



Characterization of chemical markers for the discrimination of East Asian handmade papers using pyrolysis, gas chromatography and mass spectrometry

Ung Bin Han

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**Characterization of chemical markers for the discrimination of
East Asian handmade papers using pyrolysis, gas
chromatography and mass spectrometry**

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Abstract

This PhD was conducted to explore a new methodology for East Asian handmade paper characterization and identification using pyrolysis, gas chromatography and mass spectrometry. It employs an easy sampling process requiring a small quantity of samples (in the μg range). After pyrolysis of East Asian handmade paper samples and separation by gas chromatography of characteristic metabolites of the fibers, a featured distribution patterns (presence plus intensity) of these metabolites eluting the defined region of interest (ROI) were observed to be characteristic for handmade papers of different material origins. The method utilizes these metabolites distribution patterns as markers to discriminate between different fiber origins of traditional East Asian handmade papers.

Firstly, the problems encountered in the investigation of handmade papers were presented such as the origin of papermaking, the inconsistency in the fiber identification results (sometimes gained by different scholars), the limits of microscopy in identifying fibers from similar species and the likely imprecision of the reference sample labeling. All these problems showed the necessity to explore a new method in order to (i) make precise fiber identification of handmade papers and (ii) to validate or confirm the identification results obtained by microscopy.

Then, modern reference East Asian handmade papers were firstly studied. The results revealed that different plant fibers used for papermaking have different marker distributions in the ROI, for instance, the *Moraceae* family with a featured distribution of terpene compounds and the *Thymelaeaceae* family with a featured distribution of stigmasta compounds. The fibers from the *Ma* group usually revealed few compounds in the ROI. This metabolites difference in the ROI was attested from the plant tissues with their similar distribution in handmade papers and raw plant fibers. Thus, the investigated methodology offers promise as a method of chemotaxonomy for unknown handmade paper fiber identification. With the examples of applications provided during the experimental work, the coupling of pyrolysis, gas chromatography and mass spectrometry (through the use of Py-GC/MS and Py-GC \times GC/MS) showed its ability to distinguish fibers from the same plant family (that may present similar microscopic features) and thus, can constitute an effective method for fiber identification as well as to validate the identification results of the microscopic observation.

In the present thesis, the features of GC \times GC, its benefits for cultural heritage applications and its help for the 1D data treatment were discussed. The tested Py-GC \times GC/MS methodology

has been for the first time proposed in the cultural heritage field and it reveals the potential to promote the research in this domain, enhancing our capacity to handle small quantities of complex samples while providing an exhaustive response on its composition.

Résumé

Cette thèse a été conduite afin d'explorer le potentiel d'une nouvelle méthodologie utilisant la pyrolyse, la chromatographie gazeuse et la spectrométrie de masse pour la caractérisation et l'identification des fibres papetières utilisées dans la fabrication des papiers asiatiques traditionnels à partir de la caractérisation des métabolites de ces fibres. Cette méthodologie utilise un processus d'échantillonnage facilité nécessitant une très petite quantité d'échantillons (de l'ordre de quelques dizaines de μg). Après la pyrolyse des échantillons de papiers et la séparation chromatographique des composés formés, des distributions caractéristiques pour les métabolites des fibres papetières (considérant leur présence et leur intensité) ont été observées dans une région définie comme région d'intérêt dans les chromatogrammes: ces distributions se sont révélées spécifiques pour la caractérisation des papiers fabriqués à partir de différents types de fibres et ont été utilisées pour distinguer l'origine des différentes fibres papetières couramment utilisées dans la fabrication de papiers asiatiques traditionnels.

Premièrement, les problèmes rencontrés dans l'étude des papiers faits à la main ont été présentés, comme l'origine de la fabrication du papier, l'incohérence de certains résultats dans l'identification des fibres (reportés dans différentes études scientifiques), les limites de la microscopie pour l'identification des fibres papetières d'origines botaniques similaires et les risques d'imprécision dans le référencement des échantillons. Tous ces problèmes montrent la nécessité d'explorer de nouvelles méthodes pour (1) améliorer la fiabilité de l'identification des fibres papetières des papiers asiatiques traditionnels, (2) valider et confirmer les résultats obtenus par l'analyse microscopique.

À cette fin, dans un premier temps, des papiers asiatiques de référence ont été étudiés. Les résultats expérimentaux ont montré que les différentes fibres papetières utilisées pour la fabrication des papiers étudiés montraient des différences dans les distributions de leurs marqueurs spécifiques : par exemple, les fibres d'origine de la famille *Moraceae* montrent une distribution caractéristiques de composés triterpéniques alors que les fibres d'origine de la famille *Thymelaeaceae* montrent une distribution caractéristiques de composés de type stigmastanes. De leur côté, les fibres des plantes appartenant au groupe *Ma* montrent peu de métabolites caractéristiques. Les différences observées dans la distribution de ces métabolites ont été attestées par la comparaison entre distributions obtenues à partir des fibres végétales et celles des papiers faits à la main attestant de l'origine commune de ces métabolites issus des tissus végétaux d'origine. Ainsi, la méthodologie étudiée se révèle prometteuse en tant que

méthode de chimiotaaxonomie pour l'identification des fibres inconnues de papiers faits à la main. Avec les exemples d'applications fournies au cours du travail expérimental, le couplage de la pyrolyse, de la chromatographie en phase gazeuse et de la spectrométrie de masse (avec l'utilisation de la Py-GC/MS et de la Py-GCxGC/MS) a montré sa capacité à distinguer les fibres d'une même famille (qui peuvent présenter des caractéristiques similaires en microscopie) et peut ainsi constituer une méthode efficace d'identification des fibres et de validation des résultats d'identification obtenus par l'observation microscopique.

Dans la présente thèse, les caractéristiques de la chromatographie gazeuse intégralement bidimensionnelle GCxGC, ses avantages pour les applications dans le domaine du patrimoine culturel et son apport potentiel pour le traitement des données 1D ont été discutées. La méthodologie testée basée sur l'utilisation de la Py-GCxGC/MS a été proposée pour la première fois dans le domaine du patrimoine culturel et révèle le potentiel de la recherche dans ce domaine, puisqu'elle renforce notre capacité à étudier de petites quantités d'échantillons complexes tout en apportant une réponse exhaustive sur leur composition.

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List of abbreviations

AD.....	after Jesus was born
AKDs.....	alkylketene dimers
AMDIS.....	Automated Mass Spectral Deconvolution and Identification System
ASA.....	alkenylsuccinic anhydride
BC.....	before Jesus was born
Ca.	approximately
CTMP	chemithermomechanical pulp
DP.....	degree of polymerization
EICs	extracted ion chromatograms
H-.....	p-hydroxyphenyl monomeric unit
HRMS.....	high resolution mass spectrometer
G-	guaiacyl unit
GC/MS	gas chromatography/mass spectrometry
GCxGC/MS.....	comprehensive two-dimensional gas chromatography/mass spectrometry
ISO.....	International Organization for Standardization
LCxLC.....	Comprehensive Two-Dimensional Liquid Chromatography
LG.....	levoglucosan
LM.....	light microcopy
MDGC.....	multidimensional gas chromatography
ML.....	middle lamella
MW.....	molecular weight
m/z.....	mass-to-charge ratio
NA.....	not attributed
NIST.....	National Institute of Standards and Technology (USA)
PCA.....	principal component analysis
PLM.....	polarized light microcopy
PLS-DA.....	partial least squares discriminant analysis
Py-GC/MS.....	pyrolysis- gas chromatography/mass spectrometry
Py-GCxGC/MS.....	pyrolysis-comprehensive two-dimensional gas chromatography/mass spectrometry
P1.....	primary wall
P2	secondary wall

qMS.....	quadrupole mass spectrometer
ROI.....	region of interest
RT.....	retention time
S-	syringyl unit
SIM.....	selected ion monitoring
SEM.....	scanning electron microscopy
SPME.....	solid-phase microextraction
STD.....	standard deviation
S1.....	outer layer of P2 (in Chapter 1); handmade paper samples (in chapter 3)
S2	middle layer of P2 (in Chapter 1); handmade paper samples (in chapter 3)
S3	inner layer (S3) of the P2 (in Chapter 1); handmade paper samples (in chapter 3)
TIC.....	Total Ion Chromatogram
TMAH.....	tetramethylammonium hydroxide
ToF	time-of-flight mass spectrometer
UNESCO.....	The United Nations Educational, Scientific and Cultural Organization
1D.....	gas chromatography/mass spectrometry
2D.....	comprehensive two-dimensional gas chromatography/mass spectrometry
¹ t _R	first dimension retention time
² t _R	second dimension retention time

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Chapter 1 Handmade paper: Combining social and science perspectives

The development of free discussion in Europe during this age of fermentation was enormously stimulated by the appearance of printed books. It was the introduction of **paper** from the East that made practicable the long latent method of printing.

It is scarcely too much to say that **paper** made the revival of Europe possible.

H. G. Wells

The Outline of History

Paper, the light, absorbent, strong and portable material is now indispensable to our daily life. It has a glorious history of more than two thousand years and has great influence on human civilization. Customs and cultures derived from papers have attracted tremendous interest from science and social science studies.

This thesis deals with the analytical characterization of East Asian handmade papers which the scientific analysis results (chapter 2 and chapter 3) will be mainly discussed. Before reporting the scientific analysis, it is not without importance, in fact, to know what exactly these papers are. And for that it is important to come back to the origin of paper. Moreover, the theme of paper, in a general sense, generates great social and cultural significance. So, as an introduction, the discussion of its social and cultural parts shall bring a broader interest for readers of various disciplines.

As a result, chapter 1 organized to present handmade papers with combination of its social and science perspective: It starts with the definition of paper followed by a review of the paper origin as well as the chronological and geographical material utilization in papermaking. The history and development for scientific analysis of handmade paper has been reviewed followed by an introduction of its chemical composition.

As for papers of medieval China (5th to 10th century), morphological study drew attention of scholars, but the scientific analysis of the constituents of paper as well as the manufacturing process of paper sheets are not many. More over, the most ancient remains about papermaking are very rare and their study is incomplete and unsatisfactory.

As for hand papermaking today, the undeniable fact is that the number of craft mills as well as the craftsmen has greatly declined. The aesthetic value of hand papermaking remains unchanged and poses an even greater value in the background of industrialization.

1.1. The definition of paper

From a starting point, it is interesting and also essential to present the basic question: What is paper? It is a question too simple to answer but difficult to give a precise description to reveal its essence. As an important term both in culture, science and engineering, it has been defined by various dictionaries, standards and paper historians.

1.1.1 *Cihai* (dictionary)

In *Cihai*, a large-scale dictionary and encyclopedia of Standard Mandarin Chinese¹, paper is defined as:

Paper is a sheet-like fiber product used for writing, printing, painting or packaging. It is generally made of plant fibers that undergo the processes of pulping, sheet formation (on a mould), initial dehydration and then processed by pressing and drying.

1.1.2 ISO 4046-3:2016 (standard)

From the International Organization for Standardization, in ISO 4046-3:2016, part 3, papermaking terminology, paper is defined as:

Paper, generic term for a range of materials in the form of a coherent sheet or web, excluding sheets or laps of pulp as commonly understood for paper making or dissolving purposes and non-woven products, made by deposition of vegetable, mineral, animal or synthetic fibers, or their mixtures, from a fluid suspension onto a suitable forming device, with or without the addition of other substances.

1.1.3 Dard Hunter (paper historians)

The American paper historian Dard Hunter cited Noah Webster's definition of paper as a substance made in the form of thin sheets or leaves from rags, straw, bark, wood, or other fibrous material for various uses. But he also gave opinion on the definition of 'true paper' [1]:

¹ The Literal meaning of *Cihai* (辞海) is "sea of words". The definition for paper (纸) in Chinese is provided in Annex 1.

To be classed as true paper the thin sheets must be made from fiber that has been macerated until each individual filament is a separate unit; the fibers intermixed with water, and by the use of a sieve-like screen, the fibers lifted from the water in the form of a thin stratum, the water draining through the small openings of the screen, leaving a sheet of matted fiber upon the screen's surface. This thin layer of intertwined fiber is paper.

1.1.4 Pan Jixing (paper historians)

The Chinese paper historian Pan Jixing also gave a definition for paper after considering paper definitions from various sources²:

Paper, traditionally refers to the sheet-like product made of raw plant fiber that go through mechanical and chemical treatment to disperse the fiber, put up in the water to form the pulp, drain through a mould to form a wet thin layer and dried to form hydrogen bonds that bears strength. It is used for writing, printing, packaging etc. [2].

1.1.5 Summary

Although the definitions of paper from section 1.1.1 to section 1.1.4 have minor differences³, the essence of paper concerning the raw material, making process and usages were all mentioned. Combining all these definitions, for paper, especially traditional handmade paper, the following elements must be present to be defined as paper:

- It must use plant fibers as the papermaking materials
- It must go through processes such as fiber preparing, pulping and sheet formation
- It must present like a thin layer of intertwined fiber with the usage of writing, printing, packaging etc.

The definition is a key point for the following discussions especially regarding its origin. The secret as well as the aesthetic of paper also contains in its definition that will unfurled in the following parts. Moreover, one can easily rule out the 'paperlike' craft products such as tapa (bark cloth), felt and papyrus. In the following, unless specified, the paper refers to handmade paper and applies the definition discussed above.

² The original Chinese text is provided in Annex 2.

³ For example, the mineral, animal or synthetic fibers were mentioned in ISO 4046-3:2016 with consideration to various modern papers.

1.2 The origin of paper

Though, as Pliny truly says, the remembrance of past events depends upon paper (papyrus) with the irony of fate, paper has failed to record its own origin, and much of the early history of the papermaking is left to conjecture.

The Encyclopedia Americana

As for papers of medieval China, morphological study drew attention of scholars, but the scientific analysis of the constituents of paper as well as the manufacturing process of paper sheets are not many. I will add that the most ancient remains about papermaking are very rare and their study is incomplete and unsatisfactory.

Jean-Pierre Drège

‘Conjecture’ and ‘hypothesis’ are words suitable to describe the current situation for the topic of paper origin.

The author

The invention of paper is undoubtedly one of the most important inventions of mankind associated with human culture and civilization. It is commonly acknowledged that papermaking has Asiatic origin: papermaking was first developed and used in China and later diffused to the other part of the world. Paper was the most advanced carrier of information at a time which made the transfer of knowledge, education, art, and information to a larger part of society became possible.

Commonly, there are two ways to study the origin of papermaking: (i) Study and analyze the historical records; (ii) Analyze the unearthed samples (museum collections). The historical records, mostly, were written in ancient Chinese and the unearthed samples were almost all in the domain of Chinese territory. In other words, the Chinese scholars have more material accessibility to study the origin of papermaking. However, there are some shortcomings in the study of paper origin through these years:

- Almost all the articles were written in Chinese and published in Chinese journals that prevent easy access for scholars in other countries
- Divergent opinions and debates last for over 40 years for the topic of paper origin in Chinese academic circles

In view of its importance and the current situation, the paper origin from the Chinese records, archaeological discoveries is reviewed. Further interpretations from the collected data were also provided for open discussion.

1.2.1 Stories of conjecture from ancient Chinese records

This section is organized in the following order:

- The making of paper utilizes plant fibers that come from the plant which means a sufficient knowledge for plant cultivation is a prerequisite for the fiber treatment for utilization. Section 1.2.1.1 is written in this sense of plant cultivation
- The plant fiber has to undergo several treatments before it can be utilized (whether for textile or for paper) which is the focus of fiber treatment in section 1.2.1.1 and section 1.2.1.2
- Also, if a new thing appears, simultaneously and most probably, there would be a word or sign for its correspondence. It has been discussed for paper in section 1.2.1.3 and section 1.2.1.4
- The description of the use of paper before 105 AD and the worldwide story of Cai Lun is discussed in section 1.2.1.5 and section 1.2.1.6
- A sketching of the time table for Chinese history is provided in Fig.1.2.1

1.2.1.1 Poetic description of fiber treatment and silkworm breeding

The *Classic of Poetry* is the oldest existing collection of Chinese poetry comprising 305 works (including folk songs, odes, and sacrificial psalms) dating from the 11th to 7th century BC⁴. A large number of plant names are mentioned in the poems and several are selected below⁵.

(1) Guofeng·Odes of Wei·Among their ten acres

Among their ten acres, the mulberry-planters stand idly about. Come, (says one to another), I will go away with you.

Beyond those ten acres, the mulberry-planters move idly about. Come, (says one to another), I will go away with you.

(2) Guofeng·Odes of Bin·July

In the seventh month, the Fire Star passes the meridian. In the ninth month, clothes are given out. With the spring days the warmth begins, and the oriole utters its song. The young women take their deep baskets, and go along the small paths, looking for the tender (leaves of the) mulberry trees. As the spring days lengthen out, they gather in crowds the white southernwood. That young lady's heart is wounded with sadness, for she will (soon) be going with one of our princesses as his wife.

In the seventh month, the Fire Star passes the meridian. In the eighth month are the sedges and reeds. In the silkworm month they strip the mulberry branches of their leaves, and take their axes and hatchets, to lop off those that are distant and high; only stripping the young trees of their leaves. In the seventh month, the shrike is heard; in the eighth month, they begin their spinning. They make dark fabrics and yellow. Our red manufacture is very brilliant, it is for the lower robes of our young princes.

.....

⁴ The *Classic of Poetry* is one of the 'Five Classics' traditionally said to have been compiled by Confucius (Ca. 551-Ca. 479 BC) and was frequently quoted in his *Analects*. It has been studied and memorized in China and neighboring countries over two millennia. The *Classic of Poetry* was destroyed (around 213 BC) by the Emperor Qin Shi Huang's edict for the destruction of classic works and was fortunately recovered decades later.

⁵ The Chinese texts for record (1), (2), (3) and (4) are provided in Annex 3, Annex 4, Annex 5 and Annex 6, respectively.

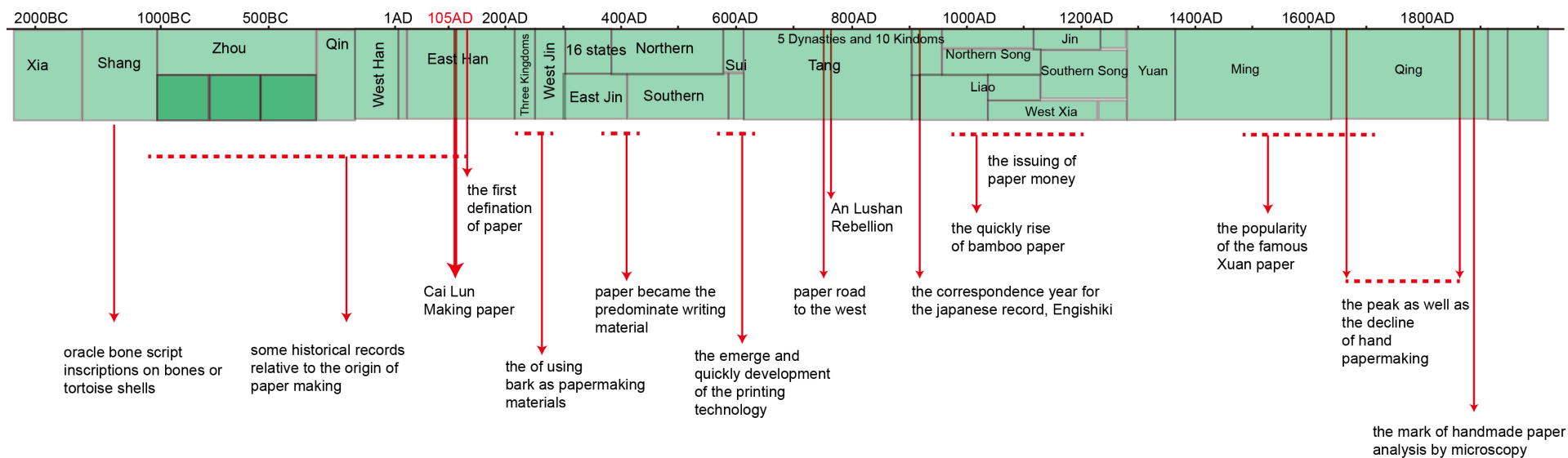


Figure 1.2.1 The time table for Chinese history and some relative contents mentioned in this thesis.

(3) Guofeng·Odes of Wei·Meng

A simple-looking lad you were, carrying cloth to exchange it for silk. (But) you came not so to purchase silk, you came to make proposals to me. I convoyed you through the Qi, as far as Dunqiu. 'It is not I who would protract the time, but you have had no good go-between' (I said). I pray you be not angry, and let autumn be the time.

.....

Before the mulberry tree has shed its leaves, how rich and glossy are they! Ah! thou dove, Eat not its fruit (to excess). Ah! thou young lady, Seek no licentious pleasure with a gentleman. When a gentleman indulges in such pleasure, something may still be said for him. When a lady does so, nothing can be said for her.

When the mulberry tree sheds its leaves, they fall yellow on the ground. Since I went with you, three years have I eaten of your poverty. And (now) the full waters of the Qi, Wet the curtains of my carriage. There has been no difference in me, but you have been doubled in your ways. It is you, sir, who transgress the right, thus changeable in your conduct.

.....

(4) Guofeng·Odes of Chen·the moat at the east gate

The moat at the east gate, is fit to steep hemp in. That beautiful, virtuous, lady, can respond to you in songs.

The moat at the east gate, is fit to steep the boehmeria (ramie) in. That beautiful, virtuous, lady, can respond to you in discourse.

The moat at the east gate, is fit to steep the rope-rush in. That beautiful, virtuous lady, can respond to you in conversation.

It has been estimated that over one hundred and thirty different kinds of plant names had been mentioned of which, the mulberry tree, was by far the most frequent one that appears in twenty poems (followed by the major crop millet mentioned in thirteen poems, see Fig.1.2.2) [3]. The mulberry tree is among the very few trees first brought into cultivation in ancient China. As can be seen from the selected odes, the mass plantation of mulberry trees (Ode 1), the picking of mulberry for silkworm breeding (Ode 2) and the trade between cloths with silk (Ode 3) has been mentioned. It is reasonable (also indicated by the second odes) to believe that there were already large areas of mulberry tree plantation in existence. Another similar

species, paper mulberry (which later become one main bark material for papermaking) is mentioned 2 times in the poems. Paper mulberry is closely related to mulberry tree that its leaves can be used as a substitute for the latter's in silkworm breeding.

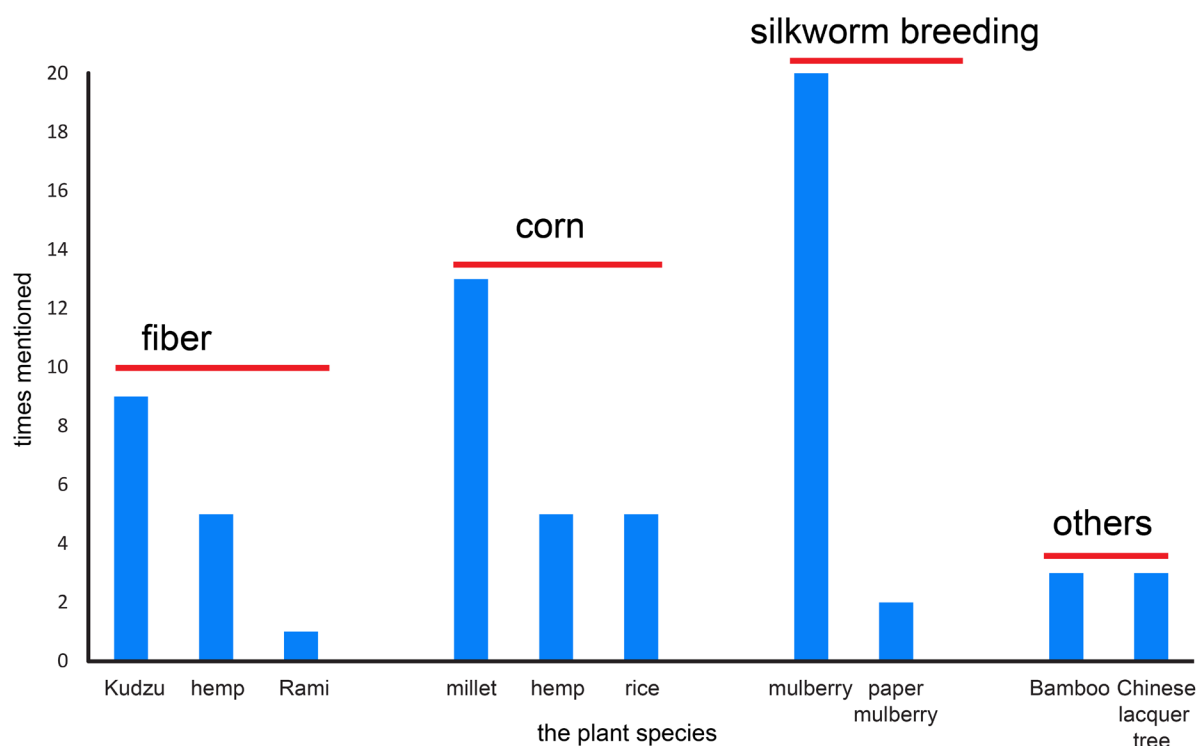


Figure 1.2.2 The times the plant mentioned in the *Classic of Poetry* according to their use.

The selected odes also pointed out that silk manufacture had been a mature and commonly practiced handcraft in North China. Besides being an aristocratic material, silk also served as a unique writing material preceding paper. The way of making silk from the silkworm cocoons (boiling silkworm with wood ash and silk reeling) bears high similarity for the manufacturing of paper. Another evidence sourced from the ancient Chinese characters in the oracle inscriptions for mulberry, silkworm, silk and silk manuscripts (Fig.1.2.3) also indicates that the silk manufacturing had become a common practice.

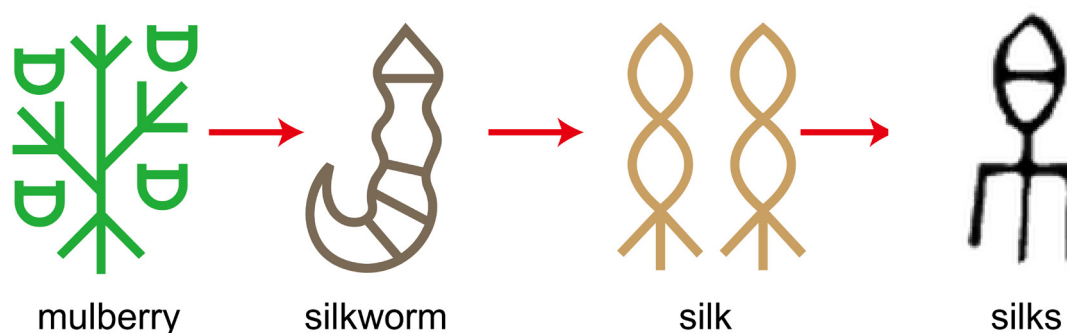


Figure 1.2.3 The oracle characters for mulberry, silkworm, silk and silks.

Another fibrous material hemp (Chinese name, *Ma*, 麻) has been mentioned in 5 poems. It was generally classified as a cereal until the 1st or 2nd century BC when many new crops were introduced. As has been indicated in Ode 2, hemp fibers had been used for making clothes. The Ode 4 showed that, there was mature technique for retting⁶ the hemp and ramie fibers in the Spring and Autumn period (771 to 476 BC). After being retting, hemp and ramie can produce relatively long and wearable fibers and become the main raw materials for clothing.

In the *Classic of Poetry*, the basic procedure for the fibrous material treatment such as retting was mentioned. The ancestors have mastered the practice by submerging bundles of stalks in the water, (the water) penetrates to the central stalk portion, swells the inner cells, bursts the outermost layer, thus increasing absorption of both moisture and decay-producing bacteria. From a biological point, the retting process employed the action of micro-organisms and moisture on plants to dissolve or rot away much of the cellular tissues and pectin surrounding bast-fiber bundles to facilitate separation of the fiber from the stem.

From the description of the *Classic of Poetry* the knowledge can be gained that during the 11th to 7th century BC:

- Mass plantation of mulberry trees appeared
- The silkworm breeding and silk manufacture has become a mature technique
- The practice of silk making (boiling the cocoon with wood ash) may have indications for the origin of papermaking
- Elements for papermaking: raw material (plant fiber, water), basic fiber pretreatment (retting) has been mentioned

In the *Classic of Poetry* there is no description of papermaking scenes or processes. So the usefulness of the above literary and historical sources which cite mulberry cultivation and sericulture to explain the invention of paper is open to discussion. Although further investigation needs to be conducted, some scholars (e.g. Yang Juzhong) have tentatively raised the opinion that the silkworm breeding and the silk making directly lead to the first stage paper making: cocoon paper (茧絮纸).

⁶ Ramie is mentioned in only 1 poem (Ode 4). Other plant that later involved in the papermaking include rice (mentioned in 5 poems), bamboo (mentioned in 3 poems) see Fig.1.2.2.

1.2.1.2 Pound wadding in the water

The fiber pretreatment process of retting has been practiced as described above. After the retting, the fiber needs to be further processed for practical use. One concern is the description of pounding wadding in the water (Some scholars believe it may directly or more closely relate to the origin of papermaking). Here three selected historical descriptions are provided⁷.

(5) Yu Jue Shu

Wu Zixu (after become a fugitive) arrived in Li Yang. He saw a woman pound wadding in the water. Zixu asked: can you give me something to eat. The woman replied with yes. She immediately opened the basket that contains food. Zixu eat all the food and prepared to leave. He told the women to cover the basket in order not to expose his whereabouts. The woman replied with yes. After several steps away, Zixu turned around and saw her commit suicide by jumping into the river (to show that his whereabouts will not be leaked).

(6) Seeds for a giant gourd in the book Zhuangzi

Zhuangzi said: "... Among the Song people there were some who were good at making a salve that prevented the chapping of the hands. They used it for generations in their family business for bleaching silk. A traveler heard of it, and offered to buy the formula for one hundred pieces of gold. The people of the clan go together and discussed it. One said: "We've been bleaching silk for generations, never making more than several pieces of gold. Now, in one morning we can make a sale of the technique for one hundred pieces. I say we go for it." The traveler took it, and told the king of Wu about it. There was trouble with Yue, and the king of Wu made him a commander. It was winter, and there was a naval battle with Yue, in which they routed the Yue. So the king took a piece of the conquered land and enfeoffed him. Now, the ability to prevent the chapping of the hands is one, but one fellow was able to turn this into a fiefdom, while another could not get out of bleaching silk.

(7) Records of the Grand Historian, the marquis of Han Xin

Xin fished outside of the city where he witnessed many women washing rags in the water. A woman saw the hungry Xin and gave him some food. These women continuously worked for several tens of days (as well as gave him food). Xin was happy, he said to the women. "I will repay the obligation". The woman was angry and

⁷ The corresponding Chinese text for record (5) is provided in Annex 7, the complete stories for record (6) and (7) are provided in Annex 8 and Annex 9, respectively.

said: you being a man but cannot support yourself, I was pity on you to give you food,
how do I expect you repay the obligation?

From the above descriptions, it can be seen that: (i) Record 5 told of Wu Zixu saw a women ‘pound wadding in the water’ when he was on his way escaping from Chu state to Wu state; (ii) Record 6 told of Zhuang Zi cited a man whose work is ‘bleaching silk’; (iii) Record 7 told of the marquis Han Xin saw women ‘washing rags’ in the water.

The ‘pound wadding in the water’, ‘bleaching silk’ and ‘washing rags’ are all modern translation of ancient Chinese characters in the old context. As for the things being ‘pounded’, ‘bleached’ or ‘washed’, scholars have different interpretations to be silk, hemp or even to be paper mulberry and consequently, different interpretations have different technical evolution pathways that leading to the origin of papermaking: it is quite different with silk bleaching and rags washing which perhaps led to the first attempts of papermaking. But one thing for sure is, after the retting of the fiber, further treatment such as pounding, washing or bleaching has been practiced. In a sense, pounding ‘thing’ in the water bears high similarity to the hand pulping process of plant fibers.

1.2.1.3 Search for the earliest Chinese character for paper

If a thing or product becomes circulate in the society, possibility goes that a word or character shall emerge to describe it. If there is a Chinese character to describe ‘paper’, it means ‘paper’ has become a common product (at last) by the time it emerged. So, the search for the earliest Chinese character for paper is one way to explore its origin.

During the 1975-1976, lots of bamboo slips were excavated in Shuihudi (Hubei province, China) which was called Shuihudi Qin bamboo texts. Dr. Donald Harper first discovered there was a Chinese character for ‘paper’ in a Shuihudi Qin bamboo texts (ca. 300 BC) when he translating a medical remedy. Dr. Donald Harper thought this remedy is significant for the history of papermaking because it has the earliest occurrence of the word for ‘paper’ (zhi, used verbally in the text) and a step more, he even provides a rudimentary description of the papermaking process [4].

(8) ‘paper’ on the Shuihudi Qin bamboo slip⁸

Without cause a person’s hair lifts up like bugs and chin or cheek whiskers. This is the qi of the Anthropophagous Bug inhabiting it. Then boil grass shoes and use them to paper (the hair), and then it will stop.

Dr. Donald Harper told this discovery to Dr. Qian Cunxun (钱存训, Tsien, Tsuen-hsuei) while Qian paid great attention to this discovery to regard it as the first Chinese character of paper [5]. Mr. Zheng Yefu also use this character as one of the evidences for the emergence of the character of paper before 105AD. He thought that the Chinese characters on this bamboo slip revealed the origin of papermaking from boil grass shoes [6]. One point to state is, in a letter written to Qian, this character has been lately explained (modified) as ‘抵’ (to throw) instead of ‘纸’ (paper, to cover) which the bamboo slip character interpretation scholars thought this explanation (to throw) is (comparatively) more reasonable than to cover.

Another report is from scholar Chen Zhi. On a late West Han dynasty bamboo slip unearthed in Juyan (1 century BC), there is a sentence “官写氏” (translated on the literal meaning: official, write, name). Chen thought that ‘氏’ is the abbreviation for ‘纸’ (paper). So the meaning of ‘官写氏’ could be interpreted as ‘official wrote on paper’. Juyan is in the northwest on the boarder when West Han had war with Xiongnu (匈奴). If the sentence “official wrote on paper” appeared at Juyan (on the boarder), it can be inferred that in the central China the use of paper would be even earlier. Pan thought the paper emerged around 2 century BC (use Chen’s hypothesis as one of the supports).

However, the reading of the Chinese character as zhi (paper) in the Shuihudi manuscript is not accepted by the whole community of scholars. And the abbreviation for paper raised by scholar Chen is an only example without further confirmation. Although these examples are often mentioned in regarding the paper origin, further evidence shall be searched and analyzed for its validation.

1.2.1.4 The first definition of paper

In section 1.2.1.3 the attempt to find and interpret the early character for paper was described. Nevertheless, the first people regarded to give the definition of paper was Xu Shen (许慎, ca. 58-147AD) in the first Chinese dictionary Shuowen Jiezi (说文解字, 121AD) with only nine characters to define what paper is: Paper, wadding on the felt, has the meaning of silk and pronounce as zhi (纸, 絮一箔也。从系。氏聲). Another dictionary, Tongsuwen (通俗文,

⁸ The Chinese context is provided in Annex 10.

ca. 180AD) uses four characters to define paper: square wadding is paper (方絮曰紙). From the definition of paper, two elements can be inferred to form a paper:

- 絮 (wadding, the raw material) from plant fiber or may from silk as from different interpretations
- 簾 (square felt, the tools)

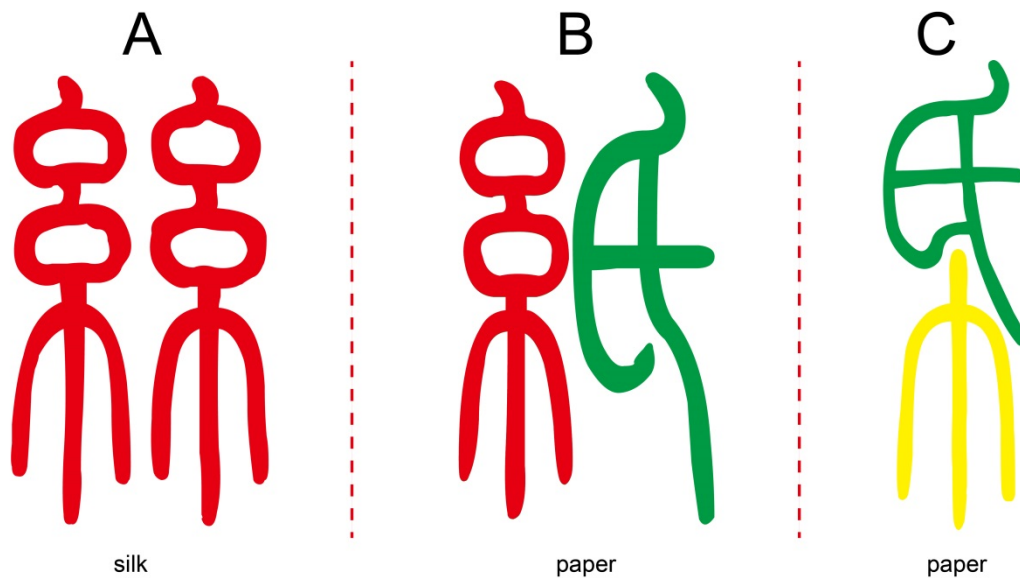


Figure 1.2.4 The Chinese character for silk and paper.

As can be seen from the character for paper (Fig.1.2.4B.), the red part may be representative for the raw materials (fibers hanging on a rock) and the green part may be representative for the tools (felt) to make the paper. Scholars like Zhang Yi (190-254 AD) thought that the red part in paper (Fig.1.2.4B) represents silk (Fig.1.2.4A) which means in the ancient time (before Cai Lun invented paper), the silk was called ‘paper’⁹. Cai Lun invented papermaking using plant fibers that the paper should use parts representative of cloth (yellow part in Fig.1.2.4C) instead of silk. The two characters for paper (Fig.1.2.4B and Fig.1.2.4C) were used simultaneously between 5-10 century and the latter (fig.1.2.4C) was abandoned.

⁹ The word zhi (紙) may first designated silk pieces. It was gradually used to designate paper as is learned from the etymology of the Chinese character as well as from written sources.

1.2.1.5 Historical records for paper use before 105 AD

When talking about the origin of papermaking, some records which mentioned the use of paper before 105 AD were often discussed. Some were selected below¹⁰.

(9) San Fu Gu Shi:

On the fourth year of Taishi (Emperor Wu, 93 BC). Emperor Wu (157-87 BC) got sick. Prince Wei (has big nose) came to see Emperor Wu. Jiang Chong said (to Prince Wei): Emperor Wu do not like (your) big nose, you should use **paper** to cover your nose when you enter to see him. (Prince Wei did as was told) Emperor Wu was angry.

(10) Book of Han:

(12BC): Wu discovered there were two small pills in the box, write on the **Haotie**: drink this medicine (to commit suicide).

(11) Feng Su Tong Yi

Emperor Guangwu (5BC-57AD) use the carriage to go to the new capital Luo Yang. There are two thousand carriages that were packed with silk, bamboo slip and **paper**.

(12) Book of the Later Han:

Emperor Guangwu (5 BC- 57AD) set the official in charge of **paper**, pen, ink etc.

The first year of Jianchu (76AD): the scholar was given bamboo slip and **paper** for use.

102AD, the queen doesn't like jewelry, she only demands the supply of **paper** and ink.

From the records, it can be seen that

- In Record 9, paper was mentioned to use to cover the (big) nose of Prince Wei and was regarded by Dr. Qian Cunxun to be the record of the earliest usage of paper (in 93 BC) [7]. However, to use Record 9 as the earliest use of paper should be very cautious. The book Sanfugushi was re-edited in Qing Dynasty (the original edition was not quite clear) (1644-1912 AD) and was highly similar to a story about King Huai of Chu (楚怀王) in Zhan Guo Ce (战国策). (See Annex 12)
- In Record 10, it was mentioned that some characters were written on a small Haotie (paper?). Historical annotation for this text is given which explained Haotie as either

¹⁰ For record (9) The Chinese texts and a similar story San Fu Jiu Shi are provided in the Annex 11; for record (10), (11) and (12), Chinese text is provided in Annex 13, Annex 14 and Annex 15, respectively.

silk or paper (See some historical annotation in Annex 13). Even modern scholars have different opinions (as silk, cocoon paper, plant fiber paper, etc.).

- In Record 11, the materials for writing were listed together as silk, bamboo slip and paper. It could be an indication for the use of paper in that period. However, Different interpretation could be also plausible such as 素 (silk) is refer to unwritten silk while 纸经 (paper texts) refer to the silk with writings.
- In Record 12, it mentioned the set of officials for govern of paper as well as the story of a queen in demand of only paper.

The records selected in this part are proceeding yet near to the year 105AD which could be high indicative. It is plausible to interpret the ‘paper’ as paper (in supportive of those who believe paper was invented before Cai Lun) or silk (in supportive of those who believe paper was invented by Cai Lun). A way for confirmation may come from corresponding archaeological excavations. However, until now, no such information has been revealed to prove these records.

1.2.1.6 Biography of Cai Lun

The worldwide story for the origin of papermaking largely relies on one description from Book of the Later Han. In this book, there is one chapter about Cai Lun who invented papermaking:

At the close of the reign of Yong Ping (106 AD), Cai Lun was employed at the court and later he was made a member of the Imperial Guard. The Emperor He, upon his accession, learning of Cai Lun’s superior qualities and talents, named him private counsellor and he was not spared by His Majesty in either praise or criticism. In the ninth year of the reign of Yong Yuan (97 AD), Cai Lun was made inspector of works and through his efforts, the engineers and workmen by the use of fine materials and skill, produced swords and arms that served as models for future generations.

In ancient times writing and inscriptions were generally traced upon pieces of bamboo, or upon strips of silk, which were given the name zhi (paper). But silk being costly and bamboo heavy, these two materials could not be used conveniently. It was Cai Lun who conceived the idea of making paper from the bark of trees, hemp waste, old rags, and fish nets.

He made a report to the Emperor, the first year of the reign of Yuan Xing (105 AD), upon his researches in papermaking and was highly commended upon his competency.

In the first year of the reign of Yuan Chu (114 AD), the Imperial Mother gave Cai Lun the honorable title of Marquis for his lengthy service at the palace; the government accorded him the ground-rent taxes and the proceeds from three hundred dwellings. Cai Lun became one of the chiefs of the palace.

In the fourth year of his reign (117 AD), the Emperor, finding that there were some faults in the books of history, ordered two competent scholars to attempt the correction of these faults, according to the rules adopted by the Han Dynasty. It was Cai Lun who was placed in charge of this important work of correction.

Cai Lun received from the Empress a secret order to invent slanders against a member of the Imperial family. After the demise of the Empress the successor to the throne was an Emperor of less animosity and he ordered Cai Lun to give himself up to the Minister of Justice so that he might be judged. Cai Lun experienced such profound remorse and shame that after bathing and dressing himself in his finest and most elaborate robes, he drank poison to commit suicide¹¹.

Actually, prior to the Book of the Later Han, another historical book edited by the Eastern Han (25-220AD) authority, Dongguanhanji had mentioned Cai Lun. Some scholar inferred that the biography Cai Lun in Dongguanhanji was written around 151AD (30 years after the death of Cai Lun), indicating it could be reliable record. The divergence of the reliability of these two records lies in the argument of presence and absence of ‘invention’ (造意) [8]. Nevertheless, the divergence does not influence its publicity worldwide as well as the importance of Cai Lun (listed as one of the most influence people in the world of natural science). Almost all of the publications that talk about the origin of papermaking will talk about Cai Lun as well as this record.

1.2.1.7 A summary

In this part, the records regarding the fiber plant cultivation, preliminary fiber treatment, the definition and character for paper, the early use of paper and the biography of Cai Lun have been described. From the description some clues can be drawn:

- A strong implication can be inferred that the silk making technique has great influence on the origin of papermaking. It may shed light on the idea (directly or indirectly) for the making of paper.

¹¹ This is the translation from Dard Hunter, modified. The Chinese text is provided in Annex 16.

- The pound wadding in the water could be a further fiber processing technique as the initial stage of papermaking. It is quite different with silk bleaching and rags washing which perhaps led to the first attempts of papermaking, though there was currently no direct evidence.

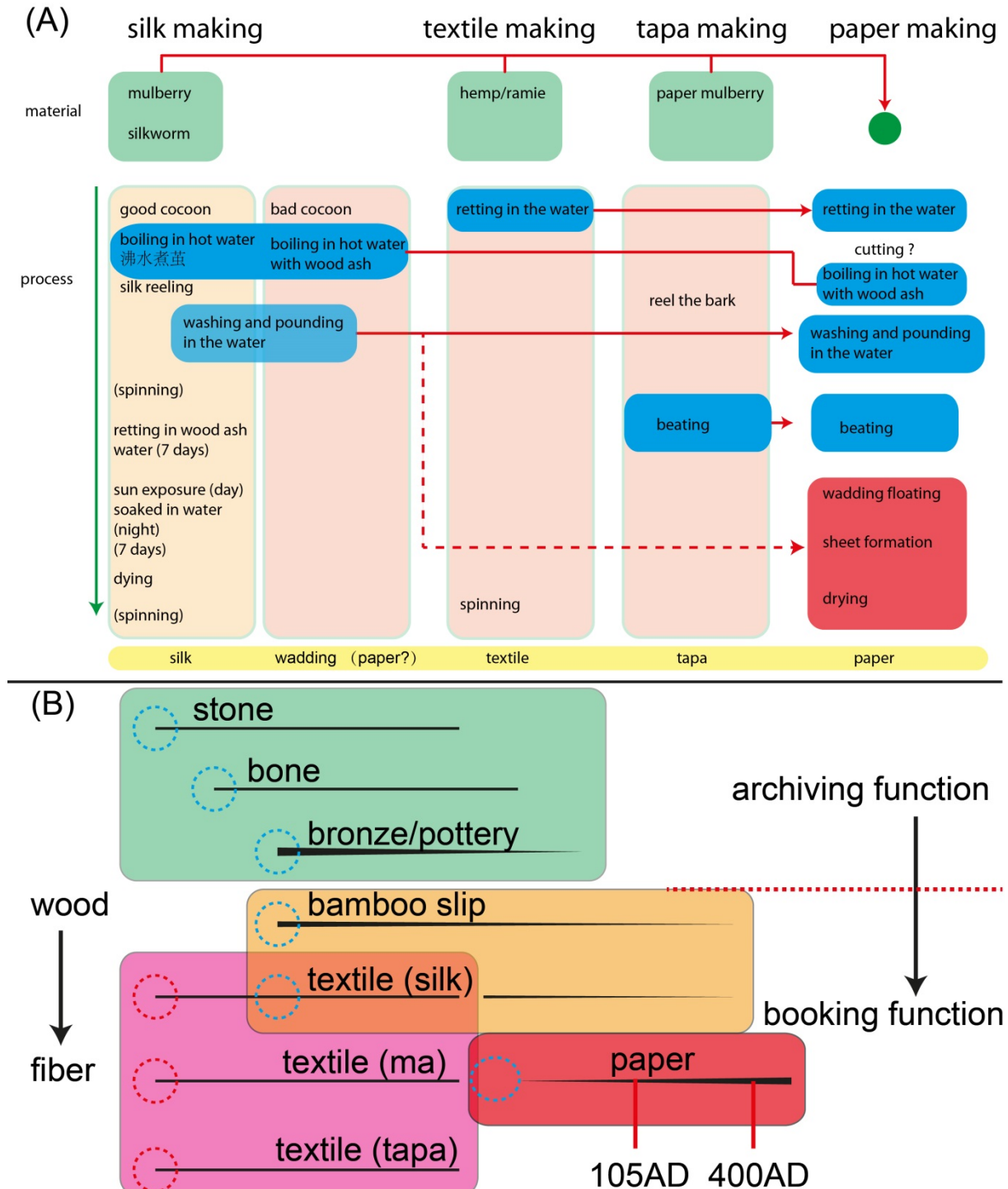


Figure 1.2.5 (A) The possible influence of the silk making, textile making, tapa making for the origin of papermaking with regard to the material and process, the influence is also indicated in (B); (B) the chronological change of the material used as predominate writing material (The relation with tapa making is further explained in section 1.3.2).

The possible influence of these techniques like silk making, textile making, tapa making is depicted in Fig.1.2.5. It can be seen that these materials (mulberry, paper mulberry, hemp, ramie etc.) can be directly used as fiber sources for papermaking. The process for the fiber treatment of these crafts can also be enlightening for the paper origin such as retting, cutting, boiling in the hot water, washing and ponding in the hot water and beating etc. the key process to add on the base is by adding the suspension of the fibers in the water and sheet formation using a sieve-like frame. Actually, it is currently not clear about when, where and how these process took place. However, one point to consider is the social need to find a suitable writing material. As can be seen from Fig.1.2.5, the early materials (e.g. stone, bone, bronze and pottery) can only serve as archiving functions with limited information that can be recorded. The transformation of writing as archiving to booking function started with the use of wood (e.g. bamboo slip) and silk as writing materials. However, the weakness in the portability and accessibility of these materials had created a need to invent a portable and not expensive material for the purpose and a matter like ‘paper’ well suits these requirements.

For the paper origin, the historical records give some hints, however, as a feature of ancient Chinese, a very complex process or matter can be explained within several characters (such as the definition of paper). On one aspect, it is elegant to create a way for imagination (and material saving for writing) while on the other hand it posed difficulties for descendant to interpret the predecessor’s meanings that different interpretations are possible and may sound plausible. Divergent annotation and interpretation for the same text had long appeared in history. The debate on the origin of papermaking was a long lasting topic: it can be traced back to ancient times when scholars began to question the reliability of some historical records. Scholars such as Zhang Ji (190-245 AD), Fan Ye (379-445 AD) deem that Cai Lun invented paper while scholars such as Zhang Huaijin (686-758 AD) and Shi Shengzu (1204-1278 AD) deemed that paper had existed before Cai Lun and Cai greatly improved it.

In the historical period, the discussion of paper origin was mainly based on the historical records while modern archaeological science should be a strong evidence to contribute to this topic, for instance, the discovery of ancient papers from the 1900s has greatly triggered the re-discuss of this topic. However, paper origin has evolved into a dispute for 40 years as is reviewed below.

1.2.2 The paper from archaeological context¹²

1.2.2.1 The early three discoveries

The early discovery of ancient paper started from 1900 by Sven Hedin and Paul Pelliot in Xinjiang, China. The change of the situation started for the discovery of the ‘earliest paper’ (the Sogdian letter) in 1907 by Aurel Stein. These letters have been attributed to the middle of the second century of our era, on the strength of archaeological evidence: if the date originally proposed by Sir Aurel Stein (between A.D. 105 and 137, or in 153) could be substantiated, the Letters, which are on paper, would have to be regarded as the oldest paper documents in existence (some scholars had expressed their doubts). And this claim to such eminence has already been widely admitted for a long time until, in 1948, it was found to be at the beginning of the fourth century, to be precise in 312 and 313 AD by palaeography study [9].

About twenty years later, in 1933, archaeologist Huang Wenbi found another handmade paper (Lobnor paper) and based on this discovery, Huang became the first to conclude that paper made from plant fibers had already exist before Cai Lun (105 AD). In fact, Huang’s conclusion has large influence on the debate in the 1950s. In 1965, Pan Jixing visited Huang, Huang confirmed that the dating of Lobnor paper as West Han paper is reliable. On the other hand, based on the description, Wang Juhua thought this paper does not going through papermaking processes such as pounding, pulping and sheet formation. It should be classified as rudiment or primitive paper (strictly by the paper definition, it’s not paper). Unfortunately, this paper has been destroyed during the war and no scientific analyze (microscopic fiber analysis) had been conducted. Conjecture can only be conducted based on the description and picture of this sample.

In 1942, Lao Gan discovered another paper (Erjinahe paper). In 1948, Lao deemed that the dating of this paper has the possibility to be earlier than Cai Lun (105AD), however, the possibility of later than Cai Lun (105AD) cannot be ruled out. By principle, it could be contemporaneous. In 1954, Lao said in a letter that the meaning of the discovery of Erjinahe paper, is the paper itself, it is not a sufficient evidence for proving the existence of paper before Cai Lun (105 AD).

This is the three main findings with paper fragments before 1950s, for these three papers: (i) The Sogdian letter has been proved to be in the Jin dynasty which cannot contribute to the topic of paper origin; (ii) Lobnor paper had lost in the war without detailed analysis carried

¹² The details for the ancient papers discussed in this part have been provided in Annex 17- Annex 30.

out; (iii) Erjinahe paper cannot largely contribute to the question due to its dating near the 105AD.

However, the contribution of these papers is that they make the debate of ‘origin of papermaking’ from the analyzing historical records to the seeking and analyzing the presence of ancient paper samples. The point of Aurel Stein and Paul Pelliot’s discovery and the following publications showed another way for the inquiry of the origin of papermaking that inspired Chinese scholars in the search of ancient paper fragment remains.

It also sparked a wide discussion in 1950s. In 1954, some scholar regard Cai Lun as the first people to use plant fibers for papermaking [10] while other scholars thought papermaking was invented in the West Han dynasty by using animal fibers followed by plant and bark: Cai Lun made some technique improvement for papermaking which accelerated the development of papermaking [11]. This dispute further excited by the discovery of the Baqiao paper which is a turning point in the discussion of paper origin. But, unfortunately, it does not solve the problem instead of making the discussion fiercer.

1.2.2.2 The Baqiao Paper

A big event in paper history is the discovery of Baqiao Paper in 1957. It was dated by the archaeologist to be in the range of West Han and was identified as the material of silk in the first archaeological report. Except Zhang Zigao’s article confirmed the silk origin (without microscopic examination) [12], no scientific analysis (mainly microscopic observation) had been reported until 1964 when scholar Pan Jixing (Pan for short) analyzed and concluded it was a hemp paper and consequently regarded it as the earliest paper made from plant fibers in the world [13]. One year later Pan did the second analysis in cooperation with Sichuan University which came with the same result (repeated in 1974, and confirmed by simulation experiment in 1977). Based on the discovery of Baqiao paper, Pan remarked on the contribution of Cai Lun as an improver of papermaking technique (1973) [14].

Different opinions arose. The early doubts appeared in 1980 following to 1982 by scholar Rong Yuankai [15, 16]. In the same year, scholar Wang Juhua (Wang for short) also published her first report dealing with the paper history that had completely different conclusions with Pan. Wang analyzed 61 samples and found: the papers were by material origin of hemp or ramie, had twisted thread but no cutting, and didn’t go through sheet formatting processes. They are not paper but a wadding of hemp used for padding [17]. In short, Wang concluded that the Baqiao paper is hemp waddings instead of paper and

consequently the archaeological findings cannot deny the fact that Cai Lun invented papermaking [18, 19] (communicated again in 1985, 2002, 2003 and 2005).

In 1985 the journal Paper Historian issued completely different conclusions in the same volume. In 1987, a meeting organized by the light industry announced the end of the dispute (actually more dispute began as no side give up their opinions). As one part to mention, Pan and Wang are two important people in the study of the ancient paper samples who usually have divergent results. They first met each other in 1964 for the analysis of Baqiao paper. According to Wang's interview, in 1972, Wang reviewed Pan's article on the Baqiao paper, and rejected it but still the article was published. In 1979, Pan's book was published that make Baqiao paper more famous. At the same time, a task was issued for the analysis of all the excavated papers which the results did not consistent with each other (Wang and Pan):

- Wang: even if the agreement was made on the nature of the fibers composing the material of the fragments (hemp and/or ramie). they differ in the existence of the entanglement (fibrillation) which was a sign of advanced papermaking material [20, 21]. it was still only a kind of primitive paper (bundles of paddings) which indicated a process still rudimentary of fiber treatment
- Pan: The fragments of Baqiao were already real paper

The general dispute of the Baqiao paper concerns lies in: (i) the dating (assumed to be disturbed by bulldozer before excavation, and by the fact that dating by pottery is not reliable etc.); (ii) the classification as paper or not (is it paper or just hemp wadding?); (iii) the material, hemp or ramie?

1.2.2.3 Paper unearthed in 1970s

In 1973, two pieces of paper were discovered (Jingguan paper). In 1979 Pan analyzed and concluded it to be hemp paper, meanwhile in the same year, Wang analyzed and concluded it to be paper with the procedure of cut with the possibility to be the first stage of 'paper'. More doubt was pointed to its dating which was thought to be not reliable [22].

In 1978, another paper (Zhongyan Paper) was unearthed. In the same year, Pan analyzed it to be hemp paper [23] while Wang identified as with the process of cut but still doubt the dating [22]. In 2012, Prof. Li Xiaoceng (Li for short) did fiber analysis of this paper and identified the raw material as ramie with the papermaking method of pouring technique (pouring the pulp onto the felt instead of using the felt to take the pulp in the pool) [24].

In 1979, Maquanwan paper was discovered in Dunhuang. In 1981, Pan analyzed it to be hemp paper while Wang think it is paper made from Ma group (see section 1.3.1) while the dating of Maquanwan paper was more reasonable to be in the range of East Han dynasty [22] (some scholar even think it could be paper from Tang dynasty).

These three unearthed papers could be an opportunity for solving the dispute about the Baqiao paper as well as further interpretations regarding other parts of the paper. Nevertheless, the dispute goes on as:

- From a basic point, the Jingguan paper is paper (advanced or primeval stage) from both side while argue goes to its dating
- The Zhongyan paper was identified as paper while discussion also goes to its dating
- The Maquanwan paper was identified as paper and again, the problem goes to dating

1.2.2.4 Paper unearthed in the 1980s

In 1983, a paperlike fragment was discovered in the far southern part of China (Xianggang paper, Guangdong Province). The first identification was done by Wang in 1984 based on two samples of 0.5 cm² and 1.0 cm². By using the average width, they were identified as ramie while no evidence of fibrillation under SEM was found. The final conclusion regards the paperlike fragment under the bronze Yi was that it is not paper but wadding of ramie used for packaging and padding the objects. Because of the water logging, the ramie wadding drifting and depositing repeatedly and finally formed a paperlike fragment. However, Pan had quite different identification result. He observed that the thickness is 0.1 mm and the fibers intertwined together as a web with several unfined lines or thread. It is hemp with possible mix with ramie. Because the core question: whether it is paper or not cannot be solved. No further interpretation has ever been conducted.

Three years later, in 1986, the famous Fangmatan map was discovered. Considering its importance, it has been written into some middle school textbook as the earliest paper in the world. In 1989, Pan analyzed this paper and identified it as high quality hemp paper. In 1990, Pan examined again with two Japanese scholars Nomura Shozo and Okawa Akinori and again confirmed the result [2]. However, at the same time doubts arose on the dating of the Fangmatan paper: The use of the similarity of the burial object for dating should be cautious [25]. On the opposite, scholar Zhang Mingwu reviewed and thought that the dating by archaeologist is reliable [26]. Wang thought there was water and humidity in the tomb and the presence of the paper (the material be silk cannot be ruled out) in the tomb is unreasonable

with the bones and clothes degraded [22]. Wang tried to analyze this paper in 1990 and 1992 but did not carry out due to some restrictions.

In 2009, Li, by his first examination (by eyes), found this paper seems to be produced by a mature technique and it is incredible to be unearthed from a West Han dynasty tomb. Also, due to the observation condition, the laid line cannot be observed to judge its making method [27]. However, in his second examination (2016) with microscopy, Li found more details: it is made from the Ma group (hemp or ramie, has to be further identified, *Ma* group is defined in section 1.3.1). It does not have laid lines thus it was made of pouring method, it has been sized on the map side (the material has to be identified). Consequently, Li regarded the Fangmatan map as the earliest paper remains in the world [28].

In the same year (1986), a paperlike fragment was discovered in Gaojiabao tomb (ca. 1000 BC). In 1992, Wang analyzed and identified it as material like the ‘papyrus’, it is from a plant material without specification. In 1989, in Daheiting tomb, a paperlike pad (ca. 300 BC) was discovered and currently no scientific analysis and interpretation were reported.

Considering the dating (1000 BC & 300BC), the so-called ‘papyrus’ and the paperlike pad could be important materials for the discussion of plant material utilization in the tomb. However, it seems no interpretation was made. In this period, more paper or paperlike material were discovered with the ongoing topic of paper origin:

- Geographically speaking, the discovery of Xianggang paper is important as it is the only early paper unearthed in the southern China. The materiality of the paper is more with concern of forming by handmade or by natural water logging
- The Fangmatan map can be classified as paper according to Li’s study although doubts pointed to its unreasonable presence in the tomb
- The first ‘papyrus and the Daheiting Fragment has not been adequately studied

1.2.2.5 Paper unearthed since 1990s

One year later in 1990, ca. 460 pieces *Ma* paper (for *Ma* group, see section 1.3.1) were discovered in Xuanquanzhi site, Dunhuang. In 1991, Pan analyzed some samples and found them to be hemp paper and concluded that West Han is the time for the origin of papermaking. In 1992 and 1993, Wang went to the site for investigation (using magnifying glass, 6 samples on the archaeological site and 10 samples in the museum) and took 6 samples back to Beijing for examination. She concluded that the dating cannot be fully rely on archaeological stratigraphy due to the climate conditions and the use of the date of the character on the

bamboo for dating is also not reliable. Some techniques for papermaking shall not be appeared in West Han dynasty, so all this West Han dynasty paper were supposed to be later ones: within the two hundred years, why there were not a single paper with dated characters?
It means there is no paper at that time.

At the same time (1992) doubts such as the character type style were discussed [29]. In 2010, Li analyze 49 Xuanquan paper (fiber analysis of four Xuanquan paper) and found most of them were made of hemp and using pouring method and sizing technique [30]. In 2011, scholar Han Fei analyzed the physical properties of 41 samples of Xuanquan paper from different layers and found that these papers tend to be thick, high grammage with laid lines [31]. Opposite to Wang, scholar Han Hua (2014) concluded that the use the bamboo slip for dating is reliable¹³ [32]. In 1995, a paper sample was found in Shuangbaoshan tomb in Sichuan Province. In 1998, it was given to Wang for analysis. Wang found it to be a rice paper, so concluded it is not an early paper.

In 2006, a paper was discovered in a necropolis of Gol Mod, in the province of Arkhangai, Mongolia [33]. The necropolis is attributed to the confederation of the half-nomadic tribe of Xiongnu. This graveyard includes 393 graves of which grave T20 is not only the largest but also surpasses the size of most of the graves found at any previously discovered Xiongnu necropolis. The grave adopts the traditional shape of the graves of the elite, and the funerary room of the monument has been excavated down to the bedrock. An archaeological piece of paper was discovered in chariot n°2, which was lying on the roof of the funerary chamber of the grave. More than 50 elements belonging to this vehicle were found, thus allowing for a clear understanding of the structure. The restoration of its appearance shows that the vehicle perfectly corresponds to Chinese models of that period. It can be divided in four parts: body shell, axle and wheels, shaft and yoke, and parasol. Simultaneously, a lacquered object inscribed with Chinese characters “Yongshi era” (永始), the period between 16 and 13BC, was found in the T20 grave. This enabled to date the paper-like fragment from the same period. Examination of the surface of the fragment with a binocular microscope confirmed that the material has all of the visual characteristics of paper: It consists of a mat of tangled fibers exhibiting no particular order. The fibers are up to several millimeters long and have a diameter ranging from 20 to 50 μm . The fiber was identified as hemp [34] and the meaning of this object was discussed elsewhere [35].

A feature for this period can be summarized as:

¹³ the archaeologist who discovered these papers also made some notes in 1989 to support their archaeological dating.

- Large quantity of handmade papers was found in Xuanquan (definitely paper) which provide more materials for paper origin studies. As previously, the dating of the samples is a point for discussion among scholars
- The Shuangbaoshan paper has been identified as rice paper and thus ruled out as early handmade papers
- The Gol Mod paper found in Mongolia, in considering of its dating and location, could be a lost link to form a chain for the usage of paper and papermaking before 105AD. However, no such interpretation has ever been made

1.2.3 The opinions and arguments

The dispute of the origin of papermaking originated through history and currently still going on. The physico-chemical analyzes made it possible to specify the fragments of ancient paper samples, but different scholars resulted in divergent observations and thereby contradictory interpretations (detailed in the review from 1979 to 2007 [36, 37]). The dispute mainly focused on whether Cai Lun invented or improved the papermaking technology with the aspects of (i) the definition of paper, (ii) the reliability of historical records, (iii) The reliability of archaeological excavation and dating, (iv) the reliability of testing and the reliability of simulation experiment. Although various scholars discussed the paper origin, the main viewpoints can be summarized below:

- Pan Jixing and Qian Cunxun: Papermaking already exist (at last) from 2 century BC before the improvement of the papermaking technique by Cai Lun (supported by the archaeological findings)
- Wang Juhua: Cai Lun invented papermaking and papermaking originated from the year 105AD (based on historical records and argue with the archaeological findings)
- Yang Juzhong: there are two origins for the paper origin, one is the utilization of natural wadding originated from the Spring and Autumn period, and the other origin is the start of manmade wadding that originated from east Han dynasty (with the mark of Cai Lun)
- Li Xiaocen: the movable type originated from china while the fixed type was possibly originated from Indian subcontinent¹⁴ [38]. However, in 2011, Li upgraded his theory

¹⁴ based on historical and field survey materials, in 2001, Li first report his found that there are two method of making paper, the fixed-mould papermaking and the movable-mould papermaking method, These two papermaking methods are totally different in technical process and key processes, which lead the products to have obviously different traits, so these two methods belong to different papermaking method systems.

that fixed-mould papermaking and the movable-mould papermaking method were all originated in China. Fixed-mould papermaking method belongs to non-Cai Lun system, while moveable-mould papermaking method belongs to Cai Lun system. The relics of these two papermaking methods have different geographical distributions

- Leng Chunsheng: papermaking was thought to be derived from the practice of tapa [6].

A fact is that the most ancient remains about papermaking are very rare and their study is incomplete and unsatisfactory. These theories were all plausible to some extent yet they failed to integrate into one theory for the origin of papermaking. Also, other important aspects such as interpretations on the function of early handmade papers for initial use have not been well addressed.

1.2.4 The re-think of paper for initial use

The paper origin is a so big topic that it is difficult to reveal its overall perspective with limited data collected. Nevertheless, there are several entry points among which the question can be asked: what is the initial aim of making paper (or what is the function of paper in the very early stage?). The early ancient papers discussed in section 1.2.3 is summarized in Table 1.2.1. Their function is discussed in this section with a geographical belt and chronological line provided for discussion.

Table 1.2.1 The list of the early paper or related samples unearthed.

No.	Dating	Unearthed	Site	Location	Identification	usage	Ref
1	~1000BC	1986	gaojiabao	Shaanxi	papyrus	padding	Annex 17
2	657~615BC	1959	shuangcheng	Anhui	cloth/silk	—	Annex 18
3	~300BC	1989	daheiting	Hebei	paperlike	padding	Annex 19
4	179-142BC	1986	fangmatan	Gansu	paper?	map	Annex 20
5	150BC	1995	sichuan	Sichuan	paper	—	Annex 21
6	140-7BC	1990	xuanquan	Gansu	paper?	writing/ padding	Annex 22
7	122-121BC	1983	xianggang	Guangzhou	paper? wadding?	padding	Annex 23
8	118BC	1957	baqiao	Shaanxi	paper?	padding	Annex 24
9	65-23BC	1979	maquanwan	Gansu			Annex 25
10	52-3BC	1973	jiguan	Gansu			Annex 26
11	49BC	1933	lobnor	Xinjiang			Annex 27
12	16-13BC	2006	gol mod	Mongolia		padding	Annex 28
13	1-5AD	1978	zhongyan	Shaanxi			Annex 29
14	109-110AD	1942	erjinahe	Gansu			Annex 30

1.2.4.1 The hypothesis for paper use

More and more evidence has demonstrated to question the invention of paper for writing. Based on logical deduction, scholar Lao Gan in the 1970's had pointed out that the origin of paper was not all for writing, writing might be incidental use and its main function still need to be discussed. In 2004, prof. Qian Cunxun also deemed that in general, paper might be initially used for packaging and padding and only at the beginning of East Han dynasty it has been used as writing materials. Recently, in 2015, based on the nine archaeological excavation, scholar Zheng Yefu calculated that only 33.3% paper were written with characters (3 out of 9, 5 out of 15), which means most of the paper were not with characters on it. Zheng thought burial is important ceremony and the paper without characters being in the burial is puzzling: only by supposing that paper was not for writing make it reasonable to be present with other objects in the tomb. Zheng's hypothesis with supporting examples¹⁵ moves a step forward for the explanation of paper in the initial use. But he also said that the function of paper in initial was still a challenge in the origin of papermaking.

On the other hand, the method and examples used by Zheng, somehow, were questionable: (i) use the percentage of have character/don't have character on the paper is at some risk for misinterpretation; (ii) the lacquer horse was in private collections and its origin is not clear; (iii) the Baqiao, Zhongyan, Xuanquan paper was under debate as reviewed above. To make this hypothesis more standing, more evidence or linkages are needed.

1.2.4.2 The Function of the unearthed paper

In gaojiabao tomb (~1000BC), a paperlike fragment was discovered under the bronze and was identified as material like 'papyrus'. It is no doubt that it is used as a padding material. Based on this preliminary identification result, it can be inferred that plant material had been used as padding material for bronze object in the burial from a very early stage. In another tomb (~300BC), A paperlike fragment, several sheet overlying together (formed like a wadding) was also discovered¹⁶. These two findings may make it clear that in the 1000~300BC, materials such as 'papyrus', paper-like fragment (wadding, filler, paper?) made from plants had been utilized as functions of filler or padding for burial objects.

¹⁵ Zheng use some examples to support his idea: one is a lacquer horse mentioned by Qian Cunxun that it has paper on it, the baqiao paper that seems to be packaging, the zhoingyan paper that was used as padding and xuanquan paper that seems to use as medicine packing.

¹⁶ But its location and use has not been reported and no investigation has ever been carried out.

The discovery of Xianggang paper is important. Geographically speaking, it is the only ‘paperlike’ fragment excavated in the far south in an early stage (122-121BC). Pan and Wang both analyzed this ‘paperlike’ fragment: Pan identified as hemp paper while Wang identified it as a wadding of hemp and naturally formed a paperlike web because of the water loggings of the tomb. It is a pity that after this dispute, no further interpretation has ever been carried out. Regardless of whether it is paper or wadding, the function of this ‘fragment’ as a pad (packaging) for the bronze object is of no doubt. The function of Xianggang paper can be linked to the Gaojiabo (~1000BC) and Daheiting (~300BC) fragments for the discussion of plant material used in the tomb. Its geographical importance and inclined to be paper will be discussed with the Gol Mod paper in the following.

Among Xuanquan papers, only 10 out of 460 have characters on it (2.2%). Although using the percentage has some risk, it gave some hint for their usage. In the Xuanquan papers, of the 10 with characters, 3 have characters related to the medicine and were interpreted as packing for the medicine (157-74 BC)¹⁷. The Baqiao paper (118 BC) discovered in 1957 has been interpreted by paper historians as packing and padding functions for the bronze mirror. However, the main discussion has been focused on the precision of the dating and whether it is paper or not, no emphasis has been well stressed on its functions. Apart from dating and identification (whether paper or not), the use of Baqiao paper for padding is of no doubt. The use of Zhongyan paper (1-5AD) is also for filler and padding.

All the 6 papers (or paperlike fragment) stated above (Gaojiabao paperlike fragment, Daheiting paperlike fragment, Xuanquan paper, Xianggang paperlike fragment, Baqiao paper, Zhongyan paper) have all pointed to the use of paper as padding or filler (of the bronze). However, problem remains as listed in Table 1.2.2. In a word, all these findings need a key point to confirm which could come from the Gol Mod paper.

Table 1.2.2 The summery of the problems for the selected papers.

No.	Dating	Site	Location	Dating	Paper or not
1	~1000BC	gaojiabao	Shaanxi	OK	‘Papyrus’
3	~300BC	daheiting	Hebei	OK	No study
6	140-7BC	xuanquan	Gansu	Questioned	Questioned
7	122-121BC	xiangguang	Guangzhou	OK	Questioned
8	118BC	baqiao	Shaanxi	Questioned	Questioned
13	1-5AD	zhongyan	Shaanxi	Questioned	Questioned

¹⁷ About the question of written paper and non written paper, it is sure that the first use of paper was not to be used as writing material. It was used for packing, for instance in wrapping funerary items, and only later for writing. But the question is when and how this transition took place. The Further study of the coexistence of the two kinds of paper in tombs, paper for writing and paper for packing may give more hints.

The Gol Mod paper was discovered in 2006. Dr. Guilhem Andre (2010) make an archaeological report (in Chinese) in a very high influence archaeological journal in China. Nevertheless, in his report, he only used one sentence stating that ‘a paper-like fragment was discovered that need to be analyzed and identified’. In the same year, Dr. Guilhem Andre and Prof. Jean-Pierre Drege published the identification of the paper-like fragment to be a ‘paper’ used as padding in the West Han dynasty with the mention of some early papers excavated in China. The problem is: (i) it seems this article has not been cited by other articles for the discussion of paper origins¹⁸; (ii) although it mentioned the other archaeological papers in China, it did not make a strong link with the detailed information for further interpretations.

In 2013, the finding of Gol Mod paper was also reported in a conference proceeding, yet it seems it doesn’t cause much attentions. In a word, the importance of the Gol Mod paper has not been fully interpreted. I provided some re-thinking of the importance of the Gol Mod paper and provided here for discussion.

1.2.4.3 Function of paper in initial

Firstly, Gol Mod paper could be an indirect source to confirm the essence of Xianggang paper (unearthed in Guangzhou, south of China). From Gol Mod paper, we gain the knowledge that during the period of West Han dynasty (16-13 BC), paper made from hemp/ramie had been used as pad (or filling) materials for the manufacturing of objects, especially bronze objects. The discovery of paper in Mongolia proved that the use of paper for padding had propagated far into the northern part¹⁹. With the proof from:

- The discovery of the Gol Mod paper in Mongolia dating from the West Han dynasty
- Its function is padding used for bronze objects

Based on logical deduction as well as the chronological time line, if the paper product had propagated (from central China?) into the far north, it is logical to suppose that the paper product (or making technology) had probably traveled to the far south. Thus, the discovering of the ‘paper’ in the Xianggang in the far south could be possibly made by humans. In fact, due to the humidity and raining environmental conditions, Xianggang paper was the only early fragment unearthed in the south China as has been stressed above.

¹⁸ This article has been cited by 4 articles discuss of magnolia tomb studies. Never did it being used for the discussion of origin or function of paper in Han dynasty. Information get from google scholar, accessed on 15, January, 2018, published in French maybe one of the reasons for the uneasy accessibility for other scholars.

¹⁹ The paper product if it is not the papermaking techniques. The exact production production place is unknown (probably in central China). The fact is, it has been discovered in a tomb in Monglia.

From far north-central-to far south, a geographical belt of paper product. As has been stated above, all the current theory of origin of papermaking reposed on hypothesis whether based on historical records or archaeological findings or the combination of both. The more reliable information we used for analysis, the closer the hypothesis will be to the reality. The discovery of Xuanquan, Baqiao and Zhongyan is one geographical point in the ancient central China. The discovery of Xianggang ‘paper’ in the far south is a good opportunity for a possible link for the distribution of paper product (may as well as papermaking technique) from central to the far southern part²⁰. As stated above, the discovery of Gol Mod paper give strong implication that Xianggang paper is more of paper of handmade. With this, further interpretation is possible and reasonable.

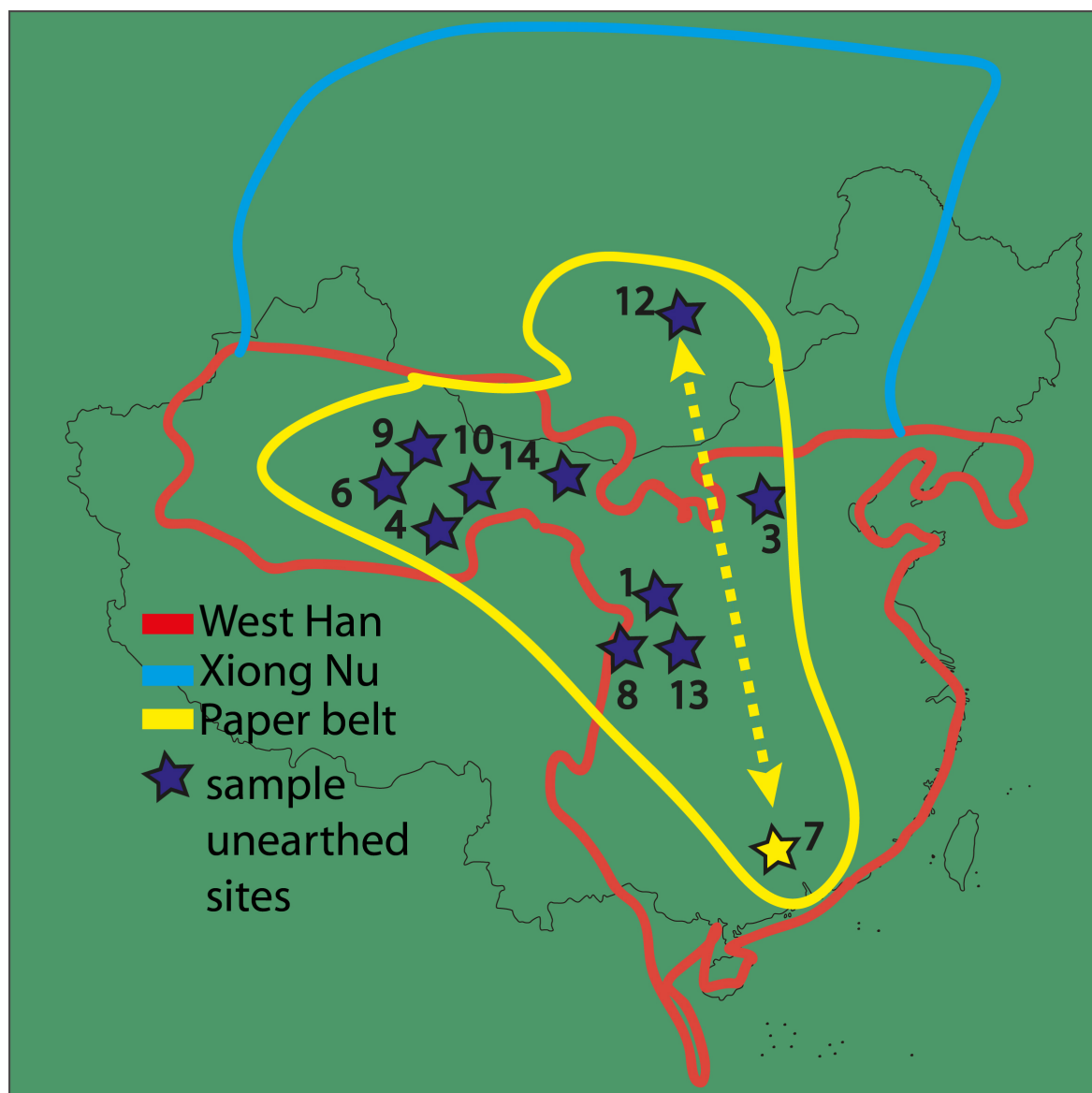


Figure 1.2.6 The distribution of the geographical belt of paper product. The numbers within are corresponding to those in Table 1.2.1.

²⁰ However, the discussion was stagnated with whether it is paper or not (in fact, no interpretation can be made if this problem cannot be solved or implied).

From above, in the West Han dynasty, handmade paper from plant fibers used for padding (filling, packaging) has been discovered in Mongolia Gol Mod (far north)---Xuanquan, Baqiao, Zhongyan (central)--- Xianggang (far south) (Fig.1.2.6). From this belt²¹, it can be inferred that:

- In the West Han dynasty, the use of paper (made from plant fibers) could have been a common practice;
- The use of paper as padding material could have been spread to far north and far south that people living in these regions may get familiar with the paper use (if it is not the papermaking technique);
- For a certain probability, the papermaking technology may also go behind the paper product (especially to the southern part which has more intimate relationship with central government).

In the historical records, Emperor Xuan of Han (91-49BC) has ever given ‘eight thousand silk and six thousand silk flosses to Xiongnu (the northern part). So, the trade (or giving) between Han and Xiongnu was quite large thus the paper product within bronze object was possible to spread to the far north part of Mongolian Plateau. And the south Yue state also had a close relation with the Han dynasty. It is possible that the paper product reached the south Yue area²². Although there is weak point in the stated geographical belt of paper product based on limited archaeological excavation, it is the most reasonable one by current collected data.

Chronological material involved for padding: the evidence of paper in initial. The discovery of the Chinese ‘papyrus’ in 1000 BC is of great importance while it has not been paid great attention. We know that the Egyptian papyrus was initially invented for writing, however, from the archaeological context, this Chinese ‘papyrus’ was used for placing the bronze object in the burial. The discovery of this papyrus is important in considering of its dating. In regard to its identification²³, it can be inferred that for an early stage (as late as 1000BC), there is a need or had been a common practice for padding the bronze object in the burial and some kinds of plants materials had been used for this practice and purpose. Within this period, other material such as cloth and silk had also been utilized for this purpose indicated from the Shucheng fragments (657-615 BC) (see Annex 18).

²¹ This distribution is based on the currently collected data. It gave hints on the travelling of paper products. The actual distribution maybe more complicated if the climate, hazard of the discoveries, social change etc. were taken into account. More difficulties is the correspondence of the finding place and the making place as the paper products can travel easily.

²² The manufacture of the paper locally was also possible with lots of ramie resources in the south.

²³ Current discuss use the identification of Wang, that is, this fragment is not paper, it is some plant material manufactured without essential papermaking process, or it is possible that the plant material has not been process and the shape was formed due to the weight of the bronze.

In the Daheiting tomb (~300 BC), the presence of cellulosic paper-like wadding is described in the archaeological report as: A paper-like fragment, several sheets overlying together (formed like wadding). A possible function could be also used as padding or filling functions²⁴. The presence of the paper-like fragment in these tombs could be inferred that

- The natural wadding from the retted material (hemp?) had been utilized as the function of padding;
- The possibility of papermaking using the waddings of the retted (hemp?) materials could be possible.
- The plant wadding (hemp?) has been produced purposefully for padding functions.

This hypothesis may directly point to the fact that early papermaking was initially used for padding/package functions. These two archaeological findings indicate that plant material has been used for padding functions in the burial. In this sense, I pointed out that the Daheiting paper (~300 BC) is a key point in the chronological time line for the evolution of plant materials used as padding function in the burial: a point that make the chronological time line ‘Chinese papyrus---wadding---paper’ (or ‘Chinese papyrus---paper’) (Fig.1.2.7).

When time goes to the West Han dynasty²⁵, It is sure to deem that the paper made from plant fibers (mainly hemp and ramie) excavated geographically from far north (gol mod)---central (Xuanquan, Baqiao, Zhongyan)---far south (Xianggang) has been utilized in the tomb for the function of packaging and filling. From this point we can infer that the papermaking was strongly related to the padding (packaging) function (no late than this period). Chinese characters began to appear on the paper such as the Xuanquan paper that was used for the packaging of the medicine (has the medicine name on it). One explanation could be that the ability of the paper (writing adaptability) might be poor and cannot compete with bamboo slip, wood, silk as a writing material at that time. From the above analysis, a chronological time line for plant material used as padding can be drafted (Fig.1.2.7):

- ~1000BC (‘Chinese’ papyrus?)---~300BC (paper?)---~100BC (paper)---300AD (paper)
- It can be reasonably stated that paper has been used before Han dynasty

²⁴ This point needs further confirmation, its usage and location has not be detailed in the archaeological report.

²⁵ The tradition of use paper as padding materials even goes into east han, jin dynasty as can be observed in the burial. Later, from tang dynasty, paper money has become popular.

Like other hypothesis, the geographical belt of paper product and the evidence of paper in initial are written based on limited data²⁶, it is possible to modify according to new archaeological discoveries and new scientific analysis on the unearthed paper samples²⁷.

1000BC	Padding/packaging/covering	'papyrus'
600BC	Padding/packaging/covering	cloth/silk
300BC	?	plant material, paper-like
100BC	Padding/packaging/covering	paper
5AD	Padding/filling	paper




Figure 1.2.7 The time development for plant material used in the tomb as filler or padding

1.2.5 The paper road

The origin of papermaking has also been a topic for the western scholars after paper traveled across the Arabic world to arrive in Europe, a road often refers as Paper Road (which was less known than the Silk Road²⁸). However, silk was only one (maybe representative one) of the commodities that traded along the routes among others that include paper. Although the paper road has been studied bits and pieces by numerous scholars from different background and created a conventional timeline for the spread of the technique, certain details were not revealed yet. The outline of the paper road is briefly discussed with special interest to the unearthed handmade papers.

One of the most quoted passages is the 'honey-fragrance paper' from the account of the flora of the southern regions (南方草木状) regarded as the earliest Chinese treaties dealing with plants and bearing a purely botanical character [39]. It mentions that in the year 284 AD the Roman Empire presented 30,000 rolls of "honey-fragrance paper" to the Chinese emperor

²⁶ For example, the two fragments discovered in Gaojiabao (Shaanxi) and Daheiting (Hebei), little information has been reported: The data currently given in the archeological reports may be tenuous for the discussion. The place of these fragments in the genealogy of paper is tentative attempt which wait for further investigations.

²⁷ For instance, the incomplete analysis of the laid lines has prevented to go further in the discussion. The existence of laid lines reveals some characteristics of paper, for instance from which kind of mould the sheets were made, and this could help us for dating the fragment of paper. Systematical examination shall be carried out on the most ancient fragments.

²⁸ The Austrian geologist and explorer Ferdinand von Richthofen (1833-1905) first coined the term Silk Road to refer to the network of land routes that linked China and Europe from the 3rd century BC to the 15th century.

who then bestowed Du Yu (222-284 AD) with a third of the tribute to him²⁹. Although few scholars today cling to this theory of a western origin of paper, it is still quoted by paper historians as an evidence of contact between China and the western countries in the third century.

The first mention of paper by Arab writer is in the year 650 AD [40] while a more popular explanation stated that in the aftermath of a battle on the banks of the Talas River in the Khangali region of central Asia, allied muslim forces routed a Chinese army, capturing and among others, Chinese papermakers³⁰. The later was taken to Samarqand, where the first papermaking center in the Islamic world was established in the year 751AD [41]. However, this anecdote (story about the captured papermakers) has been questioned³¹.

Besides the historical records, there are several unearthed paper samples that may contribute to the origin of the Paper Road. In 2006, scholar Guitty Azarpay reported the study of the Pahlavi Archive and found the textile used as writing material in the Middle Persian documents is linen (sized with gypsum), rather than silk as earlier believed³². It may point to the preparation of linen as writing material in early post-Sasanian Iran as well as links on the development of paper technology in the early Islamic world.

The most famous unearthed early papers were the Mount Mugh paper discovered in 1932³³. Although there is no way to know where the Mount Mugh papers were made, it is likely that they were local and that, as elsewhere, Buddhist monks, who had been active in the region might have brought papermaking to Transoxiana well before the Muslim conquest. Other factors have also led to the transfer of papermaking from China to Transoxiana before the mid-eighth century, such as the role played by merchants, especially by several generations of Sogdians³⁴.

A most recent finding was the Sanjar-Shah paper unearthed in Sanjar-Shah and indicated to be a Chinese origin³⁵. Besides the Mount Mugh paper, Sanjar-Shah paper provide further evidence that the use of paper by the Arabs in Sogdiana was already widespread in the 2nd/8th century.

²⁹ The original text is provided in Annex 31.

³⁰ Other times like 704AD has also been recorded as shown in Annex 32.

³¹ Some opinions are provided in Annex 33.

³² A description is provided in Annex 34.

³³ A description is provided in Annex 35. The problem of the Mount Mugh paper is the material for the paper has not been systematically studied. Further scientific analysis needed be conducted.

³⁴ A short discussion of the contribution of the sogdians is provided in Annex 37.

³⁵ A description is provided in Annex 36.

Historical records implied that al-Fadl, who had been governor of Khurasan in 794 AD introduced papermaking to the government, that is, to Baghdad [42]. It took several hundred years for paper to disperse in the Arabic world and its conquered area and by the middle of ninth century; the Egyptians (the birthplace of papyrus) began to produce more paper than papyrus which was confirmed by archaeological finding of the shift from writing on papyrus to paper in the Islamic lands. And by the thirteenth century, the manufacturing of papyrus in Egypt was already quite forgotten [43].

The outline of the paper road (Fig.1.2.8) is: in 751AD, the Talas war between Tang dynasty and Arabic became the beginning of the papermaking from China to Arab world (other possiblities has been discussed above), in 794AD, Baghdad setup the first papermaking mill, at around the 10th century, the Damascus became a center for papermaking and lots of paper was imported to the Europe. In ca.900AD, the papermaking technology arrived at Cairo (North Africa). At the 12th century, papermaking arrived at Spain (1151AD) and France (1189AD) and at the 13th century (1276AD) arrived in Italy [44, 45].



Figure 1.2.8 A sketching of the Paper Road.

1.3 The fibers of paper

In ancient times, paper was a valuable product, and papermaking was an art that was often kept secret. For the Egyptians, they were not afraid of other people learn the secret of papyrus making as the raw material was grown in a limited and restricted areas within Egypt. For the paper, the situation is different: once people master the technique of papermaking, various plant fibers can be tested for its potential interest of making paper. In a sense, the papermaking process is a material dependent engineering. The main raw plants that have been historically utilized for mass production of handmade papers are discussed in this section.

1.3.1 The *Ma* group

Linguistically, the character *Ma*, was derived from ideographic components representing fibers hanging on a rack and placed under a roofed shack (Fig.1.3.1). Having evolved from the ancient to the later styles, it remains the character for hemp. When in later times, other fiber plants from the warmer regions of the south, as well as introduced plants from foreign countries became known, the character *Ma* developed into a generic name for fibers, and hemp itself was known as *Da-Ma* (大麻) [46]. Some plants that have been commonly referred as *Ma* or *Ma* group fibers have been listed in Table 1.3.1.

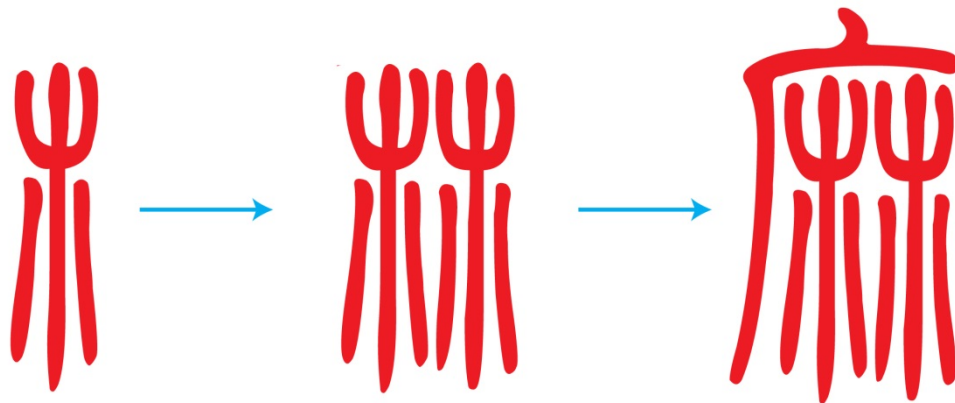
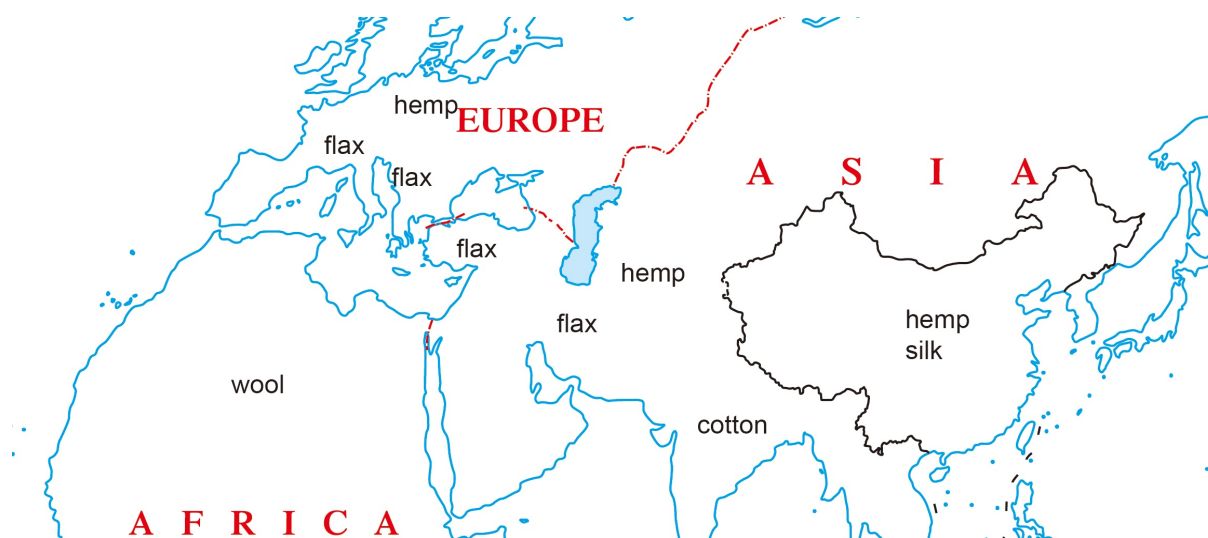


Figure 1.3.1 The Chinese character for *Ma* (麻).

Table 1.3.1 The list of plant common referred as *Ma*.

common name	scientific name	family	Genus
<i>hemp</i>	<i>Cannabis sativa</i>	Cannabaceae	Cannabis
<i>flax</i>	<i>Linum usitatissimum</i>	Linaceae	Linum
<i>ramie</i>	<i>Boehmeria nivea</i>	Urticaceae	Boehmeria
<i>jute</i>	<i>Corchorus capsularis</i>	Malvaceae	Corchorus
<i>nettle</i>	<i>Urtica ardens</i>	Urticaceae	Urtica

Some aspects for the plant in the *Ma* group are listed as: (i) hemp was originated and cultivated in northeastern of Asia; (ii) Ramie was originated in China, distributed mainly in the southern part of China; (iii) flax has been cultivated for oil and fiber and the likely place of origin was within the Near East region [47] and presents geographical diversities [48]; (iv) jute probably originated in China and now distributes across much of tropical Africa; (v) nettle distributed in temperate and tropical regions throughout the world. In China, nettle is mainly distributed in the southwest provinces. Nettle fiber has been traditionally used for the manufacture of paper.

**Figure 1.3.2** Principal fibers used in 3rd millennium BC.

Almost all the early unearthed papers were made from hemp/ramie (rags or used materials of hemp). Since Ming dynasty, due to the cotton plantation, the plant of hemp dropped. And nowadays, handmade papers from *Ma* group are not that common than handmade papers made by barks (e.g. paper mulberry). Comparing Fig.1.3.2 (principal fiber distribution) and Fig.1.2.8 (the paper road), it is more illustrative that the papermaking process is a material-based engineering: in north China, papermaking mainly utilized hemp while papermaking in central Asia, Middle East and Europe mainly utilized flax [41, 49].

1.3.2 The *Moraceae* family (group)

The *Moraceae* are a family of flowering plants comprising about 38 genera and over 1180 species [50]. The *Morus spp* (commonly refer as mulberry³⁶) and the *Broussonetia spp* (commonly refer as paper mulberry) are the mainly plant fibers used for papermaking. Their bark was an important source for paper-manufacture in the papermaking history as well as for nowadays of hand papermaking in China, Japan and South Korea due to its wide distribution (Fig.1.3.3).



Figure 1.3.3 The distribution of paper mulberry and mulberry.

Another point for paper mulberry lies in the flourished craft of bark-cloth (tapa cloth) that may possibly shed light on the invention of papermaking. In archaeological context, a kind of stone object is discovered and combining archaeological and ethnological investigation, it was identified as stone beater, a tool to make tapa (bark from paper mulberry and other plants). Tapa culture may originate from 3500 years ago while a 7900 years stone beater was

³⁶ Aspects of its origin and historical use are provided in Annex 38.

unearthed [51]. Recently, a tapa from a Pacific island dated from the 1650s has been scientifically studied and revealed the use of paper mulberry as main fiber source [52].

Scholar Ling chunsheng was among the first scholars (in 1960s) to link the tapa to the invention of papermaking [53] (later Qian Cunxun in 1973 [54] and Zheng Yefu in 2015 [6] also have similar viewpoint): The use of paper mulberry bark for papermaking may have originated (or been influenced) from the craft of making tapa, a beaten bark cloth that was very popular throughout the Pacific³⁷. This kind of bark cloth appears often in early Chinese literature as a local product of the non-Chinese tribes in the south and southwest regions. The paper origin from the (paper) mulberry trees also have some linguistic argues³⁸.

The earliest reference to the use of tree bark for papermaking is recorded in the biography of Cai Lun in the History of the Later Han Dynasty. Although it does not specify the kind of tree bark, it is reasonable to suppose it to be mulberry or paper mulberry. Tang Pan of the early third century said that The Eastern Capital (Loyang) has the paper of Marquis Cai, which was the paper made by Cai Lun; that made of used hemp is called hemp paper; that of tree bark gu paper mulberry paper; and that of used nets net paper. The earliest literature which describes the methods of planting and harvesting the shrub and the treatment of its bark is included in an ancient work on agriculture and farming by agronomist Jia Sixie (Northern Wei dynasty). He wrote a short passage that pointed out the major purpose of planting the paper mulberry was to produce raw materials for papermaking.

