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ORIGINAL PAPER

Dynamic changes in physicochemical, antioxidant, and aromatic profiles during fermentation of pea extract (*Pisum sativum* L.) by lactic acid bacteria

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Abstract

The higher demand for functional foods, combined with the growing adoption of vegan diets, requires the food industry to develop alternative foods that are not animal-based, utilizing plant-based matrices. Peas, like most legumes, are sources of protein, carbohydrates, and other nutrients. However, this matrix is still poorly explored for the development of fermented non-dairy-like products. The objective of this study was to investigate the potential of pea extracts as a base matrix for the development of fermented and potentially probiotic plant-based products by evaluating their physicochemical and technological properties. Five strains of potentially probiotic lactic acid bacteria (LAB) were evaluated. Fermentation results showed that all strains were able to ferment the pea aqueous extracts, with *Lactococcus lactis* 32B4-1 (9.51 log CFU/mL) and *Lactiplantibacillus plantarum* BL011 (9.19 log CFU/mL) presenting the highest growth rates in 12 h. The lactic acid production was observed along with cell growth, reaching a maximum concentration of 4.2 g/L by *L. lactis* 32B4-1. After fermentation, the antioxidant activity increased by 30.71 to 78.27% and the extracts changed from bright green colour to olive. The changes in volatile compounds were strain dependent. *L. lactis* 32B4-1 and *L. plantarum* BL011 showed beneficial changes, altering volatile metabolites, including aldehydes, known to impart an undesirable flavour to legumes. *L. lactis* 32B4-1 and *L. plantarum* BL011 showed potential for industrial applications for the preparation of fermented pea extracts and could be further explored to ferment other plant matrices.

Keywords Plant-based foods · Peas · Fermentation · Lactic acid bacteria · Volatile compounds

Introduction

Changes in lifestyle and consumption patterns of people have shifted research focus to the development of functional foods and beverages that improve human health. The most widely used functional ingredients are microbial probiotics, defined as live microorganisms that, when administered in adequate amounts, confer a health benefit to the host, human or animal [1].

Probiotics are often found in dairy products [2]. However, with the increasing number of people who are allergic to milk proteins or with severe lactose intolerance, it became necessary to search for new matrices free of animal-based products or ingredients. Fruits, vegetables, and cereal juices may be an ideal vehicle for delivering probiotics [3]. The application of probiotic bacteria in different food matrices presents challenges, since each bacterial species has a different metabolism towards the type of substrate, acidity, dissolved oxygen, metabolic products, temperatures, and gastrointestinal tract conditions [4].

The use of legumes in the development of plant-based products has been growing in popularity, reinforcing the

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idea of diversifying protein sources in the human diet and adopting more sustainable diets [5]. According to FAO/Biodiversity International, sustainable diets "protect and respect biodiversity and ecosystems; are culturally acceptable, accessible, economically fair and affordable; nutritionally adequate, safe and healthy, while optimizing natural and human resources" [6].

Legumes are plants taxonomically classified in the *Fabaceae* family. Peas (*Pisum sativum* L.) are an important source of protein, vitamins, minerals, carbohydrates, and insoluble dietary fibre, and are low in fat [7]. Pea grains are also rich in phytochemicals, phenolic compounds that have strong antioxidant activities [8]. Matrices with high antioxidant potential are of interest for application in functional foods, as they oxidize compounds that inhibit, prevent, or slow down cell damage caused by free radicals [9].

However, peas and other *Fabaceae*, such as soybeans, might often have undesirable physicochemical properties related to flavours, texture, and nutrient bioavailability. Plant-based beverages have limited acceptance due to the beany flavour and bitter taste characteristic of many vegetable matrices [10]. These undesirable flavours often result from volatile compounds like hexanal, which imparts the herbaceous flavour of the product [11].

LAB fermentation of vegetable sources may offer potential solutions to overcome the above problems. Bioprocess based on these bacteria, fermenting legumes, will alter in positive ways their nutritional qualities, and may contribute to maintaining the perception of products as natural and additive-free [12].

In this study, we investigated the effects of the fermentation of five LAB strains on pea extracts. The physicochemical characteristics, fermentation metabolites, total phenolics, antioxidant activity, and volatile profile of the obtained products were analysed. This study provides a scientific basis to guide the potential application of peas as a non-dairy matrix, fermented functional ingredient for plant-based food products.

Materials and methods

Reagents and chemical standards

Folin–Ciocalteu reagent was obtained from ACS Científica (São Paulo, Brazil); potassium persulfate from Comercial Neon© (São Paulo, Brazil); sodium carbonate, gallic acid, and 2,20-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS)) diammonium salt were obtained from Sigma-Aldrich (St. Louis, MO, USA). Standards of organic acids (lactic and acetic acids) and sugars (sucrose, glucose, fructose, raffinose, and stachyose) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemical reagents were of analytical grade.

Legume matrix

In natura frozen green peas (*Pisum sativum* L.), obtained from producers in the region of Atibaia, São Paulo, Brazil, were used to produce the legume-based extracts for fermentation. Moisture, ash, protein, and lipid analyses were performed according to the standards of the Adolfo Lutz Institute [13] to initially characterise the pea extract. The estimation of total carbohydrate content was obtained by difference (100 - moisture - ash - protein - lipid).

Bacteria strains, cell maintenance, and pre-inocula Preparation

Five strains of LAB were used: a commercial strain of *Lacticaseibacillus rhamnosus* FAGRON™ (Fagron, São Paulo, SP, Brazil); a strain of *Lactiplantibacillus plantarum* BL011, isolated by our group and kept in the BiotecLab collection, Federal University of Rio Grande do Sul (UFRGS) [14]; *Lactococcus lactis* 32B4-1, *Lacticaseibacillus paracasei* 12B0-2 [15], and *Lacticaseibacillus paracasei* M1A3 [16] were isolated from buffalo whey and buffalo raw milk, respectively, and were kindly provided by the Microbiology, Immunology, and Parasitology Department, UFRGS. Cells were kept frozen as a cell suspension at -20 °C in a 40% glycerol solution.

Pre-inocula were prepared by transferring 0.5 mL of the cell suspension of glycerol (20% v/v) stocks of each microorganism to 250 mL conical flasks containing 50 mL of de Man, Rogosa, and Sharpe (MRS) (Kasvi, São Paulo, Brazil). The flasks were incubated until a final optical density (OD) of 1.0 at 600 nm under the following conditions: static cultivation at 30 °C for *L. lactis* 32B4-1 [17], static cultivation at 37 °C for *L. rhamnosus* FAGRON™, *L. paracasei* M1A3, and *L. paracasei* 12B0-2 [16]; and at 180 rpm in a rotatory shaker (MA 830 Marconi, Piracicaba, Brazil) at 37 °C for *L. plantarum* BL011 [18]. After cultivation, cultures were centrifuged at 5,000 *g*, 4 °C, 5 min [17], and the cell pellets were resuspended directly in the pea extract (Sect. Production and fermentation of the pea extracts).

Production and fermentation of the pea extracts

The production of pea extract (Fig. 1) was performed based on the Ma et al. [19] methodology, with modifications. The extract was produced by blending green pea grains with water at a ratio of 1:3 (w/v). If not treated properly, minimally processed and refrigerated foods, such as plant extracts, may pose a risk to human health. Therefore, a heat

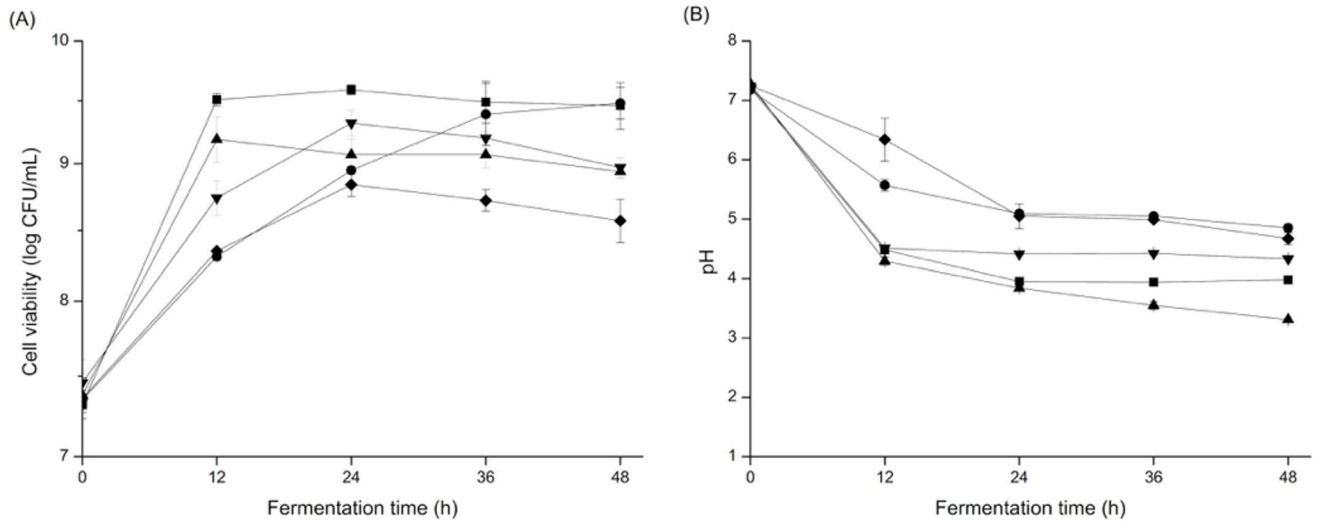


Fig. 1 Changes in values of cell count (log CFU/mL) (A) and pH (B) of pea extracts fermented with five different strains after incubation at 30 °C for 48 h. (■) *L. lactis* 32B4-1, (●) *L. rhamnosus* FAGRON™, (▲) *L. plantarum* BL011, (▼) *L. paracasei* M1A3, (◆) *L. paracasei* 12B0-2. Experiments represent the mean of duplicates

treatment of the pea extract was conducted at 95 °C for 16 min, followed by rapid cooling in ice to stabilize the extracts. After that, extracts were kept refrigerated at 4 °C until the moment of fermentation. Pea extracts (100 mL) were singularly inoculated (initial density of 2×10^7 CFU/mL) with *L. lactis* 32B4-1, *L. rhamnosus* FAGRON™, *L. plantarum* BL011, *L. paracasei* M1A3, or *L. paracasei* 12B0-2, in 500 mL conical flasks. The flasks were incubated for 48 h at 30 °C and 130 rpm under anaerobiosis.

