Characterization and Identification of Traditional Chinese Handmade Paper *via* Pyrolysis-Gas Chromatography-Mass Spectrometry

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The traditional method for the identification of paper from different origins relies on microscopy to observe the fiber morphology, which requires professional experience. In this paper, pyrolysis-gas chromatography-mass spectrometry was utilized to analyze five types of traditional Chinese handmade papers, *e.g.*, ramie paper, bamboo paper, bark fiber paper (mulberry paper and kozo paper), and Langdu paper. The results demonstrated that this method can be used to differentiate the four types of handmade papers. A high phenolic compound content was detected in bamboo paper; macromolecular triterpenoids, *e.g.*, β - amyrin, α - amyrin, and stigmastan- 3,5-diene were found in mulberry paper and kozo paper; while those compounds were not found in ramie paper. In particular, a large quantity of phytosterol compounds were found for the first time in Langdu paper. This not only can be used to differentiate Langdu paper from others, but also it helps to explain why Langdu paper can resist insect and mildew activities. Results of the study can provide experimental reference for characterization of traditional handmade Chinese papers via pyrolysis-gas chromatography-mass spectrometry.

Keywords: Py-GC/MS; Chinese handmade papers; Fiber resource; Biochemical compounds

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INTRODUCTION

Ramie paper fragments were found in a Western Han Dynasty tomb on the eastern outskirts of Xi'an Baqiao brick factory and are the earliest handmade papers found in China (Pan 1964). According to the *Book of the Later Han* (Cai Lun's biography), the raw materials for papermaking included ramie rags, fishing nets, and paper mulberry (kozo) during the Eastern Han Dynasty (Wang and Li 1980). Ramie was the most important raw material in the early stage of papermaking. During the Tang Dynasty, kozo, mulberry bark, and rattan bark were the primary sources for papermaking. The use of bamboo to make paper was primarily developed during the Song Dynasty. Although handmade paper was gradually replaced by the emergence of mechanized paper in the late Qing Dynasty, handmade paper is still in use in China and is greatly appreciated for its cultural value nowadays. In addition, there are some characteristic ethnic handmade papers in China. This is especially true for Langdu paper, which is made from a local wild plant (*Stellera chamaejasme*). This paper, from the Tibet Autonomous region in China, has anti-insect and antimicrobial properties and is one of the most characteristic ethnic handmade papers (Li *et al.* 2009).

Identifying traditional handmade paper is crucial in order to better preserve paper relics. The most commonly used method for identifying the raw materials of a paper is microscopy, which is done by observing the fiber morphological characteristics. The method requires a high level of experience as well as professional skills in order to accurately identify the paper. This is especially true when the paper is seriously aged, as it becomes more difficult to identify the origin of the paper according to the morphology of the fibers (Missori *et al.* 2004).

Microscopy is unable to identify the chemical composition of the plant raw materials in paper. Cellulose, hemicellulose, lignin, pectin, ash, and some specific triterpenoid compounds are the basic chemical components of the plant raw materials for papermaking. Of great importance are the triterpenoid compounds, which can be used as biochemical marker compounds for allowing their botanical origin to be defined with a high degree of certainty (Van Bergen *et al.* 1997). Ali and Ali (2013) studied new triterpenoids (α-amyrin acetate, β-amyrin-β-D-glucopyranoside, lup-12,20(29)-dien-3β-ol-26-oic acid, lanst-5,24-dien-3β-yl acetate) from mulberry bark (*Morus alba* L.). Cai (2013) reported that there were β-amyrin and α-amyrin in mulberry bark. Ma (2007) studied kozo bark (*Broussonetia papyrifera* Vent.), in which triterpenoid compounds were found. However, the characteristic biochemical compounds in ramie raw materials were not reported. Assuming that the paper fibers may still retain the specific biochemical constituents of their raw materials after the papermaking process, this study was conducted. It has been reported that pyrolysis-gas chromatography-mass spectrometry (Py-GC/MS) was successfully used for the differentiating East Asian handmade paper (kozo paper, mitsumata paper, and gampi paper) according to the biochemical markers of origin of the plant raw materials, since each paper has characteristic pyrolysis fingerprints (Avataneo and Sablier 2016; Han *et al.* 2016, 2017). In addition, Py-GC/MS was used to analyze the thermal cracking behaviors of cigarette papers made of wood pulp and hemp pulp (Zhang *et al.* 2017; Sun *et al.* 2008); and to estimate the age of documents through paper analyses (Keheyan 2008; Ortiz-Herrero *et al.* 2018). However, this method has not been used for the analysis of traditional Chinese handmade paper.

