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# Retention of minerals, antioxidants, pigments, and glucosinolates by broccoli florets and green bean pods boiled in alkaline, neutral and acidic waters

Vânia S. Ribeiro [a,b,c,d,*], Pedro F. Cruz [e], Zaida L. Almeida [e], Cândida G. Silva [a,b], Maria Isabel Silva [a], Inês S. Silva [a], Liliana R. Santos [a], Marta T. Santos [a], Carla Guimarães [a,b], Rui M.M. Brito [e], Mauro Guerra [f], Fernando Reboredo [g], Cidália D. Pereira [a,b,c,d], Daniela C. Vaz [a,c,d,e,**]

[a] School of Health Sciences, Polytechnic Institute of Leiria, 2411-901, Leiria, Portugal
[b] CiTechCare - Centre for Innovative Care and Health Technology, Polytechnic Institute of Leiria, 2411-901, Leiria, Portugal
[c] LSRE-LCM - Laboratory of Separation and Reaction Engineering – Laboratory of Catalysis and Materials, Polytechnic of Leiria, 2411-901, Leiria, Portugal
[d] ALiCE - Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, 4200-465, Porto, Portugal
[e] Coimbra Chemistry Centre, Institute of Molecular Sciences, Department of Chemistry, University of Coimbra, 3004-535, Coimbra, Portugal
[f] LIBPhys, Laboratory for Instrumentation, Biomedical Engineering and Radiation Physics, Department of Physics, NOVA University of Lisbon, 2829-516, Caparica, Portugal
[g] GeoBioTec, GeoBioSciences, GeoTechnologies and GeoEngineering, Earth Sciences Department, NOVA University of Lisbon, 2829-516, Caparica, Portugal

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ABSTRACT

Given the poor palatability and chewability of crude green bean pods (*Phaseolus vulgaris*) and broccoli florets (*Brassica oleracea* var. italica Plenck) these vegetables are generally microwaved, steamed or water boiled. Hence, here, we evaluated the contents of minerals, antioxidants, phenolics, glucosinolates, pigments, colour, texture and flavour of water-boiling broccoli and green bean pods, with four mineral waters with variable composition and pH. Plant matrices were characterized by High Resolution Magic-Angle Spinning (HR-MAS) and plant extracts were analysed by pseudo-2D Diffusion-ordered nuclear magnetic resonance (NMR) spectroscopy. Mineral waters ranging from acidic to neutral and alkaline were used and the colour properties (lightness, greenness/yellowness, colour saturation and hue angles), minerals and bioactive contents retained by the vegetables were compared to microwaved and steamed material. Boiling bean pods for 5 min extracted more polyphenols and antioxidants, particularly with more acidic waters, than 5 min of microwaving or steaming. However, even if boiling broccoli with more acidic water could better preserve glucosinolates, the food material presented lower retention of pigments and poor palatability. Cooking with more alkaline water increased mineral retention and broccoli greenness, also leading to highest scores in "colour", "texture", and "flavour". Hence, samples cooked in more alkaline water presented higher acceptability.

## 1. Introduction

Common green bean pods and broccoli are highly consumed vegetables that everyday bring nutrients, colour, and flavour to millions of meals around the world. Broccoli (*Brassica oleracea*) is a cruciferous vegetable low in calories but high in fibre (~24 % in dry weight (dw)), protein (~26 % dw), vitamins (A, C, and K), and essential minerals (Ca, K, Mg, Na and Fe) (Favela-González et al., 2020; Syed, Moni, Break, Khojali, Jafar, Alshammari et al., 2023). Broccoli is also a good source of bioactive compounds (flavonoids, hydroxybenzoic acids, hydroxycinnamic acids, carotenoids, chlorophylls, glucosinolates and sulforaphane) with attested antioxidant, antimicrobial, anti-inflammatory, and anticancer properties (Hwang & Lim, 2015; Syed et al., 2023). Common green beans (*Phaseolus vulgaris*) are leguminous vegetables also

* Corresponding author. School of Health Sciences, Polytechnic Institute of Leiria, 2411-901, Leiria, Portugal.
** Corresponding author. School of Health Sciences, Polytechnic Institute of Leiria, 2411-901, Leiria, Portugal.
*E-mail addresses:* vania.ribeiro@ipleiria.pt (V.S. Ribeiro), dvaz@uc.pt (D.C. Vaz).

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particularly rich in fibre (~34 % dw), protein (~24 % dw), vitamins (C) and minerals (Ca, Cu, Fe, K, Mg and Na), as well as in antioxidants (phenolic acids, flavonoids, and anthocyanins) with anti-inflammatory, antibacterial, antiaging, and antimutagenic activities (Ganesan & Xu, 2017; Kong et al., 2024). However, crude broccoli and common green beans present poor palatability and chewability given their high content in dietetic fibres, pungent aroma and bitter taste (Syed et al., 2023). Hence, these vegetables are generally submitted to processing/cooking prior consumption via stir-frying, microwaving, steaming or water-boiling. Yet, each cooking method, time, water volume and temperature interfere with the chemical composition, texture, flavour and nutritional value retained by the plant material (Palermo et al., 2014; Xu et al., 2014). When submitted to different cooking methods, matrix softening, chemical modifications and/or nutritional loss can occur, either through the leaking of water-soluble compounds into the boiling water, and/or through chemical, thermal or high-pressure modifications. Thus, whereas some compounds can be lost (e.g.: antioxidants, pigments, vitamins and minerals) other compounds (e.g.: carotenoids and sulforaphane) and properties (flavour, colour, bioavailability, digestibility and microbiological safety) can be gained (Giambanelli et al., 2016; Palermo et al., 2014; Sun, Wang, Pang, Tian, Hu, Li et al., 2021). Short-time microwaving (1000 W for 1 min) or steaming (<5 min) Brassica vegetables (e.g.: broccoli, cauliflower, Brussels sprouts and red cabbage), for instance, promotes the conversion of glucoraphanin (an aliphatic glucosinolate) into its hydrolysed component, sulforaphane; while water boiling (>15 min) leads to a large loss of glucoraphanin due to myrosinase (thioglucoside glucohydrolase) inactivation and compound leaching into the water (Sun et al., 2021). In turn, water-boiling cauliflower (200 g of florets/1000 mL of water, >5 min) or green beans (1200 g of plant material/2400 g of water, 5 min) reduces by 50 % the antioxidant capacity and total phenolic content of the plant material, since most of these compounds are water-soluble and thermally unstable (Ahmed & Ali, 2013; Baardseth et al., 2010). Thus, the use of suitable cooking methods and appropriate conditions for each type of vegetable is crucial to produce the desired organoleptic properties and retain the essential nutrients in the plant matrices. Yet, even if various research studies have already investigated the effect of different cooking methods and conditions on the composition of certain vegetables (e.g.: Brassica and Fabaceae vegetables) (AlJuhaimi et al., 2024; Armesto et al., 2016; Armesto et al., 2017; Armesto et al., 2019; Baardseth et al., 2010; Palermo et al., 2014; Turkmen et al., 2005) few is still known about the effect of water composition/pH on the sensory quality and dietary profile of these food matrices.

Natural mineral waters can present variable compositions and pHs. The practice of alkali diets or ingestion of bicarbonate-rich alkali mineral waters has shown to produce beneficial effects in humans, by decreasing bone resorption and parathyroid hormone levels (Burckhardt, 2011; Wynn et al., 2010). In what regards cooking, water-boiling vegetables in alkaline, neutral or acidic waters with different compositions and under different cooking conditions (of water volume, chopping degree, food density, cooking time, temperature or pressure) will determine the nutritional value and sensory properties presented by the food material. Moreover, even if some *chefs* and kitchen professionals add bicarbonate salts to the cooking water to brighten the colours of certain vegetables, little is still known on how these treatments affect the nutritional composition and dietary quality of food.

Hence, the objective of this work was to evaluate the retention level of minerals and bioactive compounds of broccoli florets and common green bean pods boiled in water with different compositions and pHs. To do so, we determined the metabolic fingerprint of food matrices and extracts as well as contents of minerals, antioxidants, phenolics, pigments (chlorophyll *a*, chlorophyll *b*, and carotenoids), colour (lightness, redness-greenness, yellowness-blueness), and glucosinolates of samples boiled with four mineral waters, with variable ionic strength, chemical composition and pH. Tap water (pH 7.6) and mineral waters ranging from acidic (pH 5.7) to neutral (pH 6.8) and alkaline (pH 9.5) were tested and the mineral and bioactive contents retained in the vegetable matrices were determined and compared to microwaved and steamed samples. In addition, a sensory analysis accomplished by a focus group through a 9-point hedonic scale for testing the "colour", "flavour", and "texture" presented by the cooked vegetables was also carried out to evaluate overall acceptance. In sum, the present work allowed to systematically study the effect of water composition/pH on the retention of nutrients and dietary quality of two widely consumed vegetables (broccoli and green bean pods) as well as to predict preferences and to simulate a real kitchen environment with various cooking times and cooking methods, close to those experienced and practiced by the consumers.

