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Original article

Impact of oleuropein on rheology and breadmaking performance of wheat doughs, and functional features of bread

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Summary Oleuropein (OP) is a polyphenol present in drupes and leaves of olive tree with health benefits and, as antioxidant, potentiality to alter gluten functionality. Effects of OP inclusion to wheat flour (0.01% and 0.02% on flour weight basis) on dough rheology and breadmaking performance, and bread features were investigated. Farinograph, uniaxial extension and shear rheometry (oscillatory and creep-recovery) were applied. Doughs containing OP were stronger, more elastic, and less sticky indicating the ability of OP to act as flour improver. The strengthening effect of OP on gluten led to the increase in bread volume and softer crumb compared to control. A lower crumb density of bread with the addition of OP was related to a higher *in vitro* glycaemic response. An increase in the antioxidant capacity of bread made with the phenolic compound was also found.

Keywords Antioxidant, dough, flour improver, rheology, starch digestion.

Introduction

Oleuropein (OP) is the most abundant polyphenol in olives and olive leaves, conferring the bitter principal taste and the resistance against the development of oil rancidity (Visioli *et al.*, 1998; Guinda *et al.*, 2015). During years, oleuropein intake has been correlated with several pharmacological properties, including anti-cancer, hepatoprotective, neuroprotective, anti-viral, and anti-inflammatory effects (De La Puerta *et al.*, 1999; Micol *et al.*, 2005; Daccache *et al.*, 2011; Park *et al.*, 2011).

Furthermore, the European Food Safety Authority established that the daily consumption of 5 mg of oleuropein and its derivates contributes to the protection of blood lipids from oxidative damage (EFSA, 2011). Recently, Carnevale *et al.* (2018) carried out a randomised, double-blind, placebo-controlled, cross-over study where healthy subjects received 20 mg of oleuropein before lunch. Their findings suggested that OP also improves postprandial glycaemic profile via hampering Nox2-derived oxidative stress (Carnevale *et al.*, 2018).

Phenolic compounds are well-known in literature for their antioxidant activity, which makes them interesting to be used by the food industry as functional ingredients with healthy properties (Balasundram *et al.*, 2006). In addition, the polyphenols incorporation into starchy-food products could interact with carbohydrates and/or protein molecules by forming reversible and irreversible complexes, which can be stabilised by covalent and non-covalent interactions (Sivam *et al.*, 2010; Świeca *et al.*, 2014). This aspect could represent a promising intervention for bakery industries to manufacture innovative baked food products with attractive technological properties, in terms of dough handling, mechanical stability, product texture, and good nutritional quality at the same time (Ou *et al.*, 2019). However, the addition of phenolic compounds to cereal-based products, like baked products, represents an ongoing challenge for the food sector because of the low thermostability and aptitude to interact with nutritional components and compounds formed during the baking process, affecting the final colour, texture, and flavour of products (Ou *et al.*, 2019). Breadmaking mainly consists of three major stages, notably kneading, fermentation, and baking (Cappelli *et al.*, 2021). The mixing step required sufficient mechanical energy to blend all ingredients and to develop the viscoelastic properties of gluten, which will impact dough rheology (Dobraszczyk & Morgenstern, 2003). Rheological properties of enriched-bread doughs were studied by several authors after the addition of different phenolic acids, such as caffeic and ferulic acids (Koh & Ng, 2009; Han & Koh, 2011), anthocyanins (Sui *et al.*, 2016), quercetin (Lin & Zhou, 2018), and tannic acid (Zhang *et al.*, 2010), to bread formulations. These

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authors observed that the inclusion of caffeic and ferulic acids, quercetin, and anthocyanins from black rice decreased mixing stability and maximum resistance to extension in enriched doughs, while the incorporation of tannic acids improved dough mixing properties resulting in a stronger and more elastic dough. During fermentation and baking steps, dough rheological properties are involved in the control and stabilisation of expanding gas bubbles, determining bread final expansion and its mechanical properties (Dobraszczyk & Morgenstern, 2003). Thus, to ensure a good volume increase and crumb softness, the gluten-starch network needs to be extensible and resistant to respond to gas pressure and to avoid structure collapse (Sroan *et al.*, 2009). In several studies, wheat bread textural properties were studied upon the incorporation of natural polyphenols, from quinoa leaves (Świeca *et al.*, 2014), green tea powder (Ning *et al.*, 2017), grape pomace (Hayta *et al.*, 2014), and quercetin (Lin & Zhou, 2018), showing lower loaf-specific volumes and higher values of crumb hardness, suggesting that polyphenols influence dough viscoelasticity and bread mechanical properties. Moreover, as reported by several authors, the addition in bread formulations of different polyphenol compounds from green coffee flour (Świeca *et al.*, 2017), tea powder (Culetu *et al.*, 2016; Ning *et al.*, 2017), and grape pomace (Hayta *et al.*, 2014) enhanced the nutritional profile of bread products by increasing total polyphenol content and antioxidant capacity. *In vitro* digestibility studies showed that fortified wheat bread with green tea catechins (Goh *et al.*, 2015) and anthocyanins from black rice (Sui *et al.*, 2016) could be a promising strategy to reduce bread glycaemic potential, by the inhibitory effect on α-amylase digestive enzymes. In spite of the beneficial effects previously reported, breadmaking for the food industry might represent a critical technological step for polyphenols stability in bread products, due to the thermal degradation of these phytochemical compounds, as recently reported by Lin & Zhou (2018) and Sui *et al.* (2015) after the addition of quercetin and anthocyanins in bread samples, respectively.

The objective of this study was to understand the impact of oleuropein addition to wheat flour on breadmaking performance by evaluating dough rheology, handling properties, and bread characteristics. Dough mixing properties, uniaxial extensional properties, linear and non-linear rheology in shear (dynamic and creep tests) were evaluated to obtain information on its structure and technological potential. Moreover, antioxidant activity and *in vitro* glycaemic response of bread were determined.

