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Research Article

Physical, Chemical, and Antioxidant Characterization of Nano-Pomegranate Peel and Its Impact on Lipid Oxidation of Refrigerated Meat Ball

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Pomegranate peel (CPP), enriched with bioactive constituents, had potent antioxidant features. Therefore, it is worth finding out functional and antioxidant features of the nanoscale pomegranate peel. The nanoscale of pomegranate peel was prepared by ultrafine grinding in a ball mill for 45 min (NPP45) and 90 min (NPP90). The physical (SEM, TEM, FTIR, and XRD) and chemical characteristics (phenolics, flavonoids, DPPH scavenging activity, FRAP, and reducing power) of nanoparticles were studied. The quality aspects of cold stored ($5 \pm 2^{\circ}C$) meatballs formulated with 0.5% (W/W) of nano-peel powder were evaluated. Similarly, FTIR spectra and XRD patterns were recorded for nano and crude pomegranate peel samples. Generally, grinding the crude peel for 45 and 90 min enhanced its scavenging activity, reducing power, FRAP, total phenolic, and flavonoid by a range of 12.58 to 20.37 and 20.57% to 35.18%, respectively. The addition of crude/nanosized peel to the meat ball diminish ($p < 0.05$) formation of thiobarbituric acid (TBARS), peroxide (PV), and volatile nitrogen and kept the sensory attributes up to 9 days of cold storage. No significant differences were noticed in PV and TBARS of meatballs formulated with 0.5% NPP90 and 0.1% BHT, which suggests the potential use of nanoscale pomegranate peel as natural substitutes to BHT in meat products.

1. Introduction

The pomegranate (*Punica granatum L.*), originating in the Middle East, is one of the rising tree crops grown worldwide. Its peel constitutes about 50 of a whole fruit's weight and remains a waste product in the pomegranate juice production process. Pomegranate peel extract, enriched with phenolic and flavonoid constituents, had potent antioxidant features [1, 2]. Even though the pomegranate peel's antioxidant and health benefits were upheld, scientific research should be done to improve the peel extract's effectiveness.Therefore, it is necessary to improve the efficiency of pomegranate peel extract via modern emerging food processing technologies, i.e., superfine grinding [3] and nanotechnology [2]. Over the past few decades, nanotechnology increasingly attracted most researcher in the food processing sector such research achieved a promising result in food preservation which might cause a revolution in food processing and preservation [4]. Decreasing particle sizes might increase the particle surface area and consequently release bioactive compounds [5]. Therefore, it is worth finding out the functional characteristics and antioxidant features of nanoscale pomegranate peel. Meatballs have a short shelf life due to lipid oxidation, protein decomposition, and microbial contamination during storage [6]. Lipid oxidation results in comprehensive changes in flavor, color, and structure, which minimize sensory traits and consumer acceptability of meat products. Such oxidative rancidity could be eliminated by using synthetic or natural antioxidants [7]. Although synthetic antioxidants have been

effectively applied to prevent meat products oxidation, it has negative health effects. Therefore, increased demand for natural antioxidants has been noticed in the recent decades [8]. Pomegranate peel extracts displayed extraordinary antioxidant properties with high competence in free radical scavenging and lipid oxidation suppression activity [9]. The antioxidant impact of pomegranate peel extracts has been scrutinized in cooked chicken products [10], in ground pork meat [11], beef sausage [12], and white shrimp [13]. On the other side, powdered pomegranate peel [14] as well as nano-pomegranate peel [15] has been used as a promising natural antioxidant to prolong the shelf life of meat products.

Although diverse investigations were carried out on the bioactive compounds and health merits of pomegranate peel, there was little research focused on the impact of the nanosizing of pomegranate peel on its physicochemical properties and antioxidant activity, furthermore, the impact of using nano-pomegranate peel as a preservative to prolong the shelf life of meat products. Therefore, the present study aimed to evaluate the effect of nanosized pomegranate peel (by mill ball grinding) on its physicochemical and antioxidant activities as well as its impact on retarding the lipid oxidation of cold-stored meat ball.

