



Valorization of Melissa Pomegranate Peels and Seeds Waste to Obtain Extracts for Increasing the Shelf-Life of Chicken Patties During Cold Storage

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Abstract

Purpose This study aims to valorize pomegranate seeds and peels (as food waste) to develop a natural preservative for extending chicken patties' shelf-life.

Methods Bioactive compounds from pomegranate waste were obtained by green solvent extraction and quantitatively analyzed by high-performance liquid chromatography (HPLC). Antimicrobial efficacy was also determined. Chicken patties enriched with extracts to monitor thiobarbituric acid (TBA), total volatile nitrogen (TVN), water holding capacity (WHC), pH, and cooking properties during 12 days of 4 °C storage, and half-life was determined.

Results The major bioactive compounds in seed and peel extracts were tannic (18.95 and 41.25 mg/kg) and gallic acid (16.49 and 36.59 mg/kg). Phenolics and flavonoids reached 66.71 and 9.72 mg/mL in seeds and 187.60 and 25.53 mg/mL in peels. Extracts showed antimicrobial activities against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* with considerable inhibition zones for seed (10, 9, 8 µm/mL) and peel (12, 10, and 10 µm/mL). Formulating chicken patties with 1%(w/w) extracts reduced lipid oxidation (TBA), TVN, and cooking loss by 48.2, 49.2, and 6.6% but increased WHC by 14.9% at the end of storage.

Conclusion The study enlightened the feasibility of using peels and seeds of pomegranate as a natural and economical alternative to chemical preservatives to prolong the shelf-life of meat products. The proposed valorization methodology converted food waste to natural preservatives and reduced food waste by extending patties' shelf-life. Implementing such a “waste valorization” and “waste reduction” approach could significantly enhance resource efficiency to meet sustainable development goals (SDGs) and net zero.

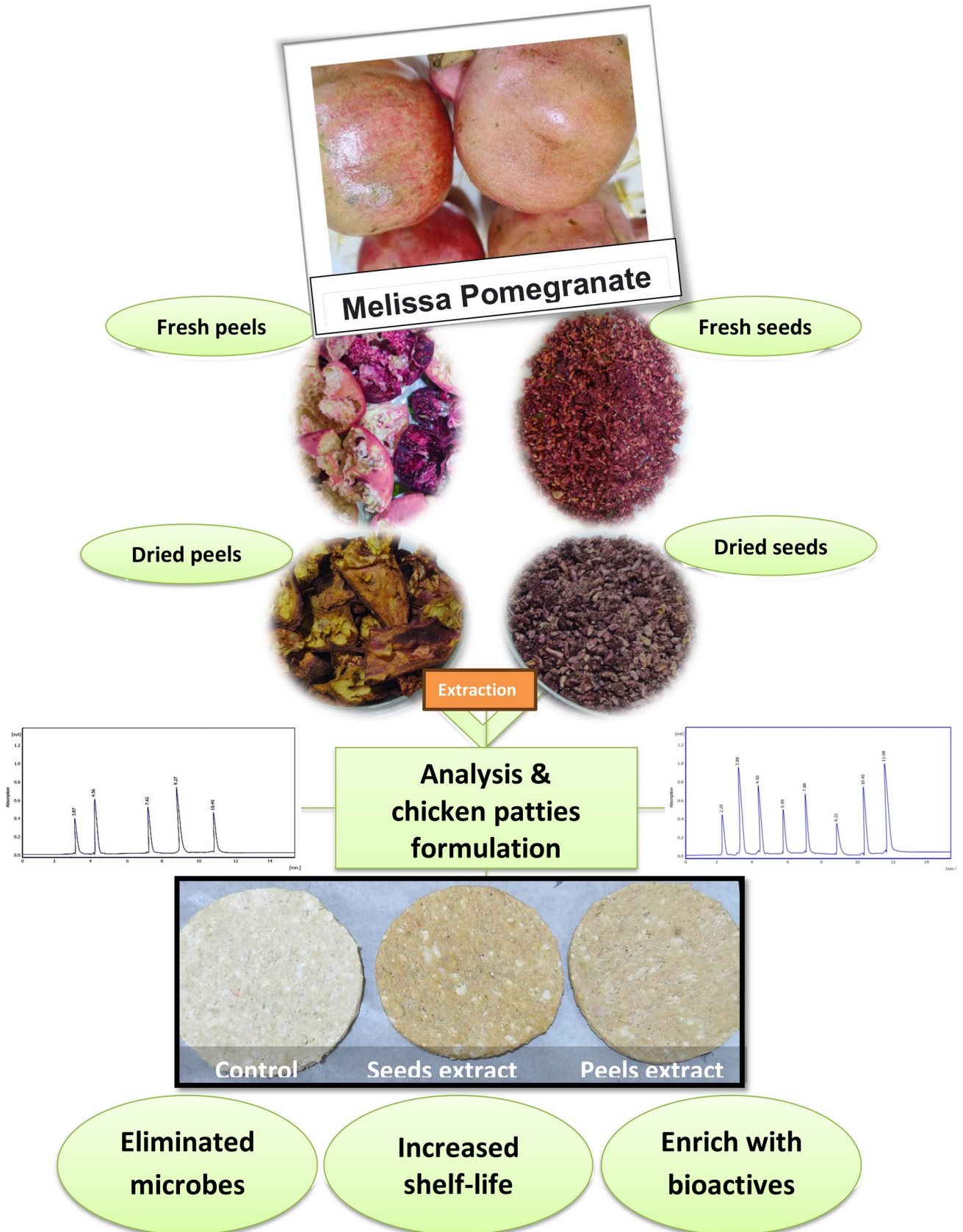
Recommendation Future research might consider using emerging technologies (e.g., ultrasound and electric field) to increase extraction efficiency and nanotechnology to enhance the stability of seed and peel extracts. Also, establishing a well-planned collecting and transporting methodology for fresh pomegranate waste to limit microbial contaminations could be considered for successful practical applications.

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Graphical Abstract



Keywords Bioactive compound · Extraction · *Punica granatum* · Zero waste · New product development · Waste bioprocess · Carbon footprint

Statement of Novelty

This work's novelty includes providing a platform based on combined "waste valorization" and "waste reduction," i.e., valorizing an agro-food waste (pomegranate processing by-products) into a natural food preservative and reducing the chicken patties waste through enhancing its shelf-life using the valorized products from pomegranates waste. At the same time, this work is based on green extraction technology, minimizing the environmental impact and carbon footprint of the proposed waste valorization process. Besides, this study provides new information on the bioactive composition of waste from the "Melissa" variety, indicating the opportunities for valorization and future application of valorized extract.

