Evaluation of Physicochemical Properties, Phytochemical Composition, Antioxidant Activity, Mineral Content, and Sensory Characteristics of Crackers Fortified with Olive Pomace

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Olive pomace (OP), a by-product of olive oil production, is a rich source of various bioactive compounds. OP can be used as a value-added product for the development of functional food. The research aimed to produce functional crackers incorporating different levels of OP powder (ranging from 0% to 20%). Subsequent analysis involved evaluating the physicochemical and phytochemical compositions, antioxidant activity, mineral content, and sensory characteristics of all samples. In light of the findings, the samples containing OP powder are rich in phytochemicals (total phenolic and flavonoid contents), with higher antioxidant activities (total antioxidant content, DPPH radical scavenging activity, ABTS radical scavenging activity, and ferric reducing power), as well as a high amount of minerals compared to the control sample ($P<0.05$). Moreover, as the concentration of OP increased, there was a decline in the moisture content, water activity, and color values of the crackers. Conversely, the ash, fat, total flavonoid, total phenolic, and mineral contents, as well as antioxidant activity and hardness values, exhibited an increase. Sensory analyses revealed that the addition of 10% OP powder produced highly acceptable crackers compared to the higher concentration of OP (15 and 20%). Overall results indicated that functional crackers can be produced by the addition of powder and a maximum of 10% OP powder fortification is recommended for consumer acceptability.

Keywords: Olive pomace; cracker; phytochemical; antioxidant activity; mineral composition; sensory properties

1. INTRODUCTION

Olive pomace (solid waste from olive oil extraction) and olive mill wastewater (aqueous discharge of the process) are by-products of the olive oil industry. The amount of OP produced varies depending on the method used to extract olive oil, but the average is 35-40 kg OP /100 kg of processed olives [1,2]. OP, which contains fragments of skin, pulp, kernel pieces, and oil, is one of the main by-products of the olive oil industry [3]. OP contains valuable components from olive oil. More than 98% of olive oil is made up of acylglycerols, and triacylglycerols (TAGs) make up the majority of these acylglycerols. TAG composition is a crucial factor in the quality of fats and oils since it can influence both their physiological and nutritional features, such as sensitivity to lipase-hydrolysis and melting point and crystallization [4]. In addition, olive oil contains sterols [5]; fatty alcohols and waxes [6]; phenols [7]; alkanes and alkenes [8]; carotenoids, and chlorophylls [9] in its structure. OP is rich in polysaccharides (cellulose) and lignin, fatty acids, minerals (potassium, calcium, magnesium, etc.), polyalcohols, pigments, polyphenols, and a quantity of proteins that can be considered a promising source of value-added products or antioxidants [3,10-12]. Natural antioxidants have health advantages because they can stop biological

deterioration from causing damage [13]. In OP obtained from the areas of Perdifumo and Acquamela di Casalvelino (Salerno, Italy), the predominant phenolic compounds have been identified as tyrosol, caffeic acid, and gallic acid. [14]. Researchers reported that the environmental, agronomic, and technological properties of virgin olive oil production have a significant impact on the concentration of these chemicals [5, 9, 14]. Olive tree irrigation can change the phenolic composition of virgin olive oil depending on the cultivar, ripening stage, and geographic origin of the olive. In other studies, it was reported that the origin of the fruit, olive tree irrigation, geographic origin of the olive, cultivation, harvest date, extraction method, ripening stage, climate, and rainfall all have an impact on biosynthesis within the fruit, and, consequently, the composition and quality of the oil [4, 8, 9, 15].
Researchers stated that the phenolic extracts and isolated polyphenols from olive processing by-products such as pomace can also be used as a natural antioxidant and antimicrobial in food products [12, 16]. For example, the biological characteristics of different olive powder extracts were produced using conventional heating and ohmic heating-assisted extraction and they reported using a green technique such as ohmic heating to recover the bioactive chemicals found in olive powder [12]. Sinrod et al. [16] reported that the use of drum drying at low speed resulted in the lowest reduction in the antioxidant activity of extract when compared to other drying methods (freeze, hot air, and drum drying), even though all drying methods decreased the antioxidant activity relative to extract derived from fresh pomace.
OP is generally used as an animal feed additive, fertilizer, soil strengthener, alternative fuel, etc. [17-19]. In addition, studies related to the production of lipase enzyme-activated carbon and biodiesel from OP are available [20, 21]. Moreover, there are studies in which pomace is used as a food additive. It was stated that pomace is used to increase the phenolic and fiber content, as well as antioxidant activity, in cereal products such as biscuits, pasta, bread, and breadsticks [22-25]. There are numerous investigations on phenolic compound recovery using various technologies [26, 27].
Pomace drying is an important process in terms of waste evaluation. Drying increases the durability of the pomace and makes it easier to process. Furthermore, in order to obtain more oil from the pomace, it is necessary to dry the pomace. For the mentioned reasons, the literature includes several studies on the drying of 2-phase or 3-phase OPs [28-31].
Fruits, vegetables, or by-products are used to develop functional products with higher added value than the cereal products that are consumed extensively all over the world [22-25; 32-35]. It is important for the evaluation of OP, which has a low cost and can be obtained in high quantity as a by-product during olive oil production, while pomace oil or powder can be used as a functional food ingredient in food formulations. Crackers, one of the snack foods consumed by consumers of all age groups [36, 37], were preferred in this study.
The objective of this investigation was to create crackers by incorporating varying concentrations (0-20%) of OP powder. The study aimed to assess how the incorporation of OP powder influences the physical, chemical, and phytochemical characteristics, antioxidant activity, and mineral content of crackers. Additionally, the research examined the effect of substituting OP powder on the sensory properties and overall acceptance of the crackers by consumers.

