Stabilization of Acidified Whey Drink Made from Buffalo (Buffalo bubalis) Milk with the Addition of Pectin from Mango (Mangifera indica) Peels

Mina P. Abella¹, Ma. Cristina B. Gragasin², Patrizia Camille O. Saturno¹, Teresita M. Baltazar¹*, and Sheryll May M. Villota²

¹Philippine Carabao Center National Headquarters and Gene Pool, Science City of Muñoz, Nueva Ecija 3120 Philippines
²Philippine Center for Postharvest Development and Mechanization, Science City of Muñoz, Nueva Ecija 3120 Philippines

Different levels (0.15, 0.20, and 0.25%) of commercial pectin (CP) and mango peel pectin (MPP) were used in the quality improvement of acidified whey drink (AWD) made from buffalo milk. Samples were evaluated for physico-chemical properties, microbiological qualities, sedimentation stability, and sensory characteristics. The treatment found to be the most acceptable and with the least percentage of sedimentation was subjected to product stability evaluation during storage for 27 d. No significant differences were observed among the treatments in terms of pH and total soluble solids (TSS), whereas viscosity increased as the level of pectin was increased. All the treatments for both CP and MPP had an aerobic plate count (APC) of < 10 cfu/mL, yeast and molds count of < 1cfu/mL, coliform count of < 1cfu/mL, and negative in E. coli. Moreover, both CP and MPP at the 0.25% level showed the lowest sedimentation with 0.76%. Results showed that AWD with 0.25% MPP can be stored for about 20 d at ambient temperature.

Keywords: acidified whey drink, buffalo, mango peel pectin, stabilization, whey

INTRODUCTION
Whey – a by-product of cheese production – is a green-yellowish, semi-translucent liquid (Chatterjee et al. 2015). The food industry has been focusing on addressing health concerns and requirements in food by consumers while maximizing the use of ingredients and decreasing food waste. There are numerous researches on the utilization of whey for the production of different products. One of the most promising and convenient products for this, apart from it being in a powder form, is whey beverage (Djurić et al. 2004; Jelić et al. 2008; Pereira et al. 2015). Whey, in a raw form, is a protein-rich liquid with good and healthy nutritional qualities (Gimhani and Liyanage 2019; León-López et al. 2020).

Acidified milk drinks are made by souring the product with one or more acidifying ingredients, with or without the addition of characterizing microorganisms. This includes beverages that contain both milk (as the neutral base) and fruit juice (as an acidic medium), yogurt and yogurt products (acid dairy phase), and soft drinks that contain milk solids (Du et al. 2007; Liu et al. 2020). Specified acidulants are food-grade citric acid, fumaric acid, glucono-delta-lactone, hydrochloric acid, lactic acid, malic acid, phosphoric acid, succinic acid, and tartaric acid (Du et al. 2007).
The low pH of acidified milk drinks results in the aggregation of milk products causing sedimentation and syneresis problems (Liu et al. 2020). This defect is linked to the collapse of the native stabilization mechanism of milk proteins, particularly casein. The extended conformation of the k-casein chains collapses, causing it to aggregate. At below pH 5.0, the charges are distributed along the pectin chain causing it to be absorbed onto the casein micelle, forming a kinetic barrier as the result of an electrostatic interaction that stabilizes the solution (Flutto 2003; Tromp et al. 2004). At the normal pH of milk (pH 6.7), casein micelles are stable due to their steric repulsive interactions with each other (Tromp et al. 2004). Therefore, in order to avoid this, the addition of a stabilizer is essential (Du et al. 2007).