In this paper, traditional Chinese handmade papers made from ramie (*Boehmeria nivea* Gaud.), bamboo (*Phyllostachys pubescens* Mazel ex H. de Lehaie), mulberry bark (*Morus alba* L.), and kozo (paper mulberry) (*Broussonetia papyrifera* Vent.) as well as Langdu (*Stellera chamaejasme*) were studied *via* Py-GC/MS. Through the analysis of their chemical compounds, the biochemical marker compounds were determined. The results demonstrated that this method can be used to differentiate traditional Chinese handmade papers including ramie paper, bamboo paper, bark fiber paper, and Langdu paper. In addition, it is the first time that the phytosterol compounds, *e.g.*, (3β)-ergost-5-en-3-ol, (3β)-stigmasta-5,22-dien-3-ol acetate, β-sitosterol acetate, and β-sitosterol were found in Langdu paper, which revealed the reason that Langdu paper has the function of preventing insect and mildew activities.

EXPERIMENTAL

Materials

Four traditional Chinese handmade papers made from ramie (*Boehmeria nivea* Gaud.), bamboo (*Phyllostachys pubescens* Mazel ex H. de Lehaie), mulberry bark (*Morus alba* L.), and kozo (paper mulberry) (*Broussonetia papyrifera* Vent.) were investigated. Two kinds of ramie paper samples were from Pingyang county and Xiangfen county in the Shanxi province. One bamboo paper sample was from Fuyang county in the Zhejiang province, and another bamboo paper sample was from Ruijin city in Jiangxi province. Two kinds of mulberry bark paper were from Qianshan county in the Anhui province and Qianan city in Hebei province. Two kinds of kozo paper were from Zhenfeng county and Danzhai county in Guizhou province, respectively. Langdu paper samples (made from *Stellera chamaejasme*) were from the Tibet Autonomous region.

Experimental Methods and Parameters

The Py-GC/MS analysis was performed using a vertical micro furnace-type pyrolyzer (PY-3030D, Frontier Laboratories, Fukushima, Japan) directly connected to the injection port of a Shimadzu QP2010Ultra gas chromatograph mass spectrometer (Shimadzu, Kyoto, Japan). The size of the paper used in this work for the development of the analytical method was 2.5 by 2.5 mm. The sample was placed in a stainless steel sample cup, which was placed on top of the pyrolyzer at near ambient temperature. The sample cup was introduced into the furnace at 600 °C, and then the temperature program of the gas chromatograph oven was started. The Py-GC interface was held at a temperature of 320 °C. The chromatographic separation was carried out on an UA+-5 Frontier Lab (Fukushima, Japan) 5% dimethyl diphenyl polysiloxane column (30 m length, 0.25 mm inner diameter, and coated with a 0.25-μm thick film). The oven temperature was initially held at 40 °C for 3 min, and then increased by 5 °C min^{-1} to 325 °C, where it was held for 5 min. The total duration of the GC analysis was 65 min. The helium carrier gas was used in the linear velocity mode (1 mL min^{-1}). The injector was held at a temperature of 280 °C and used in split mode (1 to 10 ratio of the total flow). A scan range of 50 to 750 was used in the mass spectrometer, with an electron ionization of 70 eV. The interface was kept at a temperature of 280 °C, and the MS source was kept at a temperature of 200 °C. Identifications were achieved on the basis of EI mass spectra *via* the interpretation of the main fragmentations using the NIST14 and NIST14s MS library. The same amount of each sample was pyrolyzed three times, and the variability between the replicate programs was minimal. A blank run (sometimes two or three) was inserted between each pair of actual analyses to be able to rule out such influences.

