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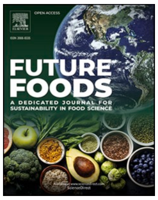

Effect of bread-making process on the quality of acorn flour-enriched bread

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ABSTRACT

Acorn flour has been recently proposed as an innovative and sustainable ingredient in bread production with potential nutritional and health benefits. This work aims to evaluate the inclusion of acorn flour in bread and to optimize acorn bread production process by optimizing acorn flour and dough hydration levels as well as production method (direct, sourdough, gel, and pre-hydration methods). The optimized breads were characterized in terms of specific volume, texture, bioactive compounds, acrylamide, and volatile organic compounds.

The results show that increasing acorn flour levels (0–50%) in wheat sourdough bread progressively reduced loaf volume and hardened crumb texture. These undesired effects were significantly minimized by modulating dough hydration level and pre-hydrating acorn flour during dough preparation, allowing for obtaining bread with acceptable specific volume and texture up to 40% acorn flour inclusion. Acorn flour inclusion and sourdough fermentation significantly increased total polyphenol content and antioxidant activity, decreased acrylamide levels and improved aroma complexity, compared to the wheat control.

This study provides a systematic investigation to identify the best production process to obtain acorn-enriched wheat sourdough bread, with an insight into physico-chemical profiles of the optimized products, offering an important reference for future studies on sustainable food development.

1. Introduction

The growing consumer demand for sustainable and “natural” food, characterized by a preference for environmentally sustainable, transparent sourcing, and traditionally processed ingredients, is significantly shaping food product development and purchasing decisions of consumers across global markets (Dooren et al., 2024; Kirby and Teixeira, 2025). Acorn, a forgotten plant-based natural food and source of nutrients in many cultures, has recently been the object of interest of the scientific community as an innovative food ingredient, due to its sustainability (Inácio et al., 2024) and richness in fibre and bioactive constituents (i.e., antioxidants) (Vinha et al., 2016). Acorn flour is considered a new and promising ingredient in bread-making, and its incorporation has been reported to increase free phenolic content and volatile compounds in bread (Suo et al., 2025) although it may negatively affect dough rheology and final product quality. Specifically, acorn-enrichment in dough shortened development time and extended stability, while in bread it decreased crumb density and cohesiveness, increased hardness (Gonzaga et al., 2015), as well as reduced the specific volume of bread loaves (Suo et al., 2025; Szabłowska and Tańska, 2025). However, the bread volume reduction was shown to be modulated by varying water addition in the formulation (Skendi et al., 2018). Indeed, acorn flour is rich in fibers and phenolic compounds that have high water-binding capacity, which alters dough hydration compared to conventional wheat systems (Forouhar et al., 2024; Suo et al., 2025). Therefore, adjusting the amount of added water directly influences gluten development, dough viscosity, gas retention, and ultimately loaf expansion during baking. In addition, to the authors’ best knowledge,

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the effect of acorn flour inclusion in bread has been studied only in products produced with a direct production method (i.e., fermentation by means of *Saccharomyces cerevisiae*), while the application of sourdough processing has not been investigated yet.

Sourdough processing is one of the oldest methods of bread leavening, dating back over 5000 years, originating from the natural fermentation of flour and water by wild yeasts and lactic acid bacteria (Chavan and Chavan, 2011; Stolz, 1999). Over the past several decades, sourdough technology has gained increasing attention in both academic and industrial sectors, driven by the growing demand for clean-label foods (Do Nascimento et al., 2018) and the potential benefits to bread quality and human health associated to its consumption. Sourdough bread is characterized by a complex and peculiar flavor and, if properly applied can soften bread crumb, slow staling during storage, and increase bread volume (Islam and Islam, 2024; Seis Subaşı and Ercan, 2024). A systematic review of clinical trials about sourdough bread consumption reported a general positive impact on reducing postprandial glycemic response (Rolim et al., 2024), mainly due to the lower pH (3.5 – 4.5) of the dough (organic acids were formed) that favors an increase in resistant starch content, thus reducing starch digestibility (D'Amico et al., 2023; Rolim et al., 2024).

Besides leavening, baking also plays a key role in defining bread quality as it involves multiple physical and chemical transformations that must be optimized to favour retention of beneficial compounds and to minimize the formation of potentially harmful substances, including acrylamide and furanic compounds. Acrylamide is defined as "probably carcinogenic to humans" by the International Agency for Research on Cancer (IARC) and the European Commission (European Commission, 2017), while furan content should be minimized to be As Low as Reasonably Achievable (ALARA principle) because of their potential toxicity (EFSA CONTAM Panel, 2017).

Up to now, the combination of sourdough and acorn flour has been investigated only in gluten-free bread production, and the results highlighted a decrease in starch hydrolysis, an enhancement of mineral content, total phenolic compounds, and antioxidant activity, and a better overall nutritional profile compared to a gluten-free rice control bread (Beltrão Martins et al., 2022). Moreover, Levent and Aktaş (2023) demonstrated that fermenting acorn flour prior to incorporation not only enriched gluten-free bread with higher protein, phenolic content, and mineral bioavailability, but also influenced staling kinetics.

As stated earlier, to the authors' best knowledge, no studies have investigated the effects of acorn flour enrichment in sourdough wheat bread systems. Although acorn flour has been explored in breadmaking, its application has been mainly limited to the straight-dough method or gluten-free sourdough systems. A systematic assessment of processing strategies for acorn flour-enriched sourdough bread is still lacking. Given the high fiber content and water-binding capacity of acorn flour (Suo et al., 2025), its incorporation into wheat-based doughs may significantly alter water distribution and gluten network development (Curti et al., 2013). The competition for water between gluten proteins and fiber-rich particles can negatively affect dough rheology, gas retention, and final bread structure (Li et al., 2025). Therefore, this study represents the first comprehensive attempt to define suitable production conditions through a structured experimental approach. Throughout process optimization, technical acceptability was defined by evaluating key quality indicators, including specific volume and crumb hardness, to ensure the final formulations achieved adequate loaf expansion and the desirable soft texture. In the first phase, two breadmaking techniques (straight-dough vs. sourdough) were compared at three acorn flour enrichment levels (10%, 20%, and 30%) and three hydration levels (75%, 77%, and 80%). This screening step allowed the identification of the most suitable formulation and processing conditions. In the second phase, the selected formulation was further investigated by evaluating the effect of different technological approaches [namely straight-dough, sourdough, gel incorporation (practice used by the bakery), and acorn flour pre-hydration (Cai et al., 2015; Wu et al., 2025)] on bread quality. These approaches were specifically tested to enhance flour hydration, modulate gluten development, and mitigate the technological drawbacks associated with high-acorn enrichment. The resulting breads were comprehensively characterized in terms of bioactive compounds, process contaminants (acrylamide and furanic compounds), and volatile organic profile.

Overall, this study provides the first systematic evaluation of acorn flour-enriched sourdough bread, integrating technological optimization and safety assessment. The findings are expected to offer practical insights to guide both research and industry in developing high-qualityand safe bakery products based on alternative flours.

