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Analysis of Aroma Components of Enzymatic Hydrolysis Products of Oak Lignin from Different Producing Areas

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ABSTRACT

The morphology and structure of lignin in French oak, American oak and Chinese Mongolian oak were observed and compared by scanning electron microscopy (SEM). The results showed that the surface of lignin after enzymatic hydrolysis was more irregular and the contact area was obviously increased. GC-MS detected 3 kinds of lignin, 4 kinds of components and 30 kinds of volatile substances. The lignin content of French oak was the highest (8911.5 ± 512.2 μg/L, 29 kinds), followed by that of American oak (8071.7 ±541.9 μg/L, 26 kinds), and that of Mongolian oak was the least (7903.3 ± 492.5 μg/L, 26 kinds). The results of aroma characteristics analysis, principal component analysis and heat map analysis showed that the aroma components of the three kinds of oak lignin were significantly different. French oak lignin could provide more volatile phenols, with obvious smoky, woody and sweet flavor. Mongolian oak lignin carries more phenolic and ester compounds, providing a relatively soft floral, fruity flavor. American oak lignin has a relatively average aroma substance content, which can bring strong and complex complex aroma characteristics.

KEYWORDS

Lignin; Volatile components; Oak; Enzymolysis.

1. INTRODUTION

The oak genus Fagaceae (Quercus L.) is also known as quercus. It grows in Europe, Asia, America and other regions, and is commonly found in the North temperate zone [1]. The original use for wine aging dates back to the Roman Empire. For hundreds of years, because oak can significantly improve the taste and quality of wine, it has been used as a material for wine storage containers and has been widely used in various regions[2]. The common oak products are mainly from American white oak (Q. alba), summer oak (Q. robur), and the stem oak (Q. petraea), the former is generally called American oak and the latter is called French oak. In addition, many types of oak are used to make oak products such as Portuguese oak (q.foinea) and Hungarian oak (q.fonetto)[3]. In China, Q. mongolica, Liaodong Quercus and Cyclobalanopsis quercus are used for aging research [4].

Lignin is a high molecular weight amorphous aromatic polymer[5]. Lignocellulose is composed of cellulose and lignin, lignin is the second most abundant component of lignocellulose after cellulose, and the global annual production is about 20 billion tons. Lignin is usually composed of three subunit structures: p-hydroxyphenylpropane (H), guaiacyl (G), and syringyl (S)[6]. In the lignin network, monomers of coumarol, coniferol and sinapol are randomly constructed by intermolecular C-C and C-O bonds[7]. The depolymerization of lignin into small molecule compounds is an important step to realize its value, and the lignin depolymerization mainly includes three methods: biological method[8], chemical method and physical method[9]. Biological depolymerization is the use of lignin degrading enzymes and other additives to break the connections between lignin molecules,

environmental protection and high selectivity of target products. lignin degrading enzymes mainly include three kinds of enzymes: lignin peroxidase (LiP), mangnase peroxidase (MnP), laccase (Lac), lignin peroxidase (LIP), Mangnase peroxidase (MNP), and Laccase (LAC). These three lignin degrading enzymes can degrade lignin alone, in combination with each other, or in combination with all three enzymes[10]. All the three enzymes are extracellular enzymes[11]. LiP can catalyze the oxidation of aromatics and the breaking of C-C bonds (ring opening and side chain breaking). MnP can catalyze the oxidation of phenolic compounds and the break of C-O bond. Lac can also catalyze the oxidation of various compounds and the break of C-C bond[12].

In this experiment, the structural differences of lignin before and after enzymatic hydrolysis were observed by scanning electron microscopy and gas chromatography-mass spectrometry (GC-MS) was used to analyze the volatile components of lignin extracted from American oak, French oak and Mongolian oak after enzymatic hydrolysis. To provide theoretical support and reference for oak in research and use.

2. MATERIALS AND METHODS

2.1. Materials and instruments

French oak chips (Q. acutissima Caruth), American oak chips (Q. alba), Mongolian oak chips (Q. mongolica Fisch) are provided by Gaomi Xincheng Oak Barrel Co., LTD.Anhydrous ethanol, glacial acetic acid, sodium acetate, dioxane, methanol Sinopharm Group Chemical Reagent Co., LTD. 2-octanol was purchased from Shanghai Maclin Biochemical Technology Co., LTD. Lignin peroxidase (294 U/L) and manganese peroxidase (301 U/L) were purchased from Shanghai Yuanye Biotechnology Co., LTD. Laccase (322 U/L) was purchased from Sigma-Aldrich.

Soxhlet extractor; LY-645C Precision Oven: Guangdong Liyi Technology Co., LTD. Himac CR 22N High Speed refrigerated centrifuge: Japan Co., LTD. RE-52AA rotary evaporator: Shanghai Yarong Biochemical Instrument Factory; Freeze dryer: UNIEQUIP, Germany; Vibrating cell level ultrafine mill: Jinan Da Micromachinery Co., LTD. HP-5 column (30 m×250 μm×1 μm) : Agilent Corporation; 7890B Gas Chromatograph: Agilent Corporation, USA; 5977B Mass Spectrometer: Agilent Corporation, USA; PHS-3C pH meter: Shanghai Leimi Co., LTD. Ultraviolet spectrophotometer: Shanghai Angla Instrument Co., LTD.

