ABSTRACT

CARTER, BRANDON GARY. The Efficiency of Whey Protein Production and Improvements on Whey Product Applications. (Under the direction of Dr. MaryAnne Drake).

Production of whey protein continues to increase due to their unique functional and nutritional properties. The objective of this dissertation was to investigate how filtration affects the processing efficiency of whey protein, how vacuum cooling affects the flavor of dairy beverages, and how whey proteins characteristics impact the perception of astringency of whey protein. Four different studies were conducted: the effect of vacuum cooling on the flavor of protein beverages using skim milk as a model, the effects of ceramic microfiltration on the removal of whey proteins from sweet whey, the effects of removal of whey protein from sweet whey using polymeric membranes, and the influence of oral movement, particle size, and zeta potential on astringency of whey protein.

In the first study, steam-infused pasteurized milk was sampled after heating but before it reached the vacuum chamber with the use of a liquid sample port. Milks cooled by the vacuum cooler were lower in sweet aromatic, sulfur/eggy and cooked flavors than milk sampled before the vacuum cooler. The vacuum cooler applied during DSI UP of milk is effective at removing steam and cooling UP milk, but this process may also remove important flavor compounds from fluid milk or whey protein beverages.

In a second study, our research objective was to measure percent removal of whey protein from separated sweet whey using 0.1 UTP ceramic microfiltration (MF) membranes in a 3 stage 3x process at 50°C. About 84 to 85% of the total nitrogen in the whey feed passed though the membrane into the permeate. There was some passage of IgG, bovine serum albumen, glycomarcopeptide, and casein proteolysis products into the permeate. β-LG was in
higher concentration in the retentate indicating that it was partially blocked from passage through the ceramic MF membrane, while α-LA had a higher rate of passage through the membrane than β-LG. The percentage of the serum proteins in sweet whey that were removed in permeate by ceramic MF was about 85% on a total nitrogen basis at 50°C.

In a third study, whey protein removal percentage from separated sweet whey using spiral wound (SW) polymeric microfiltration (MF) membranes using a 3-stage, 3x process at 50°C was investigated and compared to the performance of ceramic membranes. There was no detectable level of lactoferrin (LFR) and no intact αs or β-casein detected in the MF permeate from the 0.3 μm spiral wound PVDF membranes used in this study. About 69% of the crude protein present in the pasteurized separated sweet whey was removed using a 3X, 3-stage, 0.3 μm spiral wound PVDF MF process at 50°C compared to 0.1 μm ceramic graded permeability MF that removed about 85% of crude protein from sweet whey.

In a fourth study, the contribution of oral movement and particle size as well as zeta potential on the perception of whey protein astringency was investigated. Acidification of WPI increased astringency, but astringency was documented in a neutral pH range (pH 6.0-7.0). Oral movements increased the perception of astringency, suggesting that part of the astringent sensation was due to the interaction of whey proteins with receptors on oral tissues. Commercial WPI and WPC80 varied widely in particle size, zeta potential and astringency. Astringency of WPI solutions were correlated with zeta potential. WPC80 were astringent, but astringency of WPC 80 was not correlated with particle size or zeta potential.
The Efficiency of Whey Protein Production and Improvements on Whey Product Applications

by

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A dissertation submitted to the Graduate Faculty of North Carolina State University in partial fulfillment of the requirements for the degree of Doctor of Philosophy

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DEDICATION

I would like to dedicate this work to my wife Nicole who has always been there to love and support me. We did it! Secondly, to my children, the goal of my whole life is to provide a safe and comfortable place for you to live, learn and play.
BIOGRAPHY

Brandon Gary Carter was born on June 10th, 1991 to Gary Frank Carter and Marci Carter. Brandon grew up in Bakersfield California and he lived there through high school. Brandon attended Brigham Young University starting in 2009 and received his Bachelors of Science in Food Science with a minor in Chemistry. During his undergraduate degree he served a two year mission for the Church of Jesus Christ of Latter-day Saints in Mexico City, Mexico. Brandon married his sweetheart Nicole in the summer of 2013.

Brandon completed 2 internships during his undergraduate degree with Glanbia Nutritionals and Bolthouse Farms. To further his education Brandon started graduate studies at North Carolina State University with Dr. MaryAnne Drake in 2015. Brandon completed a Master degree in Food Science at NC State under the direction of MaryAnne Drake. Brandon hopes to use expertise gained through graduate school to help innovate the dairy industry and mentor young professionals.
ACKNOWLEDGMENTS

I would like to acknowledge the wonderful mentorship of Dr. MaryAnne Drake for helping me becoming a better version of myself. She has taught me valuable lessons in Food Science, business and life. Thank you for your patience and wisdom. I am grateful for all of the hours spent correcting my writing, and all the dollars spent to develop my talents, skills and knowledge. You have truly become a pillar in my life and I look forward to our continued relationship and collaboration. I would also like to thank my lab mates who spent countless hours in the pilot plant helping me make samples and conducting my research. I have benefitted from strong willed workers, great thinkers, and inspirational creatives around me to help me accomplish this goal. I would also like to thank Dr. Hansen, Dr. Stevenson and Dr. Poole for being on my committee and there continual support and constructive comments and encouragement.

Most importantly, I want to thank my amazing wife, who had to put up with early mornings, late nights, and long days. A wonderful woman who would wake up at 5:00 am in the morning to drive me to school so that I could make cheese. Your love and constant support have been the fuel to get me through this degree. Ultimately this degree was to help give our family a bright future, and I appreciate your hard work and sacrifice to support our family. To my daughter, Grace, your smiles have made all the hard work worth it and I love you with my whole heart.
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CHAPTER 1: Literature Review: Astringency in Whey Protein Beverages
Astringency in Whey Protein Beverages

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Abstract

Astringency is the sensation of mouth drying and puckering and has also been described as a loss of lubrication in the mouth. Astringency is perceived as an increase in oral friction or roughness. Astringency caused by tannins and other polyphenols has been well documented and studied. Whey proteins are popular for their functional and nutritional quality, but exhibit astringency, particularly under acidic conditions popular in high acid (pH 3.4) whey protein beverages. Acids cause astringency, but acidic protein beverages have higher astringency than acid alone. Whey proteins are able to interact with salivary proteins which removes the lubricating saliva layer of the mouth. Whey proteins can also interact directly with epithelial tissue. These various mechanisms of astringency limit whey protein ingredient applications as astringency is undesirable to consumers. A better understanding of the causes of whey protein astringency will improve our ability to produce products with high consumer liking that deliver excellent nutrition.
Introduction

Astringency is the sensation of mouth drying and puckering. The American Society for Testing and Materials Committee E-18 has defined astringency as “complex sensations due to shrinking, drawing or puckering of the epithelium as a result of exposure to substances such as alums or tannins” (ASTM, 2004). It is thought that the human body’s response to astringency was an early warning sign of foods that could be potentially harmful.

Astringency has been proposed to be more complicated than the mechanisms that elucidate G protein-coupled taste receptors for the sensations of sweet, bitter and umami tastes (Zhang et al., 2003; Bradbury, 2004; Zhao et al., 2003; Damak et al., 2003) and the ion channels that contribute to salty and sour tastes (DeSimone and Lyall, 2006; Chandrashekar et al., 2006). Astringency is tremendously important to the sensory perception of common foods including red wine, teas, chocolate, fruits and nuts, and protein products (Lesschaeve and Noble, 2005). Some of the compounds that cause astringency in foods are alum (Peleg et al., 1998), acids (Lawless et al., 1996), polyphenols (Lesschaeve and Noble, 2005) as well as dehydrating agents (ethanol and acetone), and some organic acids (Bajec and Pickering, 2008). The sensation of astringency may not only be a consequence of the somatosensory system, but a combination of taste and oral tactile mechanisms (Bajec and Pickering, 2008). For this reason, astringency has also been described as the loss of lubrication in the mouth which is perceived as an increase in oral friction or roughness (Upadhyay et al., 2016).

Whey proteins are popular for their functional and nutritional quality. Once only typically consumed by athletes and bodybuilders, there is now a segment of U.S. consumers that have a stated interest in increasing the amount of protein from a variety of sources that they consume (Nielsen, 2015). With the consumer demand for more protein in products, the market
has answered with protein beverages, bars, confections, cookies, and cereals. Dairy proteins, specifically whey proteins, remain the most desirable protein source to consumers (Harwood and Drake, 2019). Whey proteins aid in greater muscle protein synthesis than casein or soy protein following exercise or at rest in both young and older individuals (Tang et al., 2009; Pennings et al., 2011). Whey protein also aids in the prevention of sarcopenia, or muscle break down of 3-8% per decade after the age of 30 (Paddon-Jones and Rasmussen, 2009; Liao et al., 2019). In order to achieve the latter benefit (maintenance of muscle mass), constant supplementation of 20-25 grams of protein per meal must be maintained, especially from high quality protein sources like whey. This constant supplementation can be difficult to maintain, especially if the protein source is unpalatable.

Whey proteins are non-volatile macromolecules with no inherent aroma; however, whey protein ingredients have other molecules as a result of processing that are flavor active (Majahan et al., 2004; Carunchia Whetstine et al., 2003; Carunchia Whetstine et al., 2005; Wright et al., 2006; Drake et al., 2007; Wright et al., 2009). Each step in the production of whey protein ingredients potentially imparts flavors (Carter and Drake, 2018). Further, these flavors can carry over into finished products which can limit their application and acceptability (Drake, 2006; Wright et al., 2009; Evans et al., 2010; Oltman et al., 2015). Astringency is a sensation documented in whey protein which also can carry through into the final application (Oltman et al., 2015; Childs and Drake, 2010; Zhang et al., 2020). Protein astringency may further limit protein ingredients use in protein-dense products as astringency can be an undesired oral sensation in some foods (Beecher et al., 2008).

Whey protein beverages can be formulated as either neutral pH or low pH. Neutral beverages (pH 6.8-7.0) are cloudy and are typically formulated with sweet flavors like vanilla,
chocolate or coffee. Acidic whey protein beverages (pH < 3.5) are clear and tend to be formulated with fruity flavors that are congruent with low pH (Ahmade et al., 2018). Acidic whey protein beverages are an appealing protein delivery system as they can be shelf stable with relatively mild heat treatment (compared to neutral beverages) and hot-filled. High intensities of astringency have been noted in high acid whey protein beverages (Beecher et al., 2008; Childs and Drake, 2010) but perceivable astringency is still common in neutral beverages (Withers et al., 2014), and was recently documented as a driver of consumer dislike in neutral pH ready-to-mix whey protein beverages (Zhang et al., 2020). The ability to create protein beverages with lower or no astringency would greatly improve consumer acceptance of these products. The ability to reduce astringency in acidic beverages would also enable the dairy industry to make a high quality nutritional product with simpler processing and shelf stability.

Little published work has directly addressed the role of dairy protein astringency on consumer acceptance. Childs et al. (2010) investigated consumer perception of astringency in clear acidic whey protein beverages. Consumers were unfamiliar with the terminology of astringency but understood the concept. Consumers were familiar with astringency in certain foods like wine, juice and tea. Astringency was a driver of dislike in ready to mix whey protein beverages (Zhang et al., 2020).

The ability to produce protein products with low astringency is extremely important for the success of whey protein applications. The exact mechanism of astringency produced by dairy proteins (casein, whey) is still unclear. This literature review will summarize the current status of the literature, as well as discuss the proposed mechanisms and factors that control and contribute to astringency in whey protein beverages.
Methods for measuring astringency

First, in order to understand the causes of astringency it is important to understand the different techniques to quantify it. Astringency is a sensory perception. As such, most research focused on astringency has used some form of descriptive analysis (DA) as a way to quantify astringency (Sano et al., 2005; Lee and Vickers, 2008; Beecher et al., 2008; Kelly et al., 2010; Vardhanabhuti et al., 2010; Ye et al., 2011; Ye et al., 2012; Bull et al., 2017). This method requires training a small group of individuals on reference solutions to be able to scale and differentiate samples (Drake, 2007). Most studies have used a DA panel to document astringency intensities, although temporality has also been characterized (Kelly et al., 2010; Beecher et al., 2008). Not all descriptive analysis methods are the same, and most scales are not related or differ in reference types which makes it difficult to make comparisons across studies. However, since astringency is a sensory sensation, humans, and objective sensory methods, remain the optimum way to measure the perception of astringency. Consumers are aware of the concept of astringency (Childs and Drake, 2010), but not ideal to quantify astringency. Consumers do not have the training to identify and objectively quantify astringency or to differentiate astringency from other basic tastes such as sour or bitter.

Other indirect methods of measuring astringency included using a taste sensor, which measures a change in membrane potential caused by sample adsorption on a probe, have been used (Sano et al., 2005). Some studies have used the rate of saliva flow, collecting saliva after exposure to a stimulus for 2 minutes, as an indirect indicator of astringency (Kelly et al., 2010). Saliva flow can used as an indicator of astringency since more saliva is produced in an attempt to clear the astringent compounds and return the mouth to normal conditions. It has been
demonstrated that astringent compounds, like those found in tea, can have different perception of astringency that have been correlated with different saliva flow rates (Dawes et al., 2000).

Since astringency has been associated with interactions between proteins found in saliva and on oral surfaces, *in vitro* analysis of interactions is used alone or alongside of sensory analysis to determine astringency. Changes in turbidity has been used as an indicator of aggregation, either whey protein aggregates alone or whey protein-saliva protein aggregates (Kelly et al., 2010; Beecher et al., 2008; Ye et al., 2011). Individual proteins can be determined by electrophoretic analysis, and this approach has been used to characterize composition of protein aggregates formed in the mouth (Vardhanabhuti et al., 2010; Ye et al., 2011). Beside composition, particle size distribution of the aggregates may be important (Ye et al., 2011).

Aggregates can be formed by various forces including electrostatic interactions. A common way to analyze proteins in regards to their propensity for electrostatic interactions is to measure the Zeta potential. This provides an average protein charge (positive or negative) at a given pH and ionic conditions. Therefore, it can be useful to understand the potential interaction between charged molecules. Zeta potential was used to understand electrostatic interactions between saliva and whey proteins and showed that astringency was correlated with a decrease in zeta potential in the range tested (pH 3-5) which suggests greater interactions between whey protein and saliva when the charge states encourage interaction (Ye et al., 2011; Ye et al., 2012).

Tribology is the science of interacting surfaces in relative motion and therefore a measure of the lubrication element of astringency (Li et al., 2018). Tribology can been used to understand the frictional component of astringency (Rossetti et al., 2008, 2009; Upadhyay et al., 2016). Reduction of friction alone does not appear to be the sole explanation of astringency. Rossetti et al. (2009) used tribology (preadsorbing saliva on elastic hydrophobic substrate) in conjunction
with descriptive analysis to compare astringent polyphenols. Although the sensory of epigallocatechin gallate (EGCG) and epicatechin (EC) scored similar in astringent intensity, the effect of each on the model saliva surface differed. Epicatechin disrupts the saliva layer less than EGCG, which doesn’t track with sensory data and suggests astringency differs from compound to compound and multiple mechanisms are occurring simultaneously.

Tribology has provided important insight in wine and has been reviewed by Laguna and Sarkar (2017). Useful in this review is the effect of astringents on lubrication in the boundary regime. Correlations have been made between the friction coefficient and astringency of saliva wine complexes pointing to the ability of astringents to deplete salivary protein lubrication (Brosard et al., 2016). Another study showed that tannic acid found in wine was able to disrupt the boundary lubrication of adsorbed mucins (Ma et al., 2016). The disruption most likely lead to change in hydration and conformational changes in bound mucins that lead to a rise in friction coefficients. Whey protein and wine may not interact with oral tissues in the same manner, but there is evidence that disrupt of factors associated with the boundary regime lubrication leads to a sensation of astringency which appears to be caused by both astringents.

Tribology has also been used to evaluate the role of heat treatment on astringency of milk. Friction coefficients quantify the frictional force between two moving surfaces that are lubricated with the material under investigation (e.g., milk or whey protein beverage). A higher value friction coefficient means greater friction between the surfaces. In ultrapastuerized milk, friction coefficients and astringency were positively correlated (Li et al., 2018a; Li et al., 2018b). Since astringency is often described as roughness in the mouth, a complete understanding of how protein solutions alter friction may give us more information on how the mechanical processing of food in the mouth may influence astringency perception. Most of the interaction of food and
lubricating oral surfaces is governed by the principles of bulk rheology, the surface properties of the constituents, and the confinement or exclusion of microstructural components in the contact zone (Vicente et al., 2006).

While the methods just discussed are essential to understanding the molecular mechanism for astringency, a well-trained DA panel with a specific protocol is the most direct tool to quantify astringency. After exposure to an astringent food, it takes several minutes for the mouth to return to a normal mouthfeel (Hans et al., 2016). In the sensory sciences this is referred to as the carry-over effect. It takes longer for the mouth to clear sensations like astringency, which requires either a rinse solution or proper rest time between samples (Torrico et al., 2018). The human mouth produces more saliva in response to astringency. As new saliva is secreted into the mouth, it helps to clear the astringent compounds and return the mouth to a normal pH and level of hydration. This is why astringency lingers and takes several minutes to fully clear from the mouth. Important in the evaluation of astringency is proper protocol to prevent carryover of astringency from sample to sample. During sensory evaluations of astringency, a rinse solution is often used to help the mouth return to normal between samples. Many rinses are good to minimize astringency carryover with a variety of foods. Moreover, it supports the idea that astringency is associated with some change in oral surfaces that needs to be restored. Vickers et al. (2008) evaluated palate cleansers for evaluating sourness and reported that crackers worked better than water. The best palate cleansers reported for astringency in red wine were crackers, carboxymethyl cellulose (0.55%) and pectin (0.1%) (Brannan et al., 2001; Colonna et al., 2004; Ross et al., 2007). A rest time of 2-5 min between samples is also recommended. Ultimately, the standard for the quantification of astringency is a highly trained sensory panel. General sensory practices for trained panels have been previously reviewed (Drake, 2007).
Mechanism for Astringency

The normal model for astringency proposed by better studied food applications like tea and wine is that astringency is caused by polyphenols. This mechanism was described by Bate-Smith (1973) as an association between proline-rich salivary proteins and polyphenols to form insoluble complexes. The complexes can then precipitate or aggregate causing loss of lubrication in the mouth and deformation of the mucosal pellicle (Ployon et al., 2018). The mucosal pellicle is a thin biological component made of salivary proteins attached to the oral epithelial by covalent and non-covalent bonds (Gibbins et al., 2014). Certain salivary proteins including, proline-rich protein (Hay and Oppenheim, 1974), mucin (Tabak et al., 1982), alpha-amylase (Aguirre et al., 1989), and statherin (Douglas et al., 1991) have been shown to provide lubrication in the mouth. Complexation or aggregation of these proteins with astringents could therefore reduce the lubricity experienced in the mouth and lead to increased friction and roughness in the mouth. This has led to the theory proposed by Horne et al. (2002) that astringency is at least initially caused by delubrication via removal of the slippery coating on oral surfaces, exposing mechanoreceptors resulting in roughening, tightening, and drying sensations.

Most recently, it has been concluded that there are many factors and possibilities that contribute to astringency. Most notably the proposed mechanisms of polyphenol/protein aggregates that disrupt the salivary film and different interactions with mechanoreceptors on the tongue and oral surfaces, aided by the loss of lubricity, makes astringency difficult to define and control (Gibbins and Carpenter, 2013). An example of the mechanisms of astringency caused by polyphenol is summarized in Figure 1.
There are recent reviews on the mechanism of astringency caused by tannins and polyphenols (Bajec and Pickering, 2008; Gibbins and Carpenter, 2013). This review will focus on the astringent sensation caused by whey proteins.

**Mechanisms for Whey Protein Astringency**

Some of the same mechanisms that drive astringency by polyphenols may also the cause of astringency with whey proteins. There are several proposed mechanisms of astringency caused by or contributed by the addition of whey protein that provide some explanation. Some common proposed mechanisms from several research groups include astringency of whey protein beverages is caused solely by the acidity of the solution, whey protein saliva interactions delubricating oral surfaces, whey protein aggregate interaction directly with oral receptors, and an increase in oral friction caused by whey protein saliva aggregates.

**Astringency due to Acid Alone**

Some researchers believe that astringency is cause by the acidity of the solution and is not influenced by the concentration or presence of protein. The acidity alone cause the loss of lubrication in the mouth that leads to the mouth drying sensation of astringency. Lee and Vickers (2008) tested this hypothesis directly by preparing 1 or 6% (w/w) whey protein isolate (WPI) solutions at pH 3.4. In addition, control beverages with the same amount of phosphoric acid by volume that were required to lower the pH to 3.4 in the whey protein beverages were added to water as well as a water control that was adjusted to a pH of 3.4. A trained panel quantified astringency intensities. Acid control samples had higher astringency then the acidic whey protein solutions. The researchers concluded that astringency was caused by high acidity rather than by the whey proteins. Acids have been shown to cause astringency (Lawless et al., 1996) and clearly have a major impact on overall astringency, but to make the conclusion that acid is
the sole cause of astringency ignores research that demonstrates that protein contribute directly
to astringency. The whey protein solutions in the previously mentioned study differed in
titratable acidity, but it is pH not titratable acidity that dictates the unique functionality, solubility
and flavor of whey protein (Pelegrine and Gasparetto, 2005). Perhaps titratable acidity is not the
best measure of a solution predicted astringency but is more related to the solution buffering
capacity. The study presented by Lee and Vickers (2008) had some limitations. Panelist weren’t
highly trained, only attending two 1 hour sessions to distinguish between acidity and astringency
compared to other studies which included > 30 h of training over several months for panelists to
differentiate and scale these sensations (Beecher et al., 2008). The mere fact that they were not
highly trained does not discredit their findings, however extensive training is generally required
to generate consistent data between sessions, particularly for a complex sensation like
astringency (Lawless and Heymann, 2010).

In an experiment of different organic acids (lactic, malic, tartaric, citric) and the relative
astringency at different acid concentrations, lowering the pH of acid solutions while holding the
acid concentrations the same, resulted in increases in astringency. Therefore, astringency is
correlated with pH not TA (Sowalsky and Noble, 1998). Acids themselves cause astringency and
the astringency lingers (Sowalsky and Noble, 1998), but sourness as a sensation does not linger
after expectoration (Rubico and McDaniel, 1992; Hartwig and McDaniel, 1995). This suggests
that acids induce some changes in mucosal proteins which causes the lingering astringency, not
merely the presence of acid.

Sano et al. (2005) reported that 5 mM sodium phosphate buffer at pH 3.5 did not elicit
astringency but once 5% (wt/wt) whey protein was added, there was an increase in astringency.
Whey protein at that pH caused the astringent sensation. Other research (Beecher et al., 2008)
showed that astringency of whey protein solutions (6% w/v) decreased between the pH of 3.4 and 2.6 although there was a concurrent increase in sour taste. This phenomenon seems to contradict most of the previous research that has reported that as pH lowers, astringency increases. The shift in astringency which occurs at pH 3.4 is possibly explained by changes in associative interactions among the complex mixture of whey proteins and those found in the oral cavity (saliva proteins and those associated with oral mucosa). Apparent in these results is that astringency is not solely dependent on pH or TA. Certain whey proteins including lactoferrin are positively charged at neutral pH and cause astringency although the solution is at neutral pH, suggesting that protein along with pH contribute to astringency (Vardhanabhuti et al., 2010).

**Whey Protein Saliva Interaction Delubricating Oral Surfaces**

Oral lubrication is extremely important to normal mouthfeel conditions (Sarkar et al., 2019). Loss of lubrication plays a role in many of the other proposed mechanisms of astringency and is part of the reason astringency is so difficult to define. Salivary proteins provide a slippery coating to oral surfaces that aid in breakdown of the food and the formation of a bolus that facilitates swallowing. Saliva flow rates for a normal individual are approximately 0.3-0.4 ml/min without a stimulus (Iorgulescu, 2009). Meaning, our body is constantly replenishing the slippery surface of our mouth as we swallow, eat and drink. During the consumption of a food substance, saliva flow rates increase at the anticipation of breaking down food. Salivary flow rates may also increase after swallowing to help the mouth return to a normal state and to protect the oral cavity (Iorgulescu, 2009; Hans et al., 2016).

Acidic beverages produce some of the fastest salivary flow rates, as the continued exposure of the acid could harm the teeth (Hans et al., 2016). Salivary flow rates are therefore increased with acidic foods to return the mouth to a normal pH. This salivary film is the first
barrier of defense between our oral epithelium and food. Disruption of the salivary layer would then allow different interactions to occur in the oral cavity and potentially expose oral receptors that are normally protected by the salivary film. Astringent compounds disrupt the normal mouthfeel, which has been described as a tactile feeling with no abrasion between rubbing surfaces aided by hydrated surfaces maintained by thin film of saliva (Gibbins and Carpenter, 2012). Disruption of the salivary film layer by the previous definition would lead to abrasion and rubbing of unhydrated surfaces in the mouth. Delubrication via removal of this slippery thin layer of saliva on oral surfaces could be the cause of exposure of epithelial tissues and receptors causing roughening, tightening and drying sensations often associated with astringency.

Saliva is rich in mucins and therefore mucins are most studied to understand interactions with saliva. Mucins are large highly glycosylated glycoproteins that play a major role in the maintenance of viscoelastic properties of saliva and the oral mucosal coating as a protective barrier (Solmiany et al., 1996). Mucins typically have negatively charged groups from sialic acid residues and thus mucins have a low isoelectric point, estimated to be between 2 and 3 (Celebioglu et al., 2019). Whey protein complexation with saliva (possibly mucin) would bind the salivary film and allow whey protein to interact directly with the mucosal pellicle as well as newly exposed receptors.

Alternatively, astringency could be due to interactions between the positively charged whey proteins (pH < pI) and negatively charged proteins dispersed in saliva (Beecher et al., 2008). If salivary proteins have isoelectric points below the isoelectric points of whey proteins, there will be a pH range that results in a net electrostatic attraction causing aggregation between whey and saliva proteins. Therefore, rather than precipitation of whey proteins themselves due to pH changes, astringency of whey proteins could be related to interactions with oppositely
charged proteins in saliva, such as Proline Rich Proteins (PRP) or mucins. This fits one aspect of
the polyphenol-astringency shown in Figure 1. Hsein et al. (2015) made solutions of whey
protein (0.25-4.0% w/v) and mucins (1%, sourced from porcine stomach) to understand the
mucoadhesive properties of whey protein. Mucoadhesion is the adhesion between two materials,
one of which is a mucosal surface (Skaikh et al., 2011). Whey protein exhibited mucoadhesive
properties and interaction with mucin across a wide pH range of 1.2-4.5 in a model solution,
suggesting binding with unbound salivary proteins or salivary proteins attached to oral surfaces.
An important unanswered question is which mucins interact with whey protein first; bound or
unbound mucins. If whey proteins are in large supply both groups of mucins would interact with
whey proteins until the mouth produces enough saliva to clear the mouth. If astringents interact
directly with salivary proteins found on oral surfaces, then their detection may not require oral
movement as the response would be related to binding and not friction. However, this does not
rule out roles for surface binding and friction in astringency.

Different research groups have investigated potential factors that could overcome the loss
of lubrication mainly by changing viscosity, hydrocolloid complexation, and the effect of fat
addition. Beecher et al. (2008) investigated the role of viscosity of whey protein beverages on
the sensory perception of astringency. Beverages contained 6% w/v whey protein, phosphoric
acid to decrease pH to 3.4, and sucralose for sweetness. Maltodextrin was used to manipulate
viscosities with 0, 10, 15, 20% w/v or 0.27% methyl cellulose (w/v) (1.6- 7.7 mPa s, shear rate
10 and 100 s⁻¹). There were no differences in astringency among the beverages with increases in
instrumental viscosity. Withers et al. (2014) made a range of protein beverages (7.0-8.7 % wt/wt,
pH 7) with different casein (Milk protein concentrate, sodium caseinate, calcium caseinate) and
whey protein (WPC75) ratios and viscosities (15.1- 42.8 mPa s ,shear rate 44. s⁻¹), and subjected
the samples to pasteurization (60°C, 15 min). There was minimal to no effect of viscosity of
different treatments on the perception of astringency. These results suggest that, at least within
the viscosity range tested, viscosity doesn’t have an effect on the perception of astringency in
dairy protein beverages at high or low pH. Both studies only focused on a fluid range of
viscosities. Most whey proteins are consumed as beverages, however perhaps viscosity may
possibly effect astringency as we proceed into thicker fluids, or even semi-solid foods like bars
which are another common protein food. Viscosity has been shown to manipulate astringency
with polyphenol, therefore, a comparison of studies of viscosity on the effect of polyphenols is
important. Grapeseed tannin astringency (1.9-45.3 mPa s, Smith et al., 1996) and aluminum
sulfate and citric acid (1-29 mPa s, Smith and Noble, 1998) were investigate to seem how
viscosity effect astringency. The greatest reduction of astringency happened during an increase
from 1 to 5 mPa s and 5 to 10 mPa s. After 10 mPa s viscosity only had a minor influence
(Smith and Noble, 1998). Therefore in order to reduce astringency caused by polyphenols,
increasing the viscosity of the solution to 10 mPa s provides the greatest impact. Increased
viscosity appears to reduce astringency from polyphenols and acids, but has little effect on whey
protein solutions.

Certain hydrocolloids including high methoxy pectin, have been used to form more heat
soluble complexes with whey protein (Wagoner et al., 2016). Previous work has shown that the
addition of pectin to astringent fruit juices can reduce the perceived astringency as the pectin
complexes with the soluble tannins preventing the interaction of salivary protein and tannins
(Soares et al., 2012; Taira et al., 1997). No work has been done to quantify the astringency of
whey protein pectin complexes, but if the mechanism functions the same as has been
demonstrated with fruit juices, it presents the chance of reduced astringency by complexing the
whey protein with pectin. Whey protein-pectin complexes however create turbid beverages, which may limit their acceptability in final application (Wagoner and Foegeding, 2017).

The presence of fat decreased astringency in ultra-pasteurized milk (Lee et al., 2017; Li et al., 2018b). It has been proposed that fat acts as a lubricant that reduces perceived roughness and friction in the mouth. It is also possible that the fat competes with protein via the mucoadhesion mechanism limiting the perceptions of astringency. In contrast, the addition of fat to higher protein beverages (7-11.8%) appeared to have little effect and may have even increased astringency (Withers et al., 2014). This is different from the concept proposed by Gachons et al. (2012), which suggested that oral fatty sensation and astringency were opposite sides of the sensory perspective. Fat provides lubrication and has been called slippery, while astringent compounds create a dry and rough mouthfeel. The addition of fat may help reduce astringency in neutral whey protein beverages. Adding fat to a beverage would increase its turbidity and decrease its clarity. However, acidic whey protein beverages are characteristically clear and the addition of lipid would make them turbid, most likely lowering their acceptance. More work is needed to confirm the role of fat addition in acidic conditions to perceived astringency.

**Whey Protein Aggregates that Interact Directly with Oral Surfaces**

Different whey proteins contribute differently to astringency. The majority of whey proteins have similar charge states at varying pH values. Beta-lactoglobulin has a reported isoelectric point of pH 5.2 (Cannan et al., 1945). Alpha-lactalbumin has a reported isoelectric point of pH 4.8 (Zittle, 1956). Lactoferrin differs from the other whey proteins and has an isoelectric point of 8.8, meaning that it has a net positive charge in the normal neutral protein beverage range (6.6-7.0). Lactoferrin had a much lower astringency threshold compared to beta-lactoglobulin at pH 3.5 - 0.5% versus 3.0% (Ye et al., 2012). Lactoferrin had significant
astringency at a 0.1% (w/v) protein concentration at both pH 3.5 and 7.0. Ye et al. (2012) demonstrated that the perception of astringency may involve binding to oral epithelial cells with an ELISA test with HSC-2 or NO-1-N-1 cells. They then followed a series of dilution with both beta-lactoglobulin and lactoferrin to determine what concentration of these proteins remained bound to the cells. These results do not preclude the possibility of multiple mechanisms including interactions between whey proteins and salivary proteins. Lactoferrin had much stronger binding of oral epithelial cells than B-LG, which may explain the high level of astringency associated with lactoferrin. Astringency of lactoferrin was displayed at pH < 7.0 without the formation of precipitated saliva (Vardhanabhuti et al., 2010). Because lactoferrin carries a net positive charge at pH values below 8.8, it can potentially interact with negatively charged proteins found in the saliva or on oral surfaces under neutral and acidic beverage conditions.

Since the composition of whey protein changes with different cheese make procedures, or method of purifying protein streams, it is possible that differences in whey protein composition may contribute to perception of astringency. For example, the protein profile of whey protein isolate made by a process based on microfiltration differs from one prepared by ion exchange. Glycomacropeptide (GMP) is the resulting peptide from rennet cleavage of kappa casein during cheese manufacture. Ultrafiltration retains this peptide and it composes a large portion of the whey protein powder, approximately 20% (Farias et al., 2010). During ion exchange, the GMP is not retained in the final powder. No research has been conducted to evaluate the direct impact of GMP on astringency, but GMP serves as an example that whey protein powders can differ in protein composition. Astringency of specific whey proteins lactoferrin and B-LG have been
documented, presumably due to their unique PI and prevalence, respectively. To our knowledge, the specific contributions of other whey proteins have not been specifically addressed.

Neither whey proteins nor salivary proteins precipitate on their own without changes in heat or pH. Mixing whey proteins with saliva produces a precipitated phase that contains salivary and whey protein (Vardhanabhuti et al., 2010). Measuring the turbidity of saliva and beta-lactoglobulin mixtures at different pH values demonstrated that the maximum turbidity of the mixture was observed between pH 4.6-5.2. Beecher et al. (2008) also reported that high levels of astringency in whey protein beverages were correlated with increased turbidity of saliva and protein mixtures.

Whey protein aggregates may contribute directly to astringency. Larger size whey protein aggregates change the texture of the salivary film in the mouth and as surfaces rub may increase sensation of astringency. Turbidity in whey protein beverages increases with an increase in the size of the whey protein aggregates from 0.1 to 3 um (LaClair and Etzel, 2009). If the size of particles further change as a result of oral conditions, this would change the fluid saliva composition and could create greater oral friction increasing the perception of astringency, especially accounting for delubrication of oral surfaces considered in the previous section. These whey protein aggregates may be the cause of tactile changes without any contribution by salivary protein. Interactions between whey protein and salivary proteins will be discussed later. Sano et al. (2005) described that most whey proteins, including beta-lactoglobulin, the most abundant whey protein, precipitate at around pH 5. When an acidic whey protein solution (pH 3.5) is mixed with saliva (pH ~ 7.0) the average pH of the mixture would be ~ pH 5 causing precipitation of protein (Sano et al., 2005). The precipitated protein particles would create
astringency in a similar way that polyphenols and salivary proteins create complex precipitants that reduce lubrication and create roughness.

Whey protein aggregation can also be caused due to excessive heating. The most abundant whey protein, beta-lactoglobulin, denatures and aggregates at around 70°C (Briggs and Hull, 1945). During the production of whey protein powders, the whey proteins pass through various heat processes that may cause denaturation and aggregation. Bull et al. (2017) found that heating WPC solutions (10% w/v, pH 6.5-6.7) for longer periods of time (70°C for 5, 10, or 20 minutes) lead to increased astringency. The researchers also concluded that an increase in astringency was documented with repeated consumption, suggesting mucoadhesion of whey proteins. This phenomenon of increased denaturation or aggregation increasing mucoadhesive strength of whey proteins has been noted before (Hsein, 2015). This result is consistent with the results of Lee et al. (2017) and Li et al. (2018b) where ultra-pasteurized milk had higher astringency scores than high temperature short time pasteurized milk. Previous research has shown that whey proteins are primarily responsible for astringency in milk and milk beverages (Withers et al., 2014). Heat treatment has a major effect on whey protein which translates into changes in the perception of astringency.

Ye et al. (2011) determined that heated (90 °C for 10 minutes) and unheated beta-lactoglobulin solutions (2%) mixed with saliva had higher particle size and turbidity at pH 3.4 than beta-lactoglobulin solutions alone. This observation was likely due to beta-lactoglobulin and salivary proteins having opposite charges at this pH, which may lead to complexation through electrostatic interactions. Protein and saliva mixtures that were heated had higher turbidity and larger particle sizes at pH >5.0 than unheated samples. The heat treatment most likely denatured and aggregated some of the whey protein which increased particle size. Increases in astringency
and particle size were consistent and positively correlated over the pH range of 3.0 to 3.6, suggesting that denaturation/aggregation adds to the astringency associated with native proteins. Whey proteins and salivary proteins interact via electrostatic interactions at pH 3.4, whereas only salivary proteins undergo aggregation at pH 2.0. It appears that forming protein aggregates alone doesn’t necessarily correlate to astringency, as high particle size and turbidity noticed at pH 5.0 did not cause the perception of astringency (Ye et al., 2011) even though charge interactions between whey protein and saliva were demonstrated.

