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Compositional and microstructural changes in faba bean (*Vicia faba* L.) during soaking and boiling

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ABSTRACT

Faba bean is increasingly cultivated in Nordic regions due to its nutritional value and agronomic adaptability, but its use is limited by anti-nutritional factor (ANF) content and functional properties. This study evaluated soaking and boiling as treatments to modulate composition and functional properties. Beans were soaked in water, sodium bicarbonate, *Lactiplantibacillus plantarum* (LAB), or a combination of both, followed by heat treatment as boiling. Microbiology analysis showed that LAB inoculation during soaking lowered pH and maintained microbial safety, potentially influencing the breakdown of ANFs. Protein content ranged from 28 to 37% dry matter (DM). Resistant starch decreased from 12.8% in raw beans to 3.7–6.0% DM after processing, with a corresponding increase in digestible starch. The highest reduction of vicine, convicine, and phytic acid occurred in samples soaked only with *L. plantarum* and boiled, with decreases of 45.7%, 47.1%, and 71.3%, respectively. Free amino acids in treated samples ranged from 2.9 to 3.6 mg/g DM, and total phenolic content ranged from 1.0 to 1.7 mg GAE/g. Microscopy confirmed that sodium bicarbonate loosened the plant matrix, while LAB softened the cell wall structure. To evaluate functional implications, an unfiltered plant-based beverage model (12% w/v solids) was prepared. LAB-treated beverages exhibited improved low-shear viscosity, higher water-holding capacity, reduced phase separation, and lighter colour compared with other samples. These findings demonstrate that soaking strategy influences not only compositional and structural properties but also techno-functional performance relevant for plant-based beverage applications.

1. Introduction

Faba bean (*Vicia faba* L.) is rapidly emerging as one of the most relevant plant protein crops in Nordic agriculture. Its agronomic suitability to cool climates, short growing season, and high nitrogen fixation, which lowers fertilizer demand, make it a key crop for reducing dependency on imported soy (Auer et al., 2023; Jensen et al., 2010). The crop is widely cultivated globally, particularly in China and Ethiopia, the leading producers, and is also important in the Middle East and North Africa. In Sweden the 2024 harvest reached 51,700 tonnes, a 7 % increase over the previous year, highlighting its expanding domestic production (Jordbruksverket, 2024; Martineau-Côté et al., 2022).

The Nordic Nutrition Recommendations (Nordic Council of Ministers, 2023) state that pulses should be a regular part of the diet in the Nordic and Baltic countries due to their contribution of dietary fibre, protein, iron, and zinc. In a Danish cohort, replacing red meat with pulses was associated with a reduced risk of colorectal cancer, type 2 diabetes, and ischaemic heart disease (Fabricius et al., 2021). Faba bean (*Vicia faba* L.) contains 28–37% protein (≈85% globulins: 55% legumin, 30% vicilin, 3% convicilin), 47–68% carbohydrates (22–45% starch, plus soluble sugars like RFOs), 11–30% dietary fibre (hemicellulose, cellulose, lignin), and ≈1.5% fat. It also provides iron, zinc, B-group vitamins, and phenolic compounds, making it a nutrient-dense legume with functional health benefits (Labba et al., 2021; Martineau-Côté

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et al., 2022). Its composition makes it an ideal candidate for Nordic plant-based product development. However, its utilization remains restricted by anti-nutritional factors (ANFs) such as vicine, convicine, and phytic acid (Dhull et al., 2022; Labba et al., 2021). Vicine and convicine, upon hydrolysis, release their aglycones divicine and isouramil, which are associated with favism in individuals with glucose-6-phosphate dehydrogenase (G6PD) deficiency (Pulkkinen et al., 2019). Phytic acid chelates minerals, limiting absorption (Oomah et al., 2011).

Pulses are not consumed raw; soaking followed by thermal treatment is essential to reduce cooking time, improve texture, and decrease anti-nutrients. During soaking, hydration triggers diffusion of soluble compounds and partial activation of endogenous enzymes such as phytases and β-glucosidases (Shi et al., 2018). The soaking medium critically affects these processes; sodium bicarbonate increases water soaking pH and promotes ion exchange between Na^{+} and Ca^{2+}/Mg^{2+} in pectins, accelerating softening and reducing cooking time (Fernandez Castaneda et al., 2024; Huma et al., 2008). In contrast, *Lactiplantibacillus plantarum* (LAB) soaking lowers pH and introduces microbial β-glucosidases and phytases that can hydrolyse ANFs in a fermented paste/dough (Rizzello et al., 2016). Together, soaking conditions and heat treatment define the physicochemical state of the pulse matrix, ultimately influencing its extractability, functional performance, and suitability for further processing.

The impact of various soaking treatments, including sodium bicarbonate, LAB, and their combination followed by boiling, on the composition and structure of faba beans is still poorly understood, limiting their optimized use in plant-based foods. Beans were soaked in water (control), sodium bicarbonate, LAB, and analysed for microbial changes during soaking, macronutrients, ANFs and microstructural analysis of cooked beans. To assess functional relevance, a plant-based beverage model was prepared to evaluate viscosity, sedimentation, water-holding capacity, and colour. We hypothesize that LAB treatment further reduces anti-nutritional factors (ANFs), increases total phenolic content, and improves functional properties through enzymatic disruption of the cell wall, as observed by microscopy, with consequent effects on viscosity and colour relevant to subsequent food applications.

2. Materials and methods

2.1. Materials

Dried organic faba beans (*Vicia faba* L., var. Gloria) cultivated in Västmanland, Sweden, were purchased from Nordisk Råvara AB (Stockholm, Sweden). The beans were harvested in 2020 and dehulled in 2021. Food-grade sodium bicarbonate was sourced from a local retailer in Uppsala (Bohus, Sweden). A freeze-dried culture of *Lactiplantibacillus plantarum* (Harvest LB-1) was supplied by Chr. Hansen (Bagsvaerd, Denmark) for use during the soaking pre-treatment.

All analytical-grade chemicals and reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA) or Merck (Darmstadt, Germany). Analytical-grade chemicals and reagents used in this study included sodium sulfate (Na_2SO_4), a hexane:isopropanol mixture (HIP, 3:2 v/v), hydrochloric acid (HCl), ferric ions (Fe^{3+}), deionized water (H_2O), calcium chloride ($CaCl_2$), sodium hydroxide (NaOH), potassium chloride (KCl), sulfuric acid (H_2SO_4), sodium phosphate (Na_3PO_4), osmium tetroxide (OsO_4), ruthenium (Ru), fluorescein isothiocyanate (FITC), rhodamine, and iodine. Deionized water was produced in-house using a Milli-Q purification system (Millipore, Bedford, MA, USA).

