



# Impact of pomegranate peel nanoparticles on quality attributes of meatballs during refrigerated storage

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## ABSTRACT

This investigation was performed to evaluate the impact of lyophilized pomegranate peel nanoparticles (LPP-NPs) as an antioxidant and antimicrobial in meatballs during storage at  $4 \pm 1$  °C up to 15 days. LPP-NPs were incorporated into freshly minced beef meat at 1 and 1.5% and compared with 0.01% butylated hydroxytoluene (BHT) as a reference and control sample (without antioxidant). The LPP-NPs showed a high phenolic content and antioxidant capacity. In LPP-NPs-treated samples, contents of peroxide, thiobarbituric acid reactive substances (TBARS), and total volatile base nitrogen (TVB-N) were lower than the control (over 15 days). Cooking characteristics in meatball including LPP-NPs were improved during the storage. The microbial load of samples treated with LPP-NPs was lower than the control during storage. Sensory evaluation of color and rancid odor in treated meatballs were accepted with a high score up to 15 days. The results demonstrated that LPP-NPs were more effective in retarding lipid oxidation, improving the microbial quality, and cooking characteristics of meatballs.

## 1. Introduction

Meatballs are one of the most important meat products in many countries and highly consumed in the world (Oz & Cakmak, 2016). However, meatballs have a short shelf-life due to deterioration during storage (Turp, 2016). Lipid oxidation, protein decomposition, and microbial contamination are the main factors for reducing the quality and shelf-life of meat, particularly minced meats (Lund, Heinonen, Baron, & Estevez, 2011). These alterations result in extensive flavor changes, color loss, and protein structure damage (Fernández, Pérez-Álvarez, & Fernández-López, 1997), which reduce sensory parameters and consumer acceptability of meat products.

The main reason for quality loss in meat products is lipid and protein oxidation (Vuorela et al., 2005). The rate of oxidative changes can be reduced through synthetic or natural antioxidants (Ledesma, Rendueles, & Díaz, 2015). Although synthetic antioxidants have been successfully utilized to prevent meat products oxidation, like butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and gallate propyl (GP), they have been found to exhibit various health effects (Shahidi, Janitha, & Wanasundara, 1992). Therefore, the demand of natural antioxidants has been increased in the recent years such as rosemary (Guo et al., 2016), potato peel (Jeddou et al., 2016), and

pomegranate peel (Kazemi, Karim, Mirhosseini, & Hamid, 2016).

Pomegranate (*Punica granatum* L.) peels are by-products of pomegranate juice processing, which constitute about 40% of the whole fruit (Çam & Hışıl, 2010). Pomegranate peel extracts (PPE) have remarkable antioxidants with high efficiency free radical scavenging and inhibiting LDL oxidation (Akhtar, Ismail, Fraternal, & Sestili, 2015), as well antibacterial (Negi & Jayaprakasha, 2003). The antioxidant effect of PPE has been investigated in cooked chicken products (Kanatt, Chander, & Sharma, 2010), in ground pork meat (Qin et al., 2013), in beef sausage (El-Nashi, Fattah, Rahman, & El-Razik, 2015), and in white shrimp (Yuan, Lv, Tang, Zhang, & Sun, 2016). Also, the PPE has been reported as antibacterial against *L. monocytogenes* and *E. coli* (Al-Zoreky, 2009; Wu, Jahncke, Eifert, O'Keefe, & Welbaum, 2016), *S. aureus*, and *B. cereus* (Kanatt et al., 2010), and *A. flavus* and *A. parasiticus* (Rosas-Burgos et al., 2016).

Recently, nanotechnology has been utilized to create new products with numerous benefits for the food industry sector (Rodrigues et al., 2017) such as prolonging shelf-life and improving food quality and safety (Morsy, Khalaf, Sharoba, El-Tanahi, & Cutter, 2014). One study showed that nano-encapsulation of phenolic compounds has a fine delivery system in the preventing of degenerative diseases (Esfanjani & Jafari, 2016).

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In Egypt, pomegranate fruits are widely processed into juice and the obtained peels are discarded. There is no literature on the influence of LPP-NPs on the shelf-life and quality of meat products. The aim of this study was to investigate (I) the antioxidant and antimicrobial activity of LPP-NPs and (II) the impacts of the addition of LPP-NPs to prolong the shelf-life and improve the safety of beef meatballs during cold storage.

## 2. Materials and methods

### 2.1. Preparation of lyophilized pomegranate peel nanoparticles (LPP-NPs)

Fresh pomegranate fruits (*Punica granatum* L., cv. Manfaloty) were purchased from a local market in Egypt. The fruits were transported to the laboratory and surface-disinfected by immersing gently with 200  $\mu\text{L L}^{-1}$  sodium hypochlorite solution for 2 min, and then washed, peeled, and the edible portion was manually separated. The cleaned peels were cut into 1 cm  $\times$  1 cm pieces. Afterwards, the pieces were frozen at  $-40^\circ\text{C}$ , then dehydrated for 72 h in a freeze dryer (Labconco 74200, USA) at 0.12 mbar, with a chamber temperature of  $18 \pm 1^\circ\text{C}$  and a condenser temperature of  $-85 \pm 1^\circ\text{C}$ . The lyophilized pomegranate peel (LPP) was reduced to nanoparticle size according to (Khataee, Fathinia, & Fathinia, 2017) with some modifications. Briefly, LPP was ground with a Moulinex grinder (Model MC300, France) to micro-particles range of 100–150  $\mu\text{m}$ , and crushed by a high-energy planetary ball-mill (Model PM 2400, Iran) at a rotation speed of 320 rpm for 2 h to prepare LPP nanoparticles. The ball-milling process was implemented under atmospheric conditions ( $25^\circ\text{C}$  and 1 atm), and the ratio of ball mass to powder mass was 10:1. Finally, the homogenous LPP-NPs were measured using zetasizer (Nano Sight NS300, UK) with an average size of  $80 \pm 5$  nm. Then the LPP-NPs were packaged in a dark glass bottle without presence of oxygen, and used freshly in the experiments. All processes done for producing of LPP-NPs were under controlled conditions, to minimize the antioxidant loss.

