Composition, texture and microstructure appraisal of *paneer* coagulated with sour fruit juices

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Abstract The investigation studied the effect of sour fruit juices as coagulants on the physico-chemical properties, phytonutrients, sensory scores, texture profile analysis (TPA) and microstructure using scanning electron microscopy (SEM) of *paneer*. *Paneer* was coagulated using citric acid solution (control), lemon or *amla* juice. The total solids and protein recovery were higher using *amla* juice while fat recovery was more in control than with the other two coagulants. The mineral profile indicated that calcium content was higher in lemon and *amla paneer* than citric whereas sodium was highest in citric sample. *Paneer* collected using fruit juices exhibited higher quantity of functional components studied i.e. antioxidant activity, ascorbic acid, total phenols and tannins than control. Also, it scored high for sensory attributes of appearance/color, body and texture, mouthfeel, flavor and overall acceptability but exhibited disparate values for TPA (hardness, adhesiveness, gumminess and resilience) and discrete structures under SEM.

Keywords Fruit acidulants · *Paneer* · Phytochemical · Mineral profile · Sensory score · TPA · SEM

Introduction

The health issues coupled with growing demand for food products fortified with nutritional additives and improved sensory properties have vastly attracted the consumers' attention in the recent past. The food industry is perpetually getting innovative to satisfy these demands by developing products based on multiple functional ingredients. Therefore, it is frequently seeking to supplement foods and beverages with phytochemicals, soluble and insoluble fiber, vitamins, minerals, essential fatty acids etc. The importance of fruits and their inclusion in processed foods is also witnessing an upsurge as they are rich in phytochemicals, vital for achieving good health and prevention of chronic diseases, including cancer and cardiovascular diseases (Steinmetz and Potter 1991).

Acidified milk products are one of the oldest and most popular foodstuffs that are produced throughout the world to conserve milk solids and for value addition (Lucey 2003). Acid-coagulated cheeses are either prepared by coagulating milk at ambient temperatures using lactic acid bacteria or acid-temperature coagulation. *Paneer* is a popular product making use of the latter technique. It is an unripened soft cheese mostly used by vegetarians in India for culinary dishes, snacks and sweetmeats apart from its direct consumption. Several coagulants for *paneer* manufacture have been tested i.e. citric acid, tartaric acid, lactic acid, malic acid, hydrochloric acid, phosphoric acid, acetic acid, alum, vinegar, sour whey and yoghurt (Sachdeva et al. 1985). Chandan (2007) documented that lime (*Citrus limon* L.) have been traditionally used in the preparation of *paneer* in India and Pakistan or Queso Blanco cheese in Latin America/Central America/Caribbean. Lemon fruit (*Citrus limon* L.) is among the most important citrus fruits rich in several nutrients and phytochemicals which are

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essential for normal human growth and help to reduce the risk of many chronic diseases. The phytochemicals present in lemon include vitamin C, minerals, monoterpenes, limonoids (triterpenes), flavonoids, carotenoids and hydroxycinnamic acid (González-Molina et al. 2008). Indian gooseberry/*Amla* (*Embllica officinalis*) is a sour fruit of Indian native deciduous tree, rich in bioactive compounds namely alkaloids, flavonoids, tannins, and vitamin C besides many others (Patel and Goyal 2012). It is used in ayurvedic medicines to reduce cough, common cold and to treat pulmonary tuberculosis, hemorrhoids, diarrhea, dysentery, anemia, jaundice, and dyspepsia. Several scientists have reported the effect of a number of synthetic acids on the quality characteristics and sensory attributes of *paneer* (Sachdeva et al. 1985; Khan et al. 2014). However, Rekha and Vijayalakshmi (2010) used natural coagulants viz. lemon, tamarind, garcinia, gooseberry and passion fruit and found enhanced antioxidant activity of tofu. Therefore, use of natural acids needs to be comprehensively explored as a source of vital bioactive compounds in coagulated dairy products. Hence, the present investigation envisaged the coagulation of *paneer* using acidic fruit juices (lemon and *amla*) and assessed its physico-chemical properties, phyto-nutrients, sensory scores, texture profile analysis (TPA) and microstructure (SEM).

