ABSTRACT

WHITE, SAMANTHA SEVYNNE. The Role of Different Sweeteners and Instantization on WPI Flavor Formation during Beverage Processing Steps. (Under the direction of Dr. MaryAnne Drake.)

Whey protein isolate (WPI) for beverage manufacture is generally instantized by agglomeration with lecithin to increase solubility and wettability. Additionally, beverage manufacturers utilize different sweeteners to create products that have high nutritional value without sacrificing quality taste and flavor. Previous research established that the acidification and heat treatment steps in WPI beverage manufacture increased the formation and release of undesirable flavors from non-instantized WPI. The first objective of this study was to compare the effects of two processing steps inherent to manufacture of acidic protein beverages, acidification and heat treatment, on the flavor of WPI with and without instantization. The second objective was to characterize the impact of different sweeteners on the flavor of model WPI beverages after acidification and heat treatment.

To meet the first objective, duplicate samples of instantized (I) and non-instantized (NI) WPI from two commercial suppliers were rehydrated at 10% solids (w/v) and evaluated as-is (control, C), acidified to pH 3.2 (A), heated to 85°C for 5 min (H) or acidified and heated to 85°C (AH). For objective two, duplicate samples of commercial WPI were rehydrated to 10% WPI solids (w/v) and sweetened with fructose, sucralose or stevia before being acidified to pH 3.2 and heated to 85^{o}C for 30 sec. The experimental controls, for objective 2, included unsweetened WPI as well as aqueous solutions of fructose, sucralose and stevia; all AH processed in the same manner. All treatments were evaluated by descriptive sensory analysis and volatile analysis was conducted using solid phase microextraction gas chromatography-mass spectrometry and gas chromatography-

olfactometry. Statistical differences were analyzed using analysis of variance and principal component analysis.

I WPI were higher than NI WPI in linoleic and linolenic fatty acids ($p \leq 0.05$). Rehydrated C-I WPI displayed cucumber flavors not present in C-NI WPI. After acidification, A-I WPI were distinguished from A-NI WPI by a higher intensity of cucumber flavor and higher concentrations of 2-phenethanol and E-2-nonenal ($p \leq 0.05$). There were no perceivable differences between H-I and H-NI WPI after heating; sulfur and eggy flavors increased in both types of WPI. After acidification and heating, AH-I WPI were differentiated from AH-NI by grainy and grassy/hay flavors and increased concentrations of methanethiol, E,E-3,5-octadien-2-one and 2-phenethanol ($p \leq 0.05$). Results suggest that instantization with lecithin contributes to off-flavors in WPI subjected to typical whey protein beverage processing conditions.

After acidification and heating, model WPI beverages made with sucralose and stevia had higher soapy flavor and bitter taste than those sweetened with fructose ($p \leq 0.05$); beverages with sucralose were also metallic. Compared to unsweetened WPI, model beverages containing sucralose and stevia had decreased intensities of cabbage/brothy and potato/brothy flavors ($p \leq 0.05$). However, these sensory differences were not confirmed by volatile analysis suggesting additional cross-modal effects on sensory perception. Comparison of sweetened water and sweetened WPI indicated that processing effects (acidification and heating) on the sweetener alone did not promote the formation of soapy flavors. Beverage processing steps likely enhanced interactions between WPI and the high-intensity sweeteners to generate long-chain free fatty acids that imparted soapy flavors to these WPI solutions. Sweeteners influenced both sweet taste quality and enhanced the flavors

contributed by WPI. These results demonstrate that sweetener type influences both aromatics and basic taste that contribute to the flavor profiles of whey protein beverages.

The Role of Different Sweeteners and Instantization on WPI Flavor Formation during Beverage Processing Steps

by
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DEDICATION

To the one who loved me through all my fears, every misstep and all my tears.

For you I am truly grateful.

Amen

BIOGRAPHY

Samantha White's passion for science began with a chemistry set and physics experiments on the kitchen counters of her childhood home in Chesterfield, Virginia. Samantha's parents allowed her to explore every aspect of her personality by encouraging her in academics as well as sports, photography and performing arts. These dual interests continued through the end of high school when Samantha stumbled upon a career that would require strengths in both science and art; flavor chemistry. As there was no undergraduate program for flavor chemistry, Samantha joined NC State in 2006 to earn her Bachelor's in Food Science. Her desire to become a flavor chemist was cemented through her work with Dr. MaryAnne Drake and after an opportune internship with Hertz Flavor House. After earning her Bachelor's in 2010, Samantha began her Master's degree researching the interactions between flavor compounds and food matrices. Samantha aspires to become a certified flavorist and to work diligently to create a suitable low-fat, low-carb, sugar-free alternative to her G-ma's peach cobbler.

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Firstly, I want to acknowledge that I have not earned, deserved or achieved any amount of success through my own will, strengths or talents. My hands, my health and even my mind were graciously entrusted to me.

I have also been shown grace through the wonderful people brought into my life; from which I have had the pleasure to walk beside and learn. I am immensely grateful to my parents for the childhood lessons that strengthened and motivated me throughout adulthood. You shaped my sense of justice and my integrity in ways that I can only hope to instill in my children. Thanks to all my family and friends, who have also shaped the person I am today. Especially to my G-ma who showed me the beauty of a strong and courageous woman. I also want to thank the other courageous women in my life who have been a source of refuge and strength over the years; Amanda, Gillian, Karen, KT, Em and Jess.

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CHAPTER 1: LITERATURE REVIEW

In order to meet new consumer demands and stay ahead of trends, food manufacturers are constantly seeking to develop new products. These products must be optimized with the proper ingredients, proper concentrations, and proper unit processes to construct a well accepted and successful product. However, these changes sometimes impart undesired off flavors and textures to the final products. Often, there may even be antagonistic interactions where the combination of two variables will drastically decrease liking responses from consumers. Therefore, research is needed to understand the fundamentals about how each of these variables contributes to flavor and texture of the final product. As flavor and texture are important drivers for consumer liking, the objective is to improve overall liking of the product and increase sales.

Understanding what consumers experience as they are consuming a food product is of great concern to manufacturers. This not only accounts for overall flavor and texture profiles, but the temporal duration and intensity of these attributes. Sensory and rheological tools have been extensively used to profile and differentiate foods. However replicating the consumer experience over time remains a challenge as so many variables exist. Oral processing is complex and dynamic. Any research efforts to understand it must attempt to mimic this complexity.

The term process cheese refers to various categories of cheese that differ based on fat and moisture content, final pH, and quantity of optional ingredients that can be used (Kapoor and Metzger, 2008). There are 3 major categories of process cheese made in the United States (Table 1); pasteurized process cheese, pasteurized process cheese food, and pasteurized process cheese spread. A fourth, undefined category exists called pasteurized

process cheese products; which contain other ingredients that are not specified by the CFR regulations. These ingredients may be milk protein concentrate (MPC) or whey protein isolate (WPI).

I. Partitioning and flavor release

The unit process and ingredient formulation of processed cheese greatly impacts the textural properties of the final cheese product. In general, the cheese is made by combining natural cheese, emulsifying salts and other ingredients. After blending the mixture is cooked, packaged and stored (Figure 1). Research has shown that processing conditions including, “cook time, cook temperature, extent of agitation (mixing) during cooking, and the rate at which the cooked process cheese is cooled, have a significant effect on the functional properties of process cheese" (Kapoor and Metzger, 2008). Kapoor and Metzger (2008) also noted that an increase in cook temperature increased cheese firmness by increasing the strength of the cheese emulsion. Another study, by Garimella Purna (2006), found that high mixing speeds produced a firmer cheese due to the increased uniformity of the fat globules in the emulsion. In this instance the high mixing speeds served as a partial homogenizer of the oil-in-water emulsion, and decreased the size of the fat globules. Increasing the strength of the cheese emulsion increased firmness of the product.

Process changes can also result in a product with a different flavor profile. The changed food matrix will more rapidly release certain volatiles and have a binding effect on others based on chemical and physical properties. Heat-treated milk was used as a control to understand the effect of high-pressure-moderate-temperature processing on the volatile profile of milk (Vazquez-Landaverde et al., 2006). The control samples were subjected to

temperatures of 25, 60 and 80°C for 1, 3 and 5 minutes to mimic the temperature conditions of loading the sample, pressurization, and then unloading. At 25°C, aldehyde concentrations were the same as in raw milk. However at 60 and 80°C, nonanal increased the most in concentration followed by octanal and decanal. Increased holding time, at these temperatures, caused an increase in the formation of all aldehydes. Methyl ketones showed the same patterns, where no concentration changes occurred at 25°C but up to a 4-fold increase occurred at temperatures of 60 and 80°C. Methyl ketones (2-nonanone, 2-undecanone, 2-hexanone, 2-decanone, 2-heptanone) along with some sulfur compounds (dimethyl sulfoxide and dimethyl sulfone) and diacetyl increased in concentrations above threshold levels. Milk samples with increased concentrations of methyl sulfide, methyl ketones and aldehydes; displayed cooked off notes (Vazquez-Landaverde et al., 2006). The following year, Lozano et al. (2007) investigated heat-induced volatiles of soy milk and reported that the concentration of volatile sulfur compounds increased in treatments that underwent UHT processing. Methional has been shown to be the highest aroma active compound in UHT soy milk (Colahan-Sederstrom and Peterson, 2005; Lozano et al., 2007). Lozano et al. (2007) also found a 5 log increase of diacetyl (2,3-butanedione) and an increase in 3-hydroxy-2-butanone; a reduction product of diacetyl. An increase in volatile sulfur compounds (methanethiol, dimethyl sulfide, and dimethyl trisulfide), an increase in roasted aromatic compounds (2-acetyl-1-pyrroline, 2-acetylthiazole, and 2-acetyl-2-thiazoline) as well as an increase in sweet/buttery notes (2-methylbutanal, 3-methylbutanal, and 2,3-pentanedione) were defining characteristics of the heat treated soy milk (Lozano et al., 2007). Packaging is known to cause aroma loss in model process cheeses (Pionnier et al., 2004). There were

significant losses of diacetyl (99%), heptan-2-one (58%), ethyl hexanoate (36%) and heptan-2-ol (32%) after packaging and cooling of processed cheese (Pionnier et al., 2004).

The release of volatiles from a food product is also dependent on the composition of the food matrix. Different chemical and physical properties will influence how the volatiles are released. For example, an increase in macromolecules such as lipids, polysaccharides and proteins can have a binding effect on flavor molecules and result in decreased flavor perception. Partition coefficient has been known to correlate to sensory odor intensity (Guyot et al., 1996) and has been a useful tool to predict sensory response. The partition coefficient of the headspace and a food matrix (khs/matrix = Chs/Cmatrix) describes what fraction of a compound is in the headspace versus what fraction still remains in the food once the system reaches equilibrium (Leksrisompong et al., 2010). In this instance, a higher partition coefficient correlates to a higher volatile concentration in the headspace and perhaps a higher perceived aroma intensity. Guyot et al. (1996) reached such a conclusion; partition coefficients were positively correlated with sensory odor intensity. In addition to predicting sensory response, partition coefficient can be used to describe the hydrophobicity of a compound when it is in an oil and water mixture. Therefore, Ko/w is also known as the hydrophobicity of a compound or Log P. Hydrophobic compounds have positive values and hydrophilic compounds have negative values (Taylor, 2002). While this is a useful tool to understand the release of volatile compounds due to changes in the food matrix, the partition coefficient can only be applied to systems that are in equilibrium such as stored food products. Product developers must also understand flavor release as food is consumed. However, food consumption is a dynamic process that never quite reaches equilibrium; so the

partition coefficient is not an applicable measurement. Alternatively, one could continuously measure volatile release during oral processing or with a device that simulates oral processing.

Effect of fat concentration

Because of consumer desire and government regulations for reduced fat options, manufacturers are decreasing the fat content in process cheese. These ingredient substitutions and decreased fat concentrations impart off-flavors and undesirable textures to low-fat and reduced-fat Cheddar cheese. Low-fat Cheddar can contain higher levels of homofuraneol and phenyl compounds and lower levels of butanoic and hexanoic acids (Banks et al., 1989; Drake et al., 2010; Milo and Reineccius, 1997; Carunchia Whetstine et al., 2006) while reduced-fat cheeses may be chewy, hard, waxy and springy (Brown et al., 2003; Gwartney et al., 2002; Yates and Drake, 2007). These attributes are characterized by consumers as bad flavor and bad texture and give the perception of not being natural (Childs and Drake, 2009).

Weel et al. (2004b) noted that the aroma release of relatively hydrophilic ethyl acetate was not affected by oil concentrations of 0-5% and compounds that were more hydrophobic were more bound by the matrix as oil concentration increased. This study explained that the increased oil content decreased the aroma concentration in both oil and water phase, creating a smaller concentration gradient and thus a smaller driving force for the release of the compound into the gas phase. As a result, the compounds have a lower gas phase concentration and longer release times (Weel et al., 2004b). Furthermore, increasing the oil concentration decreased the amount of emulsifying agent on the surface of the oil droplets (Leksrisompong et al., 2010). The hydrophobic compounds become bound in the now larger

oil droplets. However, hydrophilic compounds will be less affected by increasing oil concentrations because are they are partitioned in the water phase (Leksrisompong et al., 2010; Weel et al., 2004b). Compounds with both hydrophobic and hydrophilic side groups are least affected by increases in oil concentration and instead are driven into the headspace by protein interactions (Leksrisompong et al., 2010).

The sources of problems in fat reduced cheese are changes to the microenvironment, removal of hydrophobic flavor compounds, and changes to aroma thresholds in the lower fat, water-based matrix. Reduced-fat cheeses are characterized by decreased fat, increased protein, increased salt and increased moisture concentrations which alter the microstructure and microenvironment of the cheese (Carunchia Whetstine et al. 2006). The environment and growth conditions of full-fat cheese are different from low-fat cheese which promotes the growth of different microbes. Milo and Reineccius (1997) concluded that the flavor profile of low-fat cheese had different compounds at different concentrations than full-fat Cheddar cheese and that these flavor profiles were produced by microbial, enzymatic, and biochemical reactions. Also changes to the microstructure, such as increased protein to fat ratios, caused low-fat or reduced-fat cheeses to have more dense protein networks and therefore a firmer texture (Rogers et al. 2009). Often, the removal of the fat from the milk also removes flavor compounds that are carried in the fat phase (Carunchia Whetstine et al., 2006). Nelson and Barbano (2004) found that fatty acids and low molecular weight triglycerides were removed along with the fat. It was suggested by Carunchia Whetstine et al. (2006) that low molecular weight compounds were liquid at the temperature of fat removal and were removed with the fat; leaving behind higher molecular weight compounds. Higher

molecular weight compounds have higher melting points, and perhaps this is a source of undesirable breakdown patterns in the low-fat cheeses. Rogers et al. (2009) conceived that full fat cheeses have weak spots at the protein-fat interface, and manipulation of these weak spots creates smaller particles and a more desirable breakdown. Aged full-fat cheeses are known to be fruity and were differentiated from low-fat cheeses by distinct concentrations of ethyl butanoate and ethyl hexanoate suggesting these chemicals were in the fat phase when the fat was removed (Carunchia Whetstine et al., 2006).

Also, fat is important to the flavor release of aromatic chemicals. The same chemical will have a different threshold concentration whether in a predominantly oil or water matrix. Full-fat cheese is most like an oil matrix and will bind hydrophobic compounds as noted by Weel et al. (2004a). Low-fat cheeses are mostly water and will release hydrophobic volatiles more rapidly into the headspace causing a lower threshold of these volatiles. This is because lowering the fat content decreases solubility of hydrophobic compounds and releases them into the headspace. As such, lower concentrations of those compounds are needed in low-fat cheeses to elicit a sensory response similar to full-fat cheeses. For example, the thresholds for γ-octalactone and δ-decalactone, both hydrophobic compounds, are 10 times higher in oil than in water (Carunchia Whetstine et al. 2006). The differences in low-fat and full-fat cheeses are that some of the flavor is removed by the fat reduction process, different microbes produce different flavors at different concentrations, and what flavors are still in the low-fat cheese will be released differently.

The flavor of processed cheese is impacted by fat concentration of the natural cheese because the flavors of the natural cheese contribute to the flavor profile of the processed

cheese (Drake et al. 2010). However one cannot assume that adding a natural cheese, high in flavor compounds, as an ingredient will produce a flavorful processed cheese. Other ingredients of the processed cheese may hinder the flavor release; no matter the flavor concentrations of the natural cheese. Flavor release depends on mass transfer, matrix structural hindrance, flavor-matrix interactions and other reactions within the food system (Seuvre et al., 2000). Differences in fat concentrations can also change the rate of volatile release. Delahunty and coworkers (1996) determined that 2-butanone and 2-heptanone were released faster from a reduced-fat cheese than from a full-fat cheese. This is because these hydrophobic compounds are less soluble in the high moisture, reduced-fat cheese.

Effect of whey and milk protein concentration

The use of different protein concentrations in product formulation will change the properties of processed cheese. Nonfat dry milk (NDM) and whey protein concentrate (WPC) will increase whey protein in the final product and increase curd firmness, decrease moisture content and increase flavor binding (Guinee et al., 2006; Kapoor and Metzger, 2008; Kühn et al., 2006). Whey protein products such as WPC and whey protein isolate (WPI) have a strong affinity for volatile compounds (Kühn et al., 2006). The few studies conducted on this topic have shown that 2-nonanone and nonanal bind to WPC and vanillin to WPI (Jasinski and Kilara, 1985; McNeill and Schmidt, 1993). The two major whey proteins are beta-lactoglobulin and alpha-lactalbumin. Few studies have investigated the binding abilities of alpha-lactalbumin, but it is believed to have a lower affinity for flavor compounds (Kühn et al., 2006). Beta-lactoglobulin is reported to have 2 separate binding

sites for aroma compounds. For example, fatty acids and retinol bind to the hydrophobic, central cavity of the molecule whereas others bind to weaker, secondary sites along the outer surface of the protein (Kühn et al., 2006; Weel et al., 2003). Denaturing the whey protein (60-70°C) will lead to protein-protein binding, cross-linking (Kapoor and Metzger, 2008; Kühn et al., 2006) and allow the release of hydrophobic odor compounds that were once bound in the native protein configuration. In contrast, this protein aggregation and configuration changes may make more binding sites available to trap and retain compounds such as ethyl hexanoate and nonan-2-one (Gierczynski et al., 2007; Kühn et al., 2006). Weel and colleagues (2003) concluded that the presence of whey protein, however, did not cause retention of aldehydes in vivo and that there was less binding than what they observed using static headspace.

Bovine serum albumin (BSA), another whey protein, has 2 high-affinity binding sites and several weaker sites for binding aromatics (Guth and Fritzler, 2004; Kühn et al., 2006). Of the milk proteins, it has the highest ability to bind volatile compounds. Land and Reynolds (1981) found that an increase of 0.5% BSA protein caused a 25% decrease in the volatility of diacetyl in an aqueous solution. The binding of BSA to certain flavor compounds, such as vanillin or 2-octanone, also has a stabilizing effect and can increase the temperature required to denature the protein (Burova et al., 1999, Burova et al., 2003). There are some discrepancies in the true value of the binding constant of BSA. Researchers have calculated several different binding constants which may be due to varying amounts of fatty acids tightly bound within the protein (Kühn et al., 2006).

Casein is the major protein in cheese that contributes much to its structure (O'Mahony et al., 2008). It is composed of α_{s1}-casein and β-casein. O'Mahony and associates (2008) manipulated the ratio of alpha and beta casein to determine their effect on directly-acidified fat free cheese. The fat-free cheeses were made from MPC solutions with the same total casein concentration, just different in α_{s1}: β-casein ratios. After 3 days storage, the cheese with the higher β-casein concentration (1.00:1.08) was more firm and harder to melt than the lower β-casein formulation (1.00:1.00). Storage of the product for 30-60 days improved the cheese texture by allowing protein hydrolysis. Hydrolysis of these proteins, which occurs during ripening, can improve the texture and mouthfeel properties of cheese. Proteolytic coagulants, that accelerate the breakdown of β-casein, may improve melt and flow but can cause off-notes and bitterness (O'Mahony et al., 2008).

Traditionally, to standardize milk for cheese making, one removes milk fat or adds casein in the form of skim milk powder (SMP) (Rehman et al., 2003a). However, adding SMP increases the lactose concentration and therefore allows unwanted fermentation and flavors in the final product (Rehman et al., 2003a). Another alternative, MPC, has been used to improve cheese consistency and increase product yield (Harvey, 2006; Rehman et al., 2003a). Milk protein concentrate (MPC) is made by spray drying the retentate from ultrafiltered skim milk and therefore contains the same casein-to-whey ratio as fresh milk (Harvey, 2006). It is also important to note that in addition to having the same casein-to-whey ratio, the casein micelles are not altered during MPC production (Martin et al., 2009). However, it differs from fresh milk in its rennet gelation properties. When rennet is added to MPC the viscosity decreases, as the κ-CN is cleaved, but the solution does not coagulate as

fresh milk does (Martin et al., 2009). The addition of 2 mM calcium chloride, however, allowed MPC to gel in a manner similar to fresh milk (Martin et al., 2009). The presence of calcium chloride in the milk serum is important to curd formation.

MPC has been shown to function as well as fresh milk in making cheese. However, it is known to change the cheese texture and aroma profile. In Harvey (2006), MPC-85 was added to cheese milk to increase the protein levels to approximately 5 and 6% to make Cheddar cheese. Cheese made from the MPC fortified milk was cut at higher levels of firmness to ensure moisture retention in the final cheese. While the samples were only evaluated by a cheese grader, this person found no sensory defect at the 4.56% protein level and commented that the 6.48% protein cheddar was dry and like flour (Harvey, 2006). Also, the use of MPC may lead to a firmer cheese texture because conditions during MPC manufacture cause whey protein to denature. This denatured whey protein will settle into the cheese curd during cheese making (Guinee et al., 2006) and result in a higher protein concentration and firmer texture in the final product. The flavor profile of cheese made from MPC is also altered. An earlier study found that the addition of liquid MPC (9.9% protein) to cream produced a Cheddar cheese characterized by lower milk fat aroma, fruity aroma and bitterness than cheese made from skim and whole milk (Rehman et al., 2003a). However, the MPC cheeses were more sour and showed an increase in sulfur and brothy intensities. When compared to the control cheese, the MPC cheese seemed less mature and showed slower proteolysis after 250 days storage (Rehman et al., 2003a).

In general, a greater level of protein-flavor binding can also occur if fat concentration is increased (Leksrisompong et al., 2010). The surface area of the fat globule increases,

allowing more surface area for interfacial proteins which then bind volatile compounds that contain carbonyls, alcohols and esters (Leksrisompong et al., 2010). A general rule is that the binding capabilities of protein increase from alcohols to ketones to esters due to the presence or absence of certain functional groups (Kühn et al., 2006).

The addition or reduction of emulsifying salts also causes changes in the functional characteristics of processed cheese by changing both protein interactions and fat interactions. El-Bakry and colleagues (2010) determined that reducing the concentration of emulsifying salts created a problem during cheese making; more time was needed to properly emulsify the fat globules in the processed cheese blend. A decrease of 40% in emulsifying salts led to a 3-fold increase in total production time; whereas a decrease of 20% increased production time by 25%. The researchers proposed that the reduction caused a decrease in casein hydration and since casein is also an emulsifier, the oil phase was harder to stabilize in the cheese matrix. Therefore, the reduction of emulsifying salts (at both 25 and 40%) caused an increase in production time needed to shear the fat globules. As a result, the fat globules were smaller in diameter and this change created defects in the final product.

