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A functional food perspective on sweet bakery products: effect of *Moringa oleifera* leaves on fermentation, bioactivity, product quality, and sensory attributes of sourdough brioche

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ABSTRACT

This study evaluated the enrichment of sourdough brioches with *Moringa oleifera* leaf powder (MLP) at inclusion levels of 5% and 10% (w/w) as a functional ingredient. Brioches were produced using a multi-strain sourdough starter and characterized through microbiological, physicochemical, functional, volatile organic compounds (VOCs), and sensory analyses. MLP addition did not affect the fermentation process, as evidenced by a comparable pH decrease from 5.6 to 4.9 after 2 h of fermentation and a final microbiota dominated by *Lactobacillus* (>90% relative abundance). Functional properties were markedly improved by MLP, with total polyphenol content in doughs rising from ca. 29 mg GAE/100 g fresh weight (FW) in the control trial to ca. 211 mg GAE/100 g FW in 10% MLP dough. However, antioxidant activity reached only approximately 30% of the theoretical values, likely due to interactions with the dough matrix. Total polyphenols increased approximately three- and six-fold in 5% and 10% MLP brioche formulations, respectively, while ferric reducing antioxidant power exceeded theoretical expectations. The addition of MLP affected quality attributes, leading to a reduction in specific volume from 3.31 to 2.59 cm^3/g and an increase in crumb firmness of up to fourfold. VOC analysis revealed the presence of plant-derived terpenes and esters due to MLP. Sensory evaluation identified the 5% MLP brioche as the optimal formulation, representing the best balance between enhanced functional properties and consumer acceptability. Overall, these findings support the potential of MLP as a valuable ingredient for the development of functional sweet leavened bakery products with enhanced bioactive properties.

1. Introduction

The global bakery sector represents one of the most dynamic branches of the food industry, encompassing a wide variety of products ranging from staple foods, such as bread, to sweet baked goods including cakes, muffins, cookies, pastries, and brioches. In Europe and, particularly, in Italy sweet leavened bakery products hold a central position in daily dietary habits. Among these, brioches are especially appreciated for their soft texture, rich aroma, and suitability for breakfast or as a snack (Garofalo et al., 2024). Their traditional preparation is typically based on sourdough fermentation, a complex ecosystem of lactic acid bacteria (LAB) and yeasts developing in the mixture of flour and water. The metabolic activities of these microorganisms are responsible for the development of characteristic flavour profiles, improved digestibility, and extended shelf life. Although commercial baker's yeast is often added to enhance leavening performance, particularly in high-fat doughs, sourdough remains a key technological driver in sweet leavened bakery products due to its ability to enhance both nutritional and

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sensory quality (Islam & Islam, 2024; Palomba et al., 2011).

In recent years, increasing scientific and industrial interest has been directed toward the functionalization of bakery products through the incorporation of agricultural plant-derived materials rich in bioactive compounds. Among emerging plant-based ingredients with promising technological and nutritional potential, *Moringa oleifera* leaf powder has gained considerable attention. *Moringa* leaves are widely recognized as a rich source of proteins, vitamins, minerals, essential amino acids, and diverse antioxidant phytochemicals, including polyphenols, flavonoids, and carotenoids (Milla et al., 2021).

From a functional perspective, the antioxidant compounds naturally present in *Moringa* leaves contribute to the mitigation of oxidative stress and may offer preventive benefits in relation to metabolic disorders and chronic diseases (Trigo et al., 2023). These potential health-promoting effects are primarily attributed to the ability of phenolic compounds to scavenge reactive oxygen species and modulate key metabolic pathways, including lipid and carbohydrate metabolism (Mhawish & Komarnytsky, 2025). In this context, MLP represents a strategic ingredient for improving the nutritional and functional value of widely consumed sweet bakery products, such as brioches.

Although several studies have explored the incorporation of *M. oleifera* leaf powder into different bakery matrices (Chinchilla et al., 2020; Kumar, 2025), the literature still lacks a comprehensive and multidisciplinary approach. In cookie formulations, MLP supplementation between 1.5% and 5% has been associated with increased bioactive compounds and antioxidant capacity, and mineral enrichment, while maintaining acceptable sensory properties at low-to-moderate concentrations (Nuzzo et al., 2021). Indeed, they demonstrated that MLP-enriched cookies retained significant levels of phenolic compounds after baking and exhibited improved antioxidant functionality together with good dough processability and consumer acceptance up to selected fortification levels. An integrated framework that integrates physicochemical characterization, detailed aromatic profiling through volatile organic compounds (VOCs) analysis, antioxidant and bioactive compound identification, and in-depth microbiological evaluations supported by both culture-dependent methods and next-generation sequencing, is essential to fully elucidate the role of *Moringa* supplementation on the technological performance, sensory attributes, metabolic pathways, and microbial ecology in sweet leavened bakery products. Furthermore, the use of Sicilian-grown *Moringa*, adapted to local pedoclimatic conditions, may represent a sustainable strategy to support disadvantaged rural areas by promoting crop diversification and resilience in challenging environments (Conte et al., 2024; Mashamaite et al., 2024; Peres et al., 2023). Accordingly, the aim of the present study was to evaluate the effects of fortifying a traditional sweet leavened bakery product, specifically a brioche-type baked good, with powdered *M. oleifera* leaves obtained from dried plant material harvested in the rural area of Grotte (Sicily, Italy). The study focused on: (i) assessing the physicochemical and sensory effects of *Moringa* incorporation; (ii) characterizing changes in antioxidant capacity and bioactive compound content; (iii) evaluating the technological feasibility of using *Moringa* powder as a functional ingredient in sweet leavened bakery matrices; (iv) profiling volatile organic compounds (VOCs) to investigate the influence of *Moringa* enrichment on the aroma and overall flavour chemistry; and (v) monitoring sourdough fermentation dynamics through culture-dependent methods combined with next-generation sequencing (NGS) to elucidate microbial succession and community shifts induced by *Moringa* supplementation.

2. Materials and methods

2.1. Strains and sourdough propagation and powder production

In order to formulate a multi-strain starter culture, lactic acid bacteria (LAB) strains of sourdough origin were selected from the culture collection of the Department of Agricultural, Food and Forest Sciences (SAAF), University of Palermo (Italy), based on their technological performance and complementary metabolic traits, as previously described by Alfonzo et al. (2013). The selected strains included *Fructilactobacillus sanfranciscensis* RC-UNIPA SAAFM01100, *Lactiplantibacillus pentosus* RC-UNIPA SAAFM01112, *Lactiplantibacillus plantarum* RC-UNIPA SAAFM01108, *Lentilactobacillus diolivorans* RC-UNIPA SAAFM01099, and *Levilactobacillus brevis* RC-UNIPA SAAFM01101. These species were chosen for their complementary functional roles within sourdough ecosystems. In particular, *F. sanfranciscensis* is a key species responsible for acidification and flavor development (Yang et al., 2024), whereas *Lp. plantarum* and *Lp. pentosus* contribute to rapid acidification and adaptability to different environmental conditions (Martorana et al., 2018; Reffai & Fechtali, 2025). Additionally, *Lv. brevis* and *Lnt. diolivorans* are heterofermentative LAB involved in the production of aroma compounds and improvement of dough characteristics (Yılmaz et al., 2025). The strains were reactivated from glycerol stocks at −80 °C in modified de Man Rogosa and Sharpe (mMRS) medium, prepared according to Corsetti et al. (2008), and sub-cultured twice by overnight incubation at 30 °C. Before sourdough preparation, each LAB strain was individually inoculated and cultivated in sterile semolina extract (SSE) broth, a medium supporting LAB growth above 10^9 CFU/mL (Alfonzo et al., 2016). After three successive subculturing steps, the strains were combined by pooling equal volumes of each culture to obtain the multi-strain starter, thus ensuring a balanced contribution of each strain. Subsequently, a 500 g dough was prepared with a dough yield (DY = dough weight/semolina weight × 100) of 160, using 312.5 g of semolina and 187.5 mL of the mixed LAB suspension. The inoculum was adjusted by diluting the mixed SSE cultures in sterile tap water to achieve a final LAB density of approximately 10^6-10^7 CFU/g of dough (Xu et al., 2019). The dough was fermented at 28 °C for 16 h and subjected to daily back-slopping for 7 days to obtain a mature sourdough suitable for bakery production, following the procedure reported by Corona et al. (2016). The fermentation temperature (28 °C) was selected as an optimal compromise for the growth and metabolic activity of mesophilic LAB typically associated with sourdough, allowing stable fermentation and balanced microbial interactions (Corona et al., 2016; Corsetti et al., 2008). In addition, *Moringa* leaves powder (MLP) was prepared as previously reported by Greco et al. (2025). The moisture content of the powder was determined by gravimetric analysis by drying the sample at 105 °C until constant mass was achieved, defined as two consecutive weighing showing no significant difference, with weighing intervals of 20 min (AOAC International, 2023).

2.2. Brioches dough preparation and baking

The production of sourdough brioches was designed according to three experimental formulations: CTR, control trial without MLP addition; 5-MLP, containing 5% (w/w) MLP on the weight of flour; and 10-MLP, containing 10% (w/w) MLP on the weight of flour. The three productions were carried out at the laboratories of SAAF Department (University of Palermo) using “00” type soft wheat flour (Barilla G. e R. Fratelli S.p.A., Parma, Italy), butter (Granarolo S.p.A., Bologna, Italy), granulated sugar (Eridania Italia S.p.A., Bologna, Italy), powdered milk (Inalpi S.p.A, Cuneo, Italy), salt (Italkali S.p.A., Palermo, Italy), baker’s yeast (Cameo S.p.A., Desenzano del Garda, Italy) tap water, and the sourdough propagated with selected LAB. The complete recipes are reported in Table 1. The wheat flour used is characterized by a proximate composition (per 100 g) of: carbohydrates 72.5 g, proteins 10 g, total fat 1.0-1.5 g, and dietary fibre 2.5 g, thus representing a refined flour with low fibre and fat content, commonly used in bakery products.

Briefly, CTR dough consisted of 240 g of flour, 64 g of sourdough, 12 g of baker’s yeast, 7.2 g of butter, 14.4 g of sugar, 14.8 g of powdered milk, 3.6 g of salt, and 144 mL of water. In the 5-MLP formulation, flour was partially replaced by MLP, resulting in 228 g of flour and 12 g of MLP, while the amount of the other ingredients was kept unchanged. Similarly, in the 10-MLP formulation consisted of 216 g of flour and 24 g

Table 1
Recipes of sourdough brioches.