The astringency of whey protein beverages increased as protein load increased under high acid, pH 3.5, (Sano et al., 2005) and low acid, pH 6.8 (Beecher et al., 2008) processing conditions. Kelly et al. (2010) reported that astringency of whey protein beverages increased with increasing protein concentration from 0.25% to 4% (w/w) at pH 3.5. Higher protein levels had a lower dose response with little increase of astringency from 4 to 13% w/w even though the acid concentration increase with increased buffering capacity. Although, experimentally there was no increase of astringency beyond 4% protein w/w, it is reasonable to assume that depending on the mechanism, more stimuli would create more astringent sensation. Childs et al. (2010) also showed an increase in astringency with increasing concentration of protein up to 6% w/w in a model whey protein beverage at pH 3.4. Oltman et al. (2015) reported that increasing the protein load from 4% to 8% w/w also increased the perceived sensory astringency in acidic whey protein beverages (pH 3.2). An experimental maximum of protein astringency at pH 3.5 has not been determined.
Whey Protein Saliva Aggregates That Overcome Lubrication in the Mouth Increasing Oral Friction

Astringency values of whey protein solutions rated by a descriptive analysis panel increased with increasing turbidity (an indicator of aggregation) of the solutions (Beecher et al., 2008). This result suggests that saliva proteins are not only able to interact with whey proteins, but that the end results creates larger sized particles that scattered more light and appear to increase the perception of astringency even though the exact mechanism is not known. A more direct experiment needs to be conducted with whey protein aggregates created over a range of sizes to see the direct impact on perceived astringency.

Ye et al. (2011) combined mixtures of saliva and beta-lactoglobulin (2%) at various pH values from 2-7. Whey and saliva mixtures were turbid between pH 3-4. The particle size of human saliva had a particle size of 100-500 nm and increased gradually as pH decreased from pH 7 to 3. Below a pH of 3, saliva rapidly increased in size to over 2,000 nm. The particle size of beta-lactoglobulin and saliva were similar at pH 7-4.7, however upon mixing the particle size of the mixture was larger than saliva or beta-lactoglobulin alone in the range of pH 3.0- 4.7 (Ye et al., 2011). Astringency results collected by descriptive analysis also showed that peak astringency occurred at pH 3.4, which was also the most turbid saliva protein mixture and the largest particle size (3.5 um). At pH of 2.5 there was less perceived astringency which can possibly be explained by the salivary protein (mucin) having minimal charged at that pH.

Saliva flow rates have also been studied as a possible method to assess astringency increase and plateau at higher beta-lactoglobulin concentrations (10% w/v). Saliva flow rates for individuals increased with increasing protein concentrations from 0.5 to 10% (w/w) (Kelly et al., 2010). This fact becomes important because if saliva interacts with whey protein and form
complexes, a higher flow rate of saliva will produce more substrate for more astringent complexes (Kelly et al., 2010). Higher flow rates of saliva also more quickly replace astringent complexes bound to oral mucosal and can help return the mouth back to normal conditions.

Vardhanabhuti et al. (2011) investigated the effect of beta-lactoglobulin at pH 3.5 and 7.0 on the lubricating property of saliva as related to astringency perception. Saliva was adsorbed onto surfaces of a rotating poly dimethyl siloxane ball and disc to form a film under conditions that mimic the rubbing contacts in the oral cavity (Bongaerts, Rossetti, & Stokes, 2007), and the lubricity of saliva films upon exposure to astringent compounds was measured. While addition of beta-lactoglobulin at pH 7.0 slowly increased friction of saliva film between tribopair surfaces, beta-lactoglobulin at pH 3.5 rapidly increased the friction coefficients of saliva. This effect was similar to other astringent compounds like epigallocatechin gallate and alum. This result supports the hypothesis that astringency of beta-lactoglobulin arises from the loss of lubrication of saliva which is in agreement with the well-accepted astringency model of polyphenols. Without the aid of the hydrating salivary film, large particles could be perceived by the tongue by mechanoreceptors. With typical oral movement, this would cause exaggerated oral friction as a combination of loss of lubrication and increased number of perceived particles on the tongue and oral surfaces.

Since the mucosal pellicle is a region where salivary proteins are bound to the oral epithelium, upon interaction with protein, new bonds can create a deformation of the mucosal pellicle causing it to transition from a largely smooth surface to a rough surface. In a state of delubricated oral surfaces, this newly deformed oral epithelium can further aggravate oral friction. The combinations of multiple mechanisms further exaggerate the sensation of astringency.
All of the aforementioned mechanisms of whey protein astringency contribute and exacerbate each other. Targeted solutions to the lowering of astringency in whey protein solutions should be aimed at reducing or preventing all mechanisms. The mechanism for astringency also appears to be related to different ranges of pH, so the most probable mechanisms for astringency depend on the pH.

**Acidic Whey Protein Beverage Solutions**

As mentioned earlier there is great interest in improving the palatability of acidic whey protein beverages due to their lack of need of refrigeration and mild heating requirements. However, the biggest obstacle to their popularity is astringency (Oltman, et al., 2015). After summarizing some of the possible mechanisms of whey protein astringency, there are possible solutions to limit or minimize the perceived astringency in high acid whey protein beverages based on the mechanisms.

The first consideration is acidification source. Since acids themselves can contribute astringency to beverages, it is important to choose an acidulant that has the smallest effect on astringency. Inorganic acids are more astringent than organic acids (Rubico and McDaniel, 1992). The best solutions appear to be a blend of acidulants that provide the correct pH adjustment, flavor profile, and sourness (Rubico and McDaniel, 1992). Acid selection is certainly important given the buffering of whey protein. Due to the buffering capacity of whey protein, increasing the protein load of the beverage requires the addition of more acid to hit the target pH. Creating products with high levels of protein and using organic acids to adjust the pH may be problematic as the beverage becomes more sour due to the amount of acid needed to adjust target pH.
The second consideration is prevention of whey and saliva proteins interactions. Since the saliva is provided by the consumer, the innovation responsibility is for the whey protein beverage manufacturer. As pH values decrease below the isoelectric points (pIs) of whey proteins, they become positively charged and can interact with saliva proteins that have lower pIs. If whey proteins were electrostatically complexed with a charged polysaccharide, then in theory they will not be able to interact with salivary protein. The strategy would also prevent whey proteins from binding or interacting with mechanoreceptors or the oral epithelial surface.

The third option is adding ingredients that could increase the oral lubricating properties in the beverage. With a beverage that provides lubrication in the mouth, oral friction should be minimized which would limit the perceived astringency. The effect of viscosity on astringency perception has been studied, but most were studied in relatively fluid ranges (1.6-7.7 mPa s$^{-1}$, shear rate 10 and 100 s$^{-1}$) (Beecher et al., 2008). Thicker beverages would theoretically slow the rate of diffusion of astringent compound to oral surfaces. Acidic whey proteins do not typically use stabilizers since whey proteins are readily soluble < pH 4.6. Perhaps there are polysaccharides that be effective due to a combination of mechanisms, such as forming electrostatic-based complexes and altering viscosity; however, any approach to decreasing astringency must not change the viscosity and clarity of the beverages such that consumer acceptance is decreased.

**Conclusion**

Astringency in whey protein beverages is a complex phenomenon. Astringency in foods has multiple mechanisms and several contributing factors. This is why defining the exact mechanism for astringency remains a challenge for a product like whey protein beverages. The precise molecular mechanism for astringency of whey protein remains unclear, although there
are factors known to alter the degree of astringency. Similar to polyphenol astringency, if the complexation of whey and salivary proteins can be reduced or prevented, astringency should be reduced. Astringency is generated by both whey protein and the acidity of the solution. More research is needed to understand how whey protein ingredient astringency can be minimized, which will in turn increase the consumer liking of the product and allow manufacturers to deliver superior nutrition through whey products.

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References


**Figure 1.** Possible mechanisms of astringency occurring simultaneously in the oral cavity. Adapted from Gibbins and Carpenter (2013) 1. Aggregation of salivary proteins creating grittiness 2. Salivary film disruption 3. Reduced salivary lubrication 4. Exposure of receptors 5. Nociceptors/mechanoreceptors or nerve innervation
CHAPTER 2: Microfiltration Derived Casein and Whey Proteins from Milk
Microfiltration Derived Casein and Whey Proteins from Milk

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Abstract

Milk, a rich source of nutrients, can be fractionated into a wide range of components for use in foods and beverages. With the advancement in filtration technologies, micellar caseins and milk derived whey proteins are now produced from skim milk using microfiltration. Microfiltered ingredients offer unique functional and nutritional benefits that can be exploited in new product development. The process offers promise in cheese making where microfiltered milk can be used for protein standardization to improve yields and consistency of cheese operations throughputs. Micellar casein concentrates (MCC) and milk whey proteins (mWP) are ingredients that could offer unique functional and flavor properties as an ingredient in various food applications. Consumer desires for a safe, nutritious and a clean label ingredient could be potential growth opportunities for these new ingredients for application in new products. The application of MCC in protein standardization could offer a window of opportunity to U.S. cheese makers by improving yields and throughputs of manufacturing plants.
**Introduction**

Concurrent with the increase in per capita income globally, there is an increase in demand for proteins, including dairy proteins. Dairy proteins are generally superior in nutritional quality compared to most plant-based proteins (Hoffman and Falvo, 2004; Rutherfurd et al., 2015). Some of the common dairy protein applications include its use in infant formula, canned milk, dairy products and baked goods. Dairy proteins are also sought after for their functional properties and ingredients are now widely used as clean label solutions. Dairy proteins isolated from filtration processes are among the top clean label alternatives.

Milk is an important source of macronutrients like fat, proteins, sugar in the form of lactose, and 10 other important micronutrients (including minerals) (Haug et al., 2007). Two of the next-generation dairy protein ingredients that can be isolated from milk are micellar casein and milk derived whey protein which are isolated from skim milk using microfiltration. These ingredients are gaining considerable attention from food and beverage manufacturers due to their unique protein profile and functionality. The purpose of this review is to summarize the current state of science on micellar casein and milk derived whey protein ingredients. This document will also highlight the unique characteristics of these protein ingredients and outline opportunities for their use of in food and beverage applications.

**Membrane Processing to Separate Milk Components**

The 1960s were marked as the beginning of modern membrane technology when Sourirajan and Loeb invented a reverse osmosis (RO) membrane for water desalination at the University of California in Los Angeles (Loeb and Sourirajan, 1963). The dairy industry adopted membrane technology in the 1970s and continues to add value to dairy components/ingredients by further advancing the adaptation of membrane technology.
There are four major pressure driven membranes used in dairy processing. Based on pore size and molecular weight cut off, the separation process can be classified as microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO) (Figure 1). MF has the largest pore size (0.1-10 µm) and the lowest processing pressure (0.01-0.2 MPa) and was originally introduced in the dairy industry as a pretreatment to reduce microbial load (Pouliot, 2008). Since MF covers a wide pore size range, it can separate different macromolecules depending on the particle size. When a 1.4 µm MF membrane is used, both bacteria (10-100 µm) and large milk fat globules (10 µm) are rejected by the membrane (called retentate) while micellar casein (50-500 nm) and whey proteins (3-6 nm), lactose (1 nm), minerals and water permeate through the membrane (called permeate) (Adams, 2012; Pouliot, 2008; Smith, 2013).

When a 0.1 µm MF is used with skim milk, casein micelles are retained while milk serum protein or whey protein pass through the membrane, effectively creating MCC and milk derived whey protein. A simplified schematic of the Micellar Casein and Milk derived whey protein manufacture is represented in Figure 2.

UF predates MF in the dairy industry and is currently widely used in manufacture of concentrated milk proteins and milk for cheese making (Maubois, 1986). UF membranes have a pore size ranging from 10-100 nm with processing pressures of 0.1-1.0 MPa (Adams, 2012; Pouliot, 2008; Smith, 2013). During UF of skim milk, lactose, minerals and water partition into the membrane whereas the proteins and fat partition into the retentate. The partitioning of components yields a protein rich retentate and a carbohydrate and mineral rich permeate.

Reverse osmosis (RO) is a concentration technique and thus has a smaller pore size and (<1nm) a higher processing pressure (3.0-5.0Mpa) (Adams, 2012; Pouliot, 2008; Smith, 2013). NF is a “loose” RO, with a pore size of 1-10 nm and lower processing pressure 1.5-3.0 MPa (Adams,
NF is a fractionation technique and rejects components besides water and monovalent salts.

Membrane technology provides several options for dairy processing. Membrane materials can be polymers (cellulose acetate, polysulphone/polyethersulphone, polyamide, polyvinylidene fluoride and polypropylene) or inorganic materials (ceramic, metallic) (Jensen and Kønigsfeldt, 2000). A more hydrophilic and negatively charged membrane is desired to avoid protein fouling. Besides a consideration of material choice, the cost of installation and operation often dictate membrane choice. The capital cost fee of the polymer membranes is reported as 1/10th of ceramic membranes, while ceramic membranes have a wider pH and temperature range and a longer life (at least 10 years when compared to 0.5 years typically from a polymeric membrane) (Park et al., 2014; Gitis and Rothenberg, 2016). Generally, polyamide is used for RO, polyvinylidene fluoride and polypropylene for MF and UF and ceramic for MF in dairy (Carvalho and Maubois, 2009). Membrane technology also provides several modules to house membranes. Polymer membranes are commonly housed in plate and frame, spiral wound and hollow fiber modules, and inorganic membranes have their own tubular designed module.

Selection of membranes is a balance between target products, raw material, processing efficiency, and cost of installation, operation, and maintenance.

Membrane materials also differ in method and efficiency of separation. Ceramic MF membranes are designed for high cross flow velocity (CFV) while maintaining low transmembrane pressure (TMP). This keeps permeate passage consistent down the length of the membrane and prevents major fouling with a high rate of permeate passage (Karasu et al., 2010). Uniform transmembrane pressure allows maximum passage of permeate and is achieved by using an additional pump to recirculate the permeate leveling out the TMP. Ceramic membranes
have a very uniform pore size distribution which allows the separation of molecules by size. Polymeric membranes on the other hand, form a foulant or concentration polarization layer which in reality dictates the separation as opposed to the declared molecular weight cutoff. Depending on system design, polymeric systems may have unfavorable processing condition of high TMP and low CFV leading to lower performance and efficiency of separation compared to an optimized ceramic system (Karasu et al., 2010).

**Microfiltration**

As indicated earlier, there are two sizes of MF used in dairy processing: 1.4 µm for bacterial removal done with ceramic membranes and 0.1 µm for fat removal and casein and whey protein separation. MF membranes are available in both ceramic and polymeric spiral wound forms. As MF membranes have a larger pore size, they can foul faster than other types of membranes. Generally, to prevent fouling, a high cross flow velocity can be applied to create lift of the particles, keeping the pores of the membrane clear. Spiral wound polymeric membranes can’t handle high cross flow velocity because it causes the membranes to telescope (mechanical deformation of the center of the membrane pulling out of the outer wraps). Most systems have anti telescoping devices to hold the membrane shape. To reduce fouling on ceramic MF, a higher cross flow velocity (5-7m/s) is applied on the retentate side by increasing retentate recirculation pump rate. This introduces high wall shear stress and washes away the foulant on the membrane surface. Two modified ceramic membranes have been developed to achieve the optimal transmembrane pressure to and prevent fouling; either by 1) changing the thickness of the supporting material; 2) or by changing the thickness of the membrane along the length of the membrane. These modification are meant to mimic the performance of uniform transmembrane pressure (UTP) membranes. Both of these modified ceramic membranes are more economical
options because they can operate without the need of a permeate recirculation pump, reducing operating costs.

Although MF can be used for many functions including bacteria removal, fat removal and milk fat globule membrane separation, this review will focus on the separation of casein and whey proteins from skim milk. Both polymeric and ceramic membranes can be used to achieve this, although, as indicated earlier, they vary greatly in membrane performance and process efficiency. Early studies on separation of micellar casein and milk whey protein from skim milk by MF were conducted in the 1990s. The pore size of MF membranes that work for the separation of casein and whey proteins are usually 0.1 or 0.14 µm for ceramic MF and 0.5 µm for spiral wound MF. These pore sizes are based on the size of protein, micellar casein (50-500 nm) is rejected by the MF membrane as retentate while whey protein (3-6 nm) passes through the membrane as permeate (Saboya and Maubois, 2000; Fox and McSweeney, 2003). However, if the skim milk is pasteurized at high temperatures (>78°C), whey proteins will denature and form large particles (aggregates) or the denatured whey protein will adhere to the casein micelle itself which will not pass through the membrane (Saboya and Maubois, 2000). As a result, the retentate will not be micellar casein but a product more similar to milk protein concentrate in terms of casein to whey protein ratio.

A 70-80% whey protein transmission was achieved by a 0.1 µm ceramic MF applied to skim milk (Le Berre and Daufin, 1996). Samuelsson, Dejmek, Trägårdh, and Paulsson (1997) reported that a higher processing temperature and higher cross flow velocity favored whey protein removal from skim milk via MF. An 88% whey protein removal was obtained from skim milk at 55°C with circulation velocity of 8m/s by a 0.14µm ceramic MF (Samuelsson et al., 1997). Several studies have addressed micellar casein and whey protein separation by both
ceramic (UTP, Graded Permeability (GP) and Isoflux) and spiral wound (SW- with PVDF as membrane material) MF. In a single stage (no diafiltration or dilution) at 3x (x=concentration factor= total volume/retentate volume, CF) MF of skim milk at 50°C, ceramic UTP MF had the highest whey protein removal (64.40%) followed by GP (61.04%) and SW (38.62%) (Zulewska, Newbold, & Barbano, 2009). The whey protein removal from skim milk was significantly increased in UTP (achieved 95%) and SW (achieved 70.3%) when engaging diafiltration (DF) (Beckman, Zulewska, Newbold, & Barbano, 2010). The low whey protein removal rate in SW could be due to fouling caused by the higher hydrophobicity of polymeric membranes and lower cross flow velocity (Beckman et al., 2010). For SW, a 95% whey protein removal from skim milk was achieved through an 8 stage MF with 7 stages of DF at 50 °C (Beckman et al., 2010). The membrane choice by the processor, apart from purity of the casein and whey protein stream needed is also dictated by capital and running costs of the membrane.

**Micellar Casein**

There is no standard of identity of micellar casein concentrates in the US or Europe. However, the American Dairy Product Institute defines a microfiltered milk product as either microfiltered milk protein (MMP) or micellar casein (MC) that has an adjusted ratio of casein to whey proteins (ADPI, 2018a). Other milk protein ingredients like milk protein isolate (MPI) and concentrate (MPC) retain the same natural ratio of casein to whey protein found in milk (80:20) (ADPI, 2018a). Compositionally, the protein content as a percentage of the total solids determines if the powder or liquid is a concentrate or isolate. Micellar casein products that have been generated or researched to-date are concentrates (ca 85% protein) and thus MCC is the most commonly referenced name. An MCC will have a different ratio of casein to whey protein
with a higher proportion of casein than an MPC. MCC as an ingredient evolved with the development of cost effective MF membranes with improved separation efficiencies.

In the United States, MCC is primarily used as a high quality protein source to fortify food applications. Although FDA exercises enforcement discretion in the use of UF milk in the manufacture of standardized cheese, the use of microfiltered milk (and thus MCC) is not permitted. In the EU, microfiltered milk is commonly used for standardization of cheese milk, thus improving cheese yields (Culhane, 2016; Neocleous et al., 2002). The use of MF milk has several potential benefits for cheese manufacture. Firstly, cheese yield is increased by increasing the percentage of casein per unit volume (Neocleous et al., 2002). Cheeses made from non-fortified milk typically generates 1 part cheese and 9 parts whey. Fortifying the casein content of cheese milk increases the cheese yield per hundred weight of milk used. Govindasamy-Lucey et al. (2007) made pizza cheese with cold polymeric MF milk and found increased cheese yields without compromising quality or functional attributes of the cheese. Whey protein ingredients from the permeate stream of ultrafiltered skim milk has no cheese ingredient residuals and glycomacropeptide that may enable the use of these protein ingredients in specific food applications. A more detailed explanation of the difference of whey protein produced by microfiltration of skim milk versus cheese making (whey) will be covered in later sections. Third, it allows for more accurate casein standardization of cheese milk. Currently, fat and protein in cheese milk are adjusted to a specific ratio depending on the desired composition in the final cheese. However, protein standardization may not be the most accurate technique since total protein includes whey proteins which is lost during the manufacture of most cheese varieties. Cheese milk standardization with MF involves fat separation of incoming milk into skim milk and cream followed by MF of skim milk into a casein rich retentate stream. Cream is
added to the retentate for fat standardization for final cheese manufacture (Brandsma and Rizvi, 2001).

Microfiltration used to increase the solids and casein content of cheese milk has shown promise as a novel alternative to the traditional cheese making process. Make procedures need to be adjusted to overcome, changes to gelation kinetics (Lu et al., 2016; Brandsma et al., 2001; Amelia and Barbano 2013a). Protein and fat retention is improved by 4–5% (Garem et al., 2000), and the starter and rennet quantity can be reduced. Lu et al. (2017) investigated cheesemaking with cheese milk enriched with MCC to a casein content of 3.2-10.9%. The study concluded that cheesemaking with MF and UF enriched milk had to address cheese make procedure to address changes related to: 1.) Impact on downstream processing due to less whey generation 2.) Changes to ionic strength and buffering of the milk that could influence gelation kinetics 3.) Potential changes to rennet and culture usage rate. Casein-enriched cheese milk can be pasteurized at higher time temperature combinations (110–140°C, 10 s) than conventionally pasteurized milk with minimal effects on gelation kinetics. Decreased micellar interactions of κ-casein and β-lactoglobulin (β-LG) is hypothesized to minimize impact of heat treatment on cheese making (Dalgleish, 1990). Papadatos et al. (2003) conducted an economic feasibility analysis of the use of microfiltration of skim milk prior to cheesemaking including the cost and production of coproducts. They found based on data from 1998 to 2000 that the net revenue was higher in 30 out of the 36 months examined. The net revenue compared to conventional cheesemaking was increased by $0.41 for Cheddar and $0.45 for mozzarella per 100 lb. of standardized milk and thus strengthening the economic argument of allowing MF milk in the cheese making process.
Importance of casein purity to define and name MCC

Although, ADPI provides a standard way to define the total protein of an MCC powder but this naming mechanism doesn’t provide information on casein purity. Since the purity of MCC is one of the key intrinsic factors that affects its flavor and functionality (discussed later in the paper), it should be an important criterion in the naming of MCC. Therefore, one of the important calls to action for the dairy industry is to devise a way to name MCC ingredients based on their purity. Traditionally, nomenclature of Dairy protein concentrates produced from whey and milk list the type of protein using a short 3-letter code followed by a number that distinguishes the percent protein of the dry matter. For instance, WPC 34 is a whey protein concentrate (WPC) with 34% protein on a dry basis. MCC, however, is different because in addition to the total protein content, it needs to communicate its purity via either the amount of casein as percentage of true protein that is present in the MCC or the amount of milk derived whey protein that has been removed from the original milk in addition to the percentage of protein. Without an appropriate standard or label, there is no way to economically or functionally differentiate MCC from MPI or MPC. Analytically, to correctly measure the purity of MCC it would require high performance liquid chromatography or SDS-PAGE of the MCC to correctly measure the amount of whey protein. Kjeldahl analysis following heat treatment is an inaccurate technique to measure undenatured whey proteins and will be unable to confirm the extent of whey protein removal during MC manufacture (Verdi, 1987). Table 1 summarizes the nutrition composition of protein ingredients from milk.

MCC Purity: Casein as a percent of true protein or percent casein

As discussed earlier, different membrane types allow different levels of whey protein transmission through the MF membrane. This means that the ratio of casein and whey of an
MCC is dependent on the type and extent of filtration (i.e. number of stage, diafiltration). For reference, it is important to note that fresh milk contains approximately to 82% (Verdi et al. 1987; Barbano et al., 1991) of its true protein as casein, meaning that raw skim milk starts off as an MCC of 80 to 82% purity. Summarized in Table 2 are the various protein purities by membrane type. Without a major adjustment to the casein:whey protein ratio, an MCC product may be functionally indistinguishable from a milk protein isolate in food systems. Describing MCC purity as a percentage of casein of true protein follows the style of the other protein concentrates in the industry like WPC and MPC. However, labeling MCC as a percentage of casein of the true protein doesn’t clearly communicate the total protein amount. There is a trace amount of whey protein in MCC powders of high purity. Therefore an MCC powder labeled as MCC 92 would actually have a higher total protein percentage, since the 92 only refers to casein percentage and not the total protein content of the powder. By this labeling convention, an MCC XX would declare the amount of casein but the amount of whey protein removed would be unknown. Therefore, an improved naming mechanism is required to communicate casein as a percentage of total protein as well as the total protein content.

**MCC Purity: Percent whey protein removal**

MCC can also be distinguished by the amount of milk derived whey protein that has been removed from the original skim milk by the MF process. The remainder of the milk whey protein is in the MCC as either undenatured or denatured milk derived whey protein. MCC named in this way may be called MCC with XX% reduced whey protein. This type of designation indirectly expresses the protein purity of the MCC. A comparison of MCC purity expressed that can be produced by spiral or ceramic membrane results in different concentrations of residual whey protein in the MCC (Table 2). This method also allows declaration of the
protein content, for example MCC82 with 95% reduced whey protein. This method declares the protein percentage, 82, with the casein purity as 95% reduced in whey protein.

**Functional properties of MCC**

Casein exist as colloidal complexes and compose 80% of the proteins in bovine milk (Schmidt, 1980). Classical separation techniques of casein from milk involve isoelectric precipitation and enzymatic hydrolysis of the proteins (by the action of rennet). Microfiltration is a physical separation process that can isolate caseins in the native form thus providing the protein ingredients with a unique set of functionalities (Lu et. al., 2015) At the same time, there is a significant opportunity to improve the functional properties of MCC such as its heat stability and hydration, as these properties are the cornerstone of any high protein ingredient in food applications.

**Heat Stability**

Food and beverage launches with “added protein” or “high/source of protein” saw a CAGR of 20.7% in the four year period of 2014-2018 (Innova Market Insights, 2019). MCC is an ingredient that can capitalize on the protein trend. These beverages on the shelf are primarily ultra-high temperature processed and have a shelf life of greater than 6 months at room temperature. Sauer and Moraru (2012) investigated the application of MCC in beverages processed though UHT and retort, and their results emphasized a limitation with powdered MCC: aggregation and coagulation. MCC is difficult to disperse into solutions and when exposed to the high process temperatures of UHT and retort sterilization aggregation and coagulation is observed. Beliciu et al. (2012) achieved similar results; however, that experiment concluded that retort processing had less of an effect on the casein micelles and produced a more homogenous beverage with both liquid and powdered MCC. Studies have shown that decreasing
the temperature or increasing the pH prevents the aggregation and coagulation of MCC during processing (Sauer and Moraru, 2012).

Heat stability of liquid MCC (5-10% wt/wt protein) was poor at UHT and retort temperatures (Beliciu et al., 2012). Probable causes attributed to poor heat stability could be 1) casein instability at high temperatures due to calcium phosphate precipitation; 2) Heat induced dissociation of κ-casein leading to casein instability. Liquid MCC (5-10% wt/wt protein) had a greater heat stability than rehydrated micellar casein concentrate (RMCC) powder under UHT processing (142°C, 3 seconds) (Beliciu et al., 2012; Lu et al., 2015). Stabilizers often used in protein beverages, to improve heat stability may offer solutions to improve heat stability.

**Solubility**

Solubility during rehydration of MCC powders has been the limiting factor in its use in food and beverage products (Zhang et al., 2018; Burgain et al., 2016). Proteins need to be rehydrated to ensure maximum functionality and heat stability (Crowley et al., 2014). The main theory to explain the poor rehydration properties of MCC powder is that the casein micelles are slow to release into solution from the powder particles (Schokker et al., 2011). Mimouni et al. (2009) proposed that this mechanism of casein micelle dissolution should in fact be considered the rate limiting step of MCC solubility rather than wetting of the actual powder. MCC powder, with a protein content of about 80% on dry basis, had lower solubility compared with “low heat” milk powder (Schuck et al., 1994a). Time (30-40 mins), temperature (35-60 °C) and high shear are effective in ensuring proper hydration of MCC powders (Fang et al., 2010; Mimouni et al., 2010; Schuck et al., 1994b). Increasing ionic strength of water for dispersion and adding a calcium chelator (60 mM trisodium citrate) enhanced the dispersion and solubility of highly concentrated-MCC powder in water (Lu et al., 2015).
Higher storage temperature and longer time negatively impacted rehydration of MCC powders (Gaiani et al., 2007). Storage conditions of powder need to be considered a crucial step in minimizing the loss of solubility of MCC powders over time (Burgain et al., 2016; Nasser et al., 2017a). Multiple phenomena occur during storage of MCC powder that directly correlate to its ability to rehydrate. Burgain et al. (2016) was the first to discover that the surface of the individual MCC powder particles became rougher and hardened over shelf life, forming a skin-like surface layer. This formation of a skin layer at the surface is what is thought to be inhibiting the release of casein micelles into solution. Storage time and temperature were key components in affecting the severity of the surface hardening. Powders stored constantly at \( \leq 20^\circ C \) showed little change in rehydration rate and maintained a minimum of 80% solubility compared with a control for up to 12 months (Nasser et al., 2017b). This is probably a similar mechanism to the poor solubility of milk protein concentrate powder, especially with prolonged and elevated storage (Smith et al., 2016). However, MPC has the benefit of having a portion of its protein source coming from highly soluble whey proteins.

Another phenomenon observed over shelf life of MCC powder is the change in the secondary structure of the proteins with an observable loss of \( \alpha \)-helix structure and potential increase in \( \beta \)-sheet formation. The loss of \( \alpha \)-helix structure is directly correlated to loss of solubility (Nasser et al., 2018). These changes are thought to be due to protein-protein interactions and cross linkage causing increased aggregation. The protein aggregation is driven by hydrophobic interactions resulting in an increase in powder particle size which has a direct and detrimental effect on the solubility (Havea, 2006; Schokker et al., 2011). Despite all of these factors that contribute to poor rehydration of MCC powder, there are steps and treatments that can be done to improve the solubility of MCC powder. The main treatments currently
involve treatment with calcium chelating salts (citrates, phosphates) and increasing ionic strength (addition of salts NaCl) prior to spray drying (Schuck et al., 2002; Schokker et al., 2011).

Addition of UF permeate from skim milk MF permeate (primarily lactose and minerals) and NaCl to MCC (liquid) before spray drying significantly helped MCC rehydration (Gaiani, Banon, Scher, Schuck, & Hardy, 2005; Schuck et al., 2002). This could be due to the hygroscopic nature of lactose and lowering of Ca$^{2+}$ ion activity due to changes to the ionic strength of the medium (Schuck et al., 2002). Poor rehydration properties have also been noted with other high casein powders like MPC (Smith et al., 2016; Sikand et al., 2011). Non agglomerated MCC powder showed faster rehydration than agglomerated MCC powder which was the opposite for WPI powders (Gaiani et al., 2007). A recent study by Zhang et al. (2018) showed promising potential in the use of High Intensity Ultrasound to decrease the rehydration time of MCC.

**Cold Gelation**

Due to difficulties that MCC powder presents in the rehydration process, there has been development of liquid highly concentrated micellar casein concentrate (HC-MCC). This product has a protein content of ≥ 18% wt/wt (Amelia and Barbano, 2013a; Lu et al., 2016). While this product avoids the challenge of rehydration, it does present a unique challenge of its own in the form of a strong gel at lower temperatures (~ 20°C) (Irma and Barbano, 2013; Lu et al., 2016). It is important to note that HC-MCC is pourable and pumpable at room temperature (22°C) (Irma and Barbano, 2013). However, the gel formed by the cooling of HC-MCC can only be broken up by heating to around 50°C (Lu et al., 2016). The properties and mechanisms of this cold gelation phenomena still require further study and research, but Lu et al. (2016) have done preliminary work in this area. Lu et al. (2015) proposed that HC-MCC cold gelation was caused
by casein micelles being so close together, due to the high concentration, that there was an increase of steric hindrance from the protein tendrils, which prevented movement of particles in the solution and caused a gel structure. When there is not enough energy (heat) to overcome this repulsion, the gel becomes especially strong. It is important to note that this theory has not been directly tested or confirmed and will require further study and research to verify.

HC-MCC is an ingredient that could be used for cheese making. The HC-MCC is combined with cream and skim milk to create a recombined concentrated milk (RCM) that has high casein and low whey protein which makes it ideal for cheese making. Lu et al. (2016) additionally investigated whether an RCM would retain the gelation properties of HC-MCC even with a decreased protein content (≈12%) and at a casein to fat ratio of 0.8, 1.0, and 1.2. They found that a 12% RCM still had cold gelation (≤12°C), and the temperature at which the gel formed depended on a variety of factors such as protein level, pH, and calcium and salt additions. Interestingly, cold gelation has the opposite thermal properties as that of rennet coagulated milk, namely that an increase in temperature causes an increase in gel strength of the rennet gel, whereas high temperature causes a decrease in gel strength of HC-MCC and RCM. This property is thought to be because at high temperatures, in HC-MCC, the casein micelles stay more tightly bound due to increase of hydrophobic interactions with an increase of temperature. Whereas, at colder temperatures the protein strands are less tightly bound to the micelle and can extend further outward allowing for intermolecular interactions. This process is different when compared to rennet treated milk that has an increased speed of aggregation with an increase in temperature as the micelles have had κ-casein cleaved and are driven together through entropic and hydrophobic interactions (Lu et al., 2016). The mechanism for gelation as proposed by Lu et al. (2015) becomes less apparent when using an RCM because the casein
micelles are spaced further apart and consequently should experience less steric hindrance. It is hypothesized that the increase in free protein strands might result in entanglements, which could restrict movement of particles (Lu et al., 2016).

Protein content, pH, and addition of calcium and citrate salts are known to influence the temperature at which gelation occurs. The cold gelation temperature of the RCM was directly correlated to pH between pH 6.4 - 7.0. For every 0.1 reduction in pH, the temperature of cold gelation decreased by \( \approx 7^\circ C \), and this affect was consistent across all protein levels (8-12%). A probable reason for an increase in temperature at which cold gelation occurs with an increase in pH is thought to be casein dissociation from the micelle and thus a lower steric hindrance (Lu et al., 2016). Calcium addition can have differing effects, depending on the levels of calcium added. If added at \( \leq 0.12 \text{mmol/g casein} \), then it has no significant effect; however, if added at \( \geq 0.17 \text{mmol/g casein} \), then temperature at which cold gelation occurs increases. Citrate addition also showed a significant and linear relation to cold gelation temperature, of every 0.1 mmol/g casein increase of added citrate, the average cold gelation temperature increased about 6\(^\circ\)C. As mentioned previously, citrate addition is linked to the dissociation of the casein micelle which would result in the release of more free protein strands into solution, which, in turn, could lead to the entanglement of protein strands that can restrict movement. The study of this area is relatively new and more research is needed to substantiate these hypotheses.

**Sensory properties**

Flavor studies on MCC are limited. Rehydrated MCC powder was characterized by sweet aromatic, cooked/milky and cardboard/doughy flavors and had a distinct corn chip flavor compared to liquid MCC (Smith et al., 2016; Carter et al., 2016). Liquid MPC and MCC were similar in flavor, however in freshly made powders that were rehydrated, MCC had a tortilla
flavor while the MPC did not (Carter et al., 2016). Tortilla flavor has been noted in rehydrated MPC and rehydrated MPI after storage (Smith et al., 2016). Several flavor compounds have been identified in MPC that correspond with tortilla flavor which may result by Maillard browning, lipid oxidation or amino acid degradation (Smith et al., 2016). As the protein concentration of MPCs increase from skim milk powder to MPI, sweet aromatic flavor decreases and cardboard flavor increases, presumably due to aromatic flavor compounds passing through the UF membranes during continuous stages of diafiltration required to achieve high levels of protein purity (Drake et al., 2009; Park et al., 2016; Smith et al., 2016). Cheddar cheese made using powder ceramic membrane filtered MCC (95% reduced serum protein) had undesirable off-flavors (tortilla flavor, bitter taste) and some typical aged Cheddar flavors were missing (Amelia et al., 2013b).