2.2. Pre-treatment process

Each treatment consisted of 100 g of washed faba beans (Fb) soaked in 250 mL of tap water at 25 °C for 12 h in a convection incubator (Binder BF series, class 3.1, Tuttlingen, Germany). Four soaking media were evaluated: (i) tap water only (control), (ii) sodium bicarbonate (Sb; 1 g per 250 mL of water), (iii) *Lactiplantibacillus plantarum* (LAB; 0.1 g freeze-dried culture per 250 mL of water), and (iv) a combined Sb + LAB solution (1 g Sb and 0.1 g LAB per 250 mL of water). Samples were taken at time 0 and 12 h for microbiology analysis. Previous studies showed no significant additional pH reduction between 12 h and 24 h of soaking, while off-odours developed with longer soaking. Therefore, 12 h was selected (Fernandez Castaneda et al., 2024).

After soaking, all samples were subjected to boiling as a heat treatment. Soaked and washed faba beans were added to approximately 400 mL of vigorously boiling tap water. Beans soaked in water (control) or LAB were boiled for 16 min, whereas those soaked in sodium bicarbonate or the combination of sodium bicarbonate and LAB were boiled for only 8 min. The presence of sodium bicarbonate while soaking softens the beans, reducing the time required to reach an edible texture. Our previous studies have shown that different soaking media, combined with boiling time, affect bean texture, pH, and colour. Therefore, to achieve a consistent final hardness across treatments, the boiling time must be adjusted according to the type of soaking medium used (Fernandez Castaneda et al., 2024). All samples were cooled by rinsing with cold tap water, allowed to reach room temperature, and then frozen for microscopy analysis and freeze-dried for chemical analysis.

A plant-based beverages model was prepared to evaluate viscosity, sedimentation, water-holding capacity, and colour. The production was adapted to Duarte et al. (2022) and Lopes et al. (2020), with modifications. After soaking, boiling, cooling, and draining, the cooked seeds were blended with tap water to obtain 12% (w/v) total solids. The mixture was homogenized using an Ultra-Turrax T25 at 13,500 RPM for 2 min, rested for 1 min, and homogenized again for 1 min (total homogenization time: 3 min). The beverage was not subjected to sieving or filtration to preserve its full nutritional composition. The beverage was bottled in sterilized containers and heat-shocked for 1 min in boiling water. Samples were cooled and stored at 4 °C for 24 h before analysis.

2.3. Microbiological analyses

Soaking water samples were obtained from each soaking pre-treatment described in Section 2.2 at two sampling points: at the start of soaking (0 h) and after 12 h of soaking (12 h). Prior to each sampling, the soaking beakers containing both beans and soaking water were mixed using a magnetic stirrer at 100 rpm for 5 min at room temperature to ensure uniform distribution of microorganisms within the suspension. Soaking water (5 mL) was aseptically collected from each treatment and serially diluted in sterile 0.1% (w/v) peptone solution (Merck, Germany) and spread-plated for enumeration of the following microbial groups: total aerobic bacteria (TAB) on Plate Count Agar (tryptone 0.5%, yeast extract 0.25%, glucose 0.1%, agar 1.5%), incubated at 35 °C for 2 days; lactic acid bacteria (LAB) on de Man-Rugosa Sharpe agar (AppliChem, Germany) with Delvocid (0.1% w/v, DSM, The Netherlands) to suppress fungi, incubated anaerobically at 30 °C for 2-3 days; and yeasts on Malt extract agar (Merck, Germany) with chloramphenicol (0.1% w/v, Sigma-Aldrich), incubated at 30 °C for 2 days. The presence of spore-forming bacteria was also assessed by holding sample aliquots at 80 °C for 30 min to inactivate vegetative cells, prior to plating on Tryptone Glucose Extract agar (Merck, Germany), incubated at 35 °C for 2 days. The pH of the soaking water from each pre-treatment was measured at 0 h and 12 h using a calibrated pH meter (PHM92, Radiometer Analytical A/S, Copenhagen, Denmark).

2.4. Analysis of chemical composition

Faba bean samples raw and pre-treated (soaking followed by boiling) were frozen and then freeze-dried. The dried material was ground into a fine powder using a blender (3 cycles of 30 s). All analyses were conducted in triplicate on freeze-dried samples, except for moisture, which was measured on fresh samples to determine water uptake.

Moisture content was determined according to AOAC 934.01 by

measuring the mass difference before and after drying overnight at 105 °C in a hot-air oven (Model, 2000655, J.P. Selecta, Barcelona, Spain). Moisture content was also performed in freeze-dried samples, and the results were used to correct subsequent analyses and express data on a dry matter basis (DM).

Ash content was quantified following AOAC 942.05 using a muffle furnace at 550 °C for 12 h (Model 62700, Barnstead Thermolyne Corporation, Ramsey, USA).

Crude protein was determined by the Kjeldahl method (AOAC 978.04). Raw samples were ground and homogenized; pre-treated and tempeh samples were thawed and homogenized prior to digestion. Nitrogen was quantified using a Kjeltec 8200 analyser (FOSS Analytical, Hillerød, Denmark), and protein was calculated using the factor N × 5.4.

Total fat was quantified according to AOAC 996.06 using a hexane: isopropanol mixture (3:2, v/v) (HIP). Freeze-dried samples (2 g) were homogenized with HIP, treated with Na_2SO_4 (6.67 percent w/v), and centrifuged. The upper lipid phase was collected, the solvent was evaporated under nitrogen, and the residue was weighed gravimetrically.

Resistant and non-resistant starch were analysed using a Megazyme assay kit following AOAC 2002.02 and AACC 32-40.01 (Megazyme, Bray, Ireland).

2.5. Free amino acids

Free amino acids (FAAs) were analysed in raw and pre-treated samples using ultra-performance liquid chromatography (UPLC) with a pre-column derivatization protocol (AccQ-Tag™ Ultra, Waters Corporation) following Graça et al. (2022). Approximately 0.1 g of freeze-dried material was extracted with 1 mL of 70% ethanol, vortexed for 1 min, sonicated at room temperature for 20 min, and centrifuged at 10,000 rpm for 10 min; the extraction was repeated twice. Soluble proteins were removed with 250 μL of 6 M 5-sulfosalicylic acid, followed by sonication, centrifugation at 8000 rpm for 10 min, neutralization to pH 7–8 with 2 M NaOH, and dilution to 5 mL.

Derivatization involved mixing 10 μL of sample or standard with 60 μL borate buffer, 10 μL L-norvaline internal standard, and 20 μL AccQ-Tag™ reagent, vortexing, and heating at 55 °C for 10 min. Samples were analysed on an Acquity UPLC system with an AccQ-Tag™ Ultra C18 column (2.1 × 100 mm, 1.7 μm) at 55 °C and 0.7 mL/min flow, with detection at 260 nm. Amino acids were identified by retention time matching and quantified using L-norvaline as internal standard.

