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Effect of Guava Leaves Powder on the Oxidative and Microbial Stability of Chicken Meat Nuggets during Refrigerated (4±1°C) Storage

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ABSTRACT

The present study was carried out to evaluate the efficacy of different levels (0.5 and 1 per cent) of guava leaf powder (GLP) (*Psidium guajava* L) with synthetic antioxidant ie) butylated hydroxy toluene (BHT) at 0.01 % was studied on physico-chemical, microbial and sensory quality characteristics of chicken meat nuggets under aerobic packaging conditions during refrigerated storage (4±1°C). The chicken nuggets added with 1 % GLP had significantly ($P < 0.05$) lower 2-thiobarbituric acid reactive substance (TBARS) values and free fatty acid (FFA) content compared to control nuggets and other samples during refrigerated storage (4±1°C). A significant ($P<0.05$) lower total plate count and total psychrophilic count were recorded in chicken nuggets added with GLP. Addition of 1 % GLP significantly ($P<0.05$) reduced the coliform count and yeast and mould counts than control and BHT added chicken nuggets. 1 per cent GLP added chicken meat nuggets rated significantly ($P<0.05$) superior scores of colour, flavor, tenderness, juiciness and overall acceptability than control, BHT and 0.5 % GLP added chicken meat nuggets. Based on the obtained results, it was concluded that 1 % GLP had excellent antioxidant and antimicrobial properties compared to control and BHT added chicken meat nuggets during refrigerated storage under aerobic conditions and application of GLP as natural antioxidant and anti microbial agent in chicken meat nuggets which extends shelf life up to 20 days in refrigerated storage without any quality deterioration.

Keywords: Guava leaf powder, antioxidants, antimicrobial agents, chicken meat nuggets, storage stability.

INTRODUCTION

Chicken meat has desirable nutritional characteristics, as it has low lipid levels and is rich in polyunsaturated fatty acids (Cagdas and Kumcuoglu, 2015). However, chicken meat is susceptible to oxidative damage of lipids and proteins, which are the main causes of reduced shelf life and nutritional value in meat products (Estevez et al. 2019). Lipid oxidation leads to the generation of a wide range of degradation products that may be responsible for undesirable odors and flavors in meat and meat products (Andres et al. 2017). These compounds can promote the onset of protein oxidation, during processing and storage, which leads to the loss of nutrients, such as essential amino acids (degradation of tryptophan, histidine, methionine and cysteine), resulting in decreased protein digestibility, color and texture degradation, and formation of potentially toxic compounds (Ferreira et al. 2018). The use of synthetic antioxidants is one of the main strategies of the food industry to prevent the oxidation of lipids and proteins. However, the food industry has not measured efforts to reduce or replace the use of artificial additives in the preparation of processed products, given recent studies relating to the negative health effects of continuous consumption of synthetic compounds (Jiang and Xiong, 2016). In this segment, the meat industry has invested in the search for natural ingredients that minimize oxidation reactions in meat products, thus increasing their shelf life. In addition, the concern about sustainable and environmentally-friendly production is forcing the meat industry to continuously evolve. In this sense, the search for phenolic antioxidants from natural sources has received much attention. Now a days, consumer preferences for "natural" products have resulted in increased interest in the use of natural antioxidants including rosemary, sage, aloe vera, mustard, tea catechins, whey protein concentrate, and cottonseed meals. Several types of natural plant derived antioxidants have been studied in various raw and cooked meat systems, including rosemary extract, sage, thyme, rice bran, white peony, red peony, sappanwood, moutan peony, rehmania or angelica, sedge, marjoram, wild marjoram, caraway, basil extract, ginger, plum concentrates, aloe vera, mustard, and tea catechins (Bhaskar Reddy et al. 2013).

Guava (*Psidium guajava* L.) stands as a year-round fruit tree with antioxidant-rich fruits and leaves (Chiari-Andreo et al. 2017). Its fruit, leaves, and seeds have garnered attention due to their substantial antioxidant potential (Gaber et al. 2023) attributed to their robust polyphenolic and flavonoid contents (Garcia-villegas et al. 2022). Guava boasts a repertoire of food and nutritional values along with potent bioactive properties, including anti-oxidative (Chiari-Andreo et al. 2017), anti-diabetic (Shabbir et al. 2020), anti-microbial, anti-inflammatory, anti-cancer, anti-diarrheal, anti-mutagenic, hepato-protective, anti-hemolytic anti-malarial, anti-tussive, anti-genotoxic, wound healing and cardio vascular protective effects (Delorino et al. 2020).

