Journal of Food Science and Technology (Iran)

Homepage:www.fsct.modares.ir

Scientific Research

Evaluation of antioxidant and antimicrobial effects of free and nanoencapsulated extracts of *pelargonium graveolens* leaves and flowers on the shelf life and sensory properties of mutton fillet during storage

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ARTICLE INFO

Article History:

Received:2025/2/18

Accepted:2025/4/5

Keywords:

Antioxidant,

Antimicrobial,

Pelargonium Extract,

Mutton fillet,

Storage

DOI: **10.22034/FSCT.22.163.270.**

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ABSTRACT

Due to the proven carcinogenic effects of synthetic preservatives, there has been an increasing focus on finding natural alternatives. In this study, extracts from *Pelargonium graveolens* leaves and flowers were extracted using ultrasound as a natural antioxidant and antimicrobial compound and nanoencapsulated in fenugreek seed gum and soy protein isolate coatings. Mutton fillets were treated with nanoencapsulated and free extracts of *Pelargonium* leaves and flowers at a concentration of 2000 ppm and stored at 4°C for 12 days. A control sample (without preservatives) and a sample containing 100 ppm of the synthetic preservative BHT were included as references. Results of lipid oxidation tests (peroxide value and thiobarbituric acid value), psychrotrophic bacterial counts, and total volatile nitrogen bases measured at 2-day intervals indicated that both free and nanoencapsulated extracts delayed lipid oxidation reactions and microbial growth. The lowest oxidative and microbial spoilage levels were observed in the meat samples treated with nanoencapsulated extracts. Additionally, these samples achieved higher sensory evaluation scores throughout the storage period. Considering that the *Pelargonium* leaf and flower extracts contain phenolic and flavonoid compounds, they not only extended the shelf life of lamb meat but also enhanced its nutritional value. The findings of this research recommend using nanoencapsulated extracts of *Pelargonium* flowers at a concentration of 2000 ppm in fenugreek seed gum and soy protein isolate walls to enhance the shelf life of mutton meat.

[DOI: 10.22034/FSCT.22.163.270]

1.Introduction

Mutton is a perishable product with high moisture content, providing favorable conditions for the growth of spoilage and pathogenic microorganisms [1]. One of the main causes of mutton spoilage under refrigerated conditions is the growth of microorganisms that lead to undesirable organoleptic changes, unpleasant flavors, discoloration, and pH variations [2]. In this regard, the use of freezing and cooling methods alone may not be sufficient to preserve meat quality and maintain it in storage or on store shelves for more than five days. This results in increased food waste and negatively impacts marketing and food security [2, 3].

Active packaging includes active compounds such as antioxidants, antimicrobial agents, and absorbers of moisture, gases, and ultraviolet radiation, which interact with the packaged food or its surrounding environment [3]. Active packaging can inhibit or slow down microbial growth and reactions that typically occur on the surface of food and initiate spoilage [4]. This technology has great potential to extend shelf life and enhance food safety. Among the active compounds recently gaining attention are edible gums, which have been widely used in the production of various coatings for different products [5].

Fenugreek seed gum is a natural hydrocolloid extracted from the seeds of the fenugreek plant. This gum is primarily composed of galactomannan and is widely used in the food and pharmaceutical industries due to its desirable rheological properties, gelling ability, emulsifying and stabilizing features. Fenugreek seed gum, as a wall material in encapsulation, helps protect sensitive compounds against adverse environmental conditions such as oxidation, moisture, and heat. Furthermore, due to its bioactive properties, including antioxidant and antimicrobial activities, this gum can be used in the production of bioactive coatings that improve the quality and shelf life of food products [6].

Soy protein isolate is one of the most valuable soy-derived products, containing highly purified proteins. Due to its functional properties such as solubility, gel formation, and film-forming ability, it has attracted attention in various food and biotechnological applications. Soy protein isolate can serve as an effective matrix for encapsulating bioactive compounds, protecting them from degradation caused by light, oxygen, and heat. The combination of proteins with polysaccharides can enhance the functional properties and stability of encapsulation systems. Numerous studies have focused on the antioxidant and antimicrobial properties of extracts encapsulated in protein–polysaccharide complexes [7–11].

Essential oils and extracts from aromatic and medicinal plants are natural substances that, due to their nutritional value, food preservation potential, and therapeutic properties, have attracted significant interest as potential natural agents in the food, medical, agricultural, and cosmetic industries [12]. *Pelargonium graveolens* L. is a species belonging to the *Pelargonium* genus. This plant is a valuable, aromatic, perennial shrub. Pharmacological studies have shown that geranium extract exhibits strong antioxidant activity, immune-boosting effects, and antibacterial and antifungal properties. Therefore, it is commonly used in the treatment of inflammation, hemorrhoids, dysentery, heavy menstruation, and even cancer [12, 13].

Among the natural compounds found in plants, polyphenols represent one of the largest groups of known natural substances. The beneficial effects of polyphenols—such as the prevention and treatment of cancer, inflammatory diseases, cardiovascular conditions, and neurodegenerative disorders—have received considerable attention [12]. Natural antioxidants and antimicrobials extracted from this genus offer more advantages compared to

[DOI: 10.22034/FSCT.22.163.270]

synthetic preservatives. Therefore, the development and use of naturally derived antioxidants from more effective plant sources are highly desirable in preventive medicine and the food industry due to their role in protecting the body against oxidative stress and managing various diseases. These compounds also complement antimicrobial activities [14, 15].

Nanoencapsulation of extracts is an emerging technology in the food industry aimed at preserving and enhancing the stability of bioactive compounds. Many plant extracts and bioactive ingredients, such as polyphenols and antioxidants, have strong odors and bitter or unpleasant tastes that can negatively affect the sensory properties of food products. In addition, these compounds are typically sensitive to environmental factors such as light, oxygen, moisture, and temperature, and may degrade during processing or storage. Nanoencapsulation, using suitable wall materials such as proteins and polysaccharides, can protect these extracts from oxidation and degradation, thereby enhancing their shelf life and efficacy. Moreover, this technology reduces the strong odor and unpleasant tastes of the extracts, helping to ensure better sensory acceptance and improved shelf stability of the food product. As a result, the use of nanoencapsulation not only extends the shelf life and nutritional value of food products but also improves their overall sensory quality and consumer acceptance [16–18].

It is hypothesized that both free and nanoencapsulated extracts of *Pelargonium* leaves and flowers may enhance the shelf life of mutton. Considering that no previous study has compared the effects of free versus nanoencapsulated *Pelargonium* leaf and flower extracts on the shelf life of mutton fillets, this research aims to address that gap.

