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Pomegranate Peel extract in Edible films: Impact on Microbial Growth Oxidative Stability, and Sensory Quality of Beef

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I. Abstract

This study assessed the antimicrobial, antioxidant and organoleptic effects of pomegranate peel powder (POP) incorporated into sodium alginate films and the mechanical and chemical properties of films enriched with pomegranate peel extract (PPE). Bovine samples were coated with films containing 0% (control), 2.5%, 5%, 7.5% and10% POP, and their quality was analyzed during 14 days of refrigerated storage. Microbial counts, including the aerobic plate count (APC), psychrotrophic count (PTC), and Enterobacteriaceae count (EC), significantly decreased with increasing POP concentrations. The 10% POP (Pop4) treatment demonstrated robust antimicrobial activity, maintaining APC at 3.503 log CFU/g compared to 7.630 log CFU/g in the control by day 14. Oxidative stability was significantly improved, with peroxide value (PV) and thiobarbituric acid reactive substance (TBA) levels in Pop4 reduced by 53.6% and 76.7%, respectively, compared to the control. Sensory analysis indicated superior preservation of color, odor, and overall acceptability in POP-treated samples. Concurrently, films enriched with PPE exhibit enhanced mechanical and barrier properties. Increased PPE concentrations improved film thickness (0.062 mm in T4, 10% PPE), tensile strength (46.450 MPa in T4), and water vapor barrier properties (0.005–0.007 g/m² over 24 h), while reducing moisture content and enhancing elongation at break (4.57% in T4 compared to 3.11% in control films). These findings indicate that edible films enriched with POP and PPE demonstrate significant potential for extending the shelf life, enhancing safety and improving the sensory characteristics of fresh meat. Furthermore, these films

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exhibit enhanced functionality, offering a natural and environmentally sustainable approach for food preservation.

Key word: Antimicrobial- Antioxidant- Organoleptic- Sodium alginate films- Mechanical properties.

II. INTRODUCTION

Packaging technology plays a crucial role in safeguarding food throughout production, storage, transportation, and distribution by preventing physical, biological, microbial, and chemical contamination. This ensures that the food reaches consumers without significant sensory or quality deterioration (1- 3). Active food packaging, a prominent and innovative technology in the field of smart packaging, utilizes biological components in natural polymers to protect foods against anomalies and microbial proliferation. This methodology considers the interaction between food and its packaging environment to preserve food quality and prolong its shelf life (4, 5). By doing this, the customer and the environment are both protected. As sodium alginate already has antibacterial and antioxidant properties and is biodegradable and environmentally benign, pomegranate husk extract helps sodium alginate films work in concert with it (6, 7). Alginic acid sodium salt (sodium alginate) is a natural, harmless, and non-toxic polysaccharide that is added to food. When calcium cations are present, they can create a translucent elastic gel (8). Therefore, the creation of edible coatings and films is a technological approach that helps the food industry value co-products because they can preserve their nutritional value and sensory qualities for longer periods, thereby acting as a barrier to mitigate the effects of packaging during storage (9, 10). These films solve the issues of degradability and limitations while utilizing natural ingredients, such as proteins and polysaccharides, to provide crucial advantages for food safety and preservation (11). As sodium alginate can integrate bioactive substances and improve antibacterial activity against microbes, it is a particularly beneficial choice (12). Of the entire fruit, 48–50% of the pomegranate skin that remains after the juice of the arils is extracted is waste material (13). It is used as an antibacterial, preservative, and antioxidant in the food processing and medical sectors owing to its hydrolyzable tannins and phenolic compounds (14- 16).The food industry, particularly the meat sector, grapples with microbial contamination and food-related illnesses, prompting the need for novel preservation techniques. Edible films incorporating bioactive compounds, such as PPE, present a

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sustainable and environmentally favorable alternative. These films possess the capability to extend product shelf life, preserve sensory and nutritional characteristics, and diminish the necessity for synthetic preservatives (17, 18). Recent investigations have demonstrated that films enriched with PPE can efficaciously inhibit bacterial proliferation, mitigate oxidation, and enhance the stability of meat products (19, 20). Consequently, this study sought to examine the physical, chemical, and mechanical characteristics of sodium alginate films fortified with pomegranate peel extract. Furthermore, this study assessed the antimicrobial efficacy of these films and their influence on the shelf life, sensory properties, and overall quality of ground meat during cold storage. The research aimed to investigate the viability of PPE-enriched edible films as a sustainable and efficient method for enhancing food preservation and safety.

III. MATERILAS AND METHODS

Sodium alginate (SA), calcium chloride /CaCl2, Glycerol as plasticizers were purchased from a local chemical market, gallic acid and Folin-Ciocalteu as reagents from Sigma-Aldrich, Sodium Carbonate, Pomegranate peel, Ethanol, Deionized water, and distilled water.