The salt reduction caused a longer mixing time which produced a harder cheese that was more difficult to melt than the standard control. Compared to the standard control, the hardness nearly doubled and the experimental sample was less viscoelastic; perhaps a result of higher protein interactions. El-Bakry and others (2010) tried increasing the moisture content of the cheese in an attempt to improve the texture defects. They found that the increase in moisture did not significantly decrease hardness or cohesiveness. Cohesiveness was, however, inversely correlated with emulsifying salt reduction. The 40% reduction-

cheese increased cohesiveness, as measured by the uniaxial compression test, significantly from 0.4 to 0.43; meaning the sample held together better after the emulsifying salt reduction. The researchers also hypothesized that the lower emulsifying salt concentration could lead to a different structure organization during cooling. The different matrix structure could also increase protein-protein interactions and produce a harder, less elastic processed cheese.

Effect of oral processing

Food quality is not only optimization of ingredients and processes to produce desired flavor and texture profiles. Along with their magnitude, the temporal coordination of these events during oral processing greatly impacts liking (Foegeding et al., 2010). There are also interactions in the mouth and throat of the consumer that can impact the duration and intensity of flavor release. After intake the food matrix looses structure, more surface area becomes available, saliva dilutes the food, and air flows through the airways (Bakker et al., 1996). Foegeding and colleagues (2010) commented that food undergoes continuous structural reorganization and, during this time, sensory signals from the bolus cause a change in mastication pattern. For example, crunchy foods are chewed more vigorously after the first bite but chewing decreases as the consumer feels the bolus has become more suitable for swallowing. These factors of decreasing structure, saliva production, mastication procedure and air flow impact in vivo volatile release.

Structural breakdown of food releases aromatics and taste stimuli while preparing the food for enzymatic degradation. Lubrication of the food occurs from the saliva but it may also come from water and fat released from the food product; an element affected by the

food's protein content (Foegeding et al., 2010). Harrison and Hills (1997) determined that the reversible binding between active aroma compounds and macromolecules was the rate-limiting step for aroma release in most situations. Some researchers conclude that this binding occurs so fast in vivo that the initial states of release will be the same with or without the presence of macromolecules (Harrison and Hills, 1997; Weel et al., 2003). Carunchia Whetstine and others (2006) suggested that protein-bound aromatics may be detected instrumentally, but have no impact on flavor perception because they are not released in the mouth. As such, flavor binding by macromolecules may not play as big a role in flavor perception as instrumental results suggest. However, saliva can have an enzymatic effect on esters, thiols and aldehydes after only 1 minute of exposure (Hussein et al. 1983; Weel et al., 2003). To combat this issue, one can use an instrument that allows saliva addition to the sample immediately before extracting the volatiles. Conversely, in vivo, losses in the mouth are inevitable and cannot be controlled (Pionnier et al., 2004). Weel and others (2004c) concluded that water works as well as saliva for in vitro measurements because the main function is dilution of the sample. Therefore, the use of water can serve as an alternative for saliva.

Bolus formation and the act of swallowing can impact the sensory perception of aroma intensity. After a food reaches the bolus state at about 0.8-3.0 mm (Foegeding et al., 2010), the structure does not change significantly enough to induce aroma release (Gierczynski et al., 2007). For example, a softer cheese will reach the bolus state faster than a harder cheese and this alone will change the time of volatile release. Swallowing displaces about 5-15 mL of air, charged with aromatics, into the pharynx and the first exhalation

afterward transports that air to the nose creating a maximum intensity signal (Gierczynski et al., 2007; Land, 1996; Linforth and Taylor, 2000; Weel et al., 2003). Instruments wishing to simulate oral processing should account for the aroma release that occurs due to swallowing and exhalation (Weel et al., 2004c).

Cross-modality between texture and aroma perception can also impact oral processing and the perceived aroma release. Flavor, texture, and even visual stimuli are combined together in the consumer's mind to determine product quality and liking (Foegeding and Drake, 2007). So naturally, studies attempting to evaluate texture and aroma release separately can risk halo dumping effects. Halo dumping is when panelists are asked to scale one attribute in a sample with many other attributes and they over compensate for the missing attributes (Viscchers et al., 2006). This can lead to panelists over-scaling aroma intensity in order to compensate for texture differences between the products. Therefore, it is a challenge to control for the effects of cross-modality when scaling the intensity of flavor or texture using human subjects. There are conflicting data as to whether increased firmness of a product results in an increased or decreased aroma perception (Viscchers et al., 2006; Gierczynski et al., 2007). Therefore, the true effect of firmness may be unique to each food type. The discrepancies can also be due to different mastication procedures, given to panelists, which cause increased or decreased release rates. Limited research exists on the volatile release from solid or semi-solid foods; and this may be due to the complexity of mastication (Hills and Harrison, 1995). Mastication varies due to anatomy, food texture, bite force, number of teeth, muscle activity, saliva production and swallowing threshold; to name a few causes (Foegeding et al., 2010). However, Viscchers and others (2006) concluded that

cross-modal effects were decreased when the aroma event could be delivered later in oral processing. Viscchers and others (2006) let subjects chew odorless gels with different textures and delivered aroma separately; either orthonasally or retronasally. The researchers experimented with different times of aroma delivery and discovered that aroma delivery after swallowing decreased cross-modal effects.

II. Human physiology of flavor

Aromatics

Aroma perception occurs when volatile compounds reach the olfactory epithelium at the top of the nasal cavity (Weel, 2004a). Orthonasal aroma flows through the nasal passage to the olfactory epithelium and retronasal aroma flows from the mouth through the nasopharnx and is transported to the nasal cavity (Weel, 2004a). No matter which pathway, the volatiles bind to receptor cites on the mucosal surface of the olfactory epithelium (Weel, 2004a). The aroma compounds penetrate the mucus layer with the help of odorant binding proteins (Weel, 2004a) and bind to the proper receptors to generate a signal to the brain.

Basic Taste

Gustation, or taste, occurs when chemical compounds stimulate the receptors on the tongue and back of the mouth (Veldhuizen et al. 2010). The 5 basic tastes are sweet, salty, bitter, sour and umami. There are receptor cites in the oral cavity that bind specifically to compounds to elicit each basic taste. In fact, scientists that discovered umami had to identify the specific umami taste receptor before it could be accepted as a basic taste (McCabe and Rolls, 2007). These non-volatile compounds must be soluble in saliva before they are able to bind to taste-receptor cells and elicit a response (Weel, 2004a).

Feeling Factors

Perception of food texture and feeling is the result of food characteristics interacting with processes in the mouth, and interpreted by the brain (Engelen and VanderBilt, 2008). In the oral cavity, food is mechanically broken down and manipulated by the tongue, teeth and saliva. The numerous receptors in the oral cavity and nose respond to the initially ingested food and monitor the changes during processing. Engelen and VanderBilt (2008) define texture as a sensory perception of food structure and how that structure reacts to forces in the mouth. Humans use senses of touch, sight, and hearing to perceive texture and feeling factors. Feeling factors, such as heating sensations, occur when the receptors in the trigeminal system are stimulated; called oral somatosensation (Veldhuizen et al., 2010). However, there are no specific receptors for texture attributes (Weel, 2004a).

III. Established lexicon of processed cheese

Trained panels

There are several methods of using a trained panel to evaluate food samples and profile their differences. These methods include descriptive analysis (DA), time intensity (TI) and temporal dominance of sensation (TDS). All methods provide qualitative (lexicon) and quantitative (intensity) information about the samples.

Descriptive analysis is basically a static measurement or sensory rating of the attributes in a product. It involves trained panelists evaluating a sample by marking the maximum intensity for each attribute (Weel, 2004a). There are different approaches to DA; Flavor Profile Method, Texture Profile Method, Quantitative Flavor Profiling, Spectrum Method™, Free Choice Profiling, Quantitative Method™, and generic DA (Murray et al.,

2001). The most commonly used method is generic DA; which combines aspects of the different approaches (Murray et al., 2001). No matter what approach best meets the project objectives, each begins with selecting and training panelists to become familiar with the product. Panelists can be selected based on screening tests, availability, motivation, personality etc. (Murray et al., 2001). In all the methods except Free Choice Profiling, training begins by developing a common language or lexicon to describe the attributes in a wide variety of the products. For panelists to use a common scale, it is important that panelists share a common point of reference for the sensory language. The Spectrum Method ™ uses published references as external standards to calibrate and align panelists (Murray et al., 2001). However the panel may perform better when references are in included in the product matrix and not given as external standards (Murray et al., 2001). Researchers must decide which DA approach best suits their needs based on time, experience, purpose of the scale, importance of relating to consumer data, importance of relating to other products categories, and a number of other variables. For example, one benefit of the Flavor Profile Method is the panel operates very fast since there are only 4-6 panelists (Murray et al., 2001). A disadvantage of the Texture Profile Method is attributes are rated on scales that are anchored by specific food products (Murray et al., 2001). This becomes an issue when some of the anchors are no longer commercially available. Quantitative DA™ (QDA) takes less time to train panelists and uses ordinary, non-technical language which may relate better to consumer language (Murray et al., 2001). The disadvantage here is that panelists are permitted to score attributes differently; meaning consensus is not encouraged (Murray et al., 2001). This becomes problematic if, for example, the project objective is to compare the

intensity of the grapey flavor in wine to the grapey flavor in stored whey products. It is not possible to compare the absolute intensities of these products. An advantage of QDA over Spectrum™ method is, a Spectrum™ panel must be led by a moderator to encourage consensus and proper use of the scale. This requires some expertise in descriptive analysis, panelist training, and use of the 15-point line scale. The project objectives and resources available to the researcher will determine which DA approach is most appropriate to profile the given samples.

Unlike the static measurements of descriptive analysis, TI and TDS profile sample attributes over the time of consumption. Time-intensity (TI) has been used to measure flavor and texture attributes over time to better represent the dynamic nature of sensory perception (Weel, 2004a). In this method, trained panelists are asked to give the intensity score of an attribute over the course of the evaluation period (Pineau et al., 2009). As only one attribute can be measured at a time, TI is limited to only a few samples or products with few attributes (Pineau et al., 2009). TI generates time-release curves, for each attribute, that can be analyzed for maximum intensity, time to maximum intensity and initial slope (Weel, 2004a). Temporal Dominance of Sensations (TDS) was developed to determine which sample attribute is most dominant at any time over the evaluation period. When training the panelists, Pineau and colleagues (2009) described the dominant attribute as which sensation is commanding attention at any given time. During the evaluation period, panelists record the intensity of the dominant attribute. When the dominant attribute changes, in intensity or to another attribute, a new rating is recorded. This method also generates curves that describe the sequence of flavor and texture over time. This is determined by the proportion of runs

where the given attribute was selected as dominant; independent of the intensity ratings (Pineau et al., 2009). Pineau and associates (2009) concluded that the average intensity score for TI significantly correlated with the TDS dominance rate. However, the time to reach this point and other results were not correlated between the methods; suggesting TI and TDS do not satisfy the same needs. TI is better suited to show the evolution of one attribute over time, and TDS is better designed to characterize the most important attributes over time (Pineau et al. 2009).

Flavor profiling

Although processed cheese products are made from natural cheese, a product whose lexicon is well established, the lexicon for natural cheese cannot fully describe the variety of attributes in processed cheese products. Processed cheeses have added ingredients and an additional heating process that are likely sources of flavors and textures specific to processed cheese. A study published recently sought to profile the flavor of different processed cheese types as defined by CFR regulations (Drake et al., 2010).

Processed cheeses differed from natural cheeses in only a handful of attributes. The intensities of cooked/milky, whey and milkfat flavor did not distinguish natural cheeses from processed cheeses. The term brothy, included in the lexicon for natural cheese, was sub-divided into potato/brothy and brothy/fish food. One treatment had noticeable vitamin and doughy notes, but the descriptive panel determined that these terms and brothy/fish food were unique terms not characteristic of the majority of processed cheese. The natural mild Cheddar cheeses did not have sweet aromatic or caramelized/toasted flavors and were less salty when compared to the mild processed cheeses. It was proposed that these flavor

differences were due to the heating process and the addition of emulsifying salts. This was further supported when a cold-packed cheese product lacked cooked and caramelized/toasted flavors found in the other treatments. The other treatments went through an additional heat-treatment of blending the ingredients and pasteurization. The category of cheese closest in flavor profile to natural cheese was, expectedly, pasteurized processed cheese which is required to have the highest percentage of natural cheese of all the processed cheese types.

There were clear distinctions between processed cheese categories, and the different ages of the natural cheese ingredient imparted flavors typical of young, mild or aged cheese. The pasteurized processed cheese treatments were characterized by flavors typical of young or moderately aged Cheddar; whey, milkfat, sulfur and brothy flavors (Young et al., 2004; Drake et al., 2008, 2009). Only one pasteurized processed cheese spread contained a malty/nutty flavor typical of aged Cheddar. Pasteurized processed cheese products contained the flavors of young and mild-flavored cheeses. Both the pasteurized processed cheese foods and spreads were characterized by sweet aromatic, caramelized/ toasted cheese, cooked and brothy flavors. However the spreads also had distinct free fatty acid aroma and higher salt intensities than pasteurized processed cheese. Because there is no legal definition of ingredients for pasteurized processed cheese products, there was much variance between samples of this category. Whey products are typically used in this category and so whey flavor was common. Some fat-free cheese products had fruity, sweet aromatic and cardboard/stale flavors. The researchers proposed that these fat free cheeses used other non-fat ingredients to increase solids content and therefore increased the chance of those flavors coming through (Drake et al., 2010). There was another distinction made based on the flavor

claim on the label. Cheddar products labeled as sharp Cheddar contained higher intensities of butyric acid flavor while a lower intensity of butyric acid gives a cheesy flavor (Carunchia-Whetstine et al., 2006). This study also profiled Swiss cheese products which were characterized by fruity and sweaty flavors (Drake et al., 2010). In general, processed cheeses were characterized by their formulations including heat treatments, quantity and age of natural cheese, and the addition of whey or non-fat ingredients.

Texture profiling

Evaluation of cheese texture involves attributes experienced at initial contact, first bite, after chewing and after swallowing (Foegeding and Drake, 2007). Attributes from the first two oral processing steps can be predicted by mechanical tests because there are minimal saliva interactions at this point. At this stage panelists evaluate hand firmness, springiness, and recovery as well as firmness and fracturability after the first bite (Foegeding and Drake, 2007). Firmness for example is well correlated with the mechanical measurement of hardness because both the mouth and rheological instruments can measure force. However, mechanical tests poorly correlate to the human perception during chewing and swallowing (Foegeding and Drake, 2007). Therefore a descriptive panel is better at determining the mouth evaluation terms of the cheese product. Recently, cheese texture profile has been used to differentiate between low and full-fat cheeses (Brown et al., 2003; Gwartney et al., 2002; Yates and Drake, 2007). In addition to different mouthfeel attributes, low-fat cheeses tend to have different breakdown patterns when compared to full-fat cheeses (Foegeding et al., 2010) and degree of breakdown is a defined term in the cheese lexicon (Foegeding and Drake, 2007).

Rogers et al. (2009) used sensory profiling and rheological measurements to determine texture differences in Cheddar cheeses with different fat contents over a period of aging. Protein hydrolysis during aging and density of the protein-fat structure contributed to many of the observed differences between treatments. Up to 20% of the casein in cheese was hydrolyzed in the first 14 days of storage (Lawrence et al., 1987). As the cheeses aged, the full fat treatments showed higher decreases in firmness and hand springiness between 0.5 and 3mo followed by a more steady decrease (Rogers et al., 2009). The low fat cheeses maintained a steady decrease in firmness, and the reduced fat cheeses had behaviors in the middle of the other two treatments (Rogers et al., 2009). Protein hydrolysis caused degradation of the casein network and decreased structure and firmness (Rogers et al., 2009). The researchers concluded that fat concentration impacts the stability of the protein-fat network and texture profile of the product.

IV. In vitro volatile release

The effect of macromolecules on the volatility of flavor compounds has been extensively studied. However, these effects are often different when observed in vivo. Furthermore, to predict flavor release while eating, one cannot simply use a combination of equilibrium measurements (Deibler et al., 2001). Foods are diverse and complex systems that are more than the sum of their compositional characteristics. Therefore previous researchers have used instruments to replicate the oral processing that occurs when consumers eat a food product (Deibler et al., 2001; Genovese et al., 2009; Gierczynski et al., 2007; Pionnier et al., 2005; Rabe et al. 2004; Roberts and Acree, 1995; Visschers et al., 2006; Weel et al., 2003; Weel, 2004a, Weel et al., 2004b; Weel et al., 2004c). Artificial throats and artificial mouths

have been compared to in vivo analysis including nosespace and mouthspace. All of these techniques have been used to better understand volatile release during food consumption.

Nosespace and mouthspace

In this type of analysis, the panelist's mouth creates the flavor release. Volatiles in the breath pass retronasally to aroma receptors. In nosespace, measurement of the compound concentration occurs near these receptors; only accounting for volatiles that are available to signal the brain. Comparatively, in mouthspace techniques the volatiles are sampled directly from the mouth while the subject is chewing. Often under strict protocol, the panelist consumes the sample while exhaled breath continuously enters a tube connected to the MS sampling line (Rabe et al., 2004) or exhaled breath is trapped and analyzed later. The technique in which the exhaled breath is sampled directly into an MS is also called breath-by-breath analysis. In a study conducted by Rabe and others (2004), the panelists were instructed to press flavored water underneath the tongue. Air containing volatiles was then sampled through the mouth or through the nose.

The sensorial perception of food gels better correlates to in-mouth measurements (Roberts et al., 2003) even though the nosespace technique most closely represents the consumer experience during consumption. Also, direct measurement of the volatiles in the expired breath appears to be the only method able to account for the impact of human physiology on release (Pionnier et al., 2004). However, these results lose reproducibility as they are greatly impacted by person-to-person variability if panelists are not extensively trained (Rabe et al., 2004). Even with training, subconscious variability can occur. A study using nosespace on model cheese gels, found that panelists compensated for increased gel

texture by increasing the intensity of chewing (Gierczynski et al., 2007). This resulted in a greater release of aromatics with increased gel firmness; contrary to previous findings. Also, different breathing rates will impact volatile release as a higher rate will transfer volatiles from the food matrix faster and give the perception of a higher intensity (Hanaoka et al. 2001; Pionnier et al., 2004).

Retronasal aroma simulator

The theory behind the retronasal aroma simulator (RAS) is that it mimics the conditions in the human mouth using blades for shearing, artificial saliva, temperature control and gas flow rates similar to human breathing (Figure 2). Especially with the analysis of solid foods, the addition of a blending device more closely, as compared to the artificial throat (mentioned below), mimics eating while improving sensitivity and decreasing sample loss from handling (Page and Avon, 1989). In this system, sample and artificial saliva are added into a 1L stainless steel, temperature-controlled blender (Roberts and Acree, 1995). The variable speed motor controls the blades which shear the sample. As the nitrogen gas constantly sweeps over the food, the released volatiles are extracted and trapped on the SPME fiber located above. Earlier models used Sep-Pak traps to increase sensitivity to detect volatiles at 10^{-9} g/g in foods (Roberts and Acree, 1995). Mouth simulators have been used to characterize the time-intensity release of volatiles from many foods including model fresh cheese, wine and raspberries (Deibler et al., 2001; Genovese et al., 2009; Gierczynski et al., 2007; Roberts and Acree, 1996).

In 2001, Deibler and others verified the RAS mouth simulator by comparing it to breath-by-breath analysis of volatiles released from imitation cheese. The RAS had less variation, when compared to using human subjects, and had increased sensitivity as the sample size was much larger than sample taken in the mouth. In the breath-by-breath analysis, also known as nosespace, the panelists ate 5g of cheese as they would normally. While they ate, expired air went through an API source (mentioned below) and then an MS-nose (mentioned below). Deibler et al. (2001) found that the volatile concentrations from the RAS had correlation coefficients from 0.97-0.99 with the breath-by-breath analysis. Variation for the RAS was 1-5% where variation of the panelists was between 41-49%. This study is proof of the concept that an open system that allows volatiles to return to the matrix is an adequate representation of in vivo release while eating (Deibler et al. 2001).

The use of RAS with SPME has been investigated to determine the correct fiber coating and appropriate sampling period. Deibler and others (2001) determined 95% of the maximum total peak area was extracted after 10min of exposure. However, a study conducted 4 years later used a sampling time of 5 seconds (Pionnier et al., 2005). In the later study, the researchers allowed the sample to equilibrate at 25^0C for 1 hour and then sampled the headspace for 5 seconds using a 100μm PDMS, a 65μm PDMS/DVB, a 75μm CAR/PDMS (carboxen), a 65μm CW/PDMS and an 85μm polyacrylate (PA) fiber. The objective was to evaluate the SPME technique compared to nosespace analysis with an API-MS. Therefore, a smaller sampling time had to be used since panelists only consumed the sample for a little over 3 minutes. However, Pionnier and others (2005) found that a 5 second sampling time led to a large variability between replications. A time error of plus or minus 1

second would change the volatile quantity by 20% (Pionnier et al., 2005). So, Pionnier and colleagues chose 8 seconds to ensure a measureable aroma signal while maintaining time accuracy. Next was the determination of the appropriate fiber coating. The Pionnier study concluded that PDMS was the ideal fiber coating for nosespace sampling (Pionnier et al., 2005). However this study used static headspace SPME to determine extraction efficiency. The PDMS, CAR-PDMS and the PDMS-DVB fibers produced acceptable results, but the PDMS fiber was ultimately selected due to its stability and repeatability in sampling gas mixtures (Pionnier et al. 2005). Deibler and others (2001) concluded that the carbowax/divinyl benzene (CW/DVB) fiber in combination with a divinyl benzene/poly(dimethylsiloxane) (DVB/PDMS) fiber was optimal for volatile compound recovery. The fibers were chosen because they extracted the greatest intensity of odor compounds, as determined by gas-chromatography olfactometry (GC-O).

A drawback to the RAS is that the air flow represents continuous exhaling and therefore is not completely appropriate for measuring time release of volatiles (Deibler et al., 2001). This configuration is more a measure of the volatiles at equilibrium. However, it may be possible to adapt the air flow controller to replicate normal breathing patterns of inhalation and exhaling and to create multiple ports for SPME fibers that can be taken out at different time points. Roberts and Acree (1995) used six silica traps over a 15 minute period to understand the dynamics of volatile release. Also, the blades of the modified blender limit the food sample to something that has low tear resistance (Deibler et al., 2001). Finally, the RAS cannot simulate the action of swallowing and does not account for the sample residue that covers the throat and back part of the tongue after swallowing (Rabe et al. 2004).

