Development of a shelf-stable dairy protein beverage
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ABSTRACT
The purpose of this project was to create a shelf-stable high protein beverage using whole milk and milk protein isolate (MPI) with added coffee flavor and non-caloric, natural high-intensity sweeteners. This product fills a gap in the market and improves upon the quality of current dairy protein beverages. Potassium citrate was also added to reduce sedimentation by preventing protein coagulation during heat sterilization. Effect of different levels of citrate were measured in beverages of different protein contents and pH levels. Citrate was added to beverages in levels between 0-0.75% by weight. Protein levels were adjusted to 8% (equivalent to 28g protein/12oz serving), 9%, and 10%. pH was adjusted to 6.8 or 7.0. Beverages were filled into size 300x407 (15oz) cans and sterilized in a still retort at 250°F for 20 minutes. Particle size analysis was performed as an indicator of degree of protein coagulation. At 0.25% citrate, particle size decreased 13-25% for the protein levels tested. Above 0.25%, particle size increased. On average, particle size was smaller in beverages at pH 6.8 than pH 7.0. Sensory analysis was performed on chilled samples containing 8% protein using a panel of 80 adults, primarily males age 21-34. Three samples with different sweetener profiles were tested; a combination of stevia and monkfruit was preferred based on overall liking score of 5.9 on a 9-point scale. Added citrate did not diminish creaminess or mouthfeel liking, which scored 6.0. Although sweetness was acceptable at 5.5, data shows that a higher level of sweetness would be preferred. Coffee flavor was liked by panelists with a score of 6.0. These findings show that this beverage containing 28g protein/serving, coffee flavor, and natural sweetener is liked by target consumers and with minor adjustments to sweetness is ready for commercialization.

INTRODUCTION
In the last ten years, there has been a significant increase in consumer interest for high protein products. More and more products each year are being released that tout the “high-protein” label (Archibald, 2004), and consumers are responding favorably to them. With the recent trends in health and wellness, those looking to consume nutrient-dense foods while increasing satiety and building muscle have found protein-fortified snacks and beverages (Mintel, 2013). A number of different protein sources are employed in these protein-rich foods including soy, whey, rice, pea, and casein (Fishel, 2012). In beverages with milk proteins specifically, milk proteins are generally added to water-based formulations to create shelf-stable products, while those made with milk are refrigerated (Ensure, Muscle Milk). Furthermore, these products that use milk protein often contribute fat with canola, soy, corn, or other oils rather than milk fat, which has recently been linked to certain health benefits (Juarez et al, 2015). With liquid milk prices increasing and consumption decreasing, dairy producers need new ways of attracting consumers to milk beverages. Considering their rising popularity, protein beverages are an obvious way to do so. However, in order to compete with other shelf-stable products, the milk-based beverages should be UHT (ultra-high temperature) processed and aseptically packaged, rather than ultra-pasteurized or pasteurized and refrigerated. This commercial sterilization will prolong shelf life and erase the need for refrigeration in shipping and storage (Walstra, 2006).
One problem with UHT processing on milk, however, is that it can cause protein coagulation, which leads to sedimentation, or settling of proteins in the beverage (Walstra, 2006; others). This creates an undesirable gritty mouthfeel and non-homogeneous appearance. A number of studies (Udabage et al, 2000; Chandrapala et al, 2010; Ward et al, 1997; Griffin et al, 1988) have shown that adding calcium-chelating agents such as EDTA or citrate salts will increase casein solubility and decrease protein coagulation during heat processing. Some (McSweeney et al, 2004; Sauer and Moraru, 2012) have also studied the effect of pH on milk protein coagulation under heat processing conditions. These studies have only examined MPI and calcium-chelating salts in isolation, however, and have not examined the effects of adding them to milk/milk protein solutions. This study aims to look at the effects of citrate in a high-protein milk-based beverage with coffee flavor and sweetener to see if the effects are the same in a complete beverage system. This study will examine how different protein levels and pH levels interact with added citrate when commercial sterilization is applied to milk. It will also decide if a high-protein coffee-flavored milk beverage with natural sweeteners is acceptable to consumers who use high-protein beverages or products on a regular basis.

