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How does the phytochemical composition of sprouts and microgreens from Brassica vegetables affect the sensory profile and consumer acceptability?

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ABSTRACT

Pre- and postharvest strategies have been developed during the last decades to increase yield, quality, and bioactive compounds of sprouts and microgreens. Depending on the growth stage, especially in the first stages, the phytochemicals amount differs and usually affects the sensory profile and consumer acceptability. The aim of this work was to compare key biocompounds content (organosulfur compounds and total phenolic content) with their sensory profile in two growing stages (sprouts and microgreens) of five *Brassica* species (kale, radish, rocket, broccoli, and mustard). A penalty analysis with consumer study data was also performed to optimize their harvest time by avoiding undesirable intensity of sensory descriptors. An online survey was conducted in two Mediterranean basin populations (Italy and Spain) to fix these product intentions and consumption preferences. Brassica sprouts were generally richer in organosulfur compounds than microgreens of the same species. This study confirms that some organosulfur compounds and/or their hydrolyzed compounds are linked with spicy/pungent sensory attributes in brassicas sprouts and microgreens. The results reported provide useful information for microscale vegetable farmers as a tool to determine their harvest time according to the content and composition of phytochemical compounds.

1. Introduction

Microscale vegetal production has been developed during the last decades obtaining new trendy formats from their first growing stages called sprouts, microgreens, and baby-leaves or baby-greens (Artés-Hernández et al., 2022; Du et al., 2022; Ebert, 2022; Marchioni et al., 2021; Sharma et al., 2022; Turner et al., 2020; Wojdyło et al., 2020; Verlinden, 2020). Sprouts (S) are immature vegetables, at the first growing stage of a plant, grown from seed germination, which growth cycle is around 4–10 days, and they are commonly consumed with root and shoot intact. Microgreens (M) can be defined as vegetables which have a longer growth cycle than sprouts (10–28 days), and the edible portion is constituted by stem and cotyledons, and often by the emerging first true leaves (di Gioia et al., 2017; Du et al., 2022; Ebert, 2022; Marchioni et al., 2021; Sharma et al., 2022; Turner et al., 2020; Verlinden, 2020; Wojdyło et al., 2020). Baby-leaves are young vegetables with an optimum leaf length of 8–10 cm and a leaf width of 7–8 cm, and the petiole must be present with a maximum length of 35 % of leaf length, but it depends on species and variety (Gil and Garrido, 2020). Both sprouts and microgreens (S&M) have gained popularity due to their being easy to grow, their sensory attributes, and their richness in bioactive compounds, reaching more than 20-fold phytochemicals content regarding the adult plant.

Among the most popular seeds used to produce S&M (cereals, legumes, oilseed, aromatic herbs) (Du et al., 2022; Ebert, 2022; Wojdyło et al., 2020), *Brassica* ones (Kale, Radish, Rocket, Broccoli, and Mustard, among others) a gaining great popularity for consumers due to their high content in key bioactive compounds. The main ones are organosulfur compounds, like glucosinolates (GLSs), from which isothiocyanates are biosynthesized, evidencing to be potent anticarcinogens and antimutagens (Ho et al., 2009; Kamal et al., 2020; Kyriacou et al., 2019; Vanduchova et al., 2019; Zhang et al., 2021). *Brassica* S&M also contain

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antioxidant bioactive compounds like ascorbic acid, carotenoids, anthocyanins, and phenolics, as well as minerals (macro and micro-elements) with high bioaccesibility and bioavailability (de la Fuente et al., 2019; Kyriacou et al., 2019; Zhang et al., 2021). Although the predominant GLS depends on the specie, and growth/development stage, among other variables, the main GLSs in kale are sinigrin, and glucobrassicin (Hahn et al., 2016), while in radish are glucoraphenin and glucoraphasatin (Wang et al., 2017; Yi et al., 2016), while glucoerucin and glucoraphanin are in rocket (Martínez-Zamora et al., 2022). Glucoraphanin and glucobrassicin were previously quantified as the main GLSs in Broccoli (Li et al., 2021), while glucosinalbin has been reported as the main GLS in mustard (Guijarro-real et al., 2022).

Considering the growth/development stage, brassica S&M contain higher nutritional/functional quality than their respective brassica vegetables' edible parts at the mature stage (Ebert, 2022). Between S&M, several studies reported that S contain more phytochemicals than M (Baenas et al., 2017; di Bella et al., 2020). Abiotic stress strategies have also been developed to enhance key bioactive compounds such as pre and postharvest UV-B and LED lighting, among others (Artés-Hernández et al., 2022a, 2022b; Zhang et al., 2020).

However, a high phytochemical content should be considered in microscale vegetables, as well as sensory profile and consumer acceptance. In this sense, does the phytochemical composition affect the sensory profile and consumer acceptability? It is well-known that the sensory chemistry of *Brassicaceae* species is complex (mainly due to the organosulfur compounds and phenolics), reaching gustatory, olfactory and pain nerves and receptors. Among organosulfur compounds, glucosinolates, their hydrolysis compounds and sulfur-volatile compounds are responsible for their taste, aroma, and trigeminal characteristics (mainly, bitter taste, sulfurous aroma, and pungency/spicy sensations, respectively) (Bell et al., 2018; Di et al., 2023; Sansom et al., 2015; Zeng et al., 2021; Zhou et al., 2018). Previous studies investigated consumer sensory perception and acceptability of several brassica microgreens species, and potential flavor attributes drivers/barriers to consumer acceptance (Caracciolo et al., 2020; Michell et al., 2020; Xiao et al., 2015). However, there is a lack of studies comparing the sensory profile and consumer acceptance between S vs M of the same species under the same growing conditions correlating them with the phytochemical composition, which is the main novelty of this work.

Therefore, the aim of this work was to compare organosulfur bioactive compounds and total antioxidant activity, with the sensory attributes and consumer acceptance in two growing stages (S and M) of five different Brassica species (Kale, Radish, Rocket, Broccoli, and Mustard). Relationships between identified and quantified organosulfur compounds and sensory attributes of S&M were also analyzed. Finally, an online survey concerning the intentions and consumers' preferences for these products was conducted in two populations of the Mediterranean basin (i.e., Italy and Spain). As far as we are concerned, this research is the first one in which S&M are compared in their organosulfur compounds, total phenolic compounds, and sensory attributes.

2. Materials and methods

2.1. Plant material, seed germination, minimal processing, and experimental design

Kale (*Brassica oleracea* var. *sabellica*), Radish (*Raphanus sativus*), Rocket (*Eruca vesicaria*), Broccoli (*Brassica oleracea* var. *italica*) seeds were supplied by Intersemillas S.A. (Valencia, Spain) and Mustard (*Sinapis alba*) seeds by Semillas Batlle S.A. (Barcelona, Spain). As previously described (Martínez-Zamora et al., 2021), three grams of seeds from each specie were weighed and washed with 40 mL of autoclaved distilled water. A laminar flow cabinet (Telstar Bio-II-A/M, Japan) was used for sowing, where seeds were arranged in 500 mL polypropylene trays (TR-500; 118 × 96 × 44 mm). Subsequently, ecological substrate (70 % brown peat, 25 % blond peat, and 5 % green compost; Geolia brand) was used as a support at the bottom of the tray, which was moistened with autoclaved distilled water. The seeds were placed under a thin layer of substrate, and a 40-μm film was used to partially close the tray and ensure high relative humidity (RH) in the trays. These trays were previously sterilized on a UV-C light equipment for 30 min. Conditioned trays with seeds were kept in the germination chamber (Sanyo MLR-350 H, Tokio, Japan) for 3 days at 20 °C, 90 % RH, and under darkness conditions. Then, growing for 3–17 days at 20 °C under a photoperiod of 16 h light/8 h darkness in a chamber. After 7 days at 20 °C, the film was removed to allow the sprouts to grow into microgreens (up to 17 days). During this period, S&M were irrigated twice per day. On each sampling day (3, 5, 7, 12, 15, and 17 days), S&M were harvested, frozen, and freeze-dried for further analysis. Three replicates per sampling time of each sample were carried out. For sensory analysis (10 replicates/trays per sample), samples harvested at 7 days for sprouts (Kale^S, Radish^S, Rocket^S, Broccoli^S, and Mustard^S) and 17 days for microgreens (Kale^M, Radish^M, Rocket^M, Broccoli^M, and Mustard^M) were minimally processed at 12 °C where they were disinfected for 1 min with a 150-ppm NaClO solution at 5ºC, rinsed in cold water for 1 min and drained.

