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Research Article

Variability in Phytochemical Contents and Biological Potential of Pomegranate (*Punica granatum*) Peel Extracts: Toward a New Opportunity for Minced Beef Meat Preservation

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Extraction of Tunisian pomegranate peels was employed with different solvents such as ethyl acetate, acetonitrile, and water. Total phenolic and flavonoids contents, antioxidant activity, and antibacterial capacity against five foodborne pathogenic bacteria were evaluated. The highest values of polyphenols (351 mg gallic acid equivalent/g), flavonoids (104 mg quercetin/g), and DPPH and ABTS inhibition were recorded in the ethyl acetate extract followed by the aqueous extract. The latter present the maximum antibacterial potential against *S. enterica*, *P. aeruginosa*, and *E. coli*. The potential use of the lyophilized aqueous extract (AE), used for safety reason and being rich in phenolic, as biopreservative in minced beef meat was described. AE was incorporated at 0.1, 0.5, and 1% and compared with 0.1% butylated hydroxytoluene (BHT). During 21 days at 4°C, AE at 1% could appreciably retard the microflora proliferation ($p < 0.05$), the accumulation of MetMb and the carbonyl group ($p < 0.05$), slowing down the loss of sulphydryl proteins ($p < 0.05$), and led to a decrease ($p < 0.05$) in primary (peroxide value and conjugated dienes) and secondary lipid oxidation (TBARS) in treated meat. By the 14th day, AE-treated minced meat obtained higher sensory scores than untreated and BHT samples. Based on these results, lipid and protein oxidation changes and sensorial attributes were useful in discriminating meat samples by overall acceptability prediction. Generally, AE at 1% presented the potent preservative effect that could be utilized as an application on meat-substituting synthetic antioxidant.

1. Introduction

The minced meat has been targeted as one of the main foods that deserve expanded attention. Consequently, the safety of meat is of major concern to consumers, food industries, government agencies, public health professionals, researchers, and the general public locally, nationally, and internationally [1, 2]. On the other hand, it is well known that minced meat was judged as principle substrate maintaining the development of several spoilage and pathogenic bacteria, leading then to a loss of product quality [3]. Equally, lipid and protein oxidation in meat mincing conducted to a quick quality deterioration causes changes in nutritional and sensory quality (flavor, texture, and color) [4, 5]. To prevent oxidative degradation, food industry used synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) demonstrating a toxical effect as reported in recent studies [6]. For this reason, current scientific research studies tried to find alternative antioxidants obtained from natural sources [7, 8] and tested as strong preservative to be incorporated in different meat products [6, 7, 9–15].

Pomegranate fruit parts, such as arils, peels, and rinds, were famous by their elevated antioxidants concentration

showing various activities [6]. Pomegranate peel application has been studied in beef meat [8, 16, 17], cooked chicken products [18–21], cooked goat meat [22], and pork meat [23, 24].

In Tunisia, more than sixty pomegranate cultivars have been identified and biologically characterized [25–28], but unfortunately, little data are existing on their biological potential and application in food systems. This study aimed to investigate (i) the impact of three different extracting solvents ethyl acetate, acetonitrile, and water on phytochemical contents and antibacterial potency in Tunisian pomegranate peel, and (ii) the evaluation of the aqueous pomegranate peel extract at different levels on the raw minced beef and their effect on overall acceptability.

2. Materials and Methods

2.1. Plant Material and Extraction.
Pomegranate peels, collected from farms located in Sfax, Tunisia (N: 34.4426°, E: 10.4537°), were dried and powdered. During 24 h, powdered peel (100 g) was extracted with ethyl acetate, acetonitrile, and water at a ratio of 1 : 3. Therefore, supernatants were concentrated in a rotary evaporator gaining a yellow–brown residue that was immediately analyzed.

2.2. Phytochemical Analysis.
Total phenolic content (TPC) was assessed by the Folin–Ciocalteu method according to Singleton and Rossi [29]. Moreover, gallic acid (GA) was used as a standard, and TPC was expressed as mg GA equivalents/g sample.

Total flavonoid content (TFC) was evaluated according to the method reported by Quettier-Deleu et al. [30]. Besides, quercetin (QE) was utilized as a standard, and the results were expressed as milligrams of quercetin equivalents (mg QE)/g of the peel extract.

2.3. Antioxidant Activity

2.3.1. DPPH Assay.
According to Bersuder et al. [31], DPPH radical scavenging activity of the extract at 50 μg/mL was evaluated by using a spectrometer at 517 nm. The control was handled in the same manner with the exception of using distilled water in place of the extract. Moreover, the chosen standard was butylated hydroxyanisole (BHA). Free radical scavenging activity (%) was calculated as follows:

$$\%\text{DPPH-scavenging activity} = \left[\frac{\left(A_{\text{Control}} - A_{\text{Sample}} \right)}{A_{\text{Control}}} \right] \times 100, \tag{1}$$

where A_{Control} is the initial DPPH solution's absorbance at 517 nm, and A_{Sample} is the concentration of DPPH when the extract and positive control were present.

2.3.2. ABTS Cation Radical Decolorization Assay.
The ABTS$^{\bullet+}$ scavenging activity was determined by the method described Re et al. [32]. Twenty microliters of each extract at 50 μg/mL were supplemented to 180 μL of working ABTS$^{\bullet+}$ solution and subsequently incubated at room temperature for 6 min in the dark. Absorbance was measured at 734 nm using ethanol as a control and ascorbic acid as an antioxidant standard:

$$\%\text{ABTS}^{\bullet+}\text{-scavenging activity} = \left[\frac{\left(A_{\text{Control}} - A_{\text{Sample}} \right)}{A_{\text{Control}}} \right] \times 100, \tag{2}$$

where A_{Control} is the initial concentration of the ABTS$^{\bullet+}$ and A_{Sample} is the absorbance of the extract sample.

2.4. Antibacterial Activity

2.4.1. Bacterial Strains and Culture Conditions.
Purchased from American Type Culture Collection, five target bacteria strains, namely, *Staphylococcus aureus* ATCC 6538, *Listeria monocytogenes* ATCC 19117, *Salmonella enterica* ATCC 43972, *Escherichia coli* ATCC 8739, and *Pseudomonas aeruginosa* ATCC 4912 were used. Indicator bacteria were grown overnight in Muller–Hinton broth (MH) at 30°C for *L. monocytogenes*, *P. aeruginosa*, and *S. enterica* and at 37°C for *S. aureus* and *E. coli*. For each bacterium, 10^6 CFU/mL was the final inoculum concentration appropriate to antagonist tests.

2.4.2. Agar Diffusion Method.
As described by Güven et al. [33], antibacterial activity of each extract was evaluated by agar well diffusion assays. A volume of 50 mL of the molten agar were transferred into sterile Petri (Ø90 mm) dishes. Then, 100 μl of this suspension was spread on MH plates. Once the plates had been aseptically dried, wells of 6 mm diameter were punched in the seeded agar using sterile Pasteur pipette. Each well was loaded with 50 μl of each extract at 0.6, 1.25, 2.5, 5, 10, 20, 30, and 40 mg/mL, and plates were incubated at 37°C during 24 h. Antibacterial activity was evaluated by assessing the diameter of circular inhibition zones around the well.

2.4.3. Determination of MIC.
Minimum inhibitory concentrations (MICs) of each extract against all target strains were assessed based on the microdilution method [34]. Expressed by mg/mL, MIC was the lowest concentration that inhibited the visible growth of each tested bacterium.

