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## **Fortified wheat bread with dried three-phase olive pomace: effect on physicochemical, antioxidant, nutritional, and sensory characteristics**

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## Abstract

Olive pomace (OP), a key by-product of olive oil production, is recognized as a sustainable and cost-effective source of natural antioxidants. Rich in polyphenols with proven health benefits, OP shows considerable potential for application in functional foods to improve nutritional quality and oxidative stability. This study aims to evaluate the effect of dried OP (dOP) addition on the nutritional, physicochemical, rheological, antioxidant, and sensory properties of fortified wheat bread. Four different replacement levels (1%, 2%, 5%, and 7%) of dOP obtained from the three-phase process of extra-virgin olive oil production were incorporated into the bread. OP exhibited high levels of fiber (38.5%), carbohydrates (33.22%), monounsaturated fats (67.02%), oleic acid (67.78%), phenolic content (23.39 mg GAE/g dw), flavonoids (13.02 mg QE/g dw), and antioxidant activity (15.25 mg Trolox/g dw). The addition of dOP significantly increased phenolic content, flavonoids, and antioxidant activity of breads; the highest values were recorded at 7% dOP (2.34 mg GAE/g dw, 0.32 mg QE/g dw, and 1.10 mg Trolox/g dw, respectively). No significant differences were observed in rheological and most sensory attributes between the control bread and samples fortified with 1% and 2% dOP. However, fortification at high levels (5% and 7%) significantly affected the bread's texture, color, and sensory properties. Additionally, the sensory evaluation showed that bread fortified with 1%, 2%, and 5% dOP exhibited higher overall acceptability compared to the 7% formulation, which demonstrated a moderate acceptability due to a slight bitterness and sandy mouthfeel. The results suggest that OP can be utilized as a sustainable and functional ingredient in the formulation of high-value bakery products when added at moderate levels.

## Introduction

The olive oil production sector plays a significant role in the agricultural and economic development of Mediterranean countries (Calvano and Tamborrino, 2021). This sector has promoted production growth due to well-known health benefits and great nutritional properties of olive oil, including bioactive and phenolic compounds, as the major contributors to antioxidant stability and health-enhancing effects, reaching approximately 32 million tons of olive oil during crop year 2024-25, covering 95% of the world's total production (FAO, 2024). Olive oil production represents a vital agricultural sector in Albania's economy, with substantial potential growth and competitiveness in global markets (Pikuli and Devolli, 2024; Zantedeschi *et al.*, 2025). According to FAO (2021), the country's olive oil production was estimated at approximately 11,500 tons.

Despite its health benefits and economic value, olive oil production has a notable negative environmental impact (Rapa and Ciano, 2022). This impact comes from the large quantity of by-products it generates, especially olive pomace (OP) and olive mill wastewater (OMWW), which vary in amount and composition depending on the extraction technology used, such as three-phase, two-phase, or semi-modern centrifugal systems (Centrone *et al.*, 2021; Katsinas *et al.*, 2021).

The two-phase olive oil extraction systems generate a semi-solid waste called OP, with a slightly acidic pH, high organic matter, fibers, minerals, and phenolic compounds. On the other hand, the three-phase olive oil production system generates solid waste, known as OP, and large volumes of liquid waste (OMWW), due to the high amount of added water compared to pressing and two-phase systems (Medouni-Haroune *et al.*, 2018; Alkhalidi *et al.*, 2023). However, OP produced from a two-phase system contains a large amount of water (65-70%), compared with OP from a three-phase decanter, which consists of a lower amount of water (45-50%) (Benaddi *et al.*, 2023). Moreover, despite this fact, olive oil production in Albania is still dominated by three-phase extraction systems, with limited application of two-phase and pressing technology systems (Topi *et al.*, 2014; Beqaj *et al.*, 2025).

Discharges from olive oil processing biomass are characterized by high levels of organic matter, causing toxicity, colored waters due to phenolic compounds, land degradation, and air emissions (Pampuri *et al.*, 2021). However, olive oil by-products also contain valuable nutrients (Quero *et al.*, 2022). OP, the main

solid by-product of olive oil production (Zhao *et al.*, 2022), is composed of a rich lignocellulosic matrix, abundant phenolic compounds, and residual oil fractions (Nunes *et al.*, 2021). Furthermore, it is increasingly recognized as a sustainable and cost-effective source of bioactive compounds, especially polyphenols with strong antioxidant activity (Gullón *et al.*, 2020), which can be used to develop high-value functional products (Difonzo *et al.*, 2021).

Recently, there has been growing interest in the sustainable valorization of the olive oil production chain, including the formulation of novel food products from low-value sources to reduce waste and conserve resources (Paulo and Santos, 2021; Contreras-Angulo *et al.*, 2025). Additionally, the recovery of high-value bioactive compounds and the utilization of olive oil by-products in the development of functional foods present significant opportunities from both economic and practical standpoints (Cedola *et al.*, 2020; Gill *et al.*, 2021).

Due to its high content of bioactive compounds, dietary fibers, monounsaturated and polyunsaturated fats, and antioxidants, OP represents a sustainable and cost-effective source for agro-food supply chains (Vitali *et al.*, 2018). Its utilization not only contributes to waste reduction but also responds to the growing consumer demand for functional, and healthier food products (Galanakis *et al.*, 2018; Foti *et al.*, 2022). Several studies have reported the potential use of OP as a functional ingredient in different food matrices, including the fortification of meat (Jovanovichs *et al.*, 2025), the enhancement of antioxidant stability of dairy products (Ribeiro *et al.*, 2021), and the enrichment of bakery goods (de Gennaro *et al.*, 2022).