Introduction

The pomegranate from the Punicaceae family is native to Iran and northern India and has been cultivated throughout the Mediterranean region, the Middle East, Africa, and Europe since ancient times. Spanish settlers then brought pomegranate to California, among the primary producers of this nutritious fruit in the United [1]. Commercially grown pomegranate fruit (*Punica granatum* L.) includes various cultivars with different properties (e.g., color, size, and taste). This fruit has a high consumption, processing, and export rate, making it high-priced in pomegranate importing countries such as Taiwan. Due to inappropriate disposal, the pomegranate processing sector produces enormous by-products, particularly peels and seeds, which account for about 50% of the fresh fruit weight [2].

Pomegranate seeds have nutritionally essential substances, including sugars, proteins, fats, fiber, vitamin C, calcium, phosphorous, iron, thiamine, and riboflavin [3]. Similarly, pomegranate peels contain valuable components such as tannin acid, an astringent substance with antidote properties for treating diarrhea Pellettierine [4]. They also contain tannin, volatile alkaloids, and phenolics that hinder the oxidation processes of low-density cholesterol-holding lipoproteins [5]. Also, its bioactive compounds can inhibit *Escherichia*, *Klebsiella*, *Shigella*, and *Salmonella*, preventing intestinal infections, gastric ulcers, infectious ulcers, and diarrhea. These properties promoted research on the bioactive components of pomegranate peels in medical and pharmaceutical fields [4, 6, 7].

The Food and Agriculture Organization (FAO) estimated that 60% of total fruit production would be lost, with 25–30%

resulting from fruit processing. Accordingly, governments are working on sustainable approaches for agro-food waste management so that bioactive components recovery and application (e.g., as a component in a product) can provide further economic value [8, 9]. The need for pomegranate waste valorization has recently attracted the attention of food scientists [10]. For example, it has been used as a storage enhancer in buffalo meat [11], a nitrite replacement in sausage [12], an antibacterial in coated chicken meat [13], and a quality improver in chicken meat emulsions [14]. Sharma & Yadav [15] found that pomegranate peels and seeds are rich sources of dietary fiber. Due to valuable pharmaceutical and nutritional compounds, these by-products can be better utilized in the food industry instead of being exploited as feed for animals. The poultry industry is attaining more significance worldwide due to its better consumer recognition and freedom from religious hindrances. The fast food market's massive growth has escalated demand for ready-to-eat snack foods. Chicken patties are one of the favorite comminuted products, which have a noticeable position because of their distinctive flavor and palatability. The efficiency of plant-derived antioxidants against lipid and protein oxidation has not yet been thoroughly established despite promising findings from in-vitro and in-situ investigations. Moreover, work must be done to verify natural antioxidants' usefulness, marketability, usability, and possible health advantages [16]. Finding new sources of natural antioxidants and practical ways to utilize them should be a top priority for the various food industry sectors, such as meat processing [17, 18].

Considering the environmental impacts of food waste mis-handling, the industry needs scalable and practical methodologies to utilize these wastes to benefit from developing new products enriched with waste-originated bioactive compounds [17, 19]. Therefore, this study aims to valorize pomegranate waste into a natural preservative through a green extraction and analyze the extracts' bioactive profile, antimicrobial activity, and chemical properties. It also aims to develop extract-enriched chicken patties with enhanced shelf-life to assess the practicability of this valorization process and valorized product for the food industry. This research also aims to investigate the changes in the physicochemical properties of patties enriched with waste extract during cold storage to assess practical applications of the proposed waste valorization and utilization methodology.

Materials and Methods

Pomegranate Samples and Patties

The intact freshly harvested Salimi' pomegranates (*Punica granatum* var. Melissa) were collected from Owainat vilage (Tikrit, Iraq). Experts from the Horticultural Department of Basrah University verified the identity of the variety. Seeds and peels were separated manually and dried at 55 °C (Ychem Electrical Oven, China) for about 48 h to reach 10% moisture content. Dried samples were ground, sieved at 60 MSH, packed in airtight glass containers, and kept at -18 °C. At the same time, the breasts of 6-month-old white Leghorn chicken (average weight of 1.2 kg) were minced to prepare the patties according to the commercial process. Chicken patties with various percentages of seed/peel extracts were stored at 4 ± 1 °C. Figure S1 illustrates the flow chart of the study.

Analysis of Chemical Composition

The AOAC [20] procedures were used to determine samples' moisture, protein, fat, ash, and fiber content. The moisture content of pomegranate peels and seeds was estimated by taking 3 g and placing them in a dried and weighed Ceramic thimble. They were then placed in the oven at a temperature of 105 °C (Schutzart-Din 4005 D-IP Germany) for three hours. The protein was estimated using the Kjedel method, where the waste was digested with a Kjedel device (Buchi Model B-324 Malente, Germany), and then the percentage of nitrogen was estimated, through which the percentage of protein was measured. Crude fat was extracted from pomegranate wastes using petroleum ether solvent in a Soxhlet device, and the device was operated for 16–18 h. Then, the samples were placed in a drying oven at a temperature of 135 °C (Schutzart-Din 4005 D-IP Germany) for 2 h for drying, and the percentage of extracted fat was calculated.

As for ash determination, the dry sample was burned in a muffle furnace (Carbolite-S30.2AU England) at a temperature of 525 °C for 16–18 h, then the remainder was weighed and reported as the ash content. The crude fiber content was measured by digesting pomegranate seeds and peels with 1.25% H₂SO₄ for 30 min at 100 °C, and then the mixture was filtered using a cloth (Muscle or gauze) and washed with hot distilled water to get rid of residues. It was further digested with 1.25% NaOH, and the residues were transferred to a ceramic bowl and placed in the oven (Schutzart-Din 4005 D-IP Germany) to be dried at 101 °C until the weight stabilized. They were then weighed and placed in an incinerator muffle furnace (Carbolite- S30.2AU England) for 12 h at a temperature of 550 °C.

Green Extraction

The method mentioned in Lapornik et al. [21] was used to extract seeds and peels with minor modifications while following the guidelines for the green extraction process. Briefly, 10 g of crushed and dried samples were dissolved in 0.1 L of distilled water and mixed at 3500 rpm for 24 h in a vertical shaker (Sartorius Stedim CERTOMAT RM Type 8,864,942 Orbital Shaker Germany) at a constant temperature of 30 °C. The aqueous part was then filtered and centrifuged at 7871 g for ten min. The centrifuged extracts were then concentrated to 25% of their original weight at 30 °C using a rotary evaporator (SH-PK-50L SH Scientific, Korea). These concentrated samples were stored at -18 °C (Concord Chest Freezer FC 1100, China) until use.

