East African Journal of Science, Technology and Innovation, Vol. 4 (Special Issue 2): September 2023

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# Effect of fruit acidulants and storage conditions on the physicochemical, microbiological and sensory characteristics of *paneer*

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#### Abstract

*Paneer* is an unripened soft cheese mainly used by Indians and Pakistans, and it does not require the rennet enzyme. It is prepared by coagulating heated milk with acid. However, fresh *paneer* has a short shelf life of up to 2 days at ambient temperature. In this study, *paneer* was prepared from lemon and amla fruit acidulants and stored at room and refrigeration temperatures. Then, the *paneer* samples were analysed for their physicochemical properties, texture profile, microbiological counts and sensory attributes during storage at room and refrigeration temperatures. The type of acidulant, storage temperature and time significantly affected the *paneer*'s physicochemical properties (p<0.05). However, the changes were rapid in samples stored at room temperature. Moreover, *paneer* coagulated using fruit acidulants had a higher shelf life and retention of functional properties. Furthermore, the *paneer* from fruit acidulants had high acceptability, comparable to the control, usually made from citric acid. Therefore, preparing *paneer* from fruit acidulants with improved functionality, shelf life, and acceptability is possible. Thus, adapting the technology of making *paneer* with acidulant fruits would reduce milk and fruit postharvest loss, promote a sustainable diet and create market potential for the novel milk product in India and the world at large.

Keywords:	Fruit acidulants, postharvest loss, paneer, shelf life, sensory	Received:	27/06/23
evaluation		Accepted:	06/07/23
		Published:	14/09/23
Cite as: Ahmed	et al. (2023). Effect of fruit acidulants and storage conditions on the		

physicochemical, microbiological and sensory characteristics of paneer. East African Journal of Science, Technology and Innovation 4(special issue 2).

#### Introduction

Increased consumer awareness about their health and wellness has resulted in high demand for natural foods (Kumarasamy *et al.*, 2002; Srinivasan *et al.*, 2001). Many natural products are rich in several phytochemical compounds, including antioxidants, which play important roles in the human body tissues (Jayasinghea *et al.*, 2013). One such example is Indian gooseberry (*amla*), which is rich in vitamins (folic acid, niacin, pantothenic acid, pyridoxine, riboflavin, thiamine, vitamins C and A) and minerals (sodium, potassium, calcium, copper, iron, magnesium, manganese and zinc (Ahmed & Bajwa, 2019; Thankitsunthorn *et al.*, 2009). Thus, Indian gooseberry is routinely used in food as a natural remedy for various disorders, including diabetes, atherosclerosis, asthma, constipation, inflammation, and obesity (Thankitsunthorn *et al.*, 2009). On the other hand, lemon fruit is also rich in numerous nutrients and phytochemicals.



There are several phytochemicals present in lemon. such as vitamins, minerals, flavonoids. monoterpenes, limonoids. carotenoids, and hydroxycinnamic acid (Gonzalez-Molina et al., 2008; Del Río et al., 2004). In addition, lemon is used widely as a natural acidulant in food preparations. Citric acid is the dominant acid in citrus fruits, contributing to their tartness, sourness, and acceptability (Sharma et al., 2014; Sharon-Asa et al., 2003).

Ahmed and Bajwa (2019) used amla and lemon juices to prepare paneer. Paneer is a type of unripened soft cheese in India used mainly by vegetarians as its preparation does not require the rennet enzyme (Ahmed & Bajwa, 2019; Kanawjia & Singh, 2000). Paneer is generally prepared by acid-coagulating hot milk to form a curd (Chandan, 2007). Several coagulants include lemon juice, synthetic acids such as citric acid, lactic acid, malic acid, hydrochloric acid, fermented milk, sour/cultured whey, yoghurt and lactic cultures have been used in paneer preparation (Deshmukh et al., 2009). Paneer is consumed alone or with several staple foods. Moreover, several recipes, including culinary dishes, snacks and sweetmeats such as rasogolla, rasamalai and sandesh, could also be prepared from paneer (Khan et al., 2012; Yadav et al., 2009).

However, paneer is perishable and prone to physiochemical and microbiological changes during storage, limiting its shelf life (Kumar et al., 2011). As per FSSAI (2009) specifications, paneer should not contain more than 50,000 total bacterial counts, not more than 90 coliform counts, and 250 yeast and mould counts per g of paneer. Usually, paneer stays fresh within two days at room temperature (Vishweshwaraiah and Anantakrishnan, 1985) or refrigerated for 7 to 10 days (Shukla et al., 1984). Therefore, various additives and treatments have been used to extend the keeping time of *paneer*. For example, vacuum packing (Sachdeva and Prokopek, 1992), dipping paneer in a brine solution (Sachdeva, 1983), using milk preservatives such as sorbic acid and gamma rays (Singh and Kanawjia, 1990; Modi and Jain, 1988) have been found successful in extending the shelf life of *paneer*.

Nonetheless, substances like aldehydes, phenols, and coumarines in *amla* and lemon may help

prevent or delay food spoilage to a certain extent (Jadhav et al., 2020; Aruoma et al. al., 2012; Karimi et al., 2012; Nerurkar, 2007). Al-Ani et al. (2010) observed an effective antibacterial activity of citrus extracts against Staphylococcus aureus, Proteus vulgaris and Pseudomonas aeruginosa. Patil et al. (2012) also reported the antimicrobial activity of amla against E.coli, P. multocida and S. aureus. Therefore, in the present study, paneer was prepared using lemon and amla extracts and investigated for physicochemical changes, microbiological changes, texture profile changes and sensory properties during storage at ambient and refrigeration temperatures.

# Material and methods

The investigation was conducted in the Food Science and Technology Department at the Punjab Agricultural University, Ludhiana, India. The factorial design method was used to study the effect of the type of acidulant and storage time on *paneer's* physicochemical, microbiological, sensorial properties and texture profile.

# Extraction of Fruit Acidulants and Paneer Preparation

Standardised milk (Verka brand), citric acid, lemon and amla were obtained from the local market in Ludhiana, Punjab. First, fruit acidulants were extracted, according to Ahmed and Bajwa, 2019. Then, paneer was prepared per the standard procedure by De (2015), and whey was collected. Next, milk was heated to 85°C for 5 minutes, cooled and coagulated at 72°C using a 2% citric acid (control) solution or fruit extracts until a clear whey was separated. After that, the coagulum was strained, pressed, chilled, packed in LDPE bags, heat-sealed and stored at ambient (26±0.5C°) and refrigeration  $(4.5 \pm 0.5 C^{\circ})$ temperatures.

# Analysis of Moisture, Titratable Acidity and Total Soluble Solids

The moisture, total soluble solids (TSS) and titratable acidity contents were determined according to AOAC (2000) method. A weighed sample of *paneer* was dried in a hot air oven at 50±1°C for 8 h, followed by drying at 100±1°C till constant weight. The loss in weight was used to calculate moisture content. Fresh and stored *paneer* samples were analysed for total soluble solids using a hand refractometer of 0-32°B (ERMA, Japan). Distilled water was added to a 10 g sample, swirled, and the final volume was made to 100 ml. After 30 min, it was filtered, from which 10 ml aliquot was used for titration against 0.1 N NaOH. For acidulants, acidity was calculated as anhydrous citric acid, while for milk and *paneer* was calculated as lactic acid, as given in Equation 1.

*Determination of Non-protein Nitrogen Content* The non-protein nitrogen (NPN) was determined

as per the given method by FSSAI (2015). About 0.5g of sample was taken in a preweighed beaker, warmed with water at 40°C, made up to 100 ml and noted the weight. Ten ml was pipetted into a preweighed 125 ml Erlenmeyer flask, and weight was noted. About 40 ±0.5 ml of TCA (15%) was added to the flask with the sample mixture, and weight was noted again. The mixture was swirled and left to precipitate, and the protein components were to settle for 5 minutes. The mixture was filtered through Whatman No1 paper (Nitrogen free), and the entire filtrate was collected. The precaution was that the filtrate should be clear and free from particulate matter. The filtrate was swirled to mix the content, and subsequently, 20 ml was pipetted into a 50 ml beaker and weighed. The filtrate was digested using the Kjeldahl procedure (AOAC 2000). The digestion, distillation and titration were carried out as in protein estimation. Blank was prepared with 16 ml of 15% TCA, and NPN was calculated as per the given formula in Equation 2.

#### **Determination of Free Fatty Acid Content**

The fat content of the *paneer* was extracted using the Rose-Gottlieb method (AOAC 2000). Then, to determine free fat acid content (FFA), the extracted fat was mixed with 15ml ethanol and diethyl ether in a 1:1 ratio and titrated against 0.02N KOH in the presence of a phenolphthalein indicator until the light pink colour was observed (AOAC 2000). The content of FFA was calculated as shown in Equation 3.