1.1. Extraction of Pomegranate peel (POP)

Sazan hamlet in Halabja city, which is north of Suliamni, is where the pomegranates were purchased. After cleaning the dust and other contaminants with distilled water, the pomegranates were allowed to air-dry. After manually separating the distinct parts of the fruit (skin and arils), the pomegranate peel was chopped into tiny pieces with a sharp knife and dried for 22–24 h at 55 °C in a hot air oven. Before use, the dried pomegranate peel was kept in a glass bottle at 4 °C (Refrigeration) after being finely mashed using a laboratory blender (Chico, First Austria) and sieved to a fine powder (500 µm) using a laboratory test sieve (Prufsieb 500 µm) (21). Pomegranate peel extract (POPE) was extracted by mixing 250 mL of 80% ethanol with 10 g of finely ground dried powder. Overnight, it was shaken constantly at room temperature while it was on a magnetic stirrer. Whatman filter paper no.1 was used to filter the solvent. After dying, they escaped from the glass ptri dish that had been sanitized and placed in an

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incubator set at 50°C for 48 h. Subsequently, they were placed in airtight bottles and maintained at 4°C until further examination (22).

1.2. Preparation of Na alginate- (POPE) Edible film

Edible films were synthesized from alginate and pomegranate peel extract utilizing a casting technique. The process commenced with the preparation of a 1% (w/v) sodium alginate solution by combining sodium alginate powder with 100 mL of deionized water and agitating for 30 min at 70 °C. Subsequently, the solution was cooled to 55 °C, and 0.75% (v/v) glycerol was incorporated, followed by 15 min of homogenization. Powdered pomegranate peel extract was then introduced to the film solution at four distinct concentrations (w/v): 2.5, 5, 7.5, and 10%. Subsequently, 50 mL aliquots of the prepared solutions were transferred to 10 × 10 cm square Petri plates and subjected to desiccation in an oven (Memmert, Germany) for 24 h at 50 °C (23). The resultant sodium alginate film was immersed in 45 mL of calcium chloride solution and further desiccated for one minute. Finally, the films were carefully extracted using forceps and stored in desiccators for subsequent analysis.

1.3. Films characterizations

1.3.1. Physical properties of Na alginate- (POPE) Edible film

1.3.1.1. Film Thickness

A handheld digital micrometer (Mitutoyo Tester Sangyo Co Ltd.. Ltd., Tokyo, Japan) to measure the thickness of the alginate-pop extract films. The thickness was determined using the average values derived from five randomly selected locations on the films (23).

1.3.1.2. Moisture Content (MC)

The edible film composed of alginate and pomegranate peel extract was evaluated for its moisture content utilizing a method replicated a minimum of three times, adhering to the guidelines established by (24) Samples of the film weighing 0.5 g were measured prior to and following a drying process at 105◦C for 24 hours. The moisture content (MC) was subsequently calculated using a specific equation. This procedure for determining the moisture content of the edible film aligns

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$$Moisture\% = \frac{W1 - W2}{W1 - W0} \text{x} 100$$

1.3.1.3. Water vapor permeability (WVP)

A small glass container was utilized to assess WVP, with uniform dimensions of 4, 4.1, and 4.5 cm for internal, external, and length depths, respectively. Films were affixed and sealed to the container openings using O-rings. Each container was subsequently placed in a desiccator with 55 ± 1% relative humidity, filled with 10 ml of distilled water, and weighed using silica dioxide. Eight measurements were recorded over a 24-hour period. The water vapor transmission rate was determined by dividing the slope of the weight gain versus time graph by the exposed film area. WVP measurements of the films were conducted in triplicate using a modified version of the ASTM E96-01 (25) Films were sealed on glass containers (4 cm o.d., 3.8 cm i.d., 4 cm depth) filled with silica gel using an O-ring lastic. The containers were stored at 2 °C in electronically regulated incubators within desiccators containing saturated Mg (NO3)26HNO3 solution (50 percent RH). A 50:0 RH gradient (outside RH: inside RH) was maintained. Containers were weighed (0.0001 g) at predetermined intervals once weight changes stabilized. WVP was calculated as follows:

$$WVP = \frac{WVTR.x}{P0.(RH2 - RH1)}$$

x is the film thickness (mm); P0 is the vapor pressure of pure water (25C, 3.159 kPa); and (RH1 :RH2) is the relative humidity gradient used in the experiment (25).

1.3.2. Mechanical properties of Na-alginate-POPE edible film

Tensile strength (TS) and Elongation at the break (EB)

Based on the ASTM D882-12 method, (25) used an electronic universal tensile testing machine (Biobase, China) to measure mechanical parameters such as tensile strength (TS) and elongation at break (EB). The film was cut into rectangular pieces (2 cm × 7 cm), and the clamp distance and tensile speed were

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adjusted to 40 mm and 10 mm/min, respectively. Based on the displacement and tension, the following formula was used to determine the TS (MPa) and EB (%) of the film:

$$TS(MPA) = \frac{MaximunTensileForceWhenTheFilmBroke(N)}{CrossSectionalArea(mm2)}$$

$$EAB\% = \frac{MaximumLenghtReachedWhenTheFilmBroke(mm)}{InitialLengthOfFilm(mm)} \times 100$$

