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Effect of ferulic acid grafted walnut shell hemicellulose B on the flavor of traditional pickle fermentation

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Abstract

To improve the flavor of traditional pickles, hemicellulose B (HCB) was extracted from walnut shell, the protein of which was detected by ultraviolet-visible (UV-Vis) light full wavelength scanning. Then ferulic acid was grafted in the absence of oxygen, and UV-Vis and Fourier transform infrared (FT-IR) were used to verify the branching. HCB and ferulic acid grafted hemicellulose B (FHB) were added to pickles, respectively, and the aroma substances were detected by gas chromatography and mass spectrum (GC-MS) to evaluate the effect of FHB on the aroma substances of pickles. The results showed that the protein in HCB was basically removed after UV-Vis scanning. After UV-Vis and FT-IR identification, FHB was obtained. The GC-MS analysis of aroma substances showed that HCB caused more derivatives of aroma substances in the fermentation process of pickles, and FHB made pickles produce more unique aroma substances during fermentation. This study provided a certain research basis for improving the flavor substances of traditional pickles.

Keywords: ferulic acid; walnut shell; hemicellulose B; flavor.

Practical Application: FHB can improve the flavor of traditional pickle fermentation.

1 INTRODUCTION

Walnuts are very popular nuts in daily life, and large amounts of walnut shell are produced and thrown away every year. However, the hard shell of walnut has many application values, and its main components are lignin, cellulose, and hemicellulose. Among them, hemicellulose is composed of xylan, and xylan and its derivatives are widely used in the medical field (Liu et al., 2019). In addition, xylan can improve acid production and fermentation by improving the activity of lactic acid bacteria (Haokok et al., 2023). Therefore, extracting hemicellulose from walnut shell has great prospects, especially in the fermentation of lactic acid bacteria.

Ferulic acid, which is also known as 4-hydroxy-3-oxy cinnamic acid, belongs to phenolic acids and exists in plant cell walls (Wang et al., 2022). It has various functions such as antiviral, radiation resistant, antiapoptotic, and anticancer (Antonopoulou et al., 2022; He et al., 2019; Li et al., 2018; Zheng et al., 2019); therefore, it has good physiological and health care effects. In addition, ferulic acid also has a good antioxidant effect. In vivo, ferulic acid has a strong antioxidant capacity, which can effectively quench free radicals and regulate the biological activities of various enzymes (Srinivasan et al., 2007). In terms of food, it can inhibit the peroxidation of fatty acids and can be used as a natural food preservative (Kose et al., 2022). As an additive in sausages, ferulic acid can reduce the concentration of histamine during fermentation and maturation (Zhao et al., 2018). In addition, ferulic acid can also be used for the preservation and storage of fruits and vegetables. It can reduce the incidence rate and spot diameter of Penicillium that damage tomato fruits, reduce the content of soluble solids in fruits, and increase the content of ascorbic acid and Lycopene (Hu et al., 2019), which indicates that ferulic acid treatment is a new method for the preservation and storage of fruits and vegetables. Overall, ferulic acid has a very broad application in the food industry. It has been approved to be used as an additive in food, medicine, cosmetics, and other fields in the United States, Europe, and Japan.

As ferulic acid rarely exists in the cell wall in free form (Ou & Kwok, 2004), it is often covalently bound to xylan in the form of side chains (Chen et al., 2021; Saulnier & Thibault, 1999). Moreover, ferulic acid-modified xylan had better biological activity (Fröhlich et al., 2022), but the natural ferulic acid grafted hemicellulose B (FHB) content is less. Therefore, hemicellulose B (HCB) was extracted from walnut shell in this study and first grafted with ferulic acid to obtain FHB polymer, which was first applied to the traditional fermentation of pickles to evaluate its impact on the flavor substances.

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2 MATERIALS AND METHODS

2.1 Extraction of hemicellulose B

After drying, the walnut shell was crushed and sieved through a 50-mesh sieve. The fine powder was subjected to 1% alkaline solution and 0.8% ammonium oxalate boiling water bath to remove protein and pectin, respectively, to obtain dietary fiber, which was then treated by 4% alkaline solution at 45°C to extract hemicellulose. After filtering, the pH of the filtrate was adjusted to 5 by glacial acetic acid, and then the precipitate was removed by centrifugation. The supernatant was precipitated with alcohol to obtain HCB and then freeze-dried for later use.