Calibration used a certified amino acid hydrolysate diluted to six levels (7.5–125 μM, cystine 1.25 mM), each containing 80 μM L-norvaline, processed with the same derivatization. Calibration curves were plotted as the analyte/internal standard peak area ratio. All measurements were done in duplicate.

2.6. Total phenolic content (TPC)

TPC was determined using the Folin–Ciocalteu assay. Freeze-dried raw and pre-treated materials (0.5 g; triplicate extractions) were suspended in 5 mL of methanol–water (80:20, v/v; 0.1% HCl), following a modified protocol from Arora et al. (2022). The suspensions were vortexed (10 s), subjected to ultrasound in an ice bath for 1 min (2 × 30 s with a 5-min interval) and stirred at ambient temperature (1 h). Extracts were then centrifuged (9500 rpm, 12 min, 4 °C), and supernatants were clarified using 0.45 μm membrane filtration.

For TPC quantification, 20 μL of extract was combined with 1.58 mL water and 100 μL Folin–Ciocalteu reagent and allowed to react for 8 min. Subsequently, 300 μL sodium carbonate was added, and samples were incubated for 30 min at 40 °C. Absorbance was recorded at 765 nm (UV-1800, Shimadzu, Kyoto, Japan). Results were reported as mg gallic acid equivalents (GAE) per g dry matter. All measurements were done in triplicate.

2.7. Analysis of anti-nutritional factors

2.7.1. Phytic acid analysis using the colorimetric method

Phytate was extracted from raw and pre-treated samples by suspending the material in deionized water and continuously stirring for 16 h at room temperature, according to (Latta and Eskin, 1980; Lu et al., 2024). The pH of the slurry was then adjusted to 0.75 using 3.5% HCl. After centrifugation, the supernatant was collected and purified using an AG1-X8 anion-exchange resin (Bio-Rad, Solna, Sweden) to separate phytate-bound phosphorus from inorganic phosphorus. The collected phytate fraction was subsequently adjusted to pH 3.0 using 0.1 M HCl.

For colorimetric quantification, 3 mL of the purified extract was mixed with 1 mL of Wade reagent, vortexed, and centrifuged at 3000×*g* for 10 min. Absorbance of the supernatant was measured at 500 nm using a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan). A reagent blank prepared with deionized water was used for baseline correction, and phytate concentration was determined using a sodium phytate calibration curve. All measurements were performed in triplicate.

2.7.2. Vicine and convicine assay

Vicine and convicine were extracted from raw and pre-treated materials following a modified version of Kantanen et al. (2024) & Pulkkinen et al. (2019). Briefly, 0.1 g of sample was dispersed in 4.5 mL of Milli-Q water and vortexed for 1 min. After standing for 1 min, the suspension was centrifuged at 12,000×*g* for 10 min at room temperature. The resulting supernatant was heated in a boiling water bath for 5 min to denature soluble proteins, cooled to room temperature, and 500 μL of the clarified extract was transferred into Amicon Ultra 0.5 mL Ultracel 10K centrifugal filter units (Merck, Darmstadt, Germany). Filtration was performed at 12,000×*g* for 10 min. The filtrate (350 μL) was diluted with 650 μL Milli-Q water, vortexed thoroughly, and transferred into 2 mL UHPLC vials.

Chromatographic analysis was conducted on a Waters Acquity UPLC system (Waters, Milford, USA) equipped with an HSS T3 C18 column (2.1 × 150 mm, 1.8 μm). Separation was achieved isocratically using Milli-Q water with 0.5% formic acid at a flow rate of 0.35 mL/min and a column temperature of 25 °C. After each 7-min isocratic run, the column was washed using a gradient delivering 70% acetonitrile. The injection volume was 5 μL. Vicine and convicine were detected using a photodiode array detector at 273 nm and identified by matching retention times and UV spectra. Quantification of vicine was based on an external calibration curve (Sigma-Aldrich, St. Louis, MO, USA), and convicine was quantified using the same calibration function. Data acquisition and processing were performed using Empower® 3 software (Waters, Milford, MA, USA). All analyses were carried out in triplicate.

2.8. Microstructure analysis

Following soaking and boiling, a whole piece of faba bean from each condition (Fb-W, Fb-Sb, Fb-LAB, Fb-SL) was cut into smaller fragments and then frozen. Samples of the small fragments were embedded in optimal cutting temperature (OCT) compound and directly frozen in a Leica CM1850 cryostat (Leica Microsystems, Wetzlar, Germany) at a chamber temperature of −25 °C. Sections (20 μm) were cut using a disposable steel blade. The sections were collected on glass slides and air-dried at room temperature for 30 min prior to light microscopy.

For light microscopy, starch was stained with Lugol's solution (0.05 g/L iodine) and cell walls with Toluidine blue. Bright-field and epifluorescence images were acquired on a Nikon Eclipse Ni-U microscope with 10 × and 20 × objectives and a Nikon DS-Fi2 camera (2560 × 1920 pixels). Confocal laser scanning microscopy (CLSM) was performed using a Zeiss LSM 780 (Jena, Germany) equipped with a GaAsP detector and a 40 × oil-immersion objective (1.30 NA). Sections were stained with fluorescein 5-isothiocyanate (FITC, 0.25% w/v), Rhodamine B (RhB, 0.025% w/v), and calcofluor white (CW, 0.1% v/v),

targeting starch, protein, and dietary fibre, respectively. Excitation wavelengths were 488 nm (FITC), 568 nm (RhB), and 410 nm (CW); signals were visualized as green, red, and blue. Imaging was performed with the pinhole set to 1 Airy unit, and acquisition parameters were adjusted to avoid pixel saturation. Images were captured at 1024 × 1024 pixels with line averaging for improved signal-to-noise ratio. Image processing and analysis were performed in ImageJ (Fiji, NIH, Bethesda, MD, USA), and files were exported as TIFF.

2.9. Plant-based beverages model

2.9.1. Viscosity

Viscosity changes of the plant-based beverages model after 24 h of preparation were determined using a Discovery Hybrid Rheometer (DHR-3, TA Instruments, New Castle, DE, USA) equipped with a 40 mm parallel-plate geometry based on the methodology described by (Lopes et al., 2020). The temperature was controlled at 25 ± 1 °C using a Peltier plate system. Approximately 1.26 mL of sample was carefully loaded onto the lower plate, and the measuring gap was set to 1.0 mm. Excess sample was trimmed after gap adjustment. The sample was allowed to equilibrate for 30 s prior to measurement. Apparent viscosity was measured using a logarithmic shear-rate sweep from 0.01 to 150 s^{-1}, with 15 points per decade. The instrument control parameters were set as follows: maximum equilibration time per point of 30 s, sampling period of 2 s, tolerance of 3%, and 3 consecutive points within tolerance required before data acquisition. All measurements were done in duplicate.