2. MATERIALS AND METHODS

2.1. MATERIALS

The wheat flour (Söke Milling Industry and Trade Inc.), yeast (Dr. Oerker Food Ind. and Commerce Inc.), sunflower oil (Kucukbay Oil Inc.), and salt (Billur Boiled Salt Trade Inc.) were obtained from a local market in Alanya, Antalya, Turkey.
The OP was obtained during the production of olive oil (dual-phase decanter centrifugation system) from different olive varieties harvested in the 2021/2022 crop season from the Bornova Olive Research Institute garden (38° 27´ 45.9936” and 27° 22´ 11.0964”). The location, which belongs to the governorate of İzmir, is located in the west of Turkey. Its climate is characterized by hot, dry summers and mild, rainy winters. The average annual temperature and precipitation of the location (Bornova) are 16.0°C and 742 mm, respectively.
The fresh OP was dried at 55°C in an oven (Memmert UN110, Schwabach, Germany) for 24 hours. The dried pomace was ground using a grinder (Premier, PRG259, Turkey) and subsequently subjected to a sieving process (<1000 μm) to remove olive pit fragments. To ensure the effectiveness of this procedure, sieving was performed three consecutive times, and the retained fractions were visually examined to verify the complete elimination of residual stone particles. Only the fine powder passing through the sieve was collected and used in subsequent analyses. This multiple-step approach has been reported as an effective strategy for reducing the presence of stone residues in two-phase olive pomace (De Leonardis et al., 2022). In addition, before incorporation into the formulations, the sieved pomace powder was carefully inspected to guarantee homogeneity and the absence of hard fragments, thus ensuring its safety and suitability for food applications. The dried samples were stored at 4°C for further analysis.

2.2. METHODS

2.2.1 Cracker production

Crackers were prepared in the bakery kitchen at Alanya University. The following procedure was adopted after several preliminary trials (in 100 g): wheat flour (62.15 g/100 g), salt (1 g/100 g), OP powder (0 (Control), 5, 10, 15, and 20% substitution for wheat flour), and yeast (0.55 g/100 g) were weighed, placed in a bowl, and mixed. Sunflower oil (6 g/100 g) and drinking water (30.3 g/100 g) were then added to it and kneaded with a mixer (Electrolux Mixer 5 lt, Sweden) for 10 min to obtain a homogeneous mixture. The dough obtained was placed in a holding container, covered, and fermented at 30±2°C for 15 min. At the end of the fermentation period, the dough was rolled out in 1.5 cm thickness using a rolling pin, cut into 1x1 cm pieces, and placed on a baking tray lined with cooking paper at intervals. It was then baked in a preheated oven at 180±2°C (Air-o-convect bakery, Electrolux, Sweden) for 10 min. The baked products were removed from the oven and crackers were obtained.

2.2.2 Physicochemical composition

The moisture, ash, and fat contents, as well as pH analyses, were performed according to American Association of Cereal Chemists methods [39]. The water activity value of the cracker samples was determined using a water activity measuring device (LabTouch, Novasina, Switzerland) with an accuracy of ±0.001. In addition, the moisture content and water activity of wheat flour and OP powder were determined.

2.2.3 Extraction Method and Total Flavonoid, Phenolic, and Antioxidant Content Analysis

Five-gram samples and 25 ml methanol were mixed for 24 h in a mechanical shaker (IKA, Germany). The mixture was filtered and used for flavonoid, phenol, and antioxidant analyses. Antioxidant activity was measured using three different methods: DPPH assay, ABTS assay, and FRAP assay. All analyses were made in triplicate.

Total flavonoid content

Using the aluminum chloride method, the total flavonoid content of crackers was determined according to Kasangana et al. (2015)'s method, where catechin was used as the standard [40]. Sample (500 µl), methanol: water (70:30 by volume) solution (3200 µl), 0.5 M $NaNO_2$ (150 µl), and 0.3 M $AlCl_3$ (150 µl) were mixed in a test tube. NaOH (1000 µl 1 M) was added after 5 min incubation. The same procedure was applied for standard catechin solutions (dissolved in ethanol; 25, 50, 100, 200, and 400 mg/kg) and blank samples (distilled water, 39). A UV-vis spectrophotometer (Shimadzu Spectrophotometer UV-1700 PharmaSpec, Japan) was used to measure the absorbance of crackers at 506 nm. The total flavonoid contents of samples were calculated using the calibration curve ($y = 0.0005x + 0.0098$ ($R2 = 0.990$)). The obtained results are expressed in mg CE/100 g.

Total phenolic content

The total phenolic content of the crackers was measured according to the Folin-Ciocalteu method studied by Kasangana et al. [40]. The sample (300 µl), distilled water (3.4 ml), methanol (500 µl), and Folin-Ciocalteu's reactive (200 µl) were mixed in a glass tube. Na_2CO_3 (600 µl, 10% weight/volume) solution was added to the mixture after 10 min incubation at room temperature. The absorbance of samples was measured at 760 nm using a UV-vis spectrophotometer (Shimadzu Spectrophotometer UV-1700 PharmaSpec, Japan) after 120 min incubation. The same procedure was applied for standard gallic acid (GA) solutions (20, 40, 60, 80, 120, and 160 mg/kg) and blank samples (distilled water, 39). The total phenolic content of the crackers (mg GAE/100 g) was calculated with the calibration curve ($y = 0.0063x + 0.00002$ ($R2 = 0.999$)). The results were presented as mg GAE/ 100 g.

