Current Research in Food Science 9 (2024) 100854

Contents lists available at ScienceDirect

Current Research in Food Science

journal homepage: www.sciencedirect.com/journal/current-research-in-food-science

Characterization and discrimination of volatile organic compounds and α-glucosidase inhibitory activity of soybeans (*Glycine max* L.) during solid-state fermentation with *Eurotium cristatum* YL-1

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ARTICLE INFO

Handling Editor: Dr. Quancai Sun

Keywords:
Flavor characteristics
Headspace-gas chromatography-ion mobility spectrometry
Beany flavor
α-glucosidase inhibitory activity
Molecular docking analysis

ABSTRACT

In this study, the influence of solid-state fermentation (SSF) using probiotic *Eurotium cristatum* on the change of volatile organic compounds (VOCs) and α-glucosidase inhibition activity of soybeans was investigated. A total of 46 VOCs were characterized via headspace gas chromatography-ion mobility spectrometry (HS-GC-IMS), the majority of which were aldehydes (17), alcohols (10), and ketones (7). SSF by *E. cristatum* drastically affected the flavor characteristics of soybeans, and the levels of unpleasant beany flavor components, such as hexanal-D, 1-octen-3-ol, 1-hexanol-D, 1-hexanol-M, heptanal-M, 1-pentanol, heptanal-D, and 2-pentyl furan were all substantially decreased by 50% after 15 days of SSF, while volatiles with floral, caramel, and desirable flavors such as pentanal-D, methylpropanal, 2-propanol, and propyl acetate drastically ($p < 0.05$) increased by 1.1-, 19.2-, 3.6-, and 2.6-fold, respectively. Key aroma-active compounds analysis revealed that 18 VOCs (ROAV, relative odor activity value ≥ 1) play a great role in shaping the flavor characteristics of the soybean samples. After 15 days of SSF, the ROAV values of methylpropanal, 2-propanol, and propyl acetate drastically ($p < 0.05$) increased to 8.48, 63.88, and 11.29, respectively, which greatly contributed to the desirable flavor characteristics of fermented soybeans. Furthermore, *E. cristatum* greatly improved the α-glucosidase inhibitory activity of soybean by 22.4% after 15 days fermentation, which was closely correlated with the accumulated phenolic compounds during SSF. Molecular docking showed that genistein and daidzein have high binding affinity for α-glucosidase active sites, primarily driven by hydrogen bonds and hydrophobic interactions. These results demonstrated that soybeans fermented with *E. cristatum* substantially improved the flavor characteristics and α-glucosidase inhibitory effect, and were greatly helpful to promote the application of soybeans in food products.

1. Introduction

Soybean (*Glycine* max L.) has been consumed in Asian countries for centuries and now attracted great attention in Western countries (An et al., 2023; Chen et al., 2022b). Soybean is rich in protein, dietary fiber, vitamins phenolic compounds, isoflavones, and other nutritional components. Many early studies reported that soybean has diverse health-promoting effects on chronic diseases, including anti-oxidant, anti-cancer, anti-atherosclerosis, prevention of diabetes and obesity, and cholesterol-reducing activities (Chen et al., 2023; Lee et al., 2019; Liu et al., 2022). In the last decades, the consumption of soybean products has gained increasing attention due to its potential health benefits (Liu et al., 2022). However, one barrier to greater human consumption of soybean products is the off-flavor attributes, which are often described as the so-called "beany flavor" that has a great negative effect on the acceptance of consumers (Wang et al., 2021). Earlier

Abbreviations: SSF, solid-state fermentation; VOCs, volatile organic compounds; respectively, SB0, SB2, SB4, SB8, SB10, and SB15 represented soybeans fermented with *E. cristatum* for 0, 2, 4, 8, 10, and 15 days; PCA, principal components analysis; ROAV, relative odor activity value; GIA, α-glucosidase inhibitory activity.

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https://doi.org/10.1016/j.crfs.2024.100854
Received 14 June 2024; Received in revised form 31 August 2024; Accepted 13 September 2024
Available online 14 September 2024

studies have reported that the beany flavor was mainly ascribed to 1-octen-3-ol, 1-pentanol, 2-pentyl furan, hexanol, hexanal, and other related volatile organic compounds (VOCs) (Wang et al., 2022; Xiang et al., 2023; Yang et al., 2021), which have a great impact on the sensory characteristics of soybean foods. As a result, there is growing research attention on improving the flavor characteristics of soybean products.

Several processing methods have been reported to eliminate the off-flavors of soybean products including germination, high pressure, and heat treatment (Kaczmarska et al., 2018; Wang et al., 2021; Xiang et al., 2023). For instance, Cai et al. (2021) reported that heat treatment could effectively eliminate the undesirable flavors of soybeans by inactivating the endogenous lipoxygenases and peroxidases. These approaches can decrease the levels of undesirable beany flavors to some extent. Nonetheless, some drawbacks also existed in the above strategies, for instance, heat treatment caused texture deterioration and loss of nutritional components. Recently, studies have reported that solid-state fermentation (SSF) is an attractive technology for decreasing the off-flavors of soybean products and also producing favorable aromas (Chen et al., 2022b; Wang et al., 2022). SSF is well recognized as an efficient technology for improving the flavor characteristics and biological activities of legumes, which is a simple operation, economical cost, consecutiveness selectivity and environmental friendliness approach that received increasing attention (Chen et al., 2023; Keong et al., 2023). Many traditional fermented soybean products, such as natto, sufu, and douchi in Asian countries, were achieved by SSF, which are widely received in popularity by consumers worldwide due to their good flavor and diverse health benefits (An et al., 2023; Liu et al., 2022; Zhang et al., 2024). Dajanta et al. (2011) reported that soybean fermented by *Bacillus subtilis* TN51 showed a significant increase of pleasant odors 2,6-dimethylpyrazine, 2,3,5-trimethylpyrazine, 2, 5-dimethylpyrazine, 2-methylpyrazine, 2-methylbutanoic acid, methylpropanal, 2-methylbutanoic acid, and acetic acid. Vong and Liu (2017) found that soybean residue (okara) processed by yeasts remarkably decreased the content of beany and grassy odors such as heptanal, *trans*-2-hexenal, 1-hexanol, and greatly increased the desirable aromas such as 2-methylpropanal, 2-propanol, 2-propanone, ethyl acetate, ethyl propanoate, 2-phenylethyl acetate, isoamyl aldehyde and propyl acetate. Additionally, the off-flavors 1-hexanol and hexanal of soybean degraded 100% by fermentation with *Aspergillus oryzae* for 72 h, and the 2-propanol imparts fruit, floral and sweet odors increased 43.3% (Hwang and Kim, 2023). Thus, soybeans processed by SSF might be an effective approach for improving the aroma characteristics.

Eurotium cristatum is a dominant fungal applied in the post-fermentation of Chinese Fu brick tea (Xiao et al., 2022a, 2022b). For the past few years, many health-promoting bioactive phytochemicals have been accumulated in plant-based food materials by SSF with *E. cristatum* (e.g., okara, buckwheat, tea, and soybean), thus enhancing their bioactivities (Chen et al., 2020b; Xiao et al., 2021a). Furthermore, previous studies reported that *E. cristatum* can secret a variety of extracellular hydrolase catalyzes the transformation of tea substances, thus greatly improving the flavor characteristics of dark tea (Xiao et al., 2022b). Our earlier study found that a wide range of extracellular enzymes were also generated by *E. cristatum* during soybeans fermentation, including cellulase, β-glucosidase, α-amylase, and protease, which biotransformation of the metabolites and greatly improved the antioxidant activity and isoflavone aglycones of soybeans (Chen et al., 2020b). Previous studies demonstrated that the beany flavors could be degraded by microorganisms for the produced extracellular enzymes (Du et al., 2022; Xiang et al., 2023). Therefore, *E. cristatum* might have the capacity to transform the off-flavor of soybeans. However, there is still no investigation available on SSF with *E. cristatum* affects the flavors of soybeans.

Thus, the objective of this work was to examine the alteration of VOCs and α-glucosidase inhibitory activity (GIA) of soybeans during SSF with *E. cristatum*. A new method for analyzing flavors, known as headspace gas chromatography-ion mobility spectrometry (HS-GC-IMS), was used in conjunction with multivariate statistical analysis to thoroughly identify and differentiate the volatile organic compounds (VOCs) present in soybeans during fermentation. The result of this study will demonstrate how *E. cristatum* affects the VOCs and GIA of soybeans during SSF, and will also provide a theoretical foundation for the greater utilization and processing of soybeans.

2. Materials and methods

2.1. Materials and chemicals

Ethanol, *p*-nitrophenyl glucopyranoside (*p*NPG), α-glucosidase enzyme, and the C4-C9 n-Ketones were provided by Sinopharm Chemical Reagent (Beijing, China). The other substances or reagents that were used in this investigation were all of analytical grade. A local supermarket (Dongzhiyuan, Changsha, China) provided the soybean seeds. The experimental strain *Eurotium cristatum* YL-1 was isolated from Fu-brick dark tea in Anhua, Hunan Province (Chen et al., 2020b; Xiao et al., 2021a). The isolation method of *Eurotium cristatum* YL-1 was adapted from Mao et al. (2017). Briefly, 5.0 g of Fu brick tea was added to a flask with 95.0 mL of sterile water and sterile glass beads, then vortexed at 25 °C for 30 min at 200 rpm. The suspension was serially diluted 10-fold with sterile saline solution, and the dilution containing yellow cleistothecia of "golden flora" fungi was plated onto M40Y medium (400 g/L sucrose, 20 g/L malt extract, 5 g/L yeast extract, and 20 g/L agar). Colonies appeared after 7 days of incubation at 28 °C. Hyphal tips from the M40Y medium plates were excised and transferred to fresh medium for further cultivation. A purified strain was obtained by selecting and propagating the well-growing strain for 3 to 4 generations. The isolated stain was identified as *Eurotium cristatum* according to the morphological and molecular characterizations. The strain was maintained at 4 °C and periodically sub-cultured for further use.

2.2. Preparation of inoculum starter

The following procedure was utilized to prepare the inoculum starter that was used for soybean fermentation. Briefly, the preserved *E. cristatum* was transplanted twice consecutively in M40Y agar medium (400 g/L sucrose, 20 g/L malt extract, 5 g/L yeast extract, and 20 g/L agar) to fully activate the strain. *E. cristatum* was first inoculated into M40Y medium at 28 °C and grown for 7 days. The culture was then transferred to the M40Y plate and a further cultivation was carried out at the same temperature. A large number of *E. cristatum* mycelia and spores in the activated culture were washed with sterile water and filtered into a sterilized flask. After stirring with small glass beads, the spore concentration was calculated and adjusted to 10^6-10^7 spores per milliliter. Every microbiological experiment was carried out in a sterile setting.

2.3. Solid-state fermentation of soybeans

The solid-state fermentation of soybeans was prepared according to our previous study (Chen et al., 2020b). Briefly, deionized water was used to wash the soybeans. The seeds were then submerged in deionized water at room temperature for 1 h. After pouring out the water, the obtained soybeans underwent a 25-min autoclave sterilization process at 121 °C. The soybeans were cooled to 25 °C, inoculated with the spore of *E. cristatum* (5 mL/100 g), and then kept at 28 °C for 15 days. The soybean samples fermented with *E. cristatum* for different time, namely, 2 days (SB2), 4 days (SB4), 8 days (SB8), 10 days (SB10) and 15 days (SB15) and then taken out. Soybean collected on day 0 (SB0) was used as a control for comparison. All collected samples were lyophilized in a freezer and smashed in an electric grinder. Crushed soybean powders were sieved at 60 mesh and stored at −20 °C for further analysis.

