Applied Food Research 6 (2026) 102380

Contents lists available at ScienceDirect

Applied Food Research

journal homepage: www.elsevier.com/locate/afres

Effect of *Moringa oleifera* leaves powder on quality and functional characteristics of sourdough bread

Graziella Serio [a], Giuliana Garofalo [b,*], Enrico Viola [b], Marcella Barbera [c], Michele Massimo Mammano [d], Simone Italia [e], Santo Orlando [b], Elena Franciosi [f], Hasan Tangüler [g], Daniela Piazzese [c], Carlo Greco [b], Carla Gentile [a], Luca Settanni [b], Raimondo Gaglio [b]

[a] *Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Viale delle Scienze, Bldg. 16, Palermo, 90128, Italy*
[b] *Department of Agricultural, Food and Forest Sciences, University of Palermo, Viale delle Scienze, Bldg. 5, Palermo, 90128, Italy*
[c] *Department of Earth and Marine Sciences, University of Palermo, Via Archirafi, 22, Palermo, 90123, Italy*
[d] *CREA - Research Centre for Plant Protection and Certification, Via Titina de Filippo 21, Palermo, 90135, Italy*
[e] *Le Liccumie S.r.l., Contrada Fargione Aggl. Ind., Modica, 97015, Italy*
[f] *Research and Innovation Centre, Fondazione Edmund Mach, Via E. Mach 1, San Michele all'Adige, 38098, Italy*
[g] *Food Engineering Department, Niğde Ömer Halisdemir University, Niğde, 51240, Türkiye*

A R T I C L E I N F O

Keywords:
Moringa oleifera leaves powder
Sourdough
Lactic acid bacteria
Quality characteristics
Functional bread
Consumer acceptability

A B S T R A C T

This study presents the first assessment of *Moringa oleifera* leaf powder (MOLP) derived from plants cultivated in Sicily as a functional ingredient in semolina sourdough bread. Bread formulations were enriched with MOLP at 5 % and 10 % (w/w) and fermented using a selected starter culture of lactic acid bacteria (LAB). The inclusion of MOLP did not impair dough fermentation, which reached final pH values of approximately 4.3, fermentation quotient values ranging from 3.19 to 3.33, and showed a microbiota largely dominated by *Fructilactobacillus* spp. (>90 % relative abundance). Technological characteristics were influenced in a concentration-dependent manner, evidenced by a decrease in specific volume (from 3.26 to 2.71 cm^3/g), and an increase in crumb firmness. From a functional perspective, MOLP enrichment led to a pronounced enhancement of bread antioxidant properties, with the total phenolic content increasing by 155.25 % (2.55-fold) compared to the control, alongside significant improvements in reducing power and radical scavenging activity, which increased by 94.77 % and 65.55 %, respectively. The addition of MOLP also modified the volatile profile by increasing the relative abundance of terpenes (16.4–18.0 % of the total volatile fraction) and introducing esters, thereby contributing to greater aromatic complexity. Sensory analysis identified the 5 % MOLP bread as the most acceptable in terms of overall acceptability. Furthermore, a consumer survey (n = 201) showed strong market appeal, with more than 80 % of respondents willing to pay a 10 % price premium for MOLP-enriched bread. Overall, *M. oleifera* leaf powder from plants cultivated in Sicily emerges as an effective and sustainable ingredient for improving the functional properties and consumer-perceived value of semolina sourdough bread.

1. Introduction

Bread making is widely recognized as one of the oldest and most enduring applications of food biotechnology (Das et al., 2023). It represents a complex bioprocess in which the controlled interaction among flour, water, and structured microbial communities drives the biochemical conversion of simple substrates into fermented products characterized by distinct sensory and nutritional properties (Dutta et al., 2024). Within this biotechnological framework, leavening is a key microbiologically driven process in which the metabolic activities of microorganisms that ferment dough carbohydrates are defining in the formation of carbon dioxide, organic acids, higher alcohols, and a wide variety of aromatic compounds (Taglieri et al., 2021). All together these metabolites exert physicochemical and biochemical effects that are pivotal in determining bread quality, shaping crumb structure, crust formation, and post-baking stability (Heitmann et al., 2018).

* Corresponding author.
E-mail address: giuliana.garofalo01@unipa.it (G. Garofalo).

https://doi.org/10.1016/j.afres.2026.102380
Received 11 May 2026; Received in revised form 30 June 2026; Accepted 7 July 2026
Available online 7 July 2026

However, the final characteristics of bread are largely shaped by the composition and functional activity of the microbial consortia involved in fermentation. Accordingly, distinct leavening systems can be distinguished based on the metabolic capabilities of these microbial communities. Among them, *Saccharomyces cerevisiae* is widely used as commercial baker's yeast due to its efficiency, rapid fermentation kinetics, and high reproducibility, attributes that make it particularly suited to standardized industrial productions (Heitmann et al., 2018). In contrast, sourdough fermentation is driven by a complex and dynamic ecosystem of endogenous lactic acid bacteria (LAB) and yeasts, in which alcoholic and lactic fermentations occur simultaneously. This dual fermentation process generates a broader spectrum of organic acids and volatile compounds, leading to bread with enhanced flavor complexity, improved digestibility, and extended shelf-life (Fang et al., 2023).

Bread is one of the most widely consumed foods worldwide and represents an important source of carbohydrates, minerals, and proteins. However, its contribution to dietary fiber and bioactive compounds is generally limited. As a result, growing research efforts have focused on enriching cereal-based products with plant-derived ingredients to enhance their functional properties and potential health benefits (Baker et al., 2022). In this context, sourdough fermentation, owing to its complex microbial consortium, offers a suitable platform for the incorporation of plant-based ingredients and agri-food by-products. Several studies have demonstrated that bread enrichment with plant-derived ingredients leads to higher levels of bioactive compounds and increased antioxidant capacity (Hernández-López et al., 2023; Maidana et al., 2020; Onacik-Gür et al., 2022; Saddique et al., 2024; Tolve et al., 2021).

Among such ingredients, *Moringa oleifera* leaves have gained particular attention due to their high contents of proteins, minerals, vitamins, polyphenols, and antioxidant molecules (Chhikara et al., 2021). Native to tropical and subtropical regions of Asia, Africa, and Latin America, *M. oleifera* has only recently been introduced into conventional Sicilian cropping systems. This introduction has been driven by the species adaptability to climate change-related conditions affecting the region, as well as its potential application both as a forage crop for livestock and as a functional ingredient in health-oriented food products (Greco et al., 2025). Although the use of *M. oleifera* leaves in bread making to increase protein and dietary fibre content and to enhance antioxidant capacity is not novel (Sánchez-Ortiz et al., 2024), existing studies have been limited to white wheat bread and have relied exclusively on baker's yeast as the leavening agent. To the best of our knowledge, no investigations have reported the production of sourdough semolina breads enriched with *M. oleifera* leaves cultivated in Sicily.

Based on these considerations, the present study aimed to investigate, for the first time, the addition of *M. oleifera* leaves in powder form in the formulation of a novel functional sourdough bread. The specific objectives of this work were: (i) to characterize the microbial dynamics throughout bread production using both culture-dependent methods and next-generation sequencing approaches; and (ii) to assess the effect of MOLP enrichment on physicochemical characteristics, functional aspects, volatile profile, sensory traits, and consumer acceptance.

2. Materials and methods

2.1. Raw materials and strains

Fresh leaves of *M. oleifera* were collected at the cultivation site located in Grotte (Agrigento, Italy; 37°22′52.284″ N, 13°40′24.067″ E; 380 m a.s.l.). Sampling was carried out in triplicate over three consecutive weeks, from late October to mid-November 2024. A voucher specimen has been deposited in the Herbarium Mediterraneum Panormitanum (PAL) of the Botanical Garden of the University of Palermo under the accession number 109787.

The powder was produced following the procedure described by Greco et al. (2025), which includes leaf harvesting, washing, smart solar-assisted drying, and subsequent milling to achieve a particle size of 250 μm.

Semolina used for bread production (La Molisana S.p.A., Ripalimosani, Italy) presented the following nutritional composition per 100 *g*: 70.0 *g* carbohydrates, 14.0 *g* proteins, 1.0 *g* total fats (of which 0.3 *g* were saturated fatty acids), and 3.0 *g* dietary fibre.

A mixed sourdough starter culture was formulated using three LAB strains of sourdough origin: *Fructilactobacillus sanfranciscensis* RC-UNIPASAAFM01100, *Weissella cibaria* RC-UNIPASAAFM01109, and *Leuconostoc citreum* RC-UNIPASAAFM01118. These strains were previously isolated from traditional sourdoughs used in Sicilian bakeries or from regionally sourced raw materials and were selected based on their technological relevance in sourdough fermentation (Viola et al., 2024).

2.2. Sourdough preparation

Sourdough was prepared following the procedure described by Restivo et al. (2025). Briefly, all LAB strains were re-activated from glycerol stocks stored at −80 °C and cultivated in modified de Man-Rogosa-Sharpe (mMRS) medium (Corsetti et al., 2008). After incubation for 24 h at 30 °C, each strain was propagated in sterile semolina extract (SSE) and sub-cultured three consecutive times until a final cell density of approximately 10^9 CFU/mL was achieved.

The resulting cell suspension was diluted with sterile tap water to a final volume of 187.5 mL and mixed with 312.5 *g* of semolina to obtain a total dough weight of 500 *g*. The dough, characterized by a dough yield (DY = dough weight/semolina weight × 100) of 160, reached an initial microbial load of approximately 10^6–10^7 CFU/g. Fermentation was conducted at 28 °C for 16 h, after which the dough was refreshed daily for seven consecutive days following the protocol of Corona et al. (2016), yielding a mature sourdough suitable for bread making.

2.3. Breadmaking and experimental design

Bread making trials were carried out under laboratory conditions according to the methodology described by Gaglio et al. (2023), with sourdough used as the fermenting agent. To evaluate the effect of MOLP on bread making performance, three experimental formulations were prepared, each with a final dough weight of 500 *g* and a DY of 160. The experimental design included a control dough (CTR) and two experimental doughs in which semolina was partially replaced with MOLP at 5 % (EXP-5) and 10 % (EXP-10). CTR formulation consisted of 285.75 *g* of semolina, 142.80 mL of sterile tap water, and 71.45 *g* of mature sourdough. In the EXP-5 trial semolina was reduced to 271.46 and supplemented with 14.29 *g* of MOLP, while the amounts of sterile tap water and sourdough remained unchanged. The EXP-10 trial was prepared using 257.18 g of semolina and 28.57 *g* of MOLP, together with the same amounts of sterile tap water and sourdough of the previous formulations.

All ingredients were blended using a planetary mixer (model XBM10S; Electrolux Professional S.p.A., Pordenone, Italy) operating at speed level 4 for 15 min. Salt was deliberately omitted from all formulations including CTR to allow a more accurate assessment of the effect of MOLP on the metabolic activity and stability of LAB during fermentation. Dough portions of 100 *g* were placed in stainless-steel trays shaped according to the trapezoidal design specified in ACC Method 10-10B (AACC, 2000), covered with aluminium foil, and fermented at 28 °C for 8 h. An additional 100 *g* of dough from each formulation was transferred into sterile glass jars (Vetreria Borgonovo S.p.A., Borgonovo Val Tidone, Italy) to enable fermentation under strictly aseptic conditions. Baking was carried out in a semi-industrial Compact Combi oven (Electrolux, Pordenone, Italy) following a two-phase baking program: 5 min at 200 °C with combined steam and hot air, followed by 15 min of convection baking at the same temperature. All bread making trials were performed in triplicate over three consecutive weeks.

2.4. Fermentation parameters

The progress of sourdough fermentation, as well as that of the baking doughs, was monitored by assessing pH, total titratable acidity (TTA), lactic and acetic acid production, and the development of the fermentative microbiota. These parameters were determined for both CTR and EXP doughs immediately after mixing (t_0) and at the end of fermentation (t_8). Measurements of pH and TTA were performed on MOLP, 7-day mature sourdough, and the three bread dough formulations following the protocol described by Francesca et al. (2019). Concentrations of D + L lactic acid and acetic acid were determined by high-performance liquid chromatography (HPLC) according to the method described by Gaglio et al. (2020).

Microbiological analyses were carried out using serial dilution technique and plating on selective and non-selective culture media. Plate count agar (PCA) was used for enumerating total mesophilic microorganisms (TMM) after aerobic incubation at 30 °C for 72 h. Rod-shaped mesophilic LAB on mMRS agar after anaerobic incubation at 30 °C for 48 h, while sourdough-associated LAB on sourdough bacteria (SDB) agar after aerobic incubation at 30 °C for 48 h. Yeasts were counted on yeast peptone dextrose (YPD) agar supplemented with chloramphenicol (0.1 g/L), incubated at 28 °C for 48 h. Hygiene-related microbial groups were assessed exclusively in sourdough samples. Enterobacteriaceae were enumerated on violet red bile glucose agar (VRBGA), and total coliforms on violet red bile agar (VRBA), with both media incubated at 37 °C for 24 h. The presence of aerobic spore-forming microorganisms was evaluated by subjecting sourdough cell suspensions to heat treatment at 85 °C for 15 min, followed by plating 0.1 mL aliquots on nutrient agar (NA) and incubation at 32 °C for 48 h, according to the methodology of Messina et al. (2019). All culture media were supplied by Oxoid (Basingstoke, UK).

All analyses were performed in duplicate for each independent bread production.

