Effect of different coagulants on physicochemical properties of paneer made from buffalo milk.

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Abstract

This study was carried out to develop paneer with buffalo milk under four different coagulants: citric acid (T1), Lime (T2), Vinegar (T3), and Yoghurt (T4). The paneer samples were analyzed for nutritional composition, titratable acidity, pH and colour during the storage. Sensory evaluation was done for fresh paneer samples using a seven-point hedonic scale. The result revealed that the pH of developed paneer under T1, T2, T3 and T4 were 1.67, 2.09, 2.54, and 4.16, respectively. Titratable acidity and pH of paneer were ($p<0.05$) varied with 5 days storage at 7 ^oC. The highest yield ($p<0.05$), the whitest and hardest body of paneer was observed under T3. Moisture content was ($p<0.05$) higher under T3, and total solids, ash and fat were significantly ($p<0.05$) higher in T1. Sensory evaluation revealed that the different types of coagulants were significantly influenced ($P<0.05$) by sensory attributes. The overall acceptability was highest under T1. Conclusively, paneer produced with buffalo milk using vinegar and citric acid coagulants resulted in the most suitable products compared to other coagulants used in the present study.

Keywords: *Buffalo milk, Citric acid, Coagulants, Paneer, Sensory*

Introduction

Paneer is an indigenous product from India, basically processed under heat treatment and acid coagulation, and is a good source of fat (22-25 %) and protein (16-18 %). Also, it contains 53 - 55 % moisture, 2 - 2.5 % lactose, and 1.5 - 2.0 % minerals (Kanawjia and Singh, 1996; Kumar *et al.*, 2014). It is a soft cheese that could not ordinarily be held for more than one day and processed neither rennet nor fermentation and ripening, which is closely similar to Latin American white cheese (Torres and Chandan, 1981). Paneer is widely consumed in South Asian countries, predominately Afghanistan, Bangladesh, India, and Pakistan (Kumar *et al.*, 2014). Even though it is predominately prepared by cow milk, other milk types such as buffalo milk, goat milk, camel milk, and sheep milk could also be used to process paneer (Agnihotri and Pal, 1996; Karadbhajne and Bhoyarkar, 2010; Shanaziya *et al.*, 2018; Meena *et al.*, 2020). Moreover, reconstituted with warm water, low-heat spray-dried whole milk powder was also used for paneer preparation (Khan *et al.*, 2014).

Various acids including citric acid, tartaric acid, lactic acid, alum, sour whey, turmeric, malic acid, ascorbic acid, Calcium lactate, Glucono-delta-lactone are mainly used for the acid coagulation process of paneer making (Karadbhajne and Bhoyarkar, 2010; Buch *et al.*, 2014; Kumar *et al.*, 2014, 2019; Suthar *et al.*, 2018). Even though the paneer can be easily produced by using locally available household-level coagulants at a low cost of production, it is not very popular among Sri Lankans. Moreover, several researches were done to find the effect of different coagulants on the quality of paneer made from cow milk (Buch *et al.*, 2014; Shanaziya *et al.*, 2018; Kumar *et al.*, 2019). Karadbhajne and Bhoyarkar (2010) experimented to explore the effect of different coagulants on paneer

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texture prepared by buffalo milk. Therefore, this study was mainly focused on processing paneer with locally available coagulants such as lime juice, vinegar, citric acid and yoghurt with buffalo milk to assess the effect of different coagulants on physio-chemical composition and sensory characteristics of paneer made by buffalo milk.

Material and Methods

Raw materials

Fresh cow milk was obtained from the Livestock Farm, Department of Animal Science, Faculty of Agriculture, Eastern University, Sri Lanka. Four different coagulants (lime juice, vinegar, citric acid and yoghurt) were purchased from a local market.

Preparation of paneer

Paneer preparation was done according to the previously described procedure by Buch *et al.* (2014) and Khatkar *et al.* (2017) with minor modifications. The vat was thoroughly cleaned and sterilized. Then, filtered and standardized (6.1 % fat and 9 % SNF) milk was taken in the vat. Occasional stirring was done during the heating of milk in order to prevent layer formation. The temperature of the milk was raised to 82 ^{0}C and maintained for 30 min, followed by cooling to 70 °C, and then different types of coagulants, namely citric acid, lime juice, vinegar and yoghurt at the rate of 0.25 % of milk were added in a thin continuous stream till complete coagulation was achieved as evidenced from the clarity of whey. The time taken for adding the coagulant was approximately 60-80 sec. The pH of whey ranged from 5.6 to 5.5. Before draining the whey, the contents of the vat were left undisturbed for 15 min. The coagulated mass was then collected in muslin cloth. The curd was moved to hoops lined with muslin cloth and pressed (40 g /cm^2 for 10 min) to get a compact block of paneer. The pressed paneer was removed from the muslin cloth and cut into 2-3" pieces, which were then immersed in chilled water (7 ^{o}C) for 2-3 hours. Then the product was removed from the chilled water and drained out the excess water. The paneer samples were wrapped in polythene sheets and stored in the refrigerator at 7 ^{o}C until further analysis.