Biochemical, nutritional, and microbial characterization of the extracts

Determination of cell viability

The cell viability was evaluated using the single plate dilution spotting method, in samples collected every 12 h up to 48 h of fermentation [20]. Aliquots (100 µL) of each sample were diluted with 900 µL of sterile saline solution in serial dilutions, and they were spread-plated in MRS agar for enumeration and incubated at 30 °C for 48 h to 72 h in anaerobic jars. The results were expressed as log CFU/mL.

Analyses of colour, pH, soluble proteins, and electrophoresis SDS-PAGE of fermented pea extracts

Instrumental colour evaluation was performed using a CM-400 Konica Minolta (Japan) colorimeter, obtaining the values of the coordinates L* (measurable in terms of white to black intensity), a* (measurable in terms of red and green intensity), and b* (measurable in terms of yellow and blue intensity). The total difference of colour (ΔE) was obtained by the expression:

$$\Delta E = \left[(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2\right]^{1/2} \quad (1)$$

Depending on the value of ΔE, the colour difference between pea extract non-fermented and fermentation samples can be estimated such as imperceptible difference (0–1), lightly noticeable (1-1.5), an inexperienced observer also notices the difference; (1.5–3.5), a clear colour difference is noticed (3.5-5.0), and the observer perceives two different colours. (> 6.0) [21].

The changes in soluble proteins during fermentation were determined by the Bradford method [22] using bovine serum albumin (BSA) as the standard. Also, SDS-PAGE electrophoresis [23] was performed to investigate the degradation of proteins. The Bio-Rad Mini-PROTEAN TGX system (Bio-Rad, Hercules, CA, USA) was used. Gels with 12% (v/v) acrylamide were used for resolution, and 4% (v/v) for stacking. Pre-stained molecular mass marker (Thermo Scientific) was used as a reference. Electrophoresis was conducted at a constant current of 60 V for 20 min, followed by 150 V for 1 h, and the bands were stained with Coomassie Brilliant Blue R250.

Determination of fermentation metabolites

The quantification of metabolites produced during fermentation (lactic acid, acetic acid, and ethanol) and the consumption of sugars (verbascose, stachyose, raffinose, sucrose, glucose, and fructose) was performed using

high-performance liquid chromatography (HPLC). All chromatographic assays were conducted in an HPLC equipped with a RID-20 A refractive index detector (Shimadzu, Japan). Fermentation metabolites were detected using a Bio-Rad Aminex® HPX 87 H column (300 × 7.8 mm, 9 µm particle size, Bio-Rad, USA), applying a flow rate of 0.6 mL/min, column oven temperature of 45 °C, injection volume of 20 µL, and using sulfuric acid 0.005 mol/L as eluent. The chromatographic assays for quantification of sugars were carried out using a Hi-plex Na column (300 × 7.7 mm, 10 µm particle size, Agilent Technologies, UK), at a flow rate of 0.2 mL/min, column oven temperature of 85 °C, injection volume of 3 µL, and pure Milli-Q water as mobile phase.

Determination of total phenolic content and antioxidant activity

The total phenolic content (TPC) of the pea extracts was determined using the Folin-Ciocalteu method, with a gallic acid standard curve to determine the TPC. The change in absorbance was measured using a spectrophotometer (SpectraMax ID3, Molecular Devices, Diadema, SP) at 760 nm. The concentration TPC was calculated as gallic acid equivalents mg GAE/mL. All assay determinations were conducted in duplicate.

To determine antioxidant activity, the ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging assay was conducted following the protocol of Rufino et al. [24] with adaptations. Initially, to generate free radicals, a stock solution of ABTS (0,007 mol/L) and potassium persulfate (0,14 mol/L) was prepared 16 h before the analysis. Working solutions of the radicals were prepared by dilution to reach an absorbance of 0.70 ± 0.02 at 734 nm. Reactions were performed in 96-well plates. After incubation for 6 min in the dark, the absorbance was measured at 734 nm, in duplicate. The results were expressed as a percentage of ABTS radical removal. Antioxidant effectiveness was calculated using the following equation:

$$\%\ \text{ABTS scavenging activity} = [1 - (\text{As} / \text{Ac})]\ \text{x}\ 100 \quad (2)$$

Where: A_s represents the absorbance of the sample, and A_c represents the absorbance of the control.

Volatile organic compounds (VOCs) analysis

The extraction of volatile organic compounds (VOCs) was performed using headspace solid-phase microextraction (HS-SPME). For this, a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibre (Supelco, 50/30 µm × 20 mm) was used, conditioned according to the manufacturer's specifications. Non-fermented and fermented samples (2 mL) were stored in 20 mL glass vials, to which 0.6 g of sodium chloride and 10 µL of an internal biphenyl standard solution were added. The vials were sealed with magnetic caps containing silicone septa coated with polytetrafluoroethylene.

The sealed vials were transferred to an HS-Combi-PAL autosampler (CTC Analytics), stirred at 350 rpm, and kept at 65 °C for 5 min to promote volatilization equilibrium. After this period, the fibre was exposed to the static headspace for 40 min to adsorb volatile compounds. Then, the fibre was inserted into the injector of the chromatographic system, where the analytes were desorbed for 10 min.

The separation and identification of VOCs were performed using gas chromatography-mass spectrometry (GC-MS) in a GC-MS-QP2010 Ultra Plus equipment (Shimadzu, Japan). A DB-5MS capillary column (60 m × 0.32 mm × 0.25 µm, Agilent) was used. The temperature program consisted of an initial step of 65 °C for 1 min, followed by heating to 240 °C at a rate of 5 °C/min, and finally to 270 °C at 55 °C/min, maintaining this temperature for 1 min, resulting in an analysis time of 38 min.

The mass spectrometer was operated in full scan mode covering the m/z range 45 to 500. The data obtained were processed by the LabSolution program (GC-MS solution Version 4.11 SU2). The identification of the compounds was based on comparisons with the NIST11 database, using spectral similarity indices and mass spectra.

Statistical analysis

All results were calculated as means of duplicates, and data were analysed employing one-way ANOVA. Pairwise comparison of treatment means was done by Tukey's test at $p < 0.05$, with GraphPad Prism 8 statistical software. Data expressed as means ± standard deviation (SD).

Results

The main objectives of this study were to evaluate the growth of five LAB strains in a pea matrix and analyse changes in phenolic compounds and antioxidant activities, as well as volatile compounds in the extracts after fermentation. To analyse the real potential of peas as matrices for fermentation, a unique extract of the legume was used without additional supplementation. The pea extract presented the following approximate centesimal composition: pH 7.2; 90% of moisture; 1.79% of protein; 0.36% of lipids; 0.05% of ashes; and 7.80% of carbohydrates, including fibres.

Fermentation characteristics of pea extracts

Lactic acid strains are known to survive and improve dairy products through fermentation, but their biotechnological applications in plant-based matrices are still underexplored. In this work, all LAB strains exhibited survival and growth in pea extract. Along with cell growth, a decrease in the pH was observed for all strains, which is related to the production of metabolites, especially lactic acid. Growth and pH changes of strains in pea extracts are shown in Fig. 1.

After 12 h of fermentation, *L. lactis* 32B4-1 and *L. plantarum* BL011 reached 9.59 ± 0.04 log CFU/mL and 9.19 log CFU/mL (Fig. 1A), respectively, and maintained their viability up to 48 h. *L. rhamnosus* FAGRON™ and *L. paracasei* 12B0-2 showed the same growth patterns up to 24 h of fermentation ($p < 0.05$); however, after 24 h, *L. rhamnosus* FAGRON™ continued to grow and reached the same viability as *L. lactis* 32B4-1 at 48 h (9.59 ± 0.13 log CFU/mL), while *L. paracasei* 12B0-2 decreased viability at 48 h. Among all the strains, *L. paracasei* 12B0-2 showed the lowest growth in the extract, reaching a maximum of 8.80 ± 0.14 log CFU/mL at 24 h.

