Food & Function

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal

Cite this: DOI: 10.1039/d6fo00493h

Metabolomic insights reveal bioactive enhancement in fermented oat-based beverages *via Lactobacillus plantarum*

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This study evaluates the impact of thermal processing and fermentation with *Lactobacillus plantarum* on the bioactive composition and metabolic profile modulation of an oat-based (*Avena sativa*) beverage. An oat base beverage (OBB), a heat-treated variant (OBB-H) and, based on this, a fermented drink (OBB-F) were produced. Processing-induced changes were monitored using global bioactivity indices, including total phenolic content (TPC) and the half-maximal inhibitory concentration (IC_{50}) against the DPPH$^{\bullet}$ radical, together with targeted chromatographic analyses and untargeted metabolomics by UHPLC–QTOF. Thermal treatment led to a significant increase in TPC, associated with the release of glycosylated phenolic compounds, including avenanthramides and hydroxycinnamic acids. Following fermentation, TPC remained at levels comparable to the previous stage, while IC_{50} decreased by 49.6%. This effect was linked to phenolic biotransformation processes that redistributed the phenolic pool towards compounds with a potentially greater bioactive response. Targeted analysis enabled the tracking of this reorganisation during fermentation, characterised by the consumption of hydroxycinnamic acids: ferulic, caffeic and *p*-coumaric acids, and the concomitant accumulation of lower-molecular-weight phenolic derivatives, notably protocatechualdehyde. Untargeted metabolomic analysis revealed a profound fermentation-driven reconfiguration of the metabolic profile, including the formation of oxylipins (9-HODE and 13-HODE), oxygenated fatty acids and lysophospholipids, as well as the emergence of lactoylated carbohydrate derivatives and exopolysaccharide fragments not previously reported in fermented oat beverages. In addition, the fermentative process resulted in a marked increase in phosphorylated nucleotides, such as inosine 5′-monophosphate, associated with the enhancement of umami taste. Fermentation also promoted the consumption of aromatic amino acids and the generation of tryptophan-derived metabolites, including indole-3-lactic acid. Overall, these findings show that functional fermentation with *L. plantarum* acts as a key metabolic modulator, inducing specific biotransformations that preserve total phenolic content while amplifying antioxidant response and diversifying the bioactive profile of oat-based plant beverages.

Received 30th January 2026,
Accepted 13th May 2026

DOI: 10.1039/d6fo00493h

rsc.li/food-function

Introduction

The accelerated pace of life characterising contemporary societies has significantly shaped dietary habits, promoting the predominance of fast and ultra-processed foods designed to meet the modern consumer's demand for immediacy, ease of preparation and convenience.[1] Although these products offer a practical solution aligned with current lifestyles, a substantial proportion of them exhibits low nutritional value and an unbalanced composition high in saturated fats, sugars, and additives but low in microbiota-supporting components, which has been associated with a progressive deterioration of the intestinal microbiota, thereby compromising bacterial balance and digestive health.[2] In parallel, consumers are

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showing growing interest in functional foods aimed at counteracting the adverse effects derived from this dietary pattern.[3] Within this context, prebiotic and probiotic ingredients emerge as key nutritional tools for the modulation and restoration of the intestinal microbiota, contributing to the improvement of digestive well-being.[4]

Fermented milky drinks such as yoghurt, kefir and other whey-based products enriched with *Lactobacillus* have become benchmarks among the so-called functional foods, as they combine ease of consumption, high nutritional value and proven prebiotic and probiotic effects.[5] Nevertheless, despite their widespread recognition and acceptance, the consumption of these products is limited for a significant portion of the global population.[6] On the one hand, lactose intolerance limits access to these foods in certain population groups; on the other hand, growing interest in diets free from ingredients of animal origin has driven the search for plant-based functional alternatives capable of reproducing, or even enhancing, the benefits associated with traditional fermented dairy products.[7]

In this regard, cereals have gained relevance as alternatives to dairy beverages, with oats (*Avena sativa*) standing out due to their balanced nutritional profile, characterised by a high content of dietary fibre, high-quality proteins, unsaturated fatty acids, vitamins and minerals.[8] Several studies have associated their consumption with a reduction in postprandial glycaemic response, a decrease in plasma cholesterol levels, positive modulation of the intestinal microbiota and regulation of blood pressure, among other health benefits.[9,10] Among their most relevant bioactive compounds are avenanthramides, a family of phenolic compounds unique to oats, mainly composed of 5-hydroxyanthranilic acid conjugated with hydroxycinnamic acids such as *p*-coumaric, caffeic or ferulic acids, to which antioxidant, anti-inflammatory and cardioprotective properties have been attributed.[11]

The bioavailability of these bioactive compounds may be limited by their association with the plant matrix, which has prompted the application of different processing strategies.[12–14] Among these, thermal treatment has been shown to promote the disruption of the oat grain cell wall and the release of bound phenolic acids, thereby enhancing their extractability.[15] However, uncontrolled heat application may lead to losses of vitamins and minerals, alterations in the fatty acid profile, and the degradation or polymerisation of thermolabile phenolic compounds, ultimately compromising the functional value of the final product.[15,16]

Complementarily, microbial fermentation has been proposed as an effective strategy to improve the bioavailability and functionality of bioactive compounds in oat-based beverages.[17] Inoculation with lactic acid bacteria, such as *L. plantarum*, can induce biotransformation processes that convert complex phenolic compounds into lower-molecular-weight metabolites, potentially more bioavailable and with enhanced antioxidant capacity.[18,19] Despite extensive investigation of the global effects of fermentation through bioactive indices and detailed characterisation of numerous *Lactobacillus* metabolic mechanisms in the digestive context, fermentation within the food matrix has received considerably less attention. This highlights the scarcity of recent studies focused on the metabolism of phenolic compounds by *Lactobacilli* during food fermentation.[20]

Among the most used approaches to assess the functionality of oat-based beverages are bioactive indices, such as total phenolic content (TPC) and antioxidant activity (AA).[21–24] However, traditional spectrophotometric methods employed for their determination have shown significant limitations. The Folin–Ciocalteu assay,[25] widely used to estimate TPC, responds to a broad range of reducing compounds beyond phenolics, including certain amino acids, reducing sugars, and other non-phenolic agents, which may lead to biased interpretations.[26] Similarly, radical scavenging assays, such as DPPH$^{\bullet}$, tend to underestimate the activity of hydrophilic compounds, including some flavonoids.[24] Therefore, these indices are considered general indicators, which have driven the development of more advanced approaches for the precise characterisation of the metabolites involved.

In this context, new strategies based on high-resolution metabolomics have been developed to elucidate the processes involved in the modulation of functional drinks.[27] Nevertheless, in the case of oat-based beverages, the characterisation of their bioactive profiles, as well as the available information on specific fermentation pathways, remains limited.[28]

Within this framework, the present study aims to comprehensively evaluate the impact of thermal processing and *L. plantarum*-induced fermentation on the bioactive profile of an oat-based beverage. To this end, the quantification of global bioactivity indices (TPC and IC_{50}) is combined with high-resolution MS-based metabolomics, employing both targeted and untargeted approaches, in order not only to determine the changes in the bioactive content but also to elucidate the underlying metabolic mechanisms involved in the generation of new functional compounds.

Materials and methods

Standards, reagents and materials

For 60 target polyphenols, their CAS numbers, molecular mass and retention time are shown in Table S1. MS-grade water, methanol, and ethanol were supplied by Scharlab (Barcelona, Spain). The Folin–Ciocalteu's phenol reagent (2 M), sodium carbonate, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox®), formic acid, sodium carbonate and silica oxide (SiO_2) (200–300 μm mesh) were supplied by Sigma-Aldrich (Darmstadt, Germany).

Oat-based beverage preparation

The oat-based beverage was prepared using two samples of commercially available organic oat flakes (*Avena sativa*): fine organic oat flakes from Aurion (gluten-free) and Bio El Graneo (containing gluten). Both exhibited a similar nutritional profile, with approximately 5–7 g fat, 60–70 g carbohydrates,

10–11 g fibre, and 10–13 g protein per 100 g. Mineral water and commercial white sugar were used as complementary ingredients. The overall production process is illustrated in Fig. 1.

For the preparation of the oat base beverage (OBB), oat flakes were initially blended with 20% of the total water content using a Thermomix® (Vorwerk, Germany) for 5 min until complete particle disintegration was achieved. This initial low-hydration milling step was performed to enhance mechanical breakdown and facilitate subsequent homogenization. The remaining water and sugar were then added, and the mixture was processed at 65 °C for 12 min at speed 5.

White sugar was included as a readily available source of fermentable carbohydrates to promote the rapid onset of fermentation. In future studies, enzymatic hydrolysis of oat starch could be explored as an alternative strategy to generate fermentable sugars *in situ*, thereby reducing or eliminating the need for added sugars.