Studies on oleuropein in breadmaking were not found in literature, making this work interesting and innovative for a better comprehension of the interactions between oleuropein, and gluten in dough, and bread properties.

Materials and methods

Materials

For dough preparation and breadmaking, common wheat flour (11.5% protein, 13.7% moisture) (Molino Rachello, Roncade, Italy), fresh compressed yeast (Lessafre Italia, Parma, Italy), commercial butter (Despar, Bolzano, Italy), and sucrose were used. The following reagents were purchased from Sigma Aldrich (Milan, Italy): oleuropein (OP) (purity ≥80% for HPLC), α-amylase from porcine pancreas (EC 3.2.1.1; 9 U mg^{-1}), pepsin from porcine gastric mucosa (EC 3.4.23.1; 4472 U mg^{-1}), bile extract from porcine (B8631), pancreatin from porcine pancreas (EC 232-468-9; 11.28 U mg^{-1}), amyloglucosidase from *Aspergillus niger* (EC 3.2.1.3; 1500 U mL^{-1}), L-(+)-arabinose, D-(+)-glucose, DPPH-free radical (purity ≥95% for HPLC), and Trolox® (purity ≥97% for HPLC). Sodium chloride (NaCl) was supplied by Carlo Erba Reagents (Milan, Italy).

Mixing properties

Six doughs were prepared using wheat flour, sodium chloride, and OP at different amounts as reported in Table 1. The selection of OP amounts to add to flour (100 g, on 14% moisture basis) was based on preliminary tests at 5, 10, and 20 mg OP. The former corresponds to the daily consumption of OP suggested by EFSA (2011), and this was excluded from the experimental work since it did not alter dough mixing properties. An amount higher than 20 mg OP was not used based on the observation that 20 mg OP made dough sensitive to over-mixing. Mixing properties were evaluated using a farinograph Promylograph T6 (Max Egger, Austria) equipped with a 100 g bowl, according to AACC Approved Method 54-21 (AACC, 2000). Dough water absorption (WA), which corresponds to the amount of water per 100 g flour (on 14% moisture basis) to reach a consistency of 500 PU, was evaluated.

Table 1 Weight fraction of ingredients used in the preparation of different samples

Samples	Flour (g)[a]	Oleuropein (g)	NaCl (g)
No salt			
C	100	0	0
OP1	100	0.01	0
OP2	100	0.02	0
With salt			
C-S	100	0	1.5
OP1-S	100	0.01	1.5
OP2-S	100	0.02	1.5

[a]On 14% moisture basis.

From the farinogram, stability (ST) and softening degree (SD) at 20 min of mixing were determined. Data are the average of three replicates.

Extensional properties of dough

Uniaxial extensional properties of dough at 25 °C were determined using a TA-XT Plus Unit Texture Analyzer (Stable Micro Systems, Godalming, UK) equipped with a 5 kgf load cell and a Kieffer dough system and gluten extensibility rig according to Peighambardoust *et al.* (2006) with a modification of dough resting time. Doughs were mixed in the farinograph bowl at the optimum development time (2.0–2.5 min) and farinograph absorption of C (55.1%) for samples without salt or C-S (52.9%) for samples with salt. Uniaxial extension test on doughs was carried out at 3.3 mm s^{-1} after 75 min of resting time (20 min before moulding and 55 min inside the mould). The following parameters were considered: fracture stress (σ_{max}) for resistance to extension, the Hencky strain (ε_H) at fracture stress for extensibility, and the integrated area under the stress-strain curve for energy required to extension. An apparent strain hardening index (dln σ $d\varepsilon_H{}^{-1}$, SH) was computed in the strain interval of 20-95% of the fracture strain of samples. Results are the average of three replicates, where each replicate represents a separately mixing batch. Six analytical measurements were performed for each replicate.

Dough stickiness

Dough was mixed in the farinograph bowl as previously described. A dough sample (120 g) was left relaxing for 5 min, and then analysed using TA-XT Plus Unit Texture Analyzer equipped with a 5 kgf load cell. A dough stickiness system provided with a narrow blade (HDP/BS), a sample box (8.5 cm × 12.5 cm × 3 cm), and a sample restriction plate (9 cm × 10 cm) was used for the analysis. The blade was driven through a slot in the restriction plate to a 30 mm distance, with a compression speed of 2 mm s^{-1} and a trigger force of 0.1 N. As the blade returned upwards the adhesion area (N s) was calculated as force per second to separate the probe from the dough. Data are the average of three replicates, where each replicate represents a separately mixing batch.

Dough rheological properties in shear

Dough was prepared using farinograph as previously described. Rheological tests were performed at 25 °C by using a controlled stress rheometer (Haake RheoStress 6000, Thermo Scientific, Karlsruhe, Germany) equipped with a parallel plate geometry (35 mm diameter, 2 mm gap). Each dough was loaded between the plates, the excess was gently removed, and sample edge coated with silicon grease to avoid moisture loss. Sample was left relaxing for 5 min before testing. A dynamic stress sweep test was conducted from 0.5 to 300 Pa at constant frequency (1 Hz) to identify the linear viscoelastic region (LVR). A frequency sweep test at constant shear stress was carried out within the LVR from 0.1 to 10 Hz. Storage modulus (G′), loss modulus (G″), and tan δ (G″/G′) were evaluated.

A creep-recovery test was performed consecutively on the same dough sample, according to Stone *et al.* (2017). Dough was subjected to a constant shear stress outside the LVR (σ_0 = 250 Pa) for 180 s (creep). After σ_0 removal, dough recovery was measured for 360 s (recovery). Changes in shear strain (γ) were recorded over time (t) and creep-recovery curves were expressed in terms of compliance (J) using the following equation:

$$J(t) = \gamma(t) \times \sigma_0^{-1} \quad (1)$$

From creep-recovery J (Pa^{-1}), the relative elasticity of dough (J_{el}) was calculated as follows:

$$J_{el} = (J_{max} - J_r) \times J_{max}^{-1} \quad (2)$$

where J_{max} (Pa^{-1}) is compliance at 180 s of creep and J_r (Pa^{-1}) is compliance at 360 s of recovery.