2.5. Minced Beef Meat Samples Preparation.
Fresh beef meat was delivered by a regional slaughterhouse located in Sfax (Tunisia), was minced by a sterile grinder, and was divided in 5 lots. Lyophilized powder of the water extract (AE) was added at 0.1% (15.256 mg GAE/g): AE1; 0.5% (76.28 mg GAE/g): AE2, and 1% (152.56 mg GAE/g): AE3. Controls (without antioxidant and BHT at 0.01% [35]) were equally studied. Eventually, all samples were kept for 21 days at 4°C, and quality characteristics were examined in days 0, 3, 7, 14, and 21.

2.6. Analysis of Meat Samples

2.6.1. Microbiological Analysis.
Twenty-five grams of meat sample were homogenized in 225 mL of sterile buffered peptone water solution at 0.1%. 100 μL of serial decimal dilutions was prepared and plated onto the corresponding agar. To enumerate selected colonies, aerobic plate counts (APC) was determined on plate count agar (PCA, Oxoid, UK) and incubated at 30°C for 48 h [36]. Psychrotrophic total counts (PTC) was determined as described above for APC and incubated at 7°C for 10 days [37]. *Enterobacteriaceae* counts was calculated on violet red bile glucose medium (VRBG, Oxoid, UK) and incubated at 37°C for 24 h [38].

2.6.2. Physicochemical Analysis Methods

(1) pH Analysis. According to Özyurt et al. method, pH of all meat samples was assessed [39]. At each sampling point, the pH was determined using a pH meter.

(2) Evaluation of Protein Oxidation. Based on these absorbance values, MetMb (%) was evaluated by using the following formula [7]:

$$\text{MetMb} (\%) = \left[-2.51\left(\frac{A_{572}}{A_{525}}\right) + 0.777\left(\frac{A_{565}}{A_{525}}\right) + 0.8\left(\frac{A_{545}}{A_{525}}\right) + 1.098 \right] \times 100. \tag{3}$$

Carbonyl groups evaluation was assessed according to Jiménez-Martín et al. [40] method. Carbonyl content was identified in accordance with a molar extinction coefficient equal to $22000\,M^{-1}\cdot cm^{-1}$ and expressed in nmol carbonyl/mg of protein. As described by Cando et al. [41], sulphydryl groups were evaluated by using the of 5,5′-dithio-bis-(2-nitrobenzoic acid) method. Sulphydryl groups were calculated using a molar extinction coefficient of $13600\,M^{-1}\cdot cm^{-1}$, and results were expressed in mmol sulphydryl/g of protein.

(3) Evaluation of Lipid Oxidation in Beef Meat. According to Hur et al. [42], peroxide value (PV) was assessed and expressed in meq of peroxide/kg of meat.

The TBARS experiment method was evaluated by Eymard et al. [43] method. TBARS values were expressed as mg of malonaldehyde equivalent per kg of sample (mg MDA eq/kg of meat) by using molar extinction coefficient of the MDA-TBA adducts at 532 nm ($1.56 \times 10^5\,M^{-1}\cdot cm^{-1}$) [43].

According to Juntachote et al. [44], conjugated dienes (CDs) were evaluated, and results were expressed as μmol/mg of meat sample.

2.6.3. Sensory Evaluation.
A twenty-member panel trained on the classifying beef meat color, appearance, odor, and overall acceptability evaluated sensory traits. Sensory attributes were evaluated using nine-point (1 = very bad and 9 = very good) scores scales. A score below 5 indicated the sample being unacceptable.

2.7. Statistical Analysis.
For each parameter, a one-way ANOVA with 2 factors, treatments and storage time, was realized by using SPSS 19. To test the statistical significance, Tukey's post hoc was used. By using Durbin–Watson statistic tests at ($p < 0.05$), relationships between overall acceptability and sensory characteristics were established.

3. Results and Discussion

3.1. Phytochemical Content, Antioxidant Activity, and Antibacterial Activity

3.1.1. TPC and TFC.
Studied phytochemical content of extracts from pomegranate peel is displayed in Table 1. The greatest concentration of phenolic compounds (391.515 ± 10.58 mg of GAE/g) was presented in ethyl acetate fraction. Belkacem et al. [45] and Barathikannan et al. [46] reported an ethyl acetate TPC equal to 597.08 ± 3.9 and 218.152 ± 1.73 mg GAE/g of the extract, respectively.

The ethyl acetate extract revealed the highest values of TFC with 104.128 ± 3.69 mg QE/g. In fact, TFC/TPC represents a range between 24.93 and 27.89% (Table 1). Our data approve the studies compared to total polyphenols in pomegranate peel, which was, respectively, 24% and 30%.

3.1.2. Evolution of Antioxidant Properties.
The most widely used methods for antioxidant capacity evaluation were the ABTS and DPPH radicals. According to Rufino et al. [47], aqueous/organic extracts with hydrophilic and lipophilic compounds were generally treated by the DPPH method, and antioxidant activity of hydrophilic compounds was commonly analyzed by the ABTS method.

(1) DPPH Free Radical Scavenging Activity. DPPH free radical scavenging activities are exposed in Table 1. The scavenging activity of the three fractions analyzed was evaluated to those of BHA, used as a positive control, and was found to be concentration-dependent. DPPH scavenging activity of ethyl acetate fraction (88.56%) was superior to acetonitrile (84.24%) followed by water (80.12%). Consecutively, DPPH free radical scavenging activity of pomegranate peel extracts was ranged as follows: ethyl acetate > BHA > acetonitrile > water (Table 1). It has been reported that DPPH activity is deeply influenced by the phenolic compounds of pomegranate peel extracts [48–50]. In previous studies, when studying the relationship between the DPPH scavenging activity and total phenolic content, Wang et al. [51] have reported that pomegranate peel extracts with elevated total phenolic content reveled an advanced DPPH scavenging activity and vice versa.

(2) ABTS Radical Scavenging Activity. The different solvents of pomegranate peel extraction showed a concentration-dependent manner (65.26–77.12%) in the ABTS radical scavenging assay with the greatest level illustrated in the ethyl acetate extract (Table 1). This activity was ranged as BHA > ethyl acetate > acetonitrile > water in all pomegranate peel extracts. This result proposes that maximum of the

TABLE 1: Total phenolic and flavonoids contents and antioxidant activity of the ethyl acetate, acetonitrile, and aqueous extracts of *Punica granatum* (pomegranate) peel.

| | TPC (mg GAE/g) | TFC (mg QE/g) | Antioxidant activity | |
			DPPH assay (%)	ABTS assay (%)
Ethyl acetate	391.51 ± 10.58^d	104.12 ± 3.69^c	88.56 ± 1.52^d	77.12 ± 1.57^c
Acetonitrile	281.13 ± 19.25^c	76.88 ± 6.39^b	84.24 ± 1.25^b	71.89 ± 0.32^b
Water	178.25 ± 6.22^b	44.45 ± 2.05^a	80.12 ± 1.88^a	65.26 ± 1.96^a
BHA	—	—	86.28 ± 0.97^c	81.19 ± 2.12^d

$\pm$, standard deviation of three replicates; values with a different letter (a–d) within a column are significantly different ($p < 0.05$) (Tukey's test).

antioxidant capacity of pomegranate extracts results from the contribution of TPC and TFC.