Determination of the Total Content of Phenolics

Folin-Ciocalteu reagent and 4.5 ml of distilled water were added to about 0.1 ml of the extract (seeds and peels). The contents were agitated vigorously to estimate the total phenol content using this reagent, as described by Bashir et al. [10]. Three minutes of shaking were followed by adding 0.3 ml of 2% Sodium carbonate (Na₂CO₃). The mixture remained at room temperature for two hours. A UV–VIS (Thermo Fisher Scientific, United States) spectrophotometer was used at 760 nm to determine absorbance. **Figure S2** showed the connection between acid content and absorbance at 760 nm to determine how many phenols were present in the extracts. Gallic acid was used as a reference, and an equation derived from the titration curve for calcic acid was used to determine the number of total phenols in gallic acid equivalent.

The Total Amount of Flavonoids

Total flavonoids in pomegranate wastes were determined based on the aluminum nitrate method described by Lachguer et al.[22]. The standard distribution curve was created (Figure S3), and the absorbance was measured at 415 nm using a Thermo Fisher Scientific UV–VIS spectrophotometer.

Quantification of Phenolic Compounds

Reversed-phase high-performance liquid chromatography (HPLC) was used to quantify the important phenolic compounds according to Radovanović et al., (2015). In this sense, a SYKAMN HPLC chromatographic (SYKAMN, S 155-A/C Plus, Germany) system equipped with a UV detector, Chemstation, a Zorbax Eclipse Plus- Column C18-ODS, diameter 4.6, length 25 mm was used. The column temperature was 30 °C, and the gradient elution method was

employed. Eluent A (methanol) and eluent B (1% formic acid in water (v/v)) were utilized as described in the following: initial 0–4 min, 40% B; 4–10 min, 50% B; and 0.7 mL/min flowrate. The injected volume was 100 μ L for standards and samples. The autosampler mode performed the injections. The spectra were acquired in the 280 nm. Eight standard compounds were used: kaempferol, gallic acid, tannic acid, catechine, chlorogenic acid, ferulic acid, quercetin, and p-coumaric acid (Sigma-Aldrich Darmstadt, Germany).

Determination of Antimicrobial Efficacy

Bacterial strains were *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus* (local isolates of bacteria were obtained from the microbiology laboratory in the Department of Food Sciences—College of Agriculture—University of Basrah, Iraq). The activated bacteria were spread by loop on the solidified Nutrient Agar. The antibacterial agar disc diffusion technique (Bioanalyse Company, Turkey) was utilized. At 37 °C, after being cultured for 18–24 h in a nutrient broth medium, degrees Celsius, the bacteria were triggered—medium in Petri dishes. Sterile filter paper discs were immersed in pomegranate extract (peels and seeds) with dimensions of 6 mm. It was compared with the following antibiotic discs: Nalidixic acid (NA/30), Meropenem (MEM/10), and Spectinomycin (SPT/100). The dry discs were placed on a nutrient agar inoculated with bacteria spread over a Petri dish and incubated at 37 °C for 24 h to report the inhibition zone diameter in millimeters [23].

Preparation and Storage of Chicken Patties

The chicken patties were prepared according to Thanoun & Al-Jammaas [24]. Briefly, 1700 g of meat was minced with a three mm diameter using a shredding machine, and the treatments were divided into three treatments. T1 and T2 had 1% (w/w) seed and peel extracts, respectively. The control treatment (C) was prepared without waste extracts. According to Thanoun & Al-Jammaas [24], the meat patties were manufactured, placed in vacuumed polyethylene bags, and separated from one disc to the last piece of wax paper. The bags were sealed and refrigerated at 4 ± 1 °C for 12 days.

Quality Indicators of the Patties During Storage

Chemical Indicators

Total Volatile Nitrogen (TVN) A Macro Kjeldahl apparatus determined the chicken meat patties' TVN, according to Mousavi-Nasab et al. [25], and expressed as TVN per 100 g sample. 100 gm of chicken patties were mixed with 100 ml of distilled water. The mixture was transferred quantitatively with 30 ml of distilled water to a round bottom flask and dis-

tilled after adding 20 g of MgO, then 100 ml of 20% aqueous solution of formaldehyde (HCHO) and five Drops of silicone antifoaming agent, and then the same procedure for TVN determination was used, and 125 ml of the distillate was collected. The distillate was titrated using an aqueous solution of hydrochloric acid 0.05 nitrogen, and the amount of TVN was calculated in mg/100 g of pie meat from the volume (V) of the added hydrochloric acid and its concentration (C) according to Eq. 1.

$$\text{TVN} = \frac{(V_1 - V_2) \times C \times 14}{(5m/100)} \times 100 \quad (1)$$

where in V_1 is the titration volume for the tested sample (mL); V_2 is the titration volume of the blank sample (mL); C is actual HCl concentration (mol/L); m is chicken meat patties weight (g).

Thiobarbituric Acid (TBA)

Based on the intensity of the produced pink chemicals, total burger acidity TBA was calculated for chicken patties utilizing the strategy determined by Lee et al. [26], which involves measuring the concentration of malonaldehyde (MA) accumulated with a spectrophotometer at a wavelength of 532 nm. The numbers were reported as the amount of malonaldehyde per kilogram of chicken or mg MA/kg.

Physical Indicators

Water Holding Capacity

According to the procedure described by Sharma & Yadav [15], the WHC of chicken was determined using 15 g of chicken patties that were weighed and placed in 2.8×11 cm centrifuge tubes, 22.5 ml of 0.6 M NaCl solution was added, and the contents were stirred for 1 min with a glass rod. It was stirred after keeping the meat slurry for 15 min at 4 °C. It was then centrifuged at $12,000 \times g$ for 15 min. The supernatant layer was decanted, and the volume was recorded. The amount of added solution retained by the meat is determined as the water holding capacity in ml per 100 g of chicken patties. The WHC of chicken was determined using Eq. 2.

$$\text{WHC} = \text{TWV}_1 - \text{TWV}_2 \quad (2)$$

where WHC is water holding capacity (ml), TWV_1 is Total water volume (ml), and TWV_2 is the amount of water in the included cylinder (ml).

pH Value

The method Qin et al. [27] described was used to measure the pH of chicken meat patties using a pH meter (AZ Model:8685,

China). A 5 g of minced meat was combined with 100 ml of distilled water and placed in a beaker. After about five minutes, the pH value was determined.