## 2. Materials and methods

### 2.1. Plant materials

Fresh broccoli florets (3.5 Kg, *Brassica oleracea* var. italica Plenck) and snap green bean unripe pods (3.5 Kg, *Phaseolus vulgaris*) were purchased from local suppliers (Leiria, Portugal). Plant specimens presented similar matureness, consistency and colour. Vegetables were handled at the experimental kitchen of the School of Health Sciences of Leiria. Samples were cleansed with tap water (pH 7.6, 2.0 mmol/L minerals) and cut into small pieces with stainless steel kitchen scissors (Cuyfor 21 cm, Portugal). Green bean pods were trimmed and cut into identical square portions of 2 × 2 cm, while broccoli florets were cut in small portions of 3–4 cm in length. Fresh samples were randomly divided in fractions of 150 g (Nahita Precision digital scale, max. 1000 ± 0.01g - Auxilab, Navarra, Spain) for each cooking treatment, in triplicate. One portion was kept raw, stored at 4 °C (Magnus - Catering equipment, Aveiro, Portugal), and used as reference material.

### 2.2. Cooking treatments

Broccoli florets and common green bean pods were submitted to different processing treatments: water-boiling, steaming, microwaving and crude fresh samples (control). To select the minimum cooking time to reach the adequate texture and palatability, different cooking times of 3, 5 and 10 min were used for microwaving, steaming and water-boiling.

Steaming and boiling were carried out in cooking vessels (16x8 Alum Fund 1.3 L non-stick casserole for induction) with boiling water by making use of hot plates (Teka -Shott Ceran®, Germany). Time counting was monitored with a chronometer and only began after boiling. Temperature was checked with a thermometer (TFI-250 digital thermometer, Ebro Electronic GmbH, Germany). Water volumes were measured with a graduated glass cylinder (Normax, Marinha Grande, Portugal). Water-boiling was conducted by immersing 150 g of plant material in 600 mL (1:4 ratio) of the different types of mineral water with different compositions and pH of 5.7, pH 6.8, and pH 9.5, as well as in tap water (pH 7.6), as described in Table 1. Mineral waters were acquired from various water companies. The chemical compositions and pHs of the waters were determined by the certified laboratories of the water companies, in accordance with the European standard (EU), Directive 2009/54/EC, as displayed by the water labels.

Basket steaming was conducted by suspending 150 g of each sample over a steamer with 600 mL of boiling water (tap water, pH 7.6) in a cooking pan with a lid. Microwaving was conducted by placing 150 g of sample on a ceramic plate with 30 mL of water (tap water, pH 7.6), to prevent food burning, covered with a plastic cover, at full power (650 W) in a microwave oven (MS-1924W, LG Electronics, Seoul, South Korea). Upon cooking, samples were drained off in stainless steel sieves and let to cool down. Small portions were prepared for the hedonic testing and for the colour measurements. The remaining samples were freeze-dried and stored at −20 °C. Freeze dried samples were used to characterize the main components of the food matrices by HR-MAS NMR

**Table 1**
Total phenolic content (TPC) and antioxidant capacity (AC) of raw and cooked green bean pods and broccoli florets (dry weight, dw).

| cooking time (min) | | | TPC (mg GAE/g) FC | AC (μmol VCE/g) ABTS | cooking time (min) | | | TPC (mg GAE/g) FC | AC (μmol VCE/g) ABTS |
|---|---|---|---|---|---|---|---|---|---|
| Green bean pods | | | | | Broccoli florets | | | | |
| raw | | | 2.43 ± 1.32 [b] | 2.89 ± 0.45 [b] | raw | | | 30.49 ± 1.04 [b] | 14.26 ± 0.36 [e] |
| steamed | | 3 | 3.21 ± 0.77 [b] | 4.39 ± 2.13 [a] | steamed | | 3 | 41.26 ± 2.56 [b] | 31.26 ± 0.33 [c] |
| | | 5 | 4.20 ± 0.76 [b] | 7.28 ± 0.87 [a] | | | 5 | 38.54 ± 3.18 [b] | 31.30 ± 1.77 [c] |
| | | 10 | 4.07 ± 1.37 [b] | 8.10 ± 2.23 [a] | | | 10 | 33.75 ± 1.67 [b] | 26.16 ± 0.11 [d] |
| microwaved | | 3 | 2.83 ± 0.48 [b] | 4.86 ± 0.24 [a] | microwaved | | 3 | 47.67 ± 3.75 [a] | 35.49 ± 2.55 [b] |
| | | 5 | 4.42 ± 0.58 [b] | 5.59 ± 1.14 [a] | | | 5 | 60.50 ± 8.77 [a] | 42.80 ± 0.14 [a] |
| water-boiled | tap pH 7.6 | 3 | 9.27 ± 3.51 [a] | 10.43 ± 0.54 [a] | water-boiled | tap pH 7.6 | 3 | 29.39 ± 4.01 [b] | 24.33 ± 0.11 [d] |
| | | 5 | 4.42 ± 1.54 [b] | 10.19 ± 3.94 [a] | | | 5 | 21.27 ± 1.99 [c] | 18.10 ± 1.00 [e] |
| | | 10 | 3.80 ± 0.90 [b] | 8.41 ± 0.01 [a] | | | 10 | 19.33 ± 0.25 [c] | 15.73 ± 0.57 [e] |
| | pH 5.7 | 5 | 5.39 ± 1.06 [a] | 7.72 ± 0.96 [a] | | pH 5.7 | 5 | 18.90 ± 3.11 [b] | 16.70 ± 0.17 [c] |
| | pH 6.8 | 5 | 6.74 ± 0.84 [a] | 7.54 ± 0.87 [a] | | pH 6.8 | 5 | 22.85 ± 1.67 [b] | 19.98 ± 2.90 [c] |
| | pH 9.5 | 5 | 3.50 ± 1.24 [b] | 6.71 ± 0.55 [a] | | pH 9.5 | 5 | 19.19 ± 0.88 [b] | 14.47 ± 0.30 [d] |

| Water composition[a] | $HCO_3^-$ (mmol/L) | $Cl^-$ (mmol/L) | $Na^+$ (mmol/L) | $NO_3^-$ (μmol/L) | $Ca^{2+}$ (μmol/L) | $Mg^{2+}$ (μmol/L) | $K^+$ (μmol/L) | Total (mmol/L) |
|---|---|---|---|---|---|---|---|---|
| tap pH 7.6 | – | 0.9 | 0.9 | 60 | – | 148 | – | **2.0** |
| pH 5.7 | 0.1 | 0.2 | 0.3 | 34 | 20 | 21 | 54 | **0.7** |
| pH 6.8 | 0.6 | 0.3 | 0.6 | 15 | 155 | 66 | 20 | **1.8** |
| pH 9.5 | 1.9 | – | 5.0 | – | 27 | – | – | **6.9** |

Values are mean ± standard deviation (sd) of triplicates. [(a-e)]Different superscripts in the same column indicate statistical differences ($p < 0.05$) between cooking treatments. Post-hoc test outcomes were derived from Tukey's test for cases of homogeneous variance.
[a] Values on the water labels given by the water companies.

and determine their mineral profile, as well as to prepare acetonic extracts to evaluate contents in phenolics, antioxidants and pigments, and methanolic extracts to quantify glucosinolates.

### 2.3. Hedonic testing

A preference hedonic test was applied to a small focus group of six volunteers recruited from the School of Health Sciences of Leiria. Participants were informed about the various steps and objectives of the test. Vegetables were served directly after cooking. Individual portions of 30 g of each sample (the amount recommended by the Society of Sensory Professionals to test for organoleptic properties (Meilgaard et al., 2015)) were prepared in separate. Samples were randomly labelled and presented to the test group. The preference tests were carried out in a separate quiet room, maintained at a constant temperature of 21 °C. Three attributes were analysed: "colour", "flavour", and "texture" using a 9-point hedonic scale (Meilgaard et al., 2015): 1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely. Fresh water was provided for rinsing between tastings. The hedonic testing enabled to select the ideal cooking time as well as to determine preferences in terms of cooking treatments/waters.

### 2.4. Total phenolic content and antioxidant activity

Acetone ≥99.5 % (v/v), gallic acid (GA), ascorbic acid (vitamin C) and the [2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)]-diammonium salt (ABTS) were purchased from Sigma-Aldrich, (Merck KGaA, Darmstadt, Germany). The Folin-Ciocalteu solution was obtained from Biochem Chemopharma (Nevers, France). Sodium carbonate was purchased from Scharlab (Barcelona, Spain). Ultrapure Milli-Q Type 1 water (<0.060 μS/cm) was used in all experiments (Milli-Pore, Darmstadt, Germany). Macerated samples (1g fresh weight) were extracted with 10 mL of acetone 70 % (v/v), for 2 h under magnetic stirring, at 20 °C. Samples were then filtered through qualitative filters (11 μm-pore, Whatman, Maidstone, UK) and protected from light. Subsequently, supernatants were tested for phenolic compounds and antioxidant capacity.

Total phenolic contents (TPC) were obtained by the Folin-Ciocalteu assay, as described previously (Pinteus, Neves, Tecelão, Silva, Cruz, Bernardino et al., 2024). In brief, aliquots (250 μL) of controls, vegetable extracts and gallic acid standards (0.3, 0.9, 1.5 and 2.4 mmol/L) were mixed with the Folin-Ciocalteu reagent (250 μL). Sodium carbonate was added to the mixture, and let to incubate for 60 min, at 20 °C, before registering absorbances at 750 nm. Total phenolic content (TPC) was expressed as milligrams of gallic acid equivalents per gram of vegetable material (mg GAE/g).