Breadmaking

Bread was produced using an optimised straight-dough breadmaking procedure (AACC Approved Method 10-10B) (AACC, 2000). Doughs were mixed at optimum development (8.5 min) in the farinograph bowl using the following ingredients: wheat flour (100 g, on 14% moisture basis), sucrose (6 g), salt (1.5 g), fresh compressed yeast (5.3 g), butter (3 g), OP at 0% (CB), 0.01% (OP1B) or 0.02% on flour basis (OP2B) and water (based on WA of flour or flour added with OP).

Then, each dough was kept for 52 min at 30 ± 1 °C and 85% relative humidity (RH). After the first fermentation, the dough was punched for 2 min, placed in a greased baking pan and fermented at 30 ± 1 °C and 85% RH for 50 min. Finally, the fermented dough was baked at 160 °C for 30 min with RH from 80 to 30% in a professional oven (Lainox, model HME061X, Treviso, Italy). After removing from oven, bread loaf was left cooling for 1 h and placed in sealed plastic bag until further analysis (1 h). Three batches were produced for each formulation.

Bread moisture and specific volume

Moisture of bread crumb was measured by oven drying at 105 °C until a constant weight was achieved (Venturi *et al.*, 2022). Loaf specific volume (Vs) was determined by the rapeseed displacement method

according to AACC Approved Method 10-05 (AACC, 2000). Data are the average of three replicates.

Crumb image analysis

Image of bread slices was acquired using a professional digital camera Canon reflex EOS 550D (Canon Inc., Tokyo, Japan) equipped with an EF-S 60mm f/2.8 Macro USM lens. The distance between the slice surface and the camera lens was fixed at 48 cm. For each bread loaf, four central slices were used for image analysis. Crumb grain was evaluated by using the Image-Pro Plus 6.3 software (Media Cybernetics Inc., USA) according to Sapirstein *et al.* (1994) with some modifications. Images were converted to 8-bit greyscale and a rectangular field of view (32.5 × 32.5 mm) was assessed. A digital image of a ruler was used for spatial calibration. Cell density (number cm^{-2}) and mean cell area (MCA, mm^2) were provided by the software. Determinations were performed in triplicate. For each replicate three analytical measurements were conducted.

Bread crumb firmness

A TA-XT Plus Unit Texture Analyzer supplied with a 5 kgf load cell was used to evaluate bread crumb firmness according to AACC Approved Method 74-09 (AACC, 2000). Loaf was sliced by hand and four slices (25 mm thickness) were taken from the central portion. A penetration test was conducted (P/36R cylinder probe) and stress at 25% penetration was used as a measure of crumb firmness. Data are reported as the average of twelve measurements from three different loaves.

Crumb extract preparation

Freeze-dried and fresh bread slices were used to evaluate the antioxidant capacity and OP content, respectively. The crumb was separated and ground with an electric grinder machine for 15 s to obtain a particle size lower than 500 μm. Three grams of ground crumb were mixed with ethanol 80% (v/v) and ultrasonically treated for 15 min at 120 W (Sonoplus HD 2200, Bandelin electronic, Berlin, Germany) to promote OP extraction. The sample temperature was kept lower than 40 °C with an external water-ice bath. Then, the mixture was centrifuged at 7750 × ***g*** for 15 min at 5 °C using an Avanti JA-18 high-performance centrifuge (Beckman Coulter Inc., California, USA). The supernatant was collected and stored at −18 °C overnight before OP chromatographic quantification and DPPH antioxidant activity test.

Oleuropein content of bread

Oleuropein was determined in bread crumb samples after cooking and solvent extraction, as described in paragraph 2.10, using an Agilent Poroshell 120 EC-C18 reversed-phase column (2.7 μm particle size, 4.6 × 150 mm) on a Shimadzu Nexera UHPLC System (Shimadzu Nexera, Kioto, Japan) equipped with dual pump LC-30AD, column oven CTO-30A, autosampler SIL-30AC, and diode array detector (SPD-M20A). Gradient separation was performed using solvent A (2% acetic acid in water) and solvent B (acetonitrile) as follows: 0–1 min, isocratic condition at 60% A; 1–12 min linear gradient from 40 to 100% B; isocratic condition kept up to 14 min; 14 min back to initial condition at 60% A; isocratic step kept up to 18 min. An aliquot of 1 μL of extract solution was injected into the column while a mobile phase flow rate of 450 μl min^{-1} was used. The column temperature was 30 °C while the detector was set at 280 nm. A calibration curve ($R^2 = 0.999$) prepared with oleuropein standard solutions at different concentrations (0.3–65 μg mL^{-1}) was used for the quantification of the compound. The instrumental limit of detection (LOD) for OL was 0.07 μg mL^{-1} (calculated at a signal-to-noise ratio of 3:1), while the limit of quantification (LOQ) was 0.25 μg mL^{-1} (calculated at a signal-to-noise ratio of 10:1).

Determination of DPPH radical scavenging capacity

DPPH radical scavenging capacity was evaluated according to Brand-Williams *et al.* (1995) with few modifications. DPPH solution (78 μM) was daily freshly made in ethanol 95% (v/v). A volume of 160 μL of crumb extract was mixed with 3.04 mL of DPPH solution. Absorbance at 515 nm for 30 min was measured using a Shimadzu spectrophotometer (Shimadzu Corporation, Kyoto, Japan). The antiradical activity (AA, %) was calculated with the following equation (4):

$$\mathrm{AA}(\%) = \left[1 - \left(\frac{A_{t=30}}{A_{t=0}}\right)\right] * 100$$

where $A_{t\,=\,0}$ and $A_{t\,=\,30}$ correspond to absorbance values at 0 and after 30 min.