# Analysis of Ascorbic Acid Content

The standard method given by Ranganna (2001) was followed for ascorbic acid determination. About 2 g was macerated in the presence of 0.06% meta-phosphoric acid, filtered, and volume was made to 100 ml with meta-phosphoric acid. First,

ten ml of freshly prepared standard ascorbic acid in a meta-phosphoric acid solution was titrated against 2, 6-dichlorophenol indo-phenol dye. The titre value was used to calculate the dye factor. Then, ten ml aliquot of each sample solution was titrated against dye, and ascorbic acid was calculated, as shown in Equation 4.

# Determination of Total Phenols by Folin-Ciocalteau's Method

Total phenol content was determined by the Folin–Ciocalteau method by Cindrić *et al.* (2011) with slight modification as indicated by Ahmed and Bajwa (2019). The concentration of phenols in a sample was determined using a standard curve prepared with gallic acid, and total phenol content was calculated using Equation 5. The results were expressed as mg of gallic acid equivalents (GAE) per 100 ml.

# Determination of Tannins by Folin-Denis's Method

Tannins were estimated using Folin–Denis' method (Ranganna, 2001) with some modifications, as indicated by Ahmed and Bajwa (2019). The concentration of tannins in a sample was determined using a standard curve prepared with tannic acid, and Tannin content was calculated using Equation 5.

# Determination of Antioxidant Activity by the DPPH Method

The Antioxidant activity of both raw materials and the paneer was determined by Alma et al. (2003) using DPPH (2, 2-diphenyl-1-picrylhydrazyl-hydrate) free radical scavenging method based on electron transfer that produces a violet solution in 80% extract-methanol solution. Two grams of each sample were refluxed with 50 ml of 80% methanol solution for two hours, filtered, collected, and refluxed with 50 ml of 80% methanol solution for one hour. Subsequently, the extract was again filtered, and the volume was made to 100 ml with 80% methanol. The reaction mixture consisted of 1 ml of sample, 1 ml of tris buffer and 2 ml of DPPH in methanol solution. The changes in colour (from deep violet to light yellow) after 100 min of reaction were read at 517 nm using a visible spectrophotometer (Hoverlabs, India). Control

was prepared by mixing 1 ml distilled water, 1 ml tris buffer and 2 ml DPPH. The blank solution was prepared by mixing 1 ml of distilled water, 1 ml of tris buffer and 2 ml of 80% methanol solution. DPPH Scavenging activity of samples was measured as a decrease in the absorbance and was calculated using the following Equation 6.

#### Measurement of Colour Values

The colour values of the raw materials, *paneer*, were measured using Mini Scan Xe Plus, USA (Hunter colour lab) in the hunter colour mode and expressed as 'L', 'a' and 'b' values. In the Hunter scale, 'L' measures the brightness band varying from 100 for perfect white to 0 for black. The chromaticity dimensions ('a' and 'b') give a clear designation of colour, i.e. the value 'a' determines redness when positive, grey when zero and green when negative. The value 'b' determines when negative.

#### Microbiological Analyses

The paneer samples were packaged in LDPE bags, heat-sealed and stored at ambient  $(26\pm0.5C^{\circ})$  and refrigeration  $(4.5\pm0.5C^{\circ})$  temperatures. The fresh and stored samples were analysed for microbiological quality at intervals of one day at room temperature and five days for refrigeration temperature for Standard Plate Count (SPC), yeast and mould and psychrophilic count using the APHA method (1992).

#### Estimation of Texture Profile Analysis

Texture profile analysis (TPA) was accomplished on *paneer* samples using Texture Analyser (Model TAXT2i, Exponent Stable Micro Systems, United Kingdom; Software Texture Expert Exceed version 7.1.6) following settings: load cell capacity-25 kg, displacement-0.1–524 mm and

speed-20-0.01 mm/s. To place the sample for analysis heavy-duty platform (HDP/90) was used. Stainless steel compression platen (P/75), with a 75 mm diameter, was used throughout the experiments. The paneer was cut into  $(1.5 \times 1.5 \times 1.5)$  cm cubes for TPA analysis. The cubes were exposed to TPA using a pre-test of 1 mm/s, 5 g surface trigger force, and 65 % strain, and after that, the probe returned to its original position at a constant speed (5 mm/s). The texture profile curve was interpreted in terms of adhesiveness, springiness, hardness, cohesiveness, gumminess and chewiness.

#### **Evaluation of Sensory Properties of Paneer**

The sample of fresh, cooked and stored *paneer* samples prepared using citric acid, *amla* and lemon extracts were assessed by a semi-trained panel of judges on a nine-point hedonic scale (Larmond, 1977) for the appearance/colour, flavour, body and texture, flavour and overall acceptability.

#### Statistical Analysis

The data obtained were analysed for analysis of variance (ANOVA) using the SPSS® software package with all possible interactions. They were presented as mean  $\pm$  standard deviation, and the mean difference was compared using the Least Significance Difference (LSD) test at a 95% confidence interval.

Acidity (%Citric acid or lactic acid) Titre value x Normality of NaOH x eq.wt. of acid x Vol. madex100Weight of sample taken x aliquot taken x 1000x100

Equation 1

 $Wn = \underline{1.4007 x (Vs-VB) x NWf x Wm}$ 

(Wt-0.080 Wm)

#### Where by:

Wn = NPN content of a sample, expressed as a percentage by mass;

VS = volume in ml of the standard hydrochloric acid used for the sample;

VB = volume in ml of the standard hydrochloric acid used for the blank test;

N = Normality of the standard hydrochloric acid expressed to 4 decimal places;

Wm = mass of test portion in g expressed to nearest 0.1 mg.

Wf = mass of 20 ml filtrate in g, expressed to the nearest 0.1 mg.

Wt = mass of the test portion plus 40 ml of TCA solution in g, expressed to the nearest 0.1 mg.

Note: The factor 0.080 in the denominator is derived from per cent fat and true protein (thus 0.045 + 0.035).

**Equation 2** 

FFA (%Oleic acid) = (Volume of alkali x Normality of alkali x28.2)/sample weight

**Equation 3** 

Ascorbic acid  

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

**Equation 4** 

Phenols/Tannins=
$$\frac{\text{Concentration in a sample (mg) x dilution x vol. made (ml)}}{\text{ml of sample taken x wt. of sample (g) x 1000}} x 100$$
Equation 5

#### Results

# *Effect of Acidulant and Storage Conditions on the Physico-Chemical Properties of Paneer*

Results on the effect of acidulants and storage conditions on the physicochemical properties of the paneer are presented in Table 1. The type of acidulant, storage temperature and time affected significantly the paneer's physicochemical properties (p < 0.05). The total solids significantly increased while moisture content decreased in all paneers during room and refrigeration temperatures storage. The moisture loss was highest in control, followed by amla and lemon for the *paneer* stored at room temperature

# Equation 6

and in the first five days of those stored in the refrigerator. The coarseness of the *paneer* increased with acidity and was higher in lemon than in *amla* and citric acid. The acidity was highest in control, followed by lemon and *amla paneer*. The NPN significantly increased throughout the storage period of *paneer* at room and refrigeration temperatures. The increase of NPN was highest in control, followed by lemon and *amla paneer*. The free fatty acids (FFA) significantly increased throughout the storage of *paneer* at room and refrigeration temperatures. Again, the increase in FFA was highest in control, followed by *paneer* from lemon and *amla*.

Table 1
Effect of acidulants and storage conditions on the physico-chemical properties of <i>paneer</i>