Before fermentation, pea extracts had a pH of 7.23 ± 0.04; this value decreased to a range between 3.86 ± 0.08 and 4.85 ± 0.07 after 48 h of fermentation with all strains (Fig. 1B). This reduction in pH is related to the production of organic acids such as lactic acid (0.67 ± 0.13 to 4.23 ± 0.05 g/L; Fig. 2A), which was detected in all the pea extracts after fermentation, while acetic acid (0.25 ± 0.02 to 1.78 ± 0.14 g/L) was produced by all strains, except for *L. lactis* 32B4-1(Fig. 2B). Ethanol was not produced by the heterofermentative strains (*L. rhamnosus* FAGRON™; *L. plantarum* BL011; *L. paracasei* M1A3; and *L. paracasei* 12B0-2).

The results of sugar consumption show that sucrose (Fig. 3B) was the most abundant sugar in unfermented pea extracts, followed by raffinose (Fig. 3C) and stachyose (Fig. 3D). Verbascose was not detected. The antinutrients stachyose and raffinose decreased after 48 h of fermentation. All strains reduced raffinose concentration between 39.1% and 53.85%, with *L. rhamnosus* and *L. plantarum* showing the highest consumption of this sugar. Regarding stachyose, the strains *L. paracasei* M1A3 and *L. paracasei* 12B0-2 could not significantly reduce this antinutrient. However, the strains *L. lactis*, *L. rhamnosus*, and *L. plantarum* reduced stachyose content by 43.2%, 33.3%, and 72%, respectively.

Organic acids and substances such as alcohol and aldehydes react to form several compounds that enhance the flavour of the fermented product. The fermentation of the extract with *L. paracasei* M1A3 reached the highest production of lactic acid in 24 h (2.06 ± 0.20 g/L), which was subsequently consumed until 48 h (0.09 ± 0.01 g/L) (Fig. 2A). Acetic acid imparts a characteristic vinegar taste. An adequate concentration of this acid may be desirable to increase the acidity of foods, such as in kombucha, but high concentrations may cause undesirable sensorial characteristics, decreasing the food's acceptability. Except for the fermentation with *L. paracasei* 12B0-2 in 36 h (1.64 ± 0.25 g/L) and with *L. paracasei* M1A3 in 48 h (1.78 ± 0.14 g/L), the other strains produced low concentrations (< 1 g/L) of acetic acid (Fig. 2B). *L. lactis* 32B4-1, a homofermentative bacterium, had lactic acid as the single product of fermentation.

The differences in colour values between the extracts are reported in Table 1. The effect of fermentation on colour

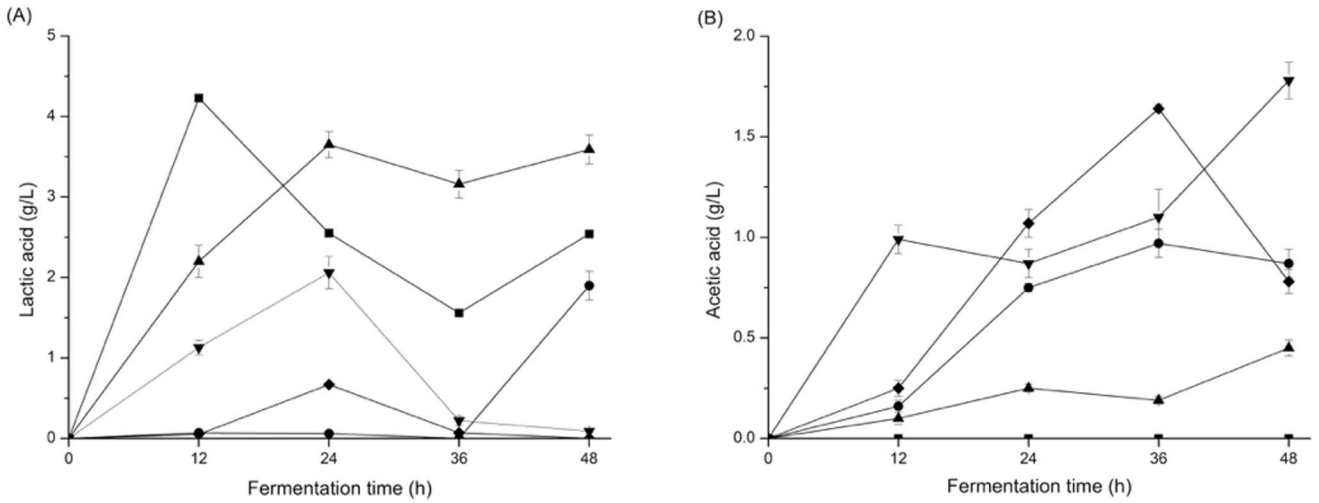


Fig. 2 Concentration of (A) lactic acid (g/L) and (B) acetic acid (g/L) of pea extracts fermented with five different strains after incubation at 30 °C for 48 h. (■) *L. lactis* 32B4-1, (●), *L. rhamnosus* FAGRON™, (▲) *L. plantarum* BL011, (▼) *L. paracasei* M1A3, (◆), *L. paracasei* 12B0-2. Experiments represent the mean of duplicates

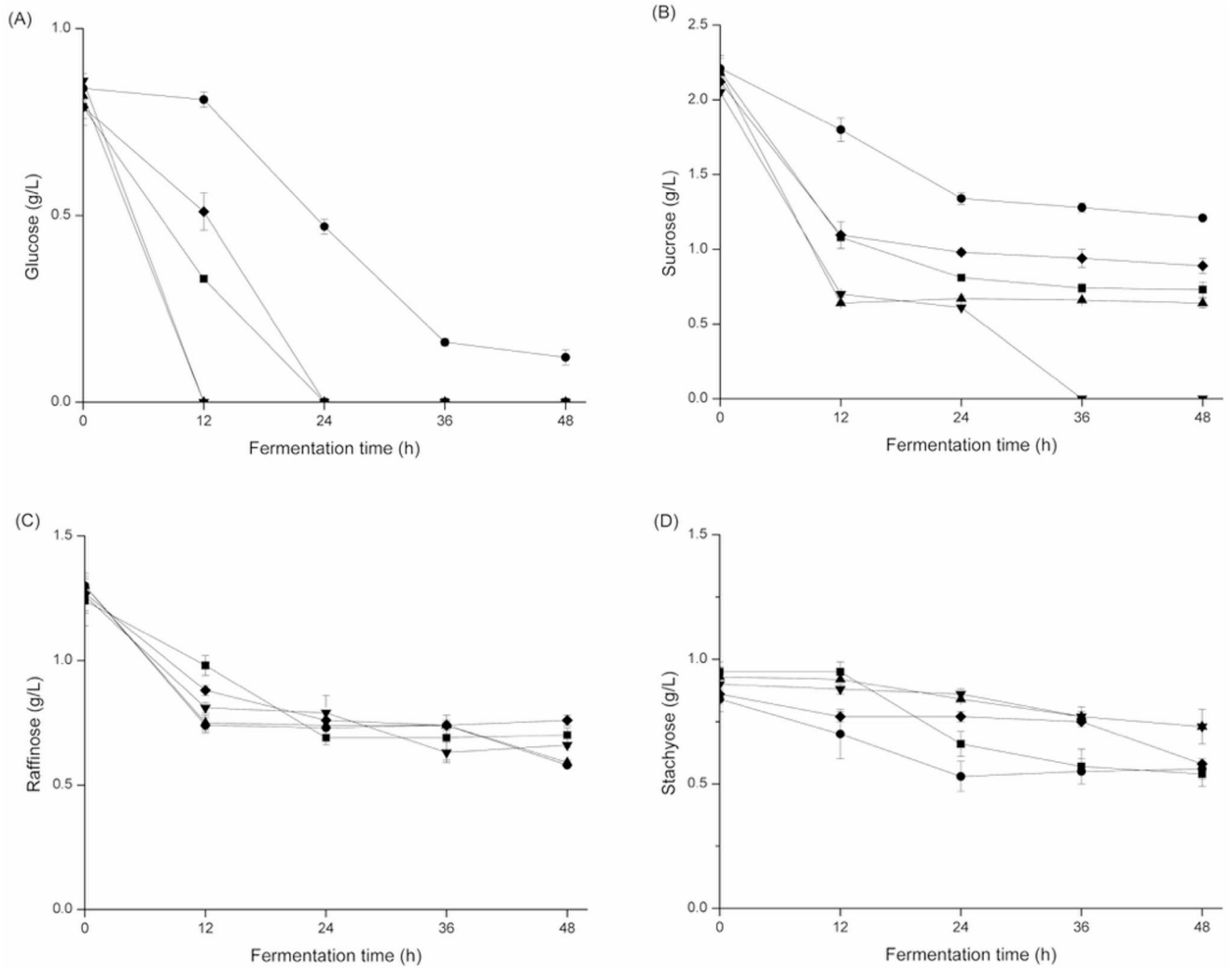


Fig. 3 Sugar concentration (g/L) in pea extracts fermented for 48 h at 30 °C. Glucose (A), sucrose (B), raffinose (C), and stachyose (D). (■) *L. lactis* 32B4-1, (●), *L. rhamnosus* FAGRON™, (▲) *L. plantarum* BL011, (▼) *L. paracasei* M1A3, (◆), *L. paracasei* 12B0-2. Experiments represent the mean of duplicates

properties was examined considering the colour characteristics (L*, a*, and b*) and the total colour difference (ΔE). Colour changes in processed foods may visually represent a loss of quality. Hence, it is important to analyse colour changes throughout fermentation. Results showed that the process increased the luminosity values (L*), with *L. lactis* 32B4-1, *L. plantarum* BL011, and *L. paracasei* M1A3 showing the greatest increases in this parameter. The a* values increased at 48 h, while the b* values decreased in all samples. This shows that throughout the fermentation, the extracts lost the intensity of the green and yellow hues.