The studies conducted by researchers showed that guava leaves are a great source for phyto-chemicals with antioxidant properties (Dewage et al. 2023). Growther and Sukirtha (2018) study showed that both guava leaf methanol extract and bark ethanol extract have significant antimicrobial activity against against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Shigella dysenteriae*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans* using the agar well diffusion method. According to Dewage et al. (2023) guava peel and pulp methanolic extracts displayed enhanced antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. Notably, *Pseudomonas aeruginosa* exhibited heightened susceptibility to the antibacterial effects of guava pulp extract. By understanding the potential anti oxidant and anti microbial benefits of guava leaf powder, an investigation was carried out to determine the anti oxidant and anti microbial efficacy of guava seed powder on storage stability of chicken meat nuggets during refrigerated storage (4±1°C) comparing with synthetic antioxidant i.e) BHT.

MATERIALS AND METHODS

Preparation and anti oxidant components determination of guava leaves powder

Guava (*Psidium guajava* L. variety "*Anakapalle*") leaves procured and all the immature and fully mature leaves were discarded. Selected leaves were examined uniformity and then thoroughly washed and air dried until the weight of the leaves became constant (moisture content 7-9 %) and ground through heavy duty grinder (Model: Turquoise, Butterfly, 1000 W Mixer Grinder, India) and powder sieved. The guava leaves powder was prepared in triplicate and all analysis was carried out in duplicates for nutritive composition were estimated by AOAC (2002) and aqueous extracts of guava leaves powder was determined for total phenolic contents (TPC) by Singleton et al. (1999), total flavonoids contents (Kim et al. 2003) and 1, 1 diphenyl-2-picrylhydrazil (DPPH) radicals scavenging activity by Singh et al. (2002).

Processing of functional chicken meat nuggets

The fresh broiler boneless chicken meat cut into small chunks and minced in a meat mincer (Sirman, TC 12 E, Italy) through 6 mm plate. The emulsion was prepared by chopping the minced meat along with other non-meat ingredients in a bowl chopper (Scharfen, Model No: TC 11, Germany). The minced chicken meat was mixed with salt @1.8 %, STPP @0.4 %, sodium nitrate @150 ppm, sodium ascorbate @ 0.5 %, sugar @ 1%, corn flour @ 4 %, and ice flakes @ 8 %, and chopped for one min followed by addition of oil @8 % and again chopped for one min and added spice mix @ 2 %, condiment mix (onion and garlic: 3:1) @ 4 %, and finally chopped for 3 min. The temperature of the emulsion was maintained between 12 to 15°C. The obtained emulsion was divided into four equal parts and one part is considered as control, emulsion was added with butylated hydroxyl toluene (BHT) @ 0.01 was treated as T1, emulsion was added with GLP @ 0.5 % was treated as T2 and emulsion was added with GLP @ 1 % was treated as T3. The emulsion was separately filled in a rectangular steel mould and steam cooked for 40 minutes to an internal temperature of 75±2°C as indicated by the temperature probe. The meat blocks were immediately chilled and sliced into nuggets of uniform size i.e., 4 x 1.5 x 1.5 cm and nuggets were packed separately in LDPE film by aerobic packaging and stored at 4 ± 1 °C and evaluated every 5 days interval up to 20 days. At every 5 interval, the nuggets were subjected for various quality attributes like physico-chemical characteristics, microbial counts and sensory characteristics.

Physico-chemical characteristics

pH: The pH of chicken meat nuggets were determined by homogenizing 10 g of sample with 50 ml distilled water with the help of tissue homogenizer (Daihan Scientifics, WiseMix, HG-15D, Korea) for 1 min. The pH was recorded using micro controlled based pH system with electrode (Model: 361, Systronics, India).

2-TBARS value: 2-Thiobarbuteric acid reactive substance (2-TBARS) value was determined based on the procedure of Witte et al. (1970). Trichloroacetic acid (TCA) extract of the chicken meat nuggets was prepared by homogenizing 4 g of sample with 20 ml of pre cooled 20% TCA solution for 2 min in an ultra turrex homogenizer. The contents were allowed to extract for 10 min and then centrifuged at 3000 g (CPR-24, Remi Instruments, Mumbai, India) for 10 min. 3 ml of supernatant was mixed with an equal volume of 0.1% TBA reagent. The mixture was boiled in water bath for 30 min, cooled and the absorbance measured at 532 nm using a UV–VIS spectrophotometer (model: UV-1700 Pharma Spec, SHIMADZU, Japan) and the 2-TBARS values were calculated using a TBA standard and expressed in mg malonaldehyde/kg. For the blank, the same procedure was followed except that 3 ml of 20 % chilled TCA solution was added instead of TCA extract.

