in WPC powders compared to bleaching with higher concentrations of BP. The results of experiment 2 suggest that another avenue of BA residue reduction could be to remove the fat phase of liquid whey after BP bleaching, and subsequently a portion of the remaining BP, when norbixin destruction levels have reached desired levels. In Experiment 1, 5 mg/kg soluble BP at 50°C bleached 40% of norbixin in liquid whey in 15 min. Removing the fat phase of liquid whey after 15 min of bleaching could result in lower BA residue in final WPC powder and decrease total processing time. Future work should address residual BP in WPC80 retentate and the fat phase of liquid whey to confirm this hypothesis.
Conclusions

Bleaching of fluid whey is an important processing step in colored liquid whey. Benzoyl peroxide is more effective and efficient bleaching agent than hydrogen peroxide, but residual benzoic acid is a concern, especially in foreign markets. Benzoyl peroxide dispersed in a soluble carrier has been developed for liquid applications, but had not been previously directly compared to traditional powdered BP. Soluble BP bleaches liquid whey faster and at lower concentrations. Using soluble BP, less BP is necessary to achieve bleaching equivalent to insoluble BP, resulting in the ability to reduce benzoic acid residues in WPC80 powder. Bleaching liquid whey at a cold temperature (4°C) resulted in more lipid oxidation and subsequent increase in cardboard flavor, and more residual benzoic acid in the final powder than bleaching at 50°C. These results demonstrate that as little as 5 mg/kg soluble BP can be used to effectively bleach fluid whey with optimum norbixin destruction and minimum BA residues by bleaching at 50°C.

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Accessed April 4, 2015


Accessed April 11, 2015.


Table 1. Mean (n=3) color (L, a, and b values) of liquid (10% wt/vol powders) and spray-dried whey protein concentrate (WPC80) from liquid whey bleached with 5 mg/kg insoluble (InsolBP) or soluble (SolBP) benzoyl peroxide or unbleached (no bleach) at 4 or 50°C.

<table>
<thead>
<tr>
<th></th>
<th>L (lightness)</th>
<th>a (red-green)</th>
<th>b (yellow-blue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liquid</td>
<td>Powder</td>
<td>Liquid</td>
</tr>
<tr>
<td>No Bleach</td>
<td>64.5^c</td>
<td>88.6^b</td>
<td>8.32^a</td>
</tr>
<tr>
<td>InsolBP 4°C</td>
<td>65.4^c</td>
<td>89.5^b</td>
<td>6.05^b</td>
</tr>
<tr>
<td>SolBP 4°C</td>
<td>67.4^bc</td>
<td>89.2^b</td>
<td>5.93^b</td>
</tr>
<tr>
<td>InsolBP 50°C</td>
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<td>93.2^a</td>
<td>2.53^c</td>
</tr>
<tr>
<td>SolBP 50°C</td>
<td>71.4^a</td>
<td>95.2^a</td>
<td>2.13^c</td>
</tr>
</tbody>
</table>

^a-cMeans in a column not sharing a common superscript are different (p<0.05).
Figure 1. Percent norbixin destruction in liquid whey bleached by 5, 10, 25, or 50 mg/kg insoluble (InsolBP) or soluble (SolBP) benzoyl peroxide at 4°C for 16 h.
Figure 2. Percent norbixin destruction in liquid whey bleached by 5, 10, 25, or 50 mg/kg insoluble (InsolBP) or soluble (SolBP) benzoyl peroxide at 50°C for 1 h.
Figure 3: Response surface model of bleaching of fluid whey with insoluble BP at 4°C. Prediction equation: Percent destruction = 100 - (INL (9.88 + (conc x -0.07) + (time x -0.003) + (conc^2 x 0.0006) + (conc x time -0.00004) + (time^2 x 0.000004)) + 1))/161.18
Figure 4: Response surface model of bleaching of fluid whey with soluble BP at 4°C. Prediction equation: Percent destruction = 100 - (INL(9.97 + (conc x -0.10) + (time x -0.006) + (conc² x 0.001) + (conc x time x -0.00005) + (time² x 0.00001)) + 1)/161.18
Figure 5: Response surface model of bleaching of fluid whey with insoluble BP at 50°C
Prediction equation: Percent destruction = 100 - (INL (9.36 + (conc x -0.0004) + (time x 0.03) + (conc^2 x 0.0002) + (conc x time x -0.002) + (time^2 x -0.0006)) + 1)/161.18
Figure 6: Response surface model of bleaching of fluid whey with soluble BP at 50°C. Prediction equation: Percent destruction = 100 - (INL (10.23 + (conc x -0.07) + (time x -0.03) + (conc x 0.001) + (conc x time x -0.003) + (time² x 0.00003)) + 1)/161.18
Figure 7. Percent norbixin destruction in WPC80 powders from liquid whey bleached by 5 mg/kg insoluble (InsolBP) or soluble (SolBP) benzoyl peroxide at 4 or 50°C. Letters denote statistical differences (p<0.05).
Figure 8. Mean cardboard and sweet aromatic flavor intensities vs. total aldehyde relative abundance concentrations (µg/kg) for rehydrated (10% wt/vol) WPC80 powders from unbleached (con) or bleached at 5 mg/kg soluble (SolBP) or insoluble (InsolBP) benzoyl peroxide at 4 or 50°C. Letters denote statistical differences (p<0.05).
Figure 9. Benzoic acid concentrations (mg/kg) in WPC80 powder and UF permeate (solids basis) from liquid whey bleached by 5 mg/kg insoluble (InsolBP) or soluble (SolBP) benzoyl peroxide at 4 or 50°C. Letters denote statistical differences within each product (WPC80, permeate) (p<0.05). Note: Statistical lettering does not compare across products (WPC80 and permeate).
CHAPTER 4: FLAVOR AND FUNCTIONAL CHARACTERISTICS OF WHEY PROTEIN ISOLATES FROM DIFFERENT WHEY SOURCES

Flavor and Functional Characteristics of Whey Protein Isolates from Different Whey Sources

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Abstract:
Whey protein isolate (WPI) is a highly functional food ingredient used in a variety of applications. Most WPI produced in the United States is a byproduct of Mozzarella or Cheddar cheese manufacture; however, rennet casein and Cottage cheese production may produce WPI with superior flavor or functional characteristics. A greater understanding of the flavor and functional characteristics of WPI from all four whey sources is necessary to investigate new applications for whey and whey protein. The objective of the study was to determine the flavor and functional characteristics of WPI from Cheddar, Mozzarella, Cottage cheese, and rennet casein whey. WPI were manufactured in triplicate. Powders were rehydrated and evaluated in duplicate by descriptive sensory analysis. Volatile compounds were extracted by solid phase microextraction (SPME) followed by gas chromatography–mass spectrometry (GC-MS). Functional properties were evaluated by measurement of foam stability, heat stability, and protein solubility. WPI from Cheddar and Cottage cheese whey had the highest cardboard flavor while sweet aromatic flavor was highest in Mozzarella WPI, and rennet casein WPI had the lowest overall flavor and aroma. Distinct sour taste and brothy/potato flavor were also noted in WPI from Cottage cheese whey. Consistent with sensory results, aldehyde concentrations were also highest in Cheddar and Cottage cheese WPI. Overrun, yield stress, and foam stability were not different (p>0.05) among Cheddar, Mozzarella and rennet casein WPI, but WPI foams from Cottage cheese whey had a lower overrun and air phase fraction (p<0.05). Heat stability was not different among WPI. Cottage cheese WPI was less soluble at pH 4.6 compared to other WPI (p<0.05) and also exhibited higher turbidity loss at pH 3, 4, 5, 6 and 7 compared to other
WPI (p<0.05). This study suggests that WPI produced from non-traditional whey could be used in new applications due to distinct functional and flavor characteristics.

**Keywords**: Whey protein isolate, acid whey, functionality, sensory

**Practical Applications**

WPI is often used for its functional properties, but whey sources vary and differences in processing influence the flavor and functionality of whey. This study identified the functional and flavor characteristics of WPI from Cheddar cheese, Mozzarella cheese, Cottage cheese, and rennet casein wheys, and provides insights for the product applications which may be better suited for WPI made from different whey sources.

**Introduction**

WPI as a functional ingredient is often used in dairy, bakery, meat, snack, and confectionery products. WPI is useful as a high-quality protein for supplementation, and has emulsifying, fat binding, water binding/thickening, gelling, and whipping properties (USDEC 2013). Typical WPI process includes fluid storage, clarification/fat separation, pasteurization, bleaching, microfiltration or ion exchange, ultrafiltration, diafiltration, and spray drying (Varnam and Sutherland 1994; Drake and others, 2009a). While a large portion of dried whey protein produced in the US is the byproduct of Cheddar cheese manufacture, many whey sources exist. Different categories of whey include “sweet” whey (pH ≥ 5.8) obtained from the manufacture of natural rennet produced cultured cheeses (Cheddar, Mozzarella, etc.) or rennet casein production, medium acid whey (pH 5.0 to 5.8) obtained from the production of fresh acid cheeses (Danbo, queso blanco, etc.), and acid whey (pH < 5.0)
obtained from the manufacture of fresh acid cheeses (Quarg, Cottage cheese, cream cheese, etc.), and whey source influences flavor of the subsequent whey ingredient (Gallardo-Escamilla and others, 2005; Wright and others, 2009). Other WPI processing variables, such as fluid storage (Drake and others, 2009a; Wright and others, 2009; Liaw and others, 2010, Whitson and others, 2011), bleaching (Kang and others 2012; Campbell and others 2012; Croissant and others 2009; Listiyani and others 2011; Jervis and others 2012; Fox and others, 2013; Smith and others, 2015), and spray drying (Park and others, 2013) also have significant effects on the flavor of whey protein ingredients. Starter culture effects on the flavor of liquid whey (Tomaino and others, 2004: Campbell and others, 2011b; Liaw and others, 2010) and whey protein concentrate (Wright and others, 2009; Campbell and others, 2011a) have also been confirmed. Campbell and others (2011b) demonstrated that starter culture increased lipid oxidation in fluid whey with differences observed among starter strains and that mesophilic strains promoted lipid oxidation more so than thermophilic strains. Given that lipid oxidation is the source of cardboard flavor in dried whey ingredients (Whitson and others, 2010), lipid oxidation is a critical process to minimize to maximize dried whey ingredient quality.

Processing steps during manufacture also affect functionality of WPI. The type of culture used and the cheese-make process affect the composition of whey proteins (Schmidt and others, 1984; Hurley, 1990). Functionality of whey protein concentrates depend on the composition and cheese source (Caric, 1994; Huffman and Harper, 1999), heat treatment during processing (Sullivan and O’Connor, 1971; Regester and others, 1992), bleaching (Jervis and others, 2012; Campbell and others, 2013; Smith and others, In press), acid or
rennet coagulation, filtration technologies (membrane filtration or ion exchange), and spray
drying (Hurley, 1990). Jervis and others (2012), Campbell and others (2013), and Smith and
others (In Press) reported that bleaching fluid whey with hydrogen peroxide improved the
heat stability of rehydrated WPC80, serum protein concentrate (SPC, 80% protein), and
Cheddar WPI, respectively. It is possible that formulators could target specific whey
products based on their unit processes and process parameters (e.g., bleaching with hydrogen
peroxide), for their specific functional characteristics (e.g., increased heat stability). By so
doing, value can be added to the whey product where previously differences in functionality
may have been ignored or even a detriment to purpose in the final product. In order to target
specific unit processes, parameters, and whey products, research is necessary to elucidate the
flavor and functional differences caused by them.

Relatively little research has been done on the flavor and functionality of acid whey or dried
whey ingredients from acid whey. Acid whey has a different chemical composition (Durham
and others, 1997; Ji and Haque, 2003) and flavor (McGugan and others, 1979; Gallardo-
Escamilla and others 2005) from sweet whey. “Sweet” cheese liquid whey and whey proteins
(Cheddar and Mozzarella) have been widely characterized (Liaw and others, 2011;
Carunchia-Whetstine and others, 2005; Wright and others, 2009; Evans and others, 2010;
Whitson and others, 2010; Jervis and others, 2012). Previous studies reported bitter, stale,
rancid, chemical, acidic, and brothy flavors in fluid acid whey (McGugan and others, 1979;
Gallardo-Escamilla and others, 2005). Mortenson and others (2008) characterized the flavor
of WPC 34 from acid whey as caramel, acidic, milky, sweaty, buttery, oxidized, stale,
sulfurous, cooked and butterfat. Understanding the flavor characteristics of WPI from acid
whey is necessary to identify its most appropriate applications. The objective of the current study was to compare the functional and sensory properties of WPI from Cheddar cheese, Mozzarella, Cottage cheese, and rennet casein whey.

**Methods and Materials:**

**Whey production**

Raw whole milk or skim milk, 720 kg, was obtained from the North Carolina State University Dairy Research and Education Farm. Milk was HTST pasteurized (720 kg/h) with a plate heat exchanger (model T4 RGS-16/2, SPX Flow Technology, Greensboro, NC) at 72°C with a hold time of 16 sec. The milk was then cooled to 31°C and transferred to a cheese vat (Kusel Equipment, Watertown, WI) or chilled to 10°C and refrigerated at 4°C for no longer than 20 h for subsequent cheesemakes. All cheese wheys were made from the same lot of milk within replications over 2 consecutive days. Cheese wheys were manufactured in triplicate and order of cheese manufacture was randomized among replications.

*Cheddar Cheese Whey Manufacture*

A standard Cheddar cheese-make procedure was followed as described by Park and others (2013) with modifications, and liquid whey obtained. Mesophilic starter culture containing *Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. *cremoris* (Danisco Choozit MA11 LYO, Dairy Connection Inc., Madison, WI) was added to the pasteurized, whole cheesemilk (31°C) at the rate of 50 dairy culture units (DCU)/454 kg of milk. A calcium chloride solution (50% wt/vol, Dairy Connection Inc.) was then added at a rate of
0.39 mL/kg of milk. The milk was then allowed to ripen for 60 min. After the ripening period, the double-strength recombinant rennet (Dairy connection Inc.) was added at a rate of 0.09 mL/kg of milk (diluted 80 times in deionized (DI) water). The milk was allowed to coagulate for 30 min, after which the curd was cut and allowed to rest for 5 min. The curd and whey were then stirred for 30 min while the temperature was gradually increased to 39°C. The pH was monitored until a pH of 6.35 was achieved, after which the whey was drained through a sieve and cheese cloth (Dairy Connection, Inc.) to remove cheese fines. Liquid whey was then separated using a hot bowl centrifugal separator (model SI600E, Agri-Lac, Miami, FL). After separation, the whey was HTST pasteurized at 72°C for 16 sec.

**Mozzarella Cheese Whey Manufacture**

A standard Mozzarella cheese-make procedure was followed as described by Campbell and others (2011b) with modifications, and liquid whey obtained. Thermophilic starter culture containing *Lactobacillus helveticus* and *Lactobacillus delbueckii ssp. lactis* (Danisco Choozit LH100, Dairy Connection Inc., Madison, WI) and *Streptococcus thermophilus* (Danisco Choozit TA61) was added to the pasteurized, whole cheesemilk (35°C) at the rate of 20 (DCU)/454 kg of milk and 50 DCU/454 kg of milk, respectively. A calcium chloride solution (50% wt/vol, Dairy Connection Inc.) was then added at a rate of 0.39 mL/kg of milk. The milk was then allowed to ripen for 60 min. After the ripening period, double-strength recombinant rennet (Dairy connection Inc.) was added at a rate of 0.09 mL/kg of milk (diluted 80 times in deionized (DI) water). The milk was allowed to coagulate for 30 min, after which the curd was cut and allowed to rest for 5 min. The curd and whey were then stirred for 30 min while the temperature was gradually increased to
42°C. The pH was monitored until a pH of 6.35 was achieved, after which the whey was drained through a sieve and cheese cloth (Dairy Connection, Inc.) to remove cheese fines. Liquid whey was then separated using a hot bowl centrifugal separator (model SI600E, Agri-Lac, Miami, FL). After separation, the whey was HTST pasteurized at 72°C for 16 sec.