RESULTS AND DISCUSSIONS

Four Typical Chinese Handmade Papers Analyzed *via* Py-GC/MS

Four typical Chinese handmade papers made from bamboo, ramie, mulberry bark, and kozo were analyzed *via* Py-GC/MS using the conditions previously described, the results of the same kind of handmade papers produced in two different places (in the Materials section) were basically the same. The chromatograms obtained from four types of handmade papers are shown in Fig. 1. The primary compounds identified are listed in Table 1. As shown in Fig. 1, the four types of papers had some peaks in common and some unique peaks, the peaks in common to all the specimens were primarily identified as the following compounds: furans (2,5-dimethyl-furan, 4-ethyl-3-methylenedihydrofuro [3,4-b] furan-2,6-dione, and 3-ethyl-2-hydroxy-2-cyclopenten-1-one), alcohols (1,3-propanediol, and 2,3,6-trimethylhept-6-en-1-ol), and carbohydrates (3,4-anhydro-d-galactosan, hydroxymethylfurfural, and 1,6-anhydro- beta -D-glucopyranose). According to Wang *et al.* (2012), the above-mentioned products are the pyrolysis products of cellulose and hemicellulose.

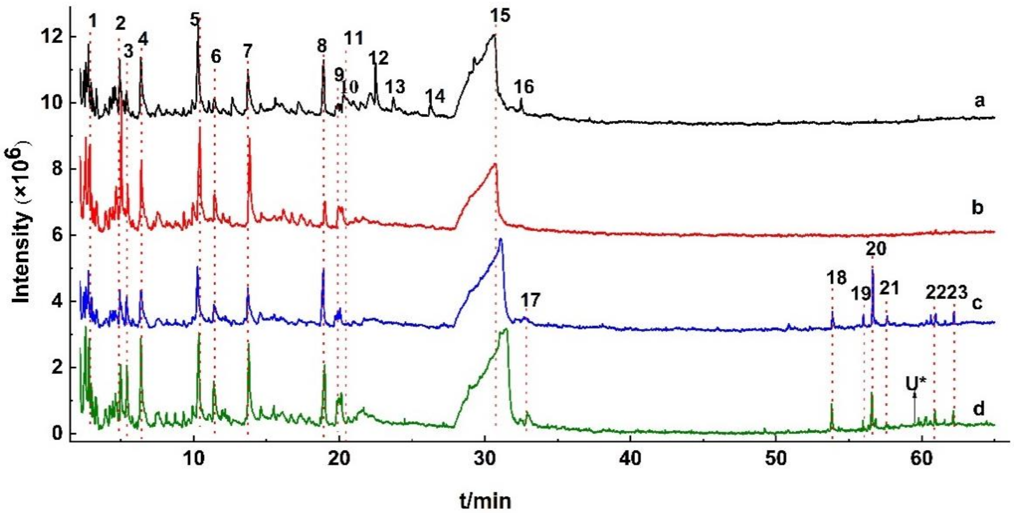

Fig. 1. The chromatograms (TIC) obtained *via* Py-GC/MS analysis of (a) bamboo, (b) ramie, (c) mulberry bark, and (d) kozo paper. Note: the peak numbers correspond to the numbers in Table 1

Table 1. List of Compounds for Bamboo, Ramie, Mulberry Bark, and Kozo Paper Analyzed *via* Py-GC/MS

No	Retain Time (t/min)	Main Ions (m/z)	Compounds Identified	Samples
1	2.86	(74)	Unknown	a, b, c, d
2	4.98	(58)	1,3-Propanediol	a, b, c, d
3	5.45	(54), 84	Unknown	a, b, c, d
4	6.37	54, 81, (96)	Furan, 2,5-dimethyl-	a, b, c, d
5	9.72	(55), 69, 98	Cyclohexanone	a, b, c, d
6	10.81	(57), 96, 110	4-Ethyl-3-methylenedihydrofuro[3,4-b]furan-2,6-dione	a, b, c, d
7	13.13	55, 69, (112)	Cyclotene	a, b, c, d
8	18.39	(70), 85, 123, 138	2,3,6-Trimethylhept-6-en-1-ol	a, b, c, d
9	19.38	57, 71, (97)	3,4-Anhydro-d-galactosan	a, b, c, d
10	19.88	91, (120)	(ethenyloxy)-Benzene	a
11	20.99	69, (97), 126	5-Hydroxymethylfurfural	a, b, c, d
12	21.92	77, 107, (135), 150	2-Methoxy-4-vinylphenol	a
13	23.15	96, 111, 139, (154)	2,6-Dimethoxy-phenol	a
14	25.70	77, 91, 131, 149, (164)	Phenol, 2-methoxy-4-(1-propenyl)-	a
15	30.26	(60), 73	β-D-Glucopyranose,1,6-anhydro-	a, b, c, d
16	31.91	91, 119, 179, (194)	2,6-Dimethoxy-4-(2-propenyl)-phenol	a
17	32.42	57, 60, (73)	1,6-Anhydro- α-d-galactofuranose	c, d
18	53.23	(57), 97, 125	1-Docosene	c, d
19	55.66	95, 189, 203, (218)	β-Amyrin	c, d
20	55.98	95, 122, 189, 203, (218)	α-Amyrin	c, d
U*	59.17	(93), 147, 189, 297	Unknown	d
21	56.52	147, 213, 255, (396)	Stigmastan-3,5-diene	c, d
22	60.87	95, 122, 189, 203, (218)	α-Amyrin	c, d
23	61.52	135, 189, 203, 365, (408)	Unknown	c, d
Note: samples are as follows: a - bamboo; b - ramie; c - mulberry bark; and d - kozo paper				