2. Materials and Methods

2.1. Materials. Pomegranate (*Punica granatum L.*) fruits were purchased from the local market in Taif City, Kingdom of Saudi Arabia. All chemicals and reagents used in this investigation were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Methods

2.2.1. Preparation and Characterization of Pomegranate Peel Nanoparticles. The pomegranate peels were manually cut into small pieces and dried at room temperature ($25 \pm 2^{\circ}$C) for five days. Dried peels were ground to prepare the CPP and sieved through a 50-mesh sieve. Planetary ball mill (PQ-N2, Gear Drive 4-station, 220V, Retsch, Germany) was used to prepare pomegranate peel nanoparticles. CPP (100 g) was milled in 250 ml vessel containing 250 stainless steel beads (100, 75, 50, and 25 beads with diameters 1.0, 1.5, 3.0, and 5.0 mm, respectively). The milling conditions were 4000 rpm, high energy frequency (40 Hz), room temperature ($25 \pm 2^{\circ}$C), and times (45 and 90 min.). The prepared nano pomegranate powders were classified according to the milling time to NPP45 and NPP90, respectively [16].

2.2.2. Physical Characterization of Nano-Pomegranate Peel Particles

(1) Particle Size. The particle size of NPP45 and NPP90 were measured in nanosuspension using Zetasizer (Malvern, Model: Zetasizer nano-series, Nano ZS, United Kingdom). A dispersion of 20 mg NPP45 or NPP90 in 4 ml deionized water and 0.5 ml dimethyl sulfoxide was prepared and homogenized by stirring for 30 min, then centrifuged for 15 min at 5000 rpm. Serial concentrations were prepared by diluting the supernatant in deionized water. Samples were measured after 5 min and equilibration at 25°C based on electrophoretic mobility under an electric field. The dynamic laser scattering angle was 173°, size ranged between 0.6 and 6000 nm, zeta potential ranged between −200 and 200 mV: the average of runs was at 16 and 10 seconds intervals [17].

(2) Scanning Electron Microscope (SEM). The images of scanning electron microscopy (SEM) were taken by using SU8020 SEM system (Hitachi, Japan). The samples (PP, NPP45, and NPP90) were mounted onto a specimen holder (under reduced pressure) with gold sputtered.

(3) Fourier-transform Infrared Spectroscopy (FTIR). Fourier transform infrared (FTIR) spectroscopy was used to disclose the active groups of pomegranate peel nanoparticles. A defined weight (A 0.002 gm) of the powder samples (CPP, NPP45, and NPP90), blended with 0.02 gm KBr and fully grinded, then turned to pellet via intense compress in a spatial mound and put in the apparatus to accomplish the FTIR spectra.

(4) X-Ray Diffraction (XRD). The X-ray diffraction (XRD) pattern of PP, NPP45, and NPP90 was tested by D8 X-ray diffractometer (Bruker, Germany). The sample diffractions were recorded between 5 and 100° (2θ) with a counting time 1 s/step and step size 0.02°.

2.2.3. Chemical Characterization

(1) Total Phenolic and Total Flavonoids. Total polyphenols and flavonoids were quantified as previously described by Slinkard and Singleton [18] using Folin–Ciocalteu and Dowd method [19].

(2) Antioxidant Characteristics. Radical scavenging activities of CPP, NPP45, NPP90 ethanolic extracts, and BHT were determined as described by Brand–Williams et al. [20] using the 2, 2-diphenyl-1- picrylhydrazyl (DPPH). The reducing power (RP) of the CPP NPP45, NPP90 ethanolic extracts, and BHT solutions was determined by the method of Oyaizu [21] with some modifications. CPP, NPP45, NPP90 ethanolic extracts, and BHT solutions were evaluated for their ferric-reducing antioxidant power (FRAP) as described by Benzie and Strain [22].

2.2.4. Formulation of Meatballs. Raw beef (top round) and fat were supplied from the Faculty of Agriculture farm (Shebin El-Kom, Egypt) and slaughtered (in the same frame slaughterhouse), cleaned, and deboned following standard commercial procedures. The meat (15% fat) was conveyed to the laboratory in an ice box to the laboratory, and then minced for 5 min. The meatballs were formulated by mixing 86.75% minced beef (~15% fat content) with 5.5% breadcrumbs, 3.25% onion powder, 0.5% garlic powder, 0.25% black pepper, 0.5% cumin, 0.5% coriander, 1.75% salt, and 1.0% water [15]. The previous ingredients were mixed well to