**Rennet Casein Whey Manufacture**

Calcium chloride solution (50% wt/vol, Dairy Connection Inc.) was added to pasteurized skim milk (29°C) at a rate of 0.39 mL/kg of milk. Double-strength recombinant rennet (Dairy connection Inc.) was added at a rate of 0.09 mL/kg of milk (diluted 80 times in deionized (DI) water). The milk was allowed to coagulate for 1 h, after which the curd was cut and allowed to rest for 5 min. The curd and whey were then stirred for 30 min while the temperature was gradually increased to 55°C. The whey was drained through a sieve and cheese cloth (Dairy Connection, Inc.) to remove cheese fines. Liquid whey was then separated using a hot bowl centrifugal separator (model SI600E, Agri-Lac, Miami, FL). After separation, the whey was HTST pasteurized at 72°C for 16 sec.

**Cottage Cheese Whey Manufacture**

Cottage cheese was manufactured as described by McAulliffe and others (1999) with modifications, and liquid whey obtained. Mesophilic starter culture (F-DVS Fresco 1000-21, Chr Hansen, Milwaukee, WI, USA) was added to the pasteurized, skim cheesemilk (21°C) at the rate of 5 g/26 kg of milk. After 30 min, double-strength recombinant rennet (Dairy Connection, Inc.) was added at a rate of 0.2 mL/103 kg milk. The pH of the milk was then monitored until a pH of 4.65-4.75 was reached (approximately 4.5 h). The curd was then cut
into 2.5 cm cubes and allowed to rest for 15 min after which the temperature was gradually increased to 55°C over 90 min. The whey was then drained through a sieve and cheese cloth (Dairy Connection, Inc.) to remove cheese fines. Liquid whey was then separated using a hot bowl centrifugal separator (model SI600E, Agri-Lac, Miami, FL). After separation, the whey was HTST pasteurized at 72°C for 16 sec.

**WPI Manufacture**

For each whey source, liquid whey was microfiltered and ultrafiltered using a pilot scale membrane system (Model Lab 46, Filtration Engineering, Champlin, MN). Remaining fat was removed from the whey by two 800,000 Da spiral wound microfiltration (MF) membranes (Synder, Vacavile, CA; nominal cutoff: 800,000 Da, surface area 7.15 m²) running at 0.052 Mpa transmembrane pressure. Temperature for MF was 50°C. After 5x concentration of the whey, diafiltration (DF) commenced. Deionized (DI) water (30% of original whey weight) was added during DF. MF run time was approximately 1 h. MF permeate was collected and used for ultrafiltration (UF). MF permeate was concentrated via UF and DF (40% w/w) using a UF membrane (Synder filtration, Vacavile, Ca; nominal cutoff: 10,000 Da, surface area 5.0 m²) running at 0.086 Mpa transmembrane pressure to a target composition of 18% solids (w/v) and 90% protein (w/w). UF run time was approximately 2 h.

Once target solids (18%) and protein (90%) levels were achieved, the WPI retentate was spray dried (model Lab 1, Anhydro Inc., Soeberg, Denmark) at an inlet temperature of 200°C and outlet temperature of 95°C. All samples were collected in mylar bags (TF-4000, Impak Corp., Central City, SD) and stored at -80°C until analysis. Spray drying of each WPI
took approximately 4 h. WPI were manufactured from separate lots of each whey in triplicate.

**Composition analysis**

The WPI powders were analyzed for total solids (TS), crude protein, fat, and minerals. Total solids were determined by air oven drying (AOAC 2000; method number 990.20; 33.2.44). Fat was quantified by ether extraction (AOAC 2000; method number 989.05; 33.2.26). Protein was determined using the Kjeldahl method (AOAC 2000; method number 991.20; 33.2.11). Analysis of phosphorus, calcium, magnesium, potassium, sulfur, sodium and iron was performed by the North Carolina State University analytical services laboratory (Raleigh, NC) using a standard dry ash method with inductively coupled plasma optical emission spectroscopy (Lloyd and others, 2009). All samples were measured in duplicate.

**Gas chromatography/mass spectrometry**

Selected volatile compounds were extracted from WPI and analyzed by headspace solid-phase microextraction (SPME) following the method used by Campbell and others (2011b) with modification. Each WPI was rehydrated to 10% solids (w/v) using HPLC water (EMD Chemicals Inc., Gibbstown, NJ), and 5 mL were added to 20 mL SPME vials (MicroLiter Analytical Supplies, Inc., Suwanee, GA) along with 10% (w/v) salt. Sample was placed into 20mL autosampler vials with steel screw tops containing silicone septa faced in Teflon (Microliter analytical, Suwanee, FL). Ten μL of 81 mg/kg 2-methyl-3-heptanone in methanol (Sigma Aldrich., Milwaukee, WI) was added as an internal standard. Samples were injected by a CombiPal autosampler (CTC Analytics, Zwingen, Switzerland) into an
Agilent 6890N GC with 5973 inert MSD (Agilent Technologies Inc., Santa Clara, CA). Samples were held at 10ºC before fiber exposure and brought up to 40ºC for 25 min before 30 min fiber exposure of a 1 cm DVB/CAR/PDMS (divinylbenzene/carboxen/polydimethylsiloxane) fiber at 31 mm with 4 sec pulsed agitation at 250 rpm. The fibers were injected for 5 min at a depth of 50 mm. The GC method used a start temperature of 40ºC for 3 min with a ramp rate of 10ºC/min until 250ºC was reached. The sample was then held for 5 min. The column used was a Zb-5ms (Zb-5ms, 30 m length x 0.25 mm inner diameter x 0.25 µm film thickness, Phenomenex) with a constant flow rate of 1 mL/min in SIM and SCAN mode. Purge time was 1 min. Compounds were identified using the National Institute of Standards and Technology (2002) (Gaithersburg, MD) mass spectral database and comparison of mass spectra of authentic standards. Retention indexes (RI) were based on an alkane series (Sigma Aldrich). Internal standard concentration was used to calculate the relative abundance of each compound.

**Descriptive Sensory Analysis**

Descriptive sensory analysis (DA) was conducted in compliance with North Carolina State University Institutional Review Board for Human Subjects guidelines. Samples were rehydrated to 10% solids (w/v) and dispensed into 59 mL cups with lids (Solo Cup Co., Champaign, IL) labeled with random 3 digit codes. Eight panelists (23-45 y, 5 females, 3 males), each with more than 100 h of experience with descriptive analysis of dried dairy ingredients and the Spectrum™ 0 to 15 point universal scale (Meilgaard and others 2007), evaluated the samples using an established sensory language (Wright and others 2009). Each
sample replicate was evaluated by each panelist in duplicate. Compusense five version 5.6 (Compusense, Guelph, Canada) was used for data collection.

**Foam Generation**

An Artisan KitchenAid Mixer (KitchenAid, St. Joseph, MI) with a 4.5-quart stationary bowl and a rotating wire beater was used to produce WPI foams. Solutions (10% wt/vol) of each WPI were rehydrated for 6 h at room temperature (20-24°C) on a stir plate and refrigerated overnight (4°C). Before foaming, the temperature was brought back up to 25°C and pH adjusted to 7 using 1 N NaOH (VWR International). Samples (200 mL) of the solution were whipped at speed 10 (beater rpm of 752) for 19 min and 36 s (Davis and Foegeding 2007). Foams were prepared in triplicate.

**Yield Stress**

Yield stress of foams was tested using vane rheometry (Brookfield 25xLVTDV-CIP viscometer, Brookfield Engineering Laboratories Inc., Middleboro, MA; Pernell and others 2000; Davis and Foegeding 2007). Tests were conducted at a speed of 0.3 rpm with a vane diameter of 10 mm and 40 mm length. Maximum torque response ($M_o$) was determined in each sample in triplicate. The following formula was used to convert $M_o$ to yield stress (Dzuy and Boger 1983, 1985; Steffe 1996):

$$T_o = M_o / [(h/d) + (1/6)][(\pi d^3)/2]$$

$T_o$ is yield stress and $h$ and $d$ are the height and diameter of the vane respectively. Torque measurements were taken in triplicate.
Overrun

Overrun of the foams was measured after yield stress of the foams was determined. Foam was removed from the mixing bowl with a rubber spatula and placed into a 100 mL weighing dish. Air phase fraction and overrun were determined using the following formulas:

\[ \text{Overrun} = \frac{\text{wt of 100 mL solution} - \text{wt of 100 mL of foam}}{\text{wt of 100 mL of foam}} \]

\[ \text{Air phase fraction} (\Phi) = \frac{\% \text{ overrun}}{\% \text{ overrun} + 100} \]

Treatments were measured in triplicate (Dickinson 1999; Wilde 2000; Davis and Foegeding 2007).

Foam Stability

Foam stability was determined by measuring the time necessary for half of the prefoam mass to drain through a hole in the whipping bowl (Phillips and others 1990; Luck and others 2001). Starting time for the measurements began immediately after foam formation (Davis and Foegeding 2007).

Protein Solubility

Protein solubility was performed as described by Jervis and others (2012). Solutions (10% wt/vol) of WPI were made by rehydrating to 80% of the desired volume for 6 h at room temperature (20-24°C) on a stir plate set to 200 rpm (Campbell and others, 2013). Solutions were then refrigerated overnight (4°C). After bringing the temperature to 25°C, the solutions were adjusted to a pH of 3, 4, 5, 6, or 7 using 1 N HCl or 1 N NaOH, and then brought to a total volume of 100 mL with deionized water for a final protein concentration of 10% (wt/vol). Solubility, using the micro bicinechoninic acid (micro-BCA) assay, and turbidity
were performed on the solutions, after which they were centrifuged at 16,500 x g for 10 min (model RC5B centrifuge; Thermo Scientific) and the turbidity and solubility were performed again on the supernatants.

Micro-BCA was performed using a kit from Thermo Fisher Scientific/Pierce (Rockfor, IL). Protein solutions were diluted 1:100 in deionized water and added at a ratio of 1:8 to a working reagent and pipetted into a 96-well plastic plate. Micro-BCA was performed in triplicate. The plate was placed on a shaker for 30 s and incubated at 37ºC for 30 min. The plate was allowed to return to room temperature and read on a Tecan Safire plate reader spectrophotometer at wavelength 562nm (Tecan, Durham, NC). Solubility was calculated using the following equation (Abs = absorbance):

\[
\text{Protein solubility} = 100 - \left\{ \frac{(\text{Abs}_{\text{before}} - \text{Abs}_{\text{after}})}{\text{Abs}_{\text{before}}} \right\} \times 100
\]

Analyses were performed in quadruplicate.

Turbidity was measured using a Hach 2100AN Turbidimeter (Loveland, CO). A tungsten filament lamp was used as a light source focused by a lens and measured transmitted light, and back, forward, and 90º angle scattering light. Centrifuged and uncentrifuged samples were pipetted into glass cuvettes and measured in quadruplicate. Turbidity was calculated using the following equation:

\[
\text{Turbidity} = \left\{ \frac{(\text{Turbidity}_{\text{before}} - \text{Turbidity}_{\text{after}})}{\text{Turbidity}_{\text{before}}} \right\} \times 100
\]

**Heat Stability**

Heat stability was measured using the methods described by Jervis and others (2012). Solutions were prepared at 10% (wt/vol) protein and then brought to pH 7 using 1 N NaOH. The solutions were then heated in a water bath at 90°C for 0 and 10 min and then placed in
an ice bath until reaching an internal temperature of 25°C. All solutions gelled at 10 min. Micro-BCA measurements were then taken before and after centrifugation as described above. Solubility calculations were then made as described above. The supernatant was then collected and brought to a pH of 4.6 using 1 N HCl. Solubility were then measured as previously described. All measurements were measured in triplicate.

**Statistical Analysis**

Data was analyzed by analysis of variance with means separation (ANOVA) (XLSTAT, version 2013.5.03, Addinsoft, New York, NY). Differences were analyzed by Tukey’s honestly significant difference (HSD).

**Results and Discussion**

**Composition Analysis**

There were differences in moisture and pH between WPI (p<0.05), but not differences between WPI in protein or fat composition (p>0.05). The WPI from Cottage cheese had a higher moisture content than the Cheddar and Rennet WPI, but was not different from Mozzarella WPI (Table 1). The higher moisture content of Cottage cheese WPI was likely due to the higher concentration of hygroscopic organic acids, which also caused a large difference in the pH of the reconstituted WPI. The pH of WPI from Cottage cheese whey was 4.67, while Cheddar, Mozzarella, and rennet WPI were 6.49, 6.44, and 6.66 respectively. Fat content (dry basis) was 0.42% ± 0.09%, and protein (dry basis) was 90.5% ± 0.95%. The pH differences among the WPI are of interest, because one would expect the buffering capacity of the increased protein levels of WPI to offset the acidity produced by the starter
culture in the liquid whey. It is likely that the buffering capacity of the whey protein was
overcome before the liquid whey was concentrated during UF and DF. One would also
expect low molecular weight organic acids, produced by the starter culture, to be removed by
UF and DF. In the case of this study, acid from starter culture fermentation remained in the
UF retentate and was not all removed into the permeate. Liquid whey was pasteurized after
draining and all starter culture was inactivated. The pH of the WPI (4.67) from acid whey
did not change appreciably from the pH of the liquid whey before pasteurization. This result
was also consistent with the higher pH of the WPI from rennet whey (6.66) compared to
Cheddar (6.49), Mozzarella (6.44), and Cottage cheese (4.67) WPI. Rennet whey was
manufactured without starter culture, and would naturally have a higher pH than the other
liquid wheys from Cheddar, Mozzarella, and Cottage cheese. Clear acidic beverages are a
common application of WPI, and the low pH of Cottage cheese WPI could be useful in
acidified beverages, removing the need to add additional acids to lower the pH.
Mineral content among WPI from Cheddar, Mozzarella, Cottage, and rennet wheys were
different (p<0.05, Table 2). Mineral content of WPI was consistent with the mineral content
of past studies on sweet whey WPI (Carunchia Whetstine and others, 2005). Cottage cheese
WPI mineral content has not been previously studied, but mineral content differences
between sweet and acid fluid whey in previous studies are consistent with the differences
between mineral content of the Cottage cheese WPI and Cheddar, Mozzarella, and rennet
WPI (Wong and others, 1978). Cottage cheese WPI was higher than Cheddar, Mozzarella,
and rennet WPI in phosphorus, calcium, potassium, and iron. This is likely due to the pH
when draining the whey during the respective cheese-makes. In rennet-set cheeses, whey is
drained at a pH of approximately 6.4, whereas Cottage cheese whey is drained at a pH of 4.65-4.75. At a lower pH, minerals are more highly ionized, for example, in rennet-set cheeses, calcium is precipitated as a calcium caseinate complex and most of the calcium is in the curd rather than the whey, while in acid set curds (Cottage cheese), calcium is more highly ionized and is expelled into the whey at a higher rate (Wong and others, 1978). Mineral content, especially calcium, is an important selling point for dairy powders and could be an important characteristic of Cottage cheese WPI powders.

Volatile compounds and descriptive analysis

Volatile compound profiles were distinct among WPI (p<0.05) (Table 3). All volatile compounds identified have been previously reported in whey products (Wright and others, 2006; Croissant and others, 2009; Evans and others, 2010; Whitson and others, 2010; Listiyani and others, 2011; Jervis and others, 2012; Campbell and others, 2013; Smith and others, in press). Total aldehyde levels were higher in WPI from Cottage and Cheddar cheese WPI than Mozzarella and rennet WPI (Figure 1). All Mozzarella and rennet WPI volatile compound concentrations were at parity with (p>0.05) or below (p<0.05) volatile compound concentrations in Cheddar and Cottage cheese WPI. Cheddar WPI was highest in 3 methyl butanal (p<0.05). Mozzarella WPI was higher in diacetyl, acetic acid, 2-pentyl furan, Z-4-heptenal, and 1-octen-3-one than rennet WPI. Rennet WPI volatile compound concentrations were at parity with (p>0.05) or lower than (p<0.05) Mozzarella WPI volatile compound concentrations.

