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Biotechnological approaches for reducing antinutrients and enhancing lentil (*Lens culinaris*) flours quality

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ABSTRACT

This study explores the effects of germination and cooking as pretreatments, followed by fermentation using different starter cultures on the physicochemical, nutritional, and techno-functional properties of Beluga and Du Puy lentil flours, focusing on reducing antinutritional factors and enhancing nutrient bioavailability. Fermentation was conducted using lactic acid bacteria (*Furfurilactobacillus rossiae* and *Lactobacillus brevis*) and a probiotic yeast (*Saccharomyces boulardii*) as starter cultures, individually and in combination. Results showed significant improvements in protein content, which increased by up to 38.4 g/100 g dry weight (dw) in Beluga lentils, and γ-tocopherol levels, which reached 8.15 mg/100 g dw after fermentation with *L. brevis*. Also, in Beluga lentils, germination followed by fermentation with *F. rossiae* and *S. boulardii* reduced carbohydrates to 57.7 g/100 g dw. Germination alone enhanced sucrose concentrations to 4.09 g/100 g dw and 4.44 g/100 g dw both Beluga and Du Puy lentils, respectively, while fermentation reduced its levels and promoted glucose production, reaching up to 1.02 g/100 g dw. Reduction in antinutritional factors was notable, with decreased phytic acid and condensed tannins concentration. Techno-functional properties such as water and oil holding capacity, and emulsifying capacity also improved significantly across treatments, enhancing the versatility of lentil flours in diverse food applications. These results highlight the potential of tailored processing techniques to enhance the physicochemical profile of lentils, providing a foundation for their use in developing nutrient-dense, plant-based food products. Such innovations offer sustainable alternatives to traditional food sources, aligning with the growing demand for high-yield, environmentally friendly options.

1. Introduction

The increasing global population feeds a corresponding expansion in the demand for enhanced food production. Alongside this, effective processing of agricultural products has become vital for maintaining food and nutritional security in the modern era (Nkhata et al., 2018). In this context, biotechnology has become particularly significant, addressing global challenges in food production, environmental sustainability, and health, serving as a powerful tool for developing sustainable foods with enhanced nutritional value (Xing et al., 2020).

Recently, there has been increasing awareness of the nutritional benefits of various pulses, leading to a higher market demand for these products (Arshad et al., 2023a). Among them, lentils (*Lens culinaris* spp.) have gained prominence as a valuable crop due to their rich nutritional profile, including high levels of protein (20 %–30 %), fiber (6 %–27 %), iron (Fe), zinc (Zn), potassium (K), folate (vitamin B9), and polyphenols, among others (Khazaei et al., 2019; Liberal, Almeida, et al., 2024; Liberal et al., 2023). Nevertheless, the application of most legume grains, including lentils, in different food formulations remains limited due to the presence of antinutritional factors, lower digestibility of plant-based proteins, and potential changes in sensory characteristics of the final product, which significantly reduce their overall acceptability and consumption (Kumar et al., 2022). Some of the main antinutrients present in lentils include phytic acid, which forms insoluble complexes essential minerals (*e.g.*, calcium, magnesium, iron, and zinc) hindering their absorption, tannins and trypsin inhibitors, which intake is associated with reduced digestibility and bioavailability of proteins, carbohydrates, and minerals by limiting the activity of digestive enzymes

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(Patterson et al., 2017).

Various processing techniques have been explored to enhance the physicochemical profile of pulses and improve the accessibility and bioavailability of their nutrients by inactivating or eliminating antinutrients, prompting their flavor and palatability, and modifying their functionality (Liberal, Almeida, et al., 2024). These may include thermal (*e.g.,* boiling, extrusion, roasting, steaming) and non-thermal treatments (*e.g.,* soaking, elicitation, germination, fermentation), which can be used alone or in combination to further improve their effect. Like other pulses, lentils must be processed before consumption to soften the seeds and improve their overall quality (Singla et al., 2020). One of the most common/traditional processing methods for this end is cooking through boiling, which improves protein digestibility while lowering the concentration of carbohydrates and fat, primarily due to the denaturation of heat-labile molecules (Liberal, Almeida, et al., 2024). Germination, in turn, is an ancient method that enables the activation of endogenous enzymes in legume grains, leading to the breakdown of large molecules, such as carbohydrates and proteins, and the release of essential minerals, which become more bioaccessible for assimilation. This process alters the nutritional, physicochemical, and textural characteristics of the grains, ultimately enhancing their overall quality (Ali & Elozeiri, 2017). Fermentation, in turn, is a bioprocessing technique that has been used for centuries to improve the nutritional profile and quality of several food products, such as beverages (*e.g.,* beer and wine), meat, and dairy products (*e.g.*, cheese, yogurts, kefir) while creating new flavors, aromas, and textures, reducing undesirable compounds, and enriching the food matrices with compounds of high added-value (Liu et al., 2011; Xu et al., 2019).

Fermentation is defined as a biological process carried out by microorganisms (bacteria, molds, yeasts) that convert substrates into new products, including enzymes, biomass, primary and secondary metabolites (O. A. Adebo et al., 2017). Specifically, enzymes like amylases, proteases, and lipases hydrolyze polysaccharides, proteins, and lipids, respectively, producing compounds that prevent spoilage and modify the food's nutritional, functional, and sensory properties (Steinkraus, 2018). Controlled fermentation using specific microorganisms is preferred over natural fermentation to enhance these attributes in pulses. Currently, lactic acid fermentation of pulses is gaining increasing attention mainly due to its potential to create healthier, more flavorful, and technologically enhanced products (Emkani et al., 2022). Lactic acid bacteria (LAB) have been used for this purpose, as they are considered safe and capable of providing specific nutritional, functional, and sensory, benefits to food products (Klupsaite et al., 2017). The primary product of LAB is lactic acid, which promotes the acidification of foods, hinders the development of other potentially pathogenic microorganisms, and enhances sensory characteristics by producing aromatic compounds and reducing off-flavors. Additionally, when used as probiotics, they offer nutritional and health benefits through the synthesis of bioactive molecules (Harlé et al., 2020; Reuben et al., 2020; Xing et al., 2020). Among pulses, some LAB strains, namely *Furfurilactobacillus rossiae*, *Lactiplantibacillus plantarum*, *Levilactobacillus brevis*, and *Fructilactobacillus sanfranciscensis* have been used to improve their nutritional quality, with some studies reporting the decrease in several antinutrients and an increase in their overall digestibility (De Pasquale et al., 2020; Montemurro et al., 2019). In parallel, the yeast *Saccharomyces cerevisiae* var. *Boulardii* has been investigated as a starter culture for developing functional foods with probiotic properties (Chandrasekar Rajendran et al., 2017; Knez et al., 2023; Romero-Espinoza et al., 2020; Swieca et al., 2019). While many studies focus on its benefits for specific health conditions, its application as a supplement in food systems remains underexplored (Lazo-Vélez et al., 2018). Notably, the use of *S. boulardii* in food matrices has been shown to enhance the synthesis of bioactive compounds (Değirmencioğlu et al., 2016; Rekha & Vijayalakshmi, 2010), improve the bioavailability of essential minerals and vitamins (Chandrasekar Rajendran et al., 2017), and increase the concentration of protein, fiber, amino acids, and fatty acids, while simultaneously reducing antinutrient concentrations. However, in fermentation, it is essential to scrutinize the antagonistic interactions between different types of microorganisms and yeasts, as they can compete for available micronutrients in the food matrix and adapt differently to pH levels, high ethanol concentrations, and the presence of various nutraceutical compounds (Hatoum et al., 2012).

There is a clear lack of studies evaluating the combined effect of different processing methods used to maximize pulses' nutritional benefits and functional characteristics. These methods and their variables must be optimized for each pulse type as their physicochemical profile differs significantly and can be impacted to different degrees. Moreover, the mechanisms by which microbial strains, alone or combined, affect specific pulse varieties are scarce and must be investigated to further improve the overall quality of the flours.

Thus, this work aims to explore the effects of germination and cooking as pretreatments, followed by fermentation using specific LAB strains (*F. rossiae* and *L. brevis*) and a probiotic yeast (*S. boulardii*), either individually or in combination (processes and microorganisms), on improving the nutritional and chemical qualities of two lentil varieties (Beluga and Du Puy), simultaneously evaluating their impact on reducing antinutrients and enhancing the techno-functional properties of the flours. This pioneering research examines the synergistic effects of diverse processing techniques and microbial cultures on lentils, providing novel insights into their capacity to alter the physicochemical and functional attributes of pulses. These methods are particularly appealing due to their natural, cost-effective, and easily implementable nature, which can enhance the nutritional quality and safety of lentils while reducing antinutritional factors. Ultimately, this study contributes to improving the sensory characteristics of lentils, potentially increasing their consumption and incorporation into everyday diets.

2. Materials and methods

2.1. Samples

Lentil samples (*Lens culinaris* spp.), specifically the Beluga and Du Puy varieties, were purchased (5 kg) from markets in two Spanish cities, Zamora and Salamanca—regions known for high lentil production. All samples were harvested in the 2023 season, and analysis was conducted one-year post-harvest. The seeds had black and mottled brown seed coats, respectively. The samples were thoroughly cleaned to remove foreign materials and damaged grains. After pre-treatments (germination and cooking), the grains were milled using a Foss Knifetec™ 1095 mill at a controlled temperature of 20 °C and stored for further analysis.

2.2. Pre-treatments

2.2.1. Boiling

The lentils were soaked in distilled water at a seed-to-water ratio of 1:5 (*w/v*) at room temperature (25 °C) for 12 h (Khandelwal et al., 2010). After soaking, the seeds were drained, washed, and placed in a beaker with distilled water at a seed-to-water ratio of 1:6.7 (*w/v*), and boiled for 35 min at 95 °C (Vidal-Valverde et al., 1994). Following boiling, the cooking water was drained, and the seeds were dried in an air oven at 50 °C until a constant weight was achieved.

2.2.2. Germination

Lentils were soaked in distilled water at a ratio of 1:5 (*w/v*) for 12 h at room temperature (~25 °C), shaking every 30 min. After soaking, the water was drained, and the lentils were transferred to glass flasks covered with gauze and kept in the dark for 3 days to germinate (Vidal-Valverde et al., 1994). The flasks were tilted at approximately 45° to allow excess water to drain. Every 24 h, the seeds were moistened with distilled water, gently shaken, and drained (Fouad & Rehab, 2015). Once sprouted, the seeds were dried in an air oven at 50 °C until a constant weight was achieved.

2.3. Microorganisms and fermentation

Two LAB strains and a probiotic yeast were selected as starter cultures for the fermenting of the different lentil flours. *Furfurilactobacillus rossiae* was purchased from the Collection de l'Institut Pasteur (CIP; Paris, France) (109910T – *Furfurilactobacillus rossiae*; batch 35208_20), and *Levilactobacillus brevis* (ATCC 14869; batch 1262-06-6) was acquired from Frilabo. *Saccharomyces boulardii,* in turn, was purchased as a supplement capsule (UL-250) in a local pharmacy. A commercial baking powder (Fermipan®) was used as control. LAB strains were cultured in MRS broth at 37 °C until they reached the late exponential growth phase. The cells were then washed twice with 50 mM phosphate buffer at pH 7.0 and resuspended in tap water to a cell density of 1.5×10^8 CFU/mL. *S. boulardii*, in turn, was cultivated in Malt Extract Broth medium at 25 °C, following the above-mentioned procedures. These suspensions, individually or in combination (Table 1), were used as starter cultures for fermenting germinated and boiled lentils, aiming to investigate their effects on the physicochemical composition, antinutrient levels, and techno-functional properties of the resulting flours. One hundred grams of each lentil flour were mixed with distilled water in a water-to-flour ratio previously determined by evaluating their water absorption capacity [9] and 5 mL of each cell suspension with a continuous high-speed mixer. Fermentation was conducted in duplicate at 30 °C for 24 h, after which the doughs was freeze-dried and stored for further analysis.

2.4. Proximate composition

The proximate composition of various lentils samples (Table 1) was evaluated following AOAC methods (AOAC, 2016). In summary, crude protein content was determined using the macro-Kjeldahl method (N x 6.25) with an automatic distillation and titration system (model Pro-Nitro-A, JP Selecta, Barcelona). Crude fat content was measured using Soxhlet extraction, where a known sample weight (3 g) was extracted with petroleum ether for 7 h. Ash content was determined by incinerating samples at 550 ± 5 °C. Total carbohydrates were estimated by difference using the formula:

Total carbohydrates = 100 – (g ash + g proteins + g fat)

The energy content was calculated using the equation:

Energy (kcal/100g) = 4 x (g proteins + g carbohydrates) + 9 x (g fat)

Table 1
Lentil samples characterization according to variety, pre-treatments, and fermentation microorganisms.

Variety	Samples	
Beluga (B) Du Puy (DP)	Raw	R
	Germinated	G (+)
	Baking powder	BP
	F. rossiae	Fr
	L. brevis	Lb
	S. boulardii	Sb
	F. rossiae + *L. brevis*	Fr + Lb
	F. rossiae + *S. boulardii*	Fr + Sb
	L. brevis + *S. boulardii*	Lb + Sb
	F. rossiae + *L. brevis* + *S. boulardii*	Fr + Lb + Sb
	Boiled	B (+)
	Baking powder	BP
	F. rossiae	Fr
	L. brevis	Lb
	S. boulardii	Sb
	F. rossiae + *L. brevis*	Fr + Lb
	F. rossiae + *S. boulardii*	Fr + Sb
	L. brevis + *S. boulardii*	Lb + Sb
	F. rossiae + *L. brevis* + *S. boulardii*	Fr + Lb + Sb

Total dietary fiber content was determined using an enzymatic-gravimetric method, involving enzymatic digestion with α-amylase, protease, and amyloglucosidase. In brief, dried lentil samples were incubated with α-amylase at ~100 °C to promote starch gelatinization, hydrolysis, and depolymerization, followed by incubations with protease and amyloglucosidase at 60 °C. The samples were then treated with ethanol, filtered, washed with 78 % and 95 % ethanol and acetone, dried, and weighed. One duplicate sample was analyzed for protein content, while another was incinerated at 525 °C to determine ash content. The results were expressed as relative percentages, and total dietary fiber content was calculated using the following equation:

%Total dietary fiber = [(R–P–A)/SW] x 100

Where:

R – average residue
P – average protein
A – average ash
SW – average weight of samples

2.5. Chemical composition

2.5.1. Free sugars

Free sugars were analyzed using high-performance liquid chromatography with a refractive index detector (HPLC-RI; Knauer, Smartline 1000 and Smartline 2300 systems). The extraction procedure followed the method described by Spréa et al. (2020). Briefly, to each dried sample powders (1.0 g) melezitose was added as an internal standard (IS, 5 mg/mL) and extracted using 40 mL of 80 % aqueous ethanol at 80 °C for 90 min. The resulting suspension was centrifuged at 4000 *g* for 10 min at 10 °C. The supernatant was concentrated at 60 °C under reduced pressure and then defatted by washing three times with 10 mL of ethyl ether. After further concentration at 40 °C, the solid residues were dissolved in water to a final volume of 5 mL and filtered through 0.2 μm Whatman nylon filters. Peak identification and quantification were carried out by comparing the retention times (Rt) with those of authentic standards and calibration curves. The data were processed using Clarity Software (Data Apex, Prague, Czech Republic), and the results were expressed in grams per 100 g of dry weight (g/100 g dw).