Sample	Flour (g)	Sourdough (g)	Baker's yeast (g)	Butter (g)	Sugar (g)	Powdered milk (g)	Salt (g)	Water (mL)	*Moringa* leaves powder (g)
CTR	240.00	64.00	12.00	7.20	14.40	14.80	3.60	144.00	0.00
5-MLP	228.00	64.00	12.00	7.20	14.40	14.80	3.60	144.00	12.00
10-MLP	216.00	64.00	12.00	7.20	14.40	14.80	3.60	144.00	24.00

Abbreviations: CTR, control dough; 5-MLP, experimental dough enriched with 5% (w/w) of MLP; 10-MLP, experimental dough enriched with 10% (w/w) of MLP.

of MLP, with the other ingredients kept as per CTR formulation.

The doughs were kneaded using a planetary mixer (model XBM10S, Electrolux Professional, SpA, Pordenone, Italy) equipped with a paddle, operating at speed 2 for 15 min followed by reverse rotation at speed 3 for 5 min. After kneading, the doughs were manually shaped into "Bombolone" brioche (100 g), a classical round and slightly flattened product of Sicilian patisserie (Garofalo et al., 2024) and placed on stainless-steel oven trays. These trays were transferred to a Lbx INCR-070-001 incubator (Labbox Italia s.r.l., Milan, Italy), where the doughs were allowed to leaven for 2 h at 28 °C. Baking was carried out in a ventilated Compact Combi oven (Electrolux, Pordenone, Italy) at 180 °C for 10 min. The production was performed over two consecutive weeks, corresponding to two independent experimental replicates, with each trial conducted in triplicate (three technical repeats per production).

2.3. Monitoring of acidification process

The fermentation process of sourdough and the different dough formulations was monitored immediately after the mixing of the ingredients and after 2 h of fermentation by measuring pH and total titratable acidity (TTA). For pH determination, a pH-meter (XS Instruments, Carpi, Italy) was directly immersed into a 10 g aliquot of sourdough or dough, aseptically collected immediately before analysis. Subsequently, TTA was determined using the same samples used for pH measurement. The samples were transferred into stomacher bags, diluted with 90 mL of distilled water, and homogenized with a stomacher BagMixer® 400 (Interscience, Saint Nom, France) operated at the maximum speed for 2 min. The homogenates were then titrated with 0.1 N NaOH, and the results were expressed as mL of 0.1 N NaOH per 10 g of dough. In addition, the concentrations of (D + L) lactic acid and acetic acid were also determined. These determinations were performed by high performance liquid chromatography (HPLC) as described by Gaglio et al. (2020) on 10 g of dough from each trial.

The microbiological monitoring was performed to determine the levels of the main viable microbial groups of interest throughout the production process, as previously reported by Garofalo et al. (2024). Each sample was firstly homogenized in Ringer's solution (Sigma-Aldrich, St. Louis, USA) to prepare decimal serial dilutions. The analysis targeted total mesophilic microorganisms (TMM), mesophilic LAB rods, and total yeasts. TMM were enumerated on Plate Count Agar (PCA), incubated aerobically at 30 °C for 72 h. Mesophilic LAB rods were detected on mMRS agar, incubated anaerobically using the AnaeroGen AN25 system into hermetically sealed jars. Total yeasts were enumerated on Yeast Peptone Dextrose (YPD) agar supplemented with chloramphenicol (0.1 g/L) and incubated at 28 °C for 48 h. Except sourdough, the other samples (brioche doughs, unprocessed flour, and MLP) were further analysed for *Enterobacteriaceae* on Violet Red Bile Glucose Agar (VRBGA) and for total coliforms on Violet Red Bile Agar (VRBA), both incubated at 37 °C for 24 h. All culture media and the anaerobic generation system were supplied by Oxoid (Basingstoke, UK). Analyses were performed in triplicates and the results were expressed as log Colony Forming Units (CFU)/g.

2.4. Metataxonomic characterization of the microbiota in sourdough brioches

Genomic DNA was extracted using the QIAamp® DNA Investigator Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. DNA yield and quality were assessed as described by Busetta et al. (2024). The hypervariable V3–V4 region of the 16S rRNA gene was amplified using primers 341F and 805R. PCR reactions were performed in a Verity™ 96-Well Thermal Cycler (Applied Biosystems, Foster City, CA, USA) under the following conditions: an initial denaturation at 95 °C for 5 min, followed by 25 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 40 s, with a final extension step at 72 °C for 5 min. Amplicons were verified by agarose gel electrophoresis and purified using Agencourt AMPure XP magnetic beads (Beckman Coulter, Brea, CA, USA) according to the manufacturer's instructions.

Amplicon library preparation, pooled library quality assessment and quantification, and paired-end sequencing on an Illumina MiSeq platform (Illumina, San Diego, CA, USA) were performed at the Sequencing and Genotyping Platform of the Fondazione Edmund Mach (FEM, San Michele all'Adige, Italy). Raw paired-end FASTQ files were demultiplexed using idemp (https://github.com/yhwu/idemp/blob/master/idemp.cpp; last accessed 28 November 2025) and processed through the QIIME2 bioinformatics framework on the Galaxy Europe platform. Sequence data were quality filtered, denoised, merged and screened for chimeras using the DADA2 algorithm, generating amplicon sequence variants (ASVs). To standardize sequencing effort among samples, ASV richness was estimated after rarefaction to 20,000 sequences per sample. Sequencing statistics, including raw read counts, quality-filtered read counts and observed ASV richness for each sample, are reported in Supplementary Table S1. All downstream bioinformatic analyses were performed at FEM.

All FASTQ files generated in this study have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA1458071.

2.5. Qualitative attributes

Following baking, the sourdough brioches were left cooling at room temperature for 30 min prior to quality assessment. Texture properties were initially evaluated by measuring crumb resistance to compression (N/mm^2) using an Instron-5564 texture analyser (Instron Corp., Canton, MA), following the method described by Corsetti et al. (2000). Crumb structure was subsequently analysed in terms of void fraction (percentage of total area occupied by gas cells), cell density (number of cells per cm^2), and mean cell area (mm^2). Crumb slices were scanned with an Epson Perfection 4180 Photo scanner (Seiko Epson Corp., Japan) at 350 dpi resolution. The resulting TIFF images were processed with ImageJ software (National Institutes of Health, Bethesda, MD, USA). Each image was cropped to a 207 × 207 pixel area (corresponding to 15 × 15 mm of crumb), converted to 8-bit grayscale, and then binarized using Otsu's automatic thresholding algorithm.

Additional morphogeometric parameters were also determined. Weight loss (WL) after baking, attributed to moisture evaporation, was calculated according to the equation:

$$WL = [(wd - wb) / wd] \times 100$$

where wd represents the dough weight (g) and wb the bread weight (g),

as reported by Purlis and Salvadori (2007). The volume of the final brioches was measured using a 2 L volumeter designed for bakery products (ErreCi s.r.l., Merate, Italy). To provide a comprehensive assessment of product quality, colour measurements were also carried out on both crust and crumb. The analysis was performed with a Chroma Meter CR-400 (Minolta, Osaka, Japan), based on Hunter colour scale parameters (L*, a*, b*). Three measurements were taken on the crumb of the central slice, while four measurements were recorded on the upper crust surface of each brioche.

2.6. Preparation of the extracts from MLP, doughs and brioches

MLP and samples from the three brioche trials (CTR, 5-MLP, and 10-MLP), including doughs and final brioches, were subjected to analysis. Dough samples were collected at two fermentation stages: the initial step (t0), after ingredient mixing, and at the end of fermentation (t2). For each experimental condition, three aliquots were prepared, accurately weighed, and extracted at room temperature using an ethanol: water solution (70:30, v/v). Extraction was carried out using solid-to-solvent ratios of 1:20 (w/v) for MLP and 1:3 (w/v) for dough and brioches samples.

The extraction protocol included vortex mixing (5 min), sonication (30 min), and incubation for 24 h under constant agitation in the dark. Samples were then centrifuged at 2000 rpm for 10 min at 4 °C. This procedure was repeated three times, and all supernatants were combined and filtered using a 0.45 μm Millex HV membrane filter (Millipore, Billerica, MA). The filtered extracts were aliquoted and stored at 20 °C until further analysis. Following the second extraction, the residual insoluble fraction was subjected to an additional extraction step, and the resulting supernatant (exhausted extract) was retained separately.

2.7. Total polyphenol content (TPC)

The total phenolic content (TPC) of extracts was determined using the Folin–Ciocalteu colorimetric assay. The analysis was conducted according to the method previously described by Mannino et al. (2022), with minor modifications where applicable. TPC values were expressed as mg of gallic acid equivalents (GAE) per 100 g of dried weight (DW) for MLP samples, and per 100 g of fresh weight (FW) for dough and brioches samples. All measurements were performed in triplicate, and results are reported as the mean of three independent experiments.

2.8. Total flavonoids

The total flavonoid content (TFC) of extracts was quantified by the aluminum chloride colorimetric method, based on the formation of flavonoid–aluminum complexes and the nitration of aromatic rings bearing catechol groups (Shraim et al., 2021). The method was slightly adapted for microplate reader analysis as previously reported (Gatti et al., 2025). In brief, 20 μL of sample was mixed with 20 μL of 5% (w/v) sodium nitrite solution and incubated for 5 min at room temperature. Then, 20 μL of 10% (w/v) aluminum chloride was added, and the mixture was allowed to react for 6 min. Subsequently, 40 μL of 4% (w/v) sodium hydroxide was added, and the volume was brought to 200 μL with distilled water. After 15 min of color development, absorbance was recorded at 510 nm.

Quercetin (Sigma-Aldrich, St. Louis, MO, USA) was used as the reference standard to generate an external calibration curve. TFC values were expressed as mmol of rutin equivalents (RE) per 100 g of fresh weight of dried weight (DW) for MLP samples, and per 100 g of fresh weight (FW) for dough and brioches samples. All analyses were performed in triplicate, and results are presented as mean ± standard deviation.

2.9. Antioxidant activity

The antioxidant activity of extracts was evaluated using the DPPH radical scavenging assay and the ferric reducing antioxidant power (FRAP) assay, as previously described (Mannino et al., 2022).

