Indian J Dairy Sci 77(1): 1-9
https://doi.org/10.33785/IJDS.2024.v77i01.001

RESEARCH ARTICLE

Effect of incorporation of guava leaf powder on storage stability of curd balls under aerobic packaging condition at refrigeration temperature

Varsha Vihan, VP Singh, Akhilesh K Verma (✉), Pramila Umaraw, Chirag Singh and Shardanand Verma

Received: 12 January 2023 / Accepted: 04 June 2023 / Published online: 23 February 2024
© Indian Dairy Association (India) 2024

Abstract: The present study has undertaken for the evaluation of the impact of incorporating guava leaf powder (GLP) on the storage stability of developed curd balls. Four groups of curd balls were formulated with the addition of different levels of guava leaf powder: C (control without GLP), T1 (with 1.5% GLP), T2 (with 3.0% GLP), and T3 (with 4.5% GLP). The results revealed that pH and titratable acidity were significantly (P≤0.05) lower in the treated groups than in control. Peroxide value, thio-barbituric acid reactive substances, and free fatty acid content were significantly (P≤0.05) lower in GLP incorporated curd balls than in control. Guava leaf powder added curd balls has significantly (P≤0.05) higher DPPH, ABTS, and total phenolic content than the control. Among all samples, T3 (4.5%) recorded significantly (P≤0.05) lower microbial growth than the others groups of curd balls. However, sensory panelists rated significantly (P≤0.05) higher scores for T2 than T3. The sequent of the study concluded that the curd balls prepared with the inclusion of 3.0 % guava leaf powder prevent Physico-chemical quality deterioration, improve antioxidant capacity, reduced lipid oxidation and microbial growth with acceptable sensory attributes.

Keywords: Antioxidant activity, lipid oxidation, antimicrobial activity, sensory quality, guava leaf powder

Department of Livestock Products Technology, College of Veterinary and Animal Sciences, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut-250110, (Uttar Pradesh) India

Akhilesh K Verma (✉)
Email: vetakhilesh@gmail.com

Introduction

Curd is considered as a well-known traditional fermented milk product of Indian origin and as the most important dairy product in human diet. It is a semi-solid product obtained from boiled or pasteurized milk by souring, natural or using harmless lactic acid or other bacterial cultures. Besides direct consumption, curd also utilised in preparation of "chakka" by straining whey from curd. "Chakka" is an indigenous fermented dairy product and refers to a white to pale yellow semi-solid product of good texture and uniform consistency obtained after draining off the whey from the curd.

Natural products are of great interest for the integration of health-promoting substances in the diet as natural food additives, for the prevention of diseases and also for the improvement of general well-being. Consumers have additional considerations and recommendations to use natural antioxidants from food sources instead of artificial antioxidants that are restricted due to their toxic and carcinogenic effects (Abdel-Hameed et al. 2014). Dairy products are one among the foremost fascinating and promising foods with reference to their potential inhibitor activity, because of their wide diversity of antioxidant molecules like milk caseins and whey proteins. Medicinal plants abundant in natural antioxidants and phenolics compounds are gradually applied in the manufacturing of dairy foods to enhance their nutritional and therapeutic properties.

Guava is known as *Psidium guajava* from the Myrtaceae family, is a globally well-liked tropical fruit contains high amount of vitamins and phytochemicals. Guava is a natural product that possesses dietary fibre as well as antioxidant compounds. The extracts and metabolites of this plant, especially from the leaves, possess beneficial activities such as antioxidant properties, antimicrobial properties compared to other herbs. Studies have shown that guava leaves are advantageous as antibacterial agents Biswas et al. (2013), antioxidants (Chen and Yen, 20017). The strong antioxidant mechanisms acquired by guava leaf could be attributed to their free radical- scavenging ability. Additionally, phenolic compounds appear to be liable for the antioxidant activity of guava leaf.

Considering the above facts, the present study is conducted to improve the shelf life of curd balls by incorporating different levels of guava leaf powder with the following objectives:To optimize the levels of guava leaf in prepared curd balls, to assess the physico-chemical and sensory parameters of the prepared product and to study the storage stability at refrigeration temperature (4 ±1ºC) under aerobic packaging condition.

Materials and methods

Chemicals and media

All chemicals and media utilised during the study were of analytical class and procured from standard firms like Hi-media, SRL, CDH and Merck, etc. Raw buffalo's milk was purchased from nearby village dairy plant of Modipuram, Meerut. Freeze-dried Lactic Culture was purchased from CHR Hensen, Denmark. Low density polyethylene films (200µm gauge) were procured from local market and were sterilised by exposing to U.V. light for 30 minutes before use.

Preparation of guava leaf powder

Guava leaves were collected from Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut campus. Guava leaves were first cleaned with tap water and damaged, unwanted leaves were discarded. To remove the wash-water from the surface the leaves were then air dried for 1 hour and then leaves were oven (Meta-Lab Scientific Industries) dried for 48 hours at 50±1ºC. Dried guava leaves were then grounded in a food mixer and strained through a stainless-steel sieve. It was packed in air tight container and stored in cool and dry place until used.

Preparation of chakka and curd balls

In summary, fresh buffalo milk was heated to 85°C±5 for 20 minutes, followed by cooling to a temperature range of 43±2°C. Milk was then inoculated with 1.5% starter lactic culture to commence the fermentation process and incubation was allowed to be carried out at 37±2°C for 5-6 hours. After that curd was strained with cheese cloth for 4 hours. The bulk was subsequently stored at 4°C overnight.

Curd balls were prepared by incorporation of three different levels of guava leaf powder *viz*., (T1) 1.5%, (T2) 3% and (T3) 4.5%, the levels of guava leaf powder and control without guava leaf powder (C). All ingredients were weighed and thoroughly mixed till uniform batter formation and then shaped into balls (Table 1). These curd balls were then cooked by convection cooking in a preheated oven (Meta-Lab Scientific Industries) at 75°C for 30 minutes and then turned and again cooked for 15 minutes. The cooked curd balls were then cooled to room temperature and then each group was separately packed under aerobic packaging in low density polyethylene bags (LDPE) and stored under refrigeration temperature at 4±2 ºC for further study.