2.5. Characterization of bread microbiota by metataxonomic analysis

Genomic DNA was isolated using the QIAamp® DNA Investigator Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. The concentration and integrity of the extracted DNA were assessed following the procedures reported by Busetta et al. (2021). The hypervariable V3-V4 region of the 16S rRNA gene was amplified using primers 341F and 805R, adopting the amplification conditions described by Muturi et al. (2021). PCR products were subsequently purified and subjected to high-throughput sequencing on an Illumina MiSeq platform at the Fondazione Edmund Mach (FEM, San Michele all'Adige, Italy). Sequence reads were processed following the workflow outlined by Busetta et al. (2025). Taxonomic assignment was performed using the Greengenes 13_8 database at 99 % Operational Taxonomic Units (OTUs) similarity. All corresponding FASTQ files have been deposited in the NCBI Sequence Read Archive (SRA) under the project PRJNA1246356.

2.6. Quality parameters of breads

After baking, all breads were allowed to cool at room temperature for 30 min prior to the assessment of their technological quality. Crust and crumb colour were evaluated using a Chroma Meter CR-400 (Minolta, Osaka, Japan), based on the Hunter L*, a* and b* coordinates. Four readings were taken from the upper crust surface, while three measurements were collected from the central crumb area of the middle slice of each bread. Weight loss (WL), associated with water evaporation during baking, was calculated according to Purlis and Salvadori (2007) using the formula:

$$WL = \left(\frac{\mathrm{wd} - \mathrm{wb}}{wd}\right) \times 100$$

where wd is the dough weight (g) and wb is the final bread weight (g). Bread volume was measured using a 2 L volumeter designed for bakery products (ErreCi s.r.l., Merate, Italy).

Crumb microstructure was examined by quantifying void fraction (percentage of alveolar area), cell density (number of cells per cm^2), and mean cell area (mm^2). Crumb slices were scanned at 350 dpi with an Epson Perfection 4180 Photo scanner (Seiko Epson Corp., Japan). The images, saved in TIFF format, were processed using ImageJ software (National Institutes of Health, Bethesda, MD, USA). Each image was cropped to a 207 × 207 pixel square (corresponding to 15 × 15 mm), converted to 8-bit grayscale, and binarized using Otsu's automatic thresholding algorithm. Textural properties were determined by measuring crumb resistance to compression (N/mm^2) using an Instron-5564 texture analyser (Instron Corp., Canton, MA), following the procedure described by Corsetti et al. (2000).

2.7. Preparation of the extracts from MOLP, doughs and breads

MOLP samples, together with those obtained from the three experimental formulations (CTR, EXP-5, and EXP-10), including both doughs and corresponding baked products, were processed for extraction. Dough samples were collected at two distinct fermentation times: the beginning (t_0) and the end (t_8). For each condition, three independent aliquots were weighed and extracted at ambient temperature using an ethanol-water mixture (70:30, v/v). The extraction was performed using matrix-specific solid-to-solvent ratios, namely 1:20 (w/v) for MOLP samples and 1:3 (w/v) for dough and bread samples. Each sample underwent sequential vortex mixing for 5 min, ultrasonic treatment for 30 min, and prolonged extraction for 24 h under constant agitation in dark conditions. Subsequently, samples were centrifuged at 2000 rpm for 10 min at 4 °C. The extraction cycle was repeated three times, after which all supernatants were combined and clarified by filtration through a 0.45 μm Millex HV membrane filter (Millipore, Billerica, MA). The resulting extracts were portioned into aliquots and stored at –20 °C pending further analyses. After completion of the second extraction step, the remaining solid residue was further extracted, and the supernatant obtained from this final step (referred to as the exhausted extract) was collected and maintained separately.

2.8. Total polyphenol content and antioxidant properties

Total phenolic content (TPC) was evaluated in extracts obtained from MOLP, as well as from the dough matrices and the corresponding breads of the CTR, EXP-5, and EXP-10 samples. The procedure followed the protocol reported by Mannino et al. (2022c), with slight adjustments where necessary. TPC was reported as mg of gallic acid equivalents (GAE) per 100 g, calculated on a dry weight (DW) basis for MOLP samples and on a fresh weight (FW) basis for dough and bread samples.

The antioxidant capacity of extracts was assessed by means of the DPPH radical scavenging assay and the ferric reducing antioxidant power (FRAP) assay, following a previously reported method with minor adjustments (Mannino et al., 2022b). For the DPPH assay, a 10 mM ethanolic solution of DPPH was mixed with appropriately diluted extracts and incubated in the dark at room temperature for 40 min. Absorbance was measured at 517 nm. For the FRAP assay, samples were reacted with a freshly prepared FRAP reagent (acetate buffer 300 mM, pH 3.6; TPTZ 10 mM; $FeCl_3$ 20 mM; 8:1:1, v/v/v) and incubated at 37 °C for 30 min before measuring absorbance at 593 nm. Results from both assays were calculated using Trolox calibration curves and expressed as mmol of Trolox equivalents (TE) per 100 g (FW for dough and bread; DW for MOLP). All determinations were conducted in triplicate, and results are expressed as mean values obtained from three independent experiments. Unless otherwise stated, all reagents and chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.9. Estimation of theoretical functional values

To assess the impact of fermentation and baking on the functional characteristics of the fortified products, theoretical values for TPC, DPPH, and FRAP were estimated and subsequently compared with the corresponding experimental values.

For dough samples, theoretical values were calculated based on the actual amount of MOLP incorporated into 100 g of dough and the functional properties experimentally determined for the MOLP. The theoretical value of each parameter was calculated using the following equation:

$$TV_{dough} = V_{CTR} + \frac{MOLP_{actual}}{100} \times V_{MOLP}$$

where TV_{dough} is the theoretical value of the fortified dough, V_{CTR} is the value determined in the control dough, $MOLP_{actual}$ is the amount of MOLP contained in 100 g of dough (2.86 and 5.72 g for the 5 % and 10 % formulations, respectively), and V_{MOLP} is the experimentally measured value of the corresponding functional parameter in 100 g of MOLP.

For the baked bread, theoretical values were estimated from the functional parameters measured in the corresponding fermented dough immediately before baking (after 8 h of fermentation). Under the assumption that no losses of bioactive compounds or antioxidant activity occurred during baking, the theoretical values were adjusted exclusively to account for the concentration effect resulting from moisture loss during thermal processing, according to the following equation:

$$TV_{bread} = V_{dough} \times \frac{100}{100 - WL}$$

where TV_{bread} is the theoretical value of the baked product, V_{dough} is the value measured in the fermented dough before baking, and WL represents the water loss during baking, expressed as grams of water lost per 100 g of dough.

The comparison between theoretical and experimental values was used to estimate the effect of processing on the functional properties of the fortified products. Deviations between the two values were interpreted as possible consequences of processing-related phenomena, including degradation of bioactive compounds, changes in their extractability, interactions with the food matrix, and/or the formation of compounds with antioxidant activity during fermentation and baking.

2.10. Analysis of volatile organic compounds

The volatile organic compounds (VOCs) of MOLP and of CTR, EXP-5 and EXP-10 bread samples were analysed by headspace solid-phase microextraction coupled with gas chromatography-mass spectrometry (HS-SPME-GC/MS). For each analysis, 5 g of bread sample were placed in a sealed vial and subjected to headspace extraction using a 30 µm DVB/CAR/PDMS fiber (divinylbenzene/carboxen/polydimethylsiloxane). The fiber was exposed to the headspace for 60 min at 25 °C to allow adsorption of volatile compounds. After extraction, analytes were thermally desorbed in the gas chromatograph injection port at 250 °C for 5 min, in splitless mode. Chromatographic separation was performed on a DB-624 capillary column (Agilent Technologies; 60 m × 0.25 mm i.d., 1.40 µm film thickness). The oven temperature program started at 40 °C and was increased to 230 °C at a rate of 4 °C/min, followed by an isothermal hold for 40 min. Mass spectra were acquired in full-scan mode over a mass range of *m/z* 40–400, with the interface temperature maintained at 230 °C. Each bread sample was analysed in duplicate for each independent production. The identification of major volatile compounds was achieved by comparing the obtained mass spectra with those contained in the NIST05 mass spectral library, while also considering the expected elution order of the identified compounds. The relative abundance of each compound was expressed as a percentage of the total chromatographic peak area, calculated by normalizing the areas of all detected peaks.

2.11. Sensory assessment

A descriptive sensory analysis was conducted on the final bread samples using a trained panel of 20 assessors (13 women and 7 men), aged between 25 and 63 years. The evaluation was performed in individual tasting booths equipped with standardized white lighting at the sensory laboratory of the Department of Agricultural, Food and Forest Sciences of the University of Palermo (Italy), in accordance with the ISO 13299:2003 (ISO, 2003). Prior to the sensory sessions, all panel members underwent specific training to ensure a consistent and standardized evaluation of bread sensory attributes. During the assessment, the panel evaluated a set of descriptors related to appearance (crust colour, crumb colour, crust thickness, porosity, alveolation and regularity of alveolation), aroma (odour and aroma intensity, bread odour and aroma, strange aroma, astringency, bitterness, and taste persistence), and texture (crumb elasticity, crispy crust, and mouth adhesiveness), following the methodological approach described by Martins et al. (2015). Each sensory attribute was scored using a 9-point structured scale, where 1 corresponded to “extremely poor” and 9 to “extremely good”. Data were collected electronically using iPads connected to the Smart Sensory Box software (Smart Sensory Solutions S.r.l., Sassari, Italy). In addition to scoring individual descriptors, panellists were asked to provide an overall quality rating for each sample, integrating their perception of all evaluated attributes.

2.12. Survey-based assessment of consumer perception and acceptability

Consumer perception and acceptability of the innovative functional bread enriched with MOLP were evaluated by means of a cross-sectional survey. The study was conducted in two different retail settings located in the province of Ragusa (Italy) and involved a total sample of 201 consumers. Of these, 103 participants (51.2 %) were recruited at the convenience-oriented “Conad” premium supermarket, while the remaining 98 respondents (48.8 %) were surveyed at the “Superstore Le Liccumie”. Data collection was performed through direct, face-to-face interviews using a structured questionnaire. The survey protocol was developed in accordance with the methodological framework proposed by Garofalo et al. (2026) and comprised items related to socio-demographic profile, dietary habits, attitude toward innovative food products, purchasing intentions, and willingness to pay for the novel bread. The socio-demographic profile of the respondents, which included sex, age category, educational attainment, and annual income, is summarized in Table 1. Before starting the survey, respondents received a comprehensive explanation of the health and environmental benefits of the bakery products enriched with plant-derived products following the approach of Miolla et al. (2023).

Table 1
Socio-demographic profile of the respondent consumers (% by retail store).

Characteristic	Categories	Conad		Superstore Le Liccumie	
		Total (n)	%	Total (n)	%
Gender	Female	77	74.8	69	70.4
	Male	26	25.2	29	29.6
Age	18–30	20	19.4	14	14.3
	31–50	53	51.5	33	33.7
	50+	30	29.1	51	52.0
Education	Primary school	13	12.6	7	7.1
	Secondary school	67	65.0	39	39.8
	University	23	22.3	52	53.1
Annual income	< 15.000 €	20	19.4	6	6.1
	15.000–30.000 €	51	49.5	17	17.3
	30.000–50.000 €	21	20.4	49	50.0
	> 50.000 €	11	10.7	26	26.5

2.13. Statistical analysis

All data were analyzed using one-way analysis of variance (ANOVA). Significant pairwise differences were identified using Tukey's post hoc test, with statistical significance set at $p < 0.05$. Questionnaire response frequencies, aimed at assessing consumer perception and acceptance of the novel MOLP-enriched functional sourdough bread, were evaluated using the Chi-square test. Statistical analyses were performed using XLStat software (Addinsoft, New York, NY, USA) evaluating only the effect of MOLP addition.

3. Results and discussion

3.1. Acidification parameters during dough propagation

The acidification kinetics of the doughs, assessed by pH, TTA, and organic acid concentrations, are reported in Table 2. The mature sourdough used as fermenting agent exhibited a pH of 3.83 ± 0.02 and a TTA of 12.25 ± 0.10 mL NaOH 0.1 N/10 g, values consistent with those typical of sourdoughs fermented with selected LAB starter cultures (Atfaoui et al., 2025). At both the initial and final stages of fermentation, no significant differences ($p > 0.05$) were observed between CTR and EXP doughs in terms of pH, TTA, and organic acid content. The similarity among all trials may be attributed to the intrinsic pH of MOLP (6.10), which closely matches that of durum wheat semolina (Corona et al., 2016). Specifically, all doughs showed initial pH values of approximately 5.3, which decreased to about 4.3 by the end of fermentation, a range commonly reported for sourdough breads enriched with plant-derived ingredients (Gaglio et al., 2023). Throughout fermentation, TTA values increased linearly in all doughs in parallel with the decrease in pH (Siepmann et al., 2019), reaching values between 9.60 and 9.90 after 8 h of fermentation. This increase was associated with a concomitant rise in organic acid production. Specifically, after 8 h of fermentation, concentrations of D + L lactic acid and acetic acid ranged from 3.71 to 3.87 and from 0.76 to 0.81, respectively, reflecting the general trend reported for semolina sourdoughs (Alfonzo et al., 2016). Moreover, the fermentation quotient (FQ), expressed as the molar ratio of lactic to acetic acid, ranged from 3.19 to 3.33, falling within the optimal range interval proposed for sourdoughs (1.5–4.0) (Spicher & Rabe, 1983).

Microbiological analyses were performed along the entire dough-making process, from MOLP to the doughs fermented for 8 h (t_8). In agreement with Greco et al. (2025), no spoilage or pathogenic microorganisms were detected in MOLP, confirming its microbiological suitability for breadmaking applications. The sourdough starter exhibited a mature and stable microbial community, characterized by high viable counts of TMM (7.19 log CFU/g), LAB (7.49 log CFU/g), sourdough-associated LAB (7.05 log CFU/g), and yeasts (5.43 log CFU/g). These levels are comparable to those commonly observed in mature semolina-based sourdoughs produced in southern Italy (Gaglio et al., 2023; Minervini et al., 2012). Moreover, the absence of total coliforms, Enterobacteriaceae, and aerobic spore-forming bacteria confirmed the hygienic quality and technological suitability of the sourdough (De Vuyst, et al., 2023; Reffai & Fechtali, 2025).