Cooking Loss and Cooking Yield

The approach outlined by Kim et al. [12] was used with transgenesis. To calculate cooking loss and cooking yield, the initial weight of chicken patties was recorded before the samples were fried at 130 °C for 3 min and cooled to approximately 21 °C. After refrigeration for 3 h, the cooking loss was calculated by comparing the weight loss after heating. To calculate the cooking loss and cooking yield, Eqs. 3–4 have been used.

$$CL = \frac{W_r - W_c}{W_r} \times 100 \quad (3)$$

$$CY = \frac{W_r}{W_c} \times 100 \quad (4)$$

where CL = Cooking loss (%), W_r = weight of patties before cooking (g), W_c = weight of cooked patties (g), and CY is cooking yield.

Cooking Shrinkage

The percentage of shrinkage in diameter was calculated by measuring the diameter of chicken nuggets before and after cooking. Equation 5 was used to determine cooking shrinkage [28].

$$S = \frac{D_i - D_o}{D_i} \times 100 \quad (5)$$

where S = Shrinkage (%), D_i = Uncooked diameter (cm), D_o = Cooked diameter (cm).

Half-Life Calculation Kinetic Model for TBA Increment During Storage

Led at “Emerging Food Processing Technology” of National Pingtung University of Science and Technology, half-life calculation was performed based on a kinetic model for TBA increment during storage. Zero and first kinetics models were used to calculate increment kinetic TBA (mg Malonaldehyde/kg oil) during the storage period as given in Eqs. 6–7 [29]:

$$TBA = TBA_0 - k_0 t \quad (6)$$

$$TBA = TBA_0 \exp(-k_1 t) \quad (7)$$

where TBA is the thiobarbituric acid (mg Malonaldehyde/kg oil) at any given time, TBA_0 is thiobarbituric acid at zero

time, k_0 is the constant rate of the zero-kinetic model (mg Malonaldehyde/kg oil. day), k_1 is the constant rate of the first kinetic model (1/day), and t is the time (day). The half-life to an increment of TBA in chicken meat was calculated from Eq. 8 [30].

$$t_{1/2} = \frac{-\ln(0.5)}{k} \quad (8)$$

k is the constant rate (1/day).

Statistical Analysis

A complete randomized design (one-way experiment) was used in this study. Data analysis was executed using SPSS (version 25, IBM Corp, Canada). Treatments were performed in triplicate, and the least significant differences were used to compare among means at a significant level of $p < 0.05$.

Results and Discussion

Chemical Content of Pomegranate Seeds and Peel

Table 1 illustrates that the inedible components of the pomegranate (the seeds and peels) have a high nutritional value, suggesting that they might be used to produce novel foods. Table 1 displays the estimated ash content, crude fiber, protein, fat content, and moisture content based on the dry weight. Moisture and ash levels in the seeds (0.0869, 0.0155 $\text{kg}_{\text{water}}/\text{kg}_{\text{db}}$, respectively) and peels (0.1111, 0.0176 $\text{kg}_{\text{water}}/\text{kg}_{\text{db}}$, respectively), indicating significant differences ($p < 0.05$).

The protein and crude fat percentages in seeds were 0.1788, 0.1876 $\text{kg}_{\text{water}}/\text{kg}_{\text{db}}$, respectively, significantly higher than those of peels (0.0333, and 0.0176 $\text{kg}_{\text{water}}/\text{kg}_{\text{db}}$, respectively). As for the crude fiber, it was significantly ($p < 0.05$) higher in the pomegranate seeds (0.2140 $\text{kg}_{\text{water}}/\text{kg}_{\text{db}}$) compared to peels (0.1293 $\text{kg}_{\text{water}}/\text{kg}_{\text{db}}$).

This study showed that fiber and protein in the seeds were higher than in peels by 53.97 and 371.11%, respectively. The results agreed with Jalal et al., [4] when they studied the chemical content of pomegranate seeds and peels, as the moisture, protein, fat, and ash in the seeds were 0.0616 (5.81%), 0.1583 (13.67%), 0.4206 (29.61%), and 0.0148 $\text{kg}_{\text{water}}/\text{kg}_{\text{db}}$ (1.46%), respectively, while in the pomegranate peels were 0.1426 (12.48%), 0.0337 (3.26%), 0.0176 (1.73%), and 0.0342 $\text{kg}_{\text{water}}/\text{kg}_{\text{db}}$ (3.31%), respectively, as the moisture and ash in the peels were higher than in the seeds. Table 1 indicates that seeds contained higher protein and fat concentrations than peels.

Total Phenols and Flavonoids in Pomegranate Peels and Seeds

According to Fig. 1, extracts of seeds and peels contained 66.71 and 187.60 mg/ml of total phenolics, respectively. These distinctions were statistically significant ($p < 0.05$). The total flavonoid content of the extracts of the seeds (9.72 mg/ml) and peels (25.53 mg/ml) was also shown to differ significantly ($p < 0.05$). According to these observations, peels have higher phenolic (181.12%) and flavonoid (162.65%) concentrations than seeds. Knowing the overall level of phenols and flavonoids can be a crucial indicator of the extract's antioxidant ability whenever an extract is considered a natural antioxidant source in various meals. It is a complex combination of organic components. The quality and amount of those chemicals vary widely depending on factors, including growth stage, environment, extraction method, and extraction solvent. This study agreed with Antony and Farid [31], who found that the peels' total phenols were 53.65 mg/g, more than the seeds' 7.94 mg/g. Therefore, Sharma and Yadav [15] findings align with these results. Peels had 0.88 mg GAE/g of total phenols, but seeds only managed 0.66 mg GAE/g. According to the literature [11] and [10] the content of the water extract of pomegranate peels of total phenols and total flavonoids were 149.75 ± 8.48 mg/g and 13.13 ± 1.73 mg/g, respectively, which are values similar to our current study. On the other hand, Setlhodi et al. [32] found that the content of total phenols and total flavonoids in the peels reached 66 and 7 mg/g, while in the seeds, these values reached 2.2 and 2 mg/g, respectively. These were lower than the values reported in the present study, which might be due to differences in the fruit variety and extraction conditions.