MATERIALS AND METHODS
Beverages were assembled using commercial whole milk (3.25% milk fat, pasteurized and homogenized), 85% milk protein isolate (MPI) (Idaho Milk Producers, Jerome, ID), coffee extract (Sensient Flavors, Hoffman Estates, IL), natural sweetener—either stevia (Truvia -Cargill, Minneapolis, MN) or monk fruit (In the Raw-Brooklyn, NY)—and anhydrous tripotassium citrate (Jungbunzlauer, Switzerland).

Effect of Protein Level
Milk was mixed with citrate (if applicable) before adding MPI. Enough MPI was added to achieve 8%, 9%, or 10% total protein by weight. These protein levels are in line with current protein beverages on the market (Muscle Milk, Ensure, Special K). For each protein level, citrate was added in levels of 0%, 0.25%, or 0.50% by weight, similar to levels in other studies and from results of preliminary lab work. The milk was heated to 40°C in a water bath to aid in MPI hydration and dissolving. A handheld emulsion blender (KitchenAid, St. Joseph, MI) was used to homogenize the beverage. The beverage was cooled before adding coffee extract (2.0% by weight) and coffee essence (0.10% by weight).

Effect of pH
Beverages were assembled as described above. All were adjusted to 8% protein by weight. Citrate was added in levels of 0%, 0.25%, 0.50%, and 0.75% by weight. pH was adjusted to 6.8 or 7.0 dropwise using 1M NaOH or 1M HCl according to Udabage et al (2000). pH was checked with a benchtop pH meter.

Heat Processing
Due to resource limitations, beverages were not able to be UHT processed via steam injection followed by aseptic packaging. Instead, an equivalent commercial sterilization method was carried out using a retort. Beverages were poured into size 300x407 (15-oz) cans, sealed, and processed in a still retort (Dixie Canner Co, Bogart, GA) at 250°F for 20 minutes to achieve commercial sterility as defined by Walstra (2006). Cans were cooled to 100°F using water, then
transferred to a refrigerator for storage. Beverages were stored for at least 24 hours but no more than one week before sensory evaluation or particle size analysis.

Particle Size Analysis
Beverages were analyzed using a Mastersizer 2000 particle analyzer (Malvern Instruments, Worcestershire, UK) at ambient temperature (25°C). For pH, three samples were measured and the average of the three was reported. For protein level, five samples were measured and the average reported.

Sensory Evaluation
Beverages were prepared and heat processed as described above in three batches. Batches were identical except for the sweetener used. Batch 1 used stevia, batch 2 used monk fruit, and batch 3 used a combination. All batches had 0.25% citrate. The amount of sweetener in each batch was adjusted so that all three had approximately the same sweetness level.

80 panelists from Columbus, OH were recruited to perform sensory evaluation on the beverages. 90% were age 21-41 and 90% were male. Panelists indicated that they regularly (at least once/month) consumed protein-rich beverages. Testing took place in the Ohio State University Department of Food Science and Technology sensory booths. Samples were presented one at a time in 2oz plastic cups with 3-digit codes. They were served at refrigeration temperature (40°F). For each sample, panelists were instructed to take at least 2 sips before answering questions. Hedonic tests with a 1-9 scale (dislike extremely(1)-like extremely(9)) were used to evaluate beverages for overall liking, aroma, flavor, sweetness, coffee flavor, texture/mouthfeel/creaminess, bitterness, and aftertaste. A Just About Right (JAR) test using a 1-5 scale (much too little(1), slightly too little (2), Just About Right (3), slightly too much (4), much too much(5)) was used to evaluate amount of flavor, bitterness, sweetness, coffee flavor, creaminess, and aftertaste. Panelists were then asked to rank the three beverages by liking.

