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Assessment of the effects of olives leaf and guava seed extracts on quality and shelf life of chilled chicken meatballs

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ABSTRACT

The contamination of food due to the occurrence of foodborne pathogens is a public health concern and a threat to the quality and safety of animal feed and human food. Considering the increasing demand for more natural and less heavily processed foods, specific requirements and preservation techniques are needed to minimize the activity of spoilage microorganisms. Thus, the purpose of this study was to evaluate the effects of OLE (olive leaf extracts) used at concentrations of 2%, guava seed extracts (GSE) at concentrations of 2%, and a mixture of them, OLE and GSE (1:1), on the quality and shelf life of chilled, stored chicken meatballs. The chemical (pH, Thiobarbituric Acid (TBA), and total volatile basic nitrogen (TVB-N)), microbiological (total bacterial, psychotrophic, coliform, Staphylococcus aureus, and total mold counts), and sensory characteristics (color, odor, texture, and overall acceptability) of the treated groups differed significantly ($P < 0.05$) from the untreated control group. Among the treated samples, OLE (2%) showed the highest reduction in aerobic plate count, psychrotrophic count, coliform count, and *Staphylococcus aureus* count, while the total mold count was not detected at the 9th of the storage period, followed by a mixture of OLE and GSE, then GSE (2%), and they increased the shelf-life of the chicken meatballs, which lasted up to 18 days when kept properly refrigerated compared to the control group, which went bad by the ninth day of storage. According to the study's findings, GSE and OLE can be utilized to naturally preserve chilled chicken meatballs at room temperature as well as to act as an antibacterial preservative.

1. INTRODUCTION

Chicken meat is a preferred and necessary component of people's daily diets because of its excellent organoleptic qualities, higher nutritional content, and great digestion (Özlü et al., 2023). However, products derived from chicken flesh are highly vulnerable to oxidation reactions of lipids and proteins, microbiological contamination, and spoiling (Pellissery et al., 2020). Owing to their efficacy, safety, and potential to enhance the physiological processes and storage stability of chicken meat products, natural plant-based preservatives are becoming increasingly popular among consumers. This is in contrast to synthetic additives, which have questionable suitability as well as toxicological and detrimental effects (Filipčev, 2020; Nadeem et al., 2022). Many studies have been done on the antibacterial and antioxidant qualities of various plants that are utilized as natural preservatives in chicken meat products to prevent microbial deterioration and to slow down oxidative processes. Because of the existence of bioactive chemicals, several plants have been shown to have some antibacterial and antioxidant capabilities. A class of phenolic compounds that can inhibit or decelerate the growth of pathogenic bacteria and spoiling agents in the food chain are the main source of this bioactivity (Beya et al., 2021). Guava seeds make up 6–12% of fruit weight and are high in dietary fiber (63.94 g/100 g), iron (13.8 mg/100 g), zinc (3.31 mg/100 g), and protein (11.19 g/100 g). Calorie content is modest (182 kcal/100 g) (Khalifa et al. 2016). Antioxidants include terpenoids, anthroquinones, polyphenols, myricetin, and coumarins, which are abundant in guava fruit seeds, as well as antinutritional flavonoids like tannin, saponins, and phytic acid. (El Anany, 2015; Ilakkiya et al., 2020). Numerous bioactive substances, such as ascorbic acid (vitamin C), total phenolics, total carotenoids, dietary fiber, and minerals, are all naturally present in guava (Sahu et al., 2020). Phenolics in particular are thought to be naturally occurring in olive leaf extracts (OLE). Taghvaei et al. (2014). Numerous phytochemicals, including rutin, luteolin 4-O-glucoside, hydroxytyrosol, tyrosol, oleuropein, and phenolic acids, may be naturally occurring in olive leaf extract (Beshbishy et al., 2019). Phenolic substances may prevent *Staphylococcus aureus* from growing, proliferating, and producing enterotoxins. Bulotta et al. (2014). OLE's polyphenolic components scavenge free radicals and interrupt the free radical chain reaction. (Lee and Lee 2010). By preventing metal ion chelation, these chemicals' bioactivation may also enhance their antioxidant potential. Hayes et al. (2011). As a natural antioxidant, OLs are a substance that is often examined and highlighted. By stopping or postponing the oxidation of oxidative substrates, it has demonstrated strong antioxidant properties. When compared to other olive tree sections, OLs have the strongest antioxidant qualities due to their high oleuropein and hydroxytyrosol content. Dekanski et al., 2011). Some