Acidulants	0	Ro	om temperature	1 1	1	Refri	geration temperatu	ıre	
		Storage time (d	lays)						
	Fresh	1	2	Mean	5	10	15	20	Mean
					Moisture (%)				
Citric acid	$58.31 \pm 0.15$	$55.79 \pm 0.02$	$53.54 \pm 0.03$	55.88×	$57.04 \pm 0.27$	$56.67 \pm 0.20$	$55.68 \pm 0.08$	$52.14 \pm 0.04$	55.97×
Lemon	$56.72 \pm 0.07$	$54.15 \pm 0.03$	$53.66 \pm 0.02$	54.84y	56.09 ±0.20	56.08 ±0.30	55.69 ±0.36	46.59 ±0.03	54.24y
Amla	56.77 ±0.24	$55.03 \pm 0.02$	53.42 ±0.02	55.07 <sup>z</sup>	56.33 ±0.50	$54.58 \pm 0.80$	$51.64 \pm 0.18$	46.43 ±0.05	53.15 <sup>z</sup>
Mean	57.26 <sup>a</sup>	54.99 <sup>b</sup>	53.54°	55.26 <sup>A</sup>	56.47 <sup>b</sup>	56.65°	54.47 <sup>d</sup>	<b>48.39</b> <sup>e</sup>	54.45 <sup>B</sup>
				]	Fotal solids (%)				
Citric acid	$41.69\pm0.15$	$44.21 \pm 0.02$	$46.46\pm0.03$	44.12 <sup>x</sup>	$42.96 \pm 0.27$	$43.33 \pm 0.20$	$44.32\pm0.08$	$47.86\pm0.04$	44.03×
Lemon	$43.28 \pm 0.07$	$45.85 \pm 0.03$	$46.34 \pm 0.02$	45.16 <sup>y</sup>	43.91 ±0.20	43.92 ±0.30	44.31 ±0.36	53.41 ±0.03	45.76y
Amla	43.23 ±0.24	44.97 ±0.02	46.58 ±0.02	44.93 <sup>z</sup>	43.67 ±0.50	$45.42 \pm 0.08$	$48.36 \pm 0.18$	53.57 ±0.05	46.85 <sup>z</sup>
Mean	<b>42.74</b> <sup>a</sup>	45.01 <sup>b</sup>	<b>46.46</b> <sup>c</sup>	47.74 <sup>A</sup>	43.51 <sup>b</sup>	<b>44.35</b> °	45.53 <sup>d</sup>	51.61 <sup>e</sup>	45.55 <sup>B</sup>
				Titratabl	e acidity (Lactic aci	d %)			
Citric acid	$0.408 \pm 0.009$	$0.430\pm0.009$	$0.531 \pm 0.007$	0.456×	$0.428 \pm 0.008$	$0.481 \pm 0.008$	$0.509 \pm 0.008$	$0.564 \pm 0.010$	0.478×
Lemon	$0.510 \pm 0.005$	$0.517 \pm 0.008$	$0.551 \pm 0.006$	0.494y	$0.518 \pm 0.005$	$0.522 \pm 0.008$	$0.560 \pm 0.008$	$0.570 \pm 0.010$	0.536y
Amla	$0.417 \pm 0.008$	$0.421 \pm 0.007$	$0.459 \pm 0.000$	$0.432^{z}$	$0.429 \pm 0.005$	$0.437 \pm 0.007$	$0.547 \pm 0.007$	$0.552 \pm 0.103$	0.476 <sup>z</sup>
Mean	<b>0.445</b> a	0.456 b	0.482c	0.460 <sup>A</sup>	0.458 <sup>b</sup>	0.480c	0.539d	0.562e	0.497 <sup>B</sup>
					pН				
Citric acid	$5.87 \pm 0.06$	$5.57 \pm 0.06$	$4.47\pm0.06$	5.63×	5.77 ±0.58	5.63 ±0.58	5.33 ±0.58	$5.03 \pm 0.58$	5.53×
Lemon	$5.57 \pm 0.06$	$5.47 \pm 0.06$	$5.43 \pm 0.06$	5.49y	5.47 ±0.58	5.37 ±0.58	5.13 ±0.58	$4.80 \pm 0.104$	5.27y
Amla	5.77 ±0.06	$5.67 \pm 0.10$	$4.63\pm0.06$	5.69×	5.63 ±0.58	$5.53 \pm 0.58$	5.28 ±0.58	4.93 ±0.58	5.43 <sup>z</sup>
Mean	5.73 <sup>a</sup>	5.57 <sup>b</sup>	5.51 <sup>b</sup>	5.60 <sup>A</sup>	5.62 <sup>b</sup>	5.51°	5.24 <sup>d</sup>	<b>4.92</b> <sup>e</sup>	5.41 <sup>B</sup>
				Non-j	protein nitrogen (%	<b>)</b>			
Citric acid	$0.094 \pm 0.03$	$0.105 \pm 0.006$	$0.180 \pm 0.008$	0.126×	$0.112 \pm 0.07$	$0.178 \pm 0.004$	0.195 ±0.009	$0.220 \pm 0.003$	0.160×
Lemon	$0.099 \pm 0.04$	$0.102 \pm 0.004$	$0.102 \pm 0.005$	0.101y	$0.106 \pm 0.004$	$0.116 \pm 0.004$	$0.123 \pm 0.004$	$0.134 \pm 0.004$	0.116y
Amla	$0.099 \pm 0.01$	$0.106 \pm 0.002$	$0.108 \pm 0.002$	0.104y	$0.110 \pm 0.002$	$0.119 \pm 0.004$	$0.133 \pm 0.007$	$0.149 \pm 0.036$	0.122 <sup>z</sup>
Mean	0.097ª	0.104 <sup>b</sup>	0.130°	0.110 <sup>A</sup>	0.110 <sup>b</sup>	0.137°	0.150 <sup>d</sup>	0.168e	0.132 <sup>B</sup>
				Free fat	ty acids (Oleic acid	%)			
Citric acid	$0.047 \pm 0.001$	$0.074 \pm 0.004$	$0.083 \pm 0.003$	0.068×	$0.051 \pm 0.003$	0.057±0.002	$0.070 \pm 0.002$	$0.084 \pm 0.001$	0.062×
Lemon	$0.044 \pm 0.001$	$0.051 \pm 0.002$	$0.055 \pm 0.003$	0.050y	$0.048 \pm 0.001$	$0.051 \pm 0.001$	$0.061 \pm 0.002$	$0.076 \pm 0.001$	0.056y
Amla	$0.043 \pm 0.001$	$0.051 \pm 0.001$	$0.060 \pm 0.001$	0.051y	$0.047 \pm 0.002$	$0.054 \pm 0.002$	$0.066 \pm 0.002$	$0.077 \pm 0.001$	0.058 <sup>z</sup>
Mean	0.045ª	0.051 <sup>b</sup>	0.066c	0.056 <sup>A</sup>	<b>0.049</b> ь	0.054c	0.066 <sup>d</sup>	0.079e	0.058 <sup>B</sup>

Mean  $\pm$  standard deviation. The values with a different superscript in column (x, y and z) or row (a, b, c, d and e) indicated that the mean difference significantly at p<0.05 based on the least significance difference test.

