LWT - Food Science and Technology 241 (2026) 119054

Contents lists available at ScienceDirect

LWT

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

Fermented beverages enriched with quinoa or chestnut–persimmon: Nutritional, functional, and anti-nutritional assessment

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ARTICLE INFO

Keywords:
Plant-based beverages
Lacticaseibacillus casei
Quinoa
Chestnut
NMR spectroscopy

ABSTRACT

In this study, chestnut-persimmon and quinoa flours were used to formulate non-dairy beverages (CNP and Q, respectively) fermented with a strain of *Lacticaseibacillus casei*. Fermentability (microbial counts and pH) and biochemical, polyphenolic, volatile, and nutritional profiles were evaluated using ^{1}H NMR, UPLC-QqQ-MS/MS, and HS-SPME/GC-MS. Both matrices sustained high LAB viability and stable acidification, confirming their suitability as fermentation substrates. NMR spectroscopy revealed matrix-dependent metabolic shifts: CNP beverages retained higher sugar levels and accumulated methanol and scyllo-inositol, whereas Q beverages exhibited enhanced nucleotide release. Lactic and acetic acids increased in both beverages. Among the 21 phenolic compounds identified, chlorogenic acid isomers, mainly derived from black carrot, were predominant. Total polyphenols content remained above 80 % of baseline values, with greater stability observed in CNP, while Q retained a more diversified flavonoid profile. VOC analysis showed suppression of aldehydes in fermented CNP, associated with reduced grassy notes, whereas fermented Q accumulated lipid-derived alcohols and fatty acids, resulting in more pronounced earthy and fatty aromas. Fermentation reduced anti-nutritional factors and improved *in vitro* protein digestibility, particularly in CNP. Overall, these findings demonstrated that both beverages are highly fermentable, nutritionally advantageous, and compositionally distinct, offering gluten-free, nutrient-rich options with complementary functional properties.

1. Introduction

Lactose intolerance, milk protein allergies, vegan diets, and sustainability concerns are driving rapid growth of plant-based beverages (Rathod et al., 2025).

These products often rely on cereals/pseudocereals, however gluten-containing grains can exclude consumers with celiac disease (Khairuddin & Lasekan, 2021). To ensure broader accessibility and health benefits, it is therefore a key-challenge to prioritize formulations that are both nutritionally adequate and free from major dietary allergens or intolerances. This challenge requires careful selection and combination of the ingredients, prompting the exploration of different and underutilized plant matrices.

Unlike wheat, barley, and rye, rice flour is gluten-free, making rice-based beverages among the most hypoallergenic dairy-free alternatives (Najman et al., 2024). Their widespread use is supported by their naturally sweet and neutral flavor profiles, arising from carbohydrate breakdown during processing, as well as by the presence of antioxidant compounds, despite their low protein and fat contents (Najman et al., 2024). Similarly, quinoa, a pseudocereal providing complete proteins and a balanced amino acid profile, has gained interest in the plant-based beverage sector for its nutritional and techno-functional advantages

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https://doi.org/10.1016/j.lwt.2026.119054
Received 23 October 2025; Received in revised form 9 January 2026; Accepted 16 January 2026
Available online 19 January 2026

(Hurtado-Murillo et al., 2025). Nevertheless, the wide range of ingredients suitable for plant-based beverage fortification has left many promising matrices poorly explored. For instance: chestnut is a Mediterranean origin ingredient rich in bioactive compounds with several reported biological activities (e.g., anti-inflammatory, cardioprotective effects) and provides gluten-free carbohydrates alongside other functional components (Barreira et al., 2020). Persimmon is another bioactive rich ingredient containing phenolics, carotenoids, dietary fibers, and natural sweetness (González et al., 2021). Beyond its nutritional value, the inherent sweetness of persimmon has promoted its incorporation into a wide range of food applications, including pork liver pâté, pasta, dairy products, vinegar, wine, and snacks (Jokar & Azizi, 2022). Black carrot, common Indian root vegetable, traditionally used in the fermented beverage *Kanji,* exhibits remarkable antioxidant activity attributed to its high anthocyanin and polyphenol contents (Akhtar et al., 2017).

Another major challenge for the development of high-quality plant-based beverages is the persistence of limitations affecting nutritional and sensory quality (Popova et al., 2023; Xie et al., 2023). These include the presence of anti-nutritional factors such as phytic acid, raffinose family oligosaccharides and fructans, which impair mineral bioavailability and may cause gastrointestinal discomfort. In addition, plant proteins often display lower digestibility and less balanced essential amino acid profile compared to dairy proteins. From sensory perspectives, many cereal-, legume- and seed-based beverages are prone to flavor defects, including grassy, beany, earthy or oxidized notes associated with lipid degradation and endogenous enzyme activity.

To enhance functional value while maintaining consumer acceptability, strategic combination of complementary ingredients may yield synergistic improvements which can be further amplified through fermentation. Lactic fermentation has long been employed in traditional plant-based fermented foods (Hurtado-Murillo et al., 2025). Lactic acid bacteria (LAB), through different enzymatic pathways, may enhance nutrient and bioactive compounds bioavailability, generate desired aromatic profiles, and reduce or eliminate anti-nutritional factors (Viretto, Tlais, Arora, et al., 2025). These transformations can contribute to improved organoleptic quality and digestibility (Viretto, Tlais, Tuccillo, et al., 2025). Moreover, viable LAB retained in the fermented matrix may exert probiotic effects after ingestion, including survival through gastrointestinal transit, adhesion to mucosal surfaces, inhibition of pathogens, and contribution to gut homeostasis and immune modulation (H. Liu et al., 2023).

In this context, the present study investigated two distinct plant-based beverage prototypes, both incorporating rice and black carrot as common ingredients, but differing in their complementary components: one enriched with quinoa, and the other with chestnut and persimmon. Fermentation was carried out with *Lacticaseibacillus casei*, and a comprehensive characterization, including microbial stability, metabolomic changes, polyphenol retention, volatile compounds profile, anti-nutritional factors, and protein quality, was performed to evaluate their nutritional and functional potential for consumer acceptance.

2. Materials and methods

2.1. Raw materials and fermenting culture

Rice flour (RF), chestnut flour (CF), fresh persimmons, and quinoa seeds were purchased from a supermarket near Fondazione Edmund Mach (San Michele a/A, Trento, Italy), transported to the laboratory, and immediately stored at 4 °C. The black carrot powder was obtained from Molino Bongiovanni S.r.l. (Villanova Mondovì, CN, Italy). The fermenting strain, a freeze-dried *Lacticaseibacillus casei* Lyofast BGP 93, was kindly donated by SACCO S.r.l. (Cadorago, CO, Italy) and stored at −20 °C until use, according to the manufacturer's instructions.

2.2. Beverage formulation and fermentation

Flour and powder materials were used without any pretreatment. Quinoa seeds were washed at least twice to remove saponins, dried (24 h, 40 °C), milled, and sieved (<250 μm). Persimmon was sliced (10 mm), dried (45 °C, 48 h), milled, and sieved (200 μm). Two recipes were selected following preliminary trials in which different ingredient ratios were tested, with the final combinations chosen to ensure suitable consistency, stability, and overall sensory acceptability. These formulations were prepared using the rice flour base (20 g/L) and black carrot base (10 g/L). Rice flour was used at 20 g/L to balance fermentability and viscosity aligning with previous work (Moiseenko et al., 2024).

The quinoa-based beverages (Q) contained quinoa flour (QF, 40 g/L), while the chestnut-persimmon-based beverages (CNP) included chestnut flour (CF, 40 g/L) and dried persimmons powder (PP, 30 g/L). The inclusion levels of chestnut flour (40 g/L) and quinoa flour (40 g/L) were the maximum dose compatible to maintain beverage-like viscosity, color stability, flavor, and robust fermentation based on preliminary formulation trials. Both formulations were pasteurized (90 °C, 5 min), cooled at room temperature under stirring, and inoculated with Lyofast BGP 93 at a final cell density of $7{\cdot}10^8$ CFU/L. Control (CTR) and fermented formulations underwent exactly the same thermal treatment and handling steps; the only difference was that CTR beverages were not inoculated with the starter culture. Fermentation was carried out at 30 °C for 48 h, followed by storage at 4 °C for another 5 d. The fermentation time of 48 h was chosen based on preliminary observations indicating that *L. casei* Lyofast BGP 93 reached stable high viability and completed the main acidification phase within this window. The 5 d as refrigeration period was selected to reflect a typical short, refrigerated shelf-life for fresh plant-based fermented beverages, allowing assessment of early storage stability and metabolite evolution. Three biological replicates per condition were sampled at 0 h (T0), 48 h (T2d), and 7 d (T7d). CTR samples were subjected to the identical procedures. The following identifiers were used: fermented chestnut-persimmon beverage (CNP-F), non-inoculated chestnut-persimmon control (CNP-CTR), fermented quinoa-based beverage (Q-F), and non-inoculated quinoa-based beverage control (Q-CTR).

2.3. Microbiological counts and pH measurement during fermentation

Beverage samples were serially diluted in sterile peptone water and placed on the following agar media: Plate Count Agar (PCA) supplemented with skim milk (10 g/L) for total bacterial count (TBC), incubated under aerobic conditions at 30 °C for 24 h; and de Man, Rogosa and Sharpe (MRS) agar for mesophilic lactobacilli, incubated anaerobically at 30 °C for 48 h using AnaeroGen ™ system. All culture media and anaerobic systems were purchased from Oxoid (Thermo Fischer, Waltham, MS, USA). The pH of all samples was measured using a pH meter (Hanna Instruments, model N° HI99191).

2.4. NMR spectroscopy analysis

Fresh beverage samples were centrifuged at 4000 rpm for 15 min. From each sample, 900 μL of the supernatant were transferred into cryovials and mixed with 100 μL of D_2O buffer. The pH of each group of samples, according to the fermentation stage, was adjusted accordingly prior to analysis (Bruker BTPH titrator). Subsequently, 600 μL of the resulting solutions were transferred into 5 mm NMR tubes (509-UP, Norell Inc., USA), and ^{1}H NMR spectra were recorded within 24 h of sample preparation. Acquisition of the NMR spectra as well as data analysis and metabolite quantification were carried out following the procedure detailed in Brigante et al. (2025). Compound identification was carried out using AssureNMR software (Bruker BioSpin GmbH) in automation mode with the aid of Human Metabolome Database (HMDB; Wishart et al., 2022) and the BBIOREFCODE database (v.2.01, Bruker BioSpin GmbH, Rheinstetten, Germany), and in manual mode basing on

literature database (Isern et al., 2013; Lalaleo et al., 2020). Metabolite concentrations were calculated from ^{1}H NMR spectral areas and expressed as mg/L of beverage, as reported in the corresponding Table 1.

2.5. Determination of total polyphenols and phenolic compounds profile

Total polyphenol content (TPC) was determined using the Folin-Ciocalteu colorimetric method, with minor modifications. Results were reported as mg catechin equivalents/L.

Phenolic compound profiling was performed using Ultra Performance Liquid Chromatography coupled with triple quadrupole tandem mass spectrometry (UPLC-QqQ-MS/MS), following the procedure described by Vrhovsek et al. (2012), with adaptations. Freeze-dried beverage samples were extracted with acidified methanol (1 mL/L formic acid), filtered (0.22 μm), and injected into a Waters Acquity UPLC H-Class system equipped with a BEH C18 column (2.1 × 100 mm, 1.7 μm; Waters). The mobile phases consisted of 1 mL/L formic acid in water (A) and acetonitrile with 1 mL/L formic acid (B). The gradient elution was as follows: 0–1 min, 1 % B; 1–10 min, 1–25 % B; 10–12 min, 25–95 % B; 12–14 min, 95 % B; 14.1–16 min, 1 % B (re-equilibration). Flow rate: 0.3 mL/min; column temperature: 40 °C. Detection was carried out on a Waters Xevo TQ-S triple quadrupole mass spectrometer with an electrospray ionization (ESI) source operated in both positive and negative modes, using Multiple Reaction Monitoring (MRM). Compound identification was based on retention times, parent-to-daughter ion transitions, and fragmentation patterns, using authenticated standards and comparison with databases (e.g., BBIOREFCODE, HMDB). Quantification was performed using external calibration curves with standard compounds and reported as mg/L of beverage. Details of the targeted MS^2 identification, including exact mass, retention time, MS^2 transition, and the analytical standard used for confirmation, are provided in Supplementary Table S1.