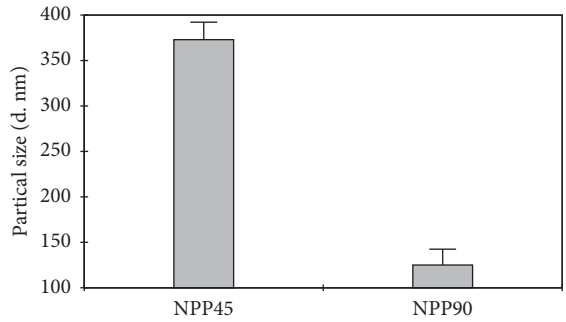


Figure 1: Means of particle size of nano-pomegranate peel powder (NPP45 and NPP90).

form a homogeneous mixture, and then divided into 5 portions. The first portion was used as control (without adding any antioxidants). CPP (0.5%), NPP45 (0.5%), and NPP 90 (0.5%) were added to the sample for three groups. The fifth group was formulated with 0.1% BHT as a reference. Meatballs (20 ± 2 g) were packed into polypropylene pages, sealed, and stored at $4 \pm 1^{\circ}$C for 12 days. The samples were analyzed at three day intervals (0, 3, 6, 9, and 12).

2.2.5. Quality Traits of Meatballs Formulated with Nano-Pomegranate Peels

(1) Peroxide Value (PV). Peroxide value of cold-stored meatballs formulated with different nano-pomegranate peel was determined according to the IDF method [23]. The results were expressed as milliequivalents of peroxide/kg fat.

(2) Thiobarbituric Acid Reactive Substances (TBARS). TBARS were determined spectrophotometrically as described by Vyncke [24] and expressed as mg of malonaldehyde (MDA)/kg sample.

(3) Total Volatile Base Nitrogen (TVB-N). The TVB-N contents were determined by the method of Egan et al. [25]. The results were expressed as mg·N/100 g of the sample.

2.2.6. Sensory Evaluation. Uncooked samples were placed randomly in coded covered cups. Fifteen semi-trained panelists were asked to evaluate the refrigerated stored meatballs for rancid odor three times repeatedly for 15 s with an interval of 30 s in the analysis on days 0, 3, 6, 9, and 12. Then, the color attributes were evaluated using the same manner [26].

2.2.7. Statistical Analysis. The data were analyzed in one way using SPSS version 19.0 (Chicago, IL, USA) to test the variance by one-way analysis of variance (ANOVA). Tukey's honest (HSD) test was followed to determine the differences in relative abundance. The results were expressed as the mean ± SEM, and $p < 0.05$ was considered statistically significant.

3. Results and Discussion

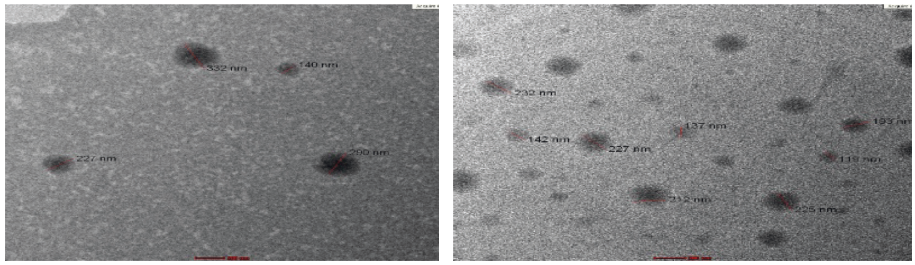
3.1. Physical Properties of Nano-Pomegranate Powder

3.1.1. Particle Size Distribution. The mean values of nano-pomegranate milled for 45 min (NPP45) was 373 nm these mean values decreased to 125 nm with increasing the milling time to 90 min (Figure 1).

3.1.2. Transmission Electron Microscopy (TEM). High resolution transmission electron microscopy (HRTEM) is a sophisticated technique used to characterize the shape, size, and distribution of nanoscale materials [27]. The nanoparticle scale of pomegranate peel showed uniform distribution and smaller in size was detected in the NPP90 than those obtained in NPP45 with a prevalent spherical morphological shape (Figure 2).

