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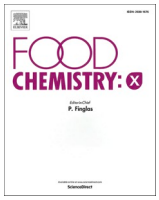

Identification of volatile and flavor metabolites in three varieties of broccoli sprouts

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ABSTRACT

Broccoli sprouts are promising functional food sources and their taste and flavor play a pivotal role in the acceptance of consumers. In this study, the flavor profiles of three varieties of broccoli sprouts, namely Bi Lv, You Xiu, and Lv Hua, were comprehensively characterized using HS-SPME-GC/MS analysis. A total of 364 volatile and flavor components across 15 chemical classes were successfully identified. The results revealed a majority of volatile metabolites exhibiting upregulation during the germination process, leading to an enhancement in taste intensity after germination, particularly for umami and sweet tastes, which was associated with an increase in associated amino acids and sugar content. Although the total glucosinolate content in broccoli sprouts has decreased compared to seeds, it remains the primary contributor to the bitterness of broccoli sprouts. The present study elaborated on the flavor contribution of broccoli sprouts, supporting the cultivation and consumption of them as a nutritious food.

1. Introduction

Broccoli (*Brassica oleracea var. italica*) belongs to the *Brassicaceae* family and is indigenous to Asia and the Mediterranean (Li, Xia, et al., 2022). The total global production of broccoli reached approximately 6.24 million tons in 1980, but experienced a significant increase to around 26.06 million tons in 2022 (FAOSTAT, 2024), with China and India as the primary producers. This significant increase can be attributed to the multitude of bioactive substances (e.g., glucosinolates and phenolic compounds) and nutritional constituents (e.g., vitamins, dietary fiber, and minerals) present in broccoli. Broccoli has potential health-promoting properties to enhance the immune system and reduce the incidence of chronic diseases, including diabetes mellitus, obesity, cancers, and cardiovascular diseases (Gan et al., 2017).

Broccoli seeds and sprouts are considered to be good sources of glucosinolates and sulforaphane. The content of glucosinolates undergoes dynamic changes during the germination process of broccoli seeds due to its enzymatic breakdown by myrosinase into thiocyanate, isothiocyanate, or nitrile compounds. Among these, sulforaphane is the predominant isothiocyanate present in broccoli (Li et al., 2024). As the main functional component in seeds and sprouts, glucosinolates and their hydrolysates exhibit chemopreventive, antiproliferative, anticancer, antioxidative, and antibacterial properties (Jo et al., 2022). However, The presence of glucosinolates and their degradation products

Abbreviations: BL, Bi Lv; YX, You Xiu; LH, Lv Hua; FAAs, free amino acids; HS-SPME-GC/MS, headspace solid-phase microextraction gas chromatography–mass spectrometry; TAV, taste active value; QC, quality control; PCA, principal component analysis; OPLS-DA, orthogonal partial least squares discriminant analysis; VIP, variable importance in projection; FC, fold change; Val, valine; Ile, isoleucine; Leu, leucine; Arg, arginine; Met, methionine; His, histidine; Asp, aspartic acid; Glu, glutamic acid; Lys, lysine; Ser, serine; Thr, threonine; Gly, glycine; Ala, alanine; Pro, proline; Cys, cystine; Tyr, tyrosine; Phe, phenylalanine..

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is commonly associated with off-flavor attributes characterized by bitter and sulfurous sensory qualities, which can negatively impact consumer acceptance (Koutidou et al., 2017). Studies indicate that sprouts provide superior nutritional value compared to seeds and offer a simple and efficient production process (Martinez-Villaluenga et al., 2010). This is due to the fact that during germination, the endosperm reserves in the seed are utilized to support seedling growth and are transported to sprouting tissues. Germination leads to the breakdown of anti-nutritional and indigestible substances such as protease inhibitors and lectin, accompanied by an increase in free amino acids (FAAs) and simple sugars (Aguilera et al., 2013; Shi et al., 2010). Amino acids are the main substances responsible for umami taste in broccoli, while sugars are the main substances that produce sweetness (Li et al., 2002). The investigation of 20 distinct cultivars of broccoli revealed significant variations in their metabolites and flavor profiles, with amino acids playing a pivotal role in taste determination (Chevilly et al., 2023). FAAs not only function as key precursors for the synthesis of various primary and secondary metabolites, but also play a crucial role as a significant class of taste-active ingredients (Amir et al., 2018). Apart from taste caused by non-volatile compounds, volatile compounds such as nitriles, aldehydes, and alcohols, also contribute to the flavor profile due to their odor characteristics or as precursors for multiple odorants (Zou et al., 2020).

The sense of taste is a crucial factor that influences consumers' selection of food. While sprouts are considered a cost-effective and relatively novel source of functional foods, broccoli sprouts are notably bitter, which may not appeal to certain consumers, particularly children (Wieczorek et al., 2018). Understanding the sensory and chemical attributes of broccoli sprouts is essential to meet consumer' preferences and promote healthy food consumption. The existing literature reports on the flavor profiles of mature broccoli vegetables under different conditions, yet there remains a dearth of research on the flavor characteristics of broccoli sprouts (Chevilly et al., 2023; Johansen et al., 2017; Mølmann et al., 2015). Only one study has investigated the sensory characteristics and consumer acceptance of Brassica vegetable sprouts, revealing that broccoli sprouts exhibit a bitter and spicy taste profile, which is not well-accepted by populations in the Mediterranean basin (Cano-Lamadrid et al., 2023). Hence, it is imperative to identify the compounds responsible for its flavor and conduct a comprehensive analysis of the aroma and taste of broccoli sprouts, in order to establish a solid groundwork for future enhancement in flavor. Recently, the combination of gas chromatography–mass spectrometry (GC/MS) technology and headspace solid-phase microextraction (HS-SPME) has gained widespread application in analyzing volatile components due to its exceptional sensitivity, simplicity and efficiency, as well as excellent reproducibility. Therefore, HS-SPM*E*-GC/MS is an excellent approach to investigating volatile metabolites in broccoli sprouts, particularly during their germination process.

This study employed HS-SPME-GC/MS-based metabolomics to explore the volatile metabolite classes and abundance among various cultivars of broccoli sprouts, particularly focusing on metabolite variations before and after germination. By combining FAAs, taste active value (TAV) evaluation, glucosinolates and sugar analysis, E-nose and E-tongue, the flavor and taste profiles of three broccoli varieties were comprehensively characterized. The aim was to identify the main metabolites and potential bioactive compounds that influence the aroma and taste of broccoli sprouts. The obtained results may serve as a valuable reference for the utilization of broccoli sprouts as raw materials of functional foods.

2. Materials and methods

2.1. Plant material and germination conditions

Three cultivars of broccoli seeds, namely Bi Lv (BL), You Xiu (YX), and Lv Hua (LH) were sourced from Jingyan Yinong Seed Science Technology Co., Ltd. (Beijing, China), Sakata Seed Corp. (Tokyo, Japan), and Silver Lake Garden Vegetable Seed Co., Ltd. (Xiamen, China). The broccoli seeds were germinated following the procedure described by a prior study (Li et al., 2023). Following disinfection and immersion, broccoli seeds were evenly distributed in trays with absorbent gauze, each containing 5 g of seeds. Subsequently, seeds were germinated under controlled conditions in incubators set at a consistent temperature of 25 °C, humidity at 75 %, and white light intensity of 40 μmol m^{-2} s^{-1}. The germination process involved a lighting schedule of 16 h of light followed by 8 h of darkness. After 7 days, the sprouts were harvested and stored at −80 °C for subsequent analysis. The LH, YX, and BL seeds and sprouts are denoted as *Z*-LH and Y-LH, Z-YX and Y-YX, Z-BL and Y-BL in the following experiments.

