

Effect of different physical pre-treatments on physicochemical and techno-functional properties, and on the antinutritional factors of lentils (*Lens culinaris* spp)

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ABSTRACT

Lentils have a valuable physicochemical profile, which can be affected by the presence of antinutrients that may impair the benefits arising from their consumption. Different treatments can be used to reduce these undesirable compounds, although they can also affect the general composition and behaviour of the lentils. Thus, the effect of different processing methods on the physicochemical and techno-functional properties, as well as on the antinutritional factors of different lentil varieties was studied. Phytic acid was eliminated during germination, while tannins and trypsin inhibitors are mostly affected by cooking. Functional properties were also altered by processing, these being dependent on the concentration of different nutrients in lentils. All the studied treatments affected the physicochemical profile of lentils and their functional properties. Cooking and germination appear to be the most effective in reducing antinutritional factors and improving the physicochemical profile of the lentils, meeting the current nutritional demands of today's society.

1. Introduction

Lentils (*Lens culinaris* spp.) are one of the oldest leguminous crops cultivated worldwide, with >70 countries listed as producers and consumers of this agricultural product, which can be consumed in its whole, dehulled, and/or split form (Nosworthy et al., 2017). Although lentils are an established nutritious leguminous, their composition and nutritional value diverge between different varieties, with these differences being mostly due to genetic influences combined with agroecological factors, production practices, and their ability to deal with the limitations of different cultivation environments (Grusak, 2009). Still, overall, raw lentils are characterized by their low-fat content (<1%), and significant amounts of carbohydrates (40–50%), including fiber (6–27%), proteins (20–30%), and essential minerals, such as iron (Fe), zinc (Zn) and selenium (Se) (Bhatty, 1988; Thavarajah et al., 2011), being a nutrient-rich option in vegetarian diets as an alternative to meat products. Additionally, over the years, health-improving benefits arising

from lentil consumption have been widely reported, with positive effects being verified in the reduction of cholesterol levels, hypertension, cardiovascular disease, diabetes, and others (Khazaei et al., 2017; Patterson et al., 2017). However, despite this, the general composition of lentils and their health benefits may be conditioned by the presence of other components, termed antinutritional factors (ANF), substances generated in food by the normal metabolism of the species and by different mechanisms through which they exert opposite effects from those of ideal nutrition (Gulewicz et al., 2014; Rochfort & Panozzo, 2007). ANFs accumulate in lentils in the pre-harvest phase, remaining until harvest and post-harvest phases (Sharma et al., 2022). These may include protease inhibitors, lectins, saponins, phytates, non-digestible oligosaccharides, and different classes of phenolic compounds, among others, which can interfere with the assimilation of proteins, carbohydrates, and enzymes, reducing the bioavailability of essential minerals and the digestibility of food (Haileslassie et al., 2016; Wang, Yang, et al., 2009). The presence of ANFs can be modulated through different processing

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methods capable of minimizing these limiting biotic factors and, consequently, providing great opportunities for the effective use of lentils as an ingredient in different food products (Sharma et al., 2022). Lentils processing can be carried out through thermal treatments, such as wet and dry heating, roasting, steaming, boiling, extrusion, microwave, and infrared heating, among others, which promote a reduction in the inhibitory activity of legumes, improve gelatinization, and water and fat retention capacity, among other features. However, undesirable effects can be also observed during heat treatments, namely protein denaturation, structural deformations, decreased emulsion capacity, soluble fiber and minerals, and others following from exposure to extreme temperatures (Baik & Han, 2012; Jeong et al., 2019; Meda et al., 2018; Pal et al., 2017). To overcome this difficulties, alternative non-heat traditional processing methods have been explored, namely elicitation, soaking, germination, and fermentation. More developed techniques include high-processing techniques, irradiation, ultrasonication, ultrafiltration, and isoelectric precipitation, which are also known to retain the quantity and quality of nutrients and antinutrients (Baik & Han, 2012; Bubelová et al., 2018; Fouad & Rehab, 2015; Patterson et al., 2017; Yadav et al., 2018). Some of these practices have been associated with the improvement of the nutritional value of leguminous by partial or total suppression of different ANFs, as well as with the increase of some nutrients, digestibility of proteins and fibers, and bioavailability of minerals, consequently enhancing the flavor and palatability and modifying the functional properties of leguminous (De Almeida Costa et al., 2006; Ghavidel & Prakash, 2007; López et al., 2017; Rehinan et al., 2004; Świeca et al., 2013). These techniques can be used alone or combined, depending on the expected outcomes. To this end, processing parameters must be adjusted to the objective of each food matrix, to accomplish a physicochemical profile suitable to consumer's demands (Asif et al., 2013). By influencing the overall composition of pulses, processing methods will, consequently, influence their techno-functional properties, which are mainly prompted by their nutritional constituents (Meda et al., 2018). Thus, their analysis makes it possible to predict and evaluate how new proteins, fats, carbohydrates (starch and sugar), and fibers may behave in specific food systems, as well as to demonstrate whether these components can be used to stimulate or replace conventional ones (Awuchi et al., 2019).

Thus, the aim of this work is to investigate the effect of different physical pre-treatment techniques, namely soaking, cooking, germination, and microwave roasting, alone and combined, on the nutritional, chemical, mineral, and techno-functional properties of different lentil flours, also assessing their influence on specific ANFs (phytates, condensed tannins, and trypsin inhibitors) that may negatively impact the overall beneficial compounds and specific characteristics of lentils, namely minerals, protein, and fiber composition. Through this study it is intended to infer which pre-treatments best preserve and/or improve the nutritional, chemical, mineral, and techno-functional properties of lentils, also resulting from the reduction of ANFs, so that they can be used as a partial replacement for plant-based meat products with improved bioavailability of minerals and biodigestibility of proteins and fibers, thus fitting into the current parameters of a healthy and balanced diet for consumers around the world.

2. Materials and methods

2.1. Samples

Lentil (*Lens culinaris* spp.) samples - Armaña, Beluga, Du Puy, and Red - were purchased (2 kg) in Spanish markets from two cities (Zamora and Salamanca). These are two regions with a high production of lentils. All samples were collected in the 2021 season and analysis was performed 1 year after harvest. The seed coat is brown, black, mottled-brown, and red, respectively, covering the whole grain. The lentils of the Red variety were marketed without seed coat. All samples were cleaned to remove foreign materials and damaged grains. After pre-

treatments, grains were reduced in size in a Foss Knifetec™ 1095 mill at a controlled temperature (20 °C) and stored until further analysis.

2.2. Pre-treatments

2.2.1. Soaking

Lentils were soaked in distilled water at a ratio of 1:5 (seed:water; w/v) (Khandelwal et al., 2010a) and 0.03% sodium bicarbonate solution (1:10) (Ibrahim et al., 2002) at room temperature for 12 h, except red lentils (4 h) (Shi et al., 2017). After soaking, the seeds were drained, washed, and left in an air oven at 50 °C until constant weight.

2.2.2. Cooking

Pre-soaked lentils (12 h in distilled water; 1:5 w/v) were placed in a beaker with distilled water (seed:water ratio 1:6.7 w/v) and boiled for 35 min at 95 °C (Vidal-Valverde et al., 1994). Afterwards, the cooking water was drained, and the seeds were left in an air oven at 50 °C until constant weight.

2.2.3. Germination

Lentils were soaked in distilled water (1:5 w/v) for 12 h at room temperature (~25 °C) and shaken every 30 min. After this period, the water was drained, and lentils were transferred to glass flasks covered with gauze and kept in the dark to germinate for 3 days (Vidal-Valverde et al., 1994). The flasks should be tilted at approximately 45° so that the remaining water drains. Every 24 h, the seeds were moistened with distilled water and carefully shaken and drained (Fouad & Rehab, 2015). Sprouted seeds were then left in an air oven at 50 °C until constant weight.

2.2.4. Microwave roasting

Lentils were roasted in a conventional microwave oven (TEKA MW 20 BF, Germany) at 900 W for 15 min. Additionally, another batch of lentil seeds was soaked in distilled water (2:1 w/v) for 45 min (15 min for Red lentils) and further subjected to the same microwave treatment (Joghalli et al., 2017).

2.3. Proximate composition

The proximate composition of the different raw and processed lentils was assessed using AOAC procedures (AOAC, 2016). In brief, crude protein concentrations were determined using the macro-Kjeldahl method (N x 6.25) using an automatic distillation and titration unit (model Pro-Nitro-A, JP Selecta, Barcelona). Soxhlet extraction was used to determine the crude fat of a known weight of each sample with petroleum ether for 7 h. Incineration at 550 ± 5 °C was used to determine ash content. Total carbohydrates were further estimated by difference: Total carbohydrates = 100 - (g ash + g proteins + g fat). The energetic value was calculated using the following equation:

$$\text{EnergyKcal}/100 \text{ g} = 4 \times (\text{g proteins} + \text{g carbohydrates}) + 9 \times (\text{g fat})$$

Total dietary fiber content was assessed through an enzymatic-gravimetric method, using α -amylase, protease, and amyloglucosidase for enzymatic digestion. Briefly, dried lentil samples were incubated with α -amylase at ~100 °C, to promote gelatinization, hydrolysis, and depolymerization of the starch, followed by further incubations at 60 °C with protease and amyloglucosidase. After that, samples were treated with four volumes of ethanol and the residues were filtered, washed with 78% and 95% ethanol and acetone, dried, and weighted. One duplicate was analyzed for protein and the other was incinerated at 525 °C to assess ash content. The results were presented in relative percentages and the total dietary fiber content were estimated using the equation:

$$\% \text{Total dietary fiber} = [(R-P-A)/SW] \times 100$$

Where:

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

2.4. Chemical composition

2.4.1. Free sugars

Free sugars were determined by high performance liquid chromatography coupled to a refraction index detector (HPLC-RI; Knauer, Smartline 1000 and Smartline 2300 systems, respectively) after an extraction procedure previously described by Spréa et al. (2020). Peaks identification and quantification were performed by comparison of their relative retention times (Rt) with authentic standards and calibration curves and using melezitose as an internal standard (IS), respectively. The obtained data were processed in Clarity Software (Data Apex, Prague, Czech Republic) and the results were expressed in g per 100 g dw.

2.4.2. Organic acids

The organic acids profile of raw and processed lentils was determined by ultra-fast liquid chromatography coupled to a photodiode array detector (UFLC-PDA; Shimadzu Corporation, Kyoto, Japan) (Barros et al., 2013). The compounds separation was done in a 18 SpherClone (Phenomenex) reverse phase column (5 µm, 250 × 4.6 mm id) thermostated at 35 °C, using 3.6 mM sulfuric acid solution as an eluent at a flow rate of 0.8 mL/min. The identification of each compound was accomplished by comparing of the obtained chromatograms with those from commercial standards, and quantification was achieved by relating the peak areas, recorded at 215 nm, with calibration curves from commercial standards of each compound. The results are presented in g per 100 g dw.

2.4.3. Tocopherols

Tocopherols were determined using a methodology described by Barros et al. (2013) using a HPLC system coupled to a fluorescence detector (P-2020; Jasco, Japan) programmed for excitation at 290 nm and emission at 330 nm. Tocopherol isoforms separation was done in a normal phase column of Polyamide II (250 mm × 4.6 mm i.d.) from YMC Waters (Japan), operating at 30 °C. The mobile phase used 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. Identification was performed by comparing the obtained chromatograms with those from authentic standards. Quantification was based on the response of the fluorescence signal, using tocol as internal standard, and by chromatographic comparison with commercial standards. The attained results were expressed in mg per 100 g dw.

2.4.4. Fatty acids

Fatty acid methyl esters (FAME) profile was assessed after transesterification of the lipid fraction obtained by Soxhlet extraction, as described by the authors (Barros et al., 2013), and determined by gas-liquid chromatography with flame ionization detection, using a YOUNG IN Chomass 6500 GC system instrument (Gyeonggi, South Korea) supplied with a split/splitless injector set at 250 °C with a 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). Identification and quantification were performed by comparing the relative retention times of the FAME peaks from lentil samples with those from commercial standards (standard mixture 47,885-U, Sigma, St Louis, MO, USA). The results were recorded and processed in Clarity DataApex 4.0 software (Prague, Czech Republic) and expressed as a relative percentage of each fatty acid.