The Concentration of Bioactive Compounds in the Extracts

The chromatograms of standard compounds and pomegranate extracts are presented in Figures S4 and S5. According to Table 2, HPLC of pomegranate seed extracts, tannic,

Table 1 Chemical content of pomegranate peels and seeds

Quantity (kg _{water} /kg db.)	Chemical Content	
	Pomegranate seeds	Pomegranate peels
Moisture	0.0869 ± 0.004^a	0.1111 ± 0.002^b
Ash	0.0155 ± 0.005^a	0.0176 ± 0.004^b
Protein	0.1788 ± 0.002^b	0.0333 ± 0.006^a
Crude Fat	0.1876 ± 0.004^b	0.0176 ± 0.002^a
Crude Fiber	0.2140 ± 0.007^b	0.1293 ± 0.0040^a

The deferent letters refer to significant differences at level of 0.05
Db dry basis

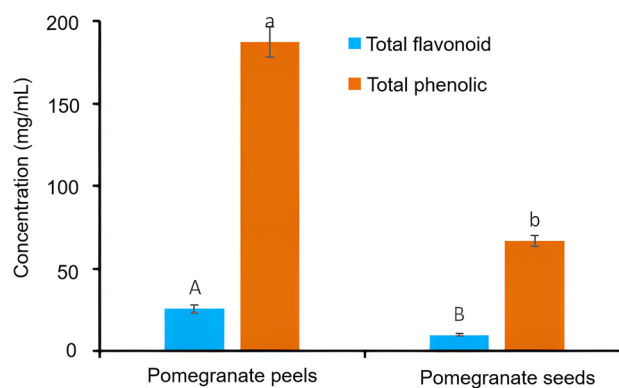


Fig. 1 Total content of phenolics and flavonoids of pomegranate peels and seeds. Different small letters refer to significant difference in total phenolic, and different capital letters refer to significant difference in the total flavonoids

and gallic acid were the major components, with 18.95 and 16.49 ppm, respectively. Seed extracts also contained 12.5, 11.25, and 5.05 mg/kg ferulic, chlorogenic, and quercetin. As for pomegranate peel extract, it was revealed that tannic acid had the highest concentration (~41.25 mg/kg), followed by Gallic (36.59 mg/kg) and Chlorogenic acid (20.14 mg/kg). The peel extract also contained ferulic acid, kaempferol, catechine, p-coumaric, and quercetine at 18.20, 14.25, 13.88, 10.56, and 9.25 mg/kg, respectively. Results showed that both extracts contain valuable bioactive compounds. However, different chemical profiles were observed for peel and seed extracts. For example, the peel extract was rich in kaempferol, catechine, and p-coumaric acid, but these compounds were not identified in the seed extract.

The results converged with what was reached by Ali et al. [30] when studying pomegranate peel, as it contained the effective compounds chlorogenic acid, catechin, ferulic acid, and kaempferol, as these compounds were also found in our study. The difference in the proportions and type of phenolic compounds may be due to environmental and genetic conditions, cultivation and storage conditions, and soil type, as explained by Setlhodi et al., [32]. El-Hadary and Taha [33] also found 25, 32.75, 15.62, 0.86, 4.92, and 35.10 ppm of gallic acid, catechein, chlorogenic, p-coumaric, ferulic, and quercetrin in pomegranate peels. Also, Abd-El Raouf et al., [34] reported the presence of gallic acid and p-coumaric in pomegranate seeds, with a concentration of 4.89 and 2.66 ppm. These differences in the presence of phenolic compounds in pomegranate waste may be due to differences in pomegranate variety, cultivation, and extraction conditions.

Antimicrobial Efficacy

Compared with the antibiotic Spectinomycin (SP/100), pomegranate peel extract had the most potent antibacterial activity (12 μ /ml) against *Staphylococcus aureus* (10 /ml) followed by the extract as an antimicrobial against *Escherichia coli* (10 μ /ml) (Table 3). Nalidixic acid (NA/30) comes from the pomegranate peel extract, preventing *P. aeruginosa* growth, indicating that the antibacterial effect of pomegranate peel extract was comparable to the antibiotic Meropenem (MEM/10). Nozohour et al. [35] findings were in line with these findings, who studied the inhibitory effect of extracts of pomegranate peels and seeds against various kinds of bacteria, the most important of which is *P. aeruginosa* (clinically isolates), *S. aureus* (clinically isolates) and *S. aureus* (PTCC 1112). The inhibitory value of the peel extract of the above bacterial species was (27.3, 27.5, 25.3) 9 mg/disc, respectively, while the seed extract was (19.3, 19.2, 22) 9 mg/disc, respectively. Results were also in line with El-Moujahed report [36]. The present study revealed that pomegranate peel and seed powder extracts are effective natural preservatives, as their incorporation reduced total bacterial count, highlighting their antibacterial activity. **Figure S6** illustrates the antimicrobial efficacy of seed and peel extracts.

Chemical Indicators

Total Nitrogen Volatile

Results presented in Table 4 clarified significant differences ($p < 0.05$) in the amount of TVN when adding pomegranate seed and peel extracts to the refrigerated chicken patties. The results showed increased TVN values in chicken patties treated with extracts. However, the increase was slight compared to the sample used as a control (C), in which the rise was evident, as the values of TVN in the patties

treated with T1 seed extracts were 8.24 mg/100 g on the first day and reached 18.66 mg/100 g at the end of the 12-days of refrigeration period, as for the second treatment with peels extracts T2 was 8.21 mg/100g on the first day and reached 18.28 mg/100 g at the end of the storage period. As for the control sample C, the increase was evident, from 8.47 mg/100 g to 32.87 mg/100 g.

Nitrogenous substances decomposition by microbial activity increased the TVN content during refrigeration. Dakheli, [37] indicated the positive effect of adding pomegranate waste extracts to meat products in inhibiting the growth of microbes, especially proteolytic microorganisms, which causes protein breakdown and leads to the volatilization of nitrogen compounds; they found that the TVN value on the last ninth day for the sample treated with pomegranate waste was 23.04 (Mg/100 g), while the control sample reached 33.80 (Mg/100 g), and just instructions of Public Health's Bureau of the National Veterinary Office If the amount of TVN in poultry exceeds 27 mg/100 g, the product

Table 3 Results of the antimicrobial activity test of pomegranate Peels and Seeds in comparison with antibiotics

Microorganism type	Diameter of inhibition zones of bacteria test / (mm)		
	Antibiotics# μ m/mL)	Pomegranate peel μ m/mL)	pomegranate seeds μ m/mL)
<i>Escherichia coli</i>	14 \pm 0.12 ^{a*}	10 \pm 0.63 ^b	9 \pm 0.24 ^c
<i>Staphylococcus aureus</i>	16 \pm 0.22 ^{a**}	12 \pm 0.33 ^b	10 \pm 0.54 ^c
<i>Pseudomonas</i>	12 \pm 0.25 ^{a***}	10 \pm 0.45 ^b	8 \pm 0.65 ^c

*The different letters in each row refer to significant differences at a level of 0.05