Antioxidant capacities (AC) were determined by the ABTS assay, as described in previous work (Silva et al., 2025). In brief, the ABTS●+ radical cation was produced by reacting 7.0 mmol/L ABTS with 2.5 mmol/L potassium persulfate ($K_2S_8O_2$) (1:1, v/v). The mixture was let to rest in the dark for 16 h, at room temperature, filtered (11 μm-pore, Whatman, Maidstone, UK) and diluted with Milli-Q ultrapure water, to obtain an absorbance of 0.700 units at 734 nm. Final samples were prepared by adding 20 μL of controls, vegetable extracts or vitamin C standards (5.6, 28.3, 56.7, 283.8, 567.7, 851.6, 1135.5 μmol/L) to 1 mL of the ABTS solution. Cuvettes were properly stoppered for mixing and to avoid oxidation by atmospheric $O_2$. Absorbances were read at 734 nm, after 6 min of reaction. Antioxidant capacity (AC) was expressed as milligrams of vitamin C equivalents per gram of vegetable material (mg VCE/g).

TPC and AC values were expressed in dry weight (dw), by taking in account the moisture presented by all samples. Moisture was determined by dehydrating 5.0 g of the raw and cooked samples in porcelain crucibles, in an oven at 105 °C (Digitheat-TFT, Germany), until constant weight.

### 2.5. X-ray fluorescence analysis

Freeze-dried samples were ground and pressed for 2 min under 10 tons to produce compact pellets (∅ = 2 cm) of 1 mm in thickness. Pellets were placed on mylar sheets and submitted to the X-ray beam. General elemental evaluation was performed with a micro-Energy Dispersive X-Ray Fluorescence (μ-EDXRF) equipment operating with a Rh-anode X-ray tube (Bruker M4 TornadoTM, Bruker, Germany). A polycapillary lens was used to obtain a spot size down to 25 μm (Pedrosa et al., 2021) and fluorescence detection was performed with an energy resolution of 142 eV at 5.9 keV (Cardoso, Mateus, Velu, Singh, Santos, Carvalho et al., 2018). Quantification was performed considering the fundamental parameters' method for bulk samples (Guerra et al., 2016; Gallardo, Queralt, Tapias, Guerra, & Carvalho, M.L., Marguí, 2016) and spectral quantification was accomplished with the built-in ESPRIT software. Several standard materials (NCS, China) were used as reference to span over various vegetable matrices with different chemical and physical properties: ZC73011 (soybean), ZC73012 (cabbage), ZC73013

(spinach), ZC73032 (celery), ZC73033 (scallion), ZC73036 (green tea), DC73348 & DC73349 (bush branches and leaves), DC73350 (poplar leaves), DC73351 (tea). Additional standards from NMIJ (Japan) - NMIJ7405a (seaweed) and INCT (Poland) - TL-1 (tea leaves), PVTL-6 (Polish Virginia tobacco leaves) and OBTL-5 (Oriental Basma Tobacco leaves) were also used. Recover rates for the studied elements were better than the certified uncertainty intervals of the reference materials. Detection limits for Cu, Ni and Zn were 2 μg/g, for S, Mn and Cl were 4 μg/g and for P, Fe, Ca, and K were 8, 15, 35 and 55 μg/g, respectively.

### 2.6. Atomic absorption spectroscopy

Amounts of sodium (Na) and magnesium (Mg) were determined by atomic absorption spectroscopy (AAS) with an air and acetylene oxidizing flame (Varian-SpectrAA-55B, Grenoble, France). AAS determinations were performed by making use of standard solutions in the range of 0.0–0.2 mmol/L, with a detection limit of 0.02 mmol/L and mono-elemental lamps operating at 589.6 nm and 285.2 nm, for Na and Mg, respectively. Freeze-dried plant material (152 mg) was digested in a total of 10 mL of nitric-hydrochloric acid mixture (1:3). All samples were filtered with a cellulose nitrate filter membrane with a pore size of 0.45 μm (Sartorius, Göttingen, Germany) before analysis. Results were expressed as micrograms of Na and Mg per gram of dry weight (μg/g dw) as performed in previous work (Correia, Antunes, Tecelão, Neves, Pires, Cruz et al., 2024). Due to evaporation issues during digestion, a correction factor was determined and applied to each sample to ensure accuracy.

### 2.7. Colour measurements

Colour measurements were performed in three replications on raw and cooked samples, using a Chroma Meter CR-400 (Konica Minolta, Osaka, Japan), with an 8-mm diameter measurement port, by registering average values of three independent readings at three different points, at room temperature. Equipment calibration was conducted with a standard white plate CR-400 (Y = 89.3, x = 0.3159, y = 0.3225). Colour parameters were determined according to the CIE (International Commission on Illumination) $L^*a^*b^*$ colour space system (CIE, 1978) and reported as $L^*$ (lightness from 0 to 100: 0-black/100-white), $a^*$ (from −60 = greenness to +60 = redness, with $a^*$ negative for green and positive for red) and $b^*$ (from −60 = blueness to +60 = yellowness, with $b^*$ negative for blue and positive for yellow) using the D65 illuminant. Colour saturations ($C^*$) and hue angles ($H^\circ$, $a^* < 0$ and $b^* > 0$) were calculated according to equations (1) and (2), respectively:

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (1)$$

$$H^\circ = \tan^{-1}\frac{b^*}{a^*} + 180^\circ \quad (2)$$

Total colour differences $\Delta E^*$ were obtained, using the raw vegetables as reference, according to equation (3):

$$\Delta E^* = \sqrt{\left[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2\right]} \quad (3)$$

### 2.8. Quantification of pigments

Total pigment concentrations, chlorophylls and carotenoids were quantified as described previously (Correia et al., 2024). In brief, extracts were prepared from macerated freeze-dried material (0.1 g) added to 10 mL acetone 70 % (v/v), followed by overnight solubilization and centrifugation at 4000×*g* (Heraeus, Waltham, USA). Visible spectra of the various plant extracts were collected from 400 to 700 nm in 1-cm pathlength quartz cuvettes (Hellma, Müllheim, Germany). Absorbances at 470 nm, 645 nm, and 662 nm were registered (Varian Cary 50 UV–Visible spectrophotometer), and the concentrations of chlorophyll *a*, chlorophyll *b* and carotenoids (xanthophylls and carotenes) were calculated as described in previous work (Primitivo, Neves, Pires, Cruz, Brito, Rodrigues et al., 2022).

### 2.9. Quantification of glucosinolates

A spectrophotometric method (Mawlong et al., 2017) with minor modifications, was used to estimate the total glucosinolate content in all samples. Briefly, 0.1 g of freeze-dried plant material was dissolved in 1.5 mL of 80 % (v/v) methanol and let to rest overnight at room temperature. Then, samples were centrifuged in a benchtop Eppendorf centrifuge, for 8 min, at 6000 rpm. Supernatants were collected, and the volumes were adjusted to 2 mL with 80 % (v/v) methanol. This extract was used for the further analyses, by mixing 100 μL of extract with 300 μL of Milli-Q water and 3 mL of 2 mmol/L sodium tetrachloropalladate ($PdCl_4$). Upon incubation at room temperature for 1 h, absorbance was measured at 425 nm using a SpectraMax iD5 multi-mode microplate reader (Molecular Devices, San Jose, USA) operated with the SoftMax Pro software v7.1.0, in clear-bottom 96-well microplates. Control samples were prepared without plant extracts. Total contents in glucosinolates were calculated according to equation (4):

$$y = 1.40 + 118.86 \times A_{425\ nm} \quad (4)$$

### 2.10. NMR experiments

NMR samples were prepared as previously described (Sebastião, Vaz, Pires, Cruz, Moreno, Brito et al., 2024). Isotope-labelled solvents were used (Cambridge Isotope Laboratories Inc, Tewksbury, USA). Plant extracts were prepared by adding the freeze-dried material (0.1 g) to deuterated acetone-$d_6$/$D_2O$ (1 mL), followed by overnight solubilization and centrifugation at 4000×*g* (Heraeus, Waltham, USA). 1D $^1$H NMR spectra were acquired with zgpr pulse sequences with 32 k complex points, 128 scans, a spectral width of 4262.04 Hz, and a 1 s relaxation delay, at 25 °C. Diffusion-ordered spectroscopy (DOSY)-NMR experiments were acquired with the diffusion bipolar pulse pair stimulated echo and LED (LEDBPGP) method (Wu, Chen, & Johnson, 1995), with 32 k complex points, 16 scans per increment, and a spectral width of 4562.04 Hz, at 25 °C, in a 9.4 T Bruker AVANCE 400 spectrometer (Bruker, Billerica, USA) equipped with a 5 mm high power diffusion BBI DiffBB probe. Data was obtained with a diffusion delay of 50 ms and 4 ms small delta, with linear gradient pulse amplitudes ranging from 2 to 95 % during 2 ms with a total of 16 steps, and an eddy current delay of 5 ms. Temperature calibration was performed using standard samples. Bruker Topspin3.5 was used to acquire and process data to extract diffusivities, according to the Stokes-Einstein Gierer-Wirtz method (SEGWE) for dilute solutions and/or pure solvents (Evans et al., 2018).

HR-MAS experiments were conducted in an 11.7 T Bruker AVANCE III HD 500 spectrometer (Bruker, Billerica, USA) equipped with a Bruker 4 mm HR-MAS TXI probe. Two-dimensional phase sensitive $^1$H-$^{13}$C HMQC experiments were collected using the Sates-TPPI method in the indirect detected dimension, with 2k complex points in t2 and 512 increments (64 scans each) in t1 using digital quadrature detection, a spectral width of 7002 Hz (14 ppm) in the direct dimension, and 15092 Hz (120 ppm) in the indirect dimension. Two-dimensional phase sensitive $^1$H–$^1$H TOCSY experiments were collected using the MLEV17 sequence for mixing (100, 250 and 300 ms mixing times were tested) a data matrix of 512 × 2048 points covering 7002 × 7002 Hz with 32 scans per increment with a relaxation delay of 2 s. Bruker Topspin3.7 was used for data acquisition and processing.