2.5. Mineral composition

The mineral profile of the raw and processed lentils was determined following the AOAC procedure (AOAC, 2016). Samples were digested with 10 mL of nitric acid within a microwave extraction system, operating at 200 °C and 1600 watts for 30 min. The mixture was subsequently adjusted 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 investigated using atomic absorption spectrophotometry (Perkin Elmer 1100B, Waltham, MA, USA) as described by the authors (Paschoalinotto et al., 2023). The results were expressed in g per Kg for K, Ca, and Mg, and as mg per Kg for Na, Fe, Mn, Cu, and Zn.

2.6. Antinutritional factors

2.6.1. Phytic acid

Phytic acid content was assessed according to a previously described protocol (Fitriani et al., 2021). Briefly, ~0.1 g of each raw and processed lentil samples were extracted with 20 mL of HNO₃ 0.5 M and kept in a water bath with continuous shaking for 4 h at 30 °C. The extracts were then filtered through a Whatman n°4 paper and 1 mL of the obtained extract was added into 0.4 mL of distilled water, followed by 1 mL of FeCl₃ 0.005 M. The mixture was boiled for 20 min and left to cool until room temperature. Five millilitres of amyl alcohol and 0.1 mL of ammonium thiocyanate 0.1 M were added. The solution was thoroughly mixed and centrifuged at 4000g and the top layer was subsequently subjected to a UV-vis spectrophotometer at 495 nm. Standard curves were prepared using 0.4, 0.2, 0.1, 0.05, and 0.025 mg/mL of phytic acid (SIGMA-P8810) diluted with HNO₃. The equation of the standard curve was used to determine the concentration of phytic acid in the studied lentil samples, and the results are presented in mg per g dw.

2.6.2. Condensed tannins

The occurrence of condensed tannins in the studied raw and processed lentils was assessed using the “modified vanillin assay” method as described by Dykes (2019). Before sample analysis, a standard curve using 0.0, 0.2, 0.4, 0.6, 0.8, 1 mL of catechin solution in 1 mL of methanol was prepared. Tubes were placed in a water bath at 30 °C, and 5 mL of vanillin reagent was added to the first tube. After that, a timer was set to 20 min and immediately started. Another 5 mL of vanillin reagent were added to the other tubes at 30s intervals since this is a time-dependent analysis. After incubation, absorbance was measured at 500 nm. For sample analysis, ~0.3 g were weighted in triplicate, and 8 mL of HCl 1% in methanol added to each tube. The blend was thoroughly mixed in a vortex and placed in a water bath at 30 °C for 20 min. Samples were centrifuged at 4000g for 10 min. Then, 1 mL of each tube supernatant was transferred into new test tubes (one labelled as “blank” and two as “samples”) and placed again in a water bath at 30 °C. While in a water bath, 5 mL of HCl 4% in methanol were added to “blank” tubes and 5 mL of vanillin solution to “samples” tubes in 30s intervals. The absorbance of each tube was measured exactly after 20 min at 500 nm. Tannin concentration in each sample was calculated using the following equation:

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

Where:

- V – Volume of extract (mL)
- A – Absorbance at 500 nm (“sample” - “blank”)
- m – slope of the standard curve
- W – Weight of the sample (g)

2.6.3. Trypsin inhibitors

Trypsin inhibitors (TI) activity measurement was performed as previously described by Kakade et al. (1974) with some modifications

(Coscueta et al., 2017; Malomo et al., 2011). Briefly, TI extracts were prepared by mixing ~1 g of the powdered lentil samples with 50 mL of NaOH 0.01 M. The mixture was agitated for 3 h at room temperature and the resultant TI extract was subsequently subjected to centrifugation for 10 min at 4500g. The filtrate was then separated as TI extract to assess its activity. BAPNA (SIGMA-B4875) stock solution was prepared by dissolving 40 mg of BAPNA in 1 mL of dimethyl sulphoxide (DMSO), and subsequently diluted to 100 mL with 0.05 M tris-buffer pH 8.2. The resultant solution was kept at 37 °C until use. Trypsin stock solution was prepared by dissolving 4 mg of trypsin in 200 mL of HCl 0.001 M, this solution being stored in an ice bath until use. Both BAPNA and trypsin solutions must be prepared only before TI assay. TI activity assessment begin with the preparation of a control solution (BAPNA and trypsin) and sample solution (BAPNA, trypsin, and sample). The first was prepared by the addition of 2 mL of distilled water and 5 mL BAPNA into 2 mL of trypsin solution in a water bath at 37 °C for 10 min. After this period, 1 mL of acetic acid 30% was added to the mixture (stop trypsin's reaction). The solution was then centrifuged at 4500g for 10 min and the absorbance read by UV–vis spectrophotometry at 410 nm. Likewise, the samples solutions were prepared by adding trypsin and BAPNA into 2 mL of sample extract. TI results were expressed as trypsin units inhibited (TUI)/mg sample using the equation:

$$TUI / mg \text{ sample} = \frac{(A_1 - A_2) \times (100/df)}{w/df}$$

Where:

A₁ – control absorbance

A₂ – sample absorbance

w – weight of the initial sample (mg)

df – dilution factor (mL)

2.7. Tecno-functional properties

2.7.1. Water and oil holding capacity

The water and oil absorption capacity of raw and processed lentils were assessed accordingly to a previous described protocol (Wani et al., 2015) with some modifications. In brief, ~1 g of each sample was weight into 25 mL of pre-weight centrifuge tubes. For each sample, 10 mL of distilled water or sunflower oil, respectively, were added and the mixtures thoroughly mixed in a vortex for 30s. Then, the samples were allowed to stand for 30 min at 20 °C, being subsequently centrifuged at 4500g for 30 min. The water and oil released by centrifugation were drained and the tubes weighted. Results of water and oil capacities were expressed as grams of water and oil absorbed per gram of each sample.

2.7.2. Swelling capacity

The swelling capacity of the studied lentils was determined using the modified method of Yu et al. (2012). Each sample (~250 mg) was mixed with 25 mL of distilled water in pre-weighted 50 mL centrifuge tubes. These were placed in a water bath at 90 °C for 30 min with constant agitation. After this period, the solutions were left to cool until room temperature and subsequently centrifuged at 4000 g for 15 min. The supernatant was decanted, and the wet sediment fraction weighted for swelling capacity determination. The results were estimated as the ratio of wet sediment weight (g) to its dw (g).

2.7.3. Emulsifying capacity

Emulsifying capacity was determined based on the method previously described by Ahmedna et al. (1999). Briefly, ~500 mg of each lentil samples was mixed with 5 mL of distilled water in 15 mL falcon tubes. The suspension was vortexed for 30s every 5 min for 30 min. Subsequently, 3 mL of sunflower oil were added to each tube, homogenised for 2 min in a vortex, and then centrifuged at 4000 g for 20 min. Results on the emulsion capacity (%) were calculated as the ratio of the height of the emulsified phase to the height of whole mixture.

2.8. Statistical analysis

A total of twenty-three samples were analyzed for all experiments, with samples being analyzed in duplicate in each of them. The results are expressed as mean values ± standard deviation (SD). The differences between processing methods were analyzed using a one-way analysis of variance (ANOVA), followed by Tukey's significant difference post-hoc test with $\alpha = 0.05$ combined with Welch's statistic, using a SPSS v.23.0 program.

3. Results and discussion

3.1. Proximate composition

The effect of various processing methods (soaking, cooking, germination, and microwave roasting, alone and combined with soaking) on the nutritional composition of different varieties of lentils (Armaña, Beluga, Du Puy, and Red) are presented in Table 1. Overall, all processing methods exerted some influence on the analyzed variables, with statistically significant differences ($p \leq 0.05$) being identified between raw lentil samples and those subjected to processing. The results showed that the fat content of lentils significantly diminished with all processing methods when compared to their raw form. Regarding soaking and cooking, this decrease can be attributed to the diffusion of fats into the processing water (Hefnawy, 2011), while in germination, the decrease may be related to the increase in the activity of lipolytic enzymes during the process (Uvere & Orji, 2002), the increase in the hydrolysis of complex organic compounds insoluble in the seeds, the loss of solids during soaking before germination (Wang et al., 1997), and the use of fat as a source of energy for the development of the process (El-Adawy, 2002). Similar results were accomplished by Gandhi et al. (2022), who also analyzed the effect of soaking, boiling, and germination on two varieties of lentils, and by Fouad and Rehab (Fouad & Rehab, 2015), who detected a decrease in fat content over six days of germination, with the lowest value being detected on the last day of the process. Similarly, microwave roasting can also alter the physical state of fats (Meda et al., 2018), diminishing their concentration on lentil seeds, with the Armaña (0.39 ± 0.02 g/100 g dw) and Beluga (0.51 ± 0.07 g/100 g dw) varieties showing the lower concentrations among all treatments. Regarding proteins, different patterns were observed between treatments and lentil varieties. In both Armaña and Beluga samples, the protein content after germination significantly ($p \leq 0.05$) increased compared to their raw form, with the highest protein concentration being detected in this field (26.9 and 27.7 g/100 g dw, respectively), followed by also a significant protein content increase after cooking (26.1 and 27.7 g/100 g dw, respectively). The increase in protein content after germination has been attributed to the loss of carbohydrates through respiration during the process (Mahmoud & El Anany, 2014; Uppal & Bains, 2012), synthesis of new enzyme proteins, such as proteases, and compositional changes following the degradation of other constituents (Bau et al., 1997; Gujral et al., 2011). Ghumman et al. (2016) also studied the effect of germination on the protein content of three lentil varieties and their results showed a progressive increase in this parameter throughout 96 h of processing, attributing this rise to the synthesis of new enzymes, resulting in the production of some amino acids, decomposition of components and degradation of proteins into simple peptides. On the other hand, the increase in protein concentration after cooking seems to be related to the loss of minerals, soluble fibers, and sugars (Baik & Han, 2012). As for the Du Puy variety, the greatest amounts of proteins were found after the microwave roasting with soaking treatment (25.2 g/100 g dw) when compared to its raw form, contrasting with the red variety, in which the lowest protein concentration was found in the same treatment (24.5 g/100 g dw). This lowest value may be related to the Red variety itself, as well as to that this was in its peeled form, being more susceptible to heat and, consequently, promoting more alterations in its constituents. Although this treatment promotes changes in

Table 1
Proximate composition of raw and processed Armaña, Beluga, Du Puy, and Red lentil varieties (mean ± SD; n = 3).

