Role of protein concentration and protein–saliva interactions in the astringency of whey proteins at low pH

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ABSTRACT

Whey protein beverages are adjusted to pH <4.5 to enhance clarity and stability, but this pH level is also associated with increased astringency. The goal of this investigation was to determine the effects of protein concentration on astringency and interactions between whey and salivary proteins. Whey protein beverages containing 0.25 to 13% (wt/wt) β -lactoglobulin and 0.017% (wt/wt) sucralose at pH 2.6 to 4.2 were examined using descriptive sensory analysis. Controls were similar pH phosphate buffers at phosphate concentrations equivalent to the amount of phosphoric acid required to adjust the pH of the protein solution. Changes in astringency with protein concentration depended on pH. At pH 3.5, astringency significantly increased with protein concentration from 0.25 to 4% (wt/wt) and then remained constant from 4 to 13% (wt/wt). Conversely, at pH 2.6, astringency decreased with an increase in protein concentration [0.5–10% (wt/wt)]. This suggests a complex relationship that includes pH and buffering capacity of the beverages. Furthermore, saliva flow rates increased with increasing protein concentrations, showing that the physiological conditions in the mouth change with protein concentration. Maximum turbidity of whey protein–saliva mixtures was observed between pH 4.6 and 5.2. Both sensory evaluation and in vitro study of interactions between β -LG and saliva indicate that astringency of whey proteins is a complex process determined by the extent of aggregation occurring in the mouth, which depends on the whey protein beverage pH and buffering capacity in addition to saliva flow rate.

Key words: astringency, β -lactoglobulin, saliva

INTRODUCTION

Whey protein is increasingly being used in beverages because of its wide variety of nutritional and health

benefits, including antimicrobial activity, increasing muscle strength, and reducing the risk of cardiovascular disease (Marshall, 2004). Protein beverages are formulated at a low pH to increase stability and clarity (Phillips et al., 1994); however, at low pH, whey protein exhibits astringency (Sano et al., 2005; Beecher et al., 2008). According to the American Society for Testing and Materials Committee E-18 on Sensory Evaluation, astringency is defined as “the complex of sensations due to shrinking, drawing, or puckering of the epithelium as a result of exposure to substances such as alums or tannic acids” (ASTM, 1995).

The mechanism of astringency observed in beverages containing whey proteins is still unknown; however, 3 possibilities have been suggested. Sano et al. (2005) attribute the astringency to direct precipitation of the whey protein in the mouth caused by a pH shift. They proposed that when saliva in the mouth (pH ~7.0) is mixed with an acidic (pH 3.5) whey protein solution, the mixture decreases in pH to approximately the isoelectric point of whey proteins, causing precipitation. Astringency is generally considered a tactile sensation; therefore, their model would suggest that whey protein aggregates are directly responsible for tactile changes because no direct involvement from salivary proteins was mentioned by Sano et al. (2005). It has also been proposed that the acid used to lower the protein beverage pH is the direct cause of astringency, with no specific involvement of the whey proteins (Lee and Vickers, 2008). This is based on previous work showing acids alone can cause astringency (Thomas and Lawless, 1995; Lawless et al., 1996; Sowalsky and Noble, 1998). These 2 models have vastly different implications on adjusting astringency in whey protein beverages. If it is whey protein precipitation alone, then factors increasing solubility when mixed with saliva should decrease astringency. If acid alone, then the only factor important is the amount and type of acid.

Another possible astringency mechanism is that it is caused by interactions between negatively charged saliva proteins and positively charged whey proteins (Beecher et al., 2008). In this case, the important factor is the charge on the proteins, which will depend on the

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pH of the solution relative to the isoelectric point of the proteins. The whey protein–saliva protein interaction model proposed by Beecher et al. (2008) is similar to the generally accepted mechanism for astringency of polyphenols, where there is a complex formation with saliva proline-rich proteins and polyphenols (Sarni-Manchado et al., 1999; Gambuti et al., 2006).