# Table 2

Acidulants	Room temperature Refrigeration temperature								
		Stor	age time (days)				Storage time (da	ys)	
	Fresh	1	2	Mean	5	10	15	20	Mean
Antioxidant activity (DPPH inhibition %)									
Citric acid	$9.59 \pm 0.24$	$12.55 \pm 0.14$	13.63 ±0.21	11.92 <sup>x</sup>	$10.16 \pm 0.14$	$12.55 \pm 0.24$	$14.15 \pm 0.22$	$21.72 \pm 0.50$	13.63×
Lemon	24.17 ±0.21	43.11 ±0.45	$41.70 \pm 0.72$	36.33 <sup>y</sup>	$46.92 \pm 0.21$	$36.39 \pm 0.14$	$33.57 \pm 0.14$	34.27 ±1.10	34.65 <sup>y</sup>
Amla	59.38 ±0.37	87.73 ±0.14	$86.74 \pm 0.14$	77.95 <sup>z</sup>	$85.85 \pm 0.30$	$72.54 \pm 0.21$	$69.82 \pm 0.37$	46.69 ±0.29	66.86 <sup>z</sup>
Mean	<b>31.05</b> <sup>a</sup>	47.80 <sup>b</sup>	47.36 <sup>c</sup>	42.07	47.64 <sup>b</sup>	40.50°	<b>39.18</b> <sup>d</sup>	33.54°	38.38
			1	Ascorbic ac	cid (mg/100g)				
Citric acid	ND	ND	ND	ND×	ND	ND	ND	ND	ND×
Lemon	$1.59 \pm 0.16$	$1.13 \pm 0.15$	0.91 ±0.20	<b>1.21</b> y	$1.18 \pm 0.13$	$0.82 \pm 0.18$	$0.68 \pm 0.23$	$0.487 \pm 0.21$	0.946 <sup>y</sup>
Amla	15.26 ±0.16	$13.65 \pm 0.15$	13.36 ±0.23	14.09 <sup>z</sup>	$14.92 \pm 0.25$	$12.88 \pm 0.17$	9.37 ±0.25	$6.94 \pm 0.63$	11.88 <sup>z</sup>
Mean	<b>5.62</b> <sup>a</sup>	<b>4.93</b> <sup>b</sup>	4.76 <sup>c</sup>	5.10	5.37 <sup>b</sup>	4.57°	3.34 <sup>d</sup>	<b>2.47</b> <sup>e</sup>	4.27
			]	Fotal phene	ols (mg/100g)				
Citric acid	56.41 ±4.43	37.44 ±+2.23	17.93 ±1.55	37.26×	$38.56 \pm 2.27$	$27.20 \pm 2.10$	21.12 ±2.11	$17.58 \pm 2.20$	32.18 <sup>x</sup>
Lemon	206.78 ±11.15	179.03± 3.14	92.33 ±2.89	150.09y	186.96 ±3.01	148.86 ±3.46	134.47 ±9.57	78.21 ±5.88	151.06 <sup>y</sup>
Amla	2507.70 ±15.39	2105.10 ±11.75	1917.90 ±11.75	2177.00 <sup>z</sup>	2117.2 ±7.33	1744.10±7.62	1151.50±10.88	853.75±13.13	1674.85 <sup>z</sup>
Mean	<b>923.63</b> <sup>a</sup>	773 <b>.</b> 87 <sup>b</sup>	666.77°	788.09	780.91 <sup>b</sup>	640.05°	<b>435.69</b> <sup>d</sup>	<b>316.51</b> <sup>e</sup>	619.359
				Tannins	(mg/100g)				
Citric acid	ND	ND	ND	ND×	ND	ND	ND	ND	ND×
Lemon	57.57 ±1.34	$48.54 \pm 1.15$	$16.81 \pm 0.71$	40.97 <sup>y</sup>	$50.76 \pm 2.42$	$40.13 \pm 2.23$	36.79 ±1.68	$20.95 \pm 1.58$	41.24 <sup>y</sup>
Amla	2198.40 ±42.04	1861.00 ±152.87	1810.90 ±192.61	1884.00 <sup>z</sup>	1952.40±8.25	1308.00±9.95	816.00 ±8.86	618.40 ±5.69	1379.00 <sup>z</sup>
Mean	<b>751.99</b> <sup>a</sup>	639.96 <sup>b</sup>	553.47°	641.81	667.71 <sup>b</sup>	<b>449.37</b> °	284.26 <sup>d</sup>	213.12 <sup>e</sup>	473.29
				Colou	r values				
				L* v	value				
Citric acid	$86.49 \pm 0.97$	$89.73 \pm 1.06$	$90.98 \pm 0.75$	89.06×	90.85±0.18	91.30±0.10	90.82b±0.66	90.70b±0.29	90.03×
Lemon	86.11 ±0.37	89.67 ±1.15	90.36 ±0.33	88.72×	$89.85 \pm 0.26$	89.31 ±0.21	$90.44 \pm 0.34$	$90.87 \pm 0.27$	<b>89.32</b> <sup>y</sup>
Amla	78.92 ±0.35	84.47 ±0.85	$85.10 \pm 1.18$	82.83y	$84.40 \pm 0.71$	83.86 ±1.20	82.98 ±0.55	84.43 ±1.47	82.92 <sup>z</sup>
Mean	<b>83.84</b> <sup>a</sup>	87.96 <sup>b</sup>	88.82 <sup>c</sup>	86.87	88.37 <sup>b</sup>	88.16 <sup>b</sup>	88.08 <sup>b</sup>	88.67 <sup>b</sup>	87.42
				a* v	value				
Citric acid	-0.087±0.100	-1.42±0.26	-1.37±0.12	-0.96×	$-1.67 \pm 0.03$	$-1.63 \pm 0.61$	$-1.49 \pm 0.05$	$-1.41 \pm 0.04$	-1.26×
Lemon	-0.367 ±0.153	$-1.41 \pm 0.20$	-1.35 ±0.10	-1.04×	-1.57 ±0.13	-1.49 ±0.08	-1.43 ±0.02	-1.13 ±0.12	-1.20×

Effect of fruit acidulant and storage condition on the phytochemical components and colour values of *paneer* 

Amla	2.00 ±0.17	1.36 ±0.16	0.900 ±0.329	1.42 <sup>y</sup>	1.23 ±0.21	$1.85 \pm 0.35$	$1.84 \pm 0.47$	$1.42 \pm 0.42$	<b>-1.67</b> y
Mean	0.514ª	-0.490 <sup>b</sup>	-0.609°	-0.195	-0.670 <sup>ь</sup>	-0.422 <sup>c</sup>	-0.358°	-0.371°	-0.261
				b* ,	value				
Citric acid	13.28±0.44	11.44±0.35	$12.85 \pm 0.62$	12.52×	11.69 ±0.55	11.62±0.46	12.17±0.50	12.24±0.32	12.20×
Lemon	$15.66 \pm 0.70$	13.31 ±1.20	$12.42 \pm 0.60$	13.80y	$13.06 \pm 0.47$	13.15 ±1.17	$13.28 \pm 0.48$	$12.94 \pm 0.45$	13.62 <sup>y</sup>
Amla	$10.77 \pm 0.25$	9.69 ±0.15	10. 04 ±0.35	10.17 <sup>z</sup>	$10.02 \pm 0.04$	8.99 ±0.26	9.78 ±0.87	9.99 ±0.10	9.91 <sup>z</sup>
Mean	<b>13.24</b> <sup>a</sup>	<b>11.48</b> <sup>b</sup>	<b>11.77</b> <sup>b</sup>	12.162	11.59 <sup>b</sup>	11.25 <sup>b</sup>	11.74 <sup>b</sup>	11.72 <sup>b</sup>	11.91

Mean  $\pm$  standard deviation. The values with a different superscript in column (x, y and z) or row (a, b, c, d and e) indicated that the mean differed significantly at p<0.05 based on the least significance difference test. ND indicated that the content of a particular compound was not detected.

#### Table 3

# Effect of storage temperature and time on the microbial count (Log No. CFU g<sup>-1</sup>) of *paneer*

Acidulants		Ro	om temperature	2	Refrigeration te	mperature			
		Sto	rage time (days)	)	-	Storage time (days)			
	Fresh	1	2	Mean	5	10	15	20	Mean
				Standard p	late count (SPC)				
Control	2.69 ±0.09	$3.30 \pm 0.02$	$3.92 \pm 0.02$	3.31×	$2.90^{a} \pm 0.05$	3.56 ª ±0.71	$4.34^{a} \pm 0.05$	$4.63^{a} \pm 0.41$	3.67 <sup>x</sup>
Lemon	$2.62 \pm 0.03$	$3.24 \pm 0.02$	$3.46 \pm 0.11$	3.11y	$2.63^{b} \pm 0.02$	$3.32^{b} \pm 0.73$	$4.05^{b} \pm 0.06$	$4.45^{b} \pm 0.04$	3.39y
Amla	$2.67 \pm 0.06$	$3.27 \pm 0.01$	3.73 ±0.10	3.22 <sup>z</sup>	$2.73^{ab} \pm 0.04$	$3.43^{ab} \pm 0.64$	$4.22^{ab} \pm 0.05$	$4.53^{ab} \pm 0.06$	3.55 <sup>×y</sup>
Mean	<b>2.66</b> <sup>a</sup>	3.27 <sup>b</sup>	<b>3.71</b> °	3.21	<b>2.75</b> <sup>a</sup>	3.37 <sup>b</sup>	<b>4.20</b> <sup>c</sup>	<b>4.54</b> <sup>d</sup>	3.54
				Yeast and	d mould count				
Control	ND	$1.36 \pm 0.10$	$1.79 \pm 0.10$	1.05×	$1.75^{a} \pm 0.05$	$1.92^{a} \pm 0.08$	2.20 <sup>a</sup> ±0.03	$2.30^{a} \pm 0.20$	1.68×
Lemon	ND	$1.10 \pm 0.17$	$1.75 \pm 0.05$	0.95y	$1.10^{b} \pm 0.17$	$1.56^{b} \pm 0.07$	$1.90^{b} \pm 0.05$	$2.06^{b} \pm 0.06$	1.40y
Amla	ND	$1.42 \pm 0.10$	$1.92 \pm 0.08$	1.11×	1.73 <sup>c</sup> ±0.05	$1.95^{\circ} \pm 0.05$	$2.25^{\circ} \pm 0.04$	2.32 <sup>c</sup> ±0.20	1.67×
Mean	$ND^a$	<b>1.29</b> <sup>b</sup>	<b>1.82</b> <sup>c</sup>	1.04	1.53 <sup>b</sup>	<b>1.81</b> °	<b>2.12</b> <sup>d</sup>	2.23 <sup>e</sup>	1.58
				Psychro	ophiles count				
Control	ND				$1.36^{a} \pm 0.10$	$1.78^{a} \pm 0.08$	$2.14^{a} \pm 0.06$	$2.25^{a} \pm 0.04$	1.88×
Lemon	ND				$1.10^{b} \pm 0.17$	$1.26^{b} \pm 0.04$	$1.69^{b} \pm 0.09$	$1.95^{b} \pm 0.05$	1.50y
Amla	ND				$1.30^{ab} \pm 0.00$	$1.63^{ab} \pm 0.06$	$1.86^{ab} \pm 0.08$	$2.05^{ab} \pm 0.05$	1.71 <sup>z</sup>
Mean	$ND^a$				1.25 <sup>b</sup>	1.56°	<b>1.90</b> <sup>d</sup>	2.08 <sup>e</sup>	1.13

Mean  $\pm$  standard deviation. The values with a different superscript in column (x, y and z) or row (a, b, c, d and e) indicated that the mean differed significantly at p<0.05 based on the least significance difference test. ND indicated that the content of a particular compound was not detected.