2.9.2. Water-holding capacity

The water-holding capacity (WHC) of the plant-based beverages was determined according to (Hurtado-Murillo et al., 2024). Briefly, 35 g of each sample were weighed into 50 mL Falcon centrifuge tubes and centrifuged at 4000×*g* for 30 min at 4 ± 1 °C using a Hettich refrigerated centrifuge (model information should be specified). After centrifugation, the supernatant was carefully decanted, and the remaining pellet (sediment) was weighed. All measurements were performed in triplicate. The WHC was calculated as follows:

$$WHC(\%) = \left(\frac{Weight\ of\ the\ pellet\ after\ centrifugation}{Initial\ sample\ weight}\right) *100$$

2.9.3. Colour

Colour of the plant-based beverages models on the upper surface was assessed based on CIELab colour values (L*, a*, and b*), which were measured using at room temperature using a Minolta Chroma Meter CR-300 (Minolta Co., Ltd., Osaka, Japan). After calibration with a standard white plate, according to the manufacturer's instructions. All measurements were performed in triplicate.

2.9.4. Stability (phase separation analysis)

The stability of the plant-based beverages samples was evaluated by monitoring phase separation during storage at 4 °C, according to the method described by (J. Tang et al., 2023). Approximately 35.0 ± 0.1 g of each sample was transferred into graduated glass tubes (16 mm internal diameter), sealed to prevent evaporation, and stored at 4 °C. Phase separation was evaluated by measuring the height of the separated layer relative to the total sample height at 0 h, 24 h, 48 h, and 7 days of storage. Representative photographs were taken at each time point. All measurements were performed in duplicate.

2.10. Statistical analysis

A one-way analysis of variance (ANOVA) was performed on the four treated samples only, as raw beans represent an unprocessed baseline and were not part of the treatment comparison. To assess differences between samples, post hoc comparisons were performed using Tukey's test, and group differences were indicated using letter-based notation. Means and standard deviations were calculated for each sample and reported alongside the significance groupings. Using RStudio software (version 4.4.2, Boston, USA). For the microbiological analysis the data were subjected to two-way ANOVA with treatment and soaking time as fixed factors. Tukey's HSD test was used for post-hoc comparisons. Results are presented as mean ± SD (n = 3), and differences were considered significant at $p < 0.05$.

3. Results and discussion

3.1. Microbiological analyses

Over the 12 h pre-treatment soaking, the total aerobic bacteria (TAB) count increased significantly, and the pH decreased significantly in all treatments (Table 1), consistent with trends from soaking of soybeans for tempeh reported by (Mulyowidarso et al., 1989). The addition of sodium bicarbonate to the soaking water raised the initial pH to 7.3-7.4, compared with unbuffered water and beans (with or without LAB starter) at pH 6.3-6.4. The treatment with addition of LAB alone yielded the lowest final pH (4.9) as well as the greatest reduction in pH (−1.4) after 12 h of soaking; and the treatment with water alone resulted in the least reduction in pH (−0.2), with treatments containing sodium bicarbonate (with or without LAB starter culture) yielding a reduction in pH of around −0.6 after 12 h soaking.

The decrease in pH is thought to be due to the production of organic acids by LAB either naturally present on the faba beans (Verni et al., 2017), or inoculated as the starter culture. Treatments inoculated with the LAB starter culture had, as expected, very high initial levels of LAB (*ca* 10^8 CFU/g) compared with LAB naturally present (*ca* 10 CFU/g); however, the LAB counts did not increase significantly in any of the treatments over the 12 h period. Such increases are likely to be more pronounced if soaking time is extended and the temperature raised, as reported by (Mulyowidarso et al., 1989). However, preliminary trials with soaking longer than 12 h tended to yield slimy, odorous beans (unpublished data), hence the choice of 12 h soaking.

Yeasts were not detected (<10 CFU/mL) in any of the soaking water samples, and the occasional colonies of spore-forming bacteria observed were too few to be included in a statistical analysis; their presence was determined to be negligible after 12 h soaking.

Table 1
Impact of soaking pre-treatment on total aerobic bacteria (TAB), lactic acid bacteria (LAB) and pH in raw faba beans (Fb).

Treatment	Soaking time (h)	Total Aerobic Bacteria (TAB) (log_{10} CFU/mL)	LAB (log_{10} CFU/mL)	pH
W: Water	0 h	2.30 ± 0.27 (a)	0.70 ± 0.00 (a)	6.40 ± 0.05 (c)
	12 h	3.10 ± 0.32 (b)	1.36 ± 0.69 (a)	6.20 ± 0.03 (d)
Sb: Sodium bicarbonate	0 h	2.56 ± 0.17 (a)	1.36 ± 0.70 (a)	7.40 ± 0.04 (a)
	12 h	3.28 ± 0.11 (b)	1.69 ± 0.84 (a)	6.90 ± 0.05 (b)
LAB: *L. plantarum*	0 h	8.24 ± 0.11 (c)	8.23 ± 0.06 (b)	6.30 ± 0.02 (d)
	12 h	9.43 ± 0.86 (d)	9.35 ± 0.83 (b)	4.90 ± 0.04 (e)
SL: Sodium bicarbonate + *L. plantarum*	0 h	8.28 ± 0.04 (c)	8.19 ± 0.07 (b)	7.30 ± 0.03 (a)
	12 h	8.74 ± 0.13 (d)	7.83 ± 1.14 (b)	6.60 ± 0.06 (c)

Values are means ± SD (n = 3, replicates below detection level were assigned a value of 5 CFU/mL, half the limit of detection) Different letters in parentheses indicate significant differences according to Tukey's post hoc test ($p < 0.05$) after two-way ANOVA.

3.2. Nutritional content

Table 2 summarizes the composition of the raw faba bean cultivar Gloria and the effect of soaking and boiling on its macronutrients. Moisture content differed among treatments, indicating variation in hydration capacity. Beans soaked in sodium bicarbonate absorbed the most water (58.55 %wm), and this value was significantly higher than in the sodium bicarbonate + LAB treatment. Ash content was highest with sodium bicarbonate, mainly due to sodium incorporation from the soaking medium.

The protein content of raw beans was 33.13 g/100 g DM. Previous research has reported a protein content of 28.3 g/100 g DM for the same variety grown in Sweden (Labba et al., 2021), while (Jezierny et al., 2010) reported 33.7 g/100 g DM cultivated in Austria. After soaking and boiling, protein content ranged from 29 to 36 g/100 g DM. The variation may be caused by the loss of non-protein components during treatment, which makes the protein proportion appear higher without increasing the actual protein content.

Raw beans contained 2.10 g/100 g DM of fat. After processing, fat values ranged between 1.10 and 1.37 g/100 g DM, with no significant differences between treatments. The slight decrease in measured lipid content likely reflects analytical dilution effects caused by increased hydration and partial loss of surface-associated lipids during soaking, rather than a true reduction of lipids in the dry biomass.