2.Materials and Methods

2.1. Materials

Mutton carcasses (with 56.9% moisture, 20.6% protein, 8.9% fat, and 1% ash) were obtained from the Protein Plus Meat Supermarket (Sari, Mazandaran, Iran). Soy protein isolate (with protein content ≥ 90%) was sourced from Linyi Company (Shandong, China). All chemicals used were of analytical grade and purchased from Scharlau (Barcelona, Spain). Fenugreek seeds were purchased from a local herbal store. The aromatic *Pelargonium* plant was obtained from the Agricultural Research Center in Karaj (Alborz, Iran), and its identification and confirmation were based on morphological and botanical characteristics.

1.2. Methods

1.2.1. Extraction of *Pelargonium* Leaf and Flower Extracts

The leaves and flowers of the *Pelargonium graveolens* plant were separately dried in an oven (VO200, Memmert, Germany) at 35 °C and ground into powder using a grinder (Pars Khazar, Iran). Dried plant powder (20 g) was immersed in 400 mL of deionized water (dH_2O). The extraction was carried out using aqueous solvent extraction assisted by ultrasonic probe (HD3100, Bandelin, Germany) by placing an Erlenmeyer flask (150 W, 25 kHz) at room temperature (23 °C) for 30 minutes. The mixture of solvent and plant material was filtered using Whatman No. 1 filter paper. The resulting extract was concentrated using an oven at 35 °C. The dried extracts were stored in a refrigerator for further use [19].

1.2.2. Identification of Bioactive Compounds in the Extract

To identify the bioactive compounds present in the extract, reverse-phase high-performance liquid chromatography (RP-HPLC; Waters, USA) was used according to the method described by Esmaeilzadeh Kenari and Razavi (2022). Phenolic compounds were analyzed using an HPLC system equipped with a photodiode array detector (M-2998) and a C18

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column (4.6 mm × 25 µm × 5 cm). For the separation of phenolic compounds, a mobile phase consisting of 0.01% (v/v) acetic acid in acetonitrile (Solvent 1) and acidified water containing 0.1% (v/v) acetic acid (Solvent 2) was used. The flow rate was maintained at 0.5 mL/min. For the separation of flavonoid compounds, 0.1% (v/v) methanol (Solvent 2) was used. Gradient and washing programs were applied according to the method reported by Mäki et al. [20, 21].

2.2.3. Encapsulation of the Extract

Fenugreek seed gum and soy protein isolate were used in combination as wall materials in a 1:1 ratio. The gum and protein isolate were dissolved in deionized water to reach a total solid content of 0.5% (w/w). The solution was stirred with a magnetic stirrer for 15 minutes at room temperature to ensure proper dissolution. The resulting solution was refrigerated for 24 hours to complete hydration [22]. To enhance hydration, the mixture was cooled and stored overnight at 4 °C. Subsequently, 40 mL of Tween 80 (with HLB value of 15) as an emulsifier and 50 mL of sunflower oil were combined with 10 mL of *Pelargonium* leaf and flower extracts at a concentration of 2000 ppm. The mixture was homogenized using a high-speed homogenizer (T50, IKA, Germany) at 12,000 rpm for 5 minutes to form an emulsion. The final emulsion was microencapsulated using the wall materials at a ratio of 2:5. To further reduce the particle size, an ultrasonic probe device was used at 94% amplitude, 37 °C, 150 W, and 25 kHz for 5 minutes [5].

2.2.4. Meat Coating

Initially, fresh lamb meat was thoroughly washed with sterile distilled water. The meat was then cut into uniform pieces of 2 × 2 × 2 cm under strictly sterile conditions using a sterile knife. The lamb meat cubes were immersed for 1 minute in the prepared coating solutions, with a coating-to-meat ratio of 3:1. After immersion, the coated meat pieces were dried for 1 minute at room temperature under a laminar airflow to prevent any contamination. The coated samples were packaged in sterile zip-lock plastic bags and stored at 4 °C for 12 days. This storage period was selected based on our previous experiments. To evaluate the effectiveness of the coatings during refrigerated storage, various analyses were performed on samples immediately after preparation and on days 2, 4, 6, 8, 10, and 12 of storage. Two additional samples were prepared: one containing 2000 ppm of the free extract and another containing 200 ppm of synthetic antioxidant BHT [13]. The particle sizes of the nanocapsules containing *Pelargonium* leaf and flower extracts in soy protein isolate–fenugreek gum coatings (MIXL and MIXF) were found to be 172.75 nm and 178.79 nm, respectively. The encapsulation efficiency for MIXL and MIXF samples was 88.06% and 89.59%, respectively [24].

2.2.5. Meat Analyses

2.2.5.1. Peroxide Value Measurement

The peroxide value was measured using the AOCS official method Cd 8-53. The fat was first extracted from the samples using a Soxhlet apparatus [25]. Then, 5 g of the extracted fat was weighed into a 250 mL Erlenmeyer flask and mixed thoroughly with 30 mL of a chloroform–acetic acid solution (2:3, v/v). After that, 0.5 mL of saturated potassium iodide, 30 mL of distilled water, and 0.5 mL of 1% starch solution were added. The liberated iodine was titrated with 0.01 N sodium thiosulfate until the yellow color disappeared. The mixture was vigorously shaken throughout the titration to separate iodine from the chloroform layer. All steps were repeated with a blank (without fat). Finally, the peroxide value was calculated using the standard formula [26].

2.2.5.2. Thiobarbituric Acid (TBA) Value Measurement

[DOI: 10.22034/FSCT.22.163.270]

To determine the TBA value, 5 g of meat sample was homogenized with 20 mL of 5% trichloroacetic acid for 5 minutes. The homogenate was centrifuged at 12,000 rpm for 10 minutes at 4 °C. Four milliliters of the supernatant were mixed with 4 mL of 0.02% TBA solution and incubated in a water bath at 100 °C for 60 minutes. After incubation, the mixture was centrifuged at 2500 rpm for 10 minutes at 4 °C. The absorbance of the supernatant was measured at 532 nm. The TBA value was calculated based on a standard curve of malondialdehyde (MDA) and expressed as micromoles of MDA per gram of sample [27].

2.2.5.3. Total Psychrotrophic Bacterial Count

A 5 g portion of the meat sample was aseptically transferred into a stomacher bag and homogenized for 1 minute using a stomacher (3500, Tekmar, Ohio, USA). Serial decimal dilutions were prepared from the initial dilution. Each dilution was then carefully spread on the surface of agar plates. The inoculated culture media were incubated at 30 °C for 72 hours to allow bacterial growth [28]. Tryptic Soy Agar (TSA) was used as the culture medium for counting these bacteria. A 0.1 mL aliquot of each diluted sample was surface-plated. Plates for psychrotrophic bacteria were counted after 10 days of incubation at 4 °C [5]. Table 1 presents the treatments evaluated in this study.