2.2. Experimental methods

2.2.1. Lignin extraction

The oak pieces were superfine crushed and screened over 20 mesh to obtain oak powder. The oak powder was wrapped in filter paper, placed in Soxhlet extractor and extracted with ethanol for 6 h. The sample of degreased and dewaxed oak powder was obtained by drying the filter residue at 40 ℃. Weigh 10 g degreased dewaxed oak powder into 250 mL conical bottle, add 1.5 g cellulase, add 100 ml Buffer. After enzymolysis at 48 ℃ for 3 days, the sample solution was centrifuged, the supernatant was removed, and 100 ml enzyme solution was added to the precipitation for 2 days, and the enzymolysis was repeated for a third time[13]. Centrifuge the sample solution after 7 days of enzymatic hydrolysis, remove the supernatant, add 100 mL sterile water to stir well, and remove the supernatant by centrifugation for three times. Add 100 mL dioxane solution, stir for 16 h, centrifuge and collect the supernatant in 500 mL triangle bottle, and repeat for 3 times to collect samples[14]. The collected sample liquid was steamed, frozen at -80 ℃, and freeze-dried until solid to obtain lignin.

2.2.2. Enzymatic hydrolysis and aroma extraction of lignin

Configure 1 mg/L lignin solution to adjust pH to 5. The lignin solution was absorbed from 1 mL to 2 mL in a centrifuge tube, and 10 μL lignin peroxidase, manganese peroxidase and laccase were added

and enzymolized at 30 ℃ for 8 h. After enzymatic hydrolysis, dry and add chromatographic pure methanol (1 mL). The samples were ultrasonically extracted for 30 min, centrifuged at 10000 r· Min^{-1}, and the supernatant was obtained, and 20 μL of 2-octanol with a concentration of 1 mg/L was added as the internal standard for GC-MS analysis[15], and two groups were repeated.

2.2.3. Scanning electron microscope analysis

The morphology of the lignin was observed by scanning electron microscopy (SEM) after drying, and the lignin was treated with gold spray. Resolution: 0.8 mm@1KV; Magnification: 100 ~ 300000X; Acceleration voltage: 1KV ~ 20KV; Spectral undetectable elements: H, He, Li, Be, B.

2.2.4. GC-MS analysis

GC: HP-5 column (30 m×250 μm×1 μm); Gas chromatograph inlet temperature: 250 ℃, carrier gas was helium, flow rate 1mL/min; The sample size was 1 μL and the shunt ratio was 6:1. Heating procedure: the initial temperature of the column temperature box was 50 ℃, maintained for 2 min, then increased to 82 ℃ at 4 ℃·min^{-1}, then increased to 122 ℃ at 10 ℃·min^{-1}, and continued to rise to 170 ℃ at 4 ℃·min^{-1}, maintained for 5 min. Then the temperature rose to 280 ℃ at 8 ℃·min^{-1} and held for 5.25 min.

MS conditions: electron ionization (EI) source, electron energy 70 e V; Ion source temperature 230 ℃; Quality scanning range 30m/z: 30-350; Solvent removal time: 3 min.

Qualitative analysis: Mass spectrograms of unknown objects to be determined were matched and searched in the standard spectrum library (NIST14), compounds with matching factor greater than 80 were retained, and RI reported in literature were compared and identified[16].

$$RI=100_n+100[(RT-RTn)/(RT_{n+1}-RT_n)]$$

In the formula, $_n$ and $_{n+1}$ are the number of carbon atoms of normal alkane before and after the peak; RT_n and RT_{n+1} are the normal alkane retention time before and after the retention time of the component to be measured, respectively.

Quantitative analysis: Quantitative analysis with internal standard method. According to formula (1) :

$$C\ /(\mu g/L)= (\text{Peak area of the object to be measured}\times C_1\times V_1)/(\text{Internal standard peak area}\times m_1) \quad (1)$$

In the formula, C are Compound mass concentration; C_1 are internal standard mass concentration; V_1 are internal standard volume; m_1 are sample mass to be measured.

2.2.5. Aroma component evaluation method

Odor activity value (OAV) method was used to analyze the relative contribution of certain aroma components to lignin overall aroma in terms of aroma content and aroma threshold. Aroma components with OAV > 1 significantly contributed to the overall aroma, and the higher the OAV value, the greater the contribution. OAV < 1 was considered to have a potential contribution to the overall aroma. OAV is the ratio of the aroma component content (C) to the odor threshold (T). According to formula (2) :

$$OAV= C/T \quad (2)$$

2.3. Data processing

GraphPad Prism 9.5.1 was used to map and process the obtained basic data, IBM SPSS Statistics 20.0 software was used to conduct multivariate analysis of variance (Duncan's method, P<0.05), and

aroma data were analyzed by aroma analysis, PCA analysis and heat map analysis. The result of aroma substance content is expressed as ±s.