2.2. Morphological and color parameters

S&M were placed and photographed on a white piece of paper next to a ruler to determine their length using the ImageJ program (Wayne Rasband, Maryland, USA). The sprouts were characterized by the length of the hypocotyl and roots, while microgreens were only characterized by the length of the hypocotyl. The results were expressed in cm. The coordinates (L*, a*, and b*) from CIELab color space were measured in hypocotyl and leaf of sprouts and microgreens using a colorimeter (CR-400, Konica Minolta, Tokyo, Japan). For that, 10 S&M hypocotyls were placed together with no gaps, which constituted a replicate and in the same manner with 5 S&M leaves. Three replicates were performed for each sampling day, specie type, and growth stage.

2.3. Glucosinolate and isothiocyanate extraction and analysis

The extraction of desulfoglucosinolates and identification of individual GLS (GLS$_i$) content was carried out following the method previously described by Martínez-Zamora et al. (2021b). Briefly, 10 mL of ethanol/water (70:30, v/v) previously heated at 70 °C in a bath were added to sprouts/microgreen powder. To ensure myrosinase inactivation, samples were incubated at 70 °C for 30 min and vortexed at 0, 10, and 20 min. The extracts were removed from the water bath, rapidly cooled on an ice bath, and centrifuged at 18,000 g for 10 min at 4 °C (L-90 K Ultracentrifuge Beckman Coulter, rotor type 45 70Ti). The supernatant was recovered for analysis. Immediately after the extraction, GLS were desulphated and purified using disposable polypropylene columns (Thermo Fisher Scientific, Waltham, MA, USA). 3 mL of clarified ethanolic extract were added into a prepared column and allowed to drip through slowly. Columns were washed with 2 × 0.5 mL of MilliQ water followed by 2 × 0.5 mL of 0.02 M sodium acetate. Purified sulfatase (75 μL) was added to each sample and left at room temperature overnight (16 h). Desulfoglucosinolates were eluted with a total of 1.25 mL of water and kept at − 80 °C until UHPLC analysis. An Ultra High Performance Liquid Chromatography (UHPLC) instrument (Shimadzu, Kyoto, Japan) equipped with a DGU-20A degasser, LC-30CE quaternary pump, SIL-30AC autosampler, CTO-10AS column heater, and SPDM-20A photodiode array detector was used. Chromatographic analyses were carried out into a Gemini C18 column (250 mm x 4.6 mm, 5 μm particle size; Phenomenex, Torrance CA, USA). Separation of desulfoglucosinolates in the UHPLC system was achieved using water (phase A) and acetonitrile (phase B) as mobile phases with a flow rate of 1.5 mL min−1 and a gradient of 0/100, 28/80, 30/100 (min/% phase A) with an injection volume of 20 μL. Desulfoglucosinolates were detected at 227 nm. The individual identified GLS were i) GLS$_1$: dsf-sinigrin, ii) GLS$_2$:

Table 1
Morphological and color parameters of sprouts (after *7* growing days at 20ºC; gray columns) and microgreens (1*7* growing days at 20ºC; red columns) of Brassicas species (*n* = 3).

Type	Length			Color coordinates					
	Hypocotyl	Roots	Total	Hypocotyl			Leaves		
	cm			L*	a*	b*	L*	a*	b*
	***[††]	***	***	***	***	***	**	***	**
Kale^S	5.6 cd[‡‡]	3.5bc	9.1c	62.5cde	-5.4ab	18.1d	45.3cde	-12.4a	24.3de
Kale^M	7.0b	nd	7.0d	60.5de	-5.5ab	20.6 cd	42.0e	-13.1ab	23.7e
Radish^S	5.9bcd	10.4a	16.3a	70.1a	-4.3a	13.5e	51.6a	-17.6c	35.9a
Radish^M	11.7a	nd	11.7b	59.6e	-12.1d	29.1a	48.2abcd	-17.1c	30.6ab
Rocket^S	3.1 g	3.7b	6.8d	66.5b	-6.1ab	20.9 cd	46.3bcde	-14.7b	28.0bcd
Rocket^M	4.7de	nd	4.7ef	63.7bcd	-8.5c	25.4ab	44.7de	-14.7b	25.9cde
Broccoli^S	3.5fg	2.7bc	6.3de	62.0cde	-6.8bc	21.8bcd	46.1cde	-14.7b	24.7de
Broccoli^M	3.8 fg	nd	3.8 f	65.0bc	-8.2c	23.5bc	45.5cde	-14.8b	26.2cde
Mustard^S	4.3e	2.4c	6.7d	66.8ab	-6.1ab	20.6 cd	50.9ab	-14.6b	29.0abc
Mustard^M	6.3bc	nd	6.3de	61.4de	-11.8d	29.1a	49.9abc	-17.2c	28.9abc

^{S}: sprouts (7 growing days); ^{M}: microgreens (17 growing days); [††]NS: not significant at $p > 0.05$; * , * *, and * ** , significant at $p < 0.05$, 0.01 and 0.001, respectively; [‡‡]Values (mean of 3 replications, a replicate was formed by 10 sprouts) followed by the same letter, within the same column, were not significantly different ($p > 0.05$); different letters in the same column denotes significant differences ($p < 0.05$) among samples; nd = no determined

dsf-glucoraphanin; iii) GLS_3: dsf: glucosinalbin; iv) GLS_4: dsf: hydroxyglucobrassicin; v) GLS_5: dsf-glucoerucin; vi) GLS_6: dsf-glucoraphasantin; vii) GLS_7: dsf.glucobrassicin, viii) GLS_8: dsf-methoxy-glucobrassicin; and, ix) GLS_9: dsf-neoglucobrassicin. Identification of GLS_i was done following Table 2 details. Glucoraphanin (PhytoLab GmbH & Co, Germany) was used as a standard to quantify, and the results were expressed as g kg^{-1} dry weight (dw). Each sample was extracted and analyzed in triplicate.

Sulforaphane was extracted and analyzed following the method previously described by Martínez-Zamora et al. (2021b). For that, 5 µL sulforaphane extracts were injected in a U-HPLC (Shimadzu, Kyoto, Japan) equipped as described in the method with a Gemini C18 column (250 mm × 4.6 mm, 5 µm particle size; Phenomenex, Torrance CA, USA). The mobile phases, 0.02 mol L^{-1} ammonium formate (A) and acetonitrile (B) with a 0.6 mL min^{-1} flow rate were prepared to detect sulforaphane at 196 nm. Sulforaphane was quantified using DL-sulforaphane standard (Sigma-Aldrich, St. Louis, MO, USA), and results were expressed as mg kg^{-1} dw. Identification of n-SFN (sulforaphane nitrile) and SFN (sulforaphane and sulforaphene) were done following Table 1 details. Each sample was analyzed in triplicate.

2.4. Total polyphenolic content (TPC)

Total polyphenolic content (TPC) was determined as previously described (Singleton et al., 1999). Briefly, 19 µL sample extract were placed on a 96-well plate (Greiner Bio-One; Frickenhausen, Germany) and 29 µL of 1 mol L^{-1} Folin–Ciocalteu reagent were added. After 3 min incubation in darkness, 192 µL of 0.4 % Na_2CO_3 2 % NaOH were added. The absorbance was measured at 750 nm using a microplate reader (Tecan Infinite M200, Männedorf, Switzerland) after 1 h incubation in darkness. The TPC was expressed as g gallic acid equivalents (GAE) kg^{-1} dw. Each sample was analyzed in triplicate.

2.5. Descriptive sensory analysis

A trained panel consisting of 5 trained judges from the Institute of Plant Biotechnology -IBV- of Universidad Politécnica de Cartagena -UPCT- (Murcia, Spain) conducted the descriptive sensory analysis. The panel was selected and trained following the ISO standard 8586–1. For the present study, the panel discussed the main sensory characteristics of brassica S&M. Table S1 shows the lexicon used for describing sensory attributes (n = 6) with references and intensities based on previous works (Baik et al., 2003; Bell et al., 2018; Clemente-Villalba et al., 2021, 2020; Prieto et al., 2019; Sansom et al., 2015) with some modifications. The scale ranged from 0 (no intensity) to 10 (extremely high intensity) with 0.5 increments. Samples were served into odor-free, disposable 50 mL covered plastic cups at room temperature, and coded with 3-digit randomized numbers. Each judge tested ~10 g of each sample, and each panelist tested the samples in a randomized order. Water and unsalted crackers were provided to the judge.