2.2 Ferulic acid grafted hemicellulose B

Solution A: An amount of 125 mg of polysaccharides was dissolved in 15 mL of distilled water. Solution B: A volume of 0.51 mL of H_2O_2 (30%) was added to 5 mL of distilled water and then 50 mg Vc was dissolved in this solution by stirring. This mixture was allowed to react for 10 min. Solution C: An amount of 200 mg of ferulic acid was added to 5 mL of distilled water and mixed. Solution B was added dropwise to solution A and allowed to react in an anaerobic environment for 30 min; afterward, solution C was added in the above-mentioned mixture and allowed to react for 24 h. After the reaction, the mixture was centrifuged at 5,000 rpm for 5 min, and the supernatant was then dialyzed using a 3,500 D dialysis bag for 24 h. Dialysate was freeze-dried and ready for use.

2.3 Pickles fermentation

Totally, 500 g of white radish and 50 g of carrots were washed and cut into strips, which were then treated with 6% saline water for 1.5 h. After removing the drained water, the strips were mixed with 10 g of garlic, 15 g of pepper, 20 g of chili, 3 g of star anise, 2 g of fragrant leaves, and 1 g of brown sugar in 6–8% saline water. After inoculation with *Lactiplantibacillus plantarum*, FHB was added to make the concentration into 0.1 mg/mL, using without/with the addition of HCB at the same concentration as two controls. Samples were fermented at room temperature for 7 days.

2.4 Instrument characterization

Protein analysis: With distilled water as the control, the HCB solution was scanned at 200–600 nm by TU-1901 spectrometer (Ma et al., 2022).

Graft analysis: Based on the ultraviolet-visible (UV-Vis) analysis of HCB, the TU-1901 spectrometer was also used to analyze ferulic acid and FHB. Ferulic acid, HCB, and FHB were mixed with KBr and ground, respectively. The transmittance of 4000–500 cm^{-1} was determined by Fourier transform infrared (FT-IR) (Nicolet 670) (Yang et al., 2003).

Flavor analysis: The model of the microextraction probe was 55 μM SPME-C-02 PA. With 20 μL 1-octanol (5 μg/mL) as the internal standard, 1 g of NaCl and 5 g of crushed samples were placed in a 20-mL headspace injection bottle. After inserting the extraction head and extracting at 50°C for 30 min, the sample was desorbed at 250°C for 5 min before injection. The GC separation column was a DB-5MS elastic quartz capillary column (30 m × 0.25 mm × 0.25 μm); The temperature of the injection port was 250°C. The heating program was as follows: the initial column temperature was maintained at 40°C for 3 min and then increased to 140°C at 3°C/min; after maintaining for 1 min, it was then increased to 260°C at 20°C/min. Without splitting the sample, the gas flow rate was 1.5 mL/min. The MS conditions were as follows: EI ionization source, electron energy of 70 eV, electron multiplier voltage of 1,153 V, ion source temperature of 230°C, interface temperature of 280°C, and mass scanning range of 35–350 amu (Lee et al., 2021).

3 RESULTS

3.1 Protein and nucleic acid testing

Due to the influence of protein and nucleic acid on the determination after grafting, this study used UV-Vis to scan the wavelength of purified HCB in the range of 200–600 nm. As shown in Figure 1, there was no obvious absorption peak at 260 and 280 nm.

3.2 Grafting reaction

In this study, ferulic acid was grafted onto walnut HCB in the absence of oxygen, and the mechanism is shown in Figure 2. First, hydrogen peroxide oxidized the hydroxyl group on VC to form free radical, which in turn induced the hydroxyl group on HCB to form a free radical. Finally, ferulic acid was grafted with HCB via a covalent bond to form an FHB copolymer.

3.3 Grafting identification

To analyze the grafting effect of walnut shell HCB, UV-Vis was first used to characterize FHB. The results are shown in Figure 3A. There was no obvious absorption peak of polysaccharides in the range of 200–600 nm, which was relatively smooth, while ferulic acid has two obvious characteristic peaks at about 290 and 315 nm, and there was an absorption trough at 256 nm. The absorption curve of FHB was obviously affected by the grafting

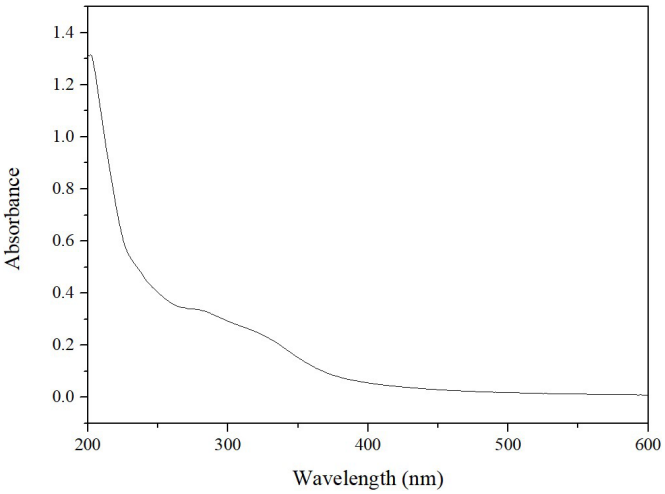

Figure 1. Full wavelength UV-Vis scanning of walnut HCB.

