Indian J Dairy Sci 77(5): 441-447
https://doi.org/10.33785/IJDS.2024.v77i05.007

RESEARCH ARTICLE

Effect on quality of *paneer* using unripe mango powder as a natural coagulant

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Received: 10 August 2023 / Accepted: 16 December 2023 / Published online: 23 October 2024

Abstract: Paneer, an important traditional dairy product is manufactured by different types of coagulants, however, use of natural coagulants reported are few. In this study, unripe mango powder was used as the natural coagulant in paneer manufacturing. Three variables employed were unripe mango powder (12-16 g/kg), coagulation temperature (70 to 85 °C), and fat content of milk (2.00 to 4.50 %); These variables were studied using Face-centered central composite design in design expert 13.0.5.0. The proximate compositional analysis of the optimized paneer revealed 59.55 ± 0.16 % moisture, 14.68 ± 0.03 % fat, while protein content was 18.94 ± 0.06 %, total carbohydrate content 4.76 ± 0.23 %, ash content 2.07 ± 0.04 % and fiber content 0.5 %. The physicochemical properties studied include titratable acidity (0.43 ± 0.02 % LA), pH (6.0 ± 0.01), water activity (0.98 ± 0.01) and Free fatty acid content (0.16 ± 0.03 %). The rheological characteristics of the developed paneer indicated values for hardness as 7.29 ± 0.60N, springiness 5.83 ± 0.15mm, cohesiveness 0.31 ± 0.01, chewiness of 13.23 ± 1.57Nmm, gumminess of 2.27 ± 0.22N, and adhesiveness of 0.03 ± 0.01Nmm. Fresh paneer sample had APC (Aerobic Plate Count) of 4.72 ± 0.47 $\log_{10}$ cfu/g, while Yeast and Mold count and Coliform count were absent/g. The sensory scores for the optimized paneer were 43.80 ± 0.51 for flavour (out of 50), 30.15 ± 0.77 for body and texture (out of 35), 8.16 ± 0.11 for colour and appearance (out of 10) and 87.11 ± 1.20 for the total sensory score (out of 100). The yield of the optimized paneer obtained was 17 %.

Keywords: Paneer, unripe mango, natural coagulant, coagulation, reconstitution, texture profile

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Introduction

Paneer is the omnipresent traditional Indian dairy product in the country. Acceptable in all households, celebrations without paneer are considered to be incomplete. Paneer consumes 7 per cent of the total milk production of India. Indian paneer market valued [1] 494 billion in 2022. Between 2023- 2028, It is expected that the paneer market will grow at a CAGR of 15.7 per cent or to [1] 1,173 billion (IMARC,2023). FSSAI (2020) has defined and classified Paneer. It consists of a pleasant mild acidic nutty and sweet flavour with a smooth and compact body and texture; white colour with a greenish tinge.

In Mango, a bio active component, Mangiferin serves as potential antioxidant, anti-lipid peroxidant, immunomodulator, cardiotonic, and hypotensive, helps in wound healing and acts as an antidegenerative and anti-diabetic compound. Another compound glucosyl xanthone also acts as a polyphenolic antioxidant. The mango fruit is regarded as energising and refreshing. Mango can be used in the treatment of different types of ailments viz. piles, leucorrhoea, haemorrhage, bronchitis, cough, asthma, hypertension, rheumatism and insomnia. It also functions as an antiseptic, stomachic, laxative, vermifuge, tonic and diuretic (Lauricella et al. 2017).

Mango acidity varies to the content of citric (0.13 to 0.71% fresh weight (FW)) and malic acids, although common organic acids are also found like oxalic, succinic and pyruvic as well as tartaric, muconic, galipic, glucuronic, and galacturonic acids as well as ascorbic acid (Maldonado-Celis et al. 2019). Unripe mango consists of 36.4 mg ascorbic acid, 1.6g dietary fibre, 0.111mg copper and 0.16 mg iron per 100 g , which all lack in the milk so the addition of unripe mango can increase the ascorbic acid, fibre, copper and iron content of milk (USDA, 2019). Thus mango in the form of unripe mango powder, with its medicinal and therapeutic

properties can be a potential coagulant in the manufacture of Paneer.