2.5.2. Organic acids

The organic acids profile of the lentil samples was analyzed using ultra-fast liquid chromatography coupled with a photodiode array detector (UFLC-PDA; Shimadzu Corporation, Kyoto, Japan). Samples (2 g) were extracted by stirring with 25 mL of meta-phosphoric acid at 25 °C and 150 rpm for 25 min, followed by filtration through Whatman No. 4 paper (Barros, Pereira, & Ferreira, 2013). Prior to analysis, the extract was further filtered through 0.2 μm nylon filters. The compounds were separated on a reverse-phase 18 SphereClone column (Phenomenex) (5 μm, 250 mm × 4.6 mm i.d.), maintained at 35 °C. A 3.6 mM sulfuric acid solution was used as the eluent at a flow rate of 0.8 mL/min. Each compound was identified by comparing the chromatograms with those of commercial standards, and quantification was done by correlating peak areas, recorded at 215 nm, with calibration curves from commercial standards. The results were expressed in grams per 100 g of dry weight (g/100 g dw).

2.5.3. Tocopherols

Tocopherols were analyzed following the method described by Barros, Pereira, Calhelha, et al. (2013), using an HPLC system coupled with a fluorescence detector (P-2020; Jasco, Japan), set to an excitation wavelength of 290 nm and an emission wavelength of 330 nm. In brief, prior to the extraction procedure, 100 μL of BHT solution in hexane (10 mg/mL) and 400 μL of an internal standard (tocol; 50 μg/mL in hexane) were added to the samples (500 mg). These were then homogenized

with 4 mL of methanol by vortex mixing for 1 min. Hexane (4 mL) was subsequently added, followed by another 1-min vortex mix. Afterward, 2 mL of a saturated NaCl aqueous solution was added, the mixture was homogenized for 1 min and centrifuged at 4000 *g* for 5 min at 10 °C. The clear upper layer was carefully transferred to a vial. The sample was re-extracted twice with hexane, and the combined extracts were evaporated to dryness under a nitrogen stream. The residue was redissolved in 2 mL of n-hexane, dehydrated with anhydrous sodium sulfate, filtered through 0.2 μm Whatman nylon filters, and transferred to a dark injection vial for analysis. The tocopherol isoforms were separated on a Polyamide II normal-phase column (250 mm × 4.6 mm i.d.) from YMC Waters (Japan), operated at 30 °C. The mobile phase was a mixture of hexane and ethyl acetate (7:3, *v/v*) with a flow rate of 1 mL/min and an injection volume of 20 μL. Tocopherols were identified by comparing chromatograms with those of authentic standards. Quantification was based on fluorescence signal response, using tocol as an internal standard and commercial standards for comparison. The results were expressed in milligrams per 100 g of dry weight (mg/100 g dw).

2.5.4. Fatty acids

The fatty acid methyl esters (FAME) profile was determined following the transesterification of the lipid fraction obtained through Soxhlet extraction, as outlined by Barros, Pereira, Calhelha, et al. (2013). The analysis was conducted using gas-liquid chromatography with flame ionization detection on a YOUNG IN Chromass 6500 GC system (Gyeonggi, South Korea), equipped with a split/splitless injector set at 250 °C (split ratio of 1:80), a flame ionization detector set at 260 °C, and a Zebron-FAME column (30 m × 0.25 mm i.d. × 0.20 μm df; Phenomenex, Lisbon, Portugal). FAME identification and quantification were achieved by comparing the relative retention times of sample peaks with those of commercial standards (standard mixture 47,885-U, Sigma, St. Louis, MO, USA). Data were recorded and processed using Clarity DataApex 4.0 software (Prague, Czech Republic), and the results were expressed as the relative percentage of each fatty acid.

2.6. Mineral composition

The mineral profile of the lentil samples was determined according to the AOAC procedures (AOAC, 2016). Samples were digested with 10 mL of nitric acid using a microwave extraction system, operating at 200 °C and 1600 W for 30 min. The resulting mixture was diluted to a final volume of 50 mL with distilled water. The mineral content, including potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), copper (Cu), and zinc (Zn), was analyzed using atomic absorption spectrophotometry (PerkinElmer 1100B, Waltham, MA, USA) (Paschoalinotto et al., 2023). Results were expressed in g per kg for K, Ca, and Mg, and in mg per kg for Na, Fe, Mn, Cu, and Zn.

2.7. Antinutritional factors

2.7.1. Phytic acid

Phytic acid content was determined following the protocol described by Fitriani et al. (2021). Approximately 100 mg of lentil samples were extracted with 20 mL of 0.5 M HNO_3 and incubated in a water bath with continuous shaking at 30 °C for 4 h. The extracts were then filtered through Whatman No. 4 paper. To 1 mL of the filtrate, 0.4 mL of distilled water and 1 mL of 0.005 M $FeCl_3$ were added. The mixture was boiled for 20 min, then cooled to room temperature. Five milliliters of amyl alcohol and 0.1 mL of 0.1 M ammonium thiocyanate were added, mixed thoroughly, and centrifuged at 4000 *g* for 20 min at 10 °C. The upper layer was analyzed using a UV–vis spectrophotometer at 495 nm. Standard curves were prepared using phytic acid concentrations of 0.4, 0.2, 0.1, 0.05, and 0.025 mg/mL (SIGMA-P8810) in HNO_3. The equation from the standard curve was used to calculate phytic acid concentrations in the lentil samples, with results expressed in mg per g dry weight (dw).

2.7.2. Condensed tannins

The presence of condensed tannins in lentil samples was evaluated using the "modified vanillin assay" method described by Dykes (2019). A standard curve was first prepared with catechin solutions in methanol at concentrations of 0.0, 0.2, 0.4, 0.6, 0.8, and 1 mL. The tubes were incubated in a water bath at 30 °C, and 5 mL of vanillin reagent were added to the first tube, starting a 20-min timer. Additional tubes received 5 mL of vanillin reagent at 30-s intervals for time-dependent analysis. After incubation, absorbance was measured at 500 nm.

For sample analysis, approximately 0.3 g of lentil samples were weighed in triplicate, and 8 mL of 1 % HCl in methanol was added to each tube. The mixture was vortexed and incubated in a water bath at 30 °C for 20 min, followed by centrifugation at 4000*g* for 5 min at 10 °C for 10 min. One milliliter of each tube's supernatant was transferred to new test tubes, labeled as "blank" and "samples," and placed back in the water bath. In 30-s intervals, 5 mL of 4 % HCl in methanol was added to the "blank" tubes, and 5 mL of vanillin solution was added to the "samples" tubes. Absorbance was measured exactly 20 min later at 500 nm. The tannin concentration in each sample was calculated using the equation:

$$\text{Tannin concentration (mg catechin eq/g)} = (V * A) / (m * W)$$

Where:

V - Volume of extract (mL)
A - Absorbance at 500 nm ("sample" - "blank")
m - Slope of the standard curve
W - Sample weight (g)

2.7.3. Trypsin inhibitors

The activity of trypsin inhibitors (TI) was measured following the method described by Kakade et al. (1974), with modifications from Coscueta et al. (2017) and Malomo et al. (2011). TI extracts were prepared by mixing approximately 1 g of powdered lentil samples with 50 mL of 0.01 M NaOH. The mixture was agitated for 3 h at room temperature, then centrifuged at 4500g for 10 min. The supernatant served as the TI extract for further analysis.

A stock solution of BAPNA (SIGMA-B4875) was made by dissolving 40 mg of BAPNA in 1 mL of dimethyl sulfoxide (DMSO), then diluting it to 100 mL with 0.05 M Tris-buffer at pH 8.2, keeping the solution at 37 °C. A trypsin stock solution was prepared by dissolving 4 mg of trypsin in 200 mL of 0.001 M HCl, which was stored in an ice bath. Both BAPNA and trypsin solutions were freshly prepared before the TI assay.

To assess TI activity, a control solution (BAPNA and trypsin) and a sample solution (BAPNA, trypsin, and sample extract) were prepared. The control solution consisted of 2 mL of distilled water, 5 mL of BAPNA, and 2 mL of trypsin solution, incubated in a water bath at 37 °C for 10 min. After incubation, 1 mL of 30 % acetic acid was added to stop the trypsin reaction. The mixture was centrifuged at 4000 *g* for 10 min at 10 °C, and absorbance was measured at 410 nm using UV–vis spectrophotometry. The sample solutions were prepared similarly, using the sample extract instead of water. TI activity was expressed in trypsin units inhibited (TUI) per mg of sample, calculated using the following equation:

$$\text{TUI/mg sample} = (A1 - A2) \times (100 / df) / w$$

Where:

A1 - control absorbance
A2 - sample absorbance
w - weight of the initial sample (mg)
df - dilution factor (mL)

2.8. Techno-functional properties

2.8.1. Water and oil holding capacity

Water and oil holding capacity (WHC and OHC, respectively) was conducted following a previously described protocol (Wani et al., 2015), with some modifications. Briefly, approximately 1 g of each sample was weighed into pre-weighed 25 mL centrifuge tubes. For each sample, 10 mL of distilled water or sunflower oil was added, and the mixtures were thoroughly vortexed for 30 s. The samples were then allowed to stand for 30 min at 20 °C before being centrifuged at 4000 *g* for 30 min at 10 °C. The water and oil that separated during centrifugation were drained, and the tubes were reweighed. The results for WHC and OHC were expressed as grams of water and oil absorbed per gram of each sample.

2.8.2. Swelling capacity

The swelling capacity of the lentil samples was evaluated using a modified method based on Yu et al. (2012). Approximately 250 mg of each sample was combined with 25 mL of distilled water in pre-weighed 50 mL centrifuge tubes. The tubes were placed in a water bath at 90 °C for 30 min with constant agitation. After this period, the solutions were allowed to cool to room temperature and then centrifuged at 4000 *g* for 15 min at 10 °C. The supernatant was decanted, and the weight of the wet sediment was recorded to determine the swelling capacity. The results were expressed as the ratio of the weight of the wet sediment (g) to its dry weight (g).

2.8.3. Emulsifying capacity

Emulsifying capacity was assessed using a method described by Ahmedna et al. (1999). Approximately 500 mg of each lentil sample was mixed with 5 mL of distilled water in 15 mL Falcon tubes. The suspension was vortexed for 30 s every 5 min over a total duration of 30 min. Following this, 3 mL of sunflower oil was added to each tube, which was then homogenized for 2 min using a vortex mixer and centrifuged at 4000 *g* for 20 min at 10 °C. The emulsion capacity (%) was calculated as the ratio of the height of the emulsified phase to the height of the entire

Table 2
Proximate composition of flours from germinated and boiled Beluga and Du Puy lentils, fermented with different combinations of lactic acid bacteria (*F. rossiae* and *L. brevis*) and a probiotic yeast (*S. boulardii*) (mean ± SD; $n = 3$).