of ferulic acid. However, the difference in peak height between 290 and 315 nm has decreased. In addition, the grafting of polysaccharide with ferulic acid was further analyzed by FT-IR, the results of which are shown in Figure 3B. HCB and FHB had the same characteristic peaks at about 2,028 and 3,419 cm^{-1}, while the peak at 3,231 cm^{-1} was relatively lower. Ferulic acid and FHB had the same characteristic peak at 1,548 cm^{-1}, but HCB did not.

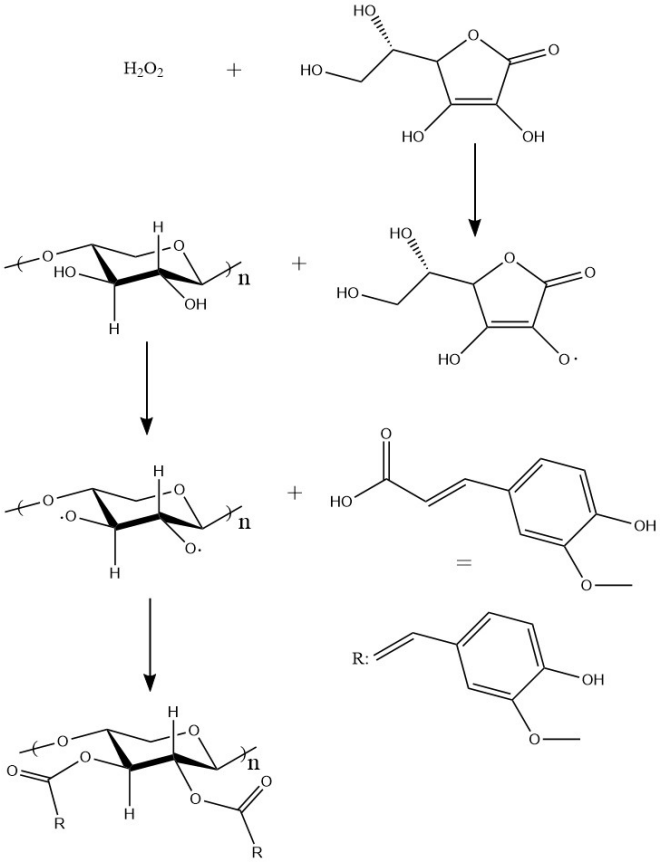

Figure 2. Grafting ferulic acid onto walnut HCB.

3.4 Flavor substance detection

Gas chromatography and mass spectrum (GC-MS) was used to detect the aroma components in pickle fermentation. From the GC spectrum of aroma (Figure 4), it can be observed that the aroma components in the three experimental samples were basically the same. However, compared with the non-add pickle sample, the addition of HCB and FHB also resulted in some specific components (Table 1). Ten substances were detected in the three samples (Table 1, No. 1–10). The aroma concentration of the polysaccharide-added sample was lower than that of the control. Compared with these two samples, except for β-Myrcene, the various components in the pickle samples of FHB have been improved. (E)-1-Methyl-2-(prop-1-en-1-yl) disulfane was only present in the non-add sample. D-Limonene existed in the samples of non-added and HCB but did not appear in the samples of FHB. Dimethyl trisulfide, (E)-1-allyl group-2-(allyl-1-allyl) disulfane, and β-myrcene appeared in the samples of non-added and FHB, while the sample of HCB did not have these three substances during fermentation. Benzofuran, 2,3-dihydro, and 1,6-octadien-3-ol,3,7-dimethyl-formate only existed in HCB samples. Five unique substances were found in the samples of FHB (Table 1, No. 18–24; Figure 5).