A complicating factor is that all of the suggested mechanisms of astringency involve saliva, at varying degrees; therefore, some key properties of saliva must be considered. Saliva is a clear, mucoserous excretion composed of a complex mix of molecules, including proteins (Humphrey and Williamson, 2001). Some of the proteins found in saliva are, but are not limited to, mucins (Masson et al., 1965); acidic, basic, and glycosylated proline-rich proteins (Mehansho et al., 1985), lactoferrin (Dodds et al., 2005), α -amylase (Ghafouri et al., 2003), and histatins (Yan and Bennick, 1995). One of saliva's main roles is lubrication between oral surfaces (Schipper et al., 2007). Proline-rich proteins (Hay and Oppenheim, 1974), mucin (Tabak et al., 1982), α -amylase (Aguirre et al., 1989), and statherin (Douglas et al., 1991) are shown to contribute to lubrication. When astringent substances are consumed, it is assumed that the natural lubrication property of saliva is diminished (Lemieux and Simard, 1994). This could be caused by the removal of lubricating proteins or formation of a complex that increases friction.

The association between binding of salivary proteins and astringency of polyphenols is well known. Bate-Smith (1973) found that salivary proline-rich proteins interact with polyphenols and form insoluble complexes. Formation of protein–tannin complexes results in the precipitation, or aggregation, or both, of salivary proteins, causing them to lose their lubrication properties. This has led to a 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, resulting in the roughening, tightening, and drying sensations. Although it is clear that polyphenols can interact with salivary protein-rich proteins, how this interaction translates into the physiological sensation of astringency is still not clear. Tannins reduce the viscosity of saliva, thus possibly increasing the friction in the mouth, resulting in higher astringency ratings and loss of lubrication (Prinz and Lucas, 2000). Other investigations found that saliva viscosity does not play a role in lubrication (Levine et al., 1987; de Wijk and Prinz, 2005), whereas others suggest the astringency mechanism is not simple tactile sensation, but rather may involve a receptor (Schiffman et al., 1992; Schwarz and Hofmann, 2008).

Some studies have investigated the effect of astringent compound concentration on the perceived amount of astringency. Doubling the concentration of alum from 0.5 and 1.0 g/L caused a significant increase in the maximum intensity (Smith and Noble, 1998). Thomas and Lawless (1995) found an increase in astringency as acid concentration increased (hydrochloric acid: 0.46–1.38 g/L; citric acid: 1.25–3.74 g/L; phosphoric acid: 0.5–1.5 g/L). A subsequent study compared acids based on hydrogen ion equivalents and showed that increasing the concentration of lactic, malic, tartaric, or citric acid 5-fold (0.02–0.10 *N*) had no effect on the maximum astringency rating for any of the acids at the same pH (Sowalsky and Noble, 1998). They conclude that astringency of acids is a function of pH rather than type of acid. Increasing polyphenol concentrations from 0.94 to 2.13 g/L causes an increase in astringency (Monteleone et al., 2004); however, as polyphenol concentration increases at higher concentration (0.39–4.48 g/L), the astringency plateaus (Condelli et al., 2006). This conflicts the findings of Gambuti et al. (2006), who reported that an increase in commercial tannin concentration (0–10 g/L) caused an increase in astringency with no plateau occurring. Clearly, the relationship between concentration of astringent compounds (acid and polyphenols) and perceived astringency does not show consistent concentration dependence and suggests that factors other than concentration may be involved.

The only study to investigate the effects of whey protein concentrations on astringency was that of Sano et al. (2005), who showed that increasing from 0.0 to 0.5% (wt/vol) coincided with increased astringency. This concentration range has limited value to food applications. The United States FDA labeling law states that in order for a food to be considered a good or excellent source of protein, it must contain 10 to 19% or more than 20% of the reference daily intake for protein, respectively (21CFR101.54; FDA, 2009a). For a beverage with a 250-mL reference serving and reference daily intake value of 50 g (21CFR101.9; FDA, 2009b), the protein concentration required is 2 to 4% (wt/vol), well above the concentrations investigated by Sano et al. (2005).

From the above discussion it is clear that there are conflicting reports on astringency intensity and astringent compound concentration. Also, little is known about the concentration effects of protein-based astringency. Therefore, the objectives of our study were to understand how protein concentration affects the overall astringency in acidic beverages and to understand the role saliva plays in the astringency mechanism.