Physico-chemical analysis

pH and titratable acidity value

The pH of the sample was determined by dipping the combined glass electrode of digital pH meter (ESICO, Model-1012). The titratable acidity in terms of percent lactic acid was determined by method as described by Shelef and Jay (1970).

Antioxidant activity

Total phenolic content

The total phenolic content of control products and treated groups was analyzed by Folin-Ciocalteu's method as prescribed by Zhang et al. (2006) with slight modification and gallic acid was used as standard.

2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

Antioxidant activity of curd balls were analyzed using stable radical (2, 2-diphenyl-1-picrylhydrazyl) as determined by Brand-Williams et al. (1995).

$$\text{DPPH Scavenging activity (\% inhibition)} = 100 - \left[\frac{At_{20}}{At_0} X \ 100 \right]$$

2-2-azinobis-3ethylbenthiazoline-6-sulphonic acid ($ABTS^+$) radical scavenging activity

$ABTS^+$ radical scavenging activity was determined as per the procedure outlined by Umaraw et al. (2023) using spectrophotometry. The $ABTS^+$ activity was expressed as % inhibition using formula –

$$\text{ABTS}^+ \text{ activity (\% inhibition)} = \frac{0.7 - At_{20}}{0.7} X \ 100$$

Lipid oxidation

Peroxide value, Thiobarbituric acid reacting substances (TBARS) value and Free fatty acids (FFAs)

Peroxide value was analyzed as per the procedure of Koniecko (1979). The peroxide value expressed as meq/kg of sample was calculated using the formula:

$$\text{PV (meq/kg sample)} = \frac{0.1 \times \text{mL 0.1N sodium thiosulphate}}{\text{Sample weight (g)}} X \ 100$$

Thiobarbituric acid reacting substances value was analyzed as

per the procedure of Witte et al. (1970).

TBARS value (mg malonaldehyde/ kg of sample) = O.D. of the sample × 5.2

Free fatty acids value of the sample was determined by modified Koniecko (1979). The amount of potassium hydroxide consumed for titration was noted and then the free fatty acids content of the sample was calculated as follows:

$$\text{Free fatty acid (\% oleic acid)} = \frac{0.1 \times \text{mL 0.1 N alcoholic KOH} \times 0.282}{\text{Sample weight (g)}} \text{ X } 100$$

Microbiological analysis

Standard plate count, psychrophilic count, coliforms count and yeast and moulds count of the samples were conducted as per the method prescribed by American Public Health Association (1992).

Sensory evaluation

The sensory quality of the samples was evaluated by using 9-point hedonic scale as presented in sensory evaluation scoring sheet. A nine-point hedonic scale, varying from extremely undesirable (score 1) to extremely desirable (score 9) was used. Sensory parameters such as colour, taste, aroma, texture and overall acceptability were used to assess the curd balls. Precooked curd balls from each batch were heated in microwave oven for 1-2 minutes and then presented to sensory panelist with 2-digit random code for evaluation. A sensory panel (semi-trained) was drawn from post-graduates' students and staff of college. After briefing properly about the product, the panelists were requested to evaluate the product to determine their organoleptic characteristics in terms of their colour, taste, aroma, texture and overall acceptability.

Statistical analysis

Experiment was carried out three times and data were collected two times for every attributes. Two-way ANOVA was used for the analysis of recorded data using SPSS 22 statistical software (SPSS Inc., Chicago, IL, USA). Means of attributes were correlated using Duncan's multiple range test (DMRT), at the (Pdd0.05) level of significance.

Results and Discussion

Change in pH and titratable acidity

The pH value varied significantly (P≤0.05) among the groups (Table 2). The decrease in pH value and a corresponding increase in titratable acidity were observed in all groups. However, the pH values decreased significantly (P≤0.05) during storage which might be due to proliferation of *Lactobacillus sp*. of the microbes. Titratable acidity value among the groups differed significantly from the 5th day of storage to end of the storage time (Table 2). Titratable acidity values increased significantly (P≤0.05) during the storage. The decrease in pH value of the curd ball samples might be due to an increase in production of the acidic compounds during the proliferation of microorganisms. However, the rate of decrement in pH value of treated samples was lower than the control which might due to slower rate of growth of spoilage microbes. Similar results were also reported by Najgebauer-Lejko et al. (2011) for yoghurt prepared with incorporation of tea polyphenols during storage. Our findings were in accordance with the results of Qureshi et al. (2019) who reported decreasing trend of pH value in paneer prepared with the extracts during storage. The increase in titratable acidity value might be due to growth of lactic acid producing microbes during the storage. Our finding is in harmony with the results reported by Ahuja and Goyal (2013). Kumar et al. (2019) also reported increasing trend for the titratable acidity during the storage study of milk products.

Change in antioxidant parameters

Total phenolic content

Table: 1 Formulation for the preparation of curd balls incorporated with guava leaf powder

Ingredients	Control	T1	T2	T3
Chakka	77.0	75.5	74.0	72.5
Refined oil	3.0	3.0	3.0	3.0
Flour	3.0	3.0	3.0	3.0
Salt	1.5	1.5	1.5	1.5
Spices	1.0	1.0	1.0	1.0
Condiments	2.0	2.0	2.0	2.0
Carrot	12.5	12.5	12.5	12.5
Guava leaf	0.0	1.5	3	4.5

C: Control curd balls without guava leaf powder; T1: curd balls with 1.5 % guava leaf powder; T2: curd balls with 3.0 % guava leaf powder; T3: curd balls with 4.5 % guava leaf powder.

