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Yashavantha R
SMC College of Dairy Science, Anand Agricultural University, Anand, Gujarat, India

Suneeta Pinto
SMC College of Dairy Science, Anand Agricultural University, Anand, Gujarat, India

Dasharath Patel
SMC College of Dairy Science, Anand Agricultural University, Anand, Gujarat, India

Preeti Paul
SMC College of Dairy Science, Anand Agricultural University, Anand, Gujarat, India

Mamata Chaudhary
SMC College of Dairy Science, Anand Agricultural University, Anand, Gujarat, India

Corresponding Author:
Yashavantha R
SMC College of Dairy Science, Anand Agricultural University, Anand, Gujarat, India

Development of lemon flavoured paneer

Yashavantha R, Suneeta Pinto, Dasharath Patel, Preeti Paul and Mamata Chaudhary

Abstract
The present investigation was planned and conducted to develop a technology for the manufacture of lemon flavoured paneer (LFP). Processed debittered lemon rind shreds were added at three stages viz. before heat treatment of milk, after heat treatment of milk (before addition of coagulant) and directly into coagulum, when about 90% of whey was drained (DC). Based on sensory scores, it was found that the most suitable stage of addition of debittered lemon rind was after heat treatment of milk (before addition of coagulant). To select the most suitable temperature of coagulation for manufacture of LFP, four batches of paneer was prepared by adding of lemon rind shreds (@ 1.0% w/w of milk) after heat treatment of milk (before addition of coagulant) (AH) and coagulating at different temperature viz. 70 °C (T1), 75 °C (T2), 80 °C (T3) and 85 °C (T4). T2 was preferred the most with respect to all the attributes studied from amongst all the experimental samples. The total score of T2 was significantly ($P \leq 0.05$) higher than other experimental samples scoring 87.15. Hence, temperature of 75 °C was selected for coagulation of LFP. Based on the results obtained in this study it can be concluded that the optimum parameters for manufacture of lemon flavoured paneer are incorporation of debittered lemon rind shreds (@ 1.0% w/w of milk) after heat treatment of milk but just before addition of coagulant i.e. 1% citric acid solution and using a coagulation temperature of 75 °C.

Keywords: Lemon, paneer, citrus, soft cheese

Introduction
Paneer is a soft variety of cheese which is very popular in the Indian subcontinent. It is used in a variety of culinary dishes. Paneer consists of more than 90% protein and almost all the fat present in milk. Because of the rising demand of paneer by consumers it is necessary to develop new varieties of paneer. Incorporation of functional ingredients like essential oils (lemon, orange, clove, etc.), fibers (soy fiber, inulin etc.), proteins (groundnut protein isolate etc.), hydrocolloids, herbs and other such ingredients will help to improve the quality, flavour and shelf-life of paneer thus making it attractive to health-conscious people.

Citrus limon L. belongs to family *Rutaceae*. On the bases of fruits and tree characters, true lemons are divided into four groups i.e. Eureka, Lisbon, Anomalous and sweet lemon. Most common variety grown in India is Kagzikalan lemon, which is characterized by its spherical, yellow, with apex slightly nippled, base rounded; rind thin, smooth, flesh acidic, light yellow, juicy, seedy (8-13 seeds). Commercial production of lemon results in large amounts of by-products. The industrial utilization of these by-products has increased significantly since the 1980s (Dilas *et al*., 2009) [10]. Albedo is the major component of lemon rind and is a spongy and cellulosic layer which is present under the flavedo. It is rich in dietary fiber (Aleson-Carbonell *et al*., 2005) [1]. Albido is rich in bioactive compounds like flavonoids and vitamin C which have antioxidant properties (Marin *et al*., 2002) [21]. The lemon oil is known to have antimicrobial effect. It consists of several components. About 40 components have been identified in lemon peel oil. Limonen is one of the major components which is present in lemon oil. Other minor constituents of oil include citral, α-terpineol, α-pinene, β-pinene, citronellal, linalyl and geranyl acetate, p-cymene, γ-pinene, β-myrcene, cumarins, bioflavonoids and pectins (Ortuno *et al*, 2006) [23].