Materials and methods

Extraction of fruit juice and paneer preparation

Standardized milk (Verka brand), citric acid, lemon and *amla* were procured from the local market. Sound lemon and *amla* fruits were washed and drained. Lemons were cut into two halves and juice extracted with manual squeezer. *Amla* was grated using a hand grater and juice extracted by pressing. Both the juices were filtered using cheese cloth and heated to 75 °C prior to use for *paneer* preparation. *Paneer* was prepared as per procedure given by De (2015). Milk was heated to 85 °C for 5 min, allowed to cool to 72 °C and coagulated using heated (75 °C) 2% citric acid solution or fruit juices till a clear whey was separated. Thereafter, whey was drained, coagulum pressed for 20 min, immersed in chilled water, packaged in polyethylene (LDPE) bags and stored in a refrigerator.

Physico-chemical analyses

Milk and *paneer* were analyzed for total solids, fat, protein, ash and titratable acidity as per AOAC (2000) methods. The total solids of milk were determined by drying to a constant weight at 98 ± 2 °C and *paneer* at 50 ± 1 °C for 8 h followed by drying at 100 ± 1 °C. The total nitrogen

was determined by the Macro-Kjeldahl method and converted into protein by multiplying by a factor of 6.38 for milk and *paneer* and 6.25 for *amla* and lemon juice. The fat content of milk was estimated using Gerber's method (BIS 1981) and that of *paneer* using Rose-Gottlieb method (AOAC 2000) with some modifications. One gram of *paneer* was digested with hydrochloric acid followed by extraction with mixed ethers and alcohol. Fruit juices were analyzed for total solids, total soluble solids, titratable acidity and specific gravity (AOAC 2000). The pH of samples was measured using pocket pH meter (Hanna, Italy) by making a paste of 10 g *paneer* with 10 ml distilled water. The titratable acidity was determined by titration against standard NaOH and expressed as the anhydrous citric acid in fruit juices and lactic acid in *paneer*. The total soluble solids were estimated by using hand refractometer (ERMA, Japan). The antioxidant activity of juices and *paneer* was determined by the method of Alma et al. (2003) using DPPH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate) free radical scavenging method that produced a violet color in 80% extract-methanol solution. The standard methods were followed for ascorbic acid and tannins (Ranganna 1997). For liquids 10 ml and for *paneer* 2 g sample was macerated with 0.06% meta-phosphoric acid, filtered, volume made and 10 ml and an aliquot titrated against standard 2,6-dichlorophenol indophenol solution until the pink color developed.

The total phenol content was determined by the Folin–Ciocalteu method by Cindrić et al. (2011) with slight modification. Two grams of sample was refluxed with 50 ml of 80% methanol for 2 h, filtered, residue collected and refluxed again for 1 h, filtered and volume made to 100 ml with methanol. Thereafter, 0.2 ml of extract in 0.8 ml of distilled water was mixed with 5 ml of Folin–Ciocalteu's reagent. After 5 min 4 ml of 20% sodium carbonate was added and incubated in dark for 90 min. The absorbance was recorded at 760 nm using visible spectrophotometer (Hoverlabs, India) and quantified using the standard curve of Gallic acid. Tannins were estimated using Folin–Denis's method (Ranganna 1997) with some modifications. The samples of lemon and *amla* juice, milk and *paneer* were refluxed with 75 ml water for 1 h, filtered and volume made to 100 ml with distilled water. To 0.5 ml aliquot of sample 37.5 ml of distilled water was added followed by 2.5 ml of Folin Denis reagent and 5 ml of saturated sodium carbonate and volume made to 50 ml. After 30 min of incubation, absorbance was read at 760 nm. Tannins were calculated using the standard curve of tannic acid. All the determinations were conducted in triplicate.