3.2. Antimicrobial Activities of Punica granatum Extracts.
For Gram-positive bacteria, the results presented that the highest diameters of the inhibition zone (IZ) at the concentration of 40 mg/mL pertained to the ethyl acetate extract at 24.00 ± 0.50 and 25.50 ± 1.00 mm, respectively (Table 2). Thus, the highest anti-*L. monocytogenes* activity and anti-*S. aureus* activity were recorded for the ethyl acetate extract, followed by the water and acetonitrile peel extracts. As presented in Table 2, a high correlation ($R^2 > 0.703$) between IZ and concentrations was revealed. For *Listeria monocytogenes*, the regression coefficients of the ethyl acetate extract inhibition zone (IZ_{EA}) were 1.31 and 1.34 times higher than IZ_A (aqueous extract inhibition zone) and IZ_A (acetonitrile extract inhibition zone), respectively. Furthermore, for *Staphylococcus aureus*, the regression coefficients of IZ_{EA} were 1.05 and 1.26 times higher than IZ_W and IZ_A, respectively.

In the case of *Pseudomonas aeruginosa* and *Escherichia coli*, Gram-negative bacteria, the highest inhibitory effect was noted for the peel extracts obtained using the water extraction at 40 mg/mL. In fact, the measured inhibition zones were 21.75 ± 0.75 and 23.25 ± 1.25 mm, respectively (Table 2). The pomegranate peel extracts showed the anti-*P. aeruginosa* and *E. coli* activities in the order of water > ethyl acetate > acetonitrile (Table 2). Our results approved with those previously described by Voravuthikunchai et al. [52]. The water peel extracts of pomegranate peel showed antibacterial activity against six strains of *E. coli*.

In the case of *S. enterica*, at 40 mg/mL, the observed inhibition zones ranged from 18.75 ± 0.75 (acetonitrile extract) to 22.75 ± 1.25 mm (water extract). In the case of *S. enterica*, at 40 mg/mL, the observed inhibition zones ranged from 18.75 ± 0.75 (acetonitrile extract) to 22.75 ± 1.25 mm (water extract). It should be noted that the regression coefficients of IZ_W were 1.18 and 1.33 times higher than IZ_{EA} and IZ_A, respectively (Table 2). The results of this study exhibited thus that the extracts obtained from pomegranate revealed antibacterial activity against the five foodborne pathogens and tested bacteria. The activity of *Punica granatum* extracts against *Salmonella* was also established in the studies by Choi et al. [53] and Hayrapetyan et al. [54].

3.3. MIC Determination of Extracts.
The effects of pomegranate peel extracts on *L. monocytogenes*, *S. aureus*, *S. enterica*, *P. aeruginosa*, and *E. coli* proliferation are listed in Table 3. They revealed different extends of antibacterial potential against all tested strains, and they were most active against Gram-positive bacteria *S. aureus* and *L. monocytogenes* with a MIC ranged between 0.60 and 2.50 mg/mL. At the same time, Naz et al. reported that the activity against Gram-positive organisms was more elevated compared to Gram-negative species [55]. This low CMI found in Gram-negative bacteria was explained by their outer lipopolysaccharide membranes that makes them more resistant to various antimicrobials [56]. In fact, in our study, Table 3 shows that the MIC against *E. coli* was at least two-fold less than that of *S. enteric*. Others studies reported the MIC against several strains of *E. coli* which ranged from 0.39 to 25 mg/mL [52, 57]. Also, Voravuthikunchai et al. investigated that the peels aqueous extract at 0.5–3 mg/mL (MIC) can inhibit enterohemorrhagic *E. coli* O157: H7 [58].

Changes in results of peel extracts profile may be clarified by the extraction solvents differentiation, the plant variety, and then geographical and the climatic conditions variations [49]. Indeed, authors recorded MIC against Gram$^+$ *S. aureus* and Gram$^-$ *Salmonella* ranged, respectively, from 0.62 to $\geq$250 mg/mL [53–55] and from 10.75 to 12.5 mg/mL [18] when using *Punica granatum* extracts.

3.4. Effect of the Pomegranate Peel Aqueous Extract on Conservation of Raw Minced Beef Meat at 4°C.
Between the organic solvents used for the extraction from peel pomegranate, water was preferred in the food application over ethyl acetate and acetonitrile for safety reasons. At the same potential in antioxidant and antibacterial activities, the less safe use made ethyl acetate and acetonitrile undesirable solvents, so the subsequent experiments; for preservation of meat sample, the water extract at different concentrations was used. In this second part, the aqueous extract (AE) was supplemented to the meat sample at 0.1, 0.5, and 1%.

3.4.1. Physicochemical Analyses

(1) pH. Figure 1 presents the effect of AE used in raw minced beef meat samples on the pH during 21 days of storage at 4°C. Statistically, pH values showed a slightly significant increase ($p < 0.05$) in treated samples during 21 days of storage with the lowest recorded in samples treated with AE at 1% (6.14 ± 0.17) and the highest in control samples (7.11 ± 0.20). This augment of pH ($p < 0.05$) may be due the degradation of amino acid by bacteria [59].

TABLE 2: Average diameter of the inhibition zone (mm) of three extractives from *Punica granatum* (pomegranate) peel against food spoilage pathogens and determination of regression equations.