Free fatty acid (%): Free fatty acids percent was determined according to Koniecko (1979). Exactly 5 g of chicken meat nuggets was blended for 2 min with 30 ml of chloroform in the presence of about 5 g anhydrous sodium sulfate. Then it was filtered through Whatman no. 1 filter paper into a 150 ml conical flask. 2-3 drops of 0.2 % phenolphthalein indicator was added to the chloroform extract and titrated against 0.1 N alcoholic potassium hydroxide till a pale pink color was obtained. The quantity of potassium hydroxide consumed during the titration was recorded. Free fatty acid content was calculated and expressed as a percentage as follows.
Free fatty acids (% oleic acid): 0.1 × ml of 0.1 N alcoholic KOH × 0.282/weight of sample (g) × 100.

Microbiological analysis: Bacterial counts were determined by the pour plate method as per (ICMSF, 1983). Chicken meat nuggets samples (10 g) were homogenized with 90 ml, 0.1% sterile peptone water. Serial 10-fold dilutions were prepared by diluting 1 ml of homogenate in 9 ml of 0.1% peptone water. Appropriate serial dilutions were duplicate plated with plate count agar for total plate counts and total psychotropic (PPC) and incubated the plates at 37 °C for 48 h and 7 °C for 10 days respectively. Duplicate plates with violet red bile agar (VRBA) for coliform counts were used and incubated at 37 °C for 48 h for coliforms. For enumeration of yeast and mold counts, about 20 ml of Potato Dextrose agar (M096) melted and maintained at 44–46 °C was poured gently. The plates were incubated at 25 °C for 7 days. The colonies with black, white, yellow, red or greenish black colors were counted and expressed as log CFU/g.

Sensory characteristics: The chicken meat nuggets were warmed and served to trained panelists and evaluated for sensory characteristics like appearance, flavour, juiciness, tenderness and overall acceptability using a 8-point hedonic scale (where, 8=extremely desirable, 1=extremely undesirable) as described by Keeton (1983).

Statistical analysis

The experiments were repeated four times and each time duplicate samples were analyzed and the data was analyzed by using General Linear Model procedure of statistical package for social sciences (SPSS) 22 version.

RESULTS AND DISCUSSION

Nutritive composition and anti oxidant components determination of guava leaves powder (GLP)

The nutritive composition and anti-oxidant components of the guava leaves powder (GLP) is presented in Table 1. The per cent mean±S.E values of moisture content in guava leaves powder was 4.19±0.49. These findings are in agreement with Pawar et al. (2024) who found that moisture content in guava leaves powder was 2.77 g/100 g. It had found good amount of protein per cent in guava leaves powder i.e. 14.63. It can be preferred as valuable source of protein compared to other plant originated phyto foods. These values were comparable with the value 22.98 g reported by Kumar et al. (2021) in guava leaves. Fat content per cent in guava leaves powder was 4.96. It was low in amount as compare to other nutritive contents. It can be used as supplement for weight loss included as low fat diet. These results are in confirmation with Pawar et al. (2024) in guava leaves powder. The present study found that total ash per cent content in guava leaves powder was 17.93. It shows that guava leaves are rich in mineral content. These values were comparable with the value of Gurusamy et al., (2020) i.e. 10.15 % and 9.92 gm/100 g ash in guava leaves.
Guava leaves powder had fair amount of fibre i.e. 11.35 per cent. It can be considered as good ingredient of fibre in diet. This fibre value was comparable with the value of Pawar et al. (2024) who reported in guava leaves powder. Verma et al. (2013) reported that Guava powder is rich in dietary fibre content and contained 43.21% total dietary fibre which is mostly contributed by insoluble dietary fibre (98.4%). The dietary fibre content in the present study is in agreement with the findings of Jimenez-Escrig et al. (2001) who reported insoluble, soluble and total dietary fibre content in dried guava as 46.72 to 47.65%, 1.77 to 1.83% and 48.55 to 49.42%,

respectively. According to them peel and pulp of *Psidium guajava* fruit has high levels of dietary fibre, indigestible fraction, and phenolic compounds. Nahar et al. (1990) found a similar relative value for insoluble dietary fibre (91 % of total dietary fibre) in the edible portion of *Psidium guajava.* The minor fraction in the samples was soluble dietary fibre (3 to 4% of total dietary fibre). Guava leaves powder had abundant amount of carbohydrate i.e. 20.44 per cent. It shows that guava leaves powder is a good source of carbohydrate and these results are in agreement with Pawar et al. (2024) in guava leaves powder.

Table 1: Mean ± S.E of nutrient content and anti oxidants components composition in guava leaves powder (n=6).

