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ORIGINAL PAPER

Freeze-dried pomegranate extract as a natural and novel ingredient in cemen paste and pastırma quality during refrigerated storage

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Abstract

This work aimed to determine the effect of freeze-dried pomegranate water extract (FDPWE) on the quality and technological properties of cemen paste and pastırma. Firstly, FDPWE was produced and its antioxidant, physical and physicochemical properties were determined. Secondly, cemen paste was produced with different levels of FDPWE (Control, 1.0%, 2.0%, 3.0%, 4.0%, and 5.0%) and its quality characteristics were determined. Afterwards, pastırma production was carried out with cemen paste produced by adding FDPWE (Control, 3.0%, 4.0% and 5.0%) and its effects on quality properties and storage stability (at 4 °C for 150 days) of pastırma were investigated. FDPWE had high total anthocyanin content and a^* values. The use of FDPWE in cemen paste production caused a decrease in pH value, thereby increasing the effectiveness of anthocyanins. It also caused an increase in a^* value ($P < 0.01$), FRAP-reducing activity, metal chelating activity, color and overall acceptability of cemen. Increasing FDPWE content in cemen paste reduced salt, pH and lipid oxidation ($P < 0.01$) of pastırma, while both external and internal surface a^* color ($P < 0.01$) and sensory scores ($P < 0.01$) increased according to the control sample. In addition, the storage stability of the pastırma with FDPWE was maintained better than control samples. The results indicated the potential of FDPWE, as a new clean label additive, containing natural functional components to enhance the quality and stability of pastırma.

Keywords Freeze-dried pomegranate water extract · Cemen paste · Pastırma · Quality · Antioxidant · Protein and lipid oxidation

Introduction

Anthocyanins are water-soluble plant pigments with a biological activity called "color cell fluid". They are responsible for the bright color of the plants. Until now, more than 700 anthocyanins have been determined in plants such as fruits, and vegetables, and they are the glycosylated form of anthocyanidins and the different structures arise from the hydroxylation and methoxylation patterns in ring B. Anthocyanins are products of the phenylpropanoid metabolism from phenylalanine. Anthocyanins have important effects on both food preservation (antioxidant, bacteriostatic etc.) and human health (anticancer, anti-diabetes, etc.). Additionally, the stability and intensity of these compounds are dependent on several factors, such as concentration of the pigments, metal ions, pH, sugars, oxygen, temperature, enzymes and structure. The conducted studies showed that anthocyanins from various sources are in different contents, they have different biological activities and structures [1–4]. In this context, one of the most important fruits that attract attention is the pomegranate. Pomegranate, *Punica granatum* L. (*Punicaceae*), is a fruit rich in anthocyanins and its juice contains various anthocyanins such as cyanidin 3-glucoside (Cy-3-G), cyanidin 3,5-diglucoside (Cy-3-DG), pelergonidin 3-glucoside (Pg-3-G), pelergonidin 3,5-glucoside (Pg-3-DG), delphinidin 3-glucoside (Dp-3-G), and delphinidin 3,5-diglucoside (Dp-3-DG) [5]. The colors of the pomegranate, pomegranate juice, and pomegranate extracts depend on

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the composition and amount of anthocyanins they contain. Pomegranate has also significant antioxidant properties. The group of phenolic substances with the highest antioxidant effect in pomegranate is anthocyanins, hydrolysable tannins and ellagic acid derivatives are also effective [6]. On the other hand, pomegranate is also important in terms of the amount and variety of sugar, mineral substance, protein and amino acid it contains. In addition, it has been reported that pomegranate has more antimicrobial and antifungal effects compared to some other fruits such as banana, grapefruit and quince [7].

Pastırma is a meat product obtained by dry-curing, drying, cemen treatment and re-drying pieces of meat removed from certain parts of bovine carcass [8]. Cemen treatment is one of the most important stages in pastırma production and it is a process performed to give pastırma special quality properties such as aroma, flavor, appearance and color. Main components used in the cemen production fenugreek seed flour, garlic, ground red pepper and water [9, 10]. In this context, the composition of cemen and the quality and properties of the substances used in the cemen production affect the quality of pastırma, especially its color and sensory properties. The color quality of the cemen paste used in pastırma production varies depending on the characteristic/quality of chili and sweet red pepper used. It is not possible to achieve the desired quality of cemen color using these peppers in small amount and/or with low quality. Therefore, synthetic dyes have been added to cemen dough used in pastırma production for years to improve the color properties of pastırma. In addition, it is known that the use of synthetic dyes in pastırma production is have been used for a long time. Therefore, it was recommended that safe and natural colorants should be used in cemen production for improving color properties of pastırma [10]. Moreover, in the Communiqué on colours for use in foodstuff of the Turkish Food Codex Regulation [11], the use of color additives such as riboflavin, riboflavin-5-phosphate (E 101), carmines (E 120) and curcumin (E 100), is permitted for the edible outer coatings of pastırma. However, since the use of synthetic additives in food production increases the health concerns of consumers, the use of natural colorants obtained from foodstuffs instead of synthetic additives in the production of these foodstuffs has begun to be investigated. In this context, it is important to investigate the possibilities of using natural colorants, which are rich in red color pigments (anthocyanins and betalains), in cemen production for the color of pastırma [10, 12–15]. Until now, there has been no study in the literature investigating the effect of using pomegranate water extract in cemen production on the quality of both cemen paste and pastırma produced using this cemen. Therefore, the aims of the present study were: (i) to determine properties of freeze-dried pomegranate water extracts (FDPWE) (total phenolic, total anthocyanin, DPPH radical scavenging activity, FRAP activity, metal chelating activity, pH, acidity and color), (ii) to determine properties of cemen paste (total phenolic, total anthocyanin, DPPH radical scavenging activity, metal chelating activity, FRAP activity, pH, acidity, color and sensory attributes) produced with FDPWE (0.0%, 1.0%, 2.0%, 3.0%, 4.0% and 5.0%), (iii) to determine the effects of cemen paste produced with FDPWE (0.0%, 3.0%, 4.0% and 5.0%) as a new additive on the quality of pastırma (external and internal color, pH, moisture, microbial, lipid and protein oxidation, and sensory) during storage at 4 ± 1 °C for 120 days.