### 2.2. Meatball manufacture

Raw beef (top round) and fat were supplied from a local store in Touth city, Egypt. The meat was transported in an ice box to the laboratory within 10 min of purchase, and then the meat's fat contents were adjusted to 15% with the fat (inter-muscular and/or sub-cut fat) before the ground. The meatballs were produced according to the formula: 86.75% minced beef ( $\sim 15\%$  fat content), 5.5% bread crumbs, 0.25% black pepper, 0.5% cumin, 0.5% coriander, 3.25% onion powder, 0.5% garlic powder, 1.75% salt, and 1.0% water. All ingredients were mixed to obtain homogeneous dough and divided into 4 groups. LPP-NPs were added to sample for two groups at a concentration of 1 and 1.5%. The third group included BHT at 0.01% as a reference and the last one was without any antioxidant (control). Meatballs ( $20 \pm 2$  g) were formed by hand, placed into plastic trays, sealed with one layer of a wrapping film, and stored at  $4 \pm 1^\circ\text{C}$  for 15 days. The samples were taken for analysis at 0, 3, 6, 9, 12, and 15 days of storage.

### 2.3. Determination of the phenolic content

Total phenolic content of the LPP-NPs methanolic extract was determined by the Folin–Ciocalteu colorimetric method (Singleton & Rossi, 1965), and calculated as mg of gallic acid equivalents (GAE) per 100 g of dw (dry weight) of the sample.

### 2.4. Determination of the flavonoid content

Total flavonoid content of the LPP-NPs methanolic extract was determined using a colorimetric method as described previously by Zhishen, Mengcheng, and Jianming (1999). Total flavonoid contents were expressed as mg rutin equivalents per gram of dried sample. Rutin

was used as standard to produce the calibration curve. Total flavonoid contents were calculated using a standard curve equation and the formula as follows;

$$\text{Total flavonoid content (\% w/w)} = \text{RE} \times \text{V-sa} \times \text{D} \times 10^{-6} \times 100/\text{W}$$

Where, RE: Rutin equivalent ( $\mu\text{g/mL}$ )

V-sa: Total volume of sample (mL)

D: Dilution factor

W: Weight of sample (g)

### 2.5. Chromatography analysis using (HPLC) of phenolic compounds

The HPLC analysis was carried out according to Kim and Thomas (2007) with slight modifications using an Agilent Technologies 1100 series liquid chromatograph equipped with an auto sampler and a diode-array detector. The analytical column was Agilent Eclipse XDB-C18.  $4.6 \times 150$  mm, 5  $\mu\text{m}$  with a C<sub>18</sub> guard column. The mobile phase consisted of acetonitrile (solvent A), 0.5% acetic acid in 99.5% acetonitrile (solvent B). The elution was by linear gradient starting with 100% (A) and ending with 100% (B). Using DAD detector set at a wavelength 254 nm. The flow rate was kept at 0.8 mL/min for a total run time of 70 min. There were 10 min of post-run for reconditioning. The injection volume was 10  $\mu\text{L}$  and peaks were monitored simultaneously at 280, 320, and 360 nm. Samples were filtered through a 0.45  $\mu\text{m}$  Acrodisc syringe filter (Gelman Laboratory, MI) before injection. The peaks were identified by congruent retention times and UV spectrum and compared with those of the standards punicalagin, p-hydroxybenzoic, catechine, chlorogenic, caffeic, syringic, vanillic, ferulic, sinapic, rutin, kaempferol, and chrysin. All the standards were purchased from Sigma-Aldrich (St. Louis, MO, USA).

### 2.6. Determination of DPPH radical scavenging activity

The ability of LPP-NPs to scavenge free 2, 2-diphenyldrazyl (DPPH) radicals was determined according to the method of Brand-Williams, Cuvelier, and Berset (1995). The DPPH scavenging effect (%) was calculated by the following equation:

$$\text{DPPH scavenging effect (Inhibition \%)} = [(\text{Ac} - \text{AS}/\text{Ac})] \times 100$$

Where Ac is the absorbance of the control reaction and the absorbance in the presence of the plant methanolic extract. Values of IC<sub>50</sub> denote the concentration of sample required to scavenge 50% of DPPH radicals. Results were expressed as IC<sub>50</sub> values ( $\mu\text{g/mL}$ ). The IC<sub>50</sub> values were calculated by a sigmoid non-linear regression model using plots, where the abscissa represented the concentration of tested plant extracts and the ordinate represented the average percent of scavenging capacity.

### 2.7. Peroxide value (PV)

The peroxide value of meatball samples was determined according to the IDF method (Shantha & Decker, 1994). The results were expressed as milli-equivalents of peroxide/kg fat.

