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Effect of Olive Pomace on the Nutritional, Technological, and Sensory Properties of Gluten-Free Sourdough Bread

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Abstract

Olive pomace (OP), a by-product of olive oil extraction consisting of seed fragments, pulp residues, peels, and water, is an underutilised source of bioactive compounds with potential applications for improving the nutritional quality of gluten-free foods. This study investigated the effects of OP addition on the nutritional composition, antioxidant compounds, technological properties, and sensory quality of gluten-free sourdough bread. Rice flour, OP, and breads containing 0%, 5%, 10%, 15%, 20%, 25%, and 30% OP were analysed for protein, lipids, ash, soluble and insoluble dietary fibre, free phenolics, tocols, and carotenoids. Technological characteristics, colour, and sensory quality were also assessed. OP contained higher levels of most analysed compounds than rice flour. Consequently, enrichment improved the nutritional composition of the breads. Protein content decreased slightly (from 10.41 to 10.03 g/100 g DM), but lipid and ash contents increased from 1.47 and 1.31 g/100 g DM in the control to 4.84 and 3.85 g/100 g DM in bread containing 30% OP. Soluble, insoluble, and total dietary fibre increased from 0.54, 3.15, and 3.69 g/100 g DM to 1.94, 13.70, and 15.64 g/100 g DM, respectively. Phenolic content rose from 10.8 to 227.4 mg/kg DM, tocols content increased from 38.9 to 58.2 mg/kg DM, while carotenoids made a minimal contribution. Enriched breads exhibited lower volume and greater firmness than the control, whereas bread containing 20% OP achieved the best sensory characteristics. These findings highlight OP as a promising ingredient for valorising olive oil processing by-products while enhancing the nutritional quality of gluten-free sourdough bread.

Keywords: carotenoids; colour; *Olea europaea*; phenolics; sensory evaluation; tocols

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1. Introduction

The olive (*Olea europaea* L.) is a fruit tree native to Asia Minor [1]. Globally, the main producers of olive drupes (a fruit composed of a thin epicarp, a mesocarp or pulp,

and a woody endocarp or stone) are Spain, Italy, Turkey, and Portugal. Of the more than 20 million tonnes of olives produced in 2023, almost 12 million tonnes were from Europe [2]. Table olives are consumed whole or pitted, while olives for oil production undergo crushing (or milling), malaxation, and extraction of virgin oil through mechanical pressing, centrifugation, or percolation. Olive pomace, the solid by-product remaining after the extraction of virgin olive oils, consists of stone fragments, pulp residues, peels, and water in varying percentages [3]. Depending on the extraction method, olive pomace represents 35–40% of the mass of the pressed olives [4] and can be further treated with solvents to extract more oil, added to animal feed [5], used in biogas production [6], or disposed of as fertiliser [7].

Every year the oil industry produces millions of tonnes of olive pomace, which is often difficult and expensive to dispose of, despite being a potential source of low-cost bioactive compounds [8]. Olive pomace contains significant quantities of carbohydrates, minerals, cellulose, hemicellulose, and lignin [9], as well as antioxidants (e.g., α-tocopherol), monounsaturated fatty acids (MUFA) and phenolic compounds, especially hydroxytyrosol and tyrosol derivatives [10]. Therefore, growing interest in its reutilisation has been driven by increased awareness of waste reduction, fostering research into new uses for this by-product in the food, pharmaceutical, and cosmetics industries.

Demand for gluten-free (GF) foods is increasing rapidly, driven not only by the medical needs of people affected by coeliac disease (a life-threatening and chronic autoimmune disease, triggered by gluten consumption in genetically predisposed people), but also by the spreading misconception that gluten-free products are healthier [11]. Gluten is the main protein component of flour in cereals such as durum and bread wheats, triticale, barley, and rye. Following gluten ingestion, coeliac disease leads to inflammation and villi damage in the small intestine, impairing the normal absorption of several essential nutrients (e.g., calcium, iron, and fat-soluble vitamins) [12]. The only therapy is absolute and permanent abstinence from gluten; hence, food for people with coeliac disease is prepared using gluten-free cereals such as maize, rice, millets, pseudocereals, or other gluten-free species (potatoes, legumes, etc.).

Unfortunately, the nutritional composition of many gluten-free foods is often inferior to that of corresponding traditional gluten-containing products, because of a lower concentration of proteins and some micronutrients, such as B vitamins, iron, and calcium. Additionally, their content of carbohydrates, lipids, and sodium is generally higher [13], increasing the risks of obesity, diabetes, and cardiovascular conditions [14], and their flavour and texture are sometimes unpleasant [15]. To alleviate some of the nutritional drawbacks in leavened products such as bread, sourdough starters can be employed to create a microbiological environment where lactic acid bacteria (LAB) and yeast act synergistically, providing functional and nutritional benefits over foods prepared only with baker's yeast [16]. In sourdough fermentation, LAB are primarily responsible for dough acidification through the production of lactic and other organic acids, whereas yeasts contribute mainly to leavening through the production of carbon dioxide. In addition, heterofermentative LAB may contribute to leavening through CO_2 production, although their primary role remains associated with acidification and flavour development [17]. GF sourdough breads have better porosity, limited staling, and superior sensory acceptability than breads from non-fermented flours [18], thus helping to overcome some technological drawbacks while at the same time enhancing the nutritional profile.

The use of olive pomace to improve the nutritional quality of oven-baked products has been suggested and tested by several researchers, including Simsek and Süfer (2021) [19], Cardinali et al. (2024) [20], Dahdah et al. (2024) [21], and Kovač et al. (2025) [22]. More challenging, from a technological perspective, has been the integration of olive pomace

in gluten-free products, because the gluten that triggers coeliac disease also influences several technological characteristics and the sensory quality of foods, while the ingredients currently used as substitutes in GF products often do not have the same properties. Some innovative experiments have been carried out on GF pasta [23], breadsticks [24,25], and cakes [26] but, to the best of current knowledge, no information is available on the effects of olive pomace on GF sourdough breads.

Therefore, considering the steadily increasing interest in novel GF products and the drive within the circular economy towards waste reduction and valorisation of by-products from the food industry, we prepared gluten-free sourdough breads in which the rice component was replaced by 5%, 10%, 15%, 20%, 25%, or 30% olive pomace and evaluated their chemical composition, antioxidant compound profile (phenols, tocopherols, and carotenoids), and textural and sensory characteristics.

2. Materials and Methods

2.1. Materials

The ingredients used in the preparation of the gluten-free sourdough bread enriched with olive pomace were: rice flour (Doves Farm, Foods Ltd., Hungerford, UK), water, a commercial mix (trade name LBB BR, produced by LB Bulgaricum, Sofia, Bulgaria) of thermophilic and mesophilic bacteria (*Lactobacillus bulgaricus*, *Lactobacillus brevis*, *Lactobacillus helveticus*, *Lactobacillus plantarum*, *Lactococcus lactis* ssp. *cremoris*, *Lactococcus lactis* ssp. *lactis*, *Lactococcus lactis* ssp. *lactis* var. *diacetylactis*, and *Leuconostoc mesenteroides* ssp. *cremoris*), unflavoured whey protein (Everbuild Whey Build, New Zealand), sugar (Zahira, Agrana Bulgaria, Sofia, Bulgaria), salt (Izzi, Global Food Company IZZI, Sofia, Bulgaria), dry yeast (Dr. Oetker, Bielefeld, Germany), xanthan gum (ZoyaBG, Sofia, Bulgaria), sunflower oil (Kaliakra, Dobrich, Bulgaria), corn starch (Dr. Oetker Kft, Budapest, Hungary), and olive pomace, prepared under laboratory conditions.

2.2. Methods

2.2.1. Production of Olive Pomace (OP)

Olives of the Kalamata variety were purchased from a local market in Kamena Vourla, Central Greece. The olives were cleaned and inspected to ensure consistent quality before processing. Cold pressing was performed at 37 °C using a 650 W screw press (Camry CR 4011, Riga, Latvia), yielding extra virgin olive oil and, as a by-product, semi-defatted olive pomace (OP). The freshly obtained OP had a moisture content of 65% and a residual oil content of 8.9%. The pomace was subsequently dried at 45 °C for 48 h to reach a final moisture content of 8.68% (8.678 ± 0.002 g/100 g). After drying, the OP was milled and sieved to obtain a particle size of <500 µm. The prepared OP was stored at −18 °C until further use in the bread formulations. These standardised processing conditions, together with the initial moisture and residual oil content, particle size and storage conditions, were recorded to improve the reproducibility of OP preparation and its subsequent application in gluten-free bread.