The selected temperature (65 °C) was chosen to promote partial starch gelatinization, a process known to occur in oats within the range of 60–70 °C. This contributes to increased viscosity and improved mouthfeel, mimicking the texture of dairy-based beverages. Continuous agitation during heating ensured uniform heat distribution and prevented sedimentation. For the thermally treated variant (OBB-H), the mixture was maintained under the same conditions (65 °C, 12 min, constant agitation).

To obtain the fermented beverage (OBB-F), the thermally treated sample was cooled to room temperature (25–30 °C) and inoculated at 0.1 g L^{-1} with *Lactobacillus plantarum* (Christian Hansen, strain 718316; 1.0 × 10^{11} CFU g^{-1}). The inoculated mixture, with an initial pH of 7.0–7.2, was vacuum-packed in sterile individual bags to ensure anaerobic conditions and fermented at 25 °C for 3 days, reaching a final pH value of 3.2–3.3. This temperature was selected as it falls within the optimal growth range for *L. plantarum*, promoting efficient lactic acid fermentation and metabolite production.[29] Following processing, all samples were filtered to remove insoluble fractions and stored at −20 °C until further analysis.

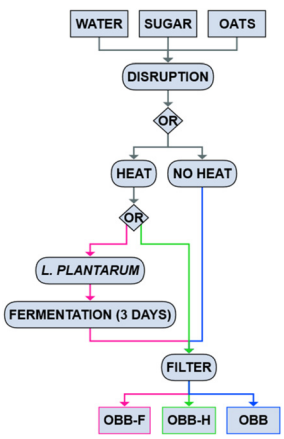

Fig. 1 Schematic representation of the oat beverage production process, including the base beverage (OBB), the thermally treated variant (OBB-H), and the fermented beverage (OBB-F), obtained following inoculation with *L. plantarum* and a 3-day fermentation.

Oat grain extraction by matrix solid-phase dispersion (MSPD)

The bioactive profile of oat grains used for the preparation of the plant-based beverages was obtained using the Matrix Solid-Phase Dispersion (MSPD) technique, as described by Gonzalez-Iglesias *et al.* (2025), with slight modifications.[30] Briefly, 1 g of sample was homogenised and mixed with 4 g of SiO_2. The resulting mixture was transferred into a polypropylene extraction cartridge containing a PTFE cellulose frit at the bottom, previously conditioned with 1 g of SiO_2 to improve filtration and elution. Subsequently, an additional 1 g of SiO_2 was added to the top of the cartridge to ensure an appropriate distribution of the extraction solvent. The extraction solvent (ethanol–water, 80 : 20, v/v) was then added, ensuring homogeneous contact with the matrix inside the cartridge. Once the solvent reached the bottom of the cartridge, a resting period of 5 min was allowed before opening the valve and collecting 5 mL of the eluate. The obtained extract was filtered through 0.22 μm hydrophilic filters and stored at −20 °C until further analysis. All extractions were performed in triplicate.

Ultrasound-assisted extraction (UAE) of oat-based beverages

For liquid samples, 1 g of sample was placed in a 10 mL glass vial and 4 mL of an ethanol–water mixture (80 : 20, v/v) were added. The vial was then sealed and immersed in an ultrasonic bath (JP Selecta, Barcelona, Spain) for 10 min at room temperature. Then, the extract was collected and centrifuged (Orto Alresa, Barcelona, Spain) at 3500 rpm for 15 min. Subsequently, the supernatant was filtered through 0.22 μm PTFE filters and stored at −20 °C until further analysis. All extractions were performed in triplicate.

Total polyphenol content (TPC)

TPC was determined using the Folin–Ciocalteu (FC) colorimetric method described by Zhang *et al.* (2006) for microtitration in 96-well plates.[31] Briefly, 20 μL of each diluted extract were mixed with 100 μL of the Folin–Ciocalteu reagent (1 : 10, v/v) and 80 μL of an aqueous sodium carbonate solution (7.5 g L^{-1}). The mixture was gently shaken and incubated in the dark for 30 min. Subsequently, absorbance was measured at 760 nm using a microplate reader (BMG LABTECH, Ortenberg, Germany). TPC was quantified using a gallic acid calibration curve over a concentration range of 20–160 mg L^{-1} (absorbance: 0.200–0.800 AU). The results were expressed as milligrams of gallic acid equivalents per gram of sample on a dry weight basis (mg GAE per g).

Antioxidant activity

The antioxidant activity (AA) of the samples and their half-maximal inhibitory concentration (IC_{50}) were evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) radical scavenging assay described by Symes *et al.* (2018).[32] Briefly, 100 μL of each extract, at eight different dilutions, were dispensed into a

96-well plate and mixed with 100 μL of the DPPH• reagent prepared in methanol. The mixture was incubated in the dark for 10 min, and absorbance was measured at 515 nm using a microplate reader. AA was quantified using a Trolox calibration curve over a concentration range of 3–31 mg L^{-1} (0.200–0.800 AU), and the results were expressed as grams of Trolox equivalents per gram of dry matter (g TE per g DW).

The IC_{50} of the samples, expressed as milligrams of dry sample per litre of extract (mg L^{-1}) required to inhibit 50% of DPPH• radicals, was obtained by linear interpolation in the range close to 50% inhibition.

UHPLC-QTOF-MS/MS analysis

The chromatographic strategy employed for the detection and quantification of metabolites was based on a protocol previously described by Castillo *et al.* (2025), with minor adaptations.[33] Analyses were performed using ultra-high-performance liquid chromatography (UHPLC) coupled to a compact quadrupole time-of-flight mass spectrometer (QToF) (Bruker Daltonics). Chromatographic separation was achieved using an Intensity Solo column (2.0 μm, 100 × 2.1 mm), maintained at 40 °C.

The mobile phase consisted of an aqueous solution of 4 mM formic acid (phase A) and methanol (phase B). Prior to each analysis, a standard calibrant (1×10^{-3} M NaOH in a 1 : 1 H_2O : 2-propanol mixture containing 0.2% formic acid) was injected to correct for potential mass deviations during data acquisition. The total run time was 20 min, with a constant flow rate of 0.20 mL min^{-1}. The elution program started with an initial composition of 95% A and 5% B for 0.4 min (calibration interval), followed by a gradual increase in the proportion of the organic phase according to the following intervals (% B: min): 30 : 4.5; 37 : 8.0; 50 : 9.0; 90 : 11.0; 90 : 14.0. The system then returned to the initial conditions at 16 min, which were maintained until completion of the total acquisition time. Each sample was injected in triplicate.

Detection was carried out using an electrospray ionisation (ESI) source operating in the negative mode. Targeted analysis was performed using data-independent MS/MS acquisition in the broadband collision-induced dissociation (bbCID) mode. Data were acquired at a spectral rate of 1 Hz over a mass range of 20–1000 *m*/*z*. For bbCID acquisition, the collision energy and acquisition time factor were set to 0, 10, and 1 for MS and 5, 30, and 1 for MS/MS, respectively.

For quantification purposes, stock solutions of phenolic compounds were prepared at different concentration levels ranging from 0.1 to 10 ppm, with each level being injected in triplicate. The method showed a direct proportional relationship between compound concentration and chromatographic response. Quantification of real samples was carried out using external calibration. Data processing and quantification were performed using TASQ software (version 2024.1.3, build 12175; Bruker Daltonics).

Untargeted analysis was conducted using data-dependent MS/MS acquisition in the Auto MS/MS mode, allowing the detection of deprotonated pseudomolecular ions $[M - H]^-$. Data were acquired at a spectral rate of 8 Hz, with a total cycle time of 1 s, over a mass range of 20–1000 *m*/*z*. Fragmentation was achieved using dynamic collision energy through the Multi-CE function (20–80%), dependent on the precursor *m*/*z*. Each sample was injected five times in this analysis. Data acquisition was performed using Compass HyStar software, while data pre-processing was carried out using DataAnalysis (version 5.1, Build 201.2.4019).

Metabolomic profiling analysis

The chromatograms obtained were screened using the T-Rex 3D® algorithm. A retention time window between 0.5 and 20 min was established for analyte filtering, considering only signals with intensities above 1×10^3 and a minimum recurrence of 25% across the analysed samples. Untargeted metabolite annotation was performed using MetaboScape 4.0.4 (Build 19).

A comprehensive example of untargeted detection calculations is presented in SI section S1. In brief, molecular formula assignment was carried out with the SmartFormula tool, applying criteria based on the signal intensity, isotopic pattern (mSigma) and mass error ($\Delta m/z$). Maximum acceptable thresholds were set at 5 ppm for $\Delta m/z$ and 50 mSigma for isotopic pattern deviation. Tentative metabolite identities were further validated through comparison with online databases from the National Center for Biotechnology Information (NCBI)[34] and the Chemical Entities of Biological Interest (ChEBI).[35] In addition, fragmentation spectra were evaluated using *in silico* tools (Compound Crawler and MetFrag) and automatically compared with reference spectral libraries, including the MassBank of North America (MoNA)[36] and MassBank Europe.[37] The mass spectra obtained through untargeted analysis are summarized in Fig. S1, offering a detailed view of the identified metabolites and their fragmentation patterns.