Materials and methods

Materials

In the current research, *Longissimus thoracis et lumborum* muscles, removed from male cattle carcasses (24 h after the slaughter) obtained from the Meat and Dairy Board (Erzurum, Türkiye) were used as meat material in pastırma production. Salt, sodium nitrite, fenugreek seed flour, ground chili red pepper, ground sweet red pepper, and garlic were purchased from local markets in Erzurum, pomegranate (*Punica granatum* L., İzmir-1499 variety) fruit was obtained from the Aegean Agricultural Research Institute, İzmir, Türkiye.

Obtaining freeze-dried pomegranate water extracts (FDPWE) and properties

As summarized in Fig. 1a, the pomegranate seeds (20 g) were homogenized with water (400 mL) using ultra-turrax, then left at room temperature for 15 min and filtered through Whatman No: 1. The 25 mL of the obtained filtrate was placed in glass balloons, frozen at – 38 °C, and lyophilized at – 50 °C. FDPWE powders were stored in light-proof jars at 4 °C until used. Total phenolic and anthocyanin content, FRAP, DPPH radical scavenging and metal chelating activity, pH, acidity and color analyzes were performed on the powders produced.

Cemen production with FDPWE and properties

For the cemen dough production (Fig. 1b), 500 g fenugreek seed flour, 350 g garlic, 75 g ground chili red pepper, 75 g ground sweet red pepper, and 1200 mL water were used [16]. Six different cemen pastes were prepared using this recipe. To one group chosen by chance, FDPWE was not added, and this group was evaluated as the control cemen group. To the other five cemen pastes, FDPWE was added at the levels of 1, 2, 3, 4, and 5% (w/w). Total phenolic and anthocyanin content, FRAP, DPPH radical

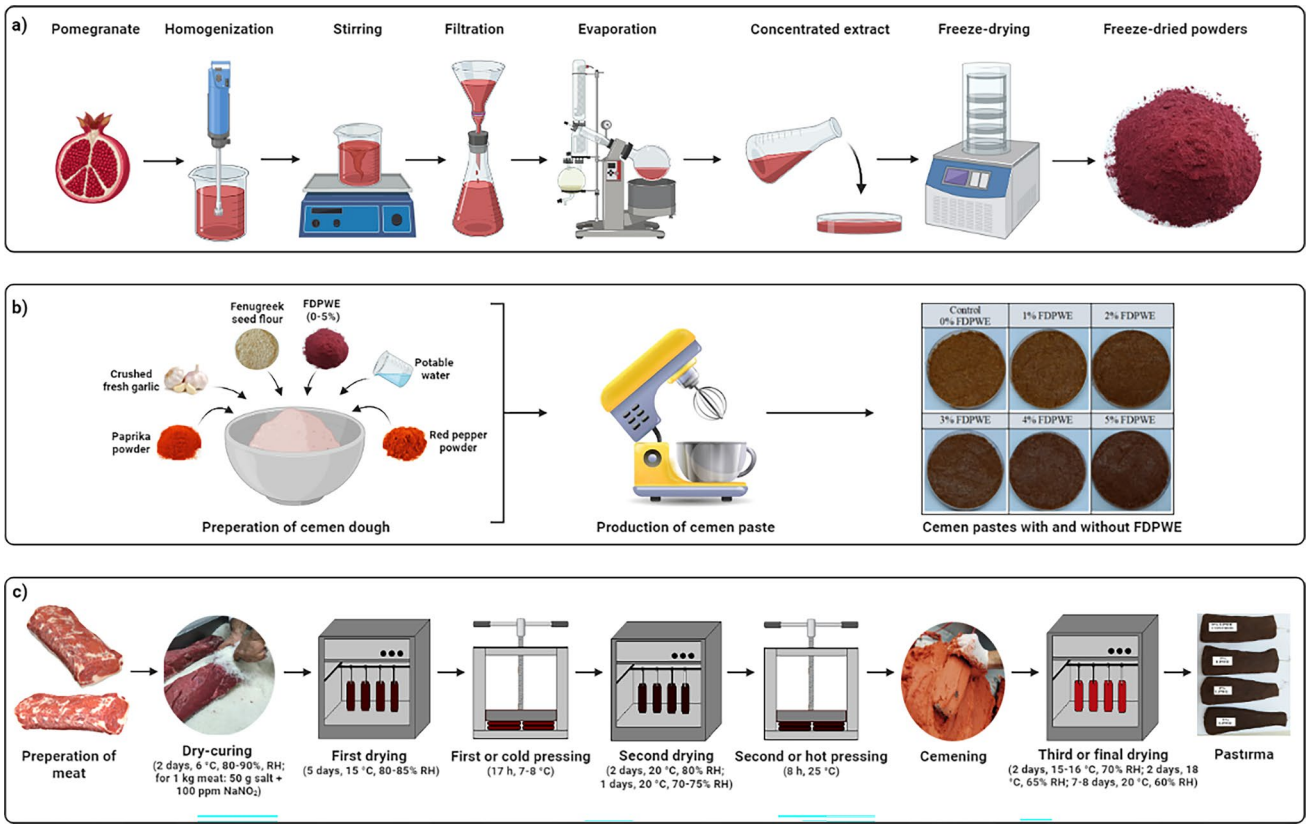

Fig.1 Flow chart indicating the production of freeze-dried pomegranate water extract (FDPWE) (**a**), cemen paste (**b**), and pastırma (**c**) (Created with BioRender.com)

scavenging and metal chelating activity, pH, acidity, color and sensory analyzes were performed on the cemen paste produced. However, as a result of sensory evaluations and color values, pastırma production was carried out using the best three FDPWE levels. The production of each cemen was done in triplicate.

FDPWE and cemen paste analyses

Determination of pH, titratable acidity and color values

The pH values of the cemen and FDPWE samples were measured using a desktop pH-meter (Thermo Scientific, Orion 3-Star, Singapore), and the titratable acidity was determined by following the potentiometric titration method [17]. L^* (lightness), a^* (redness), and b^* (yellowness) color values of cemen and FDPWE samples were measured using a Minolta CR-400 colorimeter (Osaka, Japan) equipped with an 11 mm aperture at 2° standard observer conditions and D_{65} illuminant. A standard ceramic plate was used for instrument calibration.