Samples		Fat (g/100 g dw)	Proteins (g/100 g dw)	Fibers (%)	Ash (g/100 g dw)	Carbohydrates (g/100 g dw)	Energy (kcal/100 g dw)
Armaña	Raw	0.70 ± 0.02 ^A	25.7 ± 0.5 ^C	25.8 ± 1.5 ^C	2.1 ± 0.1 ^C	72.2 ± 0.4 ^C	391.6 ± 0.3 ^B
	Soaking	0.43 ± 0.03 ^D	23.9 ± 0.1 ^E	24.9 ± 3.4 ^E	1.86 ± 0.09 ^D	74.23 ± 0.05 ^A	392.6 ± 0.2 ^C
	Soaking+cooking	0.52 ± 0.04 ^C	26.1 ± 0.7 ^B	27.1 ± 0.4 ^A	1.79 ± 0.02 ^E	73.2 ± 0.5 ^B	392.8 ± 0.1 ^A
	Germination	0.60 ± 0.09 ^B	26.9 ± 0.3 ^A	25.2 ± 1.2 ^D	2.78 ± 0.04 ^A	70.3 ± 0.2 ^D	388.9 ± 0.1 ^D
	Microwave roasting	0.39 ± 0.02 ^D	25.7 ± 0.4 ^{BC}	26.7 ± 1.6 ^B	2.21 ± 0.07 ^B	72.1 ± 0.2 ^C	391.2 ± 0.2 ^C
	Microwave roasting + soaking	0.68 ± 0.06 ^A	24.3 ± 0.5 ^D	24.8 ± 0.5 ^F	2.1 ± 0.1 ^C	73.6 ± 0.4 ^{AB}	391.8 ± 0.3 ^B
Beluga	Raw	0.84 ± 0.06 ^A	25.4 ± 0.1 ^B	25.4 ± 0.9 ^E	1.64 ± 0.04 ^D	72.93 ± 0.05 ^C	393.4 ± 0.1 ^C
	Soaking	0.62 ± 0.02 ^B	24.9 ± 0.4 ^D	26.7 ± 0.7 ^D	1.55 ± 0.01 ^E	73.5 ± 0.3 ^B	393.8 ± 0.1 ^B
	Soaking+cooking	0.54 ± 0.03 ^C	27.7 ± 0.3 ^A	34.4 ± 2.8 ^A	1.0 ± 0.1 ^F	71.2 ± 0.1 ^D	396.0 ± 0.3 ^A
	Germination	0.63 ± 0.05 ^B	27.7 ± 0.4 ^A	23.0 ± 0.2 ^F	2.01 ± 0.08 ^A	71.2 ± 1.3 ^E	392.0 ± 0.3 ^F
	Microwave roasting	0.63 ± 0.06 ^B	25.2 ± 0.3 ^{BC}	28.8 ± 3.6 ^C	1.88 ± 0.07 ^B	73.7 ± 1.2 ^C	392.7 ± 0.2 ^D
	Microwave roasting + soaking	0.51 ± 0.07 ^C	22.8 ± 0.6 ^E	29.0 ± 3.5 ^B	1.82 ± 0.07 ^C	74.9 ± 1.2 ^A	392.1 ± 0.9 ^E
Du Puy	Raw	0.71 ± 0.05 ^A	24.1 ± 0.3 ^C	27.4 ± 0.2 ^C	2.21 ± 0.01 ^C	74.2 ± 0.7 ^B	391.6 ± 0.7 ^C
	Soaking	0.53 ± 0.09 ^B	23.5 ± 0.5 ^D	28.4 ± 1.5 ^B	1.8 ± 0.1 ^E	74.7 ± 0.3 ^A	393.8 ± 1.7 ^B
	Soaking+cooking	0.46 ± 0.05 ^D	24.2 ± 0.3 ^C	31.0 ± 0.6 ^A	1.07 ± 0.01 ^F	74.2 ± 0.7 ^B	394.0 ± 3.1 ^A
	Germination	0.42 ± 0.01 ^D	24.2 ± 0.1 ^C	24.8 ± 0.8 ^D	2.44 ± 0.05 ^A	73.3 ± 0.9 ^C	390.2 ± 0.1 ^E
	Microwave roasting	0.59 ± 0.07 ^C	24.9 ± 0.5 ^B	27.3 ± 2.4 ^C	2.4 ± 0.1 ^B	72.4 ± 0.1 ^D	390.6 ± 0.3 ^D
	Microwave roasting + soaking	0.65 ± 0.08 ^A	25.2 ± 0.1 ^A	27.6 ± 1.0 ^C	2.1 ± 0.2 ^D	72.22 ± 0.02 ^D	390.3 ± 1.2 ^E
Red	Raw	0.95 ± 0.02 ^A	25.5 ± 0.3 ^A	24.4 ± 0.7 ^D	2.24 ± 0.01 ^A	72.8 ± 0.4 ^C	392.6 ± 1.4 ^C
	Soaking	0.84 ± 0.03 ^C	25.1 ± 0.5 ^B	26.6 ± 3.2 ^B	1.44 ± 0.01 ^D	73.4 ± 0.5 ^{AB}	394.9 ± 1.1 ^A
	Soaking+cooking	0.95 ± 0.01 ^A	25.8 ± 0.1 ^A	25.1 ± 0.9 ^C	1.03 ± 0.09 ^E	73.1 ± 0.3 ^B	394.3 ± 2.5 ^B
	Microwave roasting	0.93 ± 0.01 ^A	24.6 ± 0.6 ^C	28.0 ± 0.9 ^A	2.13 ± 0.03 ^B	73.5 ± 0.2 ^A	391.8 ± 0.6 ^E
	Microwave roasting + soaking	0.87 ± 0.07 ^B	24.5 ± 0.2 ^C	24.2 ± 4.5 ^E	1.9 ± 0.1 ^C	73.55 ± 0.04 ^A	392.1 ± 0.2 ^D

In each column and variety, different uppercase letters present statistically significant differences ($p \leq 0.05$).

proteins, the greatest influence is exerted on amino acids during Maillard browning, without, however, altering the quality of the proteins (Tekgül Barut et al., 2023). The diminishing of the total protein content after soaking, in turn, may be attributed to the leaching of proteins into the processing water, the degree of loss being affected by the duration of treatment, type of media/solution, temperature, and pH (Acquah et al., 2021). Total dietary fibers significantly ($p \leq 0.05$) increased with cooking, the highest concentrations being identified after this process in both Armaña, Beluga, and Du Puy varieties (27.1, 34.4, and 31.0%, respectively). This increment has been related to the formation of protein-fiber complexes after possible chemical modifications induced by soaking and cooking the dry seeds (Hefnawy, 2011). In parallel, a significant increment in fiber concentration was also detected after the microwave roasting treatment in all varieties, which has been associated with the time and high temperatures reached in the seeds during processing, causing the evaporation of free water and some volatile compounds, these results being in line with other studies in different matrices (Lawal et al., 2021; Liao et al., 2019; Wiyeh et al., 2023). Contrarywise, germination tends to diminish the concentration of total dietary fibers in lentil varieties, presenting the lowest values in both Beluga and Du Puy samples (23.0 and 24.9%, respectively), this being

related to the breakdown of complex polysaccharides (celluloses and hemicelluloses) into simple sugars (Bubelová et al., 2018). Consequently, there is also a decrease in total carbohydrates, which are disrupted due to the increase in alpha-amylase activity during the germination process, with the resulting simple sugars being used by the growing seedlings during the early stages of the process (Lasekan, 1996). Regarding ashes, a significant increase was detected via the germination of lentil seeds, this being the processing method that most improved this parameter when compared to the same raw samples. Here, Armaña lentils present the highest improvement (from 2.1 to 2.78 g/100 dw), followed by Beluga (1.64 to 2.01 g/100 g dw), and Du Puy (2.21 to 2.44 g/100 g dw) varieties. The increase of ashes during germination could be due to the increase in the phytase enzyme activity, which hydrolyses the bound between proteins and minerals, releasing them and increasing their bioavailability (Narsih et al., 2012). On the other hand, when subjected to cooking after soaking, all lentil varieties experienced a big decrease in ash content, which may be related to the leaching of minerals into the processing water, the same results being verified by Baik and Han (2012) and Gandhi et al. (2022) in lentils, and by Alajaji and El-Adawy (2006) in chickpeas.

3.2. Chemical composition

The occurrence of free sugars in raw and processed Armaña, Beluga, Du Puy, and Red lentils was also analyzed and the results are presented in Table 2. Fructose and sucrose were detected in all the studied lentil samples and processing methods, with sucrose standing out as the one present in higher concentrations. Among the studied lentil varieties glucose and raffinose were also identified, except for the Armaña lentils, and trehalose, except for the Red variety, these being present in lower concentrations when compared to fructose and sucrose. Regarding fructose, glucose, and sucrose, the higher concentrations were detected after the germination process, this growth being much more noticeable in sucrose, where values vary from 2.07 to 3.93 g/100 g dw between the raw and germinated Armaña lentils, from 1.28 to 5.03 g/100 dw in the Beluga, and from 1.44 to 4.15 g/100 g dw in Du Puy lentils. Generally, the increase in free sugars during germination is directly related to the activity of alpha-amylase (Lasekan, 1996), which is responsible for the breakdown of complex carbohydrates into simple and more absorbable sugars due to the energy needs of the growing plants (De Ruiz & Bresnani, 1990; Hooda & Jood, 2003). Similar trends were observed by Fouad and Rehab (2015), who analyzed the effect of germination on the total soluble, reducing, and non-reducing sugars of lentils, their concentrations being increasingly higher throughout the six days of the process. Inversely, after cooking with prior soaking, the concentration of sugars tends to decrease, with the lowest amounts being detected after this treatment. In this field, relatively to trehalose, this was completely eliminated in Armaña and Beluga varieties, being also absent in all of the studied samples of the Red variety and detected in very low amounts in Du Puy lentils. As for raffinose, similar tendencies were observed, this being mostly present after germination and lower concentrations being detected after cooking. The decrease in free sugars after cooking has been attributed to their diffusion into the processing water (Coffigniez et al., 2018) and to further enzymatic degradation of sugars due to the existence of more beneficial conditions for the expression of alpha-galactosidase activity which, in lentils, are active in the temperature range of 20–50 °C and up to 65 °C (Çelem et al., 2009). The decrease in sugars after cooking was also observed by Wang, Hatcher, et al. (2009) in eight Canadian lentil varieties. Their results showed that cooking caused a significant ($p < 0.05$) reduction in sucrose, raffinose, and

stachyose concentrations, attributing the decrease in raffinose and stachyose to heat hydrolysis of disaccharides and monosaccharides or to the formation of other compounds. As for microwave roasting treatment, with or without prior soaking, although some of the results achieved show small reductions in the concentration of identified sugars, in some treatments and varieties of lentils these decreases are statistically significant ($p < 0.05$). Here, this loss may be related to Maillard's browning in which the heating of food products results in a reaction between sugars and amino acids, resulting in their caramelization (Meda et al., 2018).

Organic acids are mainly responsible for the sensorial characteristics of foods, and their occurrence and concentration may vary depending on the type of matrix, geographic origin, type of culture, degree of development and maturation, and type of processing (Aires et al., 2011). The concentration of the identified organic acids in the studied raw and processed lentil varieties is presented in Table 3. Oxalic and malic acids were the major organic acids found in the overall raw lentil samples, ranging from 1.48 to 1.84 g/100 g dw and from 1.62 to 2.42 g/100 g dw, respectively, followed by also good amounts of quinic and citric acids. Different concentration patterns were observed after the different processing methods, particularly after soaking and cooking. Here, the concentration of organic acids significantly ($p < 0.05$) decreased after processing when compared to raw samples, which can be due to the leaching of organic acids into the cooking water, this loss being increased with longer cooking times (Armesto et al., 2019). Additionally, some authors have reported that heat treatments can cause important modifications in cell permeability and an increase in the chemical extraction of organic compounds (Yuan et al., 2009). To some extent, the decrease in oxalic acid can be beneficial, since large amounts of this acid have been associated with the risk of kidney stones (Dolores Arias-Carmona et al., 2014) and with negative effects on some minerals absorption, since these may form insoluble oxalate complexes (Heaney & Weaver, 1989). To the best of our knowledge, this is the first report about the influence of different processing methods on the organic acids profile of lentils and other pulses. However, similar trends were observed by Armesto et al. (2019) in a study conducted on *Brassica oleracea* (galega kale), in which the authors observed a decrease in oxalic, citric, and ascorbic acids after boiling. On the other hand, after germination, the concentration of all organic acids, except citric acid,

Table 2

Free sugars composition of raw and processed Armaña, Beluga, Du Puy, and Red lentil varieties (mean \pm SD; $n = 3$).

