DEVELOPMENT OF WHEY BEVERAGES FROM *PANEER* PREPARATION USING FRUIT COAGULANTS: A COMPARATIVE STUDY ON PHYSICOCHEMICAL AND SENSORY PROPERTIES

Amina Ahmed[1,2*] and Usha Bajwa[2]

[1]Department of Food Sciences and Technology, Mbeya University of Science and Technology, Mbeya, Tanzania.

[2]Department of Food Science and Technology, Punjab Agricultural University, 141001-Ludhiana, Punjab, India.

[*]Corresponding Author's Email: mimomed88@gmail.com

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ABSTRACT: *Whey is a by-product of milk generated during the preparation of coagulated milk products. However, whey disposal results in serious environmental pollution due to its high biological oxygen demand. Therefore, whey generated from the preparation of Indian soft cheese (paneer) by coagulating heated milk with 2% citric acid solution (control) lemon or Indian gooseberry (amla) extracts was collected and mixed with sugar, colour, and flavour were used to develop whey beverages. The whey and whey beverages were analysed for physicochemical and sensory properties. Whey from lemon extract contained higher total solids, fat and protein content than citric acid and amla extract. The ascorbic acid content was higher in amla (56.5 mg/100g) than in lemon whey (6.76 mg/100g) and was not detected in control. The total phenols (GAE mg/100g) and tannins (mg/100g) respectively were higher in amla (550; 394.45) than in lemon (69.23; 4.08) and citric acid (5.86; 0.00). The antioxidant activity was higher in whey from lemon extract (88.77%) than amla (81.38%) and citric acid (14.39%). The whey beverages from both fruit extracts were highly acceptable. Therefore, whey generated from paneer preparation can be used to make beverages with improved health benefits, hence minimising environmental pollution.*

KEYWORDS: Environmental pollution, Indian gooseberry, lemon, sensory evaluation, whey beverages.

INTRODUCTION

Whey is a valuable byproduct liquid obtained during the cheese-making process when milk proteins precipitate (Naik et al., 2023). It contains approximately 93% water, and 7% contains about 50% of the total solids present in the milk, including lactose, whey proteins, minerals and vitamins (Jeličić *et al.*, 2008). Whey protein constitutes approximately 0.7% of the whey (about 9-11% of the dry matter) (Tratnik, 2003). Therefore, improper disposal of whey poses significant environmental challenges for the dairy industry.

Improper disposal of whey, particularly acid whey, leads to severe pollution issues in water bodies due to its high biological oxygen demand (BOD) and chemical oxygen demand (COD) (Cristiani-Urbina et al., 2000; Das et al., 2016; Zhao et al., 2022). Whey's BOD ranges from 30 to 50 g L^{-1}, and its COD ranges from 60 to 80 g L^{-1}, which is categorised as a high pollutant to be disposed of without treatment (Sarenkova et al., 2022). This is a common problem in developing countries where only a small portion of whey is used for value-added products. The disposal of whey not only presents environmental concerns but also economic challenges for dairy industries, especially for small to medium enterprises that lack proper treatment facilities. Therefore, strategies focusing on efficient waste management practices and utilisation of by-products for food are essential to address the challenges posed by whey disposal.

Nonetheless, the food industry has started focusing on biodiversity as a tool to ensure food and nutritional security by maximising the use of by-products and underutilised food resources (Panghal et al., 2018; FAO, 2011). As a result, several researchers started to work on the development of whey beverages to maximise nutrition needs and overcome potential environmental pollution from whey disposal (Raoaf et al., 2014; Girsh, 2001; Jeličić *et al.*, 2008). Production of whey-based beverages started in the 1970s, and a wide range of different whey beverages have been developed. Whey beverages are becoming increasingly popular due to their refreshing, light, and healthful nature, making them a genuine thirst quencher that is less acidic than fruit juice and beneficial for health (Panghal et al., 2018; Yonis et al., 2014). These beverages are formulated using whey and other ingredients such as fruits, isolates of vegetable proteins, chocolates, cocoa, vanilla extracts and other aromatising agents, stabilisers, and acidulates to create acceptable and nutritious drinks (Raoaf et al., 2014; Jeličić *et al.*, 2008). Whey beverages can be classified into various types, including mixtures of whey with fruit or vegetable juices, dairy-type beverages, thirst-quenching carbonated drinks, and even alcoholic beverages (Chavan et al., 2015; Jeličić *et al.*, 2008). Several formulations such as whey and soy proteins (Childs et al., 2007), probiotic whey with pineapple juice (Shukla *et al.*, 2013), whey with papaya, lemongrass and cardamom extracts (Kumar *et al.* (2015), whey with oats (Herrera-Ponce et al., 2022) and whey with aloe vera juice, coconut water, honey, and black salt (Sharma et al., 2022).