RESULTS
Effect of Protein and Potassium Citrate Levels

![Figure 1-Effect of potassium citrate on particle size of protein beverage at various protein levels](image)
At 0% potassium citrate (control), the average particle size of beverages with 8%, 9%, and 10% protein ranged from 0.54-0.64 µm (Figure 1). The 0.25% potassium citrate treatment caused particle sizes to decrease 13-25% in volume (Table 1) to 0.44-0.50 µm. The 10% protein beverage decreased the most, from 0.59µm to 0.44µm. When the potassium citrate treatment was increased to 0.50%, the particle sizes increased significantly to 1.24-2.25µm. At 0% and 0.25% potassium citrate, the range of particle sizes between the three protein levels was fairly small. At 0.50%, they differentiated more with the 10% protein beverage having the highest particle size, followed by 9% and 8%, respectively.

**Table 1**-Particle size changea (increase [+]) or decrease [-]) in protein beverages from added potassium citrate

<table>
<thead>
<tr>
<th>Protein Level</th>
<th>0.25% K-Citrate</th>
<th>0.50% K-Citrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>8% Protein</td>
<td>-25.4%</td>
<td>+93.8%</td>
</tr>
<tr>
<td>9% Protein</td>
<td>-21.9%</td>
<td>+202%</td>
</tr>
<tr>
<td>10% Protein</td>
<td>-13.0%</td>
<td>+281%</td>
</tr>
</tbody>
</table>

aComparison was made between sample at given potassium citrate level and control (0%)

Beverages of all three levels of protein decreased in average particle size with an added 0.25% potassium citrate. 8% decreased the most at -25%, followed by 9% and 10%, respectively. Beverages with 0.50% citrate caused particle size in all three levels of protein to increase. 10% increased the most at +281%, while 8% increased the least at 94%. The increase at 0.50% citrate was significantly larger than the decrease at 0.25% citrate.

**Effect of pH and Potassium Citrate Levels**

![Figure 2](image)

**Figure 2**- Effect of pH and potassium citrate addition on protein beverage particle size

The addition of 0.25% potassium citrate reduced the particle size in protein beverages (8% total protein by weight) with pH values of 6.8 and 7.0 (Figure 1), though 6.8 decreased considerably more (67% vs 4% reduction in volume) (Table 1). Above 0.25%, particle size of the pH 7.0 beverage continued to increase. At 0.50%, particle size of the pH 6.8 beverage was about the same as at 0.25%, but at 0.75% it increased. The pH 7.0 beverage increased much more in
particle size than the pH 6.8 beverage; its particle size at 0.75% was double the size of the pH 6.8 beverage and more than a ten-fold larger than its size in the control treatment.

**Table 2**—Particle size change\(^a\) (increase [+] or decrease [-]) in protein beverages from added potassium citrate

<table>
<thead>
<tr>
<th></th>
<th>0.25% K-Citrate</th>
<th>0.50% K-Citrate</th>
<th>0.75% K-Citrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH=6.8</td>
<td>-67.2%</td>
<td>-66.2%</td>
<td>+142%</td>
</tr>
<tr>
<td>pH=7.0</td>
<td>-4.4%</td>
<td>+299%</td>
<td>+1218%</td>
</tr>
</tbody>
</table>

\(^a\)Comparison was made between sample at given potassium citrate level and control (0%)