One of the most common stabilizers used in acidified milk drinks is pectin, particularly the high methoxyl pectin (HMP) (Du et al. 2007; Liu et al. 2020; Tromp et al. 2004). Pectin is classified as HMP if it has an esterified acid unit of more than 50%, whereas below is classified as low methoxyl pectin (Gragasin et al. 2014). Pectin is a natural hydrocolloid found in fruits and it is commonly used as a texturizing and stabilizing agent for processed foods (Flutto 2003). HMP can be extracted from identified parts of various fruits and vegetables such as mango, passion fruit, banana, chickpea, dragon fruit, and many others (Gragasin et al. 2014; Vanitha 2020). HMP gels are obtained at low pH in the presence of high soluble solids or high sucrose concentration or similar co-solute (Edwards 2007). A high sucrose concentration reduces water activity which is necessary to promote chain-chain interactions rather than chain-solvent interactions. Hydrogen bonding is the main interaction that sustains the HMP gel structure and hydrophobic interactions are essential for HMP gelation. pH is an important factor in gelation. Pectin is an anion polysaccharide and lowering the pH protonates carboxylic groups, reducing electrostatic repulsions along and between pectin chains. Moreover, at low pH, non-dissociated carboxylic groups form inter-and intramolecular hydrogen bonds with secondary alcohol groups (Chan et al. 2017). The most common source of CP is citrus peel and apple pomace (Edwards 2007). However, increasing research is arising on the utilization of other fruit peels as a source of pectin (Abboud et al. 2020; Abou-Elseoud et al. 2021; Muñoz-Almagro et al. 2021; Thu Dao et al. 2021). The physico-chemical properties of pectin produced from mango peel and the standard for CP are shown in Table 1.

Philippine carabao mango (Mangifera indica) is considered to be the best mango variety in the world (Caparino et al. 2012). The Philippines is the ninth major mango producer in the world with more than 800 thousand metric tons of annual production. It is sweet and has non-fibrous flesh and about 15–20% of the mango fruit is composed of peels that are wasted and disposed of. Initial research was found on the use of the peel of other mango varieties as a source of pectin (Chaiwarit et al. 2020; Deng et al. 2020; Geerkens et al. 2015; Gragasin et al. 2014). This would support the decrease of pectin importation and the disposal problems of mango peels. Hence, the use of locally produced MPP is used in this study. The objective of the study was to determine the level of the MPP that will stabilize the AWD through decreased sedimentation, in addition to which level would provide the best sensory attributes.

**MATERIALS AND METHODS**

**Materials**

The MPP was made from the peels of Philippine carabao mango (Mangifera indica) produced and provided by the Philippine Center for Postharvest Development and Mechanization (PHILMECH), Science City of Muñoz, Nueva Ecija, Philippines. The commercially available pectin, citrus pectin, was purchased from IMCD Philippines, Makati City, Philippines. Whey, or preferably called “sweet whey,” from the production of kesong puti – a traditional Filipino soft white cheese using buffalo milk – was obtained from the Central Dairy Collecting and Processing Facility of the Philippine Carabao Center (PCC) National Headquarters and Geneepool in Science City of Muñoz, Nueva Ecija, Philippines. Sweet whey samples were analyzed for chemical components using a milk analyzer (FOSS Milkoscan FT-1, Denmark). It contained 0.94% fat, 1.10% protein, 5.44% lactose, 8.26% total solids, and 7.32% solids non-fat. The sweet whey samples were stored in a freezer, at < 0°C, before usage. Refined white sugar and canned pineapple juice were purchased from the local market.

**Table 1. MPP properties and the specification for CP (Gragasin et al. 2014).**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MPP</th>
<th>USP specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Degree of esterification</td>
<td>76–79</td>
<td>Not specified</td>
</tr>
<tr>
<td>Methoxyl content, %</td>
<td>12.65–12.84</td>
<td>Not less than 6.7%</td>
</tr>
<tr>
<td>Galacturonic acid content, %</td>
<td>92.82–98.65</td>
<td>Not less than 74%</td>
</tr>
<tr>
<td>Total ash content, %</td>
<td>3.32</td>
<td>Not more than 10%</td>
</tr>
<tr>
<td>Acid-insoluble ash, %</td>
<td>0.80</td>
<td>Not more than 1%</td>
</tr>
<tr>
<td>Loss on drying, %</td>
<td>8</td>
<td>Not more than 10%</td>
</tr>
<tr>
<td>Total soluble solids, %</td>
<td>0.40</td>
<td>Not specified</td>
</tr>
</tbody>
</table>
Processing of AWD
Two (2) L of whey from kesong puti making was used in each treatment for every batch of AWD processed. Whey was heat-treated to 85–90 °C for 2 min to destroy the pathogenic microorganisms and filtered, then was set aside to cool to 40–45°C. Pectin was mixed with sugar before dissolving in hot water. CP and MPP were added to the mixture at different levels: 0.15, 0.20, and 0.25%. The mixture with the three different levels of CP was labeled as Treatment 1, 2, and 3, respectively. While the mixture with the three different levels of MPP was labeled as Treatment 4, 5, and 6, respectively. It was allowed to swell and was mixed into the whey and blended for 5–10 min. Thereafter, the whey-pectin mixture was set aside to swell, then the flavoring (pineapple juice) was added. The pH of the mixture was adjusted to 3.8–4.2, using the citric acid solution and glucono-delta-lactone solution. Water was then added to make up the desired volume. The mixture was heated to 90–95 °C for 15–30 s then filled into PET bottles at 80–85 °C and the closures were applied immediately after. Thereafter, the bottles were inverted to ensure that the closures are also sterilized with the hot liquid touching the inside surface of the bottles. The filled bottles were cooled rapidly under running water until the temperature was at 30–35 °C.