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studies on the toxicity of olive leaf extracts revealed that they are generally safe and do not exhibit any harmful effects, even at large dosages. (Acar-Tek and Ağagündüz, 2020). Additionally, a plethora of studies conducted over the past ten years have detailed the impact that polyphenols derived from plant extracts have on spoilage bacteria and foodborne pathogens. (Wang et al., 2023). Therefore, the objectives of the present study were to assess plant extracts, such as leaves of olive leaves (*Olea europaea*) and seeds of guava (*Psidium guajava*), on the physiochemical and microbiological properties of chicken meatballs during the storage period.

2. MATERIALS AND METHODS

2.1. Preparation of extracts:
Ethanolic extracts from olive leaves (*Olea europaea*) and guava seeds (*Psidium guajava*) were prepared according to Saleh et al. (2020) and Kapoor et al. (2020) at the National Research Centre, Dokki, Cairo, Egypt.

2.2. Chicken meatballs
Chicken fillets were purchased from poultry markets in El Menofiya governorate and were directly packed in sterile polyethylene bags. The bags were then transported directly in an insulated ice container to the food microbiology lab, Animal Health Research Institute, Shibin Elkom, Egypt. Chicken meatballs were prepared using fresh chicken meat, garlic pest, onion pest, ginger pest, meat spices, garam masala (spices), soybean oil, refined vegetable oil, refined wheat flower, salt, and sauces.

2.3. Experimental design
Five sets of chicken meatballs were created. The first group was untreated (control), the 2nd group was treated with OLE (2%), the 3rd group was treated with GSE (2%) and the 4th group was treated with a mixture of OLE and GSE (1:1). The samples were tagged, individually packed in polyethylene bags, and kept at 4 ±1ºC. The samples were analyzed for microbiological, physicochemical, and sensory properties during the chill storage period (at zero, 3^{rd}, 6^{th}, 9^{th}, 12^{th}, 15^{th}, and 18^{th} days), and the experiment was conducted in triplicate.

2.4. Microbiological quality evaluation
Chicken meatballs samples were examined throughout the storage period in order to evaluate the total bacterial count (TBC) using the pour plate method on plate count agar (HIMEDIA, M091S) at 35°C (ISO 4833-1, 2013), Staphylococcal aureus count on Baird Parker ager base (HIMEDIA, M043) (incubated at 37°C for 48 hours (FDA, 2001), psychotrophic bacterial count (PBC) on plate count agar (HIMEDIA, M091S) at 4°C (FDA, 2001), and total coliform count according to ISO 4832 (2006) on violet red bile agar media (HIMEDIA, M049) then incubated at 37 °C for 24 hours. and mold & yeast count according to ISO 21527-1:2008 on SDA media (HIMEDIA, M084) then incubated at 30 °C for 5-7 days.

2.5. Physicochemical evaluation
The pH values were determined according to the method described in Gharibzahedi and Mohammadnabi (2017), the content of TVB-N was performed following the method described by Shokri et al. (2015) and expressed as mg N/100 g of sample. While, the oxidative state of the samples was evaluated by the determination of the thiobarbituric acid reactive substances (TBARS) values according to Gharibzahedi and Mohammadnabi (2017).