Determination of total phenolic content

For total phenolic content analysis in FDPWE and cemen, 500 µl of Folin-Ciocalteu reagent, 250 µl of sodium carbonate solution (20%) and sample extract (1000 µl) were mixed in a glass tube. Distilled water was added to equalize the total volume of the mixture to 10 ml. After vortexing, 30 min incubation was carried out in the dark at room temperature. Absorbance readings were taken in a spectrophotometer at 760 nm versus the control. A standard gallic acid curve prepared daily was used to calculate the results in mg gallic acid equivalent (GAE)/kg [18].

Determination of total anthocyanin content

The pH-differential procedure based on pH-dependent color changes of anthocyanins was followed to determine the total anthocyanin content of cemen and FDPWE samples. Briefly, the sample extracts were mixed with 0.025 M potassium-chloride and 0.4 M sodium-acetate solutions whose pH values were adjusted to 1.0 and 4.5, respectively. The absorbances of the mixtures were recorded separately at 520 nm

and 700 nm wavelengths. The results were calculated as mg Cy-3-G/kg by the formula in the method [19].

Determination of antioxidant potential

The antiradical activity of the samples was detected as percent inhibition according to the DPPH procedure [20]. The scavenging of the purple-colored DPPH (2,2'-diphenyl-1-picrylhydrazyl) radical by different concentrations of the sample extracts and the transformation of the solution color from purple to yellow after 30 min of incubation in the dark at room temperature were followed. The absorbance measurement against the control was performed at 517 nm with the aid of a spectrophotometer.

For ferric reducing antioxidant power, sample extracts were reacted with FRAP stock solution containing 1 part $FeCl_3.6H_2O$, 10 part acetate buffer (0.3 M, pH 3.6) and 1 part 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ). The absorbance of the blue color, denoting the conversion of Fe^{3+}-TPTZ to the Fe^{2+}-TPTZ complex, was recorded at 593 nm versus the control. Trolox-equivalent (TE) FRAP activity was calculated as µmol TE/g [21].

Total antioxidant activity was determined according to Yen and Chen [22]. Briefly, FDPWE powder and cemen extracts were mixed with phosphate buffer and linoleic-acid emulsion. Fe^{2+} and SCN^- were added to the mixture at certain intervals to color the mixture. Absorbance values were measured at 500 nm by following certain times of the incubation at 37 °C in the dark. The results were calculated as percent inhibition.

For metal chelating activity, the sample extracts were reacted with 0.2 mM $FeSO_4.7H_2O$ (0.125 ml), 0.5 ml each of Tris–HCl buffer (pH 7.4) and 2,2'-bipyridine solution, and then the final volume was adjusted to 3 ml. Following the incubation of the mixtures for 10 min in the dark at room temperature, absorbance measurements were made at 522 nm. Metal chelating activity results were expressed as % inhibition [23].

Sensorial analysis of cemen paste with FDPWE

Cemen samples were subjected to sensorial evaluation by 12 assessors who are experts in food technology. Selected assessors familiar with pastırma and cemen were given a briefing about the study and were asked to sign the consent form. Six different cemen (Control, 1.0%, 2.0%, 3.0%, 4.0%, and 5.0% FDPWE) were coded with random numbers and presented to assessors on white disposable plates. Assessors were asked to rate the cemen samples for color, consistency, odor, taste, and overall acceptability using a hedonic scale (1–3 = not acceptable; 4–5 = fairly acceptable, 6–7 = good, and 8–9 = very good). The palate left by the samples in the mouth was cleaned by offering bread and water to the assessors.

Pastırma production with cemen paste added FDPWE

Pastırma production was performed according to the method of Aksu et al. [24], as shown in Fig. 1c. A total of three carcasses (individual for each replication) were used, and the same production procedure was followed for all three replications. By paying attention to the cold chain and strict hygiene, the muscles were transferred to the laboratory and subjected to the process of removing the surface connective tissues and fat layers. Four pieces of meat were obtained from a carcass by dividing each muscle with a horizontal cut to ensure minimal discrepancies between treatment groups for the same repetition. In other words, 3 carcasses, 6 muscle and 12 meat pieces were used for the production of pastırma in 3 repetitions consisting of 4 treatments. A dry-curing process was applied at 6 °C. Cemen process was done at 7 °C for 4 days. Both curing and cemen treatments were performed in different cuvettes for each treatment group. The final drying process of pastırma was continued until the moisture content of the pastırma fell below 50%, which is mentioned in the Turkish Food Codex Meat Products Communiqué. In the curing process, 50 g of salt (NaCl) and 100 ppm of nitrite ($NaNO_2$) were used per 1 kg of meat. Although cemen pastes including five different levels of FDPWE were produced, four different pastırma productions were carried out including the three FDPWE levels (3, 4, and 5%) giving the best cemen sensorial analysis and color results and one control group. Each pastırma group was produced as three replications. All pastırma treatments were stored at 4 ± 1 °C for 5 months.

Pastırma analysis

Determination of moisture, pH, residual nitrite and salt amounts

Moisture content was determined based on weight loss by drying 5 g samples in nickel dishes in a drying oven at 103–105 °C until a constant weight was reached. The pH measurements were made with a benchtop pH-meter after homogenization of the mixture of 10 g sample (with cemen layer) and 100 ml distilled water with an Ultra-turrax. Residual nitrite (mg/kg) and salt (%) analyzes were carried out using spectrophotometric and titration methods, respectively [25].

Determination of color values

Color measurements of meat and pastırma samples during production and storage were made with a pre-calibrated Minolta colorimeter (CR-400, Osaka, Japan) with 2°, Ø11 mm, D_{65} specifications for the standard observer, illumination area and illuminant condition, respectively. The averages of at least eight measurements were used for color readings of the outer surface with cemen layer and the inner surfaces of 2–3 mm thick slices.