In the detection of GC-MS, some unresolved substances were found (Table 1). In the sample of HCB, two substances have not been resolved to the molecular formula (Figures 6B and 6D). According to the values and retention time of characteristic peaks (Table 1:1, No. 10), it can be inferred that they correspond to disulfide, methyl 2-propenyl, and trisulfide,di-2-propenyl (Figures 6A and 6C) of the non-added sample, respectively. The presence of distinct differences in molecular fragments suggests that their corresponding molecules have variations in molecular weight and structure. Two types of β-myrcene appeared in the non-added samples with consistent characteristic peaks (Figures 6E and 6F; Table 1, No. 2 and 15). The retention time and characteristic peaks of β-myrcene in FHB sample (Figures 6G; Table 1, No. 2) were basically the same as those in the sample of non-added (Table 1, No. 2). The substance (No. 15; Figures 6H) of FHB sample had similar characteristic

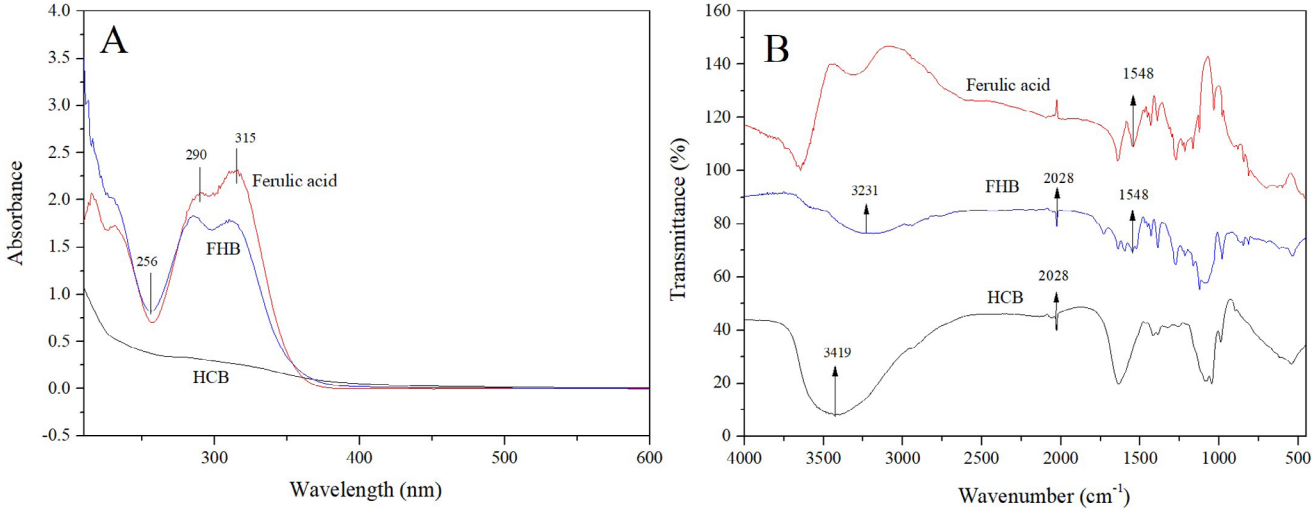

Figure 3. (A) UV-Vis and (B) FT-IR analysis of grafting.