For mineral profile analysis, 1 g sample was digested with 10 ml of di-acid until about 3 ml of the colorless residue was left. Thereafter, distilled water was added to

the residue, heated, washed and filtered with Whatman No. 1 filter paper, until 80 ml of filtrate was obtained. The filtrate was re-filtered, and volume made to 100 ml. The sample was analysed for mineral content using Inductivity Coupled Plasma Emission Spectrophotometer (ICP-AES, Thermo Electron Corporation, ICAP 600 series, England).

Sensory evaluation

The samples of *paneer* were evaluated by a semi-trained panel of judges for the sensory attributes of appearance/color, flavor, body and texture, flavor and overall acceptability on nine points hedonic scale where 1 indicated disliked extremely and 9 liked extremely (Poste and Mackie 1991).

Texture profile analysis

Texture profile analysis (TPA) was performed on *paneer* samples using Texture Analyzer (Model TAXT2i, Exponent Stable Micro Systems, United Kingdom, Software Texture Expert Exceed version 7.1.6) using the following settings: load cell capacity 25 kg, displacement 0.1–524 mm and speed 20–0.01 mm/s. For sample placement, heavy duty platform (HDP/90) was used and Compression platen (P/75) made of stainless steel (75 mm dia) was used. *Paneer* was cut into cubes ($1.5 \times 1.5 \times 1.5$ cm) and analysed by using pre-test speed 1 mm/s, surface trigger force 5 g, strain 65% and probe return speed 5 mm/s. The texture profile curve was interpreted in terms of hardness, adhesiveness, springiness, cohesiveness, gumminess and chewiness.

Scanning electron microscopy

The Scanning electron microscopy (SEM) of *paneer* samples was performed as per the method of Allan-Wojtas et al. (2008) with modifications. Samples (approx. $1 \times 1 \times 1$ cm) of *paneer* were fixed in 2.5% glutaraldehyde solution, sealed in vials and kept overnight at 4 °C. The fixative was drained and samples rinsed with 0.1 M cacodylate buffer. Subsequently, samples were incubated with 1% osmium tetroxide (OsO₄) solution for 150 min. This fixative was also rinsed thrice with caco buffer. The samples were dehydrated by incubation of samples with 30–100% ethanol. Later the samples were critical-point dried from carbon dioxide, mounted on aluminium SEM stubs, sputter coated with gold in a Hitachi E-1010 ion sputter coater, and examined under Scanning Electron Microscope (HITACHI, S-3400N, Japan).

Statistical analysis

The data obtained were analyzed by analysis of variance (ANOVA) using SPSS® 16.1 software and the mean difference was compared using least significance difference (LSD) at $p < 0.05$.

Results and discussion

Physico-chemical properties and functional constituents

The standardized milk contained 13% total solids comprising of 3.5, 4.8 and 0.2% protein, carbohydrate and ash content, respectively. The antioxidant activity and total phenols of milk sample were 28.30% and 71.19 mg GAE/100 g (Table 1). On heat treatment of juices, the solids increased due to moisture loss as vapors resulting in a concentration of both the juices. The total soluble solids (TSS) also increased slightly due to biochemical changes in pectin and other carbohydrates. The titratable acidity (TA) acidity increased significantly on heating of both the juices (Table 1). The rise in acidity showed a positive relationship with the drop of pH due to the formation of acidic compounds on the degradation of reducing sugars. The antioxidant activity, ascorbic acid, total phenols and tannins declined significantly upon heat treatment. The lowered antioxidant activity of juices might be associated with the effect of temperature on degradation of heat labile vitamins and other phytochemicals. The overall effect of antioxidant activities of *amla* has been attributed to ascorbic acid and polyphenols (Bhattacharya et al. 1999). Yu et al. (2005) correlated the antioxidant capacity of lemon juice with the presence of citrate, vitamin C, vitamin E and flavonoids. The tannin content declined in heat treated juice.