### 2.8. Thiobarbituric acid reactive substances (TBARS)

TBARS were determined according to the spectrophotometric method described by Vyncke (1970). TBARS values were expressed in mg of malonaldehyde (MDA)/kg sample.

### 2.9. Total volatile base nitrogen (TVB-N)

The content of TVB-N were performed according to the method

described by Egan, Kirk, and Sawyer (1981). The TVB-N was expressed as mg N/100 g of sample.

### 2.10. Water holding capacity (WHC) and pH value

WHC of meatball samples was measured according to the method described by (Egan et al., 1981). The results were expressed as cm<sup>2</sup> measured by planimeter per 0.3 g of raw sample. The pH measurements were carried out using a digital pH-meter (model P107, Consort, Belgium).

### 2.11. Cooking loss and cooking yield

The cooking loss (%) of the meatball samples was calculated as follows:  $\{[\text{Fresh sample weight (g)} - \text{cooked sample weight (g)}] / \text{fresh sample weight (g)} \times 100$  (Mitchell et al., 1991). Cooking yield was calculated as follows:  $[100 - \text{cooking loss (\%)}]$ .

### 2.12. Microbiological assay

Ten grams of meatball sample were taken aseptically and transferred to 90 mL of sterilized peptone water (Becton, Dickinson and Co., Le Pont de Claix, France). The mixture was vortex for 2 min., then performed ten-fold serial dilutions. A (0.1 mL) of serial dilutions of a sample was spread on a surface of the appropriate agar for determination of total viable bacterial count (TVCs), psychophilic bacteria, and lipolytic bacteria on a plate count agar (Oxoid, CM325, UK). All agar plates were incubated at 37 °C for 48 h for (TVCs), and 7 °C for 5 days for psychophilic bacteria. Regarding lipolytic bacteria, the plates were incubated at 37 °C for 5 days, then flooded with copper sulfate solution 20% and the typical bacteria colonies with blue zone were counted (Yousef & Carlstrom, 2003).

### 2.13. Sensory evaluation

A ten-member trained panel from the Food Technology Department evaluated the meatball samples during the refrigerated storage. The samples were placed in covered cups coded with 3-digit random numbers and allowed to stand at room temperature few minutes prior to evaluation. The panelists were asked to evaluate all samples using a seven-point hedonic scale for odor or color with scores being as follows; 1 = unacceptable or extremely dark brown, 2 = very strong or very dark brown, 3 = moderately strong or dark brown, 4 = slightly strong or dark red, 5 = perceptible or slightly dark red, 6 = barely perceptible or cherry red, and, 7 = none for rancid odor or light cherry (Turgut, Soyer, & Işıkçı, 2016).

### 2.14. Statistical analyses

The experiments were run in triplicate for the antioxidant activity, two factors (extracts with two levels: LPP-NPs and BHT, and replication with two levels) were applied. For the meatball analysis (PV, TBARS, TVB-N, WHC, pH, cooking quality, and sensory properties), factorial design ANOVA with two factors (treatment with four levels: control, BHT, 1% LPP-NPs and 1.5% LPP-NPs and storage time with five levels: 0, 3, 6, 9, 12, and 15 days) were applied for each parameter by using SPSS software (version 18). The Duncan multiple comparison test was used to determine the significance of mean values for multiple comparison at  $P < 0.05$  (Steel & Torrie, 1980, p. 633).

## 3. Results and discussion

### 3.1. Phenolic, flavonoid compounds, and antioxidant capacity of LPP-NPs

Total phenolic and flavonoid have effective attributes like antibacterial and/or antioxidant capacities Table 1. The LPP-NPs extract

**Table 1**

Total phenolic, total flavonoid (mg/g dw) contents and radical scavenging activity of pomegranate peel.

Extract	Phenolics (mg/g dw)	Total flavonoid (mg/g dw)	IC50 (µg/ml)
Peel	215.2 ± 2.23	70.4 ± 2.69	17.77 ± 0.4
Pulp <sup>a</sup>	29.6 ± 3.82	32.1 ± 3.82	122.20 ± 0.8
BHT	–	–	5.07 ± 0.5

Each value is represented as mean ± SD (n = 3).

Phenolics as Gallic acid equivalents; flavonoids as rutin equivalents.

<sup>a</sup> Pulp: the edible portion of pomegranate fruit.

had a high phenolic content about 215.2 mg/g (on dry basis) as gallic acid equivalent. Hasnaoui, Wathelet, and Jiménez-Araujo (2014) and Y. Li et al. (2006) reported that phenolic contents of 205.07 and 261.70 mg/g in terms of gallic acid equivalent, respectively in aqueous extract of pomegranate peel. However, Turgut et al. (2016) found that the phenolic content was 165.4 mg gallic acid/g of pomegranate peel water extract. This diversity might be because of the extraction technique or the solvent utilized. The current investigation shows a concentration of 215.2 mg/g phenolic content which is a higher than flavonoid content (70.4 mg/g) in LPP-NPs. Also, contents of phenolic or flavonoid in peel was higher than the pulp (edible portion) of pomegranate one. These obtained results are in agreement with the data reported by Salgado, Ferreira, de Oliveira Biazotto, & dos Santos Dias (2012).