2.4. Analysis of volatile organic compounds (VOCs) by HS-GC-IMS

The VOCs of various soybean samples were analyzed using a GC-IMS apparatus (Flavourspec ®, G.A.S, Dortmund, Germany) equipped with an MXT-5 capillary column measuring 15 m in length, 0.53 mm in diameter, and 1 μm in thickness. In brief, 2.0 g of soybean samples were immediately placed into an empty 20 mL headspace vial, which was then incubated for 15 min at 60 °C with 500 rpm of agitation. After incubation, a heated syringe heated to 65 °C automatically injected 300 μL of headspace into the injector (splitless mode). The following programmed flow was used with high-purity nitrogen (purity 99.99%) as the carrier gas: beginning at 2 mL/min for 2 min, then increasing to 100 mL/min over 18 min. Nitrogen (purity ≥99.99%) flowed through the drift tube at a rate of 150 mL per minute. IMS and the column were maintained at 45 °C and 60 °C, respectively. Three parallel determinations were made for each sample. In order to determine the retention index (RI) of VOCs, N-ketones C4–C9 were employed as an external reference. Then, VOCs were identified and characterized by comparing RI and the drift time of the standard in the GC-IMS library database (G.A.S, Dortmund, Germany). The RI and drift time of the standards in the database (G.A.S, Dortmund, Germany) were used to identify and characterize the VOCs. The relative odor activity value (ROAV) analysis of VOCs was also evaluated according to our previous report of Chen et al. (2024).

2.5. Assay of α-glucosidase inhibitory activity (GIA)

The GIA of soybean samples was evaluated based on our previous study (Xiao et al., 2021a). Soybean sample powders were fully suspended in 80% (v/v) aqueous ethanol with a ratio of 1: 40 (w/v). After 40 min of ultrasonic treatment (40 kHz) at 40 °C, the supernatants were obtained by centrifuging the mixture at 10000×*g* (4 °C) for 15 min. The residues were subsequently extracted twice following the same procedure. All of the supernatants were then mixed and concentrated to dryness at 40 °C in a vacuum, after which it was reconstituted in 25 mL of 80% aqueous ethanol. The obtained extracts were used for GIA investigation and kept in the dark at −20 °C. Before the assay of GIA, the extracts were diluted with four times and performed as follows. Briefly, 1.0 U/mL α-glucosidase enzyme and 5 mM *p*-nitrophenyl glucopyranoside (*p*NPG) solution were firstly prepared in 0.1 M phosphate buffer (pH 6.9), respectively. Then, a 2.0 mL centrifuge tube was filled with 100 μL of extract and 100 μL of α-glucosidase enzyme solution. After pre-incubating for 10 min at 37 °C in a water bath, 200 μL of 5 mM *p*NPG was added. Then, 500 μL of Na_2CO_3 solution (1.0 mol/L) was added to interrupt the reaction after the mixture was incubated at 37 °C for 20 min, and the absorbance in 405 nm was detected. The GIA was calculated and displayed as a percentage of inhibition. GIA (%) = (1- A/A_0) × 100%, where A_0 and A stand for the absorbance of enzyme control (without test sample but with enzyme) and the reaction mixture (both with test sample and enzyme), respectively.

2.6. Correlation analysis and molecular docking

OriginPro 8.1 statistical software was performed to construct the linear correlation relationship between the levels of total phenolic compounds, isoflavone aglycones, and GIA. Then, the molecular docking analysis was performed based on the procedure of Hou et al. (2021) with minor modifications. Autodock 4.2.6 Tools were utilized to simulate the interaction between daidzein, genistein, and GIA based on the correlation analysis. The α-glucosidase simulation model (PDB ID: 3A4A) (https://www.rcsb.org/) was obtained from the Protein Data Bank. The substrate binding pocket for the enzyme active site was encased in an AutoDock grid box with a grid spacing of 1 Å and dimensions of 60 Å × 60 Å × 60 Å. The spatial interactions between the inhibitors and the enzyme were examined using the conformation of daidzein and genistein with the lowest binding energy. The binding energy, as well as the binding affinity between the compounds and enzyme was estimated and calculated via molecular docking. Discovery Studio 4.5 was used to examine the docking results of the binding affinity and interaction between daidzein, genistein, and α-glucosidase.

2.7. Statistical analysis

The results of measurement were performed three times and reported as mean ± standard deviation. The significance of the one-way analysis of variance (ANOVA) was assessed using SPSS 26.0 to compare the means, with a *p*-value of less than 0.05 indicating statistical significance. Gallery Plot analysis, Dynamic Principal Components analysis (PCA), Laboratory Analytical Viewer (LAV), Reporter plug-ins, and GC-IMS Library Search are some of the analytical tools that use GC-IMS to assist with data collection.

3. Results and discussion

3.1. VOCs analysis of soybeans during SSF with E. cristatum

3.1.1. Topography plots of HS-GC-IMS results

GC-IMS for food flavor analysis has developed rapidly in recent years, with major applications in the areas of classification, adulteration studies, food spoilage and off-flavor detection (Wang et al., 2020; Yang et al., 2021). This technique is rapid, convenient and accurate, and shows great potential application in food industry (Wang et al., 2020). Recent studies have applied GC-IMS for flavor detection of fermented products (Chen et al., 2024; Xiao et al., 2022b; Yang et al., 2021). The VOC data of soybeans during SSF obtained by GC-IMS are presented by three-dimensional (3D) topographical visualizations (Fig. 1A). The peak signal intensity of SB15 differs significantly from that of SB0, indicating substantial changes in some VOCs during SSF. To facilitate observation, the overhead view was utilized to compare VOC differences, given that the GC-IMS 3D topographic plots are relatively coarse, and the result is revealed in Fig. 1B. SB0 was taken as a reference, the spectrum of fermented soybean samples was obtained by subtracting the reference spectral background. The blue dot indicates that the substance is less in the sample than in SB0, and the red dot indicates that the substance is higher in the sample than in SB0 (Xuan et al., 2022). It could be seen that the concentration of VOCs in the diverse soybean samples could be clearly noted in the differential contrast model plot. Specifically, the majority of the signals appeared between 1.0 and 1.8 ms of drift time (RIP relative) and 100–400 s of retention time. Additionally, Fig. 1B shows that the VOC concentration in soybeans was dramatically influenced by *E. cristatum* fermentation, especially during the middle (day 2 to day 4) and later (day 10 to day 15) stages. This observation is further supported by PCA and hierarchical cluster analysis (HCA) (Fig. 1C and D), which are based on VOCs signal strength. In Fig. 1C, it has been observed that three test samples of each group are very closely distributed, implying the results have high repeatability and reliability (Yang et al., 2021). It can be observed that the VOCs of soybeans have an obvious alteration as the fermentation time progressed. Among them, SB0 and SB2, SB8 and SB10 are closely distributed, indicating that the VOCs of these pairwise soybeans are similar (Fig. 1C). However, SB2 and SB4, SB10 and SB15 are greatly separated, indicating that the VOCs of soybeans change distinctly during 2–4 days and 10–15 days of fermentation. In addition, although the difference in ion mobility spectra of SB4 and SB8-SB10 is not obvious enough (Fig. 1B), PCA result revealed that the VOCs intensities of SB4 and SB8-SB10 were separated into two sections, implying that the flavor profiles of SB4 and SB8-SB10 are different. The hierarchical cluster analysis (Fig. 1D) and loading plot (Fig. 1E) further supported the above phenomenon. The loading plot could also reveal the contribution of various VOCs to the flavor profile of different samples (Chen et al., 2024). It was noted that soybean samples during fermentation exhibit a close relationship with varying VOCs (Fig. 1E). The above results indicated that the VOCs of soybeans were

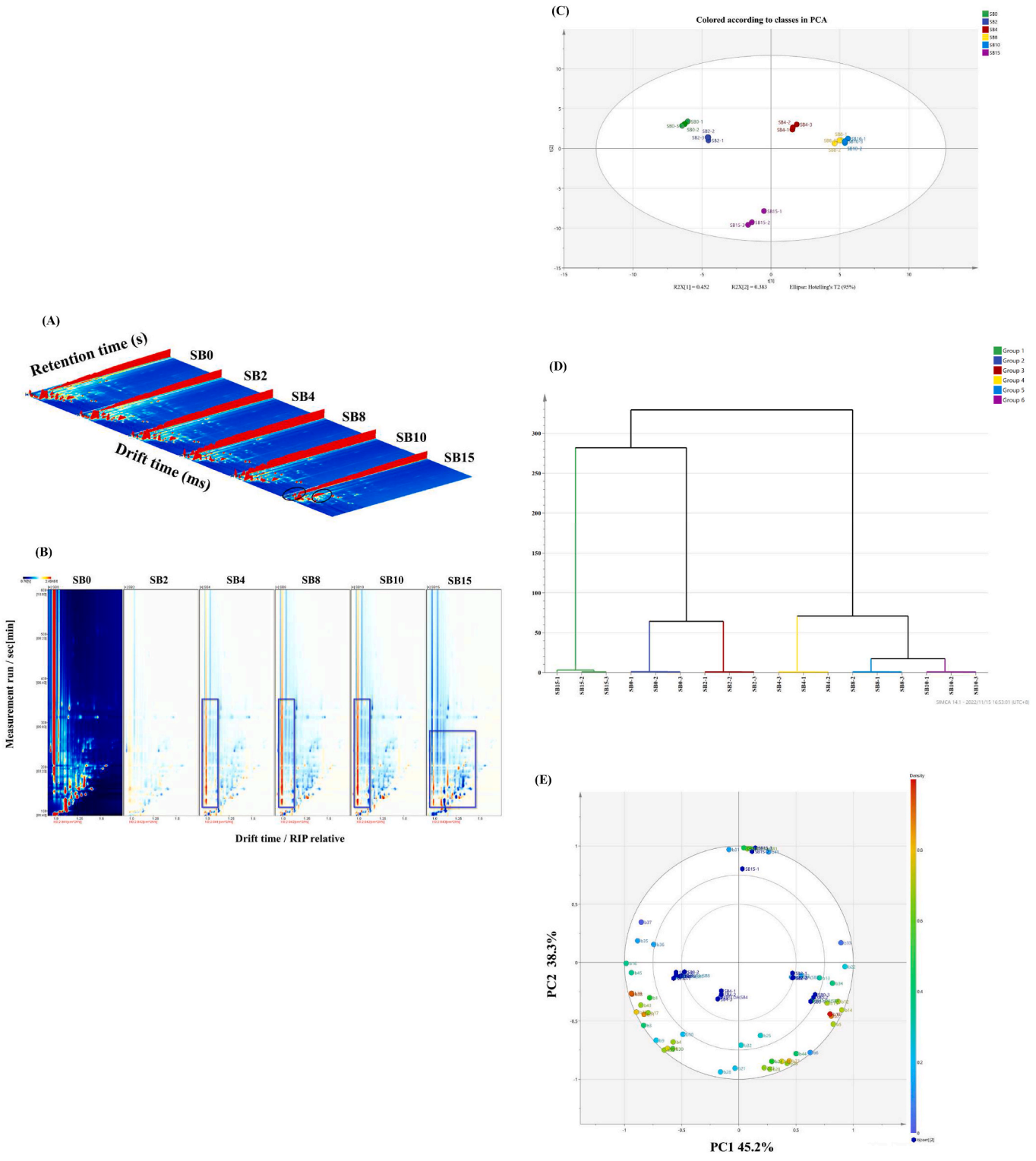


Fig. 1. (A) 3D–topographic and **(B)** 2D–topographic subtraction plots of the VOCs of soybeans during SSF with *E. cristatum*. **(C)** Principal component analysis (PCA), **(D)** hierarchical cluster analysis (HCA), and **(E)** loading plot based on the signal intensity of VOCs of soybeans at different fermentation stages.

drastically altered by *E. cristatum* fermentation.