Product Evaluation
Physicochemical characteristics. The pH of the drink was measured at room temperature by a pH meter, HI 9024 (Eutech pH 150). The TSS was determined using an Atago refractometer. Viscosity was determined using a Cannon viscometer at 100 rpm with spindle number 61.

Sedimentation stability. A 50-mL plastic centrifuge tube was filled, and sedimentation of the system was performed by centrifugation at 3000 g for 15 min. The total height of the AWD sample (HD) and the height of the sedimentation (HS) was measured. The extent of instability was characterized by sedimentation in percent (%) = (HS/HD) x 100 (Abedi et al. 2014).

Sensory evaluation. A total of fifty consumer panelists consisted of random students of the Central Luzon State University (CLSU) with an age group of 17–20 yr old participated in the consumer acceptability test. The test was held in the Food Science Laboratory of CLSU.

A consumer acceptability test was carried out with four treatments of AWD. The samples, with a volume of 30–35 mL, were placed in a white cup pre-labeled with a three-digit random number, served at room temperature together with room temperature water as a palate cleanser. The participants were asked to taste the four AWD treatments served in a fully randomized order and answer the questions in the form. The participants rated each of the AWD on the overall acceptability, color, clarity, viscosity, aroma, taste, sweetness, flavor, mouth-feel, and after-taste using the nine-point hedonic scale (1 – dislike extremely, 2 – dislike very much, 3 – dislike moderately, 4 – dislike slightly, 5 – neither dislike nor like, 6 – like slightly, 7 – like moderately, 8 – like very much, and 9 – like extremely).

Microbiological analysis. AWD was tested for total APC, coliform count, Eschericha coli, and yeast and molds count (USFDA 2002).

Product stability during storage. The treatment that was found to be the most acceptable in the sensory evaluation and with the least percentage of sedimentation was subjected to shelf-life testing and was stored at ambient temperatures (28–32 °C). The treatment was evaluated for sensory evaluation and microbiological quality (APC, yeasts and molds count, coliform count, E. coli) at 6, 13, 20, and 27 d at ambient temperature.

Data Analysis
Data on the physico-chemical composition, viscosity, and sedimentation were subjected to analysis of variance (ANOVA) in a completely randomized design. Sensory quality data were analyzed using ANOVA in a randomized complete block design, with the panel as the blocking factor. Significant differences in the treatments were compared using Duncan’s post hoc test. All the data were analyzed using the SPSS (Version 9.1). Descriptive statistics were used in the consumer acceptability data.

RESULTS
Physico-chemical Characteristics
In terms of pH, 0.25% MPP had the highest value, followed by 0.20% MPP and 0.20% CP, whereas 0.25% CP had the lowest pH. However, there were no significant (p < 0.05) differences found in the pH of all the treatments – either the CP and MPP, which had a range of 3.64–3.70 (Table 2).