Materials and Methods

Materials

Raw milk was procured from Anubhav dairy, Anand and Unripe mango (Rajapuri) powder was procured from Aum Agri Foods Pvt. Ltd., Vadodara.

The basic process of unripe mango powder coagulated paneer preparation.

Fresh good quality raw milk was procured from Anubhav Dairy. It was filtered and standardized as per the runs suggested by design software 13.0.5.0. The milk was poured into the S.S. vessel and the heating of milk inside the vessel was carried out by indirect heating. The milk was heated up to 90°C for 10 minutes and then the temperature of the milk was lowered to coagulation temperature. At this stage, 10% of the mango powder solution prepared by rehydration and reconstitution with distilled water was brought to the coagulation temperature and added to milk as a coagulant. After holding the coagulated curd for 4-5 minutes, the whey was separated by straining. Transfer of the coagulum into the S.S. paneer hoops and subjected for pressing at 6 kg/cm^2 for 30 mins. After de-hooping, the paneer block was submerged in pasteurised cold water (2-4°C) for two hours. The yield of paneer was measured and it was packed in multi layer (12µ polyester+ 50µ LD/LLDPE) pouches and stored at 7±1°C.

Experimental Design

The Face-centered central composite design was used for designing the experimental combination. The experiment was designed using the software Design Expert version 13.0.5.0 (Table 4). The independent variables were the rate of addition of unripe mango powder (12 to 16 g), coagulation temperature (70 to 85°C) and fat content of milk (2 to 4.5%). These ranges were entered into Design Expert 13.0.5.0. It suggested treatments involving various combinations or levels of the chosen process parameters and based on these data and the number of the selected process parameters or factors, a total of 20 trial combinations were suggested by the Statistical programme.

Analysis

Compositional analysis

The moisture and fat content of the developed paneer was evaluated as per FSSAI (2022). Total protein content was determined by using the micro Kjeldhal method given by AOAC (1980). The ash content was measured according to Indian Standards (1981).

Physico-chemical analysis

The titratable acidity of unripe mango powder coagulated paneer was analysed by using the method suggested by Ahmed and Bajwa (2019). pH was determined by a digital pH meter by following the process given by Franklin and Sharpe (1963) for cheese. The water activity of the paneer sample was determined using the Rotronic Hygroskop Model: Hygrolab-3 (M/s. Rotronic ag, Switzerland) connected to a sensing element (AW-DIO) with a measuring range of 0-100% relative humidity at 25°C. For the analysis of free fatty acids method given by Deeth et al. (1975) was followed. The tyrosine value of paneer was determined by a method suggested by Lowry et al. (1951).

Texture profile/Rheological analysis

Using a Food Texture Analyzer of Lloyd Instruments LRX Plus material testing machine, England, equipped with a 0-500 kg load cell, five samples from each of the experimental paneer were subjected to uniaxial compression to 40% of the initial sample height. The cross-head speed was 50 mm/min, the trigger was 10 gf, and a 40 per cent force was applied to obtain the force-distance curve for a two-bite deformation cycle. To study the paneer‘s textural attributes samples were held for 1 hour at 23°C and 55% RH.

Sensory Evaluation

More than 8 judges were chosen for the paneer’s organoleptic assessment. Using the 100-point scale outlined in Indian Standards (IS 15346, 2003), the paneer samples were assessed. The judges were also requested to give criticism for each attribute of the samples.

Microbiological analysis

All of the paneer samples were examined using the BIS (Indian Standards 5550, 2005) method with minor modifications for the Aerobic Plate Count (APC), Coliform count, and Yeast and Mold count.