Samples		Fat (g/100g dw)	Proteins (g/100g dw)	Fibers (g/100g dw)	Ash (g/100g dw)	Carbohydrates (g/100g dw)	Energy (kcal/100g dw)
Beluga	Raw	1.15 ± 0.10^{p}	26.8 ± 0.5^{m}	23.5 ± 0.8^{e}	1.86 ± 0.04^{efg}	70.2 ± 0.3^{f}	398.3 ± 0.5^{opq}
	Germination						
	G	1.05 ± 0.06^{no}	28.5 ± 0.2^{jk}	19.8 ± 1.0^{q}	2.1 ± 0.1^{de}	68.4 ± 0.3^{h}	396.9 ± 0.1^{qr}
	BP	1.6 ± 0.2^{lmn}	31.9 ± 0.4^{c}	20.1 ± 0.3^{p}	2.4 ± 0.1^{bc}	64.1 ± 0.3^{q}	398.3 ± 0.8^{op}
	Fr	0.95 ± 0.01^{rs}	33.5 ± 0.2^{b}	21.0 ± 0.1^{k}	2.30 ± 0.06^{cd}	63.3 ± 0.2^{r}	395.6 ± 0.1^{rs}
	Lb	1.43 ± 0.09^{o}	33.3 ± 0.5^{b}	20.8 ± 0.1^{m}	2.4 ± 0.4^{bcd}	62.9 ± 0.1^{r}	397.6 ± 0.7^{pq}
	Sb	1.5 ± 0.2^{n}	33.8 ± 0.8^{b}	22.8 ± 0.2^{g}	2.5 ± 0.1^{bc}	62.2 ± 0.8^{s}	397.8 ± 0.2^{pq}
	Fr + Lb	1.9 ± 0.1^{hij}	33.6 ± 0.2^{b}	22.7 ± 1.1^{h}	2.58 ± 0.08^{bc}	62.0 ± 0.2^{s}	399.0 ± 0.6^{nop}
	Fr + Sb	1.17 ± 0.08^{p}	38.4 ± 0.5^{a}	23.6 ± 0.6^{d}	2.7 ± 0.1^{ab}	57.7 ± 0.3^{u}	395.3 ± 0.5^{ijk}
	Lb + Sb	1.02 ± 0.06^{qr}	31.5 ± 0.7^{c}	18.8 ± 0.5^{w}	2.6 ± 0.1^{bc}	64.9 ± 0.4^{p}	395.8 ± 0.1^{s}
	Fr + Lb + Sb	2.62 ± 0.02^{d}	33.5 ± 0.2^{b}	20.9 ± 0.6^{l}	2.4 ± 0.3^{bcd}	61.5 ± 0.1^{t}	403.5 ± 1.0^{ghij}
	Boiling						
	B	0.9 ± 0.1^{s}	30.5 ± 0.2	19.2 ± 0.9^{u}	1.6 ± 0.2^{gh}	67.1 ± 0.2^{lm}	398.2 ± 0.9^{pq}
	BP	1.5 ± 0.1^{no}	31.6 ± 0.8^{c}	18.3 ± 0.6^{x}	1.84 ± 0.09^{efg}	65.1 ± 0.6^{p}	400.2 ± 0.6^{mn}
	Fr	1.98 ± 0.08^{g}	28.1 ± 0.2^{kl}	16.1 ± 0.8^{aa}	1.76 ± 0.02^{fg}	68.2 ± 0.1^{h}	402.9 ± 0.4^{hijk}
	Lb	1.8 ± 0.2^{j}	28.9 ± 0.5^{ghij}	17.0 ± 0.8^{z}	1.8 ± 0.2^{efg}	67.5 ± 0.3^{jkl}	401.9 ± 1.3^{kl}
	Sb	1.55 ± 0.09^{mn}	29.6 ± 0.5^{e}	14.7 ± 0.2^{cc}	1.89 ± 0.06^{ef}	66.9 ± 0.5^{mn}	400.2 ± 0.2^{m}
	Fr + Lb	1.69 ± 0.01^{kl}	29.0 ± 0.4^{fghi}	13.4 ± 0.3^{dd}	1.64 ± 0.03^{fg}	67.6 ± 0.3^{ijk}	401.9 ± 0.1^{kn}
	Fr + Sb	1.95 ± 0.04^{ghi}	31.5 ± 0.1^{c}	15.6 ± 0.4^{bb}	1.73 ± 0.07^{fg}	64.9 ± 0.1^{p}	402.8 ± 0.3^{ij}
	Lb + Sb	1.84 ± 0.06^{ij}	29.5 ± 0.2^{ef}	13.8 ± 0.1^{dd}	1.2 ± 0.2^{hi}	67.5 ± 0.2^{jkl}	404.3 ± 0.8
	Fr + Lb + Sb	3.4 ± 0.1^{a}	29.8 ± 0.9^{e}	14.0 ± 0.2^{dd}	1.7 ± 0.1^{fg}	65.1 ± 0.6^{p}	410.3 ± 0.8^{b}
Du Puy	Raw	1.13 ± 0.01^{pq}	24.5 ± 0.2^{r}	25.8 ± 2.3^{a}	2.57 ± 0.07^{bc}	71.8 ± 0.1^{bc}	395.4 ± 0.2^{s}
	Germination						
	G	0.67 ± 0.05^{t}	25.4 ± 0.4^{pq}	23.2 ± 0.5^{f}	2.95 ± 0.05^{a}	71.0 ± 0.3^{e}	391.5 ± 0.3^{t}
	BP	2.0 ± 0.1^{ghi}	29.4 ± 0.1^{efg}	23.8 ± 1.8^{c}	2.21 ± 0.08^{bcd}	66.4 ± 0.1^{no}	400.9 ± 0.6^{lm}
	Fr	1.65 ± 0.01^{lm}	27.8 ± 0.8^{l}	20.8 ± 0.8^{n}	2.5 ± 0.1^{bc}	68.1 ± 0.5^{hi}	398.4 ± 0.4^{op}
	Lb	2.75 ± 0.08^{c}	28.9 ± 0.1^{hij}	16.4 ± 1.0^{aa}	2.45 ± 0.07^{bc}	65.9 ± 0.1^{o}	403.9 ± 0.5^{ghi}
	Sb	2.7 ± 0.1^{cd}	28.5 ± 0.2^{ijk}	15.2 ± 0.2^{bb}	2.3 ± 0.1^{cd}	66.5 ± 0.3^{n}	404.2 ± 0.1^{gh}
	Fr + Lb	1.79 ± 0.01^{jk}	28.4 ± 0.1^{jk}	19.8 ± 0.2^{r}	2.43 ± 0.05^{bc}	67.4 ± 0.1^{klm}	399.3 ± 0.1^{no}
	Fr + Sb	1.61 ± 0.08^{lmn}	28.1 ± 0.2^{kl}	21.0 ± 0.1^{j}	2.34 ± 0.03^{bcd}	67.9 ± 0.2^{hij}	398.7 ± 0.2^{op}
	Lb + Sb	1.79 ± 0.01^{jk}	29.3 ± 0.6^{efgh}	22.3 ± 2.0^{i}	1.71 ± 0.04^{efg}	67.2 ± 0.4^{klm}	402.1 ± 0.1^{jkl}
	Fr + Lb + Sb	2.1 ± 0.2^{f}	28.2 ± 0.1^{kl}	19.8 ± 0.1^{s}	2.3 ± 0.2^{cd}	67.4 ± 0.3^{klm}	401.2 ± 0.4^{lm}
	Boiling						
	B	0.91 ± 0.07^{rs}	25.9 ± 0.6^{op}	24.6 ± 0.3^{b}	1.56 ± 0.02^{fg}	71.6 ± 0.5^{cd}	398.3 ± 0.3^{op}
	BP	1.66 ± 0.02^{lm}	26.3 ± 0.6^{no}	20.7 ± 1.2^{o}	2.54 ± 0.04^{c}	69.5 ± 0.5^{g}	398.2 ± 0.1^{opq}
	Fr	1.97 ± 0.06^{g}	26.3 ± 0.2^{no}	17.8 ± 0.4^{y}	1.0 ± 0.3^{ij}	70.8 ± 0.1^{e}	406.0 ± 0.6^{ef}
	Lb	1.9 ± 0.1^{ghij}	26.4 ± 0.4^{mn}	18.4 ± 0.4^{t}	0.6 ± 0.2^{lm}	71.1 ± 0.3^{e}	407.2 ± 0.8^{de}
	Sb	1.87 ± 0.01^{ghij}	27.9 ± 0.7^{l}	19.28 ± 0.1^{u}	0.8 ± 0.2^{kl}	69.3 ± 0.4^{g}	405.7 ± 3.4^{f}
	Fr + Lb	2.36 ± 0.09^{e}	25.5 ± 0.1^{p}	15.9 ± 0.4	0.86 ± 0.02^{jkl}	71.2 ± 0.1^{de}	408.2 ± 1.1^{cd}
	Fr + Sb	2.0 ± 0.1^{g}	24.8 ± 0.8^{r}	19.1 ± 0.3^{v}	0.28 ± 0.06^{m}	73.0 ± 0.2^{a}	408.7 ± 2.1^{c}
	Lb + Sb	2.0 ± 0.1^{gh}	25.0 ± 0.4^{qr}	16.4 ± 1.0^{aa}	0.92 ± 0.04^{jkl}	72.2 ± 0.2^{a}	406.4 ± 1.1^{ef}
	Fr + Lb + Sb	3.13 ± 0.09^{b}	27.8 ± 0.2^{l}	16.4 ± 0.5^{aa}	0.9 ± 0.1^{jk}	68.1 ± 0.1^{hi}	412.1 ± 0.9^{a}

In each column and variety, different letters present statistically significant differences ($p \leq 0.05$). G – germination; B – boiling; BP – baking powder; Fr – *F. rossiae*; Lb – *Lactobacillus brevis*; Sb – *Saccharomyces boulardii*.

mixture.

2.9. Statistical analysis

A total of thirty-eight samples were analyzed across all experiments, with each sample assessed in duplicate. The results are presented as mean values ± standard deviation (SD). Differences between processing methods were evaluated using a one-way analysis of variance (ANOVA), followed by Tukey's significant difference post-hoc test with $\alpha = 0.05$, incorporating Welch's statistic, all conducted using SPSS version 23.0.

3. Results and discussion

3.1. Proximate composition

The present study investigated the effects of germination and boiling combined with fermentation using LAB (*F. rossiae* and *L. brevis*) and a probiotic yeast (*S. boulardii*), either alone or combined, on the overall composition of two varieties of lentils (*Lens culinaris*; Beluga and Du Puy). The proximate composition of the raw and processed lentil samples is presented in Table 2.

Carbohydrates surge as the primary macronutrient found in raw (70.2 and 71.8 g/100g dw, respectively, for Beluga and Du Puy varieties) and processed (57.7–73.9 g/100g dw) lentil samples. However, their concentration seems strongly influenced by the processing methods and fermentation of microorganisms. In both Beluga and Du Puy varieties, germination and boiling alone promote a slight decrease in total carbohydrate content (68.4 and 67.1 g/100g dw for Beluga; 71.0 and 71.6 g/100g dw for Du Puy, respectively), this decrease being more pronounced after their further processing by fermentation. Regarding Beluga lentils, germination followed by fermentation with *F. rossiae* and *S. boulardii* promoted a higher reduction of total carbohydrates (57.7 g/100g dw), the same occurring after boiling and fermentation with the same strains (64.9 g/100g dw). In the Du Puy variety, in turn, the lower carbohydrate concentrations were found after germination and fermentation with *L. brevis* and boiling with subsequent fermentation with the three combined microbial strains in study (68.1 g/100g dw). The decrease in total carbohydrates after germination and boiling can be attributed to increased α-amylase activity, and potential polysaccharide leaching, hydrolysis, and starch gelatinization (Liberal, Almeida, et al., 2024). Additionally, some studies have reported the activation of starch-hydrolyzing enzymes (Osman, 2011) and the secretion of microbial enzymes, such as glucosidases and amylases, during fermentation, which break down carbohydrates into monosaccharides that are subsequently utilized by microorganisms as an energy source (Adebo et al., 2022).

Regarding fibers, their concentration in both lentil varieties is lowered after germination, boiling, and fermentation related to their raw form (23.5 and 25.8 %, respectively, for Beluga and Du Puy varieties), reaching concentrations between 13.4 and 23.8 %. No specific pattern was observed regarding the different microorganisms combined for fermentation purposes in the overall fiber concentration; however, the lower fiber concentrations were always found when *L. brevis* and *S. boulardii*, alone or combined, were used as starter cultures. Reduction of fiber content after fermentation was also reported by other authors in *Phaseolus vulgaris* (black beans) (Granito & Álvarez, 2006), which attributed this reduction to the hydrolysis of pectic compounds, as well as the utilization of cellulose and hemicellulose as substrates by the microorganisms responsible for fermentation (Gobbetti et al., 2014; Martín-Cabrejas et al., 2004).

Proteins follow, with all processing methods leading to an increase in their concentration in lentil samples compared to their raw form, peaking after germination and fermentation with *F. rossiae* and *S. boulardii* in the Beluga variety (38.4 g/100g dw). Likewise, the same strains promoted the highest increase when this variety was pre-treated with boiling water (31.5 g/100g dw), suggesting a stronger influence of these starter cultures in this field. Distinct patterns were observed for the Du Puy variety, which achieved its highest protein concentration after germination and fermentation with baking powder and *L. brevis* combined with *S. boulardii* (29.4 and 29.3 g/100g dw, respectively). Additionally, using *S. boulardii* and the three combined microbial strains following prior boiling resulted in 27.9 and 27.8 g/100g dw of protein, respectively. Despite these differences, it is evident that not only does the Beluga variety show more significant increases in protein content but also that the presence of *S. boulardii* is a key factor contributing to this rise among the two analyzed varieties. It is well established that fermentation, particularly with lactic acid bacteria (LAB), can alter proteins' content and composition due to the presence of enzymes and other microbial components (Klupsaite et al., 2017). The well-characterized proteolytic system of LAB comprises cell envelop-associated proteinases (*e.g.*, PrtP in *Lactococcus*), peptidases, and specific transport proteins, with proteinases cleaving proteins into peptides, which are then degraded by intracellular peptidases into amino acids and smaller peptides. Peptides formed due to protein degradation by cell wall-bound proteinases (PrtP) are then transported into the cells systems, where they undergo further degradation by endopeptidases and aminopeptidases. This process provides LAB with free amino acids needed for their own protein synthesis and growth thus increasing protein concentration (Hansen & Marcatili, 2020; Kieliszek et al., 2021). For example, Toor et al. (2022) reported a rise in the protein content of pigeon peas, chickpeas, and red beans after fermentation with *Rhizopus oligosporus*, attributed it to microbial enzyme synthesis (lipases and proteases). Similarly, Asensio-Grau et al. (2020) observed an 18.5 % increase in protein content in lentils fermented with *P. ostreatus* as a starter culture, attributing this to the bioconversion of carbohydrates into proteins, as the first serve as an energy source for fungal growth during fermentation. Furthermore, other studies have shown that co-culture inoculation leads to a significant increase in protein content than individual strains, such as in lupine fermented with *Aspergillus longae* and *Aspergillus ficuum* (Olukomaiya et al., 2020). These findings align with our results, though different starter cultures were used, which is critical in the outcomes observed.

Regarding crude fat, our results indicated that fermentation contributed to its increased concentration, with only statistically significant reductions ($p \leq 0.05$) being observed after germination and boiling in both lentil varieties, as noted in our previous study (Liberal, Almeida, et al., 2024). Overall, the highest crude fat concentrations were achieved after fermentation with the three combined starter cultures in both varieties (2.62, 3.4, and 3.13 g/100g dw), except for the Du Puy variety with prior germination, where the highest increase was observed when fermenting the flour with *L. brevis* and *S. boulardii* alone (2.75 and 2.7 g/100g dw, respectively). This increase in fat content may be attributed to heightened activity of lipolytic enzymes, leading to the production of more fatty acids during fermentation. This process likely involves the breakdown of large fat molecules into simpler fatty acids, contributions from the fat in dead microbial cells, and the assumption that microorganisms do not rely on fat as an energy source (Adebo et al., 2022). Some authors have reported increased concentration in crude fat after fermentation of different pulses, such as African yam-bean (*Sphenostylis stenocarpa*) (Onoja & Obizoba, 2010), *Tamarindus indica* (Oluseyi & Temitayo, 2015), and mahogany bean (*Afzelia africana*) (Igbabul et al., 2014) flours, attributing this to the increased activity of lipolytic enzymes which produced more fatty acids during fermentation, also pointing that fats concentration is correlated with the fermentation time.

Finally, the ashes concentration displayed different behaviors among the studied lentil flours, with all the germinated Beluga samples improving their overall concentration in relation to their raw form (2.1–2.7 g/100g dw). However, the same was not noticed in the remaining treatments, where the vast majority of the lentil flours lowered their overall ahes concentration. The increase in ash content in fermented legumes has been related to the metabolic activity of

microorganisms, as well as the breakdown of complex chelated compounds during fermentation, which is also related to longer fermentation times (Obadina et al., 2013; Olagunju et al., 2018; Onwurafor et al., 2014). On the other hand, the reduction in ash content has been attributed to the use of mineral elements for the growth of microorganisms during fermentation, as observed by some authors in African yam fermented for 24 h (Onoja & Obizoba, 2010).