Cellulose is a linear homopolysaccharide composed of β-D-glucopyranoside units linked by (1→4) bonds (Keheyan 2008). The thermal decomposition of cellulose is the result of two competing reactions: a dehydration reaction which yields anhydrocellulose and the depolymerisation of cellulose which yields primarily levoglucosan and minor anhydrosugar components (Avataneo and Sablier 2016).

As shown in Fig. 1, the obvious differences between the four papers are a large quantity of phenolic compounds detected in bamboo paper, including 2-methoxy-4-vinylphenol (Fig. 1, labelled as 12), 2,6-dimethoxy-phenol (Fig. 1, labelled as 13), 2-methoxy-4-(1-propenyl)-phenol (Fig. 1, labelled as 14), and 2,6-dimethoxy-4-(2-propenyl)-phenol (Fig. 1, labelled as 16), which were not found in the other three samples. Those phenolic compounds are the pyrolysis products of lignin (Lima *et al.* 2015). It is known that paper primarily consists of cellulose, hemicellulose, and a small amount of lignin. Lignin is a stereoscopic network polymer that are composed of monomeric phenylpropanoid units, *i.e.*, p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) (Ralph *et al.* 2004), the chemical structures were shown in Fig. 2. Different plants have different lignin composition. The high lignin content in bamboo paper primarily occurred for two reasons: (i) the bamboo raw material has a higher lignin content compared to other raw materials, *e.g.*, the relative content of lignin in bamboo is about 30%, while the relative content of lignin in ramie, mulberry bark, and kozo is less than 15% (Pan 1979); (ii) the bamboo paper is formed with a high guaiacyl units (G) (Fig. 1, labelled as 12, 14) and syringyl (S) (Fig. 1, labelled as 13, 16) lignin content, which are difficult to delignified (Lima *et al.* 2015). Lignin compounds were not detected in other three kinds of handmade papers, because the lignin content of their raw materials is low. The results suggest that the fibers had been delignified completely in the traditional preparation for the papermaking process. This phenomenon can be used to differentiate bamboo paper from other paper types.

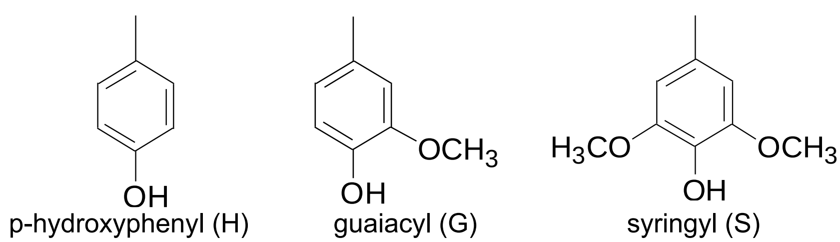

Fig. 2. There monomeric phenylpropanoid units of lignin

In the last region of the chromatograms, from a retention time of 55 min to 65 min, numerous triterpenoid compounds were found in the bark fiber papers (mulberry bark paper and kozo paper), but neither in ramie paper nor in bamboo paper. A series of compounds characterized by a base peak at 218 *m/z*, which corresponds to the beta-amyrin (Fig. 1, labelled as 19; the chemical structure is shown in Fig. 3a) and alpha-amyrin (Fig. 1, labelled as 20; the chemical structure is shown in Fig. 3b) were detected, which are in agreement with the studies by Cai (2013) and Ali and Ali (2013). In addition, stigmastan-3,5-diene (Fig. 1, labelled as 21; the chemical structure is shown in Fig. 3c), with the characteristic of a base peak at 396 *m/z*, was also found in mulberry bark paper and kozo paper. The above triterpenoid components are the biochemical marker compounds of mulberry bark and kozo fibers. These triterpenoid biochemical constituents allow their botanical origin to be defined with a high degree of certainty, often to the genus level (Van