The most pronounced changes occurred in starch fractions. Raw beans contained 12.81 g/100 g DM of resistant starch. After soaking and boiling, resistant starch decreased markedly to 4.54–5.33 g/100 g DM, whereas non-resistant starch increased from 20.18 g/100 g DM in the raw material to as high as 47.50 g/100 g DM observed in beans treated with only water and in sodium bicarbonate + LAB, while not significant differences were found between those two groups (Jezierny et al., 2010). reported an average total starch content of 43.58 g per 100 g dry matter across several varieties.

The significant changes observed after soaking and boiling, such as the increase in protein content after sodium bicarbonate treatment, likely reflect protein denaturation and structural modifications that enhance nitrogen recovery during analysis. Alkaline conditions may also promote limited proteolysis and starch–protein interactions. In contrast, the decrease observed in the *L. plantarum* treated samples is attributed to microbial utilization of amino acids, proteolytic conversion of proteins into smaller nitrogenous compounds, and leaching losses during fermentation and boiling (Wu et al., 2015). For starch, soaking hydrates granules, causing swelling and leaching of soluble carbohydrates, while boiling induces gelatinization that disrupts granular and crystalline structures, decreasing resistant starch and increasing digestible starch. Mild alkaline treatment causes partial amylose leaching and slight loss of crystallinity similar to limited gelatinization enhancing enzymatic digestibility. Heat and alkaline conditions further modify starch organization through protein–starch interactions, influencing the ratio of resistant to digestible starch (Wang and Copeland, 2012).

The total phenolic content in raw faba beans was 2.66 mg GAE/g DM. After soaking and boiling, this value decreased to a range of 1.07–1.54 mg GAE/g DM. Other studies have reported values of 1.8 mg GAE/g DM for the variety Gloria and around 2.9 mg GAE/g DM for unspecified varieties (Dhull et al., 2022). The decrease in phenolic content from raw to soaked and boiled samples is attributed to leaching of water-soluble phenolic compounds and thermal degradation during processing.

3.3. Free amino acids

Fig. 1 shows the free amino acid (FAA) composition of raw and treated faba bean samples. Soaking and boiling affected the amino acid profile; however, no significant differences ($p > 0.05$) were found among the treated samples. In general, arginine, glutamic acid, and aspartic acid were the most abundant, while proline and phenylalanine showed the lowest levels in all raw and treated samples.

Raw beans showed the highest total FAA content (5.63 mg/g DM), which is consistent with the value reported by (Tuccillo et al., 2022) of 5.6 mg/g DM on faba bean flour. Treated samples averaged 3.25 mg/g DM. All treatments reduced total FAA, indicating leaching losses or reduced extractability due to protein denaturation. Aspartic acid showed the largest reduction (from 0.71 to 0.09 mg/g DM), while leucine was least affected (0.08 to 0.05 mg/g DM). Arginine remained predominant, consistent with previous reports identifying faba bean as rich in arginine and glutamic acid (Labba et al., 2021). Many studies report amino acid composition using acid hydrolysis, which measures total amino acids and generally yields higher values. Nevertheless, comparison of individual free amino acids is limited because methodologies differ: acid hydrolysis with LC-MS chemically breaks peptide

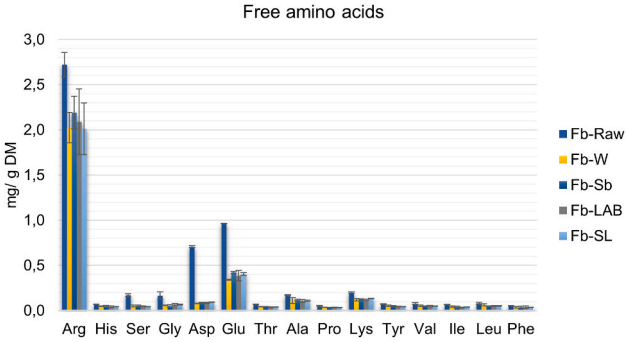

Fig. 1. Free amino acid composition of the raw faba beans and treated samples are expressed as expressed as mg/g DM. No significant differences ($p > 0.05$) were found among the treated samples.

Table 2
Proximate composition, starch fractions, total phenolic content, and free amino acids of raw faba bean (Fb) variety Gloria and samples subjected to different pretreatments of soaking + boiling.

Parameter	Raw Fb	W: Water	*Sb: Sodium bicarbonate*	*LAB: L. plantarum*	SL: Sodium bicarbonate + *L. plantarum*
Moisture (% wm)	12.7 ± 0.0	56.8 ± 1.5 (ab)	58.6 ± 0.8 (b)	55.7 ± 0.5 (ab)	53.7 ± 2.3 (a)
Ash (% DM)	3.3 ± 0.0	0.9 ± 0.0 (b)	1.0 ± 0.0 (b)	0.8 ± 0.1 (a)	0.7 ± 0.0 (a)
Protein (% DM)	33.1 ± 1.0	32.2 ± 1.8 (a)	36.2 ± 0.5 (b)	29.2 ± 1.2 (a)	32.0 ± 0.6 (a)
Fat (% DM)	2.1 ± 0.1	1.3 ± 0.2 (a)	1.4 ± 0.4 (a)	1.1 ± 0.2 (a)	1.1 ± 0.6 (a)
RS (% DM)	12.8 ± 1.4	5.3 ± 0.2 (a)	4.9 ± 1.2 (a)	4.5 ± 0.0 (a)	5.0 ± 0.3 (a)
NRS (% DM)	20.2 ± 0.8	47.5 ± 1.2 (b)	46.0 ± 0.3 (ab)	43.7 ± 1.5 (a)	47.3 ± 1.8 (b)
TPC (GAE mg/g DM)	2.7 ± 0.2	1.2 ± 0.1 (ab)	1.5 ± 0.2 (c)	1.1 ± 0.1 (a)	1.5 ± 0.1 (bc)
FAAs (mg/g DM)	5.6 ± 0.2	3.2 ± 0.3 (a)	3.4 ± 0.2 (a)	3.2 ± 0.5 (a)	3.2 ± 0.3 (a)

Wet matter (wm), dry matter (DM), resistant starch (RS), non-resistant starch (NRS), total phenolic content (TPC), sum of free amino acids (FAAs).
Values are given as mean ± standard error.
Different letters indicate significant differences compare only between treated samples excluding raw faba bean (Tukey's HSD, $p < 0.05$).

bonds, releasing all amino acids from proteins, whereas UPLC with derivatization quantifies only pre-existing soluble free amino acids without hydrolysis, resulting in lower measured values (Graça et al., 2022; Labba et al., 2021).