Statistical analysis

The experiment was designed and responses were analysed using software design expert version 13.0.5.0. All the compositional, Physicochemical, Rheological, Sensory evaluation and microbial analyses done for optimized products obtained by the software were repeated seven times. FCCD independent variables were the addition of unripe mango powder (g/kg of milk), temperature of coagulation (°C) and fat content of the milk (%).

Results and Discussion

The effect of the variables on the unripe mango powder coagulated paneer and the responses and actual values observed are reported in Table 3. A quadratic model was fitted for all the responses. R^2 > 0.80 for sensory attributes is statistically adequate for developing a model or equations (Henika,1982). In the present study R^2 was > 0.80 for all sensory responses as well as moisture and yield was observed. The probability value (p) showed the adequacy of the models so used to describe the effect of variables on different responses. The effect of the rate of addition of unripe mango powder, the temperature of coagulation and the fat content of the milk on the responses (Flavour, Body and Texture, Colour and Appearance, Total score, Yield and Moisture) are shown in the equations below and Table 2. The sign and magnitude of coefficients indicate the effect of the variable on the responses. The models thus developed with coded variables are as follows:

Flavour = $42.18 - 0.8129A - 0.0476B - 1.23C + 0.0119AB + 0.3810AC - 0.4048BC - 0.1937A^2 - 1.48B^2 + 0.0838C^2$.

Body and Texture = $29.64 - 0.6899A - 0.8280B - 1.09C - 0.0655AB + 0.2798AC - 0.3274BC - 1.19A^2 + 0.7155B^2 - 0.5617C^2$.

Body and Texture = 29.64- 0.6899A -0.8280B -1.09C -0.0655AB + 0.2798AC -0.3274BC - $1.19A^2$ +$0.7155B^2$ -$0.5617C^2$.

Table 1: Partial Coefficient of Regression Equations of Suggested Model for Sensory Scores, Yield and Moisture of Paneer

	Intercept	A	B	C	AB	AC	BC	A^2	B^2	C^2
Flavour Score	42.18	-0.8130*	-0.0476	-1.2267*	0.0119	0.3810	-0.4048	-0.1937	-1.4844*	0.0838
Body & Texture Score	29.64	-0.6899*	-0.8280*	-1.0892*	-0.0655	0.2798	-0.3274	-1.1892*	0.7155	-0.5619
Colour & Appearance Score	7.78	-0.1054	-0.1071	-0.4977*	0.0223	0.1384	-0.2009	0.0051	-0.4681	-0.2657
Total Score	84.60	-1.6081*	-0.9827	-2.8136*	-0.0313	0.7991	-0.9330	-1.3778	-1.2370	-0.7436
Yield	16.94	-0.5690	0.1290	2.336**	0.5513	0.0613	-0.2862	1.2290	-0.1709	0.3540
Moisture	53.70	-0.782	-0.031	4.658**	0.7175	0.0375	0.0225	3.2027*	0.4516	0.3768

*Significant at 5 per cent level (Pd”0.05), **Significant at 1 per cent level (Pd”0.01),

Note- A, B and C refer to the three factors studied viz. unripe mango powder, coagulation temperature and fat per cent of milk respectively

Table 2: Experimental Design Matrix and Sensory Scores, Yield and Moisture of Paneer