Determination of lipid oxidation

Thiobarbituric acid reactive substance (TBARS) values were determined by the method based on spectrophotometric monitoring of the intensity of the red color formed by malonaldehyde (MDA) and thiobarbituric acid (TBA) [26, 27]. Briefly, 1 g of meat or pastırma samples were subjected to homogenization and filtration (Whatman No: 1) processes after adding 6 ml trichloroacetic acid (TCA) based mixture (7.5% TCA, 0.1% propyl gallate, 0.1% EDTA). The homogenate was then mixed with an equal volume of TBA (0.02 M) solution and incubated in a boiling water bath. The absorbance of the cooled mixtures was recorded at 532 nm after centrifugation at 2.000 *g* for 5 min. The 1,1,3,3-tetraethoxypropane standard was used to calculate TBARS values as µmol MDA/kg.

Determination of protein oxidation

The derivatization procedure of protein carbonyl groups with 2,4-dinitrophenylhydrazine (DNPH) was followed for protein oxidation expressed by total carbonyl content. The absorbance measurements were performed at 370 nm and bovine serum albumin was used as the standard for calculating the total carbonyl content in nmol/mg [28].

Determination of microbiological counts

After aseptically mixing 25 g of sample and 225 ml of saline solution (NaCl 0.85%) in sterile sample bags, homogenization was done by a stomacher (Seward Medical 400-BA 7021, London, UK). The serial decimal dilutions of homogenates were used for microbiological analyzes performed using the spreading plate technique. Total aerobic mesophilic bacteria (TAMB) were enumerated on Plate Count Agar after 48 h of incubation at 30 °C. For the enumeration of yeast mold, incubation was performed on Potato Dextrose Agar for 5 days at 25 °C. *Enterobacteriaceae* and lactic acid bacteria (LAB) were counted after 48 h of anaerobic incubation at 30 °C on Violet Red Bile Dextrose (VRBD) and de Man Rogosa Sharpe (MRS) Agar, respectively. *Staphylococcus / Micrococcus* counts on Mannitol Salt Phenol Red Agar were performed by enumerating catalase (+) cocci after 48 h of incubation of inoculated plates at 30 °C under aerobic conditions. Microbiological analyzes were performed on days 0, 30, 60, 90, 120 and 150 of the storage period.

Sensorial analysis of pastırma

A panel of 10–12 assessors (30–50-year-old staff and researchers from the Department of Food Engineering, Atatürk University) experienced in food technology evaluated the sensorial properties of pastırma samples. An information session was held to present the details of the study to selected assessors familiar with pastırma and was asked to sign the consent form. Three separate sensorial sessions were organized on different days during the storage period for pastırma samples produced in triplicate. Pastırma samples for each sensorial session were randomly coded with 3-digit numbers and presented to assessors on white disposable plates, along with bread and water for palate neutralization. The assessors rated samples using a hedonic scale (9: extremely liked, 6–7: liked, 4–5: moderately liked, 1–3: disliked) for section surface color, cemen quality, texture and structure, flavor, and overall acceptability. The average of the scores obtained from each sample and session was taken into account.

Statistical analysis

All experiments and all analyzes were performed in triplicate. FDPWE characteristic data were summarized using descriptive statistics. All cemen results were subjected to one-way analysis of variance (ANOVA) by considering the FDPWE levels as a fixed factor. Regarding the pastırma analyses, moisture, color, pH, lipid and protein oxidation analyzes were conducted in a randomized complete block design with a 4×10 factorial arrangement including four FDPWE treatments (Control, 3% FDPWE, 4% FDPWE, and 5% FDPWE), ten production-storage periods (raw material, before the addition of cemen, after the addition of cemen, the surface with cemen, pastırma (0), 30, 60, 90, 120 and 150 days), and three replications. Microbiological and sensorial analyzes, in which the production stages were not included in the model, were designed in a 4×6 factorial arrangement. All data of pastırma samples were analyzed using the General Linear Model procedure, except the residual nitrite and salt content subjected to one-way ANOVA. Treatment, production-storage periods (for moisture, color, pH, lipid and protein oxidation data), storage period (for microbiology and sensorial analysis results), interactions among them, and assessors (for sensorial data) were accepted as fixed effects, while sessions (for sensorial data) and replications were evaluated random effects. The comparison of significant differences

($P < 0.05$) between mean values was performed by Duncan's test. The significance was considered at the 95% confidence level. SPSS 22.0 package program was used for all statistical analyses.

Results and discussion

FDPWE properties

Analysis results of FDPWE are given in Table 1. It is known that the amount of phenolic substances contained in fruits and vegetables affects the antioxidant capacity of that product [29]. As can be seen from Table 1, it was observed that the antioxidant capacity of the FDPWE was high. Researchers found that the anthocyanin (as Cy-3-G) and total phenolic contents of ethanol extract of pomegranate fruit were 13.1 ± 0.8 µg/g dw and 324.41 ± 0.76 mg GAE/g dw, respectively [30]. Salehi et al. [31] stated in their studies that pomegranate extracts have antioxidant capacity, can delay lipid oxidation, and can be considered as an alternative to synthetic antioxidants in the food industry as natural and cheap antioxidant sources. In addition, the average pH, titration acidity and L^*, a^* and b^* color values were found as 3.43 ± 0.01, 5.68 ± 0.10, 32.75 ± 2.04, 43.67 ± 1.71, and 10.48 ± 0.28, respectively. The use of cemen in pastırma production not only contributes to the sensorial properties of pastırma but also contributes to microbiological quality, especially yeast mold. In this context, it is thought that the properties of FDPWE obtained from pomegranate fruit positively affected the quality of cemen and pastırma.

Table 1 Some properties, bioactive compound content and antioxidant capacity of the freeze-dried pomegranate water extract (FDPWE)

Properties	Mean ± SE
Total phenolic content (µg/mg)	29.21 ± 1.21
FRAP activity (µmol/g)	52.23 ± 0.80
DPPH radical scavenging activity (%)	48.65 ± 0.83
Total antioxidant activity (%)	48.00 ± 2.19
Metal chelating activity (%)	82.46 ± 0.25
Total anthocyanin content (mg Cy-3-G/kg)	401.08 ± 84.64
pH	3.43 ± 0.01
Titration acidity (%, citric acid)	5.68 ± 0.10
L^* color	32.75 ± 2.04
a^* color	43.67 ± 1.71
b^* color	10.48 ± 0.28

Values are presented as the mean ± SE of three replicates. Cy-3-G: Cyanidin-3-glucoside

Cemen paste analysis

The average values of pH and titration acidity of the cemen produced without and with FDPWE are given in Table 2. It was determined that the use of FDPWE in the cemen production significantly decreased the pH value and increased the titration acidity of the cemen. In cemen production, as the level of FDPWE increased, the pH value of the cemen decreased. This effect is thought to be due to the low pH value of FDPWE. The use of FDPWE in the cemen production caused a significant decrease in L^* value and a significant increase in a^* value of cemen. On the other hand, there was no change in b^* value in cemen produced by adding FDPWE ($P > 0.05$). It was determined that there is no difference between a^* values of the cemen groups with FDPWE at different levels. It is desirable that FDPWE added to cemen dough increases a^* value of cemen.

