Impact of Active Packaging Film with Natural Plant on the Quality and Safety of Chilled Poultry Meat

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ABSTRACT

Poultry meat remains the most popular around the world. The total phenolic and flavonoid contents of pomegranate peel powder (PPP), paprika powder (PP) and guava leaves extract (GE) were determined. Active packaging enables the interaction between the packaging, the food product and the internal and external environments to improve the food quality and safety of the food product. Fresh chicken legs were treated with active packaging films of pomegranate peel powder (PPP) and paprika powder (PP) (2%PPP+1%PP; 3%PPP+1%PP; 3%PPP+2%PP and 3%PPP+3%PP). Microbiological quality, TVBN and pH were assessed during refrigerated storage at 4°C. Results showed that Pomegranate peel exhibits the highest levels of both phenolic and flavonoid contents, indicating its potential as a rich source of bioactive compounds. Paprika powder extract, characterized by a moderate phenolic content of 220.83 ± 3.06 mg/g and a concurrent flavonoid content of 153.38±2.40 mg/g, reflects a balance of antioxidant constituents to be used in active packaging film. The results indicated that psychotropic aerobic bacteria; *Enterobacteriaceae*; Yeast; and TVBN were strongly inhibited by all active packaging film applied. The highest inhibition was observed with active packaging film of 3%PPP+3%PP. A reduction in pH values was observed by all the active packaging films as compared with blank chicken samples. Chicken legs packed in active film of 2%PPP+1%PP or 3%PPP+1%PP could be refrigerated safely for 8 and 9 days respectively, as compared to 6 days for blank samples. Increasing pomegranate peel powder (PPP) and paprika powder (PP) concentration to 3% PPP + 2%, PP and 3%PPP+3%PP in the active packaging film will increase the shelf life to 10 and 12 days, correspondingly. The results indicated that packaging fresh poultry in the active film of pomegranate peel powder (PPP) and paprika powder (PP) would enhance safety, and quality and prolong the shelf life of poultry meat during storage at 4°C.

Keywords: Poultry, active packaging film; natural antimicrobial and antioxidant; quality and safety of poultry; storage at 4°C.

INTRODUCTION

The poultry industry has developed from a non-existent industry in the early 20th century to a main industrial 21st-century powerhouse. It is significant to consider the diverse segments of the poultry industry, present production targets, and meat consumption to know the place of poultry in the world meat market (Hosny, 2006; Castro *et al*., 2023; Khalifah *et al*., 2023; Bist *et al*., 2024; Dong *et al*., 2024; Grasso *et al*., 2024; Harajin *et al*., 2024 and Mozdziak, 2024).

Poultry meat remains the major popular worldwide with 15 kg/per capita each year tracked by pork (11 kg/per capita each year) and beef/veal (6 kg/per capita each year) (Soare *et al*., 2023; Grasso *et al*., 2024 and Mozdziak, 2024). A huge percentage of the world population classifies poultry meat products as vital components of a healthy diet (Abdelmonem *et al.*, 2023; Barszcz *et al*., 2024; Dong *et al*., 2024; Dutta *et al.*, 2024; Grasso *et al*., 2024 and Mozdziak, 2024).

Poultry is highly perishable due to microbial activity, natural enzymes, and the degradation of chemical lipids. Microorganisms primarily contribute to the spoilage of poultry meat by affecting nitrogenous (NPN) content, causing mild acidity (pH > 6), and increasing water activity (Wa) (Mead, 2004; Rouger *et al.*, 2017; Katiyo *et al.*, 2020; Tsaloumi *et al.*, 2023; Chen *et al.*, 2024; Dong *et al.*, 2024). Various chemical preservatives have been employed to inhibit spoilage and maintain the quality of food products (Gokoglu, 2019). Synthetic antioxidant preservatives such as butylated hydroxy anisole (BHA), tert-butylhydroquinone (TBHQ), butylated hydroxy quinone (BHT), potassium sorbate (BS), calcium sorbate (CS), monosodium and disodium glutamate (M&DS), sorbic acid (SA), benzoic acid (BA), sodium benzoate (SB), propyl gallate (PG), and sodium or potassium nitrate and nitrite are commonly used as antioxidants and antimicrobials in various products (Brewer, 2011). Antioxidants can inhibit or prevent rancidity caused by oxidation, which extends the period during which products remain edible and safe for consumption (Hussain *et al.*, 2021). The rising demand for safe, high-quality meals and increasing consumer concerns regarding the use of chemical preservatives present significant challenges for the food industry (Xu *et al.*,

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2021; Presenza *et al.*, 2023; Dong *et al.*, 2024 and Grasso *et al.*, 2024).

Pomegranate (*Punica granatum* L.) peel is an affluent supply of polyphenols, especially tannins and anthocyanins. Pomegranate peels are rich in natural antioxidants and anti-microbial agents (Hanani *et al*., 2019; Kaderides *et al*., 2021 and Al-Moghazy & El-Sayed 2023). According to Baenas *et al*. (2019) and Oswell *et al*. (2018) phenolic compounds, Carotenoids, capsaicinoids, capsinoids, vitamins (E, C) and minerals (Potassium, Phosphorus and Magnesium) make up most of the bioactive chemicals found in paprika powder of *Capsicum annum* L.

Recently there has been an increase in the active food-packaging concept, as it exceeds the unique function of traditional food packaging (Karakuş *et al*., 2023; Ahmad *et al*., 2024 and Soleimanzadeh *et al*., 2024). Active packaging is developed by intentionally adding certain compounds, for instance, natural extracts to the packaging system. In addition, active packaging allows the interaction among the packaging, the food products and the internal and outside environments to develop sensory characteristics and food safety (Hanani *et al*., 2019; Karakuş *et al*., 2023; Ahmad *et al*., 2024 and Soleimanzadeh *et al*., 2024). Therefore, the objective of the current research is to evaluate the anti-microbial and anti-oxidant activities of pomegranate peel (PPP) and paprika powders (PP) and use them as natural edible active packaging film to enhance the quality, and safety and extend the shelf life of poultry legs during storage at 4°C.