Run	Coagulant (g/kg)	Coagulation Temperature (°C)	Fat (%)	Flavour Score (50)	Body and Texture Score (35)	Colour and Appearance (10)	Total Score* (100)	Yield (%)	Moisture (%)
1	14	70	3.25	40.00	30.47	7.25	82.72	16.80	53.13
2	14	85	3.25	41.43	30.71	7.43	84.57	17.60	54.36
3	16	70	2	40.57	30.00	7.36	82.93	13.70	50.00
4	12	70	4.5	40.57	29.71	7.00	82.29	22.00	63.32
5	14	77.5	2	42.94	29.88	7.58	85.40	16.15	52.00
6	14	77.5	3.25	42.43	29.65	7.71	84.79	18.00	55.96
7	16	70	4.5	39.14	27.71	6.57	78.43	20.00	60.55
8	14	77.5	3.25	41.57	29.69	7.86	84.12	17.60	54.82
9	12	77.5	3.25	42.26	28.88	7.50	83.64	18.30	57.56
10	16	77.5	3.25	41.75	28.50	8.13	83.38	18.90	58.00
11	14	77.5	3.25	42.67	29.83	7.83	85.33	16.15	52.10
12	14	77.5	3.25	41.50	29.69	7.42	83.61	15.90	51.50
13	12	85	4.5	39.00	26.00	6.00	76.00	21.00	62.20
14	16	85	4.5	38.14	25.86	6.14	75.14	20.10	60.99
15	14	77.5	3.25	42.43	29.00	7.86	84.29	16.15	52.30
16	14	77.5	3.25	42.43	29.00	7.86	84.29	16.15	52.00
17	16	85	2	40.67	27.33	7.25	80.25	16.05	51.66
18	14	77.5	4.5	41.63	28.75	7.50	82.88	19.30	59.16
19	12	70	2	43.00	31.00	7.86	86.86	17.05	54.23
20	12	85	2	43.57	30.71	8.14	87.43	16.09	51.71

* Total score also includes package score (5)

Body and Texture = 29.64- 0.6899A -0.8280B -1.09C -0.0655AB + 0.2798AC -0.3274BC - 1.19A^2 +0.7155B^2 -0.5617C^2.

Colour and Appearance = 7.78 -0.154A -0.1071B -0.4976C + 0.0223AB +0.1384AC - 0.2009BC +0.0051A^2 - 0.4681B^2 -0.2657C^2.

Total Score = 84.60 -1.60A -0.9827B -2.81C -0.0312AB +0.7991AC -0.9330BC -1.38A^2- 1.24B^2 -0.7436 C^2.

Yield = 16.94 - 0.5690A + 0.1290B +2.34C +0.5512AB +0.0612AC- 0.2863BC +1.23A^2 -0.1709B^2 +0.3541C^2.

Moisture = 59.04- 0.7820A -0.0310B +4.66C +0.7175AB +0.0375AC +0.0225BC + 3.20A^2 – 0.8323B^2 + 0.9827C^2.

Sensory scores of developed paneer for flavour varied from 38.14 to 43.57 out of 50, Body and texture varied from 25.86 to 31.00 out of 35, Colour and appearance varied from 6.00 to 8.14 out of 10, Total score varied from 75.15 to 87.43 out of 100. Similarly, Moisture ranges from 50.00 to 63.32 per cent and yield varied from 13.70 to 22.00 % as shown in Table 2.

The Flavour scores (Figure 1) of the paneer was affected significantly (P<0.05) in a negative way by unripe mango powder and fat content linearly and coagulation temperature at quadratic levels (Table 1). The flavour score of paneer ranged from 38.14 to 43.57 (out of 50). The paneer prepared by using unripe mango powder at the rate of 12 g/kg of milk and coagulation temperature at 85p C, having 2% fat in milk was rated the best for its flavour score by the selected panellists. However, paneer prepared by using unripe mango powder at the rate of 16 g/kg of milk at 85p C of coagulation temperature having 4.5% fat in milk was rated the lowest for its flavour score. Joseph and Rao (2019) though in different types of paneer, reported a similar decreasing trend for flavour. They observed that when an increase in the amount of lemongrass oil (in milk) from 0.015 to 0.025% and varying levels of incorporation of crushed extract of the lemongrass in milk from 4 to 6% resulted in a significant decrease in the flavour score of the paneer. Khandagale et al. (2022) prepared herbal paneer using turmeric (0.1%) and black pepper (0.1 to 0.3%) and observed that as the amount of turmeric and black pepper increased the flavour score decreased.