Statistical analysis

The pre-processed data were exported to different statistical software packages for multivariate analysis. A comprehensive description of the statistical parameters is provided in SI section S2. In brief, unsupervised principal component analysis (PCA) with Pareto scaling was performed to evaluate the oat-based beverages and their thermal and fermentative treatments, as well as the oat grains used, employing MetaboAnalyst 6.0.[38] In parallel, supervised analyses were carried out using partial least squares discriminant analysis (PLS-DA), together with the identification of the most influential variables through the variable importance in projection (VIP) scores. Model robustness and predictive accuracy were assessed by permutation tests with 2000 replicates, allowing the exclusion of random separation in the generated models ($p < 0.05$). Heat maps with dendrograms were generated using OriginPro® 10.3.0.180 following cluster analysis based on the Ward linkage method and the Euclidean distance as the similarity metric. Five clusters corresponding to the analysed sample groups were defined.

Results

Effects of thermal processing and fermentation on the target bioactive compounds in oat-based beverages

To evaluate the effects on the modulation of an oat-based beverage (OBB), a base formulation was initially developed and subjected to a heating stage at 65 °C during production, yielding the OBB-H sample. Subsequently, the impact of a fermentation stage involving inoculation with *L. plantarum* was investigated, generating the fermented variant (OBB-F). Given the sequential nature of these treatments, the analyses were conducted considering the potential overlap of effects to elucidate and interpret the individual contribution of each process. The bioactive content of the different formulations was analysed using global indices, including TPC and IC_{50}. Fig. 2 illustrates the evolution of these overall indices across the three production stages evaluated.

As shown in Fig. 2, TPC increased significantly ($p < 0.05$) following the thermal treatment, increasing from 3.51 mg GAE per g in OBB to 5.63 mg GAE per g in OBB-H. This increase may be associated with a higher extraction efficiency, resulting from the disruption of the plant matrix induced by thermal treatment,[39] which on one hand releases reducing compounds that may interfere with the overall measurement of TPC. On the other hand, these effects have also been linked to the hydrolysis of cell wall polysaccharides, facilitating the release of previously bound phenolic compounds.[15] Consistent with these observations, targeted analysis (Fig. 3) revealed an overall increase in several phenolics after heating, including avenanthramides, catechin, and azelaic acid, together with a notable increase in the hydroxycinnamic acids – ferulic, *p*-coumaric, and caffeic acids.

Subsequently, the fermentation step did not induce marked changes in TPC, which remained at comparable levels in the fermented beverage (OBB-F; 5.62 mg GAE per g). Nevertheless, targeted analysis revealed that fermentation was associated with a redistribution of the phenolic profile, characterised by an overall decrease in higher-molecular-weight phenolic compounds, including avenanthramides and hydroxycinnamic acids, alongside a relative increase in lower-molecular-weight phenolic compounds, particularly protocatechualdehyde. With respect to antioxidant activity, a progressive improvement was observed across the different processing stages. As shown in Fig. 2, thermal treatment led to a reduction in IC_{50} from 208.8 mg per 100 g in OBB to 185.4 mg per 100 g in OBB-H, indicating an enhancement of antioxidant capacity, consistent with the increase in TPC and the release of phenolic compounds upon heating. More pronouncedly, fermentation with *L. plantarum* resulted in a significant decrease in the IC_{50} in the base beverage to 105.24 mg per 100 g in OBB-F, corresponding to a 49.6% reduction.

Despite the reduction in both the content and complexity of phenolic compounds following fermentation, the overall antioxidant response increased, indicating that this effect is not solely dependent on TPC variation. This response may be associated with the interaction of the DPPH$^{\bullet}$ radical with the structural conformation of antioxidants. It is known that smaller antioxidants, being able to access the radical more easily, exhibit higher antioxidant activity, considering that DPPH$^{\bullet}$ is sterically hindered.[24] Therefore, the accumulation of low-molecular-weight phenolic compounds, particularly derivatives such as protocatechualdehyde, likely contributes substantially to the observed increase in antioxidant capacity. This behaviour has been previously reported in *Lactobacillus*-mediated fermentations, such as kiwi pulp fermentation, where the activity of *L. plantarum* led to an increase in protocatechuic acid and protocatechualdehyde concomitant with a decrease in *p*-coumaric acid content.[40] This process, schematically illustrated in Fig. 4, follows a catabolic pathway based on the decarboxylation of the hydroxycinnamic acids (*p*-coumaric, ferulic, and caffeic acids), yielding intermediates such as 4-vinylphenol, 4-vinylguaiacol, and 4-vinylcatechol, which are subsequently converted into protocatechualdehyde through successive hydroxylation, demethylation, and oxidation steps.

These transformations have been described as the result of the activity of different bacterial enzymes, with the catabolic pathway of ferulic acid being one of the most complex due to the requirement for a specific demethylation step.[41] This observation is consistent with the individual concentrations detected in the present study (Fig. 3), where, despite a marked consumption of ferulic acid during fermentation, a residual concentration remained after the fermentative process. In contrast, for simpler catabolic pathways, such as those associated with caffeic and *p*-coumaric acids, no residual concentrations were detected in the fermented samples, suggesting a more selective conversion into lower-molecular-weight derivatives, such as protocatechualdehyde.

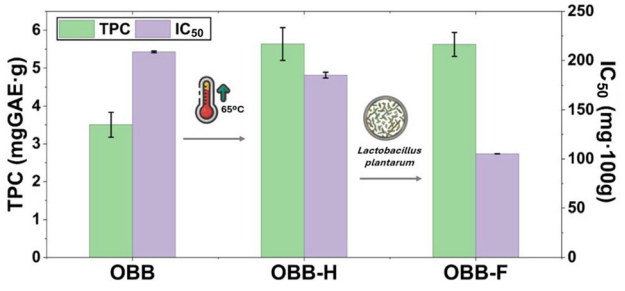

Fig. 2 Evolution of the total phenolic content (TPC) and half-maximal inhibitory concentration (IC_{50}) during the production of the oat-based beverage: base beverage (OBB), after thermal treatment (OBB-H), and fermented beverage with *L. plantarum* (OBB-F).

Untargeted metabolomic profiling of processing-induced biotransformation in oat-based beverages

In order to perform a comprehensive assessment of the metabolic enrichment of the oat-based beverage throughout the different processing stages from the incorporation of oat grains, through thermal treatment, to the fermentation step, an untargeted metabolomic study was conducted. All samples were analysed by ultra-high-performance liquid chromato-

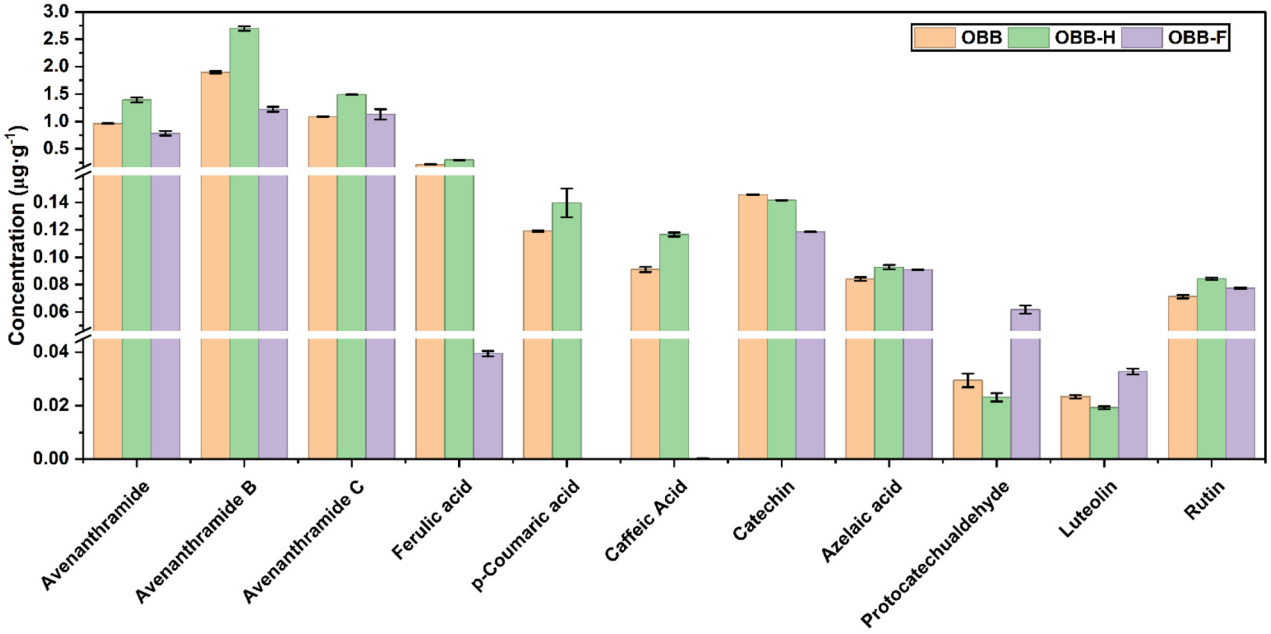

Fig. 3 Profile of individual phenolic compounds in the oat beverage (OBB), after thermal treatment (OBB-H), and after fermentation with *L. plantarum* (OBB-F).