2.2. Determination of FAAs

The broccoli seeds and sprouts samples were subjected to freeze-drying for 64 h using a SJIA-5S vacuum freeze-dryer (Shuangjia Instrument Co., Ltd., Ningbo, China). Subsequently, the dried samples were finely ground into powders using an MM400 grinder (Retsch, Laichi, Germany) operating at 30 Hz for 2 min. FAAs composition was determined following the described protocol with minor adjustments (Chen et al., 2024). 2 g of sample powder was added into 20 mL sulfosalicylic acid (5 g/100 mL) and thoroughly mixed. After standing for 1 h, 1 mL EDTA disodium (1 g/100 mL) and 1 mL hydrochloric acid (0.06 mol /L) were added and filtered with a 0.45 μm membrane. Subsequently, 1 mL supernatant was evaporated to dryness and dissolved in 2 mL sodium citrate buffer (pH 2.2). The FAAs contents were measured by an amino acid analyzer (MembraPure A300, German), with triplicate measurements for accuracy.

2.3. TAV calculation

TAV was calculated to determine each flavor substance's contribution to taste perception by comparing content to its taste threshold (Li, Ma, et al., 2022). The formula for computing TAV is as follows:

$$TAV = C/T$$

In the formula, C stands for taste substances concentration (mg/100 g); T denotes the threshold value of taste substances (mg/100 g), which refers to the minimum concentration at which a taste can be detected or recognized.

2.4. Identification of glucosinolates and sugar

The determination of glucosinolates and sugar was conducted using a UPLC-ESI-MS/MS system (SHIMADZU Nexera X2, Kyoto, Japan). The mobile phase program was carried out as previously described (Li et al., 2023). Mass spectrometry was performed using MS/MS with electrospray ionization. Experiments were set up in both positive and negative ion modes with an ion spray voltage of 5500 V for positive ion mode and − 4500 V for negative ion mode. The experimental conditions were set as follows: temperature, ion source GasI, GasII and curtain gas were 550 °C, 50, 60, and 25 psi, respectively; collision gas was high.

2.5. Analysis of volatile metabolites present in broccoli seeds and sprouts

2.5.1. Sample preparation

Sample preparation was determined based on a previous report with minor adjustments (Wu et al., 2022). Broccoli seeds and sprouts were finely pulverized into powder using a ball mill (MM400, Retsch) containing liquid nitrogen. Sample powder (0.5 g) was transferred into a 20 mL head-space vial (Agilent, Palo Alto, CA, USA) filled with a saturated NaCl solution (Aladdin Bio-Chem Technology Co. LTD, Shanghai, China). The vials for solid-phase microextraction analysis were equilibrated in a water bath of 60 °C, followed by exposing a fiber (120 μm

DVB/CAR/PDMS, Agilent) to the sample's headspace for 40 min. The preparation of quality control (QC) samples involved the blending of sample extracts to ensure the reproducibility of the analysis, typically incorporating one QC sample for every five samples.

2.5.2. GC–MS conditions

Volatile metabolomic analysis was conducted using GC–MS (8890-7000D, Agilent) equipped with an Agilent DB-5MS column (30 m × 0.25 mm × 0.25 μm). The volatile organic compounds were desorbed from the fiber at a temperature of 250 °C for 5 min in splitless mode. Helium was utilized as the carrier gas and the linear speed was 1.20 mL/min. The injector temperature was maintained at 250 °C and the detector at 280 °C. The oven temperature started at 40 °C during the initial 3.5 min, with an increase of 10 °C/min to 100 °C, 7 °C/min to 180 °C, followed by a further increase of 25 °C/min to 280 °C and kept for 5 min. The electron impact ionization mode was set at 70 eV, with the quadrupole ion source, mass detector, and transfer line maintained at temperatures of 230 °C, 150 °C and 280 °C respectively. Selected ion monitoring mode was employed for the identification and quantification of analytes. The retention time of the C8-C20 alkane standard solution was determined using identical chromatographic conditions. The volatiles were annotated by comparing their deconvoluted spectra and retention indices with those in the NIST 17 library, utilizing a reverse match score > 850 and a retention index ±30 as the criteria for identification (Yuan et al., 2022).

2.6. E-nose and E-tongue analyses

The E-nose odor analysis was conducted using the E-nose device (iNose, Shanghai Bosin Tech, China), which is equipped with an array of 14 metal-oxide semiconductor sensors. Based on the method reported previously (Zhang, Yang, et al., 2022), 5 g of sample were transferred to a 40 mL headspace vial and equilibrated at 50 °C for 20 min. Subsequently, the analysis of each sample lasted for 60 s at an air flow rate of 1 L/min and the system underwent two rounds of 60 s cleaning in between samples. The maximum values of response curves were chosen for data analysis. The details of each sensor are presented in Table S1.

The E-tongue analysis was conducted using a Taste Sensing System (SA402B, Insent Intelligent Sensor Technology, Inc., Japan) equipped with five chemical sensors for astringency (AE1), umami (AAE), sourness (CA0), saltiness (CT0) and bitterness (C00). Following a previously reported method with minor adjustments (Zhou et al., 2022), sample preparation involved mixing 15 g of sample with 60 mL distilled water to extract the taste substances, followed by centrifugation. After centrifugation at 4000 rpm for 10 min, the supernatant was filtered by a 0.45 μm membrane filter (Tianjin JinTeng Experiment Equipment Co., Ltd., China) for subsequent analysis. Three replicated measurements were performed for each sample.

2.7. Statistical analysis

All statistical analyses were performed using the SPSS software version 22.0 (SPSS, Chicago, IL, USA). Multivariate statistical analysis including unsupervised principal component analysis (PCA), hierarchical clustering analysis, orthogonal partial least squares discriminant analysis (OPLS-DA) and Venn graphs were performed using R to classify volatile compounds in broccoli sprouts and their respective seeds. The identification of differential metabolites was based on Variable importance in projection (VIP) from the OPLS-DA model, fold change (FC) criteria and statistical significance. Metabolites meeting the criteria (VIP $\geq$ 1, FC $\geq$ 2 and FC $\leq$ 0.5, and $p < 0.05$) were considered statistically different. The identified metabolites were annotated using the KEGG Compound Database (http://www.kegg.jp/kegg/pathway.html).

3. Results and discussion

3.1. FAAs content and TAV value in broccoli seeds and sprouts

FAAs play a crucial role as taste-active components and serve as essential precursors in the formation of aroma compounds (Liang et al., 2022). The content, thresholds, flavor profiles, and TAV of FAAs in broccoli seeds and sprouts are presented in Table 1. All tested samples contained 17 FAAs, with the total amount ranging from 85.03 to 521.67 mg/100 g. The leucine (Leu) content in sprouts showed a slight increase compared to that in seeds, while the contents of other FAAs significantly increased. Overall, the total FAA content in broccoli sprouts exhibited an approximately fourfold increase compared to the seed. The germination of buckwheat sprouts was found to exhibit a similar phenomenon, characterized by an elevation in the levels of FAAs (Kim et al., 2004). The rise in FAA levels has been ascribed to protein hydrolysis that may take place during the growth process, where enzymes (such as oligopeptidase, proteinases, peptidase, and aminopeptidases) hydrolyze storage proteins in the endosperm into FAAs (Barba et al., 2017).