**Sensory Evaluation**

**Table 3**—Hedonic test results for protein beverages sweetened with different natural sweeteners

<table>
<thead>
<tr>
<th>Hedonic Liking 9 Pt. Scale</th>
<th>Stevia</th>
<th>Monk Fruit</th>
<th>Combination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall Liking (First Impression)</td>
<td>5.58 b</td>
<td>5.61 ab</td>
<td>5.89 a</td>
</tr>
<tr>
<td>Aroma Liking</td>
<td>6.21 a</td>
<td>5.90 b</td>
<td>6.18 a</td>
</tr>
<tr>
<td>Flavor Liking</td>
<td>5.54 a</td>
<td>5.66 a</td>
<td>5.78 a</td>
</tr>
<tr>
<td>Sweetness Liking</td>
<td>5.56 a</td>
<td>5.35 a</td>
<td>5.45 a</td>
</tr>
<tr>
<td>Coffee Flavor Liking</td>
<td>5.84 a</td>
<td>5.86 a</td>
<td>6.03 a</td>
</tr>
<tr>
<td>Texture Liking (Mouthfeel/Creaminess)</td>
<td>6.09 a</td>
<td>5.94 a</td>
<td>5.99 a</td>
</tr>
<tr>
<td>Bitterness Liking</td>
<td>5.34 a</td>
<td>5.45 a</td>
<td>5.46 a</td>
</tr>
<tr>
<td>Aftertaste Liking (If present)</td>
<td>5.28 a</td>
<td>5.00 a</td>
<td>5.21 a</td>
</tr>
<tr>
<td>Ranking</td>
<td>1(^{st})</td>
<td>3(^{rd})</td>
<td>2(^{nd})</td>
</tr>
</tbody>
</table>

*a and b indicate significant differences. Green=highest score Teal =no sig dif from the high score. Yellow=sig dif (low score).

Panelists’ hedonic scores for the three beverages were similar for each attribute tested (Table 3). Only overall liking and aroma liking scores were significantly different from one another; the rest were not significantly different. The stevia beverage was liked most for aroma, sweetness, texture, and aftertaste and ranked 1\(^{st}\) overall. The combination beverage was liked most for overall, flavor, coffee flavor and bitterness and ranked 2\(^{nd}\) overall. Monk fruit was not liked most for any attributes, but was still scored close to one of the other beverages for each attribute, except for aroma which was scored significantly lower.

**Table 4**—Just about right (JAR) test results for protein beverages sweetened with different natural sweeteners

<table>
<thead>
<tr>
<th>Just About Right 5 Pt. Scale</th>
<th>Stevia</th>
<th>Monk Fruit</th>
<th>Combination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweetness</td>
<td>42 – 50 – 8</td>
<td>45 – 46 – 9</td>
<td>48 – 46 – 6</td>
</tr>
</tbody>
</table>
Bitterness | 12 – 59 | 20 – 54 | 17 – 54 – 29

Just About Right questions are divided into 3 segments of percent. A well balanced response would have 20% - 60% - 20%. When numbers of greater than 20% occur on either side of the middle score, the indication is for potentially greater risk that the attribute is less or more undesirable. Green is Slightly too Little, Teal is Much too Little, Orange Slightly too Much, Yellow Much Too Much. Best balanced are in BOLD.

Figure 3-Penalty drop for stevia-sweetened dairy protein beverage

Figure 4-Penalty drop for monk fruit-sweetened dairy protein beverage
Figure 5-Penalty drop for dairy protein beverage sweetened with stevia and monk fruit

The stevia-sweetened beverage had the best balanced score for flavor, sweetness, bitterness, and aftertaste (Table 4). All samples had the best balance of scores for creaminess. The least-balanced score was sweetness, followed by flavor. Interestingly, it was noted that almost the same number of panelists which found the beverage sweet enough also found it not sweet enough. This close ratio may indicate that part of the population would prefer a less sweet product.

The penalty drop analyses (Figures 3, 4, and 5) do not indicate any attributes which have a large mean drop for a large percentage of consumers. For all three sweeteners, a large number of consumers had a small mean drop for too little coffee flavor and too little sweetness, which a small number of panelists had a large mean drop for too much sweetness and too much coffee flavor. Again, this indicates a split in preferences amongst consumers in terms of flavor and sweetness. A possible solution for the future would be to offer an unsweetened or lightly sweetened version of the beverage.