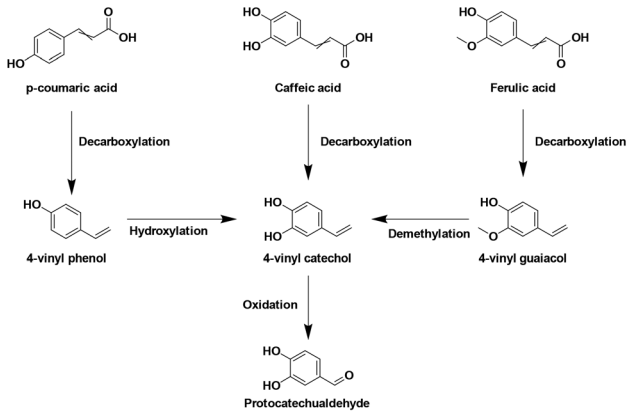

Fig. 4 Proposed metabolic pathways involved in the biotransformation of caffeic, ferulic, and *p*-coumaric acids into protocatechualdehyde during fermentation of the oat beverage with *L. plantarum*.

graphy coupled to a QTOF detector, leading to the identification of a total of 107 analytes (File S1), with their respective mass spectra (Fig. S1), classified into 13 compound families. Principal component analysis (PCA) (Fig. 5A) revealed a clear biochemical differentiation among the evaluated raw materials and beverages, resulting in three well-defined clusters. Oat grains used as raw material formed a distinct group, while the OBB and its thermally treated counterpart OBB-H clustered together. In contrast, the fermented beverage OBB-F showed a marked separation from the other samples.

To identify the metabolites responsible for this separation and to gain deeper insight into processing-induced changes, the evolution of the main compound families was examined in detail. In this context, a pronounced modification of the lipid profile was observed, characterised by a substantial loss of long-chain essential fatty acids in OBB-H and OBB-F compared with OBB. A decrease in the signal intensity of oleic and palmitic acids was detected, together with a marked reduction of the compound tentatively denoted as FA 18:1;O (mid-OH), accompanied by an increase in FA 18:1;O (ω-OH).

FA 18:1;O (mid-OH) and FA 18:1;O (ω-OH) were tentatively identified as monohydroxylated fatty acid isomers differing in the position of the non-carboxylic hydroxyl group along the aliphatic chain. For FA 18:1;O (mid-OH), the observed MS/MS fragmentation pattern (Fig. 6A), including a product ion at *m*/*z* 255 attributed to the loss of C_3H_7 (cleavage between C16–C18), suggests that the hydroxyl group is located at an intermediate position along the chain (between C1 and C15). The decrease in this isomer following thermal exposure may be related to the higher reactivity of a hydroxyl group positioned close to the double bond, favouring dehydration, oxidation or thermally induced structural rearrangements.

In contrast, FA 18:1;O (ω-OH) did not exhibit the characteristic *m*/*z* 255 ion (Fig. 6B), indicating that the hydroxyl group is located at the terminal ω-position of the aliphatic chain. The relative increase of this isomer during fermentation may be associated with selective terminal hydroxylation processes, potentially mediated by enzymes with ω-hydroxylase-like activity, or alternatively, with changes in the physicochemical conditions of the fermentative environment, such as pH variations, which may favour this type of transformation.

Stereochemical and positional selectivity in fatty acid hydroxylation by *Lactobacillus* species has been widely reported. Takeuchi *et al.* (2016) described a pronounced pos-

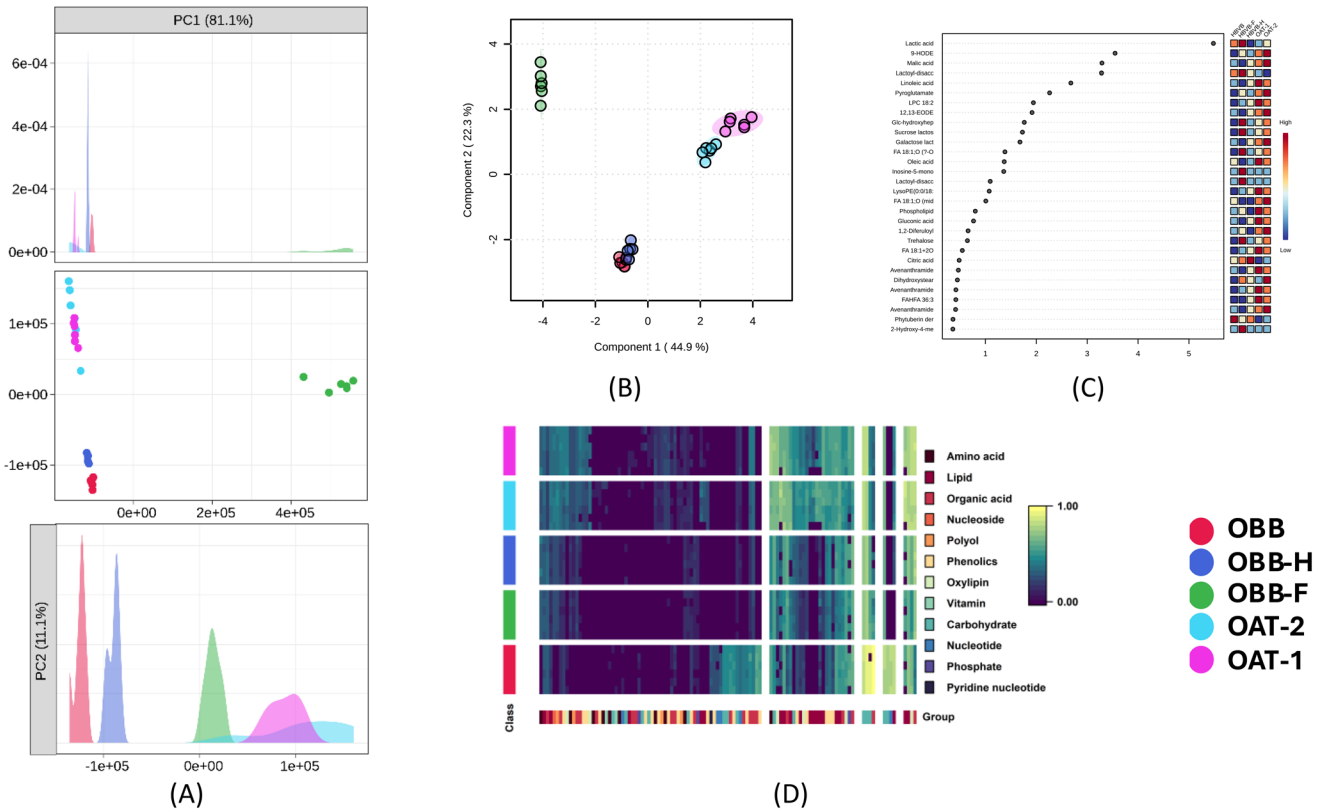

Fig. 5 Multivariate metabolomic analysis revealing sample discrimination across processing stages. (A) Principal component analysis (PCA); (B) supervised partial least squares-discriminant analysis (PLS-DA); (C) variable importance in projection (VIP) scores highlighting key discriminant metabolites; and (D) heat map visualising relative metabolite abundance patterns among oat grains, base beverage (OBB), thermally treated beverage (OBB-H) and fermented beverage (OBB-F).