3.1.2. Qualitative analysis and fingerprint profile comparisons of VOCs in soybeans fermented with E. cristatum

The qualitative analysis of VOCs in soybean samples at various fermentation stages is shown in Fig. S1. GC-IMS detected 64 peaks and successfully identified 46 typical VOCs (Fig. S1, Table 1), nevertheless, the lack of information in the library databases prevented the identification of the remaining 18 peaks. The detected VOCs can be grouped into eight categories, in which aldehydes (17), alcohols (10), and ketones (7) accounted for the three most dominant groups in these soybean samples (Fig. 2A). This result is consistent with the previous research results that aldehydes, alcohols, and ketones are the main volatile groups in soybean products (Yang et al., 2021). The 64 detected peaks

Table 1
GC-IMS integral parameters and peak intensities of volatile components in soybean fermented by *E. cristatum* were analyzed by HS-GC-IMS.

No	Compounds	Odor description[a]	CAS	Formula	MW	RI[b]	Rt[c] [sec]	Dt[d] [RIPrel]	Peak intensity					
									SB0	SB2	SB4	SB8	SB10	SB15
Esters														
b1	Methyl benzoate	Herb, Lettuce, Prune, Violet	93-58-3	C8H8O2	136.1	1096.6	484.635	1.2103	205.45 ± 15.31b	196.48 ± 1.53b	259.69 ± 8.64a	218.29 ± 21.42b	190.7 ± 9.04b	162.88 ± 26.95c
b2	Methyl 3-methylbutanoate	Apple, Fruit, Pineapple	556-24-1	C6H12O2	116.2	774.2	196.158	1.53241	463.3 ± 2.99c	470.33 ± 14.26c	721.51 ± 4.45a	761.89 ± 13.57a	676.98 ± 17.37b	315.56 ± 46.84d
b3	Ethyl Acetate	Aromatic, Brandy, Contact Glue, Grape	141-78-6	C4H8O2	88.1	577.4	138.181	1.34144	890.13 ± 10.51a	413.21 ± 15.14c	372.94 ± 5.32c	412.91 ± 3.1c	474.54 ± 11.23b	225.52 ± 48.91d
b4	Propyl acetate	Celery, Floral, Pear, Red Fruit	109-60-4	C5H10O2	102.1	722	176.11	1.16408	242.22 ± 14.91c	182.32 ± 3.03d	275.06 ± 0.91b	295.75 ± 0.28b	251.13 ± 6.55b	863.95 ± 61.81a
b5	Butyl acetate-M	Apple, Banana	123-86-4	C6H12O2	116.2	805.9	210.878	1.24089	69.39 ± 1.84e	83.31 ± 3.11d	136 ± 11c	155.11 ± 1.67b	183.05 ± 0.57a	65.04 ± 6.01e
b6	Butyl acetate-D	Apple, Banana	123-86-4	C6H12O2	116.2	803.8	209.824	1.62046	47.15 ± 2.93a	34.66 ± 2.55c	52.13 ± 3.99a	45.02 ± 2.24b	51.58 ± 3.81a	13.89 ± 1.39d
Aldehydes														
b7	Benzaldehyde-M	Bitter Almond, Burnt Sugar, Cherry, Malt, Roasted Pepper	100-52-7	C7H6O	106.1	963.3	313.165	1.15519	457.37 ± 25.09a	381.13 ± 7.27b	291.65 ± 22.37c	216.09 ± 3.64d	181.96 ± 8.9e	126.34 ± 3.38f
b8	Benzaldehyde-D	Bitter Almond, Burnt Sugar, Cherry, Malt, Roasted Pepper	100-52-7	C7H6O	106.1	963.3	313.165	1.47669	388.62 ± 12.07a	197.73 ± 5.44b	98.7 ± 2.16c	68.15 ± 0.71d	59.09 ± 3.76d	53.05 ± 12.27d
b9	Heptanal-M	Citrus, Fat, Green, Nut	111-71-7	C7H14O	114.2	902.8	262.92	1.32816	93.83 ± 9.6b	77.39 ± 3.75c	107.47 ± 5.74a	99.76 ± 5.33a	97.64 ± 3.93a	43.54 ± 6.48d
b10	Heptanal-D	Citrus, Fat, Green, Nut	111-71-7	C7H14O	114.2	899.7	260.38	1.69531	63.64 ± 6.39b	41.94 ± 2.88c	74.1 ± 2.15a	43.66 ± 2.59c	61.25±4b	28.97 ± 1.15d
b11	Hexanal-M	Apple, Fat, Fresh, Green, Oil	66-25-1	C6H12O	100.2	791.7	203.778	1.25644	195.21 ± 8.71e	188.44 ± 5.28e	441.38 ± 18.01c	568.72 ± 10.57b	604.15 ± 5.64a	320.01 ± 21.74d
b12	Hexanal-D	Apple, Fat, Fresh, Green, Oil	66-25-1	C6H12O	100.2	791.7	203.778	1.56523	564.85 ± 18.09c	362.96 ± 14.86d	528.88 ± 24.75c	750.68 ± 12.75b	838.41 ± 6.88a	235.35 ± 49.67e
b13	Acetal	Creamy, Fruit, Pleasant, Tropical Fruit	105-57-7	C6H14O2	118.2	745.8	185.273	0.97074	123.13 ± 5.14a	103.75 ± 0.98b	78.91 ± 2.83c	73.76 ± 1.7c	70.32 ± 1.98d	35.22 ± 2.38e
b14	2-Methylbutanal	Almond, Cocoa, Fermented, Hazelnut, Malt	96-17-3	C5H10O	86.1	640.4	152.404	1.40809	500.2 ± 47.88a	270.88 ± 21.51c	361.79 ± 17.96b	216.14 ± 6.86d	216.22 ± 5.07d	44.68 ± 10.98e
b15	3-Methylbutanal	Chocolate, Peach, Fatty	590-86-3	C5H10O	86.1	627.6	149.507	1.4126	255.37±7a	121.41 ± 2.17c	194.15 ± 5.9b	98.34 ± 3.14d	82.73 ± 0.71e	14.48 ± 4.19f
b16	Butanal	Banana, Green, Pungent	123-72-8	C4H8O	72.1	570.4	136.6	1.29399	915.69 ± 5.77a	746.55 ± 10.86b	615.47 ± 7.84c	490.61 ± 11.25d	519.45 ± 5.63d	338.37 ± 45.14e
b17	Furfural-M	Almond, Baked Potatoes, Bread, Burnt, Spice	98-01-1	C5H4O2	96.1	827.9	221.941	1.085	194.58 ± 9.68b	232.16 ± 3.05a	137.2 ± 9.15c	84.75 ± 3.02d	74.73 ± 4.51d	143.25 ± 2.1c
b18	Furfural-D	Almond, Baked Potatoes, Bread, Burnt, Spice	98-01-1	C5H4O2	96.1	828.5	222.204	1.33692	124.6 ± 2.88b	203.19 ± 2.67a	40.68 ± 5.85c	22.81 ± 1.28d	19.76 ± 1.67d	42.56 ± 5.76c
b19	Pentanal-D	Almond, Bitter, Malt, Oil, Pungent	110-62-3	C5H10O	86.1	685.9	162.677	1.42955	24.59 ± 3.12c	18.44 ± 0.67d	33.72 ± 3.21c	86.48 ± 7.05a	81.69 ± 5.89a	52.26 ± 5.9b
b20	Pentanal-M	Almond, Bitter, Malt, Oil, Pungent	110-62-3	C5H10O	86.1	688.2	163.203	1.18215	159.86 ± 1.44b	80.9 ± 5.94d	93.92 ± 13.56c	219.69 ± 3.01a	223.79 ± 1.47a	152.1 ± 2.77b
b21	Methylpropanal	Burnt, Caramel, Cocoa, Green, Malt	78-84-2	C4H8O	72.1	541.3	130.016	1.28608	32.47 ± 2.07d	37.47 ± 2.44d	308.18 ± 9.79c	697.32 ± 10.48b	987.45 ± 9.16a	656.15 ± 128.77b
b22	(E)-2-Hexenal	Green, Banana, Aldehydic, Fatty, Cheesy	6728-26-3	C6H10O	98.1	849	232.476	1.18441	35.89 ± 0.49c	31.1 ± 1.06c	62.42 ± 5.64b	89.26 ± 2.46a	86.16 ± 4.35a	28.86 ± 3.16d
b23	Nonanal	Fat, Floral, Green, Lemon	124-19-6	C9H18O	142.2	1112.3	507.209	1.47236	195.95 ± 19.13a	203.34 ± 4.62a	230.88 ± 11.29a	227.91 ± 22.26a	234.96 ± 30.79a	143.99 ± 19.61b
Alcohols														

(*continued on next page*)

Table 1 (*continued*)