3.2. Chemical composition

3.2.1. Free sugars

The free sugars profile of the various raw and processed lentil flour samples was investigated, and the results are presented in Table 3. Distinct patterns were observed across the different processing methods. Germination alone led to a significant increase in sucrose concentration in both lentil varieties (4.09 and 4.2 g/100 g dw, respectively) compared to their raw form (1.2 and 1.3 g/100g dw, respectively), reaching its highest levels after this process. In contrast, boiling caused a decline in sucrose content, rendering it undetectable in the Beluga variety and reducing it to 0.49 g/100 g dw in the Du Puy samples. Similar trends were observed in our previous study (Liberal, Fernandes, et al., 2024), in which the free sugars composition of four lentil varieties were analyzed after different processing methods. It has been suggested that the rapid increase in sucrose after germination is due to a fast hydrolysis of oligosaccharides when α-galactosidases reach their maximum activity (Goyoaga et al., 2011), while its decrease after boiling is linked to its leaching into the processing water and further enzymatic degradation of sugars at higher temperatures (Çelem et al., 2009). Fermentation, in turn, tends to diminish the concentration of sucrose, this being effectively eliminated in the majority of the lentil samples treated with prior boiling. Inversely, the concentration of glucose increased after germination combined with fermentation in both Beluga and Du Puy varieties (0.5–1.0 and 0.13–0.40 g/100g dw), compared to their raw form (0.12 g/100 g dw and not detected, respectively), and after boiling with further fermentation with baking powder and *S. boulardii* of the Du Puy lentils. The increased glucose concentrations after fermentation are

Table 3
Free sugars profile of the flours derived from germinated and boiled Beluga and Du Puy lentils, fermented with various combinations of lactic acid bacteria (*F. rossiae* and *L. brevis*) and a probiotic yeast (*S. boulardii*) (mean ± SD; n = 3).

Samples		Sugars (g/100g dw)				
		Glucose	Sucrose	Trehalose	Raffinose	Total sugars
Beluga	Raw	0.12 ± 0.01^{o}	1.02 ± 0.02^{c}	nd	nd	1.14 ± 0.03^{k}
	Germination					
	G	0.5 ± 0.1^{j}	4.09 ± 0.01^{b}	nd	nd	4.6 ± 0.1^{b}
	BP	0.67 ± 0.08^{g}	0.21 ± 0.03^{o}	0.13 ± 0.03^{f}	0.48 ± 0.01^{j}	1.49 ± 0.03^{j}
	Fr	0.62 ± 0.02^{h}	0.27 ± 0.04^{n}	0.16 ± 0.03^{e}	0.57 ± 0.08^{i}	1.6 ± 0.2^{i}
	Lb	0.86 ± 0.09^{e}	0.42 ± 0.05^{j}	0.22 ± 0.03^{bc}	0.89 ± 0.04^{f}	2.39 ± 0.05^{f}
	Sb	0.99 ± 0.03^{c}	0.38 ± 0.03^{k}	0.22 ± 0.02^{bc}	0.95 ± 0.04^{e}	2.5 ± 0.2^{e}
	Fr + Lb	0.97 ± 0.01^{d}	0.36 ± 0.03^{l}	0.22 ± 0.01^{bc}	1.1 ± 0.1^{c}	2.6 ± 0.1^{e}
	Fr + Sb	0.88 ± 0.06^{e}	0.32 ± 0.05^{m}	0.20 ± 0.03^{d}	0.73 ± 0.09^{g}	2.1 ± 0.2^{g}
	Lb + Sb	0.97 ± 0.01^{d}	0.38 ± 0.04^{k}	0.23 ± 0.03^{b}	0.91 ± 0.03^{e}	2.50 ± 0.05^{e}
	Fr + Lb + Sb	1.02 ± 0.07^{d}	0.35 ± 0.01^{l}	0.21 ± 0.01^{d}	0.85 ± 0.06^{f}	2.4 ± 0.1^{ef}
	Boiling					
	B	0.04 ± 0.01^{p}	nd	nd	nd	0.04 ± 0.01^{r}
	BP	0.05 ± 0.01^{p}	0.10 ± 0.04^{q}	nd	nd	0.14 ± 0.08^{p}
	Fr	Nd	nd	nd	0.76 ± 0.04^{g}	0.76 ± 0.04^{m}
	Lb	0.11 ± 0.05^{o}	nd	nd	nd	0.11 ± 0.05^{q}
	Sb	0.14 ± 0.01^{n}	nd	nd	nd	0.14 ± 0.01^{p}
	Fr + Lb	Nd	nd	nd	0.8 ± 0.2^{f}	0.8 ± 0.2^{l}
	Fr + Sb	Nd	nd	nd	0.70 ± 0.02^{gh}	0.70 ± 0.02^{mn}
	Lb + Sb	Nd	nd	nd	0.69 ± 0.01^{h}	0.69 ± 0.01^{n}
	Fr + Lb + Sb	Nd	nd	nd	nd	–
Du Puy	Raw	Nd	1.3 ± 0.2^{c}	nd	nd	1.3 ± 0.2^{k}
	Germination					
	G	0.43 ± 0.04^{n}	4.44 ± 0.02^{a}	nd	1.45 ± 0.04^{a}	6.32 ± 0.01^{a}
	BP	0.44 ± 0.04^{k}	0.23 ± 0.01	nd	0.76 ± 0.01^{g}	1.43 ± 0.04^{j}
	Fr	0.63 ± 0.01^{h}	0.54 ± 0.04^{f}	nd	nd	1.17 ± 0.05^{k}
	Lb	0.77 ± 0.02^{f}	0.49 ± 0.05^{h}	nd	1.2 ± 0.1^{b}	2.4 ± 0.2^{ef}
	Sb	0.36 ± 0.01^{l}	0.45 ± 0.02^{i}	nd	1.04 ± 0.05^{cd}	1.84 ± 0.02^{h}
	Fr + Lb	1.40 ± 0.08^{b}	0.51 ± 0.04^{g}	nd	1.02 ± 0.01^{cd}	2.9 ± 0.1^{d}
	Fr + Sb	0.36 ± 0.03^{l}	0.52 ± 0.05^{f}	nd	0.86 ± 0.08^{f}	2.1 ± 0.2^{g}
	Lb + Sb	0.67 ± 0.01^{g}	0.73 ± 0.02^{e}	nd	0.86 ± 0.08^{f}	2.1 ± 0.2^{g}
	Fr + Lb + Sb	0.6 ± 0.2^{i}	0.48 ± 0.01^{g}	nd	0.99 ± 0.06^{c}	2.1 ± 0.4^{g}
	Boiling					
	B	Nd	0.49 ± 0.01^{h}	nd	nd	0.49 ± 0.01^{o}
	BP	0.27 ± 0.01^{m}	0.18 ± 0.02^{p}	nd	nd	0.44 ± 0.01^{o}
	Fr	Nd	nd	nd	nd	–
	Lb	Nd	nd	nd	nd	–
	Sb	1.76 ± 0.01^{a}	0.75 ± 0.02^{d}	nd	1.09 ± 0.02^{cd}	3.83 ± 0.07^{c}
	Fr + Lb	Nd	nd	nd	nd	–
	Fr + Sb	Nd	nd	nd	nd	–
	Lb + Sb	Nd	nd	nd	nd	–
	Fr + Lb + Sb	Nd	nd	nd	nd	–

nd – not detected; In each column and variety, different letters present statistically significant differences ($p \leq 0.05$); G – germination; B –boiling; BP – baking powder; Fr – *F. rossiae*; Lb – *Lactobacillus brevis*; Sb – *Saccharomyces boulardii*.

related with the amplified activity of α-amylase during this process which would cause de breakage of polysaccharides into this sugar (Kaczmarska et al., 2017). Similar trends were observed by Granito and et al. (Granito et al., 2002) when analyzing the free sugars profile of beans (*Phaseolus vulgaris*) after natural fermentation, which reported an increase in glucose concentration up to 750 %. The glucose released during fermentation serves as a preferred substrate for the microorganisms involved in the fermentation process, which may partly explain the reduction in total carbohydrates after 24 h of fermentation (Osman, 2011). Raffinose, in turn, although not present in both Beluga and Du Puy raw samples, increased its concentration mainly after germination with further fermentation (0.48–1.1 and 0.76–1.2 g/100g dw for Beluga and Du Puy, respectively). Although present in some lentil samples after boiling and fermentation, mainly in the Beluga variety, in most of the remaining samples raffinose was undetectable. Kaczmarska et al. (2017) also found increased raffinose concentrations after a natural and yogurt mix fermentation of lupin (74.6 and 49.4 mg/g dw, respectively), compared to unprocessed lupin flour (19.7 mg/g dw), the same not being observed in soy samples of the same study, in which raffinose concentration decreased 69 %. Finally, trehalose was only detected in the Beluga variety after germination with further fermentation (0.13–0.22 g/100g dw), highlighting the influence of the different starter cultures used, specifically in this variety, in promoting the breakage of polysaccharides into different sugars.

3.2.2. Organic acids

The organic acids profile of raw and processed Beluga and Du Puy lentils, as shown in Table 4, exhibiting distinct patterns depending on the variety and processing technique. Oxalic and citric acids were the only organic acids consistently detected across all analyzed samples, though at lower concentrations (0.09–0.84 g/100 g dw and 0.1–2.04 g/100 g dw, respectively). Quinic acid, in turn, was the predominant organic acid in Beluga lentils, particularly when these were previously germinated and cooked, with concentrations ranging from 1.06 to 5.18

Table 4
Organic acids composition of flours from germinated and cooked Beluga and Du Puy lentils fermented with different combinations of lactic-acid bacteria (*L rossiae* and *L. brevis*) and a probiotic yeast (*S. boulardii*) (mean ± SD; $n = 3$).

Samples		Organic Acids (g/100g dw)						
		Oxalic acid	Quinic acid	Malic Acid	Citric acid	Succinic acid	Fumaric acid	Total organic acids
Beluga	Raw	0.27 ± 0.02^{i}	1.9 ± 0.2^{j}	nd	0.71 ± 0.02^{n}	nd	nd	2.9 ± 0.3^{m}
	G	0.27 ± 0.02^{i}	2.1 ± 0.2^{i}	nd	0.66 ± 0.01^{o}	nd	nd	3.0 ± 0.1^{m}
	BP	0.28 ± 0.01^{i}	2.4 ± 0.1^{h}	nd	0.56 ± 0.01^{p}	nd	nd	3.3 ± 0.1^{l}
	Fr	0.33 ± 0.01^{gh}	2.6 ± 0.1^{f}	nd	0.3 ± 0.1^{r}	0.73 ± 0.02^{f}	nd	3.98 ± 0.06^{i}
	Lb	0.40 ± 0.07^{f}	4.1 ± 0.2^{c}	nd	0.58 ± 0.03^{p}	1.03 ± 0.02^{d}	nd	6.1 ± 0.2^{d}
	Sb	0.53 ± 0.04^{d}	4.02 ± 0.06^{d}	nd	0.1 ± 0.06^{t}	1.91 ± 0.02^{b}	nd	6.97 ± 0.01^{c}
	Fr + Lb	0.52 ± 0.01^{d}	4.9 ± 0.1^{b}	nd	0.95 ± 0.01^{n}	0.90 ± 0.02^{e}	nd	7.3 ± 0.2^{ab}
	Fr + Sb	0.67 ± 0.01^{bc}	1.56 ± 0.01^{m}	nd	1.17 ± 0.01^{i}	1.01 ± 0.02^{d}	nd	4.42 ± 0.04^{g}
	Lb + Sb	0.32 ± 0.01^{gh}	5.18 ± 0.06^{a}	nd	1.22 ± 0.01^{gh}	0.95 ± 0.02^{de}	nd	7.7 ± 0.1^{a}
	Fr + Lb + Sb	0.37 ± 0.01^{p}	1.06 ± 0.01	nd	0.21 ± 0.01^{s}	0.18 ± 0.01^{g}	nd	1.2 ± 0.03^{v}
	C	0.12 ± 0.01^{mno}	1.9 ± 0.1^{j}	nd	0.55 ± 0.02^{p}	nd	0.99 ± 0.07^{c}	3.5 ± 0.2^{k}
	BP	0.36 ± 0.01^{g}	1.8 ± 0.1^{k}	nd	1.27 ± 0.01^{g}	nd	1.70 ± 0.07^{b}	5.2 ± 0.2^{e}
	Fr	0.29 ± 0.02^{hi}	2.4 ± 0.3^{h}	nd	1.13 ± 0.01^{ij}	nd	nd	3.8 ± 0.3^{j}
	Lb	0.41 ± 0.02^{ef}	2.5 ± 0.4^{g}	nd	1.04 ± 0.02^{lm}	nd	nd	2.9 ± 0.4^{m}
	Sb	0.33 ± 0.02^{gh}	2.5 ± 0.3^{g}	nd	1.12 ± 0.02^{ij}	2.01 ± 0.06^{a}	nd	6.0 ± 0.4^{d}
	Fr + Lb	0.32 ± 0.04^{gh}	3.5 ± 0.4^{e}	nd	0.92 ± 0.02^{n}	1.80 ± 0.04^{c}	nd	6.5 ± 0.5^{cd}
	Fr + Sb	0.42 ± 0.01^{ef}	2.6 ± 0.3^{f}	nd	1.16 ± 0.02^{i}	nd	nd	4.1 ± 0.3^{gh}
	Lb + Sb	0.38 ± 0.02^{g}	1.72 ± 0.04^{l}	nd	1.74 ± 0.02^{b}	nd	nd	3.84 ± 0.08^{j}
	Fr + Lb + Sb	0.35 ± 0.01^{g}	2.48 ± 0.03^{gh}	nd	2.04 ± 0.04^{a}	nd	nd	4.88 ± 0.06^{f}
Du Puy	Raw	0.26 ± 0.02^{ij}	1.19 ± 0.04	0.22 ± 0.02^{h}	1.37 ± 0.04^{f}	nd	nd	3.0 ± 0.1^{m}
	G	0.09 ± 0.01^{p}	2.5 ± 0.3^{g}	nd	1.23 ± 0.04^{gh}	nd	1.7 ± 0.2^{b}	5.5 ± 0.5^{e}
	BP	0.35 ± 0.04^{g}	nd	nd	1.47 ± 0.02^{d}	0.87 ± 0.02	nd	2.64 ± 0.08^{p}
	Fr	0.15 ± 0.01^{klm}	2.4 ± 0.3^{h}	nd	1.8 ± 0.1^{c}	nd	nd	4.3 ± 0.4^{g}
	Lb	0.49 ± 0.02^{e}	nd	nd	1.36 ± 0.04^{f}	nd	nd	1.86 ± 0.06
	Sb	0.68 ± 0.04^{bc}	nd	nd	1.52 ± 0.04^{c}	nd	nd	2.20 ± 0.07^{r}
	Fr + Lb	0.72 ± 0.04^{b}	nd	nd	2.0 ± 0.3^{a}	nd	nd	2.7 ± 0.3^{o}
	Fr + Sb	0.14 ± 0.01^{lm}	nd	nd	1.40 ± 0.04^{e}	nd	nd	2.13 ± 0.07^{s}
	Lb + Sb	0.84 ± 0.02^{a}	nd	nd	1.48 ± 0.01^{d}	nd	nd	2.32 ± 0.04^{q}
	Fr + Lb + Sb	0.13 ± 0.02^{mn}	nd	nd	0.43 ± 0.01^{q}	nd	nd	0.57 ± 0.04^{x}
	C	0.16 ± 0.01^{kl}	nd	0.72 ± 0.01^{b}	1.20 ± 0.01^{h}	nd	nd	2.08 ± 0.01^{t}
	BP	0.17 ± 0.02^{k}	nd	0.57 ± 0.02^{f}	1.31 ± 0.02^{f}	nd	2.0 ± 0.1^{a}	4.1 ± 0.2^{h}
	Fr	0.14 ± 0.01^{lm}	nd	0.79 ± 0.01^{a}	1.07 ± 0.01^{kl}	nd	0.76 ± 0.07^{d}	2.8 ± 0.1^{n}
	Lb	0.15 ± 0.01^{klm}	nd	0.28 ± 0.01^{g}	1.09 ± 0.01^{k}	nd	nd	1.3 ± 0.1^{w}
	Sb	0.15 ± 0.01^{klm}	nd	0.28 ± 0.01^{g}	1.09 ± 0.01^{k}	nd	nd	1.53 ± 0.04^{v}
	Fr + Lb	0.18 ± 0.01^{k}	nd	0.70 ± 0.01^{bc}	1.22 ± 0.02^{gh}	nd	0.18 ± 0.07^{f}	2.3 ± 0.1^{qr}
	Fr + Sb	0.15 ± 0.01^{klm}	nd	0.62 ± 0.01^{de}	0.93 ± 0.01^{nn}	nd	0.48 ± 0.07^{e}	2.2 ± 0.1^{qr}
	Lb + Sb	0.17 ± 0.01^{k}	nd	0.64 ± 0.01^{d}	1.35 ± 0.01^{f}	nd	nd	2.17 ± 0.04^{rs}
	Fr + Lb + Sb	0.65 ± 0.01^{bc}	nd	nd	1.15 ± 0.01^{i}	nd	nd	1.80 ± 0.03^{u}