	Punica granatum extract concentrations (mg/mL)								Regression equations	R^2
	0.6	1.25	2.5	5	10	20	30	40		
L. monocytogenes										
IZ_EA	0.00 ± 0.00[aA]	0.00 ± 0.00[aA]	10.75 ± 0.50[bB]	14.75 ± 0.50[bC]	17.50 ± 0.50[bD]	19.75 ± 0.50[bE]	23.25 ± 0.50[bF]	24.00 ± 0.50[bF]	12.816ln (x) − 6.008	0.848
IZ_A	0.00 ± 0.00[aA]	0.00 ± 0.00[aA]	0.00 ± 0.00[aA]	9.00 ± 0.75[aB]	12.50 ± 0.75[aC]	14.00 ± 0.50[aC]	16.25 ± 1.25[aD]	19.50 ± 0.25[aD]	9.5232ln (x) − 5.6294	0.731
IZW	0.00 ± 0.00[aA]	0.00 ± 0.00[aA]	0.00 ± 0.00[aA]	9.00 ± 0.25[aB]	13.50 ± 0.50[aC]	14.25 ± 1.75[aC]	16.75 ± 0.50[aD]	20.25 ± 1.25[aD]	9.7746ln (x) − 5.8203	0.728
S. aureus										
IZ_EA	0.00 ± 0.00[aA]	9.75 ± 0.75[bB]	14.75 ± 0.75[cC]	17.50 ± 0.50[cD]	21.75 ± 0.75[cE]	23.25 ± 0.25[cF]	24.75 ± 0.25[cG]	25.50 ± 1.00[cG]	13.484ln (x) − 3.9296	0.937
IZ_A	0.00 ± 0.00[aA]	0.00 ± 0.00[aA]	8.25 ± 1.25[aB]	10.50 ± 0.25[aC]	14.25 ± 0.50[aD]	17.00 ± 1.50[aE]	19.00 ± 1.75[aF]	20.25 ± 0.75[aG]	10.632ln (x) − 5.2059	0.840
IZ_W	0.00 ± 0.00[aA]	0.00 ± 0.00[aA]	10.75 ± 0.25[bB]	13.75 ± 0.50[bC]	18.75 ± 0.50[bD]	20.75 ± 1.00[bE]	22.75 ± 0.75[bF]	23.25 ± 0.75[bF]	12.789ln (x) − 5.9698	0.845
S. enterica										
IZ_EA	0.00 ± 0.00[aA]	0.00 ± 0.00[aA]	0.00 ± 0.00[aA]	0.00 ± 0.00[aA]	13.50 ± 1.00[bB]	16.50 ± 0.75[bC]	18.75 ± 1.75[bD]	20.25 ± 1.50[aD]	10.059ln (x) − 6.6419	0.709
IZ_A	0.00 ± 0.00[aA]	0.00 ± 0.00[aA]	0.00 ± 0.00[aA]	0.00 ± 0.00[aA]	11.25 ± 1.25[aB]	12.75 ± 1.75[aB]	16.75 ± 0.25[aC]	18.75 ± 0.75[aC]	8.9262ln (x) − 6.0025	0.703
IZ_W	0.00 ± 0.00[aA]	0.00 ± 0.00[aA]	0.00 ± 0.00[aA]	11.75 ± 0.25[aB]	15.75 ± 1.50[cC]	18.50 ± 0.50[cD]	21.25 ± 0.75[cE]	22.75 ± 125[bE]	11.909ln (x) − 6.9394	0.736
P. aeruginosa										
IZ_EA	0.00 ± 0.00[aA]	0.00 ± 0.00[aA]	0.00 ± 0.00[aA]	9.25 ± 0.25[bB]	12.00 ± 1.00[bC]	14.25 ± 1.75[bD]	17.50 ± 1.00[bE]	19.75 ± 0.25[bE]	9.7667ln (x) − 5.8091	0.729
IZ_A	0.00 ± 0.00[aA]	0.00 ± 0.00[aA]	0.00 ± 0.00[aA]	0.00 ± 0.00[aA]	9.50 ± 0.25[aB]	11.50 ± 0.50[aC]	14.75 ± 1.75[aD]	17.00 ± 1.25[aE]	7.8264ln (x) − 5.2714	0.719
IZ_W	0.00 ± 0.00[aA]	0.00 ± 0.00[aA]	0.00 ± 0.00[aA]	10.75 ± 0.50[bB]	14.50 ± 1.25[cC]	17.50 ± 1.75[cD]	19.75 ± 1.75[cE]	21.75 ± 0.75[cF]	11.2ln (x) − 6.5705	0.739
E. coli										
IZ_EA	0.00 ± 0.00[aA]	0.00 ± 0.00[aA]	0.00 ± 0.00[aA]	10.00 ± 1.00[bB]	14.25 ± 0.75[bC]	16.25 ± 0.50[bD]	19.00 ± 0.75[bE]	21.00 ± 1.75[bF]	10.727ln (x) − 6.3132	0.733
IZ_A	0.00 ± 0.00[aA]	0.00 ± 0.00[aA]	0.00 ± 0.00[aA]	8.00 ± 0.75[aB]	12.25 ± 1.25[aC]	14.75 ± 0.05[aD]	16.75 ± 1.75[aE]	18.75 ± 0.75[aF]	9.4895ln (x) − 5.6648	0.728
IZ_W	0.00 ± 0.00[aA]	0.00 ± 0.00[aA]	8.75 ± 0.50[bB]	12.25 ± 1.50[cB]	16.00 ± 1.75[cD]	18.75 ± 1.50[cE]	21.50 ± 1.25[cF]	23.25 ± 1.25[cG]	12.042ln (x) − 5.9626	0.839

IZ_EA, ethyl acetate extract inhibition zone; IZ_A, acetonitrile extract inhibition zone; IZ_W, aqueous extract inhibition zone. ±, standard deviation of three replicates; values with a different letter (A–G) within a row of the same storage day are significantly different ($p < 0.05$); values with a different letter (a–g) within a column of the same concentration are significantly different ($p < 0.05$) (Tukey's test).

Table 3: MIC values of three extractives from *Punica granatum* (pomegranate) peel.

Microorganism	MIC (mg/mL)		
	Ethyl acetate	Acetonitrile	Water
L. monocytogenes	1.25 ± 0.00^a	2.50 ± 0.00^b	2.50 ± 0.00^b
S. aureus	0.60 ± 0.00^a	1.25 ± 0.00^b	1.25 ± 0.00^b
S. enterica	5.00 ± 0.00^a	5.00 ± 0.00^a	2.50 ± 0.00^b
P. aeruginosa	2.50 ± 0.00^a	5.00 ± 0.00^b	2.50 ± 0.00^a
E. coli	2.50 ± 0.00^b	2.50 ± 0.00^b	1.25 ± 0.00^a

±, standard deviation of three replicates; values with a different letter (a–d) within a column of the same microorganism are significantly different ($p < 0.05$) (Tukey's test).
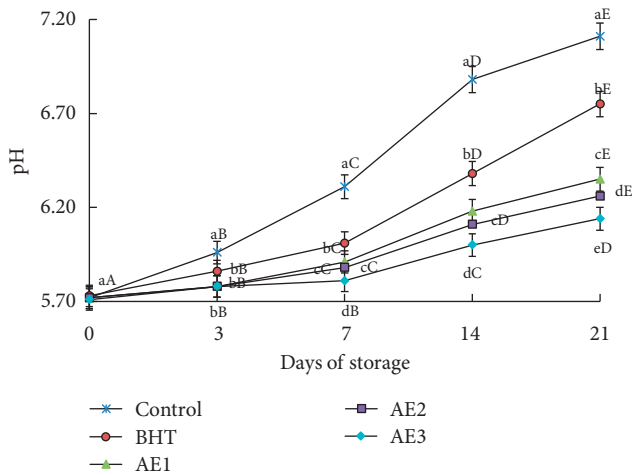

Figure 1: Effect of the aqueous pomegranate peel extract on pH of minced beef meat during storage at 4°C.

(2) The MetMb Content. The formation rate of MetMb during storage at 4°C is shown in Figure 2. During preservation, meat discoloration of meat is frequently recognized due to the myoglobin oxidation in MetMb [60]. For control samples (C), MetMb% augmented quickly in the first 7 days and extended values above 50.94%, whereas for treated samples (BHT, AE1, AE2, and AE3), the MetMb% ranged from 32.27 (BHT) to 22.64 (AE3). Limit level of MetMb leading the consumer arise rejection of meat products was determined to 40% [61]. In our case, this limit value was reached approximately after 14 days of storage for BHT samples. For AE3, MetMb% values were 37.59, which under the detection limits till the end of storage.