1.4. BEEF SAMPLE ANALYSIS

1.4.1. Microbiology analysis

- In this study, each sample (10 g) was combined with 90 mL of sterilized peptone water and homogenized using a stomacher. The resulting mixture was serially diluted and plated on solid culture media. Microbial enumeration was conducted for three categories: (1) Aerobic plate counts (APC), cultured on plate count agar (PCA) at 30 ◦C for 48 h; (2) Psychrotrophic total counts (PTC), grown on PCA at 7 ◦C for 10 days (26) and (3)Enterobacteriaceae counts at 37°C for 24 hours in violet red bile glucose agar (27). All microbial assays were conducted in triplicate to ensure data reliability and accuracy.

1.4.2. Chemical Analysis

1.4.2.1. pH determination

For each raw minced beef sample, 10 g was combined with 100 mL of water. The resulting mixtures were homogenized and filtered. Subsequently, the pH of the filtrate was determined using a pH meter. Calibration of the pH meter was conducted at pH 7.0 and 4.0, employing standard buffers maintained at room temperature (20 ◦C). This procedure adheres to the methodology described by (28) for accurate pH assessment in meat products.

1.4.2.2. Assessment of lipid oxidation

Evaluation of lipid oxidation. The peroxide values (PV) of beef samples were assessed using the Folch method as outlined by (20).PV was measured in milliequivalents of peroxide per kilogram of beef. A 1 g sample of beef was mixed in 10 mL of distilled water and homogenized for the analysis of conjugated dienes (CD). A 0.5 mL aliquot of this combination was mixed with 5 mL of a hexane and isopropanol

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solution in a 3:1 (v/v) ratio. After spinning for 5 minutes, the absorbance of the supernatant was assessed at 233 nm. CD concentrations were quantified in µmol/mg of meat sample (29). Secondary lipid oxidation products were measured using the TBARS (thiobarbituric acid reactive substances) test. A solution comprising 2 g of meat, 100 µL of butylated hydroxytoluene (1 g/L), and 16 mL of trichloroacetic acid (50 g/L) was subjected to filtration. Subsequently, 2 mL of the filtrate was combined with 2 mL of a 20 mol/L thiobarbituric acid solution. The resultant mixture was heated to 100 °C and then cooled. TBARS values were quantified as mg of malonaldehyde (MDA) per kg of the sample.

2. Sensory Evaluation

A cohort of 22 untrained individuals (11 females and 11 males) from the University of Sulimani participated in the evaluation of the sensory attributes of raw minced beef packaged with POP films. The participants, ranging in age from 20 to 48 years, were non-smokers who regularly consumed beef. They assessed three subsamples (control, POP1, POP2, and POP3) at different time intervals (0, 7, and 14 days). The evaluation focused on color, appearance, odor, and overall acceptability, utilizing a 9-point hedonic scale. This scale ranges from 9 (like extremely) to 1 (dislike extremely), with intermediate values representing varying degrees of preference or aversion. Any sample receiving a score below 5 was deemed unacceptable (30).

3. Statistical Evaluation

Data analysis was conducted utilizing Microsoft Excel 2000 and Origin 6.1 software. To compare the means of properties. A one-way analysis of variance (ANOVA) was used to assess statistical differences among group means, and the least significant difference (LSD) post was employed to identify significant differences between treatments.The results were statistically analyzed by applying the statistical analysis system (31) (32).

I. Results and Discussion

3.1. Thickness of Edible film

According to the edible film thickness values (Table 1), the edible film made with sodium alginate without pomegranate peel had a significantly different thickness than the edible film made with pomegranate peel at varying concentrations. Every recipe in this study contained an edible film that satisfied the Japanese Industrial Standard for Thickness (0.25 mm). When compared to the work by (33) , the edible film without pomegranate peel in this study had a lower thickness value, measuring between 0.14 and 0.71 mm. Variations in the thickness values are caused by the printing surface area and heating application technique. The edible thickness of the sodium alginate film containing pomegranate peel in

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this study was lower than that reported by (34). Preparation techniques, including drying, solvent evaporation duration, relative humidity, and dish surface, have a direct impact on the film material thickness (35). According to (36), the matrix is supplemented with a variety of substances, including plasticizers, antioxidants, and antibacterial chemicals. In contrast to the other evaluated samples, the control film samples in this investigation displayed a lower thickness (0.04–0.029 mm). T4 (0.062-0.029 mm) film samples had the maximum thickness, followed by T3 (0.056-0.046 mm) and T2 (0.051-0.040 mm) film samples (Table 1). According to these findings, the intermolecular interactions between the functional groups caused the edible film thickness to increase as the pomegranate peel extract concentration increased (20). Pomegranate peel extracts had no discernible effect on the thickness of the Na-alginate films ($p < 0.05$). These findings are consistent with those of earlier research conducted by (37) . According to their findings, the thickness of the film was not considerably affected by the addition of natural antioxidants, such as cinnamon, cloves, and star anise. According to (38), the addition of pomegranate peel extract to edible films had no discernible effect on the final film thickness. (39, 40), studied the effects of pomegranate peel extract on the thickness of films composed of mung bean protein, fish gelatin, and gluten. The findings of this study are in agreement with these findings.