Bergen *et al.* 1997). From the chromatograms, the compounds of mulberry bark paper and kozo paper obtained *via* Py-GC/MS were similar, since they both belong to the Moraceae family. However, there were still some differences between the mulberry bark paper and the kozo paper chromatograms. An unknown peak at a RT of 59.17 min (Fig. 1, compound U*) was only found in kozo paper, which could provide a clue in differentiating kozo paper from mulberry bark paper.

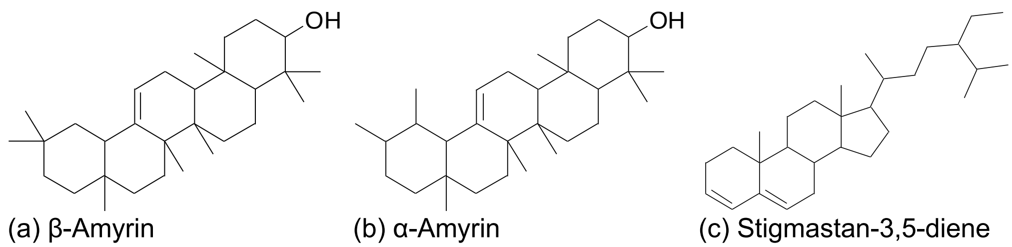

Fig. 3. The biochemical marker compounds of the pyrolysis products of the mulberry bark paper and kozo paper

Analyzing Langdu Paper *via* Py-GC/MS

The Langdu paper was analyzed using the Py-GC/MS conditions previously described. The chromatogram obtained is presented in Fig. 4, while the primary compounds identified are listed in Table 2. The pyrolysis products of Langdu paper are primary furans, carbohydrates, phenols, a large quantity of phytosterol compounds, and fatty acids and their derivatives. Similar to the above four traditional Chinese handmade papers, the furan-based structures (2,5-dimethyl-furan and 5-hydroxymethylfurfural), carbohydrates (3,4-anhydro-d-galactosan and 1,6-anhydro-beta-D-glucopyranose), and linear carbonyls (5-tetradecene, (E)-, 7-hexadecene, (Z)-, 5-octadecene, (E)-, 5-eicosene, (E)-, and 1-docosene) are the pyrolysis products of cellulose and hemicellulose. The detected pyrolysis products of lignin were 2-methoxy-4-vinylphenol (Fig. 4, labelled as 9) and 2,6-dimethoxy-phenol (Fig. 4, labelled as 10), which indicated that the lignin was not eliminated completely during the cooking process of the Langdu papermaking. Apart from stigmastan-3,5-diene (Fig. 4, labelled as 25; the chemical structure is shown in Fig. 3c), the biochemical marker compounds of Langdu paper that differed from mulberry bark paper and kozo paper were as follows: (3.beta.)-ergost-5-en-3-ol (Fig. 4, labeled as 24, chemical structure shown in Fig. 5a), (3.beta.)-stigmasta-5,22-dien-3-ol,acetate (Fig. 4, labelled as 26; the chemical structure shown in Fig. 5b), beta.-sitosterol acetate (Fig. 4, labelled as 27; the chemical structure shown in Fig. 5c), beta-sitosterol (Fig. 4, labelled as 29; the chemical structure shown in Fig. 5d), and stigmasta-3,5-dien-7-one (Fig. 4, labeled as 30; the chemical structure shown in Fig. 5e), which were detected the first time in Langdu paper. In addition, fatty acids and their derivatives were found in Langdu paper, which primarily included 9-hexadecenoic acid methyl ester, (Z)- (Fig. 4, labeled as 15), cyclopentaneundecanoic acid methyl ester (Fig. 4, labeled as 16), hexadecenoic acid, Z-11- (Fig. 4, labeled as 17), n-hexadecanoic acid (Fig. 4, labeled as 18), 9,12-octadecadienoic acid methyl ester (Fig. 4, labeled as 21), and palmitic acid vinyl ester (Fig. 4, labeled as 28).