2.2.2. Production of Gluten-Free Sourdough Bread

Gluten-free sourdough breads containing different amounts of OP (0%, 5%, 10%, 15%, 20%, 25% and 30%) were produced in the laboratories of the Technical University of Sofia, College Sliven, Bulgaria. The sourdough was initiated by incubating at 30 °C for 24 h a mixture of 10 g rice flour, 20 mL water and 0.25 g starter culture (described in Section 2.1). The sourdough was prepared by direct inoculation and used without back-slopping or successive refreshment steps. After 24 h of incubation, the pH of the sourdough was

4.24 ± 0.01. Then, 90 g rice flour (control) or a mixture of rice flour and olive pomace (85:5, 80:10, 75:15, 70:20, 65:25 or 60:30), whey protein (5 g), corn starch (10 g), salt (1.8 g), sugar (2 g), and dry yeast (6 g) were blended with a 1900 W spiral mixer (RL-PKM1900.7BG, China) for 2 min. The incubated sourdough, sunflower oil (5 mL), xanthan gum (4 g) and water (100 mL) were added to the dry mixtures and kneaded for 10 min. After kneading, the doughs were placed in rectangular moulds (12 × 8 × 5 cm) and fermented for 45 min at 30 °C. Baking was carried out in a preheated oven using a two-stage temperature profile: initially at 200 °C for 5 min, followed by 175 °C for 40 min. This two-stage baking approach was used to ensure rapid setting of the gluten-free dough structure at the initial higher temperature, followed by uniform baking and moisture removal without excessive surface browning. Seven types of bread were produced: one control (0% OP) and six with increasing OP contents (5%, 10%, 15%, 20%, 25% and 30%). All breads were prepared in triplicate batches and cooled for 120 min at room temperature before being weighed and analysed.

2.2.3. Chemical Analyses

The moisture and ash contents of rice flour, olive pomace and breads were determined according to methods 44-15.02 [27] and 08-01.01 [28] respectively. Protein content was determined using an automatic Kjeldahl apparatus (VELP Scientifica, Usmate (MB), Italy, model UDK-129) according to AOAC Method 920.87 [29]. Lipid content was measured according to method ISO 6492:1999 [30]. Soluble dietary fibre (SDF), insoluble dietary fibre (IDF) and total dietary fibre (TDF) were measured following AACC Method 32-05.01 [31], using a dedicated kit (Megazyme, Bray, Ireland). Total digestible carbohydrates were calculated by difference, using the following formula:

$$[100 - (moisture + ash + protein + fat + dietary\ fibre)] \quad (1)$$

The energy value was calculated using the following conversion factors: protein 17 kJ/g, lipid 37 kJ/g, available carbohydrates 17 kJ/g, and dietary fibre 8 kJ/g, in accordance with the conversion factors specified in Regulation (EU) No. 1169/2011 [32] and considering the relevance of the dietary fibre conversion factor discussed by Menezes et al. (2016) [33]. Water activity (Aw) was determined at 25 °C with an Aqualab (Series 3 Quick Start, Decagon Device, Inc., Pullman, WA, USA). The pH of the fermented sourdough was measured after 24 h of fermentation at 30 °C using a portable pH meter (MW102 PRO, Milwaukee Instruments Kft., Szeged, Hungary). In addition, pH was determined for all dough formulations containing 0, 5, 10, 15, 20, 25 and 30% olive pomace (OP) at two stages: immediately after the incorporation of OP during dough mixing and after the main dough fermentation period of 45 min. All analyses were performed on two loaves obtained from three independently produced batches for each bread formulation.

2.2.4. Antioxidant Analysis

For the antioxidant analysis (DPPH method), bread samples containing 10%, 20%, and 30% OP were selected to represent low, intermediate, and high levels of OP enrichment, respectively, allowing the effect of increasing OP incorporation on the concentration of antioxidant compounds in the bread to be evaluated.

2.2.5. Free Phenolic Compounds

The free soluble fraction of phenolic substances was analysed by HPLC [34]. Briefly, 1.0 g of raw material or bread was weighed into a centrifuge tube and 15 mL of an 80:20 v/v methanol-water HPLC solution was added. After stirring with a TX4 Digital IR Vortex Mixer (VELP Scientifica, Srl, Usmate, Italy), the sample was ultrasonicated in an FS200b bath (Decon, Hove, UK) for 5 min and then shaken in a PTR-35 shaker (Grant, Instruments

(Cambridge) Ltd., Shepreth, UK) for 30 min in a light-proof refrigerated environment. After centrifugation at 15,000× *g* for 10 min at 8 °C in a LISA refrigerated centrifuge (AFI Groups, Château-Gontier, France), the supernatant was recovered and the extraction was repeated twice by adding 15 mL of extraction solvent to the remaining sediment. The three extracts (total volume 45 mL) were combined and evaporated under vacuum for 30 min at 35 °C with a Laborota 4000 Efficient rotavapor (Heidolph Instruments GmbH & Co. KG, Schwabach, Germany). The residue was then thoroughly dried under a nitrogen stream and quantitatively resuspended in 2 mL of methanol (80:20, *v*/*v*; chromatographic grade). Thus, the 2 mL represented the final volume of the extract used for quantification, rather than a dilution of the initial 45 mL extraction volume. The extract was then filtered through a 0.45 µm PTFE membrane (Diana Beck Scientific, Angera, Italy), transferred to an amber glass vial, and stored at −20 °C until injection.

The system used for chromatographic analysis consisted of a Rheodyne injector with a 20 µL loop, an L-2130 Elite LaChrom pump (Hitachi High-Technologies Corporation, Tokyo, Japan), an Adamas C18 column (250 mm × 4.6 mm, 5 µm) coupled with an Adamas C18 guard column (10 mm × 4.6 mm, 5 µm) (Sepachrom SRL, Rho, Italy) thermostatted at 30 °C with an L-2300 Elite LaChrom oven (VWR, Hitachi, Japan), a Diode Array Detector L-2450 Elite LaChrom detector (Merck, Hitachi, Japan), and EZChrom Client/Server software version 3.1.7. Elution was carried out with a gradient of formic acid in water and acetonitrile (99:1, *v*/*v*) at a flow rate of 1 mL/min. The gradient program was as follows: 10.0–25.0% A from 0 to 10 min, 25.0–60.0% A from 10 to 20 min, and 60.0–70.0% A from 20 to 30 min. The mobile phase was then returned to the initial composition (10.0% A) over 10 min, followed by a 5 min re-equilibration period. Phenolic compounds were quantified at 280 nm, except for caffeic acid, rosmarinic acid, and apigenin, which were quantified at 320 nm, using external standards and calibration curves. Compounds were identified by comparing retention times and spectra with those of the standards; unidentified peaks were grouped based on the similarity of their absorbance spectra to those of the standards and tentatively classified as their derivatives, as proposed by Estivi et al. (2022) [35]. Analyses were performed on two loaves obtained from three independently produced batches of each bread formulation, and mean results are presented as mg/kg dry matter (DM).

2.2.6. Tocopherols and Carotenoids

Tocol and carotenoid extracts were obtained after thermal saponification as described in Brandolini et al. (2022) [36]. Briefly, 1 g of sample was placed in a glass tube and stirred with a TX4 Digital IR Vortex Mixer (VELP Scientifica, Srl, Usmate, Italy) upon the addition of each of the following reagents: 2.5 mL 60 g/L pyrogallolic ethanol, 1 mL 95% ethanol, 1 mL 10 g/L sodium chloride, and 1 mL 600 g/L potassium hydroxide. Saponification was carried out in the presence of nitrogen at 70 °C for 45 min in the dark. During saponification, the tubes were vortexed every 10 min. After cooling in an ice bath, 7.5 mL of 10 g/L sodium chloride was added, and the mixture was transferred into 250 mL flasks, where it underwent liquid–liquid extraction twice by stirring with 15 mL of n-hexane: ethyl acetate 90:10 (*v*/*v*) and centrifuged at 15,000× *g* and 8 °C for 10 min. The supernatants were pooled; the solvent was removed under vacuum in a Laborota 4000 Efficient rotavapor (Heidolph Instruments GmbH & Co. KG, Schwabach, Germany) at 35 °C for 5 min; and the extracts were dried under nitrogen flow for 1 min, resuspended in 2 mL of n-hexane:2-propanol 99:1 (*v*/*v*), and filtered with a 0.45 µm PTFE membrane (Diana Beck Scientific, Italy). The analysis was performed by normal-phase HPLC [35], in a system composed of a Rheodyne injector with a 50 µL loop, a non-thermostated Adamas® Silica column (250 mm × 4.6 mm, 5 µm) with a pre-column (10 × 4.6 mm, 5 µm) (Sepachrom SRL, Rho, Italy), a L-2130 Elite LaChrom pump (VWR, Hitachi, Japan), and an 821 FP fluorimetric detector (Jasco,

Hachioji, Tokyo, Japan), set at excitation wavelengths of 290 nm and emission wavelengths of 330 nm, controlled by Empower 2 software (Waters Chromatography Division, Milford, MA, USA) via the SAT/IN interface (Waters). The mobile phase was a 97.3:1.8:0.9 mixture of n-hexane: ethyl acetate: acetic acid ($v/v/v$), at a flow rate of 1.6 mL/min.

The tocol peaks were quantified with α-tocopherol, β-tocopherol, γ-tocopherol, and δ-tocopherol external standards, and the tocotrienols with the standard curves of the corresponding tocopherols. The carotenoids were quantified using the same instrumentation, but with a PDA 2996 detector (Waters Chromatography Division, Millipore, Milford) at a wavelength of 445 nm and a mobile phase of 95:5 n-hexane:2-propanol (v/v) at a flow rate of 1.5 mL/min.

For carotenoid and tocopherol quantification, calibration curves were built using standards of lutein (Fluka, St. Louis, MO, USA), β-carotene (Sigma, St. Louis, MO, USA), zeaxanthin and β-cryptoxanthin (Extrasynthese, Genay, France), α-tocopherol (Fluka Bio-Chemika, Buchs, Switzerland), and β-tocopherol, γ-tocopherol, and δ-tocopherol (Supelco, Bellefonte, PA, USA). The tocotrienols were quantified using the standard curves of their corresponding tocopherols. All analyses were performed on two loaves obtained from three independently produced batches of each bread formulation. The mean results are reported as mg/kg dry matter (DM).