# Table 4

Acidulants	Fresh	Roo	m temperature			Refrige	eration temperat	ture	
	Day	Stora	age time (days)			Sto	rage time (days)	1	
	0	1	2	Mean	5	10	15	20	Mean
				Har	dness (Kg)				
Control	$1.29 \pm 0.12$	$0.61 \pm 0.07$	$0.56 \pm 0.05$	0.825 <sup>a</sup>	$1.38 \pm 0.06$	$17.00 \pm 2.78$	17.33 ±2.75	17.33 ±2.51	10.89 <sup>x</sup>
Lemon	2.31 ±0.19	$1.33 \pm 0.37$	$0.86 \pm 0.12$	$1.50^{b}$	6.61 ±0.70	$20.33 \pm 1.04$	27.00 ±1.73	62.47 ±13.06	23.74 <sup>y</sup>
Amla	$1.88 \pm 0.45$	$0.86 \pm 0.05$	$0.960 \pm 0.10$	1.24 <sup>c</sup>	2.54 ±0.16	$18.16 \pm 1.44$	33.33 ±2.52	56.23 ±10.25	22.43 <sup>y</sup>
Mean	<b>1.83</b> ª	0.939 <sup>b</sup>	0.795 <sup>b</sup>	1.19	<b>3.51</b> <sup>a</sup>	18.5 <sup>b</sup>	<b>25.88</b> <sup>c</sup>	45.34 <sup>d</sup>	19.01
			Fracturabi	lity (g)					
Control	$10.27^{a} \pm 1.72$	7.95 ±3.07	$8.49 \pm 3.25$	8.91ª	8.94 ±0.55	8.67 ±1.26	10.33 ±0.32	$7.47 \pm 0.55$	7.27×
Lemon	$11.58^{a} \pm 0.110$	$8.05 \pm 0.57$	$1.99 \pm 0.59$	7.21ª	8.73 ±0.53	$9.77 \pm 1.04$	13.00 ±1.73	9.23 ±0.39	10.44 <sup>y</sup>
Amla	$19.66^{b} \pm 4.7$	$8.42 \pm 5.05$	$6.42 \pm 4.25$	11.50 <sup>ab</sup>	17.02 ±2.53	17.02 ±2.53	17.83 ±3.01	8.74 ±4.91	14.48 <sup>z</sup>
Mean	<b>13.84</b> <sup>a</sup>	8.14 <sup>b</sup>	5.63 <sup>b</sup>	9.20	11.56 <sup>b</sup>	9.17°	10.62 <sup>bc</sup>	8.48 <sup>cd</sup>	10.73
				Adhesi	veness (g.sec)				
Control	$-1.43^{a} \pm 0.141$	-33.52 ±8.71	-24.61 ±12.70	-19.85 <sup>a</sup>	-5.54 ±2.46	-5.42 ±2.25	-5.00 ±3.51	$-1.47 \pm 0.04$	-3.77×
Lemon	$-4.40^{b} \pm 1.56$	$-5.58 \pm 0.35$	-6.43 ±1.93	-5.47 <sup>b</sup>	-25.85 ±10.09	-5.52 ±2.88	-25.85 ±10.09	-6.00 ±1.14	-13.52y
Amla	$-4.78^{b} \pm 1.057$	-7.24 ±1.17	-5.64 ±1.70	-5.89 <sup>b</sup>	$-4.38 \pm 0.54$	-5.73 ±3.99	-5.73 ±3.99	-2.13 ±1.08	-5.97×
Mean	-3.54ª	-15.45 <sup>b</sup>	-12.23c	-10.40	-11.92 <sup>b</sup>	-7.93 <sup>abc</sup>	-12.19 <sup>b</sup>	-3.20ac	-7.76
				Coł	nesiveness				
Control	$0.497^{a} \pm 0.091$	$0.425 \pm 0.037$	$0.433 \pm 0.027$	0.452×	$0.829 \pm 0.024$	$0.449 \pm 0.68$	$0.364 \pm 0.108$	$0.496 \pm 0.093$	0.527×
Lemon	$0.467^{a} \pm 0.015$	$0.497 \pm 0.035$	$0.410 \pm 0.016$	$0.458^{x}$	$0.462 \pm 0.064$	$0.483 \pm 0.110$	0.462. 0.64	$0.764 \pm 0.011$	0.528×
Amla	$0.413 \text{ a} \pm 0.032$	$0.424 \pm 0.063$	0.370 ±0.019	0.403 <sup>y</sup>	$0.416 \pm 0.031$	$0.453 \pm 0.578$	$0.504 \pm 0.167$	$0.765 \pm 0.107$	0.511×
Mean	<b>0.459</b> ª	<b>0.449</b> <sup>a</sup>	0.404 <sup>b</sup>	0.437	0.569 <sup>b</sup>	0.462 <sup>ac</sup>	0.444 <sup>ac</sup>	0.675 <sup>d</sup>	0.522
				Sp	ringiness (mm)				
Control	$0.777^{a} \pm 0.085$	$0.760 \pm 0.017$	$0.710 \pm 0.11$	0.790×	$0.370 \pm 0.108$	$0.496 \pm 0.032$	$0.750 \pm 0.043$	$0.776 \pm 0.085$	0.634×
Lemon	$0.767^{a} \pm 0.080$	$0.803 \pm 0.04$	$0.753 \pm 0.25$	0.774×	$0.586 \pm 0.281$	$0.780 \pm 0.096$	$0.586 \pm 0.281$	$0.620 \pm 0.010$	0.668×
Amla	$0.860^{a} \pm 0.026$	$0.867 \pm 0.04$	$0.817 \pm 0.152$	0.848y	$0.860 \pm 0.02$	$0.846 \pm 0.011$	$0.470 \pm 0.121$	$0.366 \pm 0.850$	0.681×
Mean	<b>0.801</b> ª	<b>0.810</b> ª	0.760ª	0.790	0.606 <sup>b</sup>	0.708 <sup>abc</sup>	0.602 <sup>cbd</sup>	0.588 <sup>bd</sup>	0.661
					Gumminess				
Control	$0.647 \pm 0.145$	$0.263 \pm 0.55$	$0.240 \pm 0.100$	0.383×	$1.14 \pm 0.03$	$7.68 \pm 1.81$	6.21 ±1.62	8.75 ±2.93	4.88×
Lemon	$1.08 \pm 0.12$	$0.673 \pm 0.244$	$0.353 \pm 0.058$	0.703 <sup>y</sup>	$3.09 \pm 0.76$	9.97 ±2.60	$12.42 \pm 0.88$	47.63 ±9.19	14.82 <sup>y</sup>
Amla	$0.783 \pm 0.25$	$0.370 \pm 0.072$	$0.357 \pm 0.416$	0.503×	$1.05 \pm 0.03$	$8.19 \pm 0.74$	16.79 ±5.71	42.32 ±3.69	13.83 <sup>y</sup>
Mean	<b>0.838</b> ª	<b>0.436</b> <sup>b</sup>	0.317 <sup>b</sup>	0.530	<b>1.76</b> <sup>a</sup>	8.58 <sup>b</sup>	<b>11.80</b> <sup>c</sup>	32.91 <sup>d</sup>	11.18

Effect of fruit acidulant and storage conditions on the texture profile of *paneer* 

					Chewiness				
Control	$0.493 \pm 0.81$	$0.200 \pm 0.46$	0.173 ±0.35	0.289×	$0.423 \pm 0.126$	3.78 ±0.81	$4.63 \pm 1.02$	6.62 ±1.49	3.91×
Lemon	$0.840 \pm 0.182$	$0.537 \pm 0.184$	$0.267 \pm 0.55$	0.548y	1.71 ±0.53	$7.85 \pm 2.84$	$7.20 \pm 3.14$	29.60 ±5.73	9.44 <sup>y</sup>
Amla	0.677 ±0.223	$0.320 \pm 0.07$	$0.287 \pm 0.032$	0.428 <sup>y</sup>	0.910 ±0.051	6.93 ±0.57	7.70 ±2.97	$15.40 \pm 3.08$	6.32 <sup>z</sup>
Mean	0.670ª	0.352 <sup>b</sup>	<b>0.242</b> ь	0.421	<b>1.07</b> <sup>a</sup>	6.19 <sup>b</sup>	6.51 <sup>b</sup>	<b>17.20</b> <sup>c</sup>	6.32
				Res	ilience (%)				
Control	$0.172^{a} \pm 0.027$	0.117 ±0.115	$0.140 \pm 0.017$	0.143×	$0.403 \pm 0.035$	$0.143 \pm 0.032$	$0.113 \pm 0.032$	0.173 ±0.025	0.201×
Lemon	$0.144^{ab} \pm 0.013$	$0.157 \pm 0.021$	$0.117 \pm 0.006$	0.139×y	0.163 ±0.032	$0.156 \pm 0.038$	$0.163 \pm 0.032$	$0.350 \pm 0.017$	0.195×
Amla	$0.126^{b} \pm 0.011$	0.133 ±0.023	$0.117 \pm 0.006$	0.126 <sup>y</sup>	0.127 ±0.011	$0.130 \pm 0.020$	$0.136 \pm 0.031$	0.380 ±0.121	0.180×
Mean	<b>0.148</b> ª	0.136 <sup>ab</sup>	0.124 <sup>b</sup>	1.36	0.231 <sup>b</sup>	<b>0.143</b> ª	<b>0.138</b> <sup>a</sup>	0.301c	0.192

Mean  $\pm$  standard deviation. The values with a different superscript in column (x, y and z) or row (a, b, c, d and e) indicated that the mean differed significantly at p<0.05 based on the least significance difference test.