The differences between Cheddar and Mozzarella WPI are consistent with results demonstrated by Campbell and others (2011a,b) and Liaw and others (2011) with Cheddar
and Mozzarella fluid whey and WPC. Campbell and others (2011b) demonstrated that Cheddar and Mozzarella liquid whey differed in volatile compound profiles and that liquid whey from Cheddar starters had more volatile lipid oxidation products than liquid whey from Mozzarella starters. Cheddar WPC had more decanal, heptanal, and acetic acid than Mozzarella WPC. Liaw and others (2011) demonstrated similar results comparing Mozzarella and Cheddar fluid whey and also showed that Cheddar whey was more prone to lipid oxidation than Mozzarella whey. These differences were attributed to the different starter cultures used. In the current study, rennet and Mozzarella WPI were not different in many lipid oxidation products (pentanal, hexanal, heptanal, octanal, nonanal, decanal, 2 and 3 methyl butanal, E, 2 nonenal, and methional), but Mozzarella WPI was higher in diacetyl, acetic acid, 2-pentyl furan, Z-4-heptenal, and 1-octen-3-one than rennet whey WPI. These volatiles (lipid oxidation and Strecker degradation compounds) are attributed to the presence of the starter culture.

Cottage cheese WPI was higher than Cheddar, Mozzarella, and rennet WPI in diacetyl, acetic acid, dimethyl disulfide, 2-pentyl furan, and methional. Methional is a Strecker degradation product formed from the degradation of methionine, while dimethyl disulfide is formed from the degradation of methionine into dimethyl sulfide and methanethiol and then into dimethyl disulfide (Bendall, 2001). Cottage cheese milk is allowed to ferment at 21°C for approximately 4.5 h after which the temperature is increased to 55°C over 90 min. Strecker degradation is a heat induced process, and an increase in dimethyl disulfide and methional are all explained by the increased heat load during the Cottage cheese-make procedure.
compared to the other wheys manufactured in this study. The increased diacetyl and acetic acid concentrations are probably due to the mesophilic starter culture.

Rennet WPI had a lower volatile load than Cheddar, Mozzarella, and Cottage cheese WPI. This is consistent with past studies on the volatile composition of rennet wheys (Campbell and others, 2011b). Starter cultures propagate lipid oxidation in liquid whey (Campbell and others, 2011a,b; Liaw and others, 2011). Rennet whey manufacture does not include starter culture addition and lipid oxidation was reduced compared to lipid oxidation in Cheddar, Mozzarella, and Cottage cheese liquid wheys.

Flavor was also distinct among WPI (p<0.05) (Table 4). Cottage cheese WPI had a potato/brothy flavor and a sour taste concurrent with the highest astringency of the WPI. Cheddar and Cottage cheese WPI had more cardboard flavor than the Mozzarella and rennet WPI. Whey protein isolate from rennet whey had the lowest aroma intensity and less cardboard flavor than WPI from Cheddar or Cottage cheese whey. Cardboard flavor is a common off-flavor in liquid whey and whey protein concentrates sourced to lipid oxidation products (Wright and others, 2009; Whitson and others, 2010). In general, WPI from Cheddar and Cottage cheese whey were higher in lipid oxidation products, which is consistent with the increased cardboard flavor intensity in these WPI (Figure 1). The WPI from Cottage cheese whey was unique among treatments in potato/brothy flavor. The presence of this flavor in the Cottage cheese WPI was concurrent with increased levels of the volatile compound methional.

Dried ingredient flavor impacts the flavor of the final product in which it is included, and even seemingly small differences in flavor can negatively impact acceptability of an
ingredient application (Evans and others 2010; Caudle and others 2005; Wright and others 2009). It is interesting to note that overall aroma intensity of Cottage cheese WPI was lower than WPI from Mozzarella and Cheddar wheys. However, potato brothy flavor, astringency, and sour taste would all need to be taken into account during formulation. Whey protein isolate is often incorporated into beverages at many different acidity levels including neutral (pH 6-7), mildly acidic (pH 4-5), or highly acidic (pH < 3.5). Astringency and off-flavors in acidic and neutral pH protein beverages reduce consumer acceptance (Beecher and others, 2008; Childs and Drake, 2010; Evans and others, 2009, 2010). However, in certain applications, what would be considered an off-flavor in other products might be acceptable as flavor carry-through from whey protein ingredients is different for different ingredient applications (Caudle and others, 2005; Drake and others, 2009b).

Whey protein isolate manufacture includes many processing steps which may potentially introduce off-flavors, including whey source, starter culture, fluid storage, bleaching, and spray drying (Wright and others, 2009; Campbell and others, 2011b; Whitson and others 2011; Park and others 2013; Croissant and others 2009; Jervis and others 2012). The pH differences between the different WPI may also play a role in the flavor and volatile compound profiles (White and others, 2013), but starter culture and manufacture of the different wheys are also responsible for differences in flavor and volatile compounds in the WPI. It is important to note that the temperature never exceeded 39 or 42°C during the Cheddar and Mozzarella cheese-make processes, respectively. Both Cottage cheese and rennet whey processing involved heating the whey to 55°C over the course of 1 h in the case of rennet whey, and 1.5 h in the case of Cottage cheese whey. Heat and acidification likely
had a role in the increased heat-induced flavor volatiles methional and dimethyl disulfide in Cottage cheese whey as well as the distinct flavor of Cottage cheese WPI. The Cottage cheese-make process also has an extended period of time (4.5-5 h) when the starter culture was allowed to ferment and reduce the pH. Differences in temperature and time profile during processing could also explain differences in lipid oxidation levels in the WPI. Campbell and others (2011b) and Liaw and others (2011) both demonstrated that lipid oxidation was enhanced by starter cultures, and storage time of fluid whey which suggests that the longer period of starter culture fermentation of Cottage cheese whey manufacture (7 h) compared to Cheddar (2 h), Mozzarella (2 h), and rennet (no starter culture, 2 h) was also a cause of higher lipid oxidation in Cottage cheese WPI.

**Functionality**

All WPI foamed and differences were detected among WPI (p<0.05) (Table 5). Cottage cheese WPI had a lower percent overrun and air phase fraction than all other WPI (p<0.05), however, the foam made from Cottage cheese WPI had a higher foam stability and yield stress than Cheddar WPI. There were no differences between Cheddar, Mozzarella, and rennet WPI foams in percent overrun, percent air phase fraction, but foam stability and yield stress of Cheddar WPI foams was lower than Mozzarella and Cottage cheese WPI foams (p<0.05). The foamability of proteins is related to their film-forming ability at the air-water interface (Mita and others, 1977, 1978). Past studies have demonstrated that WPI that has been heat denatured possesses the molecular properties required to form a denser foam with smaller average bubble size than a native WPI (Zhu and Damodaran, 1994). This may
explain why the Cottage cheese WPI, which was heated to 55°C over the course of 90 min, formed foams with a lower overrun (%) and air phase fraction (%).

All 10% WPI solutions gelled after 10 min of heating at 90°C at pH 4.6 and 7. Percent solubility of protein in Cottage cheese WPI was lower (82%) than Mozzarella, rennet, and Cheddar cheese WPI at pH 4.6, but no differences were observed between protein solubility at pH 7 (Figure 2). Percent solubility at pH 3, 4, 5, 6, and 7 was not different among WPI (p>0.05, results not shown). Average percent solubility at pH 3 was 97.7% ± 2.4%, pH 4 was 92.8% ± 2.1%, pH 5 was 78.4% ± 3.4%, pH 6 was 94.4% ± 1.2%, and pH 7 was 98.9% ± 0.5%. Differences in turbidity at pH 3, 4, 5, 6, and 7 between WPI were observed (Figure 3) (p<0.05). Turbidity loss in Cottage cheese WPI was higher at each pH compared to all other WPI (p<0.05). Turbidity loss in Mozzarella WPI was lower at each pH compared to all other WPI, and Cheddar and rennet WPI were not different from each other (p>0.05).

Turbidity of a solution is a measurement of light scattering due to large particles. In 10% solutions of WPI, which have very little fat, these large particles (the source of turbidity) are likely protein aggregates formed due to pH or heat induced aggregation. The high turbidity and subsequent loss of turbidity due to centrifugation in Cottage cheese WPI suggests a greater degree of pH or heat induced aggregation during the Cottage cheese-make procedure. It is somewhat surprising that Cottage cheese WPI would have a greater number of insoluble aggregates than Cheddar, Mozzarella, and rennet WPI. Large protein aggregates formed during the Cottage cheese-make procedure due to pH or heat presumably would be removed during microfiltration and remain in the retentate. However, the current study also determined that acids formed during the Cottage cheese-make procedure were not completely
removed by UF and DF, so the pH of the UF retentate remained low during processing. Aggregation due to low pH may have continued after the MF process or small aggregates formed during Cottage cheese whey manufacture made it through MF and formed larger particles over time.

Whey protein isolate from Mozzarella whey had lower overall turbidity and turbidity loss compared to Cheddar, Cottage and rennet WPI at pH 3, and Cottage WPI at pH 4, 5, 6, and 7, suggesting less damage to the proteins during processing, resulting in less protein aggregation. This is likely due to a difference in oxidative stability due to starter culture. Rennet WPI had no starter culture, but had a higher heat load during whey manufacture than Mozzarella and Cheddar WPI (55°C over 1 h vs. 39 and 42°C over 1 h for rennet, Cheddar, and Mozzarella manufacture, respectively). This could explain why protein aggregation, measured as WPI (10% solutions) turbidity, was not lower than Cheddar and Mozzarella WPI. The oxidative stability of Mozzarella cheese whey is greater than that of Cheddar cheese whey due to starter cultures (Liaw and others 2010; Campbell and others 2011b). Oxidation through bleaching of various protein products (Cheddar WPC 80, SPC 80, and Cheddar WPI) and its effect on functional properties has been well studied (Jervis and others, 2012; Campbell and others, 2013; Smith and others, In press). The WPC 80, SPC 80, and WPI bleached by hydrogen peroxide were shown to be more heat stable in all cases (Jervis and others, 2012; Campbell and others, 2013; Smith and others, In press). No difference in heat stability among WPI was observed in this study, but the relatively low levels of protein oxidation in the unbleached WPI of this study compared to WPI bleached by hydrogen peroxide (250 mg/kg) or benzoyl peroxide (50 mg/kg) likely account for that.
Conclusions

Whey source has an effect on both the sensory and functionality of WPI. Whey protein isolate from Cheddar and Mozzarella whey had the highest aroma intensity compared to Cottage cheese and rennet WPI, while WPI from Cheddar and Cottage cheese whey had the highest cardboard flavor intensity and the highest levels of lipid oxidation. Whey protein isolate from Cottage cheese whey was also characterized by potato brothy flavor and sour taste. The potato/brothy flavor, sour taste, and astringency of Cottage WPI would limit use in many foods, but in sour, high acid applications (e.g. clear acidic beverages, yogurt, kefir) it could be a good alternative to WPI from other sources. Cottage cheese WPI had the highest turbidity loss at all pH levels tested (3, 4, 5, 6 and 7), although soluble protein across each pH was not different among WPI except for at pH 4.6. Overrun and air phase fraction of foams made from Cottage cheese WPI were lower than all other WPI. Compositional, sensory, and functional differences between WPI suggest the need for formulators to use targeted whey protein products for specific applications. For instance, Cottage cheese WPI could be used for foam formation in a food when low foam volume is needed, but foam strength is important. Due to differences in starter culture and process parameters, WPI from different sources (Mozzarella, Cheddar, Cottage, rennet whey) exhibit different levels of lipid oxidation, have distinct flavor characteristics, and variability in turbidity, solubility, and foam forming properties. These differences create a potential for targeted applications for which specific WPI sources would be best suited to meet the needs of product formulators.
Acknowledgements

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References


Table 1: Mean (n=3) moisture (%, wt/wt), protein (%, wt/wt), fat (%, wt/wt), and pH (10% solutions, wt/wt) of spray-dried whey protein isolate (WPI) from Cheddar, Mozzarella, and Cottage cheese, and rennet casein wheys.

<table>
<thead>
<tr>
<th></th>
<th>Cheddar</th>
<th>Rennet</th>
<th>Mozzarella</th>
<th>Cottage</th>
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<tr>
<td>Moisture (%)</td>
<td>3.58&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.42&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.74&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>4.02&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Protein (%)</td>
<td>90.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>91.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>90.5&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Fat (%)</td>
<td>0.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.45&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>pH</td>
<td>6.49&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.44&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.67&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a-c</sup> Means in the same row not sharing a common superscript are different (p<0.05)
Table 2: Mean (n=3) mineral composition (mg/100g, calculated on a dry basis) of spray-dried whey protein isolate (WPI) from Cheddar, Mozzarella, and Cottage cheese, and rennet casein wheys.

<table>
<thead>
<tr>
<th></th>
<th>P</th>
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<th>K</th>
<th>Mg</th>
<th>Na</th>
<th>Fe</th>
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<td>492.1b</td>
<td>722.5b</td>
<td>0.082a</td>
<td>191.4a</td>
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<td>524.3b</td>
<td>715.1b</td>
<td>0.079a</td>
<td>187.2a</td>
<td>0.901b</td>
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<td>Rennet</td>
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<td>487.7b</td>
<td>751.6b</td>
<td>0.084a</td>
<td>190.0a</td>
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</tr>
<tr>
<td>Cottage</td>
<td>647.6a</td>
<td>941.4a</td>
<td>812.7a</td>
<td>0.097a</td>
<td>180.1a</td>
<td>1.012a</td>
</tr>
</tbody>
</table>

a-c Means in a column not sharing a common superscript are different (p<0.05)
Table 3: Relative abundance table of selected volatile compounds in WPI from different whey sources. Mean (n=3) concentrations of selected volatile compounds (µg/L) of spray-dried WPI from Cheddar, Mozzarella, and Cottage cheese, and rennet casein wheys.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cheddar</th>
<th>Mozzarella</th>
<th>Rennet</th>
<th>Cottage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diacetyl</td>
<td>1.24(^{c})</td>
<td>2.86(^{b})</td>
<td>0.41(^{d})</td>
<td>3.45(^{a})</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>0.41(^{b})</td>
<td>0.72(^{b})</td>
<td>0.14(^{c})</td>
<td>4.57(^{a})</td>
</tr>
<tr>
<td>Pentanal</td>
<td>10.87(^{a})</td>
<td>4.17(^{b})</td>
<td>4.56(^{b})</td>
<td>12.61(^{a})</td>
</tr>
<tr>
<td>Hexanal</td>
<td>271.16(^{a})</td>
<td>163.24(^{b})</td>
<td>156.37(^{b})</td>
<td>275.57(^{a})</td>
</tr>
<tr>
<td>Heptanal</td>
<td>10.55(^{a})</td>
<td>9.32(^{a})</td>
<td>7.69(^{a})</td>
<td>13.21(^{a})</td>
</tr>
<tr>
<td>Octanal</td>
<td>2.42(^{a})</td>
<td>1.11(^{a})</td>
<td>0.73(^{a})</td>
<td>1.88(^{a})</td>
</tr>
<tr>
<td>Nonanal</td>
<td>5.15(^{a})</td>
<td>5.82(^{a})</td>
<td>5.16(^{a})</td>
<td>5.83(^{a})</td>
</tr>
<tr>
<td>Decanal</td>
<td>2.41(^{b})</td>
<td>2.05(^{b})</td>
<td>3.04(^{ab})</td>
<td>4.01(^{a})</td>
</tr>
<tr>
<td>DMDS</td>
<td>0.03(^{b})</td>
<td>0.05(^{b})</td>
<td>0.01(^{b})</td>
<td>0.15(^{a})</td>
</tr>
<tr>
<td>DMTS</td>
<td>0.03(^{b})</td>
<td>0.05(^{b})</td>
<td>0.02(^{b})</td>
<td>0.09(^{a})</td>
</tr>
<tr>
<td>2-Pentyl furan</td>
<td>14.87(^{b})</td>
<td>17.25(^{b})</td>
<td>9.81(^{c})</td>
<td>26.78(^{a})</td>
</tr>
<tr>
<td>Z-4-Heptenal</td>
<td>0.17(^{a})</td>
<td>0.21(^{a})</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>E,2 Nonenal</td>
<td>0.04(^{a})</td>
<td>0.08(^{a})</td>
<td>0.07(^{a})</td>
<td>0.04(^{a})</td>
</tr>
<tr>
<td>Methional</td>
<td>1.34(^{b})</td>
<td>0.82(^{b})</td>
<td>0.72(^{b})</td>
<td>10.45(^{a})</td>
</tr>
<tr>
<td>3 methyl butanal</td>
<td>12.41(^{a})</td>
<td>8.43(^{b})</td>
<td>7.22(^{b})</td>
<td>8.91(^{a})</td>
</tr>
<tr>
<td>2 methyl butanal</td>
<td>1.98(^{a})</td>
<td>1.72(^{a})</td>
<td>2.11(^{a})</td>
<td>2.12(^{b})</td>
</tr>
<tr>
<td>1-octen-3-one</td>
<td>0.08(^{b})</td>
<td>0.56(^{a})</td>
<td>0.14(^{b})</td>
<td>0.64(^{a})</td>
</tr>
<tr>
<td>Benzaldehyde</td>
<td>0.54(^{a})</td>
<td>0.15(^{a})</td>
<td>0.52(^{a})</td>
<td>0.21(^{a})</td>
</tr>
</tbody>
</table>

\(^{a-c}\)Means in a row not sharing a common superscript are different (p<0.05)

ND = not detected.
Table 4. Mean sensory attributes of spray-dried WPI from Cheddar, Mozzarella, and Cottage cheese, and rennet casein wheys.