2.6. Analysis of volatile organic compounds (VOCs) by HS-SPME/GC-MS

The technique Head Space - Solid Phase Microextraction/Gas Chromatography - Mass Spectrometry (HS-SPME/GC-MS) was used to analyze volatile organic compound (VOCs). Freeze-dried samples were suspended in water at 50 g/L concentration and stirred overnight. This reconstitution level was selected based on preliminary trials indicating that this dilution provided a reproducible headspace equilibrium without fiber saturation and in alignment with existing HS-SPME/GC-MS methods applied to freeze-dried plant-based matrices that allow robust semi-quantitative comparisons. Aliquots of the aqueous solutions (3 mL) were transferred into headspace vials (20 mL) capped with PTFE/silicone septa (Agilent, Cernusco sul Naviglio, Italy) and stored at −80 °C until the day of analysis. Three replicates were prepared from each sample. After defrosting, 0.10 mL of an internal standard (aqueous solution of 2-octanol 0.50 mg/L) was added, and the samples were placed into the autosampler of the GC (MPSxt XL, Gerstel, Germany), stored at 10 °C until the analysis. After 10 min at 40 °C, SPME fiber (2 cm DVB/CAR/PDMS, Supelco, Bellefonte, PA, USA) was exposed for 30 min in the vial headspace. The analytes were then desorbed from SPME fiber at 240 °C in the injector port of a GC (Agilent 7890B, Agilent Technologies, USA) and separated on a DB-Wax UI fused silica capillary column (30 m x 0.25-mm ID x 0.25 μm film thickness; Agilent Technologies, USA). The carrier gas was helium, at a constant flow rate of 1.30 mL/min. The GC oven temperature program began at 40 °C for 1 min, then increased to 240 °C at 5 °C/min and finally was held at 240 °C for 4 min. The GC was interfaced with a mass detector operating in electron ionization (EI) mode (internal ionization source; 70 eV) with a scan range of m/z 33–350 (Agilent MS 5977A MSD, Agilent Technologies, USA). Compound identification was based on mass spectra matching with the standard NIST/EPA/NIH (NIST 14), and linear retention indices (LRI) compared with the literature. LRI were calculated under the same chromatographic conditions after injection of a C7-C30 *n*-alkane series (Supelco). Results are reported as semi-quantitative concentrations, expressed in μg/L eq. of 2-octanol.

2.7. Anti-nutritional factors quantification

Residual concentrations of phytic acid and fructans were determined using the Phytic Acid (Phytate)/Total Phosphorous (K-PHYT 05/19) Megazyme kit (Megazyme International, Ireland) and the Fructo-oligosaccharides (K-FRUC 11/12) Megazyme kit (Megazyme International, Ireland), based on the AOAC method 999.03 and the AACC method 32.32.01, respectively. Raffinose-series oligosaccharides (RFOs) contents were assessed using the Raffinose/D-galactose (K-RAFGA) assay kits (Megazyme International, Ireland) according to the manufacturer's instructions.

2.8. In vitro protein digestibility (IVPD) and protein digestibility corrected amino acid score (PDCAAS)

All beverages were subjected to three separate hydrolysis sessions to ensure complete release of individual amino acids. Most amino acids were quantified following acid hydrolysis based on AOAC Method 994.12. For the determination of cysteine and methionine, a performic acid oxidation method (AOAC 994.12) was followed. Due to its degradation under acidic conditions, tryptophan was analyzed separately using alkaline hydrolysis (AOAC 988.15). Quantification of individual amino acids was carried out using a Biochrom 30 series Amino Acid Analyzer (Biochrom Ltd., Cambridge Science Park, England) with a Na-cation exchange column (20 by 0.46 cm internal diameter), except for Tryptophan, which was determined via LC-MS platform composed of an Ultimate 3000 RSLCnano system, coupled to a QExactive Plus Hybrid Quadrupole-Orbitrap mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) (Viretto, Tlais, Tuccillo, et al., 2025). The limiting essential amino acid was identified as the one with the lowest value compared to FAO-recommended values for essential amino acids. The IVPD of each beverage was estimated using the Protein Digestibility Assay kit (Megazyme International, Wicklow, Ireland) according to the manufacturer's instructions. PDCAAS was calculated by multiplying the amino acid score of the limiting amino acid and the corresponding IVPD.

2.9. Statistical analysis

Although the experimental design included two factors (fermentation status and time), each beverage matrix (CNP and Q) was analyzed independently using one-way ANOVA. This choice was motivated by the limited number of conditions per matrix (five in total: CTR-T0, CTR-T48h, CTR-T7d, F-T48h, F-T7d), which rendered multifactorial models statistically underpowered and unnecessarily complex. In addition, the use of Welch's t-tests for pairwise comparisons increases the robustness of the statistical inference, as this test is specifically tolerant to heteroscedasticity and small, uneven group variances, providing greater reliability than classical t-tests or Tukey-type procedures under these conditions. Treating each condition as a distinct group enabled straightforward comparison of practically relevant fermentation and storage stages while maintaining interpretability. When the omnibus test indicated significance ($p < 0.05$), Welch's t-tests were applied for pairwise comparisons, as they are more robust to variance heterogeneity and small sample sizes than traditional post-hoc tests. Statistical analyses were conducted in R software (version 4.3.1; https://www.r-project.org/).

3. Results and discussion

3.1. Monitoring of fermentation

The evolution of microbial viability and acidification in both CNP

Table 1
Levels of identified metabolites as calculated from the ^{1}H NMR spectral areas in fermented and non-fermented chestnut–persimmon (CNP) and quinoa (Q) beverages. Fermentation was performed at 30 °C for 48 h (T2d), followed by storage at 4 °C for 5 days (T7d). All data were expressed as mg related to 1 L of beverage.

	Chestnut-Persimmon (CNP)					Quinoa (Q)				
		CTR		Fermented (F)			CTR		Fermented (F)	
	T0	**T2d**	**T7d**	**T2d**	**T7d**	**T0**	**T2d**	**T7d**	**T2d**	**T7d**
Free Amino Acids (mg/L)										
Ala	82.9 ± 0.79c	79.2 ± 0.070b	71.6 ± 1.74b	62.4 ± 2.53a	61.4 ± 1.19a	43.1 ± 3.31c	19.1 ± 0.49a	19.4 ± 0.64a	30.7 ± 0.68b	31.2 ± 3.9b
Asn	262 ± 2.36d	251 ± 2.27c	247 ± 4.25c	205 ± 1.94a	215 ± 1.65b	79.8 ± 1.59c	85.2 ± 2.16c	89.9 ± 3.03d	71.4 ± 1.03b	67.4 ± 1.69a
Asp	357 ± 3.72	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Leu	19.9 ± 0.16b	n.d.	n.d.	14.2 ± 0.060a	14.7 ± 0.57a	n.d.	21.6 ± 2.81a	28.5 ± 3.62b	n.d.	n.d.
Phe	20.9 ± 0.64c	17.7 ± 0.79b	15.7 ± 1.01a	13.0 ± 0.24a	13.7 ± 0.34a	23.8 ± 0.11c	14.8 ± 2.39b	18.6 ± 4.07b	10.1 ± 0.83a	10.6 ± 3.62a
Val	14.7 ± 0.28c	12.5 ± 0.28b	12.3 ± 0.46b	10.9 ± 0.067a	11.3 ± 0.45a	22.9 ± 0.30b	19.4 ± 0.15a	40.1 ± 0.33c	19.3 ± 0.25a	19.7 ± 0.77a
Ile	n.d.	13.4 ± 0.27b	5.9 ± 0.13a	n.d.	n.d.	20.9 ± 0.23d	9.9 ± 3.22abc	13.5 ± 3.82c	6.1 ± 0.093b	5.7 ± 0.056a
GABA	n.d.	161 ± 2.12a	157 ± 3.57a	n.d.	n.d.	n.d.	54.4 ± 1.41a	90.4 ± 2.36b	n.d.	n.d.
Trp	n.d.	n.d.	n.d.	n.d.	n.d.	49.5 ± 0.18b	47.7 ± 2.16b	51.2 ± 3.39b	42.1 ± 0.33a	41.0 ± 1.62a
Organic Acids (mg/L)										
Acetic acid	48.8 ± 2.21a	131 ± 5.17a	130 ± 11.1b	156 ± 2.46c	177 ± 4.08d	30.4 ± 0.57a	149 ± 28.2b	140 ± 20.6b	181 ± 10.3c	189 ± 18.6c
Formic acid	7.1 ± 0.10c	13.4 ± 0.76d	8.1 ± 1.17c	3.8 ± 1.34a	5.0 ± 0.28b	7.1 ± 0.085a	39.3 ± 9.78d	27.7 ± 4.10cd	24.8 ± 2.38c	18.6 ± 2.23b
Pyruvic acid	10.2 ± 0.16d	8.3 ± 0.71c	n.d.	6.3 ± 0.11b	5.7 ± 0.33a	15.1 ± 0.42c	n.d.	n.d.	6.8 ± 0.09b	6.5 ± 0.13a
Citric acid	n.d.	752 ± 13.1b	726 ± 6.78b	545 ± 42.6a	587 ± 1.05a	n.d.	n.d.	n.d.	n.d.	n.d.
3-Hydroxybutyric acid	18.0 ± 0.16a	n.d.	n.d.	35.0 ± 1.33b	38.8 ± 0.59c	n.d.	n.d.	n.d.	35.9 ± 0.83a	36.4 ± 6.28a
Lactic acid	n.d.	2900 ± 171a	3320 ± 335 ab	3680 ± 191b	4200 ± 48c	n.d.	2830 ± 124a	3150 ± 122 ab	3310 ± 185 ab	3460 ± 675b
Sugars (mg/L)										
Fructose	2350 ± 80a	2590 ± 384a	2090 ± 766a	2960 ± 665a	3050 ± 528b	174 ± 0.16	n.d.	n.d.	n.d.	n.d.
D-Glucose	9160 ± 355b	6990 ± 1550a	9180 ± 4,600 ab	7800 ± 2,360a	7537 ± 2,060a	423 ± 3.58	n.d.	n.d.	n.d.	n.d.
Sucrose	27,460 ± 885b	n.d.	18,860 ± 4170a	31,600 ± 6050b	23,080 ± 5460 ab	7435 ± 65 ab	9113 ± 55c	8100 ± 429b	6490 ± 145a	6464 ± 82a
Others (mg/L)										
Acetaldehyde	0.86 ± 0.11a	1.37 ± 0.040b	1.33 ± 0.22 ab	1.84 ± 0.26d	1.57 ± 0.13c	0.21 ± 0.014a	1.08 ± 0.21b	1.09 ± 0.33b	0.63 ± 0.24a	0.93 ± 1.22a
2,3-Butanediol	5.4 ± 0.063a	8.0 ± 0.32c	8.5 ± 0.21c	5.5 ± 0.035a	5.9 ± 0.049b	30.4 ± 0.38a	69.5 ± 2.44d	57.7 ± 2.27c	38.4 ± 1.85b	37.4 ± 0.60b
Choline	16.5 ± 9.84bc	6.9 ± 0.20b	8.0 ± 0.32c	7.9 ± 0.21b	6.3 ± 0.24a	4.0 ± 0.057b	2.6 ± 0.056a	2.8 ± 0.29a	2.8 ± 0.055a	2.7 ± 0.066a
Ethanol	7.9 ± 0.10a	9.1 ± 0.34c	18.9 ± 14.5abc	8.0 ± 0.055a	8.4 ± 0.18b	n.d.	n.d.	n.d.	n.d.	n.d.
Methanol	77.0 ± 3.72b	98.9 ± 1.40d	13.4 ± 4.22a	86.9 ± 0.71c	89.5 ± 0.71c	7.5 ± 0.057c	6.0 ± 0.24b	8.3 ± 0.75c	6.1 ± 0.22b	5.6 ± 0.076a
Scyllo-inositol	58.9 ± 1.09a	63.1 ± 0.59b	278.3 ± 10.2c	59.3 ± 0.73a	60.6 ± 0.86 ab	17.1 ± 0.28a	29.6 ± 0.17b	30.4 ± 1.51b	29.9 ± 0.14b	29.5 ± 0.45b
Trigonelline	1.8 ± 0.23a	2.7 ± 0.19b	2.6 ± 0.20b	2.5 ± 0.12b	2.5 ± 0.13b	3.0 ± 0.16a	3.1 ± 0.085a	2.9 ± 0.067a	3.0 ± 0.050a	2.9 ± 0.14a
Acetoin	n.d.	10.9 ± 1.15a	14.2 ± 5.00b	22.8 ± 3.11b	18.3 ± 0.51b	25.2 ± 0.085b	20.3 ± 1.17a	21.1 ± 1.71a	27.1 ± 0.31b	26.6 ± 4.32 ab
Methylamine	n.d.	14.7 ± 0.34b	13.1 ± 0.85a	13.1 ± 1.08a	12.7 ± 0.085a	n.d.	n.d.	n.d.	9.9 ± 0.39a	9.8 ± 0.80a
Uridine	n.d.	8.6 ± 0.28a	7.7 ± 1.10a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Adenine	n.d.	n.d.	n.d.	n.d.	n.d.	28.8 ± 0.085a	30.8 ± 0.83a	31.7 ± 1.55 ab	33.9 ± 0.35c	32.7 ± 0.48b
Inosine triphosphate	n.d.	n.d.	n.d.	n.d.	n.d.	46.5 ± 0.049a	46.1 ± 0.86a	49.5 ± 2.87 ab	50.9 ± 0.50b	49.4 ± 0.61b
Uracil	n.d.	n.d.	n.d.	n.d.	n.d.	16.3 ± 0.62d	11.9 ± 1.09b	5.1 ± 0.48a	13.1 ± 0.43bc	13.7 ± 0.40c
Guanosine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	57.3 ± 2.91a	55.1 ± 1.64a	n.d.	n.d.

Values are expressed as mean ± standard deviation. For each beverage (CNP and Q), statistical comparisons were performed independently across fermentation times. For each compound: different lowercase letters in the same row indicate a significant difference between samples at 0, 2 and 7 days ($p < 0.05$).
n.d.: not detected.

and Q beverages confirmed successful fermentation using *Lacticaseibacillus casei* as starter culture (Fig. 1). After 48 h, LAB counts on MRS agar reached 8.7 and 8.5 log CFU/mL in CNP-F and Q-F, respectively. These values remained relatively stable after 7 days of refrigerated storage (8.4 and 8.3 log CFU/mL, respectively), confirming that both formulations provided a suitable environment for LAB growth and persistence.