Chemical analysis

The paneer samples stored at 7 ^{o}C were analyzed for chemical characteristics at regular intervals of 5 days during storage. Moisture content in paneer was determined in triplicate for dry matter by oven drying at 105 ^{o}C to get constant weight according to the method described by AOAC (2005). The percentage of dry matter was calculated. Ash content was determined by using a muffle furnace at 550 ^{o}C for 4 h as described by AOAC (2005). The titratable acidity of paneer samples was determined by following the method prescribed for cheese by the AOAC (1975). The fat content of paneer was estimated using the method described by AOAC (2005). The pH of the paneer was determined using a pH meter, as described by Arora and Gupta (1980).

Determination of colour

The colour of the paneer was analyzed using the Konica Minolta Colorimeter (Osaka, Japan) according to the procedure previously described by Pathare *et al.* (2013). The most popular RGB (Red, Green and Blue) system was used for the analysis and converted it into CIE L*a*b (International Commission on Illumination. L* indicates the intensity of light in black from 0 to 100 scales; a* (+) red or (-) green and b* (+) indicates yellow or (-) blue) and HSV (Hue, Saturation and Value) systems by using RGB converter.

Sensory evaluation

Each block of paneer was cut into rectangular pieces of approximately 1 cm × 2 cm. The prepared paneer samples were subjected to sensory evaluation by a panel of 30 judges when fresh and at regular intervals of 5 days during storage using a 9-

point hedonic scale according to the methods of Buch *et al.* (2014). The panellists judged the sensory characteristics, such as colour, flavor, taste, texture and overall acceptability of the paneer samples on days 1-5 of the storage period.

Statistical analysis

Samples were randomly collected; parametric data were analyzed using Multivariate Analysis of Variance (MANOVA) to determine the treatments' significance level. Duncan's Multiple Range Test (DMRT) was used for mean separation. The sensory analysis was carried out using Friedman's test for non-parametric data analysis.

Results and Discussion

Yield and nutritional composition of developed paneer

The pH of the fresh panner produced by different were 1.67 ± 0.01, 2.09 ± 0.01, 2.54 ± 0.01 and 4.16 ± 0.02 for citric acid, lime juice, vinegar and yoghurt, respectively. Yields of prepared paneer according to each treatment are shown in Table 1. According to our observation, paneer developed with vinegar had a ($p < 0.05$) higher yield compared to other coagulants, where the lowest was observed in the coagulant used as a yoghurt. The present values obtained for citric acid coagulant were aligned with the previous findings of paneer reported by Kumar *et al.* (2019). But Shanaziya *et al.* (2018) and Kumar *et al.* (2019) reported that citric acid had a higher yield than lime juice and vinegar. This could be due to paneer being treated with alum, calcium lactate, and glucono-delta-lactone.

Table 1: Yield and nutritional composition of paneer with different coagulants

	Treatment			
Parameter	**T1**	**T2**	**T3**	**T4**
Yield (g/Lit of milk)	156.19 ± 0.41^{b}	157.01 ± 0.66^{b}	169.21 ± 0.36^{a}	126.26 ± 0.87^{c}
Total solids (%)	53.53 ± 0.94^{a}	50.44 ± 0.23^{b}	48.56 ± 0.72^{c}	47.12 ± 0.14^{c}
Ash (%)	2.72 ± 0.01^{a}	2.57 ± 0.06^{b}	1.58 ± 0.02^{d}	2.43 ± 0.01^{c}
Fat (%)	27.20 ± 0.31^{a}	26.68 ± 0.06^{a}	25.68 ± 0.21^{b}	26.08 ± 0.02^{a}

T1-Citric acid, T2- Lime, T3- Vinegar, T4-Yourghut. The values were expressed as means ± standard deviations of replicate determination mean with different letters were significantly different ($p < 0.05$).