Total antioxidant content

The total antioxidant analysis was performed according to Prieto et al. [41]. Sample (500 µl), distilled water (2.5 ml), and molybdate reactive (1 ml, 28 mM $NaH_2PO_4.H_2O$, 4mM $H_{24}Mo_7N_6O_{24}.4H_2O$) were mixed in the screw-capped tubes. The samples were placed into the water bath (Memmert GmbH + Co. KG, Germany, 95°C for 90 min) for incubation. The incubation tubes were then cooled at 20-22°C. The same procedure was applied for standard ascorbic acid (AA) solutions (25, 50, 100, 150, 200, 400, and 800 mg/kg) and blank samples (distilled water). The absorbance of the mixtures was measured at 695 nm using a UV-vis spectrophotometer (Shimadzu Spectrophotometer UV-1700 PharmaSpec, Japan, *40*). The total antioxidant content of the samples (mg AAE / 100g) was calculated using the calibration curve ($y = 0.0035 x -0.075$ ($R2 = 0.995$)).

DPPH assay

The antioxidant activity was assessed using the DPPH assay following the method of Ahmed et al. [42]. The stock solution of DPPH (2,2-diphenyl-1-picrylhydrazyl) (10 mM) was prepared by dissolving in methanol and kept in a refrigerator until further use. The DPPH solution was diluted in methanol until the absorbance of the solution reached 0.980±0.02 at 517 nm using a UV-vis spectrophotometer (Shimadzu Spectrophotometer UV-1700 PharmaSpec, Japan). 100 µl sample and 3 ml DPPH were mixed in a test tube. The same procedure was applied for standard ascorbic acid solution (dissolved in methanol) (20, 50, 100, 150, 200, and 400 mg/kg) and blank samples (methanol). The absorbance of samples was measured at 517 nm using a UV-vis spectrophotometer. Measurements were carried out in triplicate and the results are given in mg AAE / 100 g.

ABTS Assay

Antioxidant activity was determined using the ABTS assay as described by Ahmed et al. [42]. The ABTS solution (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)) was prepared by mixing 9.5 mL ABTS (7 mM) with 245 µL potassium persulfate (100 mM). The mixture was kept in the dark at room temperature for 18 h. The resulting solution was diluted with potassium phosphate buffer (0.1 M, pH 7.4) until its absorbance reached 0.706±0.001 at 734 nm, measured with a UV-vis spectrophotometer (Shimadzu UV-1700 PharmaSpec, Japan). A volume of 150 µL of sample was mixed with 2850 µL of ABTS solution in a test tube. The same procedure was applied to ascorbic acid standards (dissolved in methanol; 2, 5, 10, 15, 20, 50, and 100 mg/kg) and ethanol blanks. Measurements were conducted in triplicate, and the results were expressed as mg AAE/100 g.

FRAP Assay

The ferric reducing antioxidant power (FRAP) of the samples was measured according to the method described by Benzie and Strain [43]. One milliliter of sample extract was mixed with 1 mL of FRAP reagent, which was prepared by combining 30 mM acetate buffer (pH 3.6), 10 mM TPTZ (in 40 mM HCl), and 20 mM $FeCl_3 \cdot 6H_2O$. A total of 250 µL of sample was mixed with 2750 µL of the FRAP reagent. Standard solutions ($FeSO_4 \cdot 7H_2O$; 5, 10, 25, 50, 75, and 100 mg/kg) and distilled water blanks were used for calibration. Absorbance was recorded at 593 nm using a UV-vis spectrophotometer (Shimadzu UV-1700 PharmaSpec, Japan). FRAP values were expressed as mg $FeSO_4$/100 g, calculated using the standard curve equation (y = 0.0127 x -0.0716, R^2 = 0.993).

2.2.4 Mineral composition

The mineral content (Fe, Ca, Zn, Mg, Cu, Al, Mn, and K) of the cracker samples was determined by inductively coupled plasma atomic emission spectrometry (ICP-AES, Varian Vista, Australia), following a modified method by Bubert and Hagenah [44]. Cracker samples (0.5 g) were placed in Teflon perfluoroalkoxy vessels and digested in a microwave oven (Berghoff Instruments, Germany) with 5 mL HNO_3 and 1 mL H_2SO_4, sequentially heated at 180°C, 200°C, and 220°C for 5 minutes each. After digestion, the solution was diluted with distilled water to 50 mL and then topped off to final volume with 5% HCl. Measurements were done in triplicate and results were expressed as mg/kg.

2.2.5 Hardness and color measurements

The hardness of the crackers was determined using a Texture Analyzer (Brookfield CT3, USA) equipped with a TA7 probe, with a trigger load of 5 g and a test speed of 3.0 mm/s. Pre- and post-test speeds were set to 10.0 mm/s. Surface color values (*L**, *a**, *b**) were measured using a Konica Minolta CR-400 Chroma Meter (Konica Minolta Sensing Inc., Japan). The total color change (ΔE) was calculated using the following equation:

$$\Delta E = \sqrt{\left(L^* - L_0\right)^2 + \left[\left(a^* - a_0\right)^2 + \left(b^* - b_0\right)\right]^2} \quad (2)$$

where L_0, a_0, and b_0 represent the color parameters of the control sample.