Whey beverages combined with natural ingredients, including fruits, have been explored for their health properties (Purkiewicz & Pietrzak-Fiećko, 2021; Herrera-Ponce et al., 2022). Many natural ingredients are rich in several phytochemical compounds, including antioxidants, which play important roles in the food system and human body tissues (Jayasinghea *et al.*, 2013). For example, regular consumption of phytochemicals, such as phenolic compounds from several fruits, helps prevent chronic diseases, including cancer and cardiovascular diseases (Steinmetz & Potter, 1991). Lemon (*Citrus limon L*) and Indian

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gooseberries (*Phyllanthus emblica/ Emblica officinalis*), also known as *amla*, are among the fruits that are rich sources of several phytochemicals (Ahmed & Bajwa, 2019). In our previous study (Ahmed & Bajwa, 2019), we prepared *paneer*, Indian soft cheese, using lemon and *amla* fruit extracts and found improved nutritional and health properties in the *paneer*. However, the properties of the whey were not reported. Therefore, the objective of this study was i. to analyse the physicochemical properties of whey obtained from the preparation of *paneer*, which coagulated using lemon and *amla* fruit extracts, ii. to develop whey beverages from the collected whey and iii. to analyse the sensory properties of the developed whey beverages.

MATERIAL AND METHODS

Preparation of Whey and Whey Beverages

In accordance with our prior study conducted by Ahmed and Bajwa (2019), whey was obtained from the paneer preparation process. The collected whey was then utilised to create a variety of whey beverages, as depicted in Figure 1. Six different formulations were produced, including three plain and three orange-flavoured beverages, each from the whey of citric acid, lemon and *amla*.

Raw whey
⇓
Removal of fat and addition of sugar (10%)
⇓
Addition of citric acid (0.3% acidity)
⇓
Preheating
⇓
Addition of flavour and colour
⇓
Filling in bottles
⇓
Pasteurisation (63°C; 30 min)
⇓
Cooling and storage

Figure 1. Flow diagram for the preparation of whey beverages

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Analysis of Total Soluble Solids, pH and Instrumental Colour Values

Fresh whey was analysed for total solids, total soluble solids (TSS), specific gravity and titratable acidity (TA) content per standard methods (AOAC, 2000). Total TSS were measured as °Brix using the hand Refractometer (Erma, Japan) at 20°C (AOAC, 2000). The pH of each whey was determined using a digital pH meter (Hanna, Italy). Total solids were determined using a hand refractometer of 0-32ºB (ERMA, Japan). The fresh and processed whey instrumental colour values were measured using Mini Scan Xe Plus, U.S.A. (Hunter colour lab) in the hunter colour mode and expressed as 'L', 'a' and 'b' values. In the Hunter scale, 'L' measures the brightness band varying from 100 for perfect white to 0 for black. The chromaticity dimensions ('a' and 'b') give a clear designation of colour, i.e. the value 'a' determines redness when positive, grey when zero and green when negative. The value 'b' determines yellowness when positive, grey when zero and blueness when negative.

Determination of Fat and Ascorbic Acid Content

The fat content of collected whey was estimated using Gerber's method (BIS, 1977). The ascorbic acid was determined using 2, 6-dichlorophenol indo-phenol dye described by Ranganna (2001). First, 10 ml of freshly prepared standard ascorbic acid in a meta-phosphoric acid solution was titrated against 2, 6-dichlorophenol indo-phenol dye. The titre value was used to calculate the dye factor. Then, 10 ml aliquot of each whey sample solution was titrated against dye, and ascorbic acid was calculated as follows:

$$\text{Ascorbic acid (mg/100g)} = \frac{\text{Titre (ml) x dye factor (mg/ml) x Vol. made (ml)}}{\text{Aliquot taken for estimation (ml) x wt. of a sample (g)}} \text{ x 100}$$

Determination of Protein Content

For protein estimation, the total nitrogen in the sample was determined by the Macro-Kjeldahl method (AOAC, 2000). Nitrogen was converted into per cent protein by multiplying with a conversion factor of 6.38 for milk, whey and paneer and 6.25 for amla and lemon extracts

%Nitrogen = [(ml of blank-ml of sample) x Normality of alkali x Nitrogen equivalent weight]/Sample weight x 100

%Protein = %Nirogen x conversion factor

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Determination of Antioxidant Activity Using the DPPH Method

The antioxidant activity of whey was determined using the DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) method, as described by Ahmed et al. (2023).