1.3.3 The *Thymelaeaceae* family

The *Thymelaeaceae* are a family of flowering plants which are mostly trees and shrubs. Several genera are of economic importance, for instance, the barks of *Daphne*, *Edgeworthia* and *Wikstroemia* are commonly used for papermaking. *Thymelaeaceae* are distributed in the south, southwest part (Yunnan province and Tibet) of China [55]. Tibetan papers are typically made from the *Thymelaeaceae* family of which *Stellera chamaejasme* has been established as a paper component used exclusively in Tibetan papermaking³⁹. *Thymelaeaceae* family plants such as (mitsumata and gampi) are commonly used for papermaking in Japan [56].

³⁷ The bark cloth culture had existed in China long before the manufacture of paper through a process of felting. The name tapa is very close to the Chinese term for bark cloth: ta-bu, which may mean a cloth made by beating, or gu-bu, a cloth made of paper mulberry.

³⁸ Some information is provided in Annex 38.

³⁹ According to Dr. Agnieszka Helman-Ważny, *Stellera chamaejasme* is more readily distinguishable from the *Daphne* and *Edgeworthia* plants: *Stellera* fibers are distinguished by their ribbon-like shape and an impression of transparency, similar to flabby cotton fibers. The fiber walls and lumen are much more irregular when compared to *Daphne* and *Edgeworthia*.

1.3.4 Bamboo

Bamboo was extensively cultivated in China, except for the extreme northern part of the country. It was grown probably as far north as the provinces along the Yellow River in ancient times, but was driven much further south by change of weather or deforestation⁴⁰. It is now abundantly grown in the Yangtze valley and the provinces to the south. Because of its long fibers, rapid growth, and low cost, it has been a major source of raw fibers for papermaking since the middle of the Tang dynasty.

1.3.5 Other barks

Barks from other plants have also been utilized. For instance, the seitan (*Pteroceltis tatarinowii*) is a tree, indigenous to China (especially in the central part). It has been traditionally used for making *Xuan* paper. The *Teng* (a climbing plant) was known to have been used for making paper in certain regions of China, especially in the southeastern part.

1.3.7 Grass

Paper made from grass mainly from rice straw (*Oryza sariva*) and wheat straw (*Triticum aestivum*). Rice has been mentioned in 5 poems in the Classic of Poetry (Fig.1.2.2) and on the basis of archeological evidences demonstrates that rice was in cultivation in China as early as 3000 B.C. And by Han dynasty, rice (mainly in the southern part) and wheat (mainly in the northern part) had been commonly planted. However, the quality of the paper manufactured from grass is not suitable for writing, painting and calligraphy and it never been used as the main material in the historical period. However, rice has been successfully mixed with seitan fiber to produce *Xuan* paper, a famous Chinese handmade paper.

1.3.8 The material change in papermaking

The secret of paper lies in the nature of cellulosic fibers: hand papermaking has a history of more than 2000 years and is a highly material dependent engineering. Due to the local fiber

⁴⁰ Some annotators in the past were rather skeptical about the possibility of bamboos being grown in North China, although it is mentioned in 3 poems in the Classic of Poetry (Fig.1.2.2). Reliable historical records indicate, however, that there was a bamboo garden belonging to the Royal Court of the Shang Dynasty (traditionally dated as ending at 1133 B.C.) in the central part of the present Henan Province.

plant resources and fiber characteristic, papermakers have explored various plant materials for hand papermaking through centuries both chronologically and geographically [54, 57, 58]. Although fibers from a wide range of plants are suitable for papermaking, yet only certain plant fibers were conventionally used for large scale production of papers, and they appear to be in an ordinal sequence if carefully examined:

- Han Dynasty (202 BC–220 AD) is the period when papermaking originated in China. Historical document revealed the mainly use of rags from hemp and ramie as the fiber sources
- Jin dynasty and Northern and Southern dynasties (265–589 AD) were a period that fiber sources began to expand from hemp/ramie to bast fibers mainly of paper mulberry and mulberry
- Sui and Tang period (581–907 AD) created a diversity of fiber sources. Apart from rags, fibers from raw hemp/ramie materials are directly used; bark fibers from various plant families such as *Moraceae*, *Thymelaeaceae*, *Malvaceae* have been used in the papermaking; bamboo fibers were used in papermaking for the first time, marking the beginning of utilization of fibers from the whole plant stem, which is one-upped until 1875 when westerners succeeded in mastering the use of bamboo fibers
- Song and Yuan period (960–1368 AD) witnessed the thriving of bamboo and straw fibers and the decline of hemp and ramie fibers
- Ming and Qing period (1368–1911 AD) is the highest state for the old manual craft yet followed by the downhill as the modernization began

The change of material for the papermaking is associated to the political or society changes, fiber resource exhaustion and technical advances which contains rich information for history and social studies. Nevertheless, little attention was paid to the paper material itself in the early studies of the old manuscripts (until the beginning of the 20th century) as it cannot be read directly from the manuscript (will be discussed in section 1.4).

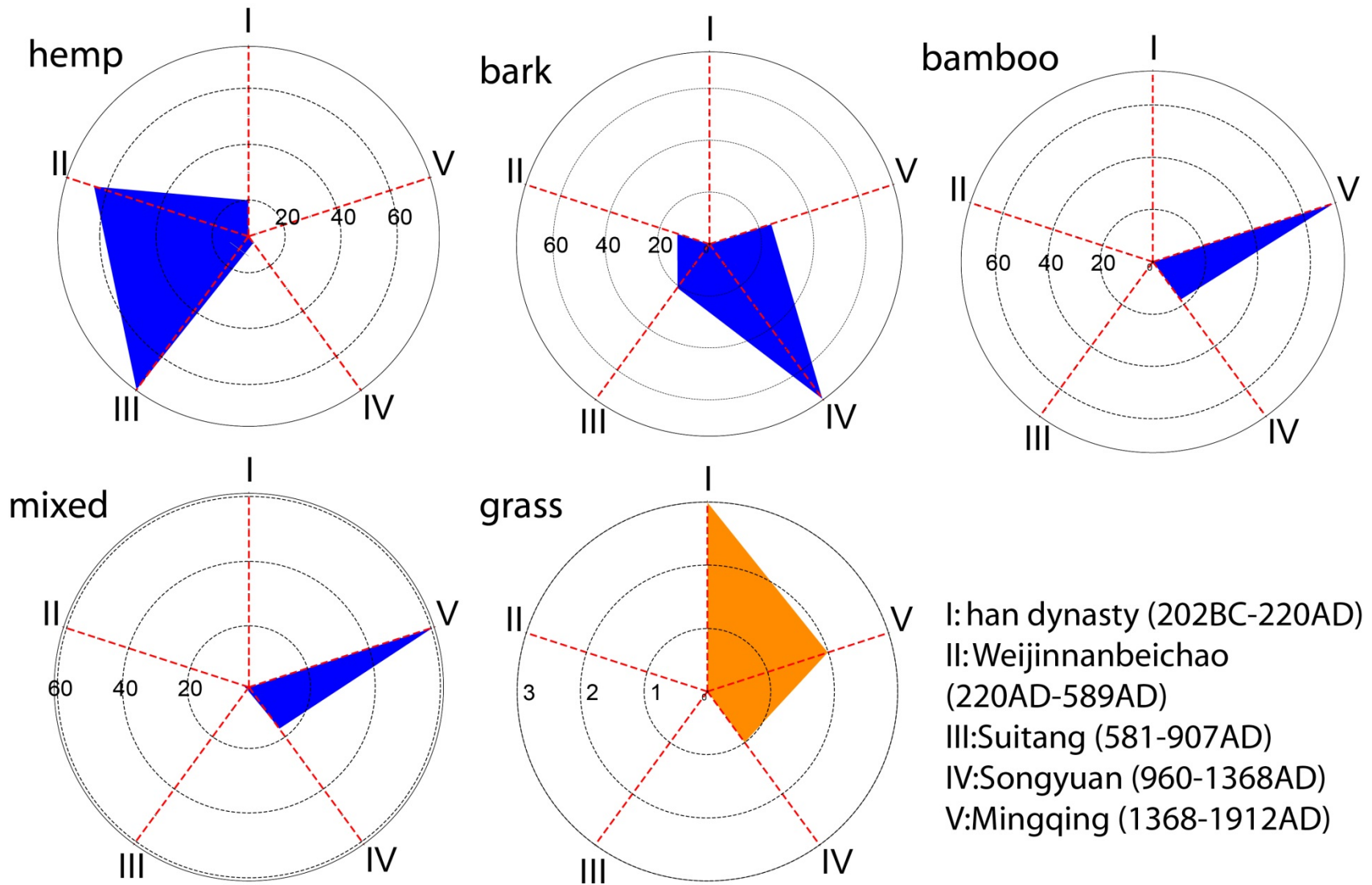


Figure 1.3.4 The fiber utilization during different period of times in Chinese history.

The main materials used in the ancient times have been introduced above and their geographic distributions were discussed. Due to the locally availability of plant resources and technique advances, a temporal change in the use of plant fibers occurred in the ancient times. For example, a statistical analysis (by Dr. Li Tao) of 493 fiber identification results of Chinese ancient papers published between 1966 and 2016 (Fig.1.3.4) showed that⁴¹:

- Hemp paper initialed in period I, got popular in period II, peaked at period III, and declined from the period III
- Bark paper get its popularity since period IV
- Bamboo paper begins to take propositions from period IV and get a large proportion in period V
- In period IV and period V, papers utilizing fibers from different plant sources with mixed fiber getting popular
- The sample amount for grass paper is less for statistical interpretations. From one aspect, it also pointed out that rice/wheat straw has not been the main manufacturing fiber source for writing purpose

The Fig.1.3.4 showed the trend using currently analyzed data, but in considering the large amount of ancient document that has not be analyzed, the figure could be upgraded upon new analyzed results. For example, fewer examples were exampled in the period I. also, the term ‘bark’ is a general term, if more detailed classification such as bark for mulberry, bark from paper mulberry is available, and the result could be more interesting.

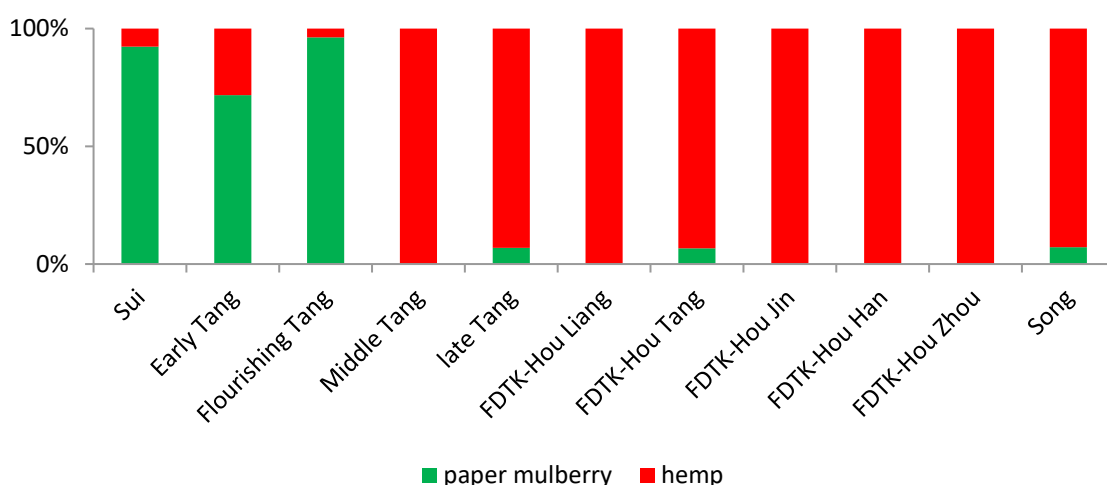


Figure 1.3.5 The material change of Dunhuang manuscript around Tang Dynasty (FDTK= Five Dynasties and Ten Kingdoms).

⁴¹ The data used in Fig.1.3.4 is from 493 fiber identification results of Chinese ancient papers published between 1966 and 2016 and collected by Dr. Li Tao (Max Planck Institute for the Science of Human History). For more information, please see his article in reference 58.

Fig.1.3.4 depicted the material change during larger time scale while in a comparatively short period (e.g. within a dynasty) the papermaking materials can also dramatically changed. For instance, Dr. Sakamoto Shouji (RyuKoku University) had noticed a material change (paper mulberry to hemp) from the middle Tang Dynasty due to the social instability (The An Lushan Rebellion) (Fig.1.3.5) [59]⁴².

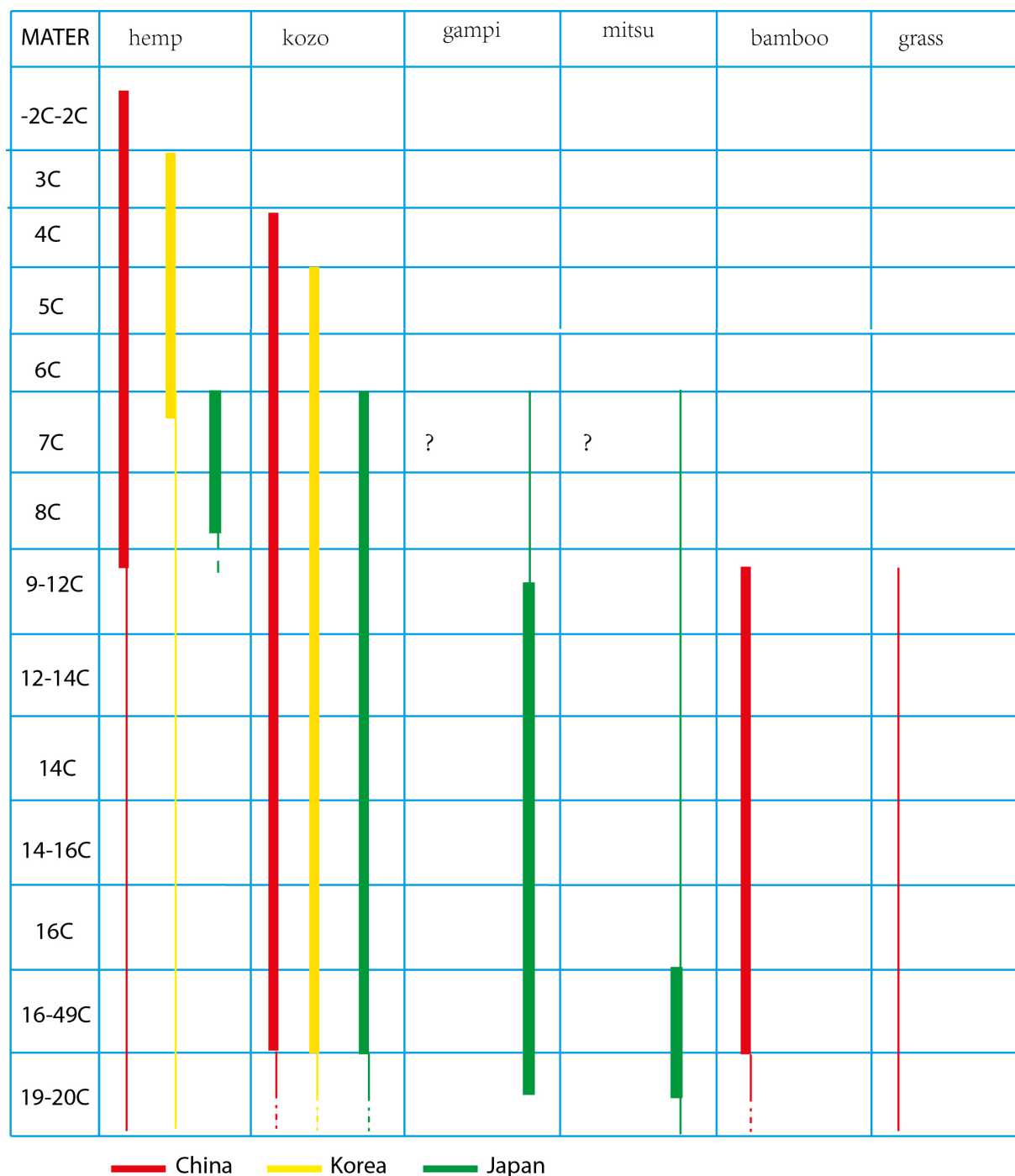


Figure 1.3.6 A sketching of the chronological material utilization in China, Korean and Japan.

⁴² The data used in Fig.1.3.5 is provided by Dr. Sakamoto Shouji (RyuKoku University). For more information, please see his article in reference 59.

Such material changes in papermaking also underwent after it distributed to the neighboring areas such as Korea [60] and Japan [61] as is shown in Fig.1.3.6. Hemp fiber has been utilized in the early stage of papermaking (before the prevalence of the papers made from the barks). The bark fiber (mainly from the paper mulberry) got its popularity as early as 3 century AD and continued to be the main material for hand papermaking into present days (especially for South Korea and Japan). The bark fibers from the *thymelaeaceae* family (e.g. gampi and mistumata) have also been utilized for papermaking in China and Japan⁴³. The prevalence of kozo papers (a sub-specie of paper mulberry) can be tested from some sample books collecting famous Japanese handmade papers (ca. 180 samples⁴⁴) through history (Fig.1.3.7).

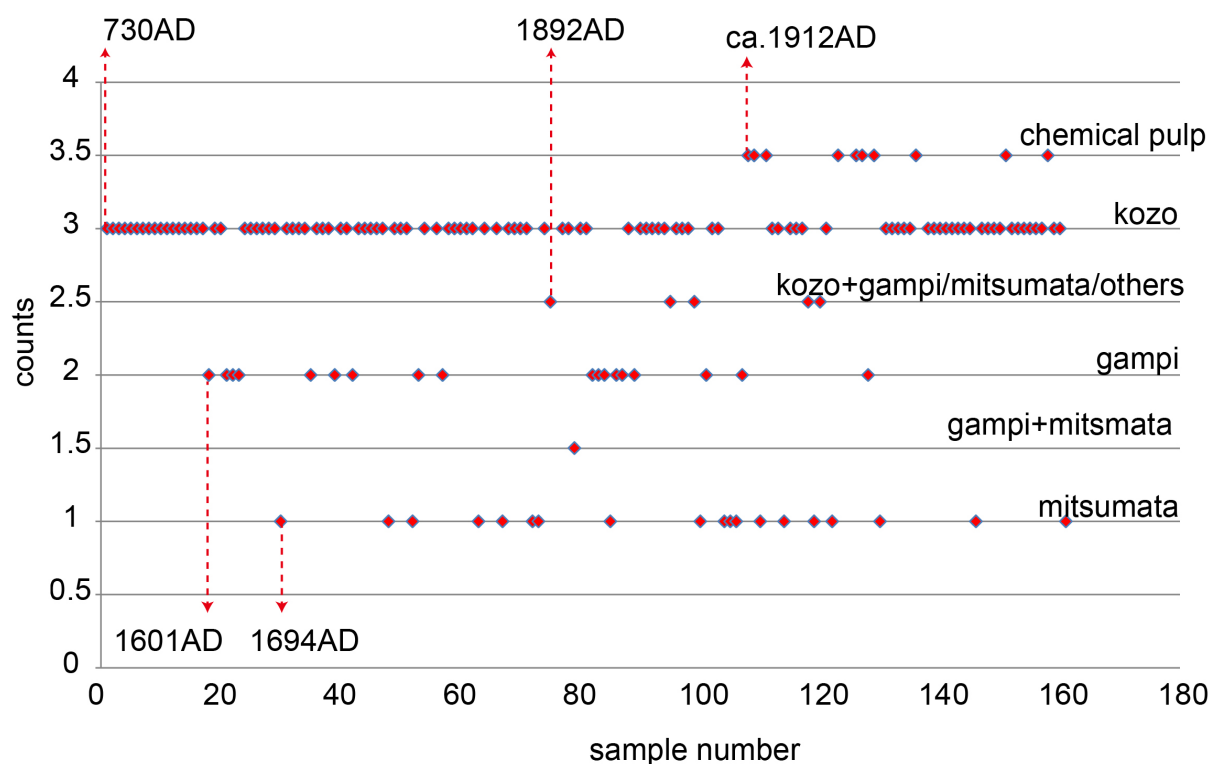


Figure 1.3.7 The statistic of the material change in a sample book collecting famous Japanese handmade papers (ca. 180 samples) through history from 730 AD to 1961AD.

The use of bamboo for papermaking was mainly occurred in ancient China as early as middle Tang dynasty and get popular from Song Dynasty⁴⁵. The popularity of bamboo paper can be interpreted as:

- Social demand: the popularity of printing makes paper in a larger demand than ever before

⁴³ They are mainly in the south and southwest parts like Yunnan province and Tibet. In Japan, kozo, mistumata and gampi are the three main raw materials for hand papermaking.

⁴⁴ This sample book is collections of Dr. Sakamoto Shouji and was kindly provided by him.

⁴⁵ Although Japan has plenty bamboo resources, making bamboo paper was seldomly practiced as the other fiber resources (kozo, mistumata and gampi) is abundant and (possibly) Japan may not be familiar with the bamboo papermaking process as the communication between China and Japan declined after Tang dynasty.

- Social change: Song dynasty (especially Southern Song dynasty) lost the fiber sources in the northern China
- Fiber resources availability: excessive utilization of bark fibers caused fiber resource exhaustion in which the abundant bamboo fiber could be an alternative substitute

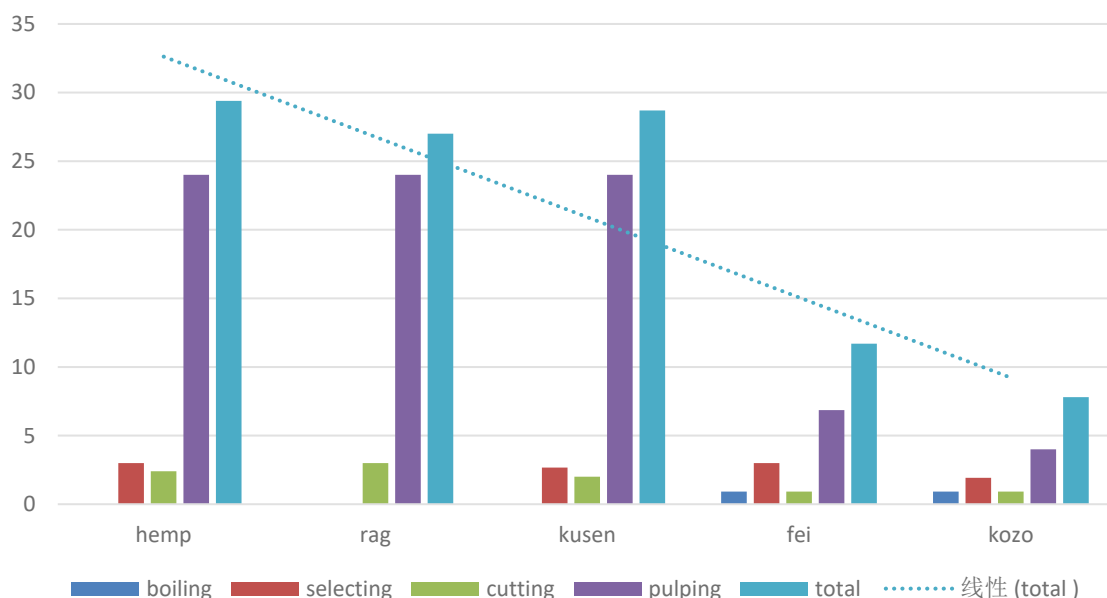


Figure 1.3.8 The workdays spend for treatment of 1.8 kg paper with different materials (kusen, *Sophora flavescens*; fei, gampi).

Hand papermaking, as indicated by its name, includes the ‘hand’ process procedures. It has to be understood that hand papermaking is a group social production behavior and the technique part shall be taken into consideration during analysis [62, 63]. The basic procedure of the hand papermaking practically remained almost the same as it was more than 2,000 years ago. The point to realize is that the output of handmade paper is kind of limited. The efforts spent for the fiber treatment is also one concern for the drive of the material change in papermaking. For instance, the Japanese historical record Engishiki⁴⁶ recorded that the annual production of paper was 20,000 sheet (1.2*2.2 chi, 1 chi=33.33cm) with the raw material of paper mulberry (1560 jin, 1 jin = ca.0.6 kg), gampi (1040 jin). The workdays for getting 1.8kg paper for rag (27 days), hemp (29.4days), kusen (28.7 days), gampi (11.7days) and kozo (7.8 days) (Fig.1.3.8). The most time-consuming process is the pulping. From the economically aspect, the bark fibers of kozo and gampi were surely getting popular as it takes less efforts to get the same amount of paper.

⁴⁶ Procedures of the Engi Era, started in 905AD, finished in 927AD and published in 967AD. Comparatively, the Chinese ancient records seldom reveal such detailed quantification information. This record was told by Dr. Sakamoto Shouji (Ryukoku University) and in discuss with him.

1.4 The scientific analysis of paper

Handmade papers as well as the carried information have long been the interest of historical research. The value of a document is generally judged by the information it carried, that is, text, graph or symbols. The traditional way of literature research cannot reveal the material from which a paper was made. Later material study became the interest of scientific analysis. Bibliophiles, historians and paleographers in Europe began to take a serious interest into the material of the manuscript on which information was written.

1670 marked the decline of the hand papermaking and the rise of machine papermaking when the Dutch first applied machine beater⁴⁷. However, at an early stage of machine-made papers, its quality cannot match those with handmade method. Under this background, the drive for setting up of the paper testing method, in turn, contribute to the scientific analysis of handmade paper in the early stage.

The handmade paper was invented in China while the early analytical techniques for its investigation were developed in Europe by applying microscopy. This section reviews the scientific approaches employed for handmade paper analysis since 1900s which has started with microscopy.

1.4.1 Microscopy-a new tool for discovery

The existence of microscopic organisms was discovered during the period 1665–1683 by two Fellows of the Royal Society, Robert Hooke and Antoni van Leeuwenhoek [64, 65]. Hooke presented the first published depiction of a microorganism, the microfungus *Mucor*, in his publication *Micrographia* (1665), later, Leeuwenhoek observed and described microscopic protozoa and bacteria [66]. By using simple microscopes that magnified objects from ca. 25x to 250x, Hooke and Leeuwenhoek discovered the important revelations from the microscopy observations. In the preface of Robert Hooke's *Micrographia*, he wrote: By the means of Telescopes, there is nothing so far distant but may be represented to our view; and by the help of Microscopes, there is nothing so small as to escape our inquiry; hence there is a new visible world discovered to the understanding.

⁴⁷ Some scholar also thought the year 1866 marked the decline of the hand papermaking when the Chinese *xuan* paper got the gold medal in world's fair in Panama which was the peak of hand paper making.

With the invention of microcopy and the establishment of the cell theory, the microscopy achieved successes in different scientific research field. In view of these great successes, microscopy found its application on the examination of the papermaking fibers which give microscopic observation a greater value in this domain: At a time, there is no other method to gain knowledge of the papermaking materials then the microscopic observation that exam different kinds of fibers as well as the mechanical state of fiber in the paper.

1.4.2 Paper identification in the early stage

1.4.2.1 The aware of the importance of natural science (microscopy)

Material origins of the manuscript cannot be directly revealed due to the pulping process. And identifying the fibers from the (historical) records was not always reliable⁴⁸. Before the late stage of the 19th century, the researchers for handmade paper document studies concentrated their investigations on the writing materials historically and linguistically. However, as far as the material investigation was concerned, mistake and errors might occur since historians and paleographers at that time were unaware of the scientific and technical methods for investigation for this purpose.

In the 1870s, some historians had declared themselves incompetent to be able to solve questions on identifying the true nature of the writing materials. For instance, in 1875, Prof. Clem. Lupi (Manuals di Palaeogratia delle carte, Firenze) directly pointed out that only botanists could judge the quality of paper fibers [67]. Nevertheless, the fact is, at that time, the botanists did not treat the questions with necessary scientific depth occasionally designed for handmade papers and they do not go beyond for the data interpretation for historical interpretations.

At the end of the 19th century, some researchers in the historical domain had realized the importance of natural science in the investigation of ancient documents. For example, Prof. Hoernle, the learned scholar in the ancient document studies, was guided by the idea that the material investigation of the antiquities could only be carried out by a natural scientist skilled in scientific investigations. Prof. Joseph von Karabacek, is also the pioneer to realize the importance of the connection of historico-antiquarian and scientific studies⁴⁹. The scientific technique pointed out by Prof. Karabacek was microscopic observation. A fact is, until the

⁴⁸ A case is provided in Annex 39.

⁴⁹ A translation of his words was provided in Annex 40.

19th century, there is no other method to gain knowledge of the papermaking materials than the microscopic observation.

1.4.2.2 A drive for paper test

A general background for the paper industry in the 19th century is the decline of hand papermaking and the rise of machine papermaking⁵⁰ as well as the advance of cellulose chemistry. The impetus to establish testing methods for paper during the last two decades of the nineteenth century may have come for the reality that the utilization of the wood pulp, especially mechanical pulp result in poor durability and permanence of paper. The rapidly expanding utilization of new materials which had different inherent properties from the traditionally used materials marked the first phase of paper testing. This point can also be revealed by the focus on the use of stain methods and microscopic observation for identification of various papermaking fibers.

Eventually, at the end of 19 century, there comes the era for standardization of paper testing method. The early recorded recognition of the importance of paper testing was point to a letter written by Carl Hoffman to the German Postmaster-General on December 11, 1882⁵¹. He suggested to the German government the necessity of establishing definite standards for paper test as well as creating a government testing department. As has been noted by Hoffman, other countries had already started works on paper test: an official British testing of this kind already established [68]. As a result of the effort of people who favors the idea of establishing the standard testing method for paper, a government laboratory was set up at Charlottenburg. One of the most famous works from this lab is the publication of the famous book on paper testing written by Herzberg in 1888 which marked the milestone in paper testing⁵² [69].

At that time, the (chemical) methods and equipment for testing paper were available that makes a rapid development of the chemical test. From 1885-1913, a total of ca.257 of papers on paper testing standard was issued from the Royal Material Audit Department as well as 7

⁵⁰ Use of wood pulp to make paper appeared at 1800 while large-scale wood paper production began with the development of mechanical pulping in Germany by Friedrich Gottlob Keller in the 1840s. In 1867, chemical pulping was invented using calcium bisulfite ($\text{Ca}(\text{HSO}_3)_2$). In 1874, the first commercial sulfite pulp mill was built in Sweden.

⁵¹ Undoubtedly, earlier paper testing in various forms can be trace back though there are not descriptions in historical records.

⁵² This publication was authorized by the Royal Commission for superintending Technical Research on July 5, 1886. And probably represented the first attempt to organize and collate paper testing procedures. A procedure for the stain is provided in Annex 41.

books published on the analysis of papers⁵³ [70]. One fact is, by 1900, paper testing was already on a firm ground [71].

1.4.2.3 A burning topic: oriental paper made of cotton

The microscopic observation of fiber was firstly conducted for the purpose of anatomy and histology in botanical studies and with case studies of fibers for papermaking. At the early stage, the research on handmade papers only served as appendix part for the botanical studies. The use of flax fiber for papermaking was firstly documented by Siegfried Reissek in 1852 with microscopic study of flax fibers with one case in papermaking (describing six different papers in total) [72].

The use of microscopic observation for handmade paper studies related to historical problems started from Charles-Moïse Briquet, Julius Ritter von Wiesner and Joseph von Karabacek [73, 74]. The initial work started in 1884 trying to find out the answer for a burning topic at that moment: was oriental paper made of cotton?⁵⁴ [75, 76]. From their own research, Briquet (1886) and Wiesner (1887) come to almost the same conclusion. After examined more than 100 samples with microscopic observation from museum and collections in France, England, Germany, Switzerland and Italy, Mr. Briquet concluded that paper entirely made with cotton were not existed. Mr. Briquet was regarded as the first to use the microscope to examine old papers and to deny the existence of pure cotton fiber [77]. However, almost at the same time, Dr. Wiesner realized that Arab paper was not made of cotton, but of similar recycled flax and hemp bast fibres as European rag paper from worn out textiles. After examination of the fiber material of more than 500 Arab manuscripts, Wiesner concluded that papers made from pure cotton fibers did not exist as previously claimed by the western palaeographers [74].

1.4.2.4 The works of Dr. Julius Ritter von Wiesner

Dr. Julius Wiesner is the pioneer for the use of microscopy to study the East Asian handmade papers. From 1886–1911, he had been involved in scientific analysis of ancient Arab paper and of archaeological papers excavated in Northwest China and Central Asia. Wiesner documented his microscopic observation of specimens of fibers with detailed drawings. The

⁵³ The historical records were only indicative of the multitude which have occurred while the complete history of the pioneers in the development of paper testing method in the 19th century were certainly more than summarized here.

⁵⁴ At that time, it was believed that the rag paper was invented in the fourteenth century of cotton according to other accounts in the eleventh to thirteenth centuries which has been proved by Wisner to be wrong and greatly shook the paleographical results of that time.

earliest publication of Wiesner's research on paper can be trace back to 1864 where he described fibers characteristic for seven fibers used for papermaking. Among these fibers, he mentioned Japanese papers from the bast fibers [78]. They are characterized by cylindrical cells, long-fiber and tissue-like softness. This microscopic description could be the first characterization of East Asian handmade papers. In 1867, Wiesner published a book relevant to the application of the microscopic-technical investigations [79]. Under the section of microscopic technology investigations (Mikroskopisch-technische Untersuchungen), microscopic examination of the paper (Die mikroskopische Untersuchung des Papiers) is one of the three applications. In this part, Wiesner concluded that the microscope was an indispensable tool to investigate the nature of a fiber and to be able to make an indisputable judgment. In Wiesner's book (1867), he mentioned to use the chemical reagent for fiber identification.

Wiesner presented the results of his scientific analysis with a lecture at the "Kaiserliche Akademie der Wissenschaften" in Vienna that marked a turning point in the paper analysis: paper history in Europe until 1887 had been based entirely on the paleographic studies of written sources (except the works by Mr. Briquet). Julius von Wiesner's scientific examination completely changed European and Western paper history on the paleographic interpretation of written sources. At the end of the 19th century Wiesner founded the scientific analysis of paper, built on optical microscopic observation, chemical tests and identification of the fibre materials through comparative analysis with a reference material [80].

1.4.3 Microscopic fiber analysis from 1940s

After Wiesner's work and the setup of standard test for paper analysis, more work has been published concerning the fiber identification as can be seen from the list in Table 1.4.1. A series of (color) fiber atlas had been published which can be used as reference for fiber identification. Another feature of this period for cellulosic fiber analysis is the further study of the polarized light microcopy (PLM)⁵⁵. The organized structures of the cell-walls of the plant fibers with different Refractive Indices were further studied for identification purpose [81]. Another way for identifying textile fibers by using the Michel-Lévy interference colour charts was also studied and showed more consistent and characteristic for synthetic fibers than for natural fibers [82].

⁵⁵ The discovery of the double reflection by Nageli in 1862 is provided in the Annex 42.

Table 1.4.1 The publications concerning the fiber identification (1940s).

date	author	content	ref
1930	Kelaney	chemical method for transverse section	[83]
1934	Clapperton	book with fiber identification	[84]
1940	Graff	color atlas for fiber identification	[85]
1942	Graff	Pulp and paper microscopy	[86]
1942	Hock	Microscopic structure of bast fibers	[87]
1943	Armitage	An atlas of papermaking fiber	[88]
1943	Dard Hunter	hand papermaking	[89]
1943(5)	Armitage	new stains	[90]
1953	Luniak	identification of textile fibres	[91]

1.4.4 Microscopic fiber analysis from 1960s

One big technical innovation in this period is the development of Scanning Electron Microscope (SEM) in 1930s, improved in 1950s and commercially available in 1965 [92]. The use of SEM in the study of wood and wood products began almost immediately after it became a practical tool and long before the system was marketed commercially. This was due partially to the ideal surface and structural makeup of wood that lends itself well to this type of investigation. For the initial studies using the SEM, specimens of single fibers were taken directly from a mill grinder pit and floated down on to a specimen stub, allowed to dry in air and metallised with a layer of silver. It was found that this simple procedure permitted the examination of intact fibres without collapse of the outer membrane. The studies were later extended to the surfaces of chemical fibres, woodchips, and newsprint with first application of SEM in this field in a study of groundwood pulp fiber [93, 94].

The main goal in these works was to exploit the capabilities of SEM in studying pulp fiber produced by different methods, with the intent of characterizing fiber morphology, nodes of structural damage and other aspects of pulp technology. However, comparatively little pertaining to techniques in preparation and handling of wood for viewing in the SEM was included in these studies. The first work that took into account the hygroscopic nature of wood was done by Washburn and Buchanan (1964). By comparison of air-dried pulp fiber webs with specimens freeze-dried from a range of moisture levels, the degree of surface modifications was evaluated [95]. This is most of the early SEM applications in the wood science. However, SEM instrumental availability for researchers is a problem up to 1969 [96].

Rezanowich (1968) noted that during the preceding years about 25,000 micrographs, mainly of wood or paper surfaces, have been accumulated by the Pulp and Paper Research Institute of

Canada. Much useful information on vessel and tracheid structure, wall thickening and pit structure as well as on the effects of chemical and physical treatment on fibers has been obtained from this applied research [97]. In 1969, in what is probably the most wide-ranging demonstration of surface topography capabilities of SEM, a Finnish publication showed photomicrographs of various types of paper [98].

At this period, more attention was paid to East Asian handmade papers for its materiality. Such as Qian Cunxun and Thomas Collings' works. The fiber identification has also been linked with the conservation practice (Table 1.4.2).

Table 1.4.2 The publications concerning the fiber identification (1960s).

date	author	content	ref
1965	Esau	Book Plant anatomy	[99]
1967	Isenberg	Pulp and paper microscopy	[100]
1970	Peterlin	Secondary wall fibrils in cotton	[101]
1973	Bole	Scanning electron microscopy of the internal cellular structure of plants	[102]
1973	Cunxun	Raw materials for old papermaking in China	[54]
1974	Parham	Papermaking materials: an atlas of electron micrographs	[103]
1976	McCrone	Authenticity of medieval document tested by small particle analysis	[104]
1978	Collings	fiber characteristic of oriental papermaking fibers	[105-107]
1978	Meynell	Fungal infection of paper	[108]
1981	Schaffer	Fiber identification in textile artifacts	[109]
1982	Catling	identification of vegetable fibers	[110]
1982	Parham	The practical identification of wood pulp fibers	[111]
1984	Collings	Identification of cotton fibers	[112]
1985	Wai	Bamboo pulp fibers	[113]
1987	Goodway	Fiber identification in practice	[114]

1.4.5 Microscopic fiber analysis since 1990s

In this period, more attention was pay to cultural heritage field applications. Microscopic analysis has become a routine analysis in museum conservation laboratories. Microscopic fiber analysis has been combined with other techniques for paper analysis. Besides fibers, other parts (crystals, phytolith) that bears information has been explored for paper analysis. Crystals are found in tissues adhering to some bast and leaf fibres and may be used to aid identification [115, 116] ⁵⁶. The continuous exploration of polarizing microscope has been applied for the differentiation of flax and hemp. Some online data base has been constructed

⁵⁶ However, because they are associated with the tissues of the plant, they will not necessarily be found adhered to processed fibres such as in totally fiber washed kozo papers.

for the fiber identification purpose (some were provided in the Annex 43). The major publications concerning the topic have been listed in Table 1.4.3.

Table 1.4.3 The publications concerning the fiber identification (1990s).

date	author	content	ref
Conservation			
1991	Florian	conservation of artifacts of plant materials	[117]
1997	Tsai	Paper conservation with various test	[118]
Fiber analysis			
1995	Xu	Simons' stain	[119]
1996	Mayer	Paper and Fiber Microscopy	[120]
2000	Jakes	Analysis of prehistoric textile fibers	[121]
2002	Woodward	Analysis of paper componets and defects by stains	[122]
2005	Cruthers	Optimized parameters for LM and SEM observations	[123]
2010	Han	Treatment of natural fiber	[124]
2010	Lowe	LM fiber analysis	[125]
2012	Ma	Fiber identification	[126]
2013	Smith	Identification of historical plant material using micro-CT	[127]
2014	Zhang	LM+SEM+XRAY characterization of kozo fibers	[128]
2016	Jablonsky	Fiber identification using its color properties	[129]
Atlas (book)			
1995	Ilvessalo	Fiber Atlas	[130]
1995	Greaves	Microscopy of textile fibers.	[131]
Review			
2000	Grieve	Fiber analysis in forensic science	[132]
2004	Biagiotti	natural fibre-based composites	[133]
2006	Zervos	Paper conservation	[134]
2008	Carr	method for vegetable textile fiber identification	[135]
2009	Hubbe	review of history, craft, and science of handmade paper	[62]
2013	Day	Botany & archaeology	[136]
2015	Kostadinovska	Non-destructive analysis of paper	[137]
Chemical composition			
1994	Srebotnik	wood delignification by white rot fungi	[138]
2005	Day	Lignin in bast fibers	[139]
PLM			
1991	Valaskovic	The Herzog effect	[140]
1994	Ye	fibril angle and the relative phase retardation	[141]
1998	Gorski	Birefringence of fibers (not accurate for natural fibers)	[142]
2000	Treacy	Comparison of mechanical and physical wood properties	[143]
2003	Wiener	Differences between flax and hemp	[144]
2005	Thygesen	quantification of dislocations in hemp fibers	[145]
2007	Garside	polarized spectroscopy of cellulosic textile fibers	[146]
2010	Bergfjord	identify flax, nettle/ramie, hemp and jute	[147]
2013	Haugan	Determining the fibrillar orientation of bast fibres	[148]
2015	Van	Spectrometric Imaging of Polarization Colors	[149]
2017	Paterson	Polarized Light Microscopy on Māori Textile Plants	[150]
Phytolith analysis			
2009	Li	Phytolith for paper analysis	[151]
Others			
2002	Chingo	Microscopy and computerized image analysis	[152]
2013	Seki	Database of traditional papermaking centers	[153]
2016	Villafana	High resolution mapping	[154]
2016	Kasiri	Dating unknown samples (written in Arabic)	[155]
2017	Brown	Characterization	[156]

1.4.6 Other techniques for paper analysis

Besides microscopic analysis, other techniques like spectroscopy based techniques and chromatographic based techniques ⁵⁷ have also been applied for the paper analysis. Spectroscopy techniques such as Raman, Nuclear Magnetic Resonance, X-Ray spectroscopy, Laser-based Spectroscopy for paper analysis have been reviewed elsewhere [157].

1.4.7 The limitations of microscopy

As underlined previously, the dating of manuscripts from their morphology and not only from their internal criteria is gradually becoming more important in manuscript studies at the end of 20th century. Furthermore, the morphological analysis can be supplemented by a microscopic or physicochemical analysis: in particular, it has been proposed that an analysis of the fibers used in the composition of the papers can reasonably make possible to confirm or invalidate a manuscript dating.

In the field of handmade paper analysis, the identification of the fibers is not necessarily easy and may lead sometimes to different interpretations. A case is reported by sinologist Prof. Jean-Pierre Drège, a great connoisseur of Chinese papers, in his study of the manuscript known as ‘Dunhuang manuscripts’. He found out that by comparing the results of the analysis (Table 1.4.4) made by Robert H. Clapperton in the 1930s and then by Thomas Collings and Derek Milner in the 1970s on the 13 manuscripts of the Stein Collections at the British Museum and dated between 406 and 991 AD, only 4 results were perfectly consistent [158]. The difference may be due to the use of polarized light by Thomas Collings and Derek Milner which made it possible to differentiate mulberry fibers from those of ramie and hemp, and to distinguish between the latter two which are often in mixture in these manuscripts. It is clear that the comparison of these results is difficult: Clapperton most often finds paper mulberry (*Broussonetia papyrifera*) (surprisingly, no hemp was found by Claperton.), whereas Collins and Milner detect more frequently fiber mixtures.

⁵⁷ The chromatographic based techniques (mainly pyrolysis) for paper analysis is introduced in the beginning of the next chapter.

Table 1.4.4 The fiber analysis of some Dunhuang manuscripts by different scholars.

Date	Period	reference	Location	Text	Clapperton	Collings	Milner	Pan	Leclerc	Chiaverina	Vidal
406	Xi Liang	S. 797	DH	vinaya	Br. pap.	Ramie + hemp					
506	Nan Liang	S.81	荊州	Sutra	Ramie	Ramie	+				
519	Nan Liang	P. 2196	建康	vow					Br. pap.	hemp	
513	Bei wei	DLG-1	DH	Sutra				hemp			
		P. 2110	DH	sutra					Br. pap.	hemp	
548	Xi Wei	P. 2273		commentary						Br. pap.	
561	Bei Zhou	S. 2082		sutra	Ramie pap.	+	Br.	Ramie pap.	+	Br.	
587	chen	P.2160	(sud)	sutra						hemp	
589	sui	p.2413	CA	sutra						Br.	
593		S.227		sutra	Br.		Br.				
600		DSB-1		sutra				Br.			
630	Tang	DTB-1	CA	vinaya				hemp			
663		P.2486		I.confucianiste					Br.	Br.	
663		p.2090	雍州	sutra					Br.		
672		P. 2644	CA	sutra						hemp	
673		S. 312	CA	sutra	Br. pap.	Ramie + hemp					

710		S.2424		sutra	Br.	Br.		
718		DTB-2	DH	t.taoique			Br.	
718		P.2371	DH	t.taoique				Br.
753		P.2257		t.taoique			Br.	
756		S.717		sutra	Br.	Ramie + Br.		
783		P. 2132		Boudhist text			Br. pap.	
803	tib	S.912	(DH)	Sutra apocryphe	Br.	Ramie + hemp		
(822)		P. 2298	(DH)	Boudhist text				Hemp
846		P. 2073	甘州	sastra			Br. pap. + Edg.	
v.850	GYJ	P.2112	(DH)	sutra			Br.	
875		S.4476	(DH)		?	Ramie + hemp		
876		P. 2681	DH	Confucianism text			Br. pap.	
909		P. 3764	(DH)	literary text				Br. pap. + hemp
929		S.4012	(DH)		Br.	Br.		
967		DWL-1		sutra				hemp
968		S.4632	(DH)	letter	Br.	Ramie + hemp		
982		P.3912		Sutra			Br.	
986		P.3403	(DH)					hemp

989	S.3985			<i>Br.</i>	<i>Ramie + hemp</i>	<i>Br.</i>
989	S. 3576	DH	letter			Br. pap.

Abbreviations:

Br. pap.: *Broussonetia papyrifera*, paper mulberry CA: Chang'an

DH: Dunhuang Edg.: *Edgeworthia papyrifera*

1.5 The chemical composition of paper

The processes of papermaking are aiming at disintegrate of the barks (or wood matrix in the modern papermaking) to yield liberated fibers proceeding to the sheet formation process. The wood and bark fibers mainly consist of three macro-molecules (cellulose, hemicellulose and lignin) that composed with the elements of carbon, oxygen and hydrogen. These macro-molecules formed interlink:

- The cellulose being an important structural component of the cell wall (crystalline, strong, and resistant to hydrolysis)
- The hemicelluloses functioned as a matrix (random, amorphous structure) between lignin and cellulose.
- The Lignin is a class of complex organic polymers functioned as an encrusting and cementing component for the rigidity of the wood and barks.

The relative content of these three polymeric constituents vary with different species, ages, and natural habitat etc. It also presents deviating values between softwood and hardwood. Besides the above three macro-molecules, certain low molecular-weight substances (usually constitute a minor fraction and classified as extractives) as well as inorganic compounds were found in wood and barks [159].

1.5.1 Cellulose

In 1838, cellulose was discovered by a French chemist Anselme Payen by isolating it from plant matter and determined its chemical formula [160, 161]. Cellulose is the most abundant organic polymer on Earth. It is the main constituent of wood (approximately 40-45% of the dry substance) located predominately in the secondary cell wall and functioned as supporting. Cellulose is a homopolysaccharide composed of β -D-glucopyranose units which are linked together by 1-4-glycosidic bonds with cellobiose being the smallest repeating unit (Fig.1.5.1). The average degree of polymerization (DP) of wood cellulose is ca. 10000. Cellulose is insoluble in most solvents due to its fibrous structure and strong hydrogen bonds. In the fiber structures, cellulose mainly deposits on the secondary wall layer (P2) (Fig.1.5.1).

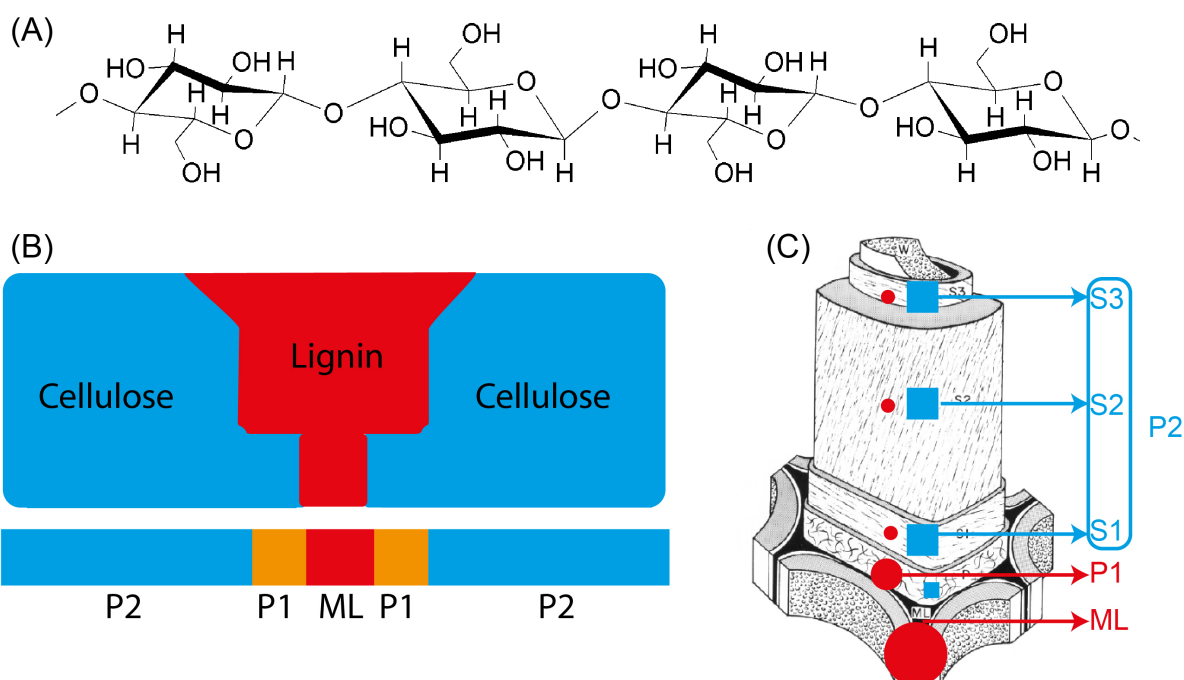


Figure 1.5.1 (A) shows the cellulose structure while (B) and (C) demonstrates the distribution of cellulose in the fiber layers (for different layers, see section 1.5.6). In (C) blue square represents cellulose and red circle represents lignin.

1.5.2 Hemicellulose

Hemicelluloses include a group of different amorphous heteropolysaccharides and are relatively easily hydrolyzed by acids to their monomeric components (D-glucose, D-mannose, D-xylose, D-galactose, L-arabinose, D-glucuronic acid, 4-O-methylglucuronic acid, D-galacturonic acid or L-rhamnose in small amount). Hemicelluloses have branched structures and have a comparatively lower DP (ca. 200). Hemicelluloses constitute 20~30% of the dry weight of wood.

1.5.3 Lignin

Lignins are polymers of phenylpropane units which can be found in all grasses, hardwoods and softwoods. Lignin is a polyphenol produced by oxidative condensation of three phenylpropanoid monomers: p-coumaryl, coniferyl and sinapyl alcohols. The phenyl moieties of such compounds differ in the hydroxy and methoxy substituents and are called p-hydroxyphenyl, guaiacyl and syringyl units (abbreviated with the acronyms H-, G- and S-units, respectively) (Fig.1.5.2). The content and ratio of the three phenyl units in different plant species varies, for instance, softwood lignin consists almost only of G-units, hardwood lignin of S- and G-units and only in grasses high amounts of H-units are found next to S- and

G-units. Their deposition in different fiber layers are also different (Fig. 1.5.1 & Fig.1.5.2). In the study of modern pulping process, the ratio of syringyl- to guaiacyl-units (S/G ratio) is often studied and a high S/G ratio is attributed to a low amount of condensed lignin structures [162]. Lignin can be hydrolyzed and extracted from the wood or converted to a soluble derivative.

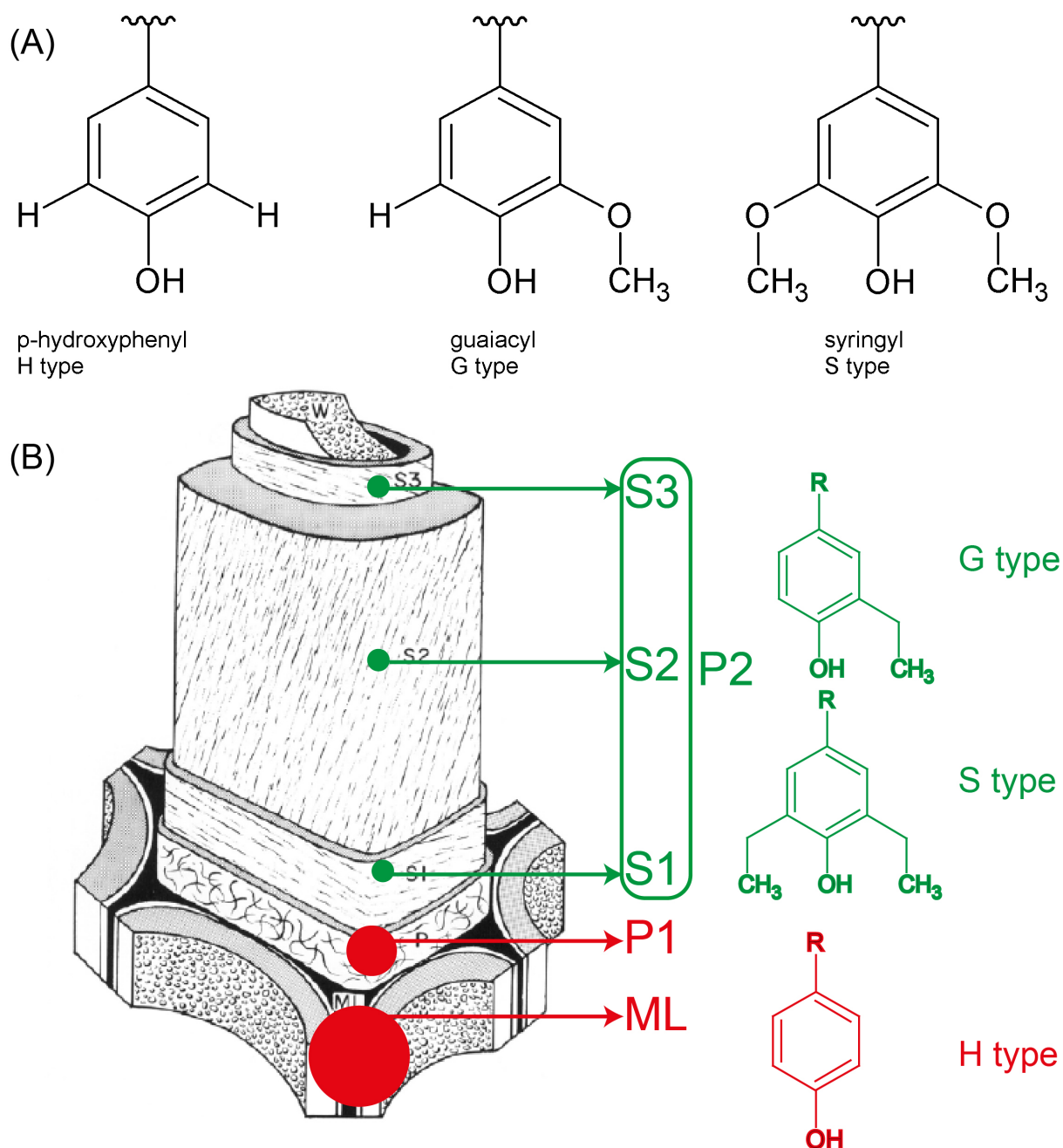


Figure 1.5.2 (A) structures of lignin precursors p-hydroxyphenyl monomeric unit (H), guaiacyl unit (G) and syringyl unit (S). (B) represents the distribution of different types of lignin in different fiber layers (for different layers, see section 1.5.6). In (B) red circle represents H type while green circle represents G and S type.

1.5.4 Extractives

The extractives refer to a large variety of wood components: a minor fraction (ca. 10%) and almost exclusively composed of extracellular and low-molecular-weight compounds soluble in neutral organic solvents or water. The extractives comprise an extraordinarily large number of individual compounds of both lipophilic and hydrophilic types. Although there are similarities in the occurrence of wood extractives within families, distinct differences in the composition even between closely related wood species with respect to both amount and composition were observed. The compounds presented in the extractives are necessary to maintain the diversified biological functions of the trees. For instance, fats can provide energy for the wood cells, whereas lower terpenoids, resin acids, and phenolic substances can protect the wood against microbiological damage or insect attacks. For analytical identification of individual components, chromatographic methods in combination with mass spectrometry play a key role.

1.5.5 Plant cells and their metabolites

Plant cells fall into two main categories: (i) undifferentiated meristematic cells capable of continuous cell division and (ii) mature or differentiated cells. The plant cells are distinguished from the animal cells by three major cytological features:

- Plastids and assimilating pigments (chlorophylls, carotenoids). Plastids are organelles limited by two membranes. They all derive from proplastids and are of two types: (i) the leucoplasts, without pigments, such as amyloplasts which accumulate starch or reserve substance, (ii) the chromoplasts, in particular chloroplasts, including pigments, chlorophylls and carotenoids, which ensure the absorption of solar energy. Of these, only chlorophyll makes it possible to convert light energy into chemical energy in the photosynthetic reaction. Other chloroplasts accumulate carotenoid pigments that give certain organs their yellow, orange, or red color.
- The wall of the cells. The wall is the outermost shell of the plant cell. It is essentially composed of carbohydrate polymers, cellulose and pectin, parietal proteins and possibly other compounds of phenolic nature (lignin). The wall is composed of three parts: (i) *the primary wall* of pecto-cellulosic nature (the primary wall exists only in the juvenile cells, extensible, it allows the cell growth or elongation), (ii) *the secondary wall*, which appears during the differentiation of the cell (it is made of cellulose and hemicellulose and is enriched in phenolic compounds like lignin to

reinforce its rigidity, cutin and suberin to waterproof the cell) (iii) *the middle lamella* is the outermost part of the wall and is common to two contiguous cells (formed the first, it consists of pectic materials).

The wall maintains and defines the size and shape of the plant cell. It participates in the regulation of relations with other cells and with the outside, and, passively, in the transport, absorption, and secretion of multiple substances. To allow communication between cells, directly from cytoplasm to cytoplasm, the walls are finely punctuated with plasmodesma.

- The vacuole. The vacuole occupies 80 to 90% of the cell volume. Young cells have several small vacuoles while the adult cell is characterized by a single vacuole. The vacuole is limited by a membrane: the tonoplasm. It contains the vacuolar juice whose composition varies according to the state of the plant. In general, its role is dedicated to the storage of water, organic solutes, mineral ions and sometimes pigments (e.g. anthocyanins). As such, the vacuole plays a major role in the regulation of the physiological functions of the plant cell (pH, osmotic pressure, ionic concentrations).

The compounds produced by plants are subdivided into two groups of molecules: primary metabolites and secondary metabolites:

- Primary metabolites are ubiquitous compounds that are necessary for vital processes, such as simple sugars, amino acids, lipids and nucleic acids. They have a vital role for metabolism and plant development. They are found in all species.
- Secondary metabolites are molecules that do not participate directly in the development of plants but rather intervene in relationships with biotic stress, abiotic or improve reproductive efficiency. They are different in different plant species. Secondary metabolites have very different functions as for examples: (i) a role of protection against the attack of pathogens or herbivores; (ii) a role of pollinator attraction; (iii) a participation in allelopathic responses (competition between plants for germination and growth)

Higher plants produce a very large range of chemicals, and the estimated number of secondary metabolites ranges from more than 25,000 for terpenoids, 12,000 for alkaloids and 8,000 for phenolic substances for those having been identified and reported in the literature at the beginning of the 2010s.

Among the biosynthesized secondary metabolites of plants, terpenoids: a diverse, widespread and numerous families of natural products constructed from an isoprene building block. Terpenoids consist of compounds with C₅ (hemiterpenoids), C₁₀ (monoterpenoids), C₁₅

(sesquiterpenoids), C₂₀ (diterpenoids), C₃₀ (triterpenoids), C₄₀ (carotenoids) skeletons including the subgroup of steroids. They are issued from the so-called Biosynthesis I channel (Krebs cycle) and are chemically generated from the all-trans squalene and 2,3-oxidosqualene through a sequence of cation-olefin cyclization generating the final complex skeleton of 4-5 hydrocarbon rings. In the course of the present work, Py-GC/MS analysis of handmade papers conducted to the detection of plant markers in the resulting chromatograms, which finally revealed to belong to triterpenoids and phytosterols.

1.5.6 Fiber

The wood cell consists mainly of cellulose, hemicelluloses, and lignin. A simplified demonstration is that cellulose forms a skeleton which is surrounded by hemicelluloses functioning as matrix and lignin as encrusting materials. The smallest building element of the cellulose skeleton is considered to be an elementary fibril and consists of 36 parallel cellulose molecules held together by hydrogen bonds [159]. The microfibrils (ca. 10~20nm) are visible in the electron microscope. Disordered cellulose molecules, hemicelluloses and lignin are located in the spaces between the microfibrils.

The cell wall is composed of several layers: middle lamella (ML), primary wall (P1), outer layer (S1) of the secondary wall (P2), middle layer (S2) of the secondary wall, inner layer (S3) of the secondary wall, and warty layer (W).

- The middle lamella M (0.2-1 μm in thickness) is located between the cells to binding the cells together. It is mainly composed of pectic substances in early stage of growth and lignified when mature.
- The primary wall P is a thin layer (0.1-0.2 μm in thickness) consisting of cellulose, hemicelluloses, pectin, and protein and completely embedded in lignin. The cellulose microfibrils form an irregular network in the outer portion of the primary wall while in the interior they are oriented nearly perpendicularly to the cell axis⁵⁸.
- The outer layer⁵⁹ (S1) (0.2-0.3 μm in thickness) contains 3-4 lamellae where the microfibrils form either a Z helix or S helix⁶⁰.

⁵⁸ The middle lamella, together with the primary walls on both sides, is often referred to as the compound middle lamella with high lignin content, however, due to the thinness of the layer, only 20-25% of the total lignin in wood is located in this layer.

⁵⁹ The secondary wall consists of three layers: thin outer (S1) and inner layers (S3) and a thick middle layer (S2). They are built up by lamellae formed by almost parallel microfibrils where lignin and hemicelluloses are located.

⁶⁰ The microfibril angle of the crossed fibrillar network varies between 50 and 70° with respect to the fiber axis.

- The middle layer (S2) (1-5 μm in thickness, varies with cell types) forms the main part of the cell wall. S2 layer have much influence on the fiber stiffness and other properties in papermaking.
- The inner layer (S3) (0.1 μm in thickness) contains microfibrils in both Z helices and S helices. Great variations are noted among different wood species.
- The warty layer (W) is a thin amorphous membrane located in the inner surface of the cell wall. Each species has its own, characteristic warty layer.

These layers differ from one another in structure and chemical composition. The microfibril winds around the cell axis in different directions and can be to the right (Z helix) or to the left (S helix). Differences in the angular directions can be observed with a microscope under polarized light and can be associated with fiber identification [159].

1.5.7 Bark

Bark is the layer external to the cambium that surrounds the stem and can be divided into living inner bark (phloem) and dead outer bark (rhytidome) (Fig.1.5.3). It is composed of different cell types and each species is characterized by specific features of its bark structure and component. Bark can roughly be divided into the following fractions: fibers, cork cells, and fine substance including the parenchyma cells. The fiber fraction is chemically similar to that of the wood fibers and consists of cellulose, hemicelluloses, and lignin. The other two fractions contain large amounts of extractives. The walls of the cork cells are impregnated with suberin, whereas the polyphenols are concentrated in the fine fraction. The diversity of bark utilization derives from the variety of the bark of different species and from the possibilities encoded in the material [163].

The chemical composition of bark varies with different tree species as well as the morphological elements involved. Bark has comparatively high content of certain soluble constituents (extractives) that can be roughly divided into lipophilic and hydrophilic constituents. The lipophilic fraction⁶¹, consists mainly of fats, waxes, terpenoids, and higher aliphatic alcohols and the hydrophilic fraction⁶² contains large amounts of phenolic constituents.

⁶¹ Extractable with nonpolar solvents (ethyl ether, dichloromethane, etc.)

⁶² Extractable with water alone or with polar organic solvents (acetone, ethanol, etc.)

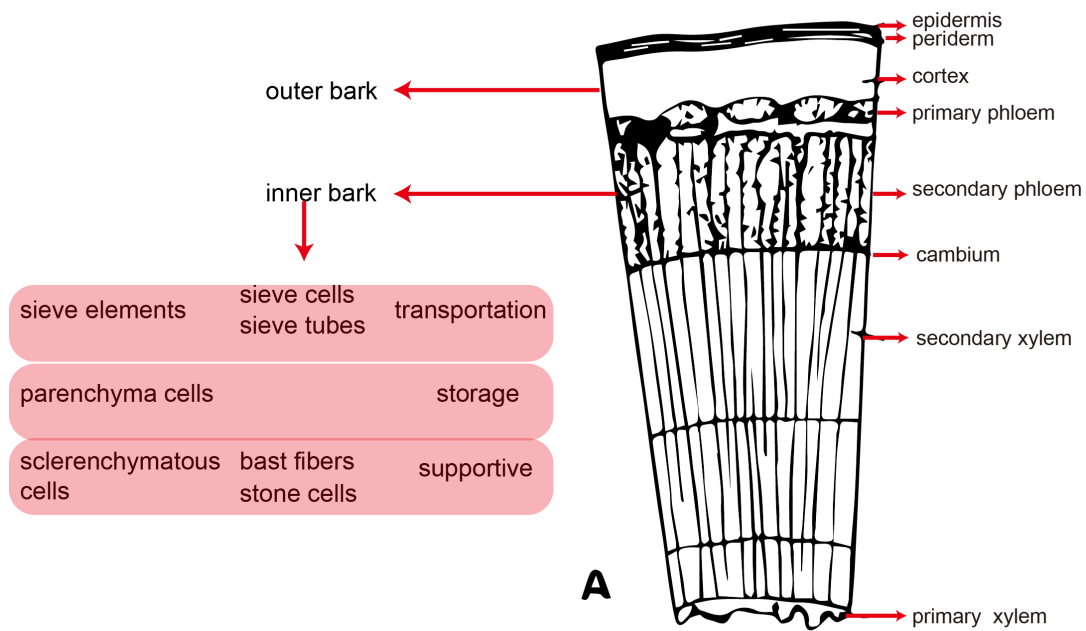


Figure 1.5.3 Main bark tissues⁶³.

⁶³ The primary growth means direct production of embryonal cells at the growing points of the stem apex and their further development to primary tissues, The formation of secondary tissues takes place in two special meristems, in vascular cambium, which produces the secondary phloem, and in the cork cambium (phellogen), which generates periderm.

1.6 The situation of handmade paper

1.6.1 The general situation as cultural heritage

As stated above, some scholar thought the year 1866 marked the decline of the hand papermaking when the *Xuan* paper got the golden medal in world's fair in Panama which was a sign of the peak of hand papermaking. Like other tradition craft practice, hand papermaking also faced with the reality of rapid growth towards modernization, in which there is little room for the valuable tradition to be reinvented and hundreds of mills had disappeared. It also faces the problem of inheritance with finance problem and lack of young people willing to master the skills which cost vigorous physical labor. This can be testified by the current remaining hand papermaking mills in different countries. For instance, in China, when Prof. Tang Shukun (University of Science and Technology of China), conducting a field survey of the current papermaking mills in China, the problem happens as there are small number of papermaking mills dispersed especially in the remote areas.

As an ancient craft, hand papermaking is a well tradition to keep and formed a link with our past history. It is a practice well deserved to keep. In fact, it has drawn attention from the UNESCO and government for the preservation of the hand craft. Hand papermaking practice has been listed as Intangible Cultural Heritage by UNESCO (Table 1.6.1) as well as different countries (Table 1.6.2).

The hand papermaking takes lots of human labor while the productivity is far behind the machine. Its comparatively low consistency of each sheets prevented its wide application in the daily life. However, hand papermaking is a tradition linked to the past, to the history as well as to the ancestor. Hand papermaking has been listed as intangible cultural heritage, which is sign of its culture importance as well as the rare fact. With the raised attention from the UNESCO, the government, the institutions and the public, hand papermaking may have the opportunity to come from a corner to the center stage.

Table 1.6.1 The papermaking related in the UNESCO list of the Intangible Cultural Heritage.

Representative List of the Intangible Cultural Heritage of Humanity	Country	Year
Washi, craftsmanship of traditional Japanese hand-made paper	Japan	2014
Chinese paper-cut	China	2009
China engraved block printing technique	China	2009
Chinese calligraphy	China	2009
Traditional handicrafts of making Xuan paper	China	2009

Table 1.6.2 The papermaking related in the Chinese list of the Intangible Cultural Heritage.

No.1	No.2	content	Location
415	VIII—65	the technique for making xuan paper	Jingxian, Anhui province
416	VIII—66	The technique for making qianshan liansizhi (bamboo paper)	Qianshan county, Jiangxi province
417	VIII—67	the technique for making bark paper (longyou bark paper, pingyang bark paper)	Guiyang City, Zhenfeng county, Danzhai county, Guizhou province Longyou county, Zhejiang province Xiangfen county, Shanxi province
418	VIII—68	The papermaking from Dai and Naxi minority	Lincang city, Xianggelila city, Yunnan province
419	VIII—69	The papermaking of Tibetan	Tibet Autonomous Region
420	VIII—70	the technique for making mulberry paper	Turpan, the Xinjiang Uygur Autonomous Region Qianshan county, Yuexi county, Anhui province
421	VIII—71	the technique for making bamboo paper	Jiajiang county, Sicuan province, Fuyang county, Wenzhou city, Zhejiang province Jiangle county, Fujian province Leiyang county, Longhui county, Hunan province
914	VIII—131	the technique for making paper mulberry paper	Xi'an city, Shaaxi province

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Chapter 2 pyrolysis of papers: the experimental considerations

In chapter 1, the history and origin of papermaking and the method for manuscript study (in related to the material study) have been introduced. The problem encountered such as different fiber identification results from different researchers has been noted. In exploring and providing a new method for handmade paper fiber identification (or more generally a new approach on chemotaxonomy since the plant markers used were related to the metabolites from different plant species), the capacity of Py-GC/MS and Py-GCxGC/MS has been studied in this chapter. This chapter defines the experimental conditions of the analysis that includes:

- Section 2.1: a short review of the application of analytical pyrolysis in cultural heritage application, more specifically, for (handmade) paper analysis
- Section 2.2: the identification of the compounds from the pyrolysis of handmade papers and the origin of the compounds confirmed by liquid extraction and the comparison with raw plant fibers
- Section 2.3: the introduction of GCxGC and the discussion of its principal and settings
- Section 2.4: the introduction and benefit of GCxGC for cultural heritage material studies illustrated using the example of handmade papers
- Section 2.5: following the discussion in section 2.4, the re-thinking and exploring 'how these benefits of GCxGC can be used for 1D data treatment' make it an evaluation carried out using GCxGC and AMDIS software

In all, this chapter demonstrated that pyrolysis is a quick and reliable way for fiber characterization and identification on the base that different plant species have different metabolites synthesis pathway in which these metabolites can be used as characteristic markers for handmade papers from different plant sources.

Another emphasis in this chapter lies in the introduction of GCxGC (Py-GCxGC) methodology into the field of cultural heritage where 1D analysis is most commonly performed while the 2D application is rare.

2.1 The role of pyrolysis in cultural heritage and in paper analysis

2.1.1 The general of chromatographic techniques

The basic procedure for chromatographic analysis mainly involves sampling, separation, detection and interpretation. On the first step, sampling aims to introduce the analytes into the chromatographic system while different ways can be applied:

- The most common and traditional method is to use a solvent to extract the target or potential compounds for injection. Sometimes, it can require comparatively large amount of quantity for extraction protocol and usually proceed to a dilution process afterwards to avoid saturation of the detection. The choice of the solvent is another concern for an effective extraction.
- Solid-phase microextraction (SPME) employs a solid phase extraction sampling technique which uses a fiber coated with an extracting phase (liquid polymer or solid sorbent) for extracting different kinds of analytes. For instance, it has been applied for the paper analysis especially for the volatile organic compounds from the books and manuscripts [1, 2].
- Pyrolysis is a thermal decomposition of materials (commonly organic polymers) at elevated temperatures within an inert atmosphere that produces volatile products as well as solid residues.

The separation procedure is applied after the introduction of the samples. Normally, the introduced samples were often comprise/include mixed compounds and the separation procedure applied benefits for their detection. Gas chromatography is the type of chromatographic technique for analyzing compounds that can be vaporized (without decomposition). The basic function of separation is to separate different compounds with further ability to determine the relative amount as well as the purity of the compound(s). It basically utilizes two phases: mobile phase (usually inert gas helium) and stationary phase (microscopic layer of liquid or polymer on an inert solid support). The introduced compounds have different characteristic in interacting with the stationary phase that causes them to elute at a different time, defined as retention time. The retention time as well as the retention index showed significant analytical usefulness especially when comparing result between different platforms and laboratories. For an improved separation, multidimensional techniques such as GCxGC have been observed with increasing applications.

After the separation of the compounds, the samples enter the detector for recording the signals, which mass spectrometer is usually used. Detector records the physical response which corresponds to the intensity of the compounds. There are different kinds of mass analyzer instrumentations such as quadrupole mass spectrometer (qMS), high resolution mass spectrometer (HRMS), and time-of-flight mass spectrometer (ToF) that are suitable for different applications.

2.1.2 Analytical pyrolysis and its cultural heritage applications

The principle of GC/MS lies in the volatility of samples: only the samples that can be volatilized are suitable for GC/MS analysis. In this sense, the samples in the catalog of non-volatiles (such as polymers, cellulose etc.) are difficult for GC/MS application especially those insoluble substances difficult to analyze by any chromatographic techniques. The analytical pyrolysis, as one sampling method, in combination with GC/MS solved this intractable problem.

Analytical pyrolysis is a technique that applies enough thermal energy to cause bond cleavage and analyzes the resulting fragments by coupling techniques such as gas chromatography, mass spectrometry, or infrared spectroscopy, among which GC/MS coupling prevails. The intrinsic value of analytical pyrolysis lies in the decomposition of the macromolecule to the greatest possible extent whilst maintaining the size of the fragments as large as possible. The beginning of modern analytical pyrolysis can be traced back to 1950s with the early reports on polymer analysis using both off-line and on-line pyrolysis-mass spectrometry (Py-MS). In 1948, Madorsky Samuel and Sidney Straus reported the first application of off-line pyrolysis-MS (Py-MS) for polymer analysis [3] and in 1953, on-line Py-MS applications was described by Paul Bradt et al [4]. In 1966, the coupling of Py-GC/MS was firstly reported by Simon et al[5]. It is interesting to note that cultural heritage material studies were among the early applications of analytical pyrolysis in 1960s [6-8]. The technical development of analytical pyrolysis is associated with other techniques and the chronicle for the development can be found in the historical review from Prof. Shin Tsuge [7].

The importance of pyrolysis lies in that it expanded the application of GC/MS for material characterization when the modern techniques of mass spectrometry (such as electrospray ionization (ESI), matrix-assisted laser desorption/ionization (MALDI)) were not available. It expended the application limit by employing thermal decomposition of macromolecules in the absence of oxygen into volatile and semi-volatile molecules that can be easily analyzed by

GC/MS. The observed fragments can be used as fingerprints of the spectral characteristic of a particular sample, in terms of fragment nature and relative concentration.

After more than 60 years of development, analytical pyrolysis has become a mature technique and it is interesting to note its applications in various fields. For this purpose, the core collection database of web of science (current producer of Clarivate Analytics and previously Thomson Reuters Corporation) was searched using ‘pyrolysis’ and ‘review’ as keywords in title. A total of 137 literatures (until March, 2017) were retrieved and analyzed in Histcite (a software package used for bibliometric analysis) where the links reported between references express citation by other authors. As can be seen from Fig.2.1.1, among the analysis review works in pyrolysis, the earliest appeared in 1960 by Manuel Gomez and Charles Prien for pyrolysis of coal and oil shale [9]. Unfortunately, this work has not been cited afterwards by the analyzed review works (node 1 in Fig.2.1.1). It is not until late 1990s that a citation network began to form with Prins and Meier’s review article on rotating cone technology for flash pyrolysis and fast pyrolysis of lignocellulosic materials [10, 11] (node 26, 29 in Fig.2.1.1). In 2006 and 2007, Mohan and Zhang’s review work on pyrolysis of wood/biomass for bio-oil has been largely cited and it is clearly indicated that pyrolysis of biomass for energy research is the hot topic in this domain [12, 13] (node 32, 33 in Fig.2.1.1). This is also attested by Bridgwater’s review article on fast pyrolysis of biomass in 2012 with nearly 900 citations [14] (until March, 2017).

In Fig.2.1.1, it is also very interesting to note that there are two lines not connected to the central network. The two lines with only three nodes (node 20, 31 and 85) are representing the application of pyrolysis in the field of cultural heritage, arts and archaeology. The early review can be found with Shedrinsky who made the first review of the application of pyrolysis in the cultural heritage field citing 37 articles in 1989 [15]. The Fig.2.1.1 indicated a situation that cultural heritage field is small enough to be overlooked as a source of commercial applications within pyrolysis research. Nevertheless, it has contributed to foundational developments in cultural heritage studies to solve important problems such as interpretation of our past history and protection of our current relics.

Nearly 30 years have passed after Shedrinsky’s first review of pyrolysis in the field of cultural heritage, lots of new applications of pyrolysis had been carried out in this field which is greatly helpful for the interpretation of archaeological context and for the conservation of cultural relics. However, there has not so many review works been reported except a mini review of pyrolysis for lacquer research and a review for binding agent in paintings [16, 17].

Until recently, more comprehensive reviews of pyrolysis in the cultural heritage applications have been reported [18, 19].

2.1.3 Analytical pyrolysis in paper analysis

As one of the main cultural heritages, cellulose based documents are a kind of special material subjected to environmental contamination and natural degradation. Pyrolysis is a way to permit the analysis of paper by GC/MS with minimum quantity and a simple sampling process. In practice, pyrolysis with controlled conditions has proved to be a fast and reproducible method for the characterization of cellulosic materials.

The earlier application of pyrolysis to investigate cellulose based ancient documents was found to be by scholar Hans G. Wiedemann in 1983 to study the age, composition, and processing of ancient Egypt papyrus, a writing material made from the pith of the papyrus plant, *Cyperus papyrus* [20]. At that time, pyrolysis coupling to mass spectrometry allows the integrating registration of all volatile pyrolyzates generated in the complete pyrolysis procedure. The amount of sample necessary for such studies is 10-50 µg. Typical mass spectra recorded during the pyrolysis of cotton cellulose, lignin, and the ancient papyrus were provided. The spectrum of cellulose indicates the presence of levoglucosan (MW 162) and cellulose decomposition products, as furan (MW 98), acetic acid (MW 60), and ionized methanol (MW 32). When present, the spectrum of lignin shows the presence of coniferyl alcohol, a lignin monomer (MW 180), as well as other substituted lignin phenols. The effect of age on cellulose structure was also investigated by recording mass spectra of pyrolysis products from cotton cellulose and papyrus of various ages. Peaks with MW at m/z 162 and 144 decreased or disappeared with increasing age indicating that levoglucosan undergoes single or multiple dehydration with time. Eventually, it is transformed to levoglucosenone (MW 126) as a result of dehydration. MS results of analytical pyrolysis on dated ancient papyrus show that with increasing age the levoglucosan (m/z at 162) becomes dehydrated to levoglucosenon (m/z at 126). This technique was further used to investigate the origin of old documents by the same author [21, 22]. Experimental results obtained on a variety of ancient objects prove that the combination of analytical pyrolysis and thermal analysis was a powerful tool for ancient document analysis at that time.

In early applications, Py-GC was also applied for the characterization of paper sizing agents for alkylketene dimers (AKDs) and alkenylsuccinic anhydride (ASA) [23]. The peaks of intact AKDs and related ketones in the pyrograms were used as the key peaks for the determination of AKDs in paper samples containing between 0.025 and 1% of AKDs.

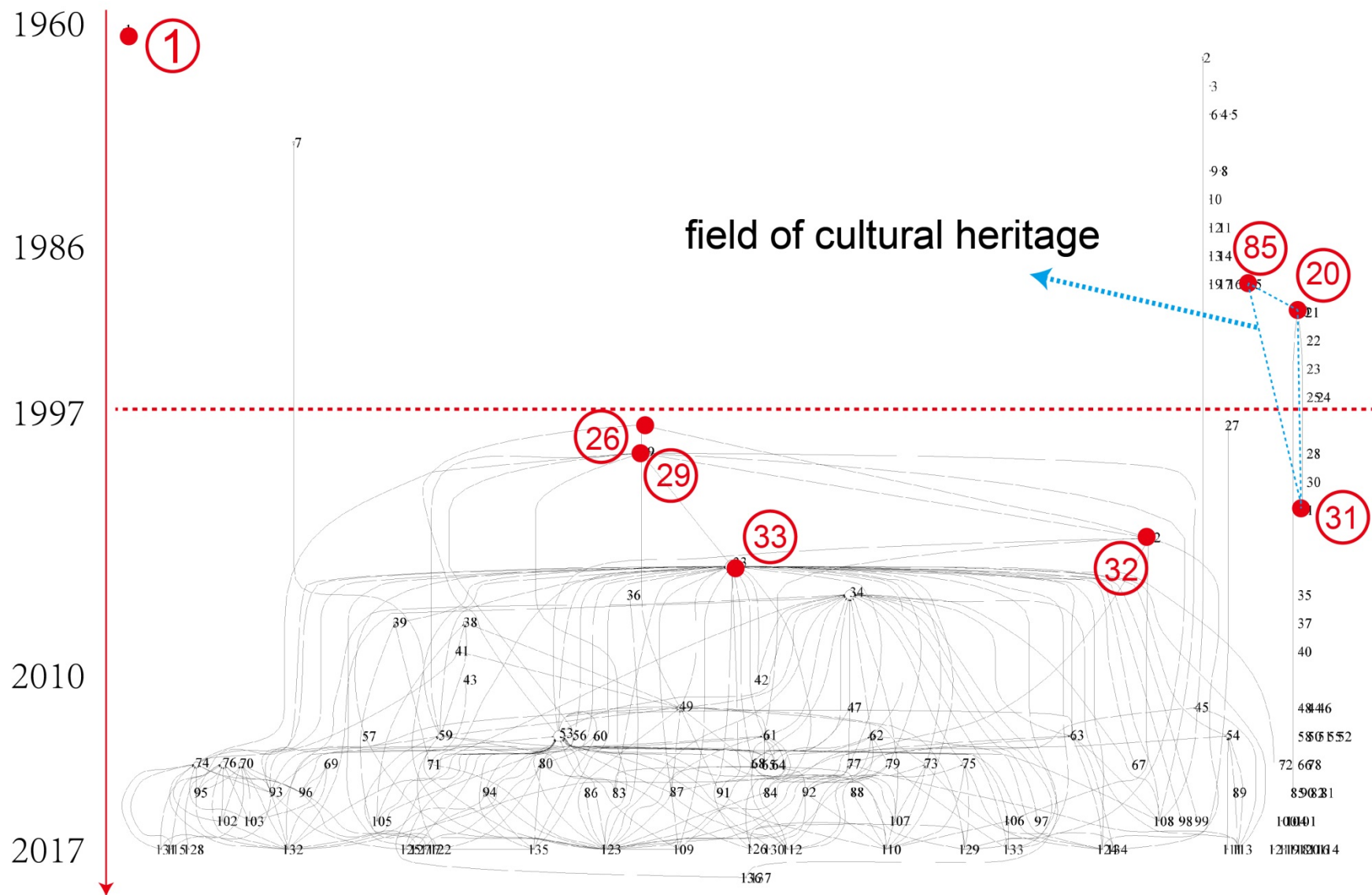


Figure 2.1.1 The bibliometric analysis of 137 articles retrieved from the core collection database of Web of Science (Thomson Reuters Corporation) using 'pyrolysis' and 'review' as keywords in title (until March, 2017).

The results obtained suggest that almost 75% of the AKDs added are retained in all the paper samples. Further, the relationship between the AKD content and the degree of sizing is interpreted in terms of the possible paper sizing mechanisms. The ASA content in paper was also determined in essentially the same way as for the AKDs, and is discussed in relation to the degree of sizing. The characterization of sizing resins and styrene copolymer was also reported [24-26].

As has been discussed in the above paragraph, the examples for the early application of pyrolysis coupling with MS or GC have been focused on the characterization of ancient document and handmade paper sizing agent. It showed its advantage as a useful tool for paper-based cultural heritage studies. However, due to the limit of technology at that time, pyrolysis coupling with MS without separation techniques cannot provide pure mass spectra for each compound, and pyrolysis coupling with GC without mass spectra information for identification also hinders wider application of pyrolysis for cellulose cultural heritage studies.

By the 2000s, computerized GC/MS instruments using quadrupole technology had become essential to chemical research and one of the foremost instruments used for organic analysis. This largely contributed to the wide application of pyrolysis: Py-GC/MS became a routine analysis within analytical laboratories and it has been used to identify the structure of thermal degradation products with the aim to characterize different materials including paper.

In a work by scholar Teruyuki Seine et al [27], the authors propose a new method to analyze quinone/hydroquinone species known as chromophores/leuco-chromophores derived during photo-irradiation by Py-GC combined with mass spectrometry (MS) using chemithermomechanical pulp (CTMP) paper samples. They found that the photo-yellowing of CTMP paper was closely related to the formation of quinone/hydroquinone without any methoxyl groups which was produced during photo-degradation of lignin structure.

Scholar Yeghis Keheyan [28, 29] studied the cellulose pyrolysis products with and without the methylating reagent tetramethylammonium hydroxide (TMAH). By comparison with model samples, pyrolysis was proved to be a good technique in characterizing modern paper and naturally aged papers due to its possibility to observe the changes in aged and unaged samples. The influence of inks on the degradation of papers was also noticed.

Analytical pyrolysis has also been applied to the characterization and evaluation of the deteriorated acidic paper in old books. One problem of document conservation is the gradually deterioration especially for those with rosin-alum additive used for sizing since the late 19th century. To preserve and restore appropriately such degraded materials, evaluating

the degree of deterioration is required. In the work carried out by Prof. Ohtani Hajime et al the result indicated that levoglucosan and its dehydrated form levoglucosenone were typical degradation products while a recent control paper gave rise mainly to levoglucosan. The relative yield of levoglucosenone was much higher for the paper sample taken at the heavily deteriorated marginal part than that in the center of the same page of the old book. Accordingly, levoglucosenone observed in the pyrogram can be used as a marker to evaluate the degree of the deterioration of the acidic paper [30]. The formation of levoglucosenone can be attributed to the slow deterioration process of paper, in which dehydration reaction play an important role accompanied by the chain scission of cellulose. The defined method for paper evaluation was also applied to clarify the effect of storage conditions and chemical compositions of paper materials to their deterioration processes. Besides ancient and museum collection papers, pyrolysis has been also frequently used as an analytical method for the characterization of machine-made papers [31]. For instance, pyrolysis-GC/MS has been applied for quantification purpose of traces of two types of bisphenols (bisphenol A and bisphenol S) in machine made paper samples for their related health and the environment concern [32].

In short, compared with the ‘hot topic’ (demonstrated in Fig.2.1.1), pyrolysis of paper (especially handmade papers) have limited reports (excluding those of pyrolysis of cellulose for biomass and energy purpose). The application of pyrolysis in the study of ancient documents and handmade papers can be summarized into three stages:

- In the early stage, pyrolysis coupling with MS or GC for an imprecise material characterization
- Later, Py-GC/MS became a routine analysis method for document characterization
- Recently, Py-GC×GC/MS has been reported and showed its advantages in handmade paper characterization as will be discussed in this chapter and in chapter 3.

2.2 Pyrolysis of handmade papers: compounds identification

2.2.1 Experimental conditions

2.2.1.1 Pyrolysis

During these three years, Py-GC/MS analyses were performed using a vertical micro-furnace-type pyrolyzer PY-2020iD (Frontier Lab, Japan) directly connected to the injection port of a Shimadzu QP2010 gas chromatograph linked with a Shimadzu QP2010Plus quadrupole mass spectrometer (Shimadzu, Champs-sur-Marne, France) for 1D Py-GC/MS analysis. As depicted later, 2D Py-GCxGC/MS analysis was conducted with the upgraded vertical micro-furnace-type pyrolyser PY-3030D (Frontier Lab, Japan). By principle, the setting of the two models is similar. The model PY-3030D proposed a higher heating rate for the oven due to an improved conception of the heating ceramic, a quicker cooling efficiency, and a simplified maintenance. A schematic drawing (Fig.2.2.1) reports the main features of the PY-3030D pyrolyzer.

At the opposite to flash pyrolysis where the sample is thermally degraded by the heating of a wire filament calibrated in temperature, oven pyrolysis results from the introduction of a sample in a heated zone controlled by a ceramic heater (Fig.2.2.1). The main differences between the two systems reside in the heating rate (faster in the flash pyrolysis system) and the residence time (increased in the oven pyrolysis system).

For the experiments, the sample is placed in a stainless-steel sample cup and the sample cup is positioned on the top of the pyrolyzer at near ambient temperature (sample standby position in Fig.2.2.1). The cooling fan system maintains the sample in the cup at roughly room temperature. The sample cups and the paper samples were weighted with an XP2U Ultra Micro Balance (Mettler Toledo, Viroflay, France). Typical quantities of reference paper used in the course of our work for the development of the analytical method ranged from 80 to 110 μg weighted with the microbalance. For the balance's capacity used (2.1 g), readability and repeatability of the microbalance were 0.0001 and 0.0002 mg, respectively.

When the analysis started, the sample cup is introduced into the furnace at 500 °C for the pyrolysis of paper samples, and then the temperature program of the gas chromatograph oven was automatically started by the electronic servo control of the gas chromatograph to the pyrolyzer. To avoid recombination of pyrolysis products and their deposition on the surfaces between the oven and the entrance of the column, all the transfer parts are kept heated. During

analysis, the Py-GC interface was held at 320 °C. The injector of the chromatograph was held at 280°C.

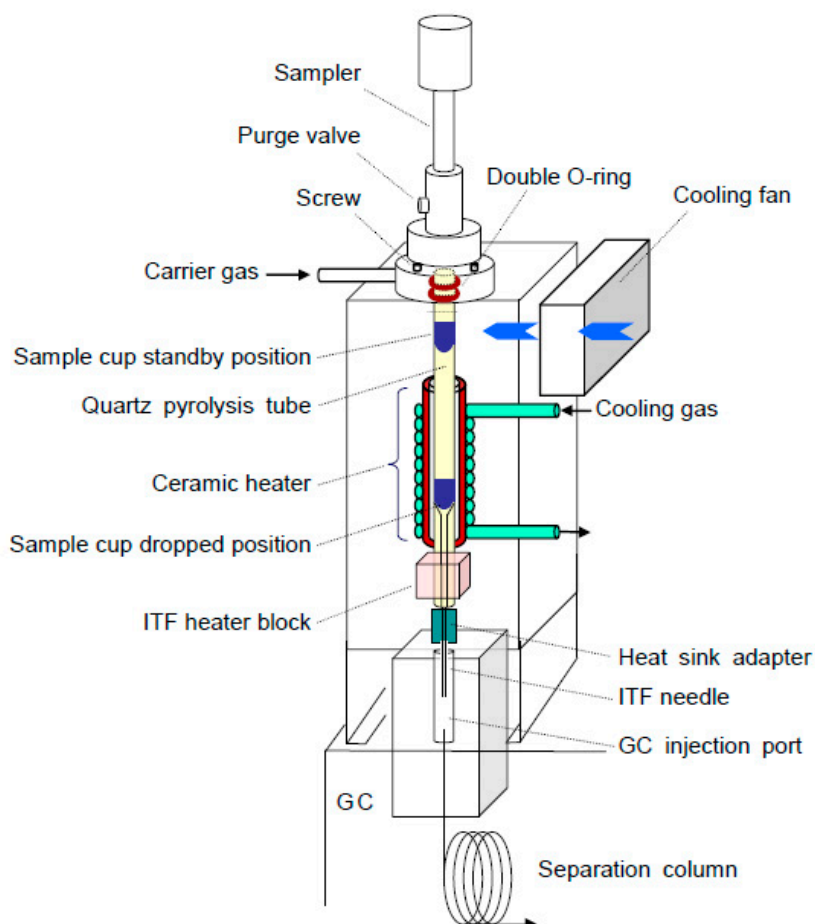


Figure 2.2.1 Schematic drawing of the PY3030D pyrolyzer with its main components.

Chromatographic separation was typically carried out on a DB5-ms 5% phenyl–95% dimethyl polysiloxane fused silica capillary column (30 m length, 0.25 mm inner diameter and coated with a 0.25 μm film thickness) from Agilent (Courtaboeuf, France) or Frontier Lab (Koriyama, Japan). The oven temperature was initially held 3 min at 40 °C, and then ramped at 5 °C min^{-1} to 325 °C, where it was held for 10 min. The total duration of GC analysis was 70 min. The helium carrier gas Alphagaz 1 (Air Liquide, France) was used in the linear velocity mode (1 $\text{mL}\cdot\text{min}^{-1}$). The injector, held at 280°C, was used in split mode (1:10 of the total flow) as the pyrolysis would not be compatible with a splitless injection, which would favor recombination of the pyrolysis products or their loss by deposition on the liner. The mass spectrometer was usually operated at 2500 u s^{-1} , with a scan range from 40 to 500 u and 50-500 u (to avoid the influence of m/z at 44 which is intense at the beginning), using electron ionization at 70 eV (for checking the possible presence of higher molecular weight

components, experiments have been conducted with an extended mass range of 50-800 u). The interface was kept at 300 °C and the MS source at 200 °C. The mass spectra of the triterpene pyrolysis products were recorded using the average spectrum + average & subtract spectrum modes in the Shimadzu GCMSsolution software. Identifications were achieved on the basis of EI mass spectra by interpretation of the main fragmentations and using the NIST MS library (2011).

2.2.1.2 Liquid injection

For GC/MS analyses, we used a Trace GC Ultra DSQ II ThermoScientific instrument (Thermoscientific, Courtaboeuf, France). Around 100-150 mg of reference samples were extracted with methanol (3 mL) during 24 h by agitation with a magnetic stirring bar. After filtration, the solution was gently evaporated under nitrogen at room temperature until a one half reduction of volume for obtaining a solution of ca. 1mg.mL⁻¹. The sample was diluted in methanol (v:v 1:10) before analysis. Chromatographic separation was carried out on a Phenomenex ZB-5HT Inferno 5% phenyl-95% dimethyl polysiloxane fused silica capillary column (30 m length, 0.25 mm inner diameter and coated with a 0.25 µm film thickness) at a constant flow rate of 1 mL.min⁻¹. The oven temperature was initially held 3 min at 40 °C, and then ramped at 5 °C min⁻¹ to 325 °C, where it was held for 10 min. The total duration of GC analysis was 70 min. The injector was held at 280°C and used in split mode (1:10 of the total flow). The mass spectrometer was operated at 3000 u.s⁻¹, with a scan range from 50 to 900 u, using electron ionization at 70 eV. The interface was kept at 300 °C and the MS source at 200 °C. Identifications of products were achieved on the basis of EI mass spectra by interpretation of the main fragmentations and using the NIST MS library (2011).

2.2.1.3 The samples

Materials used in the present study consisted of three types of handmade papers made from kozo (*Broussonetia kazinoki* Sieb.), mitsumata (*Edgeworthia papyrifera* Sieb. et Zucc.) and gampi (*Wikstroemia sikokiana* Franch. et Sav.). These modern reference papers were prepared following the Japanese traditional processes⁶⁴. A reference papers made from cotton cellulose and prepared by traditional European technique was used for comparison with the Japanese reference papers. This paper is referenced as Whatman1. The raw plant fibers are provided by Prof. Masamitsu Inaba (Tokyo University of the Arts).

⁶⁴ For detailed process, refer to Timothy Barrett's book *Japanese Papermaking: Traditions, Tools, and Techniques*.