Yield and recovery of solids

The yield of *paneer* differed significantly ($p < 0.05$) being highest with citric acid (Table 2). This variation might be due to coagulant type and its strength on the retention of moisture, fat and protein (Chandan 2007; Sachdeva et al. 1985). Rekha and Vijayalakshmi (2010) reported a yield of 17.6 and 15.6% for tofu prepared with lemon and gooseberry juice.

The solids recovery was highest with *amla* juice that is attributed to the contribution of juice solids. The protein recovery was lower in lemon due to a higher acid concentration that caused greater denaturation of milk proteins thus appreciably affecting the bonding between them and consequently loss of the milk proteins as fines into the

Table 1 Physico-chemical properties and functional constituents of milk and fruit juices

Parameter	Fresh milk	Fresh lemon juice	Heated lemon juice	Fresh <i>amla</i> juice	Heated <i>amla</i> juice
Moisture	87.00 ± 0.070	94.42 ^a ± 0.165	94.37 ^a ± 0.153	85.83 ^x ± 0.361	85.77 ^x ± 0.351
Total solids	13.00 ± 0.070	6.93 ^a ± 0.115	7.30 ^b ± 0.100	15.13 ^x ± 0.115	16.33 ^y ± 0.115
TSS (°Brix)	9.50 ± 0.100	5.58 ^a ± 0.165	5.63 ^a ± 0.153	14.17 ^x ± 0.361	14.23 ^x ± 0.351
Protein	3.5 ± 0.02	0.992 ± 0.025	–	0.763 ± 0.022	–
Specific gravity	1.028 ± 0.001	1.052 ^a ± 0.005	1.053 ^a ± 0.004	1.068 ^x ± 0.010	1.07 ^x ± 0.002
Titrateable acidity (% citric acid)	0.151 ± 0.002	7.15 ^a ± 0.092	8.11 ^b ± 0.167	2.99 ^x ± 0.049	3.57 ^y ± 0.092
pH	6.53 ± 0.058	1.83 ^a ± 0.058	1.5 ^b ± 0.100	2.53 ^x ± 0.153	1.73 ^y ± 0.058

Values in a row with different superscript (a and b, x and y) differ significantly at $p < 0.05$

Table 2 Effect of juice coagulants on yield, recovery and physicochemical composition of fresh *paneer*