The ΔE values of the fermented samples were calculated and compared with those of the control experiment, which excluded microorganisms, to evaluate better the colour differences caused by fermentation. The ΔE results of the fermented extracts showed a noticeable colour difference ($\Delta E > 5$) in the first 12 h of fermentation of *L. lactis* 32B4-1 (9.85 ± 0.4), *L. plantarum* BL011 (9.69 ± 0.6), *L. paracasei* M1A3 (9.50 ± 0.7), and *L. paracasei* 12B0-2 (5.73 ± 0.6). The higher values were detected in the extracts that presented higher cell growth in 12 h.

On the other hand, the control extract did not show noticeable changes up to 24 h (4.7 ± 0.6). After this time, the control reached a maximum ΔE of 7.9 ± 0.4 in 48 h, which is a lower value compared to those found for *L. lactis* 32B4-1 and for *L. plantarum* BL011 extracts, for example. These findings suggest that temperature and time may impact the extract's colour, but fermentation intensifies the visual alterations due to different reasons, such as cell growth and/or enzyme activity.

After 12 h of fermentation (Table 2), the protein content was reduced, whereas after 48 h, soluble protein was not detected in any of the fermented pea extracts. This result might be explained by the precipitation of larger peptides,

Table 1 Colour indexes, lightness (L*), red/green (a*), yellow/blue (b*), and total colour difference (ΔE) of extracts of non-fermented and fermented green peas

	Time (h)	Control	LL	LR	LP	LPM	LP12
L*	0	52.26±0.2 ^{c}	52.88±0.2 ^{c}	52.32±0.2 ^{b}	52.74±0.4 ^{c}	52.40±0.4 ^{d}	52.80±0.2 ^{d}
	12	54.03±0.4 ^{b}	54.15±0.3 ^{b}	52.82±0.3 ^{b}	56.06±0.5 ^{a}	55.95±0.5 ^{bc}	53.50±0.3 ^{bcd}
	24	54.45±0.4 ^{b}	55.98±0.4 ^{a}	52.39±0.3 ^{b}	55.98±0.3 ^{a}	56.95±0.6 ^{a}	54.62±0.5 ^{ab}
	36	55.25±0.3 ^{a}	55.67±0.8 ^{a}	53.96±0.3 ^{a}	55.65±0.3 ^{b}	55.59±0.5 ^{c}	53.94±0.7 ^{bc}
	48	55.35±0.1 ^{a}	56.28±0.2 ^{a}	53.69±0.4 ^{a}	55.43±0.6 ^{a}	56.42±0.3 ^{ab}	54.95±0.6 ^{a}
a*	0	-17.91±0.4 ^{c}	-18.14±0.4 ^{d}	-19.77±0.2 ^{d}	-17.25±0.6 ^{c}	-15.98±0.8 ^{c}	-18.41±0.5 ^{d}
	12	-18.27±0.1 ^{c}	-8.38±0.1 ^{c}	-18.34±0.2 ^{c}	-8.49 ± 0.1 ^{b}	-8.20±0.1 ^{ab}	-13.19±0.5 ^{c}
	24	-14.86±0.2 ^{b}	-7.64±0.3 ^{b}	-11.60±0.5 ^{b}	-7.74±0.1 ^{a}	-8.70±0.9 ^{b}	-13.32±0.1 ^{c}
	36	-11.37±0.1 ^{a}	-7.16±0.1 ^{a}	-11.29±0.3 ^{b}	-7.38±0.4 ^{a}	-7.75±0.2 ^{a}	-11.24±0.4 ^{b}
	48	-11.04±0.4 ^{a}	-8.01±0.1 ^{bc}	-8.63±0.5 ^{a}	-7.50±0.1 ^{a}	-7.63±0.6 ^{a}	-10.40±0.2 ^{a}
b*	0	34.69±0.3 ^{c}	35.01±0.3 ^{a}	36.59±0.1 ^{a}	33.56±0.6 ^{a}	35.01±0.5 ^{a}	36.73±0.5 ^{a}
	12	36.67±0.4 ^{b}	34.59±0.1 ^{a}	35.74±0.7 ^{a}	31.34±0.7 ^{b}	30.99±0.3 ^{b}	34.62±0.3 ^{b}
	24	37.63±0.5 ^{a}	30.52±0.8 ^{c}	33.95±0.7 ^{b}	29.93±0.4 ^{c}	29.51±0.2 ^{c}	34.88±0.4 ^{b}
	36	36.86±0.1 ^{b}	31.83±0.5 ^{b}	35.90±0.5 ^{a}	32.11±0.7 ^{b}	30.02±0.4 ^{c}	34.71±0.8 ^{b}
	48	37.20±0.4 ^{ab}	31.87±0.5 ^{b}	34.69±0.8 ^{b}	29.38±0.9 ^{c}	28.02±0.7 ^{d}	35.22±0.5 ^{b}
ΔE	0	-	-	-	-	-	-
	12	2.7±0.4 ^{Cc}	9.85±0.4 ^{Ab}	1.80±0.4 ^{Cc}	9.69±0.6 ^{Ab}	9.50±0.7 ^{Ab}	5.73±0.6 ^{Bb}
	24	4.7±0.6 ^{Db}	11.82±0.5 ^{Aa}	8.60±0.4 ^{Cb}	10.69±0.5 ^{Aa}	10.08±0.8 ^{Bb}	5.74±0.6 ^{Db}
	36	7.4±0.4 ^{Da}	11.76±0.4 ^{Aa}	8.67±0.2 ^{Cb}	10.21±0.8 ^{Bab}	9.96±0.7 ^{Bb}	7.63±0.9 ^{Da}
	48	7.9±0.4 ^{Ba}	11.15±0.4 ^{Aa}	11.41±0.4 ^{Aa}	10.99±0.4 ^{Aa}	11.65±0.7 ^{Aa}	8.44±0.4 ^{Ba}

Data are the mean of two independent experiments±standard deviation. Different lowercase letters within a row indicate significant differences (Tukey, $p<0.05$) over time for a single strain; different uppercase letters within a column indicate significant differences (Tukey, $p<0.05$) between strains at a single time point. LL: *L. lactis* 32B4-1; LR: *L. rhamnosus* FAGRON™; LP: *L. plantarum* BL011; LPM: *L. paracasei* M1A3; LP12: *L. paracasei* 12B0-2

Table 2 Changes in soluble protein (mg/L) of pea extracts fermented by the LAB strains after incubation at 30 °C for 48 h

Fermentation time (h)	0	12	24	36	48
L. lactis 32B4-1	0.82±0.03^{Aa}	0.14±0.08^{Cb}	n.d.	n.d.	n.d.
L. rhamnosus FAGRON™	0.89±0.07^{Aa}	0.35±0.03^{Bb}	0.24±0.05^{Ac}	0.09±0.01^{Ad}	n.d.
L. plantarum BL011	0.84±0.03^{A}	n.d.	n.d.	n.d.	n.d.
L. paracasei M1A3	0.82±0.05^{Aa}	0.14±0.02^{Cb}	0.12±0.01^{Bb}	n.d.	n.d.
L. paracasei 12B0-2	0.82±0.07^{Aa}	0.40±0.07^{Ab}	0.13±0.04^{ABc}	0.07±0.02^{Ad}	n.d.

Data are the mean of two independent experiments±standard deviation. Different lowercase letters within a row indicate significant differences (Tukey, $p<0.05$) over time for a single strain; different uppercase letters within a column indicate significant differences (Tukey, $p<0.05$) between strains at a single time point. nd: not detected

leaving only small water-soluble peptides after 12 h, or the limitations of the method used to quantify the proteins, which are below the detection threshold.

SDS gels were performed to visualize the changes in the molecular mass of soluble proteins (Fig. 4). Band identities were compared with the literature.

Antioxidant activity

The total phenolic content of the pea extract throughout fermentation is shown in Fig. 5A. The unfermented extract exhibited the highest levels (7.22±0.75 to 8.00±0.43 mg GAE/mL), which decreased significantly ($p<0.05$) after fermentation. Although the total phenolic content declined over time, the antioxidant activity increased when compared to the unfermented extract (Fig. 5B). At the beginning of fermentation, the antioxidant capacity was approximately 30%. This value increased markedly, reaching 70.87% in the *L. lactis* 32B4-1 fermentation at 12 h and 78.27% in the *L. rhamnosus* FAGRON™ fermentation at 24 h. This interesting correlation between the variations in phenolic content and antioxidant activities is further discussed in the Discussion section.