Determination of Total Phenols by the Folin–Ciocalteau Method

Total phenol content was determined using the Folin–Ciocalteau method, as described by Ahmed and Bajwa (2019).

Determination of Tannins by Folin–Denis Method

Tannins were estimated using the Folin–Denis method (Ranganna, 2001). Measured whey was refluxed with 75 ml of water for one hour, filtered, and the volume was made to 100 ml with distilled water. First, 0.5 ml of sample was added to 37.5 ml of distilled water. Then, 2.5 ml of Follin Denis reagent was added, followed by 5 ml of saturated sodium carbonate, and the volume was made to 50 ml with distilled water. After 30 min of incubation, absorbance was read at 760 nm. Next, tannins were calculated using a standard tannic acid curve.

Evaluation of Sensory Properties of Whey Beverages

The samples of cooled plain and flavoured whey beverages were evaluated by a semi-trained panel of judges using a 9-point hedonic scale (Larmond, 1977) for the sensory attributes of appearance/colour, consistency, mouthfeel, flavour and overall acceptability. The value 1, indicated dislike extremely, 5, neither like nor dislike, and 9, liked extremely.

Statistical Analysis

The data obtained were analysed for analysis of variance using the SPSS® 16.1 software package and were presented as mean and standard deviation. The mean differences were compared using the Least Significance Difference (LSD) test at a 95% confidence interval.

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RESULTS AND DISCUSSION

Physicochemical Properties of the Whey

The results on the physicochemical properties of whey are shown in Table 1. The whey from lemon extract contained higher total solids than *amla* extract and citric acid solution ($p<0.05$). The TSS were highest in the whey from lemon, followed by citric acid solution and *amla* extract ($p<0.05$). The specific gravity of whey differed significantly ($p<0.05$), though the specific gravity of lemon and *amla* whey were similar. Similarly, TS and the specific gravity were highest in whey from lemon, followed by *amla* extracts and citric acid solution. For the TA, the content was highest in lemon whey, followed by *amla* and citric acid solution. Similarly, lemon whey had the lowest pH, followed by *amla* and citric acid solution. The TS content reported in this study is lower compared to other studies. For example Khan and Pal (2010) observed total solids of 23% for whey collected from reconstituted milk. Later, Khan et al. (2014) reported the total solids of 8.9%, 9.1% and 9.4% for whey collected by coagulating reconstituted milk with 2%, 3% and 5% citric acid, respectively. However, the pH values were similar to the values reported by Khan and Pal (2010), Khan *et al.* (2012), and Khan et al. (2014). The variation in TS content in whey can be attributed to several factors, including the type and composition of the milk (Macedo et al., 2018), processing methods (Sarenkova et al., 2019), and the presence of specific components (Yadav et al., 2015). Moreover, factors like pH, temperature, and calcium concentrations can affect whey protein denaturation during processing, subsequently influencing the TS content of the whey (Rashid et al., 2017).

The fat content was higher in whey from lemon extract than *amla* extract and citric acid solution ($p<0.05$). The observed fat content in whey was close to those reported by Khan and Pal (2010) (1.3% for whey collected from reconstituted milk), Khan et al. (2012) (0.18% for whey collected from fresh cow milk with 3.73% fat), both coagulated with 2% citric acid. Nonetheless, the fat content of whey depends on the type of milk used and the strength of the acidulant (Khan et al., 2014).

The protein content of each whey type of acidulant differed significantly ($p<0.05$). The protein content was highest in whey from lemon, followed by *amla* extract and citric acid solution. The protein content of lemon and amla whey was close to that (0.38% and 0.537%) reported by Khan et al. (2014). The whey proteins may supply the body with all the essential amino acids metabolised directly into muscle tissue, unlike other amino acids metabolised into the liver; thus why, its consumption is increasing, especially among people engaged in sports (German *et al.*, 2001).