For the calibration curve, Trolox was dissolved in 80% (v/v) ethanol at concentrations ranging from 100 to 500 {M. The DPPH free radical scavenging activity of crumb extracts was expressed as micromoles of Trolox equivalent (TE) per g of sample (dry weight basis).

In vitro digestion and glucose determination

The digestion of bread samples was simulated using the *in vitro* digestion method proposed by Minekus *et al.* (2014). Briefly, the bread crumb was ground for 30 s using an electric grinder. After weighing 1 g of sample and 0.1 g of L-(+)-arabinose as the internal standard for sugars determination, the oral phase (2 min) started

with the addition of α-amylase from porcine pancreas solution (75 U mL^{-1} in the final mixture). The gastric phase (2 h) started with the addition of pepsin from porcine gastric mucosa solution (2000 U mL^{-1} in the final mixture). Then, the intestinal phase (2 h) started with the addition of pancreatin from porcine pancreas solution (100 U mL^{-1} in the final mixture) and bile extract from porcine solution (160 mM in the final mixture). For the glucose quantification, 100 μL of amyloglucosidase from *Aspergillus niger* was added to the digested sample. Digestive reactions after 20, 60, 90, and 120 min were stopped with ethanol 98% (1/4, v/v) to estimate the amount of glucose released during the *in vitro* digestion. High-Performance Liquid Chromatography system (Varian ProStar, model 230, Varian Chromatography Systems, California, USA) equipped with a chromatographic column (Robusta 100A, NH2, 5 μL, 250 mm × 4.6 mm, Sepachrom Srl, Rho, Italy) in combination with a refractive index detector RID-10A (Shimadzu, Kyoto, Japan) were used for glucose separation and evaluation. Acetonitrile/water (3/1, v/v) was used as the mobile phase at a flow rate of 1 mL min^{-1}. Glucose released during *in vitro* digestion was plotted as a function of digestion time. Incremental areas under the curves (IAUCs) were calculated using the trapezoid method proposed by FAO/WHO (1998).

Statistical analysis

Results are expressed as mean ± standard deviation of three replicates ($n = 3$). Bartlett test for the homogeneity of variances, one-way analysis of variance (ANOVA), and Tukey's HSD test for the statistical significance of data ($P < 0.05$) were performed using Statistica software package, version 8.0 (StatSoft, Inc., Tulsa, OK, USA).

Results and discussion

Dough properties

Dough mixing properties, uniaxial extensional properties, linear and non-linear rheology in shear (dynamic and creep tests) were evaluated to obtain information on its structure and technological potential.

Farinograph water absorption (WA) was 55.1% for control dough without salt (C) and 52.9% for control with salt (C-S). The former WA value was used to prepare OP samples without NaCl (OP1 and OP2), while the latter was used for doughs with salt (OP1-S and OP2-S). As expected, C-S gave higher stability and lower softening degree than C indicating a higher dough strength due to an increase in hydrophobic interactions in the gluten network (Table 2). This is the result of the charge shielding effect of salt on gluten proteins (Preston, 1989; He *et al.*, 1992). OP individually added (0.01 and 0.02% on flour weight basis) or in combination with salt increased stability and decreased the degree of softening suggesting an improvement in dough mixing properties. Previously, a similar behaviour was observed for wheat doughs enriched with tannic acid (Zhang *et al.*, 2010; Wang *et al.*, 2015) and sorghum seed proanthocyanidins (Girard *et al.*, 2016). In contrast, mixing tolerance decreased with the inclusion of caffeic, ferulic, syringic and gallic acids (Han & Koh, 2011), and catechin (Girard *et al.*, 2016), which promoted the reduction of gluten disulphide bonds to free thiols weakening the dough. Although OP also altered covalent cross-links between glutenin chains, the dough became more tolerant to mechanical stresses during mixing suggesting the increase in other types of molecular interactions, which led to a stabilisation of gluten structure.

Table 2 Effect of oleuropein (OP) addition on mixing and extensional properties, and stickiness of wheat doughs with and without sodium chloride

Samples	WA (%)[§]	ST (min)[§]	SD (PU)[§]	σ_{max} (kPa)[†]	ε_{max} (–)[†]	$\sigma_{max}\,\varepsilon_{max}^{-1}$	Area (kPa)[†]	$d\ln\sigma\, d\varepsilon_H^{-1}$ (–)[†]	Stickiness (N s)
No salt									
C	55.1 ± 0[a]	2.8 ± 0.1[c]	80.0 ± 0[a]	27.3 ± 2.4[b]	2.23 ± 0.04[a]	12.3	24.8 ± 2.7[b]	1.29 ± 0.03[a]	3.21 ± 0.27[a]
OP1	55.2 ± 0.2[a]	4.0 ± 0.2[b]	50.0 ± 4.1[b]	38.9 ± 2.4[a]	1.91 ± 0.05[b]	20.4	33.9 ± 1.9[a]	1.27 ± 0.02[a]	1.69 ± 0.12[b]
OP2	54.5 ± 0.3[b]	15.4 ± 0.1[a]	52.5 ± 3.5[b]	38.2 ± 0.8[a]	1.68 ± 0.04[c]	22.0	33.4 ± 0.9[a]	1.23 ± 0.05[a]	1.86 ± 0.11[b]
With salt									
C-S	52.9 ± 0.2[a]	15.8 ± 0.4[b]	40.0 ± 0[a]	51.7 ± 2.7[b]	2.04 ± 0.09[a]	25.3	43.2 ± 2.7[a]	1.33 ± 0.03[a]	1.72 ± 0.13[a]
OP1-S	53.1 ± 0.3[a]	17.8 ± 1.0[a]	17.5 ± 3.3[b]	49.9 ± 1.8[b]	1.86 ± 0.08[a]	26.8	44.0 ± 1.6[a]	1.28 ± 0.04[a]	1.81 ± 0.06[a]
OP2-S	52.8 ± 0.2[a]	18.1 ± 0.2[a]	0[c]	57.9 ± 1.2[a]	1.36 ± 0.05[b]	42.5	47.1 ± 1.8[a]	1.34 ± 0.01[a]	1.77 ± 0.11[a]