2.2.6 Sensory properties

Sensory evaluation was conducted by a panel of 20 trained individuals (12 females and 8 males; 3 smokers and 17 non-smokers) aged between 20-35 years from Alanya Hamdullah Emin Paşa University, Alanya, Antalya, Turkey. The panelists were trained to assess six sensory attributes (color, appearance, flavor, taste, texture, and overall acceptability) using a 5-point hedonic scale (1 = strongly dislike, 5 = strongly like).

2.2.7 Statistical analysis

Cracker production was carried out in duplicate and all analyses were performed in triplicate. Statistical analyses were conducted using SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). Differences among samples were evaluated by one-way ANOVA and Duncan's multiple range test at P<0.05. The effect of oil pomace on phytochemical content and antioxidant activity was modelled using linear (y = ax + b), power ($y = ax^b$), logarithmic (y = a ln(x) + b), and quadratic ($y = ax^2 + bx + c$) regression equations in Microsoft Excel 2019. Goodness of fit was assessed using the coefficient of determination (R^2).

3. RESULTS AND DISCUSSION

3.1. CHEMICAL COMPOSITION, WATER ACTIVITY AND PH VALUES OF CRACKERS

The moisture content and water activity of the wheat flour and dried olive pomace powder were found as 11.76±0.16 % (wet basis, wb) and 0.322±0.010 and 2.45±0.21% (wb) and 0.233±0.010, respectively. It was stated that the moisture content of pomace samples dried with a fluidized bed, drum, combined (microwave+convectional oven), roller, and tray dryers varied between 4.6-8% [45-49]. It is thought that the reason why the moisture content value of the OP in our study is lower than in other studies may be due to many different factors such as geographical area, environmental conditions, the origin of the olive, cultivar, harvest date, stage of ripening, climate, method of extraction, and drying conditions [4,5,8,9].

The moisture, ash, and fat contents, water activity, and pH values of crackers are given in Table I. Compared to the control, the moisture content and water activity values of the OP powder-added crackers were found to be low (P<0.05). This may be due to the lower moisture content of OP (2.45%) compared to wheat flour (11.76%). Lekjing and Venkatachalam [50] reported that the ingredients significantly affect the final moisture content of the crackers. The moisture content (2.59-3.06%) and water activity (0.185-0.192) values of breadsticks with 5 and 10% red grape pomace reported by Rainero et al. [33] were compatible with our results. The moisture content of crackers with OP was found to be lower than green gram flour crackers (5.76%) [50]. This may be due to different cracker formulations and production processes. The addition of the OP to the cracker formulation resulted in a significant decrease in the moisture content value of the samples up to a 10% OP powder substitution level (P<0.05). However, the further increase did not cause a significant decrease in the moisture content of the crackers (P>0.05).

According to the ash analysis results, it can be said that the ash content of the crackers significantly increased with the addition of OP powder (P<0.05). The ash content of OP was reported as 4.55±0.22 g/100 g (dry weight, dw) [12]. The high ash content of the OP may be the reason for the high ash content of crackers with OP. The highest fat content (11.26±0.07 %, dw) was determined in the cracker sample with 20% OP. With the addition of pomace, the fat content of the crackers generally increased (P<0.05). It has been stated that this is due to the residual oil in the pomace [25]. Since olive pomace is rich in oil, it is expected that increased OP powder concentration will increase the fat content of crackers. Supporting our findings, the same study observed a significant increase in fat content with an increase in the amount of OP in the breadsticks.

Water activity values of the cracker samples were found to be lower than 0.254 and the crackers can be considered microbiologically stable. The water activity values of the crackers significantly decreased with the addition of OP (P<0.05). This is expected due to the lower water activity value of OP than wheat flour and the low moisture content of OP-added crackers. The pH values of the cracker samples ranged between 5.02-5.17. With the addition of OP, the pH value of the cracker samples decreased. However, this decrease was found to be insignificant up to the 20% OP powder replacement ratio (P>0.05). Similar to our results, Rainero et al. [33] reported that increasing the amount of red grape pomace decreased the pH value of breadsticks and the acidic character may cause a more tenacious and less extensible structure in the dough. In addition, previous studies have reported that high pH values cause weakening of gluten, a decrease in consistency, and undesirable brown spots [51,52].

3.2. PHYTOCHEMICAL CONTENT AND ANTIOXIDANT ACTIVITY OF CRACKERS

Antioxidants may react differently to various radical or oxidant sources. The mechanisms of action of all radical sources and antioxidant chemicals in a complex system cannot, therefore, be accurately reflected by a single technique. Nowadays, it is crucial to assess the antioxidant content of food. To determine the overall antioxidant potential of any food matrix, estimation of the total antioxidant using several assays is crucial. Using three in vitro assays—DPPH, ABTS and FRAP—the total antioxidant activity of the food products was measured. Many researchers have investigated the antioxidant activities of bakery products using these methods [25,53–55].