Nevertheless, the ascorbic acid and tannins were not detected in whey from citric acid but were higher in *amla* than in lemon whey ($p<0.05$). The total phenols were highest in *amla*, followed by lemon and citric whey ($p<0.05$). The antioxidant activity was highest in whey from lemon, followed by *amla* extract and citric acid solution ($p<0.05$). The high antioxidant activity of whey from lemon and *amla* whey could be explained by the presence of ascorbic acid and phenolic compounds, highly presented in the fruits, which leached in whey during the separation of coagulum and pressing of *paneer* (Ahmed & Bajwa, 2019).

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Table 1. Physicochemical Properties of the Whey Collected from Different Acidulants

Parameter	**Citric acid solution**	**Lemon extract**	***Amla* extract**
Yield (%)	81.27[a] ±2.53	73.99[b] ±0.14	77.42[c] ±0.50
Moisture (%)	95.04[a] ±0.25	94.44[b] ±0.31	94.72[ab] ±0.09
Total solids (%)	4.96[a] ± 0.25	5.56[b] ± 0.31	5.28[ab] ± 0.09
TSS (°Brix)	4.60[a] ± 0.20	5.20[b] ± 0.35	4.33[a] ± 0.12
Specific gravity	1.016[a] ± 0.001	1.044[b] ± 0.002	1.042[b] ± 0.04
Titratable acidity (Lactic%)	0.218[a] ± 0.003	0.423[b] ± 0.024	0.312[c] ± 0.05
pH	5.20[a] ± 0.100	4.37[b] ± 0.25	5.03[a] ± 0.06
Fat (%)	0.583[a] ±0.029	1.33[b] ±0.06	1.27[b] ± 0.06
Protein (%)	0.183[a] ±0.016	0.399[b] ±0.024	0.356[c] ±0.05
Ascorbic acid (mg/100g)	0.00[a] ±0.00	6.76[b]± 0.064	56.50[c] ± 0.65
Total phenols (GAE mg/100g)	5.86[a] ±3.36	69.23[b] ±3.85	550.00[c] ±3.85
Tannin (mg/100g)	0.00[a] ±0.00	4.08[b] ±0.88	394.45[c] ±4.84
Antioxidant activity (%)	14.39[a] ± 0.14	88.77[b] ± 0.08	81.38[c] ± 0.14
Colour values			
Fresh whey			
‘L’	56.03[a] ±0.10	64.63[b] ± 0.04	52.92[c] ± 0.13
‘a’	-2.90[a] ± 0.41	-4.30[b] ± 0.02	-3.23[a] ± 0.12
‘b’	5.21[a] ± 0.08	6.98[b] ± 0.02	6.40[c] ± 0.13
Plain whey beverage			
‘L’	33.71[a] ±0.220	31.18[b] ±0.250	35.93[c] ± 0.510
‘a’	-0.850[a] ±0.303	-0.29[a] ±0.997	-1.19[a] ± 0.12
‘b’	0.09[a] ± 0.556	0.19[a] ±0.118	-1.51[b] ± 0.130
Flavoured whey beverage			
‘L’	31.53[a] ±0.260	29.35[b] ±0.480	34.03[c] ± 0.110
‘a’	0.67[a] ±0.115	0.51[a] ±0.282	-1.24[b] ± 0.180
‘b’	8.30[a] ±0.070	3.86[b] ±0.180	9.64[c] ± 0.160

‘L’ measures the brightness band varying from 100 for perfect white to 0 for black, ‘a’ determines redness when positive, grey when zero and green when negative, and ‘b’ determines yellowness when positive, grey when zero and blueness when negative. The values with a different superscript in a row differed significantly at p<0.05

On the other hand, the ‘L’ values (brightness) of the raw whey differed significantly among the acidulants (p<0.05). It was highest in lemon whey, followed by citric acid and *amla*. However, the ‘L’ value for the plain whey beverage was highest in whey from *amla* in plain beverages, followed by citric acid and lemon (p<0.05). Also, the greenness (‘a’ value) was highest in fresh whey and plain whey beverages from lemon, followed by *amla* and citric acid. The ‘b’ value (yellowness) differed significantly (p<0.05) with acidulants. It was higher in whey from lemon and *amla* than citric acid for fresh whey. However, the plain whey beverage from amla exhibited a negative ‘b’ value, which implied blueness. The high ‘a’ value of whey from fruit extracts might be attributed to yellow pigments such as flavonoids and carotenoids retained from the fruit extracts. Normally, the greenish-yellow translucent colour of the whey is due to the water-soluble and heat-stable riboflavin (De, 1977).

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Nonetheless, the intense green colour of lemon and *amla* whey might be due to plant pigments such as chlorophyll naturally present in the fruit extracts.