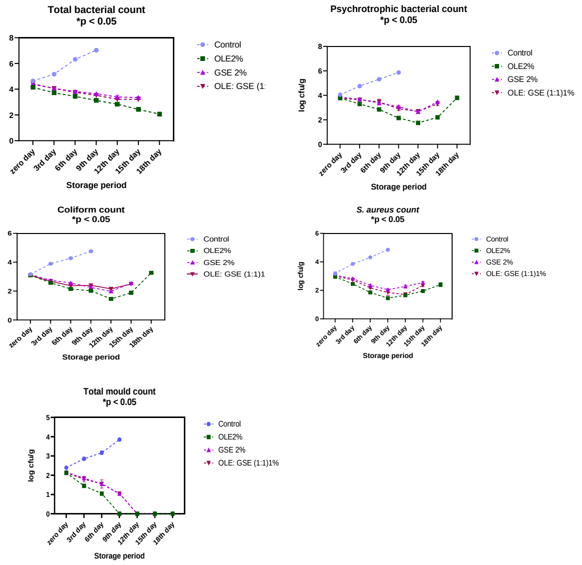
2.6. Sensory evaluation
It was performed according to ISO 13299 (2003).
Sensory attributes of the chicken meatball samples were assessed by 10 well-trained panelists. The color, odour, texture, and overall acceptability are all examples of sensory attributes. Sensory evaluation was based on a ten-point scale (1–10) to determine.

2.7. Statistical analysis
Data was analyzed using the graph pad prism application (Version 8.0.2) for Windows. The analysis of variance was performed on all data using one way ANOVA. Values were expressed as means and SD. Significant p-values were found at $P \leq 0.05$ at confidence level 95%.

3. RESULTS

Data in Fig. 1 showed the effect of natural extracts (OL, GS, and OL: GS) applied on chicken meatballs to evaluate their impacts on microbiological attributes. The results revealed a significant variation ($P < 0.05$) between samples in the control and treated groups with natural extract. The mean total bacterial count values increased from 4.64 ± 0.11 to 7.03 ± 0.09 in control chicken meatballs. ls. While, values of TBC were decreased in OL (2%), GS (2%), and OL: GS treated groups from 4.15 ± 0.04, 4.38 ± 0.09, and 4.42 ± 0.03 to 2.07 ± 0.10, 3.35 ± 0.07, and 3.21 ± 0.01 (log cfu/g), respectively. At the end of the storage period, control chicken meatballs exhibited an increase in TBC, whereas the count of treated chicken meatballs decreased continuously until 12^{th} day of storage, then increased. Also, the mean values of total psychotrophic count of OL, GS, and mixture of OL and GS treated groups decreased throughout the chill storage period till 12th of storage from 3.77 ± 0.03, 3.85 ± 0.05, and 3.77 ± 0.03 to 1.75 ± 0.03, 2.65 ± 0.07, and 2.69 ± 0.01 (log cfu/gm), respectively, and then the count increased as a result of bacterial growth. While, the mean values of the psychotrophic count of the control group increased from 4.05 ± 0.03 to 5.8 ± 0.10.
The coliform count increased in the control group throughout the chilled storage period, while the coliform count of the treated group decreased till 12th of chilled storage, then increased. The mean value of coliform decreased in OLE 2%, GSE 2%, and mixtures of OLE and GSE from 3.09 ± 0.01, 3.14 ± 0.04, and 3.08 ± 0.09 to 1.45 ± 0.08, 1.95 ± 0.02, and 2.15 ± 0.08 (log cfu/g), respectively. It was observed that *S. aureus* count decreased till 9th of chill storage in treated groups. While *S. aureus* count increased in the control group throughout the chill storage period.
Total mold count was completely inhibited at 9th day of refrigerated storage, which was treated with OLE 2%, and at 12th which was treated with GSE 2% and a mixture of OLE and GSE (1:1).
The results in figure (2) showed the significance variation ($P<0.05$) between the physicochemical characters of chilled chicken meatballs in the control group and those in the treated groups with natural plant extracts. The 2% extract of olive leaf significantly improved the values of TVB-N, TBA, and PH, which are indicators of the freshness and shelf life of the samples. As, control group showed higher values of pH, TBA, and TVB-N, this reflects its incipient spoilage from day 9th of chilling storage, while, the groups treated with guava seed extract and the mixture of OLE and GSE (1:1) showed spoilage at 15th day of spoilage. The treated samples with olive leaf extract 2% stayed in the accepted range till 18^{th} day of refrigerated storage.

Concerning sensory attributes of chilled chicken meatballs, the results in Table 1 showed the positive impacts of OLE, GSE, and mixture extracts on sensory characters (odor, color, texture, and overall acceptability) and shelf life of chilled chicken meatballs.



Fig(1) Impacts of natural bioactive extracts (*olive leaf* and *guava seed*) on microbiological status of chilled chicken meatballs. Values are shown as the mean of triplicates ±SD. The significance at $P < 0.05$ between groups and time of storage.