Acidification profiles further supported active fermentation. In the CNP formulations, the pH decreased from 5.63 to 3.65 within 48 h and stabilized at 3.74 during storage. In Q beverages, pH dropped from 6.07 to 4.51 after 48 h and reached 3.95 by day 7. This acidification reflects the metabolic activity of the starter LAB and creates conditions that restrict the growth of spoilage organisms. Notably, CNP beverages acidified more rapidly and maintained slightly higher LAB counts, likely due to the higher availability of fermentable sugars and prebiotic fibers from chestnut and persimmon, which are known to promote LAB metabolism (Markowiak & Ślizewska, 2017).

Non-inoculated beverages (CTR) also showed microbial proliferation during storage, although with higher variability and larger standard deviations compared to fermented samples. This instability suggests the presence of indigenous microbiota originating from raw flours or the processing environment, consistent with spontaneous fermentations reported in cereal-based products (Meena et al., 2023). Such uncontrolled growth highlights the importance of inoculation with selected starter LAB, to ensure predictable acidification, microbial stability, and safety while avoiding undesirable sensory shifts.

These findings are consistent with previous studies. Magala et al. (2015) reported that rice flour slurries fermented with *Lactiplantibacillus plantarum* and *Levilactobacillus brevis* exhibited a pH decrease from ~5.1 to 3.7–4.3 within 24 h, with LAB counts reaching 8.5 log CFU/mL. Similarly, Khan et al. (2021) observed gradual acidification (pH 5.97 to 3.68) in a rice-barley-wheat blend with LAB counts reaching 6 log CFU/mL. Overall, both formulations proved suitable as carriers for LAB fermentation. The CNP matrix supported higher LAB viability and faster acidification, attributable to chestnut- and persimmon-derived fermentable carbohydrates and bioactive compounds. This interpretation is supported by NMR data (Table 1), which showed substantially higher levels of fructose, glucose, and especially sucrose in CNP than in Q beverages at T0, providing a readily available carbohydrate pool for LAB metabolism. These findings also align with earlier reports on chestnut and persimmon based fermentations, highlighting their capacity to sustain LAB growth and acid production (Zou et al., 2023). Meanwhile, Q beverages also sustained strong LAB growth and stable acidification, in agreement with reports indicating that LAB enhance phenolic content, antioxidant capacity, and microbial stability in pseudocereal matrices (Cerdá-Bernad et al., 2022). In contrast, quinoa is mainly starch based with a relatively low free sugar fraction, which may limit rapid acidification despite sustaining microbial growth (Barreira et al., 2020; González et al., 2021). Nevertheless, both CNP and Q beverages consistently maintained high LAB counts and achieved stable acidification.

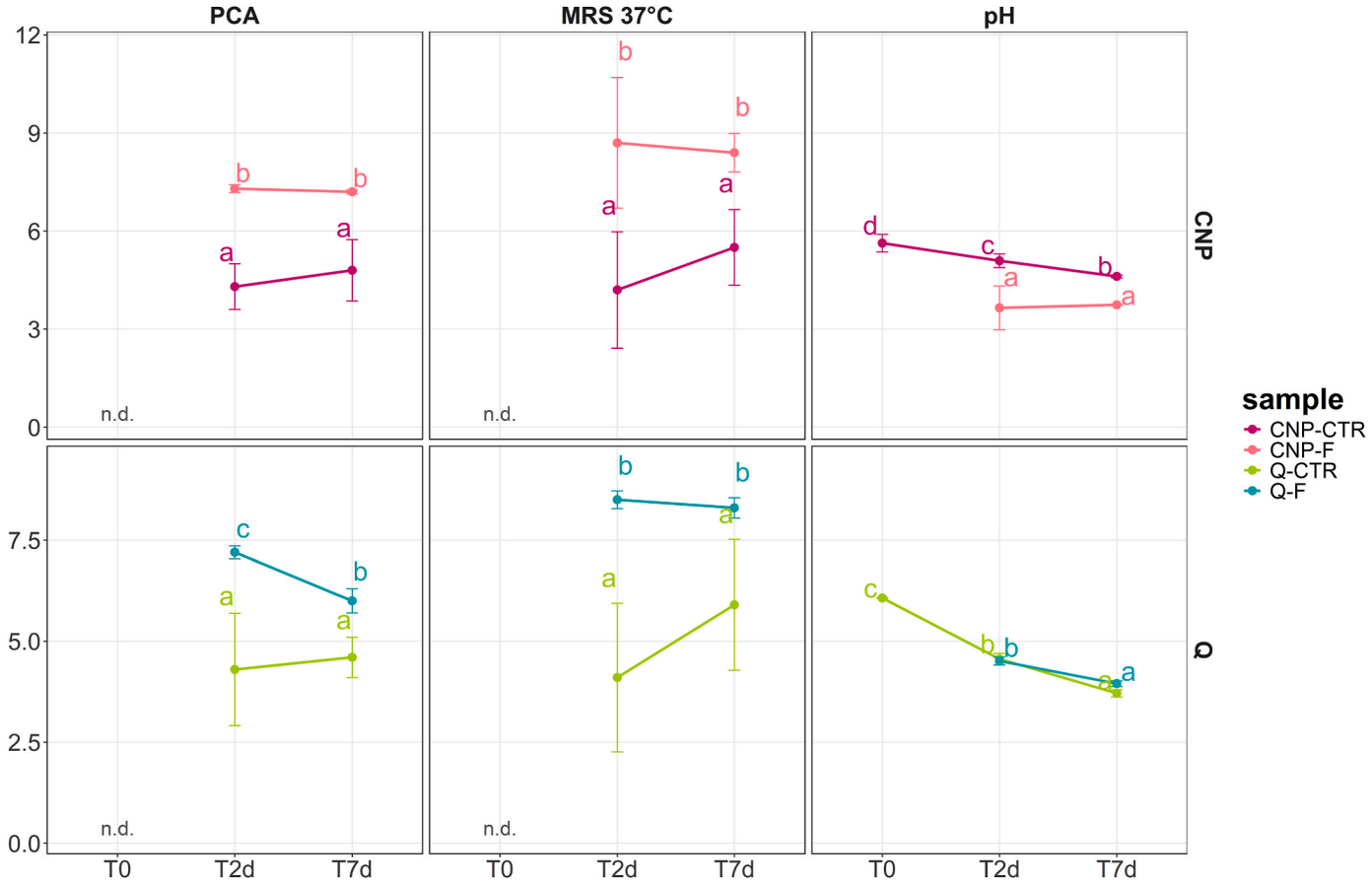

Fig. 1. Changes of viable counts and pH. Fermentation was performed at 30 °C for 48 h (T2d), followed by storage at 4 °C for 5 days (T7d). Values are expressed as mean ± standard deviation. Different lowercase letters indicate significant differences within each beverage prototype ($p < 0.05$).

3.2. Biochemical profiling of fermented beverages by ^{1}H NMR spectroscopy

^{1}H NMR spectroscopy enabled comprehensive metabolite profiling of CNP and Q beverages, highlighting clear differences between controls and fermented samples across storage (Table 1). In CNP, concentration of alanine, asparagine, phenylalanine, and valine consistently declined during fermentation, while alanine remained more stable in Q beverages. These trends are typical of lactic fermentations, where amino acids are incorporated into microbial biomass or catabolized via deamination and decarboxylation (Filannino et al., 2018). γ-Aminobutyric acid (GABA) accumulated in CTR (157 and 90.4 mg/L in CNP-CTR and Q-CTR, respectively) but was absent in fermented beverages. This pattern can be explained by differences in microbial metabolism. GABA detected in CTR likely originates from indigenous microbiota naturally present in the raw materials, which may possess active glutamate decarboxylase (GAD) systems (Diana et al., 2014). In fermented beverages, the inoculated *L. casei* BGP 93 strain may have consumed the GABA produced, leading to its disappearance. Similar decreases in GABA, linked to GABA-transaminase activity, have been reported in other LAB species during extended fermentation (Carafa et al., 2019; Lai et al., 2025). Strategies to enhance GABA accumulation could include increasing the glutamate availability (e.g., via monosodium glutamate or protein hydrolysates), and selecting LAB strains with strong GAD activity, such as certain *L. brevis* (Carafa et al., 2019). Tryptophan was detected only in Q beverages and declined modestly during fermentation. Branched-chain amino acids exhibited matrix-dependent trends: leucine persisted in CNP-F and Q-CTR after 48h and 7d but disappeared in CNP-CTR and Q-F, while isoleucine was present in CNP-CTR and all Q samples, though at lower levels in fermented beverages.

Organic acids profiles confirmed LAB activity. Lactic acid, absent in controls, reached 4200 and 3460 mg/L in CNP-F and Q-F respectively by T7d. Acetic acid also increased, while formic acid was more variable, peaking in Q-CTR at 48h (39 mg/L) before declining. Citric acid, detected only in CNP, decreased after fermentation, suggesting microbial consumption. 3-Hydroxybutyric acid was detected only in fermented samples, pointing to LAB-driven acetyl-CoA metabolism (Filannino et al., 2018).

Sugar metabolism showed pronounced matrix effects. In CNP beverages, derived from chestnut and persimmon, sucrose remained abundant throughout fermentation (23,000–31,600 mg/L) and declined modestly during fermentation, indicating partial microbial utilization. In contrast, Q beverages, dominated by starch-rich quinoa, contained lower sucrose levels (~7400 to 6460 mg/L), which declined modestly during fermentation, indicating also partial microbial utilization. These patterns reflect the intrinsic differences in raw materials composition. Sweet chestnut fruits are characterized by high free sugar contents, with sucrose levels reported up to ~29.9 g/100 g dry weight (Musilová et al., 2024), while persimmon flesh accumulates sucrose, glucose, and fructose as dominant soluble sugars (Dong et al., 2024). This large initial sugar pool in CNP beverages is only partially consumed by *L. casei*, explaining the persistently high sucrose and monosaccharide levels during fermentation and storage. In contrast, quinoa seeds allocate storage primarily to protein and starch rather than soluble sugars (Yang et al., 2024). These sugar trends align with broader compositional differences among the flours: chestnut and persimmon provide abundant soluble carbohydrates and simple phenolics, whereas quinoa contributes higher protein, starch and flavonoid glycosides but a lower free-sugar fraction. Rice flour mainly supplies starch with limited phenolic content, and black carrot is the principal source of chlorogenic acid isomers and anthocyanins (Dong et al., 2024; Hur et al., 2018; Kammerer et al., 2004). Together, these ingredient specific traits provide a biochemical rationale for the matrix-dependent differences in sugar utilization, phenolic retention, and anti-nutrient evolution observed in the fermented beverages.

Additional metabolites have further differentiated the matrices. Acetoin accumulated in both fermented beverages, consistent with pyruvate diversion (Li et al., 2023). Methanol concentrations were consistently higher in CNP than Q, even at T0, reflecting chestnut- and persimmon-derived methylated polysaccharides and pectin (Barreira et al., 2020). Scyllo-inositol was detected mainly in non-fermented samples, (e.g., in CNP-CTR at T7d it was 278 mg/L) likely reflecting its natural presence in plant tissues and gradual release during storage rather than microbial metabolism. Similar behavior has been reported for inositol isomers in fresh fruit juices (Paolini et al., 2023), suggesting that scyllo-inositol may serve as a marker of native plant matrix integrity rather than fermentation activity.

Several compounds highlight potential health relevance. GABA is associated with antihypertensive and glucose-regulating effects (Ngo & Vo, 2019), lactic acid contributes to gut health and mineral bioavailability (Markowiak & Ślizewska, 2017), and scyllo-inositol exhibits neuroprotective potential against amyloid aggregation (López-Gambero et al., 2020). Overall, NMR profiling demonstrated that fermentation reshaped amino acid, sugar, and organic acid pools in a matrix-dependent manner. CNP beverages retained higher sugar levels and showed greater methanol and scyllo-inositol accumulation, whereas Q beverages displayed richer nucleotide release. Both matrices supported lactic and acetic acid production, confirming effective LAB activity. These findings highlight that substrate composition, more than fermentation alone, drives the balance between nutrient preservation and functional enhancement. The higher levels of nucleic acids, purine bases, and ribonucleotides in Q beverages may result from combined microbial hydrolysis and endogenous quinoa enzyme activity, alongside limited cell-wall disruption, which facilitates nucleotides and nucleosides release during fermentation and storage (e.g., adenine, uracil, inosine phosphate; Verni et al., 2019).

3.3. Total polyphenols and polyphenols profile by UPLC-QqQ-MS/MS

Table 2 reports the 21 phenolic compounds identified in CNP and Q beverages at T0, T48h, and T7d by UPLC-QqQ-MS/MS together with TPC determined by Folin-Ciocalteu method. Overall, TPC remained above 80 % of baseline values, indicating substantial phenolic stability during fermentation and storage. In CNP beverages, the initially high TPC (1689 mg/L) declined moderately in both control and fermented samples by T7d (1425 and 1376 mg/L, respectively). In Q beverages, TPC remained stable up to 48h (1479 and 1468 mg/L in control and fermented samples, respectively) but decreased significantly by day 7 (1241 and 1291 mg/L in CTR and fermented samples, respectively). This indicates that both chestnut-persimmon and quinoa phenolics were also sensitive to storage. Similar declines in levels during storage and fermentation have previously been reported for chestnut- and quinoa-based matrices (Filannino et al., 2015).