β -Lactoglobulin, the major protein in whey, was used to prepare model beverages in this study.

MATERIALS AND METHODS

Materials

The β -LG was a gift from Davisco Foods International (Le Sueur, MN). Sucrose (purchased at a local grocery store; Imperial Sugar Company, Savannah, GA), sodium chloride (Sigma, St. Louis, MO), and citric acid (Tate and Lyle, London, UK) were used as references for sweet, salty, and sour, respectively. Alum (McCormick, Sparks, MD) was used as astringency reference solution (da Conceicao et al., 2007). Carboxymethyl cellulose (CMC) was donated by Hercules-Aqualon (Wilmington, DE).

Effect of Protein Concentration on Astringency of Whey Proteins

Because of the high number of samples, sensory evaluation was divided into 3 studies. The first study investigated the effect of protein concentrations ranging from 0.25 to 4%. Protein concentration in the second study ranged from 4 to 13%. In the third study, samples were selected to cover the whole range of concentration, and time-intensity analysis was chosen to provide more comprehensive data (i.e., time to reach maximum astringency, astringency duration).

Sample Preparation. β -Lactoglobulin solutions were prepared by dissolving protein powder in deionized water overnight at approximately 80% final weight. Once hydrated, the solution pH was adjusted to 3.5 using 1 M phosphoric acid. Final protein concentrations were 0.25, 0.5, 0.75, 1, 2, 4, 7, 10, and 13% (wt/wt). Sucralose was added to the beverages at 0.017% (wt/wt). Phosphate buffer solutions were prepared as controls from stock solutions of 1 M phosphoric acid and 1 M dibasic sodium phosphate. The amount of acid used to adjust each protein solution [i.e., 0.5–13% (wt/wt)] to pH 3.5 was determined and expressed as molarity. Aliquots of 1 M phosphoric acid, 1 M dibasic sodium phosphate, and water were mixed such that the final solution pH was 3.5 and the total phosphate molarity was equal in both the protein solutions and phosphate buffer solutions. Solutions for sensory analysis were dispensed using a dispensette (Brandtech Scientific Inc., Essex, CT) at 20 mL ($\pm$ 0.1 mL) per 59.1-mL plastic soufflé cup. Containers were labeled with a 3-digit random code to prevent bias. Samples were prepared the day before and held overnight at refrigerated temperatures. The samples were taken out before evaluation and equilibrated to $23 \pm 1^\circ\text{C}$.

For the time intensity study of protein concentration effects, β -LG solutions were prepared as above to contain 0.5, 1, 4, 7, and 10% (wt/wt) protein and adjusted to pH 3.5 using 1 M phosphoric acid.

Subjects. Ten healthy subjects (2 males and 8 females, 23–41 yr of age) were chosen to participate in the study based on their availability and interest. All panelists were students, staff, or faculty of North Carolina State University (Raleigh). Panelists were trained for 8 h (eight 1-h sessions) to familiarize them with astringency and basic taste evaluation (sour, sweet, salty, and bitter tastes) using the Spectrum method (Meilgaard et al., 1998; Beecher et al., 2008) and a 0 to 15-point universal intensity scale (Meilgaard et al., 1998; Beecher et al., 2008). For training of basic tastes, subjects were familiarized with sweet, sour, and bitter tastes by tasting 5% sucrose (sweet 5), 0.08% citric acid (sour 5), and 0.05% caffeine (bitter 2) solutions. Samples of known intensities (0 to 15 Spectrum intensity scale) were presented to panelists at each training session in 60-mL plastic cups randomly numbered with 3-digit codes. Samples and references were stored at $4 \pm 1^\circ\text{C}$ and tempered to room temperature 2 h before evaluation. During the training sessions, panelists indicated the maximum intensities for each sample using a 15-point scale labeled none at number 0 on the left and very strong at number 15 on the right. Ballots were collected and monitored for panelist accuracy and consistency. For astringency training, astringency was defined for the subjects as “the complex of sensations due to the shrinking, drawing, or puckering of the epithelium as a result of exposure to substances such as alums or tannins” (ASTM, 1995). Alum solutions of astringent intensities 2 (0.02% alum) and 10 (0.1% alum) were provided to panelists as examples of low and high intensities of astringency. Samples of known intensities of alum were presented to panelists at each training session. Panelists were instructed to take the entire sample (20 ± 0.1 mL) into their mouth and swish for 5 s before expectorating. Maximum astringent intensity was noted on paper ballots using 15-point category scales as described above. The carryover effect was minimized by a rinse protocol. After maximum intensity was reached and recorded, panelists went through the following rinse protocol to prevent carryover: first a bite of cracker (Ross et al., 2007), then a sip of water, a CMC rinse (0.55% CMC), and finally a sip of water (Brannan et al., 2001; Colonna et al., 2004). A 2-min wait period between samples was also enforced. Analysis of variance of data collected from preliminary sessions confirmed that the panel and panelists could consistently differentiate astringency and basic tastes.