Samples	Free sugars (g/100 g dw)						
	Fructose	Glucose	Saccharose	Trehalose	Raffinose	Total sugars	
Armaña	Raw	0.11 \pm 0.01 ^C	nd	2.07 \pm 0.08 ^C	0.30 \pm 0.01 ^A	nd	2.54 \pm 0.09 ^C
	Soaking	0.14 \pm 0.01 ^B	nd	1.99 \pm 0.01 ^D	0.29 \pm 0.01 ^B	nd	2.46 \pm 0.01 ^C
	Soaking+cooking	0.10 \pm 0.02 ^C	nd	1.52 \pm 0.09 ^F	nd	nd	1.7 \pm 0.1 ^E
	Germination	0.35 \pm 0.07 ^A	nd	3.93 \pm 0.02 ^A	nd	nd	4.34 \pm 0.08 ^A
	Microwave roasting	0.14 \pm 0.03 ^B	nd	1.33 \pm 0.08 ^B	0.21 \pm 0.01 ^C	nd	1.7 \pm 0.1 ^B
Beluga	Microwave roasting + soaking	0.13 \pm 0.01 ^{BC}	nd	1.91 \pm 0.06 ^E	0.16 \pm 0.02 ^D	nd	2.19 \pm 0.04 ^D
	Raw	0.31 \pm 0.03 ^{BC}	nd	1.28 \pm 0.04 ^B	0.29 \pm 0.06 ^A	nd	1.9 \pm 0.1 ^C
	Soaking	0.35 \pm 0.04 ^B	nd	1.00 \pm 0.01 ^C	0.16 \pm 0.03 ^C	0.16 \pm 0.03 ^D	1.7 \pm 0.1 ^E
	Soaking+cooking	0.27 \pm 0.01 ^C	nd	0.41 \pm 0.07 ^D	nd	0.07 \pm 0.03 ^E	0.8 \pm 0.1 ^F
	Germination	1.06 \pm 0.09 ^A	1.06 \pm 0.09 ^A	5.03 \pm 0.09 ^A	0.19 \pm 0.05 ^C	0.66 \pm 0.07 ^A	8.0 \pm 0.1 ^A
Du Puy	Microwave roasting	0.28 \pm 0.04 ^C	0.28 \pm 0.04 ^B	0.27 \pm 0.03 ^E	0.32 \pm 0.04 ^A	0.62 \pm 0.07 ^B	1.78 \pm 0.02 ^D
	Microwave roasting + soaking	0.27 \pm 0.07 ^C	1.03 \pm 0.09 ^A	1.30 \pm 0.01 ^B	0.25 \pm 0.02 ^B	0.56 \pm 0.06 ^C	3.4 \pm 0.1 ^B
	Raw	0.14 \pm 0.01 ^C	0.09 \pm 0.01 ^C	1.44 \pm 0.06 ^C	0.15 \pm 0.01 ^A	0.33 \pm 0.03 ^B	2.2 \pm 0.1 ^C
	Soaking	0.51 \pm 0.01 ^B	0.14 \pm 0.02 ^B	1.52 \pm 0.07 ^B	0.15 \pm 0.01 ^A	0.36 \pm 0.05 ^B	2.7 \pm 0.1 ^B
	Soaking+cooking	0.13 \pm 0.02 ^C	0.08 \pm 0.01 ^C	0.65 \pm 0.02 ^E	0.05 \pm 0.01 ^C	0.02 \pm 0.03 ^D	0.94 \pm 0.04 ^E
Red	Germination	1.00 \pm 0.07 ^A	0.25 \pm 0.06 ^A	4.15 \pm 0.05 ^A	nd	0.44 \pm 0.04 ^A	5.8 \pm 0.1 ^A
	Microwave roasting	0.10 \pm 0.03 ^D	0.23 \pm 0.01 ^A	0.16 \pm 0.09 ^F	nd	0.25 \pm 0.07 ^C	0.75 \pm 0.02 ^F
	Microwave roasting + soaking	0.12 \pm 0.01 ^C	0.10 \pm 0.03 ^C	1.31 \pm 0.01 ^D	0.13 \pm 0.01 ^B	0.33 \pm 0.04 ^B	1.99 \pm 0.01 ^D
	Raw	0.10 \pm 0.01 ^C	1.55 \pm 0.03 ^B	0.14 \pm 0.04 ^E	nd	0.43 \pm 0.04 ^A	2.22 \pm 0.02 ^C
	Soaking	0.15 \pm 0.01 ^A	0.20 \pm 0.01 ^D	0.95 \pm 0.05 ^B	nd	nd	1.31 \pm 0.04 ^D

In each column and variety, different uppercase letters present statistically significant differences ($p \leq 0.05$), nd - not detected.

Table 3

Organic acids composition of raw and processed Armuña, Beluga, Du Puy, and Red lentil varieties (mean \pm SD; $n = 3$).

Samples		Organic Acids (g/100 g dw)				Total organic acids
		Oxalic acid	Quinic acid	Malic acid	Citric acid	
Armuña	Raw	1.62 \pm 0.04 ^D	1.55 \pm 0.04 ^C	2.42 \pm 0.02 ^B	1.63 \pm 0.01 ^B	7.22 \pm 0.07 ^C
	Soaking	1.86 \pm 0.02 ^B	1.76 \pm 0.02 ^B	2.24 \pm 0.04 ^C	2.40 \pm 0.07 ^A	8.26 \pm 0.08 ^A
	Soaking+cooking	1.34 \pm 0.01 ^E	0.87 \pm 0.03 ^F	1.43 \pm 0.04 ^F	1.16 \pm 0.01 ^F	4.79 \pm 0.01 ^F
	Germination	2.33 \pm 0.04 ^A	1.90 \pm 0.02 ^A	2.60 \pm 0.03 ^A	1.33 \pm 0.01 ^E	8.16 \pm 0.09 ^B
	Microwave roasting	1.65 \pm 0.06 ^D	1.31 \pm 0.08 ^D	2.09 \pm 0.02 ^D	1.51 \pm 0.01 ^C	6.55 \pm 0.04 ^D
	Microwave roasting + soaking	1.82 \pm 0.03 ^C	1.24 \pm 0.03 ^E	1.95 \pm 0.03 ^E	1.39 \pm 0.07 ^D	6.41 \pm 0.01 ^E
	Raw	1.84 \pm 0.04 ^C	1.08 \pm 0.07 ^B	1.95 \pm 0.06 ^B	2.21 \pm 0.08 ^A	7.09 \pm 0.07 ^A
Beluga	Soaking	1.83 \pm 0.06 ^C	1.14 \pm 0.02 ^A	1.74 \pm 0.04 ^D	1.27 \pm 0.07 ^D	5.98 \pm 0.08 ^E
	Soaking+cooking	1.38 \pm 0.01 ^E	0.90 \pm 0.03 ^D	0.85 \pm 0.05 ^E	1.06 \pm 0.08 ^F	4.18 \pm 0.06 ^F
	Germination	2.45 \pm 0.01 ^A	1.12 \pm 0.01 ^A	2.25 \pm 0.02 ^A	1.21 \pm 0.03 ^E	6.87 \pm 0.03 ^B
	Microwave roasting	1.75 \pm 0.04 ^D	0.97 \pm 0.03 ^C	1.84 \pm 0.01 ^C	2.04 \pm 0.02 ^B	6.60 \pm 0.08 ^C
	Microwave roasting + soaking	1.93 \pm 0.02 ^B	0.76 \pm 0.02 ^E	1.86 \pm 0.02 ^C	1.72 \pm 0.08 ^C	6.27 \pm 0.07 ^D
	Raw	1.69 \pm 0.02 ^D	0.82 \pm 0.01 ^B	1.62 \pm 0.02 ^B	1.71 \pm 0.01 ^B	5.84 \pm 0.06 ^B
	Soaking	1.65 \pm 0.01 ^E	0.85 \pm 0.01 ^B	1.39 \pm 0.04 ^E	1.81 \pm 0.09 ^A	5.70 \pm 0.02 ^C
Du Puy	Soaking+cooking	1.27 \pm 0.01 ^F	0.42 \pm 0.06 ^D	0.58 \pm 0.03 ^F	0.71 \pm 0.06 ^E	2.99 \pm 0.09 ^E
	Germination	2.31 \pm 0.01 ^A	1.03 \pm 0.02 ^A	2.72 \pm 0.07 ^A	1.08 \pm 0.02 ^D	7.14 \pm 0.09 ^A
	Microwave roasting	1.79 \pm 0.03 ^C	0.79 \pm 0.01 ^C	1.53 \pm 0.02 ^D	0.71 \pm 0.06 ^E	4.83 \pm 0.05 ^D
	Microwave roasting + soaking	2.08 \pm 0.01 ^B	0.76 \pm 0.08 ^C	1.59 \pm 0.01 ^C	1.45 \pm 0.02 ^C	5.88 \pm 0.09 ^B
	Raw	1.48 \pm 0.04 ^B	0.74 \pm 0.01 ^A	1.99 \pm 0.01 ^A	1.75 \pm 0.01 ^A	5.96 \pm 0.03 ^A
	Soaking	1.26 \pm 0.09 ^D	0.40 \pm 0.01 ^D	1.10 \pm 0.05 ^D	0.63 \pm 0.01 ^D	3.39 \pm 0.05 ^D
	Soaking+cooking	0.74 \pm 0.01 ^E	0.22 \pm 0.02 ^E	0.37 \pm 0.01 ^E	0.37 \pm 0.05 ^F	1.62 \pm 0.03 ^E
Red	Microwave roasting	1.39 \pm 0.08 ^C	0.60 \pm 0.03 ^C	1.78 \pm 0.03 ^B	0.96 \pm 0.01 ^B	4.74 \pm 0.09 ^C
	Microwave roasting + soaking	1.80 \pm 0.01 ^A	0.64 \pm 0.01 ^B	1.75 \pm 0.02 ^C	0.69 \pm 0.04 ^C	4.78 \pm 0.04 ^B

In each column and variety, different uppercase letters present statistically significant differences ($p \leq 0.05$).

experienced an increase relatively to the raw samples. Organic acids are predominantly produced during oxidative metabolic processes, namely in the TCA and the glyoxylate cycles, the latter becoming active during the germination of seeds, which can explain the increase in oxalic, quinic, and malic acids during this process (Ma et al., 2016). The lower concentrations of citric acid, in turn, can reflect its rapid utilization during the storage reserve accumulation process (Fait et al., 2006), however, this is a topic that has been little studied and requires greater investment from the scientific community.

Vitamin E is an organic and fat-soluble compound that includes all of the natural tocopherols and tocotrienols, whose physiological activity is linked to their ability to eliminate free radicals in cell membranes and other lipid environments, preventing polyunsaturated fatty acids from undergoing lipid oxidation (Bramley et al., 2000). The tocopherol profile of the studied lentil samples, before and after processing, is presented in Table 4. Two tocopherol isoforms were detected in all samples, namely α - and γ -tocopherol, with the latter being present in larger amounts in the overall lentil varieties, ranging from 1.82 to 3.71 mg/100 g dw in the raw ones. The results achieved showed that, in general, all processing methods promoted an increase in the concentration of the two isoforms, except in the Red variety, in which a decrease in their concentration was detected after all treatments when compared with their raw form. Germination appears as the processing method that mostly increased the concentration of both tocopherol isoforms, the greatest rise being achieved by the Beluga variety regarding γ -tocopherol (from 2.42 to 4.52 mg/100 g dw), followed by Armuña (from 1.98 to 3.71 mg/100 g dw), and Du Puy varieties (from 1.82 to 3.06 mg/100 g). The influence of the germination process on the concentration of tocopherols is still unclear and little studied, not only in pulses but in the overall agricultural products. Zhang et al. (2007) studied the enrichment of tocopherols in *Brassica napus* (canola) during germination, observing that, in dark conditions, as in our study, an interconversion between the α - and γ -tocopherol isoforms occurs, this being nearly complete after 5 days of germination. This interconversion isomers may be due to the activation of the enzyme γ -methyltransferase (γ -MTF) during germination, which catalyzes the final step of the α -tocopherol synthesis pathway for which γ -tocopherol is a direct precursor (Shintani & Delapenna, 1998). However, through their results, it was possible to observe an increase in both tocopherol isoforms between the second and

third days of germination, which is in accordance with our study, where lentils were left to germinate for only three days in the dark. In parallel, important increases were also observed in the remaining treatments, specifically after soaking and cooking. Jené and Munné-Bosch (2023) studied the influence of the cultivar, origin, and processing on the tocopherol composition of different varieties of lentils. Although these authors detected the same isoforms as in our study, their concentration greatly varies among the different varieties and after processing. Their results showed a decrease in γ -tocopherol contents after boiling in Castellana and Pardina varieties (55% and 32%, respectively), an increase of the same isoform in the Red variety, and no significant differences in Beluga and Verdina lentils. Also, important differences considering cooking time were observed, with increased processing times being linked with lower tocopherol concentrations. Regarding microwave roasting, the increased concentrations of tocopherols, mainly that without prior soaking, may be related to their release by disruption of the membrane and the bond between tocopherols and phospholipids or proteins (Vaidya & Eun, 2013).