Volatile compounds

The flavour-related metabolites in pea extract (unfermented and fermented with LAB for 24 h and 48 h) were

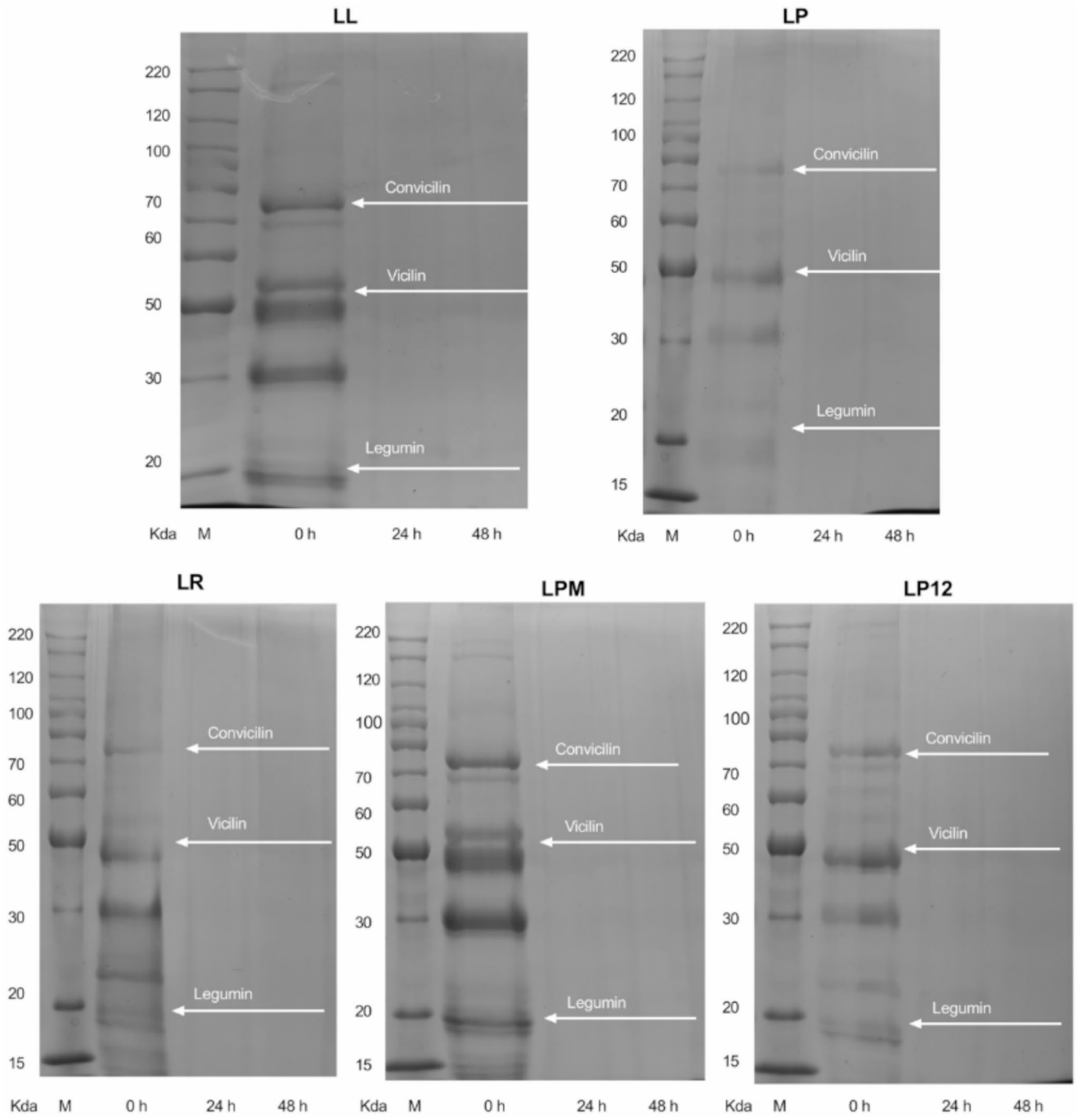


Fig. 4 Gel electrophoresis of pea extract fermented with the five bacteria at 0 h, 24 h, and 48 h. M = molecular mass marker. LL: *L. lactis* 32B4-1; LP: *L. plantarum* BL011; LR: *L. rhamnosus* FAGRON™; LPM: *L. paracasei* M1A3; LP12: *L. paracasei* 12B0-2

analysed by GC-MS, and 28 volatile compounds were identified (Table 3). These compounds were grouped into seven classes, including 10 aldehydes, 5 alcohols, 3 acids, 2 esters, 1 furan, 5 alkanes, and 2 ketones. The concentrations of volatile compounds found in the samples, calculated by the ratios of the peak surface areas, are presented in Table 4.

Discussion

LAB are easily grown in most food substrates, and they lower the pH rapidly to a point where other competing organisms are no longer able to grow. LABs exhibited either homofermentative or heterofermentative metabolisms, which directly influence the type and amount of organic

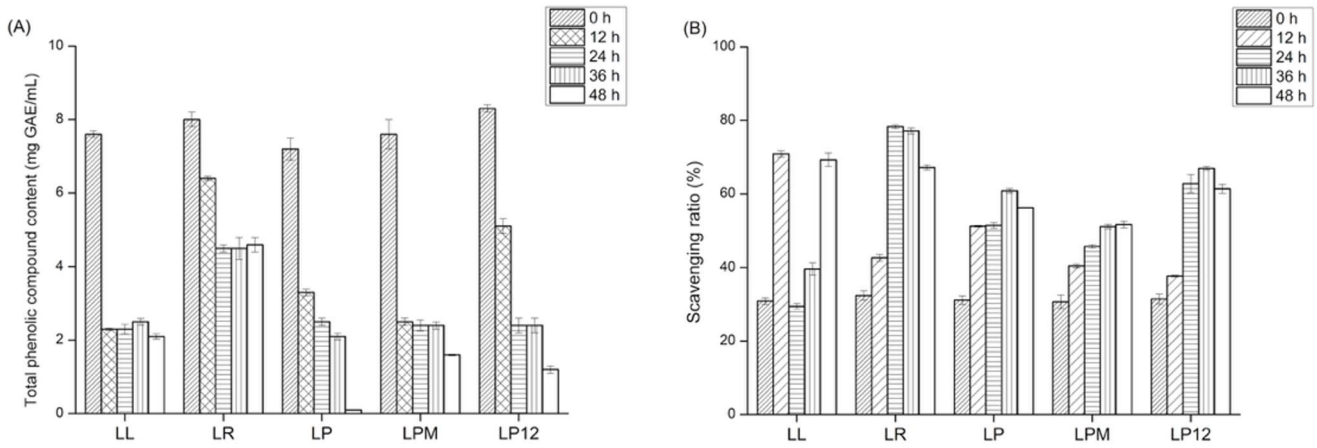


Fig. 5 (A) Changes in total phenolic compounds content (mg GAE/mL) during fermentation, and (B) ABTS radical scavenging capacities (%) during fermentation. LL: *L. lactis* 32B4-1; LR: *L. rhamnosus* FAGRON™; LP: *L. plantarum* BL011; LPM: *L. paracasei* M1A3; LP12: *L. paracasei* 12B0-2

acids produced and, consequently, the extent of pH reduction. For example, *L. lactis* is a homofermentative strain and produces lactic acid as the sole metabolite from glucose fermentation through the Embden-Meyerhof route [25]. The other strains studied in this work, *L. plantarum*, *L. rhamnosus*, and *L. paracasei*, present heterofermentative metabolism. These strains utilize the pentose-phosphate route, producing other metabolites in addition to lactic acid, such as acetic acid or ethanol. The fermentation of plant matrices depends on the microorganism's ability to adapt quickly and metabolize available nutrients. Adaptation is specific to each strain, as well as determined by the plant material [3]. Essentially, LAB ferment carbohydrates into lactic acid, and sugars are fermented mainly under anaerobic conditions.

The differences observed in fermentation time, growth kinetics, and pH values are attributable to the specific physiological and metabolic profiles of each strain. This could explain the longer logarithmic phase exhibited by *L. rhamnosus*, with a slower growth rate. This does not necessarily represent an inability to grow, but rather a gradual adaptation process, taking 48 h to reach maximum growth and not acidifying the medium as much. Even so, all strains were able to grow at least 1.4 log CFU/mL in this substrate, demonstrating that this plant matrix can be fermented using selected LAB strains. Similar growth results have been reported in pea flour extract fermented with *L. rhamnosus* under fermentation conditions similar to those in our work [26].

Legume-based extracts may contain variable levels of raffinose family oligosaccharides (RFO), including raffinose, stachyose, and verbascose, which are not fully digested by the small intestine [12] due to the lack of α-galactosidase enzyme activity in human metabolism. This can lead to symptoms of gastrointestinal discomfort [27]. Fermentation can be used as a strategy to decrease these antinutrients.