### 2.11. Statistical analysis

Experiments were performed at least in triplicate. Data was reported as mean ± standard deviation (sd). Data were analysed by one-way analyses of variance (ANOVA) to determine significant differences

between means, considering the Tukey's test deemed statistically significant at $p < 0.05$. A principal component analysis (PCA) was used to relate the quantitative variables (colour, pigments, mineral profile, total phenolic content, antioxidant capacity and glucosinolates) with the cooking treatments and water pH. Calculations were performed with the *R* software (R Foundation for Statistical Computing, Vienna, Austria).

## 3. Results and discussion

### 3.1. Metabolite fingerprinting of the raw food matrices

The metabolite fingerprints of the raw matrices of broccoli florets and green bean pods were obtained by unidimensional (1D) and bidimensional (2D) HR-MAS NMR. Green bean pods are essentially composed (>87 % dry weight, dw) of carbohydrates (~67 g/100g dw) and peptides (~20 g/100g dw) (Favela-González et al., 2020; Quizhpe et al., 2024). Hence, the $^{1}$H HR-MAS spectrum of broccoli florets and green bean pods (Fig. 1) presented typical proton signals of saccharides and amino acids, as primary metabolites (Ward, Baker, Miller, Deborde, Maucourt, Biais et al., 2010), as also confirmed by the correlations found in the 2D $^{1}$H-$^{13}$C Heteronuclear Multiple Quantum Coherence (HMQC) spectrum and in the 2D $^{1}$H–$^{1}$H total correlation spectroscopy (TOCSY) spectrum (Fig. 1). The NMR signals of the 2D $^{1}$H-$^{13}$C HMQC spectrum of green bean pods were in the expected range for monosaccharides such as glucose and fructose, and disaccharides, such as saccharose (α-D-glucopyranosyl-(1 → 2)-β-D-fructofuranoside), with chemical shifts (δ) for the pyranose and furanose rings between 3.0 and 4.5 ppm for $^{1}$H and of 60–100 ppm for $^{13}$C nuclei (Fig. 1). In addition, the spin-system correlations detected in the 2D $^{1}$H–$^{1}$H TOCSY spectrum of green bean pods were attributed to aromatic amino acids such as tyrosine and phenylalanine, as well as to other amino acids such as leucine, isoleucine, valine, threonine and alanine (according to the chemical shifts of their side chain protons βH, γH and δH).

Likewise, a similar pattern was observed in the NMR spectra of broccoli, also mainly composed (>95 %, dw) of carbohydrates (~65 g/100g dw) and peptides (~30 g/100 g dw) (Kong et al., 2024; Sinković et al., 2024). Typical signals of saccharide protons were also clearly identified in the carbohydrate region of the $^{1}$H-$^{13}$C HMQC and $^{1}$H–$^{1}$H TOCSY spectra.

### 3.2. Degree of cooking and dietary quality

To select a standard cooking time that would guarantee the best organoleptic properties, along with the retention of dietary quality, different processing times and treatments were tested and the colour, texture, flavour, and contents of phenolics and antioxidants of the cooked legumes were analysed.

Total phenolic contents (TPC) and antioxidant capacities (AC) were determined for acetonic extracts of green bean pods and broccoli florets obtained from the 3, 5, and 10 min microwaved, steamed, and tap water boiled samples. Table 1 and Fig. S1 (supplementary material) present the results in dry weight (dw). In general, raw and cooked broccoli presented approximately 5-fold more TPC and AC than raw and cooked green bean pods, and the values obtained for both vegetables are within the range reported by previous studies for raw and cooked material (Le et al., 2020; Mahn & Rubio, 2017; Zhou & Liangli, 2006).

In the case of microwaved broccoli and green bean pods, the AC and TPC detected in the extracts increased with cooking time (from 3 to 5 min), when compared to uncooked vegetables, as seen previously (Kim, Ediriweera, Boo, Kim & Cho, 2021). However, longer microwaving times (10 min) were found to be overly disruptive, and destabilisers of the structural integrity of the food matrices and were not considered for

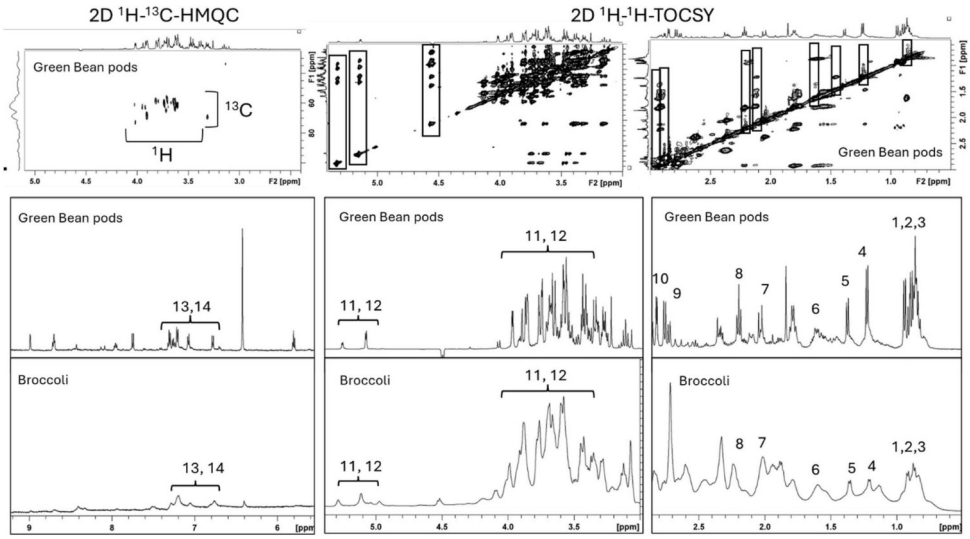


**Fig. 1.** HR-MAS NMR metabolite fingerprints of the raw matrices of green bean pods and broccoli florets. Given their composition resemblance in primary metabolites, the two vegetables show 1D 1H NMR signals in the aliphatic, carbohydrate and aromatic regions of the spectra assigned to amino acids, such as valine (1), isoleucine (2), leucine (3), threonine (4), alanine (5), leucine (6), glutamate (7,8), asparagine (9,10), phenylalanine, tyrosine and phenols (13,14), and to saccharides, such as glucose and fructose (11,12). Likewise, the 2D 1H-13C HMQC spectrum of green bean pods shows typical signals of pyranose and furanose rings of hexoses, between 3.0 and 4.5 ppm for 1H nuclei and of 60–100 ppm for 13C, and the 2D 1H–1H TOCSY spectrum allows to identify the spin-systems of monosaccharides and amino acids. Broccoli presented identical 2D 1H-13C HMQC and 2D 1H–1H TOCSY spectra (not shown).

comparison. Likewise, similar results were obtained for the extracts of the 3, 5 and 10 min steamed green bean pods, which presented a gradual increase of AC and TPC, as the cooking time was raised. This increase in TPC and AC can be associated with cell-matrix softening, (particularly needed for very fibrous legumes, such as green bean pods) as a way to release both free and bound phenolics and other antioxidants, as previously described for 1.5 min microwaved and 7.5 min (Turkmen et al., 2005) and 10 min steamed vegetables (Gliszczyńska-Swigło et al., 2006).

In opposition, a gradual loss of AC and TPC was observed for both legumes when the water-boiling time increased from 3 to 5, and from 5 to 10 min. Even if after 3 min of cooking, the AC and TPC of the extracts of water-boiled material increased relatively to the extracts of raw material, a gradual loss in polyphenols and antioxidants was observed when the cooking time was raised beyond 5 min (Table 1, Fig. S1), as previously seen for 8 min boiled broccoli (Pellegrini, Chiavaro, Gardana, Mazzeo, Contino, Gallo et al., 2010), and 10 min (Preti, Rapa, & Vinci, 2017) and 20 min (Burgos-Edwards, Miño, Nina, Plaza, Daza, Theoduloz et al., 2023) boiled green bean pods. This data indicates that even if a short heat treatment (<5 min) can help to soften the fibrous cell walls of the plant tissues and increase the release of bioactive compounds, longer boiling times (>5 min) can lead to greater compound losses by thermal degradation and diffusion to the decoction water (Baardseth et al., 2010). Similarly, in the case of broccoli florets, which are composed of a less fibrous matrix, an increase in TPC and AC was observed relatively to raw material after 3, 5 and 10 min of steaming, but with a gradual reduction of the TPC and AC values, as the cooking time augmented (Table 1, Fig. S1).