The taste properties of each FAA vary depending on their characteristic functional groups and spatial structure, resulting in categorizations as bitter, sweet, umami, and aromatic amino acids (Yang et al., 2020). The results presented in Table 1 demonstrate that both broccoli seeds and sprouts exhibit the highest concentration of bitter amino acids, particularly arginine (Arg), which constitutes more than 12 % of the total amino acid content. The umami amino acid content ranked second and the synergy effect between alanine (Ala) and glutamic acid (Glu) can enhance the intensity of the umami taste (Zhang, Chen, et al., 2022). Umami not only mitigates the perception of salty, sour, or bitter tastes but also enhances the perception of sweetness (Liu et al., 2022).

The absolute content and relative ratio of FAAs can significantly influence the sensory perception of food taste. Therefore, TAV for 17 different amino acids in 6 test samples was analyzed and compared. The TAV values of broccoli sprouts ranged from 8.88 to 11.16, while the TAV of broccoli seeds ranged from 2.32 to 2.92, indicating that the germination process enhances its taste intensity. Generally, a TAV value greater than 1 indicates that the taste-producing substance has a major impact on the overall taste effect of the sample, while a TAV value less than 1 suggests that the substance does not have a significant taste-producing effect (Li, Ma, et al., 2022). According to the TAV value, the most significant FAAs that affect broccoli seeds and sprouts were glutamic (Glu) in the umami amino acid, followed by histidine (His) in bitter amino acids and cystine (Cys) in aromatic amino acids. The bitterness of broccoli seeds and sprouts was found to be remarkably prominent, with contribution ratios exceeding 40 %. Interestingly, germination enhances the ratio of umami and sweet taste contributions by about 60 % and 40 % respectively, while diminishing the contribution ratio of aromatic taste by about 40 %. Among the three sprout varieties, Y-BL exhibited the most pronounced flavors, particularly in terms of its sweetness, the TAV value of its sweetness was two to three times that of other varieties. Especially proline, the TAV value of Y-BL was 8.6 times and 3.7 times greater than that of Y-YX and Y-LH, respectively.

3.2. Glucosinolates and sugar analyses in broccoli seeds and sprouts

The presence of 39 glucosinolates was detected in both broccoli seeds and sprouts (Table S2). Total glucosinolate content in all sprouts exhibited a decrease compared to that in seeds (Fig. 1A), which is consistent with a previous study. The total glucosinolate content of broccoli from the Lucky, Tiburon, and Belstar varieties exhibited a significant decrease on the 3rd, 4th, and 5th day of germination (Martinez-Villaluenga et al., 2010). This can be attributed to the dilution of glucosinolates in the sprouts as the tissue increases in size and its water content increases during the germination process. Additionally, degradation of glucosinolates may also contribute to this phenomenon (Brown

Table 1
The FAAs content and TAV value in broccoli seeds and sprouts.

FAA	Taste characteristics	Taste threshold (mg/100 g)	Content (mg/100 g)						TAV value					
			Z-YX	Z-BL	Z-LH	Y-YX	Y-BL	Y-LH	Z-YX	Z-BL	Z-LH	Y-YX	Y-BL	Y-LH
Val	Bitter	40	9.13 ± 0.66^{e}	11.53 ± 1.08^{d}	10.43 ± 1.17^{de}	30.73 ± 1.27^{c}	48.13 ± 1.23^{a}	45.90 ± 0.70^{b}	0.23	0.29	0.26	0.77	1.20	1.15
Ile	Bitter	90	5.43 ± 0.91^{e}	7.87 ± 0.75^{d}	5.20 ± 0.78^{e}	18.77 ± 0.98^{c}	32.83 ± 1.56^{a}	24.73 ± 0.90^{b}	0.06	0.09	0.06	0.21	0.36	0.27
Leu	Bitter	190	10.97 ± 0.82^{d}	18.63 ± 0.63^{b}	14.33 ± 0.34^{c}	11.60 ± 1.02^{d}	21.10 ± 1.18^{a}	15.03 ± 0.05^{c}	0.06	0.10	0.08	0.06	0.11	0.08
Arg	Bitter	50	10.30 ± 0.16^{e}	17.20 ± 2.69^{d}	15.90 ± 1.13^{d}	36.60 ± 1.37^{c}	73.67 ± 0.60^{a}	52.03 ± 0.46^{b}	0.21	0.34	0.32	0.73	1.47	1.04
Met	Bitter	30	0.33 ± 0.05^{d}	0.70 ± 0.08^{c}	0.73 ± 0.12^{bc}	0.73 ± 0.17^{bc}	0.93 ± 0.12^{b}	1.23 ± 0.05^{a}	0.01	0.02	0.02	0.02	0.03	0.04
His	Bitter	20	10.67 ± 0.25^{f}	13.77 ± 1.20^{d}	12.17 ± 0.74^{e}	41.03 ± 0.39^{c}	52.97 ± 0.33^{a}	44.60 ± 0.96^{b}	0.53	0.69	0.61	2.05	2.65	2.23
Bitter amino acids		–	46.93 ± 0.74^{f}	69.70 ± 3.69^{d}	58.77 ± 2.73^{e}	139.47 ± 1.28^{c}	229.63 ± 3.19^{a}	183.53 ± 2.50^{b}	1.10	1.53	1.34	3.85	5.82	4.81
Asp	Umami	100	3.47 ± 0.19^{d}	6.17 ± 1.34^{c}	5.57 ± 0.82^{c}	14.03 ± 0.66^{b}	35.30 ± 0.70^{a}	36.43 ± 0.62^{a}	0.03	0.06	0.06	0.14	0.35	0.36
Glu	Umami	30	11.47 ± 1.82^{c}	13.80 ± 0.45^{c}	13.23 ± 0.90^{c}	73.83 ± 0.91^{b}	106.43 ± 1.69^{a}	107.23 ± 1.00^{a}	0.38	0.46	0.44	2.46	3.55	3.58
Lys	Umami	50	2.93 ± 0.34^{d}	2.03 ± 0.12^{d}	1.67 ± 0.09^{d}	16.07 ± 1.81^{c}	26.03 ± 1.38^{a}	20.97 ± 1.43^{b}	0.06	0.04	0.03	0.32	0.52	0.42
Umami amino acids		–	17.87 ± 1.815^{e}	22.00 ± 1.85^{d}	20.47 ± 0.17^{de}	103.93 ± 2.24^{c}	167.77 ± 3.19^{a}	164.67 ± 0.86^{b}	0.48	0.56	0.53	2.92	4.42	4.36
Ser	Sweet	150	2.30 ± 0.29^{d}	2.60 ± 0.14^{d}	2.03 ± 0.25^{d}	11.87 ± 1.23^{c}	14.30 ± 1.53^{b}	16.93 ± 0.62^{a}	0.02	0.02	0.01	0.08	0.10	0.11
Thr	Sweet	260	7.33 ± 0.76^{e}	8.43 ± 0.34^{e}	11.30 ± 0.99^{d}	20.23 ± 1.02^{b}	18.40 ± 0.96^{c}	26.30 ± 0.75^{a}	0.03	0.03	0.04	0.08	0.07	0.10
Gly	Sweet	130	1.13 ± 0.12^{e}	2.40 ± 0.14^{d}	3.87 ± 0.48^{c}	2.77 ± 0.34^{d}	6.73 ± 0.60^{a}	5.20 ± 0.24^{b}	0.01	0.02	0.03	0.02	0.05	0.04
Ala	Sweet	60	2.43 ± 0.12^{de}	2.47 ± 0.29^{d}	1.73 ± 0.21^{e}	9.50 ± 0.70^{c}	33.33 ± 0.38^{a}	17.90 ± 0.14^{b}	0.04	0.04	0.03	0.16	0.56	0.30
Pro	Sweet	300	0.90 ± 0.08^{d}	1.17 ± 0.12^{d}	1.30 ± 0.08^{d}	3.13 ± 0.58^{c}	25.83 ± 0.90^{a}	6.83 ± 0.79^{b}	0.03	0.04	0.04	0.10	0.86	0.23
Sweet amino acids		–	14.10 ± 1.16^{e}	17.07 ± 0.21^{de}	20.23 ± 0.61^{d}	47.50 ± 2.97^{c}	98.60 ± 3.19^{a}	73.17 ± 0.85^{b}	0.12	0.15	0.16	0.44	1.64	0.78
Cys	Aromatic	2	1.13 ± 0.21^{c}	1.23 ± 0.06^{c}	0.73 ± 0.17^{d}	2.97 ± 0.21^{a}	2.67 ± 0.19^{a}	1.93 ± 0.29^{b}	0.57	0.62	0.37	1.48	1.33	0.97
Tyr	Aromatic	90	2.13 ± 0.25^{c}	2.47 ± 0.05^{c}	1.33 ± 0.16^{c}	6.23 ± 0.58^{a}	6.57 ± 0.74^{a}	5.07 ± 1.00^{b}	0.02	0.03	0.01	0.07	0.07	0.06
Phe	Aromatic	90	2.97 ± 0.53^{c}	3.30 ± 0.22^{c}	2.80 ± 0.22^{c}	10.50 ± 0.54^{b}	16.43 ± 0.74^{a}	16.53 ± 0.74^{a}	0.03	0.04	0.03	0.12	0.18	0.18
Aromatic amino acids		–	6.23 ± 0.60^{de}	7.00 ± 0.23^{d}	4.87 ± 0.38^{e}	19.70 ± 0.92^{c}	25.67 ± 1.31^{a}	23.53 ± 1.58^{b}	0.62	0.68	0.41	1.67	1.58	1.21
Total FAAs		–	85.03 ± 0.87^{f}	115.77 ± 3.07^{d}	104.33 ± 3.12^{e}	310.60 ± 7.02^{c}	521.67 ± 7.00^{a}	444.90 ± 2.04^{b}	2.32	2.92	2.45	8.88	13.46	11.16