Although lentils are a characteristically low-fat agricultural product, with concentrations usually around 1 g/100 g dw (Liberal et al., 2023, 2024, 2021), their profile in fatty acids methyl esters (FAME) upholds an interesting composition in compounds of great interest to human health and well-being. The effect of soaking, cooking, germination, and microwave roasting on the FAME of different varieties of lentils has been investigated and the results are expressed in Table 5 (Suppl. 1). Different patterns were detected not only among processing methods but also between the different varieties. Linoleic acid (C18:2n6c) was the predominant fatty acid found in all lentil varieties, ranging from 27.7 to 42.2% in the raw samples, except Armuña, in which oleic acid prevails (C18:1n9c). Slightly different results were accomplished by our investigation group in a previous study (Liberal et al., 2023), in which linoleic acid appears as the major fatty acid in Armuña lentils. However, through the same study, we were able to detect the influence of the harvest year on the FAME profile of this lentil variety, which could be the reason for the small divergence observed. Interesting concentrations of α -linolenic, palmitic, and stearic acids were also found in all lentil samples and among different processing techniques. During germination, the concentration of palmitic (C16:0) and stearic (C18:0) acids experienced a decrease in their concentrations except in Du Puy, which remains equal

Table 4
Tocopherols composition of raw and processed Armaña, Beluga, Du Puy, and Red lentil varieties (mean ± SD; n = 3).

Samples		Tocopherols (mg/100 g dw)		
		α-tocopherol	γ-tocopherol	Total tocopherols
Armaña	Raw	0.15 ± 0.01 ^F	1.98 ± 0.05 ^F	2.13 ± 0.06 ^D
	Soaking	0.21 ± 0.02 ^E	3.20 ± 0.10 ^D	3.40 ± 0.10 ^C
	Soaking+cooking	0.26 ± 0.08 ^D	3.30 ± 0.10 ^C	3.58 ± 0.04 ^B
	Germination	0.51 ± 0.02 ^A	3.71 ± 0.04 ^B	4.21 ± 0.06 ^A
	Microwave roasting	0.28 ± 0.01 ^C	3.18 ± 0.02 ^E	3.46 ± 0.01 ^C
Beluga	Microwave roasting + soaking	0.33 ± 0.08 ^B	3.87 ± 0.04 ^A	4.20 ± 1.10 ^A
	Raw	0.17 ± 0.02 ^C	2.42 ± 0.08 ^C	2.59 ± 0.06 ^D
	Soaking	0.14 ± 0.02 ^{CD}	3.09 ± 0.01 ^B	3.22 ± 0.01 ^B
	Soaking+cooking	0.08 ± 0.01 ^E	3.33 ± 0.01 ^F	3.41 ± 0.01 ^F
	Germination	0.66 ± 0.09 ^A	4.52 ± 0.08 ^A	5.19 ± 0.01 ^A
Du Puy	Microwave roasting	0.37 ± 0.02 ^B	2.30 ± 0.08 ^D	2.67 ± 0.09 ^C
	Microwave roasting + soaking	0.12 ± 0.01 ^D	2.03 ± 0.05 ^E	2.15 ± 0.06 ^E
	Raw	0.10 ± 0.02 ^D	1.82 ± 0.05 ^F	1.92 ± 0.07 ^F
	Soaking	0.12 ± 0.02 ^D	2.63 ± 0.04 ^D	2.76 ± 0.02 ^E
	Soaking+cooking	0.12 ± 0.02 ^D	3.70 ± 0.05 ^A	3.82 ± 0.05 ^B
Red	Germination	0.87 ± 0.01 ^A	3.06 ± 0.01 ^C	3.93 ± 0.01 ^A
	Microwave roasting	0.43 ± 0.05 ^B	2.38 ± 0.06 ^E	2.80 ± 0.10 ^D
	Microwave roasting + soaking	0.20 ± 0.04 ^C	3.10 ± 0.06 ^B	3.30 ± 0.02 ^C
	Raw	0.26 ± 0.01 ^A	3.71 ± 0.01 ^A	3.97 ± 0.01 ^A
	Soaking	0.04 ± 0.01 ^D	1.15 ± 0.02 ^D	1.19 ± 0.04 ^D
Red	Soaking+cooking	0.01 ± 0.01 ^D	0.54 ± 0.02 ^E	0.55 ± 0.02 ^E
	Microwave roasting	0.18 ± 0.01 ^B	3.20 ± 0.05 ^B	3.39 ± 0.05 ^B
	Microwave roasting + soaking	0.10 ± 0.01 ^C	2.07 ± 0.01 ^C	2.17 ± 0.03 ^C

In each column and variety, different uppercase letters present statistically significant differences ($p \leq 0.05$).

when compared to the raw sample (16.3%). This decrease in palmitic and stearic acids may be related to lipolytic activity and to breakdown of triglycerides and polar lipids into simpler compounds during germination (Al-Taher & Nemzer, 2023). Here, a limited hydrolysis of fat occurs, which releases one or two fatty acids from glycerin generating two diglycerides or mono-glycerides, which can be used directly for energy (Jan et al., 2018). Inversely, an increasing trend in concentrations of oleic, linoleic, and α-linolenic acids was detected upon germination, this increase being mainly related to the needs for the seedlings to establish membrane structures, mainly composed by polyunsaturated fatty acids (PUFAs), for cell growth and development during this process. As the seed germinates and the embryo begins to grow, new cell membranes are synthesized to support the expanding and dividing cells (Bareke, 2018). On the other hand, despite heating may potentially degrade fatty acids, our results showed that most lentil samples increased their concentration regarding oleic, linoleic, and α-linolenic acids, which can be explained by the coexistence of tocopherols, which concentrations also increased during cooking, and their ability to prevent lipid peroxidation by acting as peroxy radical scavengers that end chain reactions in membranes and lipoprotein units (Traber & Atkinson, 2007). Similar

results were accomplished by Zhang et al. (2014) and Alkaltham et al. (2022), who studied the effect of cooking and germination on the fatty acids profile of lentils. PUFAs remain the main class of fatty acids in the analyzed lentil samples, before and after processing (37.8 to 60.6%), followed by monounsaturated fatty acids (MUFAs; 17.5 to 32.8%) and saturated fatty acids (SFAs; 17.2 to 31.7%). The potential health benefits of PUFAs include the prevention of some acute and chronic diseases, such as cardiovascular issues (Alkaltham et al., 2022), which makes lentils a highly valuable pulse for food applications.

3.3. Mineral composition

The mineral profile of pulses is highly dependent on their variety, growing conditions, processing, and other features. Although pulses, and particularly lentils, are considered a rich source of minerals, the consumption of 100 g of their raw form did not reach the daily recommended intake (RDA) (Avezum et al., 2023). Several differences were detected in the identified minerals of the studied lentil samples, not only between varieties but also regarding the applied processing methods, and the results are presented in Table 6. Among the raw samples, calcium (Ca) was the prevalent mineral, with concentrations ranging from 205.1 to 556 mg/kg, followed by also high concentrations of sodium (Na; 36.0 to 533.9 mg/kg), zinc (Zn; 59.4 to 88.6 mg/kg), magnesium (Mg; 58.3 to 87.4 mg/kg), and iron (Fe; 68.1 to 72.7 mg/kg), and lower contents of manganese (Mn; 11.5 to 14.8 mg/kg), copper (Cu; 6.6 to 10.6 mg/kg), and potassium (K; 6.8 to 8.0 mg/kg). After germination, the concentration of some minerals increased significantly ($p < 0.05$) when compared to their raw form, these increases being also influenced by the variety of the studied lentils. Fe and Zn were the ones that most increased after this process, namely on Beluga and Du Puy varieties, their concentration rising from 67.3 to 83.5 mg/kg and 79.7–93.0 mg/kg, respectively regarding Fe, and from 59.4 to 72.0 mg/kg and 73.0–86.3 mg/kg, respectively, regarding Zn. In these varieties, it was also possible to detect increased concentrations of Ca, Mn, and Cu after germination. On the other hand, in the same conditions, only Ca e Mg increases after the germination of Armaña lentils, which evidences the influence of variety on the concentration of minerals. The improvement of minerals after germination has been linked with the activation, during this process, of the phytase enzyme, which degrades phytate, a compound that is responsible for a strong chelating affinity with cations such as Zn^{2+} , $Fe^{2+/3+}$, Ca^{2+} , Mn^{2+} , and Cu^{2+} , reducing their solubility and availability to be absorbed by the human organism (Benincasa et al., 2019). In this sense, as the germination process progresses, phytate content decreases, improving the bioavailability of key minerals, such as Fe, essential for the maintenance of human health and well-being, and enhancing the nutritional value of germinated lentils and other pulses. The concentration of K and Na, in turn, decreases in all varieties after this process, indicative of the influence of germination in this effect. Gandhi et al. (2022) also analyzed the influence of germination on the mineral content of two varieties of lentils, and their results showed that, after processing, the concentration of the overall detected minerals increased, which is not completely in accordance with our results. These differences may be attributed not only to the different varieties of lentils used in both studies but also concerning the different conditions of germination.

Similarly, after soaking and cooking, the concentration of most of the detected minerals decreased, with only slight increases being detected in the Na and Ca content of the Beluga and Red varieties, in the Mg of Beluga, and in the Fe and Cu concentrations of Du Puy and Red varieties, highlighting, once again, the influence of the variety on the overall concentrations of minerals. Similar trends were also observed by Alkaltham et al. (2022), who also only detected some small improvements in the mineral content of lentils after boiling, namely in Na, Ca, Cu, and Mn. These authors connected the decrease of minerals after cooking with their possible leaching into the processing water. Regarding microwave roasting, with or without prior soaking, several

Table 5

Fatty acids methyl esters composition of raw and processed Armuña, Beluga, Du Puy, and Red lentil varieties (mean ± SD; n = 3).