The total solids content of paneer was different ($p < 0.05$) with different types of coagulant (Table 1). The total solids content of the paneer was higher in citric acid-added paneer and lower in yoghurt-added paneer. The value of total solids was higher than reported by Karadbhajne and Bhoyarkar (2010) and Shanaziya *et al.* (2018). The similar trend of the results was in accordance with those previously reported by Meena *et al.* (2020) for panner development from buffalo and cow milk.

The ash content was the highest in the case of citric acid paneer, followed by lime juice on the other, ash contents of yogurt and vinegar added paneer was showing lower ash contents (Table 1). The ash content of product depends upon the mineral content in the raw material. Fat content significantly ($p < 0.05$) influenced between treatments that was indicated in Table 1 With different coagulants fat content of paneer changes (Arya and Bhaik, 1992). When citric acid used as a coagulant fat content is higher in paneer than other coagulants. The flavor of paneer directly related with fat content of milk. These values were fallen within the acceptable range (Chawla *et al.*, 1987).

pH changes in developed paneer during storage

The pH of the developed paneer was significantly affected ($P < 0.05$) by different coagulants (Table 2). In addition, the treatment and storage time interacted with pH and were within the acceptable range of up to four days of storage at 7 ^{0}C. In most of the developed paneer, pH decreased with storage time. It might be the breakdown of lactose into lactic acid by microorganisms during the fermentation process. The decrease in the pH of paneer during storage was also reported earlier by various research workers Shanaziya *et al.* (2018).

Table 2: pH in paneer during storage with different coagulants

Treatments	Day 2	Day 3	Day 4	Day 5
T_1	5.84 ± 0.02^{edfg}	5.72 ± 0.14^{gh}	5.51 ± 0.01^{i}	5.46 ± 0.05^{i}
T_2	5.90 ± 0.14^{cdef}	5.80 ± 0.01^{efgh}	5.79 ± 0.02^{fgh}	5.68 ± 0.01^{h}
T_3	6.26 ± 0.04^{a}	6.14 ± 0.01^{b}	5.92 ± 0.03^{cde}	6.01 ± 0.06^{bc}
T_4	6.02 ± 0.01^{bc}	5.94 ± 0.04^{cd}	5.95 ± 0.01^{cd}	5.85 ± 0.01^{defg}

T1-Citric acid, T2- Lime, T3- Vinegar, T4-Yourghut. The values were expressed as means ± standard deviations of replicate determination mean with different letters were significantly different ($P < 0.05$).

Change in paneer Titratable acidity during storage

The titratable acidity of the developed paneer was significantly affected ($P < 0.05$) by different coagulants (Figure 1). There might be an interactive effect of different coagulants used as treatments and the storage time on the titratable acidity of the developed paneer, and the shelf life could be acceptable for four days after preparation under 7 ^{o}C. Generally, the microbial count increases with the time of storage and the temperature of storage. The increasing population of microbial count leads to ferment lactose, specifically into lactic acid. Therefore, the titratable acidity levels are increased with the storage time (Pal *et al.*, 1991).

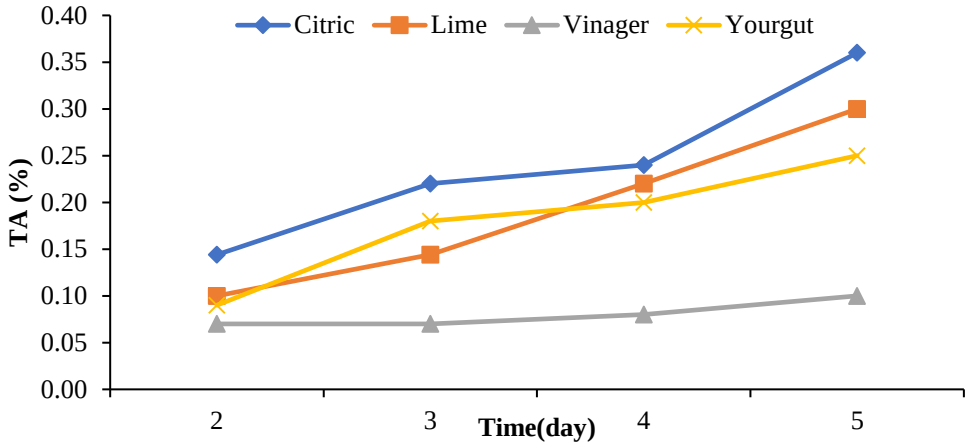

Figure 1: Changes in titratable acidity in paneer with the storage period

Determine the colour parameters of paneer with different coagulants.