In both matrices, chlorogenic acid was the predominant phenolic compound (231 and 154 mg/L at T0 in CNP and Q, respectively), together with neochlorogenic and cryptochlorogenic acids. The similar abundance of these chlorogenic acid isomers in both beverages likely reflects the contribution of black carrot, which is exceptionally rich in hydroxycinnamic acids such as chlorogenic acid (657 mg/kg; Kammerer et al., 2004). By contrast, quinoa generally contains only trace levels of chlorogenic derivatives (<0.5 mg/g DW; Hur et al., 2018), while chestnut and persimmon contribute only modest amounts (Donno et al., 2024; El Makhzangy et al., 2023). Taken together, these comparisons indicate that black carrot represents the primary source of chlorogenic acids in beverage formulations. CNP beverages exhibited a broader (17 out of the 21 compounds) and more concentrated phenolic profile than Q beverages. Ferulic acid was the only compound showing significant decline in CNP (from 21.0 to 5.3 and 7.2 mg/L in CTR and fermented beverages, respectively), consistent with possible LAB driven transformations such as ester hydrolysis or phenolic acid decarboxylation (Filannino et al., 2015). Other minor phenolics (e.g., gallocatechin, syringic acid) fluctuated without clear trend, suggesting limited

Table 2
Levels of identified polyphenols and total polyphenol content (TPC) in fermented and non-fermented chestnut–persimmon (CNP) and quinoa (Q) beverages. Fermentation was performed at 30 °C for 48 h (T2d), followed by storage at 4 °C for 5 days (T7d). Identified compounds were quantified and expressed as mg related to 1 L of beverage. TPC was expressed as mg of catechin equivalents per liter of beverage.

mg/L	Chestnut-Persimmon (CNP)					Quinoa (Q)				
		CTR		Fermented (F)			CTR		Fermented (F)	
	T0	T2d	T7d	T2d	T7d	T0	T2d	T7d	T2d	T7d
Neochlorogenic acid	28.2 ± 7.60a	23.1 ± 12.3a	25.1 ± 3.50a	25.7 ± 1.70a	22.3 ± 2.00a	27.6 ± 1.26a	27.9 ± 4.23a	19.9 ± 3.24a	28.9 ± 0.34a	24.4 ± 2.57a
Cryptochlorogenic acid	52.4 ± 12.8a	41.9 ± 30.1a	45.1 ± 8.40a	48.1 ± 2.10a	40.4 ± 4.10a	45.1 ± 1.13a	43.7 ± 6.45a	29.0 ± 8.77a	44.2 ± 2.19a	41.7 ± 5.40a
Chlorogenic acid	231 ± 64a	188 ± 16a	202 ± 21a	200 ± 13a	176 ± 17a	154 ± 5.5a	147 ± 22 ab	104 ± 24b	156 ± 3.6a	134 ± 16 ab
Fertaric acid	0.17 ± 0.04a	0.16 ± 0.08a	0.23 ± 0.06a	0.31 ± 0.20a	0.14 ± 0.03a	n.d	n.d	n.d	n.d	n.d
t-coutaric acid	0.62 ± 0.15a	0.38 ± 0.03a	0.63 ± 0.09a	0.28 ± 0.10a	0.34 ± 0.11 a	0.14 ± 0.00a	n.d	n.d	n.d	n.d
Gallocatechin	2.40 ± 1.11a	3.22 ± 0.00a	2.53 ± 1.66a	1.6 5 ± 0.00a	1.93 ± 0.35a	n.d	n.d	n.d	n.d	n.d
Procyanidins	4.10 ± 1.99a	3.61 ± 1.79a	4.32 ± 1.85a	4.65 ± 0.98a	5.03 ± 0.56a	1.59 ± 0.82a	2.37 ± 2.11a	0.68 ± 0.17a	0.52 ± 0.74a	1.37 ± 0.55a
Rutin	n.d	n.d	n.d	n.d	n.d	10.1 ± 1.31b	8.75 ± 1.76b	4.88 ± 0.43a	8.11 ± 0.15b	5.57 ± 0.63a
Quercetin-3-glucuronide	n.d	n.d	n.d	n.d	n.d	0.65 ± 0.44a	1.00 ± 0.16a	0.50 ± 0.37a	0.70 ± 0.56a	0.41 ± 0.35a
Vanillic acid	n.d	n.d	n.d	n.d	n.d	2.75 ± 0.15b	n.d	n.d	7.58 ± 0.03c	1.24 ± 0.00a
Syringic acid	0.19 ± 0.18a	0.12 ± 0.05a	0.084 ± 0.03a	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Caffeic acid	11.8 ± 4.80b	8.0 ± 1.95b	6.4 ± 1.68b	19.3 ± 6.67a	12.8 ± 4.97b	5.72 ± 1.86b	0.63 ± 0.00a	3.72 ± 0.00b	n.d	1.00 ± 0.45a
Ferulic acid	21.0 ± 5.7b	n.d	5.25 ± 4.49a	5.37 ± 4.22a	7.17 ± 3.10a	4.28 ± 0.32a	n.d	1.69 ± 0.57a	n.d	1.21 ± 0.16a
Gallic acid	332 ± 198a	347 ± 167a	302 ± 230a	278 ± 11a	283 ± 17a	n.d	n.d	n.d	n.d	n.d
Rosmarinic acid	38.3 ± 9.8a	32.4 ± 18.7a	32.4 ± 3.6a	23.8 ± 0.8a	22.9 ± 3.1a	n.d	n.d	n.d	n.d	4.44 ± 0.00
Myricitrin	0.13 ± 0.01a	0.13 ± 0.08a	0.15 ± 0.05a	0.19 ± 0.03a	0.076 ± 0.01a	n.d	n.d	n.d	n.d	n.d
Quercetin-3-Glc-Ara	0.12 ± 0.01a	n.d	0.15 ± 0.00a	0.12 ± 0.00a	0.14 ± 0.03a	3.28 ± 0.46b	3.44 ± 1.13b	1.41 ± 0.53a	3.33 ± 0.23b	3.30 ± 0.10b
2,4-diOH-benzoic acid	n.d	18.7 ± 0.23b	5.23 ± 0.58a	n.d	n.d	n.d	n.d	n.d	n.d	n.d
Protocatechuic acid	4.43 ± 2.55a	4.41 ± 5.69a	3.94 ± 2.06a	n.d	1.62 ± 0.00a	4.13 ± 2.02a	4.68 ± 2.16a	3.65 ± 1.75a	6.26 ± 2.22a	2.96 ± 1.46a
Sinapyl alcohol	45.6 ± 22.1b	27.2 ± 18.0a	22.5 ± 10.6a	20.0 ± 6.3a	11.5 ± 7.3a	1.98 ± 0.05a	3.56 ± 0.08a	4.23 ± 0.12a	n.d	1.95 ± 0.17a
Robinina	n.d	n.d	n.d	n.d	n.d	33.4 ± 1.9a	32.3 ± 7.8a	25.1 ± 6.5a	33.1 ± 0.4a	29.9 ± 5.7a
TPC (mg eq/L)	1689 ± 2.6c	1546 ± 55.4b	1425 ± 21.1 ab	1565 ± 70.8b	1376 ± 50.1a	1512 ± 37.4b	1479 ± 13.2b	1241 ± 67.0a	1468 ± 73 ab	1291 ± 29.0a

Values are expressed as mean ± standard deviation. For each beverage (CNP and Q), statistical comparisons were performed independently across fermentation times. For each compound: different lowercase letters in the same row indicate a significant difference between samples at 0, 2 and 7 days ($p < 0.05$).
n.d.: not detected.

enzymatic turnover. Q beverages displayed a narrow phenolic profile (15 out of 21 compounds) and a moderate decrease in rutin and quercetin-3-glucuronide, which may reflect β-glucosidase–mediated deglycosylation, an enzymatic reaction commonly reported in LAB fermentations and known to increase the bioaccessibility of the resulting aglycones (De Montijo-Prieto et al., 2023).

Several detected phenolics are associated with recognized health benefits. Chlorogenic acid exhibits antioxidant and glycemia-modulating properties (Naveed et al., 2018), and its metabolite, caffeic acid, is readily absorbed in the small intestine, contributing to increased plasma antioxidant capacity (Lafay et al., 2006). Rutin, present only in Q beverages, is associated with cardiovascular protection (Rahmani et al., 2023). Ferulic acid, more abundant in CNP, contributes to antioxidant activity and may generate bioactive microbial derivatives (Purushothaman & Rizwanullah, 2024), while protocatechuic acid is associated with anti-inflammatory and hepatoprotective effects (Zhang et al., 2021). Overall, fermentation with *L. casei* preserved over 80 % of total polyphenols in both matrices with CNP retaining higher absolute levels, and Q maintaining a richer but lower-intensity profile. These modest yet matrix dependent changes suggest that LAB-mediated bio--transformations may slightly influence the phenolic bioactivity and bioavailability, even when the overall concentrations remain largely stable.

3.4. Volatile organic compound (VOC) profile

HS-SPME/GC-MS analysis revealed that fermentation markedly reshaped the VOC profiles of both CNP and Q beverages, with aldehydes, ketones, alcohols, organic acids, terpenes, and minor volatiles showing distinct matrix dependent trends (Table 3). In general, fermentation suppressed lipid oxidation-derived aldehydes while enhancing alcohols, ketones, and acids, thereby increasing overall aroma complexity. In CNP beverages, aldehydes were strongly suppressed after fermentation, with hexanal decreasing from 334 to <24 μg/L and similar reductions

Table 3
Volatile organic compounds (VOCs) (μg/L eq of 2-octanol) detected in fermented and non-fermented chestnut–persimmon (CNP) and quinoa (Q) beverages by HS-SPME/GC-MS. Fermentation was performed at 30 °C for 48 h (T2d), followed by storage at 4 °C for 5 days (T7d).