MATERIALS AND METHODS

Materials

Raw materials

Fresh chicken legs (24 kg each 220 ±12 grams) were obtained from Alasania Poultry, Wadi Natrun, Egypt, then transported in ice boxes to the Faculty of Agriculture (Saba Basha), Food Science Department, Alexandria University within 3 h of slaughtering. Chicken legs were washed with tap water and left to drain for 30 min at 4℃. Tow plant materials, pomegranate (*Punica granatum* L.), and red (*Capsicum annuum*) and Guava (*Psidium guajava* L) leave, were collected from the local markets in Alexandria, Egypt. All chemicals and Media used in the Analytical and Microbiological analysis were obtained from Merck and Oxoid companies respectively.

Methods

Plant powders preparation

The cleaned pomegranate peel, and red paprika and Guava leave were dried at 50-55°C in an oven for 72 hours, the plants were pulverized using a blender and sieved at 40-mesh sieve (420 µm). Each powder was stored at -18˚C in a glass bottle for further use.

Preparation of plant extracts

Plant powders have been extracted according to the method of Kim *et al.* (2013).

Chemical analysis

Total phenolic content (TPC) of plant extracts:

TPC was measured spectrophotometrically using the Folin–Ciocalteu method according to the methods of Dewanto *et al*. (2002); Kim *et al*. (2013) and El Sohaimy & Masry (2014).

Total Flavonoid Content (TFC) of plant extracts:

Calculation of TFC in the plant extracts of pomegranate peels, paprika powder and Guava leaves were calculated by the modified method recorded by Radha Krishnan *et al*. (2014).

DPPH radical scavenging activity of plant extracts

The DPPH (1,1-diphenyl-2- picrylhydrazyl) method was used to calculate the inhibition concentration (IC50) of plant extracts for the free radical scavenging with some modifications. Different concentrations (5–1000 µg/ml) of standard and extracts. 1 ml from the extract and ascorbic acid solution each concentration (as a positive control) was assorted separately with 1mL of DPPH (0.2 mM) % solution (0.0078 g / 100 ml) in ethanol, the mixtures were kept for 30 min in the dark, then the absorbance at 517 nm was measured. For positive control, all reagents were added except for the plant extract, and the measurements were performed in three replicates. The percentage of DPPH radical-scavenging activity was measured by the current formula:

$$= \frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{\text{Abs}_{\text{control}}} \times 100$$

Where.

Abs $_{\text{Control}}$: Blank absorbance (DPPH Radical+methanol)

Abs $_{\text{sample:}}$ The sample / standard absorbance.

$$(IC50\%) = [\frac{Abs\ control - Abs\ sample}{Abs\ ontrol]}\ x\ 100$$

pH values: The pH values were determined using the method of Zeitoun *et al.* (1994).

Determination of TVB-N: To determine the total volatile basic nitrogen content, a customized method by Jinadasa (2014) as TVB-N(mg/100g) $=\frac{14 \times a \times b \times 300}{25}$

a = mL volume of sulfuric acids, b = Normality of sulfuric acid

Active packaging treatments

Chicken legs were divided into five groups for treatments as follows: The first one remains without any additions, as a control group (Blank) (uncoated), the second group coated with 2% pomegranate peel powder + 1% paprika powder, the third group coated with 3% pomegranate peel powder + 1% paprika powder, the fourth group coated with 3% pomegranate peel powder + 2% paprika powder, the fifth group coated with 3% pomegranate peel powder + 3% paprika powder, Chicken legs in all groups were aerobically packed in polyethene bags and stored at 4 °C.

Microbiological assay

Three samples (10 g each) were aseptically homogenized for two minutes in 90 ml of treated saline (w/v: 0.85% NaCl). Decimal dilutions of this homogenate were prepared in sterile physiological saline containing 0.1% peptone (Radha Krishnan *et al*., 2014 and Berizi *et al*., 2018). After homogenization, serial dilutions were prepared. Colony forming units (CFU) of psychotrophic aerobic bacteria were measured in plate count agar (Oxoid CM 325), and incubated for 5 days at 20 °C. Yeast and mold CFU were measured on Rose Bengal Chloramphenicol agar (Oxoid CM 549) supplemented with chloramphenicol antibiotic (Oxoid SR 78). The colonies were incubated at 30 °C for 5 days. *Enterobacteriaceae* were calculated on Violet Red Bile Glucose agar (VRBG) (Oxoid CM 485) with an overlay of the same agar incubated for 18h at 37 °C. The microbial population of each plate was measured and presented as log10 CFU/g.

Statistical Analysis

Data analysis involved employing two-way Analysis of Variance (ANOVA) and Duncan's test to ascertain the significance of the correlation between independent and dependent variables. Descriptive statistics and frequency tables were utilized to illustrate the data. A statistically significant variance was acknowledged when the p-value was below 0.05 (Calinski *et al*., 1981).

RESULTS AND DISCUSSION

TPC of plant extracts

The analysis of total phenolic and flavonoid contents in various plant extracts, as presented in Table (1), provides valuable insights into the bioactive compounds and their potential health benefits. The paprika powder extract displays a moderate phenolic content of 220.83 ± 3.06 mg/g alongside a flavonoid content of 153.38 ± 2.40 mg/g, indicating a well-balanced composition of antioxidant constituents. This aligns with existing knowledge regarding paprika powder as a source of health-promoting phytochemicals. Previous studies have identified ellagic acid and other polyphenols in pomegranate peels, contributing to their antioxidant and anti-inflammatory properties (Oswell *et al.,* 2018 and Baenas *et al.,* 2019). Notably, the pomegranate peel extract stands out with a higher phenolic content of 288.59 ± 4.18 mg/g and a significant flavonoid content of 242.19 ± 2.40 mg/g. These elevated levels suggest a potential for enhanced antioxidant activity, supporting the view of pomegranate peels as a rich source of polyphenols. This is consistent with research highlighting the peel as a source of polyphenols such as punicalagin and anthocyanins, which exhibit notable antioxidant and anti-inflammatory effects (Hanani *et al.,* 2019; Kaderides *et al.,* 2021 and Al-Moghazy & El-Sayed, 2023). Nag and Sit (2018) reported a phenolic content of 140 mg/g for pomegranate peel extract (PE), which aligns with similar findings. Lee *et al.* (2016) documented a flavonoid content of PE at 7.57 mg/g in catechin equivalents. Importantly, Lee *et al.* (2016) also noted that flavonoids represent only a small fraction of the total phenolic content in pomegranate peel extract. Furthermore, Seeram *et al.* (2005) identified PE as a rich source of ellagitannins, a type of hydrolysable tannin. These findings underscore the diversity and abundance of polyphenolic compounds in pomegranate peel extract, highlighting its potential health benefits and antioxidant properties. Guava leaves, with a moderate phenolic content (117.21 ± 2.05 mg/g) and a comparable flavonoid content (128.10 ± 1.39 mg/g), showcase a balanced phytochemical composition. This equilibrium may contribute to the multifaceted health-promoting properties traditionally attributed to guava leaves reflects the known antioxidant potential of guava leaves. Studies attribute these effects to the presence of quercetin, catechins, and other flavonoids in guava leaves (Amer, 2014). These results highlight the substantial variability in phenolic and flavonoid compositions among the plant extracts studied. Pomegranate peel extract exhibits the maximum levels of flavonoid and phenolic contents, indicating its potential as a rich source of bioactive compounds. Guava leaf extract, although moderate, showcases a balanced profile of flavonoid and phenolic. In conclusion, the diverse phenolic and flavonoid profiles presented in this experiment underscore the intricate composition of bioactive compounds in plant extracts. From the obtained results, it can be stated that pomegranate peel is a good source of natural flavonoids and phenolic compounds which encourage its use in food processing and storage to get its health benefits**.**