Among cereal-based products, bread is the most widely consumed and is often considered nutritionally poor due to its common ingredients such as flour, water, yeast, salt, sugar, and fat (Toumi *et al.*, 2022). Recently, there has been a growing awareness of consumers regarding the relationship between diet and health, leading to an increased interest in bread that offers enhanced nutritional benefits (Dahdah *et al.*, 2024a). Therefore, incorporation of OP in the baking industry represents a promising approach for developing functional bakery goods rich in bioactive compounds, dietary fibers, antioxidants, vitamins, and minerals, by enhancing both sensory and nutritional values (Elkatry *et al.*, 2022). The beneficial nutritional properties of polyphenols and dietary fiber render OP an attractive functional ingredient for bakery products (Gill *et al.*, 2021). Kamiloglu *et al.* (2022) have reported that the addition of functional ingredients into bread presents several challenges, particularly concerning antioxidant stability and potential effects on the bread's sensory attributes. Furthermore, the addition of OP in bread formulation, even in low percentages, can influence the fiber content and bioactive components, which affect dough rheology and texture profile, and extend the shelf life (Falsafi *et al.*, 2025).

Although several studies have explored bread fortification with OP, further research is needed to clarify its impact on physicochemical, textural, nutritional, and sensory characteristics.

In the present study, wheat bread was fortified with dried OP (dOP), obtained from the three-phase production of extra-virgin olive oil, at four replacement levels (1%, 2%, 5%, and 7%) to enhance its nutritional value. The effects of dOP incorporation on the bread's physicochemical, nutritional, textural, and sensory characteristics were investigated.

The study also aims to optimize the level of dOP incorporated in bread fortification, enhance nutritional quality and antioxidant stability, and simultaneously improve technological qualities without compromising overall sensory acceptability.

Additionally, this study contributes to the development of high-added-value bakery products that meet both consumer demands for functional products and technological performance.

## **Materials and Methods**

### ***Sampling and treatment of olive pomace***

OP was obtained during the 2024/25 crop year from a three-phase centrifugal extraction of extra-virgin olive oil produced from the Kalinjot cultivar, the most important autochthonous olive variety in Albania, predominantly cultivated in the southern regions (Thomaj and Panajoti 2003; Ismaili, 2017). The

collected OP samples were homogenized and oven-dried at 45-50°C for 72 hours to prevent the degradation of phenolic compounds (Pikuli and Devolli, 2025). The dOP was grounded into a fine powder using a laboratory mill, sifted through a flour sieve, and stored in airtight containers at 4°C until further use in bread formulation.

### ***Reagents and chemicals***

All chemicals and reagents, purchased from Sigma-Aldrich (Germany), were of analytical grade, and doubly distilled water was used throughout the study.

### ***Physicochemical analysis***

Moisture content of OP was determined using a forced-air oven at 105±1°C under vacuum for 48 hours, or until a constant weight was reached, following the procedure described by Moya *et al.* (2010). Ash content was measured gravimetrically following AOAC (2019) guidelines. Total protein content was quantified using the Kjeldahl method and expressed as nitrogen, multiplied by a conversion factor of 6.25 (Nunes *et al.*, 2018). Lipid content was determined gravimetrically through automated Soxhlet extraction with hexane as the solvent, according to the method reported by Azadmard-Damirchi and Dutta (2007). Total dietary fibers were analyzed using the enzymatic-gravimetric method described by AOAC (2019), and the total carbohydrate content was calculated by the difference method. Meanwhile, the moisture content of bread was determined by weight difference at 105°C, after drying for 24 hours in a ventilated oven LBX OVF series at 35°C and grinding with a domestic blender (Kenwood Blend-X Fresh BLP41.A0WH, 650 W, Germany), in accordance with the AACC (2000). Water activity (aw) of bread samples was determined using an electronic hygrometer (Rotronic HP23-AW-A HygroPalm, Bassersdorf, Switzerland), calibrated with standard solutions ranging from 0.10 to 0.95, in accordance with ISO 18787 (2017). Measurements were performed at 21 °C.

### ***Extraction of phenolic compounds from olive pomace***

Ultrasound-assisted extraction with ethanol:water (80:20, v/v) was employed to recover phenolic compounds from both bread and OP samples. Dried and finely ground samples (5 g) were placed in 50 mL centrifuge tubes and combined with 25 mL of ethanol:water (80:20, v/v). The mixtures were vortexed for 1 min at 400 rpm and sonicated at room temperature for 60 min using an ultrasonic bath (Cole-Parmer 8893, 47 kHz, 230 W). Following sonication, the samples were centrifuged at 6000 rpm for 15 min (Hettich EBA 21, Tuttlingen, Germany), and the resulting supernatants were filtered through 0.45 µm PTFE membranes (Sigma-Aldrich, Berlin, Germany) and stored at -20°C until further analysis.

### ***Determination of total phenolic content, total flavonoid content, and total antioxidant activity***

The total phenolic content (TPC) and total antioxidant activity (TAA) of the extracts were determined spectrophotometrically using the Folin-Ciocalteu and DPPH (2,2-diphenyl-1-picrylhydrazyl) methods, respectively, following the analytical procedures described by Pikuli and Devolli (2024). All analytical tests were carried out in triplicate. The experimental procedures are briefly described below.

The TPC content was quantified using a previously calibrated curve of gallic acid as a standard (50-500 mg GA/mL;  $y = 2.6276x - 0.0168$ ;  $R^2 = 0.9997$ ). Specifically, 250 µL of phenolic extract was mixed with 1250 µL Folin reagent (10% v/v) in a test tube, vigorously vortexed, and kept in the dark for 8 minutes. After that, 1000 µL of Na<sub>2</sub>CO<sub>3</sub> (7.5%) was added, and the mixture was vortexed again and placed in a water bath (for 30 minutes at 40°C). After incubation, the test tubes were allowed to reach room temperature. The absorbance was measured at a wavelength of  $\lambda=750\text{nm}$  using a UV/Vis spectrophotometer (Biochrom Libra S22 UV/Vis Shimadzu, Cambridge CB4 0FJ, UK). The results of TPC content were expressed as mg gallic acid equivalents/g dry weight (mg GAE/g dw).