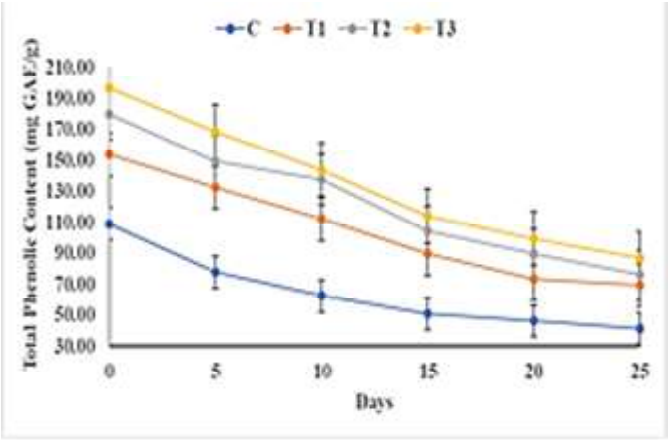

Fig. 1 Change in total phenolic content of curd balls prepared with guava leaf powder

C: Control curd balls without guava leaf powder; T1: Curd balls with 1.5 % guava leaf powder; T2: Curd balls with 3.0 % guava leaf powder; T3: Curd balls with 4.5 % guava leaf powder. n=6

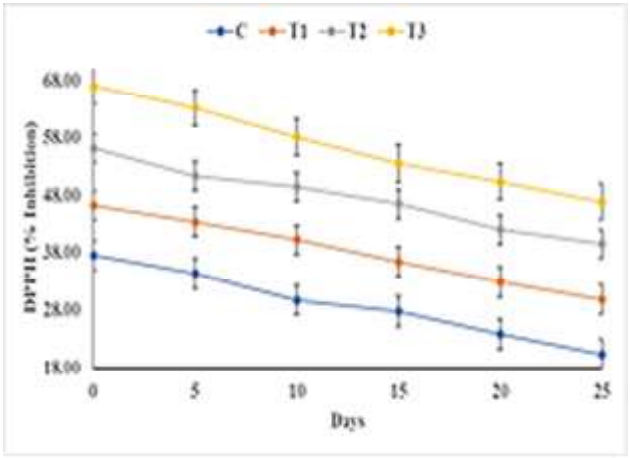

Fig. 2 Change in DPPH (% inhibition) of curd balls prepared with guava leaf powder

C: Control curd balls without guava leaf powder; T1: Curd balls with 1.5 % guava leaf powder; T2: Curd balls with 3.0 % guava leaf powder; T3: Curd balls with 4.5 % guava leaf powder. n=6

On day 0, total phenolic content exhibited by treated curd balls followed the order T3>T2>T1>C as depicted in Fig. 1. The total phenolic content of guava leaf powder added curd balls was considerably (P≤0.05) higher than that of control curd balls, 109.17 (C), 154.00 (T1), 180.00 (T2), and 196.67 (T3) mg GAE/g, respectively. The total phenolic content of the groups varied significantly (P≤0.05) across the storage days. Refrigerated curd balls prepared with inclusion of guava leaf powder and control curd balls showed significantly (P≤0.05) declining trends for total phenolic content during the storage. The decreased total phenolic content during storage of curd balls might be due to lipid oxidation and microbial degradation of phenolic compounds during storage. However, T3 showed highest total phenolic content amongst treatments at the end of storage. Phenolic content has linear association between scavenging activity. Lee et al. (2016) also reported decreased total phenolic content with increased storage time.

2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

Da Porto et al. (2000) reported that 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical activity has been widely used to assess the free

Table: 2 Change in pH and titratable acidity of curd balls incorporated with guava leaf powder during refrigeration (4±1°C) storage

Groups	0 Day	5 Days	10 Days	15 Days	20 Days	25 Days
			pH			
C	5.50^{Yf}±0.05	5.29^{Ye}±0.03	5.13^{Yd}±0.03	4.89^{Xc}±0.02	4.72^{XYb}±0.03	4.53^{Wa}±0.05
T1	5.27^{Xe}±0.03	5.11^{Xd}±0.02	4.92^{Xc}±0.03	4.85^{Xbc}±0.03	4.80^{Yb}±0.03	4.58^{WXa}±0.02
T2	5.18^{Xe}±0.02	5.01^{Wd}±0.03	4.86^{WXc}±0.02	4.67^{Wb}±0.02	4.68^{WXb}±0.04	4.59^{WXa}±0.03
T3	5.06^{We}±0.03	4.95^{Wd}±0.03	4.81^{Wc}±0.02	4.72^{Wb}±0.02	4.62^{Wab}±0.03	4.66^{Xa}±0.02
			Titratable acidity (% lactic acid)			
C	0.74^{a}±0.003	0.77^{Ya}±0.004	0.87^{Zb}±0.003	0.94^{Zc}±0.003	1.05^{Zd}±0.004	1.24^{Ye}±0.048
T1	0.74^{a}±0.002	0.76^{Xa}±0.003	0.85^{Yb}±0.003	0.92^{Yc}±0.003	1.03^{Yd}±0.002	1.11^{Xe}±0.031
T2	0.74^{a}±0.002	0.75^{Xa}±0.003	0.84^{Xb}±0.002	0.90^{Xc}±0.003	0.97^{Xd}±0.003	1.06^{WXe}±0.031
T3	0.74^{a}±0.003	0.73^{Wa}±0.004	0.82^{Wb}±0.002	0.88^{Wc}±0.004	0.93^{Wd}±0.002	0.96^{Wd}±0.029

Means values bearing small letters (a, b, c, d…..) days wise and capital letters (W, X, Y and Z) groups wise indicate differ significantly (P≤0.05) n=6; C: Control curd balls without guava leaf powder; T1: curd balls with 1.5 % guava leaf powder; T2: curd balls with 3.0 % guava leaf powder; T3: curd balls with 4.5 % guava leaf powder.

radical scavenging capacity of several compounds and has been recognized as a method for free radicals originating in lipids during oxidation. 2,2-diphenyl-1-picrylhydrazyl (DPPH) activity of curd balls prepared with inclusion of guava leaf powder are presented in Fig. 2. Among all group's DPPH value differed significantly (P≤0.05) across the all-storage days. However, the DPPH value decreased significantly (P≤0.05) for all groups during the entire storage. Guava leaf powder exhibited concentration-based DPPH radicals scavenging activity. The DPPH free radical scavenging activity of guava leaf powder might be due to their hydrogen donating capacity. Presence of higher quantity of hydroxyl groups, resulted greater ability of free radical scavenging capacity. Guava leaf is rich source of phenolic compounds, like gallic acid, ellagic acid, ferulic acid, pyrocatechol and taxifolin Chen and Yen (2007) and presence of these phyto-active compounds is primarily responsible for the antioxidant activity (Farag et al. 2020).