Parameter	Citric acid	Lemon juice	Amla juice
Yield (%)	19.31 ^a ± 0.14	17.34 ^b ± 0.28	19.08 ^a ± 0.28
Milk solids recovery (%)	61.92 ^a ± 0.68	57.72 ^c ± 0.86	63.46 ^b ± 0.56
Protein recovery (%)	95.91 ^b ± 0.65	90.85 ^c ± 1.54	99.51 ^a ± 1.91
Fat recovery (%)	90.01 ^a ± 1.43	77.27 ^b ± 1.33	78.46 ^b ± 0.27
Moisture (%)	58.31 ^a ± 0.15	56.72 ^b ± 0.07	56.77 ^b ± 0.24
Protein (%)	17.39 ^b ± 0.05	18.34 ^a ± 0.05	18.25 ^a ± 0.05
	(41.70 ^b ± 0.22)	(42.38 ^a ± 0.17)	(42.21 ^a ± 0.13)
Fat (%)	20.98 ^a ± 0.2	20.06 ^b ± 0.57	18.51 ^c ± 0.26
	(50.32 ^a ± 0.34)	(46.35 ^b ± 1.24)	(42.71 ^c ± 0.37)
Ash (%)	1.30 ^a ± 0.03	1.24 ^c ± 0.012	1.27 ^b ± 0.01
	(3.11 ^a ± 0.07)	(2.87 ^c ± 0.26)	(2.94 ^b ± 0.01)
Element/mineral profile (ppm)			
Boron	0.172 ^a ± 0.0	0.153 ^b ± 0.0	0.096 ^c ± 0.0
Calcium	77.32 ^c ± 0.0	80.15 ^a ± 0.0	79.97 ^b ± 0.0
Copper	0.034 ^b ± 0.0	0.046 ^a ± 0.0	0.026 ^c ± 0.0
Iron	0.365 ^c ± 0.0	0.441 ^a ± 0.0	0.387 ^b ± 0.0
Potassium	38.20 ^a ± 0.0	25.91 ^b ± 0.0	24.73 ^b ± 0.0
Magnesium	14.44 ^a ± 0.0	14.86 ^a ± 0.0	12.11 ^b ± 0.0
Manganese	0.022 ^a ± 0.0	0.021 ^a ± 0.0	0.025 ^a ± 0.0
Sodium	41.65 ^a ± 0.0	36.82 ^b ± 0.0	24.26 ^c ± 0.0
Phosphorus (ppm)	84.49 ^c ± 0.0	94.20 ^b ± 0.0	97.00 ^a ± 0.0
Sulphur	8.54 ^a ± 0.0	9.07 ^a ± 0.0	5.80 ^c ± 0.0
Zinc	0.708 ^c ± 0.0	0.802 ^a ± 0.0	0.720 ^b ± 0.0
Carbohydrate (by difference), (%)	2.03 ^a ± 0.13	3.63 ^b ± 0.47	5.21 ^c ± 0.09
	(4.87 ^a ± 0.31)	(8.40 ^b ± 1.2)	(12.05 ^c ± 0.26)
Titrateable acidity (% lactic acid)	0.401 ^a ± 0.003	0.522 ^b ± 0.016	0.417 ^a ± 0.08
pH	5.60 ^a ± 0.10	5.33 ^b ± 0.058	5.80 ^c ± 0.100
Phytonutrients			
Antioxidant activity (% inhibition of DPPH)	9.59 ^a ± 0.24	24.17 ^b ± 0.22	59.38 ^c ± 0.37
Ascorbic acid (mg/100 g)	ND ^a	1.59 ^b ± 0.16	15.26 ^c ± 0.16
Total phenol (GAE mg/100 g)	43.17 ^a ± 9.38	207.52 ^a ± 35.17	2507.70 ^b ± 154.33
Tannin content (mg/100 g)	ND ^a	57.57 ^b ± 1.34	2198.40 ^c ± 42.03

ND non detectable, $n = 3$, values are mean ± SD, values with different superscript in a row differ significantly at $p < 0.05$, values in parentheses are presented on dry weight basis

whey. The fat recovery was lowered in both, lemon and *amla*. Earlier Khan et al. (2014) reported a lower fat recovery in *paneer* from 3 and 5% citric acid, also solids recovery varied with the concentration of the coagulant.

Proximate composition and mineral profile

The type of coagulants caused variations in most of the constituents of *paneer*. The moisture content lowered with fruit juices which might be attributed to the high acid strength. Rao et al. (1984) and Khan et al. (2014) observed a decline in moisture content with increased strength of coagulant above 2%. The protein content varied significantly ($p < 0.05$) with the type of acidulant being highest in *paneer* from lemon juice (Table 2). The fat content was higher in *paneer* prepared from citric acid than lemon and *amla* juice, however, Khan et al. (2014) observed augmentation in fat with increased strength of the acid. The ash content also differed significantly ($p < 0.05$). Bajwa et al. (2005b) reported ash content of 4.02% (DWB) for *paneer* made by coagulating buffalo milk with 2% citric acid. However, Khan et al. (2014) reported lower ash content with citric and malic acid. Rekha and Vijayalakshmi (2010) observed the higher ash content (DWB) in tofu made from *amla* (2.8%) than lemon juice (2.6% DWB). Hence, type of milk and nature and strength of coagulant effect the ash recovery of acid-coagulated milk products. The mineral profile of *paneer* varied appreciably with the type of acid/juice used as coagulant. It indicated that calcium content was higher in lemon and *amla paneer* than citric whereas sodium was maximal in the citric sample. The values of other minerals and trace elements (Table 2) reveal the improvement of juice *paneer* over that of control with respect to some of the studied elements. Copper content was highest in lemon whereas iron quantity improved in both the juice *paneer* over control. *Amla paneer* encompassed enhanced phosphorus whereas lemon juice boosted zinc. The elemental composition was highly variable in all the three *paneer* types due to differences in composition of juices and their interaction with various components of milk during processing for *paneer* manufacture.