Table 1. TPC and TFC contents in the plant extracts (mg GAE/g) of pomegranate peel, paprika powders and guava leave extracts used in the current study

Plant Extracts	TPC (mg/g)	TFC (mg/g)
Pomegranate peel extract	288.59 ^{A}± 3.06	242.19 ^{A} ± 2.59
Paprika powder extract	220.83 ^{B} ± 4.18	153.38 ^{B} ± 2.40
Guava leaves extract	117.21^{C}± 2.05	128.10^{C} ± 1.39

* Each value reported represents the mean ± SD calculated from three replicate measurements.
* Values in the similar column with the equal letter are not significantly different (P≥ 0.05).

DPPH and ABTs scavenging activity of plant extracts

The investigation of the antioxidant properties of extracts involved the use of DPPH radical and ABTS radical cation assays, and the findings are found in Table (2). The DPPH assay, a widely adopted and preferred method for calculating the radical scavenging activity of plant extracts, relies on the reactivity of DPPH a stable nitrogen-centred free radical that imparts a violet color to the methanol solution. Once DPPH radicals interact with effective reducing agents like antioxidants, the solution undergoes a color change, the magnitude of which depends on the number of electrons absorbed by the system (Umamaheswari and Chatterjee, 2008). The obtained results indicated that the examined extracts showed varied scavenging capacities, highlighting the diversity in their antioxidant capabilities. The presented Table (2) offers a comprehensive overview of the antioxidant activities of diverse plant extracts as assessed through DPPH and ABTS assays, with IC50 values reported in micrograms per milliliter (µg/mL). The IC50 values signify the concentration at which a plant extract can scavenge 50% of free radicals, providing crucial insights into their potential health-promoting properties. Pomegranate peel extract stands as the unequivocal leader, boasting the lowest IC50 values in both DPPH (5.13 ± 0.15 µg/mL) and ABTS (2.25 ± 0.10 µg/mL) assays, signifying its outstanding potential to counteract free radicals.

The standout performer in this analysis is pomegranate peel extract, which consistently exhibits the lowest IC50 values in both DPPH and ABTS assays. This aligns with existing literature, such as the study conducted by Malviya *et al.* (2014), which reported an IC50 of 4.9 µg/mL for pomegranate peel extract. Similar results were obtained by Okonogi *et al*. (2007) who reported that pomegranate peel extract had the highest DPPH scavenging activity between the eight-fruit peel extracts. Kanatt *et al*. (2010) also recorded that IC50 of pomegranate peel extract was 4.9 µg/mL.

Each plant extract demonstrates unique antioxidant profiles, with notable variations in their IC50 values. Wang *et al.* (2011) stated that pomegranate peel powder extracts with higher TPC exhibited enhanced DPPH scavenging activity, and vice versa. Specifically, pomegranate seed extract demonstrated moderate activity, while pomegranate peel extract was notably effective in scavenging free radicals. This suggests a correlation between the TPC and the antioxidant activity of pomegranate extracts, with the peel extract showing pronounced efficacy in neutralizing free radicals.

Paprika powder extract and guava leaf extract showcase commendable radical-scavenging capacity, with IC50 value of 22.12 ± 0.23 µg/mL and 36.49 ±0..31µg/mL for DPPH, and 9.01 ± 0.19 µg/mL and 23.01 ± 0.24 µg/mL for ABTS, contributing to its potential applications in functional foods or nutraceuticals. Guava leaf extract, while displaying comparatively higher IC_{50} values, still demonstrates substantial antioxidant activity. Rounding out the overall spectrum of antioxidant effectiveness among the examined plant extracts.

The findings of the current results are in agreement with the suggestion of Fernandes *et al.* (2018) who recorded the antioxidant capacity of spray-dried guava leaf extracts. The consistency in these results enhances their credibility and underscores the importance of pomegranate peel as a rich source of bioactive compounds with potent antioxidant properties. Additionally, the observed antioxidant activities are supported by the analysis of TPC and TFC. The linear relationship between TPC and antioxidant activity supports the notion that the presence of phenolic compounds contributes significantly to the observed antioxidant potential of these plant extracts. The implications of these findings extend to potential applications in food processing and storage, where the incorporation of plant extracts with high antioxidant activities could mitigate oxidative processes and improve the shelf life of food products. This aligns with the broader context of utilizing natural resources to progress the stability and nutritional value of processed foods.