2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay

Perusal of Fig. 3 results revealed that guava leaf powder added groups had shown significantly (P≤0.05) higher ABTS scavenging activity as compared to control. Comparatively higher ABTS scavenging activity of treated groups might be due to presence of higher phenolics contents. All guava powder added curd balls groups showed significantly (P≤0.05) higher ABTS scavenging activity than control. However, ABTS values followed a decreasing pattern in all the samples during the storage study. Olatunde et al. (2021) reported that the antioxidant activity of guava leaf is due to presence of various phyto-active compounds

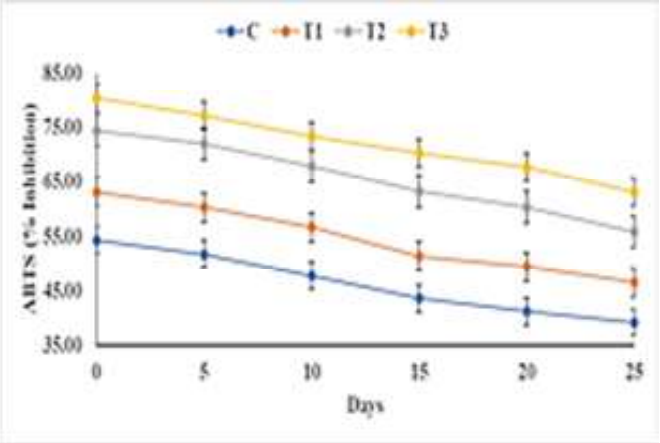

Fig. 3 Change in ABTS (% inhibition) of curd balls prepared with guava leaf powder

C: Control curd balls without guava leaf powder; T1: Curd balls with 1.5 % guava leaf powder; T2: Curd balls with 3.0 % guava leaf powder; T3: Curd balls with 4.5 % guava leaf powder. n=6

Table: 3 Effect of guava leaf powder incorporation on lipid oxidation of curd balls during refrigeration (4±1°C) storage

Groups	0 Day	5 Days	10 Days	15 Days	20 Days	25 Days
			Peroxide value (meq/kg)			
C	2.06^{Xa}±0.05	2.66^{Yb}±0.06	3.60^{Yc}±0.08	7.45^{Yd}±0.05	9.27^{Ze}±0.05	13.56^{Zf}±0.05
T1	1.95^{WXa}±0.04	2.47^{Yb}±0.05	3.19^{Xc}±0.08	6.88^{Xd}±0.08	7.68^{Ye}±0.06	10.87^{Yf}±0.06
T2	1.79^{Wa}±0.04	2.24^{Xb}±0.09	2.84^{Wc}±0.06	6.44^{Wd}±0.07	7.05^{Xe}±0.07	9.79^{Xf}±0.14
T3	1.86^{Wa}±0.08	1.91^{Wa}±0.07	2.65^{Wb}±0.05	6.27^{Wc}±0.04	6.52^{Wd}±0.06	8.90^{We}±0.06
			TBARS (mg malonaldehyde/ kg)			
C	0.17^{a}±0.01	0.40^{Zb}±0.01	0.50^{Yc}±0.01	0.63^{Zd}±0.01	0.83^{Ze}±0.02	1.14^{Yf}±0.08
T1	0.16^{a}±0.01	0.30^{Yb}±0.01	0.37^{Xc}±0.01	0.54^{Yd}±0.01	0.76^{Ye}±0.01	0.90^{Xf}±0.02
T2	0.16^{a}±0.01	0.25^{Xb}±0.01	0.33^{Wc}±0.01	0.48^{Xd}±0.01	0.55^{Xe}±0.01	0.75^{Wf}±0.01
T3	0.15^{a}±0.01	0.21^{Wb}±0.01	0.34^{WXc}±0.02	0.36^{Wc}±0.01	0.49^{Wd}±0.01	0.66^{We}±0.01
			FFA (% oleic acid)			
C	0.08^{Xa}±0.00	0.16^{Yb}±0.01	0.24^{Zc}±0.01	0.36^{Yd}±0.01	0.48^{Ze}±0.01	0.74^{Zf}±0.01
T1	0.08^{WXa}±0.00	0.13^{Xb}±0.01	0.20^{Yc}±0.01	0.30^{Xd}±0.01	0.37^{Ye}±0.01	0.58^{Yf}±0.01
T2	0.07^{Wa}±0.00	0.10^{Wb}±0.01	0.16^{Xc}±0.01	0.27^{Xd}±0.02	0.32^{Xe}±0.01	0.51^{Xf}±0.01
T3	0.07^{Wa}±0.01	0.09^{Wa}±0.00	0.13^{Wb}±0.01	0.18^{Wc}±0.01	0.26^{Wd}±0.02	0.43^{We}±0.02

Means values bearing small letters (a, b, c, d…...) days wise and capital letters (W, X, Y and Z) groups wise indicate differ significantly (P≤0.05) n=6; C: Control curd balls without guava leaf powder; T1: curd balls with 1.5 % guava leaf powder; T2: curd balls with 3.0 % guava leaf powder; T3: curd balls with 4.5 % guava leaf powder.

like piceatannol 40-galloylglucoside, quercetin 3-(23 -galloyl-alpha-Larabinopyranoside), epicatechin, 8-hydroxyluteolin 8-sulphate, and aclurin 3-C-(63 -p-hydroxybenzoyl-glucoside). In addition to these compounds Taha et al. (2019) also identified various flavonoid compounds in guava leaf such as quercetin, kaempferol, hesperetin, catchin, quercitrin, rutin and apigenin and postulated that these compounds are accountable for the antioxidant activity. The decreased ABTS values during the storage might be due to the decrease in the concentration of the phyto-active compounds during the neutralizing of the free radicals which was formed during oxidation.