Table 1. Code and formulation of coatings Examined in the Study

Treatment Code	Treatment Description	Type of Extract	Type of Coating	Antioxidant Concentration (ppm)
CON	Uncoated meat sample	None	None	0
BHT	Meat treated with synthetic antioxidant (BHT)	None	None	200
LFRE	Meat treated with free (non-encapsulated) extract	Geranium leaf	None	2000
FFRE	Meat treated with free (non-encapsulated) extract	Geranium flower	None	2000
MIXL	Meat treated with nanoencapsulated extract	Geranium leaf	Fenugreek seed gum and soy protein isolate	2000
MIXF	Meat treated with nanoencapsulated extract	Geranium flower	Fenugreek seed gum and soy protein isolate	2000

2.2.5.4. Determination of Total Volatile Basic Nitrogen (TVB-N)

The method described by Khalili et al. (2024) was used to measure total volatile basic nitrogen. For this purpose, 10 g of meat sample, 2 g of magnesium oxide, 300 mL of water, and a few boiling stones were added to a Kjeldahl distillation flask. A 500–700 mL Erlenmeyer flask containing 25 mL of 2% boric acid solution and a few drops of methyl red indicator was placed as the receiving container beneath the condenser of the distillation unit. After distillation, the distillate was titrated with 0.1 N sulfuric acid, and the amount of acid consumed was multiplied by 14 to calculate the TVB-N content [1].

2.2.5.5. Color Evaluation

The color of meat samples was assessed using a HunterLab colorimeter (Hunter Lab, USA), based on the measurement of L*, a*, and b* values. The L*, a*, and b* indices represent lightness, redness, and yellowness, respectively. Five different points on each meat sample were selected and measured. Barium sulfate white was used as the standard reference [29].

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2.2.5.6. Sensory Evaluation

Meat fillet samples (100 g) were individually cooked in a microwave oven (NN-SD9675, Panasonic, Japan) at 700 W for 4 minutes. Sensory properties of the cooked lamb fillets were evaluated by a six-member panel using a 5-point hedonic scale. The parameters evaluated included taste, color, texture, odor, and overall acceptability. Scoring was based on a scale from 1 to 5 (1 = poor, 2 = fair, 3 = average, 4 = good, 5 = excellent) [30, 31].

1.3. Statistical Analysis

The data obtained from the different tests were analyzed using a completely randomized design and one-way analysis of variance (ANOVA) at a 95% confidence level. Means were compared using SPSS software version 20. All experiments were conducted in triplicate to minimize experimental error.

2. Results and Discussion

3.1. Bioactive Compounds of the Extract

Table 2 presents the phenolic and flavonoid compounds identified in the leaf and flower extracts of *Pelargonium graveolens*, along with their concentrations (expressed in mg per gram of dry extract). The concentration of phenolic and flavonoid compounds in the flower extract was higher than that in the leaf extract, indicating a stronger antioxidant potential of the flower. Quercetin and kaempferol were the predominant flavonoids detected in both extracts, with significantly higher levels in the flower. Among phenolic compounds, gallic acid and caffeic acid showed the highest concentrations. The HPLC analysis results were in relative agreement with the total phenolic, flavonoid, and flavanol content previously measured. In our earlier study, the total phenolic content of the flower and leaf extracts was reported as 74.97 and 67.46 mg GAE/g DM, respectively [24]. However, the sum of phenolics identified by HPLC was lower, which may be due to the presence of other phenolic compounds not detected by this method.

Similarly, the total flavonoid content was measured as 31.93 and 23.04 mg QE/g DM for flower and leaf extracts, respectively, whereas the sum of identified flavonoids by HPLC was 18.86 and 9.93 mg/g, respectively. This suggests the possible presence of other unidentified flavonoids in the extracts. Additionally, the total flavanol content was found to be higher in the leaf extract than in the flower extract, which aligns with the HPLC-determined levels of quercetin, kaempferol, and isorhamnetin. Overall, these findings indicate that the compounds identified via HPLC represent only a portion of the total bioactive compounds in the extracts. More comprehensive analyses are required for a complete understanding of their phytochemical profiles. In a study by Achchouna and Hamdi (2012), the presence of myricetin in *Pelargonium graveolens* leaves was confirmed [32]. Similarly, Abdelbaki et al. (2022) reported the presence of gallic acid, chlorogenic acid, and caffeic acid in the leaf extract of *P. graveolens* [19].

Table 2. Phenolic and flavonoid compounds

Compounds	Leaf(mg/g)	Flower (mg/g)
Phenolic compounds		
Gallic acid	2.31 ± 0.12^{b}	3.45 ± 0.18^{a}
Caffeic acid	1.87 ± 0.10^{b}	2.94 ± 0.15^{a}
Chlorogenic acid	1.45 ± 0.08^{b}	2.67 ± 0.12^{a}
Ferulic acid	0.98 ± 0.05^{b}	1.76 ± 0.09^{a}
Sinapic acid	0.75 ± 0.04^{b}	1.32 ± 0.06^{a}
p-Coumaric acid	0.68 ± 0.03^{b}	1.21 ± 0.05^{a}
Flavonoid compounds		
Quercetin	3.12 ± 0.16^{b}	5.89 ± 0.22^{a}

[DOI: 10.22034/FSCT.22.163.270]

Kaempferol	2.67 ± 0.14[b]	4.75 ± 0.20[a]
Isorhamnetin	1.32 ± 0.07[b]	2.84 ± 0.11[a]
Myricetin	1.14 ± 0.06[b]	2.15 ± 0.09[a]
Luteolin	0.92 ± 0.05[b]	1.78 ± 0.07[a]
Apigenin	0.76 ± 0.04[b]	1.45 ± 0.06[a]

Different letters indicate statistically significant differences ($p<0.05$) between leaf and flower extracts

2.2. Peroxide Value (PV) and Thiobarbituric Acid (TBA) Index

Lipid oxidation is one of the main limiting factors affecting the quality and acceptability of lamb meat. It leads to undesirable changes in the sensory, chemical, and nutritional properties of meat and results in the formation of potentially toxic compounds [1]. The results of peroxide value (PV) and thiobarbituric acid (TBA) index in different lamb meat treatments during the storage period (day 0 to 12) indicated that both indicators increased in all treatments over time. However, the type of treatment significantly affected the rate of this increase.