Microbial counts obtained for the three dough samples are shown in Fig. 1. At each sampling point, no significant differences ($p > 0.0001$) were observed among doughs for any of the microbial groups analyzed. However, significant time dependent variations were detected within each trial between 0 h and 8 h of fermentation, indicating that MOLP inclusion at levels up to 10 % did not interfere with microbial growth dynamics during leavening (Ebrahim, 2024). The evolution of TMM (Fig. 1a), total LAB (Fig. 1b), and sourdough-associated LAB (Fig. 1c) showed nearly overlapping trends across all formulations, highlighting the dominance of LAB within the microbial consortia. After 8 h of fermentation, these populations converged toward final levels of approximately 6 log CFU/g, demonstrating that MOLP addition did not detrimentally affect the adaptability or competitiveness of the indigenous sourdough LAB consortium, thereby preserving the characteristic microbial ecology of sourdough systems (Suo et al., 2021).

Yeast populations (Fig. 1d) which also play a key role during sourdough fermentation (De Vuyst, et al., 2023), steadily increased throughout the 8 h fermentation period, reaching levels close to 6.0 log CFU/g in all trials. These results suggest that the dough environment supported yeast growth and adaptation despite the presence of MOLP, in line with previous findings demonstrating the resilience of sourdough yeasts in formulations enriched with plant-derived bioactive ingredients (Pérez-Alvarado, et al., 2022). Overall, the results clearly demonstrated that MOLP incorporation up to 10 % does not adversely affect sourdough fermentation dynamics or microbial safety. On the contrary, MOLP appears to selectively promote LAB development while maintaining balanced yeast growth, supporting its suitability as a functional ingredient in sourdough based products.

3.2. Microbial community dynamics during dough fermentation

The microbial communities associated with the different doughs were examined through a next-generation sequencing (NGS) approach, which is widely applied to investigate microbial composition and succession, including viable but non-cultivable and subdominant taxa (Settanni et al., 2020). Illumina MiSeq analyses were performed at the beginning of fermentation and after 8 h and the resulting profiles are shown in Fig. 2. Only OTUs with a relative abundance (RA) ≥ 0.1 % were considered, according to Logares et al. (2014).

Overall, the bacterial community displayed low taxonomic diversity, comprising only three main groups: the class of Alphaproteobacteria, the family of Enterobacteriaceae, and the genus *Fructilactobacillus* spp.. In all samples, *Fructilactobacillus* spp. was the dominant taxon already in the initial doughs, with RA of 84 % in CTR, 86 % in EXP-5, and 89 % in EXP-10. Its dominance further increased during the first 8 h of fermentation. This pattern is consistent with the known ecology of Italian sourdoughs, which are typically characterized by the prevalence of lactobacilli (Pino et al., 2022). The strong agreement observed between NGS profiles and culture-dependent data suggests that MOLP may create favourable conditions for lactobacilli proliferation, potentially facilitating a more

Table 2
Kinetics of acidification during dough fermentation.

Samples	t_0					t_8				
	pH	TTA	D + L Lactic acid	Acetic acid	FQ	pH	TTA	D + L Lactic acid	Acetic acid	FQ
CTR	5.28	7.68	0.83	0.16	3.46	4.31	9.60	3.71	0.76	3.25
EXP-5	5.25	7.73	0.84	0.17	3.29	4.27	9.70	3.80	0.76	3.33
EXP-10	5.21	7.98	0.84	0.17	3.29	4.24	9.90	3.87	0.81	3.19
SEM	0.04	0.10	0.035	0.005	n.e.	0.02	0.07	0.091	0.055	n.e.
p value	0.117	0.178	0.994	0.745	n.e.	0.085	0.098	0.814	0.920	n.e.

Results indicate mean value of six measurements (carried out in duplicate for three independent productions). Abbreviations: TTA, total titratable acidity; FQ, Fermentation Quotient (molar ratio between lactic and acetic acids); CTR, control dough; EXP-5, experimental dough enriched with 5 % of (*Moringa oleifera* leaves powder (MOLP); EXP-10, experimental dough enriched with 10 % of MOLP; SEM, standard error of the mean; n.e., not evaluated.

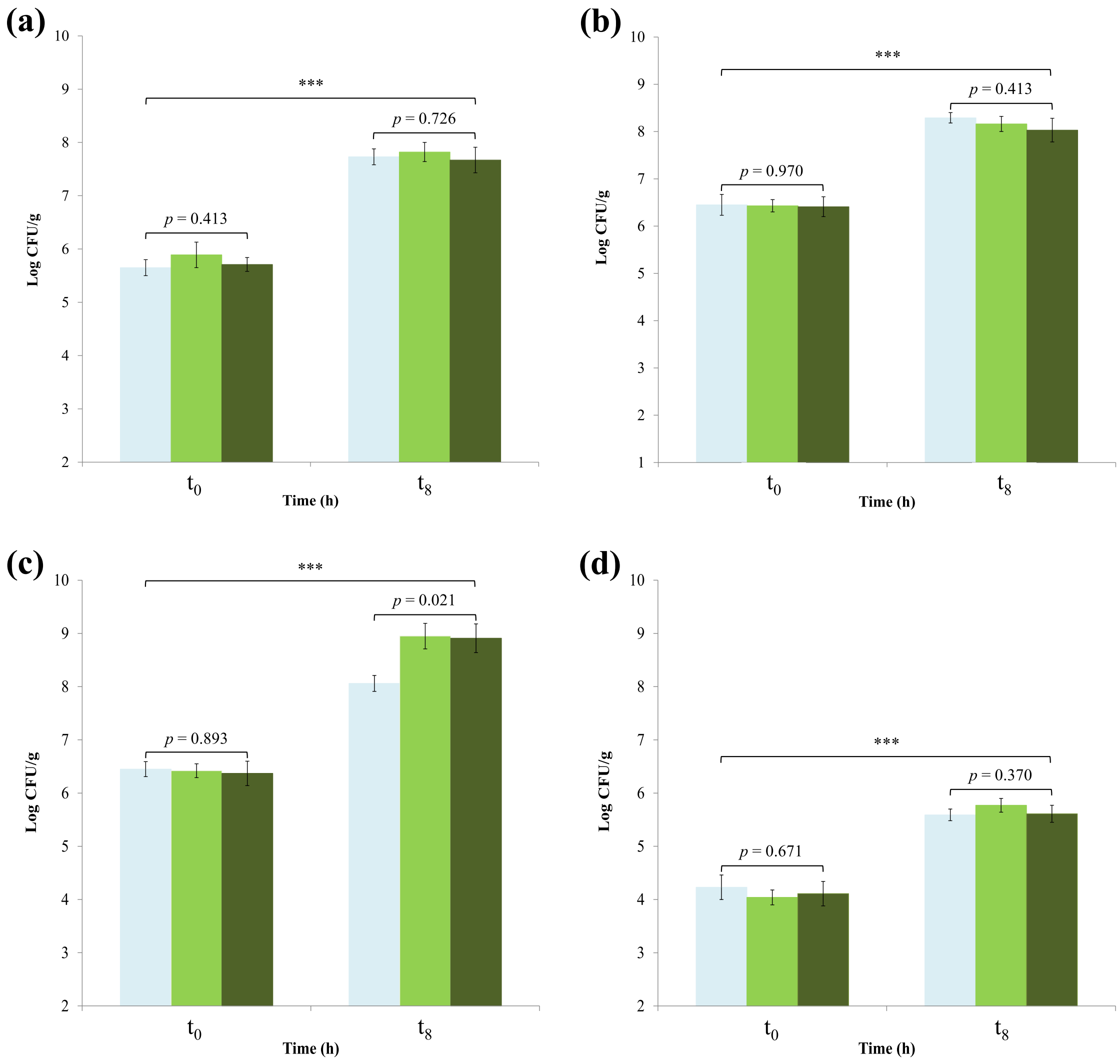


Fig. 1. Microbiological counts (log CFU/g) of doughs immediately after mixing (t_0) and at the end of fermentation (t_8). CTR, control dough (light blue); EXP-5, experimental dough enriched with 5 % of *Moringa oleifera* leaves powder (MOLP) (light green); EXP-10, experimental dough enriched with 10 % of MOLP (dark green). (a) total mesophilic microorganisms; (b) total LAB; (c) sourdough LAB; (d) yeasts. Results indicate mean values ± standard deviation of six plate counts (carried out in duplicate for three independent productions). Asterisks indicate significant differences between the same trial (CTR, EXP-5, and EXP-10) at t_0 and t_8, as determined by Student's *t*-test. ***$p < 0.001$.

rapid acidification process (Ebrahim, 2024).

Alphaproteobacteria were detected in all the samples analyzed, albeit at varying levels. This taxonomic group showed the highest RA in CTR (15.36 %), whereas in the experimental doughs it ranged from 13 % to 10 % at the beginning of fermentation and decreased to 5–3 % at the end of the process. Members of this class are generally associated with environmental contamination (Mannino et al., 2022a). The higher RA observed in the CTR dough suggests that these bacteria originated primarily from the raw materials used for sourdough propagation rather than from MOLP. In fact, the lowest levels were detected in EXP-10, indicating that higher MOLP inclusion may even exert a limiting effect on their growth.

Enterobacteriaceae were detected only at trace levels in all samples. Their presence is not surprising, as this family is frequently reported in flours and doughs (Khlestkin et al., 2022). Importantly, no other spoilage-associated or potentially undesirable microbial groups were identified, supporting the existence of a highly selective fermentation environment dominated by acid-tolerant and fermentative microorganisms. These findings are in full agreement with the results obtained through culture- based microbial analyses.

Taken together, the observed microbial dynamics follow the typical succession pattern of sourdough ecosystems, in which LAB rapidly outcompete environmental microorganisms as acidification progresses (Ercolini et al., 2013). The progressive decline of non-LAB taxa, together with the successful establishment of lactobacilli in doughs supplemented with MOLP, suggests that *Moringa* supplementation may accelerate the stabilization of a technologically desirable and ecologically robust fermentation community.

3.3. Bread quality attributes

The main quality parameters of the bread are shown in Table 3. The incorporation of MOLP induced significant modifications in several key attributes, with effects that were generally dose-dependent. Color alterations were the most evident impact of the enrichment: both crust and crumb exhibited a marked reduction in lightness. This darkening can be attributed to the intrinsic colour of *Moringa* powder, particularly its phenolic content and chlorophyll pigments, which have been reported to lower L* values in bakery products enriched with plant-derived powders (Ferreira et al., 2023). Similarly, a progressive decrease in a* and b* values was detected in both crust and crumb, indicating reduced redness and yellowness. These shifts suggest that MOLP influenced not only the

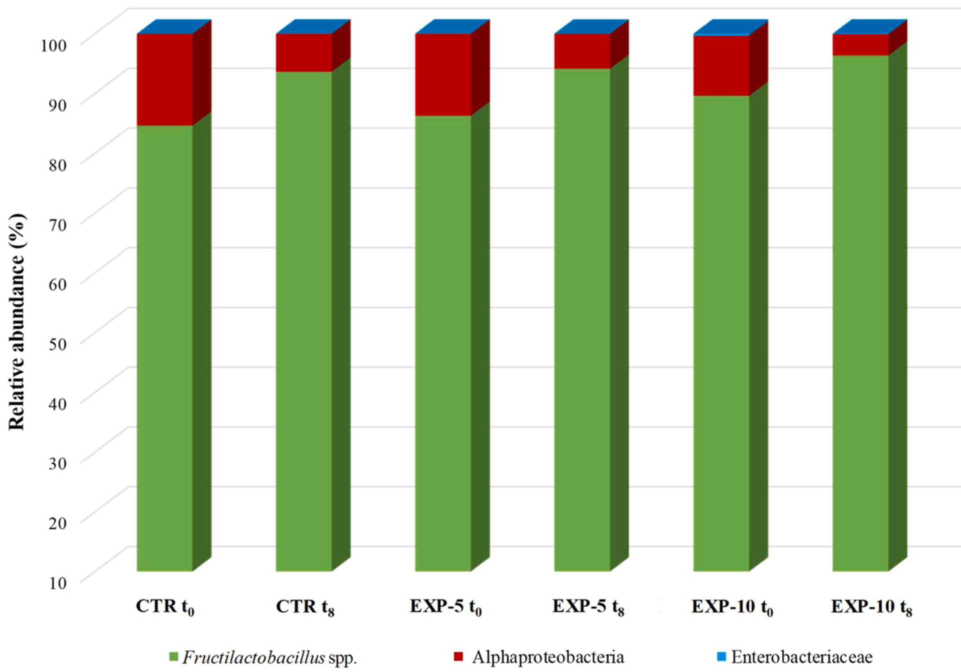


Fig. 2. Relative abundances (%) of bacterial taxa identified in doughs immediately after mixing (t_0) and at the end of fermentation (t_8) using Illumina MiSeq high-throughput sequencing. Abbreviations: CTR, control dough; EXP-5, experimental dough enriched with 5 % of *Moringa oleifera* leaves powder (MOLP); EXP-10, experimental dough enriched with 10 % of MOLP.

Table 3
Key quality parameters of breads.