(3) Carbonyl Contents. Aqueous extract-treated raw minced beef meat showed significantly lower ($p < 0.05$) protein carbonyl contents (Figure 3). The protein carbonyl contents displayed that the aqueous extract of pomegranate peel addition could retard protein oxidation in raw minced beef meat. At all sampling days, control samples have significantly higher ($p < 0.05$) amounts of protein carbonyls than treated samples. Initial carbonyl concentrations of control, BHT, 0.1, 0.5, and 1% AE groups are 0.71, 0.58, 0.51, 0.43, and 0.36 nmol/mg proteins, respectively. Figure 3 shows, in all samples, an increase of carbonyl levels, which is
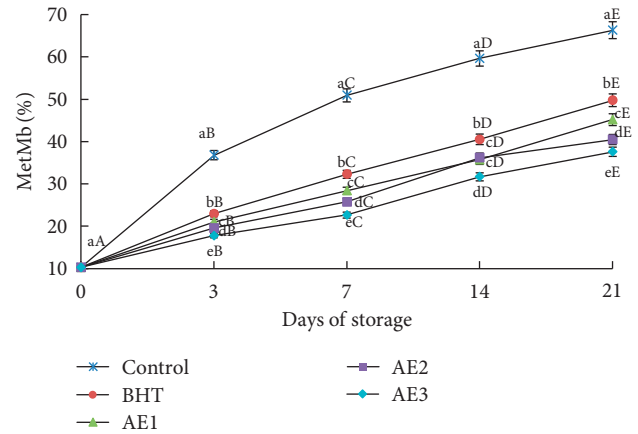

Figure 2: Effect of the aqueous pomegranate peel extract on MetMb (%) of minced beef meat during storage at 4°C.
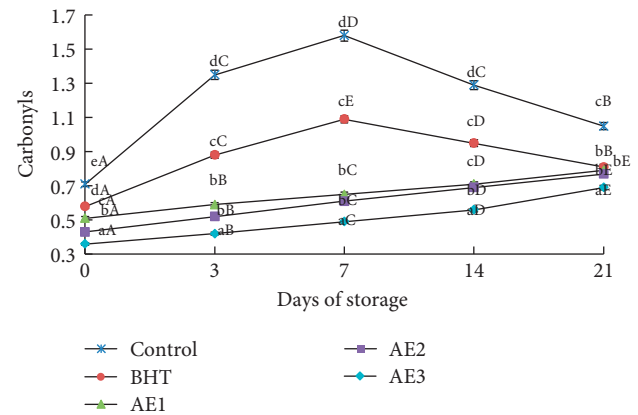

Figure 3: Effect of the aqueous pomegranate peel extract on protein carbonyl (nmoles carbonyl/mg protein) of minced beef meat during storage at 4°C.

demonstrated by the generation of oxidative reactions during 7 days. Until 21 days of storage, AE1-3 and BHT samples showed a significant ($p < 0.05$) effect on final carbonyl concentrations. These results showed that antioxidant sources were able to preventing carbonyl formation. Similarly, the decrease in carbonyl groups was reported for various type of meat [62–64].

(4) Sulphydryl Contents. Storage time resulted in a significant ($p < 0.05$) decrease in sulphydryl contents. As shown in Figure 4, at 0 day, treated samples with 1% AE (AE3) presented the lowest % reduction of sulfhydryl content (16.63%), followed by the AE2 (21.79%), AE1 (23.25%), BHT (32.58%), and control samples (45.49%). At the end of storage, the maximum decrease and minimum decrease were found in the control group and AE3, respectively, at 24.11 and 40.39 nmol/mg proteins. SH occupied an essential role in the enhancement of functional properties of proteins. In this way, the reduction of SH is associated to the development of disulphide bonds, protein aggregates, and loses its functionality [65].
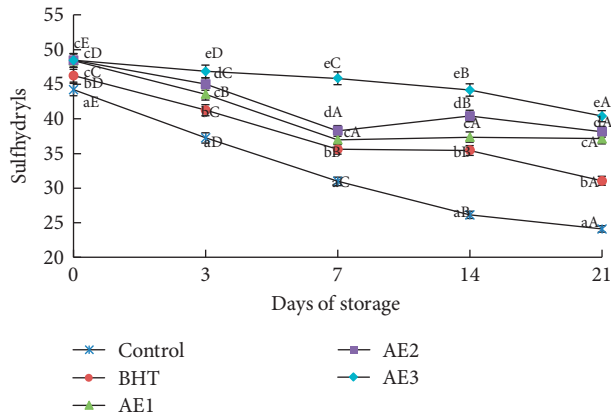
FIGURE 4: Effect of the aqueous pomegranate peel extract on sulfhydryl (nmoles sulfhydryl/mg protein) of minced beef meat during storage at 4°C.

(5) Peroxide Value. To be acceptable in fatty foods, PV should not reach the limit of 25 meq peroxide/kg of meat [66, 67]. Figure 5 demonstrates that AE and storage time (days) have a significant ($p < 0.05$) effect on PV. In fact, at the twenty-first day of storage, lower PV was significantly ($p < 0.05$) investigated in treated AE 3 samples (14.75 meq peroxide/kg of meat) compared to the control (28.59 meq peroxide/kg of meat). The high PV explained the high accumulation of peroxide, which will degrade into ketone and aldehyde [68, 69]. AE phenolic compounds properties will possibly participate in the peroxides decomposition leading to the peroxide value decreasing [69].

(6) CD. As shown in Figure 6, CD increased significantly ($p < 0.05$) during the first 3 days of storage and then decreased until the end of storage. Remarkably, meat samples containing the aqueous extract (AE1-3) demonstrated CD values significantly ($p < 0.05$) lower than those of control and BHT samples during storage. This decrease could be explained by the fact that the decomposition rate of the hydroperoxides was higher than the formation rate of CD [70]. Many works reported the lower CD values when different types of meat were treated with plant extracts [44, 71–73].

(7) TBARS Value. The reaction between thiobarbituric acid and malonaldehyde (MAD), formed by lipid hydroperoxides decomposition, is employed as the basis for the measurement of secondary oxidation products [74]. Together with peroxide value and conjugated dienes data, a more comprehensive picture on the oxidation of raw minced beef meat can be developed. At the end of the storage period, AE treatments demonstrated a significant ($p < 0.05$) effect on TBARS values. As shown in Figure 7, TBARS values were superior in control samples (C) than AE-treated samples, with the lowest showed at AE2 and AE3 with 1.79 ± 0.015 and 1.56 ± 0.031 mg of malonaldehyde/kg of sample, respectively. These latter levels respected the detection limit (2 mg MDA/kg) recommended by Botsoglou et al. [71]. Equally, a similar positive effect of sage extracts [75, 76],
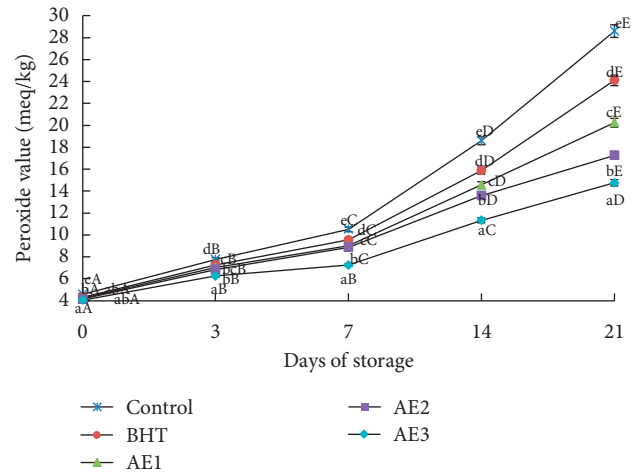

FIGURE 5: Effect of the aqueous pomegranate peel extract on peroxide values (meq peroxide/kg of meat) of minced beef meat during storage at 4°C.

FIGURE 6: Effect of the aqueous pomegranate peel extract on conjugated dienes (μmol/mg of meat) of minced beef meat during storage at 4°C.

curry, and mint leaf extracts [77] in pork meat systems was found.