3. RESULTS AND DISCUSSION

3.1. Observation and analysis by scanning electron microscope

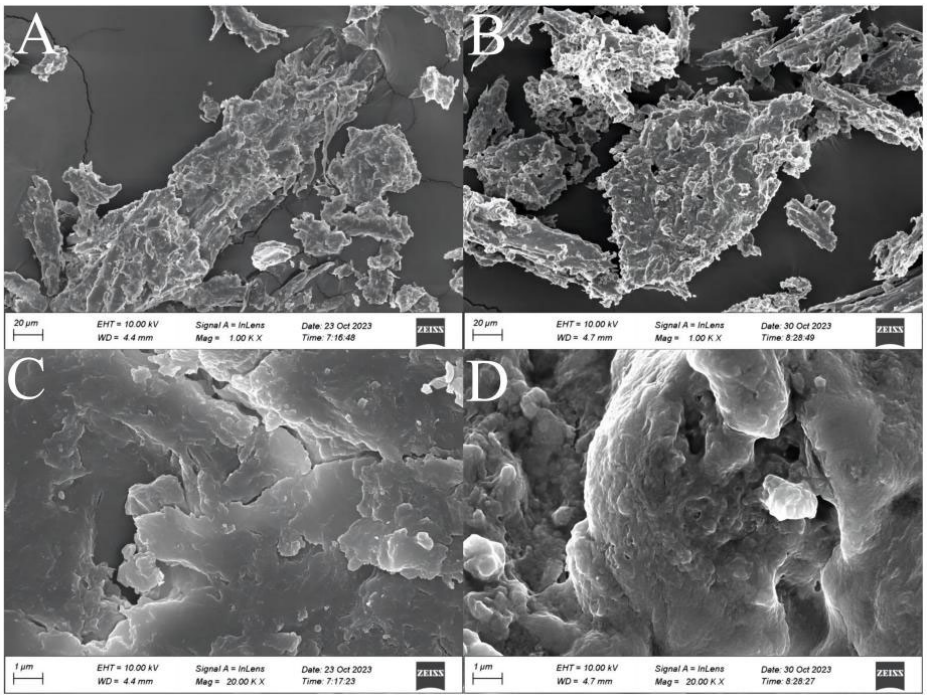

Figure 1. Scanning electron microscopy lignin digestion results

Note :A and C are unenzymolysis lignin; B and D are enzymolysis lignin.

Scanning electron microscopy can observe the changes and differences in the morphology and structure of substances. It can be seen from Figure 1 that the surface of lignin without enzymatic hydrolysis is smooth and wrinkle-free, and its shape is relatively regular. The lignin as a whole was in the shape of polymerized large particles, maintaining a complete geometry. The surface of lignin after enzymatic hydrolysis has more folds and cracks, which can effectively increase the unit contact area and dissolved oxygen volume. The chemical bonds between lignin are broken, splitting into many small particles, which can also increase the overall surface area. The surface of lignin treated by enzymatic hydrolysis becomes more irregular, and the lignin is decomposed into many small particles, which increases the contact area and improves the dissolved oxygen effect.

3.2. Analysis of volatile components of lignin by enzymatic hydrolysis

A total of 30 volatile substances were detected from three kinds of oak lignin by GC-MS, including 6 volatile phenols, 9 phenolic, 9 ester and 6 other substances. A total of 29 aroma substances were detected in lignin of French oak, including 6 volatile phenols, 9 phenolic substances, 9 ester substances and 5 other substances. A total of 26 aroma substances were detected in American oak lignin, including 6 volatile phenols, 9 phenolics, 6 esters and 5 others. A total of 26 aroma substances, including 6 volatile phenols, 9 phenolic substances, 6 ester substances and 5 other substances, were detected in lignin species of Quercus mongolica. The lignin content of French oak was the highest (8911.5 ± 512.2 μg/L), which was 1.1 times (8071.7 ±541.9 μg/L) and 1.13 times (7903.3 ± 492.5 μg/L) of American oak and Mongolian oak. Literature has shown that oak aroma is mostly derived from the volatile aromatic substances produced by lignin cracking[17], variety, origin[18,19],

processing methods[20] and other factors related to oak aroma. The lignin aroma components of three kinds of oak showed significant difference (P<0.05).

3.2.1. Volatile phenols

Table 1. Volatile phenolic compounds in lignin of 3 oak species

Number	RI	Aroma component	Threshold value /(μg/L)	contentα/ (μg/L)		
				French oak	American oak	Mongolian oak
A1	1170	Guaiacol	9.5	202±11.2a	188.4±9.5b	164.4±7.6c
A2	1190	4-methylguaiacol	20	93.8±4.3a	44.7±4.4c	64.5±2.7b
A3	1316	4-ethyl guaiacol	33	168.2±8.8a	99.7±6.9b	67.7±4.3c
A4	1362	eugenol	6	236.5±13.1a	204.6±15.4b	142.5±9.3c
A5	1512	2, 4-di-tert-butylphenol	200	169.3±9a	49.3±5.1b	46.8±4.6c
A6	1604	4-allyl-2, 6-dimethoxylphenol	1200	573.1±22.5a	562.3±16.8b	379.1±17.2c
		total		1442.9±68.9a	1149±58.1b	865±45.7c

Note: 2-octanol was the internal standard for α-quantification. The lower case letters of the same shoulder mark indicated significant difference (P<0.05) ; - Not detected; / Not found; See Table 1-4.

During the degradation of lignin, volatile phenolic substances will be produced, and more phenolic substances will be immersed in the aging process of wine[21]. Phenolic substances will bring unique odor and are an important volatile aroma component[22]. Table 1 shows that the content of volatile phenols in lignin of French oak is higher than that of American oak lignin and Mongolian oak lignin. Compared with French oak, the lignin content of American oak and Mongolian oak decreased by 20.4% and 40.0%, and the total lignin content of the three oak trees was significantly different (P<0.05). Three kinds of guaiacol and its alkyl derivatives were detected (4-methyl guaiacol, 4-ethyl guaiacol). Guaiacol has a distinctive smoky and roasted aroma[23], while its alkyl derivatives have a more pronounced herbal aroma[24]. Eugenol substances have sweet, woody and medicinal aroma[25], and are important aroma compounds in brandy. Eugenol is the main product of lignin degradation, and the difference of its content may be one of the reasons for the difference of different oak trees.