S.No	Nutritive and anti-oxidant components composition	Quantity (%)
1.	Moisture	4.19±0.49
2.	Crude protein	14.63±1.25
3.	Crude fat	4.96±0.37
4.	Total ash	17.93±2.68
5.	Crude fibre	11.35±1.75
6.	Total carbohydrate	20.44±0.25
7.	Total phenolic content (mg GAE/g)	48.34±1.44
8.	Total flavonoids content (mg CE/Kg)	3.58±0.38

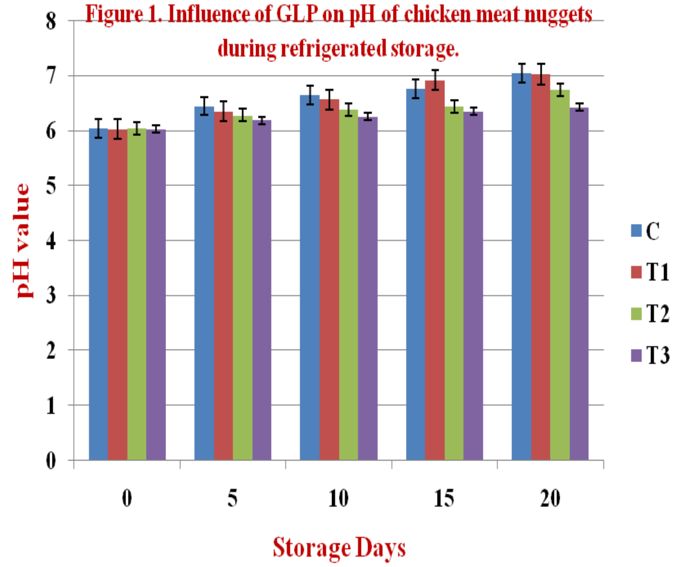
Guava leaves powder (GLP) which has a unique collection of phytochemicals, has attracted attention as a potent anti-oxidant and anti microbial components to be used commercially in many applications. The present study found that total phenolics content of GLP was 48.34 mg GAE/g and total flavonoids content was 3.58 mg CE/Kg which indicates strong anti oxidant activity of GLP. Determination of total phenolics is one of the important parameters to estimate the amount of antioxidants. Guava powder is rich in phenolic compounds which act as natural antioxidants. Similar to these results Verma et al. (2013) found Total phenolics in guava powder were found to be 44.04 mg GAE per gram. The total phenolics (on fresh mass basis) was 344.9 mg GAE/100 g in 'Allahabad Safeda' and ranged from 170.0 to 300.8 mg GAE/100 g in the pink pulp clones (Thaipong et al. 2006). According to Correa et al. (2011) total phenolics, expressed as equivalent of gallic acid (GAE), varied from 158 to 447 mg GAE/100 g in guava powder. DPPH radical scavenging activity of GLP is depicted in Figure 1. Aqueous GLP extract showed concentration based scavenging activity. As the concentration of aqueous extract of GLP is increasing from 0 to 200 µl, the DPPH radical scavenging activity (%) increased from 0 to 83.11. A study by Shabbir et al. (2020) revealed that the highest DPPH scavenging activity was found in guava pulp methanolic extract than in guava leaf and seed methanolic extracts and suggested that because guava pulp contains Vitamin C, it may be the reason for the higher DPPH scavenging activity. According to the study conducted by Yousaf et al. (2020), the antioxidant activity of guava fruits varies in different indigenous guava cultivars81. Research conducted by Liu et al. (2018) found that compared to guava fruit and seed ethanol aqueous extracts, guava peel extract shows significant antioxidant potential compared to other samples in both DPPH, ABTS+, and FRAP assays.

Physico-chemical characteristics

pH

Addition of different levels (0.5 and 1.0 %) of GLP significantly ($P<0.05$) influenced the overall mean pH values of the aerobic (Figure 1) packaged chicken meat nuggets during refrigerated storage. Chicken meat nuggets added with 1 % GLP significantly ($P<0.05$) lower pH during entire storage period. Similar to these findings, Verma et al. (2013) reported that the pH values of emulsion and sheep meat nuggets with guava powder were significantly lower ($P<0.05$) than control nuggets. The lower pH values of chicken meat nuggets during storage could be attributed to the added guava leaves powder which is a good source of ascorbic acid. Guava (*Psidium guajava* L.) fruit contains a high level of ascorbic acid (50 to 300 mg/100 g fresh weight), which is three to six times higher than oranges (Mercadante et al. 1999). However, storage period significantly ($P<0.05$) influenced the pH of the chicken meat nuggets at 4 °C. As storage period progressed, significant ($P< 0.05$) increases in pH were noticed, from 6.04 (0 day) to 7.05 (20 days) in control nuggets and 6.03 (0 day) to 6.43 (20 days) in T3 nuggets. This increase in pH during storage could be due to protein breakdown and liberation of protein metabolites, mainly amines due to bacterial activity during storage. In addition, polyphosphates in the curing solution may also cause increased pH during storage. A similar increase in pH during storage was also reported in restructured mutton slices (Bhaskar Reddy et al. 2013).