The R, G, B values, H, S, V values and L*, a* and b* values of paneer prepared from different coagulants were delineated in Table 3. The lightness values (L*) of paneer were 86.44, 91.36, 95.46, and 86.31 for citric acid, lime, vinegar and yoghurt as coagulants, respectively. The higher values for L* indicate the whiter colour paneer, which might be the formatting of finer casein particles due to the mechanism of coagulation rather than the casein destabilization. More whitish paneer was obtained by using vinegar as the coagulant, followed by lime, citric acid, and yoghurt. Various research workers also observed similar results for L* values for prepared paneer using cow milk with the citric acid coagulant (Kumar *et al.*, 2019).

Table 3: Colour parameters of Paneer with different coagulants

Treatments	RGB value	HSV value	L*a*b value
T1	**R** 223.69±0.45^{c} **G** 215.79±0.30^{c} **B** 197.87± 3.02^{b}	**H** 41.54±2.17^{a} **S** 11.54± 1.17b^{c} **V** 87.67±0.17^{b}	**l** 86.44±0.18^{b} **a** -0.31±0.49^{a} **b** 9.79±1.33^{b}
T2	**R** 239.67±0.47^{b} **G** 229.74±0.37^{b} **B** 204.18± 0.25^{a}	**H** 43.21±0.18^{a} **S** 14.89±0.28^{ab} **V** 93.99±0.19^{a}	**l** 91.36±0.13^{ab} **a** -0.79±0.06 ^{a} **b** 13.66±0.32^{ab}
T3	**R** 243.88±0.18^{a} **G** 234.61±0.56^{a} **B** 204.72±0.40^{a}	**H** 59.32±19.63^{a} **S** 17.87±2.46^{a} **V** 97.80±3.12^{a}	**l** 95.46±3.74^{a} **a** -6.69±7.23^{a} **b** 19.18±5.03^{a}
T4	**R** 221±1.41^{d} **G** 216±2.83^{c} **B** 199±1.41^{b}	**H** 46.37±3.85^{a} **S** 9.96± 0.06^{c} **V** 86.67±0.56^{b}	**l** 86.31±0.86^{b} **a** -1.19±0.73^{a} **b** 8.98±0.50^{b}

T1- Citric acid used as a coagulant, T2- Lime used as a coagulant, T3- Vinegar used as a coagulant, T4-Yogurt used as a coagulant. Values are means ± standard deviations of replicate determination mean with same letters are not significantly different at (P > 0.05)

Dhankhar (2014) also observed that the colour of paneer is also white. Moreover, Masud *et al.* (2007) found that the colour of the paneer varied from fairly white to bright white when there was a reduction in the coagulation temperature of the paneer. The * values are explained as the red to green range of colours where the positive values represent red colours and negative values represent the green colours. Similarly, b* values are extrapolated as the blue to the yellow range, where the positive values represent yellow and blue colours are represented by negative values (Choudhury and Kumar, 2014). Therefore, the findings revealed that those all-prepared paneers were in pale colours that varied between white to pale yellow, considering all the obtained values for L*, a*, and b*.

Determine the texture parameters of paneer with different coagulants

Paneer has a compact to slightly opened texture associated with slight porosity and sponginess (Springiness). This depends on several factors, including the type of milk, the quality of milk, heat treatments provided during processing, the amount, type, and temperature of coagulants used, and the period of storage (Patel and Jayaraj Rao, 2012).

The obtained hardness values of the panner were significantly different under four different coagulants. The hardest body of paneer was prepared under the vinegar coagulant, followed by lime, citric acid and yoghurt coagulant, respectively (Table 4). In this study, the values of the hardness were very low compared to previous findings of paneer prepared using different coagulants like citric acid, malic acid, Alum, Ca-lactate and Glucono-delta-lactone while higher and lower springiness values were obtained panner made from Citric acid and Lime, respectively (Kumar *et al.*, 2019). However, those obtained springiness values were significantly lower than the previous springiness values of prepared paneers (Karadbhajne and Bhoyarkar, 2010). The cohesiveness and chewiness values were closely similar to those of the previous findings. The paneer prepared using vinegar was more cohesive among the four coagulants used during the present study.