In each column and variety, different letters present statistically significant differences ($p \leq 0.05$). G – germination; C – cooking; BP – baking powder; Fr – *F. rossiae*; Lb – *Lactobacillus brevis*; Sb – *Saccharomyces boulardii*.

g/100 g dw and 1.72–3.5 g/100 g dw, respectively. In contrast, for Du Puy lentils, quinic acid was only present in raw, germinated, and germinated samples further fermented with commercial baking powder, being undetectable in the remaining processing conditions. Malic acid was only detected in Du Puy lentils, specifically in raw samples and those cooked before fermentation, with concentrations ranging from 0.22 to 0.79 g/100 g dw. The improvement of malic acid concentrations upon cooking may be related to its release from cell wall components or with the breakdown of complex carbohydrates (Domizio et al., 2017). Succinic acid was primarily identified in germinated Beluga samples and was absent from most of the remaining samples, which may be related to the individual genetic characteristics of each variety. The occurrence of succinic acid in germinated lentils can be attributed to the activation of metabolic processes during germination, particularly the tricarboxylic acid (TCA) cycle. During seed germination, there is a transition from a dormant state to active metabolism, which involves increased activity of the TCA cycle to generate energy in the form of ATP. Succinic acid is an important intermediate in the TCA cycle, and its accumulation may occur as a result of this increased metabolic activity (Liu et al., 2022; Zhang & Lang, 2023). When compared to their raw forms, both lentil varieties majorly experienced an increase in organic acid concentration after processing. Notably, the highest concentrations of total organic acids were observed when both lentil varieties were fermented with combinations of two or more microbial cultures, highlighting the significant impact of microbial metabolism on organic acid production.

Few studies have explored the impact of fermentation with different microbial cultures on the modulation of the organic acid profile in various agricultural products. However, Shukla et al. (2015) observed that microbial enzymes break down proteins, starches, and fats into smaller molecules, such as amino acids, organic acids, and other small organic compounds during fermentation. This process likely explains the observed increase in organic acid concentrations in processed samples compared to their raw counterparts.

3.2.3. Tocopherols

The tocopherol profile of the various lentil samples was investigated, and the results are presented in Table 5. Two isoforms, α- and γ-tocopherol, were detected in both raw and processed lentil varieties, with γ-tocopherol being present in significantly higher concentrations. Our findings indicate that different processing methods greatly influence the overall γ-tocopherol concentration in both Beluga and Du Puy varieties. Notably, γ-tocopherol levels increased mainly after boiling followed by fermentation with the starter cultures studied, reaching 6.4–8.15 mg/100g dw in Beluga and 3.4–6.9 mg/100g dw in Du Puy lentils, compared to 3.5 and 3.3 mg/100g dw in their raw forms, respectively. In the Beluga variety, the highest γ-tocopherol concentration was observed after fermentation with *L. brevis* (8.15 mg/100g dw), *F. rossiae* with *S. boulardii* (7.74 mg/100g dw), and *L. brevis* with *S. boulardii* (7.4 mg/100g dw). In Du Puy lentils, in turn, the highest levels were achieved using *L. brevis* with *S. boulardii* (6.9 mg/100g dw) and *L. brevis* alone (6.6 mg/100g dw) as fermentation starters. These results emphasize the greater effect of *L. brevis* and *S. boulardii* in enhancing γ-tocopherol concentrations after boiling, likely due to the impact of processing on tocopherol release, metabolism, and degradation. The increase in tocopherols can be attributed to the disruption of cell walls and membranes in the lentil samples during boiling, which makes fat-soluble compounds more accessible for extraction (Liberal, Fernandes, et al., 2024). Additionally, certain microorganisms used in fermentation can produce antioxidant compounds that may protect tocopherols from oxidative degradation, contributing to the rise in their concentration after processing (Verni et al., 2019). Conversely, during germination followed by fermentation, the tocopherol concentration tends to decrease in both Beluga and Du Puy varieties, ranging from 1.22 to 3.74 and 1.6–3.73 mg/100g dw, respectively. Frias et al. (2005) investigated the effect of germination and fermentation on the tocopherol composition of whole lupin and lupin flour. Their findings revealed

Table 5
Tocopherols composition of flours from germinated and boiled Beluga and Du Puy lentils, fermented with different combinations of lactic acid bacteria (*F. rossiae* and *L. brevis*) and a probiotic yeast (*S. boulardii*) (mean ± SD; $n = 3$).

Samples		Tocopherols (mg/100g dw)		
		α-tocopherol	γ-tocopherol	Total tocopherols
Beluga	Raw	0.41 ± 0.02^{l}	3.5 ± 0.2^{lm}	3.9 ± 0.3^{jkl}
	Germination			
	G	0.76 ± 0.01^{d}	3.5 ± 0.1^{lm}	4.2 ± 0.1^{j}
	BP	0.52 ± 0.02^{ij}	2.30 ± 0.08^{pq}	2.82 ± 0.05^{m}
	Fr	0.46 ± 0.01^{k}	3.4 ± 0.2^{n}	3.9 ± 0.2^{n}
	Lb	0.27 ± 0.01^{p}	1.22 ± 0.01^{t}	1.49 ± 0.01^{r}
	Sb	0.58 ± 0.01^{fgh}	2.70 ± 0.01^{no}	3.28 ± 0.01^{m}
	Fr + Lb	0.90 ± 0.01^{a}	3.74 ± 0.01^{l}	4.63 ± 0.01^{i}
	Fr + Sb	0.35 ± 0.01^{no}	2.5 ± 0.2^{qr}	2.9 ± 0.2^{nop}
	Lb + Sb	0.49 ± 0.01^{jk}	2.12 ± 0.06^{pqr}	2.61 ± 0.06^{no}
	Fr + Lb + Sb	0.56 ± 0.01^{ghi}	2.14 ± 0.09^{pqr}	2.71 ± 0.08^{n}
	Boiling			
	B	0.52 ± 0.03^{ij}	5.3 ± 0.4^{ij}	5.8 ± 0.4^{g}
	BP	0.80 ± 0.01^{bcd}	7.0 ± 0.2^{d}	7.8 ± 0.2^{b}
	Fr	0.61 ± 0.01^{f}	6.41 ± 0.01^{fg}	7.02 ± 0.01^{cd}
	Lb	0.60 ± 0.01^{fg}	8.15 ± 0.06^{a}	8.76 ± 0.06^{a}
	Sb	0.8 ± 0.2^{b}	6.8 ± 0.7^{de}	7.7 ± 0.5^{b}
	Fr + Lb	0.59 ± 0.02^{fgh}	7.1 ± 0.4^{cd}	7.7 ± 0.3^{b}
	Fr + Sb	0.78 ± 0.06^{cd}	7.74 ± 0.21^{b}	8.5 ± 0.3^{a}
	Lb + Sb	0.41 ± 0.02^{lm}	7.4 ± 0.2^{c}	7.8 ± 0.3^{b}
	Fr + Lb + Sb	0.67 ± 0.05^{e}	7.2 ± 0.6^{de}	7.9 ± 0.6^{b}
Du Puy	Raw	0.25 ± 0.02^{p}	3.3 ± 0.4^{h}	3.6 ± 0.4^{lm}
	Germination			
	G	0.53 ± 0.02^{ij}	4.8 ± 0.2^{k}	5.3 ± 0.2^{h}
	BP	0.33 ± 0.01^{o}	2.48 ± 0.01^{op}	2.81 ± 0.01^{n}
	Fr	0.38 ± 0.02^{lmn}	3.73 ± 0.07^{l}	4.11 ± 0.09^{jk}
	Lb	0.37 ± 0.03^{lmn}	2.26 ± 0.09^{pqr}	2.6 ± 0.1^{no}
	Sb	0.19 ± 0.04^{q}	1.97 ± 0.09^{s}	2.17 ± 0.05^{qr}
	Fr + Lb	0.40 ± 0.01^{lm}	1.9 ± 0.1^{rs}	2.3 ± 0.2^{op}
	Fr + Sb	0.40 ± 0.01^{lm}	1.7 ± 0.2^{s}	2.11 ± 0.2^{pq}
	Lb + Sb	0.39 ± 0.01^{lmn}	1.90 ± 0.01^{rs}	2.29 ± 0.01^{op}
	Fr + Lb + Sb	0.36 ± 0.01^{mno}	4.0 ± 0.2^{lm}	4.4 ± 0.2^{jk}
	Boiling			
	B	0.22 ± 0.02^{q}	1.72 ± 0.02^{s}	1.94 ± 0.01^{k}
	BP	0.47 ± 0.01^{k}	4.9 ± 0.1^{jk}	5.4 ± 0.1^{h}
	Fr	0.60 ± 0.06^{fg}	6.3 ± 0.1^{gh}	6.9 ± 0.2^{de}
	Lb	0.57 ± 0.08^{fgh}	6.6 ± 0.1^{ef}	7.2 ± 0.2^{c}
	Sb	0.84 ± 0.05^{b}	4.73 ± 0.02^{k}	5.58 ± 0.02^{gh}
	Fr + Lb	0.38 ± 0.03^{lmn}	3.4 ± 0.2^{lm}	3.8 ± 0.2^{kl}
	Fr + Sb	0.54 ± 0.01^{hi}	5.58 ± 0.05^{i}	6.13 ± 0.06^{f}
	Lb + Sb	0.80 ± 0.02^{bc}	6.9 ± 0.3^{de}	7.7 ± 0.3^{b}
	Fr + Lb + Sb	0.56 ± 0.07^{fghi}	6.3 ± 0.2^{h}	6.8 ± 0.2^{e}

In each column and variety, different letters present statistically significant differences ($p \leq 0.05$). G – germination; B – boiling; BP – baking powder; Fr – *F. rossiae*; Lb – *Lactobacillus brevis*; Sb – *Saccharomyces boulardii*.

that γ-tocopherol concentration also dropped by 13 % after three days of germination and by about 90 % following the fermentation of lupin flour, with a less pronounced reduction when fermenting whole lupin. There are very few reports on the influence of germination and fermentation on the overall concentration of tocopherols; however, some authors have attributed this decrease to the sensitivity of tocopherols when in contact with oxygen during agitation in the fermentation process. Additionally, during germination, the production of reactive oxidative species (ROS) increases due to greater metabolic activity, which can cause tocopherols to be used as antioxidants during this process, reducing their availability (Carciochi et al., 2016). Regarding α-tocopherol, our results revealed an increase in the great majority of both processed Beluga and Du Puy lentil samples, its concentration ranging from 0.27 to 0.8 and 0.19–0.84 mg/100g dw, respectively, comparatively with their raw form (0.41 and 0.25 mg/100g dw, respectively). Here, once again, the improvement in

α-tocopherol may be related to the synthesis of phenolic compounds with antioxidant capacity by the fermentative microorganisms', which protect tocopherols from oxidative damage during processing (Chou et al., 2008; Verni et al., 2019). Examining the impact of various processing methods, particularly fermentation, on tocopherol concentrations in food matrices is crucial. This is not only due to their role as potent antioxidants but also to understanding their behavior during processing. Such insights enable the manipulation of food to develop functional products with enhanced physicochemical and bioactive properties.

3.2.4. Fatty acids

The main fatty acid methyl esters (FAME) profile of the studied raw and processed lentil samples, presented in Table 6 (and Supplementary Material, Table SM 1), unveils the great influence of the different processing methods and starter cultures on the concentration of individual compounds. Linoleic acid (C18:2n6c) was the dominant fatty acid in both Beluga and Du Puy raw samples (27.98 and 38.7 %), followed by oleic (C18:1n9c; 15.80 and 21.3 %), palmitic (C16:0; 16.96 and 18.2 %), α-linolenic (C18:3n3; 11.02 and 10.4 %) and stearic (C18:0; 5.97 and 3.05 %, respectively) acids. In addition to processing methods, distinct patterns were observed between the lentil varieties. In the Beluga samples, germination alone led to an increase in linoleic acid (36.9 %) compared to the raw form. However, after boiling, concentrations of palmitic (18.72 %), oleic (20.2 %), linoleic (36.3 %), and α-linolenic (12.3 %) acids improved. Fermentation further elevated the linoleic acid levels in most Beluga samples, reaching a peak of 37.91 % when cooking was combined with fermentation using the three microbial cultures

Table 6
Main fatty acids methyl esters composition of flours from germinated and boiled Beluga and Du Puy lentils, fermented with different combinations of lactic acid bacteria (*F. rossiae* and *L. brevis*) and a probiotic yeast (*S. boulardii*) (mean ± SD; $n = 3$).