2. Materials and Methods

2.1. Materials

Hard wheat flour was (Manitaly provided by Molino Paola Mariani, Italy) characterized, according to the product's technical datasheet, by the following parameters: W = 360, P/L = 0.70, 15.5% proteins, 3.3% fibres; 68.3% carbohydrates [of which 2.4% sugars], fat 1.0% [of which 0.3% saturated fat], ashes 0.58%. Acorn flour was an organic product supplied by Dary Natury Sp. z. o., Poland and contained 4.1% proteins, 16.8% fibres, 73.4% carbohydrates [of which 9.9% sugars]; fat 5.1% [of which 0.9% saturated fat], according to the product's technical datasheet.

The sourdough used was the one maintained daily by Forneria Marchigiana and produced with 30% Manitaly wheat flour type 0, 70% organic wheat flour type 2 (W = 190, P/L = 0.65, 11.9% proteins, 3.5% fibres; 67.8% carbohydrates [of which 1.9% sugars]; fat 1.0% [of which 0.2% saturated fat]; Molino Agostini, Massignano AP, Italy), and 47% water, and it was refreshed every 24 h. Water was obtained from the city aqueduct and treated with a reverse osmosis filter. Yeast (*Saccharomyces cerevisiae*, compressed yeast) and salt were purchased from a local supermarket.

2.2. Bread production

Acorn bread production was carried out in the facilities of a commercial bakery (Forneria Marchigiana, Morrovalle [MC], Italy) whose machinery allows for 5 Kg batches of wheat flour to be processed. Bread was produced at first using a direct method with the use of yeast (*Saccharomyces cerevisiae*), and then, based on the success of the direct method, a sourdough process was implemented. Multiple trials were carried out to optimize the sourdough bread production process and, in particular, to identify the best way to incorporate acorn flour to form a *"wheat-acorn dough"* suitable for further processing into bread, as summarized in Fig. 1. Bread recipes used are reported in Tables 1 and 2. Recipes differed for both acorn flour substitution (direct and sourdough method: 0–30%; gel and pre-hydration method: 0–50%) and water addition (direct method: 75%; sourdough: 75–80%; gel: 94–137%; pre-hydration: 65–89%), and are the outcome of multiple (unreported) preliminary trials aiming to increase acorn flour inclusion and optimize water addition to improve bread quality. Samples were named following the logic: the letter refers to the bread-making method (D = direct, S = sourdough, G = gel, P = pre-hydration); the first number refers to the percentage of acorn flour in the sample; the second number is the hydration level (% water).

2.2.1. *"Wheat-acorn dough" production methods*

Direct method

Wheat (80% of total wheat flour) and acorn flours were blended to obtain a homogeneous mixture that was then inserted into a planetary mixer (IBT5, Kosmitech s.r.l., Morrovalle MC, Italy) where water (80% of total water) was added prior to kneading at 60 rpm for 8 min to obtain a wheat-acorn dough that was allowed to rest in a stainless-steal container sealed with polyethylene wrap at 30 °C for 90 min (Stratos

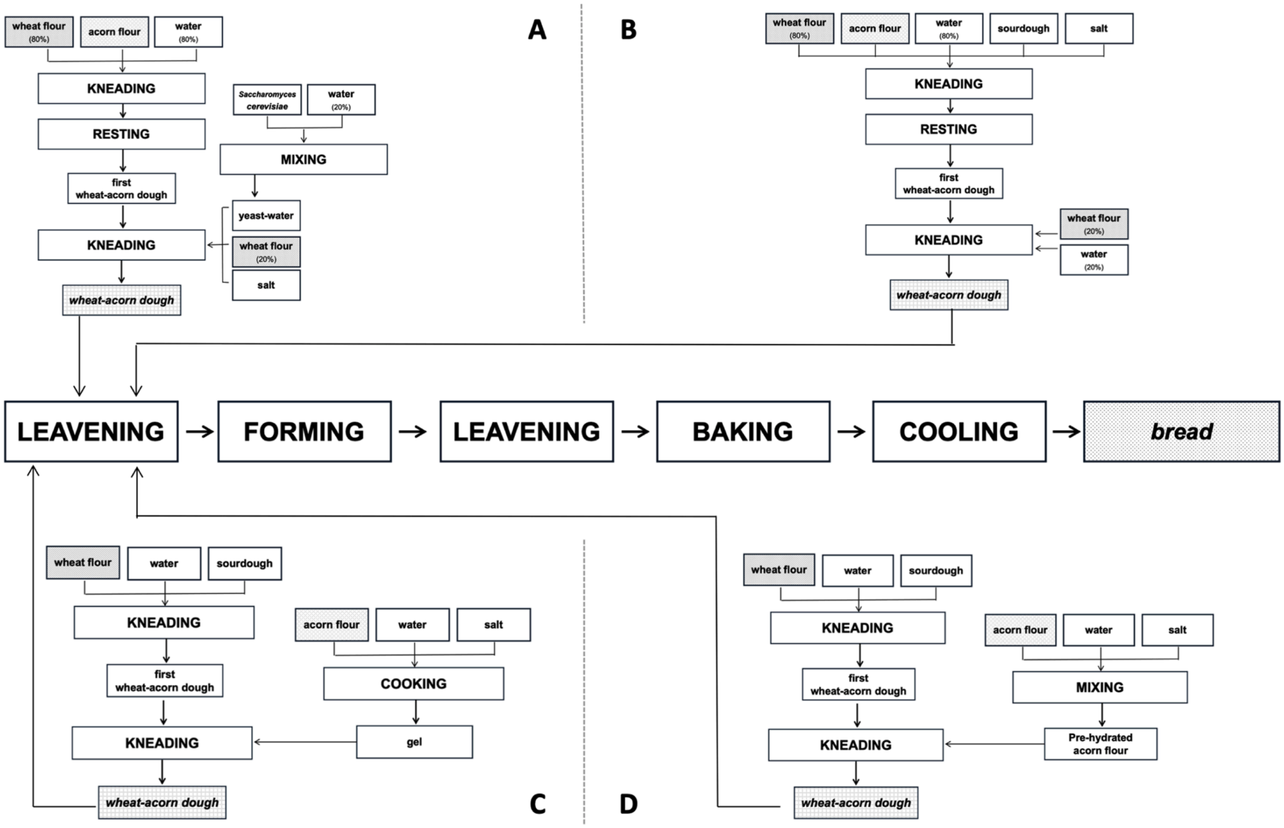

Fig. 1. Bread production flowchart (using different production methods: A- Direct, B- Sourdough, C- Gel, D- Pre-hydration).

Table 1
Bread recipes used in the direct and sourdough methods.

Bread recipe	Acorn substitutions									
A - Direct method	0%	10%			20%			30%		
Wheat flour (g)	100	90	-		80	-		70	-	
Acorn flour (g)	-	10	-		20	-		30	-	
Yeast (g)	3	3	-		3	-		3	-	
Water (g)	75	75	-		75	-		75	-	
Salt (g)	1.5	1.5	-		1.5	-		1.5	-	
Sample name*	**D-0–75**	**D-10–75**	-		**D-20–75**	-		**D-30–75**	-	
B - Sourdough method										
Wheat flour (g)	100	90	90	90	80	80	80	70	70	70
Acorn flour (g)	-	10	10	10	20	20	20	30	30	30
Sourdough (g)	30	30	30	30	30	30	30	30	30	30
Water (g)	75	75	77.5	80	75	77.5	80	75	77.5	80
Salt (g)	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Sample name*	**S-0–75**	**S-10–75**	**S–10–77.5**	**S-10–80**	**S-20–75**	**S–20–77.5**	**S-20–80**	**S-30–75**	**S–30–77.5**	**S-30–80**

Sample name.
* **:** the letter refers to the bread-making method (D = direct, S = sourdough); the first number refers to the percentage of acorn flour in the sample; the second number is the hydration level (% water).