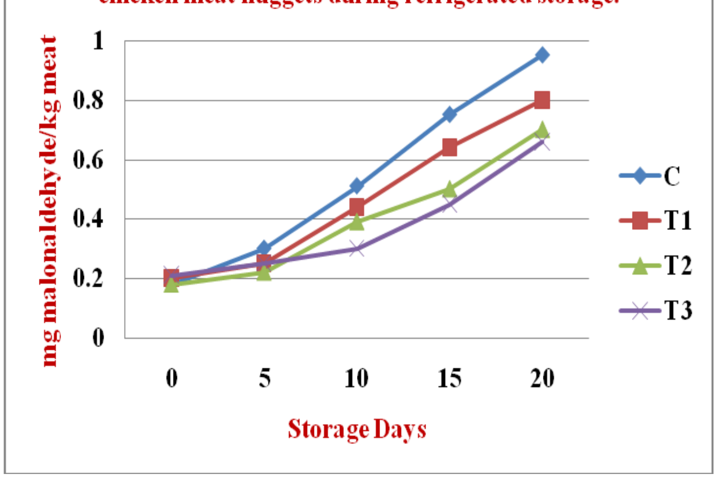
Figure 1. Influence of GLP on pH of chicken meat nuggets during refrigerated storage.



TBARS value

Guava leaves powder (GLP) and storage period significantly ($P < 0.05$) affected the 2-TBARS values of chicken meat nuggets packaged in aerobic (Figure 2) system during refrigerated storage. Chicken meat nuggets added with 1 % GLP showed lower 2-TBARS values compared to controls, BHT and 0.5 % added GLP. This might be due to the potential anti-oxidative property of GLP. The GSE used in this experiment contained total phenolics content of GLP was 48.34 mg GAE/g and total flavonoids content was 3.58 mg CE/kg which indicates strong anti oxidant activity of GLP and also potent free radical scavengers that reduce free radical concentration and block their propagation. Furthermore, total phenolics and total flavonoids are direct scavengers of reactive oxygen species and have the ability to chelate metals such as iron with their o-diphenol groups (Kumar et al. 2021). The storage period significantly ($P<0.05$) increased the TBARS values, from 0.18 mg malonaldehyde/kg meat (0 day) to 0.95 mg malonaldehyde/kg (20 days) in control nuggets and 0.21 mg malonaldehyde/kg (0 day) to 0.66 mg malonaldehyde/kg (20 days) in chicken nuggets added with 1 % GLP (T3). This could be due to the fact that as storage progresses lipid oxidation increased with the production of more secondary products of lipid oxidation formed from the decomposition of oxidized lipid molecules. These results are in agreement with Mahapatra et al. (2019) who reported that addition of guava powder at 1 %, 1.5 % and 2.0 % to the meatball mixture resulted in reduction in TBA values by 8.23 %, 13.36 % and 21.43 %, when noted on 21st day of refrigerated storage.

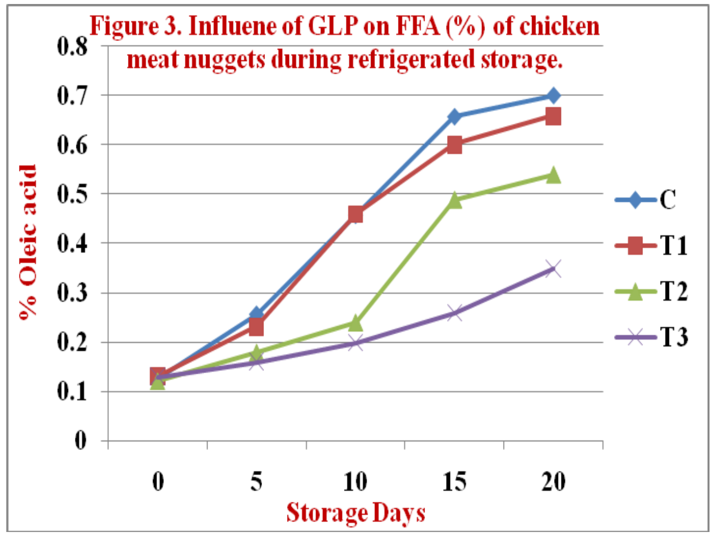
Figure 2. Influence of GLP on 2-TBARS value of chicken meat nuggets during refrigerated storage.