No	Compounds	Odor description[a]	CAS	Formula	MW	RI[b]	Rt[c] [sec]	Dt[d] [RIPrel]	Peak intensity					
									SB0	SB2	SB4	SB8	SB10	SB15
b24	1-Octen-3-ol	Cucumber, Earth, Fat, Floral, Mushroom	3391-86-4	C8H16O	128.2	988.5	334.06	1.16284	978.58 ± 17.92a	906.67 ± 16.24b	558.39 ± 9.15c	184.44 ± 10.58d	144.46 ± 4.11e	154.79 ± 5.17e
b25	3-Methylpentanol	Fruit	589-35-5	C6H14O	102.2	857.6	236.80	1.31479	482.81 ± 80.92a	73.4 ± 27.25b	36.32 ± 3.21b	33.8 ± 3.77b	34.23 ± 2.44b	99.39 ± 3.24b
b26	1-Hexanol-M	Banana, Flower, Grass, Herb	111-27-3	C6H14O	102.2	872	244.05	1.3233	91.24 ± 5.26a	62.6 ± 5.76b	25.96 ± 1.65c	8.53 ± 0.74d	9.68 ± 2.17d	12.92 ± 0.23d
b27	1-Hexanol-D	Banana, Flower, Grass, Herb	111-27-3	C6H14O	102.2	869.1	242.60	1.63818	90.8 ± 3.16a	43.13 ± 2.6b	16.87 ± 2.22c	16.58 ± 2.75c	19.39 ± 7.09c	17.73 ± 1.02c
b28	1-Pentanol	Balsamic, Fruit, Green, Pungent, Yeast	71-41-0	C5H12O	88.1	758.1	189.99	1.51174	55.02 ± 1.29a	28.34 ± 4.01b	14.23 ± 1.12c	9.68 ± 1.81d	10.79 ± 0.3c	9 ± 1.15d
b29	1-Butanol	Fruit	71-36-3	C4H10O	74.1	650.9	154.78	1.17424	357.19 ± 22.37a	229.94 ± 28.84b	41.95 ± 2.85d	49.79 ± 4.89d	43.78 ± 2.16d	124.03 ± 8.92c
b30	Ethanol	Sweet	64-17-5	C2H6O	46.1	400.1	98.15	1.05337	798.42 ± 36.39b	807.34 ± 3.62b	998.34 ± 22.92a	939.94 ± 25.86a	952.84 ± 14.72a	251.06 ± 68.43c
b31	2-Methyl-1-pentanol	Nutty	105-30-6	C6H14O	102.2	846.9	231.459	1.30071	27.91 ± 1.81b	24.67 ± 0.32b	22.88 ± 3.33b	20.66 ± 0.74c	22.5 ± 4.74c	87.41 ± 1.83a
b32	2-Methylbutanol	Wine, Onion	137-32-6	C5H12O	88.1	722.8	176.42	1.47655	141.79 ± 3.56a	146.87 ± 11.37a	119.93 ± 8.99b	72.82 ± 1.35c	52.26 ± 1.26d	30.52 ± 4.05e
b33	2-Propanol	Floral, Woody, Alcoholic	67-63-0	C3H8O	60.1	461	111.89	1.09059	1068.39 ± 23.4b	1141.69 ± 20.89b	1111.38 ± 5.73b	1045.19 ± 16.58b	1033.69 ± 15.06b	4871.24 ± 693.5a
Ketones														
b34	Furaneol	Burnt, Caramel, Cotton Candy, Honey	3658-77-3	C6H8O3	128.1	1075.2	453.91	1.20724	103.74 ± 6.8a	38.41 ± 5.3b	39.99 ± 5.32b	34.2 ± 4.95b	37.05 ± 8.14b	43.05 ± 7.03b
b35	2-Heptanone-M	Blue Cheese, Fruit, Green, Nut, Spice	110-43-0	C7H14O	114.2	896.6	257.84	1.26251	201.18 ± 13.71c	218.7 ± 11.09c	330.24 ± 11.82a	250.13 ± 11.83b	315.61 ± 8.16a	139.76 ± 3.16d
b36	2-Heptanone-D	Blue Cheese, Fruit, Green, Nut, Spice	110-43-0	C7H14O	114.2	894.9	256.39	1.63453	52.7 ± 3.69a	42.65 ± 0.9b	16.45 ± 3.28c	12.22 ± 2.6c	14.81 ± 4.91c	14.95 ± 0.57c
b37	3-Hydroxy-2-butanone	Butter, Creamy, Green Pepper	513-86-0	C4H8O2	88.1	669.5	158.99	1.32788	2871.27 ± 48.3c	3547.62 ± 19.64a	3094.33 ± 68.55b	1930.78 ± 26.41e	2449.87 ± 17.53d	438.57 ± 94.56f
b38	2-Butanone	Fragrant, Fruit, Pleasant	78-93-3	C4H8O	72.1	556.4	133.44	1.25106	3515.23 ± 17.65b	3712.22 ± 22.09a	2851.54 ± 32.39c	1846.15 ± 28.82d	1393.45 ± 14.97e	469.5 ± 39.9f
b39	Acetone	Ethereal, Apple, Pear	67-64-1	C3H6O	58.1	458.4	111.31	1.11776	17403.12 ± 159.53b	17930.66 ± 11.11a	15834.64 ± 16.53c	14483.57 ± 113.93d	12785.24 ± 18.04e	7695.21 ± 515.87f
b40	2-Pentanone	Fruit, Pungent	107-87-9	C5H10O	86.1	687	162.94	1.3629	173.52 ± 3.74c	188.55 ± 4.18b	289.96 ± 6.43a	112.77 ± 2.27d	98.47 ± 2.11e	39.53 ± 6.23f
Phenols														
b41	Phenol	Phenolic, Plastic, Rubber	10-89-52	C6H6O	94.1	997	341.44	1.08017	797.15 ± 20.21d	867 ± 7.97c	1227.55 ± 58.56a	1196.16 ± 16.24a	1095.11 ± 32.23b	459.68 ± 13.54e
Acids														
b42	Acetic acid	Acid, Fruit, Pungent, Sour, Vinegar	64-19-7	C2H4O2	60.1	672.1	159.56	1.15953	824.86 ± 37.99b	605.93 ± 14.88c	367.68 ± 45.51d	283.26 ± 10.9d	299.85 ± 22.48d	2182.32 ± 241.73a
Hydrocarbon														
b43	Styrene	Balsamic, Gasoline	100-42-5	C8H8	104.2	894.6	256.117	1.50898	23.96 ± 4.33b	24.36 ± 2.75b	30.66 ± 2.09a	31.4 ± 1.97a	30.08 ± 2.45a	22.35 ± 1.41b
Heterocyclics														
b44	2-Pentylfuran	Butter, Floral, Fruit, Green Bean	3777-69-3	C9H14O	138.2	999.2	344.51	1.25623	170.53 ± 2.93b	145.74 ± 2.97c	183.34 ± 7.16a	182.73 ± 5.1a	132.6 ± 3.27d	85.21 ± 9.34e
b45	2,5-Dimethylpyrazine	Cocoa, Roast Beef, Roasted Nut	123-32-0	C6H8N2	108.1	919.4	276.71	1.11298	113.87 ± 5.15a	122.45 ± 1.59a	100.87 ± 9.52b	61.29 ± 2.96c	63.39 ± 3.74c	32.39 ± 2.68d
b46	2-Ethylfuran	Butter, Caramel	3208-16-0	C6H8O	96.1	691.8	164.52	1.04772	271.95 ± 7.06c	250.11 ± 7.14d	325.9 ± 10.56b	361.94 ± 7.98a	254.81 ± 4.85c	68.94 ± 15.75e

[a] Odor descriptions were from the FEMA database. Different small letters in the same row indicated significant differences ($p < 0.05$). D: Dimer, M: Monomer.
[b] Represents the retention index in the capillary GC column.
[c] Represents the retention time in the capillary GC column.
[d] Represents the drift time in the drift tube.

were constructed in the form of fingerprints to further assess the variations of VOCs in soybean samples during fermentation (Fig. 2B and C). The results of fingerprint analysis showed that *E. cristastum* fermentation significantly changed the VOCs of soybeans, and the levels of off-flavors such as 2-pentylfuran, 1-octen-3-ol, 1-hexanol-M, and 1-hexanol-D were highest in the unfermented soybeans (SB0), and their concentration gradually decreased after SSF with *E. cristatum* (red box a in Fig. 2B). Additionally, it was found that a large part of VOCs greatly increased after fermentation, and varied VOCs displayed diverse alteration trends (Fig. 2B).

It can be seen from Fig. 2A that aldehyde is the largest category of VOCs in soybean. After SSF by *E. cristatum*, the concentration of aldehydes, such as pentanal, methylpropanal, and (E)-2-hexenal with bitter almond, caramel, and fat aromas increased significantly. It was found that pentanal-D and methylpropanal of soybean increased by 1.1- and 19.2-fold, respectively, after 15 days of SSF. Previous studies have showed that these aldehydes contribute to flavors that overlap with various other compounds, imparting soy sauce, nut, and milk-like flavors, thus enhancing the overall flavor complexity (Ling et al., 2022). For instance, the increased level of methylpropanal during SSF could contribute to enhancing the caramel aroma of soybeans. Additionally, raw soybeans are found to be rich in hexanal, which is the main volatile substance in raw soybeans (Table 1). Hexanal is one of the main off-flavors in soybean, imparting green and grassy odors (Wang et al., 2022). It is noted that the level of hexanal-D greatly decreased by 58.3% after SSF with *E. cristatum* for 15 days. A substantial reduction in the level of hexanal concentration is desirable for the production of soybean food. The reduction in hexanal content after fermentation is also consistent with previous studies reported for the fermentation of legume products with other strains, such as soybean meal and okara fermented with yeast, and soybean milk fermented with lactic acid bacteria (Blagden and Gilliland, 2005; Shi et al., 2020). Furthermore, we have found that another typical off-flavor aldehyde compounds heptanal-M and heptanal-D drastically decreased by 53.6% and 54.5%, respectively, after 15 days of fermentation. During fermentation, microbial extracellular enzymes catalyze a series of biological transformations of soybean, facilitating the conversion of numerous metabolites and influencing the flavor characteristics of soybeans (Du et al., 2022; Xiang et al., 2023). In our previous study, *E. cristatum* produced abundant hydrolytic enzymes like protease, cellulase (CMCase), β-glucosidase, and α-amylase during SSF of soybeans (Chen et al., 2020b). Correlation analysis was conducted to precisely reveal the connection between the change in VOCs and hydrolytic enzymes (Fig. 3A). The finding showed that the increased desirable aromas pentanal-D and methylpropanal are highly positively correlated ($p < 0.05$) with protease ($r = 0.841$ and 0.949, respectively) and β-glucosidase ($r = 0.830$ and 0.915, respectively) (Fig. 3A). Protease hydrolyzes soybean protein into amino acids during fermentation, which then undergo a series of biochemical reactions similar to Strecker degradation, leading to the formation of VOCs (An et al., 2023). In addition, it is also reported in earlier studies that β-glucosidase is critical for the formation of aldehydes, which can be formed by the degradation of lipids and hydrolysis of glycoside through the action of β-glucosidase (Supriyadi et al., 2021). Besides, we have also noted that the hydrolytic enzymes were negatively correlated with heptanal and hexanal, which implies that these enzymes played an important on the transformation of soybean off-flavors. In addition, it has been demonstrated that the phenolics and flavonoids are the precursors of the VOCs (Xiao et al., 2022b). In our previous study, total phenolics and aglycone isoflavone significantly increased during the SSF of soybean by *E. cristatum*, while glycoside isoflavones drastically decreased (Chen et al., 2020b), thus, further network correlation relationship between the change of main non-volatile metabolites and VOCs

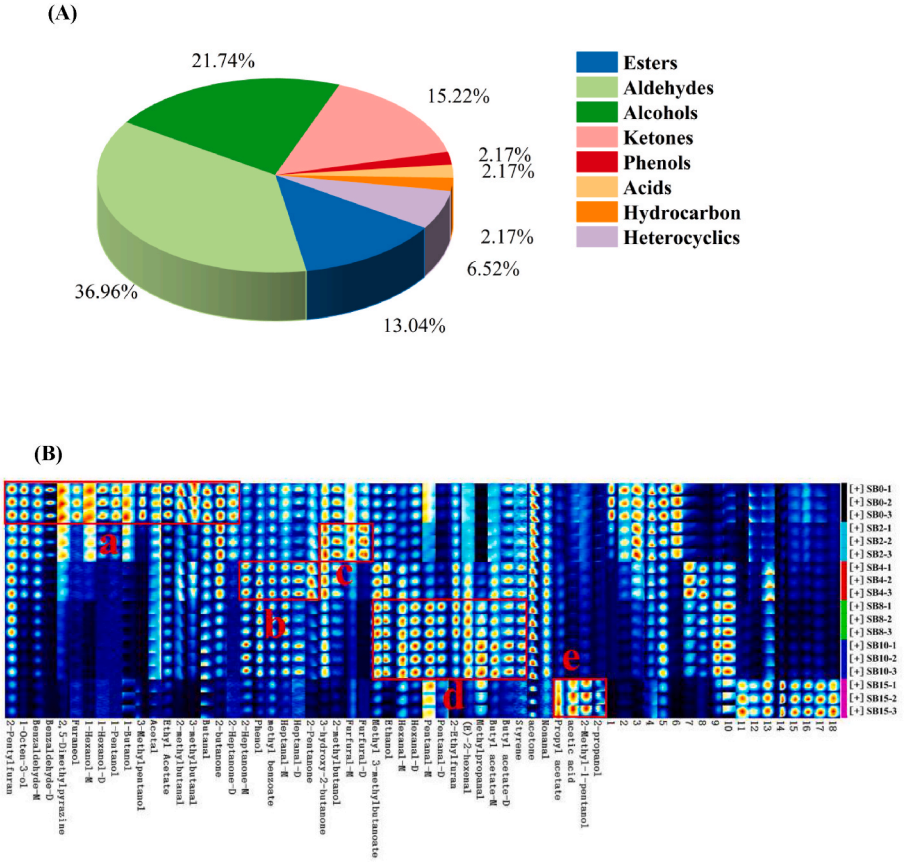


Fig. 2. (A) Classification of all VOCs and **(B)** VOCs fingerprint comparisons of soybeans during SSF with *E. cristatum*. **(C)** Heatmap analysis of all VOCs during the fermentation of soybeans with *E. cristatum*.