3.2. Moisture

As the concentration of pomegranate peel extract increased, the edible films exhibited a decreasing trend in moisture content (Table 2). The control (TC) film exhibited a higher moisture content when compared to the other films evaluated in the study, ranging from 37.667% to 30.267%. The T4 film samples had the lowest moisture content in the fourth week (20.667%), followed by the T2 film samples (21.6%).

The experimental findings demonstrate that an increase in the concentration of pomegranate peel extract within the matrix resulted in a corresponding decrease in the water content of the sodium alginate-based film. This was most likely caused by molecular interactions and modifications to the hygroscopic properties of the sodium alginate matrix. Contradictory findings were reported by (41), who discovered that the addition of pomegranate peel extract to a gluten-based matrix considerably increased the moisture content of the film (43.53%) compared to regular film (35.91%). The findings of this study are in conflict with those of a prior work by (42), who found that adding Codium tomentosum seaweed extract increased the moisture content of a film made of chitosan and alginate.

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3.3. Water vapor permeability (WVP)

Pomegranate peel administration differs significantly depending on whether it is edible or film-based, according to the water vapor transmission value. When pomegranate peels are added to edible alginate films, the water vapor transmission is significantly different from that when they are not. According to the water vapor transmission test findings, the film formulation that includes pomegranate peels has the highest water vapor transmission value among the three formulae and complies with the Japan Industrial Standard. When pomegranate peels were added to the film formula, the water vapor transmission value was the same as that found in the study by Sunardi and (43) ,who found that the water vapor transmission value of films containing pomegranate peels ranged from 0.005 to 0.007 g/m2.24 h a day (Table 3). Subsequently, a movie without a pomegranate peel was released. This is because the hydrophilic constituents of the film, sodium alginate, and pomegranate peels can accelerate the passage of water vapor (44). Of the three formulae, the film containing casein had the lowest moisture transmission value and satisfied the Japan Industrial Standard. The addition of pomegranate peels may result in flexible films with relatively high permeability to water vapor and low permeability to oxygen and carbon, which would change the low value of water vapor transmission in the films (45). The rate of water vapor transmission in edible films can be increased by a variety of factors, such as film thickness (the thinner the film, the higher the water vapor transmission), composition of the materials used to form the film, swelling caused by water vapor, and humidity of the surrounding environment during testing (46).

3.4. Mechanical tests

In Table 4, sodium alginate films containing pomegranate peel extract had tensile strengths ranging from 46.450 MPa in T4 in the first week to 43.4 in T2 in the fourth week. The elongation rate was 4.57% in T4 during the first week in the extract-supported film, which was higher than that of the sodium alginate film with 3.11% extract. Every week and transaction, it outperformed the control group. Regarding the tensile strength, it was discovered that adding pomegranate extract to the film enhanced its tensile strength, which was 45.01 MPa for the control and 46.45 MPa for the first week in T4. According to (46), the chitosan film's tensile strength was 20.8 MPa; however, after applying pomegranate extract. The value rose to 29.9 MPa. The addition of pomegranate peel extract to sodium alginate membranes likely improved their tensile strength and elongation due to the presence of phenolic compounds. These compounds, containing multiple OH groups, can form hydrogen bonds with sodium alginate, potentially enhancing its mechanical properties. Furthermore, the interaction between tissue and polyphenolic

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chemicals in the pomegranate peel extract increased. The tensile strength of the sodium alginate membrane supported by the extract may be enhanced by the increased thickness of the membrane (47). Elongation, or elongation percentage, is a crucial factor in determining film flexibility. When the elongation values are less than 10%, 10-50%, and more than 50%, the JIS classifies the edible film as bad, good, or very good, respectively. The observed enhancement in tensile strength and elongation of chitosan films incorporating pomegranate peel extract may be attributed to the interaction between the extract's polyphenolic compounds and the structural composition of the tissue. As well as the presence of several OH groups in the phenolic compounds that form hydrogen bonds with sodium alginate. The tensile strength of the sodium alginate film supported by the extract may be enhanced by increased film thickness (48).