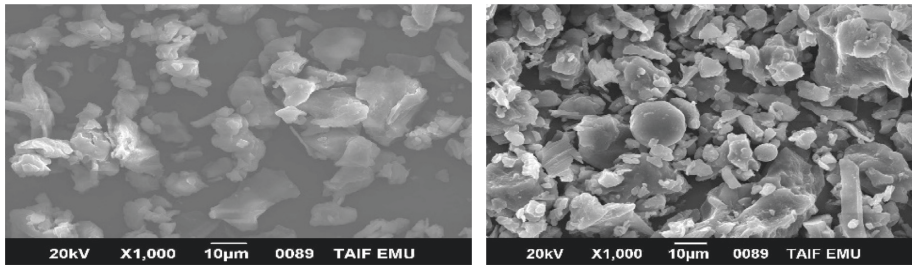
3.1.3. Scanning Electron Microscopy (SEM). The microphotographs of nano-pomegranate peel powder showed the morphology of CPP, NPP45, and NPP90 nanoparticles (Figure 3). Milled pomegranate peel particles depicting the granular mechanical damage. Increasing the ball milling time increased the broken particles into smaller fractions, and the presence of various pomegranate peel particle shapes might be resulted from the combination of flattening, aggregation, and fracture of particles. At the beginning of crack proliferation and fracture, the aggregation of pomegranate peel particles might lead to an increase in size. The morphology of ball milled peel considerably changed, which might have a remarkable impact on physicochemical properties. The SEM images of CPP, NPP45, and NPP90 confirm well with that detected by XRD and maintain the particle's random amorphous structure. Similar trend was detected by Zhong et al [3] for superfine particles of pomegranate peel.

3.1.4. FTIR Spectra. The FTIR spectra of crude (CPP) and nanoparticles (NPP45 and NPP90) of pomegranate peel powder are illustrated in Figure 4. Similar patterns of structures were detected for different pomegranate peel nanoparticles. However, a decrease in the transmittance intensity of bands was recorded by increasing the size of particles. No significant changes in FTIR spectra of six particle-sized pomegranate peel powders [3]. The relatively high number of bands might be correlated with the complexity of the peel constituents (proteins, carbohydrates, cellulose, and pectin) which contains different types of bonds. Similar results were detected by Zhong et al. [3] who found that grinding of pomegranate peel to different fine and superfine powder did not affect their chemical composition and consequently their FTIR spectra. Complex nature with wide variety of compounds in pomegranate peel powder was previously proved by Salih et al. [28]. Also, many active groups, which are mostly aldehyde compounds, ketones, amines, amides, alcohols, or aromatic or phenolic compounds were detected in pomegranate peel [29, 30].



NPP45 NPP90

Figure 2: TEM of nano-pomegranate peel fractions (NPP45 and NPP90).



NPP45 NPP90

Figure 3: SEM of nano-pomegranate peel fractions (NPP45 and NPP90).

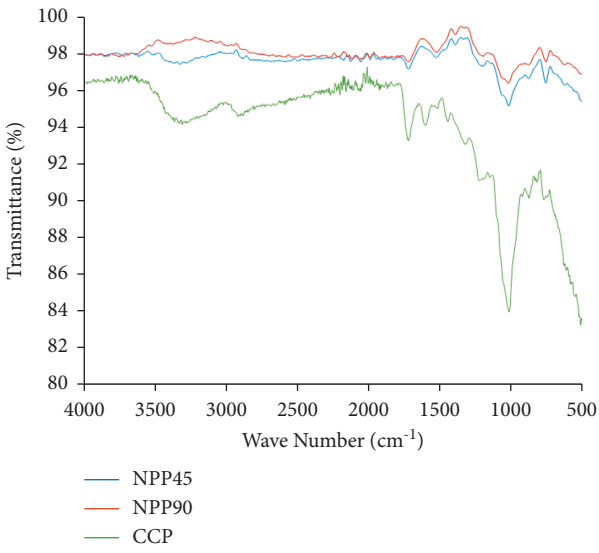


Figure 4: IR spectra of CPP, NPP 45, and NPP 90.