The average total phenolic content (µg/mg) and FRAP-reducing activity (µmol/g) values of the cemen produced without and with FDPWE are given in Table 2. The use of FDPWE in the cemen production did not have a significant effect on the total phenolic content of the samples, but it had a significant effect on the FRAP reducing activity, and the use of FDPWE at the levels of 3, 4 and 5% significantly increased the FRAP reducing activity compared to the control group. The average DPPH* free radical scavenging activity (%, inhibition), metal chelating activity (%) and total antioxidant activity (%) values of the cemen produced without and with FDPWE are also given in Table 2. Although the use of FDPWE in the cemen production significantly increased the metal chelating activity, it was determined that it did not have a significant effect on total antioxidant and DPPH* free radical scavenging activity. The average anthocyanin content as Cy-3-G of cemen produced without and with FDPWE is also given in Table 2. Although the use of FDPWE in the cemen production increased anthocyanin content, this increase was statistically insignificant.

The average sensorial analysis results of the cemen produced without and with FDPWE are given in Table 2. The use of FDPWE in the cemen production significantly increased the color value of cemen, and as the level of FDPWE used in the production of cemen increased, the color value of cemen increased. The color liking determined in the sensorial analysis results and the a^* color values given in Table 2 supported each other. On the other hand, the use of FDPWE did not have a significant effect on the consistency, odor and taste values of the cemen. However, the use of FDPWE caused an increase in the overall acceptability value of the cemen samples, and it was determined that the overall acceptability value of the cemen with 4 and 5% FDPWE was significantly higher than that of the control group cemen.

Table 2 The average values of pH, titration acidity (as citric acid), color (L^*, a^* and b^*), total phenolic content, FRAP activity, metal chelating activity, DPPH radical scavenging activity, total antioxidant activity, and total anthocyanin concent of the cemen produced without and with FDPWE

	FDPWE levels						
	Control (0%)	1%	2%	3%	4%	5%	Significant
pH	5.96 ± 0.04 [a]	5.77 ± 0.05[b]	5.61 ± 0.04[c]	5.42 ± 0.04[d]	5.23 ± 0.02[e]	5.13 ± 0.01[e]	**
Titration acidity (%)	0.77 ± 0.02 [d]	0.92 ± 0.01 [cd]	0.97 ± 0.03[bc]	1.02 ± 0.07[abc]	1.15 ± 0.08[ab]	1.21 ± 0.09[a]	**
L^*	47.84 ± 0.58 [a]	46.49 ± 0.87[ab]	43.82 ± 0.91[b]	44.29 ± 1.10[bc]	44.32 ± 0.07[bc]	43.88 ± 0.09[b]	**
a^*	15.50 ± 0.57 [b]	16.94 ± 0.12[a]	16.82 ± 0.20[a]	16.85 ± 0.09[a]	17.21 ± 0.08[a]	17.39 ± 0.13[a]	**
b^*	32.17 ± 1.47	33.51 ± 0.60	31.96 ± 0.75	31.80 ± 1.12	30.20 ± 0.49	29.44 ± 1.02	ns
Total phenolic content (µg/mg)	2.73 ± 0.08	3.01 ± 0.35	2.75 ± 0.54	3.13 ± 0.40	3.29 ± 0.35	3.44 ± 0.27	ns
FRAP activity (µmol/g)	143.50 ± 0.59 [c]	143.89 ± 4.81[c]	134.75 ± 4.22[c]	163.58 ± 3.94[b]	177.32 ± 3.86[a]	186.89 ± 4.48[a]	**
Metal chelating activity (%)	10.74 ± 0.69 [e]	13.35 ± 0.72[d]	14.80 ± 0.34 [cd]	16.13 ± 0.95[bc]	17.69 ± 1.03[b]	20.43 ± 0.70[a]	**
DPPH radical scavenging activity (Inhibition%)	42.26 ± 0.93	44.45 ± 2.61	44.38 ± 0.27	45.17 ± 0.66	48.25 ± 1.44	46.63 ± 2.08	ns
Total antioxidant activity (%)	38.00 ± 3.01	39.33 ± 6.01	44.44 ± 3.74	43.78 ± 2.56	54.89 ± 4.04	46.00 ± 1.39	ns
Total anthocyanin content (mg cyanidin-3-glucoside/kg)	146.89 ± 0.72	170.77 ± 17.29	191.75 ± 10.21	197.31 ± 13.79	195.62 ± 7.59	171.50 ± 15.01	ns
Sensory attributes							
Color	4.89 ± 0.19[d]	5.48 ± 0.11[c]	5.63 ± 0.14[c]	5.94 ± 0.10[bc]	6.36 ± 0.16[ab]	6.76 ± 0.22[a]	**
Consistency	6.76 ± 0.04	6.76 ± 0.13	6.94 ± 0.14	6.94 ± 0.12	7.06 ± 0.12	7.12 ± 0.03	ns
Odor	6.53 ± 0.34	6.49 ± 0.29	6.64 ± 0.11	6.59 ± 0.02	6.36 ± 0.12	6.59 ± 0.05	ns
Taste	5.87 ± 0.17	6.04 ± 0.33	5.89 ± 0.27	6.08 ± 0.30	6.64 ± 0.13	6.71 ± 0.21	ns
Overall acceptability	5.89 ± 0.22[b]	6.11 ± 0.27[b]	6.19 ± 0.20[ab]	6.40 ± 0.21[ab]	6.82 ± 0.11[a]	6.87 ± 0.21[a]	**

FDPWE Freeze-dried pomegranate water extract

[a–e]Any means in the same column with the same letters are not significantly different at $P < 0.05$; ns: not significant ($P > 0.05$); **$P < 0.01$