Treatments containing natural and synthetic antioxidants, particularly nanoencapsulated extracts (MIXL and MIXF), significantly slowed the rise in PV and TBA values. The BHT treatment also showed good performance in controlling peroxidation but was less effective than the nanoencapsulated extracts. In contrast, the free extracts of *Pelargonium* (LFRE and FFRE), despite their antioxidant properties, were less effective than BHT and nanoencapsulated forms. The peroxide value results demonstrated that treatments with nanoencapsulated extracts significantly inhibited PV increase, likely due to the stabilization of antioxidant compounds. Similar results were observed for the TBA index, which reflects the formation of malondialdehyde (MDA) as a secondary lipid oxidation product. Nanoencapsulated treatments performed best in reducing MDA formation, especially after day 6 of storage.

Overall, the findings suggest that nanoencapsulation of *Pelargonium* extracts improves antioxidant stability and efficacy, playing a crucial role in reducing lipid oxidation and enhancing the storage quality of lamb meat. Significant differences among treatments, particularly on days 6 and 12, underscore the importance of using nanoencapsulated antioxidants compared to free and synthetic forms. The effectiveness of plant phenolic compounds in reducing lipid oxidation is mainly attributed to their free radical scavenging activity and metal chelating properties. These compounds delay the oxidation process by breaking down lipid hydroperoxides. However, the antioxidant capacity varies among different phenolic compounds [33]. Previous studies have also demonstrated that both free and encapsulated plant extracts incorporated into edible coatings can reduce lipid oxidation in meat samples during storage [3, 31]. These results are consistent with the findings of Hass et al. (2017), who reported that buckwheat hull extract effectively reduced lipid oxidation in frozen meat [34].

Further research has confirmed that lipid oxidation in minced or ground red meat can be significantly inhibited by plant extracts such as wild thyme, sweet basil, *Alata*, and pomegranate peel [35–37]. Moreover, encapsulated extracts—such as those derived from pomegranate peel [1, 38], avocado peel [39], bay leaves [31], and pineapple peel [40]—have shown strong lipid oxidation inhibitory effects. This inhibition is mainly attributed to the high ability of plant bioactive compounds to neutralize reactive radicals generated during lipid oxidation [41]. The phenolic compounds present in both forms of *Pelargonium* extract are responsible for the inhibition of lipid

[DOI: 10.22034/FSCT.22.163.270]

oxidation, as they interrupt radical chain reactions during the oxidative process and exert potent antioxidant effects.

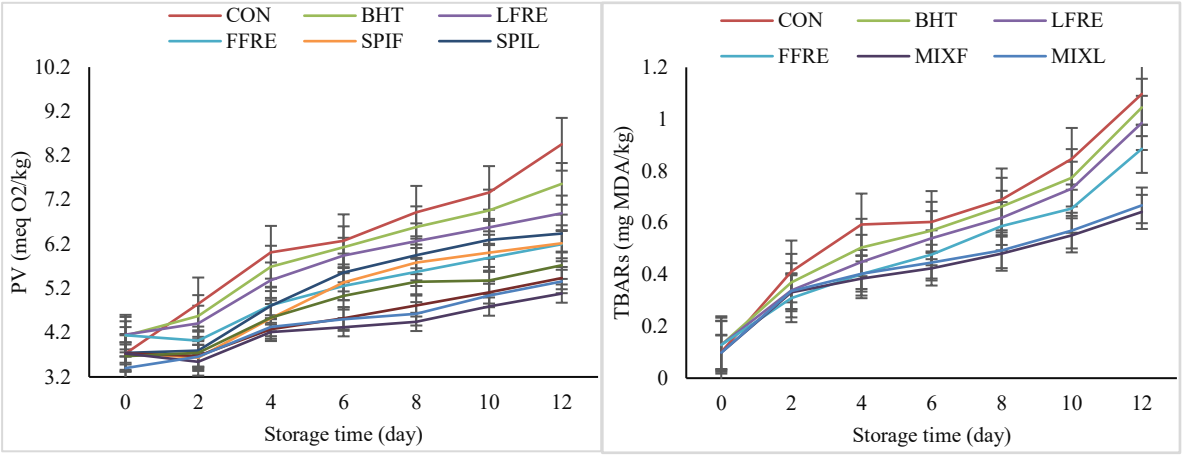

Fig. 1. Changes in the peroxide value and thiobarbituric acid of different mutton samples during storage

2.3. Total Microbial Count

The quality of meat deteriorates during storage due to microbial and chemical spoilage, which can be evaluated through parameters such as psychrotrophic bacteria count (PTC). Rapid microbial growth is the main cause of spoilage in meat products. Generally, when the total viable count of bacteria in meat products exceeds 10^6 CFU/g, the meat is considered unfit for consumption [42]. The results of psychrotrophic bacteria count, expressed as Log CFU/g, are presented in Figure 2. As shown, in the control sample (CON) with no protective treatment, the number of psychrotrophic bacteria continuously increased, consistent with findings from other studies [28]. On day 12 of storage, the PTC in the control sample reached 7.18 Log CFU/g, indicating severe microbial spoilage.

The BHT-treated group, containing a synthetic antioxidant, partially inhibited bacterial growth and reduced the PTC to 5.71 Log CFU/g by the end of the storage period. However, this was still less effective than the other treatments. Treatments containing free extracts from *Pelargonium* leaves and flowers (LFRE and FFRE) also significantly inhibited psychrotrophic bacterial growth, reducing the PTC on day 12 to 4.76 and 4.55 Log CFU/g, respectively. These results highlight the potential of *Pelargonium* extracts in mitigating microbial growth.

On the other hand, treatments with nanoencapsulated extracts showed superior antimicrobial effects compared to free extracts and synthetic antioxidants. The most effective treatments were the combined formulations (MIXF and MIXL), which utilized fenugreek seed gum and soy protein isolate as carriers for the nanoencapsulated extracts. In these groups, the PTC on day 12 dropped to 2.97 and 3.12 Log CFU/g, respectively, indicating a strong synergistic effect between the gum and protein in microbial inhibition. Statistical analysis also revealed significant differences among the treatments. The nanoencapsulated formulations, especially MIXF and MIXL, significantly reduced psychrotrophic bacterial growth compared to the other groups ($P < 0.05$). These findings indicate that nanoencapsulation of natural extracts with appropriate carriers can be an effective approach for inhibiting microbial growth at low temperatures and extending the shelf life of food products.

[DOI: 10.22034/FSCT.22.163.270]

Applying edible coatings acts as a physical barrier, preventing direct contact between meat and the external environment while also limiting nutrient transfer to microbial cells [43]. The delay in microbial growth can be attributed to the presence of bioactive compounds, particularly polyphenols, in the coated samples. Previous studies have shown that *Pelargonium* polyphenols exhibit antioxidant and antibacterial properties [44]. These compounds possess broad-spectrum antimicrobial activity, inhibiting both Gram-positive and Gram-negative bacteria, inducing morphological changes in microbial cells, and disrupting bacterial cell walls [32, 45, 46]. These findings are consistent with the study by Zhou et al. (2021), who applied a coating containing camellia oil combined with carrageenan and glucomannan to improve chicken meat quality. The coating significantly reduced microbial counts ($P < 0.05$) and extended the shelf life of the meat by inhibiting microbial growth [47].