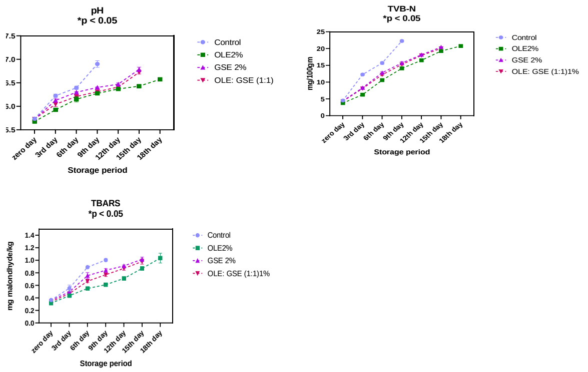


Fig (2) Impacts of natural bioactive extracts (*olive leaf and guava seed)* on physic-chemical characteristics (pH, TBA, TVB-N) in chilled chicken meatballs. Values are shown as the mean of triplicates ±SD. The significance at $P < 0.05$ between groups and time of storage.

Table (1) Impacts of natural bioactive extracts (*olive leaves and guava seed*) on sensory attributes (color, odor, texture, over all acceptability) in chicken meatballs.

groups / storage period	Control	OLE2%	GSE 2%	OLE:GSE (1:1)1%
zero day	9.50 ± 0.30^{a}	9.50 ± 0.30^{a}	9.50 ± 0.30^{a}	9.50 ± 0.30^{a}
3rd day	6.5 ± 0.14^{a}	8.70 ± 0.14^{b}	7.90 ± 0.10^{c}	8.10 ± 0.13^{c}
6th day	4.6 ± 0.21^{a}	8.00 ± 0.35^{b}	6.80 ± 0.14^{c}	7.00 ± 0.10^{c}
9th day	4.00 ± 0.10^{a}	7.20 ± 0.10^{b}	5.9 ± 0.13^{c}	6.20 ± 0.10^{c}
12th day	S	6.40 ± 0.14^{a}	4.9 ± 0.10^{b}	5.10 ± 0.14^{b}
15th day	S	5.10 ± 0.14^{a}	4.00 ± 0.10^{b}	4.00 ± 0.10^{b}
18th day	S	4.00 ± 0.10^{a}	3 ± 0.10^{b}	3.2 ± 0.10^{b}

S= Spoiled. The results are considered significant ($p < 0.05$) when the same row contained different small letters

4. DISCUSSION

Unrecognizable food spoilage can lead to food safety issues that endanger the health and lives of customers (Liu et al., 2022). Consumers are becoming increasingly conscious of the importance of nutrition for good health, and studies show that foods should be selected for their high nutritional value as well as the kinds and amounts of nutrients they contain. Natural preservatives with antibacterial properties are increasingly being used in food processing to enhance food safety and extend shelf life (Elsabagh et al., 2023). Many studies have been done on the antibacterial and antioxidant qualities of many plants that are utilized as natural preservatives. Because of the existence of bioactive chemicals, several plants have been shown to have some antibacterial and antioxidant capabilities. The primary cause of this bioactivity is a group of phenolic chemicals that have the power to stop or slow the growth of harmful bacteria and spoiling agents in the food chain (Beya et al. 2021). Olives and Guava extract are two natural ingredients that have been studied for their potential use as preservatives. On physicochemical evaluation, the control sample's pH increased significantly compared to the treated samples, and this could be related to the active phenolic components in the GSE and OLE extract. The slow increase in pH seen in samples containing natural extracts during cold storage could be ascribed to the phenolic compounds' suppression of bacterial growth. This phenomenon leads to the breakdown of amino complexes, contributing to the pH increase over time. Amiri et al. (2019) support this explanation, highlighting the impact of phenolic compounds on bacterial growth and subsequent pH changes during cold storage.