Because of its high dietary fiber, lemon powder which is obtained from lemon by-products has been used in several food formulations such as meat, dairy and bakery products (Lario *et al.*, 2004) [20]. Sendra *et al.*, (2008) [26] reported that lemon fiber (obtained from citrus by-product) enhanced the growth and survival of the probiotic *Bifidobacterium bifidum* CECT 870, *L. casei* CECT 475 and *L. acidophilus* CECT 903 in model system and in fermented milk during cold storage. In this study lemon rind of Kagzikalan variety was added in paneers as a flavouring and functional ingredient. In preliminary studies it was seen that addition of lemon rind shreds in paneer resulted in product with hard pasty body with uneven lemon rind distribution therefore hence, it becomes imperative to develop a suitable method for

manufacture of lemon flavoured paneer. This study was carried out to select a suitable stage of addition of debittered lemon rind and to select a suitable coagulation temperature for manufacture of acceptable quality LFP.

Materials and method

Fresh, raw mixed (cow and buffalo) whole milk was procured from Anubhav Dairy, Anand was standardized by mixing required quantity of skim milk and cream. The average composition of milk was 4.6±0.05% fat and 8.6±0.05% MSNF. Citric acid, β-cyclodextrin and zinc sulphate were procured from Loba-Chemical Pvt. Ltd. D-limonen and orange oil were supplied by Hi-Media Laboratories Pvt. Ltd. Yellow coloured, Kagzi variety lemon were procured from local market, in Anand.

Debittering and Processing of lemon rind shreds

Tender, firm lemons of round or oval shape lemons of Kagzi variety having yellowish to slightly greenish but uniform in colour were selected. The average weight of lemons ranged from 60 to 75 g. After thoroughly washing the lemons with potable water the lemons were soaked in water containing 25 to 50 ppm chlorine for 10 min followed by peeling. During peeling, care was taken to use a sharp, clean, dry stainless steel peeler to prevent the discolouration of the plant tissues. Lemon rinds were immediately transferred in to a 5% NaCl solution and kept for 1 h at 7±1 ^{0}C. Quantity of peels obtained was 140 to 160 g per kg lemon and after soaking drained through muslin cloth, add pasteurized chilled water (4X) by weight of peel thereafter add zinc sulphate (40 mg/ 100 ml of water) and β-cyclodextrin (250 mg/ 100 ml of water) and keep it for overnight at 7±1 ^{0}C followed by draining to get debittered lemon rinds (110-115 g). To obtain shreds, lemon rinds were shredded in a clean and sanitized grater attachment of Boss Food Processor (Model – Boss Food Processor, Boss Electrical, Solan, Himachal Pradesh). The shreds had an average length of 0.8 ± 0.1cm, average width and thickness of 0.3 ± 0.1 cm.

Preparation of paneer

Three kg milk (4.5% fat/8.5% MSNF) was taken in a stainless steel vessel was heated to 90 °C for 5 min and cooled to 80 °C. At this stage lemon rind shreds were added followed by coagulation at 80 °C. Citric acid (in the form a 1% solution) was used as a coagulant in all the experimental samples. The coagulum was allowed to settle for 5 min and the whey was drained through a clean, sterile muslin cloth. Care was taken so that the temperature of whey was maintained above 70 °C. The curd was then collected and transferred to a rectangular shaped sterilized stainless steel hoop (15x10x9 cm^3) lined with a clean sterile muslin cloth. The coagulum was pressed for 15-20 min by applying a pressure of 2 to 3 kg/cm^2. The pressed block of paneer were removed from the hoop and immersed in pasteurized chilled water (3 to 5 °C) for 2 h. The paneer blocks were removed from chilled water placed in a clean stainless steel dish for allowing the water to drain off for 10 min. On completion of draining, paneer blocks were weighed and their representative samples drawn as per method given in IS: 5162 (1969) and subjected to compositional, sensory and rheological evaluation. The products were vacuum packed in the 12 µ polyester + 50 µ LD/LLDPE laminated pouches using a vacuum-packaging machine Make: Saurabh Engg. Ltd., Ahmedabad and stored at 7±1 °C.