3.2.2. Phenolic substances

Table 2. Phenolic compounds in 3 kinds of oak lignin

Number	RI	Aroma component	Threshold value / (μg/L)	contentα/ (μg/L)		
				French oak	American oak	Mongolian oak
B1	1357	hydroxybenzaldehyde	/	498.7±23.1a	438.9±33.5b	346.2±16.3c
B2	1620	2,4, 5-trimethoxybenzaldehyde	/	40.6±2.6b	38.5±2.4c	47.3±2.4a
B3	1537	4-hydroxy-2-methylacetophenone	/	147.2±6.9b	127.3±10.3c	167.4±9.9a
B4	1402	Vanillic aldehyde	60	336.9±25.6c	365.4±19b	384.2±15.6a
B5	1555	3, 5-dimethoxyacetophenone	/	78.4±4.8c	112.5±7.3a	104.4±11.9b
B6	1405	Vanillyl ketone	1000	48.6±1.8a	36.1±2.6c	37.8±4b
B7	1533	4-hydroxy-3-methoxyphenylacetone	/	106.3±10.3c	167.3±12.1a	112.5±8.5b
B8	1666	syringaldehyde	25000	468.5±33.9b	443.6±23.9c	587±33.7a
B9	1743	coniferaldehyde	/	890.7±73.7c	1077.2±66.4a	930.3±49.7b
		total		2615.9±182.7c	2806.8±177.5a	2717.1±151.8b

Similar to volatile phenols, phenolic substances are also one of the main substances for lignin degradation, which can give wine a strong vanilla odor[26]. As can be seen from Table 2, a total of 9 kinds of phenolic substances were measured in this experiment, and the contents of phenolic substances in three kinds of oak lignin were similar. Vanillin source is composed of lignin degradation and oak hydrolysis, and its typical vanilla and vanilla flavor[27] is an important aroma characteristic of wine. Vanillin threshold is lower in ethanol, which makes a significant contribution to wine flavor. The content of syringal is related to the degree of baking and is an important degradation product of lignin[28]. The substance has a rich sweet aroma and almond odor, which is considered to be the characteristic aroma substance in oak. Although the content of syringaldehyde is high, it is difficult for syringaldehyde to directly affect the aroma of wine due to its high sensory threshold, so it affects the aroma through superposition and synergistic effect[29.30]. All three kinds of oak lignin contain a high content of coniferaldehyde, which may be related to the presence of more precursors of dimethoxyphenol in the lignin structure[31]. Four phenolic ketones were also detected in the experiment, and the main way to produce these substances is the degradation of lignin[32]. Vanilla acetone, also known as acetylvanillone, is a product of thermal degradation of carbohydrates and has a woody and pandan aroma[33].

3.2.3. Ester substance

Table 3. Ester compounds in lignin of 3 kinds of oak

Number	RI	Aroma component	Threshold value / (μg/L)	contentα/ (μg/L)		
				French oak	American oak	Mongolian oak
C1	1272	Ethyl caprylate	12.87	126.6±3.5b	114.2±12.8c	148.4±8.9a
C2	1395	Ethyl caprate	200	98.7±5.6b	117.4±7.3a	—
C3	1368	Ethyl laurate	400	698.2±27.5b	528.1±44.6c	718.4±52.5a
C4	1727	Methyl myristate	/	293.3±13.1b	313.6±21.2a	290.9±12.1c
C5	1658	Octyl ethylene glycol mono-n-dodecyl ester	/	128.4±8.4a	—	97.7±8.7b
C6	1463	Glycol laurate	/	85.3±4.7a	—	—
C7	1994	Methyl palmitate	/	230.1±16.3a	117.9±8.4c	199±19.4b
C8	1532	Dibutyl phthalate	/	103.7±6.2a	33.2±2.8b	—
C9	2206	Methyl stearate	/	91.5±3.3a	—	53.2±5.7b
		total		1855.8±88.6a	1224.4±97.1c	1507.6±107.3b

Table 3 shows that French oak lignin has the most ester types (9 kinds) and the highest content (1855.8±88.6 μg/L). Compared with Mongolian oak lignin, American oak lignin is similar in species but the content is lower. Ethyl caprylate, ethyl decanoate and ethyl laurate are all medium chain fatty acid ethyl esters, which are important components of wine flavor[34]. Other esters that are lower in content can also increase the complexity of memory. Esters bring rich floral and fruity flavor to wine and are indispensable aroma compounds[35].

3.2.4. Other substances

As can be seen from Table 4, a total of 6 other compounds were detected in the three lignins, including 1 acid and 5 alcohols. In the detected alcohols, the copolymers formed by sinapol and coniferol can form corresponding aldehyde compounds (sinapol and coniferaldehyde) after the depolymerization and oxidation process[36]. Lauric acid can be esterified to the corresponding medium chain fatty acid ethyl ester, and the esterification reaction is an oxidation reaction, which is also a reversible reaction, and hydrolysis will occur if the concentration of the product is high[37].

Table 4. Other compounds in lignin of 3 oak species

Number	RI	Aroma component	Threshold value / (μg/L)	contentα/ (μg/L)		
				French oak	American oak	Mongolian oak
D1	747	hexanediol	/	200.3±9.8a	126.3±11.5b	—
D2	1733	Sinapyl alcohol	/	60.9±2.2b	77.2±6.4a	54.8±4.1c
D3	1106	citronellol	/	—	233.1±12.9b	245.8±13.8a
D4	1150	coniferol	/	1729.4±103.4a	1699.6±122.7b	1532.3±95.3c
D5	876	Lauric acid	9153.8	148.3±7.4a	—	112.8±10.2b
D6	1369	Trans-coniferol	/	857.9±49.2b	755.3±55.7c	868.2±64.3a
		total		2996.8 ±172a	2891.5±209.2b	2813.9±187.7c