The total flavonoid, total phenolic, and total antioxidant contents, as well as antioxidant activities of crackers are given in Table II. With the addition of OP to the cracker dough, total flavonoid, phenolic, and antioxidant contents and antioxidant activity (DPPH, ABTS, and FRAP) of cracker samples significantly increased compared to the control sample (P<0.05). This is thought to be due to the fact that pomace is

Table I - Proximate composition of crackers

Sample (%)	Moisture Content (%, Wet Basis)	Ash Content (%, Dry Basis)	Fat Content (%, Dry Basis)	Water Activity	pH
0 (Control)	3.18±0.49 ^{c}	1.75±0.03 ^{a}	7.68±0.21^{a}	0.254±0.00^{d}	5.17±0.01^{b}
5	2.12±0.35 ^{b}	2.23±0.04 ^{b}	8.91±0.10^{b}	0.252±0.00^{d}	5.13±0.01^{b}
10	1.55±0.32 ^{a}	2.42±0.02 ^{c}	9.12±0.15^{b}	0.241±0.00^{c}	5.11±0.01^{b}
15	1.47±0.17^{a}	2.44±0.06 ^{c}	10.98±0.03^{c}	0.228±0.00^{b}	5.10±0.01^{b}
20	1.47±0.13 ^{a}	2.57±0.02 ^{d}	11.26±0.07^{c}	0.217±0.00^{a}	5.02±0.01^{a}

Mean ± SD (n=3). Different letters (a-e) in the columns represent the statistical difference (*P*<0.05).

rich in phytochemicals, which contribute to its antioxidant activities [3,10,11]. With the addition of 5% OP to the crackers, the total phenolic content and antioxidant activity (DPPH and ABTS) of the samples increased approximately two times, while the total flavonoid content increased four times compared to the control. It can also be said that the addition of OP most affects the amount of total flavonoid content, and least affects the total antioxidant content and FRAP.

The total flavonoid ($y = 1.0705x^2 + 7.4377x + 42.751$, $R^2 = 0.9866$), total phenolic ($y = -0.0729x^2 + 3.7371x + 15.688$, $R^2 = 0.9956$), and total antioxidant ($y = 0.0913x^2 + 0.4861x + 70.984$, $R^2 = 0.9815$) contents, and ABTS ($y = -0.0947x^2 + 4.6929x + 21.214$, $R^2 = 0.9467$, graphs were not given) of crackers followed a quadratic trend depending on the increase of OP concentration, whereas the antioxidant activities of the crackers followed a linear trend (DPPH AA Eq.: $y = 2.082x + 12.732$, $R^2 = 0.9949$, and FRAP: $y = 3.91x + 57.052$, $R^2 = 0.97$, graphs were not given).

Similarly, Simonato et al. [24] reported that an increase in the OP percentage increased both the total phenolic content and antioxidant activity of the cooked and uncooked pasta samples. In addition, it was also claimed that DPPH ($R^2 = 0.90$) and ABTS ($R^2 = 0.99$) assay strictly correlated to the OP percentage. Simsek and Sufer [25] also reported that an increase in the replacement ratio of OP with wheat flour increased the total phenolic content and antioxidant activity (DPPH and FRAP) in breadsticks fortified with OP.

Phenolic compounds such as flavonoids, phenolic acids, and diterpenes contribute to the antioxidant activity of the foods. As a result of the study, in order to determine the relationship between total flavonoid and total phenolic contents and antioxidant capacity, the correlation graphs were drawn and shown in Figure 1. It can be stated that both the total flavonoid and phenolic contents showed a linear relationship with antioxidant activity. The correlation coefficient values were generally found higher than 0.90, except for the ABTS-total flavonoid and ABTS-total antioxidant graphs. It can also be stated that the antioxidant activity of the crackers can be expressed with their total flavonoid and total phenolic contents. Antioxidant activity may also come from the presence of volatile oils, carotenoids, vitamins, etc. [56].

3.3. MINERAL COMPOSITION OF CRACKERS

Table III provides the mineral composition of cracker

Table II - Total flavonoid, total phenolic, and total antioxidant contents and antioxidant activity (DPPH, ABTS, and FRAP) of cracker samples with OP (Wet basis)

Sample (%)	TFC (mg CE /100 g)	TPC (mg GAE /100 g)	TAC (mg AAE /100g)	DPPH (mg AAE /100 g)	ABTS (mg AAE /100 g)	FRAP (mg $FeSO_4$ Eq. /100 g)
0 (Control)	30.38±4.98[a]	15.02±0.40[a]	69.36±1.07[a]	13.38±0.29[a]	20.62±2.13[a]	51.54±0.58[a]
5	125.50±5.00[b]	33.73±1.60[b]	78.93±1.80[b]	21.86±1.16[b]	46.01±2.14[b]	83.62±0.10[b]
10	242.00±7.50[c]	46.24±0.80[c]	85.02±0.36[c]	33.35±0.58[c]	51.14±0.46[c]	99.30±2.01[c]
15	352.60±14.90[d]	53.54±0.00[d]	95.53±1.42[d]	45.62±0.87[d]	76.64±0.76[d]	110.40±0.10[d]
20	638.00±17.50[e]	62.10±0.60[e]	118.87±0.35[e]	53.55±3.20[e]	75.27±0.61[d]	135.90±2.00[e]

Mean ± SD (n=3). Different letters (a-e) in the columns represent statistical differences (*P*<0.05).
TFC: Total Flavonoid Content, CE: Catechin Equivalent,TPC: Total Phenolic Content, GAE: Gallic Acid Equivalent, TAC: Total Antioxidant Content, AAE: Ascorbic Acid Equivalent, DPPH: 1,1-diphenyl-2-picrylhydrazyl, ABTS: 2,2-Azino-Bis (3-Ethylbenzthiazoline-6-Sulfonic Acid), Diammonium Salt, FRAP: Ferric Reducing Antioxidant Power Assay, Eq: Equivalent.