Samples	C16:0	C18:0	C18:1n9c	C18:2n6c	C18:3n3	SFA	MUFA	PUFA	
Armuña	Raw	19.2 ± 0.6	6.2 ± 0.1	30.5 ± 0.2	27.7 ± 0.4	10.1 ± 0.4	31.7 ± 0.3 ^A	30.5 ± 0.2 ^B	37.8 ± 0.1 ^F
	Soaking	16.7 ± 0.3	4.8 ± 0.3	30.2 ± 0.2	35.6 ± 0.1	9.51 ± 0.08	24.7 ± 0.1 ^B	30.2 ± 0.2 ^B	45.1 ± 0.1 ^E
	Soaking+cooking	14.2 ± 0.7	3.4 ± 0.2	30.0 ± 0.1	38.5 ± 0.6	10.9 ± 0.4	20.7 ± 0.9 ^F	29.9 ± 0.9 ^B	49.4 ± 0.1 ^B
	Germination	11.4 ± 0.3	3.4 ± 0.3	30.56 ± 0.05	40.9 ± 0.2	11.4 ± 0.2	17.2 ± 0.1 ^F	30.6 ± 0.1 ^B	52.3 ± 0.1 ^A
	Microwave roasting	16.4 ± 0.7	3.35 ± 0.06	31.5 ± 0.6	35.3 ± 0.2	10.13 ± 0.02	21.7 ± 0.7 ^D	32.8 ± 0.5 ^A	45.5 ± 0.2 ^D
	Microwave roasting + soaking	17.1 ± 0.6	3.6 ± 0.4	26.4 ± 0.2	37.7 ± 0.4	10.3 ± 0.6	23.8 ± 0.01 ^C	28.2 ± 0.8 ^C	48.0 ± 0.2 ^C
Beluga	Raw	16.5 ± 0.4	2.8 ± 0.1	21.5 ± 0.6	41.5 ± 0.3	10.48 ± 0.05	24.1 ± 0.1 ^B	21.0 ± 0.3 ^D	52.0 ± 0.3 ^E
	Soaking	14.24 ± 0.06	2.57 ± 0.01	20.7 ± 0.3	40.4 ± 0.2	11.1 ± 0.1	24.9 ± 0.1 ^A	25.8 ± 0.1 ^A	54.2 ± 0.3 ^B
	Soaking+cooking	12.9 ± 0.7	1.78 ± 0.01	22.0 ± 0.2	40.5 ± 0.1	10.7 ± 0.2	20.8 ± 0.1 ^D	19.0 ± 0.9 ^E	53.2 ± 0.3 ^D
	Germination	9.6 ± 0.6	1.73 ± 0.03	17.3 ± 0.9	45.9 ± 0.1	12.3 ± 0.6	20.4 ± 0.5 ^D	23.3 ± 0.1 ^B	60.6 ± 0.4 ^A
	Microwave roasting	12.67 ± 0.01	2.58 ± 0.01	19.95 ± 0.02	39.93 ± 0.05	11.95 ± 0.04	25.9 ± 0.1 ^A	23.5 ± 0.4 ^B	51.9 ± 0.1 ^E
	Microwave roasting + soaking	12.25 ± 0.01	2.72 ± 0.04	19.2 ± 0.5	40.4 ± 0.4	10.9 ± 0.7	22.9 ± 0.1 ^C	20.7 ± 0.1 ^C	53.6 ± 0.4 ^C
Du Puy	Raw	16.3 ± 0.2	2.75 ± 0.07	16.5 ± 0.1	37.6 ± 0.4	12.09 ± 0.02	27.5 ± 0.4 ^B	20.9 ± 0.3 ^B	51.7 ± 0.4 ^C
	Soaking	15.7 ± 0.4	2.43 ± 0.06	17.2 ± 0.3	37.5 ± 0.5	12.1 ± 0.2	26.1 ± 0.9 ^D	20.9 ± 0.2 ^B	53.1 ± 0.6 ^B
	Soaking+cooking	16.7 ± 0.2	2.2 ± 0.1	17.2 ± 0.2	38.2 ± 0.3	12.4 ± 0.2	27.3 ± 0.3 ^B	17.5 ± 0.1 ^D	51.8 ± 0.6 ^C
	Germination	16.3 ± 0.4	1.9 ± 0.1	16.02 ± 0.02	37.6 ± 0.4	15.8 ± 0.5	28.1 ± 0.4 ^A	20.2 ± 0.1 ^C	54.4 ± 0.2 ^A
	Microwave roasting	16.4 ± 0.4	2.09 ± 0.02	17.4 ± 0.1	37.2 ± 0.4	15.7 ± 0.3	26.5 ± 0.3 ^C	17.6 ± 0.2 ^D	53.2 ± 0.1 ^B
	Microwave roasting + soaking	15.7 ± 0.4	2.2 ± 0.1	15.7 ± 0.3	38.2 ± 0.2	13.7 ± 0.6	28.2 ± 0.5 ^A	22.2 ± 0.2 ^A	54.2 ± 0.6 ^D
Red	Raw	23.8 ± 0.1	4.2 ± 0.3	19.8 ± 0.2	42.2 ± 0.6	0.2 ± 0.1	30.7 ± 0.1 ^B	26.7 ± 0.5 ^A	42.6 ± 0.6 ^D
	Soaking	20.6 ± 0.4	3.1 ± 0.2	19.3 ± 0.2	37.3 ± 0.3	6.8 ± 0.3	31.6 ± 0.2 ^A	22.2 ± 0.2 ^B	46.2 ± 0.1 ^C
	Soaking+cooking	18.3 ± 0.5	2.8 ± 0.1	18.1 ± 0.1	41.5 ± 0.3	8.1 ± 0.5	27.8 ± 0.4 ^E	19.8 ± 0.1 ^D	52.3 ± 0.3 ^A
	Microwave roasting	19.6 ± 0.2	2.9 ± 0.2	17.8 ± 0.3	44.01 ± 0.04	6.8 ± 0.3	28.4 ± 0.1 ^D	19.3 ± 0.3 ^E	52.3 ± 0.3 ^A
	Microwave roasting + soaking	15.6 ± 0.3	3.0 ± 0.1	17.91 ± 0.06	41.4 ± 0.1	7.20 ± 0.01	29.7 ± 0.2 ^C	20.1 ± 0.1 ^C	50.1 ± 0.2 ^B

In each column and variety, different uppercase letters present statistically significant differences ($p \leq 0.05$). C16:0 – palmitic acid; C18:0 – stearic acid; C18:1n9c – oleic acid; C18:2n6c – linoleic acid; C18:3n3 – α -linolenic acid; SFA – Saturated fatty acids; MUFA – Monounsaturated fatty acids; PUFA – Polyunsaturated fatty acids.

Table 6

Mineral composition of raw and processed Armuña, Beluga, Du Puy, and Red lentil varieties (mean ± SD; n = 3).

Samples	[K]/(g/Kg)	[Na]/(mg/Kg)	[Ca]/(mg/Kg)	[Mg]/(mg/Kg)	[Fe]/(mg/Kg)	[Mn]/(mg/Kg)	[Cu]/(mg/Kg)	[Zn]/(mg/Kg)	
Armuña	Raw	8.0 ± 0.1 ^{AB}	533.9 ± 0.7 ^B	556.1 ± 0.1 ^B	66.7 ± 0.1 ^{DE}	66.9 ± 0.8 ^C	13.9 ± 0.9 ^B	10.6 ± 0.5 ^A	85.4 ± 0.2 ^B
	Soaking	5.7 ± 0.6 ^C	679.9 ± 0.3 ^A	353.3 ± 0.7 ^F	67.2 ± 0.3 ^D	83.4 ± 0.5 ^A	15.6 ± 0.1 ^A	10.3 ± 0.5 ^A	87.7 ± 0.2 ^A
	Soaking+cooking	3.9 ± 0.2 ^D	110.9 ± 0.8 ^D	434.2 ± 0.6 ^D	67.2 ± 0.3 ^D	62.7 ± 0.2 ^D	13.7 ± 0.3 ^B	5.5 ± 0.07 ^E	68.8 ± 0.7 ^F
	Germination	8.55 ± 0.04 ^A	450.5 ± 0.1 ^C	580.2 ± 0.6 ^A	87.7 ± 0.7 ^B	55.7 ± 0.3 ^F	12.0 ± 0.1 ^C	7.4 ± 0.2 ^D	70.3 ± 0.1 ^E
	Microwave roasting	7.9 ± 0.5 ^B	68.8 ± 0.7 ^E	483.0 ± 0.7 ^C	88.3 ± 0.1 ^A	61.5 ± 0.7 ^E	10.8 ± 0.5 ^E	8.0 ± 0.4 ^B	74.2 ± 0.5 ^D
	Microwave roasting + soaking	8.1 ± 0.5 ^{AB}	64.0 ± 0.5 ^F	364.9 ± 0.7 ^E	74.8 ± 0.7 ^C	72.9 ± 0.8 ^B	11.8 ± 0.1 ^D	7.7 ± 0.9 ^C	76.6 ± 0.4 ^C
Beluga	Raw	7.0 ± 0.4 ^A	82.3 ± 0.1 ^C	405.6 ± 0.5 ^D	63.5 ± 0.6 ^D	67.3 ± 0.1 ^E	11.5 ± 0.8 ^C	7.6 ± 0.4 ^B	59.4 ± 0.7 ^E
	Soaking	6.64 ± 0.04 ^A	106.1 ± 0.8 ^B	416.6 ± 0.8 ^C	65.1 ± 0.3 ^D	71.4 ± 0.6 ^D	12.4 ± 0.4 ^{BC}	6.6 ± 0.5 ^C	62.2 ± 0.2 ^D
	Soaking+cooking	4.5 ± 0.2 ^B	110.5 ± 0.4 ^A	679.7 ± 0.8 ^A	69.4 ± 0.9 ^C	62.4 ± 0.2 ^F	9.7 ± 0.7 ^D	6.35 ± 0.06 ^C	51.5 ± 0.2 ^F
	Germination	7.1 ± 0.6 ^A	64.0 ± 0.5 ^E	395.9 ± 0.7 ^E	64.6 ± 0.3 ^D	83.5 ± 0.9 ^A	14.3 ± 0.3 ^A	9.74 ± 0.03 ^A	79.0 ± 0.4 ^A
	Microwave roasting	7.5 ± 0.2 ^A	69.29 ± 0.01 ^D	432.8 ± 0.8 ^B	72.1 ± 0.5 ^B	75.9 ± 0.7 ^C	11.8 ± 0.7 ^{BC}	8.0 ± 0.2 ^B	64.2 ± 0.5 ^C
	Microwave roasting + soaking	6.6 ± 0.1 ^A	56.6 ± 0.1 ^F	382.6 ± 0.1 ^F	97.7 ± 0.4 ^A	79.9 ± 0.3 ^B	12.7 ± 0.7 ^B	9.5 ± 0.2 ^A	71.8 ± 0.7 ^B
Du Puy	Raw	8.0 ± 0.6 ^{AB}	107.4 ± 0.3 ^B	362.4 ± 0.7 ^D	87.4 ± 0.1 ^C	72.7 ± 0.8 ^C	14.78 ± 0.04 ^B	6.6 ± 0.5 ^{BC}	73.0 ± 0.3 ^C
	Soaking	6.6 ± 0.7 ^C	111.4 ± 0.7 ^A	457.4 ± 0.6 ^A	92.8 ± 0.5 ^B	89.8 ± 0.8 ^B	19.9 ± 0.3 ^A	7.6 ± 0.6 ^{AB}	92.2 ± 0.1 ^A
	Soaking+cooking	2.9 ± 0.6 ^D	47.8 ± 0.5 ^D	361.2 ± 0.2 ^D	71.0 ± 0.5 ^E	91.9 ± 0.8 ^{AB}	18.8 ± 0.5 ^A	5.0 ± 0.1 ^D	67.3 ± 0.6 ^D
	Germination	6.9 ± 0.1 ^{BC}	51.9 ± 0.7 ^C	415.5 ± 0.7 ^C	72.7 ± 0.1 ^D	93.0 ± 0.4 ^{AB}	19.6 ± 0.1 ^A	7.8 ± 0.3 ^A	86.3 ± 0.5 ^B
	Microwave roasting	9.1 ± 0.1 ^A	29.4 ± 0.9 ^E	427.1 ± 0.5 ^B	102.6 ± 0.6 ^A	94.7 ± 0.4 ^A	17.6 ± 0.1 ^A	7.5 ± 0.7 ^{AB}	83.8 ± 0.1 ^B
	Microwave roasting + soaking	8.7 ± 0.3 ^A	25.6 ± 0.1 ^F	454.3 ± 0.5 ^A	89.3 ± 0.1 ^{BC}	69.2 ± 0.6 ^C	13.0 ± 0.5 ^C	5.89 ± 0.01 ^{CD}	62.8 ± 0.1 ^E
Red	Raw	6.8 ± 0.2 ^B	36.0 ± 0.3 ^B	205.1 ± 0.4 ^D	58.3 ± 0.3 ^C	68.1 ± 0.3 ^C	13.4 ± 0.2 ^{AB}	10.1 ± 0.2 ^A	88.6 ± 0.6 ^A
	Soaking	5.0 ± 0.3 ^C	35.7 ± 0.1 ^B	235.8 ± 0.3 ^B	53.9 ± 0.1 ^D	82.6 ± 0.9 ^B	12.3 ± 0.5 ^B	7.3 ± 0.3 ^C	73.6 ± 0.2 ^C
	Soaking+cooking	2.0 ± 0.3 ^D	51.2 ± 0.6 ^A	263.2 ± 0.4 ^A	37.5 ± 0.7 ^E	87.9 ± 0.6 ^A	14.1 ± 0.6 ^A	4.4 ± 0.1 ^D	73.0 ± 0.8 ^C
	Microwave roasting	8.8 ± 0.2 ^A	28.3 ± 0.1 ^C	262.8 ± 0.8 ^A	71.4 ± 0.2 ^A	57.3 ± 0.2 ^E	12.3 ± 0.4 ^B	8.1 ± 0.4 ^B	74.0 ± 0.5 ^C
	Microwave roasting + soaking	7.19 ± 0.08 ^B	15.5 ± 0.6 ^D	218.2 ± 0.5 ^C	64.0 ± 0.5 ^B	65.0 ± 0.1 ^D	11.8 ± 0.5 ^C	7.7 ± 0.1 ^{BC}	83.8 ± 0.8 ^B

In each column and variety, different uppercase letters present statistically significant differences ($p \leq 0.05$).

improvements in the overall concentration of minerals were observed, such as Ca, Mg, and others, with also slight differences between the different lentil varieties in study. In this field, this increase is followed by thermal damage of antinutritional factors which complexes with minerals, leading to their release during roasting (Mohamed Ahmed et al., 2020).