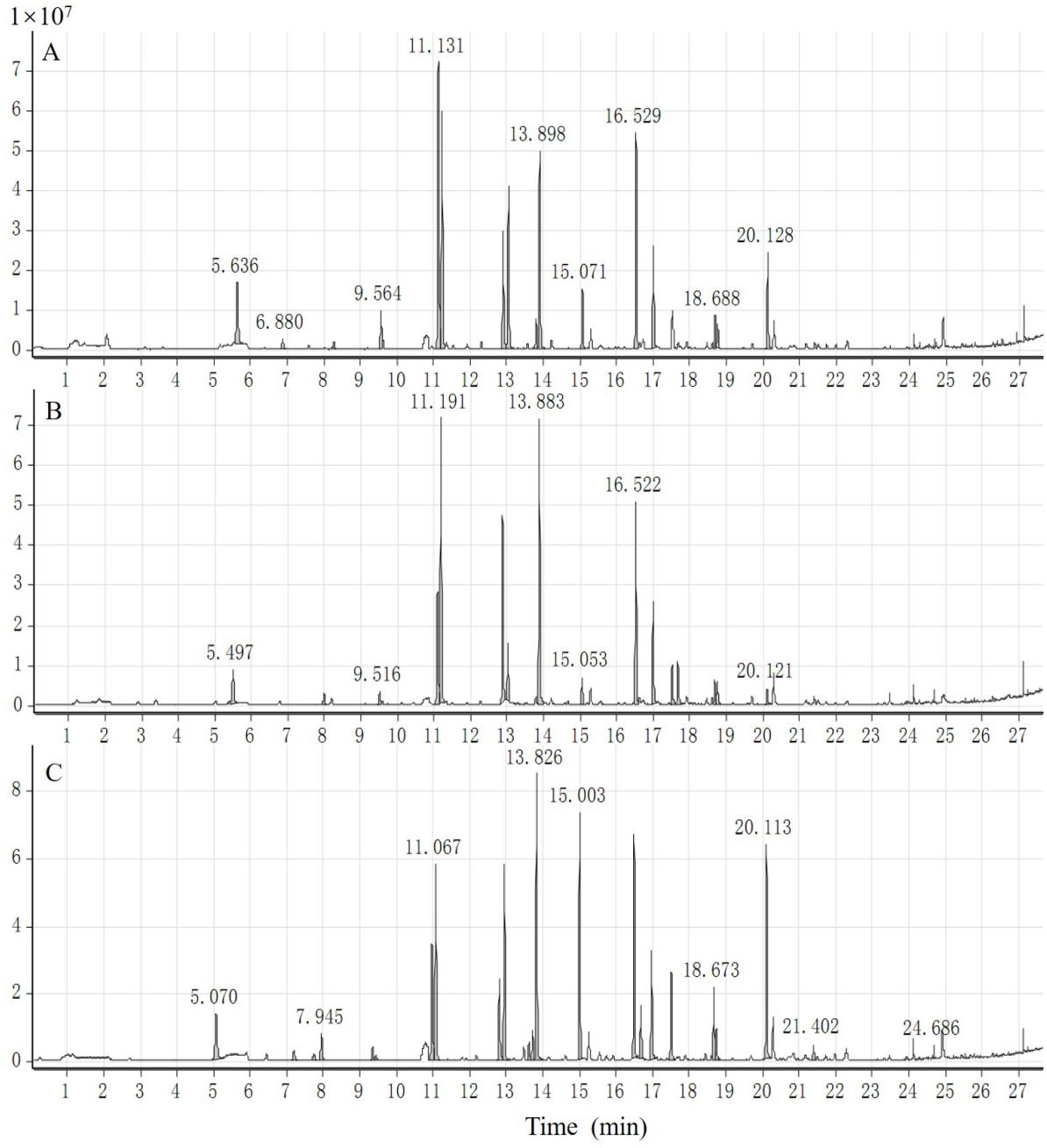

Figure 4. (A) GC spectrum of non-added, (B) HCB-added, and (C) FHB-added pickles.

peaks in mass spectrum and retention time with the samples of non-added and FHB. In addition, substances that could not be predicted by mass spectrometry were also present in the HCB sample (No. 17), which exhibited some of the same characteristic peaks on the mass spectrometry fragments as 2-vinyl-4H-1,3-dithiine in the FHB sample, and the retention time was basically the same (Figures 6I and 6J).

4 DISCUSSION

Protein and nucleic acid will affect the determination of grafting. This study used UV-Vis to scan the wavelength of purified HCB in the range of 200–600 nm. There was no obvious absorption peak at 260 and 280 nm, indicating that the protein and nucleic acid in the polysaccharide have been completely removed (Sun et al., 2021). After grafting, UV-Vis was first used to characterize FHB. There was no obvious absorption peak of polysaccharides, while ferulic acid has two obvious characteristic peaks at 290 and 315 nm, and the absorption curve of FHB was obviously affected by the grafting of ferulic acid. However, due to the influence of polysaccharides, the difference in peak heights between 290 and 315 nm has decreased. The above analysis showed that HCB was grafted with ferulic acid. FT-IR was widely used for the analysis of molecular composition and

Table 1. Analysis of flavor substance composition.