3.5. Microbial tests

The figure illustrates the variations in microbial populations for Aerobic Plate Count (APC), Psychrotrophic Plate Count (PTC), and Enterobacteriaceae Count (EC) under diverse treatment conditions and storage durations.The treatments included a control group and four concentrations of pomegranate peel powder (Pop1–Pop4) incorporated into a sodium alginate matrix. These microbial counts provide critical insights into the antimicrobial efficacy of pomegranate peel powder (POP). APC is a key indicator of bacterial growth. The control group exhibited the highest microbial load, starting at 2.023 on day 0 and increasing to 7.630 log CFU/g by day 14, slightly exceeding the acceptable limit of 6.0 log CFU/g as established by AFNOR standards (49) . This highlights the insufficient microbial control in untreated systems and underscores the necessity for effective antimicrobial interventions. In contrast, treatment with increasing concentrations of POP resulted in a progressive reduction in bacterial counts. The most effective treatment, Pop4 (10% POP), began with an APC of 2.013 and increased to only 3.503 log CFU/g by day 14, which was less than half of the microbial load observed in the control group. This indicates robust microbial inhibition at higher POP concentrations. These findings align with previous studies (50, 51), which reported exponential microbial growth in untreated systems during storage. The significant reduction in APC for Pop4 corroborates the findings of (52, 53), who highlighted the antimicrobial properties of polyphenols such as ellagic acid and punicalagin in pomegranate peel extracts. These compounds disrupt bacterial cell membranes, thereby inhibiting their growth. The LSD value for APC (0.352 log CFU/g) showed statistically significant differences across the treatments,

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emphasizing the concentration-dependent antimicrobial efficacy of POP; consequently, the application of coatings containing antimicrobial agents could prove more efficacious, as they are gradually released at the food's surface (54). Furthermore, the utilization of sodium alginate packaging successfully impeded and/or restricted microbial proliferation due to the protective protein biofilm layer surrounding meat samples, which inhibited oxygen penetration and microbial expansion (54).

PTC analysis revealed similar trends to APC, with bacterial growth increasing over time, especially in the control group, which increased from 1.843 log CFU/g on day 0 to 8.530 log CFU/g on day 14which exceeded the limit approved by (48) (6.0 log CFU/g).. Notably, Pop1 (2.5% POP) exhibited slightly higher final values (8.773) than the control, likely due to the reduced inhibitory effect of lower POP concentrations and the adaptability of psychrotrophic bacteria to cold environments(54). However, higher concentrations of POP (Pop3 and Pop4) consistently suppressed psychrotrophic bacterial growth. Pop4 demonstrated the strongest inhibition, with PTC values initiating at 1.823 log CFU/g and increasing to only 5.397 log CFU/g after 14 days, a 36.7% reduction compared to the control.

This is consistent with findings by (55), who highlighted the inhibitory effects of higher polyphenol concentrations on psychrotrophic bacteria, which are major contributors to spoilage in refrigerated foods. The LSD value for PTC (0.256 log CFU/g) confirmed significant differences among the treatments, reinforcing the efficacy of POP in reducing psychrotrophic bacterial adaptation to cold storage. The EC results showed slower bacterial proliferation than the APC and PTC results did. The control group showed a steady increase from 0.800 on day 0 to 2.417 on day 14. Pop4, despite starting with a slightly higher initial value (0.867 log CFU/g), achieved the lowest growth rate, reaching only 1.240 log CFU/g by Day 14. This was significantly lower than that of the control, indicating the antimicrobial potential of higher POP concentrations.

Interestingly, minimal growth of Enterobacteriaceae was observed during the firacross all treatments, indicating the presence of additional inhibitory mechanisms. On the first day, the Enterobacteriaceae counts in all meat samples were approximately 0.8 log CFU/g. In the control sample, bacterial counts reached the minimal spoilage level (2.417 log CFU/g [49], after 14 days. However, meat packaged withfilms showed significantly ($P < 0.05$) lower Enterobacteriaceae counts compared to the control.This finding is consistent with previous research (56), which demonstrated that tannins in pomegranate peel suppress the proliferation of Enterobacteriaceae by forming protein complexes that reduce microbial viability. Recent studies have highlighted the notable antimicrobial properties of pomegranate peel

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powder, reinforcing its broad-spectrum effectiveness. For instance, both (57, 58) reported significant reductions in APC, PTC, and EC levels in food systems treated with pomegranate peel extracts, particularly at concentrations exceeding 7%. The antimicrobial effects of pomegranate peel powder (POP) can be attributed to its high content of elliptical tannins and polyphenols, which compromise bacterial cell membranes and inhibit microbial growth.

Lower concentrations of POP (Pop1 and Pop2) occasionally showed sublethal bacterial adaptation, as suggested by (58, 59). This underscores the importance of using higher concentrations, such as Pop3 (7.5% POP) or Pop4 (10% POP), to consistently suppress microbial activity and prevent adaptation. The LSD values for all microbial counts confirmed statistically significant differences among the treatments, further validating the concentration-dependent antimicrobial efficacy of pomegranate peel powder.

3.6. Oxidative tests

In table 6, we can see some information regarding Pops 1 to 4 and their effects on the peroxide value (PV), thiobarbituric acid reactive substances (TBA), and pH during and after the 14 days of storage. For the controls, we see a more substantial PV of 8.850 after a week, which signifies that there was significant lipid oxidation due to a lack of protective antioxidants. On the other hand, the treated sample Pop4 showed significantly lower PV of 4.103 after 14 days. The reason why Pop4 has lower lipid oxidation is probably because it has a higher percentage of pomegranate peel 10% which is rich in polyphenols that neutralizes free radicals, which stablilizes lipids, supporting the previous studies (37, 38). The decrease in PV by day 14 likely resulted from hydroperoxide decomposition exceeding conjugated diene (CD) formation (61). These results highlight the superior antioxidant capacity of polyphenols in retarding lipid deterioration.