Data are presented as mean ± SD (n = 3, for FAAs). a-f: different letters within a row are significantly different ($P < 0.05$). FAAs, free amino acids; TAV, taste activity value; Val, valine; Ile, isoleucine; Leu, leucine; Arg, arginine; Met, methionine; His, histidine; Asp, aspartic acid; Glu, glutamic acid; Lys, lysine; Ser, serine; Thr, threonine; Gly, glycine; Ala, alanine; Pro, proline; Cys, cystine; Tyr, tyrosine; Phe, phenylalanine.

et al., 2003). Glucosinolates are considered the most important secondary metabolite in broccoli, undergoing initial degradation into volatile isothiocyanates and nitriles. These compounds contain nitrogen-containing groups that contribute to the pungent odor and bitter taste (Zheng et al., 2023). The main glucosinolates in broccoli are glucobrassicin, neoglucobrassicin and glucoraphanin, which have been proven to exhibit a significant correlation with bitterness. 3-Indolylmethyl glucosinolate and 2-(*R*)-2-Hydroxy-3-butenyl glucosinolate are also regarded as the main source of bitterness (Li et al., 2021).

A total of 63 sugar components were identified in broccoli seeds and sprouts (Table S2), with a significantly observed increase in the overall sugar content during germination (Fig. 1B). The same phenomena have also been observed in legumes, such as kidney beans, mung beans, soybeans, and peanuts, and total sugar content increased in the germinated legume samples (Benitez et al., 2013; Megat et al., 2016). During germination, the hydrolysis of carbohydrate storage in the form of starch and oligosaccharides leads to an increase in sugar levels. The increased activity of α-galactosidase leads to the cleavage of α-1,6-galatosidic bonds, which may be another reason for the increase in total sugar content (Megat et al., 2016). The most predominant sugar components in broccoli are sucrose, glucose, and fructose. However, they exhibit distinct sweet tastes, with fructose being the sweetest among the three (Chevilly et al., 2023; Keutgen & Pawelzik, 2007). The sweetness of broccoli seeds and sprouts is primarily attributed to these sugars, which are also likely to inhibit bitterness (Bell et al., 2018).

3.3. Volatile metabolites analysis of broccoli seeds and sprouts

The analysis of volatile metabolites was conducted to investigate the flavor distinction between broccoli seeds and sprouts. The total ion current of QC samples (Fig. S1) exhibits a high degree of curve overlap, indicating stable signal detection across different times using mass spectrometry (Rong et al., 2024). A total of 364 volatile metabolites were identified and categorized into 15 groups in seeds and sprouts of three broccoli cultivars (Table S3). Heterocyclic compound (19.83 %), ester (17.63 %), and terpenoids (16.25 %) constituted significant proportions among the volatile metabolites (Fig. 2A). Previous studies have identified 43 compounds in broccoli sprouts, including heterocyclic

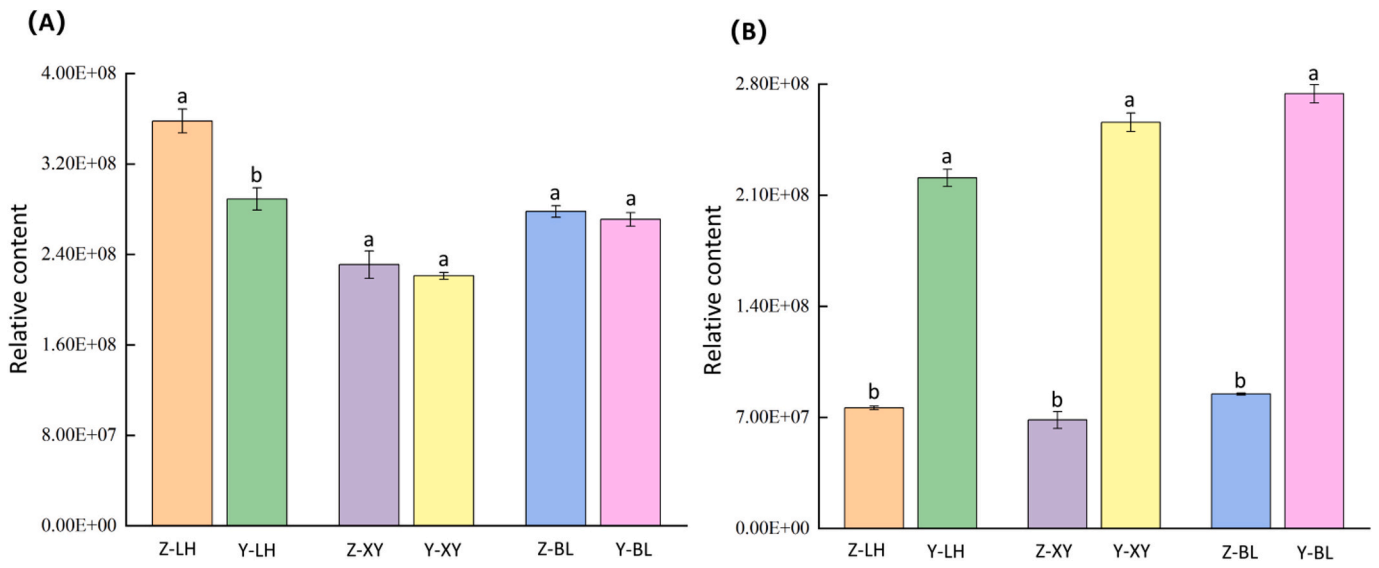

Fig. 1. (A) Total glucosinolates and (B) sugar relative content in different varieties of broccoli seeds or sprouts Values are expressed as mean ± SD ($n = 3$). a-b: Bars with different lowercase letters represent significant differences between seeds and sprouts of the same variety ($p < 0.05$).