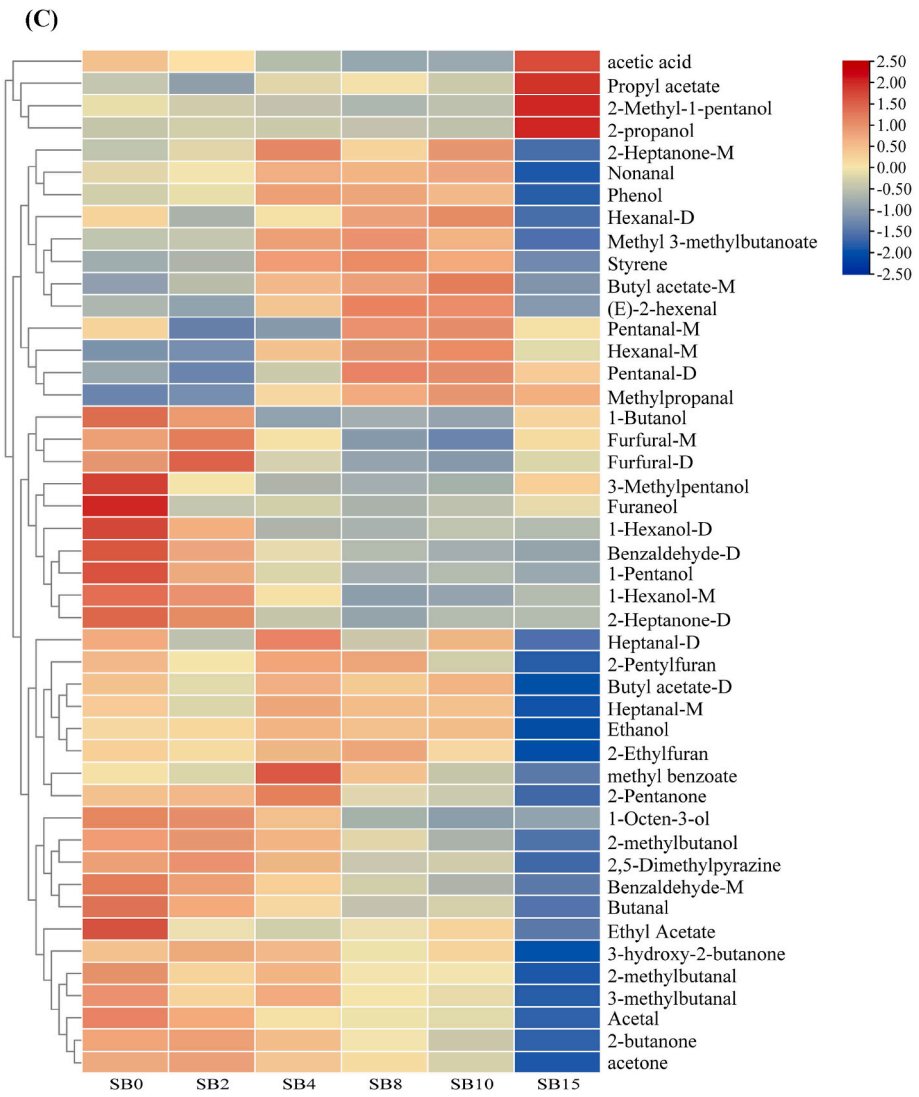


Fig. 2. (*continued*).

has been analyzed (Fig. 3B). It was observed that glycoside isoflavones are highly correlated with certain aldehydes. This correlation may be attributed to the hydrolysis of soybean isoflavone glycosides into acetylglucosides by β-glucosidase, which may facilitate aldehydes formation.

Alcohol compounds are the second largest group of VOCs in soybeans. GC-IMS qualified 10 alcohols and almost all of these alcohols decreased (except for 2-propanol and 2-methyl-1-pentanol) during SSF. Among them, 1-octen-3-ol and 1-hexanol, are the typical off-flavor compounds with beany flavor in soybean products (Cai et al., 2021; Chen et al., 2022b; Wang et al., 2022). The result revealed that 1-octen-3-ol and 1-hexanol were significantly decreased as the fermentation progressed. We found that the content of 1-octen-3-ol, 1-hexanol-D and 1-hexanol-M was reduced by 84.2%, 80.5% and 85.8%, respectively, compared with the non-fermented soybeans after 15 days of fermentation. Thus, the undesirable flavor of soybeans caused by 1-hexanol and 1-octen-3-ol was remarkably weakened by SSF with *E. cristatum*. Earlier studies also found that the unpleasant beany flavor compounds could be reduced by fermentation. For example, Chen et al. (2022b) verified that SSF with *Bacillus subtilis* natto significantly declined 1-hexanol and 1-octen-3-ol of soybeans. *E. cristatum* produces highly active hydrolytic enzymes, which may play a critical role in the conversion of volatiles. The correlation analysis revealed that the reduction of 1-octen-3-ol was strongly correlated ($p < 0.05$) with cellulase ($r = 0.822$), protease ($r = 0.904$) and β-glucosidase ($r = 0.947$), respectively (Fig. 3A). The decrease of 1-hexanol-M was closely correlated ($p < 0.05$) with β-glucosidase ($r = 0.820$) and protease ($r = 0.907$), respectively. 2-Propanol is well known for its fruit, woody and alcoholic aroma, which imparts soybean products a desirable odor (Hwang and Kim, 2023). Its level was greatly increased by 3.6-fold after 15 days of SSF, which was greatly correlated with α-amylase ($r = 0.914$) and protease ($r = 0.822$), respectively. In addition, we also found that the some of alcohol volatiles were closely related to the change of non-volatiles (i.e., phenolics and flavonoids) by the network correlation analysis (Fig. 3B). The generation of flavor compounds was involved in direct and indirect processes, i.e., the flavors were directly formed by catalysis of their flavor precursors by enzymes, or oxidase catalyzes the production of oxidation products, which in turn catalyze the oxidation of flavor precursor components to generate flavor metabolites (Xiao et al., 2022b).

A total of 7 ketone VOCs were identified during SSF of soybeans, which are mainly composed of 2-butanone, 3-hydroxy-2-butanone, and acetone. The content of these three compounds showed decreased trends during fermentation, which reduced by 86.6%, 84.7% and 55.8% after 15 days of fermentation, respectively. It was revealed in Fig. 3A that the alteration of these three volatiles negative closely correlated with α-amylase, β-glucosidase and cellulase with r ranging from 0.846 to 0.980. Among them, although the concentration of 2-butanone is high, it has no contribution to the flavor of soybean due to the high aroma

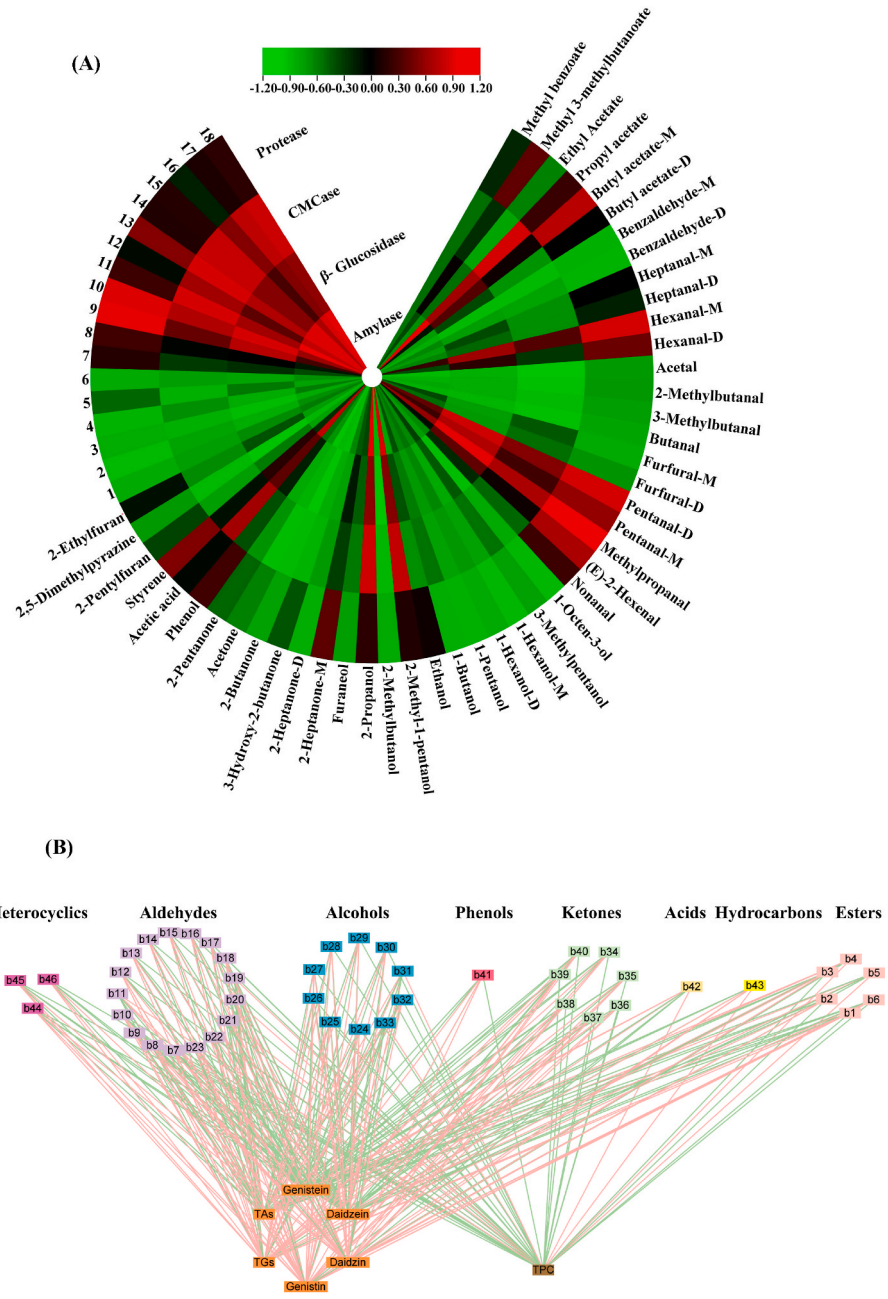


Fig. 3. (A) Correlation analysis between the changes of VOCs and hydrolytic enzymes during SSF of soybeans by *E. cristatum*. **(B)** Network correlation analysis of the VOCs related to the main non-volatile metabolites during SSF with *E. cristatum*. The red and green lines represent positive and negative correlations, respectively. Note: the detail names of the VOCs are shown in Table 1. TPC, total phenolics; TAs, total aglycones isoflavones; TGs, total glucosides isoflavones. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

threshold. However, the aroma threshold of 3-hydroxy-2-butanone is low, and it has a great contribution to the flavor of soybean. It has been reported that 3-hydroxy-2-butanone is a common flavor substance in fermented products such as soybean paste, soy sauce, and Japanese natto (Liu et al., 2018). The content of 3-hydroxy-2-butanone revealed a fluctuation change trend, which increased in the early fermentation and then drastically decreased in the final stage. Similarly, we have found that the content of 2-heptanone-M also showed a fluctuation change trend during SSF, which increased to the maximum after 4 days of fermentation. 2-Heptanone elicits an appealing, creamy scent and soft fragrant flavor that can cover up unpleasant flavors, and is presented in many fermented soybean products (Yang et al., 2021; Zong et al., 2022).

Esters are typically regarded as one of the most critical volatiles that affect the aroma of foods (Niu et al., 2018). Esterification of organic acids and alcohols is the main process used to form esters. The generation of alcohols might spontaneously interact with acetyl coenzyme (Mahapatra et al., 2009), leading to ester production and conferring a pleasant fruity aroma (Liu et al., 2013). It was found that most of the esters showed a fluctuation alteration, which drastically elevated in the early stage of fermentation and then reduced in the final stage. Notably, propyl acetate imparts floral, pear, celery, and red fruit aromas remarkably increased by 256.7% after 15 days of fermentation. Hence, *E. cristatum* fermented soybean might have more fruity and floral aroma characteristics. We have found that the amylase and cellulase played a critical role in the accumulation of propyl acetate (Fig. 3A). The esters were probably produced by their respective alcohols by the action of acyltransferase and/or alcohol acetyltransferase (Keong et al., 2023). In this study, we found that most of alcohol compounds declined during

fermentation (Table 1), which further supported the above statement. In addition, fungal lipases catalyzed fatty acids could also lead to esters production. Previous studies revealed that microbial enzymes can catalyze the generation of flavor-active ester volatiles by utilizing their amino acids as substrates/precursors (An et al., 2023; Zhang et al., 2023). *E. cristatum* has been verified to show a strong ability to metabolism of amino acids (Xiao et al., 2022a). Soybean is rich in protein and amino acids, thus, it is reasonable to speculate that the amino acid metabolism by *E. cristatum* played a crucial role in the alteration of esters.