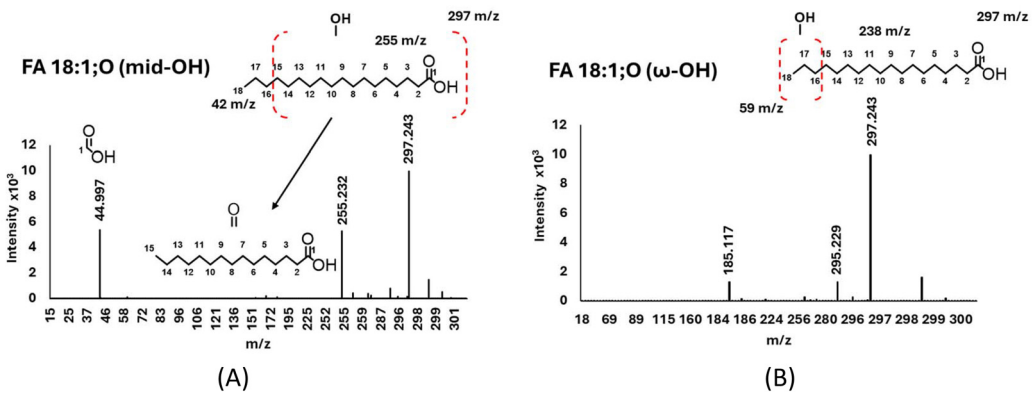

Fig. 6 MS/MS fragmentation patterns of isomeric FA 18:1;O according to hydroxyl group localisation: (A) mid-chain (mid-OH) and (B) terminal ω-position (ω-OH).

itional and stereochemical selectivity in fatty acid hydroxylation systems mediated by *L. plantarum*, targeting C16 and C18 fatty acids, with enzymatic activity enhanced by the presence of the reduced cofactor nicotinamide adenine dinucleotide (NADH).[42] This observation was consistent with the findings of the present study, in which a marked response of NAD-related metabolites was detected exclusively in OBB-F, suggesting a fermentation-induced redox environment favourable to enzymatic lipid transformations. Notably, the selectivity described for *L. plantarum* has been predominantly associated with hydroxylation at mid-chain positions of aliphatic fatty acids.[43] In this context, the higher relative abundance of FA 18:1;O (ω-OH) observed in OBB-F points to the involvement of secondary, environment-dependent processes rather than

the direct action of the canonical fatty acid hydratase pathways classically attributed to this microorganism.

Beyond their structural specificity, hydroxy fatty acids have been associated with beneficial biological properties, including anti-inflammatory, immunomodulatory and metabolic effects, highlighting the potential functional relevance of the lipid transformations observed during oat beverage fermentation.[44]

Oleic and palmitic acids showed a moderate reduction after both thermal treatment and fermentation, whereas azelaic acid remained largely unchanged throughout processing. This behaviour was consistent with the results obtained in the targeted analysis (Fig. 3), where azelaic acid exhibited the highest relative stability among the quantified analytes. This stability could be attributed to the chemical nature of azelaic acid, a saturated dicarboxylic acid lacking double bonds, which confers enhanced resistance to degradation processes induced by heat or microbial metabolism. Azelaic acid is known to originate from the oxidation of unsaturated fatty acids such as oleic acid, yet its fully saturated structure renders it more stable under processing conditions.

The formation of dihydroxystearic acid was specifically detected in OBB-F, together with an increase in oxygenated fatty acids (FA 18:1 + 2O and FA 18:2 + 2O) and lysophospholipids, particularly LPC 18:2, in both OBB-H and OBB-F beverages. Oxygenated fatty acids may arise from lipid peroxidation processes, which are likely promoted by thermal stress during processing. Similarly, the increase in lysophospholipids may be attributed to partial phospholipid hydrolysis induced by temperature.

Regarding the fermentation stage, an increased formation of the oxylipins 9-HODE and 13-HODE was observed. Although these compounds can be generated *via* non-enzymatic pathways, their accumulation during lactic fermentation with *L. plantarum* has been previously reported.[45,46] Several *L. plantarum* strains have been associated with increased levels of 9-S-HODE, 9-R-HODE and 13-R-HODE, as well as lysophosphatidic acids (LysoPA), compounds linked to anti-inflammatory effects and improved intestinal mucosal integrity, potentially contributing to epithelial repair and the alleviation of diarrhoeal disorders.[45]

As expected, lactic acid was the most abundant metabolite detected in OBB-F, while it was not detected in OBB-H and was present only at residual levels in OBB. This observation was consistent with the fermentative activity of *L. plantarum*, whose primary metabolic pathway leads to lactic acid production from fermentable carbohydrates.

The second most intense metabolite corresponded to a compound tentatively identified as a lactoyl-disaccharide, consisting of disaccharide structures conjugated with lactic acid. The presence of this compound was consistent with the fermentative process, as the high production of lactic acid may favour its esterification with polysaccharides or oligosaccharides present in the OBB-F matrix, giving rise to lactoylated derivatives.

Negative ion mode MS/MS fragmentation (Fig. 7A) of the lactoyl-disaccharide showed a precursor ion at *m/z* 773, yielding characteristic fragments at *m/z* 683, 431, 341 and 179. The transition *m/z* 773 → 683 corresponds to a neutral loss of 90 Da, attributed to the elimination of a lactic acid residue ($C_3H_6O_3$), supporting the proposed structural assignment. In addition, the fragment at *m/z* 341, approximately half of *m/z* 683, suggests cleavage of the polysaccharide backbone into simpler structural subunits, while the transition *m/z* 431 → 341 may be attributed to a second lactic acid loss, further reinforcing the identification of a lactoylated disaccharide.

This lactoyl-disaccharide has not previously been reported in fermentations mediated by *L. plantarum*. However, a structurally related molecule has been described in a metabolomic study of pineapple (*Ananas comosus*, cultivar Vitória), suggesting that the formation of lactoylated carbohydrate derivatives may occur in polysaccharide-rich plant matrices subjected to fermentation processes.[47] Moreover, fragments at *m/z* 683 and 431 were detected as major components in OBB-F, appearing both in their free form and conjugated with a lactic acid moiety. This group of molecules may be interpreted as fragments of exopolysaccharides (EPS). EPS are bioactive macromolecules produced by lactic acid bacteria and have

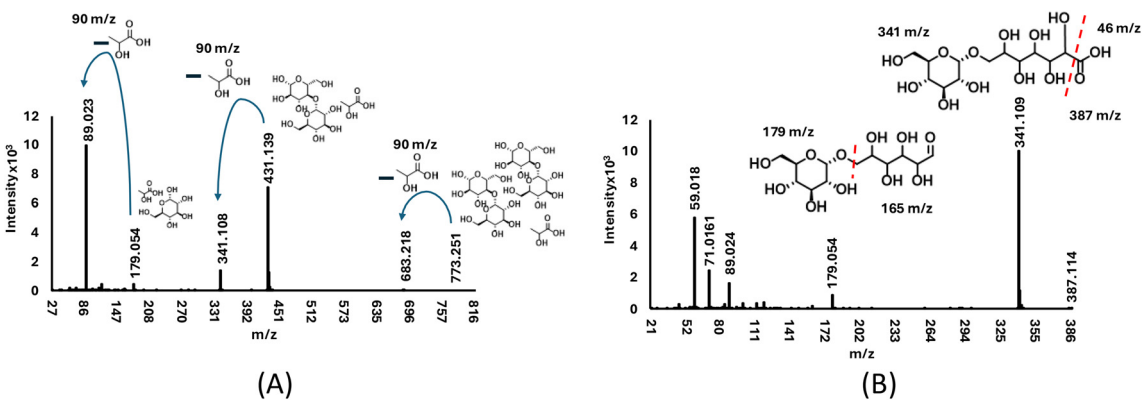

Fig. 7 Negative ion mode MS/MS fragmentation patterns of (A) lactoyl-disaccharide showing successive losses of lactic acid ($C_3H_6O_3$) and (B) 7-(α-D-glucopyranosyloxy)-2,3,4,5,6-pentahydroxyheptanoic acid showing losses of HCOOH and $C_7H_{12}O_7$.

been associated with antioxidant, antitumour and gut microbiota-modulating activities.[48] Notably, EPS typically exhibit molecular weights exceeding 10 kDa, which precludes their intact detection by UHPLC-QTOF, making their identification possible only through lower-molecular-weight fragments.

Another metabolite showing a high response in OBB-F was identified as 7-(α-D-glucopyranosyloxy)-2,3,4,5,6-pentahydroxyheptanoic acid. Its negative ion mode fragmentation spectrum (Fig. 7B) displayed a precursor ion at *m*/*z* 387, with a fragmentation pattern similar to that observed for the lactoyl-disaccharide. This compound can be interpreted as a structure formed by lactic acid-derived units conjugated to a monosaccharide, further supporting its association with the fermentation process. This metabolite has previously been reported in ethanolic extracts of fungi and plants, showing characteristic fragments at *m*/*z* 341, attributed to the loss of HCOOH (formic acid), and at *m*/*z* 179, corresponding to the loss of $C_7H_{12}O_7$ (4-*O*-methylglucuronic acid),[49] as observed in Fig. 7B.

A metabolite of particular interest detected in OBB-F was inosine-5′-monophosphate (IMP), a purine ribonucleotide whose nitrogenous base is hypoxanthine. Although direct evidence linking *L. plantarum* to IMP production is limited, increased levels of this metabolite have been reported in association with *Lactobacillus sakei*[50] and other probiotic microorganisms.[51] IMP, like glutamate, acts as an umami flavour enhancer, and a synergistic effect between monosodium glutamate (MSG) and IMP has been described, whereby IMP amplifies the gustatory nerve response to MSG.[52] Therefore, the detection of IMP in OBB-F could be associated with a potential improvement in the sensory profile during fermentation, although further studies are needed to confirm this relationship.