Consumers expect great flavor in fluid milk and protein beverages (Harwood and Drake, 2018; Oltman et al., 2015). Cooked and sulfur flavors are disliked by consumers (Lee et al., 2017). Research has suggested that high heat processes like ultra-pasteurization lead to sulfur and eggy flavors in milk and protein beverages. These off flavors have been sourced to whey proteins (Jo et al., 2019). Therefore, depending on the purity (i.e., lack of milk whey protein) of the MCC prepared from skim milk, in a high heat process, sulfur and eggy flavors may be decreased. This potential flavor change (sulfur/eggy flavor) following ultrapasteurization or retort processing might be a potential marketable point of difference for high purity MCC compared to MPI.

**MCC Applications in Milk Based Beverages**

MCC has many qualities that make it ideal for food application: heat-stable, capable of binding large amounts of calcium associated with casein, lactose reduced, bland in flavor,
provides mouthfeel that is similar to fat, and white color (Barbano, 2009; Amelia, 2012; Carter et al., 2016). Literature cites various applications using MCC: shelf-stable beverages, Greek-style yogurt, cheese, and nutrition bars.

Vogel (2019) manufactured 15 and 25 g protein per 240 mL serving vanilla-flavored milk protein beverages from liquid MCC (95% whey protein reduced), liquid MPC, and liquid blends of MPC and serum protein isolate (SPI, also called milk whey protein isolate). Beverages were ultrapasteurized by direct steam injection and evaluated through 8 weeks refrigerated storage. Sulfur/eggy flavors were documented in MPC and MPC/SPI beverages, but not in MCC beverages. Sulfur/eggy flavors are drivers of dislike for consumers (Lee et al., 2017). Vanilla flavor was also higher in MCC beverages compared to MPC and MPC/SPI beverages by both sensory and instrumental measurement. The reduction of sulfur/eggy flavors and higher vanilla flavor represent a potential advantage for the application of MCC in the expanding beverage market. However, the specific concentration or concentration range of whey protein that causes detectable (unpleasant) sulfur/eggy flavors is unknown and consequently, the purity necessary for MCC to manufacture off flavor-free protein beverages is currently unknown.

When increasing the protein concentration in a beverage, microfiltration offers the opportunity to selectively produce a beverage that is either high in milk derived whey protein or milk casein. The influence of these two groups of protein on beverage color, viscosity, and cooked sulfur eggy flavor are different. In general, protein beverages with higher casein content will be lighter, show a temperature dependent viscosity profile, and have less sulfur/eggy flavor than a milk based beverage with higher milk derived milk whey protein when ultrapasteurized (Lee et al., 2017; Jo et al., 2019; Cheng et al., 2019a,b). Viscosity of milk based beverages changes with increasing fat and increasing casein at a percentage of protein at 4 and 25 °C.
(Rudan and Barbano, 1999). The viscosity of protein beverages will largely depend on protein amount, but can be influenced by protein type. Achieving the correct viscosity, texture and mouthfeel requires understanding of individual protein types.

Color also influences consumer preferences for fluid milks and presumably, milk beverages (Quiñones et al., 1997, 1998). McCarthy et al. (2017) reported that better color or color/whiteness was an important reason that skim milk, 2% fat milk and whole milk drinkers all preferred higher fat level milks. As such, appearance is a critical parameter for fluid milk. Phillips et al. (1995) found that increasing milk fat level (0.06 to 2.0%) increased L-value (whiteness) and decreased a-value (greenness to redness) and b-value (blueness to yellowness) of milk, which were well correlated with perceived color of lowfat milk by sensory analysis with trained panels. More recently, Cheng et al. (2018) documented that temperature had a large impact on perceived color (appearance) of skim milk and fat free milk beverages but not for milks or beverages containing fat. Increasing true protein level (Quiñones et al., 1997, 1998) and casein as a percentage of true protein (Misawa et al., 2016) increased whiteness of low fat milks and caused the sensory perception of these low fat milks to be more similar to higher fat level milks. Rudan and Barbano (1999) also reported that increasing casein as a percentage of true protein increased milk based beverage whiteness. Milk whiteness increased with increasing casein as a percent of true protein causing lower fat beverages to have similar whiteness to higher fat beverages. Increased whiteness is a positive attribute that consumers value in fluid milk. This means that a milk beverage with the same protein as milk, with the protein all coming from casein (MCC) will be whiter. For this reason, MCC has been used as the base ingredient for coffee creamers (Merrill and Jiancai, 2017).
Bong and Moraru (2014) reported that MCC powder could be used to fortify Greek style yogurt (GSY) protein content and eliminate the acid whey removal in conventional Greek style yogurt processing. In this study, MCC 58 and MCC 88 were used, the number signifying the total nitrogen protein (both MCCs were 85-88% casein as a percentage of true protein). While the acidification rate of the GSY was faster than the traditional control, MCC 58 proved to be a promising alternative to the conventional make process of GSY as opposed to MCC 88 (Bong and Moraru, 2014). Powdered MCC was combined with skim milk to increase the yogurt base protein content, the MCC 58 powder dispersed easily in the milk with no visible aggregation or coagulation. However, they also reported differences in physicochemical properties between the MCC fortified and regular Greek style yogurt (Bong & Moraru, 2014). Sensory properties were not addressed.

Another food application for MCC is nutrition bars. Simple protein bars were manufactured with a mixture of protein ingredients, high fructose corn syrup and glycerol for comparison of different dairy protein powders and hardening behavior over shelflife (Hogan et al., 2012). MCC based nutrition bars were softer in texture compared to bars made of whey protein hydrolysates, WPI or sodium caseinate after 10 days of storage at 37°C (Hogan et al., 2012). Banach et al. (2016) found that high protein nutritional bars made with MPC and MCC, MPC bars were harder and more cohesive than MCC. More research needs to be done to better understand MCC unique functionality on the physiochemical properties of protein bars.

**Milk derived whey protein**

Up to this point, we have reviewed MCC which is the retentate stream obtained during MF of skim milk. The major component of the permeate stream of the same MF process are serum proteins or whey proteins. Traditionally, the liquid substance obtained by separating the
coagulum from milk, cream, or skim milk in cheesemaking has been called whey (FDA, 2019) and proteins isolated thereof whey protein. Since the GRAS notification from FDA also specifies the origin of whey (from cheese manufacture), this has led to a debate in the industry around the nomenclature of whey proteins obtained from MF of fluid skim milk. Tradition and previous scientific literature has called this protein stream serum proteins and serum protein concentrates and isolates. This wording may be confusing to consumers and the industry requires a way to differentiate whey proteins separated directly from milk by filtration compared to those isolated from cheese or rennet/acid casein manufacture. To help with this, ADPI has created an industry standard for this protein stream called “milk whey proteins” (ADPI, 2018b). Although there are many terms used in the literature, we will refer to milk whey protein concentrate (mWPC) and milk whey protein isolate (mWPI) as having 34-89% and 90% protein respectively.

The milk whey protein market is growing. There is still no available data on production numbers to distinguish from traditional cheese whey products. Milk whey protein is similar to traditional whey protein and therefore fits into many of the same product applications. Some key items differentiate milk whey proteins from traditional cheese whey protein. Since milk whey protein isn’t derived from cheese manufacture, it is free from the residues of cheese making. These residues include starter culture residues, dead or living cells, thermoduric bacteria or bacteriophages, enzyme, colorants or bleaching agents (Maubois, 2002; Kang et al., 2010; Campell et al., 2013). This makes milk whey protein particularly useful in applications like infant formula, where there are regulations in regard to ingredients that can be present in whey ingredients from carryover from the previous processes. It also means that a higher premium can be demanded.
Composition/industry standards/labelling

WPC and mWPC are similar in composition but differ in fat content and mWPC has no glycomacropeptide (no rennet is used). Since there is no glycomacropeptide (GMP) in mWPC, the yield of whey protein per unit volume is lower than whey protein isolated from cheesemaking as GMP is retained by ultrafiltration and composes a large portion of whey protein powder, approximately 20%, from traditional membrane fractionation (Farias et al., 2010). Percentage of casein in WPC and mWPC also vary, with WPC having lower amounts of casein, as rennet is more selective than a membrane (Evans et al., 2010).

Evans et al. (2009, 2010) compared whey protein and milk whey protein concentrates (34% and 80%) made by either cheese whey or by ceramic microfiltration of skim milk. The whey protein made from cheese manufacture had a higher fat content. This is due to the ultrafiltration step that retains the fat while the microfiltration step of mWPC completely removes the fat as it is retained with the casein. Fat content on a dry weight basis of WPC80 ranges between 6-8% while mWPC80 had a fat content of 0.5% (Evans et al., 2009). The fat content is more similar to WPI which typically has 0.5-1.0% lipid. Reconstituted WPC and mWPC 34% at 10% solids also had different appearance. The mWPC was clear while the WPC was cloudy, presumably due to the fat content. One of the other key differences is pH. The pH of cheese whey WPC is lower due to the lactic acid produced by the starter culture. Due to the slight difference pH, it is possible that there is a mineral content difference (WPC 80 pH 6.51, mWPC 80 pH 6.87; Evans et al., 2010). WPC 34 and 80 had a higher calcium content than mWPC (Evans et al., 2009; Evans et al., 2010).

mWPC composition can change based on the membrane used and the temperature of filtration. Coppola et al. (2014) compared mWPC processed at different temperatures with
polymeric membranes and the residual casein as a percentage of true protein in the mWPC changed with temperature. The residual casein percentage in the mWPC varied from 1.0% to over 20% in mWPC depending on the temperature of microfiltration with higher temperatures retaining more casein (Coppola et al., 2014). Even 1% casein has a dramatic effect on the clarity of the milk whey protein stream. Milk whey protein with 1% casein had a light transmission rate of 94%, compared to traditional WPC which had a light transmission rate of 15% (Coppola et al., 2014). Meaning that even with a small amount of casein contamination, mWPC is less turbid than traditional WPC due to the higher fat in WPC.

It has been proposed that the casein that passes through the MF membrane at cold temperatures is beta casein (Coppola et al., 2014). At 4°C, β-casein exists in solution as monomers of molecular mass 25 kDa (Fox and McSweeney, 1998) and disassociates from the casein micelle (Davies and Law, 1983; Famelart et al., 1989) with decreasing temperature from 50 to 4°C. Use of cold MF has been successfully adapted to fractionate caseins from skim milk by Terre et al. (1987), Woychik (1992), and Van Hekken and Holsinger (2000). Filtration conducted at higher temperature retains more casein and makes a purer milk whey protein stream. Perhaps allowing β-casein transmission during MF is beneficial for making unique casein fractions and a unique permeate stream that may be beneficial for infant formula, however the appearance and composition limit milk whey protein with high casein in typical whey protein applications. Whey protein products with high amounts of casein will scatter more light and at a given percentage will cause turbidity as well as have decreased solubility at low pH.

The amount of β-casein transmission also seems to be a function of membrane type used. Zulewska et al. (2018) found that regardless of different heat treatments (1.4 µm MF filtration, thermization, and pasteurization at 72°C for 15 sec) the ceramic MF membrane used in the study
(0.1 µm) yielded less than 1% β-casein at 6°C in the permeate. Cold MF at 7°C done by Coppola et al. (2014) with a spiral wound polymeric membrane (0.08 µm) resulted in 22% casein in the protein fraction of the milk whey ingredient. This study did not identify the casein type or discuss membrane performance (leakage) at the processing temperatures employed.

**Functional properties**

Several studies have addressed the physical and functional properties of milk whey protein. mWPC have good clarity, heat gelation, solubility and foaming properties (Heino et al., 2007; Luck et al., 2013). mWPC and mWPI obtained from MF had lower fat, less heat treatment since cheese whey is usually pasteurized 2 times, less protein denaturation and no GMP compared with whey protein concentrate (WPC) or whey protein isolate (WPI) (Evans et al., 2009, 2010; Heino, et al., 2007; Coppola et al., 2014; Qiu et al., 2015). However, mWPI processed from lower temperature MF (7°C) contained a high amount of β-casein while mWPI processed from 50°C MF had no β-casein (Coppola et al., 2014).

The color of liquid mWPI (10.88% protein content on w/w) was reported as amber to reddish due to the high concentration of lactoferrin and lactoperoxidase (Misawa et al., 2016). Instrumental color of mWPC34 powder and WPC34 powder were identical (Evans et al., 2009). Rehydrated mWPC34 and mWPC80 powders, both with a fat content of 0.02%, were clear while rehydrated WPC34 and WPC80 were opaque due to the higher fat content (Evans et al., 2009, 2010).

Solubility of mWPC34 and mWPC80 powders were the same as WPC powder while rehydrated mWPC34 and mWPC80 powders were less turbid than rehydrated WPC34 and WPC80 powder (pH3-7) (Bacher & Kønigsfeldt, 2000; Heino et al., 2007; Luck et al., 2013). mWPC34 and mWPC80 showed better gelation and foaming compared with WPC due to lower
GMP and fat content and heat induced protein denaturation (Heino et al., 2007; Luck et al., 2013; Morr & Ha, 1993; Mangino et al., 1987). A milk whey protein solution (the skim milk MF permeate was concentrated and dialyzed to 5% protein content) showed an identical overrun volume as an egg white protein (5% protein content) at pH=7 (Punidasas & Rizvi, 1998). Coppola et al. (2014) reported that mWPI processed at 7°C MF with a high amount of beta-casein produced a more stable foam than mWPI processed from 50°C MF. The unfolding of beta-casein would expose more hydrophilic groups (Swaisgood, 2003). The unfolding and annealing of beta-casein on the interface of air and water could decrease the surface tension and stabilize the foam. Drying methods (spray dry and freezing dry) had no effect on turbidity, gelation, or foaming of mWPC34 and mWPC80 (Luck et al., 2013).

**Sensory properties**

Rehydrated spray dried mWPC34 powder had no buttery (diacetyl) or cardboard flavors compared to WPC34 powder (spray dried and freeze dried) presumably due to less fat content, processing time and lipid oxidation compounds (Evans et al., 2009). The WPC 34 had a low intensity of cardboard flavor while the mWPC had none. Cardboard flavor has been documented in whey protein products and is associated with secondary lipid oxidation products (Mahajan et al., 2004; Carunchia Whetstine et al., 2005; Evans et al., 2009, 2010; Whitson et al., 2010, 2011; Carter and Drake, 2018). Whey protein isolated from colored cheese manufacture requires bleaching to remove residual colorant that leaches into the whey stream. Bleaching of whey has negative impacts on whey protein flavor (McDonough et al., 1968; Croissant et al., 2009; Listiyani et al., 2011; Kang et al., 2012; Kang et al., 2010; Li et al., 2012; Campbell et al., 2013; Fox et al., 2013; Jervis et al., 2012; Listiyani et al., 2012; Jervis et al., 2015). Bleaching chemicals had a similar impact on milk derived whey protein (Jervis et al., 2012). Increased
cardboard/fatty and cabbage flavors and higher lipid oxidation compounds are present from chemical and enzymatic bleaching of fluid whey (Campbell et al., 2013). Clear acidic protein beverages were formulated with commercial WPC80 powders and pilot plant spray dried mWPC80 (Evans et al., 2010). Beverages made with mWPC80 had equal or higher liking scores for aroma, appearance, and mouthfeel compared to the WPC80 formulated beverages. In contrast, mWPC80 beverages had lower flavor and overall liking scores compared with beverages made with 3 of the 4 commercial WPC80 powders (Evans et al., 2010). These differences were attributed to higher intensities of soapy flavor and bitter taste in mWPC80 beverages compared to WPC80 beverages.

**Food applications**

The applications of milk derived whey protein are similar to that of cheese derived whey protein. These applications include ready to mix and ready to drink beverages, snacks, bars, yogurt, sour cream, baked goods and infant formula. The high level of protein purity and the absence of cheese making by products make it an ideal protein source in any of these applications. Its high clarity at low and high pH also makes it a very versatile ingredient for ready to mix and ready to drink protein beverages.

**Future needs**

From a functional perspective, micellar casein concentrates, and milk whey protein have differences from MPC and WPC. A wide array of dairy ingredients can offer the industry formulation tools to meet a product claim, clean label solutions and desired sensorial properties. For both micellar casein concentrates and milk whey protein there needs to be better differentiation from MPC and WPC. Both products offer unique flavor and functional properties which still need to be further explored in more applications. More data on production
volumes and market analysis would help the industry better position these new dairy ingredients and showcase their high quality and flexibility to compete with other protein ingredients. Industry standards for nomenclature of MCC need to be clearly established to permit both processor and customer better communication of what products are being delivered. The use of MCC in cheese making could allow the industry greater flexibility and offer improved operational efficiencies. Regulations needs to be loosened in the US to allow cheese makers more flexibility to use MF milk permitting the US to compete more directly with international cheese producers. Finally, more studies need to be done to compare the functional and sensory properties of MCC and mWPC/mWPI with emerging alternative proteins to highlight the uniqueness of these ingredients in food applications.

**Conclusion**

Advancements in filtration technology have allowed for the advancement in dairy ingredient production and technology. The primary protein fractions in milk can now effectively be separated directly by filtration, creating two highly nutritious and distinct functional protein streams. A direct application of microfiltered milk is cheese making that could help processors with improved flexibility and operational efficiencies. Micellar casein concentrate is growing in popularity in the U.S. as an ingredient in different food applications. Micellar casein composition and functionality can vary depending on its purity, and the method of production and labeling guidelines need to account for these differences. Milk derived whey protein is a highly valuable co product of the microfiltration of milk. Being similar to whey protein derived from cheese making, it easily fits into any whey protein application. Since it is fractionated from skim milk, the ingredient is free of cheese by product residues making it a suitable protein source for products like infant formula. As the market grows, milk derived whey protein will be an
extraordinary ingredient with a wide range of function with dairy proteins. As more protein ingredients emerge to compete, it is important to demonstrate the strengths of dairy ingredients in the protein market. These ingredients allow the US dairy industry more flexibility to create unique products for an ever-changing consumer base.

**Acknowledgement**

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References


Food and Drug Administration, Office of Nutrition and Food Labeling. 2019. Direct food substance affirmed as generally recognized as safe: whey. 21CFR part 184.1979


Figure 1. Passed and rejected dairy components based on membrane pore size (modified from Adams, 2012).
**Figure 2.** Schematic of Micellar Casein and Milk Derived Whey Protein Manufacture.
### Table 1. Composition difference of Casein Concentrates

<table>
<thead>
<tr>
<th></th>
<th>MCC</th>
<th>MPC</th>
<th>Acid Casein</th>
<th>Calcium Caseinate</th>
<th>Sodium Caseinate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>83.0</td>
<td>82.5</td>
<td>92.0</td>
<td>88.0</td>
<td>92.7</td>
</tr>
<tr>
<td>Lactose</td>
<td>1.0</td>
<td>2.5</td>
<td>0.5</td>
<td>0.7</td>
<td>0.3</td>
</tr>
<tr>
<td>Fat</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>2.0</td>
<td>0.7</td>
</tr>
<tr>
<td>Ash</td>
<td>7.8</td>
<td>6.6</td>
<td>2.4</td>
<td>5.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Calcium</td>
<td>2.3</td>
<td>2.1</td>
<td>0.03</td>
<td>0.9</td>
<td>0.03</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>1.7</td>
<td>1.6</td>
<td>1.3</td>
<td>1.1</td>
<td>1.2</td>
</tr>
<tr>
<td>Sodium</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>1.1</td>
</tr>
<tr>
<td>Moisture</td>
<td>5.0</td>
<td>5.0</td>
<td>10.0</td>
<td>5.0</td>
<td>4.3</td>
</tr>
</tbody>
</table>

Adapted from Dairy Export Council 2015

### Table 2. Composition and MCC purity at various milk whey protein removal percentages that reflect typical spiral wound and ceramic membrane system performance of a 3X, 3 stage MF process

<table>
<thead>
<tr>
<th>Metric</th>
<th>Skim Milk</th>
<th>MF Ret 1</th>
<th>MF Ret 2</th>
<th>MF Ret 3</th>
<th>MF Ret 4</th>
<th>MF Ret 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCC purity*</td>
<td>82%</td>
<td>93.05%</td>
<td>94.21</td>
<td>96.81%</td>
<td>98.91%</td>
<td>99.56%</td>
</tr>
<tr>
<td>SP Removal (%)</td>
<td>0%</td>
<td>66%</td>
<td>72%</td>
<td>85%</td>
<td>95%</td>
<td>98%</td>
</tr>
<tr>
<td>Residual SP (g/100g MCC)</td>
<td>1.152</td>
<td>0.392</td>
<td>0.323</td>
<td>0.173</td>
<td>0.058</td>
<td>0.023</td>
</tr>
<tr>
<td>Membrane Type</td>
<td>Spiral</td>
<td>Spiral</td>
<td>Spiral/Ceramic</td>
<td>Ceramic</td>
<td>Ceramic</td>
<td>Ceramic</td>
</tr>
</tbody>
</table>

*Casein as a percent of true protein. Adapted from Barbano and Drake, 2018
CHAPTER 3: Short Communication: The Influence of Flash Vacuum Cooling on the Flavor of Ultrapasteurized Milk
Short Communication: The Influence of Flash Vacuum Cooling on the Flavor of Ultrapasteurized Milk

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Abstract

Ultrapasteurization (UP) extends the shelf life of milk. Direct steam injection (DSI) is commonly used for UP because milk is quickly heated and cooled. During this process, steam is directly injected into milk and removed by a vacuum cooler. Consumers do not prefer the flavor of DSI UP milk compared to traditional high temperature short time (HTST) milk. The objective of this research was to characterize the influence of the vacuum cooler on the flavor of DSI UP milk. Raw skim milk was pasteurized at 140°C for 2.3s by DSI and homogenized at 20.7 MPa. Steam-infused pasteurized milk was sampled after heating but before it reached the vacuum chamber with the use of a liquid sample port. A septa was installed in the vacuum chamber to allow sampling of the removed volatiles by solid phase microextraction (SPME) fiber followed by gas chromatography-triple quadrupole mass spectrometry (GC-MS/MS) combined with a sulfur selective flame photometric detector. Steam infused milk and vacuum cooled milk diluted to the same solids were evaluated by descriptive sensory analysis and volatile compound analysis. The entire experiment was replicated three times. Milks cooled by the vacuum cooler were lower in sweet aromatic, sulfur/eggy and cooked flavors than milk sampled before the vacuum cooler (p<0.05). Volatile compounds removed by the vacuum cooler included sweet aromatic flavor contributors furaneol, maltol, furfural, sotolon, gamma-octalactone, gamma-dodecalactone, gamma-decalactone, delta-decalactone as well as cooked and sulfur/eggy contributors, hydrogen sulfide and dimethyl sulfide. The vacuum cooler applied during DSI UP of milk is effective at removing steam and cooling UP milk, but this process may also remove important flavor compounds from fluid milk.

Key Words: Milk, Ultrapasteurization, Vacuum Cooling
Short Communication

The dairy industry has been shifting toward extended shelf-life (ESL) milk. Traditional high temperature short time (HTST) pasteurization (72°C for 15 s) milk has a relatively short refrigerated shelf-life of 2-3 weeks. The approximate 18 day refrigerated shelf life puts strain on the dairy industry to distribute product quickly and get it into the hands of the consumer.

Ultrapasteurized (UP) milk is a high heat pasteurization process where milk is heated and held at 138 °C for at least 2 seconds. The higher heat treatment of UP milk extends the refrigerated shelf-life to 60-70 days and these milks are also called ESL milks. Shelf-life can be extended with UP, however, there are adverse flavor effects that occur.

There are two main methods of UP used in the dairy industry. Indirect (IND) UP involves the indirect heating of the milk by either a plate, tube in tube or tube in shell heat exchanger. This process requires a lot of come up and cool down time which leads to greater overall heat load on the milk (Lee et al., 2017). The second method is direct steam injection (DSI). DSI milk has steam injected directly into the milk to heat it rapidly, limiting the heat load on the milk with the same level of lethality. The added water by the steam is later removed by a vacuum chamber which also serves to cool the product (Datta et al., 2002). In recent years, DSI has become more popular due to the efficient heat transfer as well as the lower total heat exposure which leads to a higher quality product.

These two heating methods for UP produce different flavor profiles in the UP milk. Lee et al. (2017) demonstrated that DSI milk was lower in sweet aromatic flavor and higher in sulfur/eggy flavor than indirect UP milk (Lee et al., 2017). Both UP milks had higher cooked flavor and distinct sulfur flavor compared to HTST milk, regardless of fat content. The
differences in the flavor profile of IND and DSI milk suggest that the vacuum chamber may play a key role in the flavor differences of these two milks.

During DSI-UP production, a vacuum chamber is used to remove excess water added by the steam and to quickly cool the milk. Vacuum is often used in the food industry because it lowers the boiling point of water, allowing evaporation of water at lower temperature. The vacuum pressure also affects the boiling point and vapor pressure of volatile flavor compounds. Under vacuum, these compounds would more easily volatilize into the headspace, which would make them more susceptible to removal by the vacuum pump. This effect has been noted before in the vapor separator of an evaporator for the production of milk powder (Park et al., 2016). Milk concentrates produced without vacuum evaporation to the same solids and heat load were higher in typical milky, sweet aromatic flavors and volatile compounds than concentrates produced with vacuum evaporation. Analysis of the vapor removed from the milk revealed that many milky and sweet aromatic compounds were removed by the vacuum.

Even though the use of vacuum gives the industry better processing control, it may remove important flavor components. This problem may be exaggerated in flavored milks and protein beverages. Flavors are often the most expensive ingredients included in beverage formulations and may be the most affected by vacuum cooling. Milk processed by DSI-UP already has intense cooked and sulfur flavors due to the extreme heat treatment, and the vacuum may be selectively removing desirable flavor compounds (sweet aromatic) and intensifying undesirable flavors (sulfur). The purpose of this research was to determine the roles of the vacuum cooler on the flavor of skim DSI-UP milk.

For this study, on three different occasions raw skim milk (3.4% protein, 0.08% fat, coliforms <10^1 CFU/ml, APC <10^2 CFU/ml, somatic cell count <50,000) was obtained from the
North Carolina State University Dairy Education System. A Microthermics EHVH pasteurization unit (Microthermics, Raleigh, NC) with a 2-stage homogenizer (model NS2006H, GEA Niro Soavi, Parma, Italy) was used to pasteurize the milk. Prior to processing milk, the system was sterilized by heating the unit to 121.1 °C recorded by the last thermocouple and held at that temperature for 20 minutes. Raw skim milk was processed at a flow rate of 1.4 L/min. The milks were preheated to 90°C, then heated to 140°C for 2.3 s under 330 kPa pressure by direct culinary steam injection (model LG30, Electro-Steam Generator Corp., Alexandria, VA) using a Microthermics Steam Injection Module with Cub 5 software (version 3.1), then cooled to 85°C by vacuum cooling under a 1,040-mm Hg vacuum to remove both heat and added water. The DSI-UP milk was then homogenized (20.7 MPa total, 3.4 MPa second stage) and cooled to 10°C and collected into glass jars (500 ml, VWR, Wayne, PA) and cooled to 4°C in an ice bath and stored at 4°C in the dark until analysis. Analyses were conducted in less than 24 h. Hold times were calculated under laminar flow assumptions. DSI-UP represents a normal processing condition for ultrapasteurized milk by DSI.

In order to isolate the direct effect of the vacuum chamber, but still maintain the same heating profile, modifications were made to the Microthermics to sample milk after the hold tube and before the vacuum chamber. Milk was diverted through a cooling coil place into an ice bucket to produce not vacuum cooled (NVC) milk which was cooled indirectly and filled into glass jars. This method did not remove the added water caused by steam injection. Both DSI and NVC milks were diluted to 8.6% solids (w/v) with DI water as determined by a CEM microwave moisture analyzer (SMART 5, CEM, Mathews, NC) and a calibrated mid-FTIR (Lactoscope FTA, Delta Instruments, The Netherlands).
In order to better understand flavor compounds removed directly by the vacuum chamber, a sampling septa (Fisher Scientific, Waltham, MA) was installed on the entrance of the vacuum pump to allow sampling of the vapor. Before collection of fluid milk samples, 9 solid-phase micro-extraction fibers (2 cm divinylbenzene/carboxen/polydimethylsiloxane fiber, Supelco, Bellefonte, PA) were exposed for 2 min through a silicone septa (Fisher Scientific, Waltham, MA) in the vacuum exhaust of the vacuum chamber to collect qualitative information about which flavor compounds were removed in the vapor. The entire experiment was repeated in triplicate.

Furosine (FUR) was measured as described by Resmini et al. (1990) with modifications. 2 ml of milk were dissolved in 6 ml of 10.6 M HCl (Sigma Aldrich, St. Louis, MO) into a glass tube. Nitrogen gas was bubbled into the tube for 2 minutes followed by sealing glass tubes with Teflon-lined lids. The samples were hydrolyzed at 110°C for 24 h and then cooled to room temperature. Next, 100 µl of hydrolysate was added to 900 µl of HPLC grade water (Fisher Scientific, Waltham, MA). The diluted hydrolysate was centrifuged at 14,000 x g for 10 min and the supernatant was placed in HPLC autosampler vials with Teflon lined lids (Waters Corporation, Milford, MA).

Separation and detection of FUR was done by reversed phase UPLC-ESI-MS (Acquity H Class, Waters Corporation). One µl was injected on a C18 column (HSS T3, 2.1 x 100 mm, 1.7 µm; Waters Corporation) with a temperature of 40°C and a flow rate of 0.5 ml/min. The mobile phase consisted of 0.1% formic acid in water. ESI/MS analysis was performed with a single quadrupole mass spectrometer (SQ Detector 2, Waters Corporation) operated in ES+ mode. The source temperature was 150°C with a desolvation temperature of 650°C and nitrogen gas flow of 1200 L/hr. Capillary and cone voltages were 0.50 and 38 V respectively. FUR was quantified
using the charged ion 255.06 in selective ion monitoring mode. A 5 point standard curve was constructed ranging from 15 µg/L to 150 µg/L for quantification.

Sensory testing was conducted in accordance with the North Carolina State University Institutional Review Board for the Protection of Human Subjects in Research regulations. A trained sensory panel documented sensory attributes of milk on the day of manufacture. Each panelist (3 females, 3 males, ages 21 to 55 y) had a minimum of 100 h of prior experience evaluating the sensory properties of fluid milk using the Spectrum method and an established lexicon for fluid milk (Lee et al., 2017; Meilgaard et al., 2007). Milks (30 mL) were dispensed into lidded 59-mL soufflé cups (Dart Container Corp., Mason, MI) with random 3-digit blinding codes. Samples were prepared with overhead lights off to prevent light oxidation. Milks were tempered to 10°C and evaluated using paper ballots. Each panelist evaluated each milk in duplicate.

Volatile compound analysis was evaluated from DSI and NVC milk as well as from the SPME fibers collected during milk processing in the vacuum chamber. Volatile compounds were extracted from liquids using sorptive stir bar extraction (Prieto et al., 2010). Milks were extracted in triplicate using the following methods adapted from Park et al. (2016). Prior to analysis, the stir bars and thermal desorption unit (TDU) tubes were conditioned for 1 h at 300°C. First, 5 ml of milk was added to a 10 ml glass vial. Next, 20 µL of internal standard was added (0.81 mg/kg of 2-methyl-3-heptanone in water, Sigma Aldrich, St. Louis, MO). Sequential stir bar extraction was used because the adsorption of compounds onto the polydimethylsiloxane (PDMS) layer is affected by the addition of salt. Polar compounds are extracted more effectively with salt, whereas hydrophobic compounds are extracted more efficiently without salt (Prieto et al., 2010). One PDMS-coated stir bar (10 mm × 0.5 mm thickness, Gerstel Inc.) was placed in the vial,
sealed, and stirred for 1 h at 1000 rpm. After 1 h, the stir bar was briefly rinsed in HPLC-grade water, dried, and placed in an autosampler tube (Gerstel Inc.) and 1 g of NaCl was added along with another PDMS stir bar into the sample vial. This stir bar was stirred for 1 h at 1,000 rpm, rinsed, dried, and placed in the same TDU tube previously mentioned. Stir bars were injected using an autosampler (MPS Autosampler, Gerstel Inc.). The stir bars were desorbed at 250°C for 10 min (TDU, Gerstel Inc.) and the volatile compounds were cryogenically trapped at −120°C (CIS 4, Gerstel Inc.). Volatile compounds were analyzed by GC-MS. An Agilent 7890B GC (Agilent Technologies Inc., Santa Clara, CA) with an inert mass selective detector (model 5970A, Agilent Technologies Inc.) with a ZB-5MS column (30 m × 0.25 mm × 0.25 μm; Phenomenex, Torrance, CA) was used to identify and quantify volatile compounds of interest (Karagul-Yuceer et al., 2001; Karagul-Yuceer et al., 2002; Drake et al., 2007; Park et al., 2016; Jo et al., 2018). Initial GC oven conditions were 40°C for 3 min with ramp rates of 10°C/min to 90°C, 5°C/min to 200°C held for 10 min, and 20°C/min to 250°C held for 5 min. Purge time was set to 1.2 min using helium as the carrier gas at a constant flow rate of 1 mL/min. Compounds were identified by comparison with the 2014 NIST mass spectral library (NIST, 2014), retention index, and retention time of authentic standards injected under identical conditions. Relative abundance of selected compounds was calculated using recovery of the internal standard.

Relative abundance of selected compounds in liquids were made on an Agilent 7890B GC applied to an Agilent 7000C triple quad MS (MS/MS) and sulfur selective FPD (Agilent Technologies Inc.) equipped with a ZB-5ms (30 m length × 0.25 mm i.d. × 0.25 μm film thickness; Phenomenex) column. Five milliliters of milk along with 20 μL of internal standard (2-methyl-3-heptanone in ethyl ether at 81 mg/kg) was added to 20-mL SPME autosampler vials with steel screw tops containing silicone septa faced in Teflon (Microliter Analytical, Suwanee,
GA) in triplicate. Sample introduction was accomplished using a CTC Analytics CombiPal Autosampler (CTC Analytics). Vials were equilibrated for 25 min at 35°C with 4 s of pulsed 250 rpm agitation. A single 50/30 um DVB/CAR/PDMS (Supelco) 1-cm fiber was used for all analyses. The SPME fiber was exposed to the samples for 40 min at a depth of 31 mm. The fiber was retracted and injected at 50 mm in the GC inlet for 5 min. The GC oven was initially held at 35°C for 3 min with a gradual increase of 10°C/min to 150°C, held for 1 min, then raised at a rate of 20°C/min to 250°C and maintained for 5 min. The MS transfer line was maintained at 250°C with the quad at 150°C and source at 250°C. The flow rate of helium quench gas and nitrogen collision gas was 1.0 mL/min and 2.5 mL/min, respectively. Effluent from the capillary column was split 1:1 between the MS and FPD. The FPD for sulfur compounds was set to 325°C for transfer line, air 100 mL/min, and hydrogen 60 mL/min. Compounds were identified by comparison with the 2014 NIST mass spectral library (NIST, 2014), retention index, and retention time of authentic standards injected under identical conditions. Relative abundance of selected compounds was calculated using recovery of the internal standard.

Volatile compounds that were extracted in the vacuum chamber by SPME fibers during milk processing were identified qualitatively by GC-olfactometry (GC-O) as well as by GC-MS. The GC-O analysis was performed with a gas chromatograph with a flame ionization detector and olfactometry port (model 6850, Agilent Technologies Inc.) and the same nonpolar column as stated above. Three highly trained sniffers (>50 h experience each) sniffed each sample once per replication and recorded aroma active events. Oven temperature program was as follows: 40°C held for 3 min, 10°C/min gradual increase to 150°C, and 30°C/min gradual increase to 200°C held for 10 min. The GC-MS analysis was performed on an Agilent 7820A GC (Agilent Technologies Inc.) with an inert mass selective detector (model 5975 MSD, Agilent
Technologies Inc.). Fibers were manually injected for GC-O. Column and oven temperature program were the same as above. All fibers were exposed into the column inlet at 250°C. Helium carrier gas was used on both GC-O and GC-MS at a rate of 1 mL/min with a purge time of 1.5 min.