2.2.7. Physical Characteristics, Colour and Texture

The specific volume of the different breads was assessed with a Volscan Profiler (Stable Micro Systems Ltd., Godalming, Surrey, UK). Measurements were performed on two loaves obtained from three independently produced batches of each bread formulation. Crust and crumb colours were evaluated with a Chroma Meter CR-400 colorimeter (Konica Minolta, Tokyo, Japan). Colour measurements were performed on two loaves obtained from three independently produced batches. The crust and central cross-section colour of the loaves were measured at ten randomly selected points.

The texture profile analysis (TPA) of the bread samples was performed using an EZ Test Texture Analyzer (EL-LX, Shimadzu Corp., Kyoto, Japan). Central slices of the bread loaves underwent double compression to 40% thickness (10 mm). Compression was applied using an aluminium cylindrical attachment with a diameter of 36 mm. Trapezium X software (v2.0.2, Shimadzu Corp., Kyoto, Japan) was used to collect force–time data at a constant crosshead rate of 1 mm/s. A 5 s delay separated the two compression cycles. Analysis of the resulting curves provided values for hardness, springiness, cohesiveness, resilience, and chewiness. Each measurement was performed on twelve bread slices obtained from three independently produced batches of each bread formulation. One loaf was selected from each independent batch, and four slices were taken from each loaf for TPA. The results are reported as mean ± standard deviation.

2.2.8. Sensory Analysis

The preliminary sensory analysis was conducted in a quiet, comfortable testing area, with the samples presented under consistent conditions. It involved twenty-six semi-trained panellists to provide preliminary information on differences among the breads [37], rather than a large-scale consumer hedonic test. The test was approved by the Ethics Committee of the Technical University of Sofia, Bulgaria (Approval No. 3, dated 30 June 2025) and was carried out at the Technical University of Sofia, College of Sliven, Bulgaria. Twenty-six semi-trained panellists, after reading and signing informed consent in accordance with the ethical guidelines for food-related research defined by the European Union [38], participated in the trial. The bread loaves were baked on the day of testing, cooled to room temperature, and cut into 2-cm slices. The samples were coded with three-digit random numbers and

presented in randomised order to minimise potential order effects. Each panellist evaluated the samples independently, without discussion with other panellists. Water was provided for palate cleansing between samples. Before the evaluation, the panellists received a brief orientation regarding the sensory attributes, use of the 9-point hedonic scale, and the evaluation procedure [39]. The evaluation followed a structured 9-point hedonic scale, assessing volume, smell, crust colour, crumb colour, chewability, texture, taste and aftertaste, where 1 = extreme dislike and 9 = extreme like [40].

2.2.9. Statistical Analysis

Bread samples were prepared in three independent production batches, which served as the experimental units for all chemical, physical, colour, and texture analyses. For each production batch, determinations were performed using the appropriate number of analytical replicates. These replicate measurements were averaged within each independent batch before statistical analysis. Thus, the sample size for statistical comparisons among the bread formulations was $n = 3$ independent batches, rather than the total number of analytical determinations. Results are expressed as mean ± standard deviation (SD), with the SD calculated from the three independent batches. Data were analysed using a one-way analysis of variance (ANOVA), and significant differences between means were identified using Fisher's least significant difference (LSD) test at $p < 0.05$. For the sensory evaluation, 26 independent panellists evaluated all seven bread formulations. A repeated measures ANOVA, followed by Fisher's LSD test ($p < 0.05$), was applied to account for the panellist effect and the repeated-measures structure of the data, with each panellist treated as an independent subject. The sensory statistical analysis therefore accounted for the panellists' effect and the repeated-measures structure of the data. Statistical analyses were performed using software XLSTAT 2019 (Addinsoft Inc., Long Island City, NY, USA) and Microsoft Office Excel 2019 (Microsoft, Redmond, WA, USA).

3. Results and Discussion

3.1. Rice Flour and Olive Pomace Composition

ANOVA revealed significant differences between rice flour and olive pomace for all the compounds analysed. The moisture content of rice flour (8.838 ± 0.003 g/100 g) was slightly but significantly higher ($p = 0.0009$) than that of olive pomace (8.678 ± 0.002 g/100 g), while its protein content (7.49 ± 0.06 g/100 g DM) was markedly higher than that of olive pomace (5.90 ± 0.01 g/100 g DM). The OP protein content was lower than the values reported by Caponio et al. (2022) [24] and De Gennaro et al. (2022) [25], as well as those (9.83 and 12.00 g/100 g DM) reported by Balbinot Filho et al. (2022) [26] and Simsek and Süfer (2021) [19], whereas rice flour showed a higher protein content than the value (5.55 g/100 g DM) stated by Ren et al. (2024) [41] and a similar value (7.29 ± 0.02 g/100 g DM) to that reported by Altındag et al. (2014) [42].

The lipid and ash contents of olive pomace (11.24 ± 0.02 and 12.06 ± 0.42 g/100 g DM, respectively) were significantly higher than those of rice flour (1.42 ± 0.02 and 0.87 ± 0.01 g/100 g DM). Several OP lipid contents are reported in the literature, probably due to different olive varieties, climatic conditions, maturity, cropping practices, oil extraction methods, presence of stone fragments, etc., and range from 8.43 g/100 g DM in an industrial by-product [43] and or 15.78 g/100 g DM in olive pomace flour [19] up to 22.78 g/100 g DM [25] and 23.53 g/100 g DM [26] in freeze-dried olive cake powder. Olive oil extraction technology is one of the most important factors, as two-phase, three-phase, and traditional pressing systems differ in their efficiency of oil separation and consequently in the amount of residual oil retained in the pomace. Furthermore, the relative proportions of pulp, skin, and stone in the resulting pomace can also influence its lipid content. Therefore, the

differences between the lipid content observed in the present study and those reported in previous studies can be attributed to variations in olive characteristics, oil extraction technology, and subsequent processing and drying conditions of the OP samples [44]. The same considerations apply to ash content, which reportedly may vary from 4.54 g/100 g DM [26] to 9.43 g/100 g DM [24].

Olive pomace contained far more soluble, insoluble and total dietary fibre (6.08 ± 0.11, 43.79 ± 0.33, and 49.87 ± 0.22 g/100 g DM, respectively) than rice flour (0.63 ± 0.04, 3.55 ± 0.07, and 4.18 ± 0.04 g/100 g DM). Similarly, high dietary fibre concentrations in OP (48.00–57.96 g/100 g DM) have been reported by Simsek and Süfer (2021) [19] and Simonato et al. (2019) [45], while rice flour, mainly composed of starch, is notoriously poor in such compounds [46]. Conversely, the maximum digestible carbohydrate content was found in rice flour (87.56 ± 0.02 g/100 g DM), while olive pomace showed significantly lower levels (17.46 ± 0.05 g/100 g DM). The rice results are marginally lower than those (89–91 g/100 g DM) reported by Abdelsalam et al. (2025) [47], while the literature reports OP values ranging from 28.5 g/100 g DM (computed from Simonato et al., 2019) [45] to 58.07 g/100 g DM [24].

Among the phenols, only ferulic and *p*-coumaric acids were detected in the rice flour, as already observed by Irakli et al. (2012) [48] and Mattila et al. (2005) [49]; in contrast, in olive pomace seven phenolic acids (cinnamic, ferulic, gallic, *p*-coumaric, protocatechuic, vanillic, and *p*-coumaric der) and five flavonoids (diosmin, luteolin, apigenin der, catechin der, rutin der), as well as catechol, were found (Table 1). Despite being typical phenolic compounds of olives, oleuropein, tyrosol, and hydroxytyrosol were not identified in the pomace. Their absence could be related to transformations occurring during processing and the subsequent prolonged drying stage at mild temperature (45 °C) in the presence of oxygen. In particular, oleuropein may undergo enzymatic hydrolysis, mainly catalysed by β-glucosidase [50], while drying conditions have been reported to affect the phenolic profile and antioxidant capacity of olive pomace [51]. Therefore, enzymatic and oxidative reactions occurring during processing and drying could have contributed to the absence of these compounds in the analysed pomace. However, since enzymatic activity was not directly assessed in the present study, this explanation should be considered a hypothesis.

Overall, in OP total flavonoids were more abundant than total phenolic acids (998.9 ± 30.8 vs. 265.5 ± 3.7 mg/kg DM, respectively).

Table 2 reports the tocol and carotenoid contents of rice flour and olive pomace. The rice flour contained little β-tocopherol and γ-tocotrienol, while the olive pomace was characterised by a substantial amount of α-tocopherol (almost 200 mg/kg DM) and smaller quantities of γ-tocopherol, δ-tocotrienol and β-tocopherol. Minimal β-tocopherol and γ-tocotrienol contents have been reported in rice flour [52]. Nunes et al. (2018) [10] detected traces of β-tocopherol, γ-tocopherol and α-tocotrienol in OP, along with 7.64 ± 0.81 mg/100 g DM of α-tocopherol. The total tocol content of OP (222.8 ± 8.8 mg/kg DM) was approximately twenty times greater than that of rice flour (11.28 ± 0.30 mg/kg DM).

Carotenoids were present only in the olive pomace (8.2 ± 0.4 mg/kg DM); the most abundant compounds were β-carotene and lutein, followed by β-cryptoxanthin and zeaxanthin.