XP, Polin, Verona Italy). Yeast was dispersed into the remaining water (20% of the total water). The remaining 20% of wheat flour, yeast-water dispersion, and salt were then slowly incorporated into the wheat-acorn dough (in the planetary mixer) by kneading at 60 rpm for 3 min, and at 120 rpm for 60 s to obtain the *"wheat-acorn dough"* that was further processed. A schematic representation of the process is shown in Fig. 1A.

Sourdough method

Wheat (80% of total wheat flour) and acorn flours were blended to obtain a homogeneous mixture and were then inserted in the planetary mixer (IBT5, Kosmitech s.r.l., Morrovalle MC, Italy) where water (80% of total water) and sourdough were then added prior to kneading at 60 rpm for 8 min to obtain a wheat-acorn dough that was allowed to rest in a stainless-steal container sealed with polyethylene wrap at 30 °C for 90 min (Stratos XP, Polin, Verona Italy). The remaining 20% of wheat flour and water were then slowly incorporated into the wheat-acorn dough (in the planetary mixer) by kneading at 60 rpm for 3 min, and at 120 rpm for 60 s to obtain the *"wheat-acorn dough"* that was further processed. A schematic representation of the process is shown in Fig. 1B.

Gel method

Wheat flour, water and sourdough were at first kneaded in the planetary mixer (IBT5, Kosmitech s.r.l., Morrovalle MC, Italy) at 60 rpm for 8 min to obtain a wheat dough. Acorn flour was preliminarily mixed into boiling water (in a ratio of 1:2.25), cooked for 30 s, cooled to room temperature to obtain a gel that was then incorporated into the wheat-dough (in the planetary mixer) by kneading at 60 rpm for 3 min, and at 120 rpm for 60 s to obtain the *"wheat-acorn dough"* that was further

Table 2
Recipes used in the gel and pre-hydration methods.

Bread recipe	Acorn substitution							
C - Gel method	20%		25%	30%		40%		50%
Wheat flour (g)	-		75	70	-	-	60	50
Acorn flour (g)	-		25	30	-	-	40	50
Sourdough (g)	-		30	30	-	-	30	30
Water (g)	-		94	103	-	-	120	137
Salt (g)	-		1.5	1.5	-	-	1.5	1.5
Sample name	-		**G-25–94**	**G-30–103**	-	-	**G-40–120**	**G-50–137**
D - Pre-hydration method								
Wheat flour (g)	80	80	-	70	70	60	60	50
Acorn flour (g)	20	20	-	30	30	40	40	50
Sourdough (g)	30	30	-	30	30	30	30	30
Water (g)	65	70	-	72.5	80	80	89	87.5
Salt (g)	1.5	1.5	-	1.5	1.5	1.5	1.5	1.5
Sample name*	**P-20–65**	**P-20–70**	-	**P–30–72.5**	**P-30–80**	**P-40–80**	**P-40–89**	**P–50–87.5**

Sample name.
* : the letter refers to the bread-making method (G = gel, P = pre-hydration); the first number refers to the percentage of acorn flour in the sample; the second number is the hydration level (% water).

processed. A schematic representation of the process is shown in Fig. 1C.

Pre-hydration method

Wheat flour, water, and sourdough were at first kneaded in the planetary mixer (IBT5, Kosmitech s.r.l., Morrovalle MC, Italy) at 60 rpm for 8 min to obtain a wheat dough. Acorn flour was pre-hydrated with water (1:1.25 flour:water ratio) at room temperature for 15 min and the pre-hydrated acorn flour was then incorporated into the wheat-dough (in the planetary mixer) by kneading at 60 rpm for 3 min, and at 120 rpm for 60 s to obtain the *"wheat-acorn dough"* that was further processed. A schematic representation of the process is shown in Fig. 1D.

2.2.2. *Bread-making process*

"Wheat-acorn dough" obtained from section 2.2.1 was allowed to leaven at 30 °C for 90 min (Stratos XP, Polin, Verona Italy), was then divided into 600 g aliquots that were placed into plastic baskets (21.5 cm diameter, 9.5 cm height), and covered with a linen bakery cloth, and then allowed to leaven at 30 °C for 90 min before baking at 250 °C for 50 min in a forced convection stone oven (Italforni, Avanzini model ANGASMATIC, methane powered). Baked bread was then taken out of the oven, cooled to room temperature, and analyzed on the day after production. For each bread type, three productions were repeated, and two bread replicates were produced in each production (6 replicates for each bread type).

2.3. *Bread characterization*

2.3.1. *Physical properties*

All breads were characterized for specific volume, water content, and hardness. Specific volume of each bread loaf was measured using the rapeseed displacement method according to AACC Method 10–05.01 (AACC, 2001) for baking quality. Specific volume was calculated by dividing loaf volume by loaf weight and was expressed as g/cm^3. Results were expressed as mean ± standard deviation of six independent trials.

Water content of bread crumb was measured by drying the crumb in an oven at 105 °C to constant weight and was calculated as the percentage of weight loss (%, g H_2O/ 100 g bread crumb), following the AACC method 44–15.02 (AACC, 1999). Results were expressed as mean ± standard deviation of six independent trials.

Hardness of bread crumb was measured as previously described (Rinaldi et al., 2015) with slight modification. Briefly, bread crumbs were cut into crumb cubes (2 × 2 × 2 cm) and were compressed with a double compression test using a TA1 Texture Analyzer (Ametek, USA) equipped with a 100 N load cell and a cylindrical probe (25 mm × 100 mm): each cube was compressed to 40% of its height at 5 mm/s, then the probe was released; the holding time between the two compressions was 10 s. The maximum force during the first compression cycle was recorded as bread hardness (N). Results were expressed as mean ± standard deviation of eight measurements of three independent trials.

2.3.2. *Chemical properties of breads made with the optimized process*

Chemical composition of breads was analyzed only for the breads produced with the optimized process (i.e., P-20–70, P-30–80, P-40–89) as well as for a 100% wheat sourdough sample (sample S-0–75), a 100% wheat direct method bread (sample D-0-75), and the 20% AF (sample D-20-75) direct method product, for comparison.

Antioxidant activity (DPPH method) was carried out using the methodology set by Piatti et al. (2024). The decrease in DPPH radical concentration was then measured at 517 nm using an Agilent Cary 8454 UV–Vis spectrophotometer. Trolox was used as the reference antioxidant, and the results were expressed as mg trolox equivalent/g dry weight (DW) of sample ± standard deviation of two independent trials.

Total polyphenolic content (TPC) was determined spectrophotometrically, following the method described by Pompei et al. (2025) with some modifications. It was measured using Agilent Technologies (Cary 8454 UV–Vis, Woburn, Massachusetts, USA). TPCs were expressed as milligrams of gallic acid equivalents (GAE)/g dry weight (DW) of the sample. Analytical determinations of TPC were performed in duplicate for each sample, and all results are expressed as mean values ± standard deviation.