### 3.3. Degree of cooking, palatability and water pH

In addition, to choose an appropriate cooking time that would guarantee vegetable chewability and adequate palatability, a preference trial with a 9-point scale was carried out. Results regarding the 3, 5 and 10-min microwaved, steamed and water-boiled broccoli and green bean pods are illustrated in Fig. 2. Scores varied between 3 and 8 points (pts). In general, water boiled vegetables achieved the highest scores across all evaluating attributes (colour, texture and flavour), while 3 min microwaved, and 3 min steamed vegetables achieved the lowest (~3 pts) in texture and flavour. Regarding green bean pods, 3-min tap water-boiled

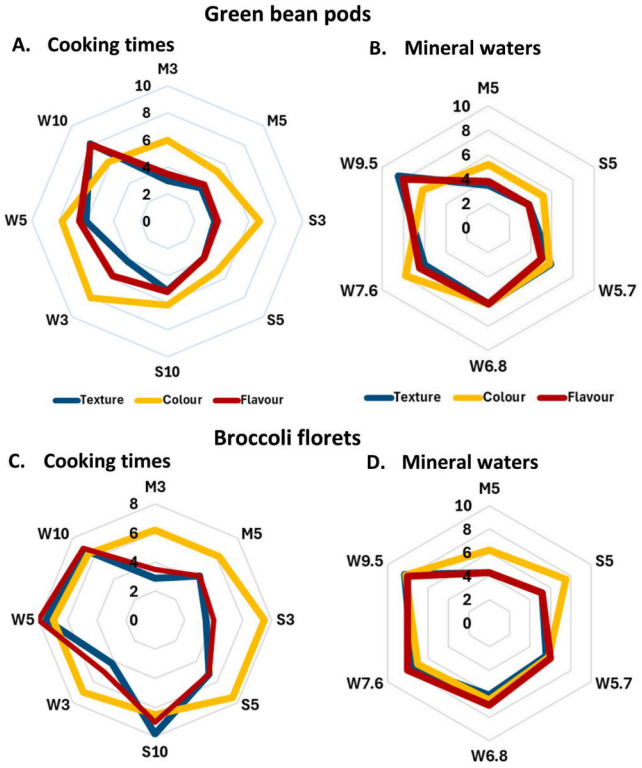


**Fig. 2.** Sensory properties of cooked green bean pods and broccoli florets tested by a focus group via a 9-point hedonic scale for "texture", "colour" and "flavour": 1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely. Left panels: results for the samples of 3-min (S3), 5-min (S5) and 10-min (S10) of steamed, 3-min (M3) and 5-min (M5) of microwaved and 3-min (W3), 5-min (W5) and 10-min (W10) of water boiled (tap water, pH 7.6) green bean pods (A) and broccoli florets (C). Right Panels: results for the 5-min cooked samples of green bean pods (B) and broccoli florets (D). via steaming (S5), microwaving (M5) and water boiling in mineral waters with pH 5.7 (W5.7), pH 6.8 (W6.8), pH 7.6 (W7.6) and pH 9.5 (W9.5).

pods were the most preferred (8 ~pts) in colour, and the 10 min tap water-boiled samples achieved the highest scores (~8 pts) in flavour and texture. In what respects broccoli, vegetables submitted to 5 min (~7.5 pts) and 10 min (~8 pts) of steaming, were preferred in colour and texture respectively, while 5 min water-boiled broccoli (~8 pts) were preferred in flavour. Therefore, to find a compromise between dietary quality and good palatability, a cooking time of 5 min was chosen to carry out the further analyses and evaluate the effect of water composition and pH on the retention of minerals, antioxidants, polyphenols, glucosinolates, pigments, lightness, colour, texture and flavour.

Moreover, to relate the influence of water composition and pH with texture, flavour and colour, 5-min vegetables water-boiled with water of different composition and pH (5.7, 6.8, 7.6 and 9.5) were also presented and tested by the focus group (Fig. 2). Vegetables boiled in alkaline water (pH 9.5) reached the highest scores across all parameters, [texture (~8 pts); colour (~8 pts); flavour (~8 pts)], whereas vegetables boiled in acidic water (pH 5.7) reached the lowest [texture (~5 pts); colour (~5 pts); flavour (~5 pts)]. Hence, even if cooking the vegetables in more acidic water may better preserve polyphenols and other antioxidants, when vegetables are cooked in alkaline water, highest preference scores are reached for texture, flavour and colour.

### 3.4. Bioactive compounds and water pH

The stability of polyphenols and other antioxidants is highly determined by pH, oxygen availability, metal ions, UV light, temperature, enzymes, and other constituents. Polyphenols are generally more stable at acidic pH, as they can remain neutral, while in more alkaline environments they tend to suffer oxidation and dimerization, also affecting food colour (El-Saadony, Yang, Saad, Alkafaas, Elkafas, Eldeeb et al., 2024). Hence, total phenolic contents (TPC) and antioxidant capacities (AC) were determined for the various acetonic extracts obtained from green bean pods and broccoli florets boiled for 5 min in different mineral waters (Table 1) with varied composition and pH (pH 5.7, 6.8, 7.6 and 9.5, Fig. 3). In the case of broccoli, few differences were found between the different types of water, indicating that the amount of antioxidants and polyphenols extracted from the cooked material is more influenced by the cooking method than by the type of water used. Indeed, steaming and microwaving broccoli for 5 min led to higher values of TPC and AC (statistically significant, $p < 0.05$) than 5 min of water boiling, due to diffusion of compounds to the decoction water and/or thermal instability. Conversely, in what respected green bean pods, a different pattern was observed. Some heat treatment is necessary to soften the fibrous matrix of the pods and release the bioactive compounds. Boiling bean pods for 5 min enables to extract more compounds, such as polyphenols

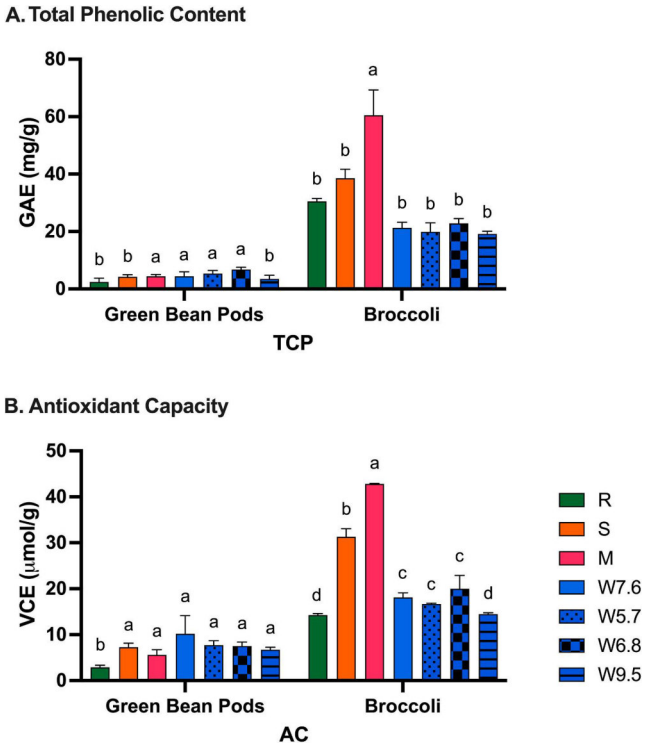


**Fig. 3.** Total phenolic contents (TPC) (A) and antioxidant capacities (AC) (B) of the acetonic extracts (1:10) (m/v) obtained from raw and 5-min steamed (S), 5-microwaved (M) and 5-min boiled (W) samples with the different mineral waters at the various pH values (W5.7, W6.8, W7.6 and W9.5). Experiments carried out in triplicates of three independent samples. Different superscripts among columns indicate statistical differences ($p < 0.05$).

and antioxidants ($p < 0.05$), particularly when using waters with lower pH (pH 5.7, 6.8 and 7.6), than 5 min of microwaving or steaming, as also stated by the pseudo 2D $^{1}$H DOSY-NMR data (Fig. S2).

### 3.5. $^{1}$H DOSY-NMR analysis of the food extracts

2D $^{1}$H DOSY-NMR spectra were also collected for the acetonic extracts of the 5 min microwaved, steamed and water-boiled vegetables in the various mineral waters (pH 5.7, 6.8, 7.6 and 9.5). A DOSY analysis was carried out to generate pseudo-2D plots of chemical shifts *versus* diffusion coefficients. Fig. S2 shows the molecular weights estimated from the diffusion coefficients. Small molecules (<1000 g/mol) typically show diffusion coefficients in the range of $1 \times 10^{-10}$ to $1 \times 10^{-8}$ $m^2$ $s^{-1}$, as seen for the diffusion coefficients obtained. Even if the thermal treatments led to some matrix softening, facilitating the extraction of bioactive compounds, the cooked material kept its main compounds and integrity, as judged by the similar profile in sugars, amino acids and phenolics freely diffusing in the extracts of cooked legumes, when compared to raw material. The NMR signals of the pseudo-2D plots were assigned to aromatic and aliphatic protons, associated with diffusion coefficients of low weight compounds/moieties such as of hydroxybenzene (94.11 g/mol), glycine (75.07 g/mol), alanine (89.09 g/mol), threonine (119.12 g/mol), leucine/isoleucine (131.18 g/mol), valine (117.15 g/mol), tyrosine (181.19 g/mol), phenylalanine (165.15 g/mol), hexoses (180.16 g/mol), and sucrose (342.30 g/mol), as well as quercetin (302.24 g/mol), and kaempferol (286.23 g/mol).

### 3.6. Colour measurements

Colour measurements based on the CIE parameters (lightness ($L^*$), redness-greenness ($a^*$), and yellowness-blueness ($b^*$), colour saturation ($C^*$) and hue angle ($H^\circ$)), were also carried for the raw and 5-min cooked broccoli and green bean pods (Table 2). Upon cooking, all vegetables presented a reduction in lightness ($L^*$) and total colour differences ($\Delta E^*$) ranging from 14 to 16 for broccoli and from 12 to 19 for green bean pods, when compared to raw material (Table 2, Fig. S3).