There is an increase in viscosity with an increase in pectin concentration in both the CP and MPP treatments. 0.25% CP and 0.25% MPP – which both have the highest concentration of pectin from CP and MPP, consecutively – had the highest viscosity and are not significantly (p > 0.05) different from each other. However, 0.25% MPP is not significantly different from 0.15% CP and 0.20% CP that use CP. In terms of sedimentation, as the pectin concentration increases from either the CP or the MPP, the occurrence of sedimentation decreases. However, it was found that there were no significant differences among the treatments.
Sensory Evaluation
The hedonic ratings showed that there were significant differences (ANOVA; \( p < 0.05 \)) between treatments on three of the ten sensory attributes, which are overall acceptability, color, and viscosity. In the overall acceptability, 0.15% CP was the most liked treatment with a rating of 7.24, whereas 0.15% MPP was the least liked obtaining a mean score of 6.22. Data analysis showed that 0.20% CP, 0.25% CP, 0.20% MPP, and 0.25% MPP were not significantly \( (p > 0.05) \) different from each other, whereas 0.15% CP and 0.15% MPP are significantly \( (p < 0.05) \) different. To describe appearance in this study, the sensory attributes – color, clarity, and viscosity – of the whey drink were analyzed, wherein it was found that there is a significant difference \( (p < 0.05) \) between treatments in terms of color and viscosity. For the viscosity, 0.25% MPP got the highest rating, whereas 0.25% CP got the lowest rating (Figure 1).

Microbiological Analysis
The total APC of the treatments was < 10 cfu/mL, no found *E. coli*, a count of < 1 cfu/mL for coliform, and a range of < 1 to 1 cfu/mL yeast and molds count in all treatments – which are in line with the Philippine Food and Drug
Administration (FDA) microbiological standards (FDA Circular 2013-010) for a non-alcoholic beverage, which is 10–100 cfu/mL and negative (Table 3) (FDA 2013).

**Product Stability during Storage**
Based on the product evaluation, treatment with 0.25% MPP showed the lowest percent sedimentation of 0.76%, which answered one of the objectives on which level of MPP could stabilize AWD. This treatment was subjected to product stability monitoring from Days 6–27 at ambient storage.

**Sensory Evaluation**
From all the sensory attributes, only viscosity found a significant difference ($p < 0.05$) between the storage period. The viscosity decreases over time with a mean score of 6.60 on Day 6 and eventually a mean score of 5.98 on Day 27. It can also be seen that the rating for color, clarity, viscosity, aroma, taste, flavor, sweetness, mouthfeel, and aftertaste decreases – but is not significantly different – during storage.

![Figure 2](image-url) 

**Table 3.** Microbial count in AWD with varying pectin type and levels.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Aerobic plate count</th>
<th>Coliform count</th>
<th><em>Escherichia coli</em></th>
<th>Yeast and molds count</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.15% CP</td>
<td>&lt; 10 cfu/mL</td>
<td>&lt; 1 cfu/mL</td>
<td>Negative</td>
<td>&lt; 1 cfu/mL</td>
</tr>
<tr>
<td>0.20% CP</td>
<td>&lt; 10 cfu/mL</td>
<td>&lt; 1 cfu/mL</td>
<td>Negative</td>
<td>&lt; 1 cfu/mL</td>
</tr>
<tr>
<td>0.25% CP</td>
<td>&lt; 10 cfu/mL</td>
<td>&lt; 1 cfu/mL</td>
<td>Negative</td>
<td>1 cfu/mL</td>
</tr>
<tr>
<td>0.15% MPP</td>
<td>&lt; 10 cfu/mL</td>
<td>&lt; 1 cfu/mL</td>
<td>Negative</td>
<td>&lt; 1 cfu/mL</td>
</tr>
<tr>
<td>0.20% MPP</td>
<td>&lt; 10 cfu/mL</td>
<td>&lt; 1 cfu/mL</td>
<td>Negative</td>
<td>1 cfu/mL</td>
</tr>
<tr>
<td>0.25% MPP</td>
<td>&lt; 10 cfu/mL</td>
<td>&lt; 1 cfu/mL</td>
<td>Negative</td>
<td>&lt; 1 cfu/mL</td>
</tr>
</tbody>
</table>
Table 4. Microbial count in AWD with 0.25% MPP during storage at ambient temperature.