Table III - Mineral composition of cracker samples with OP (Wet basis)

Sample (%)	Minerals (mg/kg)							
	Fe	Ca	Zn	Mg	Cu	Al	Mn	K
0 (Control)	49.52±0.65[a]	386.46±2.15[a]	8.18±0.23[a]	342.74±2.80[a]	3.56±0.36[a]	14.39±1.11[a]	9.48±0.47[a]	1852.12±12.23[a]
5	49.52±1.33[a]	421.93±2.31[b]	8.67±0.29[a]	369.94±2.64[b]	4.34±0.52[b]	14.48±0.43[a]	9.77±0.12[a]	2853.75±8.25[b]
10	55.19±0.43[b]	552.17±6.90[c]	14.93±0.42[c]	420.17±2.39[c]	9.65±0.31[c]	20.38±0.58[b]	9.64±0.32[a]	3777.55±18.45[c]
15	58.28±1.20[c]	805.43±1.84[d]	8.60±0.33[a]	436.65±2.22[d]	9.80±0.19[c]	23.66±1.03[c]	9.61±0.44[a]	4721.40±17.11[d]
20	59.29±0.45[c]	832.72±1.79[e]	11.13±0.25[b]	485.49±2.53[e]	9.75±0.32[c]	48.46±1.20[d]	9.65±0.46[a]	5888.50±6.01[e]

Mean ± SD (n=3). Different letters (a-e) in the columns represent the statistical difference (*P*<0.05).

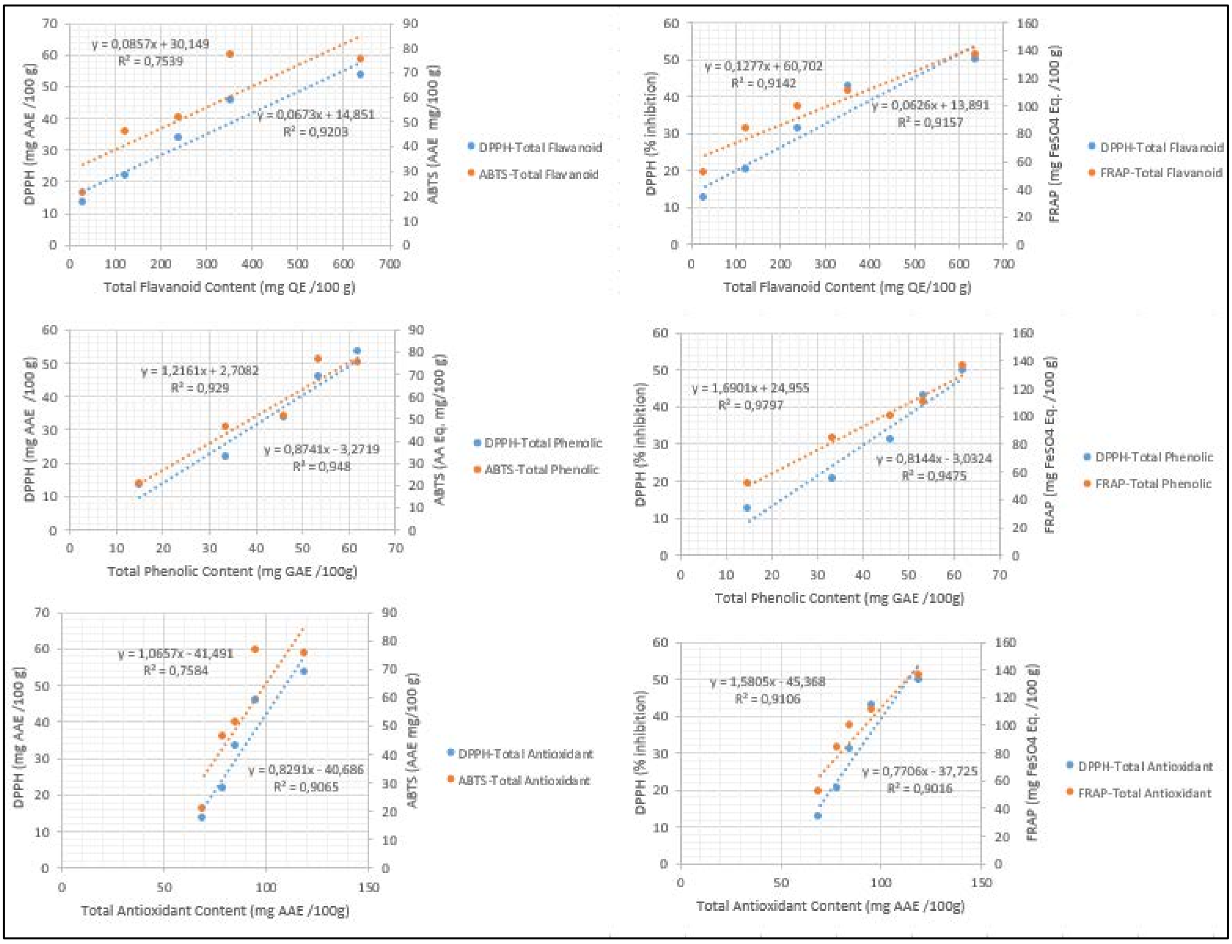

Figure 1 - Linear correlation between total flavonoid, phenolic, and antioxidant contents and antioxidant activity of the crackers

samples containing OP. The addition of OP powder influenced the mineral composition of the resulting crackers ($P<0.05$). When examining the mineral content of the samples, there was no statistically significant difference between the control and the sample enriched with 5% OP in terms of iron (Fe), zinc (Zn), aluminum (Al), and manganese (Mn) content ($P>0.05$). Generally, the addition of OP at concentrations of 10% and above led to a statistically significant increase in mineral content ($P<0.05$). However, manganese (Mn) content was not found to be dependent on the concentration of OP ($P>0.05$).