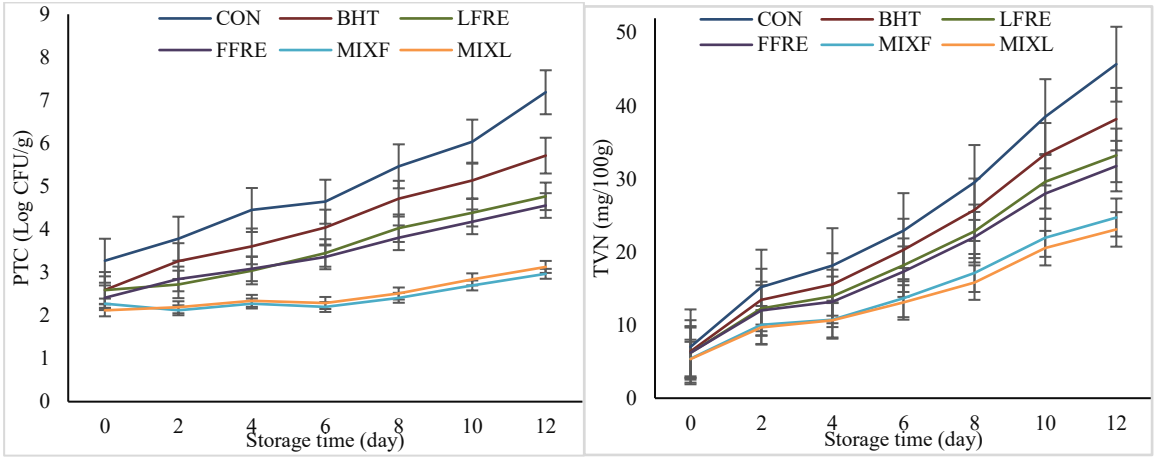

Fig. 2. Changes in the total psychrophilic bacteria and total volatile nitrogen basic of different mutton samples during storage

2.4. Total Volatile Basic Nitrogen (TVB-N)

One of the key chemical indicators for determining spoilage in seafood products is the level of total volatile basic nitrogen (TVB-N). This index refers to the total amount of volatile nitrogenous compounds such as trimethylamine (TMA), dimethylamine (DMA), ammonia, and other nitrogen-containing substances. These compounds are produced during various processes, including bacterial spoilage activity, spontaneous enzymatic reactions in meat during storage, and the catabolism of amino acids and nucleotides. TVB-N content is directly associated with microbial activity and the extent of spoilage. Both endogenous enzymes and bacterial activity in meat play a significant role in increasing TVB-N levels, making it a sensitive indicator for evaluating freshness and quality in seafood products [48].

The results related to TVB-N levels are shown in Figure 2. As can be seen, the levels of these compounds increased in all treatment groups during the storage period. This increase is attributed to protein degradation and the microbial production of alkaline compounds such as ammonia and volatile amines. In the control group (CON), TVB-N values significantly increased ($P < 0.05$), rising from an initial value of 7.03 mg/100 g to 45.71 mg/100 g on day 12, representing the highest level among all treatments. In the BHT group, the use of a synthetic antioxidant moderately slowed the increase in TVB-N, which reached 38.19 mg/100 g by day 12—a significant reduction compared to the control group ($P < 0.05$). In the treatments containing free *Pelargonium* leaf and flower extracts (LFRE and FFRE), TVB-N values reached 33.23 and 31.77 mg/100 g, respectively, on day 12. Due to the antioxidant and antimicrobial properties

[DOI: 10.22034/FSCT.22.163.270]

of the extracts, these treatments significantly inhibited the accumulation of volatile nitrogenous bases ($P < 0.05$).

The best results were observed in the combined formulations MIXF and MIXL, which utilized soy protein isolate and fenugreek seed gum along with nanoencapsulated extracts. On day 12, the TVB-N values in these groups were 24.73 and 23.10 mg/100 g, respectively—significantly lower than in the other treatments ($P < 0.05$). These findings highlight the strong impact of nanoencapsulation technology and the use of natural extracts in controlling microbial spoilage and reducing the production of volatile nitrogenous compounds.

These results are in agreement with the findings of Khalili et al. (2024), who reported a gradual increase in TVB-N levels in all minced meat samples during storage. The meat sample treated with nanoencapsulated pomegranate peel extract exhibited the lowest TVB-N content compared to both free pomegranate peel extract and TBHQ-treated samples. Their results demonstrated the beneficial effects of plant extracts in suppressing microbial growth, particularly proteolytic bacteria responsible for converting proteins into volatile nitrogenous compounds [1]. Similarly, Elbeltagi et al. (2022) reported that TVB-N levels increased over time in meat samples, with the sample treated with 0.5% raw pomegranate peel extract showing lower TVB-N levels than the one treated with 0.1% BHT [49].

2.5. Color Changes

The results of color parameter measurements during the storage period are presented in Table 3. The lightness index (L*) showed a significant decrease ($P < 0.05$) in all treatments. The greatest reduction occurred in the control group (CON), where L* declined from 39.82 on day 0 to 35.54 on day 12. The treatment containing the synthetic antioxidant BHT showed a smaller decrease in lightness, and the treatments with free extracts (LFRE and FFRE) also had a positive effect, though their performance was inferior to BHT and nanoencapsulated treatments. The combined treatments (MIXL and MIXF), which utilized soy protein isolate and fenugreek seed gum, exhibited the best preservation of lightness.

The redness index (a*) also decreased across all treatments during storage. The control group showed the greatest reduction in redness (from 16.82 to 13.12). BHT performed better in maintaining redness (from 16.88 to 14.31), and the LFRE and FFRE treatments showed less reduction compared to the control. The nanoencapsulated treatments (MIXL and MIXF) had the smallest decrease and were most effective in preserving the red color of the samples. These results align with findings by Deus et al. (2017), who studied the changes in lightness (L*) of turkey meat samples coated with silver nanoparticles during 12 days of storage and observed a time-dependent decline in lightness [2].