<table>
<thead>
<tr>
<th></th>
<th>Aroma Intensity</th>
<th>Sweet Aromatic</th>
<th>Cardboard</th>
<th>Potato Brothy</th>
<th>Astringency</th>
<th>Sour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheddar</td>
<td>1.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>1.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND</td>
</tr>
<tr>
<td>Mozzarella</td>
<td>1.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ND</td>
<td>1.7&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>ND</td>
</tr>
<tr>
<td>Rennet</td>
<td>1.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.0&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ND</td>
<td>1.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ND</td>
</tr>
<tr>
<td>Cottage</td>
<td>1.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ND</td>
<td>1.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.0</td>
<td>3.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.5</td>
</tr>
</tbody>
</table>

<sup>a-c</sup>Means in the same column not sharing a common superscript are different (p<0.05).

<sup>1</sup>Intensities were scored on a 0-15 universal scale, where 0 = none detected and 15 = very high intensity (Meilgaard and others 1999). Dried whey ingredient intensities usually fall between 0 and 4 on this scale (Drake and others 2003; Wright and others 2009).
Table 5. Mean (n=3) overrun (%), air phase fraction (%), foam stability (min), and yield stress (Pa) of foams made from 10% WPI solutions from Cheddar, Mozzarella, and Cottage cheese, and rennet casein wheys.

<table>
<thead>
<tr>
<th></th>
<th>Cheddar</th>
<th>Rennet</th>
<th>Mozzarella</th>
<th>Cottage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overrun (%)</td>
<td>1332(^a)</td>
<td>1308(^a)</td>
<td>1383(^a)</td>
<td>984(^b)</td>
</tr>
<tr>
<td>Air Phase Fraction (%)</td>
<td>92.9(^a)</td>
<td>92.8(^a)</td>
<td>93.1(^a)</td>
<td>90.7(^b)</td>
</tr>
<tr>
<td>Foam Stability (min)</td>
<td>15.9(^b)</td>
<td>19.6(^a)</td>
<td>18.2(^a)</td>
<td>19.1(^a)</td>
</tr>
<tr>
<td>Yield Stress (Pa)</td>
<td>32.6(^b)</td>
<td>33.1(^{ab})</td>
<td>36.5(^a)</td>
<td>35.4(^a)</td>
</tr>
</tbody>
</table>

\(^{a-b}\) Means in the same row not sharing a common superscript are different (p<0.05)
Figure 1: Total aldehyde relative abundance (µg/L) vs. cardboard flavor intensity scores of spray-dried WPI from Cheddar, Mozzarella, and Cottage cheese, and rennet casein wheys.
Figure 2. Mean percentage solubility of whey protein isolate (WPI) from Cheddar, Mozzarella, and Cottage cheese, and rennet casein wheys at pH 4.6 and 10% (wt/vol). a-b Means in the same row not sharing a common superscript are different (p<0.05)
Figure 3. Mean percentage turbidity loss of 10% (wt/vol) solutions of whey protein isolate (WPI) from Cheddar, Mozzarella, and Cottage cheese, and rennet casein wheys at pH 3,4,5,6, and 7.

a-b Means not sharing a common superscript are different (p<0.05)
CHAPTER 5: FLAVOR AND FLAVOR CHEMISTRY OF MILK PROTEINS

Flavor and Flavor Chemistry of Milk Proteins

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Abstract

A greater understanding of the nature and source of dried milk protein ingredient flavor(s) is required to characterize flavor stability and identify the sources of flavors. The objective of this study was to characterize the flavor and flavor chemistry of milk protein concentrates (MPC 70, 80, 85), isolates (MPI), acid and rennet caseins, and micellar casein concentrate (MCC) and to determine the effect of storage on flavor and functionality of milk protein concentrates using instrumental and sensory techniques. Spray dried milk protein ingredients (MPC, MPI, caseins, MCC) were collected in duplicate from 5 commercial suppliers or manufactured at NCSU. Powders were rehydrated and evaluated in duplicate by descriptive sensory analysis. Volatile compounds were extracted by solid phase microextraction (SPME) followed by gas chromatography−mass spectrometry (GC-MS) and gas chromatography−olfactometry (GC-O). Compounds were identified by comparison of retention indices, odor properties, and mass spectra against reference standards. A subset of samples was selected for further analysis using direct solvent extraction (DSE) with solvent assisted flavor extraction (SAFE), and aroma extract dilution analysis (AEDA). External standard curves were created to quantify select volatile compounds. Pilot plant manufactured MPC were stored at 3, 25, and 40°C (44% relative humidity). Solubility, furosine, sensory properties, and volatile compound analyses were performed at 0, 1, 3, 6, and 12 mo. Milk proteins and caseins were diverse in flavor and exhibited sweet aromatic and cooked/milky flavors as well as cardboard, brothy, tortilla, soapy, and fatty flavors. Key aroma active compounds in milk proteins and caseins were 2-aminoacetophenone, nonanal, 1-octen-3-one, dimethyl trisulfide, 2-acetyl-1-pyrroline, heptanal, methional, 1-hexen-3-one, hexanal,
dimethyl disulfide, butanoic acid, and acetic acid. Stored milk proteins developed animal and burnt sugar flavors over time. Solubility of MPC decreased and furosine concentration increased with storage time and temperature. Solubility of MPC80 was reduced more than that of MPC45, but time and temperature adversely affected solubility, with storage temperature having the greatest effect. Flavor and flavor sources of milk proteins provide a foundation of knowledge to improve the flavor and shelf-life of milk proteins.

**Interpretive Summary**

Flavor of emerging dried dairy ingredients such as milk protein concentrate (MPC), micellar casein concentrate (MCC), milk protein isolate (MPI) and caseins is an important factor for success in the market. This study characterized the flavor, flavor chemistry, and shelf life of commercially available milk protein concentrates, isolates, acid and rennet caseins, and micellar casein concentrate (MCC) using instrumental and sensory techniques.

**Introduction**

Milk protein concentrates (MPC) and isolates (MPI) are a relatively new category of dried dairy ingredients. Milk protein concentrate is a complete dairy protein ingredient containing both caseins and whey proteins at a concentration ranging from 40-89% while MPI contains a protein concentration of 90% or greater. Milk protein concentrates and isolates are manufactured by concentrating milk proteins (both whey proteins and caseins) from fluid skim milk by membrane filtration followed by spray drying. Globally, MPC production has quadrupled to 184,000 tons from 2000-2011 (USDEC, 2012). The United States is both the single largest user in the world and the single largest importer, and the
USDEC predicts significant growth through the end of the decade to satisfy rising demand. Micellar casein concentrate (MCC) is a product for which commercial production initiated a few years ago so there is not yet any production data. MCC is produced by first microfiltration of skim milk, followed by ultrafiltration to further concentrate proteins prior to spray drying. In the production of MCC, serum proteins are removed via microfiltration. In production of MPI, filtration or dialysis is used to remove lactose from milk, but both casein and whey proteins are retained in their original proportions found in milk, without combining separately produced casein (caseinate) and whey proteins (ADPI, 2015). The flavor of these ingredients is an important quality parameter as ingredient flavor can influence consumer liking and acceptability of the finished product.

Most of the research conducted on MPC, MCC, or caseins has been focused on functionality and very little research has been conducted on flavor or elucidating the compounds responsible for the characteristic flavors of these proteins. Functional characteristics such as solubility during storage (Le et al., 2011), the effect of NaCl addition during diafiltration on solubility, hydrophobicity, and soluble protein composition (Mao et al., 2012; Sikand et al., 2013), change in protein structure upon oxidation (Dalsgaard et al., 2007), and the influence of the Maillard reaction on functionality (Le et al., 2011; Thomas et al., 2010) have all been studied in recent years, however, the effect of low (45%) and high (80%) protein content, and low temperature storage (3°C) on solubility and flavor have not been investigated.

Rennet and acid casein have been described as having an animal/wet dog (Karagul-Yuceer et al., 2003) or gluey flavor (Rankin, 2009) and characteristic aroma components of
rennet casein were 2-aminoacetophenone, hexanoic acid, indole, guaiacol, and p-cresol (Karagul-Yuceer et al., 2003). The sensory profiles of higher protein MPC (MPC 77, MPC 80, and MPI) were characterized by tortilla, brothy, cardboard, and animal flavors (Drake et al., 2009) and these authors suggested that differences in flavor in MPC with increasing protein content were due to changes in relative abundance of compounds rather than the evolution of new compounds. The flavor of MCC has not been reported, and the flavor and flavor chemistry of milk proteins have not been fully investigated. The objectives of this study were to characterize the flavor and flavor chemistry of milk protein concentrates (MPC 70, 80, 85), isolates (MPI), acid and rennet caseins, and micellar casein concentrate (MCC) (experiment 1), and to determine and compare the effects of storage temperature and time on the sensory and functionality (shelf-life) of low (45%) and high (85%) protein MPC over an extended period (1 y) (experiment 2) using instrumental and sensory techniques.

Methods

Experimental Overview

Two experiments (experiments 1 and 2) were included in this study. The purpose of experiment 1 was to determine the sensory profiles and key aroma active volatile compounds in milk proteins. To carry out this objective, nine commercial milk protein products were collected and one manufactured in the North Carolina State University (NCSU) dairy pilot plant. Products were collected or manufactured in duplicate. Descriptive analysis, headspace extraction and quantification of volatile compounds by gas chromatography-mass spectroscopy (GC-MS) and gas chromatography-olfactometry (GC-O), and solvent assisted
flavor evaporation (SAFE) followed by aroma extract dilution analysis (AEDA) were performed on the dried milk protein products. The purpose of experiment 2 was to determine the flavor and functional properties of milk protein concentrates across storage. To conduct this objective, MPC 45 and 80 were produced in the NCSU dairy pilot plant and stored at constant relative humidity (44%) at 3, 25, or 40°C. Samples were measured after 0, 1, 3, 6 and 12 mo. Descriptive analysis, furosine content, solubility, and flavor volatiles by GC-MS were measured on all samples at each time point.

Sample Collection

Commercial Samples and chemical standards

Nine different products were obtained in duplicate lots from 5 different manufacturers across the United States and Canada (Table 1). Upon arrival, samples were store at -80°C until analysis. Chemical standards were obtained from Sigma Aldrich (Milwaukee, WI) and ChemStep (Montesquieu, France).

Pilot Plant Production of MPC

Raw skim milk was obtained from the North Carolina State University Dairy Research and Education Unit on two different occasions. The milk was high temperature short time (HTST) pasteurized (720 kg/h; hold time 15s at 72C) using a plate heat exchanger (model T4 RGS-16/2, SPX Flow Technology, Greensboro, NC). The milk was the ultrafiltered and diafiltered using a lab scale ultrafiltration unit (Model Lab 46, Filtration Engineering, Champlin, MN) equipped with four spiral wound membranes (Snyder Filtration, Vacaville, CA; nominal cutoff: 10,000 Da; surface area 5.0 m^2). The temperature
for ultrafiltration was 50°C. For the MPC45, the skim milk was ultrafiltered and protein
monitored by Sprint™ Rapid Protein Analyzer (CEM, Matthews, NC) and Smart System 5
moisture/solids analyzer (CEM) until 45% protein (wt/wt) was achieved. Total filtration
time was approximately 25 min. For WPC80, diafiltration water was added at a rate of 40%
of the original weight of the milk. Total filtration time was approximately 3 h. Once the
MPC reached 45 or 80% protein (w/w), the MPC retentate was then spray dried (Model Lab
1, Anhydro Inc., Soeborg, Denmark). Inlet temperature was 200°C and the outlet
temperature was 90°C. MPC80 was then weighed and separated into lots for flavor analysis
or storage. MPC80 used in the flavor study were collected and stored in mylar bags (Uline,
Pleasant Prairie, WI) at -80°C following production. MPC45 and 80 used in the storage
study were weighed, and heat sealed in laminated poly-nylon vacuum pouches (30NV0608,
Elkay Plastics, Austell GA) at a relative humidity of 44%, boxed (to protect from light
exposure), and stored in temperature controlled incubators at predetermined temperatures
(3°C, 25°C, or 40°C).

Headspace Extraction of Volatile Compounds

Gas Chromatography – Mass Spectrometry (GC-MS)

Volatile compounds in caseins, MPC/MPI, and MCC powders were extracted by
solid phase microextraction (SPME) using selective ion monitoring (SIM). Volatile
compounds for SIM were selected from previous literature (Liaw et al., 2011; Carunchia
Whetstine et al., 2003; Karagul-Yuceer et al., 2003; Drake et al., 2009; Campbell et al.,
2013), gas chromatography olfactometry (described below) and by evaluation of
chromatograms collected in scan mode. Compounds were then separated and identified by gas chromatography-mass spectrometry (GC-MS) using a modified method of Liaw et al. (2010). Dried powders were reconstituted at 10 % solids (w/v). All samples contained 10 % (w/v) sodium chloride (Fisher Scientific), and 10 µl internal standard solution (2-methyl-3-heptanone in methanol at 81 mg/kg (Sigma Aldrich) in 20 ml autosampler vials with steel screw tops containing silicone septa faced in Teflon (Microliter Analytical, Suwanee, GA). Samples were injected using a CombiPal autosampler (CTC Analytics, Zwingen, Switzerland) attached to an Agilent 7820A GC with 5975B MSD (Agilent Technologies Inc., Santa Clara, CA). Samples were maintained at 5°C prior to fiber exposure. Samples were equilibrated at 40°C for 25 min before 30 min fiber exposure of a 1 cm DVB/CAR/PDMS fiber (Supelco, Bellefonte, PA) at 31 mm with 4 sec pulsed agitation at 250 rpm. Fibers were injected for 5 min at a depth of 50 mm. Each sample from each lot was evaluated in triplicate.

The GC method used an initial temperature of 40°C for 3 min with a ramp rate of 10°C/min to 250°C held for 5 min. SPME fibers were introduced into the split/splitless injector at 250°C. An Zb-5ms column (Zb-5ms 30 m length × 0.25 mm i.d. × 0.25 µm film thickness; Phenomenex, Torrance, CA) was used for all analyses at a constant flow rate of 1 ml/min. Purge time was set at 1 min. The MS transfer line was maintained at 250°C with the quad at 150°C and source at 250°C. Compounds were identified using the NIST 2005 library of spectra and comparison of spectra of authentic standards injected under identical conditions. Relative abundance for each compound was calculated using the calculated recovery of the internal standard concentration to determine relative concentrations of each
compound. Retention indices were calculated using an alkane series (Sigma Aldrich) (Van den Dool and Kratz, 1963).