3.3. Analysis of flavor characteristics

Table 5. Attribute table of fragrance characteristics

Aroma characteristic	Aroma component	Threshold value /(μg/L)	OAV value (Odor activity value)		
			French oak	American oak	Mongolian oak
Floral scent	4-methylguaiacol	20	4.69	2.24	3.23
	4-allyl-2, 6-dimethoxylphenol	1200	0.48	0.47	0.32
	Guaiacol	9.5	21.26	19.83	17.31
	Ethyl laurate	400	1.75	1.32	1.80
	Vanillic aldehyde	60	5.62	6.09	6.40
	syringaldehyde	25000	0.019	0.018	0.023
	Vanillyl ketone	1000	0.049	0.036	0.038
	Ethyl caprylate	12.87	9.84	8.87	11.53
Fruity, nutty aroma	Ethyl caprylate	12.87	9.84	8.87	11.53
	Vanillic aldehyde	60	5.62	6.09	6.40
	syringaldehyde	25000	0.019	0.018	0.023
	Ethyl laurate	400	1.75	1.32	1.80
Wood fragrance, medicinal fragrance	4-ethyl guaiacol	33	5.10	3.02	2.05
	eugenol	6	39.42	34.10	23.75
	Vanillyl ketone	1000	0.049	0.036	0.038
Bake, smoke	4-methylguaiacol	20	4.69	2.24	3.23
	Guaiacol	9.5	21.26	19.83	17.31
Sweet and fragrant	4-allyl-2, 6-dimethoxylphenol	57	0.48	0.47	0.32
	eugenol	6	39.42	34.10	23.75
	Guaiacol	9.5	21.26	19.83	17.31
	Vanillic aldehyde	60	5.62	6.09	6.40
	syringaldehyde	25000	0.019	0.018	0.023
	Vanillyl ketone	1000	0.049	0.036	0.038

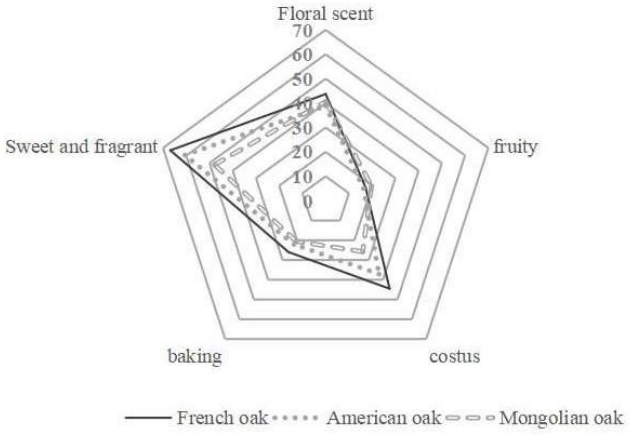

Figure 2. Aroma outline of aroma components in lignin

The five aromas were taken as five coordinates, and the OAV values of the three oak lignin were added and connected at each coordinate to get the aroma outline, as shown in Figure 2. The hollow line is the aroma outline of the Mongolian oak lignin. The Mongolian oak lignin has the richest fruit aroma, but it is insufficient in sweetness and wood aroma. The 5 aroma contents of lignin in Mongolian oak were sweet > floral > woody > roasted > fruity from most to least. The main aroma of Mongolian oak lignin is fruity and floral. The content of lignin in 5 flavors of American oak was sweet > floral > woody > roasted > fruity, which was consistent with that of Mongolian oak. Compared with Mongolian oak lignin, American oak lignin has obvious advantages in sweetness and wood flavor, but it is slightly inferior in floral and fruity flavor. Black solid lines represent the aroma outline of French oak lignin, French oak lignin has the richest sweet, floral, roasted, woody aroma, with the most complete and complex aroma of the three lignin. The five aromas of French oak lignin were: sweet > woody > floral > roasted > fruity from most to least.

3.4. PCA and heat map analysis

According to published literature[38,39] and considering the threshold, aroma, content and other factors of each volatile substance, 10 common volatile compounds were selected as the focus for PCA and heat map analysis.

Table 6. Main aroma substances in lignin of 3 kinds of oak

Mumber	Aroma component	Threshold value / (μg/L)	CAS number	Aroma description
A1	Guaiacol	9.5	90-05-1	Smoky, burnt, sweet
A2	4-methylguaiacol	20	93-51-6	Cloves, smokes
A3	4-ethyl guaiacol	33	458-36-6	Wood fragrance, medicinal fragrance
A4	eugenol	6	97-53-0	Sweet, clove, wood
A6	4-allyl-2, 6-dimethoxylphenol	1200	6627-88-9	Sweet, floral
B4	Vanillic aldehyde	60	121-33-5	Cream, vanilla, almonds
B6	Vanillyl ketone	1000	498-02-2	Wood, pandan
B8	syringaldehyde	25000	134-96-3	Sweet, almond
C1	Ethyl caprylate	12.87	106-32-1	Pineapple, floral, fruity
C3	Ethyl laurate	400	106-33-2	Fruity, floral