Change in lipid oxidation (PV, TBARS and FFA) values

The peroxide value differed significantly (Pd≤0.05) among the groups and comparatively higher peroxide value was estimated in control than the treatment (Table 3). All the groups showed significantly (P≤0.05) increasing trends for peroxide value with progression of the storage period. The increased peroxide value during storage might be attributed primary oxidation of fat molecules and formation of hydroxy-peroxide molecules during storage. The comparatively lower peroxide value in guava leaf powder added sample was due to the presence of phyto-active compounds in guava leaf powder such as poly-phenolic compounds, triterpenoids, flavonoids, alkaloids, saponins and sesquiterpenes (Kumar et al. 2021). These phyto-active compounds have capacity to inhibit the generation of hydroperoxide reducing the formation of free radicals and/or terminating the free radicals, therefore, lower peroxide value recorded in treated groups.

Thiobarbituric acid reactive substances value varied significantly (P≤0.05) among groups throughout storage and highest value was observed for control at last day of storage (Table 3). The increased TBARS value was observed on increase in storage time for all the curd ball samples. The rate of upsurge in TBARS formation was normally lower for guava leaf treated groups in a dose-dependent manner than control. Guava plant enriched with various natural antioxidant substances such as phenolic compounds, alkaloids, chlorophyll derivatives, carotenoids, and ascorbic acid. Paganga et al. (1999) reported that antioxidant action of phenolic compounds was due to their redox activity and play significant role in sequestering and deactivating free radicals or disintegrating peroxide substances. Lower TBARS values in treated groups might be due to the presence of high antioxidant compounds of guava leaf powder.

Free fatty acid content of all samples increased during refrigerated storage (Table 3). However, the incorporation of guava leaf powder at different levels in the curd balls had a significant effect on the FFA formation with concentration dependent manner. Chen and Yen (2007) reported that the guava leaf is rich source of antioxidants and have capacity to reduce the lipid oxidation in food products. Various phyto-active compounds were also isolated by Nantitanon and Okonogi (2012) like morin, quercetin and quercetin-3-O-glucopyranoside from leaf of guava and

Table: 4 Microbiological changes in curd balls incorporated with guava leaf powder during refrigeration (4±1 °C) storage

Groups	0 Day	5 Days	10 Days	15 Days	20 Days	25 Days
			SPC count (cfu/g)			
C	2.12^{a}±0.42	3.02^{Yb}±0.11	4.11^{Xc}±0.09	5.00^{Yd}±0.06	5.34^{Yd}±0.04	6.27^{Ye}±0.07
T1	2.08^{a}±0.42	2.89^{XYb}±2.80	3.81^{Wc}±3.59	4.80^{XYd}±0.09	4.96^{Xd}±0.07	5.90^{Ye}±0.02
T2	1.70^{a}±0.54	2.76^{WXb}±0.06	3.67^{Wc}±0.08	4.73^{Xd}±0.07	4.83^{Xd}±0.08	5.71^{Xe}±0.07
T3	1.67^{a}±0.52	2.64^{Wb}±0.06	3.71^{Wc}±0.07	4.08^{Wc}±0.05	4.34^{Wc}±0.03	5.40^{Wd}±0.03
			Psychrophilic count (cfu/g)			
C	ND	ND	1.25^{a}±0.56	2.12^{b}±0.42	2.78^{Xbc}±0.09	3.28^{Xc}±0.08
T1	ND	ND	ND	1.67^{a}±0.53	2.25^{WXab}±0.46	2.74^{WXb}±0.06
T2	ND	ND	ND	1.30±0.58	1.70^{WX}±0.54	2.22^{WX}±0.45
T3	ND	ND	ND	0.83^{ab}±0.53	1.28^{Wb}±0.57	1.77^{Wb}±0.56
			Coliform count (cfu/g)			
C	ND	ND	ND	ND	0.88^{ab}±0.55	1.70^{b}±0.54
T1	ND	ND	ND	ND	0.83^{ab}±0.53	1.26^{b}±0.57
T2	ND	ND	ND	ND	ND	0.94±0.59
T3	ND	ND	ND	ND	ND	0.87±0.55
			Yeast and moulds count (cfu/g)			
C	ND	ND	ND	1.26^{a}±0.56	2.10^{b}±0.42	2.56^{b}±0.03
T1	ND	ND	ND	0.90^{ab}±0.57	1.68^{bc}±0.53	2.08^{c}±0.42
T2	ND	ND	ND	0.84^{ab}±0.53	1.27^{b}±0.57	1.72^{b}±0.54
T3	ND	ND	ND	0.87^{ab}±0.55	1.26^{ab}±0.56	1.38^{b}±0.62

Means values bearing small letters (a, b, c, d…...) days wise and capital letters (W, X, Y and Z) groups wise indicate differ significantly (P≤0.05) n=6; C: Control curd balls without guava leaf powder; T1: curd balls with 1.5 % guava leaf powder; T2: curd balls with 3.0 % guava leaf powder; T3: curd balls with 4.5 % guava leaf powder.

confirmed that quercetin is the most effective antioxidant substance among all phyto-active substances. Similar finding was also reported by Tachakittirungrod et al. (2007) that the presence of flavonoids, morin and quercetin-3-oglucopyranoside in guava leaf possessed scavenging activity.

Change in microbial quality parameters

Microbial quality (standard plate count, psychrophilic count, coliform count and yeast and mould count) of curd balls was evaluated and the data is depicted in Table 4. Among groups, during initial day of storage, data for SPC did not differ significantly (P≥0.05) and further it was found that from 5th day of storage onward the SPC count increased significantly (P≤0.05). The SPC value increased significantly (P≤0.05) throughout storage in all groups. The SPC counts was observed significantly (P≤0.05) lower in T3 than T2, T1 and control group. The antimicrobial activity of guava leaf might be due to presence of phenolic compounds in the leaves, primarily gallic acid. Similarly, Rattanachaikunsopon and Phumkhachorn (2007) reported the antibacterial activity of guava leaf. Ozcelik et al. (2008) postulated that phenolic compounds act as inhibition of nucleotides and depolarization of microbial membrane followed by inhibition of macromolecular synthesis. Ahuja et al. (2012) also found increased total plate count during the storage of paneer tikka.