3.4.2. Microbiological Evaluation. After 14 days of storage, APC showed a rapid increase in control sample reaching the minimal spoilage level (5.10^6 CFU/g~$\log_{10}$ 6.7 CFU/g) [78]. In the first day of storage (day 0) (Figure 8), APC and TPC of different samples was above 2.35 log CFU/g. In the twenty-first day, APC of AE1, AE2, and AE3 samples was gradually increased ($p < 0.05$) and reached 7.18, 6.59, and 6.23 log CFU/g, respectively. As well for TPC, the levels were AE1 (5.84 log CFU/g), AE2 (5.71 log CFU/g), and AE3 (5.11 log CFU/g). It should be mentioned that limit levels of both APC and TPC leading raw minced beef meat unsuitable for use was log 6.7 CFU/g [79].

Comparable to our results, preceding reports verified that the APC of meat products were considerably reduced by

FIGURE 7: Effect of the aqueous pomegranate peel extract on TBARS (mg MDA/kg meat) of minced beef meat during storage at 4°C.

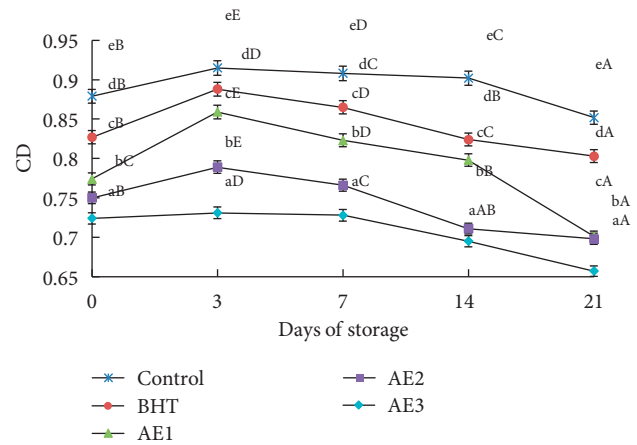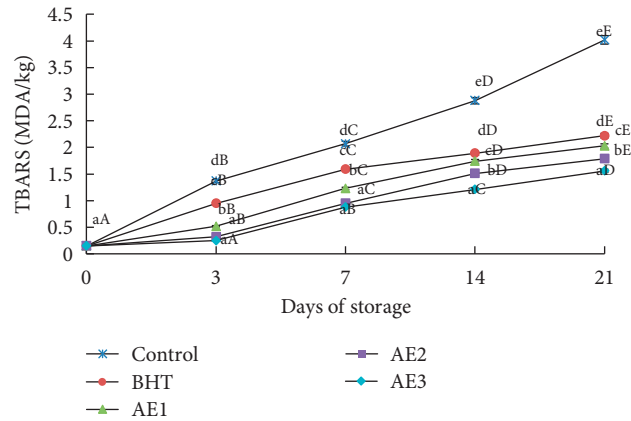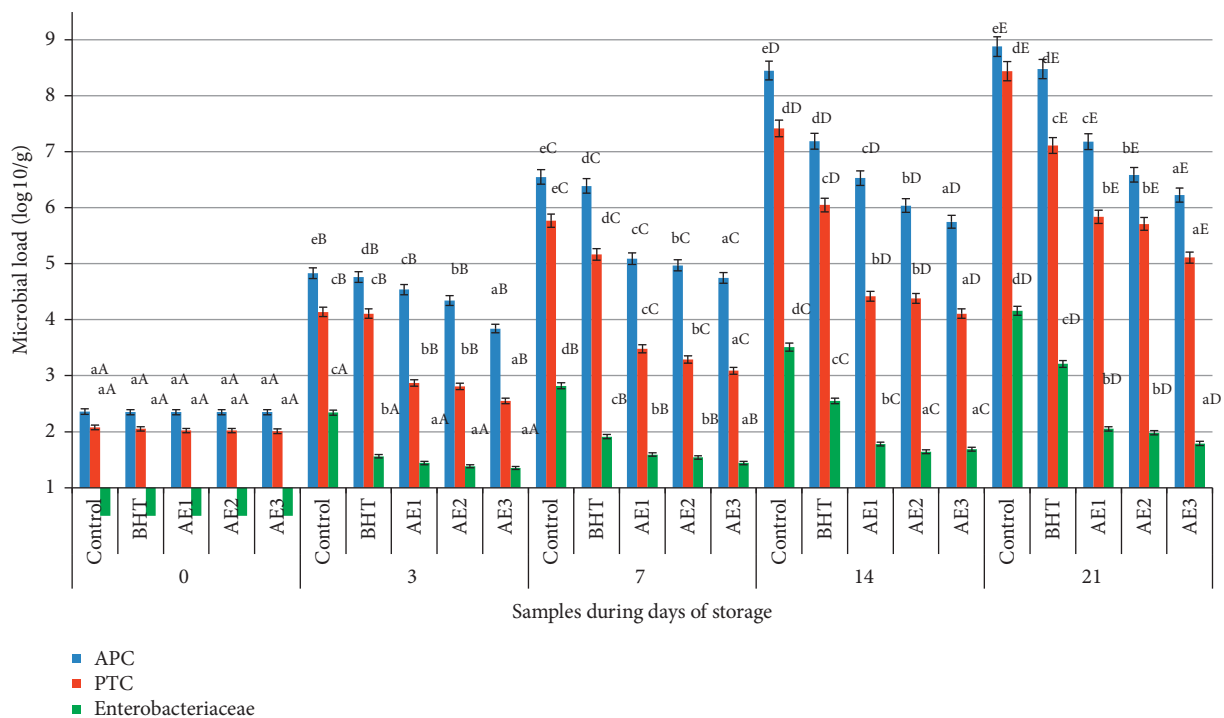FIGURE 8: Effect of the aqueous pomegranate peel extract on the microbial load of aerobic plate count (APC), psychrotrophic count (PTC), and *Enterobacteriaceae* count of raw minced meat beef during storage at 4°C.

the addition of Moringa leaves in beef burgers [80] and herbal chicken sausage [81] kept at 4°C for five weeks. Besides, Hawashin et al. [82] stated that destoned olive cake powder tardy bacterial growth in beef patties during 14 days.

3.4.3. Sensory Evaluation. During the storage time, all the sensory characteristics were significantly ($p < 0.05$) decreased, whereas the treated samples exposed exceptional stability until 14 days due to the fact that the limit of rejection is 5. As shown in Table 4, the overall acceptability of minced beef meat treated with BHT, AE1, AE2, and AE3 was acceptable until 14 days ($p < 0.05$) but unacceptable for untreated samples (C) from day 7

($p < 0.05$). A dissimilar trend has been reported by Hawashin et al. [82], Al-Juhaimi et al. [80], and Muthukumar et al. [83] who found an insignificant decrease or changes in sensory attributes.

3.5. Linear Regression Analysis

3.5.1. Relationship between Overall Acceptability and Physicochemical Parameters. In the beginning of storage at 4°C, pH and sulfhydryl contents showed that a positive effect on the overall acceptability (OA) with regression coefficients was higher in treated samples compared to control (Table 5). In day 3, it should be noted that levels higher than 6.25 meq peroxide/kg of

TABLE 4: Effect of the aqueous pomegranate peel extract on color, appearance, odor, and overall acceptability of raw minced meat beef stored at 4°C.