**Gas Chromatography – Olfactometry (GC-O)**

Solid-phase microextraction (SPME) followed by gas chromatography-olfactometry (GC-O) for characterization of aroma active compounds was conducted on all caseins, MPC/MPI (time 0 only), and MCC. Twenty mL of each sample (10% solids w/v) along with 10% sodium chloride (wt/wt) was dispersed into six 40-mL amber vials (28 × 98 mm; Supelco Inc.) with a polytetrafluoroethylene (PTFE)/silicone septum (Supelco Inc.) and a stir bar. The vials were heated at 40°C for 20 min with constant stirring. A SPME fiber [divinylbenzene/Carboxen/polydimethylsiloxane (DVB/CAR/PDMS); Supelco Inc.] was exposed in each sample at 1 cm for 1 h. The fiber was then injected on an Agilent 6850 gas chromatography-flame ionization detector (FID) equipped with an olfactometer port (Agilent Technologies Inc.). The GC method used an initial temperature of 40°C for 3 min. The temperature was then increased at a rate of 10°C/min to 150°C, followed by 30°C/min to 200°C, and held for 5 min. Samples were evaluated in duplicate on 2 different columns: polar ZB-WAX (30-m length × 0.25-mm i.d. × 0.25-µm film thickness; Zebron; Phenomenex Inc.) and a nonpolar ZB-5ms (30-m length × 0.25-mm i.d. × 0.25-µm film thickness; Zebron; Phenomenex Inc.). Effluent was split 1:1 between the FID and sniffing port using deactivated fused-silica capillaries (1-m length × 0.25-mm i.d.; Phenomenex Inc.). The FID sniffing port was held at a temperature of 300°C, with a helium carrier gas flow of 1018.6 cm/s, and the port was supplied with humidified air at 30 mL/min. Each sample was evaluated on each
column by at least 2 highly experienced sniffers (each with > 50 h previous experience with
GCO) who recorded aroma character and perceived intensity.

**Solvent Extraction of Volatile Compounds**

**Direct Solvent Extraction**

A representative of subset of milk proteins (n=8; Manufacturer A: Rennet Casein, Acid Casein, Manufacturer B: MPC85, MPI, Manufacturer C: MCC, Manufacturer D: MPC80, Manufacturer E: MPC80, MPC85, duplicate lots) was selected based on sensory and SPME results for further analysis via direct solvent extraction with solvent assisted flavor evaporation (DSE SAFE). Direct solvent extraction (DSE) was conducted on powders following the methods of Evans et al. (2009). DSE was performed in duplicate on each selected sample. 100 g of powder was divided among two 250 mL Teflon bottles with Tefzel™ closures (Nalgene®, Rochester, NY). Thirty mL of water was added to each bottle and was allowed to rehydrate for 30 min. Then 100 mL of diethyl ether (EMD Chemicals Inc., Gibbstown, NJ) was added to the powder/water mixture along with 20 µl of internal standard at 81 mg/kg each (2-methyl-3-heptanone, 2-methyl-pentanoic acid in ether, Sigma Aldrich). The bottles were agitated using a Roto mix (Tyle 50800; Thermolyne, Dubuque, Iowa) on high speed for 30 min. The solvent phase was separated from the mixture by low-speed centrifugation (1450 x g) for 10 min. After centrifugation, the ether phase containing the volatile components was collected into an amber glass jar with a Teflon™ lined lid. Diethyl ether addition, agitation, and centrifugation were repeated three more times each with
50 mL diethyl ether added to each bottle each time. The extracts were then concentrated under a stream of nitrogen gas to 200 mL. Solvent extracts were stored at -20ºC.

**Solvent Assisted Flavor Evaporation (SAFE)**

Solvent extracts were distilled by solvent-assisted flavor evaporation (SAFE) (Ace Glassware, Vineland, NJ). The assembly was similar to the methods of Evans et al. (2009) and Whitson et al. (2011). The glass SAFE head, with the 2 L round bottom flask attached, was connected to two glass traps. The primary trap for receiving the final distilled solvent extract and the secondary trap for waste were both submerged in liquid nitrogen via separate dewar flasks. The round bottom flask was kept at 50ºC using a water bath. Vacuum was reached in the glassware using a rough pump/diffusion pump combination. The solvent extract was poured into the top of the SAFE head and introduced into the vacuum system drop-wise until all sample was introduced. Distillation was carried out for 10 min under vacuum (1.33 KPa) and kept at 50ºC with a circulating water bath. After distillation, the sample was concentrated under a stream of nitrogen gas to 20 mL and transferred to a 50 mL screw top glass tube for phase separation.

**Phase Separation**

To separate solution into neutral/basic and acid fractions, the concentrated distillate was first washed twice with 3 mL sodium bicarbonate (0.5M; Fisher Scientific) and mixed vigorously. The concentrate was washed a total of three times and after each wash, the bottom layer (water phase) was placed into another glass tube. The resulting concentrate was the neutral/basic fraction and was dried over anhydrous sodium sulfate (VWR International)
to remove any residual water. The neutral/basic fraction was then concentrated to 0.5 mL under a stream of nitrogen gas. The water phase was acidified by adding hydrochloric acid (18% w/v; Sigma Aldrich) until a pH of 2-2.5 was reached. Acidic volatiles were then extracted by the addition of 5 mL diethyl ether, mixing vigorously, and removing the top ether phase. This process was repeated 2 more times. The acidic volatiles were then dried over anhydrous sodium sulfate and concentrated to 0.5 mL under a stream of nitrogen gas.

**Volatile Analysis of Solvent Extracts**

*Gas Chromatography – Mass Spectrometry (GC-MS)*

Selected volatile compounds in solvent extraction fractions were detected using both scan and selective ion monitoring (SIM) simultaneously. Analysis was conducted on an Agilent 7890B with a 5977A mass selector detection (Agilent Technologies Inc.). Separations were performed on a fused silica capillary column Zb-5ms 30 m length × 0.25 mm i.d. × 0.25 µm film thickness; Phenomenex Inc.). Helium was utilized as the carrier gas at a constant flow of 1.5 mL/min. The GC-MS oven program started with an initial oven temperature of 40°C for 3 min, increasing to 90°C at a rate of 10°C per min, then increasing 5°C per min to 200°C, then 20°C per min to 250°C hold for 5 min and a final hold time of 10 min. MSD conditions included: capillary direct interface temperature, 280°C; source temperature 230°C, quad temperature 150°C. The injector temperature was 250°C.

One microliter of each solvent extract was injected in duplicate in the pulsed splitless (25 psi air for 0.5 min) mode. A 3.75 min solvent delay was programmed into the MS
acquisition parameters for solvent injections. Retention indices were calculated using an alkane series (Sigma Aldrich) (Van den Dool and Kratz, 1963).

Gas Chromatography – Olfactometry (GC-O) with Aroma Extract Dilution Analysis (AEDA)

One microliter of solvent extract was injected into the GC injection port, maintained at 250°C, fitted with a direct injection liner (Supelco; Sigma Aldrich). Column conditions and GC-O parameters were identical to those described previously for SPME. Both neutral/basic and acidic fractions from solvent extracts were analyzed in duplicate on both columns. Post peak intensity of aroma active compounds were evaluated and recorded by two experienced sniffers, each with > 100 h experience with GC-O of dairy proteins.

AEDA was conducted on solvent extracts under similar conditions as those used for post peak intensity GC-O. A step wise dilution of 1:3 (v/v) with diethyl ether was followed until two experienced sniffers detected no odorants. The neutral/basic fractions were injected onto the non-polar Zb-5ms capillary column and acid fractions were injected onto the polar ZB-WAX capillary column. The highest dilution, for which an aroma was still detected, was reported as the flavor dilution factor (Grosch, 1993).

Compound Identification and Quantitation

Mass spectra, retention index, and aroma properties of authentic standards injected under identical conditions on the GC-O and GC-MS were used for aroma active compound identification. Nineteen compounds, 9 of which were fatty acids, were selected for quantification using external standard curves. These compounds were selected due to their high Log3 FD values, aroma character, and previous literature (Drake et al., 2009; Karagul-
Yuceer et al., 2003). Due to retention times and the mandatory solvent delay on the GCMS after SAFE, 3 of these compounds (hexanal, 1-hexen-3-one, and DMDS) were quantified via SPME. The remaining 16 compounds (2-acetylpyroline, 1-octen-3-one, 2-aminoacetophenone, heptanal, nonanal, DMTS, methional, and butyric, pentanoic, hexanoic, heptanoic, octanoic, nonanoic, decanoic, dodecanoic, and tetradecanoic acids) were quantified by SAFE. Quantification of selected volatile compounds in SAFE extracts and SPME vials was executed using five point external standard curves ranging from 0.50 μg/kg to 2 mg/kg (depending on selected compound) and integrated to an internal standard (81 mg/kg 2-methyl-3-heptanone, 2-methyl-pentanoic acid in ether (SAFE extracts) or water (SPME)). For SAFE extracts: external standard curves were prepared by adding selected compounds to 30 mL water which was then added to 50 g of powder in a 250 mL Teflon bottles with Tefzel™ closures (Nalgene®, Rochester, NY) and held at 21°C for 30 min. Direct solvent extraction, solvent assisted flavor evaporation, and phase separation proceeded as previously described. Extracts were injected onto the GC-MS using previously described parameters. For SPME, the selected compounds were diluted in water and then added to the milk powder, prepared as described previously. Relative abundance of other selected compounds was calculated relative to internal standard recovery.

**Descriptive Analysis**

Descriptive sensory analysis was conducted on MPC/MPI, MCC, and caseins using a trained descriptive sensory panel and an established dairy flavor language (Drake et al., 2003, 2009). Panelists (n =8) each had more than 50 h of training and 150 h of previous experience with the sensory analysis of dried dairy ingredients using the Spectrum™ descriptive analysis
method (Meilgaard et al., 2007). All sensory testing was conducted in accordance with NCSU Institutional Review Board for Human Subjects guidelines.

Powders were rehydrated to 10% solids (w/v) with deionized water and dispensed into three-digit-coded 60 mL lidded cups for evaluation (Solo Cup Company, Champaign, IL). Preparations were conducted with overhead lights off to avoid exposure to light. Samples were evaluated by each panelist in duplicate. Sensory data were collected using Compusense™ five, release 4.8 (Compusense, Guelph, Canada).

**Solubility Analysis**

Solubility analysis was performed on MPC45 and MPC85 produced in the NCSU pilot plant at all time points. Solutions of 10% protein (wt/vol) were made by rehydrating the MPC to 80% of the total volume desired, for 6 h at room temperature on a stir plate set to 300 rpm (Campbell et al., 2011). Solutions were then refrigerated overnight at 4°C. The solutions were then brought back up to 25°C and the pH was adjusted using 1 N NaOH to a pH of 7 and brought to a total volume of 100 mL with deionized water, resulting in 10% (wt/vol) solids solutions. Solubility using the micro bicinchoninic acid (micro-BCA) assay were measured on the uncentrifuged solutions. Solutions were then centrifuged at 16,500 x g for 10 min using a model RC5B centrifuge (Thermo Scientific) and the supernatants were measured for solubility. Solubility measurements were recorded before and after centrifugation. Solubility was measured by the micro-BCA assay using a kit from Thermo Fisher Scientific/Pierce (Rockford, IL). Protein solutions before and after centrifugation were diluted 1:100 in deionized water. Solutions were added to working reagent in a ratio of 1:8 and pipetted in triplicate into a 96-well plastic plate. Deionized water with working
solution was used as a reference blank. The plate was put on a shaker for 30 s and incubated at 37°C for 30 min. The plate was brought to room temperature and read on a Tecan Safire plate reader spectrophotometer at wavelength 562 nm (Tecan, Durham, NC). Analysis was performed in quadruplicate. Solubility was calculated using the following equation (where ABS = absorbance):

\[
\text{Protein solubility} = 100 - \left\{ \frac{(\text{Abs}_{\text{before}} - \text{Abs}_{\text{after}})}{\text{Abs}_{\text{before}}} \right\} \times 100
\]

Furosine analysis

Furosine analysis, adapted from Baptista and Carvalho (2004), was performed on MPC45 and MPC85 at each time point. Approximately 30 mg of MPC was placed into a screw-cap (PTFE faced septa) glass tube (VWR). Water (HPLC grade, 2.5 mL, EMD Chemicals) was added and vortexed. Two milliliters of 8N HCl (EMD Chemicals) was added and mixed well. The solution was placed into an oven at 110°C and incubated for 24 h. A 400 µl aliquot was placed into a glass tube and the HCl was blown down with nitrogen until completely dry. Two milliliters of HPLC water was added and mixed. The solution was then filtered through a 0.45µg syringe filter into an HPLC vial (Phenomenex). Furosine was quantified by HPLC (Breeze HPLC, Waters, Milford, MA) using a mobile phase of 0.06M sodium acetate, pH 4.3 with acetic acid at a flow rate 1 mL/min pumped through a binary pump (Waters 1525, Waters, Milford, MA). Twenty microliters of sample was injected (Waters 2707 autosampler) onto the column (Lunca 5u C8 100A 250x4.6mm) at 35°C. Sample was analyzed using a photodiode array detector (Waters 2998) with a maxima of 280 nm. Sample run time was 10 min. All MPC were extracted and evaluated in
triplicate. Furosine increase was determined by calculating the percent increase of furosine concentration in the stored MPC to the furosine concentration of the time 0 MPC.

**Statistical Analysis**

Data in experiment 1 was analyzed by a one-way analysis of variance (ANOVA) using a general linear model with Fisher’s least significant difference for means separation (XLSTAT, version 2015.1.01; Addinsoft Inc., New York, NY). All data in experiment 2 was analyzed by 3-way ANOVA (protein x temperature x storage) with Tukey’s HSD for means separation (XLSTAT, version 2015.1.01; Addinsoft Inc., New York, NY).

**Results and Discussion**

**Experiment 1**

*Instrumental volatile and descriptive analysis*

Forty two odor active events were detected in dried milk proteins by SPME GC-O. Of the 42, 30 were identified by aroma, retention index, and mass spectra, and 12 were tentatively identified by retention index on ZB5 or wax column and odor quality (Table 2). Predominant odorants of the MPC, MCC, and casein samples were also identified by solvent extraction with aroma extract dilution analysis (AEDA). Forty three odor active events were detected in dried milk proteins by solvent extraction with AEDA. Twenty two neutral/basic and acid odorants were identified by retention index, aroma, and mass spectra, with 16 tentatively identified by retention index and odor quality, and 5 unknowns (Table 3). All of the odor active compounds listed in Tables 2 and 3 were previously reported as odor-active
components of milk, cheese, casein, skim milk powder (SMP), fluid whey, whey powder, whey protein concentrates, or whey protein hydrolysates (Brewington et al., 1974; Ferretti and Flanagan, 1971; Hammond and Hill, 1994; Preininger and Grosch, 1994; Shiratsuchi et al., 1994a,b; Karagul-Yuceer et al. 2001, 2002, 2003; Noriaki et al., 2008; Mahajan et al., 2004; Carunchia Whetstine et al., 2005; Leksrisompong et al., 2010).