The psychrophilic count was not detected up to 5th day of storage in control sample and up to 10th day of storage in treated groups (Table 4). Among the groups psychrophilic count differed significantly (P≤0.05) at 20th and 25th day of storage. Psychrophilic count increased significantly (P≤0.05) during the storage of curd balls in each group. However, comparatively lower psychrophilic count was observed in guava leaf powder added curd balls than control. Olatunde et al. (2018) reported that polyphenolic compounds, especially quercetin 32 -xyloside present in guava leaf extract was attributed the antimicrobial activity. Some other antimicrobial phyto-active compounds also present in guava leaf such as quercetin and its glycosides having an antimicrobial activity (Gorniak et al. 2019).

The coliform count was not detected up to 15th day of storage in control and T1 sample and it was not detected in T2 and T3 up to 20th days of storage (Table 4). Among the groups coliform count did not differ significantly (P≥0.05) during storage. Coliform count

Table: 5 Sensory attributes of curd balls incorporated with guava leaf during refrigeration (4±1°C) storage

Groups	0 Day	5 Days	10 Days	15 Days	20 Days	25 Days
			Colour and appearance			
C	$8.18^{e}\pm0.19$	$7.96^{We}\pm0.09$	$7.25^{Wd}\pm0.09$	$6.93^{Wc}\pm0.09$	$6.57^{Wb}\pm0.12$	$5.96^{Wa}\pm0.11$
T1	$8.36^{d}\pm0.14$	$8.04^{Wcd}\pm0.14$	$7.68^{WXc}\pm0.19$	$7.25^{WXb}\pm0.11$	$6.64^{Wa}\pm0.14$	$6.25^{WXa}\pm0.12$
T2	$8.46^{e}\pm0.14$	$8.39^{Xde}\pm0.11$	$8.14^{Ycd}\pm0.07$	$7.93^{Yc}\pm0.09$	$7.36^{Xb}\pm0.11$	$6.93^{Ya}\pm0.07$
T3	$8.39^{e}\pm0.13$	$8.11^{WXde}\pm0.07$	$7.86^{Xd}\pm0.09$	$7.36^{Xc}\pm0.18$	$6.86^{Wb}\pm0.07$	$6.46^{Xa}\pm0.16$
			Taste			
C	$8.11^{e}\pm0.07$	$7.96^{We}\pm0.09$	$7.21^{Wd}\pm0.09$	$6.89^{Wc}\pm0.12$	$6.57^{Wb}\pm0.12$	$5.93^{Wa}\pm0.13$
T1	$8.36^{e}\pm0.14$	$7.89^{Wd}\pm0.13$	$7.64^{WXd}\pm0.18$	$7.21^{WXc}\pm0.10$	$6.64^{Wb}\pm0.14$	$6.14^{WXa}\pm0.09$
T2	$8.46^{d}\pm0.14$	$8.39^{Xd}\pm0.11$	$8.11^{Yc}\pm0.07$	$8.04^{Yc}\pm0.07$	$7.43^{Xb}\pm0.09$	$6.90^{Ya}\pm0.07$
T3	$8.39^{d}\pm0.13$	$8.00^{Wcd}\pm0.12$	$7.86^{Xc}\pm0.09$	$7.32^{Xb}\pm0.18$	$6.79^{Wa}\pm0.14$	$6.46^{Xa}\pm0.16$
			Aroma			
C	$8.18^{d}\pm0.09$	$7.96^{Wd}\pm0.09$	$7.14^{Wc}\pm0.07$	$6.86^{Wc}\pm0.11$	$6.54^{Wb}\pm0.11$	$5.86^{Wa}\pm0.14$
T1	$8.39^{e}\pm0.13$	$8.00^{Wde}\pm0.15$	$7.68^{Xd}\pm0.19$	$7.25^{Xc}\pm0.11$	$6.71^{Wb}\pm0.10$	$6.18^{WXa}\pm0.12$
T2	$8.46^{d}\pm0.14$	$8.39^{Xd}\pm0.11$	$8.11^{Yc}\pm0.07$	$8.04^{Yc}\pm0.06$	$7.43^{Xb}\pm0.09$	$6.86^{Ya}\pm0.09$
T3	$8.36^{d}\pm0.14$	$8.04^{WXcd}\pm0.14$	$7.75^{Xc}\pm0.16$	$7.25^{Xb}\pm0.14$	$6.75^{Wa}\pm0.14$	$6.43^{Xa}\pm0.18$
			Texture			
C	$8.29^{f}\pm0.09$	$7.86^{We}\pm0.13$	$7.21^{Wd}\pm0.10$	$6.86^{Wc}\pm0.07$	$6.39^{Wb}\pm0.11$	$6.00^{Wa}\pm0.09$
T1	$8.11^{d}\pm0.13$	$7.89^{Wcd}\pm0.13$	$7.57^{Xc}\pm0.16$	$7.14^{Xb}\pm0.09$	$6.71^{Wa}\pm0.18$	$6.32^{WXa}\pm0.12$
T2	$8.39^{e}\pm0.11$	$8.54^{Xe}\pm0.04$	$8.14^{Yd}\pm0.07$	$7.79^{Yc}\pm0.11$	$7.29^{Xb}\pm0.09$	$6.82^{Ya}\pm0.07$
T3	$8.25^{d}\pm0.09$	$7.89^{Wcd}\pm0.16$	$7.71^{Xc}\pm0.16$	$7.18^{Xb}\pm0.09$	$6.71^{Wa}\pm0.15$	$6.39^{Xa}\pm0.15$
			Overall acceptability			
C	$8.11^{d}\pm0.07$	$7.82^{Wd}\pm0.12$	$7.11^{Wc}\pm0.07$	$6.75^{Wb}\pm0.13$	$6.50^{Wb}\pm0.11$	$5.82^{Wa}\pm0.09$
T1	$8.14^{e}\pm0.13$	$7.93^{WXe}\pm0.13$	$7.54^{Xd}\pm0.14$	$7.11^{Xc}\pm0.07$	$6.71^{Wb}\pm0.15$	$6.21^{Xa}\pm0.10$
T2	$8.43^{e}\pm0.12$	$8.29^{Xde}\pm0.10$	$8.07^{Yd}\pm0.05$	$7.64^{Yc}\pm0.13$	$7.25^{Xb}\pm0.09$	$6.75^{Ya}\pm0.12$
T3	$8.25^{e}\pm0.08$	$8.00^{WXde}\pm0.14$	$7.79^{Xd}\pm0.09$	$7.25^{Xc}\pm0.12$	$6.82^{Wb}\pm0.09$	$6.43^{XYa}\pm0.13$