Sample Evaluation. Panelists were asked to evaluate the samples for astringency and sour, sweet, salty,

and bitter tastes. Reference solutions were provided as a calibration. Samples were evaluated under red light conditions to eliminate the difference in the perception of color intensity. Four replications of each sample were evaluated by each panelist. Before each formal evaluation, panelists were provided with 3 reference solutions: sweet 5 (5% sucrose), sour 5 (0.08% citric acid), and salty 2 (0.325% sodium chloride). Panelists were required to taste a solution of alum (0.08% alum) to warm up the palate and then tasted the reference of astringent 4 (0.04% alum) or astringent 8 (0.08% alum) for low and high protein concentration studies, respectively. Four samples were evaluated each session using the Williams Latin squares method to reduce carryover effects (Schlich, 1993). After each sample, panelists went through the rinse protocol with a 2-min wait time as described previously.

Astringency was also evaluated based on time intensity using Compusense (version 5.0, Compusense Inc., Guelph, Ontario, Canada). Panelists were familiarized with the computer program with a step-by-step presentation followed by 3 training sessions specifically on this topic. Before evaluation, panelists tasted the astringency warm-up solution (0.08% alum) and a reference solution (astringent 4). During each sitting, maximum intensity (relative units), time to maximum intensity (s), duration of the astringency (s), and area under the curve (relative units) were obtained and used in the evaluation of astringency.

Combined Effect of pH and Protein Concentrations on Astringency of Whey Proteins

β -Lactoglobulin solutions [0.5, 2, and 10% (wt/wt)] were prepared and adjusted to pH 2.6, 3.4, and 4.2 to cover the range of protein concentration and pH. Sample astringency was evaluated using time intensity as described previously. Four replications were performed.

Saliva Flow Rates

Ten subjects (2 males and 8 females, 23–41 yr of age) were used to determine stimulated saliva flow rates. In preparing for saliva collections, volunteers rinsed the mouth out with deionized water and obtained a comfortable position, keep their head tilted downward to ease in the expectoration of saliva (Navazesh and Christensen, 1982). Different stimulants [water; 0.08% (wt/wt) alum; phosphate buffer at pH 3.5 with the equivalent concentration of phosphate in 4% protein solution; and 0.5, 4, and 10% protein solutions] were

evaluated. The stimulants were in 59.1-mL plastic soufflé cups and each contained 20 ± 0.1 mL. Samples were presented in a random order. Panelists were asked to taste the stimulant, swish in the mouth for 5 s, and expectorate. Saliva was then collected for a period of 2 min and flow rates were measured in g/min.

Saliva and β -LG Interactions

Three volunteers (3 females) were asked to participate in the study based on their availability and saliva production. People with stimulated saliva flow rates in the range of 1.7 to 2.4 g/min were selected to normalize flow rates. Saliva collection was done in the morning and used within 1 h. Subjects were required to refrain from eating or drinking anything but water at least 2 h before saliva collection (Fenoll-Palomares et al., 2004). In preparing for saliva collections, volunteers rinsed the mouth out with deionized water, obtained a comfortable position, and kept their head tilted downward to ease in the expectoration of saliva (Navazesh and Christensen, 1982). To stimulate saliva flow, 1 ± 0.1 g of parafilm was chewed. Saliva collected during the first 30 s was discarded (Moritsuka et al., 2006) and the remainder was collected into glass beakers sitting on crushed ice for rapid cooling. Saliva was collected for a period of 10 min, pooled, and centrifuged at $10,000 \times g$ for 10 min and stored at $4 \pm 1^\circ\text{C}$ until use.