Furthermore, Table 1 presents the antioxidant capacity of pomegranate peel (scavenge DPPH free radical). The LPP-NPs show a high free radical scavenging capacity (IC50; 17.77 µg/mL) contrasted with BHT (IC50; 5.07 µg/mL), considering that there is an inverse relationship between the IC50 value and the antioxidant content. (Devatkal, Narsaiah, & Borah, 2010) found that peel extract (PE) showed highest free radical scavenging capacity compared with other fruits. Other studies demonstrated that peel extracts had stronger or similar antioxidant activity as compared with popular antioxidant additives, i.e. BHT (Kanatt et al., 2010) and ascorbic acid (Naveena, Sen, Vaithyanathan, Babji, & Kondaiah, 2008). These results indicate that LPP-NPs could be utilized as an alternative of synthetic antioxidant. The antioxidant activity of LPP-NPs would be mainly attributed to the compounds such as phenolic hydroxyl groups and double bonds including hydrolysable tannins and flavonoids. The antioxidant activity of LPP-NPs is a multi-factorial impact and chemical synergistic action of multiple compounds (Wang, Ding, Liu, Xiang, & Du, 2010).

### 3.2. HPLC fingerprint of LPP-NPs

Results shown in Table 2 confirm that pomegranate peel extract exhibited the high amount of total phenolics. The HPLC recognized the

**Table 2**

Polyphenolic profile of pomegranate peel extract.

Compound	Rt (min)	Area %
Punicalagin	5.87	48.21
p-hydroxybenzoic	9.11	0.54
Catachine	16.18	1.30
chlorogenic	20.99	1.36
Caffeic	22.32	1.70
Syrngic	23.43	0.71
Vanillic	24.92	0.44
Ferulic	31.35	3.51
Sinapic	32.57	1.17
Rutin	34.03	1.05
Kaempferol	41.40	0.74
Chrysin	45.29	3.92

Rt = Retention time.

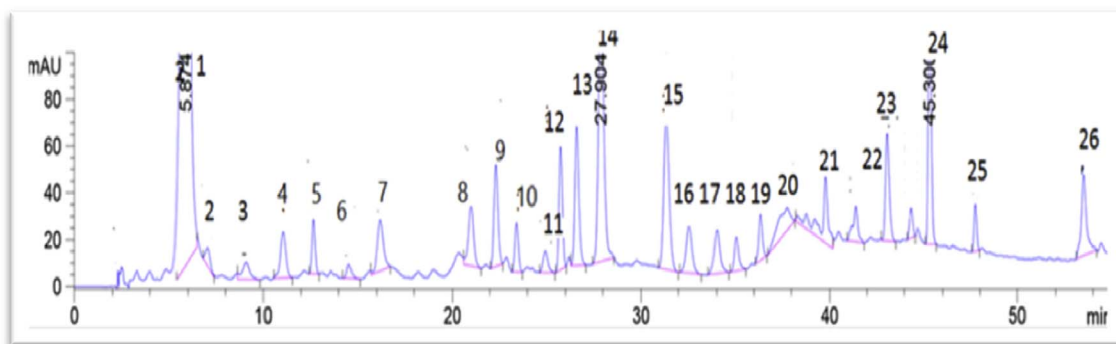


Fig. 1. HPLC fingerprints of pomegranate peel extract- Twelve components were identified in pomegranate peel, as punicalagin (peak 1), p-hydroxybenzoic (peak 3), Catechine (peak 7) chlorogenic (peak 8), caffeic (peak 9) syringic (peak 10), vanillic (peak 11), ferulic (peak 15), sinapic (peak 16), rutin (peak 17) Kaempferol (peak 22), Chrysin (peak 24).

main phenolic compounds (~26 peaks) in pomegranate extracts. Based on Fig. 1 twelve components were identified in pomegranate peel having the following order Punicalagin > Chrysin > Ferulic > Caffeic > Chlorogenic > Catechin > Sinapic > Rutin > Kaempferol > Syringic > p-hydroxybenzoic > Vanillic. Punicalagin represented approximately 73.4% of the twelve phenols, and was the predominant component of them. These results are in agreement with those reported by Gullon, Pintado, Pérez-Álvarez, and Viuda-Martos (2016) who found that punicalagin was the most abundant phenolic compound in pomegranate peels. However, Cai, Luo, Sun, and Corke (2004) noted the presence of quercetin and vanillic acid in pomegranate peel. The presence of specific phenolics may be influenced by different pomegranate cultivars and other factors such as soil quality and sunlight (Li et al., 2015).

### 3.3. Effect of LPP-NPs on lipid peroxidation of meatball

Fig. 2 illustrates that addition of antioxidant and storage period had a significant effect ( $P \leq 0.05$ ) on PV. The meatballs contained polyphenol constituents from other added ingredients i.e. onions, garlic, pepper, and cumin, the control group (without any antioxidants addition) was performed. The highest initial PV was recorded in the control sample, while the lowest value was in meatballs with 1.5% LPP-NPs. In the control sample, the PV reached to the threshold limit value on day 6, and then a rapid decrease was observed. However, the PV in meatball samples containing antioxidant (BHT), 1% LPP-NPs, and 1.5% LPP-NPs were below the limit value until the 9th, 12th, and 15th day, respectively during refrigerated storage. The results suggest that the control sample has a noticeable lipid-oxidation until day 6 of storage and maximum PV at the end of the primary auto-oxidation. After 6 days of storage, the PV decreased, possibly due to hydroperoxides decomposition to form secondary lipid oxidation products (Ladikos & Lougovois, 1990). A slight increase was noticed when antioxidants were added in meatballs after 9 days of storage. In the sample with 1.5% LPP-NPs, a small rise in PV was recorded. This demonstrates that the progress of

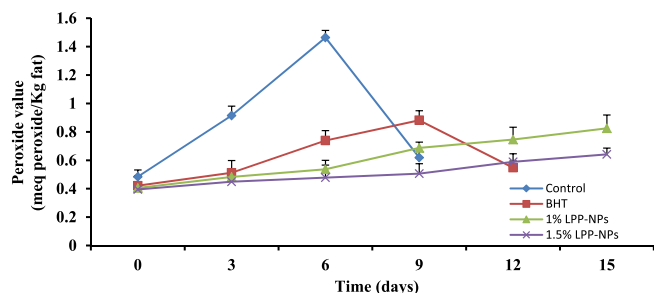


Fig. 2. Effect of the addition of LPP-NPs on peroxide value in meatball samples during storage at (4 °C). Error bars represent standard deviation, (n = 3).