2.6. Consumer study

A sample group of 60 consumers was recruited within the UPCT and consisted of 31 men and 29 women aged between 18 and 69 years who regularly consume leafy vegetables. It is a relevant number but that above 100 consumers are needed to have more solid conclusions. The consumer study was conducted at the IBV facilities, during 1 session of 5 h. Consumers tested 5 brassicas species (S&M) samples. Each consumer was served ~ 10 g of each sample coded with 3-digit numbers and the questionnaire. Water and unsalted crackers were provided between samples for palate cleaning. Consumers were asked to taste each sample, express their overall liking and "attribute" liking using a 9-point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely). The following question was used for global satisfaction/overall, and different attributes (appearance, leaves color, hypocotyl color, size, bitterness, spicy, astringency, herbal-vegetal and ID flavor): How much do you like the "attribute" of this sample?. Data were subjected to ANOVA after checking the normality and homogeneity of the variance and to Tukey's multiple-range test to compare means. Also, questions regarding attributes intensity using a Just About Right (JAR) scale for the above attributes were asked: "Considering the intensity of the "attribute", you would say…". The data curation was done following the penalty analysis tool and the XLSTAT software as it is mentioned in the statistical section.

In addition, a convenience sample of 436 consumers was recruited to take part in the online survey through emails sent by UPCT (Spain) and University of Milan (Italy) listservs for faculty, staff, and students, flyer distribution, and word of mouth. Participants were divided into two groups based on their native language (Spanish or Italian). Of the 436 interviewed participants, 136 failed to complete the survey, giving a final sample of 300 subjects: i) Italian group (n = 148, women: 61.5 %; men: 38.5 %) and Spanish group (n = 152, women: 69.7 %; men: 30.3 %). As regards age, a higher proportion of subjects between 18 and 39 years old were found in the Italian group (71.6 %), while Spanish participants were more likely to be older (54.6 %). The majority of participants in both groups declared to have obtained a university degree (> 80 %). The level of neophobia (Food Neophobia Scale, FNS) was calculated as an average score and by dividing the subjects into three classes, based on cut-offs previously defined and validated on a representative sample of the Italian population. The subjects were divided into neophilic (FNS $\leq$25), moderate (25 $<$ FNS $<$35), and neophobic (FNS $\geq$35) (Laureati et al., 2018). To study the visual preference to buy and consume sprouts and/or microgreens, different randomized coded pictures were presented to the survey respondents, as shown in Fig. S1. After selection, the respondents had to choose from a list of reasons which had led them to choose that image, being those: "I like the color of the hypocotyl", "I like the color of the leaves", "I like the number of leaves", "I like the size", "I like the elongated shape of the leaves", "I like the shape of the leaves to be rounded", "The leaves are whole (not broken)", "they are in good condition (not yellowed)".

Both the consumer study and online survey were performed according to the principles established by the Declaration of Helsinki, and the survey was approved by the UPCT research ethics committee (Expedient nº CEI22_004 and nº CEI22_006 for hedonic test and online survey, respectively).

2.7. Statistical analyses

Statistical analysis and comparison among means were carried out using the statistical package. Table 1 and Table 3 shows the one–way ANOVA test using "day" for each brassica specie during growth/development cycle (n = 6 days for each specie: 3, 5, 7, 12, 15, and 17d). Fig. 1, Fig. 2, Table S2, and Table S4 shows the one–way ANOVA test using "sample" (n = 10 samples: 5 brassica species * 2 growth stage: Kale^{S}, Radish^{S}, Rocket^{S}, Broccoli^{S}, Mustard^{S}, Kale^{M}, Radish^{M}, Rocket^{M}, Broccoli^{M}, and Mustard^{M}). Tukey test was used for means comparison (95 % confidence level). Principal component analysis (PCA regression map) was conducted to project the samples depending on the phytochemicals (glucosinolates, sulforaphane, and total phenolic content) and descriptive sensory analysis based on Pearson correlation. Penalty analysis using XLSTAT Premium 2016 (Addingsoft, Barcelona, Spain) was conducted thanks to hedonic and JAR questions to provide information about the possible improvements of some samples. The independent samples t-test was used to determine whether a between-group effect occurs for food neophobia scores. Results of paired preference test (two-

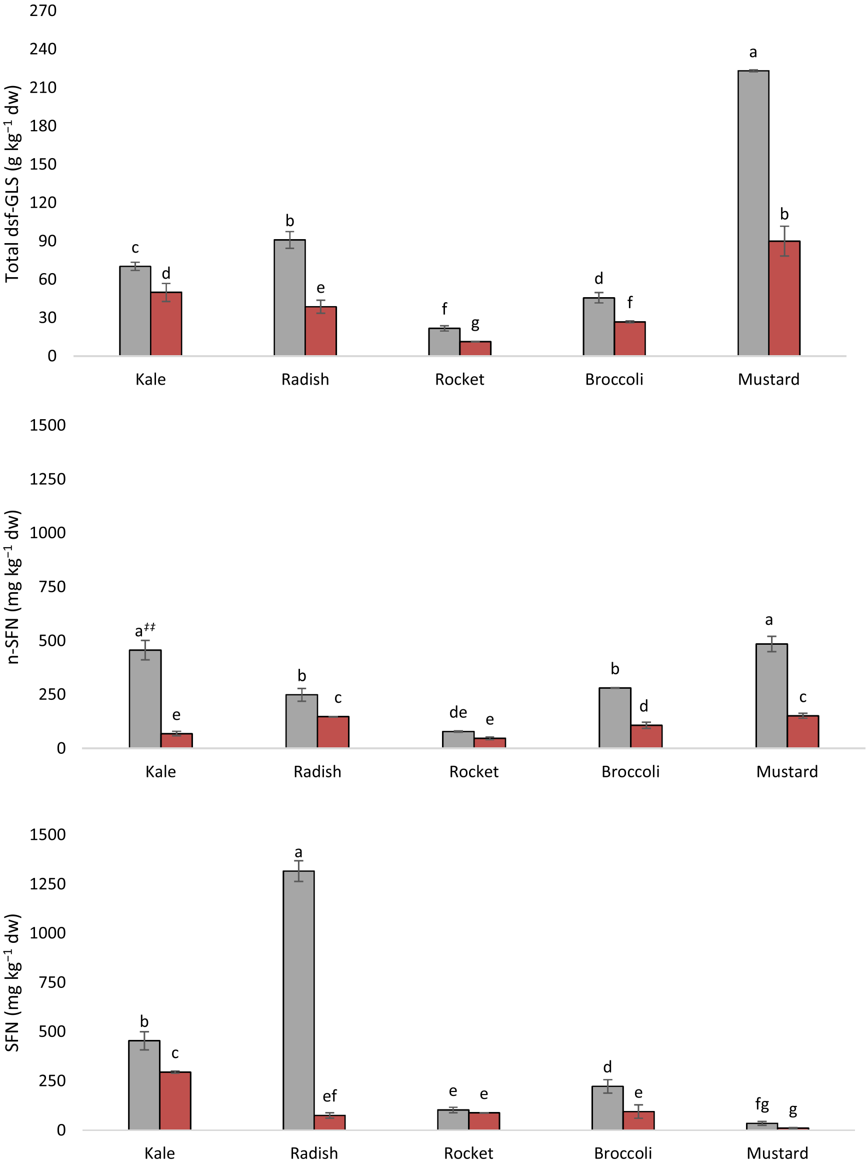

Fig. 1. Sulfur compounds between sprouts (after 7 growing days at 20ºC; gray columns) and microgreens (17 growing days at 20ºC; red columns) of Brassicas species: a) ∑GLS; b) SFN; and, c) n-SFN. ‡‡*Columns with the same letter were not significantly different ($p > 0.05$).* (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

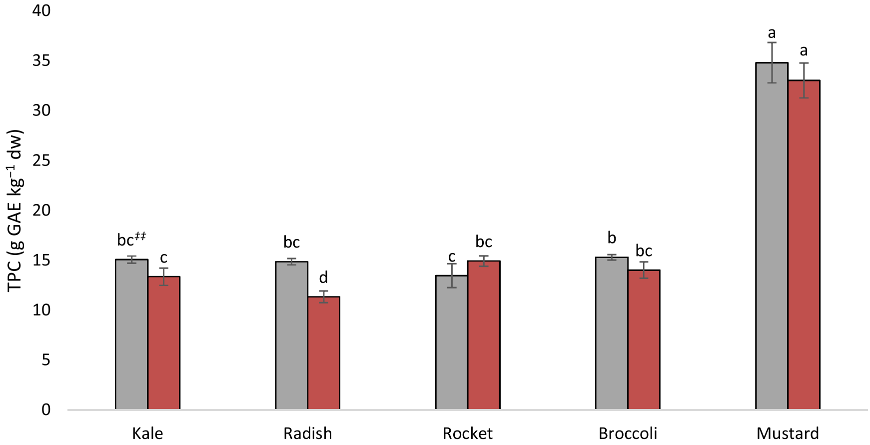

Fig. 2. Total phenolic compounds between sprouts (7 growing days at 20ºC; gray columns) and microgreens (after 17 growing days at 20ºC; red columns) of Brassicas species. *‡Columns with the same letter were not significantly different ($p > 0.05$).* (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

tailed) were analyzed based on the minimum value required for a significant preference between two samples, as previously reported (Lawless Harry and Heymann, 2010).