Solvent extraction and AEDA of milk proteins and caseins detected many compounds not detected by SPME headspace extraction. These volatile compounds included 2-methyl-1-propanol, heptanal, 1-octen-3-one, E,E 2,4 heptadienal, 2-acetyl thiazole, benzyl alcohol, gamma terpinene, 2-ethyl-3,5-dimethylpyrazine, sotolon, homofuraneol, 2,4-dimethylphenol, 4-ethylphenol, decanal, E,Z-2,4-nonadienal, benzothiazole, acetic acid, beta ionone, propionic acid, 5-methyl furfural, butanoic, pentanoic, hexanoic, and decanoic acids, and 1-octadecanol. Similarly, SPME headspace extraction detected many compounds not detected by solvent extraction (SAFE) and AEDA of milk proteins and caseins. These volatile compounds included dimethyl sulfide, diacetyl, methyl ethyl sulfide, 2 and 3 methyl butanal, ethyl-2-methylpropanoate, 1-hexanol, furfuryl alcohol, 2-heptanone, dimethyl sulfone, 1-octen-3-ol, maltol, 3-nonenal, 2,6 –dimethylphenol, Z-2-nonenal, E,Z-2,6-nonadienal, E-2-nonenal, 2,3-dimethylphenol, 2,4,6-trimethylphenol, E,E-2,4-nonadienal, E-2-decenal, eugenol, E-2-undecenal, E,Z-2,4-decadienal, E-2-dodecenal, δ-decalactone, α-curcumene, and δ-undecalactone. No volatile extraction approach is unbiased. Headspace extraction generally recovers more highly volatile compounds while solvent extraction tends to favor higher molecular weight compounds. For these reasons, both extraction approaches were applied in the current study.
The greatest number of odorants (neutral/basic and acid) were identified in acid and rennet caseins. Acidic odorants consisted mainly of short-chain volatile acids (Table 3). On the basis of log$_3$FD (>2) factors, the most potent odorants in rennet casein were 1-hexen-3-one, heptanal, methional, 2-acetyl-1-pyrroline, dimethyl trisulfide, 1-octen-3-one, octanal, 2-methoxyphenol, benzothiazole, acetic, propionic, butanoic, and pentanoic acids, and 1-octadecanol. In acid casein, compounds with high log$_3$FD factors included hexanal, homofuraneol, methional, 1-octen-3-one, and butanoic acid. The compounds with the highest log$_3$FD factors in milk proteins (MPC 70,80, 85, MCC, and MPI) were dimethyl disulfide, hexanal, 1-hexen-3-one, sotolon, homofuraneol, 4-hexen-3-one, heptanal, methional, 1-cyclohexen-3-one, 2-acetyl-1-pyrroline, dimethyl trisulfide, 1-octen-3-one, octanal, guaiacol, nonanal, decanal, benzothiazole, 2-aminoacetophenone, gamma-decalactone, acetic, propionic, butanoic, pentanoic, and hexanoic acids, and delta-dodecalactone.

Among the MPC, MPI, and MCC, the MPC had greatest number of neutral/basic and acid odorants identified with high (>2) log$_3$FD factors. Volatile compounds with the highest log$_3$FD factors (>3.5) in MPC were dimethyl disulfide, hexanal, 2-acetyl-pyrroline, 1-octen-3-one, acetic acid, 2-methoxy phenol (guaiacol), nonanal, methional, 2-methyl-1-propanal, and butanoic acid. Aroma active compounds with high log$_3$FD factors (>2) in MPC which were not high in MPI or MCC were dimethyl disulfide, hexanal, 1-hexen-3-one, heptanal, 1-octen-3-one, octanal, homofuraneol, decanal, and pentanoic acid. Aroma active compounds with high log$_3$FD factors in MPI which were not in MPC or MCC were dimethyl trisulfide, 2-aminoacetophenone, propionic acid, and delta-dodecalactone. MCC had relatively few
aroma active compounds with high log$_3$FD factors, and only β-ionone had a high log$_3$FD factor in MCC and not in MPC or MPI.

Sensory profiles of the casein, MPC, and MCC samples varied (Table 5). Tortilla/corn chip and cardboard flavors were predominant in all samples, with the exception of acid casein, which was highest in tortilla/corn chip flavor followed by brothy/animal flavor. Only acid and rennet caseins exhibited animal flavor. Tortilla/corn chip flavor has previously been identified as a primary flavor of rennet casein and MPC 80/MPI by descriptive analysis (Karagul-Yuceer et al., 2003; Drake et al., 2009). The flavor of caseins has been characterized by a variety of descriptors including animal/wet dog, cooked, potato/brothy, sulfur, glue, tortilla, and cardboard (Karagul-Yuceer et al., 2003; Drake et al., 2009). Karagul-Yuceer et al. (2003) identified the most potent odorant in rennet casein (based on log$_3$FD factors) as 2-aminoacetophenone which contributed a tortilla and grapelike odor. This compound was previously reported to contribute to a glue/burnt feathers flavor in stored casein (Ramshaw and Dunstone, 1969), stored dry milk (Parks et al., 1964), and sterilized concentrated milk (Arnold et al., 1966). Despite its high aroma activity, Karagul-Yuceer et al. (2003) concluded that the typical odor of rennet casein was primarily caused by hexanoic acid, indole, guaiacol, and p-cresol based upon odor activity values (OAV) and sensory analysis of model mixtures of flavor compounds. In the current study, the significance of 2-aminoacetophenone is consistent with past studies.

Milk proteins (MPC and MPI) have been characterized by descriptors including cooked/milky, sweet aromatic, tortilla, potato/brothy, cardboard, and animal (Drake et al., 2009). Drake et al. (2009) also reported that MPC with lower protein content (56% protein
by dry weight) were characterized by fluid milk types of flavor (cooked/milky, sweet aromatic, sweet taste and cereal, and as protein content increased, the flavor profiles changed and MPC70, MPC80, and MPI were characterized by tortilla, brothy, cardboard and animal flavors and higher astringency. The current study characterized milk proteins using the same descriptors, and the MPC 70 and 80 were characterized by low but distinct intensity of tortilla and cardboard flavor, cooked/milky and sweet aromatic flavors (Table 5). It is likely that as protein level decreases and MPC composition becomes more similar to that of skim milk powder, sensory profile differences become more evident. This effect was observed between the MPC 45 and MPC 80 in experiment 2.

Seven odor active volatile compounds (2-acetylpyrroline, 1-octen-3-one, 2-aminoacetophenone, nonanal, heptanal, DMTS, and methional) from the neutral/basic portion of SAFE extracts were quantified by standard curves. Nine volatile fatty acids (butyric, pentanoic, hexanoic, heptanoic, octanoic, nonanoic, decanoic, dodecanoic, and tetradecanoic) from the acid portion of the SAFE extracts were quantified by standard curves. Three other odor active volatile compounds (hexanal, 1-hexen-3-one, and DMDS) were quantified via SPME. Relative abundance of 15 other volatile compounds was determined by SPME headspace GC-MS (Table 4). Sensory profiles of milk proteins and caseins were generally consistent with volatile compounds concentrations and relative abundance (Tables 4 and 5). A correlation between concentration of 2-aminoacetophenone and tortilla/corn chip flavor was observed (Figure 1, \( r^2 = 0.87 \), \( p<0.05 \)). The importance of 2-aminoacetophenone to tortilla/corn chip flavor is well established in both corn tortillas and taco shells (Karahadian and Johnson, 1993; Buttery et al., 1994; Buttery and Ling, 1995, 1998) and
casein (Badings, 1991; Karagul-Yuceer et al., 2003). The current study confirms the importance of 2-aminoacetophenone to the tortilla flavor of dried milk proteins. However, tortilla flavor in milk proteins may also be caused by a number of volatile compounds and not just 2-aminoacetophenone based on previous studies (Karagul Yuceer et al., 2003) and on aroma active compounds detected in the current study. Volatile compounds with the highest probability to contribute to the aroma and flavor of corn tortillas, corn tortilla chips, and taco shells were E,E and E,Z 2,4 decadienal, acetic acid, 3-methylbutanal, 2-furfurylthiol, 2-acetyl-1-pyrroline, 2-acetyltetrahydropyridine, 6-acetyltetrahydropyridine, 2-aminoacetophenone, 4-vinylguaiacol, E-2-nonenal, 2-methylbutanal, 2-propionyl-1-pyrroline, E-2-decenal, methional, β-ionone, hexanal, 1-octen-3-ol, 4-vinylphenol, E-2-heptenal, α-ionone, and 2-ethyl-3,5-dimethylpyrazine (Buttery and Ling, 1995, 1998). Many of the odor active compounds in corn tortillas, chips, and taco shells have also been identified in the milk protein products of the current study, including E,E and E,Z 2,4 decadienal, acetic acid, 3-methylbutanal, 2-acetyl-1-pyrroline, 2-aminoacetophenone, E-2-nonenal, 2-methylbutanal, E-2-decenal, methional, β-ionone, hexanal, 1-octen-3-ol, and 2-ethyl-3,5-dimethylpyrazine (Tables 2,3). This result is consistent with the results of Karagul-Yuceer et al. (2003) which concluded that while 2-aminoacetophenone was important to the flavor of rennet casein, it was not the only flavor component.

Acid casein was characterized by high aroma intensity, sour aromatic, cardboard, animal flavor, and tortilla/corn chip flavors. Acid casein also had high concentrations of 2-aminoacetophenone, E-2-heptenal, and E-2-nonenal. Rennet casein was also characterized by high aroma intensity, cardboard, and animal flavors, but was high in phenylacetaldehyde,
sotolon, E-2-heptenal, 2,4 decadienal, 1-hexen-3-one, hexanal, DMDS, DMTS, and 2-pentyl furan. Despite similar sensory profiles, compound concentrations and relative abundance were highly variable likely due to distinct differences in manufacture and processing. It is interesting to note that hexanoic acid was identified as an important flavor contributor to rennet casein in past studies (Karagul Yuceer et al., 2003) but hexanoic acid was not a factor in the flavor of rennet casein in the current study. This result is consistent with the SAFE GCO data, which also lacked a hexanoic acid aroma event. The effects of raw materials, processing and storage all impact flavor and flavor chemistry of dairy ingredients. It is likely this discrepancy is due to one or a number of those factors. Volatile compound differences were also observed among the MPC80 and MPI from different suppliers. For instance, MPI-B and MPC80-E were characterized by fatty flavor and were also high in many lipid oxidation products that contribute to fatty flavor. The MPI-B was high in undecanal, E,Z 2,6 nonadienal, nonanoic acid, and 2-pentyl furan,. The MPC80-E was high in phenylacetaldehyde, heptanal, 2-pentylfuran, decanal and butyric, and hexanoic acids. The MPC80-D, MPC80-E, and MPC85-E were characterized by a soapy flavor and were also high in different volatile compounds. The MPC85-B and MPI-B had potato/brothy flavor and had high levels of methional, a source of potato flavor in dairy products (Lekrisompong et al., 2010), although all milk proteins contained some concentration of methional and didn’t necessarily display potato/brothy flavor. Flavor of the milk proteins and caseins were likely caused by a wide variety of compounds and are likely dependent on volatile compound concentrations and interactions with other aroma active compounds.
Many of the volatile compounds related to flavor in milk proteins are either formed by lipid oxidation or Maillard browning. The production of 2-aminoacetophenone in corn tortilla/chip products occurs when corn is heated and exposed to an alkali (Buttery and Ling, 1998), but past studies have also suggested that alkaline conditions catalyzed air oxidation of the amino acid tryptophan to the compound kynurenine, which is then degraded to 2-aminoacetophenone (Tabone et al., 1951). It is possible that in milk protein powders, 2-aminoacetophenone is formed by the oxidation of tryptophan in solution. However, this seems unlikely due to the lack of a strong oxidative step during the manufacture of milk proteins necessary to catalyze the oxidation of tryptophan. It also seems unlikely due to the lack of 2-aminoacetophenone content, and tortilla off-flavor in whey protein concentrates, which often undergo oxidative bleaching during processing (Croissant et al., 2009; Listiyani et al., 2011; Jervis et al., 2012; Fox et al., 2013). The tryptophan content of β-lactoglobulin and α-lactalbumin (whey proteins) is much higher than that of α and β-caseins (Spies, 1967), which would suggest that if the alkali breakdown of tryptophan was the primary source of 2-aminoacetophenone in milk proteins, it would most certainly also be a major flavor component in whey protein concentrates. Past studies (Parks et al., 1964; Spacek, 1954) have demonstrated that alkaline degradation of kynurenine results in a >90% yield of 2-aminoacetophenone, while the yield of 2-aminoacetophenone from tryptophan by alkaline degradation is less than 0.1%. Parks et al. (1964) also determined that kynurenine occurred naturally in bovine milk. It is possible that the 2-aminoacetophenone concentration in milk and milk protein powders is due to the free kynurenine in milk which may be broken down
by non-alkaline means or remains in the cheese during the cheese-make procedure of liquid whey manufacture preventing its presence in whey protein.

Alkanals and alkenals, such as decadienal, methylbutanal, nonenal, decenal and hexanal (all flavor compounds which are found in both corn tortilla/chip products and milk proteins) are typical products of lipid oxidation. Increased lipid oxidation products over storage is expected and has been demonstrated in pasteurized fluid milk, skim milk powder, whole milk powder, and dried whey protein ingredients (Karatapanis et al., 2006; Lloyd et al., 2009; Karagul Yuceer et al., 2002; Wright et al., 2009). The antioxidative mechanisms of unsaturated fatty acids in the milk lead to the formation of many aldehydes (Loury, 1971; Paquette et al., 1985). They have low flavor thresholds and are commonly characterized by oily, fatty, or tallow odor (Azzara and Campbell, 1992). Whitson et al. (2010) demonstrated that a combination of lipid oxidation products and DMTS caused cardboard flavor in whey proteins. In certain concentrations and combinations with 2-aminoacetophenone, some or all of these compounds may also contribute to tortilla flavor in milk proteins. The alcohol, 1-octen-3-ol was important to both the flavor of the milk proteins in this study and corn tortillas and corn chips (Buttery and Ling, 1995, 1998). The compound 1-octen-3-ol is characterized by a mushroom/metallic flavor and it has been proposed that it is the autoxidation product of linoleic acid (Stark and Forss, 1964). Linoleic acid occurs naturally in milk and is dependent on the diet of the cow (Dhiman et al., 1999).

The compound 2-acetyl-1-pyrrrole is characterized by a popcorn aroma and is a result of the reaction between the amino acids proline or ornithine and the reactive sugar-degradation product 2-oxopropanal (Schieberle, 1995). The compound 2-acetyl-1-pyrrrole is
commonly found in many fresh dairy ingredients such as skim milk powder (Karagul Yuceer et al., 2001, 2002) and fluid and dried whey powder (Karagul Yuceer et al., 2003; Mahajan et al., 2004) and contributes a popcorn and cooked flavor. This compound is attributed to sweet aromatic and cooked /milky flavors in milk proteins.

Methional has an aroma similar to boiled potatoes. Methional formation is a result of Strecker degradation of methionine (Tressl et al., 1989) or is a result of methionine breakdown when milk is exposed to light (Badings, 1991). It is also a common flavor-active component of dairy protein powders including liquid whey (Karagul Yuceer et al., 2003), WPC and WPI (Carunchia Whetstine et al., 2005), milk powders (Karagul Yuceer et al., 2001), and rennet casein (Karagul Yuceer, 2003). The compound 2-ethyl-3,5-dimethylpyrazine is also a Maillard browning product, specifically produced by Strecker degradation (Dawes and Edwards, 1966; Manley and Fagerson, 1970) and has a roasted earthy aroma (Grosch and Schieberle, 1997). Products formed from Strecker degradation are heat-induced and would likely have higher concentrations in products with high heat treatments.

It is interesting to note the similarities and differences between the volatile compounds and sensory properties of milk and whey protein concentrates. The sensory language for each is relatively similar, with a few exceptions. The milk proteins in this study were characterized by sweet aromatic, cooked milky, sour aromatic, cardboard, potato/brothy, animal, tortilla/corn chip, soapy, fatty, astringency, and sour taste (Table 5). Whey proteins are characterized by sweet aromatic, sour aromatic, cooked milky, doughy, fatty, metallic, cucumber, grassy, cardboard, potato brothly, cabbage, raisin, animal, cereal,
soapy, and astringency (Russell et al., 2006; Carunchia Whetstine et al., 2003; Liaw et al., 2011; Drake et al., 2009; Wright et al., 2009). In this study, the only sensory characteristic which differentiated milk protein concentrates and caseins from the whey protein concentrates was the inclusion of tortilla/corn chip flavor. Volatile compound profiles were also very similar. Many of the same odor active compounds in milk proteins were also odor active in studies on WPC and WPI. Acetic acid, dimethyl disulfide, hexanal, butanoic acid, methional, 2-acetyl-1-pyrroline, dimethyl trisulfide, 1-octen-3-one, octanal, hexanoic acid, nonanal, E,Z-2,6-nonadienal, E-2 nonenal, decanal, 2-aminoacetophenone, E,E-2,4-decadial, delta-decalactone, delta-dodecalactone, pentanoic acid, and decanoic acid are all compounds found in WPC and WPI (Carunchia Whetstine et al., 2005) and the milk proteins of the current study.