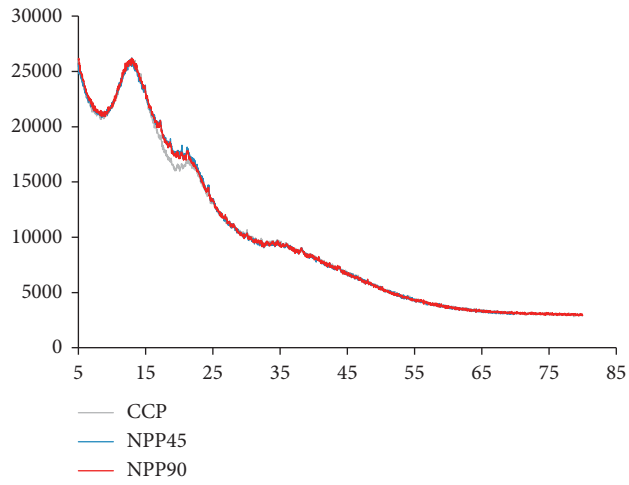


Figure 5: XRD pattern of CPP, NPP45, and NPP 90.

3.1.5. X-Ray Diffraction (XRD). X-ray diffractometry (XRD) was applied to assess the crystallinity features of crude and nano-pomegranate peel (Figure 5). The diffractograms represented typical beaks for crude and nanosized pomegranate samples. The peak broadening revealed no crystallinity of crude and nano-size samples and maintain the random amorphous structure. These results suggesting the inhomogeneous composition of the tested peels which is matching with the results previously stated by Vinay et al. [31] for pomegranate peel silver nanoparticles.

3.2. Antioxidant Characteristics and Phenolic and Flavonoid Contents. Reducing power, ferric reducing power, and DPPH scavenging activity as well as, phenolic and flavonoid contents of crude and nano-pomegranate peel ethanolic extracts compared with BHT are illustrated in Table 1. Generally, decreasing the particle size of pomegranate peels by milling for 45 min (NPP45) and 90 min (NPP90) enhanced their DPPH-scavenging activities by 15.77% and 20.57%, respectively, and their FRAP by 20.37% and 35.18%, respectively. These results maintained by the noticed increase in total phenolic (12.58% and 35.17%) and total flavonoids (14.12% and 24.51%) in the same table. This might be due to the decrease in particle size which increased the surface area via decreasing the size into nanoscale and

Table 1: Oxygen scavenging activity, reducing power, and ferric reducing antioxidant power of ethanolic extract of nano-pomegranate peel ethanolic extract.

Antioxidants/bioactive compounds	Crude and nano-pomegranate ethanolic extracts			
	CPP	Npp 45	Npp 90	BHT (0.1%)
DPPH-scavenging activity	76.12	88.13	91.78	80.25
Reducing power	0.55	0.64	0.68	0.81
FRAP	0.54	0.65	0.73	0.86
Total phenolic	72.69 mg·GAE/gm·d. w.	81.84 mg·GAE/gm·d. w.	98.26 mg·GAE/gm·d. w.	
Total flavonoids	76 mg·rutin/gm·d. w.	87.12 mg/gm·d. w.	94.63 mg/gm·d. w.	

Table 2: Impact of nano-pomegranate peel on the peroxide value (meq peroxide/kg lipid) of cold stored meatballs.

Meatball blends	Storage period (d)					Means[1]
	0	3	6	9	12	
Control (0.0)	0.47 ± 0.04	0.77 ± 0.06	1.03 ± 0.07	0.94 ± 0.05	0.98 ± 0.05	0.84[a]
0.5% CPP	0.45 ± 0.03	0.58 ± 0.03	0.72 ± 0.03	0.86 ± 0.07	0.95 ± 0.06	0.71[b]
0.5% NPP45	0.44 ± 0.03	0.54 ± 0.04	0.62 ± 0.02	0.77 ± 0.05	0.88 ± 0.03	0.65[c]
0.5% NPP90	0.41 ± 0.05	0.51 ± 0.03	0.63 ± 0.04	0.72 ± 0.05	0.84 ± 0.07	0.62[c]
0.1% BHT	0.40 ± 0.04	0.52 ± 0.05	0.67 ± 0.03	0.75 ± 0.03	0.86 ± 0.05	0.64[c]
Mean	0.43[e]	0.58[d]	0.73[c]	0.81[b]	0.91[a]	

[1]Means in the same column with different letters are significantly different ($p < 0.05$), LSD = 0.05. [2]Means in the same row with different letters are significantly different ($p < 0.05$), LSD = 0.06.

consequently facilitate releasing of phenolic and flavonoids [2, 3]. The DPPH-scavenging activity of crude pomegranate peel was lower than that of 0.1% BHT. Meanwhile, decreasing the pomegranate peel size into nanoscale enhanced its DPPH radical inhibition efficiency compared with BHT. On the other side, all pomegranate peels (crude and nanoscale) had a lower FRAP and reducing power compared to 0.1% BHT.