Means values bearing small letters (a, b, c, d…..) days wise and capital letters (W, X, Y and Z) groups wise indicate differ significantly (P≤0.05) n=21; C: Control curd balls without guava leaf powder; T1: curd balls with 1.5 % guava leaf powder; T2: curd balls with 3.0 % guava leaf powder; T3: curd balls with 4.5 % guava leaf powder.

increased during storage of curd balls from 20th to 25th days in control and T1 but the value did not differ significantly (P≥0.05). Phyto-active compounds present in the guava leaf powder was attributed to lower count of coliform in treated curd balls than control. Chanda and Kaneria (2011) reported that extract of guava leaf exhibited antimicrobial activity against *E. coli* and other microbes.

The yeast and mould count were absent up to 10th days of storage among all the groups (Table 4). Among the groups yeast and mould count did not differ significantly (P≥0.05) throughout storage. Yeast and mould count were increased in curd ball groups from 15th day of storage onwards though the rate of their increase did not differ significantly (P≥0.05). Treated curd balls showed lower yeast and mould count than control which might be due to antifungal activity of guava leaf powder. Morais-Braga et al. (2017) reported that guava leaf extracts exhibited antifungal activity against *C. albicans* and *C. tropicalis* by significant reduction in percentage of viability of yeast and mould during their study. Beatriz et al. (2012) stated in their study that guava leaf showed antifungal activity against various fungi.

Change in sensory attributes

All sensory attributes among the groups did not differ significantly (P≥0.05) at 0 day of storage and varied significantly (P≤0.05) from 5th day to 25th day of storage (Table 5). Among all samples, T2 sample was rated highest score than control, T3 and T1. The sensory score was lower for T3 group than T2 which might be due to higher level of guava leaf powder and formation of oxidative compounds during storage which could be responsible for bitter taste. Sensory scores for colour and appearance, taste, aroma, texture and overall acceptability exhibited decreasing trend during the storage. The decreased taste, aroma and overall acceptability scores of sensory attributes might be due to oxidation of lipid and formation of volatile free fatty acid content in the aforementioned curd ball samples on day 25th than initial day of storage. Kumar et al. (2019) also reported declined trend of sensory parameters during the storage of milk smoothies prepared with the addition of tulsi, lemon grass and aloe vera. The production of lactic acid due to growth of lactic acid producing as well as non-starter lactic acid bacteria during the storage of curd balls producing repulsive odour in curd balls resulted decrease in overall acceptability score by the evaluators. Oxidation of lipid and fat molecule of curd balls further attributed to decreased textural quality of the products. Ahuja et al. (2012) also reported decreased sensory score during the storage of paneer tikka.

Conclusions

The results revealed that curd ball prepared with addition of guava leaf powder T3 (4.5%) were recorded significantly lower lipid oxidation, microbial growth however, sensory panelist rated comparatively lower sensory score for T3 than control (0 %), T1 (1.5 %) and T2 (3.0 %) during refrigerated storage. Also, treated curd balls with GLP were significantly higher in antioxidant profiles (Total phenolics content, DPPH % inhibition and ABTS % inhibition) than control, therefore guava leaf powder could be utilized as potential natural antioxidant. Results concluded that the developed curd balls incorporated with 3.0 % guava leaf powder could be successfully stored under aerobic packaging for 25 days at 4±1°C with an acceptable physico-chemical, antioxidant parameters, lipid oxidation microbiological quality and sensory attributes.

Conflict of interest: None

References

Abdel-Hameed ESS, Nagaty MA, Salman MS, Bazaid SA (2014) Phytochemicals, nutritionals and antioxidant properties of two prickly pear cactus cultivars (*Opuntia ficus indica Mill.*) growing in Taif, KSA. Food Chem 160: 31-38. doi:10.1016/j.foodchem.2014.03.060.

Ahuja KK, Goyal GK (2013) Combined effect of vacuum packaging and refrigerated storage on the chemical quality of paneer tikka. J Food Sci Technol 50(3): 620-623. doi: 10.1007/s13197-012-0688-x.

Ahuja KK, Goyal S, Goyal GK (2012) Shelf life prediction of paneer tikka by artificial neural networks. Cientific. J Agri 1(6): 145-149

APHA (1992) Microbiological methods for dairy products. In Standard methods for examination of dairy products.16th edition. Marshall, RT. (ed.). *American public health association*, *Washington,* DC 287-307

Beatriz PM, Ezequiel VV, Pilar CR (2012) Antifungal activity of Psidium guajava organic extracts against dermatophytic fungi. J Med Plant Res 6(41): 5435-5438. doi: 10.5897/JMPR12.240.

Biswas B, Rogers K, McLaughlin F, Daniels D, Yadav A (2013) Antimicrobial activities of leaf extracts of guava (*Psidium guajava L.*) on two gram-negative and gram-positive bacteria. Int J Microbiol Article ID 746165. doi.org/10. 1155/2013/746165.

Brand-Williams W, Cuvelier ME, Berset, C (1995) Use of a free radical method to evaluate antioxidant activity. LWT- Food Sci Technol 28(1): 25–30. doi.org/10.1016/S0023-6438(95)80008-5.

Chanda S, Kaneria M (2011) Indian nutraceutical plant leaves as a potential source of natural antimicrobial agents. Sci. Against Microbial. pathogens: Science Against Microbial Pathogens: Communicating Current Research and Technological Advances. 2: 1251-1259. doi.org/10.1016/S2221-6189(13)60143-2.

Chen HY, Yen, GC (2007) Antioxidant activity and free radical-scavenging capacity of extracts from guava (*Psidium guajava L.*) leaf. Food Chem 101(2): 686-694. doi.org/10.1016/j.foodchem.2006.02.047.