**DISCUSSION**

*Effect of Protein and Potassium Citrate Levels*

It was expected that with an increased level of protein in the milk beverages, the protein would be more prone to aggregation upon heating. It was also expected that increasing the level of citrate would reduce the average particle size, based on previous work with casein and calcium-chelating salts (Udabage et al, 2000; Chandrapala et al, 2010). It was observed that at 0.25% potassium citrate the aggregation did decrease, presumably due to calcium ion chelation which allowed for a higher degree of protein solubility. However, above 0.25%, the citrate caused a significant jump in particle size, above the degree of coagulation of the control. Although these findings are not in line with those of Udabage et al (2000) and Chandrapala et al (2010), this may be due to the harsher heating conditions of retort sterilization than steam-injection. Additionally, pH was not controlled in this portion of the study. Adding citrate increased pH ~0.1-0.2 per
0.25% increase in citrate, so this slight change could have influenced coagulation as well (see below: *Effect of pH and Potassium Citrate Levels*).

Findings show low risk in increased protein coagulation with increased protein levels in a shelf-stable product. Similar findings were presented by Chandrapala et al (2010) in concentrations up to 21% non-fat milk solids. Furthermore, adding 0.25% potassium citrate can help reduce protein coagulation for all protein levels tested.

**Effect of pH and Potassium Citrate Levels**
The pH of the beverages was adjusted to 6.8 and 7.0 to compare the degree of coagulation, if any, of the proteins. Initially the pH 6.8 beverage had a higher average particle size than pH 7.0. Other studies (Sauer and Moraru, 2012; Chandrapala et al, 2010; McSweeney et al, 2004) were in agreement and found higher stability, or reduced aggregation, in casein when pH was increased. When potassium citrate was added, pH 6.8 had a lower average particle size than pH 7.0. Yourvaong et al (2003) had similar findings: at a fixed level of citrate, the particle size increased with increasing pH.

Findings in this study show that as potassium citrate was added, particle size was lower in the pH 6.8 solution, implying a lower degree of protein coagulation. There was no significant difference in average particle size for 0.25% and 0.50% citrate at pH 6.8, though it increased quite a bit from 0.25% to 0.50% for pH 7.0. It is recommended that for a shelf-stable protein beverage, formulators adjust pH to 6.8 and use 0.25% citrate to minimize protein aggregation and subsequent sedimentation.

**Sensory Evaluation**
Panelists of this study overall were satisfied with the attributes of the beverages they evaluated. Although the aim of testing three formulations with differing sweeteners was to find one that was most well-liked, other findings revealed that coffee flavor could be adjusted to be stronger, though not more bitter. In terms of differences between sweeteners, they appear to be manifested through differences in likings of attributes besides sweetness, such as aftertaste. Over 90% of panelists believed the beverages to be either sweet enough or not sweet enough; less than 10% found them too sweet. Between those who rated it as having “too little sweetness” or “just enough sweetness,” the split was close to 50/50, illustrating a potential for multiple products—one with the level of sweetness evaluated and one with more. Future testing should look at levels of sweetness to decide the best level in this beverage. There highest score for overall liking/first impression was for the combination stevia/monk fruit beverage. This was a significant difference, but did not match the ranked scores panelists gave after trying all three beverages. The ranked scores did not show significant differences, however, indicating that panelists found them to be fairly similar. Considering that only 2 of 9 attributes had significant differences in liking and the rest had very similar scores, this result seems reasonable. Formulators, then, could safely adopt any of the tested sweeteners for this type of beverage at this sweetness level. However, it should be noted that if sweetness is increased, these results may change, especially with stevia which is known to have a bitter aftertaste among some consumers. Ultimately, any reformulation should be re-evaluated by a consumer panel prior to product launch.

**CONCLUSIONS**
At pH 6.8 and 0.25% potassium citrate, a shelf-stable dairy protein beverage can control protein coagulation, preventing excess sedimentation and undesirable mouthfeel. Using Stevia and monk fruit showed similar results for attribute liking and JAR scores, so either could be used in formulations.

FURTHER STUDY
This study may be repeated with UHT steam-injection processing to see if there are similar increases in coagulation/particle size at higher levels of potassium citrate. Changes in sweetness and coffee flavor may help increase liking from consumers.

ACKNOWLEDGEMENTS
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REFERENCES