TBA values also indicated advanced secondary lipid oxidation in the control group 3.000 at 14 days, whereas Pop4 achieved substantially lower values of 0.700. This supports the protective effects of pomegranate peel compounds against oxidative by-products, possibly through a combination of radical-scavenging mechanisms and the reduction of hydroperoxide breakdown products, as corroborated by studies on plant-based polyphenols (62) Malondialdehyde (MDA), a key marker generated during lipid degradation, aligns with the observation The TBARS values of all samples increased during storage(63, 64), but were significantly lower in samples treated with antioxidant-rich films (64). Similar results were observed in studies utilizing dill essential oil with Plantago major seed mucilage films, which demonstrated reduced TBARS values compared to controls (66, 67).

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The pH levels in the control group steadily increased during storage, peaking at 8.123 on day 14, which was associated with protein breakdown and microbial activity. However, the treatments, particularly Pop4, maintained stable pH levels (6.290 on day 14). This stabilization can be attributed to the antimicrobial properties of pomegranate peel, which inhibits microbial growth and enzymatic degradation. These findings align with earlier studies where antioxidant-enriched compositions minimized microbial spoilage and stabilized pH (63, 66). The observed pH increase indicates the degree of spoilage due to protein breakdown, which was partially mitigated in samples treated with antioxidant films, supporting their protective role (66).

The low LSD values for PV (0.185), TBA (0.33), and pH (0.347) indicated statistically significant differences among treatments and storage times. These findings collectively demonstrate that increasing the concentration of pomegranate peel powder (particularly in Pop4) effectively mitigates oxidative and microbial spoilage, extending product shelf life by stabilizing lipids and reducing protein degradation. These results align with previous research emphasizing the importance of natural antioxidants in improving food stability and quality during storage (68- 70).

4. Sensory

The results of appearance and odor analyses indicated significant effects of treatment and storage duration on beef quality, with notable variations observed among treatments. With respect to appearance, the control group exhibited a significant decline from 8.125 on day 0 to 5.250 on day 14, falling below the acceptability threshold, presumably due to oxidative discoloration and microbial proliferation.(29, 71) In contrast, Pop4 exhibited higher stability, with values remaining constant at 6.750 on day 14, attributed to the antioxidant effects of pomegranate peel extract. Studies such as (29, 58, and 72) additionally, similar enhancement of visual quality in food products treated with bioactive compounds was observed, corroborating the current findings (73).

Regarding odor, the superior efficacy of treatments was evident, as the control group exhibited a significant decrease in odor scores from 8.750 on day 0 to 3.625 on day 14, accompanied by the development of rancid aromas, corroborating findings by (73) who reported rapid odor deterioration in untreated meat. Similarly, Pop4 demonstrated the highest efficacy in delaying off-odor development, with relatively stable values (7.500 on day 0 to 7.250 on day 14), aligning with the results of (55). Notably, Pop1 and Pop2 treatments, despite their initial high odor scores (8.250 and 7.625, respectively), exhibited significant reductions over time (5.125 and 4.750 by day 14), indicating diminished efficacy

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compared to higher concentrations, such as Pop4. These variations can be attributed to the differing antioxidant capacities and interactions with storage factors (74).

In accordance with numerous studies elucidating the role of polyphenols in mitigating oxidative and microbial degradation(60, 62, 63), this study consistently demonstrated that higher concentrations of pomegranate peel (Pop4) effectively preserved appearance and odor, indicating its potential to extend shelf life and maintain sensory quality.

Table 1. Thickness of edible films with and without pomegranate peel at different concentrations

Properties	Treatments	Storage				
		W1	**W2**	**W3**	**W4**	**LSD valve**
Thickness	**TC**	**0.041** bcd	**0.04** bcd	**0.037** cde	**0.029** f	**0.006**
	T1	**0.046** ab	**0.041** ab	**0.036** abc	**0.034**abc	
	T2	**0.051** abc	**0.05** abc	**0.047** abc	**0.04** bc	
	T3	**0.056** abc	**0.054** bcd	**0.05** def	**0.046** ef	
	T4	**0.062** a	**0.053** abc	**0.049** abc	**0.029** f	
LSD value		**0.011***				

- Values with different letters (a, b, c, etc.) indicate statistically significant differences based on LSD (Least Significant Difference) tests, and if there has same letter means not significant differences at $p < 0.05$**.** TC: Sodium alginate film as control, T1: Sodium alginate film with 2.5% PoPE, T2: Sodium alginate film with 5% PoPE, T3: Sodium alginate film with 7.5% PoPE, T4: Sodium alginate film with 10% PoPE. *: Numbers in a table representing an average of triplicate**.**

Table 2. Moisture contents of edible films with and without pomegranate peels at different concentrations.