Mean ± standard deviation ($n = 3$). For each dough type (no salt or with salt), values within a column followed by the same letter are not significantly different, Tukey test ($P > 0.05$). OP addition (on flour weight at 14% moisture): 0 (C), 0.01% (OP1), or 0.02% (OP2) for doughs without NaCl; 0 (C-S), 0.01% (OP1-S), or 0.02% (OP2-S) for doughs with NaCl.

[§]Farinograph water absorption (WA), stability (ST), and softening degree (SD).

[†]Uniaxial extensional properties of doughs at water absorption of 55.1% (no salt) and 52.9% (with salt): fracture stress (σ_{max}), Hencky strain (ε_H) at fracture stress, integrated area under the stress-strain curve (Area), and apparent strain hardening index ($d\ln\sigma\, d\varepsilon_H^{-1}$).

Previous studies reported that non-covalent interactions (hydrogen, ionic, or hydrophobic bonds) (Wang *et al*., 2015) and covalent bonds between hydroxyl and amino groups (Zhang *et al*., 2010) were increased by tannic acid, resulting in gluten aggregation. Moreover, tannin-protein interactions were shown to promote crosslinks in the gluten network leading to stronger doughs (Girard & Awika, 2020).

For unsalted dough, OP showed a dose-dependent effect for stability, while the degree of softening was not influenced by OP level. Surprisingly, the stability of OP2 was about four times that of OP1, but no difference in the degree of softening was observed between these samples indicating that the antioxidant action of OP at 0.02% made gluten-sensitive to over-mixing. Based on softening degree values, OP1 and OP2 showed 34-38% higher tolerance to over-mixing in comparison with C (control). Probably, more than 0.02% polyphenol decreases this gap and at a certain dose induces dough weakening in over-mixing. The addition of OP in combination with salt exhibited a synergic effect on dough tolerance to mechanical stresses.

Table 2 shows uniaxial extensional properties of doughs, which are determinants of bread quality (Dobraszczyk & Morgenstern, 2003; Peressini *et al*., 2017). OP1 and OP2 showed significantly higher fracture stress (σ_{max}) and energy required for extension (area), and lower Hencky strain at break (ε_H) than C (control), confirming a strengthening effect of OP on gluten and a decrease in extensibility ($P < 0.05$). Hencky strain at fracture stress decreased with OP level. Girard *et al*. (2016) reported a similar effect on a weak dough upon incorporation of sorghum seed proanthocyanidins, but when catechin was added resistance to extension decreased. In contrast, Han & Koh (2011) found that phenolic acids reduced dough resistance to extension and increase extensibility since their reducing action on disulphide crosslinks in gluten network induced a decrease in high molecular weight glutenins.

When OP was added in combination with salt, σ_{max} significantly increased and ε_H decreased only at the maximum level compared to C-S ($P < 0.05$). OP2-S gave the lowest extensibility among samples. Consequently, $\sigma_{max}\ \varepsilon_H^{-1}$ ratio ranged from 12.3 for control without salt (C) to 42.5 for OP2-S. Since large loaf volume is the result of high σ_{max} and high ε_H of the dough, OP addition at 0.02% could be critical.

Further investigation was performed using shear oscillatory and creep-recovery tests. Fig. 1 shows mechanical spectra from oscillatory frequency sweep test and creep-recovery curves of unsalted doughs. In order to obtain information on dough viscoelastic properties, storage modulus (G′) and loss tangent (tan δ) at a frequency of 1 Hz, maximum compliance (J_{max}) and relative elasticity (J_{el}) were evaluated (Table 3). Doughs without salt exhibited significantly higher G′ and lower tan δ values for OP samples than C (control), which are related to more elastic interactions and solid-like behaviour (stronger gluten) ($P < 0.05$). Similar trends were found for grape seed and sorghum

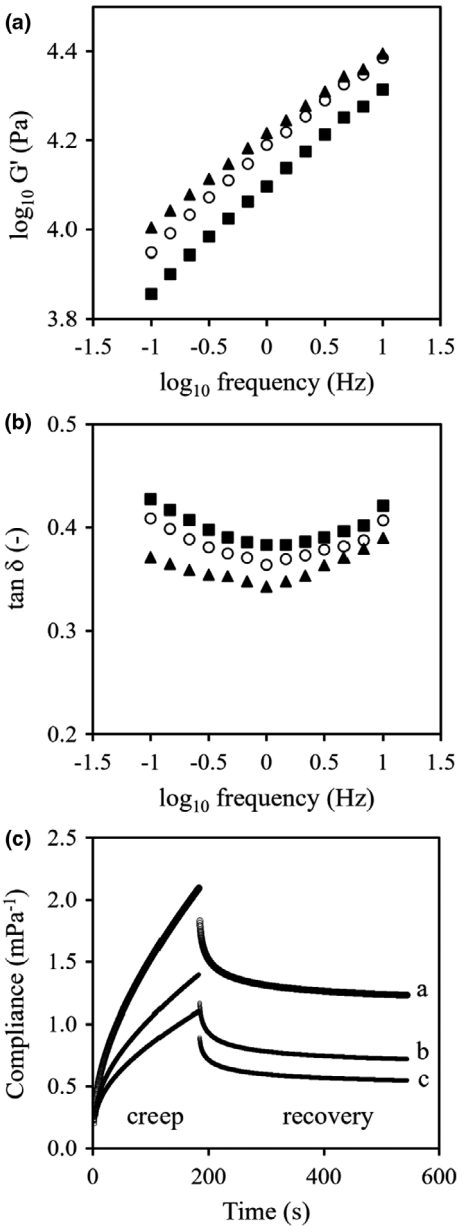

Figure 1 Viscoelastic properties of unsalted wheat doughs with and without oleuropein (OP) from oscillatory frequency sweep and creep-recovery tests. Storage modulus (G′) and loss tangent (tan δ) as a function of frequency (a, b). Compliance as a function of time (c). OP addition (on flour weight at 14% moisture): 0 (square, a), 0.01% (circle, b), or 0.02% (triangle, c).