From a food safety perspective, the use of olive pomace (OP) as a food ingredient requires appropriate control of its microbiological and chemical quality. Previous research has shown that olive pomace and related olive-processing by-products may harbour microorganisms, including Enterobacteriaceae, yeasts and moulds, highlighting the importance of adequate processing and storage conditions before their incorporation into food products [53]. More recently, the microbiological stability of OP has been shown to depend on storage temperature and duration, with higher microbial counts, particularly of lactic

acid bacteria and yeasts, observed under favourable temperature conditions [54]. In the present study, the freshly obtained OP was dried at 45 °C for 48 h to reduce its moisture content and subsequently stored at −18 °C until use. These controlled conditions were applied to reduce moisture availability and limit microbial proliferation during storage. However, microbiological analyses were not performed in the present study; therefore, the microbiological safety of the prepared OP cannot be directly confirmed.

Table 1. Free phenolic acids, flavonoids, and catechol content (mg/kg DM ± standard deviation) in rice flour (RI), olive pomace (OP), gluten-free sourdough control bread (Ctrl) and gluten-free sourdough breads enriched with 10% (O10), 20% (O20) or 30% (O30) olive pomace. Different capital letters indicate statistical differences ($p < 0.05$) between raw materials (RI and OP), whereas different lowercase letters indicate statistically significant differences ($p < 0.05$) among the bread formulations (Ctrl, O10, O20, and O30), according to Fisher's LSD test.

	Raw Materials			Breads		
	RI	**OP**	**Ctrl**	**O10**	**O20**	**O30**
Phenolic acids						
Cinnamic		13.88 ± 0.62		21.12 ^{a} ± 0.36	19.73 ^{a} ± 0.17	16.84 ^{b} ± 1.29
Ferulic	3.30 ^{B} ± 0.29	16.41 ^{A} ± 1.33	3.19 ^{c} ± 0.13	3.32 ^{c} ± 0.20	4.10 ^{b} ± 0.20	5.39 ^{a} ± 0.50
Gallic		32.54 ± 1.51				2.93 ± 0.26
p-coumaric	0.69 ^{B} ± 0.15	19.43 ^{A} ± 0.95	1.27 ^{d} ± 0.05	3.42 ^{c} ± 0.08	4.68 ^{b} ± 0.34	5.67 ^{a} ± 0.37
p-coumaric *		47.94 ± 1.23	2.58 ^{c} ± 0.39	3.30 ^{c} ± 0.34	7.84 ^{b} ± 0.58	10.94 ^{a} ± 0.18
Protocatechuic		86.16 ± 5.41		5.33 ^{c} ± 0.24	10.42 ^{b} ± 0.22	32.17 ^{a} ± 1.36
Vanillic		49.14 ± 2.13	1.90 ^{c} ± 0.34	7.57 ^{b} ± 0.21	11.17 ^{a} ± 0.59	12.30 ^{a} ± 1.05
Flavonoids						
Apigenin *		30.25 ± 2.60		1.68 ^{c} ± 0.06	3.28 ^{b} ± 0.17	4.10 ^{a} ± 0.21
Catechin *		113.6 ± 0.9		45.15 ^{c} ± 1.80	52.06 ^{b} ± 2.61	59.82 ^{a} ± 0.78
Diosmin		200.6 ± 16.3		18.59 ^{c} ± 1.35	34.59 ^{b} ± 0.35	44.35 ^{a} ± 0.88
Luteolin		175.5 ± 0.03		23.70 ^{b} ± 1.36	24.38 ^{b} ± 0.48	29.89 ^{a} ± 0.70
Rutin *		65.25 ± 4.01		1.59 ^{c} ± 0.05	2.13 ^{b} ± 0.01	2.98 ^{a} ± 0.12
Catechol		413.7 ± 7.0		24.37 ^{c} ± 0.97	68.28 ^{b} ± 4.93	78.20 ^{a} ± 1.17

*: tentatively identified derivative.

Table 2. Tocol and carotenoid contents (mg/kg DM ± standard deviation) in rice flour (RI), olive pomace (OP), gluten-free sourdough control bread (Ctrl) and gluten-free sourdough breads enriched with 10% (O10), 20% (O20) or 30% (O30) olive pomace. Different capital letters indicate statistical differences ($p < 0.05$) between raw materials or among breads (lowercase), according to Fisher's LSD test.

	Raw Materials			Breads		
	RI	**OP**	**Ctrl**	**O10**	**O20**	**O30**
Tocols						
α-tocopherol		199.66 ± 10.16	30.68 ^{c} ± 0.39	41.05 ^{b} ± 1.21	43.68 ^{b} ± 2.04	52.66 ^{a} ± 0.18
β-tocopherol	5.04 ^{A} ± 0.35	2.21 ^{B} ± 0.15	3.93 ^{a} ± 0.32	3.95 ^{a} ± 0.47	3.80 ^{a} ± 0.25	3.12 ^{b} ± 0.03
γ-tocopherol		15.87 ± 1.01				
γ-tocotrienol	6.03 ± 0.24		4.65 ^{a} ± 0.29	4.32 ^{a} ± 0.10	2.91 ^{b} ± 0.22	2.42 ^{c} ± 0.05
δ-tocotrienol		5.00 ± 0.22				
Carotenoids						
β-carotene		3.98 ± 0.05		0.18 ^{c} ± 0.01	0.29 ^{b} ± 0.01	0.45 ^{a} ± 0.01
β-cryptoxanthin		0.34 ± 0.06				0.06 ± 0.01
Lutein		3.50 ± 0.21		0.32 ^{c} ± 0.02	0.53 ^{b} ± 0.04	0.64 ^{a} ± 0.04
Zeaxanthin		0.10 ± 0.009		0.04 ^{a} ± 0.003	0.04 ^{a} ± 0.003	0.05 ^{a} ± 0.003

In addition to microbiological hazards, the agricultural and processing history of olives should be considered when OP is intended for food applications. Olive-derived products may be exposed to contaminants such as pesticide residues, mycotoxins and polycyclic aromatic hydrocarbons (PAHs), and their occurrence may depend on raw-material origin and processing conditions [4]. Therefore, the use of market-derived OP for food applications should be accompanied by appropriate microbiological and chemical quality-control measures. The present study demonstrates the technological and nutritional potential of OP for bread enrichment under the controlled processing and storage conditions applied, but it does not establish its universal microbiological or chemical safety. Future studies should therefore include microbiological characterisation and screening for relevant chemical contaminants before OP is considered for broader industrial or commercial food applications.

3.1.1. Composition of the Breads

The ANOVA revealed significant differences among the bread samples for all the compounds analysed. Gradual addition of olive pomace increased moisture (Figure 1) from 40.2 ± 0.7 g/100 g in the control bread to 42.5 ± 0.4 g/100 g in O30. Dietary fibres are well known for their high water-holding capacity, which can significantly influence the moisture content of baked goods. By binding free water within the dough matrix, fibres reduce water mobility and limit water evaporation during baking, resulting in a final product with higher moisture [55]. In the present study, the amount of added water was kept constant at 100 mL for all formulations. Therefore, the observed increase in moisture content may be associated with the water-binding capacity of the fibre-rich OP; however, this mechanism cannot be confirmed directly, as water absorption and dough consistency were not measured. Water activity (Aw) values of the gluten-free sourdough breads ranged from 0.84 to 0.86 (Figure 1). No statistically significant differences were observed between the control bread and the formulations containing 5, 10, and 15% olive pomace, all of which showed an Aw value of 0.86. At higher incorporation levels, a slight reduction in Aw was observed, with values of 0.85, 0.84, and 0.84 for breads containing 20, 25, and 30% olive pomace, respectively. According to Fisher's LSD test, the 30% OP formulation showed a significantly lower Aw than the control ($p < 0.05$), whereas the 20 and 25% formulations exhibited intermediate values. These results are consistent with previous studies showing that incorporation of plant-derived by-products or dietary fibre can modify water availability and water binding in bread. Azadfar et al. (2023) [56] reported Aw values of 0.70–0.72 in wheat bread containing 0–15% olive pomace dietary fibre, with no significant differences between the control and breads containing 5 and 10% fibre. The authors attributed the behaviour of water in fibre-enriched breads to the ability of hydrophilic groups in dietary fibre to bind water and form bound-water fractions. In another study, Dahdah et al. (2024) [21] investigated bread supplemented with freeze-dried olive pomace and monitored Aw during storage, demonstrating that water activity is an important quality parameter affected by olive pomace incorporation and storage conditions. The differences between the Aw values observed in the present study and those reported previously may be related to differences in bread formulation, type and concentration of olive pomace or dietary fibre, moisture content, and processing conditions.