Table 2. Inhibition values (IC_{50}) of pomegranate peel, paprika powder and guava leave extracts determined by DPPH and ABTS Tests

Plant Extracts	Antioxidant activity IC_{50}	
	DPPH (µg/mL)	ABTS (µg/mL)
Pomegranate peel extract	$5.13^{A} \pm 0.15$	$2.25^{A} \pm 0.1\ 0$
Paprika powder extract	$22.12^{B} \pm 0.23$	$9.01^{B} \pm 0.19$
Guava leave extract	$36.49^{C} \pm 0..31$	$23.01^{C} \pm 0.24$

* DPPH: The 2,2-Diphenyl-1-picrylhydrazyl; ABTS: (2,2'-azino-di (3-ethylbenzothiazoline-6-suslfonic acid).
* Each value reported represents the mean ± standard deviation (SD) calculated from three replicate measurements.
* Values in the same column with a similar letter are not significantly different (P≥ 0.05).

pH value

Table (3) illustrates the impact of the active packaging film of pomegranate peel and paprika powders during packing at 4°C on the pH values of chicken legs samples over different storage times. The initial pH values (day 0) indicate that the control (Blank) sample started at higher pH level (6.41±0.11) compared to the packaging film of pomegranate peel and paprika powders. Over the storage period, the blank sample showed a gradually increased value in pH, reaching 6.75±0.08 by day 6. In contrast, the active packaging film of pomegranate peel and paprika powders generally exhibited a more stable pH trend, with variations observed among the different concentrations of packaging film of pomegranate peel powder and paprika powder.

Notably, with the increased period of using packaging film of pomegranate peel and paprika powders, the pH value of these treated samples showed significant fluctuations compared to the blank group, suggesting a potential role of active packaging film of pomegranate peel and paprika powders in maintaining the acidity of the meat during storage. The values of 3%PPP+2%PP and 3%PPP+3%PP at day 0 were 5.24±0.12 and 5.13±0.14 at day 0, respectively and increased to 5.65 ±0.05 and 5.44 ±0.08 at day 10, respectively while the 3%PPP+3%PP showed a pH value 5.54 ±0.1.7 at day 12. This study demonstrates the effectiveness of the active packaging film of pomegranate peel and paprika powders, especially when combined with 3%PPP+3%PP, in conserving the pH of chicken legs samples through storage. The observed differences in pH values among the treatments highlight the potential of active packaging film of pomegranate peel and paprika powders in increasing the shelf life and maintaining the quality of chicken legs. Further research could delve into the specific mechanisms underlying these observed effects and explore additional quality parameters influenced by this innovative coating approach.

Table 3. The changes in pH of chicken legs treatment with active packaging film of pomegranate peel and paprika powders during storage at 4°C

Treatment	Days of Storage						
	0	3	6	8	9	10	12
Blank	6.41^{Aa} ±0.11	6.53^{Ba} ±0.12	6.75^{Ca} ±0.08	N.D.	N.D.	N.D.	N.D.
2%PPP+ 1%PP	5.55^{Ab} ±0.13	5.64^{Bb} ±0.20	5.70^{Bb} ±0.10	5.84^{Ca} ±0.11	N.D.	N.D.	N.D.
3%PPP+ 1%PP	5.30^{Ac} ±0.20	5.36^{Ac} ±0.10	5.47^{Bc} ±0.02	5.61^{Cb} ±0.05	5.76^{Da} ±0.01	N.D.	N.D.
3%PPP+ 2%PP	5.24^{Ac} ±0.12	5.29^{Ac} ±0.13	5.31^{Ad} ±0.09	5.46^{Bc} ±0.11	5.47^{Bb} ±0.05	5.65^{Ca} ±0.05	N.D.
3%PPP+ 3%PP	5.13^{Ad} ±0.14	5.18^{Ad} ±0.11	5.20^{Ae} ±0.08	5.19^{Ad} ±0.12	5.32^{Bc} ±0.16	5.44^{Cb} ±0.08	5.54^{D} ±0.1.7

* Each value stated represents the mean ± standard deviation (SD) calculated from 3 replicates.
* PPP pomegranate peel powder.
* PP paprika powder.
* N.D: Not Determined because of spoilage.

Similar observations were made regarding pH levels, where the control (blank) samples exhibited a significantly higher pH value at the end of the storage period compared to the other chicken thigh samples, including those treated with packaging films and preserved samples. These treated samples consistently showed lower pH values throughout the study period, indicating that the treatments had an inhibitory effect on pH increase, even keeping it below the initial levels. The addition of pomegranate peel powder was noted to reduce the pH of the meat, likely due to the powder's acidic nature. For instance, incorporating pomegranate rind powder into buffalo meat nuggets significantly lowered pH levels at various concentrations (2%, 4%, and 6%) (Habib *et al.,* 2018). Essid *et al.* (2020) reported a substantial decrease in pH of fish fillets after 24 hours of marination. Similarly, Firuzi *et al.* (2019) found that the inclusion of pomegranate juice (PJ) concentrates and pomegranate rind powder (PRP) resulted in lower pH values compared to the control. Additionally, Naveena *et al.* (2008) noted that there was no significant change in the pH of cooked chicken patties when pomegranate rind powder extract was added. Overall, the pH of muscle foods containing pomegranate by-products remained within the acceptable range. Interestingly, the use of pomegranate peel and seed powder, rather than extracts, has been shown to improve emulsion stability and cooking yield in muscle foods (Abdel Fattah *et al.,* 2016 and Sharma *et al.,* 2020**).**

Total Volatile Basic Nitrogen (TVB-N)

Table (4) presented the impact of active packaging film of PPP and PP on TVB-N, in chicken legs samples through storage at 4°C. In the control sample (Blank), the TVB-N value started at 8.63±0.16 on day 0. Subsequently, the TVB-N values increased progressively, reaching 27.14±0.28 on day 6, indicating significant degradation in the absence of any protective coating. For the 2% PPP+1%PP sample, the TVB-N values were consistently lower compared to the control during the storage period. On day 0, the 2% PPP+1%PP sample had a TVB-N value of 8.10±0.19, and by day 8, it increased to 26.21±0.30. This suggests that the packaging film of pomegranate peel powder and paprika powder coating alone contributed to mitigating protein oxidation, although to a lesser extent than the blank treatment. In the case of 3%PPP+1%PP, the TVB-N values were notably lower than both the control and 2% PPP+1%PP samples. Starting at 7.81±0.13 on day 0, the TVB-N value increased to 24.89±0.27 on day 9, indicating that the inclusion of a lower concentration of pomegranate peel had a positive influence on preserving protein quality over the storage period. 3%PPP+2%PP demonstrated a more substantial effect on protein preservation, with TVB-N values starting at 7.74±0.11 on day 0 and increasing to 25.20±0.24 on day 10 (Table 4).