3.4. Anti-nutritional content

As shown in Fig. 2, raw faba beans contained 7082 μg/g vicine and 2300 μg/g convicine, in agreement with values reported for the cultivar Gloria (7014 and 1906 μg/g, respectively). Soaking and boiling significantly reduced both compounds, with the strongest effect in *L. plantarum* soaking followed by boiling: 3846 μg/g vicine and 1216 μg/g convicine (reductions of 46% and 47% compared to raw faba bean). No significant difference was found between water and *L. plantarum* soaking alone. Sodium bicarbonate treatment showed the lowest reduction (33% and 32%).

Vicine and convicine are polar, water-soluble glycosides that can cause hemolytic anemia in individuals with glucose-6-phosphate dehydrogenase (G6PD) deficiency. Although no safe intake threshold exists, reducing these compounds is critical, as low-vicine lines contain <500 μg/g DW (Khazaei et al., 2019). Reducing vicine and convicine content may also improve sensory quality, as these compounds have been associated with bitterness, astringency, and high taste intensity in faba beans (Tuccillo et al., 2025).

Reductions during soaking and boiling are primarily driven by diffusion into the surrounding medium. This process can be enhanced by pH-dependent changes in solubility, which increase compound mobility and leaching. Hydration increases permeability and facilitates diffusion into the soaking medium, while heating enhances this by softening cell structures (Dhull et al., 2022). In treatments involving *L. plantarum*, microbial or endogenous β-glucosidases may hydrolyse vicine and convicine into aglycones (divicine and isouramil) (Pulkkinen et al., 2019), which degrade more readily, further reducing toxicity.

Phytic acid in raw beans was 843 mg/100 g DM, in agreement with previous values reported for the Gloria variety (820 mg/100 g DM)

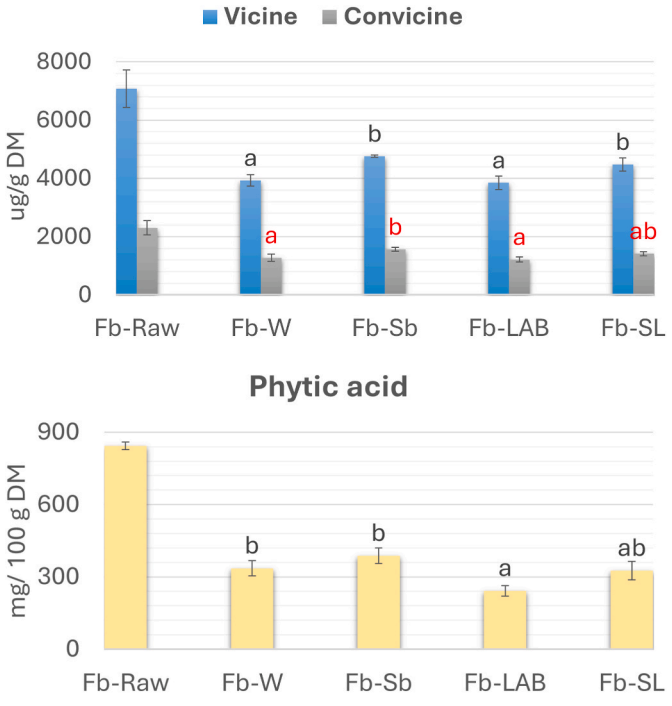

Fig. 2. Vicine, convicine, and phytic acid contents are presented as means of triplicate analyses. Different letters indicate significant differences among treated samples (Tukey's HSD, $p < 0.05$); raw faba bean was not included in the comparison.

(Labba et al., 2021). All treatments significantly ($p < 0.05$) reduced phytate content. The largest decrease occurred in the *L. plantarum* soaking + boiling treatment (71%), while soaking + boiling in only water reduced phytate by up to 60%. Our decrease was greater than in Canadian faba beans (2285 mg/100 g DM), where cooking lowered phytate by 38% and soaking by 3.2% (Shi et al., 2018).

This likely reflects a combined effect from the soaking increasing the tissue permeability, allowing soluble phytate to diffuse out, while boiling disrupted protein–phytate and mineral–phytate complexes (Vadivel and Pugalenthi, 2009). In *L. plantarum* treatments, microbial phytases likely hydrolysed myo-inositol hexakisphosphate to lower inositol phosphates (IP_5–IP_3) with reduced mineral-binding ability (Adeyemo and Onilude, 2013). Microbial phytases are likely to come from LAB (Verni et al., 2017) rather than yeasts (Georgiev et al., 2024; Olstorpe et al., 2009) as yeasts were not detected in the soaking water (see section 3.1).

3.5. Microstructure analyses

Light and confocal microscopy showed distinct structural (Fig. 3) differences among the treatments. Water-soaked seeds maintained intact cellular organization, with well-defined cell walls and evenly distributed starch. Sodium bicarbonate soaking caused visible cell swelling and partial loosening of the cell walls, with a more heterogeneous distribution of starch and protein, especially observed in CLSM with the red parts representing protein content.

LAB-treated seeds showed partial cellular disruption: starch granules appeared larger and more separated, and protein signals were more aggregate. Moreover, in the light microscopy was weaker Lugol's staining in all the individual cells. The last row of the combined sodium bicarbonate + LAB treatment resulted in the greatest structural breakdown. Cells appeared collapsed, and starch, protein, and fibre signals were highly redistributed throughout the tissue.

These observations had similarities compare with findings in kidney beans (Rovalino-córdova et al., 2019), where intact cotyledon cells maintained protein matrices around starch granules, limiting enzyme access, similar to the well-defined cell walls in water-soaked faba beans. Treatments that disrupted the matrix, such as protease pre-incubation, increased starch hydrolysis, paralleling the partial cell loosening and structural breakdown seen in sodium bicarbonate or LAB–treated faba beans. Nevertheless, in this study it was not possible to establish a relationship with *in vitro* starch or protein digestibility because these analyses were outside the scope.

3.6. Plant-based beverages model

3.6.1. Viscosity

All samples exhibited shear-thinning behaviour observed in Fig. 4, as viscosity decreased with increasing shear rate. However, the magnitude differed markedly. LAB showed the strongest shear-thinning and highest structural organization at rest, followed by W and SL. In contrast, SB displayed the lowest viscosity and only weak shear-thinning, approaching near-Newtonian behaviour at shear rates above ~1 s^{-1}.

The apparent viscosity at high shear rates (0.02–0.07 Pa s) was comparable to values reported for commercial plant-based beverages, where $\eta\infty$ typically ranges between 0.007 and 0.03 Pa s. The slightly higher values observed in the present study likely reflect the use of whole, unfiltered seed dispersions at relatively high solid content (12%, w/v) (Lopes et al., 2020).