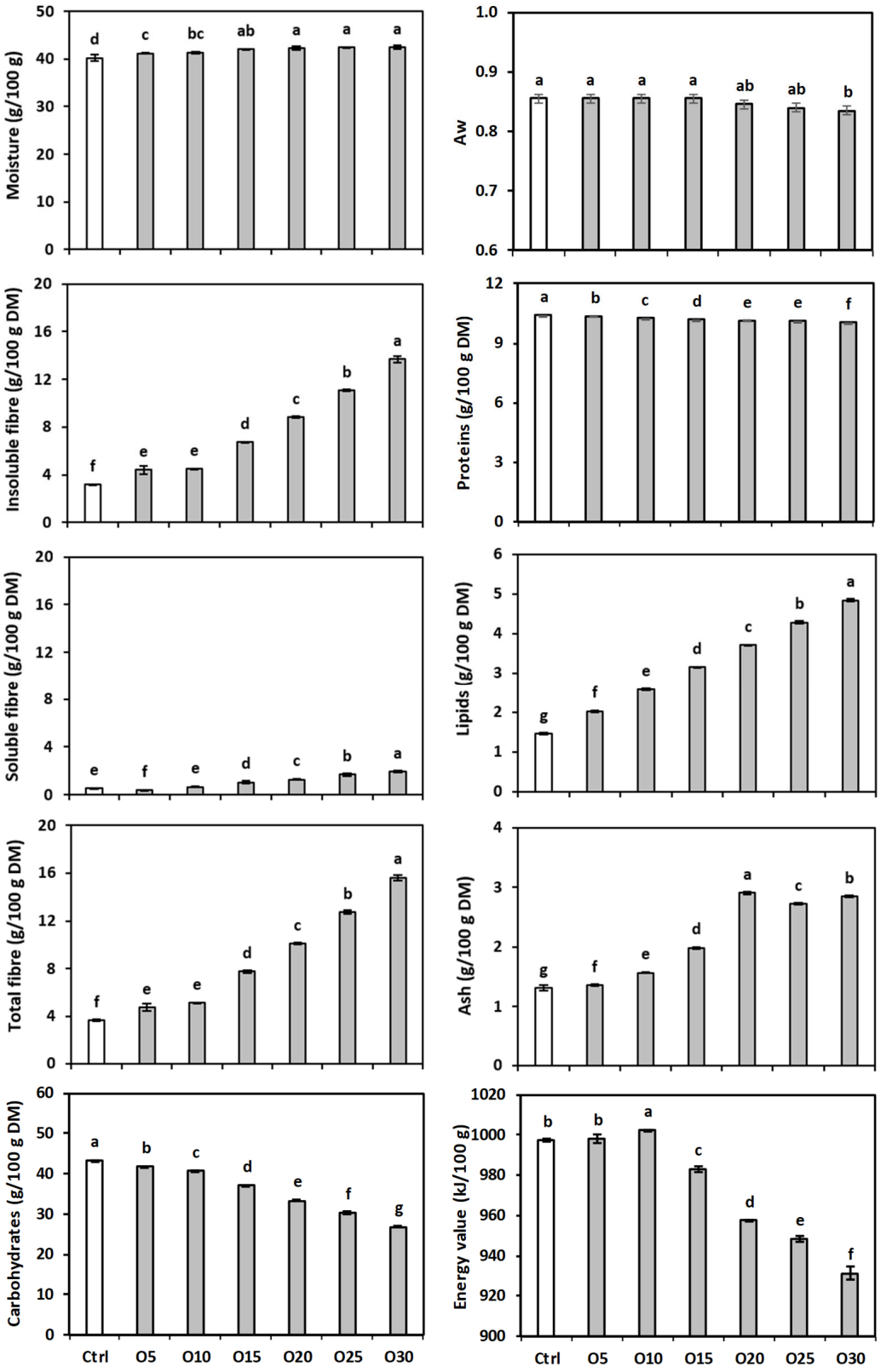


Figure 1. Chemical composition of gluten-free sourdough control bread (Ctrl) and gluten-free sourdough breads enriched with 5% (O5), 10% (O10), 15% (O15), 20% (O20), 25% (O25) or 30% (O30) olive pomace. The available carbohydrates were calculated by difference. The bars represent the standard deviation. Different letters indicate statistical differences ($p < 0.05$) according to Fisher's LSD test.

Enrichment with olive pomace slightly reduced protein content (−4%) and increased lipid and ash contents in line with the quantity of by-product added (Figure 1), rising by 230% and 193%, respectively, in the O30 breads compared with the control. In contrast, the available carbohydrates in the control bread, whose main ingredient was rice flour, were significantly higher than in the enriched breads, decreasing from 45.5% to 28.2% in O30 (i.e., −38.05%), as OP in the dough increased. Another notable aspect is that fibre content rose in the OP-enriched breads according to the percentage addition. Dietary fibre fractions showed strong positive linear relationships with OP incorporation. SDF, IDF and TDF increased progressively with increasing OP level, with coefficients of determination (R^2) of 0.946, 0.954 and 0.954, respectively. Total dietary fibre, mainly insoluble (>85%), was 7.8% in O15, 10.1% in O20, 12.7% in O25 and 15.6% in O30, values comparable to those of gluten-free sourdough breads enriched with hemp press cake meal [57]. The increase in dietary fibre can be attributed to the high fibre content of OP and the increasing proportion of OP incorporated into the bread formulation. Overall, the addition of 30% OP resulted in increases of 259%, 335%, and 324% in soluble, insoluble, and total dietary fibre, respectively, compared with the control bread [58]. The energy value of the breads increased slightly at lower OP levels (up to 1002.5 kJ/100 g in O10) but progressively declined at higher substitution levels, reaching its lowest value in O30 (931.4 kJ/100 g). This trend is directly linked to the marked reduction in available carbohydrates and the higher fibre content, which outweighed the caloric contribution of the increased lipid content. This reduction in energy value represents a 6.6% decrease compared with the control, improving the nutritional profile of the enriched gluten-free breads.

3.1.2. Phenolics Content of the Breads

Only ferulic, *p*-coumaric, a *p*-coumaric derivative and vanillic acids were present in the control bread (Table 1). Evidently, ferulic acid and, to some extent, *p*-coumaric acid were provided by the rice flour, while the *p*-coumaric derivative and vanillic acid, along with some *p*-coumaric acid, were derived from the sunflower oil used in the formulation [58]. The total phenolic concentration in the control bread was 8.94 ± 0.13 mg/kg DM (Figure 2). In the breads enriched with olive pomace, ferulic and *p*-coumaric acids increased in line with the by-product addition, and in O30 their level were +63.5% and +150.5% higher than in the control, respectively. An even more pronounced increase was observed for the *p*-coumaric derivative (+281.8%) and vanillic acid (+415.6%). Protocatechuic acid, not detected in the control, increased progressively in the enriched breads, reaching 32.2 mg/kg DM in O30. Gallic acid from OP was detected only in the O30 breads; in O10 and O20 it was below the detection limit. In general, phenolic acids from the olive pomace increased in the breads in line with the enrichment level, with the notable exception of cinnamic acid, whose decrease may be due to the combined effect of higher olive pomace incorporation and thermal processing during baking, which could have promoted selective degradation and/or reduced extractability through interactions with the fibre-rich bread matrix [59–61]. As mentioned above, flavonoids and catechol were present only in the OP; therefore their concentration increased rapidly in the enriched breads according to the by-product percentage. Overall, the addition of olive pomace increased the total phenolic content from 8.94 mg/kg DM (control) to 133.3 ± 5.7 mg/kg DM (O10), 242.7 ± 9.6 mg/kg DM (O20) and 305.6 ± 5.3 mg/kg DM (O30).

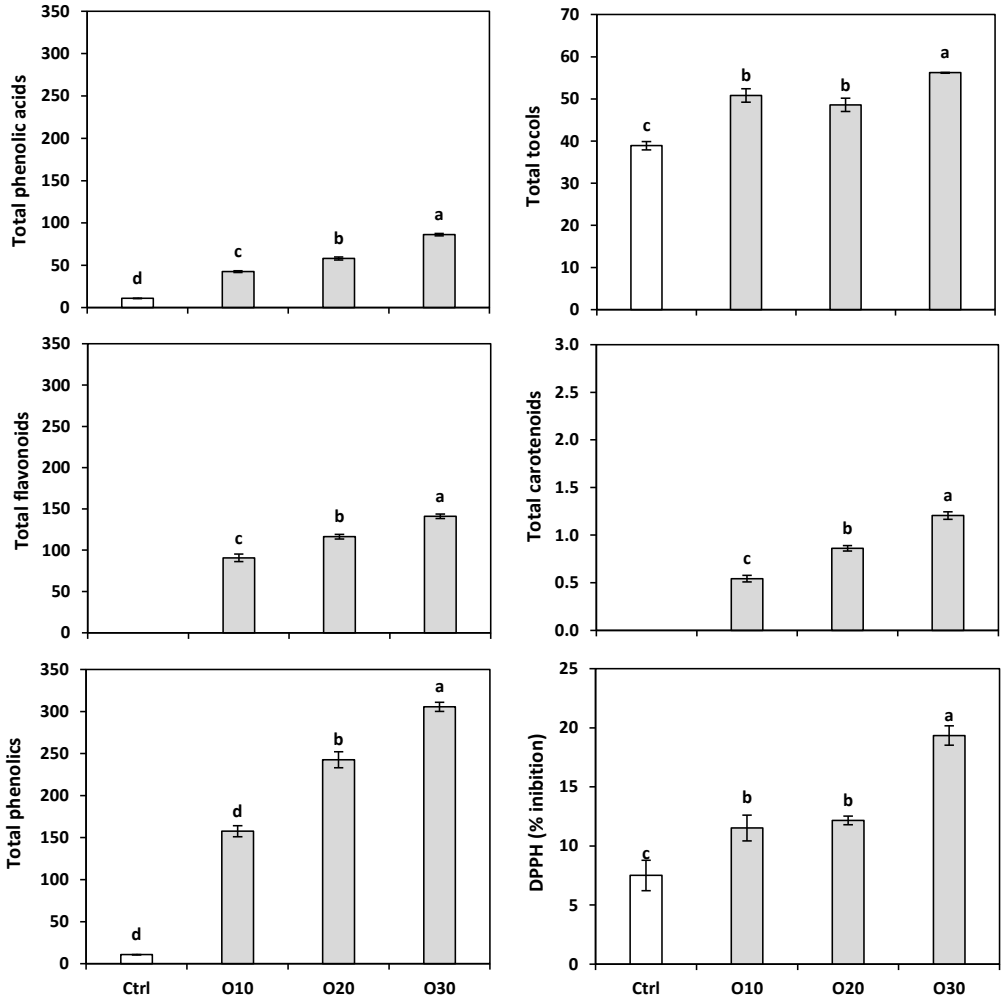


Figure 2. Antioxidant content (mg/kg DM) in gluten-free sourdough control bread (Ctrl) and gluten-free sourdough breads enriched with 10% (O10), 20% (O20) or 30% (O30) olive pomace. The bars represent the standard deviation. Different letters indicate statistical differences ($p < 0.05$) among breads according to Fisher's LSD test.