This suggests that a moderate concentration of 3%PPP+2%PP contributed to a significant decrease in protein oxidation related to both the control and other samples. In the case of 3%PPP+3%PP, the TVB-N values were consistently the lowest among all treatments. Starting at 7.51±0.12 on day 0, the TVB-N value reached 24. 12±0.19 on day 12. This concentration-dependent response indicates that the higher concentration of pomegranate peel and paprika powder had a remarkable protective effect on protein degradation, showcasing the potential of this treatment in preserving protein quality during refrigerated storage. The results from Table (4) underscore the potential of the active packaging film of pomegranate peel powder and paprika powder on TVB-N of chicken legs through storage at 4°C in inhibiting protein oxidation in chicken meat through refrigerated storage.

The concentration-dependent outcome and the lower TVB-N values in the treated samples, particularly 3%PPP+3%PP, suggest that this innovative active packaging film of pomegranate peel powder and paprika powder may play a vital role in preserving the quality of chicken meat by minimizing protein degradation. Previous studies revealed that in the early stages of storage, all five groups had low TVB-N values ranging from 7.96-8.32 mg N/100 g with no major differences between the treated samples (Bazargani-Gilani *et al.,* 2015). However, from day 9 onwards, the TVB-N content of the blank group improved significantly and continued to increase during storage. By the end of storage, the control samples had the highest TVB-N values of 22. 2 mg, while the T2 group (Ch + PPE3) had the lowest TVB-N value of 11.80 mg N/100 g. The TVB-N value of the control samples improved significantly from day 9 compared to the samples treated with diverse concentrations of PPE ($P < 0.05$). Although the reduction was less in the T2 group compared to the Ch and T0.5 groups, it was not significant ($P < 0.05$) (Bazargani-Gilani *et al.,* 2015).

The study also established a continuous rise in TVB-N levels in the control group due to improved growth of microorganisms and other chemical changes such as the production of TVB-N compounds during the storage. However, treatment with diverse concentrations of PPE and chitosan considerably reduced the TVB-N values. This significant variance in TVB-N content qualified to the antioxidant and antimicrobial properties of PPE, and phenolic compounds in PPE that could hinder bacterial-induced protein decomposition and oxidative

Table 4. Influence of active packaging film of pomegranate peel powder and paprika powder on TVB-N of chicken legs during storage at 4°C

Treatment	Days of storage						
	0	3	6	8	9	10	12
Blank	8.63 ^{Aa} ±0.16	16.37 ^{Ba} ±0.21	27.14 ^{Ca} ±0.28	N.D.	N.D.	N.D.	N.D.
2% PPP+ 1%PP	8.10 ^{Ab} ±0.19	11.23 ^{Bb} ±0.21	18.10 ^{Cb} ±0.23	26.21 ^{Da} ±0.30	N.D.	N.D.	N.D.
3%PPP+1%P P	7.81 ^{Ac} ±0.13	9.16 ^{Bc} ±0.09	13.10 ^{Cc} ±0.31	19.46 ^{Db} ±0.20	24.89 ^{Ea} ±0.27	N.D.	N.D.
3%PPP+2%P P	7.74 ^{Ac} ±0.11	8.15 ^{Bd} ±0. 18	10.16 ^{Cd} ±0.20	15.15 ^{Dc} ±0.26	19.18 ^{Eb} ±0.23	25.20 ^{Fa} ±0.24	N.D.
3%PPP+3%P P	7.51 ^{Ad} ± 0.12	7.59 ^{Ae} ±0.09	8..13 ^{Be} ±0.19	11.84 ^{Cd} ±0.19	13.05 ^{Dc} ±0.14	16.10 ^{Eb} ±0.18	24. 12^{F} ±0.19

* PPP pomegranate peel powder.
* PP paprika powder.
* N.D: Not detected because of spoilage.

breakdown of nitrogen compounds from other sources (non-protein). Berizi *et al.* (2018) revealed identical outcomes in terms of the impact of using chitosan and pomegranate peel extract together on TVB-N levels in coated rainbow trout throughout its frozen storage period.

The findings were agreed with the results obtained from the TVN analysis, which demonstrated that the levels of TVBN and NH2-N increased over time. TVN increased rapidly and reached a total N level of 114 mg/g after 10 days of storage**.** In a study on fresh meagre fillets stored on ice, it was found that the average TVBN values (16.7-20.4 mg N/100 g) remained relatively stable throughout the storage period, despite significant variances among certain days of the experiment. The initial TVB-N levels in meagre fillets averaged at 14.63 ± 2.76 mg N/100 g of sample (Table 4). The average initial TVB-N values reported by Genç *et al.* (2013) were 14.63 ± 2.76 mg N/100 g of sample of meagre fillets. After a storage period of 8 days for fillets stored in air packaging (AP) and 13 days for those stored in vacuum packaging (VP), the TVB-N levels increased to 18.84 ± 0.95 mg N/100 g of sample for AP samples and 21.56 ± 2.64 mg N/100 g of sample for VP fillets. While, Shahamirian *et al.* (2019), recorded that pomegranate rind powder extracts were compared to pomegranate juice in inhibiting surface discoloration and maintaining the desirable color of burgers during frozen storage.

Microbiology Analysis:

Psychrotrophic aerobic bacteria

The key element reducing poultry meat shelf life is the activity of microbes. In standards, guidelines, and specifications, an estimation of psychotropic aerobic bacteria is utilized as an acceptability index (Olafsdottir *et al*., 1997 and Nie *et al*., 2022).

Data in Table (5) delineates the influence of active packaging film with PPP and PP on the *Psychrotrophic aerobic* bacteria (log CFU/g) of chicken legs stored during a 12-day storage period at 4°C. In the Blank sample (Bl), the *Psychrotrophic aerobic* bacteria (log CFU/g) of chicken legs started at 3.98 ±0.06 (log CFU/g) on day 0, and over the storage period, it increased significantly to 6.84 ±0.12 (log CFU/g) by day 6. This observed increase in Psychrotrophic aerobic bacteria reflects the natural microbial proliferation in untreated chicken meat during refrigerated storage.