It has been reported that OP is rich in potassium (2798.44±29.64 mg/kg dw), calcium (352.03±23.01 mg/kg dw), and magnesium (145.41±16.54 mg/kg dw). It also contains iron (12.24±2.71 mg/kg dw), sodium (16.42±0.67 mg/kg dw), aluminum (16.93±2.08 mg/kg dw), manganese (1.56±0.22 mg/kg dw), zinc (2.92±0.36 mg/kg dw), copper (2.73±0.29 mg/kg dw), boron (4.02±0.52 mg/kg dw), and barium (0.18±0.03 mg/kg dw) [12].

Similarly, our findings demonstrated that the incorporation of OP powder into crackers resulted in increased content of several minerals, including Fe, Ca, Zn, Mg, Al, and K. The elevated mineral levels may be attributed to the inherent richness of OP in mineral compounds compared to wheat flour. Studies in the literature also support these results. For example, high dietary fiber biscuits enriched with OP exhibited a calcium content of 731.15±5.32 mg/kg and a magnesium content of 301.16±3.45 mg/kg [23]. Another study involving muffins enriched with olive pomace flour, extra virgin olive oil, and hydrolysed soy protein reported an improved mineral composition of 1.09±0.01%, which was attributed to the composition of both olive pomace flour (3.02±0.08%) and hydrolyzed soy protein (9.87±0.25%). The study also suggested that interactions between fibers and certain proteins may result in the formation of complexes with minerals, thereby increasing the micronutrient content in the final product [57].

3.4. HARDNESS AND COLOR VALUES OF CRACKERS

Hardness is a crucial quality parameter in crackers, as consumers generally prefer a crisp texture. The hardness values of the crackers exhibited an upward trend with the incorporation of higher levels of OP (Figure 2). The increase was evident up to a 15% OP powder inclusion level; beyond this concentration, no further increase in hardness was observed. A similar trend was

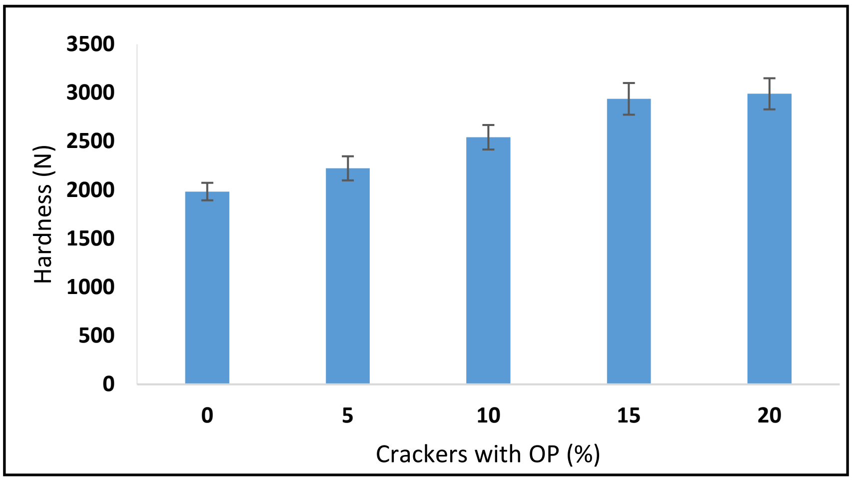

Figure 2 - Hardness levels of crackers Mean ± SD (n=3)

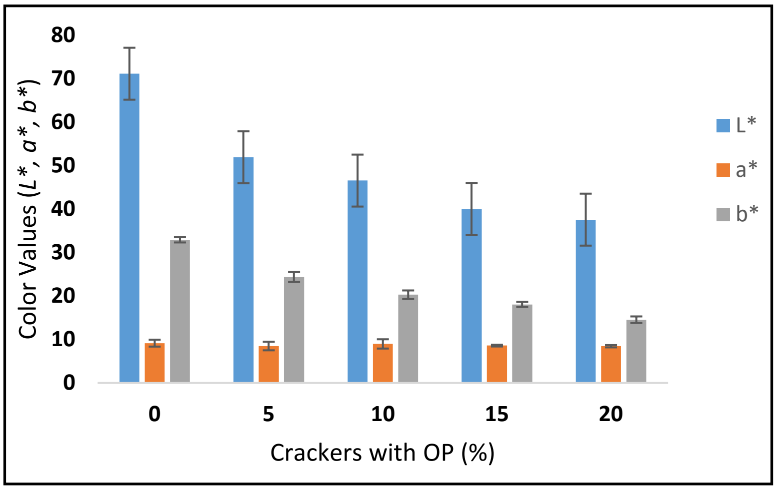

Figure 3 - Color values of crackers Mean ± SD (n=3)