Samples	Weight loss (g)	Specific volume (cm^3/g bread)	Crust colour			Crumb colour			Firmness (N)	Void fraction (%)	Cell density (n/cm^2)	Mean cell area (mm^2)
			L*	a*	b*	L*	a*	b*				
CTR	16.17 a	3.26 a	58.56 a	11.37	39.88 a	69.67 a	−3.5 c	23.58 c	15.27 c	28.78 b	79.56 c	0.47 a
EXP-5	14.73 b	2.86 ab	40.43 b	9.30	27.1 b	47.83 b	−2.07 b	29.84 a	28.25 b	39.53 a	84.15 b	0.36 a
EXP-10	13.68 c	2.71 b	37.18 b	8.63	22.34 b	39.82 c	−1.37 a	27.44 b	36.41 a	41.14 a	89.78 a	0.31 a
SEM	0.456	0.113	4.190	0.918	3.299	5.502	0.390	1.142	3.820	2.392	1.831	0.036
p value	0.002	0.035	0.001	0.489	0.001	< 0.0001	< 0.0001	0.001	< 0.0001	< 0.0001	< 0.0001	0.102

Results indicate mean value of six measurements (carried out in duplicate for three independent productions). Abbreviations: L*, lightness; a*, redness; b*, yellowness; CTR, control bread; EXP-5, experimental bread enriched with 5 % of (*Moringa oleifera* leaves powder (MOLP); EXP-10, experimental bread enriched with 10 % of MOLP; SEM, standard error of the mean. On the column: a, b, c = $p < 0.05$.

base color of the dough, but also the extent of Maillard and caramelization reactions occurring during baking (Liu et al., 2024).

From a morpho-geometric perspective, increasing levels of MOLP resulted in a progressive reduction in baking weight loss, which was highest in CTR trial and lowest in EXP-10 trial. This trend may be explained by the elevated water-holding capacity of fiber-rich matrices such as MOLP, which can limit moisture evaporation during the baking process (El-Gammal et al., 2016). In agreement with this observation, specific volume significantly decreased as *Moringa* concentration increased, from 3.26 cm^3/g in CTR to 2.71 cm^3/g in EXP-10. Such reductions in loaf volume are commonly reported in breads containing non-gluten or fiber-rich powders, which may interfere with the development and stability of the gluten network and gas retention (Zhou et al., 2021).

Textural analysis further revealed an increase in crumb firmness in MOLP-enriched breads. The higher hardness values measured in EXP-5 and EXP-10 indicate a more denser crumb structure, consistent with the observed decrease in specific volume and with the dilution and weakening of gluten proteins caused by MOLP addition (Giuberti et al., 2021).

Interestingly, crumb porosity analysis revealed that increasing MOLP concentrations led to higher void fraction and cell density, while mean cell area remained comparable between EXP-5 and EXP-10. This suggests that although overall dough expansion was limited by MOLP inclusion, a finer and denser alveolar structure was promoted. This behavior may arise from interactions between dietary fibers, proteins, and polysaccharide components in MOLP, which may modulate dough viscosity and gas-retention capacity (Sardabi et al., 2021).

3.4. Functional attributes of MOLP and doughs

The functional attributes of MOLP used for bread production are reported in Table 4. MOLP showed high levels of TPC (3.02 g GAE/100 g DW) and TFC (701.0 mg RE/100 g DW), together with marked antioxidant activity as assessed by DPPH (5.71 mmol TE/100 g DW) and FRAP (14.97 mmol TE/100 g DW) assays. These values are consistent with

Table 4
Antioxidant properties of MOLP used for bread production.

Properties	Value	Unit
TPC	3.02 ± 0.34	g GAE/100 g DW
TFC	701.0 ± 61.8	mg RE/100 g DW
DPPH	5.71 ± 0.27	mmol TE/100 g DW
FRAP	14.97 ± 1.02	mmol TE/100 g DW

Results indicate mean values ± standard deviation of six measurements (carried out in duplicate for three independent productions). Abbreviations: TPC, total phenolic content; TFC, total flavonoid content; DPPH, 2,2-diphenyl-1-picrylhydrazyl assay; FRAP, ferric reducing antioxidant power; GAE, gallic acid equivalent; RE, rutin equivalent; DW, dry weight; TE, Trolox equivalent.

those previously reported for MOLP (Greco et al., 2025), confirming its reliability and reproducible source of bioactive compounds for functional food applications.

To investigate the evolution of bioactive compounds during dough processing, TPC and antioxidant activity were monitored in CTR and EXP-5 and EXP-10 doughs immediately after mixing (t_0) and at the end of fermentation (t_8), as shown in Fig. 3. In the enriched formulations, the effective MOLP incorporation corresponded to 2.85 and 5.71 g per 100 g of dough in EXP-5 and EXP-10, respectively. A relevant outcome was the relatively high baseline functional value of CTR. At t_0, this dough (Fig. 3a) showed a TPC of 69.45 mg GAE/100 g FW, a value notably higher than those reported for doughs made with highly refined wheat flours (Ferreira et al., 2023). This finding reflects the use of semolina as the cereal matrix. Although semolina remains a refined product, it retains a higher phenolic fraction compared with soft wheat flour. Durum wheat-based products are known to contain appreciable amounts of phenolic acids, particularly in bound form, with ferulic acid derivatives being prominent (Nicoletti et al., 2013). Consequently, semolina provides a comparatively rich endogenous phenolic background.

The incorporation of MOLP resulted in a clear dose-dependent increase in TPC at t_0, reaching 190.52 mg GAE/100 g FW in EXP-5 and 288.36 mg GAE/100 g FW in EXP-10, corresponding to nearly three- and more than four-fold increases over CTR, respectively. Interestingly, these experimental values exceeded the theoretical TPC predicted based solely on the MOLP content added to the dough. This discrepancy indicates that MOLP addition does not act merely as an additive, but seems to enhance the extractability of phenolics already present in the system. Dough mixing and hydration likely promote the release of matrix-bound phenolics from both semolina and MOLP, facilitating their solubilization and analytical detection.

Fermentation further modulated the phenolic profile. In CTR dough, TPC increased by approximately 1.5-fold after fermentation, supporting the hypothesis that fermentation promoted the release of endogenous phenolics from the semolina matrix. This behaviour aligns with the known occurrence of bound phenolic acids in durum wheat products and their susceptibility to biochemical changes during fermentation that increases their extractability. In contrast, in MOLP-enriched doughs, TPC remained relatively constant over fermentation, despite a marked decrease in the contribution attributable to MOLP from 121.07 to 92.48 mg GAE/100 g FW in EXP-5 and from 234.55 to 180.84 mg GAE/100 g FW in EXP-10. The stability of total TPC therefore reflects a compensatory increase in the endogenous semolina-derived phenolic fraction. This behaviour suggests that MOLP-derived phenolics underwent transformations during fermentation that were not fully captured by the Folin-Ciocalteu assay. Several mechanisms may be involved, including partial oxidation, microbial conversion into compounds with reduced Folin reactivity, or interactions with gluten proteins and other dough components. Polyphenol-gluten interactions have been well documented and may alter extractability and chemical accessibility within the dough matrix (Girard & Awika, 2020). In this context, semolina not only provides phenolics but also acts as an interactive matrix influencing the availability of MOLP-derived compounds.

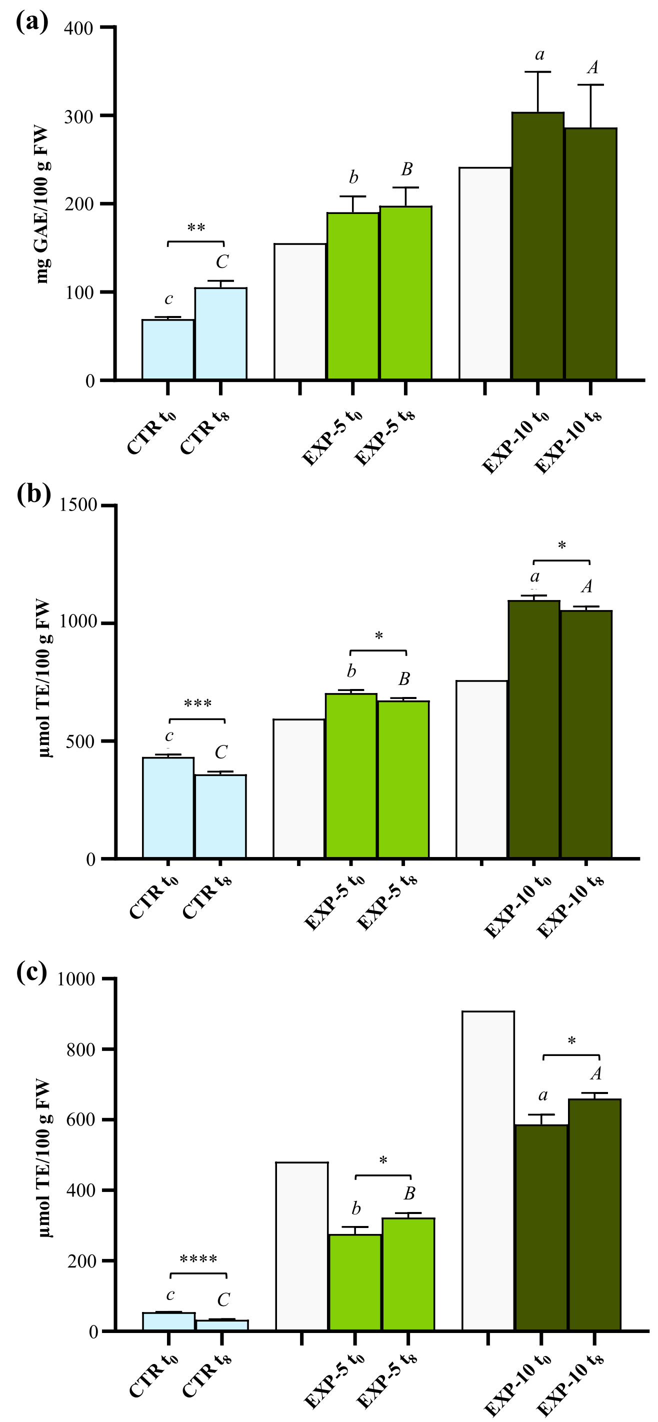


Fig. 3. Functional properties of doughs measured immediately after mixing (t_0) and at the end of fermentation (t_8). CTR, control dough (light blue); EXP-5, experimental dough enriched with 5 % of *Moringa oleifera* leaves powder (MOLP) (light green); EXP-10, experimental dough enriched with 10 % of MOLP (dark green). White bars represent the corresponding theoretical values calculated from the functional properties of MOLP and its actual concentration in the dough formulations. (a) total phenolic content; (b) antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl assay (DPPH); (c) ferric reducing antioxidant power (FRAP) assays. Results indicate mean values ± standard deviation of six measurements (carried out in duplicate for three independent productions). Different letters (lowercase for t_0 and uppercase for t_8) indicate significant differences among the samples as measured by one-way ANOVA followed by Tukey's post hoc test, while asterisks show significant differences between t under the same experimental condition (CTR, EXP-5 or EXP-10), as evaluated by student *t*-test. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$.

The antioxidant assays highlighted a mechanism-dependent response (Fig. 3c). In CTR dough at t_0, DPPH activity (433.22 μmol TE/100 g FW) was much higher than FRAP (54.38 μmol TE/100 g FW), suggesting that semolina provides compounds with stronger radical-scavenging than ferric-reducing capacity. MOLP supplementation enhanced both activities in a dose-dependent manner. DPPH values increased to 673.47 and 1099.16 μmol TE/100 g FW in EXP-5 and EXP-10, respectively, while FRAP increased to 276.57 and 587.38 μmol TE/100 g FW, respectively.

At t_0, the quantitative contribution of MOLP to DPPH and FRAP was comparable in the enriched doughs, indicating that MOLP supplies bioactive compounds effective through both radical scavenging and reducing mechanisms. However, DPPH activity exceeded theoretical predictions, mirroring the behaviour observed for TPC, whereas FRAP values were lower than expected. This divergence suggests that dough matrix interactions preferentially preserve or enhance radical scavenging capacity, while partially masking ferric-reducing functionality, possibly through protein complexation or structural modification of reducing agents.

Fermentation accentuated these differences. DPPH activity decreased or remained unchanged in all doughs, whereas FRAP values increased, indicating a shift toward compounds with stronger reducing power. The enhanced antioxidant capacity observed in the MOLP-enriched doughs may also be considered biologically relevant, as dietary antioxidants can contribute to the overall antioxidant potential of the diet. While the actual health effects of these compounds depend on their bioaccessibility, metabolism, and bioavailability, the observed increase in antioxidant activity supports the potential of MOLP as a functional ingredient for the development of bakery products with improved functional properties. Overall, the functional profile of semolina-based doughs results from a dynamic interplay between endogenous cereal phenolics and MOLP-derived compounds, with fermentation influencing not only their concentration but also their chemical behaviour and antioxidant mechanisms.

3.5. Functional properties of MOLP-enriched breads

To evaluate the impact of baking on functional properties, TPC and antioxidant activity were determined in CTR and MOLP-enriched breads and compared with theoretical values calculated from fermented doughs after correction for baking weight loss (Fig. 4). Semolina bread retained a relevant basal functional value even without addition, consistent with the phenolic background already observed in the dough. MOLP incorporation significantly enhanced the functional profile in a dose-dependent manner, with TPC values increasing up to approximately 2.5-fold in EXP-10 compared with CTR (Fig. 4a), confirming the ability of MOLP to significantly improve the polyphenolic value of the final bread. However, experimental TPC values were consistently lower than those expected, indicating partial loss, transformation, or reduced extractability of phenolic compounds during baking. This effect was more pronounced for the MOLP-derived fraction than for endogenous semolina phenolics: only about 60 % of the expected MOLP contribution was retained in the final breads (60.46 *vs.* 109.96 mg GAE/100 g FW in EXP-5; 133.56 *vs.* 219.43 mg GAE/100 g FW in EXP-10). This suggests a lower thermal stability and/or lower extractability of the MOLP-derived phenolics compared with the endogenous phenolics of semolina. Although the relatively simple bread matrix should limit interactions, baking still imposes oxidative and thermal stress capable of affecting the fortifying fraction (Schefer et al., 2021).