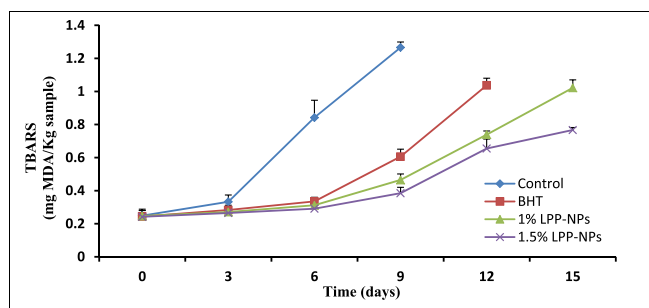


Fig. 3. Effect of the addition of LPP-NPs on TBARS (mg MDA/Kg) in meatball samples during storage at (4 °C). Error bars represent standard deviation, n = 3.

the pre-oxidation stage and the degradation of the peroxides created were moderate (Turgut et al., 2016).

Fig. 3 shows that the effect of antioxidant and storage time on malondialdehyde (MDA) concentration in meatball samples. A significant difference ( $P \leq 0.05$ ) in TBARS was noticed between the control and the treated samples. The lowest value of TBARS was observed in meatballs samples 1.5% LPP-NPs, while the highest value was in the control sample during cold storage. The initial TBARS in the meatball sample was ~0.25 mg MDA/kg, and was rapidly increased in the control sample up to 1.27 mg MDA/kg after 9 days of storage, followed by BHT (1.04 mg MDA/kg) after 12 days, then 1% LPP-NPs (1.03 mg MDA/kg) after 15 days, and 1.5% LPP-NPs (0.77 mg MDA/kg) after 15 days. Addition of synthetic and/or natural antioxidants decreased significantly TBARS' values even on the 1st day of the storage. However, the lipid oxidation-reduction was highest in 1.5% LPP-NPs containing meatballs as compared with BHT and 1% LPP-NPs containing samples. There was a significant difference in the percentage of antioxidant when BHT is compared with the LPP-NPs extract, but these results demonstrate that the use of natural antioxidant sources LPP-NPs can be effective in preventing lipid oxidation of meatballs at cold storage. Other studies demonstrated that pomegranate peels inhibited the oxidation of lipids in chicken patties (Kanatt et al., 2010) and goat patties (Devatkal et al., 2010). The inhibitory effect of LPP-NPs on lipid oxidation is attributed to its phenolic compounds which show an antioxidant activity by blocking radical chain reactions in the oxidation process (Negi & Jayaprakasha, 2003).

### 3.4. Effect of LPP-NPs on protein degradation

Fig. 4 presents the effect of LPP-NPs on TVB-N contents in meatball during storage at 4 °C. Addition of antioxidants and storage time had significant effects ( $P \leq 0.05$ ) on protein quality. In the control sample, the TVB-N gradually increased from 7.47 to 30.80 mg N<sub>2</sub>/100 g after 9 days of storage. The meatballs treated with 1.5% LPP-NPs showed the lowest value of TVB-N compared with LPP-NPs 1% and BHT during cold



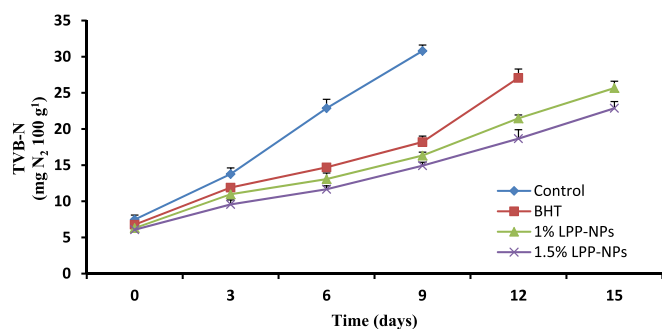


Fig. 4. Effect of the addition of LPP-NPs on TVB-N ( $\text{mg N}_2/100 \text{ g}^1$ ) in meatball samples during storage at ( $4^\circ\text{C}$ ). Error bars represent standard deviation,  $n = 3$ .

storage. These results demonstrate the positive effect of LPP-NPs on microbial growth inhibition, especially the proteolytic microbes which cause a breakdown of a protein to volatile nitrogen compounds. The increase in TVB-N during cold storage of meatball (control) might be attributed to a breakdown of nitrogenous substances by microbial activity (El-Nashi et al., 2015).