Artificial throat simulator

It is theorized that swallowing food leaves a thin layer that coats the throat; and during consumption the retronasal aromas released are volatiles extracted from this thin coating (Visschers et al., 2006; Weel et al., 2003) . The artificial throat (Figure 3) consists of vertical glass tubing with 3mm of rubber tubing in the middle. The rubber tubing is closed upon sample loading. The system is temperature-controlled with a water mantle and the sample can exit from the bottom of the tube. Sample and saliva are loaded with syringes and allowed to combine. The middle clamp is opened, allowing the liquid to flow down the tube and exit. After the tube is covered with the thin layer of sample, the air flow starts and is sampled at the top by the MS-Nose (mentioned below); also known as the electronic nose (Weel et al., 2004c). This model is designed to simulate the aroma release upon exhalation after the act of swallowing.

One drawback of this instrument is the sample must be a liquid. To the best knowledge of the author, no studies have been conducted comparing the artificial throat to the RAS (which mainly has solid food applications). In Weel (2004a), the artificial throat and the artificial mouth were compared to *in vivo* aroma release measurements. For liquid foods the mouth-model consistently overestimated the degrees of flavor binding by protein and oil; resulting in much lower flavor release than *in-vivo* measurements (Weel, 2004a). The throat simulator is better used to analyze liquids which are swallowed after intake and do not require much oral processing (Weel et al., 2004c). The addition of a chewing device could make the artificial throat applicable to solid samples. Also, the viscosity of the liquid impacts the extraction as the sample adheres more to the glass tubing. Higher viscosity liquids are

less consistent with the in vivo measurements (Weel et al., 2004c). This may be because the saliva does not homogeneously dilute the thicker solution. An advantage of the artificial throat is the dynamic air flow that better mimics oral processing. Weel and colleagues suggested using a tidal flow to better imitate breathing (Weel et al., 2004c).

V. Volatile Quantification

The above tools were used to release volatiles from a food. Next the compounds must be captured on extraction devices such as a SPME fiber or Sep-Pak Trap and then introduced to another set of instruments for quantification and identification. Several instruments, including MS-Nose and APCI-MS, have been used to quantify and identify volatile compounds.

MS-Nose; electronic nose

Gardner and Bartlett described the MS-nose as "an instrument, which comprises an array of electronic chemical sensors with partial specificity and an appropriate pattern recognition system, capable of recognizing simple or complex odours" (Dirinck et al., 2009). It consists of an autosampler, perhaps a GC-MS, and pattern recognition software designed to characterize a volatile mixture (Dirinck et al., 2009). Also, the MS-nose is easily calibrated by introducing the headspace of an aromatic solution with a known concentration and integrating the area under the curve (Weel, 2004a). This electronic nose mimics the way humans take an aroma, which may be comprised of thousands of volatiles, and interpret the pattern as one experience. Similarly, the MS-Nose generates a fingerprint or total ion chromatogram with one broad peak for all co-eluting volatiles (Dirinck et al., 2009). For this reason, the MS-nose cannot be used to identify aromatic compounds. It is has been used to

determine time-intensity of overall volatile release; where the objective was not to understand what volatiles contribute to the overall aroma (Dirinick et al. 2009). However, the instrument is not sensitive enough to profile the time-intensity release of individual volatile compounds (Dirinck et al., 2009).

Atmospheric pressure (chemical) ionization- mass spectrometer

The atmospheric pressure chemical ionization mass spectrometer (APCI-MS) samples extracted volatiles from the RAS or throat simulator through a capillary tube. The APCI-MS is an ion trap mass spectrometer that can target compounds of interest using their *m/z* values corresponding to protonated or dehydrated protonated molecular ions (Gierczynski et al., 2007). The mobile phase, which can be a polar or non-polar solvent, is generally heated above 400^0C and sprayed with nitrogen at a high flow rate (Zaikin and Halket, 2006). The gas is subjected to an electrical discharge that ionizes the sample. One advantage of APCI-MS over gas chromatography-MS is that the APCI-MS can measure the release of compounds that elute simultaneously (Weel, 2004a). The ionization forms mainly molecular ions which are easily interpreted in the spectra results (Weel, 2004a). The APCI-MS is also capable of generating fragment ions if the research purpose is to analyze individual compounds in a mixture (Weel, 2004a).

The APCI-MS often is seen coupled with several different extraction methods. This instrument has been used to sample the breath from the nose of people during consumption; a method called nosespace APCI-MS. (Gierczynski et al., 2007). Weel and associates (2004b; 2003) used the APCI-MS to study time release of esters at different fat concentrations and the release of aldehydes over time from whey protein solutions. Visschers and others (2006)

used the APCI-MS to understand the cross-modality of texture and aroma perception. The APCI-MS has also been paired with a mouth simulator (Gierczynski et al., 2007). In this instance, a model fresh cheese sample was broken down by a rotor with 45^0 angled blades to simulate the forces between the tongue and palate. The released volatiles were then sampled by the APCI-MS inlet tube. In another study with model cheese, the instrument was coupled with a SPME fiber to increase sensitivity (Pionnier et al., 2004). The researchers connected the SPME fibers by Y-junction to the expired air in order to take samples at different, overlapping, time intervals. Later, Pionnier et al. (2005) verified that SPME-GC-MS produced the same patterns of release as API-MS. The patterns of release considered time to reach max intensity, greatest chromatographic peak area for SPME, highest intensity for API, area under the curve, and initial slope of the curve from 0-10 seconds. SPME and API are both adequate tools for investigating in vivo volatile release.

VI. Conclusion

Processed cheese, by definition incorporates a variety of ingredients. Macromolecules in the food matrix affect the partitioning of flavor active compounds as well as functionality and texture of the cheese product. Changing the formulation or unit processing steps also affects the flavor and texture profile of processed cheese and may cause perceived differences to consumers. Some of these attributes are best evaluated by a trained descriptive panel. However, sensory and instrumental methods have been developed to characterize the time-intensity of volatiles from several food matrices. Nosespace and mouthspace techniques account for all the variables within the human mouth, however the resulting data often varies widely among panelists. One wishing to profile a solid or semi-solid food, instrumentally,

should use some sort of shearing device and attempt to mimic breathing, temperature, saliva conditions in the mouth. This apparatus must contain multiple sampling ports or must be coupled with a quantification device that will give a continuous spectrum.

VII. Objectives

The general objective of this research was to characterize the interactions between food ingredients and flavor compounds after post-processing of the finished product by acidification and heat treatment. Specifically, this research sought to understand how lecithin agglomeration and sweeteners may interact with WPI in a high protein beverage and to further investigate the potential for volatile compound production after acidification and pasteurization.

TABLES

Table 1- CFR[a] definition of the 3 major categories of process cheese in the United States

Category	Major ingredients and other optional ingredients (and their permitted levels)	Moisture (% w/w)	Fat (% w/w)	pH
PC[b]	* Cheese *Emulsifying agent (≤3% (w/w) of the final product) *Acidifying agent *Cream, anhydrous milk fat, dehydrated cream (weight of the fat derived is ≤5% (w/w) of the final product) *Water, salt, colors, spices or flavorings, enzyme-modified cheese, mold inhibitors (≤0.2% (w/w) or ≤0.3% (w/w) of the final product), anti-sticking agent (≤0.03% (w/w) of the final product)	≤ 40	≥30	≥5.3
PCF[c]	*Cheese (≥ 51% (w/w) of the final product) *Other optional ingredients and their permitted levels include all of the ingredients allowed in PC in addition to milk, skim milk, buttermilk, and cheese whey	≤44	≥23	≥5.0
PCS[d]	*Cheese (≥ 51% (w/w) of the final product) *Other optional ingredients and their permitted levels include all of the ingredients allowed in PCF in addition to food gums, sweetening agents, and nisin (≤250ppm of the final product)	44 to 60	≥20	≥4.0

[a]CFR= Code of Federal Regulations (FDA 2006). [b]PC= pasteurized processed cheese (21CFR133.169). [c]PCF=pasteurized processed cheese food (21CFR133.173). [d]PCS=pasteurized processed cheese spread (21CFR133.179).
(Kapoor and Metzger, 2008)

FIGURES

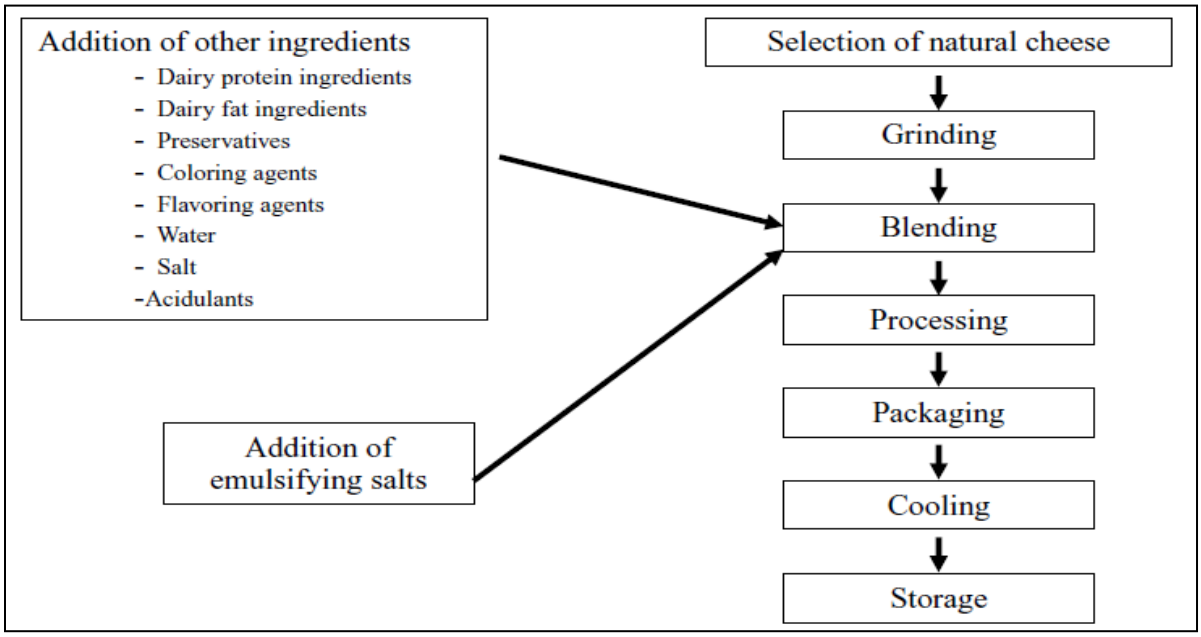

Figure 1. Schematic flow chart of process cheese manufacture (Kapoor and Metzger, 2008)

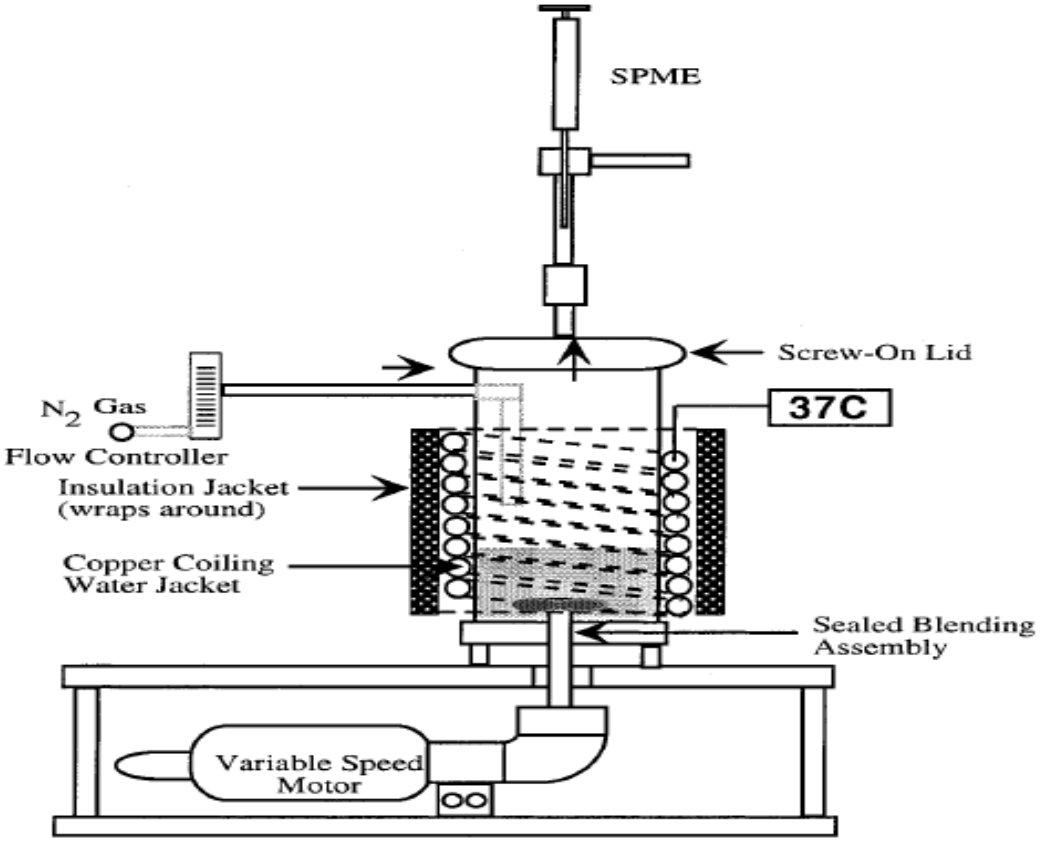

Figure 2. RAS mouth simulator (Deibler et al., 2001)

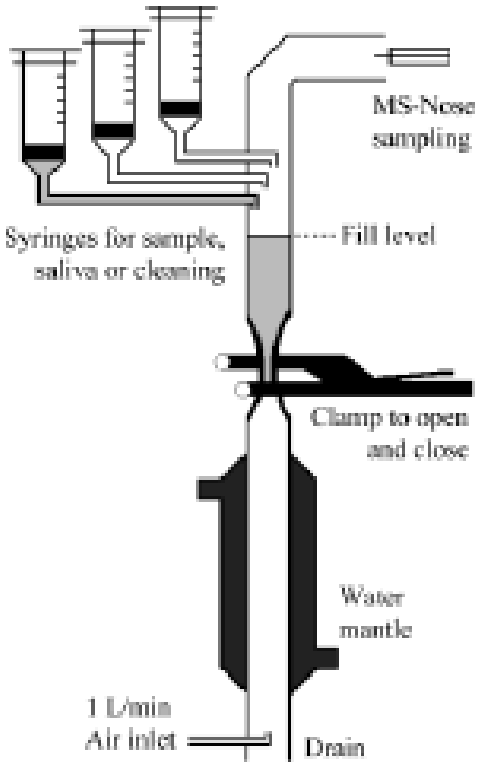

Figure 3. APCI-MS artificial throat simulator (Weel et al., 2004c)

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CHAPTER 2: THE ROLE OF INSTANTIZATION ON WPI FLAVOR FORMATION DURING BEVERAGE PROCESSING STEPS

The Role of Instantization on WPI Flavor Formation during Beverage Processing Steps

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Abstract

Previous research has established that the acidification and heat treatment steps in WPI beverage manufacture increase formation and release of undesirable flavors from non-instant WPI. Additionally, whey protein isolate (WPI) for beverage manufacture is generally instantized by agglomeration with lecithin to increase solubility and wettability. The objective of this study was to compare the effects of two processing steps [acidification and pasteurization] inherent to manufacture of acidic protein beverages, on the flavor of WPI with and without instantization. Duplicate samples of instant (I) and non-instant (NI) WPI from two commercial suppliers (n=8) were subjected to proximate and free fatty acid analyses for base line establishment. WPI were then rehydrated to 10% solids (w/v) and evaluated as-is (control, C), acidified to pH 3.2 (A), heated to 85°C for 5 min (H) or acidified and heated to 85°C (AH). Flavor of rehydrated treatments was evaluated by descriptive sensory analysis and volatile compounds were evaluated by solid phase microextraction gas chromatography-mass spectrometry and gas chromatography-olfactometry. Statistical differences were analyzed using one-way analysis of variance and principal component analysis. Rehydrated C-I WPI displayed cucumber flavors not present in C-NI WPI. After acidification, A-I WPI were distinguished from A-NI by a higher intensity of cucumber flavor and higher concentrations of 2-phenethanol and E-2-nonenal ($p \leq 0.05$). There were no perceivable differences between H-I and H-NI after heating; sulfur and eggy flavors increased in both types of WPI. After acidification and heating, AH-I WPI were differentiated from AH-NI by grainy and grassy/hay flavors and increased concentrations of methanethiol, E,E-3,5-octadien-2-one, and 2-phenethanol ($p \leq 0.05$). Results suggest that

instantization with lecithin contributes to off-flavors in WPI subjected to typical whey protein beverage processing conditions.

Key Words: Whey protein isolate, acidification, heat treatment, instantization, flavor

INTRODUCTION

Advances in processing technology and increased whey production have led to an increase in different types of whey ingredients. In 2010, over 28 million kilograms of whey protein isolate (WPI), with 90.0 percent or higher protein content, was produced in the US (USDA, 2011). Whey ingredients add not only protein to beverage applications, but provide essential amino acids and functional properties. For this reason, whey protein readily lends itself to sports drinks, infant formula and meal replacement beverages. As an ingredient, whey protein is expected to have a bland flavor. Undesirable off flavors are generated from multiple variables in the whey manufacture process; such as milk type, cheese starter culture, processing and storage conditions (Carunchia Whetstine et al., 2003; Carunchia Whetstine et al., 2005; Croissant et al., 2009; Mahajan et al., 2004; Gallardo-Escamilla et al., 2005; Wright et al., 2006; Wright et al., 2009; Whitson et al., 2011). Cardboard, animal, soapy, cucumber and brothy off-flavors have been previously reported in whey protein ingredients (Carunchia Whetstine et al., 2005; Drake, 2006; Wright et al., 2006; Wright et al., 2009). This poses a challenge for food manufactures, because the flavor variability of dried whey protein negatively impacts the flavor profile and consumer acceptance of products containing whey protein (Childs et al., 2007; Wright et al., 2009; Evans et al., 2010).

WPI for beverage manufacture may also be agglomerated to increase wettability and solubility of the protein ingredient. In the process of agglomeration, the dried powder forms

clumps and particles which create pores in the powder and increase dispersibility. When lecithin is chosen as the binder for agglomeration, this process is termed instantization. Methods of instantization include re-wet and single pass agglomeration. In re-wet agglomeration, dry product is placed on a fluidized bed and steam or lecithin is applied (Wright et al., 2009; Henning et al., 2006). The partial wetting allows sticking and particle formation. An alternate method is single pass agglomeration. In this process, instantization occurs as the product is spray dried; using a multinozzel system and redirecting dry product into the path of wet product (Henning et al., 2006). A common form of agglomeration includes addition of soy lecithin to further increase wettability and solubility by utilizing the amphiphilic properties of lecithin. Soy lecithin is also desired because it is classified as a nutraceutical food due to its phospholipid constituents (Colbert, 1998). However, lecithin-associated volatiles have been documented in instantized products and might contribute cucumber, fatty and cardboard flavors (Mortenson et al., 2008; Suriyaphan et al., 2001; Wright et al., 2009). Wright et al. (2009) documented that WPI instantized with lecithin had decreased shelf stability due to increased lipid oxidation products. Fortunately, researchers have reported fewer to no off-flavors in cheese and fermented milk made with hydrogenated soy lecithin or oat lecithin (Drake et al., 1998; Suriyaphan et al., 2001). Hydrogenated soy and oat lecithin do not contain linoleic or linolenic fatty acids, and dairy products made with these ingredients exhibited fewer off-flavors than those containing unmodified soy or rice lecithin.

Additionally, WPI may be further subjected to processing when incorporated into a food product. One challenge of creating a whey protein beverage is that heat treatment causes

denaturation and aggregation of the protein; increasing the turbidity of the beverage. Etzel (2004) explored varying methods to reduce the turbidity of heated whey protein beverages. When beverages at pH 3.0 or lower were heated, the samples remained clear regardless of the ingredients used. Dried whey protein is subjected to additional beverage post-processing steps; including acidification (pH<4.6) for clarity and heat treatment (typically 88^{o}C for 120s) for shelf-stability (Prendergast, 1985; Rittmanic, 2006). In addition to solubility concerns, flavor is an essential component in the consumer acceptance of whey protein beverages. Cardboard, soapy, cabbage, and potato are documented flavors associated with whey protein beverages, and post-processing of the beverage have been shown to further impact the flavor and acceptance of the finished product (Childs et al., 2007; Fox, 2011; Wright et al., 2009; Evans et al., 2010).

To our knowledge, no studies have documented the impact of instantization on the flavor of WPI and its possible role in the flavor of acidified whey protein beverages. The purpose of this study was to investigate the contribution of lecithin instantization to off-flavor development in WPI under processing conditions typical for acidified whey protein beverages. This objective was met through sensory and instrumental analyses of instantized (I) and non-instantized (NI) WPI under various treatments (control, acidified, heated, and acidified and heated).

MATERIALS AND METHODS

Sample Acquisition and Preliminary Assessments

Natural variance between commercial suppliers of WPI is common and expected due to processing variation and equipment differences (Carunchia Whetstine et al., 2005). To account for this variance, two commercial WPI manufacturers provided two lots of WPI manufactured from Cheddar whey (Figure 1). Two lots of instantized (I) and two lots of non-instantized (NI) WPI were provided. These suppliers were selected for their ability to instantize WPI using single-pass agglomeration. Single pass-agglomeration, as opposed to re-wet agglomeration, was chosen to minimize extraneous variables; such as initial product age. The samples were stored at -20°C upon receipt.

Chemical standards were obtained from Aldrich (St. Louis, MO) with some exceptions: dimethyl sulfide, Z-4-heptenal, phenylacetaldehyde, octanal, and o-aminoacetophenone were obtained from Acros Organic (Morris Plains, NJ); nonanal, 1-hexen-3-one, 2-pentyl furan, δ-decalactone, and δ-dodecalactone were obtained from Alfa Aesar (Ward Hill, MA). 2-acetyl-2-thiazoline was obtained from Astatech, Inc (Bristol, PA); 2-nonanone, and ethyl octanoate were obtained from SAFC Supply Solutions (St. Louis, MO). P-cresol, butyric acid and benzaldehyde were obtained from Fluka (Buchs, Switzerland); 2,5-octanedione was obtained from Frinton Laboratories (Vineland, NJ).

Proximate Analysis. WPI obtained from each supplier was analyzed for protein, fat, moisture, ash and mineral content in duplicate. Total protein was measured by Kjeldahl (AOAC, 2000: method number 991.20; 33.2.11) and total nitrogen was multiplied by a 6.38

conversion factor. Total fat was determined by Mojonnier extraction (AOAC, 2000: method number 989.05; 33.2.26) and total moisture by vacuum oven drying (AOAC, 2000; method number 990.20: 33.2.44). Total ash and minerals were measured by North Carolina State University Analytical Services Laboratory (Raleigh, NC) using a dry ash method paired with inductively coupled plasma optical emission spectroscopy (Lloyd et al., 2009).