Furosine, an indicator of heat treatment, was measured to ensure that the two milks received similar heat treatments and that flavor differences were not attributed to difference in heat load. Both milks had similar furosine values (55.37 vs. 52.94 mg of FUR/ 100 g of protein) (p>0.05). Flavor differences measured between NVC and DSI milk in flavor were therefore attributed to the effect of the vacuum cooler as opposed to a difference in heat treatment. DSI milk was lower in sweet aromatic and cooked milky flavors by descriptive analysis compared to NVC milk (p<0.05, Table 1). Aroma intensity, viscosity and astringency intensities were not different between the two milks (p<0.05). These results are consistent with the hypothesis that DSI milk is lower in sweet aromatic flavor due to compounds stripped from the milk by the vacuum cooler. Sulfur eggy flavor was higher in DSI milk compared to NVC milk (p<0.05). Analysis of the SPME fibers exposed to the exit of the vacuum chamber showed that many odor active compounds were removed during the ultrapasteurization process (Table 2). Of the compounds removed by vacuum cooling, important flavor volatiles can be organized into three categories: sulfur/eggy, cooked, sweet aromatic volatiles. Hydrogen sulfide, dimethyl sulfide and dimethyl disulfide have sulfur/eggy aromas (Jo et al., 2019) that were detected by GC-MS and GC-O. Cooked compounds like carbon disulfide and 2-methylbutanal (Jo et al., 2018) were also detected by SPME in the vacuum chamber. Sweet aromatic compounds included furaneol, maltol, delta-decalactone, and diacetyl (Karagul-Yuceer et al., 2001) were also detected. These results suggest that beyond the direct measurement of the sensory and volatile profiles of the two
liquid samples, we can qualitatively see that many important milk flavor compounds were removed by vacuum cooling.

Analysis of GC data of NVC and DSI further revealed the effect of vacuum cooling on milk flavor. DSI milk was lower in sweet aromatic flavor compounds delta-decalactone, delta-dodecalactone, gamma-dodecalactone than NVC milk (p<0.05, Table 3). DSI milk was also lower (p<0.05) in hydrogen sulfide, dimethyl sulfide, carbon disulfide and 2-methyl butanal than NVC milk. Both GC techniques demonstrated that vacuum cooling removed aroma active compounds.

DSI milk was higher in sulfur/eggy flavor than NVC milk. This result seems to contradict the results obtained from the GC, which showed that DSI milk was lower in sulfur/eggy volatile compounds than NVC milk. This discrepancy may be due to the complexity of sensory perception of flavor. The presence of other compounds can modulate and change detection thresholds of compounds (Green et al., 2010). Suppression of sulfur flavors may occur due to the presence of other background flavor compounds. After removal of other background flavor compounds by the vacuum chamber, sulfur-eggy flavor increases even though sulfur-eggy flavor compounds were decreased by vacuum cooling.

The flavor of UP milk varies between the methods used to pasteurize the milk (Lee et al., 2017). Flavor changes that occur during IND UP have been associated with Maillard reactions and include sulfurous, cooked cabbage, and caramelized notes (Colahan-Sederstrom and Peterson, 2005; Potineni and Peterson, 2005; Kokkinidou and Peterson, 2014). Recently, Jo et al. (2018) confirmed that DSI UP milk was higher in sulfur compounds hydrogen sulfide, dimethyl trisulfide, and methional compared to IND UP milk. Our research also suggests that these sulfur
compounds may be more influential to milk flavor partially due to the effect the vacuum cooler has on these volatile compounds.

Park et al. (2016) investigated the effect of the vacuum pressure of an evaporator during the evaporation of nonfat dry milk (NFDM) and milk protein concentrate 70 (MPC70). They concluded that poor flavor of NFDM and MPC70 was a combination of heat treatment followed by vacuum applied during evaporation. The heat treatment of milk forms undesirable flavor compounds followed by evaporation that removes characteristic milk flavor compounds. Our work demonstrates that the same characteristic milk flavor compounds that were removed by evaporation reported by Park et al. (2016) were also removed during vacuum cooling of DSI UP milk. Our work clearly demonstrates that the vacuum cooler caused distinct flavor between DSI and NVC milks. Analysis of the exiting vapor also shows that many important milk flavor compounds were removed by the vacuum cooler.

Vacuum cooling of milk during ultrapasteurization removes aroma active compounds and changes the flavor profile of the milk. Sweet aromatic, sulfur-eggy and cooked flavors are removed as measured by both analysis of the milk and the vapor removed by vacuum. The resulting DSI UP milk has decreased sweet aromatic flavor and higher sulfur-eggy flavor. Flavor removal by DSI UP becomes a further problem with highly flavored ESL beverages. Flavors for beverages make up a large percentage of the formulation costs. Losing costly flavor compounds in the form of a vapor through the vacuum chamber would be less than ideal. Vacuum chamber coolers can be removed from the process and exchanged with a different type of cooler, however there will be dilution of the product. Therefore, it cannot be done with standard of identity dairy products, but could be done for a formulated beverage that is batched at higher solids to meet the
target solids with the dilution of steam. Although DSI allows for a lower heat load on milk, the effect of the vacuum cooler may reduce desirable milk flavors and highlight less desired flavors.

**Acknowledgements**

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References


### Tables and Figures

**Table 1.** Descriptive sensory means for DSI and NVC milks diluted to 8.6% solids

<table>
<thead>
<tr>
<th></th>
<th>Aroma Intensity</th>
<th>Sweet Aromatic</th>
<th>cooked milky</th>
<th>Sulfur/eggy</th>
<th>viscosity</th>
<th>astringency</th>
</tr>
</thead>
<tbody>
<tr>
<td>DSI</td>
<td>4.0 a</td>
<td>0.7 b</td>
<td>4.4 b</td>
<td>2.7 a</td>
<td>1.9 a</td>
<td>2.3 a</td>
</tr>
<tr>
<td>NVC</td>
<td>3.3 a</td>
<td>2.1 a</td>
<td>4.6 a</td>
<td>1.8 b</td>
<td>1.9 a</td>
<td>2.3 a</td>
</tr>
</tbody>
</table>

^a-b Means in the same column no sharing a common superscript are different (P<0.05). Sensory attributes were scored on a 0 to 15 scale. Most fluid milk flavors fall between 0 and 5 on this scale (Lee et al., 2017). Means from descriptive analysis of three experimental replications.
Table 2. Aroma active volatile compounds identified in the vacuum chamber outlet during DSI pasteurization of skim milk by gas chromatography olfactometry (GC-O)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Identification</th>
<th>Odor description</th>
<th>RI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogen sulfide</td>
<td>RI, MS</td>
<td>Sulfur/egg</td>
<td>544</td>
</tr>
<tr>
<td>Dimethyl sulfide</td>
<td>RI, MS, O</td>
<td>Chemical/sulfur</td>
<td>564</td>
</tr>
<tr>
<td>Carbon disulfide</td>
<td>RI, MS</td>
<td>Cooked</td>
<td>568</td>
</tr>
<tr>
<td>2-Butanone</td>
<td>RI, MS</td>
<td>Plastic</td>
<td>596</td>
</tr>
<tr>
<td>Diacetyl</td>
<td>RI, MS, O</td>
<td>Diacetyl</td>
<td>607</td>
</tr>
<tr>
<td>2-Methylbutanal</td>
<td>RI, MS</td>
<td>Cooked/malty</td>
<td>645</td>
</tr>
<tr>
<td>3-Methylbutanal</td>
<td>RI, MS</td>
<td>Cooked/malty</td>
<td>665</td>
</tr>
<tr>
<td>Acetoin</td>
<td>RI, MS, O</td>
<td>Sweet/milky</td>
<td>701</td>
</tr>
<tr>
<td>Dimethyl disulfide</td>
<td>RI, MS, O</td>
<td>Earthy/sulfur</td>
<td>752</td>
</tr>
<tr>
<td>Dimethyl sulfoxide</td>
<td>RI, MS</td>
<td>Sulfur/garlic</td>
<td>767</td>
</tr>
<tr>
<td>Hexanal</td>
<td>RI, MS, O</td>
<td>Grass</td>
<td>808</td>
</tr>
<tr>
<td>Furfural</td>
<td>RI, MS, O</td>
<td>Barny/brothy</td>
<td>857</td>
</tr>
<tr>
<td>2-Heptanone</td>
<td>RI, MS</td>
<td>Cooked</td>
<td>897</td>
</tr>
<tr>
<td>Heptanal</td>
<td>RI, MS</td>
<td>Earthy/fatty</td>
<td>898</td>
</tr>
<tr>
<td>Methional</td>
<td>RI, MS, O</td>
<td>Potato/earthy</td>
<td>920</td>
</tr>
<tr>
<td>Benzaldehyde</td>
<td>RI, MS</td>
<td>Cooked/nutty</td>
<td>944</td>
</tr>
<tr>
<td>Dimethyl trisulfide</td>
<td>RI, MS, O</td>
<td>Garlic/cabbage</td>
<td>984</td>
</tr>
<tr>
<td>1-Octen-3-one</td>
<td>RI, MS</td>
<td>Mushroom</td>
<td>990</td>
</tr>
<tr>
<td>Octanal</td>
<td>RI, MS</td>
<td>Grass</td>
<td>995</td>
</tr>
<tr>
<td>1-Octen-3-ol</td>
<td>RI, MS</td>
<td>Mushroom</td>
<td>1003</td>
</tr>
<tr>
<td>Furaneol</td>
<td>RI, O</td>
<td>Sweet</td>
<td>1061</td>
</tr>
<tr>
<td>Guaiacol</td>
<td>RI, O</td>
<td>Earthy</td>
<td>1091</td>
</tr>
<tr>
<td>Maltol</td>
<td>RI, MS, O</td>
<td>Sweet</td>
<td>1110</td>
</tr>
<tr>
<td>Sotolon</td>
<td>RI, MS</td>
<td>Cooked/spicy</td>
<td>1120</td>
</tr>
<tr>
<td>2-Aminoacetophenone</td>
<td>RI, O</td>
<td>Tortilla/grain</td>
<td>1354</td>
</tr>
<tr>
<td>γ-Decalactone</td>
<td>RI, MS</td>
<td>Sweet/caramel</td>
<td>1486</td>
</tr>
<tr>
<td>δ-Decalactone</td>
<td>RI, MS, O</td>
<td>Fruity</td>
<td>1494</td>
</tr>
<tr>
<td>δ-Undecalactone</td>
<td>RI, MS</td>
<td>Butter sweet</td>
<td>1553</td>
</tr>
<tr>
<td>Dodecanoic acid</td>
<td>RI, MS</td>
<td>Soapy</td>
<td>1593</td>
</tr>
<tr>
<td>γ-Dodecalactone</td>
<td>RI, MS</td>
<td>Sweet/grain</td>
<td>1649</td>
</tr>
<tr>
<td>δ-Dodecalactone</td>
<td>RI, MS, O</td>
<td>Sweet/grain</td>
<td>1779</td>
</tr>
</tbody>
</table>

RI= retention index; O= odor; MS= mass spectra
Table 3. Relative abundance (µg/kg) of selected volatile compounds of ultrapasteurized milk with and without vacuum cooling.

<table>
<thead>
<tr>
<th>Volatile Compounds Identified by SBSE-GC-MS</th>
<th>NVC</th>
<th>DSI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butanal</td>
<td>0.074 a</td>
<td>0.016 a</td>
<td>0.072</td>
</tr>
<tr>
<td>Hexanal</td>
<td>0.042 a</td>
<td>0.013 a</td>
<td>0.397</td>
</tr>
<tr>
<td>Heptanal</td>
<td>0.038 a</td>
<td>0.016 a</td>
<td>0.141</td>
</tr>
<tr>
<td>Methional</td>
<td>0.52 a</td>
<td>0.086 b</td>
<td>0.016</td>
</tr>
<tr>
<td>1-octen-3-one</td>
<td>0.034 a</td>
<td>0.057 a</td>
<td>0.153</td>
</tr>
<tr>
<td>Octanal</td>
<td>0.031 a</td>
<td>0.02 a</td>
<td>0.393</td>
</tr>
<tr>
<td>E-2-octenal</td>
<td>0.074 a</td>
<td>0.052 a</td>
<td>0.325</td>
</tr>
<tr>
<td>Guaiacol</td>
<td>0.103 a</td>
<td>0.09 a</td>
<td>0.157</td>
</tr>
<tr>
<td>Nonanal</td>
<td>0.005 a</td>
<td>0.004 a</td>
<td>0.346</td>
</tr>
<tr>
<td>Phenylethanol</td>
<td>0.174 b</td>
<td>0.295 a</td>
<td>0.021</td>
</tr>
<tr>
<td>2-nonenal</td>
<td>0.013 a</td>
<td>0.008 a</td>
<td>0.645</td>
</tr>
<tr>
<td>E-2-decenal</td>
<td>0.16 a</td>
<td>0.144 a</td>
<td>0.374</td>
</tr>
<tr>
<td>Z-2-decenal</td>
<td>0.145 a</td>
<td>0.143 a</td>
<td>0.306</td>
</tr>
<tr>
<td>Dodecanal</td>
<td>0.054 a</td>
<td>0.021 b</td>
<td>0.098</td>
</tr>
<tr>
<td>E-2-dodecenal</td>
<td>0.012 a</td>
<td>0.011 a</td>
<td>0.834</td>
</tr>
<tr>
<td>Decanal</td>
<td>0.009 a</td>
<td>0.005 a</td>
<td>0.970</td>
</tr>
<tr>
<td>Delta-decalactone</td>
<td>0.032 a</td>
<td>0.0146 b</td>
<td>0.032</td>
</tr>
<tr>
<td>Delta-dodecalactone</td>
<td>0.131 a</td>
<td>0.101 b</td>
<td>0.029</td>
</tr>
<tr>
<td>Gamma-dodecalactone</td>
<td>0.147 a</td>
<td>0.003 b</td>
<td>0.031</td>
</tr>
<tr>
<td>Gamma-decalactone</td>
<td>0.068 a</td>
<td>0.054 a</td>
<td>0.009</td>
</tr>
<tr>
<td>Gamma-octalactone</td>
<td>0.134 a</td>
<td>0.138 a</td>
<td>0.008</td>
</tr>
<tr>
<td>1-octen-3-ol</td>
<td>0.039 a</td>
<td>0.045 a</td>
<td>0.035</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Volatile Compounds Identified by SPME Triple Quad GC-MS/MS</th>
<th>NVC</th>
<th>DSI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogen sulfide</td>
<td>15.48 a</td>
<td>9.668 b</td>
<td>0.002</td>
</tr>
<tr>
<td>Dimethyl sulfide</td>
<td>7.405 a</td>
<td>1.852 b</td>
<td>0.036</td>
</tr>
<tr>
<td>Carbon disulfide</td>
<td>5.423 a</td>
<td>2.859 b</td>
<td>0.047</td>
</tr>
<tr>
<td>Diacetyl</td>
<td>0.51 a</td>
<td>0.42 b</td>
<td>0.011</td>
</tr>
<tr>
<td>3-methyl butanal</td>
<td>1.3 a</td>
<td>0.935 b</td>
<td>0.048</td>
</tr>
<tr>
<td>2-methyl butanal</td>
<td>2.193 a</td>
<td>1.145 b</td>
<td>0.008</td>
</tr>
<tr>
<td>Dimethyl disulfide</td>
<td>0.385 a</td>
<td>0.076 b</td>
<td>0.029</td>
</tr>
<tr>
<td>1-octen-3-one</td>
<td>1.4 a</td>
<td>0.344 b</td>
<td>0.038</td>
</tr>
<tr>
<td>Benzoaldehyde</td>
<td>1.225 a</td>
<td>0.587 b</td>
<td>0.026</td>
</tr>
<tr>
<td>Furaneol</td>
<td>0.489 a</td>
<td>0.434 a</td>
<td>0.094</td>
</tr>
<tr>
<td>Maltol</td>
<td>0.106 a</td>
<td>0.106 a</td>
<td>0.073</td>
</tr>
<tr>
<td>Dimethyl trisulfide</td>
<td>0.142 a</td>
<td>0.085 b</td>
<td>0.029</td>
</tr>
</tbody>
</table>

a-b Means in the same column no sharing a common superscript are different (P< 0.05). Each mean represents triplicate evaluations from three experimental replications.
CHAPTER 4: Efficiency of Ceramic Microfiltration Removal of Whey Protein from Sweet Whey
Efficiency of removal of whey protein from sweet whey with ceramic microfiltration membranes

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Abstract

Our research objective was to measure percent removal of whey protein from separated sweet whey using 0.1 UTP ceramic microfiltration (MF) membranes in a 3 stage 3x process at 50°C. Cheddar cheese whey was separated at 72°C and pasteurized (72°C for 15 s), cooled to 4°C, and held overnight. Separated whey (375 kg) was heated to 50°C with a plate heat exchanger and microfiltered using a pilot-scale ceramic 0.1 µm uniform transmembrane pressure MF system in bleed-and-feed mode at 50°C in a 3X, 3-stage (2 diafiltration stages) process to produce a 3X MF retentate and MF permeate. Feed, retentate and permeate samples were analyzed for total nitrogen, noncasein nitrogen, and nonprotein nitrogen using Kjeldahl methods. Sodium dodecyl sulfate-PAGE analysis was also performed on the whey feeds, retentates, and permeates from each stage. A flux of 54 kg/m²h was achieved with 0.1µ ceramic UTP microfiltration membranes at 50°C in accordance with the membrane manufacturer’s specifications. About 84 to 85% of the total nitrogen in the whey feed passed through the membrane into the permeate. No passage of lactoferrin from the sweet whey feed of the MF into the MF permeate was detected. There was some passage of IgG, bovine serum albumen, glycomarcopeptide, and casein proteolysis products into the permeate. β-LG was in higher concentration in the retentate indicating that it was partially blocked from passage through the ceramic MF membrane, while α-LA had a higher rate of passage through the membrane than β-LG. The percentage of the serum proteins in sweet whey that were removed in permeate by ceramic MF was about 84% on a total nitrogen basis at 50°C.

Key Words: Ceramic microfiltration, serum proteins, sweet whey
Introduction

Whey protein isolate (WPI) contains greater than 90% protein. In order to achieve this level of protein purity, a defatting step, either by microfiltration (MF) or anion-exchange, is necessary (Morr and Ha, 1993; Daufin and Merin, 1990). In the dairy industry, spiral wound polymeric membranes are typically used for MF because of their lower capital cost per unit of membrane surface area and lower operation energy, but they have a relatively low chemical resistance to cleaning compounds, a short life (Jost and Jelen, 1997; Cheryan, 1998), and can only withstand low cross flow velocity surface and operating temperatures compared to ceramic membranes. Membranes are typically differentiated and sold based on a mean pore size, which in theory separates components based on size. The pore size of MF (0.1 to 0.2 µm) should allow whey proteins to pass through the MF membrane and casein micelles to be retained.

Ceramic membranes can be operated over a wide range of pH and temperatures and have higher mechanical strength to resist damage due to high cross flow velocity at the membrane surface. Ceramic membranes operated with uniform transmembrane pressure (UTP) achieve less fouling and longer processing runs (Saboya and Maubois, 2000). However, ceramic membranes have higher initial capital cost per unit of surface area than polymeric membranes. Milk and whey are typically processed at 50°C with ceramic membranes, while polymeric membranes are typically run at 4 to 7°C in the dairy industry. The flux per unit of membrane surface area is much higher with ceramic membranes than polymeric membranes, particularly when they are operated at different temperatures. In a comparison of ceramic and spiral wound polymeric membranes filtering skim milk under the same processing conditions (50°C) the 0.1µ pore size UTP membrane had a flux of about 72 kg/m² while the polymeric spiral membrane operated at 50°C had a lower flux of 16.2 kg/m² (Zulewska et al., 2009). Skim milk microfiltered at 5°C using 0.1µ pore size ceramic membranes was reported to have a flux of approximately 17 kg/m².
(Zulewska et al., 2018). The authors are not aware of a systematic published study on the impact of processing temperature on polymeric MF membranes, but a study of this type with polymeric UF membranes has been reported. Ng et al. (2018) found that the flux for skim milk at 50, 30, 10°C after 240 min of processing time were 42, 35, and 22 L/m²h, respectively. The foulant and resistant of the foulant was higher at 50°C, but the over flux and amount of product that would be processed per unit of membrane area was higher by about 91% at 50°C than at 10°C. The primary foulants on the membrane reported by Ng et al. (2018) were α-LA and casein proteolysis products.

Most studies that have investigated milk serum protein removal using MF have been done with spiral wound polymeric membranes. There is limited published research on the use of ceramic membranes for MF of cheese whey, but extensive research has been done on serum protein removal with ceramic MF of skim milk (Jost et al., 1997; Nelson et al., 2005; Zulewska et al., 2009; Beckman et al., 2010; Hurt et al., 2010). Ceramic membranes have been shown to be more effective at removing serum proteins from skim milk compared to spiral membranes (Zulewska et al., 2009; Beckman et al., 2010). Ceramic membranes removed 95% of serum proteins from skim milk with a 3 stage 3x process using a 0.1 µ UTP membrane at 50°C (Hurt et al., 2010). Spiral membranes under similar conditions were only able to remove about 70% of serum proteins from skim milk during 3 stages at 50°C (Beckman et al., 2010). Nelson and Barbano (2005) demonstrated that 95% of whey proteins could be removed from milk for aged Cheddar cheese making using diafiltration with UF permeate to keep the lactose, soluble mineral and nonprotein nitrogen composition of the milk for cheese making constant using conventional cheese making equipment. Ceramic MF of separated sweet whey may allow higher protein passage and be an effective alternative to polymeric spiral wound membranes.
Filtration systems with a high cross flow velocity have less fouling. Ceramic membrane design allows for a higher cross flow velocity (6 to 7 m/s) while spiral wound polymeric membranes have a much lower cross flow velocity (0.5 to 1.5 m/s) which leads to formation of a concentration polarization foulant layer when operating spiral wound membranes. There is minimal foulant layer formation at the high surface shear in ceramic membranes (Adams et al., 2015b). In a polymeric membrane system, the concentration polarization layer composition and solute rejection characteristics is a function of the feed material composition (e.g., skim milk versus whey) and the crossflow velocity. The lower the crossflow velocity of the feed material the thicker the concentration polarization layer and the lower the flux. It is typical to see flux decline during a processing run with polymeric membranes (Beckman et al., 2010) and this is due to the increase in resistance to permeate flow through the foulant layer as the thickness and compactness of the foulant layer increases during the processing run.

To demonstrate the importance of foulant layer in polymeric MF of skim milk, skim milk and casein-free skim milk (CFSM) that was made from the same skim milk with ceramic membranes were both processed through a 0.3-μm polyvinylidene fluoride spiral-wound membrane (Zulewska and Barbano, 2013). The flux of the CFSM was 4.6 times higher than that of the skim milk. In the absence of fouling material contributed by the skim milk, nearly all of the milk serum proteins were passed through the same polymeric membrane showing clearly the importance of the foulant layer in controlling the separation achieved with polymeric MF membranes.

Differences in protein concentration and type of proteins in skim milk versus sweet whey may change the composition and porosity of the foulant layer which may impact SP passage through the membrane. Skim milk contains about 3.3% true protein (TP) which is about 80%
casein and 20% milk derived whey proteins, while sweet whey contains about 0.7 to 0.8% TP that is made up of primarily β-LG and α-LA plus some glycomacropeptide (GMP) produced by the rennet hydrolysis of κ-casein during cheese making and some level of proteolysis products from β and α-caseins that are formed during cheese making. As a result, when a concentration polarization layer forms at a membrane surface with a feed material of skim milk versus sweet whey, the composition and possibly the porosity of the foulant layer may be quite different. The difference in overall concentration of protein in the starting sweet whey versus skim milk will cause a difference in the viscosity of the feed material and the difference in protein type may also have an impact on viscosity near the membrane surface impacting fouling and flux. Sweet whey will also have a high concentration (10^7 to 10^9 cells/mL) of dead starter culture bacteria cells that may contribute to fouling when processing sweet whey but not when processing skim milk. The pH of the sweet whey will be lower than skim milk (5.9 to 6.2 versus 6.5 to 6.6, respectively) due to lactic acid production by the starter culture during cheese making. The lower pH of sweet whey will result in a higher concentration of soluble mineral, which has been found to be a major contributor to limiting flux and a cause of reduced SP removal in MF of skim milk and milk protein concentrates (Adams et al., 2015a). MF feeds with higher soluble mineral had lower limiting fluxes, which means that the presence of more soluble mineral potentially leads to greater fouling and lower SP transmission. Therefore, ceramic MF of whey may have lower transmission of SP than for skim milk due to the increased amount on soluble mineral.

Prior to MF of skim milk, the skim milk is pasteurized not much higher than the minimum temperature and time requirements (72°C for 15s) to prevent the thermal denaturation of whey protein (primarily β-LG) which becomes covalently bound to casein micelles and then will not pass through a 0.1µ MF membrane. The amount of milk derived whey protein that is
bound to casein micelles can be measured as an apparent increase in casein as % to TP (CN%TP) of the milk measured by the Kjeldahl method (Lynch et al., 1998) before and after pasteurization. Thermal denaturation of milk derived whey proteins will decrease recovery when microfiltering skim milk (Hurt and Barbano, 2010). During the production of cheese whey, there are typically 2 pasteurization steps, one to kill any pathogenic bacteria in the initial cheese milk, and the second to deactivate the starter culture and residual rennet. Whey proteins are more thermolabile than casein (Considine et al., 2007) and as the whey proteins denature during pasteurization of sweet whey they would form larger whey protein aggregates or interact with milk fat globule membrane, which may cause them not to pass through an MF membrane.

Therefore, when processing cheese whey instead of skim milk, any protein rejection that occurs at the membrane surface will lead to lower yield of WPI that is produced from MF permeate. The retentate from MF of sweet whey creates a co-product (MF retentate), whey protein phospholipid concentrate (WPPC), which contains at least 50% protein (ADPI, 2015). WPI is sold at a premium, and manufacturers lose potential profit with every kilogram of protein that does not pass through the MF membrane and is lost in the WPPC product that has lower value than WPI. Therefore, there is a need to further investigate how to improve protein removal from whey during MF of separated sweet whey. Ceramic MF of separated sweet whey may be an effective alternative to polymeric membranes that may allow increased protein passage. The objective of this research was to measure whey protein removal percentage from separated sweet whey using 0.1µ uniform transmembrane pressure (UTP) MF ceramic membranes in a 3-stage, 3x process at 50°C.
Materials and Methods

Liquid Sweet Whey Manufacture

Raw whole milk, 600 kg, was obtained from the North Carolina State Univ. Dairy Research and Education System. Milk was high temperature short time (HTST) pasteurized (720 kg/h) with a plate heat exchanger (model T4 RGS- 16/2, SPX Flow Technology, Greensboro, NC) at 72°C with a hold time of 16 s. The milk was then cooled to 31°C and transferred to a cheese vat (Model TH0041, Kusel Equipment, Watertown, Wis., U.S.A.). Mesophilic starter culture containing Lactococcus lactis ssp. lactis (CHR Hansen R-604, Milwaukee, Wis., U.S.A.) was added at the rate of 50 Danisco Culture Units (DCU)/454 kg of milk. A calcium chloride solution (32 to 33% w/v, Dairy Connection Inc., Madison, Wis., U.S.A.) was also added at the rate of 0.39 mL/kg of milk. The milk was then allowed to ripen for 60 min. Next, the milk was coagulated with double strength microbial rennet (DCI Supreme - Mucor mehei microbial rennet, Dairy Connection Inc., Madison, Wis., U.S.A.) for 30 min at a rate of 0.09 mL/kg of milk diluted 80 times in DI water. The coagulum was then cut and the curd was allowed to rest for 5 min. The curd was stirred for 30 min while the temperature was increased gradually to 39 °C. The pH was monitored with a pH meter (Orion Model 0290, Thermo Scientific, Waltham, MA) and probe (VWR Model 89231-572, VWR, Radnor, PA) and the whey was drained through a sieve to remove cheese fines once a pH of 6.35 was achieved. Drained whey was HTST pasteurized (750 kg/h) at 72°C for 15 s and fat separated using an inline (after the regeneration section and before the hold tubes of the pasteurizer) hot bowl centrifugal separator (Model J5-OSCP-1, JSC PLAVA, Savery, Orlando, FL, U.S.A.) at 65°C. Separated whey was cooled to 4°C and stored for 24 h before filtration.
Pasteurized separated whey (375 kg) was heated to 50°C with a plate heat exchanger and microfiltered using a pilot-scale ceramic 0.1 μ UTP MF system (Tetra Alcross M7, TetraPak Filtration Systems, Aarhus, Denmark) in bleed-and-feed mode at 50°C to continuously produce a 3X (mass/mass) MF retentate and MF permeate. After the first stage, the MF retentate was diluted back to a 1× concentration (2 kg of water for every 1 kg of retentate) with deionized (DI) water, heated to 50°C, and diafiltered with the ceramic MF system. This diafiltration procedure was repeated to complete a 3-stage process. All of the retentate and permeate from each stage was collected separately, weighed, mixed and sampled for mass balance calculations of whey protein removal for each stage of the process. The cheese making and whey processing was replicated in 3 different weeks starting with a separate batch of milk.

**Microfiltration Operation**

The operation of the filtration unit followed the method outlined in Hurt et al. (2010) with slight modification. Briefly, A pilot-scale UTP MF system (Tetra Alcross M7, TetraPak Filtration Systems, Aarhus, Denmark) equipped with ceramic Membralox (EP1940GL0.1μA, alumina, Pall Corp., Cortland, NY) membranes (pore diameter: 0.1 μ; surface area: 1.7 m²) was used. The membranes in a tubular stainless module consisted of 7 ceramic tubes, with 19 channels each with a channel diameter of 4-mm. The UTP MF system consisted of a feed pump (type LKH 10/110 SSS 1.75 kW), a retentate recirculation pump (type LKH 20/125 SSS 6.3 kW), and a permeate recirculation pump (type LKH 10/130 SSS 2.5 kW) all from Alfa Laval (Kansas City, MO). The membranes were 1.02 m long and were mounted vertically in the MF system with permeate and retentate flow co-current from the top to the bottom of the module. Because the membrane was mounted vertically, the inlet and outlet gauge pressures have a correction factor applied to correct for the difference due to the weight of the vertical column of
liquid. The correction factor was determined as follows: with 50°C DI water in the system and only the feed pump turned on, the retentate and permeate outlet valves were closed. Retentate inlet pressure ($R_{pi}$), permeate inlet pressure ($P_{pi}$), retentate outlet pressure ($R_{po}$), and permeate outlet pressure ($P_{po}$) were measured under these conditions. A correction factor for calculating transmembrane pressure was calculated for each gauge pressure as follows: the $R_{pi}$ gauge pressure correction was $P_{po} - R_{pi}$, the $R_{po}$ gauge pressure correction was $P_{po} - R_{po}$, the $P_{pi}$ gauge pressure correction was $P_{po} - P_{pi}$, and the $P_{po}$ gauge pressure correction was zero. This correction factor was determined at the beginning of each run of each stage. Next, retentate and permeate recirculation pumps were turned on and the retentate bleed flow was set to 45 L/h and the permeate bleed flow was set to 90 L/h. The elevation-corrected inlet and outlet pressures were measured and the transmembrane pressure from the retentate to the permeate side of the membrane at retentate inlet (TMPi) and outlet (TMPo) ends of the membrane were calculated. The goal was to have a pressure differential ($\Delta P = TMPi - TMPo$) of $25 \pm 3$ kPa for a membrane length of 1.02 m. A diaphragm valve in the permeate recirculation loop was used to adjust the recirculation flow rate on the permeate side of the membrane. The permeate recirculation flow rate was adjusted with the diaphragm valve until the $\Delta P$ was $25 \pm 3$ kPa.

**Cleaning Before Processing.** Immediately before processing storage solution (0.55% vol/vol solution of nitric acid) was flushed out of the system with room temperature DI water until the pH was neutral. The MF flow system was heated with a heat exchanger on the recirculation loop with 65°C water while recirculating until the system temperature was 50°C and the initial clean water flux was determined. The following conditions were applied during the flux measurement: the retentate outlet valve was closed and permeate outlet valve was fully open with only the feed pump running. Clean water flux was typically about 750 to 800 L/m² h.
**First Stage.** Cheddar whey (about 375 kg) was processed to approximately a 3X CF at 50°C using a pilot-scale UTP MF system described above (a 3× CF being 2 kg of permeate removed for every 1 kg of retentate). The system was started on 50°C DI water and was transitioned from water to whey with all the pumps running; the retentate recirculation rate was approximately 685 L/min with a linear velocity of approximately 6.5 m/s. To flush the 50°C water out of the system with whey at the beginning of the process, both retentates and permeate line were flushed until the first visible appearance of product, about 2.5 kg of retentate and 5.5 kg of permeate were collected, the weights were recorded, and both were discarded (mostly water). After this start up, retentate and permeate were collected continuously. Retentate and permeate removal rates were 45 and 90 L/h, respectively. The ΔP of the transmembrane pressure was adjusted to 25 ± 3 kPa after switching from water to whey with the permeate recirculation diaphragm valve while processing whey to achieve and maintain this TMP difference between the outlet and inlet ends of the membrane. Flux (kg/m² per hour) was measured every 15 min, and samples of permeate and the retentate were taken for analysis using an infrared spectrophotometer (Lactoscope FTIR, Delta Instruments, Drachten, the Netherlands) to monitor retentate and permeate composition for process control. At the end of each stage of the MF run, the collected retentate and permeate were mixed separately and sampled as a representative sample for each stage. In-between stages, retentate and permeate exit hoses were placed in the feed tank for approximately 20 min to recirculate product to set processing conditions and allow time for dilution of previous stage retentate with DI water. Once the feed tank was filled with diluted retentate, retentate and permeate collection continued and stage 2 of filtration had commenced.
**Second Stage and Third Stage.** The second-stage feed of the 3-stage process was the retentate from the first stage diluted by weight: 2 kg of DI water for every 1 kg of retentate (about 120 kg of retentate and 240 kg of water). Retentate and water were mixed before heating to 50°C and processed with the MF UTP system using the same operating conditions as described for the first stage. The ΔP was adjusted to 25 ± 3 kPa after starting stage 2 with the permeate recirculation diaphragm valve while processing whey to achieve and maintain this TMP difference between the outlet and inlet ends of the membrane. Final stage 2 retentate and permeate samples were collected, cooled, mixed, and sampled as described above. The third stage of the 3-stage process was as described for the second stage. Stage 2 retentate was diluted with DI water as described above and was filtered as stage 3. The complete batch of stage 3 retentate and permeate were weighed, mixed and sampled as described above.

**Cleaning After Processing.** Immediately after processing, DI water (150 to 200 L) was flushed through the system with all pumps on. The MF system was flushed until no whey retentate was visible in the flush water exiting the retentate side. The membrane was cleaned as described by Zulewska et al. 2009.

**Chemical Analyses**

Samples of cheese milk, feed whey, permeate, and retentate collected during processing were analyzed using an infrared spectrophotometer (Lactoscope FTIR, Delta Instruments) for fat, lactose, and TP content (Kaylegian et al., 2006). This was done to quickly monitor the composition of retentate and permeate during the run to detect if the system was running as expected. Feed whey, and retentate and permeate for each stage were analyzed in duplicate for TS, total N (TN), and NPN content using forced-air oven drying (AOAC, 2000; method 990.20; 33.2.44), Kjeldahl (AOAC, 2000; method 991.20; 33.2.11), and Kjeldahl (AOAC, 2000; method
The noncasein nitrogen (NCN) content of retentates was determined using Kjeldahl (AOAC, 2000; method 998.05; 33.2.64). TP was calculated by subtracting NPN from TN and multiplying by 6.38; CN was calculated by subtracting the NCN from TN and multiplying by 6.38; and whey content was calculated by subtracting NPN from NCN and multiplying by 6.38. The whey protein concentration in the permeate portion of the whey (expressed as a percentage by weight) was calculated by dividing mass of SP in 1 kg of whey by the permeate portion of the whey multiplied by 100, where the permeate portion of whey is 1 kg minus the weight of casein in 1 kg of whey.