The interpretation of rheological results depends on the intended final product, as desirable viscosity characteristics vary by application. Plant-based milk alternatives should exhibit low apparent viscosity at high shear rates (~0.01–0.05 Pa s at 100 s^{-1}) to ensure good pourability and drinkability comparable to dairy milk (J. Tang et al., 2023). At the same time, moderate shear-thinning behaviour is beneficial to help reduce sedimentation during storage. In contrast, plant-based yogurt

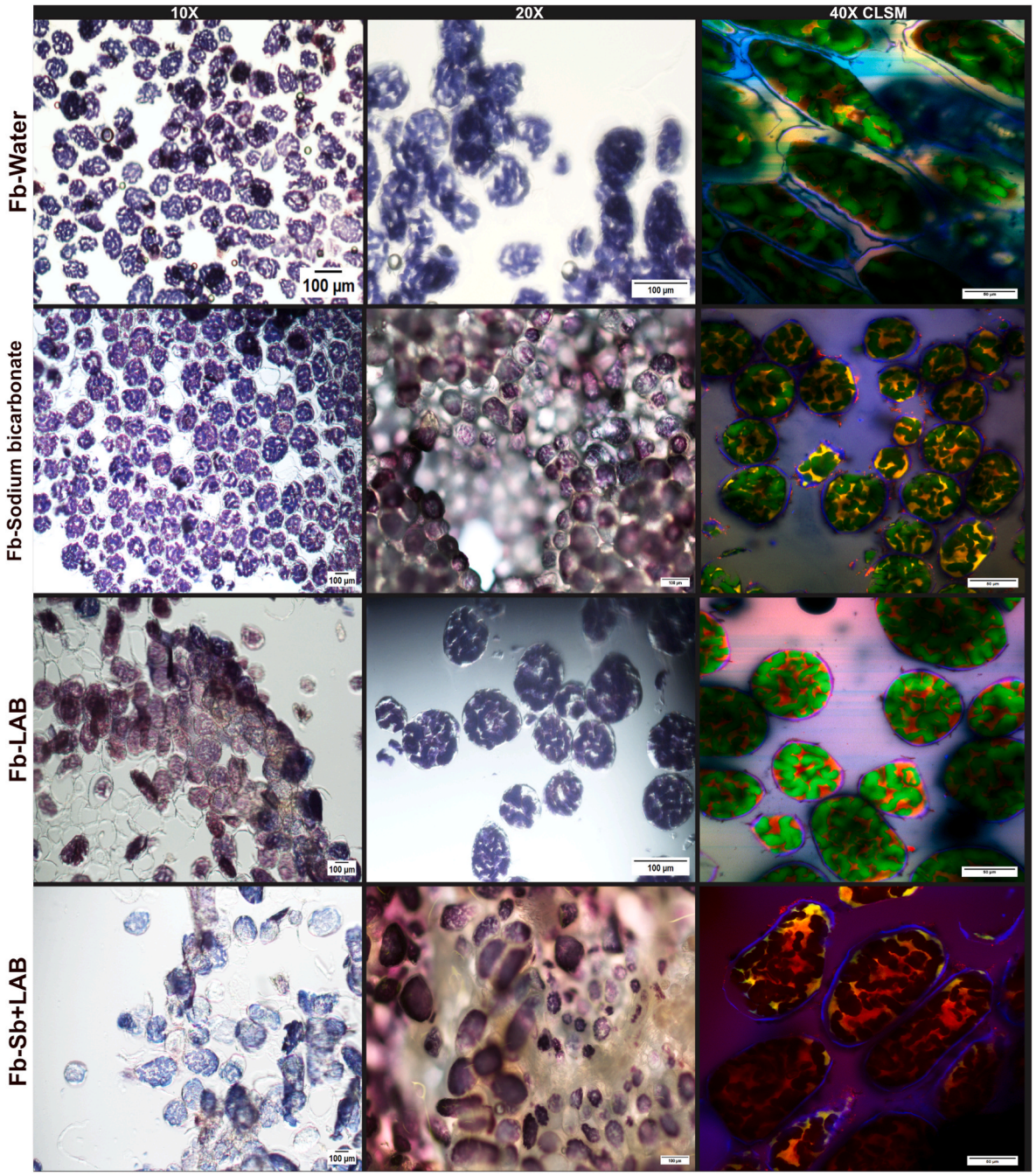

Fig. 3. Microstructure of faba bean samples subjected to different pre-treatments. Seeds were soaked for 12 h at 25 °C in tap water (control), sodium bicarbonate, *Lactiplantibacillus plantarum*, or a combined sodium bicarbonate + LAB solution, followed by boiling. Light microscopy images (first and second columns) were acquired with 10 × and 20 × objectives using Lugol's iodine to visualize starch; scale bar = 100 µm. Confocal laser scanning microscopy images (third column) were acquired with a 40 × objective; scale bar = 50 µm. Samples were stained with Calcofluor White to visualize fibres (blue), Rhodamine B to visualize proteins (red), and FITC (fluorescein isothiocyanate) to visualize starch (green).

bases require high zero-shear viscosity and gel-like viscoelastic behavior ($G' > G''$) to achieve thicker texture, creaminess, and resistance to syneresis (Duarte et al., 2022).

3.6.2. *Water-holding capacity (WHC) and colour*

WHC varied markedly among treatments (Table 3), with LAB showing the highest value (68.4 ± 2.5%), followed by SL and S, and W showing significantly lower retention (26.2 ± 1.4%). The higher WHC observed in LAB treated faba bean beverage was maybe by the modified protein functionality through mild acidification rather than strong enzymatic degradation. The pH reduction during soaking (pH 4.9, Table 2) may have altered protein charge and subsequent thermal denaturation behaviour during boiling (Tang et al., 2023). Since no major differences were observed in phytic acid reduction, total phenolics, or microstructural disruption, the higher value of WHC is more plausibly attributed to changes in protein functionality rather than extensive cell wall degradation.

L* values ranged from 58.1 to 68.8, with the LAB treated sample showing significantly higher lightness (68.8 ± 1.3; $p < 0.05$), while W, SB, and SL did not differ. Previous studies reported higher L* values for filtered lupin and chickpea beverages 79 and 68, respectively; (Lopes et al., 2020), emphasizing the influence of filtration and particle

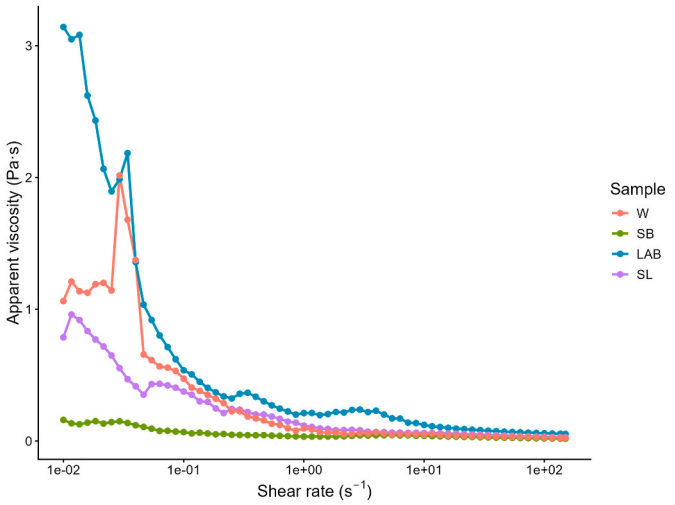

Fig. 4. The rheological properties of faba bean beverages depending on treatment as soaking from W: water, Sb: sodium bicarbonate, LAB: *L. plantarum* and SL: sodium bicarbonate + *L. plantarum*.