<table>
<thead>
<tr>
<th>Day</th>
<th>Aerobic plate count</th>
<th>Yeast and molds count</th>
<th>Coliform count</th>
<th>Escherichia coli count</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>&lt; 10 cfu/mL</td>
<td>1 cfu/mL</td>
<td>&lt; 1 cfu/mL</td>
<td>Negative</td>
</tr>
<tr>
<td>20</td>
<td>&lt; 10 cfu/mL</td>
<td>1 cfu/mL</td>
<td>&lt; 1 cfu/mL</td>
<td>Negative</td>
</tr>
<tr>
<td>27</td>
<td>&lt; 10 cfu/mL</td>
<td>2 cfu/mL</td>
<td>&lt; 1 cfu/mL</td>
<td>Negative</td>
</tr>
</tbody>
</table>

**Microbiological Analysis**

Table 4 shows the mean scores of microbial qualities of AWD during storage. The microbial qualities (< 10 cfu/mL APC, 1 cfu/mL yeasts and molds count, < 1cfu/mL coliform count, negative E. coli) of AWD are within the standards based on the Revised Guidelines for the Assessment of Microbiological Quality of Processed Foods for Non-alcoholic Beverages (FDA Circular No. 2013-010) up until the 20th day of storage at ambient temperature (FDA 2013).

**DISCUSSION**

**Physico-chemical Characteristics**

Treatment with the highest MPP content had the highest pH value among the treatments. This is in contrast to the results observed by Rehmantalpur et al. (2016), wherein buffalo milk whey with HMP provided a low pH than the treatments with no HMP. Furthermore, the type and concentration of hydrocolloids found no significant effect on the pH of fermented whey beverages from cow, goat, and buffalo milk (Krasaekoopt and Cabraal 2011).

The TSS of the MPP used in the present study is 0.40%, and the sugar content is 4.8% (Gragasin et al. 2014). However, there were no observed differences in the TSS from all the treatments. This might be due to the minute amount of pectin added per treatment to provide an evident change.

Viscosity increases with the increase of the concentration of stabilizers such as pectin when added to acidified milk drinks and other drinks (Akkarachaneeyakorn and Tinrat 2015; Arioui et al. 2017; Karimi et al. 2016; Liu et al. 2020). This can also be observed in the present study. The gel strength of the MPP used in this study is 0.074 N (Gragasin et al. 2014). This is comparable to the gel strength of pectin that is commercially bought, thus explaining the similarity of results on the viscosity and sedimentation of the two kinds of pectin used in this study. Furthermore, viscosity behavior influences the sensory aspect when assessing the final products. Viscosity has an effect on sedimentation, as it gives the body to the beverage and prevents the particles from aggregating with each other.

According to Tasneem et al. (2014), low levels of stabilizer present results in an increase in sedimentation, which is similar to the present study. This is due to the interaction of pectin molecules with milk proteins through ionic and steric stabilization of protein in acidic milk beverages (Liu et al. 2020). Aggregation or sedimentation is the cause of protein-protein interaction present in a solution with a pH value near the isoelectric point of the whey proteins (Kováčová et al. 2009). At low pH, complex formation occurs but if the pH is far below the isoelectric point, extensive complex formation occurs that leads to precipitation (Salminen and Weiss 2014). However, the complex formation is also temperature, ionic strength, protein: pectin ratio, and pectin concentration dependent (Oduse et al. 2018; Santos et al. 2018).

**Sensory Evaluation**

Appearance is one of the important parameters, which can influence the acceptability of a food product, including in this parameter is the appearance of sediments and sometimes associated with viscosity in terms of beverages. The addition of pectin prevents the settling of sediments as it serves as a thickening and stabilizing agent; thus, 0.25% MPP and CP had the lowest sedimentation and highest viscosity. It was found by Rehmantalpur et al. (2016) that the addition of 0.2 % HMP to whey beverages had the highest acceptability compared to the treatments with no addition, lower and higher concentration than 0.2 %. The sensory characteristics, particularly, appearance, color, body, and texture, of whey beverage with 0.2% HMP were better than that of the control. Similarly, Krasaekoopt and Cabraal (2011) found that the concentration of hydrocolloids added to fermented whey beverage affects its sensory properties. As the concentration increases the preference for the sensory attributes also increases until the maximum concentration is reached.