3.1.3. Tocols and Carotenoids Content of the Breads

The control bread (Table 2) contained a relevant quantity of α-tocopherol, likely provided by the sunflower oil in the formulation [62], along with small amounts of β-tocopherol and γ-tocotrienol, originating from the rice flour. The α-tocopherol content of the OP-enriched breads increased in line with the by-product addition, while γ-tocotrienol (entirely from rice flour) decreased significantly and the β-tocopherol (mainly from rice flour) was slightly lower only after the addition of 30% OP. By contrast, γ-tocopherol and δ-tocotrienol (from the olive pomace) were not detected in the enriched breads, either because they were too diluted or susceptible to degradation. Overall, the addition of the olive oil by-product led to a 25.8%, 27.8% and 49.7% increase in O10, O20 and O30 breads, respectively (Figure 2).

The four carotenoids detected in the OP increased progressively in the enriched breads according to by-product content. However, the total carotenoid content of the samples was still minimal (Figure 2).

3.1.4. Bread Colour

The addition of olive pomace significantly affected the colour characteristics of both the crust and the crumb (Table 3), showing progressive changes in line with the level of OP incorporation. In the crust, the luminosity (L^*) decreased significantly ($p < 0.05$)

with increasing OP content (from 71.1 in the control to 39.7 at 30% OP), indicating visible darkening. At the same time, the red component (a^*) increased from 1.3 to 7.2, and the yellow component (b^*) decreased from 33.4 to 19.5, reflecting a transition to darker, reddish-brown tones. A similar trend was observed in the crumb, where L^* decreased even more (from 79.3 to 36.6), indicating substantial darkening of the internal structure. The a^* values increased from −5.1 (greenish tint in the control sample) to 5.5, demonstrating a shift towards reddish tones, while b^* decreased from 22.1 to 17.6, reflecting a reduction in the yellow tint.

Table 3. Colour of gluten-free sourdough bread enriched with different percentages of olive pomace (OP). Values are presented as mean ± standard deviation ($n = 3$). Values within the same column followed by different letters are statistically significantly different ($p < 0.05$), according to Fisher's LSD test.

OP (%)	Crust			Crumb		
	L*	***a****	***b****	***L****	***a****	***b****
0	71.1 ^{a} ± 1.9	1.3 ^{d} ± 0.5	33.4 ^{a} ± 0.6	79.3 ^{a} ± 0.5	−5.1 ^{f} ± 0.1	22.1 ^{a} ± 0.1
5	57.5 ^{b} ± 1.1	4.9 ^{c} ± 0.0	29.7 ^{b} ± 0.5	59.0 ^{b} ± 0.7	1.8 ^{e} ± 0.1	21.0 ^{b} ± 0.2
10	49.6 ^{c} ± 1.8	6.2 ^{b} ± 0.7	25.6 ^{c} ± 0.7	49.4 ^{c} ± 0.4	3.9 ^{d} ± 0.1	20.6 ^{bc} ± 0.2
15	46.5 ^{d} ± 1.4	6.4 ^{b} ± 0.8	23.4 ^{d} ± 1.0	45.9 ^{d} ± 0.2	5.0 ^{c} ± 0.0	20.5 ^{c} ± 0.2
20	42.6 ^{e} ± 1.9	6.7 ^{ab} ± 0.4	21.5 ^{e} ± 1.4	38.3 ^{e} ± 1.5	5.6 ^{b} ± 0.1	18.3 ^{d} ± 0.5
25	42.1 ^{e} ± 0.4	6.8 ^{ab} ± 0.3	20.2 ^{ef} ±1.9	38.3 ^{e} ± 0.6	5.5 ^{ab} ± 0.1	17.8 ^{e} ± 0.3
30	39.7 ^{f} ± 0.9	7.2 ^{a} ± 0.1	19.5 ^{f} ± 0.8	36.6 ^{f} ± 0.4	5.5 ^{a} ± 0.1	17.6 ^{e} ± 0.4

Olive pomace is rich in natural pigments and phenolic compounds (see above), which directly affect the colour of the final product. A similar effect on fortified breads has been observed by Saura-Martínez et al. (2025) [63] with dried olive, by Cantero et al. (2022) [64] with apple pomace, and by Muñoz-Bernal et al. (2024) [65] with grape pomace: the enriched breads were significantly darker than the control. The reduction in L^* and b^* values and the increase in a^* values observed with increasing OP addition can be related to several chemical mechanisms occurring during baking. Regression analysis confirmed systematic colour changes as a function of OP incorporation level. Quadratic regression models fitted the colour parameters effectively for both crust (L^*: $R^2 = 0.9793$; a^*: $R^2 = 0.9113$; b^*: $R^2 = 0.9981$) and crumb (L^*: $R^2 = 0.9761$; a^*: $R^2 = 0.9403$; b^*: $R^2 = 0.9323$). These quantitative relationships demonstrate that the reduction in lightness (L^*) and yellowness (b^*), together with the increase in redness (a^*), progressed systematically with higher OP substitution levels. First, Maillard reactions occur between reducing sugars and amino groups of amino acids and proteins. The initial condensation reactions form Amadori products, which subsequently undergo dehydration, fragmentation, and further reactions, generating reactive carbonyl compounds and, at advanced stages, high-molecular-weight brown nitrogen-containing pigments known as melanoidins. These melanoidins are important contributors to the brown colour developed in bread during baking and therefore can contribute to the observed decrease in L^* values [66,67]. The intensity of Maillard browning is strongly influenced by baking temperature, time, moisture, water activity, pH, and the availability of reducing sugars and amino groups [67].

In addition to Maillard browning, phenolic compounds introduced by OP may undergo oxidation during thermal processing. Phenolic oxidation can result in the formation of quinones, which are highly reactive intermediates capable of undergoing further condensation and polymerisation reactions with phenolic compounds and other nucleophilic constituents, including proteins and amino groups. The resulting higher-molecular-weight products can contribute to the formation of darker-coloured compounds in plant-derived food matrices. Moreover, phenolic compounds can interact with carbonyl compounds and amino groups and may modify Maillard reaction pathways, potentially affecting final colour

development [68,69]. In olive-pomace-fortified bread, the incorporation of OP has been reported to modify L^*, a^*, and b^* values, with the colour changes attributed to both the intrinsic pigments and the thermal reactions occurring during baking [68]. Thus, the progressive decrease in L^* and b^* values and the increase in a* values observed in the present study likely reflect the combined effects of the naturally dark-coloured phenolic/pigment fraction of OP and the formation and transformation of Maillard- and phenolic-derived colour compounds during baking [25]. The decrease in b^* values (yellow hue) and the simultaneous increase in a^* values (red hue) indicate a change in the dominant colour tone—from light and yellowish to darker, reddish-brown, often observed because of enrichment with plant by-products [68].

3.1.5. Technological Quality of the Breads

The incorporation of olive pomace (OP) significantly affected the pH of the dough (Figure 3). Before the main fermentation, the pH decreased progressively with increasing OP, from 6.50 in the control dough to 4.90 in the dough containing 30% OP. After 45 min of fermentation, the pH values ranged from 5.32 to 5.01 for the control and 30% OP formulations, respectively. Although the pH remained significantly different among several formulations, the differences between samples were less pronounced after fermentation. In all formulations, fermentation resulted in a significant change in pH, as indicated by the different capital letters. These results indicate that OP incorporation was associated with changes in dough acidity during fermentation.