Pastırma analysis

External surface color

The average external surface L^*, a^* and b^* values of pastırma including cemen without and with FDPWE determined during the production-storage periods are given in Table 3. It was determined that the use of FDPWE in the cemen production did not have a significant effect on the external surface L^* value of pastırma, but it significantly increased a^* and b^* values. One of the important quality indicators in pastırma is a^* value, and in the current study, the highest a^* value was determined in pastırma including cemen produced with 5% FDPWE. The average external surface a^* color value, which was determined as 7.18 in the control group pastırma, increased to 8.51 in pastırma sample with 5.0% FDPWE. It was determined that a^* value of the pastırma samples increased as the amount of FDPWE added to the cemen increased. The fact that this difference was determined during storage is an important finding, and it is remarkable in terms of maintaining the quality characteristics of pastırma. Color values were also significantly ($P < 0.01$) affected by the production and storage time of pastırma, and the values, which were the highest after cemen treatment, decreased during storage.

Internal surface color

The averages internal surface L^*, a^* and b^* values of pastırma including cemen without and with FDPWE determined during the production and storage are also given in Table 3. The results were obtained from the sliced pastırma (2–3 cm). Similar to the external color properties, it was determined that the use of FDPWE in the cemen production did not have a significant effect on the internal surface L^* value of pastırma, but it significantly increased a^* ($P < 0.01$) and b^* ($P < 0.05$) values. The average internal surface a^* value, which was determined as 33.89 in the control group pastırma, increased to 36.40 in the pastırma sample with 5.0% FDPWE.

In various studies, it has been shown that adding extracts with rich phenolic content causes a decrease in the L^* value of meat and food products. Similarly, researchers found that fruits rich in anthocyanin improve the color of meat products and can delay metmyoglobin formation by inhibiting lipid oxidation, thereby preventing discoloration [31, 32]. Unal et al. [33] reported that the addition of pomegranate juice

Table 3 The averages external and internal surface color results of pastırma including cemen without and with FDPWE determined during the production and storage

	External Surface Color			Internal Surface Color		
	*L**	*a**	*b**	*L**	*a**	*b**
FDPWE treatments (FT)						
Control (0%)	32.42	7.18^{c}	9.77^{b}	40.77	33.89^{c}	18.11^{b}
3%	33.15	7.89^{b}	10.50^{a}	39.65	35.09^{b}	18.72^{ab}
4%	33.70	8.06^{b}	10.96^{a}	40.26	35.60^{ab}	19.33^{a}
5%	33.61	8.51^{a}	11.07^{a}	40.18	36.40^{a}	19.63^{a}
SEM	0.382	0.160	0.248	0.413	0.373	0.370
P	ns	**	**	ns	**	*
Production and storage time (PS)						
Raw	39.29^{b}	18.02^{a}	9.11^{c}	40.57^{abc}	20.05^{d}	7.18^{d}
Before cemen	28.09^{f}	3.04^{f}	2.86^{e}	38.55^{d}	32.94^{c}	16.75^{c}
After cemen	28.88^{f}	6.28^{e}	6.90^{d}	41.60^{b}	34.79^{b}	19.67^{b}
External with cemen	41.03^{a}	10.92^{b}	20.99^{a}			
Pastırma (Day 0)	33.24^{cd}	7.32^{c}	11.79^{b}	42.29^{a}	37.58^{a}	21.86^{a}
Day 30	33.87^{c}	7.38^{c}	12.92^{b}	41.60^{ab}	38.31^{a}	21.52^{a}
Day 60	33.56^{c}	7.09^{cd}	12.12^{b}	40.03^{bcd}	38.57^{a}	21.41^{a}
Day 90	31.31^{e}	6.50^{de}	10.15^{c}	39.05^{cd}	38.07^{a}	20.65^{ab}
Day 120	31.29^{e}	6.20^{e}	9.29^{c}	39.20^{cd}	38.40^{a}	20.81^{ab}
Day 150	31.66^{de}	6.37^{de}	9.62^{c}	39.04^{cd}	38.49^{a}	20.69^{ab}
SEM	0.605	0.253	0.392	0.620	0.559	0.554
P	**	**	**	**	**	**
Interaction						
FT x PS	ns	ns	ns	ns	ns	ns

FDPWE Freeze-dried pomegranate water extract

[a–f]Any means in the same column with the same letters are not significantly different at $P<0.05$

ns: not significant ($P>0.05$); *$P<0.05$; **$P<0.01$; SEM: Standard error of the mean

increased the average *L**, *a**, and *b** values of poultry (turkey and chicken) breast meats. Aksu et al. [12] found results similar to the results of our study and stated that pastırma production stages and storage time have very important effects on the external and internal surface *L**, *a**, and *b** values of the final product.

Moisture and pH

The averages of the moisture, pH and TBARS values of the pastırma produced with cemen with and without FDPWE during the storage stages and production are given in Table 4. The use of FDPWE did not have a significant effect on the moisture content of pastırma ($P>0.05$), but the production and storage time had a very significant effect on the moisture content ($P<0.01$). During storage, the moisture content of the pastırma samples decreased, and the moisture content, which was determined as 42.81% in pastırma (before storage), decreased to 38.68% at the end of storage. In various studies, it has been reported that during the production and storage of pastırma, there is a decrease in moisture value [12, 13].

The use of cemen containing FDPWE in the production of pastırma caused a significant decrease in pH value of the pastırma samples. It is thought that the low pH value of FDPWE has contributed to this result. However, no difference was found between pH values of the pastırma samples produced with cemen including FDPWE at different levels. On the other hand, an increase in the pH value of the pastırma samples was detected during storage. Unal et al. [33] determined that the pomegranate juice has a significant effect on the pH values of chicken and turkey meat samples, and the pH values of the meat samples marinated with the pomegranate juice generally decreased significantly compared to controls. The nitrogenous compounds in the samples are decomposed by the activity of proteolytic enzymes during storage and cause an increase in pH [31, 34].