Cooked broccoli presented significant ($p < 0.05$) reductions in lightness of ~40 %, 38 % and 39 % for water boiled, steamed and microwaved samples, respectively. This data agrees with previous works, that also reported a decrease in lightness caused by the heat treatment, due to cell disruption and conversion of chlorophylls (bright green) into pheophytins (olive greyish brown), as seen for broccoli (Czarnowska-Kujawska, Draszanowska, & Starowicz, 2022) and other Brassica vegetables, such as galega kale (Armesto et al., 2016, 2017). In addition, regarding greenness ($a^*$) and yellowness ($b^*$), cooked broccoli increased their levels of greenness ($-a^*$) and yellowness ($+b^*$) relatively to crude material, particularly when microwaving, steaming and water-boiling samples with more alkaline waters (Table 2 and Fig. S3). Likewise, a more greenish-yellow colour, related to a decrease of the hue angle ($H^*$) from 124.4° (raw), and an increase in colour saturation ($C^*$), indicating higher colour intensity perception by humans, was obtained for the boiled samples in alkaline waters (pH 7.6 and pH 9.5), in agreement with the highest scores (~8 pts) obtained for "colour" in the 9-point preference test. Overall, all cooking methods caused a change in the colour of broccoli when compared with raw material, with total colour differences ($\Delta E^*$) ranging from 14 to 16 (Table 2 and Fig. S3), particularly in the case of the broccoli water-boiled with the mineral waters with varied pH ($\Delta E^* > 15$), with waters with pH 9.5 and pH 6.8 leading to $\Delta E^*$of 15.6 and 16.3, respectively.

Regarding the colour parameters of green bean pods, microwaved (↓17 %) and steamed (↓20 %) samples presented lower and similar reductions in lightness ($L^*$), whereas water-boiled samples presented higher reductions (~↓28 %), particularly the sample cooked with mineral water with pH 9.5 (↓30 %). Conversely, unlike for broccoli, cooked green bean pods showed a reduction in their levels of greenness ($-a^*$) and yellowness ($+b^*$), as well as a decrease in colour saturation ($C^*$) and an increase in hue angles ($H^\circ$) from 120.1° (raw) related to more greenish-blue tones (Fig. S3), especially for water-boiled samples ($\Delta E^*$ from 16 to 19) with more alkaline waters (pH 9.5; ↓$a^*$ 30 %, ↓$b^*$ 47 %, ↓$C^*$ 43 %), as also stated by the overall total colour difference ($\Delta E^*$ of 19) obtained (stronger green colours). These colour differences between boiling with more acidic (pH 5.7); neutral (pH 6.8) or basic (pH 7.6 and pH 9.5) waters relate to mineral contents, polyphenol oxidation and antioxidant/anthocyanin colour change to colourless and greenish-yellow tones at more alkaline pH (7–10), as well as to the chemical changes occurring with chlorophylls and other pigments. Hence, the total amount of chlorophylls, xanthophylls and carotenes present in the samples of the 5-min cooked vegetables was also analysed.

### 3.7. Pigments

Total pigment concentrations, chlorophylls and carotenoids (xanthophylls and carotenes) were quantified in the raw and 5 min-cooked samples of broccoli and green bean pods. Results are shown in Fig. 4. All cooked samples presented a decrease in pigment concentration when compared to uncooked vegetables. In addition, all samples are richer in chlorophylls, especially in chlorophyl *a*, than in carotenoids (*c*), as expected for dark-green vegetables. Moreover, water-boiled samples led to

**Table 2**
Colour parameters of green bean pods and broccoli florets according to ICI: $L^*$, $a^*$ and $b^*$, colour saturation $C^*$, hue angle $H^\circ$, and overall difference $\Delta E^*$.

| Colour parameter | | $L^*$ | $a^*$ | $b^*$ | $C^*$ | $H^\circ$ | $\Delta E^*$ |
|---|---|---|---|---|---|---|---|
| **Green bean pods** | | | | | | | |
| raw | | 47.98 ± 1.67 $^{a}$ | −13.13 ± 0.52 $^{b}$ | 22.63 ± 1.47 $^{a}$ | 26.17 ± 1.52 $^{a}$ | 120.14 ± 0.72 $^{b}$ | – |
| steamed | | 38.52 ± 5.98 $^{a}$ | −12.15 ± 0.59 $^{a}$ | 14.07 ± 2.04 $^{b}$ | 18.60 ± 1.90 $^{b}$ | 131.00 ± 3.00 $^{a}$ | 12.95 ± 5.85 $^{a}$ |
| microwaved | | 39.67 ± 7.12 $^{a}$ | −11.08 ± 1.99 $^{a}$ | 14.81 ± 5.24 $^{b}$ | 18.54 ± 5.35 $^{b}$ | 127.71 ± 5.03 $^{a}$ | 11.75 ± 8.76 $^{a}$ |
| water-boiled | tap pH 7.6 | 34.39 ± 2.42 $^{b}$ | −11.88 ± 0.62 $^{a}$ | 14.00 ± 1.57 $^{b}$ | 18.37 ± 1.60 $^{b}$ | 130.43 ± 1.63 $^{a}$ | 16.15 ± 2.90 $^{a}$ |
| | pH 5.7 | 35.52 ± 2.15 $^{b}$ | −10.91 ± 1.12 $^{a}$ | 12.91 ± 4.29 $^{b}$ | 17.06 ± 3.40 $^{b}$ | 131.24 ± 9.87 $^{a}$ | 16.11 ± 4.12 $^{a}$ |
| | pH 6.8 | 34.58 ± 0.66 $^{b}$ | −10.71 ± 0.59 $^{a}$ | 12.74 ± 0.24 $^{b}$ | 16.65 ± 0.35 $^{b}$ | 130.05 ± 1.82 $^{a}$ | 16.84 ± 0.64 $^{a}$ |
| | pH 9.5 | 33.42 ± 0.65 $^{b}$ | −9.05 ± 1.30 $^{a}$ | 11.92 ± 1.70 $^{b}$ | 14.98 ± 2.09 $^{b}$ | 127.23 ± 1.73 $^{a}$ | 18.58 ± 1.43 $^{a}$ |
| **Broccoli florets** | | | | | | | |
| raw | | 36.44 ± 3.55 $^{a}$ | −10.25 ± 1.11 $^{a}$ | 14.98 ± 2.00 $^{b}$ | 18.16 ± 2.27 $^{b}$ | 124.45 ± 1.02 $^{a}$ | – |
| steamed | | 22.65 ± 1.84 $^{b}$ | −11.26 ± 1.39 $^{b}$ | 18.69 ± 1.34 $^{a}$ | 21.83 ± 1.75 $^{a}$ | 121.00 ± 2.10 $^{a}$ | 14.43 ± 1.45 $^{a}$ |
| microwaved | | 22.22 ± 2.09 $^{b}$ | −11.64 ± 0.53 $^{b}$ | 18.30 ± 0.69 $^{a}$ | 21.69 ± 0.76 $^{a}$ | 122.46 ± 1.10 $^{a}$ | 14.70 ± 1.86 $^{a}$ |
| water-boiled | tap pH 7.6 | 22.33 ± 1.68 $^{b}$ | −11.87 ± 0.37 $^{b}$ | 18.77 ± 1.32 $^{a}$ | 22.22 ± 1.08 $^{a}$ | 122.37 ± 2.19 $^{a}$ | 14.73 ± 1.82 $^{a}$ |
| | pH 5.7 | 21.67 ± 2.98 $^{b}$ | −10.99 ± 0.95 $^{b}$ | 17.85 ± 1.24 $^{a}$ | 20.96 ± 1.54 $^{a}$ | 121.59 ± 0.68 $^{a}$ | 15.18 ± 2.57$^{a}$ |
| | pH 6.8 | 20.52 ± 2.97 $^{b}$ | −11.22 ± 1.26 $^{b}$ | 17.18 ± 1.96 $^{a}$ | 20.52 ± 2.25 $^{a}$ | 123.17 ± 1.62 $^{a}$ | 16.26 ± 2.61$^{a}$ |
| | pH 9.5 | 21.31 ± 0.71 $^{b}$ | −11.97 ± 0.81 $^{b}$ | 17.74 ± 1.60 $^{a}$ | 21.41 ± 1.67 $^{a}$ | 124.06 ± 1.73 $^{a}$ | 15.55 ± 0.47 $^{a}$ |

Values are mean ± standard deviation (sd) of triplicates. $^{(a\text{-}b)}$Different superscripts in the same column indicate statistical differences ($p < 0.05$) between the 5-min cooking treatments applied (steaming, microwaving and water boiling with different mineral waters). Post-hoc test outcomes were derived from Tukey's test for cases of homogeneous variance.