### 3.5. Effect of LPP-NPs on physical properties of meatballs

The pH value as a quality criterion of meatballs is presented in Fig. 5. There was no significant differences ( $P \geq 0.05$ ) in pH of the different meatball samples containing BHT, 1% LPP-NPs, and 1.5% LPP-NPs compared with the control at time zero. During the storage period at  $4^\circ\text{C}$  the significant differences ( $P \leq 0.05$ ) between the samples were observed. The pH value of the control sample increased rapidly up to 6.73 after 9 days of cold storage. On the other hand, samples incorporating BHT and LPP-NPs at different concentration revealed a slight increase in pH during cold storage. The pH value of meatball samples containing BHT was 6.65 after 12 days, while samples, including 1% LPP-NPs, and 1.5% LPP-NPs had pH values of 6.44 and 6.32, respectively after 15 days of storage. Results elucidate an increase in pH due to decomposition of nitrogenous compounds by endogenous or microbial enzymes (Reza Gheisari, Aminlari, & Shahram Shekarforoush, 2009; Yuan et al., 2016).

The WHC of raw meatballs is shown in Fig. 6. Results revealed higher WHC in samples containing LPP-NPs than the control sample ( $P \leq 0.05$ ). The addition of different concentrations of LPP-NPs in meatballs improved the WHC while in the control sample the WHC decreased gradually during the storage period. The positive effect of LPP-NPs on meatball samples could be attributed to the properties of pomegranate peels powder as a water-binding agent. The current results are in an agreement with those reported by (Li et al., 2015).

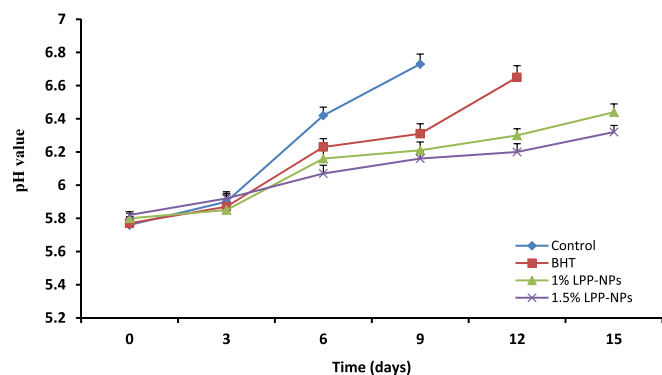


Fig. 5. Changes in the pH value in meatball samples with LPP-NPs and BHT during storage at ( $4^\circ\text{C}$ ). Error bars represent standard deviation, ( $n = 3$ ).

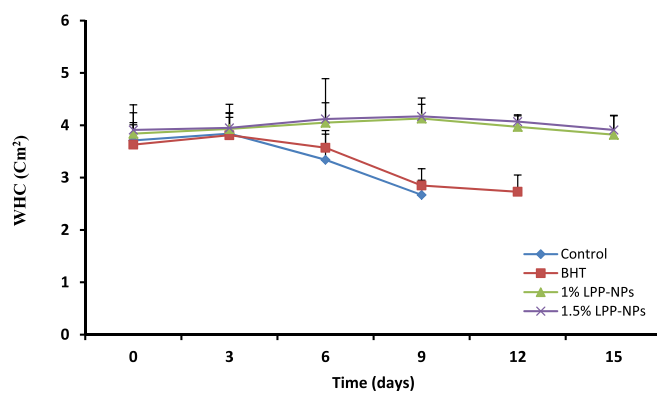


Fig. 6. Effect of the addition of LPP-NPs on WHC property in meatball samples during storage at ( $4^\circ\text{C}$ ). Error bars represent standard deviation, ( $n = 3$ ).

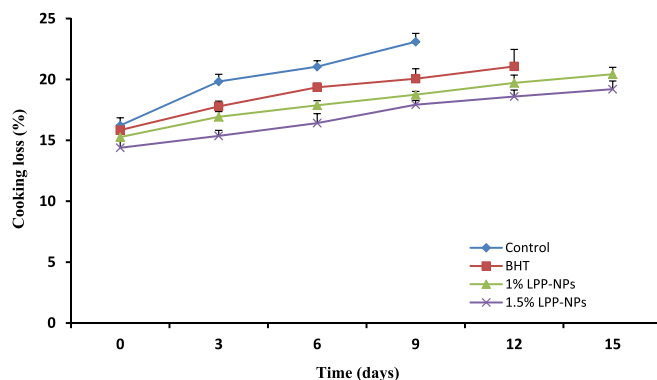


Fig. 7. Effect of the addition of LPP-NPs on cooking loss (%) in meatball samples during storage at ( $4^\circ\text{C}$ ). Error bars represent standard deviation, ( $n = 3$ ).

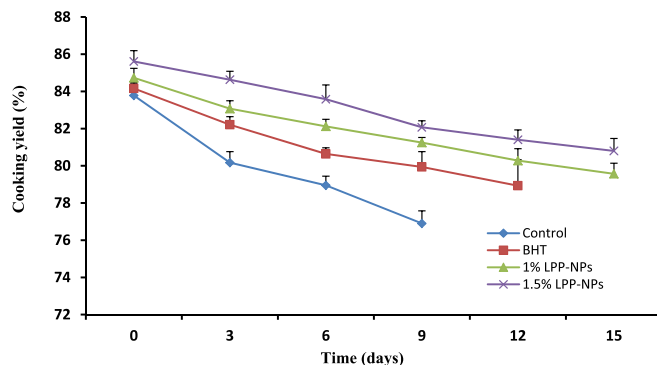


Fig. 8. Effect of the addition of LPP-NPs on cooking yield (%) in meatball samples during storage at ( $4^\circ\text{C}$ ). Error bars represent standard deviation, ( $n = 3$ ).