No.	Compound	Non-added		HCB		FHB	
		Relative concentration (μg/L)	Retention (min)	Relative concentration (μg/L)	Retention (min)	Relative concentration (μg/L)	Retention (min)
1	Methyl 2-propenyl disulfide	15.68	5.636	6.01 *	5.497	18.95	5.07
2	β-Myrcene	7.18	9.564	1.61	9.516	3.77	9.347
3	Eucalyptol	46.40	11.229	35.14	11.191	54.70	11.067
4	Diallyl disulfide	27.21	13.047	6.06	13.021	48.18	12.945
5	Linalool	33.14	13.898	31.54	13.883	67.59	13.826
6	Trisulfide, methyl 2-propenyl	10.19	15.071	3.05	15.053	54.55	15.003
7	Terpinen-4-ol	35.80	16.529	21.98	16.522	51.73	16.492
8	α-Terpineol	17.34	17.002	11.09	16.994	26.44	16.968
9	2-Cyclohexen-1-one,3-methyl-6-(1-methylethyl)	4.01	18.76	2.81	18.760	8.07	18.741
10	Di-2-propenyl trisulfide	15.68	20.128	1.76 *	20.121	46.45	20.113
11	(E)-1-Methyl-2-(prop-1-en-1-yl)disulfane	2.27	6.880	——	——	——	——
12	D-Limonene	54.91	11.131	14.52	11.090	——	——
13	Dimethyl trisulfide	1.65	8.271	——	——	8.45	7.945
14	(E)-1-Allyl-2-(prop-1-en-1-yl)disulfane	5.16	13.800	——	——	7.03	13.718
15	β-Myrcene	5.48	18.688	——	——	1.53*	9.433
16	Benzaldehyde	——	——	1.74	7.983	2.25	7.742
17	2-Vinyl-4H-1,3-dithiine	——	——	4.37*	17.515	23.24	17.511
18	Benzofuran, 2,3-dihydro	——	——	5.04	17.673	——	——
19	1,6-Octadien-3-ol,3,7-dimethyl-formate	——	——	2.71	18.685	——	——
20	3H-1,2-Dithiole	——	——	——	——	3.51	7.199
21	Cyclohexene, 1-methyl-5-(1-methylethenyl)-,(R)-	——	——	——	——	31.67	10.966
22	3-Methyl-3H-1,2-dithiole	——	——	——	——	4.31	13.616
23	3-Vinyl-1,2-dithiacyclohex-4-ene	——	——	——	——	13.05	16.675
24	Linalyl acetate	——	——	——	——	16.28	18.673

*The substance not resolved by mass spectrometry and speculated as a derivative; ——, not detected.

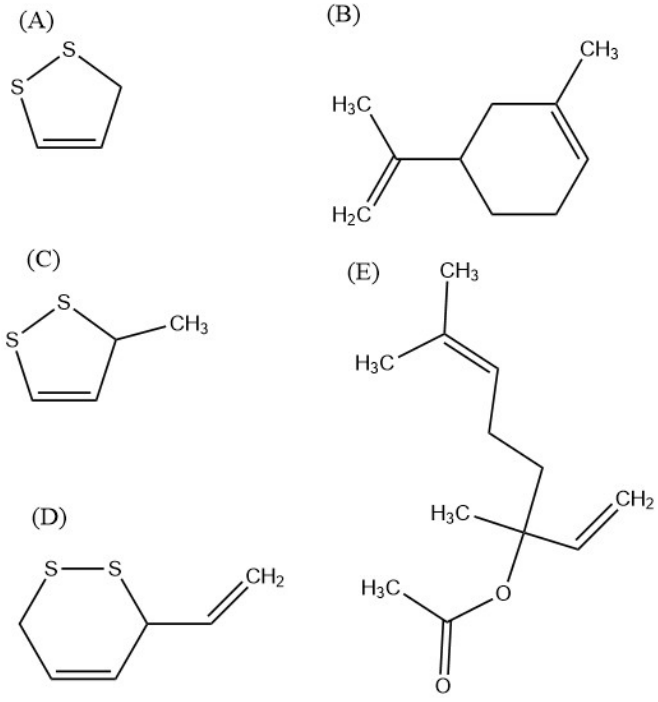

Figure 5. Special aroma components of ferulic acid HCB sample.

structure due to its high sensitivity, accurate wavenumber, and good repeatability. In this study, the grafting of polysaccharides with ferulic acid was further analyzed by FT-IR. HCB and FHB had the same characteristic peak at 2,028 cm^{-1}, indicating that they both have xylan molecular chains. The absorption peak of HCB at 3,419 cm^{-1} was the stretching vibration absorption peak of –OH (Zhang et al., 2020) because part of -OH on HCB formed free radicals and then connected with ferulic acid. Therefore, the peak value of FHB near it was relatively lower (3,231 cm^{-1}). Ferulic acid and FHB had the same characteristic peak at 1548 cm^{-1}, but HCB did not have this characteristic peak, which belongs to the vibration of aromatic ring C = C (Thirukumaran et al., 2016). The analysis of FT-IR further proves that ferulic acid and HCB had formed a copolymer.

GC-MS can detect the molecular weight of compounds and infer the molecular structure information, which was often used for the detection of aroma substances (Guo et al., 2022). It can be observed that the aroma components in the three experimental samples were basically the same from the spectrum. However, compared with the non-add pickle sample, the addition of HCB and FHB also resulted in some specific components. Ten substances including disulfide ethyl 2-propenyl were detected in the three samples (Table 1, No. 1–10).