Physico-chemical analysis

Fat content in milk was estimated by Gerber's method (IS: 1479, Part I, 1960) [15]. The total solids of milk was determined by the standard procedure IS: 12333 (1997) [14]. The titratable acidity of milk was determined by the method described in the IS: 1479 (Part II) (1961) [16]. Moisture content in paneer was determined by according to IS: 10484 (1983) [13]. The fat content of paneer samples was determined by the Mojonnier method as described in IS: 2785-1979 (Reaffirmed 1995) [18]. Protein content in paneer was determined by kjeldahl method as per AOAC (1980) [2], using Kjel-plus digestion system (Model-KPS 006L) and Kjel-plus semi-automatic distillation system (Model-Distil M) of M/s. Pelican Instruments, Chennai. Ash content of all the samples was determined by procedure described in BIS (1981) [4]. Titratable acidity of paneer was determined by the procedure as described by Boghra and Rajorhia (1982) [5]. pH of paneer was determined as described by O' Keefe *et al.* (1976) [22] using a Handheld digital pH meter (Mettler Toledo, Fivego)

Sensory Evaluation of Paneer

Each block of paneer was cut into approximately 25 g rectangular pieces and served in petri dishes which were labelled with three digit codes in randomized order. The paneer samples were tempered to 10±2 °C before judging. Sensory analysis of paneer samples was performed in a sensory evaluation laboratory. The sensory panel (n=10) was composed of staff members and post graduate students working in the department. Panellists were selected based on their consistent performance in triangle test. The samples were evaluated using 100 point score card as described in Indian Standards (IS: 15346, 2003) [17].

Statistical analysis

The data was analysed using Response Surface Methodology (RSM). To carry out their optimization in the final product formulation, an advanced statistical software program named Design Expert (Version 8.0.3) was employed. Completely randomized design (CRD) was also used for analysis of data.

Results and Discussion

In order to select a suitable stage of addition of lemon rinds, four batches of paneer was prepared by adding of lemon rind shreds (@ 1.0% w/w of milk) at different stage viz. before heat treatment of milk (BH), after heat treatment of milk (before addition of coagulant) (AH), directly to the coagulum when about 90% of whey was drained (DC) and coagulating at 80 °C and citric acid (in the form a 1% solution) was used as a coagulant in all the experimental samples. Control (C) was prepared without addition of lemon shreds.

As seen in Table 1 flavour profile wise the preferred sequence was C > AH > BH > DC. Statistically the flavour score of control was significantly (P<0.05) higher than BH and DC but it was found to be par (P>0.05) with experimental sample AH. The decrease in flavour scores of BH could be due to high heat treatment resulting in reduction of volatile flavour components and DC had uneven distribution of lemon rind and no uniformity in flavour. The body and texture scores for control and AH were significantly (P<0.05) different from DC and BH. Least body and texture score of DC and BH could be due to uneven mixing of lemon rind shreds and adverse effect on body and texture of the product. Colour and appearance scores for DC and BH were significantly (P<0.05) different from C and AH but DC got least score (7.30). The decreases in colour and appearance scores of experimental

samples were due to addition of lemon rind shreds at different stage during manufacture of LFP.

The overall acceptability score of for all samples are significantly (P<0.05) different from each other. The overall acceptability scores of AH was significantly (P<0.05) higher than other experimental samples scoring 83.75 marks which indicates that AH is of good quality according to BIS grading system for paneer. Hence it was selected for manufacture of LFP.

The results obtained in this part of study are similar to those reported by Buch *et al.* (2012) who prepared turmeric flavoured paneer. These authors also advocated that the best stage of addition of turmeric was after heat treatment and just before coagulation i.e. at 70 ℃.

Effect of coagulation temperature

To study the effect of coagulation temperature on quality of lemon flavoured paneer, four batches of paneer was prepared by adding of lemon rind shreds (@ 1.0% w/w of milk) after heat treatment of milk (before addition of coagulant) using different coagulation temperatures viz. 70 ℃, 75 ℃ 80 ℃ and 85 ℃.