	Days of storage at 4°C				
	0	3	7	14	21
Color					
Control	6.33 ± 0.25^{aE}	6.15 ± 0.15^{aD}	5.55 ± 0.25^{aC}	3.25 ± 0.15^{aB}	2.22 ± 0.02^{aA}
BHT	6.51 ± 0.23^{bD}	6.55 ± 0.17^{bD}	6.15 ± 0.25^{bC}	5.37 ± 0.11^{bB}	3.75 ± 0.04^{bA}
AE1	6.51 ± 0.18^{bD}	6.55 ± 0.10^{bD}	6.33 ± 0.14^{bcC}	5.87 ± 0.16^{cB}	3.83 ± 0.08^{bA}
AE2	6.83 ± 0.11^{cD}	6.61 ± 0.28^{bC}	6.55 ± 0.21^{cC}	5.93 ± 0.22^{cB}	3.83 ± 0.09^{bA}
AE3	6.92 ± 0.22^{dD}	6.83 ± 0.25^{cCD}	6.77 ± 0.20^{dC}	6.12 ± 0.18^{dB}	4.25 ± 0.14^{cA}
Appearance					
Control	6.83 ± 0.15^{aE}	6.25 ± 0.14^{aD}	5.11 ± 0.11^{aC}	3.25 ± 0.25^{aB}	2.83 ± 0.02^{aA}
BHT	6.92 ± 0.10^{aD}	6.33 ± 0.13^{aC}	6.33 ± 0.19^{bC}	5.25 ± 0.24^{bB}	3.11 ± 0.04^{bA}
AE1	6.92 ± 0.20^{aE}	6.50 ± 0.19^{bD}	6.33 ± 0.28^{bC}	6.00 ± 0.11^{cB}	3.11 ± 0.09^{bA}
AE2	7.50 ± 0.19^{bE}	6.71 ± 0.12^{cD}	6.50 ± 0.25^{cC}	6.00 ± 0.19^{cB}	3.50 ± 0.08^{cA}
AE3	7.50 ± 0.15^{bE}	6.83 ± 0.23^{dD}	6.66 ± 0.24^{dC}	6.33 ± 0.11^{dB}	3.83 ± 0.05^{dA}
Odor					
Control	6.17 ± 0.27^{aE}	6.00 ± 0.22^{aD}	5.78 ± 0.18^{aC}	3.55 ± 0.09^{aB}	2.22 ± 0.02^{aA}
BHT	6.25 ± 0.20^{abE}	6.00 ± 0.19^{aD}	5.83 ± 0.18^{aC}	5.25 ± 0.22^{bB}	2.75 ± 0.08^{bA}
AE1	6.33 ± 0.25^{bD}	6.17 ± 0.22^{bCD}	6.00 ± 0.22^{bC}	5.37 ± 0.22^{bB}	3.25 ± 0.02^{cA}
AE2	6.66 ± 0.22^{cC}	6.50 ± 0.16^{cC}	6.50 ± 0.29^{cC}	5.62 ± 0.19^{cB}	3.50 ± 0.09^{dA}
AE3	6.83 ± 0.19^{dD}	6.75 ± 0.15^{dCD}	6.66 ± 0.11^{dC}	5.87 ± 0.15^{dB}	3.75 ± 0.07^{eA}
Overall acceptability					
Control	6.50 ± 0.22^{aE}	6.25 ± 0.25^{aD}	5.83 ± 0.19^{aC}	3.37 ± 0.15^{aB}	2.50 ± 0.05^{aA}
BHT	6.50 ± 0.19^{aE}	6.33 ± 0.26^{aD}	6.00 ± 0.17^{bC}	5.75 ± 0.25^{bB}	2.75 ± 0.02^{bA}
AE1	6.66 ± 0.19^{bD}	6.33 ± 0.28^{aC}	6.17 ± 0.10^{cB}	6.12 ± 0.11^{cB}	2.83 ± 0.08^{bA}
AE2	6.66 ± 0.15^{bC}	6.66 ± 0.22^{bC}	6.33 ± 0.11^{dB}	6.25 ± 0.27^{dB}	3.00 ± 0.07^{cA}
AE3	6.77 ± 0.21^{cC}	6.71 ± 0.27^{bC}	6.50 ± 0.18^{eB}	6.50 ± 0.22^{eB}	3.33 ± 0.04^{dA}

±, standard deviation of three replicates; values with a different letter (a–e) within a row of the same storage day are significantly different ($p < 0.05$) (Tukey's test). Values with a different letter (A–E) within a column of the same concentration are significantly different ($p < 0.05$) (Tukey's test).

meat (PV of AE3) and 0.731 μmol/mg of meat (CD of AE3), respectively, for PV and CD (Table 5) affected negatively the OA of the raw minced beef meat, whereas predicted OA was not influenced by MetMb up to 36.76% (MetMb of control samples). Equally, the loss of sulphydryl groups of treated samples AE3 (3.302%), AE2 (7.079%), and AE1 (9.977%) showed a strong positive correlation, respectively, with OA_3, OA_2, and OA_1, while a percentage of 15% of the loss of sulphydryl groups (control) had a negative influence on the OA_C (−0.014) (Table 5).

A negative effect of protein oxidation parameters on the overall acceptability was distinguished in control samples after 7 days of storage. This latter was probably explained by an endogenous reducing enzyme's action in MetMb [24] and a higher degree of protein oxidation in case of lower sulfhydryl [84]. Equally, secondary lipid oxidation products (TBARS) correlated negatively with the OA_C. Smaoui et al. [64] have reported that both CD and PV influence TBARS: these relationships were established through Bayesian networks and Pearson coefficients correlation. In fact, after 7 days of storage, primary (PV and CD) and secondary (TBARS) lipid oxidation products of control sample acted a negative correlation on OA_C. For BHT samples, loss of sulphydryl groups at 23.048% contributed a negative effect on OA_{BHT} (Table 5). Furthermore, for AE1, the sulphydryl groups had a negative effect on OAC_{AE1}, while for AE1 and AE2, the loss up to 16.594% (loss of sulphydryl groups of AE2) acted a positive effect on OA. After 14 days, all treated samples except AE3 presented a negative effect of TBARS. Thereby, predicted OA of AE3 appeared to be the most remarkable (Table 5) and could be presented as follows:

$$OA_{AE3} = 2.947 + 0.314 \times pH + 0.352 \times MetMb + 0.122 \times Sulph - 0.114 \times PV - 1.128 \times CD + 0.127 \times TBARS. \tag{4}$$

3.5.2. Relationship between Overall Acceptability and Sensory Parameters. In day 0, for all samples, a positive effect of color was shown on the predicted overall acceptability (Table 6). The predicted overall acceptability was found to be the same for control and BHT samples, while the treatment by the aqueous extract improved significantly the color compared to control and BHT samples. In fact, OA_{AE3} was 1.7 times higher than OA_C and OA_{BHT} (Table 6). After 3 days, compared to control and BHT samples, we noticed that the ethanolic extract significantly improved the color and the odor. Like the 7th day of storage at 4°C, color and odor parameters remained the same (Table 6). The aqueous

Table 5: Multiple linear regression equations of overall acceptability versus physicochemical parameters of raw minced beef meat stored at 4°C.