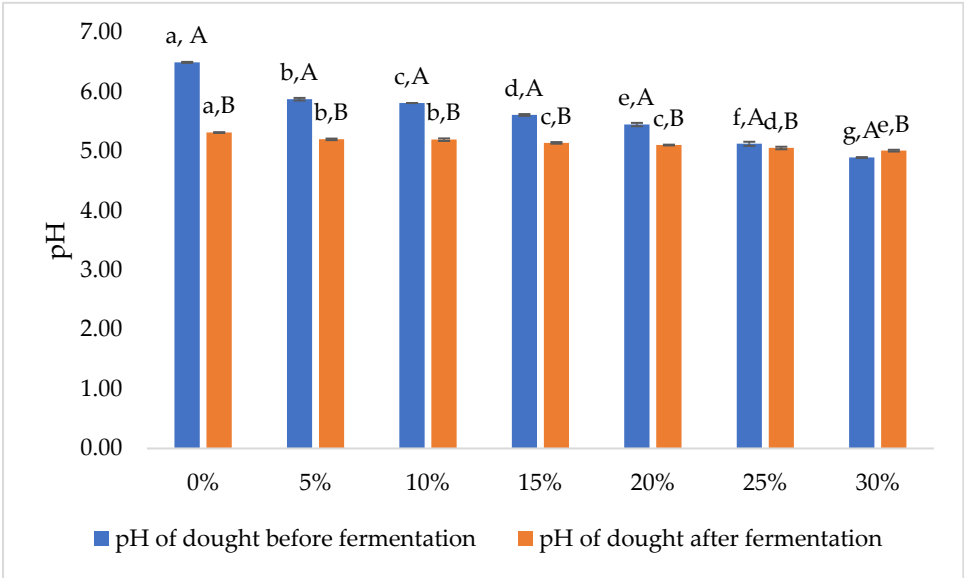


Figure 3. pH value of the dough before and after fermentation of gluten-free sourdough bread enriched with different percentages of olive pomace (OP). The values are presented as mean ± standard deviation (n = 3). Different lowercase letters indicate statistically significant differences ($p < 0.05$) among the different OP concentrations within the same fermentation phase. Different uppercase letters indicate statistically significant differences ($p < 0.05$) between pH values before and after fermentation for the corresponding OP concentration. Different letters indicate statistically significant differences ($p < 0.05$) according to Fisher's LSD test.

In the present study, the progressive reduction in initial dough pH with increasing OP may be partly related to the acidic character of OP. Similar reductions in dough pH with increasing OP addition have been reported previously [21]. The further changes observed after the 45-min fermentation indicate that OP incorporation was associated with modifications of dough fermentation conditions. However, pH measurements alone do

not allow direct conclusions regarding CO_2 production, gas retention, or the underlying rheological mechanisms.

The specific volumes of gluten-free bread enriched with different OP contents (Table 4) showed a statistically significant ($p < 0.05$) decrease with increasing OP, from 1.72 cm^3/g (control) to 1.55 cm^3/g (30% OP). The volume of breads prepared with bread wheat flour depends on multiple factors, including protein content and composition, amylose/amylopectin ratio, yeast type, leavening time and temperature, dough viscosity, etc.; however, in gluten-free bread, the lack of a protein network limits CO_2 retention, leading to products with low specific volume and compact crumb. This effect is often associated with the addition of ingredients rich in dietary fibre [70,71], which can affect dough expansion and may limit CO_2 retention during fermentation [72]. Furthermore, fibre has a high water-binding capacity, thus limiting the water available for adequate dough hydration and potentially affecting expansion during baking [73]. Moreover, the addition of OP, which is rich in lipids (11.24%), may have contributed to the reduction in volume, as higher fat levels in dough have been reported to affect gas retention and may contribute to structural weakening during baking [74].

Table 4. Specific volume and texture characteristics (mean ± standard deviation) of gluten-free bread enriched with different percentages of olive pomace (OP). The values are presented as mean ± standard deviation ($n = 3$). Values within the same column followed by different letters are statistically significantly different ($p < 0.05$) following Fisher's LSD test.

OP (%)	Specific Volume (cm^3/g)	Hardness (N)	Springiness	Resilience	Chewiness (N)	Cohesiveness
0	1.72 ^{a} ± 0.03	18.90 ^{b} ± 2.21	0.81 ^{a} ± 0.08	0.30 ^{a} ± 0.02	9.28 ^{a} ± 0.96	0.61 ^{a} ± 0.03
5	1.68 ^{a} ± 0.01	19.67 ^{ab} ± 1.78	0.78 ^{a} ± 0.04	0.30 ^{a} ± 0.03	9.23 ^{a} ± 0.89	0.61 ^{a} ± 0.03
10	1.62 ^{b} ± 0.01	20.50 ^{ab} ± 2.27	0.72 ^{b} ± 0.05	0.28 ^{ab} ± 0.02	8.70 ^{ab} ± 0.98	0.59 ^{a} ± 0.03
15	1.59 ^{bc} ± 0.01	20.73 ^{ab} ± 2.65	0.69 ^{bc} ± 0.03	0.26 ^{bc} ± 0.02	7.75 ^{b} ± 0.92	0.55 ^{b} ± 0.04
20	1.56 ^{bc} ± 0.03	20.48 ^{ab} ± 2.28	0.68 ^{bc} ± 0.05	0.26 ^{bc} ± 0.02	7.66 ^{b} ± 1.26	0.55 ^{b} ± 0.02
25	1.56 ^{c} ± 0.01	21.12 ^{a} ± 2.00	0.66 ^{c} ± 0.03	0.25 ^{bc} ± 0.03	7.72 ^{b} ± 1.09	0.55 ^{b} ± 0.02
30	1.55 ^{c} ± 0.08	20.64 ^{ab} ± 1.93	0.66 ^{c} ± 0.07	0.25 ^{c} ± 0.03	7.58 ^{b} ± 1.72	0.55 ^{b} ± 0.04

Texture is a critical factor influencing consumer acceptance of bakery products, particularly in gluten-free formulations which are often prone to rapid staling and unsatisfactory mouthfeel. The results of the Texture Profile Analysis (TPA) for the experimental breads are presented in Table 4. Hardness showed a slight numerical increase with OP incorporation, rising from 18.90 N in the control to 21.12 N in the OP-enriched samples, with the significantly highest value observed at 25% OP ($p < 0.05$). Unlike many studies where the addition of food by-products significantly increases crumb hardness [21,74,75], the breads in this study did not show statistically significant differences ($p > 0.05$). Thus, although OP addition tended to increase crumb hardness, the magnitude of this increase was not sufficient to produce a statistically detectable difference among samples. The absence of a significant increase in hardness, despite the numerical trend observed, may be related to the specific composition of the rice-based sourdough matrix and the olive pomace used in the present study. The OP contained 11.24% lipids (dry matter basis), which may have exerted a plasticising and lubricating effect within the crumb matrix, thereby attenuating the increase in firmness associated with incorporating a fibre-rich ingredient. Lipids can also interact with starch and influence starch retrogradation, one of the main mechanisms contributing to crumb firming during bread storage [75]. Therefore, the lipid fraction of OP may have partially counteracted the structural effects expected from OP incorporation, including the lower specific loaf volume observed in OP-enriched breads. This interpretation should be considered a possible mechanism rather than a direct causal effect,

since starch retrogradation and lipid–starch interactions were not directly measured in the present study.

In contrast to hardness, springiness, resilience, and cohesiveness were significantly affected by OP addition. Springiness, which indicates the ability of the bread to recover its height after compression, significantly decreased from 0.81 in the control to 0.66 in the 30% OP sample, in agreement with the findings of Azadfar et al. (2023) [56], who reported that the addition of 15% olive pomace fibre significantly decreased springiness. A significant decrease in resilience (from 0.30 to 0.25) and cohesiveness (from 0.61 to 0.55) was also observed. These results agree with the data reported by Pycia and Juszczak (2025) [75], Martinez et al. (2025) [74] and Oliveira et al. (2023) [76], in which the addition of nut and oilseed by-products often reduced elasticity and cohesiveness. These parameters describe different aspects of the crumb's ability to recover after deformation and maintain its internal structure, rather than the maximum force required for compression. The different response of hardness compared with springiness, resilience, and cohesiveness is therefore not necessarily contradictory. Hardness represents the maximum force recorded during the first compression, whereas springiness, resilience, and cohesiveness characterise different aspects of the crumb's recovery and structural integrity. Consequently, the OP-enriched breads could require a similar force to reach the specified compression level while exhibiting a reduced ability to recover their original structure and a greater tendency to fracture after deformation. In other words, OP addition appears to have affected the mechanical behaviour of the crumb, particularly its structural recovery and internal cohesion, rather than its overall resistance to compression. This effect may be associated with the high insoluble dietary fibre content of OP (>85% of total dietary fibre in the material used). In gluten-free bread, where gluten is absent, the continuous starch-based matrix plays a major role in the structural integrity and viscoelastic properties of the crumb. Incorporating a high proportion of insoluble fibre may dilute and physically disrupt this continuous matrix and alter water distribution during dough development and baking. Such structural disruption may reduce the ability of the crumb to recover after deformation and increase its susceptibility to fracture and crumbling, thereby explaining the observed decreases in springiness, resilience, and cohesiveness [56,74,75].

Both chewiness and cohesiveness showed a gradual numerical decrease from the control to the 10% OP sample (from 9.28 to 8.70 N and from 0.61 to 0.59, respectively). With further OP addition, a statistically significant decrease was observed, with the lowest values recorded in the 30% OP sample (7.58 N for chewiness and 0.55 for cohesiveness) ($p < 0.05$). This trend is consistent with the observed reductions in springiness and cohesiveness, as chewiness is a derived TPA parameter influenced by hardness, cohesiveness, and springiness. Thus, the combination of relatively unchanged hardness with lower springiness and cohesiveness would be expected to result in lower chewiness values. However, variability among samples was sufficient to prevent this numerical trend from reaching statistical significance.

Overall, the incorporation of OP did not significantly increase crumb hardness, although a slight numerical rise was observed, indicating that the softness of the gluten-free bread was largely retained even at the highest substitution level (30%). In contrast, the significant decreases in springiness, resilience, and cohesiveness indicate that OP addition primarily altered the elastic recovery and internal structural integrity of the crumb. At higher OP levels, the resulting bread can therefore be characterised as relatively soft but less elastic and less cohesive, with a greater tendency towards crumbliness.