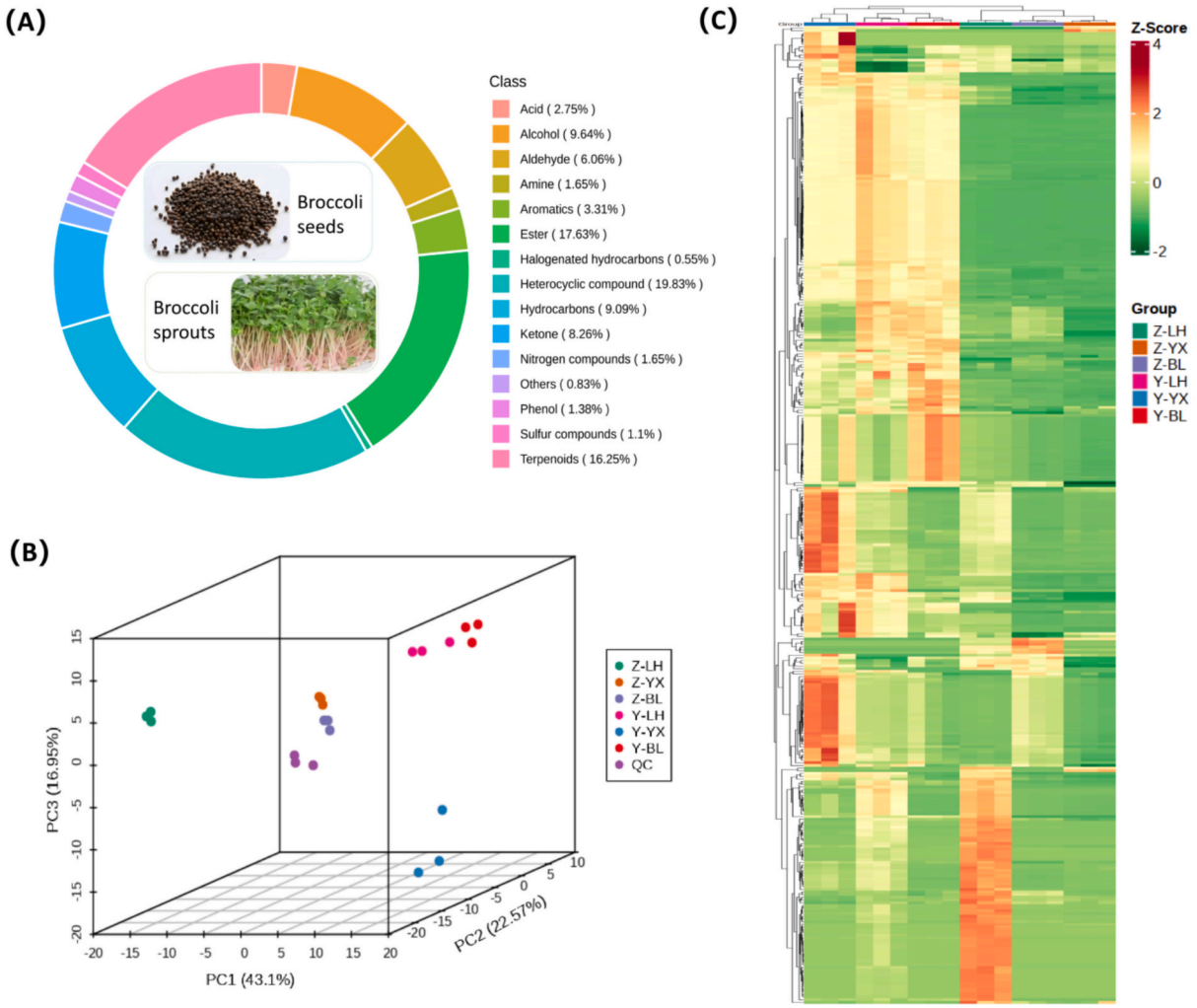

Fig. 2. Volatile metabolites analysis of three cultivars of broccoli seeds and sprouts. (A) Classification of volatile metabolites; (B) PCA score plot; (C) clustering heat map.

compounds, aromatic compounds, nitriles, thiocyanate and isothiocyanates, alkyl-hydrocarbons, carbonyls, alcohols, and esters (Jang et al., 2015). Cinnamyl acetate and 5-(methylthio)-valeronitrile were identified as key odorants in both seeds and sprouts, contributing spicy and cabbage-like aromas, respectively (Lasekan & See, 2015). The PCA analysis provided insights into the overall metabolite differences between groups and the extent of variability within each group. The first three PCs of PCA accounted for 82.60 % of the total variance (Fig. 2B). The PCA analysis demonstrated distinct separation among the three cultivars and QC samples, with all QC samples tightly clustered together, which highlights the high reliability and reproducibility of the measured data. Broccoli seeds and sprouts of the same cultivar exhibited differences in volatile components. The heatmap (Fig. 2C) clearly segregates the two into distinct categories indicating significant differences in metabolite composition.

3.3.1. *Analysis of volatile metabolites among different varieties of broccoli seeds or sprouts*

To monitor the differences in volatile metabolites among different varieties of broccoli seeds or sprouts, OPLS-DA analyses were performed for several comparisons, including *Z*-LH vs Z-YX vs Z-BL, and Y-LH vs Y-YX vs Y-BL. In the OPLS-DA score plot (Fig. S2A ~ B), the broccoli seeds and sprouts of various cultivars also demonstrated distinct segregation, suggesting that the variety of broccoli exerted a substantial impact on volatile metabolites. As shown in Fig. S2C ~ D, the OPLS-DA model validation result of broccoli seeds was $R^2X = 0.896$, $R^2Y = 0.989$, and $Q^2 = 0.98$. For broccoli sprouts, $R^2X = 0.789$, $R^2Y = 0.99$, and $Q^2 = 0.977$. The R^2Y and Q^2 values surpass 0.9, indicating an exceptional model with excellent predictability and high accuracy of fit.