Antibiotics: Nalidixic acid 30 μ m, Spectinomycin 100 μ m, Meropenem 10 μ m

Table 2 Polyphenols composition (mg/kg) from pomegranate peel and seed extracts

No	Component	Formula	pomegranate peel extract			pomegranate seed extract		
			RT* (min)	RPA (%)	C (mg/kg)	RT (min)	RPA (%)	C (mg/kg)
1	Kaempferol	C ₁₅ H ₁₀ O ₆	2.25	10.25	14.25	–	–	–
2	Gallic acid	C ₇ H ₆ O ₅	3.88	16.25	36.59	3.87	18	16.49
3	Tannic acid	C ₇₆ H ₅₂ O ₄₆	4.50	13.44	41.25	4.56	22	18.95
4	Catechine	C ₁₅ H ₁₄ O ₆	5.99	10.25	13.88	–	–	–
5	Chlorogenic acid	C ₁₆ H ₁₈ O ₉	7.88	12.44	20.14	7.82	19	11.25
6	Ferulic acid	C ₁₀ H ₁₀ O ₄	9.22	8.98	18.2	9.27	22	12.05
7	Qurcetine	C ₁₅ H ₁₀ O ₇	10.45	12.65	9.25	10.49	18.98	5.05
8	p-Coumaric acid	C ₉ H ₈ O ₃	11.98	15.55	10.56	–	–	–
Total			56.15	99.81	153.56	36.01	99.98	63.79

*RT retention time, RPA relative peak area, C concentration

is not consumable. The product could be considered consumable if it meets the maximum limit of 20–27 mg/100 g. Bacterial activities and enzymes in meat could increase the amount of TVN. Al-Baidhani & Al-Mossawi [38] reported that the TVN of meat burgers was increased during storage, which was similar to the trends observed in the current study.

Thiobarbituric Acid

One of the most common ways to evaluate oxidative stress in meals is by analyzing TBA metabolites produced by the food. TBA levels in refrigerated chicken patties are shown in Table 4. While all samples showed an increase in TBA levels throughout storage, the control sample C showed the most significant increase, reaching a peak value of 2.21 mg MA/kg after 12 days in the fridge. While T1 and T2 samples treated with seed and peel extracts had smaller increases (0.637 and 0.632 mg MA/kg after 12 days of storage, respectively), T3 samples not treated with extracts showed no change. This observation demonstrates the impact of these extracts on the product's oxidative stability. The natural antioxidant components in pomegranate seeds, skins, and phenolic compounds may be responsible for the reduced TBA levels. The results agreed with what was found by Sharma and Yadav [15] during their study on adding aqueous extract of pomegranate seeds and peels to chicken patties and storing them in the refrigerator, where they found that there was an increase in TBA values for all treatments. However, this increase was less in the samples treated with seed and peel extracts compared to the control sample, and the increase was also less than in the samples to which the artificial antioxidant BHT was added, as the TBA values reached at the end of the 16-day refrigerator storage period for the added chicken patty samples. The water extracts of the peels and seeds were 0.83 and 1.00, while in the artificial antioxidant (butylated hydroxytoluene) was 1.40, which is less than the control samples, i.e., 1.95. The researchers explained the results of their

study that the presence of active compounds in aqueous extracts of pomegranate waste could be used as natural antioxidants in chicken patties instead of using industrial antioxidants.

Physical Indicators

pH

As shown in Fig. 2a, chicken patties' pH was affected by adding aqueous extracts of pomegranate waste (seeds and peels) at a concentration of 1% and varied storage durations. The patties treated with T1 seed extract had a pH of 5.66 before storage and reached 6.97 after 12 days in the fridge, while those treated with pomegranate peel extracts had a pH of 5.63 before storage increased to 7.27 after 12 days. The control treatment had a higher pH than the patties treated with the extracts. Gram-negative bacteria, such as *Pseudomonas* and *Acinetobacter*, operate to break down proteins and separate amine groups, which raises pH. Therefore, this may be the case during storage [37].

Similarly, Sharma and Yadav [15] investigated the impact of pomegranate waste on the physical markers of refrigerated meat products. They found that pH rose at the end of storage due to bacterial activity, protein breakdown, and increased amine. They found that at the end of the 16-day refrigeration storage period for the chicken patty samples treated with pomegranate seeds and peel extract, the values reached 6.39 and 6.40, respectively. In contrast, the same value in the control sample and samples treated with the synthetic antioxidant BHT reached 6.42.

Water Holding Capacity

Figure 2b shows how the WHC of chicken meat patties changed after being enriched with extracts. WHC decreased from 18.22 ml on the first day to 15.47 ml after 12 days of refrigeration of the sample treated with pomegranate seed extract, decreased from 18.78 ml on the first day of storage to

Table 4 Total Volatile Nitrogen and Thiobarbituric acid of chicken patties during cold storage

Storage period (day)	(mg/100 g)		TBA (mg MA/kg)			
	C	T1	C	T1	T2	C
0	8.47±1.05 ^a	8.24±1.31 ^a	8.21±1.60 ^a	0.135±0.01 ^a	0.131±0.02 ^a	0.131±0.01 ^a
4	15.71±1.16 ^c	12.42±2.40 ^b	12.38±2.22 ^b	0.381±0.02 ^c	0.362±0.01 ^b	0.36±0.04 ^b
8	20.82±3.28 ^d	16.82±2.98 ^c	16.71±3.02 ^c	0.638±0.01 ^e	0.453±0.03 ^d	0.45±0.05 ^d
12	32.87±5.19 ^e	18.66±4.77 ^a	18.28±6.49 ^a	1.221±0.04 ^f	0.637±0.04 ^e	0.632±0.07 ^e

*Different letters refer to significant differences at level of 0.05 (interaction between storage periods and treatments)

TVN: Total Volatile Nitrogen, and TBA: Thiobarbituric acid; C: control samples without adding the pomegranate aqueous extract; T1: samples contain 1%(w/w) pomegranate seeds extract; T2: samples contain 1%(w/w) pomegranate peels extract

15.65 ml after 12 days of storage for the samples treated with peel extract. Meat's WHC, or its ability to keep the water when subjected to external pressures, is directly related to its quality: the longer it is stored, the lower its pH and WHC [39]. It was gradual as the values of WHC during the storage periods of 4 and 8 days for the seed extract sample were 17.31 ml and 16.23 ml, respectively, while in the sample treated with peels extract, it was 17.6 ml and 13.53 ml, respectively, for the same two storage periods above. As for the control sample, the decrease was apparent. The WHC

values were 17.42 ml, 15.37 ml, 13.8 ml, and 11.77 ml for storage periods of 0, 4, 8, and 12 days, respectively.