	Days of storage at 4°C			
	0	3	7	14
C	$3.055 + 0.542 \times pH + 0.162 \times$ Sulph ($R^2 = 0.951$)	$-4.055 + 0.484 \times pH + 0.457 \times MetMb + 0.041 \times$ Carb $- 0.014 \times Sulph - 0.457 \times PV - 1.257 \times CD$ ($R^2 = 0.924$)	$-1.25 + 0.125 \times pH - 0.101 \times MetMb - 0.031 \times$ Carb $- 0.298 \times Sulph - 0.598 \times$ PV $- 1.333 \times CD - 0.457 \times TBARS$ ($R^2 = 0.907$)	
BHT	$3.152 + 0.556 \times pH + 0.211 \times$ Sulph ($R^2 = 0.977$)	$-2.258 + 0.425 \times pH + 0.485 \times MetMb + 0.058 \times$ Carb $+ 0.015 \times Sulph - 0.325 \times PV - 1.114 \times CD$ ($R^2 = 0.821$)	$-0.689 + 0.175 \times pH + 0.252 \times MetMb - 0.124 \times$ Sulph $- 0.447 \times PV - 1.247 \times CD$ ($R^2 = 0.833$)	$7.638 - 0.148 \times MetMb - 0.368 \times Sulph - 0.658 \times$ PV $- 1.335 \times CD - 0.585 \times TBARS$ ($R^2 = 0.816$)
AE1	$3.330 + 0.598 \times pH + 0.257 \times$ Sulph ($R^2 = 0.925$)	$-3.228 + 0.511 \times pH + 0.488 \times MetMb + 0.062 \times$ Carb $+ 0.133 \times Sulph - 0.148 \times PV - 0.689 \times CD$ ($R^2 = 0.859$)	$-0.857 + 0.333 \times pH + 0.298 \times MetMb + 0.058 \times$ Sulph $- 0.311 \times PV - 1.114 \times CD$ ($R^2 = 0.825$)	$-0.477 + 0.298 \times MetMb - 0.138 \times Sulph - 0.311 \times$ PV $- 1.114 \times CD - 0.358 \times TBARS$ ($R^2 = 0.988$)
AE2	$3.352 + 0.601 \times pH + 0.266 \times$ Sulph ($R^2 = 0.926$)	$-4.144 + 0.533 \times pH + 0.491 \times MetMb + 0.066 \times$ Carb $+ 0.142 \times Sulph - 0.137 \times PV - 0.598 \times CD$ ($R^2 = 0.845$)	$1.258 + 0.384 \times pH + 0.321 \times MetMb + 0.111 \times$ Sulph $- 0.298 \times PV - 1.044 \times CD$ ($R^2 = 0.819$)	$1.258 + 0.321 \times MetMb + 0.198 \times Sulph - 0.298 \times$ PV $- 1.044 \times CD - 0.324 \times TBARS$ ($R^2 = 0.928$)
AE3	$3.207 + 0.628 \times pH + 0.274 \times$ Sulph ($R^2 = 0.901$)	$-0.625 + 0.548 \times pH + 0.505 \times MetMb + 0.079 \times$ Carb $+ 0.215 \times Sulph - 0.098 \times PV - 0.117 \times CD$ ($R^2 = 0.914$)	$-0.477 + 0.458 \times pH + 0.498 \times MetMb + 0.138 \times$ Sulph $- 0.311 \times PV - 1.114 \times CD$ ($R^2 = 0.829$)	$2.947 + 0.314 \times pH + 0.352 \times MetMb + 0.122 \times$ Sulph $- 0.114 \times PV - 1.128 \times CD + 0.127 \times TBARS$ ($R^2 = 0.921$)

TABLE 6: Multiple linear regression equations of overall acceptability versus sensorial parameters of raw minced beef meat stored at 4°C.

	Days of storage at 4°C			
	0	3	7	14
C	$3.129 + 0.516 \times \text{color}$ $(R^2 = 0.859)$	$-4.258 + 0.384 \times \text{color}$ $(R^2 = 0.958)$	$-11.289 + 0.125 \times \text{color}$ $(R^2 = 0.901)$	
BHT	$4.328 + 0.514 \times \text{color}$ $(R^2 = 0.856)$	$-2.521 + 0.488 \times \text{color}$ $(R^2 = 0.955)$	$-3.729 + 0.375 \times \text{color}$ $(R^2 = 0.914)$	$9.355 - 0.149 \times \text{appearance} + 0.248 \times \text{color}$ $(R^2 = 0.918)$
AE1	$3.005 + 0.537 \times \text{color}$ $(R^2 = 0.866)$	$1.29 + 0.468 \times \text{color} + 0.801 \times \text{odor}$ $(R^2 = 0.914)$	$-6.929 + 0.414 \times \text{color} + 0.521 \times \text{odor}$ $(R^2 = 0.904)$	$5.267 + 0.114 \times \text{color} + 0.324 \times \text{odor}$ $(R^2 = 0.921)$
AE2	$-2.658 + 0.667 \times \text{color}$ $(R^2 = 0.824)$	$4.119 + 0.501 \times \text{color} + 1.297 \times \text{odor}$ $(R^2 = 0.927)$	$-8.524 + 0.487 \times \text{color} + 0.787 \times \text{odor}$ $(R^2 = 0.864)$	$-1.987 + 0.333 \times \text{color} + 0.597 \times \text{odor}$ $(R^2 = 0.847)$
AE3	$-1.257 + 0.897 \times \text{color}$ $(R^2 = 0.836)$	$7.984 + 0.748 \times \text{color} + 1.547 \times \text{odor}$ $(R^2 = 0.911)$	$7.598 + 0.657 \times \text{color} + 1.247 \times \text{odor}$ $(R^2 = 0.887)$	$9.587 + 0.488 \times \text{color} + 1.107 \times \text{odor}$ $(R^2 = 0.888)$

extract at 1% (AE3) application after 7 days of storage enhanced significantly the color and odor (Table 6). The odor of OA_{AE3} was 1.584 (1.247/0.787) and 2.393 (1.247/0.521) times higher than the odor of AE1 and AE2, respectively. After 14 days of storage at 4°C, the predicted OA for AE3 was directly correlated to the increase of color and odor attributes. Similarly, the regression coefficients of these two parameters remained higher for AE3 compared with that for AE1 and AE2 (Table 6). Overall, these findings showed the strongest preservative effect of the water extract at 1% (AE3).

4. Conclusions

Pomegranate peels were extracted by three different solvents (ethyl acetate, acetonitrile, and water) and used to study their phytochemical content, antioxidant activity, and antibacterial potential against foodborne pathogenic bacteria. In our study, the ethyl acetate extract presented the most elevated content of polyphenols, flavonoids, DPPH, and ABTS inhibition followed by the aqueous extract. Equally, the three extracts demonstrated notable antibacterial activities against all analyzed bacterial strains.

The minced beef meat susceptible to spoilage and pathogenic bacteria, and lipid and protein oxidation, has limited shelf life. In this regard, in a second part of the present work, we demonstrated the big effect on microbiological, physicochemical, and sensory analyses in minced beef meat carried by the aqueous extract of pomegranate peel.

Oxidation stability of proteins, lipids, and sensory changes were well improved by the addition of AE at 0.5–1%, while using BHT at legal limit sowed less effectiveness. We have investigated two overall acceptability relationships, one with physicochemical change and other with sensory parameters. As a potential source of phenolic compounds, the AE3 extract could be effectively considered as a promising tool and secured method, used soon in meat products preservation.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

Mariam Fourati and Slim Smaoui contributed equally to this work.

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