The body and texture of paneer was significantly (P<0.05) negatively impacted by unripe mango powder, coagulation temperature and fat content of milk. Unripe mango powder also had a significant (P<0.05) negative impact at quadratic levels (Table 1) .The body and texture score of paneer ranged from 25.86 to 31.00 (out of 35). The paneer prepared with unripe mango powder at 16 g/kg milk and 85p C coagulation temperature for coagulation of milk and 4.5% fat milk received the lowest body and texture score, whereas paneer prepared using unripe mango powder at the rate of 12 g/kg milk, 70p C coagulation temperature and 2.00 % fat milk was rated the best for body and texture score by the panellists. Yashvantha et al. (2020) reported the addition of lemon rinds in paneer and such additions lead to a significant (P<0.05) negative effect on the body and texture score of the paneer. They also observed that at 75 p C coagulation temperature, paneer had a better body and texture score compared to other coagulation temperatures in the study i.e. at 70p C, 80p C and 85p C.

The colour and appearance score of the paneer as depicted in Table 2, was negatively significantly (P<0.05) affected by the fat content of the milk. The colour & appearance score of paneer ranged from 6.00 to 8.14 (out of 10). Paneer prepared from unripe mango powder at the rate of 12 g/kg milk, 85 p C coagulation temperature and 4.5% fat in milk received the lowest score, whereas paneer prepared by using unripe mango powder at the rate of 12 g/kg milk, 85 p C coagulation temperature for coagulation of milk and 2.00 % fat in milk was rated superior in relation to the colour and appearance score by the panellists. Paul et al. (2018) observed that the addition of herbal extract of mint and ginger in paneer reduced the colour and appearance score significantly compared to the control paneer. As the fat content increased in basil incorporated paneer the score of colour and appearance decreased.

Figure 2 depicts the effect on total score. It got impacted in a significant (P<0.05) negative way by unripe mango powder and the fat content of the milk (Table 1). The total score of paneer ranged from 75.14 to 87.43 (out of 100). The paneer prepared with unripe mango powder at the rate of 16 g/kg milk, 85 p C coagulation temperature and 4.5% fat in milk had the lowest total score, whereas paneer prepared by using unripe mango powder at the rate of 12 g/kg milk, 85 p C coagulation temperature for coagulation of milk and 2.00 % fat in milk was rated the best and recived highest total score by the panellists. A similar negative trend for total score was observed by Yashvantha et al. (2020) for paneer containing lemon rind in lemon-flavoured paneer. Paul et al. (2018) also reported that overall acceptability score decreased in basil paneer when the concentration of basil increased from 1 to 1.5 % and fat per cent increased from 1.5 to 2.5 %.

As indicated in Table 2, yield and Moisture content of paneer was significantly (p<0.05) impacted by the fat content of the milk linearly. Moisture content was affected by unripe mango powder significantly at quadratic levels (Table 1). The values of the yield of paneer ranged from 13.70 to 22.00 %. However Yield values higher than 20.0 % had the moisture content higher than the legal limit. The paneer prepared by using unripe mango powder at the rate of 16 g/kg milk, a coagulation temperature of 70°C and fat per cent of milk 2% had lowest yield. The paneer prepared by using unripe mango powder at the rate of 12 g/kg milk, a coagulation temperature of 70 °C and fat per cent of milk 4.5% had highest value of yield. The moisture content of paneer ranged from 50.00 to 63.32 per cent. The paneer prepared by using unripe mango powder at the rate of 16 g/kg milk, a coagulation temperature of 70°C and 2% fat in milk had lowest moisture. The