Table 3 Effect of oleuropein (OP) addition on viscoelastic properties of wheat doughs with and without sodium chloride obtained from shear oscillatory and creep-recovery tests

Samples	G′ (kPa)[§]	tan δ (–)[§]	J_{max} (mPa^{-1})[†]	J_{el} (–)[†]
No salt				
C	12.5 ± 0.1[b]	0.38 ± 0.00[a]	2.28 ± 0.11[a]	0.42 ± 0.03[b]
OP1	15.5 ± 0.4[a]	0.36 ± 0.01[b]	1.47 ± 0.01[b]	0.48 ± 0.02[a]
OP2	16.5 ± 0.4[a]	0.35 ± 0.01[c]	1.23 ± 0.02[c]	0.50 ± 0.01[a]
With salt				
C-S	16.1 ± 0.5[a]	0.36 ± 0.00[a]	2.12 ± 0.17[a]	0.36 ± 0.02[b]
OP1-S	15.7 ± 0.1[a]	0.35 ± 0.01[b]	1.10 ± 0.03[b]	0.42 ± 0.06[ab]
OP2-S	15.6 ± 0.6[a]	0.36 ± 0.01[ab]	1.19 ± 0.23[b]	0.52 ± 0.05[a]

Mean ± standard deviation ($n = 3$). For each dough type (no salt or with salt), values within a column followed by the same letter are not significantly different, Tukey test ($P > 0.05$). OP addition (on flour weight at 14% moisture): 0 (C), 0.01% (OP1) or 0.02% (OP2) for doughs without salt; 0 (C-S), 0.01% (OP1-S) or 0.02% (OP2-S) for doughs with salt.

[§]Values at 1 Hz obtained from oscillatory frequency sweep test on doughs at water absorption of 55.1% (no salt) and 52.9% (with salt).

[†]Parameters obtained from creep-recovery test on doughs.

proanthocyanidins added to a weak flour (Girard *et al.*, 2016). No differences in these parameters were recorded for salted doughs except for OP1-S, which gave a lower tan δ value than C-S (control).

OP individually added or in combination with salt decreased J_{max} and increased J_{el} compared to control samples, indicating that dough is more resistant to deformation in creep and its elastic recovery of deformation is higher. This confirms that the dough containing OP was stronger and more elastic. It was reported that a greater elastic recovery of the dough is correlated to a large bread volume (Wang & Sun, 2002). Differences in viscoelasticity related to the phenolic compound became more apparent using creep-recovery test (large strain) than frequency sweep (small strain), in particular for salted doughs. Sensitivity of creep-recovery to detect small alterations in the gluten network was in agreement with Avramenko *et al.* (2020), who evaluated doughs containing different salts.

Finally, the impact of OP on the prevention of dough stickiness was determined. Sodium chloride is usually included in dough formulation to make it less sticky (Avramenko *et al.*, 2020). Interestingly, OP addition significantly lowered the stickiness of unsalted dough to values comparable to the dough containing salt ($P < 0.05$) (Table 2). Therefore, OP could be a NaCl-alternative useful to improve handling properties (machinability) of doughs in a low sodium diet.

Bread characteristics

The effects of OP supplementation on loaf specific volume (Vs) and crumb firmness are reported in Table 4. The addition of OP in both enriched samples significantly increased Vs compared to the control ($P < 0.05$). Rheological results showed that doughs containing OP were stronger due to more elastic interactions in the gluten network (Tables 2 and 3), which conferred to the gluten matrix more resistance to collapse during dough expansion leading to the increase in loaf volume. No significant difference in Vs was observed between OP bread samples ($P > 0.05$). OP addition at 0.02% gave a dough with the lowest Hencky strain (extensibility), but this seems to be not detrimental for dough expansion. A possible explanation could be a partial breakdown of disulphide bonds during the leavening of bread dough since an antioxidant action of OP at 0.02% was observed in dough over-mixing (Table 2). Crumb firmness significantly decreased with the increase in OP level added in bread dough, exhibiting a reduction of values by 19-30% compared to the control ($P < 0.05$) (Table 4). Changes in mechanical properties of OP-breads are mainly due to higher Vs and crumb moisture. Similar findings were reported by Zhang *et al.* (2010) for tannic acid, which made doughs stronger promoting an increase in loaf expansion and reduced firmness. Besides, tannic acid delayed amylose and amylopectin retrogradation

Table 4 Effect of oleuropein (OP) addition on bread characteristics and functionality

Samples	Moisture (%)	Firmness (g)	Vs ($cm^3\ g^{-1}$)	Cell density (cells cm^{-2})	MCA (mm^2)	OP (mg 100 g^{-1})[†]	AA (%)	RSC (μmol TE g^{-1})[†]	IAUC (mg g^{-1} min)[†]
CB	40.16 ± 0.94[b]	242 ± 23[a]	4.37 ± 0.02[b]	85 ± 4[a]	0.38 ± 0.05[b]	nd*	4.67 ± 0.06[c]	0.345 ± 0.004[c]	6200 ± 170[b]
OP1B	42.51 ± 0.24[a]	196 ± 19[b]	4.67 ± 0.04[a]	81 ± 1[ab]	0.44 ± 0.07[ab]	nd*	5.21 ± 0.16[b]	0.378 ± 0.009[b]	7210 ± 251[a]
OP2B	42.86 ± 0.50[a]	171 ± 17[c]	4.71 ± 0.03[a]	78 ± 1[b]	0.53 ± 0.02[a]	0.95 ± 0.09	6.97 ± 0.20[a]	0.478 ± 0.016[a]	6860 ± 109[a]

Mean ± standard deviation ($n = 3$). Values within a column followed by the same letter are not significantly different, Tukey test ($P > 0.05$). OP addition (on flour weight at 14% moisture): 0 (CB), 0.01% (OP1B) or 0.02% (OP2B) for bread products.