Da Porto C, Calligaris S, Celotti E, Nicoli, MC (2000) Antiradical properties of commercial cognacs assessed by the DPPH test. J Agri Food Chem 48(9): 4241-4245. doi: 10.1021/jf000167b.

Farag RS, Abdel-Latif MS, Abd El Baky HH, Tawfeek LS (2020) Phytochemical screening and antioxidant activity of some medicinal plants' crude juices. Biotechnol Rep 28: e00536. doi.org/10.1016/j.btre.2020.e00536.

Gorniak I, Bartoszewski R, Kroliczewsk J (2019) Comprehensive review of antimicrobial activities of plant flavonoids. Phytochem Rev 18(1): 241-272. doi.org/10.1007/s11101-018-9591-z.

Koniecko R (1979) Handbook for Meat Chemists. *Wayne, NJ: Avery Publishing Group Inc., Wayne, New Jersey*, USA 68-69

Kumar B, Singh VP, Pathak V, Verma AK (2019) Shelf-life assessment of natural antioxidant-treated milk smoothies stored under refrigeration at 4±2° C. Nutr Food Sci 49(6): 1000-1013. doi.org/10.1108/NFS-10-2018-0291.

Kumar M, Tomar M, Amarowicz R, Saurabh V, Nair MS, Maheshwari C, Sasi M, Prajapati U, Hasan M, Singh S, Changan S (2021) Guava (*Psidium guajava*) leaf: Nutritional composition, phytochemical profile, and health-promoting bioactivities. Foods 10(4): 752. doi: 10.3390/foods10040752.

Lee NK, Jeewanthi RKC, Park EH, Paik HD (2016) Physicochemical and antioxidant properties of Cheddar-type cheese fortified with *Inula britannica* extract. J Dairy Sci 99(1): 83-88. doi.org/10.3168/jds.2015-9935.

Morais-Braga MF, Carneiro JN, Machado AJ, Sales DL, Dos Santos AT, Boligon AA, Athayde ML, Menezes IR, Souza DS, Costa JG, Coutinho HD (2017) Phenolic composition and medicinal usage of Psidium guajava Linn.: Antifungal activity or inhibition of virulence?. Saudi J Biol Sci 24(2): 302-313. doi.org/10.1016/j.sjbs.2015 .09.028.

Najgebauer-Lejko D, Sady M, Grega T, Walczycka M (2011) The impact of tea supplementation on microflora, pH and antioxidant capacity of yoghurt. Int Dairy J 21(8): 568-574. doi.org/10.1016/j.idairyj.2011.03.003.

Nantitanon W, Okonogi S (2012) Comparison of antioxidant activity of compounds isolated from guava leaf and a stability study of the most active compound. *Drug Discov Ther* 6(1): 38-43. doi: 10.5582/ddt.2012.v6.1.38.

Olatunde OO, Benjakul S, Vongkamjan K (2018) Antioxidant and antibacterial properties of guava leaf extracts as affected by solvents used for prior dechlorophyllization. J Food Biochem 42(5): e12600. doi.org/10.1111/jfbc.12600.

Olatunde OO, Della Tan SL, Shiekh KA, Benjakul S, Nirmal NP (2021) Ethanolic guava leaf extracts with different chlorophyll removal processes: Anti-melanosis, antibacterial properties and the impact on qualities of Pacific white shrimp during refrigerated storage. Food Chem 341: 128251. doi.org/10.1016/j.foodchem.2020.128251.

Ozcelik Berrin, Orhan DD, Ozgen S, Ergun F (2008) Antimicrobial activity of flavonoids against extended-spectrum â-lactamase (ESâL)-producing Klebsiella pneumoniae. Trop J Pharm Res 7(4): 1151-1157. doi**:** 10.4314/tjpr.v7i4.14701**.**

Paganga G, Miller N, Rice-Evans CA (1999) The polyphenolic content of fruit and vegetables and their antioxidant activities. What does a serving constitute?. Free Radic Res 30(2): 153-162. doi: 10.1080/10715769900300161.

Qureshi TM, Amjad A, Nadeem M, Murtaza MA, Munir M (2019) Antioxidant potential of a soft cheese (paneer) supplemented with the extracts of date (*Phoenix dactylifera L.*) cultivars and its whey. Asian-Australas J Anim Sci 32(10): 1591-1602. doi: 10.5713/ajas.18.0750.

Rattanachaikunsopon P, Phumkhachorn P (2007) Bacteriostatic effect of flavonoids isolated from leaf of Psidium guajava on fish pathogens. Fitoterapia 78(6): 434-436. doi: 10.1016/j.fitote.2007.03.015.

Shelef LA, Jay JM (1970) Use of a titrimetric method to assess the bacterial spoilage of fresh beef. Appl Microbiol 19(6): 902-905. doi: 10.1128/am.19.6.902-905.1970.

Tachakittirungrod S, Ikegami F, Okonogi S (2007) Antioxidant active principles isolated from Psidium guajava grown in Thailand. Sci Pharm 75(4): 179–193. doi.org/10.3797/scipharm.2007.75.179.

Taha TF, Elakkad HA, Gendy AS, Abdelkader MA, Hussein SE (2019) In vitro bio-medical studies on Psidium guajava leaf. Plant Arch19(1): 199-207

Umaraw P, Verma AK, Singh, VP, Fahim A (2022) Effect of Turmeric and Aloe Vera Extract on Shelf-Life of Goat and Buffalo Admixture Milk Paneer during Refrigeration Storage. Foods, 11(23): 3870. doi.org/10.3390/foods1123387.

Witte VC, Krause GF, Bailey ME (1970) A new extraction method for determining 2-Thiobarbituric acid values of pork beef during storage. J Food Sci 35(5): 582–585. doi.org/10.1111/j.1365-2621.1970.tb04815.x.

Zhang Q, Zhang J, Shen J, Silva A, Dennis DA, Barrow CJ (2006) A simple 96-well microplate method for estimation of total polyphenol content in seaweeds. Journal Appl Phycol 18(3): 445-450. doi.org/10.1007/s10811-006-9048-4.