3. Results and discussion

3.1. Sprouts and microgreens characterization

According to results shown in Table 1, the S hypocotyl length ranged from 3.1 to 5.9 cm after 7 growing days at 20ºC, while M hypocotyl length ranged from 3.8 to 11.7 cm after 17 days at 20ºC (Table 3). The longest sprout hypocotyl length corresponded to Radish^{S}, and even these showed the highest growth rate. The lowest growth rate corresponded to Broccoli^{M}, which only increased its hypocotyl length by 8.6 % compared to Broccoli^{S}. The root length was just characterized in sprouts since, according to their definition, the sprouts are harvested, including their root. In this case, the greatest root length corresponded to Radish^{S}. However, no correlations were observed between hypocotyl and root lengths. Other authors reported similar morphometric characteristics in broccoli sprouts, with hypocotyl length ranging from 36.0 to 38.8 mm, although this may vary according to variety (di Bella et al., 2021).

The color of cotyledons/leaves and hypocotyl in two growth stages (S&M) were also characterized (Table 3). The leaf color of the 5-brassica species is green, and the main color parameters ranged from 42 to 51.6 for L* , from − 12.4 to − 17.6 for a* , and from 23.7 to 35.9 for b* , with no differences between S&M. The hypocotyl color of S&M ranged from 59.6 to 66.8, from − 4.3 to − 12.1, and from 13.5 to 29.1 for L* , a* and b* , respectively. There was no difference between the hypocotyl color of kale and broccoli S&M. However, Radish^{M}, Rocket^{M}, and Mustard^{M} showed hypocotyls with lower lightness and greener color than the sprouts of those species. This may have contributed to making microgreens more visually appealing to consumers.

3.2. Changes on bioactive compounds during growing

The main GLS, n-SFN, and SFN were identified and quantified in the Brassica species studied, with their main chromatographic characteristics shown in Table 2 by order of elution. The evolution of the bioactive compounds content during growth is shown in Table 3. As all species studies belong to the *Brassicaceae* family, they are characterized by their $\sum$GLS content. Samples with 7 and 17 days of growth are considered Sprouts (S) and Microgreens (M), respectively.

After 3 days of growth, Mustard^{S} and Radish^{S} were the species with the highest $\sum$GLS content (179 and 120 g kg^{-1}, respectively), while Kale^{S}, Brocoli^{S} and Rocket^{S} were the species with the lowest content (58.4, 44.0 and 29.8 g kg^{-1}, respectively). This amount decreased by 27 % and 27.5 % for 7-day Radish^{S} and Rocket^{S} compared to day 3, respectively, except for Kale^{S}, Broccoli^{S}, and Mustard^{S} whose content remained the same during this period. After 17 growing days, the $\sum$GLS content decreased by 39.1 %, 49.8 %, 61.7 %, and 67.8 % for 17-day Brocoli^{S}, Mustard^{S}, Rocket^{S}, and Radish^{S}, respectively. Nevertheless, Kale^{S} was the only specie that maintained its $\sum$GLS content compared to day 3.

The main individual GLS was different depending on each species, i.e. glucoraphasantin (GLS$_6$) for radish, glucoerucin (GLS$_5$) for rocket, 4-hydroxyglucobrassicin (GLS$_4$) for broccoli, and glucosinalbin (GLS$_3$) for mustard, and was the same during the different growing periods. However, glucoraphanin (GLS$_2$) was the main GLS in 3-day Kale, although it changed during its growth, i.e., GLS$_4$ was for Kale^{S} and glucobrassicin (GLS$_7$) for Kale^{M}.

Isothiocyanates are the biologically active product of the hydrolysis of GLS by the enzyme myrosinase (Martínez-Zamora et al., 2021) and sulforaphane (or sulforaphene in case of radish), the studied isothiocyanate in the present work, is derived from glucoraphanin (or glucoraphenin in case of radish) (GLS$_2$). Therefore, the species which the highest SFN content was 3-day Radish with 2296 mg kg^{-1}, as it had the highest GLS$_2$ content, 35.5 g kg^{-1}, followed by Kale, Broccoli, Rocket, and Mustard. This conversion process also forms a non-bioactive compound called sulforaphane nitrile (n-SFN). The highest n-SFN was found in 3-day Radish, followed by Broccoli, Mustard, Kale, and Rocket. SFN and n-SFN content decreased during the growth of all species.

TPC was preserved during the growing of the species from day 3 (sprout initiation) to 17 (microgreens) for Kale, Rocket, and Mustard. TPC decreased by 14.2 % for Radish^{M} and increased by 42.9 % for Broccoli^{M} compared to day 3. The *Brassicaceae* family, which includes a broad variety of vegetables, was previously described (Ebert, 2022). Their principal bioactive compounds are polyphenols and GLS, which have been extensively investigated due to their well-known health-promoting benefits. Other authors previously revealed that the activation of numerous enzymes during this process could enhance phenolic compounds (Pająk et al., 2014). Germination is related to several notable alterations in phytochemical content. Additionally, it is thought that seeds primarily serve as reservoirs for the growth of sprouts (Ebert, 2022). Previous studies reported the decrease of GLS and SFN during germination as activation of the metabolism from day 4 of germination, where it reached the maximum value after cotyledon disclosure (di Bella et al., 2021).

Table 2
Identification of individual organosulfur compounds and sensory descriptors.

Code	Chemical compound	Rt (min)	λ_{UPLC}	$\lambda^{\dagger\dagger}_{spectrum}$	Sensory descriptor	Sensory descriptor of the hydrolysis product	References
GLS_1	*dsf-sinigrin*	3.897	227	194, 211, 222	Bitter taste	Bitter taste, pungent/spicy, sulfurous, mustard-like, horseradish-like (Allyl)	(Baik et al., 2003; Bell et al., 2018; Prieto et al., 2019; Sansom et al., 2015; Wieczorek et al., 2018)
GLS_2	*dsf-glucoraphanin*	5.777	227	191, 211, 222	No taste Negative correlation with green aroma	No taste or flavor (sulforaphane) No bitterness	(Wieczorek et al., 2018)
GLS_3	*dsf-glucosinalbin*	8.934	227	205, 225, 277	-	spicy, pungent (4-hydroxybenzyl ITC)	(Baik et al., 2003; Bell et al., 2018; Sansom et al., 2015)
GLS_4	*dsf-4-hydroxyglucobrassicin*	11.551	227	222, 266	-	-	-
GLS_5	*dsf-glucoerucin*	15.100	227	207, 220	-	Radish-like, cabbage-like (erucin)	(Baik et al., 2003; Bell et al., 2018; Prieto et al., 2019; Sansom et al., 2015)
GLS_6	*dsf-glucoraphasantin*	16.141	227	225, 245	-	Pungent, spicy (Raphasatin)	(Baik et al., 2003; Bell et al., 2018; Sansom et al., 2015)
GLS_7	*dsf-glucobrassicin*	17.911	227	221, 280	Bitter taste	“unpleaseant” taste (Indole-3-carbinol)	(Wieczorek et al., 2018; Prieto et al., 2019; Zeng et al., 2021)
GLS_8	*dsf-4-methoxy-glucobrassicin*	21.852	227	221,269	-	-	-
GLS_9	*dsf-neoglucobrassicin*	27.282	227	223, 277, 289	Bitter taste	“unpleaseant” taste (Indole-3-carbinol)	(Baik et al., 2003; Bell et al., 2018; Sansom et al., 2015)
n-SFN	Sulforaphane nitrile	4.010	196	224, 267, 314	-	-	-
SFN†	Sulforaphane Sulforaphene	6.207	196	229, 348	-	-	-

Rt: Retention time; †Sulforaphane in all species except in radish in which sulforaphene was found; ††Peaks of spectral profile of each GLS.