### 3.6. Effect of LPP-NPs on cooking characteristics of meatballs

As can be observed in Fig. 7 and 8, the cooking loss was significantly increased ( $P \leq 0.05$ ) in the control sample compared with samples containing LPP-NPs. The highest value of cooking loss (23.1%) was present in the control sample, while the lowest value was observed in samples containing 1.5% LPP-NPs (19.20%) after the storage period. However, the cooking yield slightly decreased in meatball samples prepared with LPP-NPs compared with the control sample during the cold storage period. The positive impact of the LPP-NPs addition in improving the cooking attributes of the prepared meatball samples was observed. These results could be associated with the functional properties of pomegranate peels powder as a water-binding agent and not polyphenols in particular (El-Nashi et al., 2015).

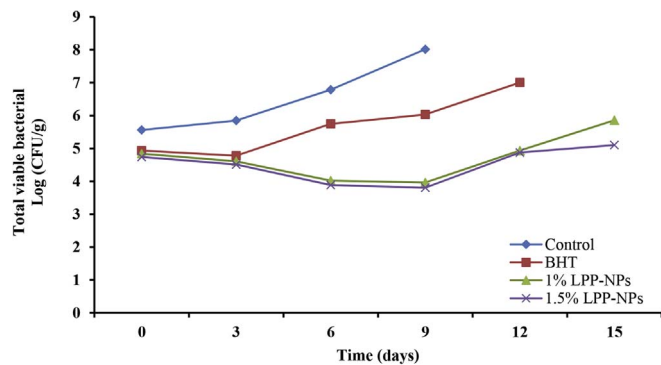


Fig. 9. Effect of the addition of LPP-NPs on Total viable bacteria in meatball samples during storage at (4 °C).

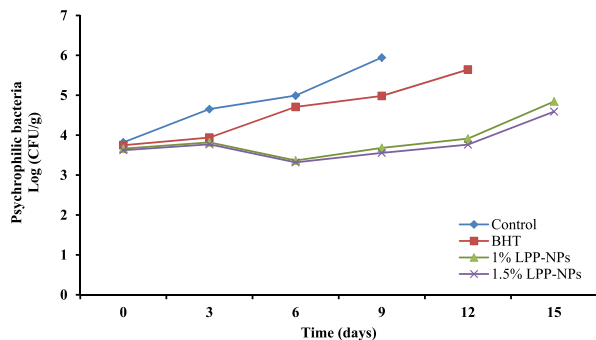


Fig. 10. Effect of the addition of LPP-NPs on psychrophilic bacteria in meatball samples during storage at (4 °C).

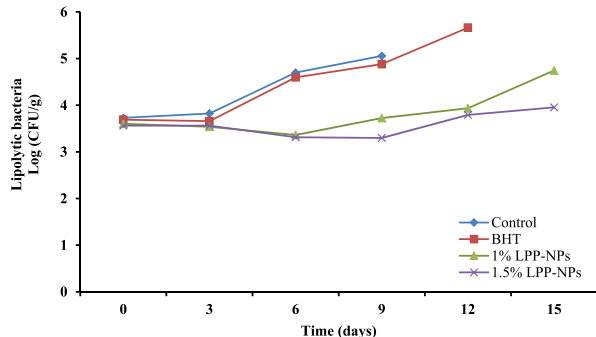


Fig. 11. Effect of the addition of LPP-NPs on lipolytic bacteria in meatball samples during storage at (4 °C).

### 3.7. Microbiological quality of meatballs

Data in Fig. 9 illustrate the TVCs of meatball samples during storage for 15 days. The TVCs of meatball were 4.91 log<sub>10</sub> CFU/g at day 0 and increased to 8.01 log<sub>10</sub> CFU/g after 9 days in the control sample. However, samples including BHT, 1% LPP-NPs and 1.5% LPP-NPs exhibited counts of 7, 5.78, and 5.1 log<sub>10</sub> CFU/g, respectively. The psychrophilic bacteria were approximately 3.71 log<sub>10</sub> CFU/g at day 0, but increased to approximately 5.94 log<sub>10</sub> CFU/g (C), 5.64 log<sub>10</sub> CFU/g (BHT), 4.84 log<sub>10</sub> CFU/g (LPP-NPs 1%), and 4.59 log<sub>10</sub> CFU/g (LPP-NPs 1.5%) (Fig. 10). As shown in Fig. 11, lipolytic bacteria were approximately 3.64 log<sub>10</sub> CFU/g at day 0, but increased to approximately 5.05 log<sub>10</sub> CFU/g (C), 5.66 log<sub>10</sub> CFU/g (BHT), 4.74 log<sub>10</sub> CFU/g (LPP-NPs 1%), and 3.95 log<sub>10</sub> CFU/g (LPP-NPs 1.5%). From above-mentioned results, the meatball samples incorporated with LPP-NPs had the lowest microbial load during the storage period compared to the control. These results could be correlated with the results of TVB-N as reported in (Fig. 4). This may be attributed to tannins and other phenolics in the LPP-NPs, which have antioxidant and antimicrobial activity (Reddy, Gupta, Jacob, Khan, & Ferreira, 2007). The results are in an agreement with Al-Zoreky (2009).