Samples		Fatty acids (%)							
		Palmitic acid (C16:0)	Stearic acid (C18:0)	Oleic acid (C18:1n9c)	Linoleic acid (C18:2n6c)	α-linolenic acid (C18:3n3)	SFA	MUFA	PUFA
Beluga	Raw	16.96 ± 0.01	5.97 ± 0.01	15.80 ± 0.01	27.98 ± 0.01	11.02 ± 0.01	40.2 ± 0.1^{f}	16.9 ± 0.1^{p}	42.9 ± 0.1^{m}
	Germination								
	G	16.6 ± 0.1	5.8 ± 0.1	14.6 ± 0.3	36.9 ± 0.4	9.46 ± 0.4	34.3 ± 0.1^{k}	15.8 ± 0.3	49.9 ± 0.4^{g}
	BP	37.7 ± 0.4	10.5 ± 0.5	18.4 ± 0.4	8.45 ± 0.06	1.89 ± 0.08	67.6 ± 0.1^{a}	19.6 ± 0.5^{k}	12.8 ± 0.5^{s}
	Fr	22.02 ± 0.03	6.22 ± 0.01	17.2 ± 0.1	31.59 ± 0.04	6.25 ± 0.03	41.9 ± 0.1	18.2 ± 0.2	39.9 ± 0.1
	Lb	21.8 ± 0.2	5.5 ± 0.2	18.6 ± 0.9	32.1 ± 0.2	6.5 ± 0.4	39.2 ± 0.1^{g}	19.7 ± 0.8^{k}	41.1 ± 0.9^{p}
	Sb	21.9 ± 0.8	5.4 ± 0.5	14.9 ± 0.3	26.6 ± 0.8	6.3 ± 0.3	43.3 ± 0.1^{e}	16.1 ± 0.8^{q}	40.6 ± 0.3^{p}
	Fr + Lb	21.90 ± 0.04	4.7 ± 0.2	18.4 ± 0.5	28.00 ± 0.06	5.2 ± 0.2	35.1 ± 0.1^{j}	24.1 ± 0.3^{f}	40.8 ± 0.5^{p}
	Fr + Sb	35.5 ± 0.7	7.79 ± 0.01	23.0 ± 0.1	10.36 ± 0.07	1.4 ± 0.1	63.6 ± 0.3^{b}	24.6 ± 0.1^{d}	11.8 ± 0.1^{t}
	Lb + Sb	25.2 ± 0.4	6.42 ± 0.02	16.3 ± 0.1	34.01 ± 0.02	7.07 ± 0.04	40.4 ± 0.1^{f}	17.5 ± 0.2^{o}	42.2 ± 0.2^{o}
	Fr + Lb + Sb	22.9 ± 1.6	5.6 ± 0.2	16.2 ± 0.5	30.1 ± 0.5	7.7 ± 0.1	46.0 ± 0.1^{c}	16.2 ± 2.1^{pq}	37.8 ± 0.5^{q}
	Boiling								
	B	18.72 ± 0.08	3.7 ± 0.1	20.2 ± 0.4	36.3 ± 0.1	12.3 ± 0.1	28.4 ± 0.4^{o}	21.4 ± 0.4^{i}	50.2 ± 0.1^{ef}
	BP	19.43 ± 0.06	4.51 ± 0.08	17.83 ± 0.09	33.4 ± 0.1	12.68 ± 0.05	31.8 ± 0.1^{m}	19.2 ± 0.1^{l}	49.9 ± 0.1^{g}
	Fr	19.3 ± 1.4	4.8 ± 0.4	17.8 ± 0.1	35.0 ± 2.1	12.6 ± 0.5	31.6 ± 1.9^{m}	18.8 ± 0.2^{m}	49.9 ± 2.6^{g}
	Lb	18.93 ± 0.07	3.92 ± 0.01	18.3 ± 0.3	32.7 ± 0.2	11.4 ± 0.3	33.0 ± 0.2^{l}	19.0 ± 0.3^{lm}	48.0 ± 0.5^{h}
	Sb	18.7 ± 0.2	5.25 ± 0.02	17.0 ± 1.1	30.7 ± 0.7	12.1 ± 0.2	35.6 ± 0.8^{ij}	18.3 ± 1.2^{n}	46.1 ± 0.5^{jk}
	Fr + Lb	15.89 ± 0.02	3.30 ± 0.01	17.13 ± 0.07	34.6 ± 0.2	13.29 ± 0.04	25.8 ± 0.1^{s}	21.3 ± 0.1^{ij}	52.8 ± 0.1^{c}
	Fr + Sb	17.8 ± 0.3	3.56 ± 0.02	20.40 ± 0.03	35.2 ± 0.4	10.7 ± 0.4	27.0 ± 0.2^{pq}	25.2 ± 0.3^{bc}	47.8 ± 0.1^{h}
	Lb + Sb	16.9 ± 0.2	3.5 ± 0.3	23.0 ± 0.3	32.8 ± 0.4	9.8 ± 0.3	26.1 ± 0.1^{rs}	27.1 ± 0.1^{a}	46.8 ± 0.1^{i}
	Fr + Lb + Sb	17.8 ± 0.1	3.09 ± 0.01	22.3 ± 0.3	37.91 ± 0.09	10.27 ± 0.02	26.0 ± 0.2^{rs}	23.4 ± 0.3^{g}	50.7 ± 0.1^{e}
Du Puy	Raw	18.2 ± 0.3	3.05 ± 0.06	21.3 ± 0.2	38.7 ± 0.7	10.4 ± 0.7	24.6 ± 0.4^{t}	23.6 ± 0.3^{g}	51.8 ± 0.1^{d}
	Germination								
	G	15.4 ± 0.8	4.0 ± 0.2	13.4 ± 0.7	38.1 ± 0.2	14.6 ± 0.8	27.2 ± 0.7^{p}	14.5 ± 0.7	58.3 ± 1.4^{b}
	BP	22.9 ± 0.1	6.0 ± 0.3	13.4 ± 0.3	32.9 ± 0.1	9.73 ± 0.04	36.8 ± 0.6^{g}	17.4 ± 0.6^{o}	45.8 ± 0.1^{k}
	Fr	24.9 ± 0.1	5.05 ± 0.01	3.58 ± 0.01	42.57 ± 0.01	11.14 ± 0.01	33.9 ± 0.1^{k}	4.9 ± 0.1^{r}	61.2 ± 0.2^{a}
	Lb	23.5 ± 0.5	5.2 ± 0.2	16.6 ± 0.4	32.9 ± 0.7	7.9 ± 0.4	34.3 ± 0.8^{k}	18.3 ± 0.5^{n}	47.5 ± 1.3^{h}
	Sb	21.60 ±0.08	4.66 ± 0.08	15.7 ± 0.2	36.1 ± 0.1	8.95 ± 0.04	32.9 ± 0.2^{l}	16.8 ± 0.3^{p}	50.2 ± 0.1^{ef}
	Fr + Lb	23.8 ± 0.2	5.6 ± 0.1	16.3 ± 0.2	33.6 ± 0.2	7.0 ± 0.3	36.2 ± 0.1^{h}	16.3 ± 0.2^{pq}	47.5 ± 0.1^{h}
	Fr + Sb	30.1 ± 0.1	5.95 ± 0.02	17.67 ± 0.04	26.9 ± 0.2	6.50 ± 0.07	44.6 ± 0.2^{d}	18.4 ± 0.1^{n}	37.0 ± 0.2^{r}
	Lb + Sb	22.2 ± 0.1	4.29 ± 0.02	16.7 ± 0.1	33.5 ± 0.2	7.6 ± 0.1	35.7 ± 0.2^{hi}	18.1 ± 0.1^{n}	46.2 ± 0.3^{j}
	Fr + Lb + Sb	18.09 ± 0.02	3.50 ± 0.07	20.5 ± 0.3	36.5 ± 0.6	10.6 ± 0.2	28.5 ± 0.1^{o}	21.5 ± 0.3^{i}	50.0 ± 0.2^{fg}
	Boiling								
	B	17.58 ± 0.07	3.57 ± 0.01	21.37 ± 0.01	37.6 ± 0.1	10.38 ± 0.07	26.0 ± 0.1^{q}	23.3 ± 0.1^{gh}	50.7 ± 0.1^{ef}
	BP	17.82 ± 0.08	3.3 ± 0.1	20.30 ± 0.09	37.6 ± 0.1	11.1 ± 0.05	27.0 ± 0.1^{pq}	22.8 ± 0.1^{h}	50.2 ± 0.1^{f}
	Fr	21.3 ± 0.2	3.5 ± 0.2	20.32 ± 0.02	36.37 ± 0.06	9.2 ± 0.3	31.6 ± 0.2^{m}	22.9 ± 0.1^{h}	45.5 ± 0.2^{l}
	Lb	19.1 ± 0.1	3.5 ± 0.3	20.6 ± 0.4	35.5 ± 0.4	9.91 ± 0.06	28.4 ± 0.7^{o}	24.3 ± 0.2^{e}	47.4 ± 0.5^{hi}
	Sb	18.53 ± 0.02	3.4 ± 0.2	20.5 ± 0.5	36.12 ± 0.07	10.4 ± 0.2	27.4 ± 0.2^{p}	25.0 ± 0.5^{c}	47.6 ± 0.3^{h}
	Fr + Lb	18.9 ± 0.2	3.8 ± 0.2	19.6 ± 0.7	36.6 ± 0.1	11.4 ± 0.8	26.6 ± 0.1^{qr}	23.6 ± 0.7^{g}	49.8 ± 0.7^{g}
	Fr + Sb	15.1 ± 0.1	1.93 ± 0.01	18.8 ± 0.1	33.1 ± 0.1	8.91 ± 0.01	35.2 ± 0.1^{ij}	21.5 ± 0.1^{i}	43.3 ± 0.1^{m}
	Lb + Sb	17.8 ± 0.01	2.77 ± 0.01	20.37 ± 0.01	36.8 ± 0.1	9.57 ± 0.01	30.2 ± 0.1^{n}	21.1 ± 0.1^{j}	48.7 ± 0.1^{g}
	Fr + Lb + Sb	17.4 ± 0.5	3.0 ± 0.2	21.9 ± 0.7	34.4 ± 0.5	10.5 ± 0.2	28.5 ± 0.9^{o}	25.4 ± 0.5^{b}	46.1 ± 0.3^{jk}

In each column and variety, different letters present statistically significant differences ($p \leq 0.05$). G – germination; B – boiling; BP – baking powder; Fr –*F. rossiae*; Lb – *Lactobacillus brevis*; Sb – *Saccharomyces boulardii*; SFA – Saturated fatty acids; MUFA – Monounsaturated fatty acids; PUFA – Polyunsaturated fatty acids.

studied. This increase was also seen in palmitic and oleic acids after fermentation. For Du Puy lentils, different trends were observed. Germination alone increased stearic (4.0 %) and α-linolenic (14.6 %) acids, while boiling caused only a slight rise in stearic acid (3.57 %). In this variety, fermentation generally led to a decrease in oleic (only with prior boiling), linoleic, and α-linolenic acids compared to the raw, germinated, or boiled samples, while palmitic and stearic acid concentrations increased. Ziarno et al. (2020) examined the effects of lactic acid fermentation with pure monocultures of ten *Lactobacillus* species on the fatty acid profile of a fermented beverage made from germinated white beans (*Phaseolus vulgaris*). Their findings highlighted that fermentation with different *Lactobacillus* species altered individual fatty acid concentrations, with increases or decreases depending on the specific culture. In particular, linoleic and α-linolenic acids increased to varying degrees across all cultures, while the behavior of palmitic, stearic, and oleic acids differed depending on the microbial species used. Overall, polyunsaturated fatty acids (PUFAs) dominate across the analyzed lentil varieties and processing methods, with their concentration notably increasing after boiling and fermentation in Beluga lentils compared to their raw form. Saturated fatty acids (SFAs) are the next most prevalent, with their levels rising across all treatments and varieties, except in boiled Beluga samples. Lastly, monounsaturated fatty acids (MUFAs) show varying behavior depending on the variety, with concentrations increasing in Beluga samples but decreasing in Du Puy samples. Fermentation alters the fatty acid profile of pulses plants due to the metabolic activity of the microbial cultures involved. These microbes produce enzymes, such as lipases and esterases, that interact with lipids in the legume matrix, leading to changes in the concentration of individual fatty acids. These changes occur not only through modification of existing fatty acids but also through mechanisms like *de novo* biosynthesis and selective utilization, among others (Collins et al., 2004; Holland et al., 2005). Further studies using various pulses species and

Table 7
Mineral composition of flours from germinated and cooked Beluga and Du Puy lentils fermented with different combinations of lactic-acid bacteria (*L rossiae* and *L. brevis*) and a probiotic yeast (*S. boulardii*) (mean ± SD; $n = 3$).