OBB-F showed a significant consumption of fermentable sugars and polyols, including melezitose, glucose, xylitol, and sorbitol, with no appreciable variations observed in OBB-H or OBB. This pattern suggests a preferential utilization of these carbon sources by *L. plantarum* during fermentation. Concomitantly, an increase in glutamic acid and sucrose levels was observed. In the case of sucrose, this increase could be attributed to metabolic carbon redistribution processes, possibly associated with transglucosylation reactions originating from melezitose, involving partial cleavage of the glycosidic chain and subsequent sucrose formation. Consistently, this fermentative metabolism was associated with a markedly higher formation of pyroglutamate, which was detected at substantially higher levels in OBB-F. This phenomenon has been previously described in fermentations mediated by lactic acid bacteria and has been attributed to the microbial conversion of glutamate under fermentative conditions.[53]

Pyroglutamate, a cyclic derivative of L-glutamic acid, occurs naturally in animal and plant tissues and has been associated with potential beneficial effects on the gut microbiota.[53] In oat grains, its formation may occur through non-enzymatic cyclisation of glutamate[54,55] during certain stages of industrial processing under specific conditions. In the case of OBB-H, although elevated temperatures could promote pyroglutamate formation *via* dehydration, the presence of an aqueous medium and moderate temperatures, well below those required for this reaction, may explain the lack of a significant increase.[56] Conversely, in OBB-F, fermentation with *L. plantarum* significantly elevates pyroglutamate levels, likely through glutamate consumption and acidification of the medium. In addition to the non-enzymatic pathway, pyroglutamate formation can also be catalysed by γ-glutamylcyclotransferase, a mechanism whose activity may be enhanced during fermentation.[57]

During fermentation with *L. plantarum*, a decrease in 3-hydroxybenzaldehyde is observed, which can be attributed to metabolic biotransformation processes towards simpler phenolic structures. Within bacterial metabolism, microbial esterases have been reported to exhibit specificity towards phenolic acid esters, with *L. plantarum* showing relatively high esterase activity, particularly against protocatechuic acid.[58] In this context, although a direct hydroxylation process mediated by *L. plantarum* does not represent a direct metabolic route, fermentative conditions and esterase activity may indirectly favour an increase in protocatechuic acid associated with the consumption of 3-hydroxybenzaldehyde. These findings are supported by the results obtained from the targeted analysis (Fig. 4). Moreover, protocatechuic acid is widely recognised as a phenolic compound with high antioxidant capacity compared with other hydroxybenzoic structures,[59] which could explain the notable radical-scavenging activity against the DPPH$^{\bullet}$ reagent observed in OBB-F.

In the same line, OBB-F exhibits a consumption of amino acids such as tryptophan and cyanoalanine. Amino acid consumption is commonly observed in metabolic pathways promoted by *L. plantarum*;[60] indeed, the marked increase in indole-3-lactic acid (ILA) detected in OBB-F, derived from the tryptophan metabolic pathway, clearly reflects the relationship between the utilisation of this amino acid and the production of this metabolite. Overall, these findings highlight the key role played by *L. plantarum* in the fermentation processes of oat-based beverages, steering them towards a profile with greater bioactive potential. However, the validation of specific metabolic pathways requires further targeted studies to confirm the underlying mechanisms. Furthermore, comparisons need to be made with controls in which a single treatment pathway (fermentation or heating) is applied, including untreated samples, to support the observed effects.

Conclusions

This work addressed the development of oat-based beverages subjected to thermal processing and fermentation with *L. plantarum* using a comprehensive approach aimed at quantifying bioactive indices, characterising individual phenolic profiles and conducting untargeted metabolomic analysis.

The application of heat treatment to oat-based beverages promoted the release and extractability of phenolic compounds, particularly ferulic, *p*-coumaric and caffeic hydroxy-

cinnamic acids. Meanwhile, the fermentation processes driven by *L. plantarum* revealed a metabolic reconfiguration characterised by the consumption of these hydroxycinnamic acids and their conversion into derivatives of lower molecular weight, such as protocatechualdehyde. This process resulted in maintaining the overall phenolic content and reducing the half-maximal inhibitory concentration (IC_{50}) of the DPPH$^{\bullet}$ radical by 49.6% compared to the base drink.

Beyond phenolic compounds, untargeted metabolomic analysis revealed a profound metabolic reconfiguration induced by *L. plantarum*, including the formation of oxygenated fatty acids, oxylipins, lysophospholipids, lactoylated carbohydrate derivatives and exopolysaccharide fragments not previously described in this type of fermented oat beverage. Furthermore, fermentation significantly modulated nitrogen and nucleotide metabolism, promoting the accumulation of tryptophan-derived metabolites, such as ILA, and phosphorylated nucleotides, including inosine 5′-monophosphate.

Taken together, these results broaden our understanding of the metabolic changes induced during the processing of oat-based beverages and reinforce the potential of *L. plantarum*-mediated fermentation as a tool for modulating their functional profile.

Future research should focus on the specific validation of the proposed metabolic pathways, as well as on the evaluation of the bioactive and organoleptic properties associated with the described metabolic profiles, with a view to optimising their application in the development of functional foods.

Author contributions

Conceptualization, M. L., C. M., J. O. and B. O.; methodology, C. M., M. L., and J. O.; software, A. C. and M. C.; formal analysis, A. C.; investigation, A. C. and M. C.; resources, J. O., F. D. and M. L.; writing – original draft preparation, A. C.; writing – review and editing, A. C., G. A-R., and M. L.; supervision, M. L. and G. A-R.; project administration, M. L., C. M., J. O., F. D., and B. O.; funding acquisition, M. L., J. O. and B. O. All authors have read and agreed to the published version of the manuscript.

Conflicts of 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.

Data availability

Data supporting this article have been included in the supplementary information (SI). See https://doi.org/10.57760/sciencedb.36364.

Supplementary information is available. See DOI: https://doi.org/10.1039/d6fo00493h.

Acknowledgements

The authors thank Eva García Pérez, Pablo Casas and Francisco J. Novás from Culler de Pau (high cuisine) for their contribution to the development and preparation of the oat-based beverages used in this study. Their involvement was key to the formulation and standardization of the products evaluated. This work has been developed in the context of the regional research project: AveaMoura_Recuperación y valorización de avenas negras autóctonas gallegas. Xunta de Galicia. Ref. FEADER 2023/038B. Dates: 2023–2026. G. Alvarez-Rivera would like to acknowledge Ministerio de Ciencia e Innovación (MICINN/AEI, Spain) for his RYC2022-037949-I contract. Castillo A. acknowledges the support of the Program and the Ministry of Science, Innovation and Universities, Spain (MCIN) of Spain for his contract – part of the grant DIN2021-011976 funded by the MCIN/AEI/10.13039/501100011033. This work was also funded by project ED431 2020/06 (Galician Competitive Research Groups, Xunta de Galicia).

References

1 L. Castrillón-Ruiz, A. Estrada-Restrepo, G. Cediel, D. Cárdenas-Sánchez, J. Barona-Acevedo and J. C. Aristizábal, Association between ultra-processed food intake, diet quality, and cardiovascular risk factors among adolescents in Antioquia, Colombia, *Front. Public Health*, 2025, **13**, 1710967, DOI: 10.3389/fpubh.2025.1710967.

2 A. Atzeni, A. Hernández-Cacho, N. Khoury, N. Babio, C. Belzer, J. Vioque, D. Corella, M. Fitó, C. Clish, J. Vidal, P. Konstanti, S. Gonzales-Palacios, O. Coltell, A. Goday, I. Moreno Indias, S. Carlos Chillerón, M. Ruiz-Canela, F. J. Tinahones, F. B. Hu and J. Salas-Salvadó, The link between ultra-processed food consumption, fecal microbiota, and metabolomic profiles in older Mediterranean adults at high cardiovascular risk, *Nutr. J.*, 2025, **24**, 62, DOI: 10.1186/s12937-025-01125-5.

3 C. Roberto, N. Lorenzo, S. Daniela, M. D'Amico and C. Gaetano, Functional foods at the agrifood interface: consumer knowledge, trust and socio-economic drivers for market uptake and policy in Italy, *J. Agric. Food Res.*, 2026, **25**, 102566, DOI: 10.1016/j.jafr.2025.102566.

4 J. Ji, W. Jin, S. J. Liu, Z. Jiao and X. Li, Probiotics, prebiotics, and postbiotics in health and disease, *MedComm*, 2023, **4**, e420, DOI: 10.1002/mco2.420.

5 C. R. Kok and R. Hutkins, Yogurt and other fermented foods as sources of health-promoting bacteria, *Nutr. Rev.*, 2018, **76**, 4–15, DOI: 10.1093/nutrit/nuy056.

6 F. Cosme, A. Inês and A. Vilela, Consumer's acceptability and health consciousness of probiotic and prebiotic of non-dairy products, *Food Res. Int.*, 2021, **145**, 110842, DOI: 10.1016/j.foodres.2021.110842.