Effect of Acidulants and Storage Conditions on The Phytochemicals and Colour Values of Paneer The effect of acidulants and storage at room temperature on the functional components and colour values ('L', 'a' and 'b') of paneer are presented in Table 2. The type of acidulant, temperature, and storage storage time significantly affected the paneer's functional components and colour values (p<0.05). Therefore, the DPPH scavenging capacity was used to test the antioxidant activity of paneer during storage. Generally, the antioxidant activity was highest in amla, followed by lemon and control paneer. Despite the type of acidulant and storage conditions, ascorbic acid, total phenols, and tannins significantly reduced during the storage of the paneer. However, the loss was more in the paneer stored at room temperature than in the refrigerator. Generally, the 'L' values of the paneer increased during storage at all temperatures, even though the increase was higher in refrigerated samples. This is because the increase correlated with the content of phytochemicals, which depleted over time. Although lemon paneer had a high initial 'a' value, it was noticed that the greenness increased more in control than lemon paneer during storage. On the other hand, the amla paneer was characterised by redness ('-a'), and the value declined as storage progressed.

### *Effect of Fruit Acidulant and Storage Conditions on the Microbial Count of Paneer*

The type of acidulant, storage condition and time significantly affect (p <0.05) the microbial count of paneer (Table 3). The number of SPC colonies increased significantly daily in all paneer samples during storage despite the types of acidulants used. However, the number of bacteria colonies were highest in paneer made from citric acid, followed by amla and lemon extract. For the storage condition, the increase in SPC was higher in paneer stored at room temperature than at refrigeration temperature.

Yeast and mould growth was not detected in all fresh paneer samples. However, the yeast and mould were highest in paneer made from amla, followed by citric and lemon during the storage of paneer at room temperature for three days. A comparable trend was observed for the paneer stored at refrigeration temperature. The number of psychrophiles significantly differed with the acidulant and storage days at 5°C. The highest psychrophile count was found in paneer made from citric acid, followed by amla and lemon.

# *Effect of Fruit Acidulant and Storage Conditions on the Texture Profile of Paneer*

The results on the effect of acidulants and storage conditions on the texture profile of paneer are presented in Table 4. The type of acidulant, storage temperature and time significantly affected the texture profile of paneer. For example, hardness significantly (p<0.05) decreased when the paneer was stored at room temperature. On the other hand, the hardness of all paneer stored refrigeration temperature significantly in increased with storage time. For both storage temperatures, the hardness was highest in lemon, followed by amla and control. In addition, there was a decrease in the cohesiveness of the paneer temperature. stored at room However, cohesiveness increased for those paneer stored in refrigeration temperature except for the control.

The springiness was highest in amla paneer, followed by control and lemon. Generally, the springiness of treatments increased after one day and decreased the following days. There was a significant decrease in gumminess, chewiness and resilience in all types of *paneer* throughout the storage period. The gumminess was highest in lemon paneer, followed by amla and control. However, the change of gumminess in lemon and amla paneer was almost similar (Table 4). The chewiness was highest in lemon paneer, followed by amla and control. The chewiness was significantly increased in all types of paneer and showed a linear relationship with hardness, cohesiveness and gumminess. Resilience was highest in control, followed by paneer from lemon and amla acidulants.

There was a significant variation in the springiness of the *paneer* during the storage of all *paneer* types (Table 4). The springiness of the *amla paneer* decreased continuously, whereas variable change was observed in the lemon and control. There was a marked increase in gumminess of all types of *paneer* during the storage at refrigeration temperature. However, the highest increase of

gumminess was found in lemon *paneer*, followed by *amla* and control. Nonetheless, the change of gumminess between lemon and *amla paneer* was almost similar.

#### Table 5

Effect of fruit a	acidulant and storage	e conditions on the se	nsory scores of <i>paneer</i>

Acidulants	Fresh	Room temp	erature		<b>Refrigeration temperature</b>				
	Day	Storage time	e (days)		Storage t	ime (days)			
	0	1	Mean	5	10	15	Mean		
			Appeara	nce/ colour					
Citric acid	$8.50 \pm 0.60$	$8.06 \pm 0.56$	8.28×	$8.06 \pm 0.50$	$7.88 \pm 0.52$	$7.82 \pm 0.56$	8.06×		
Lemon	$8.50 \pm 0.60$	$8.06 \pm 0.68$	8.28×	$7.88 \pm 0.79$	7.86 ±0.83	$7.68 \pm 0.84$	8.00×		
Amla	7.36 ±0.52	$7.13 \pm 0.64$	7.25 <sup>y</sup>	$7.56 \pm 0.77$	7.38 ±0.92	$6.63 \pm 0.74$	7.21 <sup>y</sup>		
Mean	<b>8.13</b> <sup>a</sup>	<b>7.75</b> <sup>a</sup>	7.94	<b>7.83</b> <sup>a</sup>	7.71 <sup>b</sup>	7.38 <sup>b</sup>	7.76		
			Body a	nd texture					
Citric acid	$8.38 \pm 0.58$	7.87 ±0.99	8.13×	$8.06 \pm 0.73$	7.93 ±0.68	$7.25 \pm 0.76$	7.90×		
Lemon	8.19 ±0.53	7.69 ±0.75	7.94×	$8.06 \pm 0.42$	7.75 ±0.53	7.19 ±0.99	7.78×		
Amla	$8.00 \pm 0.71$	$7.94 \pm 0.50$	7.97×	7.56 ±0.73	7.50 ±0.16	$7.44 \pm 0.73$	7.69×		
Mean	<b>8.19</b> <sup>a</sup>	<b>7.83</b> <sup>a</sup>	8.01	7.90 <sup>ab</sup>	7.73ь	7.29°	7.79		
			Mou	ıthfeel					
Citric acid	$8.25 \pm 0.71$	$7.88 \pm 0.13$	8.06×	$8.06 \pm 0.50$	7.81 ±0.75	7.19 ±0.37	7.84×		
Lemon	$8.56 \pm 0.50$	8.31 ±0.59	8.44 <sup>xy</sup>	$8.12 \pm 0.35$	$8.06 \pm 0.56$	$7.25 \pm 0.69$	8.06 <sup>xy</sup>		
Amla	7.88 ±0.69	$7.50 \pm 0.80$	7.67 <sup>x</sup>	7.63 ±0.92	$7.56 \pm 0.90$	$7.50 \pm 0.10$	7.61×		
Mean	8.23 <sup>a</sup>	<b>7.90</b> <sup>a</sup>	8.06	<b>7.94</b> <sup>a</sup>	<b>7.81</b> <sup>a</sup>	7.31 <sup>b</sup>	7.84		
			Fla	ivour					
Citric acid	$8.06 \pm 0.68$	$7.69 \pm 0.70$	7.94×	$7.94 \pm 0.72$	7.93 ±0.68	$6.63 \pm 0.92$	7.68×		
Lemon	$8.44 \pm 0.62$	$8.13 \pm 0.64$	8.28 <sup>xy</sup>	$8.06 \pm 0.68$	7.69 ±0.75	$7.00 \pm 0.88$	7.86×		
Amla	$7.88 \pm 0.69$	$7.50 \pm 0.80$	7.69 <sup>x</sup>	$7.68 \pm 0.75$	$7.46 \pm 0.54$	$7.25 \pm 0.93$	7.56×		
Mean	<b>8.16</b> <sup>a</sup>	7 <b>.</b> 77ª	7.97	7.90 <sup>ab</sup>	<b>7.70</b> ь	7.00 <sup>c</sup>	7.70		
			Overall a	cceptability					
Citric acid	$8.20 \pm 0.57$	$7.80 \pm 0.71$	8.00×	$8.02 \pm 0.29$	7.91 ±0.57	$7.06 \pm 0.64$	7.80×		
Lemon	$8.52 \pm 0.48$	$8.14 \pm 0.72$	8.33y	$8.00 \pm 0.59$	$7.88 \pm 0.47$	7.31 ±0.67	8.00 <sup>xy</sup>		
Amla	$7.81 \pm 0.50$	$7.56 \pm 0.66$	7.69 <sup>xy</sup>	$7.58 \pm 0.78$	$7.58 \pm 0.86$	$7.09 \pm 0.93$	7.53×		
Mean	<b>8.18</b> ª	<b>7.83</b> <sup>a</sup>	8.05	7.85 <sup>ab</sup>	7.79 <sup>b</sup>	<b>7.26</b> <sup>c</sup>	7.76		

Mean  $\pm$  standard deviation. The values with a different superscript in column (x, y and z) or row (a, b, c, d and e) indicated that the mean differed significantly at p<0.05 based on the least significance difference test.