3.3. *Sprouts vs microgreens: functionality*

Fig. 1 shows the statistical differences of ∑GLS and isothiocyanates (n-SFN and SFN) of S&M of studied brassica species. The highest value of ∑GLS was found in Mustard^{S}, followed by Radish^{S} and Mustard^{M}. Significant differences depending of the growing period (S&M) of each specie were found, reporting higher values in sprouts than microgreens. The ∑GLS content in kale, radish, rocket, broccoli, and mustard sprouts was 1.4-, 2.3-, 1.9-, 1.7- and 2.1-fold more than that of the respective microgreens, respectively. This behavior can be compared to previous authors, who have already reported these differences during the growth of several Brassicas. For instance, di Bella et al., (2021) showed as broccoli and kale sprouts presented higher amounts of the same GLS identified (glucoiberin glucoraphanin, 4-hydroxyglucobrassicin, glucobrassicin, glucoerucin, 4-methoxyglucobrassicin, and neoglucobrassicin) than broccoli and kale microgreens.

As to isothiocyanates, the highest content on n-SFN was found in Kale^{S} and Mustard^{S}, followed by Radish^{S} and Broccoli^{S}. The content of Radish^{M} and Mustard^{M} was similar and higher than Rocket^{S} and Broccoli^{M}, followed by Kale^{M} and Rocket^{M}. The values of n-SFN of kale, radish, rocket, broccoli, and mustard sprouts were 6.6-, 1.4-, 1.7-, 2.5- and 3.2-more folders than the respective microgreens, respectively. As to SFN, the highest value was found in Radish^{S,} being at least 2-folder times higher than Kale^{S}, followed by Kale^{M} and Broccoli^{S}. Also, SFN values of kale, radish, rocket, broccoli, and mustard sprouts were 1.5-, 15.8-, 1.2-, 2.3-, 3.3-more folder than the respective microgreens, respectively. Therefore, it is essential to highlight that those sprouts of each brassica species presented higher values of n-SFN, and SFN than their respective microgreens. This fact is comparable to the previously described above regarding GLS content, and previous findings shown by other authors in broccoli sprouts (Guo et al., 2014), in which the content of glucoraphanin and sulforaphane decreased through the time of germination.

Fig. 2 shows the TPC of S&M of the studied brassica species. Both Mustard^{S} and Mustard^{M} presented the highest values, followed by Broccoli^{S}. The lowest TPC was found in Radish^{M}, followed by Kale^{M} and Rocket^{S}. A previous study indicated that Broccoli^{S} presented higher content of compounds with oxygen radical scavengers as polyphenols than Radish^{S} (Wojdyło et al., 2020). In such study, higher compounds with oxygen radical scavengers as polyphenols were detected in Kale^{M} than in Radish^{M}. No differences were found between S&M in all species, except between S&M in radish species, being higher values in sprouts than microgreens. According to Wojdyło et al. (2020), sprouts accumulate higher amounts of oxygen radical scavengers (ORAC assay; correlated to polyphenols, flavan-3-ols, and L-ascorbic acid) than microgreens. The antioxidant capacity (FRAP assay: correlated to the content of L-ascorbic acid and polyphenols, particularly flavan-3-ols) was higher in sprouts than in microgreens. Microgreens also show higher anti-diabetic and anti-cholinergic activity than sprouts. It is worth mentioning that previous studies compared sprouts against microgreens, but the species and the growing conditions were not the same. While high nutrient density and high phytochemical content are considered a must in S&M, these microscale vegetables must also have a higher complex sensory profile consumer acceptance of flavor attributes and visual appearance. Therefore, the following sections of this manuscript are related to this fact, and how they can be affected by the changes in their main bioactive compounds.

Previous studies related to clinical applications incorporating cruciferous foods with health-promoting benefits mentioned that the studies including sprouts are of major interest, followed in the degree of importance by interventions with fresh mature plants. No information was added related to the differences between sprouts and microgreens (Costa-Pérez et al., 2023). A recent review about the potential of glucosinolate-hydrolysis products and their biologically formed metabolites, with clinical relevance in a broad spectrum of health problems indicated that it is important to continue researching how processing

Table 3

Changes of glucosinolates (GLS*i*; $\sum$GLS), nitrilo-sulforaphane (n-SFN), sulphoraphane (SFN[††]) and total phenolic compounds (TPC) during growing at 20ºC under a photoperiod of 16 h light/8 h darkness of different brassica species ($n = 3$).

Day	GLS_1[‡]	GLS_2[‡]	GLS_3	GLS_4	GLS_5	GLS_6	GLS_7	GLS_8	GLS_9	$\sum$GLS	n-SFN	SFN[†]	TPC
Kale													
	***[††]	***	-	***	***	-	**	***	***	**	***	***	*
3	6.6a[‡‡]	16.1a	Nd	14.4a	7.6^{a}	Nd	4.7d	3.6d	5.4c	58.4bc	775^{a}	267c	12.9b
5	7.2a	17.4ab	Nd	18.1a	8.6a	Nd	8.7abc	5.0cd	10.1ab	75.2a	409b	380b	15.1a
7^{S}	5.7b	13.7b	Nd	15.3a	7.3^{a}	Nd	10.3ab	6.1bc	11.8a	70.2ab	456b	455ab	15.1a
12	4.1c	8.9c	Nd	8.6b	5.4b	Nd	7.3cd	9.8a	11.3a	55.3bcd	173c	485a	13.9b
15	3.8c	8.0c	Nd	8.3b	3.4c	Nd	8.2bc	5.2c	5.6c	42.3d	83d	407ab	15.1a
17^{M}	3.5c	6.6c	Nd	9.7b	3.6c	Nd	11.0a	7.3b	8.1bc	49.8cd	69d	296c	13.3b
Radish													
	-	***	-	***	-	***	-	*	***	***	***	***	***
3	nd	35.4a	Nd	11.6a	Nd	70.3^{a}	Nd	3.0c	nd	120a	2101^{a}	2296a	12.9c
5	nd	17.6b	Nd	9.1b	Nd	69.9^{a}	Nd	4.2ab	nd	101b	501b	2013b	17.4a
7^{S}	nd	7.5c	Nd	5.1c	Nd	70.8^{a}	Nd	4.1b	nd	87.6c	249c	1204c	14.8b
12	nd	4.8cd	Nd	3.7cd	Nd	41.4b	Nd	5.4a	nd	55.3d	241c	237d	11.9cd
15	nd	4.4cd	Nd	3.1d	Nd	34.1bc	Nd	4.5ab	nd	46.1de	227c	157d	11.9cd
17^{M}	nd	3.9d	Nd	3.1d	Nd	27.7c	Nd	3.9bc	nd	38.7e	175c	76e	11.3d
Rocket													
	-	***	-	NS	***	-	-	**	-	***	***	**	***
3	nd	5.6a	Nd	2.4	23.6a	Nd	Nd	3.8b	nd	29.8a	166^{a}	99ab	14.2ab
5	nd	5.5a	Nd	2.3	23.2a	Nd	Nd	3.7b	nd	29.2a	147^{a}	110a	13.9ab
7^{S}	nd	5.5a	Nd	2.1	16.1b	Nd	Nd	3.4bc	nd	21.6b	79b	104ab	13.4b
12	nd	3.7b	Nd	2.0	8.2c	Nd	Nd	4.5a	nd	14.8c	55bc	66d	11.1c
15	nd	2.7c	Nd	2.0	6.3c	Nd	Nd	2.9c	nd	11.1c	44c	82cd	14.7ab
17^{M}	nd	3.0bc	Nd	2.1	5.3c	Nd	Nd	3.9b	nd	11.4c	47c	89bc	14.9a
Broccoli													
	**	***-	-	***	***	-	***	***	***	***	***	***	***
3	4.2^{a}	8.0a	Nd	11.4a	5.7a	Nd	3.1d	4.3bc	7.3b	44.0ab	1488^{a}	108b	9.8d
5	3.6ab	6.4b	Nd	9.0b	4.3b	Nd	6.0b	5.0b	11.4a	45.7a	531b	122b	12.7c
7^{S}	3.5ab	6.3b	Nd	7.7b	3.8b	Nd	7.4a	6.5a	10.5a	45.6a	266c	223a	15.3a
12	2.9b	4.4c	Nd	5.2c	2.9c	Nd	5.7bc	6.5a	10.2a	37.8b	114c	97b	12.8c
15	2.7b	4.1c	Nd	5.9c	2.3c	Nd	7.1a	4.0c	5.7c	31.9c	88c	98b	15.7a
17^{M}	2.4b	3.7c	Nd	5.4c	2.2c	Nd	4.9c	3.7c	4.3c	26.8c	107c	95b	14.0b
Mustard													
	**	-	***	**	-	-	-	**	**	**	***	***	NS
3	4.3a	nd	166a	3.3a	Nd	Nd	Nd	3.1cd	2.7c	179ab	937^{a}	32b	32.6
5	3.3ab	nd	122b	3.2ab	Nd	Nd	Nd	5.0abc	5.4a	139bc	573b	64a	33.4
7^{S}	4.2a	nd	172a	4.0ab	Nd	Nd	Nd	6.4a	5.7a	193a	485b	36b	34.8
12	2.8b	nd	99.3bc	2.9b	Nd	Nd	Nd	6.1ab	4.5ab	116cd	302c	38b	33.8
15	2.1b	nd	77.4c	2.4b	Nd	Nd	Nd	2.9d	2.5c	87.3d	284c	15c	32.5
17^{M}	2.3b	nd	77.6c	2.8b	Nd	Nd	Nd	4.3bcd	2.8bc	89.8d	151c	11c	34.8