FFA per cent value

Free fatty acid (FFA) % values were significantly ($P<0.05$) influenced by addition of different levels of GLP and storage temperature during storage of chicken meat nuggets (Figure 3). The chicken meat nuggets added with 1.0 % GLP significantly ($P<0.05$) lower FFA % than control and BHT added nuggets. This might be due to the antimicrobial activity of GLP which causes reduction in microbial growth and subsequent microbial lipolytic activity and generation of free fatty acids. GLP owing to the presence of different organic and inorganic antioxidants and anti-inflammatory compounds, are known to possess antimicrobial properties (Naseer et al. 2018). GLP essential oils display strong antimicrobial properties against P*seudomonas aeruginosa, Escherichia coli, Streptococcus faecalis, Staphylococcus aureus,* and *Bacillus subtilis* (Soliman et al. 2016). Storage period also ($P<0.05$) affected FFA formation during refrigerated storage, FFA levels significantly increased from 0 to 20 days during refrigerated storage. The increased FFA levels during storage might be due to microbial lipolytic activities. Bhaskar Reddy et al. (2013) observed a similar trend in FFA during storage of restructured mutton slices. In another study, it was revealed that guava leaves extracts at 4000 ppm or higher can prevent the oxidation of fresh pork sausages, suggesting its application as a functional food ingredient (Tran et al. 2020). To release insoluble bound polyphenol components, guava leaves powder were co-fermented with yeast and bacterial strains and it was observed that fermentation enhanced the antioxidant ability of soluble guava leaf polyphenols (Wang et al. 2017). In an advanced study, silver nanoparticles were synthesized by utilizing crude polysaccharides of guava leaves powder, and showed high DPPH radical- and ABTS radical cation-scavenging activity. It is evident from the findings that guava leaves extracts can be a useful antioxidant material in the food preservation.

Figure 3. Influene of GLP on FFA (%) of chicken meat nuggets during refrigerated storage.



Microbiological analysis

Addition of guava leaves powder (GLP) significantly ($P<0.05$) influenced the total plate counts, total psychrophilic counts, coliform counts and yeast and mould counts of aerobic packaged chicken meat nuggets during refrigerated storage (Table 2). Incorporation of GLP reduced the overall mean values of total plate counts and total psychrophilic counts from 4.89 (control) to 3.53 (T3) log CFU/g at the end of 20 days of storage. Coliforms were not detected at 0 day in BHT and GLP treated nuggets including control nuggets which might be due to the antimicrobial activity of BHT and GLP. Absence of coliforms initially might be due to good hygienic practices and effective thermal processing. Also, chilling and the packaging environment might have prevented the initial growth of coliforms in chicken meat nuggets. The reduction of total plate counts, coliform counts and psychrophilic counts in GLP added is probably due to the antimicrobial activity of GLP (Kumar et al. 2021). Qualitative analysis of aqueous and organic extracts of guava leaves revealed the presence of phenolic acids, flavonoids, terpenoids, glycosides, and saponins, in which their presence is positively correlated with antimicrobial activity (Kumar et al. 2021). Similarly, water-soluble tannins present in guava leaves act as bacteriostatic agents, with mechanisms of actions like withholding substratum, hampering oxidative phosphorylation, and extracellular enzyme inhibition and inhibitory effect on antibiotic-resistant clinical isolates of *Staphylococcus aureus* (Das and Goswami, 2019). Irrespective of treatment, refrigerated storage (4±1°C) also significantly (P<0.05) influenced the total plate counts and toal psychrophilic counts of both chicken meat nuggets during storage. As storage increased, microbial counts significantly (P<0.05) increased which might be due to conducive water activity, changes in pH and type of environment (Bhaskar Reddy et al. 2013). The overall mean yeast and mould counts were significantly (P < 0.05) influenced by addition of GLP (Table 2). A significant (P < 0.05) increase in yeast and mould counts of chicken meat nuggets was noted with the increased storage period. HPLC–TOF–ESI/MS analysis of fermented guava leaves confirmed the presence of gallic acid, chlorogenic acid, rutin, isoquercitrin, avicularin, quercitrin, kaempferol, morin, and quercetin. These compounds have the property of inhibiting ergosterol, which is a fungal cell membrane component, and glucosamine, which is a fungal cell growth indicator (Kumar et al. 2021).

Table 2: Mean ± S.E values of microbial counts of chicken meat nuggets added with guava leaves powder (GLP) during refrigerated storage (4±1°C) *.