For the DPPH assay, a solution of DPPH radical (10 mM) prepared in ethanol was mixed with appropriately diluted samples and incubated in the dark at room temperature for 40 min. The decrease in absorbance was measured at 517 nm. For the FRAP assay, the reaction mixture, consisting of acetate buffer (300 mM, pH 3.6), TPTZ solution (10 mM), and $FeCl_3$ (20 mM) in an 8:1:1 (v/v/v) ratio, was added to the sample. After incubation at 37 °C for 30 min, absorbance was recorded at 593 nm. Calibration curves for both assays were constructed using Trolox as a standard antioxidant. Results were expressed as mmol of Trolox equivalents (TE) per 100 g of fresh weight (FW) for dough and bread samples, and per 100 g of dry weight (DW) for MLP. All measurements were performed in triplicate, and results are reported as mean ± standard deviation.

2.10. Calculation of theoretical values for functional parameters

To evaluate the effects of fermentation and baking on the functional properties of the fortified products, theoretical values of TPC, TFC, DPPH, and FRAP were calculated and compared with the corresponding experimental values.

For dough samples, theoretical values were estimated from the amount of MLP actually present in 100 g of dough and from the experimentally determined functional properties of the MLP. The theoretical value of each parameter was calculated according to the following equation:

$$TV_{dough} = V_{CTR} + \frac{MLP_{actual}}{100} \times V_{MLP}$$

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

For baked brioches, theoretical values were calculated from the values measured in the corresponding fermented dough after 2 h of fermentation and before baking. Assuming complete retention of the measured compounds and antioxidant activities during baking, the theoretical values were corrected only for the concentration effect associated with water loss during thermal processing according to the following equation:

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

where $TV_{brioche}$ is the theoretical value of the baked product, V_{dough} is the value measured in the corresponding fermented dough before baking, and WL is the water loss during baking, expressed as g water lost from 100 g of dough.

Differences between theoretical and experimental values were interpreted as indicators of processing-induced modifications, including degradation of bioactive compounds, changes in their extractability, interactions with matrix components, and/or the formation of newly generated antioxidant-active compounds.

2.11. Volatile organic compounds determination

The volatile organic compounds (VOCs) of CTR and MLP enriched brioches were determined by headspace solid-phase microextraction coupled with gas chromatography–mass spectrometry (HS-SPME-GC/MS). Briefly, 5 g of each sample were transferred into hermetically sealed vials and subjected to headspace extraction using a 30 μm DVB/

CAR/PDMS fiber (divinylbenzene/carboxen/polydimethylsiloxane). The extraction was carried out by exposing the fiber to the sample headspace for 60 min at 25 °C to allow the adsorption of volatile compounds. After extraction, the fiber was inserted into the GC injection port, where thermal desorption of the analytes was performed at 250 °C for 5 min in splitless mode. Separation of volatile compounds was achieved using a DB-624 capillary column (Agilent Technologies; 60 m × 0.25 mm i.d., 1.40 μm film thickness). The oven temperature was initially set at 40 °C and then increased to 230 °C at a rate of 4 °C min^{-1}, followed by a final holding time of 40 min. Mass spectrometric detection was carried out in full-scan mode within a mass range of m/z 40–400, with the transfer line temperature maintained at 230 °C. All samples were analysed in triplicate. Identification of volatile compounds was performed by comparing the obtained mass spectra with those available in the NIST05 mass spectral library. The relative abundance of each compound was expressed as a percentage of the total peak area by normalization of the chromatographic signals.

2.12. Sensory evaluation

The final brioches were evaluated by descriptive sensory analysis carried out in individual booths in accordance with ISO 8589 (2007). The three samples were served at room temperature on black dish and they were presented in a randomised way. The evaluation was performed by a trained panel of 17 assessors (9 women and 8 men), aged between 25 and 65 years, who were specifically trained for the sensory evaluation of brioche products. The samples were identified with three random 2-digit codes and water was provided to cleanse the assessor palates between sample evaluations. Within two separate sessions, one for each independent experiment, judges were asked to evaluate the samples considering three main sensory domains: visual features, olfactory sensations, and taste sensations, following approaches previously described in the literature (Comendador et al., 2012; Martins et al., 2015; Rodrigues et al., 2014).

Visual attributes included crust and crumb colour, crust thickness, porosity, and alveolation, including its regularity. Olfactory evaluation considered odour intensity, typical bread odour, and the presence of any strange odour. Taste and mouthfeel descriptors comprised parameters related to texture and flavour perception, such as crumb elasticity, aroma intensity and bread aroma, possible strange aroma, basic taste perceptions (sweetness, saltiness, acidity, bitterness), as well as astringency, taste persistency, crust crispness, and in-mouth adhesiveness. Before starting the evaluation, a reference standard was supplied, and the panellists were asked to refer to it when assigning scores to each attribute. All attributes were rated using a 9-point hedonic scale (1 = extremely bad; 9 = extremely good). Finally, judges provided an overall assessment for each sample, representing a comprehensive evaluation based on the combined perception of all considered attributes.

2.13. Statistical analysis

In order to discriminate significance differences between samples for all the different analyses performed, a one-way variance analysis (ANOVA) or student t-test were applied. The Tukey's test was applied for multiple mean comparisons (statistical significance $p < 0.05$). All the statistical data were processed through the use of the XLStat software version 7.5.2 for Excel (Addinsoft, New York, USA) or SPSS Statistics 24 (SSPSS, Chicago, IL, USA) evaluating only the effect of MLP enrichment. Pearson correlation analysis was performed to investigate the relationships among sensory attributes, physicochemical parameters (colour and texture), and selected volatile compounds of the analysed samples. Correlation coefficients (r) were calculated using Microsoft Excel (Microsoft Corp., Redmond, WA, USA). The strength and direction of linear relationships between variables were assessed based on Pearson's correlation coefficients, with values close to +1 or −1 indicating strong positive or negative correlations, respectively, and values close to 0 indicating weak or no correlation.

3. Results and discussion

3.1. Sourdough and sweet leavened dough acidification parameters

The results of the physicochemical characterization of the brioches are reported in Table 2. The sourdough starter exhibited a pH of 3.97 ± 0.08 and a total titratable acidity (TTA) of 12.00 ± 0.07 mL NaOH, which is consistent with previous studies reported in literature for sourdough starters (Arora et al., 2021). Indeed, the primary function of sourdough is to act as a microbial inoculum capable of promoting rapid acidification and leavening of the dough (Gobbetti et al., 2008).

Following the incorporation of all ingredients, all dough formulations showed comparable initial pH values (about 5.60), without significant differences among samples. In contrast to previous studies exploring the addition of plant derived powders, where the added doughs usually showed higher pH due to the lower acidity of the added raw materials (Gaglio et al., 2023; Viola et al., 2023), this trend was not observed in the present work. This behaviour is imputable to the pH of *M. oleifera* leaves which is around 6.10 (practical observation). Similarly, also TTA showed comparable values across formulations (ca. 7.30 mL NaOH/10 g). Moreover, the moisture content of the powder was 8.63 ± 0.31%.

After 2 h of fermentation, a duration shorter than that typically employed in sourdough processes, all dough samples exhibited a significant decrease in pH. However, no statistically significant differences were observed among the samples, with pH values of 4.86 (CTR), 4.92 (5-MLP), and 4.93 (10-MLP). A similar pattern was observed for TTA, which did not differ significantly among doughs, yielding values of 8.15 mL NaOH/10 g for CTR, 8.10 mL NaOH/10 g for 10-MLP, and 8.08 mL NaOH/10 g for 5-MLP. These results indicate a uniform acidification process across all doughs. Overall, the incorporation of the powder did not impair dough acidification, as observed by the consistent drop in pH. Indeed, this decrease is commonly regarded as a reliable indicator of successful fermentation, reflecting the accumulation of organic acids produced by LAB (Hernández-Figueroa et al., 2024).

Table 2 also reports the concentrations of lactic acid (D + L isomers), acetic acid, and the fermentation quotient (FQ) for all doughs at 0 h and 2 h of fermentation. At the beginning of fermentation, lactic acid concentrations were similar among samples, with values of 0.71 mg/g in CTR and little lower for 5-MLP and 10-MLP. Also acetic acid concentrations were comparable across formulations (0.11-0.12 mg/g). Correspondingly, FQ ranged from 4.30 in CTR to 3.67 in 10-MLP. After 2 h of fermentation, an increase in organic acid concentration was observed in all doughs. Lactic acid increased to 1.14-1.20 mg/g while acetic acid 0.21-0.23. These increase in both organic acids confirmed the activation of sourdough microbiota and the progression of fermentative metabolism in all dough formulations. The fermentation quotient decreased in all doughs, reaching comparable final values (3.62 for CTR, 3.48 for 5-MLP, and 3.51 for 10-MLP), indicating that *M. oleifera* addition up to 10% (w/w) did not hinder LAB fermentative activity. Moreover, FQ values across all trials fell within the optimal range (1.5-4.0) reported by Spicher and Rabe (1983) for sourdough products.

The results of plate counts are reported in Table 3. Raw materials recorded low or undetectable levels of all analysed microbial groups. Flour showed low levels of TMM, yeasts, and LAB rods (2.71, 3.14, and 3.26 Log CFU/g, respectively), while total coliforms and *Enterobacteriaceae* were not detected. Similarly, *Moringa* powder showed low TMM levels (3.25 Log CFU/g), with the remaining microbial groups below the detection limit, in agreement with previous findings (Greco et al., 2025). The sourdough starter was characterized by TMM at 8.09 Log CFU/g, yeasts at 6.43 Log CFU/g, and mesophilic LAB rods at 8.49 Log CFU/g. These values indicate a well-established microbial community, with a LAB/yeast ratio of about 100:1, typical of active sourdough systems (Dertli et al., 2016).

Table 2
Doughs acidification parameters.

Samples	0 h					2 h				
	pH	TTA (mL NaOH/10 g)	D + L Lactic acid (g/100 g dough)	Acetic acid (g/100 g dough)	FQ	pH	TTA (mL NaOH/10 g)	D + L Lactic acid (g/100 g dough)	Acetic acid (g/100 g dough)	FQ
CTR	5.55	7.38	0.71	0.11	4.30	4.86	8.15	1.14	0.21	3.62
5-MLP	5.62	7.23	0.65	0.11	3.94	4.92	8.10	1.20	0.23	3.48
10-MLP	5.61	7.33	0.66	0.12	3.67	4.93	8.08	1.16	0.22	3.51
p-value	0.846	0.540	0.877	0.924	n.d.	0.837	0.725	0.872	0.908	n.d.
SEM	0.055	0.067	0.054	0.053	n.d.	0.059	0.040	0.053	0.020	n.d.

Results indicate mean values of four determinations (carried out in two technical repeats for two independent experiments). Abbreviations: TTA, total titratable acidity; CTR, control dough; 5-MLP, experimental dough enriched with 5% (w/w) of MLP; 10-MLP, experimental dough enriched with 10% (w/w) of MLP; SEM, standard error of the mean; n.d., not determined.