Effect of Coagulation Temperature on Quantity of Citric Acid Used in Paneer

Table 2 depicts the average quantity of coagulant used in lemon flavoured paneer in terms of g of citric acid /kg milk. There was a progressive decrease in quantity of citric acid with increase in coagulation temperature however, coagulation temperature had no significant effect (P>0.05) on quantity of citric acid used in LFP. The results are in agreement with Bhatt (2013) [3] who observed that there was significant ($P\leq$0.05) differences in quantity of citric acid with increase in coagulation temperature. Chawla *et al.,* (1987) [9] also reported that amount of coagulant required decreases with increase in the coagulation temperature.

Effect of Coagulation Temperature on Compositional Attributes of LFP

As seen in Table 2, coagulation temperature had significant effect (P<0.05) on moisture and FDM content of paneer. No significant (P>0.05) effect was observed in fat content of paneer. The moisture and FDM content of T1 and T2 was significantly (P<0.05) higher compared to all other samples (T3 and T4). The moisture and FDM content of T3 and T4 were found to be at par (P>0.05) with each other. The results obtained are in corroboration with those obtained by Sachdeva and Singh (1988) [25] who reported that the moisture of paneer decreased consistently with an increase in temperature of coagulation. This is due to the fact that the moisture absorption increased with decrease in coagulation temperature. They reported that the penetration values i.e. a measure of hardness decreased markedly with increasing temperatures of coagulation. Thus, the result of study is in agreement with the reported literature.

Bhatt (2013) [3] also reported that in low-fat paneer prepared with different coagulation temperature FDM and moisture were affected significantly ($P\leq$0.05) by coagulation temperature at all the levels studied. There was a gradual decline in FDM and moisture content of paneer with increase in temperature of coagulation. The results obtained are similar to those reported by Sachdeva and Singh (1988) [25] who observed that increase in coagulation temperature from 70 to 85 °C, decreased the moisture content in paneer. According to Food safety and standards authority of India (FSSAI 2011) [11], Paneer means the product obtained from cow or buffalo milk or a combination thereof by precipitation with sour milk, lactic acid or citric acid. It shall not contain more than 60.0% moisture and milk fat content shall not be less than 50.0% of the dry matter. Therefore, all the samples of LFP prepared in the present study fulfilled both the FSSAI and the BIS requirements for the chemical composition.

Effect of coagulation temperature on pH, acidity and yield of LFP

As seen in Table 2, the coagulation temperature had significant effect (P<0.05) on yield of paneer. No significant (P>0.05) effect was observed in pH and acidity of paneer. The yield of T1 and T2 was significantly ($P\leq$0.05) higher compared to T3 and T4. Sachdeva and Singh (1988) [25] and Chandan (2007) [7] also reported that the yield of paneer decreases with increase in the coagulation temperature. Use of coagulation temperature exceeding 72 ℃ is reported to yield paneer having dry appearance and hard body (Sachdeva and Singh, 1988) [25]. Smitha *et al.* (2014) [27] also reported that yield of paneer obtained from milk coagulated at 70 °C and 75 °C was 22.69 and 22.85% respectively. Roy and Singh (1994) studied the effect of coagulation temperature and the effect of certain additives (pre-gelatinized potato starch and sodium alginate) on production and quality of filled paneer. They found hard and dry paneer at 90 °C of coagulation temperature. Sachdeva and Singh (1988) [25] and Chandan (2007) [7] also reported that the yield of paneer decreases with increase in the coagulation temperature.