There were varying numbers of differential metabolites between different comparisons in broccoli seeds (Fig. 3A~B), with heterocyclic compound (11), ester (9) and terpenoids (9) being key categories. It is worth noting that the expression levels of differential metabolites showed both up- and down-regulation trends in different varieties (Table S4). The top 15 metabolites with the largest Variable Importance in Projection (VIP) values were mainly ketone (5) and ester (4) (Fig. 3C), all of which have the highest content in Z-BL. Among these, 2-phenoxyethyl propionate acted as a perfuming agent and exhibited a strong fruity taste (Api et al., 2022), while geranylacetone and cinnamyl acetate are characterized by floral notes (violet and rose)and sweet odor, respectively (Bhatia et al., 2007; Bonikowski et al., 2015). Dihydro-β-ionone is the main aroma compound in *Osmanthus* oil, which imparts a mellow, sweet, and fresh cedar scent. Interestingly, this compound has also been discovered in broccoli seeds for the first time (Xu et al., 2019).

The number of differential metabolites between Y-YX vs Y-BL, Y-LH vs Y-YX and Y-LH vs Y-BL were 135, 158, and 155 respectively (Fig. 3D), and 36 differential metabolites were identified in three broccoli sprouts (Fig. 3E), predominantly comprising terpenoids (7), hydrocarbons (6) and alcohol (5). Compared with Y-BL, the expression levels of all differential metabolites were up-regulated in both Y-YX and Y-LH (Table S6). The top 15 differential metabolites with the highest VIP values were predominantly terpenoids (4), hydrocarbons (3) and alcohol (3) (Fig. 3F). Among these differential metabolites, citronellal was the main component of citronella and Lemongrass oil and lemon scent (Quintans-Junior et al., 2011). The compounds (E, Z)-3,6-nonadien-1-ol and (Z)-3-nonen-1-ol were primarily found in melons and possess a green and fresh fruity aroma. Due to their low odor detection threshold of 0.2 ppb, these compounds can be considered highly potent odorants. The presence of these two compounds has been newly discovered in broccoli sprouts (Perry et al., 2009).

The comparison of broccoli seeds and sprouts revealed a significant enrichment of 9 and 8 differential metabolites in the metabolic pathways, respectively. Furthermore, other pathways such as phenylpropanoid biosynthesis, monoterpenoid biosynthesis, fatty acid biosynthesis, and butanoate metabolism were also found to be enriched both in broccoli seeds and sprouts (Table S5 and S7). A previous study also reported that volatile compounds in apples were primarily synthesized through the fatty acid, phenylpropanoid, and other metabolic pathways. Among these, fatty acid metabolism plays a crucial role in the synthesis of aldehydes, alcohols, and esters (Liu et al., 2021).

3.3.2. *Analysis of volatile metabolites between broccoli seeds and sprouts of the same varieties*

The abundance of metabolites varies significantly among broccoli seeds and sprouts of the same varieties, but the relative ratios of different metabolite abundance remain remarkably consistent (Fig. S3A). The OPLS-DA score plot (Fig. S3B) demonstrated the pronounced differences between seeds and sprouts of the same varieties, as

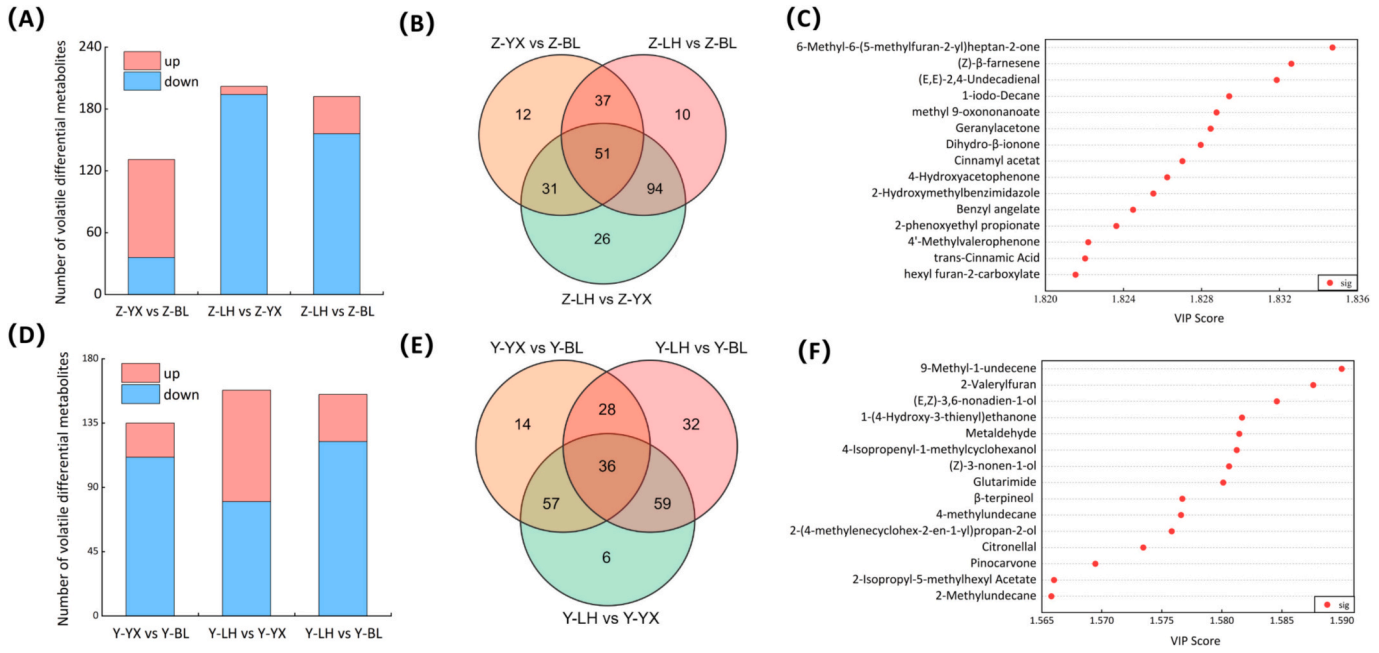

Fig. 3. Analysis of volatile metabolites between seeds and sprouts of different varieties. The number of differential metabolites among (A) seeds and (D) sprouts; Venn diagram of (B) seeds and (E) sprouts; differential metabolites in the top 15 of VIP values of (C) seeds and (F) sprouts.

well as a high level of intragroup reproducibility, surpassing that observed in PCA. The well-explained variance ($R^2X = 0.821$, $R^2Y = 0.995$) and cross-validated predictive capability ($Q^2 = 0.988$) confirm the model's reliability (Fig. S3C).

In addition, volcano plot analysis was applied to acquire a comprehensive understanding of the metabolite differences during the germination process of broccoli. The volcano plot (Fig. 4A~C) revealed substantial differences in metabolite profiles during the germination process of broccoli seeds, demonstrating the dynamic change in the volatile profile. A significant number of differential metabolites were observed between broccoli seeds and sprouts of identical varieties. The broccoli flavor was significantly enhanced after germination, with a majority of metabolites exhibiting upregulation during the germination process (Table S8), which may be attributed to the macronutrient composition in the seeds. Macronutrients such as starch and protein exhibit a pronounced affinity for flavor compounds, and can effectively capture them through hydrophobic interactions and reversible hydrogen bonding, resulting in a higher polarity aroma compared to inclusion complexes. During the process of germination, protein and starch are hydrolyzed into small molecules by a protease and α-amylase respectively, leading to the release of trapped flavor compounds (Lv et al., 2017). Additionally, endogenous lipases hydrolyzed the stored lipids in the seeds to free fatty acids during germination, while lipoxygenase facilitates the oxidation of unsaturated fatty acids to generate hydroperoxides. These hydroperoxides were subsequently degraded by hydroperoxide lyase and alcohol oxidoreductase into volatile compounds such as alkanes, ketones, aldehydes, and alcohols (Keawkim et al., 2021).