	Odor characteristics[c]	Chestnut-Persimmon (CNP)					Quinoa (Q)				
			CTR		Fermented (F)			CTR		Fermented (F)	
		T0	T2d	T7d	T2d	T7d	T0	T2d	T7d	T2d	T7d
Aldehydes (μg/L eq of 2-octanol)											
Butanal	Green, pungent	7.4 ± 1.3a	0.7 ± 0.0b	1.4 ± 0.3b	3.3 ± 1.1b	4.0 ± 1.9b	5.3 ± 1.6a	0.9 ± 0.6b	1.1 ± 0.3b	1.1 ± 0.4b	1.1 ± 0.8b
2-methyl butanal	Malty, roasted/nutty	55.4 ± 22.5a	3.9 ± 1.0b	1.9 ± 1.2b	1.7 ± 0.3b	5.4 ± 2.0b	31.5 ± 20.4a	1.6 ± 0.7b	1.8 ± 0.3b	1.4 ± 0.4b	1.8 ± 0.9b
3-methyl butanal	Roasted, nutty, chocolate-like	18.4 ± 3.4a	1.2 ± 0.1b	1.03 ± 0.6b	0.6 ± 0.2c	2.2 ± 0.7b	19.2 ± 9.2a	0.7 ± 0.2b	0.7 ± 0.2b	0.7 ± 0.2b	0.7 ± 0.2b
Pentanal	Grassy, green	41.9 ± 5.2a	5.2 ± 3.0b	3.1 ± 2.0b	4.5 ± 1.3b	4.0 ± 1.3b	92.9 ± 33.2a	9.2 ± 6.7bc	20.0 ± 6.6b	8.2 ± 1.6c	21.0 ± 9.9b
Hexanal	Grassy, green, leafy	334 ± 25.3a	6.6 ± 1.4c	7.9 ± 2.1c	6.0 ± 1.4c	23.8 ± 10.6b	62.8 ± 28.5c	178.8 ± 137abc	355.3 ± 80.3a	164.5 ± 20.5b	368.5 ± 159a
Octanal	Citrus, pungent	7.2 ± 0.9a	0.7 ± 0.2b	0.5 ± 0.3b	0.6 ± 0.2b	1.1 ± 0.6b	2.2 ± 0.8b	3.3 ± 2.1b	7.1 ± 2.3a	2.8 ± 0.8b	6.1 ± 1.1a
(Z)-2-heptenal	Heavy, planty green odor, nutty	1.6 ± 0.3°	0.5 ± 0.1b	0.4 ± 0.1b	0.7 ± 0.3b	0.7 ± 0.3b	0.8 ± 0.4b	3.8 ± 1.3a	2.1 ± 0.8a	3.5 ± 1.5a	3.1 ± 1.2a
Furfural	Caramel-like, almond-like	7.0 ± 0.4a	1.8 ± 0.3c	2.6 ± 0.9bc	4.4 ± 1.0b	2.4 ± 1.2bc	3.1 ± 1.0 ab	3.4 ± 1.3 ab	1.9 ± 0.8b	6.6 ± 2.9a	3.2 ± 1.4 ab
Ketones (μg/L eq of 2-octanol)											
Diacetyl	Buttery, creamy	16.5 ± 3.1a	16.9 ± 13.2a	18.5 ± 5.4a	20.2 ± 6.3a	9.5 ± 1.9b	347.0 ± 122.7a	3.1 ± 0.5b	3.8 ± 2.0b	8.8 ± 4.4b	3.8 ± 1.3b
2-heptanone	Fruity, cheesy	21.0 ± 0.4a	1.3 ± 0.5b	2.2 ± 0.8b	2.4 ± 1.1b	1.9 ± 1.1b	2.9 ± 1.9b	2.6 ± 2.0 ab	7.9 ± 3.6 ab	3.0 ± 0.9b	8.2 ± 3.0a
4-methyl-2-heptanone	Fruity, green	2.8 ± 0.3a	2.9 ± 1.5a	1.6 ± 1.0a	2.4 ± 1.7a	2.6 ± 1.6a	1.3 ± 0.8b	3.1 ± 1.1a	2.2 ± 0.9 ab	1.8 ± 1.2 ab	1.5 ± 0.4b
6-methyl-5-hepten-2-one	Green-citrus	21.8 ± 0.2a	2.4 ± 0.3b	3.8 ± 1.0b	3.1 ± 0.7b	5.8 ± 1.4b	8.1 ± 2.9a A	5.2 ± 2.5a	6.9 ± 1.5a	4.9 ± 1.3a	7.0 ± 1.5a
Acetoin	Creamy, buttery	n.d.	57.6 ± 46.8a	4.6 ± 3.7b	22.7 ± 12.4 ab	18.2 ± 6.0 ab	94.8 ± 49.6a	6.8 ± 2.8 cb	7.8 ± 2.6 cb	9.1 ± 1.7b	3.6 ± 1.5c
Alcohols (μg/L eq of 2-octanol)											
2-methyl-1-butanol	Malty, fruity	2.4 ± 0.5a	2.4 ± 1.4a	0.9 ± 0.6a	2.7 ± 2.2a	2.4 ± 0.9a	1.2 ± 0.0a	2.6 ± 1.1 ab	1.7 ± 0.6a	3.2 ± 0.2b	1.2 ± 0.4a
3-methyl-1-butanol	Malty, fruity	3.1 ± 0.5 ab	8.4 ± 5.8a	1.5 ± 1.4b	9.9 ± 5.9a	3.2 ± 1.6 ab	1.1 ± 0.7a	5.0 ± 3.2a	2.7 ± 1.3a	10.0 ± 1.6c	1.9 ± 1.1a
2-methyl propanol	Green	n.d.	1.9 ± 0.6a	2.5 ± 1.5a	2.2 ± 1.6a	1.5 ± 1.2a	1.2 ± 0.7a, A	1.7 ± 0.7a	1.4 ± 0.7a	1.0 ± 0.9a	1.1 ± 0.6a
1-octen-3-ol	Earthy, mushroom-like	39.9 ± 0.5a	3.4 ± 1.3b	1.6 ± 1.2b	3.1 ± 1.9b	3.9 ± 2.0b	4.8 ± 3.1c	16.0 ± 4.4b	37.6 ± 14.0a	13.9 ± 1.9b	35.5 ± 9.1a
1-octanol	Fatty, green	5.1 ± 0.5a	2.3 ± 1.0b	1.4 ± 0.4b	2.3 ± 0.6b	2.2 ± 0.8b	1.4 ± 0.5c	4.9 ± 0.7b	9.5 ± 2.9a	4.7 ± 0.8Bb	9.7 ± 1.7ba
Hexanol	Green, grassy	14.8 ± 0.6a	8.5 ± 1.8b	2.6 ± 1.8c	7.7 ± 2.5b	7.0 ± 2.7b	11.9 ± 5.3a	20.7 ± 9.1a	22.5 ± 5.4a	16.9 ± 2.7a	16.5 ± 7.6a
2-ethyl-1-hexanol	Floral, green	n.d.	27.3 ± 14.5a	40.8 ± 14.9a	49.7 ± 12.7a	58.5 ± 28.6a	11.2 ± 5.4b	47.3 ± 9.8a	44.6 ± 11.3a	36.2 ± 13.3a	33.3 ± 10.3a
1-pentanol[a]	Green, fruity	39.6 ± 4.6a	2.0 ± 0.4b	1.4 ± 0.5b	2.0 ± 0.6b	2.8 ± 1.3b	3.9 ± 1.5a	20.5 ± 19.9b	70.4 ± 16.1c	20.5 ± 4.1b	73.9 ± 31.5a

(*continued on next page*)

Table 3 (*continued*)

	Odor characteristics[c]	Chestnut-Persimmon (CNP)					Quinoa (Q)				
			CTR		Fermented (F)			CTR		Fermented (F)	
		T0	T2d	T7d	T2d	T7d	T0	T2d	T7d	T2d	T7d
Organic Acids (μg/L eq of 2-octanol)											
Acetic acid	Sour, pungent	60.7 ± 2.2a	49.5 ± 18.2a	55.2 ± 23.7a	80.3 ± 28.0a	61.0 ± 20.8a	15.9 ± 6.7b	87.3 ± 34.6a	84.9 ± 53.6a	86.9 ± 48.8a	43.9 ± 45.6 ab
Propanoic acid	Cheesy, rancid	3.9 ± 0.6a	0.8 ± 0.4b	0.6 ± 0.2b	1.1 ± 0.5b	1.2 ± 0.5b	0.7 ± 0.3b	1.6 ± 0.9 ab	5.4 ± 2.1a	2.2 ± 1.0a	4.5 ± 3.1a
3-methyl butanoic acid	Cheesy	n.d.	2.4 ± 1.0a	1.0 ± 0.5a	1.5 ± 0.4a	2.3 ± 0.5a	39.7 ± 29.7a	2.2 ± 1.2b	2.2 ± 1.0b	4.1 ± 1.2b	0.5 ± 0.0c
Pentanoic acid	Sweaty, pungent	74.5 ± 1.4a	2.4 ± 1.0b	1.9 ± 0.5b	2.6 ± 0.8b	3.5 ± 1.2b	1.3 ± 0.8c	4.1 ± 3.0bc	34.2 ± 16.3a	6.6 ± 2.8b	31.3 ± 21.0a
Hexanoic acid	Fatty, cheesy	1628.4 ± 22.4a	37.6 ± 15.3bc	21.4 ± 8.3c	34.1 ± 11.1bc	59.3 ± 16.2b	6.8 ± 0.8c	149.6 ± 81.8b	679.6 ± 209.3a	193.5 ± 85.6 ab	707.6 ± 506.2a
Heptanoic acid	Fatty	106.9 ± 8.1a	4.0 ± 1.7b	3.7 ± 1.6b	4.0 ± 1.6b	6.8 ± 1.9b	2.4 ± 0.9c	4.9 ± 2.6bc	23.0 ± 8.2a	8.1 ± 2.4b	30.7 ± 17.5a
Octanoic acid	Fatty, oily	87.8 ± 9.4a	6.8 ± 2.2b	4.8 ± 1.9b	7.1 ± 3.5b	9.0 ± 4.0b	1.4 ± 0.6b	4.2 ± 2.1a	11.8 ± 3.5a	6.3 ± 1.7a	13.0 ± 7.5a
Nonanoic acid	Fatty, waxy	22.7 ± 3.2a	2.3 ± 0.8b	3.2 ± 1.0b	3.1 ± 2.0b	3.3 ± 1.0b	1.4 ± 0.8b	2.4 ± 1.8b	15.4 ± 7.6a	10.1 ± 8.9a	19.8 ± 15.9a
Terpenes and Lactones (μg/L eq of 2-octanol)											
Limonene	Citrus-sweet	n.d.	1.2 ± 0.8a	0.9 ± 0.3a	1.0 ± 0.3a	1.6 ± 0.9a	1.8 ± 0.6 A	1.2 ± 0.5a	1.1 ± 0.2a	1.4 ± 0.4a	1.0 ± 0.5a
α-ionone	Violet-like, sweet	n.d.	0.5 ± 0.2b	0.5 ± 0.2b	0.6 ± 0.2b	1.2 ± 0.3a	0.5 ± 0.2a	0.4 ± 0.2a	0.5 ± 0.2a	0.3 ± 0.0a	0.3 ± 0.1a
β-ionone	Sweet, woody, floral	1.9 ± 0.2a	0.8 ± 0.4b	0.4 ± 0.2b	0.8 ± 0.3a	1.3 ± 0.7 ab	n.d.	0.1 ± 0.1a	0.1 ± 0.1a	0.1 ± 0.0a	0.1 ± 0.0a
β-ionone epoxide	Sweet, floral	6.0 ± 1.9a	0.6 ± 0.3b	0.3 ± 0.1b	0.3 ± 0.2b	0.9 ± 0.4b	0.4 ± 0.2a	0.2 ± 0.2a	0.3 ± 0.2a	0.3 ± 0.2a	0.3 ± 0.1a
γ-butyrolactone	Creamy, buttery	5.0 ± 0.9a	3.5 ± 1.3a	4.1 ± 3.2a	8.1 ± 4.9a	5.5 ± 2.7a	1.7 ± 0.9a	1.4 ± 0.6a	1.9 ± 1.7a	1.8 ± 1.1a	1.9 ± 1.8a
γ-nonalactone	Coconut-like, sweet, creamy	43.2 ± 8.7a	2.1 ± 0.9b	1.9 ± 1.0b	2.4 ± 0.9b	3.4 ± 1.3b	1.8 ± 0.7a	1.9 ± 0.9a	3.3 ± 1.6 ab	2.7 ± 0.9 ab	3.6 ± 0.9b
γ-caprolactone	Sweet, milky	9.7 ± 1.5a	0.7 ± 0.4b	0.4 ± 0.2b	0.8 ± 0.3b	1.0 ± 0.4b	0.5 ± 0.2b	1.0 ± 0.5b	4.3 ± 1.7 ab	2.5 ± 1.5b	6.2 ± 2.7a
Others (μg/L eq of 2-octanol)											
2-pentyl furan	Green, beany	6.1 ± 1.0a,	1.4 ± 0.6b	0.6 ± 0.3b	0.9 ± 0.6b	1.8 ± 1.1b	3.1 ± 1.8b	5.7 ± 2.9 ab	13.0 ± 5.5a	5.7 ± 1.9 ab	6.8 ± 3.2a
α-terpinyl acetate	Sweet, fruity	6.4 ± 0.6	1.4 ± 0.5b	0.8 ± 0.6b	1.2 ± 0.4b	2.0 ± 1.0b	2.4 ± 0.8	1.7 ± 0.8a	1.5 ± 0.6a	1.9 ± 0.6a	1.4 ± 0.6a
tetrahydro-3-methyl-5-oxo-2-furancarboxylic acid or hexanoic anhydride[b]	Caramel-like, sweet	1.6 ± 0.1a	n.d.	n.d.	0.9 ± 0.1b	0.8 ± 0.2b	n.d.	4.2 ± 2.2b	13.7 ± 7.2a	5.7 ± 3.3 ab	21.7 ± 10.5a
2-methoxy phenol	Smoky, toasted	1.5 ± 0.3a	5.9 ± 3.7a	0.4 ± 0.3b	2.8 ± 1.5a	2.5 ± 1.2a	63.0 ± 23.4	37.0 ± 23.1a	33.8 ± 12.3a	4.5 ± 1.4b	3.5 ± 2.9b
2-acetylpyrrole	Nutty, roasted	15.0 ± 1.8a	6.6 ± 3.8 ab	3.6 ± 2.8b	8.6 ± 5.0 ab	10.5 ± 6.5 ab	5.3 ± 1.5	4.6 ± 2.3a	4.2 ± 2.1a	6.5 ± 2.3a	4.4 ± 3.8a
Phenol	Smoky	8.1 ± 1.4	4.6 ± 2.3b	4.5 ± 2.3b	4.1 ± 1.8a	5.5 ± 3.0a	13.6 ± 5.5a	6.9 ± 2.3ba	8.6 ± 5.2 ab	4.0 ± 1.9b	4.0 ± 3.0b

(*continued on next page*)

Table 3 (*continued*)

	Odor characteristics[c]	Chestnut-Persimmon (CNP)					Quinoa (Q)				
			CTR		Fermented (F)			CTR		Fermented (F)	
		T0	T2d	T7d	T2d	T7d	T0	T2d	T7d	T2d	T7d
2-phenoxy ethanol	Sweet–floral	1.4 ± 0.2	1.4 ± 0.8a	1.1 ± 1.5a	1.3 ± 1.1a	1.8 ± 1.5a	1.3 ± 0.1a	1.2 ± 1.0a	1.7 ± 1.6a	1.5 ± 1.4a	1.0 ± 0.4a

Values are expressed as mean ± standard deviation. For each beverage (CNP and Q), statistical comparisons were performed independently across fermentation times. For each VOC: different lowercase letters in the same row indicate a significant difference between non-fermented sample at 0, 2 and 7 days ($p < 0.05$).

[a] Tentative identification.

[b] Two or more tentative identifications present. n.d.: Not detected.