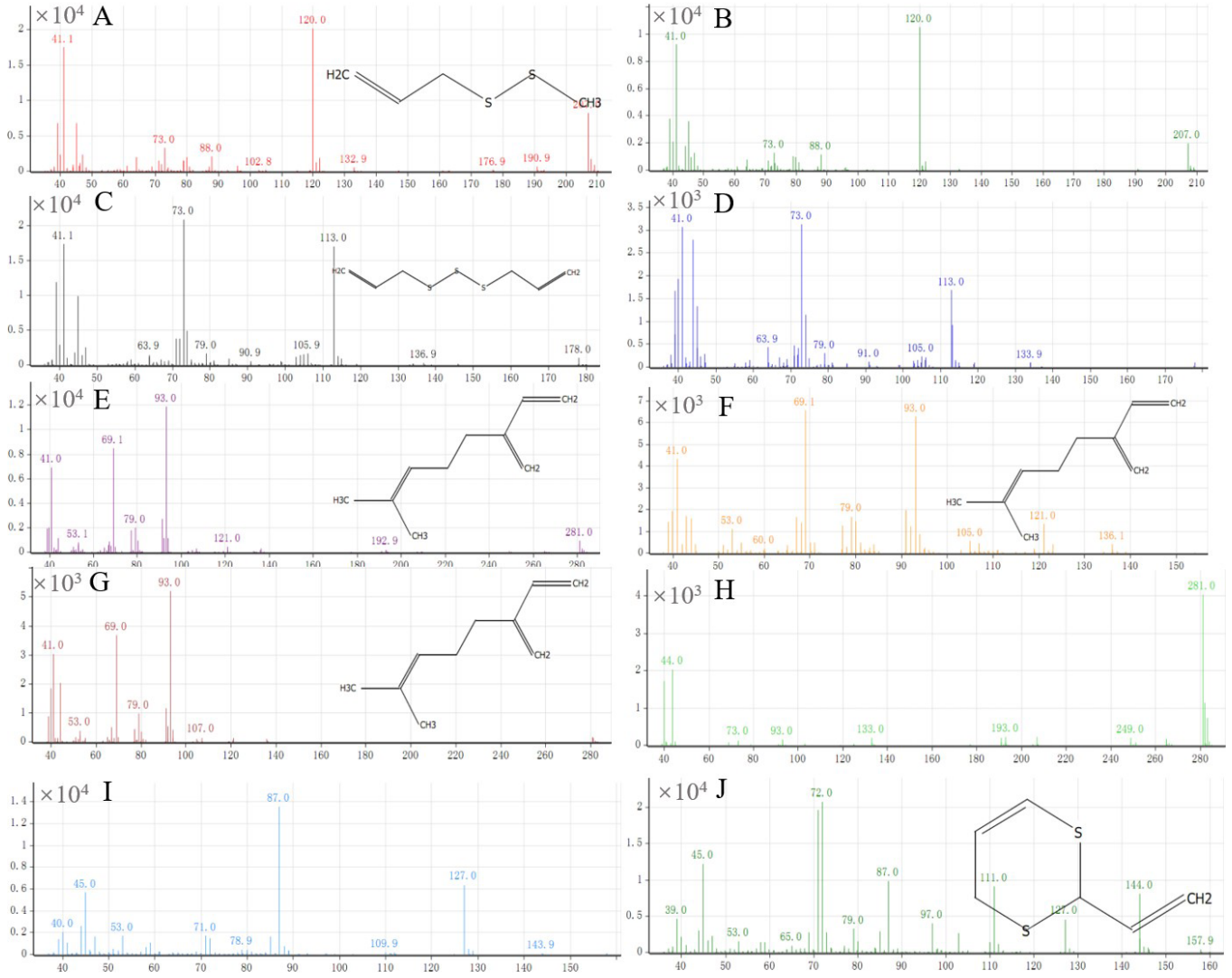

Figure 6. MS analysis of (A and B) methyl 2-propenyl disulfide, (C and D) di-2-propenyl disulfide, (E–H) β-myrcene, and (I and J) 2-vinyl-4H--1,3-dithiine.