^{S}: *sprouts (7* growing days*);* ^{M}*: microgreens (17* growing days*);* GLS_i and $\sum$GLS were expressed as g kg^{-1} dw; n-SFN and SFN were expressed as mg kg^{-1} dw; and, TPC were expressed as g GAE kg^{-1} dw [††]*SFN: sulforaphane in all species except in radish in which sulforaphene was found;* [‡]*GLS_1: dsf-sinigrin; GLS_2[‡]: dsf-glucoraphanin, in all species except in radish in which glucoraphenin was found; GLS_3: dsf-glucosinalbin; GLS_4: dsf-4-hydroxyglucobrassicin; GLS_5: dsf-glucoerucin; GLS_6: dsf-glucoraphasantin; GLS_7: dsf-glucobrassicin; GLS_8: dsf-4-methoxy-glucobrassicin; GLS_9: dsf-neoglucobrassicin; $\sum$GLS: sum of individual dsf-glucosinolates; nd: no detected.* [††]*NS: not significant at $p > 0.05$; *, **, and ***, significant at $p < 0.05$, 0.01 and 0.001, respectively;* [‡‡]*Values followed by the same letter, within the same column, were not significantly different ($p > 0.05$).*

techniques affects the quality and dosage of bioactive compounds from vegetables (including microscale brassicas) in the context of dietary-compatible consumption. In this processing techniques can be included the harvesting time as this research is focused on. Also, in this recent review indicated that a recommendation of incorporating various portions of cruciferous vegetables (including sprouts and microgreens) every week because of the positive effect on a more balanced diet (Costa-Pérez et al., 2023). Moreover, it is important to mention that although cruciferous foods are in the spotlight to be considered within multiple preventive and active nutrition and wellness programs as mentioned by the cited authors, consumer acceptability must be taken into account and that is why this work is of high importance.

3.4. Sprouts vs microgreens: bioactive compounds linked with descriptive sensory evaluation

Flavor chemistry of brassicas is complicated due to the dynamic release of isothiocyanates and other compounds by the myrosinase activity during growth, processing, and consumption, which gives spicy/pungency at pain receptors, plus volatile aroma compounds reaching olfactory receptors (Marchioni et al., 2021). Principal component analyses (PCAs) were done to get a better understanding of the relationships among the five-brassica species (n = 10, sprout and microgreens of each species), using (i) organosulfur compounds (GLS_i, $\sum$GLS, and isothiocyanates) and (ii) descriptive sensory attributes (Fig. 3). The F1 axis explained 35.95 % of the total data variance, while the axis F2 explained 39.56 % of the total variance. Table S2 of the supplementary material shows descriptive sensory analysis data. Here are the most relevant associations:

- Both Mustard^{S} and Mustard^{M} were linked to $\sum$GLS (especially GLS_3), TPC and n-SFN, and with the spicy sensory descriptor. Table 5S shows the Pearson`s correlation used for the PCA graph in which $\sum$GLS and TPC were positive correlated to spicy (R = 0.701 and 0.695). GLS_3 was also correlated to the spicy sensory descriptor (R = 0.713). A negative correlation was found between $\sum$GLS and vegetal flavor and odor (R = −0.819 and −0.769, respectively), as it can be observed in Fig. 3. These results were in accordance with previous studies in which GLS_3 (glucosinalbin) was quantified as the highest GLS in mustard (Artés–Hernández et al., 2022a, 2022b; Guijarro-real et al., 2022). Although no sensory descriptor has been reported about GLS_3, the sensory descriptor of the hydrolysis product (4-hydroxybenzyl ITC) has previously been defined as spicy

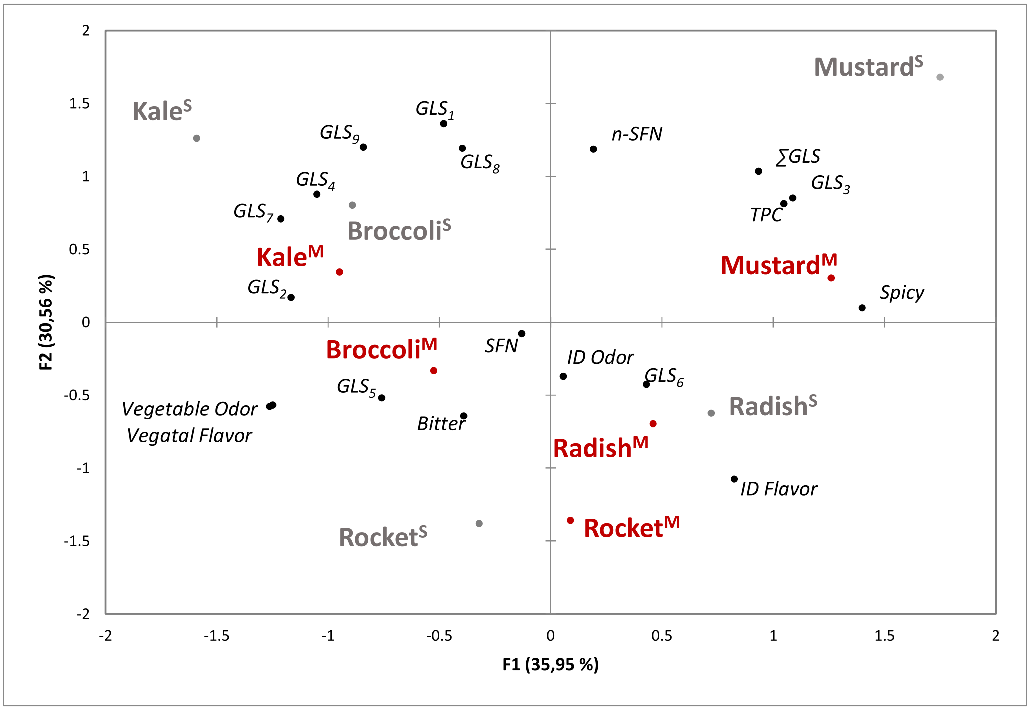

Fig. 3. Principal Component Analysis (PCA) of sprouts (S, gray color) and microgreens (M, red color) bioactive compounds and descriptive sensory attributes (63.47 %). ^{S}: *sprouts (7 growing days at 20°C); ^{M}: microgreens (17 growing days at 20°C);* GLS$_i$ and ∑GLS were expressed as g kg^{-1} dw; n-SFN and SFN were expressed as mg kg^{-1} dw; and, TPC were expressed as g GAE kg^{-1} dw ††*SFN: sulforaphane in all species except in radish in which sulforaphene was found;* ‡*GLS$_1$: dsf-sinigrin; GLS$_2^{\ddagger}$: dsf-glucoraphanin, in all species except in radish in which glucoraphenin was found; GLS$_3$: dsf-glucosinalbin; GLS$_4$: dsf-4-hydroxyglucobrassicin; GLS$_5$: dsf-glucoerucin; GLS$_6$: dsf-glucoraphasantin; GLS$_7$: dsf-glucobrassicin; GLS$_8$: dsf-4-methoxy-glucobrassicin; GLS$_9$: dsf-neoglucobrassicin; ∑GLS: sum of individual dsf-glucosinolates.* (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

and/or pungent (Baik et al., 2003; Bell et al., 2018; Sansom et al., 2015) as Table 2 shows.