Sensory Scores of the Whey Beverages

The sensory scores of the whey beverages are shown in Table 2. The appearance/ colour of the whey beverage was comparable to the plain whey beverages from lemon, citric acid and *amla*. The appearance/ colour score was highest in flavoured lemon whey beverages, followed by citric and *amla*. The slightly low score from *amla* extract could be due to the high content of tannin, which imparted the distinctive darkish colour, sedimentations and haze. Normally, the hazes are caused by suspended insoluble particles of colloidal or larger particle size, most often due to protein-polyphenol interaction (Siebert, 1999; Siebert, 2009). Therefore, clarification of the whey is recommended to remove all sedimentable casein fine particles. The high acceptability of various whey beverages with various colours is reported (Landge & Gaikwad, 2013). However, the reduced scores for the coloured whey beverages were evidence that natural products with their native colour could have high acceptability in the market.

There was no significant difference in consistency and mouthfeel scores among the whey beverages. Similarly, Landge and Gaikwad (2013), Khan and Pal (2010) and Khan et al. (2012) have reported the high acceptability of consistency of several whey beverages. For the flavour, the beverages from plain citric acid and lemon whey beverage scores were similar but differed significantly from *amla* ($p<0.05$). The lowest sensory score in plain *amla* whey beverages might be attributed to astringency, a complex of whey proteins and tannins (Rawel *et al.*, 2001).

There was no significant difference in overall acceptability among the whey beverages ($p>0.05$). However, overall acceptability was highest in lemon, followed by citric acid and *amla* whey beverages. Landge and Gaikwad (2013) also reported the high overall acceptability of *jeera*-flavoured whey beverages added with C.M.C. and sugar. Based on the sensory scores and overall acceptability, plain whey beverages and those flavoured with lemon and citric acid were highly acceptable. The *amla*-flavoured whey beverage, on the other hand, received slightly lower scores due to its darkish colour and astringency. The study suggests that whey beverages with their native colour could have high acceptability in the market.

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Table 2. Sensory Scores of the Whey Beverages

Whey type	Sensory attribute				
	Appearance/ colour	**Consistency**	**Mouthfeel**	**Flavour**	**Overall acceptability**
Plain whey beverage					
Citric	8.25^{a} ±0.49	7.75^{a} ±0.35	7.75^{a} ±0.49	7.95^{a} ±0.69	7.90^{a} ±0.41
Lemon	8.35^{a} ±0.47	7.90^{a} ±0.57	7.80^{a} ±0.59	7.85^{a} ±0.67	7.90^{a} ±0.56
Amla	8.00^{a} ±0.71	7.70^{a} ±0.54	7.50^{a} ±0.75	7.20^{b} ±0.63	7.45^{a} ±0.65
Flavoured whey beverage					
Citric	7.95^{a} ±0.76	7.60^{a} ±0.46	7.75^{a} ±0.49	7.62^{a} ±0.74	7.74^{a} ±0.47
Lemon	8.05^{a} ±0.72	7.70^{a} ±0.48	7.95^{a} ±0.60	8.40^{b} ±0.57	8.10^{a} ±0.51
Amla	7.10^{b} ±0.74	7.65^{a} ±0.53	7.60^{a} ±0.70	7.50^{a} ±0.58	7.45^{b} ±0.52

The value 1 indicated dislike extremely, 5, neither like nor dislike, and 9 liked extremely. The values with different superscripts in the column differed significantly at $p<0.05$

Plate 1. Pictures of whey beverages

Plain: C- Citric acid, L- lemon, A- *amla*, Orange flavoured: CC- Citric acid, L.L.- lemon and A.A.- *amla*

CONCLUSION

In conclusion, whey generated from paneer preparation can be used to make naturally fortified beverages with improved health benefits. The whey beverages prepared from lemon and Indian gooseberry extracts were highly acceptable, and their physicochemical and sensory properties were analysed. The whey from lemon extract contained higher total solids, fat, and protein content than citric acid and amla extract. However, amla extract had higher total phenols, tannins, and ascorbic acid content than lemon whey. The antioxidant activity was also higher in whey from lemon extract than amla and citric acid. Therefore, the use of whey for developing beverages can help to minimise environmental pollution while maximising the utilisation of byproducts of food resources. Further research can explore the

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development of more nutritious and acceptable whey-based beverages to promote sustainable and efficient waste management practices in the dairy industry.

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