Volatile Free Fatty Acid Analysis. A method adapted from Tomaino et al. (2004) was used to measure volatile free fatty acids after soapy flavors were observed in untreated control WPI. This analysis was conducted on untreated WPI to identify potential intrinsic differences in volatile free fatty acids that may contribute to flavor differences initially and after treatments (Campbell et al., 2011). All WPI were rehydrated to 10% (w/v) solids and the pH was adjusted to pH 2.0 with 3.3M HCl (Sigma Aldrich, St. Louis, MO). After equilibration to room temperature (25^{o}C) in a water bath, 5 g of sample was added to a 20-mL screwcap vial (Microliter Analytical, Suwanee, GA) with 5 μL of internal standard [1 mg of heptadecanoic acid (C17)/ mL of methanol (Sigma Aldrich, St. Louis, MO)] and a stir bar (Fisher Scientific, Pittsburgh, PA). The vials were heated to 110^{o}C for 40 min with the stir module on high. During the 40 min heating time, volatiles were extracted by a 30-μm-thick polydimethylsiloxane fiber (Supelco, Bellefonte, PA) attached to a fiber holder (Supelco) set to a depth of 1 cm. Volatiles were analyzed, in triplicate, by an Agilent 5975 GC-MS (Agilent Technologies, Santa Clara, CA) with a ZB-FFAP column (30-m length x 0.25-mm i.d. x 0.25-µm df) (Phenomenex Zebron, Torrance CA) and the FID set at 250°C. The oven temperature, initially 100°C, was held for 2 min and then increased at 10°C/min to 245°C, and was held for 10 min. Compounds were identified by comparison of retention time to

authentic standards. Relative abundance for each free fatty acid was calculated based on the internal standard calculated recovery.

Processing Treatments

Acidified (A) samples. Samples were rehydrated to 10% (w/v) solids with deionized water. The rehydrated whey was then acidified to pH 3.2 using a 2M 1:1 blend of phosphoric (Mallinkrodt Chemical, Phillipsburg, NJ) and citric (J.T. Baker, Mallinkrodt Baker, Phillipsburg, NJ) acids; to replicate industry practices (Prendergast, 1985; Rittmanic, 2006). Preliminary experiments were conducted to determine the volume of acid required to acidify the samples. This volume was subtracted from the total water added to retain 10% (w/v) solids (9% w/v protein) in the samples. The solids content was verified with the Smart System 5 moisture/solids analyzer (CEM, Matthews, NC).

Heated (H) samples. Due to protein coagulation observed in preliminary testing, the heat treated samples could not be passed through a high temperature short time (HTST) coil. Therefore, rehydrated samples were batch heated to 85°C in a digital shaker water bath (VWR apparatus, West Chester, PA) with an Arrow 1750 stirrer placed inside the sample (Arrow Engineering, Inc., Hillside, NJ). Once the samples reached temperature (approximately 20 min) they were held for 5 min before cooling to 4°C in an ice bath (approximately 15 min).

Acidified and Heated samples (AH). Rehydrated samples were acidified to pH 3.2 with the same methods described for the acidified (A) samples. Samples were then run through a benchtop HTST pasteurizer so that the sample maintained 85°C for 30 seconds. The benchtop HTST consisted of a peristaltic pump (Masterflex L/S, Cole Parmer, Vernon

Hills, Il) forcing sample through a 1/8" stainless steel coil (Cole Parmer, Vernon Hill, Il) that was submerged in a heated waterbath (VWR apparatus, West Chester, PA). The flow rate was 170 ml/min and the design was adapted from Cole and Jones (1990). Once the samples were heated, they were immediately cooled to 4°C in an ice bath (approximately 15 min).

Descriptive Sensory Analysis

Unprocessed controls (C) and the processed WPI samples (A, H, AH) were evaluated for flavor and aroma intensity by a trained panel (10 panelists, 9 females, 1 male, ages 23-50 y) using an established dried ingredient lexicon (Wright et al., 2009). Samples were dispensed into lidded soufflé cups with random 3-digit codes, tempered to room temperature for 1 h, and presented in a randomized order. Attributes were scaled using a 0 to 15pt universal Spectrum™ scale. Each sample was evaluated in duplicate by each panelist (Meilgaard et al., 1999). Paper ballots or Compusense five version 4.8 (Compusense, Guelph, Ontario, Canada) were used for data collection.

Solid Phase Microextraction Gas Chromatography-Mass Spectrometry (SPME GC-MS)

Volatile analysis of WPI samples was conducted in triplicate by SPME GC-MS. Sample preparation and SPME GC-MS methods were adapted from Wright et al. (2006). Five mililiters of each sample were measured into 20 ml autosampler vials with silicon septa lined steel screw tops (Microliter Analytical, Suwanee, GA). Internal standard solution (81 ppm 2-methyl-3-heptanone in methanol, Sigma-Aldrich, Milwaukee, WI) and 10% (w/v) sodium chloride (Fisher Scientific, Pittsburgh, PA) were added to the vials. Samples were introduced to the Agilent 6890N GC (Agilent Technologies, Santa Clara, CA) using a CTC Analytics CombiPal autosampler (Leap Technologies, Carrboro, NC). Samples equilibrated

at 40°C for 25 min before volatile extraction with a 3-phase fiber DVB/CAR/PDMS (Supelco, Bellefonte, PA) at 31 mm for 30 min, with 4 sec pulsed agitation at 250 rpm.

The GC method began with an initial temperature of 40°C for 3 min, then temperature was increased at a rate of 10°C/min until 90°C was reached. The rate changed to 5°C/min as temperature was raised to 200°C, which was then held for 10 min. Finally the rate increased to 20°C/min to 250°C and temperature was held for 5 min. The GC injector was programmed at 250°C and 7.06 psi with He carrier gas and a purge flow of 169737 cm/s.The GC was equipped with a ZB-5 ms column (30-m length × 0.25-mm i.d. × 0.25 μm df) (Phenomenex Zebron, Torrance, CA), the MS transfer line was set at 250°C, the quad at 150°C, and the source at 250°C.

Solid Phase Microextraction Gas Chromatography-Olfactometry (SPME GC-O)

Twenty milliliters of sample and 2 g of salt were measured into 40 ml amber vials (28 × 98 mm, Supelco, Bellefonte, PA) with a PTFE/Silicone septum (Supelco, Bellefonte, PA) and a stir bar. The vials were heated to 40°C for 30 min to allow headspace equilibration. A 3 phase (DVB/CAR/PDMS) SPME fiber (Supelco, Bellefonte, PA) was exposed to the sample for 30 min at a 2 cm depth. The fiber was injected onto an Agilent 6850 gas chromatograph–flame ionization detector (GC-FID) with an olfactometer port (Agilent Technologies, Santa Clara, CA). Samples were evaluated in duplicate by two panelists each with over 50 h experience evaluating post peak intensity. Samples were evaluated on both a polar ZB-WAX (30 –m length × 0.25-mm i.d. ×0.25-μm df) and a nonpolar ZB-5 ms (30 –m length × 0.25-mm i.d. ×0.25-μm df) (Phenomenex Zebron, Torrance, CA) column. The GC method began with an initial temperature of 40°C for 3 min, then temperature increased at a rate of

10°C/min until 150°C was reached. The rate changed to 30°C/min as temperature was raised to 200°C, which was then held for 5 min. Deactivated fused silica capillaries (1 m length × 0.25-mm i.d.) (Phenomenex Zebron, Torrance, CA) were used to split the column effluent evenly between the FID and the sniffing port. The FID sniffing port was held at 300°C with a He carrier gas flow of 1018.6 cm s -1, and humidified air passed through the port at 30 mL/min.

Identification of Odorants

Identification of compounds was based on a comparison of odor properties, retention indices, and/or mass spectra of the unknowns against authentic standards injected under identical conditions. Retention indices were calculated with the use of an alkane series (Fluka, Buchs, Switzerland) (Van den Dool and Kratz, 1963). For the identification of aroma active compounds, authentic standards were injected on both polar and nonpolar columns to compare the odor properties, retention indices and spectra of unknown compounds. Compounds, methanethiol, 3,5-octadien-2-one, and 2-octyl furan, were tentatively identified based on odor properties and retention indices from the literature since standards were not readily available during testing.

Quantification of Odorants

Compounds that showed significant differences between treatments by relative abundance were selected for quantification with external standard curves. Authentic standards were used to generate 4 point standard curves for selected compounds of interest. Compounds, dimethyl sulfide, 2,5-octanedione and 2-pentyl furan were substituted for methanethiol, 3,5-octadien-2-one and 2-octyl furan respectively; to approximate chemical

properties of the unavailable compounds. Standard curves were used to calculate compound concentration. Solutions were made to span 0 to 200 ppb in neutral and acidified (see Acidified (A) samples) liquid whey retentate (23.0% solids, 19.55% protein) diluted to 9% (w/v) protein with HPLC grade water (EMD Chemicals Inc., Gibbstown, NJ). Cold (below 10^{o}C) ultrafiltered, liquid whey retentate from unbleached Cheddar whey was provided by the North Carolina State University Dairy Research Laboratory (Listiyani et al., 2011). Cold ultrafiltered retentate was chosen as a matrix with protein composition similar to WPI that also contained substantially lower concentrations of the volatiles of interest. External standard curves were evaluated by SPME GC-MS in SIM mode.

Statistical Analysis

WPI variation was inherent to the study due to the selection of commercial WPI from two manufacturers. Therefore, data from both suppliers was averaged in descriptive, gas-chromatography olfactometry and gas-chromatography mass spectrometry analyses to better investigate how beverage processing steps impacted WPI flavor; independent of manufacturer.

Proximate analyses, sensory and instrumental results were analyzed using XLSTAT statistical software (Addinsoft, New York, NY). Analysis of variance and principal component analysis were conducted to test significant differences between NI and I WPI after treatments (no treatment, acidified, heated, and acidified and heated) and how samples were differentiated across sensory and instrumental measurements.

RESULTS AND DISCUSSION

Preliminary and Baseline Analyses

Proximate Analyses. There were compositional differences among the WPI produced by different suppliers ($p \leq 0.05$; Table 1): fat content varied between 0.7– 1.8%, phosphorous content ranged from 1700 – 2400ppm, calcium content between 670 – 7200ppm, magnesium content between 94 - 860ppm, and sodium content between 1300 – 8700ppm. There were no statistical differences in protein (92 ± 0.43), moisture (4.6 ± 0.37 %), ash (2.7 ± 0.071), potassium (2300 ± 640) or sulfur (1100 ± 430) content ($p>0.05$). The differences in proximate analysis of WPI were likely due to different processing parameters at each manufacturing facility (Carunchia Whetstine et al., 2005) all values were within expected ranges (Holt et al., 1999). Although not a significant difference, I WPI had higher fat and phosphorous content than NI WPI. These results were expected because lecithin is a phospholipid and contributes these compounds to I WPI.

Volatile Free Fatty Acid Analysis. In preliminary testing, the descriptive panel documented soapy flavors in the untreated WPI. Volatile free fatty acid analysis was conducted to document any intrinsic differences in volatile free fatty acid composition. The relative abundance of volatile free fatty acids in WPI from different manufacturers was variable (Table 2). Interestingly, when compared to NI WPI, I WPI was on average 5 times higher in linoleic and 7 times higher in linolenic fatty acids. These two readily oxidized species are abundant in lecithin derived from plant sources and unmodified soy lecithin is approximately 30% linoleic acid and 3% linolenic acid (Anonymous, 1997; Lehninger et al.,

1993). Consistent with these results, soy is the most common lecithin type used in the whey protein industry and may contribute highly oxidized species or substrates to the protein ingredient.

Supplier differences are an unavoidable reality in the large span of manufactured WPI. Therefore, to better investigate the impact of beverage processing steps and instantization on WPI flavor, data from the multiple suppliers was combined. This provided more generalized trends to better understand how these factors impact WPI flavor; independent of manufacturer differences.

Impact of Beverage Processing Steps on Non-instant and Instant WPI

Descriptive Analysis. Descriptive sensory results reiterated the large impact of beverage processing steps on WPI flavor observed by Fox (2011); increased cardboard and sulfur flavors. The greatest impact on WPI flavor was the contribution of the various processing treatments; rather than instantization (Figure 2). Cardboard, potato/fatty, soapy and bitter taste attributes did not exhibit significant differences in treated WPI (Table 3). A closer look at the impact of agglomeration on WPI flavor, however, revealed lecithin as a potential source of grainy, grassy/hay and cucumber off flavors (Table 3).

Both C-I and A-I WPI had cucumber flavors not observed in the H-I or AH-I samples. Cucumber flavor has previously been attributed to lecithin oxidation products in WPI and WPC80 (Wright et al., 2009). This flavor was also observed in reduced-fat Cheddar cheese with granular soy lecithin; and was attributed to aldehyde compounds formed from the autoxidation and degradation of soy lecithin (Suriyaphan et al., 1999).

There were no significant differences observed after heating NI and I WPI; both were characterized by high overall aroma and sulfur intensities. The thermal degradation and release of sulfur containing volatiles had an impact on WPI flavor; perhaps overwhelming the impact of lecithin. The 2 main proteins in whey, alpha-lactalbumin and beta-lactoglobulin, degrade and release sulfur-containing amino acids which are purported to contribute cooked/sulfur flavors (Linden et al., 1999).

After acidification and heating, both AH-NI and AH-I developed soapy aftertaste. Probing of the sensory panel in follow up sessions described the soapy aftertaste as medium chain free fatty acid in nature. Free fatty acids are lipolysis products and have been previously documented in WPC80 and WPI (Carunchia Whetstine et al., 2005; Karagul-Yuceer et al., 2003; Lee et al., 1996; Tomaino et al., 2001). However, protein degradation and matrix pH have been shown to enhance the perceived intensity of FFA flavor (Adda et al., 1982). Matrix pH may have also impacted the differences observed between I and NI WPI by lowering the threshold for certain compounds. Suriyaphan et al. (1999) concluded that low pH corresponded to production of new aromas as well as increased volatility of certain aldehydes. Beverage processing steps collectively promoted soapy flavor in the WPI solutions. Acidification and heating steps also promoted grainy and grassy/hay flavors in AH-I WPI. Grainy and grassy flavors were not observed before or after processing any of the NI samples. Bread-like notes have been previously documented in fermented milk and Cheddar cheese that contained lecithin (Drake et al., 1998; Suriyaphan et al., 2001). The fermented milk and Cheddar cheese were both subjected to subsequent heat treatment after the addition of lecithin; suggesting the impact of thermal processing in the formation of

grainy flavors. Suriyaphan et al. (2001) also noted hay/grassy notes in the commercial lecithin before it was included in fermented milk. This attribute likely originated from the lecithin and was enhanced by the processing steps by thermal degradation and increased aromatic volatility.

Gas Chromatography Olfactometry. Consistent with descriptive results, a large impact on flavor from the processing treatments was observed by GC-O. Fifty-four aroma active compounds were detected by SPME GC-O of NI and I WPI (Table 4) and likely contributed to the flavor profiles of these WPI. Forty-four compounds were positively identified (detected mass spectra, retention index and odor properties compared to published literature) and 10 were tentatively identified (retention index and odor properties compared to published literature). The contribution of lecithin to WPI flavor was much smaller than the contribution of the beverage processing steps. Therefore, GC-O analysis did not show consistent trends between I and NI WPI. Thirty-nine compounds were detected in C-NI; compared to 35 detected in C-I. Forty-six compounds were detected in both A-NI and A-I and 34 compounds were detected in both H-NI and H-I. Panelists detected 43 compounds in AH-NI compared to 44 detected in AH-I. However, only one compound was detected in instantized samples. Compound 2-acetyl-2-thiazoline was detected in C-I and H-I samples. This thiazoline is a cysteine degradation product (Hofmann and Scheiberle, 1995) suggesting instantized WPI is more susceptible to formation of protein degradation compounds. These results also suggest the processing steps, especially thermal processing, may be entrapping certain volatile compounds within the denatured protein matrix. Temperatures around 80^{o}C are high enough to induce WPI gelation; but all samples were stirred during processing.

Increased hydrophobic reactions between protein molecules and volatiles may have bound and prevented the release of volatile compounds (Foegeding et al., 1998). However, these treated samples were differentiated by sensory profiles. Matrix pH may have also impacted the differences observed between NI and I WPI. Research has shown that low pH corresponds to production of new aromas and increased volatility of certain aldehydes (Suriyaphan et al., 1999). Quantitative instrumental analysis (GC-MS) was conducted to better account for the sensory differences observed within these samples.

Gas Chromatography Mass Spectrometry. In the control and after acidification, C-I and A-I WPI were higher than their NI counterparts in E-2-nonenal ($p \leq 0.05$; Table 5); an autoxidation product of linoleic fatty acid known to contribute cucumber/fatty flavors in instantized dairy products (Burdock, 2010; Drake et al., 2009; Hoffmann, 1962; Suriyaphan et al., 2001; Wright et al., 2009). This result was expected as I WPI contained approximately 5 times more linoleic acid than NI WPI (Table 2) and soy lecithin is abundant in this fatty acid (Lehninger et al., 1993). Linoleic acid is a precursor for Z-3-nonenal which becomes E-2-nonenal after shifting of a double bond (Hoffmann, 1962). Commercial lecithin has been reported to contain methypropanal, hexanal, and E-2-nonenal (Stephan and Steinhart, 1999). At concentrations above its threshold of 0.1 ppb (Burdock, 2010) in water, it could account for the cucumber flavor detected in both C-I and A-I samples (Table 3). Additionally C-I was higher than C-NI in DMDS, 2-phenethanol, E,Z-2,6-nonadienal ($p \leq 0.05$). Both DMDS and 2-phenethanol are formed from the degradation of amino acids methionine and phenylalanine; respectively (Carunchia Whetstine et al., 2005; Singh et al., 2003). They are known contributors of sulfur, brothy and unclean notes to WPI (Carunchia Whetstine et al.,

2005). E,Z-2,6-nonadienal is a lipid oxidation compound previously documented in dried whey ingredients with storage and agglomeration (Carunchia Whetstine et al., 2005; Leksrisompong et al., 2010; Wright et al., 2009).

In addition to differences in E-2-nonenal, A-I WPI was lower in furaneol and gamma-octalactone, but higher than A-NI in 2-phenethanol. Furaneol and gamma-octalactone contribute sweet aromatic and milky notes to WPI (Carunchia Whetstine et al., 2005). This result is important to flavor in that sweet aromatic and milky are "dairy flavors" desired in a WPI (Drake et al., 2003); so the flavor of A-I WPI may be less desirable than the flavor of A-NI WPI. However, this trend was not observed in AH-I WPI. As observed with C-I, 2-phenethanol was higher in A-I WPI. However, the increase in concentration from the control, suggests that the acidification step further promoted the formation of this protein degradation compound.

After heating, I and NI WPI were not perceived as different by sensory analysis (Table 3); but I WPI was higher in E-2-nonenal and E-2-dodecenal ($p \leq 0.05$; Table 5). To our knowledge, E-2-dodecenal has never been documented in WPI, but is purported to contribute rancid oil and fatty notes to beef and olive oil after heat induced oxidation (Kanavouras and Hernandez, 2006; Resconi et al., 2012). E-2-dodecenal is a compound in the essential oil extracted from seeds and plants (Deterre et al., 2011; Matasyoh et al., 2009; Singh et al., 2008). Protein degradation not only releases sulfur compounds but exposes reactive sites in the protein sequences that can bind other volatile compounds (Gierczynski et al., 2007; Kapoor and Metzger, 2008; Kühn et al., 2006; Weel et al., 2003). The two major whey proteins are beta-lactoglobulin and alpha-lactalbumin. Few studies have investigated the

binding abilities of alpha-lactalbumin, but it is believed to have a lower affinity for flavor compounds (Kühn et al., 2006). Beta-lactoglobulin is reported to have 2 separate binding sites for aroma compounds. For example, fatty acids and retinol bind to the hydrophobic, central cavity of the molecule whereas others bind to weaker, secondary sites along the outer surface of the protein (Kühn et al., 2006; Weel et al., 2003). Although E-2-nonenal and E-2-dodecenal were significantly higher in H-I WPI, entrapment in the denatured protein could have increased their threshold values and hindered detection by sensory analysis.

The impact of acidification and heating on instant WPI resulted in grassy/hay and grainy notes (Table 3). AH-I WPI were higher in methanethiol, E,E-3,5-octadien-2-one and 2-phenethanol ($p \leq 0.05$; Table 5) which are perceived as sulfurous, grassy/hay and honey/yeasty; respectively (Belitz and Grosch, 1999; Burdock, 2010). Although below its threshold of 150 ppb, the grassy/hay note detected in AH-I could be contributed by the presence of 3,5-octadien-2-one; a known derivative of linoleic oxidation (Belitz and Grosch, 1999; Burdock, 2010). This compound may originate in the lecithin as Suriyaphan et al. (2001) noted hay/grassy notes in the commercial lecithin before addition to milk. However, this compound only increased between AH-NI and AH-I WPI; suggesting the combination of acidification and heating promoted lipid oxidation to a higher extent than the individual beverage processing steps alone. Both 2-phenethanol and methanethiol have been identified in dairy products previously, and are produced from the degradation of amino acids phenylalanine and methionine; respectively (Carunchia Whetstine et al., 2005; Singh et al., 2003). The grainy flavor observed in AH-I WPI could be contributed by 2-phenethanol present above its detection threshold of 0.015 ppb (Burdock, 2010). A bread-like attribute has

been detected in instantized dairy products (Drake et al., 1998; Suriyaphan et al., 2001) and this was believed to be due to increases in E,E-2,4-decadienal (Suriyaphan et al., 1999). However, this compound showed no difference between corresponding I and NI WPI. Therefore, more research is needed to verify a direct link between these compounds and the attributes observed in AH WPI.

Acid induced conformational changes to α-lactalbumin and β-lactoglobulin may explain the flavor differences observed in A-NI and A-I WPI. In its native state, Ca^{2+} is held within a high affinity binding site of α-lactalbumin. When pH is lowered to pH 3.0, Ca^{2+} dissociates leaving β-carboxy groups and hydrophobic domains available for protonation and other reactions (Hunt and Dalgleish, 1994; Permyakov et al., 1985). Acidification promotes volatile formation by exposing new reactive sites within the WPI that can interact with lipids, sugars and other proteins to produce volatiles. This could help explain increased concentrations of amino-acid degradation product, 2-phenethanol, in A-I WPI (Table 5). Instant WPI contained more fat (Table 1) and higher concentrations of highly reactive unsaturated fatty acids (Table 2). Therefore, it is likely instant WPI provided more lipid constituents to react with the denatured protein.

Protein degradation can also occur from thermal processing. In native conformation, disulfide bonds help to stabilize both α-lactalbumin and β-lactoglobulin (Monahan et al., 1995). Thermal denaturation can destroy disulfide bonds and promote the formation of sulfur containing compounds. Frankel (1998) attributed cooked flavor in dairy products to sulfhydryl reducing compounds formed from β-lactoglobulin degradation. Sulfhydryl

reducing compounds are the likely sources for the cooked/sulfur aromas detected in H-NI and H-I WPI (Table 3).

The combination of acidification and heating may have a synergistic effect on volatile formation in WPI. Lipid degradation may be induced by both acidification and heat treatments (Frankel et al., 1998). This may help to explain the higher concentrations of 3,5-octadien-2-one in AH-I (Table 5). The higher lipid content in I WPI may also contribute to the formation of new compounds after AH treatment. A model system containing ribose and cysteine produced multiple sulfur-containing compounds with the addition of phospholipids and triacylglycerols (Farmer and Mottram, 1990). This may explain the increased concentrations of methanethiol and 2-phenethanol observed in AH-I WPI (Table 5).