Samples		Minerals							
		[K]/(g/Kg)	[Na]/(mg/Kg)	[Ca]/(g/Kg)	[Mg]/(mg/Kg)	[Fe]/(mg/Kg)	[Mn]/(mg/Kg)	[Cu]/(mg/Kg)	[Zn]/(mg/Kg)
Beluga	Raw	6.9 ± 0.4^{d}	16.8 ± 0.2^{t}	0.33 ± 0.03^{ghijkl}	815.7 ± 0.7^{n}	63.7 ± 0.7	12.9 ± 0.1^{jklmn}	7.7 ± 0.4th	89.7 ± 0.8^{l}
	Germination								
	G	5.95 ± 0.04^{gh}	17.9 ± 0.4^{s}	0.31 ± 0.02^{ijklm}	817.9 ± 0.1^{m}	68.0 ± 0.3^{y}	13.3 ± 0.1^{hijk}	7.7 ± 0.1st	107.1 ± 0.4^{b}
	BP	6.1 ± 0.5^{fg}	16.5 ± 0.4^{t}	0.28 ± 0.01^{mn}	800.2 ± 0.5^{n}	67.3 ± 0.5^{z}	12.3 ± 0.4^{o}	7.64 ± 0.01^{t}	115.2 ± 0.6^{a}
	Fr	7.0 ± 0.4^{d}	20.3 ± 0.4^{r}	0.29 ± 0.04^{lmn}	764.0 ± 0.9^{w}	68.6 ± 0.5^{y}	12.5 ± 0.5^{mno}	8.21 ± 0.02^{qr}	101.1 ± 0.3^{e}
	Lb	6.4 ± 0.6^{ef}	12.1 ± 0.3^{z}	0.27 ± 0.06^{n}	773.0 ± 0.6^{v}	79.0 ± 0.5^{s}	12.5 ± 0.2^{mno}	8.5 ± 0.3^{mnopqr}	91.4 ± 0.8^{j}
	Sb	7.5 ± 0.2^{c}	28.1 ± 0.1^{m}	0.34 ± 0.01^{defghij}	990.5 ± 0.8^{e}	80.3 ± 0.7^{r}	13.5 ± 0.1^{hi}	9.00 ± 0.08^{hijkl}	99.0 ± 0.9^{f}
	Fr + Lb	7.4 ± 0.1^{c}	26.2 ± 0.1^{o}	0.30 ± 0.04^{jklmn}	956.2 ± 0.5^{i}	74.1 ± 0.3^{w}	13.5 ± 0.1^{ghi}	9.00 ± 0.08^{ijkl}	93.8 ± 0.9^{h}
	Fr + Sb	7.4 ± 0.3^{c}	35.8 ± 0.8^{h}	0.4 ± 0.1^{cdefg}	1182.1 ± 0.4^{a}	76.3 ± 0.7^{u}	12.4 ± 0.8^{mno}	8.6 ± 0.2^{mnopq}	92.6 ± 0.2^{i}
	Lb + Sb	8.1 ± 0.6^{a}	27.4 ± 0.5^{n}	0.29 ± 0.01^{klmn}	1010.3 ± 0.7^{c}	76.4 ± 0.5^{u}	14.7 ± 0.9^{c}	9.2 ± 0.7^{ghhijk}	100.4 ± 0.2^{e}
	Fr + Lb + Sb	8.01 ± 0.07^{a}	54.4 ± 0.4^{e}	0.29 ± 0.01^{lmn}	1015.3 ± 0.6^{b}	80.4 ± 0.9^{r}	14.7 ± 0.6^{bc}	9.4 ± 0.7^{fghi}	101.9 ± 0.5^{d}
	Cooking								
	C	4.2 ± 0.6^{m}	12.3 ± 0.3^{z}	0.30 ± 0.07^{jklmn}	795.1 ± 0.5^{q}	64.3 ± 0.1	11.7 ± 0.7^{pq}	7.7 ± 0.3rd	98.0 ± 0.5^{g}
	BP	4.50 ± 0.06^{l}	10.3 ± 0.8	0.29 ± 0.01^{klmn}	758.2 ± 0.1^{y}	73.2 ± 0.3^{x}	13.0 ± 0.6^{il}	9.86 ± 0.01^{cde}	103.0 ± 0.7^{c}
	Fr	3.9 ± 0.3^{no}	12.0 ± 0.8	0.28 ± 0.04^{mn}	715.7 ± 0.4^{cc}	78.2 ± 0.7^{t}	12.2 ± 0.4^{op}	8.4 ± 0.5^{nopqr}	93.9 ± 0.4^{h}
	Lb	3.8 ± 0.4^{no}	16.5 ± 0.2^{t}	0.33 ± 0.05^{efghijk}	754.2 ± 0.1^{z}	75.6 ± 0.3^{v}	12.1 ± 0.5^{opq}	8.5 ± 0.3^{mnopqr}	93.7 ± 0.6^{h}
	Sb	5.5 ± 0.5^{i}	25.4 ± 0.6^{p}	0.33 ± 0.01^{fghijkl}	740.2 ± 0.8	82.2 ± 0.5^{q}	13.47 ± 0.04^{fghi}	10.3 ± 0.9^{ab}	97.8 ± 0.6^{g}
	Fr + Lb	4.39 ± 0.05^{lm}	14.70 ± 0.07^{v}	0.41 ± 0.02^{b}	758.0 ± 0.5^{y}	85.6 ± 0.8^{o}	14.4 ± 0.6^{cde}	9.2 ± 0.3^{ghij}	84.8 ± 0.9^{pq}
	Fr + Sb	4.6 ± 0.4^{lm}	20.5 ± 0.5^{r}	0.40 ± 0.03^{bc}	784.1 ± 0.7^{t}	84.1 ± 0.3^{p}	14.7 ± 0.4^{c}	9.9 ± 0.4^{bcd}	89.1 ± 0.2^{m}
	Lb + Sb	4.4 ± 0.3^{lm}	16.69 ± 0.02^{t}	0.31 ± 0.01^{ijklm}	836.0 ± 0.9^{l}	80.4 ± 0.5^{r}	13.5 ± 0.3^{fgh}	8.7 ± 0.2^{klmn}	71.3 ± 0.1^{v}
	Fr + Lb + Sb	4.7 ± 0.2^{kl}	22.7 ± 0.7^{q}	0.32 ± 0.01^{hijklm}	883.8 ± 0.7^{k}	84.4 ± 0.3^{p}	13.5 ± 0.4^{fghi}	9.7 ± 0.3^{def}	70.78 ± 0.04^{v}
Du Puy	Raw	4.0 ± 0.2^{no}	28.7 ± 0.4^{l}	0.33 ± 0.01^{ghijkl}	730.0 ± 0.2	88.5 ± 0.6^{n}	13.5 ± 0.5^{fgh}	8.9 ± 0.7^{jklm}	81.96 ± 0.05^{r}
	Germination								
	G	5.5 ± 0.5^{i}	29.7 ± 0.4^{k}	0.34 ± 0.03^{defghij}	731.1 ± 0.2^{bb}	89.7 ± 0.3^{m}	14.5 ± 0.5^{cd}	9.42 ± 0.07^{efghi}	85.0 ± 0.8^{pq}
	BP	6.6 ± 0.2^{de}	78.6 ± 0.7^{b}	0.22 ± 0.02^{o}	793.9 ± 0.7^{r}	103.8 ± 0.4^{e}	11.7 ± 0.4^{q}	8.2 ± 0.3^{opqr}	90.6 ± 0.2^{k}
	Fr	6.12 ± 0.08^{fg}	23.1 ± 0.1^{q}	0.29 ± 0.02^{klmn}	986.9 ± 0.5^{f}	101.1 ± 0.9^{fg}	12.4 ± 0.2^{no}	9.6 ± 0.7^{defg}	86.8 ± 0.3^{o}
	Lb	7.9 ± 0.2^{ab}	33.5 ± 0.4^{j}	0.37 ± 0.07^{bcde}	955.3 ± 0.4^{j}	120.3 ± 0.9^{a}	13.3 ± 0.8^{hij}	9.5 ± 0.6^{efgh}	84.7 ± 0.5^{q}
	Sb	7.6 ± 0.4^{bc}	35.2 ± 0.3^{i}	0.34 ± 0.04^{defghi}	928.5 ± 0.2^{j}	106.5 ± 0.6^{d}	13.9 ± 0.2^{efg}	9.3 ± 0.4^{fghij}	67.1 ± 0.1^{x}
	Fr + Lb	8.06 ± 0.01^{a}	47.8 ± 0.1^{f}	0.37 ± 0.01^{bcdef}	923.5 ± 0.6^{j}	108.7 ± 0.2^{bc}	13.2 ± 0.6^{hijkl}	9.3 ± 0.3^{fghi}	67.3 ± 0.1^{x}
	Fr + Sb	8.0 ± 0.2^{a}	63.4 ± 0.1^{c}	0.38 ± 0.03^{bcd}	958.6 ± 0.2^{h}	108.3 ± 0.6^{c}	15.9 ± 0.3^{a}	10.5 ± 0.4^{a}	72.8 ± 0.3^{t}
	Lb + Sb	7.9 ± 0.6^{ab}	61.6 ± 0.1^{d}	0.36 ± 0.04^{defgh}	971.0 ± 0.1^{g}	101.6 ± 0.7^{f}	14.8 ± 0.5^{bc}	10.2 ± 0.5^{abc}	72.2 ± 0.7^{u}
	Fr + Lb + Sb	7.9 ± 0.3^{ab}	93.08 ± 0.06^{a}	0.47 ± 0.02^{a}	995.6 ± 0.8^{d}	109.29 ± 0.03^{b}	15.2 ± 0.5^{b}	9.99 ± 0.03^{bcd}	85.5 ± 0.5^{p}
	Cooking								
	C	5.6 ± 0.2^{i}	15.58 ± 0.06^{u}	0.31 ± 0.01^{ijklm}	761.2 ± 0.3^{x}	88.80 ± 0.02^{n}	12.78 ± 0.07^{lmn}	9.1 ± 0.1^{hijkl}	65.9 ± 0.2^{y}
	BP	5.48 ± 0.03^{i}	20.5 ± 0.5^{r}	0.37 ± 0.01^{bcdefg}	798.7 ± 0.8^{p}	97.8 ± 0.7^{i}	13.5 ± 0.4^{fgh}	8.64 ± 0.03^{lmnop}	88.2 ± 0.1^{n}
	Fr	5.48 ± 0.08^{i}	12.3 ± 0.3^{yz}	0.37 ± 0.04^{bcde}	776.3 ± 0.5^{u}	101.0 ± 0.3^{g}	14.11 ± 0.01^{de}	8.5 ± 0.3^{mnopqr}	77.5 ± 0.5^{s}
	Lb	5.01 ± 0.03^{jk}	9.2 ± 0.6	0.32 ± 0.04^{hijklm}	687.7 ± 0.7^{dd}	97.9 ± 0.7^{i}	12.8 ± 0.3^{klmn}	8.2 ± 0.4^{qr}	61.2 ± 0.7^{z}
	Sb	5.5 ± 0.4^{i}	12.8 ± 0.2^{yz}	0.36 ± 0.01^{defgh}	753.0 ± 0.2^{aa}	100.0 ± 0.4^{h}	13.4 ± 0.5^{hi}	8.2 ± 0.3^{qr}	64.4 ± 0.5^{w}
	Fr + Lb	5.0 ± 0.3^{jk}	13.4 ± 0.4^{x}	0.32 ± 0.01^{hijklm}	711.6 ± 0.6^{cc}	93.6 ± 0.5^{k}	12.9 ± 0.2^{lm}	8.1 ± 0.1^{rs}	57.4 ± 0.2^{bb}
	Fr + Sb	5.06 ± 0.03^{j}	15.2 ± 0.3^{u}	0.34 ± 0.07^{defghij}	686.1 ± 0.5^{dd}	95.5 ± 0.3^{j}	14.5 ± 0.1^{cd}	8.21 ± 0.04^{pqr}	57.0 ± 0.6^{bb}
	Lb + Sb	5.7 ± 0.2^{hi}	27.6 ± 0.5^{n}	0.34 ± 0.05^{defghij}	813.6 ± 0.7^{o}	95.5 ± 0.3^{j}	13.31 ± 0.08^{hijkk}	8.5 ± 0.4^{mnopr}	58.4 ± 0.7^{aa}
	Fr + Lb + Sb	5.77 ± 0.01^{ghi}	44.5 ± 0.3^{g}	0.32 ± 0.01^{hijklm}	788.3 ± 0.2^{s}	91.9 ± 0.8^{l}	14.0 ± 0.4^{ef}	8.65 ± 0.07^{lmno}	58.4 ± 0.3^{aa}

In each column and variety, different letters present statistically significant differences ($p \leq 0.05$). G – germination; C – cooking; BP – baking powder; Fr – *F. rossiae*; Lb – *Lactobacillus brevis*; Sb – *Saccharomyces boulardii*.

microbial cultures are needed to gain a deeper understanding of the mechanisms driving these changes in the fatty acid profile of agricultural products.

3.3. Mineralogical composition

Fermentation has been shown to enhance the mineral profile of legumes, primarily by improving mineral bioavailability. This improvement stems from the breakdown of antinutritional compounds and the metabolic activities of microbial cultures. Table 7 presents the mineral composition of various raw and processed lentil varieties, highlighting statistically significant differences ($p \geq 0.05$). Among all samples analyzed, potassium (K) emerged as the most abundant mineral, with concentrations ranging from 3.8 to 8.1 g/kg. Other notable minerals included magnesium (Mg; 686.1–1182.1 mg/kg), calcium (Ca; 0.27–0.47 g/kg), zinc (Zn; 57.0–115.2 mg/kg), and iron (Fe; 63.7–120.3 mg/kg). Processing methods influenced mineral composition differently across varieties and techniques. In Beluga lentils, fermentation following germination resulted in the greatest increases in mineral concentrations, particularly zinc (Zn) and iron (Fe), which rose from 89.7 to 115.2 mg/kg and 63.7–80.4 mg/kg, respectively, compared to their raw forms. Additionally, the use of two or more microbial cultures after germination yielded higher mineral concentrations in this variety. Pre-cooking, on the other hand, notably enhanced iron content, increasing from 63.7 mg/kg in raw lentils to 85.6 mg/kg. For Du Puy lentils, prior germination followed by fermentation also resulted in significant mineral improvements, surpassing those observed in Beluga lentils. Magnesium (Mg) increased from 730.0 to 995 mg/kg, and iron (Fe) rose from 88.5 to 120.3 mg/kg, this increase being also observed when fermentation was preceded by cooking. Dhull et al. (2020) investigated the effect of fermentation of three lentil cultivars—HM-1, LL-931, and Sapna—with *Aspergillus awamori* over six days. Their findings revealed significant differences in mineral content among the cultivars, with fermented samples exhibiting higher mineral concentrations compared to their unfermented counterparts. While potassium (K) remained the most abundant mineral across all varieties in their study, similar to our findings, its concentration increased by less than 0.1 % following fermentation, unlike the more pronounced changes observed in the present study. Additionally, their results showed that copper (Cu) experienced the largest increase (+46.6 %) after fermentation, followed by zinc (Zn; +9.2 %) and iron (Fe; +8.6 %). These trends partially align with our findings, highlighting both similarities and differences in the mineral enhancements achieved through fermentation.