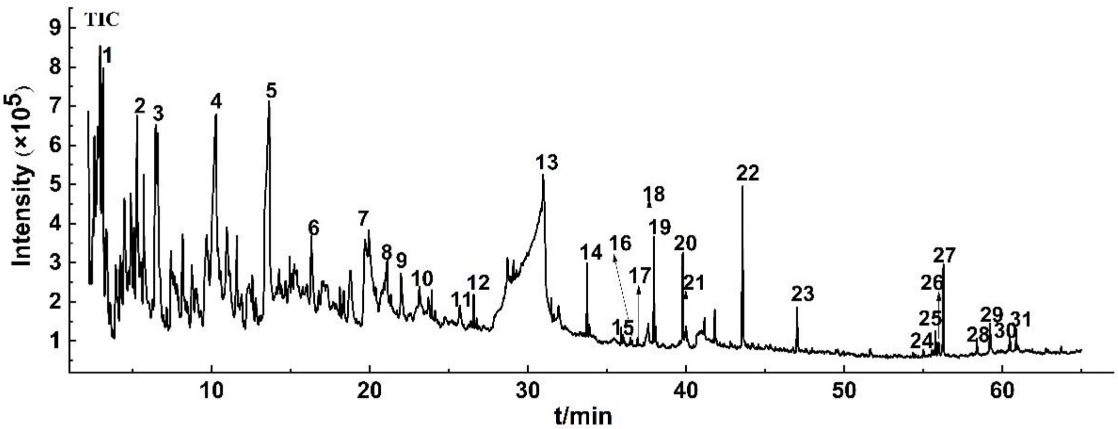

Fig. 4. Total ion current chromatogram obtained from the pyrolysis of Langdu paper

Table 2. List of Compounds for Langdu Paper Analyzed *via* Py-GC/MS

No.	RT (min)	Main Ions (m/z)	Attribution
1	3.15	57, (74)	dl-Homoserine
2	5.28	(58)	1,3-Propanediol
3	6.46	53, 82, (96)	2,5-dimethyl-Furan
4	10.27	(55), 69, 98	Cyclohexanone
5	13.64	55, 69, 83, (112)	3-Methylcyclopentane-1,2-dione
6	16.31	55, 83, 97, (126)	3-Ethyl-2-hydroxy-2-cyclopenten-1-one
7	19.70	57, 71, (97)	3,4-Anhydro-d-galactosan
8	19.94	69, (97), 126	5-Hydroxymethylfurfural
9	21.97	77, 107, (135), 150	2-Methoxy-4-vinylphenol
10	23.13	96, 111, 139, (154)	2,6-dimethoxy-Phenol
11	25.69	(77), 103, 125, 168	Unknown
12	26.57	(55), 97, 111, 125	5-Tetradecene, (E)-
13	30.96	(60), 73	β-D-Glucopyranose,1,6-anhydro-
14	33.75	(55), 97,111,125	7-Hexadecene, (Z)-
15	36.47	(55), 74, 87, 152	9-Hexadecenoic acid, methyl ester,(Z)-
16	36.59	55, (74), 87, 143	Cyclopentaneundecanoic acid, methyl
17	36.93	(55), 69, 83, 97	Hexadecenoic acid, Z-11-
18	37.61	(73), 129, 213, 256	n-Hexadecanoic acid
19	37.97	(55), 97, 111, 125	5-Octadecene,(E)-
20	39.80	55,83,97,111,125	5-Eicosene, (E)-
21	39.99	(55), 95, 123, 294	9,12-Octadecadienoic acid, methyl
22	43.57	55, 69, 83, 97, 111, 125	1-Docosene
23	47.04	(55), 97, 111, 125	1-Eicosanol
24	55.00	55, 105, 145, (382)	Ergost-5-en-3-ol, (3β)-
25	55.77	147, 213, 255, (396)	Stigmastan-3,5-diene
26	55.97	55, 81, 145, (394)	(3β)-Stigmasta-5,22-dien-3-ol,acetate
27	56.27	147, 213, 255, (396)	β-Sitosterol acetate
28	58.39	57, 83, 97, (239)	Palmitic acid vinyl ester
29	59.22	55, 105, 145, 329, (414)	β-Sitosterol
30	60.47	55, 91, (174), 410	Stigmasta-3,5-dien-7-one
31	60.87	57, 71, 123, (239)	Unknown