The results revealed no significant ($p < 0.05$) differences between seed and peel extracts in WHC, but WHC for patties treated by extracts was higher than the control. The high WHC of chicken patties enriched with pomegranate extracts is due to the presence of phenolic compounds. These bioactive compounds are natural antioxidants that contribute to the protection and stability of fats by curbing the activity of free radicals resulting from oxidation. The reason for the

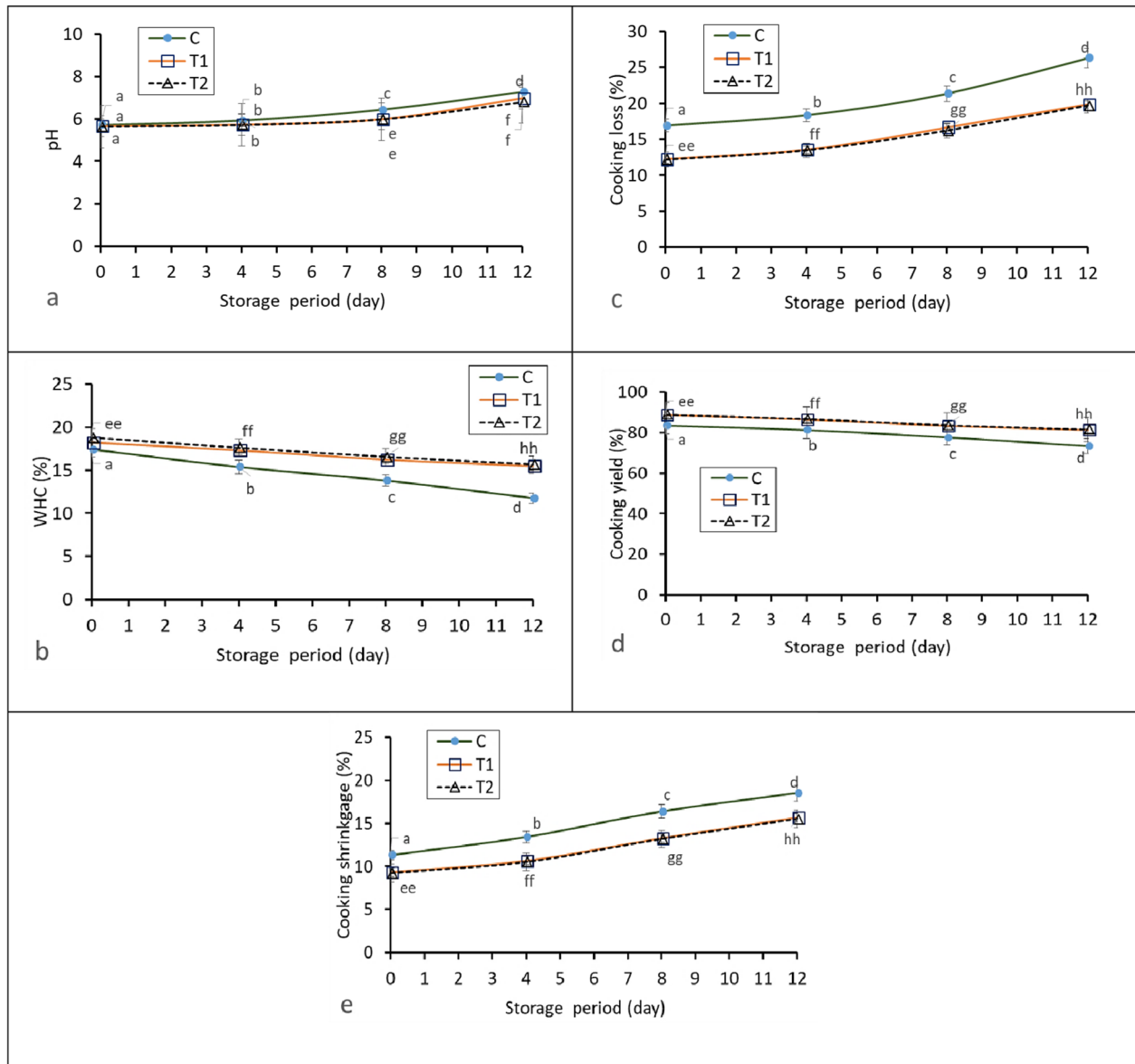


Fig. 2 Physical properties (a: pH, b: WHC, c: cooking loss, d: cooking yield, and e: cooking shrinkage) of chicken patties treated by aqueous extract of pomegranate peels at a concentration of 1% of the weight of the meat versus storage period. T1: adding aqueous extract of pomegranate seeds at a concentration of 1% (g/100g), T2: add-

ing an aqueous extract of pomegranate peels at a concentration of 1% (g/100g) and C: Without adding the aqueous extract of pomegranate seeds and peels. The different letters refer to significant differences at level of 0.05

higher WHC of chicken patties treated with extracts prepared from pomegranate waste may be attributed to its content of phenolic compounds that, as antioxidants contribute to the protection and stability of fats by curbing the activity of free radicals resulting from oxidation and limiting the rupture of cellular membranes surrounding and maintaining muscle fibers, which increases of the ability of the meat to retain water during storage, and that these extracts contributed to providing stability to the cellular structure of the meat and protecting the components of the sarcoplasm and fluids in the membranes during storage of the meat from oxidative damage, which results in less loss of exudate liquid upon thawing, and this is reflected in the susceptibility to loss during cooking and an improvement in susceptibility meat to bind water, as the safety and protection of these membranes and limiting their rupture contribute to preserving the cellular components of the meat, which leads to a decrease in exuded liquid and an improvement in the ability of the meat to hold water during storage.

At the same time, the high number of hydrogens causes or increases the binding of water to protein inside the muscle cell, which causes water penetration from outside into the inside of the cells Ibrahim et al. [40]. The protein decomposition or biochemical changes associated with the refrigeration of meat products may account for the drop in WHC in all samples during storage. In contrast, the drop in WHC values at the end of refrigeration can be attributed to water loss through evaporation. Research on the effects of adding pomegranate waste extracts to various refrigerated meat products yielded results consistent with Serdaroğlu et al. [14], who reported that using 1–3% pomegranate waste powder improved the beef burger samples WHC during 12 days of cold storage, the WHC increased and reached 74.08%, while the WHC in samples without the addition was 70.43%.

Cooking Loss

When making minced beef patties, it is essential to consider that they will shrink in size while cooking. Variables such as fat content, whether the food was refrigerated or frozen, and the presence or absence of additives impact the weight lost during cooking. Figure 1c displays the data on the weight loss percentage during cooking for the frozen chicken patties due to adding the prepared pomegranate seed and peel extracts. Patties treated with pomegranate seed extract had a 12.21% drop in weight, while patties treated with pomegranate peel extract had a 12.16% decrease in weight compared to untreated patties.