Three β -LG solutions (1, 2, and 10%) were prepared and adjusted to 7 different pH values (ranging from 2.2–4.6, increasing by 0.2-pH increments) using 1 M phosphoric acid. The saliva and β -LG solutions were mixed at a 1:1 ratio. Turbidity of the mixtures was measured using a 2100AN Turbidimeter (Hach Company, Loveland, CO). The final pH of each solution was measured with an accuTupH pH probe (Fisher Scientific, Pittsburgh, PA) and an Accumet pH meter (model AB15 plus, Fisher Scientific). The experiments were performed in duplicate.

Statistical Analysis

Linear models were considered for the response variables astringency, sour, sweet, salty, and bitter intensities. The models included fixed effects for experimental factors such as protein concentration, pH, and panelist as a blocking factor. The GLM procedure of SAS (SAS Institute Inc., Cary, NC) was used to fit these models, to obtain ANOVA tables, and to compute Tukey's honestly significant differences to control the experimentwise error rate at 0.05 when carrying out multiple comparisons.

Figure 1. Effects of protein concentration (0.25–4%) on astringency in model beverages at pH 3.5. Significant differences are denoted by different letters ($P < 0.05$). Error bars represent the standard error of the mean.

RESULTS

Effects of Protein Concentration on Astringency of Whey Proteins

Increasing protein concentration resulted in an increase in astringency ($P < 0.05$) up to 4% (wt/wt) protein, whereas phosphate buffer solutions did not differ in astringency (Figure 1). The plateau of astringency at higher protein concentrations [4–13% (wt/wt)]

Figure 2. Effects of protein concentration (4–13%) on astringency in model beverages at pH 3.5. Significant differences are denoted by different letters ($P < 0.05$). Error bars represent the standard error of the mean.

was observed, with no difference seen among samples ($P > 0.05$; Figure 2). This indicates a saturation of astringency possibly starting at 4% (wt/wt) protein. When compared with phosphate buffers, all protein samples were more astringent ($P < 0.05$; Figure 1 and 2). Sourness of β -LG samples increased as protein concentration increased because of the increased amount of acid required to adjust the pH (Figure 3). Most protein samples were more sour than buffers at equivalent phosphate levels (Figure 3 and 4).

Bitterness and saltiness of all samples were very low, with ratings of ≤ 1 for both attributes (data not shown). No difference in bitterness or saltiness was found among samples ($P > 0.05$). No difference in sweetness was found when protein samples were compared with their equivalent phosphate buffers except for 13% protein (sweet rating = 4.32), where protein solutions were less sweet than buffer solutions (sweet rating = 5.96). This may be because of the decrease in sweetness associated with increased sourness (Keast and Breslin, 2002).

Time-intensity measurements of astringency were determined to examine the temporal changes associated with protein concentration. The time-intensity plot shown in Figure 5 is the average of all data points for each panelist for every second of evaluation. If panelists reach a maximum astringency at different time points, maximum astringency will be lowered because of constant time averaging. In contrast, the data shown in Table 1 is the average maximum value from all panelists, independent of observation time. Thus, there is a numerical difference between Figure 5 and Table 1 in

Figure 3. Effects of protein concentration (0.25–4%) on sour taste in model beverages at pH 3.5. Significant differences are denoted by different letters ($P < 0.05$). Error bars represent the standard error of the mean.