For the 2% PPP+1%PP, the initial Psychrotrophic aerobic bacteria (log CFU/g) on day 0 was lower at 3.38 ±0.11 log CFU/g related to the control (Blank), suggesting a certain level of microbial inhibition by the 2% PPP+1%PP coating. However, the *Psychrotrophic aerobic* bacteria in 2% PPP+1%PP increased progressively, reaching 6.81±0.12 log CFU/g by day 8 and not detected from day 9-12 as presented in Table (5). While 3%PPP+2%PP coating initially contributed to reduced microbial counts ranging from day 0 to day 10 with values were 3.07±0.15 to 6.82 ±0.11, respectively, and ND at the day 12 as found in Table (5).

The samples treated with active film of pomegranate peel and paprika powders (3%PPP+3%PP) displayed varying effects on the *Psychrotrophic aerobic* bacteria (log CFU/g) of chicken legs, with lower count was 2.80 ±0.09 compared with Blank at day 0, while this value was increased with the increase of storage period which showed 2.85±0.12 at day three, 3.12±0.18 at day six, 3.71±0.18 at day eight, 4.10±0.32 at day nine, 4.96±1.15 at day ten and 6.65±1.19 at day twelve.

Table 5. Impact of active packaging film with PPP and PP on the Psychrotrophic aerobic bacteria (log CFU/g) of chicken legs stored at 4°C

Treatment	Days of storage						
	0	3	6	8	9	10	12
Blank	3.98 ^{Aa} ±0.06	4.81 ^{Ba} ±0.31	6.84 ^{Ca} ±0.12	N.D.	N.D.	N.D.	N.D.
2% PPP+ 1%PP	3.38 ^{Ab} ±0.11	3.85 ^{Bb} ±0.41	5.11 ^{Cb} ±0.19	6.81 ^{Da} ±0.12	N.D.	N.D.	N.D.
3%PPP+ 1%PP	3.19 ^{Ac} ±0.12	3.52 ^{Bc} ±0.21	4.32 ^{Cc} ±0.21	5.87 ^{Db} ±0.21	6.75 ^{Ea} ±0.11	N.D.	N.D.
3%PPP+ 2%PP	3.07 ^{Ad} ±0.15	3.30 ^{Bd} ±0.13	3.81 ^{Cd} ±0.24	4.50 ^{Dc} ±0.01	5.62 ^{Eb} ±0.15	6.82 ^{Fa} ±0.11	N.D.
3%PPP+ 3%PP	2.80 ^{Ae} ±0.09	2.85 ^{Ae} ±0.12	3. 12 ^{Be} ±0.18	3.71 ^{Cd} ±0.18	4.10 ^{Dc} ±0.32	4.96 ^{Fb} ±1.15	6.65 ^{G} ±1.19

* PPP pomegranate peel powder.
* PP paprika powder.
* N.D: Not Determined because of spoilage

The highest concentration of 3%PPP+3%PP, exhibited the most significant microbial inhibition, starting at 2.80 ±0.09 log CFU/g on day 0 and reaching 6.65±1.19 log CFU/g on day 12 (Table 5). Our results reveal that the combination of pomegranate peel powder and paprika powder on the *Psychrotrophic aerobic* bacteria (log CFU/g) of chicken legs, influences the *Psychrotrophic aerobic* bacteria counts in chicken meat samples during refrigerated storage. Higher concentrations of PPP and PP appear to provide more effective microbial inhibition, highlighting the potential of this combined treatment in extending the microbial shelf life of chicken meat.

In a study shown by Hayrapetyan *et al.* (2012), the antibacterial efficacy of pomegranate peel extract pomegranate peel powder in meat pâté against *Listeria monocytogenes* was investigated at diverse temperatures (4, 7, and 120 °C) for 46 days. The researchers concluded that pomegranate peel powder effectively inhibited microbial growth during the storage related to the control, Additionally, the application of pomegranate peel extract on the surface of beef steak reduced bacterial counts for antibiotic-resistant strains of *Staphylococcus aureus*, suggesting its potential for comprehensive meat decontamination and enhancement of quality attributes.

Recently, the application of pomegranate juice (PPJ), and extract in film or coating formulations has been investigated by different researchers such as Mushtaq *et al.* (2018). Nonetheless, restricted studies on the treatment of PPP have been registered. Kanatt *et al.* (2010) reported that the antioxidant and antimicrobial activity of PPP extract improves the shelf life of chicken products.

These results agree with Realini and Marcos (2014) who reported that the active agents with antimicrobial properties could help to extend the shelf life and preserve quality and food safety. Molnár *et al.* (2018) reported that Paprika is considered one of the most important ones utilized both for flavoring and enhancing other sensorial characteristics of foods. Because of their agricultural origin, spices are often genuinely contaminated with several bacteria due to poor sanitation throughout growth, harvest, drying, and storage.

***Enterobacteriaceae* (log CFU/g) of chicken legs stored at 4°C**

Table (6) illustrates the impact of active packaging film with pomegranate peel powder (PPP) and paprika powder (PP) on *Enterobacteriaceae* (log CFU/g) of chicken legs stored at 4°C. In the control sample (Blank), the *Enterobacteriaceae* (log CFU/g) counts started at 2.42 ±0.12 log CFU/g on day 0 and increased to 3.41±0.18 log CFU/g by day 3 and 6.11±0.21 log CFU/g by day 6. This indicates a natural proliferation of *Enterobacteriaceae* bacteria in untreated chicken meat during refrigerated storage. For the 2% PPP+1%PP coated sample, the initial *Enterobacteriaceae* counts on day 0 were lower at 1.91±0.17 log CFU/g compared to the control, suggesting some initial microbial inhibition by the 2% PPP+1%PP coating (Table 6), While at day 8 *Enterobacteriaceae* counts was 5.92±0.19 log CFU/g. With using of 3%PPP+1%PP the *Enterobacteriaceae* (log CFU/g) counts started at 1.65±0.15 log CFU/g on day 0 with lower value compared with blank group and increased to 2.04±0.09 log CFU/g by day 3, 5.89±0.19 log CFU/g (day 9) and ND for the days from 10 to 12 (Table, 6). The results indicated that using 3%PPP+2%PP, the *Enterobacteriaceae* (log CFU/g) counts started at 1.51± 0.19 log CFU/g on day 0 with a

lower value compared with the blank group and increased to 1.80±0.13 log CFU/g by day 3, 5.72±0.19 log CFU/g (day 10) and ND for the day 12 (Table, 6). The results indicated that using 3%PPP+3%PP, the *Enterobacteriaceae* (log CFU/g) counts started at 1.23±0.09 log CFU/g on day 0 with a lower value compared with the blank group and increased to 1.80±0.13 log CFU/g by day 3, 1.27±0.11 log CFU/g (day 3) and 5.51± 0.10 log CFU/g for the day 12 (Table, 6). The results indicated that this treatment (3%PPP+3%PP) showed great values for the impact of packaging film with pomegranate peel powder (PPP) and paprika powder (PP) on Enterobacteriaceae (log CFU/g) of chicken legs stored at 4°C in chicken meat samples during a 12-day storage period