DPPH assay (Fig. 4b) showed a fully coherent trend with the TPC data. Experimental values in breads were consistently lower than theoretical ones, and in enriched breads, were about half, or less than half, of the expected values. This indicates that radical scavenging compounds are particularly sensitive to baking, even more so than the total phenolic pool measured by the Folin assay. Thus, despite the clear enhancement provided by MOLP, a substantial fraction of DPPH-active compounds was lost or transformed during thermal processing. In contrast, FRAP values (Fig. 4c) were consistently higher than theoretical predictions. This indicates the formation of additional ferric-reducing compounds during baking, most plausibly Maillard reaction products (Delgado-Andrade, 2014). Even in the absence of added sugars or milk proteins, crust formation involves reactions between reducing sugars from starch hydrolysis and amino groups from cereal proteins, leading to the formation of melanoidins and related products. These compounds are known to contribute strongly to ferric-reducing capacity and to respond in both FRAP and Folin–Ciocalteu assays (Delgado-Andrade, 2014). The lack of a parallel increase in DPPH suggests that these processing-induced compounds predominantly enhance reducing capacity rather than radical scavenging activity.

Overall, the functional profile of MOLP-enriched semolina breads reflects a balance between partial loss of native, particularly MOLP-derived, antioxidant compounds and the concurrent formation of technologically generated ferric-reducing species during baking. Although MOLP remains an effective fortifying ingredient, the final functional outcome is shaped not only by the amount added but also by matrix-related interactions and the transformations induced by fermentation and baking. Thus, these factors should be carefully considered when designing fortified breads and interpreting antioxidant measurements in processed cereal foods.

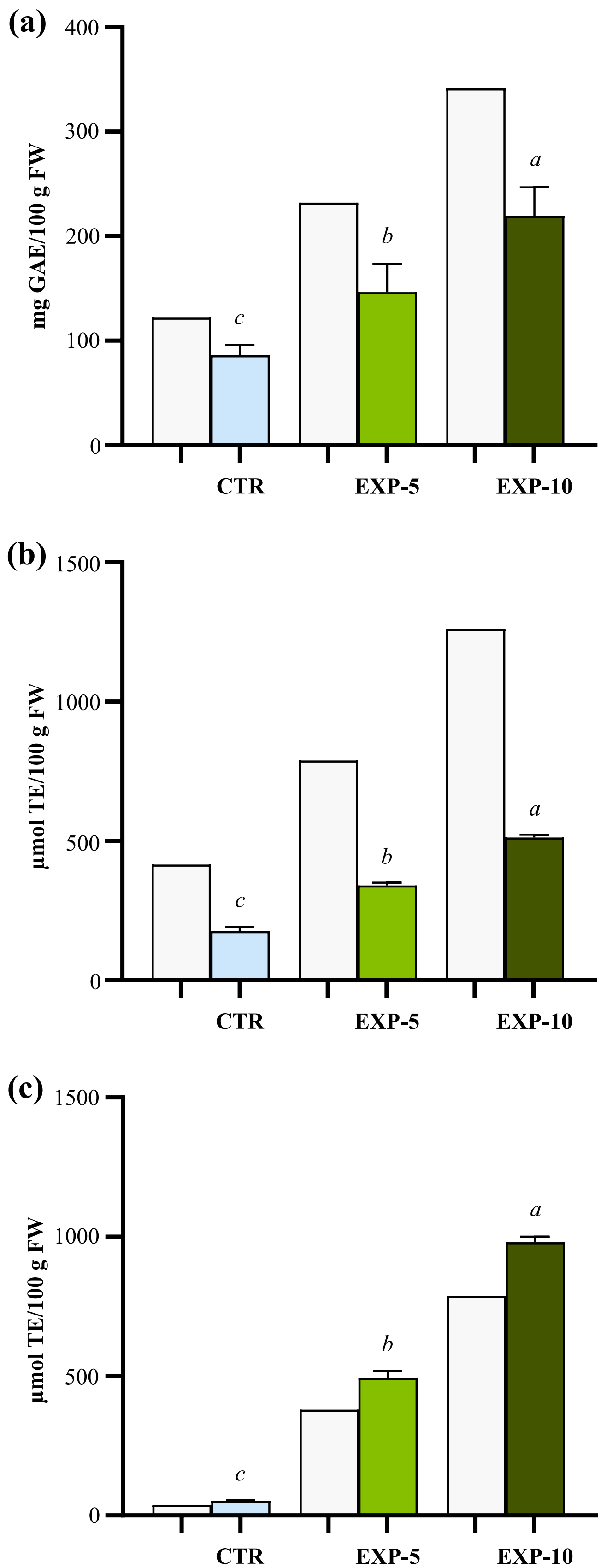
3.6. Volatile organic compounds emitted from breads

The results of the headspace SPME-GC/MS analysis performed on MOLP before its incorporation into the formulations and on all breads are reported in Table 5. The volatile profile of MOLP revealed the presence of 20 compounds distributed across seven chemical classes. Terpenes represented the dominant fraction, accounting for approximately 33.9 % of the total chromatographic peak area of all detected volatile compounds, followed by carbonyl compounds (26.3 %). Alcohols and esters represented 15.9 % and 8.8 % of the total volatile fraction, respectively, whereas acids (4.9 %) and hydrocarbons (4.2 %) were detected at lower relative abundance. Although relatively few studies have specifically investigated the volatile composition of *M. oleifera* leaves, the overall fingerprint observed herein is consistent with previous published data (Balogun et al., 2017; Greco et al., 2025; Ismael et al., 2016; Li et al., 2023; Mukunzi et al., 2011; Shunmugapriya et al., 2020).

Among the most abundant compounds detected in MOLP were phenylethyl alcohol, known for its floral aroma (Fabre et al., 1998), and geranylgeraniol, which was the most prominent terpene, followed by geranyl acetone. Geranyl acetone is associated with green, fruity, waxy, woody, pear-like, rosy, and guava-like aromas with tropical notes (Bonikowski et al., 2015). Among ketones, 3-hexen-2-one and 3,5-octadien-2-one were detected and are known contributors to the green and roasted nuances of the powder (Teng et al., 2024). Overall, these key constituents are consistent with those previously reported for *Moringa* leaves and flowers (Balogun et al., 2017; Greco et al., 2025).

Regarding breads, CTR showed a volatile profile characterized by several chemical classes, including alcohols, carbonyl compounds, acids, furans, and hydrocarbons. Carbonyl compounds represented the predominant class, with hexanal, heptanal, and benzaldehyde among the most abundant constituents. Phenylethyl alcohol was the main alcohol detected, while 2-pentylfuran, commonly associated with thermal reactions and lipid oxidation in baked products (Martínez-Anaya, 1996), was also present. Overall, the volatile profile of CTR bread was consistent with previously reported sourdough aroma patterns (Chulibert et al., 2024; Gaglio et al., 2020, 2021; Giannone et al., 2018).

The addition of MOLP markedly reshaped the volatile fingerprint of the breads, leading in both EXP-5 and EXP-10 to the detection of several terpenes, ketones, and esters that were absent in the CTR production. Terpenes accounted for 16.4 % and 18.0 % of the total volatile fraction in EXP-5 and EXP-10, respectively, whereas they were not detected in the CTR bread. Despite the introduction of MOLP-derived volatiles,



(caption on next column)

Fig. 4. Functional properties of breads. CTR, control bread (light blue); EXP-5, experimental bread enriched with 5 % of *Moringa oleifera* leaves powder (MOLP) (light green); EXP-10, experimental bread enriched with 10 % of MOLP (dark green). White bars represent the corresponding theoretical values calculated from the functional properties of MOLP and its actual concentration in the bread formulations. (a) total phenolic content; (b) antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl assay (DPPH); (c) ferric reducing antioxidant power (FRAP) assays. Results indicate mean values ± standard deviation of six measurements (carried out in duplicate for three independent productions). Different letters indicate significant differences among the samples as measured by one-way ANOVA followed by Tukey's post hoc test.

carbonyl compounds remained the predominant chemical class in both enriched breads (43.6 % in EXP-5 and 41.9 % in EXP-10), indicating that the characteristic cereal-derived volatile profile was largely preserved. Among the compounds attributable to MOLP addition, geranylgeraniol and geranyl acetone were the dominant terpenes detected in both enriched formulations. Among ketones, the odor-active compound 3-hexen-2-one was consistently present in both EXP-5 and EXP-10. The occurrence of esters, known to influence aroma perception by imparting fruity and floral notes (Li & Sun, 2019), together with terpenes associated with floral, green, and resinous odor descriptors (Alves et al., 2015; Cadwallader, 2018), further enhanced the aromatic complexity of the fortified breads.

3.7. Sensory attributes of breads

Sensory evaluation is widely used to assess consumer acceptance of novel food products prior to their market introduction (Świąder & Marczewska, 2021). In the present study, MOLP incorporation significantly affected the sensory profile of sourdough breads (Fig. 5). Visual attributes were the most markedly influenced, as evidenced by the progressive increase in crust and crumb colour scores with increasing MOLP levels, reflecting the characteristic darkening associated with *Moringa* pigments (Koriyama et al., 2025). Conversely, crumb structural features associated with openness, including porosity and alveolation, showed a slight reduction, whereas alveolation regularity remained largely unaffected. Comparable trends have been consistently reported in breads enriched with functional ingredients (Cirlincione et al., 2022).

Olfactory attributes were enhanced in the enriched breads, with increases in overall odour and aroma intensity accompanied by a reduction in typical bread notes, particularly at higher MOLP levels. This shift suggests a growing contribution of herbal and plant nuances derived from MOLP to the overall aromatic profile (Rodrigues et al., 2023). Tactile attributes were only marginally affected, with a slight reduction in crumb elasticity and no relevant changes in crust crispness, in agreement with previous studies on *Moringa*-enriched bakery products (Ogunsina et al., 2011).

Finally, taste related attributes, including strange aroma, bitterness, and astringency increased with MOLP concentration, likely due to the presence of polyphenols and other bioactive compounds in plant leaves (Teclegeorgish et al., 2021). Taste persistence also increased, indicating a more complex and longer-lasting gustatory profile. Overall satisfaction followed a non-linear trend, with the highest score recorded for EXP-5, suggesting that moderate MOLP inclusion represents the best compromise between functional enhancement, sensory complexity, and consumer acceptability.

3.8. Consumer perception and acceptance of bread

Consumer perception and acceptance of the novel functional bread enriched with MOLP were assessed through a survey conducted in two retail formats characterized by distinct market positioning. The selection of these retail formats was intended to capture potential differences in consumer profiles between a convenience-oriented supermarket and a premium retail store, in line with previous studies on consumer

Table 5
Volatile organic compounds of MOLP and breads.

Compounds	Samples				SEM	*p* value
	MOLP	CTR	EXP-5	EXP-10		
Alcohols						
Phenethyl alcohol	13.22	19.03	11.88	12.87	1.669	0.096
3-Methyl-1-butanol	n.d.	8.45 a	3.95 b	4.25 b	0.969	0.018
1-Hexanol	n.d.	1.36 a	n.d. b	n.d. b	0.283	< 0.0001
1-Octen-3-ol	n.d.	3.19	2.71	3.05	0.198	0.621
3-Hexen-1-ol	2.72	n.d.	n.d.	n.d.	n.e.	n.e.
Carbonylic compounds						
Pentanal	n.d.	2.75 a	1.15 b	1.60 b	0.311	0.008
Benzaldehyde	5.58	5.61 b	2.95 a	3.04 a	0.611	0.046
Nonanal	n.d.	4.46	4.61	2.96	0.421	0.117
Heptanal	n.d.	6.56	3.89	4.92	0.582	0.093
2-Octenal	n.d.	2.59 a	1.52 b	0.88 b	0.324	0.007
Hexanal	1.8	18.59	13.02	13.56	1.521	0.236
Acetophenone	5.35	2.68 b	6.61 a	5.11 ab	0.784	0.028
1-Octen-3-one	n.d.	2.22 a	n.d b	n.d b	0.460	< 0.0001
2.4-Octadienal	3.19	n.d. c	1.95 a	1.04 b	0.354	0.001
Decanal	1.66	n.d.	n.d	n.d	n.e.	n.e.
3-Hexen-2-one	6.08	n.d. b	4.84 a	3.46 a	0.907	0.001
3.5-Octadien-2-one	5.15	n.d. b	2.99 a	4.25 a	0.800	0.002
4-Octanone	3.51	n.d. b	n.d. b	1.06 a	0.219	< 0.0001
Acids						
Octanoic acid	n.d.	1.62 a	0.91 b	n.d c	0.295	0.001
Nonanoic acid	n.d.	2.73 a	1.93 ab	1 b	0.334	0.015
Acetic acid	n.d.	5.53 a	2.18 b	2.43 b	0.702	0.009
Hexadecanoic acid	4.87	n.d. b	2.27 a	3.05 a	0.577	0.002
Furans						
2-Pentylfuran	n.d.	8.01	6.73	5.04 a	0.698	0.157
Hydrocarbons						
Pentadecane	n.d.	3.03 a	1.25 b	1.64 b	0.350	0.007
Undecane	1.03	1.59 a	0.73 b	0.92 ab	0.178	0.028
Dodecane	3.15	n.d. c	0.77 b	1.31 a	0.239	0.001
Esters						
Benzyl acetate	2.41	n.d. b	1.25 a	1.64 a	0.313	0.002
Methyl valerate	5.04	n.d. b	3.55 a	2.95 a	0.692	0.001
Methyl palmitate	1.36	n.d.	n.d.	n.d.	n.e.	n.e.
Terpenes						
Geranylgeraniol	11.77	n.d. b	6.54 a	8.40 a	1.621	0.003
Anethole	3.75	n.d. c	1.77 b	2.92 a	0.535	0.001
Geranylacetone	4.71	n.d. b	2.81 a	2.05 a	0.532	0.002
α-Terpinene	3.92	n.d. c	2.36 a	1.06 b	0.429	0.001
Nectarol	1.82	n.d.	n.d.	n.d.	n.e.	n.e.
β-Ocimene	4.05	n.d. b	1.95 a	2.54 a	0.489	0.003
α-Linalool	2.36	n.d. b	0.93 a	1.00 a	0.203	0.001
β-Myrcene	1.5	n.d.	n.d.	n.d.	n.e.	n.e.
Summary of VOC classes (relative abundance, %)						
Alcohols	15.94	32.03	18.54	20.17	n.e.	n.e.
Carbonyl compounds	32.32	45.46	43.53	41.88	n.e.	n.e.
Acids	4.87	9.88	7.29	6.48	n.e.	n.e.
Furans	n.d	8.01	6.73	5.04	n.e.	n.e.
Hydrocarbons	4.18	4.62	2.75	3.87	n.e.	n.e.
Esters	8.81	n.d	4.80	4.59	n.e.	n.e.
Terpenes	33.8	n.d	16.36	17.97	n.e.	n.e.