7 S. S. da Cruz Nascimento, T. R. de Oliveira, M. dos Santos Lima, R. M. de Oliveira Tavares, K. S. F. da S.

C. Damasceno, C. F. de Assis, T. S. Passos and F. C. de Sousa Júnior, Oat-grape beverages enriched with anthocyanins from jambolan (*Syzygium cumini*): A novel plant-based probiotic functional food, *Food Chem.*, 2025, **487**, 144673, DOI: 10.1016/j.foodchem.2025.144673.

8 G. F. Alemayehu, S. F. Forsido, Y. B. Tola and E. Amare, Nutritional and Phytochemical Composition and Associated Health Benefits of Oat (*Avena sativa*) Grains and Oat-Based Fermented Food Products, *Sci. World J.*, 2023, **2023**, 2730175, DOI: 10.1155/2023/2730175.

9 H. Zhang, G. Önning, A. Ö. Triantafyllou and R. Öste, Nutritional properties of oat-based beverages as affected by processing and storage, *J. Sci. Food Agric.*, 2007, **87**, 2294–2301, DOI: 10.1002/jsfa.2987.

10 D. Carbajal-Padilla, M. E. Sosa-Morales, A. Cerón-García, M. Morales-De la Peña and J. A. Gómez-Salazar, Effect of Oat and Chickpea Content on the Physicochemical, Bioactive, and Sensory Properties of a Plant-Based Beverage during Chilled Storage, *ACS Food Sci. Technol.*, 2025, **5**, 942–952, DOI: 10.1021/acsfoodscitech.4c00254.

11 A. A. Pridal, W. Böttger and A. B. Ross, Analysis of avenanthramides in oat products and estimation of avenanthramide intake in humans, *Food Chem.*, 2018, **253**, 93–100, DOI: 10.1016/j.foodchem.2018.01.138.

12 C. Yang, J. Li, T. Luo, J. Tu, T. Zhong, Y. Zhang, X. Liang, L. Zhang, Z. Zhang and J. Wang, Ultrasonic-microwave assisted extraction for oat bran polysaccharides: Characterization and in vivo anti-hyperlipidemia study, *Ind. Crops Prod.*, 2024, **220**, 119229, DOI: 10.1016/j.indcrop.2024.119229.

13 S. C. Senarathna and M. Malalgoda, Impact of defatting method on oat protein isolate structure-function characteristics, *J. Cereal Sci.*, 2024, **117**, 103876, DOI: 10.1016/j.jcs.2024.103876.

14 M. S. Ibrahim, M. Nadeem, W. Khalid, A. Ainee, T. Roheen, S. Javaria, A. Ahmed, H. Fatima, M. N. Riaz, M. Z. Khalid, I. A. Mohamed Ahmed and M. O. Aljobair, Optimization of ultrasound assisted extraction and characterization of functional properties of dietary fiber from oat cultivar S2000, *LWT – Food Sci. Technol.*, 2024, **197**, 115875, DOI: 10.1016/j.lwt.2024.115875.

15 L. F. Călinoiu and D. C. Vodnar, Thermal Processing for the Release of Phenolic Compounds from Wheat and Oat Bran, *Biomolecules*, 2019, **10**, 21, DOI: 10.3390/biom10010021.

16 P. Lehtinen, K. Kiiliäinen, I. Lehtomäki and S. Laakso, Effect of Heat Treatment on Lipid Stability in Processed Oats, *J. Cereal Sci.*, 2003, **37**, 215–221, DOI: 10.1006/jcrs.2002.0496.

17 A. M. Abdelshafy, M. A. Mustafa, M. A. Hassan and F. Al-Asmari, Probiotic-fermentation of oat: Safety, strategies for improving quality, potential food applications and biological activities, *Trends Food Sci. Technol.*, 2024, **151**, 104640, DOI: 10.1016/j.tifs.2024.104640.

18 E. N. Ayar-Sümer, Y. Verheust, B. Özçelik and K. Raes, Impact of Lactic Acid Bacteria Fermentation Based on Biotransformation of Phenolic Compounds and Antioxidant Capacity of Mushrooms, *Foods*, 2024, **13**, 1616, DOI: 10.3390/foods13111616.

19 M. Liu, Y. Fang, R. Chen, M. Cai, X. Yang, Z. Fang, X. Fang and S. Dong, Effect of *Lactobacillus plantarum*, 1243 fermentation on quality properties and metabolome of *Aronia melanocarpa* (Michx.) elliott juice, *Food Chem.: X*, 2025, **29**, 102706, DOI: 10.1016/j.fochx.2025.102706.

20 G. Gaur and M. G. Gänzle, Conversion of (poly)phenolic compounds in food fermentations by lactic acid bacteria: Novel insights into metabolic pathways and functional metabolites, *Curr. Res. Food Sci.*, 2023, **6**, 100448, DOI: 10.1016/j.crfs.2023.100448.

21 A. L. Grosso, G. Cestonaro, M. Scampicchio, G. Ferrentino and E. Costanzo, Development of a protein-enriched oat beverage: Enhancing antioxidant activity, functional properties and stability with soy protein hydrolysates, *LWT – Food Sci. Technol.*, 2025, **222**, 117644, DOI: 10.1016/j.lwt.2025.117644.

22 L. Moretto, F. Tonolo, A. Folda, V. Scalcon, A. Bindoli, M. Bellamio, E. Feller and M. P. Rigobello, Comparative analysis of the antioxidant capacity and lipid and protein oxidation of soy and oats beverages, *Food Prod., Process. Nutr.*, 2021, **3**, 1, DOI: 10.1186/s43014-020-00046-6.

23 L. Chen, D. Wu, J. Schlundt and P. L. Conway, Development of a Dairy-Free Fermented Oat-Based Beverage With Enhanced Probiotic and Bioactive Properties, *Front. Microbiol.*, 2020, **11**, 609734, DOI: 10.3389/fmicb.2020.609734.

24 J. A. Carabajal Torrez, Y. Llebeili, A. Rodríguez de Olmos and C. L. Gerez, Cultivos lácticos como potenciadores de compuestos bioactivos de avena, *Rev. Argent. Microbiol.*, 2021, **53**, 333–342, DOI: 10.1016/j.ram.2020.12.002.

25 V. L. Singleton and J. A. Rossi, Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents, *Am. J. Enol. Vitic.*, 1965, **16**, 144–158, DOI: 10.5344/ajev.1965.16.3.144.

26 F. Raposo, R. Borja and J. A. Gutiérrez-González, A comprehensive and critical review of the unstandardized Folin-Ciocalteu assay to determine the total content of polyphenols: The conundrum of the experimental factors and method validation, *Talanta*, 2024, **272**, 125771, DOI: 10.1016/j.talanta.2024.125771.

27 S. Sarkar, S. P. Sha and K. Ghatani, Metabolomics of ethnic fermented foods and beverages: understanding new aspects through Omic techniques, *Front. Sustain. Food Syst.*, 2023, **7**, 1040567, DOI: 10.3389/fsufs.2023.1040567.

28 S. Bocchi, G. Rocchetti, M. Elli, L. Lucini, C. Y. Lim and L. Morelli, The combined effect of fermentation of lactic acid bacteria and in vitro digestion on metabolomic and oligosaccharide profile of oat beverage, *Food Res. Int.*, 2021, **142**, 110216, DOI: 10.1016/j.foodres.2021.110216.

29 P. Popova-Krumova, S. Danova, N. Atanasova and D. Yankov, Lactic Acid Production by *Lactiplantibacillus plantarum* AC 11S—Kinetics and Modeling, *Microorganisms*, 2024, **12**, 739, DOI: 10.3390/microorganisms12040739.

30 D. Gonzalez-Iglesias, L. Rubio, F. Martinez-Vazquez, A. Castillo, M. Celeiro, C. Garcia-Jares and M. Lores, Phytochemical and antioxidant comparison of *Quercus ilex* and *Quercus robur* acorn extracts obtained by matrix solid-phase dispersion, *RSC Adv.*, 2025, **15**, 12538–12546, DOI: 10.1039/D4RA08675A.

31 Q. Zhang, J. Zhang, J. Shen, A. Silva, D. A. Dennis and C. J. Barrow, A Simple 96-Well Microplate Method for Estimation of Total Polyphenol Content in Seaweeds, *J. Appl. Phycol.*, 2006, **18**, 445–450, DOI: 10.1007/s10811-006-9048-4.

32 A. Symes, A. Shavandi, H. Zhang, I. A. M. Ahmed, F. Y. Al-Juhaimi and A. E. D. A. Bekhit, Antioxidant Activities and Caffeic Acid Content in New Zealand Asparagus (*Asparagus officinalis*) Roots Extracts, *Antioxidants*, 2018, **7**, 52–65, DOI: 10.3390/antiox7040052.

33 A. Castillo, M. Celeiro, B. Martínez-Vallespín, L. Rubio, D. Gonzalez-Iglesias, R. Facorro, C. Garcia-Jares, J. Zentek and M. Lores, In vivo tracking of grape marc biomarkers, bioconversion, metabolic tracers, and microbiota modulation in swine fed a polyphenol-rich extract diet, *PLoS One*, 2025, **20**, e0325079, DOI: 10.1371/journal.pone.0325079.