Table 3
Microbiological plate counts.

Sample	Media					
	PCA (Log CFU/g)		YPD (Log CFU/g)		mMRS (Log CFU/g)	
	t_0	t_2	t_0	t_2	t_0	t_2
CTR	6.29	6.95	6.69	7.43	6.65	7.56
5-MLP	6.36	7.05	6.73	7.41	6.58	7.62
10-MLP	6.32	7.28	6.68	7.37	6.57	7.65
p-value	0.769	0.105	0.989	0.961	0.916	0.150
SEM	0.036	0.138	0.138	0.073	0.073	0.075

Results indicate mean values of six determinations (carried out in three technical repeats for two independent experiments). Abbreviations: PCA, plate count agar; mMRS, modified de Man, Rogosa, and Sharpe agar; YPD, yeast extract peptone dextrose agar; CTR, control dough; 5-MLP, experimental dough enriched with 5% (w/w) of MLP; 10-MLP, experimental dough enriched with 10% (w/w) of MLP; SEM, standard error of the mean.

At beginning of the fermentation process (t0), TMM levels were similar among samples (6.29-6.36 Log CFU/g). At t2, microbial growth was observed in all doughs, reaching 6.95, 7.05, and 7.28 Log CFU/g for CTR, 5-MLP and 10-MLP, respectively, with no significant differences among samples. This trend is consistent with the expected microbial dynamics during dough fermentation, particularly over short fermentation periods (Garofalo et al., 2024). Yeast counts followed a similar pattern, with initial values between 6.68 and 6.73 Log CFU/g, which increased to approximately 7.37-7.43 Log CFU/g after fermentation, without significant differences among formulations. Mesophilic LAB rods also showed comparable counts at t0 (6.57-6.65 Log CFU/g) and a marked increase after fermentation, reaching 7.56-7.65 Log CFU/g across all doughs. These results reflect the active proliferation of LAB despite the limited fermentation time.

Hygienic indicators were also assessed to evaluate the microbiological safety of the doughs, particularly in relation to MLP incorporation. *Enterobacteriaceae* and total coliforms were below the detection limit in all samples, confirming that the addition of MLP did not compromise hygienic quality. The absence of these microorganisms is probably linked to the decrease in pH driven by LAB activity, which is known to inhibit the growth of undesirable bacteria and enhance the safety of fermented products (Viola et al., 2024). Overall, all doughs exhibited comparable microbial evolution, characterized by increased levels of total mesophilic microorganisms, yeasts, and LAB during fermentation, with no significant differences among samples at the corresponding time points. These findings confirm that powder incorporation did not interfere with microbial growth dynamics and that fermentation proceeded efficiently and consistently across all formulations (Gaglio et al., 2023; Garofalo et al., 2024).

3.2. Effect of Moringa addition on the microbial community of fermenting brioche dough

The microbial communities associated with the different doughs were investigated through a NGS approach, which is widely applied to assess the microbial composition and dynamics, including viable but non-cultivable and low-abundance taxa (Settanni et al., 2020). A total of 340,505 reads were obtained, and, after quality filtering, denoising, merging and chimera removal, 216,692 high quality reads were retained, corresponding to 209 amplicon sequence variants (ASVs). Observed ASV richness was estimated after rarefaction to 20,000 sequences per sample. Raw and quality-filtered read counts, together with observed ASV richness for each sample, are reported in Supplementary Table S1. Illumina MiSeq analysis was carried out at t0 and t2 and the results are graphically presented in Fig. 1. Data included only operational taxonomic units (OTUs) with a relative abundance (RA) $\geq$ 0.1%, in agreement with the threshold proposed by Logares et al. (2014).

Overall, sequencing data revealed a relatively simple bacterial community, consisting of a limited number of dominant taxa. At t0, the family *Lactobacillaceae* was as the most abundant across all samples, accounting for approximately 81.68% RA in CTR, 83.02% in 5-MLP, and 82.52% in 10-MLP. After 2 h of fermentation, the dominance of this genus increased further, reaching 92.57%, 94.55%, and 92.56% in CTR, 5-MLP, and 10-MLP, respectively. This trend reflects the well-established dominance of LAB in sourdough ecosystems, where members of the family *Lactobacillaceae* rapidly proliferate and drive acidification (Pino et al., 2022).

Members of the class Alphaproteobacteria were detected in all samples, particularly at t0. This group showed relatively high RAs, with the highest value observed in CTR (18.32%), followed by 5-MLP (16.64%) and 10-MLP (16.36%). At the end of fermentation, a marked decrease in Alphaproteobacteria was observed in all samples. This decline is consistent with the progressive acidification of the dough, which creates unfavorable conditions for many environmental non acid-tolerant microorganisms (Ercolini et al., 2013). This trend appears to be closely linked to the microbial composition of the raw materials used in dough preparation. Previous studies have shown that Alphaproteobacteria represent the dominant microbial group in MLP, accounting for approximately 83.20% RA Greco et al. (2025). In addition, several taxa within this class have been associated with environmental contamination of flours and sourdoughs (Mannino et al., 2022). However, their progressive decline during fermentation indicates that, despite their high initial abundance in the powder, these microorganisms are poorly adapted to the competitive conditions characteristic of sourdough environment (De Vuyst et al., 2023).

Betaproteobacteria and members of the family *Enterobacteriaceae* were detected sporadically and at very low relative abundances. In particular, *Enterobacteriaceae* were observed at t0 in 5-MLP (0.14%) and 10-MLP (0.99%), while after fermentation they persisted only at trace levels in 10-MLP (0.50%). This finding is consistent with the microbiota previously described for MLP, where *Enterobacteriaceae* (4.07%) and

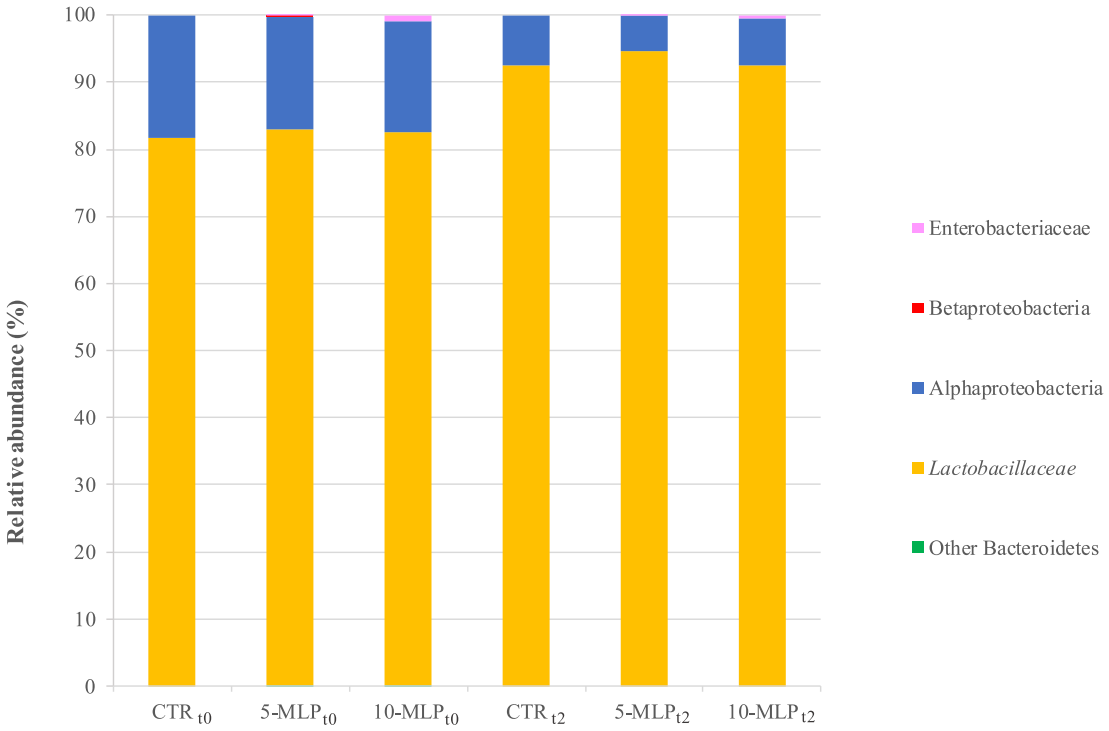

Fig. 1. Relative abundances (%) of bacterial community identified by Illumina technology. Abbreviations: CTR, control dough; 5-MLP, experimental dough enriched with 5% (w/w) of MLP; 10-MLP, experimental dough enriched with 10% (w/w) of MLP.

Betaproteobacteria (1.50%) have been reported as part of its natural, soil-associated microbial community (Greco et al., 2025). Their detection in doughs can therefore be attributed, at least in part, to the microbial load introduced with the raw materials.

The presence of *Enterobacteriaceae* detected by NGS but not by plate counts can reflect residual DNA from non-viable cells, which are not revealed by the classical culture-based methods. During sourdough fermentation, the sharp decrease in pH and the production of antimicrobial metabolites by LAB exert strong selective pressure on the microbial community, resulting in ecological succession. In this process, initially present microorganisms progressively decline, while acid-tolerant lactobacilli become dominant in the final stages of fermentation. The consistently low abundance and reduction of *Enterobacteriaceae* over time thus confirm the selective pressure exerted by fermentation. (De Vuyst et al., 2017; McKenney et al., 2023).

Minor contributions from other taxa, such as Bacteroidetes, were negligible and detected only at trace levels in a few samples at t0, disappearing completely after fermentation. This observation is in line with their previously reported presence in *Moringa* powder (Greco et al., 2025) and further supports the hypothesis that these microorganisms are introduced with the ingredients but are rapidly outcompeted during fermentation.

Overall, these findings are consistent with the typical microbial succession observed in sourdough ecosystem, where LAB rapidly prevail over less adapted microorganisms during the early stages of fermentation (Ercolini et al., 2013). Furthermore, the results highlight that although *Moringa* addition introduces a diverse, predominantly environmental microbial community, the fermentation process effectively reshapes the community, leading to the establishment of a stable ecosystem dominated by LAB.

3.3. Quality evaluation of sweet sourdough brioches

The main quality attributes of sourdough brioches formulated with different percentages of MLP are reported in Table 4. Overall, the partial replacement with MLP led to modifications in several physicochemical and structural properties of the final products. In particular, enriched brioches exhibited a slight but progressive reduction in baking weight loss, which decreased from 13.40% in the CTR to 12.16 and 11.41 in 5-MLP and 10-MLP, respectively. This behaviour may be attributed to the water-binding capacity of plant fibers of *Moringa* leaves, which can limit

Table 4
Quality attributes of sourdough brioches.