2.2.2 The Total Ion Chromatogram

The reference East Asian paper samples manufactured from different plant origins were analyzed using the Py-GC/MS settings described in section 2.2.1, after optimization of the effect of pyrolysis temperature on samples of reference papers made from cotton cellulose, and from kozo paper (cooked with woodash). The resulting chromatograms from the analysis of typical kozo, mitsumata and gampi papers are presented in Fig.2.2.2. As can be seen, each of these papers resulted in similar chromatograms for the first half of the separation while they are noticeably different in the second half of the separation. The fingerprint of cellulose, for retention times below 35 min, is characterized by numerous peaks generated from the thermal carbohydrate backbone degradation, for which the most abundant and characteristic peak is the peak of the 1,6-anhydro- β -D-glucose (levoglucosan, refer to LG for short). Two other regions were observed in the chromatograms: (i) the region of elution of fatty acid derivatives and hydrocarbons compounds at retention times comprised between 32 min and 50 min, and (ii) the region of elution of others compounds between 50 min and 65 min, finally assigned to plant markers. Noteworthy, visual inspection showed that the three reference papers could be distinguished from their chromatogram profiles in this latter region (latterly defined as ‘region of interest’ ROI). The most interesting results obtained concern this latter region of elution, 50-65 min, which showed groups of homologous compounds relevant to the biochemistry of the plants used in the process of papermaking (see section 1.5 and will be discussed below).

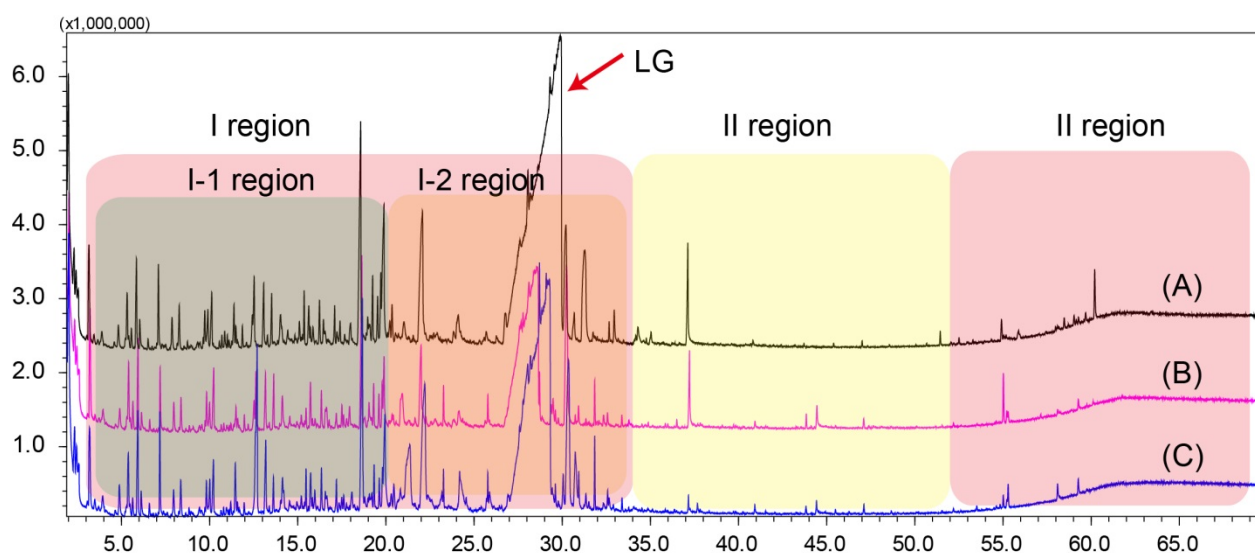


Figure 2.2.2 Total Ion Chromatogram of the pyrolysis of handmade papers with the example of kozo (A), mitsumata (B) and gampi (C). I is the region of the cellulose pyrolysis products, within I region, I-1 is the region of mainly furan based structures and I-2 is the region of mainly anhydrosugar based structures, II is the region of fatty acid derivatives and hydrocarbon compounds, III is the region of biomarker compounds.

2.2.3 The cellulose pyrolysis part

The thermal decomposition of cellulose is the result of two competing reactions: a dehydration to yield anhydrocellulose and a depolymerisation of cellulose to yield primarily levoglucosan and minor anhydrosugar components [33]. Both mechanisms lead to various fragmentation, elimination and condensation reactions which produce non-condensable volatiles, condensable vapors (liquid tar after cooling) and carbonaceous chars as solid residues. The fingerprint of cellulose can be depicted by two regions dominated by furan based structures (from 1 min to roughly 20 min) and anhydrosugar based structures (from 20 min to 35 min), as reported in Table 2.2.1 and Fig.2.2.3.

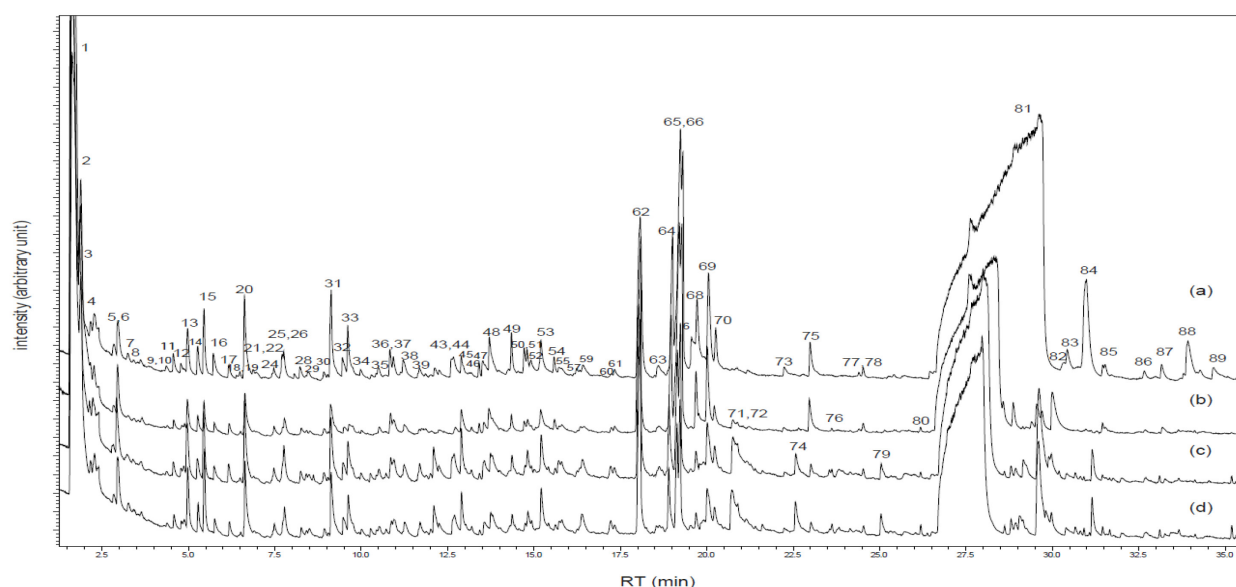


Figure 2.2.3 Detailed chromatograms of the region 1-35 min presented for (a) Whatman1, (b) kozo, (c) mitsumata, and (d) gampi reference papers.

By analyzing these chromatograms, the fingerprint of cellulose permits to distinguish roughly furan based structures and sugar derivatives. Indeed, the pyrolytic products included furan compounds specific to an arrangement of glucose units in the polymer (typically the 2-furaldehyde; 2(5H)-furanone; 5-methyl-2-furaldehyde; 3 or 4-methyl-5H-furan-2-one) as well as light linear carbonyls (mainly the 2-butenal; 1-hydroxy-2-propanone; pyruvic acid, methyl ester; 1,2-cyclopentanedione or 2-hydroxy-2-cyclopenten-1-one), various anhydrosugars (dominated by the levoglucosan), anhydrosugar derivatives (mainly the 1,3,4,6-dianhydro- α -d-glucopyranose; 3,4-anhydro-d-galactosan; 2,3-anhydro-d-mannosan), and other sugar structures for which rigorous identifications are difficult. These latter pyrolysis products may justify the presence of hemicellulose in the reference papers.

Table 2.2.1 Identification of the pyrolysis products of the cellulose region of the paper sample in the chromatograms compared to the Whatman reference paper with corresponding retention times (min), major fragment ions, corresponding molecular mass, identification of the products, and assigned formula.

No	RT	mass spectra	MW	identification	formula
1	1.62	44	44	carbon dioxide	CO ₂
2	1.88	44,43,40,42	44	acetaldehyde	C ₂ H ₄ O
3	2.27	43,55,86	86	2,3 butanedione + 3-Buten-2-one	C ₄ H ₆ O ₂
4	2.4	43,57,45,60	60	acetic acid	C ₂ H ₄ O ₂
5	2.84	70,41,69,42	70	2-Butenal	C ₄ H ₆ O
6	2.96	43,74,45	74	2-Propanone, 1-hydroxy-	C ₃ H ₆ O ₂
7	3.41	43,57,41,100,71	100	3-Hexanone	C ₆ H ₁₂ O
8	3.61	86,58,57,55	86	1,4-Dioxin, 2,3-dihydro-	C ₄ H ₆ O ₂
9	4.27	69,41,84,43	84	3-Penten-2-one	C ₅ H ₈ O
10	4.36	69,41,70,98	98	4-Hexen-3-one	C ₆ H ₁₀ O
11	4.56	82,53,55,81	82	Furan, 2-methyl-	C ₅ H ₆ O
12	4.78	84,55,56,83	114	5-(Hydroxymethyl)-2(5H)-furanone	C ₅ H ₆ O ₃
13	4.98	43,73,84,55	114	Ether, 3-butenyl propyl	C ₇ H ₁₄ O
14	5.27	58,57,43,42	86	Butanedial	C ₄ H ₆ O ₂
15	5.45	43,102,42,44	102	Pyruvic acid, methyl ester	C ₄ H ₆ O ₃
16	5.72	84,54,55,42	84 or 114	2(3H)-Furanone or 5-(Hydroxymethyl)-2(5H)-furanone	C ₄ H ₄ O ₂ or C ₅ H ₆ O ₃
17	6.17	95,96,67	96	3-Furaldehyde	C ₅ H ₄ O ₂
18	6.39	69,41,98,70	98	4-Methyl-5H-furan-2-one	C ₅ H ₆ O ₂
19	6.49	96,68,40,67	96	But-1-ene-3-yne, 1-ethoxy-	C ₆ H ₈ O
20	6.62	96,95,82,67	96	2-Furaldehyde	C ₅ H ₄ O ₂
21	6.88	96,42,54,94	96	4-Cyclopentene-1,3-dione	C ₅ H ₄ O ₂
22	6.97	69,41,100,68	100	2-Butenoic acid, methyl ester	C ₅ H ₈ O ₂
23	7.42	98,97,41,81	98	3-Furanmethanol	C ₅ H ₆ O ₂
24	7.47	43,72,57,58,73	128	3,4-Dimethyl-2-hexanone	C ₈ H ₁₆ O
25	7.71	55,98,43,69,41	98	2(3H)-Furanone, 5-methyl- (alpha angelica lactone)	C ₅ H ₆ O ₂
26	7.76	43,86,116,73	116	2-Propanone, 1-(acetyloxy)-	C ₅ H ₈ O ₃
27	8.06	44,43,74,102	102	2(3H)-Furanone, dihydro-4-hydroxy-	C ₄ H ₆ O ₃
28	8.22	96,68,42,54,94	96	2-Cyclopentene-1,4-dione	C ₅ H ₄ O ₂
29	8.48	96,57,42,68	96	2-Cyclopenten-1-one, 3-methyl- + ?	C ₆ H ₈ O
30	8.92	67,96,58,45,43	96	2-Cyclopenten-1-one, 2-methyl- + ?	C ₆ H ₈ O
31	9.13	55,84,54,42,56	84	2(5H)-Furanone	C ₄ H ₄ O ₂
32	9.46	70,42,41,98,69	98	2-Cyclohexen-1-ol	C ₆ H ₁₀ O
33	9.61	98,55,42,69,41	98	1,2-Cyclopentanedione or 2-Cyclopenten-1-one, 2-hydroxy-	C ₅ H ₆ O ₂
34	9.98	55,83,98,43,54	98	2(5H)-Furanone, 5-methyl- (beta angelica lactone)	C ₅ H ₆ O ₂
35	10.49	55,43,98,53	98	2(3H)-Furanone, 5-methyl-	C ₅ H ₆ O ₂
36	10.83	110,109,57,53,43	110	2-Furaldehyde, 5-methyl-	C ₆ H ₁₀ O ₂
37	10.93	43,96,53,67,81	96	2-Cyclopenten-1-one, 3-methyl-	C ₆ H ₈ O
38	11.21	69,98,41,40,53	98	2(5H)-Furanone, 3-methyl- or 4-Methyl-5H-furan-2-one	C ₅ H ₆ O ₂

39	11.68	94,66,65,95,63	94	phenol	C ₆ H ₆ O
40	11.86	118,89,90,63,119	118	Benzofuran	C ₈ H ₆ O
41	12.125	114,58,57,55,42	114	NA	C ₄ H ₂ O ₄
42	12.25	57,58,55,112,84	158	Formic acid, 2-propylpentyl ester?	C ₉ H ₁₈ O ₂
43	12.61	112,55,69,84,71	112	2-Cyclopenten-1-one, 2-hydroxy-3-methyl-	C ₆ H ₈ O ₂
44	12.67	82,68,96,109,55	NA	long-chain alcohol NA	C ₁₄ H ₂₈ O
45	12.9	112,69,55,41,56	112	1,2-Cyclopentanedione, 3-methyl-2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-	C ₆ H ₈ O ₂
46	13.41	83,68,126,55,85	126	4-Methyl-5H-furan-2-one	C ₇ H ₁₀ O ₂
47	13.52	69,41,98,40	98	NA	C ₅ H ₆ O ₂
48	13.71	57,85,84,116,70	NA	NA	C ₉ H ₁₈ O
49	14.35	81,82,54,53,55	138	2-Pentylfuran	C ₉ H ₁₄ O
50	14.72	68,57,85,70,81	170	Butanoic acid, 2-methyl-, 3-methyl-3-butenyl ester	C ₁₀ H ₁₈ O ₂
51	14.8	128,43,57,68,85	128	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	C ₆ H ₈ O ₃
52	14.91	124,95,123,67,43	124	2,5-Furandicarboxaldehyde	C ₆ H ₄ O ₃
53	15.2	44,57,43,69,54	NA	NA	
54	15.58	98,68,96,53,97	126	Levogluosenone (6,8-Dioxabicyclo[3.2.1]oct-2-en-4-one)	C ₆ H ₆ O ₃
55	15.72	126,71,55,43,97	126	4H-Pyran-4-one, 3-hydroxy-2-methyl- (maltol)	C ₆ H ₆ O ₃
56	15.81	126,55,71,43,69	126	2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-	C ₇ H ₁₀ O ₂
57	16.24	102,45,44,43,75	190	2-Octenoic acid, 4,5,7-trhydroxy	C ₈ H ₁₄ O ₅
58	16.33	85,53,81,57,96	170	2(3H)-Furanone, 5-hexyldihydro-	C ₁₀ H ₁₈ O ₂
59	16.42	56,114,84,57,42	114	2,4(3H,5H)-Furandione, 3-methyl-1,4-Dioxaspiro[2.4]heptan-5-one, 7-methyl- + ?	C ₅ H ₆ O ₃
60	17.25	42,70,56,41	128	2,4(3H,5H)-Furandione, 3,5-dimethyl-	C ₆ H ₈ O ₃
61	17.33	56,43,128,45,100	128	5-(2-Propynyloxy)-2-pentanol + ?	C ₈ H ₁₄ O ₂
62	18.08	43,69,41,57,70,85	142	5-Hydroxymethyldihydrofuran-2-one	C ₅ H ₈ O ₃
63	18.6	85,43,57,86,95	116	1,4:3,6-Dianhydro-.alpha.-d-glucopyranose	C ₆ H ₈ O ₄
64	19.01	69,57,70,41,98	144	3,4-Anhydro-d-galactosan	C ₆ H ₈ O ₄
65	19.24	71,73,69,97,43	144	2,3-Anhydro-d-mannosan	C ₆ H ₈ O ₄
66	19,31	71,69,97,73,43	144	2-Furaldehyde, 5-(hydroxymethyl)-	C ₆ H ₆ O ₃
67	19.57	97,126,41,69,53	126	carbohydrate structure NA	C ₆ H ₈ O ₄
68	19.72	71,69,73,44,43	144	carbohydrate structure NA	C ₆ H ₈ O ₄
69	20.05	69,71,97,73,60	144	carbohydrate structure NA	C ₆ H ₈ O ₄
70	20.26	71,73,43,57,55	144	1,5-Anhydrohexitol	C ₆ H ₁₂ O ₅
71	20.74	57,73,43,104,132	164	1,2,4,5-Cyclohexanetetrol	C ₆ H ₁₂ O ₄
72	20.91	57,73,43,69,71	148	3-Deoxy-d-mannonic acid	C ₆ H ₁₂ O ₆
73	22.25	56,57,73,43,82	180	Phenol, 2,6-dimethoxy- or Phenol, 3,4-dimethoxy-	C ₈ H ₁₀ O ₃
74	22.57	154,139,93,111,96	154	2,3-Anhydro-d-mannosan	C ₆ H ₈ O ₄
75	22.99	97,71,43,69,57	144	1-Tetradecanol	C ₁₄ H ₃₀ O
76	23.62	83,55,98,69,70	214	2-Butenoic acid, 4,4-dimethoxy-, methyl ester	C ₇ H ₁₂ O ₄
77	24.41	0	160	2-Propenal, 3-phenyl- or 4,7-	C ₉ H ₈ O or
78	24.53	131,78,132,103,10	132 or		

		4	160	Methano-1H-indene-1,8-dione, 3a,4,7,7a-tetrahydro-	C ₁₀ H ₈ O ₂
79	25.05	168,153,125,107	168	2,5-Dimethoxybenzyl alcohol	C ₉ H ₁₂ O ₃
80	26.2	83,69,97,55,43	228	n-Pentadecanol	C ₁₅ H ₃₂ O
81	29.65	60,57,73,56,70	162	1,6-Anhydro-.beta.-D- glucopyranose (levoglucosan)	C ₆ H ₁₀ O ₅
82	30.36	73,103,57,69,43	164	1,5-Anhydro-d-mannitol	C ₆ H ₁₂ O ₅
83	30,43	73,69,57,43,87	164	1,6-Anhydro-.alpha.-d- galactofuranose	C ₆ H ₁₂ O ₅
84	30.99	73,69,44,43,61	162/192	1,6-Anhydro-.beta.-D- glucofuranose	C ₆ H ₁₂ O ₅
85	31.54	69,41,57,70,73		NA	
86	32.68	69,41,73,70,98		NA	
87	33.19	69,41,70,87,98		NA	
88	33.95	60,57,73,70,69	210	d-Gluco-heptulosan	C ₇ H ₁₄ O ₇
89	34.71	73,60,99,69,43	342	4-O-Hexopyranosylhexose	C ₁₂ H ₂₂ O ₁₁

Identification of these products is supported by previous studies conducted on wood samples and pure cellulose samples pyrolysis [34-39] and confirmed by comparison of their mass spectra with those recorded in the NIST database. No clear differences could be obtained between the reference papers from the compounds characterized in this region, even if some aromatics derivatives (compounds 74, 79) were detected more specifically for the mitsumata and gampi papers, but with relatively small intensities. These differences could either be attributed to the presence of different lignin content in these papers. A more detailed analysis of the lignin monomers will be discussed later in the manuscript (section 3.7.1) in which Py-GCxGC/MS revealed to be of help in the identification and quantification analysis.

2.2.4 The fatty acid region

After the eluting of the largest peak (levoglucosan) within the TIC, another region (from retention time 32 to 50 min) could be identified on the chromatograms issued from the pyrolysis of the reference East Asian paper samples. This region corresponded to the elution of fatty acids and hydrocarbons derivatives. Fatty acid and hydrocarbons derivatives are mostly present in the case of the mitsumata and the gampi reference papers while they were hardly detected in the kozo reference paper. This observation could constitute a rough differentiation between kozo samples on one side and mitsumata and gampi samples on the other side if the eluted compounds were specific. Indeed, the compounds detected in this region, 32-50 min, are far to be representative of any specificity. In particular, (i) a series of linear monocarboxylic saturated fatty acids ranging from 12 to 24 carbon atoms with hexadecanoic acid (palmitic acid) and octadecanoic acid (stearic acid) as the most prominent components were observed in the mitsumata and gampi samples, (ii) long chain alkenes and

aldehydes, constituted the main components of this region. Attribution of the fatty acid and hydrocarbon derivatives is presented in Table 2.2.2.

Table 2.2.2 Identification of the pyrolysis products detected in the intermediate region 32-50 min resulting from the pyrolysis of the mitsumata and gampi reference paper samples, reporting retention time (RT), major fragment ions (base peak in bold), corresponding molecular mass, identification of the product, and assigned formula.

RT	mass spectra	WM	Identification	formula
33.11	83 ,97,69,55,43	252	1-octadecene	C ₁₈ H ₃₆
33.66	57 ,82,96,43,71	240	hexadecanal	C ₁₆ H ₃₂ O
34.13	43 ,58,71,85,95	268	2-pentadecanone, 6,10,14-trimethyl-	C ₁₈ H ₃₆ O
35.19	83 ,97,55,69,43	266	1-nonadecene	C ₁₉ H ₃₈
35.32	57 ,71,85,43,99	268	nonadecane	C ₁₉ H ₄₀
35.76	43,82,57,96,69	254	1-heptadecanal	C ₁₇ H ₃₄ O
35.85	74 ,87,75,143,227	270	hexadecanoic acid, methyl ester (methyl palmitate)	C ₁₇ H ₃₄ O ₂
36.58	73 ,60,73,129,256	256	n-hexadecanoic acid	C ₁₆ H ₃₂ O ₂
37.18	97 ,83,57,69,55	280	1-eicosene	C ₂₀ H ₄₀
37.29	71 ,57,85,43,99	282	eicosane	C ₂₀ H ₄₂
37.75	82 ,57,96,69,43	268	octadecanal	C ₁₈ H ₃₆ O
39.08	97 ,83,57,69,43	294	1-heinecosene	C ₂₁ H ₄₂
39.19	57 ,85,71,43,99	296	heinecosane	C ₂₁ H ₄₄
39.66	74 ,87,43,55,57	298	octadecanoic acid, methyl ester (methyl stearate)	C ₁₉ H ₃₈ O ₂
40.89	97 ,83,69,55,111	308	1-docosene	C ₂₂ H ₄₄
41	57 ,71,85,43,99	310	docosane	C ₂₂ H ₄₆
41.49	82 ,57,96,69,43	282	nonadecanal	C ₁₉ H ₃₈ O
42.65	97 ,83,57,69,43	322	1-tricosene	C ₂₃ H ₄₆
42.74	57 ,71,85,43,99	324	tricosane	C ₂₃ H ₄₈
42.9	59 ,60,71,43,85	268	2-pentadecanone, 6,10,14-trimethyl-?	C ₁₈ H ₃₆ O
43.22	74 ,87,43,57,55	326	eicosanoic acid, methyl ester	C ₂₁ H ₄₂ O ₂
44.31	97 ,83,57,69,111	336	1-tetracosene	C ₂₄ H ₄₈
44.4	57 ,71,85,99,113	338	tetracosane	C ₂₄ H ₅₀
44.91	82 ,57,96,69,43	296	eicosanal	C ₂₀ H ₄₀ O
45.93	97 ,83,57,69,111	350	1-pentacosene	C ₂₅ H ₅₀
46	57 ,71,85,43,99	352	pentacosane	C ₂₅ H ₅₂
46.47	74 ,87,43,57,55	354	docosanoic acid, methyl ester	C ₂₃ H ₄₆ O ₂
46.52	82 ,96,57,43,69	310	heinecosanal	C ₂₁ H ₄₂ O
48.09	82 ,96,57,43,69	324	docosanal	C ₂₂ H ₄₄ O
49.49	74 ,87,75,43,143	382	tetracosanoic acid, methyl ester	C ₂₅ H ₅₀ O ₂
50.62	69 ,81,95,41,121	410	squalene	C ₃₀ H ₅₀

2.2.5 The marker part region

The third region corresponds to the elution of discriminative compounds for retention time comprised between 50 to 65 min observed during the Py-GC/MS analysis (defined as ‘region

of interest', ROI). This region, where biochemical compounds present in fiber plants elutes, is shown with their corresponding labeled peaks in Fig.2.2.4. Product identifications were reported in Table 2.2.3.

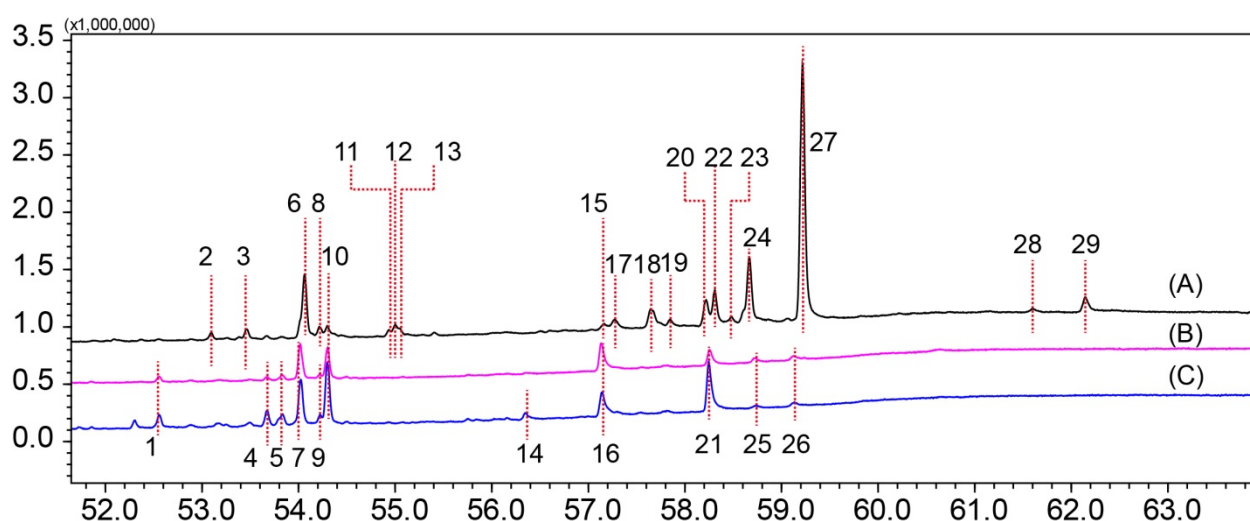


Figure 2.2.4 Detailed chromatograms of the ROI are presented for (A) kozo, (B) mitsumata, and (C) gampi reference papers.

Table 2.2.3. Identification of pyrolysis plant markers products of the kozo, mitsumata and gampi reference paper samples with retention time (RT), presence in the reference samples, expected molecular weights, main fragment ions, assigned formula and identification of the plant marker products.

No	RT	presence	MW	mass spectra	formula	Identification
1	52,56	m,g	396	121 , 145,81,105,95	C ₂₉ H ₄₈	stigmasta-3,5-diene
2	53,08	k	406	406 ,255,123,95,253	C ₃₀ H ₄₆	oleanane-2,9,12-triene
3	53,49	k	408	218 ,203,189,119,95	C ₃₀ H ₄₈	oleanane-2,12-diene
4	53,68	m,g	398	215 ,398,105,81,91	C ₂₉ H ₅₀	stigmasta-3-ene
5	53,83	m,g	414	105 ,396,91,81,145	C ₂₉ H ₅₀ O	stigmasta-4-en-3-ol
6	54,02	k	408	218 ,189,119,95,107	C ₃₀ H ₄₈	urs-2,12-diene
7	54,02	m,g	412	135 ,143,394,81,149	C ₂₉ H ₄₈ O	stigmasta-4,6-dien-3-ol
8	54,22	k	NA	218 ,189,95,119,203	C ₃₀ H ₄₈	ursane structure NA isomer of the stigmasta-3,5-diene NA
9	54,22	m,g	396	145 ,81,396,275,95	C ₂₉ H ₄₈	stigmasta-3-ol
10	54,30	m,g	414	147 ,141,81,145,396	C ₂₉ H ₅₀ O	stigmasta-3-ol
11	54,92	k	408	93 ,95,107,109,105 218 ,107,119,	C ₃₀ H ₄₈	oleanane structure NA
12	54,99	k	408	105,133	C ₃₀ H ₄₈	ursane structure NA oleanane or ursane structure NA
13	55,05	k	408	121 ,107,147,93,105 136 ,412,394,121,79 (pb sur le 136 exo cetone?)	C ₃₀ H ₄₈	stigmasta-5,7-diene-3-ol
14	56,35	g	412		C ₂₉ H ₄₈ O	stigmasta-5,7-diene-3-ol
15	57,14	k	422	135 ,407,105,95,91	C ₃₀ H ₄₆ O	urs-3,12-diene-11-one stigmast-5-en-3-ol (β-sitosterol)
16	57,14	m,g	414	145 ,81,105,95,91	C ₂₉ H ₅₀ O	stigmasta-5-en-3-ol (β-sitosterol)
17	57,25	k	424	218 ,203,95,119,69	C ₃₀ H ₄₈ O	oleanane-12-en-3-one oleanane-12-en-3-ol (β-amyrrin)
18	57,63	k	426	218 ,203,189,95,119	C ₃₀ H ₅₀ O	oleanane-12-en-3-ol (β-amyrrin)

19	57,83	k	424	218 ,95,109,107,203	C ₃₀ H ₄₈ O	urs-12-en-3-one
20	58,20	k	426	218 ,95,107,121,119	C ₃₀ H ₅₀ O	urs-12-en-3-ol (α -amyrin)
21	58,25	m,g	410	174,410,159,161,187	C ₂₉ H ₄₆ O	stigmasta-3,5-dien-7-one oleanane-12,14-diene-3- acetate
22	58,29	k	466	466 ,255,95,133,391	C ₃₂ H ₅₀ O ₂	urs-12,14-diene-3-acetate oleanane-12-ene-3-acetate (β -amyrin acetate)
23	58,47	k	466	95 ,189,119,69,215	C ₃₂ H ₅₀ O ₂	stigmasta-4-en-3-one
24	58,65	k	468	218 ,203,189,95,119	C ₃₂ H ₅₂ O ₂	stigmasta-4,6-diene-3-one urs-12-ene-3-acetate (α - amyrin acetate)
25	58,73	m,g	412	124 ,229,135,95,43	C ₂₉ H ₄₈ O	oleanane-12-ene-11-one-3- acetate
26	59,13	m,g	410	136 ,410,160,95,43	C ₂₉ H ₄₆ O	urs-12-ene-11-one-3- acetate
27	59,20	k	468	218 ,189,95,135,107	C ₃₂ H ₅₂ O ₂	acetate
28	61,60	k	482	135,232,273,175,217	C ₃₂ H ₅₀ O ₃	
29	62,14	k	482	135 ,273,232,175,95	C ₃₂ H ₅₀ O ₃	

k (kozo), m (mitsumata), g (gampi) NA means non attributed

The main results obtained during Py-GC/MS analysis of these three reference East Asian handmade papers resided in the identification of oleanane and ursane structures in the case of the kozo reference papers (whatever the cooking process and the mucilaginous additives used during the paper making), while mitsumata and gampi papers demonstrated mainly the presence of sterol structures (phytosterols). Obviously, a net differentiation between the kozo reference papers on one side and the mitsumata/gampi reference papers on the other side is obtained from the identification of these pyrolysis products, in particular because of the presence of these different metabolite structures. The discrimination between the mitsumata papers and the gampi papers is more difficult to attain due to the similarities in the plant marker region of these two papers. Fiber's analysis by microscopy usually fails in the differentiation of these two East Asian papers. However, the presence of a peak at RT 56.35 min (compound 14) attributed to MW 412 (C₂₉H₄₈O) for the gampi reference paper and comparison of the relative intensities of peaks at RT 53.68 min (compound 4) and 53.83 min (compound 5) attributed to MW 398 (C₂₉H₅₀) and 414 (C₂₉H₅₀O), respectively, may give help in the differentiation of mitsumata reference paper and gampi reference paper after pyrolysis. As will be discussed in section 3.2, the approach based on the combination of Py-GC/MS analysis and multivariate data analysis could ameliorate the discrimination of these two papers by mono-dimensional GC/MS analysis.

As can be seen from Fig.2.2.4, the signal-to-noise ratio was observed to be better for the kozo paper (A) than for the mitsumata paper (B) and the gampi paper (C) for samples of similar weights. Noticeably, more compounds issued from the pyrolysis of the paper samples were observed in this region than for the paper made from kozo fibers. Several difficulties appeared in the attribution of structures: the difficulty of structure assignments from their fragmentation

patterns, the difficulty to operate a good chromatographic separation under a classical temperature programming since even if similar retention times could be observed when comparing the different chromatograms obtained from papers of different origins, the corresponding structures were not identical.

A practical guideline was adopted in order to avoid misinterpretation (or to reduce the risk of error in the interpretation) considering in particular that oleanane structures and ursane structures were the likely dominant structures obtained from the pyrolysis of the kozo reference papers, while structures with a stigmastane-based skeleton were the likely structures obtained from the pyrolysis of the mitsumata and gampi reference papers.

This approach has been validated by liquid extraction experiments that permitted to demonstrate how triterpene structures (oleananes and ursanes) were only detected in the case of the kozo paper, while stigmastane structures were only observed in the case of the mitsumata and gampi papers. The associated skeletons of these different structures are shown in Fig.2.2.5.

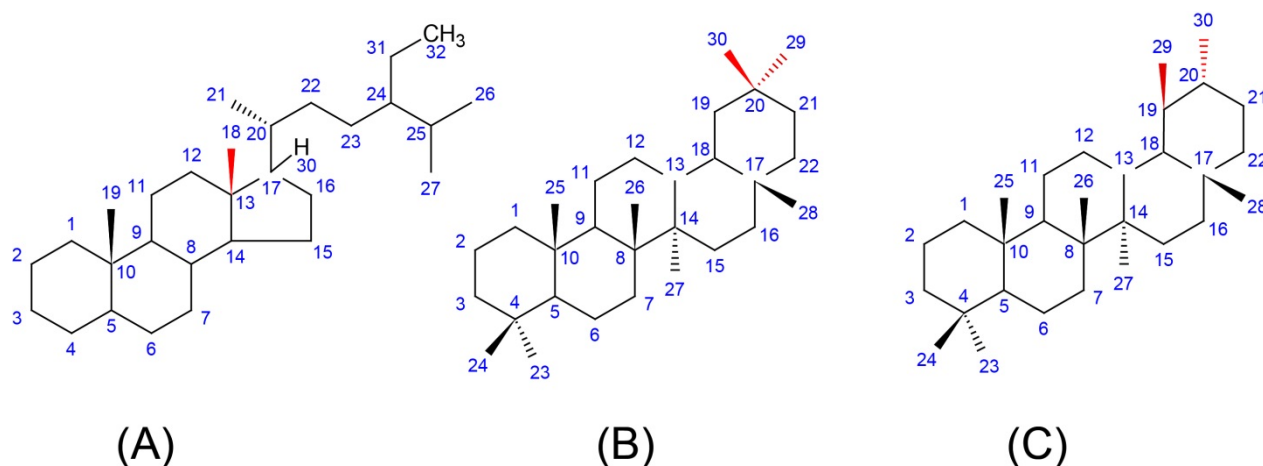


Figure 2.2.5 Structures of the observed marker plants with their respective labelling: (A) stigmastane, (B) oleanane (C) ursane structures.

Considering the mass fragmentation patterns of the plant markers, oleananes and ursanes are specifically well characterized by the presence of a base peak at m/z 218 (traditionally assigned to a retro Diels Alder fission of ring C) in their mass spectra. The occurrence of this strong peak at m/z 218 is usually assigned as a characteristic fragment of olean-12-ene or urs-12-ene derivatives which does not present functionalization on rings C, D and E, and is reported as a reliable diagnostic tool for the presence of a 12(13) double bond in α - and β -amyrins [40-42]. This first criterion was considered for the identification of ursane and oleanane compounds in the samples of kozo reference papers.

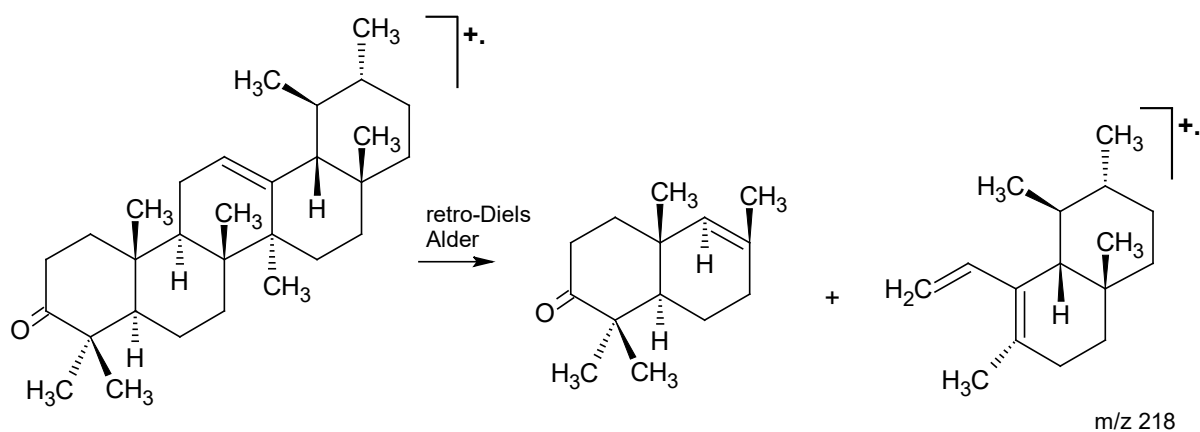


Figure 2.2.6 Fragmentation pathway of the ursane-12-ene structure with the formation of its characteristic base peak at m/z 218.

This m/z at 218 base peak ion has been largely investigated in the past [42-44] and its proposed fragmentation is reported in Fig.2.2.6. One particularity of the m/z at 218 is its further fragmentation yielding fragments at m/z 203 always accompanied by a less intense ion of 14u lower at m/z 189 [45]. Several mechanisms have been proposed to explain the formation of m/z 203 involving loss of the methyl group at C17, or loss of one of the two vicinal methyl groups at C19 and C20 [42-44]. For the fragment m/z at 189, its formation was proposed to result from the concerted cleavage of C(16)-C(17) and in the loss of the carbon at C(17) and its methyl group [43]. Even if the formation of the two fragment ions m/z 203 and 189 are a matter of discussion due to the complexity of their associated fragmentation pathways, in the mass spectra of triterpenes, comparison between peaks at m/z 203 and m/z 189 allows the distinction between oleanane and ursane structures [41] since the fragment ion at m/z 203 was observed as more intense than the peak at m/z 189 for the Δ^{12} -oleanene derivatives. Usually the opposite situation was observed in the mass spectra of an homolog Δ^{12} -ursene derivative (the peaks at m/z 203 and 189 were also reported with similar intensities in that case). The ratio between m/z at 203 and 189 was consequently considered as a second criterion for the differentiation of ursane and oleanane structures. These two criteria permitted the identification of compounds 3, 18, 20, 24, 27 (Table 2.2.3).

However, the similarities in the fragmentation patterns of oleanane and ursane triterpenes have made difficult the unequivocal assignment of their structures (with the exception of fragment peaks at m/z 203, as reported above). The comparison of experimental mass spectra with those of the NIST library 2011 (even if the triterpenes and stigmastanes under their native forms are limited in this library), analysis of the most informative fragment ions, and chromatographic retention criteria (as signaled above) were used for the tentative attribution of compounds reported in Table 2.2.3. In particular, the fact that ursane compounds are

reported as more retained on nonpolar columns than the oleanane compounds has permitted to raise some ambiguities for attribution of isomeric structures.

As a matter of fact, we can state that the observed structures from the pyrolysis of the reference kozo paper were mainly constituted of products showing a base peak at m/z 218, characterizing their triterpene origin. In complement to the fact that none of the detected C-12 unsaturated oleanane and ursane markers have shown a base peak of higher mass to charge ratio than m/z 218 permitted to preclude the presence of substituents in the rings D and E of these structures since such substituted structures would have been characterized by a base peak at m/z values corresponding to $[218 + \text{substituent}]^+$ [44, 45]. As an illustration, the presence of an *exo* ketone at C11, which impinges the previous fragment pattern leading to the characteristic base peak at m/z 218, permitted to confirm the structure of compounds 28 and 29 (Table 2.2.3). At the opposite, this 11-keto compound showed an intense fragment peak at m/z 273 [43].

Beside oleanane and ursane structures assigned to amyirin components, pyrolysis products of the kozo papers were characterized by the formation of pyrolysis products showing oxidation at C3 (compounds 17, 19), loss of acetyl group (compounds 22, 23), dehydrogenated and dehydrated structures predominant at retention times between 52 and 55 min. The pyrolysis of gampi and mitsumata reference papers showed different characteristics in particular, these papers were shown to provide less diversified structures by thermal degradation.

Compounds generated from the pyrolysis of gampi and mitsumata reference papers are associated to phytosterols for which series are characterized by the common loss of the side chain at C17 which resulted in fragment ions at m/z 255 or m/z 257 for most of them. Noticeably, such side-chain fragmentation allows in calculating the difference with the molecular mass the assignment of the series to which compounds belong in steroid series [44]. It was also reported that when the side chain is saturated, the molecular ion shows a higher intensity in the mass spectrum [46]. The saturated side chain observed here for both mitsumata and gampi reference papers corresponds to a $C_{10}H_{21}$ chain, which permitted to associate the detected phytosterol compounds to stigmastane based-structures. Comparison of their mass spectra to data library (NIST 2011) allowed the identification of compounds 1, 16, 21, 25 as stigmasta-3,5-diene, β sitosterol, stigmasta-3,5-diene-7-one and stigmasta-4-ene-3-one (Table 2.2.3).

For the pyrolysis products observed in the chromatograms of gampi and mitsumata reference papers, it is clear that the differentiation of isomers of the phytosteroid series is very difficult. In particular, fragmentation from the molecular ion may lead to similar stereochemistry for

fragment ions, a well-known difficulty in the identification of steroid series [47]. As an example, differentiation of compound 1 and the minor compound 9 was quite impossible, and only relative retention indexes considering other identified products permitted to identify compound 1 as stigmasta-3,5-diene (both of them showed very similar mass spectra with little differences in fragment relative ratios). A similar ambiguity was observed for compound 5 and compound 16 (sitosterol) which were only differentiated by the relative intensity of the molecular ion m/z 414 in the mass spectra and presence of fragment ions at m/z 342 for compound 5 and m/z 329 for compound 16.

At the opposite to the kozo reference paper, acetate derivatives for phytosterols were not detected in Py-GC/MS of mitsumata and gampi reference papers. The other compounds observed during pyrolysis were attributed to stigmasta-en, stigmasta-en-ol and keto stigmasta-en structures (compounds 4, 7, 10, 14 and 26). All of these compounds demonstrated a fragment peak attributed to the removal of the side chain in addition to three carbon groups, a well-known general fragmentation process for steroids [48, 49]. Rupture of the C ring, leading to a fragment ion composed from ring A and B at m/z 147, was used as an indication of the presence of a double bond within ring A at C3 (compound 4).

The reported rupture of ring B [49], leading to a large fragment associated to the cleavage of the C9-C10 and C7-C8 carbon bonds, gives a clue for the likely presence of an unsaturation in ring A and/or B (as well as its position). Such rupture of ring B was used to assign compound 7 which showed a peak at m/z 135, compound 4 which showed a peak at m/z 105, and for compound 14 showing a peak at m/z 121. Despite difficulties with their identification, however, these pyrolysis products were all formed reproducibly from the mitsumata and gampi reference papers, and hence could still be used effectively for their differentiation.

2.2.6 Plant components by liquid extraction and GC/MS analysis

In order to confirm the structures of the components of the three reference papers, a liquid extraction has been conducted using methanol and ethyl acetate as extraction solvents. Such procedure was also expected to confirm the relation between the extracted components and the observed pyrolysis products.

To avoid the potential problem of incomplete recovery of the triterpene and phytosterol content of reference papers, we used two solvent of different polarities: methanol and ethyl acetate, as extraction solvent for the chemicals present in the kozo, the mitsumata and the

gampi reference papers [50]. The structural identification of these compounds was performed by GC-MS and these compounds are reported in Table 2.2.4.

Table 2.2.4. Identification of plant markers products of the East Asian reference papers observed by liquid extraction with retention time, presence in the reference samples, expected molecular weights, main fragment ions, assigned formula and attribution of the products.

RT (min)	presence	MW	Major peaks	formula	Pyro	Attribution
51.59	m>g	410	69,81,68,95,67	C ₃₀ H ₅₀	-	squalene
57.2	g	400	55,57,71,79,91	C ₂₈ H ₄₈ O	-	campesterol
58.29	m,g	414	57,55,69,105,91	C ₂₉ H ₅₀ O	16	stigmast-5-en-3-b-ol (β-sitosterol)
58.86	k	426	218,203,55,69,95	C ₃₀ H ₅₀ O	18	oleanane-12-en-3-ol (β-amyirin)
59.4	m,g	410	174,55,91,207,57	C ₂₉ H ₄₆ O	21	stigmasta-3,5-diene-7-one
59.46	k	426	218,55,81,107,95	C ₃₀ H ₅₀ O	20	urs-12-en-3-ol (α-amyirin)
59.85	m	412	124,57,55,79,91	C ₂₉ H ₄₈ O	25	stigmasta-4-ene-3-one
59.86	k	468	218,203,69,95,55	C ₃₂ H ₅₂ O ₂	24	oleanane-12-ene-3-acetate (β-amyirin acetate)
60.45	k	468	218,107,189,95,81	C ₃₂ H ₅₂ O ₂	27	urs-12-ene-3-acetate (α-amyirin acetate)
61.9	m>g	428	55,57,98,81,79	C ₂₉ H ₄₈ O ₂	-	stigmasta-3,6-dione
62.23	g>m	428	55,57,91,428,93	C ₂₉ H ₄₈ O ₂	-	stigmast-3ol—4-ene-6-one
63.18	k	482	232,135,273,175,69	C ₃₂ H ₅₀ O ₃	28	oleanane-12-ene-11-one-3-acetate
63.83	k	482	273,135,232,175,95	C ₃₂ H ₅₀ O ₃	29	urs-12-ene-11-one-3-acetate

Pyro: Compound detected by pyrolysis in Table 2.2.3 MW: Molecular mass

A scale of relative retention times, relative to α-amyirin acetate for the kozo reference paper and relative to squalene for the mitsumata and the gampi reference papers have been used to confirm the identity of structures between all the sets of reference paper available, to avoid misleading attribution due to variation in the retention time by manual liquid injection. Results have been checked for correspondence with the structures identified during the pyrolysis experiments.

α- and β- amyirin structures appeared to be the principal components of the triterpenes extracts from the kozo reference paper. Sitosterol and other stigmastane-base structures appeared to be the main phytosterol components extracted from the mitsumata and the gampi reference papers. As a confirmation, this liquid extraction experiment permitted a clear differentiation between the kozo paper on one side and the mitsumata/gampi papers on the other side. Results obtained with ethyl acetate were identical to those reported for the extraction with methanol.

Triterpenes were mainly detected in the kozo reference paper and phytosterols were mainly detected in the mitsumata and gampi reference papers. This observation could reasonably be

correlated to the fact that *Broussonetia papyrifera* (koko) belongs to the family of Moraceae, a dicotyledonous plant commonly characterized by its triterpenoid saponin content. The situation might be different for *Edgeworthia chrysantha* (mitsumata) and *Wikstroemia sikokiana* (gampi)-as stated in section 1.5 of the metabolites.

Biochemistry of plant fibers may rationalize the detection of triterpene and phytosterol congeners, which finally constitutes markers of origin. As a matter of fact, these components are not totally removed during the hand papermaking process of traditional fiber's cooking. Liquid extraction of the koko reference paper provided few structures attributed to oleanane and ursane congeners. Mainly, β -amyrin and α -amyrin were eluted at retention time 58.86 and 59.46 min respectively. The two main peaks at 59.86 min and 60.45 min correspond to the acetate derivatives of these oleanane and ursane structures. Identification of these four compounds were unequivocal from the fragmentation pathways leading to fragment peaks at m/z 218 (base peak), m/z 203 and m/z 189, as discussed previously (section 2.2.5), and consecutive losses of a water molecule and a methyl group from the molecular ion at m/z 408 and m/z 393. Comparison of the experimental mass spectra with the mass NIST library comforted these identifications (NIST 2011). Two other minor peaks at 63.18 min and 63.83 min were observed in the chromatogram of koko reference paper, and were attributed to oleanane 12-ene-11-one-3-acetate and urs 12-ene-11-one-3-acetate, respectively, on the basis of their mass fragmentation patterns and comparison with mass spectra library. Moreover, presence of these oxidized compounds may be rationalized from the biochemistry of *Broussonetia* species since 12-ene oleanane structures are reported to be oxidized at the carbon C11 adjacent to the double bond [51]. All these compounds were identified in the pyrolysis of the koko reference paper and were present in close relative abundances: the strong stability of these structures may certainly justify their observation after thermo-desorption rather than pyrolysis during Py-GC/MS of the reference papers.

Liquid extraction of the mitsumata and the gampi reference papers provided β -sitosterol as a main component at retention time 58.29 min, for which identification was confirmed by comparison with the liquid injection of the standard compound. Moreover, under our conditions of chromatographic separation, the possibility of presence of γ -sitosterol was precluded by comparison of the relative order of elution of β -amyrin, γ -sitosterol and α -amyrin [52]. The presence of β -sitosterol as a major component is not surprising as β -sitosterol is strongly represented in all stages of maturation for many varieties of plants [44]. A trace of squalene was observed at 51.59 min in good correlation with the expected biochemical pathways of phytosterol synthesis in plants [53]. A small peak at 57.20 min attributed to

campesterol was only observed from the liquid extraction of the mitsumata reference paper and confirmed by its relative order of elution relative to squalene and β -sitosterol [54].

In the resulting chromatograms, four phytosterols attributed to: stigmasta-3,5-diene-7-one (59.40 min), stigmasta-4-ene-3-one (59.85 min), stigmasta-3,6-diene-7-one (61.90 min), and a compound at 62.29 min assigned to the stigmasta-3-ol-4-ene-6-one.

Liquid extraction compared to pyrolysis presents the advantage of avoiding the generation of unknowns for which attribution is rendered difficult due to the complex thermal degradation processes of large terpenic and steroid skeletons. Meanwhile, liquid extraction experiments confirmed the presence of the main initial structures observed by pyrolysis, as well as the different structures belonging to kozo (triterpenes) and mitsumata, gampi (stigmastanes). The results shown here are representative of those obtained in repeated liquid extraction experiments, and have demonstrated that liquid extraction may help for the unequivocal differentiation of mitsumata origin from gampi origin of the papers. In particular, the relative intensities of the two groups of products: stigmasta-3,5-diene-7-one at 59.40 min, stigmasta-4-ene-3-one at 59.85 min, and stigmasta-3,6-diene-7-one at 61.90 min, stigmasta-3-ol-4-ene-6-one at 62.29 min clearly differentiate mitsumata from gampi. The low level of detection of the stigmasta-4-ene-3-one under pyrolysis conditions (compound 25, Table 2.2.3) does not allow such comparison.

If, fortunately for the analytical chemist, there are many examples of lipids and terpenes in higher plants like those used in handmade papermaking that can release reliable biomarkers even though they may not be completely restricted in their distribution to specific groups of organisms. Investigations on a large set of fiber plants may help in confirming the opportunity of liquid extraction for origin's identification in the aim of East Asian paper characterization. However, one must concede that the large quantity of material required for liquid extraction (section 2.2.1.2) compared to pyrolysis, more or less condemns its efficient use for the differentiation of archaeological or museum samples.

2.2.7 The origin of the plant markers

In chapter 3, the plant markers will be used for differentiation of handmade papers while its origin shall be stressed. As the bark went through physical and chemical treatment before being the fibers used for papermaking, the plant markers discussed in section 2.2.5 and 2.2.6 may originated from:

- The plant and wasn't removed or modified during the papermaking process (chemical and physical treatment)
- The plant but has been modified during the papermaking process
- The additives during the papermaking process

To confirm the above hypothesis, the raw plant fibers (not being chemical treated) were analyzed under the same condition for the analyzing of the handmade papers. As can be visually observed from Fig.2.2.7, the pyrolysis of raw plant fibers and the handmade papers (processed fibers) result in similar compound distribution pattern in the ROI region. Besides the demonstrated mass spectra in Fig.2.2.7, other compounds have also been carefully examined which yielded well correspondence: all the plant markers discussed in section 2.2.6 and 2.2.7 can all be found in the corresponding raw fibers. This result showed that the plant markers in the ROI originated from the plant fibers and preserved through the papermaking process which provide a base for Py-GC/MS and Py-GCxGCC/MS analysis of fibers (linked with chemotaxonomy of the plant) and eventually being a tool for the identification of handmade papers from different material sources.

The raw fibers and handmade papers present identical biomarkers with raw fibers having more compounds in the ROI region (especially for the fibers from *Morus alba* and *Broussonetia kazinoki*). The can be reasonably explained that the chemical and physical treatment of the fibers eliminate certain compounds in the fibers while the markers discussed in this section was not affected. As the aim of this section is to confirm the origin of the markers detected in the ROI region of handmade papers and the compound identification resembles the process described in section 2.2.5 and 2.2.6, the compound identification result is not described here.

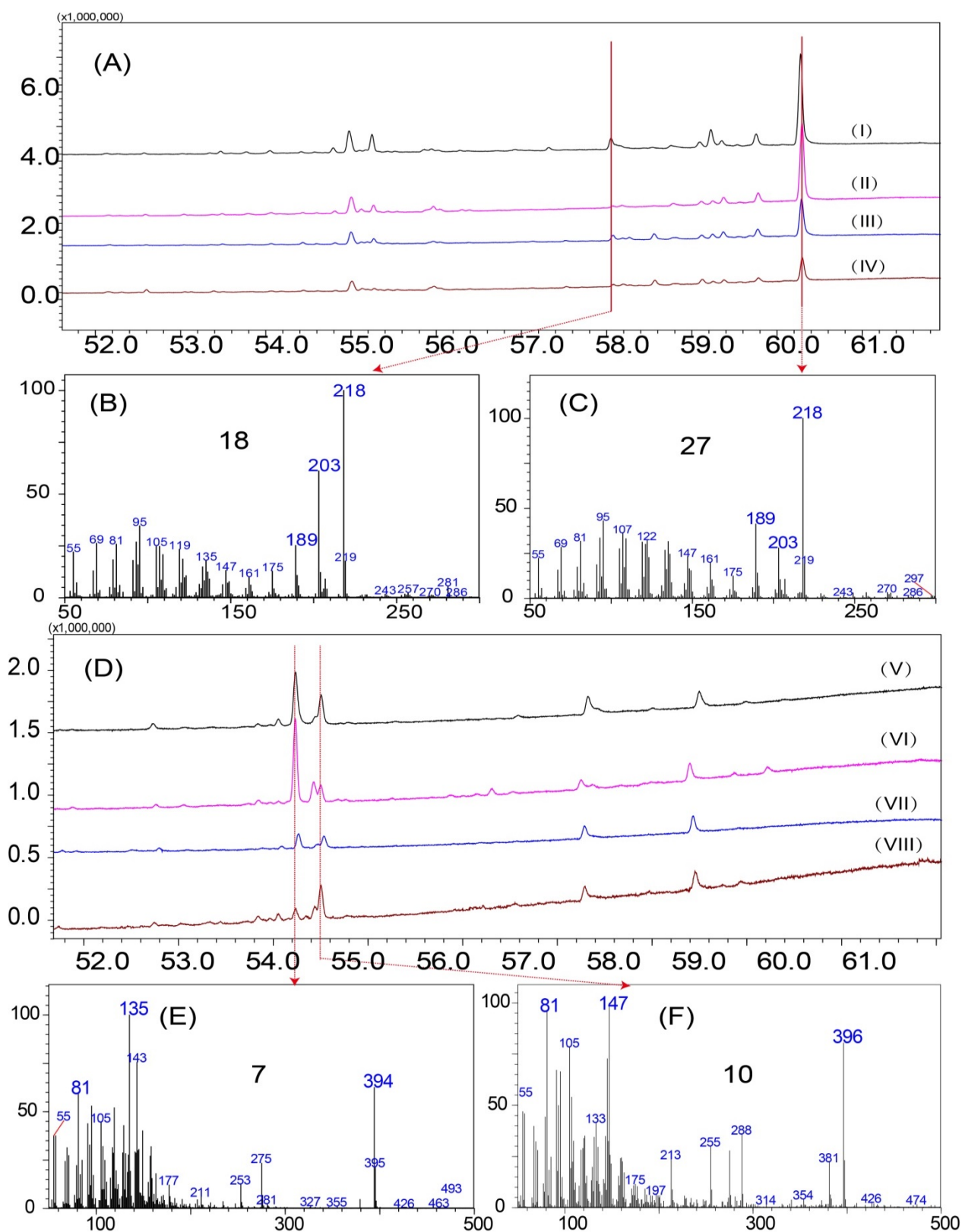


Figure 2.2.7 (A) shows the ROI region from the pyrolysis of raw plant fibers from *Morus alba* (I), handmade papers from *Morus alba* (II), raw fibers from *Broussonetia kazinoki* (III) and handmade papers from *Broussonetia kazinoki* (IV). (B) and (C) are the mass spectra for two compounds demonstrated. (D) shows the ROI region from the pyrolysis of raw plant fibers from *Mitsumata* (V), handmade papers from *Mitsumata* (VI), raw fibers from *Gampi* (VII) and handmade papers from *Gampi* (VIII). (E) and (F) are the mass spectra for two compounds demonstrated. The compound numbering refers to Table 2.2.3.

2.2.8 A summary

In this section, pyrolysis of three handmade papers was studied in focusing on the compound identification. The pyrolysis of handmade papers (the main constituent being cellulose) result in numerous of compounds. In considering the co-eluting compounds and low concentration compounds, the identification using only 1D information was tedious, time-consuming and in some cases cannot have a define attribution if high quality mass spectra cannot be retrieved. In such cases, some dedicated software such as AMDIS in focusing on the 1D data treatment can be applied. Another method is by improving the separation using method such as GCxGC to have more detailed information for compound identification or applied with statistical analysis. These two points will be discussed in the following parts. In all, this section formed the base for the following discussions.

2.3 pyrolysis-GCxGC/MS: principle and settings

2.3.1 The GCxGC principle

The introduction of gas chromatography with open-tubular capillary columns in 1958 by Golay is a milestone in analytical chemistry [55]. The application of gas chromatography jumped from hundreds per year in 1960s to ca. 4000 per year in 2000s and came to the peak of ca.7000 per year in 2017⁶⁵. In view of its wide application, a fact is that the observed complexity of the samples is regularly increasing. Normally, monodimensional gas chromatography (1D GC) can separate hundreds of peaks in one run. In some cases, 1D GC is insufficient for separating the individual constituent in complex mixtures. For example, samples encountered during the analysis of petrochemical products, perfumes or tobacco blends, 1D GC was not able to efficiently separate each compound constitutive of the samples due to the presence of co-eluting compounds [56-58].

The basic goal of GC is to separate compounds. To obtain the improved knowledge of the tested samples, enhanced resolution is needed. In fact, effects have been direction on this point. One way is by using column in series to make a 'long column' (e.g. 450 m). under this condition, better separation can be achieved at the sacrifice of instrumental complexity and acquisition time [59]. This method has not commonly been practiced with column usually being 30 m or 60 m long.

Another direction is by adding an extra separation capacity which harbored the invention of multidimensional gas chromatography (MDGC) proceeding to comprehensive gas chromatography (GCxGC). In fact, the MDGC has been introduced and discussed since 1960s by a 'heart cutting' technology to select interesting elutes from the first column and reinject into a second column for separation [60]. By the 'heart cutting' method, the total profiling of the sample is impossible.

The principles of GCxGC separation can be exemplified with a case when hand papermakers have to sort the papermaking materials (e.g. the paper mulberry trees) from different area origins shown in Fig.2.3.1. By applying only one principle (such as the size of the trees or the area where the trees originate), all samples cannot be separated totally as demonstrated with method I and II in Fig.2.3.1. However, by applying an orthogonal two-dimensional (2D) separation approach (size and origin, method III in Fig.2.3.1), the total separation of the

⁶⁵ In search of web of science (WoS), core collection with the 'gas chromatography' as the key word. Accessed 28, March 2018. The actual application shall be larger as there are articles outside the core collections of WoS.

samples can be achieved. This example can be an illustration for the benefit of GC separation with two or more properties. In the common 1D separation, usually one column is applied thus only one property is applied (polarity or boiling point, depending on the choice in choosing different columns). This separation principle resembles the method I and II shown in Fig.2.3.1: the compound co-eluting is sure to happen as commonly observed especially for complex samples. The principle of GCxGC resembles the sorting of mulberry tree by two properties: the boiling point and the polarity, two different and independent separation mechanisms, with the aim to obtain an orthogonally separation result (resembles method III in Fig.2.3.1). GCxGC take advantage of these two different separation properties by using two columns connected via a modulator.

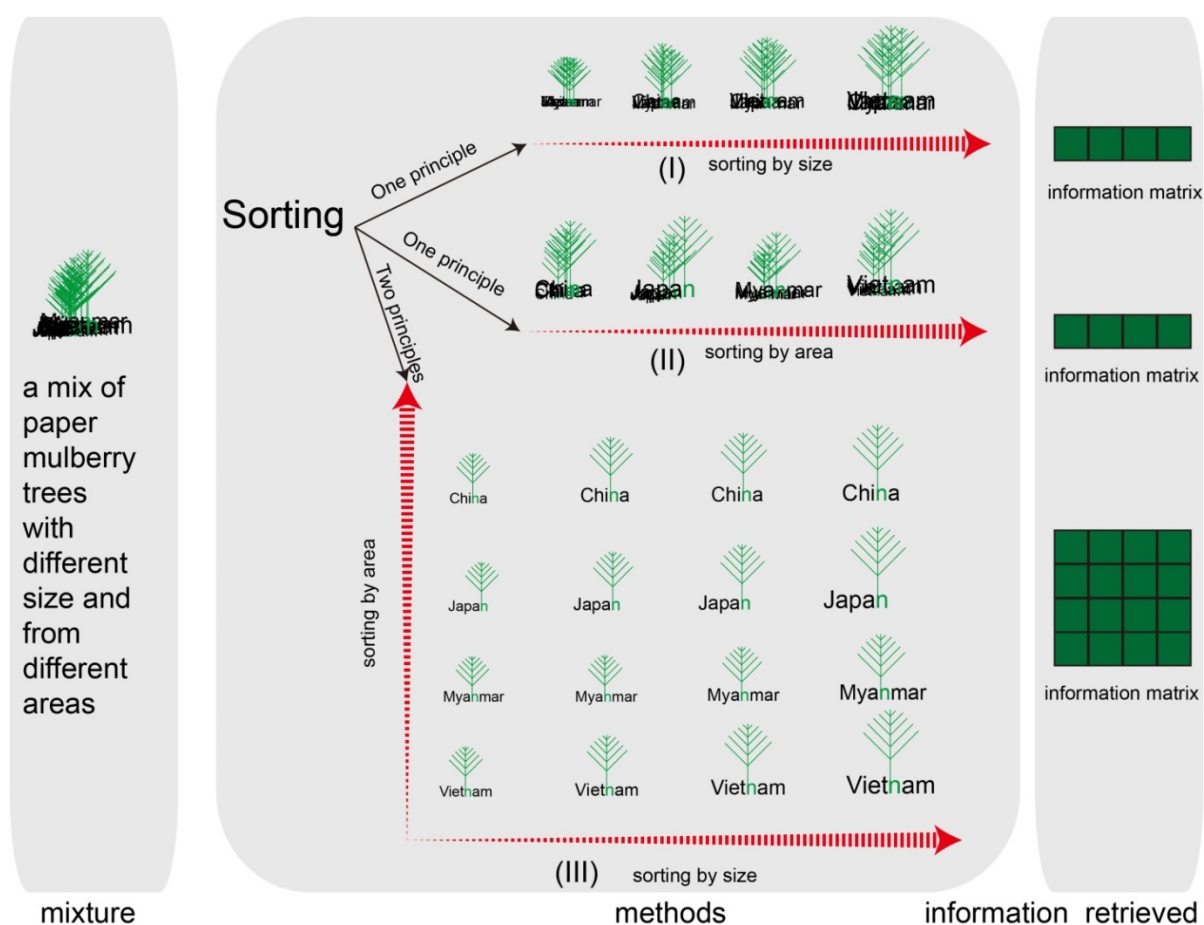


Figure 2.3.1 The GCxGC principle illustrated by an example using a case to sorting paper mulberry trees with different size and from different area origins.

GCxGC can be defined as: a chromatographic technique in which a sample is subject to two different separation processes coupled on-line. In a typical GCxGC system, two capillary columns connected via a modulation system are employed to optimize the complementary separation capabilities of columns of different polarities [61, 62]. Addition of a mass spectrometer as a detector adds another dimension in the separation capability, allowing the identification of compounds through mass spectra analysis. A schematic description of

pyrolysis coupling with GC/MS system and GC×GC/MS system is shown in Fig.2.3.2. In coupling two capillary columns, the GC×GC/MS system requires the use of a cryogenically cooled loop modulator. The modulation step permits the sequential trapping and releasing of compounds and, by the use of software imaging, the data are collected and treated under the form of a colored 2D chromatogram (Fig.2.3.2). With the additional column, the GC×GC/MS system provides information on the second dimension retention time (t_{R2}) besides first dimension retention time (t_{R1}) as well as fragment ions (m/z), peak heights and areas for the chromatographic peaks. As a result, a comprehensive two-dimensional gas chromatography/mass spectrometry (GC×GC/MS) system offers much higher resolution, peak separation capacity, selectivity and lower detection limit for the analysis of volatile organic molecules, making it one of the most powerful analytical systems [63].

The principle is quite easy to understand while it took a long time to achieve instrumentally. For instance, the difficulties in solving the system complexity as well as the long time sequence prevented the popularity of multidimensional gas chromatography (MDGC). Although the year 1991 marked the beginning of comprehensive two-dimensional gas chromatography (GC×GC) from the work of Liu and Phillips [64], it took more than two decades for its popularity in different areas.

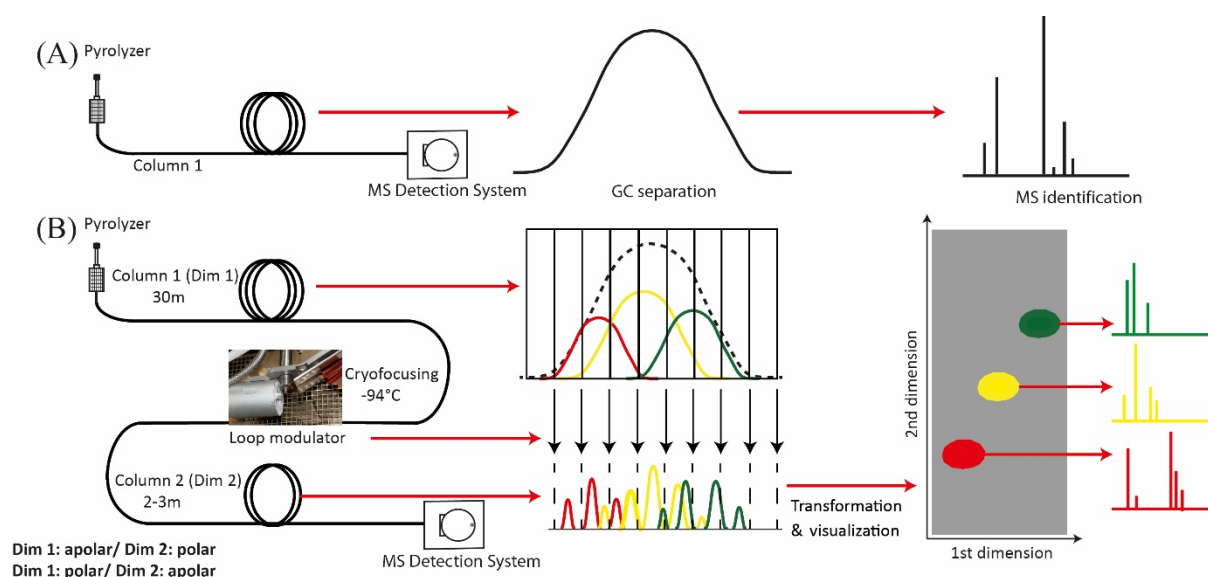


Figure 2.3.2 A schematic description of pyrolysis coupling with GC/MS system and GC×GC/MS system (from Figure 1 in section 2.4).

The core part of the GC×GC instrumentation lies in the modulation mechanism achieved by a modulator: an interface between the first column and the second column. It has been called as ‘the heart of GC×GC’ which reveals its importance in the separation system. The modulator brought about the focusing effect that can improve the sensitivity of the system with a two to five times gain in sensitivity [65, 66]. Two types of modulator are currently available: thermal

modulator and valve-based modulator (some thermal independent modulator such as stainless-steel capillary trap [67], solid state modulator [68, 69] have been reported). Thermal modulators apply the difference in temperature to achieve quick sampling and reinjection:

- Sampling mode: the effluent from the first-dimension separation column (1D) is focused by strong retention or condensation in the column by the cold flow from the cold jet
- Reinjection mode: the focused band is quickly desorbed or revaporized with thermal energy from the hot jet

The commercially available two-stage jet cooled thermal modulator are based on the works of Ledford and Billesbach [70, 71] with a further design of a loop system named delay loop [72]. The working sequence is designed as the hot jet is turned off and only activated for a short period at the end of a defined modulation time while the cold jet works continuously (Fig.2.3.3):

- The hot jet is turned off when cold spots are formed, compounds eluting from the first column are then trapped by the continuous cold flow (from the cold jet)
- The hot jet is turned on to inhibit the permanent cold jet for releasing the trapped analytes, and then released into the delay loop by a brief (usually a quick and short period, e.g. 0.35s) firing of the hot jet at a defined interval (named modulation time).
- The compound in the delay loop will be trapped again by the cold jet before they leave the delay loop (at the second cooling point in the delay loop system)
- The next firing of the hot jet releases a sharp chemical pulse into the second dimension (compound frozen at the second cooling point in the delay loop system) while at the same time releasing the next pulse into the delay loop (compound frozen at the first cooling point in the delay loop system)

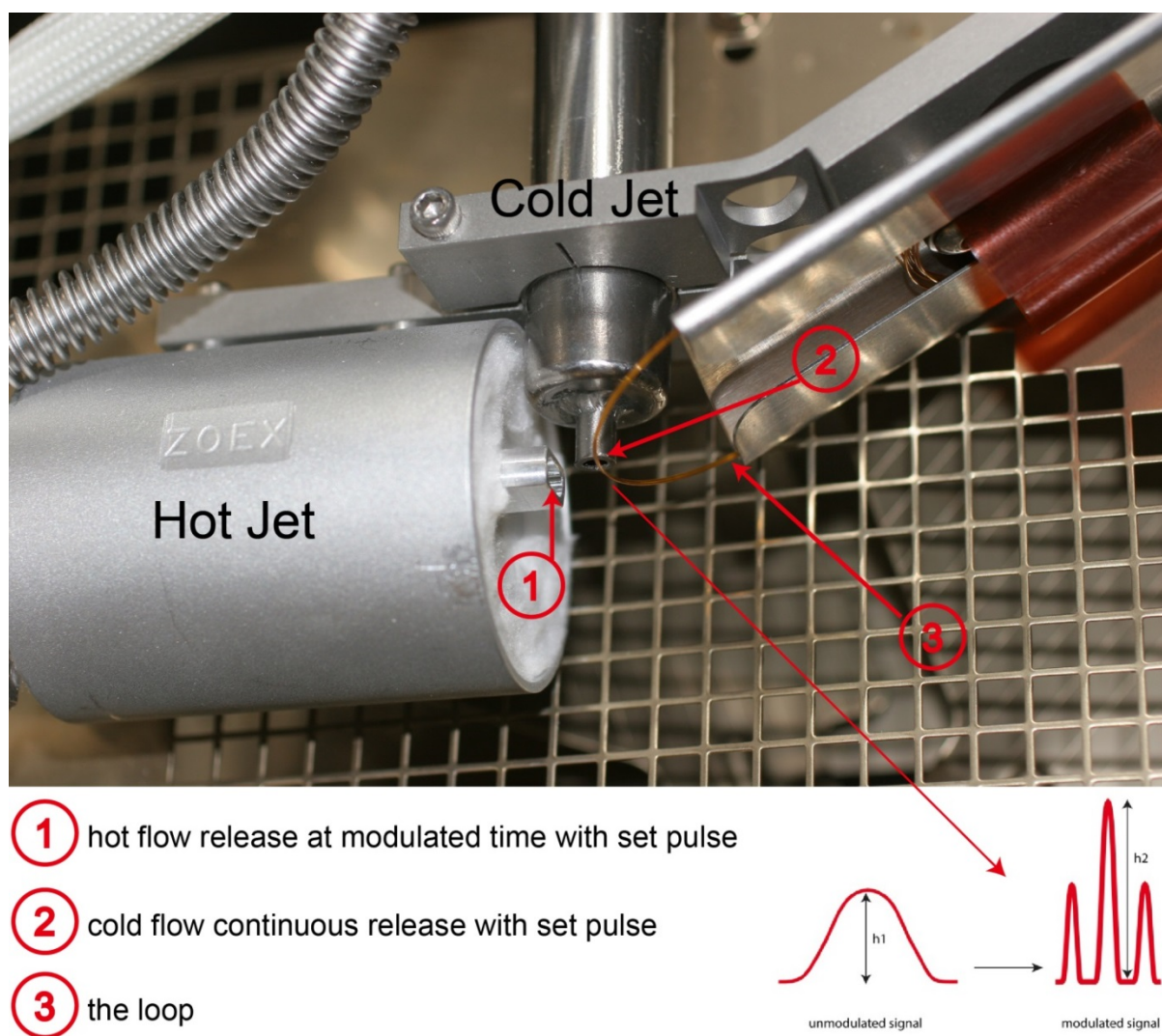


Figure 2.3.3 The set of the thermal modulator with a sketching of the re-focusing effect.

Actually, the repeat of the procedure (modulation time) above is rather rapid (usually 2-10s, long modulation time, e.g. 20s, has only occasionally reported). The fluid for the cold jet can be CO₂ (liquid) or dry N₂ (gas) while the supply of these gases to the modulator bears high operational cost. An alternative and more flexible choice is the closed-cycle cryo-refrigeration system proposed by Zoex (ZX2 Cooled Loop Modulation). It employs a closed cycle refrigerator/heat exchanger to produce -90° cold jet which can modulate volatile and semi volatile compounds over the C7+ range (the modulation type employed in this study). By permutation and combination, there could be 16 combinations while in practice the non-polar (1D) plus semi polar (2D) has been more practiced [73].

2.3.2 The GCxGC settings

The loop modulator (Fig.2.3.3) makes it possible to let the GC employ two properties for the separation but it adds more parameters to adjust within the system such as hot jet temperature, hot jet programming, pulse time, the cold jet flow as well as the adjustment of the delay loop. Any mis-setting of the parts mentioned, may lead to the undesirable separation and detection result. In this section, the effects of different parameters on the efficiency of the GCxGC separation will be discussed.

2.3.2.1 The hot jet

The most significant impact on the trapping-release process lies in the hot jet release process. The possible troubleshoot may exist in:

- The hot jet either does not have enough thermal energy to completely release the eluent being trapped (mainly hot jet temperature)
- The hot jet does not spread wide enough to cover the entire length cooled by the cold jet (also related to the loop position)

These problems may cause the modulation operation to partly or completely fail and cause some separated component to carry a long tail in the second dimensional separation (especially for the polar compounds when using nonpolar column as the 1st dimension column). As can be seen from Fig.2.3.4, at a hot jet temperature of 200°C and 220°C the efficiency to liberate the trapped effluent was not enough to completely release the eluent being trapped, which result in a long tailing effect (Fig.2.3.4). However, on the contrary, excessive thermal energy may also cause problems. For example, in Fig.2.3.5 when the hot jet was ramping from 200°C to 350°C (it took ca. 1 minute to ramp from 200°C to 350°C and ca. 2 minutes to stabilize), it makes an instant impact on the shape of the levoglucosan (the V shape, Fig.2.3.5A). Because 350°C approaches the temperature limit of use for the DB50 column, it may cause the degradation of the column as certain degradation compounds from the column can be modulated and detected: a series of the ‘bleeding compound’ with typical m/z at 73, 147, 207, 221, 281, 355 and 429 (Fig.2.3.5C). It pointed that excessive energy of hot jet in GCxGC analysis may cause damage to the column. Also the light compound has not been well trapped as has been revealed in Fig.2.3.5 with the light compounds continuously eluting without modulation (circle P, Fig.2.3.5A). The wraparound effect of the LG can also be observed when applying the hot jet temperature of 350°C.

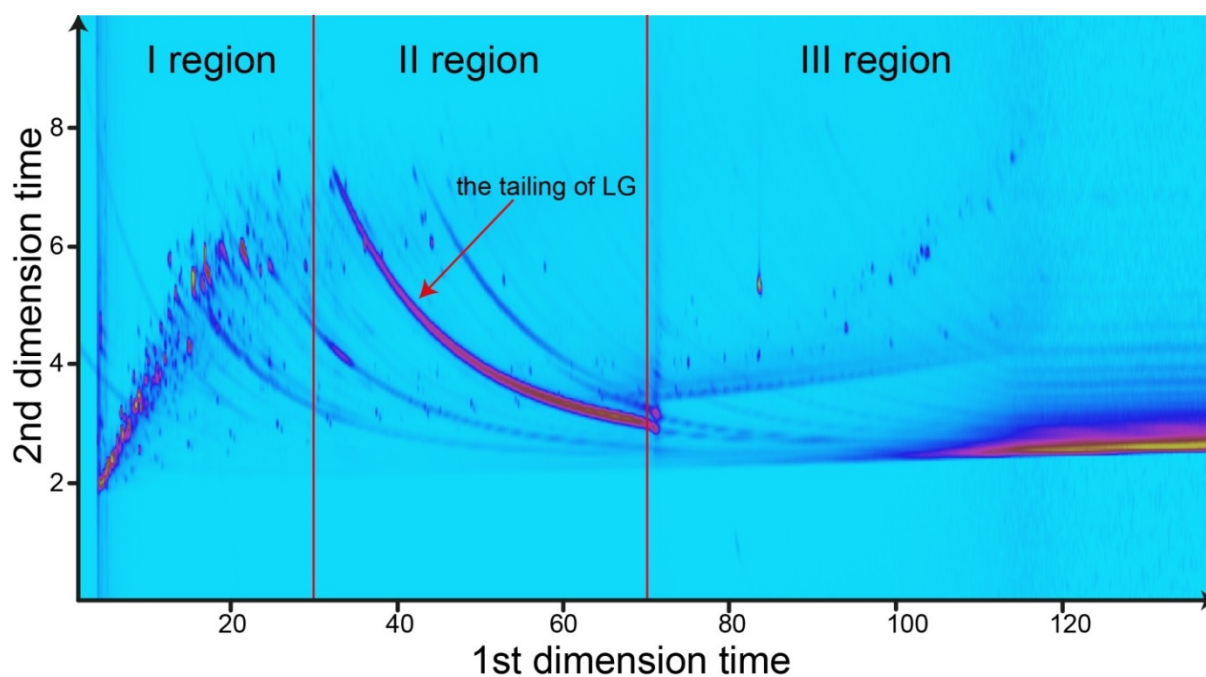


Figure 2.3.4 The hot jet temperature was initially set at 200°C (I) region, start to ramp to 220°C at 30 minutes (II) region, and start to ramp to 300°C at 70 minutes (III) region. The LG stands for levoglucosan.

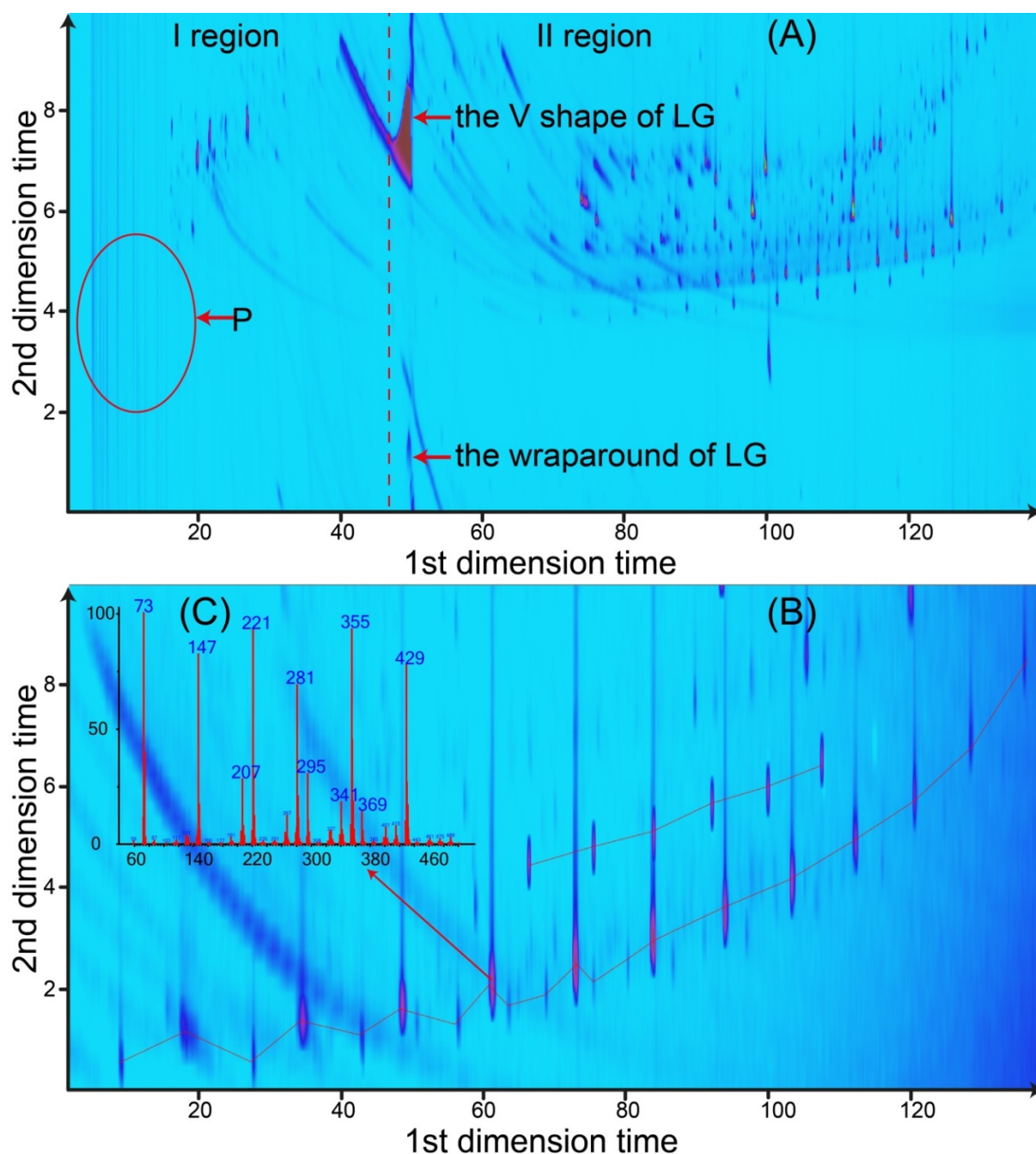


Figure 2.3.5 The hot jet temperature set initially at 200°C, (I) region in (A), ramped to 350°C at 40 minutes (II) region in (A). (B) demonstrates a series of compounds that are supposed to result from the degradation of the column with their corresponding mass spectra (C). The LG stands for levoglucosan.

2.3.2.2 The cold jet

The function of the cold jet is to continuously trap the compounds. Two parameters settings, the flow and the pressure of the cold jet can be manually adjusted (using the Zoex GCxGC settings as an example, some others like programmed automation of modulator cold jet flow has also been reported [74]). Excessive and insufficient cold flow can both affect the

separation results [74]. If the cold flow is set as low (the total cold flow value set as 1.5 L.min⁻¹), it cannot sufficiently trap the compound which means the re-focusing process has not been done properly exemplified by the re-concentration of LG when we compare (A) to (B) and (C) in Fig.2.3.6. GCxGC analysis under this total cold flow value cannot yield satisfying separation results as the modulation was not acting as can be observed from Fig.2.3.7A. To one extreme condition (the cold jet turned off), the low cold flow value makes GCxGC resembles the 1D separation process.

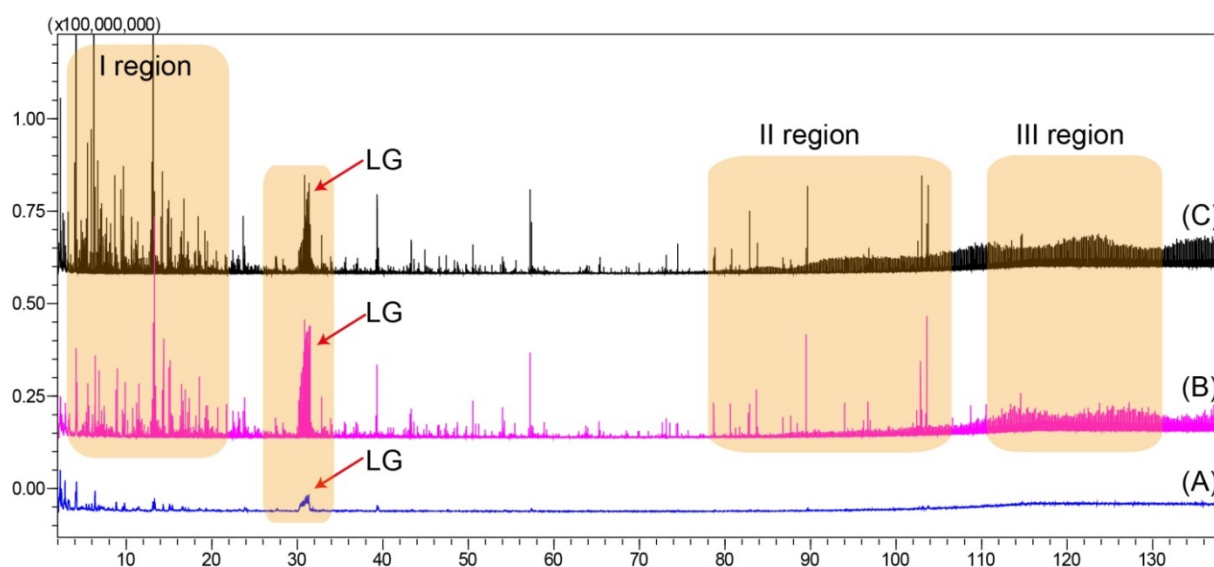


Figure 2.3.6 The chromatogram (raw data) of the test of a kozo paper sample with different total cold flow: (A) the total cold flow value set as 1.5 L.min⁻¹ (low), (B) the total flow value set as 7 L.min⁻¹ (medium), (C) the total flow value set as 15 L.min⁻¹ (high). (I) region is mainly light compounds; (II),(III) regions are mainly plant marker region plus column bleeding. The LG stands for levoglucosan.

It is also observed that with a middle cold flow (total cold flow value set as 7 L.min⁻¹), the light compounds cannot be re-focused well when comparing Fig.2.3.7B (total cold flow value set as 7 L.min⁻¹) with Fig.2.3.7C (total cold flow value set as 15 L.min⁻¹). Fig.2.3.7C presents nicer separation results for the light compounds while in Fig.2.3.7B more light compounds continuous eluting during the modulation period (the re-focusing effect for light compounds can also be observed when comparing I region in sample (B) and (C) in Fig.2.3.6). As for high boiling point compounds, the total cold flow from medium (value set as 7 L.min⁻¹) to high (value set as 15 L.min⁻¹) does not cause too much difference with only high values of total cold flow observed with higher background signals (III region, Fig.2.3.6), This medium value of the cold jet (7 L.min⁻¹) was the value currently used during the experiments.

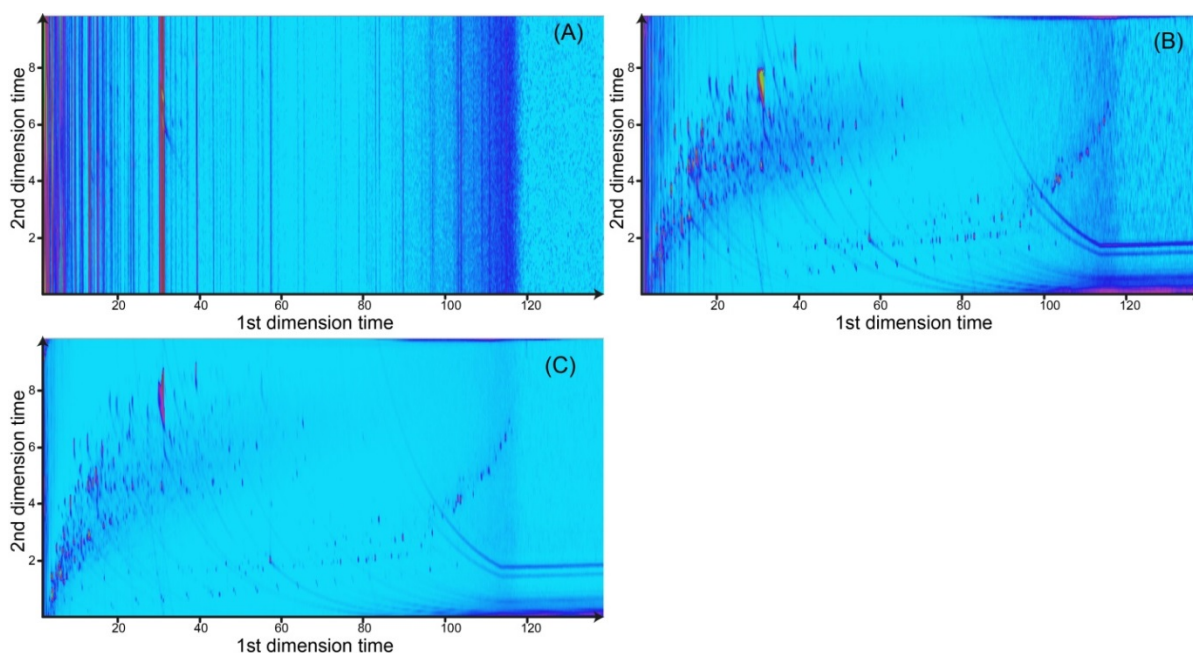


Figure 2.3.7 The chromatogram using the GC IMAGE software of the test of sample paper with different total cold flow: (A) the total cold flow value set as 1.5 L.min⁻¹, (B) the total flow value set as 7 L.min⁻¹, (C) the total flow value set as 15 L.min⁻¹.

2.3.2.3 The loop position

Although the design of the loop modulator optimizes the modulation system of GCxGC, it presents some inconvenience as observed in practice. To make the loop modulator work efficiently, it is required that the cold jet, hot jet and the ‘peak point’ of the out sketched semicircle be aligned in a single point (Fig.2.3.8). Only within good position, the cold jet has the most cooling power to trap eluents and the hot jet also in the best position efficiently blows hot gas to release the trapped compounds. If it was misaligned (the ‘peak point’ of the out sketched semicircle was not in the aligned position as is shown in Fig.2.3.8B), there will be a significant impact on the modulation process. In practice, it has been observed that the position of the loop can become loose and out of right position after a period of analysis and will cause bad separation results (Fig.2.3.9B). As can be observed from the chromatogram with misaligned loop, the major compounds have a long tailing and the light compounds are not well trapped. After the re-adjustment of the loop position, a normal GCxGC separation can be obtained (Fig.2.3.9A).

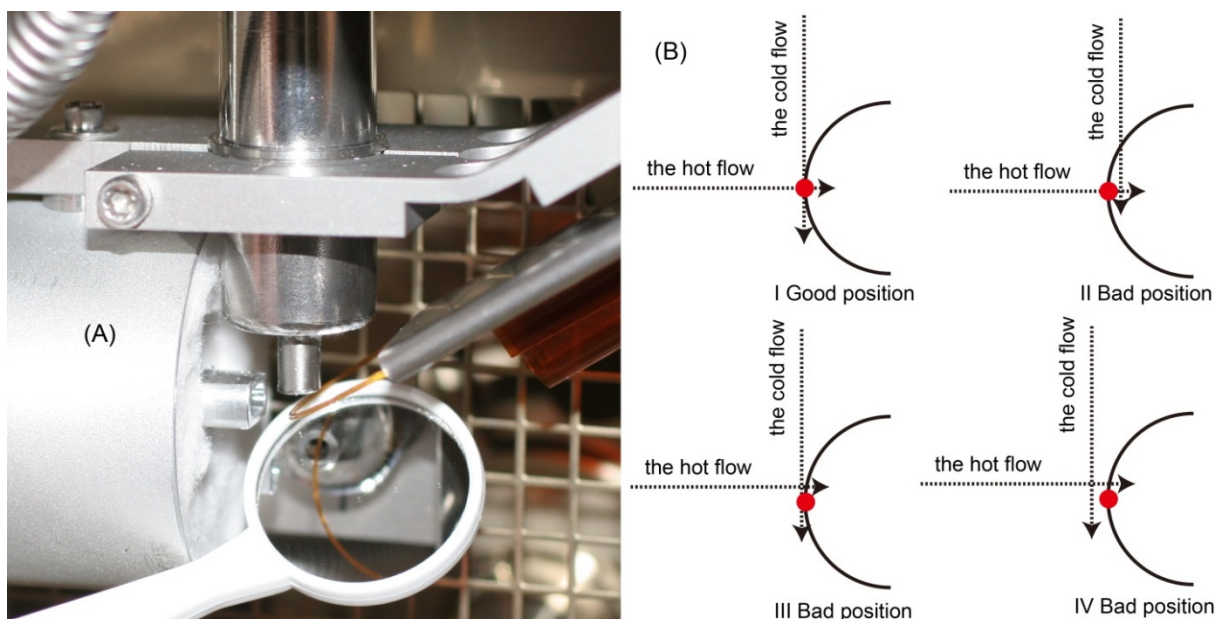


Figure 2.3.8 The actual hot jet-loop-cold jet position (A). (B) demonstrates the good positioning (I) and the bad positioning (II) to (IV).

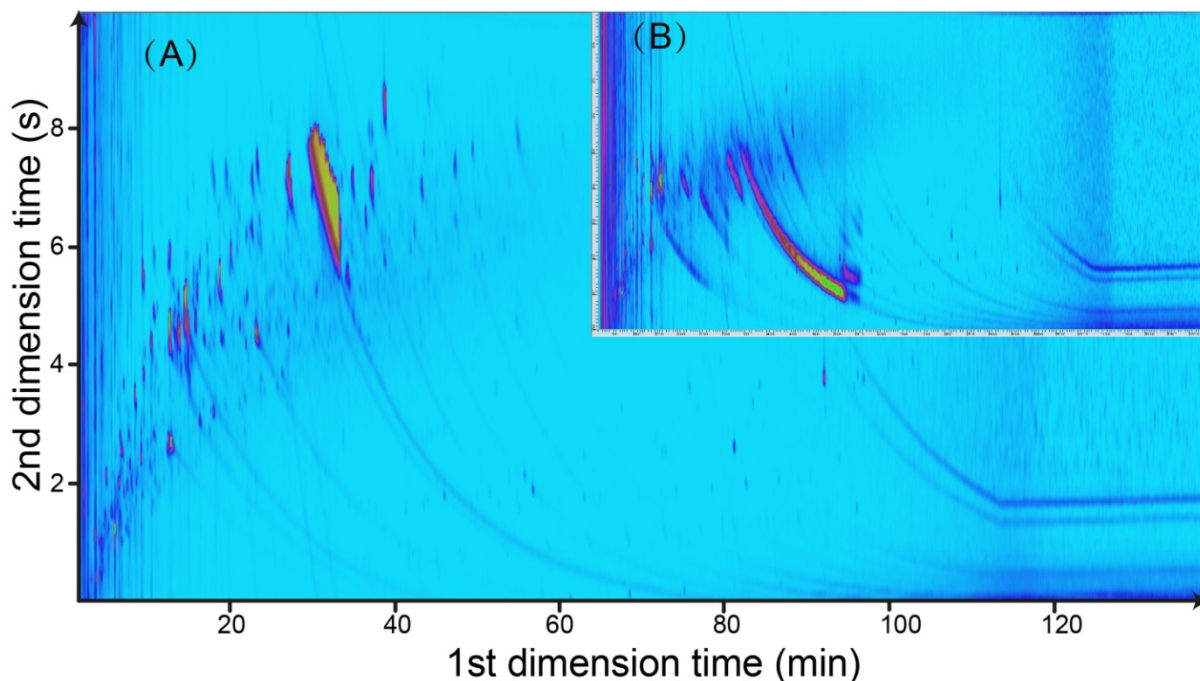


Figure 2.3.9 The chromatogram of the test of a paper sample from Astana tomb with good positioning (A) and displaced loop position (B).