After four days of storage, the loss percentage in the meat patties treated with extracts increased to 18.32%, and after eight days of storage, it reached 21.33% and continued to rise until the end of the 12-day storage period. However,

this increase was less pronounced in the meat patties treated with extracts than in the control treatment. Consistent with previous studies by Serdaroğlu et al. [14], our findings show that the weight loss during cooking increases with the time the sample is stored, while it is still less pronounced than in the control sample. The results were in line with Abdel Fattah et al. [41] who used pomegranate peel powder to improve cooking properties, including cooking loss, as they found that the percentage of cooking loss for the control sample was higher than samples containing pomegranates, as the percentage reached 28.67% at the end of the 12-days cold storage period in the control sample. The percentage improved when adding pomegranate waste powder at 1%, 2%, and 3% until the end of the storage period; the increase was 22.95%, 20.69%, and 18.44%, respectively.

Cooking Yield

Figure 1d displays how the percentage of cooking yield in chicken patties kept in the fridge changed after adding extracts generated from pomegranate waste. Regarding cooking yield, the patties treated with pomegranate seed extract achieved 88.76%, more significant than the control treatment's 83.49%. The results also showed that the cooking yield percentage dropped as the storage period progressed; in the control treatment, it dropped at the end of the 12-day storage period and reached 73.34%, while the yield percentage dropped to 81.22% and 81.55% for patties treated with extracts at the end of the storage period.

Serdaroğlu et al. [14] stated that adding pomegranate waste powder to the product improved the product's characteristics, and the oxidation and decomposition processes decreased compared to the control sample. They found that when adding 1% pomegranate seed powder, the cooking yield was 97.42%, while when the percentage was increased to 3% and 5%, the yield increased and reached 99.95 and 99.81%, respectively, while the yield was lower in the samples. Control is 96.86%. as the oxidation and decomposition processes decreased compared to the control sample. The researchers found that the cooking efficiency of the emulsions increased with the increase in the levels of waste powder. Since pomegranate extract is rich in phenolic components and flavonoids, it acts as a natural antioxidant and helps beef patties maintain more fat and moisture while cooking.

Shrinkage

In Fig. 2e, we can observe how the diameter of chicken meat patties changed after being refrigerated and treated with extracts made from pomegranate peels and seeds. The diameter shrinkage ratio in the control group was 11.32. Therefore, adding extract reduced the shrinking of the beef patties.

Table 5 Rate constants of zero and first orders kinetic models, statistics parameters, and half-life

Treatments	k0	Zero order model			First order model			
		RMSE	R ²	t _{1/2}	k1	RMSE	R ²	t _{1/2}
T1	0.043	0.020	0.987	16.12 ^a	0.140	0.036	0.948	4.95 ^a
T2	0.042	0.021	0.987	16.50 ^a	0.149	0.0006	0.949	4.651 ^a
C	0.081	0.066	0.974	8.55 ^b	0.185	0.001	0.995	3.747 ^b

T1: adding aqueous extract of pomegranate seeds at a concentration of 1% by weight of the meat, T2: adding aqueous extract of pomegranate peels at a concentration of 1% of the weight of the meat, and C: Without adding the aqueous extract of pomegranate seeds and peel. Different letters in t_{1/2} column refer to significant differences at a level of 0.05

The beef patties treated with pomegranate peel extract shrank by the least amount in diameter (9.24%), followed by those treated with pomegranate seed extract (9.32%). Because meat can retain water, the proportion of shrinkage in cooked meat products is decreased. The shrinkage percentage constantly grew, indicating that the time spent in cold storage influenced it. After four days of storage, the proportion of seed- and peel-extracted meat patties reached 10.66% and 10.52%, respectively; after eight days of storage, these percentages increased to 13.29% and 13.2%; and after 12 days of storage, they reached 15.67% and 15.52%. Compared to the control treatment, where shrinkage was 13.43% after four days, 16.39% after eight days, and 18.53% at the storage end, this increase was less severe.

The results were in line with Abdel Fattah et al. [41], who used pomegranate peel powder to improve cooking properties, including shrinkage in the diameter of meat products. According to these authors, the percentage of diameter shrinkage was less in pomegranate-containing samples than in the control treatment. The diameter shrinkage rate was lower in the samples containing pomegranates than in the control treatment, where the percentage reached 12.36% at the end of the 12-day cold storage period in the control sample. The percentage improved when adding pomegranate waste powder at 1%, 2%, and 3% at the end of the storage period and reached 10.23%, 8.89%, and 7.12%, respectively. The difference in the shrinkage rates in diameter between the untreated and extract-treated patties could be due to phenolics, which improve the capacity of chicken meat to retain water and prevent weight loss during cooking.

Mathematical Modeling of TBA Development and Half-Life

According to Table 5, the zero-order model with a rate constant of 0.042732 could be used for T1 as it yielded lower RMSE and higher R² than the first-order model. On the other hand, the first-order model can be applied for T2 and C, considering the RMSE and R² values were lower (Table 5). Results showed that C, T1, and T2 have a half-life of 3.75, 16.12, and 4.65 days, respectively.

Considerations for Practical Applications of the Proposed Waste Valorization Protocol

Despite the new information revealed in the present study, continuing research in this area could address the challenges and facilitate industrial implementation. For example, obtaining fresh and microbial-free raw material could be challenging but could be addressed well by planning a collection system and transportation platform. Also, the extracts might be unstable if stored at elevated temperatures for long times; while stability tests could be performed, there is a need for storage at controlled conditions and quick usage as a natural preservative. Furthermore, the bioactive profile of other cultivars of pomegranates might be different by pomegranate varieties, growing area, maturity, cultivation, and climate. Moreover, despite being affordable and widely available, rotary evaporator-based aqueous extraction consumes high energy and time compared to organic solvents. However, organic solvents could not meet the sustainability criteria. The possibility of using emerging extraction technologies, such as sonication and electrical fields, might be explored in future works. Providing the industry with such information could facilitate practical applications in large-scale production.

Conclusion

This research revealed that Melissa pomegranate processing residues (peels and seeds) are valuable sources for waste valorization, considering the presence of bioactive compounds (e.g., gallic acid, chlorogenic acid, tannic acid, ferulic acid, kaempferol, catechine, p-coumaric, and quercetin) with antioxidants and antimicrobial effects. Such findings give these residues promising capability as practical natural food additives for developing foods with functional ingredients. The valorized product effectively enhanced the shelf-life of a perishable food sample, i.e., chicken patties, and well-maintained quality parameters during storage, further contributing to waste reduction. Pomegranate waste extracts also

enhanced the nutritional properties of patties. Therefore, the combined waste valorization-waste reduction approach proposed in this study could be implemented in the food industry to contribute to sustainable development goals (SDGs) and zero waste. Future research may explore applying novel statistical analyses combined with in-vivo tests to determine the correlation among biological activities (e.g., anti-inflammatory effects) and using emerging energy-saving extraction technologies for further contributions to SDGs.

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Declarations

Competing Interests The authors have no conflict of interest.

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