Treatments	Storage Period (days)				
	0	5	10	15	20
			Total plate count ($\log_{10}$ CFU/g)		
C	2.46±0.47^{eA}	3.67±0.30^{dA}	3.99±0.15^{cA}	4.23±0.09^{bA}	4.89±0.25^{aA}
T1	2.51±0.02^{eA}	3.61±0.59^{dA}	3.90±0.16^{cA}	4.30±0.13^{bA}	4.80±0.18^{aA}
T2	2.40±0.36^{eA}	3.05±0.31^{dB}	3.28±0.65^{cB}	3.69±0.48^{bB}	4.09±0.27^{aB}
T3	2.43±0.18^{eA}	2.69±0.67^{dC}	3.03±0.18^{cC}	3.28±0.20^{bC}	3.53±0.12^{aC}
			Total psychrophilic count ($\log_{10}$ CFU/g)		
C	ND	2.64±0.38^{dA}	3.67±0.11^{cA}	3.99±0.17^{bA}	4.54±0.17^{aA}
T1	ND	2.69±0.22^{dA}	3.70±0.18^{cA}	3.90±0.28^{bA}	4.52±0.35^{aA}
T2	ND	ND	3.01±0.23^{cB}	3.09±0.13^{bB}	3.35±0.13^{aB}
T3	ND	ND	2.45±0.20^{cC}	2.60±0.28^{bC}	2.90±0.59^{aC}
			Coliform count ($\log_{10}$ CFU/g)		
C	ND	2.09±0.24^{dA}	2.96±0.57^{cA}	3.40±0.17^{bA}	3.98±0.17^{aA}
T1	ND	2.11±0.14^{dA}	2.90±0.38^{cA}	3.50±0.28^{bA}	3.91±0.35^{aA}
T2	ND	1.60±0.43^{dB}	2.25±0.20^{cB}	2.66±0.11^{bB}	3.21±0.13^{aB}
T3	ND	1.63±0.09^{dB}	2.10±0.49^{cC}	2.30±0.19^{bC}	2.79±0.88^{aC}
			Yeast and mould count ($\log_{10}$ CFU/g)		
C	2.05±0.29^{eA}	2.99±0.68^{dA}	3.39±0.18^{cA}	3.92±0.33^{bA}	4.38±0.69^{aA}
T1	2.09±0.07^{eA}	2.89±0.42^{dA}	3.42±0.08^{cA}	3.93±0.12^{bA}	4.16±0.42^{aB}
T2	2.04±0.31^{eA}	2.20±0.18^{dB}	2.79±0.27^{cB}	3.07±0.08^{bB}	3.94±0.07^{aC}
T3	2.11±0.24^{eA}	2.27±0.16^{dB}	2.50±0.22^{cC}	3.05±0.13^{bB}	3.26±0.13^{aD}

Note: Mean values within row and column bearing different superscripts are differ significantly (P<0.05). * n=8; ND: Not detected.

Sensory characteristics

Addition of different concentrations of guava leaves powder (GLP) significantly affected the sensory characteristics of chicken meat nuggets during refrigerated storage (Table 3). GLP significantly (P<0.05) improved the color scores compared to control and BHT added chicken nuggets during refrigerated temperature during 20 days. From 0 day to 20 days of storage, respective higher colour scores were found in the chicken meat nuggets added with 1 % GLP. This might be due to inhibition of myoglobin oxidation by GLP, which in turn delays surface color deterioration during storage. The delay in color deterioration during storage in chicken meat nuggets added with GLP is in agreement with Mahapatra et al. (2019) in chevon meat balls added with guava powder. The chicken meat nuggets treated with GLP showed significantly (P<0.05) higher flavor scores than control and BHT, whereas, BHT treated samples had better flavor scores than control nuggets. This might be due to the anti-oxidative effect of GLP and BHT which decrease the intensity of off-flavor generation, (Brannan and Mah, 2007). Similarly Brannan (2009) reported that GSE was effective in limiting the intensity of rancid or warmed over flavor in pre-cooked meat. Bhaskar Reddy et al. (2018) also reported that grape seed extract addition lowers rancid and wet cardboard off-odor scores indicating that GLP has the potential to control some of the negative sensory characteristics associated with warmed-over flavor in restructured mutton slices. A significant ($P < 0.05$) reduction was found in overall flavor scores of chicken meat nuggets, as storage progressed. The GLP treated chicken meat nuggets had higher juiciness scores than control and BHT and the juiciness scores of chicken meat nuggets treated with BHA were superior to the control. This could be because the powdery antioxidant added to product retained more water. During storage a significant ($P < 0.05$) reduction in juiciness was seen all chicken nuggets, probably due to dehydration of the product. The addition of GLP showed higher tenderness scores than control, BHT and 0.5 % GLP added chicken meat nuggets during refrigerated storage. Among all chicken meat nuggets, 1 % GLP treated chicken meat nuggets (T3) had superior overall acceptability scores which might be due to the better favorable color, flavor, juiciness and tenderness scores compared to control and BHT. The BHT treated nuggets scored significantly ($P<0.05$) higher overall acceptability than control nuggets. With storage, the overall acceptability scores were significantly ($P < 0.05$) reduced, from 6.88 to 5.65 after 20 days of refrigerated storage in control nuggets and 6.89 (0 day) to 6.09 (20 days) T3 nuggets. These results were in accordance with Mahapatra et al. (2019) in chevon meat balls added with guava powder stored under refrigerated storage and Bhaskar Reddy et al. (2018) in vacuum packaged pork patties added with natural anti oxidants. Similar trend was observed for outer texture, juiciness and overall acceptability for sheep meat nuggets treated with guava powder (Verma et al. 2013) and goat meat nuggets treated with brocolli powder (Banerjee et al. 2012).