The TAA was evaluated at 515 nm by UV-vis spectrophotometry using DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity assay as described below: A 40  $\mu$ L aliquot of phenolic extract, 1960  $\mu$ L of methanol, and 2000  $\mu$ L of DPPH solution (0.5 mM) were added to each test tube. The test tubes were mixed in a vortex and were placed in the dark for 30 minutes at room temperature. A control sample was prepared in the same way without phenolic extract, and the absorbance was measured against methanol as a blank. Trolox standard solution (25-250 mg/mL) was used to prepare the calibration curve ( $y = 0.0981x + 5.6843$ ;  $R^2 = 0.997$ ), and TAA was expressed as mg Trolox/g dry weight.

The TFC was determined using the colorimetric method with aluminum chloride, as described by Sakanaka *et al.* (2005), with some modifications, using quercetin as a standard. Phenolic extract (1 mL) was mixed with 4 mL of distilled water and 300  $\mu$ L of a 5% sodium nitrite solution, and the mixture was allowed to stand for 5 min. Then, 1 mL of aluminium chloride solution (10%) and 400  $\mu$ L of distilled water were added, and the solution was allowed to react for 6 minutes. After that, 4 mL of 1 M NaOH was added, and changes in the color were observed (from yellow to red). The solution was mixed and allowed to stand at room temperature for 15 minutes. The absorbance was measured at a wavelength of 510 nm, while the results were expressed as milligrams of quercetin/g of dry weight (mg QE/g dw).

### ***Fatty acid analysis***

The lipid fraction of OP was extracted using a Soxhlet apparatus with n-hexane as the extraction solvent. Approximately 5 g of dOP was placed in the extractor and subjected to 30 continuous cycles following the procedure described by Azadmard-Damirchi and Dutta (2007). After extraction, the mixture was filtered through filter paper to remove solids, and the lipid-containing hexane phase was collected. The solvent was subsequently evaporated under reduced pressure at 35°C using a rotary evaporator, and the recovered oils were stored at -20°C until further analysis. Meanwhile, fatty acids were converted into fatty acid methyl esters (FAMES) by acid-catalyzed trans-esterification following the procedure outlined in ISO 12966 (2017).

Chromatographic analysis of FAMES was performed on a GC-2030 gas chromatograph (Shimadzu, Tokyo, Japan) fitted with a flame ionization detector and an AOC-30i split/splitless autosampler (Shimadzu). Separation was achieved on a DB-WAX capillary column (122-7033; 30 m  $\times$  0.25 mm, 0.50  $\mu$ m, Agilent Technologies, Santa Clara, CA, USA), with a maximum operating temperature of 350 °C. Helium served as the carrier gas at a constant flow rate of 1.0 mL/min. Detector gases consisted of hydrogen (32 mL/min) and air (200 mL/min). Injections of 1.0  $\mu$ L were made in split mode with a split ratio of 1:20, and both the injector and detector were maintained at 250 °C. The oven temperature program was set as follows: initial temperature 140 °C (held for 1 min), increased to 220 °C at 10 °C/min, then to 250°C at 3°C/min, where it was held for 20 min. Fatty acids were identified by comparing retention times with those of a certified standard mixture (37-Component FAME Mix, Supelco, Bellefonte, PA, USA). All analyses were performed in triplicate, and results were expressed as the relative percentage of individual fatty acids in the total fatty acid content.

### ***Color measurement***

Color measurements of bread samples were performed using a portable colorimeter (Model NH 300/3nh, Shenzhen, China) based on the CIELAB system ( $L^*$ ,  $a^*$ ,  $b^*$ ,  $c^*$ ,  $h^*$ , and  $\Delta E^*$ ) according to the procedure described by Harasym *et al.* (2020). The instrument was standardized using white and black calibration plates. Both crust and crumb color were analyzed 24 hours after baking, and results are presented as the mean of independent triplicate measurements  $\pm$  standard deviation (SD).

The total color difference ( $\Delta E$ ) was calculated using the following equation [Eq. 1]:

$$\Delta E = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad [\text{Eq. 1}]$$

where  $\Delta L^*$ ,  $\Delta a^*$ , and  $\Delta b^*$  represent the differences between control and fortified bread samples. One-way analysis of variance (ANOVA) was performed to assess statistically significant differences in  $\Delta L^*$ ,  $\Delta a^*$ ,  $\Delta b^*$ , and  $\Delta E$ .

The whiteness index (WI) of the bread crumb and the browning index (BI) of the crust were determined using the following equations [Eq. 2]:

$$WI = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}}$$
$$BI = [100 * (x - 0.31)] / 0.17, \text{ where } x = \frac{(a^{*+1.75L^*})}{(5.645L^{*+}a^{*-0.012b^*})} \quad (\text{Nedviha \& Harasym 2025}) \quad [\text{Eq. 2}]$$

### ***Sensory evaluation***

A panel test of 33 participants, consisting of eight trained members (six females and two males, aged 30-60 years) from the Food Research Center and 25 Master's students from the Faculty of Biotechnology and Food, Agricultural University of Tirana (Albania), assessed the acceptability of breads enriched with dOP. The sensory properties of the bread samples, including taste, aroma, texture, and color, were evaluated using a nine-point hedonic scale (1 = extremely unpleasant, 5 = neither like nor dislike, and 9 = extremely pleasant), with a score of 5 representing the minimum level of acceptability.

Bread samples (without dOP and breads fortified with 1%, 2%, 5%, and 7% dOP) were cut into 1 cm slices, placed on white plates at room temperature, and randomly coded to ensure unbiased evaluation. Sensory evaluation was conducted 24 h after baking, and overall acceptability was determined by calculating the weighted average of the sensory attributes (Azadfar *et al.*, 2023).