Instantization of WPI with soy lecithin impacts WPI flavor and flavor stability. This effect has been previously documented by Wright et al. (2009) and our results confirm this initial effect on flavor. Results from the current study also suggest that soy lecithin may also contribute additional flavors in beverage processing. The use of unsaturated wetting agents or steam agglomeration may counter balance these effects. However, beverages manufacture steps had large effects on whey protein flavor, regardless of instantization. These effects may be alleviated by sweetener and flavoring ingredients within the beverages to eliminate or mask the off-flavors of treated WPI. Additionally, future research is needed to optimize processing treatments for both product functionality and flavor. Acidification of WPI before instantization and drying steps may volatilize flavor compounds by increasing matrix polarity. Hydrophobic volatiles may then be released from the matrix and create better flavored WPI ingredient for beverage application.

CONCLUSIONS

The use of instantized WPI promoted cucumber flavor and higher concentrations of E-2-nonenal and 2-phenethanol to acidified WPI. Additionally grainy aroma, grassy/hay aroma, methanethiol, E,E-3,5-octadien-2-one, and 2-phenethanol were higher in instant WPI after AH beverage processing steps. These undesirable flavors can carry over into ingredient applications, presenting a challenge to manufacturers. Further research is necessary to discover alternative processing treatments, alternative forms of lecithin and/or complementary ingredients to remove, lessen, or mask the off-flavors of treated WPI.

TABLES

Table 1. Proximate analysis of instant (I) and non-instant (NI) WPI

	WPI			
	1-NI	1-I	2-NI	2-I
Protein (%, dry weight basis)	92[a]	92[a]	93[a]	92[a]
Fat (%, dry weight basis)	0.90[ab]	1.8[b]	0.70[a]	1.2[ab]
Moisture (%)	5.1[a]	4.5[a]	4.8[a]	4.1[a]
Ash (%, dry weight basis)	2.7[a]	2.8[a]	2.6[a]	2.7[a]
Phosphorous (mg/kg)	2300[bc]	2400[c]	1700[a]	1900[ab]
Calcium (mg/kg)	7200[b]	6900[b]	670[a]	820[a]
Magnesium (mg/kg)	840[b]	860[b]	94[a]	120[a]
Potassium (mg/kg)	2800[a]	3100[a]	1600[a]	1800[a]
Sulfur (mg/kg)	11000[a]	10000[a]	11000[a]	11000[a]
Sodium (mg/kg)	1300[a]	1300[a]	8700[b]	8700[b]

Statistical analysis with means separations using Fisher's (LSD) with 95% confidence interval. [a-c] Means in a row not followed by the same letter are different ($p \leq 0.05$). Means represent duplicate lots from 2 suppliers.

Table 2. Volatile free fatty acid concentrations (ppb) in non-instant (NI) and instant (I) WPI

	WPI			
	1-NI	1-I	2-NI	2-I
Butyric	0.0020[b]	0.0020[b]	0.0050[ab]	0.0070[a]
Hexanoic	0.013[a]	0.0050[a]	0.020[a]	0.021[a]
Octanoic	0.0080[b]	0.032[a]	0.030[a]	0.028[a]
Decanoic	0.11[a]	0.10[a]	0.15[a]	0.18[a]
Dodecanoic	0.74[a]	0.60[a]	0.58[a]	0.60[a]
Tetradecanoic	3.0[a]	2.1[a]	2.4[a]	2.1[a]
Hexadecanoic	5.5[a]	5.5[a]	6.7[a]	9.1[a]
Octadecanoic	0.54[b]	0.63[b]	2.8[a]	3.2[a]
Oleic	2.1[b]	2.3[b]	5.6[a]	7.2[a]
Linoleic	0.18[b]	1.7[b]	2.0[b]	9.2[a]
Linolenic	0.045[b]	0.16[ab]	0.095[ab]	0.83[a]

Statistical analysis with means separations using Fisher's (LSD) with 95% confidence interval. [a-b] Means in a row not followed by the same letter are different ($p \leq 0.05$). Means represent duplicate lots from 2 suppliers.

Table 3. Sensory attribute means of instant (I) versus non-instant (NI) WPI after beverage processing steps

		Sensory attribute[2]											
Treatment[1] and WPI type		Aroma intensity	Cardboard	Potato/ Fatty	Cooked/ sulfur	Soapy	Grain	Grassy/ hay	Cucumber	Astringency	Sour	Bitter	Free fatty acid aftertaste
C	NI	1.7^{d}	1.7^{a}	0.5^{a}	ND	1.9^{a}	ND	ND	ND	2.3^{b}	ND	0.5^{a}	ND
C	I	1.9^{cd}	1.7^{a}	0.6^{a}	ND	1.9^{a}	ND	ND	0.5^{ab}	2.3^{b}	ND	0.6^{a}	ND
A	NI	2.6^{bcd}	2.4^{a}	0.9^{a}	ND	1.9^{a}	ND	ND	ND	3.5^{ab}	5.0^{a}	ND	ND
A	I	2.8^{bc}	2.6^{a}	ND	ND	1.6^{a}	ND	ND	1.4^{a}	3.5^{ab}	5.0^{a}	ND	ND
H	NI	4.3^{a}	2.1^{a}	ND	2.3^{a}	1.8^{a}	ND	ND	ND	3.9^{a}	ND	ND	ND
H	I	4.2^{a}	2.4^{a}	ND	1.7^{a}	1.8^{a}	ND	ND	ND	3.8^{a}	ND	ND	ND
AH	NI	2.9^{b}	2.4^{a}	ND	ND	2.9^{a}	ND	ND	ND	4.5^{a}	4.9^{a}	0.7^{a}	1.6^{a}
AH	I	3.2^{b}	2.3^{a}	ND	ND	2.4^{a}	1.9	1.7	ND	4.5^{a}	5.0^{a}	0.7^{a}	1.5^{a}

[a-d] Means in a column not followed by common letters are different (Fisher's LSD, $p \leq 0.05$). Means represent the average of duplicate lots obtained from 2 suppliers. [1]C = control; A = acidified; H = heated; AH = acidified and heated; N = non-instant WPI; I = instant WPI. [2]Attributes scored using a 0-15 pt universal intensity scale (Meilgaard et al., 1999) using an established dried ingredient lexicon (Wright et al., 2009). [3]ND = mean scores below 0.5 were noted as not detected.

Table 4. Presence of aroma active compounds detected in non-instant (NI) and instant (I) WPI samples after beverage processing steps

Aroma	Compound	Identification Method[a]	RI (ZB-5)[b]	RT (ZB-Wax)[c]	C-NI[d]	C-I	A-NI	A-I	H-NI	H-I	AH-NI	AH-I
Sulfur pumpkin	DMS	RI,O,MS	<600	<600	1.9	1.8	1.6	1.6	2.1	1.9	1.7	2.0
Sulfur	Methanethiol	RI,O	<600		1.2	0.8	1.4	1.6	1.5	ND[e]	1.6	1.4
Ether	2-butanone	RI,O,MS	<600	<600	0.8	0.8	1.9	1.9	ND	1.3	1.4	1.8
Malt	Butanal	RI,O,MS	<600		1.2	1.6	1.0	1.0	1.3	1.7	1.1	1.4
Buttery	2,3-butanedione	RI,O	<600	972	2.2	2.2	1.7	1.7	2.0	2.0	1.6	1.6
Sour vinegar	Acetic acid	RI,O	617	1340	0.8	ND	0.8	0.8	0.9	0.8	0.5	ND
Malty chocolate	3-methylbutanal	RI,O,MS	618	915	1.7	1.7	1.7	1.8	1.2	1.6	1.6	1.6
Garlic	Methylethylsulfide	RI,O	620		2.1	1.6	1.4	1.6	1.7	2.2	1.8	2.0
Fruity, grassy	Ethyl propionate	RI,O,MS	709	948	0.5	ND	1.1	0.6	ND	0.7	0.8	ND
Onion cabbage	DMDS	RI,O,MS	749	1073	ND	ND	0.6	0.6	ND	ND	ND	ND
Rubbery	1-hexen-3-one	RI,O, MS	754	1012	ND	ND	1.2	1.7	0.8	ND	1.3	1.9
Cheesy, rancid	Butyric acid	RI,O	768	1611	ND	ND	1.2	ND	ND	ND	0.5	ND
Grass, tallow, fat	Hexanal	RI,O,MS	795	1082	1.9	1.9	1.7	1.4	1.9	1.8	1.4	1.6
Cheesy, dried fruit, ffa	3-methyl butyric acid	RI,O	840	1777	0.6	0.6	1.0	1.7	ND	ND	1.5	1.3
Fruity green	Isopropyl butanoate	RI,O,MS	843	1162	0.9	ND	0.8	0.6	ND	ND	ND	ND
Cooked nutty	2-methyl-3-furanthiol	RI,O,MS	865	1317	1.8	2.0	2.1	2.0	2.0	1.7	2.1	2.3
Fishy fatty	Z-4-heptenal	RI,O	896	1191	2.4	2.1	2.2	2.0	2.0	1.5	2.1	2.5
Fat citrus rancid sour	Heptanal	RI,O,MS	900	1185	1.0	ND	ND	ND	0.6	ND	ND	ND
Potato musty	Methional	RI,O,MS	906	1468	1.3	1.3	1.8	2.0	2.1	2.1	2.0	2.2
Popcorn	2-acetyl pyrroline	RI,O	919	1343	1.5	2.1	0.7	1.3	2.0	1.5	0.6	1.1
Garlic cabbage	DMTS	RI,O,MS	966	1382	2.4	2.4	2.4	2.2	2.4	2.0	2.0	2.0
Mushroom metallic	1-octen-3-one	RI,O,MS	975	1301	1.9	2.1	1.8	2.1	1.9	1.7	1.6	1.8
Sweet fruity metallic	2-pentyl furan	RI,O	991		ND	ND	0.6	0.5	ND	ND	2.0	1.2
Citrus, fatty sweet	Octanal	RI,O,MS	1001	1294	2.1	2.2	2.1	2.0	2.3	1.7	2.2	2.0
Sweaty (brothy)	Hexanoic acid	RI,O	1014	1826	1.1	0.9	0.7	0.5	0.8	0.8	0.8	1.1

[a] Method of identification by retention indices (RI), olfactometry (O) and comparison to mass spectra (MS) of authentic standards. [b]Retention indices of the aroma event on the ZB-5 column. [c]Retention indices of the aroma event on the ZB-WAX column. [d] C=control, A= acidified, H=heated, AH=acidified and heated, NI=non-instantized, I=instantized WPI. [e] Not detected (ND) indicates the mean was below 0.5. Means represent the average of duplicate lots obtained from 2 suppliers.

Table 4 Continued

Aroma	Compound	Identification Method[a]	RI (ZB-5)[b]	RT (ZB-Wax)[c]	C-NI[d]	C-I	A-NI	A-I	H-NI	H-I	AH-NI	AH-I
Bandaid, cooked	2-acetyl pyrazine	RI,O	1023		ND[e]	ND	ND	0.5	ND	ND	ND	ND
Rosy fatty	Phenylacetaldehyde	RI,O,MS	1042	1647	0.9	0.8	0.8	1.2	1.1	ND	ND	0.8
Caramel	Furaneol	RI,O,MS	1061	1451	ND	ND	0.8	1.2	ND	0.5	0.9	1.4
Cooked, burnt rubber	2-acetyl pyrrole	RI,O	1064		1.1	0.6	1.4	0.7	0.5	ND	1.9	1.4
Smoky	Guiacol	RI,O,MS	1081	1524	1.9	1.9	1.4	1.6	2.1	1.8	1.8	1.7
Sweet fatty; fruity	2-nonanone	RI,O,MS	1092	1382	2.1	1.6	1.2	1.8	1.2	1.3	2.0	0.6
Phenol carpet	p-cresol	RI,O	1099		2.0	0.9	2.0	1.7	1.9	1.5	1.6	1.9
Citrus fatty	Nonanal	RI,O,MS	1104	1376	1.0	1.4	0.9	0.8	0.5	0.8	1.1	0.8
Roasted cooked	2-acetyl-2- thiazoline	RI,O,MS	1104	1737	ND	0.5	ND	ND	ND	0.8	ND	ND
Rosy fruity	2-phenethanol	RI,O,MS	1144	1926	2.0	1.5	1.8	0.8	1.7	0.9	1.0	0.7
Fatty	Z-2-nonenal	RI,O,MS	1144	1570	ND	ND	1.0	1.0	ND	ND	1.5	1.3
Cucumbers	E,Z-2,6-nonadienal	RI,O,MS	1153	1558	2.8	2.3	2.3	2.0	1.8	1.0	1.9	2.0
Carpets cucumbers	E-2-nonenal	RI,O,MS	1156	1527	2.8	2.7	2.7	2.7	2.2	1.9	2.5	2.6
Bell pepper earthy	2-isobutyl-3-methoxypyrazine	RI,O,MS	1164	1690	ND	ND	ND	ND	0.9	0.5	ND	ND
Brothy cooked nutty	Methyl-2-methyl-3-furyl disulfide	RI,O,MS	1173	1669	1.9	1.7	2.2	2.0	ND	0.7	1.5	1.9
Fatty, citrus, sweet	Decanal	RI,O	1194		1.8	1.1	0.6	1.0	0.6	0.7	0.6	1.4
Fatty hay	E,E-2,4-nonadienal	RI,O,MS	1215	1716	0.9	1.3	1.6	2.0	1.1	1.2	0.5	1.2
Garlic, burnt rubber	Dimethyl tetra sulfide	RI,O	1215		ND	ND	0.6	1.1	ND	ND	ND	1.0
Minty hay sweet	2-octyl furan	RI,O	1237		ND	ND	ND	ND	ND	0.5	0.5	1.3
Hay, waxy	E-2-decenal	RI,O,MS	1254	1642	0.9	0.8	1.4	0.6	0.6	0.9	1.1	0.5
Coconut, oatmeal	Gamma-octalactone	RI,O,MS	1278	1933	0.8	1.6	0.9	1.2	0.9	1.2	0.9	1.3
Fatty, fryer oil	2,4-decadienal	RI,O	1310		ND	ND	1.0	0.9	ND	ND	0.6	0.8
Grapy tortilla (glue fruity)	o-aminoacetophenone	RI,O,MS	1338	>2000	0.9	1.1	ND	ND	0.6	1.3	ND	ND
Cilantro fatty	Gamma-nonalactone	RI,O,MS	1358	>2000	ND	ND	1.0	1.5	ND	ND	1.3	1.2

[a] Method of identification by retention indices (RI), olfactometry (O) and comparison to mass spectra (MS) of authentic standards. [b]Retention indices of the aroma event on the ZB-5 column. [c]Retention indices of the aroma event on the ZB-WAX column. [d] C=control, A= acidified, H=heated, AH=acidified and heated, NI=non-instantized, I=instantized WPI. [e] Not detected (ND) indicates the mean was below 0.5. Means represent the average of duplicate lots obtained from 2 suppliers.

Table 4 Continued

Aroma	Compound	Identification Method[a]	RI (ZB-5)[b]	RT (ZB-Wax)[c]	C-NI[d]	C-I	A-NI	A-I	H-NI	H-I	AH-NI	AH-I
Cilantro	Decanoic acid	RI,O	1384		ND[e]	ND	ND	ND	ND	ND	ND	0.5
Grainy soapy (glue)	E-2-dodecenal	RI,O,MS	1443		ND	ND	0.9	0.8	ND	ND	ND	0.6
Coconut	Delta-decalactone	RI,O,MS	1495		0.6	0.5	ND	ND	ND	ND	0.8	0.8
Peach	Gamma-dodecalactone	RI,O,MS	1635		0.5	ND	1.0	1.3	0.5	ND	0.5	0.9
Fruity sweet; coconut	Delta-dodecalactone	RI,O,MS	1721		0.5	0.6	1.3	1.3	ND	ND	1.1	1.0

[a] Method of identification by retention indices (RI), olfactometry (O) and comparison to mass spectra (MS) of authentic standards. [b]Retention indices of the aroma event on the ZB-5 column. [c]Retention indices of the aroma event on the ZB-WAX column. [d] C=control, A= acidified, H=heated, AH=acidified and heated, NI=non-instantized, I=instantized WPI. [e] Not detected (ND) indicates the mean was below 0.5. Means represent the average of duplicate lots obtained from 2 suppliers.

Table 5. Concentration (ppb) of selected compounds in instant (I) and non-instant (NI) WPI by SPME GC-MS

	[1]C-NI	C-I	A-NI	A-I	H-NI	H-I	AH-NI	AH-I
Methanethiol (TID)*	1.26^{c}	0.930^{c}	14.6^{a}	16.6^{a}	4.40^{b}	6.64^{b}	8.42^{b}	13.6^{a}
2-butanone	0.0474^{a}	0.346^{abc}	0.160^{bc}	0.159^{bc}	0.122^{c}	0.152^{bc}	0.475^{a}	0.461^{ab}
Butanal	1.37^{b}	1.53^{ab}	1.63^{a}	1.58^{ab}	1.47^{ab}	1.51^{ab}	1.57^{ab}	1.62^{a}
Diacetyl	0.0610^{b}	0.0670^{ab}	0.0750^{ab}	0.0840^{a}	0.0730^{ab}	0.0570^{b}	0.0640^{b}	0.0700^{ab}
3-methylbutanal	4.22^{a}	5.14^{a}	4.78^{a}	4.56^{a}	3.92^{a}	4.44^{a}	4.42^{a}	4.48^{a}
Ethyl propionate	ND[2]	ND	0.0700^{a}	0.0690^{a}	0.0560^{ab}	0.0410^{b}	0.0570^{ab}	0.0620^{ab}
Butyric acid	0.0150^{a}	0.0150^{a}	0.0160^{a}	0.0160^{a}	0.005^{b}	0.005^{b}	0.0150^{a}	0.0130^{a}
1-hexen-3-one	0.0390^{a}	0.0390^{a}	0.0370^{a}	0.0360^{a}	0.0300^{ab}	0.0300^{ab}	0.0220^{b}	0.0280^{ab}
DMDS*	2.15^{b}	2.86^{a}	1.13^{d}	1.36^{cd}	1.85^{bc}	1.75^{bc}	0.557^{e}	0.601^{e}
Hexanal	1.93^{a}	1.63^{ab}	1.24^{abcd}	1.41^{abc}	0.503^{cd}	0.452^{d}	0.454^{d}	0.658^{bcd}
3-methylbutyric acid	0.007^{a}	0.007^{a}	0.006^{ab}	0.006^{ab}	ND	ND	ND	0.006^{ab}
Isopropyl butanoate	0.110^{a}	0.131^{a}	0.158^{a}	0.117^{a}	0.031^{b}	0.009^{b}	0.0140^{b}	0.200^{b}
Methional	7.74^{a}	7.96^{a}	7.31^{a}	7.01^{a}	0.690^{cd}	0.0250^{d}	1.00^{bc}	1.57^{b}
Z-4-heptenal	0.189^{b}	0.094^{b}	0.418^{a}	0.270^{ab}	0.238^{ab}	0.0970^{b}	0.0990^{b}	0.118^{b}
Heptanal	0.750^{a}	0.559^{ab}	0.750^{a}	0.630^{a}	0.291^{c}	0.226^{c}	0.361^{bc}	0.332^{bc}
Benzaldehyde	1.29^{abc}	1.59^{ab}	1.78^{a}	1.64^{ab}	0.378^{c}	0.403^{c}	0.619^{bc}	0.844^{abc}
DMTS	0.115^{bc}	0.231^{ab}	0.349^{a}	0.320^{a}	0.0720^{c}	0.0700^{c}	0.0880^{bc}	0.0750^{bc}
1-octen-3-one	0.111^{abc}	0.130^{a}	0.0900^{abc}	0.127^{ab}	0.0760^{bcd}	0.0640^{cd}	0.0270^{d}	0.0810^{abcd}
2-pentylfuran	4.27^{a}	4.09^{a}	4.16^{a}	4.12^{a}	2.55^{a}	1.61^{a}	2.67^{a}	3.07^{a}
Octanal	0.461^{a}	0.417^{a}	0.456^{a}	0.376^{ab}	0.260^{c}	0.141^{c}	0.181^{c}	0.224^{bc}
Phenylacetaldehyde	0.0610^{ab}	0.0450^{bc}	0.0880^{a}	0.0720^{ab}	0.0160^{cd}	ND	0.0170^{cd}	0.0280^{cd}
2-acetyl pyridine	ND	0.0100^{b}	0.0580^{a}	0.0110^{b}	ND	ND	ND	ND
E-2-octenal	0.985^{b}	3.80^{ab}	8.19^{a}	3.15^{ab}	0.481^{b}	1.79^{b}	0.846^{b}	2.28^{b}
Furaneol*	1430^{b}	6010^{b}	20700^{a}	6860^{b}	686^{b}	2860^{b}	1610^{b}	4990^{b}
E,E-3,5-octadien-2-one (TID[3])*	ND	ND	5.78^{a}	2.81^{bc}	ND	ND	0.396^{cd}	3.35^{ab}
2-nonanone	1.54^{a}	1.58^{a}	1.71^{a}	1.57^{a}	0.111^{b}	0.0900^{b}	0.464^{b}	0.605^{b}
p-cresol	ND	0.009a	ND	0.009a	ND	ND	ND	0.006b

[a-e] Means in a row not followed by common letters are different (Fisher's LSD, $p \leq 0.05$). Means represent the average of duplicate lots obtained from 2 suppliers. *Mean concentrations of volatile compounds by SPME GC-MS using SIM mode and four-point external standard curves; each made to a R^2 value of 0.92 or greater. Otherwise, means quantified with relative abundance as there were no differences between I and NI within a treatment. [1] Treatments are labeled C = control; A = acidified; H = heated; AH = acidified and heated. [2]ND = means less than 0.005ppb noted as not detected. [3]TID= compound tentatively identified; standard curve made with DMS (replaced methanethiol) and 2,5-octanedione (replaced E,E-3,5-octadien-2-one).