Except for the above-mentioned VOCs, it should be pointed out that the change of other VOCs (e.g., 2-pentylfuran, 2-ethylfuran, phenol, acetic acid, and styrene) during SSF also helps to improve the odor characteristic of soybeans. Notably, 2-pentylfuran and 2-ethylfuran impart the typically undesirable green beany flavor of soybean (Du et al., 2022), which drastically decreased by 50.0% and 74.6%, respectively, after 15 days of fermentation. Earlier studies have also shown that microorganism fermentation can remove the beany odor induced by 2-pentylfuran (Du et al., 2022). For instance, Keong et al. (2023) reported that SSF of soybean byproduct (okara) with *Bacillus coagulans* dramatically mitigated the level of 2-pentylfuran, which improves the aroma characteristic of okara. Besides, the acetic acid level of soybean increased by 1.6-fold compared to the unfermented sample after 15 days of SSF with *E. cristatum*. Acetic acid contributes to distinctive sour, sweet, and cheese-like odors and plays a significant role in flavor development in soybean paste (Zhang et al., 2022).

From the above result, the off-flavors of soybeans was greatly reduced by SSF with *E. cristatum*, while VOCs with floral, caramel, and

Table 2
The marker VOCs of soybean fermented by *E. cristatum* and their potential contribution to soybean's aroma were determined by HS-GC-IMS. The threshold (mg/kg) was obtained from the literatures.

No.	Compounds	Threshold (mg/kg)	ROAV values					
			SB0	SB2	SB4	SB8	SB10	SB15
Esters								
b1	Methyl benzoate	0.073	1.18 ± 0.06	1.1 ± 0.01	1.64 ± 0.04	1.51 ± 0.13	1.49 ± 0.06	2.11 ± 0.17
b2	Methyl 3-methylbutanoate	0.0044	2.66 ± 0.01	2.62 ± 0.07	4.56 ± 0.02	5.26 ± 0.04	5.3 ± 0.12	4.09 ± 0.27
b3	Ethyl Acetate	0.005	5.12 ± 0.08	2.3 ± 0.07	2.36 ± 0.03	2.85 ± 0.01	3.71 ± 0.08	2.91 ± 0.35
b4	Propyl acetate	2	1.39 ± 0.08	1.02 ± 0.01	1.74 ± 0.01	2.04 ± 0.01	1.96 ± 0.04	11.29 ± 1.22
b5	Butyl acetate-M	0.058	0.4 ± 0.01	0.46 ± 0.01	0.86 ± 0.06	1.07 ± 0.02	1.43 ± 0	0.84 ± 0.04
b6	Butyl acetate-D	0.058	0.27 ± 0.01	0.19 ± 0.01	0.33 ± 0.02	0.31 ± 0.01	0.4 ± 0.02	0.18 ± 0.01
Aldehydes								
b7	Benzaldehyde-M	0.3	2.63 ± 0.1	2.13 ± 0.03	1.84 ± 0.12	1.49 ± 0.01	1.42 ± 0.06	1.64 ± 0.05
b8	Benzaldehyde-D	0.3	2.23 ± 0.04	1.1 ± 0.03	0.62 ± 0.01	0.47 ± 0	0.46 ± 0.02	0.68 ± 0.09
b9	Heptanal-M	0.01	0.54 ± 0.04	0.43 ± 0.02	0.68 ± 0.03	0.69 ± 0.03	0.76 ± 0.03	0.56 ± 0.04
b10	Heptanal-D	0.01	0.37 ± 0.03	0.23 ± 0.01	0.47 ± 0.01	0.3 ± 0.02	0.48 ± 0.03	0.38 ± 0.01
b11	Hexanal-M	0.005	1.12 ± 0.04	1.05 ± 0.02	2.79 ± 0.09	3.93 ± 0.03	4.73 ± 0.03	4.16 ± 0.09
b12	Hexanal-D	0.005	3.25 ± 0.06	2.02 ± 0.07	3.34 ± 0.13	5.18 ± 0.04	6.56 ± 0.04	3.04 ± 0.36
b13	Acetal	0.0049	0.71 ± 0.02	0.58 ± 0	0.5 ± 0.01	0.51 ± 0.01	0.55 ± 0.01	0.46 ± 0
b14	2-Methylbutanal	0.001	2.87 ± 0.2	1.51 ± 0.1	2.28 ± 0.09	1.49 ± 0.03	1.69 ± 0.03	0.58 ± 0.08
b15	3-Methylbutanal	0.0002	1.47 ± 0.03	0.68 ± 0.01	1.23 ± 0.03	0.68 ± 0.01	0.65 ± 0.01	0.19 ± 0.03
b16	Butanal	0.0082	5.26 ± 0.03	4.16 ± 0.05	3.89 ± 0.04	3.39 ± 0.05	4.06 ± 0.03	4.38 ± 0.23
b17	Furfural-M	0.3	1.12 ± 0.04	1.29 ± 0.01	0.87 ± 0.05	0.59 ± 0.02	0.58 ± 0.03	1.87 ± 0.11
b18	Furfural-D	0.3	0.72 ± 0.01	1.13 ± 0.01	0.26 ± 0.03	0.16 ± 0.01	0.15 ± 0.01	0.56 ± 0.08
b19	Pentanal-D	0.2	0.14 ± 0.01	0.1 ± 0	0.21 ± 0.02	0.6 ± 0.04	0.64 ± 0.04	0.68 ± 0.04
b20	Pentanal-M	0.2	0.92 ± 0.01	0.45 ± 0.03	0.59 ± 0.07	1.52 ± 0.02	1.75 ± 0.01	1.98 ± 0.08
b21	Methylpropanal	0.0015	0.19 ± 0.01	0.21 ± 0.01	1.95 ± 0.05	4.81 ± 0.03	7.72 ± 0.05	8.48 ± 0.88
b22	(E)-2-Hexenal	0.11	0.21 ± 0	0.17 ± 0	0.39 ± 0.03	0.62 ± 0.02	0.67 ± 0.03	0.37 ± 0.01
b23	Nonanal	0.001	1.13 ± 0.08	1.13 ± 0.02	1.46 ± 0.06	1.57 ± 0.13	1.84 ± 0.2	1.87 ± 0.15
Alcohols								
b24	1-Octen-3-ol	0.001	5.62 ± 0.04	5.06 ± 0.08	3.53 ± 0.05	1.27 ± 0.07	1.13 ± 0.03	2.02 ± 0.13
b25	3-Methylpentanol	0.0075	2.78 ± 0.4	0.41 ± 0.12	0.23 ± 0.02	0.23 ± 0.02	0.27 ± 0.02	1.3 ± 0.08
b26	1-Hexanol-M	0.5	0.52 ± 0.03	0.35 ± 0.03	0.16 ± 0.01	0.06 ± 0	0.08 ± 0.01	0.17 ± 0.01
b27	1-Hexanol-D	0.5	0.52 ± 0.01	0.24 ± 0.01	0.11 ± 0.01	0.11 ± 0.02	0.15 ± 0.05	0.23 ± 0.02
b28	1-Pentanol	4	0.32 ± 0	0.16 ± 0.02	0.09 ± 0.01	0.07 ± 0.01	0.08 ± 0	0.12 ± 0.01
b29	1-Butanol	0.4592	2.05 ± 0.12	1.28 ± 0.13	0.26 ± 0.01	0.34 ± 0.03	0.34 ± 0.01	1.62 ± 0.17
b30	Ethanol	0.83	4.59 ± 0.14	4.5 ± 0.02	6.3 ± 0.12	6.49 ± 0.11	7.45 ± 0.1	3.23 ± 0.53
b31	2-Methyl-1-pentanol	950	0.16 ± 0.01	0.14 ± 0	0.14 ± 0.02	0.14 ± 0.01	0.18 ± 0.03	1.14 ± 0.08
b32	2-Methylbutanol	0.0159	0.81 ± 0.01	0.82 ± 0.05	0.76 ± 0.05	0.5 ± 0.01	0.41 ± 0.01	0.4 ± 0.02
b33	2-propanol	9.787	6.14 ± 0.14	6.37 ± 0.1	7.02 ± 0.03	7.22 ± 0.12	8.09 ± 0.11	63.88 ± 10.52
Ketones								
b34	Furaneol	0.0223	0.6 ± 0.04	0.21 ± 0.02	0.25 ± 0.03	0.24 ± 0.03	0.29 ± 0.05	0.56 ± 0.08
b35	2-Heptanone-M	0.14	1.16 ± 0.06	1.22 ± 0.05	2.09 ± 0.06	1.73 ± 0.07	2.47 ± 0.05	1.82 ± 0.07
b36	2-Heptanone-D	0.14	0.3 ± 0.02	0.24 ± 0	0.1 ± 0.02	0.08 ± 0.01	0.12 ± 0.03	0.2 ± 0.02
b37	3-Hydroxy-2-butanone	0.055	16.5 ± 0.11	19.79 ± 0.09	19.54 ± 0.35	13.33 ± 0.14	19.16 ± 0.12	5.66 ± 0.68
b38	2-Butanone	35.4	20.2 ± 0.23	20.7 ± 0.11	18.01 ± 0.16	12.75 ± 0.09	10.9 ± 0.09	6.1 ± 0.1
b39	Acetone	0.832	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
b40	2-Pentanone	1.38	1 ± 0.02	1.05 ± 0.02	1.83 ± 0.03	0.78 ± 0.02	0.77 ± 0.01	0.51 ± 0.04
Phenols								
b41	Phenol	5.9	4.58 ± 0.13	4.84 ± 0.03	7.75 ± 0.3	8.26 ± 0.13	8.57 ± 0.2	5.98 ± 0.2
Acids								
b42	Acetic acid	99	4.74 ± 0.21	3.38 ± 0.07	2.32 ± 0.23	1.96 ± 0.07	2.35 ± 0.15	28.58 ± 4.01
Hydrocarbon								
b43	Styrene	0.73	0.14 ± 0.02	0.14 ± 0.01	0.19 ± 0.01	0.22 ± 0.01	0.24 ± 0.02	0.29 ± 0.02
Heterocyclics								
b44	2-Pentylfuran	0.0048	0.98 ± 0.01	0.81 ± 0.01	1.16 ± 0.04	1.26 ± 0.02	1.04 ± 0.02	1.11 ± 0.06
b45	2,5-Dimethylpyrazine	1.75	0.65 ± 0.02	0.68 ± 0.01	0.64 ± 0.05	0.42 ± 0.02	0.5 ± 0.02	0.42 ± 0.01
b46	2-Ethylfuran	0.00129	1.56 ± 0.03	1.39 ± 0.03	2.06 ± 0.05	2.5 ± 0.03	1.99 ± 0.03	0.89 ± 0.12

pleasant flavors such as pentanal-D, methylpropanal, 2-propanol, and propyl acetate drastically enhanced. Thus, it can be reasonably verified that *E. cristatum* metabolism helped to improve the soybean flavor characteristics during SSF.