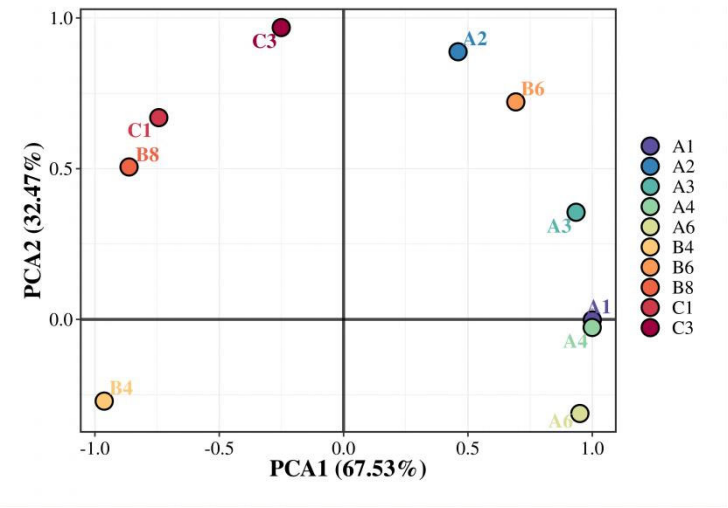

Figure 3. PCA analysis of three oak lignin volatilities

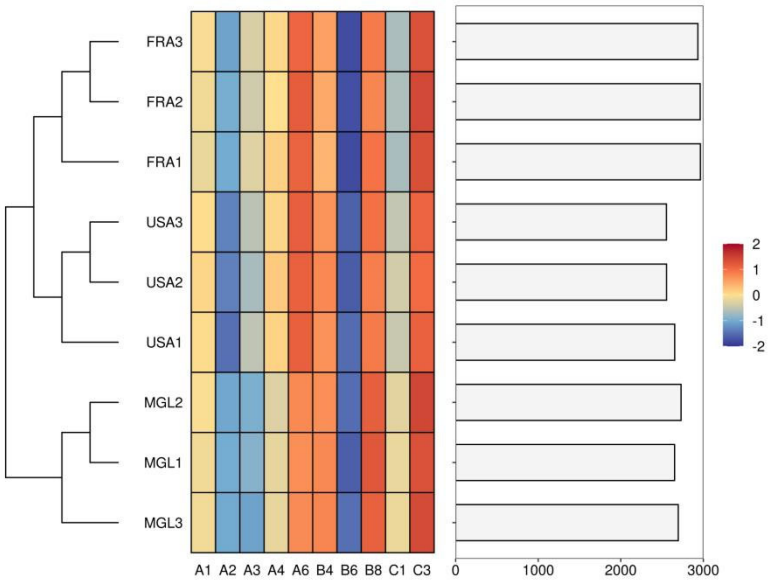

Figure 4. Thermal map analysis of the lignin volatiles of three oak species

By principal component analysis of 10 aroma substances in lignin of three oak species, the total interpretable variances of PCA1 and PCA2 were 67.53% and 32.47%, respectively. As can be seen from Figure 3, French oak lignin has a higher score in PCA1, and PCA1 mainly reflects the information of floral, baking, and woody aroma substances, indicating that the use of French oak lignin can bring rich aroma and have a more obvious baking aroma. The PCA1 negative axis of lignin in Mongolian oak has a high score, and the PCA1 negative axis mainly reflects the information of phenolic and ester aroma substances, indicating that Mongolian oak lignin has a strong floral and fruity aroma. American oak lignin has a high PCA2 negative semi-axis score, and this region mainly distributes sweet, woody, smoky and other aroma substances, indicating that the use of American oak lignin has a woody aroma.

Heat map analysis of the volatile components of three kinds of oak lignin was carried out to further explore the characteristics of aroma components. It can be seen from Figure 4 that the lignin aroma

of the three oak trees is significantly different. Oak aroma can be preliminarily divided into two categories according to different aroma substance content from top to bottom. The first type consists of French oak and American oak. The second type is composed of Mongolian oak. According to the heat map analysis, the lignin of French oak and American oak A4(eugenol) and A6(4-allyl-2, 6-dimethoxyphenol) were relatively high, which was consistent with the results of PCA. This shows that French and American oak lignin has a strong sweet, woody aroma. The content of B8(clove aldehyde), C1(ethyl caprylate) and C3(ethyl laurate) of Mongolian oak is high, and it has rich floral and fruity flavor characteristics, which is consistent with the results of PCA.

Based on PCA and heat map analysis, French oak lignin has a higher content of volatile phenols and other compounds with floral and baking aroma, with more baking smoke aroma. The content of lignin phenolic and ester compounds of Mongolian oak is relatively high, and it has more intense fruit and fruit aroma characteristics. On the other hand, the quality of American oak is more average, with complex and soft complex aroma characteristics.

4. CONCLUSIONS

The volatile aroma components and differences of lignin from American, French and Mongolian quercus were detected. The analysis of aroma characteristics showed that the lignin of Mongolian oak has more phenolic and ester compounds, so it can bring obvious floral and fruity aroma characteristics. American oak lignin and French oak lignin are relatively similar, both can provide floral, woody, sweet aroma. The French oak is more intense and the American oak is softer.

As a container material for aging, oak has been widely produced and applied in various regions of the world for hundreds of years. In the aging process, various oxidation, esterification, condensation and other physical and chemical reactions will occur, which will change the color of the wine, make the wine more full, and taste more soft and smooth. At the same time, the chemicals in the wood are also immersed in the wine as it ages, giving the wine a complex and varied aroma and unique taste. The aromatic components of phenolic and phenolic in oak mainly come from the depolymerization of lignin. The analysis of aroma components of oak lignin shows that the percentage of aroma components in oak lignin is consistent with the percentage of aroma components in oak. Compared with American and French oak lignin, Mongolian oak lignin has the highest phenolic compounds content and complex aroma, and has the possibility to replace American and French oak as oak barrel materials. There are few researches on aroma substances in oak lignin that are not conducive to wine aging and need to be improved. Therefore, these related studies are the future focus and need to be further explored.