3.4. Antinutritional factors

The presence of antinutrients in lentils limits, to some extent, their widespread consumption by the general population and their use in the food industry (Kumar et al., 2013). These compounds are produced in lentils at the pre-harvest stage, staying until the harvest and post-harvest phases, directly or indirectly interfering with the bioaccessibility and

bioavailability of nutrients in lentils (Sharma et al., 2022). The effect of processing on some of the main antinutrients occurring in lentils, namely phytic acid, condensed tannins, and trypsin inhibitors, are presented in Fig. 1. Overall, lentils belonging to the Red variety present lesser concentrations of both of the analyzed antinutrients since these are also present in the seed coat of lentils (Wang, Hatcher, et al., 2009). Regarding phytic acid, all processing methods significantly ($p < 0.05$) decreased its concentration in all the analyzed lentil varieties, mainly after germination, in which phytic acid was eliminated. Phytic acid (myoinositol 1,2,3,4,5,6 – hexakisdi-hydrogenphosphate) represents a group of phosphorous compounds that are stored in the aleurone layer and in scutellum cells of legumes, grains, and vegetables (Ali & Elozeiri, 2017), being responsible for a strong chelation affinity with different cations, proteins, and starch, reducing their solubility and bioavailability for human absorption. The decline in phytic acid concentration after germination has been attributed to its degradation by the endogenous phytase enzyme which is activated during this process (Egli et al., 2002; Fouad & Rehab, 2015). Rico et al. (2021) also reported a decrease in the phytic acid content of lentils after germination, stating that both temperature and time of germination exerted quadratic, linear, and interaction effects on this parameter. These authors observed that time caused a more distinct effect than temperature in phytic acid concentration, which decreased as germination time increased, especially at high temperatures. Soaking and cooking also significantly reduced the content of phytic acid in all lentil varieties, this effect being more pronounced after cooking. Although phytates are heat-stable and therefore do not degrade during cooking, in the early steps of this process, the

activation of endogenous phytases or phosphatases could be responsible for the decrease in phytic acid (Feizollahi et al., 2021). Similar results were accomplished by Wang, Hatcher, et al. (2009), who detected reduced concentrations of phytic acid in several varieties of lentils after cooking. Reduced concentrations of phytic acid were also recorded after microwave roasting, this decrease being more evident with prior soaking, except for Armuña lentils. Different reasons have been pointed to explain the effect of microwave roasting in reducing the concentration of phytic acid in grains, with some authors attributing it to the formation of insoluble complexes between phytic acid and other components such as phytate-protein and phytate-protein-mineral complexes (Deng et al., 2015), to decreased water extractability of phytate caused by heating processes (Xu et al., 2016), or to low levels of inositol and inositol phosphate due to the effects of free radicals produced during irradiation (Kala & Mohan, 2012).

Condensed tannins are water-soluble phenolic compounds that interfere with the digestion of proteins by inactivating several digestive enzymes, establishing multiple hydrogen bonding between tannin's hydroxyl group and protein's carbonyl group, giving rise to irreversible and reversible tannin-protein complexes (Joye, 2019). The effect of processing on the concentration of condensed tannins in different lentil varieties is presented in Fig. 1. Unlike phytic acid, not all processing methods were efficient in reducing the content of condensed tannins, with substantial increases being observed after soaking and microwave roasting, with or without prior soaking, of the vast majority of the samples when compared to their raw form. Our results are not in agreement with most studies in lentils, in which the concentration of

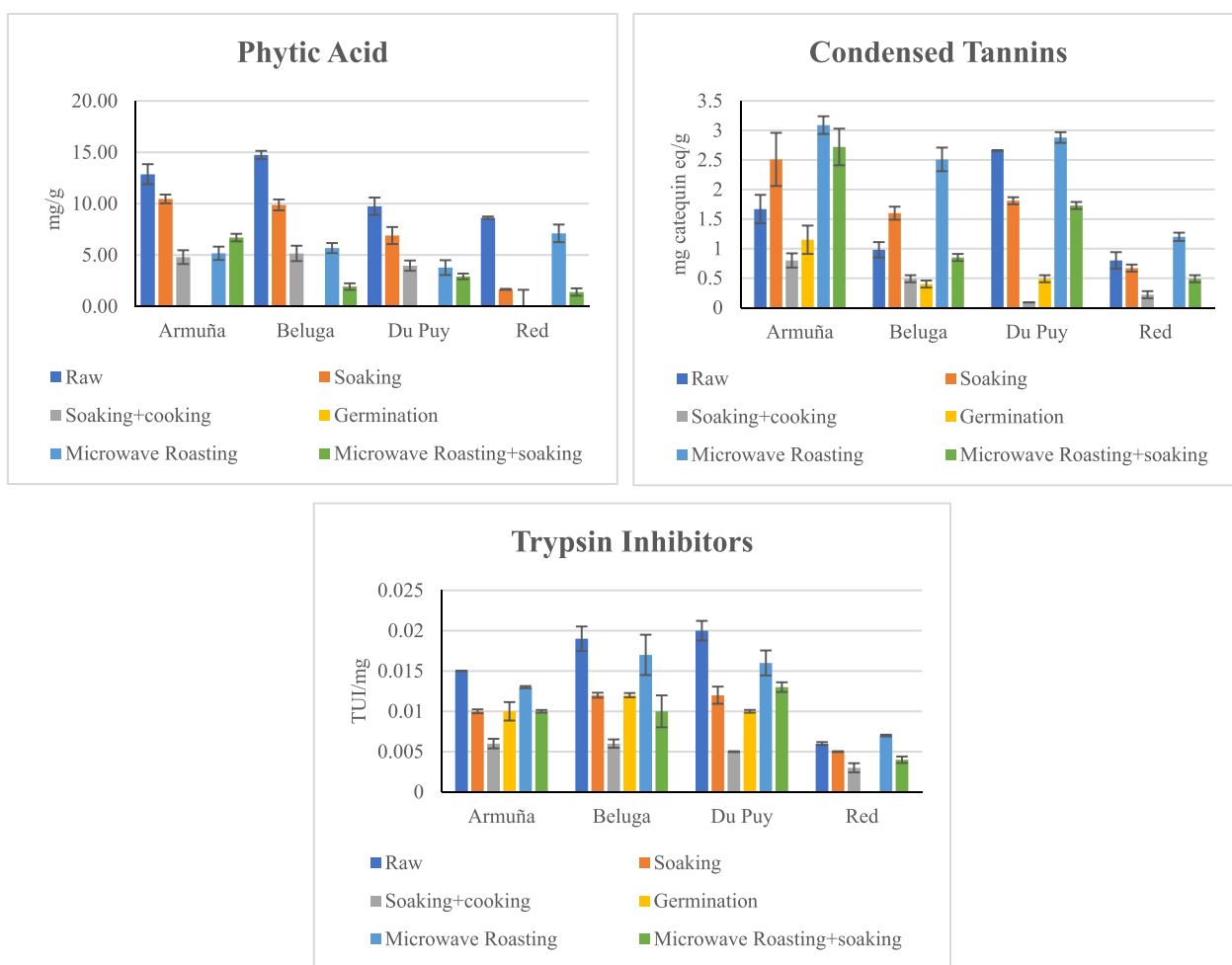


Fig. 1. Phytic acid, condensed tannins, and trypsin inhibitors composition of raw and processed Armuña, Beluga, Du Puy, and Red lentil varieties. TUI: Trypsin Units Inhibited. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

tannins significantly reduces with soaking (Gandhi et al., 2022; Khan-delwal et al., 2010b). Here, this divergence may be related to the activation of enzymes that may play a role in the breakdown of certain compounds, leading to the release or formation of tannins, as well as to hydration and swelling of lentils during soaking, which can disrupt cell structures and release tannins that were bound within the cells (Mazi et al., 2023). On the other hand, the increase in tannin content after microwave roasting can be related to the non-enzymatic reaction of the hydrolysis of anthocyanins to anthocyanidins and the subsequent polymerization with simple catechin to form complex tannins (Suazo et al., 2014). Here, although generally heat treatments have been shown to reduce the tannin content in different matrices, it appears that roasting has a marked inverse effect. Inversely, cooking has a pronounced effect in reducing the concentration of condensed tannins in all lentil samples, representing the processing technique that most reduce this antinutritional factor, except in the Beluga variety, in which germination provides a slightly greater decrease in this parameter. Here, the reducing effect of cooking on condensed tannins could be attributed to their degradation in high temperatures, these results being in agreement with others also performed in different lentil varieties (Fouad & Rehab, 2015; Gandhi et al., 2022). During germination, in turn, condensed tannins may be reduced by the activation of polyphenol oxidase and other catalytic enzymes, this process being responsible for the greatest reductions of tannins in several other studies (Aktas et al., 2022; Gandhi et al., 2022; Pal et al., 2017).

Trypsin inhibitors (TI) are protease inhibitors that limit protein digestion and restrict the metabolic use of amino acids and sulfur causing pancreatic hyperplasia (Adeyemo & Onilude, 2013). TI binds to the protease enzyme resulting in a reduction in protein digestion in the small intestine whilst favoring the faster release of proteins, thus lowering the bioavailability of sulfur-containing amino acids (Nikmaram et al., 2017). The results (Fig. 1) showed that cooking with prior soaking was the most efficient processing method in reducing the concentration of TI in all lentil varieties when compared to their raw form. This reduction has been attributed to the denaturation of TI during cooking, since these are heat-labile compounds (Vidal-Valverde et al., 1994), as well as to possible enzymatic inactivation, namely those responsible for synthesizing TI, with similar results being also accomplished by Wang, Hatcher, et al. (2009) who analyzed the influence of cooking and dehulling on the antinutritional features of several varieties of lentils. After germination, a significant decrease in TI concentrations when compared to the raw form of the different lentil varieties was also observed. El-Adawy (El-Adawy, 2002) also detected decreased levels of TI in the early stages of germination (72 h), relating this decrease with the activation of protease inhibitor degrading enzymes during this process. Regarding microwave roasting, although this method is less efficient than those previously explained, it also promotes a reduction in TI concentration when compared to the raw form of lentils, this reduction being more prominent when applying soaking after this process. Avilés-Gaxiola et al. (2018) attributed this phenomenon to the denaturation of thermally unstable proteins by the high-frequency electromagnetic energy of roasting which led to the inactivation of TI. When performed with prior soaking, the levels of TI decreased due to their possible leaching into the processing water.