**Experiment 2**

*Furosine Analysis*

As expected, furosine levels increased in MPC 45 and 80 over time (Figure 2). Protein level, storage time and storage temperature had an effect on furosine production (p<0.05). The MPC 45 at 3°C demonstrated a 31% furosine increase over 12 mo, while the MPC 45 at 25°C and 40°C demonstrated a 650 and 980% increase respectively (Figure 2). The MPC 80 at 3°C demonstrated an 82% furosine increase over 12 mo, while the MPC 45 at 25°C and 40°C demonstrated a 510 and 820% increase respectively (Figure 2). Storage temperature had a larger effect on furosine concentration than protein level.
Furosine is a commonly used measure of protein-bound Amadori products and therefore measures the extent of the Maillard reaction in dairy proteins (Erbersdobler, 1986). Reduction of solubility in milk proteins could be due to covalent cross-linking of the proteins, hydrophobic or hydrogen bonds (Anema et al., 2006; Havea et al., 2006). The Maillard reaction may not be the primary cause of solubility loss in milk proteins, but Maillard browning products such as glyoxal or methylglyoxal can react with lysine or arginine, causing cross-linking of proteins and subsequent loss of solubility (Biemel et al., 2001; Lederer and Klaiber, 1999). The Maillard reaction is also responsible for the formation of many volatile flavor compounds including pyrroles, furans, Strecker aldehydes, and pyrazines (van Boekel, 2006). Many of the important volatile compounds in this study are Maillard reaction compounds, including 2-acetyl-1-pyroline, methional, 2-ethyl-3,5-dimethylpyrazine, dimethyl disulfide, and dimethyl trisulfide. Maillard reactions play an important role in the shelf-life of milk proteins, and furosine is a useful measurement to compare the degree of Maillard browning which has taken place. The MPC 45 and 80 stored at 40°C became noticeably more brown at 3 mo whereas it was 6 mo before both MPC 45 and 80 stored at 25°C became noticeably brown. This visual change corresponded to an increase in furosine content of approximately 400-500% (Figure 2).

Solubility

Temperature, storage time and protein level had an effect on solubility of MPC powders (p<0.05) (Figure 3). The solubility of MPC 45 stored at 3°C and 25°C was reduced by 9% and 15% respectively over 12 mo while the solubility of MPC 45 stored at 40°C decreased by approximately 28% (Figure 3). Temperature had a more pronounced effect on
the solubility of MPC 80 than time. The solubility of MPC 80 stored at 3°C was reduced by approximately 15% after 12 mo, while the solubility of MPC 80 stored at 25°C was reduced by 51% and at 40°C, solubility was reduced by 88%.

The solubility of MPC has been extensively studied (Jimenez-Flores and Kosikowski, 1986; Schuck et al., 1994, 2007; Anema et al., 2006; Havea 2006; Singh, 2006; Baldwin and Truong, 2007, Lee et al., 2011) Interestingly, while past studies agree that solubility of stored MPC is an issue, the solubility of MPC during storage is highly variable and dependent on many factors. For instance, Havea et al. (2006) produced 6 MPC 85 powders, one of which had a solubility of 53% after 2 days, while another, which had had 30% of its calcium replaced by sodium ions, had a solubility of 98% after 7 weeks of storage at 20°C. Anema et al. (2006) tested the solubility of MPC 85 at temperatures ranging from 20°C-50°C of which, after 60 days of storage, the 20°C treatment retained 100% solubility, while the 50°C treatment dropped to approximately 20% solubility after 10 days. Le et al. (2011) studied the effect of relative humidity (RH) and temperature on MPC 80 solubility and demonstrated a 50% drop in solubility of MPC 85 stored at 25°C with a RH of 44% (the RH used in this study) after 12 weeks. The solubility of MPC 80 stored at 40°C with a RH of 44% decreased to approximately 20% after 4 weeks. While the variables in these previous studies vary, the current study solubility results are consistent with past studies, especially Le et al. (2011), whose storage conditions matched those of the current study (except for the lack of a 3°C storage treatment). Ultimately, the purpose of experiment 2 was to determine the shelf life of MPC 80 stored under specific conditions, and while flavor is of utmost importance to the shelf life of any food product, usability, specifically solubility in the case
of MPC 80, must also be taken into account. These results confirm that temperature abuse during storage for a short period of time renders MPC virtually unusable in liquid suspensions. Solubility and furosine content are inversely correlated ($r^2 = 0.84-0.97$), which suggests that Maillard browning products are reacting with amino acids, causing cross-linking of proteins and subsequent solubility loss.

*Instrumental volatile and descriptive analysis*

Storage time, temperature, and protein content also impacted volatile compounds and flavor of MPC powders. Aldehydes (hexanal, heptanal, octanal, nonanal, methional, 2 and 3-methylbutanal, E,E 2,4 decadienal) and other lipid oxidation products such as 2-pentylfuran and 1-octen-3-one and sulfur containing volatile compounds (dimethyl sulfide, dimethyl disulfide, dimethyl trisulfide) increased with time and as storage temperature increased in both MPC 45 and MPC 80 (Table 6).

Generally, MPC 45 stored at lower temperatures for less time were associated with sweet aromatic and cooked milky flavors and a sweet taste. As the powders aged, aroma intensity, cardboard, animal, and tortilla/corn chip flavors increased. The MPC 45 stored at 25°C exhibited a burnt sugar flavor after 6 mo, while MPC 45 stored at 40°C exhibited this after only 1 mo of storage. No burnt sugar flavor was detected in MPC 45 stored at 3°C. The MPC 45 stored at 25°C and 40°C exhibited an animal flavor after 12 mo, while this flavor was not detected in product stored at 3°C (Table 7). Since burnt sugar flavor followed the trend of Maillard reactions (furosine), it seems likely that this flavor can be attributed to Maillard reaction products such as sotolon or furaneol. These compounds are formed by thermal treatment of sugars and have distinct burnt sugary aromas, and have been previously
reported in stored skim milk powder (Karagul Yuceer, 2002; Drake et al., 2006), sweet whey powder (Mahajan et al., 2004), and whey protein (Carunchia Whetstine et al, 2005) extracted by solvent extraction. These compounds were also odor active in fresh MPC80 in experiment 1 by solvent extraction, but not by SPME, so they likely were not well-extracted by SPME. Additional work would be needed to confirm this hypothesis. Cardboard flavor in whey protein concentrates has been sourced to pentanal, heptanal, and nonanal in the presence of DMTS and 1-octen-3-one, and lipid oxidation compound increases are the likely source of cardboard flavor in the stored milk proteins (Whitson et al., 2010).

Similar to MPC 45, MPC 80 stored at lower temperatures for less time were associated with sweet aromatic and cooked milky flavors. As the powders aged, overall aroma intensity, cardboard, animal, cabbage, and tortilla/corn chip flavors increased. This result is consistent with an increase in the lipid oxidation and protein degradation volatile compounds which cause these off-flavors. Cabbage flavor in whey protein isolate has been sourced to DMTS (Wright et al., 2006). Compounds attributed to cardboard and cabbage flavor increased during storage (pentanal, heptanal, nonanal, 1-octen-3-one, and DMTS, Table 6). The compounds, E,E 2,4 decadienal, 3-methylbutanal, methional, and hexanal, possible sources of corn chip, fatty and potato flavors, increased during storage in MPC 80 and 45. The MPC 80 stored at 3°C exhibited an animal flavor after 12 mo, while MPC 80 stored at 25°C and 40°C exhibited this flavor after 6 and 1 mo respectively. The MPC 80 stored at 25°C exhibited a cabbage flavor after 6 mo, but burnt sugar flavor was not detected. The MPC 80 stored at 40°C exhibited cabbage flavor after 3 mo and burnt sugar flavor after 1 mo. These two flavors were not detected in MPC 80 stored at 3°C. Unlike the MPC 45,
MPC 80 stored at all three temperatures exhibited the tortilla/corn chip flavor immediately after production rather than following storage.

The principle component biplots generated from sensory analysis and relative abundance tables can be used to approximate similarities in the stage of shelf-life of the MPC 45 and 80 powders by the increase in animal, tortilla/corn chip, cardboard, cabbage, and aroma intensity and increase in corresponding lipid oxidation and heat-induced volatile compound concentration over time (Figures 4, 5, 6, 7). For instance, MPC 45 stored at 3°C for 6 mo was similar in flavor to MPC 45 stored at 25°C for 3 mo and MPC 45 stored at 40°C for 1 mo, while MPC 45 stored at 25°C stored for 6 mo had a similar flavor profile to MPC 45 stored at 40°C for 3 mo. It is important to note that MPC 45 stored at 3°C for 12 mo never developed the off-flavor profile of MPC 45 stored at 25°C by 6 mo and MPC 45 stored at 40°C by 3 mo. Milk protein concentrate (45%) stored at 40°C for 6 mo was more highly associated with tortilla, cardboard, animal, and burnt sugar flavors than MPC 45 stored at 25°C for 12 mo.

The effect of storage temperature on the flavor of MPC 80 was more pronounced. The MPC 80 stored at 3°C for 12 mo was similar in flavor to MPC 80 stored at 25°C for 6 mo and MPC 80 stored at 40°C for 1 mo, while MPC 80 stored at 25°C for 12 mo had a similar flavor profile to MPC 45 stored at 40°C for 3 months. The impact of temperature is especially important to consider during transport overseas, when powder may be stored at high temperatures for extended periods of time. The flavor related shelf life of MPC 45 stored at high temperatures (40°C) is half that of those stored at lower temperatures (25°C) while the shelf life of MPC 80 stored at high temperatures (40°C) is a quarter of those stored
at 25°C. Refrigerated storage (3°C) of MPC greatly decreases the development of off-flavors but does not completely suspend it.

Previous studies on MPC have focused on functionality, primarily solubility, rather than instrumental volatile and sensory properties. While there have been a few studies published on sensory properties of milk proteins, to the authors’ knowledge, the effect of storage on milk protein flavor has not been previously addressed. Compounds associated with lipid oxidation and Maillard browning are the primary source of flavor in milk proteins and storage increased the concentration of many of these compounds. Aldehydes (hexanal, heptanal, octanal, nonanal, methional, 2 and 3-methylbutanal, E,E 2,4 decadienal) and other lipid oxidation products such as 2 pentylfuran and 1-octen-3-one and sulfur containing volatile compounds increased over time and as storage temperature increased in both MPC 45 and MPC 80. While the effect of storage on the flavor of MPC has not been studied, Drake et al. (2006) compared flavor stability of SMP stored at 21°C or 35°C, and Wright et al. (2009) studied the flavor stability of whey protein concentrates (80%) and isolates across 18 mo storage at 21°C. Wright et al. (2009) documented that lipid oxidation increased during storage of whey proteins confirming the involvement of lipid oxidation in dried dairy protein ingredients during storage. Drake et al. (2006) also documented lipid oxidation compounds in SMP stored at 21°C as well as increased Maillard reaction production in SMP stored at 35°C. They documented that different flavors as well as different volatile compounds formed in milk powders stored at different temperatures. These results collectively are consistent with the results from the current study with MPC.
Conclusions

Flavor is of utmost importance to the success of milk protein products. The flavor of different milk protein products varied depending on the composition, manufacturing process, and storage. Milk proteins were characterized by sweet aromatic, sour aromatic, cardboard, potato/brothy, brothy/animal, tortilla/corn chip, soapy, and fatty flavors. Tortilla flavor is a defining flavor of milk protein ingredients and many of the odor active volatile compounds found in corn tortillas, corn chips, and taco shells were also odor active compounds in milk proteins. While 2-aminoacetophenone plays a role in the tortilla flavor of milk proteins, it is likely a combination of this compound and other lipid oxidation and heat-induced compounds that contribute to this flavor. Key flavor active compounds in milk proteins were 1-hexen-3-one, 2-acetyl-1-pyrroline, benzothiazole, 1-octadecanol, homofuraneol, 1-octen-3-one, 1-hexen-3-one, sotolon, homofuraneol, 4-hexen-3-one, 1-cyclohexen-3-one, dimethyl disulfide, dimethyl trisulfide, guaiacol, methional, hexanal, heptanal, nonanal, decanal, 2-aminoacetophenone, acetic, propionic, butanoic, pentanoic, and hexanoic acids, gamma-decalactone, and delta-dodecalactone. Volatile compound profiles of MPC 45 and 80 stored for 12 mo were different. In MPC 45, pentanal, octanal, DMS, 2-methylbutanal, and 3-methylbutanal concentrations were higher than those in MPC 80, while 1-octen-3-one, E,E 2,4 decadienal, hexanal, 2-pentylfuran, DMDS, DMTS, heptanal, methional, and nonanal were higher than those in MPC 45. MPC 45 and 80 stored at lower temperatures for less time were associated with sweet aromatic and cooked milky flavors and a sweet taste. As the powders aged, aroma intensity, cardboard, animal, burnt sugar and tortilla/corn chip flavors increased.
Storage temperature played a large role in the shelf life of MPC. At high temperature (40°C), 1 mo storage is sufficient to reduce solubility of MPC 45 and 80 below the solubility of 3 and 25°C stored MPC 45 and 80. Storage at high temperatures (40°C) also reduced the flavor related shelf-life of MPC 45 by 50%, and by 75% in MPC 80 compared to MPC 45 and 80 stored at 25°C. Special care must be taken during shipping and storage or the effective shelf-life of MPC is drastically reduced.

Acknowledgements

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References


Table 1. Commercial proteins and manufacturers (MPC: Milk Protein Concentrate; MCC: Micellar Casein Concentrate; MPI: Milk Protein Isolate). Two lots of each protein were obtained from each supplier. Letters A-E are commercial suppliers. P were manufactured in the NCSU pilot plant.

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<tr>
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<td>Acid Casein</td>
</tr>
<tr>
<td>B</td>
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</tr>
<tr>
<td>B</td>
<td>MPC 85</td>
</tr>
<tr>
<td>B</td>
<td>MPI</td>
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<tr>
<td>C</td>
<td>MCC</td>
</tr>
<tr>
<td>D</td>
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<td>P</td>
<td>MPC 45</td>
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<td>P</td>
<td>MPC 80</td>
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Table 2. Odor-active compounds detected in caseins, milk protein concentrates (MPC), milk protein isolates (MPI), and micellar casein concentrates (MCC) by gas chromatography-olfactometry using SPME (solid phase microextraction).

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<th>RI (WAX)</th>
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<th>Compound</th>
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<th>A: Rennet Case</th>
<th>B: MPC 70</th>
<th>B: MPI</th>
<th>B: MPC 85</th>
<th>C: MCC</th>
<th>D: MPC 90</th>
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*Retention Indices (RI) from GC-O data on the ZB-5 column
bRetention Indices (RI) from GC-O data on the WAX column
Method of identification by RI (retention indices), O (olfactometry), and MS (mass spectrometry) compared with authentic standards
Table 3. Log FD3 values of odor-active compounds detected in caseins, milk protein concentrates (MPC), milk protein isolates (MPI), and micellar casein concentrates (MCC) in the neutral/basic and acid fractions from solvent extraction with gas chromatography-olfactometry with aroma extract dilution analysis.