Influence of Coagulation Temperature on Sensory attributes of LFP

As seen in Table 3, there was no significant (P>0.05) in flavour score among all the samples studied. The results obtained are in corroboration with those obtained by Chawla *et al.* (1987) [9] who reported a temperature of coagulation of low-fat paneer was inversely related to the flavour quality. They reported that the product having lowest flavour score being at the highest coagulating temperature (75 °C) in contrast to the highest score at the lowest coagulation temperature (65 °C). Chawla *et al.* (1987) [9] who reported no significant difference among the paneer which were obtained by coagulating milk at 75 °C and 70 °C, respectively. Bhatt (2013) [3] also reported that there was a progressive increase in flavour score with decrease in coagulation temperature from 75 to 65 °C. Thereafter, further decrease in coagulation temperature resulted in decrease in flavour score (6.82, 6.84, 6.96 and 6.92 75 ℃, 70 ℃, 65 ℃ and 60 ℃ respectively).

From the point of view of the body and texture score, the T_2 obtained the highest score of 31.50, followed by T1 scoring 28.74 and T3 (27.70) scored less than T1. The minimum score of T4 was 25.80. Free moisture was observed on the surface of T1 and T4 was criticized for its hard and dry body. Higher temperature of coagulation viz. 85 C caused lower moisture retention (as seen in Table 2) and as a consequence contributed to hardness which was responsible for lower body and texture score. The statistical analysis reveals that there was significant difference (P<0.05) in body and texture score among all the samples studied. Statistically the flavour score of T2 was significantly (P<0.05) higher than all other samples. T1 and T3 were found to be at par (P>0.05) with each other. Similarly T3 and T4 were significantly (P<0.05) lower than all other samples. The results obtained in this part of study are in corroboration with those reported by Sachdeva and Singh (1988) [25] who observed that coagulation

temperatures higher than 70 ℃ results in hard and dry paneer and at lower temperatures free moisture on surface was observed. The results obtained are in also in corroboration with those obtained by Chawla *et al*. (1987) [9] who reported a temperature of coagulation of low-fat paneer was inversely related to the body and texture quality. They reported that the product having lowest body and texture score being at the highest coagulating temperature (75 °C) in contrast to the highest score at the lowest coagulation temperature. Bhatt (2013) [3] also reported that low fat paneer coagulated at lower coagulation temperatures viz. 60 ℃ had excessively soft body and pasty texture with free moisture on surface whereas paneer coagulated using higher coagulation temperature (75 ℃) was criticized for their hard, dry chewy and rubbery body and coarse grainy texture.

In contrast, Ghosh *et al.* (2019) [12] reported that lower coagulation temperatures viz. 55 and 60 ℃ gave acceptable body and texture in low fat paneer prepared from milk standardized to 0.5% fat, whereas the body and texture score of low fat paneer decreased significantly (p<0.05) when milk coagulation temperature was increased to 70 °C. Chawla *et al*. (1985) [8] also observed that coagulation temperature of 75 °C resulted in harder paneer which as a consequence lowered the body and texture score, while coagulation at 65 °C produced slightly softer paneer with higher body and texture score. The statistical analysis reveals that there was no significant difference (P>0.05) in colour and appearance score among all the samples studied. There was significant difference (P<0.05) in overall acceptability score among all the samples studied. Statistically the overall acceptability score of T2 was significantly (P<0.05) higher than all other samples. T1 and T3 were found to be at par (P>0.05) with each other. T2 was preferred the most with respect to all the attributes studied from amongst all the experimental samples. The total score of T2 was significantly ($P\leq$0.05) higher than other experimental samples scoring 87.15 marks which indicates that T2 is of good quality according to BIS grading system for paneer. Hence, temperature of 75 ℃ was selected for coagulation of LFP. The results obtained in this part of study are in contrast to those reported by Chandan, (2007) [7] who observed that coagulation of milk at 70 °C had the best organoleptic quality. The differences in results could be attributed to the fibre content in lemon peel and presence of zinc because of treatment of lemon rind with Zinc sulphate. Rajashekar *et al.* (2016) [24] also reported that the overall acceptability score for paneer sample coagulated at 70 ℃ was higher and it may be due to its bright colour and uniform appearance, chewy body and perfect close knit texture.