The yellowness index (b*) also decreased during storage. In the control group, the b* value dropped from 12.95 to 8.00. BHT and free extract treatments (LFRE and FFRE) showed less decrease compared to the control. However, the nanoencapsulated treatments (MIXL and MIXF) demonstrated the best performance in preserving the yellowness of the meat. Overall, the results indicate that nanoencapsulation technology—particularly with soy protein isolate and fenugreek seed gum—has a positive effect on maintaining color indices during storage and can effectively prevent oxidative deterioration. These findings are consistent with those reported by Sarvineh Baghi et al. (2021), who observed that the lightness of all tested meat samples decreased over the storage period, with a statistically significant difference between day 0 and day 20. Samples treated with the synthetic antioxidant BHT had higher values of brightness, redness, and yellowness, while those treated with free onion peel extract showed the lowest color scores. Additionally,

[DOI: 10.22034/FSCT.22.163.270]

samples coated with nanoemulsions had lower lightness and brightness values compared to those treated with BHT [5].

Table 3. Changes in the color index of different mutton samples during storage

Color index	Sample	0	2	4	6	8	10	12
L*	CON	39.82 ± 0.3^{Aa}	38.53 ± 0.2^{Bb}	37.18 ± 0.2^{Cfg}	36.70 ± 0.3^{Dg}	36.23 ± 0.8^{Eh}	35.69 ± 0.2^{Fg}	35.54 ± 0.1^{Gg}
	BHT	37.22 ± 0.2^{Ah}	37.01 ± 0.3^{Bi}	36.74 ± 0.5^{Ch}	36.55 ± 0.3^{Dh}	36.19 ± 0.4^{Eh}	35.87 ± 0.3^{Fh}	35.46 ± 0.2^{Gh}
	LFRE	39.22 ± 0.4^{Ab}	38.65 ± 0.6^{Ba}	37.47 ± 0.3^{Ce}	37.11 ± 0.4^{De}	36.63 ± 0.3^{Ef}	36.27 ± 0.4^{Ff}	35.44 ± 0.3^{Gh}
	FFRE	38.73 ± 0.2^{Ac}	37.55 ± 0.5^{Bh}	37.11 ± 0.9^{Cg}	36.83 ± 0.5^{Df}	36.42 ± 0.3^{Eg}	35.77 ± 0.6^{Fg}	35.31 ± 0.2^{Ga}
	MIXL	38.04 ± 0.5^{Ag}	37.92 ± 0.4^{Bf}	37.74 ± 0.5^{Cd}	37.49 ± 0.2^{Dc}	37.27 ± 0.4^{Ed}	37.09 ± 0.2^{Fd}	36.90 ± 0.3^{Ge}
	MIXF	38.01 ± 0.1^{Ag}	37.74 ± 0.5^{Bg}	37.49 ± 0.2^{Ce}	37.27 ± 0.2^{Dd}	37.09 ± 0.2^{Ee}	36.84 ± 0.5^{Fe}	36.49 ± 0.5^{Gf}
a*	CON	16.82 ± 0.1^{Ac}	16.27 ± 0.1^{Bfg}	15.85 ± 0.1^{Ce}	14.94 ± 0.3^{Df}	14.38 ± 0.3^{Eh}	14.28 ± 0.2^{Fi}	13.12 ± 0.4^{Gf}
	BHT	16.88 ± 0.4^{Ab}	16.01 ± 0.3^{Bh}	15.47 ± 0.1^{Cf}	14.78 ± 0.4^{Dg}	14.52 ± 0.1^{Eg}	14.43 ± 0.3^{Fh}	14.31 ± 0.1^{Ge}
	LFRE	16.75 ± 0.2^{Ad}	16.24 ± 0.2^{Bg}	15.68 ± 0.2^{Ce}	14.83 ± 0.3^{Dg}	14.66 ± 0.2^{Ef}	14.52 ± 0.1^{Fg}	14.43 ± 0.3^{Gd}
	FFRE	16.92 ± 0.1^{Aa}	16.32 ± 0.1^{Bf}	15.88 ± 0.1^{Cd}	15.36 ± 0.1^{De}	15.02 ± 0.3^{Ee}	14.63 ± 0.2^{Ff}	14.45 ± 0.1^{Gd}
	MIXL	16.73 ± 0.2^{Ad}	16.64 ± 0.1^{Bc}	16.52 ± 0.1^{Ca}	16.37 ± 0.5^{Dab}	16.22 ± 0.3^{Ea}	16.17 ± 0.2^{Fa}	16.03 ± 0.1^{Ga}
	MIXF	16.77 ± 0.2^{Ad}	16.70 ± 0.1^{Bb}	16.43 ± 0.1^{Cb}	16.25 ± 0.1^{Dc}	16.14 ± 0.1^{Eb}	16.09 ± 0.1^{Fb}	15.92 ± 0.1^{Gb}
b*	CON	12.95 ± 0.1^{Ab}	12.20 ± 0.1^{Bh}	11.57 ± 0.1^{Cg}	10.46 ± 0.3^{Df}	9.63 ± 0.3^{Ei}	9.14 ± 0.2^{Fj}	8.0 ± 0.4^{Gj}
	BHT	13.00 ± 0.4^{Aa}	12.17 ± 0.3^{Bh}	11.45 ± 0.1^{Ch}	10.49 ± 0.4^{Df}	9.87 ± 0.1^{Eh}	9.52 ± 0.3^{Fi}	8.87 ± 0.1^{Gi}
	LFRE	12.90 ± 0.2^{Ac}	12.34 ± 0.2^{Bg}	11.76 ± 0.2^{Cf}	10.68 ± 0.3^{De}	10.12 ± 0.2^{Eg}	9.73 ± 0.1^{Fh}	9.09 ± 0.3^{Gh}
	FFRE	13.03 ± 0.1^{Aa}	12.40 ± 0.1^{Bf}	11.91 ± 0.1^{Ce}	11.21 ± 0.1^{De}	10.51 ± 0.3^{Ef}	9.95 ± 0.2^{Fg}	9.25 ± 0.1^{Gg}
	MIXF	12.88 ± 0.2^{Ad}	12.65 ± 0.1^{Bc}	12.39 ± 0.1^{Ca}	12.28 ± 0.5^{Daa}	12.17 ± 0.3^{Eb}	11.97 ± 0.2^{Fb}	11.22 ± 0.1^{Gb}
	MIXL	12.91 ± 0.2^{Abc}	12.69 ± 0.1^{Bbc}	12.35 ± 0.1^{Cbc}	12.32 ± 0.1^{CDa}	12.27 ± 0.1^{Da}	12.07 ± 0.1^{Ea}	11.46 ± 0.1^{Fa}

Uppercase letters in each row indicate significant statistical differences between days of storage at $p<0.05$.
Lowercase letters in each column indicate significant statistical differences between samples at $p<0.05$.