Parameter	CTR	5-MLP	10-MLP	p-value	SEM
Weight loss (%)	13.40 a	12.16 b	11.41 c	<0.001	0.365
Specific volume (cm^3/g)	3.31 a	2.70 b	2.59 b	<0.001	0.140
Compression (N/mm^2)	0.01027 b	0.03113 a	0.03914 a	<0.001	0.005
Crust colour					
Lightness (L*)	56.25 a	45.31 b	36.45 c	<0.001	3.595
Red-green (a*)	12.86 a	7.60 b	7.96 b	<0.05	1.114
Yellow-blue (b*)	38.80 a	28.09 b	19.7 c	<0.001	3.393
Crumb colour					
Lightness (L*)	67.16 a	44.91 b	33.81 c	<0.001	6.010
Red-green (a*)	−2.34 b	−2.10 b	−0.54 a	<0.001	0.346
Yellow-blue (b*)	17.20 c	28.09 a	26.55 b	<0.001	2.089
Image analysis					
Void fraction (%)	35.08	35.98	35.15	0.821	0.623
Cell density (n/cm^2)	50.07	51.85	48.15	0.332	1.097
Mean cell aerea (mm^2)	0.70	0.70	0.73	0.770	0.020

Results indicate mean value of six determinations (carried out in three technical repeats for two independent experiments). Data within a row followed by different letters are significantly different according to Tukey's test. Abbreviations: CTR, control dough; 5-MLP, experimental dough enriched with 5% (w/w) of MLP; 10-MLP, experimental dough enriched with 10% (w/w) of MLP; SEM, standard error of the mean.

moisture evaporation during baking. Similar effects in baking loss have been observed in fiber-enriched bakery products, where the hygroscopic nature of dietary fiber restricts water migration (Pasqualone et al., 2020).

MLP addition also influenced the specific volume of the brioches, which decreased progressively from 3.31 cm^3/g in CTR to 2.59 cm^3/g in 10-MLP. Also the reduction in brioche expansion is commonly linked to the presence of dietary fibers, which can hinder gluten network formation and reduce gas retention during fermentation and baking (Rosell & Santos, 2010). Consequently, the enriched samples showed a marked increase in firmness, with compression values rising from 0.01027 N/mm^2 in CTR to 0.03113 and 0.03914 N/mm^2 in 5-MLP and 10-MLP, respectively. This increase in hardness is consistent with previous studies indicating that fiber-rich ingredients create denser and firmer crumb structures through interaction with the dough matrix (Verbeke et al., 2024). However, no rheological measurements were performed on the doughs, representing a limitation of the technological characterization of the samples. Therefore, the interpretation of the observed changes in compression behaviour, specific volume, and crumb structure remains based exclusively on final product properties rather than on direct rheological evidence.

The addition of MLP significantly impacted the colour characteristics of both crust and crumb. Crust lightness (L*) showed a marked decrease, falling from 56.25 in CTR to 45.31 and 36.45 in 5-MLP and 10-MLP, respectively, indicating a progressive darkening of the surface. A similar trend was observed in the crumb, where L* decreased from 67.16 until 33.81 in 10-MLP. Changes were also detected in the a* and b* color coordinates, suggesting shifts in colour hues likely associated with the presence of chlorophyll pigments and phenolic compounds naturally abundant in *Moringa* leaves. The incorporation of plant powders rich in pigments and antioxidants is known to significantly modify the colour profile of baked goods, often resulting in darker and greener tones (Santamaria et al., 2024). In addition, colour development, especially in the crust, may also be influenced by Maillard reactions occurring during baking (Mzoughi et al., 2024).

Image analysis of crumb structure revealed only minor variations among samples. Void fraction values remained relatively stable (around 35%), while cell density ranged between 48.15 and 51.85 $cells/cm^2$ and mean cell area between 0.70 and 0.73 mm^2. These results suggest that the addition of MLP did not markedly disrupt air cell formation during mixing and fermentation, although the reduced specific volume indicates a slightly more compact crumb structure. Similar findings have been reported for bakery products enriched with plant-derived fibers, where crumb morphology is only moderately affected despite alterations in dough rheology (Rubel et al., 2015).

Overall, MLP incorporation significantly influenced key quality attributes of the brioches, particularly specific volume, firmness, and colour, while exerting a limited impact on crumb cellular structure. These results support the feasibility of incorporating plant-based functional powders into sweet baked goods, although their fiber and pigment composition unavoidably affects product quality characteristics.

3.4. Functional attributes of powdered Moringa oleifera leaves

The enrichment of bakery products with plant-derived bioactive compounds represents an effective strategy to enhance their functional value, particularly in widely consumed foods such as bread and sweet baked goods (Dziki et al., 2014; Shah et al., 2016). In this context, *Moringa oleifera* leaves have attracted increasing attention due to their rich phytochemical profile and associated biological activities, including antioxidant, antimicrobial, and health-promoting effects (Chhikara et al., 2021).

In our previous study, the phytochemical profile of *M. oleifera* leaves from plants grown under Sicilian pedoclimatic conditions was extensively characterised by HPLC analysis (Greco et al., 2025), revealing a complex composition dominated by phenolic acids and flavonoids, mainly in glycosylated forms. Among the most abundant compounds, quinic acid and caffeoylquinic acid derivatives were identified together with flavonoids such as quercetin and kaempferol glycosides. The predominance of glycosylated flavonoids is particularly relevant, as these forms are generally more stable under processing conditions and can show improved bioaccessibility compared to aglycones (Manach et al., 2004).

Building on this evidence, in the present study the functional profile of the MLP used as a fortifying ingredient was evaluated through the determination of total polyphenols, flavonoids, and antioxidant activity. This step was essential to define the actual bioactive potential of the material incorporated into the dough and to enable a mechanistic interpretation of its evolution during processing.

MLP exhibited a high polyphenolic content (3.02 ± 0.34 g GAE/100 g DW) together with a significant flavonoid fraction (701 ± 61.8 mg RE/100 g DW). The antioxidant activity, measured through both radical scavenging and reducing capacity assays, confirmed the strong redox-active properties of the extract (DPPH: 5.71 ± 0.27 mmol TE/100 g DW; FRAP: 14.97 ± 1.02 mmol TE/100 g DW). These values are in close agreement with those previously reported for the same plant material obtained under identical extraction conditions (Greco et al., 2025), confirming the robustness of its functional profile.

Overall, these results identify MLP as a highly concentrated and reliable source of bioactive compounds. However, the functional value of the final product cannot be inferred solely from the composition of the ingredient itself, as it depends on interactions with the food matrix and on transformations occurring during fermentation and thermal processing. Monitoring these changes is therefore essential to assess the real effectiveness of the functionalization process.

3.5. Functional properties of MLP-enriched doughs

To evaluate the effectiveness of MLP incorporation as a functionalizing strategy, TPC, TFC, and antioxidant activity were monitored in control (CTR) and enriched doughs (5-MLP and 10-MLP) t0 and after 2 h of fermentation (Fig. 2). The actual amount of MLP incorporated into the dough corresponded to 2.86 and 5.72 g per 100 g of dough for the 5% and 10% formulations, respectively.

As expected, MLP incorporation resulted in a marked and dose-dependent increase in the functional profile of the doughs. At t0, TPC increased from 28.68 ± 1.58 mg GAE/100 g FW in CTR to 105.83 ± 4.33 and 211.00 ± 3.49 mg GAE/100 g FW in 5-MLP and 10-MLP, respectively. TFC increased more moderately, from 46.84 ± 0.98 to 82.10 ± 3.71 and 104.48 ± 5.88 mg QE/100 g FW, confirming that non-flavonoid phenolics represent a substantial fraction of MLP.

The comparison between experimental and theoretical values provides insight into matrix effects. For TPC, values were close to or only slightly higher than theoretical expectations, indicating that most phenolic compounds are readily extractable even before dough formation. The limited increase suggests that hydration and mixing only marginally enhance their accessibility, likely through partial disruption of plant cell structures (Jakobek, 2015).

In contrast, TFC values markedly exceeded theoretical expectations. This behaviour cannot be explained solely by a general increase in extractability, but rather indicates that flavonoids are initially less accessible, possibly due to their stronger association with the plant matrix or their occurrence in bound or compartmentalised forms. Dough hydration and mixing appear to preferentially enhance the release of this fraction, leading to a disproportionate increase compared to total phenolics. Given the short fermentation time (2 h), enzymatic hydrolysis is expected to play a minor role, and the observed effect is mainly attributable to physical matrix disruption. Despite the increased extractable phenolic fraction, antioxidant activity showed a markedly different behaviour. DPPH and FRAP values at t0 corresponded to only about 30% of theoretical values. This apparent discrepancy can be explained by interactions between phenolic compounds and dough