Figure 4. Effects of protein concentration (4–13%) on sour taste in model beverages at pH 3.5. Significant differences are denoted by different letters ($P < 0.05$). Error bars represent the standard error of the mean.

the maximum astringency values. Time to maximum astringency was not different for any of the protein concentrations ($P > 0.05$), although a numerical increase was observed (Figure 5; Table 1). Maximum astringency as well as astringency duration increased from 0.5 to 4.0% (wt/wt) protein ($P < 0.05$) and plateaued from 4.0 to 10.0% (wt/wt) protein, similar to what was observed when astringency was determined as a maximum intensity alone (Figures 1 and 2).

Saliva Flow Rates

The plateauing of astringency intensity was unexpected and required further investigation. It has been shown with other astringent compounds (tea) that saliva flow rate can affect the sensory perception of astringency (Dawes et al., 2000). Therefore, saliva flow rates were evaluated to see whether differences in flow rate could account for the plateauing of astringency values (Figure 6). The phosphate buffer and 0.5% (wt/wt) protein solution caused similar flow rates and were slightly higher than water alone. Increasing protein concentration [4–10% (wt/wt)] caused a coinciding increase in saliva flow rate. This shows that increased protein concentration alters the oral physiology and the rate of astringent clearance may be altered.

Saliva and β -LG Interactions

Saliva and β -LG interactions, as determined by turbidity measurement, are shown in Figure 7. As the protein concentration increased, the turbidity and pH

Figure 5. Time intensity of astringency at different protein concentrations in model beverages at pH 3.5. Zone I represents time to maximum astringency, zone II represents maximum astringency, and zone III represents the duration of astringency. Legend: 0.5% protein ($\diamond$), 1.0% protein ($\blacklozenge$), 4.0% protein (Δ), 7.0% protein ($\blacktriangle$), 10.0% protein ($\bullet$), A6 (astringency 6)($\circ$).

at maximum turbidity both increased. When the data are normalized based on the final pH of the saliva and β -LG mixtures (Figure 8), it can be seen that maximum turbidity occurred at the final pH of 4.6 to 5.2. This is around the isoelectric point of β -LG. This suggests that concentration effects may in part be caused by buffering of the protein beverage–saliva mixture.

Combined pH and Protein Concentrations Effect on Astringency of Whey Proteins

Astringency of 3 protein concentrations [0.5, 2, and 10% (wt/wt)] and 3 pH values (2.6, 3.2, and 4.2) were evaluated to investigate the combined pH–protein concentration effects (Table 2). There was a general trend for the time to maximum astringency to increase

Table 1. Effects of protein concentration on time-intensity analysis of astringency in model beverages at pH 3.5

Protein (%)	Time to maximum astringency (s)	Maximum astringency ¹	Duration (s)
0.5	17.1 ^a	3.47 ^b	41.6 ^c
1	17.5 ^a	4.32 ^b	45.9 ^{bc}
4	18.4 ^a	5.25 ^a	61.9 ^{ab}
7	20.4 ^a	5.37 ^a	59.3 ^{ab}
10	21.9 ^a	5.36 ^a	63.2 ^a

^{a–c}Means within a column with different superscript letters differ significantly ($P < 0.05$).

¹Maximum astringency adjusted from 100-point scale to a 0 to 15-point scale.

Figure 6. Effects of saliva flow rate after tasting astringent samples. Significant differences are indicated by different letters ($P < 0.5$).

with protein concentration, but this was only significant when comparing among concentrations at pH 4.2. Another general trend was that maximum astringency decreased as pH increased, except for 10% protein. This was most apparent in the 0.5% protein samples. The most striking trends were observed when comparing the effects of protein concentration at each pH. At pH 2.6, maximum intensity decreased when protein concentration increased, whereas at pH 4.2, the maximum intensity increased with protein concentration. There were no significant changes at the intermediate pH of 3.2. This shows a strong pH–protein concentration effect.