Acrylamide content was determined according to a previous report (Schouten et al., 2024). Ground, freeze-dried acorn bread samples (1 g) were spiked with 0.2 mL of AA-d3 (2500 ng/mL), mixed with 9.8 mL water, stirred, sonicated for 30 min at 40 kHz and centrifuge at 5000 rpm, 10 min. The supernatant was filtered, collected at 4 °C, then purified by solid-phase extraction (SPE) using Oasis HLB and Bond Elut-Accucat cartridges. Eluates were filtered (0.2 μm) and analysed by UHPLC-MS/MS (Agilent 1290 Infinity with 6420 Triple Quad, ESI+, MRM mode). Separation was performed using a water/acetonitrile mobile phase (0.1% formic acid) in gradient mode at 0.8 mL/min. Instrument settings included a 2 μL injection volume, 350 °C drying gas, 12 L/min flow, 45 psi nebulizer, and 4000 V capillary voltage. Quantification used the most abundant transition; LOQ and LOD were 10 and 3 times the signal-to-noise ratio, respectively, and the present method possessed the same sensitivity as that already developed and reported (Schouten et al., 2022). Each sample was analysed in duplicate.

Furanic compounds were quantified using HS-SPME-GC–MS (Acquaticci et al., 2024). Briefly, 1 g of each sample was placed in a 20 mL vial and rapidly the internal standard 100 μg/mL was added into. The DVB/PDMS/CWR/PDMS fiber (Agilent Technologies, Santa Clara, CA, USA) was used. GC–MS analysis was performed on an Agilent 7890B GC coupled to a 5977B MSD and equipped with a PAL RSI 85 autosampler. Separation was achieved on a DB-WAX column (60 m ×

250 μm, 0.25 μm film) under splitless injection (Ultra Inert liner, 0.75 mm i.d.), with helium as the carrier gas (1 mL/min) and an injector temperature of 250 °C. Compounds were identified using the NIST library in SIM mode with time-windowed acquisition, and quantification was based on the most abundant ions. Data were processed with MSD ChemStation (Agilent). All analyses were conducted in triplicate with % RSD < 20%.

The volatile profile of acorn bread crumb was identified by SPME-GC/MS (Acquaticci et al., 2024). Finely ground bread crumb (1 g) was placed in a 20 mL vial and an AgilentChem workstation and a DVB/PDMS/CWR/PDMS fiber (Agilent Technologies, Santa Clara, California, USA) were used for the GC–MS system. Volatile compounds were analyzed using an Agilent 8890 GC–5977B MSD system with a PAL RTC 120 autosampler. Separation was achieved on a DB-WAX column (60 m × 250 μm, 0.25 μm film) under splitless mode with helium (99.5%) as carrier gas at 3 mL/min, and injector/detector temperatures of 260 °C. Mass spectra were acquired in full-scan mode (35–400 m/z, 70 eV), and compounds were identified by comparison with the WILEY275 and NIST08 libraries (match quality > 60%).

2.4. Data statistical analysis

Data were statistically analyzed using XLSTAT (Addinsoft 1995–2024, VERSION 2021.4.1, France) and the MetaboAnalyst 5.0 tool (https://www.metaboanalyst.ca). The one-way analysis of variance (one-way ANOVA) with Bonferroni's correction for multiple comparisons was used to compare mean values and to identify significance among samples at $p < 0.05$. Principal Component Analysis (PCA) was performed to explore the relationships between VOCs and bread samples. The PCA allowed for the identification of correlations between specific compounds and sample groups, highlighting the impact of acorn flour substitution and leavening agent type on the volatile profile. PERMANOVA (permutational multivariate analysis of variance) was applied to test the significance of experimental factors: acorn flour substitution level and leavening agent type on VOCs profile variation. Model quality for PCA was assessed via cumulative explained variance (R^2) and cross-validated predictive ability (Q^2). VOCs with absolute loading values $|L| > 0.30$ on principal components were considered major contributors to sample discrimination.

3. Results and discussion

This study was designed to optimize the acorn-enriched bread production process and this goal was achieved by, at first, identifying the optimal acorn flour substitution and water addition levels in bread made from direct (yeast-based, Fig. 1A) and sourdough (sourdough-based, Fig. 1B) methods and, then, by investigating the effect of different acorn flour hydration methods on bread quality.

3.1. Bread produced from the direct and sourdough methods

The effects of acorn flour addition and water hydration levels on bread quality, in terms of specific volume, crumb moisture content, and crumb hardness, are shown in Fig. 2A, B, and C, respectively, for breads produced with direct and sourdough methods. Wheat bread produced with the direct fermentation process and 75% hydration (D-0–75) was taken as a reference. As shown in Fig. 2A, the inclusion of acorn flour, water addition, and production method had a slight impact on the moisture content of the final products, which was considered comparable among samples with values ranging from 46% to 48%. In 100% wheat breads, sourdough fermentation led to the development of bread loaves with significantly lower specific volume compared to loaves produced with the direct method (Fig. 2B), as expected, in line with other reports (Verdonck et al., 2023). The negative impact of sourdough on specific volume is probably due to the lower CO_2 production and strong acidification during sourdough breadmaking, which reduces gas retention, but this mechanism is not fully understood yet (Verdonck et al., 2023). Sourdough processing was also found to significantly harden crumb texture ($p < 0.05$; 1.9 N for D-0–75 and 4.6–6.0 N for sourdough bread), in line with previous reports (Katsi et al., 2021; Verdonck et al., 2023), and possibly due to elasticity reduction of the gluten network associated with increased proteinase activity and dough acidification (Arendt et al., 2007).

Acorn flour inclusion in bread formulation resulted in a constant and significant reduction in bread specific volume by increasing acorn substitution from 0% to 30% ($p < 0.05$, Fig. 2B), regardless of bread production method, but less importantly when the sourdough method was used. A remarkably low loaf specific volume was found in samples with 30% acorn flour ($p < 0.05$), regardless of production method, similarly to previous reports (Szabłowska and Tańska, 2021, 2025), and it was