Table 5 Continued

	[1]C-NI	C-I	A-NI	A-I	H-NI	H-I	AH-NI	AH-I
Nonanal*	52.2^{a}	43.3^{b}	11.7^{d}	7.61^{d}	34.7^{c}	39.5^{bc}	6.62^{d}	7.13^{d}
2-phenethanol*	0.428^{d}	0.964^{c}	1.62^{b}	2.49^{a}	0.205^{d}	0.206^{d}	0.440^{b}	1.03^{c}
E-2-nonenal*	7.71^{de}	25.7^{bc}	16.1^{cd}	27.5^{b}	23.4^{bc}	62.4^{a}	5.31^{e}	7.90^{de}
E,Z-2,6-nonadienal*	33.7^{b}	45.7^{a}	0.394^{e}	0.577^{e}	10.6^{cd}	15.8^{c}	0.174^{e}	0.706^{de}
Ethyl octanoate	0.877^{bc}	0.884^{bc}	1.56^{a}	1.25^{ab}	0.947^{bc}	0.564^{cd}	0.204^{d}	0.616^{cd}
Decanal	0.308^{c}	0.309^{c}	0.621^{a}	0.515^{ab}	0.268^{c}	0.252^{c}	0.197^{c}	0.327^{bc}
2,4-nonadienal	0.018^{bc}	0.008^{cd}	0.0320^{a}	0.0230^{ab}	ND	ND	0.009^{cd}	0.0150^{bc}
2-octylfuran (TID[3])	ND	0.008^{a}	ND	0.007^{a}	ND	0.006^{ab}	ND	ND
Gamma-octalactone*	0.0953^{c}	0.113^{c}	0.526^{a}	0.342^{b}	0.199^{bc}	0.194^{bc}	0.112^{c}	0.247^{bc}
2,4-decadienal*	10.7^{a}	9.13^{ab}	0.0363^{c}	0.0221^{c}	9.49^{ab}	5.91^{b}	0.0158^{c}	0.0151^{c}
Gamma-nonalactone	0.133^{ab}	0.106^{abc}	0.164^{a}	0.122^{ab}	0.0920^{bc}	0.0550^{c}	0.0520^{c}	0.0870^{bc}
2-dodecanone	0.724^{ab}	0.559^{abc}	1.02^{a}	0.652^{ab}	0.392^{bc}	0.133^{c}	0.288^{bc}	0.259^{bc}
E-2-dodecenal*	14.6^{bc}	23.6^{b}	0.772^{c}	0.417^{c}	17.9^{b}	48.2^{a}	0.261^{c}	0.222^{c}
Beta-ionone	0.0200^{a}	0.0150^{ab}	0.0180^{ab}	0.0130^{b}	0.007^{c}	0.005^{c}	ND	0.005^{c}
Delta-decalactone	0.139^{a}	0.0820^{ab}	0.0180^{b}	0.0130^{b}	0.008^{b}	0.005^{b}	0.009^{b}	0.007^{b}
Gamma-dodecalactone	0.005^{a}	ND	0.005^{a}	ND	0.005^{a}	ND	ND	ND

[a-e] Means in a row not followed by common letters are different (Fisher's LSD, $p \leq 0.05$). Means represent the average of duplicate lots obtained from 2 suppliers. *Mean concentrations of volatile compounds by SPME GC-MS using SIM mode and four-point external standard curves; each made to a R^2 value of 0.92 or greater. Otherwise, means quantified with relative abundance as there were no differences between I and NI within a treatment. [1] Treatments are labeled C = control; A = acidified; H = heated; AH = acidified and heated. [2]ND = means less than 0.005ppb noted as not detected. [3]TID= compound tentatively identified; standard curve made with 2-pentylfuran.

FIGURES

Objective: Determine effect of lecithin on WPI flavor after acidification and heating

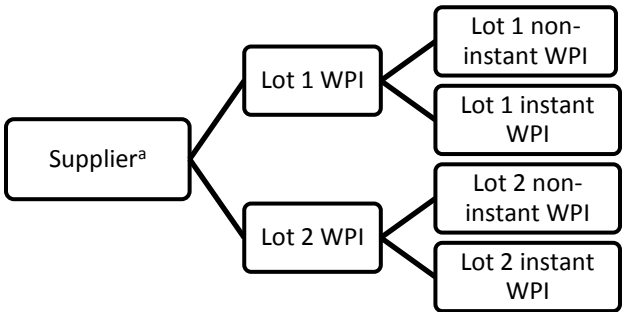

Figure 1. Obtained experimental samples of instant and non-instant WPI
[a]WPI obtained from 2 commercial suppliers (n=8).

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CHAPTER 3: THE ROLE OF DIFFERENT SWEETENERS ON WPI FLAVOR CONTRIBUTIONS IN ACIDIC PROTEIN BEVERAGES

The Role of Different Sweeteners on WPI Flavor Contributions in Acidic Protein Beverages

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Abstract

The modern consumer demands that food products have high nutritional value without sacrificing flavor. Among these products are high protein beverages that may contain low calorie sweeteners. Previous research has documented that processing steps in protein beverage manufacture may increase off flavors in the finished product. The objective of this study was to determine if different sweeteners impacted whey protein isolate (WPI) flavor contributions in acidified whey beverages after acidification and pasteurization. Duplicate lots of WPI were rehydrated to 10% solids (w/v) and sweetened with fructose, sucralose, or stevia followed by acidification to pH 3.2 and heat treatment at 85^{o}C for 30 sec. The experimental controls included unsweetened WPI, as well as deionized water sweetened with fructose, sucralose and stevia; all processed in the same manner. All treatments were evaluated by descriptive sensory analysis, solid phase microextraction gas chromatography-mass spectrometry and gas chromatography-olfactometry. The experiment was repeated in duplicate and statistical differences were analyzed using one-way analysis of variance. WPI with different sweeteners were distinct in sensory and volatile profiles ($p<0.05$). After acidification and heating, model beverages made with sucralose and stevia had higher soapy flavor and bitter taste than those sweetened with fructose; beverages with sucralose were also metallic. Model beverages made with sucralose and stevia also had decreased intensities of cabbage/brothy and potato/brothy flavors. However, these sensory differences were not confirmed by volatile analysis suggesting additional cognitive effects on sensory perception. After treatment, fructose AH WPI was the most similar to unsweetened AH WPI.

Fructose imparted sweetness without promoting additional off-flavor formation from the WPI. A comparison of sweetened water and sweetened WPI indicated that processing effects (heat and acidification) on the sweetener alone did not impart unique flavors. Sweeteners influenced sweet taste quality and enhanced soapy flavors contributed by WPI. These results demonstrate that sweetener type influences sensory properties (aromatics and basic tastes) of whey protein beverages.

Key Words: sweeteners, whey protein isolate, acidification, heat treatment, flavor

INTRODUCTION

The modern consumer demands that food products have high nutritional value without sacrificing flavor. The need for health and convenience has inspired food manufactures to create a new product category - functional foods (Burrington, 2005). Functional foods and beverages frequently contain a high quality protein, such as whey protein isolate (WPI; >90% protein), for its nutritional and functional properties. In addition to being a source of all essential amino acids, WPI can also reduce the need for emulsifiers and stabilizers in beverages (Burrington, 2005; Gottschalk, 2006; Haines, 2005); thereby reducing beverage manufacture costs. However, sensory properties of these foods and beverages are commonly influenced by protein-derived off flavors that carry through to the finished product. WPI can display cardboard, animal, soapy, cucumber and brothy flavors (Carunchia Whetstine et al., 2005; Drake, 2006; Wright et al., 2006; Wright et al., 2009) which are attributed to multiple variables along the whey manufacture process (Carunchia Whetstine et al., 2003; Carunchia Whetstine et al., 2005; Croissant et al., 2009; Mahajan et

al., 2004; Gallardo-Escamilla et al., 2005; Whitson et al., 2011; Wright et al., 2006; Wright et al., 2009). Not surprisingly cardboard, soapy, cabbage and potato flavors have also been documented in whey protein beverages (Childs et al., 2007; Evans et al., 2010; Wright et al., 2009). Childs and Drake (2010) concluded that flavors contributed by WPI were more offensive than astringency and basic tastes of acidic whey protein beverages and that these off-flavors decreased consumer acceptance. Despite health claims of meal replacement bars and beverages, products containing whey and soy protein were generally disliked by consumers (Childs et al., 2007).

Another challenge in creating a whey protein beverage is that heat treatment causes protein denaturation and aggregation; increasing the turbidity of the beverage. Consumers may perceive this turbidity as spoilage or decreased product quality. Methods to reduce the turbidity of heated whey protein beverages include acidification below pH 3.6 (Etzel, 2004). At pH above 3.8, careful ingredient selection is required to prevent turbidity. Therefore, whey beverages are typically acidified (pH < 4.6) for clarity and heat treated (88^{o}C for 120 s) for shelf stability (Prendergast, 1985; Rittmanic, 2006). While product clarity and heat stability have been optimized, previous research has documented that processing steps in acidic protein beverage manufacture promoted potato/brothy, cooked, cardboard and malty flavors derived from oxidation and protein degradation in the finished product (Fox et al., 2011). Post-processing of the beverage may further impair the flavor and acceptance of acidic whey protein beverages.

Whey protein beverages may also contain non-nutritive, low-calorie sweeteners to

further benefit calorie and sugar-conscious consumers. An array of sweeteners can be used and are typically selected to for desired calorie and flavor targets (Rittmanic, 2006). Beverage manufacturers may choose from sugars, sugar alcohols, artificial high-intensity sweeteners and natural high-intensity sweeteners (Rittmanic, 2006). In the current study, we selected a sugar (fructose), an artificial high-intensity sweetener (sucralose) and a natural high-intensity sweetener (stevia). As a monosaccharide, fructose readily participates in Maillard reactions once it is heated in the presence of protein. Giroux et al. (2010) demonstrated the generation of Maillard compounds in dairy beverages (pH 6.7) heated to 110^{o}C for 10 min. Fructose is approximately 1.2-1.8 times sweeter than sucrose (Vaclavik and Christian, 2008). Sucralose is a polychlorinated synthetic sweetener that is derived from sucrose to contain one monomer of 1-6-dichloro-1,6-dideoxyfructose and one monomer of 4-chloro-4-deoxygalactose (Rahn and Yaylayan, 2010). Sucralose is a chlorinated galacto-fructose because the stereochemistry of C-4, in the fructose moiety, reverses when it is chlorinated (Lee, 2000). It is approximately 400-800 times sweeter than sucrose (Vaclavik and Christian, 2008). Aqueous sucralose can undergo hydrolysis of the glycosidic bond under acidic conditions (Goldsmith and Merkel, 2001) and completely degrades at pH values of 3, 7 and 11 when heated to 180^{o}C (Hutchinson, 1996). The release of chloride ions as sucralose degrades was linked to the production of volatile compounds by Hutchinson et al. (1999). Stevia, a mixture of steviol glycosides extracted from the leaves of *Stevia rebaudiana*, is approximately 200-300 times sweeter than sucrose (Guggisberg et al., 2011; Soejarto et al., 1983). Stevia was shown to be quite heat and pH stable. Kroyer (1999) did not observe

degradation of stevia until temperatures exceeded 140^{o}C. Stevia was also pH stable; only degrading up to 5% over the range of pH 2-10. Kroyer observed the potential for degradation after heating aqueous stevia (5% stevia) to 80^{o}C and acidifying the solutions. After storing the aqueous stevia solutions for 4 months, Kroyer observed a 30% stevia loss in solutions containing phosphoric acid (pH 2.2) and a 22% loss in solutions containing citric acid (pH 2.1).

In the manufacture of acidic whey beverages, sweeteners are added before the post-processing steps (Rittmanic, 2006) with the assumption that they do not impart or contribute to off-flavor development. However, few studies have investigated the impact of sweeteners on flavor of finished products (Drake et al., 2000; Giroux et al., 2010; Hewson et al., 2008). Fewer still have studied the impact of high intensity sweeteners on the flavor of finished products (Cardoso and Bolini, 2008; Guggisberg et al., 2011). Hewson et al. (2008) added glucose, fructose and citric acid to a citrus-flavored model beverage. Perceived citrus flavor was enhanced with the addition of sweeteners and citric acid, although volatile concentrations decreased compared to an unsweetened control. Researchers suggested that the increased presence of solutes in the matrix changed the amount of free water and changed volatile partitioning. Hewson et al. (2008) also noted that the addition of citric acid suppressed the perceived sweetness of the model beverage made with fructose. Contrastingly, low levels of citric acid enhanced the sweetness of glucose sweetened beverages. Glucose- fructose blends were also shown to produce Maillard reaction products that served as antioxidants and decreased production of hexanal and propanal in heated milk

beverages (Giroux et al., 2010). Another study concluded that sweetener type (including sucrose, fructose and a sucrose-fructose blend) had no effect on the sensory properties of soy fortified yogurt (Drake et al., 2000). However, this product was not further processed after the addition of sweeteners. Cardoso and Bolini (2008) compared flavor profiles of peach nectar containing stevia, sucralose and sucrose. Although sweetener was added after batch pasteurization, an impact of sweetener was observed. Compared to the sucrose model, peach flavor and sweet intensity was decreased in both sucralose and stevia models; while stevia also contributed astringency, herb aroma and bitterness. Bitterness was also noted in stevia sweetened yogurt in addition to metallic, cardboard and musty off flavors (Guggisberg et al., 2011). However this study was able to mask some of the off-notes with the use of a product called Actilight™ (a fructooligosaccharide).

A search of the literature indicated that no studies have investigated the stability and possible interactions of different sweeteners during post-processing of acidified whey beverages. It is essential to understand the impact of sweeteners in functional foods for future formulation of a more appetizing product. Taste-aroma interactions as well as interaction with food ingredients may contribute to off-flavor enhancement or development in acidic whey protein beverages. This study sought to determine the impact of different sweeteners on WPI flavor contributions in beverages after acidification and pasteurization. Sensory and instrumental analyses were utilized to profile the flavors in unsweetened WPI as well as WPI sweetened with fructose, sucralose and stevia.

MATERIALS AND METHODS

Sample Acquisition and Manufacture

Duplicate lots of spray dried, non-instantized WPI manufactured from Cheddar whey, were supplied by a commercial WPI manufacturer. These samples were stored at -20°C upon receipt. All chemical standards used for identification were purchased from Sigma Aldrich (St. Louis, MO) with some exceptions: dimethyl sulfide, Z-4-heptenal, phenylacetaldehyde, octanal, and *o*-aminoacetophenone were obtained from Acros Organic (Morris Plains, NJ); nonanal, 1-hexen-3-one, 2-pentyl furan, δ-decalactone, and δ-dodecalactone were obtained from Alfa Aesar (Ward Hill, MA). 2-acetyl-2-thiazoline was obtained from Astatech, Inc (Bristol, PA); 2-acetyl-thiazole and 2-nonanone were obtained from SAFC Supply Solutions (St. Louis, MO). P-cresol, butyric acid and benzaldehyde were obtained from Fluka (Buchs, Switzerland).

To determine whether the use of fructose, sucralose and stevia impacted flavor of WPI, samples were formulated according to Table 1. Preliminary experiments were conducted to determine concentrations of fructose (Spectrum Chemical MFG Corp, New Brunswick, NJ), SPLENDA® sucralose (Tate and Lyle Sucralose LLC, Decatur, IL) and PureCircleAlpha Stevia (PureCircle Sdn, Bhd., Oak Brook, IL) that were equisweet to a sucrose sweet 10 intensity (Meilgaard, 1999) in deionized water. WPI were rehydrated with deionized water to 10% WPI solids (w/v) and were either left unprocessed (NT) or were acidified and heat treated (AH). Unsweetened WPI and sweetened deionized water (Table 1) were controls to determine the impact of AH treatment on the WPI and each sweetener alone.

Acidified and Heated (AH) WPI. To replicate typical practices in the functional beverage industry (Prendergast, 1985; Rittmanic, 2006), rehydrated WPI (approximately pH 6.5) was acidified to pH 3.2 using a 2M 1:1 blend of phosphoric (Mallinkrodt Chemical, Phillipsburg, NJ) and citric (J.T. Baker, Mallinkrodt Baker, Phillipsburg, NJ) acids. It was vital for comparison to retain 10% (w/v) WPI solids (9% w/v protein) in both the control and AH treated samples. Therefore, preliminary experiments were conducted to determine the volume of acid required; this volume was then subtracted from the total water added.

Samples were pasteurized at 85°C for 30 seconds using a benchtop high temperature short time (HTST) pasteurizer (Cole and Jones, 1990). The benchtop HTST contained a peristaltic pump (Masterflex L/S, Cole Parmer, Vernon Hills, Il) to pump sample through a 1/8" stainless steel coil (Cole Parmer, Vernon Hill, Il) submerged in a heated waterbath (VWR apparatus, West Chester, PA). The flow rate was 170 ml/min. Once the AH samples were heated, they were immediately cooled to 4°C in an ice bath (approximately 15 min).

Descriptive Analysis

All formulations were evaluated in duplicate by a trained panel (10 panelists, 8 females, 2 males, ages 23-50 yr) for flavor and aroma intensity using an established dried ingredient lexicon (Wright et al., 2009). Samples were poured into 60 mL soufflé cups (Solo Cup, Highland Park, IL), lidded and tempered to room temperature for 1 h before evaluation. Samples were assigned 3-digit codes and presented in a random order. Attributes were scaled using a 0 to 15pt universal Spectrum™ scale. Paper ballots or Compusense five version 4.8 (Compusense, Guelph, Ontario, Canada) were used for data collection.

Solid Phase Microextraction Gas Chromatography-Olfactometry (SPME GC-O)

Twenty milliliters of sample and 2g of salt were measured into 40ml amber vials (28 × 98 mm, Supelco, Bellefonte, PA) with a PTFE/Silicone septum (Supelco, Bellefonte, PA) and a stir bar. The vials were heated to 40°C for 30min to allow headspace equilibration. A 3 phase (DVB/CAR/PDMS) SPME fiber (Supelco, Bellefonte, PA) was exposed to the sample for 30min at a 2cm depth. The fiber was injected onto an Agilent 6850 gas chromatograph–flame ionization detector (GC-FID) with an olfactometer port (Agilent Technologies, Santa Clara, CA). Samples were evaluated in duplicate by two panelists each with over 50h experience evaluating post peak intensity. Samples were evaluated on both a polar ZB-WAX (30 –m length × 0.25-mm i.d. ×0.25-µm df) and a nonpolar ZB-5 ms (30 –m length × 0.25-mm i.d. ×0.25-µm df) (Phenomenex Zebron, Torrance, CA) column. The GC method began with an initial temperature of 40°C for 3min, then temperature increased at a rate of 10°C/min until 150°C was reached. The rate changed to 30°C/min as temperature was raised to 200°C, which was then held for 5min. Deactivated fused silica capillaries (1 m length × 0.25-mm i.d.) (Phenomenex Zebron, Torrance, CA) were used to split the column effluent evenly between the FID and the sniffing port. The FID sniffing port was held at 300°C with a He carrier gas flow of 1018.6 cm s -1, and humidified air passed through the port at 30 mL/min.

Solid Phase Microextraction Gas Chromatography-Mass Spectrometry (SPME GC-MS)

Volatile analyses of WPI samples and controls were conducted in triplicate by SPME GC-MS. Sample preparation and SPME GC-MS methods were adapted from Wright et al.

(2006). Five milliliters of each sample were measured into 20ml autosampler vials with silicon septa lined steel screw tops (Microliter Analytical, Suwanee, GA). An internal standard solution (81ppm 2-methyl-3-heptanone in methanol, Sigma-Aldrich, Milwaukee, WI) and 10% (w/v) sodium chloride (Fisher Scientific, Pittsburgh, PA) were added to the vials. Samples were introduced to the Agilent 6890N GC (Agilent Technologies, Santa Clara, CA) using a CTC Analytics CombiPal autosampler (Leap Technologies, Carrboro, NC). Samples equilibrated at 40°C for 25min before volatile extraction with a 3-phase fiber DVB/CAR/PDMS (Supelco, Bellefonte, PA) at 31mm for 30min, with 4sec pulsed agitation at 250rpm.

The GC method began with an initial temperature of 40°C for 3min, then temperature increased at a rate of 10°C/min until 90°C was reached. The rate changed to 5°C/min as temperature was raised to 200°C, which was then held for 10min. Finally the rate increased to 20°C/min to 250°C and temperature was held for 5min. The GC injector was programmed at 250°C and 7.06psi with He carrier gas and a purge flow of 169737cm/s. The GC was equipped with a ZB-5 ms column (30-m length × 0.25-mm i.d. × 0.25 μm df) (Phenomenex Zebron), the MS transfer line was set at 250°C, the quad at 150°C, and the source at 250°C.

Identification of Odorants

Compounds were identified by comparing retention indices, odor properties and/or mass spectra of the unknown compounds versus authentic standards measured under identical conditions. An alkane series (Fluka, Buchs, Switzerland) was used to calculate retention indices (Van den Dool and Kratz, 1963). Compounds not commercially available,

methanethiol and 2-octyl furan, were tentatively identified using retention index and odor properties from literature.

Quantification of Odorants

Compounds that showed significant differences, between sweetened AH WPI, by relative abundance were selected for further quantitation with external standard curves. Authentic standards were used to generate 4 point standard curves for selected compounds of interest. Solutions of each compound of interest were made within the range of 0 to 200ppb in neutral and acidified (pH 3.2) liquid whey retentate (23.0% solids, 19.55% protein) diluted to 9% (w/v) protein with HPLC grade water (EMD Chemicals Inc., Gibbstown, NJ). The North Carolina State University Dairy Research Laboratory provided cold (approximately 10^{o}C) ultrafiltered, liquid whey retentate from unbleached Cheddar whey (Listiyani et al., 2011). Retentate was chosen as a matrix with protein composition similar to WPI but contained substantially lower levels of the compounds of interest. External standard curves were evaluated by SPME GC-MS in SIM mode.

Statistical Analysis

Analysis of variance and principal component analyses were conducted on instrumental and sensory results to determine if treated samples were statistically different. Statistical analysis was performed using XLSTAT statistical software (Addinsoft, New York, NY).

RESULTS AND DISCUSSION

Descriptive Analysis

Fructose. Sensory analysis was conducted on fructose sweetened WPI after AH treatment (Fructose AH). As expected, fructose AH was sweeter and less sour than unsweetened AH WPI ($p \leq 0.05$; Table 2). This could be explained by the psychophysical interactions of modality and their impact on taste. In studies of human psychophysics, Moskowitz (1972) concluded that highly concentrated mixtures of sweet (glucose or fructose) or salty (NaCl) compounds developed "unblended" or "clashing" tastes in which the attributes seemed to compete to dominate perception. An increase in sweet intensity most likely decreased the perception of sour taste. However, fructose AH WPI did not differ from unsweetened AH WPI in any aromatic attribute ($p \leq 0.05$). Fructose may be a desired sweetener because it contributes sweet taste without enhancing off-flavors in the whey protein.

Descriptive analysis was also conducted on sweetened deionized water samples to document the effect of acidification and heating on each sweetener – separate from the whey protein. Aqueous fructose solution was characterized by high aroma intensity; sweet in nature (Table 3). However, all other documented flavor attributes were basic tastes and mouthfeel attributes. After processing, aqueous fructose solution was characterized with low astringency and high sweet taste. Results suggest aqueous fructose does not contribute off-notes after AH treatment.

Other flavor impacts of fructose were observed, although they did not carry over into the AH treated WPI. Fructose NT WPI had higher sweet aromatic and lower soapy flavor

than unsweetened NT WPI ($p \leq 0.05$; Table 2). This result is congruent with enhanced citrus flavor reported by Hewson et al. (2008) in fructose sweetened, citrus model beverages. Researchers also noted decreased volatile concentration and attributed this to an increase in the total solutes in the matrix. An increase in the amount of solutes available to interact with free water in the system may hinder volatile partitioning. The addition of fructose solids to the WPI matrix could have enhanced sweet aromatic perception while hindering the perception of soapy flavor.