Table 3
Effect of soaking + boiling treatments on water-holding capacity (WHC) and colour parameters (L, a, b*) of plant-based beverages.

Treatment	WHC (%)	L	a	b
W	26.2 ± 1.4 (c)	61.6 ± 1.0 (b)	−10.6 ± 0.2 (a)	28.0 ± 2.0 (b)
SB	53.3 ± 1.0 (b)	58.6 ± 1.2 (b)	−10.4 ± 0.1 (a)	28.5 ± 0.6 (b)
LAB	68.4 ± 2.5 (a)	68.8 ± 1.3 (a)	−10.9 ± 0.2 (a)	34.0 ± 0.5 (a)
SL	56.5 ± 0.9 (b)	58.1 ± 2.4 (b)	−10.1 ± 1.0 (a)	26.2 ± 1.1 (b)

Values are given as mean ± standard error.
Different letters indicate significant differences compare between plant-based beverage (Tukey's HSD, $p < 0.05$).

refinement on colour. As the present beverages were produced from whole seeds without filtration, lightness is mainly governed by particle dispersion, hydration, and suspension stability. The higher L* in the LAB sample may therefore be linked to enhanced particle hydration and increased light scattering, resulting in a lighter appearance, which may be beneficial for beverage applications.

3.6.3. Stability (phase separation analysis)

Visual observations in Fig. 5 showed some differences in phase separation during storage. The water-soaked samples exhibited the highest separation, with a distinct serum layer and sediment formation after 24 h. In contrast, the LAB and SL treated samples showed the least separation and maintained a more homogeneous appearance. However, after 48 h and 7 days the differences in phase separation were not significant, while colour remain significantly different among the beverages. These findings align with the rheological results as LAB samples had the highest low-shear viscosity, which reduces particle sedimentation according to Stokes' law, whereas the lower viscosity of W samples explains their greater phase separation at time 0 h and time 24 h.

Soaking treatments markedly influenced the functional performance of the plant-based beverages. LAB treatment resulted in higher low-shear viscosity, greater water-holding capacity, higher lightness, and reduced phase separation, indicating enhanced structural stability. In contrast, water-soaked samples showed the lowest viscosity and stability.

4. Conclusions

Faba bean cultivar Gloria showed compositional changes after soaking and boiling. *L. plantarum* inoculation reduced pH, sodium bicarbonate buffers acidification, and after 12 h, counts remained within acceptable levels. Moisture uptake was highest with sodium bicarbonate, while protein content varied depending on the treatment, reflecting structural changes and the effects of enzymatic and microbial activity. Starch was the most responsive component, with resistant starch decreasing and digestible starch increasing, indicating gelatinization and partial leaching, due to soaking and boiling. Total phenolic content and free amino acids decreased moderately, suggesting limited solubilization and thermal effects. Soaking and boiling also reduced vicine,

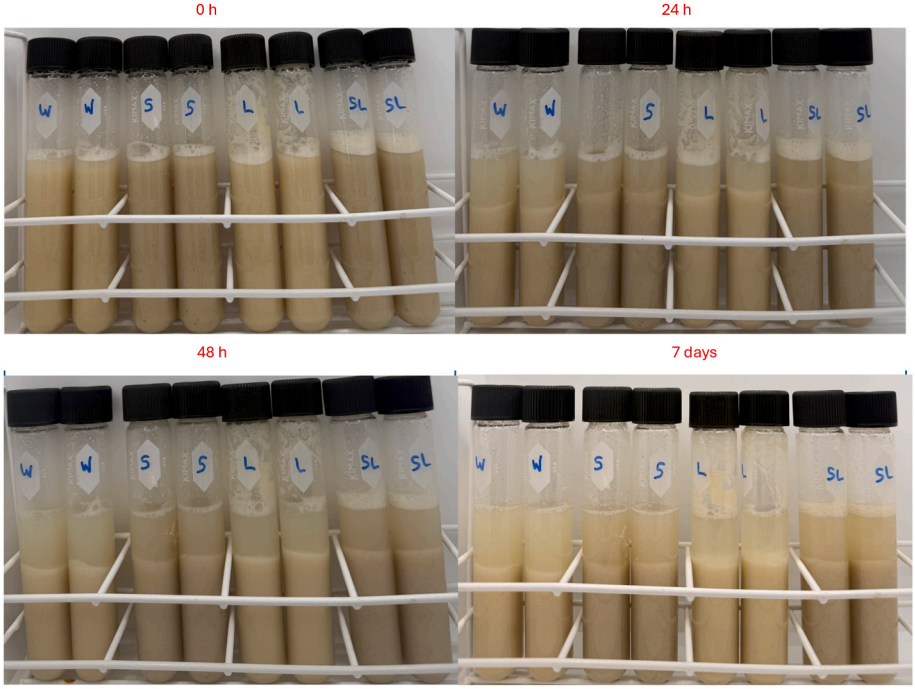

Fig. 5. Visual assessment of the sedimentation rate of faba bean beverages. Tubes are in duplicate per treatment from W: water, Sb: sodium bicarbonate, LAB: *L. plantarum* and SL: sodium bicarbonate + *L. plantarum*.

convicine, and phytic acid, with *L. plantarum* soaking and boiling treatment showing the greatest reductions. In the beverage model, LAB treatment gave the most favourable physical performance (higher low-shear viscosity, higher WHC, lighter appearance, and less phase separation), suggesting functionality was driven mainly by changes in protein–particle interactions and water immobilization rather than major compositional changes. These results indicate that specific processing methods can modulate the composition and improve functional properties.

CRediT authorship contribution statement

LAFC: Writing – original draft, Writing – review & editing, Methodology, Investigation, Formal analysis. OK, PM, JL, FT: Investigation. MA: Writing – review & editing, Formal analysis. ME: Methodology, Investigation and Supervision. KK: Project administration, Funding acquisition. S-Lll: Writing – review & editing, Methodology, Investigation, Formal analysis, Supervision. ML: Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization. GZ: Writing – review & editing, Supervision, Methodology, Conceptualization.

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Declaration of competing interest

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

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Data availability

Data will be available in Zenodo.

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