REFERENCES

[1] Robert B C, Dane A J, Dawson-Andoh B, et al. Rapid classification of White Oak (Quercus alba) and Northern Red Oak (Quercus rubra) by using pyrolysis direct analysis in real time (DART) and time-of-flight mass spectrometry[J]. 2012, 95: 134-137.

[2] Zierer B, Schieberle P, Granvogl M. Aroma-Active Compounds in Bartlett Pears and Their Changes during the Manufacturing Process of Bartlett Pear Brandy. J Agric Food Chem. 2016 Dec 21;64(50):9515-9522.

[3] Martínez-Gil AM, Del Alamo-Sanza M, Gutiérrez Gamboa G, Moreno-Simunovic Y, Nevares I. Volatile composition and sensory characteristics of Carménère wines macerating with Colombian (Quercus humboldtii) oak chips compared to wines macerated with American (Q. alba) and European (Q. petraea) oak chips. Food Chem. 2018 Nov 15;266:90-100.

[4] Smailagić A, Stanković DM, Vranješ Đurić S, Veljović S, Dabić Zagorac D, Manojlović D, Natić M. Influence of extraction time, solvent and wood specie on experimentally aged spirits - A simple tool to differentiate wood species used in cooperage. Food Chem. 2021 Jun 1;346:128896.

[5] Chen YA, Zhou Y, Qin Y, Liu D, Zhao X. Evaluation of the action of Tween 20 non-ionic surfactant during enzymatic hydrolysis of lignocellulose: Pretreatment, hydrolysis conditions and lignin structure. Bioresour Technol. 2018 Dec;269:329-338.

[6] Zirbes M, Graßl T, Neuber R, Waldvogel SR. Peroxodicarbonate as a Green Oxidizer for the Selective Degradation of Kraft Lignin into Vanillin. Angew Chem Int Ed Engl. 2023 Mar 27;62(14):e202219217.

[7] Shao Y, Xia Q, Dong L, Liu X, Han X, Parker SF, Cheng Y, Daemen LL, Ramirez-Cuesta AJ, Yang S, Wang Y. Selective production of arenes via direct lignin upgrading over a niobium-based catalyst. Nat Commun. 2017 Jul 24;8:16104.

[8] Chio C, Sain M, Qin W. Lignin utilization: A review of lignin depolymerization from various aspects. Renewable & Sustainable Energy Reviews, 2019, 107: 232-249.

[9] Kamimura N, Sakamoto S, Mitsuda N, Masai E, Kajita S. Advances in microbial lignin degradation and its applications. Curr Opin Biotechnol. 2019 Apr;56:179-186.

[10] Zhu X, Zhou Z, Guo G, Li J, Yan H, Li F. Proteomics and metabolomics analysis of the lignin degradation mechanism of lignin-degrading fungus Aspergillus fumigatus G-13. Anal Methods. 2023 Feb 23;15(8):1062-1076.

[11] Zhu N, Zhu Y, Li B, Jin H, Dong Y. Increased enzyme activities and fungal degraders by Gloeophyllum trabeum inoculation improve lignocellulose degradation efficiency during manure-straw composting. Bioresour Technol. 2021 Oct;337:125427.

[12] Esakkimuthu ES, Ponnuchamy V, Mikuljan M, Schwarzkopf M, DeVallance D. Fungal enzyme degradation of lignin-PLA composites: Insights from experiments and molecular docking simulations. Heliyon. 2023 Dec 17;10(1):e23838.

[13] Lu X, Zheng X, Li X, Zhao J. Adsorption and mechanism of cellulase enzymes onto lignin isolated from corn stover pretreated with liquid hot water. Biotechnol Biofuels. 2016 Jun 3;9:118.

[14] Zhou G, Wei F, Qiu X, Xu X, Zhang J, Guo X. Influence of enhanced ultraviolet-B radiation during rice plant growth on rice straw decomposition with nitrogen deposition. Sci Rep. 2018 Sep 28;8(1):14512.

[15] Zhang S, Xiao J, Wang G, Chen G. Enzymatic hydrolysis of lignin by ligninolytic enzymes and analysis of the hydrolyzed lignin products. Bioresour Technol. 2020 May; 304: 122975.

[16] Sánchez-Gómez R, Del Alamo-Sanza M, Nevares I. Volatile composition of oak wood from different customised oxygenation wine barrels: Effect on red wine. Food Chem. 2020 Nov 1;329:127181.

[17] Hess D, Trott L. Gaschromatographische Bestimmung der höheren Ester in Weindestillat, Weinbrand, Branntwein und Wein. II [Determination of the ethyl esters of long chain fatty acids in wine distillate, brandy, and wine. II. (author's transl)]. Z Lebensm Unters Forsch. 1977 Aug 31;164(4):260-2. German.

[18] Ziyatdinova G, Antonova T, Davletshin R. Voltammetric Sensor Based on the Poly(p-aminobenzoic Acid) for the Simultaneous Quantification of Aromatic Aldehydes as Markers of Cognac and Brandy Quality. Sensors (Basel). 2023 Feb 20;23(4):2348.

[19] Ambindei WA, Kekel E, Ndasi NP, Jong NE. Optimization of the pulp ratio of Ananas comosus, Citrullus lanatus and Psidium guajava and fermentation time in the production of a "fruit brandy"[J]. Heliyon. 2023 Mar 26;9(4):e14910.

[20] Wang H, Wang X, Yan A, Liu Z, Ren J, Xu H, Sun L. Metabolomic and transcriptomic integrated analysis revealed the decrease of monoterpenes accumulation in table grapes during long time low temperature storage. Food Res Int. 2023 Dec;174(Pt 1):113601.