DISCUSSION

Increasing protein concentrations increased astringency ratings [0.25–4% (wt/wt)] and showed a clear plateau at higher concentrations [4–13% (wt/wt)]. Comparing Figure 1 and 2, differences in the astringency for 4% (wt/wt) protein is observed; however, all samples were within 1 standard deviation of each other. Sano et al. (2005) found that increasing protein concentrations over a range of 0 to 0.5% increased astringency at pH 3.5, which is similar to what was found in this study. A saturation or plateau of astringency has been observed with other astringent compounds. As grape seed tannin, tannic acid, or grape procyanidin extract increased in concentration, astringency increased and then plateaued at higher concentrations (>2.28 g/L for tannic acid; >1.48 g/L for procyanidin extract; >1.36

g/L for grape seed tannins; Condelli et al., 2006). Similarly, a saturation in perceived astringency of tannic acid in wine was observed at 0.5 g/L (Guinard et al., 1986). Whereas the saturation level of polyphenols is approximately 10 times lower than that of whey protein (~1 g/L compared with 10 g/L), the saturation of the tactile sensation suggests that some lubricating salivary compounds are maximally altered.

Binding of saliva proteins is a part of the astringency mechanism proposed by Beecher et al. (2008). Approximately 1 to 2 mL of saliva containing 0.3% protein is found in a normal mouth (Lagerlof and Dawes, 1984; Schipper et al., 2007). This would mean that the 6 mg of salivary proteins would be mixed with 100 to 2,000 mg of β -LG over the concentration range evaluated in this study. The binding stoichiometry would vary with protein concentration and pH because pH determines the charge on saliva and whey proteins. Therefore, not only the amount of proteins and pH but also the buffering capacity of protein solution will determine the extent of saliva–whey protein interactions. As seen in Figure 7, this is a complex relationship. Moreover, the saliva flow rate is increased with increasing protein concentration (Figure 6). This increase in saliva can introduce new salivary proteins that may bind with whey proteins and cause astringency, or increase the clearance of astringent aggregates. These combined processes are reflected in time-intensity measurements.

Time intensity studies provide a temporal map of the astringent sensation. Time to maximum astringency

Figure 7. Turbidity of solutions containing β -LG and saliva. Legend: 1% protein (▲), 2% protein (■), 10% protein (◆).

Table 2. Effects of protein concentration and pH on astringency rating in model beverages

Protein (%)	pH	Time to maximum astringency (s)	Maximum astringency ¹	Duration (s)	Area under curve
0.5	2.6	17.2 ^{abc}	6.20 ^{ab}	62.2 ^a	1,610 ^a
0.5	3.2	16.1 ^{abc}	4.72 ^{cd}	40.8 ^{ab}	826 ^{abc}
0.5	4.2	14.6 ^c	2.16 ^e	26.3 ^b	305 ^d
2	2.6	18.1 ^{abc}	5.60 ^{abc}	54.1 ^a	1,250 ^{ab}
2	3.2	17.5 ^{abc}	4.73 ^{bc}	45.6 ^a	902 ^{bc}
2	4.2	16.8 ^{abc}	3.53 ^d	37.2 ^{ab}	594 ^{cd}
10	2.6	21.6 ^{abc}	4.47 ^{cd}	55.4 ^a	1,170 ^{ab}
10	3.2	21.9 ^{ab}	4.70 ^{cd}	56.3 ^a	1,199 ^{ab}
10	4.2	20.1 ^a	3.87 ^{cd}	46.7 ^a	867 ^{bcd}

^{a-d}Means within a column with different superscript letters differ significantly ($P < 0.05$).

¹Maximum astringency adjusted from 100-point scale to a 0 to 15-point scale.

was not significantly different at any of the protein concentrations, although some numerical trends were observed. This has previously been shown in wine (Guinard et al., 1986) and protein beverages at lower pH levels (Beecher et al., 2008). The duration of astringency increases with increasing concentrations of tannic acid in white wine (Guinard et al., 1986). The results here showed that the astringency duration increased when protein concentration increased from 0.5 to 4% (wt/wt) and then plateaued; however, the decay in astringency (Figure 5, zone III) was slower as protein concentrations increased. As mentioned above, differences in the duration of astringency may be attributed to saliva production. Once an astringent substance is introduced to the mouth, saliva is produced at a higher rate (Figure 6). The decay in astringency (Figure 5, zone III) is therefore a combination of clearance of initial astringency and possibly addition of more salivary proteins to bind with the astringent compound. Salivary protein content is increased with stimulation using parafilm and is decreased when water is the stimulant (Nayak and Carpenter, 2008). If more of the proper salivary protein is elicited, more whey protein is able to bind with the salivary proteins.