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<th>RI/WAX</th>
<th>RI/DBS</th>
<th>Fraction&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Compound&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Odor&lt;sup&gt;c&lt;/sup&gt;</th>
<th>ID</th>
<th>Acid Casein-A</th>
<th>Retent. Casein-A</th>
<th>MPC78-B</th>
<th>MPC80-B</th>
<th>MPI-B</th>
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<sup>a</sup>Retention Indices (RI) from GC-O data

<sup>b</sup>Odor description at GC-sniffing port

<sup>c</sup>ND = not detected

<sup>d</sup>Log<sub>3</sub> flavor dilution

<sup>e</sup>A = Acid fraction, NB = Neutral/Basic fraction from solvent extraction with gas chromatography-olfactometry.
Table 4. Mean concentration or relative abundance of selected compounds in milk protein and casein powders.

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<th>Compounds extracted and quantified by SPME headspace.</th>
<th>Acid Casein-A</th>
<th>Rennet Casein-A</th>
<th>MFC 85-B</th>
<th>MPT-B</th>
<th>MCC-C</th>
<th>MPC 80-D</th>
<th>MPC 80-E</th>
<th>MPC 85-E</th>
<th>MPC 85-P</th>
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<td>0.011&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.88&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.502&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.011&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.171&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.523&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.07&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>1-hexen-3-one&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>264&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.35&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.451&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.315&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.393&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.11&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>1-octen-3-one</td>
<td>0.238&lt;sup&gt;b&lt;/sup&gt;</td>
<td>104&lt;sup&gt;b&lt;/sup&gt;</td>
<td>528&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.98&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>52.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>50.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>2-aminoacetoophenone</td>
<td>12.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.053&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.060&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.076&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.630&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.102&lt;sup&gt;de&lt;/sup&gt;</td>
<td>0.055&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.238&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hexanal&lt;sup&gt;b&lt;/sup&gt;</td>
<td>52.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>217&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.3&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>2.31&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.57&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.95&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.29&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.96&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.53&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Heptanal</td>
<td>1.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>210&lt;sup&gt;b&lt;/sup&gt;</td>
<td>190&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.50&lt;sup&gt;d&lt;/sup&gt;</td>
<td>18.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>57.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>203&lt;sup&gt;d&lt;/sup&gt;</td>
<td>30.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>63.4&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Nonanal</td>
<td>20.5&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>16.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>644&lt;sup&gt;a&lt;/sup&gt;</td>
<td>76.8&lt;sup&gt;de&lt;/sup&gt;</td>
<td>16.7&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>210&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>279&lt;sup&gt;d&lt;/sup&gt;</td>
<td>690&lt;sup&gt;a&lt;/sup&gt;</td>
<td>64.0&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>DMDS&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.27&lt;sup&gt;b&lt;/sup&gt;</td>
<td>26.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>500&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.057&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.062&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.093&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.105&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.058&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.460&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>DMTS&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.092&lt;sup&gt;c&lt;/sup&gt;</td>
<td>36.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.50&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.293&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.593&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.455&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.48&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.89&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Methionol</td>
<td>0.082&lt;sup&gt;d&lt;/sup&gt;</td>
<td>18.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.095&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.66&lt;sup&gt;d&lt;/sup&gt;</td>
<td>36.1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>8.22&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.108&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.99&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Table 5. Sensory profiles of caseins, milk protein concentrates (MPC), milk protein isolate
(MPI), and micellar casein concentrate (MCC) obtained from various manufacturers.

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Type</th>
<th>Aroma Intensity</th>
<th>Cooked/Milky</th>
<th>Sweet Aromatic</th>
<th>Sour</th>
<th>Aromatic</th>
<th>Cardboard</th>
<th>Potato/Brothy</th>
<th>Animal</th>
<th>Tortilla/Corn Chip</th>
<th>Soapy</th>
<th>Fatty</th>
<th>Sour</th>
<th>Astringency</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>Acid Casein</td>
<td>4.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>1.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>3.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>1.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>Rennet Casein</td>
<td>3.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>2.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>2.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>2.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>MPC85</td>
<td>2.6&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.3&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>2.3&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>2.1&lt;sup&gt;de&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>2.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>MPC70</td>
<td>2.3&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>1.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>2.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>MPI</td>
<td>3.2&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>ND</td>
<td>2.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>3.2&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>2.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>MCC</td>
<td>2.5&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>1.3&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
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<td></td>
</tr>
<tr>
<td>D</td>
<td>MPC80</td>
<td>1.1&lt;sup&gt;de&lt;/sup&gt;</td>
<td>1.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.1&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>ND</td>
<td>1.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>ND</td>
<td>2.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>2.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>MPC80</td>
<td>2.1&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>1.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>ND</td>
<td>1.5&lt;sup&gt;e&lt;/sup&gt;</td>
<td>ND</td>
<td>2.1&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>ND</td>
<td>2.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>MPC85</td>
<td>1.2&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>1.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>ND</td>
<td>2.0&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>ND</td>
<td>1.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>2.5&lt;sup&gt;bc&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>MPC80</td>
<td>1.8&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.7&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>ND</td>
<td>1.6&lt;sup&gt;e&lt;/sup&gt;</td>
<td>ND</td>
<td>1.5&lt;sup&gt;d&lt;/sup&gt;</td>
<td>ND</td>
<td>ND</td>
<td>2.5&lt;sup&gt;bc&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a-g</sup> Means in a column 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., 1999). Most dried ingredient flavors fall between 0 and 4 (Croissant et al., 2009; Listiyani et al., 2011).

ND – not detected
233

Table 6. Mean relative abundance (µg/kg) of select volatile compounds by protein concentration, storage time, and storage
temperature.
2pentylfuran

DMS

DMDS

DMTS

Heptanal

Methional

nonanal

2 MB

3 MB

1octen3one

2,4
decadienal

1.26efghi
1.32efgh
1.38efg
1.14fghij
1.95de
2.29cd
1.05fghijk
2.72bc
2.91bc
0.954fghijk
3.38ab
3.63a
0.503jk
0.610ijk
0.848fghijk
0.369k
0.772ghijk
1.00fghijk
0.554jk
0.931fghijk
1.15fghij
0.663hijk
0.824fghijk
1.50ef

3.47k
3.74k
5.24jk
4.57jk
6.11ijk
10.3fghijk
5.74ijk
14.1defgh
18.1bcdef
9.52ghijk
19.4bcde
23.9bc
11.0fghijk
10.4fghijk
16.0defg
7.87hijk
16.1cdefg
25.4b
12.2efghij
20.7bcd
43.4a
13.2defghi
17.7bcdef
44.5a

0.312c
0.297c
0.294c
0.309c
0.312c
0.412c
0.315c
0.353c
0.470c
0.317c
0.381c
0.543c
0.426c
0.896c
1.23c
0.428c
1.03c
3.88b
0.565c
1.11c
3.70b
6.68a
0.928c
6.25a

31.8kl
35.2jkl
31.0l
48.1hijkl
58.2fghijkl
69.2cdefghi
64.8defghij
85.0bcdef
101b
61.7efghijk
89.9bcde
136a
34.8jkl
72.0bcdefghi
85.1bcdef
29.8l
82.3bcdefg
94.3bcd
44.3ijkl
89.1bcde
98.6bc
52.4ghijkl
75.0bcdefgh
99.9b

2.30cdef
2.41bcdef
2.64abcde
1.83f
1.81f
3.07ab
1.81f
2.15def
2.96abc
1.96ef
2.78abcd
3.33a
0.025g
0.084g
0.098g
0.029g
0.067g
0.114g
0.037g
0.064g
0.099g
0.046g
0.085g
0.088g

1.52g
4.31g
15.9fg
3.16g
7.64g
49.6def
4.23g
11.4g
80.2bcd
5.79g
14.8fg
113b
2.84g
29.2efg
95.1bc
9.64g
59.1de
168a
10.3g
80.6bcd
191a
64.3cd
71.0cd
197a

0.052de
0.059cde
0.050de
0.052de
0.052de
0.060cde
0.053de
0.050de
0.060cde
0.054de
0.045e
0.061cde
0.050de
0.049de
0.080cd
0.041e
0.051de
0.091c
0.055de
0.060cde
0.158b
0.061cde
0.052de
0.223a

0.323h
0.396gh
0.346h
0.342h
0.453gh
0.578efgh
0.369h
0.546fgh
0.740cdefg
0.392gh
0.618defgh
0.927bcde
0.618defgh
0.849bcdef
0.883bcdef
0.552fgh
1.02bc
1.47a
0.733cdefg
1.11b
1.72a
0.857bcdef
0.952bcd
1.81a

0.084efg
0.073efg
0.079efg
0.031fg
0.028g
0.035fg
0.135def
0.088efg
0.091efg
0.160de
0.108efg
0.092efg
0.045fg
0.110efg
0.129defg
0.041fg
0.218cd
0.443b
0.056efg
0.273c
0.629a
0.073efg
0.297c
0.720a

0.224d
0.236d
0.196d
0.110d
0.173d
0.193d
0.214d
0.185d
0.209d
0.195d
0.227d
0.268d
0.626abc
0.571bc
0.582abc
0.458c
0.629abc
0.640ab
0.600abc
0.697ab
0.737ab
0.649ab
0.587abc
0.750a

1.53ghi
1.56gh
1.75fg
1.47ghi
2.51ef
3.03de
1.46ghi
3.62cd
3.95bc
1.43ghij
4.60ab
5.01a
0.540jk
0.708hijk
1.05ghijk
0.487k
0.790hijk
1.26ghijk
0.613ijk
0.878ghijk
1.31ghijk
0.707hijk
0.724hijk
1.35ghijk

0.432defg
0.523bcde
0.088kl
0.287ghij
0.371efgh
0.575bcd
0.348fghi
0.486cdef
0.270ghij
0.028l
0.750a
0.363efgh
0.124jkl
0.183ijkl
0.241hijk
0.147jkl
0.189ijkl
0.470cdef
0.239hijk
0.240hijk
0.611abc
0.341fghi
0.187ijkl
0.685ab

1.11ef
1.35def
1.29def
0.780f
0.919ef
0.990ef
1.05ef
1.11ef
1.78cdef
0.756f
1.35def
1.99cde
0.960ef
1.98cde
2.25cd
1.03ef
2.25cd
2.83c
1.39def
2.37cd
5.60b
1.62def
1.97cde
8.17a

0.268g
0.554fg
0.556fg
0.541fg
0.632fg
0.545fg
0.433fg
0.543fg
1.71cde
0.721fg
1.12defg
1.77cd
0.453fg
0.915defg
1.28def
0.450fg
1.14defg
3.98b
0.763efg
1.22defg
4.22b
2.58c
2.45c
7.86a

0.886f
0.969ef
1.11ef
0.755f
1.36de
1.64cd
0.803f
1.82bc
2.03bc
0.808f
2.10b
2.56a

7.24cd
7.07cd
10.6cd
6.22d
11.1cd
17.8b
8.98cd
17.4b
30.7a
11.3c
18.5b
34.2a

0.369c
0.596c
0.763c
0.369c
0.674c
2.14b
0.440c
0.731c
2.08b
3.49a
0.655c
3.39a

33.3f
53.6de
58.0d
38.9ef
70.2cd
81.7bc
54.6de
87.0bc
99.8ab
57.1de
82.5bc
118a

1.16bcde
1.25abcde
1.37abcde
0.930e
0.940e
1.59ab
0.925e
1.10cde
1.53abc
1.00de
1.43abcd
1.71a

2.18f
16.7ef
55.5c
6.40f
33.3de
109b
7.27f
46.0cd
135a
35.0cde
42.9cd
155a

0.051d
0.054d
0.065cd
0.047d
0.051d
0.075c
0.054d
0.055cd
0.109b
0.058cd
0.049d
0.142a

0.471f
0.622def
0.614def
0.447f
0.741de
1.02bc
0.551ef
0.829cd
1.23ab
0.625def
0.785d
1.37a

0.065de
0.091de
0.104d
0.036e
0.123cd
0.239b
0.095de
0.181bc
0.360a
0.117cd
0.203b
0.406a

0.425ab
0.403ab
0.389bc
0.284c
0.401ab
0.417ab
0.407ab
0.441ab
0.473ab
0.422ab
0.407ab
0.509a

1.03e
1.13de
1.40de
0.982e
1.65cd
2.14bc
1.03e
2.25b
2.63ab
1.07de
2.66ab
3.18a

0.278cde
0.353bc
0.164f
0.217def
0.280cde
0.523a
0.294cd
0.363bc
0.440ab
0.184ef
0.468a
0.524a

1.03ef
1.67cde
1.77cd
0.907f
1.58cdef
1.91c
1.22cdef
1.74cd
3.69b
1.18def
1.66cde
5.08a

0.361d
0.735d
0.920d
0.495d
0.886d
2.265c
0.598d
0.881d
2.97b
1.65c
1.79c
4.81a

Octanal

Hexanal

45*T1*3C
45*T1*25C
45*T1*40C
45*T3*3C
45*T3*25C
45*T3*40C
45*T6*3C
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45*T6*40C
45*T12*3C
45*T12*25C
45*T12*40C
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80*T1*25C
80*T1*40C
80*T3*3C
80*T3*25C
80*T3*40C
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80*T6*25C
80*T6*40C
80*T12*3C
80*T12*25C
80*T12*40C
Time x temperature
T1*3C
T1*25C
T1*40C
T3*3C
T3*25C
T3*40C
T6*3C
T6*25C
T6*40C
T12*3C
T12*25C
T12*40C

Pentanal

Protein x temperature x time


### Table 6. Continued

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### Protein x Time

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</table>

**Note:** Means in a column not sharing a common superscript are different ($P < 0.05$).

- **DMS** = Dimethyl sulfide
- **DMDS** = Dimethyl disulfide
- **DMTS** = Dimethyl trisulfide
- **2MB** = 2 methylbutanal
- **3MB** = 3 methylbutanal

**1** Means in a column not sharing a common superscript are different ($P < 0.05$).
Table 7: Sensory profiles of milk protein concentrates (MPC 45 and 80) stored for 0, 1, 3, 6, and 12 mo at 3, 25, and 40°C.

*---Means in a column 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., 1999).

Most dried ingredient flavors fall between 0 and 4 (Croissant et al., 2009; Listiyani et al., 2011).

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Figure 1. Concentration (µg/kg) of 2-aminoacetophenone compared to tortilla/corn chip flavor intensity in select milk proteins and caseins.
Figure 2. Percent increase of furosine concentration in MPC 45 and 80 stored at 3, 25, and 40°C for 1, 3, 6, and 12 mo.
Figure 3. Percent solubility of MPC 45 and 80 stored at 3, 25, and 40°C for 0, 1, 3, 6 and 12 months.
Figure 4. Principal component biplot of sensory attributes of MPC 45 stored at 3, 25, and 40°C for 0, 1, 3, 6, and 12 mo.

T0 = no storage, T1 = 1 mo storage, T3 = 3 mo storage, T6 = 6 mo storage, T12 = 12 mo storage. 3C = stored at 3°C, 25C = stored at 25°C, 40C = stored at 40°C.
Figure 5. Principal component biplot of sensory attributes of MPC 80 stored at 3, 25, and 40°C for 0, 1, 3, 6, and 12 mo. T0 = no storage, T1 = 1 mo storage, T3 = 3 mo storage, T6 = 6 mo storage, T12 = 12 mo storage. 3C = stored at 3°C, 25C = stored at 25°C, 40C = stored at 40°C.
Figure 6. Principal component biplot of relative abundance of selected volatile compounds of MPC 45 stored at 3, 25, and 40°C for 0, 1, 3, 6, and 12 mo. T0 = no storage, T1 = 1 mo storage, T3 = 3 mo storage, T6 = 6 mo storage, T12 = 12 mo storage. 3C = stored at 3°C, 25C = stored at 25°C, 40C = stored at 40°C.

DMDS = Dimethyl disulfide
DMTS = Dimethyl trisulfide
2MB = 2 methylbutanal
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Figure 7. Principal component biplot of relative abundance of selected volatile compounds of MPC 80 stored at 3, 25, and 40°C for 0, 1, 3, 6, and 12 mo. T0 = no storage, T1 = 1 mo storage, T3 = 3 mo storage, T6 = 6 mo storage, T12 = 12 mo storage. 3C = stored at 3°C, 25C = stored at 25°C, 40C = stored at 40°C.

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