Some LAB strains have the metabolic pathways to degrade RFO. In lentil extract fermentation, *L. acidophilus* ATCC 4356, *L. fermentum* DSM 20,052, and *L. paracasei* subsp. *paracasei* DM 20,312, the RFO content was lower compared to the non-fermented control [28]. The authors reported reduced concentrations of oligosaccharides during fermentation. This capacity for partial degradation of raffinose and stachyose may contribute to the reduction of gastrointestinal side effects associated with the consumption of foods rich in RFO. From the perspective of the development of functional products, this may represent an additional benefit, such as improved digestibility of legume-based foods.

The colour changes in the extracts during fermentation may be related to different phenomena. It is known that the loss of chlorophyll is a common change in green plants, from bright green to olive brown in processed foods. Chlorophyll is sensitive and degrades when exposed to light, heat and is dependent on pH. Acidification causes the conversion of chlorophylls to Mg^{2+}-free derivatives such as pheophytins, resulting in a perceptible discoloration from green to olive-brown [29]. Organic acids produced during the fermentation process, such as lactic acid, lower the pH in the medium, which may also involve changes in pigments sensitive to low pH [30]. This would explain the more pronounced colour changes found in the extracts fermented by *L. lactis* 32B4-1 and *L. plantarum* BL011, the producers of lactic acid at higher amounts, and the lesser change in fermentation with *L. paracasei* 12B0-2, which produced a low concentration of lactic acid and acetic acid.

The reported band sizes in SDS gels in the literature [31, 32, 33], which indicate that pea contains convicilin (71–75 kDa), vicilin (44–50 kDa), and legumin (20 kDa), are consistent with the bands appearing in the 0 h samples of each line. The molecular distribution profile of unfermented pea extract obtained by us is similar to the profile reported by

Table 3 Identified volatile compounds in non-fermented and fermented pea extract by the LAB, showing their chemical group and aroma attributes

Nº	Compound name[a]	RI lit[b]	RI[c]	Aroma description[d]
Aldehyde				
1	Hexanal	802	806	green, grassy
2	(E)-2-Heptenal	903	913	grassy, fruity, beany
3	Octanal	1004	1005	herbal, fruity
4	(E)-2-Octenal	1039	1013	cucumber, fatty
5	Nonanal	1102	1104	grassy
6	(E)-2-Nonenal [5]	1145	1112	green, cucumber
7	(E)-2-Decenal	1234	1212	waxy, earthy, green
8	2,4-Nonadienal [3,4,5]	1169	1120	fatty, melon
9	(E, E)-2,4-Decadienal [4,5]	1290	1220	oily, cucumber
10	2-Undecenal [4,5]	1340	1311	fresh, fruity
Alcohol				
11	1-Octen-3-ol	983	969	mushroom, earthy
12	2-Octen-1-ol	1072	1067	green, citrus
13	1-Hexanol [12]	865	860	grassy
14	1-Octanol [2,3,4,5]	1070	1059	waxy, green
15	1-Nonanol [1]	1172	1159	fresh, fatty
Acid				
16	Pentanoic acid[2]	879	875	cheesy
17	Octanoic acid[5]	1175	1173	cheesy, fatty
18	Nonanoic acid[1,2]	1280	1272	waxy, cheese
Ester				
19	Lactic acid, ethyl ester[4]	836	848	Fruity
20	Nonadecanoic acid, methyl ester[1,2,3,4,5]	2190	2177	Fruity
Furan				
21	2-Pentylfuran	996	1040	fruity, green, earthy
Alkane				
22	Hexadecane	1600	1612	-
23	Octadecane[1,3]	1800	1810	-
24	Nonadecane[1,2,3,4,5]	1900	1910	-
25	Eicosane[1,2,3,4,5]	2000	2009	-
26	Heneicosane[1,2,3,4,5]	2100	2109	-
27	2-Heptanone [3]	896	853	fruity, spicy
28	3-Octanone [4]	971	952	herbal, Sweet

[a] Suggested compound with greatest similarity between the mass spectra contained in the NIST11 library database; The superscripted number represents newly formed volatiles during fermentation of *L. lactis* 32B4-1 (1), *L. plantarum* BL011 (2), *L. rhamnosus* FAGRON™ (3), *L. paracasei* M1A3 (4), and *L. paracasei* 12B0-2 (5)

[b] RI lit: Kovats Index (DB-5MS) available at the online address of the National Institute of Standards and Technology, library in version NIST11 for the suggested compound, available at "hhttp://webbook.nist.gov/chemistry"

[c] RI: Calculated Kovats Index for DB-5MS Column

[d] Odor description was obtained from literature data at (https://www.thegoodscentscompany.com)

Ma et al. [19] for neutral pea milk. Fermentation with LAB resulted in considerable modification of the protein profile, leading to the disappearance of the bands.

The protein degradation profiles observed among the different LABs vary due to intrinsic differences in their proteolytic systems and the interaction of these microorganisms with the food matrix. Furthermore, changes in the protein profile can occur for several reasons. Among them are the low pH achieved during fermentation, which limits further protein cleavage, and the absence of additional protease production during the growth stabilization phase [34].

Legume fermentation may improve protein digestibility because of the partial degradation of complex stored proteins into simpler and soluble products [35]. The reduction in pH during the fermentation process brings proteins closer to their isoelectric point, leading to changes in their solubility. The isoelectric point of pea proteins is in a wide range of pH, from 4 to 6 [36]. In addition, LAB produce hydrolytic protease, and the action of these proteolytic enzymes during fermentation might break the peptide bonds in proteins, resulting in smaller peptides and amino acids that are readily available to bacterial metabolism [37]. This variation in protein subunits may result in beneficial changes in functional characteristics, such as improved digestibility and the creation of bioactive peptides with antioxidant activity. The subsequent antioxidant activity results reinforce this, showing high activity against ABTS radicals after fermentation.

Fermentation impacts the antioxidant properties that characterize food matrices, and the total phenolic compounds content can vary depending on the strain and the specific metabolic activities [38]. Fermentation with LAB promotes the release of phenolic compounds bound to the plant matrix, facilitating their conversion into smaller molecules and potentially increasing their bioavailability [39]. According to Gomes-Lobo et al. [39], these bacteria possess enzymatic capabilities that allow them to transform various phytochemicals into more bioavailable and bioactive compounds through mechanisms such as hydrolysis, decarboxylation, and depolymerisation of complex structures. Furthermore, LAB such as *L. rhamnosus* exhibit high β-glucosidase activity, which corroborates their ability to convert phenolic compounds bound to glycosides—a common form in plant matrices—into simple phenolic compounds with high antioxidant potential [40].

The decrease in pH that occurs during fermentation can lead to a reduction in total phenolic compounds, as verified in our results. The acidic environment can influence the structure of phenolic compounds in several ways, including structural rearrangements [41], which can make them undetectable by Folin-Ciocalteu reagents and therefore not quantified. The reduction can also indicate a metabolic use of the compounds by microorganisms.

Table 4 Profile of volatile compounds identified in unfermented and fermented pea extract by different LAB strains