[c] Almaguer et al. (2022); Zhu et al. (2018); Wang et al., 2025; Frangipane et al. (2025).

observed for butanal and branched-chain aldehydes. These compounds are typically associated with green, grassy, and fatty off-notes, therefore, their suppression is expected to improve the characteristic chestnut-like aroma. In contrast, Q beverages showed an initial decrease in aldehydes, followed by a sharp rise in hexanal during storage (>350 μg/L at T7d), indicating greater oxidative susceptibility of the pseudocereal matrix (K. Liu et al., 2017). Ketones displayed matrix specific behavior. Diacetyl and acetoin accumulated transiently in CNP-F at T48h, consistent with LAB mediated pyruvate diversion (Wang et al., 2021). These compounds are associated with buttery, creamy, and malty aromas, contributing positively to the sensory profile. In Q beverages, ketone levels were already elevated at T0, likely due to the processing related reactions (e.g., Maillard reactions during heat treatment), and declined thereafter. 2-Heptanone increased in Q during storage, consistent with lipid oxidation processes previously reported in dairy systems (Y. Xi et al., 2023). Alcohol became a prominent contributor to aroma in both beverages. Higher alcohols such as 2- and 3-methyl-1-butanol increased after fermentation, providing malty, fruity, and nutty notes in CNP, while 2-ethyl-1-hexanol accumulated during storage, associated with floral and sweet perceptions. Lipid-derived alcohols (e.g., 1-octen-3-ol and hexanol) declined in CNP-F but increased in Q-F, further reflecting enhanced oxidation in quinoa. Organic acids clearly differentiated the matrices: Q beverages accumulated high levels of medium-chain fatty acids (MCFA) (e.g., hexanoic acid up to 707 μg/L), which may impart fruity or cheesy notes at low levels but cause rancid off-flavors at higher concentrations (Chaves-López et al., 2020). In contrast CNP beverages contained substantially lower MCFA concentrations. Acetic acid increased in both beverages (~80–87 μg/L at T48h), reflecting LAB metabolism (Filannino et al., 2015) and contributing mild sour or fermented notes. Terpenes and lactones followed divergent trends: CNP showed post-fermentation release of limonene, imparting citrus-fresh notes, and α-ionone, likely derived from glycosidic precursors via microbial β-glucosidase activity. Conversely, Q beverages accumulated γ-caprolactone and γ-butyrolactone during storage, typical markers of lipid oxidation, associated with creamy and fatty nuances (H. Liu et al., 2023).

From a sensory standpoint, aldehydes suppression in CNP-F likely reduced grassy and rancid notes, while increases in higher alcohols and acetoin contributed fruity, malty, and buttery aromas. In Q beverages, accumulation of MCFA and lipid-derived volatiles such as 1-octen-3-ol is expected to enhance earthy, mushroom-like, and fatty notes, which at high levels may compromise flavor balance. Therefore, oxidation control will be critical for future product development. Potential mitigation strategies include supplementation with natural antioxidants such as tocopherols or ascorbic acid (Shahidi & Zhong, 2010), inactivation of pro-oxidant lipoxygenases via mild heat treatment or high-pressure processing (Waraho, McClements, & Decker, 2011), inert-gas flushing during storage (Shahidi & Zhong, 2010), microencapsulation of unsaturated lipids, and the use of chelating agents to suppress formation of hexanal, pentanal, and C6–C10 fatty acid-derived off-notes (McClements, 2010).

It should be noted that VOC analysis was performed on freeze-dried samples reconstituted to 50 g/L. Although this approach ensures matrix homogeneity and minimizes viscosity-related extraction bias, freeze-drying may cause partial loss of highly volatile compounds or alter matrix–volatile interactions. Consequently, VOC results should be interpreted as semi-quantitative and indicative of matrix-dependent trends rather than absolute profiles.

3.5. Anti-nutritional factors quantification

Anti-nutritional factors are presented in Fig. 2. Initial phytic acid concentrations were 1.02 ± 0.07 and 1.85 ± 0.19 g/100g DW in CNP and Q beverages, respectively. Phytic acid chelates minerals such as iron, zinc, and calcium, reducing their bioavailability, and can also impair protein digestion (Pontonio et al., 2020). Fermentation significantly reduced phytic acid levels in both beverages during incubation, with reductions persisting throughout storage in Q-F and continuing to decrease in CNP-F beverages. CTR samples showed no significant changes. These trends are consistent with literature reports, attributing phytic acid degradation to microbial phytases activity during fermentation (De Angelis et al., 2003).

Initial RFOs were 1.95 ± 0.17 and 2.37 ± 0.23 mmol/100 g DW in CNP and Q, respectively. RFOs resist digestion due to the lack of α-galactosidase in the human gastrointestinal tract, allowing them to reach the colon, where microbial fermentation produces short-chain fatty acids (SCFAs) that benefit gut health, but also generate excessive gas, leading to bloating and discomfort (Teixeira et al., 2012). LAB fermentation effectively mitigates these effects via endogenous α-galactosidase, hydrolyzing raffinose into digestible monosaccharides (Harlé et al., 2020). In CNP beverages, both control and fermented samples showed reductions after incubation, with greater depletion in fermented sample, that remained stable during storage. In Q beverages, complete RFOs hydrolysis occurred in fermented samples after incubation, whereas CTR required storage to reach similar degradation.

Fructans were initially measured at 0.68 ± 0.11 and 0.28 ± 0.05 g/100g in CNP and Q, respectively. These carbohydrates are incompletely absorbed in the small intestine and may trigger gastrointestinal symptoms, particularly in individuals with functional bowel disorders. LAB can enzymatically convert fructans into fructose, mitigating these adverse effects (Viretto, Tlais, Tuccillo, et al., 2025). In CNP beverages, fructans decreased slightly but significantly after incubation, with no further changes during storage. Conversely, in Q beverages, fructans levels remained stable in fermented samples while a slight non-significant increase was observed in CTR after the storage.

3.6. In vitro protein digestibility (IVPD) and protein digestibility corrected amino acid score (PDCAAS)

Protein quality and digestibility are shown in Fig. 3. Plant matrices generally exhibit lower digestibility than animal-derived proteins due to structural complexity and the presence of anti-nutritional factors (Gomes & Sobral, 2022). However, relatively high initial digestibility values were observed in CNP (82 %) and Q (80 %) samples when

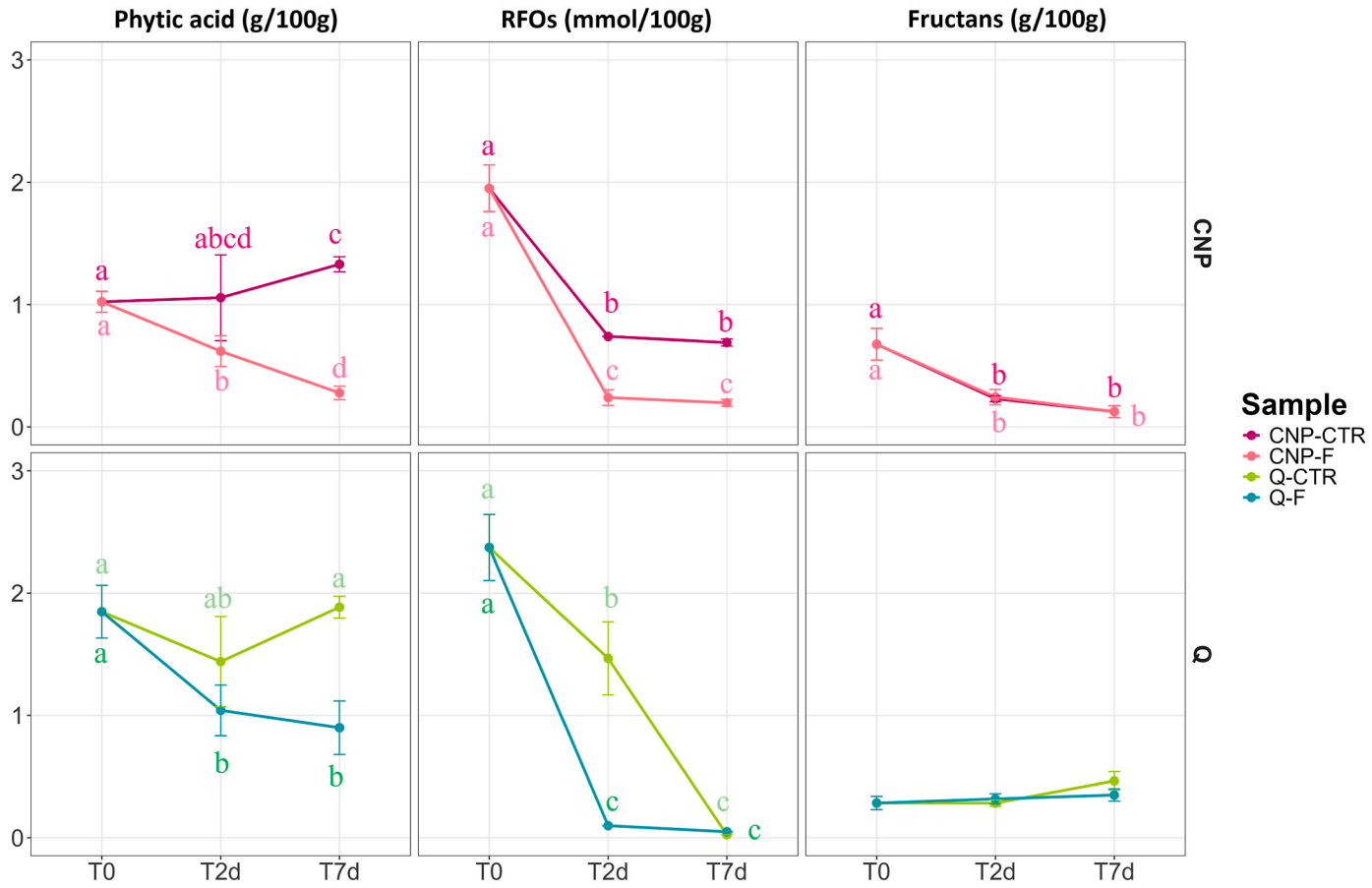

Fig. 2. Antinutritional factor values in fermented and non-fermented chestnut–persimmon (CNP) and quinoa (Q) beverages. Fermentation was performed at 30 °C for 48 h (T2d), followed by storage at 4 °C for 5 days (T7d). Values are expressed as mean ± standard deviation. Different lowercase letters indicate significant differences within each beverage prototype ($p < 0.05$).

compared with other plant-based formulations (Viretto, Tlais, Tuccillo, et al., 2025).

LAB fermentation significantly improved protein digestibility during incubation, reaching approximately 85 %, in both beverages and remained stable during storage. A similar improvement was also observed in Q-CTR, suggesting the role of LAB activity in reducing anti-nutritional compounds that otherwise could inhibit protease action and amino acid absorption (De Pasquale et al., 2020). In addition, LAB may secrete proteolytic enzymes that hydrolyze storage proteins into smaller peptides and free amino acids, enhancing gastric and pancreatic accessibility (Yousif & El Tinay, 2001). This microbial metabolism can also modulate amino acid profiles through selective degradation or *de novo* synthesis, potentially improving limiting amino acids (Mockus et al., 2024).

Based on FAO reference values, amino acids scores (AAS) and PDCAAS were calculated. The limiting amino acid differed between beverages: lysine in CNP and L-isoleucine in Q. Although incubation and storage did not alter the identity of the limiting amino acid, incubation significantly increased the AAS and, consequently, the PDCAAS in both control and fermented Q beverages, with values remaining stable during storage. In CNP, fermentation shifted the limiting amino acid to L-isoleucine and resulted in greater improvements in AAS and PDCAAS compared with the CTR. Storage further enhanced both parameters, with fermented CNP consistently showing superior protein quality.

4. Conclusions

Both chestnut-persimmon and quinoa beverages supported vigorous LAB fermentation reaching 8.5–8.7 log CFU/mL and pH values of 3.7–4.0 and maintained high microbial viability during refrigerated storage. NMR spectroscopy revealed substantial lactic acid production, matrix-dependent sugar use (high and persistent in CNP; lower and slightly declining in Q beverages), and selective remodeling of organic and amino acid pools. Polyphenols were largely preserved (>80 % TPC); with chlorogenic acid isomers remaining dominant and stable; CNP beverages retained higher absolute phenolic levels whereas Q beverages exhibited a leaner flavanols profile. VOCs evolution was strongly matrix dependent: CNP beverages showed suppression of aldehydes and modest increases in higher alcohols and acetoin contributing fruity, malty, and buttery notes, while Q beverages accumulated lipid-derived volatiles and MCFAs during storage associated with fatty notes, and potential rancidity at elevated concentrations. Fermentation also significantly reduced anti-nutritional factors and improved protein digestibility and PDCAAS in both formulations.