### ***Rheological analysis***

Rheological analysis is an important technique for evaluating dough quality, characterizing its mechanical properties, and predicting the baking performance and quality of bakery products (Nikolić *et al.*, 2022). The rheological characteristics of the control dough (without dOP) and dough enriched with dOP were assessed using a Farinograph (Brabender, Duisburg, Germany) and an Alveograph (Chopin CD-1), in accordance with AACC Method 54-21 (2000). The Farinograph measured water absorption (WA), dough development time (DDT), stability, softening degree, and mixing behavior. At the same time, the Alveograph test evaluated dough resistance, extensibility, and deformation energy, which are key indicators of baking strength and technological performance. Additionally, the effect of dOP addition on both Farinograph and Alveograph parameters was evaluated.

### ***Bread-making process***

Bread-making trials were conducted at laboratory scale using equipment provided by Atlas Mills (Tirana, Albania). Dried and pretreated OP was incorporated into the dough at four levels (1%, 2%, 5%, and 7%), while a control formulation without dOP was prepared for comparison. All dough samples were prepared using commercial wheat flour (*Triticum aestivum*), obtained from Atlas Mills Shpk, according to the formulations described below. Each formulation was prepared by mixing 1000 g of wheat flour with 600 g of water, 15 g of yeast, 20 g of salt, and the respective amount of dOP until a homogeneous dough was obtained. Mixing was performed using a Brabender Planetary Mixer (P600, Germany) at two speed levels (80 rpm and 180 rpm) for 7 minutes and 30 seconds each. After mixing, the dough was divided into 300 g portions, manually rounded, and placed in a fermentation chamber (Bongard, France) for 50–60 min at 32°C and 89% relative humidity. For each formulation, four loaves were prepared and baked in aluminium pans using an electric oven (Polin 9 Tray, Model No. 3STA4676-3, Italy) at 225°C for 20 minutes.

### ***Statistical analysis***

All analyses were performed in triplicate, and results are reported as mean  $\pm$  standard deviation using SPSS version 28.0 (IBM, Chicago, IL, USA). A one-way ANOVA was applied to assess the effect of dOP addition on the physicochemical, rheological, oxidative stability, and sensory properties of bread. Significant differences between the control and fortified samples were determined using Tukey's multiple range test at a 95% confidence level ( $p \leq 0.05$ ).

## **Results and Discussion**

### ***Physicochemical composition of olive pomace***

The physicochemical characteristics and fatty acid composition of dOP used for bread fortification are presented in Table 1. The moisture content of the crude OP and dOP was  $63.25 \pm 2.32\%$  and  $5.06 \pm 0.52\%$ , respectively. The dOP exhibited a high dietary fiber content (38.5%), followed by total carbohydrates (33.22%) and protein (6.76%), confirming its potential as a valuable ingredient for nutritional enrichment.

The total fat content of dOP was 13.78%, primarily composed of oleic acid (68%), the most abundant monounsaturated fatty acids (MUFA) and the main contributor to the health benefits (Lu *et al.*, 2024). Palmitic acid (10.6%) was the most abundant saturated fatty acids (SFA), while the overall distribution of SFA, MUFA, and polyunsaturated fatty acids (PUFA) was 14.53%, 68.25%, and 12.83%, respectively. These results are consistent with previous findings on the fatty acid composition of Kalinjot virgin olive oils (Morina & Kongoli, 2022; Zantedeschi *et al.*, 2025), confirming the characteristic lipid profile of olive-derived by-products. Moreover, dOP exhibited a considerable content of bioactive compounds, including total phenolics ( $23.39 \pm 2.32$  mg GAE/g dw) and flavonoids ( $13.00 \pm 1.95$  mg QE/g dw), along with strong antioxidant activity ( $15.23 \pm 1.02$  mg Trolox/g dw). These findings align with previous studies emphasizing the remarkable antioxidant potential of OP and its suitability for developing novel functional food products (Cedola *et al.*, 2020; Nunes *et al.*, 2021; Pikuli and Devolli, 2025).

### ***Effect of dried olive pomace addition on the rheological properties of wheat dough***

The rheological behavior of wheat dough, both unfortified and fortified with different levels of dOP, was evaluated using Farinograph and Alveograph tests to assess its technological quality and baking performance. The corresponding parameters are summarized in Table 2.

The Farinograph results provide insights into DDT, stability time (ST), and WA, which are key indicators of the dough's mixing properties and gluten strength. In contrast, the Alveograph parameters, such as resistance of deformation (P), extensibility (L), and deformation energy (W), reflect the viscoelastic balance and gas-holding capacity of the dough, which are critical for bread-making performance (Azadfar *et al.*, 2023).

Dough samples for the Farinograph tests were prepared using only wheat flour, dOP, and water to eliminate the effects of salt and yeast. Measurements were repeated until an average dough consistency of 500 Farinograph Units (FU) was achieved, with a range of 480-520 FU considered acceptable (Vizitiu & Danciu, 2011).

The obtained results revealed that the addition of dOP significantly ( $p < 0.05$ ) affected the rheological characteristics of the dough, with notable changes observed in WA, DDT, and ST compared with the control sample. Farinograph data showed that WA increased with increasing dOP content, from 58.7% in the control to 61.9% at 7% dOP, reflecting the high water-binding capacity of dietary fiber. Conversely, DDT decreased slightly, suggesting reduced gluten hydration efficiency (Selim *et al.*, 2020), while the DST increased significantly, from 9.1 min in the control to 17.8 min in the dough fortified with 7% OP, due to fiber-gluten interactions that enhance dough resistance during mixing, as reported by Abdelaleem and Al-Azab (2020). Moreover, the DS initially increased at moderate dOP levels (40-46

FU) but declined sharply at 7% dOP (27 FU). The FQN decreased from 83 to 41, reflecting compromised mixing quality (Shelton, 2004).