### 3.8. Sensory evaluation of meatball

The scores of color and odor of meatballs during storage are presented in Table 3. The sensory evaluation was carried out for 9 days for the control sample, 12 days for BHT and 15 days for the LPP-NPs treated samples due to the unpleasant odor that probably indicates spoilage and would lead to consumer rejection (Gram et al., 2002). In general, no difference was recorded at time zero of storage between the treatments ( $P \geq 0.05$ ). The scores of colors decreased for all treatments when the time of storage increased while no significant differences were noticed during the first 9 days between BHT and LPP-NPs added samples. On the other hand, the control sample revealed the lowest score and significantly results at ( $P \leq 0.05$ ). Addition of LPP-NPs in both concentrations did not negatively affect the meatball color. In general, the rancid odor development increased in all treatments as the storage time increased, which was indicative of lipid oxidation of meatballs. The samples were quite stable for 9 days while some differences became apparent between day 6 and day 9, as well as between day 9 and day 15. On a day 9, the color of the control samples was dark red and had no odor while the meatballs with LPP-NPs were slightly dark red or cherry red and had an acceptable odor. The panelists who detected the differences in color and odor in day 15 perceived LPP-NPs in meatballs. Thus, based on sensory and oxidative studies, the shelf-life of meatballs was extended up to 15 days in LPP-NPs treated samples. As well as the differences in rancidity between the control sample and treated samples with antioxidant have similar trends compared with TBARS' values at day 8. LPP-NPs did not show any negative impact on

Table 3

Sensory responses for color and rancid odor of raw meatballs with different treatments stored at  $4 \pm 1$  °C for 15 days (mean  $\pm$  SD).

Attributes	Treatments	Storage days					
		0	3	6	9	12	15
Color	Control	6.50 $\pm$ 0.53 <sup>a</sup>	5.50 $\pm$ 0.85 <sup>b</sup>	4.20 $\pm$ 0.42 <sup>c</sup>	3.30 $\pm$ 0.82 <sup>b</sup>	R*	R
	BHT	6.40 $\pm$ 0.52 <sup>a</sup>	6.10 $\pm$ 0.32 <sup>a</sup>	5.00 $\pm$ 0.47 <sup>ab</sup>	4.10 $\pm$ 0.99 <sup>a</sup>	3.40 $\pm$ 0.97 <sup>b</sup>	R
	1% LPP-NPs	6.30 $\pm$ 0.48 <sup>a</sup>	5.90 $\pm$ 0.32 <sup>a</sup>	5.20 $\pm$ 0.63 <sup>a</sup>	4.20 $\pm$ 1.03 <sup>a</sup>	4.40 $\pm$ 0.52 <sup>a</sup>	3.40 $\pm$ 0.84 <sup>b</sup>
	1.5% LPP-NPs	6.30 $\pm$ 0.48 <sup>a</sup>	6.10 $\pm$ 0.32 <sup>a</sup>	5.90 $\pm$ 0.32 <sup>a</sup>	4.60 $\pm$ 0.97 <sup>a</sup>	5.10 $\pm$ 0.32 <sup>a</sup>	4.60 $\pm$ 0.97 <sup>a</sup>
Odor	Control	7.00 $\pm$ 0.00 <sup>a</sup>	7.00 $\pm$ 0.00 <sup>a</sup>	5.70 $\pm$ 0.48 <sup>b</sup>	1.10 $\pm$ 0.32 <sup>c</sup>	R	R
	BHT	7.00 $\pm$ 0.00 <sup>a</sup>	7.00 $\pm$ 0.00 <sup>a</sup>	7.00 $\pm$ 0.00 <sup>a</sup>	4.60 $\pm$ 0.84 <sup>ab</sup>	3.25 $\pm$ 0.74 <sup>b</sup>	R
	1% LPP-NPs	7.00 $\pm$ 0.00 <sup>a</sup>	7.00 $\pm$ 0.00 <sup>a</sup>	7.00 $\pm$ 0.00 <sup>a</sup>	5.40 $\pm$ 0.70 <sup>a</sup>	4.90 $\pm$ 0.57 <sup>a</sup>	3.40 $\pm$ 0.70 <sup>b</sup>
	1.5% LPP-NPs	7.00 $\pm$ 0.00 <sup>a</sup>	7.00 $\pm$ 0.00 <sup>a</sup>	7.00 $\pm$ 0.00 <sup>a</sup>	6.10 $\pm$ 0.74 <sup>a</sup>	5.90 $\pm$ 0.32 <sup>a</sup>	5.20 $\pm$ 0.63 <sup>a</sup>

abc There is no significant difference between any two means 'in the same column' have the same superscript letter ( $P \geq 0.05$ ).

\*R: reject.

sensory properties of raw or cooked meatballs. The results are in agreement with the data reported by Devatkal et al. (2010) and Turgut et al. (2016).

#### 4. Conclusion

In the present study, phenolic content and antioxidant activity of LPP-NPs (a by-product of pomegranate juice industry) were evaluated. LPP-NPs methanolic extract exhibited a high amount of phenolic and demonstrated effective antioxidant and antimicrobial properties. The retarding capacity of LPP-NPs is obviously promising for lipid and protein oxidation in meatballs. The LPP-NPs improved the WHC and cooking yield of meatball samples during the cold storage period. As well, the LPP-NPs have improved the microbial quality of meatball samples during storage. Samples containing LPP-NPs were more sensory acceptable up to 15 days. As demonstrated in this study, the LPP-NPs could be successfully added to meatball products as a natural antioxidant and antimicrobial additives.

#### Conflicts of interest

The authors declare that there are no conflicts of interest.

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