It is worth noting that, despite the reported side effects of the analyzed antinutritional factors in the nutritional and chemical composition of pulses, these may also impair beneficial outcomes to human health. Some of these include the DPPH radical scavenger activity performed by phytic acid (Khatab et al., 2010), improvement of blood pressure conditions in people suffering from hypertension by tannins (Odai et al., 2019), and anti-inflammatory effects arising from the intake of TI (Carvalho et al., 2016), among others.

3.5. Tecno-functional properties

In pulse flours, tecno-functional properties are important features

that must be considered as they may affect the processing applications, food quality, and acceptance, and how ingredients can be used and may behave in foods and food formulations (Mahajan & Dua, 2002). These are mainly affected by protein components of foods, specifically by their composition, structure, conformation, interactions with other components, and environment (Kinsella, 1976), and by complex carbohydrates, such as starch, due to its versatile functionalities (Singh, 2001). The effect of processing on some of the most important techno-functional properties of pulse flours, namely water absorption capacity (WAC), oil absorption capacity (OAC), swelling capacity (SC), and emulsifying capacity (EC), were assessed in lentils, and the results are expressed in Fig. 2, with different patterns being detected among the different varieties. WAC measures the amount of water retained per gram of sample (Aryee & Boye, 2017). Cooking and germination stand out as the processing methods that most improve WAC, except in the Du Puy variety, in which germination promotes a decrease in this parameter. The largest increase of WAC after cooking of lentils has been attributed to the greater porosity and fluid entrapment during boiling (Catsimpooulas & Meyer, 1970), to the denaturation and unfolding of proteins caused by heat, which uncover their hydrophilic sites ensuing higher water uptake (Aguilera et al., 2011), and to starch gelatinization and swelling of crude fiber (Aguilera et al., 2009). Germination, in turn, increased WAC in lentils through the activation of endogenous protease enzymes which might expose the hydrophilic sites of proteins as well as amplify the content of low molecular weight proteins with a higher propensity to absorb water (Ghumman et al., 2016). Similar results were accomplished by Gandhi et al. (2022) who detected an increase in the WAC of lentils after boiling and germination. Microwave roasting, in turn, exerts a lesser influence on the WAC of lentils, presenting results slightly higher than their raw forms, except in Du Puy lentils, in which this parameter diminishes. The increase in WAC after microwave roasting has been also attributed to the denaturation of proteins with subsequent unfolding of water binding sites, as well as to starch gelatinization (Ouazib et al., 2016), these parameters being also influenced by the variety, time of exposure, and potency. The increment of the WAC of flours makes them good ingredients for bakery products, allowing the addition of more water to doughs, improving the handling characteristics, and preserving freshness in bakery products (Ma et al., 2011).

The OAC of flours is an important functional property that helps to improve mouthfeel and retention of flavors in the deriving products (Kinsella, 1976). Germination was the processing method that most improved the OAC of the different lentil flours when compared to their raw form, which might be related to the increase in lipophilic amino acids during this process, leading to hydrophobic interactions with lipids (Singh et al., 2017), with similar results being accomplished by Oskaybaş-Emlek et al. (2021) in green lentils from Turkey. In Armaña lentils, the remaining processing methods decreased the OAC, which did not occur in Beluga, Du Puy, and Red varieties, a decrease being also verified after soaking of Du Puy lentils and microwave roasting with soaking of Beluga and Red varieties, highlighting the influence of variety on the functional properties of lentil flours. Cooking also improved the OAC of most lentil flours by unmasking the hydrophobic sites of proteins due to high temperatures during cooking, exposing the non-polar side chains of amino acids (Kinsella, 1976). Moreover, the physical structural differences of boiled flours may induce greater porosity, allowing a greater entrapment of fats. Our results agree with others from Ma et al. (2011), who also detected increased levels of OAC after boiling in different varieties of lentils. These authors also observed decreased levels of this parameter after roasting, the same occurring in our study, except in Du Puy lentils, which effect is highly dependent on variety, time of exposure, and frequency of the applied treatment.

Swelling capacity refers to the ability of substances, such as starch or some hydrocolloids, to absorb and retain water, leading to an increase in volume or swelling (Thomas et al., 2023). The effect of processing on the swelling capacity of different varieties of lentil flours is presented in Fig. 2. The results showed that both soaking, cooking with prior soaking,

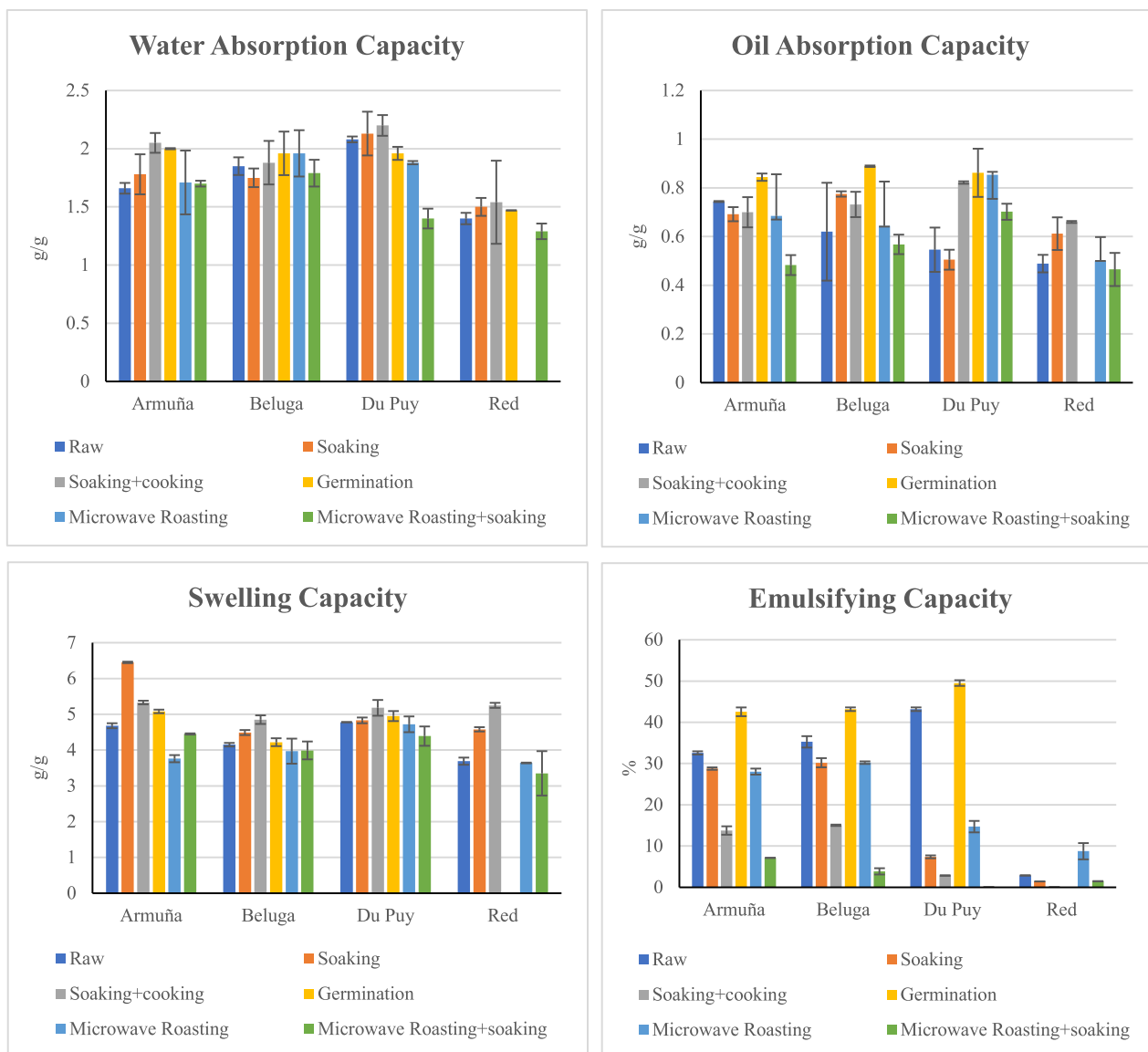


Fig. 2. Water holding capacity, oil holding capacity, swelling capacity, and emulsifying capacity of raw and processed Armuña, Beluga, Du Puy, and Red lentil varieties. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and germination increased, to an extent depending on variety, the SC of the overall lentil samples, the same not occurring after microwave roasting treatments. Microwave processing promotes the compaction of starch granules due to intra-granular rearrangement, which improves the absolute density of starches and limits the accessibility of water to their amorphous sites, reducing the SC of lentil flours (González & Pérez, 2002). During cooking, specifically, the increase in SC occurs due to specific characteristics of starch granules, since these are insoluble in cold water and gelatinization is crucial to ease water absorption, enhancing the physical and chemical reactivity of inactive starch granules in food processing (Singla et al., 2020). Thus, when lentil flours are subjected to heat and adequate moisture, gelatinization occurs and starch granules are disrupted, losing their organized structure, and facilitating swelling (Zhao et al., 2022). Regarding germination, although many studies reported a decrease in SC after this treatment due to possible hydrolysis of amylopectin chains because of metabolic activity (Benítez et al., 2013; Gandhi et al., 2022; Gutiérrez-Osnaya et al., 2020), our results showed an increase in this parameter, which may be related to the increase in soluble solids because of the breakdown of lipids, fibers, and amylose-lipid complexes that may inhibit the swelling

of starch granules (Phattanakulkaewmorie et al., 2011). Similar trends were observed by Ocheme and Chinma (2008) who studied the effect of germination on the SC of millet flours, this parameter being also amplified after this process. The increase in SC after soaking, in turn, has been related to the increased water absorption capacity of lentil flours and subsequent swelling (Aguilera et al., 2009).

The emulsifying capacity reflects the capacity of a protein to help in the formation of an emulsion and is related to the protein's capability to absorb the interfacial area of oil and water in an emulsion as a response to stress and changes (Ma et al., 2011). Here, proteins play a key role since they decrease surface tensions and provide electrostatic repulsion on the surface of oil droplets thereby emulsifying and stabilizing the emulsion (Jitngarmkusol et al., 2008) The effect of processing on the EC of different varieties of lentil flours was studied (Fig. 2) and the results showed that only germination promotes an increase in this parameter. The improvement of EC after germination occurs due to the separation and partial unfolding of polypeptides, which expose the hydrophobic sites of amino acids, enabling hydrophobic associations of peptide chains with lipid droplets, resulting in amplified volume/surface areas of protein, and improving emulsification properties (Kinsella, 1976).

After cooking, on the other hand, lentil samples present the greatest decrease in EC which has been related to the denaturation of proteins due to high temperatures, which was also observed by Gandhi et al. (2022) in two varieties of lentils. After roasting, in turn, despite presenting EC values lower than the raw forms of lentils, this decrease is not as pronounced, this being related to possible protein modifications following heat treatments, with similar results being accomplished by other authors (Benmeziane-Derradji et al., 2020; Ma et al., 2011).

4. Conclusions

Lentils hold a valuable nutritional profile, constituting rich sources of complex carbohydrates, proteins, minerals, and fiber, and their inclusion in different diets can bring several benefits to human health and well-being.

All the investigated processing methods exerted some influence on the analyzed parameters of lentils, with a greater influence and beneficial results arising from germination and cooking treatments to different extents. These showed to be highly dependent on lentils' variety and processing conditions, with some of the attained results being accomplished for the first time, not only in lentils but also in other agricultural products. Thus, the present investigation greatly contributes to the knowledge of the effects of different processing methods on the general characteristics of lentils flours, being able to infer their behaviour when used in the formulation of new food products capable of meeting the new nutritional, chemical and mineral demands and needs of today's society.

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CRedit authorship contribution statement

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

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.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodchem.2024.139293>.

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