The carbohydrate content also varied, being highest in *paneer* prepared from *amla* juice due to retention of carbohydrates present in its juice. The values of titratable acidity differed significantly ($p < 0.05$), lemon *paneer* having the highest acidity and lowest pH due to the retention of acid from the juice. Highly variable values of acidity with citric acid coagulation ranging from 0.29 to 0.893 have been reported in the literature (Bajwa et al. 2005b). Further, Khan et al. (2014) stated that higher strength of coagulant formed rapid and non-uniform curd with greater denaturation and produced more acidic *paneer*.

Functional components

The antioxidant activity varied significantly ($p < 0.05$) being higher in juice *paneer* due to the retention of phenolic compounds and ascorbic acid from the juices (Choe and Min 2009). Rekha and Vijayalakshmi (2010) reported higher DPPH scavenging activity for tofu coagulated with lemon and gooseberry juice than with synthetic coagulant. Some antioxidant activity of citric *paneer* might have been contributed by some proteinaceous compounds from milk and also of citric acid (estimated to be 22.30% in the present case). The ascorbic acid content, total phenols and tannins were highest in *amla* juice *paneer* (Table 2). A small amount (1.59 mg) of ascorbic acid was found in lemon *paneer* whereas it was not detected in the citric sample. Earlier Bajwa et al. (2005a) did not detect ascorbic acid in *paneer* made from citric acid solution.

Sensory scores

Paneer has a white appearance, slightly spongy body, close-knit texture and possesses a sweetish-acidic-nutty flavor. It has a soft meaty texture from cow milk whereas from skim milk it has undesirable chewy and rubbery texture and hard body (Aneja et al. 2002). The sensory scores for appearance/color, body and texture, mouthfeel and overall acceptability and were highest in citric followed by lemon and *amla* juice (Table 3). The scores for these attributes ranged from liked moderately (7) and liked very much (8). The appearance scores chased the deviation from whiteness. The appearance score of *amla paneer* was lowest, due to light reddish/brownish color imparted by phenolic compounds of *amla*. *Paneer* absorbs the color of the added components, for that Bajwa et al. (2005a) observed a decline in color scores due to a greenish tinge of chlorophyll on the incorporation of ground coriander and mint leaves.

The structure of *paneer* is basically the network of casein micelles entrapping other milk constituents in it and is affected mainly by retention of moisture and fat along with other milk constituents. The high moisture retention results in loose and soft body and texture (Aneja et al. 2002). The body and texture scores were higher for citric than juice samples (Table 3) because these had lower moisture and fat content as compared to control (Table 3). The mouthfeel score was higher for citric than *amla* and lemon *paneer* without any significant difference due to the difference in microstructure that has a great effect on physical properties such as elasticity, susceptibility to syneresis and mouthfeel (Aneja et al. 2002). *Paneer* should have a mildly-acid flavor. The flavor scores were lower for *paneer* made using lemon and *amla* juice that may be attributed respectively to delicate lemon flavor imparted by

Table 3 Effect of juice coagulants on sensory scores and texture profile analysis (TPA) of *paneer*