3.2. Analysis of key aroma-active compounds in soybeans during SSF

It is important to note that not all VOCs found in soybean samples had a significant effect on the flavor characteristic. The contribution of various volatiles to the aroma of fermented soybeans varied based on their concentrations and odor threshold values. Relative odor activity value (ROAV) analysis was generally used to reflect the importance of a VOC, and a higher ROAV value of a VOC implies a greater contribution to the fragrance attribute of a product (Chen et al., 2024; Huang et al., 2023b). The principle of ROAV suggests that volatiles with ROAV $\geq$1 are regarded as key flavor compounds and greatly contribute to the overall flavor of the sample. It can be seen from Table 2 that a total of 32 VOCs with ROAV $\geq$1 were found during soybean fermentation. Additionally, it was found that there are 18 VOCs with ROAV $\geq$1 across all soybeans with different fermentation times, which play a significant role in shaping the flavor characteristics of the soybean samples (Table 2). Specially, 2-propanol, 3-hydroxy-2-butanone, 2-butanone, and acetone have high ROAV, which greatly contributed to the main aroma of the soybean samples. The ROAV of desirable flavor compound 2-propanol (imparts floral, alcoholic and woody aromas) gradually raised as the fermentation time extended, suggesting that *E. cristatum* fermentation is beneficial to the development of floral odor characteristic of soybean. After 15 days of SSF, 2-propanol (ROAV = 63.88) emerged as the second most aroma-active substance in soybean samples, which played a crucial role in the overall aroma of fermented soybean. 2-Propanol is also a key flavoring compound in fermented soybean koji (Hwang and Kim, 2023), which could be generated via the acetone metabolic pathway. It was reported that 2-propanol was produced by the reduction of ketones (such as acetone) catalyzed by the action of secondary alcohol dehydrogenase (Sutak et al., 2012). 3-Hydroxy-2-butanone had buttery and creamy odors, is a characteristic flavor in Korean traditional fermented soybean pastes (Doenjang) (Dajanta et al., 2011; Yi and Hong, 2021) and could be formed through the oxidation of fatty acids (Huang et al., 2022). 2-Butanone gave an aromatic and pleasant fruity flavors, is also a characteristic flavor components of fermented milk (Wang et al., 2023). Acetone gave the fermented soybeans a pleasant fresh aroma with sweet and fruity notes (Cheng, 2010). Notably, the ROAV of 1-octen-3-ol is 5.62, a key aroma-active compound in non-fermented soybean, which is drastically decreased by SSF with *E. cristatum*. 1-Octen-3-ol content in soybean seed could produce sensory off-flavors (has mushrooms, lavender, rose and hay flavors) during the processing of soymilk (Xia et al., 2019). They reported that the most effective way to reduce off-flavors of soymilk is the screening and utilization of soybean cultivars with reduced 1-octen-3-ol content (Xia et al., 2019). Thus, the overall effect of 1-octen-3-ol on the flavor profile of soybeans remarkably decreased by fermented with *E. cristatum*, which was effective in alleviating the beany flavor. Consistent with our study, Du et al. (2022) also noted that the main bean flavor compound 1-octen-3-ol was dramatically degraded by fermentation with *Lactiplantibacillus plantarum* X7021. Acetic acid gives fermented soybeans an acidic flavor and is the third largest ROAV in SB15, which was reported to give fermented soybean products a sweet and refreshing taste (Serra et al., 2005). The production of acetic acid is associated with lipid degradation or carbohydrate metabolism (Li et al., 2018). Therefore, its release is attributed to the metabolism of soybean lipids and carbohydrates by *E. cristatum*. Furthermore, we have also noted that the ROAVs of propyl acetate, methylpropanal and pentanal-D impart floral, fruit and pleasant aromas increased during the SSF process. Propyl acetate and methylpropanal have high ROAV with 11.29 and 8.48, respectively, in 15 days fermented soybeans, which values are only 1.39 and 0.19, respectively, in non-fermented soybeans. Of these compounds, methylpropanal contribute to the nutty flavor of cheese (Chen et al., 2020a), while propyl acetate has fruity aroma of pear, a key flavor substance in baijiu, was considered as important regional markers to effectively identify the production region of sauce-aroma style baijiu (Huang et al., 2023a). A drastic increase in their values is undoubtedly favorable to the flavor of fermented soybeans, which further validated that *E. cristatum* fermentation improved the flavor characteristics of soybeans. Thus, the effects of *E. cristatum* on various volatiles led the overall flavor of the soybean to change from a strong beany flavor to a distinctive scent rich in fruity and floral aromas.

3.3. Effect of SSF with E. cristatum on the α-glucosidase inhibitory activity (GIA) of soybeans

Up to now, acarbose and other synthetic α-glucosidase inhibitors have been employed to retard glucose absorption. Nevertheless, in order to prevent the undesirable gastrointestinal side effects of synthetic inhibitors, researchers have been exploring natural α-glucosidase inhibitors that offer enhanced efficacy and reduced adverse effects (Choi et al., 2010; Dirir et al., 2022; Starzec et al., 2023; Xiao et al., 2021b). Legume plants, in particular, are recognized for containing inhibitors of α-glucosidase due to their abundance of phenolic and flavonoid compounds (Lee et al., 2019; Zheng et al., 2023). Nonetheless, there is limited information regarding the GIA of soybean that have been fermented with *E. cristatum*.

In this work, the GIA of soybean influenced by *E. cristatum* during SSF was displayed in Fig. 4A. According to the findings, the GIA of soybean was observed to be significantly ($p < 0.01$) influenced by the duration of fermentation, showing a gradual increase as the fermentation process advanced. The GIA of soybeans increased by 22.4% after 15 days of fermentation compared with non-fermented soybeans. Previous literatures have also elucidated the GIA of samples drastically enhanced during SSF, which is primarily linked to the accumulation of phenolic compounds and flavonoid aglycones (Lee et al., 2019; Xiao et al., 2021a; Yang et al., 2022). Guo et al. (2020) reported that mulberry leaves processed by *Monascus anka* greatly increased the GIA due to the improvement of flavonoid aglycones during fermentation. Our earlier study reported that total phenolic contents (TPC) and aglycone isoflavones of soybeans were greatly enhanced during SSF with *E. cristatum* (Chen et al., 2020b). Thus, the relationship between TPC, aglycone isoflavones, and GIA was accurately clarified using linear-correlation analysis (Fig. 4B–E). The results indicated a greatly positive relationship ($p < 0.01$) between TPC, genistein, daidzein, total isoflavone aglycones, and GIA, with correlation values R^2 ranging from 0.8935 to 0.9662. Previous studies have also shown that phenolic compounds and flavonoid aglycones, particularly those with multiple hydroxyl groups, strongly inhibit α-glucosidase activity (Choi et al., 2010; Lee and Lee, 2001; Wu et al., 2016). This inhibitory effect is likely due to hydrogen bonds formation facilitated by the hydroxyl groups (Liu et al., 2023; Swargiary et al., 2023). Hydrogen bonds were reported as the main driving force behind the interactions between phenolic compounds and α-glucosidase. The affinity of the compounds for α-glucosidase increases with the number of hydrogen bonds formed with its amino acid residues (Chen et al., 2022a). Apart from hydrogen bonds, hydrophobic interactions were reported to be another driving force affects the binding affinity between phenolic compounds and α-glucosidase (Xiao et al., 2009; Zheng et al., 2023). Earlier studies have noted that glycosylation of flavonoids have a negative impact on α-glucosidase inhibitory activity (Wang et al., 2017; Wu et al., 2016; Xiao et al., 2009). For instance, Zheng et al. (2023) reported that isoflavone aglycones, in the absence of glycosylation, demonstrated strong α-glucosidase inhibitory activity, whereas isoflavone glucosides showed much lower effect. This is primarily due to glycosylation of flavonoids increased spatial steric hindrance, thereby weakening the hydrophobic interactions between the flavonoids and the protein, and subsequently reducing the affinity of flavonoids for the protein (Hua et al., 2018; Wang et al., 2017). Herein, the interaction between phenolic compounds, flavonoid aglycones, and

α-glucosidase involves the spontaneous formation of a complex, primarily driven by hydrogen bonds and hydrophobic interactions (Wang et al., 2017; Wu et al., 2016; Zheng et al., 2023). Therefore, the greater level of phenolic compounds and isoflavone aglycones accumulated during SSF was the primary factor contributing to the GIA improvement in fermented soybeans.

To further unveil the correlation and potential mechanism between genistein, daidzein and GIA, molecular docking was conducted to investigate the interaction between the flavonoid aglycones and α-glucosidase, as illustrated in Fig. 4F–I. Molecular docking result revealed that both compounds entered the active site of α-glucosidase, interacting with specific amino acid residues to inhibit the enzyme's activity. As shown in Fig. 4F–I, daidzein formed two hydrogen bonds with Glu 422 and Gly 161 residues within the active pocket of α-glucosidase, while genistein established five hydrogen bonds with Lys 156, Ser 241, Pro 312, Thr 310, and Asp 307 residues. Previous studies have also reported that the formation of hydrogen bonds is one of the main binding forces between inhibitor-enzyme complexes. In addition, Chen et al. (2022a) also stated that Lys156 and Asp307 residues are the crucial amino acid catalytic active sites of the α-glucosidase (PDB: 3A4A) that formed hydrogen bonds with flavonoid compounds.

Furthermore, various hydrophobic interactions were observed,

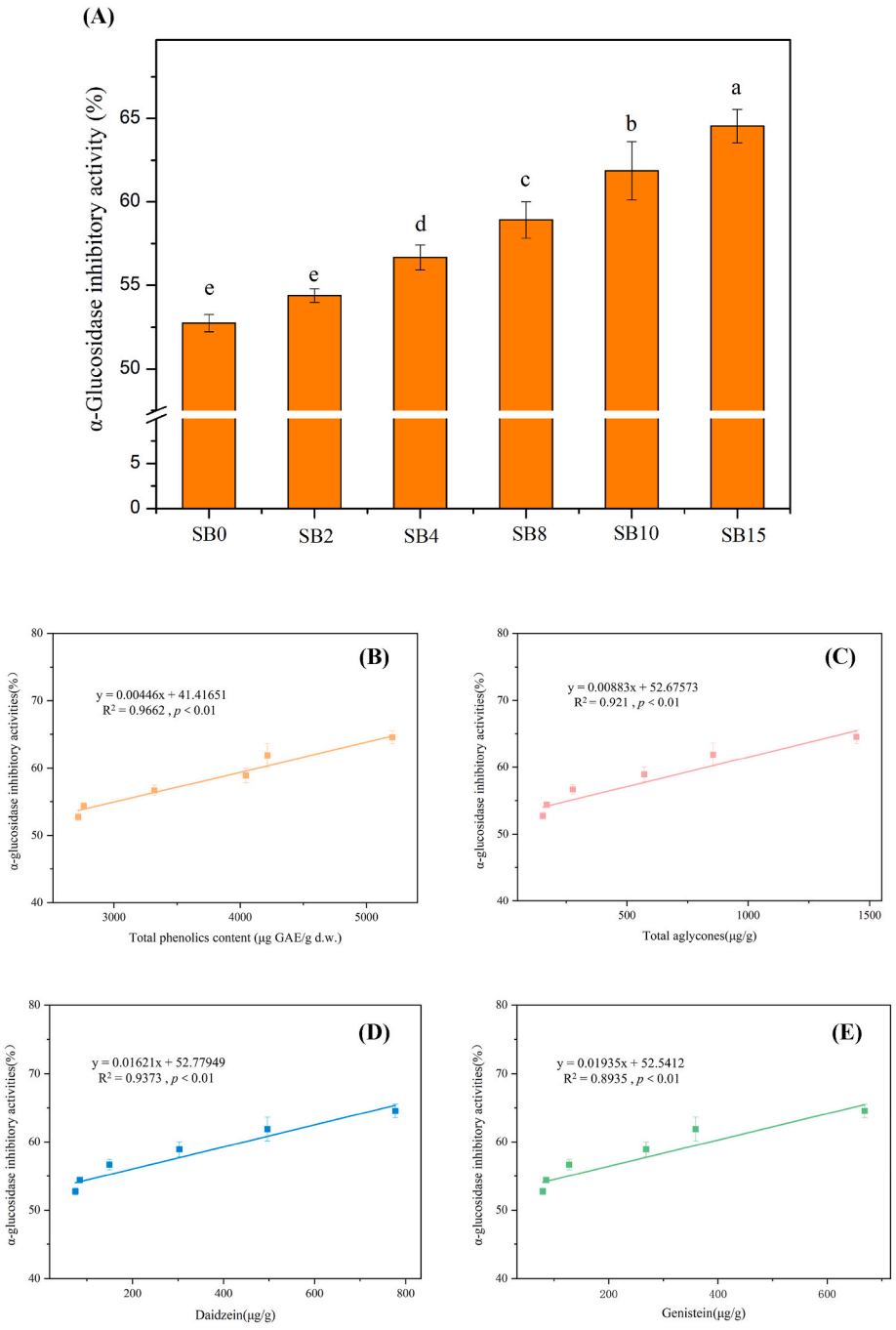
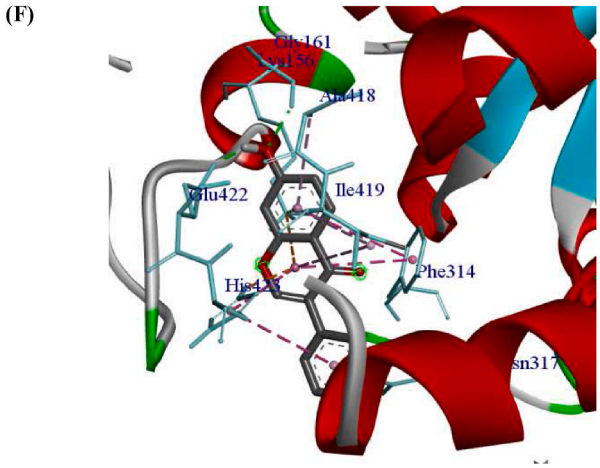