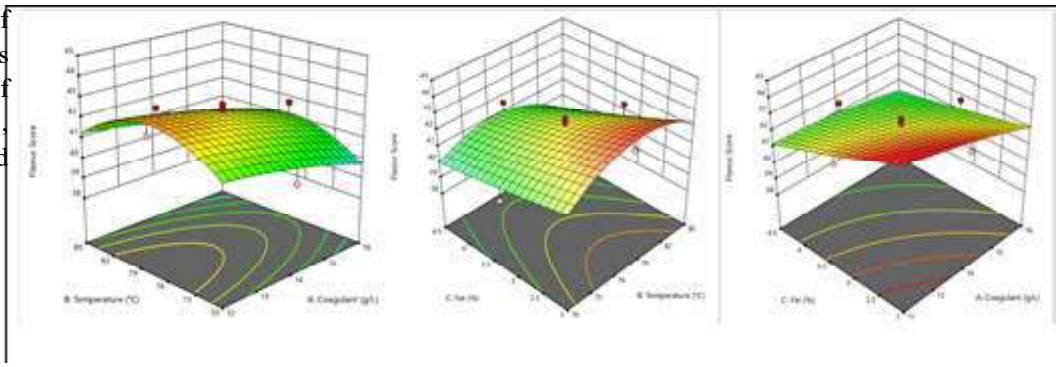

Fig. 1 Response Surface of Flavour Score (Out Of 50) as Influenced by Varying Levels of Unripe Mango Powder (A), Coagulation Temperature (B) and Fat per cent of Milk (C)

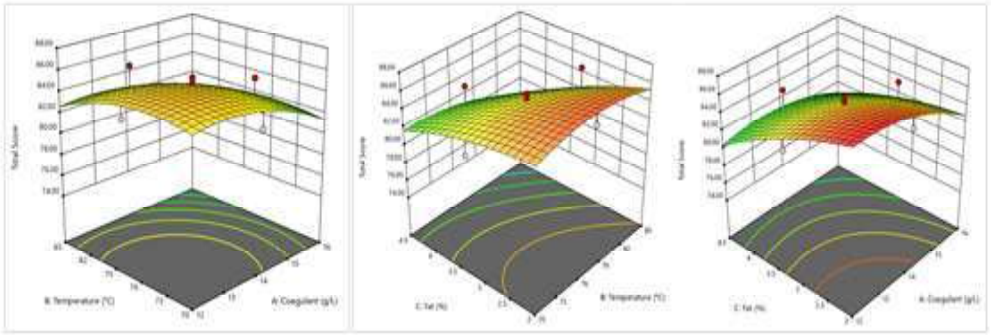

Fig. 2 Response Surface of Total Score (Out Of 100) as Influenced by Varying Levels of Unripe Mango Powder (A), Coagulation Temperature (B) and Fat per cent of Milk (C)

Table 3: Comparison of Predicted v/s Actual Values of Responses Used for Process Optimization for Paneer Manufacture

Response	R^2	Predicted Value *	Actual Value @	Cal. t-Value#
Flavour Score (Out of 50)	0.89	43.98	43.80	0.70
B & T Score (Out of 35)	0.82	30.42	30.15	0.67
C & A Score (Out of 10)	0.80	8.13	8.16	0.56
Total score** (Out of 100)	0.86	87.526	87.50	0.052
Yield %	0.88	17	17.05	1.34
Moisture %	0.90	59.56	59.40	0.94

** Total score includes maximum package score of five
* Predicted values of Design Expert 13.0.5.0 package
@ Actual values are average of five trials for optimised product
# t-values found non-significant at a 5 per cent level of significance
Tabulated t-value = 2.78 (cal. t-value less than tabulated value)

paneer prepared by using unripe mango powder at the rate of 12 g/kg milk, a coagulation temperature 70°C and fat per cent of milk 4.5% had highest moisture content. Thus signifying the negative impact of unripe mango powder and fat content of milk on moisture content of paneer.

Optimization of independent variables

Optimization of the process for the manufacture of paneer using unripe mango powder as a coagulant was carried out to determine the best possible combination(s) of the rate of addition of unripe mango powder, coagulation temperature and per cent fat content of milk, which leads to the most acceptable product in terms of compositional, physicochemical, rheological characteristics, sensory and microbial attributes. The optimum levels as suggested by the software Design expert 13.0.5.0 for the rate of addition of unripe mango powder was 12.70 g/kg of milk, coagulation temperature 76.0°C and fat content of milk was 2.00% with a desirability of 1.00. The predicted and actual response values (obtained after making the product using the optimum level of ingredients) have been presented in Table 3 from which it can be observed that both the values were statistically at par, suggesting the levels of ingredients recommended fits well in the model.