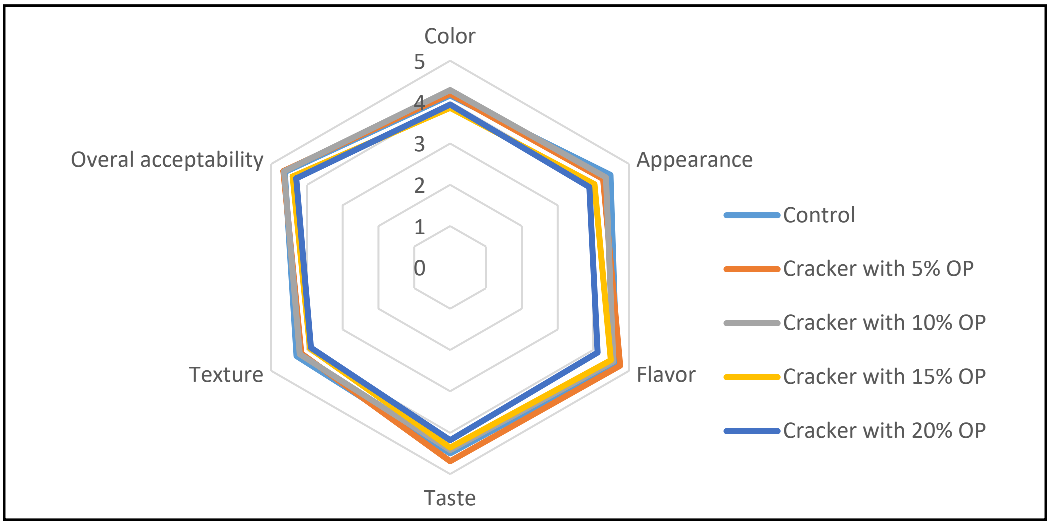

Figure 4 - The radar chart of consumer preference scores of crackers with OP Mean ± SD (n=3)

reported by Drisya et al. [58] in cookies fortified with dried Murraya koenigii leaves. The researchers noted that higher amounts of these leaves (0 to 15%) diluted the gluten by increasing the protein, dietary fiber, calcium, and iron content, resulting in a harder cookie dough with less stickiness and springiness compared to the control.
Similarly, in our investigation, increased OP levels correlated with higher mineral content, which may have contributed to the enhanced hardness. The inclusion of substantial amounts of OP could have diluted the gluten structure, leading to a more rigid cracker texture. Additionally, elevated pH values associated with OP may have weakened gluten networks, contributing further to the formation of harder crackers [51,52]. Collar et al. [59] also stated that fiber addition can affect the mechanical properties of dough by increasing hardness and decreasing cohesiveness.
Figure 3 illustrates the color values of crackers at various OP powder ratios. The L* (lightness) and b* (yellowness) values exhibited significant variations with the addition of OP ($P<0.05$), while there were no statistically significant differences in a* (redness) values among the OP-containing samples ($P>0.05$), except for the control. The L* values decreased with increasing OP content, likely due to the naturally dark color of OP powder. The a* values also declined in the OP-added samples, and b* values decreased in parallel with increasing OP concentrations. As OP ratio increased, the ΔE (total color difference) values rose significantly ($P<0.05$), ranging from 21.04±0.98 in crackers with 5% OP to 38.28±2.43 in those with 20%.
Similar to our findings, Simsek and Sufer [25] reported a significant reduction in L* and b* values with increasing OP content in breadsticks. In another study, lower L* and b* values were also reported for OP-enriched cooked pasta compared to control samples [24]. Furthermore, Qadri et al. [60] highlighted that Maillard reactions, caramelization, and thermal processing at high temperatures can lead to surface color changes in dough products.

3.5. SENSORY PROPERTIES OF CRACKERS

Figure 4 displays the sensory characteristics of crackers manufactured with varying OP ratios, along with the radar chart depicting consumer preference scores. The control samples and crackers containing 5% and 10% OP exhibited similar sensory attributes. Notably, the crackers enriched with 10% OP attained the highest overall acceptability, receiving a score of 4.66 (±0.36) in comparison to the control. Crackers with 20% OP received the lowest acceptability score among all other samples. In this recipe, in terms of sensory attributes including color, appearance, texture, and overall acceptability, crackers were found well accepted up to a ratio of 10% OP incorporation, while in terms of flavor and taste properties, crackers were found acceptable up to a ratio of 15% OP incorporation. The previous study has demonstrated that high-fiber biscuits with a 15% incorporation level with OP had the highest overall acceptability (4.58±0.50, *23*). In order to develop products with health benefits, Padalino et al. [61] produced functional spaghetti from durum wheat semolina that was enhanced with olive paste powder (10-15%), a by-product of olive oil. It was emphasized that it may be better to add optimal olive paste powder to avoid a negative impact on sensory qualities and the overall quality of the pasta. Overall, the lowest percentage of olive paste (10%) was found to be better in terms of sensory and textural aspects. In our study, OP incorporation crackers with the lowest ratios (5-10%) were found to be superior in terms of sensory properties.

4. CONCLUSIONS

This study showed that OP, which is a by-product of olive oil industries, could be used as a source of functional components to produce a high-value-added cracker. OP-fortified crackers are rich in ash, fat, flavonoid, and phenolic contents, antioxidant activities, and mineral composition compared to crackers made with only wheat flour (control). These valuable components in the crackers increased with the increase in the amount of OP (0 to 20%). With the increase in the amount of OP, the moisture content, water activity, and color values of the crackers decreased. Among the cracker samples, those incorporated with 10% OP powder had the highest overall acceptability score. These findings show that OP is a valuable functional ingredient for producing crackers high in flavonoids, phenols, minerals, and antioxidants, as well as crackers with good sensory attributes. Furthermore, there was a high correlation between phenolic and flavonoid content and antioxidant activities. In addition, functional crackers prepared using OP powder fortification could find a place in the functional food product market.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

Author's contribution
Kadriye Altay: performed the experimental work, contributed to the formatting and writing of the manuscript.
Arda Akdoğan: conducted the literature review, performed the experimental work, and carried out the statistical analysis.
Selma Lubabe Erdoğan: conducted the literature

review, performed the experimental work, and carried out the statistical analysis.

Gülşah Çalışkan Koç: conceived the research idea, supervised the analytical work, contributed to the statistical analysis, and took the lead in formatting and writing the manuscript.

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