[21] Emanuelli F, Battilana J, Costantini L, Le Cunff L, Boursiquot JM, This P, Grando MS. A candidate gene association study on muscat flavor in grapevine (Vitis vinifera L.)[J]. BMC Plant Biol. 2010 Nov 9;10:241.

[22] Chira K, Teissedre PL. Extraction of oak volatiles and ellagitannins compounds and sensory profile of wine aged with French winewoods subjected to different toasting methods: behaviour during storage. Food Chem. 2013 Sep 1;140(1-2):168-77.

[23] Guerrero-Chanivet M, Ortega-Gavilán F, Bagur-González MG, Valcárcel-Muñoz MJ, García-Moreno MV, Guillén-Sánchez DA. Influence of Oak Species, Toasting Degree, and Aging Time on the Differentiation of Brandies Using a Chemometrics Approach Based on Phenolic Compound UHPLC Fingerprints. J Agric Food Chem. 2024 Jan 31;72(4):1959-1968.

[24] Dumitriu Gabur GD, Teodosiu C, Gabur I, Cotea VV, Peinado RA, López de Lerma N. Evaluation of Aroma Compounds in the Process of Wine Ageing with Oak Chips. Foods. 2019 Dec 10;8(12):662.

[25] Yu Y, Li L, Xue R, Wang C, Chen M, Ramos J, Zhang S, Sun B. Impact of Different Oak Chips' Aging on the Volatile Compounds and Sensory Characteristics of Vitis amurensis Wines. Foods. 2022 Apr 14;11(8):1126.

[26] Kyraleou M, Kallithraka S, Chira K, Tzanakouli E, Ligas I, Kotseridis Y. Differentiation of Wines Treated with Wood Chips Based on Their Phenolic Content, Volatile Composition, and Sensory Parameters. J Food Sci. 2015 Dec;80(12):C2701-10.

[27] Reboredo-Rodríguez P, González-Barreiro C, Rial Otero R, Cancho-Grande B, Simal-Gándara J. Effects of sugar concentration processes in grapes and wine aging on aroma compounds of sweet wines—a review. Crit Rev Food Sci Nutr. 2015;55(8):1053-73.

[28] Coldea TE, Socaciu C, Mudura E, Socaci SA, Ranga F, Pop CR, Vriesekoop F, Pasqualone A. Volatile and phenolic profiles of traditional Romanian apple brandy after rapid ageing with different wood chips. Food Chem. 2020 Aug 1;320:126643.

[29] Canuti V, Conversano M, Calzi ML, Heymann H, Matthews MA, Ebeler SE. Headspace solid-phase microextraction-gas chromatography-mass spectrometry for profiling free volatile compounds in Cabernet Sauvignon grapes and wines. J Chromatogr A. 2009 Apr 10;1216(15):3012-22.

[30] Mayr Marangon C, De Rosso M, Carraro R, Flamini R. Changes in volatile compounds of grape pomace distillate (Italian grappa) during one-year ageing in oak and cherry barrels. Food Chem. 2021 May 15;344:128658.

[31] Tarko T, Krankowski F, Duda-Chodak A. The Impact of Compounds Extracted from Wood on the Quality of Alcoholic Beverages. Molecules. 2023 Jan 7;28(2):620.

[32] Sánchez-Gómez R, Del Alamo-Sanza M, Nevares I. Volatile composition of oak wood from different customised oxygenation wine barrels: Effect on red wine. Food Chem. 2020 Nov 1;329:127-181.

[33] Caldeira I, Santos R, Ricardo-da-Silva JM, Anjos O, Mira H, Belchior AP, Canas S. Kinetics of odorant compounds in wine brandies aged in different systems. Food Chem. 2016 Nov 15;211:937-46.

[34] Hess D, Trott L. Gaschromatographische Bestimmung der höheren Ester in Weindestillat, Weinbrand, Branntwein und Wein. II [Determination of the ethyl esters of long chain fatty acids in wine distillate, brandy, and wine. II. (author's transl)]. Z Lebensm Unters Forsch. 1977 Aug 31;164(4):260-2.

[35] Tarko T, Krankowski F, Duda-Chodak A. The Impact of Compounds Extracted from Wood on the Quality of Alcoholic Beverages. Molecules. 2023 Jan 7;28(2):620.

[36] Castro MC, Bortoletto AM, Silvello GC, Alcarde AR. Lignin-derived phenolic compounds in cachaça aged in new barrels made from two oak species. Heliyon. 2020 Nov 24;6(11):e05586.

[37] Cadahía E, Fernández de Simón B, Jalocha J. Volatile compounds in Spanish, French, and American oak woods after natural seasoning and toasting. J Agric Food Chem. 2003 Sep 24;51(20):5923-32.

[38] Guittin C, Maçna F, Barreau A, Poitou X, Sablayrolles JM, Mouret JR, Farines V. The aromatic profile of wine distillates from Ugni blanc grape musts is influenced by the nitrogen nutrition (organic vs. inorganic) of Saccharomyces cerevisiae[J]. Food Microbiol. 2023 May;111:104193.

[39] Qian X, Jia F, Cai J, Shi Y, Duan C, Lan Y. Characterization and Evolution of Volatile Compounds of Cabernet Sauvignon Wines from Two Different Clones during Oak Barrel Aging. Foods. 2021 Dec 29;11(1):74.

[40] Fernández de Simón B, Cadahía E, del Alamo M, Nevares I. Effect of size, seasoning and toasting in the volatile compounds in toasted oak wood and in a red wine treated with them. Anal Chim Acta. 2010 Feb 15;660(1-2):211-20.