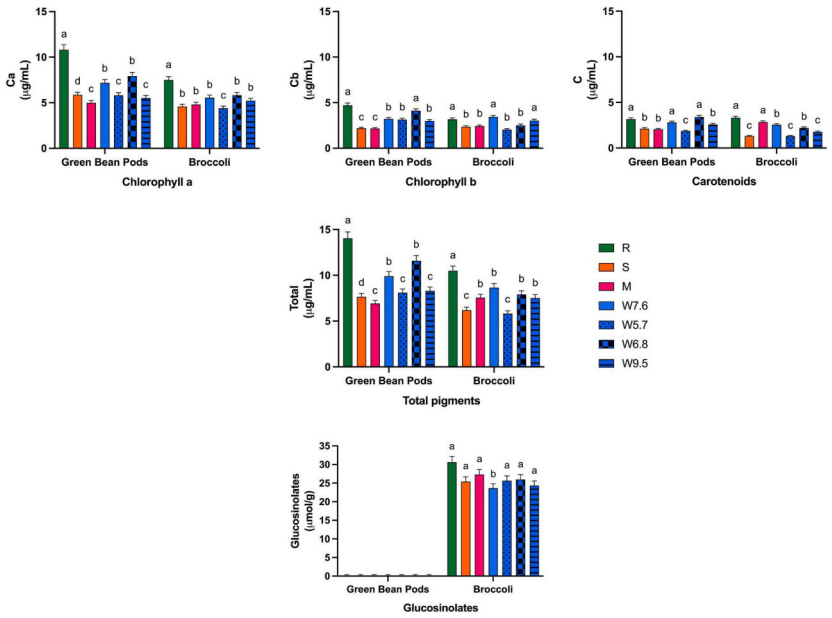


**Fig. 4.** Total pigments, chlorophyll *a* (Ca), chlorophyll *b* (Cb) carotenoids (C) and glucosinolates of raw (R), 5-min steamed (S), 5-min microwaved (M), and 5-min water-boiled (W) green bean pods and broccoli florets, by making use of the various mineral waters with variable: pH 5.7 (W5.7), pH 6.8 (W6.8), pH 7.6 (W7.6) and pH 9.5 (W9.5). Values are mean and standard deviations of triplicates. Different superscripts among columns indicate statistical differences ($p < 0.05$).

the extraction of more chlorophylls and carotenoids than microwaved or steamed samples. Differences were also found among the water-boiled legumes with the various mineral waters. Vegetables cooked with more acidic (pH 5.7), as well as with more alkaline (pH 9.5) mineral waters presented lower amounts of chlorophyl *a,* chlorophyl *b,* and carotenoids than samples cooked with waters with pH 6.8 and pH 7.6. The lower amounts of pigments detected at low and high pH can result from pigment degradation at both ranges. Under more alkaline conditions, chlorophyllase enzymes are activated and chlorophylls are oxidized and produce allomerized derivatives (Gandul-Rojas & Gallardo-Guerrero, 2014), while at acidic pH, chlorophylls and chlorophyllides lose their metallic Mg-centre and suffer conversion to pheophytins and pheophorbides, respectively, which are greyish-brown pigments (Ambra, Pastore, & Natella, 2023). Hence, given the influence of Mg and other minerals in the colour and stability of pigments and antioxidants, the mineral profile of the 5-min microwaved, steamed and water boiled broccoli florets and green bean pods was determined.

### 3.8. Mineral content

Plant metabolism depends on nitrogen (N), phosphorus (P), potassium (K), sulphur (S), calcium (Ca) and magnesium (Mg) as macronutrients (>0.1 g/kg dry weight), as well as on other minerals such as copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn), as micronutrients (<0.1 g/kg dry weight). Contents in P, S, K, Ca, Mg, Fe, Mn, Cu, Zn, Cl, and Na present in the raw and 5-min cooked vegetables were determined. Fig. 5 and Table S1 present the data obtained. The results show that broccoli florets were richer than green bean pods in most macrominerals (P, S, Ca and K), with amounts within the range of previously reported data (Quizhpe et al., 2024). In addition, regarding the cooking method, water boiling the legumes led to some significant ($p < 0.05$) loss of S (↓~15 % in bean pods and ↓~25 % in broccoli), K (↓~23 % in bean pods and ↓~50 % in broccoli), and Cl (↓~18 % in bean pods and ↓~30 % in broccoli), but to a general increase, particularly in the case of broccoli, in Ca (↑20 %), Fe (↑86 %), Zn (↑29 %), Mn (>100 %) and Cu (>100 %), when compared to raw material, as well as relatively to microwaved and steamed samples. Since these divalent minerals are cofactors of various metalloenzymes involved in several intracellular mechanisms (e.g.: redox equilibrium and mitochondrial respiration), they can remain retained/protein-bound in the plant matrix, if short boiling times are used, as also seen for other vegetables (García-Sartal et al., 2013). Regarding the different types of water used, Ca, Fe, Zn, Mn and Cu were better retained in broccoli, when using water with higher pH (6.8, 7.6 and 9.5) than when using more acidic water (pH 5.7). In what respected Cl, even if all boiled samples presented a reduction relatively to raw, microwaved and steamed material, green bean pods and broccoli boiled with alkaline water (pH 9.5) were richer in Cl than when using neutral or acidic water. Cooking with more saline (6.9 mmol/L minerals) and alkaline water (pH 9.5, 1.9 mmol/L $HCO_3^-$) also led to vegetables richer in sodium (↑57 % in green bean pods; ↑65 % in broccoli), due to the higher concentrations of Na (5.0 mmol/L $Na^+$) in the boiling water, when compared to the other mineral waters (with <0.9 mmol/L $Na^+$). Broccoli florets and green bean pods presented 248 ± 8 µg/g dw (24.8 mg/100g dw) and 148 ± 5 µg/g dw (14.8 mg/100g dw) of Na, respectively. In turn, cooking with less saline (0.7 mmol/L minerals) and acidic water (pH 5.7, 0.1 mmol/L $HCO_3^-$) led to some loss of macrominerals, particularly of K (↓30 % in bean pods and ↓52 % in broccoli), by ion diffusion, but also to some loss of S (↓20 % in bean pods and ↓32 % in broccoli) and P (↓18 % in bean pods and ↓6 % in broccoli), associated to peptides and other metabolites. Regarding Mg, the metallic centre of

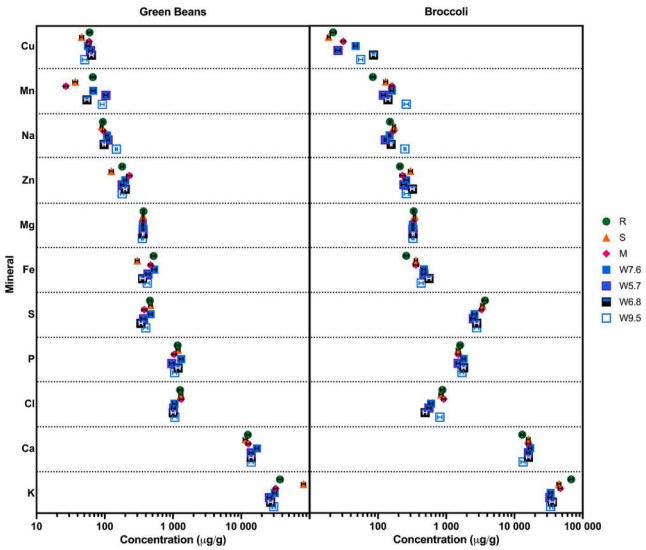


**Fig. 5.** Mineral profile of raw (R), 5-min steamed (S), 5-min microwaved (M), and 5-min water-boiled (W) green bean pods and broccoli florets by making use of various mineral waters with variable pH: pH 5.7 (W5.7), pH 6.8 (W6.8), pH 7.6 (W7.6) and pH 9.5 (W9.5). A logarithmic axis is used to simultaneously display contents in macro-minerals and trace elements. Values are mean and standard deviations (sd) of triplicates.

chlorophyll, few differences were observed among the different methods and types of water used. In addition, broccoli florets also presented some vestigial amounts of Br, Ni and Sr.

### 3.9. Principal component analysis

A principal component analysis (PCA) was conducted to correlate differences in minerals, antioxidants and polyphenols between vegetables and cooking treatments. Fig. S4 displays the PCA biplots for the two main components, explaining more than 70 % of the variance according to the PCA scree plot. Despite of the cooking treatment, the PCA analysis outlined the differences in the chemical composition of broccoli (left quadrants) and green bean pods (right quadrants). Both vegetables are rich in K and Fe, while broccoli florets are particularly rich in P, S, Ca, Na, Zn and Mn, and green bean pods in Mg, Cl and Cu.

Moreover, when analysing separately each vegetable by PCA, additional correlations were observed (Fig. 6). Green bean pods boiled in waters with pH close to neutrality (pH 6.8 and 7.6) presented higher amounts of Fe, Cu, Zn and pigments (chlorophyll *a*, chlorophyll *b* and carotenoids), when compared to samples boiled with lower (pH 5.7) or higher water pH (pH 9.5). The higher amounts of Mg also relate with the higher concentration of pigments, especially chlorophyll *a*, presented by common green bean pods. In turn, samples cooked in more alkaline water (pH 9.5) presented brighter colours and more Na, while samples cooked with more acidic water (pH 5.7) were able to better preserve polyphenols (TPC) but presented some loss of minerals. Conversely, a different pattern was found for broccoli florets, which are composed by a less fibrous matrix. Microwaved and steamed broccoli presented similar and higher amounts of antioxidants (AC) and polyphenols (TPC) when compared to water-boiled samples. Additionally, the composition presented by broccoli cooked with more acidic water showed a significant separation in the PCA biplot from raw material, as well as from the samples boiled with other waters (Fig. 6B). Broccoli florets boiled with more acidic water (pH 5.7) presented less minerals and less pigments, but more antioxidants (AC) and polyphenols (TPC) than when using water with higher pH (6.8, 7.6 and 9.5). Hence, the differences in colour detected in the CIE-$L^*a^*b^*$measurements, which also relate to lower scores in "colour" and "flavour" in the hedonic test, when using more acidic water (pH 5.7), can be associated to some loss of photosynthetic pigments, as well as to some chemical changes in anthocyanins/antioxidants.