**Protein Removal Calculation Using Kjeldahl Data**

The whey protein removal for each stage was calculated using Kjeldahl analysis (TN and NPN) of the initial whey feed and the permeates from each of stage in combination with the weights of initial whey feed and permeate removed from each of 3 stages. Total whey protein removal equaled the mass of protein removed in the sum of the 3 stages divided by the mass of protein in the original feed multiplied by 100. Protein removal was calculated by dividing the mass of TP (TP concentration was calculated from TN and NPN concentrations obtained by Kjeldahl analysis of permeates, and mass of TP was calculated by multiplying the concentration of TP by the mass of permeate) in the permeate of each stage by the mass of SP in the starting whey times 100. Theoretical values for removal were calculated using the above equations assuming that CN was 100% retained and that the concentration of SP in the permeate equaled the concentration of SP in the permeate phase of skim milk or water diluted retentate feed and that the CF and DF were both exactly 3.
A 10 to 20% polyacrylamide gradient gel (BioRad, Hercules, CA: Acrylamide/Bis Powder 37.5:1, cat. no. 1610125) was used to determine the relative proportion of protein types in retentates and permeates from 3 MF stages. Retentate samples were diluted with sample buffer consisting of 10 mM Tris-HCl pH 6.8 (Sigma-Aldrich, Milwaukee, WI: Trizma Base, cat. no. T1503), 1.0% SDS (Sigma-Aldrich, Milwaukee, WI: Sodium Dodecyl Sulfate, cat. no. L4390), 20% glycerol (Fisher Scientific, Pittsburgh, PA: Glycerol, cat. no. G331), and 0.02% bromophenol blue (BioRad, Hercules, CA: Bromphenol Blue, cat. no. 1610404) tracking dye and 50 mM dithiothreitol (Sigma-Aldrich, Milwaukee, WI: DL-dithiothreitol, cat. no. 43817) and stored frozen (–17°C) in glass vials (Target DP Vials C4000-1W, National Scientific Co., Rockwood, TN) sealed with DP Blue Cap (C4000-51B, National Scientific Co.). Diluted samples were thawed, heated to 100°C in a steam chamber, held at 100°C for 3 min, and then cooled to about 25°C. Retentates and milks were loaded onto an SDS-PAGE gel (Verdi et al., 1987), and the procedure of Verdi et al. (1987) was used for pouring of the stacking and running gel, running conditions, staining, and destaining the gels. The SDS-PAGE gels were run using a Protean II xi cell (Bio-Rad Laboratories Inc., Hercules, CA). Gels were scanned with USB GS 800 Densitometer using Quantity 1 1-D Analysis software (Bio-Rad Laboratories Inc., Hercules, CA) to obtain a relative protein composition of samples. Protein loading on the gel for each sample was adjusted for each sample to achieve an optical density of the predominant protein for each sample in the range of 1.0 to 1.4, based on Kjeldahl measured protein content of each sample. This approach to achieve uniform mass of protein loaded per slot and avoids overloading of the most prominent protein in a sample, even though the protein content from sample-to-sample varies, produces much more repeatable quantitative analysis by densitometry. A milk
sample was run on each gel as a reference for proper resolution of milk proteins and a check for consistency of quantitative analysis from gel-to-gel on the same milk sample. The background was adjusted separately for each lane using the rolling disk method of subtraction to obtain a flat base on the pop-up trace in the densitometer software. The line that defined each lane was adjusted using the lane tool function (add, adjust anchors) in the software so that the lane line crossed each band at the center. The adjust band function of the software was used with brackets to set the leading and trailing edge for each band as visually observed on the image of the gel, not based on the beginning and end of the peak in the pop-up trace. The bracket width was set to include the full width of all bands. To achieve the most consistent analysis conditions for quantitation of relative protein amounts among the 3 experimental replicates, all retentate and permeate samples were run on two gels in the Protein II system at the same time with the retentate or permeate samples from the 3 replicates within MF stage in adjacent lanes on the same gel.

**Whey Protein Removal Estimation Using SDS-PAGE**

To calculate relative percentage of whey protein removal using SDS-PAGE results, first the whey protein as a percentage of TP in each lane is calculated, which was the sum of the relative density of all whey bands divided by the sum of the relative density of all protein bands times 100. Calculation of whey removal was as follows: \[ \text{Whey removal} = 100 \times \left( \%\text{whey [feed]} - \%\text{whey [retentate]} \right) / \%\text{whey [feed]} \].

**Statistical Analysis**

Data were analyzed using XLSTAT (version 2013.5.03; Addinsoft, New York, N.Y., U.S.A.). Analysis of variance was conducted to compare treatment means using the Tukey’s honestly significant difference test at a significance level of \( P < 0.05 \).
Results and Discussion

Processing

Operational Parameters. The membrane manufacturer recommended a flux of 54 kg/m$^2$h UTP ceramic membranes and that was achieved and maintained throughout the runs in all 3 replicates. It has been reported (Hurt et al., 2015) that for processing of skim milk using 0.1µ graded permeability ceramic membranes, higher sustainable flux (i.e., >54 kg/m$^2$h) on skim milk (100 to 130 kg/m$^2$h) can be achieved depending on the protein concentration and this may also be possible with whey. The concentration factor was maintained by adjusting the retentate and permeate bleed rates to achieve the target concentration factor 3x in all 3 stages (Table 1) and the ΔTMP was near the manufacturers’ recommendation of 24 kPa in all 3 stages of filtration.

Composition of Whey, MF retentates and MF Permeates

Whey and MF retentates. The percentage of fat in the separated whey was low and typical of good removal of fat from Cheddar cheese whey (Table 2). The percentage of casein remaining in the whey reported in Table 2 was due to GMP cleavage from rennet action on κ-casein and a low level of other casein proteolysis products. The fat concentration measured by ether extraction was typical for separated sweet whey. Milk fat was concentrated in the retentate as expected and was about twice the concentration of that in the separated whey being microfiltered which would be typical with a 3x concentration factor. The fat percentage of retentate doesn’t change from stage to stage indicating fat retention by the MF membrane ($P < 0.05$). Lactose and milk solids concentration in the MF retentate decreased as expected with successive stages of diafiltration. The CN%TP increased and SP as a percentage of TP ($SP%TP$) in the retentate decreased as more SP was removed from the separated whey in stages 1 through
3. Casein as a percent of dry matter (CPDM) and fat as a percentage of dry matter (FDM) in the permeate both increased with increasing stage number as lactose was removed in MF permeate (Table 2). The goal of the MF process is to remove protein from the retentate and retain fat. Going from whey to the final stage 3 retentate, the concentration factor for crude protein was 2.01 and the concentration factor for fat was 11X (Table 2). Thus, fat was being selectively concentrated in the MF retentate while protein passed through the membrane. If the stage 3 retentate was ultrafiltered to remove 70% of the lactose then the CPDM would increase from 25.8% to 47.6% and the FDB would increase from 6.28 to 9.51%.

**MF Permeates.** Fat concentration in the permeate was very low and uniform, indicating that very little milk fat is present in the ceramic MF permeate. The ceramic MF membranes were very effective at blocking the passage of fat through the membrane. Lactose concentration in permeate decreased with each stage as expected with successive stages of diafiltration. CP, NPN, TP, NCN, SP concentration in the permeate decreased with each stage of filtration as not only is the permeate more dilute, but protein removal decreases with each stage as the percentage of the original protein in separated whey decreases with increasing stage number (Table 3). Microfiltration permeates containing about 0.6% TP were clear due to the retention of fat in the MF retentate (Figure 1).

**Serum Protein Removal from Cheddar Cheese Whey.** Most of the fat from the original separated whey did not pass through the MF membrane in permeate. The portion of fat that was recovered in the permeate may not be lipid, but it was ether soluble material that was not further characterized. All 3 methods of measuring protein removal from separated whey provide similar results, with the SDS-PAGE indicating slightly higher protein removal because the SDS-PAGE reflects TP removal while the Kjeldahl in this case reflects a TN based estimation of protein.
removal (Table 4). The calculated cumulative removal of SP was underestimated by Kjeldahl TP measured in MF permeate because a portion of what was counted as NPN in MF permeate was actually TP from the GMP. The NPN concentration in separated whey is typically higher than the milk used for cheese making because at least a portion of the GMP from κ-CN is soluble in 12% trichloroacetic acid in the Kjeldahl sample preparation for measurement of nonprotein nitrogen. When calculation of protein removal is done on a TN basis, the contribution of 12% trichloroacetic acid (TCA) soluble GMP is accounted for in the recovery calculation. Serum protein removal based on TP and TN in the permeate, as well as the cumulative SP removal as measured SDS-PAGE are summarized in Table 5. Protein removal on TN basis is probably more related to the yield of WPI because it reflects the contribution of the TCA soluble GMP from κ-CN. The SP removal from sweet whey on a TN basis and based on SDS-PAGE analysis were 84.3 and 85.0, respectively.

There was no detectable level of lactoferrin (LFR) passage into the MF permeate and no intact αs or β-CN was detected in the feed whey, MF retentate, or permeate as measured by SDS-PAGE (Figure 2, Table 6). The low level of LFR in Cheddar sweet whey before salting is not surprising and the large difference in LFR content of whey before and after salt application to Cheddar cheese curd has been reported (Blaschek et al., 2007). Some of the casein proteolysis products (CNPP) present in the whey passed through membrane and some did not (Table 6). Bovine serum albumin (BSA) and immunoglobulin G (IgG) were partially concentrated (Table 6) in the retentate (P < 0.05).

The SDS-PAGE data presented in Table 7 shifts the focus to only β-LG and α-LA and normalizes their data to 100%. This eliminates the effect of the differential partitioning of BSA, LFR and CNPP between the whey and permeate to better understand the partitioning of the two
major proteins in whey (β-LG and α-LA) between retentate and permeate by the ceramic membrane used in this study. Some β-LG was rejected by the MF ceramic membrane while there appeared to be less rejection of α-LA (Table 7). The β-LG plus α-LA was a higher ($P < 0.05$) percentage of total protein in the permeate than in the retentate (Table 7) indicating that other proteins (i.e., BSA, LFR, GMP, and CNPP) were being blocked more from passage through the membrane into permeate than α-LA and β-LG. The ratio of β-LG to α-LA was higher in the retentate than in feed and the permeate ($P < 0.05$) because β-LG did not pass through the membrane as completely as α-LA (Table 7). Less passage of β-LG through the membrane than α–LA may be due to heat induced β-LG to β-LG aggregates or aggregates of β-LG with milk fat globule membrane that were caused by pasteurization of the whey (Lee and Sherbon, 2002).

**Comparison of Serum Protein Removal: Skim Milk Versus Whey**

MF of skim milk using ceramic membranes removes more serum protein from skim milk (Zulewska et al., 2009) than polymeric membrane when the milk is processed with the same amount of diafiltration and at the same processing temperature (Zulewska et al., 2009; Hurt et al., 2010; Beckman et al., 2010). MF of sweet whey did not remove as high a percentage of the starting protein from whey as was reported for ceramic MF of skim milk (Table 8) and this was likely due to aggregation of β-LG or the retention of GMP and casein proteolysis products. Under the same processing conditions, the removal of SP from skim milk by spiral wound membranes was reported (Beckman et al., 2010; Hurt et al., 2010) to be lower than for ceramic membranes (70 versus 98% removal, respectively).

**Conclusions**

A flux of 54 kg/m$^2$h was achieved with 0.1μ ceramic UTP microfiltraton membranes at 50°C in accordance with the membrane manufacturer’s specifications. About 84 to 85% of the
total nitrogen in the whey feed passed through the membrane into the permeate. No passage of lactoferrin from the sweet whey feed of the MF into the MF permeate was detected. There was some passage of IgG, bovine serum albumen, GMP, and casein proteolysis products into the permeate. \( \beta \)-LG was in a higher concentration in the retentate indicating that it was partially blocked from passage through the ceramic MF membrane, while \( \alpha \)-LA had a higher rate of passage through the membrane than \( \beta \)-LG. The percentage of the serum proteins in sweet whey that were removed in permeate by ceramic MF was about 84% at 50°C.

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#### Tables and Figures

**Table 1.** Mean ± standard deviation (n=3) microfiltration (MF) transmembrane pressure (TMP) at the membrane inlet and outlet, delta TMP, flux, and concentration factors for each stage of the 3-stage 3x ceramic uniform trans membrane pressure microfiltration.

<table>
<thead>
<tr>
<th>MF stage</th>
<th>TMP inlet (kPa)</th>
<th>TMP outlet (kPa)</th>
<th>ΔTMP (kPa)</th>
<th>Flux (kg/m² h)</th>
<th>Concentration factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 1</td>
<td>45</td>
<td>21.6 ± 2.9</td>
<td>23.3 ± 2.9</td>
<td>54.0 ± 1.0</td>
<td>3.04 ± 0.07</td>
</tr>
<tr>
<td>Stage 2</td>
<td>54</td>
<td>30.3 ± 2.9</td>
<td>23.7 ± 2.9</td>
<td>53.9 ± 1.2</td>
<td>3.02 ± 0.08</td>
</tr>
<tr>
<td>Stage 3</td>
<td>54</td>
<td>30.3 ± 2.9</td>
<td>23.7 ± 2.9</td>
<td>54.2 ± 1.1</td>
<td>3.05 ± 0.07</td>
</tr>
</tbody>
</table>

¹Inlet TMP was set and maintained during the run by controlling the permeate removal rate.
Table 2. Mean (n = 3) composition (%w/w) of separated whey and ceramic microfiltration (MF) and diafiltration (DF) retentates produced for stages 1, 2, and 3 produced from separated Cheddar cheese whey.

<table>
<thead>
<tr>
<th></th>
<th>Fat</th>
<th>Lactose* (IR)</th>
<th>Total solids</th>
<th>CP</th>
<th>NPN</th>
<th>TP</th>
<th>NCN</th>
<th>Casein</th>
<th>SP</th>
<th>CN%TP</th>
<th>SP%TP</th>
<th>CPDB</th>
<th>FDB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Separated whey</td>
<td>0.038</td>
<td>4.868 a</td>
<td>6.638 a</td>
<td>0.851</td>
<td>0.208</td>
<td>0.643</td>
<td>0.817</td>
<td>0.034 a</td>
<td>0.609</td>
<td>5.30 c</td>
<td>94.702 a</td>
<td>12.82 d</td>
<td>0.57 d</td>
</tr>
<tr>
<td>Stage 1 - MF retentate</td>
<td>0.080</td>
<td>4.629 b</td>
<td>6.441 b</td>
<td>0.941</td>
<td>0.206</td>
<td>0.735</td>
<td>0.898</td>
<td>0.043 a</td>
<td>0.692</td>
<td>5.78 c</td>
<td>94.217 a</td>
<td>14.61 c</td>
<td>1.24 c</td>
</tr>
<tr>
<td>Stage 2 (DF) - MF retentate</td>
<td>0.080</td>
<td>1.945 c</td>
<td>2.737 c</td>
<td>0.505</td>
<td>0.097</td>
<td>0.408</td>
<td>0.466</td>
<td>0.039 a</td>
<td>0.369</td>
<td>9.62 b</td>
<td>90.385 b</td>
<td>18.45 b</td>
<td>2.92 b</td>
</tr>
<tr>
<td>Stage 3 (DF) - MF retentate</td>
<td>0.076</td>
<td>0.794 d</td>
<td>1.216 d</td>
<td>0.314</td>
<td>0.072</td>
<td>0.242</td>
<td>0.281</td>
<td>0.032 a</td>
<td>0.210</td>
<td>13.27 a</td>
<td>86.734 c</td>
<td>25.80 a</td>
<td>6.28 a</td>
</tr>
<tr>
<td>Standard error</td>
<td>0.003</td>
<td>0.066</td>
<td>0.045</td>
<td>0.018</td>
<td>0.003</td>
<td>0.017</td>
<td>0.016</td>
<td>0.005</td>
<td>0.015</td>
<td>1.106</td>
<td>1.106</td>
<td>0.60</td>
<td>0.16</td>
</tr>
<tr>
<td>R²</td>
<td>0.980</td>
<td>&gt;0.99</td>
<td>&gt;0.99</td>
<td>&gt;0.99</td>
<td>&gt;0.99</td>
<td>&gt;0.99</td>
<td>&gt;0.99</td>
<td>&gt;0.99</td>
<td>&gt;0.99</td>
<td>&gt;0.99</td>
<td>&gt;0.99</td>
<td>&gt;0.99</td>
<td>&gt;0.99</td>
</tr>
</tbody>
</table>

CP = Crude protein = total nitrogen x 6.38; NCN = noncasein nitrogen x 6.38; NPN = nonprotein nitrogen x 6.38; TP = true protein (TN - NPN); casein = CN = (TP - (NCN - NPN)); serum proteins = SP = (NCN - NPN); CN%TP = casein as a percentage of true protein; CPDB = crude protein on dry-mater basis = ((CP/TS) x 100); FDB = fat on a dry-mater basis = ((Fat/TS) x 100).

*Anhydrous lactose measured by mid-infrared spectroscopy

a, b, c, d Means not sharing a common superscript differ (P < 0.05)
Table 3. Mean (n = 3) composition in (% w/w) of ceramic microfiltration (MF) and diafiltration (DF) permeates produced from separated Cheddar cheese whey.

<table>
<thead>
<tr>
<th></th>
<th>Fat</th>
<th>Lactose*</th>
<th>Total solids</th>
<th>CP</th>
<th>NPN</th>
<th>TP</th>
<th>NCN</th>
<th>Casein</th>
<th>SP</th>
<th>CN%TP</th>
<th>SP%TP</th>
<th>CPDB</th>
<th>FDB</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stage 1 - MF permeate</strong></td>
<td>0.005</td>
<td>4.471 a</td>
<td>5.937</td>
<td>0.696</td>
<td>0.189</td>
<td>0.507</td>
<td>0.670</td>
<td>0.026 a</td>
<td>0.481</td>
<td>5.08 b</td>
<td>94.916 a</td>
<td>11.72 b</td>
<td>0.09 c</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td><strong>Stage 2 (DF) - MF permeate</strong></td>
<td>0.005</td>
<td>1.949 b</td>
<td>2.549</td>
<td>0.307</td>
<td>0.085</td>
<td>0.222</td>
<td>0.292</td>
<td>0.015 b</td>
<td>0.207</td>
<td>6.76 b</td>
<td>93.242 a</td>
<td>12.04 b</td>
<td>0.20 b</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>b</td>
</tr>
<tr>
<td><strong>Stage 3 (DF) - MF permeate</strong></td>
<td>0.005</td>
<td>0.797 c</td>
<td>0.961</td>
<td>0.122</td>
<td>0.038</td>
<td>0.084</td>
<td>0.108</td>
<td>0.014 b</td>
<td>0.070</td>
<td>17.06 a</td>
<td>82.944 b</td>
<td>12.69 a</td>
<td>0.49 a</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td>c</td>
<td>c</td>
<td>c</td>
<td>c</td>
<td>c</td>
<td>c</td>
<td>c</td>
<td>c</td>
<td>c</td>
<td>c</td>
<td>c</td>
<td>c</td>
</tr>
<tr>
<td><strong>Standard error</strong></td>
<td>0.001</td>
<td>0.083</td>
<td>0.065</td>
<td>0.013</td>
<td>0.004</td>
<td>0.006</td>
<td>0.014</td>
<td>0.002</td>
<td>0.011</td>
<td>1.162</td>
<td>1.162</td>
<td>0.206</td>
<td>0.032</td>
</tr>
<tr>
<td><strong>R²</strong></td>
<td>&gt;0.99</td>
<td>&gt;0.99</td>
<td>&gt;0.99</td>
<td>&gt;0.99</td>
<td>&gt;0.99</td>
<td>&gt;0.99</td>
<td>&gt;0.99</td>
<td>&gt;0.99</td>
<td>&gt;0.99</td>
<td>&gt;0.99</td>
<td>&gt;0.99</td>
<td>&gt;0.99</td>
<td>&gt;0.99</td>
</tr>
</tbody>
</table>

CP = Crude protein = total nitrogen x 6.38; NCN = noncasein nitrogen x 6.38; NPN = nonprotein nitrogen x 6.38; TP = true protein (TN - NPN); casein = CN = (TP) – (NCN – NPN); serum proteins = SP = (NCN – NPN); CN%TP = casein as a percentage of true protein; CPDB = crude protein on dry-mater basis = ((CP/TS) x 100); FDB = fat on a dry-mater basis = ((Fat/TS) x 100).

*Anhydrous lactose measured by mid-infrared spectroscopy

a, b, c, d Means not sharing a common superscript differ (P < 0.05)
Table 4. Mean ± standard deviation (n=3) percentage fat, nitrogen, and protein recovered in ceramic microfiltration retentate and permeate.

<table>
<thead>
<tr>
<th>Fat (%)</th>
<th>TN recovery based on permeate measured by Kjeldahl (%)&lt;sup&gt;1&lt;/sup&gt;</th>
<th>TN recovery based on retentate measured by Kjeldahl (%)&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Protein recovery measured by SDS-PAGE (%)&lt;sup&gt;3&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retentate</td>
<td>74.2 ± 5.1</td>
<td>15.2 ± 1.4</td>
<td>15.5 ± 1.1</td>
</tr>
<tr>
<td>Permeate</td>
<td>25.8 ± 5.1</td>
<td>84.8 ± 1.4</td>
<td>84.5 ± 1.1</td>
</tr>
</tbody>
</table>

<sup>1</sup> Subtraction of TN recovered from each stage of permeate from feed TN  
<sup>2</sup> Subtraction of mass of TN recovered from stage 3 retentate from starting mass of TN in the separated feed  
<sup>3</sup> Cumulative recovery of protein after 3 stages of MF measured by SDS-PAGE of the permeates

Table 5. Mean ± standard deviation (n=3) relative percentage serum protein (SP) removed by ceramic microfiltration (MF) each stage of the 3-stage uniform trans membrane pressure ceramic MF system based on measurement by SDS-PAGE, Kjeldahl serum protein (SP), and Kjeldahl total nitrogen (TN).

<table>
<thead>
<tr>
<th>MF stage</th>
<th>Cumulative SP percentage removed measured by Kjeldahl TP in Permeate&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Cumulative TN percentage removed measured by Kjeldahl TN in Permeate&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Cumulative SP percentage removed (SDS-PAGE)&lt;sup&gt;3&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 1</td>
<td>52.8 ± 1.3</td>
<td>51.8 ± 1.5</td>
<td>52.2 ± 2.0</td>
</tr>
<tr>
<td>Stage 2</td>
<td>76.4 ± 0.7</td>
<td>75.5 ± 1.3</td>
<td>75.7 ± 1.6</td>
</tr>
<tr>
<td>Stage 3</td>
<td>78.7 ± 0.7</td>
<td>84.8 ± 1.4</td>
<td>85.0 ± 1.8</td>
</tr>
</tbody>
</table>

<sup>1</sup> Subtraction of SP recovered from each stage of permeate from feed SP  
<sup>2</sup> Subtraction of TN recovered from each stage of permeate from feed TN  
<sup>3</sup> Summation of each individual protein recovered in each stage in permeate as measured by SDS-PAGE
Table 6. Mean protein composition of whey feed, retentates, and permeates for ceramic MF of sweet whey determined using SDS-PAGE.

<table>
<thead>
<tr>
<th></th>
<th>LFR (%)</th>
<th>BSA (%)</th>
<th>IgG (%)</th>
<th>β-LG (%)</th>
<th>α-LA (%)</th>
<th>CN (%)</th>
<th>CNPP (%)</th>
<th>CN+CNPP (%)</th>
<th>WP (%)</th>
<th>TOTAL (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed Whey</td>
<td>0.96 d</td>
<td>3.23 c</td>
<td>bc</td>
<td>2.47</td>
<td>69.12</td>
<td>21.30</td>
<td>2.91</td>
<td></td>
<td>97.09 b</td>
<td>100.00</td>
</tr>
<tr>
<td>Stage 1 Retentate</td>
<td>1.99 c</td>
<td>3.55 c</td>
<td>ab</td>
<td>3.22</td>
<td>67.50</td>
<td>18.98</td>
<td>0.00 a</td>
<td>4.76 c</td>
<td>95.24 c</td>
<td>100.00</td>
</tr>
<tr>
<td>Stage 2 Retentate</td>
<td>3.52 b</td>
<td>5.02 b</td>
<td>3.60 a</td>
<td>63.44</td>
<td>16.76</td>
<td>0.00 a</td>
<td>7.67 b</td>
<td>92.33 d</td>
<td>100.00</td>
<td></td>
</tr>
<tr>
<td>Stage 3 Retentate</td>
<td>6.89 a</td>
<td>7.06 a</td>
<td>ab</td>
<td>58.25</td>
<td>14.02 f</td>
<td>0.00 a</td>
<td>10.36 a</td>
<td>89.64 e</td>
<td>100.00</td>
<td></td>
</tr>
<tr>
<td>Stage 1 Permeate</td>
<td>0.00 e</td>
<td>2.26 d</td>
<td>1.70 c</td>
<td>70.12</td>
<td>23.93</td>
<td>a</td>
<td>1.98 e</td>
<td>98.02 a</td>
<td>100.00</td>
<td></td>
</tr>
<tr>
<td>Stage 2 Permeate</td>
<td>0.00 e</td>
<td>2.46 d</td>
<td>1.91 c</td>
<td>69.84</td>
<td>22.64</td>
<td>ab</td>
<td>3.80 d</td>
<td>96.20 bc</td>
<td>100.00</td>
<td></td>
</tr>
<tr>
<td>Stage 3 Permeate</td>
<td>0.00 e</td>
<td>1.89 d</td>
<td>1.83 c</td>
<td>a</td>
<td>0.00 a</td>
<td>cd</td>
<td>3.80 cd</td>
<td>bc</td>
<td>100.00</td>
<td></td>
</tr>
<tr>
<td>Standard error</td>
<td>0.26</td>
<td>0.24</td>
<td>0.36</td>
<td>0.59</td>
<td>0.14</td>
<td>0.34</td>
<td>0.34</td>
<td>0.34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R²</td>
<td>0.98</td>
<td>0.96</td>
<td>0.68</td>
<td>0.96</td>
<td>0.97</td>
<td>0.91</td>
<td>0.97</td>
<td>0.97</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

IgG= Immunoglobulin G; BSA= Bovine Serum Albumin, LFR= Lactoferrin, β-LG= beta-lactoglobulin; α-LA= alpha-lactalbumin; CN=Casein=intact α + β casein; CNPP= Casein proteolysis products; WP= Whey Proteins

a, b, c, d Means not sharing a common superscript differ (P < 0.05)
Table 7. Mean relative percentage of β-LG and α-LA in feed, retentate, and permeate during ceramic microfiltration of whey and BLG to a LA ratio determined using SDS-PAGE.

<table>
<thead>
<tr>
<th></th>
<th>β–LG (%)</th>
<th>α–LA (%)</th>
<th>% of α–LA and β–LG of total protein</th>
<th>Ratio of β–LG to α–LA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed Whey</td>
<td>76.44 c</td>
<td>23.56 b</td>
<td>90.42 c</td>
<td>3.25 c</td>
</tr>
<tr>
<td>Stage 1 Retentate</td>
<td>78.06 b</td>
<td>21.94 c</td>
<td>86.48 d</td>
<td>3.56 b</td>
</tr>
<tr>
<td>Stage 2 Retentate</td>
<td>79.10 b</td>
<td>20.90 c</td>
<td>80.19 e</td>
<td>3.79 b</td>
</tr>
<tr>
<td>Stage 3 Retentate</td>
<td>80.61 a</td>
<td>19.39 d</td>
<td>72.27 f</td>
<td>4.17 a</td>
</tr>
<tr>
<td>Stage 1 Permeate</td>
<td>74.56 d</td>
<td>25.44 a</td>
<td>94.06 a</td>
<td>2.93 d</td>
</tr>
<tr>
<td>Stage 2 Permeate</td>
<td>75.12 cd</td>
<td>24.88 ab</td>
<td>92.25 b</td>
<td>3.02 cd</td>
</tr>
<tr>
<td>Stage 3 Permeate</td>
<td>75.52 cd</td>
<td>24.48 ab</td>
<td>92.47 b</td>
<td>3.09 cd</td>
</tr>
<tr>
<td>Standard error</td>
<td>0.67</td>
<td>0.67</td>
<td>0.68</td>
<td>0.14</td>
</tr>
<tr>
<td>R²</td>
<td>0.91</td>
<td>0.91</td>
<td>&gt;0.99</td>
<td>0.91</td>
</tr>
</tbody>
</table>

β–LG= Beta-lactoglobulin; α – LA= Alpha-lactalbumin

Means not sharing a common superscript differ (P < 0.05)
**Table 8.** Comparison of mean serum protein removal from Cheddar sweet whey compared to removal of serum proteins from skim milk using ceramic and spiral wound polymeric membranes.

<table>
<thead>
<tr>
<th>MF stage</th>
<th>Skim milk spiral wound MF&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Skim milk ceramic MF&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Sweet whey ceramic MF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 1</td>
<td>38.6</td>
<td>64.8</td>
<td>52.8 ± 1.3</td>
</tr>
<tr>
<td>Stage 2</td>
<td>20.8</td>
<td>22.9</td>
<td>23.7 ± 0.6</td>
</tr>
<tr>
<td>Stage 3</td>
<td>10.9</td>
<td>10.5</td>
<td>2.3 ± 0.03</td>
</tr>
<tr>
<td>Cumulative</td>
<td>70.3</td>
<td>98.3</td>
<td>78.7 ± 0.7</td>
</tr>
</tbody>
</table>

<sup>1</sup>Beckman et al., 2010  
<sup>2</sup>Hurt et al., 2010

**Figure 1.** Separated Cheddar whey, stage 1 microfiltration retentate and stage 1 microfiltration permeate from ceramic uniform transmembrane pressure microfiltration of separated Cheddar cheese whey.
**Figure 2.** Image of a typical SDS-PAGE gel of microfiltration retentates and permeates from separated whey. IgG = immunoglobulins; BSA = bovine serum albumin; LFR = lactoferrin; CNPP = casein proteolysis products; β LG = β-lactoglobulin; α LA = α-lactalbumin. Slot 1= Milk reference, 2-4 MF Feed Whey, 5-7 stage 1 retentate, 8-10 stage 1 permeate, 11-13 stage 2 retentate, 14-16 stage 3 retentate, 17 Milk, 18-20 stage 2 permeate, and 21-23 stage 3 permeate. The three slots within each series of 3 adjacent samples of the same type represent replicates 1, 2, and 3, respectively.
CHAPTER 5: Efficiency of Removal of Whey Protein from Sweet Whey Using Polymeric Membranes
Efficiency of Removal of Whey Protein from Sweet whey Using Polymeric Membranes.

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Abstract

Our objective was to measure whey protein removal percentage from separated sweet whey using spiral wound (SW) polymeric microfiltration (MF) membranes using a 3-stage, 3x process at 50°C and compare the performance of polymeric membranes with ceramic membranes. Pasteurized, separated Cheddar cheese whey (1080 kg) was microfiltered using a polymeric 0.3 μm polyvinylidene (PVDF) fluoride spiral-wound membrane and a 3X, 3-stage MF process. Cheese making and whey processing were replicated 3 times. There was no detectable level of lactoferrin (LFR) and no intact αs or β-casein detected in the MF permeate from the 0.3 μm spiral wound PVDF membranes used in this study. Bovine serum albumen (BSA) and immunoglobulin G (IgG) were found in both the retentate and permeate. β-lactoglobulin (β–LG) and α-lactalbumin (α–LA) partitioned between retentate and permeate but β–LG passage through the membrane was retarded more than α–LA because the ratio of β–LG to α–LA was higher in the MF retentate than either the sweet whey feed or the MF permeate. About 69% of the crude protein present in the pasteurized separated sweet whey was removed using a 3X, 3-stage, 0.3 μm spiral wound PVDF MF processes at 50°C compared to 0.1 μm ceramic graded permeability MF that removed about 85% of crude protein from sweet whey. The polymeric SW membranes used in this study will achieve approximately 20% lower yield of whey protein isolate (WPI) and a 50% higher yield of whey protein phospholipid concentrate (WPPC) under the same MF processing conditions as ceramic MF membranes used in the comparison study. Total gross revenue from the sale of WPI plus WPPC produced with polymeric versus ceramic membranes will be influenced by both the absolute market price for each product and the ratio of market price of these two products. The combination of the ratio of the market price of WPPC to WPI and the influence of difference in yield of WPPC and WPI between polymeric and ceramic membranes yield a price ratio of 0.556 as the cross over point for which membrane type achieves higher total gross revenue. A complete economic engineering study
comparison of the WPI and WPPC manufacturing costs for polymeric versus ceramic MF membranes is needed to determine the impact of membrane material selection on long term processing costs, which will impact net revenue and profit when the same quantity of sweet whey is processed under various market price conditions.

**Key Words:** polymeric microfiltration, serum proteins, sweet whey
Introduction

Sweet whey is a coproduct of cheese making for cheese varieties that are made with a rennet (e.g., Cheddar and Mozzarella). After rennet coagulation of milk and cutting the curd, the whey is drained from the cheese curd, clarified, centrifugally separated, and pasteurized. Clarification removes small particulate curd fines from the whey. Centrifugal separation removes fat from the whey. Residual fat in whey products can have negative impacts on flavor (Evans et al., 2010), therefore reducing the fat content of whey as much as possible prior to filtration is desirable. The whey pasteurization step is required to thermally inactivate the starter culture to prevent continued lactic acid production and also to inactivate the proteolytic activity of residual rennet that may remain in the whey, allowing the whey to be subsequently fractionated into a whey protein concentrate (WPC) and ultrafiltration (UF) permeate. Sweet whey composition prior to UF has approximately 6.2% solids, 4.77% lactose, 0.82% protein, 0.07% fat (0.2% fat before separation), and 0.53% ash (Morr and Ha, 1993). WPC80 typically contains at least 80% protein and about 4 to 8% fat on a dry matter basis (ADPI, 2015a).

Whey protein isolate (WPI) contains > 90% protein on a dry matter basis. To achieve this level of protein purity, an additional defatting step, either by microfiltration (MF) or anion-exchange, is necessary (Morr and Ha, 1993; Daufin and Merin, 1990). In the US dairy industry, spiral wound polymeric membranes are typically used for MF because of their lower capital cost and lower operation energy than ceramic MF membranes. However, polymeric membranes have a relatively low chemical stability and have a short life (Jost and Jelen, 1997; Cheryan, 1998) compared to ceramic membranes. Filtration membranes are typically selected for a particular application based on a mean pore size, which in theory, separates solutes in a liquid feed based on their difference in size. The typical pore size of MF membranes for separation of casein from milk...
derived whey proteins (0.1 to 0.2 µ) would be expected to allow whey proteins to pass through the MF membrane into permeate and keep casein micelles in the retentate.

Any protein rejection by the membrane leads to lower yields of WPI produced from MF permeate and creates a co-product (MF retentate) that is called whey protein phospholipid concentrate (WPPC). WPI should contain a minimum of 89.5% protein and a maximum of 1.5% fat on dry basis, and WPPC should contain a minimum protein on a dry basis of 50% and a minimum fat of 12% on a dry basis (ADPI, 2015b,c). WPI typically sells for a higher price than WPPC, and manufacturers lose potential profit with every kilogram of protein that does not pass through the MF membrane. Therefore, there is a need to further investigate how to improve protein removal from sweet whey by MF.

Spiral wound polymeric membranes are operated at much lower cross flow velocity (Zulewska et al., 2009) than ceramic membranes (i.e., 0.5 to 1.5 m/s versus 6 to 7 m/s), resulting in formation of a concentration polarization foulant layer at the surface of polymeric membranes. Ceramic membranes can be operated at much higher cross flow velocity because the ceramic membrane can physically withstand much higher surface shear forces than polymeric membranes. As permeate passes through a filtration membrane, rejected solutes accumulate (i.e., the concentration polarization layer) at the membrane surface, and a high cross flow velocity and turbulent flow are needed to sweep away this layer. If the concentration of rejected solute exceeds its solubility, solute may precipitate on the membrane surface creating a barrier to flow of solutes and solvent through the membrane. Once the concentration polarization layer forms on the membrane surface, then this layer will influence the passage of both solvent and solutes through the combination of the membrane plus the surface foulant layer.
The foulant layer may influence the selectivity and flux of a polymeric membrane both for UF and MF of milk or whey. During UF of milk using polyether sulfone membranes, immediate adsorption fouling of the membrane with α-LA increases hydrolytic resistance and decreases water flux before any concentration polarization takes place during UF (Tong et al., 1988). When starting the processing of skim milk on a pilot scale polymeric system versus a ceramic MF system, there are differences in the appearance of the MF permeate during the first minute of the start-up process. For a spiral polymeric system being started up to process skim milk, the permeate for the first 30 s to 1 minute of startup looks like skim milk and then the MF permeate becomes a clear light green liquid. For ceramic system starting up the same way, the permeate is a clear light green liquid from the beginning. We think that during the first minute of running on the spiral polymeric the solute adsorption and concentration polarization layer is rapidly occurring and then that layer’s solute rejection characteristics predominate and casein micelles do not pass through the polymeric membrane + foulant layer. In a ceramic membrane, the high cross flow velocity of the feed prevents this layer from forming and therefore the pores of the ceramic membrane differentiate solute passage based size.