Table 1: Effect of stage of addition of processed lemon rind shreds on sensory score of LFP

Stage of addition	Sensory score			
	Flavour (Max.50)	Body and texture (Max.35)	Colour and appearance (Max.10)	Total score (Max.100)
Control	$45.20^{a} \pm 1.88$	$32.55^{a} \pm 2.00$	$8.69^{a} \pm 0.08$	$91.44^{a} \pm 1.55$
AH	$41.50^{ab} \pm 1.47$	$28.70^{b} \pm 2.82$	$8.55^{a} \pm 0.15$	$83.75^{b} \pm 1.70$
BH	$38.15^{bc} \pm 3.77$	$26.05^{c} \pm 2.61$	$8.34^{b} \pm 0.05$	$77.54^{c} \pm 1.44$
DC	$35.00^{c} \pm 2.16$	$23.78^{c} \pm 2.58$	$7.30^{c} \pm 0.16$	$71.08^{d} \pm 2.09$
CD (0.05)	3.82	2.46	0.19	2.64

* Full marks has been allotted to packaging i.e 5/5

Each observation is a mean ± SD of four replicate experiments (n=4)

[a-d] Superscript letters following numbers in the same column denote significant difference (P<0.05)

Table 2: Influence of coagulation temperature on quantity of citric acid used, composition, pH acidity and of yield LFP

Type of paneer (Coagulation temperature)	Quantity of citric acid used (g/kg milk)	Constituents (%)			pH	Acidity (% LA)	Yield (%)
		Fat	FDM	Moisture			
T-1	1.98 ± 0.10	24.97 ± 1.61	$56.46^{a} \pm 1.86$	$55.78^{a} \pm 2.96$	5.88 ± 0.13	0.52 ± 0.06	$18.58^{b} \pm 1.00$
T-2	1.94 ± 0.15	25.91 ± 2.08	$58.40^{a} \pm 0.65$	$55.64^{a} \pm 2.23$	5.70 ± 0.19	0.54 ± 0.07	$19.29^{a} \pm 0.50$
T-3	1.93 ± 0.06	24.65 ± 1.60	$51.27^{b} \pm 2.16$	$51.93^{b} \pm 1.07$	6.12 ± 0.23	0.48 ± 0.03	$17.23^{c} \pm 0.80$
T-4	1.90 ± 0.07	24.58 ± 2.12	$50.23^{b} \pm 1.97$	$51.07^{b} \pm 1.85$	5.91 ± 0.18	0.51 ± 0.03	$17.20^{c} \pm 0.85$
CD(0.05)	NS	NS	2.72	2.76	NS	NS	1.25

Each observation is a mean ± SD of four replicate experiments (n=4)

[a-c] Superscript letters following numbers in the same column denote significant difference (P<0.05)

Table 3: Influence of different coagulation temperature on sensory score of LFP

Type of paneer	Coagulation temperature (ºC)	Flavour (Max.50)	Body and texture (Max.35)	Color and appearance (Max.10)	*Total score (Max.100)
T-1	70	40.85 ± 1.82	$28.74^{b} \pm 1.26$	8.20 ± 0.15	$82.79^{b} \pm 2.04$
T-2	75	42.15 ± 2.74	$31.50^{a} \pm 1.87$	8.50 ± 0.16	$87.15^{a} \pm 2.24$
T-3	80	40.74 ± 2.07	$27.70^{bc} \pm 2.09$	8.00 ± 0.63	$81.44b^{c} \pm 1.55$
T-4	85	39.78 ± 2.63	$25.80^{c} \pm 1.33$	7.94 ± 0.18	$78.52^{c} \pm 1.57$
CD(0.05)		NS	2.58	NS	2.89

* Full marks has been allotted to packaging i.e 5/5

Each observation is a mean ± SD of four replicate experiments (n=4)

[a-c] Superscript letters following numbers in the same column denote significant difference (P<0.05

Conclusion

Based on the results obtained in this study it can be concluded that the optimum parameters for manufacture of lemon flavoured paneer are incorporation of debittered lemon rind shreds (@ 1.0% w/w of milk) after heat treatment of milk but just before addition of coagulant i.e. 1% citric acid solution and using a coagulation temperature of 75 °C.

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