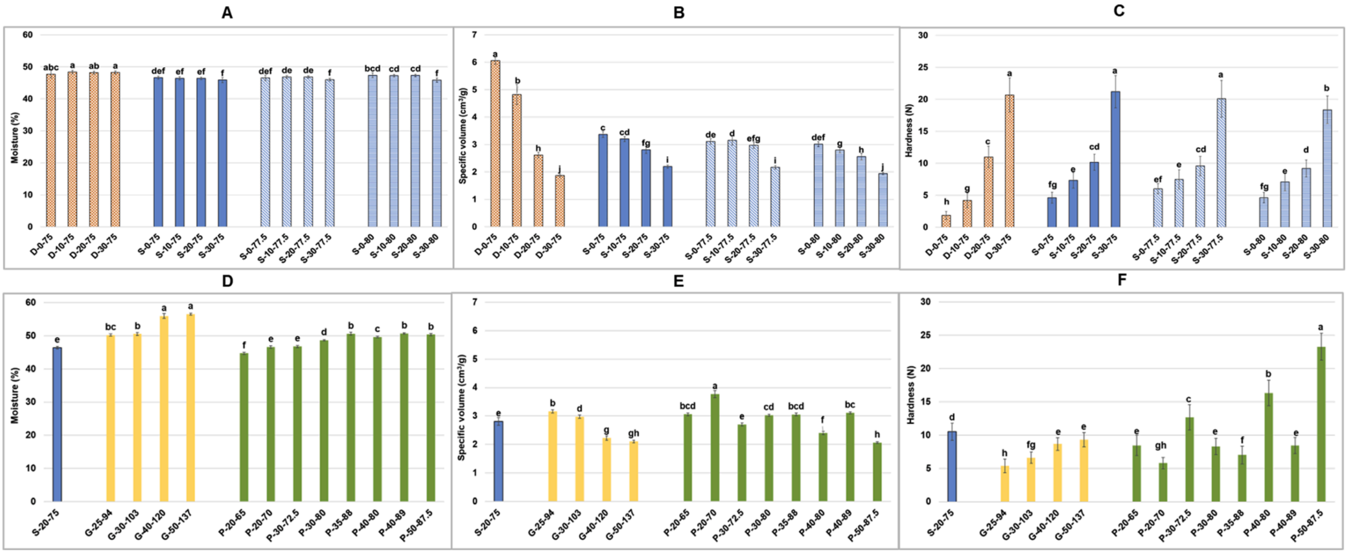

Fig. 2. Physical properties of all bread samples (moisture: A and D; specific volume: B and E; hardness: C and F. Sample name: the letter refers to the bread-making method [D = direct, S = sourdough, G = gel, P = pre-hydration]; the first number refers to the percentage of acorn flour in the sample; the second number is the hydration level [% water]. Data are presented as means ± SD. In A, B, and C: different letters represent significant differences among samples with the same hydration level; in D, E, and F: different letters represent significant differences among all samples at $p < 0.05$.

mainly attributed to the lack of gluten and high fiber and polyphenol contents in acorn flour, which are detrimental to dough structure expansion and loaf volume (Szabłowska and Tańska, 2021). However, when 20% acorn flour was added, the specific volume of bread was similar (2.6 and 2.9 cm^3/g in D-20–75 and S-20–77.5, respectively), suggesting an acceptable dough development. Bread crumb texture was also significantly hardened by increasing acorn flour content in bread formulation ($p < 0.05$, Fig. 2C), with the 30% acorn-enriched sample being twice as hard as that of the 20% acorn-enriched. Similarly to specific volume, 20% acorn sourdough bread (sourdough method) exhibited a comparative crumb hardness to the yeast-fermented counterpart (9.2–11.0 N), when the water addition was taken into consideration.

Lastly, the impact of water addition was also studied. By increasing the water addition from 75% to 80% in acorn-enriched bread, its specific volume was slightly reduced from 3.4 to 3.0, 3.2 to 2.8, 2.8 to 2.6, and 2.2 to 1.9 cm^3/g for 0%, 10%, 20%, and 30% acorn-enriched samples, respectively. Therefore, 75% of water addition was considered optimal in acorn sourdough bread. As to crumb moisture and hardness, the effect of water addition was found to be limited, as shown in Fig. 2A and C.

The above results indicate that the sourdough method with 20% acorn incorporation, and 75% water addition (sample S-20–75) can produce a bread comparable with the yeast-fermented counterpart (sample D-20–75) in terms of specific volume (2.8 vs 2.6 cm^3/g), moisture content (46.4% vs 48.2%), and crumb hardness (10.2 vs 11.0 N). Sourdough acorn-bread production process was further analyzed by investigating the effect of different acorn flour hydration methods on bread quality, taking the S-20–75 bread as a reference for comparison.

3.2. Bread produced from the gel and pre-hydration methods

As discussed in Section 3.1, sample S-20–75 was comparable to sample D-20–75 regarding specific volume, moisture, and hardness. However, the specific volume of sourdough bread with high acorn inclusions was significantly reduced, and further investigations were carried out in an effort to increase the bread loaf's specific volume and to verify the possibility of further increasing acorn flour inclusion in the product. It was hypothesized that pre-hydration of acorn flour might have a positive effect on bread loaf specific volume (Cai et al., 2015; Wu et al., 2025), and this was verified by evaluating the effect of acorn flour inclusions from 20% to 50%. Acorn flour pre-hydration was carried out both associated with thermal treatment (gel method, Fig. 1C; practice used by the artisanal bakery) or at room temperature (pre-hydration method, Fig. 1D) to further improve the sourdough acorn-wheat bread attributes. Sample S-20–75 (sourdough method, 20% acorn, and 75% water) was taken as a comparison.

Loaf moisture, specific volume, and hardness of breads produced with the gel and pre-hydration methods are presented, respectively, in Fig. 2D (crumb moisture), 2E (loaf specific volume), and 2F (crumb hardness). Notably, a very wet dough was observed for all gel-method formulations during breadmaking, and this was also reflected in a very high crumb moisture content (Fig. 2D) of baked bread, indicating that the gel method is not suitable for producing acorn sourdough bread. Breads made with the gel method were generally softer (Fig. 2F) than S-20–75 due to their high moisture content, but their crumb was also sticky and not springy. Moreover, the very high crumb moisture might also favor microbial growth, and it is not compatible with a suitable bread shelf life (Rahman et al., 2022). The P-20–70 bread was found to have a specific volume of 3.8 cm^3/g, significantly higher ($p < 0.05$) than the control S-20–75 (2.8 cm^3/g) and all other breads produced. Compared to S-20–75, samples G-25–94, G-30–103, P-20–65, P-30–80, P-35–88, and P-40–89 exhibited a slightly higher specific volume (3.0–3.2 cm^3/g; $p < 0.05$), while G-40–120, G-50–137, P-40–80, and P-50–87.5 were smaller (2.1–2.4 cm^3/g; $p < 0.05$). Sample P-30–72.5 specific volume (2.7 cm^3/g) was comparable to the control. This information suggests that the optimal acorn substitution level in sourdough bread should be lower than 50%.

Acorn flour pre-hydration was therefore considered, and it was found to significantly increase crumb moisture content and increase bread loaves' specific volume within reasonable levels (< 50%) for water content in bread formulation from 65% to 70%, 72.5% to 80%, and 80% to 89% for 20%, 30%, and 40% acorn sourdough bread in the pre-hydration method ($p < 0.05$, Fig. 2D and E), respectively. Increasing water addition significantly softened the bread crumb ($p < 0.05$, Fig. 2F), which means that in the pre-hydration production method, increasing water addition into the formulation contributes to counteract the hardening effect resulting from the increased acorn flour inclusion. Bread crumbs made with the pre-hydration procedure retained springiness and were not sticky.

For bread products, a higher specific volume means a softer and more aerated bread crumb, attributes that are important to meet consumers' expectations (García-Segovia et al., 2020; Sych et al., 1987), with loaves with larger specific volume that might also tend to stale more slowly (Sych et al., 1987). Taking these key factors into consideration, the bread samples produced using the pre-hydration method that were considered better or comparable to the control S-20–75 (specific volume: 2.8 cm^3/g; moisture: 46.4%; crumb hardness: 10.5 N), were the P-20–70 (specific volume: 3.8 cm^3/g; moisture: 46.6%; crumb hardness: 5.8 N), P-30–80 (specific volume: 3.0 cm^3/g; moisture: 48.7%; crumb hardness: 8.3 N), and P-40–89 (specific volume: 3.1 cm^3/g; moisture: 50.7%; crumb hardness: 8.4 N). These breads were considered the optimized bread samples and were used for further chemical characterizations.