Sucralose. Compared to unsweetened AH WPI, sucralose AH WPI had lower potato/brothy and cabbage/brothy flavor but was higher in soapy, bitter and metallic attributes ($p \leq 0.05$; Table 2). The decrease in brothy attributes may be attributed to cross-modal interactions where increased sweet taste can decrease the perception of certain off-notes. Many studies have concluded that taste-aroma interactions are dependent on the congruency of the stimuli (Frank et al., 1993; Schifferstein, 1996; Stevenson et al., 1999). For example, most people would perceive fruit aroma and sweet taste as congruent while brothy aroma and sweet taste are less congruent. Stevenson et al. (1999) demonstrated sweet intensity enhancement in the presence of certain odors, but depression of sweetness in the presence of others. Cross-modal interactions may explain decreased perception of brothy aromas; however sucralose could have also bound the volatiles that contribute to this aroma. Instrumental analysis was performed to confirm whether volatile concentration decreased in the presence of sucralose. Bitter and metallic notes were expected in sucralose AH WPI, as bitter and metallic attributes are frequently documented attributes of high intensity

sweeteners (Cardoso and Bolini, 2008; Schiffman et al., 2000). However, soapy flavor has not been previously attributed to sucralose. Soapy flavor in dairy ingredients has been associated with increased lipolysis by-products (Carunchia Whetstine et al., 2005). Sucralose may have enhanced lipid degradation reactions to enhance the perceived soapy flavor.

Aqueous sucralose was characterized by high aroma intensity (sweet aroma), high astringency, bitter and metallic tastes (Table 3). This analysis further supports that sucralose is a potential source of bitter and metallic tastes. Astringency is another attribute previously detected in high intensity sweeteners (Cardoso and Bolini, 2008; Schiffman et al., 2000). Aqueous sucralose was formulated to have a sweet intensity of 10; however sweet intensity decreased slightly after AH treatment. The processing steps, especially the acidification, may contribute to the degradation of aqueous sucralose. Under acidic conditions, Goldsmith and Merkel (2001) also detected degradation of sucralose.

Analysis of sucralose NT WPI suggested soapy flavor was produced from interactions between sucralose and WPI only after AH treatments. Sucralose NT WPI had higher sweet aromatic and lower cabbage/brothy than unsweetened NT WPI ($p \leq 0.05$; Table 2); thus increasing desirable dairy notes and decreasing off-notes in WPI (Drake et al., 2003). Sweet aromatic was also detected in aqueous sucralose (Table 3); suggesting the ingredient itself may impart a sweet aroma. Decreased intensity of cabbage/brothy aroma, compared to unsweetened WPI, was observed in both sucralose AH and sucralose NT (Table 2). This result alludes to the ability of sucralose to either bind or mask brothy flavors. However, sucralose NT WPI was higher than unsweetened NT WPI in cardboard flavor and metallic taste (Table 2). An increase in cardboard flavor has been attributed to increased oxidation

products (Whitson et al., 2010); suggesting that sucralose may be a catalyst for oxidation which could be further catalyzed by beverage processing steps.

Stevia. Stevia AH WPI had lower cabbage/brothy intensity compared to unsweetened AH WPI ($p \leq 0.05$; Table 2). Similar to sucralose, cross-modal interactions or volatile binding may explain the decreased intensity of cabbage/brothy flavor. The addition of stevia to WPI also decreased astringency but increased bitter taste and soapy flavor when compared to unsweetened AH WPI. Bitter taste has been frequently associated with stevia (Cardoso and Bolini, 2008; Guggisberg et al., 2011; Schiffman et al., 2000). However, decreased astringency and increased soapy flavor were interesting results and to our knowledge have not been attributed to stevia. In unsweetened, fructose and sucralose WPI, astringency increased approximately 4 fold after AH treatment. Beecher et al. (2008) suggested that astringency was due to increased interactions between salivary and whey proteins; thus low pH and heat induced protein denaturation contributed to increased astringency. Stevia WPI was the exception to this trend; suggesting the processing treatment may have a unique impact on astringency contributed by stevia. Strong intermolecular interactions between stevia, WPI, and salivary proteins could explain the decreases in astringency observed in stevia AH WPI. Stevia may have prevented the protein-protein interactions that would have contributed to astringency.

Astringent and bitter attributes were detected after processing aqueous stevia (Table 3). Increased bitter taste and astringency have been previously documented in products that contained stevia (Cardoso and Bolini, 2008; Guggisberg et al., 2011). Stevia was the likely

source of these attributes in stevia AH WPI. Trends observed in aqueous stevia were also observed in stevia NT WPI; increased bitter taste and astringency ($p \leq 0.05$; Table 2). These results suggest the importance of AH treatment to the observed changes in soapy and cabbage/brothy flavors in stevia AH WPI.

Gas Chromatography Olfactometry (GC-O)

Of the 46 aroma-active compounds detected in WPI solutions, 30 compounds were positively identified (comparison of mass spectra, retention index, odor properties from published literature), 15 were tentatively identified (comparison of retention index and odor properties to published literature) and one compound remained unknown (Table 4). The impact of sweetener and processing treatment was apparent in frequency of detected compounds.

Fructose. Sixteen aroma-active compounds were detected in aqueous fructose after AH treatment (Table 5). The number of aroma compounds increased to 36 in fructose AH WPI (Table 4); a 9.1 % increase from the number of aroma-active compounds detected in unsweetened AH WPI. Increased detection of volatiles in fructose AH WPI may be due to volatiles contributed by the protein, but results suggest compounds may also be generated from fructose – WPI interactions during AH processing steps. Compared to fructose NT WPI, there was a 5.6% increase in volatiles detected in fructose AH WPI.

Sucralose. Twelve aroma-active compounds were detected in aqueous sucralose (Table 5) but this number increased to 30 in both sucralose NT WPI and sucralose AH WPI (Table 4). Volatiles in the commercial WPI most likely contributed to the increased

frequency of aroma active compounds in sucralose sweetened WPI. The addition of sucralose to AH WPI corresponded to a 9.1% decrease in detected volatiles; compared to unsweetened AH WPI (Table 4). GC-O results suggest that during beverage processing steps, sucralose may have the ability to bind or prevent the formation of volatiles in AH WPI.

Stevia. Aqueous stevia contained the fewest detected aroma-active compounds (14) possibly indicating high stability to processing treatments (Table 5). This trend was also observed in stevia AH WPI; in which only 25 aromatic compounds were detected (Table 4). The addition of stevia to AH WPI corresponded to a 24.2% decrease in detected volatiles compared to unsweetened AH WPI. A 28.2% decrease in volatiles was also observed between unsweetened NT WPI and stevia NT WPI. These results provided support for the binding or protective capabilities of stevia even before beverage processing steps. Perhaps this binding ability was decreased by AH treatment.

Gas Chromatography Mass Spectrometry (GC-MS)

Fructose. Compared to the other AH WPI (unsweetened AH, sucralose AH, stevia AH), fructose AH WPI contained the highest concentrations of hexanoic acid, furaneol and decanal ($p \leq 0.05$; Table 6). These compounds were previously documented in dried and liquid whey ingredients (Carunchia Whetstine et al., 2005; Karagul-Yuceer et al., 2003; Lee et al., 1996; Stevenson and Chen, 1996). Short-chain fatty acids, such as hexanoic acid, have a low odor threshold (1-10ppm) and usually contribute largely to flavor (Karagul-Yuceer et al.,

2003). At concentrations of approximately 1ppm, hexanoic acid likely did not induce a sensory response. Furaneol is produced from the reaction of a reducing sugar with protein to form Maillard products (Mahajan et al., 2004). At concentrations above its detection threshold, 0.03 – 60ppb (Burdock, 2010), furaneol contributes sweet aromatic and cooked/milky flavors to dairy products (Mahajan et al., 2004). This compound may not have been detected by the sensory panel because of the overwhelming contribution of other aromatics. Decanal is a lipid oxidation compound known to contribute fatty and oxidized aromas to dried ingredients at concentrations above its detection threshold of 0.1 – 6ppb (Burdock, 2010; Carunchia Whetstine et al., 2005). However, it is likely that volatiles contributed by the acidified and heated protein overwhelmed the sensory impact of decanal. Similar trends in hexanoic acid, furaneol and decanal were not observed before processing steps (fructose NT WPI) nor in the aqueous fructose (Table 7); suggesting the importance of AH treatment and WPI in the formation of these oxidation and Maillard browning compounds.

After AH processing, aqueous solutions of fructose contained high concentrations of 3-methyl butanal (malty), octanal (citrus green) and nonanal (fatty/citrus) (Table 7). Although the HTST apparatus was cleaned according to industry practices, these results suggest residual lipids and protein may have remained in the system. Octanal and nonanal are lipid oxidation compounds (Frankel et al., 1998); indicating increased lipid oxidation when fructose is AH treated. This result was unexpected as fructose is not a known oxidant. Upon closer examination of the provided fructose, the authors noted an allowance of up to 0.018% chloride in the product. Fukayama et al. (1986) demonstrated that in aqueous solutions,

chlorine is able to oxidize fatty acids. In some instances, chlorine is considered an acceptable food additive (Fukayama et al., 1986). However, chloride could have oxidized lipids from residual the WPI and these reactions may have been further catalyzed by the acidification and heat treatment. Heat treatment can also stimulate fructose to produce Maillard products by reacting with protein. The compound 3-methyl butanal is formed from the reaction of Maillard by-products and α-amino acids (Leksrisompong et al., 2010). It is likely that fructose reacted with residual whey protein to create Maillard browning products which continued to react to form 3-methyl butanal. The presence of these compounds could explain the sweet aromatic detected in aqueous fructose.

Volatile analysis of fructose NT WPI, compared to unsweetened NT WPI, provided further evidence of lipid oxidation and degradation enhancement in the presence of fructose. Fructose NT WPI contained significantly higher concentrations of E-2-dodecenal, Z-4-heptenal, 2,4-decadienal and gamma-octalactone ($p \leq 0.05$; Table 6). E-2-dodecenal, to our knowledge, has not been observed in dairy products previously. However, it has been attributed to lipid oxidation in thermally treated olive oil and beef (Kanavouras and Hernandez, 2006; Resconi et al., 2012). Z-4-heptenal and 2,4-decadienal are lipid oxidation compounds previously documented in WPI (Carunchia Whetstine et al., 2005). Gamma-octalactone, however is formed through non-oxidative degradation of lipids after heat treatment (Dimick et al., 1969). Higher concentrations of lipid degradation compounds in fructose NT WPI versus unsweetened NT WPI implicates fructose as a potential contributor of lipid oxidation products. However, these significant differences in volatile concentrations

were not detected by sensory analysis and likely did not contribute significantly to the flavor of fructose AH WPI.

Sucralose. Sensory analysis of sucralose AH WPI documented increased soapy flavor and decreased potato/brothy and cabbage/brothy attributes; compared to unsweetened AH WPI. Soapy flavor has been attributed to increased concentrations of octanoic acid, decanoic acid and/or long-chain free fatty acids (Carunchia Whetstine et al., 2005). However, neither octanoic acid nor decanoic acid changed in concentration ($p \leq 0.05$, Table 6). Increases in long-chain free fatty acids may have contributed the soapy flavor. An experiment conducted by Hannon et al. (2007) detected soapy flavor in Cheddar cheese containing significantly higher concentrations of C-6:0, C-8:0, C-12:0 and C18:0 fatty acids. Soapy flavor was not detected in Cheddar cheese containing higher concentrations of C-4:0, C-10:0, C-14:0, C-16:0 and C-18:1 fatty acids. GC-MS requires aromatic compounds to be volatilized before ionization and detection can occur; leading to the systematic exclusion of some high molecular weight compounds. Additionally, fatty acids have low affinity for both the SPME fiber (3-phase DVB/CAR/PDMS) and MS column (ZB-5) selected for analysis. For these reasons, large molecular weight free fatty acids would not have been readily detected by GC-MS methods used in this study. Sucralose AH WPI could have contained long-chain fatty acids that were not detected instrumentally. Of all AH WPI, sucralose AH WPI had the highest concentration of acetyl pyrazine ($p \leq 0.05$; Table 6). Acetyl pyrazine is thermally generated from the reaction of a reducing sugar and protein to form Maillard browning products which contribute typical dairy, cooked flavors to WPI (Carunchia Wheststine et al.,

2005; Mahajan et al., 2004). However, sucralose does not participate in Maillard browning reactions (Cross, 2004). This volatile was likely produced from interactions of WPI and residual lactose during the beverage processing steps (Carunchia Whetstine et al., 2005). Sucralose may enhance interactions between lactose and α-amino acids of WPI; however one would also expect increases in other Maillard reaction products. Hutchinson et al (1999) linked the release of chlorine molecules from sucralose to increased volatile formation; however more research may discover another mechanism to explain how sucralose can promote the formation of acetyl pyrazine. At concentrations below detection threshold (62ppb), this compound did not contribute to flavor (Burdock, 2010). Interestingly, this sample was not distinguished from unsweetened AH WPI by any other compound.
This result is further support that the decreases in potato/brothy and cabbage/brothy aromas were most likely due to cross-modal interactions and incongruence of sweet taste with brothy attributes.

Aqueous sucralose contained high concentrations of lipid oxidation compounds; heptanal, octanal, nonanal, decanal and 2,4-decadienal (Frankel et al., 1998) (Table 7). Thus, the use of sucralose may increase lipid oxidation reactions and by-products in WPI after beverage processing steps. At similar concentrations to aqueous fructose, these aldehydes could have contributed to the sweet aroma detected by sensory panelists. A slight decrease in sweet taste intensity was observed by sensory analysis in aqueous sucralose. Sweet intensity is a product of hydrophobic groups on the molecule stimulating the hydrophobic pocket of the sweet taste receptor (Robyt, 1998). Chlorine atoms within the sucralose molecules contribute more hydrophobic properties to the sweetener. Therefore, the decrease sweet

intensity may signify that sucralose lost some of its chlorine molecules. When chlorine mixes with water, it becomes more oxidative; contributing to the formation of singlet oxygen and the oxidation of fatty acids to produce aldehydes (Held et al., 1978; Meiners and Morriss, 1964). The differences in lipid oxidation compounds, observed in aqueous sucralose, were not observed in sucralose AH WPI (Table 6). Fox (2011) concluded that beverage processing steps have a synergistic effect that promotes protein denaturation, lipid oxidation and volatile formation within the system. It is likely that the volatile compounds formed from the beverage processing steps had a muting effect on any volatile differences contributed by sucralose.

The muting effect, of AH processing to the oxidative properties of sucralose, was further supported by higher concentrations of lipid oxidation compounds sucralose NT WPI; compared to unsweetened NT WPI (Table 6). Without treatment, sucralose NT WPI contained the highest (compared to all other NT WPI) concentrations of hexanal, heptanal, 2-pentylfuran, octanal, E-2-octenal, nonanal, 2,4-nonadienal, o-aminoacetophenone, 2,4-decadienal and 2-dodecanone ($p \leq 0.05$). Heptanal, octanal, nonanal and 2,4-decadienal were detected in aqueous sucralose (Table 7) and therefore may have originated in the sucralose ingredient. However, increased concentrations of the other lipid oxidation compounds suggest additional oxidative reactions occurring between sucralose and WPI. These trends were not observed in the AH WPI and likely did not contribute to the flavor differences observed in sucralose AH WPI.

Stevia. Compared to unsweetened AH WPI, stevia AH WPI had higher concentrations of 2-butanone but lower concentrations of 2-pentylfuran and acetyl pyrazine

($p \leq 0.05$; Table 6). The volatile, 2-butanone, has an ether/solvent like aroma and is an important component to Cheddar cheese flavor (Singh et al., 2003). At concentrations above its taste threshold (5ppm), it could have contributed to the increased aroma intensity perceived in stevia AH WPI (Burdock, 2010). Thermal treatment of WPI has been documented to form acetyl pyrazine while 2-pentylfuran is produced from the autoxidation of linoleic acid (Carunchia Whetstine et al., 2005; Ho et al., 1978). These results suggest that stevia may have a protective effect on WPI flavor; either protecting WPI from thermal and oxidative

degradation or by binding the volatile compounds generated from AH processing. Several studies have focused on the antioxidant properties of *Stevia rebaudiana* extracts (Ghanta et al., 2007; Shukla et al., 2009; Tadhani et al., 2007). Ghanta et al. (2007) discovered that stevia extracts not only inhibited lipid peroxidation, but also protected DNA from scission by hydroxyl radicals. As a product with antioxidant properties to rival ascorbic acid (Shukla et al., 2009), stevia sweetener could have protected AH WPI from thermal and oxidative degradation. Congruent with sucralose AH WPI, volatile analysis did not show a significant increase in compounds that had soapy aroma characteristics. This further suggests increased concentrations of long-chain fatty acids may have contributed to the soapy flavor of stevia AH WPI.

Compared to other aqueous sweetener solutions, aqueous stevia had lower levels of lipid oxidation products ($p \leq 0.05$; Table 7). This result also suggests that aqueous stevia may be able to better protect lipids from thermal and pH degradation after AH treatment. This may also explain why sweet aromatic was not detected in this sample by sensory analysis.

Antioxidant effects were also observed in stevia NT WPI (Table 6). Compared to unsweetened NT WPI, stevia NT WPI had a lower concentration of Z-4-heptenal ($p \leq 0.05$). This lipid oxidation compound has been attributed to stale/oxidized off flavors in WPI (Carunchia Whetstine et al., 2005). However, stevia NT WPI also had higher concentrations of methional ($p \leq 0.05$). These trends were not observed after the beverage processing steps and likely did not contribute to the flavor differences observed in stevia AH.

CONCLUSIONS

After treatment, fructose AH was the most similar in flavor profile to unsweetened AH WPI. Fructose imparted sweetness without further promoting off-flavors in WPI. Higher concentrations of hexanoic acid, furaneol and decanal were detected in fructose AH; signs of lipid oxidation and Maillard reactions. However, these compounds did not contribute to flavor. They were either below detection threshold or were overpowered by other aromatic compounds. Fructose AH also lacked bitter and metallic notes detected in sucralose and stevia AH WPI. For these reasons, fructose AH WPI may be the best option for beverage manufacturers.

Analysis of sweetened water and sweetened WPI indicated that processing effects on sweetener alone did not contribute to the observed increase in soapy flavors detected in AH WPI sweetened with sucralose and stevia. The beverage processing steps likely enhanced interactions between WPI protein and the high-intensity sweeteners to generate long-chain free fatty acids that imparted soapy flavors to these solutions. Although sucralose and stevia may have promoted soapy flavors in AH WPI, they may have also masked perception of

brothy off-notes. Sucralose AH WPI had decreased intensities of cabbage/brothy and potato/brothy flavor; while cabbage/brothy was not detected in stevia AH WPI. Volatile analysis did not confirm a decrease in the volatile compounds that contribute to these flavors. Cross-modality interactions between sweet taste and brothy flavors may explain decreased perception of these attributes as sweet intensity increased. Another important factor to consider, in sweetener selection, is the sweet taste stability during beverage processing steps. Sweet intensity of each sweetener decreased after AH treatment. However stevia had the highest intensity followed by fructose then sucralose. This trend is a likely indicator of the stability of stevia, fructose and sucralose to beverage processing steps.

Beverage manufacturers must choose ingredients that best compliment the desired attributes of the finished product. Inclusion of WPI in many functional beverages, however may impart several off-notes. Careful selection of sweeteners, either alone or in appropriate blends, may mask off-flavors contributed by the protein or, in the least, impart sweetness without further promoting off-flavor development.

TABLES

Table 1. Formulations for equisweet[a] model beverages of equal volume[b]

Sample [sweetener – matrix]	WPI solids (%)	Sweetener solids (%)
Fructose – WPI	10	9.5
Sucralose – WPI	10	0.035
Stevia – WPI	10	0.07
Unsweetened – WPI	10	N/A
Fructose – H2O	N/A	9.5
Sucralose – H2O	N/A	0.035
Stevia – H2O	N/A	0.07

[a]Sweet 10 reference from Spectrum™ reference (Meilgaard, 1991). [b]Water and acid were subsequently used to hydrate each sample to equal volumes.

Table 2. Descriptive sensory means of sweetened WPI before (NT) and after (AH) acidification and heat treatment

	Unsweetened		Fructose		Sucralose		Stevia	
Attributes[1]	NT[2]	AH	NT	AH	NT	AH	NT	AH
Aroma intensity	2.6^{b}	2.7^{b}	2.4^{b}	2.7^{b}	2.3^{b}	2.6^{b}	2.2^{b}	3.4^{a}
Sweet aromatic	ND[3]	ND	1.7^{a}	ND	1.1^{b}	ND	ND	ND
Cardboard	2.3^{bc}	2.4^{bc}	2.8^{ab}	2.6^{abc}	3.1^{a}	2.5^{bc}	2.1^{c}	2.6^{abc}
Potato/ brothy	ND	1.7^{a}	ND	1.7^{a}	ND	1.4^{b}	ND	1.7^{a}
Cabbage/ brothy	1.1^{a}	1.1^{a}	0.8^{ab}	1.0^{ab}	0.5^{bc}	0.5^{bc}	0.8^{ab}	ND
Soapy	1.1^{bc}	1.5^{b}	ND	1.5^{b}	0.6^{c}	2.4^{a}	1.0^{bc}	2.3^{a}
Astringent	0.6^{c}	4.2^{a}	0.5^{c}	3.7^{a}	0.8^{c}	4.2^{a}	2.0^{b}	2.6^{b}
Sweet	ND	ND	9.5^{b}	5.0^{d}	11^{a}	2.8^{e}	10^{ab}	7.3^{c}
Sour	ND	6.8^{a}	ND	3.5^{c}	ND	4.5^{b}	ND	4.8^{b}
Bitter	0.5^{c}	ND	ND	ND	ND	1.1^{b}	1.4^{a}	1.2^{ab}
Metallic	ND	ND	ND	ND	1.6^{a}	1.7^{a}	ND	ND

[a-e] Means in a row not followed by common letters are different (Fisher's LSD; $p \leq 0.05$). Means represent the average of duplicate lots from a commercial WPI supplier. [1] Attributes were scored using a 0-15pt universal intensity scale (Meilgaard et al., 1999) and an established dried ingredient lexicon (Wright et al., 2009). [2] No treatment (NT) and acidified and heated (AH) WPI model beverages. [3]ND = mean scored below 0.5 were noted as not detected.

Table 3. Sensory means of aqueous sweetener solutions after acidification and heat treatment (AH)

	Aqueous Solutions		
Attributes[1]	Fructose[2]	Sucralose	Stevia
Aroma intensity	0.9[a]	0.6[a]	ND[3]
Sweet	11[a]	9.5[b]	8.5[c]
Sour	1.1[a]	1.7[a]	1.8[a]
Bitter	ND	0.9[b]	1.7[a]
Metallic	ND	2.2[a]	ND
Astringent	0.5[b]	1.8[a]	1.0[ab]

[a-c] Means in a row not followed by common letters are different (Fisher's LSD; $p \leq 0.05$). [1] Attributes were scored using a 0-15pt universal intensity scale (Meilgaard et al., 1999) and an established dried ingredient lexicon (Wright et al., 2009). [2] Deionized water sweetened with fructose, sucralose and stevia after acidification and heat treatments. [3]ND = mean scores below 0.5 were noted as not detected.