Fig. 4. (A) Inhibitory activity of α-glucosidase of soybeans during SSF with *E. cristatum*. Data were recorded as the mean value ± standard deviation of three replicates. Mean values marked by the different letters among the samples denoted significant differences ($p < 0.05$). **(B–E)** Linear correlation analysis between α-glucosidase inhibitory activity, total phenolics content, and aglycone isoflavones. Binding interactions between daidzein **(F, G)**, genistein **(H, I)**, and α-glucosidase predicted by molecular docking analysis.



Binding Energy: -7.07kcal/mol

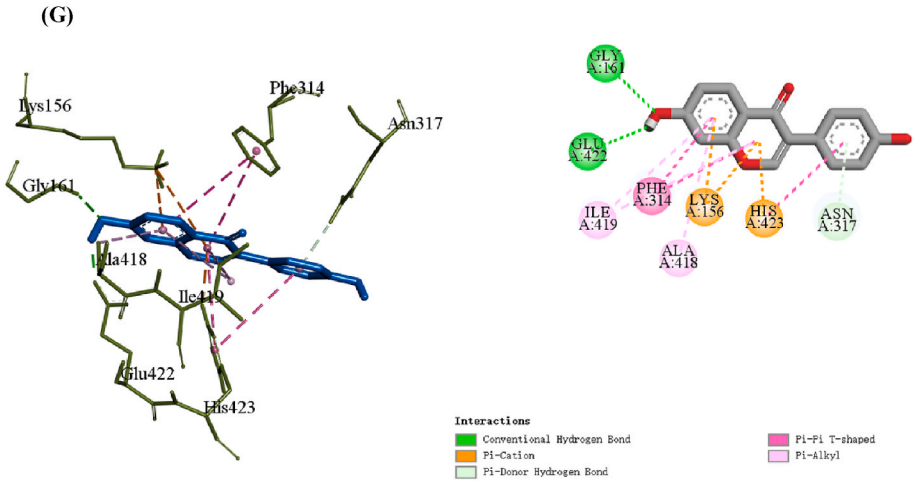


Fig. 4. (*continued*).

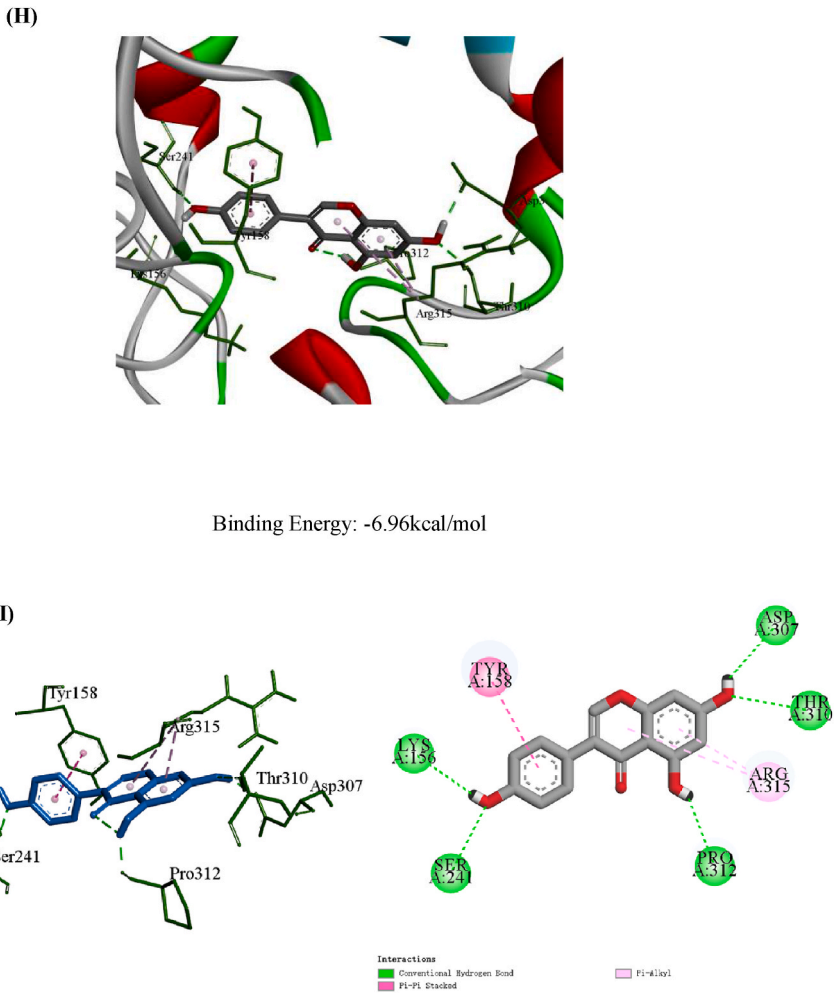


Fig. 4. (*continued*).

including Pi-Pi stacking, Pi-Pi T-shaped, and Pi-alkyl interactions. It was noted that genistein interacted with α-glucosidase through the Pi-Pi-stacked of Tyr 158, interactions with the Pi-Alkyl groups of Arg 315. Daidzein interacted with α-glucosidase through the Pi-Pi T-shaped of Phe 314 residue, interactions with the Pi-Alkyl groups of Ile 419 and Ala 418 residues, and interactions with the Pi-Cation of Lys 156 and His 423 residues. Pi interactions play a crucial role in protein-ligand recognition by significantly contributing to binding enthalpy (Meyer et al., 2003). Flavonoids bind to the hydrophobic cavity of α-glucosidase through these interactions, influencing the polarity of the amino acid residue microenvironment and inducing structural changes in the enzyme, thereby inhibiting its activity (Xiang et al., 2024). Genistein and daidzein are both hydrophobic compounds containing multiple rings that spontaneously interact with the hydrophobic amino acid residues of enzymes (Zhao et al., 2022). Besides, previous studies have also reported that flavonoids could insert into the hydrophobic region to interact with Arg 315 residue, which located at the entrance of the active site pocket, thus preventing the substrate from entering, reduce catalytic activity, resulting in changes in enzyme conformation, and finally inhibited the activity of α-glucosidase (Dong et al., 2021). Additionally, the predicted binding affinities of daidzein and genistein to α-glucosidase was calculated as −7.07 kcal/mol and −6.96 kcal/mol, respectively, which is comparable to the binding energy of a standard ligand α-D-glucose (−6.74 kcal/mol) (Seong et al., 2016). This result indicated that a strong binding of isoflavone aglycones to α-glucosidase. Furthermore, Ding et al. (2018) also reported that His280, Pro312, Tyr158, Phe314, Lys156, and Arg315 are all active sites for α-glucosidase. Isoflavone aglycones interacted with the active sites of α-glucosidase through hydrogen bonding and hydrophobic interactions, leading to reduced enzyme catalysis and subsequent inhibition of α-glucosidase activity (Xie et al., 2021).

Therefore, the result of molecular docking demonstrated that hydrogen bonding and hydrophobic interactions are the main binding force of isoflavone aglycones to α-glucosidase. These interactions collectively stabilize the inhibitor-enzyme complex, effectively inhibiting the enzyme's activity and ensuring the potency of the inhibition. The GIA improvement in fermented soybeans can be attributed to the enrichment of phenolic compounds (especially isoflavone aglycones) during SSF. Therefore, the stronger GIA effect of fermented soybeans suggests that they could be a crucially potential source of nutraceutical and hypoglycemic agents.

4. Conclusion

This study demonstrated that solid-state fermentation with *E. cristatum* greatly degraded the grassy characteristics and typical beany flavor compounds of soybeans, and remarkably increased the levels of volatile organic compounds with floral and sweet aromas. Key odor-active compounds, such as propyl acetate, 2-propanol, and methylpropanal, which impart floral and sweet odors, were found to increase drastically after 15 days of fermentation. *E. cristatum* secretes extracellular enzymes that are critical for the transformation of volatile organic compounds during solid-state fermentation. However, the mechanisms by which *E. cristatum* degrades off-flavor compounds warrant further

investigation. Bioinformatics analysis would be conducted to infer the possible transformation pathway of key flavor compounds. Then, RNA-sequence based transcriptomic analysis would be used to identify the differentially expressed genes of *E. cristatum* during the fermentation process, and clarify the differentially expressed genes related to the degradation of beany flavor compounds and synthesis of key desirable flavors. Additionally, the α-glucosidase inhibitory activity of soybeans increased by 22.4% after fermented with *E. cristatum* for 15 days, mainly ascribed to the accumulation of dadzein and genistein during fermentation. The results suggested that soybean fermented with *E. cristatum* for 15 days is the best fermentation stage to produce fermented soybean product. In conclude, this study revealed that solid-state fermentation with *E. cristatum* improved the aroma characteristics and α-glucosidase inhibitory activity of soybeans, thereby facilitating the use of fermented soybeans as ingredients in the design and development of various legume and cereal-based food products.

CRediT authorship contribution statement

Yu Xiao: Project administration, Methodology, Resources, Formal analysis, Funding acquisition, Validation, Conceptualization, Investigation, Writing – original draft, Writing – review & editing. **Hui Chen:** Writing – original draft, Data curation, Investigation. **Yajing Wang:** Investigation. **Jinrong Ma:** Investigation. **Aixiang Hou:** Resources. **Yuanliang Wang:** Resources. **Yulian Chen:** Methodology, Data curation, Validation, Supervision, Writing – original draft, Investigation. **Xingjun Lu:** Resources, Funding acquisition, Writing – review & editing, All authors have read and agreed to the published version of the manuscript.

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.

Acknowledgements

The authors appreciate the National Natural Science Foundation of China (No. 32302611), Hunan Province Key Research and Development Program (No. 2023NK2025), the Science Research Project of Education Department of Hunan Province (No. 23A0727), Postgraduate Scientific Research Innovation Project of Hunan Province (No. CX20230722) and Hunan Province Natural Science Foundation (No. 2024JJ7337).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2024.100854.

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