Table 3: Mean ± S.E values of sensory characteristics of chicken meat nuggets added with guava leaves powder (GLP) during refrigerated storage (4±1°C) *.

Treatments	Storage Period (days)				
	0	5	10	15	20
			Colour		
C	$6.83±0.54^{aB}$	$6.40±0.10^{bC}$	$6.35±0.87^{cA}$	$6.07±0.11^{dA}$	$5.85±0.67^{eB}$
T1	$6.73±0.22^{aC}$	$6.51±0.75^{bD}$	$6.31±0.58^{cC}$	$6.14±0.05^{dC}$	$5.97±0.29^{eC}$
T2	$6.97±0.88^{aB}$	$6.80±0.21^{bB}$	$6.51±0.31^{cB}$	$6.36±0.27^{dB}$	$6.14±0.44^{eA}$
T3	$6.95±0.19^{aA}$	$6.88±0.53^{bA}$	$6.69±0.30^{cB}$	$6.54±0.37^{dC}$	$6.47±0.32^{dA}$
			Flavor		
C	$7.05±0.13^{aA}$	$6.74±0.08^{bC}$	$6.59±0.43^{cC}$	$6.21±0.18^{dC}$	$5.65±0.09^{eC}$
T1	$6.99±0.44^{aB}$	$6.70±0.18^{bD}$	$6.42±0.34^{cD}$	$6.18±0.43^{dC}$	$5.62±0.16^{eC}$
T2	$7.07±0.28^{aA}$	$6.88±0.17^{bB}$	$6.73±0.28^{cB}$	$6.59±0.13^{dB}$	$5.98±0.15^{eB}$
T3	$6.98±0.18^{aB}$	$6.98±0.26^{bA}$	$6.82±0.29^{cA}$	$6.69±0.34^{dA}$	$6.10±0.15^{eA}$
			Juiciness		
C	$6.99±0.19^{aA}$	$6.54±25^{bD}$	$6.25±0.15^{cD}$	$6.12±0.37^{dD}$	$5.81±0.29^{eC}$
T1	$6.93±0.33^{aA}$	$6.62±0.30^{bC}$	$6.44±0.27^{cC}$	$6.26±0.16^{dC}$	$6.15±0.37^{eB}$
T2	$6.89±0.29^{aB}$	$6.75±0.17^{bB}$	$6.58±0.48^{cB}$	$6.35±0.25^{dB}$	$6.12±0.88^{eB}$
T3	$6.98±0.61^{aA}$	$6.81±0.38^{bA}$	$6.71±0.18^{cA}$	$6.42±0.33^{dA}$	$6.25±0.41^{eA}$
			Tenderness		
C	$7.03±0.21^{aA}$	$6.77±0.59^{bB}$	$6.59±0.32^{cB}$	$6.19±0.30^{dC}$	$5.71±0.58^{eD}$
T1	$6.98±0.49^{aB}$	$6.70±0.18^{bB}$	$6.55±0.68^{cB}$	$6.25±0.67^{dB}$	$5.93±0.19^{eC}$
T2	$6.97±0.13^{aB}$	$6.83±0.44^{bA}$	$6.67±0.44^{cA}$	$6.21±0.20^{dA}$	$6.20±0.28^{dB}$
T3	$6.99±0.22^{aB}$	$6.78±0.11^{bA}$	$6.67±0.19^{cA}$	$6.45±0.38^{dA}$	$6.26±0.19^{eA}$
			Overall acceptability		
C	$6.88±0.18^{aB}$	$6.50±0.51^{bB}$	$6.15±0.35^{cC}$	$5.71±0.25^{dC}$	$5.65±0.15^{dD}$
T1	$6.94±0.34^{aA}$	$6.53±0.27^{bB}$	$6.47±0.70^{cB}$	$6.09±0.66^{dB}$	$5.74±0.59^{eC}$
T2	$6.93±0.19^{aA}$	$6.78±0.29^{bA}$	$6.59±0.28^{cA}$	$6.17±0.32^{dA}$	$5.95±0.40^{eB}$
T3	$6.89±0.14^{aA}$	$6.79±0.18^{bA}$	$6.58±0.17^{cA}$	$6.18±0.45^{dA}$	$6.09±0.33^{eA}$

Note: Mean values within row and column bearing different superscripts are differ significantly ($P<0.05$). * n=24

CONCLUSION

Based on the above results, guava leaves powder is having excellent polyphenolics, flavonoids contents and also efficient radical scavenging activity. By comparing the comparative efficacy of both antioxidant and antimicrobial efficacy of BHT and different concentration of guava leaves powder, it can be concluded that the addition of guava leaves powder at 1 % reduced the lipid oxidation, delays the microbial organism's multiplication and improves the sensory attributes of chicken meat nuggets under aerobic environment during refrigerated storage (4±1°C) up to 20 days without any significant quality deterioration.

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