Parameter	Citric	Lemon	Amla
Sensory scores ^c			
Appearance/colour	8.35 ^a ± 0.6	8.06 ^a ± 1.1	7.13 ^b ± 1.0
Body and texture	8.17 ^a ± 0.8	7.98 ^{ab} ± 0.7	7.63 ^b ± 0.7
Mouthfeel	8.27 ^a ± 0.84	7.85 ^a ± 0.8	7.86 ^a ± 0.9
Flavour	8.08 ^a ± 0.8	7.81 ^a ± 1.1	7.58 ^a ± 0.8
Overall acceptability	8.15 ^a ± 0.7	7.89 ^{ab} ± 0.8	7.57 ^b ± 0.6
Texture profile analysis ^d			
Hardness (kg)	1.29 ^a ± 0.12	2.31 ^b ± 0.18	1.88 ^b ± 0.44
Fracturability (g)	10.27 ^a ± 1.72	11.58 ^a ± 0.11	19.66 ^b ± 4.7
Adhesiveness (g s)	-1.43 ^a ± 0.141	-4.40 ^b ± 1.56	-4.78 ^b ± 1.057
Cohesiveness	0.497 ^a ± 0.091	0.467 ^a ± 0.015	0.413 ^a ± 0.032
Springiness (mm)	0.774 ^a ± 0.083	0.769 ^a ± 0.079	0.858 ^a ± 0.023
Gumminess	0.645 ^a ± 0.147	1.08 ^b ± 0.12	0.791 ^{ab} ± 0.25
Chewiness	0.493 ^a ± 0.078	0.839.73 ^a ± 0.184	0.680 ^a ± 0.224
Resilience (%)	0.172 ^a ± 0.027	0.144 ^{ab} ± 0.013	0.126 ^b ± 0.011

[#]*n* = 8, ^{\$}*n* = 3, values are mean ± SD, values with different superscript in a row differ significantly at *p* < 0.05

lemon essential oils and astringency imparted by *amla* polyphenols and tannins that are unusual in commercial preparation used for consumption in households. Cheetham (2002) reported that citrus fruits contain characteristic flavor attributed by stereoisomers, geranyl acetate and nearly acetate, citral, g-terpinene, dimethylantramilate, a-sine-nasal, esters, aldehydes and alcohols. Earlier studies reported a decline in flavor scores on the incorporation of vegetable leaves (Bajwa et al. 2005a). Khan et al. (2014) concluded that the flavor scores of *paneer* decreased with the increased concentration of coagulants. The overall acceptability score was highest for citric followed by lemon and *amla paneer*.

Texture profile analysis (TPA)

The hardness was greater in *paneer* made from lemon and *amla* (Table 3) which might be attributed to low moisture as well as fat and higher calcium content (Table 2). Desai et al. (1991) found that the hardness was inversely related to the moisture content and directly related to the calcium content of *paneer*. Also, a positive relationship between fat and hardness have earlier been reported by Syed et al. (1992) in that the hardness was highest for skim milk *paneer* when compared to cow and buffalo milk. Also the higher acidity, presence of carbohydrate and pectin in lemon and *amla* might have attributed to the hardness. A wide variation in hardness have been reported earlier, due to type and composition of milk, type and strength of coagulant, type of treatment to *paneer* and type and setting of the texture profile analyser (Superkar et al. 2014; Singh et al. 2015). Fracturability was lowest in citric *paneer*,

attributed to its higher moisture content than the others. Luyten et al. (1991) reported that increased moisture content in Gouda cheese decreased the fracture stress. Despite the higher moisture content in *paneer* made from *amla* juice, it showed higher fracturability than lemon due to the higher carbohydrate content (Table 3) interrupting the protein matrix.

There were significant differences in adhesiveness of *paneer* prepared from different acidulants being highest in *amla* juice. The adhesiveness of the *paneer* has direct relationship with springiness, gumminess and chewiness that might be influenced by the presence of pectin and other carbohydrates in the protein matrix. Cohesiveness and springiness were comparable in all three *paneer*. Superkar et al. (2014) concluded that springiness depended on factors such as heat treatment, protein interaction and flexibility and degree of unfolding of the protein. The gumminess was higher in lemon *paneer* due to the effect of acid and pectic compounds of juice on the protein matrix. The chewiness (gumminess/springiness) was greater in lemon *paneer* than *amla* and citric acid with no significant differences in accordance to Superkar et al. (2014). Resilience, refers to the ability of the material to fight to regain its original height, was highest in citric followed by lemon and *amla paneer*.