Data are expressed as percentages [(peak area of each compound/total peak area of significant compounds) × 100] and represent the mean of six measurements (carried out in duplicate for three independent productions). The total relative abundance (%) of each chemical class was calculated as the sum of the relative chromatographic peak areas of all compounds belonging to that class. Abbreviations: CTR, control bread; EXP-5, experimental bread enriched with 5 % of (*Moringa oleifera* leaves powder (MOLP); EXP-10, experimental bread enriched with 10 % of MOLP; SEM, standard error of the mean; n.d., not detectable; n.e., not evaluated. On the column: a, b, c = $p < 0.05$.

segmentation and the acceptance of emerging food technologies (Testa et al., 2021). As shown in Table 1, participants recruited in both retail formats were predominantly female, in line with previous studies reporting a higher representation of women due to their greater responsibility in grocery shopping compared with men (Ding et al., 2015; Verbeke, 2015). Moreover, respondents recruited at Superstore Le Liccumie were more frequently older (50+ years) and characterized by higher educational levels and household income. This finding supports the observation that premium retail formats tend to attract more informed consumers who are more attentive to product quality and further highlights the influence of education and income on food-related attitudes (Gagliardi et al., 2025).

Responses to all questionnaire items are reported in Table 6. Significant differences between the two retail formats were observed only for familiarity with foods enriched with plant-derived products ($\chi^2 = 59.20$, $p < 0.0001$), which was markedly higher among respondents recruited at the premium retail store. This finding is consistent with the characteristics of premium retail formats, which typically offer a wider and more differentiated product assortment with a stronger emphasis on quality, thereby attracting consumer segments with distinct motivations and purchasing behaviours (Bonfrer et al., 2022). In addition, consumers with higher income levels and greater health awareness are generally more exposed to and familiar with innovative food products (Dhakal & Khadka, 2021). The importance attributed to the health benefits and food safety of breads enriched with plant-derived ingredients remained high across retail formats. This finding suggests that consumers recognize the functional potential of these products and exhibit comparable levels of trust in the safety of foods purchased from retail outlets, irrespective of retail store format (Sajdakowska et al., 2026; Wu et al., 2021). A similar trend was observed for openness to innovation, with 68.6 % of respondents at Conad and 71.5 % at Superstore Le Liccumie expressing a favourable attitude toward purchasing bread enriched with MOLP. This result is consistent with previous studies on consumer perception and acceptance of technologies aimed at improving the functional properties of bakery products through natural enrichment strategies with a reduced environmental impact (Baker et al., 2022; Coderoni & Perito, 2020). From an economic perspective, more than 80 % of respondents in both retail formats indicated a willingness to pay a 10 % price premium over conventional bread. This outcome supports previous evidence suggesting that perceived health benefits and product quality may outweigh price sensitivity in food purchasing decisions (Miolla et al., 2023; Nazzaro et al., 2024). Overall, these findings indicate that functional breads enriched with MOLP exhibit a strong potential for positive consumer perception.

4. Conclusions

This study demonstrates that MOLP produced from Sicilian-grown plants can be effectively used as a functional ingredient in semolina sourdough bread without compromising fermentation performance, microbial stability, or product safety. Beyond technological feasibility, the results highlight the importance of matrix-ingredient interactions and processing effects in determining the actual functional expression of plant-derived bioactive compounds in baked products. From a formulation and production perspective, a 5 % (w/w) inclusion level emerged as the most suitable compromise, providing significant functional and

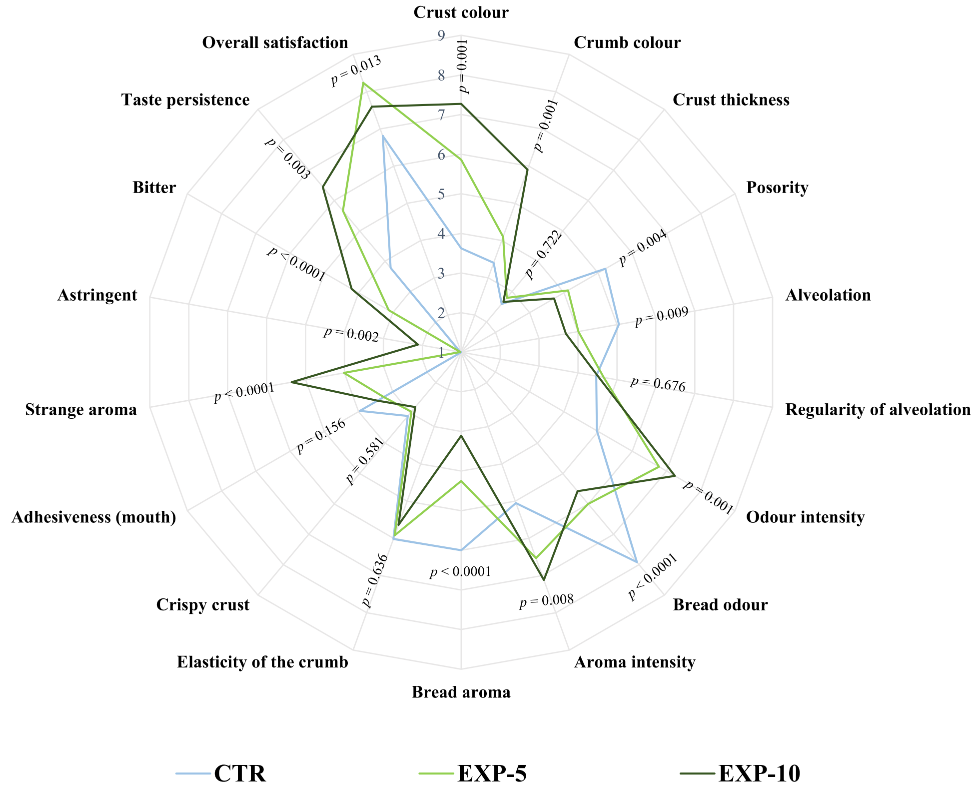


Fig. 5. Radar chart illustrating the sensory profile of the bread samples. Abbreviations: CTR, control bread; EXP-5, experimental bread enriched with 5 % of (*Moringa oleifera* leaves powder (MOLP); EXP-10, experimental bread enriched with 10 % of MOLP.

Table 6
Consumer perception and acceptance variables across retail formats.

Variables	Retail formats		χ^2	p value
	Conad	Superstore Le Liccumie		
Familiarity with foods enriched with plant by-products				
Yes	6.7	57.1	59.20	< 0.0001
No	60.0	28.6		
I am not sure	33.3	14.3		
Importance of beneficial effects of enriched bread				
Unimportant	0.0	0.0	0.28	0.597
Medium important	33.4	28.6		
Absolutely important	66.7	71.5		
Perceived food safety				
Very safe	73.3	71.5	2.00	0.368
Moderately safe	20.0	28.6		
Slightly safe	6.7	0.0		
Openness to innovation				
High openness	68.6	71.5	4.83	0.089
Conditional openness with clear explanation	26.7	28.6		
No openness	4.7	0.0		
Willingness to pay				
Affirmative	80.5	81.4	0.02	0.992
Negative	19.5	18.6		

aromatic enrichment, while ensuring sensory acceptability and positive consumer response. These findings offer practical guidance for the development of value-added sourdough breads and support the industrial applicability of MOLP as a clean-label, plant-based fortifying ingredient. Ongoing research is aimed at validating the process under industrial-scale conditions, exploring the shelf-life stability of MOLP-enriched bread, and assessing the bioaccessibility of its functional compounds. Overall, the study contributes to the valorization of locally cultivated *M. oleifera* and reinforces sourdough biotechnology as a sustainable and adaptable platform for functional bread innovation aligned with future food system and consumer demands.

Fundings

This research has been financially supported by the INTERREG VI-A NEXT Italy–Tunisia Cooperation Programme (Call 1/2024) through the project “RÉINVESTIR-Recherche et innovation dans le domaine des espèces végétales d’intérêt agroalimentaire, nutraceutique et en voie d'extinction en Sicile et en Tunisie” (Project code A1.–1.1–159; CUP B73C25000290002) and by the project funded under the Italian National Recovery and Resilience Plan (PNRR), Mission 2, Component 1, Investment 3.4, Revolving Fund for Supply Chain Contracts (FCF) to support supply chain contracts in the agri-food, fisheries and aquaculture, forestry, floriculture, and nursery sectors, financed by NextGenerationEU, Ref. position no. 110 – programme 237, Organic Supply Chain “Bio Slow”, (CUP: B17G21000110001).

Ethical statement

The present work did not involve human subjects for medical purposes.

CRediT authorship contribution statement

Graziella Serio: Writing – original draft, Formal analysis, Data curation. **Giuliana Garofalo:** Writing – original draft, Methodology, Formal analysis, Data curation. **Enrico Viola:** Software, Investigation, Formal analysis, Data curation. **Marcella Barbera:** Writing – original draft, Methodology, Investigation, Formal analysis. **Michele Massimo Mammano:** Resources, Project administration, Funding acquisition. **Simone Italia:** Methodology, Investigation. **Santo Orlando:** Software, Methodology. **Elena Franciosi:** Software, Methodology, Investigation, Formal analysis, Data curation. **Hasan Tangüler:** Writing – review & editing, Software, Methodology. **Daniela Piazzese:** Validation, Methodology. **Carlo Greco:** Resources, Project administration, Funding acquisition, Conceptualization. **Carla Gentile:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Conceptualization. **Luca Settanni:** Writing – review & editing, Supervision, Conceptualization. **Raimondo Gaglio:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Software, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that there is no conflict of interest for this research.

Acknowledgements

The authors gratefully acknowledge Fondazione Umberto Veronesi for supporting Graziella Serio.

Data availability

Data will be made available on request.

References

AACC Approved Methods of Analysis. (2000). *Approved methods of the American association of cereal chemists* (11th Edition). St. Paul, MN, U.S.A: AACC International.

Alfonzo, A., Urso, V., Corona, O., Francesca, N., Amato, G., Settanni, L., & Di Miceli, G. (2016). Development of a method for the direct fermentation of semolina by selected sourdough lactic acid bacteria. *International Journal of Food Microbiology, 239*, 65–78. https://doi.org/10.1016/j.ijfoodmicro.2016.06.027

Alves, Z., Melo, A., Figueiredo, A. R., Coimbra, M. A., Gomes, A. C., & Rocha, S. M. (2015). Exploring the *Saccharomyces cerevisiae* volatile metabolome: Indigenous versus commercial strains. *PLoS ONE, 10*, Article e0143641. https://doi.org/10.1371/journal.pone.0143641

Atfaoui, K., Lebrazi, S., Raffak, A., Chafai, Y., Kabous, K. E., Fadil, M., & Ouhssine, M. (2025). Impact of selected starter-based sourdough types on fermentation performance and bio-preservation of bread. *Fermentation, 11*, 449. https://doi.org/10.3390/fermentation11080449

Baker, M. T., Lu, P., Parrella, J. A., & Leggette, H. R. (2022). Consumer acceptance toward functional foods: A scoping review. *International Journal of Environmental Research and Public Health, 19*, 1217. https://doi.org/10.3390/ijerph19031217

Balogun, O. S., Fadare, R. Y., Fadare, O. A., Akinpelu, D. A., & Obafemi, C. A. (2017). Chemical composition and in-vitro antibacterial activity of the essential oil of Nigerian *Moringa oleifera* Lam. Flowers. *European Journal of Medicinal Plants, 18*, 1–9. https://doi.org/10.9734/EJMP/2017/31834

Bonfrer, A., Chintagunta, P., & Dhar, S. (2022). Retail store formats, competition and shopper behavior: A systematic review. *Journal of Retailing, 98*, 71–91. https://doi.org/10.1016/j.jretai.2022.02.006

Bonikowski, R., Świtakowska, P., & Kula, J. (2015). Synthesis, odour evaluation and antimicrobial activity of some geranyl acetone and nerolidol analogues. *Flavour and Fragrance Journal, 30*, 238–244. https://doi.org/10.1002/ffj.3238

Busetta, G., Garofalo, G., Mangione, G., Botta, L., Franciosi, E., Di Gerlando, R., Todaro, M., Licitra, G., Scatassa, M. L., Gaglio, R., & Settanni, L. (2021). Polyphasic characterization of microbiota of "mastredda", a traditional wooden tool used during the production of PDO Provola dei Nebrodi cheese. *Applied Sciences, 11*, 8647. https://doi.org/10.3390/app11188647

Busetta, G., Garofalo, G., Ponte, M., Barbera, M., Alfonzo, A., Franciosi, E., Francesca, N., Frusteri, G., Piazzese, D., Bonanno, A., Schicchi, R., Moschetti, G., Gaglio, R., & Settanni, L. (2025). Replacing preservative E 252 with powdered dried sumac (*Rhus coriaria* L.) fruits in "Suino Nero dei Nebrodi" salamis: Effects on microbiological, physicochemical, and antioxidant properties. *Food Microbiology, 127*, Article 104684. https://doi.org/10.1016/j.fm.2024.104684

Cadwallader, K. R. (2018). Aromas. In P. Varelis, L. Melton, & F. Shahidi (Eds.), *Encyclopedia of food chemistry* (pp. 22–29). Amsterdam: Elsevier.