AA, antiradical activity; IAUC, incremental area under the curve; MCA, mean cell area; RSC, radical scavenging capacity; Vs, specific volume.

[†]On dry basis.

*Not determined.

(Zhang *et al*., 2010). In contrast, other studies showed that the addition of caffeic acid (Han & Koh, 2011) and ferulic acid (Koh & Ng, 2009; Nicks *et al*., 2013) to wheat dough weakened the gluten network with a detrimental effect on loaf expansion, probably due to an excessive breakdown of disulphide bonds within the gluten network (Koh & Ng, 2009; Han & Koh, 2011; Sui *et al*., 2016) and/or to the yeast activity inhibition (Nicks *et al*., 2013). Moreover, Sui *et al*. (2016) showed that bread fortified with anthocyanin-rich extract (1%–4%) was firmer with a tight crumb structure due to a weaker dough and poor gas retention.

Image analysis on bread slices (Fig. 2) was performed to obtain information on crumb grain in terms of cell density and mean cell area (MCA) (Table 4). No significant differences in crumb cellular structure were observed between control and bread made from a dough added with 0.01% OP. The increase in OP level to 0.02% gave a significant reduction in cell density and higher MCA than control, revealing a coalescence of gas cells ($P < 0.05$). Green tea extract (0.5% on flour weight) gave a similar effect on crumb grain of white bread (Wang *et al*., 2007). Looking at rheological results, low extensibility (ε_H) of OP2-S did not restrict air cell expansion supporting the hypothesis of a breakdown of disulphide bonds during dough leavening due to an antioxidant action of OP at 0.02% (Tables 2 and 3). Image analysis results indicate that cell structure of crumb was not affected by the addition of OP at a level of 0.01%.

Based on these results, the following potential mechanism might be proposed to describe the effects of OP addition in breadmaking: (i) alteration of covalent cross-links (disulphide bonds) between glutenin chains and promotion of other types of molecular interactions, resulting in gluten aggregation; (ii) strengthening effect of OP on the dough and a decrease in extensibility due to more elastic interactions in the gluten network; (iii) increase in bread volume due to more resistance of gluten matrix to collapse during dough expansion. Probably, OP at 0.02% acts as an antioxidant during dough leavening leading to an improvement in extensibility, thus promoting bread dough expansion.

Bread functionality

Oleuropein content and antioxidant activity

Generally, functional ingredients undergo degradative reactions during breadmaking, as reported for high molecular weight β-glucans (Cleary *et al*., 2007) and catechins (Pan *et al*., 2022) added to bread formulations, resulting in chemical derivates with limited nutritional values.

Table 4 shows OP content of OP2B bread sample, which indicates a 97% loss in the phenolic compound in comparison to the amount included in the dough. This result suggests a degradation of most of the bioactive compounds during breadmaking. Derivates from oleuropein are phenolic alcohols, mainly hydroxytyrosol and hydroxytyrosol acetate. However, hydroxytyrosol was not found in OP2B bread. Three mechanisms were proposed to describe the loss of polyphenol compounds during the process: (i) oxidative reactions (Stamatopoulos *et al*., 2014); (ii) several yeast strains utilise OP as a carbon source for their metabolism and further biological activities (Mujdeci & Ozbas, 2020), probably causing OP degradation during dough fermentation and proofing; (iii) thermal degradation during baking (Sui *et al*., 2015; Lin & Zhou, 2018). Previously, OP thermal degradation was observed during simulation of home-cooking process at 120–170 °C for 15–60 min (Lozano-Castellón *et al*., 2020) and 80–230 °C for a sufficient period of time (Attya *et al*., 2010). Lozano-Castellón *et al*. (2020) reported an 85% reduction in phenolic alcohols (90% in hydroxytyrosol) under high temperature and long cooking time conditions. Hydroxytyrosol seems to be prone to thermal degradation more easily than other phenolic alcohols. For this reason, OP2B bread did not contain hydroxytyrosol. In contrast, Cedola *et al*. (2020) studied olive leaf extract-enriched taralli prepared from an unleavened dough and reported that baking (200 °C for 12 min) did not alter the content of polyphenols (mainly OP). The OP-enriched bread functionality was assessed in terms of antioxidant capacity. The results are reported in Table 4. Antiradical activity (AA) and DPPH radical scavenging capacity (RSC) of samples added with OP showed

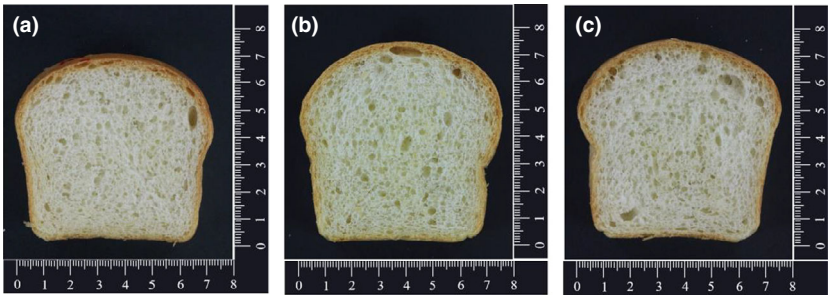

Figure 2 Slices of bread with and without oleuropein (OP). OP addition (on flour weight at 14% moisture): 0 (a), 0.01% (b), or 0.02% (c).

significantly higher values than control bread ($P < 0.05$). The improvement in antioxidant properties of enriched samples could be attributed to OP and its thermal degradative chemical derivates, such as elenolic acid and hydroxyelenolic acid (Lozano-Castellón *et al*., 2020). Generally, OP and the other olive leaf constituents have the ability to donate electrons to free radicals, converting them to more stable species and ending radical chain reactions (Lee *et al*., 2009). Control bread possessed a specific antioxidant activity probably due to the simultaneous presence of endogenous phenolic compounds in the flour and other antioxidant substances formed during baking, as previously reported by other authors (Sęczyk *et al*., 2017; Świeca *et al*., 2017).