2.3.2.4 The oven temperature

Generally, it has been observed that in 2D analysis an oven ramping rate of 1~3°C per minute is suitable. As is shown in Fig.2.3.10A, I region, the use of higher temperature ramping rate (5°C/min) together with a higher initial temperature (200°C) resulted in a bad separation for the cellulose pyrolysis products. While the use of ramping rate at 2°C/min results in comparative

nice separation result as can be observed in the I region, Fig.2.3.10B. When using the initial temperature at 100°C and a temperature ramping rate at 2°C/min, nice separation results for the cellulose pyrolysis products can be observed (I region, Fig.2.3.10B). The adding of a temperature holding (II region, Fig.2.3.10B) results in a ‘echelon’ shape for the separation of the LG, The afterwards temperature ramping at 4°C/min observed with a worse separation compared with using a ramping rate at 2°C/min (III region, Fig.2.3.10B).

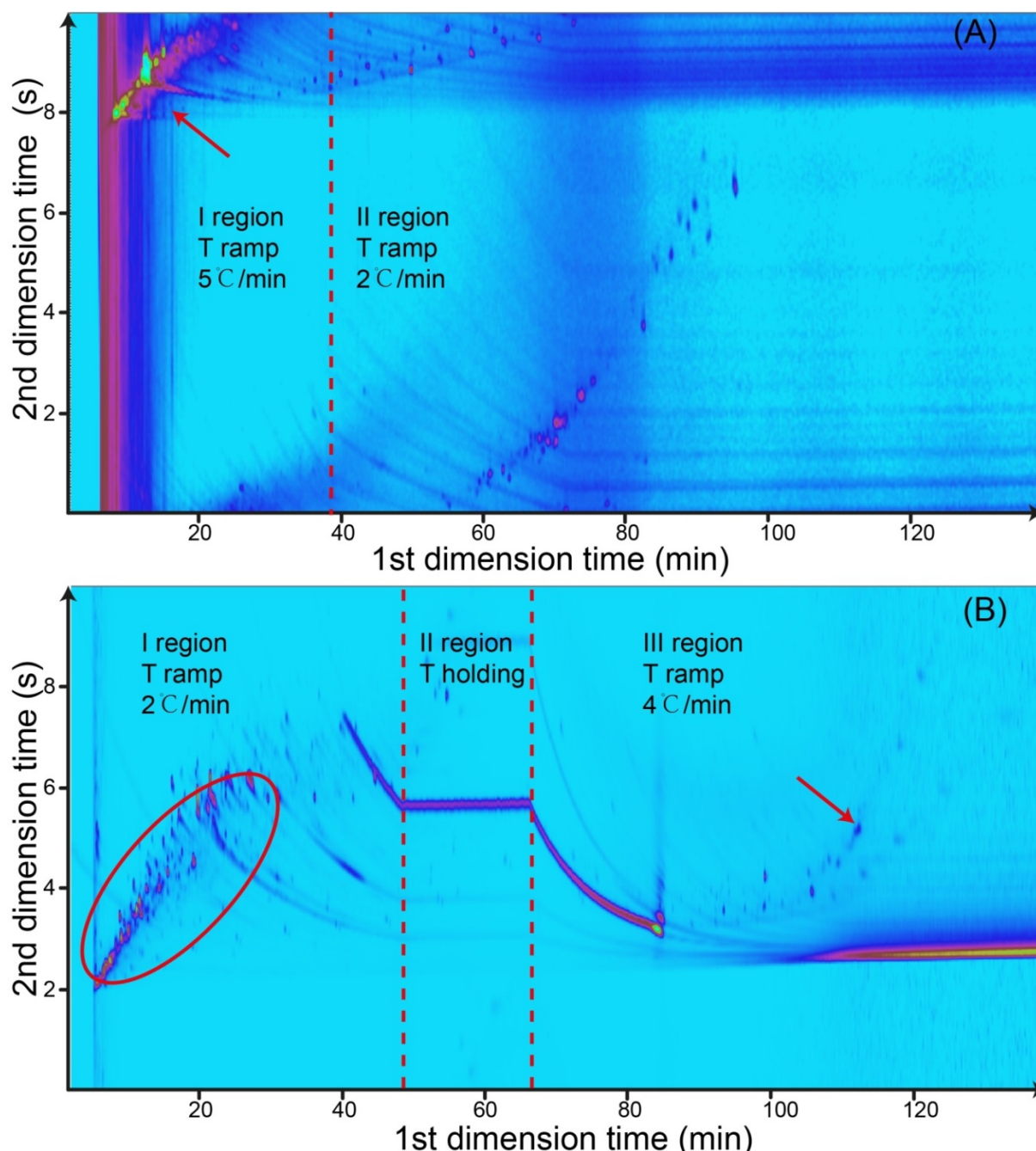


Figure 2.3.10 (A) oven temperature initiated at 200°C (hold 1 minute), ramp 5°C per minute to 290°C and then ramp 2°C per minute to 320°C (hold 30 minutes). (B) oven temperature initiated at 100°C (hold 1 minute), ramp 2°C per minute to 180°C (hold 15 minutes) and then ramp 4°C per minute to 325°C (hold 25 minutes).

2.3.2.5 The detection

GCxGC system is a sensitive system for trace compound analysis. To further explore for an increased sensitivity, one way is to use the detection gain for the detection. It can be seen from Fig.2.3.11 and Fig.2.3.12 that a normal analysis (with detection gain off) and the analysis with detection gain (0.97 kV) from 0-55 minutes and detection gain (1.35 kV) from 55-138.5 minutes, presents different TIC response as can be observed in comparing (A) with (B) (also I region and II region within B) in Fig.2.3.11. It can be seen from the raw data (Fig.2.3.11), that the detection gain (1.35 KV) greatly increase the TIC response that can be observed in comparing I region and II region in Fig.2.3.11B and the relative intensity of LG compared to the marker part compounds in II region. However, the background signal has also been enlarged in comparing the II region of (A) and (B) in Fig.2.3.11. For this reason, if the normal run (with detection gain off) satisfies the test requirement, the detection gain setting is not recommended. In cases such as in search of real trace compound, the detection gain is one setting to consider.

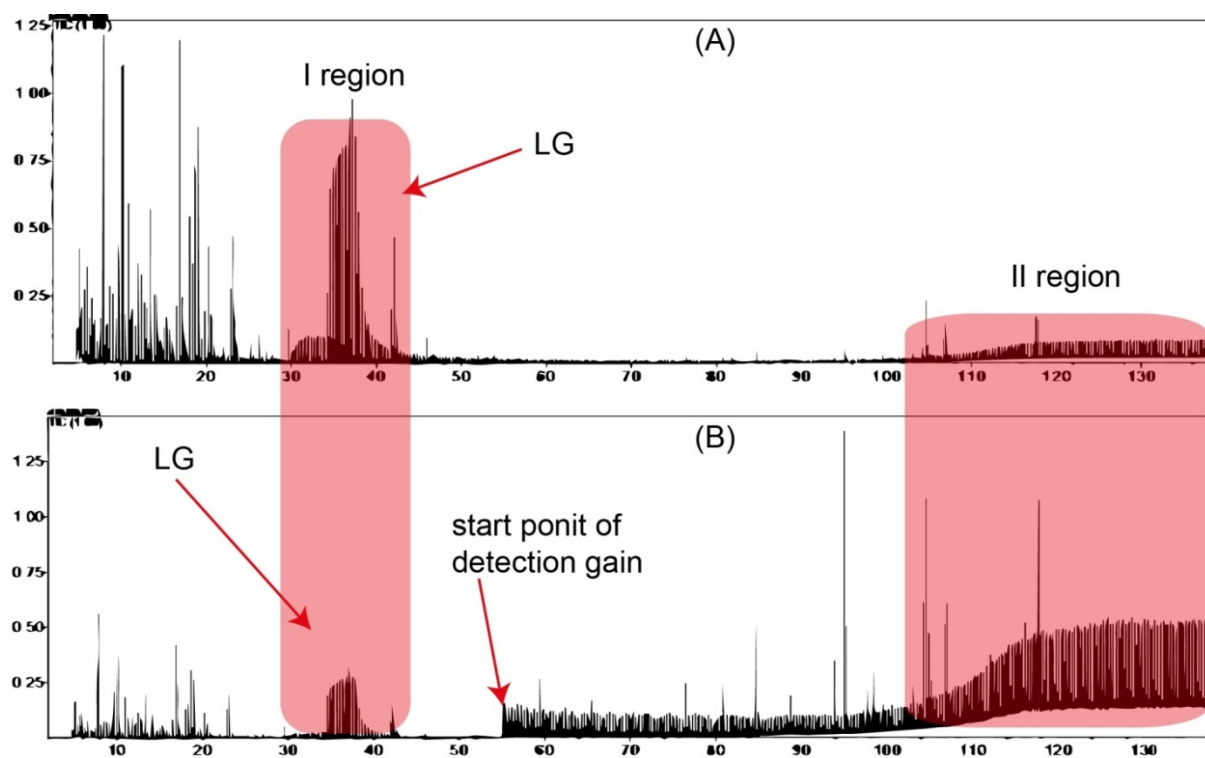


Figure 2.3.11 The raw data of an analysis with detection off (A) and detection gain=0.97 kV from 0 to 55 min and detection gain=1.35 kV from 55 min to the end of the test (B).

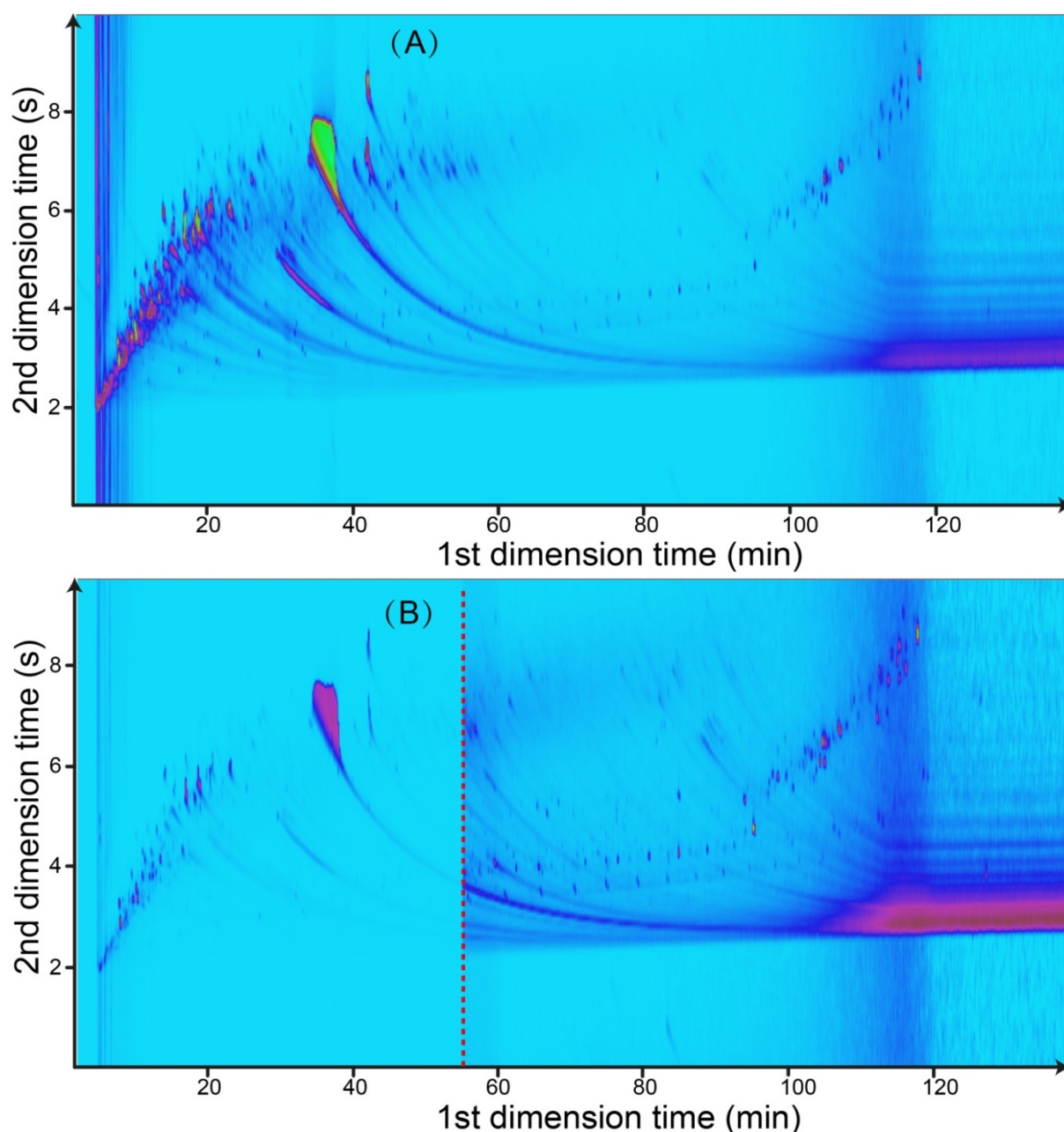


Figure 2.3.12 The result of an analysis with detection off (A) and detection gain=0.97 KV from 0 to 55min and detection gain=1.35 KV from 55 min to the end of the test (B).

Another way to increase the detection sensitivity is by employing the SIM mode which only monitors the selected ions. This has been mostly used for screening or quantifying already identified compounds. SIM mode has been commonly used in 1D analysis and some instrumentents and softwares (like the Shimadzu QP2010PLUS at disposale at CRC) permit SIM+Full Scan or first full scan then SIM (within one full program). However, in 2D analysis, some dedicated software like GC IMAGE, cannot treat SIM+Full Scan data due to the data storage and input problems. The SIM mode has to be conducted solely within all the analysis time in order to let the recorded data be interpreted using dedicated 2D software. Due to the

re-focusing effect, the SIM mode results in ‘thin and high’ peaks compared to 1D SIM model analysis (Fig.2.3.13).

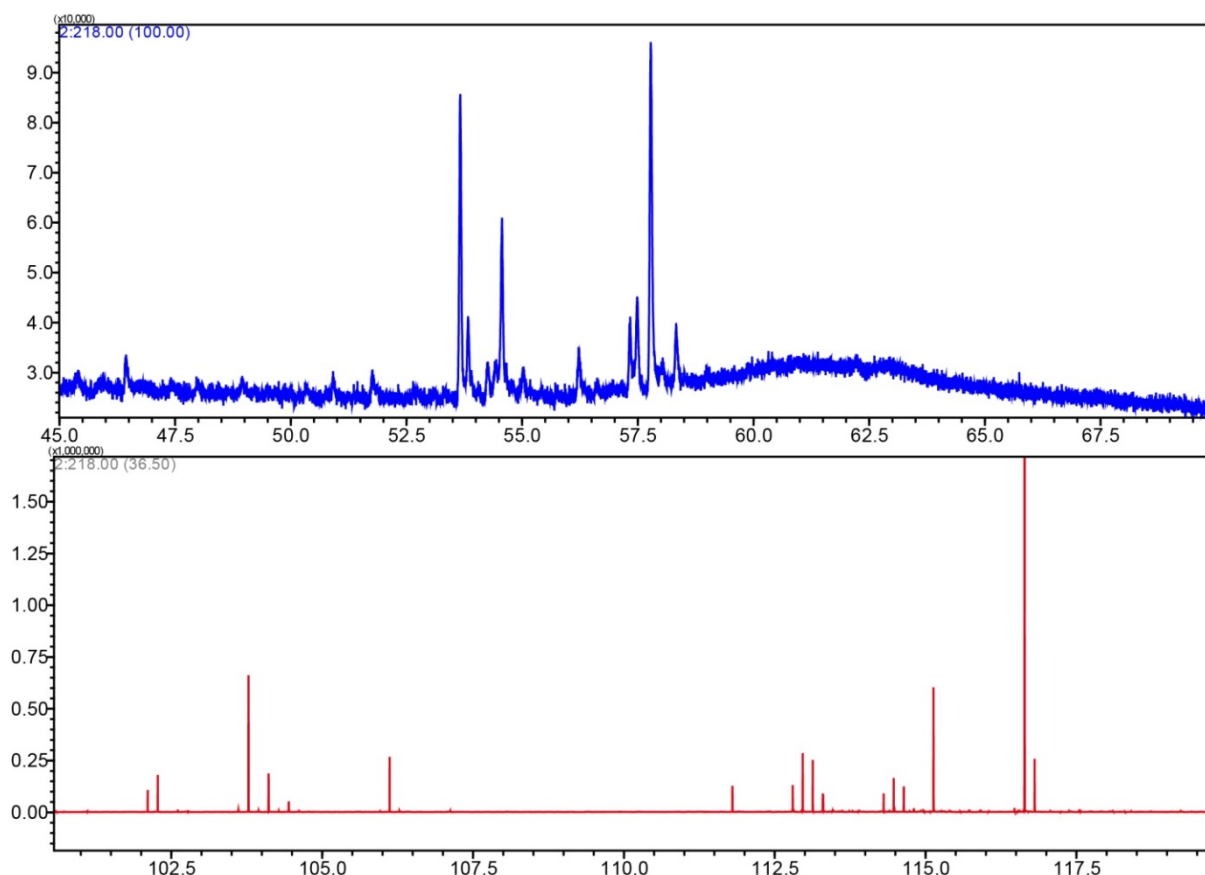


Figure 2.3.13 Examples of application the SIM Mode detection of m/z 218 in 1D (A) and 2D (B) analysis.

2.3.2.6 Modulation time

In GCxGC analysis, a short modulation time ($T_M=3\sim 10s$) are usually applied to enable a quick separation. The compounds 2nd retention time is preferred to be less than or, at the most, equal to the modulation time (a constant value normally in the case of GCxGC operating under fast GC conditions in the second column of short length). However, in real analysis, the overlap of GC profile can be observed. As is shown in Fig.2.3.14, overlap of the GC profiles of subsequent modulations may happen when the retention time of a compound in the 2nd dimension time (T_x) exceeds the modulation time (T_M) (the so-called wraparound). The elution of the compounds with T_x larger than T_M can be identified in the 2D plot during the subsequent modulation cycles (n). The location of these compounds in the 2D usually showed a broadened peak shape in the 2nd dimension.

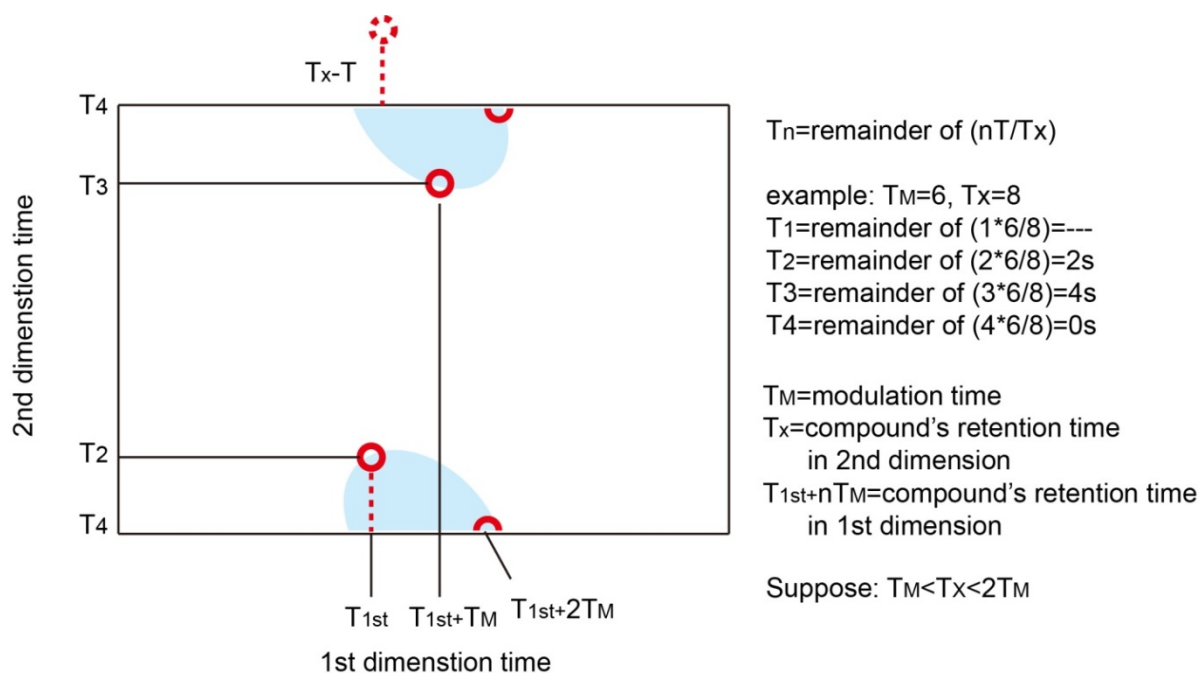


Figure 2.3.14 A simplified sketching showing the problem of wraparound.

The handmade paper was tested with different T_M settings. It can be observed that a shorter modulation period ($T_M = 4s$, Fig.2.3.15A) result in broaden peak shape along the second dimension, especially for the eluting of LG. When using the $T_M = 6s$ and $T_M = 8s$, wraparound effect still exist as can be seen from the ‘separated part’ (one part in the bottom and one part in the top of the chromatogram) of the cellulose pyrolysis products and LG, this problem can partly be solved using phrase shift functions in the GC IMAGE software for a nice presentation. When using the $T_M = 10s$, all the compounds are present nicely as can be observed in Fig.2.3.15.

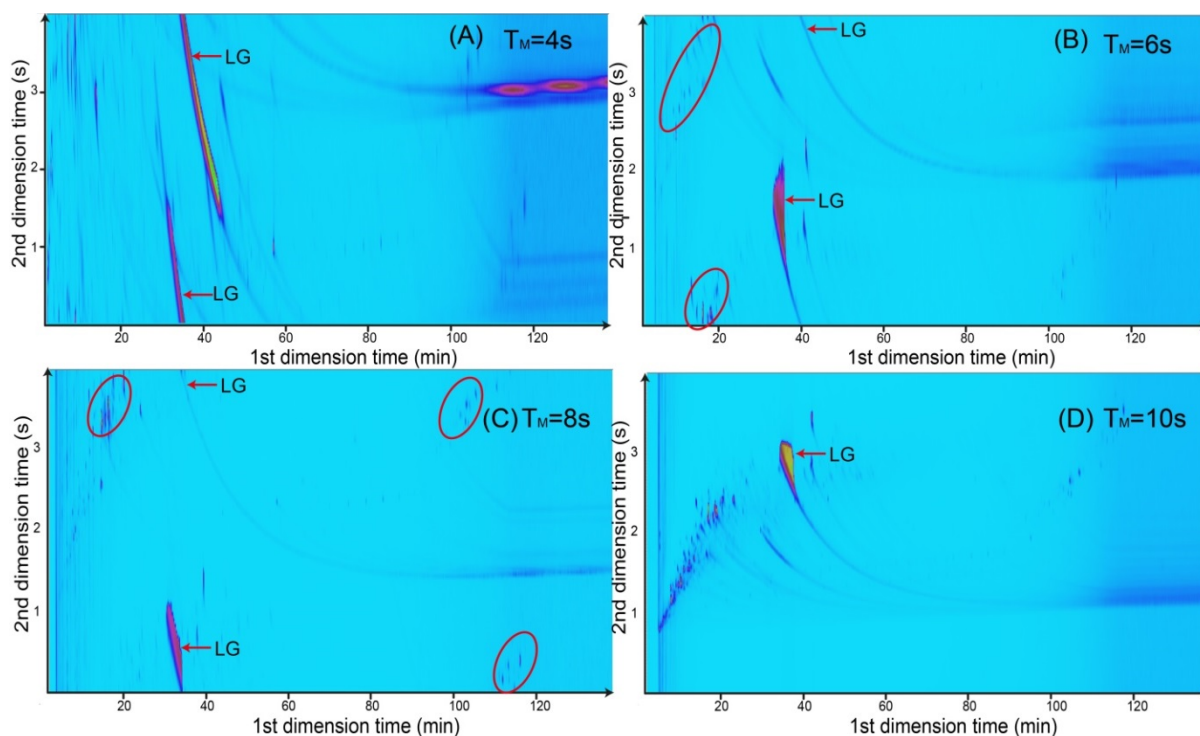


Figure 2.3.15 The test of handmade paper with different modulation time (T_M). (A) $T_M = 4s$, (B) $T_M = 6s$, (C) $T_M = 8s$, (D) $T_M = 10s$, The red circle and arrow in (A)-(D) are pointing the wraparound effect.

2.3.3 The general settings for test

After the examination of different parameters and the following method has been generally used for the test of handmade papers: Analysis was conducted with a Shimadzu QP 2010-Ultra gas chromatograph mass spectrometer (Shimadzu, Champs-sur-Marne, France) equipped with a two-stage thermal modulator ZX 2 (Zoex, Houston, USA). Pyrolysis was performed using a vertical micro-furnace-type pyrolyzer PY-3030D (Frontier Lab, Fukushima, Japan) directly connected to the injection port of the gas chromatograph. The sample was placed in a stainless steel sample cup. Typical quantities of sample paper used in this work were ca.50 μg weighted with an XP2U Ultra Micro Balance (Mettler Toledo, Viroflay, France). The pyrolysis temperature was defined to be 500 $^{\circ}C$. The pyrolyzer interface was held at 320 $^{\circ}C$. An OPTIMA-5HT column (30m \times 0.25mm I.D., 0.25 μm film thickness, Macherey-Nagel, Hoerd, France) was used as first dimension column and a Zebron ZB-50 (2.8m \times 0.1mm I.D., 0.1 μm film thickness, Phenomenex, Le Pecq, France) was used as a second-dimension column and for the loop modulator system. The separation was carried out at a constant pressure of 300 kPa using Helium Alphagaz 1 as carrier gas (Air Liquide, Bagneux, France). The ZX 2 two-stage thermal modulator was utilized with a modulation period of 10 s with a programmed hot pulse of 0.350 s. A two-step temperature program was

used for the hot jet set at 200 °C for 30 min and subsequently raised to 280 °C. The oven temperature was initially held 1 min at 100 °C, and then ramped at 2 °C min⁻¹ to 325 °C, where it was held for 25 min. The injector was held at 280°C and used in split mode (1:30 of the total flow). The mass spectrometer was operated at 20,000 u.s⁻¹, with a scan range from 50 to 500 u, using electron ionization at 70 eV. The interface was kept at 280 °C and the ion source at 200 °C.

2.3.4 A summary

The adding of the modulation in the GCxGC system added system complexity for the sample test. Apart from the factors discussed in section 2.3.3, other parameters such as sample overloading, the relation between the program of hot jet and oven temperature, gas flow rate can also affect the separation result. Sometimes, it is a multifactor effect that causes problem in GCxGC analysis. However, the effort for optimizing the parameters will be paid off in getting a nicer separation which makes the identification easier.

2.4 Py-GCxGC/MS in cultural heritage studies: an illustration using handmade papers

2.4.1 The aim of the study

The study of paper documents in the aim of identification, authenticity and provenance firstly require to identify plant materials used for the papermaking process. To obtain the information of the components of the paper, or more specifically, the fiber identification of handmade papers, microscopy observation has been mostly employed which resides in the optical characterization of the distinctive features of plant fibers. However, the identification of fibers by microscopy necessitates that the observer has a good experience in optical analysis of fibers and different researchers have different microscopic observation results for the same documents has been reported (in chapter 1). Also, visual microscopic observation even at higher magnifications may be ineffective to fully assess the nature of the fibers. For these reasons, an experimental method based on the chemical analysis of fiber constituents will be of great help for the identification of traditional handmade papers.

As has been introduced in section 2.3, the benefit of GCxGC separations has been utilized in areas where the complexity of the samples exceeds the capacity of 1D GC separation such as petroleum industry, medicine, and environmental analysis. GCxGC has really promote the comprehensive understanding of the sample's composition in the mentioned areas. Nevertheless, few applications have been reported for GCxGC's application in cultural heritage domain where the 1D separation has been mostly performed. until recently; the GCxGC methodology has only been applied for the analysis of organic residues (glues) from the stone tools [75, 76]. In considering the large archaeological remains and museum collection, the application of GCxGC is comparatively rare. Moreover, the pyrolysis has contributed to the analysis of cultural heritage material (discussed in section 2.1) due to the low quantity of sample required and an easy sampling process. Nevertheless, Py-GCxGC has not been reported in this domain. Under these circumstances, it is interesting to investigate what benefit GCxGC can bring to the analysis of cultural heritage materials. With this aim, Py-GCxGC/MS experiments were designed to test for the example of the kozo handmade papers with evaluation in the aspect of:

- A comparison of Py-GCxGC/MS data with Py-GC/MS data
- Py-GCxGC/MS characterization of kozo handmade papers
- Py-GCxGC/MS analysis of the museum samples

2.4.2 The research content

The work carried out explores the efficiency of Py-GC×GC/MS for the detection of triterpene and phytosterol plant markers for identification and characterization of a traditional Japanese handmade paper. The evaluation of the method is conducted in the aspect of the number of detected peaks, compound separation, sensitivity and identification of marker compounds. The use of Py-GC×GC/MS for the characterization of the material origin of an aged lining papers added to an ancient painting excavated from Dunhuang (China) demonstrated the potential application of this method for archaeological sample analysis. The benefits of the on-line coupling of analytical pyrolysis with a two-dimensional gas chromatography/mass spectrometry system were for the first time examined and proposed for cultural heritage studies.

The work described above was carried out during the PhD study period, presented at an international congress (21st International Symposium on Analytical and Applied Pyrolysis-Pyro 2016) and was published in a special issue of the Journal of Analytical and Applied Pyrolysis. In order to keep integration of the designed evaluation of Py-GC×GC/MS for cultural heritage applications (with the example of kozo handmade papers), the corrected proof of the work has been integrated in this section for the demonstration of the work carried out⁶⁶.

⁶⁶ For more information, refer to <https://www.sciencedirect.com/science/article/pii/S0165237016304806>



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Py-GCxGC/MS in cultural heritage studies: An illustration through analytical characterization of traditional East Asian handmade papers

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ABSTRACT

We tested the performance of pyrolysis-comprehensive two-dimensional gas chromatography/mass spectrometry in the course of our cultural heritage studies, and compared its potentialities for the characterization of traditional East Asian handmade papers with those previously obtained by pyrolysis-gas chromatography/mass spectrometry. The evaluation of the two methods was conducted in considering the number of detected peaks, the compounds separation, the sensitivity, and the identification of marker compounds. The results showed that the number of plant markers (triterpenes and phytosterols) detected in a Japanese kozo reference paper was improved in the pyrolysis fingerprint. With a gain in sensitivity in the order of six, the case study of a museum sample confirmed that pyrolysis-comprehensive two-dimensional gas chromatography/mass spectrometry is more efficient for a reliable identification of museum collections samples and/or archaeological samples where tiny quantities of materials are the rule. The present study has been successful in refining a micro-destructive method based on analytical pyrolysis for the characterization of traditional East Asian handmade papers using pyrolysis-comprehensive two-dimensional gas chromatography/mass spectrometry.

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1. Introduction

It has been more than 2000 years since the Chinese first discovered the secret of cellulosic fibers by pulping and intertwining plant fibers [1]. The predominance of paper left us tremendous amount of paper-based documents, paintings and calligraphies, which are invaluable for historical and archaeological studies. Even when modern papermaking technology prevails, the traditional hand papermaking still exists for its importance in culture, art and aesthetics [2,3].

The study of paper-based samples in the aim of identification, authenticity and provenance firstly require to identify plant materials used for the papermaking process [1,2,4,5]. Up to now, microscopy still remains as the main efficient way to distinguish paper-based cultural heritage samples from their material origin which resides in the optical characterization of the distinctive features of plant fibers [6,7]. Such fiber identification consists in careful observation of paper samples at 100 times or greater magnifications to study the fiber morphology, with the help of staining to accentuate fiber features and to identify pulping processes.

However, visual microscopic observation even at higher magnifications may be ineffective to fully assess the nature of the fibers. The use of reference materials and known fiber samples are essential for a proper identification of the raw materials [8]. The identification of fibers by microscopy necessitates that the observer has a good experience in optical analysis of fibers. Moreover, when mixture of plant fibers is used for papermaking, microscopic fiber analysis is rendered more difficult. It also appears that some plants can produce more than one typology of fibers, requiring further investigation to characterize the differences shown by varieties of the same species [7]. Additionally, the omission of certain plants in the diagnostic analysis may induce differences in interpretation which consequently influences the related accuracy of the final results [9]. For these reasons, an experimental method based on the chemical analysis of fiber constituents will be of great help for the identification of traditional handmade papers.

As an analytical method, on-line pyrolysis had been explored for its practical application as early as 1953, and later in 1965, it was applied in culture heritage studies [10–12]. Pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) became a versatile technique commonly used in the domain of cultural heritage where it was employed for the characterization of different materials: resins, gums, varnishes [13,14]. Although much analytical pyrolysis on cellulosic materials had been carried out from the per-

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spective of thermodynamics, biomass and energy research, studies concerning paper for its immaterial cultural heritage value, and more specifically, Py-GC/MS experiments on ancient East Asian papers were limited. Investigations on historical paper artefacts can be found in the literature where cellulose fingerprints revealed to be of great help for the characterization of the state of degradation [15–17]. To the best of our knowledge, only one Py-GC study for the analysis of mucilaginous compounds in traditional East Asian handmade paper was previously available in the literature [18] before the publication of our recent investigations on East Asian papers by Py-GC/MS [19].

Normally, one dimensional gas chromatography (1D GC) can separate hundreds of peaks in one run. In some cases, 1D GC is insufficient for separating the individual constituents in complex mixtures. For example, for samples encountered during the analysis of petrochemical products, perfumes or tobacco blends, 1D GC was not able to efficiently separate each compound constitutive of the samples mainly because of the presence of co-eluting compounds [20–22]. In a comprehensive two-dimensional gas chromatography system (2D GC), two capillary columns connected via a modulation system are employed to optimize the complementary separation capabilities of columns of different polarities [23,24]. Addition of a mass spectrometer as a detector adds another dimension in the separation capability, allowing the identification of compounds through mass spectra analysis. As a result, a comprehensive two-dimensional gas chromatography/mass spectrometry (GC×GC/MS) system offers much higher resolution, peak separation capacity, selectivity and lower detection limit for the analysis of volatile organic molecules, making it one of the most powerful analytical systems [25,26]. However, in practice, claims of the ability of 2D GC over 1D GC to resolve a larger number of peaks are still subject to contradictory discussions principally in relation with the difficulty to apply similar conditions of analysis [27]. Nevertheless, for culture heritage and archaeological applications, the sensitivity of 1D GC may be not enough to precisely detect the targeted compounds due to the sample degradation and low quantity of available material currently at disposal of the heritage scientists for only one analysis. Even with this limitation, pyrolysis coupling with GC/MS has demonstrated its powerfulness for polymer analysis and identification in this field [28]. However, the evaluation of the benefits of using an analytical method of higher selectivity deserves to be investigated.

GC×GC/MS system was commonly explored by the method of liquid injection. As a matter of fact, the introduction of on-line coupling of analytical pyrolysis to GC×GC/MS enlarges the scope to polymeric and/or organic sample analysis [29]. Although the advantages of GC×GC/MS over GC/MS had been extensively reviewed and their applications had been frequently reported in energy, fuel, geochemistry and medicine [30–32], available examples for the on-line coupling of pyrolysis to GC×GC/MS systems are scarce [29,33]. To our knowledge, neither GC×GC/MS nor Py-GC×GC/MS have been introduced and used in culture heritage studies. No works have been carried out to compare in detail the difference of Py-GC×GC/MS over Py-GC/MS to take advantage of GC×GC capacities for the complex organic materials currently observed in the culture heritage field meanwhile their interests have been explored in other fields [31,34,35]. This fact is likely due to the increased difficulty of data mining of tremendous number of peaks. However, with the help of chemometric and chromatographers, lots of applications are available that make the GC×GC/MS feasible as an efficient routine analysis method [29,36,37]. For example, the use of GC×GC/MS system for biomarker detections of oil samples has demonstrated its potential for the characterization of organic and/or polymeric samples [38,39]. The reported separation of oleanane and lupane congeners in Tertiary oils by 2D system for characterization of angiosperm-derived biomarkers is demonstra-

tive since angiosperm-derived plants are widely used in cultural heritage objects [40].

This article explores the efficiency of Py-GC×GC/MS for the detection of triterpene and phytosterol plant markers for identification and characterization of a traditional Japanese handmade paper. The evaluation of the method is conducted in the aspect of the number of detected peaks, compound separation, sensitivity and identification of marker compounds. The use of Py-GC×GC/MS for the characterization of the material origin of an aged lining papers added to an ancient painting excavated from Dunhuang (China) demonstrated the potential application of this method for archaeological sample analysis. The benefits of the on-line coupling of analytical pyrolysis with a two-dimensional gas chromatography/mass spectrometry system were for the first time examined and proposed for cultural heritage studies.

2. Experimental

2.1. Material

Material used as modern reference consisted of a handmade paper prepared following the Japanese traditional processes. This paper was made from kozo (*Broussonetia kazinoki* Siebold & Zucc.). An ancient sample of lining paper was also employed in this study to evaluate the efficiency of the techniques with a naturally aged sample. This lining paper was taken from a painting belonging to the Musée national des arts asiatiques–Guimet (Paris). The original artwork is dated from the first half of the Five dynasties (907–960 AD) and originates from the library cave in Mogao-ku, Dunhuang, located in northwest of China. A small sample of the lining paper, unknown origin, was removed from a paper painting showing “Two Buddha and a Bodhisattva” (EO 3642).

2.2. Py-GC/MS analysis

Py-GC/MS analyses were performed using a vertical micro-furnace-type pyrolyzer PY-2020iD (Frontier Lab, Fukushima, Japan) directly connected to the injection port of a Shimadzu QP2010 gas chromatograph linked with a Shimadzu QP2010Plus quadrupole mass spectrometer (Shimadzu, Champs-sur-Marne, France). The sample was placed in a stainless steel sample cup. The paper samples were weighted with an XP2U Ultra Micro Balance (Mettler Toledo, Viroflay, France). Typical quantities of reference paper used in this work for the development of the analytical method ranged from 80 to 110 µg weighted with the microbalance. For the balance’s capacity used (2.1 g), readability and repeatability of the microbalance were 0.0001 and 0.0002 mg, respectively. The sample cup was placed on top of the pyrolyzer at near ambient temperature. The sample cup was introduced into the furnace at 500 °C, and then the temperature program of the gas chromatograph oven was started. The Py-GC interface was held at 320 °C. Chromatographic separation was carried out on an Agilent DB5-ms 5% phenyl–95% dimethyl polysiloxane fused silica capillary column (30 m length, 0.25 mm inner diameter and coated with a 0.25 µm film thickness). The oven temperature was initially held 3 min at 40 °C, and then ramped at 5 °C min^{−1} to 325 °C, where it was held for 10 min. The total duration of GC analysis was 70 min. The helium carrier gas Alphagaz 1 (Air Liquide, France) was used in the linear velocity mode (1 mL/min). The injector was held at 280 °C and used in split mode (1:10 of the total flow). The mass spectrometer was operated at 5000 Da.s^{−1}, with a scan range from 50 to 500 Da, using electron ionization at 70 eV. The interface was kept at 300 °C and the MS source at 200 °C. The mass spectra of the pyrolysis products were recorded using the average spectrum + average and subtract spectrum modes in the Shimadzu GCMSsolution soft-

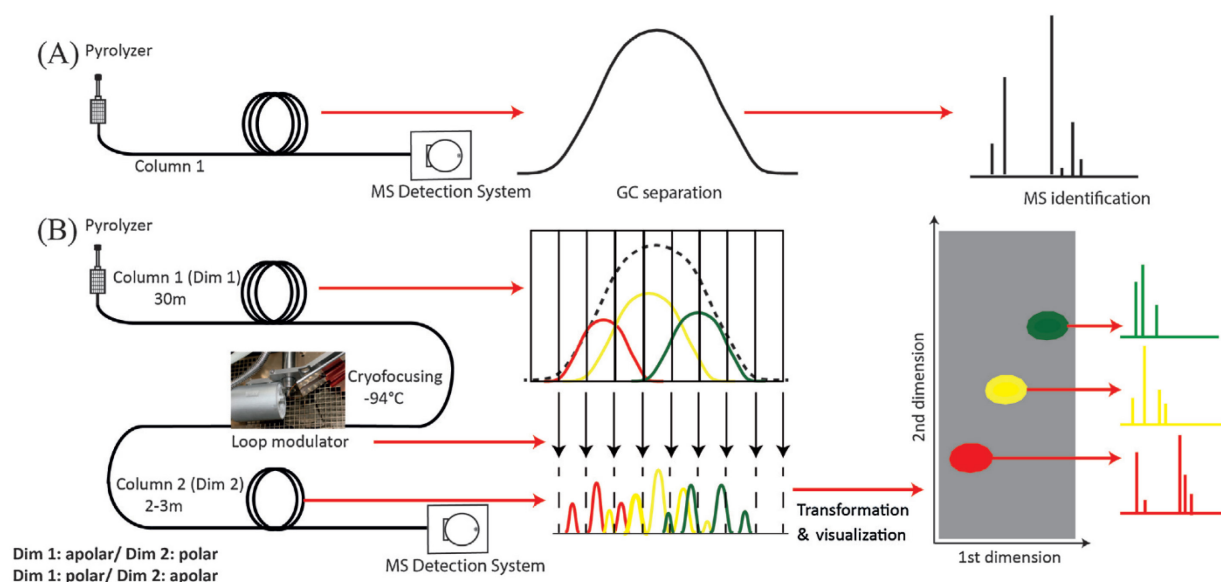


Fig. 1. Schematic representation of the experimental setup of pyrolysis coupling with GC/MS system (Fig 1A) and GCxGC/MS system (Fig 1B) with corresponding data process workflow.

ware. Identifications were achieved on the basis of EI mass spectra by interpretation of the main fragmentations [19] and using the NIST MS library (2011).

2.3. Py-GCxGC/MS Analysis

Py-GCxGC/MS analyses were conducted with a Shimadzu QP 2010-Ultra mass spectrometer (Shimadzu, Champs-sur-Marne, France) equipped with a two-stage thermal modulator ZX 2 (Zoex, Houston, USA). Pyrolysis was performed using a vertical micro-furnace-type pyrolyzer PY-3030iD (Frontier Lab, Fukushima, Japan) directly connected to the injection port of the gas chromatograph. The sample was placed in a stainless steel sample cup. Typical quantities of sample paper used in this work ranged from 20 to 50 μg weighted as described in 2.2. The sample cup was placed on top of the pyrolyser at near ambient temperature. The sample cup was introduced into the furnace at 500 °C, and then the temperature program of the gas chromatograph oven was started. The Py-GCxGC interface was held at 320 °C.

A OPTIMA-5HT column (30 m \times 0.25 mm I.D., 0.25 μm film thickness, Macherey-Nagel, Hoerd, France) was used as first dimension column and a Zebron ZB-50 (2.8 m \times 0.1 mm I.D., 0.1 μm film thickness, Phenomenex, Le Pecq, France) was used as a second-dimension column and for the loop modulator system. The separation was carried out at an initial constant pressure of 300 kPa (which resulted in a flow rate varying from 1.0 mL/min to 0.4 mL/min during the acquisition), and using Helium Alphagaz 1 as carrier gas (Air Liquide, Bagnex, France). The ZX 2 two-stage thermal modulator employs a closed cycle refrigerator/heat exchanger to produce a -94 °C cooled air jet regularly modulated with a pulsed hot air jet. The optimized modulation period was 10 s with a programmed hot pulse of 0.350 s. A two-step temperature program was used for the hot jet set at 200 °C for 30 min and subsequently raised to 280 °C in order to increase the modulation step efficiency in correlation with the volatility of pyrolysis products. The oven temperature was initially held 1 min at 100 °C, and then ramped at 2 °C min⁻¹ to 325 °C, where it was held for 25 min. The total duration of GC analysis was 138.5 min. The injector was held at 280 °C and used in split mode (1:30 of the total flow). The mass spectrometer was operated at 20,000 Da.s⁻¹, with a scan range from

50 to 500 Da, using electron ionization at 70 eV. The interface was kept at 280 °C and the ion source at 200 °C. Data processing of the Py-GCxGC/MS raw data was achieved using GC Image software, version 2.4 (Lincoln, Nebraska). Identification of paper components was performed by comparing the mass spectra of unknown components with the ones of the NIST mass spectra library, 2011 edition. All analysis has been conducted in triplicate except for the ancient lining papers from the Musée national des arts asiatiques-Guimet (Paris).

3. Results and discussions

3.1. Py-GCxGC/MS data over Py-GC/MS data

A schematic description of the experimental setup of pyrolysis coupling with GC/MS system and GCxGC/MS system is shown in Fig. 1. In coupling two capillary columns, the GCxGC/MS system requires the use of a cryogenically cooled loop modulator. The modulation step permits the sequential trapping and releasing of co-eluting compounds and, by the use of software imaging, the data are collected and treated under the form of a colored 2D chromatogram (Fig. 1B). With the additional column, the GCxGC/MS system provides information on the second dimension retention time (2t_R) besides first dimension retention time (1t_R) as well as fragment ions (m/z), peak heights and areas for the chromatographic peaks. As GCxGC is now a widely used multidimensional GC technology, readers who are interested in cultural heritage studies but not fully familiarized with Py-GCxGC/MS applications are directed to the literature if detailed information is required [26,41,42].

An illustration of the benefit of such setting is provided in Fig. 2 where the same kozo reference paper sample was analyzed by Py-GC/MS and Py-GCxGC/MS. The two chromatograms are corresponding to each other according to the elution order of 1t_R . The 2D chromatogram confirmed the same layout but, obviously, demonstrated the benefit of separation on a second dimension with an expanded distribution of the compounds generated by pyrolysis through the two-dimensional space. Interest of this 2D chromatogram resides in the clear differentiation between the constituents issued from pyrolysis which could be barely differ-

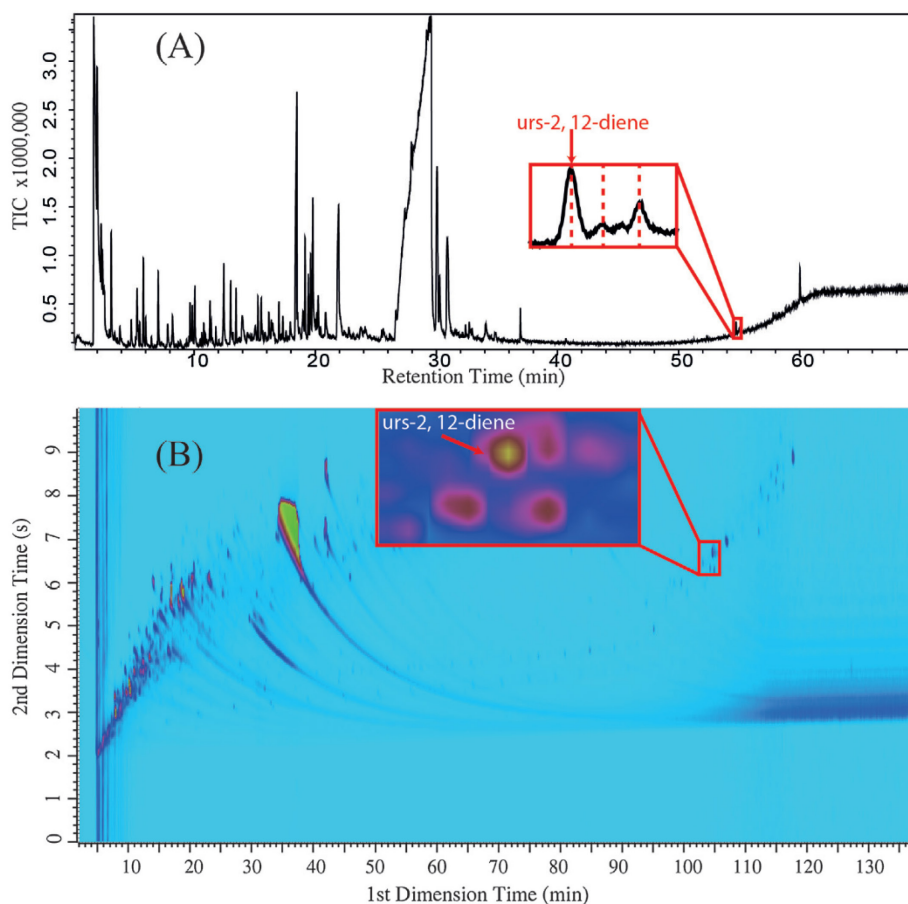


Fig. 2. Separation results of chromatograms from the kozo reference paper by Py-GC/MS (A) and Py-GC×GC/MS (B) with 109.4 μ g and 46.6 μ g, respectively. The inset photography in (A) and (B) represent the number of peaks detected around the identified peak urs-2, 12-diene.

entiated in 1D GC as illustrated for the case of the urs-2,12-diene and its neighboring co-eluting compounds. A total of six chromatographic peaks were detected by Py-GC×GC/MS analysis, while only three chromatographic peaks were detected by Py-GC/MS analysis (insets in Fig. 2A and B). These insets obtained from the pyrolysis of the kozo reference paper demonstrated the difficulty faced with the characterization of pyrolysis fingerprints of the paper samples, a problem commonly encountered in the 1D pyrolysis of natural polymers. As can be clearly seen, the peaks eluting in this portion of the chromatogram were not well separated in 1D GC, with the associated risk of missing informative peaks due to co-elution. At the opposite, the same peaks were clearly separated and nicely resolved in 2D GC. This gain in separation resulted from the modulation step which, by principle, improves the sensitivity for low abundance peaks.

Fig. 3 summarizes the total number of detected peaks from the kozo reference paper sample by Py-GC/MS and Py-GC×GC/MS analysis in applying different thresholds for spectrum deconvolution. In Py-GC/MS analysis the threshold for peak integration was modulated by the slope/min in the GCMSsolution software (Shimadzu). In Py-GC×GC/MS analysis, the various groups of datapoint pixels associated with the separated analytes were detected and quantified after correcting the baseline in GC IMAGE software. Each such group of pixels is called a blob and the operation of segmenting pixels into blobs is defined as blob detection. The threshold for peak integration of 2D GC data is modulated by the minimum area for blob detection. These two parameters, the slope/min and the minimum area for blob detection, are the acting parameters which

operate the deconvolution step in 1D GC and 2D GC, respectively. As is shown in Fig. 3, the 2D GC has much more peaks detected during the defined deconvolution process. The number of detected peaks was in hundreds range for 1D GC and in thousands range for 2D GC, which was explained by the enhanced sensitivity and resolution of 2D GC system. The thresholds for peak detection and integration can largely affect the number of identified peaks during the chromatographic analysis. For 2D GC data treatment, the background subtraction step (baseline subtraction) is important since it will contribute to the level for peak detection. After the baseline subtraction procedure, the number of detected peaks can be artificially influenced by considering the minimum area for blob detection during the deconvolution step. Additionally, increasing the minimum area for peak detection may not avoid the detection of background signals: to avoid this, careful check of the mass spectra information must be considered. Once the baseline subtraction step is refined and the peaks of interest are identified, the choice of the value for the minimum area for blob detection becomes significant. Unfortunately, one must notice that background peaks will never be totally eliminated by the automatic deconvolution process, and this drawback is also operative in 1D GC. As will be discussed (part 3.3), the use of templates helps to solve this problem in 2D GC. As illustrated in Fig. 3, the number of peaks detected, which was attributed to the improved sensitivity and improved resolution in 2D GC was more than 10 times than that of 1D GC. By manual examination and comparison of the detected peaks, a threshold of 500 (slope/min) and 25 (minimum area for blob detection) were chosen for Py-GC/MS and Py-GC×GC/MS analysis, respectively.

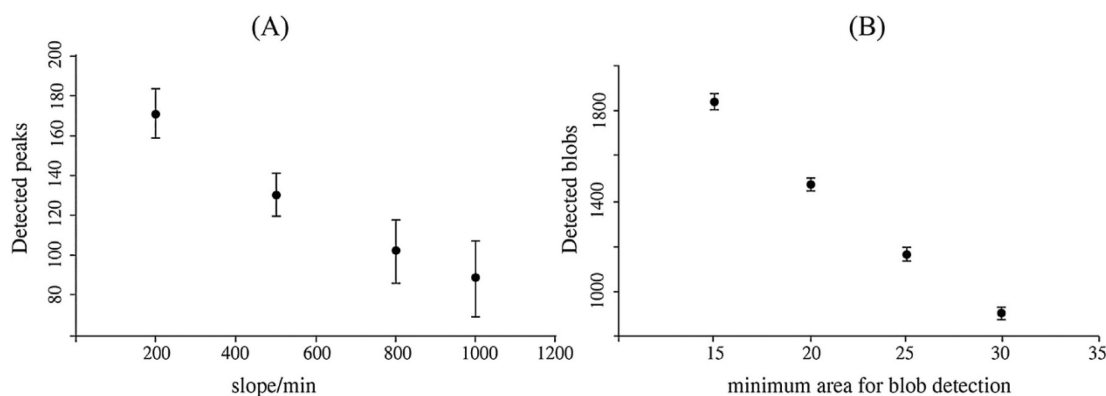


Fig. 3. Number of total peaks detected by applying different thresholds for spectrum deconvolution of Py-GC/MS analysis (A) and Py-GC \times GC/MS analysis (B). The dots are the average of the peaks detected and the error bar is the standard deviation of the triplicate of the kozo reference paper sample.

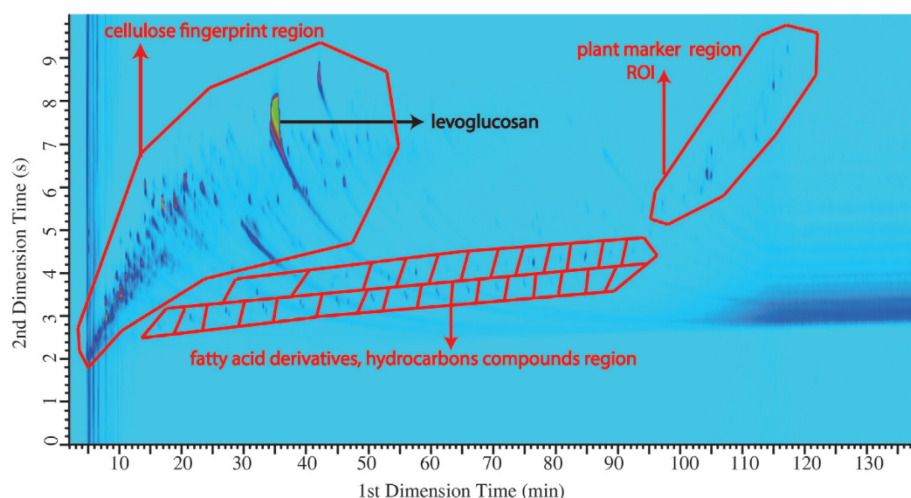


Fig. 4. The 2D chromatogram with the pattern of (i) cellulose fingerprint region, (ii) fatty acid derivatives, hydrocarbons compounds region and (iii) plant marker region (ROI).

3.2. Reference paper characterized by py-GC \times GC/MS

The plants have the capacity to synthesize and store a wide variety of low molecular weight compounds, which help in the identification of different plant materials. Specificity of the traditional East Asian hand papermaking process permits to preserve these primary metabolites initially present in the raw fiber materials used for papermaking. Our previous study had demonstrated the feasibility of using analytical pyrolysis for the characterization of traditional East Asian handmade papers of different material origins, in providing new criteria for discriminating plant species focusing on the detection of these primary metabolites [19]. However, the structural similarity of these metabolites, e.g. phytosterols, may conduct to the use of lengthy analytical methods for their identification. The application of new analytical methods succeeding in the clarification of the whole composition of the plant marker content can be of great efficiency and effectiveness [43].

The 1D chromatograms obtained from Py-GC/MS could be divided into three parts according to the retention time of eluting components, which consists of cellulose degradation products, hydrocarbons/fatty acid derivatives compounds, and the plant fiber markers at the higher retention time of the temperature program [19]. The 2D chromatogram revealed the same pattern (Fig. 4), where these three types of pyrolysis products can be nicely distinguished: the cellulose fingerprint which encompasses

levoglucosan, anhydrosugars, furan derivatives, the intermediate region of the hydrocarbons (alkanes, alkenes) and fatty acid derivatives, and the region of plant markers used to distinguish and to characterize the origin of different handmade paper fibers. In the following, only the region of plant markers that have distinguishable compounds will be studied. This region is defined as region of interest (ROI).

Chromatographic co-elution occurs when two (or more) compounds do not chromatographically separate due to the fact that both compounds have retention times that differ by less than the resolution of the method (Fig. 1). As a matter of fact, this is the point where the advantages of GC \times GC/MS systems for separating closely eluting compounds have been explored in several studies [22,44,45]. Fig. 2 has demonstrated the increased separation ability of the Py-GC \times GC/MS analysis of a kozo reference paper vs. Py-GC/MS by adding a second dimension for the separation of pyrolysis compounds. Fig. 5 depicts in more detail the gain in separation capacity for the compounds eluting between 102 min and 106 min in 1t_R and 6.2 s and 7.0 s in 2t_R in the 2D chromatogram. In this region, the Py-GC/MS analysis permitted to identify two main compounds, namely urs-2,12-diene (8), stigmasta-3-ol (9) [19]. As shown in Fig. 5, the Py-GC \times GC/MS analysis provided more peaks beside compound 8 and compound 9. In particular, it was possible to detect stigmasta-4,6-dien-3-ol (n), which was not detected during the 1D analysis due to its low concentration and its co-

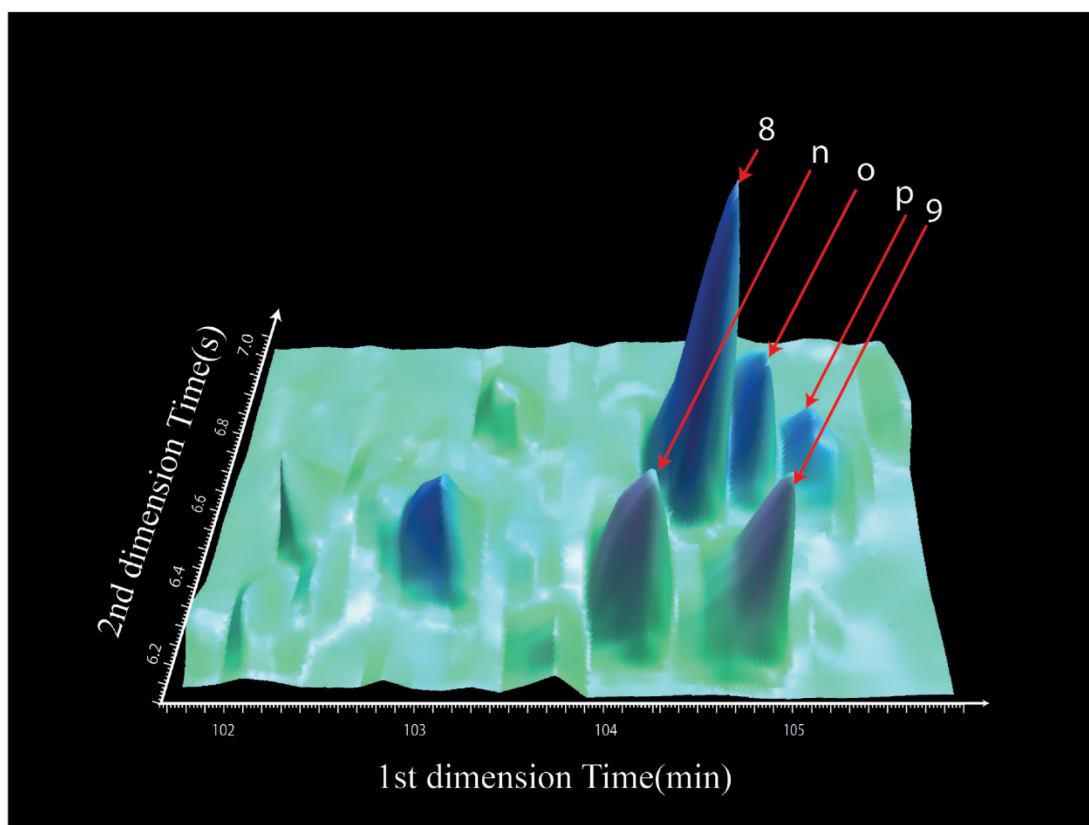


Fig. 5. The 3D chromatogram view of the distribution of compounds 8, n, o, p and 9 from kozo reference paper by Py-GC×GC/MS analysis. See Table 1 for detailed identification.

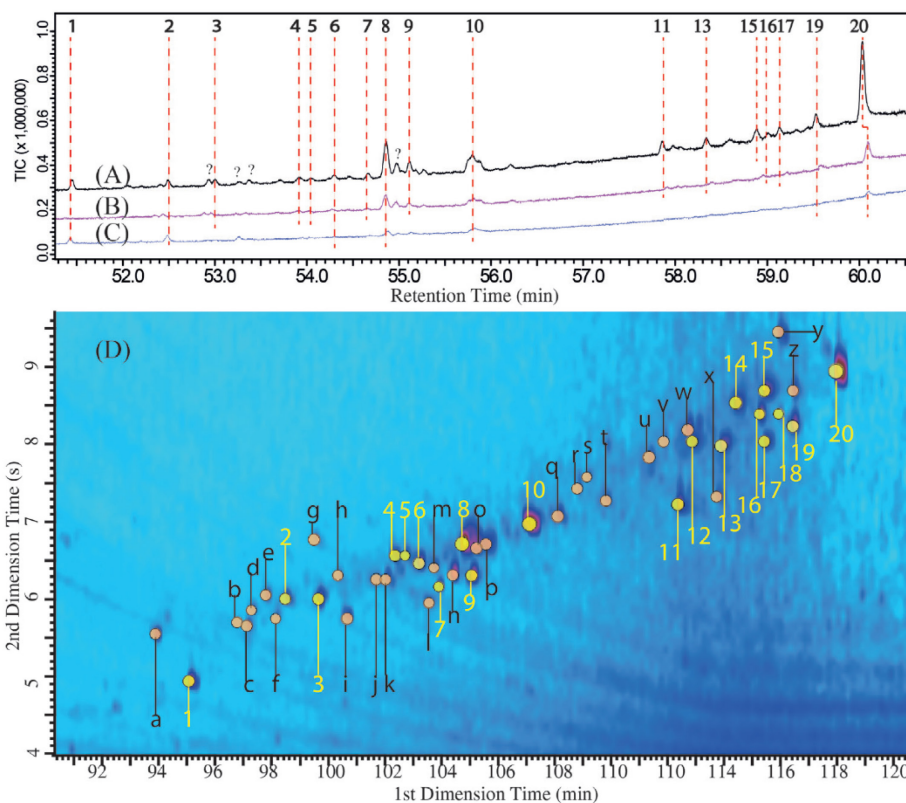


Fig. 6. Compound assignment within the ROI of the kozo reference paper. Fig. 6A–C reported the Py-GC/MS of kozo reference samples with 109.4 μg , 64.1 μg and 46.6 μg , respectively. Fig. 6D showed the Py-GC×GC/MS result with 50.0 μg .

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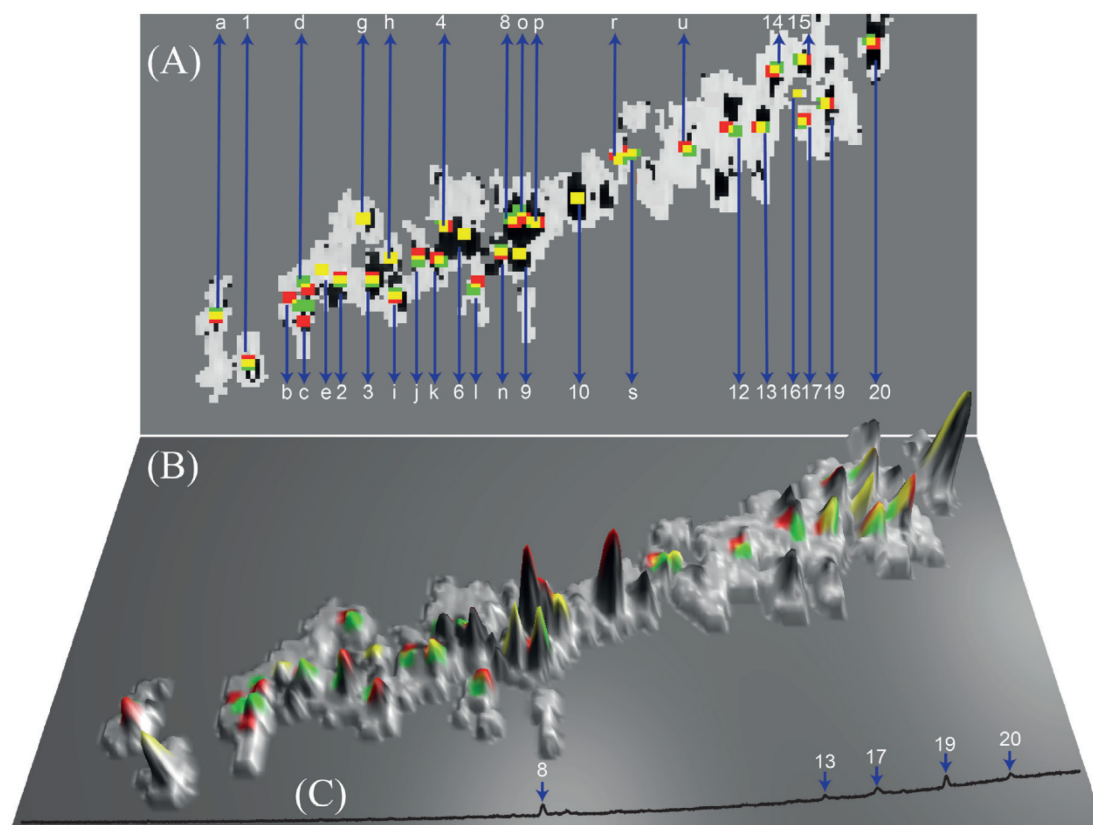


Fig. 7. Visualized Py-GC×GC/MS profile compared between analyzed sample (museum sample) and the kozo reference paper. A is the 2D view of the compounds distribution and B is the corresponding 3D view with the relative peak intensity. C reports the Py-GC/MS chromatogram of the ROI of tested museum sample.

elution with compound 8. Moreover, the stigmasta-4,6-dien-3-ol (*n*) was not characterized by Py-GC/MS because of the difficulty to extract a high quality mass spectrum under this co-eluting condition. Two other compounds *o* and *p* were nicely separated as opposed to the 1D separation where identification of the structure of these compounds was not attainable. In Py-GC/MS profile, only one compound was assigned, while now two isomers of ursane (*o*) and oleanane (*p*) structures were characterized. Detection of compound *n* during 2D analysis also revealed that the kozo fibers were not only providing ursane and oleanane structures but also minor phytosterol compounds through thermal degradation. The case of sitosterol (compound 11, Fig. 6), that was detected in 1D Py-GC/MS only with high quantity of sample, is illustrative of this fact. The 2D analysis permitted the easy detection of these phytosterol compounds, which were not easily characterized during 1D analysis because of co-elution and low level in the 1D chromatogram.

Fig. 6 depicted the gain in sensitivity and resolution of Py-GC×GC/MS compared to Py-GC/MS for the ROI where plant markers eluted. It can be concluded from this figure that when testing ca. 100 µg under Py-GC/MS conditions, a good description of the kozo reference paper with ca. 20 characteristic markers can be obtained. Lowering the quantity of material to ca. 50 µg drastically reduce the number of detected compounds: there were barely 7 compounds that could be well recognized for identification. As shown in Fig. 6A, some minor compounds, clearly detected by Py-GC×GC/MS (compound 2, 3 etc. in Fig. 6D), could also be detected but could hardly be well recognized by mass spectrometry due to a low signal to noise ratio. Obviously, under the conditions of Py-GC×GC/MS used during the present work, analysis provided more information at a level of ca. 50 µg with a better resolution as demonstrated above: a factor of gain of 2 is then obtained. Moreover, due to the re-concentration

effect of the modulation step in two-dimensional comprehensive gas chromatography, the split ratio used for the injection of samples is usually increased in 2D GC. The split ratio used in these experiments was of 30:1 for Py-GC×GC/MS analysis, compare to 10:1 for Py-GC/MS, which represents a factor of 3. Then it appeared that a rough factor of 6 times in gain of sensitivity can be expected from the use of Py-GC×GC/MS compared to Py-GC/MS in the course of the present work for the analysis of kozo reference paper. Additionally, Py-GC×GC/MS provides an amazing dynamic, a noticeable advantage which permits the detection of minor compounds if a large sample, namely a 100 µg sample, is analyzed even if the major compounds lead to saturated peaks.

Under our experimental conditions, for the kozo reference paper sample, within the ROI, finally, ca. 20 compounds were identified in the Py-GC/MS data profile while 46 compounds were identified in the Py-GC×GC/MS data profile. Consequently, the number of peaks detected and identified by Py-GC×GC/MS was more than 2 times higher than those detected by Py-GC/MS (Table 1). This improvement in peak detection increases the reliability of the characteristic fingerprint for a given sample of East Asian paper.

3.3. Application to a museum case

Py-GC/MS demonstrated the capability of targeting different biomarkers from modern handmade reference papers and therefore confirmed that ancient East Asian papers can be characterized through the detection of these basic plant fiber components [19]. Even though the Py-GC/MS analysis meet the demand for low quantity sampling with a minimum quantity of material (in the low µg range), when applied to museum collections or ancient papers that went through aging and degradation, Py-GC/MS cannot always

Table 1

List of plant marker compounds detected within the ROI in the 2D chromatogram of the Py-GC/MS analysis of the kozo reference paper with their respective retention times, assigned structures with molecular weight (MW), main fragment ions in the corresponding mass spectrum, and formula. The Arabic number correspond to compounds detected in 1D and 2D experiments and the letter correspond to the compounds mainly detected in 2D experiment.

No.	¹ t _R	² t _R	MW	main fragment ions (in decreasing intensity)	formula	assignment
a	93.83	5.5	368	145, 81, 55	C ₂₇ H ₄₄	NA* cholestane
1	95	4.9	410	69, 81, 95	C ₃₀ H ₅₀	squalene
b	96.67	5.65	370	91, 162, 316	C ₂₇ H ₄₆	cholest-2-ene or cholest-3-ene
c	97	5.6	400	133, 81, 107, 145	C ₂₈ H ₄₈ O	campesterol
d	97.17	5.8	368	105, 55, 255, 368	–	NA cholestane
e	97.67	6	384	135, 81, 366	C ₂₇ H ₄₄ O	cholesta-4,6-dien-3-ol, (3 beta)-
f	98	5.7	408	175, 135, 365	C ₃₀ H ₄₈	A-neooleana-3(5),12-diene isomer
2	98.33	5.95	368	147, 81, 368	C ₂₇ H ₄₄	cholesta-3,5-diene
g	99.33	6.7	–	294,155,211	–	NA
3	99.5	5.95	408	365, 121, 135	C ₃₀ H ₄₈	A-neooleana-3(5),12-diene isomer
h	100.17	6.25	NA	406, 255, 133	–	NA
i	100.5	5.7	396	145, 81, 95	C ₂₉ H ₄₈	stigmasta-3,5-diene
j	101.5	6.2	–	218,189, 147	–	NA ursane
k	101.83	6.2	–	218, 189, 147, 365	–	NA ursane
4	102.17	6.5	406	406, 255, 95	C ₃₀ H ₄₆	oleanane-2,9,12-triene
5	102.5	6.5	406	406,95,391	C ₃₀ H ₄₆	NA
6	103	6.4	408	218, 203,189	C ₃₀ H ₄₈	oleanane-2,12-diene
l	103.33	5.9	398	215, 55, 135	–	NA cholestan
m	103.5	6.35	–	218, 95, 203	–	NA oleanane
7	103.67	6.1	414	105, 57, 145, 396	C ₂₉ H ₅₀ O	NA stigmastan
n	104.17	6.25	412	135, 143, 394	C ₂₉ H ₄₈ O	stigmasta-4,6-dien-3-ol
8	104.5	6.65	408	218, 189, 95	C ₃₀ H ₄₈	urs-2,12-diene
9	104.83	6.25	414	147, 81, 396	C ₂₉ H ₅₀ O	stigmasta-3-ol
o	105	6.6	408	218, 189,95	C ₃₀ H ₄₈	NA ursane
p	105.33	6.65	408	218, 203, 189	C ₃₀ H ₄₈	NA oleanane
10	106.83	6.9	408	121, 147, 91	C ₃₀ H ₄₈	NA
q	107.83	7	408	218, 109, 189	–	NA
r	108.5	7.35	422	422, 95, 255	C ₃₀ H ₄₆ O	ursa-9(11),12-dien-3-one
s	108.83	7.5	422	422,208,255,133	–	NA
t	109.5	7.2	–	155, 143, 107, 55	–	NA
u	111	7.75	–	232, 217, 147, 422	–	NA
v	111.5	7.95	424	424, 95, 255	C ₃₀ H ₄₈ O	ursa-9(11),12-dien-3-ol
11	112	7.15	414	57, 55, 69	C ₂₉ H ₅₀ O	beta sitosterol
w	112.33	8.1	440	135, 273, 407	C ₃₀ H ₄₈ O ₂	oleanane-12-ene-11-one-3-ol
12	112.5	7.95	424	218, 203, 189	C ₃₀ H ₅₀ O	oleanane-12-en-3-one
x	113.33	7.25	466	466, 255, 95, 69	C ₃₂ H ₅₀ O ₂	13,27-cycloursan-11-en-3-ol, acetate
13	113.5	7.9	426	218, 203, 189	C ₃₀ H ₅₀ O	beta amyryn (Olean-12-en-3-ol)
14	114	8.45	424	218, 95, 107	C ₃₀ H ₄₈ O	urs-12-en-3-one
15	115	8.6	426	218, 55, 81	C ₃₀ H ₅₀ O	alpha-amyryn (urs-12-en-3-ol)
16	114.83	8.3	410	174, 410, 91	C ₂₉ H ₄₆ O	stigmasta-3,5-dien-7-one
17	115	7.95	466	466, 255, 95	C ₃₂ H ₅₀ O ₂	oleanane-12,14-diene-3-acetate
y	115.5	9.35	–	135, 232, 95, 55	–	NA
18	115.5	8.3	466	95, 69, 466	C ₃₂ H ₅₀ O ₂	urs-12,14-diene-3-acetate
19	116	8.15	468	218, 203, 189	C ₃₂ H ₅₂ O ₂	oleanane-12-ene-3-acetate beta-amyryn acetate
z	116	8.6	412	124, 229, 55	C ₂₉ H ₄₈ O	stigmast-4-ene-3-one
20	117.5	8.85	468	218, 189, 95	C ₃₂ H ₅₂ O ₂	alpha-amyryn acetate (urs-12-ene-3-acetate)

NA*: not attributed.

guarantee a good signal response for mass spectrometry identification. In our previous Py-GC/MS study, the presence of amyryn congeners permitted to identify the lining paper sample from the Musée national des arts asiatiques–Guimet (“museum sample” for short) as a kozo paper. Its material origin was also confirmed by microscopic fiber analysis. However, this sample of lining paper presented a relatively low signal to noise response and due to their low abundance, certain of the characteristic compounds observed in the fingerprint of the kozo reference sample cannot be detected.

As has been discussed in 3.2, Py-GC/MS revealed to be a great potential for the exhaustive characterization of traditional East Asian paper sample, providing a more complete view of the composition in one analysis sequence with a higher sensitivity. As for the Py-GC/MS data treatment, the baseline subtraction process and definition of minimum area value for blob detection are crucial in the process of 2D data treatment. Besides manually checking the mass spectra information for identification, data treatment requires the use of a dedicated software. The GC IMAGE software designed for this purpose proceeds through a template-

based chemical identification process. This process, based on a pixel-image approach, consists in creating a database associated to the sample of interest in defining the criteria that bear the chemical information. The described criteria are defined as template in the GC IMAGE software that use these sets of metadata (¹t_R, ²t_R, mass spectra information etc.) for matching the compounds in the chromatograms from unknown samples or for comparison from sample to sample. If similar chemical patterns in a subsequent chromatogram(s) were matched in the template, then the compounds were recognized and confidence of the result of the analysis was demonstrated.

Obviously, a run of 2D analysis will generate thousands of peaks and identification of all of them will be time-consuming. In order to reduce the quantity of peaks to consider in the 2D chromatogram, a template was made within the region of interest (ROI) of kozo reference paper (Fig. 4). The peaks detected and used for the construction of template was reported in Table 1, which provides the identification of plant marker compounds with their respective retention times in the first and second dimension, mass spectra information

and their tentative assignment. These compounds were identified from their comparison with MS library [46] and from their main fragmentations [19]. Once the template resulting from the 2D data treatment procedure was defined and characterized, comparison of samples can be conducted.

For the museum sample, the template procedure described above has been applied within the ROI. After the template matching procedure, the two profiles were compared between museum sample and kozo reference paper. Fig. 7A and B demonstrated the differences between the analyzed profile (museum sample) and the reference profile (kozo reference paper) with the corresponding identified peaks. According to the describe workflow in Fig. 1, in the generated image from the 2D chromatogram, each pixel value in the reference profile is subtracted from the corresponding pixel value in the analyzed profile. For the differences between the two profiles, the darker the image pixel, the higher the reference pixel value is above the analyzed pixel value. Medium grey represents no difference between the two profiles. Colored squares representing the blob peaks existing in the two profiles are drawn on top of this greyscale background image, where green squares represent peaks in the analyzed profile and red squares represent peaks in the reference profile. Yellow pixels displayed where the green and red squares overlapped.

It is depicted that 33 pairs of compounds were matched in the analyzed and reference profile (Fig. 7A). The yellow squares in Fig. 7A showed that for almost all of the matched compounds in kozo reference sample and the museum sample, their retention times were close with around 0.2 min difference in t_R and 0.1 s in $2t_R$ which can be regarded as well matching samples. It is also interesting to note that most of the matched peaks in Fig. 7A and B were within the dark pixel regions which means the peak intensities in kozo reference sample was comparatively higher than those in museum sample. This might be the result from its natural aging and degradation.

These results confirmed the previous Py-GC/MS identification of the museum sample as kozo handmade paper which was aided by microscopic fiber analysis. These results demonstrated the potentiality of Py-GC×GC/MS in providing more than 30 peaks of assigned plant fiber markers for the characterization of the museum sample. Even without microscopic fiber analysis, a reliable identification can be made which is an improvement in the analysis of handmade papers, especially for the characterization of archaeological samples mostly available in low quantity. Additionally, the comparison between Fig. 7A–C is a good demonstration of the power of the 2D GC. In the 1D GC analysis, only 5 peaks were identified while those peaks in 2D GC analysis increased more than five times. It was observed from the data processing that in somehow 2D GC can act as “peak finders” for 1D GC data treatment. Actually, some of the newly emerged compounds in 2D GC can be recognized with the information previously demonstrated by 2D GC if careful subtraction from the background was conducted in 1D GC chromatography.

4. Conclusion

The present study has been successful in defining a micro-destructive method using Py-GC×GC/MS for the characterization of traditional East Asian papers. The case study of a reference handmade paper prepared following the Japanese traditional processes and a museum collection sample tested by a Py-GC×GC/MS system and a Py-GC/MS system verified that Py-GC×GC/MS system outperformed well in the analysis of these samples with advantages of enhanced capacity, sensitivity and resolution. Although the main plant marker compounds were detected by both methods, providing a characteristic fingerprint for identification of material origin,

the Py-GC/MS analysis was limited for separating the co-eluting compounds and for detecting low abundance compounds, while Py-GC×GC/MS analysis separates and detects all of those chromatographic peaks. As a result, this improved comprehensive profile can be used as templates for quick and effective comparison and identification of samples. This profile illustrated how those benefits can be used for archaeological samples where minute quantities of samples are usually available.

The present analysis of traditional East Asian paper samples revealed that Py-GC×GC/MS analysis can detected ca. 10 times the number of peaks compared to Py-GC/MS analysis after choosing optimized parameters. Under this condition and after careful mass spectra data examination, within the ROI, 46 markers were assigned in Py-GC×GC/MS data profile while 20 markers were identified in Py-GC/MS data profile. The gain in sensitivity and in resolution in the ROI give the opportunity to increase the efficiency of using statistic tools, namely principal component analysis, in Py-GC×GC/MS analysis with the advantage of increased reliability of statistical results for a thorough comparison of samples of different material origins. Such investigations are the subject of ongoing studies in our laboratory.

Moreover, a worthy point of interest concerns the quick and complete view provided by Py-GC×GC/MS for unknown archaeological samples with (i) both a clear insight of its composition (e.g. purity of the sample), and (ii) a particular focus on a region of interest, like the ROI defined here for characteristic plant markers. These results illustrate how Py-GC×GC/MS promises to be a beneficial technique in cultural heritage studies where it is essential to further develop applications of modern technologies for the studies of our cultural heritage.

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2.5 Benefit of GCxGC/MS profiles for 1D GC/MS data treatment

2.5.1 The aim of the study

Two problems are currently pointed out for 1D GC/MS workflow analysis: one concerns the co-elution of compounds and the other concerns the detection of analytes of low concentration within the inherent background. The ways to tackle this problem lies in:

- Mass spectra deconvolution to identify low concentrated and co-eluting compounds which allows the separation of overlapping mass spectra into individual pure-mass spectra
- Adding another dimension for better separation by employing methods such as GCxGC

Some dedicated software, such as the Automated Mass Spectral Deconvolution and Identification System (AMDIS) has been routinely used for processing of GC/MS data. Deconvolution, the basis of AMDIS approach, is a critical step in the sense that deconvolution will reconstruct a pure mass spectrum for each eluted compound detected by the mass spectrometer. AMDIS works with an identity search approach: the unknowns are searched with a reference library, generally a home-made library in connection with the routine workflow. It provides fast and accurate results for identification and quantification of GC/MS results and compares well for its ability in GC/MS routine analysis. Since its introduction, AMDIS has demonstrated its ability to detect target compounds in matrices with high backgrounds. However, AMDIS may produce false peaks and may require optimized settings for an efficient use and was reported to frequently misidentify peaks (in generating false positives) or do not recognize compounds resulting in false negative assignments.

The principle and benefits of GCxGC analysis has been discussed in section 2.3 and section 2.4 and various applications can be found in petroleum, medicine and fragrance analysis. Nevertheless, a fact is, until recently, 1D GC is currently the predominant and effective analytical method within laboratories in certain areas (e.g. cultural heritage field). The enhanced resolution from GCxGC can give insight for mass spectra analysis reserved in a lower resolution data. The aforementioned approach of reintroducing optimized data obtained through 2D GC/MS into 1D GC/MS may present interesting applications for the optimized use of low resolution mass spectrometers in large workflow analysis.

For these reasons, the evaluation of the chromatographic and mass spectra information revealed by 2D GC/MS profile that can be favorably used for 1D GC/MS data mining and biomarker discovery within AMDIS deserves to be investigated. The aim of the study lies in:

- To answer the question ‘how these characteristics of GCxGC analysis are able to improve the mass spectra collection and to benefit 1D data treatment’ that has not yet been investigated
- to scrutinize how the improved knowledge obtained by Pyrolysis-GCxGC/MS profile can be beneficial for compound identification in a 1D GC/MS data collection process like the routine procedure of using AMDIS

2.5.2 The research content

The analysis was focused on low concentration and co-eluting compounds analysis, and focuses on the identification of the pyrolysis fingerprint of East Asian handmade paper samples. The method was firstly carried out with the examination of Py-GCxGC/MS data to generate a 2D profile for manual identification of low concentrated and co-eluting compounds in 1D GC/MS data. Then, an in-house AMDIS library was constructed using the mass spectra gained from a GGxGC/MS analysis and corresponding 1D GC/MS profile and their performance in the AMDIS automatic identification process was evaluated.

This designed work was carried during the PhD study period and was accepted for a publication in Journal of The American Society for Mass Spectrometry. In order to keep integration of the designed work, the full accepted content has been integrated in this section for the demonstration of the work carried out⁶⁷. The cited articles are provided within this section. For the supplementary information mentioned in the text, please refer to Annex 44.

⁶⁷ For more information, refer to: <https://link.springer.com/article/10.1007/s13361-018-1953-7>

Benefit of the use of GCxGC/MS profiles for 1D GC/MS data treatment illustrated by the analysis of pyrolysis products from East Asian handmade papers

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Abstract

In this study, we report the use of pyrolysis-GCxGC/MS profiles for an optimized treatment of data issued from pyrolysis-GC/MS combined with the automatic deconvolution software Automated Mass Spectral Deconvolution and Identification System (AMDIS). The method was illustrated by the characterization of marker compounds of East Asian handmade papers through the examination of pyrolysis-GCxGC/MS data to get information which was used for manually identifying low concentrated and co-eluting compounds in 1D GC/MS data. The results showed that the merits of a higher separation power for co-eluting compounds and a better sensitivity for low concentration compounds offered by a GCxGC system can be used effectively for AMDIS 1D GC/MS data treatment: i) the compound distribution in pyrolysis-GCxGC/MS profiles can be used as ‘peak finder’ for manual check of low concentration and co-eluting compound identification in 1D GC/MS data; ii) pyrolysis-GCxGC/MS profiles can provide better quality mass spectra with observed higher match factors in the AMDIS automatic match process. The combination of 2D profile with AMDIS was shown to contribute efficiently to a better characterization of compound profiles in the chromatograms obtained by 1D analysis in focusing on the mass spectral identification.

Keywords: pyrolysis; comprehensive two dimensional gas chromatography; AMDIS; data processing; East Asian handmade papers

Introduction

Two problems are currently pointed out for 1D GC/MS workflow analysis: one concerns the co-elution of compounds and the other concerns the detection of analytes of low concentration within the inherent background. One effective way to identify low concentrated and co-eluting compounds consists in mass spectra deconvolution. The deconvolution process allows the separation of overlapping mass spectra into individual pure-mass spectra, a practical way to unmix co-eluting compounds. Several applications have been developed for mass interpretation with the help of mathematical deconvolution employing different computing environment: Microsoft Visual Basic [1], R [2, 3] or Matlab [4]. Among them, the Automated Mass Spectral Deconvolution and Identification System (AMDIS), developed by the National Institute of Standards and Technology (NIST), is certainly the oldest stand-alone software package freely available for the routine processing of GC/MS data [5]. It was originally intended to provide unambiguous identification through deconvolution, algorithms-based quality matching, background subtraction as well as retention index comparison [6].

Deconvolution, the basis of AMDIS approach, is a critical step in the sense that deconvolution will reconstruct a pure mass spectrum for each eluted compound detected by the mass spectrometer. This deconvolution process is designed for the identification of target compounds in complex matrices. However, if extracted spectra do not match the target, the corresponding compound will be retained only if it shows dissimilar characteristics to the extracted nearby spectra. As a consequence, a risk consists in the fact that there are many extracted spectra that do not represent true chemical compounds but that are due to the effect of overlapping or the presence of noise in the collected chromatograms. Post-processing AMDIS filtering procedure have been subsequently proposed to circumvent these drawbacks and to limit the problem of co-eluting peaks [7].

AMDIS works with an identity search approach: the unknowns are searched with a reference library, generally a home-made library in connection with the routine workflow. This procedure, at the opposite of a similarity search, requires the utmost effectiveness of the deconvolution of mass spectra issued from the chromatograms. A serious limitation resides in the difficulty to distinguish clearly between compounds belonging to the same family or isomer structures. A key feature of AMDIS is that it can automatically identify components from a complex spectrum by comparing the mass spectra with the so-called reference libraries to resolve the problem of complex mixtures [8]. However, AMDIS may produce false peaks and may require optimized settings for an efficient use [9, 10] and was reported to frequently

misidentify peaks (in generating false positives) or do not recognize compounds resulting in false negative assignments [11].

Nevertheless, AMDIS can provide fast and accurate results for identification and quantification of GC/MS results [12, 13], and compares well for its ability in GC/MS routine analysis with other commercially or freely available deconvolution software platforms [9]. In particular, since its introduction, AMDIS has demonstrated its ability to detect target compounds in matrices with high backgrounds [9]. The protocols for its application in GC/MS workflow has been fully explained elsewhere [14]. One must concede that with the development of metabolomics in GC/MS, among software platforms for untargeted metabolomics based on GC–MS rapid annotation using in-house databases, AMDIS competes well with the other home-made software allowing for much tighter control and fine tuning than commercial software often characterized by their “black box” effects. Automatic searches for identification of unknowns from current libraries are still a vivid domain of development since most algorithms require constant optimization for improving their effectiveness [8].

Another way to solve the problem of co-eluting and low concentration compounds is by adding another dimension for separation. GC/MS is now a well-established method, while the seeking for higher separation and resolution has been partly fulfilled by multidimensional gas chromatography. On the criterion of 1D GC separation (e.g., different volatilities), co-elution of compounds occurred when the vapor pressures of analytes do not differ sufficiently from each other. In practice, the peak capacity of column (e.g., 30 meters) in a 1D GC is in hundreds range while values of peak capacity over 1000 is hard to attain even at the sacrifice of instrumental complexity and acquisition time. The introduction of 2D GC in 1991 largely accomplish the initial goal of separating these co-eluting compounds posed by 1D GC separation [15]. The setup of 2D GC is well know now which employs two columns of different polarity via a modulator. The entire eluent from the first column is subjected to reinjection in a second column through the modulator in a designed continuous and real-time mode. A comprehensive two-dimensional gas chromatography/mass spectrometry (GC×GC/MS) system offers much higher resolution, peak separation capacity, selectivity and lower detection limit for the analysis of volatile organic molecules [16, 17]. As a result of the separation feature and the re-concentration effect of the modulation step, peak capacity over 1000 is easily obtained [18, 19]. The merits of improved peak capacity and structure-based grouping of 2D GC over 1D GC has been extensively reported [20] (and references cited therein). One may expect a growing interest for GC×GC/MS in various fields, and one may

quote metabolomics where huge set of data are compiled as an example of its likely application.

Although 2D GC has advantages, 1D GC is currently the predominant and effective analytical method within laboratories all over the world. Improved knowledge about the composition of co-eluting compounds or low concentration compounds reserved in the testing sample demands enhanced resolution. And conversely, the enhanced resolution can give insight for mass spectra analysis reserved in a lower resolution data [21]. A fact is that, until recently, routine analysis in many fields was relying on low resolution mass spectrometry, the larger market in volume for manufacturers: the aforementioned approach of reintroducing optimized data obtained through 2D GC/MS into 1D GC/MS may present interesting applications for the optimized use of low resolution mass spectrometers in large workflow analysis. For these reasons, the evaluation of the chromatographic and mass spectra information revealed by 2D GC/MS profile that can be favorably used for 1D GC/MS data mining and biomarker discovery within AMDIS deserves to be investigated.

The aim of the present study is to scrutinize how the improved knowledge obtained by Pyrolysis-GCxGC/MS profile can be beneficial for compound identification in a 1D GC/MS data collection process like the routine procedure of using AMDIS. The analysis was focused on low concentration and co-eluting compounds analysis, and focuses on the identification of the pyrolysis fingerprint of East Asian handmade paper samples. The method was firstly carried out with the examination of Py-GCxGC/MS data to generate a 2D profile for manual identification of low concentrated and co-eluting compounds in 1D GC/MS data. Then, an in-house AMDIS library was constructed using the mass spectra gained from a GGxGC/MS analysis and corresponding 1D GC/MS profile and their performance in the AMDIS automatic identification process was evaluated.

Experimental

Material

Material used in this study consisted of handmade paper made from kozo (*Broussonetia kazinoki* Sieb.) prepared following the Japanese traditional processes. Kozo is a plant native to Asia and its inner bark fiber is commonly used in East Asian hand papermaking which accounted for a significant fiber resources in the history of hand papermaking.

Py-GC/MS Analysis

Py-GC/MS analyses were performed using a vertical micro-furnace-type pyrolyzer PY-2020iD (Frontier Lab, Fukushima, Japan) directly connected to the injection port of a Shimadzu QP2010 gas chromatograph linked with a Shimadzu QP2010Plus quadrupole mass spectrometer (Shimadzu, Champs-sur-Marne, France). The sample was placed in a stainless-steel sample cup. The paper samples were weighted with an XP2U Ultra Micro Balance (Mettler Toledo, Viroflay, France). Typical quantities of handmade paper used in this work were ca. 100 μ g weighted with the microbalance. The sample cup was placed on top of the pyrolyzer at near ambient temperature. The sample cup was introduced into the furnace at 500 °C, and then the temperature program of the gas chromatograph oven was started. The Py-GC interface was held at 320 °C. Chromatographic separation was carried out on a Frontier Lab DB5-ms 5% phenyl–95% dimethyl polysiloxane fused silica capillary column (30 m length, 0.25 mm inner diameter and coated with a 0.25 μ m film thickness). The oven temperature was initially held 3 min at 40 °C, and then ramped at 5 °C min⁻¹ to 325 °C, where it was held for 10 min. The total duration of GC analysis was 70 min. The helium carrier gas Alphagaz 1 (Air Liquide, France) was used in the linear velocity mode (1 mL min⁻¹). The injector was held at 280°C and used in split mode (1:10 of the total flow). The mass spectrometer was operated at 5000 u.s⁻¹, with a scan range from 50 to 500 u, using electron ionization at 70 eV. The interface was kept at 300 °C and the MS source at 200 °C. The mass spectra of the pyrolysis products were recorded using the average spectrum + average and subtract spectrum modes in the Shimadzu GCMS Solution software. Identifications were previously achieved on the basis of EI mass spectra by interpretation of the main fragmentations and using the NIST MS library (2011) [22].

Py-GCxGC/MS Analysis

Analysis was conducted with a Shimadzu QP 2010-Ultra gas chromatograph mass spectrometer (Shimadzu, Champs-sur-Marne, France) equipped with a two-stage thermal modulator ZX 2 (Zoex, Houston, USA). Pyrolysis was performed using a vertical micro-furnace-type pyrolyzer PY-3030iD (Frontier Lab, Fukushima, Japan) directly connected to the injection port of the gas chromatograph. The sample was placed in a stainless steel sample cup. Typical quantities of sample paper used in this work were ca.50 μ g weighted with an XP2U Ultra Micro Balance (Mettler Toledo, Viroflay, France). The pyrolysis temperature was defined to be 500 °C. The pyrolyzer interface was held at 320 °C. An OPTIMA-5HT column (30m \times 0.25mm I.D., 0.25 μ m film thickness, Macherey-Nagel, Hoerd, France) was

used as first dimension column and a Zebron ZB-50 (2.8m \times 0.1mm I.D., 0.1 μ m film thickness, Phenomenex, Le Pecq, France) was used as a second-dimension column and for the loop modulator system. The separation was carried out at a constant pressure of 300 kPa using Helium Alphagaz 1 as carrier gas (Air Liquide, Bagneux, France). The ZX 2 two-stage thermal modulator was utilized with a modulation period of 10 s with a programmed hot pulse of 0.350 s. A two-step temperature program was used for the hot jet set at 200 °C for 30 min and subsequently raised to 280 °C. The oven temperature was initially held 1 min at 100 °C, and then ramped at 2 °C min⁻¹ to 325 °C, where it was held for 25 min. The injector was held at 280°C and used in split mode (1:30 of the total flow). The mass spectrometer was operated at 20,000 u.s⁻¹, with a scan range from 50 to 500 u, using electron ionization at 70 eV. The interface was kept at 280 °C and the ion source at 200 °C.

Data treatment

Data processing of the Py-GCxGC/MS raw data was achieved using GC Image software, version 2.4 (Lincoln, Nebraska). Identification of paper components was performed by comparing the mass spectra of unknown components with the ones of the NIST mass spectra library, 2011 edition. More information about the identification refers to [22, 23]. The mass spectra from GCxGC profile and 1D profile were extracted using GC Image software, version 2.4 (Lincoln, Nebraska), GCMS Postrun Analysis (a software build within Shimadzu analysis platform) and AMDIS. The procedure is provided in Supplementary Figure S3. The deconvolution of mass spectra was carried out triplicate within AMDIS for the evaluation of the quality of the mass spectra from GCxGC profile and 1D profile. In order to evaluate the efficiency of the Py-GCxGC/MS approach, specific characteristics of the AMDIS software like filtering procedure were set at default value (“filtering off”) during the treatment of data. As AMDIS cannot handle Shimadzu data format (.qgd), the data acquired by the Shimadzu platform was previously saved as CDF format (.cdf) by the GCMS Postrun Analysis software.

Results and discussion

The pros and cons of AMDIS deconvolution for Py-GC/MS treatment of handmade paper pyrolysis products

In our study for characterization of East Asian handmade papers using pyrolysis-gas chromatography and mass spectrometry, the identification of pyrolysis product of handmade papers was performed with both Py-GC/MS and Py-GCxGC/MS analysis and the

chromatograms were shown in Figure 1. It can be seen that the GCxGC/MS and 1D GC/MS profiles correspond to each other according to their eluting order in the first dimension of separation. The part of the chromatogram associated with the cellulose pyrolysis products presents high similarities and the latter part (plant marker region) in both chromatograms present composition differences which can be used for tracing fiber origins [22-24].