Venn diagram analysis showed up to 139 differential metabolites among the three varieties (Fig. 4D), heterocyclic compound, terpenoids, and esters were identified as primary differential metabolites during broccoli germination (Fig. 4E). The expression of volatile hydrocarbons was predominantly upregulated during germination, but their contribution to flavor may be relatively limited due to a high threshold (Lv et al., 2017). The levels of all the esters' differential metabolites were upregulated after germination. Among them, 2-methyl butyl 2-methyl butyrate was widely recognized as one of the key contributors to the distinctive sweet and apple-like aroma found in broccoli (Shoko et al., 2013). The levels of all alcohol differential metabolites, except for 2-cyclopentylethanol, were observed to be upregulated following germination. The formation of alcohols primarily occurs through lipid oxidation and decomposition, as well as the dehydrogenation and decarboxylation of amino acids, resulting in a floral and pleasantly fruity aroma with significant odor-active values (Yin et al., 2021). Except for pentan-1-ol, which is the initial product of the lipoxygenase reaction, exhibits an unpleasant fermented aroma (Zhou et al., 2023). There are only two differential metabolites of acids. Among them, tiglic acid possesses a pleasant, warm spicy aroma (Lee et al., 2008), and is upregulated during the germination process of YX while being downregulated in the germination processes of BL and LH.

3.4. E-nose evaluation of broccoli sprouts

The E-nose flavor radar of different varieties of broccoli seeds and sprouts was illustrated in Fig. 5A. The characteristic sensors included S1, S2, S4, and S14. Particularly, S1 emerged as the most prominent in capturing the distinctive flavors. The identified characteristic flavors (Table S1) included alkane and sulfur compounds, as well as aromatic compounds, consistent with the flavor profile of ripe broccoli (Hong et al., 2022). Notably, the predominant pungent odor associated with broccoli is attributed to sulfur-containing compounds, which have an extremely low odor threshold (Wieczorek et al., 2020). The characteristic sensor values of broccoli sprouts were generally higher than those of seed samples, indicating that the process of germination resulted in the production and release of flavor compounds. Fig. 5B illustrates the PCA for the flavor of different varieties of broccoli seeds and sprouts. Two principal components explained more than 70.00 % of the variance. Broccoli sprouts of various varieties grouped together, as were the broccoli seeds. However, there was still a noticeable distinction between seeds and sprouts, indicating that the flavor of all the broccoli seed

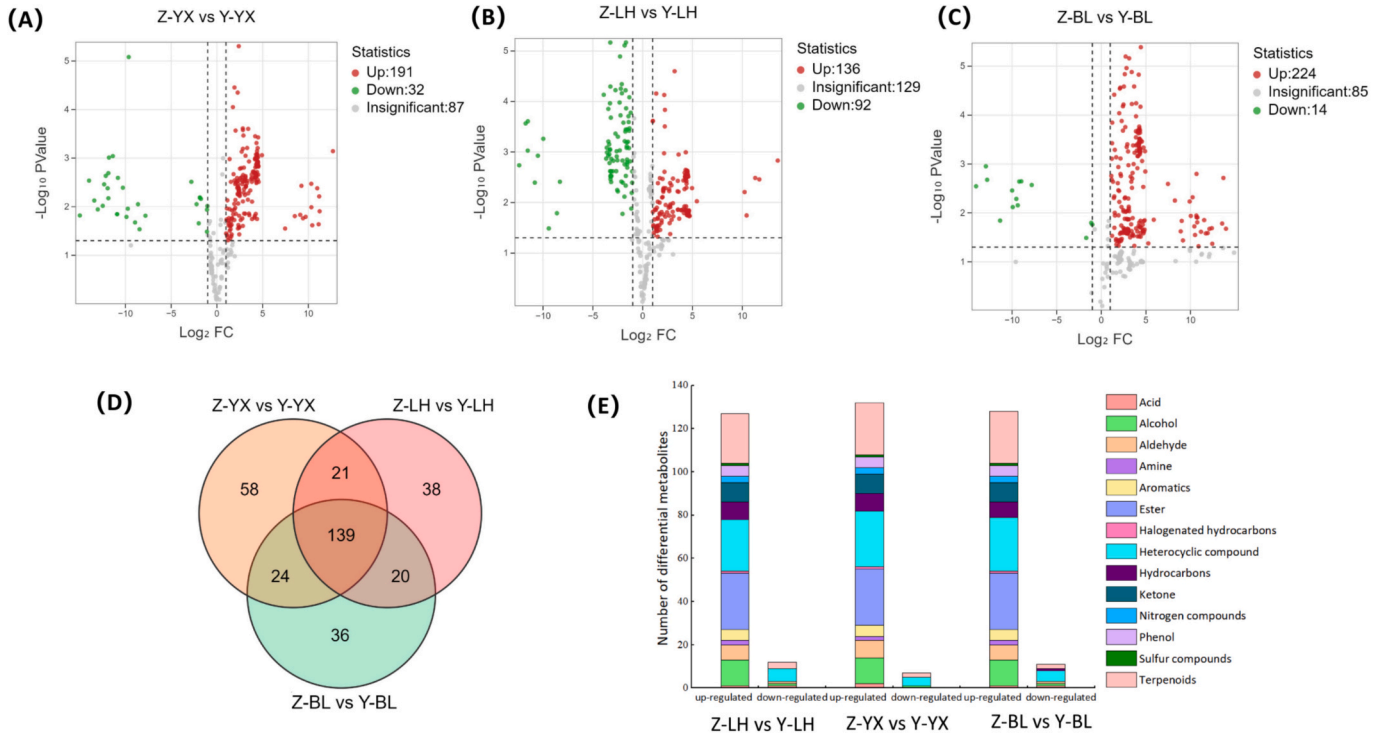

Fig. 4. Metabolomic comparison between broccoli seeds and sprouts. Volcano plots of (A) Z-LH vs Y-LH; (B) Z-YX vs Y-YX; (C) Z-BL vs Y-BL; (D) Venn diagram and (E) the number of differential metabolites among the three comparison groups.