3.1.6. Sensory Analysis of the Breads

The preliminary sensory evaluation revealed that adding olive pomace significantly ($p < 0.05$) affected all assessed sensory properties of the gluten-free sourdough bread (Figure 4 and Table S1).

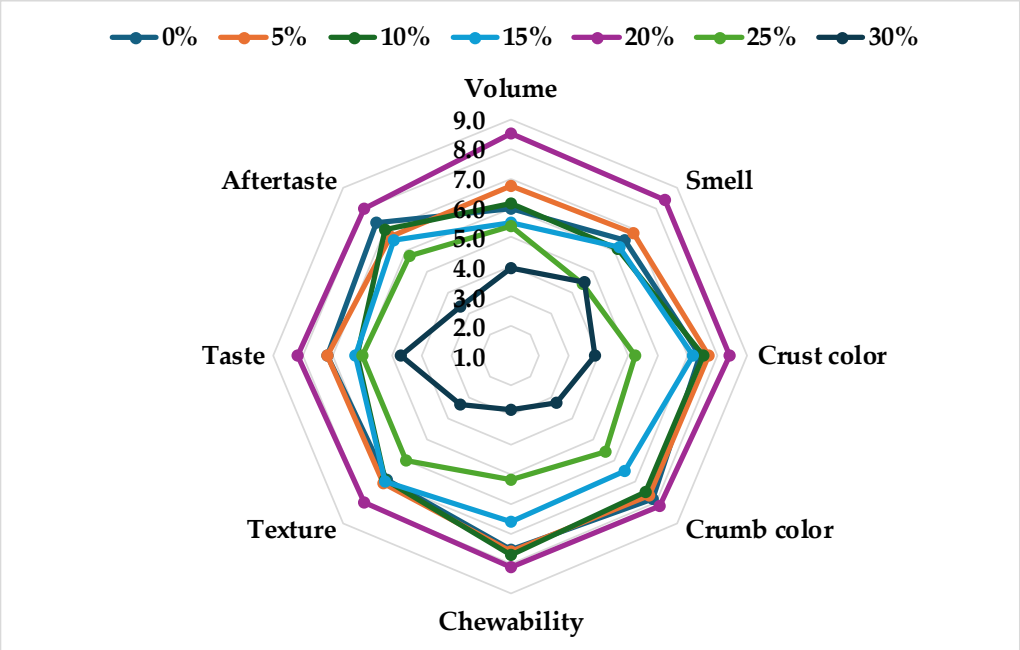


Figure 4. Sensory analysis of gluten-free bread from sourdough, enriched with olive pomace.

For volume, the 20% OP bread received the highest hedonic score (8.50 ± 0.87), which was significantly higher ($p < 0.05$) than all other formulations. The 5% OP bread scored 6.73 ± 1.91, while the 10% OP (6.15 ± 1.20), control (6.00 ± 2.21), 15% OP (5.50 ± 2.47), and 25% OP (5.38 ± 0.75) breads showed intermediate values. The 30% OP bread received the significantly lowest score (3.96 ± 1.28). The marked reduction in scores at 25–30% OP indicates that excessive OP incorporation negatively affected panellists' hedonic perception of bread volume.

For aroma, the highest score was recorded for the 20% OP sample (8.42 ± 0.91), which was significantly higher ($p < 0.05$) than all other samples. The control (6.50 ± 2.18), 5% OP (6.85 ± 1.74), 10% OP (6.12 ± 0.91), and 15% OP (6.23 ± 2.41) breads formed a statistically equivalent group with moderate hedonic scores. In contrast, the 25% and 30% OP formulations received significantly lower ratings (4.46 ± 1.10 and 4.54 ± 0.86, respectively). These findings suggest that moderate OP incorporation (up to 20%) enhanced perceived aroma, whereas higher concentrations adversely affected odour acceptability, in agreement with Al-Juhaimi et al. (2024) [77].

Crust colour acceptability was also significantly influenced by OP concentration. The 20% OP sample achieved the highest score (8.42 ± 0.82), significantly exceeding ($p < 0.05$) the control (7.42 ± 1.58), 5% OP (7.69 ± 1.35), 10% OP (7.50 ± 1.36), and 15% OP (7.15 ± 1.91) samples. An incorporation level of 30% OP (3.85 ± 1.16) resulted in a significant reduction in crust colour acceptance, reflecting a lower preference for excessively dark crusts [20].

For crumb colour, the 20% OP bread received the highest score (8.15 ± 0.93), which did not differ significantly from the control (7.81 ± 1.47) and 5% OP (7.65 ± 1.35) breads, all belonging to the same top statistical group. However, scores decreased significantly at 10% OP (7.46 ± 1.07) and 15% OP (6.50 ± 2.37), and further declined at 25% (5.54 ± 0.81) and

30% OP (3.23 ± 1.31). This indicates that high OP inclusion significantly reduced crumb colour acceptance due to pronounced darkening.

For chewability, the highest hedonic rating was obtained for the 20% OP bread (8.12 ± 1.00), which belonged to the same top statistical group as the 10% OP (7.69 ± 0.99), 5% OP (7.62 ± 1.42), and control (7.54 ± 1.75) breads. Scores dropped at 15% OP (6.58 ± 2.72) and reached the lowest values at 25% (5.19 ± 0.94) and 30% OP (2.81 ± 0.98). The marked reduction at 30% OP suggests that excessive dietary fibre disrupted the crumb matrix, leading to a compact texture that negatively affected mouthfeel [21].

Texture scores followed a similar pattern, with the 20% OP bread receiving the significantly highest score (8.00 ± 0.98). Breads containing 0% (6.88 ± 1.80), 5% (7.04 ± 1.59), 10% (6.92 ± 0.98), and 15% OP (6.96 ± 2.22) formed an intermediate statistical group. Higher incorporation levels caused a significant drop in texture acceptability to 6.00 ± 0.75 (25% OP) and 3.35 ± 0.69 (30% OP).

For taste, the 20% OP formulation obtained the highest score (8.15 ± 1.13), significantly exceeding all other formulations ($p < 0.05$). The control and 5% OP breads both scored 7.19 ± 1.70, while the 15% OP bread scored 6.19 ± 2.47. Lower scores were observed for 10% OP (6.15 ± 1.41) and 25% OP (6.00 ± 1.10), whereas 30% OP received the lowest score (4.69 ± 0.84). The reduction in taste acceptance at higher OP levels is likely attributed to increased bitterness and astringency associated with phenolic compounds [78].

Aftertaste evaluations showed a similar trend, with the control (7.38 ± 1.27) and 20% OP (8.00 ± 1.37) breads receiving the significantly highest scores. The 5% OP (6.73 ± 2.01) and 10% OP (7.00 ± 0.75) breads showed good aftertaste scores, followed by 15% OP (6.54 ± 2.27), and 25% OP (5.81 ± 1.23). The 30% OP bread scored lowest (3.35 ± 1.02), confirming that excessive OP incorporation negatively impacts aftertaste due to persistent bitterness.

Overall, the statistical analysis indicates that 20% OP represented the most favourable incorporation level among the formulations tested in the present sensory evaluation, as it generally received the highest or among the highest scores across the evaluated attributes. The results suggest that moderate OP incorporation, particularly at 20%, provides the best balance between product enrichment and sensory acceptability, while incorporation levels of 25–30% significantly compromise several important sensory characteristics. These findings are consistent with Al-Juhaimi et al. (2024) [77], who reported the highest sensory acceptability for bread containing 20% fermented OP, whereas higher concentrations negatively affected flavour and visual appearance.

4. Conclusions

The addition of olive pomace to gluten-free sourdough bread led to significant changes in its physicochemical, nutritional and sensory properties. The nutritional composition of the bread generally improved with increasing OP, with higher ash and dietary fibre contents. Phenolic and tocol levels were also higher at the investigated OP levels, although these bioactive compounds were analysed only in the 0, 10, 20, and 30% OP formulations. However, increasing OP levels also resulted in a lower specific volume and a darker crust and crumb. Although the nutritional parameters generally continued to improve at 30% OP, this higher inclusion level was accompanied by less favourable technological and sensory characteristics. Considering the combined technological and sensory results, the 20% OP formulation represented the best overall compromise under the specific experimental conditions of this study. This level should therefore be regarded as an empirical optimum for the investigated formulation rather than a universally optimal inclusion level. The sensory findings should also be considered preliminary, as they were obtained from a semi-trained panel and should not be interpreted as evidence of consumer preference or acceptance.

A limitation of the present study is that the observed increases in bioactive compounds with increasing OP incorporation should not be interpreted as evidence of a rigorously established continuous dose–response relationship across the entire 0–30% inclusion range. The observed trends are specific to the formulations and experimental conditions investigated. Further studies involving a broader range of OP incorporation levels and appropriate dose–response modelling are needed to establish the relationship between OP level and the concentration of individual bioactive compounds.

The incorporation of OP into gluten-free sourdough bread demonstrates its potential for valorisation as an olive-processing by-product and for the development of nutritionally enhanced food products. However, this study did not quantify environmental benefits, waste reduction, energy consumption, processing inputs, economic performance, or life-cycle impacts. Therefore, no direct conclusions regarding the environmental sustainability of the developed bread or its quantified contribution to a circular economy can be drawn. Further research, including life-cycle assessment, evaluation of processing and energy requirements, economic feasibility, and comprehensive food-safety assessment, is needed to substantiate the broader sustainability potential of OP valorisation in food applications.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/antiox15111456/s1. Table S1. Effect of olive pomace (OP) addition on the sensory scores of breads.

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