Lipid oxidation (TBARS)

The use of cemen containing FDPWE in the production of pastırma caused a significant decrease in the TBARS value of the pastırma samples. It is thought that the antioxidant capacity of the FDPWE has contributed to this result. However, no difference was found between the TBARS

Table 4 The averages moisture, pH, TBARS and protein oxidation results of pastırma including cemen without and with FDPWE determined during the production and storage

	Moisture (%)	pH	TBARS (μmol MDA/kg)	Protein oxidation (nm carbonyl/mg protein)
FDPWE treatments (FT)				
Control (0%)	45.88	5.63^{a}	25.09^{a}	3.74
3%	46.10	5.58^{b}	20.85^{b}	3.33
4%	46.18	5.57^{b}	20.44^{b}	3.55
5%	46.53	5.55^{b}	18.34^{b}	3.42
SEM	0.308	0.014	0.882	0.197
P	ns	**	**	ns
Production and storage time (PS)				
Raw	75.23^{a}	5.50^{e}	2.15^{f}	2.40^{b}
Before cemen	44.11^{c}	5.65^{a}	8.32^{e}	2.52^{b}
After cemen	53.90^{b}	5.54^{cde}	18.62^{d}	3.25^{bc}
Pastırma (Day 0)	42.81^{c}	5.57^{bcd}	29.61^{a}	3.74^{ab}
Day 30	41.32^{d}	5.53^{de}	29.30^{ab}	4.11^{ab}
Day 60	40.99^{d}	5.58^{abcd}	27.65^{ab}	3.87^{ab}
Day 90	39.48^{e}	5.60^{abc}	26.48^{abc}	3.53^{ab}
Day 120	39.01^{e}	5.63^{ab}	23.18^{c}	3.79^{ab}
Day 150	38.68^{e}	5.64^{a}	25.32^{bc}	4.39^{a}
SEM	0.462	0.020	1.323	0.295
P	**	**	**	**
Interaction				
FT x PS	ns	ns	ns	ns

FDPWE Freeze-dried pomegranate water extract

$^{a–f}$Any means in the same column with the same letters are not significantly different at $P < 0.05$

ns: not significant ($P > 0.05$); **$P < 0.01$; *SEM* Standard error of the mean

values of the pastırma samples produced with cemen including FDPWE at different levels. Some researchers [31, 33] reported that the reduction in the amount of TBARS was significantly associated with the high antioxidant activity and total phenolic contents of pomegranate. In the present study, there was generally a marked increase in TBARS values as the production and storage times increased. Aksu et al. [15] determined the average TBARS value as 2.65 μmol malonaldehyde/kg in beef meats used in the pastırma production and 18.17 μmol malonaldehyde/kg in the final product pastırma. The researchers found no significant changes in the TBARS values of the samples during the storage stages (30, 60, 90, 120, and 150 days). We reported similar results in our other studies [9, 12]. Also found that the average TBARS value of 3.20 μmol malonaldehyde/kg in beef muscles increased to 31.62 μmol malonaldehyde/kg in pastırma.

Protein oxidation

The amount of protein oxidation, another major cause of meat quality deterioration in addition to lipid oxidation, was determined by measuring the formation of carbonyls. The averages of protein oxidation values of pastırma including cemen without and with FDPWE during the production and storage stages are also given in Table 4. The use of FDPWE had no effect ($P > 0.05$) on protein oxidation values, while production and storage time had a very significant effect ($P < 0.01$). In this study, the inability to detect the effect of FDPWE on the formation of protein carbonyls can be attributed to the fact that lipid oxidation occurs faster than protein oxidation and the possible covalent binding of phenolic compounds to proteins, which will mask their antioxidant activity on proteins [32, 35]. Protein oxidation value showed an increasing trend during pastırma production, but there was no significant change in the value from the pastırma stage during storage ($P > 0.05$). Aksu et al. [13] also found similar results with us, and they determined that the protein

oxidation values varied between 2.00 and 4.56 nm carbonyl/mg protein on the analysis days between the raw material and 150 days of storage. On the other hand, Aksu et al. [15] found that protein oxidation values of pastırma were significantly affected by the production-storage periods, partially low values during the production stages increased until the second month of storage and relatively decreased in the following months. Aksu et al. [12] determined that the protein oxidation values, which were 1.93 nm carbonyl/mg protein in the raw materials, increased to 2.74 nm carbonyl/mg protein in the pastırma, and changed between 3.33 and 4.02 nm carbonyl/mg protein in the 150 days of storage.

Residual nitrit (RN) and salt

The amount of RN was found to be lower in the control group pastırma than the pastırma samples including cemen with FDPWE. However, the use of the FDPWE did not have a significant effect on the RN content of pastırma ($P > 0.05$). The average RN amounts for control, 3%, 4%, and 5% FDPWE were 0.79 ± 0.22, 3.16 ± 2.60, 1.27 ± 0.26, and 1.96 ± 0.70, respectively. On the other hand, the amount of salt in pastırma was affected by adding the FDPWE ($P < 0.05$). The highest % salt content was in the control (5.64 ± 0.17), and decreased as a result of treatment with 3%, 4%, and 5% FDPWE to 5.01 ± 0.09, 4.83 ± 0.15, and 5.02 ± 0.1, respectively. However, there was no difference ($P > 0.05$) in salt content between the pastırma groups with FDPWE. It was determined that the residual nitrite amount in the raw materials (nitrite-free) of pastırma cured with nitrite at different rates was 0.28 and the nitrite amount decreased during the production [36]. Researchers produced pastırma using 150 pmm nitrite and potassium lactate (0%, 0.5%, 1.0%, 2.0%) in curing, and they stated that the RN amount in the lactate-free pastırma is 9.51 ± 0.80 ppm [34].

Microbial counts

The averages of the total aerobic mesophilic bacteria, *Micrococcus/Staphylococcus*, lactic acid bacteria, yeast-mold counts of pastırma including cemen without and with FDPWE during the storage stages are given in Table 5. The use of FDPWE had no effect ($P > 0.05$) on the counts of total aerobic mesophilic bacteria, lactic acid bacteria, *Micrococcus/Staphylococcus*, and yeast-mold. On the other hand, the storage time had a significant effect ($P < 0.01$) only on the yeast-mold count. Yeast-mold count decreased after 30 days of storage, but only the yeast-mold counts of pastırma samples stored for 90th, 120th and 150th days were different from those of the pastırma and pastırma samples stored for 30th days in terms of statistical evaluation. The count of *Enterobacteriaeceae* during storage was < 2.00 log cfu/g (limit of detectable) in all pastırma samples including cemen without and with FDPWE. Tanveer et al. [37] reported that studies have shown that various pomegranate extracts have a great inhibitory effect against a wide variety of spoilage and pathogenic microorganisms, including viruses, fungi, bacteria and protozoans. Researchers have expressed that various phytochemical compounds are considered potent agents responsible for the antimicrobial activity of pomegranate extracts and that these compounds include polyphenols, including hydrolyzable tannins, anthocyanins, and other polyphenols.