Recently, Carter et al. (2020) has reported results for MF of sweet whey using a 0.1 µm ceramic membrane system and compared the efficiency of removal of whey proteins from sweet whey (84.8% on a total nitrogen basis) to the efficiency of removal of milk derived whey protein from skim milk with the same ceramic membranes (98.3% (Hurt et al., 2010)) Carter et al. (2020) also reported that the percent removal of milk derived whey protein from skim milk was lower when using spiral wound MF compared to when using ceramic MF under the same 3-stage, 3X MF process at 50°C [70.3% (Beckman et al., 2010) versus 98.3% (Hurt et al., 2010)].
To our knowledge, a comparison of ceramic and polymeric spiral wound microfiltration of sweet whey has not been investigated in the context of similar separation mechanisms like microfiltration of skim milk. Our objective was to measure whey protein removal percentage from separated sweet whey using spiral wound polymeric microfiltration membranes using a 3-stage, 3x process at 50°C and compare the performance of polymeric membranes with ceramic membranes.

**Materials and Methods**

**Liquid Sweet Whey Manufacture**

Raw whole milk, 1200 kg, was obtained from the North Carolina State University Dairy Research and Education System. Milk was high temperature short time (HTST) pasteurized (720 kg/h) with a plate heat exchanger (model T4 RGS-16/2, SPX Flow Technology, Greensboro, NC) at 72°C with a hold time of 16 s. The milk was then cooled to 31°C and transferred to a cheese vat (Model TH0041, Kusel Equipment, Watertown, Wis., U.S.A.). Mesophilic starter culture containing *Lactococcus lactis ssp. lactis* (CHR Hansen R-604, Milwaukee, Wis., U.S.A.) was added at the rate of 50 Danisco Culture Units (DCU)/454 kg of milk. A calcium chloride solution (32 to 33% w/v, Dairy Connection Inc., Madison, Wis., U.S.A.) was also added at the rate of 0.39 mL/kg of milk. The milk was allowed to ripen for 60 min. Next, the milk was coagulated with double strength microbial rennet (DCI Supreme - *Mucor mehei* microbial rennet, Dairy Connection Inc., Madison, Wis., U.S.A.) for 30 min at a rate of 0.09 mL/kg of milk diluted 80-fold with deionized (DI) water. The coagulum was then cut and the curd was allowed to rest for 5 min. The curd was stirred for 30 min while the temperature was increased gradually to 39°C. The pH was monitored with a pH meter (Orion Model 250+, Thermo Scientific, Waltham, MA) and probe (VWR Model 89231-572, VWR, Radnor, PA), and the whey was drained through a sieve to remove cheese fines once a pH of 6.35 was achieved. Drained whey was HTST pasteurized (750 kg/h) at 72°C for 15 s and fat separated.
using an inline hot bowl centrifugal separator (Model J5-OSCP-1, JSC PLAVA, Savery, Orlando, FL, U.S.A.) at 65°C. Separated whey was cooled to 4°C and stored for 24 h before filtration.

Pasteurized separated whey (1080 kg) was heated to 50°C with a plate heat exchanger and microfiltered using a pilot-scale polymeric microfiltration unit in bleed-and-feed mode at 50°C to continuously produce a 3X MF retentate and MF permeate (2 kg of permeate for every 1 kg of retentate). After the first stage, the MF retentate was diluted back to a 1× concentration (2 kg of water for every 1 kg of retentate) with DI water, heated to 50°C, and diafiltered with the MF system. This diafiltration procedure was repeated to complete a 3-stage process. All of the retentate and permeate from each stage was collected separately, weighed, mixed, and sampled for mass balance calculations of whey protein removal for each stage of the process.

**Microfiltration Operation**

The operation of the filtration unit followed the method described by Beckman et al. (2010) with slight modifications. Briefly, a polymeric polyvinylidene fluoride (PVDF) spiral-wound membrane (model FG7838-OS0x-S, 0.3 μm PVDF, Parker-Hannifin, Process Advanced Filtration Division, Tell City, IN) with a nominal pore size of about 0.3 μm and surface area of 20.5 m² was used. Membrane diameter was 198 mm, spacer thickness was 1.09 mm, and length was 96.5 cm. The membrane was placed in a stainless-steel housing (length 1.3 m) and mounted horizontally in the membrane system. The spiral wound (SW) MF system had a feed pump (model 0, Cherry Burrell, Little Falls, NY) and retentate recirculation pump (model GH30, G&H Products Corp., Kenosha, WI). Digital pressure gauges (model PI2094, IFM Efector Inc., Exton, PA) were used to monitor the retentate pressure outlet (Rpo) and retentate pressure inlet (Rpi). Permeate pressure outlet (Ppo) was assumed to be 0 kPa because the permeate exit tube was open to the atmosphere. Retentate bleed
flow and recirculation flow (L/min) were measured with magnetic volumetric flow meters (model AE202MH and model AM204DH, respectively; Yokogawa Electronic Corp., Tokyo, Japan).

Cleaning Before Processing. The soak solution (0.26% solution of Ultrasil MP; Ecolab Inc., Food and Beverage Division, St Paul, MN) was drained from the system, and the system was flushed with DI water until the water exiting the system was at neutral pH. The Rpi and Rpo were set by adjusting the speed of the retentate recirculation pump with the retentate and permeate outlets fully open. The membrane was cleaned as described by Beckman et al. (2010). The clean water flux (typically about 33 kg/m² per hour) was calculated based on the weight of permeate collected in 30 s and the total membrane surface area.

First Stage. Sweet whey (approximately 1,080 kg) was filtered through the SW MF unit at 50°C, and the permeate and retentate exiting the SW MF unit were collected during the run and weighed (kg) into sanitized milk cans. The SW MF system was started as follows: the feed pump was turned on with the permeate exit valve closed. When whey or diluted retentate started exiting the retentate outlet, the retentate recirculation loop shut-off valve was opened to allow milk to fill the recirculation loop. Then, the recirculation pump was turned on and the permeate exit valve was opened slowly to balance the pressures and flows of retentate and permeate to achieve a ΔP <100 kPa. Throughout the run, the retentate removal rate was adjusted to maintain a 3× concentration factor (CF). The CF [(permeate (kg) + retentate (kg))/ retentate (kg)] was monitored every 10 min by collecting permeate and retentate for 30 s into buckets and weighing each. Flux (kg/m² per h) was determined every 10 min using the weight of permeate collected in 30 s and the surface area of the membrane. Samples of permeate and retentate were collected every 10 min for analysis by infrared spectrophotometer (Lactoscope FTIR, Delta Instruments, Drachten, the Netherlands) to monitor their composition. At the end of the MF run for each stage, all retentate and all permeate from the
processing run was weighed to the nearest 0.01 kg (model ICS439 scale, Mettler Toledo, Columbus, OH), mixed, and sampled. A final CF was calculated at the end of the run using the final weights of permeate and retentate. After weighing and sampling, the collected permeate was discarded. The retentate was cooled and stored (4°C) in a tank overnight for the next stages of diafiltration. The small amount of unprocessed whey remaining in the feed vat at the end of the run was weighed and subtracted from the starting material weight. The pH of the retentates was measured with an electrode (model 250 A+, Orion, Waltham, MA) that was standardized at pH 4.06 and 6.97 at 50°C (pH 4.00 and 7.00 standard buffer solutions, Fisher Scientific, Fair Lawn, NJ). The membrane system was cleaned at the end of the first day of processing, so the stage 2 and 3 run on day 2 of processing started with a clean membrane.

**Second Stage and Third Stage.** On day 2 of filtration whey was weighed and diluted (1 part retentate and 2 parts DI water) back to the original weight of skim milk from stage 1 (about 1,150 kg) with heated DI water to achieve a temperature of 50°C of feed. The membrane was cleaned as mentioned previously. Final stage 2 retentate and permeate samples were collected, cooled, mixed, and sampled as described above. The third stage of the 3-stage process was as described for the second stage. Stage 2 retentate was diluted with DI water as described above and was filtered as stage 3. The complete batch of stage 3 retentate and permeate were weighed, mixed and sampled as described above.

**Cleaning After Processing.** After processing, the SW MF system was cleaned (long clean) as follows: first, the MF system was flushed with 150 L of 50°C DI water at 158 kPa Rpi and 68 kPa Rpo, with no back pressure on the permeate side. During a second rinse using 150 L of 25°C DI water, the recirculation pump was turned off and the inlet pressure was adjusted to 34.5 kPa by adjusting the retentate outlet valve. The fouled water flux (kg/m² per hour) was calculated based on
the weight of permeate collected in 30s. After determination of the fouled water flux, the membrane was cleaned as described by Beckman et al. (2010).

**Chemical Analyses**

Milk, feed whey, permeate, and retentate composition were measured as described by Carter et al. (2020). Briefly, samples collected during processing were analyzed using a mid-infrared spectrophotometer (Lactoscope FTIR, Delta Instruments) for fat, lactose, and TP content calibrated as described by Kaylegian et al. (2006) to quickly monitor the composition of retentate and permeate during the run to detect if the system was running as expected. Feed whey, retentate, and permeate for each stage were analyzed for TS, total N (TN), and NPN content using forced-air oven drying (AOAC, 2000; method 990.20; 33.2.44), Kjeldahl (AOAC, 2000; method 991.20; 33.2.11), and Kjeldahl (AOAC, 2000; method 991.21; 33.2.12), respectively. The noncasein nitrogen (NCN) content of retentates was determined using Kjeldahl (AOAC, 2000; method 998.05; 33.2.64). Analysis of feed, retentate and permeate were measured for relative protein percentages by SDS-PAGE and protein removal was calculated as described by Carter et al. (2020).

**Statistical Analysis**

Data were analyzed using XLSTAT (version 2013.5.03; Addinsoft, New York, N.Y., U.S.A.). Analysis of variance was conducted to compare treatment means using the Tukey’s honestly significant difference test at a significance level of $P < 0.05$.

**Results**

**Processing**

*Operational Parameters.* The concentration factor was maintained close to the target concentration factor by adjusting retentate bleed rate and retentate recirculation rate to maintain 3x
CF in all 3 stages. A ΔP of 115 kPa was maintained in all 3 stages of filtration (Table 1). Flux for the second and third stages was slightly higher than flux for the first stage because the membrane system was cleaned after the first stage run and foulant material accumulated on the membrane surface during stage 1 would not be present in the whey feed for stage 2 and 3 on the second day of processing within each of the 3 replicates.

Composition of Whey, MF retentates and MF Permeates

**Whey and MF retentates.** The percentage of fat in the separated whey was low and typical of good removal of fat from Cheddar cheese whey (Table 2). The percentage of casein remaining in the whey was due to glycomacropeptide (GMP) from rennet action on kappa casein and other casein proteolysis products. Milk fat was concentrated in the retentate as expected. Lactose and total solids concentrations in the MF retentate decreased ($P < 0.05$) as expected with successive stages of diafiltration. Crude protein on a dry basis (CPDB) and fat on dry basis (FDB) both increased with increasing stage number as lactose and soluble minerals were removed in MF permeate. The goal of the MF process for whey is to remove protein from the retentate and retain fat.

**MF permeates.** Fat concentration in the permeate was very low and uniform, indicating that very little milk fat was present in the polymeric MF permeate (Table 3). The polymeric MF membranes were very effective at blocking the passage of fat through the membrane. Lactose concentration in permeate decreased ($P < 0.05$) with each stage as expected. Crude protein (CP), NPN, TP, NCN, SP concentration in the permeate decreased with each stage of filtration as the permeate became more dilute. The casein content of the permeate was very low and there was no difference detected ($P > 0.05$) in SP%TP among permeate from different stages. The CPDB in the permeate increased ($P < 0.05$) progressively from stage to stage as lactose and soluble minerals were
removed from the separated whey feed. The FDB was low and no difference ($P > 0.05$) among permeates of different stages was detected.

**Serum Protein Removal from Cheddar Cheese Whey.**

Nearly 90% of the fat from the original separated whey did not pass through the MF membrane into the MF permeate, as desired (Table 4). The portion of fat that was recovered in the permeate may not be lipid, but was ether soluble material that was not further characterized. About 69% of the crude protein present in the separated whey was removed in the MF permeate (Table 4). The calculated cumulative removal of whey protein was underestimated by Kjeldahl true protein measured in MF permeate because a portion of what was counted as NPN in MF permeate was actually true protein from the GMP (Table 5). The NPN concentration in separated whey is typically higher than in the milk used for cheese making because at least a portion of the GMP from $\kappa$-casein is soluble in 12% trichloroacetic acid in the Kjeldahl sample preparation for measurement of NPN. When calculation of protein removal is done on a TN basis, the contribution of 12% trichloroacetic acid soluble GMP is accounted for in the recovery calculation.

There was no detectable level of lactoferrin (LFR) passage into the permeate and no intact $\alpha_s$ or $\beta$-casein (Figure 1) in the separated whey, retentates, or permeates (Table 6). There were casein proteolysis products present in the whey but they did not pass though the membrane into the permeate. Bovine serum albumin (BSA) and immunoglobulin G (IgG) were partially retained in the retentate. Both $\beta$–LG and $\alpha$–LA passed into the permeate resulting in partial removal from the retentate. The $\beta$–LG to $\alpha$–LA ratio was higher in the stage 2 and 3 retentate ($P < 0.05$) indicating that a lower proportion of $\beta$–LG was passing through the membrane than $\alpha$–LA (Table 7). The CNPP were higher ($P < 0.05$) in the stage 3 retentate than in the feed or permeate indicating that they do not pass through the polymeric membrane and CNPP were not detected in the MF permeate.
(Table 6). The relative percentage of β–LG (Table 7) and the ratio of β–LG to α–LA (Table 6) were higher ($P < 0.05$) in the stage 2 and 3 retentates than the feed whey, indicating that β–LG was partially rejected by the MF polymeric membrane. The β–LG plus α–LA was a higher ($P < 0.05$) percentage of total protein in the permeate than in the retentate (Table 7) because more casein proteolysis products in the whey were retained than passed through membrane into the permeate.

**Impact of Ceramic and Polymeric MF on Yield of WPPC and WPI**

The WPPC MF retentates from either ceramic or polymeric MF would be further concentrated by UF and diafiltration to bring them to a minimum protein of 50% on a dry matter basis and minimum fat of 12% on a dry matter basis by removal of other solids. The yield of WPPC from a ceramic MF process would be about half that of a polymeric MF process. Ceramic MF membranes will achieve approximately 20% higher yield of WPI under the same microfiltration process conditions as polymeric SW membranes. The magnitude of the difference in total revenue will be a function of the market prices of WPI and WPPC.

**Comparison of Serum Protein Removal: Skim Milk Versus Whey**

Microfiltration using ceramic membranes removed more milk derived whey protein from skim milk [98.3 (Hurt et al., 2010) versus 70.3% (Beckman et al., 2010)] and sweet whey [84.8% (Carter et al., 2020) versus 68.8% (Table 5)] than polymeric membranes when processed with the same amount of diafiltration at the same processing temperature. The difference is protein passage between ceramic and polymeric membrane systems may be related to the very large difference in mean cross flow velocity (i.e., 6 to 7 versus 0.5 to 1.5 m/s, respectively). Lower cross flow velocity in polymeric membrane systems makes the concentration polarization driven membrane fouling more important than in ceramic membrane systems and deposits a feed material dependent layer of
rejected feed solutes on the surface of the membrane, which can change the solute rejection characteristics of the filtration system.

DISCUSSION

Polymeric Microfiltration

*Feed Material Type: Influence on Processing Parameters, Flux, and Fouling.* Numerous studies have been conducted by our research group on microfiltration of milk investigating different styles of membranes [Zulewska et al., 2009 (uniform transmembrane pressure (UTP), graded permeability (GP), SW); Hurt et al., 2010 (UTP); Hurt and Barbano, 2010 (milk preconcentration); Adams and Barbano, 2013 (Isoflux)], channel geometry (Adams et al., 2015a), channel diameter (Hurt et al., 2015b), processing temperatures (Hurt et al., 2015a), linear velocity (Zulewska and Barbano, 2014), and the effect of lactose and calcium on limiting flux (Adams et al., 2015b) and whey (Carter et al., 2020) where data has been collected using the same pilot scale membrane filtration units using the same analytical equipment and methods. This offers an opportunity to look at results across several research projects where research conditions and data collection are very similar. Mean stage 1 MF flux on skim milk for ceramic membranes was 4 to 5 times higher (Zulewska et al. 2009: 72 kg/m² per hour) than spiral wound polymeric MF membranes [Table 10:(16 kg/m² per hour)]. Mercier-Bouchard et al. (2017) report similar values for 0.1 μm polymeric membranes (22.9 kg/m² per hour) and 0.1 μm ceramic membranes (90 kg/m² per hour) in a 3X process at 50°C. Zulewska and Barbano (2013) microfiltered skim milk with ceramic membranes, and then used the permeate from the ceramic as feed material for a spiral wound polymeric membrane. For spiral wound polymeric membranes, flux was more feed material [i.e., skim milk, casein free skim milk (CFSM), and whey] dependent than for ceramic membranes. Whey in the current study had higher flux (Table 10; 52 kg/m² per h) than skim milk (Table 10: 16 kg/m² per h).
on polymeric MF membranes. In a study by Zulewska et al. (2013) ceramic MF was used to produce stage 1 MF permeate, which is skim milk with casein removed (i.e., CFSM). The CFSM was then used as the feed material for a polymeric MF system (Zulewska et al., 2013). Removal of the major foulant material (i.e., casein) from a skim milk feed used for the polymeric membrane system increased flux on polymeric spiral wound membranes 5-fold (i.e., about 64 kg/m² per h). If casein were the only foulant material then it would be expected that sweet whey would have the same flux as CFSM, however there appeared to be other foulants present in whey that caused lower flux (52.6 versus 80.19 kg/m² per h) (Table 10). After processing skim milk with a polymeric MF membrane, the fouled membrane has lost 60 to 80% of its water permeability, and after processing whey, about 40% of water permeability was lost (Table 10). After processing skim milk with a ceramic MF membrane, about 50% of the water permeability was lost, while when processing whey using ceramic membranes, the water permeability loss was only about 15%.

**Feed Material Type: Influence on Passage of β-LG and α-LA into Permeate.** The concentration of true protein in stage 1 permeate was very consistent across studies (Table 11) within membrane material type (i.e., ceramic versus polymeric). Because of the difference in concentration of protein in the stage 1 permeate between polymeric and ceramic membranes at a 3X concentration factor at 50°C, as shown in Table 11, the amount of true protein that passed from the feed (skim milk or whey) into permeate was much higher for ceramic than polymeric membranes (3.8 kg true protein versus 2.4 kg/1000 kg skim milk processed). We propose that the difference in protein passage on ceramic versus polymeric membranes was due to a difference in membrane fouling (Table 10) and the change in solute rejection characteristics of the combination of membrane plus foulant in the case of polymeric membranes. When casein was removed from skim milk prior to polymeric membrane microfiltration of skim milk (Zulewska et al., 2013), the flux was increased 5
fold and the concentration of true protein in the polymeric MF permeate was increased greatly from 0.36 to 0.52%, demonstrating that the foulant restricted the passage of milk derived whey proteins through the polymeric membranes and that type of fouling does not occur to any great extent with ceramic membranes, because of the much higher cross flow velocity, when processing skim milk at 50°C.

Ceramic MF membranes allowed more passage of true protein into permeate for both skim and whey (Table 11). It is likely that passage of both β-LG and α-LA into permeate were lower for polymeric than for ceramic membranes. The lower ratio of β-LG to α-LA in the MF stage 1 permeate for polymeric membranes versus ceramic membranes indicates that the relative passage of β-LG into permeate was less than for α-LA when processing skim milk and a similar difference in ratio β-LG to α-LA was observed when the feed for the polymeric MF was sweet whey instead of skim (Table 11). It appears that the foulant at the surface of a polymeric membrane changes the solute rejection characteristics of polymeric membrane for both skim milk and sweet whey. It is interesting how consistent this is with type of feed material in the same membrane processing system (Tables 10, 11) based on the comparison of results from three different processing studies on skim milk (Beckman et al., 2010, Zulewska et al., (2009, 2013)).

The difference in passage of β-LG between ceramic and polymeric on sweet whey was about the same as for skim milk, so again there is something in the whey that changes the rejection characteristics of the polymeric membrane. The fact that β-LG passage into permeate was lower for ceramic on whey than ceramic on skim milk may be due to the double heat treatment (milk and whey pasteurization) that happens during whey processing for both ceramic and polymeric systems. The lower β-LG passage into permeate for sweet whey than skim milk with polymeric membrane
reflects the combined impact of both the foulant plus the thermal denaturation of β-LG when compared with skim milk.

**Comparison of Processing Costs of Polymeric versus Ceramic Membranes.** An economic engineering study of whey product manufacturing costs of whey powder and WPC was published by Hurst et al. (1990), using similar economic engineering methodology to determine Cheddar cheese manufacturing costs (Mesa-Dishington et al., 1987a,b). The same economic engineering approach to estimate manufacturing costs was also applied to the use of RO and UF membrane filtration prior to cheese manufacture (Aplin et al., 1992a,b). Hurt and Barbano (2015) demonstrated how preconcentration of skim milk by UF prior to MF system for separation of casein and serum protein can reduce the ceramic membrane surface area required by as much as 40% and reduce capital costs. A similar economic engineering study of long term processing costs is needed to compare the manufacturing costs of WPPC and WPI using ceramic versus polymeric membranes, considering the sensitivity of product manufacturing costs to factors such as, production scale, operating schedule, capital costs (including depreciation and interest), utilities (electrical, fuel, and waste treatment), materials (production, packaging, membrane replacement, and cleaning), laboratory (process control analytical), property tax and insurance and other expenses.

In the current study, polymeric membranes were operated at a processing temperature of 50°C for comparison to ceramic membranes operated at the same temperature. However, it is common in the US dairy industry to operate polymeric UF and MF membranes for processing skim milk and whey at temperatures between 7 to 10°C to reduce microbial growth during processing. Some processors with excellent sanitation and cleaning practices with polymeric UF membranes are able to operate at 50°C. Because of the stronger cleaning chemicals and higher temperatures that can be used for ceramic membranes, it is easier to maintain low microbial growth with ceramic
membranes during 12 to 18 h processing runs at 50°C than with polymeric membranes. The impact of running polymeric membranes at 10 versus 50°C on membrane flux and fouling is large. Méthot-Hains et al. (2016) reported that UF polyethersulfone (PES) membranes had higher flux and lower energy input per unit of milk processed when operated at 50°C compared to at 10°C for processing skim milk due to lower pumping energy required at 50 vs 10°C. Gavazzi-April et al. (2018) determined the effect of two different diafiltration approaches for UF of skim milk and found that while continuous diafiltration at higher CF reduced the amount of water consumption for diafiltration, it also increased the amount of membrane surface area by 1.57 fold to process the same amount of milk in one day. A larger surface area increases operation, cleaning and membrane replacement cost. If the effect of low operating temperature and continuous diafiltration are combined, the amount of polymeric surface area required to process the same amount of milk per day would be expected to increase greatly. This relationship would be expected to be the same for both UF and MF using polymeric membranes. These factors need to be considered in an economic comparison of polymeric versus ceramic MF membranes to determine the impact of membrane materials on long term processing costs, which will impact net revenue and profit when the same quantity of WPI and WPPC are produced under constant market price conditions.

Influence of Difference in Crude Protein Removal on Yield and Gross Revenue from WPI plus WPPC. The impact of use of ceramic versus polymeric membranes on yield of WPI and WPPC per day is shown in Table 12. The yield per day of WPI was higher and WPPC was lower using ceramic membranes than polymeric membranes. Generally, the market price of WPI and other protein based dairy products move up and down together as the market price for protein varies with time (USDEC, 2020). While market price data was not available specifically for WPPC in the published public domain, based on discussion with industry representatives it is expected that the
ratio of the price of WPPC to WPI will be about 0.55. The effect of the ratio of the prices of WPPC to WPI has an important impact on total revenue from the sum of WPI and WPPC produced per day in factory. The gross revenue for a factory using spiral versus ceramic membranes was determined by using data from Carter et al. (2020) for ceramic membranes and using data produced in the current study for polymeric membranes, as well as pricing information collect from industry partners and USDEC (2020). All comparisons were made based on the price of protein per kilogram and the default protein content on a dry matter basis for WPPC and WPI. A range of ratios of WPPC to WPI prices (from about 0.3 to 0.79), were used to determine the impact of the ceramic versus polymeric MF of whey on gross revenue from WPPC plus WPI (Table 12). At price ratios > 0.556 the value of protein per kilogram is higher in the WPPC than WPI (Table 12). At price ratios > 0.556 the total gross revenue per day from WPI plus WPPC is higher with spiral membranes than with ceramic membranes, and at price ratio < 0.556 total gross revenue is higher for ceramic membranes. It is likely that the manufacturing cost per kilogram of these products is different with ceramic versus polymeric membranes. The results in Table 12 are gross revenues and to conduct a complete economic analysis, the product manufacturing costs using polymeric versus ceramic membranes should be determined in an economic engineering study.

Conclusions

There was no detectable level of LFR and no intact αs or β-casein detected in the MF permeate from the 0.3 μm spiral wound PVDF membranes used in this study. BSA and IgG were found in both the retentate and permeate. β–LG and α–LA partitioned between retentate and permeate but β–LG passage through the membrane was retarded more than α–LA because the ratio of β–LG to α–LA was higher in the MF retentate than either the sweet whey feed or the MF permeate. About 69% of the crude protein present in the pasteurized separated sweet whey was
removed using a 3X, 3-stage, 0.3 μm spiral wound PVDF MF process at 50°C compared to 0.1 μm ceramic graded permeability MF that removed about 85% of crude protein from sweet whey. The polymeric SW membranes used in this study will achieve approximately 20% lower yield of WPI and a 50% higher yield of WPPC under the same MF processing conditions as ceramic MF membranes used in the comparison study. Total gross revenue from the sale of WPI plus WPPC produced with polymeric versus ceramic membranes will be influenced by both the absolute market price for each product and the ratio of market price of these two products. The combination of the ratio of the market price of WPPC to WPI and the influence of difference in yield of WPPC and WPI between polymeric and ceramic membranes yield a price ratio of 0.556 as the cross over point for which membrane type achieved higher total gross revenue. A complete economic engineering study comparison of the WPI and WPPC manufacturing costs for polymeric versus ceramic MF membranes is needed to determine the impact of membrane material selection on long term processing costs, which will impact net revenue and profit when the same quantity of sweet whey is processed under various market price conditions.

**Acknowledgements**

Funding provided in part by the National Dairy Council (Rosemont, IL). The authors thank Chassidy Coon, Michelle Bilotta, and Sara Hastings for the technical assistance in chemical analyses.
References


Tables and Figures

Table 1. Mean ± standard deviation (n=3) microfiltration (MF) retentate pressure at the membrane inlet (Rpi) and outlet (Rpo), delta P (ΔP), flux, and concentration factors for each stage of the 3-stage 3x polymeric microfiltration run of approximately 60 minutes.

<table>
<thead>
<tr>
<th>MF stage</th>
<th>Rpi (kPa)</th>
<th>Rpo (kPa)</th>
<th>ΔP</th>
<th>Flux (kg/m² h)</th>
<th>Concentration factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 1</td>
<td>149.2 ± 0.8</td>
<td>87.2 ± 0.5</td>
<td>118.2 ± 0.2</td>
<td>56.2 ± 4.4</td>
<td>2.89 ± 0.12</td>
</tr>
<tr>
<td>Stage 2</td>
<td>146.9 ± 2.3</td>
<td>86.7 ± 1.2</td>
<td>116.8 ± 1.7</td>
<td>68.7 ± 1.7</td>
<td>3.02 ± 0.04</td>
</tr>
<tr>
<td>Stage 3</td>
<td>145.7 ± 2.4</td>
<td>84.9 ± 2.1</td>
<td>115.3 ± 2.2</td>
<td>66.2 ± 1.8</td>
<td>2.98 ± 0.02</td>
</tr>
</tbody>
</table>

ΔP = [(Rpi – Rpo)/2] + Rpo
Table 2. Mean (n = 3) composition (%w/w) of separated whey and polymeric microfiltration (MF) and diafiltration (DF) retentates for stages 1, 2, and 3 produced from separated Cheddar cheese whey.

<table>
<thead>
<tr>
<th></th>
<th>Fat</th>
<th>Lactose</th>
<th>Total solids</th>
<th>CP</th>
<th>NPN</th>
<th>TP</th>
<th>NCN</th>
<th>Casein</th>
<th>SP</th>
<th>CN%TP</th>
<th>SP%TP</th>
<th>CPDB</th>
<th>FDB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Separated whey</td>
<td>0.036 c</td>
<td>4.937 a</td>
<td>6.651 b</td>
<td>0.910 b</td>
<td>0.231 b</td>
<td>0.679 c</td>
<td>0.896 b</td>
<td>0.013 a</td>
<td>0.665 c</td>
<td>1.990 a</td>
<td>98.010 a</td>
<td>13.681 d</td>
<td>0.544 d</td>
</tr>
<tr>
<td>Stage 1 - MF retentate</td>
<td>0.097 a</td>
<td>4.889 b</td>
<td>7.397 a</td>
<td>1.592 a</td>
<td>0.275 a</td>
<td>1.317 a</td>
<td>1.567 a</td>
<td>0.025 a</td>
<td>1.292 a</td>
<td>1.887 a</td>
<td>98.113 a</td>
<td>21.523 c</td>
<td>1.310 c</td>
</tr>
<tr>
<td>Stage 2 (DF) - MF retentate</td>
<td>0.088 b</td>
<td>1.643 c</td>
<td>3.010 c</td>
<td>0.978 b</td>
<td>0.137 c</td>
<td>0.841 b</td>
<td>0.951 b</td>
<td>0.027 a</td>
<td>0.814 b</td>
<td>3.269 a</td>
<td>96.731 a</td>
<td>32.474 b</td>
<td>2.916 b</td>
</tr>
<tr>
<td>Stage 3 (DF) - MF retentate</td>
<td>0.085 b</td>
<td>0.642 d</td>
<td>1.639 d</td>
<td>0.759 c</td>
<td>0.098 d</td>
<td>0.661 c</td>
<td>0.733 c</td>
<td>0.025 a</td>
<td>0.635 c</td>
<td>3.763 a</td>
<td>96.237 a</td>
<td>46.230 a</td>
<td>5.215 a</td>
</tr>
<tr>
<td>Standard error</td>
<td>0.003</td>
<td>0.014</td>
<td>0.073</td>
<td>0.052</td>
<td>0.005</td>
<td>0.05</td>
<td>0.047</td>
<td>0.008</td>
<td>0.045</td>
<td>0.916</td>
<td>0.916</td>
<td>1.119</td>
<td>0.131</td>
</tr>
<tr>
<td>R²</td>
<td>0.980</td>
<td>&gt;0.99</td>
<td>&gt;0.99</td>
<td>&gt;0.98</td>
<td>&gt;0.99</td>
<td>0.97</td>
<td>0.98</td>
<td>0.31</td>
<td>0.97</td>
<td>0.44</td>
<td>0.44</td>
<td>0.99</td>
<td>&gt;0.99</td>
</tr>
</tbody>
</table>

CP = Crude protein = total nitrogen x 6.38; NCN = noncasein nitrogen x 6.38; NPN = nonprotein nitrogen x 6.38; TP = true protein (TN - NPN); casein = CN = ((TP) – (NCN – NPN)); serum proteins = SP = (NCN – NPN); CN%TP = casein as a percentage of true protein; CPDB = crude protein on dry-mater basis = ((CP/TS) x 100); FDB = fat on a dry-mater basis = ((Fat/TS) x 100).

a, b, c Means in the same column that are followed by a different superscript differ (P < 0.05).
Table 3. Mean (n = 3) composition in (%w/w) of polymeric microfiltration (MF) and diafiltration (DF) permeates produced from separated Cheddar cheese whey.

<table>
<thead>
<tr>
<th></th>
<th>Fat</th>
<th>Lactose</th>
<th>Total solids</th>
<th>CP</th>
<th>NPN</th>
<th>TP</th>
<th>NCN</th>
<th>Casein</th>
<th>SP</th>
<th>CN%TP</th>
<th>SP%TP</th>
<th>CPDB</th>
<th>FDB</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stage 1 - MF permeate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MF permeate</td>
<td>0.003</td>
<td>4.922</td>
<td>6.225</td>
<td>0.527</td>
<td>0.203</td>
<td>0.324</td>
<td>0.517</td>
<td>0.010</td>
<td>0.314</td>
<td>3.199</td>
<td>96.801</td>
<td>8.461</td>
<td>0.056</td>
</tr>
<tr>
<td><strong>Stage 2 (DF) - MF permeate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MF permeate</td>
<td>0.002</td>
<td>1.651</td>
<td>2.205</td>
<td>0.293</td>
<td>0.078</td>
<td>0.215</td>
<td>0.283</td>
<td>0.010</td>
<td>0.205</td>
<td>4.898</td>
<td>95.102</td>
<td>13.303</td>
<td>0.091</td>
</tr>
<tr>
<td><strong>Stage 3 (DF) - MF permeate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MF permeate</td>
<td>0.001</td>
<td>0.639</td>
<td>0.875</td>
<td>0.132</td>
<td>0.034</td>
<td>0.098</td>
<td>0.126</td>
<td>0.006</td>
<td>0.092</td>
<td>6.662</td>
<td>93.338</td>
<td>15.102</td>
<td>0.095</td>
</tr>
<tr>
<td><strong>Standard error</strong></td>
<td>0.001</td>
<td>0.017</td>
<td>0.033</td>
<td>0.028</td>
<td>0.004</td>
<td>0.026</td>
<td>0.028</td>
<td>0.003</td>
<td>0.026</td>
<td>2.101</td>
<td>2.101</td>
<td>0.691</td>
<td>0.088</td>
</tr>
<tr>
<td><strong>R²</strong></td>
<td>0.640</td>
<td>&gt;0.99</td>
<td>&gt;0.99</td>
<td>0.97</td>
<td>&gt;0.99</td>
<td>0.93</td>
<td>0.97</td>
<td>0.26</td>
<td>0.92</td>
<td>0.31</td>
<td>0.31</td>
<td>0.94</td>
<td>0.039</td>
</tr>
</tbody>
</table>

CP = Crude protein = total nitrogen x 6.38; NCN = noncasein nitrogen x 6.38; NPN = nonprotein nitrogen x 6.38; TP = true protein (TN - NPN); casein = CN = ((TP) – (NCN – NPN)); serum proteins = SP = (NCN – NPN); CN%TP = casein as a percentage of true protein; CPDB = crude protein on dry-mater basis = ((CP/TS) x 100); FDB = fat on a dry-mater basis = ((Fat/TS) x 100).

a, b, c Means in the same column that are followed by a different superscript differ (P < 0.05).
Table 4. Mean ± standard deviation (n=3) percentage of fat and total nitrogen (TN) recovered in polymeric microfiltration retentate and permeate.

<table>
<thead>
<tr>
<th>Fat (%)</th>
<th>TN recovery based on permeate measured by Kjeldahl (%)¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retentate</td>
<td>88.82 ± 2.23</td>
</tr>
<tr>
<td>Permeate</td>
<td>11.18 ± 2.23</td>
</tr>
</tbody>
</table>

¹ Subtraction of the mass TN recovered in permeate from each stage from the mass of TN in the feed whey.

Table 5. Mean ± standard deviation (n=3) of the mean cumulative relative percentage serum protein (SP) removed by polymeric microfiltration (MF) by stage of the 3-stage polymeric MF process based on measurement by Kjeldahl true protein (TP) and Kjeldahl total nitrogen (TN).

<table>
<thead>
<tr>
<th>MF stage</th>
<th>Cumulative SP percentage removed measured by Kjeldahl TP in Permeate¹</th>
<th>Cumulative TN percentage removed measured by Kjeldahl TN in Permeate²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 1</td>
<td>29.95 ± 5.51</td>
<td>39.23 ± 3.59</td>
</tr>
<tr>
<td>Stage 2</td>
<td>48.94 ± 4.45</td>
<td>59.56 ± 1.83</td>
</tr>
<tr>
<td>Stage 3</td>
<td>57.83 ± 3.11</td>
<td>68.79 ± 0.65</td>
</tr>
</tbody>
</table>

¹ Subtraction of TP recovered from each stage of permeate from feed TP
² Subtraction of TN recovered from each stage of permeate from feed TN
Table 6. Mean (n=3) Protein composition of whey feed, retentates, and permeates for polymeric MF of sweet whey determined using SDS-PAGE.