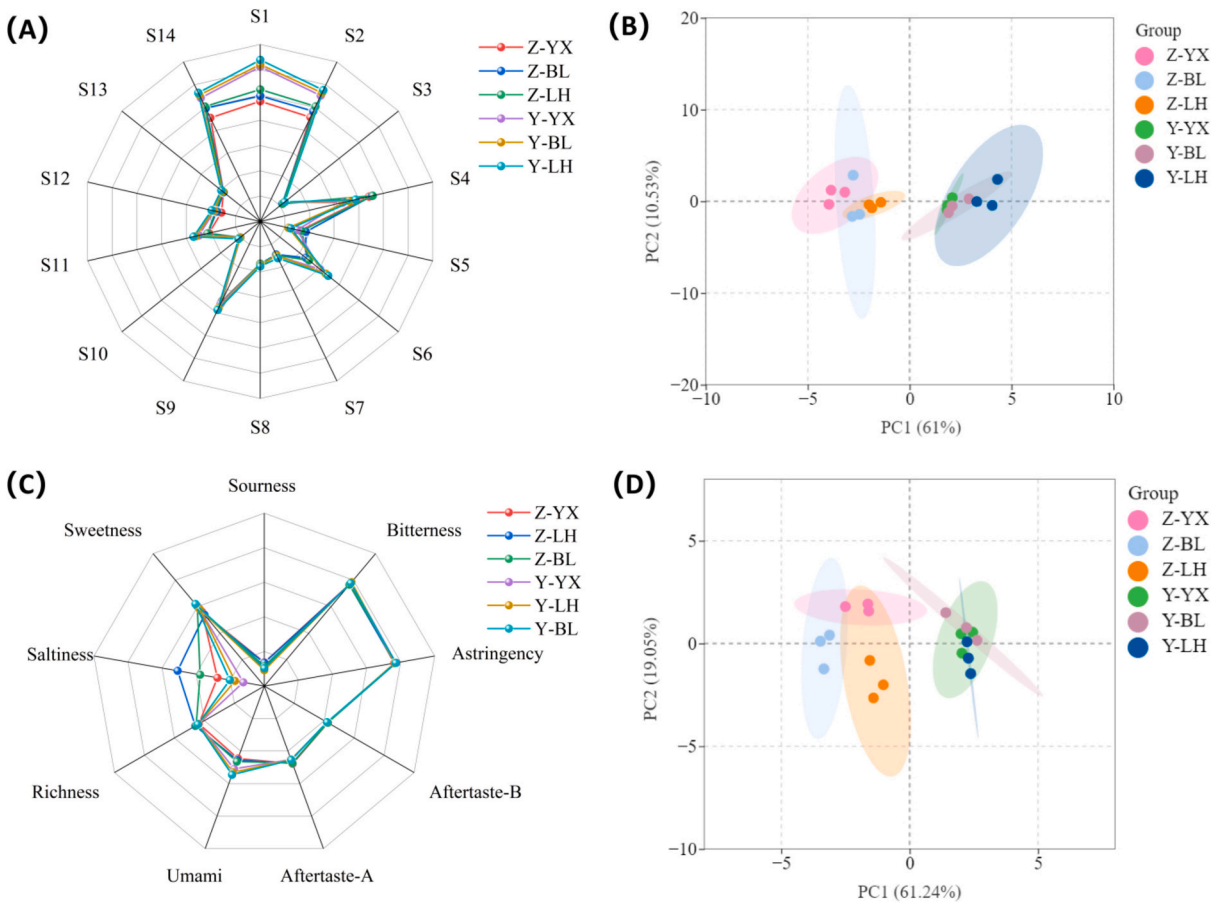

Fig. 5. Statistical analysis of E-nose and E-tongue dataset in broccoli seeds and sprouts. Radar chart (A) and PCA score plot (B) for E-nose. Radar chart (C) and PCA score plot (D) for E-tongue.

samples had changed after germination. The phenomenon is also observed in legumes and quinoa, where the flavor undergoes significant changes during germination. Specifically, germination enhances the floral aroma of quinoa and the woody aroma of legumes (Kaczmarska et al., 2018; Peng et al., 2024).

3.5. E-tongue evaluation of broccoli sprouts

The radar map for taste determination of broccoli by the E-tongue is presented in Fig. 5C. Bitterness, astringency, and sweetness were prominent flavors observed in broccoli seeds and sprouts, while sourness and richness were not distinctly evident, this observation was consistent with the flavor characteristics typically found in mature broccoli (Hong et al., 2022). Bitterness and astringency, often considered undesirable attributes, are identified as the key features of broccoli, that arise from compounds like glucosinolates and their degradation products (Hennig et al., 2014). Compounds such as phenols, vitamin C and quinine also contribute to the bitterness of broccoli (Zeng et al., 2021). The sweetness is prominent derived from the high sugar (glucose, fructose and sucrose) content of broccoli (Schonhof et al., 2004). There were no significant differences in the intensities of sourness and richness among these groups, whereas the broccoli seeds group exhibited higher saltiness taste values and the broccoli sprouts showed higher umami taste values. Besides, the Y-BL variety of broccoli sprouts demonstrated the highest levels of sweetness and umami compared to other varieties, which was consistent with the findings of the FAA and sugar analysis. As can be seen from Fig. 5D, the contribution rates of PC1 and PC2 were 61.24 % and 19.05 % respectively, with a cumulative contribution rate of 80.29 %. The PCA results revealed significant differences in the taste profiles between broccoli seeds and sprouts, indicating that the germination process greatly changes the taste of broccoli.

3.6. KEGG metabolic pathway analysis

The differential metabolites were clustered and analyzed to investigate potentially significant metabolic pathways, specifically investigating the patterns of changes in substance content during broccoli germination. The KEGG enrichment analysis of differential metabolites between the same cultivars seeds and sprouts was shown in Table S9, the enriched metabolic pathways in broccoli seeds during germination varied among cultivars. BL, LH and YX were mapped to 6, 8 and 14 core metabolic pathways, respectively. The six common metabolic pathways were identified, including tyrosine metabolism, monoterpenoid biosynthesis, biosynthesis of secondary metabolites, metabolic pathways, limonene and pinene degradation, as well as tropane, piperidine and pyridine alkaloid biosynthesis. The metabolic pathways involving terpenoids, aromatics, and nitrogen compounds exhibited varying levels between seeds and sprouts, with the majority of these related compounds being up-regulated in all three sprouts. These predicted pathways were in alignment with the changes in the relative abundance of differential metabolites during broccoli germination (Fig. 4E). During germination, the activation of endogenous enzymes facilitates the breakdown of macromolecules such as proteins and carbohydrates, thereby enhancing the accessibility of nutrients in seeds. Additionally, a range of bioactive compounds is also synthesized (Ma et al., 2022). Secondary metabolites, such as terpenes, alkaloids and nitrogen/sulfur-containing compounds were enriched in the biosynthesis of secondary metabolites pathway, which significantly affects the flavor of

cauliflower sprouts (Albaladejo-Marico et al., 2024). Besides, KEGG analysis showed differential metabolites were also mapped to tyrosine metabolism. As previously reported, tyrosine plays a crucial role in energy metabolism, signal transduction, and the biosynthesis of proteins and glucosinolates, thereby elucidating its status as an active metabolite during broccoli germination (Lyu et al., 2023).

4. Conclusions

The present study conducted a comprehensive analysis of the volatile component composition, flavor characteristics, and sensory evaluation of three distinct broccoli varieties seeds and sprouts. The levels of volatile metabolites in broccoli sprouts vary between variety and pre- and post-germination, with a majority of volatile components demonstrating an increase during the process of broccoli germination. The germination process enhanced the total FAAs by approximately fourfold, particularly elevating the TAV value of umami and sweet FAAs, the significant increase in total sugar content after germination may be another source of sweet taste in broccoli sprouts. Despite a decrease in total glucosinolates after broccoli germination, the bitterness and astringency remain the predominant flavors of broccoli sprouts. Future research is needed to further enhance the flavor profile of broccoli sprouts to satisfy the dual preferences of consumers with both taste appeal and nutritional benefits.

CRediT authorship contribution statement

Yu Xia: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Conceptualization. **Ming-Yue Li:** Investigation. **Syed Abdul Wadood:** Writing – review & editing. **Han-Jun Hong:** Conceptualization, Supervision. **Yi Liu:** Methodology. **Yu-Xuan Luo:** Investigation. **Yi-Yan Wang:** Investigation. **Hong-Yan Liu:** Writing – review & editing, Supervision, Conceptualization. **Ren-You Gan:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

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

Data availability

Data will be made available on request.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101862.

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