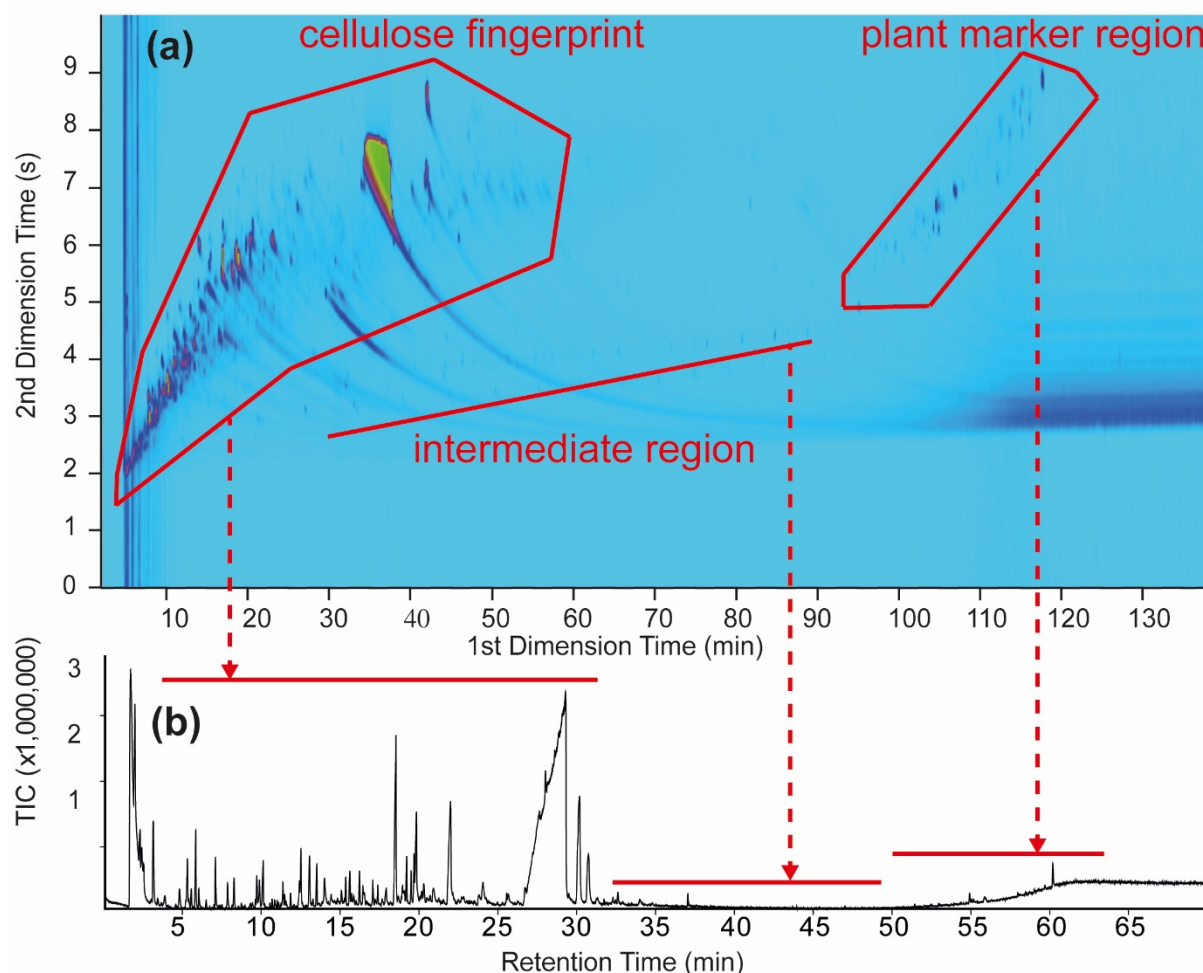


Figure 1. Py-GCxGC/MS (a) and Py-GC/MS (b) chromatograms obtained from the pyrolysis of a kozo handmade paper sample

The manual examination of the mass spectra of the plant marker region in Figure 1 was first carried out treating Py-GC/MS data with the commercially dedicated software GCMS Postrun Analysis (GC/MS Postrun Analysis is the commercial software build within the Shimadzu analysis platform) using subtraction of background from nearby baseline for peak investigation. For example, the base peak mass spectra, m/z 218 (Figure 2c), generated by a retro Diels Alder fission of the triterpenic ring structures was considered here as a characteristic fragment ion in the mass spectra of such structures [25-27]. In the mass spectra of triterpenes, comparison between fragment peak intensities at m/z 203 and m/z 189 allows the distinction between the oleanane and ursane skeleton [26]: the fragment ion at m/z 203 is

more intense than the peak at m/z 189 for the 12-oleanene derivatives series, while usually the reverse occurs in the mass spectra of an 12-ursene derivative (fragment peaks at m/z 203 and m/z 189 may have similar intensities in that case). Variation of the ratios between the fragment ions at m/z 203 and m/z 189 may be considered as a second criterion for the identification of ursane and oleanane structures. Mass spectra analysis following this procedure permits to assign the peak (compound II in Figure 2a) as an ursane structure (urs-2,12-diene, MW 408) with a base peak of m/z 218 and the corresponding ratio between m/z 189 and m/z 203 in favor of m/z 189 (Figure 2b and Figure 2c). However, the presence of peaks at m/z 135, 394 was noticed. This latter peak might be assigned to co-eluting compound(s) presenting another skeleton structure than the previous triterpene.

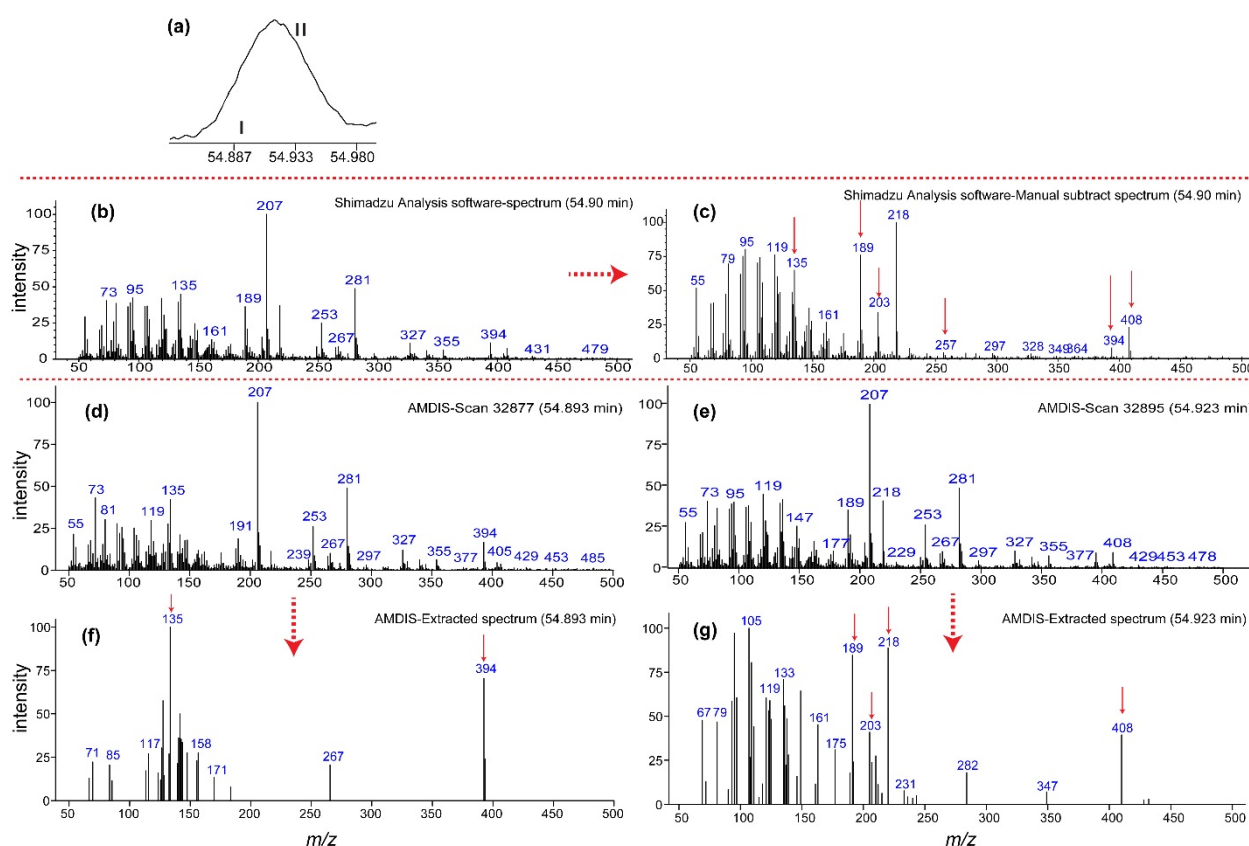


Figure 2. The examined peak issued from Py-GC/MS analysis (a) with its corresponding scanned mass spectrum (b) and manually subtracted spectrum (c) using the GCMS Postrun Analysis software (Shimadzu) for peak identification. AMDIS automatic mass spectra deconvolution was conducted and resulted in the scanned spectra (d and e) and automatic extracted spectra (f and g)

Besides the commercially dedicated software, AMDIS provide a way of deconvolution for mass examination. To illustrate this point, AMDIS deconvolution was carried out for examination of co-eluting compounds around compound II in Figure 2a. As is known, the AMDIS deconvolution ability largely rely on the analyze settings. The following deconvolution settings in AMDIS with shape requirements set as ‘medium’, resolution set as

‘high’, and sensitivity set as ‘very high’ were applied here. Under these conditions, AMDIS generated ca. 50 peaks within the retention time range 54.8 min–55.1 min. Among these compounds, most of them revealed to be false peaks, but a compound assigned to a stigmastane structure was located and identified (compound I, Figure 2d, Figure 2f) together with the previously identified peak in GCMS Postrun Analysis software (Shimadzu) (Figure 2e, Figure 2g). Actually, the number of detected compounds with AMDIS can be found dependent on the chosen analysis settings. With the parameters: resolution, sensitivity and shape requirement all set as ‘medium’ for deconvolution, AMDIS deconvolution revealed only five peaks in the same region. Of the five automated identified peaks, only the compound urs-2,12-diene was detected and other extracted spectra were not conclusive for compound identification.

In the above example from the pyrolysis product of East Asian handmade papers, AMDIS demonstrated its ability to locate the co-eluting compounds but the drawbacks of AMDIS lies in the fact that: i) the number of returned compounds by AMDIS largely depends on the analysis settings; ii) when using lower resolution and lower sensitivity settings for analysis of GC/MS data, AMDIS produces less peaks to check but poses the risk of losing the co-eluting peaks; iii) while when using optimized settings, AMDIS produces many false peaks and checking the AMDIS identified peaks is tedious and time-consuming. The observed false peaks were in accordance with the previous report that AMDIS may produce artifact components corresponding to noise or give multiple peak assignments that correspond to a single chromatographic peak [9].

The separation of compounds in mixtures is the main aim of GC/MS, meanwhile, an efficient separation is a guarantee for a reliable mass spectral identification. However, as observed in routine 1D workflow analysis, it is not always easy to obtain an ideally ‘pure’ mass spectrum in consideration of mutual interference from other mass spectra (chromatographic peaks overlapping) as well as interference with the background. Even by adding another dimension for further separation, compound co-elution is still predicted to occur and can be observed in real applications [28, 29]. Commercial mass spectrometer-based platform software usually proposes solutions for identifying and integrating compounds based on peak area or height using total ion count (TIC), or peaks from the extracted ion chromatograms (EICs) by selecting m/z characteristic values for given compounds. Meanwhile, most of the currently applied manual identification approaches use simple subtraction of background from nearby baseline or a shoulder of a given peak of interest: an approach that can reveal insufficient to handle overlapping and/or co-eluting peaks. For this reason, the increased resolution

capabilities of the analytical method could clearly constitute a plus for the AMDIS automatic data treatment as will be discussed below.

The benefit of GCxGC profile use for 1D data treatment

Our previous study revealed that by using Py-GCxGC/MS, a factor of gain in sensitivity of ca. 6 can be expected for the analysis of East Asian handmade papers [23]. The aspect of how this benefit can be used for 1D data mining is explored here. Since the tested samples are identical, the presence of compounds in 2D profile may lead to quick location of co-eluting compounds or new compounds difficult to attribute in the corresponding 1D GC/MS profile. Also, a well-known feature of 2D analysis is the structure-based (or structure grouping) compound distribution in the 2D chromatogram that may be helpful for analysis of a series of compounds with similar structures in 1D GC/MS data.

The region of elution of five pyrolysis products presented as an example and analyzed in the Py-GCxGC/MS profile and the corresponding 1D Py-GC/MS profile (Figure 3). The compounds I-V showed close volatilities as indicated by their retention time t_R . However, due to their difference in structure, they presented different polarities and by the benefit of a GCxGC separation, they were separated into two different groups (Figure 3a). The first group consists of phytosterol structure compounds eluting first around $t_R = 6.5$ s (compound I and IV) followed by the second group mainly consisting of terpenic structure compounds eluting at $t_R = 6.95$ s (compound II, III and V). From the structure layout in the 2D analysis, we gained the knowledge that within the analyzed region, there were two different groups of eluting compounds. For a quick location of these compounds in the 1D data, fragment ions selected from their 2D mass spectra were used (namely, m/z at 143, 189, 203, 218, 394 and 396), which is convenient and a real time-saving method compared to a manual check for the identification of peaks deconvoluted by AMDIS. The order of the selected ions in Figure 3b clearly depicts all the compounds in the 1D data. It can be seen that compounds I and II are closely co-eluting within a retention time difference of less than 2 s and the other three compounds are also easily located by the selected ions within AMDIS after the help of 2D separation for a reliable assignment.

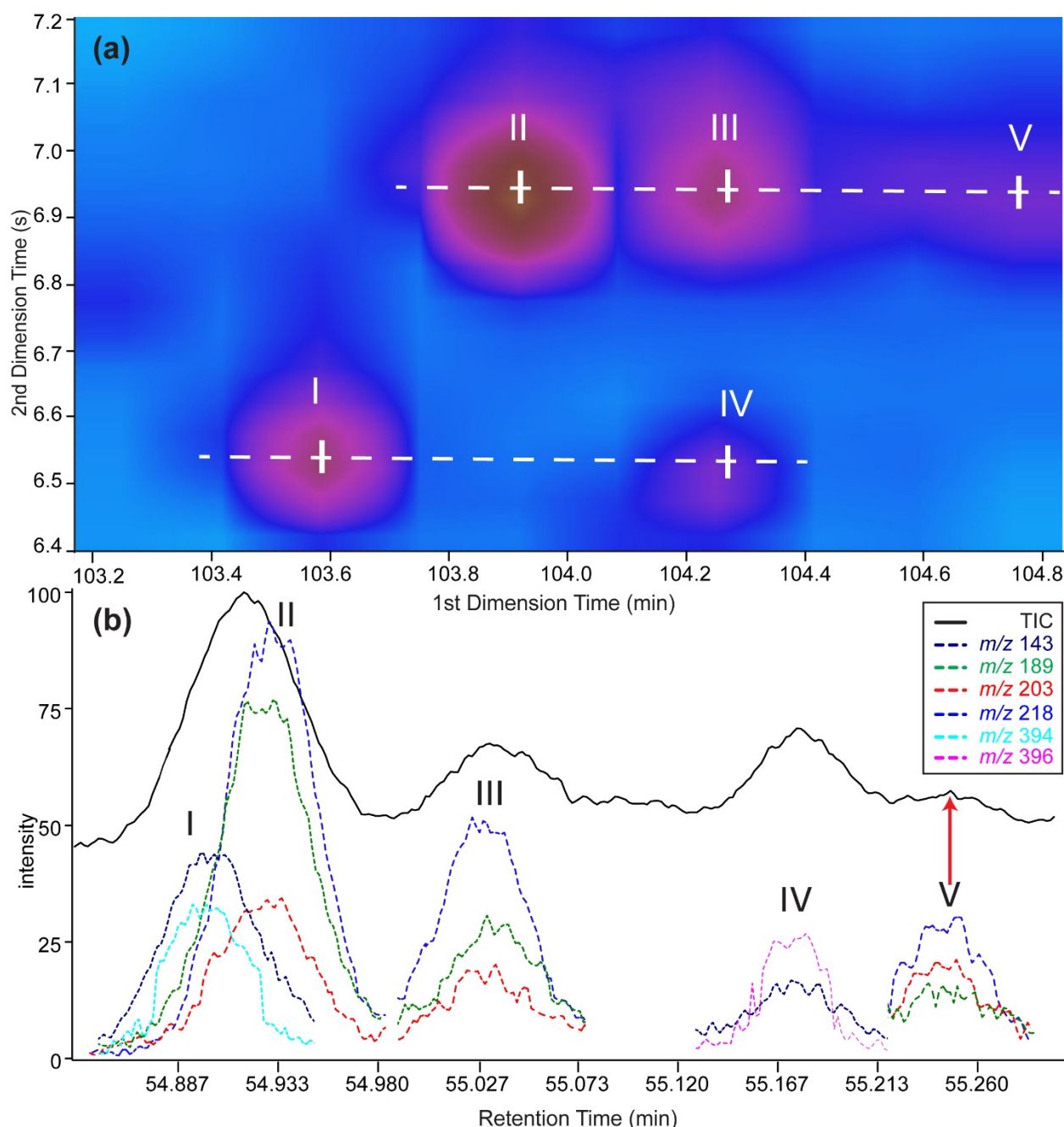


Figure 3. Py-GCxGC/MS profile for five pyrolysis products within the plant marker region distribution (a) and examination of the corresponding 1D data (b) revealed with an extracted ion chromatograms (EICs) procedure

The procedure involving the acquired compound distribution knowledge seems quick and simple, but without the improved separation obtained by GCxGC, AMDIS alone would be inefficient or even unsuccessful in targeting all these compounds. In particular, the direct deconvolution of compounds III, IV and V using AMDIS with the previously reported parameter settings during the 1D Py-GC/MS analysis was not conclusive. For the terpenic compounds III and V, AMDIS did not extract the m/z 218, 203 from the mass spectra, which adversely were showing a high level of noise (Supplementary Figure S1-1 and Supplementary Figure S1-3), a critical point for their identification (only m/z 189 was extracted for compound

V). For compound IV, although the m/z 396 is extracted, ions such as m/z 147 and 95 were missing (Supplementary Figure S1-2). On the contrary, the GCxGC separation even with less sample quantity clearly presents better mass spectra (Supplementary Figure S1). In considering interference from background and co-eluting compounds, reliability of AMDIS for detecting unknown and low concentration of compounds shall be taken into account for biomarker discovery.

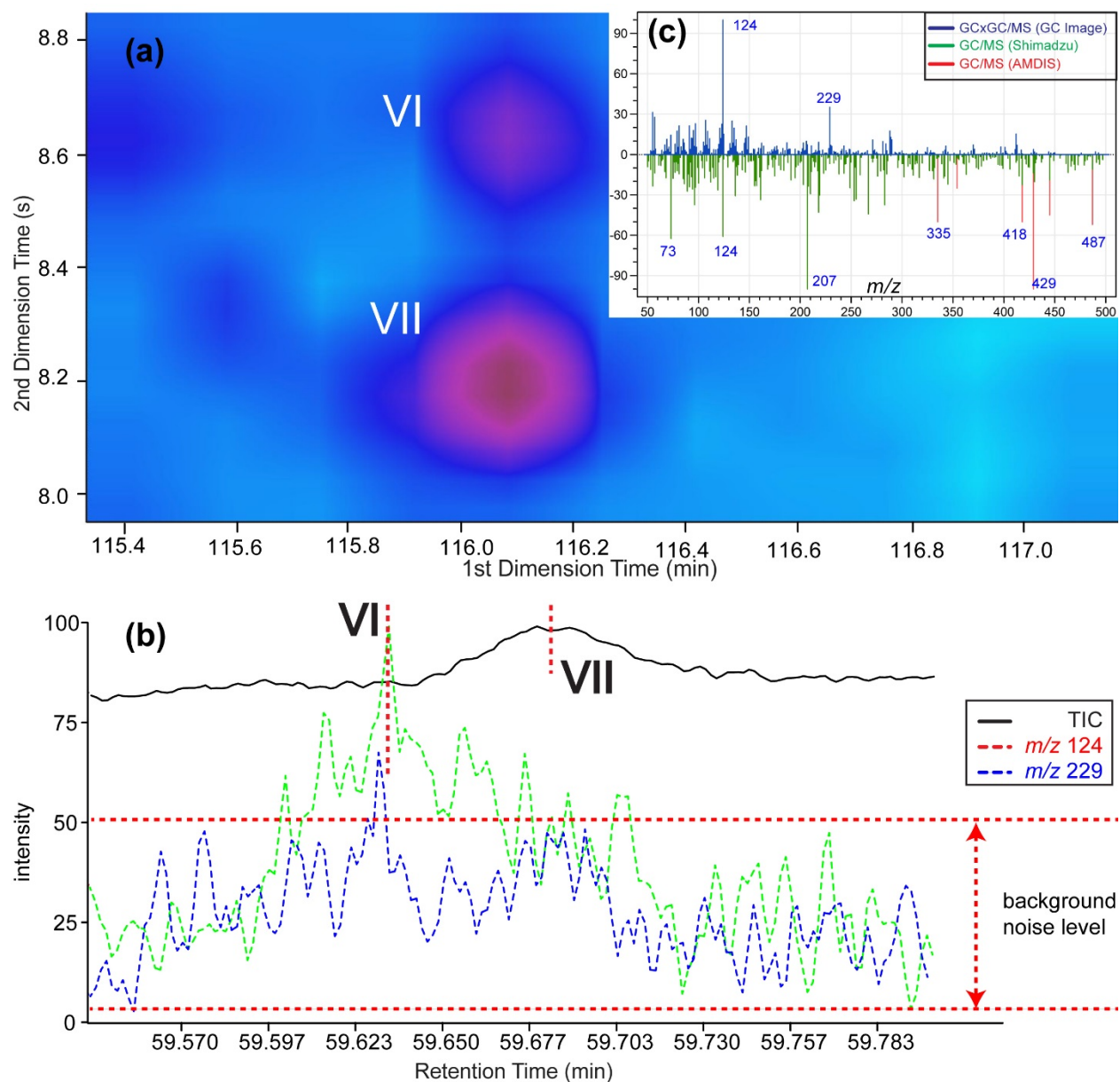


Figure 4. Py-GCxGC/MS profile (a) distribution for examination of the corresponding 1D data revealed with an extracted ion chromatograms (EICs) procedure (b). Insert reports (c) were compound VI's mass spectra from the Py-GCxGC/MS profile (blue), while GCMS Postrun Analysis (Shimadzu) manual check (green) and AMDIS automatic extraction (red)

Due to the detection limit of 1D GC/MS and the interference from the background, using AMDIS to detect signals barely above background is not easy or even impossible in some cases. For example, in Figure 4a, compounds VI and VII presented almost the same retention

times in 1t_R and their differentiation relied largely on their separation in the second dimension 2t_R for their detection. After their identification in the 2D profile, the 1D profile is re-examined using AMDIS. Previously the compound VI (Figure 4b) was not taken into account as it does not have a sufficient signal/noise ratio to emerge from the background. Even after knowing the existence of compound VI in the chromatogram, it is not easy to locate it in the GCMS Postrun Analysis software (Shimadzu) using subtraction of background from nearby baseline as its intensity is too low and the background mass spectra (with predominant m/z at 73, 207, 281) were still influent after the subtraction procedure (Figure 4c). Also, AMDIS deconvolution cannot satisfactorily extract the characteristic ions such as m/z 124 and m/z 229 as revealed in Figure 4c. The co-elution of compound VI and compound VII in the 2D profile considering their retention time in the first dimension 1t_R confirmed that compound VI is eluting near compound VII or even within compound VII in the corresponding 1D profile. From the mass spectrum of compound VI extracted from the 2D analysis (Figure 4c), the intensive ions at m/z 124 and m/z 229 were selected to reinspect the 1D profile. From the AMDIS display of m/z 124 and m/z 229, the presence of VI around compound VII was confirmed (Figure 4b). In 1D data, the compound VI firstly eluted straight before the elution of compound VII and continued to elute within the peak of compound VII. However, the intensity of VII is comparatively higher and compound VI was hidden in the background as was illustrated by Fig.4b. In this typical case where a compound at low concentration elutes closely with a compound at a higher concentration, AMDIS cannot work properly. Only after the insight of compounds eluting from the 2D profile, AMDIS using the selected EIC procedure can locate the compound of lower intensity. The success of separating compounds VI and VII in Figure 4 is largely due to their difference in structure resulting in a difference in polarity allowing their efficient separation in the second dimension. Identification of compounds VI and VII is finally accomplished after reinjection of 2D fragment ion identification. This example illustrated the expected contribution of a GCxGC analysis for resolution of unfavorable cases in a monodimensional separation.

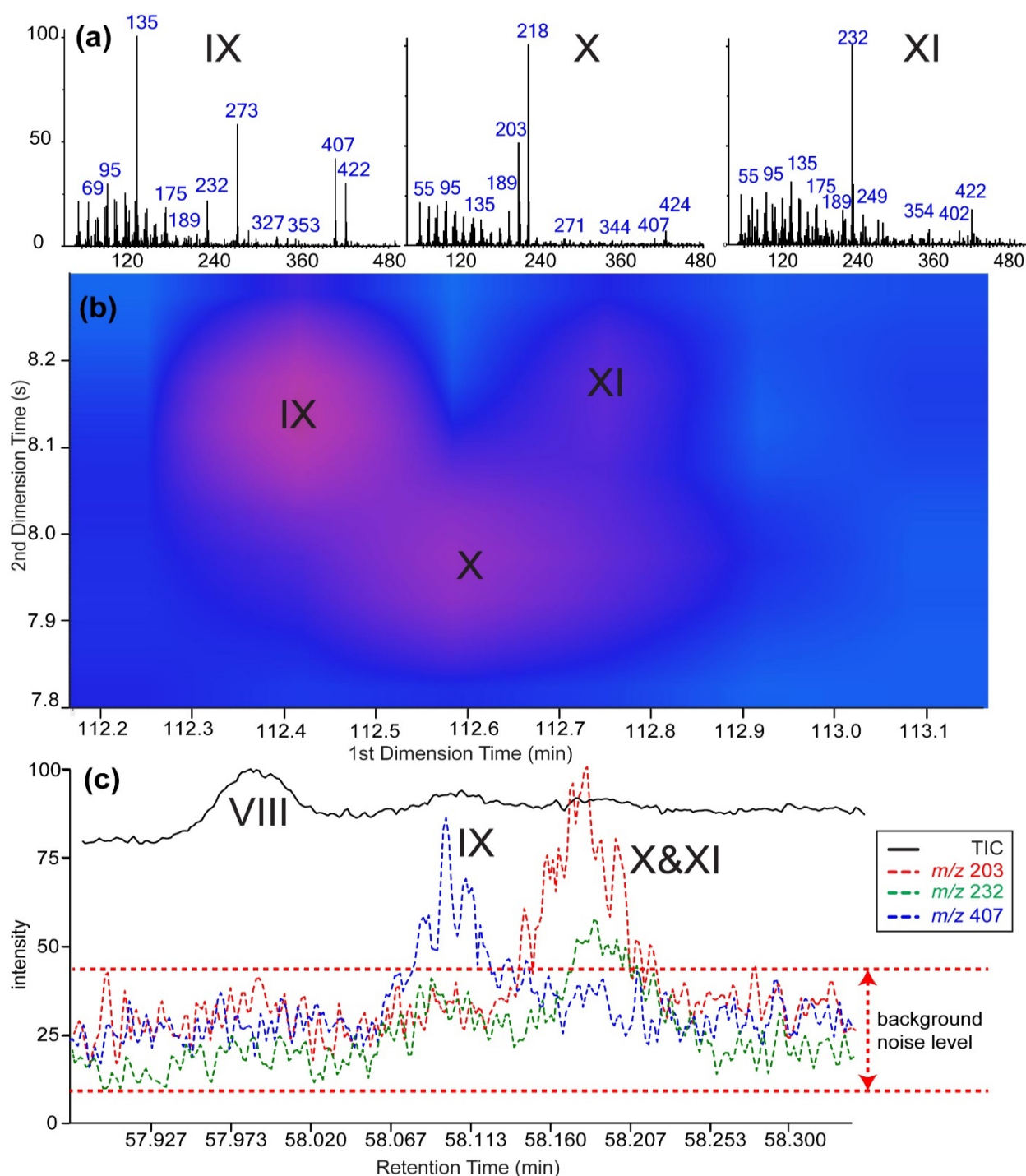


Figure 5. Mass spectra for compounds IX, X and XI (a) and their Py-GCxGC/MS profile (b) for examination of the corresponding 1D data (c) revealed with an extracted ion chromatograms (EICs) procedure

To illustrate the benefit of use of Py-GCxGC/MS profiles for other pyrolysis products detected in the plant marker region, Figure 5 reports the identification process of another three compounds. In this example, AMDIS deconvolution worked well only with compound VIII, which is characterized by a satisfying signal to noise ratio in the 1D profile, while it cannot extract informative ions for compounds IX, X and XI since their signals were very low and submerged in the background (Figure 5c). From the 2D profile, it is clear that three peaks

were clearly detected in the region $^1t_R = 112.3 \text{ min} - 112.8 \text{ min}$, $^2t_R = 7.8 \text{ s} - 8.3 \text{ s}$ (Figure 5b). And the corresponding mass spectra are reported in Figure 5a. The main fragments m/z at 203, 407 and 232 were selected for their display in AMDIS for the treatment of 1D GC/MS data (for compound XI, the base peak m/z 232 was chosen while for compound IX, X the m/z of lower intensity 203, 407 was selected to compensate the difference of concentration between compounds IX, X vs compound XI). It is shown that the three compounds were present in the 1D profile with comparatively low intensity (especially for compound XI) with compound IX eluting first and compounds X, XI co-eluting together.

The three above examples illustrated that by using the 2D profile and reinjecting the mass spectra information for an EIC identification procedure, co-eluting compounds in 1D analysis can be efficiently detected and located within AMDIS. Instead of spending time in choosing the right ions (EICs) for the optimization of the AMDIS identification procedure (sometimes in vain due to the low resolution and low mass spectra quality in 1D profile), the 2D profile can act as ‘peak finder’ for the 1D GC/MS data analysis. Also, considering that the required quantity of sample for 2D analysis is at least half than that of the 1D analysis (ca. 50 μg vs ca. 100 μg), it can be seen from their mass spectra that the 2D improved separation offering more reliable mass spectra and consequently, the identification of products during 1D analysis can be confirmed by their corresponding 2D spectra. On our opinion, an important feature reported for AMDIS, which lays in its suitability at detecting very low levels of metabolite compounds in biological samples [6], may be noticeably increased by the combination of 1D analysis with mass spectral information derived from a 2D analysis. The 2D profile, when analyzing complex samples, can also contribute to improve the reliability of the mass spectra for comparison with a comprehensive library or even in the case of unidentified compounds [30].

GCxGC/MS profiles combined to AMDIS automatic identification process in 1D GC/MS data analysis

The advantage of AMDIS is indicated by its name: Automated Mass Spectral Deconvolution and Identification System. In routine analysis, AMDIS is a powerful tool for quick peak identification. One important part of AMDIS is its library search function which was frequently used for match and identification of recognized compounds. Although several libraries have been embedded in AMDIS, such as NISTFF (for flavours and fragrances analysis), NISTTOX (for toxicity analysis) and NISTCW (for chemical weapon analysis) [31], the main drawback is still evident as the library provided may not contain enough compounds,

enough mass spectra information and even, in some specific domains, standard libraries may be missing. In practice, lots of AMDIS users are intend to build in-house library which proved to be effective and convenient for their specific studies [32, 33].

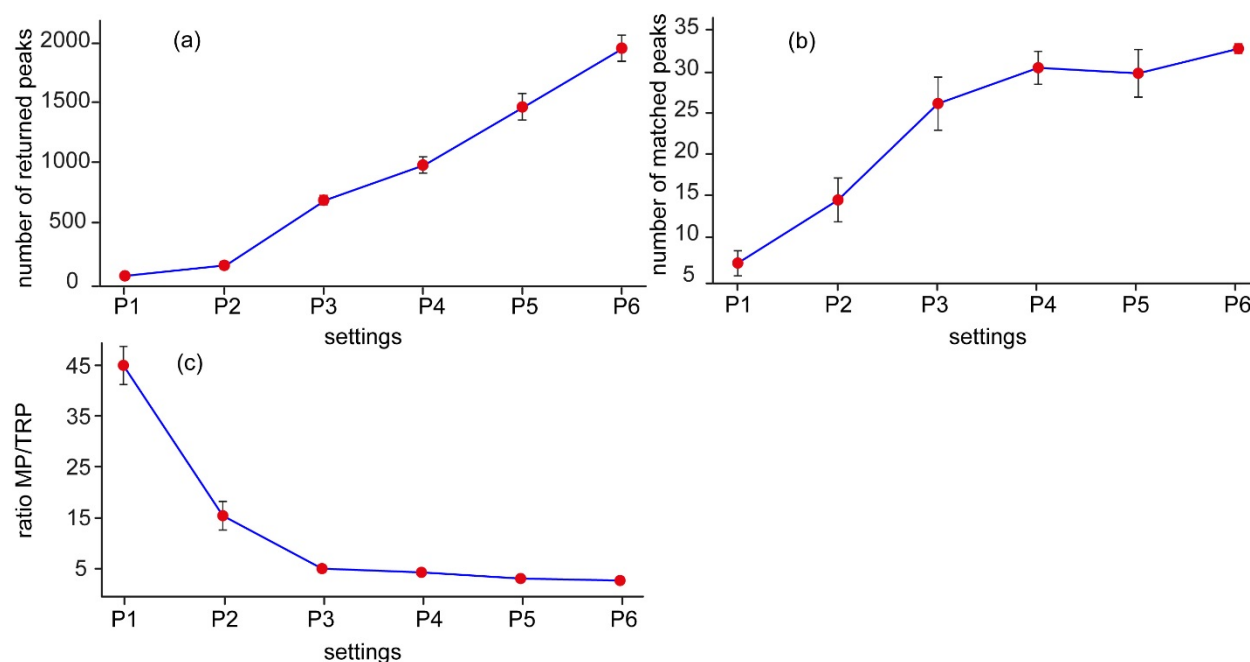


Figure 6. Total number of peaks returned (a) and number of matched peaks with target library (b) by AMDIS deconvolution process with different settings applied to the parameters “resolution” (RE), “sensitivity” (SE), and “peak shape requirement” (PE) (P1:all Low; P2:all Medium; P3: all High; P4: RE,SE High, PE Medium; P5: all Very high; P6: RE,SE Very high, PE Medium). (c) ratio of matched peak (MP)/total returned peaks (TRP)

As has been mentioned above, the number of peaks returned by AMDIS deconvolution process largely depends on the analysis settings. To define an optimized setting, an in-house target compound library was built using the assigned compounds in the plant marker regions (for compound identification, refer to [23]) and tested with different parameter settings (Figure 6). Even considering optimized parameter settings in AMDIS, one must be aware of the influence of these settings during the AMDIS deconvolution procedure. It can be seen from Figure 6 that the setting of parameters for the parameters “resolution”, “sensitivity” and “shape requirement”, greatly influences the final results. As an illustration, when changing parameter values from low to medium (P1 to P2), the total number of peaks returned increases slowly, but when using the ‘high’ settings (P3), the number of generated peaks rise noticeably rendering time consuming the treatment of data after deconvolution (Figure 6a). The deconvolution returned peaks continued to increase when using ‘very high’ settings (P3 to P5). The influence of peak shape is also depicted in Figure 6. The number of returned peaks also increased when set the peak shape requirement as medium (P3 to P4, P5 to P6). The number of peaks matched with the library search also varies with these different settings (Figure 6b): the number of matched peaks with library search using high settings increased by 2~3 times

compared with those using low and medium settings (P3 compared to P1 and P2) while continue to use ‘very high’ settings did not have much influence in the number of matched peaks. The problem raised when using the high settings for AMDIS deconvolution process is that it will generate a large number of false peaks, to be eliminated during the post-processing of data. It can be seen from Figure 6c that the number of matched peaks was less than 5% of the total peaks returned from the deconvolution when use ‘high’ settings. In the following, deconvolution settings with “high resolution”, “high sensitivity” and “medium shape requirement” were used as optimized settings for 1D GC/MS data analysis.

Table 1. List of compounds used for building up an in-house target library for the evaluation the quality of the GCxGC/MS and 1D GC/MS mass spectra.

Retention Time	<i>m/z</i>	Assigned formula	Molecular mass	Attribution	TIC response	Label*
53.023	365, 121, 135, 93	C ₃₀ H ₄₈	408	A-neooleana-3(5),12-diene	72305	S2
53.394	145, 81, 121, 95	C ₂₉ H ₄₈	396	stigmasta-3,5-diene	48551	S3
53.953	406, 255, 95, 123	C ₃₀ H ₄₆	406	oleanane-2,9,12-triene	118881	S4
54.343	218, 203,189, 95	C ₃₀ H ₄₈	408	oleanane-2,12-diene	117283	T4
54.712	105, 57, 145, 396	C ₂₉ H ₅₀ O	414	NA stigmastan	91922	S1
54.917	135, 143, 394, 81	C ₂₉ H ₄₈ O	412	stigmasta-4,6-dien-3-ol	631803	S5*
54.917	218, 189, 93, 95	C ₃₀ H ₄₈	408	urs-2,12-diene	631803	T5*
55.178	147, 81, 145, 396	C ₂₉ H ₅₀ O	414	stigmasta-3-ol	130403	S6
58.465	218, 203, 189, 95	C ₃₀ H ₅₀ O	426	beta amyrin (Olean-12-en-3-ol)	119246	T3
58.726	218, 95, 107, 219	C ₃₀ H ₄₈ O	424	urs-12-en-3-one	82013	T1
59.023	218, 95, 81, 189	C ₃₀ H ₅₀ O	426	alpha-amyrin (urs-12-en-3-ol)	151263	T2
59.277	466, 255, 121, 95	C ₃₂ H ₅₀ O ₂	466	oleanane-12,14-diene-3-acetate	62445	S7
59.678	218, 203, 189, 69	C ₃₂ H ₅₂ O ₂	468	oleanane-12-ene-3-acetate beta-amyrin acetate	249653	T6
60.196	218, 189, 95, 69	C ₃₂ H ₅₂ O ₂	468	alpha-amyrin acetate (urs-12-ene-3-acetate)	1438017	T7

Label* is corresponding to the match factors illustrated in Figure 7

S5 and T5* are co-eluting, the same TIC area was used for both.

As a benefit of the increased resolution and sensitivity of the GCxGC system, both the chromatographic separation and resulting pyrolysis product identifications were improved. It also resulted in an increased mass spectra quality even with a lower quantity of sample. However, the question “how these characteristics of GCxGC analysis are able to improve the mass spectra collection and to benefit 1D data treatment” has not yet been investigated. To

test the performance of reintroducing the GCxGC mass spectra in the 1D mass spectra data treatment procedure of AMDIS, two categories of compounds in the plant marker region were selected: one category constituted mainly of stigmasta compounds (reported as S type) and another category constituted of terpenic compounds (reported as T type). The compound information is listed in Table 1 and their distribution in the 1D Py-GC/MS chromatogram is presented in Supplementary Figure S2. Their respective mass spectra were obtained following the procedure described in Supplementary Figure S3 which involved the GCxGC data collection of mass spectra using GCxGC Image, and the 1D GC/MS data collection of mass spectra using the GCMS postrun analysis software as well as AMDIS. Finally, compilation of this set of mass spectra formed an AMDIS in-house target library assembling 56 mass spectra (Supplementary Figure S4). For the purpose of mass spectra comparison, the AMDIS analysis was conducted with a simple type of analysis model (a minimum match factor was set at 20% and multiple identifications per compound was allowed).

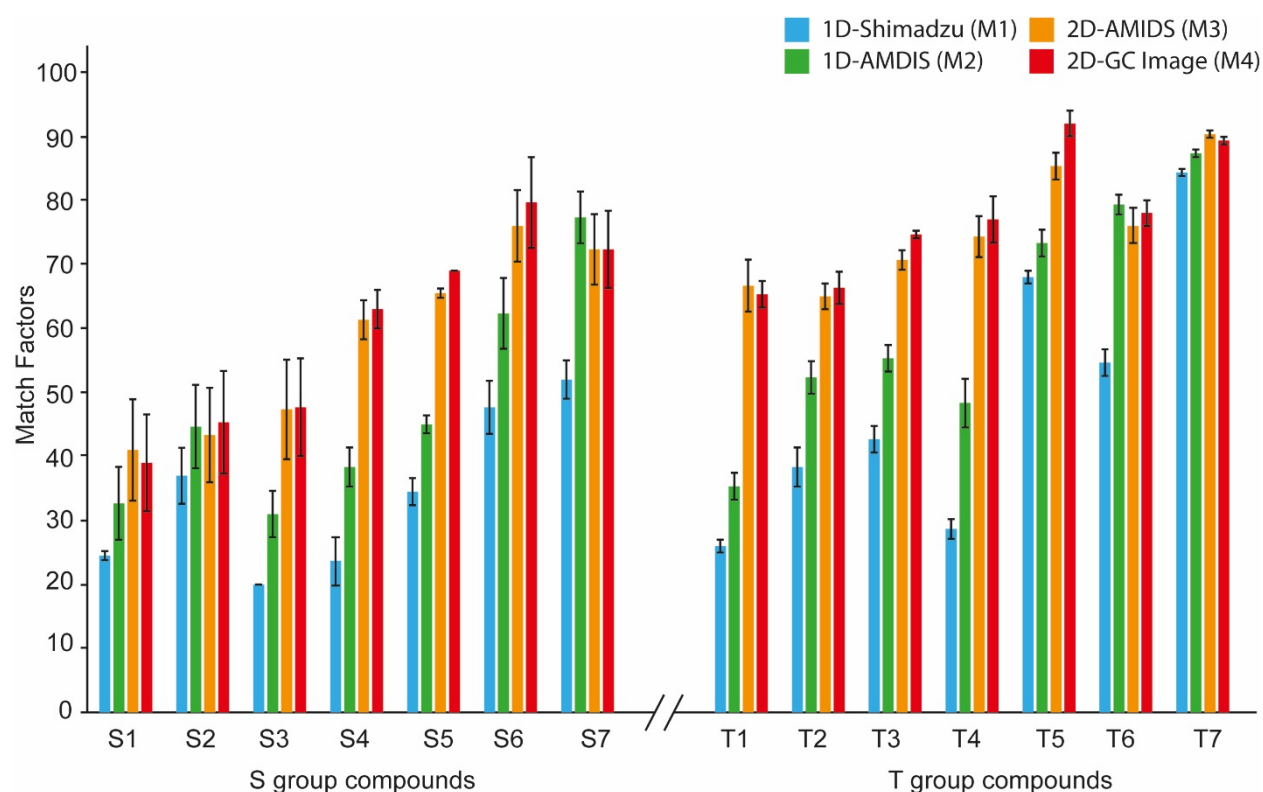


Figure 7. Report of the match factors for each products with the in-house target library consisting of mass spectra derived from GCxGC/MS and 1D GC/MS data collection. For the collecting of mass spectra using M1, M2, M3 and M4, refers to methods in Supplementary Figure S2

The match factors for each category of compounds with mass spectra collected from Py-GCxGC/MS and 1D Py-GC/MS analysis were shown in Figure 7. As all the applied parameters were the same during the procedure of deconvolution, the differences observed in

the match factor was mainly caused by the quality of the mass spectra (the ability to extract characteristic ions from the test chromatograms is also a matter of interest). As can be seen in Figure 7, the mass spectra collected from the GCxGC profile generally provided a 10% to 20% higher match factor than the mass spectra collected from the 1D GC/MS data. For S1~S7, The GCxGC mass spectra have less interference from the background (see Supplementary Figure S4-1 to Supplementary Figure S4-28) and presented a higher match factor except for S1 and S2. This could be correlated to the fact that the AMDIS deconvolution missed to extract enough characteristic ions of S1 and S2 for comparison with the mass spectra in the in-house target library, which can be rationalized considering the low concentration and background interference of these two compounds. The T type compounds, which all have characteristic fragment ions m/z at 218, 203 and 189 of high intensities were more illustrative for a series of compounds with similar structure as they are belonging to the same family with high similarity observed in their mass spectra patterns (Supplementary Figure S4-29 to Supplementary Figure S4-56). The chromatographic response for T1~T4 in 1D analysis is comparatively lower compared to the corresponding GCxGC/MS profile, which resulted in mass spectra containing high intensity of background ions (column bleeding) (Supplementary Figure S4-29 to Supplementary Figure S4-44). On the contrary, the GCxGC/MS profile provided a higher detection level associated to a increased resolution of chromatographic peaks which resulted in an improved quality of the mass spectra and a higher match factor in the AMDIS automatic identification process when using GCxGC reintroduced mass spectra. As can be exemplified from the mass spectrum of compound T5, the intensity of m/z 218 and 189 were comparatively higher in the collected GCxGC mass spectrum than the 1D mass spectrum and their difference were mainly residing in the content of low m/z fragment ions from 50 to 180 u (Supplementary Figure S5). This can be explained by the co-elution of compound T5 with compound S5: in the 1D mass spectrum, the m/z 50-180 fragment ions result from the fragmentation of both T5 and S5, which cannot be fully separated by AMDIS deconvolution, while in the GCxGC profile, as T5 and S5 have been separated in the two dimensions, m/z 218 and 203 ions have comparatively higher intensities with the decrease of m/z 50-180. As for compound T6, using the GCMS Postrun Analysis software (Shimadzu) for background subtraction can still contain high background and column bleeding ions like m/z at 207 (Supplementary Figure S4-49) which may cause its comparatively lower match factor, while background subtraction using AMDIS can have the same math factor with mass spectra from the GCxGC profile. It is also observed that for the compounds present in comparatively high concentration, like compound T7, both the 1D and GCxGC method can produce mass spectra with lower background interference (Supplementary Figure S4-53 to Supplementary

Figure S4-56, Supplementary Figure S5), which demonstrated that in such case, the 1D chromatographic detection level and resolution are satisfactory for proper identification.

Among the four way of getting mass spectra for target library construction, the manual background subtraction using the GCMS Postrun Analysis software did not produce high match factors as there were still high level of background interference (Supplementary Figure S4). The algorithm for background subtraction in AMDIS reduced the level of background interference compared to the use of GCMS Postrun Analysis software but has the limitation of detection and resolution reported above during 1D data collection. The mass spectra produced from a GCxGC/MS profile has fewer contaminations from the background (column bleeding) and are observed with higher match factors in the AMDIS automatic identification procedure. This result constitutes a plus for the AMDIS automatic data treatment to provide a complementary way of analysis for AMDIS use. Moreover, to our opinion, the approach adopted here demonstrated its potential to circumvent the inability to reliably extract weak signals in AMDIS automatic deconvolution procedure due to the inherent difficulty of effectively establishing thresholds for distinguishing significant signal from noise.

Conclusions

The potential use of GCxGC/MS profiles to conduct an improved AMDIS procedure for compound identification in 1D GC/MS data was reported in this study with application to the identification of pyrolysis products of East Asian handmade papers. The benefits of GCxGC profiles use for manual and automatic 1D GC/MS data mining with AMDIS was explored. The result showed that the presence of compounds in 2D profile may lead to the quick location of co-eluting compounds or the quick identification of new compounds in 1D GC/MS chromatograms eluding background contamination in the mass spectra and avoiding mass spectra mixing due to ineffective chromatographic separation. Clearly, the structured layout of products in a 2D chromatogram may help for 1D analysis of a series of compounds with similar structures. As a consequence, the compound distribution in GCxGC/MS profiles can be used as ‘peak finder’ for manual check of low concentration and co-eluting compound identification in 1D GC data. GCxGC/MS profiles improved the identification of unresolved patterns in 1D GC/MS analysis, as well as permitted to improve the EICs identification procedure for low signal peak detection and unresolved peak patterns showing a high dynamic in their corresponding chromatographic signals.

The performance of an in-house library constructed with mass spectra collected from GCxGC/MS profiles and corresponding 1D profiles for AMDIS automatic identification process is also evaluated. The result showed that the mass spectra from the GCxGC profile

generally have 10% to 20% higher match factors than the mass spectra obtained from the 1D profile. Also, the way of collecting mass spectra was shown to affect the quality of the mass spectra with the influence of background ions interference. The algorithm for background subtraction in AMDIS reduced the level of background interference but with a limitation in its effectiveness for identification in 1D analysis. The mass spectra produced from a GCxGC/MS profile have higher matches observed in the AMDIS automatic identification process which may further contribute to the 1D data analysis when combined with parameters such as retention time and retention indexes.

The present study demonstrated how 2D profiles provide a complementary way of analysis for AMDIS use: a future application may consist for a given sample system of interest, firstly, in a scrutinizing application of 2D GCxGC/MS to appropriately identify the components of this system, and, second, in applying a routine use of AMDIS in a subsequent 1D GC/MS analysis. Extension of the application of the present method may be done through the use of an in-house library, which can be easily shared by scientists working in the same domain of interest. Similarly, a m/z table of interest build from the EIC issued from the 2D GCxGC analysis can be provided for routine 1D GC/MS analysis where 2D GCxGC/MS are not at disposal.

The ability of AMDIS for identifying spectra with very low levels of signals once high-quality spectrum is provided and operated with optimized parameters (filtering procedure in the post-processing of data, retention index use) were previously reported and currently effective in routine AMDIS uses. The further investigation of the optimized AMDIS parameters with the described 2D method (the automated in-house library search together with an EIC examination procedure), may contribute to the identification of similar plant species that have resemble major compounds and may only be differentiated by minor compounds. By definition, there must be a potential usage of the proposed method and in-house libraries collected from 2D analysis in an extended field of applications like cultural heritage, botany or material science.

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Chapter 3 Pyrolysis of handmade papers: the applications

In chapter 1, the methods for paper analysis have been described and the problem has been pointed out that microscopy observation results may vary with different observers (depending on their experience), which presents the limits of microscopy. The limits of microscopy have already been pointed out by researchers in this field: for instance, Dr. Agnieszka Helman-Ważny has deemed the difficulties to distinguish fibers that have similar morphological features [1].

In chapter 2, the feasibility of Py-GC/MS and Py-GC_xGC/MS for analysis of handmade papers has been tested. The results indicate that certain metabolites preserved in the plant fiber for papermaking (a point proved in section 2.2.7) may provide plant chemotaxonomy indications. This make it possible to link the pulped fibers (handmade papers) to the plant species via different metabolite profiles (example shown in section 2.4).

Another point to make it feasible for handmade paper studies lies in that only certain plants has been favorably used by papermakers for papermaking (e.g. the material change depicted in section 1.3) which greatly reduced the range for reference sample testing to get the metabolite profiles.

As the experimental conditions has been set (discussions in chapter 2), the forthcoming question is ‘how the analytical method proposed based on pyrolysis, gas chromatography and mass spectrometry can contribute to the fiber identification (more broadly, material characterization) in the cultural heritage field’. This chapter is written to answer this question.

Pyrolysis (Py-GC/MS, Py-GC_xGC/MS) is a methodology, as will be demonstrated, that can provide more detailed informations if precise identification is needed (in cases, it could be important for archaeological and historical interpretations if a restricted or specified material origin can be indicated). Moreover, the value of the Py-GC/MS provides an objective and reliable verification of microscopic observation results. In a broad sense, Py-GC_xGC/MS may yield interesting results when applying to related topics such as ink, glue and lacquer analysis.

3.1 Pyrolysis: a tool to read ‘what is written’ and ‘what is not written’

3.1.1 Pyrolysis to read ‘what is written’

In a common sense, the word ‘read’ is to understand written words while here it bears the meaning of ‘to check the material with a written label’. In a general sense and standard procedure, the use of (modern) reference material to setup a database is commonly practiced prior to the characterization and identification of museum collections or archaeological materials and examples can be found in various cultural heritage applications such as Raman spectrometry analysis [2], phytolith analysis [3], pyrolysis analysis [4] and microscopy analysis [5].

Within this procedure, the reference samples play a key role. In analytical chemistry domain, there are companies which provide standard analytes with well-labeled information that can be directly used (varnishes, resins, lacquers etc...), while in some cases (take the handmade paper research for example), there are no such companies. Other issues also make it more difficult to get reliable reference handmade paper samples in considering of:

- Hand papermaking has been really less practiced since it is labor-intensive and the efficiency cannot compete with machine made papers
- It is usually practice in some rural areas with different of materials and methods applied (not a unified procedure defined and some procedures were kept as secrets)
- There are various kinds of handmade papers available on the market originating from different sources (hand papermakers, paper historians, provided in sample books and private collections)
- Some of them were not well labeled (not precise). In some cases, the papers were just labeled using the making places such as Chinese handmade paper, Japanese handmade paper, Korean handmade paper. In some other cases, the papers were labeled using a general name without specific information such as bark paper, grass paper
- In some occasions, the labeling may be not correct

Some chaos also comes from the naming. For example, paper mulberry was commonly and historically used as a raw papermaking material, which played a significant role in hand papermaking in East Asian regions. Nevertheless, the historical record of paper mulberry was far earlier than the scientific naming system originated from the Europe. The scientific taxonomic relation of paper mulberry belongs to *Broussonetia* sp. Three species of *Broussonetia* sp. distributed in East Asia and continental Southeast Asia: *Broussonetia*

papyrifera (L.) L'Hér. Ex Vent, *Broussonetia kaempferi* Siebold, and *Broussonetia kazinoki* Siebold. In fact, the taxonomic study of *Broussonetia* took a long time and until nowadays its naming was still a bit confusing [6-9]. For example, paper mulberry has been deemed as both *Broussonetia kazinoki* Siebold and *Broussonetia papyrifera* in different publications for the study of the same handmade paper, *Hanji* [10-12].

And also, in the study of handmade paper documents, the study of written text ('what is written') is usually performed by paleography, codicology and technical art history studies which do not involve scientific fiber analysis (the situation stated in chapter 1). For example, there was a period in ancient China when the Korean handmade papers were thought to be made from silk just sourcing from one scholar's statement (the original story provided in Annex 39).

With the situation stated above, the reliability of the reference sample is a real concern in scientific analysis of handmade papers. When analyze samples with a label (state the fiber source), one basic question is 'is this labeling (identification) precise or not?' two examples are provided here to stress these concerns.

3.1.1.1 the 'gampi' paper

The kozo, mitsumata and gampi handmade papers from reliable sources provided by Professor Masamitsu Inaba (Conservation Science Laboratory, Tokyo University of the Arts) have been illustrated in section 2.2. As can be observed from the result, the main results obtained during Py-GC/MS analysis of these reference East Asian handmade papers resided in the identification of oleanane and ursane structures in the case of the kozo papers, while mitsumata and gampi papers demonstrated mainly the presence of sterol structures (phytosterols).

In order to enlarge the sample database, a gampi paper (with fiber sources labeled as gampi from a published book with handmade paper samples provided in the annex) has been sampled. After the analysis, it is surprising and unexpected to find the presence of the metabolite profile pattern of a series of terpene compounds with characteristic m/z at 218, 203 and 189 which resemble the compounds from the inner barks of *Moraceae* family (Figure 3.1.1).

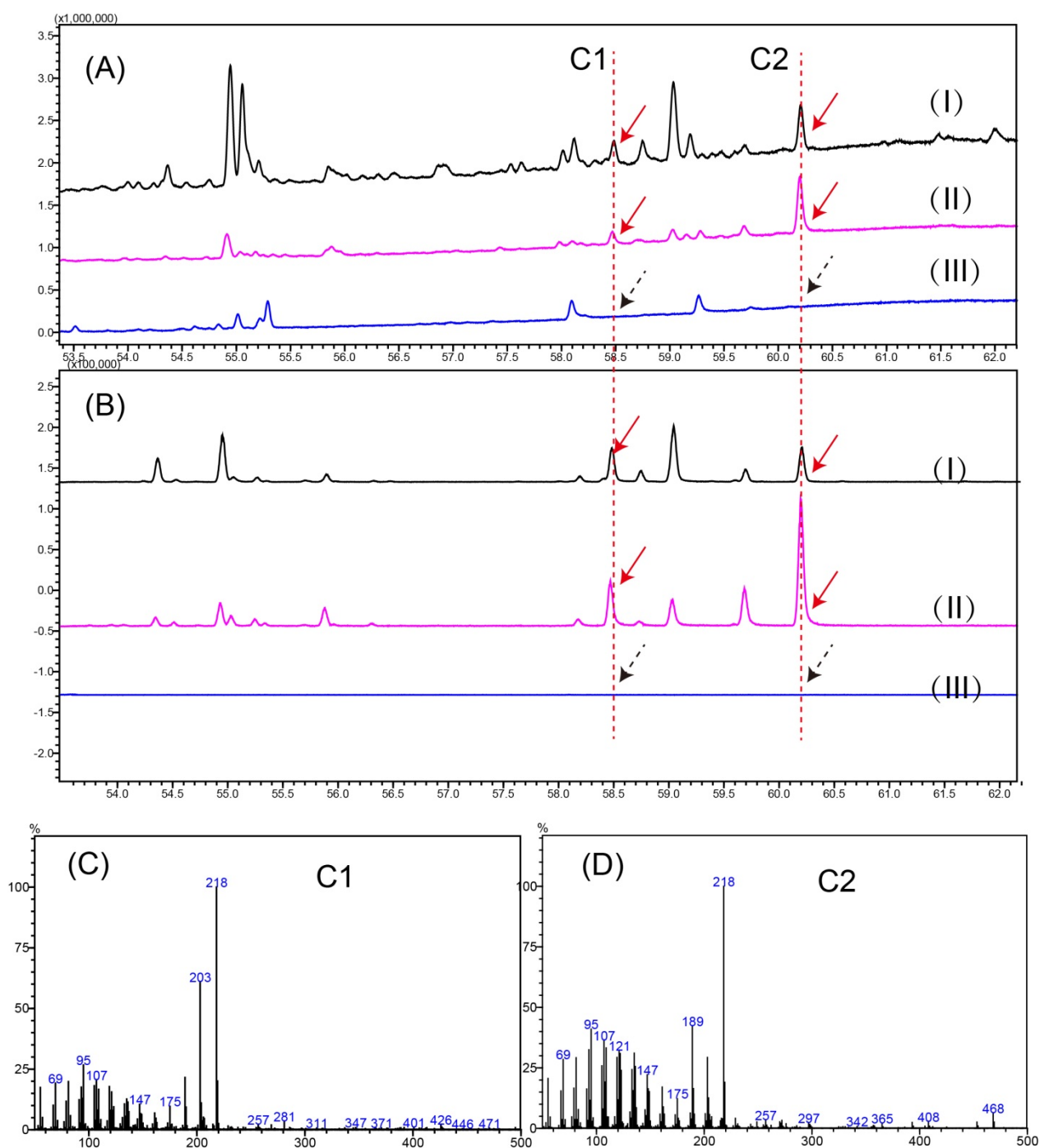


Figure 3.1.1 (A) is the TIC chromatogram of the ROI and (B) is the EIC chromatogram illustrated with m/z at 218. In (A) and (B), (I) is the tested 'gampi' paper, (II) is the kozo reference paper and (III) is the gampi reference paper. (C) and (D) are the mass spectra after background subtraction for C1 and C2, respectively.

The test result showed in Fig.3.1.1 strongly indicated the presence of fibers from Moraceae family. To further confirm and verify the identification result obtained by the Py-GC/MS. Digital microscopic observation has been carried out⁶⁸. The observation verified the result

⁶⁸ The microscopic observation within this section was conducted with Dr. Sakamoto Shouji (RyuKoku University) who is currently visiting scholar in CRCC.

demonstrated that fibers resemble the morphological features of fibers from the Moraceae family in large quantity (Fig.3.1.2).

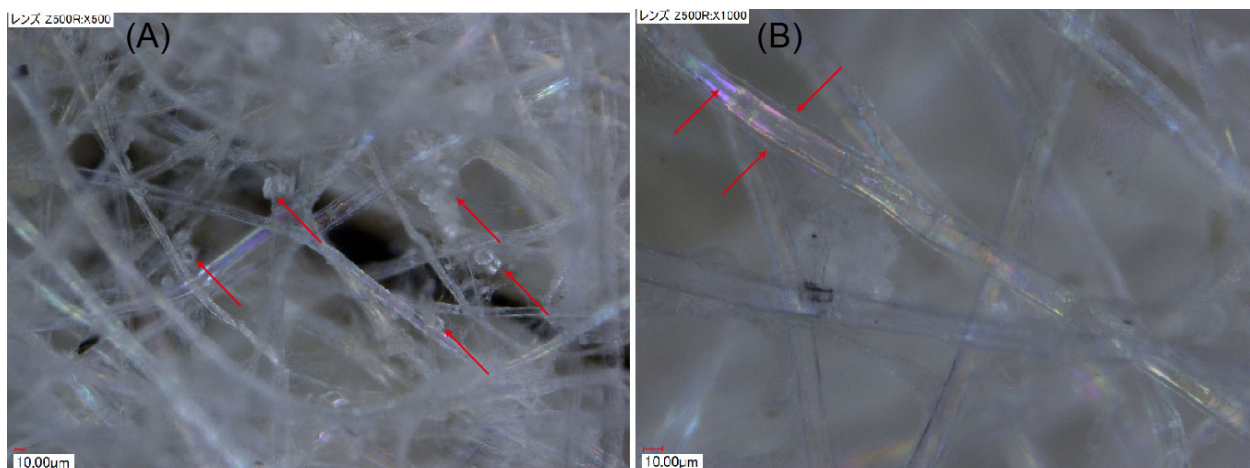


Figure 3.1.2 (A) and (B) are the microscopic observation of the test ‘gampi’ paper.

With the ‘fingerprints’ from Py-GC/MS (metabolite profiling) and the ‘fingerprints’ from digital microscopic observation (morphological features), it can be safely concluded that the label for ‘gampi’ paper is not precise. The tested type of handmade paper has two material origins (mixed origins), one is from Moraceae family and the other is from *Thymelaeaceae* family. This precise labeling might be an overlook issue by the sample providers. As the metabolite profiling for the terpene compounds resembles those described in section 2.2, two mass spectra has been illustrated in Figure 3.1.1 while others has been carefully check but not detailed here.

3.1.1.2 The ‘ramie’ sample

With the same aim to enlarge the sample database, a sample labeled ‘ramie’ fiber has been sampled. After the liquid extraction (method described in section 2.2). contradictory to the knowledge gained by the pyrolysis of ramie handmade paper (II in Fig.3.1.3) which present less metabolite markers, the tested ‘ramie’ sample present quite complex fingerprint maker patterns (II in Figure 3.1.3). As can be seen from Fig.3.1.3B, the test samples EIC profile with m/z at 218 is similar to that of kozo’s expect for one peak (point 4 in Fig.3.1.3). however, it might not be the characteristic terpene compounds in the defined method in section 2.2 as can be seen from its mass spectra (Figure 3.1.3F) with lower mass ion such as m/z at 109 as the base peak (compound co-eluting could also be possible in considering the complexity of this sample).

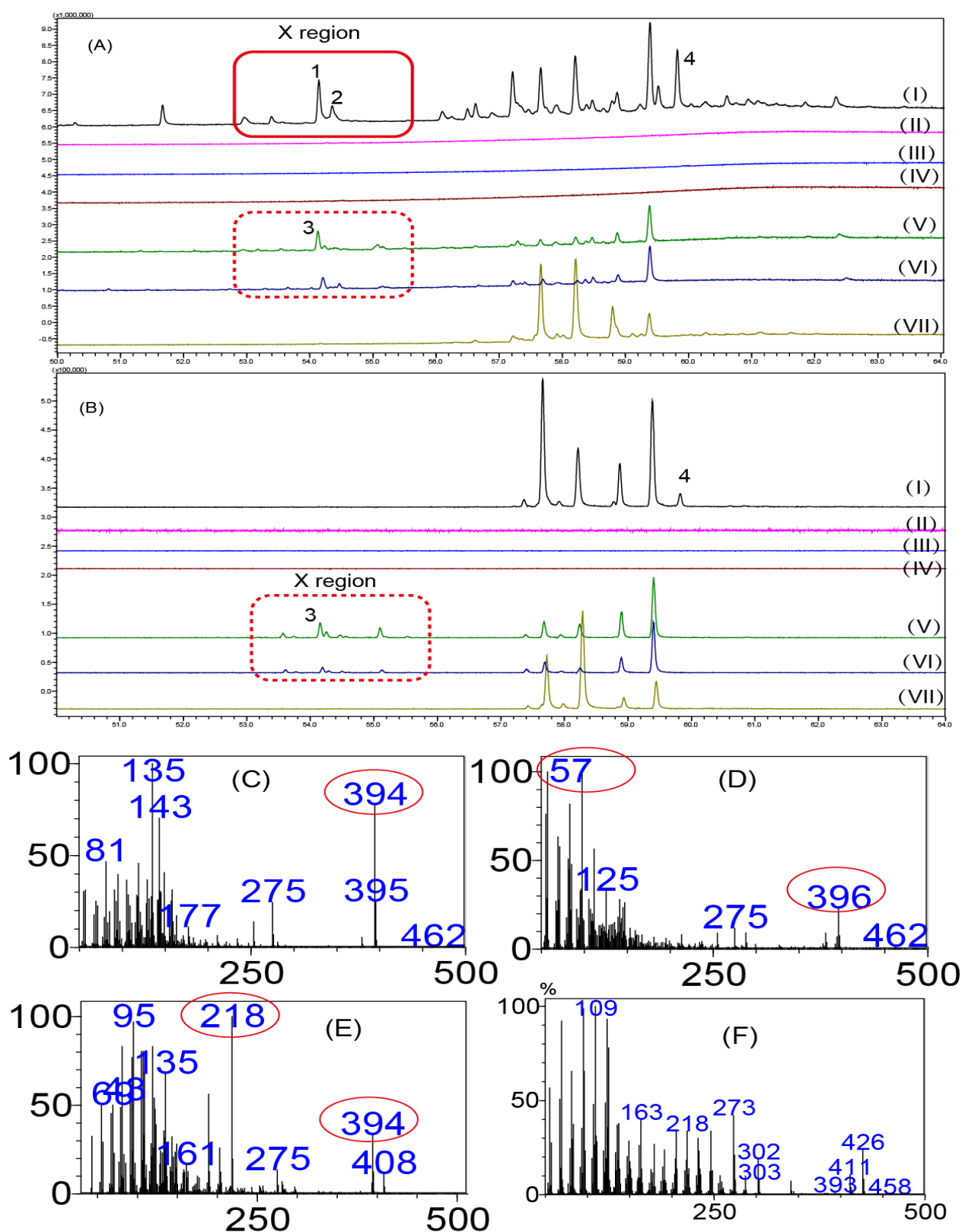


Figure 3.1.3 (A) is the TIC chromatogram and (B) is the EIC chromatogram at m/z 218. In (A) and (B), only the ROI region is shown, (I) is the tested 'ramie' fiber sample with liquid extraction, (II) is the ramie handmade paper pyrolysis, (III) is hemp fiber sample with liquid extraction, (IV) is hemp handmade paper pyrolysis, (V) is kozo handmade paper pyrolysis, (VI) is raw kozo fiber pyrolysis, (VII) is pulped fiber from the Moraceae family with liquid extraction. (C)-(F) are the associated mass spectra after background subtraction for peaks 1-4 in (A) and (B).

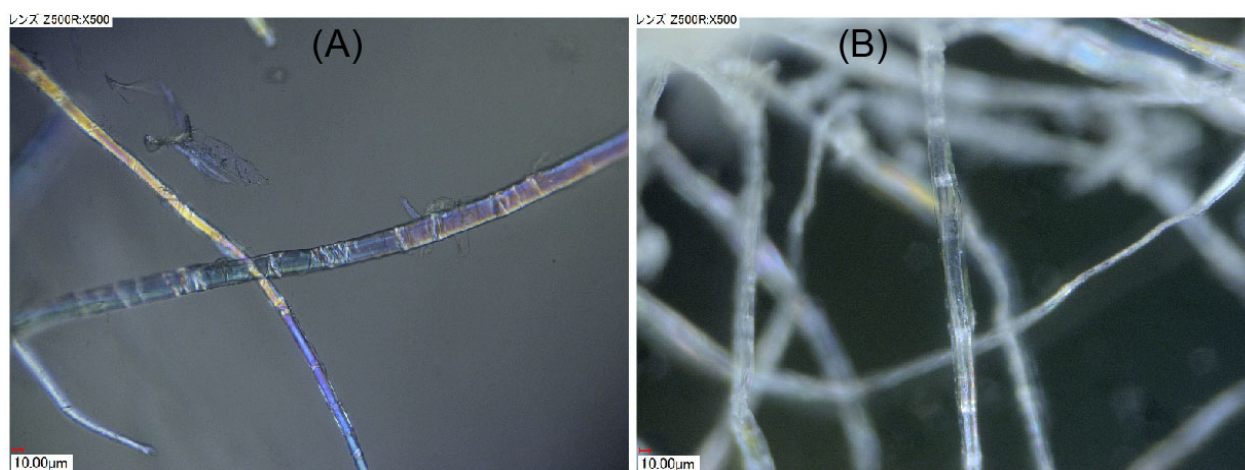


Figure 3.1.4 The evidence showing the presence of the kozo fiber from the microscopic observation for the presence of hemp/ramie fiber (A) and kozo fiber (B).

It can be observed from sample V, VI, VII in Fig.3.1.3 that the X region indicated the presence of mainly terpene compounds only observed with pyrolysis (not in liquid extraction) which means these compound was not initially present in the fibers but generated in the pyrolysis process (discussed in section 2.2). However, several peaks were observed in the chromatogram of the tested sample (e.g. peak 1, 2 in Fig.3.1.3). From their mass spectra, it can be seen that these compounds present mainly a sterol structure similar to those previously identified (Fig.3.1.3C, stigmasta-4,6-dien-3-ol, and Fig.3.1.3D, stigmasta-3-ol), and do not correspond to terpenic compounds (Fig.3.1.3E). Actually, peak 3 in Fig.3.1.3 contains two co-eluting compounds as can be observed with the present of m/z at 218 and m/z at 394, a point that has been discussed in section 2.5.

The Py-GC/MS analysis result strongly suggest the tested sample was a rather a complex mixture rather than the label 'ramie' sample or in a sense, it is not constitutes with pure ramie. To further verify the result, digital microscopic observation has been carried out. The observation found the existence of hemp/ramie fiber together with kozo fiber (Fig.3.1.4B). The indication of complex constitute was also tested by finding several unknown fibers.

3.1.1.3 A summary

At the beginning of this chapter, the problem of the reliability of reference samples from various sources has been stressed. This pointed out again the importance of the reliability of the sample sources: the fiber source labeled shall not be taken for granted and has to be carefully checked. From these two cases, the defined Py-GC/MS method was proved to provide objective and clear evidence for material origins where this point, in some case, has been overlooked by some scholars as well as by microscopic observations.

3.1.2 To read ‘what is not written’

If read ‘what is written’ described in section 3.1.1 bears the meaning of ‘to check the material with a written label’, to read ‘what is not written’ may employ here a mere general sense of material characterization and identification with no (little) information previously acquired (often the case in cultural heritage studies). This point is what analytical chemistry can and has already done to promote the deep understandings of the cultural heritage materials: studies such as paleographical studies use text records and experiences for sourcing while chemistry directly goes to the nature of the materials. In fact, the theme ‘material characterization’ goes through all this thesis in which the application of the defined method scatters in different sections.

3.1.3 The illustration of benefits from material and method well-defined samples

3.1.3.1 The aim of the designed work

The concerns for the reliability of reference handmade papers have been raised in section 3.1.1. It pointed out a situation that although various handmade papers can be commercially available, the material origin, papermaking methods, function and usages, physical property samples, especially in large quantities, are rare in the field of scientific analysis of handmade papers. From the authors' knowledge, only one handmade paper sample book edited by Professor Prof. Hyoung-Jin Kim (Kookmin University, Seoul) currently meet all these requirements to be classified as ‘well-defined’ sample book providing large quantity of handmade papers with detailed information⁶⁹.

The Centre de recherche sur la conservation des collections (CRCC) was honorably granted by one copy of the sample book edited and donated by Prof. Hyoung-Jin Kim⁷⁰. In a general situation where well-defined samples are lacking, with well edited handmade paper samples, one question has been raised that ‘what benefit can be derived from well edited samples’. In answering this question, a work was carried out for the analysis of the handmade papers together with the information provided within.

⁶⁹ For a general situation, the quality of some Japanese edited sample books is also well-defined and similar work has currently in progress by prof. Tang Shukun (University of Science and Technology of China).

⁷⁰ 200 copies in total and 50 copies distributed world widely.

3.1.3.2 The work content

This work first analyzed these data using methods such as regression and principal components analysis followed by a direct fiber observation using a digital microscope. It provides the advantage not necessary to take the materials from the paper and it allows to exam all parts of a sample, thus being beneficial when dealing with cultural heritage artefacts in the conservation process. As the results, correlation is shown between fiber orientation and papermaking technique (*Gadumtteugi* vs *Heulimtteugi*). Fiber identification revealed the characteristic features of Hanji made of *Dak* fibers with the non-sampling method (*Dak* is the Korean name for *Broussonetia* sp.). In parallel, pyrolysis-gas chromatography/mass spectrometry is performed for the first time on *Hanji* samples with 18 pyrolysis product from lignin identified (H/G/S type) as well as the pattern of terpenic fingerprint compounds that constitute biomarkers for *Hanji* with the ions m/z 218, 203, 189 being characteristic for identification.

The work described above was carried out during the PhD study period and in order to demonstrate the pyrolysis analysis, only Py-GC/MS analysis has been incorporated here while the full text is provided in Annex 45.

The Py-GC/MS characterization of *Hanji* fibers

Since *Hanji* and *Washi* are made from quite similar species, microscopic observation cannot distinguish paper fibres from these two areas. The similarity can also be observed from their similar compound distribution patterns in the ROI demonstrated in Fig.3.1.5A and Fig.3.1.5B. Nevertheless, a difference of relative intensity ratio of compound X1 and compound 31 (31 in Table 1, refers as C31) has been noticed with hanji have relative higher ratio. As can be seen from the mass spectra and the NIST library search result, X1 could possibly be a sterol compound. Its structure was assigned to lanosta-8,24-dien-3-ol, acetate. The difference noticed in the chromatogram such as the difference of the relative intensity observed in the EIC of m/z at 218 (Fig.10B and Fig.10C), can be a way to indicate their change in composition.

As can be observed from Fig.3.1.6, the X1/C31 ration (EIC area at m/z 218) was averaged to 12% for hanji while the average value for washi is 3%. As can be observed from the box plot, this ratio differs largely in hanji and washi which could be one information used for their differentiation. One point for the noticed ratio difference lies in that the m/z at 218 (compound X1) is in general a minor peak in the EIC plot and depending on the testing

conditions, it is occasionally observed to be too low to integrate its area both in hanji and washi paper analysis. Under these conditions, this criterion cannot systematically be applied, but opens the way for the differentiation of handpaper of different origins in their papermaking processes.

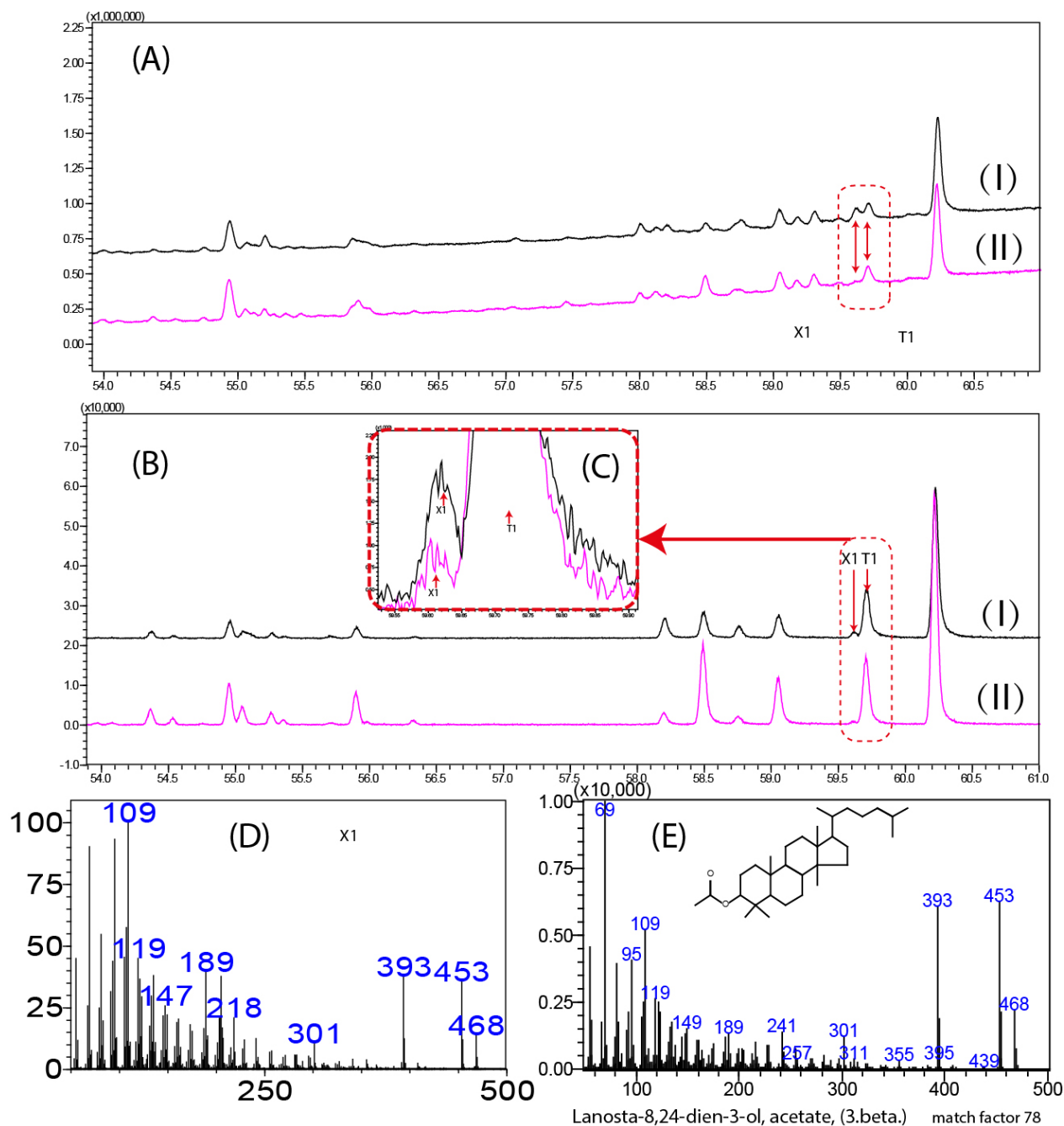


Figure 3.1.5 (A) is the TIC chromatogram while (B) is the EIC m/z at 218. In (A) and (B), (I) is hanji and (II) is washi. (C) is the enlarged demonstration of the region in (B), (D) is the mass spectra of compound X1 after background subtraction and (E) is the NIST library search results with high match factor.

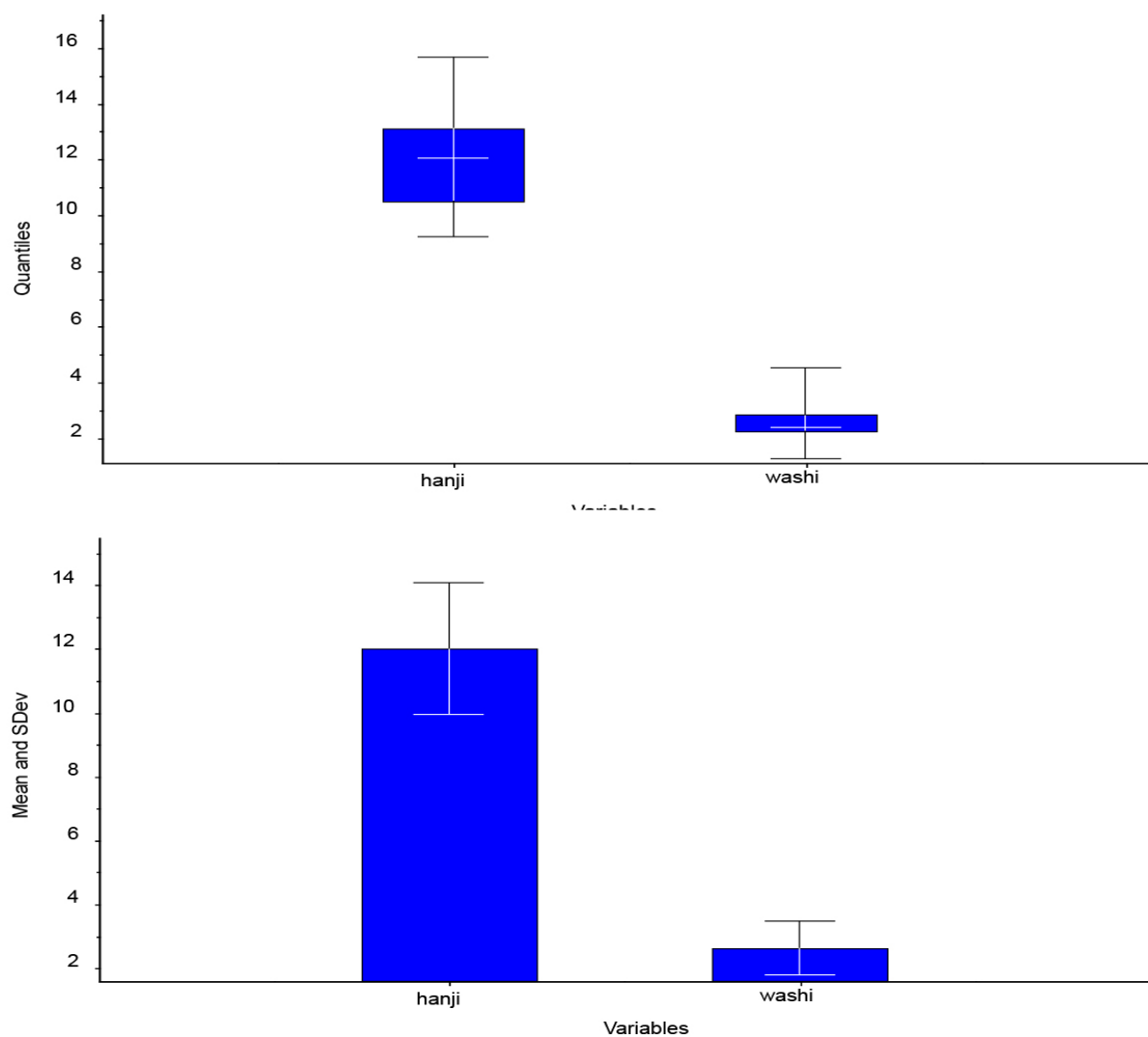


Figure 3.1.6 The ration of the EIC (m/z at 218) area of X1/C31 (in percentage). The data are from 12 test of hanji papers and 12 test of kozo papers that have signals of X1 and C31 at EIC m/z at 218 and respective retention time at 59.6 min and 59.7 min, respectively.

3.2 Identification of traditional East Asian handmade papers through multivariate data analysis of pyrolysis-GC/MS data

3.2.1 The aim of the study

The problem of fiber identification has been demonstrated with some cases where there presence some limits of the microscopic observation and the identification results don't have a feasible method for validation. The technical part of Py-GC/MS and Py-GC \times GC/MS for characterization of fibers from different material origins has been discussed in chapter 2. And by using the analyzed metabolite distribution patterns, a new method for fiber identification is proposed.

The new approach is based on the multivariate analysis of on-line pyrolysis-gas chromatography/mass spectrometry data for the identification of traditional East Asian handmade papers from different material origins. The approach utilized different biomarkers in the defined region of interest (ROI) for handmade papers' identification and discrimination.

3.2.2 The research content

An analytical approach based on the multivariate analysis of on-line pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) data is proposed for the identification of traditional East Asian handmade papers from different fiber material origins. This approach utilized several biomarkers detected during Py-GC/MS analysis of the paper samples. At first, the total ion chromatogram (TIC) was taken as the response and then the extracted ion chromatograms (EICs) were considered to improve the discrimination of papers. The influence of different data pretreatment (raw responses vs normalized values) including different weighting of the variables (weighting as 1 vs weight as 1/STD, where STD stands for standard deviation) for principal component analysis was also investigated. The results showed that compared to the commonly used microscopy techniques, the Py-GC/MS technique proved to be able to discriminate against handmade paper materials that have similar microscopic morphologies such as *Morus* species vs. *Broussonetia* species. The data pretreatment influenced the PCA modeling: the analysis based on normalized values showed more interpretable PCA group features for *Moraceae* species. PCA without weighting resulted unsurprisingly in discrimination through the presence of high intensity response biomarkers, while when applying weight as 1/STD, PCA loading plot was shown to provide a

group of compounds, most of them being present at low levels, to be discriminating. Additionally, the characteristic EICs can provide data matrix for statistical analysis avoiding the interference from co-eluting compound and background compared to the data matrix obtained from the TIC. As a result, a quick Py-GC/MS based handmade paper identification procedure using PCA modeling of characteristic EICs was for the first time proposed in the identification of traditional East Asian handmade papers. This procedure could be very beneficial in cultural heritage applications.

The current version of this work will be presented here under the form of its forthcoming submission for publication. For the supplementary materials mentioned within the text, please refer to Annex 46.

Identification of traditional East Asian handmade papers through multivariate data analysis of pyrolysis-GC/MS data

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ABSTRACT An analytical approach based on the multivariate analysis of on-line pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) data is proposed for the identification of traditional East Asian handmade papers from different fiber material origins. This approach utilized several biomarkers detected during Py-GC/MS analysis of the paper samples. At first, the total ion chromatogram (TIC) was taken as the response and then the extracted ion chromatograms (EICs) were considered to improve the discrimination of papers. The influence of different data pretreatment (raw responses vs normalized values) including different weighting of the variables (weighting as 1 vs weight as 1/STD, where STD stands for standard deviation) for principal component analysis was also investigated. The results showed that compared to the commonly used microscopy techniques, the Py-GC/MS technique proved to be able to discriminate against handmade paper materials that have similar microscopic morphologies such as *Morus* species vs. *Broussonetia* species. The data pretreatment influenced the PCA modeling: the analysis based on normalized values showed more interpretable PCA group features for *Moraceae* species. PCA without weighting resulted unsurprisingly in discrimination through the presence of high intensity response biomarkers, while when applying weight as 1/STD, PCA loading plot was shown to provide a group of compounds, most of them being present at low levels, to be discriminating. Additionally, the characteristic EICs can provide data matrix for statistical analysis avoiding the interference from co-eluting compound and background compared to the data matrix obtained from the TIC. As a result, a quick Py-GC/MS based handmade paper identification procedure using PCA modeling of characteristic EICs was for the first time proposed in the identification of traditional East Asian handmade papers. This procedure could be very beneficial in cultural heritage applications.

Keyword: Py-GC/MS, chemometrics, data pretreatment, East Asian handmade papers, fiber identification, cultural heritage, plant markers.

1. Introduction

Handmade paper has a history of more than 2000 years and became the principal vehicle in civilization to carry and keep information mankind has ever wanted to communicate. The credit for the invention of papermaking was given to a Chinese official Cai Lun at the year of 105 AD, while new archaeological evidences indicated earlier origin for papermaking [1]. From that time on, papermakers have explored various plant materials for hand papermaking through centuries both chronologically and geographically [1, 2]. With time, material changes were observed in papermaking and such material changes also underwent after papermaking technique was distributed to the neighboring areas such as Korea [3, 4] and Japan [5]. The change of material for the papermaking was associated to the political or society changes, and may contain rich information for history studies. Nevertheless, little attention was paid to the paper itself for its materiality in the early studies of the old manuscripts (at least until the beginning of the 20th century) as it cannot be identified directly from the manuscript by historians and paleographers [6]. In fact, as far as the material investigation was concerned, misinterpretation of fibers origin could occur when the scientific and technical methods for material investigation were not robustly available. For instance, in the 1870s, some historians pointed out that only botanist could judge the type and quality of papermaking fibers [7]. However, at that time, the botanists did not deal with the questions with necessary scientific depth for handmade papers analysis and they do not go beyond to treat the data for historical interpretations.

At the beginning of the 20th century, the importance of natural science in the investigation of ancient documents has been realized by proposing the connection of historical and scientific studies in order to turn to the starting point of the question: the material origin itself. The first analytical technique for handmade paper analysis appeared by applying microscopy. The pioneer work of handmade paper microscopic observation was carried out by Julius Ritter von Wiesner and Charles-Moïse Briquet [8, 9]. They founded the scientific analysis of paper on optical microscopic observation, chemical tests and identification of the fiber materials through comparative analysis with a reference material [10].

Handmade paper identification benefits from those stain methods and microscopic observation, which have a greater value in this domain up to now as can be found in practice [11-17]. However, microscopic observation is sometimes insufficient to assess unambiguously the nature of the fibers especially when the plants involved present a close taxonomy relationship and present high similarities in their morphological features [11, 18, 19]. By means, microscopy observation can only retrace part of the fingerprint information

which presents limitations especially in identifying plant fibers that have quite similar morphological features (usually plant within a close taxonomy relationship): a dilemma situation commonly confronted in culture heritage studies [11, 18, 19]. For instance, during the study of Tibetan manuscript, it was found that fiber analysis allow for clear-cut identification only in a limited number of cases with difficulties in separating the fibers within the *Thymelaeaceae* family. Although efforts has been done to solve this problem since 1970s, it seems no success has been achieved until now [20]. Furthermore, A systematic microscopy study has revealed that typical characteristic traits (such as cross-section diameter, lumen diameter, dislocations) cannot be taken as unique identification criteria (with the example of flax, nettle and hemp fibers) [18]. Another example lies in the identification of fibers from *Moraceae* family: associated parenchymal cells or calcium oxalate crystals has been used as an indicator to identify paper mulberry fibers (*Broussonetia* species) from mulberry fiber (*Morus* species) [2]. However, this indicator is not always reliable as they both exist in paper mulberry and mulberry fibers. To our knowledge, currently there is no feasible and reliable methods for fiber identification within *Moraceae* family utilized in hand papermaking.

As an analytical method designed for polymer analysis, on-line pyrolysis had been explored for its practical application as early as 1953 and was quickly applied in culture heritage studies [21]. Since then, pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) has been widely used for the characterization of organic materials used in artworks and archaeological samples [22-24]. Also, Py-GC/MS is among the most frequently used techniques for cellulosic material studies due to its ability to provide information on their molecular structure. However, pyrolysis process can generate large numbers of compounds in varying quantities which posed difficulties in quantitative measurement and data treatment. Chemometric methods such as principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) have demonstrated their effectiveness for Py-GC/MS data treatment and interpretation [25].

Papers produced on a large scale in East Asian regions are made of vegetable fibers of which the main constituent is always the same: cellulose. Investigations on historical paper artefacts by pyrolysis revealed the cellulose fingerprints to be of great help for the characterization of the state of degradation [26, 27]. Besides cellulose, the presence of terpenic and stigmasta compounds residue in East Asian handmade papers was found to be biomarkers for their differentiation and served as a basis for a new approach for fiber identification [28]. Also, the use of improved chromatographic separations combined with multivariate data analysis for analysis of handmade papers having a priori similar pyrogram was proposed for a more reliable identification [29]. However, a technique like comprehensive gas

chromatography/mass spectrometry (GCxGC/MS) is not always available in laboratories involved in Cultural Heritage studies, it is then worthwhile to assess the capacity of the Py-GC/MS to discriminate different handmade papers using a novel chemometrics approach.

It is important to illustrate the specificity in the art of traditional East Asian papermaking which resides in the rational choice of fiber plants adequate to obtain the required quality for the final paper formation. This point constitutes an opportunity to chronologically and geographically locate the origin of manuscript if a reliable analytical method is available. However, similarities in the structure of plant fibers render tedious identification by conventional microscopy method, especially for species having close botanical relations. Noteworthy, phytochemistry has demonstrated that different plant species may present specificities in their bio-synthesis pathways for certain low molecular weight metabolites, which provides the foundation to establish new criteria for their characterization [30].

In the aim of gaining a thorough knowledge on the capacity of Py-GC/MS for the identification of East Asian handmade papers, different data pretreatment within PCA analysis were considered for the investigation of the plant marker region (defined as region of interest, ROI) of the Py-GC/MS data and their efficiency to discriminate handmade papers were compared. Such approach based on the application of Py-GC/MS associated to multivariate data analysis is for the first time explored for the fiber identification of East Asian handmade papers.

2. Materials and methods

2.1 Materials

Table 1. Summary of the tested samples of known origin used in the present study.

No.	fiber origin	plant family	common name	made in
S1	<i>Morus mongolica</i>	<i>Moraceae</i>	sang pi zhi	China
S2	<i>Morus alba</i>	<i>Moraceae</i>	sang pi zhi	China
S3	<i>Broussonetia papyrifera</i>	<i>Moraceae</i>	gou pi zhi	China
S4	<i>Broussonetia kazinoki</i>	<i>Moraceae</i>	kozo	Japan
S5	<i>Broussonetia papyrifera</i>	<i>Moraceae</i>	hanji	South Korea
S6	<i>Pteroceltis tatarinowii maxim</i>	<i>Ulmaceae</i>	seitan	Japan
S7	<i>Edgeworthia papyrifera</i>	<i>Thymelaeaceae</i>	mitsumata	Japan
S8	<i>Wikstroemia sikokiana</i>	<i>Thymelaeaceae</i>	gampi	Japan
S9	<i>oryza sativa</i>	<i>Poaceae</i>	rice paper (grass)	Japan
S10	<i>Phyllostachys pubescens</i>	<i>Poaceae</i>	bamboo paper	Japan
S11	<i>Cannabis sativa</i>	<i>Cannabaceae</i>	ma paper (hemp)	Japan
S12	<i>Linum usitatissimum</i>	<i>Linaceae</i>	ma paper (flax)	Japan
S13	<i>Boehmeria nivea</i>	<i>Urticaceae</i>	ma paper (ramie)	Japan
S14	<i>Corchorus capsularis</i>	<i>Malvaceae</i>	ma paper (jute)	Japan

Materials used in the study consisted of fourteen types of handmade papers from different plant material origins listed in Table 1. These papers were prepared following the traditional hand papermaking processes and will be reported in the text and figures under the denomination of 'S1' to 'S14'.

2.2 Py-GC/MS Analysis

Py-GC/MS analyses were performed using a vertical micro-furnace-type pyrolyzer PY-2020iD (Frontier Lab, Fukushima, Japan) directly connected to the injection port of a Shimadzu QP2010 gas chromatograph linked with a Shimadzu QP2010Plus quadrupole mass spectrometer (Shimadzu, Champs-sur-Marne, France). The sample was placed in a stainless steel sample cup. The paper samples were weighted with an XP2U Ultra Micro Balance (Mettler Toledo, Viroflay, France). Typical quantities of paper used in this work were ca. 100 μ g weighted with the microbalance. The sample cup was placed on top of the pyrolyzer at near ambient temperature. For analysis, the sample cup was introduced into the furnace at 500 °C, and then the temperature program of the gas chromatograph oven was started. The Py-GC interface was held at 320 °C. Chromatographic separation was carried out on an Frontier Lab DB5-ms 5% phenyl–95% dimethyl polysiloxane fused silica capillary column (30 m length, 0.25 mm inner diameter and coated with a 0.25 μ m film thickness). The oven temperature was initially held 3 min at 40 °C, and then ramped at 5 °C min⁻¹ to 325 °C, where it was held for 10 min. The total duration of GC analysis was 70 min. The helium carrier gas Alphagaz 1 (Air Liquide, France) was used in the linear velocity mode (1 mL.min⁻¹). The injector was held at 280°C and used in split mode (1:10 of the total flow). The mass spectrometer was operated at 5000 u.s⁻¹, with a scan range from 50 to 500 u, using electron ionization at 70 eV. The interface was kept at 300 °C and the MS source at 200 °C. The mass spectra of the pyrolysis products were recorded using the average spectrum +average and subtract spectrum modes in the Shimadzu GCMSsolution software. Identification of pyrolysis products was performed by comparing the mass spectra of unknown components with the NIST mass spectra library (2011 edition) and by interpretation of the main fragmentations [28]. All the analysis has been conducted in triplicate.

2.3 data pretreatment and PCA analysis

In the chromatograms, only the compounds within the defined region of interest ROI (see below) have been considered for the multivariate data analysis. This ROI usually encompasses plant marker compounds eluting from t_r = 50 min to t_r = 63 min (Figure 1B) but

the ROI has been extended from $t_r = 44.7$ min to $t_r = 63$ min for S9-S14 (Fig.1C) for which less compounds were detected in the marker part region of their corresponding chromatograms. Most of the detected plant marker compounds were assigned to triterpene and phytosterol structures [28]. The cellulose pyrolysis products have not been considered in the modeling due to their high similarity within samples. The compound identification and area integration in the total ion chromatogram (TIC) has been done manually within the ROI using the GCMS Postrun analysis software (Shimadzu); consequently, no retention time alignment was necessary. The TIC peak areas (either raw or normalized values) were used as responses for the multivariate data analysis. The selected ions (m/z) for different handmade paper groups were constructed in the extracted ion chromatograms (EICs) as it will be depicted below. The EIC peak area (both raw and normalized values) were used for the multivariate data analysis to explore the most characteristic features of each handmade papers. In the data matrix for PCA modeling, when one compound is absent, its corresponding descriptor value was set at 0 in the matrix. After the data pre-treatment procedure, the data were submitted to principal component analysis (PCA) modeling. Each analysis was considered as an individual sample in PCA analysis and the peak areas of the compounds distributed within the ROI acted as variables. The PCA analyses were performed using the Unscrambler X (Version 10.4) software from CAMO Software AS (Oslo, Norway). In the process of PCA modeling, data were centered and different variable weighting parameters (weight as the same value 1 and weight as $1/STD$) were performed. Here, STD is defined as the standard deviation calculated among each variable, which indicates how much the data are scattered for this response. So the weight of each variable is no longer related to its value but to the way it differs among samples. The data were mean centered in the course of PCA analysis.