Table 4. Presence of aroma active compounds detected in sweetened WPI before (NT) and after (AH) acidification and heat treatment

					Unsweetened WPI		Fructose WPI		Sucralose WPI		Stevia WPI	
Aroma	Compound	Identification Method[a]	RI (ZB-5)[b]	RI (ZB-Wax)[c]	NT[d]	AH	NT	AH	NT	AH	NT	AH
Sulfur pumpkin	Dimethyl sulfide	RI,O,MS	<600	<600	1.9	1.8	2.1	2	2.3	1.8	1.9	1.3
Sulfur	Methanethiol	RI,O	<600		1.5	ND	2	ND	1.5	1	ND	ND
Ether	2-butanone	RI,O,MS	<600	<600	1.3	1.3	ND	1.8	1.8	1.4	1.8	1.5
Sulfur eggs	Unknown		<600		1	ND	1	1	ND	ND	1	ND
Malt	Butanal	RI,O,MS	<600		1.5	1.5	1.8	1	1	0.5	2	ND
Buttery	2,3-butanedione	RI,O	<600	972	1.7	1.5	1.5	1.8	1.5	1.3	1	1.3
Sour vinegar	Acetic acid	RI,O	617	1340	ND	1.3	ND	0.5	1	ND	ND	ND
Malty chocolate	3-methylbutanal	RI,O,MS	618	915	1.4	0.8	1.1	2	1	1.3	0.5	0.8
Garlic	Methylethylsulfide	RI,O	620		1.8	ND	1.5	1.2	1	0.8	2	ND
Fruity grassy	Ethyl propionate	RI,O,MS	709	948	1	0.5	ND	1.3	ND	ND	ND	1
Onion cabbage	Dimethyl disulfide	RI,O,MS	749	1073	1	1	0.5	ND	1.2	0.8	0.8	ND
Rubbery	1-hexen-3-one	RI,O,MS	754	1012	0.5	ND	1.5	0.5	0.5	ND	ND	1
Grass tallow fat	Hexanal	RI,O,MS	795	1082	0.8	1.1	0.5	1	1	0.8	0.7	1
Cheesy free fatty acid	3-methylbutyric acid	RI,O	840	1777	1	0.5	ND	1	ND	ND	ND	ND
Fruity green	Isopropyl butanoate	RI,O,MS	843	1162	0.7	ND	0.5	0.5	ND	ND	0.8	ND
cooked nutty	2-methyl-3-furanthiol	RI,O,MS	865	1317	1.8	1.5	1.5	1.2	1.6	1.5	1.2	1
Fishy fatty	Z-4-heptenal	RI,O	896	1191	1.1	1.8	1	1.3	1	1.3	0.5	1
Potato musty	Methional	RI,O.MS	906	1468	1.5	2	0.9	2	1.3	1.3	0.5	1.9
Popcorn	2-acetyl pyrroline	RI,O	919	1343	2.1	1.2	1.7	ND	1.7	ND	1.1	0.5
Cabbage garlic	Dimethyl trisulfide	RI,O,MS	966	1382	2.2	1.7	2	1.6	1.9	1.4	1.8	1.7
Mushroom metallic	1-octen-3-one	RI,O,MS	975	1301	1	0.5	1.1	1.1	2	1	1.8	1

[a] Method of identification by retention indices (RI), olfactometry (O) and comparison to mass spectra (MS) of authentic standards. [b]Retention indices of the aroma event on the ZB-5 column. [c]Retention indices of the aroma event on the ZB-WAX column. [d] No treatment (NT) and acidified and heated (AH) WPI model beverages. [e] Not detected (ND) indicates the mean was below 0.5. Means represent the average of duplicate lots of WPI.

Table 4 Continued

Aroma	Compound	Identification Method[a]	RI (ZB-5)[b]	RI (ZB-Wax)[c]	Unsweetened WPI		Fructose WPI		Sucralose WPI		Stevia WPI	
					NT[d]	AH	NT	AH	NT	AH	NT	AH
Sweet fruity metallic	2-pentyl furan	RI,O	991		ND[e]	ND	ND	1.8	ND	1	ND	ND
Citrus fatty	Octanal	RI,O,MS	1001	1294	1.7	1.6	1	1.9	1.5	1.3	1	1.4
Rosy fatty	Phenylacetaldehyde	RI,O,MS	1042	1647	1	1	ND	1	ND	0.5	0.5	0.8
Cooked burnt rubber	2-acetyl pyrrole	RI,O	1064		0.9	ND	0.8	ND	0.8	1.5	1	1
Smoky	Guaiacol	RI,O,MS	1081	1524	1.1	1.5	1	1	1.2	1.3	1.4	0.8
Sweet fatty fruity	2-nonanone	RI,O,MS	1092	1382	0.5	1	0.8	1	1	1	ND	1
Phenol carpet	p-cresol	RI,O	1099		1.5	1.7	1.7	1.5	2	1.3	0.8	ND
Citrus fatty	Nonanal	RI,O,MS	1104	1376	1	ND	0.5	1.3	2	0.5	ND	1.3
Roasted cooked	2-acetyl thiazoline	RI,O,MS	1104	1737	ND	1	ND	1.2	ND	ND	ND	ND
Rosy fruity	2-phenethanol	RI,O,MS	1144	1926	1.5	1	0.5	1	ND	1.3	0.5	ND
Cucumbers	E,Z-2,6-nonadienal	RI,O,MS	1153	1558	1	ND	ND	0.8	1	ND	0.5	1.8
Carpets cucumbers	E-2-nonenal	RI,O,MS	1156	1527	1.6	1	1.5	1.2	1.5	0.9	0.7	1.5
Brothy cooked nutty	Methyl-2-methyl-3-furyl disulfide	RI,O,MS	1173	1669	1.8	0.9	1.2	1.5	1.5	1	1	0.8
Fatty citrus sweet	Decanal	RI,O	1194		1	1	0.5	ND	1	1	0.5	ND
Fatty hay	E,E-2,4-nonadienal	RI,O,MS	1215	1716	2	1	1	0.8	1	0.8	ND	0.5
Garlic burnt rubber	Dimethyl tetra sulfide	RI,O	1215		ND	ND	0.5	ND	ND	ND	ND	ND
Minty hay sweet	2-octyl furan	RI,O	1237		0.8	1	0.5	ND	1	ND	ND	ND
Coconut oatmeal	Gamma-octalactone	RI,O,MS	1278	1933	ND	0.5	0.5	0.8	0.8	1	1	0.8
Fatty fryer oil	2,4-decadienal	RI,O	1310		0.9	0.8	ND	ND	ND	ND	0.5	ND
Grapy tortilla	o-aminoacetophenone	RI,O,MS	1338	>2000	0.8	ND	1	ND	ND	ND	ND	ND
Cilantro fatty	Gamma-nonalactone	RI,O,MS	1358	>2000	ND	ND	0.8	0.5	ND	ND	ND	0.8

[a] Method of identification by retention indices (RI), olfactometry (O) and comparison to mass spectra (MS) of authentic standards. [b]Retention indices of the aroma event on the ZB-5 column. [c]Retention indices of the aroma event on the ZB-WAX column. [d] No treatment (NT) and acidified and heated (AH) WPI model beverages. [e] Not detected (ND) indicates the mean was below 0.5. Means represent the average of duplicate lots of WPI.

Table 4 Continued

					Unsweetened WPI		Fructose WPI		Sucralose WPI		Stevia WPI	
Aroma	Compound	Identification Method[a]	RI (ZB-5)[b]	RI (ZB-Wax)[c]	NT[d]	AH	NT	AH	NT	AH	NT	AH
Cilantro	Decanoic acid	RI,O	1384		0.5	ND[e]	ND	0.7	ND	ND	ND	ND
Grainy soapy	E-2-dodecenal	RI,O,MS	1443		1	1	0.5	0.5	ND	0.5	ND	ND
Peach	Gamma-dodecalactone	RI,O,MS	1635		0.8	0.5	ND	ND	ND	ND	ND	ND
Fruity sweet coconut	Delta-dodecalactone	RI,O,MS	1721		ND	0.5	ND	0.5	ND	0.5	ND	ND

[a] Method of identification by retention indices (RI), olfactometry (O) and comparison to mass spectra (MS) of authentic standards. [b]Retention indices of the aroma event on the ZB-5 column. [c]Retention indices of the aroma event on the ZB-WAX column. [d] No treatment (NT) and acidified and heated (AH) WPI model beverages. [e] Not detected (ND) indicates the mean was below 0.5. Means represent the average of duplicate lots of WPI.

Table 5. Presence of aroma active compounds detected in aqueous sweetener solutions after (AH) acidification and heat treatment

					Aqueous Solutions[d]		
Aroma	Compound	Identification Method[a]	RI (ZB-5)[b]	RI (ZB-Wax)[c]	Fructose	Sucralose	Stevia
Sulfur pumpkin	Dimethyl sulfide	RI,O,MS	<600	<600	0.8	0.5	0.5
Ether	2-butanone	RI,O,MS	<600	<600	1.8	1.4	1.3
Malt	Butanal	RI,O,MS	<600		ND	ND	0.5
Buttery	2,3-butanedione	RI,O	<600	972	0.8	ND	ND
Garlic	Methylethylsulfide	RI,O	620		ND	ND	0.5
Fruity grassy	Ethyl propionate	RI,O,MS	709	948	ND	1	0.5
Onion cabbage	Dimethyl disulfide	RI,O,MS	749	1073	0.5	ND	ND
Rubbery	1-hexen-3-one	RI,O,MS	754	1012	1	ND	0.7
Grass tallow fat	Hexanal	RI,O,MS	795	1082	0.5	ND	ND
Cheesy free fatty acid	3-methylbutyric acid	RI,O	840	1777	0.5	ND	ND
cooked nutty	2-methyl-3-furanthiol	RI,O,MS	865	1317	ND	ND	0.5
Cabbage garlic	Dimethyl trisulfide	RI,O,MS	966	1382	ND	0.5	ND
Mushroom metallic	1-octen-3-one	RI,O,MS	975	1301	1.5	ND	1.5
Sweet fruity metallic	2-pentyl furan	RI,O	991		ND	0.5	ND
Citrus fatty	Octanal	RI,O,MS	1001	1294	1	1	1
Rosy fatty	Phenylacetaldehyde	RI,O,MS	1042	1647	0.5	ND	ND
Cooked burnt rubber	2-acetyl pyrrole	RI,O	1064		1	ND	ND
Smoky	Guaiacol	RI,O,MS	1081	1524	1.3	1.2	ND
Sweet fatty fruity	2-nonanone	RI,O,MS	1092	1382	2	ND	ND
Phenol carpet	p-cresol	RI,O	1099		ND	1	ND
Citrus fatty	Nonanal	RI,O,MS	1104	1376	0.9	ND	0.7
Rosy fruity	2-phenethanol	RI,O,MS	1144	1926	ND	1	ND

[a] Method of identification by retention indices (RI), olfactometry (O) and comparison to mass spectra (MS) of authentic standards. [b]Retention indices of the aroma event on the ZB-5 column. [c]Retention indices of the aroma event on the ZB-WAX column. [d] Deionized water sweetened with fructose, sucralose and stevia after acidification and heat treatments. [e] Not detected (ND) indicates the mean was below 0.5.

Table 5 Continued

					Aqueous Solutions[d]		
Aroma	Compound	Identification Method[a]	RI (ZB-5)[b]	RI (ZB-Wax)[c]	Fructose	Sucralose	Stevia
Cucumbers	E,Z-2,6-nonadienal	RI,O,MS	1153	1558	ND[e]	ND	1
Carpets cucumbers	E-2-nonenal	RI,O,MS	1156	1527	ND	1.1	1
Brothy cooked nutty	Methyl-2-methyl-3-furyl disulfide	RI,O,MS	1173	1669	ND	0.5	ND
Fatty citrus sweet	Decanal	RI,O	1194		ND	ND	0.5
Fatty hay	E,E-2,4-nonadienal	RI,O,MS	1215	1716	1.5	0.5	ND
Fatty fryer oil	2,4-decadienal	RI,O	1310		ND	ND	1
Cilantro fatty	Gamma-nonalactone	RI,O,MS	1358	>2000	0.5	ND	ND

[a] Method of identification by retention indices (RI), olfactometry (O) and comparison to mass spectra (MS) of authentic standards. [b]Retention indices of the aroma event on the ZB-5 column. [c]Retention indices of the aroma event on the ZB-WAX column. [d] Deionized water sweetened with fructose, sucralose and stevia after acidification and heat treatments. [e] Not detected (ND) indicates the mean was below 0.5.

Table 6. Concentration (ppb) of selected compounds in sweetened WPI beverages by SPME GC-MS

	Unsweetened		Fructose		Sucralose		Stevia	
Compound	NT[1]	AH	NT	AH	NT	AH	NT	AH
Dimethyl sulfide	0.0220^{a}	0.0170^{a}	0.0120^{a}	0.0150^{a}	0.007^{a}	0.011^{a}	0.017^{a}	0.011^{a}
Methanethiol (TID[3])	0.0800^{a}	0.0160^{a}	0.0530^{a}	0.0450^{a}	ND[2]	0.009^{a}	0.0660^{a}	0.0600^{a}
2-butanone*	24.1^{b}	ND	ND	ND	ND	ND	15.5^{b}	87.4^{a}
Butanal	1.92^{ab}	0.707^{b}	1.09^{ab}	1.62^{ab}	0.629^{b}	1.14^{ab}	1.98^{a}	1.49^{ab}
Acetic acid	0.610^{a}	0.0550^{ab}	0.0570^{ab}	0.0520^{b}	0.0340^{b}	0.0420^{b}	0.128^{ab}	0.0350^{b}
3-methylbutanal*	21.2^{ab}	4.44^{b}	43.3^{a}	0.221^{b}	25.0^{ab}	15.5^{b}	12.4^{b}	14.2^{b}
Ethyl propionate	0.460^{a}	0.184^{a}	0.182^{a}	0.346^{a}	0.456^{a}	0.168^{a}	0.344^{a}	0.358^{a}
Butyric acid*	70.7^{ab}	65.2^{b}	99.7^{ab}	77.0^{ab}	179a	123^{ab}	89.2^{ab}	158^{ab}
1-hexen-3-one*	102^{b}	9.62^{b}	152^{b}	6.56^{b}	389^{a}	37.1^{b}	173^{ab}	10.9^{b}
Dimethyl disulfide*	18.0^{ab}	5.61^{c}	19.6^{ab}	10.7^{bc}	22.4^{a}	2.39^{c}	17.0^{ab}	4.00^{c}
Hexanal*	18.7^{b}	6.23^{b}	29.5^{b}	10.2^{b}	221^{a}	10.6^{b}	21.1^{b}	4.58^{b}
3-methylbutyric acid	ND	0.0100^{b}	ND	0.0690^{a}	ND	ND	ND	ND
Isopropyl butanoate*	0.030^{ab}	0.013^{b}	0.023^{b}	0.056^{ab}	0.078^{a}	0.025^{b}	0.039^{ab}	0.038^{ab}
2-methyl-3-furanthiol	0.122^{a}	0.0240^{ab}	0.115^{a}	0.0390^{ab}	0.121^{a}	0.0180^{ab}	0.0910^{ab}	0.0140^{b}
Methional*	36100^{b}	6460^{c}	12900^{bc}	8390^{bc}	14900^{bc}	5030^{c}	67200^{a}	4050^{c}
Z-4-heptenal*	21.3^{bc}	0.129^{c}	131^{a}	0.139^{c}	79.3^{ab}	0.180^{c}	11.2^{c}	0.0760^{c}
Heptanal*	133^{bc}	0.918^{c}	179^{b}	1.25^{c}	481^{a}	1.08^{c}	142^{bc}	0.599^{c}
Benzaldehyde*	233^{ab}	798^{ab}	615^{ab}	1050^{a}	346^{ab}	441^{ab}	205^{ab}	43.1^{b}
Dimethyl trisulfide*	105^{b}	8.08^{c}	140^{ab}	12.7^{c}	181^{a}	9.15^{c}	109^{b}	5.10^{c}
1-octen-3-one	0.0600^{a}	0.217^{a}	0.0810^{a}	0.349^{a}	0.0910^{a}	0.340^{a}	0.0760^{a}	0.476^{a}

[a-e] Means in a row not followed by common letters are different (Fisher's LSD; $p \leq 0.05$). Means represent the average of duplicate lots of WPI. *Mean concentrations of volatile compounds by SPME GCMS using SIM mode quantified using four-point external standard curves; each made to R2 value of 0.92 or greater. Otherwise, means quantified with relative abundance as there were no differences between sweetened AH WPI. [1] No treatment (NT) and acidified and heated (AH) WPI model beverages. [2]ND = means less than 0.005ppb noted as not detected.[3]TID= compounds tentatively identified.

Table 6 Continued

	Unsweetened		Fructose		Sucralose		Stevia	
Compound	NT[1]	AH	NT	AH	NT	AH	NT	AH
2-pentylfuran*	0.809^{b}	0.778^{b}	0.842^{b}	0.721^{b}	1.37^{a}	0.639^{b}	0.841^{b}	0.363^{c}
Octanal*	45.1^{b}	3.90^{c}	39.9^{b}	3.49^{c}	70.5^{a}	3.78^{c}	45.4^{b}	3.85^{c}
Hexanoic acid*	9.14^{c}	389^{bc}	24.8^{c}	1110^{a}	17.3^{c}	182^{bc}	10.3^{c}	463^{b}
Acetyl pyrazine*	0.206^{d}	2.37^{b}	0.185^{d}	1.96^{bc}	0.523^{d}	5.53^{a}	0.244^{d}	0.759^{cd}
Phenylacetaldehyde*	26.6^{a}	6.36^{b}	21.7^{a}	8.80^{b}	26.7^{a}	2.10^{b}	24.6^{a}	0.634^{b}
2-acetyl pyridine	ND[2]	ND	ND	ND	ND	0.0200^{a}	0.005^{a}	0.0210^{a}
E-2-octenal*	71.3^{b}	1.38^{c}	74.4^{b}	0.891^{c}	136^{a}	0.772^{c}	77.3^{b}	0.805^{c}
Furaneol*	610^{b}	689^{b}	873^{b}	2180^{a}	986^{b}	413^{b}	567^{b}	300^{b}
3,5-octadien-2-one (TID[3])	0.007^{b}	ND	ND	ND	0.006^{bc}	ND	0.019^{a}	ND
2-nonanone*	0.297^{bcd}	0.236^{cde}	0.437^{ab}	0.376^{bc}	0.594^{a}	0.180^{de}	0.305^{bcd}	0.0940^{e}
Nonanal*	73.8^{b}	12.4^{c}	81.9^{b}	15.6^{c}	155^{a}	12.7^{c}	76.5^{b}	8.45^{c}
2-phenethanol	0.0430^{a}	0.0380^{a}	0.0460^{a}	0.0340^{a}	0.0680^{a}	0.0200^{a}	0.0730^{a}	0.0480^{a}
E-2-nonenal	0.222^{a}	0.0900^{a}	0.258^{a}	0.254^{a}	0.250^{a}	0.0410^{a}	0.174^{a}	0.0270^{a}
E,Z-2,6-nonadienal*	15.5^{b}	0.150^{c}	20.7^{ab}	0.211^{c}	30.6^{a}	0.129^{c}	14.6^{b}	0.031^{c}
Decanal*	6.25^{abc}	1.38^{bc}	6.95^{abc}	11.5^{a}	8.53^{ab}	0.902^{c}	5.47^{abc}	0.506^{c}
2,4-nonadienal*	1640^{b}	0.111^{c}	1460^{b}	0.291^{c}	4570^{a}	0.0520^{c}	1260^{b}	0.0390^{c}
o-aminoacetophenone*	0.714^{b}	1.57^{b}	0.566^{b}	0.337^{b}	5.20^{a}	0.145^{b}	0.798^{b}	0.0460^{b}
g-octalactone*	0.193^{b}	0.172^{bc}	0.371^{a}	0.0620^{bc}	0.400^{a}	0.0840^{bc}	0.193^{b}	0.005^{c}
2,4-decadienal*	43.3^{c}	0.043^{d}	58.8^{b}	0.0320^{d}	69.7^{a}	0.0360^{d}	47.6^{c}	0.0150^{d}
g-nonalactone*	19.5^{a}	2.10^{b}	20.0^{a}	3.84^{b}	28.8^{a}	2.57^{b}	21.2^{a}	0.484^{b}

[a-e] Means in a row not followed by common letters are different (Fisher's LSD; $p \leq 0.05$). Means represent the average of duplicate lots of WPI. *Mean concentrations of volatile compounds by SPME GCMS using SIM mode quantified using four-point external standard curves; each made to R2 value of 0.92 or greater. Otherwise, means quantified with relative abundance as there were no differences between sweetened AH WPI. [1] No treatment (NT) and acidified and heated (AH) WPI model beverages. [2]ND = means less than 0.005ppb noted as not detected.[3]TID= compounds tentatively identified.

Table 6 Continued

	Unsweetened		Fructose		Sucralose		Stevia	
Compound	NT[1]	AH	NT	AH	NT	AH	NT	AH
Decanoic acid	ND[2]	0.0190^{a}	0.0110^{a}	0.009^{a}	0.0140^{a}	0.007^{a}	ND	ND
2-dodecanone*	7.20^{b}	2.12^{cd}	7.20^{b}	2.70^{c}	9.18^{a}	1.43^{cd}	7.42^{b}	0.557^{d}
E-2-dodecenal*	720^{b}	5.68^{c}	1950^{a}	3.92^{c}	2480^{a}	4.18^{c}	790^{b}	1.19^{c}
Beta-ionone	0.009^{abc}	0.0130^{abc}	0.0280^{ab}	0.0130^{abc}	0.0280^{a}	0.007^{bc}	0.0150^{abc}	ND
d-decalactone*	11.4^{a}	3.11^{c}	3.22^{bc}	7.45^{abc}	4.33^{abc}	1.80^{c}	10.0^{ab}	2.66^{c}
g-dodecalactone*	4.78^{ab}	0.774^{c}	5.97^{a}	0.985^{bc}	6.78^{a}	0.915^{bc}	4.65^{ab}	0.505^{c}

[a-e] Means in a row not followed by common letters are different (Fisher's LSD; $p \leq 0.05$). Means represent the average of duplicate lots of WPI. *Mean concentrations of volatile compounds by SPME GCMS using SIM mode quantified using four-point external standard curves; each made to R2 value of 0.92 or greater. Otherwise, means quantified with relative abundance as there were no differences between sweetened AH WPI. [1] No treatment (NT) and acidified and heated (AH) WPI model beverages. [2]ND = means less than 0.005ppb noted as not detected.[3]TID= compounds tentatively identified.

Table 7. Relative abundance (ppb) of significantly different volatile compounds in aqueous sweetener solutions after acidification and heating treatment

	Aqueous Solutions[1]		
	Fructose	Sucralose	Stevia
3-methylbutanal	1.75^{a}	0.741^{b}	0.456^{b}
Heptanal	0.0630^{b}	0.0800^{a}	0.0380^{c}
Octanal	0.146^{a}	0.147^{a}	0.112^{b}
Nonanal	1.66^{a}	2.05^{a}	0.975^{b}
Decanal	0.277^{b}	0.501^{a}	0.127^{b}
2,4-decadienal	0.0240^{b}	0.0490^{a}	0.0210^{b}

[a-c] Means in a row not followed by common letters are different (Fischer's LSD; $p \leq 0.05$). Mean concentrations detected by SPME-GCMS and quantified with relative abundance. [1] Deionized water sweetened with fructose, sucralose and stevia after acidification and heat treatments.

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