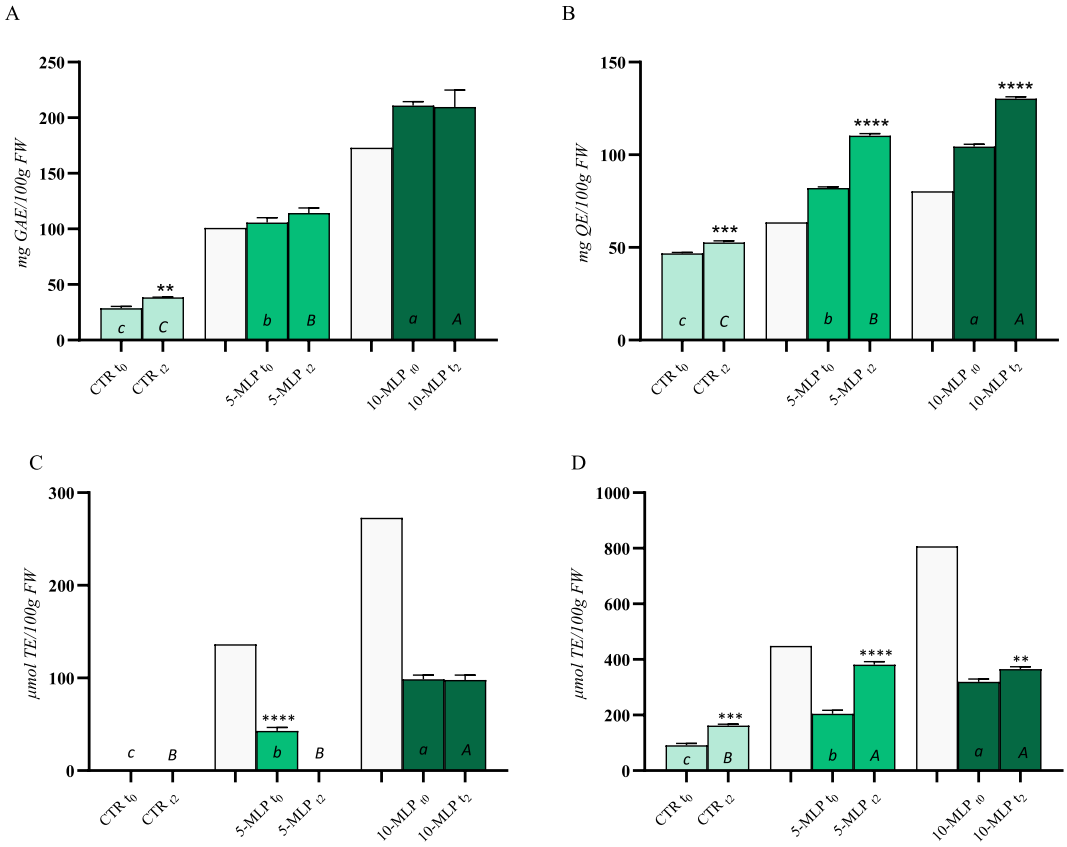

Fig. 2. Total phenolic content (TPC) (Panel A), total flavonoid content (TFC) (Panel B), and antioxidant activity determined by DPPH (Panel C) and FRAP (Panel D) assays in CTR (light green bars) and MLP-fortified doughs containing 5% (green bars) or 10% (dark green bars) MLP. Analyses were performed immediately after MLP addition (t0) and after 2 h of fermentation (t2). White bars represent the corresponding theoretical values calculated from the functional properties of MLP and its actual concentration in the dough formulations. Within each panel, different letters (lowercase for t0 and uppercase for t2) indicates significant differences among the samples as measured by one-way ANOVA followed by Tukey's post hoc test ($P \leq 0.001$), while asterisks show significant differences between t under the same experimental condition (CTR, 5-MLP or 10-MLP), as evaluated by student t-test. Abbreviations: CTR, control dough; 5-MLP, experimental dough enriched with 5% (w/w) of MLP; 10-MLP, experimental dough enriched with 10% (w/w) of MLP.

components.

In this system, the presence of milk proteins is particularly relevant. Polyphenol–protein interactions, driven by hydrogen bonding and hydrophobic forces, can reduce the accessibility and reactivity of phenolics without necessarily affecting their quantification by Folin-based assays (Jakobek, 2015; Ozdal et al., 2013). Additionally, lipids from butter may further limit extractability by promoting partitioning of hydrophobic compounds. These combined effects explain why TPC remains high while antioxidant activity is reduced.

Fermentation introduced moderate but consistent changes. In CTR, increases in TPC and FRAP indicate the release of endogenous compounds and formation of reducing species during yeast metabolism. In enriched doughs, the MLP contribution to TPC remained essentially stable, confirming the relative stability of MLP-derived polyphenols under short fermentation conditions.

TFC increased during fermentation, suggesting improved extractability and partial transformation of conjugated flavonoids. Importantly, this increase does not contradict the stable TPC values, as flavonoids represent only a fraction of total phenolics and their relative changes may not significantly affect the overall TPC.

Antioxidant activity showed a concentration-dependent behaviour. The loss of DPPH activity in 5-MLP and its persistence in 10-MLP can be interpreted as a threshold effect, where lower concentrations of radical scavengers are more susceptible to interaction or degradation. The increase in FRAP in 5-MLP, but not in 10-MLP, may reflect differences in compound accessibility rather than true saturation, suggesting that matrix effects become more limiting at higher enrichment levels. On the other hand, the different behaviour observed for DPPH and FRAP in 5-MLP dough may also reflect the different chemical sensitivities of the two assays. DPPH is more strongly influenced by the presence of compounds able to directly quench free radicals, whereas FRAP reflects the overall reducing capacity of the sample. Consequently, fermentation-induced transformations may reduce the availability of some radical-scavenging molecules while simultaneously generating or releasing compounds with reducing properties, resulting in opposite trends for the two assays.

Overall, these results demonstrate that, in a complex matrix such as brioche dough, the functional profile is governed not only by the amount of added bioactive compounds but also by their accessibility, which is strongly influenced by protein and lipid interactions.

3.6. Functional properties of MLP-enriched brioches

To evaluate the impact of baking, TPC, TFC, and antioxidant activity were determined in control and enriched brioches and compared with theoretical values calculated considering water loss (Fig. 3). MLP incorporation led to a strong increase in functional properties, with TPC values increasing approximately threefold (5-MLP) and sixfold (10-MLP) compared to control, and TFC increasing 2.5- and 3.6-fold, respectively. Experimental TPC values corresponded to 75–80% of theoretical values, indicating partial degradation and/or transformation of phenolic compounds during baking. This behaviour is consistent with

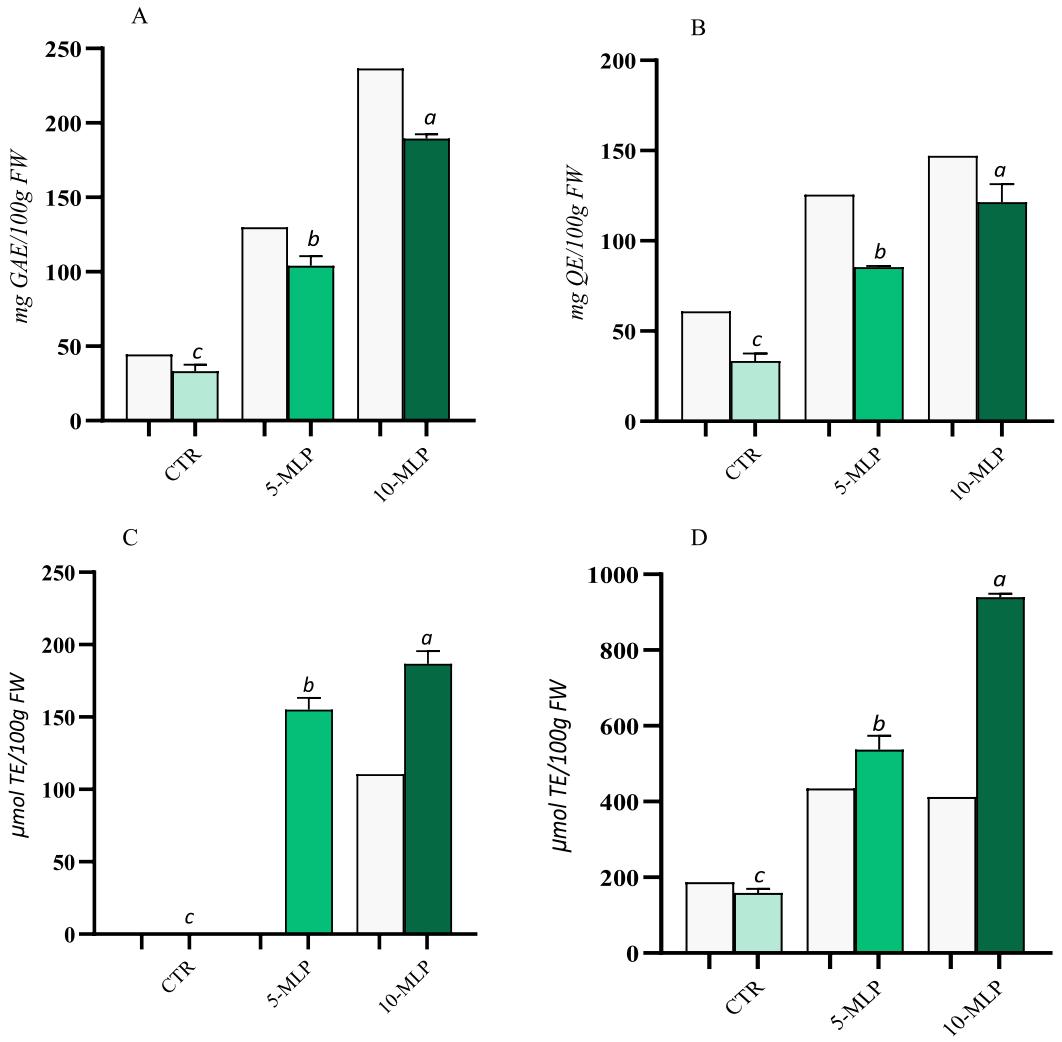

Fig. 3. Total phenolic content (TPC) (Panel A), Total Flavonoid content (TFC) (Panel B), and Antioxidant activity by DPPH (Panel C) and FRAP (Panel D) assays of CTR (light green bars) and MLP-fortified brioches with 5% (green bars) or 10% (dark green bars) of MLP as functionalizing agent. Within each panel, different lowercase letters indicate significant differences among the samples, as measured by one-way ANOVA followed by Tukey's post hoc test ($P \leq 0.001$). Abbreviations: CTR, control brioche; 5-MLP, experimental brioche enriched with 5% (w/w) of MLP; 10-MLP, experimental brioche enriched with 10% (w/w) of MLP.

the known thermal sensitivity of some phenolics, although the relatively mild baking conditions (180 °C for 10 min) limit these losses.

A different behaviour was observed for flavonoids. In control brioches, TFC was approximately 50% of theoretical values, confirming the thermal sensitivity of endogenous flour flavonoids. In enriched samples, TFC values were closer to theoretical ones, suggesting higher stability of MLP-derived flavonoids, likely related to their glycosylated forms. The most striking effect was observed for antioxidant activity. FRAP values in enriched brioches exceeded theoretical expectations, indicating the formation of additional reducing compounds during baking. This does not contradict the partial loss of native phenolics, as newly formed compounds can contribute significantly to reducing capacity.

In this matrix, the presence of sucrose, milk proteins, and lipids promotes Maillard reactions and caramelization. The hydrolysis of sucrose generates reducing sugars, which react with amino groups from proteins to form melanoidins, compounds known to exhibit strong reducing activity and to contribute to antioxidant measurements (Delgado-Andrade, 2014). These compounds can also react in Folin assays, potentially contributing to TPC values. FRAP reflects the overall reducing power of the sample rather than the contribution of phenolic compounds alone; therefore, the formation of highly reducing Maillard reaction products may offset the loss of native phenolics and result in FRAP values exceeding theoretical expectations. In contrast, DPPH activity showed a limited and non-linear increase, with similar values in 5-MLP and 10-MLP. This suggests that radical scavenging compounds are more susceptible to degradation or interaction with the matrix during baking, while Maillard reaction products contribute more strongly to reducing power than to radical scavenging activity. The appearance of DPPH activity in 5-MLP after baking, despite its absence in the corresponding dough, can be explained by the formation or release of new antioxidant compounds during thermal processing, rather than by the preservation of native ones.