Considering their distinct nutritional and aromatic profiles, the two prototypes offer different potential applications and consumer targets. The chestnut–persimmon beverage (CNP), characterized by higher sugar availability, rapid acidification, superior polyphenol retention, reduced anti-nutrient levels, and a cleaner fruity/malty aroma, is well suited for consumers seeking mild-tasting, functional, and easily digestible plant-based beverages. Its gluten-free formulation, improved PDCAAS, and fermentability stability make it attractive particularly for children, older adults, and individuals with sensitive digestion or mineral-absorption concerns. In contrast, the quinoa-based beverage (Q), with its lower sugar load, richer nucleotide release, higher protein/starch contribution, and more complex VOC signature, may appeal to health-conscious adults, athletes, and individuals following low-sugar, nutrient-dense diets. Its antioxidant stability and pseudocereal origin also further

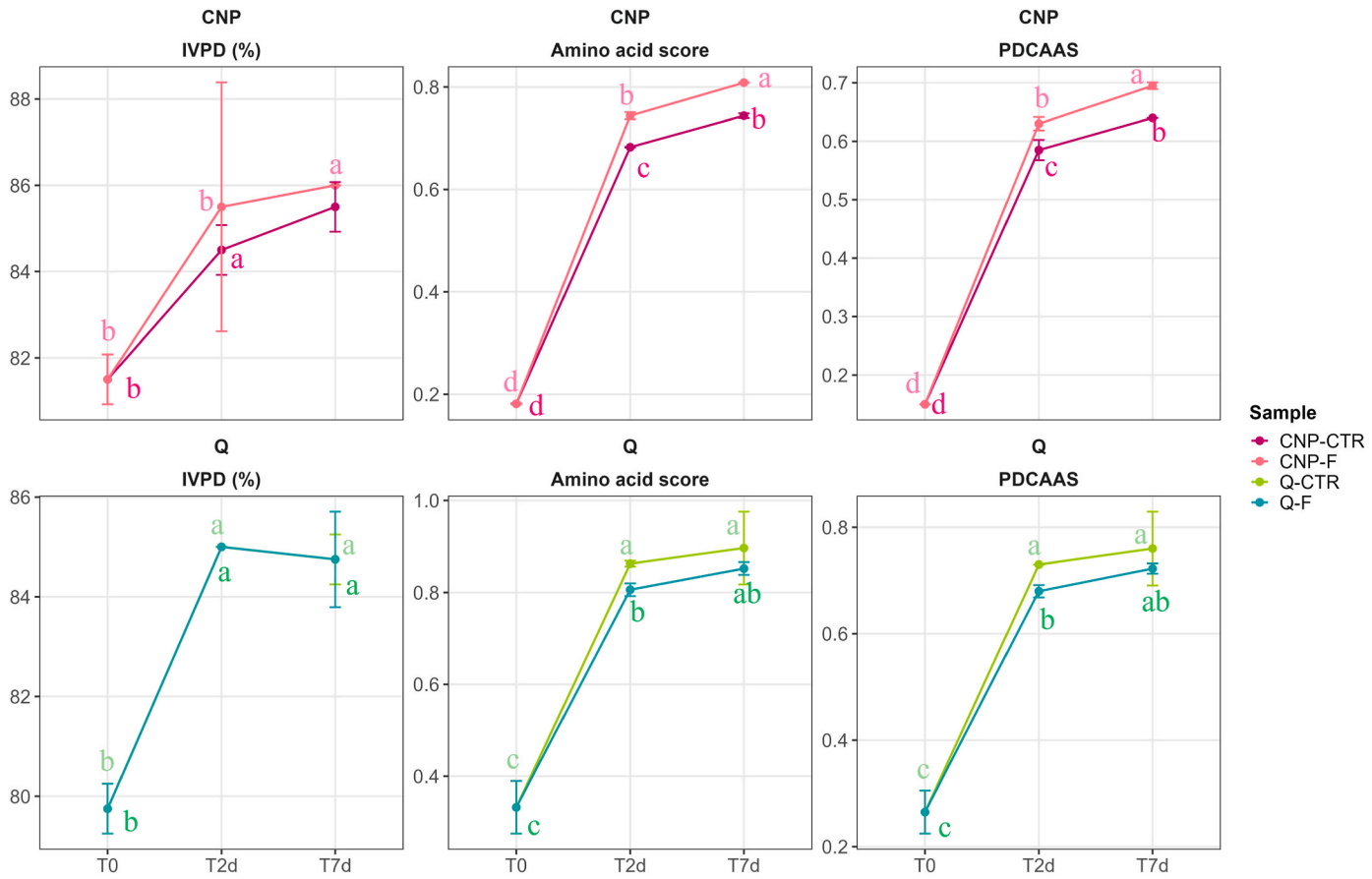

Fig. 3. Protein digestibility (IVPD %) and protein digestibility corrected amino acids score (PDCAAS) of fermented and non-fermented chestnut–persimmon (CNP) and quinoa (Q) beverages. Fermentation was performed at 30 °C for 48 h (T2d), followed by storage at 4 °C for 5 days (T7d). Values are expressed as mean ± standard deviation. Different lowercase letters indicate significant differences within each beverage prototype ($p < 0.05$).

position it as a promising base for fortified functional beverages or balanced meal-replacement products.

Overall, these complementary attributes highlight how lactic fermentation can be strategically applied to tailor plant-based beverages to different nutritional and consumer needs. As this study was conceived as a preliminary proof-of-concept, future studies will incorporate structured sensory evaluation to validate the relationship between VOC evolution and perceived flavor, color, and texture.

Consent for publication

Not applicable.

CRediT authorship contribution statement

Giorgia Secchi: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Adineh Tajmousavilangerudi:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Chiara Viretto:** Investigation, Formal analysis. **Ali Zein Alabiden Tlais:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Andrea Angeli:** Investigation, Formal analysis. **Urska Vrhovsek:** Visualization, Supervision. **Federico Brigante:** Investigation, Formal analysis. **Martina Moretton:** Writing – review & editing, Investigation, Formal analysis. **Emanuela Betta:** Writing – review & editing, Investigation, Formal analysis. **Iuliia Khomenko:** Investigation, Formal analysis. **Franco Biasioli:** Writing – review & editing, Visualization, Supervision. **Pavel Solovyev:** Writing – review & editing, Investigation, Formal analysis. **Luana Bontempo:** Writing – review & editing, Supervision. **Andrea Mancini:** Investigation, Formal analysis. **Elena Franciosi:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Disclosure statement

The authors report there are no competing interests to declare.

Data availability statement

Majority of the data are displayed in the main manuscript but also in the supplementary material. Further data will be made available on request.

Ethical statement

Not applicable.

Funding

This study was carried out within the ONFoods - Research and innovation network on food and nutrition Sustainability, Safety and Security - Working ON Foods and received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) - MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.3 – D. D. 1550 11/10/2022, PE00000003) as well as from Provincia Autonoma di Trento (FRUITOMICS, FESR, 2014–2020). This manuscript reflects only the authors' views and opinions, neither the European Union nor the European Commission can be considered responsible for them.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to express their sincere gratitude to SACCO S. r.l. (Cadorago, CO, Italy) for the generous donation of the *Lacticaseibacillus casei* Lyofast BGP 93 strain, which was essential for the development of this research.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2026.119054.

References

Akhtar, S., Rauf, A., Imran, M., Qamar, M., Riaz, M., & Mubarak, M. S. (2017). Black carrot (Daucus carota L.), dietary and health promoting perspectives of its polyphenols: A review. *Trends in Food Science and Technology, 66*, 36–47. https://doi.org/10.1016/j.tifs.2017.05.004

Almaguer, C., Kollmannsberger, H., Gastl, M., & Becker, T. (2022). Characterization of the aroma profile of quinoa (*Chenopodium quinoa Willd.*) and assessment of the impact of malting on the odor-active volatile composition. *Journal of the Science of Food and Agriculture, 103*, 2283–2294. https://doi.org/10.1002/jsfa.12418

Barreira, J. C. M., Ferreira, I. C. F. R., & Oliveira, M. B. P. P. (2020). Bioactive compounds of chestnut (Castanea sativa Mill.), 1–11 https://doi.org/10.1007/978-3-030-06120-3_18-1.

Brigante, F. I., Solovyev, P., Fabroni, S., Campanelli, G., Montemurro, F., & Bontempo, L. (2025). Classification of farming systems by NMR widely targeted metabolomics: A cauliflower case study. *Journal of Food Composition and Analysis, 138*(September 2024), Article 107018. https://doi.org/10.1016/j.jfca.2024.107018

Carafa, I., Stocco, G., Nardin, T., Larcher, R., Bittante, G., Tuohy, K., & Franciosi, E. (2019). Production of naturally γ-Aminobutyric acid-enriched cheese using the dairy strains *Streptococcus thermophilus* 84C and *Lactobacillus brevis* DSM 32386. *Frontiers in Microbiology, 10*, 93. https://doi.org/10.3389/fmicb.2019.00093. Feb 13.

Cerdá-Bernad, D., Valero-Cases, E., Pastor, J. J., Frutos, M. J., & Pérez-Llamas, F. (2022). Probiotic red quinoa drinks for celiacs and lactose intolerant people: Study of functional, physicochemical and probiotic properties during fermentation and gastrointestinal digestion. *International Journal of Food Sciences & Nutrition, 73*(1), 49–59. https://doi.org/10.1080/09637486.2021.1921707

Chaves-López, C., Rossi, C., Maggio, F., Paparella, A., & Serio, A. (2020). Changes occurring in spontaneous maize fermentation: An overview. *Fermentation, 6*(1). https://doi.org/10.3390/FERMENTATION6010036

De Angelis, M., Gallo, G., Corbo, M. R., McSweeney, P. L. H., Faccia, M., Giovine, M., & Gobbetti, M. (2003). Phytase activity in sourdough lactic acid bacteria: Purification and characterization of a phytase from Lactobacillus sanfranciscensis CB1. *International Journal of Food Microbiology, 87*(3), 259–270. https://doi.org/10.1016/S0168-1605(03)00072-2

De Montijo-Prieto, S., Razola-Díaz, M. D. C., Barbieri, F., Tabanelli, G., Gardini, F., Jiménez-Valera, M., Ruiz-Bravo, A., Verardo, V., & Gómez-Caravaca, A. M. (2023). Impact of lactic acid bacteria fermentation on phenolic compounds and antioxidant activity of avocado leaf extracts. *Antioxidants, 12*(2), 298. https://doi.org/10.3390/antiox12020298

De Pasquale, I., Pontonio, E., Gobbetti, M., & Rizzello, C. G. (2020). Nutritional and functional effects of the lactic acid bacteria fermentation on gelatinized legume flours. *International Journal of Food Microbiology, 316*. https://doi.org/10.1016/j.ijfoodmicro.2019.108426

Diana, M., Quílez, J., & Rafecas, M. (2014). Gamma-aminobutyric acid as a bioactive compound in foods: A review. *Journal of Functional Foods, 10*, 407–420. https://doi.org/10.1016/j.jff.2014.07.004

Dong, Y., Liu, C., Gong, B., Yang, X., Wu, K., Yue, Z., & Xu, Y. (2024). Analysis of the correlation between persimmon fruit-sugar components and taste traits from germplasm evaluation. *International Journal of Molecular Sciences, 25*(14), 7803. https://doi.org/10.3390/ijms25147803

Donno, D., Turrini, F., Farinini, E., Mellano, M. G., Boggia, R., Beccaro, G. L., & Gamba, G. (2024). Chestnut episperm as a promising natural source of phenolics from agri-food processing by-Products: Optimisation of a sustainable extraction protocol by ultrasounds. *Agriculture, 14*(2), 246. https://doi.org/10.3390/agriculture14020246

El Makhzangy, A., Hamad, D., & A, E.-S. (2023). Chemical and bioactive composition in persimmon (Diospyros kaki) fruits. *Mathews Journal of Nutrition & Dietetics, 6*(2). https://doi.org/10.30654/MJND.10023

Filannino, P., Bai, Y., Di Cagno, R., Gobbetti, M., & Gänzle, M. G. (2015). Metabolism of phenolic compounds by Lactobacillus spp. during fermentation of cherry juice and broccoli puree. *Food Microbiology, 46*, 272–279. https://doi.org/10.1016/j.fm.2014.08.018

Filannino, P., Di Cagno, R., & Gobbetti, M. (2018). Metabolic and functional paths of lactic acid bacteria in plant foods: Get out of the labyrinth. *Current Opinion in Biotechnology, 49*, 64–72. https://doi.org/10.1016/j.copbio.2017.07.016

Frangipane, M. T., Costantini, L., Garzoli, S., Merendino, N., Massantini, R., & Corona, P. (2025). Organic vs. conventional chestnuts (Castanea sativa Mill.): A focus on antioxidant activity, volatile compounds, and sensory profile. *Foods, 14*. https://doi.org/10.3390/foods14122013, 2013.