<table>
<thead>
<tr>
<th></th>
<th>LFR (%)</th>
<th>BSA (%)</th>
<th>IgG (%)</th>
<th>β-LG (%)</th>
<th>α-LA (%)</th>
<th>βLG:αLA</th>
<th>CN (%)</th>
<th>CNPP (%)</th>
<th>CN+CNPP (%)</th>
<th>WP (%)</th>
<th>TOTAL (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed whey</td>
<td>2.21 a</td>
<td>4.01 b</td>
<td>4.44 b</td>
<td>6.14 a</td>
<td>59.70 c</td>
<td>21.49 c</td>
<td>2.54 bc</td>
<td>0.00</td>
<td>9.22 c</td>
<td>10.16 bc</td>
<td>100.00</td>
</tr>
<tr>
<td>Stage 1 retentate</td>
<td>3.82 ab</td>
<td>6.39 a</td>
<td>6.80 a</td>
<td>56.58 d</td>
<td>14.19 d</td>
<td>4.01 a</td>
<td>0.00</td>
<td>0.00</td>
<td>12.22 b</td>
<td>12.22 b</td>
<td>89.84 b</td>
</tr>
<tr>
<td>Stage 2 retentate</td>
<td>3.91 a</td>
<td>5.62 a</td>
<td>5.24 a</td>
<td>53.47 e</td>
<td>14.07 d</td>
<td>3.80 a</td>
<td>0.00</td>
<td>0.00</td>
<td>17.68 a</td>
<td>17.68 a</td>
<td>82.32 d</td>
</tr>
<tr>
<td>Stage 3 retentate</td>
<td>0.00 c</td>
<td>2.70 c</td>
<td>2.05 b</td>
<td>66.70 b</td>
<td>28.55 a</td>
<td>2.34 c</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>100.00 a</td>
<td>100.00 a</td>
</tr>
<tr>
<td>Stage 1 permeate</td>
<td>0.00 c</td>
<td>2.45 c</td>
<td>3.36 b</td>
<td>69.80 a</td>
<td>68.52</td>
<td>25.67 b</td>
<td>2.67 bc</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>100.00 a</td>
</tr>
<tr>
<td>Stage 2 permeate</td>
<td>0.00 c</td>
<td>1.38 c</td>
<td>2.64 b</td>
<td>69.80 a</td>
<td>26.17 b</td>
<td>2.67 bc</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>100.00 a</td>
<td>100.00 a</td>
</tr>
<tr>
<td>Stage 3 permeate</td>
<td>0.79</td>
<td>0.48</td>
<td>0.79</td>
<td>1.22</td>
<td>1.00</td>
<td>0.18</td>
<td>1.15</td>
<td>1.15</td>
<td>1.15</td>
<td>1.15</td>
<td>1.15</td>
</tr>
<tr>
<td>Standard error</td>
<td>0.85</td>
<td>0.92</td>
<td>0.83</td>
<td>0.97</td>
<td>0.97</td>
<td>0.92</td>
<td>0.97</td>
<td>0.97</td>
<td>0.97</td>
<td>0.97</td>
<td>0.97</td>
</tr>
</tbody>
</table>

IgG= Immunoglobulin G; BSA= Bovine serum albumin, LFR= Lactoferrin, β–LG= Beta-lactoglobulin; α–LA= Alpha-lactalbumin; CN=Casein; CNPP= Casein proteolysis products; WP= Whey proteins (i.e., protein minus CNPP).

Means in the same column that are followed by a different superscript differ (P <0.05).
Table 7. Mean (n=3) relative percentage of β-LG and α-LA in feed, retentate and permeate during polymeric microfiltration of whey determined using SDS PAGE.

<table>
<thead>
<tr>
<th></th>
<th>β–LG (%)</th>
<th>α–LA (%)</th>
<th>% of α–LA and β–LG of total protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed whey</td>
<td>71.71 bc</td>
<td>28.29 ab</td>
<td>75.92 c</td>
</tr>
<tr>
<td>Stage 1 retentate</td>
<td>73.72 b</td>
<td>26.28 b</td>
<td>81.01 b</td>
</tr>
<tr>
<td>Stage 2 retentate</td>
<td>79.93 a</td>
<td>20.07 c</td>
<td>70.77 d</td>
</tr>
<tr>
<td>Stage 3 retentate</td>
<td>79.17 a</td>
<td>20.83 c</td>
<td>67.54 e</td>
</tr>
<tr>
<td>Stage 1 permeate</td>
<td>70.02 c</td>
<td>29.98 a</td>
<td>95.25 a</td>
</tr>
<tr>
<td>Stage 2 permeate</td>
<td>72.75 b</td>
<td>27.25 b</td>
<td>94.19 a</td>
</tr>
<tr>
<td>Stage 3 permeate</td>
<td>70.73 b</td>
<td>27.27 b</td>
<td>95.98 a</td>
</tr>
<tr>
<td>Standard error</td>
<td>1.03</td>
<td>1.03</td>
<td>1.48</td>
</tr>
<tr>
<td>R²</td>
<td>0.92</td>
<td>0.92</td>
<td>0.98</td>
</tr>
</tbody>
</table>

β–LG = Beta-lactoglobulin; α–LA = Alpha-lactalbumin

a, b, c, d, e Means in the same column that are followed by a different superscript differ (P < 0.05).
**Table 8.** Comparison of mean total nitrogen removal from Cheddar sweet whey compared to removal of serum proteins on a true protein basis from skim milk using ceramic and spiral wound polymeric membranes.

<table>
<thead>
<tr>
<th>MF stage</th>
<th>Skim milk polymeric MF(^1)</th>
<th>Skim milk ceramic MF(^2)</th>
<th>Whey ceramic MF(^3)</th>
<th>Whey polymeric MF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 1</td>
<td>38.6</td>
<td>64.8</td>
<td>51.8 ± 1.5</td>
<td>39.2 ± 3.6</td>
</tr>
<tr>
<td>Stage 2</td>
<td>20.8</td>
<td>23.0</td>
<td>23.7 ± 0.45</td>
<td>20.3 ± 1.9</td>
</tr>
<tr>
<td>Stage 3</td>
<td>10.9</td>
<td>10.5</td>
<td>9.3 ± 0.13</td>
<td>9.2 ± 1.7</td>
</tr>
<tr>
<td>Cumulative</td>
<td>70.3</td>
<td>98.3</td>
<td>84.8 ± 1.4</td>
<td>68.8 ± 0.65</td>
</tr>
</tbody>
</table>

\(^1\)Beckman et al., 2010.
\(^2\)Hurt et al., 2010
\(^3\)Carter et al., 2020

**Table 9.** Comparison of crude protein on a dry basis (CPDB) and fat on dry basis (FDB) of stage 3 retentate (whey phospholipid protein concentrate) of ceramic and polymeric microfiltration of Cheddar sweet whey.

<table>
<thead>
<tr>
<th></th>
<th>Ceramic microfiltration(^1)</th>
<th>Polymeric microfiltration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CPDB</td>
<td>FDB</td>
</tr>
<tr>
<td>Stage 3 Retentate</td>
<td>25.80 %</td>
<td>6.28 %</td>
</tr>
</tbody>
</table>

\(^1\)Carter et al., 2020
Figure 1. Image of two SDS-PAGE gels of polymeric microfiltration retentates and permeates from separated sweet whey. IgG = immunoglobulin G; BSA = bovine serum albumin; LFR = lactoferrin; CNPP = casein proteolysis products; β LG = β-lactoglobulin; α LA = α-lactalbumin. Slot 1= Milk reference, 2-4 MF Feed Whey, 5-7 stage 1 retentate, 8-10 stage 1 permeate, 11-13 stage 2 retentate, 14-16 stage 3 retentate, 17 Milk, 18-20 stage 2 permeate, and 21-23 stage 3 permeate. The three slots within each series of 3 adjacent samples of the same type represent replicates 1, 2, and 3, respectively.
**Figure 2.** Image of two SDS-PAGE gels of ceramic microfiltration retentates and permeates from separated sweet whey adapted from Carter et al. 2020. IgG = immunoglobulin G; BSA = bovine serum albumin; LFR = lactoferrin; CNPP = casein proteolysis products; $\beta$ LG = $\beta$-lactoglobulin; $\alpha$ LA = $\alpha$-lactalbumin. Slot 1= Milk reference, 2-4 MF Feed Whey, 5-7 stage 1 retentate, 8-10 stage 1 permeate, 11-13 stage 2 retentate, 14-16 stage 3 retentate, 17 Milk, 18-20 stage 2 permeate, and 21-23 stage 3 permeate. The three slots within each series of 3 adjacent samples of the same type represent replicates 1, 2, and 3, respectively.
Table 10. Mean stage 1 flux parameters for ceramic and polymeric microfiltration of skim milk, casein free skim milk (CFSM), and sweet whey at 3X concentration factor at 50°C reported from several different research studies.

<table>
<thead>
<tr>
<th>Membrane type</th>
<th>Product feed</th>
<th>Stage 1 product flux (kg/m² per h)</th>
<th>Fouled water flux as a % of clean water flux</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymeric</td>
<td>Skim¹</td>
<td>14.40</td>
<td>19.40</td>
</tr>
<tr>
<td>Polymeric</td>
<td>Skim²</td>
<td>16.21</td>
<td>34.53</td>
</tr>
<tr>
<td>Polymeric</td>
<td>Skim³</td>
<td>17.22</td>
<td>41.46</td>
</tr>
<tr>
<td>Polymeric</td>
<td>CFSM³</td>
<td>80.19</td>
<td>95.85</td>
</tr>
<tr>
<td>Ceramic UTP</td>
<td>Skim²</td>
<td>54.08</td>
<td>46.10</td>
</tr>
<tr>
<td>Ceramic GP</td>
<td>Skim²</td>
<td>71.79</td>
<td>55.40</td>
</tr>
<tr>
<td>Mean ceramic</td>
<td>Skim</td>
<td>62.94</td>
<td>48.80</td>
</tr>
<tr>
<td>Mean polymeric</td>
<td>Skim</td>
<td>15.94</td>
<td>31.72</td>
</tr>
<tr>
<td>Ceramic UTP</td>
<td>Whey⁶</td>
<td>54.00</td>
<td>84.61</td>
</tr>
<tr>
<td>Polymeric</td>
<td>Whey</td>
<td>56.20</td>
<td>57.63</td>
</tr>
</tbody>
</table>

¹Beckman et al., (2010)  
²Zulewska et al., (2009)  
³Zulewska et al., (2013)  
⁴UTP = Uniform transmembrane pressure  
⁵GP = graded permeability  
⁶Carter et al., (2020)
Table 11. β-LG and α-LA relative percentage and ratio in stage 1 permeate with different feed materials [skim milk, casein free skim milk (CFSM), and sweet whey] during ceramic and polymeric microfiltration.

<table>
<thead>
<tr>
<th>Membrane type</th>
<th>Product</th>
<th>β-LG</th>
<th>α-LA</th>
<th>Ratio (β-LG: α-LA)</th>
<th>True protein (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymeric</td>
<td>Skim¹</td>
<td>71.91</td>
<td>28.09</td>
<td>2.56</td>
<td>0.34</td>
</tr>
<tr>
<td>Polymeric</td>
<td>Skim²</td>
<td>73.28</td>
<td>26.72</td>
<td>2.74</td>
<td>0.38</td>
</tr>
<tr>
<td>Polymeric</td>
<td>Skim³</td>
<td>72.35</td>
<td>27.65</td>
<td>2.62</td>
<td>0.36</td>
</tr>
<tr>
<td>Polymeric</td>
<td>CFSM³</td>
<td>75.71</td>
<td>24.29</td>
<td>3.12</td>
<td>0.52</td>
</tr>
<tr>
<td>Ceramic UTP²</td>
<td>Skim²</td>
<td>76.04</td>
<td>23.96</td>
<td>3.17</td>
<td>0.57</td>
</tr>
<tr>
<td>Ceramic GP⁵</td>
<td>Skim²</td>
<td>76.31</td>
<td>23.69</td>
<td>3.22</td>
<td>0.57</td>
</tr>
<tr>
<td>Mean ceramic</td>
<td>Skim</td>
<td>76.18</td>
<td>23.83</td>
<td>3.20</td>
<td>0.57</td>
</tr>
<tr>
<td>Mean polymeric</td>
<td>Skim</td>
<td>72.51</td>
<td>27.49</td>
<td>2.64</td>
<td>0.36</td>
</tr>
<tr>
<td>Ceramic UTP</td>
<td>Whey⁶</td>
<td>74.56</td>
<td>25.44</td>
<td>2.93</td>
<td>0.51</td>
</tr>
<tr>
<td>Polymeric</td>
<td>Whey</td>
<td>70.02</td>
<td>29.98</td>
<td>2.34</td>
<td>0.32</td>
</tr>
</tbody>
</table>

¹Beckman et al. (2010)  
²Zulewska et al. (2009)  
³Zulewska et al. (2013)  
⁴UTP = Uniform transmembrane pressure  
⁵GP = graded permeability  
⁶Carter et al., (2020)
**Table 12.** Gross Revenue (US$/day) from WPPC plus WPI given 4 different WPPC to WPI selling price for a factory processing 1,000,000 kg of separated whey per day with a crude protein removal of 68.8% for polymeric and 84.8% for ceramic membranes.

<table>
<thead>
<tr>
<th>Membrane type</th>
<th>Product</th>
<th>Yield kg/day</th>
<th>Price $/kg</th>
<th>Price ratio</th>
<th>Income $ per day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spiral</td>
<td>WPI</td>
<td>6955</td>
<td>$6.72</td>
<td>0.788</td>
<td>46,769</td>
</tr>
<tr>
<td>Spiral</td>
<td>WPPC</td>
<td>5680</td>
<td>$5.30</td>
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<td>30,102</td>
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<tr>
<td>Spiral</td>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>76,871</td>
</tr>
<tr>
<td>Spiral</td>
<td>WPI</td>
<td>6955</td>
<td>$6.72</td>
<td>0.672</td>
<td>46,769</td>
</tr>
<tr>
<td>Spiral</td>
<td>WPPC</td>
<td>5680</td>
<td>$4.52</td>
<td></td>
<td>25,672</td>
</tr>
<tr>
<td>Spiral</td>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>72,441</td>
</tr>
<tr>
<td>Spiral</td>
<td>WPI</td>
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</tr>
<tr>
<td>Spiral</td>
<td>WPPC</td>
<td>5680</td>
<td>$3.74</td>
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<td>21,219</td>
</tr>
<tr>
<td>Spiral</td>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>67,988</td>
</tr>
<tr>
<td>Spiral</td>
<td>WPI</td>
<td>6955</td>
<td>$6.72</td>
<td>0.298</td>
<td>46,769</td>
</tr>
<tr>
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<td>11,360</td>
</tr>
<tr>
<td>Spiral</td>
<td>Total</td>
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<td>58,130</td>
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<tr>
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<td>WPI</td>
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<td>$6.72</td>
<td>0.788</td>
<td>57,654</td>
</tr>
<tr>
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<tr>
<td>Ceramic</td>
<td>Total</td>
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<td>72,314</td>
</tr>
<tr>
<td>Ceramic</td>
<td>WPI</td>
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<td>$6.72</td>
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<td>57,654</td>
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<tr>
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</tr>
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<td>Ceramic</td>
<td>WPI</td>
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<td>$6.72</td>
<td>0.556</td>
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</tr>
<tr>
<td>Ceramic</td>
<td>WPPC</td>
<td>2766</td>
<td>$3.74</td>
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<td>10,334</td>
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<tr>
<td>Ceramic</td>
<td>Total</td>
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<td></td>
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</tr>
<tr>
<td>Ceramic</td>
<td>WPI</td>
<td>8574</td>
<td>$6.72</td>
<td>0.298</td>
<td>57,654</td>
</tr>
<tr>
<td>Ceramic</td>
<td>WPPC</td>
<td>2766</td>
<td>$2.00</td>
<td></td>
<td>5,533</td>
</tr>
<tr>
<td>Ceramic</td>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>63,187</td>
</tr>
</tbody>
</table>
CHAPTER 6: Influence of Oral Movement, Particle Size and Zeta Potential on Astringency of Whey Protein
Influence of Oral Movement, Particle Size and Zeta Potential on Astringency of Whey Protein

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Abstract

Whey protein astringency is complex summation of many interacting mechanisms which has made it difficult to define and control. The contribution of oral movement and particle size as well as zeta potential on the perception of whey protein astringency was investigated. Three sources of whey protein isolates (microfiltration of cheese whey, ion exchange of cheese whey, microfiltration of skim milk) from 2 lots were evaluated by a trained panel at 10% (w/w) protein adjusted to a pH of 3.4 and 7. Trained panelists documented the astringency of these solutions with and without oral movements. In a second experiment, 67 commercial WPI and WPC80 were evaluated for astringency as well as particle size and zeta potential at neutral pH. Acidification of WPI increased astringency, but astringency was documented in a neutral pH range (pH 6.0-7.0) as well. Oral movements increased the perception of astringency (P<0.05), suggesting that part of the astringent sensation was due to the interaction of whey proteins with receptors on oral tissues and oral movement further increasing astringency perception, possibly by friction of delubricated oral surfaces. Commercial WPI and WPC80 varied widely in particle size, zeta potential and astringency. Astringency of WPI solutions were correlated with zeta potential (p< 0.05; r = -0.81). WPC80 were astringent, but astringency of WPC 80 was not correlated with particle size or zeta potential (p>0.05; r = 0.14, 0.40). Astringency is an important characteristic in whey protein products that leads to decreased liking, improvements in astringency may improve consumer acceptance of high protein products.
Practical Applications

This study established that oral movements and zeta potential are linked to the astringency of whey protein. Astringency of whey proteins is complex and a result of multiple mechanisms. These results further explain the mechanisms of astringency and may help to identify possible methodologies to reduce astringency in whey protein ingredient applications.

Key Words: Whey protein, astringency, whey protein isolate
**Introduction**

Astringency is the sensation of mouth drying and puckering. It can also be described as the loss of lubrication in the mouth, perceived as an increase in oral friction and roughness. Astringency may be a taste sensation, but may also be a tactile mechanism in the mouth during oral manipulation of the food (Bajec and Pickering, 2008). The model for astringency proposed by better studied food applications like tea and wine is that astringency is caused by polyphenols interacting with salivary proteins. Horne et al. (2002) described that astringency is at least initially caused by delubrication via removal of the slippery coating (salivary proteins) on oral surfaces, exposing mechanoreceptors on oral tissues resulting in roughening, tightening, and drying sensations. Whey proteins have also been shown to elicit an astringent response. A recent review of astringency in whey protein beverages was written by Carter et al. (2020).

Whey protein is a common ingredient because of its excellent nutritional quality. Astringency is a sensation documented in whey protein which also can carry through into the final application (Oltman et al., 2015; Childs and Drake, 2010; Zhang et al., 2020). Protein astringency may further limit protein ingredients use in protein-dense products as astringency can be an undesired oral sensation in some foods (Beecher et al., 2008; Zhang et al., 2020). High intensities of astringency have been noted in high acid whey protein beverages (Beecher et al., 2008; Childs and Drake, 2010) but perceivable astringency is still common in neutral pH beverages (Withers et al., 2014), and was recently documented as a driver of consumer dislike in neutral pH ready-to-mix whey protein beverages (Zhang et al., 2020). The exact mechanisms of whey protein astringency is still not fully understood. A few of the possible mechanisms that are relevant to this work are these: Astringency is caused by 1.) acid and lower pH 2.) whey protein interactions with salivary proteins, removing them from oral surfaces and delubricating oral...
tissues, 3.) whey protein aggregates that interact directly with bound salivary proteins on the oral epithelium or mechanoreceptors, or 4.) whey protein saliva aggregates that overcome lubrication in the mouth and the rubbing of deformed oral surfaces with perceivable particles increasing oral friction (Upadhyay et al., 2016).

Oral manipulation of food increases the perception of sensory attributes (Wijk et al., 2003; Wijk et al., 2011). Lubrication of food in the mouth is determined by viscosity and protein content of the saliva, the shape and hardness of any particles in the oral fluid and the surface properties of the oral mucosa and teeth (Wijk et al., 2006a). Larger particles in food exhibit higher friction in the mouth (Wijk et al., 2006a). Aggregates can be formed by various forces, including electrostatic interactions. Larger size whey protein aggregates may change the texture of the salivary film in the mouth and as surfaces rub, may increase the sensation of astringency. If the size of particles further change as a result of oral conditions, this would change the fluid saliva composition and could create greater oral friction increasing the perception of astringency, especially with delubricated oral surfaces. Whey protein aggregates may be the cause of tactile changes without any contribution by salivary proteins. If we also take into account precipitated protein particle interactions with salivary protein, astringency would be caused in a similar way that polyphenols and salivary proteins create complex precipitants that reduce lubrication and create roughness.

Particle size of proteins in solution relates to the rate of sedimentation of particles. To evaluate the size of particles, either laser diffraction or dynamic light scattering (DLS) methods are used. These techniques provide information based on scattering angle (laser diffraction) or diffusion of particles due to Brownian motion (DLS) (Wagoner and Foegeding, 2017). Each technique favors a particular size class. Whey proteins are small which means they are better
characterized by DLS (Ye et al., 2011). In a limited range (pH 3.0-3.6), Ye et al. (2011) reported that an increase in astringency and particle size were correlated suggesting that denaturation/aggregation adds to the astringency associated with native proteins. It appears that forming protein aggregates alone doesn’t necessarily correlate to astringency, as high particle size and turbidity noticed at pH 5.0 did not increase the perception of astringency (Ye et al., 2011) even though charge interactions between whey protein and saliva were demonstrated. However, the combination of particle size and charge interactions, which is an indication of the affinity between whey protein and salivary proteins, could help further explain astringency. There may also be a difference between large particle sizes caused by proteins near their isoelectric point versus that of heat/denatured aggregates. For example, Bull et al. (2017) found that heating WPC solutions (10% w/v, pH 6.5-6.7) for longer periods of time (70°C for 5, 10, or 20 minutes) lead to increased astringency.

A common way to analyze proteins in regards to their propensity for electrostatic interactions is to measure the Zeta potential. This measurement provides an average protein charge (positive or negative) at a given pH and ionic conditions. Therefore, it can be useful to understand the potential interaction between charged molecules. Zeta potential was used to understand electrostatic interactions between salivary and whey proteins, and astringency was positively correlated with a decrease in zeta potential in the range tested (pH 3-5) which suggests greater interactions between whey protein and salivary proteins when the charge states of whey protein encouraged interaction (Ye et al., 2011; Ye et al., 2012). The objective of this research was to better understand the effect of oral manipulations on the perception of astringency. In addition, an analysis of current whey protein products were evaluated for particle size and zeta-potential to better understand how these parameters may affect the perception of astringency.
Methods

Sample Preparation

Experiment 1. Three different whey/serum protein isolates (SPI) were sourced in duplicate lots from different processing methods (ion exchange, microfiltration of cheese whey, and direct microfiltration of skim milk). Actual protein content of liquid or powdered proteins were confirmed by an infrared spectrophotometer (Lactoscope FTIR, Delta Instruments, Drachten, the Netherlands). The SPI was produced at South Dakota State University and shipped in liquid form overnight to NC State University and held at -20 ºC. Briefly MF permeate from the microfiltration of skim milk was concentrated using ultrafiltration to a high protein (about 25% w/v) SPI, and the concentrate was packaged in half gallon plastic jugs (Upstate Niagara Cooperative, Buffalo, NY), and held at -20ºC (Cheng et al., 2018). Frozen SPI containers were placed in a 4ºC cooler to thaw slowly prior to dilution with DI water to a 10% (w/w) protein solution. For powdered samples, 10% (w/w) protein solutions were made by weighing powders and mixing in half of the total volume of DI water. Sample pH was adjusted by either NaOH [1 M] (Sigma Aldrich, St. Louis, MO) or phosphoric acid [1 M] (Sigma Aldrich) to a pH of 7 or 3.4 as measured by a calibrated pH meter (Orion Model 250+, Thermo Scientific, Waltham, MA) and probe (VWR Model 89231-572, VWR, Radnor, PA). Samples were then diluted to the final protein concentration of 10% protein (w/w) and allowed to hydrate overnight at 4ºC. Samples were tempered to 21ºC prior to sensory analysis.

A reference solution was prepared to provide trained sensory panelists with an astringency reference. An alum reference solution was used which is an ideal reference solution since it is astringent with minimal contributions to sour or bitter taste (Drobna et al., 2004). The astringency reference solution was made by mixing 0.02% (w/v) alum (purchased at a local
grocery store) in DI water which represented an intensity of 2 on a 0 to 15 point intensity scale (Beecher et al., 2008). A buffered acid solution was prepared with sodium dibasic phosphate (ICL Performances Products LP, St. Louis, MO) at 30 mM phosphate (approximately the buffering capacity of whey at 10% w/w protein) and adjusted to a pH of 3.4 as an additional experimental sample. The purpose of the inclusion of this experimental sample was to be able to separate the contribution of acid to perceived astringency. The 13 (3 protein sources, 2 lots, 2 pH values, 1 buffered acid solution) treatments were then subjected to descriptive analysis to quantify perceived astringency and sour taste intensity.

**Experiment 2.** Sixty seven commercial WPI (37) and WPC (30) samples were collected from 3 separate manufacturers representing multiple lots of production. All proteins were less than 60 days old at time of receipt and analyses. Samples were hydrated with DI water to 10% protein (w/w) and stored overnight at 4°C to fully hydrate. Whey protein solutions at 10% (w/w) protein were used for descriptive analysis and pH measurement. For particle size and zeta potential analysis, the proteins were rehydrated to a protein concentration of 1 mg/mL to reduce multiple light scattering effects. Solutions for particle size and zeta potential were made with HPLC water (Sigma Aldrich) and allowed to hydrate overnight before analysis. Before introduction to the equipment, solutions were filtered with a 0.45 um filter (Sigma Aldrich) to remove any particulate. These analyses were run prior to descriptive analysis to be able to distinguish samples and choose the most extreme in both particle size and zeta potential to continue to descriptive analysis.

**Dynamic light scattering (DLS) particle size analysis and ζ-potential**

**Experiment 2.** Intensity-weighted z-average diameter and ζ-potential were measured at ambient temperature (23 ± 1 °C) simultaneously using a Wyatt Möbiuζ (Wyatt Technology,
Santa Barbara, CA) as described by Wagoner and Foegeding (2017). To calculate particle diameter using DLS, fluctuations due to Brownian motion were measured over 30 s intervals and quantified per a second order correlation function with a regularization fit in the accompanying software (Dynamics v7.3.1, Wyatt Technology). The calculated diffusion coefficient is related to the hydrodynamic diameter per the Stokes-Einstein equation. Mass concentration (c\text{mass}) was used to approximate the mass percentage of particles in distinct size ranges based on the measured scattering intensity, where I is the intensity of scattered light, M is molar mass, P is a form factor, r is particle radius, and θ is the angular dependence of scattered light.

\[ \text{Cmass} \propto \frac{I(r, \theta)}{M(r) \times P(r, \theta)} \]

All 67 WPC and WPI samples were subjected to particle size and zeta potential analysis 6 times. These averaged results for each protein were examined and then, a sub sample (n= 12 WPI, n=13 WPC 80) was selected that represented the most extreme values of both particle size and zeta potential for descriptive analysis.

**Descriptive Sensory Analysis**

**Experiment 1.** All descriptive sensory analysis was done in compliance with the North Carolina State University Institutional Review Board for Human Subjects guidelines. Whey protein solutions were dispensed into 60 ml soufflé cups (Solo Cup, Highland Park, IL) and were assigned random 3 digit codes. They were then lidded and tempered to 21°C. Astringency was evaluated using the 0 to 15 Spectrum™ intensity scale with a highly trained panel (n=7, 7 females, 38-56y) each with >250 h of experience evaluating dairy ingredients, including astringency of whey proteins. Samples were held in the mouth for 15 seconds with or without oral movements and then expectorated. Oral movements involved moving the solution in the
mouth with the tongue on the roof of the mouth, teeth and cheeks at approximately 1 movement per sec. Sour taste intensities were also scaled using the same 0 to 15 point intensity scale. A 5 min rest time was enforced between samples during which panelists rinsed with 1% (w/v) carboxyl methyl cellulose (CMC) solution (Sigma Aldrich) (Brannan et al., 2001). No more than 4 samples were evaluated per session. Samples were evaluated in duplicate by each panelist using paper ballots.

**Experiment 2.** Ten WPI and 11 WPC were selected that represented a range of particle size and zeta potential. WPI and WPC solutions (10% w/w protein) were prepared as described previously and were dispensed into 60 ml soufflé cups (Solo Cup, Highland Park, IL), lidded and tempered to 21°C. Astringency was evaluated using the same methods and panel described previously. Samples were evaluated by each panelist in duplicate.

**Time Intensity (TI)**

The time intensity of astringency for solutions in experiment 1 was evaluated with the same trained panel of seven participants. Time intensity was conducted in separate sessions from descriptive analysis. Each panelist had at least 40h of previous experience with TI of basic tastes using a 0 to 15 point scale consistent with the Spectrum method\(^\text{TM}\). Two 1 h sessions were conducted with the panel on TI of astringent solutions of alum, as well as experimental solutions to calibrate them prior to data collection sessions. For TI, 15ml of each solution was dispensed into a lidded cup with a 3 digit code. Panelists were instructed to press the start button, immediately place the entire sample in their mouths, and begin evaluation. Samples were held in the mouth for 15 seconds with or without oral movements and then expectorated. Panelists were instructed to move a cursor to the appropriate intensity level for astringency using a 0- to 15-point Spectrum scale (Meilgaard et al., 2007). Evaluations took place over 150 s. Data was
collected on Ipads using Compusense Cloud (Compusense, Guelph, ON, Canada). A 5 min rest time was enforced between samples with 1% (w/v) carboxyl methyl cellulose (CMC) as a rinse solution. No more than five samples were evaluated per session to minimize the build-up of astringency. Order of presentation was randomized per session to prevent order of presentation carryover effects. Values determined from TI curves were: maximum intensity (relative units), time to maximum intensity (s), total duration of astringency (s), and are under the TI curve (relative units).

**pH Measurement**

Samples were rehydrated at 10% protein (w/w) with DI water in a 50 ml centrifuge tube (Sigma Aldrich). Samples were mixed for 10 min and refrigerated overnight to allow the protein to fully hydrate. Before analysis, samples were tempered to 21 °C. A calibrated pH meter (Orion Model 250+, Thermo Scientific, Waltham, MA) and probe (VWR Model 89231-572, VWR, Radnor, PA) was used and pH values were measured at 21º C and recorded in duplicate.

**Statistical analysis**

Data was analyzed using XLSTAT (version 2013.5.03; Addinsoft, New York, N.Y., U.S.A.). A 3-way ANOVA (protein type x pH x oral movement) was conducted to more fully explain the role of pH and oral movements on perceived astringency. The astringency of the external buffered acid solution was then compared to selected samples. Smoothing and statistical analysis on TI data was conducted in tempR package in R version 3.3.1 (Castura, 2016). Parameters of maximum intensity, time at maximum intensity, total time, and area under the curve were extracted from TI data using an XLSTAT macro (Addinsoft, New York, NY) and compared once again with a 3-way ANOVA (protein type x pH x oral movement). In experiment 2, a one way ANOVA with means separation was conducted for all samples. Correlation of
astringency with particle size measurements or zeta potential for each protein (WPC80, WPI) were analyzed with Pearson correlation coefficient (XLSTAT).

Results

Experiment 1

Descriptive Analysis. There was no effect of whey protein type or oral movement on the perception of sour taste (P>0.05). As expected, the acidified solutions were more sour than neutral pH samples (4.0 ± 0.2 vs. ND). There was a significant interaction among the protein source, pH and oral movement on the perception of astringency (Figure 1, Table 1, P<0.05). There were also significant interactions for protein type x pH and oral movement x pH (P<0.05). However, the main effects of pH and oral movement accounted for the majority of variance (Table 1). Acidified whey protein solutions (pH 3.4) had higher intensities of astringency regardless of oral movement than neutral pH solutions (pH 7) (Figure 1) (P <0.05). Oral manipulation increased the perception of astringency regardless of protein type or pH (Figure 1) (P< 0.05). The interaction of protein type x pH demonstrated that WPI produced by microfiltration (MF) was lower in astringency than WPI by ion exchange (IX) and serum protein isolate (SPI) at pH 3.4, but not at pH 7 regardless of oral movement (Figure 1, P<0.05). Buffered acid solution had an astringency intensity of 2.5 ± 0.05 which increased to 3.6 ± 0.1 with oral movement (P<0.05). Buffered acid solutions (pH 3.4) were less astringent than all acidified protein solutions (2.5+0.05 vs. 3.5+0.2) (P<0.05).

Time Intensity. The time to reach maximum astringency intensity was influenced by pH (P<0.05) (Table 1) with low pH samples (3.4) requiring 40.5 ± 1.0 s to reach maximum intensity vs. neutral pH samples that reached maximum intensity at 28 ± 1.2 s (P<0.05). There was a
significant interaction of protein type x pH on the maximum intensity of astringency (P<0.05) (Table 1). The maximum perceived intensity of astringency was also primarily influenced by pH (P<0.05), but there were protein x pH and pH x oral movement interactions (Table 1). Low pH whey protein solutions (pH 3.4) had higher maximum astringency intensity (6.2 ± 0.4 vs. 1.3 ± 0.1) compared to neutral pH whey protein solutions indicating that the interaction was largely explained by pH (P<0.05). Oral movement increased the intensity of maximum astringency versus no oral movement (3.9 ± 0.1 vs. 3.6 ± 0.1) (Table 2, P<0.05). There was also a significant interaction of pH x oral movement, and pH x protein type on the intensity of maximum astringency (P<0.05). The significant interaction of pH and oral movement was explained by no oral movement being lower than oral movement in maximum at pH 3.4 but not at pH 7.0 (Table 2) (P<0.05). The significant interaction of pH * protein type was explained by MF WPI having lower maximum intensity than IX or SPI at pH 3.4 but no differences in astringency were documented at pH 7.0 (Table 3) (P<0.05).

Once again, pH had the largest impact with duration of astringent intensity significantly higher in acidified samples compared to neutral samples (124 ± 2.2 s vs. 63 ± 2.5s) (P<0.05) (Table 1, Figure 2). Oral movement also impacted the duration of astringency by increasing the perception of astringency by 9 s (94.5 ± 2.2 vs. 103 ± 2.4 s) (P<0.05). There was a significant interaction of protein type, pH, and oral movement on the total area under the curve (P<0.05) (Table 1, Figure 2). There was also a significant interaction of pH * oral movement, and pH * protein type on total area under the curve (P<0.05). The significant interaction of pH and oral movement can be explained by no oral movement being lower than oral movement in total area under the curve at pH 3.4, but not at pH 7.0 (Table 2) (P<0.05). The significant interaction of pH * protein type can be explained by MF WPI having less area under the curve than IX or SPI at
pH 3.4, but not at pH 7.0 (Table 3) (P<0.05). WPI produced by MF had significantly less area under the curve compared to IX and SPI samples (278 ± 10.2 vs. 316 ± 10.6) (P<0.05). Acidified samples had more area under the curve compared to neutral pH whey protein solutions (533.3 ± 8.0 vs. 71.8 ± 9.0) (P<0.05). Oral movement increased the total area under the curve (328 ± 8.8 vs. 277 ± 8.3) (P<0.05).

Consistent with traditional descriptive analysis results, buffered acid solutions (pH 3.4) had a maximum astringency value lower than all acidified protein solutions (2.6±0.8 vs. 6.2±0.4) (P<0.05) (results not shown). Oral movement increased the maximum intensity of astringency of buffered acid solutions compared to no oral movement (2.2 ±0.4 vs. 3.2±0.6) (P<0.05). Buffered acid had less total area under the curve compared to all protein samples (186 vs. 331) (P<0.05). Time of maximum and duration were not statistically different from other samples (P>0.05).

**Experiment 2**

Commercial WPI varied widely in particle size from 30.72- 84.18 nm (Table 4). Likewise, zeta potential varied greatly from -60.02 to 0.38 mV (Table 4). The pH of WPI varied by process used to make them. WPI made by ion exchange had a pH of approximately 7, while those made by microfiltration ranged from 6.05 to 6.36, which is consistent with other reports of whey protein pH (Morr and Ha, 1993). Commercial WPC80 had a more uniform particle size from 72.58- 90.97 compared to WPI (Table 4). Similar to WPI, zeta potential varied among WPC80 samples from -57.59 to -0.15 mV (Table 4). The pH of WPC80 ranged from 6.06 to 6.44. Values of particle size and zeta potential for WPC80 and WPI are similar to values reported in literature (Ye et al., 2012; Bull et al., 2017; Wagoner et al., 2016).