Overall, these results demonstrate that the functional properties of enriched brioches result from a balance between degradation of native bioactive compounds, matrix interactions that limit their accessibility, and the formation of new antioxidant species during baking. In particular, Maillard reaction products play a key role in determining the final antioxidant capacity, especially in terms of reducing power.

3.7. Volatile organic compounds determination

The volatile profile of *Moringa* powder revealed a total of 23

compounds (Fig. 4) distributed among seven chemical classes. Terpenes represented the dominant fraction, with Geranylgeraniol being the most abundant, at 10.88%, followed by carbonyl compounds (Acetophenone and 3-Hexen-2-one at 6.35% and 6.08%, respectively), alcohols (Phenethyl alcohol at 14.33%), and esters (Methyl valerate at 5.04%), whereas acids and hydrocarbons were detected at lower RA. This volatile fingerprint is in agreement with previous reports on the aromatic composition of *Moringa* leaves (Balogun et al., 2017; Greco et al., 2025; Ismael et al., 2016; Shunmugapriya et al., 2020).

The results of headspace SPME-GC/MS analysis of brioches are reported in Table 5. CTR brioches showed a broad range of volatile compounds belonging to different chemical classes. Carbonyl compounds (31.44%) and alcohols (30.41%) represented the predominant fractions, followed by esters (15.78%), acids (14.98%), and furans (7.30%). Overall, the volatile profile of CTR brioches reflects the complexity of sourdough fermentation and baking processes, highlighting the combined effect of microbial metabolism and thermal reactions occurring during baking (Pétel et al., 2017; Pico et al., 2017).

Among alcohols, compounds such as phenethyl alcohol and isoamyl alcohol, typical metabolites of yeast activity, were identified as key contributors to bread aroma (Martínez-Anaya, 1996). These compounds are commonly associated with fermentation-derived sensory notes in sourdough products (Pico et al., 2017; Rehman et al., 2006) and are produced by various microbial groups, including yeasts and LAB (Settanni et al., 2013). The predominance of alcohols, esters, organic acids, and carbonyl compounds observed in CTR brioches is consistent with the metabolic activity of the LAB and yeast consortium used for sourdough fermentation. Indeed, these classes of volatile compounds are recognized as major contributors to sourdough bread aroma and have been largely associated with the activity of sourdough microbiota, particularly LAB (Settanni et al., 2013). Carbonyl compounds, including aldehydes such as hexanal, nonanal, and 3-methylbutanal, were also abundant and are mainly derived from lipid oxidation (Frankel, 1983), playing an important role in bread aroma development (Chang et al., 1995; Ruiz et al., 2003). Furans, which originate from Maillard and caramelization reactions during baking, were also detected (Martínez-Anaya, 1996).

In addition, a substantial portion of the volatile profile consisted of compounds associated with the characteristic buttery and creamy aroma of brioche, which can be directly linked to the presence of butter in the formulation (Table 1). Key compounds such as diacetyl, acetoin, and 2,3-butanediol, well-known metabolites of LAB involved in citrate metabolism (Hugenholtz, 1993), were detected and are widely recognized as crucial aroma constituents in dairy and bakery products. These compounds serve as important indicators of LAB activity and play a central role in imparting pronounced buttery notes (Leroy & De Vuyst, 2004; Smit et al., 2005). Moreover, the presence of short- and medium-chain fatty acids, including butyric, hexanoic, and octanoic acids, further reinforced the rich, fatty sensory perception typically associated with butter-enriched baked goods (Arora et al., 1995). Esters such as isoamyl acetate and phenethyl acetate also contributed to fruity and sweet notes, enhancing the overall aromatic complexity of the product. These compounds are formed through the enzymatic activity of both yeasts and LAB (Pétel et al., 2017; Smit et al., 2005). Globally, the aroma profile of sourdough brioches results from the combined contribution of butter-derived compounds, microbial metabolism during fermentation, and thermal reactions occurring during baking, each of which plays a distinct role in shaping the final volatile composition.

The addition of MLP to sourdough brioches markedly altered the volatile fingerprint of the final products. Both 5-MLP and 10-MLP brioches contained several terpenes, ketones, and esters that were absent in CTR, clearly indicating the direct contribution of *Moringa* powder. Among these changes, the most evident was the appearance of terpene compounds, including geranylgeraniol, anethole, β-ocimene, α-terpinene, linalool, and geranylacetone, which collectively accounted for 17.9% and 20.31% of the total volatile fraction in 5-MLP and 10-MLP, respectively. Several of these compounds are associated with characteristic plant-derived aroma notes, including floral notes for linalool, floral-green notes for β-ocimene, resinous-terpenic notes for α-terpinene, and sweet anise-like notes for anethole (El Hadi et al., 2013; Xie et al., 2023). The occurrence of these compounds is consistent with the sensory differentiation observed among formulations, particularly the progressive increase in herbal odour intensity recorded for MLP-enriched brioches. Despite these changes, carbonyl compounds remained the predominant chemical class in fortified samples, indicating that the core cereal- and lipid-derived volatilome was preserved with MLP addition.

3.8. Sensory analysis

Sensory evaluation (Fig. 5) indicated that the incorporation of MLP was generally well accepted by the judges, although it induced

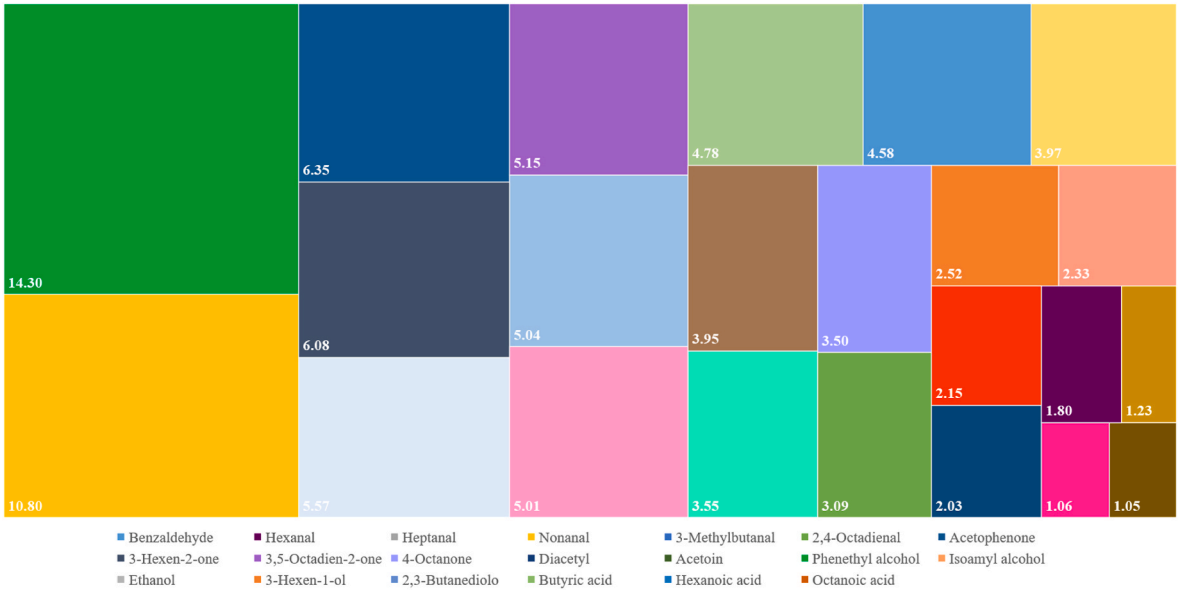

Fig. 4. Volatile Organic Compounds detected in *Moringa* Leaves Powder (MLP).

Table 5
Volatile organic compounds.

Compound	CTR	5-MLP	10-MLP	p-value	SEM
Carbonylic Compounds					
benzaldehyde	4.03	3.28	3.20	0.446	0.407
Hexanal	4.61	4.79	3.12	0.136	0.605
Heptanal	2.44 a	n.d. b	n.d. b	<0.001	0.646
Nonanal	6.34	4.33	5.19	0.204	0.690
3-Methylbutanal	2.88 a	1.28 b	1.68 ab	<0.05	0.425
2,4-Octadienal	n.d. b	n.d. b	1.02 a	<0.001	0.270
Acetophenone	n.d. b	3.34 a	3.65 a	<0.01	0.951
3-Hexen-2-one	n.d. b	4.18 a	4.21 a	<0.01	1.139
3,5-Octadien-2-one	n.d. b	2.80 a	3.12 a	<0.01	0.802
4-Octanone	n.d. c	2.16 a	1.08 b	<0.001	0.502
Diacetyl	4.80 a	2.43 b	2.56 b	<0.05	0.688
Acetoin	6.34	3.60	4.25	0.055	0.789
Alcohols					
Phenethyl alcohol	15.43	12.23	12.80	0.503	1.631
Isoamyl alcohol	7.11	5.04	4.48	0.127	0.851
Ethanol	5.38	4.01	3.21	0.099	0.653
3-Hexen-1-ol	n.d.	1.12 a	n.d.	<0.001	0.296
2,3-Butanediolo	2.49 a	1.18 b	1.25 b	<0.05	0.370
Acids					
Butyric acid	5.53 a	2.43 b	2.55 b	<0.05	0.867
Hexanoic acid	4.42	2.92	3.11	0.127	0.501
Octanoic acid	5.03 a	1.93 b	1.17 b	<0.01	0.966
Hexadecanoic acid	n.d. b	2.97 a	2.13 a	<0.01	0.716
Furans					
2-Pentylfuran	2.69	2.39	1.73	0.131	0.313
Furfural	4.61 a	1.86 b	2.14 b	<0.01	0.745
Hydrocarbons					
Undecane	n.d.	n.d.	n.d.		0.000
Dodecane	n.d. b	1.06 a	0.99 a	<0.01	0.278
Esters					
Benzyl acetate	n.d. b	0.70 a	0.80 a	<0.01	0.204
Methyl valerate	n.d. b	3.52 a	4.27 a	<0.01	1.068
Ethyl acetate	3.39 a	1.09 b	1.88 b	<0.01	0.571
Isoamyl acetate	4.61 a	1.82 b	1.96 b	<0.01	0.766
Phenethyl acetate	5.57 a	2.66 b	2.14 b	<0.01	0.908
Ethyl octanoate	2.30 a	1.01 b	n.d. c	<0.001	0.535
Terpenes					
Geranylgeraniol	n.d. b	7.27 a	8.09 a	<0.01	2.089
Anethole	n.d. b	2.58 a	3.08 a	<0.01	0.773
Geranylacetone	n.d. b	2.16 a	3.24 a	<0.001	0.770
α-Terpinene	n.d. c	2.74 a	1.11 b	<0.001	0.639
Nectarol	n.d. b	n.d. b	1.13 a	<0.001	0.299
β-Ocimene	n.d. b	2.22 a	2.58 a	<0.01	0.654
α-Linalool	n.d. b	0.90 a	1.08 a	<0.01	0.270