The biochemical marker compounds, *e.g.*, (3beta)-ergost-5-en-3-ol, beta-sitosterol, (3beta)-stigmasta-5,22-dien-3-ol, acetate, and beta-sitosterol acetate were found in Langdu paper, which belong to the phytosterol compound class and their derivatives. According to

Wu (2013), the phytosterol compound class, especially beta-sitosterol, have antimicrobial properties. A study by Ramadan *et al.* (2012) analyzed cold-pressed black cumin seed oil for their fatty acid profiles, phytosterol and tocopherol contents, antiradical properties, and inhibition of microbial growth. Roberts *et al.* (2003) reported that stigmastanol compounds have potential antimicrobial function. Additionally, fatty acids and their derivatives can also prevent the growth of fungi, *e.g.*, *Aspergillus niger*, *Penicillium chrysogenum*, and *Candida albicans*, which are the most common types of fungi found on paper materials (Kabara *et al.* 1977; Rai *et al.* 1990; Buňková *et al.* 2010). Hence the phytosterol compounds, fatty acids, and their derivatives detected in Langdu paper *via* Py-GC/MS are most likely the reason why Langdu paper is able to prevent insect and mildew activities. Furthermore, they are the biochemical marker compounds in Langdu paper made from *Stellera chamaejasme* fibers, which can be used for the identification of Langdu paper.

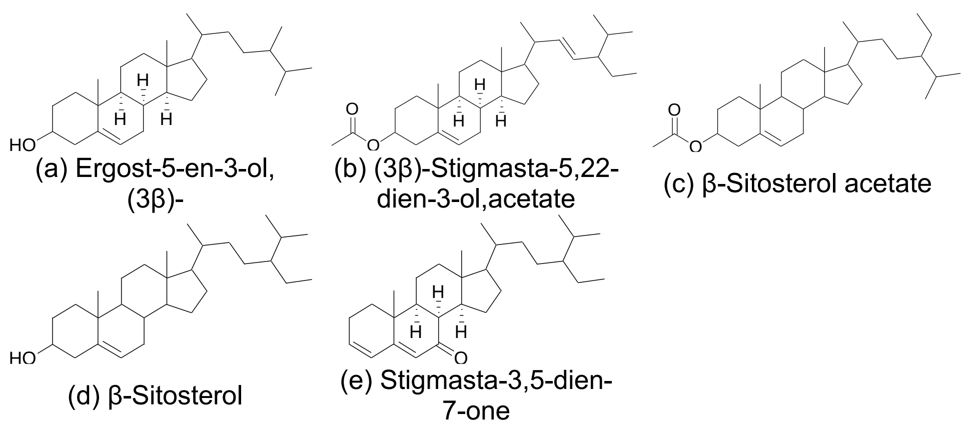

Fig. 5. The biochemical marker compounds of the pyrolysis products of Langdu paper

CONCLUSION

1. Pyrolysis-gas chromatography-mass spectrometry (Py-GC/MS) was utilized for the characterization of traditional Chinese handmade papers, *e.g.*, bamboo paper, ramie paper, mulberry bark paper, kozo paper, and Langdu paper. The results showed that Py-GC/MS is a practical method for detecting various biochemical markers, which can be used to differentiate traditional Chinese handmade papers including bamboo paper, ramie paper, bark fiber paper (mulberry bark paper and kozo paper), and Langdu paper.
2. Macromolecular triterpenoids, *e.g.*, beta-amyrin, alpha-amyrin, and stigmastan-3,5-diene, are the biochemical markers for bark fiber papers (mulberry bark paper and kozo paper); a high phenolic compound content is characteristic of bamboo paper; however, these compounds were not found in ramie paper; phytosterol compounds are the biochemical marker compounds of Langdu paper, which can be used to identify Langdu paper as well as explain why Langdu paper is able to prevent insect and mildew activities.
3. Through this study, Py-GC/MS was demonstrated to be an effective method for characterizing traditional Chinese handmade papers. The information obtained by Py-GC/MS not only can be used for the characterization of traditional Chinese

handmade papers from different origins, but also can help to understand the properties of the papers. This study could provide scientific supports for the conservation and restoration of traditional handmade paper.

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