- Radish^S and Radish^M were highly linked with GLS$_6$ and ID Odor/Flavor sensory descriptors. Previous studies indicated that no sensory descriptor has been described GLS$_6$ (glucoraphasantin), and the hydrolysis product sensory descriptor was spicy and pungent (Baik et al., 2003; Bell et al., 2018; Sansom et al., 2015) as Table 2 shows. No significant correlation between GLS$_6$ and spicy/pungent sensory descriptors was observed in this study.
- SFN content (located in the center of the chart) is not linked with any key sensory attributes, agreeing with previous literature (i.e., no taste, no flavor). As to SFN-n, a positive correlation was found between GLS$_1$ (R = 0.655), which is linked with the spicy descriptor as described above (Baik et al., 2003; Bell et al., 2018; Prieto et al., 2019; Sansom et al., 2015).
- Rocket^S and Rocket^M were characterized by the lowest values of ∑GLS, being Rocket^S presented the highest GLS$_5$ content. The compounds obtained by hydrolysis of glucoerucin have been previously described as brassica ID sensory attribute (flavor ID: example cabbage), although no significant correlation was observed in this study. Also, the alkyls GSLs such as glucoerucin (4-methylthiobutyl) do not have the bitter taste ability attributed (Baik et al., 2003; Bell et al., 2018; Prieto et al., 2019; Sansom et al., 2015).
- Kale^S, Kale^M, Broccoli^S, and Broccoli^M samples were linked to GLS$_1$, GLS$_2$, GLS$_4$, GLS$_7$, GLS$_8$ and GLS$_9$. According to Pearsońs correlation (Table S3), ID flavor was negatively correlated to GLS$_1$ (R = −0.835), GLS$_7$ (R = −0.774) and GLS$_9$ (R=−0.929) as PCA shows. Vegetal Odor sensory descriptor was negatively correlated to GLS$_4$ and GLS$_8$ (R = −0.733 and R = −0.648, respectively), while it was positively correlated to GLS$_2$ (R = 0.654). Also, glucobrassicin (GLS$_7$) was negatively correlated to spicy sensory descriptor (R = −0.758). Previous studies correlated GLS$_7$ content to bitter taste (Wieczorek et al., 2018), mainly produced by the hydrolysis products obtained from glucobrassicin (3-indolylmethyl) (Prieto et al., 2019; Zeng et al., 2021). Table 2 shows the sensory descriptor and hydrolysis product sensory descriptor of several of those GLS$_i$, being bitterness of GLS$_1$ (sinigrin), and no taste of GLS$_2$ (glucoraphanin) (Wieczorek et al., 2018). The alkyl GSLs, such as glucoraphanin (4-methylsulfinylbutyl), do not have the bitter taste ability attributed (Prieto et al., 2019). Also, GLS$_7$ (glucobrassicin) and GLS$_9$ (neoglucobrassicin) present bitterness sensory descriptor and unpleasant taste of the hydrolysis product of both GLS$_i$ (Baik et al., 2003; Bell et al., 2018; Sansom et al., 2015). Although Fig. 3 shows bitterness close to GLS$_5$ and GLS$_6$, it is essential to highlight that no statistical correlation was found in this study, as Table S3 shows. The rest of the GLS was not previously described at the sensory level.

Previous studies indicated that designing crop improvement strategies for sensory traits based on ∑GLS content would be flawed, as it does not consider the relative differences in sensory characteristics of different GLS$_i$ and isothiocyanates, nor the contribution from other GLS hydrolysis products (Bell et al., 2018) (Wieczorek et al., 2018). The Brassica group is often rejected by consumers due to the sensory characteristics linked with phytochemical composition (Baik et al., 2003; Bell et al., 2018; Prieto et al., 2019; Sansom et al., 2015). Considering this evidence, a consumer study and online survey about brassica S&M were included in this work, and results and discussion are subsequently described in the following sections.

3.5. Sprouts vs microgreens: critical sensory attributes identified by consumers

This research evaluated sensory properties, and no statistical significance was found in identifying liking drivers suggesting that more deep research is needed. Therefore, this information was not added, and research was focused on identifying critical attributes. Fig. 4 shows the penalty analysis, which is a method that helps interpret the data of the JAR questions, describing how close to the optimal intensity of key sensory attributes for consumers (Cano-Lamadrid et al., 2018; Clemente-Villalba et al., 2021; Lipan et al., 2020; Sánchez-Rodríguez

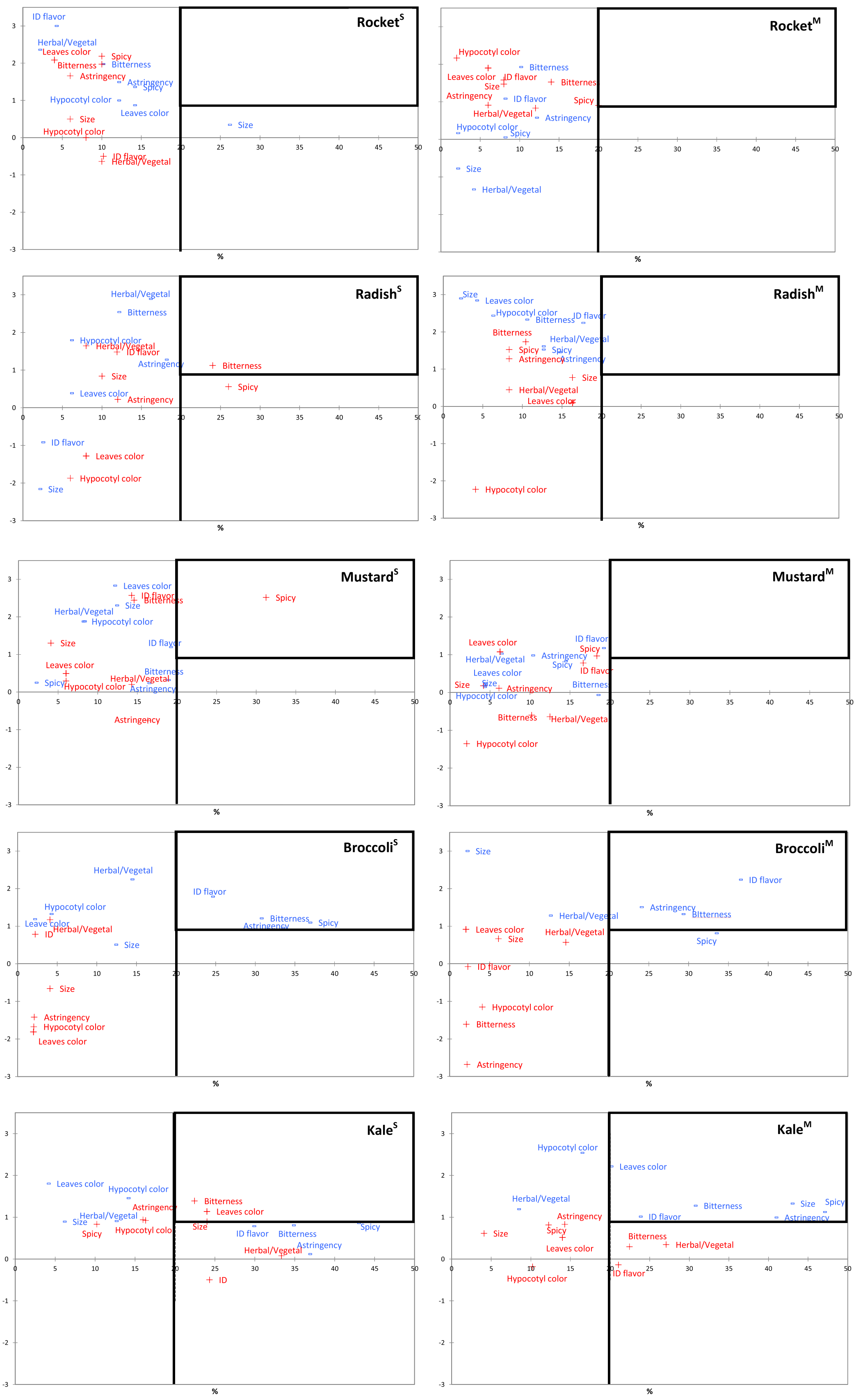

Fig. 4. Penalty analysis of attribute intensity assessed by consumers of the sprouts and microgreens samples (sample code indicated on the top right of each figure; “too low intensity” is indicated by the blue symbol “–”. and “too high intensity” is indicated by the red symbol “+“). Right column microgreens samples. left column sprouts simples; *S: sprouts (7 growing days at 20°C); M: microgreens (17* growing days at 20°C). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

et al., 2019). The attributes that would need to be improved, by excess or defect, will be those producing a drop of, at least, 1 unit of satisfaction grade for at least 20 % of consumers, reflecting a negative effect on the sensory attributes of the samples. In addition, Table S4 of the supplementary material shows data from hedonic questions of consumer study. No significance in global acceptance was noticed between samples, and no correlation was observed. A previous study indicated that the overall acceptability of microgreens (brassica and other species) was positively correlated to the acceptability of flavor, texture, and sweetness; while the overall acceptability was negatively linked with acceptability of sourness, bitterness, and astringency (Xiao et al., 2015). The study concluded that the obtained results suggested that flavor-related characteristics best predicted consumer preferences for overall eating quality, though textural and visual quality characteristics also contributed (Xiao et al., 2015). Also, the results from a previous consumer study of 12 microgreen species (amaranth, coriander, cress, green basil, komatsuna, mibuna, mizuna, pakchoi, purple basil, purslane, Swiss chard, and tatsoi) revealed that while the visual appearance of the microgreens played a role, the flavor and texture of microgreens were the main determining factors for consumer acceptance. In general, low astringency, sourness, and bitterness enhanced the consumer acceptability of microgreens (Caracciolo et al., 2020).