Chhikara, N., Kaur, A., Mann, S., Garg, M. K., Sofi, S. A., & Panghal, A. (2021). Bioactive compounds, associated health benefits and safety considerations of *Moringa oleifera* L.: An updated review. *Nutrition & Food Science, 51*, 255–277. https://doi.org/10.1108/NFS-03-2020-0087

Chulibert, M. E., Roppolo, P., Buzzanca, C., Alfonzo, A., Viola, E., Sciurba, L., Tinebra, I., D'Amico, A., Farina, V., Piazzese, D., Di Stefano, V., Barbera, M., Gaglio, R., & Settanni, L. (2024). Exploring the addition of mango peel in functional semolina sourdough bread production for sustainable bio-reuse. *Antioxidants, 13*, 1278. https://doi.org/10.3390/antiox13111278

Cirlincione, F., Venturella, G., Gargano, M. L., Ferraro, V., Gaglio, R., Francesca, N., … Mirabile, G. (2022). Functional bread supplemented with *Pleurotus eryngii* powder: A potential new food for human health. *International Journal of Gastronomy and Food Science, 27*, 100449. https://doi.org/10.1016/j.ijgfs.2021.100449

Coderoni, S., & Perito, M. A. (2020). Sustainable consumption in the circular economy. An analysis of consumers' purchase intentions for waste-to-value food. *Journal of Cleaner Production, 252*, Article 119870. https://doi.org/10.1016/j.jclepro.2019.119870

Corona, O., Alfonzo, A., Ventimiglia, G., Nasca, A., Francesca, N., Martorana, A., Moschetti, G., & Settanni, L. (2016). Industrial application of selected lactic acid bacteria isolated from local semolinas for typical sourdough bread production. *Food Microbiology, 59*, 43–56. https://doi.org/10.1016/j.fm.2016.05.006

Corsetti, A., Gobbetti, M., De Marco, B., Balestrieri, F., Paoletti, F., Russi, L., & Rossi, J. (2000). Combined effect of sourdough lactic acid bacteria and additives on bread firmness and staling. *Journal of Agricultural and Food Chemistry, 48*, 3044–3051. https://doi.org/10.1021/jf990853e

Corsetti, A., Settanni, L., Braga, T. M., Lopes, M. D. F. S., & Suzzi, G. (2008). An investigation of the bacteriocinogenic potential of lactic acid bacteria associated with wheat (*Triticum durum*) kernels and non-conventional flours. *LWT - Food Science and Technology, 41*, 1173–1182. https://doi.org/10.1016/j.lwt.2007.07.022

Das, R. S., Tiwari, B. K., & Garcia-Vaquero, M. (2023). The fundamentals of bread making: The science of bread. In R. S. Das, B. K. Tiwari, & M. Garcia-Vaquero (Eds.), *Traditional European breads: An illustrative compendium of ancestral knowledge and cultural heritage* (pp. 1–40). Cham: Springer International Publishing. https://doi.org/10.1007/978- 3-031-23352-4_1.

De Vuyst, L., Comasio, A., & Kerrebroeck, S. V. (2023). Sourdough production: Fermentation strategies, microbial ecology, and use of non-flour ingredients. *Critical Reviews in Food Science and Nutrition, 63*, 2447–2479. https://doi.org/10.1080/10408398.2021.1976100

Delgado-Andrade, C. (2014). Maillard reaction products: Some considerations on their health effects. *Clinical Chemistry and Laboratory Medicine, 52*, 53–60. https://doi.org/10.1515/cclm-2012-0823

Dhakal, C. K., & Khadka, S. (2021). Heterogeneities in consumer diet quality and health outcomes of consumers by store choice and income. *Nutrients, 13*, 1046. https://doi.org/10.3390/nu13041046

Ding, Y., Veeman, M. M., & Adamowicz, W. L. (2015). Functional food choices: Impacts of trust and health control beliefs on Canadian consumers' choices of canola oil. *Food Policy, 52*, 92–98. https://doi.org/10.1016/j.foodpol.2014.12.002

Dutta, D., Bhattacharya, S., & Nandi, S. (2024). Microbes in the baking industry: Harnessing the power of microbes in baking products. In V. Kothari, S. Ray, & P. Kumar (Eds.), *Microbial products for health and nutrition* (pp. 261–284). Singapore: Springer.

Ebrahim, E. M. (2024). Effects of *Moringa oleifera* leaf and seed powder on the quality and viability of probiotic fermented milk. *Journal of Food and Dairy Sciences, 0*, 97–102. https://doi.org/10.21608/jfds.2024.295997.1159

El-Gammal, R. E., Ghoneim, G. A., & ElShehawy, S. M. (2016). Effect of moringa leaves powder (*Moringa oleifera*) on some chemical and physical properties of pan bread. *Journal of Food and Dairy Sciences, 7*, 307–314. https://doi.org/10.21608/jfds.2016.46005

Ercolini, D., Pontonio, E., De Filippis, F., Minervini, F., La Storia, A., Gobbetti, M., & Di Cagno, R. (2013). Microbial ecology dynamics during rye and wheat sourdough preparation. *Applied and Environmental Microbiology, 79*, 7827–7836. https://doi.org/10.1128/AEM.02955-13186

Fabre, C. E., Blanc, R. J., & Gorna, G. (1998). Phenylethyl alcohol: An aroma profile. *Perfumer and Flavorist, 23*, 43–46.

Fang, L., Wang, W., Dou, Z., Chen, J., Meng, Y., Cai, L., & Li, Y. (2023). Effects of mixed fermentation of different lactic acid bacteria and yeast on phytic acid degradation and flavor compounds in sourdough. *LWT - Food Science and Technology, 174*, Article 114438. https://doi.org/10.1016/j.lwt.2023.114438

Ferreira, T., Gomes, S. M., & Santos, L. (2023). Elevating cereal-based nutrition: *Moringa oleifera* supplemented bread and biscuits. *Antioxidants, 12*, 2069. https://doi.org/10.3390/antiox12122069

Francesca, N., Gaglio, R., Alfonzo, A., Corona, O., Moschetti, G., & Settanni, L. (2019). Characteristics of sourdoughs and baked pizzas as affected by starter culture inoculums. *International Journal of Food Microbiology, 293*, 114–123. https://doi.org/10.1016/j.ijfoodmicro.2019.01.009

Gagliardi, F., Brogi, L., Betti, G., Riccaboni, A., & Tozzi, C. (2025). Italian consumer willingness to pay for agri-food sustainable certification labels: The role of sociodemographic factors. *Sustainability, 17*, 6792. https://doi.org/10.3390/su17156792

Gaglio, R., Alfonzo, A., Barbera, M., Franciosi, E., Francesca, N., Moschetti, G., & Settanni, L. (2020). Persistence of a mixed lactic acid bacterial starter culture during lysine fortification of sourdough breads by addition of pistachio powder. *Food Microbiology, 86*, Article 103349. https://doi.org/10.1016/j.fm.2019.103349

Gaglio, R., Barbera, M., Tesoriere, L., Osimani, A., Busetta, G., Matraxia, M., Attanzio, A., Restivo, I., Aquilanti, L., & Settanni, L. (2021). Sourdough "ciabatta" bread enriched with powdered insects: Physicochemical, microbiological, and simulated intestinal digesta functional properties. *Innovative Food Science and Emerging Technologies, 72*, Article 102755. https://doi.org/10.1016/j.ifset.2021.102755

Gaglio, R., Tesoriere, L., Maggio, A., Viola, E., Attanzio, A., Frazzitta, A., Badalamenti, N., Bruno, M., Franciosi, E., Moschetti, G., Sottile, F., Settanni, L., & Francesca, N. (2023). Reuse of almond by-products: Functionalization of traditional semolina sourdough bread with almond skin. *International Journal of Food Microbiology, 395*, Article 110194. https://doi.org/10.1016/j.ijfoodmicro.2023.110194

Garofalo, G., Pisana, C., Gaglio, R., Barbera, M., Settanni, L., Belvedere, G., Marino, G., Calandra Checco, G. A., Ruta, S., Caccamo, M., Schadt, I., & Caggia, C. (2026). Effect of an anti-*Listeria* whey protein-based edible coating activated with bacteriophage on