Microstructure (SEM)

The coagulation of heated milk with fruit acids resulted in curd microstructure which differed appreciably from each other. The microstructure of *paneer* consisted of the protein matrix of casein micelles forming interconnected chains

with diverse sized interstitial spaces within which the liquid phase is immobilized in the protein network. The coarser the protein network, the bigger the interstitial spaces. The scanning electron microscope (SEM) shows that the microstructure of *paneer* samples was dissimilar in different acidulants (Fig. 1a–c). The coarseness in the structure increased with increased acidity being higher in lemon (b) than *amla* (c) and citric acid (a). The heterogeneity in the structure may be explained by differences in

the pH value during the coagulation of the milk (Kalab et al. 1988). Lemon juice having higher acidity resulted in bigger clumps with irregularly shaped void spaces (Fig. 1b). On the other hand, *amla* curd appeared to be quite fine and compact with less interstitial spaces thereby retaining less moisture within these.

The milk was heated to 85 °C for 5 min before coagulation with juices that was sufficient to form protein aggregates. SEM showed that structures were uniform and

Fig. 1 Microstructure of *paneer* under scanning electron microscopy. **a** Citric acid/control *paneer*, **b** lemon juice *paneer* and **c** *Amla* juice *paneer*

fat globules were distributed throughout the protein network. The fat globule structure was not visible in all the *paneer* types as the milk used was commercially homogenized and size of the globules must have been too small and probably evenly distributed as well as fat globule membrane (FGM) reformed during homogenization. Thus big void fat globule-shaped spaces could not be observed. However there is some big irregular shaped void spaces, might be due to higher moisture in citric *paneer* and such structures were not frequently visible in either lemon or *amla paneer*. Few coarser and uneven pockets in lemon and fine ones, almost evenly distributed could be noticed in *amla paneer*. The compact protein network resembled those found in other milk products like cottage cheese and similar core and lining structure was found in all the three samples. According to Green et al. (1978), the first step in structure development comprises aggregation of individual micelles to form chains that grew into multiple strands, possibly by aggregation of adjacent chains. The acid-heat gels contained thicker fibers (Kalab and Harwalkar 1973). As the pH of cheese curd decreased below 6 towards 5.4, the curd became more flowable and had a relation with an increased ratio of soluble to bound calcium. Also, citrate played a role that it complexed with soluble calcium ions and prevented the facilitation of protein–protein interactions (Pastorino et al. 2003).

Kalab and Modler (1985) observed that the compactness of curd increased with increased temperature and denaturation of protein in *Queso Blanco* cheese. Furthermore, they noticed that the altered casein micelles were fused into large protein particles and the initial micellar outlines were not noticeable due to core and lining structure. Harwalkar and Kalab (1981) reported that core and lining structure was found only in curd (milk gels) obtained by acidulating hot milk to the final pH of 5.5 or around. Marchesseu et al. (1997) observed that small changes in ionic composition and strength modified the protein interactions substantially and had important repercussions on the final structure and quality of the protein gel formed during processing of cheese and suggested the optimization of pH during the manufacturing process to overcome the variation in the microstructure. Khan et al. (2014) stated that lower coagulant concentrations resulted in more uniform coagulum compared to milk coagulated at higher concentration.

Conclusion

Milk coagulation using lemon and *amla* juice for the manufacture of *paneer* resulted in considerable effect on the physico-chemical characteristics, functional components, sensory attributes, TPA and microstructure (SEM). Since both the juices contained a substantial quantity of

phytochemicals, *paneer* coagulated using these exhibited higher magnitude of antioxidant activity, ascorbic acid, total phenols and tannins than control (citric acid). Sensory scores indicated that good quality *paneer* with high acceptability could be manufactured both from lemon and *amla* juice.

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