Properties	Treatments		Storage			
		W1	**W2**	**W3**	***W4***	***LSD value***
Moisture %	**TC**	**37.667**a	**31.533**a	**30.267**b	**30.733** bc	
	T1	**27.867**cd	**27.067**de	**26.067**de	**25.333** de	***0.04***
	T2	**25.067**de	**23.533**fg	**22.933**gh	**21.6** hi	
	T3	**26.867**de	**25.667**de	**24.6**de	**23.933** ef	

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	T4	**24.133**ef	**23.067**d	**21.667** hi	**20.667** i	
LSD value			**2.07***			

- The decrease is statistically significant ($p<0.05$), as denoted by different superscript letters. Values with different superscripts are significantly different as per LSD of 2.07–0.04, confirming reliable statistical separation). TC: Sodium alginate film as control, T1: Sodium alginate film with 2.5% PoPE, T2: Sodium alginate film with 5% PoPE, T3: Sodium alginate film with 7.5% PoPE, T4: Sodium alginate film with 10% PoPE. *: Numbers in a table representing an average of triplicate.

Table 3. Water vapor transmission rates of edible films with or without pomegranate peels at different concentrations.

Properties	Treatments		Storage			
		W1	**W2**	**W3**	***W4***	***LSD value***
Water vapor permability (WVT) %	**TC**	**0.007** abc	**0.007** abc	**0.007** ab	**0.008**a	
	T1	**0.006** abcd	**0.006** abcd	**0.006** abcd	**0.007** abcd	**8.61**
	T2	**0.005** d	**0.005** d	**0.005** cd	**0.005**bcd	
	T3	**0.006** abcd	**0.007** abcd	**0.007** abc	**0.007** ab	
	T4	**0.006** abcd	**0.007** abc	**0.007** abcd	**0.007** ab	
LSD value	**0.002***					

- The decrease is statistically significant ($p<0.05$), as denoted by different superscript letters. Values with different superscripts are significantly different as per LSD of 2.07–0.04, confirming reliable statistical separation). TC: Sodium alginate film as control, T1: Sodium alginate film with 2.5% PoPE, T2: Sodium alginate film with 5% PoPE, T3: Sodium alginate film with 7.5% PoPE, T4: Sodium alginate film with 10% PoPE. *: Numbers in a table representing an average of triplicate.

Table 4. Physical characteristics of consumable films with varying concentrations of pomegranate peel and without

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Properties	Treatments		Storage			
		W1	**W2**	**W3**	***W4***	***LSD value***
Mechanical: Tensile strength (MPa)	**TC**	**44.89**gh	**44.65** gh	**44.11**j	**39.7**j	
	T1	**45.01**bc	**44.95** gf	**44.55**gf	**42.95** gf	
	T2	**45.62** bc	**45.46**cd	**45.38** cd	**43.4** k	
	T3	**45.91**ab	**45.76** ab	**45.44**bc	**44.8**hi	
	T4	**46.45**a	**46.27**a	**46.03**ab	**45.15**de	
LSD value	***0.619****					
Elongation %	**TC**	**3.11** j	**2.96** k	**2.75** kl	**2.03** m	
	T1	**3.76** g	**3.44**i	**3.35** i	**3.05** jk	***0.058***
	T2	**4.13**de	**4.01**f	**3.78**g	**3.61**h	
	T3	**4.36**b	**4.28**bc	**4.11**de	**4.05**ef	
	T4	**4.570** a	**4.36**b	**4.29**bc	**4.19**cd	
LSD value	***0.115****					

- The decrease is statistically significant ($p<0.05$), as denoted by different superscript letters. Values with different superscripts are significantly different as per LSD of 0.115–0.058, confirming reliable statistical separation). TC: Sodium alginate film as control, T1: Sodium alginate film with 2.5% PoPE, T2: Sodium alginate film with 5% PoPE, T3: Sodium alginate film with 7.5% PoPE, T4: Sodium alginate film with 10% PoPE. *: Numbers in a table representing an average of triplicate**.**

Table5. Microbial test, the results are presented as the mean ± standard error. POP, pomegranate peel powder; APC, aerobic plate count; PTC, psychrotrophic count; EC, Enterobacteriaceae count. Control: a combination of 100% sodium alginate. POP1: sodium alginate + 2.5% POP; POP2: sodium alginate + 5% POP. POP3: sodium alginate + 7.5% POP. POP4: sodium alginate + 10% POP. T: treatment; n.s.: Not Significant. Means in the same column with different letters are significantly different ($P < 0.05$). A-E Means in the same row with different letters indicate significant differences ($P < 0.05$). Significance: * $P<0.05$, ** $P<0.01$, and *** $P<0.001$.