Table 4: Textural properties of Paneer with different coagulants

Treatments	Hardness (N)	Cohesiveness	Springiness	Gumminess (N)	Chewiness (N)
T1	10.3 ± 0.28^{c}	0.30 ± 0.00^{b}	4.3 ± 0.28^{ab}	3.07 ± 0.06^{b}	12.60 ± 0.26^{c}
T2	14.4 ± 0.56^{b}	0.77 ± 0.02^{a}	2.6 ± 0.57^{b}	11.08 ± 0.66^{a}	28.94 ± 8.05^{b}
T3	19.7 ± 0.42^{a}	0.58 ± 0.11^{ab}	4.7 ± 0.42^{a}	11.37 ± 1.94^{a}	53.04 ± 4.30^{a}
T4	8.2 ± 0.99^{d}	0.53 ± 0.29^{ab}	3 ± 0.99^{ab}	4.46 ± 2.88^{b}	11.97 ± 4.21^{c}

T1-Citric acid is used as a coagulant, T2- Lime is used as a coagulant, T3- Vinegar is used as a coagulant, and T4-Yoghurt used as a coagulant. Values are means ± standard deviations of replicate determination mean with the same letters are not significantly different at ($P > 0.05$).

Sensory characteristics of developed paneer

Although there were no significant differences in colour and aroma among paneer prepared by four different coagulants, the texture, taste, and overall acceptability differed ($P < 0.05$). The highest taste was noticed in citric acid paneer, followed by yoghurt, lime juice and vinegar. Aghav *et al.* (2014) revealed that there were only minimal changes, even negligible, in the physio-chemical

constituent of milk fat after the preparation of paneer from cow milk as well as from buffalo milk. Therefore, it could be suggested that those who lacked significant differences in colour and aroma in developed paneers closely accepted that finding. Paneer developed with citric acid showed the best preference (Figure 2). The highest texture score was observed in the case of paneer prepared from coagulants with citric acid, followed by yoghurt, lime juice and vinegar.

The overall acceptability is based on multiple organoleptic quality parameters, including colour, flavour, and texture and shows the accumulative perception and acceptance by the panelists. Paneer developed with citric acid sample had maximum overall acceptability compared to other coagulants, and a lower overall acceptable score was observed in paneer developed with vinegar. Furthermore, those present findings for overall acceptability were closely similar to the sensory evaluation of paneer prepared by cow milk (Shanaziya *et al.*, 2018).

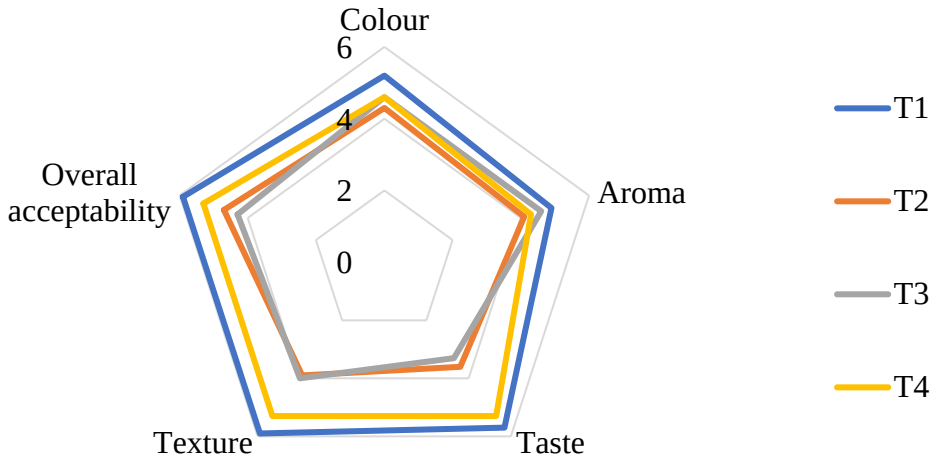

Figure 2: Sensory quality attributes of paneer with different coagulants (T1-Citric acid, T2- Lime juice, T3- Vinegar, T4-Yourghut)

Conclusion

Different coagulants such as citric acid, lime juice, vinegar, and yoghurt can be used to prepare paneer by using buffalo milk with acceptable sensory quality attributes without a disturbance to the nutritional composition as well as the organoleptic properties like colour, aroma and taste. However, the yield of paneer was greater when vinegar was used as the coagulant, while the overall acceptability of paneer was greater when citric acid was used as the coagulant. Further studies should be encouraged to evaluate the effect of various coagulants on paneer quality prepared by buffalo milk by comparing different concentrations of coagulants.

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Conflict of Interest

None of the authors of this study have any financial interest or conflict with industries or parties.

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