3.3. Quality Traits of Meatballs Formulated with Nano-Pomegranate

3.3.1. Peroxide Values. Initial oxidation of oils and fats were usually expressed by peroxide values (PV). Both nano-pomegranate addition and storage time significantly ($p < 0.05$) affected the PV of stored meatballs (Table 2). The highest ($p < 0.05$) initial PV was detected in the control samples (without pomegranate peel or BHT) followed by that formulated with crude pomegranate peel, whereas the lowest initial oxidation value was detected in meatballs formulated with 0.5% NPP45, NPP90, and 100 ppm BHT. The PV of the control sample reached to the threshold limit value on day 6 and decreased after that. Such decrease might be due to the degradation of the formed hydroperoxides and formation of secondary lipid oxidation derivatives. No significant differences were noticed among the stored meatballs formulated with 0.5% NPP45, NPP90, and 0.1% BHT, which indicated that progression of initial oxidation and the subsequent peroxides degradation was delayed with retardation of further oxidation progress. On the other side, significant ($p < 0.05$) increase in the mean of PV was detected by increasing the storage days. Similar trends were noticed in meatballs formulated with 1% crude pomegranate peel [30] and 1% nano-pomegranate peel [15].

3.3.2. Lipid Peroxidation (TBARS). Secondary lipid oxidation products, the main entrepreneur of oxidative rancidity, were determined by the TBARS assay. Generally, BHT and crude and nano-pomegranate peel as well as the storage time affected ($p \leq 0.05$) TBARS values of the formulated meatballs (Table 3). The TBA value of all formulated meatball samples was almost the same at zero time of the storage period. The TBA means of all formulated meatball samples significantly increased ($p < 0.05$) by increasing the storage period. The TBA value of meatball control was higher than other meatball samples at all storage periods. No significant differences ($P > 0.05$) were noticed in the TBA among the stored meatballs formulated with 0.5% NPP45, NPP90, and 0.1% BHT. These results confirm well with the results of the peroxide value (Table 1), which might maintain that the nanoscales pomegranate peel might delay the peroxide degradation and retard further oxidation progression that reduce the rancid odor. Morsy et al. [15] detected a lower TBA value in meatball samples formulated with 1.5% lyophilized pomegranate peels nanoparticles compared to control and BHT. As illustrated in our results, both negative and positive control reached the upper TBARS quality standard limit by the eighth day of storage.

3.3.3. Total Volatile Nitrogen (TVN). Total volatile nitrogen (TVN) is the main proof of meat protein degradation. The mean TVN value of freshly prepared meatballs was 4.21 mg·N2/100 gm and increased significantly ($p < 0.05$) by increasing the storage periods and reached to 18.65 mg·N2/100 gm after 12 day of cold storage (Table 4). The highest ($p < 0.05$) mean TVN value was noticed in control (14.27 mg·N2/100 gm) meatball samples followed by both that formulated with 0.5% crude peel (10.06 mg·N2/100 gm) and 0.1% BHT (10.20 mg·N2/100 gm). The lowest ($p < 0.05$)

Table 3: Impact of nano-pomegranate peel on thiobarbituric acid (mg malonaldehyde/kg sample) of cold stored meatballs.