The alveograph analysis further confirmed these trends, showing increased tenacity, decreased extensibility, lower swelling index, and deformation energy with rising dOP levels. The P/L ratio increased significantly (4.41), while the elasticity index ( $I_e=0$ ) at 7% dOP indicated severe disruption of the gluten network (Abdelaleem and Al-Azab, 2020).

The results of the rheological tests are in agreement with those reported by previous studies (Bolek, 2020; Dahdah *et al.*, 2024b). Rosell *et al.* (2005) reported that the incorporation of dOP in bread enhances the nutritional value by increasing the dietary fiber content; however, it affects some of the rheological properties of the dough and also the sensory profile. Azadfar *et al.* (2023) also observed that increasing the OP level in bread fortification influences dough rheology by increasing the WA rate while reducing the softening degree. This effect may be attributed to the linkage fiber-gluten due to the large number of hydroxyl groups in the fiber structure, which increases dough demand for WA, dilutes gluten, and reduces dough extensibility (Selim *et al.*, 2020; Dahdah *et al.*, 2024b).

Overall, the incorporation of dOP in the fortification of the bread significantly improved the rheological properties of the dough due to the presence of fibre and polyphenols. However, at a higher incorporation level (7% dOP), the dough exhibited an adverse effect by decreasing extensibility, elasticity, and overall dough functionality, due to the disruption of the gluten network by excessive fiber content.

### ***Physicochemical quality attributes of bread samples***

The physicochemical properties and color parameters of the bread samples are summarized in Table 3. The addition of dOP significantly affected ( $p<0.05$ ) the physicochemical characteristics of the fortified breads, including moisture content, aw, texture, color, and antioxidant stability.

Moreover, the incorporation of dOP notably enhanced the bioactive properties of the fortified bread samples compared to the control. TPC, TFC, TAA increased from 0.68 to 2.34 mg GAE/g dW, 0.07 to 0.32 mg QE/g dW, and 0.04 to 1.10 mg Trolox/g dW, respectively. Breads fortified with 7% dOP showed approximately four times higher TPC and TFC, and several times higher antioxidant capacity (DPPH) compared with the control. These improvements are attributed to the high concentration of bioactive compounds and the strong antioxidant potential of the incorporated OP, in line with previous studies on bakery products enriched with olive oil by-products (Chandrasekara and Shahidi, 2021; Marinopoulou *et al.*, 2020).

Furthermore, the results indicated that adding dOP to bread increased TPC and antioxidant activity, especially in samples containing 5% and 7% dOP. These findings are similar to those reported by other researchers who studied the impact of OP addition on the phenolic content and antioxidant activity of bakery goods, showing that the higher the levels of dOP addition into the fortification of bread, the greater the increase in both phenolic content and antioxidant activity (Marinopoulou *et al.*, 2020; Cardinali *et al.*, 2024). However, the concentrations of TPC, TFC, and TAA in the fortified bread samples were lower than expected due to the baking conditions. Delgado-Andrade *et al.* (2010) and Cedola *et al.* (2020) reported a similar trend: a decrease in phenolic concentration due to heat and moisture conditions during baking, which promote the Maillard reaction and lead to the degradation of antioxidant compounds.

Meanwhile, the reduction in bread volume is primarily attributed to the dilution of gluten-forming proteins and the disruptive effect of insoluble fiber particles on gas retention and dough expansion, in agreement with findings reported by Cardinali *et al.* (2024) and Dahdah *et al.* (2024a).

The color of the fortified bread was evaluated in both the crumb and crust, and the results are presented in Table 3. The incorporation of dOP significantly affected the crumb color compared with the control. Increasing dOP level resulted in higher redness ( $a^*$ ) and yellowness ( $b^*$ ) values, accompanied by a decrease in lightness ( $L^*$ ). Fortified breads also exhibited a noticeable color difference ( $\Delta E > 2$ ) and higher BI, indicating progressive darkening of the crumb, particularly at 7% dOP. This effect can be

attributed to the presence of natural pigments and the high phenolic content of the Kalinjoti cultivar used in this study. Similar results were reported by de Gennaro *et al.* (2022) and Kovač *et al.* (2024), who demonstrated that the addition of OP significantly affected bread color attributes.

However, in the present study, the incorporation of dOP did not significantly affect the color components of the bread crust. This may be attributed to the influence of Maillard and caramelization reactions occurring at high baking temperatures, which lead to the formation of brown pigments and the masking of potential color variations induced by OP addition (Saura-Martínez *et al.*, 2025).

### ***Sensory evaluation of fortified bread with dried olive pomace***

The results of the sensory evaluation of breads fortified with different levels of dOP are presented in Table 4. No significant differences ( $p > 0.05$ ) were observed between the control sample and the bread fortified with 1% dOP. However, increasing the fortification level of dOP caused significant ( $p < 0.05$ ) changes in color, taste, and several sensory parameters of the bread. According to the results of sensory evaluation, bread enriched with 7% dOP exhibited a noticeable decline in odor, taste, texture, and overall acceptability. Panelists also noted a slightly bitter flavor and a sandy mouthfeel, likely associated with the higher fiber and phenolic content of dOP (Marinopoulou *et al.*, 2020). Although breads with 7% dOP showed a decrease in sensory scores, they remained within the acceptable range (score  $> 5$ ).

Breads fortified with 2% and 5% dOP achieved the highest scores for texture, appearance, and overall acceptability, indicating that moderate dOP levels can improve sensory properties without compromising consumer acceptance.