3.4. Antinutritional factors

Legumes are among the most essential contributors to a balanced diet worldwide, providing a rich source of both micro- and macronutrients. While lentils are well-established as a nutritious staple food, their nutritional quality can be compromised by the presence of antinutritional factors (ANFs), which reduce the bioavailability and digestibility of key nutrients such as proteins, fibers, and minerals. The presence of specific ANFs, including phytic acid, condensed tannins, and trypsin inhibitors, was assessed in both raw and processed lentil flours, with results presented in Fig. 1 and Table SM 2. Regarding phytic acid, both germination and boiling generally reduced its concentration in both lentil varieties, mainly in Du Puy one, except for a slight increase observed in Beluga samples after boiling. These findings partially align with those of a previous study (Liberal, Almeida, et al., 2024), which found that germination completely eliminated phytic acid in the same varieties, and that boiling reduced its levels. The observed variations could be due to the intrinsic physicochemical properties of the lentils, which, despite belonging to the same variety, may have been influenced by varying environmental conditions, since they belong to different harvest years, leading to changes in their behavior (Liberal et al., 2023). Fermentation, on the other hand, generally resulted in a significant reduction of phytic acid, with a few exceptions. In Beluga samples, fermentation with *F. rossiae* and *L. brevis* combined with *S. boulardii*, or after germination and boiling, did not reduce phytic acid content. Similarly, in Du Puy lentils, prior boiling followed by fermentation with *F. rossiae*, *L. brevis* combined with *S. boulardii*, or the three microbial cultures combined, also increased phytic acid levels, highlighting the limited effectiveness of these cultures in reducing phytic acid in lentil flours. However, when Du Puy lentils were pre-treated with germination, fermentation consistently decreased phytic acid concentration, with complete elimination observed during fermentation with *F. rossiae* and *L. brevis*. The decreased phytic acid concentration after germination and fermentation are related to the activity of the enzyme phytase, which is activated and produced during processing, respectively. When activated, phytase hydrolyzes phytic acid into lower inositol phosphates

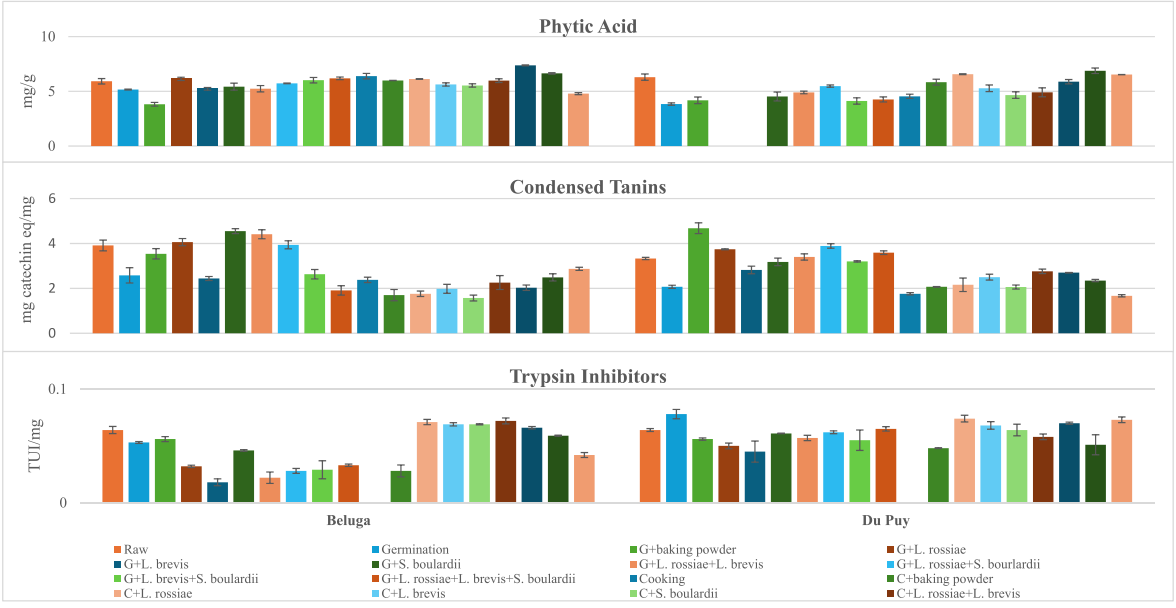

Fig. 1. Phytic acid, condensed tannins, and trypsin inhibitors composition of flours from germinated and boiled Beluga and Du Puy lentils, fermented with different combinations of lactic acid bacteria (*F. rossiae* and *L. brevis*) and a probiotic yeast (*S. boulardii*). All results show statistically significant differences ($p \leq 0.05$).

or free inositol and phosphate, lowering its concentration (Kumar & Anand, 2021). Upon cooking, this decrease may be related to the denaturation of phytic acid due to high temperatures (Godrich et al., 2023). Montemurro et al. (2019), investigated the effect of germination and fermentation with selected LAB (*F. rossiae*, *L. plantarum*, and *L. sanfranciscensis*) on the antinutrient composition of five different grains, among them lentils, also observing a decrease in phytic acid concentration upon germination alone (1.13 g/100g) and after fermentation (1.00 g/100g) when compared to their raw form (1.30g/100g). Phytic acid typically binds to essential minerals like iron, zinc, and calcium, reducing their bioavailability in the organism. However, during fermentation, microbial enzymes such as phytases, can break down these complexes. This occurs at a specific pH level, which is achieved through acid production by fermenting microorganisms, thereby reducing the concentration of phytic acid (Gibson et al., 2010).

The content of condensed tannins in both raw and processed lentil samples was also assessed (Fig. 1), revealing distinct patterns between the two varieties. In general, fermentation following germination led to a decrease in condensed tannin levels in both varieties, except when the lentils were fermented with *F. rossiae* alone or in combination with either *L. brevis* or *S. boulardii*, as well as in Beluga samples fermented with *S. boulardii* after germination. Additionally, pre-treatment with boiling resulted in greater reductions in tannin content compared to germination in both varieties, which may be related to their degradation at high temperatures (Liberal, Almeida, et al., 2024). However, while boiling followed by fermentation generally decreased tannin levels in Beluga samples compared to the boiled control, in Du Puy lentils, tannin concentrations increased after fermentation, once again emphasizing the impact of variety on the physicochemical composition of lentils. Moreover, in Beluga samples, the lowest tannin concentrations were observed after germination followed by fermentation with the three combined microbial cultures, and after boiling followed by fermentation with *S. boulardii*. In Du Puy lentils, the greatest reductions occurred with germination combined with fermentation using *L. brevis* and boiling combined with the three microbial cultures.

The reduction of tannins during germination and fermentation is attributed to the activation of enzymes like polyphenol oxidase during processing, which break down tannins, lowering their concentration (Arshad et al., 2023; Mehanni et al., 2021). De Pasquale and et al. (2020) also assessed the content of condensed tannins in both raw and fermented red and yellow lentil samples, using *Lactobacillus plantarum* and *Lactobacillus brevis* for this purpose. Although this study focused on different lentil varieties than those examined here, the authors similarly observed a reduction in tannin concentration after fermentation, emphasizing the impact of this processing method on lowering antinutrient levels. The decrease in condensed tannins during fermentation is likely due to the production of microbial enzymes, such as tannases, which hydrolyze tannins and reduce their concentration. Additionally, microbial activity may transform tannins into simpler phenolic compounds, further contributing to their reduction (Tian et al., 2019).

Protease inhibitors have been considered as antinutritional compounds due to their inhibitory activity on digestive enzymes in humans and animals (Kostekli & Karakaya, 2017). The impact of germination, boiling, and fermentation on trypsin inhibitors (TI) concentrations in both lentil varieties was examined (Fig. 1), revealing similar trends across processing methods and between varieties. Germination followed by fermentation led to a reduction in TI levels in all lentil samples. However, boiling alone completely eliminated these compounds, as thermal treatments promote the breakdown of the intermolecular bonds that holds the tertiary structure of TIs, altering their active site conformation (Avilés-Gaxiola et al., 2018). Interestingly, when boiled lentils were subsequently fermented with different microbial cultures, the concentration of trypsin inhibitors increased—not only surpassing their levels in the raw samples but also exceeding those in the boiled lentils. This increase may be related to modifications on the structure of lentil proteins that expose previously hidden trypsin inhibitor sites or create new compounds that exhibit trypsin inhibitory activity (Shi et al., 2017). Kostekli and Karakaya (2017), investigated the trypsin inhibitor activity (TIA) in wheat, rye mix, mixed cereals, and whole wheat flours, as well as in doughs and breads made from these flours. Their findings showed some similarities to ours, particularly in wheat and rye mix, where there was a significant increase in TIA between the flours and the bread (from 5.13 to 28.76 and 15.85 to 46.02 TI/mg protein, respectively), following normal bread fermentation and high-temperature baking, with no clear explanation for this increase being presented. To date, very few studies have focused on the joint influence of the action of high temperatures with subsequent fermentation with different microorganisms, and these studies are even scarcer in relation to legumes. Despite this, we can somehow assume that this increase may be related to the proteolytic activity of fermentative microorganisms, which can induce the formation of new peptides with trypsin inhibitor-like activity, and which can be erroneously quantified as TI. Furthermore, among the fermented samples, the lowest concentration of trypsin inhibitors was observed after germination and fermentation with *L. brevis* in both varieties.

3.5. Techno-functional properties

Food matrix interactions are altered or disrupted during processing, causing macronutrients like carbohydrates, proteins, and fats to undergo biochemical changes that modify their functional properties, influencing how food ingredients behave in final products (Senanayake et al., 2023). The effects of germination, boiling, and fermentation on key techno-functional properties of lentils were assessed, with the results shown in Fig. 2. Water absorption capacity (WAC) analysis revealed slight differences not only between lentil varieties but also among processing methods and microbial cultures. Overall, processing increased WAC in both varieties, with germination followed by fermentation showing the highest capacity, compared to samples subjected to prior boiling. Du Puy lentils appeared less affected by processing, as their WAC showed less improvement. During fermentation, the increase in WAC may be attributed to microbial protein hydrolysis, which exposes hydrophilic sites on proteins, enhancing water absorption (Senanayake et al., 2023). This effect was further enhanced by prior germination and boiling, as endogenous proteases activate during germination, and heat from boiling denatures proteins, exposing additional hydrophilic sites (Aguilera et al., 2011; Ghumman et al., 2016). Similar results were accomplished by Badia-Olmos et al. (2024), who studied the techno-functional properties of flours obtained from lentil grains fermented with *P. ostreatus* and verified an increase in their WAC after fermentation.

The oil absorption capacity (OAC), which indicates the amount of oil absorbed per gram of sample, exhibited more notable differences across processing methods (Fig. 2 and Table SM 3). Fermentation had the greatest impact on improving the OAC of lentils, whether they were pretreated with germination or boiling. This improvement may also be attributed to the proteolytic activity of microorganisms, which alter proteins and expose their hydrophobic sites. Lentils that underwent prior germination showed even better performance in both varieties. In Beluga lentils, samples fermented with *F. rossiae*, *L. brevis*, and their combination exhibited the highest OAC, while in Du Puy lentils, *L. brevis* and *S. boulardii* alone delivered the best results. A similar increase in OAC after fermentation was reported by Chinma et al. (2020), who observed a 39.13 % improvement in African yam beans.

Among legumes, starch is the major carbohydrate storage (Jeong et al., 2019), which are composed of amylose and amylopectin formed by polymers of -D-glucoses that are linked together, with their structure influencing the gelatinization, dextrinization, and swelling capacity of the granules (Keskin et al., 2022). The swelling capacity (SC) of both raw and processed lentil samples was analyzed (Fig. 2), highlighting the effects of processing methods and variety on this parameter. In Beluga lentils, pre-treatment with germination resulted in an increase in SC compared to their raw form. In contrast, Du Puy lentils showed a general

Fig. 2. Water and oil holding capacity, swelling, and emulsifying capacity of flours from germinated and boiled Beluga and Du Puy lentils, fermented with different combinations of lactic acid bacteria (*F. rossiae* and *L. brevis*) and a probiotic yeast (*S. boulardii*). All results show statistically significant differences ($p \leq 0.05$).

decrease in SC after germination and fermentation. Additionally, when boiling was used prior to fermentation, the SC either decreased or remained unchanged, though it still remained higher than in the raw samples. The improvement of SC after fermentation of lentils was also observed by Badia-Olmos and et al. (2024). During fermentation, the decrease in the swelling power of starch granules can be attributed to the hydrolysis of starch molecules into smaller units, such as dextrins and oligosaccharides, facilitated by organic acids and amylase. The swelling capacity of starch granules is influenced by several factors, including the original particle size of the substrate, the ratio of β-amylase to amylopectin, and the conditions of fermentation. Starches with higher levels of branched amylopectin tend to exhibit greater swelling capacity because the branching allows for more spatial bonding with water molecules (Wei et al., 2022).

Proteins play a crucial role as emulsifiers in the formation and stabilization of emulsions. When proteins interact with the oil-water interface, they quickly reduce interfacial tension, which facilitates droplet rupture, and create a viscoelastic film that helps stabilize the newly formed droplets against coalescence (Tian et al., 2022). The emulsifying capacity (EC) of the lentil samples studied was analyzed (Fig. 2), revealing significant variations among different varieties and processing methods. In Beluga lentils, fermentation following prior germination led to an increase in EC, with the highest levels achieved when fermented with *L. brevis* and *S. boulardii* alone. Conversely, when subjected to prior cooking, the EC of Beluga lentils decreased significantly, particularly when microbial cultures were combined. In the case of Du Puy samples, only germination alone resulted in an increase in EC, with little to no EC observed in the remaining treatments. The improvement of EC after fermentation may be ascribed to protein hydrolysis, which leads to the display of hydrophilic and/or hydrophobic

regions ensuing higher surface activity. The peptides can migrate easily to the oil-water interface, enhancing the emulsion stability and emulsifying capacity (Shi et al., 2021). On the other hand, the decreased EC after boiling may be related to the denaturation of proteins at high temperatures.

4. Conclusions

This study explored the impact of various processing techniques, including germination, cooking, and fermentation with different microbial cultures, individually or in combination, on the nutritional, chemical, and antinutrient profiles of two lentil varieties, as well as their techno-functional properties, with significant benefits arising from these processes. While all processing techniques influenced these parameters in both varieties, the most pronounced and consistent improvements were observed in Beluga lentils subjected to germination followed by fermentation. This approach particularly improved protein in Beluga lentils, from 26.8 g/100g dw in raw samples to 38.4 g/100g dw in those subjected to germination and fermentation with *F. rossiae* and *S. boulardii*, while also improving magnesium, iron, and zinc concentrations. Additionally, these combined processing treatments mostly reduced trypsin inhibitors and condensed tannins and improved the overall techno-functional properties of the lentils. Notably, the inclusion of *Saccharomyces boulardii* in fermentation proved to be a critical factor in achieving superior effects, highlighting its importance in future applications.

This study is the first report on the combination of different processing techniques and microbial cultures on lentils, providing pioneering insights into the potential of these methods to modulate the physicochemical and functional properties of legumes. These findings underline the significant role of integrating different processing techniques, specifically fermentation, to achieve remarkable improvements in nutrient availability, reduction of antinutritional factors, and enhancement of techno-functional properties. These results highly contribute to the knowledge of legume processing, paving the way for the development of innovative, nutrient-dense, and functional plant-based food products that meet current consumer's demands and dietary needs.

CRediT authorship contribution statement

Ângela Liberal: Writing – original draft, Validation, Software, Methodology, Investigation, Data curation. **Ângela Fernandes:** Writing – review & editing, Validation, Supervision, Investigation, Data curation, Conceptualization. **Tânia S.P. Pires:** Methodology. **Isabel C.F.R. Ferreira:** Supervision. **Ana Maria Vívar-Quintana:** Writing – review & editing, Visualization, Validation, Supervision, Conceptualization. **Lillian Barros:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Funding acquisition, Conceptualization.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fbio.2025.106720.

Data availability

Data will be made available on request.

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