Fig. 4 shows that differences among samples depend more on brassica species than the growing stage (S&M). In rocket S&M, it can be said that no attributes were found in the critical corner. On the other hand, although no differences were found in global, bitterness and spicy consumer satisfaction degree between S&M of radish and mustard species (Table S4), penalty analysis from JAR questions indicated a need to improve the excess intensity of bitterness and spicy in Radish^S and Mustard^S, while no attributes at the critical corner were found in Radish^M and Mustard^M. As to Broccoli and Kale, some sensory attributes were located at the critical corner in both S&M, meaning that they should be improved. Both broccoli samples are needed to improve several attributes because at least 20 % of consumers detected a low intensity in several sensory attributes such as ID flavor, bitterness, astringency and spicy. The trained panel scored low values of bitterness, spicy and ID flavor in broccoli samples (Table S2). Also, Kale^S was characterized by an excess of bitterness, leaves color and spicy, while Kale^M was described by a deficit of spicy, astringency, bitterness, size, ID flavor and leaves color intensity. Summarizing the results from this section, it can be stated that 4 out of 5 of the sprout samples need improvements, while only 2 out of 5 of the microgreens samples need improvements. A previous consumer study of six commonly grown and consumed microgreen species tested, three of them brassicas (rocket, broccoli, and red cabbage). On the other hand, previous studies indicated a higher appreciation of microgreens (Chinese basin) compared to mature vegetables, focusing on their aroma profile and consumer acceptance. In the future, a similar study can be carried out with brassica species (Dimita et al., 2022).

3.6. Sprouts vs microgreens: online survey

The level of food neophobia (tendency to avoid unfamiliar foods) is important to be calculated to understand its potential impact on volunteers' consumers' attitudes towards brassica S&M. Comparing the results obtained from the two populations, the level of neophobia between Italian (I) and Spanish (Sp) one resulted in identical mean values (Italian FNS = 27.4 ± 10.9; Spanish FNS = 27.7 ± 9.3; $p = 0.8$), as well as the percentage of subjects in each class (Neophilic: I = 23 %, Sp = 28 %; Moderate: I = 54 %, Sp = 47 %; Neophobic: I = 23 %, Sp = 25 %). These data agree with those reported in a recent literature review (Rabadán and Bernabéu, 2021), where the mean food neophobia scores of I and Sp resulted in comparable, reaching a low/moderate level of food neophobia. On the other hand, it can be highlighted that the frequency of brassica vegetable consumption has been investigated as well to verify that the consumers in the two populations did not present large differences in the consumption of these vegetables, which are commonly used to produce sprouts and microgreens (Michell et al., 2020).

Overall, participants reported eating this kind of vegetables with the same frequency (***never***: kale ≈ 50 % of both populations; radish ≈ 40 % of both populations; Brussels sprouts ≈ 40 % of both populations; pak-choi ≈ 75 % of both populations; mustard ≈ 40 % of both populations; wasabi ≈ 50 % of both populations; *occasionally*: red cabbage ≈ 45 % of both populations; radishes ≈ 36 % of both populations; *1–3 times per month*: broccoli ≈ 50 % of both populations; cauliflower ≈ 50 % of both populations; cabbage ≈ 37 % of both populations; rocket ≈ 35 % of both populations) and no differences were found between the two populations (all $p > 0.05$). As regards familiarity with S&M, the 'Spanish' group reported being significantly more familiar ($p < 0.05$), while the percentage of Italians (more than 50 %) who are familiar only with sprouts is significantly greater between the two groups. Because S&M are new food products and there is evidence of consumer confusion between S&M (Yeargin et al., 2023), it is important to clarify that information about sprouts and microgreens was added in the online survey. As far as consumption is concerned, data showed that more than 50 % of consumers belonging to both populations did not consume either S&M, suggesting that this category of fresh 'super food' is still scarcely accepted and consumed, because of there is no regulation in this regard, so its declaration on the label is counterproductive (Michell et al., 2020). In particular, the Italians stated to mainly consume sprouts, while the Spanish seemed to be more used to consuming both S&M. The most familiar and consumed S&M species reported by both groups were soy, peas, lentils, onion, and garlic.

The importance of some reasons impacting the likelihood of habitual consumption/purchasing of S&M was also investigated through the survey. Examples of reasons included price, availability/access, health benefits, taste, color, and tasty appearance. Looking at the two groups, it is possible to note that for Spanish, all six reasons are of great importance for their habitual consumption and purchase of S&M. For Italians, on the other hand, the only two characteristics that turn out to seem important are the 'taste' and 'health benefits' (about 40 % of the Italian consumers rated them as 'very important'), while all the other reasons are considered "moderately important". In general, it is possible to state that sensory characteristics, such as general appearance and taste, are those with the greatest importance for consumers, as has been recently reported (Ebert, 2022). Accordingly, Caracciolo et al., (2020) suggested that the visual appearance of microgreens is a characteristic that drives consumer liking, although overall acceptability is mainly determined by flavor and texture. A paired preference test was used to visually compare S vs M of the six-brassica species within the two groups (Fig. 2S). Italians and Spanish preferred the appearance of Rocket^M and Broccoli^M ($p < 0.001$) compared to the respective sprouts. When asked about the reasons for the choice, Italians declared that the samples were chosen for the color and dimension of the leaves, while Spanish declared that they especially liked the dimension of the leaves. As well, both groups preferred Radish^S compared to the respective microgreens ($p < 0.001$) for the color of leaves and hypocotyl (I) and for the color and dimension of the leaves (Sp). On the contrary, Italians selected Kale^S as preferred compared to the microgreen sample ($p < 0.01$), mainly for the color of hypocotyl, while no differences were found between Mustard samples. Spanish, otherwise, indicated a preference for Mustard^S ($p < 0.05$) for the color and dimension of the leaves compared to Mustard^M, and equally preferred kale samples. On the other hand, High ratings for the appearance acceptability of brassica microgreens were previously reported, being a change for increasing the visual appearance of meals (Michell et al., 2020).

4. Conclusion

This study carries out for the first time a comparison between S&M of different brassica species and related the main phytochemicals to the sensory perception of these novel foods, which are increasingly appreciated. It was observed that sprouts are richer in organosulfur compounds than microgreens, although it depends on the species. In addition, we confirmed that some organosulfur compounds and/or their hydrolysis compounds are linked with spicy/pungent sensory attributes in S&M brassicas. Overall, all S&M evaluated present good functional value and sensory descriptors, a good opportunity to further increase its consumption regarding the mature plant. This work provides useful information for microscale brassica vegetable farmers to determine the harvest time according to the content and composition of organosulfur compounds. The results of the penalty analysis can help to optimize the harvest time of S&M by avoiding the undesirable intensity of several sensory descriptors. Minimally processed vegetable companies will benefit from getting a deeper knowledge of key sensory attributes. Nevertheless, this research provides a series of practical tips for the food industry to understand consumer preferences related to these products, select brassica species, and improve their organosulfur health-promoting compounds. In the future, an interesting research line would be to study the odor-active compounds of these products and link them with consumer preferences, and even other vegetable crops could be included, such as cereals and legumes.

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CRediT authorship contribution statement

Conceptualization, **M.C-L., L. M-Z, C.N and F. A-H.**; Methodology, **M.C-L., L. M-Z, C.N and C. C**; Investigation, **M.C-L., L. M-Z, C.N and C. C;** Writing – original draft, **M.C-L., L. M-Z, C.N and C. C.**; Writing – review & editing, **M. C-L, F.A-H and P. E**; Visualization, **M. C-L, F.A-H and P. E.**; Supervision, Project administration, Funding acquisition: **F.A-H.** All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript.

Data availability

The data that has been used is confidential.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.postharvbio.2023.112411.

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