34 NCBI, National Center for Biotechnology Information, 1988. https://www.ncbi.nlm.nih.gov.

35 J. Hastings, G. Owen, A. Dekker, M. Ennis, N. Kale, V. Muthukrishnan, S. Turner, N. Swainston, P. Mendes and C. Steinbeck, ChEBI in 2016: Improved services and an expanding collection of metabolites, *Nucleic Acids Res.*, 2016, **44**, D1214–D1219, DOI: 10.1093/nar/gkv1031.

36 MassBank of North America, 2007. https://mona.fiehnlab.ucdavis.edu.

37 MassBank Europe, Mass Spectral Data Base, 2007. https://massbank.eu/MassBank.

38 J. Xia, N. Psychogios, N. Young and D. S. Wishart, MetaboAnalyst: a web server for metabolomic data analysis and interpretation, *Nucleic Acids Res.*, 2009, **37**, W652–W660, DOI: 10.1093/nar/gkp356.

39 T. Sharanappa, R. Chetana and G. Suresh Kumar, Evaluation of genotypic wheat bran varieties for nutraceutical compounds, *J. Food Sci. Technol.*, 2016, **53**, 4316–4324, DOI: 10.1007/s13197-016-2430-6.

40 X. Chen, M. Yuan, Y. Wang, Y. Zhou and X. Sun, Influence of fermentation with different lactic acid bacteria and in vitro digestion on the change of phenolic compounds in fermented kiwifruit pulps, *Int. J. Food Sci. Technol.*, 2022, **57**, 2670–2679, DOI: 10.1111/ijfs.15316.

41 T. R. Monisha, I. Mukram, B. Kirankumar, P. V. Reddy, A. S. Nayak and T. B. Karegoudar, Utilization of Phenylpropanoids by Newly Isolated Bacterium *Pseudomonas* sp. TRMK1, *Appl. Biochem. Biotechnol.*, 2017, **182**, 1240–1255, DOI: 10.1007/s12010-017-2396-5.

42 M. Takeuchi, S. Kishino, S. B. Park, A. Hirata, N. Kitamura, A. Saika and J. Ogawa, Efficient enzymatic production of hydroxy fatty acids by linoleic acid Δ9 hydratase from *Lactobacillus plantarum* AKU 1009a, *J. Appl. Microbiol.*, 2016, **120**, 1282–1288, DOI: 10.1111/jam.13088.

43 S. Serra, D. De Simeis, A. Castagna and M. Valentino, The Fatty-Acid Hydratase Activity of the Most Common Probiotic Microorganisms, *Catalysts*, 2020, **10**, 154, DOI: 10.3390/catal10020154.

44 K. R. Kim and D. K. Oh, Production of hydroxy fatty acids by microbial fatty acid-hydroxylation enzymes, *Biotechnol. Adv.*, 2013, **31**, 1473–1485, DOI: 10.1016/j.biotechadv.2013.07.004.

45 A. Wang, C. Guan, T. Wang, G. Mu and Y. Tuo, Changes in Intracellular and Extracellular Metabolites of Mixed *Lactobacillus* Strains Enhance Inhibition of Pathogenic Bacterial Growth and Lipopolysaccharide-Induced Alleviation of RAW264.7 Cellular Inflammation, *Probiotics Antimicrob. Proteins*, 2023, **17**, 175–192, DOI: 10.1007/s12602-023-10132-z.

46 A. Watanabe, T. Yamaguchi, K. Murota, N. Ishii, J. Terao, S. Okada, N. Tanaka, S. Kimata, A. Abe, T. Suzuki, M. Uchino and Y. Niimura, Isolation of lactic acid bacteria capable of reducing environmental alkyl and fatty acid hydroperoxides, and the effect of their oral administration on oxidative-stressed nematodes and rats, *PLoS One*, 2020, **15**, e0215113, DOI: 10.1371/journal.pone.0215113.

47 E. M. Ogawa, H. B. Costa, J. A. Ventura, L. C. Caetano, F. E. Pinto, B. G. Oliveira, M. E. S. Barroso, R. Scherer, D. C. Endringer and W. Romão, Chemical profile of pineapple cv. Vitória in different maturation stages using electrospray ionization mass spectrometry, *J. Sci. Food Agric.*, 2018, **98**, 1105–1116, DOI: 10.1002/jsfa.8561.

48 S. Liang, X. Wang, C. Li and L. Liu, Biological Activity of Lactic Acid Bacteria Exopolysaccharides and Their Applications in the Food and Pharmaceutical Industries, *Foods*, 2024, **13**, 1621, DOI: 10.3390/foods13111621.

49 F. Polito, L. De Martino, G. Mirabile, G. Venturella, M. L. Gargano, V. De Feo, H. S. Elshafie and I. Camele, Composition and antimicrobial activity of hydroalcoholic extracts of *Pleurotus eryngii* var. *ferulae* and *P. eryngii* var. *elaeoselini*, *Front. Chem.*, 2024, **12**, 1498787, DOI: 10.3389/fchem.2024.1498787.

50 X. Li, Y. Zheng, W. Cui, X. Bai, C. Zhu and G. Zhao, Comparative Effects of the Single and Binary Fermentations of *Latilactobacillus sakei* and *Staphylococcus carnosus* on the Growth and Metabolomic Profiles of Fermented Beef Sausages, *Microorganisms*, 2025, **13**, 1523, DOI: 10.3390/microorganisms13071523.

51 S. Dong, L. Li, F. Hao, Z. Fang, R. Zhong, J. Wu and X. Fang, Improving quality of poultry and its meat products with probiotics, prebiotics, and phytoextracts, *Poult. Sci.*, 2023, **103**, 103287, DOI: 10.1016/j.psj.2023.103287.

52 R. Yoshida and Y. Ninomiya, Umami and MSG, in *Umami. Food and Health*, ed. A. San Gabriel, T. M. Rains and G. Beauchamp, Springer, Cham, 2024, pp. 7–42. DOI: 10.1007/978-3-031-32692-9_2.

53 L. De Luca, F. Pizzolongo, M. Calabrese, G. Blaiotta, M. Aponte and R. Romano, Addition of glutamine to milk during fermentation by individual strains of lactic acid bacteria and the effects on pyroglutamic and butyric acid,

J. Food Compos. Anal., 2024, **130**, 106175, DOI: 10.1016/j.jfca.2024.106175.

54 D. Chelius, K. Jing, A. Lueras, D. S. Rehder, T. M. Dillon, A. Vizel, R. S. Rajan, T. Li, M. J. Treuheit and P. V. Bondarenko, Formation of Pyroglutamic Acid from N-Terminal Glutamic Acid in Immunoglobulin Gamma Antibodies, *Anal. Chem.*, 2006, **78**, 2370–2376, DOI: 10.1021/ac051827k.

55 S. Shirako, Y. Kojima, N. Tomari, Y. Nakamura, Y. Matsumura, K. Ikeda, N. Inagaki and K. Sato, Pyroglutamyl leucine, a peptide in fermented foods, attenuates dysbiosis by increasing host antimicrobial peptide, *npj Sci. Food*, 2019, **3**, 18, DOI: 10.1038/s41538-019-0050-z.

56 Z. Zhu, Y. Bian, X. Zhang, R. Zeng and B. Yang, Terahertz spectroscopy of temperature-induced transformation between glutamic acid, pyroglutamic acid and racemic pyroglutamic acid, *Spectrochim. Acta, Part A*, 2022, **285**, 121150, DOI: 10.1016/j.saa.2022.121150.

57 B. Gazme, R. T. Boachie, A. Tsopmo and C. C. Udenigwe, Occurrence, properties and biological significance of pyroglutamyl peptides derived from different food sources, *Food Sci. Hum. Wellness*, 2019, **8**, 268–274, DOI: 10.1016/j.fshw.2019.05.002.

58 Z. Liang, Y. Huang, P. Zhang and Z. Fang, Impact of fermentation on the structure and antioxidant activity of selective phenolic compounds, *Food Biosci.*, 2023, **56**, 103147, DOI: 10.1016/j.fbio.2023.103147.

59 D. Skroza, V. Šimat, L. Vrdoljak, N. Jolić, A. Skelin, M. Čagalj, R. Frleta and I. Generalić Mekinić, Investigation of Antioxidant Synergisms and Antagonisms among Phenolic Acids in the Model Matrices Using FRAP and ORAC Methods, *Antioxidants*, 2022, **11**, 1784, DOI: 10.3390/antiox11091784.

60 H. Zhang, C. You and Y. Wang, Metabolomics study on fermentation of *Lactiplantibacillus plantarum* ST-III with food-grade proliferators in milk, *J. Dairy Sci.*, 2024, **107**, 9005–9014, DOI: 10.3168/jds.2024-25017.