**Microbiological Analysis**

APC can be valuable in evaluating food quality. Large numbers of bacteria may be an indication of poor sanitation or problems with process control or ingredients. However, a low reading on APC does not also necessarily indicate that the food is safe from pathogens or toxins (Mendonca et al. 2020). Certain products, such as those produced through fermentation, naturally have high APC. Table 3 shows the
microbial quality in terms of total APC, coliform count, *E. coli*, and yeast and molds count of AWD.

The presence of coliform in the pasteurized milk and other dairy products might be due to post-pasteurization contamination of milk as it cannot survive pasteurization. Poor herd hygiene, contaminated water, unsanitary milking practices, and improperly washed and maintained equipment can all lead to higher coliform counts in raw milk (Murphy 1997).

Moreover, the large and diverse group of microscopic foodborne yeasts and molds (fungi) includes several hundred species. The ability of these organisms to attack many foods is due in large part to their relatively versatile environmental requirements. Although most yeasts and molds are obligate aerobes (require free oxygen for growth), their acid/alkaline requirement for growth is quite broad – ranging from pH 2 to above pH 9. Their temperature range (10–35 °C) is also broad, with a few species capable of growth below or above this range. Moisture requirements of foodborne molds are relatively low; most species can grow at a water activity (aw) of 0.85 or less, although yeasts generally require a higher water activity (McLandsborough 2017).

Product Stability during Storage
The AWD with the acceptable sensory score and lowest sedimentation was the treatment used to evaluate product stability during storage. The product was stored at ambient temperature and was evaluated for 27 d.

Sensory Evaluation
All attributes have a decreasing trend during storage; however, only viscosity has shown a significant decrease. The decrease in the rating of the parameters the longer a product is stored was common and similar to the result gathered by Navneet *et al.* (2010).

Pectin stability is a crucial property in the storage, particularly in the viscosity and gel strength of acidified milk drinks (Tromp *et al.* 2004). One of the factors in the stability of viscosity of HMP solutions is the temperature (Oakenfull 1991). The study of Morris *et al.* (2010) showed that increased storage time at elevated temperatures has resulted in a notable increase in pectin depolymerization in pectin solutions and gels, thus decreasing viscosity and gel strength. However, constant viscosity and gel strength were observed in the treatments stored at 4 °C, whereas there is a minimal decrease when stored at 25 °C.

Microbiological Analysis
The microbial qualities (<10 cfu/mL APC, 1 cfu/mL yeasts and molds count, <1 cfu/mL coliform count, negative *E. coli*) of AWD is within the standards based on the Revised Guidelines for the Assessment of Microbiological Quality of Processed Foods for Non-alcoholic Beverages (FDA Circular No. 2013-010) up until the 20th day of storage at ambient temperature (FDA 2013). However, on the 27th day of storage, the yeast and molds count exceeded the acceptable standard. The study of Gorachiya *et al.* (2018) showed no yeast and molds growth for the first 9 d; however, it showed growth and exceeded the standard on the 12th day. Yeast and molds best grow at conditions of 10–35 °C with a pH range of 2–9 and are obligate aerobes. Thus, food with low pH is more susceptible to yeast and molds (McLandsborough 2017). Within the 20-d storage period of the AWD, the microbial counts reveal that it is safe and acceptable to consume.

CONCLUSION
There was no significant difference in the pH, TSS, and sedimentation on treatments that used CP and locally made MPP with different concentrations. However, treatments with the highest level of pectin (0.25%) had the least sedimentation. There was a significant difference in viscosity among the treatments wherein 0.25% CP with the highest value but not significantly different from 0.25% MPP. Thus, 0.25% MPP was used for the evaluation of the product stability during storage of the acidified whey beverage through sensory evaluation and microbial analysis. Throughout the storage period, only the attribute viscosity presented a significant difference that decreases over time. The AWD can last about 20 d at ambient temperature.

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