WPI and WPC80 for descriptive analysis in experiment 2 were selected based on stepwise increases in both particle size and zeta potential, making sure to include representative
samples from each manufacturing facility. The astringency of the selected WPI solutions ranged from 1.8-3.4 on a 0 to 15 point scale (Table 6). The astringency of the selected WPC80 solutions ranged from 2.0-3.0 on a 0 to 15 point scale (Table 7). The astringency of WPI solutions was correlated with zeta potential of the proteins (P<0.05; r = -0.81) (Table 8). The particle size and zeta potential of WPI solutions were also correlated (P<0.05; r = -.78), as the particle size increased, the zeta potential of the protein decreased (Figure 3). There was no direct correlation between particle size and pH on the astringency of WPI (P>0.05; r = -0.30). There was no correlation between zeta potential, particle size or pH on the astringency of WPC (P>0.05; r = -0.40, 0.15, 0.26, respectively).

**Discussion**

The objective of this study was to evaluate possible mechanisms of whey protein astringency. As mentioned previously, one of the possible mechanisms is that astringency is in part due to acid concentration or pH. Buffered acid controls used in TI had a maximum astringency value of 2.5 without oral movement and 3.6 with oral movement, and there was a similar oral movement effect by descriptive analysis. In an experiment of different organic acids (lactic, malic, tartaric, citric) and the relative astringency at different acid concentrations, lowering the pH of acid solutions while holding the acid concentrations the same resulted in increases in astringency (Sowalsky and Noble, 1998). Acids themselves cause astringency and the astringency lingers (Sowalsky and Noble, 1998), but sourness as a sensation does not linger after expectoration (Rubico and McDaniel, 1992; Hartwig and McDaniel, 1995). This result suggests that acids induce some changes in mucosal proteins which cause or facilitate astringency, not merely the presence of acid. WPI and WPC80 have little residual acid from the cheese make, therefore the astringency caused by WPI and WPC80 at neutral pH is not likely a
result of residual acid content. In our results, acidified whey protein solution were more astringent than buffered acid solutions alone. This result confirms the results of other research groups that observed that the addition of whey protein increased astringency in acidic conditions and that astringency was not due to acid alone (Vardhanabhuti et al., 2010).

As expected, pH was a significant factor in the perception of astringency in whey protein solutions. However, in addition, protein type (MF, IX, SPI) also had a significant impact, with the impact being more pronounced at pH 3.4. These whey proteins differed in processing method which may vary the different specific whey protein proportions, presence of glycomacropeptide (GMP), mineral content and pH, all of which may have an effect on astringency (Britten and Pouliot, 1996). In experiment 1, pH of samples was adjusted to minimize the variation of pH since our primary purpose in experiment 1 was to investigate the role of oral movement. As such, differences due to protein type observed in experiment 1 were not due to pH but some other process-specific parameter. MF and IX WPI (both concentrated from cheese whey) are more similar in process to each other than SPI. Whey protein isolate made by MF results from cheese whey that has been defatted by MF. Depending on the cheese make it can vary in mineral profile and contains glycomacropeptide (GMP), which is a peptide released by action of chymosin on kappa casein. Whey protein isolate made by IX also results from cheese making but passes through an ion exchange process to remove fat. Also, GMP is not retained by resin IX beads, which produces a WPI without GMP and a different mineral profile than MF WPI. SPI is made by concentrating the microfiltration permeate of skim milk. Since SPI is not derived from cheese manufacture, it is free from the residues of cheese making (starter culture, proteolysis, colorant, etc). The different astringency intensities of these 3 sources of whey protein at similar
pH with or without oral movement warrants future research to determine if any of these differences among these 3 whey protein sources are contributors to astringency.

Oral movement also made a significant contribution to the astringency of whey protein solutions. We investigated the role of oral movement to provide more clarity on mechanisms of whey protein astringency. Other studies have evaluated oral movement effects on sensory attributes of vanilla custard (Wijk et al., 2006b) full fat and low fat custards and mayonnaise (Wijk et al., 2003). These studies were more focused on a range of oral processing motions on perception of sensory attributes such as creaminess, rough, sticky, melting and astringency as opposed to a focus on one attribute and one product category as in our study. In other words, they were focused on testing a range of oral movements on a few samples to better understand the range of movement effect on sensory attributes, as opposed to one set of oral processing conditions on a range of whey proteins to understand a single sensory attributes, astringency. There are a couple of reasons that may account for increased astringency with oral movement. Oral movement provides more exposure of whey protein to salivary proteins and/or oral receptors and/or the oral movement itself engages mechanoreceptors on oral surfaces. Oral manipulations mix food with saliva and thereby enhance mechanical and chemical breakdown of the food and with additional oral movement, food particles are moved to engage more sense organs in the mouth (Wijk et al., 2003). These results confirm that astringency is in part, caused by movement in the mouth and rubbing of oral tissues with perceived particles as a result of friction.

In experiment 2, we investigated protein astringency correlation with particle size and zeta potential at neutral pH. Particle size measures the average size of molecules in solution. We hypothesized that an increase in particle size would increase the sensation of oral friction and
therefore astringency. Particle size and astringency did not strongly correlate in our study. Bull et al. (2017) found that heating WPC80 (10% w/v) samples increased the particle sizes of the proteins and increased mouth drying (astringency). The whey proteins used in our study varied widely in particle size. In general, WPC80 had a more uniform particle size, likely because of the fat content of WPC80. The WPI varied from 30.72-84.18 nm versus 72.58-90.97 for WPC80. Many things can affect the particle size of whey protein including spray drying parameters (Park et al., 2013) and agglomeration (Gaiani et al., 2007). Each manufacturer and facility have a different approach, process and system for manufacture and spray drying which will ultimately affect the final powder product.

Bull et al. (2017) reported that increased particle size increased astringency, but our results showed no correlation (p>0.05) with particle size and astringency. There are some important differences between these studies. First, Bull et al. (2017) rehydrated one sample of WPC80 and heated it at different temperatures. In our study, we evaluated multiple samples and did no additional heating. Since only one source of WPC80 was used by Bull et al. (2017), the direct effects of heating and aggregation were observed without confounding factors of differences among manufacturers. Second, Bull et al. (2017) found no difference in zeta potential with different heating procedures. They concluded that particle size, not zeta potential, directly affected astringency. In our study, zeta potential more strongly correlated with astringency. Bull et al. (2017) did not have a range of WPC80 with varying zeta potential. Their research captured one component of astringency, but was not equipped to provide information of correlation of zeta potential and astringency. With our work, our collected samples encompassed a range of both particle size and zeta potential. Perhaps it is not the inherit size of particles, but the heating and denaturation (which increase particle size) of protein that changes protein interactions with
oral tissues. Hsein et al. (2015) found that denaturation of whey proteins increased hydrophobic interaction or disulphide bonds with the oral mucosae which increased mucoadhesive strength, increasing the perception of astringency.

Zeta potential measures the overall charge of the proteins. Zeta potential is mainly affected by pH. In whey proteins, as the isoelectric point (pI) is reached (pH 4.6), zeta potential is zero. Zeta potential is positive below pI and negative above the pI. However, pH alone doesn’t dictate zeta potential of whey proteins, as it can also be manipulated by mineral content (Ravindran et al., 2018). Bull et al. (2017) heated 10% WPC80 solutions at 70°C in a water bath for 0, 5, 10 and 20 min. They found no change in zeta potential with increased heating time, even with increasing particle size. However, at more extreme conditions (88°C, 2 min) Barrios Quant et al. (2019) reported that heat treatment increased particle size and made zeta potential more negative at both pH 3.0 and 7.0 in formulated WPI beverages (10% protein). There appears to be a relationship between particle size of whey protein and the charge, which is evident in our experiment of the two being correlated. There was no correlation between pH and zeta potential (WPI r= 0.18; WPC80 r= -0.26, p>0.05) which may be because some companies may adjust pH, as this is common in IX as the primary way to elute the protein from charged resin.

In our study, we found that as zeta potential became more negative, the astringency of the WPI protein solutions increased. These results suggest that a greater overall negative charge of the whey protein makes them more prone to interaction with either salivary proteins or oral receptors. Ye et al. (2012) found that there was a relationship between the perception of astringency of whey proteins and the binding of these proteins to oral epithelial cells. However, the impact of more negative zeta potential did not correlate with astringency in WPC80. The impact of a more negative zeta potential on astringency is only true under the same conditions of
pH. As pH decreases, zeta potential of whey proteins become more positive and astringency increases with a decrease in pH. Therefore, if two protein solutions have the same pH but different zeta potential, the solution with a more negative zeta potential is likely more astringent. There have been no studies that have compared zeta potential of whey proteins at a similar pH range.

WPC80 differs from WPI in protein content (80% vs. 90%) and fat content (5-8% vs. <1%). There has been no research on the contribution of whey fat on astringency. In this study, a 0.45 micron prefilter was used before analysis of particle size and zeta potential. The particle size of typical milk fat would be larger than 0.45 microns (Mulder and Walstra, 1974), however, during whey processing, fluid whey undergoes clarification and fat separation which removes the majority of the lipid content. The lipid that remains in the fluid whey stream is likely smaller and may be complexed with proteins such as milkfat globule membrane (MFGM) proteins. Ye et al. (2004) demonstrated that heating of whole milk (60-95 C) for 10 minutes created interactions between beta-lactoglobulin and alpha-lactalbumin with MFGM. These aggregates may create astringency similar to whey protein aggregates. There has been no research on the astringency of MFGM, or whey lipid. It is possible that complexed whey fat and proteins such as MFGM contribute to astringency and result in different direct impacts on astringency of WPC80 compared to WPI. Future research should address this topic.

It has been proposed that fat acts as a lubricant that reduces perceived roughness and friction in the mouth. The presence of milk fat decreased astringency in ultra-pasteurized milk (Lee et al., 2017; Li et al., 2018). It is also possible that the fat competes with protein via the mucoadhesion mechanism, limiting the perceptions of astringency. In contrast, the addition of fat (cream and sunflower oil) to higher protein beverages (7-11.8% w/v) appeared to have little
effect and may have even increased astringency (Withers et al., 2014). This result is different from the concept proposed by Gachons et al. (2012) which suggested that oral fatty sensation and astringency were opposite sides of the sensory perspective. Fat provides lubrication and has been called slippery, while astringent compounds create a dry and rough mouthfeel. The addition of fat may help reduce astringency in neutral pH whey protein beverages where the fat globule size is >45 um. The WPC80 solutions in this study did not exhibit a correlation between particle size or zeta potential with astringency. Fat content of the different WPC80, or the fat globule size may be too small to have an effect, or may alter oral conditions in the mouth making it difficult to correlate to a parameter like particle size or zeta potential. A better understanding of the MFGM proteins and or the actual state of whey fat in WPC80 may help us better understand astringency of WPC80 and how it may be different from WPI.

Other possible mechanisms for astringency mentioned earlier is the possibility of whey protein aggregates that interact directly with bound salivary proteins on the oral epithelium or oral mechanoreceptors. In our study, whey proteins were astringent even when the mouth was held stationary. This result suggests that whey proteins interact with salivary tissues, regardless of the contribution of friction or oral movement. These interactions would cause dryness, as hydrating salvia protein is bound with whey protein, in the mouth and tissue tightening causing deforming of the mucosal pellicle, suggestive of whey protein interacting directly with salivary proteins covalently bound to oral tissues. Also in our study, oral movement increased perceived astringency. This suggests that the initial slippery layer of saliva that is removed by whey protein interaction exposes mechanoreceptors that can further detect particle and increase friction.

Lastly, whey protein/saliva aggregates may overcome lubrication in the mouth and then the rubbing of deformed oral surfaces with perceivable particles may increase oral friction and
astringency. Ye et al. (2011) determined that heated (90 °C for 10 minutes) and unheated beta-lactoglobulin solutions (2% w/w) mixed with saliva had higher particle size and turbidity at pH 3.4 than beta-lactoglobulin solutions alone. Protein and saliva mixtures that were heated had higher turbidity and larger particle sizes at pH >5.0 than unheated samples. The heat treatment most likely denatured and aggregated some of the whey protein which increased particle size consistent with Bull et al. (2017). Increases in astringency and particle size were consistent and positively correlated over the pH range of 3.0 to 3.6, suggesting that denaturation/aggregation added to the astringency associated with proteins. It appears that forming protein aggregates alone doesn’t necessarily correlate to astringency, as high particle size and turbidity noticed at pH 5.0 did not cause the perception of astringency (Ye et al., 2011), even though charge interactions between whey protein and saliva were demonstrated. This result agrees with the current results of our study that demonstrated that particle size of the proteins was not significantly correlated with astringency of the solution for both WPI and WPC80.

The main focus of this research was to explore possible mechanisms of astringency for whey protein. We found that oral movements increased the perception of astringency regardless of pH, and zeta potential played a major role in the perception of astringency. Our results point to strong contributions of oral friction and charged electrostatic interactions in the mouth to the perception of astringency. Sourcing protein from different suppliers and processes allowed us to view the larger picture of whey protein astringency, however inherent differences among samples made it more difficult to find correlations of particle size and astringency, possibly due to unknown confounding factors inherit to the whey protein samples that also contributed to astringency. Inserting experimental variables with varying mineral content to create a range of samples with different zeta potential will help confirm the relationship between zeta potential
and astringency. It is also important to better characterize the role of whey fat in dried whey ingredients and to have a better understanding of how MFGM protein participates in reactions in the mouth. Future work should better characterize how processing parameters of whey protein effect zeta potential and possible adjustments to control the zeta potential of whey protein ingredients.

Conclusions

Whey protein astringency is complex and a summation of many interacting mechanisms which has made it difficult to define and control. In our study we determined that both WPI and WPC were astringent at neutral pH. Acidification of WPI increased astringency, but astringency was still detectable in a neutral pH range (pH 6.0-7.0). Oral movements increased the perception of astringency, suggesting that part of the astringent sensation is interaction with oral surface receptors and further aggravation is a combination of friction of delubricated oral surfaces. Commercial WPI and WPC varied widely in both particle size and zeta potential. Astringency of a wide selection of commercial WPI solutions was correlated with zeta potential but not particle size. WPC80 were astringent, but our results did not correlate astringency with particle size or zeta potential. Astringency is an important characteristic in whey protein products that leads to lower liking, improvements in astringency may improve consumer acceptance of high protein products.

Acknowledgements

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References


Tables and Figures

Table 1. Analysis of variance type III sum of squares F statistics for descriptive analysis and time intensity of whey protein solutions from 3 protein types (microfiltration of cheese whey, ion exchange of cheese whey, microfiltration of skim milk), 2 pH values (3.4, 7.0), and 2 treatments (oral movement, no oral movement)

<table>
<thead>
<tr>
<th>Factor</th>
<th>Descriptive Analysis</th>
<th>Time Intensity</th>
</tr>
</thead>
<tbody>
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<td>Astringency</td>
<td>Time of Max</td>
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<tr>
<td>Protein</td>
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<tr>
<td>pH</td>
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<td>65.370</td>
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<tr>
<td>Protein*pH</td>
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<tr>
<td>Protein* oral movement</td>
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<td>1.337</td>
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<tr>
<td>pH* oral movement</td>
<td>6.072</td>
<td>1.122</td>
</tr>
<tr>
<td>Protein<em>pH</em> oral movement</td>
<td>3.825</td>
<td>0.604</td>
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</table>

Bold values are significant (P<0.05)
Figure 1. Mean trained panel astringency intensities of 3 different whey/serum protein solutions at 2 different pH values under 2 oral movement conditions.

<table>
<thead>
<tr>
<th>Oral Movement</th>
<th>No Oral Movement</th>
<th>Oral Movement</th>
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<tr>
<td>IX 3.4</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>SPI 3.4</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>MF 3.4</td>
<td>1</td>
<td>1</td>
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<tr>
<td>Buffered Acid</td>
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</tr>
<tr>
<td>MF 7</td>
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<td>4</td>
</tr>
<tr>
<td>IX 7</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>SPI 7</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

*Means in the same column no sharing a common superscript are different (P< 0.05). MF- microfiltration, IX- ion exchange, SPI- serum protein isolate.*
Table 2. Effect of pH and oral movement on the perception of astringency by TI

<table>
<thead>
<tr>
<th></th>
<th>Time Of Max</th>
<th>Intensity at Max</th>
<th>Duration</th>
<th>Area Under Curve</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.4*Swish</td>
<td>40.6 a</td>
<td>6.6 a</td>
<td>137.1 a</td>
<td>578.7 a</td>
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<tr>
<td>3.4*Hold</td>
<td>40.5 a</td>
<td>5.9 b</td>
<td>129.8 a</td>
<td>487.9 b</td>
</tr>
<tr>
<td>7*Swish</td>
<td>26.3 b</td>
<td>1.3 c</td>
<td>68.9 b</td>
<td>77.0 c</td>
</tr>
<tr>
<td>7*Hold</td>
<td>29.5 b</td>
<td>1.2 c</td>
<td>59.1 c</td>
<td>66.6 c</td>
</tr>
</tbody>
</table>

abc Means in the same column no sharing a common superscript are different (P< 0.05).

Table 3. Effect of pH and protein type on the perception of astringency by TI

<table>
<thead>
<tr>
<th></th>
<th>Time Of Max</th>
<th>Intensity At Max</th>
<th>Duration</th>
<th>Area Under Curve</th>
</tr>
</thead>
<tbody>
<tr>
<td>IX*3.4</td>
<td>40.9 a</td>
<td>6.6 a</td>
<td>134.6 a</td>
<td>571.8 a</td>
</tr>
<tr>
<td>SPI*3.4</td>
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<td>6.4 a</td>
<td>135.5 a</td>
<td>543.5 a</td>
</tr>
<tr>
<td>MF*3.4</td>
<td>41.8 a</td>
<td>5.8 b</td>
<td>130.3 a</td>
<td>484.6 b</td>
</tr>
<tr>
<td>SPI*7</td>
<td>28.6 b</td>
<td>1.3 c</td>
<td>66.5 b</td>
<td>75.2 c</td>
</tr>
<tr>
<td>MF*7</td>
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<td>1.3 c</td>
<td>65.0 b</td>
<td>71.7 c</td>
</tr>
<tr>
<td>IX*7</td>
<td>27.0 b</td>
<td>1.3 c</td>
<td>60.5 b</td>
<td>68.5 c</td>
</tr>
</tbody>
</table>

abc Means in the same column no sharing a common superscript are different (P< 0.05). MF- microfiltration, IX- ion exchange, SPI- serum protein isolate
Figure 2. TI curves of 10 % whey protein solutions (w/w) from 3 protein types (microfiltration of cheese whey, ion exchange of cheese whey, microfiltration of skim milk and 2 treatments (oral movement, no oral movement) at pH 7 (top) or pH 3.4 (bottom).
Table 4. Radius, Zeta potential and pH of 37 commercial WPI from different manufacturers

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Sample</th>
<th>Radius (nm)</th>
<th>Zeta Potential (mV)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>WPI 1</td>
<td>78.62 ab</td>
<td>-13.47 bc</td>
<td>6.34 e</td>
</tr>
<tr>
<td>B</td>
<td>WPI 2</td>
<td>57.72 fgh</td>
<td>0.38 a</td>
<td>6.99 b</td>
</tr>
<tr>
<td>B</td>
<td>WPI 3</td>
<td>67.73 de</td>
<td>-20.38 d</td>
<td>7.07 a</td>
</tr>
<tr>
<td>C</td>
<td>WPI 4</td>
<td>41.96 lm</td>
<td>-16.94 bcd</td>
<td>6.26 g</td>
</tr>
<tr>
<td>B</td>
<td>WPI 5</td>
<td>42.78 klm</td>
<td>-18.890 bcd</td>
<td>6.11 i</td>
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<tr>
<td>B</td>
<td>WPI 6</td>
<td>47.54 jk</td>
<td>-21.09 d</td>
<td>6.97 b</td>
</tr>
<tr>
<td>C</td>
<td>WPI 7</td>
<td>45.39 jkl</td>
<td>-19.41 bcd</td>
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<tr>
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<td>WPI 8</td>
<td>60.86 efg</td>
<td>-38.77 ijk</td>
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<tr>
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<tr>
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<td>-28.10 ef</td>
<td>6.92 c</td>
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<td>WPI 11</td>
<td>31.73 o</td>
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<td>-17.46 bcd</td>
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<td>WPI 13</td>
<td>75.98 bc</td>
<td>-49.38 n</td>
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</tr>
<tr>
<td>A</td>
<td>WPI 14</td>
<td>84.18 a</td>
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<tr>
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<td>WPI 15</td>
<td>73.07 cd</td>
<td>-48.51 mn</td>
<td>6.36 e</td>
</tr>
<tr>
<td>A</td>
<td>WPI 16</td>
<td>71.96 cd</td>
<td>-48.20 lmn</td>
<td>6.30 ef</td>
</tr>
<tr>
<td>A</td>
<td>WPI 17</td>
<td>57.06 fgh</td>
<td>-34.50 fghi</td>
<td>6.33 e</td>
</tr>
<tr>
<td>B</td>
<td>WPI 18</td>
<td>35.13 no</td>
<td>-17.22 bcd</td>
<td>6.07 j</td>
</tr>
<tr>
<td>B</td>
<td>WPI 19</td>
<td>59.96 fgh</td>
<td>-41.92 jkl</td>
<td>7.0 b</td>
</tr>
<tr>
<td>C</td>
<td>WPI 20</td>
<td>43.51 klm</td>
<td>-19.69 cd</td>
<td>6.25 f</td>
</tr>
<tr>
<td>A</td>
<td>WPI 21</td>
<td>62.64 ef</td>
<td>-43.30 klmn</td>
<td>6.22 g</td>
</tr>
<tr>
<td>B</td>
<td>WPI 22</td>
<td>42.46 klm</td>
<td>-19.47 bcd</td>
<td>6.08 j</td>
</tr>
<tr>
<td>C</td>
<td>WPI 23</td>
<td>47.14 jkl</td>
<td>-22.65 de</td>
<td>6.25 f</td>
</tr>
<tr>
<td>C</td>
<td>WPI 24</td>
<td>46.90 jkl</td>
<td>-21.83 de</td>
<td>6.26 f</td>
</tr>
<tr>
<td>B</td>
<td>WPI 25</td>
<td>54.64 hi</td>
<td>-29.99 fg</td>
<td>6.09 ij</td>
</tr>
<tr>
<td>B</td>
<td>WPI 26</td>
<td>59.13 fgh</td>
<td>-42.06 jklm</td>
<td>6.98 b</td>
</tr>
<tr>
<td>C</td>
<td>WPI 27</td>
<td>45.53 jkl</td>
<td>-21.47 d</td>
<td>6.24 g</td>
</tr>
<tr>
<td>A</td>
<td>WPI 28</td>
<td>58.74 fgh</td>
<td>-40.35 ijk</td>
<td>6.29 ef</td>
</tr>
<tr>
<td>B</td>
<td>WPI 29</td>
<td>32.92 o</td>
<td>-18.16 bcd</td>
<td>6.12 i</td>
</tr>
<tr>
<td>B</td>
<td>WPI 30</td>
<td>39.14 mn</td>
<td>-19.29 bcd</td>
<td>6.10 i</td>
</tr>
<tr>
<td>B</td>
<td>WPI 31</td>
<td>50.72 ij</td>
<td>-30.21 fgh</td>
<td>6.97 b</td>
</tr>
<tr>
<td>C</td>
<td>WPI 32</td>
<td>46.05 jkl</td>
<td>-22.91 de</td>
<td>6.33 e</td>
</tr>
<tr>
<td>A</td>
<td>WPI 33</td>
<td>55.77 ghi</td>
<td>-35.19 ghi</td>
<td>6.27 f</td>
</tr>
<tr>
<td>A</td>
<td>WPI 34</td>
<td>56.68 gh</td>
<td>-36.74 ij</td>
<td>6.23 gh</td>
</tr>
<tr>
<td>B</td>
<td>WPI 35</td>
<td>42.07 lm</td>
<td>-22.94 de</td>
<td>6.10 i</td>
</tr>
<tr>
<td>B</td>
<td>WPI 36</td>
<td>30.72 o</td>
<td>-19.85 cd</td>
<td>6.10 i</td>
</tr>
<tr>
<td>B</td>
<td>WPI 37</td>
<td>57.04 fgh</td>
<td>-59.27 o</td>
<td>7.05 a</td>
</tr>
</tbody>
</table>

a-o Means in the same column no sharing a common superscript are different (P< 0.05)
Table 5. Radius, Zeta potential and pH of 30 commercial WPC80 from different manufacturers

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Sample</th>
<th>Radius (nm)</th>
<th>Zeta Potential (mV)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>WPC 1</td>
<td>85.62 cdefg</td>
<td>-11.39 bc</td>
<td>6.19 de</td>
</tr>
<tr>
<td>A</td>
<td>WPC 2</td>
<td>87.49 abcd</td>
<td>-20.41 cd</td>
<td>6.28 c</td>
</tr>
<tr>
<td>B</td>
<td>WPC 3</td>
<td>81.54 ghij</td>
<td>-0.15 a</td>
<td>6.2 de</td>
</tr>
<tr>
<td>A</td>
<td>WPC 4</td>
<td>88.22 abc</td>
<td>-38.42 gh</td>
<td>6.24 d</td>
</tr>
<tr>
<td>B</td>
<td>WPC 5</td>
<td>80.25 hijk</td>
<td>-9.56 ab</td>
<td>6.4 a</td>
</tr>
<tr>
<td>B</td>
<td>WPC 6</td>
<td>83.70 defgh</td>
<td>-27.56 def</td>
<td>6.22 d</td>
</tr>
<tr>
<td>B</td>
<td>WPC 7</td>
<td>81.93 fghij</td>
<td>-35.76 efg</td>
<td>6.2 de</td>
</tr>
<tr>
<td>B</td>
<td>WPC 8</td>
<td>83.06 efghi</td>
<td>-38.28 gh</td>
<td>6.31 b</td>
</tr>
<tr>
<td>B</td>
<td>WPC 9</td>
<td>79.98 hijk</td>
<td>-16.84 bc</td>
<td>6.27 c</td>
</tr>
<tr>
<td>B</td>
<td>WPC 10</td>
<td>80.60 hij</td>
<td>-37.47 fg</td>
<td>6.44 a</td>
</tr>
<tr>
<td>A</td>
<td>WPC 11</td>
<td>85.97 bcdef</td>
<td>-39.54 gh</td>
<td>6.23 d</td>
</tr>
<tr>
<td>A</td>
<td>WPC 12</td>
<td>86.53 bcde</td>
<td>-40.89 gh</td>
<td>6.37 b</td>
</tr>
<tr>
<td>A</td>
<td>WPC 13</td>
<td>90.97 a</td>
<td>-43.85 gh</td>
<td>6.28 c</td>
</tr>
<tr>
<td>B</td>
<td>WPC 14</td>
<td>80.76 hijk</td>
<td>-38.59 gh</td>
<td>6.37 b</td>
</tr>
<tr>
<td>B</td>
<td>WPC 15</td>
<td>79.62 hijk</td>
<td>-35.77 efg</td>
<td>6.23 d</td>
</tr>
<tr>
<td>B</td>
<td>WPC 16</td>
<td>80.06 hijk</td>
<td>-37.79 gh</td>
<td>6.17 e</td>
</tr>
<tr>
<td>A</td>
<td>WPC 17</td>
<td>90.20 ab</td>
<td>-47.69 h</td>
<td>6.29 bc</td>
</tr>
<tr>
<td>A</td>
<td>WPC 18</td>
<td>85.44 cdefg</td>
<td>-57.59 gh</td>
<td>6.26 c</td>
</tr>
<tr>
<td>B</td>
<td>WPC 19</td>
<td>72.58 l</td>
<td>-13.85 bc</td>
<td>6.18 e</td>
</tr>
<tr>
<td>A</td>
<td>WPC 20</td>
<td>85.58 cdefg</td>
<td>-41.73 gh</td>
<td>6.28 c</td>
</tr>
<tr>
<td>B</td>
<td>WPC 21</td>
<td>76.17 kl</td>
<td>-27.12 de</td>
<td>6.29 bc</td>
</tr>
<tr>
<td>B</td>
<td>WPC 22</td>
<td>80.06 hijk</td>
<td>-39.08 gh</td>
<td>6.18 e</td>
</tr>
<tr>
<td>B</td>
<td>WPC 23</td>
<td>79.14 ijk</td>
<td>-38.35 gh</td>
<td>6.2 de</td>
</tr>
<tr>
<td>A</td>
<td>WPC 24</td>
<td>85.29 cdefg</td>
<td>-44.37 gh</td>
<td>6.27 c</td>
</tr>
<tr>
<td>B</td>
<td>WPC 25</td>
<td>79.08 ijk</td>
<td>-38.90 gh</td>
<td>6.17 e</td>
</tr>
<tr>
<td>B</td>
<td>WPC 26</td>
<td>78.41 jk</td>
<td>-39.29 gh</td>
<td>6.41 a</td>
</tr>
<tr>
<td>B</td>
<td>WPC 27</td>
<td>78.93 ijk</td>
<td>-39.33 gh</td>
<td>6.21 d</td>
</tr>
<tr>
<td>B</td>
<td>WPC 28</td>
<td>78.99 ijk</td>
<td>-40.82 gh</td>
<td>6.19 de</td>
</tr>
<tr>
<td>B</td>
<td>WPC 29</td>
<td>79.56 hijk</td>
<td>-41.68 gh</td>
<td>6.19 de</td>
</tr>
<tr>
<td>A</td>
<td>WPC 30</td>
<td>73.04 l</td>
<td>-42.88 gh</td>
<td>6.06 f</td>
</tr>
</tbody>
</table>

Means in the same column no sharing a common superscript are different (P< 0.05)
Table 6. Descriptive analysis results of WPI (n=12) at 10 % protein (w/w)

<table>
<thead>
<tr>
<th>Samples</th>
<th>Radius (nm)</th>
<th>Zeta Potential (mV)</th>
<th>pH</th>
<th>Astringency</th>
<th>Fat (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WPI 6</td>
<td>47.54</td>
<td>-21.09</td>
<td>6.97</td>
<td>2.2 d</td>
<td>0.3</td>
</tr>
<tr>
<td>WPI 13</td>
<td>75.98</td>
<td>-49.38</td>
<td>6.72</td>
<td>2.5 c</td>
<td>0.4</td>
</tr>
<tr>
<td>WPI 14</td>
<td>84.18</td>
<td>-60.02</td>
<td>6.29</td>
<td>3.4 a</td>
<td>0.7</td>
</tr>
<tr>
<td>WPI 33</td>
<td>55.77</td>
<td>-35.19</td>
<td>6.27</td>
<td>3.0 b</td>
<td>0.8</td>
</tr>
<tr>
<td>WPI 4</td>
<td>41.96</td>
<td>-16.94</td>
<td>6.26</td>
<td>1.9 e</td>
<td>0.9</td>
</tr>
<tr>
<td>WPI 23</td>
<td>47.14</td>
<td>-22.65</td>
<td>6.25</td>
<td>2.0 d</td>
<td>0.3</td>
</tr>
<tr>
<td>WPI 36</td>
<td>30.72</td>
<td>-19.85</td>
<td>6.10</td>
<td>2.5 c</td>
<td>0.3</td>
</tr>
<tr>
<td>WPI 25</td>
<td>54.64</td>
<td>-30.00</td>
<td>6.09</td>
<td>2.2 d</td>
<td>0.7</td>
</tr>
<tr>
<td>WPI 18</td>
<td>35.13</td>
<td>-17.22</td>
<td>6.07</td>
<td>2.5 c</td>
<td>0.5</td>
</tr>
<tr>
<td>WPI 20</td>
<td>43.51</td>
<td>-19.69</td>
<td>6.25</td>
<td>2.1 d</td>
<td>0.3</td>
</tr>
<tr>
<td>WPI 2</td>
<td>57.72</td>
<td>0.38</td>
<td>6.99</td>
<td>1.8 e</td>
<td>0.7</td>
</tr>
<tr>
<td>WPI 16</td>
<td>71.96</td>
<td>-48.20</td>
<td>6.30</td>
<td>2.9 b</td>
<td>0.3</td>
</tr>
</tbody>
</table>

a-e Means in the same column no sharing a common superscript are different (P< 0.05). Percent fat means provided by manufacturer.

Table 7. Descriptive analysis results of WPC (n=13) at 10 % protein (w/w)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Radius (nm)</th>
<th>Zeta Potential (mV)</th>
<th>pH</th>
<th>Astringency</th>
<th>Fat (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WPC 14</td>
<td>80.76</td>
<td>-38.60</td>
<td>6.37</td>
<td>2.3 c</td>
<td>5.5</td>
</tr>
<tr>
<td>WPC 21</td>
<td>76.17</td>
<td>-27.12</td>
<td>6.29</td>
<td>2.7 b</td>
<td>5.6</td>
</tr>
<tr>
<td>WPC 7</td>
<td>81.93</td>
<td>-35.76</td>
<td>6.20</td>
<td>2.0 d</td>
<td>5.9</td>
</tr>
<tr>
<td>WPC 19</td>
<td>72.58</td>
<td>-13.85</td>
<td>6.18</td>
<td>2.3 c</td>
<td>6.2</td>
</tr>
<tr>
<td>WPC 3</td>
<td>81.54</td>
<td>-0.15</td>
<td>6.20</td>
<td>2.4 c</td>
<td>5.7</td>
</tr>
<tr>
<td>WPC 1</td>
<td>85.62</td>
<td>-11.39</td>
<td>6.19</td>
<td>2.0 d</td>
<td>6.9</td>
</tr>
<tr>
<td>WPC 17</td>
<td>90.20</td>
<td>-47.69</td>
<td>6.29</td>
<td>2.7 b</td>
<td>6.1</td>
</tr>
<tr>
<td>WPC 12</td>
<td>86.53</td>
<td>-40.90</td>
<td>6.37</td>
<td>2.5 bc</td>
<td>5.8</td>
</tr>
<tr>
<td>WPC 13</td>
<td>90.97</td>
<td>-43.85</td>
<td>6.28</td>
<td>2.6 b</td>
<td>5.9</td>
</tr>
<tr>
<td>WPC 23</td>
<td>79.14</td>
<td>-38.35</td>
<td>6.20</td>
<td>2.7 b</td>
<td>8.0</td>
</tr>
<tr>
<td>WPC 16</td>
<td>80.06</td>
<td>-37.79</td>
<td>6.17</td>
<td>2.1 d</td>
<td>7.0</td>
</tr>
<tr>
<td>WPC 30</td>
<td>73.04</td>
<td>-42.87</td>
<td>6.06</td>
<td>2.5 bc</td>
<td>7.7</td>
</tr>
<tr>
<td>WPC 24</td>
<td>85.29</td>
<td>-44.37</td>
<td>6.27</td>
<td>3.0 a</td>
<td>7.6</td>
</tr>
</tbody>
</table>

a-d Means in the same column no sharing a common superscript are different (P< 0.05). Percent fat means provided by manufacturer.
Table 8. Correlation of particle size, zeta potential, pH and astringency in WPI (n=12) and WPC (n=13)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Radius (nm)</th>
<th>Zeta Potential (mV)</th>
<th>pH</th>
<th>Astringency</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>WPI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Radius (nm)</td>
<td>1</td>
<td>-0.779</td>
<td>0.296</td>
<td>0.538</td>
</tr>
<tr>
<td>Zeta Potential (mV)</td>
<td>-0.779</td>
<td>1</td>
<td>0.184</td>
<td>-0.809</td>
</tr>
<tr>
<td>pH</td>
<td>0.296</td>
<td>0.184</td>
<td>1</td>
<td>-0.292</td>
</tr>
<tr>
<td>Astringency</td>
<td>0.538</td>
<td>-0.809</td>
<td>-0.292</td>
<td>1</td>
</tr>
<tr>
<td><strong>WPC 80</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Radius (nm)</td>
<td>1</td>
<td>-0.308</td>
<td>0.540</td>
<td>0.146</td>
</tr>
<tr>
<td>Zeta Potential (mV)</td>
<td>-0.308</td>
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<td>-0.263</td>
<td>-0.400</td>
</tr>
<tr>
<td>pH</td>
<td>0.540</td>
<td>-0.263</td>
<td>1</td>
<td>0.257</td>
</tr>
<tr>
<td>Astringency</td>
<td>0.146</td>
<td>-0.400</td>
<td>0.257</td>
<td>1</td>
</tr>
</tbody>
</table>

Values in bold are significant (P<0.05).

Figure 3. Scatterplots correlating astringency and zeta potential, and radius and zeta potential of WPI (n=12)