2.6. Sensory Properties

The results of sensory evaluation of color in different meat samples during the storage period are presented in Figure 3. The type of treatment had a significant effect on color quality. The control group (CON) showed the weakest performance, with a sharp decline in color score from 4.83 on day 0 to 2.16 on day 12. The BHT-treated samples also experienced a similar decrease, with scores dropping from 4.66 to 2.83. In contrast, the combined treatments (MIXF and MIXL) exhibited the best performance in maintaining color, with final scores of 4.16 and 4.00, respectively, on day 12. The treatment type also played a crucial role in preserving odor characteristics. Both the control and BHT samples showed a substantial decline in odor scores—from initial values of 4.83 and 5.00 to 2.00 and 2.66, respectively, by day 12—indicating poor performance. Conversely, the MIXF and MIXL treatments maintained higher odor quality, with scores reaching 3.83 and 3.66, respectively, by the end of the storage period. These results highlight the synergistic effect of the active compounds in these formulations.

As shown in Figure 3, the flavor quality of the samples declined during storage. The control group exhibited a significant drop in flavor score from 4.83 to 2.33 on day 12. The BHT treatment, while slightly better, still showed limitations in flavor preservation, with a score decrease from 4.83 to 2.83. The MIXF and MIXL treatments demonstrated superior performance, maintaining flavor scores of 4.00 on day 12. Similarly, the sensory scores for texture decreased significantly in the control group due to structural degradation, dropping from 5.00 to 2.83. The BHT treatment experienced a gradual reduction, ending with a score of 3.00. In contrast, the combined

[DOI: 10.22034/FSCT.22.163.270]

treatments MIXF and MIXL had the least decline, with final texture scores of 4.16. These findings emphasize the positive effect of antioxidant and stabilizing compounds in preserving sensory attributes during cold storage.

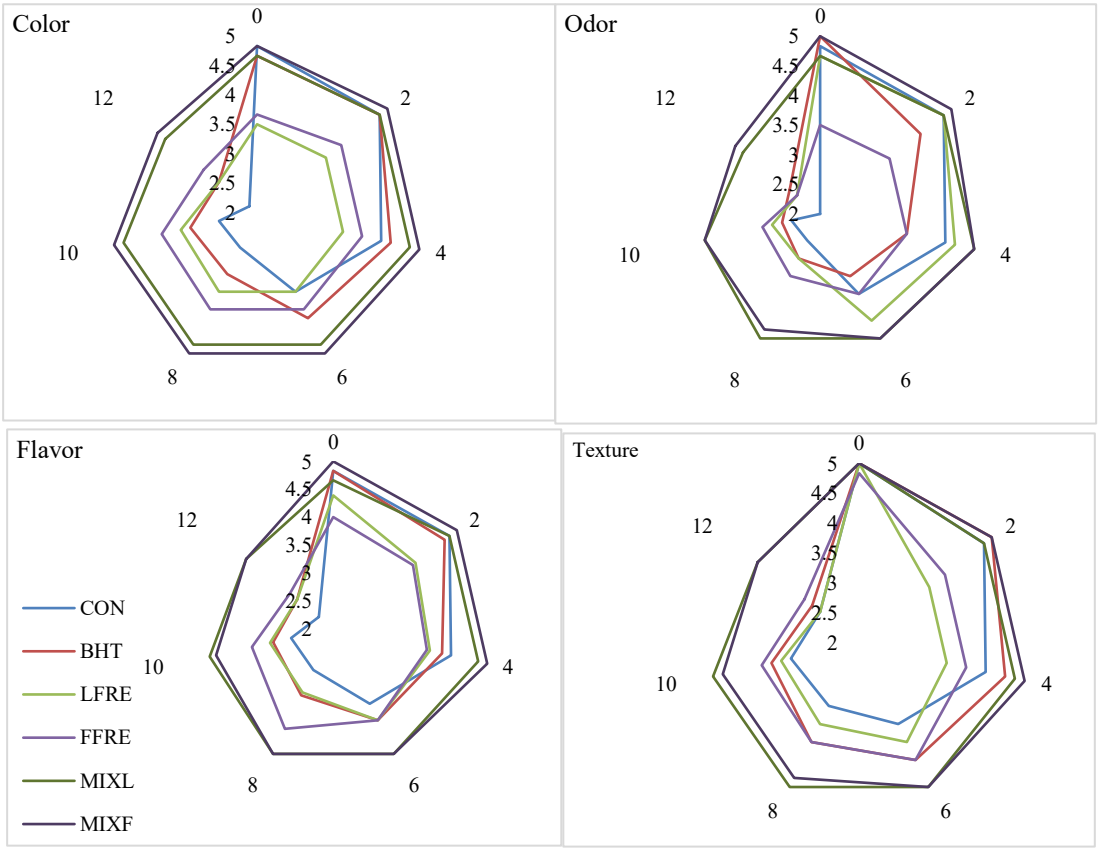

Fig. 3. Changes in sensory evaluation scores for color, odor, taste, and texture of different mutton meat samples during the storage

The trend of sensory attribute changes in different treatments during the storage period was consistent with the patterns observed in oxidation and microbial growth. This correlation is primarily due to lipid oxidation, which leads to the degradation and deterioration of sensory quality, a decrease in nutritional value (including the loss of essential polyunsaturated fatty acids), and the production of toxic oxidation products. Moreover, enhanced lipid hydrolysis and the accumulation of free fatty acids contributed to the decline in certain acceptability indicators, as free fatty acids are known to affect protein stability and cause textural deterioration through interactions with proteins. Encapsulation plays a key role in masking the undesirable flavors of phenolic compounds [31]. Sarvineh Baghi et al. (2021) attributed the higher sensory scores in onion extract-treated samples to the presence of organosulfur compounds, which are the primary contributors to the characteristic flavor and aroma of onion and significantly influence the sensory properties of treated meat samples, especially at higher extract concentrations [5, 50, 51]. Similarly, Sani et al. (2017) compared the sensory attributes—color, odor, texture, and overall acceptability—of coated and uncoated lamb meat samples. Their findings indicated that all sensory attributes declined over time, with the uncoated samples consistently receiving lower scores than the coated ones, which aligns with the findings of this study [52]. Mahmoudzadeh et al. (2010) also reported similar results for rosemary extract in fish

[DOI: 10.22034/FSCT.22.163.270]

burgers, showing extended shelf life and improved sensory quality up to day 10 [53].

As shown in Table 4, the overall acceptability score in the control group dropped significantly from 5.00 to 2.33. The BHT-treated samples followed a similar trend, with the score declining to 2.83. The combined treatments (MIXF and MIXL) demonstrated the most effective performance in preserving overall acceptability, with scores decreasing only slightly to 3.83 and 4.00, respectively, by day 12. The superior sensory performance of MIXF and MIXL treatments can be attributed to the synergistic effects of the active components, underscoring the potential of natural antioxidants and stabilizers in enhancing the shelf life and sensory quality of meat products.