		Volatile concentration (Peak area (×10^5)															
		L. lactis 32B4-1			L. plantarum BL011			L. rhamnosus FAGRON™			L. paracasei M1A3			L. paracasei 12B0-2		
	Compound	0 h	24 h	48 h	0 h	24 h	48 h	0 h	24 h	48 h	0 h	24 h	48 h	0 h	24 h	48 h
1	Hexanal	7.89±6.64^a	6.27±4.99^a	7.02±2.49^a	8.71±2,97^a	6.90±0.71^a	5.91±2.36^a	10.1±3.09^a	12.4±0.14^a	17.5±7.32^a	6.4±1.72^b	19.0±6.09^{ab}	37.9±9.09^a	11.2±0.71^a	23.6±6.12^a	15.3±4.61^a
2	(E)-2-Heptenal	27.6±13.9^a	2.63±0.27^a	n.d.	27.9±3.64^a	n.d.	n.d.	42.7±8,5^a	10.2±0,17^a	n.d.	22.7±5,72^a	14.7±0.62^a	22.3±0.27^a	23.4±0.85^a	30.4±3.72^a	11.1±2.52^a
3	Octanal	4.31±4,95^a	n.d.	3.09±2.54^a	n.d.	n.d.	n.d.	10.5±2,8^a	3.41±0.64^a	2.49±1.62	4,30±2,61^a	8.78±3.05^a	15.6±4.50^a	3.70±0.24^a	13.5±0.27^a	12.9±0.04^a
4	(E)-2-Octenal	11.9±5.48^a	n.d.	n.d.	8.06±2.94^a	n.d.	n.d.	19.9±4,14^a	5.90±1.01^a	n.d.	n.d.	17.8±6.18^a	22.6±3.34^a	8.80±0.04^a	12.3±5.85^a	10.8±5.28^a
5	Nonanal	4.69±0.80^a	2.77±0.04^a	3.18±0.89^a	4.41±0.92^a	1.53±0.41^a	1.41±0.49^a	5.93±0.69^a	6.80±0.24^a	5.07±3.40^a	3.40±1.12^a	19.1±7.41^a	19.3±0.65^a	3.62±0.94^a	27.1±1.92^a	11.5±4.26^a
6	(E)-2-Nonenal	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	11.9±9.23^a	4.63±0.14^a
7	(E)-2-Decenal	31.4±21.9^a	25.4±6.37^a	n.d.	49.2±24.8^a	22.6±2.12^{ab}	18.0±2.12^b	n.d.	n.d.	n.d.	23.1±10.7^b	178±12.7^a	183±9.15^a	24.9±2.39^b	135±38,1^a	128±55.0^a
8	2,4-Nonadi-enal	n.d.	n.d.	n.d.	18.5±6.77^a	n.d.	n.d.	n.d.	6.10±1.65^a	n.d.	n.d.	14.1±9.21^a	n.d.	n.d.	14,8±8,87^a	7.75±3.60^a
9	(E, E)-2,4-Decadi-enal	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	6.46±0.55^a	7.07±0.81^a	n.d.	18.3±0.87^a	3.87±1.20^a
10	2-Undecenal	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	29.5±2.60^a	72.2±12.9^a	34.7±16.0^a	n.d.	55.4±0.52^a	n.d.	n.d.	33.9±6.43^a	38.0±15.5^a
11	1-Octen-3-ol	22.3±10.2^a	5.53±3.54^a	3.38±1.47^a	16.2±6.29^a	n.d.	15.66±0.59^a	30.6±5.34^a	8.28±0.48^{ab}	6.99±4.57^b	15.7±0.98^a	11.8±5.05^a	18.1±2.45^a	14.3±0.29^a	10.7±7.87^a	8.26±3.94^a
12	2-Octen-1-ol	22.1±14.2^a	7.78±4.97^a	n.d.	14.5±2.43^a	n.d.	n.d.	40.5±9,82^a	n.d.	n.d.	17.3±6.66^a	n.d.	17.3±1.15^a	18.9±8.76^a	n.d.	n.d.
13	1-Hexanol	n.d.	11.7±5.01^a	8.25±3.32^a	n.d.	13.9±4.40^a	80.1±20.8^b	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.19±0.61^a
14	1-Octanol	n.d.	n.d.	n.d.	n.d.	1.97±0.68^a	n.d.	n.d.	6.46±0.45^a	n.d.	n.d.	11.8±3.53^a	n.d.	n.d.	9.52±5.60^a	n.d.
15	1-Nonanol	n.d.	n.d.	2.25±0.49^a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	10.3±5.32^a
16	Pentanoic acid	n.d.	n.d.	n.d.	n.d.	5.31±1.66^a	3.32±0.06^a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
17	Octanoic acid	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	10.5±2.42^a	n.d.
18	Nonanoic acid	n.d.	25.8±8.27^a	49.8±40.6^a	n.d.	25.2±3.17^a	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
19	Lactic acid, ethyl ester	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	42,2±1,55^a	n.d.	n.d.	n.d.	n.d.
20	Nonadecanoic acid, methyl ester	n.d.	10.4±4.11^a	11.3±1.08^a	n.d.	9.26±3.48^a	19.0±7.07^a	n.d.	4.58±1.81^a	6.95±2.87^a	n.d.	6.03±0.99^a	n.d.	n.d.	9.79±5.71^a	4.37±0.18^a
21	2-Pentylfuran	2.09±2,72^a	n.d.	n.d.	2.62±0.28^a	n.d.	n.d.	2.05±1.94^a	0.40±0.17^a	n.d.	2.62±0,28^a	5.50±1.35^a	n.d.	1.16±0.21^a	n.d.	2.63±0.96^a
22	Hexadecane	1.46±0.36^a	2.31±0.06^a	2.17±0.18^a	2.49±1.06^a	2.34±0.58^a	3.09±0.70^a	1.55±0.29^a	3.12±0.67^a	2.47±0.41^a	1.65±0.52^a	3.43±1.83^a	2.43±1.20^a	1.67±0.32^a	2.30±0.56^a	3.12±1.67^a
23	Octadecane	n.d.	1.31±0.10^a	1.47±0.23^a	n.d.	n.d.	n.d.	n.d.	1.38±0.28^a	1.34±0.08^a	0.73±0.28^a	1.39±0.77^a	n.d.	1.25±0.05^a	n.d.	n.d.
24	Nonadecane	n.d.	2.03±0.53^a	2.15±0.55^a	n.d.	3.56±0.02^a	2.66±0.42^a	n.d.	1.64±0.13^a	2.09±0.47^a	n.d.	1.50±0.28^a	1.52±0.32^a	n.d.	2.13±0.73^a	1.71±0.34^a
25	Eicosane	n.d.	1.36±0.15^a	1.77±0.44^a	n.d.	1.02±0.02^a	1.37±0.49^a	n.d.	1.36±0.31^a	1.16±0.19^a	n.d.	1.50±0.28^a	n.d.	n.d.	1.81±0.89^a	1.51±0.10^a
26	Heneicosane	n.d.	2.17±0.60^a	1.57±0.20^a	n.d.	1.62±0.21^a	2.28±0.28^a	n.d.	1.28±0.01^a	1.39±0.11^a	n.d.	1.63±1.15^a	n.d.	n.d.	2.29±1.58^a	2.25±0.43^a
27	2-Heptanone	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.11±0.3^{aa}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
28	3-Octanone	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	6.96±1.65^a	n.d.	n.d.	n.d.	n.d.

Data are the mean of two independent experiments±standard deviation. Different lowercase letters within a row indicate significant differences (Tukey, $p<0.05$) over time for a single strain. nd: not detected

Furthermore, changes in the phenolic compound profile may occur throughout the bioprocess. However, it is the type and structure of the compounds that will be associated with their antioxidant potential. Therefore, knowing the profile and type of compounds at each fermentation stage is important to associate the quantity of phenolic compounds with their antioxidant potential. The Folin method quantifies reducing species in general, not just the phenolic compounds associated with antioxidant potential.

The antioxidant activity of foods is usually attributed to phenolic compounds. However, studies have reported that proteinase and peptidase activities of LAB can release free peptides and amino acids with strong antioxidant activity [42]. This released activity could explain the significant increase in the ABTS radical scavenging antioxidant activity of fermented extracts. Furthermore, throughout the fermentation process, microorganisms and medium conditions can alter the phenolic compound profile of the original extract. These changes can lead to an increase in antioxidant activity without, however, increasing the total content of phenolic compounds, since these compounds act synergistically and, in most cases, independently of their concentration. Furthermore, processes such as enzymatic hydrolysis, fermentation, food processing, and gastrointestinal digestion can generate bioactive peptides. These compounds are formed by 2 to 20 amino acid residues and are present in different plant sources, including peas [43]. Since the Folin-Ciocalteu method is not specific for phenolic compounds but measures total reducing substances, these peptides cannot be quantified by it. This may explain the increase in antioxidant capacity during fermentation, despite the lack of increase in the total phenolic content.

The volatile compounds present in peas that cause undesirable flavours are partly inherent to the peas themselves and partly developed during harvesting, processing, and storage [44]. The flavour of legumes is characterized by "bean-like" and "green" notes, which are perceived as undesirable by consumers. Among the identified compounds in this work, hexanal, octanal, nonanal, (E)-2-Heptenal, 1-Hexanol, 1-octen-3-ol, and 2-pentyl-furan are recognised as off-flavours in peas [45, 46, 47]. Research has shown that fermentation with LAB can result in the release and degradation of volatile compounds, such as aldehydes, alcohols, and acids, in plant products, leading to a favourable alteration of the volatile profile and reduction of off-flavour molecules [7].

During fermentation, microorganisms produce enzymes that hydrolyze nutrients in food (e.g., proteins, carbohydrates, lipids, organic acids, and amino acids), thus generating precursors of non-volatile and volatile compounds that can affect aroma, texture, and flavour [48]. Three main enzymatic pathways have been identified in the metabolism of LAB, leading to the generation of flavour, namely the conversions of sugars (glycolysis), proteins (proteolysis), and lipids (lipolysis) [49]. Proteolysis of LAB is the main biochemical pathway for flavour development in fermented foods. The small peptides and free amino acids formed can be subsequently converted into various alcohols, aldehydes, acids, esters, and other compounds [49].

The non-fermented extracts were characterized by the presence of aldehydes such as hexanal, octanal, and nonanal. These compounds originate from enzymatic oxidation (via lipoxygenase) and/or auto-oxidation of unsaturated fatty acids, particularly linoleic, linolenic, and oleic acids, which are naturally present in peas [50]. After 24 h of fermentation, alcohols, acids, esters, and alkanes were the main compounds formed. Figure 6 shows the changes over the fermentation time for each microorganism. Aldehydes are commonly associated with the unpleasant taste of legumes [51]. All aldehydes identified in our study have been previously described in the literature as present in several pea products, such as *milk*, flour, and isolated protein [26, 52].