Gomes, A., & Sobral, P. J. D. A. (2022). Plant protein-based delivery systems: An emerging approach for increasing the efficacy of lipophilic bioactive compounds. *Molecules, 27*(1), 1–30. https://doi.org/10.3390/molecules27010060

González, C. M., Gil, R., Moraga, G., & Salvador, A. (2021). Natural drying of astringent and non-astringent persimmon "Rojo brillante". Drying kinetics and physico-chemical properties. *Foods, 10*(3), 647. https://doi.org/10.3390/foods10030647

Harlé, O., Falentin, H., Niay, J., Valence, F., Courselaud, C., Chuat, V., Maillard, M. B., Guédon, É., Deutsch, S. M., & Thierry, A. (2020). Diversity of the metabolic profiles of a broad range of lactic acid bacteria in soy juice fermentation. *Food Microbiology, 89*(August), Article 103410. https://doi.org/10.1016/j.fm.2019.103410

Hur, J., Nguyen, T. T. H., Park, N., Kim, J., & Kim, D. (2018). Characterization of quinoa (Chenopodium quinoa) fermented by Rhizopus oligosporus and its bioactive properties. *AMB Express, 8*(1), 143. https://doi.org/10.1186/s13568-018-0675-3

Hurtado-Murillo, J., Franco, W., & Contardo, I. (2025). Impact of homolactic fermentation using Lactobacillus acidophilus on plant-based protein hydrolysis in quinoa and chickpea flour blended beverages. *Food Chemistry, 463*(P1), Article 141110. https://doi.org/10.1016/j.foodchem.2024.141110

Isern, N. G., Xue, J., Rao, J. V., Cort, J. R., & Ahring, B. K. (2013). Novel monosaccharide fermentation products in Caldicellulosiruptor saccharolyticus identified using NMR spectroscopy. *Biotechnology for Biofuels, 6*(1), 1–11. https://doi.org/10.1186/1754-6834-6-47

Jokar, A., & Azizi, M. H. (2022). Formulation and production of persimmon milk drink and evaluation of its physicochemical, rheological, and sensorial properties. *Food Science and Nutrition, 10*(4), 1126–1134. https://doi.org/10.1002/fsn3.2772

Kammerer, D., Carle, R., & Schieber, A. (2004). Quantification of anthocyanins in black carrot extracts (Daucus carota ssp. sativus var. atrorubens Alef.) and evaluation of their color properties. *European Food Research and Technology, 219*(5), 479–486. https://doi.org/10.1007/s00217-004-0976-4

Khairuddin, M. A. N., & Lasekan, O. (2021). Gluten-free cereal products and beverages: A review of their health benefits in the last five years. *Foods, 10*(11). https://doi.org/10.3390/foods10112523

Khan, M., Singh, K., & Shruti, P. (2021). Quality aspects of rice based fermented beverage. *Biomedical Journal of Scientific & Technical Research, 34*(5), 27096–27104. https://doi.org/10.26717/BJSTR.2021.34.005609

Lafay, S., Morand, C., Manach, C., Besson, C., & Scalbert, A. (2006). Absorption and metabolism of caffeic acid and chlorogenic acid in the small intestine of rats. *British Journal of Nutrition, 96*(1), 39–46. https://doi.org/10.1079/BJN20051714

Lai, Q. D., Doan, N. T. T., & Nguyen, T. H. (2025). Enhancing gamma aminobutyric acid synthesis from defatted rice bran extract by *Lactobacillus brevis* VTCC-B397: Technical parameters analysis. *International Journal of Food Science and Technology, 60*(1). https://doi.org/10.1093/ijfood/vvaf015

Lalaleo, L., Hidalgo, D., Valle, M., Calero-Cáceres, W., Lamuela-Raventós, R. M., & Becerra-Martínez, E. (2020). Differentiating, evaluating, and classifying three quinoa ecotypes by washing, cooking and germination treatments, using 1H NMR-based metabolomic approach. *Food Chemistry, 331*(December 2018), Article 127351. https://doi.org/10.1016/j.foodchem.2020.127351

Li, Y., Zhao, X., Yao, M., Yang, W., Han, Y., Liu, L., Zhang, J., & Liu, J. (2023). Mechanism of microbial production of acetoin and 2,3-butanediol optical isomers and substrate specificity of butanediol dehydrogenase. *Microbial Cell Factories, 22*(1), 1–19. https://doi.org/10.1186/s12934-023-02163-6

Liu, K., Li, Y., Chen, F., & Yong, F. (2017). Lipid oxidation of brown rice stored at different temperatures. *International Journal of Food Science and Technology, 52*(1), 188–195. https://doi.org/10.1111/ijfs.13265

Liu, H., Ni, Y., Yu, Q., & Fan, L. (2023). Evaluation of co-fermentation of L. plantarum and P. kluyveri of a plant-based fermented beverage: Physicochemical, functional, and sensory properties. *Food Research International, 172*(May), Article 113060. https://doi.org/10.1016/j.foodres.2023.113060

López-Gambero, A. J., Sanjuan, C., Serrano-Castro, P. J., Suárez, J., & Rodríguez de Fonseca, F. (2020). The biomedical uses of inositols: A nutraceutical approach to metabolic dysfunction in aging and neurodegenerative diseases. *Biomedicines, 8*(9), 295. https://doi.org/10.3390/biomedicines8090295

Magala, M., Kohajdová, Z., Karovičová, J., Greifová, M., & Hojerová, J. (2015). Application of lactic acid bacteria for production of fermented beverages based on rice flour. *Czech Journal of Food Sciences, 33*(5), 458–563. https://doi.org/10.17221/74/2015-CJFS

Markowiak, P., & Ślizewska, K. (2017). Effects of probiotics, prebiotics, and synbiotics on human health. *Nutrients, 9*(9). https://doi.org/10.3390/nu9091021

McClements. (2010). Emulsion design to improve the delivery of functional lipophilic components Annu. *Reviews In Food Science and Technology, 1*, 241–269. https://doi.org/10.1146/annurev.food.080708.100722

Meena, K. K., Taneja, N. K., Jain, D., Ojha, A., Saravanan, C., & Bunkar, D. S. (2023). Spontaneously fermented cereal based products: An Ancient health promoting formulae for consumption of probiotic lactic acid bacteria. *Biointerface Research in Applied Chemistry, 13*(5), 1–26. https://doi.org/10.33263/BRIAC135.465

Mockus, E., Starkute, V., Klupsaite, D., Bartkevics, V., Borisova, A., Sarunaite, L., Arlauskiene, A., Rocha, J. M., & Bartkiene, E. (2024). Changes in chemical composition of lentils, including gamma-aminobutyric acid and volatile compound formation during submerged and solid-state fermentation with Pediococcus acidilactici. *Foods, 13*(8), 1249. https://doi.org/10.3390/foods13081249

Moiseenko, K. V., Glazunova, O. A., & Fedorova, T. V. (2024). Fermentation of rice, oat, and wheat flour by pure cultures of common starter lactic acid bacteria: Growth dynamics, sensory evaluation, and functional properties. *Foods, 13*(15). https://doi.org/10.3390/foods13152414

Musilová, J., Fedorková, S., Podhorecká, K., et al. (2024). Carbohydrates and mineral substances in sweet chestnuts (Castanea sativa Mill.) from important growing areas in Slovakia. *European Food Research and Technology, 250*, 565–572. https://doi.org/10.1007/s00217-023-04408-5

Najman, K., Ponikowska, P., Sadowska, A., Hallmann, E., Wasiak-Zys, G., Świderski, F., & Buczak, K. (2024). Physicochemical properties and nutritional relevance of rice beverages available on the market. *Applied Sciences (Switzerland), 14*(19). https://doi.org/10.3390/app14199150

Naveed, M., Hejazi, V., Abbas, M., Kamboh, A. A., Khan, G. J., Shumzaid, M., Ahmad, F., Babazadeh, D., FangFang, X., Modarresi-Ghazani, F., WenHua, L., & XiaoHui, Z. (2018). Chlorogenic acid (CGA): A pharmacological review and call for further research. *Biomedicine & Pharmacotherapy, 97*, 67–74. https://doi.org/10.1016/j.biopha.2017.10.064

Ngo, D.-H., & Vo, T. S. (2019). An updated review on pharmaceutical properties of gamma-aminobutyric acid. *Molecules, 24*(15), 2678. https://doi.org/10.3390/molecules24152678

Paolini, M., Perini, M., Allari, L., Tonidandel, L., Finato, F., Guardini, K., & Larcher, R. (2023). Myo-Inositol, scyllo-inositol, and other minor carbohydrates as authenticity markers for the control of Italian bulk, concentrate, and rectified grape must. *Molecules, 28*(8). https://doi.org/10.3390/molecules28083609

Pontonio, E., Raho, S., Dingeo, C., Centrone, D., Carofiglio, V. E., & Rizzello, C. G. (2020). Nutritional, functional, and technological characterization of a novel Gluten- and lactose-free yogurt-style snack produced with selected lactic acid bacteria and leguminosae flours. *Frontiers in Microbiology, 11*. https://doi.org/10.3389/fmicb.2020.01664

Popova, A., Mihaylova, D., & Lante, A. (2023). Insights and perspectives on plant-based beverages. *Plants, 12*(19), 3345. https://doi.org/10.3390/plants12193345

Purushothaman, J. R., & Rizwanullah, M. (2024). Ferulic acid: A comprehensive review. *Cureus*. https://doi.org/10.7759/cureus.68063

Rahmani, S., Naraki, K., Roohbakhsh, A., Hayes, A. W., & Karimi, G. (2023). The protective effects of rutin on the liver, kidneys, and heart by counteracting organ toxicity caused by synthetic and natural compounds. *Food Science and Nutrition, 11*(1), 39–56. https://doi.org/10.1002/fsn3.3041

Rathod, N., Soni, A., Meena, P., & Sharma, H. (2025). Non-dairy dietary approach for lactose intolerant. In *Lactose hydrolysis in dairy products* (pp. 119–137). Nature Switzerland: Springer. https://doi.org/10.1007/978-3-031-78207-7_7.

Shahidi, F., & Zhong, Y. (2010). Lipid oxidation and improving the oxidative stability. *Chemical Society Reviews, 39*, 4067–4079. https://doi.org/10.1039/b922183m

Teixeira, P. J., Carraça, E. V., Markland, D., Silva, M. N., & Ryan, R. M. (2012). Exercise, physical activity, and self-determination theory: A systematic review. *International Journal of Behavioral Nutrition and Physical Activity, 9*. https://doi.org/10.1186/1479-5868-9-78

Verni, M., Rizzello, C. G., & Coda, R. (2019). Fermentation biotechnology applied to cereal industry By-products: Nutritional and functional insights. *Frontiers in Nutrition, 6*. https://doi.org/10.3389/fnut.2019.00042

Viretto, C., Tlais, A. Z. A., Arora, K., Ameur, H., Tuccillo, F., Polo, A., Ardèvol, V. N., Verté, F., Katina, K., Di Cagno, R., & Gobbetti, M. (2025). Unraveling the functional potential of microbial resources and pulse-based matrices for sourdough breadmaking. *Future Foods, 11*, Article 100643. https://doi.org/10.1016/j.fufo.2025.100643

Viretto, C., Tlais, A. Z. A., Tuccillo, F., Polo, A., Arora, K., Verté, F., Katina, K., Di Cagno, R., & Gobbetti, M. (2025). Maximize the synergistic interactions among microbial consortia and plant-based matrices to design fermented cereal-pulse-based beverages. *Food Research International, 220*(July). https://doi.org/10.1016/j.foodres.2025.117045

Vrhovsek, U., Masuero, D., Gasperotti, M., Franceschi, P., Caputi, L., Viola, R., & Mattivi, F. (2012). A versatile targeted metabolomics method for the rapid quantification of multiple classes of phenolics in fruits and beverages. *Journal of Agricultural and Food Chemistry, 60*(36), 8831–8840. https://doi.org/10.1021/jf2051569

Wang, Y., Wu, J., Lv, M., Shao, Z., Hungwe, M., Wang, J., Bai, X., Xie, J., Wang, Y., & Geng, W. (2021). Metabolism characteristics of lactic acid bacteria and the expanding applications in food industry. *Frontiers in Bioengineering and Biotechnology, 9*. https://doi.org/10.3389/fbioe.2021.612285

Waraho, T., McClements, D. J., & Decker, E. A. (2011). Mechanisms of lipid oxidation in food dispersions. *Trends in Food Science & Technology, 22*(1), 3–13. https://doi.org/10.1016/j.tifs.2010.11.003

Wishart, D. S., Guo, A., Oler, E., Wang, F., Anjum, A., Peters, H., Dizon, R., Sayeeda, Z., Tian, S., Lee, B. L., Berjanskii, M., Mah, R., Yamamoto, M., Jovel, J., Torres-Calzada, C., Hiebert-Giesbrecht, M., Lui, V. W., Varshavi, D., Varshavi, D., … Gautam, V. (2022). HMDB 5.0: The human metabolome database for 2022. *Nucleic Acids Research, 50*(D1), D622–D631. https://doi.org/10.1093/nar/gkab1062

Xi, Y., Ikram, S., Zhao, T., Shao, Y., Liu, R., Song, F., Sun, B., & Ai, N. (2023). 2-Heptanone, 2-nonanone, and 2-undecanone confer oxidation off-flavor in cow milk storage. *Journal of Dairy Science, 106*(12), 8538–8550. https://doi.org/10.3168/jds.2022-23056

Xie, A., Dong, Y., Liu, Z., Li, Z., Shao, J., Li, M., & Yue, X. (2023). A review of plant-based drinks addressing nutrients, flavor, and processing technologies. *Foods, 12*(21), 3952. https://doi.org/10.3390/foods12213952

Yang, J., Wang, Y., Sun, J., Li, Y., Zhu, R., Yin, Y., Wang, C., Yin, X., & Qin, L. (2024). Metabolome and transcriptome association analysis reveals mechanism of synthesis of nutrient composition in quinoa (Chenopodium quinoa Willd.) seeds. *Foods, 13*(9), 1325. https://doi.org/10.3390/foods13091325

Yousif, N. E., & El Tinay, A. H. (2001). Effect of fermentation on sorghum protein fractions and in vitro protein digestibility. *Plant Foods for Human Nutrition, 56*(2), 175–182. https://doi.org/10.1023/A:1011140602122

Zhang, S., Gai, Z., Gui, T., Chen, J., Chen, Q., & Li, Y. (2021). Antioxidant effects of protocatechuic acid and protocatechuic aldehyde: Old wine in a new bottle. *Evidence-Based Complementary and Alternative Medicine*, 1–19. https://doi.org/10.1155/2021/6139308, 2021.

Zhu, Y., Lv, H.-P., Shao, C.-Y., Kang, S., Zhang, Y., Guo, L., Dai, W.-D., Tan, J. F., Peng, Q. H., & Lin, Z. (2018). Identification of key odorants responsible for chestnut-like aroma quality of green teas. *Food Research International, 108*, 74–82. https://doi.org/10.1016/j.foodres.2018.03.026

Zou, J., Hu, Y., Li, K., Liu, Y., Li, M., Pan, X., & Chang, X. (2023). Chestnuts in fermented rice beverages increase metabolite diversity and antioxidant activity while reducing cellular oxidative damage. *Foods, 12*(1). https://doi.org/10.3390/foods12010164