3. Results and discussions

3.1 PCA of all the tested samples using TIC

Identification and characterization of pyrolysis products of handmade paper was performed using Py-GC/MS (Figure 1). The tested papers resulted at first view, in similar chromatograms for the first half of the separation, which is mainly composed of the cellulose pyrolysis products generated from the thermal carbohydrate backbone degradation, the most abundant peak being the peak corresponding to the 1,6-anhydro- β -D-glucose (levoglucosan) as illustrated in Figure 1A for the kozo paper. After the elution of levoglucosan appears a region where fatty acid derivatives and hydrocarbons compounds eluted at retention times comprised between $t_r = 32$ min and $t_r = 44$ min, followed by a region of interest (ROI) for retention times $t_r \geq 44.7$ min revealing the plant markers (terpenic and stigmasta compounds)

which have been previously detailed and defined as region of interest (ROI) [28, 29, 31]. The visual comparison of the chromatograms (compound distribution pattern) revealed that the samples S1 to S5 have a global similar patterns while, comparatively, S6 to S14 showed different distributions of markers in the ROI (Figure 1B, 1C). It is noticeable that S11 to S14, which correspond to hemp, flax, ramie and jute handmade papers, presented less detectable compounds in their respective ROI. The visual check of the ROI patterns give some hint on the similarity and differences of the tested handmade papers but more definitive links between them can be only achieved using mathematical procedures which allow for statistical comparison of their chromatograms in the aim of an increased confidence for their discrimination and identification [29]. Detailed assignments of plant marker compounds in the ROI are reported in Table 2.

Principal component analysis (PCA) was applied to reduce the dimension of the multidimensional data sets and to establish which variables contribute the most to the organization of the samples in the principal component space among the assigned compounds in Table 2. In an aim of determining the most recognizable features of the chemical fingerprints issued from the pyrolysis product of different handmade papers, data pretreatment and different PCA parameters were investigated within the ROI of the handmade papers for their effective discrimination.

From a starting point, prior to focus on the ROI, the whole chromatogram has been used for multivariate data analysis but it does not yield satisfying result as the large intensity compounds in the chromatogram (namely, cellulose pyrolysis products) bear high similarities and, consequently, the differences in the distributions of compounds within the ROI were sunk in the data analysis process. Statistical analysis was therefore applied to the compound distribution within the ROI. Considering the number of compounds detected in the ROI, as can be seen in Figure 1B and 1C, the plant markers differ from paper sample to paper sample: this results in discriminating fingerprints in the chromatogram. In particular, fingerprints of handmade papers in Figure 1B report mainly triterpene structures and phytosterol structures (Table 2). Noticeably, it can be observed that for samples S9 to S14, the markers are more limited in number, and correspond to chemicals whose structures are less characteristic than previous triterpenes or phytosterols [28]; these structures consist mainly in long-chain aldehydes, alcohols or alkanes difficult to assign for their mass spectra. However, one must concede that the presence of these compounds in the associated extended ROI, expanding from $t_r = 44.7$ min to $t_r = 62$ min, permits the differentiation of these particular handmade papers by Py-GC/MS through their fingerprints. The co-elution of few compounds in the ROI was observed in the chromatograms (Table 2, compounds reported with an asterisk). During

the Total Ion Current (TIC) treatment of data by PCA, these co-eluting compounds were attributed to the paper samples in relation to their major components, which means that even if a compound of different structure was detected as co-eluent, the peak was associated with the major compound considered as a descriptor of the handmade paper for the PCA analysis. The influence of the co-eluting compounds for PCA analysis is discussed below.

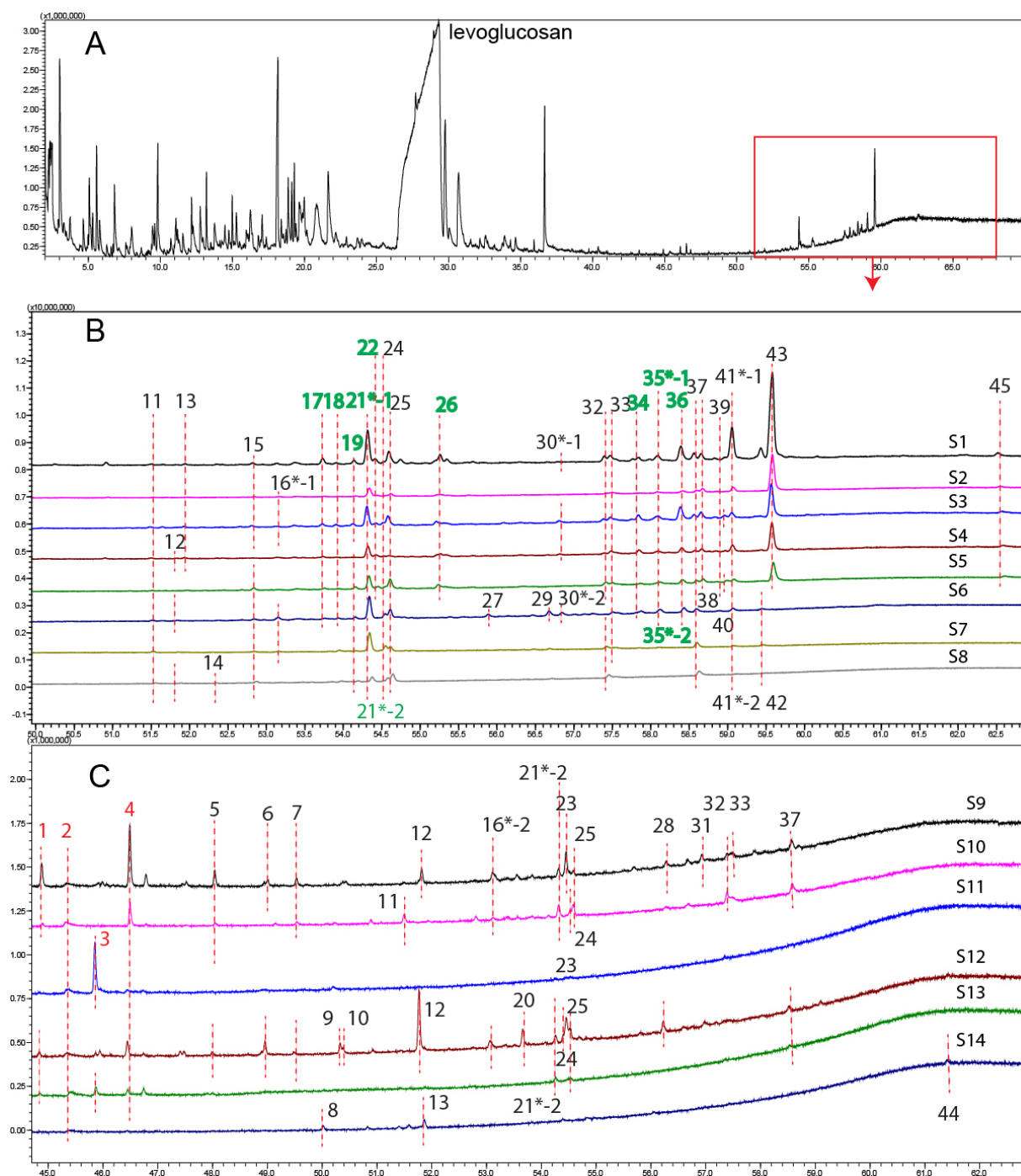


Figure 1. Total ion current chromatograms obtained from the pyrolysis of a kozo paper sample (A), with the enlargement of the corresponding regions of interest ROI obtained during Py-GC/MS analysis of sample S1 to S8 (B) and S9 to S14 (C).

Table 2. Assignment of plant markers detected by Py-GC/MS in the ROI of the investigated East Asian papers with retention time (t_r), main fragment ions, expected molecular weights, assigned formula and most likely attribution of the products.

No ¹	t_r (min)	fragment ions (in decreasing order of intensity)	MW	formula	assignment
1	44.86	82, 57, 97, 55	226	C ₁₅ H ₃₀ O	n-pentadecanal
2	45.35	115, 69, 143, 73	272	C ₁₅ H ₂₈ O ₄	pentanedioic acid, 2,2-dimethyl-, bis(1-methylpropyl) ester
3	45.85	55, 69, 83, 97	263	C ₁₈ H ₃₃ N	oleanitrile
4	46.45	82, 57, 96, 43	268	C ₁₈ H ₃₆ O	stearyl aldehyde (n-octadecanal)
5	48.10	82, 57, 96, 83	282	C ₁₉ H ₃₈ O	2-heptadecyloxirane
6	48.90	57, 71, 85, 99	282	C ₂₀ H ₄₂	eicosane
7	49.50	82, 57, 96, 83	NA	NA	aldehyde
8	50.01	81, 91, 145, 55	386	NA	cholestane derivative
9	50.38	97, 57, 83, 55	396	C ₂₇ H ₅₆ O	1-heptacosanol
10	50.43	57, 71, 85, 99	380	C ₂₇ H ₅₆	2-methylhexacosane
11	51.50	133, 81, 131, 107	382	NA	cholestane derivative
12	51.82	57, 71, 85, 99	408	C ₂₉ H ₆₀	2-methyloctacosane
13	51.90	81, 105, 91, 147	368	C ₂₇ H ₄₄	cholestane derivative
14	52.23	74, 87, 57, 75	410	C ₂₇ H ₅₄ O ₂	hexacosanoic acid, methyl ester
15	52.80	81,145, 95,105	396	NA	stigmastane derivative
16* -1	53.13	135, 81, 143, 380	380	NA	cholestane derivative
16*-2	53,12	57, 97, 83, 71	NA	NA	long chain alkene or long chain alcohol
17	53.75	218, 203, 189, 95	408	C ₃₀ H ₄₈	oleanane-2,12-diene
18	53.99	105, 95, 91, 203	398	NA	cholestane derivative
19	54.12	105, 91, 95, 396	396	NA	stigmastane derivative
20	53.66	82, 57, 96, 83	NA	NA	long chain aldehyde
21* -1	54.32	218, 189,119, 95	408	C ₃₀ H ₄₈	urs-2,12-diene
21* -2	54.26	135, 394, 81, 143	412	C ₂₉ H ₄₈ O	stigmasta-4,6-diene-3-ol
22	54.43	218, 95, 119, 189	NA	C ₃₀ H ₄₈	triterpene (ursane) structure
23	54.46	57, 71, 85, 99	NA	NA	long chain alkane
24	54.53	141, 142, 128, 81	NA	NA	cholestane derivative
25	54.59	147, 81, 105, 396	396	C ₂₉ H ₅₀ O	stigmasta-3-ol
26	55.26	107, 93, 95, 109	408	C ₃₀ H ₄₈	triterpene (ursane) structure
27	55.89	123, 95, 105, 107	412	C ₂₉ H ₄₈ O	cholestane derivative
28	56.23	57, 82,96, 71	240/268	C ₁₆ H ₃₂ O/ C ₁₈ H ₃₆ O	long chain aldehyde
29	56.67	412, 136, 95, 394	412	C ₂₉ H ₄₈ O	stigmastane or cholestane derivative
30*-1	56.81	424, 255, 95, 69	424	C ₃₀ H ₄₈ O	ursa-9(11),12-dien-3-ol
30*-2	56.84	95, 55, 412, 81	412 + 424	NA	stigmastane derivative + ursane derivative
31	56.93	57, 71, 85, 99	NA	NA	long chain alkane

32	57.39	107 283 414 329	414	C ₂₉ H ₅₀ O	stigmasta-5-ene-3-b-ol (sitosterol)
33	57.48	95, 424, 135, 109	424	NA	stigmastane structure
34	57.85	218, 203, 95, 189	426	C ₃₀ H ₅₀ O	oleanane-12-en-3-ol (b-Amyrin)
35*-1	58.10	95, 109, 218, 93	424, 466	C ₃₀ H ₄₈ O	urs-12-ene-3-one (424) + co-eluting compound (466)
35*-2	58.12	218, 95, 122, 412	412	NA	triterpene derivative
36	58.40	218, 95, 107, 109	426	C ₃₀ H ₅₀ O	urs-12-en-3-ol (α -Amyrin)
37	58.57	174, 159, 161, 410	410	C ₂₉ H ₄₆ O	stigmasta-3,5-dien-7-one
38	58.67	466, 255, 95, 69	466	C ₃₂ H ₅₀ O ₂	oleanane-12,14-diene-3-acetate
39	58.83	95, 105, 135, 91	466	C ₃₂ H ₅₀ O ₂	urs-12,14-diene-3-acetate
40	58.96	109, 95, 189, 218	466	NA	triterpene derivative
41*-1	59.06	218, 203, 189, 95	468	C ₃₂ H ₅₂ O ₂	oleanane-12-ene-3-acetate (β -amyrin acetate)
41*-2	59.07	124, 95, 229, 109	412	C ₂₉ H ₄₈ O	stigmast-4-en-3-one
42	59.45	410, 136, 95, 174	410	C ₂₉ H ₄₆ O	stigmasta-4,6-diene-3-one
43	59.58	218, 95, 107, 189	468	C ₃₂ H ₅₂ O ₂	urs-12-ene-3-acetate (α -amyrin acetate)
44	61.40	207, 57,71, 267	NA	NA	fatty acid derivative
45	62.60	273, 135, 232, 95	482	C ₃₂ H ₅₀ O ₃	urs-12-ene-11-one-3-acetate

¹ the asterisk * in the corresponding product number (also in Figure 1B and 1C) signifies the presence of a co-eluting pyrolysis products (see text for discussion)

The PCA was firstly applied using the raw (not-normalized) peak area values of the compounds generating the TIC of the ROI with each variable's weight as 1 for PCA modeling. As can be seen from the score and loading plots in Figure 2, the first principal component (PC1) describes 90% of the variance in the data and identifies three main clusters: the first cluster contains handmade papers from the *Morus* species (S1), the second group consists of handmade papers that have a series of triterpenic compounds characterized by a base peak at $m/z = 218$ in their mass spectra (*Morus* and *Broussonetia* species: S2 to S5), the third cluster contains the other paper samples (S6-S14). Actually, the distribution of the samples along PC1 was largely dependent on the presence and intensities of plant markers 43, 41*1, 21*1 as can be seen from the corresponding loadings plot (Figure 2B). The second principal component (PC2) for the TIC describes 4% of the variance and further separated the second cluster into two main groups: S2 and S5 vs S3 and S4, and the third cluster into two groups: S6, S7, S8 vs the other paper samples S9-S14 (S10 was slightly differentiated within this cluster). It can be seen from the loadings plot that the PC2 is defined by plant markers 36, 21*2, 25 which explains the cluster distribution along the PC2 on the base of discrimination within clusters given by PC1.

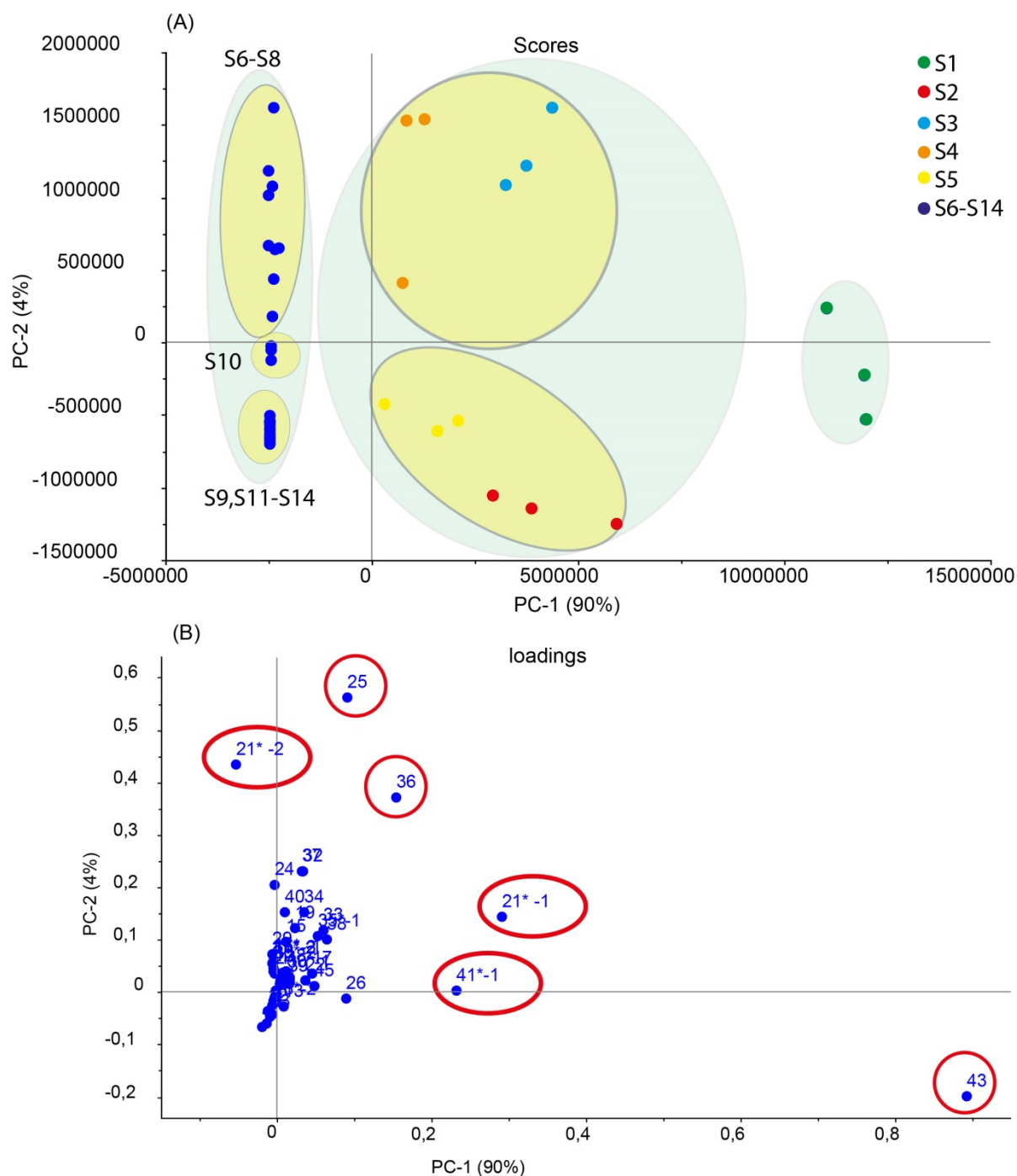


Figure 2. Score plot (A) and loading plot (B) resulting from the PCA treatment of peak areas as variables with un-normalized values in the ROI for the paper sample S1-S14. The numbering in (B) is corresponding to compounds in Table 2.

As expected, and as can be seen from Figures 1B, 1C and Figure 2, the compounds presenting the larger response (larger peak areas in the TIC of the corresponding ROI) will be identified as characteristic variables when applying the same weight (weight value as 1) for all the variables. This point is exemplified with the compounds differentiated in the loading plot (Figure 2B). PCA with this parameter is very efficient to discriminate samples showing quite different compound distribution patterns such as papers belonging to the second (S2-S5) and

the third clusters (S6-S14) for which the group location is well distanced in the PCA space (Figure 2). However, the main drawback lies in the fact that when analyzing samples with quite similar chromatographic patterns, the differentiation sometimes relies on minor peaks or relies on the slight variation of relative intensity of peaks in the ROI, otherwise subject to variation by the pyrolysis process. The present modeling approach shows limitations for the differentiation of samples of close composition in this case.

One way to overcome this limitation is to apply different weights to different variables. The modeling process using $1/\text{STD}$ for each variable were applied and illustrated in Figure 3. By applying this weight strategy, the influence of larger peaks in the chromatograms has been greatly reduced and a group of variables (instead of single large peaks) was shown to be responsible for the discrimination of samples in the loading plot (Figure 3B). The information bear by PC1 (34% here) has been reduced compared to the previous approach that used variables weight as 1, and conversely, the information bear by PC2 (18%) has been increased. In particular, clearer group characters were obtained compared to Figure 2: e.g. S6 is now well separated from S7 and S8, S3 has been separated from S4, S9 and S12 have been separated from the rest of the tested samples (S10-S14). Globally, it can be noticed that the loading plot is much more balanced, by employing this weighting strategy, the importance of each variable is observed to be of the same order of magnitude. Group of compounds rather than a few isolated compound) acts to define the directions of PC1 and PC2 in the scores plot. The grouping feature of the compounds in the loadings plot corresponds to their presence and relative high intensity in different samples and the weighting strategy ($1/\text{STD}$) make a balance of variable's weight and data values that results in a grouping feature in the loadings plot.

Another way to refine the data treatment resides in the use of normalization (normalization consisted in the normalization of all the markers peak intensity in the ROI). After data normalization, when modeling uses weight as 1 (i.e. the same weight for all markers), the use of the resulting TIC ratio provided a noticeably different distribution of the samples (Figure 4A) compared to the previously used TIC area with un-normalized values (Figure 2A). In particular, in the grouping of samples providing less intense marker peaks as exemplified for samples S11-S14, the location of sample S12 appeared as clearly separated from the three other samples S11, S13 and S14 (Figure 4A): this result could be of interest for its discrimination. Similarly, the distribution among the samples was clearly changed compared to the previous treatment with a better separation of S10 from the cluster S9-S14. However, these discriminations are to the detriment of the previous separation of samples S1-S5 with un-normalized values (Figure 2A). The observed modification in the distribution of samples S11-S14 is tentatively rationalized by the fact that for these samples providing less compound

distribution, after normalization, the relative ratio of markers became high (even though the TIC response is lower). When applying the weight as 1/STD with normalized values, unexpectedly almost the same group features were obtained as is shown in Figure 4B.

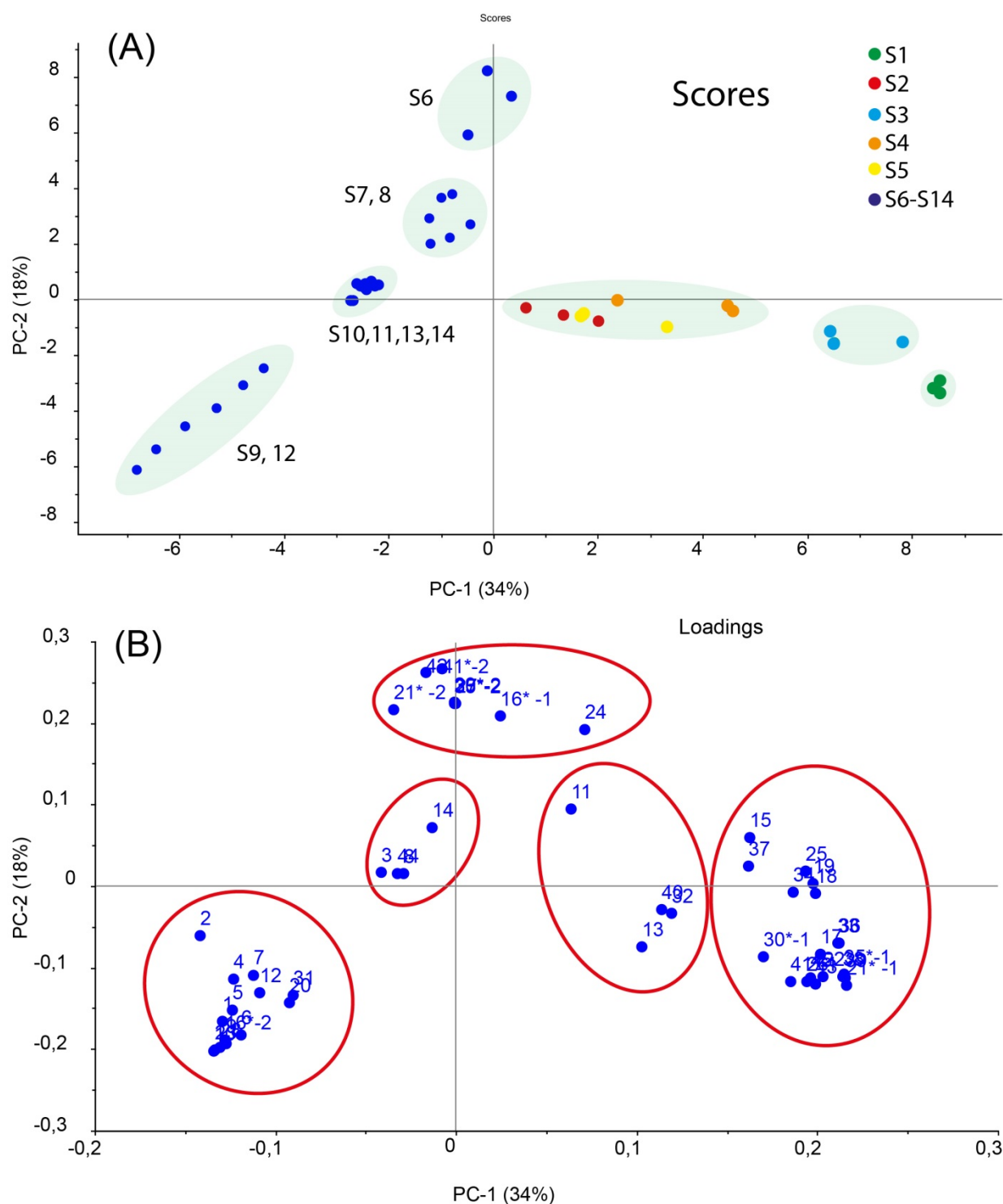


Figure 3. Score plot (A) and loading plot (B) resulting from the PCA treatment of peak areas as variables with each variable's weight as 1/STD in the PCA modeling process. The numbering in (B) is corresponding to compounds in Table 2.

By applying different PCA modeling, the compound TIC response (TIC area) showed capabilities to differentiate handmade papers of different material origins. In a similar way than microscopic observation, the traditional method for East Asian paper identification, which uses morphological features for this aim, Py-GC/MS combined to multivariate analysis create a new approach with the same purpose but in using their chemical fingerprint. Even, the Py-GC/MS demonstrated some advantages on microscopic analysis. For example, seitan fibers (S6) which show similarity in their morphological features with those of *Moraceae* species (S1-S5) are not differentiated from each other by microscopy (see Supplementary materials, Figure S1). At the opposite, Py-GC/MS combined to multivariate analysis have easily permitted this differentiation here from their chemical fingerprint (Figure 4). But the general inconvenient using TIC response lies in that: i) the used fingerprints were at a comparatively low response (compared to the cellulose pyrolysis product) for the other samples than *Moraceae* species and integration of their TIC area (either automatically or manually) are prone to induce errors that can influence the interpretation of the PCA modeling; ii) the TIC area contains all the ions monitored, consequently under the TIC area, the target compound's response cannot be easily separated from the background as well as from co-eluting compounds, even in using automatic deconvolution algorithm [32]. For instance, compounds 21*-1 and 21*-2 were co-eluting, in kozo sample (S4), the TIC area was assigned to 21*-1 (compound 21*-2 was comparatively low) while in S10 (bamboo paper), the TIC area was assigned to 21*-2 (compound 21*-1 was not present): this procedure affects noticeably the PCA modeling. On the other hand, the PCA modeling with TIC area does not allow the separation of handmade papers of similar plant origins (e.g. S1 vs. S3, S2 and S4 vs S3 and S5). Consequently, the information contained in the Py-GC/MS data has not been fully explored by investigating only the TIC response: for this reason, the extracted ion chromatograms (EICs) of marker's ions from the Py-GC/MS data were further explored in the following parts.

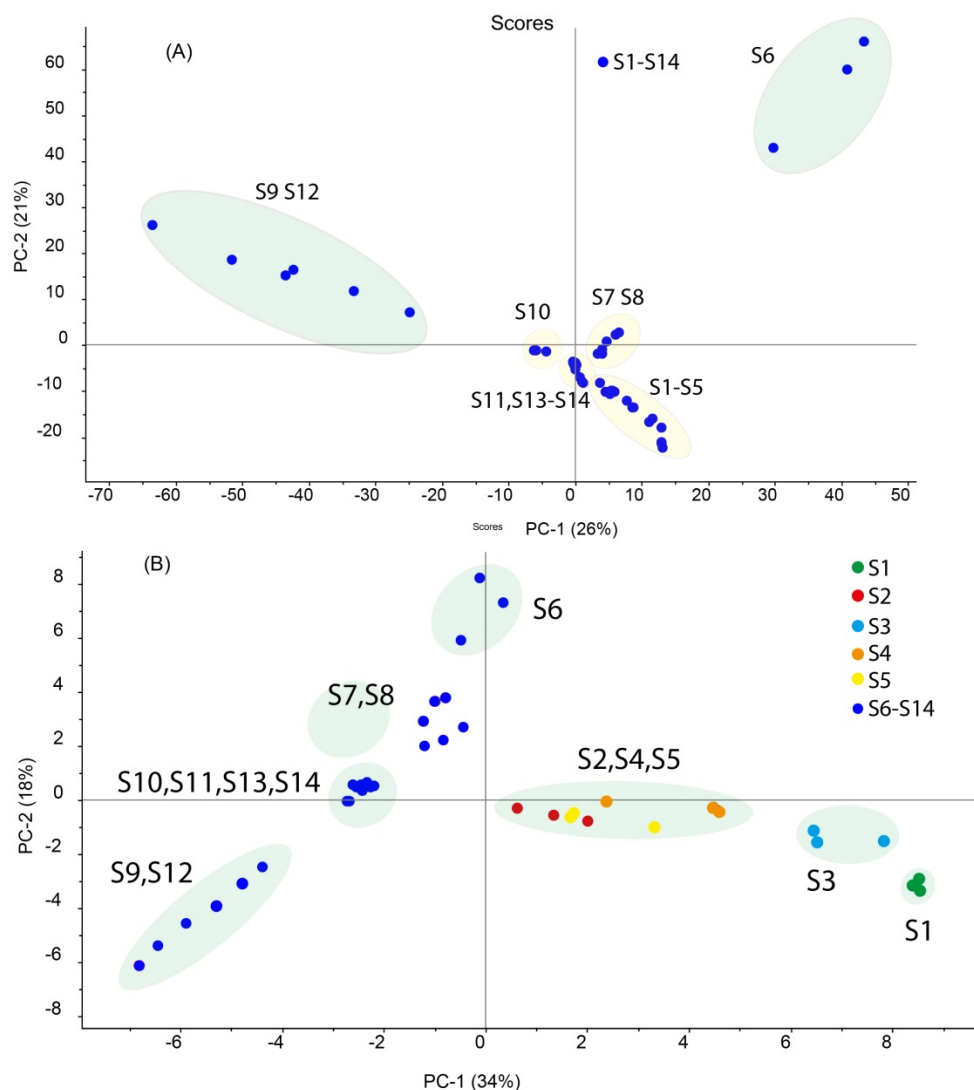


Figure 4. Score plots resulting from the PCA treatment of peak areas as variables with normalized values with the STD in the ROI for the paper samples, weight as 1 (A), weight as 1/STD (B).

3.2 PCA of tested samples using EICs

3.2.1 Detailed investigation of handmade papers of the *Ma* group

In the history of crafting, hemp, ramie, flax and jute have been long utilized for production of textile as well as papermaking. In practice, in the examination of old documents and handmade papers, sometimes the identification result was written as *Ma* group in cases where specific plant species within this group cannot be identified (“*Ma*” is the Chinese pronunciation of hemp while the *Ma* group consists in plant fibers from hemp, flax, ramie and jute et al). This is due to the morphological features of hemp, ramie, flax and jute that present high similarities, and the difficulty in discriminating them has been addressed both in practice and in literature [33]. Besides microscopic observation, their differences in chemical profiles could also contribute to their identification. However, there was less compounds detected in

the ROI, a situation which posed difficulties for their comparison. In the course of the present work, besides the use of TIC for data treatment, EICs have also been utilized for PCA analysis, for investigation of the potential advantages in providing more reliable results especially when the low intensity of pyrolysis products resulted in a relative low signal/noise ratio. EICs have been used to describe a chromatogram created by plotting the intensity of the signal observed at a chosen m/z value or a set of values in a series of mass spectra recorded as a function of retention time. EICs allow to separate both components by choosing characteristic m/z values, e.g. of the respective molecular ions or abundant fragment ions. After an examination of the compound distribution, the intensity of fragment peaks at m/z 57, 69, 145, 174, 394, 396 and 410 (chosen accordingly to their presence in the mass spectra of markers in the ROI, see Table 2) have been selected for PCA modeling of the *Ma* group.

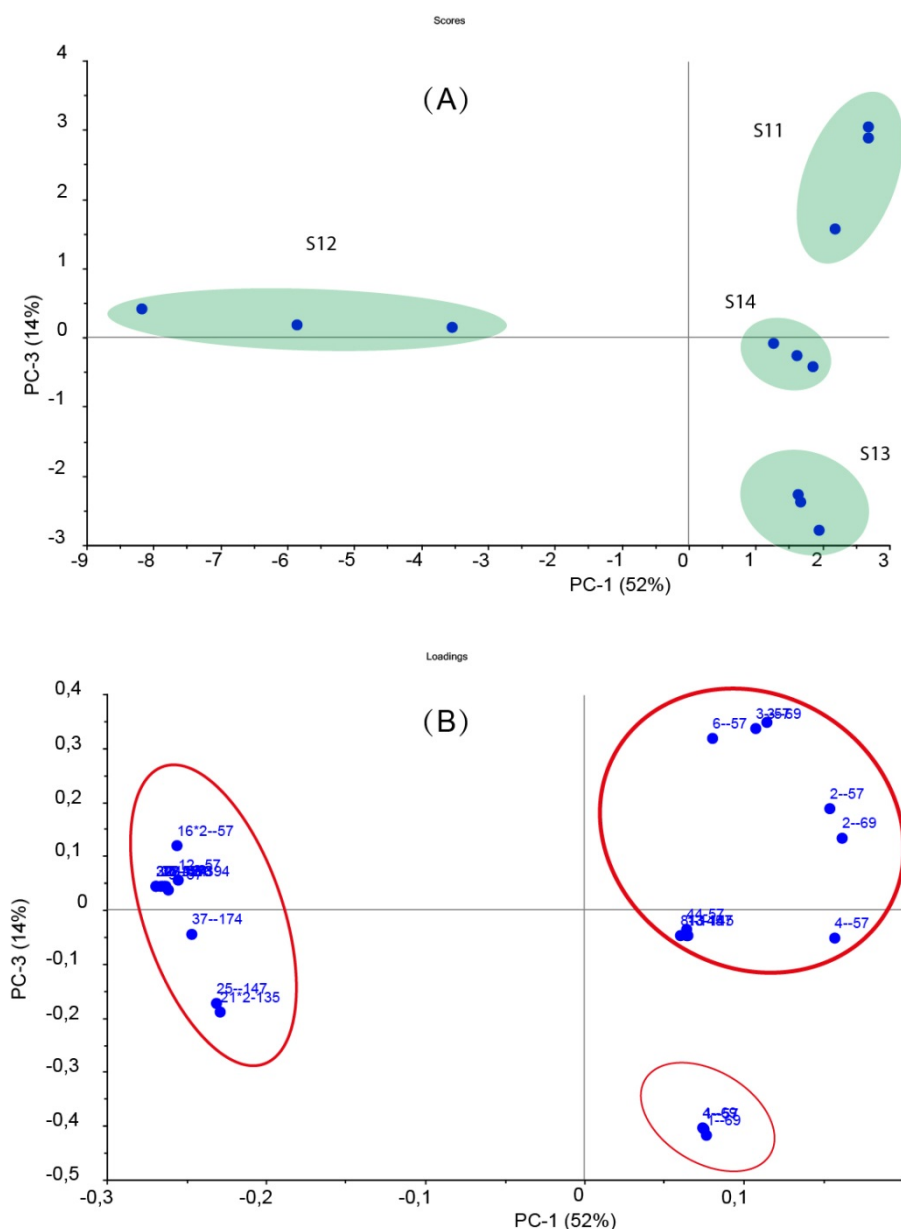


Figure 5. Score plot (A) and loading plot (B) resulting from the PCA treatment of peak areas as variables using variables weighting as 1/STD in the ROI from the EICs of the *Ma* group handmade paper samples showing the PC1 vs PC2. S11-S14 are corresponding to Table 1. In Figure 5B, the number before ‘-’ corresponding to the numbering in Table 2 and the number after ‘-’ represents the chosen EIC (m/z).

The EIC response (EIC area) (weight as 1/STD) showed a relevant grouping for each kind of papers: jute, flax, hemp and ramie (Figure 5A). When interpreting the data, considering PC1 (49%) and PC2 (35%), the hemp (S11) and ramie (S13) were still clustered together when applying the weighting strategy as 1/STD in the score plots (see Supplementary materials, Figure S2), only considering PC3 instead of PC2 permitted a nicer discrimination among the four group. It can be seen from Figure 5A that PC1 (52%) separated the space into two main groups of papers: S11, S13 and S14 in one side and S12 in the other side, while PC3 (14%) further separated the mixed papers, S11 from S13. By examining the EICs with PCA modeling, the Py-GC/MS method showed its potential to discriminate the plant fiber within the *Ma* group.

3.2.2 Detailed investigation of handmade papers of the *Moraceae* family

Fibers from the *Moraceae* family have been widely used for papermaking in East Asian regions: they are one of the main raw materials used for papermaking in China, Japan, and South Korea. Like the *Ma* group fibers, the fibers of the *Moraceae* family present similarities in their morphological features, which poses difficulties for their discrimination. Compared to the *Ma* group handmade papers, handmade papers from *Moraceae* family presented more markers in their ROI. One feature of these compounds is the detection of a series of terpenic compounds that present a base peak at m/z 218 in their mass spectra (Table 2). Taking into consideration the discussion of the TIC response in the part 3.1 above, the EIC information contained in the Py-GC/MS is explored for the *Ma* group fibers. As for the *Moraceae* family, the EIC of m/z at 218 was chosen as parameter for the PCA modeling of S1-S6 (while S6 is not belonging to the *Moraceae* family, it was chosen here for its fiber similarity to S1-S5 under microscopic observation, see Supplementary Materials Figure S1).

As can be seen in Figure 6, when using the EIC response (EIC area) with normalized values and variable's weights as 1, PC1 (93%) separated the samples into four groups: S1 that have the larger intensity for the marker compound 41*-1 (oleanane-12-ene-3-acetate), S2 presenting the larger intensity for the marker compound 43 (urs-12-ene-3-acetate), S3-S5 that have a moderate intensity for the markers compounds 41*-1 and 43, and S6 (seitan) that did not show the presence of markers 41*-1 and 43. As previously discussed, when applying the

same weight value (value set as 1), compounds that have the larger intensity will be found as discriminant variables after the PCA modeling of the pyrolysis products within the ROI. As a result, it appeared that the intensity of marker compounds 41*-1 and 43 bear most of the information as revealed in the PC1 value of 93% while PC2 was reduced to 5%: the two most important triterpene derivatives of the *Moraceae* family: □ and □ amyirin acetate respectively, permitted to separate visually the samples in four groups considering their relative intensities (S1, S2, S3-S5, S6) or considering their presence/absence (sample S1 and sample S6 from the others S2-S5). (for the result of un-normalized value with variable's weights as 1, see Supplementary materials, Figure S3).

The PCA modeling applying normalization of the variables and weight as 1/STD was also tested (Figure 7). As can be seen in Figure 7A, the PC1 (62%) separates the space into three groups: S6; S3 and S5; S1, S2 and S4, while PC2 (19%) further refined the separation of the samples into five groups: samples S1 and S2 (*Morus* species); S4 (*Broussonetia kazinoki*); S3 and S5 (*Broussonetia papyrifera*); and S6 (*Pteroceltis tatarinowii maxim*). These separations are correlated to the intensities of the corresponding groups of marker compounds in the loadings plot (Figure 7B), in particular, the high intensity value of compounds 41*-1 and 43 were comparatively higher in *Morus* species than the *Broussonetia* species making the difference after the PCA treatment.

When applying EICs ratio and PCA modeling with normalized values (weight as 1/STD), the influence of the two markers 41*-1 and 43 has been reduced compared to the previous case (Figure 6B) as revealed from the close location of S1 and S2 in Figure 7A.

By using the EIC response with different data pretreatment (normalized values with different weights), the handmade papers from the *Moraceae* family group can be discriminated in providing complementary information to microscopic observation. The discrimination of *Morus* genus (*Morus alba* and *Morus mongolica*) is easier with the intensity of the two marker compounds 41*-1 and 43. The sample seitan (S6) is also easier to discriminate since it does not present these two markers. However, the separation of S3 from S5 is comparatively not easy, whatever the PCA data pretreatment, and may necessitates a better chromatographic separation and resolution in the ROI to achieve their discrimination [29].

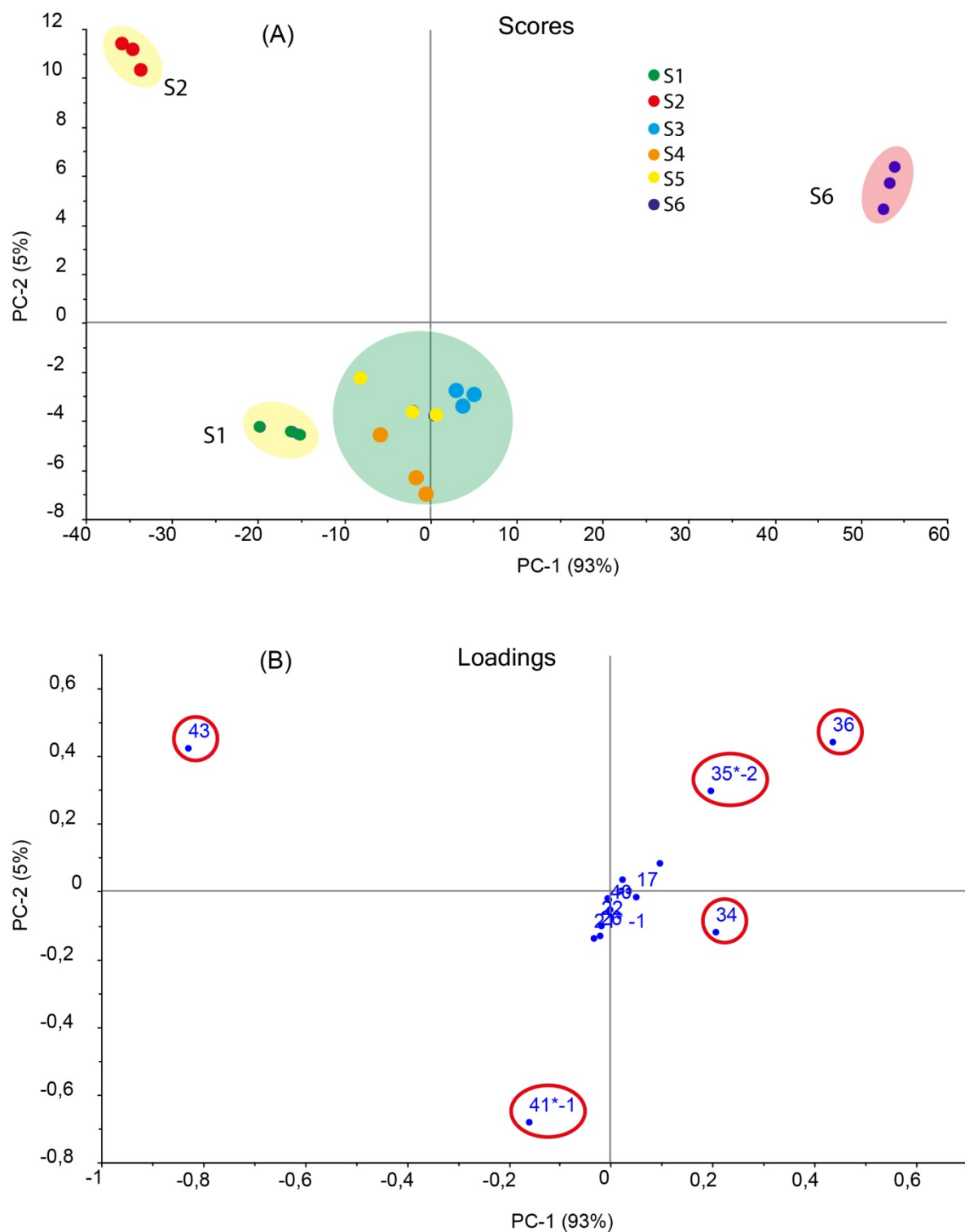


Figure 6. Score plot (A) and loading plot (B) resulting from the PCA treatment of peak areas as variables using normalized variables weighting as 1 in the ROI from the EIC (m/z 218) of the *Moraceae* family S1-S5 and S6.

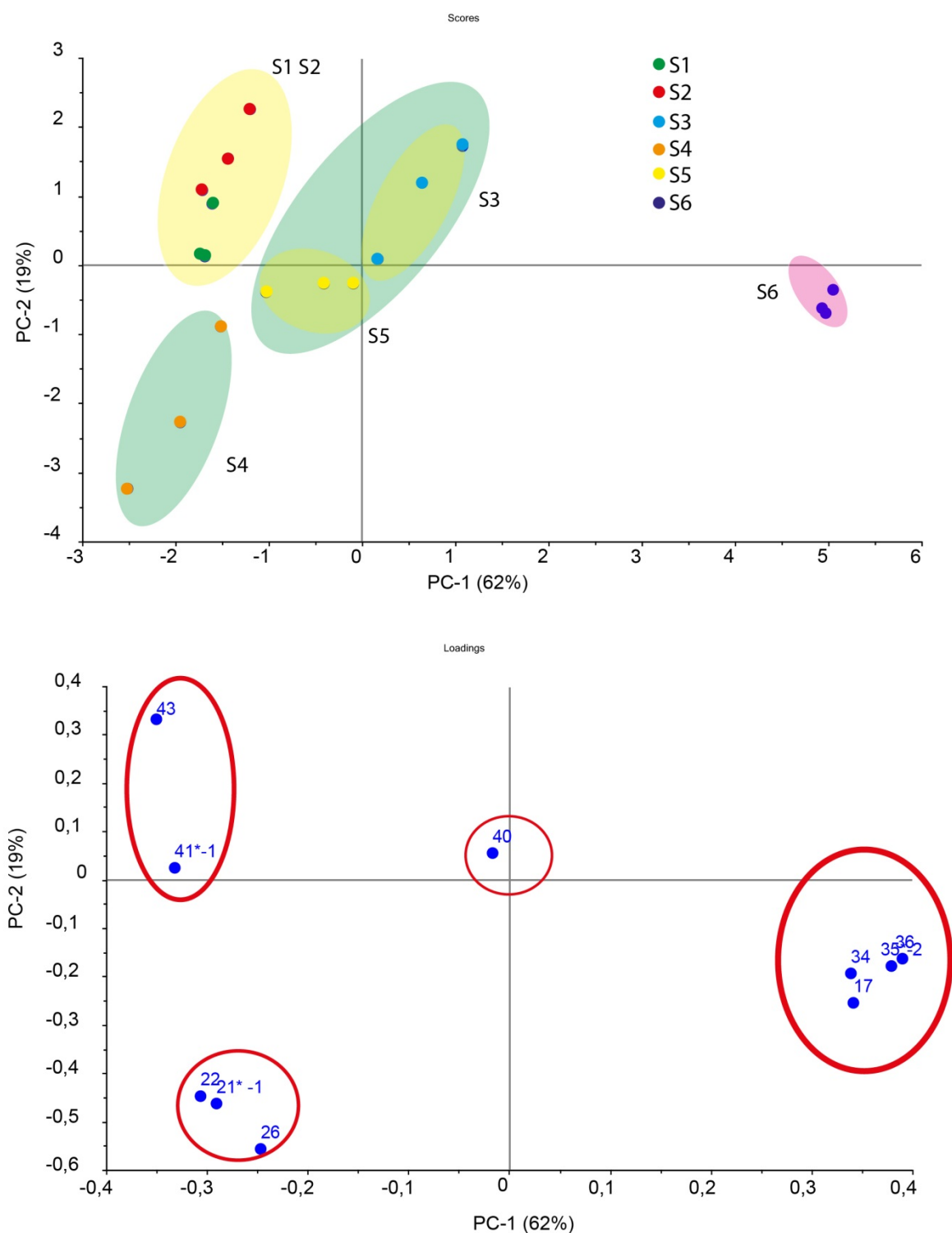


Figure 7. Score plot (A) and loading plot (B) resulting from the PCA treatment of peak ratio as variables of S1-S6 using normalized variables weighting as $1/\text{STD}$ from the EIC (m/z 218) of the *Moraceae* family S1-S5 and S6.

3.3 An overall approach

Indeed, as shown in the preceding parts, handmade papers can be characterized by their different EICs corresponding to the ions of their characteristic markers. After the investigation of handmade paper samples of the *Ma* group and handmade paper samples of the *Moraceae* family, a PCA space using all the characteristic EICs is presented in Figure 8. This PCA space utilizing different EICs can be separated into different series. The first series contains the terpenic compounds that have the m/z 218 as a base peak in the plant marker's mass spectra which is situated on the positive axis of PC1, while the other series were on the left side of this PCA space (with the EICs of 57, 145, 174, 394, 396, 410). The samples mitsumata (S7), gampi (S8) and bamboo (S9) were located on the negative PC1-positive PC3 quadrant. Although the samples mitsumata, gampi and sample bamboo can be separated in the PCA space operating descriptors with a weight as $1/STD$ (Figure 4), their location were still close and after the EIC procedure, one notice that they mixed together. In a similar way, samples of jute, hemp and ramie papers (S11, S14 and S13) have been clustered and mixed together as well as samples of rice (S9) and flax (S12) papers.

However, as stated above, by a quick and optimized EIC approach, the PCA modeling provide group information indicating fiber origins if we accept as a counterpart that some samples remain still mixed together. This point indicates how multi-approach identification conducted in conjunction is preferred to obtain the best from each analytical method. For example, microscopic analysis cannot easily differentiate samples S1 and S3 which correspond respectively to hanji paper and goupizhi paper (both made from *Broussonetia papyrifera*), while pyrolysis-GC/MS combined to PCA can easily do it (as shown in the procedures depicted in Figure 2 and Figure 3). Under the adopted overall approach, while mitsumata-gampi-bamboo were clustered (Figure 8), microscopic observation can separate bamboo papers from mitsumata and gampi papers from its morphological fiber features (noteworthy, bamboo is clearly differentiated from mitsumata and gampi through the only detection of its plant markers using Py-GC/MS). (for PCA space of PC1 vs PC2, see Supplementary materials, Figure S4)

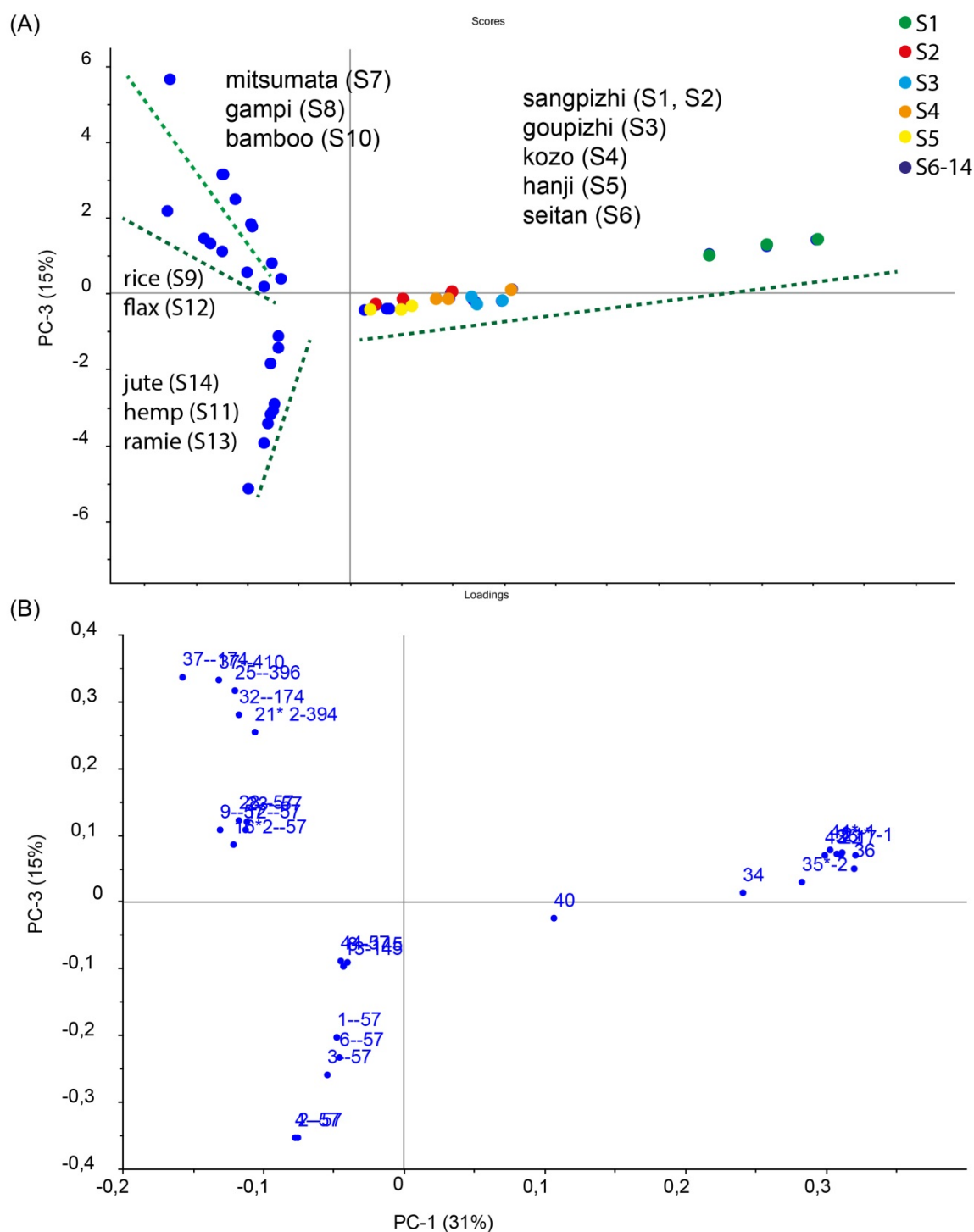


Figure 8. Score plot obtained from the PCA of all groups of samples (normalized values set as 1/STD from the EICs of markers in the ROI). S11-S14 are corresponding to Table 1. In Figure 8B, the number before '--' corresponding to the numbering in Table 2 and the number after '--' represents the chosen EIC (m/z). for the m/z at 218, the '--' was not used (located along the positive axis of PC1)

Considering the overall approach based on EICs for each East Asian handmade paper, one must concede that a prime screening of the samples can be obtained after a routine Py-GC/MS analysis. A rough classification of the unknowns can be deduced prior to a closer

investigation, which can be more exhaustive if considering specificities in plant marker presence. The conjunction of the overall approach with microscopic fiber observation may provide initial good insights on the samples, minimizing the risk of misinterpretation, known to be present during archaeological sample analysis.

As a general strategy for unknown handmade paper classification, the TIC information within the ROI has to be studied to retrieve the compound distribution pattern which provide the base for selected EIC analysis. From the TIC analysis, the general grouping information for different plant families such as *Moraceae* family, *Thymelaeaceae* family and *Ma* group can be obtained (Figure 2, Figure 3). However, the inconvenience of using TIC response has been observed. Comparatively, it is easier to get a data matrix free of problems of co-elution and background interference, especially for rather similar samples having similar TIC patterns. Since the TIC information has been revealed, in the following, a quick EIC analysis with different data pretreatment (weight as 1 or weight as 1/STD) is recommended: firstly, load the selected EICs into the PCA space (Figure 8), the PCA spacing in the loadings plot will give a first hint for unknown samples grouping classification; secondly, the EIC analysis could be performed within the groups indicated by the first step for more detailed discrimination within a sub group. If the traditional microscopic analysis is capable of classification of fibers from different plant families, the proposed Py-GC/MS method achieved the same capacity with more ability to distinguish sub-family and sub-specie (illustrated in Supplementary materials, Figure S5). Thus, it can provide more acute identification result with minor quantity required.

4. Conclusion

An analytical approach based on the multivariate analysis of on-line pyrolysis-gas chromatography/mass spectrometry data has been investigated in this paper for the identification of traditional East Asian handmade papers manufactured from different material origins. The approach was carried out with the investigation of biomarker distributions in the defined ROI with different data extraction method (TIC vs EIC), data pretreatment (raw responses vs normalized values) and different weighting for PCA modeling (weight as 1 vs weight as 1/STD). The results showed that both the TIC and EIC value can be applied for the discrimination of handmade papers while the EIC is more simple, quick and reliable to be used for PCA modeling in considering of background interference and likely compound co-elution. As a characteristic of pyrolysis analysis, semi quantitative procedures is more easy to carry out in a routine workflow and the data normalization proved to reduce the data variation and present clearer PCA grouping characteristics. When applying the weight as 1 for all descriptors during PCA modeling, within the ROI, the large intensity values will be modeled

as a discriminative factor, while taking weight as $1/STD$ will pick up a group of variables in which the influence of all compounds (major and minor) is more balanced.

A detailed investigation was given to two groups of handmade papers (*Ma* group and *Moraceae* family) that have high similarity in their morphological features and, consequently, cannot easily be distinguished by a sole microscopic observation. For the *Ma* group, few biomarkers were detected from the pyrolysis of handmade papers. EICs issued from the presence of long chain carbohydrate compounds were utilized for their PCA modeling. One concern lies in the fact that less marker compounds were detected within the ROI and the numbers of descriptors that can be used for their discrimination were consequently limited. To comfort this result and to increase the dimension of data that can be used for PCA modeling, further investigation of the cellulose pyrolysis products, could be considered to check if some characteristic components of the *Ma* group could not be included to increase the number of descriptors in the PCA, these added descriptors could be for example sub-units of the phenylpropanoid monomers in lignin such as the H/G ratio since *Ma* group fibers were revealed to be differentiated considering their lignin content [35, 36]. For the *Moraceae* family, the pattern of the base peak at m/z 218 for a series of terpenic compounds was selected and showed to be a quick and reliable way for their discrimination. As for the gampi and mitsumata, their group location was close indicating their similarities in composition, which may further require employing more compounds in the chromatogram for their representation or the use of a more discriminant method like comprehensive GCxGC as previously proposed [29].

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3.3 Analytical characterization of three traditional East Asian handmade papers: A combined approach using Py-GCxGC/MS and multivariate analysis

3.3.1 The aim of the study

The method using the Py-GC/MS and PCA analysis for different handmade papers were presented in section 3.2. From the result, it can be observed that handmade papers from similar species have closer PCA space locations in the scores plot which indicating its difficulties for their identification. The benefit of the GCxGC analysis has been discussed in section 2.4 and section 2.5, on that base, an analytical method based on the combination of on-line pyrolysis-comprehensive two-dimensional gas chromatography-mass spectrometry and multivariate analysis for a direct characterization of traditional East Asian handmade papers has been conducted. As the feature of the GCxGC separation, more compounds can be separated detected to be used as variables in the PCA analysis.

3.3.2 The research content

The method was applied to three kinds of handmade papers followed by an attempt of identification of ancient lining papers belonging to museum samples of unknown origin. The results indicated that Py-GCxGC/MS, together with multivariate data analysis, was more efficient and reliable than the usual compound presence/absence strategy to meet the challenging search for organic markers in rare cultural heritage materials. The established method showed promises as an efficient analytical tool for the characterization of artwork and culture heritage materials and in filling the lacuna for paper fiber analysis which is currently almost entirely relied on microscopy analysis. Results also revealed that two-dimensional techniques could constitute an efficient approach for the analysis of complex artwork samples to circumvent the limitation of materials at disposal in cultural heritage studies.

The work described above was carried out during the PhD study period, presented at an international congress (Le 12ème congrès francophone des sciences séparatives et des couplages SEP 2017) and was published in Journal of Analytical and Applied Pyrolysis. In order to keep integration of the designed evaluation of Py-GCxGC/MS for cultural heritage

applications (with the example of handmade papers), the corrected proof has been integrated in this section for the demonstration of the work carried out⁷¹.

⁷¹ For more information, refer to <https://www.sciencedirect.com/science/article/pii/S0165237017302747>



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Analytical characterization of East Asian handmade papers: A combined approach using Py-GC/MS and multivariate analysis

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ABSTRACT

An analytical method based on the combination of on-line pyrolysis-comprehensive two-dimensional gas chromatography/mass spectrometry and multivariate analysis for a direct characterization of traditional East Asian handmade papers is presented here. The method was applied to three kinds of widely used handmade papers in East Asia followed by an attempt of identification of ancient lining papers belonging to museum samples of unknown origin. The results indicated that Py-GC/MS, together with multivariate data analysis, was more efficient and reliable compared to the compound presence/absence analysis to meet the challenging search for organic markers in rare cultural heritage materials. The established method showed promises as an efficient analytical tool for the characterization of artwork and culture heritage materials and in filling the lacuna for papermaking fiber analysis which is currently almost entirely relied on microscopy analysis. Results also revealed that two-dimensional techniques could constitute an efficient approach for the analysis of complex artwork samples to circumvent the limitation of materials at disposal in cultural heritage studies.

1. Introduction

Paper, one of the Fourth Great Inventions of ancient China, originates approximately from 2,000 years. Historical reports agreed that paper was invented by a Chinese political official, Cai Lun (ca. 60–121 AD) in the year 105 AD. Nevertheless, new archaeological findings have demonstrated that papermaking may date back to approximately 200 BC [1]. Papermaking technology firstly spread from China throughout Asia and propagated to Islamic world and Europe through the Silk Road. Since then, paper became the prime medium for the mass transition of idea and memory into notation and later paved way for the typographical revolution spurred by printing press [2].

Until nowadays, microscopic observation of the fibrous materials used during the handcraft making process was the main technique allowing the identification, authentication and determination of provenance for historical and archaeological East Asian handmade papers. In microscopic analysis, fiber identification relies on the careful observation of the fiber morphology to identify the material origins and the pulping processes. Nevertheless, microscopy observation is sometimes insufficient to assess precisely the nature of the fibers: in particular, the use and consultation of reference materials reveals themselves as a

necessity for a proper identification of the raw material [3,4]. Moreover, the identification of fibers by optical microscopy necessitates a good experience in fiber analysis for the experimentalist. A main drawback of this technique concerns the possibility for some plants to produce more than one type of fibers, which requires further investigation to characterize the differences shown by varieties of the same species [5,6]. Even in that cases, however, the omission of certain plants in the analysis may induce misleading interpretations of the analyzed samples [5,7,8]. Consequently, we consider that the development of an experimental method based on the chemical analysis of fiber constituents deserves further investigation for the identification of traditional, historical and archaeological East Asian handmade papers.

It is commonly admitted that the application of on-line pyrolysis as a routine analytical method date back from the second half of the 20th century [9]. Since then, pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) has been widely used for the characterization of organic materials used in artworks and archaeological samples: e.g. resins and gums [10,11], Asian lacquer [12,13], or varnishes [14,15]. Although analytical pyrolysis profiles on cellulosic materials have been established previously [16,17], studies concerning East Asian handmade papers have not been reported except for the Py-GC

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characterization of the likely mucilaginous compounds present in traditional East Asian handmade paper [18] and our recent investigation on the characterization of kozo, mitsumata and gampi handmade papers by Py-GC/MS [19].

If, in the past, the introduction of on-line pyrolysis permitted to enlarge the scope of analysis of polymers and natural materials [9,20], the identification of molecular constituents within complex organic mixtures or with low quantity samples at disposal may typically not be achieved after a one dimension chromatographic separation. The introduction and development of comprehensive two-dimensional gas chromatography (GCxGC) offered greatly enhanced resolution for identification of organic analytes in complex mixtures compared to one-dimensional separation techniques [21–23]. As a consequence, the enhanced separation power and ability to gather information of GCxGC/MS system has rapidly attracted the attention of analysts from different scientific fields [24,25]. However, few application was reported in cultural heritage studies where two-dimensional techniques could constitute an efficient approach for the analysis of complex artwork samples to circumvent the limitation of materials at disposal in cultural heritage studies.

The specificity in the art of traditional East Asian papermaking resides in the rational choice of fiber plants adequate to obtain the required quality for the final paper sheets. This specificity constitutes an opportunity to chronologically and geographically locate the origin of samples if a thorough identification of plant components is available. Noteworthy, phytochemistry studies have demonstrated that different plant species may present specificities in their bio-synthesis pathways for certain low molecular weight metabolites, which provides the foundation to establish new criteria for their characterization [19]. However, similarities in the structure of these potential markers render tedious identification, especially for species having close botanical relations. The coupling of pyrolysis with GCxGC/MS (Py-GCxGC/MS) was recently demonstrated to enlarge potentialities for the analysis of fibrous materials like cellulosic materials, increasing sensitivity and resolution for the analysis of samples at disposal in minute amounts, a current situation in cultural heritage studies [26]. Compared to conventional Py-GC/MS, the information contained in a two-dimensional chromatogram is significantly larger, consequently, this increased amount of information favorably benefits the use of multivariate analysis for the interpretation of data. As such, multivariate analysis facilitated the comparison of complex chromatograms, and principal components analysis (PCA) is currently a widely used multivariate approach in combination with comprehensive two-dimensional analysis [27,28]. As an example, application for the investigation of materials from different plant origins had been illustrated in food chemistry [29–31].

In the aim of gaining a thorough knowledge on the provenance and the making technique of East Asian handmade papers, the present study explored the benefits of using Py-GC × GC/MS combined with multivariate analysis for their characterization. Three traditional Japanese handmade papers widely used in East Asia were investigated and their characterization was refined through the identification of their triterpene and phytosterol plant markers. The improved analytical fingerprints of these papers were then employed for the identification of three lining papers of unknown origin in the course of the restoration of three paper paintings belonging to the Musée national des arts asiatiques–Guimet (Paris). Such approach based on the application of Py-GC × GC/MS associated to multivariate analysis is for the first time explored and proposed in cultural heritage studies and is a continuation of our previous study initiated on the Py-GC × GC/MS characterization of traditional handmade kozo paper [26].

2. Materials and methods

2.1. Materials

Materials used in the present study consisted of three types of handmade papers made from kozo (*Broussonetia kazinoki* Sieb.), mitsumata (*Edgeworthia papyrifera* Sieb. et Zucc.) and gampi (*Wikstroemia sikokiana* Franch. et Sav.). These modern reference papers were prepared following the Japanese traditional processes. Three ancient samples of lining papers were employed in this study. All these lining papers were taken from paintings belonging to the Musée national des arts asiatiques–Guimet (Paris). These original artworks are dated from the first half of the Five Dynasties (907–960 AD) and are originating from the library cave in Mogao-ku, Dunhuang, China. Sampling on these original artworks has been conducted in the course of their restoration. A first sample of a lining paper was removed from a paper painting showing “Two Bouddha and a Bodhisattva” (reference No. EO3642, refer to sample A in the present work). The second sample of a lining paper was removed from a painting showing a “Bodhisattva Ksitigarbha” (sample EO17656, refer to sample B). A third sample of lining paper was removed from a “mandala of Vajrasattva” (sample EO1167, refer to sample C). All these samples are of unknown origin and may date from the collection of the paper paintings in the Musée national des arts asiatiques–Guimet at the beginning of the twentieth century. One set of kozo (*Broussonetia kazinoki* Sieb.) reference paper was artificially aged by thermal aging for 3 weeks at 80 °C and 60% relative humidity and gamma irradiation at 390 kGy using the methods described elsewhere [32,33].

2.2. Py-GCxGC/MS analysis

Analysis was conducted with a Shimadzu QP 2010-Ultra gas chromatograph mass spectrometer (Shimadzu, Champs-sur-Marne, France) equipped with a two-stage thermal modulator ZX 2 (Zoex, Houston, USA). Pyrolysis was performed using a vertical micro-furnace-type pyrolyzer PY-3030iD (Frontier Lab, Fukushima, Japan) directly connected to the injection port of the gas chromatograph. The sample was placed in a stainless steel sample cup. Typical quantities of sample paper used in this work were ca. 40–50 µg weighted with an XP2U Ultra Micro Balance (Mettler Toledo, Viroflay, France). The pyrolysis temperature was defined to be 500 °C. The pyrolyzer interface was held at 320 °C. An OPTIMA-5HT column (30m × 0.25 mm I.D., 0.25 µm film thickness, Macherey-Nagel, Hoerd, France) was used as first dimension column and a Zebron ZB-50 (2.8m × 0.1 mm I.D., 0.1 µm film thickness, Phenomenex, Le Pecq, France) was used as a second-dimension column and for the loop modulator system. The separation was carried out at a constant pressure of 300 kPa using Helium Alphagaz 1 as carrier gas (Air Liquide, Bagneux, France). The ZX 2 two-stage thermal modulator was utilized with a modulation period of 10 s with a programmed hot pulse of 0.350 s. A two-step temperature program was used for the hot jet set at 200 °C for 30 min and subsequently raised to 280 °C. The oven temperature was initially held 1 min at 100 °C, and then ramped at 2 °C min^{−1} to 325 °C, where it was held for 25 min. The injector was held at 280 °C and used in split mode (1:30 of the total flow). The mass spectrometer was operated at 20,000 u s^{−1}, with a scan range from 50 to 500 u, using electron ionization at 70 eV. The interface was kept at 280 °C and the ion source at 200 °C. All analysis have been conducted in triplicate except for the ancient lining papers from the Musée national des arts asiatiques–Guimet (Paris) which were sampled once due to the limitation of quantity of samples. Data processing of the Py-GCxGC/MS raw data was achieved using GC Image software, version 2.4 (Lincoln, NE, USA). Identification of pyrolysis products was performed by comparing the mass spectra of unknown components with the NIST mass spectra library (2011 edition) and by interpretation of the main fragmentations.

2.3. Principal component analysis

Twelve pyrolysis experimental data sets of the reference samples were submitted to principal component analysis (PCA) modeling. Then the three ancient museum samples were introduced in the PCA modeling for their identification. Each analysis was considered as an individual sample in PCA analysis and the normalized volumes of 72 peaks reported below within 92 min–120 min in the first dimension retention time (1t_R) and 4 s–10 s in the second dimension retention time (2t_R) in the chromatograms acted as variables. The volumes consist in the integration of modulated peaks recorded in the raw data chromatogram. Reconstruction of these modulated peaks in the 2D chromatograms corresponds consequently to the total response of each pyrolysis products in the total ion chromatogram. The corresponding volumes of the detected peaks were calculated using the GC IMAGE software, version 2.4 (Lincoln, NE, USA) before exportation to Excel for normalization to compensate for the fluctuations of sensitivity range of the instrument. The PCA analyses were performed using the Unscrambler X (Version 10.4) software from CAMO Software AS. In the process of PCA modeling, data was centered and standardization was performed to give each variable the same weight.

3. Results and discussions

3.1. Analytical profiles of kozo, mitsumata and gampi handmade papers

Previous studies demonstrated how Py-GC/MS permitted to define new criteria for discriminating plant species used for traditional East Asian handmade papers [19]. The one-dimensional (1D) chromatograms obtained from Py-GC/MS analysis could be divided into three parts according to the retention time of eluting components, which consisted of cellulose degradation products, hydrocarbons/fatty acid derivatives and the plant fiber markers [19,26]. The two-dimensional (2D) chromatogram revealed similar but more nicely distinguished patterns. In the following, only the region of plant markers that have distinguishable compounds will be studied and this region is defined as region of interest (ROI).

Fig. 1 depicted the compound distribution within the ROI of the reference paper samples of kozo, mitsumata and gampi where plant markers eluted in the 2D analysis (for assignment of the compounds, refer to Table 1). Careful mass spectrum analysis demonstrated that compounds detected in the ROI of the chromatograms were mainly triterpenes in the case of the kozo reference paper (Fig. 1A) while those issued from the mitsumata and gampi reference papers were mainly phytosterols (Fig. 1B and Fig. 1C). With no surprise, such markers are detected from the pyrolysis of traditional handmade papers since triterpenoids and phytosterols are compounds expected from the metabolism of plants [34,35]. These compounds were shown to be stable through diagenesis [36] and, as an example, triterpenes were reported as the most abundant compounds in sediment extracts and often proposed to explain the generation of aromatic biomarkers [37]. Numerous investigations on diterpenoids and triterpenoids from fossil wood or extant plants are available in the literature and have shown all the interest presented by studies on relatively ancient organic matter derived from land plants [38–40]. Nevertheless, a difficulty in the characterization of plant marker compounds in artwork materials lays in the unequivocal identification of biomarkers: a difficult condition to achieve due to a lack of substances of reference, reference mass spectra, and retention index data of unknowns [13].

Studies on the triterpenes synthesis in plants showed that hundreds of stereoisomers are potentially expected for both α - and β -amyrin structures [41]. Furthermore, pyrolysis process, by definition, is able to increase the number of products due to the complexity of the thermal degradation mechanisms. The plethora of compounds observed during the identification step of reference paper characterization (Table 1) can be rationalized by this chemical complexity. Indeed, the 1D general

plant marker fingerprint provides two class of compounds: (i) those initially present in the cellulose material which can be extracted by liquid extraction and which are not modified by pyrolysis, among those let's quote the amyirin structures and the phytosterol structures, α - and β -amyrin, α - and β -amyrin acetate, β -sitosterol and stigmasta-3,5-dien-7-one; (ii) those which are generated by pyrolysis and lead to unsaturated structures or dehydrated structures. The combination of these two categories of compounds constitutes the observed fingerprint of plant fibers employed in the traditional handmade papers. The fingerprint of kozo reference paper was mainly constituted of oleanane and ursane structures identified by their main fragmentation pathways and comparison with NIST library [19,42]. Plant markers for the mitsumata and gampi reference papers were mainly phytosterols (Table 1). As a matter of fact, the pyrolysis behavior of gampi and mitsumata reference papers appeared less diversified than the kozo papers and resulted in a lower amount of pyrolysis products.

The analytical profiles of kozo, mitsumata and gampi handmade papers were established after analysis of the ROI. Compared to the previously developed 1D analytical protocol [19], the use of Py-GC/MS demonstrated an improved resolution and a net gain in sensitivity since the number of detected compounds in the ROI was noticeably increased by a factor of ca. 6 in the example of kozo reference sample [26]. The number of detected compounds in the analytical profiles were 46, 35 and 29 for kozo, mitsumata and gampi reference papers, respectively (see Fig. 1 and Table 1). This number illustrated a total increase of 2–3 times for the number of total compounds detected compared to the 1D profiles [19,26]. If the developed Py-GC/MS protocol revealed as well suited for the differentiation of fibrous and polymeric cultural heritage materials manufactured from different plant families, it could reveal some limitations to distinguish raw papermaking materials which have close taxonomic relationship. The two investigated fiber plants from gampi and mitsumata reference papers are belonging to the same plant family, namely *Thymelaeaceae*, and one may conceive as likely hypothesis that certain plants that have close taxonomic relationship may have similar synthetic pathway which results in similarity in primary metabolites. That is, separating these metabolites by 1D GC is not always easy, especially with regard to the close structural relationship between some tetracyclic structures and their low concentration in samples [43]. Indeed, one limitations of the Py-GC/MS analytical protocol has appeared here since it was not possible to differentiate well mitsumata papers from gampi papers. Liquid extraction and GC/MS analysis has permitted this differentiation [19]. But, unfortunately, the liquid extraction consumed ca. 1000 times the mass of sample compared to pyrolysis protocol which is unrealistic in cultural heritage studies where in most cases micro-destructive analysis are required.

It is also worthy of interest to notice that phytosterol compounds were detected during the Py-GC/MS analysis of the kozo reference paper besides ursane and oleanane compounds, while these compounds were not considered during the Py-GC/MS analysis since their corresponding signals were too low to be detected from the background under similar conditions, i.e. less than 50 μ g of samples, or co-eluting with other compounds. Concomitantly, more compounds were revealed in the mitsumata profile than in the gampi profile, and were likely to be used for their discrimination (e.g. compounds 6, 26, 34, 41, 43 in Table 1). One must emphasize that all these pyrolysis compounds were formed reproducibly for the reference papers, and hence can be used as potential markers for their differentiation. From a strict chemotaxonomical sense, the common members of terpenic classes such as α , β -amyrin and α , β -amyrin acetate may be of less taxonomic value without restricted occurrence in apparently related groups of plants. Meanwhile, it has appeared that the distribution of the selected primary metabolites (phytosterols, triterpenes) were strongly indicative as biomarkers among the materials commonly preserved in the fiber materials used for East Asian hand papermaking as will be described in the following part.

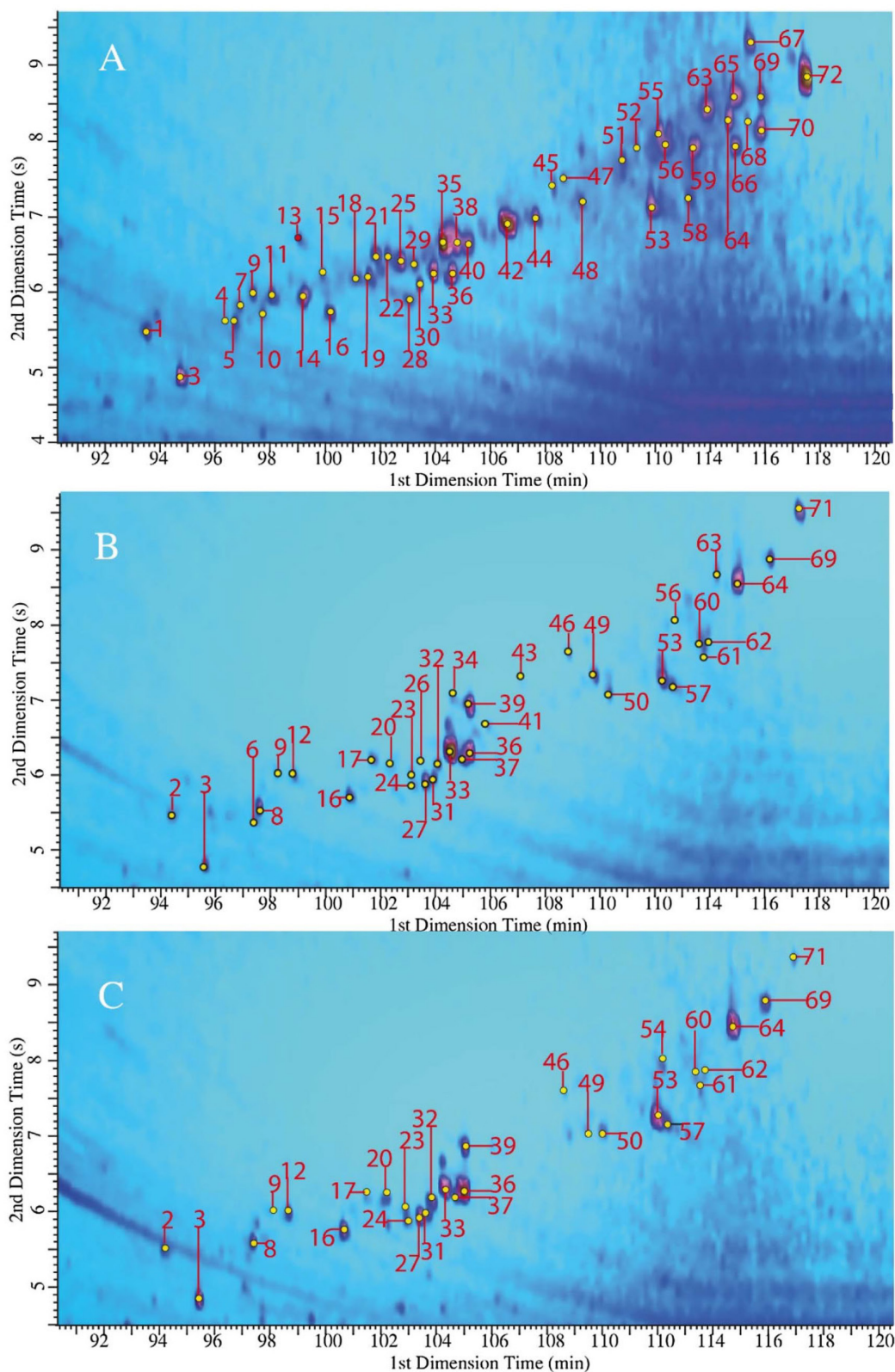


Fig. 1. Compound assignment within the ROI of the reference paper samples. (A–C) report the assignment of plant markers of kozo, mitsumata and gampi reference papers, respectively.

Despite the difficulties associated with the identification of plant markers, the comparison from sample to sample was rendered easier by the use of 2D chromatogram templates which consists in the integration

of marker peaks associated to their mass spectral identification through metadata collections (1t_R , 2t_R and base peak etc.) [26]. This approach was used to process the data of the reference paper samples and for

Table 1

List of plant marker compounds detected within the ROI in the 2D chromatogram of the Py-GC/MS analysis of the kozo, mitsumata and gampi reference paper samples with their respective retention times (t_R , t_R), assigned structures with molecular weight (MW), main peaks in the corresponding mass spectrum, formula and presence in the analytical profiles.

NO.	RI		identification				presence/absence					
	t_R	t_R	MW	m/z	formula	identification	kozo	Mitsumata	Gampi	sample A	sample B	sample C
1	93,83	5,5	368	145, 81, 55, 93	C ₂₇ H ₄₄	NA ^a cholestane	×			×	×	×
2	93,83	5,5	368	145, 81, 121, 107	C ₂₇ H ₄₄	NA cholestane		×	×			
3	95	4,9	410	69, 81, 95, 70	C ₃₀ H ₅₀	squalene	×	×	×	×	×	×
4	96,67	5,65	370	91, 117, 162, 316	C ₂₇ H ₄₆	cholest-2-ene or cholest-3-ene	×			×	×	
5	97	5,6	400	133, 81, 107, 145	C ₂₈ H ₄₈ O	campesterol	×			×	×	
6	97	5,4	384	384, 148, 369, 342	C ₂₇ H ₄₄ O	cholesten-one		×				×
7	97,17	5,8	–	105, 55, 255, 368	–	NA cholestane	×			×	×	×
8	97,17	5,6	382	133, 81, 382, 241	–	NA cholestane		×	×			
9	97,67	6	384	135, 81, 366, 119	C ₂₇ H ₄₄ O	cholesta-4,6-dien-3-ol,(3β)-	×	×		×	×	
10	98	5,7	408	175, 135, 365, 81	C ₃₀ H ₄₈	A-neooleana-3(5),12-diene	×					
11	98,33	5,95	368	147, 81, 105, 368	C ₂₇ H ₄₄	cholesta-3,5-diene isomer	×			×	×	×
12	98,5	5,95	368	147, 105, 81, 107	C ₂₇ H ₄₄	cholesta-3,5-diene		×				
13	99,33	6,7	–	294, 155, 211, 129	–	NA	×			×		×
14	99,5	5,95	408	365, 121, 135, 93	C ₃₀ H ₄₈	A-neooleana-3(5),12-diene isomer	×			×		
15	100,2	6,25	–	406, 255, 133, 135	–	NA	×			×		
16	100,5	5,7	396	145, 81, 121, 57	C ₂₉ H ₄₈	stigmasta-3,5-diene	×	×		×	×	×
17	101,3	6,15	380	135, 143, 96, 55	C ₂₇ H ₄₄ O	cholesta-4,6-dien-3-ol, (3β)-?		×	×			
18	101,5	6,2	–	218, 189, 147, 119	–	NA ursane	×			×	×	×
19	101,8	6,2	–	218, 189, 365, 147	–	NA ursane	×			×	×	×
20	102	6,05	–	253, 158, 55, 57	–	NA cholestane		×	×			
21	102,2	6,5	406	406, 255, 95, 123	C ₃₀ H ₄₆	oleanane-2,9,12-triene	×			×	×	×
22	102,5	6,5	406	406, 255, 95, 123	C ₃₀ H ₄₆	oleanane-2,9,12-triene isomer	×					
23	102,8	6	396	106, 91, 396, 69	–	stigmastane		×	×			×
24	102,8	5,85	398	108, 134, 55, 398	–	cholestane		×	×			
25	103	6,4	408	218, 203, 189, 95	C ₃₀ H ₄₈	oleanane-2,12-diene	×			×	×	
26	103,2	6,15	–	135, 149, 57, 69	–	NA stigmastane		×				
27	103,3	5,9	398	215, 105, 81, 398	C ₂₉ H ₅₀	stigmast-3-ene		×	×			
28	103,3	5,9	398	215, 55, 135, 91	–	NA cholestan	×			×	×	×
29	103,5	6,35	408	218, 203, 189, 79	–	NA oleanane	×					
30	103,7	6,1	414	105, 57, 145, 396	C ₂₉ H ₅₀ O	NA stigmastan	×					×
31	103,7	5,95	398	81, 215, 108, 398	–	stigmast-8(14)-ene		×	×			
32	103,8	6,1	414	105, 57, 71, 396	C ₂₉ H ₅₀ O	stigmast-4-en-3-ol		×	×			
33	104,2	6,25	412	135, 143, 394, 81	C ₂₉ H ₄₈ O	stigmasta-4,6-dien-3-ol	×	×	×	×	×	×
34	104,3	6,9	–	141, 142, 128, 237	–	NA		×				
35	104,5	6,65	408	218, 189, 107, 95	C ₃₀ H ₄₈	urs-2,12-diene	×			×	×	
36	104,8	6,25	414	147, 81, 396, 145	C ₂₉ H ₅₀ O	stigmast-3-ol	×		×	×	×	×
37	104,8	6,2	396	145, 81, 396, 275	C ₂₉ H ₄₈	stigmasta-3,5-diene (isomer)		×	×			
38	105	6,6	408	218, 189, 95, 81	C ₃₀ H ₄₈	NA ursane	×			×	×	
39	105	6,8	378	141, 142, 128, 378	C ₂₇ H ₃₈ O	NA		×	×			×
40	105,3	6,65	408	218, 203, 189, 95	C ₃₀ H ₄₈	NA oleanane	×			×	×	×
41	105,7	6,55	392	251, 197, 392, 155	–	NA		×				
42	106,8	6,9	408	218, 189, 95, 107	C ₃₀ H ₄₈	NA oleanane or ursane	×			×	×	×
43	107	7,05	–	195, 378, 183, 141	–	NA		×				
44	107,8	7	408	218, 109, 189, 95	–	NA oleanane or ursane	×			×	×	×
45	108,5	7,35	422	135, 107, 93, 273	C ₃₀ H ₄₆ O	Ursa-9(11),12-dien-3-one	×			×		
46	108,7	7,45	376	193, 235, 376, 141	–	NA		×	×			
47	108,8	7,5	422	174, 161, 187, 382	–	NA	×			×	×	×
48	109,5	7,2	–	155, 143, 107, 55	–	NA	×					
49	109,7	7,15	392	155, 142, 251, 392	C ₂₈ H ₄₀ O	NA		×	×			
50	110,2	6,95	412	136, 95, 412, 79	C ₂₉ H ₄₈ O	stigmasta-5,6-diene-3-ol		×	×			
51	111	7,75	–	135, 232, 95, 55	–	NA	×			×		
52	111,5	7,95	422	422, 133, 208, 107	C ₃₀ H ₄₆ O	NA	×			×		×
53	112	7,15	414	57, 55, 81, 95	C ₂₉ H ₅₀ O	β sitosterol	×	×		×	×	×
54	112,2	7,8	396	174, 161, 396, 105	C ₂₈ H ₄₆ O	NA stigmastane			×			
55	112,3	8,1	440	135, 273, 407, 95	C ₃₀ H ₄₈ O ₂	oleanane-12-ene-11-one-3-ol	×			×		×
56	112,5	7,95	424	218, 203, 95, 55	C ₃₀ H ₅₀ O	oleanane-12-en-3-one	×	×		×		×
57	112,5	7,05	416	57, 81, 95, 107	C ₂₉ H ₅₀ O	stigmastanol		×	×			
58	113,3	7,25	466	466, 255, 95, 69	C ₃₂ H ₅₀ O ₂	13,27-cycloursan-11-en-3-ol,acetate	×			×		×
59	113,5	7,9	426	218, 203, 189, 119	C ₃₀ H ₅₀ O	beta amyryn (Olean-12-en-3-ol)	×			×	×	
60	113,5	7,65	412	123, 136, 121, 79	–	NA		×	×			×
61	113,7	7,5	414	231, 55, 414, 69	–	NA stigmastane		×	×			
62	113,8	7,7	412	176, 412, 207, 136	–	NA stigmastane		×	×			
63	114	8,45	424	218, 95, 109, 55	C ₃₀ H ₄₈ O	urs-12-en-3-one	×	×		×	×	
64	114,8	8,3	410	174, 161, 410, 187	C ₂₉ H ₄₆ O	stigmasta-3,5-dien-7-one	×	×	×	×	×	×
65	115	8,6	426	218, 95, 69, 189	C ₃₀ H ₅₀ O	α-amyryn (urs-12-en-3-ol)	×			×	×	
66	115	7,95	466	466, 255, 95, 55	C ₃₂ H ₅₀ O ₂	oleanane-12,14-diene-3-acetate	×			×	×	
67	115,5	9,35	–	135, 232, 95, 55	–	NA	×					
68	115,5	8,3	466	95, 133, 209, 466	C ₃₂ H ₅₀ O ₂	urs-12,14-diene-3-acetate	×			×		
69	116	8,6	412	124, 229, 55, 57	C ₂₉ H ₄₈ O	stigmast-4-ene-3-one	×	×	×	×	×	×
70	116	8,15	468	218, 203, 219, 189	C ₃₂ H ₅₂ O ₂	oleanane-12-ene-3-acetate beta-amyryn acetate	×			×	×	
71	117,2	9,2	410	136, 95, 160, 174	C ₂₉ H ₄₆ O	stigmasta-4,6-dien-3-one		×	×			

(continued on next page)

Table 1 (continued)

NO.	RI		identification				presence/absence					
	1t_R	2t_R	MW	m/z	formula	identification	kozo	Mitsumata	Gampi	sample A	sample B	sample C
72	117,5	8,85	468	218, 189, 95, 135	$C_{32}H_{52}O_2$	α -amyrin acetate (urs-12-ene-3-acetate)	× (×)			×	×	×
total							46(39)	35	29	40	30	29

NA⁺: not attributed. ×: presence in the analytical profile. (×): presence in the artificially aged kozo sample.

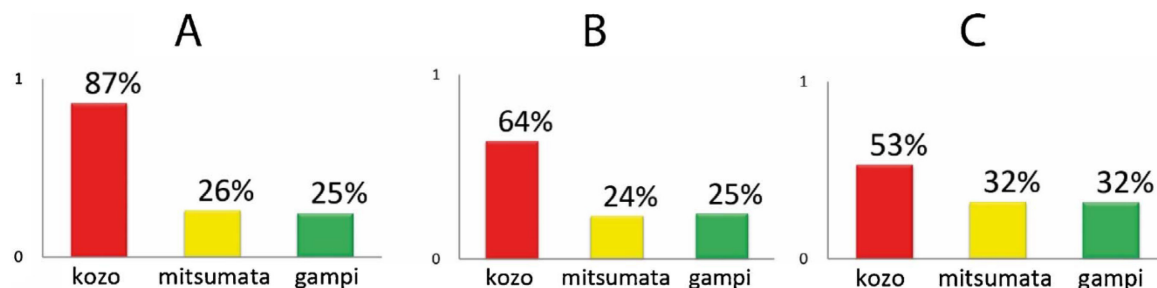


Fig. 2. Pyrolysis profiles of museum samples in the ROI compared to reference papers using presence/absence analysis. (A), sample A compared to reference papers with corresponding matching percentages; (B), sample B compared to reference papers with corresponding matching percentages; (C), sample C compared to reference papers with corresponding matching percentages.

unknown samples of the lining papers from the Musée national des arts asiatiques–Guimet (Paris) for identification and multivariate data analysis.

It was first applied to a commonly used presence/absence analysis which resides in a careful comparison of unknown sample profiles with the reference profiles [14]. As can be seen from Fig. 2, all the identified compounds in sample A and B matched correspondence fingerprints with the kozo reference profile, which accounts for 87% and 64% of the identified compounds in the kozo reference profile, respectively. For sample C, 24 compounds matched with the kozo reference profile, which accounted for 53% of the total profile compounds. With 11 compounds and 9 compounds matching with the mitsumata and gampi reference profiles, the matches were both 32%. With a match factor of nearly 90%, the origin of sample A was likely to be of kozo origin. For sample B and C, it was more difficult to decide since the highest match factor was less than 70%. Obviously, the results of the identification with presence/absence analysis compared with the reference profile only gave clear indication and high confidence for the sample A.

3.2. Multivariate data analysis

Complete quantitative comparison of the pyrograms by creating standard curves for each compound would be an ideal way, but it is time-consuming and is not suitable for a rapid screening method for routine works. Moreover, such approach applied for a strict quantitative purpose is subject to contradictory discussion due to the repeatability of the thermal degradation process itself, which can be influenced by many factors. Furthermore, in a field where micro-destructive analysis is required, it is difficult for multiple sampling or injections to fulfill this purpose. Instead of an unrealistic absolute quantification, we chose to work with the volumes of 72 peaks within ROI acted as variables and normalized by their percentage ratio. Multivariate data analysis, namely principal component analysis (PCA) was utilized to process these data and to examine differences and similarities between Py-GC/MS profiles within the ROI.

The first PCA was performed with all the assigned peaks within ROI of the three reference handmade papers. As is shown in Fig. 3A, the differences of reference profiles were visualized by the PCA scores plot along Principal Component 1 (PC1) and Principal Component 2 (PC2) with 66% and 18% of the total data variance, respectively, indicating that two thirds of the information present in the original data has been

taken into PC1. Fig. 3A depicts how the profile of kozo reference sample, compositionally different from the profiles of mitsumata and gampi, is clearly corroborated by the position along the PC1 axis. The location of mitsumata and gampi samples along PC1 indicate their similarities in their analytical profiles as discussed above. The observed distribution of kozo reference paper compared with gampi and mitsumata reference papers confirmed their different composition. Moreover, considering these three reference papers, one must notice that the mitsumata samples presented a higher variability than the kozo and gampi samples.