The aroma concentration of the polysaccharide added sample was lower than that of the control, which was due to that the polysaccharide was used by *Lactiplantibacillus plantarum* (Seong et al., 2019), thus delaying the fermentation of the internal pickle ingredients. Compared with these two samples, except for β-myrcene, various components in the pickle samples of FHB have been improved, indicating that FHB could inhibit the utilization of polysaccharides and induce the production of aroma components. (E)-1-Methyl-2-(prop-1-en-1-yl) disulfane was only present in the non-added sample, which was utilized and derived into other substances under the induction of HCB (Ma et al., 2022). D-Limonene existed in the samples of non-added and HCB but did not appear in the samples of FHB, which might be because branched chain of ferulic acid induced its derivation into other substances. Dimethyl trisulfide, (E)-1-allyl group-2-(allyl-1-allyl) disulfane, and β-myrcene appeared in the samples of non-added and FHB, while the sample of HCB did not produce these three substances during fermentation; therefore, ferulic acid changed the role of polysaccharides in fermentation. Due to the presence of polysaccharides, benzaldehyde and 2-Vinyl-4H-1,3-dithiine were co-present in the latter two samples. Benzaldehyde gave the pickle an almond flavor (Zhang & Jia, 2023), while 2-vinyl-4H-1,3-dithiophene was used as an antithrombotic substance (Moghaddam-Manesh et al., 2019); thus, pickle may have a garlic aroma (Hossain et al., 2023). Benzofuran,2,3-dihydro and 1,6-octadien-3-ol,3,7-dimethyl-formate only existed in HCB samples, so HCB may be the precursor of these two substances or has an induction effect. However, these two substances did not appear in FHB sample, indicating that ferulic acid changed the role of HCB in fermentation. Five unique substances were found in the sample of FHB. Compounds 3H-1,2-dithiole, 3-methyl-3H-1,2-dithiole, and 3-vinyl-1,2-dithiacyclohex-4-ene are sulfides; 3-methyl-3H-1,2-dithiole is the methylation product of 3H-1,2-dithiole; cyclohexene and 1-methyl-5-(1-methylethenyl) are isomers of limonene; linalyl acetate is the acetate esterification product of linalool. These five substances give the pickle more aromatic odors. This shows that the ferulic acid grafted HCB was

the essential substance for the production of these substances. Therefore, FHB can induce a variety of aroma substances during the fermentation process of pickles, which can improve the flavor of traditional pickles.

In addition to the differences in the types and concentrations of aroma substances, there were also some effects on the isomerization and derivation of aroma substances during the fermentation process. Two substances have not been resolved to the molecular formula in the sample of HCB. According to the value and retention time of characteristic peaks, it can be inferred that they correspond to methyl 2-propenyl disulfide and di-2-propenyl trisulfide of the non-added sample, respectively; the differences in molecular fragments indicated that their corresponding molecules have certain differences in molecular weight and structure. Two types of β-myrcene appeared in the non-added samples, which were speculated by MS according to the consistent characteristic peaks. However, due to the significant difference in retention time between the two types, it indicated that the two molecules may be isomers (Carius et al., 2022; Rodríguez-Pérez et al., 2013), which lead to different affinity between gas molecules and the solid phase of the column. The retention time and characteristic peaks of β-myrcene in the FHB sample were basically the same as those in the sample of non-added, so they were the same substance in molecular structure. The substance No. 15 of the FHB sample had similar characteristic peaks in the mass spectrum and retention time with the non-added samples. Therefore, the substance had a similar molecular structure to β-myrcene, and the difference was not large. It can be inferred that it was a derivative of β-myrcene (Soares-Castro et al., 2023). Substances of No. 17 that could not be predicted by mass spectrometry were also present in the HCB sample, which exhibited some of the same characteristic peaks on the mass spectrometry fragments as 2-vinyl-4H-1,3-dithiine in the FHB sample, and the retention time was basically the same. Therefore, this substance may be a small molecule derivative of 2-vinyl-4H-1,3-dithiine. From the analysis of the derivative situation, HCB produced more unknown substances in the fermentation of pickles, while FHB had less modification of flavor substances.

5 CONCLUSION

To improve the flavor substances of traditional pickles, this study used walnut shell as a substrate to extract HCB. UV-Vis full wavelength scanning was used to ensure no interference from proteins and nucleic acids. After UV-Vis analysis, the proteins and nucleic acids in the HCB extract were basically removed. The walnut shell HCB was grafted with ferulic acid in the absence of oxygen. UV-Vis and FT-IR were used to verify the grafting, the results of which showed that FHB polymer was obtained. Using a non-added pickle sample as a control, HCB and FHB were added to the pickle for fermentation. GC-MS was used to detect aroma compounds. The analysis showed that HCB induced the derivation of aroma compounds in pickles during the fermentation process, and FHB produced more unique aroma compounds. This study indicated that FHB can improve the flavor compounds of traditional pickle fermentation.

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