3.3. Chemical characterizations of the optimized bread samples

Bread produced with 0% and 20% AF, 75% water addition, and direct fermentation (Fig. 1A) were taken as "yeast controls" (D-0–75 and D-20–75), while the sample produced with 0% AF, 75% water addition, and sourdough using the sourdough method (Fig. 1B) as the "sourdough control" (S-0–75), and compared to the chemical profiles of the optimized sourdough breads (P-20–70, P-30–80, and P-40–89). In particular, the presence of positive compounds (e.g., phenols) and harmful compounds (e.g., acrylamide and furanic compounds) has been investigated, alongside their volatile profile.

AF is known to be rich in polyphenols and antioxidants (Vinha et al., 2016), and its inclusion into bread formulation can importantly boost the product's phenolic compounds content and antioxidant properties (Beltrão Martins et al., 2020; Suo et al., 2025). As expected, acorn flour addition significantly increased total polyphenol content (TPC, Fig. 3A) and antioxidant activity (DPPH, Fig. 3B) of sourdough bread samples ($p < 0.05$), compared to the relative controls (D-0–75 and S-0–75). TPC continuously increased with increasing AF from 0% to 40% in sourdough breads and was found to be the highest in the D-20-75 sample. Antioxidant activity was found to be similar in all AF containing breads, regardless of the leavening agent used, except for the bread prepared with sourdough and 30% of AF. The highest TPC detected in D-20–75 is probably due to the *Saccharomyces Cerevisiae* action that can partially degrade phenolic acid-carbohydrate complexes, boosting the soluble phenolic acids content (Tian et al., 2021).

Volatile organic compounds (VOCs) that directly contribute to the final product's overall flavor were analyzed and are reported in Table 3, where their corresponding sensory descriptors are also shown. To the authors' best knowledge, VOCs have never been analyzed in sourdough acorn-enriched breads, and limited information is available on VOCs in dry yeast acorn bread (Beltrão Martins et al., 2022; Gonzaga et al., 2015; Hrusková et al., 2019; Suo et al., 2025; Tolga Niçin et al., 2022). A total of 15 VOCs were identified, categorized into six main families: alcohols, aldehydes, esters, organic acids, ketones, and pyrazines.

VOC profiles exhibited distinct clustering patterns based on both the type of leavening agent and the level of AF substitution (Fig. 4). PERMANOVA analysis confirmed that both acorn flour inclusion level

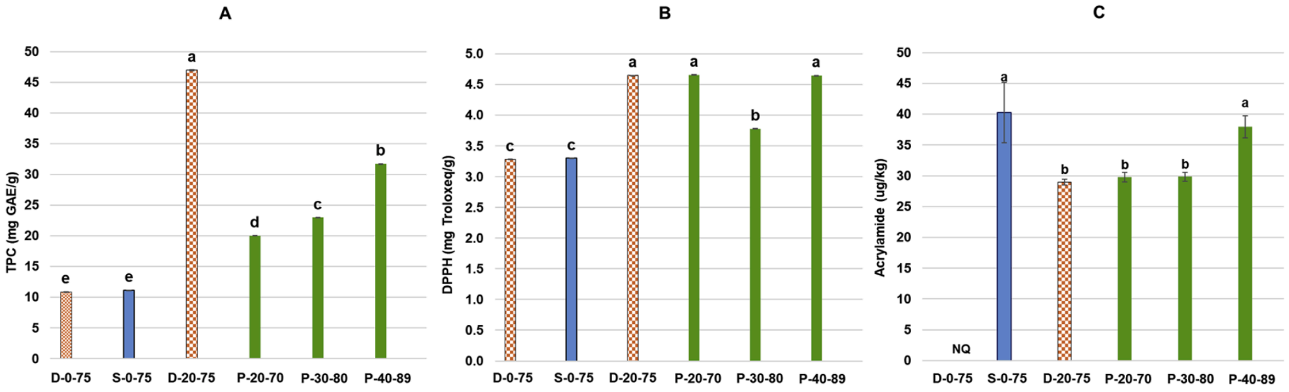

Fig. 3. Total phenolic compounds (TPC; A), antioxidant activity (DPPH; B), and acrylamide content (C) of optimized bread samples. Sample name: the letter refers to the bread-making method (D = direct method [yeast], S = sourdough method [sourdough], P = pre-hydration method [sourdough]); the first number refers to the percentage of acorn flour in the sample; the second number is the hydration level [% water]. Data are presented as means ± SD. Different letters in each figure represent significant differences among samples at $p < 0.05$. NQ: not quantified.

Table 3
Level of Key volatile organic components (GS-MS area counts x 10^5; mean ± sd, n = 3) sampled by SPME in the headspace of different bread formulations.

Family	RT	ERI	LRI	Compounds	Sensorial descriptor	Origin	D–0–75*	S-0–75	D-20–75	P-20–70	P-30–80	P-40–89
Alcohols	8.4	930.5	930	Ethanol	sweet	F	14.70 ±0.37	17.42 ±2.51	47.50 ±2.47	17.94 ±1.03	15.51 ±3.97	17.35 ±0.05
	16.4	1200.8	1211	3-methyl-butanol	wine, bitter	F	1.07 ±0.12	2.63 ±0.09	3.50 ±0.43	1.15 ±0.02	0.91 ±0.05	-
	28.3	1641.8	1655	2-Furanmethanol	burn	B	1.26 ±0.04	-	2.78 ±0.06	0.68 ±0.01	-	4.05 ±1.13
	34.1	1906.0	1954	Phenylethyl Alcohol	honey, spice rose	F	0.39 ±0.04	1.77 ±0.23	2.27 ±0.56	0.62 ±0.04	-	1.47 ±0.66
Sum							17.42	21.82	56.05	20.39	16.42	22.87
Aldehydes	23.4	1445.4	1462	Furfural	bread, almond, sweet	B	6.66 ±0.57	2.77 ±0.19	-	6.90 ±0.46	-	2.66 ±0.35
	25.1	1511.5	1510	Benzaldehyde	almond, burnt sugar	B	1.22 ±0.08	4.42 ±0.61	-	0.62 ±0.00	0.44 ±0.01	0.45 ±0.06
	25.5	1526.8	1525	2-Nonenal	fat, cucumber	B	-	-	-	0.33 ±0.01	-	-
	26.3	1559.8	1531	2-Furancarboxaldehyde,5-methl	almond, caramel, burn sugar	B	2.09 ±0.10	-	-	1.89 ±0.09	0.95 ±0.06	3.74 ±0.05
Sum							9.97	7.19	-	9.74	1.39	6.85
Organic acids	23.0	963	1122	Acetic acid	sour	F	9.97 ±2.17	-	-	8.48 ±0.90	8.27 ±0.42	7.29 ±2.73
	32.5	1830.3	1823	Hexanoic acid	sweet	F	0.40 ±0.04	-	-	0.34 ±0.00	0.79 ±0.04	-
Sum							10.37	-	-	8.82	9.06	7.29
Ketones	24.6	1490	1457	Ethanone 1-(2-furanyl)-acetylfuran (chetone)	balsamic	F	0.68 ±0.03	0.63 ±0.20	-	0.54 ±0.03	-	-
	35.3	1963.2	1954	Maltol	caramel	B	1.25 ±0.04	3.45 ±0.09	-	0.69 ±0.01	-	-
Sum							1.93	4.08	-	1.23	-	-
Pyrazine	18.1	1258.1	1270	Pyrazine, methyl-	pop corn flavour	B	1.06 ±0.04	1.66 ±0.11	0.85 ±0.19	-	-	1.70 ±0.05
	20.1	1325.6	1381	Pyrazine, ethyl	peanut butter, wood	B	-	-	-	-	-	0.82 ±0.00
	21.6	1377.7	1392	Pyrazine,2-ethyl-6-methyl	sweet	B	-	-	-	-	-	0.63 ±0.12
Sum							1.06	1.66	0.85	-	-	3.15