The interrelationship of sample groupings or differences in the scores plot was revealed by plotting the loadings. The loadings plot depicts the degree of contributions of different variables to a given PC and the correlation among the explanatory variables in the scores plot. The variable values in the loadings plot proportionally correspond to their relative contribution while variables with loadings smaller than 10% were commonly considered as having little discernible influence on the discrimination. The corresponding loadings plots of PC1 and PC2 were displayed in Fig. 3B and Fig. 3C, respectively. The loadings plot showed more qualitative information that can be used to differentiate profiles. The loadings plots in Fig. 3B and Fig. 3C showed that only a small amount of variables were attributed to the major of the compositional differences. The top three compounds largely attributed to the location of kozo reference samples along the positive PC1 axis on the scores plot were urs-12-ene-3-acetate (72), 42%, oleanane or ursane structure compound (42), 24%, urs-2,12-diene (35), 19% while stigmasta-3,5-dien-7-one (64), –45%, stigmasta-4,6-dien-3-ol (33), –33%, ergosterone structure compound (39), –19% contribute on a large scale to the distribution of mitsumata and gampi on the negative PC1 axis in the scores plot. It is interesting to note that terpenic compounds were characteristic for kozo handmade papers while steroid compounds were explanative for mitsumata and gampi. The observed scores and loadings plot indicate that PC1 is functionally effective to discriminate analytical profiles taking on obvious composition differences, i.e. kozo vs gampi and mitsumata. For the profiles with high similarity in compound presence/absence analysis (gampi and mitsumata), PC2 is further explored. It can be seen from Fig. 3C that stigmasta-4,6-dien-3-ol (33), 75%, urs-12-en-3-one (63), 18%, and stigmasta-4,6-dien-3-one (71), 17% has the larger weight and consequently determine the location of mitsumata samples in the scores plot while the stigmasta-3,5-dien-7-one (64), –39%, stigmasta-3-ol (36), –24%,

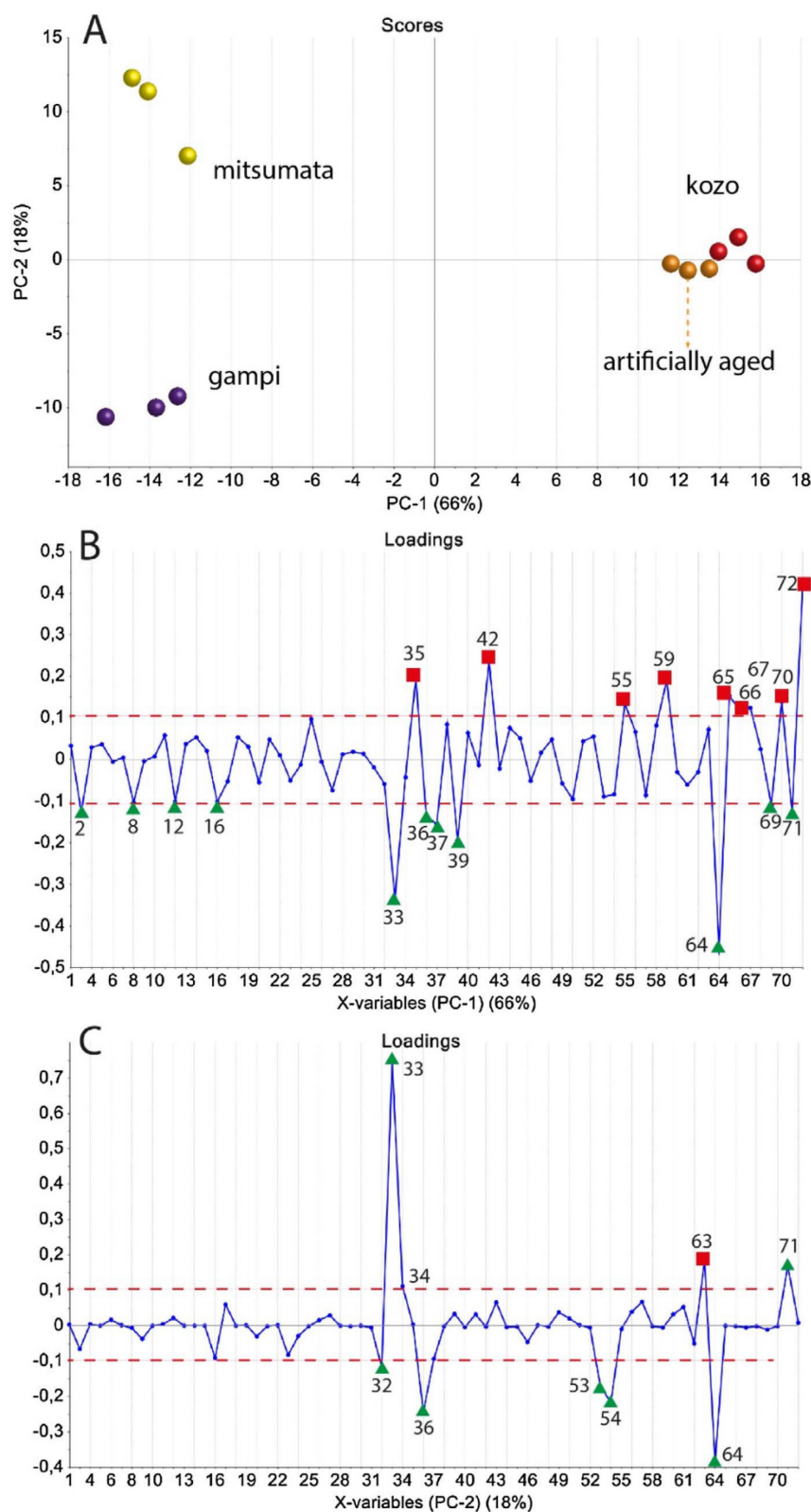


Fig. 3. Score plots (A) and the corresponding loadings plots for PC1 (B) and PC2 (C) of the PCA modeling for reference samples. In (A), orange dots represent artificially aged kozo paper, in B and C blue dots represent each variable, green triangles represent steroid compounds and red rectangles represent terpenic compounds.

and stigmastane structure (54), –22% compounds, are mainly responsible for the location of gampi samples in the scores plot. The smaller percentage of variance brought by PC2 justified that differences between mitsumata and gampi were much smaller than those between

kozo on one side and mitsumata/gampi on the other side. Here, PCA proved to be especially sensitive and powerful in determining influential variables for the discrimination of different analytical profiles, as it depicts differences among samples that are not detectable or

recognizable with the presence/absence analysis.

As stated above, one may notice that the mitsumata reference paper seems not so closely clustered. The explanation can be found from the loadings plot of PC2. From Fig. 3C, it can be seen that three compounds: 33, 63, and 71 contributed largely to the scores plot of mitsumata reference papers where compound 33 took up a loadings value up to 75%. In this case, even small variations of compound 33 may have large influences on mitsumata reference paper's scores plot. Also, it might be true that the range of percentage of compound 33 is widely distributed in the pyrolysis of mitsumata handmade papers. At the opposite, more compounds were attributed to the gampi reference paper's scores plot: compounds 64, –39%; 36, –24%; 54, –22%; 53, –17% without one taking the dominate position. Consequently, for gampi samples, one compound's variation can be buffered by the other compounds in the PCA modeling. Also, Most of the compounds detected in the reference kozo paper were still present after ageing (Table 1). The location of artificially aged kozo paper along the PC1 in Fig. 3 also indicate that the aging process does not have a great influence on the compound distribution and its relative percentage. It has been generally observed in Py-GC/MS experiments that hardening of the ageing conditions of the reference paper samples resulted in a decrease of the total ion current in the chromatograms with no noticeable effect on the global fingerprint of the ROI. Recalculating PCA in introducing the artificially aged kozo paper set in the calculation yielded the score plot reported in Fig. 3.

These results demonstrated that the relative percentage of compounds plays a great role in their discrimination while the presence/absence information is not sufficient for a proper conclusion in the identification process when their profiles present high level of similarities. It is interesting to note that compounds 33 and 64 both have negative loadings in PC1 but have different loadings in PC2 which is favorably used to differentiate mitsumata from gampi reference papers. From the perspective of material origin, *Broussonetia kazinoki* Sieb. (kozo paper) belongs to *Moraceae* family while *Edgeworthia papyrifera* Sieb. et Zucc. (mitsumata paper) and *Wikstroemia sikokiana* Franch. et Sav. (gampi paper) belong to the same *Thymelaeaceae* family. Fig. 3 demonstrated that PC1 can separate compounds derived from different families relaying on the compound distribution while PC2 may discriminate between analytical profiles derived from closely related plant families.

The three unknown samples from the Musée national des arts asiatiques–Guimet (Paris) were introduced after recalculation into the PCA plots. As can be seen from Fig. 4, the location of sample A and B along the PC1 axis confirmed that their assignment were in accordance

with a kozo paper origin. Noteworthy, with the introduction of these unknown samples, and considering that sample A–B fit with a kozo paper origin, one must observe that dispersion of all the paper is similar. Location of these samples in the space defined by the score plot confirmed that characteristics of the unknowns are included in the group defined by the three reference paper samples and the artificially aged kozo paper sample. This result was confirmed by microscopic fiber analysis. The situation encountered for sample C with a 53% match with a kozo reference paper in the presence/absence analysis is more illustrative of the difficulty in identifying mix material origins by microscopic analysis. The location along the PC1 indicate that sample C do not fit with a kozo paper origin and more likely correspond to a mitsumata or a gampi paper. By its location on the score plot (Fig. 4), this sample more likely corresponds to a gampi paper according to its proximity with the gampi reference paper. In addition, due to the poor conservation state of sample C, its microscopic observation can only provide inconclusive proof on its fiber composition where it was only possible to conclude that sample C bear mitsumata and/or gampi features with no clear differentiation. Concretely, Py-GC/MS plus PCA analysis of sample C revealed better fits with gampi reference sample without eliminating the possibility of mixture with other fibers. This ability of distinguishing mixtures of fibers in the analyzed papers needs further investigation.

4. Conclusion

The combination of Py-GC/MS as an analytical method and multivariate analysis as a chemometric tool for data treatment demonstrated its potential for the characterization of reference and ancient East Asian handmade papers. The new analytical approach based on online pyrolysis-comprehensive two-dimensional GC/MS showed its efficiency in improving the fingerprint of paper samples, and revealed its capabilities in distinguishing plant fibers which currently show similarities in morphological features and for this reason, are indiscernible by microscopic analysis as illustrated for the example of mitsumata and gampi papers.

The investigation was carried out with a multivariate data analysis of the pyrolysis results using the refined fingerprint issued from the comprehensive two-dimensional analyses. This approach confirmed the effectiveness of combining a Py-GC/MS with PCA for the differentiation of reference papers, and was extended to the application of museum cases with the characterization of lining papers of unknown origin. Such analytical strategy has been for the first time explored and

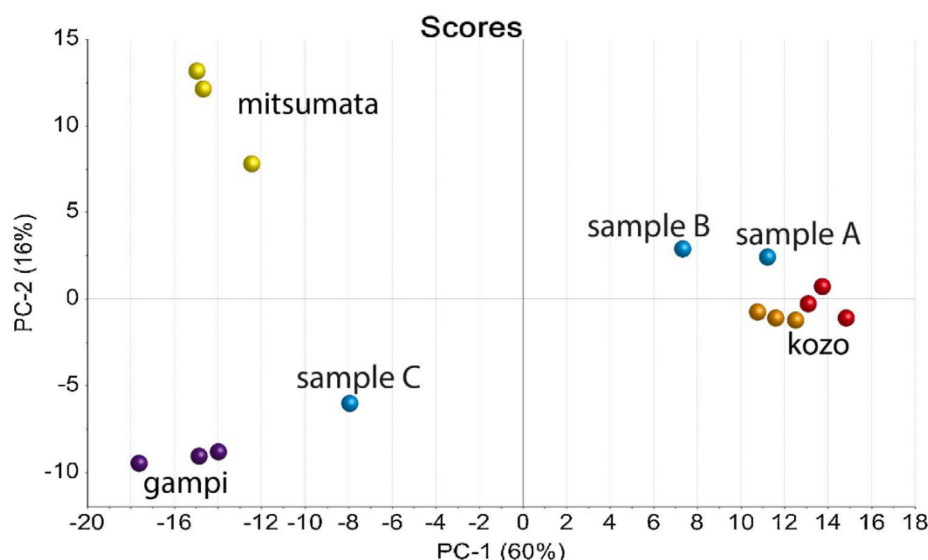


Fig. 4. Score plots of the PCA modeling for reference samples and unknown museum samples. The blue dots represent the museum samples while the orange dots represent artificially aged kozo paper.

proposed in cultural heritage studies. It showed early promise as an efficient analytical tool in the comprehensive analysis of organic markers in rare cultural heritage materials. It demonstrated its effectiveness to provide new proofs to verify the historical records concerning the history of origin and spread of papermaking techniques for East Asian handmade paper studies. Extension to handmade paper samples for other material origins will be further performed and such investigations are the subject of ongoing studies in our laboratory. In addition, the present multivariate analysis in comparing fresh reference paper samples and an artificially aged paper sample of kozo did not provide discrimination in the resulting score plot. It would be interesting in further work to check if ageing presents an influence for the discrimination of close related samples: in particular, it would be interesting to check the ability of higher PC's to separate samples on their ageing scale.

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3.4 A preliminary study among the earliest paper offerings unearthed in China at the Astana Cemeteries

3.4.1 Introduction

Ghost money, as indicated by the name, is the money used in the spirit world when people died. It is a necessity in sacrificial ceremony or at the funerals in ancient China and neighboring regions: a way to show condolence as the traditions. The appearance of ghost money as a funerary object can date back to as early as Shang Dynasty (1600-1046 BC) with the material of shell, jade or bronze. However, with the invention and popularity of paper, ghost money took transformation from real object to virtual offerings (mainly made of paper) during a period from 220 AD to 960 AD (Wei and Jin dynasty, Tang dynasty and Five Dynasties, chronologically). It gradually separates from other funerary objects and made its way into indispensable item for funeral and worship activity offerings, to gods and to ancestors.

Paper offerings, as a cellulosic material, were liable to degradation and environmental erosions in archaeological context which made them difficult to survive and to conserve [13, 14]. However, benefitting from the extremely dry environmental conditions, the ancient tombs located at the Turpan District in Xinjiang, northwestern China, preserve numerous ancient paper-based artifacts. Among these ancient tombs, the Astana Cemetery complex, located on the famous Silk Road (Fig.3.4.1), are particularly well-known for their abundant unearthed mummies, written documents, textiles, plant remains, and food residues [15, 16]. The tomb covers an area of approximately 10 km² and served as a public graveyard for the ancient Gaochang people, dating from about the 3rd to the 9th centuries.

A large number of text documents and paper offerings have been excavated from the Astana Cemetery complex. Previous studies of these materials have been conducted from the perspectives of art, literature and microscopic observation. Although the paper offerings offered a unique insight into culture and technique perspectives, however, until now, seldom comprehensive scientific research has been conducted concerning the aspect to verify botanical source of the paper fibers, to determine any possible symbolic or historical importance associated with the fibers, and to establish their relationship with the plant utilization and spread of papermaking technique.

On the other hand, the art of traditional East Asian papermaking resides in the rational choice of fiber plants adequate to obtain the required quality for the final paper formation: a

fingerprint preserved in ancient manuscript to reveal information on political/society changes, technical advances and culture exchanges [5, 17]. This point constitutes an opportunity to chronologically and geographically locate the origin of manuscript if a reliable analytical method is available.

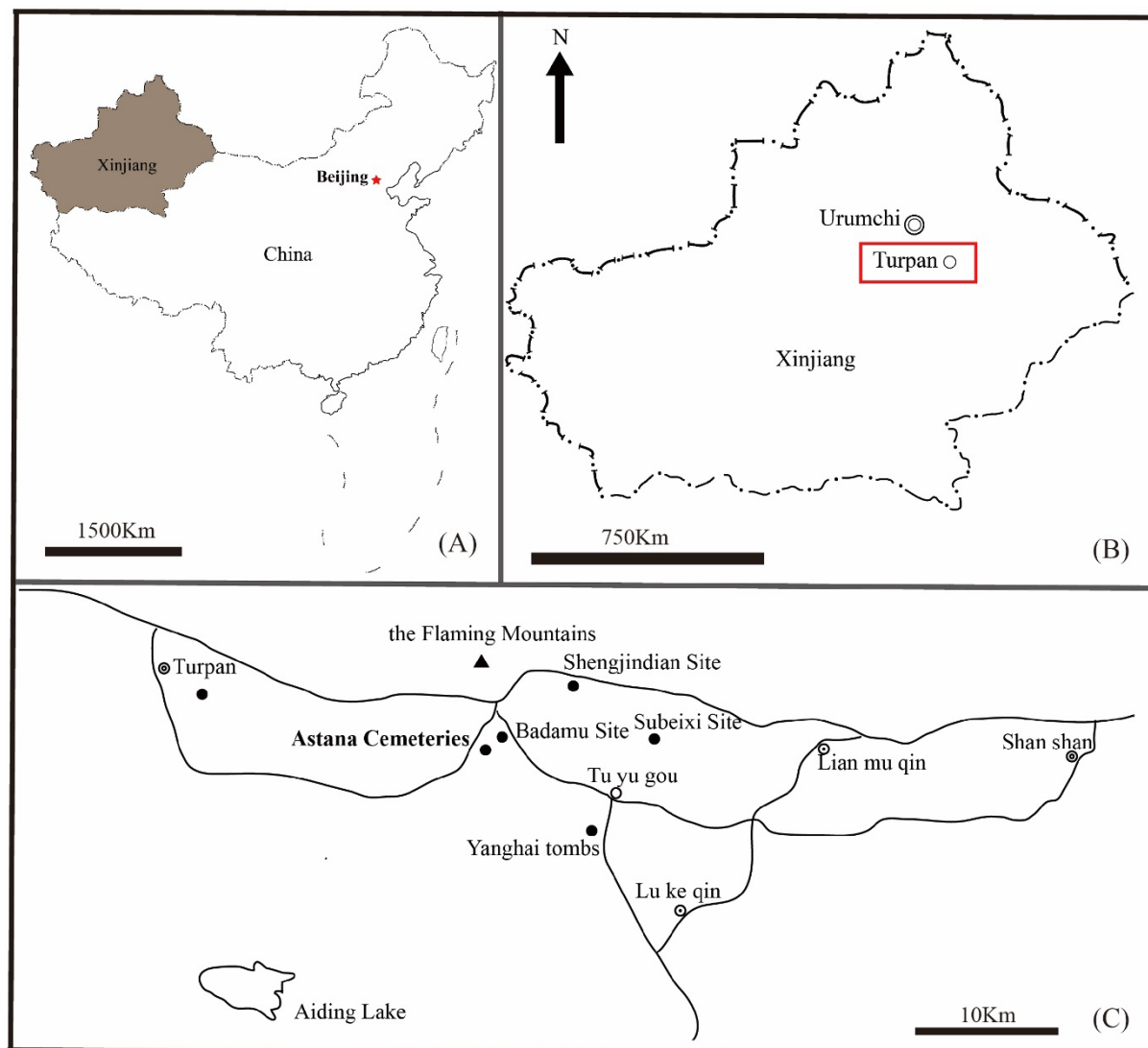


Figure 3.4.1 The map of the location of the Astana Cemeteries, Xinjiang, northwest China. (A) The location of Xinjiang in China. (B) The location of Turpan (the red rectangular area). (C) The location of the Astana Cemeteries.

By means, microscopy observation can only retrace part of the fingerprint information which presents limitations especially in identifying plant fibers that have quite similar morphological features (usually plant within a close taxonomy relationship): a dilemma situation commonly confronted in this domain [18-20]. For instance, during the study of Tibetan manuscript (by Agnieszka Helman-Ważny, University of Hamburg), it was found that fiber analysis allow for clear-cut identification only in a limited number of cases with difficulties in separating the fibers within the *Thymelaeaceae* family. Furthermore, A systematic microscopy study has

revealed that typical characteristic traits (such as cross-section diameter, lumen diameter, dislocations) cannot be taken as unique identification criteria (with the example of flax, nettle and hemp textile fibers) [18]. Although the Z and S twist has been proposed for their identification [21], seldom identification has been reported for handmade paper fiber identification using this method as it may require clear and high resolution picture as well as training to recognize the twist in the pulped fibers.

Noteworthy, phytochemistry studies have demonstrated that different plant species may present specificities in their bio-synthesis pathways for certain low molecular weight metabolites, which provide the foundation to establish new criteria for their characterization which is the theme within this thesis. In this application, an easy protocol combining Py-GC/MS and Py-GCxGC/MS analysis together with microscopic observation for more reliable fiber identification was proposed.

3.4.2 Materials and methods

Remnants of paper offerings unearthed from tombs of 72TAM184, 72TAM501 and 73TAM518 in Astana Cemeteries (Fig.3.4.2) were scientifically examined. Based on previous archaeological research and the written documents with certain chronology in each tomb, the tombs were most likely built in early and prosperous age Tang dynasty (618-755AD) (Table 3.4.1).

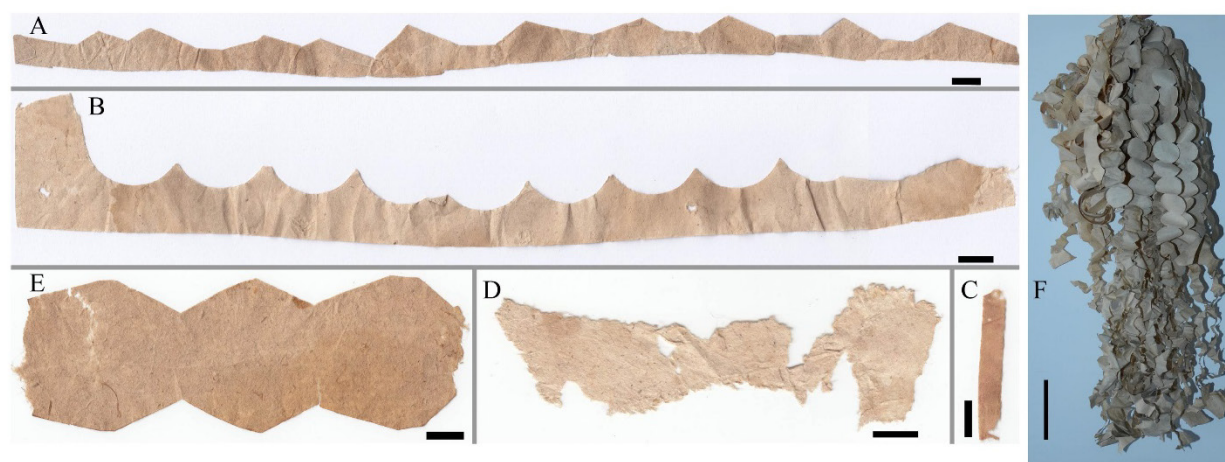


Figure 3.4.2 Remnant of paper offerings from Astana tombs. (A) offcut of paper offerings from the tomb of 72TAM184:5, scale bar = 1cm; (B) offcut of paper offerings from the tomb of 72TAM184:2, scale bar = 1cm; (C) remains of paper offerings from the tomb of 72TAM501:108, scale bar = 1cm; (D) remains of paper offerings from the tomb of 72TAM501:108, scale bar = 1cm; (E) paper offerings from the tomb of 73TAM518:9, scale bar = 1cm; (F) paper offerings from Astana Cemeteries, scale bar = 10cm.

Table 3.4.1 The dating of the tombs

No.	Dating materials	Possible span
72TAM184 (Sample A and B)	Written documents with certain chronology	714AD-724AD
73TAM518 (Sample E)	Written documents with certain chronology	666AD-706AD
72TAM501 (sample C and D)	Written documents with certain chronology	685AD-698AD

The samples were carefully placed on the objective table and then viewed under a KEYENCE VHX-600ESO light microscope at magnification of 20×, 50×, 100× and 200× for surface morphology examination and photographic documentation. Herzberg stain, an iodide-iodine solution mixed with zinc chloride solution is commonly used to identify the non-wood fiber source with considerably few paper samples. The quantity of lignin in the fiber leads to different adsorption of the reagent, which makes the fibers take on different colors. Bark fibers from hemp and ramie are nearly lignin-free and are dyed in red-wine color, with the increase of lignin content, the color of the fibers may orderly take on the color of red-wine, grey-bluish, grey-yellow-greenish, brown-yellowish and yellow. The preparation of Herzberg reagent and staining process had been described elsewhere [5]. The stained fiber samples were observed under a NIKON LV100POL polarized light microscope, at a magnification of 500×. The Py-GC/MS and Py-GCxGC/MS analyses are previously described in chapter 2.

3.4.3 Microscopic observations

Sample B is comparatively thin with distortion. The surface contains unidentified objects and bunches of clustered fibers (Fig.3a, Fig.3b); compared to Sample B, Sample A is thicker and stiffer and has comparatively rough morphology. There are black nubbles, large phloem fiber and clustered fibers on the surface (Fig.3.4.3c~Fig.3.4.3f). Although Sample C and Sample D are from the same tomb, they differ in color, intensity and morphology. Sample C is thin and loose with long fibers and clean surface, while Sample D is similar to Sample A in thickness and morphology with only a little more flexibility (Fig.3.4.3g~Fig.3.4.3l). Sample E (Fig.3.4.3m~ Fig.3.4.3o) also has rough morphology with twisted and clustered fibers and large plant phloem. All the dopants on the paper surface are ‘trapped’ in the paper ‘fiber net’ which rule out the possibility of contamination from the burial complex.

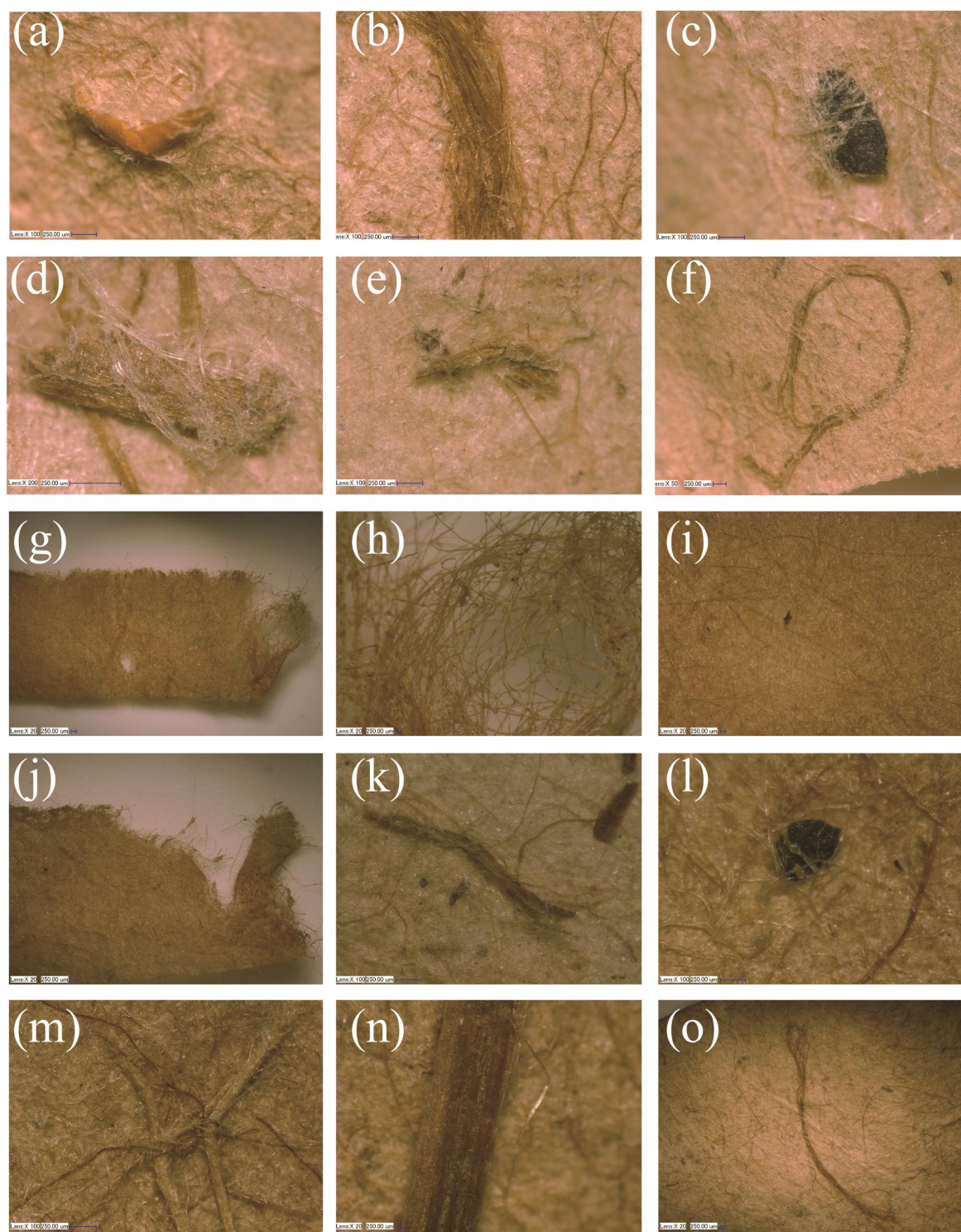


Figure 3.4.3 Micrograph of paper offerings from Astana tombs. 72TAM184:2, (a)~(b); 72TAM184:2, (c)~(f); 72TAM501:108(C), (g)~(l); 72TAM501:108(D), (j)~(l); 73TAM518:9, (m)~(o). (g),(h),(i),(j),(n)and(o) are 20×; (f) is 50×; (a),(b),(c),(e),(k),(l)and(m) are 100×; (d) and (n) are 200×.

Although the main component is cellulose, the microscopic examination is indicative of different fiber sources or manufacturing technique for the testing samples. According to historical records and previous research, most frequently utilization of plant fibers for large scale production of papers through history was: (i) bast fibers such as hemp, ramie, mulberry,

and paper mulberry, (ii) bamboo, and (iii) rice and/or wheat straw. To obtain a detailed information of the plant fibers used, stain and fiber microscopic observation was carried out.

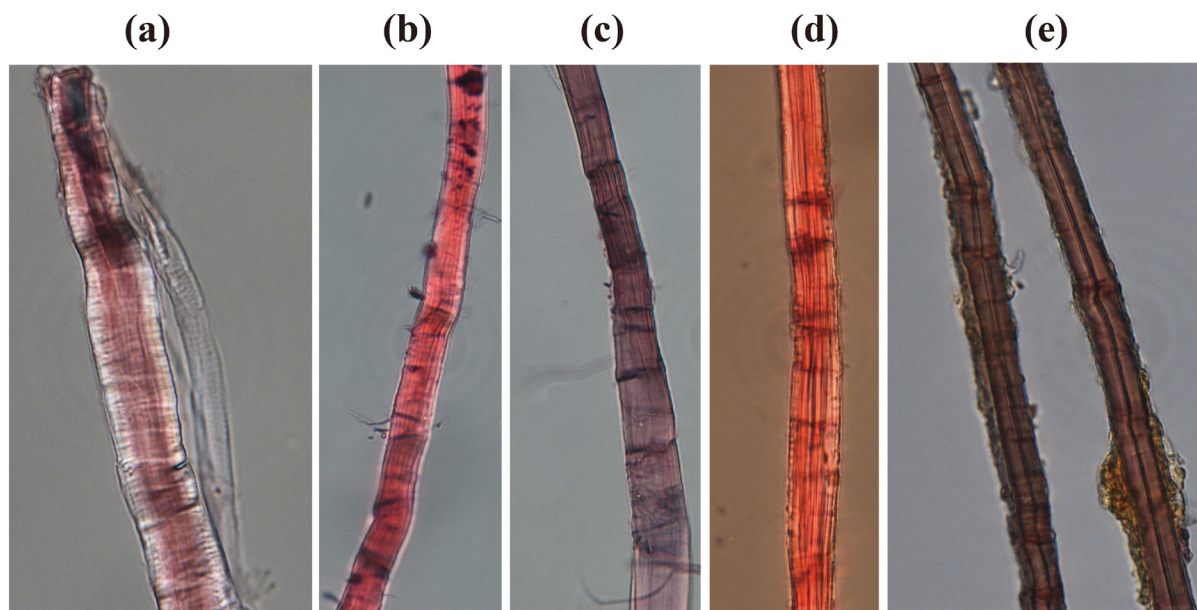


Figure 3.4.4 Microscopic morphological features of the stained fibers from: (a)-72TAM184:5, scale bar=20μm; (b)-72TAM184:2, scale bar=20μm; (c)-73TAM518:9, scale bar=20μm; (d)-72TAM501:108(D) , scale bar=20μm; (e)- 72TAM501:108(C) , scale bar=20μm.

Fig.3.4.4 displays morphological features of stained fibers from the investigated paper offerings of Astana cemeteries. Lignin, a complex phenolic polymer plays a decisive role in the presence of fiber color when stained by Herzberg reagent. Previous studies in fiber identification has examined the content of lignin changing from significantly low (0–5% of dry weight in hemp, ramie, and cotton) to low (8–15% of dry weight in mulberry and paper mulberry trees) to low-to-moderate (15–20% of dry weight in rice and wheat straw) to high (25–30% of dry weight in bamboo), may make the fiber take on the color of wine red, pink, blue, blue-grey, and yellow, accordingly [5]. The color of the stained fibers from the paper offerings varies from wine red to pink (Fig.3.4.4a to Fig.3.4.4f), parenchymal cells or coarse serrated cells which are usually seen in bamboo, rice, and wheat straw were not observed to rule out bamboo, rice or wheat straw as fiber source. The fiber characteristic features were suggestive of fiber sources coming from bast fibers. No chemical reaction between iodine solution and starch grains from sizing was noticed in the process of fiber staining, indicating the absence of starch sizing treatment.

As for the color of the fibers, all fibers show a wine-red color and/or pink when stained by Hertzberg reagent. Fiber in sample A (Fig.3.4.4a) and sample E (Fig.3.4.4c) have generally a variable thickness along its length, with cross striations at a certain interval forming the nodes, a shape characteristic of bast fibers such as hemp, ramie, mulberry and paper mulberry.

Longitudinal striations, a diagnostic feature for hemp and ramie fibers, are recognizable, though they had been worn probably due to the pulping process. Fibrillation of fibers is obvious, which is usually noticeable in ramie pulp. This suggests a fiber source from ramie. Fiber in sample B (Fig.3.4.4b) and Sample D (Fig.3.4.4d) resemble fibers in Sample E (Fig.3.4.4d) with the cross striations and longitudinal striations, but they are of comparatively constant thickness along its length. From the microscopic observation, we are inclined to believe that hemp is a possible source for fibers in Sample B and Sample D.

Fiber in Sample C (Fig.3.4.4e and Fig.3.4.4f) is of a variable thickness with pointed ends and surrounded by the cuticle, a transparent envelope wrapping around the fiber surface (a characteristic feature for mulberry and paper mulberry). Well-pronounced cross striations also occur at frequent intervals and form nodes. Wax-like clusters (yellow colored when stained with Hertzberg reagent, a feature of mulberry fiber as observed by other scholars) on the surface of the fibers is obvious, and also calcium oxalate crystals of irregular shape and size are scarcely observed. All these agree well with the characteristics of mulberry fibers, implying that the bast fibers of mulberry trees be the fiber source in Sample C (for the digital microscopic observation and Graff C stain observation, refer to Annex 47). The results of fiber identification were summarized in Table 2.

Table 3.4.2 Results of fiber identification by Hertzberg staining method

sample	Stained color	Possible fiber sources
Sample A	wine red	Ramie
Sample B	wine red	Hemp
Sample E	wine red	Ramie
Sample C	pink	Mulberry trees
Sample D	wine red	Hemp

Simulation experiment and observations have established the relevance between fracture morphology, the swell of the fiber (into a ball shape), fibrillation and the cutting, ramming and/or pulping technique in the process of papermaking. The process of cutting might cause comparative straight fracture, while ramming and/or pulping left the trace of swelling fibers, degrees of fibrillation. Traces of fibrillation have been observed in all samples, indicating a process of manual ramming or pulping (in Fig.3.4.5). The way of cutting is also partly seen in fig.6a and fig.6f. It is noted that the fibrillation of fibers in sample 72TAM501:108(C) is comparatively low, which is an inconspicuous characteristic of bark fibers from mulberry trees.

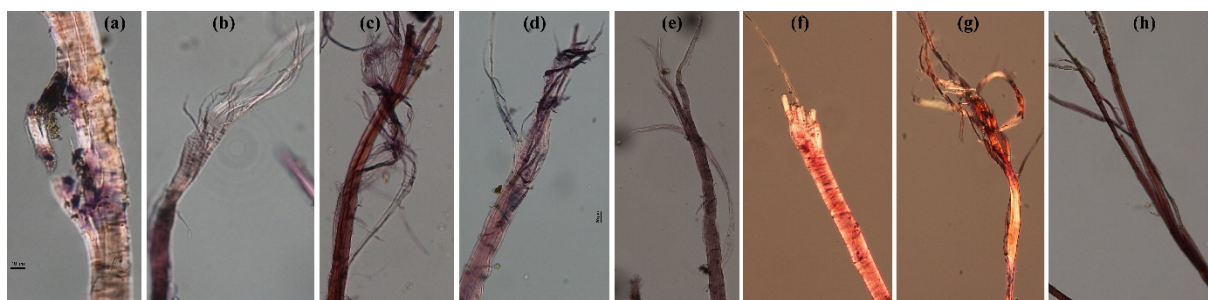


Figure 3.4.5 Microscopic morphological features of the stained fibers from: (a),(b)-72TAM184:5, scale bar = 20μm; (c)-72TAM184:2, scale bar = 20μm; (d),(e)-73TAM518:9, scale bar = 20μm; (f),(g)-72TAM501:108(D) , scale bar = 20μm; (h)- 72TAM501:108(C).

3.4.4 Analysis by Py-GC/MS

In order to have detailed information of the sample and to further verify the microscopic identification result, a Py-GC/MS analysis has been carried out. But low level of marker peaks (only C showed a higher S/N for the plant markers) was observed. After integration of sample C, the compounds reported in Table 3 are detected, but with uncertainty on their structures.

Considering the presence/absence criteria when comparing the detected compounds with those of other reference paper samples (kozo, gampi, mitsumata, morus alba, morus mongolica, seitan etc.), two groups of samples could be tentatively deduced: samples A & B on one side, samples C, D, E on the other side. The latter group presented more similarity in term of concordance of detected compounds with *Broussonetia* or *Morus* plants, but this information is not deduced from the fingerprint of the plants since the main markers are missing. Moreover, this observation is in opposition to the results of microscopy analysis.

Under these circumstances, if the chromatograms confirmed the presence of cellulose (Fig.3.4.6), and an intermediate region with hydrocarbons structures, one must note the lack of plant markers with characteristic structures for unexplained reason, only cholestane based structures are detected with a low signal to noise ratio (Fig.3.4.7). Referring to the previous investigation of reference paper samples (section 3,2), the present situation could be linked with the low level of characteristic structures observed with hemp and ramie papers.

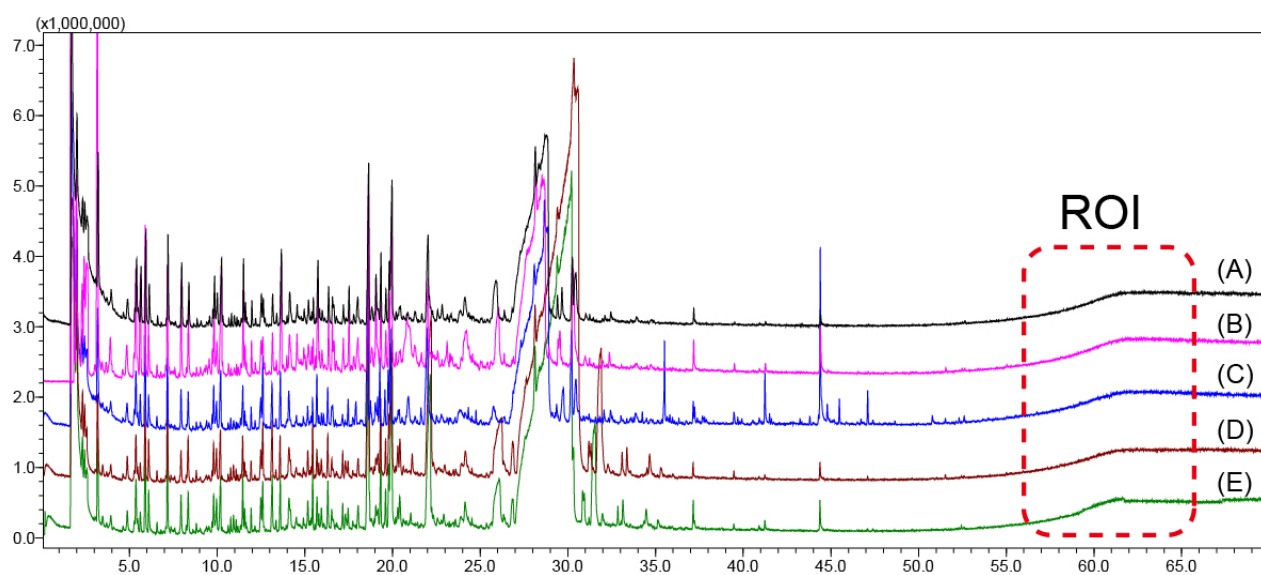


Figure 3.4.6 The pyrolysis of the Astana papers.

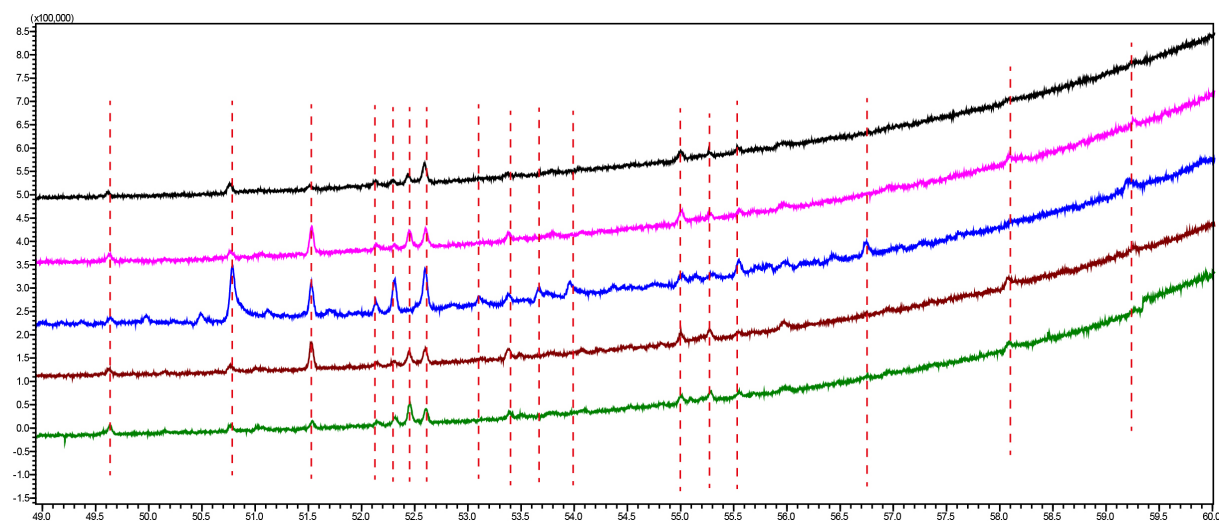


Figure 3.4.7 The ROI of the analyzed samples. Compound assignment refers to Table 3.4.3.

Table 3.4.3 Assignment of plant markers detected by Py-GC/MS in the ROI of the investigated Astana papers with retention time (RT), base peak, main fragment ions, expected molecular weights, assigned formula and most likely attribution of the products.

Ret. Time (min)	area	Base peak	Main fragment ions	MW	formula	attribution
50.788	533742	59	59,72,55,43,69	281	C ₁₈ H ₃₅ NO	9-octadecenamide (oleyl amide)
51.123	33627	59	59,72,43,69,55	NA	NA	long chain amide
51.530	189478	69	69,81,95,121,93	410	C ₃₀ H ₅₀	squalene
51.699	14071	57	57,43,97,55,85	NA	NA	long chain alkene
52.138	80423	105	105,95,91,43,81	368	C ₂₇ H ₄₄	cholesta-2,4-diene?
52.314	218366	135	135,143,81,95,366	366	C ₂₇ H ₄₄ O	cholesta-4,6-dien-3-ol,
52.599	240181	147	147,81,105,91,145,368	368	C ₂₇ H ₄₄	cholesta-3,5-diene?
53.111	36156	57	57,43,71,97,85	NA	NA	long chain alkane
53.379	50662	57	57,43,83,9,55	424	C ₂₈ H ₅₆ O ₂	dodecyl palmitate ?
53.666		59	59,72,55,43,69	NA	NA	long chain alkane amide
53.963	105104	59	59,72,43,57,60	NA	NA	long chain alkane amide
55.019	95758	135	135,81,55,95,43,119	NA	NA	cholestane structure
55.544	107274	105	105,43,107,81,95	386	C ₂₇ H ₄₆ O	cholest-5-en-3-ol (cholesterol)
56.749	107024	174	174,159,161,382,187	382	C ₂₇ H ₄₂ O	cholesta-3,5-dien-7-one
59.202	87328	137	137,43,55,109,81	NA	NA	cholestane derivative

3.4.5 A discussion on the usage of S/G ratio

In fact, the Hertzberg stain is a quick way to have a rough estimation of the lignin content in the fibers indicated by the color presentation of the stained fibers. However, its precision (resolution) may not be enough to measure minor difference in the lignin content. For instance, Lignin in ramie fiber is found in much smaller quantities (0.5–1.0%) while in hemp (5-10%) (Fig.8). Using Hertzberg stain to detect the minor lignin content in hemp and ramie and consequently use as one criterion pose certain risk.

In considering the following aspects, it is difficult by this method to distinguish hemp from ramie:

- Hertzberg stain is a rough estimation of the lignin content while the resolution of this method may not be enough to detect minor changes such as 5% change in the lignin content
- The presentation of the stained fibers was also affected by other component in paper, a point may add difficulty to associate the color change to the lignin content
- Use of the fiber characteristic features such as width, length and nodes for identification is at a risk (unless measure times in hundreds range and use statistical analysis). These potential pitfalls have been discussed elsewhere [18].

As a result, the handmade paper fibers from hemp, ramie, flax and jute were often vaguely and collectively identification identified as ‘Ma group’ fibers. Any further separation only using microscopic observation posed certain risk of mis-identification for the reasons stated above.

From the starting point, we stated that Hertzberg stain is a rough estimate of the lignin content in the analyzed fibers and may not preserve the resolution to detect the minor change in the lignin content (e.g. the case of hemp and ramie). Comparatively, Py-GC/MS is able to provide more information (molecular level) with enough resolution to detect the minor change (in structure and content) of the component in the fiber. In fact, in the lignin studies (especially the modern pulping process) the ration of syringyl- to guaiacyl-units (S/G ratio) is often studied to characterize different plant species and a high S/G ratio is attributed to a low amount of condensed lignin structures (less alkali consumption and therefore higher pulp yield) [22-24]. This could be a point to utilize for the purpose of fiber identification (e.g. hemp and ramie that have different lignin constitute).

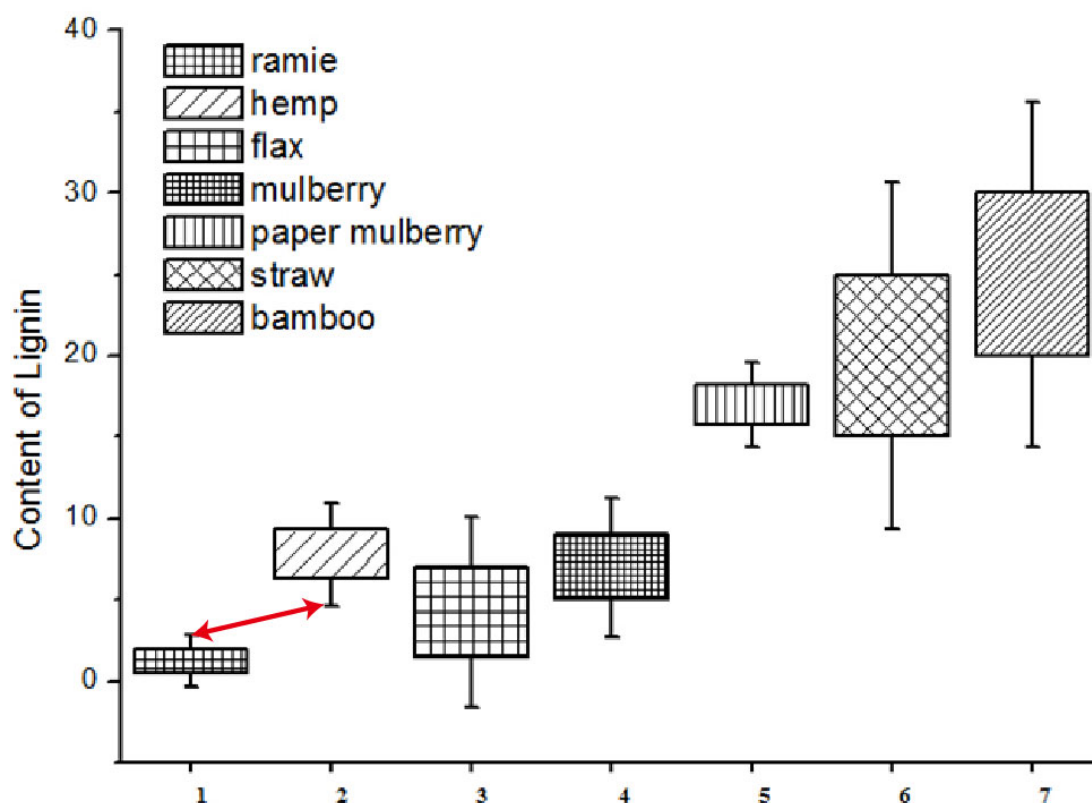


Figure 3.4.8 The approximate content range of lignin in fibers from different plant.

The presence of lignin (with different H/S/G types) in fibers was discussed in section 1.5. Lignin was observed to present as minor compounds often co-eluting with cellulose pyrolysis products:

- In the 1D pyrolysis of handmade papers, the lignin monomers and other cellulose pyrolysis product (e.g. the levoglucosan) were largely co-eluting. In a typical TIC response peaks, lignin monomers could be the minor compounds while larger response corresponds to the other compounds
- The TIC response for the lignin monomers were mostly in a low response and integrate their value is not easy (in considering some just rising above the background level). A fact that has been observed in the scan model for the trace compounds detection in 1D analysis

In such case, use of the TIC response to calculate the S/G ratio in 1D data is impossible: only by better separation (e.g. GCxGC) or SIM model in the 1D data acquisition which was observed to be more sensitivity than the full scan model (TIC and EIC information) [25] (but the influence for the co-eluting compounds cannot be ruled out).

Table 3.4.4 The identified lignin monomers in the pyrolysis of the tested samples.

Peak	Ret.Time	most intensive m/z	tpye	identification
1	21.221	137	G152	4-Ethylguaiaicol
2	27.288	137	G166	4-Propylguaiaicol
3	27.927	137	G180	Guaiaacylacetone
4	22.289	150	G150	4-Vinylguaiaicol
5	24.573	151	G152	Vanillin
6	26.816	151	G166	Acetovanillone
7	23.285	154	S154	2,6-Dimethoxyphenol
8	25.888	164	G164	cis-Isoeugenol
9	33.4	167	G210	Syringyl acetone
10	25.803	168	S168	2,6-Dimethoxy-4-methyl-phenyl
11	28.733	180	S180	2,6-Dimethoxy-4-vinylpheno
12	32.588	181	S196	Acetosyringone
13	30.947	182	S182	Syringaldehyde
14	31.86	194	S194	trans-2,6-Dimethoxy-4-propenylphenol

In order to have a first screen of the S/G ratio in the pyrolysis of handmade papers (liquid extraction is impossible due to the comparatively larger sample consumption), a method using the most intensive ions response (EIC area) in each of the identified lignin monomers has been used to avoid the influence of the co-eluting compounds (listed in Table 3.4.4). These ions have been utilized to indicate the relative content and used to calculate the S/G ratio. The method was firstly employed to same reference samples and then the Astana paper manuscript (Fig.3.4.9):

- The S/G ratio for hemp handmade paper was found to be 0.72, the value was calculated as 0.6 using other method [26]
- The S/G ratio for flax handmade papers was calculated as 0.017, a really small value. In fact, only 1 S type monomer and three G type monomers can be targeted. This value was calculated as 0.1 when applying other methods [26]
- The S/G ratio for hemp handmade paper was calculated to be 0 as only one G type lignin can be targeted. This may be in correlation to the fact that lignin in ramie fibre is found in much smaller quantities (0.5–1.0%) compared with other vegetable fibres (2–29%) [27]
- The S/G ratio for jute handmade paper was calculated to be 3.2, a value quite different from hemp, ramie and flax. This value was calculated as 2 when applying other methods [26]
- The S/G ratio for other handmade papers using the defined method was also calculated: mitsumata (3.79), gampi (3.2), hanji (2.9)

- The Astana paper's S/G ratio for sample A, B, D and E was calculated as ca. 0.6 while for sample C the value was 2.05

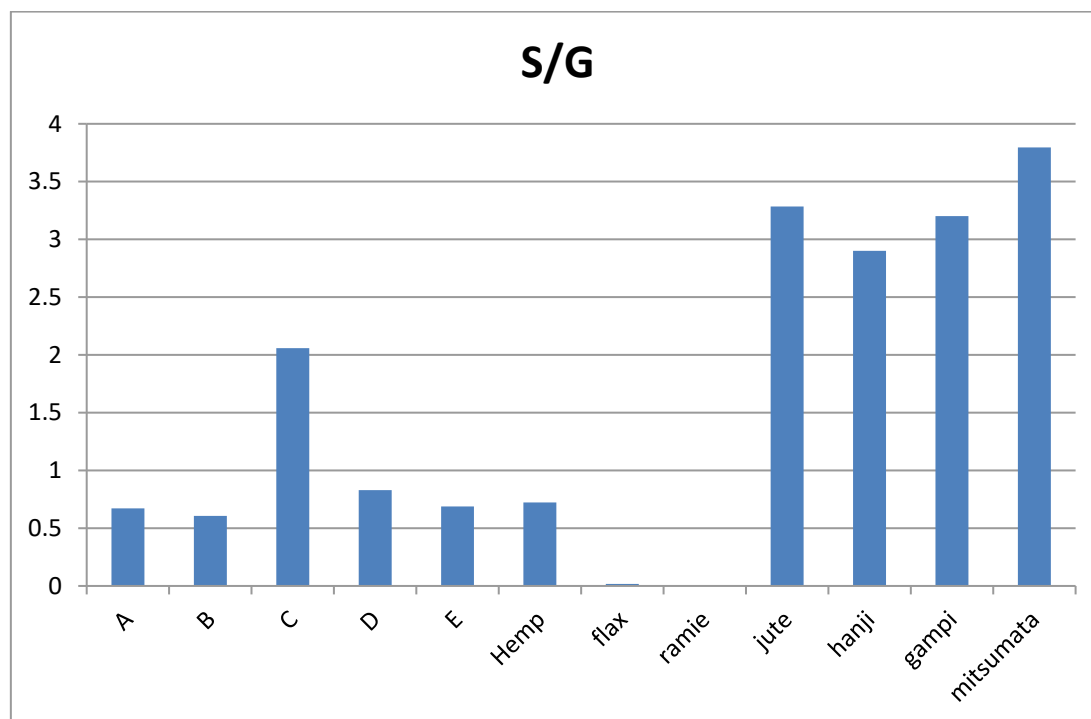


Figure 3.4.9 The S/G ratio of the tested samples.

It has been observed in the 1D analysis that flax and ramie have comparatively lower lignin monomers (four monomers detected for flax and only one monomer for ramie). Hemp was observed with more lignin monomers. The most intensive lignin content (in considering their response value) was observed to be mitsumata, gampi, and jute. From this point, jute could be differentiated from hemp, ramie and flax using the lignin content.

As can be seen from Fig.3.4.9, the S/G ratio (calculated using the method defined) for sample A, B, D and E was around 0.6, a value similar to that of hemp. Moreover, in considering of the low values of flax and ramie and the high values of jute, this might be a potential way awaiting further investigation for a reliable and easy way to separate hemp fiber from ramie fibers. The distribution of the lignin compounds in GCxGC separation is more illustrative as demonstrated in Fig.3.4.10. It provides a quick identification of the archeological sample (sample A) as hemp origins. These results are preliminary and a more precise result may come after detailed analysis of the 2D data.

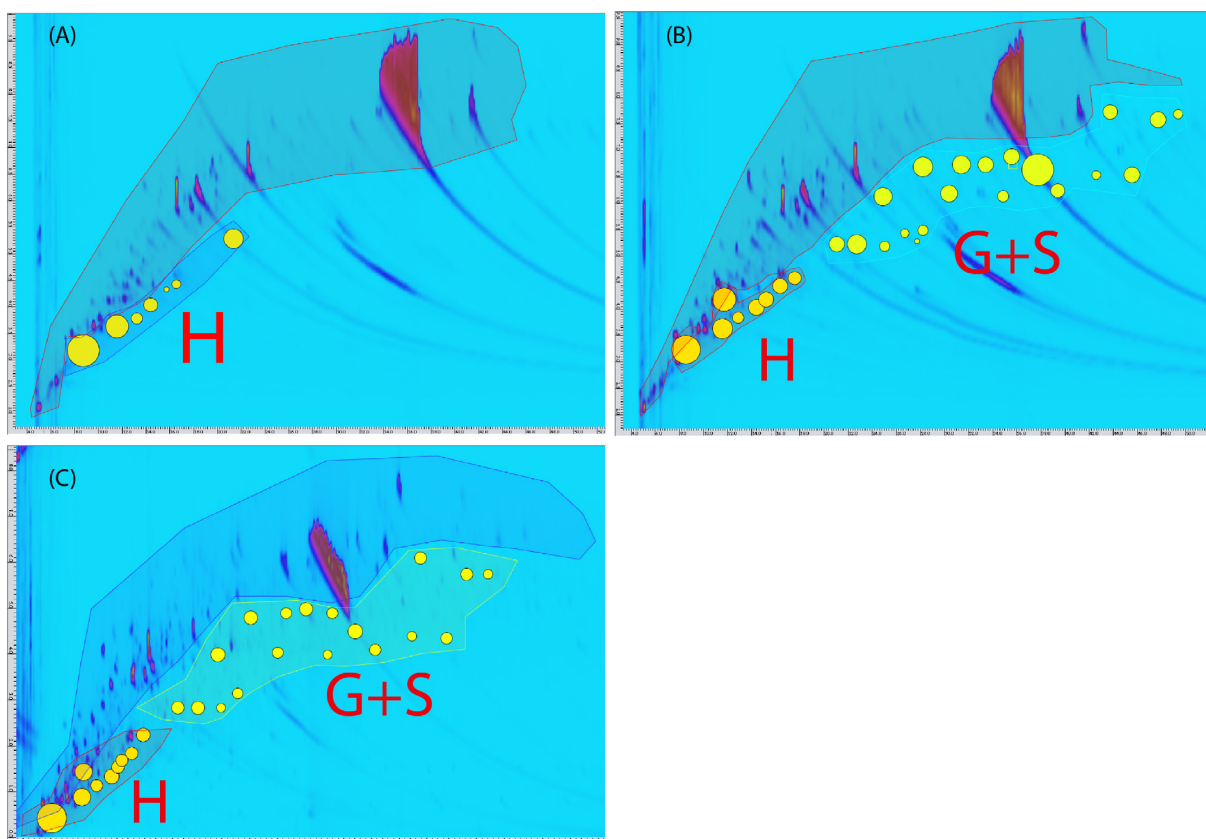


Figure 3.4.10 The types of lignin distribution in GCxGC separation of (A) hemp, (B) ramie and (C) sample A from Astana tomb. For the lignin types refer to section 1.5.

3.4.6 A discussion concerning the social background

The papermaking panorama in early Tang Dynasty in turpan, Xinjiang cannot be fully rebuilt due to the limited number of samples analyzed, yet the rule of cellulosic fiber purification and lignin elimination provide an insight into its fiber utilization, technique and environment. Besides writing, paper has been applied in ornamentation, decoration, burial and sacrifice, indicating a prosperous of paper utilization and fabrication. However, traces of predicament in paper supply in early Tang dynasty in turpan, Xinjiang were evident:

- In the Tang and Song Dynasty the social convention favored the use of high quality paper offerings for the respect of the departed and/or the ancestor, but the samples from Astana Cemeteries seems low in quality, indicating being roughly manufactured
- The evidence that waste and/or used paper (like used deeds, official record or archive) has been directly used for the fabrication of paper clothes, shoes, offerings and even paper coffin for the departed. The seemingly ‘cyclic utilization’ of paper was likely an indicator of the insufficient paper production capacity or economic hardship

Historical records documented that high quality papers were still in great demand and imported from the central part of China. Hypotheses for the godly purposes use of paper offerings stated that it was not acceptable to use either waste paper or rag as the source of fibers to be used for such purposes. The direct use of ‘used papers’ in Astana Cemeteries strongly implied that paper was still a scarce resource, directly pointing to the stagnation of the papermaking industry. The corresponding reason behind the predicament was nothing less than the technological hysteresis: relative deprivation of fiber sources or economic reasons.

Data from hitherto published researches demonstrate that fibers from hemp/ramie are still predominant and bast fibers from mulberry or paper mulberry are too rare (if sample artificial selection is ruled out). So an outline of papermaking in early Tang Dynasties in Turpan, Xinjiang, could be tentatively outlined:

- The fibers from hemp/ramie is still a main fiber sources
- Fibers from mulberry and/or paper mulberry is also utilized although the accurate percentage remains unknown
- The extremely dry environments aggravate deprivation of fiber sources severely limited the development of papermaking technique
- Certain degree of technological hysteresis existed as can be seen from the import of high quality papers from central area of China

It shall be noted that the percentage is not a conclusive criterion. For a large set of paper-based remnant has been unearthed in turpan, Xinjiang, pitifully, only a handful of them have been scientifically researched. By comparison with previous works, the predominance of hemp/ramie fibers is highly reliable but the detailed percentage still awaits further scientific investigation for completeness.

China fully embraced the ‘paper age’ during the period of Tang Dynasty (618-907AD) and the papermaking technique spread outwards radially. The Turpan District is an important bridgehead on the Silk Road in Xinjiang and the age of Astana Cemeteries is within the range of the spread of papermaking from China to Islamic world, which makes the paper-based remnant an infrequent material for the study of internal relation of papers through the Silk Road. The use of fiber sources in the initial stage of papermaking in the Islamic world may due to the deprivation of fiber sources, yet the successive technique of fiber selection and lignin elimination might be of key role for papermaking.

Although long fibers were preferred by papermakers, they are more liable to form flocs than short fibers. The Chinese craftsman learned to use mucilaginous agent extracted from various plant to retard the fiber aggregation and makes them uniformly distributed in the pulp, which

is efficiency beneficial and can produce thin paper. Doubts concerning whether the conservative of captured craftsman (technical reasons) or the lack of the certain plant (source deprivation) that makes the loss of mucilaginous technique in the Islamic remain controversy. Further scientific research concerning the use of mucilaginous technique in paper unearthed from Astana Cemeteries may provide more insight.

3.4.7 A summary

In the present work, samples of paper offerings unearthed from Astana Cemeteries in the early Tang Dynasty were investigated by microscopic examination, Hertzberg staining and Py-GC/MS analysis. As for the identification of paper fiber sources, although Hertzberg staining method is effective and currently used, it may cause ambiguity. The Py-GC/MS results pointed that the sample A, B, D and E are likely to be of hemp origins and the further investigation of the S/G ratio may provide more precise information. The 2D analysis shows a potential to quick separate the hemp/ramie fibers with their different distribution of lignin compound in the 2D space.

The low quality of paper offering examined, together with the direct use of used paper as paper offering, indicate stagnation in papermaking technique and/or insufficient fiber source. The time of Astana Cemeteries lies around the era when papermaking spread to the Islam world. The paper examined in Astana Cemeteries and that in contemporary Islam world have similarity regarding their fiber selection. Prospective of new developments in structural analysis may reveal further detailed information on discrimination and interconnection among the ancient papers along the Silk Road.

3.5 A material study of source-unknown ancient banknotes and cut sutra

3.5.1 Introduction

3.5.1.1 The issuing of the paper banknote in ancient China

The paper banknote is an indispensable part of our social life now while in ancient times it mainly characterized by the use of metal material (iron, bronze, silver and gold) for currency [28]. The heavy and cumbersome metals were inconvenient for long distance commercial activities while the trial of using handmade paper for currency showed its convenience and has been regarded as one of man's most ingenious inventions during the last 1000 years [29]. The paper banknote originated probably in the early 9th century named 'fei qian' (flying money) and by the end of Northern Song period (960AD-1127AD), over million banknotes were in circulation [30, 31].

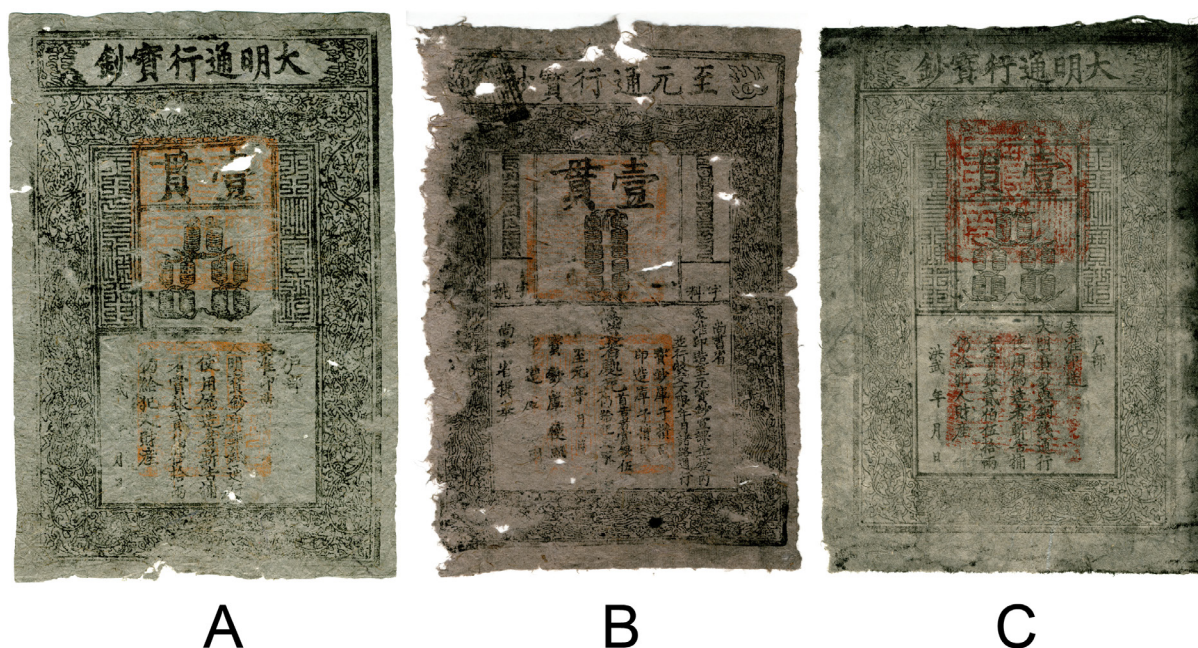


Figure 3.5.1 The analyzed paper banknote samples (A)-(C) from private collections⁷².

The Yuan Dynasty (1271-1368 AD) and Ming Dynasty (1368-1644 AD) were characterized by issuing of official paper currency (samples shown in Fig.3.5.1). From the late half of the 1270s, a steep increase in the issue of paper money can be observed in the statistics in the

⁷² All these samples are provided by Dr. Sakamoto Shouji (RyuKoku University). The microscopic analysis work was done by him and published elsewhere (reference 44 and 45). The sample images and microscopic observations were kindly provided by him; herewith he reserves the rights for other usages. For more information, please see his article in reference 44 and 45.

official history of the Yuan Dynasty. The initial paper banknote issued by the government is Zhongtong Chao (中统元宝钞, 1260-1287 AD) which became non-convertible with the issue of Zhiyuan chao (至元通行宝钞, not redeemable for metal, sample B in Fig.3.5.1) at 1287 AD [32]. The paper banknote was largely issued to supplement costly copper coins which (together with other factors) caused collapsing of the money system in the late Yuan Dynasty. The following Ming Dynasty also issued paper banknote as the dominant currency. However, problems such as uncontrolled issuing and the subsequent devaluing led to its official abandon in 1523 AD (some measures such as prohibit the use of silver as currency in 1367 AD does not improve this situation [33]). Counterfeit (forgeries), emerged from an early stage with paper banknote issuing, was another reason for its discredit during this period. As for the Zhiyuan Tongbao (至元通宝, sample B in Fig.3.5.1), it was issued in the year 1287 AD by Yuan Dynasty.

As for the Daming Tongbao (大明通宝, sample A and C in Fig.3.5.1), this paper banknote began to be issued in the eighth year of Hongwu Emperor (1375 AD) due to the lack of copper for manufacturing coins, and is the only paper banknote issued in the Ming Dynasty⁷³. The size of the banknote is commonly 30cm*20cm, to be largest banknote in size in the history. The government has issued severe punishment (death penalty) for the manufacturing of forgeries. According to Volume 31 of Ming Dynasty's legal document, this banknote was made from mulberry stems (*Morus alba*) and was made with a height of 1 chi (about 34 cm), a width of 6 cun (about 22 cm).

3.5.1.2 Background of cut sutra in ancient China

Before the invention of printing, the reproduce of books for transmission of knowledge were mainly by handwritten copy. The produce of handwritten copy is time-consuming and error-prone. With the prosperity of Tang dynasty, people have larger demand on books and sutras which contributed to the invention of wood block printing. The wood block printing technique was further developed in Song dynasty for mass production. Although the movable type printing was invented by Bi Sheng in Song dynasty, wood block printing still gains the popularity.

⁷³ According to Dr. Sakamoto Shouji, in BnF, a 'Daming Baochao' sample is preserved (Chinois 1133). It is previously held by Julian Heinrich Klaproth (1783-1835 AD) who collected a large number of books locally on the occasion of Russia dispatching delegation to China.

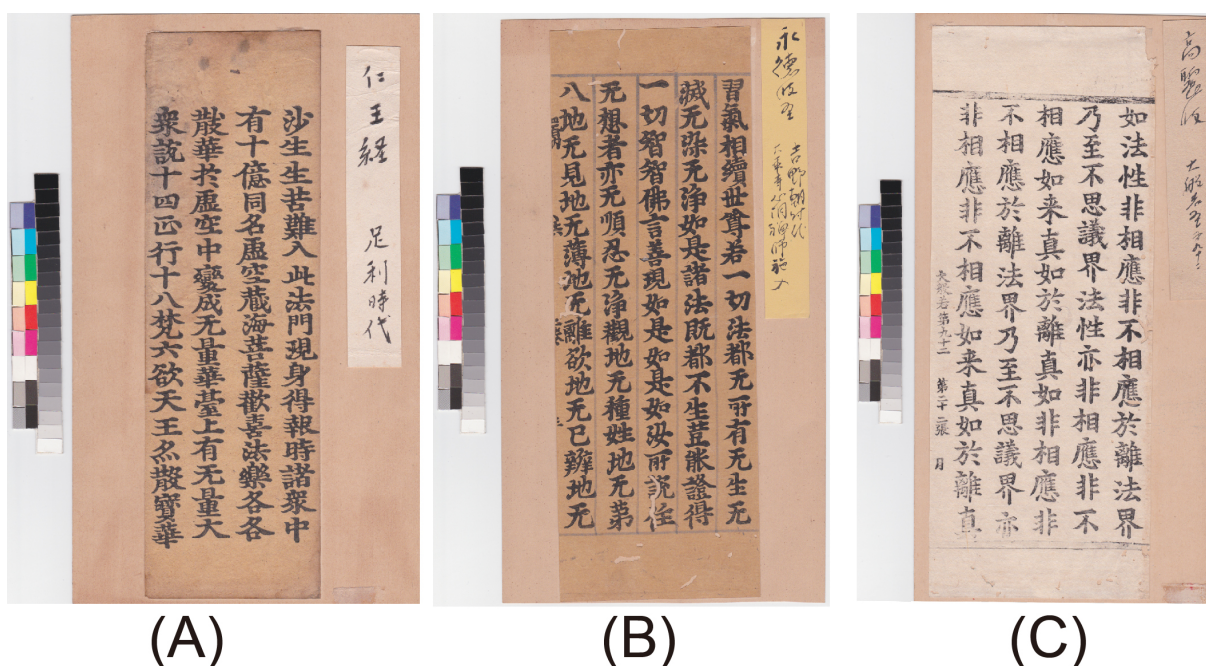


Figure 3.5.2 The analyzed cut sutra samples: (A), JP02; (B) JP03; (C) KP01.

Sutras prints of Song dynasty is of high values but they are really rare in China now. However, lots sutras prints were imported to Japan and meanwhile a process of domestication for sutras prints made in Japan was carried out. One of the famous wood prints is the Kasuga wood block print from the Kasuga Temple in Kofukuji, Nara. It was printed from the late Heian era until the Edo era, and the wood block of each era is well preserved in Kofukuji. In the Kasuga version there is a difference between the type of several versions and the era in which it was printed. It is known that various kinds of paper exist because different paper and ink are different. It has been confirmed that many such books are stored in temples in various places, and its investigation has also been carried out. Moreover, it seems that it dispersed out from a temple. The edited version is cut for purpose of viewing which has been dissipated.

3.5.1.3 The problem in the material studies

The modern made counterfeit has also caused chaos in art collection market: a fact of difficulty to identify genus and counterfeit even by the experts. The comprehensive study of the genus ones in museum collections could help nevertheless few specimens (especially the early ones from the Song Dynasty) of ancient Chinese paper currency survive today due to its tendency for degradation and environmental change. The survived samples (mainly from Ming and Qing Dynasties) were dispersed in various museum collections as well as private collections. The scientific analysis of these valuable samples could provide new insight for the technique and material usage in supplementing historical descriptions (sometimes this

historical information is missing). Nevertheless, until now there are only limited scientific analyses reported in focusing on the fiber identification and pigment utilization. [5, 34-36].

As for the fiber identification of the paper banknote, traditional staining method together with microscopic analysis was commonly applied [5, 35] as the characteristic features of different plant fibers has been described [19, 37] with various applications in cultural heritage studies [38-41]. However, the microscopic observation may be ineffective to fully assess the nature of the fibers: an easy identification of fibers from the *Moraceae* family while difficult to classify into the sub-genre such as mulberry (*Morus alba*) or paper mulberry (*Broussonetia spp*). It also necessitates that the observers to be trained as an expert for proper identification. It also appears that some plants can produce more than one typology of fibers, requiring further investigation to characterize the differences shown by varieties of the same species. For some close taxonomy related species such as above mentioned mulberry and paper mulberry have almost resemble fiber characteristics and identification by their stained color and presence of calcium oxalate crystals may, in some cases, not be determinative, or at least another analytical method is needed for verifying the identification result of microscopic observation. An identification only use microscopy may result in different conclusions, for instance, the same samples observed by different researchers generated different identification results, a situation pointed out for the Dunhuang document studies [42].

This section reports the microscopic observation of three paper banknote from private collections: two damingbaochao (sample A and sample C) and one zhiyuantongbao (sample B). The Carbon-14 dating was performed on sample A-C to determine its dating. A set of cut sutra samples were also analyzed (Fig.3.5.2). Moreover, this article emphasis on introducing a easy, quick and reliable identification of mulberry and paper mulberry fibers by Py-GC/MS which can be a quick and complementary way to verify microscopic observation results.

3.5.2 Results and discussion

3.5.2.1 Analysis of the banknote samples

Sample A and C in Fig.3.5.1 are all the ‘Daming Baochao’ banknotes while minor differences in the pattern and quality of the printed letters and patterns can be observed. For sample A, B and C, the fiber is thin with numerous rectangular crystals, there is little nonfibrous cells (such as parenchyma cells), purple bundles (crystals attached), yellow fiber bundles, trace amounts of pink color fiber can be observed.

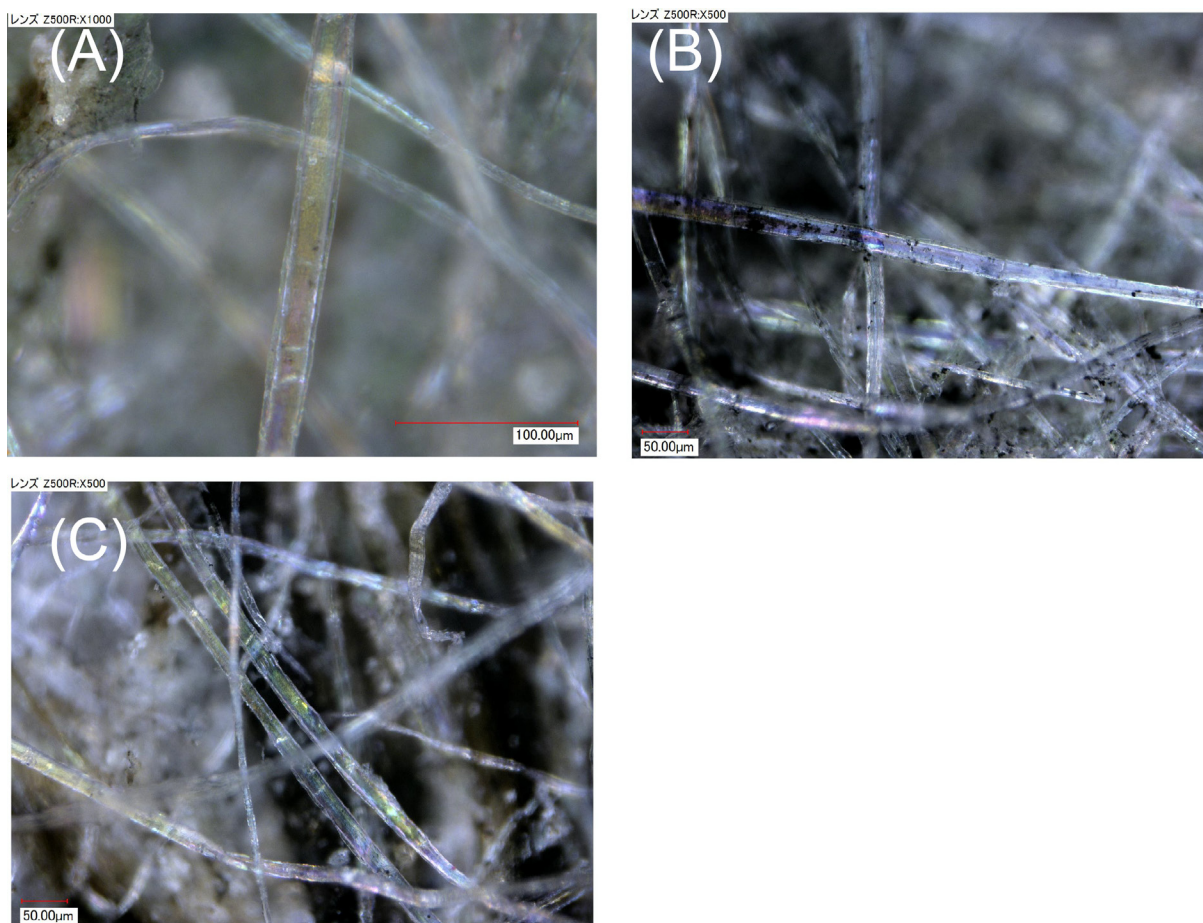


Figure 3.5.3 The microscopic observation of the tested samples, the numbering refers to Figure 3.5.1.

Table 3.5.1 The microscopic observation results

Samples	fiber identification	presence of crystals
A	mulberry or paper mulberry	yes
B	mulberry or paper mulberry	yes
C	mulberry or paper mulberry	yes

The microscopic observation showed that all the fibers are from the Moraceae family. A further study for their classification was carried out with pyrolysis analysis. The ROI region has been previously studied for the fibers from the Moraceae family: a characteristic feature of a series terpene compounds with the main m/z at 218, 203, 189. Differentiation of mulberry and paper mulberry by PCA has been demonstrated in section 3.2. The most intensive loadings indicated the importance of the intensity of the Urs-12-ene-3-acetate (α -Amyrin acetate). The PCA score plots and loadings plot is shown in Fig.3.5.4. it can be seen that sample B is close in the PCA spacing with S2 (*Morus alba*) which both have a higher content of compound 43 (α -Amyrin acetate) while Sample A and C are indicated to be of

Broussonetia spp as its location near the S3-S4 (S3-S5 are the same mentioned in section 3.2). By the Pyrolysis analysis, a more precise identification of *Morus* spp from *Broussonetia* spp has been proposed for the identification of these banknotes.

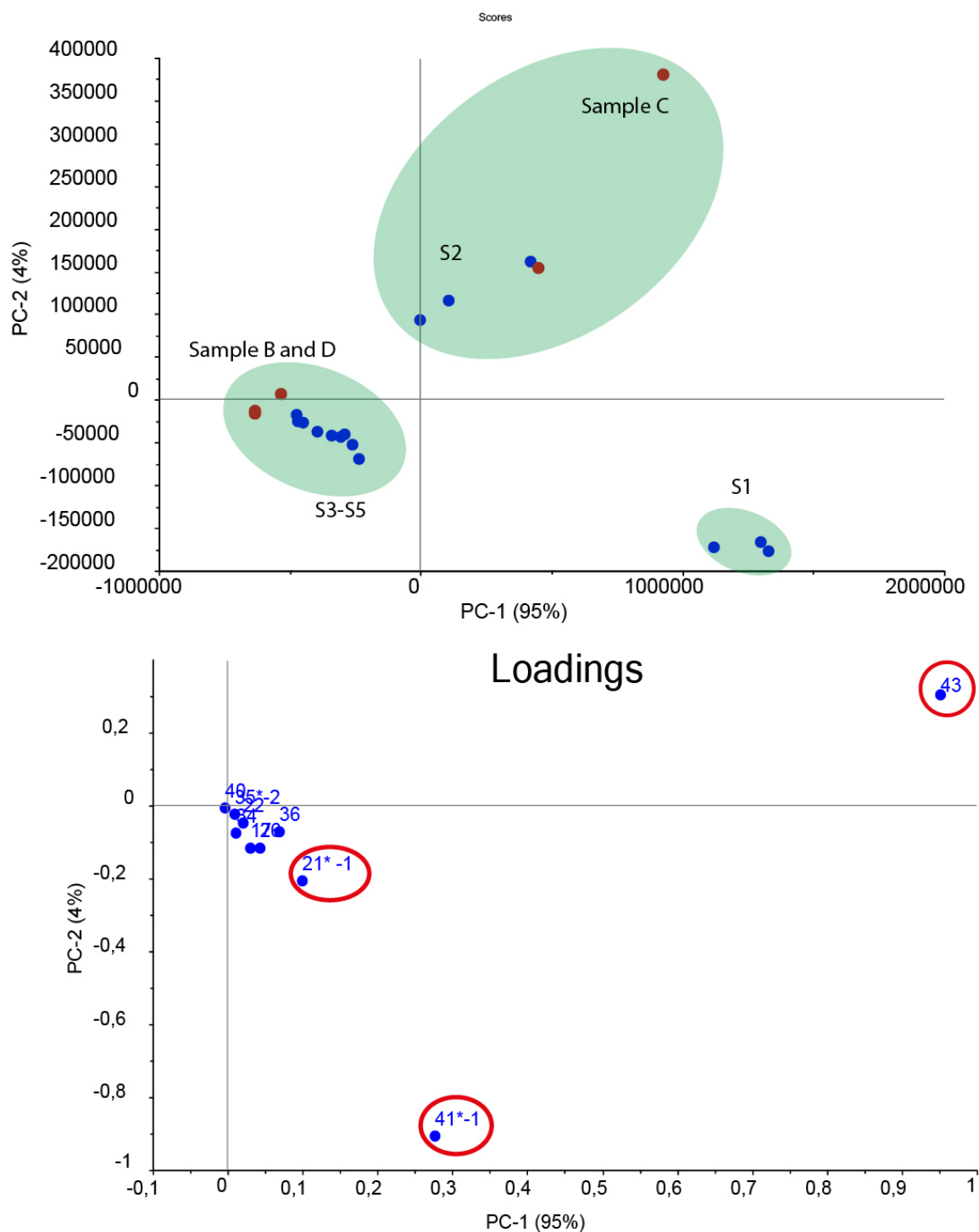


Figure 3.5.4 The PCA results of the tested banknote samples.

3.5.2.2 Analysis of the cut sutra samples

The fiber composition of the cut sutra samples was from *Moraceae* family by the microscopic analysis. For a further classification, the pyrolysis analysis was conducted. The pyrolysis of handmade papers (fibers) most commonly was observed with Urs-12-ene-3-acetate (α -Amyrin acetate) as the most intensive compounds in the ROI and the content was comparatively high in *Morus* spp than *Broussonetia* spp. besides the S1-S5 tested in section 3.2, two more species within the *Moraceae* family (common Japanese name *Taori* and *Akaso*) were tested. The profile of *Taori* is the same as S1-S5 with α -Amyrin acetate as the most intensive peaks while the profile for *Akaso* is different with Oleanane-12-en-3-ol (β -Amyrin) as the most intensive peaks. Consequently, all the tested reference samples and the cut sutras were put into the PCA modeling.

It can be seen from the PCA scores plot and the corresponding loadings plot (Fig.3.5.5) that the scores plot can be divided into two space: the left side (negative coordinate) was the samples (S1-S5 plus *Taori*) which featured α -Amyrin acetate as the most intensive peaks and the right side (positive coordinate) was the samples (*Akaso* and cut sutra samples) which featured β -Amyrin as the most intensive peaks. The tested cut sutra samples were all located on the right-side region together with the *Akaso* reference samples. From their location, it can be inferred that their material origin was more probably similar to *Akaso* in the *Moraceae* Family.

3.5.3 A summary

In this application, microscopic observation and pyrolysis have been both applied for the identification of the fiber source of some ancient documents. The result showed that microscopic observation can provide first screen result indicating the plant family information while the pyrolysis analysis can target different sub-species within the plant family.

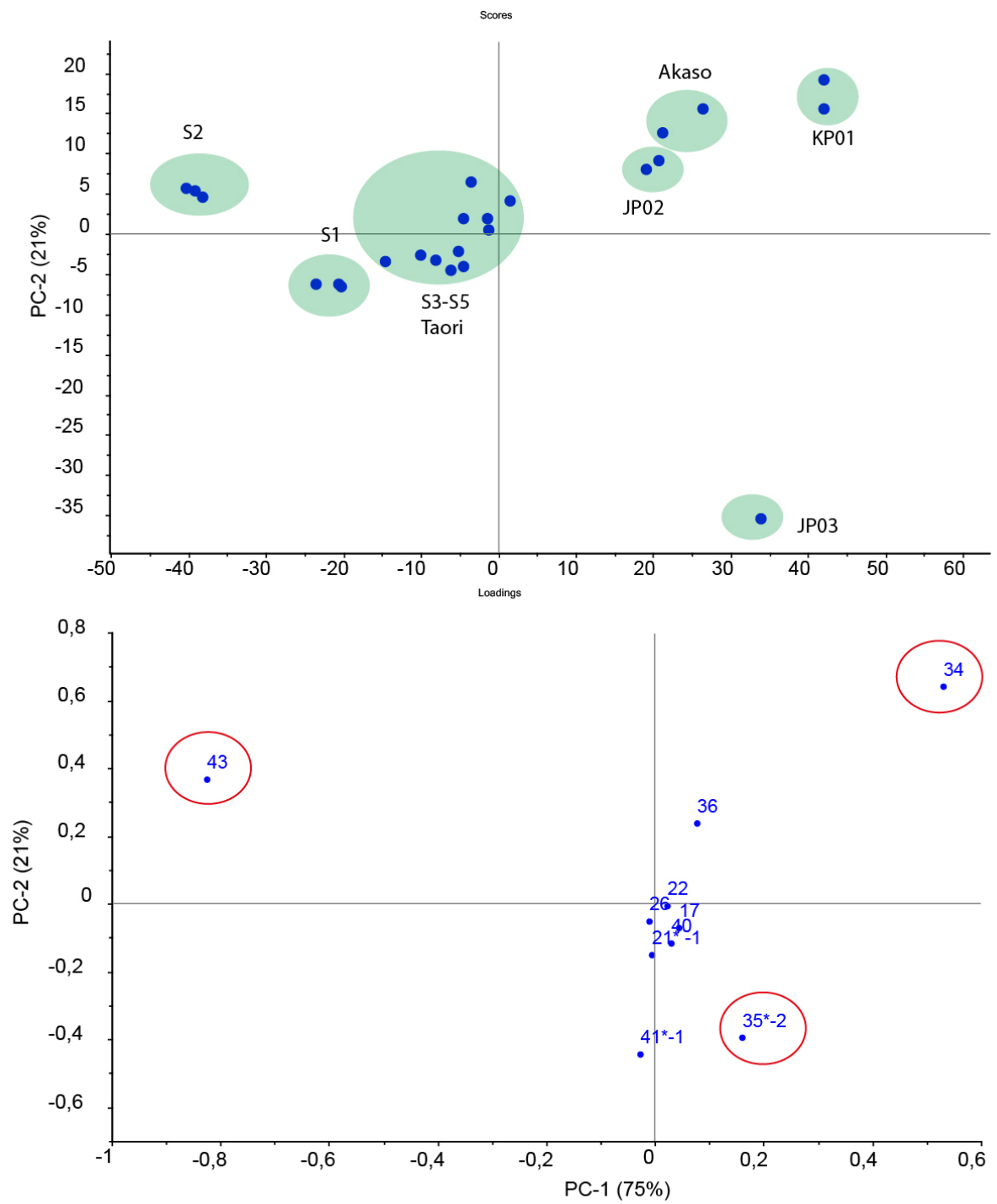


Figure 3.5.5 The PCA results of the tested cut sutra samples.

3.6 Conclusions and perspectives

3.6.1 Summary and general discussions

The hand papermaking, although has been less practiced, is an important activity incorporated into our history and culture. The handmade paper became the carrier of the information and composed numerous manuscripts and documents in museums and private collections. In this thesis, several aspects concerning the materiality of the handmade papers were discussed and some problems have been pointed out.

3.6.1.1 The origin of papermaking

In the first chapter, the social and cultural part of hand papermaking has been introduced. An interesting point is the discussion of the origin of papermaking which currently is not clear and subject to conjecture. There are two ways for its study: (i) the analysis of historical records and (ii) the analysis of the archaeological discoveries.

The thesis reviews the historical records regarding the origin of papermaking that showed (i) a strong implication that the silk making technique has great influence on the origin of papermaking and (ii) the pound wadding in the water could be a further fiber processing technique as the initial stage of papermaking. But due to the simple and brief description in ancient Chinese, it posed difficulties to precisely interpret the predecessor's meanings that different interpretations are possible and may sound plausible. Divergent annotation and interpretation for the same text had long appeared in history and even now.

The archaeological findings that are related to the origin of papermaking have been reviewed and on the base of the current data, the question of what is the initial aim of making paper (or what is the function of paper in the very early stage?) has been investigated and an hypothesis of (i) a geographical belt of paper product and (ii) chronological material involved for padding has been proposed. The hypothesis uses the God Mod paper as a key point while its academic value has currently not been fully estimated. The hypothesis also pointed out the necessity of further analytical analysis for samples such as the Daheting paper-like fragment, a key point in the time line around 300BC.

A third chaos in the topic of paper origin lies in the fiber identification. For the same sample, it can be identified as ramie from some scholars while identified as hemp from others or more collectively identified as *Ma* group fibers. It seldom been an identification results attested by

other method. For this point, the precise determination of their lignin types and content could hopefully improve the situation.

3.6.1.2 The change of material through history

The material change of handmade papers is an interesting point that may contain rich information about the social change, the economic condition and the cultural exchange. This point has been shortly discussed with the current carried out work (from some scholars such as Qian Cunxun, Li Tao and Sakamoto Shouji). For example, the social instability has been directly reflected into the materials for the handmade papers. However, this study requires large data sets for interpretations. Currently, this kind of information remains blank for certain period and areas. In considering the large amount of ancient document preserved in museums, a systematic study may reveal information that contributes to the historical studies.

3.6.1.3 The role of microscopy

Microscopy is the first and currently used method for fiber analysis of handmade papers. It provide a way in the early stage (when other analytical methods were not available) to characterize the fibers. However, as pointed, the morphological features could be similar for different species and it requires long time training to be able to appraise in fiber identification. Also, the subjective description of the characteristic features of the fibers leaves room for ‘mis-understanding’: a case that has been raised by Prof. Jean-Pierre Drège for the inconsistency of the identification result for the same Dunhang documents. Also, the microscopy is able to characterize plant fibers from a plant family such as the Moraceae family while it is difficult to distinguish the sub-species within the family such as *Morus spp* VS *Broussonetia spp*.

3.6.1.4 The novelty of the defined method

In exploring and providing a new method for handmade paper fiber identification (or more generally a new approach on chemotaxonomy since the plant markers used were related to the metabolites from different plant species), the capacity of Py-GC/MS and Py-GCxGC/MS has been studied in this thesis for this purpose. The identification of the metabolites in the pyrolysis products of different handmade papers (with the example of kozo, mitsumata and gampi in section 2.2) attested that different plants having different synthesis pathway and their metabolites distribution pattern can be utilized for fiber identification (in a more general sense, their

chemotaxonomy). The analysis and correspondence with the raw plant fibers confirmed the plant origin of these metabolites.

3.6.1.5 The introduction of Py-GCxGC into cultural heritage field

One emphasis in this thesis lies in the introduction of GCxGC (Py-GCxGC) methodology into the field of cultural heritage where 1D analysis is most commonly performed while the 2D application is rare. With the case demonstrated with the analysis of Japanese handmade papers, the performance of pyrolysis-comprehensive two-dimensional gas chromatography/mass spectrometry was tested for the characterization of traditional East Asian handmade papers compared with those previously obtained by pyrolysis-gas chromatography/mass spectrometry. The results showed that the number of plant markers (triterpenes and phytosterols) detected in a Japanese kozo reference paper was improved in the pyrolysis fingerprint. With a gain in sensitivity in the order of six, the case study of a museum sample confirmed that pyrolysis-comprehensive two-dimensional gas chromatography/mass spectrometry is more efficient for a reliable identification of museum collections samples and/or archaeological samples where tiny quantities of materials are the rule. The advantage was also obvious observed when analyze some paper-related subject such as the lignin analysis and the sizing agent analysis. It can be forecast that with more applications, GCxGC methodology will bring more insight into this field.

3.6.1.6 The data treatment

Lots of methods and applications have been created to tackle the 1D data treatment where in some cases it can be difficult in considering of the co-elution of compounds and the presence of low concentration compounds. Besides the well-developed mass spectra deconvolution method (software like AMDIS), A GCxGC based method is proposed for a plus and alternative method. The work was carried out (section 2.5) with the aim to answer the question ‘how these characteristics of GCxGC analysis are able to improve the mass spectra collection and to benefit 1D data treatment’.

The potential use of GCxGC/MS profiles to conduct an improved AMDIS procedure for compound identification in 1D GC/MS data was studied with application to the identification of pyrolysis products of East Asian handmade papers. The result showed that the presence of compounds in 2D profile will lead to the quick location of co-eluting compounds or the quick identification of new compounds in 1D GC/MS chromatograms eluding background

contamination in the mass spectra and avoiding mass spectra mixing due to ineffective chromatographic separation. Clearly, the structured layout of products in a 2D chromatogram may help for 1D analysis of a series of compounds with similar structures. As a consequence, the compound distribution in GCxGC/MS profiles can be used as ‘peak finder’ for manual check of low concentration and co-eluting compound identification in 1D GC data. GCxGC/MS profiles improved the identification of unresolved patterns in 1D GC/MS analysis, as well as permitted to improve the EICs identification procedure for low signal peak detection and unresolved peak patterns showing a high dynamic in their corresponding chromatographic signals. GCxGC also preserves the ability to provide high quality mass spectra for building up the in-house library. There must be a potential usage of the proposed method and in-house libraries collected from 2D analysis in an extended field of applications like cultural heritage, botany or material science.

3.6.1.7 The lacking of the material well defined samples

The theme of this thesis is to use the metabolites distribution patterns of fiber derived from different plant fibers and the analysis of the modern reference sample is a fundamental step. However, problems for the samples (demonstrated in section 3.1) occurs during the analysis. It has been raised and stressed for a basic question concerning the handmade paper labeling (basically is the fiber identification). In some cases, this question has been overlooked and resulted in imprecise and confusing results. It pointed out a situation that a material origin and making method well defined samples are lacking in this field. The case study of the hanji samples in large quantity was a demonstration of the benefit for well-defined samples.

3.6.1.8 The novelty in creating a new method for fiber identification

The chaos of the fiber identification of the early un-earthed paper samples, the inconsistent identification results by different researchers and the limits of the microscopic observation have been noted and discussed. It all pointed out that a new methodology shall be created to improve the situation in the field. In fill this need, an analytical approach based on the multivariate analysis of on-line pyrolysis-gas chromatography/mass spectrometry data has been investigated for the identification of traditional East Asian handmade papers from different material origins. The results showed that besides the commonly used microscopy, pyrolysis was proven to be able to discriminate common handmade paper materials with a step further to easily separate plant fibers that have similar microscopic morphologies. As a result, a quick Py-GC/MS based handmade paper identification procedure using PCA

modeling of characteristic EICs was for the first time proposed in the identification of traditional East Asian handmade papers which could be beneficial in cultural heritage applications.

3.6.1.9 The necessity of GCxGC for PCA modeling

The defined method when using 1D analysis results in close PCA spacing for similar species (e.g. mitsumata, gampi paper in section 3.2) while by using the GCxGC methodology for a detailed screen of the compound distribution, an improved separation results can be obtained (section 3.3). As demonstrated in section 3.2, when using the PCA modeling of weight as 1/STD, a feature of minor compounds will be potentially selected as influential compounds. In this case, GCxGC is recommended as it can detect more minor compounds.

3.6.1.10 The application of the defined method

In this thesis, a methodology to detect the metabolite compounds was developed and as an application used their distribution pattern (presence vs intensity) for the purpose of fiber identification. Within this method, it has been currently observed:

- The Moraceae family: a featured distribution of terpene compounds with characteristic m/z at 218, 203 and 189, the α -amyirin acetate was observed to be the largest peaks in the ROI while the intensity is comparatively high for *Morus spp* than *Broussonetia spp*. currently, a different distribution pattern which featured the β -amyirin as the most intensive peak has been observed for a sub-species of *Akaso*
- The *Thymelaeaceae* family: a featured distribution of stigmasta compounds in the ROI
- The Ma group: it presents less compounds in the ROI

The application has been provided with two examples. The first one is an analysis of the handmade paper from the Astana Tomb at around Tang Dynasty (section 3.4). In the pyrolysis analysis, the tested samples don't preserve characteristic markers which more resemble the fibers from the *Ma* group. The sample C was by microscopy analysis to be identified as mulberry paper while in the pyrolysis analysis it does not present the characteristic terpene compounds. The result of the S/G ratio has been proposed for discrimination of hemp and ramie. By further investigation, a pyrolysis-based method is expected to be defined to distinguish the fibers within the *Ma* group.

The second application was proposed with banknotes and cut sutra samples analysis (section 3.5). For the banknotes samples, the microscopy observation was able to classify their fibers as from *Moraceae* family while differentiating sub-species such as *Morus spp* and *Broussonetia spp* which is relatively difficult to distinguish by microscopy. The pyrolysis results indicate one sample from *Morus spp* and two samples from *Broussonetia spp* as the intensity of the α -amyrin acetate being picked out by PCA analysis for their differentiation. As for the cut sutra samples, their metabolite distribution pattern is similar to the sub-species of *Akaso* where the β -amyrin takes to be the most intensive peaks.