Regarding sensory appearance (Table 4), the most affected attributes of the fortified bread samples were color (crumb and crust) and texture (adhesiveness, elasticity, crumbliness, and porosity). It was observed that the addition of dOP at high levels (5% and 7%) significantly influenced ( $p < 0.05$ ) both color and bread texture profile. In addition, these results were consistent with instrumental assessment of bread color (Table 3), which showed that the incorporation of dOP at 5 % and 7% significantly affected ( $p < 0.05$ ) the color components by increasing redness and yellowness and decreasing lightness. A noticeable color difference ( $\Delta E > 2$ ) and a higher BI were also observed. In contrast, the incorporation of dOP at levels 1%, 2% and 5% significantly improved the textural properties, while a higher level of dOP (7%) addition exhibited defects in crumb porosity (bubbles homogeneity), elasticity, and appearance of the fortified bread samples (Table 4). Furthermore, dOP incorporation at 7% adversely affected dough rheological properties (extensibility, elasticity, and overall dough functionality), which can be attributed to the high fiber and phenolic content of OP disrupting the gluten network.

These findings align with previous studies showing that excessive incorporation of OP can negatively affect the sensory properties of bakery products (Cedola *et al.*, 2020; Selim *et al.*, 2020). In the present study, the overall acceptability of the fortified bread with 7% dOP was compromised by its bitter taste and sandy mouthfeel, likely due to the high phenolic content of the OP used in the wheat bread. Similarly, Marinopoulou *et al.* (2020) and Dahdah *et al.* (2024 a) found that the addition of 10–15% OP to bread formulation caused bitterness and a sandy mouthfeel.

### **Conclusions**

The present study investigated the impact of dOP addition on the nutritional and quality characteristics of wheat bread. Four incorporation levels (1%, 2%, 5%, and 7%) of dOP, obtained from the three-phase process of extra virgin olive oil production, were used in bread fortification. The physicochemical properties, antioxidant activity, rheological behavior, texture, and sensory attributes of the fortified samples were evaluated. The results showed that dOP addition significantly increased TPC, TFC, and TAA of fortified bread, with the highest values recorded in bread fortified with 7% dOP. Rheological analysis revealed that the addition of 1% and 2% dOP enhanced overall dough quality by increasing stability, deformation energy, and extensibility compared with the control. However, higher

incorporation levels (5% and 7%) led to significant differences in rheological characteristics of the dough, including increased tenacity, reduced extensibility, and loaf volume, due to the high fiber content of dOP, which interacts with the gluten matrix and modifies dough hydration. Increasing the level of dOP significantly affected the bread crumb color, indicating progressive darkening due to the presence of natural pigments and high phenolic content of dOP. Fortified breads also exhibited a significant color difference ( $\Delta E > 2$ ) and higher BI values, particularly in samples with 7% dOP. In contrast, the incorporation of dOP did not significantly alter crust color, as Maillard and caramelization reactions occurring at high baking temperatures masked color variations. Regarding sensory attributes, breads fortified with 2% and 5% dOP were rated as more acceptable than the control, whereas 7% dOP bread was

Overall, the findings showed that adding a moderate amount of dOP (2-5%) improves the nutritional value and antioxidant stability of bread without harming dough consistency, baking quality, or consumer acceptance. Additionally, the results confirmed that OP is not just a by-product of the olive oil industry but also a low-cost, nutrient-rich ingredient that can be used in food formulations to produce high-value bakery products with extended shelf life and better nutrition, including healthy fats, fiber, protein, minerals, and natural bioactive compounds. Furthermore, using OP as a functional ingredient offers a promising way to boost the nutritional profile of baked goods while supporting circular economy principles in the food industry.

From a sensory and nutritional perspective, future research is required to develop new strategies to mitigate the bitter taste and sandy mouthfeel associated with high levels of OP incorporation in bakery product formulations. In this context, further studies should focus on the extraction of phenolic compounds, the emulsification of phenolic extracts, and the encapsulation of bioactive compounds recovered from OP, to facilitate their utilisation into bakery industry and to improve nutritional quality and antioxidant stability without compromising sensory attributes.

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**Table 1. Physicochemical analysis and fatty acid composition of dried olive pomace.**

Parameters	Ash (%)	Protein (%)	Dietary fiber (%)	Carbohydrates (%)
	3.87±1.08	6.76±0.57	38.5±2.34	33.22±1.43
Parameters	Fat (%)	TPC (mg GAE/g dw)	TFC (mg QE/g dw)	TAA (mg Trolox/g dw)
	13.78±2.06	23.39±2.32	13.00±1.95	15.23±1.02
Parameters	C14:0 Myristic acid (%)	C18:1( <i>n-9</i> ) Oleic acid (%)	C20:0 Arachidic acid (%)	C22:0 Behenic acid (%)
	0.022±0.001	67.779±0.32	0.514±0.05	0.193±0.03
Parameters	C18:2 ( <i>n-6</i> ) Linoleic acid (%)	C18:0 Stearic acid (%)	C16:1( <i>n-9</i> ) Palmitoleic acid (%)	C16:0 Palmitic acid (%)
	11.916±0.84	2.608±0.12	0.379±0.041	10.623±0.74
Parameters	C17:0 Heptadecanoic acid (%)	C17:1 Heptadecenoic acid (%)	C20:1( <i>n-9</i> ) Eicosenoic acid (%)	C24:0 Lignoceric acid (%)
	0.108±0.01	0.159±0.012	0.381±0.021	0.098±0.003
Parameters	C22:1( <i>n-9</i> ) Erucic acid (%)	C18:2 ( <i>n-6</i> ) Linoleic acid (%)	C18:3 ( <i>n-3</i> ) $\alpha$ -Linolenic acid (%)	C12:0 Lauric acid (%)
	0.005±0.000	11.916±1.12	0.930±0.032	ND
Parameters	C10:0 Capric acid (%)	$\Sigma$ SFA (%)	$\Sigma$ MUFA (%)	$\Sigma$ PUFA (%)
	ND	14.53±0.82	68.25±1.05	12.83±0.34

Results are expressed as mean  $\pm$  standard deviation (n=3) on dry weight basis (g/100g dw) of OP. Fatty acid compositions are expressed as relative %. SFA, Saturated fatty acids; MUFA, Monounsaturated fatty acids; PUFA, Polyunsaturated fatty acids. ND, not detected.