### 3.10. Glucosinolates

Glucosinolates are natural thioglucosides particularly abundant in cruciferous vegetables of the Brassicaceae family (Prieto et al., 2019). Glucosinolates are synthesized from aliphatic, aromatic, or indole amino acids, and suffer enzymatic cleavage by myrosinases, enabling the formation of isothiocyanates upon removal of the glucose group (Blažević et al., 2020). These hydrolysis products are known for their beneficial effects associated with antioxidant, antiparasitic, antimicrobial and antineoplastic activity (Prieto et al., 2019). Cooking leads to thermal inactivation of plant myrosinases and to the preservation of glucosinolates in the plant material. The degree of myrosinase inactivation is influenced by the cooking method, temperature, pH and size of the cut pieces, also influencing the amount of glucosinolates retained in the vegetables. Hence, here, the total amount of glucosinolates was also determined in the raw and 5-min cooked broccoli florets and green bean pods. As expected, only broccoli presented a considerable amount of glucosinolates (>3.0 μmol/g dw), that varied according to the treatment applied (Fig. 4). Upon cooking, a reduction in glucosinolates was observed in all cases. According to previous studies, the glucosinolates found in broccoli are essentially aliphatic glucosinolates like glucoraphanin, progoitrin and sinigrin which have been described as more vulnerable to thermal degradation than aromatic glucosinolates (Abdel-Massih et al., 2023), explaining the reduction observed here

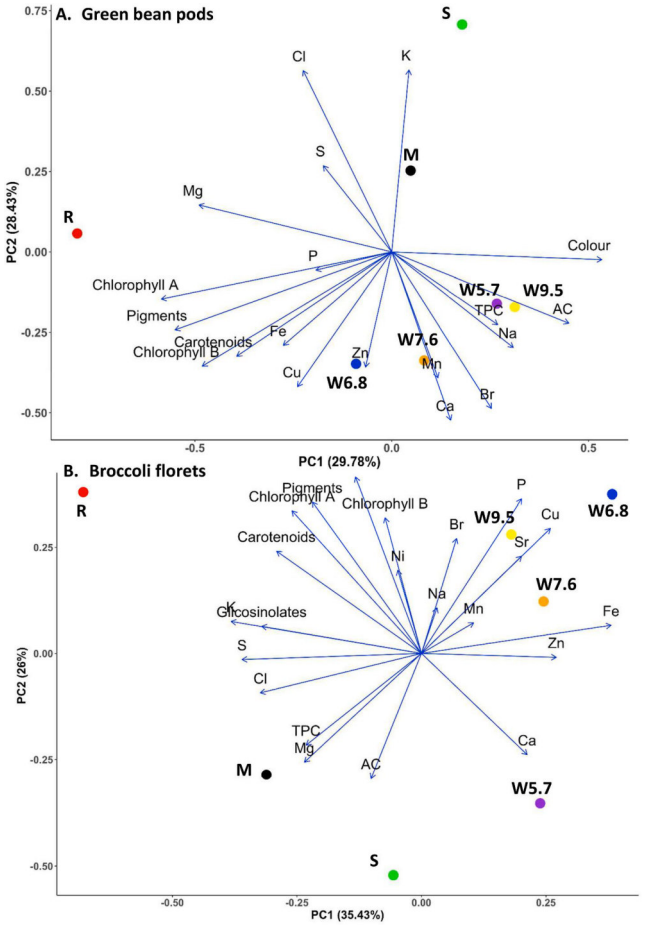


**Fig. 6.** PCA analyses relating green bean pods (A) and broccoli florets (B) overall colour, pigments (carotenoids, chlorophyll *a and b*), total phenolics (TPC), antioxidants (AC) and glucosinolates (for broccoli) of raw (R), 5-min steamed (S), 5-min microwaved (M), and 5-min water-boiled (W) vegetables in the various mineral waters with variable pH: pH 5.7 (W5.7), pH 6.8 (W6.8), pH 7.6 (W7.6) and pH 9.5 (W9.5).

upon 5-min thermal treatments. Moreover, when compared to raw material (5.39 ± 0.01 μmol/g dw), 5-min steamed, and 5-min microwaved broccoli presented lower reductions in glucosinolates (↓26 %), as corroborated by the PCA analysis, than 5-min water-boiled broccoli florets in alkaline water, pH 9.5 (↓40 %). Beyond promoting compound leaching to the decoction water, boiling broccoli at a different pH led to a differential retention of glucosinolates in the plant material. Glucosinolates are known to be more stable at more neutral pH, since at more acidic pH (pH < 4) they convert into nitrile derivates, and at more alkaline pH occurs the formation of isothiocyanates by non-enzymatic hydrolysis (Abdel-Massih et al., 2023). Hence, here, even if the acidic pH tested (pH 5.7) was not low enough to promote the formation of nitriles, the alkaline pH was high enough to promote glucosinolate hydrolysis, justifying the reductions observed, when using waters with higher pH (pH 7.6 and 9.5).

Moreover, glucosinolates and isothiocyanates are also responsible for the pungent aroma and bitter taste of the vegetables of the Brassicaceae family. Hence, the sensory properties presented by the vegetables will also be influenced by the cooking process (temperature, method, time, amount and type of water) since it influences the amount of glucosinolates in the cooked material. Therefore, the better acceptance, in terms of flavour, of broccoli florets boiled in more alkaline water (pH 9.5) can not only be related to the higher amount of sodium and other minerals present in the plant material, but also to the lower amount of glucosinolates retained in the food matrix.

## 4. Conclusions

Plant-based foods have gained worldwide attention due to their high nutritive value and environmental sustainability. However, when uncooked, some plant matrices present poor organoleptic properties and reduced acceptance by consumers. Hence, to increase palatability and chewability, some vegetables like broccoli and green bean pods are generally microwaved, steamed or boiled. Beyond increasing the sensory properties of the vegetables, the heat treatment softens the food matrix and promotes compound extraction, especially in green bean pods that hold a more fibrous texture. Moreover, even if plant composition is very different among different vegetables, and can vary according to different maturation stages and edaphoclimatic conditions, it is possible to identify differences among cooking treatments and types of boiling water used. In addition, even if waters with different compositions and pHs are not always available to consumers, and if broccoli and green bean pods can be submitted to other cooking treatments (e.g. stir-frying, stewing, pressure cooking) and longer cooking times at higher temperature and pressure, here, it was possible to identify dietary quality variations, already after 5 min of water-boiling, steaming and microwaving. Boiling bean pods for 5 min enables to extract more polyphenols (TPC) and antioxidants (AC), particularly when using waters with lower pH (pH 5.7 and 6.8), than 5 min of microwaving or steaming. In turn, steaming and microwaving broccoli for 5 min leads to higher values of TPC and AC than 5 min of water boiling, due to diffusion of compounds to the decoction water and/or thermal instability. In addition, vegetables cooked with more acidic (pH 5.7) or alkaline (pH 9.5) waters retain lower amounts of pigments, due to conversion to pheophytins at lower pH or to chlorophyllase activation at higher pH, than samples cooked with waters with more neutral pH (6.8 or 7.6). Regarding greenness, different patterns are observed depending on the vegetable. Cooked broccoli florets increase their levels of greenness relatively to crude material, particularly when boiled in more alkaline waters (pH 7.6 and pH 9.5), also leading to higher scores in “colour”, “flavour” and “texture”. Conversely, cooked green bean pods reduce their levels of greenness, especially when using more alkaline water (pH 9.5). In addition, boiling can also lead to some loss of minerals (↓15 %–50 %), especially in broccoli. However, cooking with more saline (6.9 mmol/L minerals, 5.0 mmol/L $Na^+$) and alkaline water (pH 9.5) can lead to vegetables richer in sodium (↑57 % in green bean pods; ↑65 % in broccoli) and other minerals. Moreover, even when testing consumers preferences among a small focus group, with no experience on vegetable tasting, it is possible to identify preferences. Boiling broccoli with more acidic water can better preserve glucosinolates and other bioactive compounds, but the food material presents poor palatability. In turn, samples cooked in more alkaline water (pH 9.5) can present higher acceptability by the consumers, given their less bitter taste due to glucosinolate reduction, brighter colours, higher mineral retention and overall flavour. Therefore, all these factors need to be considered when designing and preparing healthier and tastier plant-based meals.

## CRediT authorship contribution statement

**Vânia S. Ribeiro:** Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Pedro F. Cruz:** Methodology, Investigation. **Zaida L. Almeida:** Methodology, Investigation. **Cândida G. Silva:** Formal analysis, Data curation. **Maria Isabel Silva:** Methodology, Investigation. **Inês S. Silva:** Methodology, Investigation. **Liliana R. Santos:** Methodology, Investigation. **Marta T. Santos:** Methodology, Investigation. **Carla Guimarães:** Writing – review & editing, Methodology, Investigation. **Rui M.M. Brito:** Resources. **Mauro Guerra:** Resources, Methodology, Investigation. **Fernando Reboredo:** Resources, Methodology, Investigation. **Cidália D. Pereira:** Writing – review & editing, Formal analysis, Data curation, Conceptualization. **Daniela C. Vaz:** Writing – review & editing, Resources, Methodology, Investigation, Formal analysis, Data curation.

## Ethical statement

Hedonic testing was conducted according to the appropriate protocols for protecting the rights and privacy of all participants (WMA Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Participants), along with full disclosure of study requirements and risks and possibility to withdraw from the study at any time. Written informed consent was obtained from all participants. Privacy rights were guaranteed. All data was treated anonymously.

## 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.lwt.2025.118014.

## Data availability

Data will be made available on request.

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