The turbidity measurements of whey protein and saliva differed among protein concentrations. At 10% (wt/wt), turbidity was highest at a higher pH of β -LG (Figure 7). As protein concentration decreased, the turbidity decreased and was highest at lower pH values. The final pH of the mixtures at which the highest turbidity occurred was near the isoelectric point of whey proteins. These results further illustrate the numerous factors that are involved in the astringency mechanism.

Titrateable acidity was correlated to sourness when using a descriptive panel for the lemon-flavored yogurt (Barnes et al., 1991), which is similar to what is seen in this investigation (Figures 3 and 4). Lee and Vickers (2008) proposed that acid alone was causing astringency in low-pH whey protein beverages. This was based on

comparisons of equal titrateable acidity but different pH values. It is known that pH of acids (Thomas and Lawless, 1995; Lawless et al., 1996; Sowalsky and Noble, 1998) plays an important role in astringency perception. Variation among investigations may be explained based on a pH–protein concentration interaction (Table 2). Increasing protein concentration at pH 2.6 or 4.2 requires increased amount of acid to adjust the pH; however, astringency decreased with more acid at pH 2.6 and increased with more acid at pH 4.2 (Table 2). Clearly, the amount of acid alone cannot explain astringency of whey protein beverages. Beecher et al. (2008) found maximum astringency at pH 3.4 and a decrease in astringency when the pH was lowered to 2.6. This trend was not seen in this investigation (Table 2). This

Figure 8. Turbidity of solutions containing β -LG and saliva normalized by the final pH of the mixtures. Legend: 1% protein (▲), 2% protein (■), 10% protein (◆).

difference may be because of the type of protein used and the processing parameters. Beecher et al. (2008) used whey protein isolates [6% (wt/vol) protein] and heated the whey protein beverages. In this study, β -LG was used because of its higher purity. The samples were unheated to reduce the degree of protein aggregation. These factors may have contributed to the different results.

Turbidity is a simple way to observe changes in aggregate size, and this approach has been used to evaluate in vitro models for polyphenol-based astringency. Turbidity resulting from polyphenols and mucin interactions is correlated with sensory perception of astringency (Monteleone et al., 2004; Condelli et al., 2006). Peak turbidities correlate with maximum astringency of tannic acid and grape seed tannins (Horne et al., 2002; Monteleone et al., 2004; Condelli et al., 2006). We did not observe such a simple correlation with protein-based astringency. This can be explained by the differences between intermolecular binding mechanisms of proteins and polyphenols. Interactions between polyphenols and salivary proteins are a result of hydrogen bonding (Haslam, 1974) or hydrophobic interactions (Hagerman and Butler, 1980). These interactions have minor pH dependence. In contrast, because protein charge is pH dependent, interactions between saliva and whey proteins will be pH dependent. Because pH will depend on the amounts and buffering capacities of saliva and protein beverage, coupled with changes in saliva flow, a dynamic model is needed to explain the astringency of proteins.

CONCLUSIONS

Astringency of whey protein beverages increases with increasing protein concentrations from 0.25 to 4% (wt/wt) and plateaus from 4 to 13% (wt/wt). Saliva flow rate increases with increasing protein concentration from 0.5 to 10% (wt/wt). Therefore, 2 competing processes cause a temporal change in astringency. First, more saliva production would suggest a faster clearance of astringent compounds. However, if astringency is caused by interactions between whey and salivary proteins, additional saliva would also allow for more astringent complexes to form. Both sensory evaluation and in vitro study of interactions between β -LG and saliva indicate that astringency of whey proteins is a function of protein concentration and pH. These data suggest that a model to adequately describe protein-based astringency must take into account properties of the stimulant (pH, concentration, buffering capacity, and pH-related aggregation) and how that alters physiological processes occurring in the mouth. Because astringency relates to protein aggregation, future works

may include the investigation of preaggregated whey proteins or modified whey proteins having reduced aggregation at their isoelectric point.

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