**Table 2. Farinograph and alveograph parameters of control and dried olive pomace-fortified dough samples**

Samples	B-CTRL	B-OP-1	B-OP-2	B-OP-5	B-OP-7
<b>Farinograph parameters</b>					
WA (%)*	58.7 <sup>c</sup>	59.1 <sup>b,c</sup>	59.9 <sup>b</sup>	60.6 <sup>b</sup>	61.9 <sup>a</sup>
WA (%)**	58 <sup>b</sup>	58.4 <sup>b</sup>	58.6 <sup>b</sup>	59.9 <sup>a</sup>	60.5 <sup>a</sup>
DDT (min)	2.5 <sup>a</sup>	2 <sup>b</sup>	2 <sup>b</sup>	2 <sup>b</sup>	1.7 <sup>c,b</sup>
ST (min)	9.1 <sup>d</sup>	11 <sup>c</sup>	11.4 <sup>c</sup>	15.4 <sup>b</sup>	17.8 <sup>a</sup>
DS (FU)*	40 <sup>b</sup>	40 <sup>b</sup>	34 <sup>c</sup>	46 <sup>a</sup>	27 <sup>d</sup>
DS (FU)**	64 <sup>a</sup>	58 <sup>b</sup>	44 <sup>c</sup>	35 <sup>d</sup>	12 <sup>c</sup>
FQN	83 <sup>a</sup>	53 <sup>c</sup>	63 <sup>b</sup>	44 <sup>d</sup>	41 <sup>e,d</sup>
<b>Alveograph parameters</b>					
P (mm H <sub>2</sub> O)	104 <sup>d</sup>	115 <sup>c</sup>	115 <sup>c</sup>	140 <sup>b</sup>	163 <sup>a</sup>
L (mm)	91 <sup>b</sup>	106 <sup>a</sup>	85 <sup>c</sup>	44 <sup>d</sup>	37 <sup>c</sup>
G (mm)	21.2 <sup>b</sup>	22.9 <sup>a</sup>	20.5 <sup>b</sup>	14.8 <sup>c</sup>	13.5 <sup>d</sup>
W (10 <sup>-4</sup> J)	318	398	342	262	266
P/L	1.15 <sup>d</sup>	1.08 <sup>d</sup>	1.35 <sup>c</sup>	3.18 <sup>b</sup>	4.41 <sup>a</sup>
Ie (%)	57 <sup>b</sup>	58.9 <sup>a</sup>	58.8 <sup>a</sup>	58.4 <sup>a</sup>	0 <sup>c</sup>

Values in the same line marked with different letters are significantly different (p<0.05) according to Tukey's test. B-CTRL, bread without OP; B-OP-1, bread fortified with 1% OP; B-OP-2, bread fortified with 2% OP; B-OP-5, bread fortified with 5% OP; B-OP-7, bread fortified with 7% OP; WA\*, corrected for 500 FU; WA\*\*, corrected to 14% moisture; DS\*, degree of softening (10 min after begin); DS\*\*, degree of softening (12 min after max); FQN, farinograph quality number; FU, farinograph unit; G, swelling index; Ie, elasticity index.

**Table 3. Physicochemical characteristics of bread samples fortified with different levels of dried olive pomace.**

	B-CTRL	B-OP-1	B-OP-2	B-OP-5	B-OP-7
Moisture (%)	15.53±0.29 <sup>a</sup>	15.13±0.82 <sup>a,b</sup>	15.05±0.17 <sup>b</sup>	13.99±0.83 <sup>c</sup>	13.51±1.04 <sup>c</sup>
Bread volume (cm <sup>3</sup> )	285±7.5 <sup>a</sup>	281±3.2 <sup>a,b</sup>	277±5.6 <sup>a,c</sup>	270±2.8 <sup>b,c</sup>	265±3.6 <sup>c</sup>
Volume reduction (%)		1.40	2.80	5.26	7.01
Baking loss (%)	14.86	13.77	13.09	12.29	11.59
Water activity (aw)	0.930±0.12 <sup>a</sup>	0.930±0.09 <sup>a</sup>	0.932±0.11 <sup>a</sup>	0.933±0.07 <sup>a</sup>	0.935±0.14 <sup>a</sup>
TPC (mg GAE/g dw)	0.68± 0.08 <sup>d</sup>	1.44± 0.07 <sup>c</sup>	1.65± 0.09 <sup>c</sup>	2.01± 0.11 <sup>b</sup>	2.34± 0.14 <sup>a</sup>
TFC (mg QE/g dw)	0.07± 0.01 <sup>b</sup>	0.19± 0.04 <sup>a,b</sup>	0.21± 0.05 <sup>a,b</sup>	0.29± 0.08 <sup>a,b</sup>	0.32± 0.16 <sup>a</sup>
TAA (mg trolox/g dw)	0.04±0.008 <sup>c</sup>	0.23± 0.01 <sup>d</sup>	0.53± 0.02 <sup>c</sup>	0.89± 0.07 <sup>b</sup>	1.10± 0.09 <sup>a</sup>
<b>Crumb color</b>					
L*	69.54±1.32 <sup>a</sup>	60.42±0.98 <sup>b</sup>	56.11±1.02 <sup>c</sup>	45.31±0.87 <sup>d</sup>	43.26±1.31 <sup>d</sup>
a*	2.19±0.25 <sup>c</sup>	3.78±0.38 <sup>b</sup>	4.51±0.13 <sup>b</sup>	7.12±0.64 <sup>a</sup>	7.52±0.41 <sup>a</sup>
b*	13.66±0.15 <sup>c</sup>	15.78±0.17 <sup>b</sup>	16.6±0.51 <sup>b</sup>	20.27±0.37 <sup>a</sup>	20.4±0.64 <sup>a</sup>
ΔE	0 <sup>d</sup>	9.5±0.82 <sup>c</sup>	13.94±1.50 <sup>b</sup>	30.99±0.84 <sup>a</sup>	33.07±1.02 <sup>a</sup>
WI	66.55±1.08 <sup>a</sup>	57.22±1.15 <sup>b</sup>	52.86±0.87 <sup>b</sup>	41.25±1.06 <sup>c</sup>	39.24±0.89 <sup>c</sup>
BI	2.26±0.21 <sup>c</sup>	4.46±0.52 <sup>b</sup>	5.71±0.48 <sup>b</sup>	11.18±1.06 <sup>a</sup>	12.32±0.94 <sup>a</sup>
<b>Crust color</b>					
L*	47.53±1.05 <sup>a</sup>	47.25±0.25 <sup>a,b,d</sup>	49.51±1.32 <sup>a,c</sup>	46.76±0.86 <sup>a,b,d</sup>	44.88±0.38 <sup>b</sup>
a*	17.09±0.05 <sup>a</sup>	15.06±0.16 <sup>b</sup>	13.67±0.12 <sup>c</sup>	13.32±0.25 <sup>c</sup>	13.67±0.32 <sup>c</sup>
b*	33.4±0.42 <sup>a</sup>	31.79±0.09 <sup>a</sup>	28.06±0.63 <sup>c</sup>	29.83±0.84 <sup>b</sup>	29.27±0.92 <sup>b,c</sup>
ΔE	0 <sup>c</sup>	2.6±0.02 <sup>d</sup>	6.63±0.06 <sup>c</sup>	9.25±0.17 <sup>a</sup>	7.44±0.34 <sup>b</sup>