±1°C.

# *Effect of Storage at Room Temperature on the Sensory Scores of Paneer*

The sensory evaluation of the *paneer* results are presented in Table 5. The scores for appearance/colour, body and texture, mouthfeel flavour and overall acceptability decreased during the storage of *paneer*. The control and lemon *paneer* were comparable in appearance/colour, whereas the *paneer* from the *amla* extract scored less due to the reddish tinge imparted by phenolic compounds from the *amla*  extract. Buch et al. (2014) reported a decline in the

score of colour/ appearance of *paneer* added with

0.4% and 0.6% turmeric powder and stored at 7

in score might be due to moisture loss caused by

syneresis and low temperature. Buch et al. (2014)

reported a change of body and texture score at 7  $\pm$ 1°C for *paneer* added with 0.4% and 0.6% turmeric powder.

The mouthfeel and flavour scores decreased during storage in all paneer samples. However, the flavour score of lemon paneer was higher than the rest. The panel reported that the high acceptability of *paneer* from the lemon extract was due to its characteristic flavour. On the other hand, the lower flavour score in amla paneer may be attributed to the slight bitterness, as reported by the sensory panel. The bitter flavour of *paneer* is usually associated with rancidity during longterm storage and can easily be detected by the sense of taste and persists even after the sample has been removed from the mouth (Kumar, 2016). Moreover, the excessive proteolysis of curd is reported to cause bitterness in cheese (Nerurkar, 2007). For example, bitterness in cottage cheese is associated with increased insoluble nitrogen and the growth of psychotropic bacteria that produce proteolytic and lipolytic enzymes that break down beta-casein and fat into bitter compounds (Lemieux & Simard, 1991). In addition, the bitter peptides interact with the taste buds at the back of the tongue to give the sensation of bitterness (Iwaniak et al., 2016).

The overall acceptability (OA) scores decreased refrigeration and over room storage temperatures. However, the OA score of lemon paneer was higher than control and amla paneer. Earlier, Bajwa et al. (2005) recorded a lowering of OA in 10% coriander (Coriandrum sativum), and mint (Mentha piperita) incorporated paneer stored for 24 h at ambient temperature and 15 days at refrigeration temperature. The factors contributing to the reduced OA of paneer are related to changes in physicochemical and texture profiles.

# Discussion

The type of acidulant, storage temperature and time significantly affected the *paneer*'s physicochemical properties (p<0.05). Ahmad *et al.* (2014) observed the moisture decrease in *Kaladhi* (hard and dry acid-coagulated cheese variety of Jammu and Kashmir) stored between 21 to 25°C. Also, Rai *et al.* (2008) observed a

moisture loss for vacuum-packed *paneer* with 100% nitrogen gas stored for 15 days at 7  $\pm$ 1°C. Generally, the moisture loss in the *paneer* is due to syneresis. The rate of syneresis has been associated with *paneer* microstructure (Sinha, 2007). A previous scanning electron microscope study by Ahmed and Bajwa (2019) revealed that the microstructure of *paneer* samples differed with different acidulants.

On the other hand, the increase in the titratable acidity during the storage of *paneer* samples could be highly associated with the growths of microorganisms and biochemical changes causing the hydrolysis of lactose to lactic acid. Earlier, Bajwa et al. (2005) recorded increased acidity in the vegetable-impregnated *paneer* during storage for 15 days. Singh et al. (2014) also recorded increased acidity in cows, buffalo, and mixed milk *paneer* with turmeric powder stored at 27 ±1°C. Singh et al. (2015) observed that the acidity of control samples increased significantly after the first day and the first month for paneer with 0.4% formalin during the storage at 37°C for six months. The highest pH decrease in the control could be due to higher microbial growth (Table 1) than paneer prepared from fruit acidulants. Rai et al. (2008) and Buch et al. (2014) reported a decrease in pH for the vacuum-packed paneer stored at 7±1°C for 45 days and in paneer added with turmeric powder and stored at 7 ±1°C, respectively.

Generally, the milk's NPN constituents include ammonia, urea, creatine, creatinine, uric acid, peptides, amino acids, and other compounds (DePeters and Ferguson, 1992). The high increase in NPN in control during the storage possibly was due to the growth of psychrophiles (Table 3) that have the potential to induce biochemical changes in proteins even at refrigeration temperature. On the other hand, the lower acidity and functional compounds in the fruit acidulant might contribute to the lower formation of NPN in lemon and *amla paneer*. Similarly, Buch *et al.* (2014) noticed a lesser change of soluble nitrogen in *paneer* added with 0.4% and 0.6% turmeric powder than in control stored at 7±1°C.

The increased FFA could be associated with biochemical changes or microbial growth. Singh *et al.* (2015) observed the FFA increase in control

paneer after the first day and after the first month of paneer with 0.4% formalin during the storage at 37°C for six months. Earlier, Buch *et al.* (2014) reported increased FFA in paneer stored at 7±1°C for 12 days. Rai *et al.* (2008) reported an increase in FFA of vacuum-packed paneer stored for 15 days at 7±1°C. Therefore, the fruit acidulants have the potential to inhibit the physicochemical changes, such as the formation of NPN and FFA, which are highly associated with spoilage and the safety of the paneer during storage.

Phytochemicals, functional components, are plant substances such as phenols and ascorbic acid that have specific pharmacological effects on health, such antimicrobial, human as antioxidants, anti-inflammatory, anti-allergic, anti-cancer, anti-spasmodic, and anti-ageing neuroprotective, hypotensive and diabetes (Unuofin et al., 2017; Prakash et al., 2012). The high antioxidant activity in lemon and amla paneer was due to the high phytochemicals in lemon and amla fruit extract (Ahmed and Bajwa, 2019). Moreover, phenolic compounds such as tannins contribute to the antioxidant activity of fruits and plants (Li et al., 2007; Pourmorad et al., 2006). The trend showed that the antioxidant activity of all paneer stored at room temperature significantly increased, whereas it decreased in paneer stored at refrigeration temperature. Gracía-Alonso et al. (2008) found that the different concentration of antioxidant compounds was affected by the storage condition of food. Therefore, the decreased antioxidant in lemon and amla paneer on the 10th day onward might be attributed to the catalytic action of reactivated phenolases, as elaborated by Xu et al. (2015). On the other hand, amino acids or peptides may play significant roles in antioxidant and antimicrobial activities (EFSA, 2009).

Tripathi *et al.* (1988) observed decreased ascorbic acid and phenol content while storing *amla* candy. Generally, temperature and the vitamin's form most affect vitamin C's stability in foods and beverages (Stešková *et al.*, 2006). Therefore, the loss of ascorbic acid might be due to the oxidation of L-ascorbic acid to dehydro-ascorbic acid (Reynold, 1965). However, studying structural changes of ascorbic acid in *paneer* during storage was beyond the scope of the study.

On the other hand, the decrease of 'a' values of amla paneer might be due to the bleaching/decolourisation of phenolic compounds and tannins. Similarly, Shrivastava et al. (2013) observed a decrease of 'L' and 'a' and an increase of 'b' of *paneer* samples packaged under an atmosphere of vacuum, CO<sub>2</sub>, N<sub>2</sub> and a combination after ten days of storage at 3±1°C.