Properties	Treatments	Storage			
		0day	**7 day**	**14day**	**LSD value**

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APC	**Control**	**2.023 j**	**5.027 d**	**7.630 a**	**0.203**
	Pop1	**2.017 j**	**4.620 e**	**6.823 b**	
	Pop2	**2.010 j**	**3.203 gh**	**5.943 c**	
	Pop3	**2.003 j**	**3.020 hi**	**4.110 f**	
	Pop4	**2.013 j**	**2.743 i**	**3.503 g**	
LSD value		**0.352**			
PTC	**Control**	**1.843 h**	**5.467 c**	**8.530 a**	**0.148**
	Pop1	**1.820 h**	**4.743 d**	**8.773 a**	
	Pop2	**1.787 h**	**4.387 e**	**6.767 b**	
	Pop3	**1.687 h**	**3.740 f**	**5.433 c**	
	Pop4	**1.823 h**	**3.093 g**	**5.397 c**	
LSD value		**0.256***			
EC	**Control**	**0.800 g**	**1.533 cd**	**2.417 a**	**0.132**
	Pop1	**0.767 g**	**1.533 cd**	**1.853 b**	
	Pop2	**0.800 g**	**1.400 de**	**1.690 bc**	
	Pop3	**0.733 g**	**1.200 ef**	**1.333 def**	
	Pop4	**0.867 g**	**1.133 f**	**1.240 ef**	
LSD value		**0.229***			

- The decrease is statistically significant ($p<0.05$), as denoted by different superscript letters. Values with different superscripts are significantly different, confirming reliable statistical separation. Control: Minced meat packed by sodium alginate film, Pop1: Minced meat packed by Sodium alginate film with 2.5% PoPE, Pop2: Minced meat packed by Sodium alginate film with 5% PoPE, PoP3: Minced meat packed by Sodium alginate film with 7.5% PoPE, Pop4: Minced meat packed by Sodium alginate film with 10% PoPE. *: Numbers in a table representing an average of triplicate.

Table 6. Oxidative tests

Properties	**Treatments**	**Storage**			
		0day	**7 day**	**14day**	**LSD value**
	control	**0.23** h	**6.217**c	**8.85** a	

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PV	**Pop1**	**0.4** h	**5.297** d	**7.11**b	**0.107**
	Pop2	**0.34**h	**4.19**e	**7.04**b	
	Pop3	**0.31** h	**3.777**f	**5.237**d	
	Pop4	**0.34** h	**3.453**g	**4.103** e	
LSD value		**0.185**			
TBA	**control**	**0.233** h	**2.73** ab	**3**a	**0.19**
	Pop1	**0.267** i	**2.1** c	**2.67**b	
	Pop2	**0.333** hi	**1.233**e	**1.6**d	
	Pop3	**0.367** hig	**0.7**fg	**0.767**f	
	Pop4	**0.4**gh	**0.6**fg	**0.7** fg	
LSD value		**0.33**			
pH	**control**	**5.663**jk	**6.617**cd	**8.123**a	**0.2**
	Pop1	**5.62**kl	**6.63**cd	**7.297**b	
	Pop2	**5.647**kl	**6.473**ef	**6.833** cd	
	Pop3	**5.613**kl	**5.983**gh	**6.417**ef	
	Pop4	**5.563**im	**5.96**cd	**6.29**fg	
LSD value		**0.347**			

Table6. Sensory evaluation

Properties	**Treatments**	**Storage**

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Color		**0day**	**7 day**	**14day**	**LSD value**
	Control	**8.125**a	**7.125**bc	**3.625**j	**0.434**
	Pop1	**8.125**a	**8.125** a	**5.25** hi	
	Pop2	**6.375**cd	**7** cd	**4.75**i	
	Pop3	**6** cd	**6.865** cd	**6.625**fg	
	Pop4	**6**bc	**7**cd	**7.25** fg	
LSD value		**0.753**			
Appearance	**Control**	**8.125**a	**6.125**	**5.25** hi	**0.426**
	Pop1	**8.125** a	**7.875**a	**5.62**hi	
	Pop2	**6.375**cd	**6.375**cd	**4.875**i	
	Pop3	**6.125** cd	**6.5**de	**6.125**fg	
	Pop4	**6.375** bc	**6.75**cd	**6.75**fg	
LSD value		**0.738**			
Odour	**Control**	**8.75**a	**5.875**g	**3.625**j	**0.338**
	Pop1	**8.25**ab	**7.125** de	**5.125**h	
	Pop2	**7.625** cd	**6.5**f	**4.75** h	
	Pop3	**8** bc	**7.25**de	**6.875**ef	
	Pop4	**7.5**cd	**7.375**de	**7.25**de	
LSD value	**Control**	**0.586**			
Acceptability	**Pop1**	**9**a	**6.125**e	**3.5**f	**0.296**
	Pop2	**8.375**b	**7.375**cd	**4.38** h	
	Pop3	**7.125**cd	**6.375**e	**5.25**g	
	Pop4	**7.375**cd	**7.125**cd	**5.875**ef	
		7.125cd	**7**cd	**7.125**d	
LSD value		**0.513**			

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