Starch digestion

Impact of differences in structure between samples on starch digestion was investigated using a standardised *in vitro* protocol, which simulates bread human digestion. The glucose released from bread crumb was plotted against digestion time for control, OP1B and OP2B samples. The glycaemic curves and the relative incremental areas under the curves (IAUC) are reported in Fig. 3 and Table 4, respectively. Control sample exhibited the lowest IAUC value in comparison with OP-enriched breads ($P < 0.05$). In aerated products such as bread, high expansion and cellular structure of crumb increase susceptibility of gelatinised starch to amylases activity, due to the highly porous structure and direct accessibility to amylopectin and amylose chains (Mishra *et al*., 2012). In accordance with this mechanism, bread samples containing OP showed higher Vs (lower density) and IAUC values compared to control bread ($P < 0.05$) (Table 4), confirming that a less compact crumb is related to a higher glycaemic response because of multiple interactions between starch and digestive enzymes. Several authors stated a relation between highly porous structures and higher digestive enzymes accessibility (Fardet *et al*., 2006; Eelderink *et al*., 2015; Borczak *et al*., 2018; Aleixandre *et al*., 2019). Recently, Ge *et al*. (2021) reported that the addition of different amounts of pumpkin powder, rich in dietary fibre and bioactive compounds, reduced bread-specific volumes and originated more compact structures which encapsulated starch granules, retarding starch digestion. Moreover, gluten-free breads with more compact structure showed reduced *in vitro* glycaemic responses (De La Hera *et al*., 2014; Carini *et al*., 2015). In contrast, Yun *et al*. (2021) found that white bread enriched with a novel resistant starch (NRS) possessed a more compact structure in comparison with control and at the same time higher *in vitro* GI values. However, this was probably due to a complete gelatinisation of NRS upon the baking process.

Several phenolic compounds in baked products exhibited potential inhibitory effects on digestive enzymes, slowing down starch digestion (Ou *et al*., 2019). Based on IAUC data (Table 4), OP and its derivates did not inhibit digestive amylases during in *vitro* starch digestion. However, these compounds might act on other biological mechanisms leading to the reduction of the postprandial glycaemic response, as previously reported by Carnevale *et al*. (2018).

In conclusion, the product structure and available carbohydrates for digestion are important factors for modulating the postprandial glycaemic response.

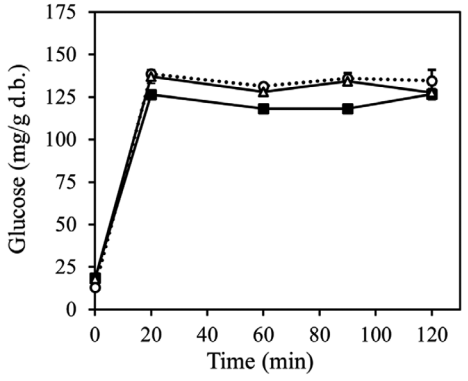

Figure 3 *In vitro* digestion of bread with and without oleuropein (OP). Glucose release as a function of digestion time. Mean ± SEM ($n = 3$). OP addition (on flour weight at 14% moisture): 0 (square), 0.01% (circle), or 0.02% (triangle).

Conclusions

This study evaluated the effects of OP inclusion on the breadmaking performance of wheat doughs and bread products. The OP addition improved the mixing properties of doughs with and without added salt, by promoting alternative hydrophobic interactions (covalent and non-covalent bonds). Extensional properties of unsalted dough showed that OP incorporation significantly increased the stress required for fracture the dough and the energy to extend it, suggesting the ingredient as a strengthener of the gluten network. For dough with salt, OP2-S exhibited the lowest extensibility between samples. Doughs containing OP were more elastic, stronger, and less sticky than the control sample. Although reduced extensibility of enriched doughs, OP in breads positively affected loaf volume expansion and reduced crumb firmness, confirming the hypothesis of new and stronger molecular connections promoted by the polyphenol. The results from the *in vitro* digestion showed higher values of IAUC for OP1B and OP2B samples, probably due to the more aerated and open structures, which facilitated the access to

digestive enzymes. After baking, in OP2B bread the oleuropein content was about 3% of the starting amount, suggesting that the process was detrimental for the compound. Alternative baking methods (such as microwave, infrared heating, or a combination) could represent a potential solution to avoid thermal degradation of OP and its derivates. However, antioxidant results showed that both enriched breads were characterised by higher antiradical activities caused by native compounds and OP chemical derivates. Oleuropein could be incorporated in bread formulation to improve dough mechanical properties and to produce bread products with a good volume expansion.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

Niccolò Renoldi: Investigation (lead), Formal analysis (lead), Methodology (equal), Visualisation (lead), Writing – original draft (lead). **Paolo Lucci:** Conceptualisation (supporting), Methodology (equal), Resources (supporting), Writing – review & editing (supporting). **Donatella Peressini**: Supervision (lead), Conceptualisation (lead), Methodology (equal), Resources, Writing – review & editing (lead).

Ethical approval

Ethics approval was not required for this research.

Data availability statement

Research data are not shared.

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