Meatball blends	Storage period (d)					Means[1]
	0	3	6	9	12	
Control (0.0)	0.32 ± 0.03	0.46 ± 0.01	0.60 ± 0.03	0.82 ± 0.02	1.31 ± 0.03	0.70[a]
0.5% CPP	0.32 ± 0.01	0.39 ± 0.02	0.50 ± 0.02	0.78 ± 0.02	0.97 ± 0.03	0.59[b]
0.5% NPP45	0.30 ± 0.02	0.38 ± 0.01	0.45 ± 0.01	0.56 ± 0.03	0.79 ± 0.02	0.49[c]
0.5% NPP90	0.30 ± 0.03	0.34 ± 0.01	0.40 ± 0.03	0.53 ± 0.02	0.73 ± 0.03	0.46[c]
0.1% BHT	0.31 ± 0.02	0.34 ± 0.01	0.41 ± 0.01	0.50 ± 0.01	0.70 ± 0.02	0.45[c]
Means[2]	0.31[e]	0.38[d]	0.47[c]	0.64[b]	0.90[a]	

[1]Means in the same column with different letters are significantly different ($p < 0.05$), LSD = 0.06. [2]Means in the same row with different letters are significantly different ($p < 0.05$), LSD = 0.05.

Table 4: Impact of nano-pomegranate peel on the total volatile nitrogen value (mg·N_2/100 gm sample) of cold stored meatballs.

Meatball blends	Storage period (d)					Means[1]
	0	3	6	9	12	
Control (0.0)	4.22 ± 0.30	7.95 ± 0.37	12.81 ± 2.39	18.75 ± 0.35	27.65 ± 0.35	14.27[a]
0.5% CPP	4.21 ± 0.35	6.75 ± 0.29	9.75 ± 0.35	12.86 ± 0.47	16.95 ± 0.60	10.06[b]
0.5% NPP45	4.22 ± 0.33	6.25 ± 0.25	8.95 ± 0.24	11.65 ± 0.35	15.85 ± 0.35	9.38[c]
0.5% NPP90	4.20 ± 0.35	5.51 ± 0.31	7.86 ± 0.40	10.55 ± 0.35	14.76 ± 0.70	8.58[d]
0.1% BHT	4.21 ± 0.32	6.45 ± 0.30	9.10 ± 0.70	13.18 ± 0.53	18.05 ± 0.80	10.20[b]
Means[2]	4.21[e]	6.58[d]	9.69[c]	13.38[b]	18.65[a]	

[1]Means in the same column with different letters are significantly different ($p < 0.05$), LSD = 0.61. [2]Means in the same row with different letters are significantly different ($p < 0.05$), LSD = 0.72.

Table 5: Impact of nano-pomegranate peel on color and odor of cold stored meatballs.

Meatball blends	Storage period (d)					Means[1]
	0	3	6	9	12	
Color						
Control (0.0)	8.0 ± 0.81	7.55 ± 0.69	5.10 ± 1.28	4.75 ± 1.33	3.45 ± 1.25	5.77[b]
0.5% CPP	8.10 ± 0.73	7.65 ± 0.51	5.45 ± 0.91	4.52 ± 1.1	3.52 ± 1.15	5.84[ab]
0.5% NPP45	8.05 ± 0.87	7.77 ± 0.91	5.68 ± 0.52	4.85 ± 0.9	4.10 ± 0.95	6.09[ab]
0.5% NPP90	8.13 ± 0.87	7.76 ± 0.69	5.78 ± 0.48	5.07 ± 1.03	4.55 ± 1.03	6.25[a]
0.1% BHT	8.0 ± 0.66	7.88 ± 0.63	5.85 ± 0.63	5.10 ± 0.87	4.32 ± 0.90	6.23[a]
Mean[2]	8.05[a]	7.75[a]	5.57[b]	4.84[c]	3.99[d]	
Odor						
Control (0.0)	8.73 ± 0.82	8.50 ± 0.78	7.24 ± 0.56	5.21 ± 1.47	3.66 ± 0.70	6.67[b]
0.5% CPP	8.72 ± 0.78	8.55 ± 0.63	7.65 ± 0.42	5.63 ± 1.05	3.94 ± 0.94	6.90[b]
0.5% NPP45	8.75 ± 0.67	8.64 ± 0.69	7.86 ± 0.70	5.92 ± 0.94	4.95 ± 0.79	7.22[ab]
0.5% NPP90	8.80 ± 0.78	8.70 ± 0.82	8.05 ± 0.67	6.63 ± 1.63	5.75 ± 0.94	7.53[a]
0.1% BHT	8.70 ± 0.69	8.70 ± 0.63	7.73 ± 0.52	6.26 ± 1.68	5.34 ± 0.82	7.34[a]
Mean[2]	8.74[a]	8.62[a]	7.71[b]	5.93[c]	4.73[d]	

[1]Means in the same column with different letters are significantly different ($p < 0.05$), LSD = 0.38 and 0.41 for color and odor, respectively. [2]Means in the same row with different letters are significantly different ($p < 0.05$), LSD = 0.72 and 0.46, respectively.