Nevertheless, the SPC was below the maximum limit that FSSAI (2009) prescribed, i.e. the total plate count should not exceed 50,000 g<sup>-1</sup> of paneer. Shashikumar and Puranik (2011) observed that SPC at 30±1°C in fresh and two days stored *paneer* increased from 3.4 to 4.8, 4.64, 4.58 and 4.25 (log CFUg-1) for control and paneer treated with 10, 15 and 20% lactoferrin, respectively. Lamdande (2012) also noted similar changes in the SPC of edible film-coated paneer stored at 5°C for 14 days. Singh et al. (2014) reported an increase in SPC from 4.48 to 5.68, 4.51 to 5.69 and 4.80 to 5.61 log CFU g<sup>-1,</sup> respectively, for cow, buffalo, and mixed milk paneer coagulated with citric acid after storage of 16 days below 5°C, and the number decreased when treated with 0.6% turmeric powder. Buch et al. (2014) observed that SPC for control and 0.4% turmeric-treated paneer with per cent increased from 4.2 to 4.9 and 4.1, respectively, at 7±1°C. According to FSSAI (2009), paneer is considered unfit if the yeast and mould count exceeds 250gm-1. The highest number of yeast and moulds count in amla paneer might be due to its lower pH. Shashikumar and Puranik (2011) observed that yeast and mould count in fresh and two days paneer stored at 30±1°C increased from 1.33 to 1.94, 1.9, 1.74 and 1.68 (log CFUg-1) for control and paneer treated with 10, 15 and 20% lactoferrin respectively. Singh et al. (2014) stored paneer for 16 days below 5°C and reported an increase in yeast and mould count from 4.50 to 5.41, 4.41 to 5.44 and 4.4 to 5.43 for cow, buffalo and mixed milk coagulated with citric acid, respectively. Furthermore, they reported a decline in the number of *paneer* treated with 0.6% turmeric powder. Buch et al. (2014) reported yeast and mould counts of 2.3 and 0.02, 3.1 and 2.5 and 3.5 and 3.7 (log. CFUg<sup>-1</sup>) for the fresh, six and 12-day storage at 7±1°C, respectively for control and paneer treated with 0.4% turmeric powder.

Psychotropic bacteria in milk and milk products include pseudomonads and related aerobic, Gram-negative, rod-shaped bacteria (Ledenbach Marshall, 2009). The psychotropic and microorganisms can survive harsh processing conditions, grow at refrigeration temperatures, and change food composition and quality (de Santana et al., 2020). In addition, they possess several adaptive mechanisms to cope with low temperatures, high UV irradiation levels, elevated pH, salt, and low water content (Aliyu et al., 2014). For example, psychrotrophs limit the shelf life of cottage cheese because the pH (4.5 to 4.7) and salt content of cottage cheese are insufficient to limit their growth (Ledenbach & Marshall, 2009; Cousin, 1982).

On the other hand, the phytochemicals in lemon and amla could be highly attributed to the inhibition of the psychrotrophs. For example, Pandey et al. (2011) reported that phytochemical compounds found in lemon, including tannins, inhibited the growth of Pseudomonas aeruginosa. Citrus fruits contain bioactive compounds such as phenolics, flavonoids, vitamins, and essential oils, which are reported to have several health benefits, including antioxidative, antiinflammatory, antitumor, and antimicrobial activities (Aruoma et al., 2012; Karimi et al., 2012). Saxena and Patil (2014) also observed that solvent fruit extract of Emblica officinalis essential oil had a strong inhibitory effect against Staphylococcus aureus compared to gentamicin. Furthermore, Oikeh et al. (2016) observed the antifungal activities of lemon juice and lemon concentrate against Candida albicans and Penicillum spp., respectively, with an inhibition zone of 24 mm and 10 mm. Therefore, it is evident that the paneer from the fruit acidulants has a great potential to extend shelf life beyond ten days at refrigeration temperature.

The previous microstructure appraisal study showed that lemon *paneer* had a firmer and more compact structure than *amla* and citric acid (Ahmed and Bajwa, 2019). The decrease in hardness of *paneer* stored at room temperature might be attributed to changes in moisture content and protein structure facilitated by the biochemical changes and growth of microorganisms. Shashikumar and Puranik (2011) observed an increase in hardness during storage of control and *paneer* treated with lactoferrin. Singh *et al.* (2014) observed that the increase of adhesiveness of *paneer*-containing turmeric powder was slower than the control. Moreover, Singh *et al.* (2014) observed increase in the cohesiveness of *paneer* containing turmeric powder was slower than in the control. Shashikumar and Puranik (2011) observed a similar trend earlier, who reported increased cohesiveness of control and *paneer* treated with lactoferrin. The difference in observations might be attributed to the type and strength of the acidulants.

Again, Singh *et al.* (2014) observed that the decrease in the springiness of *paneer* samples containing turmeric powder was slower than control *paneer*. Shashikumar and Puranik (2011) also observed the increase in chewiness of stored control samples and *paneer* preserved with lactoferrin. Resilience was highest in control, followed by lemon and *amla paneer*. Again, Shashikumar and Puranik (2011) observed a slight increase in the springiness of *paneer* treated with lactoferrin at 10 ppm. Similarly, Singh et al. (2014) reported curvilinear changes in turmeric *paneer*.

The control and lemon *paneer* were comparable in appearance/colour, whereas the *paneer* from the *amla* extract scored less due to the reddish tinge imparted by phenolic compounds from the *amla* extract. Buch *et al.* (2014) reported a decline in the score of colour/ appearance of *paneer* added with 0.4% and 0.6% turmeric powder and stored at 7±1°C. Moreover, Buch *et al.* (2014) reported a change in body and texture score at 7±1°C for *paneer* added with 0.4% and 0.6% turmeric powder.

The panel reported that the high acceptability of *paneer* from the lemon extract was due to its characteristic flavour. On the other hand, the lower flavour score in *amla paneer* may be attributed to the slight bitterness, as reported by the sensory panel. The bitter flavour of *paneer* is usually associated with rancidity during long-term storage and can easily be detected by the sense of taste and persists even after the sample has been removed from the mouth (Kumar, 2016). Moreover, the excessive proteolysis of curd is reported to cause bitterness in cheese (Nerurkar,

2007). For example, bitterness in cottage cheese is associated with increased insoluble nitrogen and the growth of psychotropic bacteria that produce proteolytic and lipolytic enzymes that break down beta-casein and fat into bitter compounds (Lemieux & Simard, 1991). In addition, the bitter peptides tend to give the sensation of bitterness when interacting with the taste buds at the back of the tongue (Iwaniak et al., 2016). Earlier, Bajwa et al. (2005) recorded a lowering of OA in 10% coriander (Coriandrum sativum), and mint (Mentha piperita) incorporated paneer stored for 24 h at ambient temperature and 15 days at refrigeration temperature. The factors contributing to the reduced OA of paneer are related to changes in physicochemical and texture profiles.

# Conclusion

The type of acidulant and storage conditions physicochemical significantly affect the properties of paneer, with an increase in the total solids and a decrease in moisture content during storage. The increase in titratable acidity, NPN, and FFA during storage is associated with microbial growth, causing several biochemical changes. Fruit acidulants have the potential to restrict these changes and improve the quality of paneer during storage. In addition, paneer's functional components and colour values were significantly affected by the type of acidulant and storage conditions. The antioxidant activity was high in amla and lemon paneer due to their high phytochemical content. Ascorbic acid, total phenols, and tannins were reduced during storage, with the loss being more significant at room temperature. The 'L', 'a' and 'b' values of paneer changed during storage, with the decrease of 'a' values of amla paneer attributed to the bleaching/decolourisation of phenolic compounds and tannins.

Nevertheless, the microbial count of *paneer* was significantly affected by the type of acidulant, storage condition, and time. Although the number of microorganisms increased daily during storage, it remained below the maximum limit prescribed by FSSAI. Nevertheless, the highest psychrophile count was found in *paneer* made from citric acid, followed by *amla* and

lemon. Therefore, the phytochemicals in lemon and *amla* could be highly attributed to the inhibition of psychrotrophs. Furthermore, the texture profile of *paneer* is significantly affected by the type of acidulant, storage temperature, and time.

Lemon paneer had the firmest and most compact structure, while amla paneer had the highest fracturability and springiness. The hardness of the *paneer* stored in refrigeration increased with storage time, while the adhesiveness and cohesiveness increased for the paneer stored in refrigeration temperature except for the control. However, all types of *paneer* showed a significant decrease in gumminess, chewiness, and resilience during storage. Therefore, it is important to carefully select acidulants and storage conditions to maintain the desired texture of paneer. Finally, the sensory evaluation of paneer showed decline in scores а for appearance/colour, body and texture, mouthfeel flavour, and overall acceptability during storage. The decrease in scores could be attributed to moisture loss, syneresis, low temperature, proteolysis, and lipolysis. However, the lemon acidulant paneer received higher flavour scores and overall acceptability due to its characteristic taste. Thus, the study suggests that adding natural fruit acidulants improves paneer's physicochemical, functional, shelf life and sensory properties.

# Acknowledgements

The first author is thankful for financial support from the iAGRI-Tanzania/Ohio State University "Feed the Future Program" to conduct research and fulfil other degree requirements for M.Sc. Food Technology at Punjab Agricultural University.

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