Data are means percentage of three replicate expressed as (peak area of each compound/total area of significant peaks) × 100. Data within a row followed by different letters are significantly different according to Tukey's test. Abbreviations: CTR, control brioche; 5-MLP, experimental brioche enriched with 5% (w/w) of MLP; 10-MLP, experimental dough brioche with 10% (w/w) of MLP; SEM, standard error of the mean; n.d., not detected.

noticeable differences among the samples. Colour attributes were the most strongly affected, with both crust and crumb colour scores increasing progressively from CTR (3.55 and 3.32, respectively) to 5-MLP (5.89 and 5.29, respectively) and reaching maximum values in 10-MLP (6.52 and 5.96, respectively). These findings indicated a darker appearance with higher MLP levels, a trend commonly observed in baked goods enriched with plant-based ingredients (Viola et al., 2023).

Conversely, structural characteristics such as porosity, alveolation size, and irregularity remained relatively stable across samples, showing only minimal variations at higher MLP supplementation levels. Odour perception was notably influenced by MLP addition: overall odour intensity increased with enrichment percentage, accompanied by a marked rise in herbal notes (from 1.00 to 6.89) and a simultaneous reduction in yeasty odour. These changes can be directly linked to the presence of polyphenols and their thermal degradation products, which are known to modulate aroma compound formation during baking (Ou et al., 2019). Moreover, they are consistent with the VOC profile of the enriched brioches, characterized by the occurrence of several MLP-derived terpene compounds associated with plant-derived aroma notes.

Taste perception was also affected. Sweetness decreased with increasing MLP concentration (from 4.23 in CTR to 3.09 in 10-MLP), while bitterness showed a clear increasing trend (from 1.00 in CTR to 5.08 in CFL10), suggesting a shift in flavour balance. This behaviour is consistent with previous reports linking polyphenol-rich ingredients to enhanced bitterness and astringency in baked products (Czajkowska–González et al., 2021). Butter and yeasty flavour notes remained relatively stable, although overall flavour intensity slightly decreased at the highest enrichment level.

Texture analysis revealed comparable springiness among samples, while hardness increased with MLP addition (from 1.59 in CTR to 2.46 in 10-MLP), indicating a firmer crumb structure. This texture modification is consistent with studies highlighting the effect of powdered additives on the structure of baked goods, where an increase in non-starch components or fiber alters the matrix and can increase the perceived texture (Giri et al., 2024). Oiliness and chewiness showed only minor variations. Overall, while moderate inclusion levels (5-MLP) maintained sensory characteristics closer to the control, higher concentrations (10-MLP) led to more pronounced changes, particularly in colour, odour profile, and taste attributes. Indeed, the overall assessment clearly highlighted the intermediate formulation as the most balanced option, with 5-MLP (7.18) achieving a level of appreciation comparable to the control sample (7.26), while the lower score observed for 10-MLP (6.07) can likely be attributed to the more pronounced shifts in sensory profiles, especially increased bitterness and herbal notes, and textural modifications at higher addition levels.

The Pearson correlation analysis (Table S2) revealed strong relationships among sensory, physicochemical, and volatile variables, outlining clear co-variation patterns across the samples. A strong positive correlation was observed among odour intensity, herbal odour, bitterness, and hardness ($r = 0.95–1.00$), indicating that more aromatic samples tend to be more bitter, herbaceous, and firm. Conversely, overall flavour was negatively correlated with these attributes ($r = -0.71$ to -0.89), suggesting that high intensity and bitterness reduce overall acceptability.

Colour parameters supported this trend: crumb lightness (L*) was negatively correlated with sensory intensity and hardness, indicating that darker samples are more intense and structured, while crumb a* showed an opposite trend and was strongly negatively correlated with overall flavour. Compression values were highly correlated with perceived hardness, confirming consistency between instrumental and sensory measurements. Regarding volatile compounds, ethanol and isoamyl alcohol were negatively associated with sensory intensity and hardness, suggesting a link with milder profiles. In contrast, hexanal showed a strong positive correlation with overall flavour, indicating its role in pleasant sensory perception. In addition, nonanal appeared less influential, showing weak correlations with flavour.

Overall, the analysis highlights two opposing profiles: an intense, bitter, and firm profile negatively affecting acceptability, and a milder, lighter profile associated with improved overall flavour and the presence of compounds such as hexanal.

4. Conclusions

This study demonstrated that MLP can be effectively incorporated into sourdough brioches to produce nutritionally enhanced sweet baked products without compromising fermentation performance or microbiological safety. While higher inclusion levels significantly affected colour, texture, and flavour, moderate supplementation (5% w/w) achieved the best balance between functional improvement and sensory acceptability. From an industrial perspective, the results highlight the

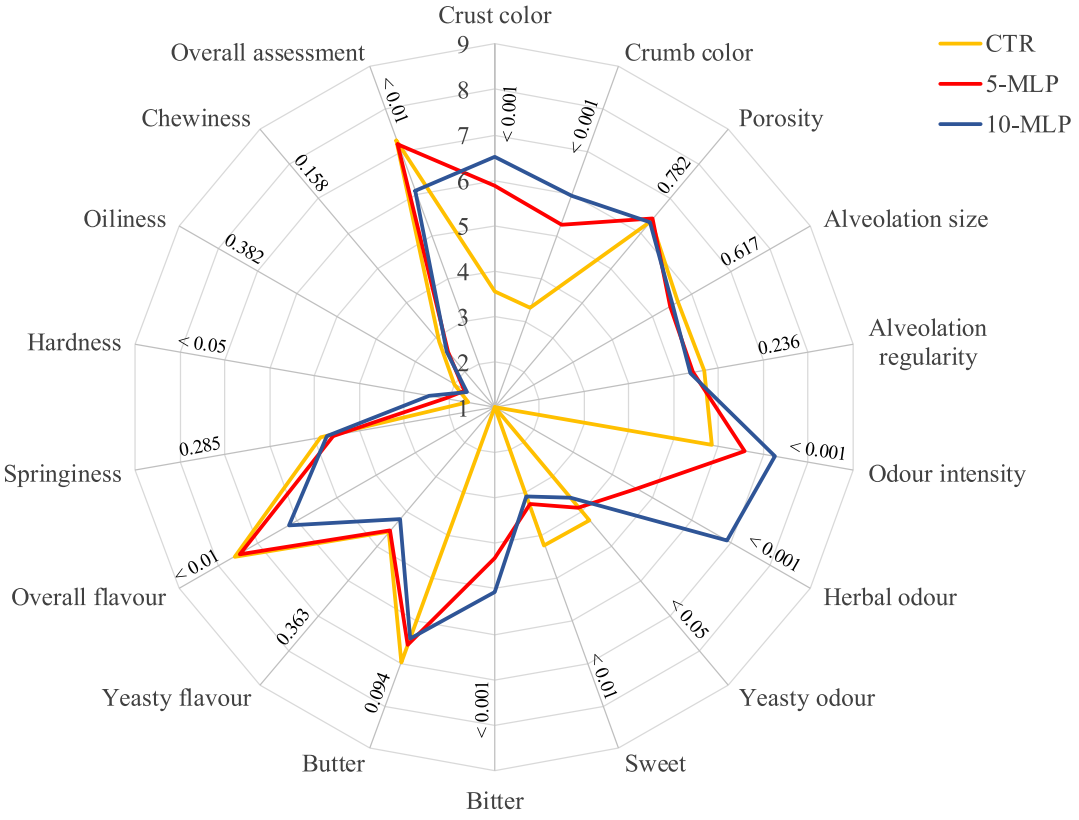

Fig. 5. Radar plot displaying sensory evaluation of the experimental brioches. Abbreviations: CTR, control brioche; 5-MLP, experimental brioche enriched with 5% (w/w) of MLP; 10-MLP, experimental brioche enriched with 10% (w/w) of MLP.

technological feasibility of integrating MLP into conventional bakery processes, as no critical limitations were observed in dough fermentation or process handling. In this context, sourdough primarily contributed as an aroma-enhancing component, consistent with its role in many industrial sweet leavened products, while yeast-driven leavening ensured adequate volume development. This suggests that MLP could be realistically adopted by the bakery industry as a functional ingredient for product innovation, particularly in the growing market segment of health-oriented baked goods. Moreover, the use of MLP derived from locally cultivated *M. oleifera* reinforces the potential for sustainable and regionally based value chains, supporting agricultural diversification. However, some challenges remain for large-scale applications, particularly related to textural modifications and sensory changes at higher enrichment levels, which may require formulation adjustments or process optimization. A limitation of this study is the relatively short sourdough fermentation time (2 h), which may have restricted microbial succession, metabolic activity, and phenolic bioconversion typically associated with longer fermentations. Consequently, the role of sourdough in this study was likely more relevant to aroma development than to extensive fermentation-driven biochemical transformations. Importantly, conclusions are restricted to compositional characteristics and *in vitro* antioxidant properties, as no digestion, bioaccessibility, or *in vivo* analyses were performed. Previous work using the same MLP showed antioxidant activity in a cell-based assay, suggesting potential intracellular effects. However, no direct health implications can be inferred for the brioche products.

Future research should therefore focus on (i) optimizing formulations to improve texture and sensory properties at higher MLP inclusion levels; (ii) investigating process scalability and industrial validation, including continuous production systems; (iii) evaluating shelf-life stability and storage behaviour of enriched products; and (iv) exploring strategies to enhance bioactive compound bio accessibility and stability during processing and digestion. Overall, MLP represents a promising plant-derived ingredient for the development of innovative bakery products with enhanced polyphenol content and antioxidant properties, good technological feasibility, and consumer appeal.

CRediT authorship contribution statement

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

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fbio.2026.109384.

Data availability

Data will be made available on request.

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