TVN mean value (8.85 mg·N2/100 gm) was recorded for meatballs formulated with 0.5% NPP90. The TVN value was increased in the control sample from 4.22 mg·N2/100 gm to 27.65 mg·N2/100 gm after 12 days of cold storage, while the samples formulated with NPP90 was increased from 4.20 mg·N2/100 gm to 14.76 mg·N2/100 gm by the twelfth day of storage which might elucidate the inhibition impact of nanoscale pomegranate peel on the proteolytic microbes and consequently reduce the protein decomposition and the volatile nitrogen. Morsy et al. [15] reported an increase in control meatballs from 7.47 to 30.80 mg·N2/100 gm after 9 days of cold storage, while the meatballs treated with 1.5% lyophilized pomegranate peel nanoparticles had the lowest value of TVN compared with that formulated with 1% lyophilized pomegranate peel nanoparticles and 0.01% BHT.

3.3.4. Sensory Properties. The mean score of color and odor of meatballs formulated with 0.5% of nano-pomegranate particles are illustrated in Table 5. Generally, both sensory properties were affected ($p < 0.05$) by the cold storage period and added nano-pomegranate peel (NPP45 and NPP90) types. No difference ($p > 0.05$) was observed among all treatments at the beginning of the storage period. Color attribute was deteriorated ($p < 0.05$) by increasing (up to 3 days) the storage time in all the formulated samples, whilst

meatball control had a lower color means compared with the samples formulated with NPP90 and BHT 0.1%. On the other side, meatball odor was beginning to deteriorate by the third day of cold storage, whereas the control meatballs and that formulated with CCP had the lowest odor mean values compared with those formulated with 0.5% NPP45, 0.5% NPP90, and 0.1% BHT. The meat ball formulated with NPP90 and BHT kept their odor acceptability till the 9th of storage, whilst the other samples showed unacceptable odor from the 6th day of storage. These results indicated that the addition of nano-pomegranate particles (specially NPP90) might significantly inhibit the oxidation process of lipids and proteins and prolong the shelf life of cold stored meatballs. Morsy et al. [15] stated that the nano-pomegranate peel had remarkable effect to retard the lipid and protein oxidation and consequently prolong the shelf life of meatballs. Jouki and Khazaei [26] reported that oxidative rancidity (TBARS) and sensory quality of cold stored (4°C) camel meat can be easily enhanced by controlling the packaging atmosphere.

4. Conclusions

Using of crude pomegranate peel powder as a source of natural antioxidant have been previously studied. However, in the present study, crude peel was grinded in a planetary ball mill for 45 min (NPP45) and 90 min (NPP90). The physical and chemical characteristics of crude and nanosized pomegranate peel were evaluated and applied as a natural antioxidant to preserve the cold stored beef meatballs. Changing the pomegranate peel into nanoscale had no impact on its physical criteria where XRD diffractogram and FTIR showed a similar pattern. Meanwhile, nanosizing the peel improved the DPPH radical scavenging activity (even higher than BHT), FRAP, reducing power, and enhanced the efficiency of its phenolic and flavonoids to retard the lipid and protein oxidation in meat products, which could both satisfy consumer requests for natural food ingredients and add commercial value to pomegranate by-products. Therefore, nanoscales of pomegranate peels (especially NPP90) could be successfully added to meatball products as a promising natural substitute of the synthetic antioxidants. For more transparency of safety issues, further mandatory testing of using nano-pomegranate peel as to retard lipid and protein oxidation in meat products is needed.

Data Availability

The data presented in this study are available on request from the corresponding author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

A. E. and Y. K. were responsible for study conceptualization; A. E. and M. E. for methodology; S. K. for software; S. A. and A. A. for validation; Y. K. for formal analysis; A. E. for investigation; M. E. for resources; S.A. for data curation; A. E. and Y. K. for original draft preparation; S. K. for review and editing; A. A. for visualization; A. E. for supervision; and S. A. for project administration; A.E. for funding acquisition. All authors have read and agreed to the published version of the manuscript.

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