In summary, Table (6) highlights that the combination of pomegranate peel powder (PPP) and paprika powder (PP) influences *Enterobacteriaceae* (log CFU/g) counts in chicken meat samples during refrigerated storage. Higher concentrations of 3%PPP+3%PP appear to provide more effective inhibition of *Enterobacteriaceae* bacteria, indicating the potential of this combined treatment in extending the microbial shelf life and ensuring the safety and quality of chicken meat.

Previous research has consistently verified the antibacterial activity of pomegranate peels (Al-Zoreky, 2009). The broad-spectrum anti-microbial compounds found in pomegranate peel extract exhibit inhibitory impact against both Gram-positive and Gram-negative bacteria. The antibacterial properties of pomegranate peel extract (PPE) are ascribed to its phenolic toxicity, which interacts with the sulfhydryl groups of proteins in microorganisms**.** The efficacy of phenolic compounds in displaying antimicrobial effects hinges on their capacity to harm and disrupt cell membranes**.** Consequently, pomegranate peel has been identified as a potential natural preservative for food items. Incorporating pomegranate peel extract into active coatings has demonstrated inhibitory and bactericidal effects (Ghorbani *et al*., 2021). Active edible coatings function to impede microbial growth by restricting essential growth factors, including oxygen, carbon dioxide, and moisture permeability.

Yeast

The current data in Table (7) outlines the impact of combining pomegranate peel powder (PPP) and paprika powder (PP) on Yeast (CFU log/g) in chicken meat samples during a 12-day storage period at 4°C. In the control sample (Blank), the yeast counts started at 2.74±0.18 log10 CFU/g on day 0 and increased to 4.59± 0.17 log10 CFU/g by day 6. This indicates a natural proliferation of yeast in untreated chicken meat during refrigerated storage (Table 7).

For the 2% PPP+1%PP, the initial yeast counts on day 0 were lower at 2.27 ±0.19 log10 CFU/g compared to the control, suggesting some initial microbial inhibition by the 2% PPP+1%PP coating. However, the Yeast counts in 2% PPP+1%PP increased gradually, reaching 4.35±0.24 log10 CFU/g by day 8. The 3%PPP+1%PP coating initially contributed to reduced yeast counts at the day 0 to 2.20±0.23 log10 CFU/g compared with the blank group and other treatments. This treatment showed a value to the day 9 with an average of 4.42±0.13 log10 CFU/g. Samples treated with 3%PPP+2%PP exhibited varying effects on yeast counts and showed the most significant inhibition of yeast, starting at 1.96±0.22 log10 CFU/g on day 0 and reaching 4.45 ±0.15 log10 CFU/g by day 10. 3%PPP+3%PP also demonstrated inhibitory effects on yeast counts compared to the control, suggesting the antimicrobial potential of pomegranate peel powder and

Table 6. Effect of active packaging film with PPP and PP on the *Enterobacteriaceae* (log CFU/g) of chicken legs stored at 4°C

	Days of Storage						
Treatment	0	3	6	8	9	10	12
Blank	2.42 ^{Aa} ±0.12	3.41 ^{Ba} ±0.18	6.11 ^{Ca} ±0.21	N.D.	N.D.	N.D.	N.D.
2% PPP+ 1%PP	1.91 ^{Ab} ±0.17	2..39 ^{Bb} ±0.17	3.75 ^{Cb} ±0.13	5.92 ^{Da} ±0.19	N.D.	N.D.	N.D.
3%PPP+ 1%PP	1.65 ^{Ac} ±0.15	2.04 ^{Bc} ±0.09	2.85 ^{Cc} ±0.11	4.45 ^{Db} ±0.15	5.89 ^{Ea} ±0.19	N.D.	N.D.
3%PPP+ 2%PP	1.51 ^{Ad} ± 0.19	1.80 ^{Bd} ±0.13	2.30 ^{Cd} ±0.17	2.98 ^{Dc} ±0.22	4.31 ^{Eb} ±0.10	5.72 ^{Fa} ±0.19	N.D.
3%PPP+ 3%PP	1.23 ^{Ae} ±0.09	1.27 ^{Ae} ±0.11	1.64 ^{Be} ±0.09	2.14 ^{Cd} ±0.17	2.63 ^{Dc} ±0.14	3.58 ^{Eb} ±0.13	5.51 ^{F} ± 0.10

* PPP pomegranate peel powder.
* PP paprika powder.
* N.D: Not Determined because of spoilage.

Table 7. Effect of active packaging film with pomegranate peel and paprika powders on the yeast (log CFU/g) of chicken legs stored at 4°C

Treatment	Days of Storage						
	0	3	6	8	9	10	12
Blank	2.74 ^{Aa} (±0.18)	3.17 ^{Ba} (±024)	4.59 ^{Ca} (± 0.17)	N.D.	N.D.	N.D.	N.D.
2% PPP+1%PP	2.27 ^{Ab} (±0.19)	2.74 ^{Bb} (±0.21)	3.48 ^{Cb} (±0.17)	4.35 ^{Da} (±0.24)	N.D.	N.D.	N.D.
3%PPP+1%P P	2.20 ^{Ab} (±0.23)	2.48 ^{Bc} (±0.19)	2.96 ^{Cc} (±0.09)	3.82 ^{Db} (±0.16)	4.42 ^{Ea} (±0.13)	N.D.	N.D.
3%PPP+2%P P	1.96 ^{AC} (±0.22)	2.21 ^{Bd} (±0.18)	2.55 ^{Cd} (±0.13)	3.44 ^{Dc} (±0.17)	3.94 ^{Eb} (±0.19)	4.45 ^{Fa} (±0.15)	N.D.
3%PPP+3%P P	1.82 ^{Ad} (±0.09)	1.87 ^{Ae} (±0.11)	2.11 ^{Be} (±0.15)	2.71 ^{Cd} (±0.13)	3.19 ^{Dc} (±0.10)	3.43 ^{Eb} (±0.13)	3.98 ^{F} (±0.19)

paprika powder on the yeast (log CFU/g) of chicken legs stored at 4°C.