P: process formation, B: baking; ERI: experimental retention index; LRI: literature retention index.
- indicates undetected compounds. The detection of the peak itself had an accuracy greater than 70% of the compounds match according to NIST 2020 Mass Spectral Library.

Sample name.

[*] : the letter refers to the bread-making method (G = gel, P = pre-hydration); the first number refers to the percentage of acorn flour in the sample; the second number is the hydration level (% water).

(Fig. 4A) and leavening agent type (Fig. 4B) significantly influenced the VOCs profile clustering, explaining 85.4% ($R^2 = 0.854$, F = 27.295, p = 0.001) and 94.0% ($R^2 = 0.940$, F = 30.726, p = 0.001) of the total variance, thereby validating that progressive acorn flour substitution (0–40%) and the choice between yeast and sourdough fermentation are the predominant drivers of the observed aromatic differentiation in acorn-enriched breads.

The loading plot for the PCA-a (Fig. 1SA) demonstrates that PC3 is

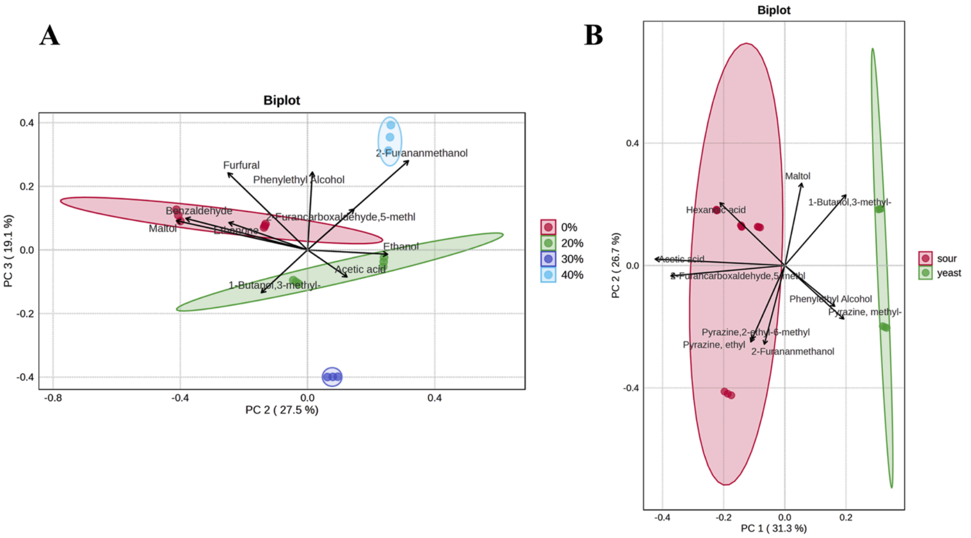

Fig. 4. (A) Principal component analysis (PCA) biplot highlighting the distribution of the main VOCs variables and the distribution of the sample grouping according to the percentage of acorn flour (0%, 20%, 30%, 40% acorn) present in optimized breads. (B) Principal component analysis (PCA) biplot highlighting the distribution of the main VOCs variables and the distribution of the sample grouping according to the leavening agent (Sour and Yeast) in optimized breads.

primarily driven by furanic and aromatic compounds (i.e., furfural, maltol, and benzaldehyde), whose strong positive loadings directly correspond to increasing acorn flour substitution levels. Fig. 1SB (PCA-b loading plot) demonstrates that PC1 is primarily driven by leavening-specific volatile markers (i.e., ethanol and phenylethyl alcohol versus acetic acid and furanic compounds), whose opposing loadings directly correspond to the distinct fermentation signatures of yeast and sourdough processes.

Ethanol and 3-methyl-butanol alcohols were present in the highest concentration in the 20% yeast bread (D-20–75), indicating that a moderate level of AF combined with dry yeast contributes to their production, and it might contribute to a slight alcoholic note and a rich aromatic profile in the product (Suo et al., 2025). The presence of these compounds positively correlated with direct method fermentation, as shown in Fig. 4B (PCA sour-yeast). In the 20% and 30% sourdough samples, the lower alcohol levels compared to the yeast sample, are likely due to a more complex fermentation pattern carried out by the variety of microorganisms present in the sourdough that result in a more complex range of metabolites beyond simple alcohols, supporting aroma complexity more than the alcoholic character (Chavan and Chavan, 2011).

Furfural, an aldehyde associated with a caramel-like aroma, was detected in both control samples. Its concentration was lower in the direct method bread compared with the sourdough control and progressively decreased with increasing AF content. Furfural is positively correlated with 0% AF (Fig. 4A), while lower AF levels resulted in less aldehyde development, while still maintaining toasted and aromatic notes, albeit at lower intensity.

Organic acids. primarily acetic and hexanoic acids, were produced by LABs in sourdough, and contribute directly to sour flavors (De Luca et al., 2021). Indeed, they were mainly identified in the sourdough bread sample with the AF substitution, as well as in D-0-75, which is further supported by their positive correlation with the sourdough sample (Fig. 4B). In the yeast sample with the addition of AF, acetic acid production decreased, suggesting a less acidic flavor in this bread compared to the sourdough samples.

Ketones were found only in D-0–75, S-0–75, and P-20–70 and are associated with balsamic notes. They were not identified in bread formulated with 30−40% acorn flour and 20% yeast. The most abundant ketone was the maltol, whose concentration varies among samples, peaking in the control yeast and decreasing at higher acorn substitution levels (Paterson, 2006). It significantly contributes to a caramel-like aroma; however, its concentration decreases as AF substitution increases.

Related to pyrazines, 2-Ethyl-6-methyl-pyrazine and 4-Pyridinamine 2,6-dimethyl have been detected, in low amounts, only in the 40% sourdough sample (P-40–89). Their presence could depend on Maillard reactions involving acorn amino acids and sugars under acidic conditions (Ashoor and Zent, 1984), and it is positively correlated with the sourdough processing (Fig. 4A).

Regarding the harmful compounds, the presence of acrylamide, known to originate from Maillard reaction (i.e. asparagine reacts with reducing sugars) at high temperatures (above 120 °C), may cause toxicological concerns, including neurotoxicity, reproductive toxicity, and genotoxicity (Adimas et al., 2024). Consequently, reducing the acrylamide content in bakery products is of significant importance for public health. Wheat flour generally contains a high amount of free asparagine across cultivars (Tafuri et al., 2023), and Italian common wheat bread was reported to have acrylamide of 31–454 μg/kg (Esposito et al., 2020). Acorn flour contains a high amount of asparagine among nonessential amino acids, specifically 6.96 mg/g (Szabłowska and Tańska, 2021).