Values in the same line marked with different letters are significantly different (p<0.05). WI, whiteness index; BI, browning index.

**Table 4. Sensory evaluation of fortified bread samples with dried olive pomace.**

Parameters	B-CTRL	B-OP-1	B-OP-2	B-OP-5	B-OP-7
Crumb color	7.00±0.58 <sup>b</sup>	7.67±0.47 <sup>a,b</sup>	7.83±0.90 <sup>a,b</sup>	8.50±0.50 <sup>a</sup>	6.33±0.94 <sup>b,c</sup>
Crust color	7.50±0.50 <sup>b</sup>	7.83±0.37 <sup>b</sup>	8.33±0.75 <sup>a,b</sup>	8.33±0.75 <sup>a,b</sup>	6.83±0.69 <sup>b,c</sup>
Crust firmness	6.83±0.69 <sup>a</sup>	7.33±0.47 <sup>a</sup>	7.67±0.47 <sup>a</sup>	8.00±0.58 <sup>a</sup>	7.50±0.96 <sup>a</sup>
Crumb firmness	6.83±0.69 <sup>c</sup>	7.33±0.47 <sup>a,c</sup>	8.00±0.58 <sup>a,b</sup>	7.83±0.37 <sup>b,c,d</sup>	8.00±0.82 <sup>a,d</sup>
Adhesiveness (crumb)	6.00±0.58 <sup>c</sup>	6.67±0.47 <sup>b,c</sup>	7.33±0.47 <sup>a,b</sup>	8.17±0.37 <sup>a</sup>	8.33±0.47 <sup>a,d</sup>
Large bubbles	8.00±0.58 <sup>a</sup>	7.67±0.47 <sup>a,b</sup>	7.00±0.58 <sup>b</sup>	6.00±0.58 <sup>b,c</sup>	5.33±0.47 <sup>c</sup>
Crumbliness	5.17±1.07 <sup>c</sup>	5.50±0.50 <sup>a,c</sup>	6.67±0.47 <sup>a</sup>	7.33±0.47 <sup>a,b</sup>	7.67±0.94 <sup>a,b</sup>
Bubbles homogeneity	7.67±0.47 <sup>a</sup>	7.67±0.47 <sup>a</sup>	6.67±0.47 <sup>b</sup>	6.33±0.75 <sup>c</sup>	5.50±0.96 <sup>c</sup>
Elasticity	8.00±0.82 <sup>a</sup>	7.50±0.50 <sup>a</sup>	7.00±0.82 <sup>a,b</sup>	6.17±0.69 <sup>b</sup>	5.33±0.47 <sup>b</sup>
Appearance	7.50±0.50 <sup>a,b,c</sup>	7.17±0.37 <sup>b</sup>	8.00±0.58 <sup>a,b</sup>	8.33±0.47 <sup>a</sup>	6.83±0.37 <sup>b,c</sup>
Texture	7.00±0.82 <sup>a</sup>	7.33±0.47 <sup>a</sup>	7.67±0.75 <sup>a,b</sup>	7.67±0.47 <sup>a,b,c</sup>	6.50±0.50 <sup>a,d</sup>
Odor/Aroma	7.33±0.47 <sup>b</sup>	7.50±0.50 <sup>b</sup>	8.17±0.37 <sup>a,b</sup>	8.83±0.37 <sup>a</sup>	7.67±0.94 <sup>b</sup>
Aftertaste	6.67±0.47 <sup>b</sup>	7.50±0.50 <sup>a,b</sup>	8.00±0.58 <sup>a</sup>	8.67±0.47 <sup>a,d</sup>	6.50±0.50 <sup>a,c</sup>
Sandy mouthfeel	0.00±0.00 <sup>d</sup>	0.83±0.69 <sup>c,d</sup>	1.17±0.37 <sup>c</sup>	2.67±0.75 <sup>b</sup>	3.83±0.69 <sup>a</sup>
Overall acceptability	7.33±0.47 <sup>b</sup>	7.33±0.47 <sup>b</sup>	7.67±0.47 <sup>a,b</sup>	8.17±0.37 <sup>a</sup>	6.50±0.50 <sup>b,c</sup>

Values in the same line marked with different letters are significantly different (p<0.05).