In summary, Table (7) highlights that the combination of pomegranate peel and paprika powders influences yeast counts in chicken meat samples during refrigerated storage. Higher concentrations of pomegranate peel and paprika powders appear to provide more effective inhibition on yeast, indicating the potential of this combined treatment in extending the microbial shelf life and ensuring the safety and quality of chicken meat. A previous study showed the ethanolic or aqueous extracts of pomegranate peel (PPE) have been identified as effective means for food preservation through methods such as dipping treatments or using edible coatings (Belgacem *et al.,* 2021).

Specifically, the usage of ethanolic PPP extract has demonstrated a significant ($p \leq 0.05$) reduction in lesion diameter and infection rate in mandarins contaminated with *Penicillium italicum* and *Penicillium digitatum* (Givi *et al*., 2019).

Pomegranate peel powder is rich in ellagitannins, which can undergo hydrolysis by bacteria to form punicalagin and ellagic acid, exhibiting prebiotic effects and promoting microbial growth, as highlighted by Akhtar *et al*. (2015) and Chen *et al.* (2020). The tannins present in pomegranate peels serve as antioxidant and antimicrobial agents in various food applications, as reported by Sandhya *et al*. (2018). Interestingly, these tannins can positively influence the growth of probiotic bacteria, particularly certain lactobacillus strains that possess the capability to degrade tannic acid and generate energy, as mentioned by Akhtar *et al.* (2015).

CONCLUSION

Poultry products are an important source of high-quality proteins, essential amino acids, and lipids. Understanding the total phenolic and flavonoid contents in various plant extracts provides a deeper insight into the bioactive compounds present and their potential health benefits. Paprika powder extract, with a moderate phenolic content of 220.83 ± 4.18 mg/g and a flavonoid content of 153.38 ± 2.40 mg/g, offers a balanced antioxidant profile. This finding aligns with existing research that highlights paprika powder as a source of health-promoting phytochemicals. Previous studies have identified ellagic acid and other polyphenols in pomegranate peels, contributing to their antioxidant and anti-inflammatory properties. Both pomegranate peel extract and paprika powder extract demonstrated significant radical-scavenging abilities, with IC50 values of 5.13 ± 0.15 and 22.12 ± 0.23 µg/mL for DPPH, and 2.25 ± 0.10 and 9.01 ± 0.19 µg/mL for ABTS, respectively. These properties support their potential applications in functional foods or as nutraceuticals. This study highlights the effectiveness of pomegranate peel and paprika powder. In conclusion, treating poultry with active packaging films, particularly those containing 3% pomegranate peel (PPP) and 3% paprika powder (PP), exhibits antimicrobial and antioxidant effects, enhances safety and quality, and extends the shelf life of poultry to 12 days at 4°C, compared to 6 days for untreated sample stored at the same temperature.

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الملخص العربي

التغليف الفعال باستخدام بعض النباتات الطبيعية واثرة على جودة وسلامة لحوم الدواجن المبردة

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تعتبر لحوم الدواجن الأكثر شعبية واستهلاكاً على مستوى العالم. خلال هذة الدراسة تم تحديد محتوى الفينولات والفلافونويدات من مسحوق قشر الرمان (PPP) ومسحوق الفلفل الحلو (PP) ومستخلص أوراق الجوافة (GE) ومن خلال النتائج تم اختيار المواد الأكثر فاعلية للاستخدام فى الدراسة الحالية. حيث تمت معاملة أوراك الدجاج الطازجة بالتغليف النشط من مسحوق قشر الرمان (PPP) ومسحوق الفلفل الحلو (PP) (٢٪ PP٪PPP + 1 ؛ ٣٪ PP٪PPP + 1 ؛ ٣٪ PPP + ٢٪PP و ٣٪ PP٪PPP + 3). تم تقييم الجودة الميكروبيولوجية و TVBN و pH أثناء التخزين على درجة ٤ مئوية. أظهرت نتائج قشور الرمان أعلى قيم من محتويات الفينولات والفلافونويدات، مما يشير إلى انه مصدر غني بالمركبات النشطة بيولوجيا. كما أظهرت النتائج لمستخلص مسحوق الفلفل (البابريكا)، بمحتوى فينولات بلغ ٢٢٠,٨٣ ± ٣,٠٦ مجم / جم ومحتوى فلافونويدات بلغ ١٥٣,٣٨ ± ٢,٤٠ مجم/ جم، حيث يعكس ذلك توازن مكونات مضادات الأكسدة المستخدمة في عملية التغليف. وأشارت النتائج عملية التغليف للحوم الدواجن بتلك المواد النباتية كان له الأثر الواضح على معدل نشاط البكتريا حيث تم تثبيطها بشدة بواسطة جميع مواد التغليف المطبقة. لوحظ أعلى تثبيط كان مع استخدام 3% PPP +PP لوحظ انخفاض في قيم pH مقارنة بالكنترول. ومن خلال النتائج نجد انه يمكن تبريد أوراك الدجاج المعبأة والمغلف بتركيزات من المواد النباتية المستخدمة بأمان لمدة ٨ و ٩ أيام على التوالي، مقارنة بـ ٦ أيام فى الكنترول. زيادة تركيز مسحوق قشر الرمان (PPP) ومسحوق الفلفل الحلو (PP) إلى ٣% PPP+ ٢% PP و ٣%PPP+ ٣%PP في غلاف التغليف النشط سيزيد من مدة الصلاحية إلى ١٠ و١٢ يومًا على التوالي. وبالتالى أشارت النتائج إلى أن تعبئة أوراك الدواجن الطازجة في غلاف نشط من مسحوق قشر الرمان (PPP) ومسحوق البابريكا (PP) من شأنه أن يعزز السلامة والجودة ويطيل مدة صلاحية لحوم الدواجن أثناء التخزين عند ٤ درجات مئوية.