Table 5 The average values (log cfu/g) of the total aerobic mesophilic bacteria, *Micrococcus/Staphylococcus*, lactic acid bacteria, yeast-mold counts of pastırma including cemen without and with FDPWE during the production and storage stages

	Total aerobic mesophilic bacteria	*Micrococcus/ Staphylococcus*	Lactic acid bacteria	Yeast-mold
FDPWE treatments (FT)				
Control (0%)	6.00	5.47	3.21	3.74
3%	6.15	5.64	3.44	3.69
4%	6.13	5.79	3.43	3.72
5%	5.92	5.61	3.01	3.80
SEM	0.109	0.137	0.207	0.232
P	ns	ns	ns	ns
Storage time (ST)				
Pastırma (Day 0)	6.21	5.78	3.36	4.43^{a}
Day 30	6.24	5.43	3.48	4.47^{a}
Day 60	5.87	5.52	2.95	3.77^{ab}
Day 90	6.01	5.69	2.97	3.29^{b}
Day 120	6.07	5.76	3.41	3.27^{b}
Day 150	5.90	5.60	3.48	3.18^{b}
SEM	0.134	0.168	0.254	0.285
P	ns	ns	ns	**
Interaction				
FT x ST	ns	ns	ns	ns

FDPWE Freeze-dried pomegranate water extract

[a–f]Any means in the same column with the same letters are not significantly different at $P < 0.05$

ns: not significant ($P > 0.05$); **$P < 0.01$; SEM: Standard error of the mean

Sensorial analysis of pastırma

The averages of sensorial evaluation scores of pastırma including cemen without and with FDPWE during the storage stages are given in Table 6. The scores of the analyzed parameters increased depending on the FDPWE level added to the cemen of the pastırma ($P < 0.05$), and the highest scores were generally found in the pastırma samples

Table 6 The averages of sensory evaluation scores of pastırma including cemen without and with FDPWE during the storage stages

	Section surface color	Cemen quality	Flavor	Texture and structure	Overall acceptability
FDPWE treatments (FT)					
Control (0%)	6.34^{c}	6.86^{b}	6.80^{c}	6.75^{c}	6.76^{d}
3%	6.85^{b}	7.02^{b}	7.04^{b}	7.09^{b}	7.12^{c}
4%	7.07^{b}	7.29^{a}	7.29^{a}	7.26^{ab}	7.34^{b}
5%	7.39^{a}	7.45^{a}	7.46^{a}	7.47^{a}	7.54^{a}
SEM	0.080	0.081	0.070	0.087	0.066
P	**	**	**	**	**
Storage time (ST)					
Pastırma (Day 0)	6.68^{c}	7.46^{a}	7.42^{a}	7.51^{a}	7.36^{a}
Day 30	6.85^{bc}	6.83^{c}	7.09^{bc}	7.00^{bc}	7.02^{b}
Day 60	6.80^{c}	7.00^{bc}	6.93^{b}	6.90^{c}	7.02^{b}
Day 90	6.80^{c}	7.18^{ab}	7.35^{a}	7.20^{abc}	7.34^{a}
Day 120	7.11^{ab}	7.23^{ab}	6.85^{b}	6.97^{bc}	7.02^{b}
Day 150	7.26^{a}	7.22^{ab}	7.26^{ab}	7.27^{ab}	7.38^{a}
SEM	0.098	0.099	0.085	0.107	0.081
P	**	**	**	**	**
Interaction					
FT x ST	ns	ns	ns	ns	ns

FDPWE Freeze-dried pomegranate water extract

[a–c]Any means in the same column with the same letters are not significantly different at $P < 0.05$

ns not significant ($P > 0.05$); **$P < 0.01$; *SEM* Standard error of the mean

including cemen with 4.0% and 5.0% FDPWE. Section surface color increased during storage ($P < 0.05$). Although other parameters were found to be statistically significant, they had scores close to the initial score at the end of storage. Therefore, the addition of FDPWE at different rates to the cemen paste caused an increase in the sensorial properties of pastırma and preserved its quality during storage. Salehi et al. [31] investigated the effect of water and ethanol extracts of pomegranate fruit on the sensory quality of trout fillets and observed that the sensory quality was the highest in the water extracts of pomegranate. In a study conducted on the appearance, texture, flavor, and general acceptance of chicken and turkey breast samples marinated with mulberry, grape, and pomegranate juices, the appearance results showed that meat samples containing juices had higher scores than non-marinated samples. Other sensory scores had close values between marinated and unmarinated samples [33].

Conclusion

The anthocyanin content and high instrumental a^* value (around 43) of the freeze-dried pomegranate water extracts (FDPWE) indicated its significant potential for coloring foods. Depending on the level of FDPWE, the redness of the cemen increased, but this increase was about 2 units. The color quality properties of pastırma produced by adding 3.0%, 4.0% and 5.0% FDPWE to cemen partially improved. Compared to the control group, a^* value increased by 1 unit on the external surface and 3 units on the section surface. In addition, the use of FDPWE decreased lipid oxidation in the production and storage of pastırma. As a result, it was determined that the quality of cemen and pastırma can be increased by using especially 4.0% or 5.0% FDPWE. Therefore, it can be suggested to use FDPWE in the production of cemen paste and pastırma as a natural and novel ingredient.

Author contributions MIA: Conceptualization, data curation, methodology, investigation, funding acquisition, project administration, writing—review & editing; ET: Resources, visualization, methodology, formal analysis; EE: Formal analysis, methodology, review & editing; FO: Methodology, writing—orijinal draft and editing.

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Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors declare no conflict of interest.

Compliance with ethics requirements The study does not involve research on human participants and/or animals.

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