quality attributes and consumer perception of sicilian Canestrato fresco cheese. *Foods (Basel, Switzerland), 15*, 689. https://doi.org/10.3390/foods15040689
Giannone, V., Giarnetti, M., Spina, A., Todaro, A., Pecorino, B., Summo, C., & Pasqualone, A. (2018). Physico-chemical properties and sensory profile of durum wheat Dittaino PDO (Protected Designation of Origin) bread and quality of re-milled semolina used for its production. *Food Chemistry, 241*, 242–249. https://doi.org/10.1016/j.foodchem.2017.08.096
Girard, A. L., & Awika, J. M. (2020). Effects of edible plant polyphenols on gluten protein functionality and potential applications of polyphenol–gluten interactions. *Comprehensive Reviews in Food Science and Food Safety, 19*, 2164–2199. https://doi.org/10.1111/1541-4337.12572
Giuberti, G., Bresciani, A., Cervini, M., Frustace, A., & Marti, A. (2021). *Moringa oleifera* L. leaf powder as ingredient in gluten-free biscuits: Nutritional and physicochemical characteristics. *European Food Research and Technology, 247*, 687–694. https://doi.org/10.1007/s00217-020-03656-z
Greco, C., Serio, G., Viola, E., Barbera, M., Mammano, M. M., Orlando, S., Franciosi, E., Ciulla, S., Alfonzo, A., Schicchi, R., Piazzese, D., Gentile, C., Settanni, L., Mannino, G., & Gaglio, R. (2025). Exploring the functional properties of leaves of *Moringa oleifera* lam. Cultivated in Sicily using precision agriculture technologies for potential use as a food ingredient. *Antioxidants, 14*, 799. https://doi.org/10.3390/antiox14070799
Heitmann, M., Zannini, E., & Arendt, E. (2018). Impact of *saccharomyces cerevisiae* metabolites produced during fermentation on bread quality parameters: A review. *Critical Reviews in Food Science and Nutrition, 58*, 1152–1164. https://doi.org/10.1080/10408398.2016.1244153
Hernández-López, I., Alamprese, C., Cappa, C., Prieto-Santiago, V., Abadias, M., & Aguiló-Aguayo, I. (2023). Effect of Spirulina in bread formulated with wheat flours of different alveograph strength. *Foods (Basel, Switzerland), 12*, 3724. https://doi.org/10.3390/foods12203724
Ismael, S., Farahat, A., Ebrahim, Y., Gohari, S., & Ibrahim, G. (2016). Fortification effect of *Moringa oleifera* leaves powder on nutritional and volatile compounds of sweet whey beverage. *Journal of Agricultural, Biological and Environmental Statistics, 3*, 1–11.
ISO. (2003). *Sensory analysis – methodology – general guidance for establishing a sensory profile*. International Organization for Standardization. ISO 13299:2003.
Khlestkin, V. K., Lockachuk, M. N., Savkina, O. A., Kuznetsova, L. I., Pavlovskaya, E. N., & Parakhina, O. I. (2022). Taxonomic structure of bacterial communities in sourdoughs of spontaneous fermentation. *Vavilov Journal of Genetics and Breeding, 26*, 385–393. https://doi.org/10.18699/VJGB-22-47
Koriyama, T., Kurosu, Y., & Hosoya, T. (2025). Enhancing bread quality with steam-treated moringa (*Moringa oleifera*) powder. *Foods (Basel, Switzerland), 14*, 927. https://doi.org/10.3390/foods14060927
Li, R., & Sun, Y. (2019). Effects of honey variety and non-*saccharomyces cerevisiae* on the flavor volatiles of mead. *Journal of The American Society of Brewing Chemists, 77*, 40–53. https://doi.org/10.1080/03610470.2018.1546072
Li, X., Shi, C., Wang, S., Wang, S., Wang, X., & Lu, X. (2023). Uncovering the effect of *Moringa oleifera* Lam. Leaf addition to Fuzhuan Brick tea on sensory properties, volatile profiles and anti-obesity activity. *Food & Function, 14*, 2404–2415. https://doi.org/10.1039/D2FO03531F
Liu, Y., Chin, F. W. L., Sun, J., Liu, S. Q., & Lu, Y. (2024). The impacts of different roasting degrees on aroma compounds profiles of moringa seed kernels and its oil. *Food Bioscience, 59*, Article 104184. https://doi.org/10.1016/j.fbio.2024.104184
Logares, R., Audic, S., Bass, D., Bittner, L., Boutte, C., Christen, R., Claverie, J. M., Decelle, J., Dolan, J. R., Dunthorn, M., Edvardsen, B., Gobet, A., Kooistra, W. H. C. F., Mahé, •. F., Not, F., Ogata, H., Pawlowski, J., Pernice1, M. C., Romac, S., Shalchian-Tabrizi, K., Simon, N., Stoeck, T., Santini, S., Siano, R., Wincker, P., Zingone, A., Richards, T. A., de Vargas, C., & Massana, R. (2014). Patterns of rare and abundant marine microbial eukaryotes. *Current Biology, 24*, 813–821. https://doi.org/10.1016/j.cub.2014.02.050
Maidana, S. D., Finch, S., Garro, M., Savoy, G., Gänzle, M., & Vignolo, G. (2020). Development of gluten-free breads started with chia and flaxseed sourdoughs fermented by selected lactic acid bacteria. *LWT-Food Science and Technology, 125*, Article 109189. https://doi.org/10.1016/j.lwt.2020.109189
Mannino, G., Cirlincione, F., Gaglio, R., Franciosi, E., Francesca, N., Moschetti, G., Asteggiano, A., Medana, C., Gentile, C., & Settanni, L. (2022a). Preliminary investigation of biogenic amines in type I sourdoughs produced at home and bakery level. *Toxins, 14*, 293. https://doi.org/10.3390/toxins14050293
Mannino, G., Serio, G., Asteggiano, A., Gatti, N., Bertea, C. M., Medana, C., & Gentile, C. (2022b). Bioactive compounds and antioxidant properties with involved mechanisms of *Eugenia involucrata* DC fruits. *Antioxidants, 11*, 1769. https://doi.org/10.3390/antiox11091769
Mannino, G., Serio, G., Bertea, C. M., Chiarelli, R., Lauria, A., & Gentile, C. (2022c). Phytochemical profile and antioxidant properties of the edible and non-edible portions of black sapote (*Diospyros digyna* Jacq.). *Food Chemistry, 380*, Article 132137. https://doi.org/10.1016/j.foodchem.2022.132137
Martínez-Anaya, M. A. (1996). Enzymes and bread flavor. *Journal of Agricultural and Food Chemistry, 44*, 2469–2480. https://doi.org/10.1021/jf960020d
Martins, Z. E., Erben, M., Gallardo, A. E., Silva, R., Barbosa, I., Pinho, O., & Ferreira, I. M. (2015). Effect of spent yeast fortification on physical parameters, volatiles and sensorial characteristics of home-made bread. *International Journal of Food Science & Technology, 50*, 1855–1863. https://doi.org/10.1111/ijfs.12818
Messina, C. M., Gaglio, R., Morghese, M., Tolone, M., Arena, R., Moschetti, G., Santulli, A., Francesca, N., & Settanni, L. (2019). Microbiological profile and bioactive properties of insect powders used in food and feed formulations. *Foods (Basel, Switzerland), 8*, 400. https://doi.org/10.3390/foods8090400
Minervini, F., Di Cagno, R., Lattanzi, A., De Angelis, M., Antonielli, L., Cardinali, G., Cappelle, S., & Gobbetti, M. (2012). Lactic acid bacterium and yeast microbiotas of 19 sourdoughs used for traditional/typical Italian breads: Interactions between ingredients and microbial species diversity. *Applied and Environmental Microbiology Journal, 78*, 1251–1264. https://doi.org/10.1128/AEM.07721-11
Miolla, R., Ottomano Palmisano, G., Roma, R., Caponio, F., Difonzo, G., & De Boni, A. (2023). Functional foods acceptability: A consumers' survey on bread enriched with oenological by-products. *Foods (Basel, Switzerland), 12*, 2014. https://doi.org/10.3390/foods12102014
Mukunzi, D., Nsor-Atindana, J., Xiaoming, Z., Gahungu, A., Karangwa, E., Mukamurezi, G., Al-Domi, H., Princewill-Ogbonna, I. L., Ogbonna, P. C., & Arief, N. J. (2011). Comparison of volatile profile of *Moringa oleifera* leaves from Rwanda and China using HS-SPME. *Pakistan Journal of Nutrition, 10*, 602–608. https://doi.org/10.3923/pjn.2011.602.608
Muturi, E. J., Njoroge, T. M., Dunlap, C., & Cáceres, C. E. (2021). Blood meal source and mixed blood-feeding influence gut bacterial community composition in *Aedes aegypti*. *Parasites & Vectors, 14*, 83. https://doi.org/10.1186/s13071-021-04579-8
Nazzaro, C., Stanco, M., Uliano, A., & Marotta, G. (2024). Consumers' acceptance and willingness to pay for enriched foods: Evidence from a choice experiment in Italy. *Future Foods, 10*, Article 100405. https://doi.org/10.1016/j.fufo.2024.100405
Nicoletti, I., Martini, D., De Rossi, A., Taddei, F., D'Egidio, M. G., & Corradini, D. (2013). Identification and quantification of soluble free, soluble conjugated, and insoluble bound phenolic acids in durum wheat (*Triticum turgidum* L. var. *durum*) and derived products by RP-HPLC on a semimicro separation scale. *Journal of Agricultural and Food Chemistry, 61*, 11800–11807. https://doi.org/10.1021/jf403568c
Ogunsina, B. S., Radha, C., & Indrani, D. (2011). Quality characteristics of bread and cookies enriched with debittered *Moringa oleifera* seed flour. *International Journal of Food Science and Nutrition, 62*, 185–194. https://doi.org/10.3109/09637486.2010.526928
Onacik-Gür, S., Szafrańska, A., Roszko, M., & Stępniewska, S. (2022). Interaction of dough preparation method, green tea extract and baking temperature on the quality of rye bread and acrylamide content. *LWT - Food Science and Technology, 154*, Article 112759. https://doi.org/10.1016/j.lwt.2021.112759
Pérez-Alvarado, O., Zepeda-Hernández, A., Garcia-Amezquita, L. E., Requena, T., Vinderola, G., & García-Cayuela, T. (2022). Role of lactic acid bacteria and yeasts in sourdough fermentation during breadmaking: Evaluation of postbiotic-like components and health benefits. *Frontiers in Microbiology, 13*, Article 969460. https://doi.org/10.3389/fmicb.2022.969460
Pino, A., Russo, N., Solieri, L., Sola, L., Caggia, C., & Randazzo, C. L. (2022). Microbial consortia involved in traditional Sicilian sourdough: Characterization of lactic acid bacteria and yeast populations. *Microorganisms, 10*, 283. https://doi.org/10.3390/microorganisms10020283
Purlis, E., & Salvadori, V. O. (2007). Bread browning kinetics during baking. *Journal of Food Engineering, 80*, 1107–1115. https://doi.org/10.1016/j.jfoodeng.2006.09.007
Reffai, Y. M., & Fechtali, T. (2025). A critical review on the role of lactic acid bacteria in sourdough nutritional quality: Mechanisms, potential, and challenges. *Applied Microbiology, 5*, 74. https://doi.org/10.3390/applmicrobiol5030074
Restivo, I., Sciurba, L., Indelicato, S., Allegra, M., Lino, C., Garofalo, G., Bongiorno, D., Davino, S., Avellone, G., Settanni, L., Tesoriere, L., & Gaglio, R. (2025). Repurposing olive oil mill wastewater into a valuable ingredient for functional bread production. *Foods (Basel, Switzerland), 14*, 1945. https://doi.org/10.3390/foods14111945
Rodrigues, J. F., Soares, C., Moreira, M. M., Ramalhosa, M. J., Duarte, N. F., Delerue-Matos, C., & Grosso, C. (2023). *Moringa oleifera* Lam. Commercial beverages: A multifaceted investigation of consumer perceptions, sensory analysis, and bioactive properties. *Foods (Basel, Switzerland), 12*, 2253. https://doi.org/10.3390/foods12112253
Saddique, M. S., Sultan, M. T., Iqbal, S. Z., Bosch, C., Akhtar, S., Raza, H., Tariq, N., & Ahmed, W. (2024). Physicochemical, rheological and sensory evaluation of herbal bread containing turmeric, ginger, and black cumin powder. *Foods (Basel, Switzerland), 13*, 555. https://doi.org/10.3390/foods13040555
Sajdakowska, M., Gniotek, D., & Gębski, J. (2026). Selected consumer behaviours in the bread market: Does dietary fibre labelling influence consumer decisions? A preliminary study. *Nutrients, 18*, 587. https://doi.org/10.3390/nu18040587
Sánchez-Ortiz, L. K., Sánchez-Quezada, V., Gaytán-Martínez, M., Cuellar-Nuñez, M. L., & Loarca-Piña, G. (2024). Influence in physicochemical, nutritional, and antioxidant properties by addition *Moringa oleifera* leaves in *Avena sativa* bread. *Food Chemistry, 460*, Article 140743. https://doi.org/10.1016/j.foodchem.2024.140743
Sardabi, F., Azizi, M. H., Gavlighi, H. A., & Rashidinejad, A. (2021). The effect of *Moringa peregrina* seed husk on the in vitro starch digestibility, microstructure, and quality of white wheat bread. *LWT - Food Science and Technology, 136*, Article 110332. https://doi.org/10.1016/j.lwt.2020.110332
Schefer, S., Oest, M., & Rohn, S. (2021). Interactions between phenolic acids, proteins, and carbohydrates—Influence on dough and bread properties. *Foods (Basel, Switzerland), 10*, 2798. https://doi.org/10.3390/foods10112798
Settanni, L., Barbaccia, P., Bonanno, A., Ponte, M., Di Gerlando, R., Franciosi, E., Di Grigoli, A., & Gaglio, R. (2020). Evolution of indigenous starter microorganisms and physicochemical parameters in spontaneously fermented beef, horse, wild boar and pork salamis produced under controlled conditions. *Food Microbiology, 87*, Article 103385. https://doi.org/10.1016/j.fm.2019.103385
Shunmugapriya, K., Vennila, P., Kanchana, S., & Vellaikumar, S. (2020). Identification of volatile flavour compounds in *Moringa oleifera* powder and soup mixes. *Chemical Science Review and Letters, 9*, 162–170. https://doi.org/10.37273/chesci.CS20510110
Siepmann, F. B., de Almeida, B. S., Waszczynskyj, N., & Spier, M. R. (2019). Influence of temperature and of starter culture on biochemical characteristics and the aromatic compounds evolution on type II sourdough and wheat bread. *LWT - Food Science and Technology, 108*, 199–206. https://doi.org/10.1016/j.lwt.2019.03.065

Spicher, G., & Rabe, E. (1983). The microflora of sourdough: XVI. Communication: The influence of the dough yield on the production of lactate and acetate in sourdough, started with lactic acid bacteria. *Zeitschrift für Lebensmittel-Untersuchung und Forschung, 176*, 190–195.

Suo, B., Chen, X., & Wang, Y. (2021). Recent research advances of lactic acid bacteria in sourdough: Origin, diversity, and function. *Current Opinion in Food Science, 37*, 66–75. https://doi.org/10.1016/j.cofs.2020.09.007

Świąder, K., & Marczewska, M. (2021). Trends of using sensory evaluation in new product development in the food industry in countries that belong to the EIT regional innovation scheme. *Foods (Basel, Switzerland), 10*, 446. https://doi.org/10.3390/foods10020446

Taglieri, I., Macaluso, M., Bianchi, A., Sanmartin, C., Quartacci, M. F., Zinnai, A., & Venturi, F. (2021). Overcoming bread quality decay concerns: Main issues for bread shelf life as a function of biological leavening agents and different extra ingredients used in formulation. A review. *Journal of the Science of Food and Agriculture, 101*, 1732–1743. https://doi.org/10.1002/jsfa.10816

Teclegeorgish, Z. W., Aphane, Y. M., Mokgalaka, N. S., Steenkamp, P., & Tembu, V. J. (2021). Nutrients, secondary metabolites and anti-oxidant activity of *Moringa oleifera* leaves and Moringa-based commercial products. *South African Journal of Botany, 142*, 409–420. https://doi.org/10.1016/j.sajb.2021.07.008

Teng, R., Ao, C., Huang, H., Shi, D., Mao, Y., Zheng, X., & Zhao, Y. (2024). Research of processing technology of Longjing tea with 'Baiye 1' based on non-targeted aroma metabolomics. *Foods (Basel, Switzerland), 13*, 1338. https://doi.org/10.3390/foods13091338

Testa, R., Schifani, G., & Migliore, G. (2021). Understanding consumers' convenience orientation: An exploratory study of fresh-cut fruit in Italy. *Sustainability, 13*, 1027. https://doi.org/10.3390/su13031027

Tolve, R., Simonato, B., Rainero, G., Bianchi, F., Rizzi, C., Cervini, M., & Giuberti, G. (2021). Wheat bread fortification by grape pomace powder: Nutritional, technological, antioxidant, and sensory properties. *Foods (Basel, Switzerland), 10*, 75. https://doi.org/10.3390/foods10010075

Verbeke, W. (2015). Profiling consumers who are ready to adopt insects as a meat substitute in a western society. *Food Quality and Preference, 39*, 147–155. https://doi.org/10.1016/j.foodqual.2014.07.008

Viola, E., Garofalo, G., Busetta, G., Supper, M., Alfonzo, A., Tolone, M., Francesca, N., Moschetti, G., Sottile, F., Gaglio, R., & Settanni, L. (2024). Selection of lactic acid bacteria from home-made sourdoughs for resistance to the main almond skin polyphenols. *Journal of Agriculture and Food Research, 15*, Article 100951. https://doi.org/10.1016/j.jafr.2023.100951

Wu, W., Zhang, A., Van Klinken, R. D., Schrobback, P., & Muller, J. M. (2021). Consumer trust in food and the food system: A critical review. *Foods (Basel, Switzerland), 10*, 2490. https://doi.org/10.3390/foods10102490

Zhou, Y., Dhital, S., Zhao, C., Ye, F., Chen, J., & Zhao, G. (2021). Dietary fiber-gluten protein interaction in wheat flour dough: Analysis, consequences and proposed mechanisms. *Food Hydrocolloids, 111*, Article 106203. https://doi.org/10.1016/j.foodhyd.2020.106203