The proximate chemical composition, physicochemical characteristics, rheological values, sensory scores and microbial attributes for the optimized paneer samples are delineated in Table 4. As depicted in Table 4, the developed paneer had 18.94 ± 0.06 % protein and 0.50 % fiber content (calculated value as obtained

from values of mango powder). Titratable acidity was 0.43 ± 0.02 (% LA) and pH was 6.00 ± 0.01. It had water acitivity value of 0.98 ± 0.01, while Free Fatty Acids content (as % Oleic acid) was 0.16 ± 0.03. Rheological characteristics was affected by coagulant (mango powder) and hardness, guminess and chewiness decreased and had values 7.29 ± 0.60 (N), 2.27 ± 0.22 (N) and 13.23 ± 1.57 (Nmm), respectively; while adhesiveness was 0.03 ± 0.01 (Nmm).

Aerobic plate count of the fresh paneer sample was 4.72 ± 0.47 (Log10 cfu/g) and was well within the legal limit as prescribed by FSSA specifications. Yeast and Mold as well as Coliform was absent per g of fresh paneer sample.

Conclusion

It has been observed that all the study parameters including unripe mango powder as coagulant (12.70 g/kg of milk), coagulation temperature (76°C) as well as fat content of the milk (2%) play a significant role in obtaining paneer of comparable quality and acceptability. The optimized paneer had yield of 17.0 % and it had calculated fiber content of 0.5 %. The developed paneer was found to have higher values of vitamin C, iron, copper and fiber content as compared to control paneer and such effect could be due to the presence of vitamin C (48.77 mg/100gm), iron (258.80 mg/kg), copper (5.15 mg/kg) and crude fiber (6.37 %) in unripe mango powder. It can be concluded that the unripe mango powder can be successfully employed as an alternative natural coagulant in the manufacture of paneer.

Table 4. Proximate Chemical Composition, Physicochemical Properties, Rheological, sensory attributed and Microbiological Quality of Paneer Manufactured by Standardized Process

Compositional Attributes	Paneer
Moisture (%)	59.55 ± 0.16
Fat (%)	14.68 ± 0.03
Total protein (%)	18.94 ± 0.06
Total carbohydrate (%)	4.76 ± 0.23
Ash (%)	2.07 ± 0.04
Fiber content (%) (Calculated value)	0.50
Vitamin C (mg/100gm)	48.77
Copper (mg/kg)	5.15
Iron (mg/kg)	258.80
Physico-Chemical Properties	
Titratable Acidity (% LA)	0.43 ± 0.02
pH	6.00 ± 0.01
Water Activity(a_w)	0.98 ± 0.01
Free Fatty Acids (% Oleic acid)	0.16 ± 0.03
Rheological Characteristics	
Hardness (N)	7.29 ± 0.60
Springiness (mm)	5.83 ± 0.15
Cohesiveness	0.31 ± 0.01
Chewiness (Nmm)	13.23 ± 1.57
Gumminess (N)	2.27 ± 0.22
Adhesiveness (Nmm)	0.03 ± 0.01
Sensory Attributes	Score
Flavour Score (out of 50)	43.80 ± 0.51
Body and Texture Score (out of 35)	30.15 ± 0.77
Colour and Appearance Score (out of 10)	8.16 ± 0.11
Package Score (out of 5)	5.00 ± 0.00
Total Score (out of 100)	87.11 ± 1.20
Microbial Count	
Aerobic Plate Count (Log_{10} cfu/g)	4.72 ± 0.47
Yeast and Mold Count (cfu/g)	Absent/g
Coliform Count (cfu/g)	Absent/g

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