In this study, bread produced with *Saccahromyces cerevisiae* as the leavening agent without AF addition (D-0–75) showed an acrylamide level that was below the quantification limit (LOQ: 5 μg/kg, Fig. 3C). Interestingly, the addition of 20% and 30% AF to sourdough bread significantly reduced the acrylamide content from 40 μg/kg in the sourdough control (S-0–75) to less than 30 μg/kg (P-20–70 and P-30–80). This reduction could be attributed to naturally occurring polyphenols in AF, which can inhibit acrylamide formation by trapping carbonyl compounds and preventing lipid oxidation (Liu et al., 2015). However, the addition of 40% AF did not lead to a reduction in acrylamide content compared to the sourdough control. Thus, the polyphenols present were possibly unable to effectively exert this protective effect due to the higher availability of asparagine.

Therefore, the combination of sourdough and acorn flour (between 20% and 30% level) significantly reduced the acrylamide content of sourdough wheat bread.

Other unwanted thermal contaminants in bakery products are furans, volatile organic compounds formed through Maillard reactions and sugar degradation during bread baking, that can cause negative health implications (Koszucka and Nowak, 2018). In this work, 4 furans were determined in bread crumb, including furfural, furfuryl acetate, 5-methylfurfural, and furfuryl alcohol (Fig. 5). In all sourdough breads, furfuryl acetate was still at a low level, while furfural and furfuryl alcohol were

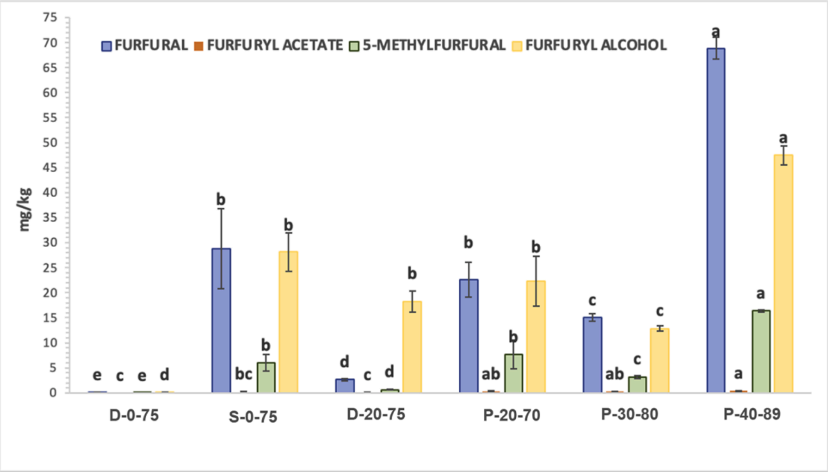

Fig. 5. Content of furan compounds in optimized bread samples. Data are presented as means ± SD. Different letters indicate statistically significant differences among the different bread samples for each furan compound.

the most representative compounds, independently of AF addition, except for the yeast control bread (D-0–75), where their present were the lowest.

Whereas, in the bread samples formulated with sourdough, even the control (S-0–75) showed furfural and furfuryl alcohol levels of around 30 mg/kg. Similar levels were also observed in the bread samples with 20% of AF addition (P-20–70). However, with the addition of 30% AF (P-30–80), the concentrations of both furfural and furfuryl alcohol decreased (to 15 and 13 mg/kg, respectively). When the AF addition was increased to 40% (P-40–89), the highest levels of these compounds were observed (68 and 47 mg/kg, respectively). Pasqualone et al. (2019) reported a similar trend in acorn-enriched biscuits, which showed higher levels of furfural and furfuryl alcohol compared to the control. These results had a similar trend to the acrylamide concentration, further supporting the acorn-enriched bread formulation at low AF substitution.

Regarding 5-methylfurfural, its concentration was observed to increase as the acorn addition increased to 30% and 40% in the sourdough bread samples, compared to the relative control.

4. Conclusion

This study confirms the key role of dough hydration level in modulating the quality of acorn–wheat bread, particularly in terms of specific volume and crumb hardness. The inclusion of acorn flour in wheat sourdough bread reduced the loaf's volume and increased crumb firmness to a level that makes the product unacceptable to be successful in the market when present at a 30% level. However, these weaknesses can be significantly minimized through modulation of dough hydration level and pre-hydrating acorn flour prior to dough preparation (pre-hydration method), which proved to be a successful strategy for improving bread quality while allowing higher inclusion levels (up to 40%). On the other hand, acorn flour inclusion in wheat sourdough bread significantly fortified products with polyphenols leading to higher antioxidant activity, together with a lower acrylamide content. From a nutritional perspective, the increase in bioactive compounds content combined with acorn's naturally high fiber content and sourdough's potential to moderate glycemic response, support the development of acorn-enriched sourdough breads as a sustainable strategy to enhance the nutritional profile of staple foods. Furthermore, the combination of sourdough fermentation and acorn flour resulted in a more complex aroma profile, such as less alcoholic but more toasted, sour, and balsamic flavour. A moderate acorn addition (< 30%) in wheat sourdough bread reduced total furan content but increased organic acids and ketone contents, which exhibit the sour and balsamic flavour, compared to the yeast counterparts. A higher inclusion (> 30%) significantly increased total furans and esters (caramel-like flavour), but reduced ketone level.

Overall, adjusting dough hydration and pre-hydrating acorn flour are suggested in acorn-wheat sourdough bread production to maintain acceptable bread volume and texture. Keep acorn inclusion below 30% when aiming for lower furan content and a more sour, balsamic profile. For toasted and caramel-like notes, higher acorn levels (> 30%) can be used, with texture adjustments applied. Future work will not only incorporate sensory evaluation and consumer acceptance of the optimized acorn-enriched breads to validate the overall perception of the formulated products, ultimately guiding market-ready product development, but also will verify their nutritional and health potential.

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Conflicts of interest

No conflict to report.

Statement for studies in humans/animals

The authors declare that NO humans nor animals were object of the present study.

CRediT authorship contribution statement

Xinying Suo: Data curation, Formal analysis, Investigation, Visualization, Writing – original draft. **Cinzia Mannozzi:** Data curation, Formal analysis, Investigation, Visualization, Writing – original draft. **Elia Gaspari:** Data curation, Investigation. **Marco Salvucci:** Investigation. **Riccardo Sturba:** Data curation, Investigation. **Francesca Pompei:** Data curation, Formal analysis, Writing – original draft. **Dario Sarracino:** Data curation, Investigation. **Giovanni Caprioli:** Formal analysis, Writing – review & editing. **Gianni Sagratini:** Writing – review & editing. **Antonietta La Terza:** Conceptualization, Funding acquisition, Writing – review & editing. **Alessandra Marti:** Writing – review & editing. **Elena Vittadini:** Conceptualization, Funding acquisition, Project administration, Supervision, Visualization, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Author Marco Salvucci works at the Forneria Marchigiana Bakery (Morrovalle [MC], Italy) where the bread production took place. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.fufo.2026.101063.

Data availability

Data will be made available on request.

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