Table 4. Changes in sensory evaluation scores for overall acceptance of different mutton meat samples during storage

Color index	Sample	0	2	4	6	8	10	12
	CON	5 ± 0.0^{Aa}	4.66 ± 0.3^{Bab}	4.16 ± 0.1^{Cb}	3.5 ± 0.1^{Db}	2.83 ± 0.3^{Ec}	2.66 ± 0.3^{Ec}	2.33 ± 0.1^{Fc}
	BHT	5 ± 0.0^{Aa}	4.5 ± 0.1^{Ba}	4.0 ± 0.1^{Ca}	3.83 ± 0.1^{Ca}	3.33 ± 0.3^{Db}	3.16 ± 0.3^{Db}	2.83 ± 0.3^{Eb}
	LFRE	4.66 ± 0.9^{Ac}	3.83 ± 0.3^{Bb}	3.83 ± 0.3^{Bba}	3.66 ± 0.3^{Bab}	3.33 ± 0.1^{CDb}	3.16 ± 0.1^{Db}	2.66 ± 0.3^{Eb}
	FFRE	4.16 ± 0.1^{Ac}	3.83 ± 0.1^{Bba}	3.72 ± 0.3^{Bab}	3.66 ± 0.3^{BCb}	3.66 ± 0.1^{BCb}	3.5 ± 0.1^{Cb}	3.0 ± 0.3^{Db}
	MIXL	5 ± 0.0^{Aa}	4.66 ± 0.3^{Bba}	4.66 ± 0.3^{BCa}	4.5 ± 0.1^{Ca}	4.5 ± 0.1^{Ca}	4.33 ± 0.1^{Da}	3.83 ± 0.1^{Ea}
	MIXF	5 ± 0.0^{Aa}	4.83 ± 0.3^{Bba}	4.83 ± 0.1^{Ba}	4.5 ± 0.1^{Ca}	4.5 ± 0.1^{Ca}	4.33 ± 0.3^{Da}	4.0 ± 0.3^{Ea}

Uppercase letters in each row indicate significant statistical differences between days of storage at $p<0.05$.
Lowercase letters in each column indicate significant statistical differences between samples at $p<0.05$.

4. Conclusion

In this study, for the first time, leaf and flower extracts of *Pelargonium* (geranium) were introduced as natural antioxidant and antimicrobial agents for extending the shelf life of lamb meat fillets. The results demonstrated that these extracts, due to their rich phenolic and flavonoid content, possess significant antioxidant and antimicrobial properties. Throughout the storage period, the extracts effectively delayed lipid oxidation reactions and microbial growth, showing a performance comparable to the synthetic preservative BHT. Moreover, nanoencapsulation of the extracts significantly enhanced their efficacy. Meat samples treated with nanoencapsulated extracts achieved the highest sensory evaluation scores, exhibited lower levels of lipid oxidation (as reflected by reduced peroxide and thiobarbituric acid values), and had slower psychrotrophic bacterial growth rates. Overall, the findings of this research suggest that nanoencapsulation is a promising technique for improving the functional performance of plant-based extracts as natural preservatives. *Pelargonium* flower extract, in particular, can be considered a competitive alternative to synthetic antioxidants such as BHT in meat preservation applications.

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[DOI: 10.22034/FSCT.22.163.270]

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مقاله علمی_پژوهشی

بررسی تاثیر آنتی‌اکسیدانی و آنتی‌میکروبی عصاره آزاد و نانوریزپوشانی شده برگ و گل شمعدانی عطری (*Pelargonium graveolens*) بر افزایش ماندگاری و خصوصیات حسی گوشت فیله گوسفند طی دوره نگهداری

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اطلاعات مقاله

تاریخ های مقاله :

تاریخ دریافت: ۱۴۰۳/۱۱/۳۰

تاریخ پذیرش: ۱۴۰۴/۱/۱۶

کلمات کلیدی:

آنتی اکسیدانی،
آنتی میکروبی،
عصاره شمعدانی،
گوشت فیله گوسفند،
نگهداری

DOI:10.22034/FSCT.22.163.270.

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چکیده

امروزه به دلیل محرز گردیدن اثرات سرطان‌زایی نگهدارنده‌های سنتزی، توجه به یافتن نگهدارنده‌های طبیعی افزایش یافته است. در این پژوهش عصاره برگ و گل شمعدانی به عنوان یک ترکیب آنتی اکسیدان و آنتی‌میکروب طبیعی با استفاده از فراصوت استخراج گردید و در پوشش صمغ دانه شنبلیله و ایزوله پروتئینی سویا نانوریزپوشانی شد. گوشت فیله گوسفند با استفاده از عصاره نانوریزپوشانی شده و عصاره آزاد برگ و گل شمعدانی با غلظت ۲۰۰۰ ppm تیمار شد و به مدت ۱۲ روز در دمای ٤ درجه سلسیوس نگهداری گردید. یک نمونه گوشت بدون نگهدارنده و یک نمونه حاوی ۱۰۰ ppm نگهدارنده سنتزی BHT به عنوان شاهد در نظر گرفته شد. نتایج آزمون‌های اکسیداسیون چربی (عدد پراکسید و تیوباربیتوریک اسید)، شمار باکتری‌های سرمادوست و بازهای ازته فرار که در فواصل زمانی ۲ روزه اندازه گیری شده بود نشان داد که عصاره‌های حاصله به هر دو شکل آزاد و نانوریزپوشانی شده توانای به تاخیر انداختن واکنش‌های اکسیداسیون چربی و رشد میکروبی را دارا بودند. کمترین میزان فساد اکسایشی و میکروبی در نمونه‌های گوشت تیمار شده با عصاره‌های نانوریزپوشانی شده مشاهده شد. همچنین این نمونه‌ها دارای امتیاز ارزیابی حسی بالاتری طی دوره نگهداری بودند. با توجه به اینکه عصاره برگ و گل گیاه شمعدانی حاوی ترکیبات فنولی و فلاونوئیدی است، لذا علاوه بر افزایش عمر ماندگاری گوشت گوسفند، می‌تواند ارزش تغذیه‌ای آن‌را نیز افزایش دهد. نتایج این تحقیق استفاده از عصاره نانوریزپوشانی شده گل شمعدانی در غلظت ۲۰۰۰ ppm در دیواره صمغ دانه شنبلیله و ایزوله پروتئینی سویا را جهت افزایش ماندگاری گوشت گوسفند پیشنهاد می‌نماید.

[DOI: 10.22034/FSCT.22.163.270]