TVB-N values increased rapidly in the control group with the chill storage period till spoilage at 9th day (22.25 mg/100 g), according to ES (2006), as the maximum permissible limit for TVB-N in poultry is 20 mg/100 g. During the chilling storage period, the TVB-N content of the treatment groups showed a significant improvement ($p < 0.05$). The findings demonstrated a substantial correlation between the TVB-N data and the microbial load because microbial enzymes play a major role in the deamination of amino acids, which produces volatile bases and contributes to spoiling. Previous studies have observed similar trends. Studies by Licciardello et al. (2018) and Laly et al. (2020) have reported similar trends.

Over the course of the storage period, the TVB-N values of the treated groups were significantly reduced ($P < 0.05$). The antibacterial properties of plant extracts (OLE and GSE) may be the cause of the treated groups' notable decrease in TVB-N readings. The quantity of secondary metabolites produced when meat oxidizes its fatty acids can be measured using TBARs (Ehsani, et al. 2019). Throughout the trial, TBA values in the treated groups were considerably lower ($P < 0.05$) than in the control group. These plants have antioxidant properties because of the high concentration of phenolic and flavonoid chemicals found in olive and guava extracts. Additionally, OLE and GSE treated samples suppressed lipid oxidation. Antioxidants such as condensed tannins, flavonols, flavonoids, and other polyphenols are abundant in guava seeds. (Lima et al., 2019). Guava seed extracts successfully stopped fat oxidation in processed chicken flesh. (Packer et al. 2010). Microbial growth increased in the control group while, it considerably decreased ($p < 0.05$) in the treated group. According to Kamal El-Sawah et al. (2024), olive leaf extracts showed antibacterial activity against tested Gram-positive bacterial strains at low concentrations, reduced total viable count, and prevented *Staphylococcus aureus* and *E. coli* growth along the storage period until 9th days under cooling. Additionally, a prior study (Oliveira et al., 2021) demonstrated that the OLE extracts had a potent antibacterial impact on *B. cereus*, *S. enterica*, *E. coli*, *S. aureus*, and *P. aeruginosa*, some of the most often found pathogens linked to bacterial foodborne illnesses. Wan et al. (2013) discovered that the aqueous extract from guava seeds had antibacterial action against *Bacillus subtilis* and *Staphylococcus aureus*, two types of Gram-positive bacteria. Hafez and Abdelrahman (2015) indicated that most pathogenic bacteria, including *S. aureus*, may be killed with lower quantities of olive leaf extract. Mohamed et al. (2011) found that the antioxidant- and phenolic-rich GSEs exhibit antibacterial action against *S. Typhimurium*, *B. cereus*, *E. coli*, *L. monocytogenes*, and *S. aureus*. Lafka et al. (2013), who stated that compounds found in OLE had strong antibacterial and fungal properties. Moreover, Özcan et al. (2017) reported that OLE is a powerful antifungal agent, particularly against C. albican. The extracts′ polyphenols, which interacted with the bacterial cell membranes and were frequently associated with the hydrophobicity of the bioactive chemicals, may have contributed to the observed antibacterial activities. It suggests that the treated groups experienced a decrease in sensory attribute scores, while the control group spoiled at 9th day rapidly due to the formation of toxic metabolites being retarded and a decrease in bacterial population because of the antibacterial and antioxidant effects of OLE and GSE. Similar results by Saleh et al. (2020), who use OLE as a natural antioxidant to delay the spoilage of chicken meat. Consumers are likely to reject putrid and bitter odors produced by microbial deterioration in chicken products because it can lead to the development of biogenic amines and volatile organic compounds, which are produced when proteins break down owing to the action of microorganisms (Hao et al. 2021). It has been demonstrated that the bioactive components in plant extract are useful in preventing the spread of foodborne infections, which can postpone the synthesis of biogenic amines and increase the shelf life of meat products. (Embuscado, 2015; Perricone et al., 2015).

Conclusion:

The sensory characteristics chicken meatball samples have been proven to be preserved after storage at freezing temperatures by extracts of 2% olive leaf and 2% guava seed. These extracts have significant levels of phenolic chemicals, which have strong antibacterial and antioxidant qualities and can prolong the shelf life of meat. Comparing the extracts to control samples, it has been demonstrated that the extracts significantly reduced the values of pH, TVN, and TBA. This implies that adding these plant extracts to meat and meat products could enhance their overall quality and meet consumer demands by providing a healthier substitute for artificial preservatives.

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