Fermentation is an alternative to reduce aldehyde concentration in foods. Shi et al. [31] showed that after 10 h of fermentation by *L. plantarum*, yellow pea flour had 42% of its aldehydes converted into carboxylic acids. Pea protein extracts fermented with *Lactiplantibacillus plantarum* L1047 or *Pediococcus pentosaceus* P113 for 48 h at pH 4.5 showed a more pleasant sensory profile by reducing or masking undesirable flavours [53]. The aldehyde content in pea protein reduced from 25.32% to 4.25% in fermentations using *Saccharomyces cerevisiae* SK1.008 and to 4.43% when fermentation was with *L. plantarum* FSB7 [43]. In our study, 24 h fermentation by *L. lactis* 32B4-1 and *L. plantarum* BL011 decreased the total area of aldehydes from 65.7% to 34.5% and 76.5% to 35.7%, respectively. Octanal, in *L. lactis* 32B4-1, and (E)-2-heptenal, and (E)-2-octenal in *L. plantarum* BL011, were not detected after fermentation. However, fermentation with *L. rhamnosus* FAGRON™, *L. paracasei* M1A3, and *L. paracasei* 12B0-2 increased the number of compounds in this group, identified at different stages of fermentation, from 57.3% to 80.4% in 24 h, 61.1% to 88.66% in 48 h, and 66.9% to 87.1% in 24 h, respectively. The higher level of these compounds may not improve on aroma profile, as aldehydes can produce unpleasant flavours described as "beany," "green," "vegetable," and "rancid" [54].

These differences in LAB behaviour, particularly with *L. rhamnosus* and *L. paracasei* increasing the amount of aldehydes, may be related to the presence, activity, and regulation of enzymes involved in the conversion of these compounds. Strains such as *L. plantarum*, possess alcohol dehydrogenase and aldehyde dehydrogenase enzymes that convert aldehydes and ketones into less odorous alcohols or

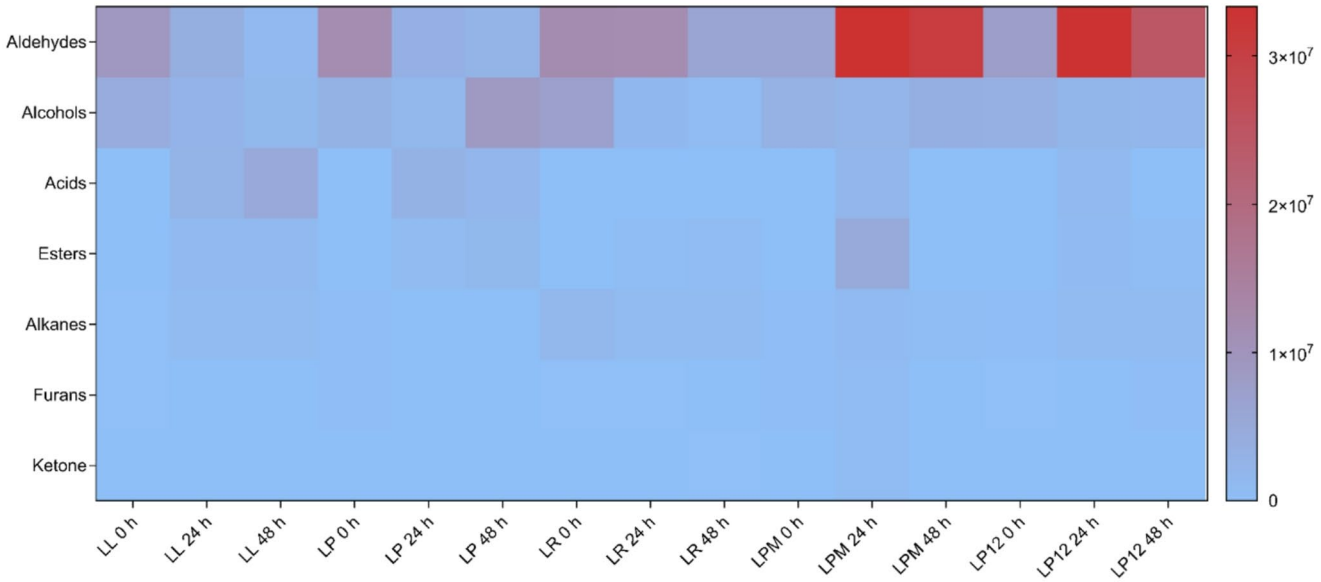


Fig. 6 Heatmap of volatile compound classes determined by GC–MS of unfermented and fermented pea extracts at 0 h, 24 h, and 48 h. The Y-2 axis represents compound abundance expressed as peak area $\times 10^7$. Colour intensity indicates relative variations in compound abundance, highlighting strain-specific effects on the volatile profile. LL: *L. lactis* 32B4-1; LR: *L. rhamnosus* FAGRON™1; LP: *L. plantarum* BL011; LPM: *L. paracasei* M1A3; LP12: *L. paracasei* 12B0-2

carboxylic acids [51]. However, other specific strains may have limited enzyme activity, favouring the accumulation of these compounds during fermentation. The results found in our work thus corroborate the hypothesis that the formation or depletion of aldehydes during fermentation depends on the overall fermentative activity, but also on specific metabolic pathways that are regulated differently among strains, particularly when fermenting plant matrices.

Hexanal and nonanal aldehydes can be reduced to corresponding alcohols and oxidized to acids by microorganisms [55]. The most typical alcohols produced by the autooxidation of linolenic acid and linoleic acid in peas are 1-pentanol and 1-octen-3-ol [51]. In this study, the different strains exhibited similar metabolism in terms of total reduction in identified alcohols, except for *L. plantarum* BL011, which increased the total area of alcohols within 48 h of fermentation, from 20% to 59.7%. Notably, the reduction of 1-octen-3-ol and 2-octen-1-ol, both associated with the beany flavour [47], was observed. Meanwhile, 1-hexanol, 1-octanol, and 1-nonanol appeared after 24 h of fermentation. The presence of these alcohols may be related to the reduction of aldehydes to their corresponding alcohols by the action of alcohol dehydrogenase, thus improving the overall odour properties [45].

Acids are relevant components produced by sugar metabolism. Three acids were identified in the fermented samples. Nonanoic acid in *L. lactis* 32B4-1 fermentation for 24 h and 48 h, octanoic acid in *L. paracasei* B120-2 fermentation for 24 h, and nonanoic and pentanoic acid in *L. plantarum* BL011 fermentation up to 48 h. These acids are described as having an odour reminiscent of cheese and fat [56].

Other significant compounds in the formation of off flavours in plant products are furans and heterocyclic compounds. The 2-pentylfuran is produced through the oxidative degradation of unsaturated fatty acids and has been previously identified as an active odour compound related to earthy and green notes in pea-based beverages [57]. In this study, the non-fermented samples presented 2-pentylfuran. The content of furan was depleted in 24 h by *L. lactis* 32B4-1 and *L. plantarum* BL011, and in 48 h by *L. rhamnosus* FAGRON™.

Esters exhibit floral and fruity aromas that can mask or attenuate undesirable flavours in foods and beverages [58]. Furthermore, esters can act synergistically with other molecules, affecting the flavour of the final product at concentrations below the perception threshold [59]. In this study, results showed the absence of esters in the unfermented pea extracts. In fermented samples, lactic acid ethyl ester was found in the fermentation of *L. paracasei* M1A3 in 24 h. Also known as ethyl lactate, it can originate from the esterification of lactic acid produced during fermentation [59]. It brings a smooth, buttery, and creamy flavour, with notes of fruit and coconut. Other ester identified in the samples was nonadecanoic acid, methyl ester. The formation of these compounds during the fermentation process may increase the complexity of pea products.

Of the five strains evaluated, only *L. rhamnosus* FAGRON™, and *L. paracasei* M1A3 produced ketones, such as 2-heptanone and 3-octanone. Ketones are produced

by the oxidation of unsaturated fatty acids, degradation of amino acids, or microbial fermentation [60]. 2-Heptanone (fruity aroma) was detected at the 48 h of fermentation of *L. rhamnosus* FAGRON™, and 3-octanone (herbaceous and sweet aroma) was observed in pea extract fermented for 24 h by *L. paracasei* M1A3.

Overall, all fermentations demonstrated changes in aroma attributes, either by reducing or increasing volatile compounds that cause off flavours. *L. lactis* 32B4-1 and *L. plantarum* BL011 showed the highest ability to reduce the area of aldehydes and to produce esters, improving the volatile profile of the pea extract, which may result in a positive effect on the "bean" notes.

Conclusion

Fermented pea extracts showed the ability to function as a carrier for potentially probiotic bacteria to be applied as a matrix for functional plant-based food products. The results showed that probiotic viability in the fermented extract is strain-dependent and reflected in colour changes and metabolite production. Although all microorganisms showed the ability to ferment peas, *L. lactis* 32B4-1 and *L. plantarum* BL011 achieved the highest cell viability, lactic acid production, and better efficiency in modulating the profile of aromatic precursors and aroma-associated marker compounds. While further research is needed to determine the sensory aspects, these findings indicate that fermentation may contribute to changes in the profile of these aromatic precursors. After fermentation, the antioxidant activity increased by up to 78.27%, suggesting that the bioavailability of compounds was enhanced with fermentation. Results obtained in this work suggest the possibility of applying fermented pea extract as a matrix or ingredient in novel plant-based products, such as fermented milk analogues, with the addition of natural flavours and thickeners. Probiotic yogurts and ice creams may also be produced using the extract, carrying the microorganisms in adequate quantities and using pea starch to improve the viscosity of the product. Further research into the biochemical reactions that occur during fermentation is recommended, especially concerning flavour formation, the reduction of antinutritional factors, the profile of phenolic compounds, and their changes throughout the bioprocess.

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Data availability Data associated to this work will be available on reasonable request.

Declarations

Conflict of interest All the authors of this work declare to have no conflicts of interest.

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