3.6.2 Conclusions

This thesis explores and provides a novel methodology on handmade paper characterization and identification. It employs easy sampling process with minor quantity of samples required, using GC/MS instrumentation for separation and detection. The method uses the metabolites distribution patterns (presence plus intensity) eluting in the defined ROI as markers for discriminating handmade papers from different material origins. The result revealed that different plant fibers used for papermaking have different marker distributions in the ROI, for instance, the *Moraceae* family with a main featured distribution of terpene compounds and the *Thymelaeaceae* family with a main featured distribution of stigmasta compounds. The fiber from the *Ma* group was currently observed with few compounds in the ROI. With the applications, the Py-GC/MS and Py-GC \times GC/MS showed its ability to distinguish fibers from the same family (presenting similar microscopic features), thus can constitute as an effective method for fiber identification as well as to validate the identification results of the microscopic observation. The tested Py-GC \times GC/MS methodology has been for the first time proposed in the cultural heritage field and it harbors the potential to promote the research in this field.

3.6.3 Perspectives

3.6.3.1 Lignin analysis

Pyrolysis hyphenated with GC/MS has been widely applied with obvious advantages: the pyrolysis fragments can be identified using their retention indices and mass spectra. However, the following situation has been frequently observed:

- Overlapping of the mass spectra due to the overlapping of the eluted compounds
- The fail or difficulty to find and identify the trace compounds thus cannot be used as fingerprint of the original material. To improve the situation, the necessary software for data processing can be applied

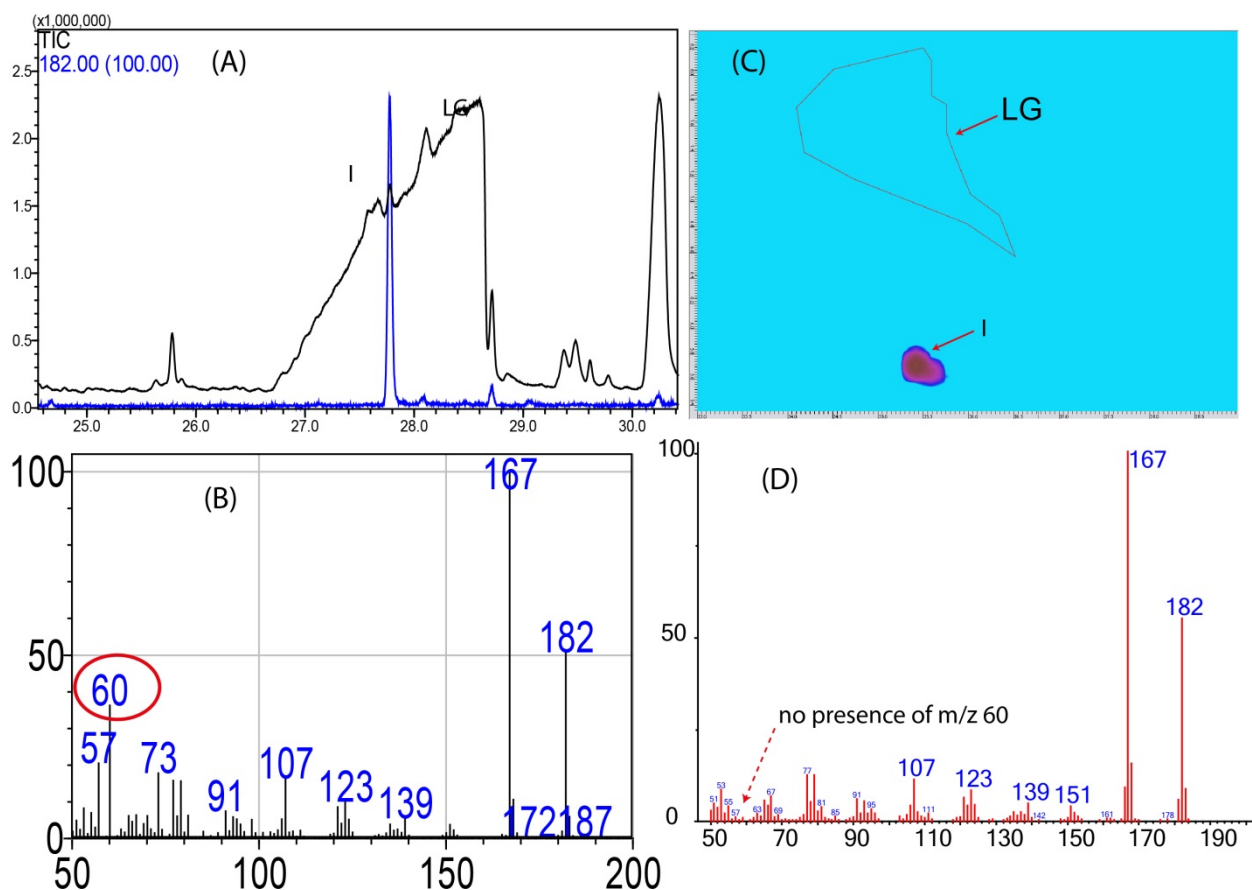


Figure 3.6.1 The chromatographic location of compound I illustrated with m/z 182 in 1D profile (A) and its mass spectra after background subtraction (B); in 2D profile (C) and its mass spectra after background subtraction (D)

The levoglucosan (LG) present high intensity and have a very broad eluting peak shape as can be obviously observed in 1D and 2D analysis where some other compounds may possibly co-elute with it. For instance, it can be seen from Fig.3.6.1 that compound I has been co-eluting with LG in 1D analysis while it has been well separated from LG in 2D analysis. After background subtraction, 1D and 2D give almost the same mass spectra (with 1D mass spectra have more intensity of m/z 60). The compound has been assigned as 4-Ethyl-2,6-dimethoxypheno (lignin structure compound) by analyzing its mass spectra and in comparison with previous studies [43]. Although 1D and 2D provide the same quality mass spectra after background subtraction, one improvement lies in that compound VII has been separated from LG. Furthermore, in considering the potential to use lignin monomers as biomarkers, the 2D separation make it possible for easy integration of compound VII as parameters used in

statistical analysis while in 1D analysis this task is difficult to perform with the influence of LG (co-eluting).

Moreover, the structure of lignin monomer group-type patterns can be exploited as another dimension for identification purposes and quantification purposes. For lignin pyrolysis product analysis, the polarity information (together with the volatility) can be practically useful when considering lignin monomers with similar skeletons but different branched chains. The compound identification of pyrolysis of lignin monomers within the mitsumata handmade paper was shown in Fig.3.6.2 and Fig.3.6.4 and compound identification has been proposed in Fig.3.6.3. As is revealed, the formation of group-type patterns can be observed with different lignin monomers resulted in a ‘structured’ chromatogram since these compounds presenting similar values for one of the two properties (boiling point vs polarity). The well grouping features in the 2D analysis can be summarized in an order of polarity:

- The H type monomers (I region in Fig.3.6.2) eluted first indicating its lower boiling point and lower polarity. A linear group feature has been observed within this group
- The G type+alkyl group elutes second (II region in Fig.3.6.2) and a horizontal elongated cluster (parallel to the 1st dimension in the 2D plot) was observed
- The S+alkyl group elutes thirdly (III region in Fig.3.6.2) and a horizontal elongated cluster was also observed
- The S+alkenyl group elutes fourthly (IV region in Fig.3.6.2) with a horizontal elongated cluster observed
- The G+keto-/aldehyde- group elutes fifthly (V region in Fig.3.6.2) with a horizontal elongated cluster observed
- The S+keto-/aldehyde- group elutes lastly (VI region in Fig.3.6.2) with a horizontal elongated cluster observed

As a result of the structured 2D chromatogram, the identification of the pyrolysis products of lignin monomers has become easier with the information available for identification: volatility, polarity and mass spectra. With the obtained results, the location of lignin monomers can be easily and visually predicted in the 2D plot and thus can be further explored for quantification purposes.

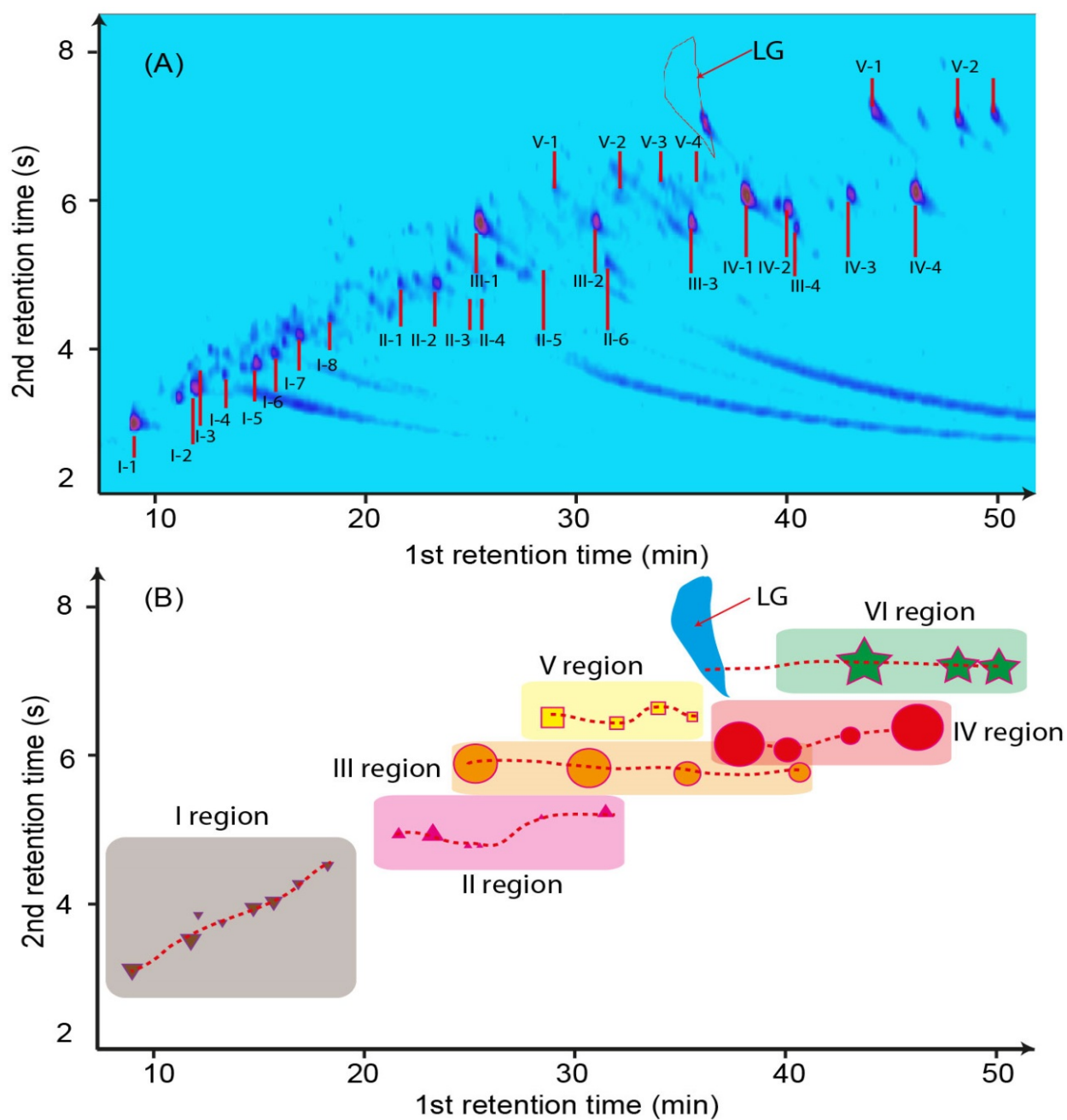


Figure 3.6.2 The 2D distribution of all the identified lignin monomers (A) illustrated with selected EICs. (B) a sketching showing the group features with compound's relative intensity.

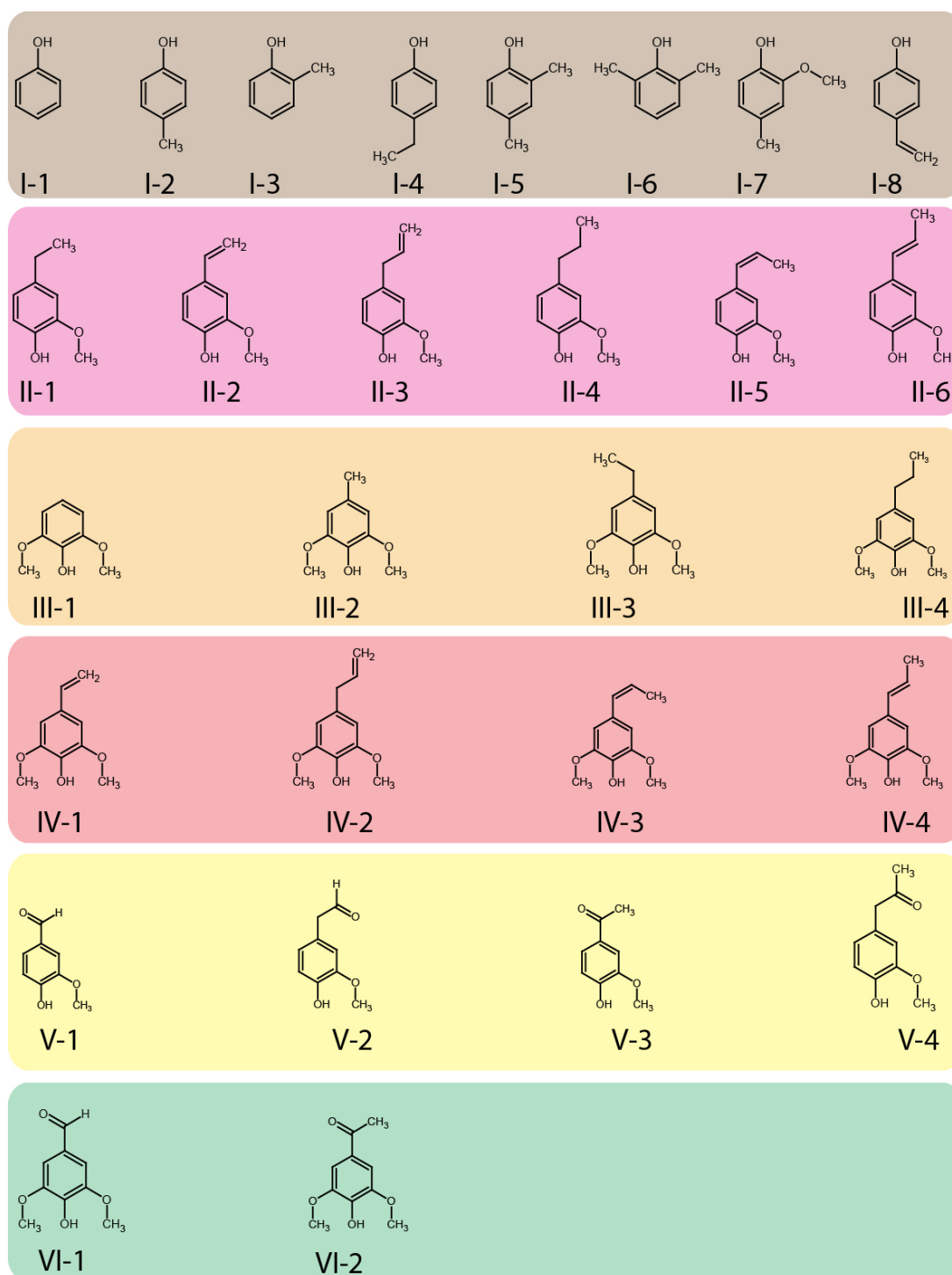


Figure 3.6.3 The identified lignin monomers.

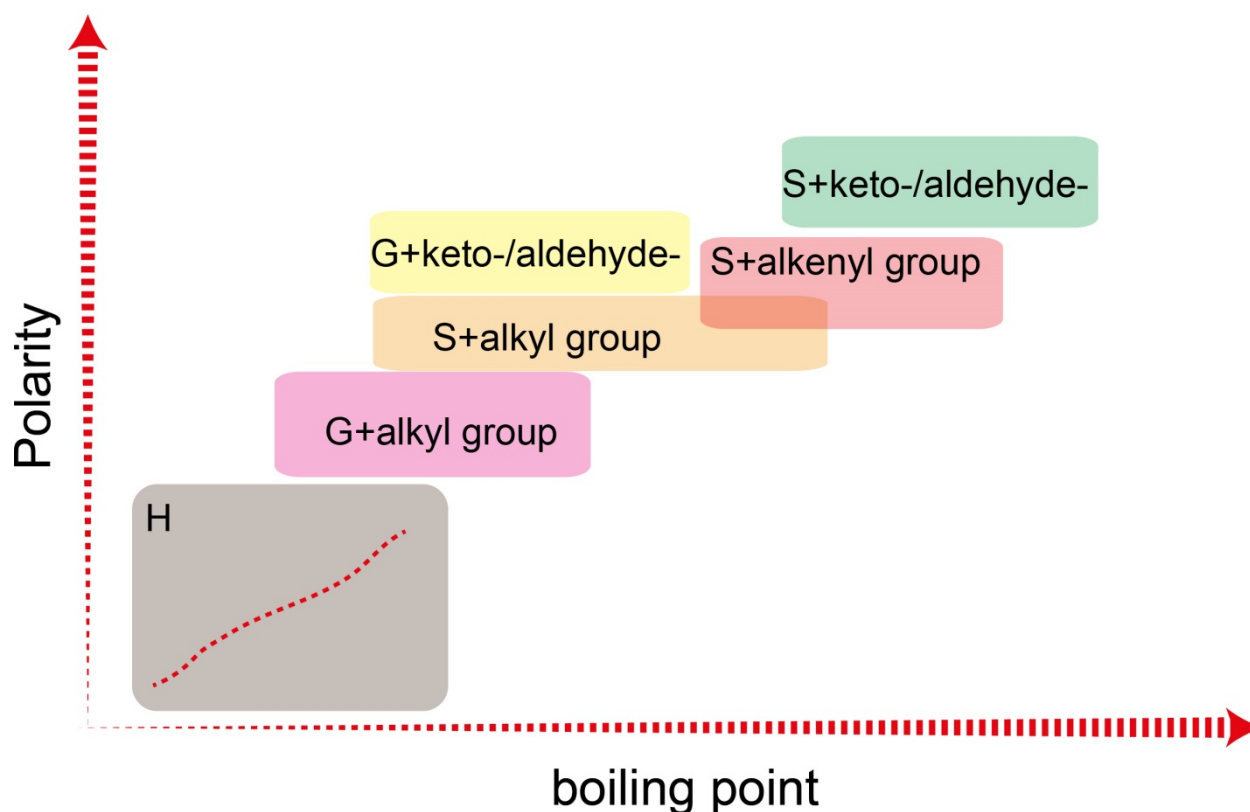


Figure 3.6.4 The identified lignin monomers.

3.6.3.2 Sizing agent analysis

The sizing agent is another paper related topic. In the hand papermaking, the starch was primarily used while the use of animal glue (protein colloid) has been commonly practiced since Song Dynasty. a Py-GC/MS profile of a collagen sample is characterized (Fig.3.6.5A):

- By the presence of light weight compounds like toluene and pyrrole at the beginning of the chromatogram
- By an intermediate region made up mainly of heterocyclic hydrocarbon derivatives
- By the presence of diketopiperazines issued from the thermal degradation process of the amino acid chains.

Comparatively, the Py-GCxGC/MS profile of the same reference collagen sample shows (Fig.3.6.5B):

- An extended distribution of the whole thermal decomposition products of the amino acid chains resulting in an improved collagen fingerprint
- A series of long chain alkanes/alkenes spanning until the C24 homologues
- Fatty acid derivatives and long chain nitriles

- A nicely resolved region of elution for the observed cholestane congeners.

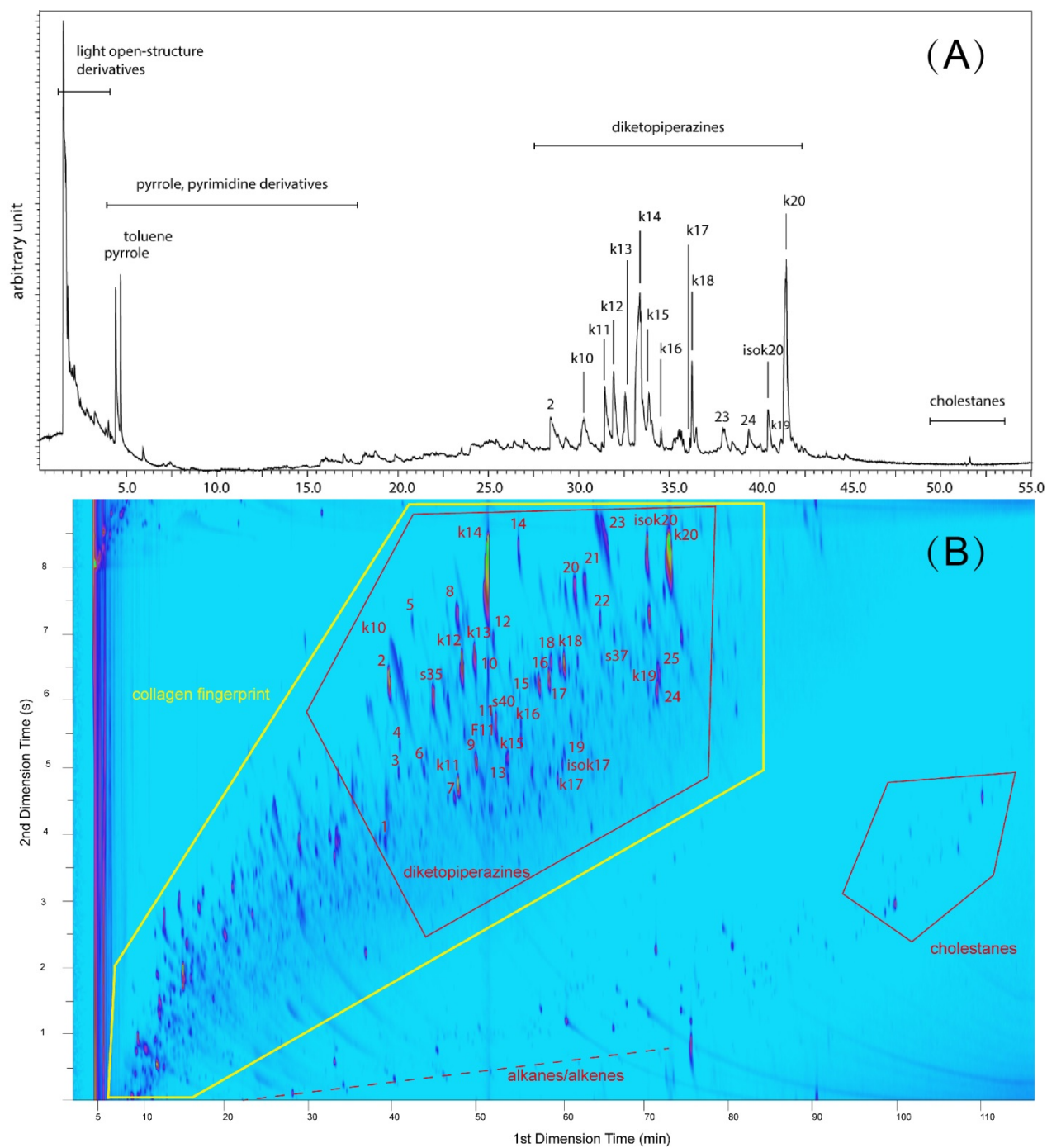


Figure 3.6.5 The pyrolysis of collagen in 1D and 2D analysis.

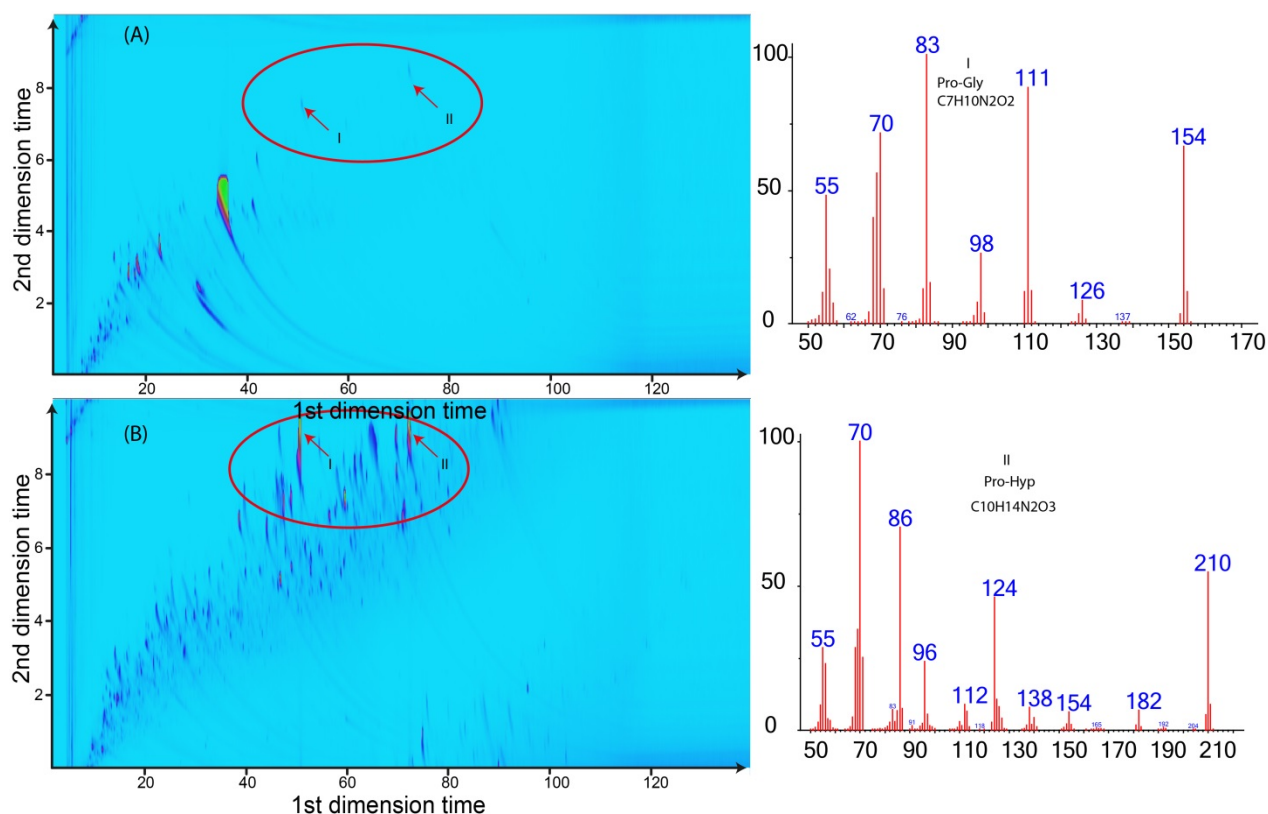


Figure 3.6.6 The pyrolysis of handmade papers with sizing agent inside (A) and the pyrolysis of collagen (B).

It clearly appeared that through two-dimensional gas chromatography separation conditions, analysis of the collagen samples was improved with a net increase in the information content of the collagen fingerprint. The most noticeable gain was obtained for the resolution of the diketopiperazine region. In the manuscript analysis, the handmade papers with sizing agent were observed with some peaks from the collagen (Fig.3.6.6). A further investigation may possibly reveal the origin of the sizing agent.

3.6.3.3 A summary

In this thesis, The importance of the Gol Mol paper unearthed in Mongolia was re-evaluated and used as a key point to form a ‘paper distribution belt’ before East Han Dynasty. Currently, it has only been analyzed by microscopic observation, if possible, the Py-GCxGC analysis may provide more information. In the timeline for the discussion of paper in the initial use as filings and waddings in the tomb, the validation of it as paper or fragment, is key information that is currently unavailable. The further analysis of the archaeological data and new archaeological evidence (if any) may give more strong evidence.

The difference in the metabolite’s distribution in the ROI with different plants must lies in the plants metabolite synthesis pathway and finally may differ in their genetic differences. In this

thesis, the metabolites have been utilized as markers to discriminate handmade papers from different material origins while the specific synthesis mechanism has only been explained generally (section 1.5). The further study on the specific case may explain the difference in their metabolite compounds distribution patterns such as the intensity difference of α -amyrin acetate between *Morus spp* and *Broussonetia spp*.

As a way to solve the problem encountered in the *Ma* group fiber that presents few markers in the ROI and the problem in the identification of the Astana paper (section 3.4), a possible way could be to extend the markers detection to the lignin monomers. However, direct pyrolysis handmade papers in 1D analysis results in the lignin monomers co-eluting with the cellulose pyrolysis products, a point hindering its precise quantification. As has been demonstrated in section 3.6.3, due to the difference in the polarity, the lignin and the cellulose pyrolysis products have been nicely separated and make it possible for quantification purpose with minor quantity required in GCxGC.

As has been briefly illustrated in section 3.6.3, the compounds from the sizing agent in the paper can be easily traced during the pyrolysis of handmade papers. In fact, a visual check of the collagen fingerprint can reveal that the complexity in the sizing agent exceeds the capacity of 1D analysis. A further analysis of the sizing agent in the handmade papers may reveal more information concerning the technique of hand papermaking.

Within this thesis, principal component analysis (PCA) has been utilized to visually depict the differences in the profile of ROI. However, the work for the method validation has not been conducted at the beginning since fewer samples were available for testing. As the collaborations go on, more and more samples were provided for testing and the method validation could be possible to conduct based on the work carried out during the PhD studies.

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List of publications and conferences

Publications

1. **Bin Han**, Ghizlène Daheur, Michel Sablier, Py-GCxGC/MS in cultural heritage studies: An illustration through analytical characterization of traditional East Asian handmade papers, **Journal of Analytical and Applied Pyrolysis**. 2016, 122, 458-467.
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Conferences

1. **Bin Han**, Michel Sablier. Elucidation of Ancient Asian Papermaking Processes using Py-GCxGC/MS. 3rd YNHM , Paris, **France**, 2-6 Feb 2016 (**poster**)
2. Ghizlène Daheur, **Bin Han**, Michel Sablier, and Léon-Bavi Vilmont. Characterization of Traditional East Asian Papers Using Py-GC/MS and Py-GCxGC/MS. 21st International Symposium on Analytical and Applied Pyrolysis. Nancy, **France**, 9-12 May 2016 (**poster**)
3. **Bin Han**, Michel Sablier. Benefits of the use of Py-GCxGC/MS compared to Py-GC/MS in cultural heritage studies. The 13th Symposium on Archaeometry. Chongqing, **China**, 16-20 Oct 2016 (**oral presentation**)
4. **Bin Han**, Michel Sablier. A new method for the characterization of botanical resources used for traditional East Asian handmade papers. 4th YNHM, Paris, **France**, 7-11 Feb 2017 (**oral presentation**)
5. **Bin Han**, Jérôme Vial, Masamitsu Inaba, Michel Sablier. Bénéfice d'une approche bidimensionnelle pour la caractérisation des matériaux du patrimoine: application au cas des papiers traditionnels asiatiques. Le 12ème congrès francophone des sciences séparatives et des couplages SEP 2017. Paris, **France**, 28-30 Mar, 2017 (**poster**)
6. **Bin Han**, Jérôme Vial, Masamitsu Inaba, Michel Sablier. Application of Py-GCxGC/MS

Combined with Chemometrics for the Elucidation of Ancient East Asian Papermaking Materials. 65th ASMS Conference on Mass Spectrometry and Allied Topics. Indianapolis, USA, 4-8 Jun, 2017 (**oral presentation**)

7. **Bin Han**. A new approach for the characterization of East Asian handmade papers. Un Papier à d'hier pour demain-La pérennité du papier à la forme en Orient et en Occident. **Musée du Louvre**, Paris, 24, Nov 2017. (**oral presentation**)
8. **Bin Han**, Jérôme Vial, Michel Sablier. Fiber identification of traditional East Asian handmade papers through multivariate data analysis of pyrolysis-GC/MS data. CHIMIOMETRIE XIX, Paris, France, 30-31 Jan, 2018. (**Poster**)
9. **Bin Han**, Silvia Lob, Michel Sablier, How the use of GCxGC/MS profiles can benefit to 1D GC/MS data treatment: Illustration with the analysis of the pyrolysis products of East Asian handmade papers. 22nd International Symposium on Analytical and Applied Pyrolysis. Kyoto, **Japan**, 3-8 Jun 2018 (**poster**)

Annex

Annex 1 Definition of paper-*Cihai* (dictionary)

辞海（1999 年版）。纸：用以书写、印刷、绘画或包装等的片状纤维制品。一般由经过制浆处理的植物纤维的水悬浮液，在网上交错组合，初步脱水，再经压榨、烘干而成。

Annex 2 Definition of paper-Chinese paper historian, Pan Jixing

纸史学者潘吉星先生：传统上所谓的纸，指植物纤维原料经机械、化学作用制成纯度较大的分散纤维，与水配成浆液，使浆液流经多孔的模具帘滤去水，纤维在帘的表面形成湿的薄层，干燥后形成具有一定强度的由纤维素靠氢键缔合而交结成的片状物，用作书写、印刷和包装等用途的材料。

Annex 3 *Guofeng•Odes of Wei•Among their ten acres*

(1) 国风·魏风·十亩之间

十亩之间兮，桑者闲闲兮。行与子还兮。

十亩之外兮，桑者泄泄兮。行与子逝兮。

Annex 4 *Guofeng•Odes of Bin•July*

(2) 国风·邶风·七月

七月流火，九月授衣。一之日觶发，二之日栗烈。无衣无褐，何以卒岁。三之日于耜，四之日举趾。同我妇子，饁彼南亩，田峻至喜。

七月流火，九月授衣。春日载阳，有鸣仓庚。女执懿筐，遵彼微行，爰求柔桑。春日迟迟，采繁祁祁。女心伤悲，殆及公子同归。

七月流火，八月萑苇。蚕月条桑，取彼斧斨，以伐远扬，猗彼女桑。七月鸣鵙，八月载绩。载玄载黄，我朱孔阳，为公子裳。

.....

Annex 5 *Guofeng•Odes of Wei•Meng*

(3) 国风·卫风·氓

氓之蚩蚩，抱布贸丝。匪来贸丝，来即我谋。送子涉淇，至于顿丘。匪我愆期，子无良媒。将子无怒，秋以为期。

.....

桑之未落，其叶沃若。于嗟鸠兮，无食桑葚！于嗟女兮，无与士耽！士之耽兮，犹可说也。女之耽兮，不可说也！

桑之落矣，其黄而陨。自我徂尔，三岁食贫。淇水汤汤，渐车帷裳。女也不爽，士贰其行。士也罔极，二三其德。

.....

Annex 6 *Guofeng•Odes of Chen•the moat at the east gate*

(4) 国风·陈风·东门之池

东门之池，可以沤麻。彼美淑姬，可与晤歌。

东门之池，可以沤纈。彼美淑姬，可与晤语。

东门之池，可以沤菅。彼美淑姬，可与晤言。

Annex 7 *Yu Jue Shu**

《越绝书》：子胥**遂行。至溧阳界中，见一女子击絮于濑水之中，子胥曰：“岂可得托食乎？”女子曰：“诺。”即发簞饭，清其壶浆而食之。子胥食已而去，谓女子曰：“掩尔壶浆，毋令之露。”女子曰：“诺。”子胥行五步，还顾女子，自纵于濑水之中而死。

*Yu Jue Shu was an unofficial history book written by Ping Wu (East Han Dynasty).

*Wu Zixu (伍子胥, ~6 Century BC-484 BC) was a general and politician of the Wu kingdom in the Spring and Autumn period (722–481 BC). Since his death, he has evolved into a model of loyalty in Chinese culture.

Annex 8 *Seeds for a giant gourd in the book Zhuangzi**

惠子谓庄子曰：“魏王贻我大瓠之种，我树之成而实五石。以盛水浆，其坚不能自举也；剖之以以为瓢，则瓠落无所容。非不呶然大也，吾为其无用而掊之。”庄子曰：夫子固拙于用大矣！宋人有善为不龟手之药者，世世以**洴澼絖**（píng pì kuàng）为事。客闻之，请买其方百金。聚族而谋曰：“我世世以**洴澼絖**，不过数金；今一朝而鬻（yù）技百金，请与之。”客得之，以说吴王。越有难，吴王使之将。冬与越人水战，大败越人，裂地而封之。能不龟手一也，或以封，或不免于洴澼絖，则所用之异也。今子有五石之瓠，何不虑以为大樽，而浮乎江湖？而忧其瓠落无所容？则夫子犹有蓬之心也夫！

Huizi, speaking to Zhuangzi: “The King of Wei gave me some seeds for a giant gourd. I planted them, and they grew to the size of five bushels. I used them as water containers, but they were so heavy that I couldn't lift them. I cut them into pieces to make ladles, but they were so flat and broad that they wouldn't hold anything. It's not that they weren't huge (as promised), but I had no use for them, and broke them into pieces.” Zhuangzi said: “Well you are certainly clumsy when it comes to using large things, aren't you? Among the Song people there were some who were good at making a salve that prevented the chapping of the hands. They used it for generations in their family business for bleaching silk. A traveler heard of it, and offered to buy the formula for one hundred pieces of gold. The people of the clan go together and discussed it. One said: “We've been bleaching silk for generations, never making more than several pieces of gold. Now, in one morning we can make a sale of the technique for one hundred pieces. I say we go for it.” The traveler took it, and told the king of Wu about it. There was trouble with Yue, and the king of Wu made him a commander. It was winter, and there was a naval battle with Yue, in which they routed the Yue. So the king took a piece of the conquered land and enfeoffed him. Now, the ability to prevent the chapping of the hands is one, but one fellow was able to turn this into a fiefdom, while another could not get out of bleaching silk. This is the difference in usage. Now you, sir, have a gourd five bushels in size. Why didn't you think of making it into a great barrel and use it to float on the rivers and lakes? Instead you suffer over the uselessness of large dippers. It's like you have brambles in your brain!”

*Zhuang Zhou (369-286 BC), often known as Zhuangzi (Master Zhuang, 庄子), was an influential Chinese philosopher who lived around the 4th century BC during the Warring States period, a period corresponding to the summit of Chinese philosophy, the Hundred Schools of Thought. He is credited with writing, in part or in whole, a work known by his name, the Zhuangzi, which is one of the foundational texts of Daoism.

Annex 9 *Records of the Grand Historian, the marquis of Han Xin**

淮阴侯韩信者，淮阴人也。始为布衣时，贫无行，不得推择为吏，又不能治生商贾。常从人寄食饮，人多厌之者。常数从其下乡南昌亭长寄食，数月，亭长妻患之，乃晨炊蓐食。食时，信往，不为具食。信亦知其意，怒，竟绝去。

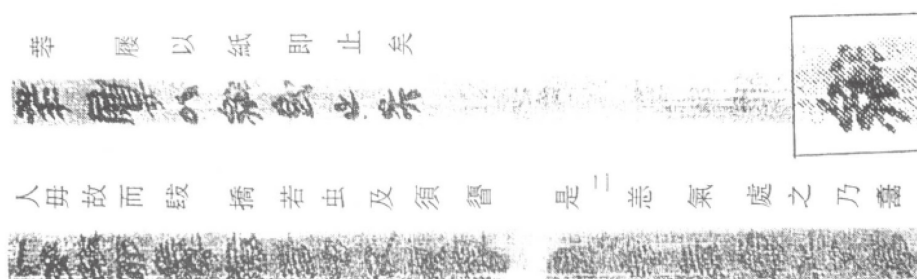
信钓于城下，诸母漂，有一母见信饥，饭信，竟漂数十日。信喜，谓漂母曰：“吾必有以重报母。”母怒曰：“大丈夫不能自食，吾哀王孙而进食，岂望报乎！”

The Marquis of Han Xin was born in Huai Yin. When he was a civilian, he was poor and had no good deeds to be recommended to be officials. He also cannot make a living by doing business. He often had meals for free in his friends' house so that people don't like him. He once had free meal in the house of Nanchang tingzhang, Xia Xiang County for several months. Tingzhang's wife detest Xin. She cooked in the morning and eat on the bed (under the quilt⁷⁴). When there was time for dinner, Xin came. However, the wife did not prepared food for him. Xin understood her meanings and became angry. He never been there again.

Xin fished outside of the city where he witnessed many women washing rags in the water. A women saw the hungry Xin and gave him some food. These women continuously worked for several tens of days (as well as gave him food). Xin was happy, he said to the women. "I will repay the obligation". The woman was angry and said: you being a man but cannot support yourself, I was pity on you to give you food, how do I expect you repay the obligation?

* Han Xin (230 BC-196 BC) was a military general who served Liu Bang during the Chu-Han Contention and contributed greatly to the found of the Han dynasty. Han Xin was named as one of the "Three Heroes of the early Han dynasty", along with Zhang Liang and Xiao He.

Annex 10 *The Chinese character on the Shuihudi Qin bamboo slip*



人毋故而拔发播，若虫及须聚署眉，是一是恙气处之，乃滋煮拿责属以纸，即止矣⁷⁵。

⁷⁴ The discription is a little exaggerated.

⁷⁵ The text and picture are in reference to 钱存训，书于竹帛书. 上海世纪出版社，上海书店出版社，2003.

Annex 11 *San fu jiu shi*

汉太始四年。卫太子大鼻。武帝病，卫太子入省，江充曰：“上恶大鼻，当持纸蔽其鼻而入。”帝怒。

卫太子岳鼻，太子来省疾，至甘泉宫。江充告太子：“勿入。陛下有诏恶太子岳鼻，尚以纸蔽其鼻。充语武帝曰：“太子不欲闻天子脏臭，故蔽鼻”。武帝怒太子，太子走还。

Prince Wei has big nose (like mountains). (Emperor Wu got sick) Prince Wei came to see him at palace Ganquan. Jiang Chong told Prince Wei: wait, Emperor Wu do not like your big nose, you should use paper to cover your nose before enter. (However,) Jiang Chong said to Emperor Wu: 'Prince Wei do not like your smell so he cover his nose with paper'. Emperor Wu got angry and let Prince Wei get out.

Annex 12 *The Han Feizi-deadly trap with cover of the nose*

夫人知王之不以己为妒也，因为新人曰：“王甚悦爱子，然恶子之鼻，子见王，常掩鼻，则王长幸子矣。”于是新人从之，每见王，常掩鼻。王谓夫人曰：“新人见寡人常掩鼻，何也？”对曰：“不己知也。”王强问之，对曰：“顷尝言恶闻王臭。”王怒曰：“剿之。”夫人先诫御者曰：“王适有言，必可从命。”御者因揄刀而剿美人。

The queen was jealous of the new concubine of King Huai. She told the concubine 'King Huai like you very much, however, he do not like your nose. When you go to see him, you should cover your nose so that he will be infatuated with you for a longer time' the concubine followed the advice. Every time she met with King Huai, she covered her nose. King Huai asked the queen: 'why dose the new concubine cover her nose when I met her?' the queen answered: 'I am not supposed to talk about it', King Huai ask again (compulsory). The queen said: 'she often said to me that she don't like the smell of you' King Huai got angry and said '(then) cut her nose off!' the queen warned the soldier 'the king has (just now) make an order to cut the concubine's nose, you must follow the order'. So the soldier went with knife and cut the concubine's nose off.

Annex 13 *Book of Han*

武发篋中有裹药二枚，赫蹏书，曰...饮此药，不可复入。女自知之！”

说文解字：絳，繫，繫蹏也。一曰惡絮。從糸，軟聲。

颜师古注：邓展曰：赫音兄弟阅墙之阅。应劭曰：赫蹏，薄小纸也。

应劭（143-198AD）：赫蹏，薄小纸也。孟康（194-250AD）：赫蹏地也，染纸、素令赤而书之，若今黄纸也。宋，赵彦卫《云麓漫钞》卷七：“《赵后传》所谓‘赫蹏’者，注云‘薄小纸’，然其寔亦缣帛。

Annex 14 *Fengsutongyi*

唐马总《意林》卷四摘引东汉应劭《风俗通义》谓：“光武车驾徙都洛阳，载素、简、纸经凡二千辆。”此话说的是东汉光武帝刘秀享国之后，将都城从长安迁往洛阳，其中所搬运的经籍就装有两千车。而所载两千车的书籍中，

有的用缣素写成，有的用竹木简写成，也有的用纸张写成。古人虽有将缣帛就说成纸的习惯。

Annex 15 *Book of houhan*

《后汉书 百官志》：光武帝（25-57AD）时，在尚书令之下设左右丞各一人“右丞假署印绶及纸笔墨诸财用库藏”

《后汉书》卷 66《贾逵传》：建初元年（76AD）诏逵入宫讲《左氏传》“令逵自选公羊、严、颜诸生高材者二十人，教以左氏，与简、纸经传各一通”

《后汉书》卷 10 上《和熹邓皇后传》：至冬，立为皇后。辞让者三，然后即位。手书表谢，深陈德薄，不足以充小君之选。是时，方国贡献，竞求珍丽之物，自后即位，悉令禁绝，岁时但供纸墨而已。帝每欲官爵邓氏，后辄哀请谦让，故兄骘终帝世不过虎贲中郎将。

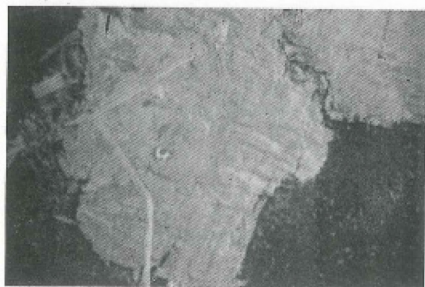
Annex 16 *Cai Lun Zhuan*

《后汉书》卷七十八 宦者列传第六十八, Biographies of Eunuchs, 445AD.

蔡伦字敬仲，桂阳人也。以永平末始给事宫掖，建初中，为小黄门。及和帝即位，转中常侍，豫参帷幄。伦有才学，尽心敦慎，数犯严颜，匡弼得失。每至休沐，辄闭门绝宾，暴体田野。后加位尚方令。永元九年，监作秘剑及诸器械，莫不精工坚密，为后世法。自古书契多编以竹简，其用缣帛者谓之纸。缣贵而简重，并不便于人。伦乃造意，用树肤、麻头及敝布、鱼网以为纸。元兴元年奏上之，帝善其能，自是莫不从用焉，故天下咸称“蔡侯纸”。元初元年，邓太后以伦久宿卫，封为龙亭侯，邑三百户。后为长乐太仆。四年，帝以经传之文多不正定，乃选通儒谒者刘珍及博士良史诣东观，各讎校家法，令伦监典其事。伦初受窦后讽旨，诬陷安帝祖母宋贵人。及太后崩，安帝始亲万机，敕使自致廷尉。伦耻受辱，乃沐浴整衣冠，饮药而死。国除。

《东关汉记》：蔡伦，字敬仲，为中常侍，有才学，尽忠重慎，每至休沐，辄闭门绝宾客，曝体田野。黄门蔡伦，字敬仲，典作上方，造意用树皮及敝布、鱼网造纸，奏上，帝善其能，自是莫不用，天下咸称蔡侯纸也。伦典上方，造纸，用故麻造者谓之麻纸，用木皮名穀纸，用故鱼网名网纸。

Annex 17 *Gaojiabao fragments (~1000 BC)*



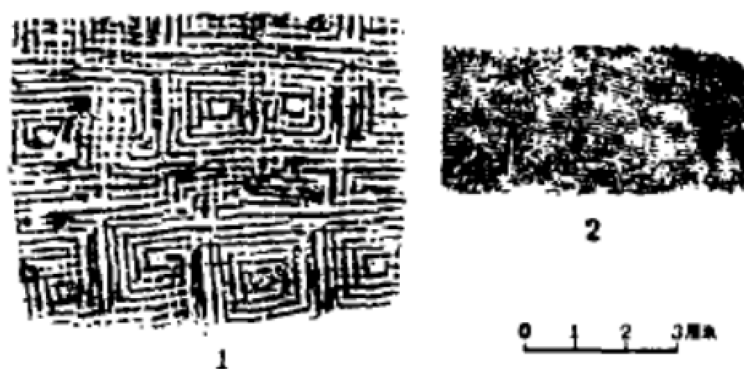
2. 采于二号墓簠下的“纸”



3. 采于二号墓簠圈足外的“纸”

In 1986, a Zhou dynasty tomb was excavated. This tomb was dated around 1000 BC and consisted of places defined as Mi. M1, M4 were identified as the tomb of kings while M2, M3 are identified as tombs of nobles, M5 of civilians and M6 of slaves. Paperlike fragments were found in M2 and M4 under the feet of the bronze object Gui (簋). In the archaeology report it is wrote: in 1992, it was identified by Wang as material similar to the papyrus of ancient Egypt. It was identified as a plant material without detail specification⁷⁶.

Annex 18 Shucheng fragments (657-615 BC)



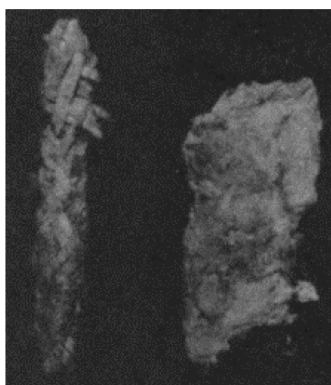
布的破片珑迹, 分粘于裁鼎、蟾、盖形器和盘形器的表面。每樱粗糊不匀, 无光泽。用放大统观察, 其粗藏有粗韧两种删布珑迹粘于盛羊兹鼎盖面上, 每平方厘米的握樟抄各为二十四樱。粗布遣迹粘于盖形器面上图四, 助和蟾的表面上者, 每平方厘米的粗樟抄各为十七旗。与长沙号战国墓中布片相比, 则后者比舒城出土铜器上的布迹要紧密些。与今之棉布比, 韧的一种几乎与之相近粗的一种仍较粗疏。即此舫藏的水平, 已很可观了。捐粗厚之林为之⁷⁷。

In 1959, in a tomb unearthed in Shucheng, Anhui province. Traces of cloth fragment was found on the surface of Ding (鼎), Chan(蟾), cover-shaper ware (盖形器) and disk-like ware(盘形器). The fragments were rough without gloss. Observed with magnifying glass, there was two different kinds of fragments that differ in thickness: the thin one was found on the surface of the cover of Ding (鼎), 24 thread per square centimeter; the thick one was found on the Chan(蟾) and cover-shaper ware (盖形器), 17 thread per square centimeter. Silk fragment was also found on the surface of bronze ge (铜鬲). The thread is thin and even with gloss. It was 25 thread \times 17 thread in square centimeter.

⁷⁶ In reference to (i) 陕西省考古研究所, 高家堡戈国墓. 1995: 三秦出版社; (ii) 王野, 《高家堡戈国墓》评介. 考古, 1997(1): p. 95-96. The sample picture is from the references and used here for better understanding of corresponding text, for more information, please refer to the original article. The purpose for the sample picture also applied to those in Annex 10, Annex 18- Annex 30.

⁷⁷ In refernce to 殷滌非, 安徽舒城出土的铜器. 考古, 1964(10): p. 498-503.

Annex 19 Daheiting fragments (~300 BC)



In 1989, a paperlike wadding was discovered in a tomb excavated in Qianxi County, Hebei province. It was described as: paperlike fragment (paper?), fragment, light yellow color, cellulosic material, several layers compressed together. No scientific analysis was reported afterwards⁷⁸.

Annex 20 Fangmatan paper (179-142 BC)

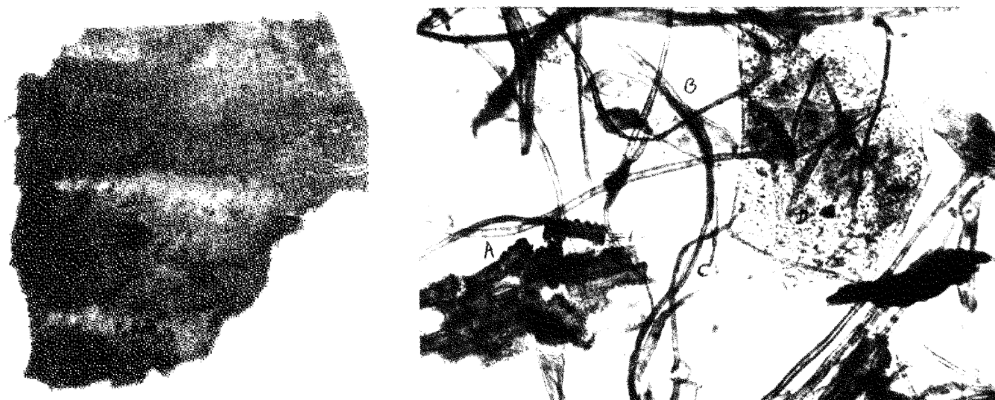


In 1986, Gansu Institute of Archeology excavated the Fangmatan tomb in Tianshui City. The tomb (M5) was dated within the period of Emperor Wen of Han and Emperor Jing of Han (180-142 BC) according to the structure of the tomb and the burial objects. In M5, a paper based map (M5:5) was discovered: It is 5.6cm*2.6cm, thin and soft, located on the chest of the dead body. Due to the humidity in the tomb, it has degraded into pieces. It presents the color of yellow when unearthed and later became light gray-yellow. It has stains on the surface. The surface is very flat. There were black lines mapping of maintains, rivers, roads. The drawing method is similar to the maps on the silk unearthed in the Mawangdui tomb⁷⁹.

⁷⁸ In reference to 顾铁山, 郭景斌, 河北省迁西县大黑汀战国墓. 文物, 1996(3): p. 4-17.

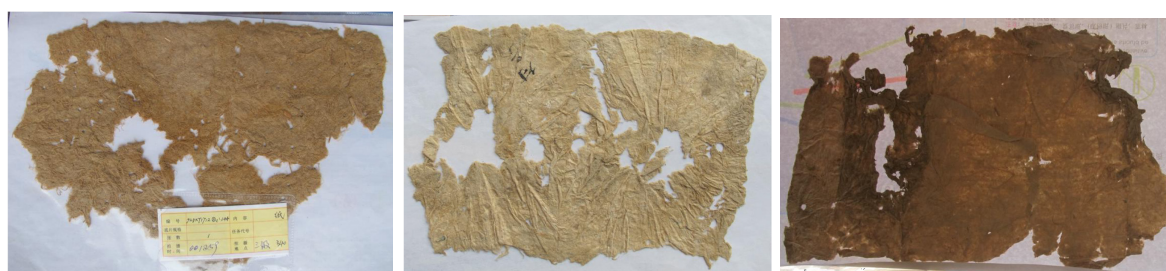
⁷⁹ In reference to (i)长沙马王堆二、三号汉墓发掘简报. 文物, 1974(7): p. 39-48; (ii) 田建, 何双全, 甘肃天水放马滩战国秦汉墓群的发掘. 文物, 1989(2): p. 1-11; (iii) 李晓岑. 甘肃天水放马滩西汉墓出土纸的再研究. 考古, 2016(10):110-114.

Annex 21 Sichuan paper (~150BC)



In 1995, a paper sample was found in Shuangbaoshan tomb in Sichuan. In 1998, Wang analyzed and concluded it to be a rice paper (made of rice straw), and consequently concluded it is not early paper as rice straw was only later used in papermaking⁸⁰.

Annex 22 Xuanquan paper (140-7 BC)



In 1990, lots of documents from Han dynasty was unearthed in Xuanquanzhi site, Dunhuang city. Among them, a total of 35000 bamboo slips was unearthed: 23000 with characters on it and 1900 with precise date on it. As for the paper, 10 papers with characters (9 from Han dynasty and 1 from Jin dynasty) as listed in table 1 below. Ca. 460 Ma (hemp) paper was discovered in this site lasting for a long period of time. They can be classified into 8 groups according to their color and thickness: black-thick, black-thin, brown-thick, brown-thin, white-thin, white-thick, yellow-thin, yellow-thick. Also, with unearth of silk, bamboo, wood and paper, it proved that they were used in the west Han dynasty for writing materials⁸¹.

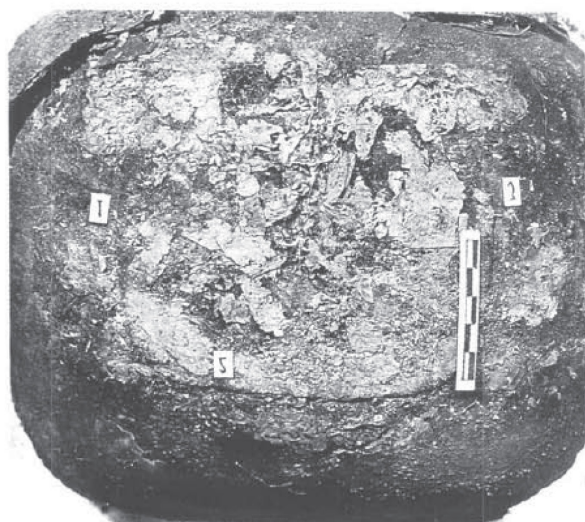
⁸⁰ In referene to (i) 何志国, 四川绵阳永兴双包山一号西汉木椁墓发掘简报. 文物, 1996(10): p. 4-12; (ii) 赵树中等, 绵阳永兴双包山二号西汉木椁墓发掘简报. 文物, 1996(10): p. 13-2; (iii) 王菊华, 陈启新. 中国古代造纸工程技术史. 山西教育出版社, 2006.

⁸¹ In reference to 何双全, 甘肃敦煌汉代悬泉置遗址发掘简报. 文物, 2000(5): p. 4-20; (ii) 李晓岑. 甘肃汉代悬泉置遗址出土古纸的考察和分析. 广西民族大学学报(自然科学版), 2010, 16(4):7-16.

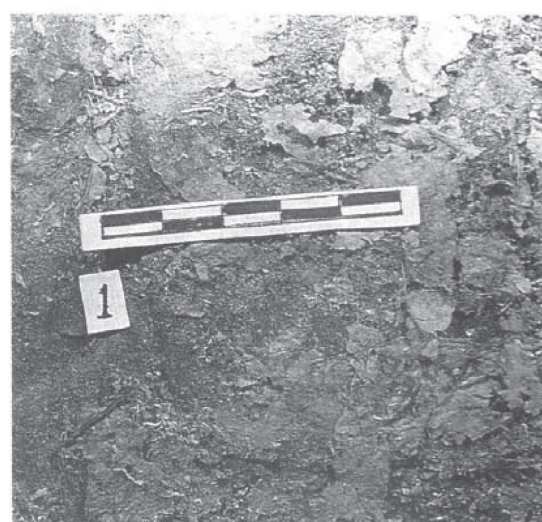
Table A1 the statistics of some samples from the reference articles.

No		size	typeface	character	Description&use
T0212④:1	汉武帝、昭帝	18×12cm	Clerical script	付子	package for medicine (based on shape and folding of the paper)
T0212④:2	时期 (157-	12×7cm	Clerical script	熏力	
T0212④:3	74BC)	3×4cm	Clerical script	细辛	
T0114③:609	汉宣帝-汉成帝 (91-7BC)	7×3.5cm	Cursive script	x 持书来 x 致嵩	irregular shape, yellow- white, thin and flat, tenacity
—		—	—	—	—
—		—	—	—	—
—		—	—	—	—
T0111①:469	东汉初期	30×32cm	Clerical script	巨阳大利上缮皂五匹	irregular rectangle, yellow-gray, thick, loose, rough
—		—	—	—	—
T0419①:15	西晋	14×7.5cm	—	—many	dark yellow brown, tight, uniform, tenacity

Annex 23 Xianggang paper (122-121 BC)



1. 两匣分开后露出纸状残片



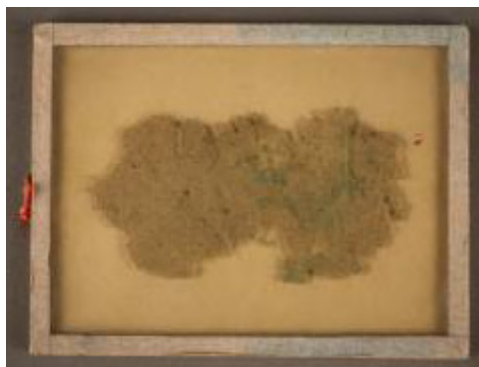
2. 纸状残片(局部)

In 1983, a tomb was excavated in Guangzhou, China. It is the tomb of Nan Yue Wang (137-122 BC). Due to its location in the southernmost part of the Western Han territory, the Nanyue state is a vassal state of the Western Han Dynasty but more like an independent kingdom. History of the Nanyue state, "Historical Records" and "Han" and other history books are documented, but the content is very simple. The excavation of the Nanyue King Tombs provided invaluable material for understanding the history of the South Vietnam State and the conditions in the Lingnan (Guang Zhou, southern China) area at that time.

A paper-like fragment was discovered in this tomb. It was discovered in the 西耳室, between two bronze Yi (铜匱) which was tight together by bamboo (竹筩). The upper Yi have lots of fragments, with a range of 18cm*15.5cm, all is fragment. The largest one being 3cm*4cm, the smaller one was like the fingernail. According to the archaeologists, there were waterlines on the stone door which indicate water has come into this tomb several times (the heavy one being on top of the tomb). It can be inferred that the water logging is severe. Due to

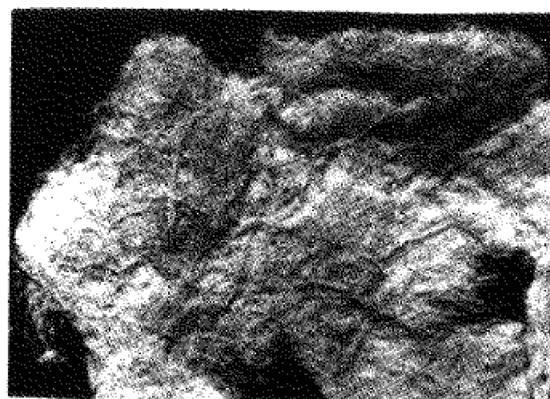
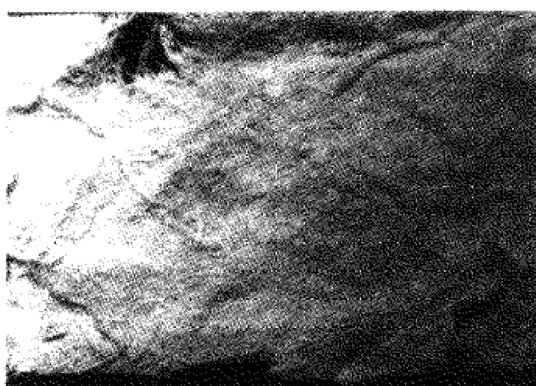
the water logging in the early years, the burial objects moves and the objects decayed some of which cannot be identified⁸².

Annex 24 Baiqiao Paper (118 BC)



In 1957, the Baiqiao paper was first reported (as silk in this report)⁸³. In this archaeological report, it is written: “they discovered three bronze mirror and under the mirror there is thin hemp cloth and under the cloth there was silk-like paper fragment, yellow and made with mature technique”.

Annex 25 Maquanwan Paper (65-23BC)



麻纸五件八片，出土时均已揉皱。T120:47 呈黄色，粗糙，纤维分布不均匀，边缘清晰，长、最宽厘米，为所有出土麻纸中最大的一片。同出的纪年简，最早为西汉宣帝元康，最晚为甘露年间。T10:06 T9:026，共四片，与畜粪堆积一起，颜色被污染，呈土黄色，质地较细匀。同出的纪年简多为成、哀、平帝时期。T9:25，呈白色，质地细匀，残边游麻纤维。T12:018 共二片，呈白色，质地细匀，系于堡内 F2 上层、烽健倒塌废土中发现，应为王莽时物。

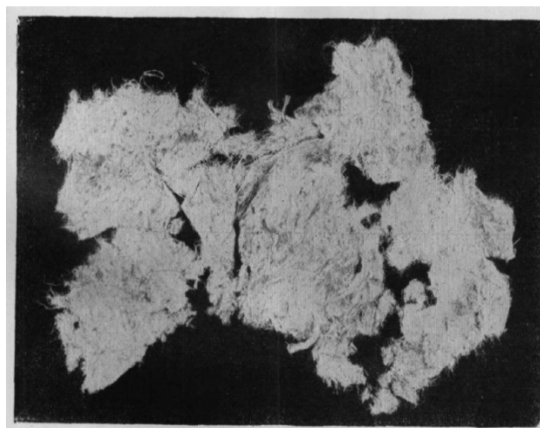
In 1979, the paper was discovered in Dunhuang. In the archaeological report it was written: Hemp paper, five item (into eight fragment), all have been crumpled upon unearthed. The T120:47 presents yellow color, rough, fiber dispersed unevenly, have clear edges, 32cm*20cm, is among the largest paper unearthed. According to the bamboo slip with written

⁸² In reference to (i) 西汉南越王墓发掘初步报告. 考古, 1984(3): p. 222-230; (ii) 西汉南越王墓. 文物出版社, 1991.

⁸³ In reference to 文物工作报导. 文物, 1957(12): p. 78-83. The picture is from Shaanxi History Museum.

date, it was within Emperor Xuan of Han (65-50 BC). T10:06 and T9:026 (four fragments) were stacked with animal excrement (contaminated by them), they present earthy yellow with fine grain. The bamboo slips with written date were mainly during (51BC-6AD). T9:25 presents white color with fine grain and have hemp fiber exposed on the edge. T12:018 (two fragments) presents white color with fine grain and probably within 8-17AD⁸⁴.

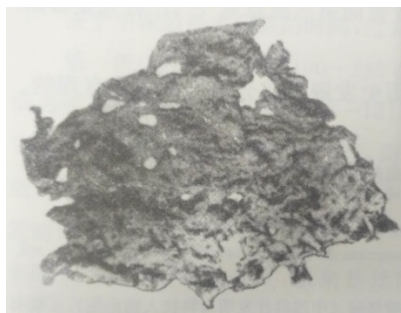
Annex 26 Jingguan paper (52-3 BC)



麻纸二种。纸 I (EJT1:011)出土 时团成一团, 经修复展平, 最大一片长宽 21*19cm 厘米, 色泽白净, 薄而匀, 一面平整, 一面稍起毛, 质地细密坚韧, 含微量细蔗线头。显微观察和化学鉴定, 只含大麻纤维。同一处出的简最晚年代是宣帝甘露二年。纸 II (EJT30:03), 长宽 11.5*9 厘米。暗黄色, 似粗草纸, 含麻筋、线头和碎麻布块, 较稀松。出土地层属于平帝建平以前。

In 1973, two pieces of paper were discovered in Juyan, Gansu province⁸⁵. The first paper (EJT1:011) has been crumpled upon unearthed. It unfolded to be 21cm*19cm (the largest one of the two unearthed). It has gloss, thin and even. Flat on one side while (a little) rough on the other side. It is strong with several threads. Microscopic observation and chemical test revealed only hemp fiber. Of the bamboo slips unearthed together, the latest one being ca. 52BC. The second paper (EJT30:03) was 11.5cm *9cm, dark yellow color looks like rough paper with hemp thread and rags, the grain is rough. The layer where it was unearthed belongs to ca. 3BC.

Annex 27 Lobnor paper (49BC)



⁸⁴ In reference to (i) 敦煌马圈湾汉代烽燧遗址发掘简报. 文物, 1981(10): p. 1-8; (ii) 王菊华, 陈启新. 中国古代造纸工程技术史. 山西教育出版社, 2006.

⁸⁵ In reference to 初仕宾, 任步云, 居延汉代遗址的发掘和新出土的简册文物. 文物, 1978(1): p. 1-25.

It was discovered in 1933, in Xinjiang by archaeologist Huang Wenbi. It is ma, 4*10cm, Huang wrote in the archaeology report: hemp-like material, thin square fragment, it is very rough, not uniform and not clean. Hemp thread was present on the surface (so that it is not a high quality paper). In the same archaeological context, a bamboo slip with date of Huanglong yuannian (49 BC) which was reign title of Emperor Xuan of Han (73-49 BC) was also unearthed. So this paper should be dated as west Han paper. This paper has been destroyed during the Second Sino-Japanese War⁸⁶.

Annex 28 Gol Mod paper (16-13 BC)



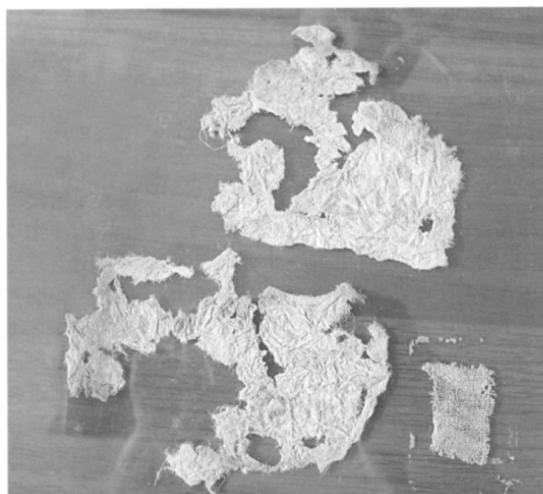
It was discovered in Mongolia in 2006. It was discovered in a necropolis of Gol Mod, in the province of Arkhangai, Mongolia. The necropolis is attributed to the confederation of the half-nomadic tribe of Xiongnu. This graveyard includes 393 graves. Among these, grave T20 is not only the largest, but it also surpasses the size of most of the graves found at any previously discovered Xiongnu necropolis. The grave adopts the traditional shape of the graves of the elite, and the funerary room of the monument has been excavated down to the bedrock. An archaeological piece of paper was discovered in chariot n°2, which was lying on the roof of the funerary chamber of the grave. More than 50 elements belonging to this vehicle were found, thus allowing for a clear understanding of the structure. The restoration of its appearance shows that the vehicle perfectly corresponds to Chinese models of that period. It can be divided in four parts: body shell, axle and wheels, shaft and yoke, and parasol. Simultaneously, a lacquered object inscribed with Chinese characters “Yongshi era” (永始)—the period between 16 and 13BC—was found in the T20 grave. This enabled us to date the paper-like fragment from the same period, that is, around the beginning of our era.

Examination of the surface of the fragment with a binocular microscope confirmed that the material has all of the visual characteristics of paper: It consists of a mat of tangled fibers exhibiting no particular order. The fibers are up to several millimeters long and have a diameter ranging from 20 to 50 µm. The fiber was identified as hemp and the meaning of this object was discussed elsewhere⁸⁷.

⁸⁶ In reference to (i) 黄文弼, 罗布淖尔考古记. 1948: 中国西北科学考察团; (ii) 潘吉星, 中国造纸史. 上海人民出版社, 2009.

⁸⁷ In reference to (i) 安伟, 奚芷芳, 蒙古匈奴贵族墓地初步研究. 考古学报, 2009(1): p. 69-88; (ii) Andre, G. and V. Rouchon, A Paper Fragment from the Gol Mod Necropolis, Mongolia (a Late First Century BC Archaeological Excavation). *Senri Ethnological Studies*, 2013; (iii) André, G., et al., L'un des plus anciens papiers du monde exhumé récemment en Mongolie—découverte, analyses physico-chimiques et contexte scientifique. *Arts Asiatiques*, 2010: p. 27-42.

Annex 29 Zhongyan paper (1-5AD)



麻纸：在标本 78FTH:051-053 三枚铜泡的扁钉上各穿一枚五株钱，盖严了铜泡口。取掉五铢钱，发现铜泡扁钉周围的空隙内塞有乳黄色的东西。原来是揉成一团的纸张。经展平，最大的一块，面积 6.8*7.2 厘米（一角已损），其余几块大小不等。其他铜泡的扁钉周围也有填塞物的痕迹，似乎也是纸张，因铜口敞开，塞物已损坏。

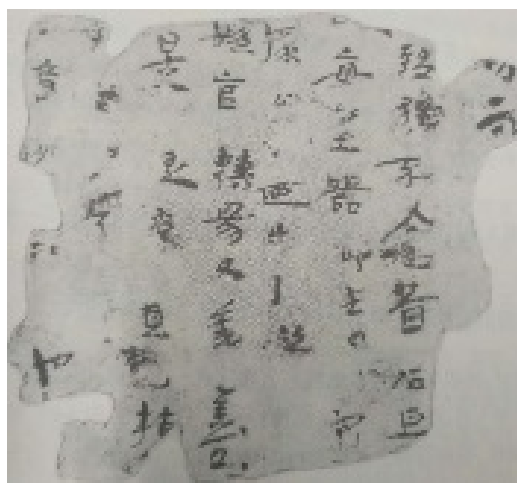
塞在铜泡内的纸张呈乳黄色，粘有不少铜锈绿斑。纸较为坚韧，耐折，色泽较好，没有虫蛀和朽坏，略精于渭桥西汉故纸，但仍然较为粗糙。用放大镜观察，有较多的纤维束和尚未完全打散的麻绳头，组织松散，纸浆分佈不匀。经中国科学院自然科学史研究所等单位分析鉴定，肯定样品是纸，不是纤维堆积物，原料为麻类纤维，而不是丝质纤维图版肆。实测纸的厚度约为 0.022-0.024 厘米。从纸的物理结构观察，属于早期纸的范畴，与先前在罗布淖尔和金关发掘的汉宣帝时期的麻纸近似。

In 1978, it was discovered in fufeng, zhongyan. On top of three bronze pao (铜泡)'s bianding (扁钉) there were wuzhuqian (五株钱), after the wuzhuqian has been removed, creamy yellow items were found on the empty space of the bronze pao. It turned out to be crumpled paper. After unfolding, the largest one being 6.8*7.2 (one corner has been worn). Other bronze pao's bianding also have the trace of filling, it seems also paper. Because it is open, the inside filler has been destroyed.

The paper was creamy yellow stuck with dots of aerugo. The paper was strong, glossy and in good condition. The quality is better than the baqiao paper although it presents rough grain. Observed with magnifying glass, it presents lots of fiber bundles and threads, the structure is loose with fibers unevenly dispersed. After the examination from Institute for History of Natural Sciences, Chinese academy of sciences (Pan), it was identified as paper (not fiber bundles), the raw material is hemp (麻类) (not silk). Its thickness is 0.022-0.024cm⁸⁸.

⁸⁸ In reference to (i) 罗西章, 陕西扶风中颜村发现西汉窖藏铜器和古纸. 文物, 1979(9): p. 17-20; (ii) 齐吉祥, 灞桥纸、中颜纸和汉代的造纸术. 历史教学, 1982(7): p. 43-44; (iii) 李晓岑. 陕西扶风出土汉代中颜纸的初步研究. 文物, 2012, (070)(7):93-96.

Annex 30 Erjinahe paper (109-110 AD)



It was discovered in 1942 in juyan, Gansu province by Lao Gan⁸⁹. Lao deemed that the dating of this paper has the possibility to be earlier than Cai Lun (105AD), however, the possibility of later than Cai Lun (105AD) cannot be ruled out. By principle, it could be contemporaneous.

Annex 31 The honey-fragrance paper

蜜香纸，以蜜香树皮、叶作之。微褐色，有纹如鱼子，极香而坚韧。水渍之，不溃烂。泰康五年，大秦献三万幅，帝以万幅赐镇南大将军、当阳侯杜预，令写所撰《春秋释例》及经传《集解》以进。未至而预卒，诏赐其家，令藏之。

This record has ever given rise to the assumption that a quantity of Roman paper, the produce of the papyrus plant, took its way to China as a present from the reigning Roman emperor. The noted western writer Dr. Edkins (1867) used this passage to support his assertion that paper was introduced to China from the west. In 1885, Hirth doubtful that i) thirty thousand rolls of paper represent a big cargo and as the Chinese at that time were already in the possession of a voluminous literature written on paper of silk and other materials which must have suited their own purpose it does not seem probable that present of such a bulky nature should have been sent on this lengthy journey in order to introduce a mission, official or commercial; ii), neither embassy nor paper was send, instead it could be the Syrian or Alexandrian merchants reached annam (vietnam?) and in order to obtain certain trading privileges, they had to send presents to the court of china and having disposed of their ta shi goods, they invested part of the proceeds of their sales in the purchase of local (annam) produce to serve in lieu of original hoe articles as a present to the emperor; the tree mentioned in the record mixiangshu is not mentioned in any of the dashi records.

Needham calls it the 'paper mission' and pinpoints its arrival at 284AD. Pan thought that is might be the first time that roman paper were in contact with paper and they may bring some paper back to roman. Some also think it is an imitation of other stories and think this story is not authentic⁹⁰.

⁸⁹ 勞幹，論中國造紙術之原始。中央研究院歷史語言研究所集刊，1948：p. 489-498.

⁹⁰ In reference to (i) Edkins, J., On the origin of paper making in China. Notes and Queries on China and Japan, 1867. 1(6): p. 67-8; (ii) 馬泰來, M.T.-L., The Authenticity of the "Nan-Fang Ts' ao-mu Chuang" 南方草木狀. T'oung Pao, 1978: p. 218-252; (iii) 罗桂环, 关于今本《南方草木狀》的思考. 自然科学史研究, 1990. 9(2): p. 165-170.

Annex 32 The start of the paper road

The first mention of paper by Arab writer is in 650 AD⁹¹. The currently traditional account of the introduction of paper into the middle east is contained in a medieval Arabic text dated 1482, it was uncovered by the Spanish orientalist Miguel Casiri (1710-1791) while cataloguing the Arabic manuscript in the library of the Escorial in Spain. This discovery was published in his book *Bibliotheca Arabico Hispana Excursalensis* (Madrid 1760-70):

In the city of Samarqand a very fine paper is used, that is found nowhere else except amongst the Chinese. The arabs, after their conquest of that city (in 704 AD⁹²), introduced amongst themselves the methods of its manufacture.

Annex 33 The 751 AD

Prisoners of war were brought from China. Among these was someone who knew (about) the manufacture of paper and so he practiced it. Then it spread until it became a main product for the people of Samarqand, from whence it was exported to all countries.

Some historian such as Hirth through historical studied concluded (1890) that papermaking was introduced into the Arabic world from China in 751AD. However, the accuracy of this explanation has been doubted. The story about the captured papermakers is probably false for several reasons: i) it is first reported three centuries after the events in question by the Arab historian Tha'alibi (961-1038), in his *Book of Curious and Entertaining Information*. Among Tha'alibi's chapters is one enumerating the specialties of different lands. One of the specialties of Samarqand, he wrote, is paper, which looks better, suppler, more easily handled, and more convenient for writing on than either Egyptian papyrus or parchment. On the authority of a certain *Book of Roads and Provinces* (a text, now lost), probably written by the vizier to the Samanid rulers of Transoxiana a century earlier, Tha'alibi reported the Chinese prisoner story. ii) Earlier Muslim historians had ignored the battle of Talas and its repercussions and instead focused their attention on other events in West Asia. There are, consequently, no contemporary Arab accounts of the battle, which turned out to be of inestimable importance: it ultimately determined that Islamic civilization would dominate in Turkestan. iii), one Chinese source, Du Huan, who was taken prisoner but returned to his homeland a decade after the battle, mentions only weavers, painters, goldsmiths, and silversmiths among the prisoners taken. iv), In any case, papermaking would have been practiced throughout Central Asia by the eighth century; captured prisoners need not be credited with introducing it. Paper was used in Samarqand-and probably made there-decades before the battle. v), the discovery of handmade paper in Mount Mugh as will be discussed below⁹³.

Annex 34 The Pahlavi Archive

The Pahlavi Archive, a collection of Middle Persian documents at the University of California's Bancroft Library at Berkeley, dated to the early post-Sasanian period in Iran, and constitutes a corpus of leather/parchment and textile documents that were recently subjected

⁹¹ In referece to Bosch, J.C. and G. Petherbridge, *Islamic bindings and bookmaking*. 1981.

⁹² Bosch thought the date of the siege and capture may not be correct, it may have been 712 AD

⁹³ (i) Hirth, F., *Die Erfindung des Papiers in China*. T'oung Pao, 1890. 1(1): p. 1-14; (ii) Quraishi, S., *A survey of the development of papermaking in Islamic countries*. Bookbinder, 1989. 3: p. 31; (iii) Bloom, J., *Paper before print: the history and impact of paper in the Islamic world*. 2001: Yale University Press.

to scientific analysis by Dr Kathleen Martin and her associates at McCrone Group Associates, Inc. One of the findings of this analysis was the revelation that the textile used as writing material in the Middle Persian documents at Berkeley is linen, rather than silk as earlier believed. Moreover, the linen cloth was evidently sized with gypsum, which made it resistant to the spreading of ink. The study explores the relevance of the use and preparation of linen as writing material in early post-Sasanian Iran, for the development of paper technology in Khorasan and Transoxiana in the early Islamic world⁹⁴.

Annex 35 The Mount Mugh paper

In the spring of 1932, a shepherd discovered a cave in Mount Mugh containing seventy-six texts in Soghdian, Arabic, and Chinese written on several kinds of material-including paper, cloth, leather, and wood. The document passed through many hands eventually attracted the attention of Soviet authorities and in early 1933 the document was confiscated and delivered to Dushanbe. The interpretation of this document has been published in Russian and English.

Although there is no way to know where the Mount Mugh papers were made, it is likely that they were local and that, as elsewhere, Buddhist monks, who had been active in the region had brought papermaking to Transoxiana well before the Muslim conquest, while on the other end in the Arabic world, papyrus was contemporarily used (Al-Tha'alibi tells us: 'al-Jahiz mentions that the papyrus of Egypt is to the western lands what the paper of Samarkand is to the East). Boom in his other books also think: after the collapse of the Western Turkish overlords and of the Sasanian Empire, in 659AD, Chinese forces reached Samarkand and Bukhara in order to fill the power vacuum in Transoxiana. He speculates that papermaking may have spread to Transoxiana even earlier, through the medium of itinerant Buddhist monks who carried the technology along the Silk Road between China and India. Other factors may have led to the transfer of papermaking from China to Transoxiana before the mid-eighth century, such as the role played by merchants, especially by several generations of Sogdians who are now known to have held a variety of posts in China⁹⁵.

Annex 36 The earliest mulberry paper in Arabic world

In 2008, fragments of paper inscribed in Arabic were found inside the round tower on the upper floor of room 5 located in the north-eastern part of the tower, to the left of the entrance. Sanjar-Shah was probably founded in the 5th century CE and abandoned at the same time as Panjikent, in the 770's, or perhaps a little later, around 780AD. All the rooms uncovered in Area 2 probably belonged to two or more different households separated by a large open courtyard and a wide street. Furthermore, it appears that this part of Sanjar-Shah housed living quarters as well as craftsmen's workshops. The fact that we now possess the fragments of at least three letters all written on paper provides further evidence that the use of paper by the Arabs in Sogdiana was already widespread in the 2nd/8th century. If the paper fragment of the Arab manuscript consisted of a mixture of recycled textile fibres, it would indicate either a

⁹⁴ In reference to Azarpay, G. and V.A. Livshits, *The MP Archive at Berkeley: a pre-Islamic Forerunner of "Samarkand" Paper?* *Journal of Inner Asian Art and Archaeology*, 2006. 1: p. 141-143.

⁹⁵ In reference to (i) Yakubovich, I., *Mugh 1. I. revisited*. *Studia Iranica*, 2005. 31(2): p. 231-253; (ii) Livshits, V.A., *Sogdian epigraphy of Central Asia and Semirech'e*. *Corpus Inscriptionum Iranicarum*, 2015(Part II); (iii) Benkato, A., *Sogdian Epigraphy of Central Asia and Semirech'e*. 2017, JSTOR; (iv) Friedman, H., et al., *Fragments of an early Islamic Arabic papyrus from Khirbet Hamrā lfdān*. *Arabian Archaeology and Epigraphy*, 2017. 28(2): p. 285-296.

Central Asian origin or an Arabic origin, especially if heavily sized with starch or coated. If the paper however, consisted of pure new bast fibres, it would indicate a Chinese origin. Textile was also found in this context⁹⁶.

Annex 37 The Sogdians

Traffic had existed between China and Central Asia since the mid-second millennium BC⁹⁷ while more stable trade routes were established under the Ancient Persians (ca.550-330 BC) during which time Sogdian involvement in commercial enterprise between east and west began in earnest. A Chinese document listing taxes paid on caravan trade in the Turpan region in the early decades of the seventh century discloses that twenty-nine out of thirty-five commercial transactions involved Sogdian merchants and that in thirteen of those operations both the buyer and seller were Sogdian. Geographically, Sogdiana was a midpoint on the trade routes: it functioned as a conduit between commercial centers in China, India, Sassanian Iran, and Byzantium.

The Ancient Letters, a group of five letters written in the Sogdian language discovered in 1907 by the British archaeologist Aurel Stein in a postbag in the ruins of a watchtower near Dunhuang contains rich information. They provide information not only about the goods exchanged and the early network of trade and commerce between Central Asia and China via the Tarim Basin but also about the Sogdian merchants and artisans who were involved (Later documents and murals reveal that traffic also involved people of faith, including monks and missionaries).

The Sogdians had learned the art of papermaking from the Chinese and were renowned for the quality of their paper (in the early years it might well have been called soghdian paper named after the region about Samarqand). This craft was put to use by the many Islamic savants in Samarqand and Bukhara during the rule of the Persian-speaking Samanids (819–999 AD) and quickly became a medium for the textual transmission of Islam.

The Sogdians had made use of paper already in the early 4th century AD, as shown in the “Ancient Letters” and shown by the Mugh documents and the Sanjar-Shah paper, paper was used in Sogdiana at least from the beginning of the 2nd/8th century (may probably even earlier). Between this two time points, there is a lapse of some 400 years during which the Sogdians could act as envoy for paper on the paper road⁹⁸.

⁹⁶ In reference to (i) Haim, O., M. Shenkar, and S. Kurbanov, The earliest Arabic documents written on paper: three letters from Sanjar-Shah (Tajikistan). *JERUSALEM STUDIES IN ARABIC AND ISLAM* 43 (2016). 2016; (ii) Kurbanov, S. and A. Teplyakova, Textiles Objects from the Citadel of Sanjar-Shah. *Bulletin of Miho Museum*, 2014. 15: p. 167-177.

⁹⁷ In the late second century BC, the Han imperial envoy Zhang Qian was sent to make an alliance with the peoples of Central Asia, in particular of Sogdian, in order to combat incursions into northern China from the Xiongnu steppe nomads. On his second diplomatic mission between 119 and 115 BC, Zhang Qian is said to have brought “tens of thousands” of cattle and sheep with him and to have offered gold and silk goods in great quantities, to bring political influence on China’s northwestern frontier.

⁹⁸ In reference to (i) Rose, J., *The Sogdians: Prime Movers between Boundaries*. Comparative Studies of South Asia, Africa and the Middle East, 2010. 30(3): p. 410-419; (ii) Frye, R.N., *Sughd and the Sogdians: A Comparison of Archaeological Discoveries with Arabic Sources*. *Journal of the American Oriental Society*, 1943. 63(1): p. 14-16.

Annex 38 The mulberry tree

Mulberry planting originated in China, during the pre-Qin period, it has begun to grow in the middle and lower reaches of the Yellow River. As mentioned earlier, among all the plant names mentioned in Shih Ching, mulberry is by far the most frequent one, which appears in 20 poems. It is reasonable to believe that the mulberry tree is among the very few trees first brought into cultivation in ancient China (in company with the silk manufacture). For example, five predicted mulberry miRNAs are found in the haemolymph and silk glands of the silkworm, suggesting interactions at molecular levels in the plant–herbivore relationship.

Whether mulberry bark was used for papermaking in China has been controversial. In commenting on Marco Polo's observation that Chinese paper money was made of "the bark of certain trees, in fact of mulberry tree, Bretschneider said: "He seems to be mistaken, paper in China is not made from mulberry-trees, but from the *Broussonetia papyrifera*." To prove that bark of the mulberry tree, as well as that of paper mulberry, was used as a material for paper, Laufer cited a number of authorities and concluded: "Marco Polo is perfectly correct: not only did the Chinese actually manufacture paper from the bark of the mulberry tree (*Morus alba*), but also it was this paper which was preferred for the making of paper money.

The paper mulberry (*Broussonetia* spp) is a species of flowering plant in the family Moraceae. It is native to Asia, where its range includes China, Japan, Korea, Indochina, Burma, and India. Currently, it has been used as a markers for the discussion of human migration in the pacific islands [39-43]. DNA analysis contribute lots to it taxonomy [44]. It was introduced intentionally throughout the world for economic and aesthetic purposes, It can be an invader on several continents and in over a dozen countries [45].

In 1890, HIRTH asserted that the Arabic word kaghid for paper The Arabic word for paper, derived from the Persian, can without great difficulty be traced to a term ku-chih 穀紙, which means 'paper from the bark of the mulberry-tree,' and was already used in times of antiquity. This was accepted by horn but was doubted by Laufer (Hirth's supposition of a former pronunciation kok cannot be accepted) [46]. However, thought that the word kou originates from the language of Chu 楚, in which it had the significance "milk" and, as the bark of this tree contained a milk-like sap, this word was transferred to the tree. It is noteworthy in this connection that Ts'ai Lun, the inventor of paper in A.D. 105, was a native of Chu⁹⁹.

Annex 39 Identify fibers from the record-not always reliable

In Ming dynasty, Tu Long (1542-1605AD) wrote in his book that 'the Korean handmade papers were made of cocoon and it is white and strong, it is unique that China does not have this paper'. His 'identification' was popular for a while when discuss the fiber material origin of Korean handmade papers. However, Kangxi Emperor (1654-1722, the fourth emperor of the Qing dynasty, 1644-1912) pointed out that: 'people always say that the Korean handmade papers were made from cocoon (which is wrong), in fact, it was made of paper mulberry. I asked the envoy and was told that the Korean use of the bark of paper mulberry with the white inner part (the black out layers peeled off) for papermaking so that it is white and strong (looks like the cocoon). The people in china has misunderstand it as cocoon paper'. The white

⁹⁹ (i) Keng, H., Economic plants of ancient north China as mentioned in Shih Ching (Book of Poetry). Economic Botany, 1973. 28(4): p. 391-410; (ii) He, N., et al., Draft genome sequence of the mulberry tree *Morus notabilis*. Nature communications, 2013. 4: p. 2445; (iii) Tsien T H. Raw materials for old papermaking in China[J]. Journal of the American Oriental Society, 1973: 510-519.

and strong outlook pointed to cocoon (which is wrong) rather than paper mulberry. Kangxi Emperor inquired the Korean envoy and made the correct 'identification'. However, in most paleographical studies, the origin of the paper may be unknown and only by visual examination (eyes) may come to wrong identification as demonstrated. From the story we can infer that before the application of microscopy, it is difficult to identify the plant origin of handmade papers as the papermaking process has removed most of the plant features with only the (pulped) plant fibers left.

Annex 40 In the publication: the Arabic paper (1887):

What the armed man, with authoritative certainty, could read from the venerable pieces of paper, is often fruitful. The results obtained by scientific research are given to the historical ones a first and secure foundation; they have shown incontestably that the previous conception of certain cardinal contributions to the preparation of paper, as well as the criteria of paper recognition, were missed. , , , It is gratifying to see how, in this case, the criterion of natural sciences, and not the scientific criterion, may arise in an eminently historical and antiquarian question with decisive weight. The importance of the co-operation of these two so divergent research methods, however, leaps even more clearly into the eye when we leave the occidental oculities which have been withdrawn to the exertion, and their doubtful results, in order to turn to the starting point of the paper question, the Oriente itself. "

Annex 41 The Herzberg stain

In chapter five of Herzberg's book, he describes methods of preparing samples for fiber identification using an iodine stain made with 20g water, 1.15g iodine, 2g potassium iodide and 1 ml glycerin, which colored jute and ground wood yellow, chemical wood pulp, straw and hemp brown.

Annex 42 The discovery of the double reflection

In 1862, Nageli published a paper named "the application of polarized light to the study of the structure of plants" that considerably advanced polarized light microscopy (PLM) for plant tissue studies. Nageli found that organized structures, such as starch granules or cell-walls, are doubly refractive and this is not affected by causing them to increase or diminish in size in consequence either of the absorption or removal of water, or by mechanical stretching or pressure. He concluded that the double refraction is not a property of the organized structure as a whole, but that it belongs to each individual micella: hence these micella must be crystalline. Again from the interference colours which these objects present when examined with polarized light, he ascertained that the crystalline micella have three axes of elasticity, that they must be bi-axial crystals. He also concluded that the micella are so arranged in the membrane of which they form part that one of their axes of elasticity is perpendicular to the surface, whereas the other two axes lie in the plane of the membrane. The Michel-Lévy interference colour charts, which relate the interference colour observed under a PLM to the

retardation and birefringence of a specimen of a particular thickness, was first introduced to the world in a book published in Paris in 1888¹⁰⁰.

Annex 43 Some online database

‘The International Plant Names Index’ (2008). <http://www.ipni.org> (accessed 30 October 2008).

United States Department of Agriculture Agricultural Research Service National Genetic Resources Program, ‘Germplasm Resources Information Network – (GRIN) [Online Database] National Germplasm Resources Laboratory, Beltsville, Maryland’, <http://www.ars-grin.gov/cgi-bin/npgs/html/index.pl> (accessed 13 June 2006).

Scientific Working Group for Materials Analysis, ‘Forensic fiber examinations guidelines, Chapter 2, Microscopy of textile fibers’, Forensic Science Communications 1 (1999) <http://www.fbi.gov/hq/lab/fsc/backissue/april1999> (accessed 30 October 2008).

¹⁰⁰ (i) Vines, S.H., The Works of Carl Von Nägeli. 1880, Nature Publishing Group; (ii) Michel-Levy, A. and A. Lacroix, Les Minéraux des Roches. 1: Application des Méthodes Minéralogiques et Chimiques à leur Étude Microscopique. 2: Données Physiques et Optiques. 1888, Librairie Polytechnique Baudry.

Annex 44 Supplementary Information for the discussion in section 2.5

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Figure S1. Py-GCxGC/MS mass spectra (blue) and 1D AMDIS extracted spectra (red) for compound III (a), IV (b) and V (c) (unassigned peaks were not used for identification)

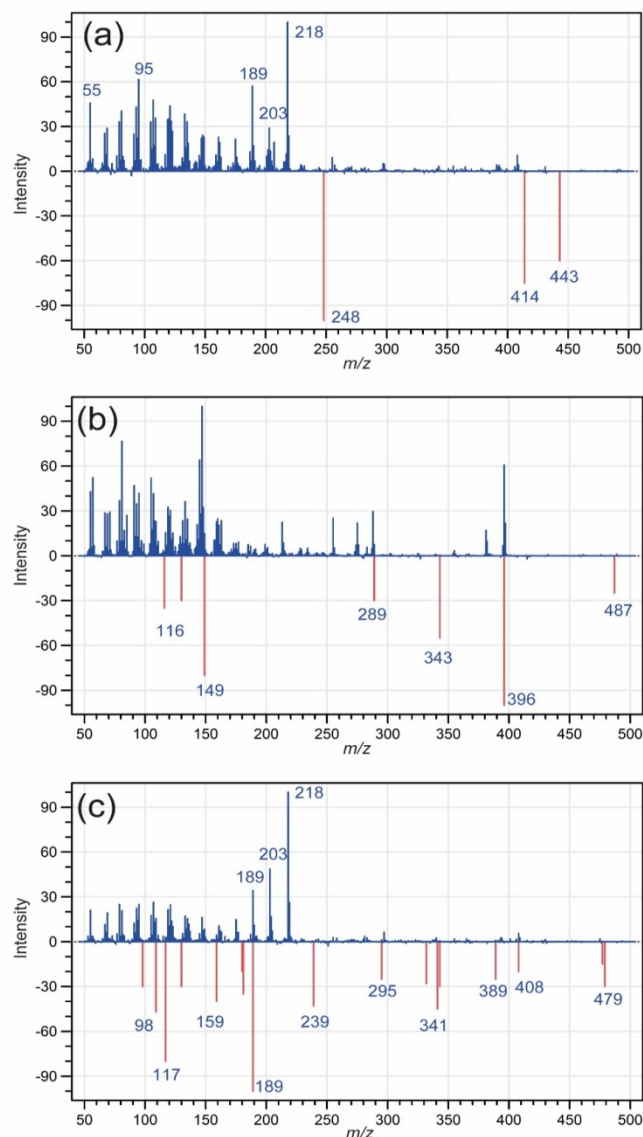


Figure S2. Compounds selected in the Py-GC/MS analysis in the marker region to test the performance of GCxGC/MS reintroduction for mass spectra analysis

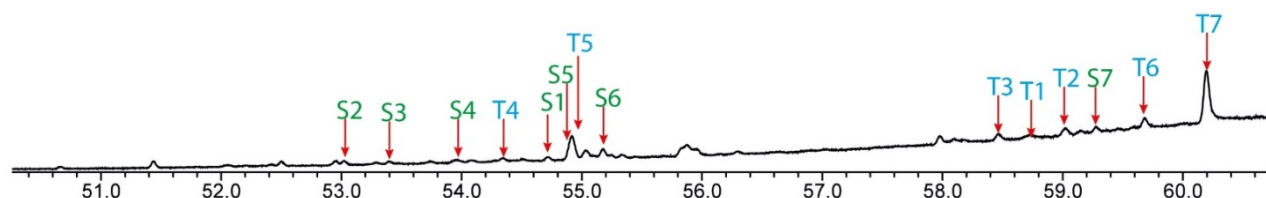


Figure S3. Procedure employed to derive the GCxGC mass spectra, 1D GC mass spectra using GCMS Postrun Analysis, AMDIS and GC Image to constitute the target library used in Part 3.3

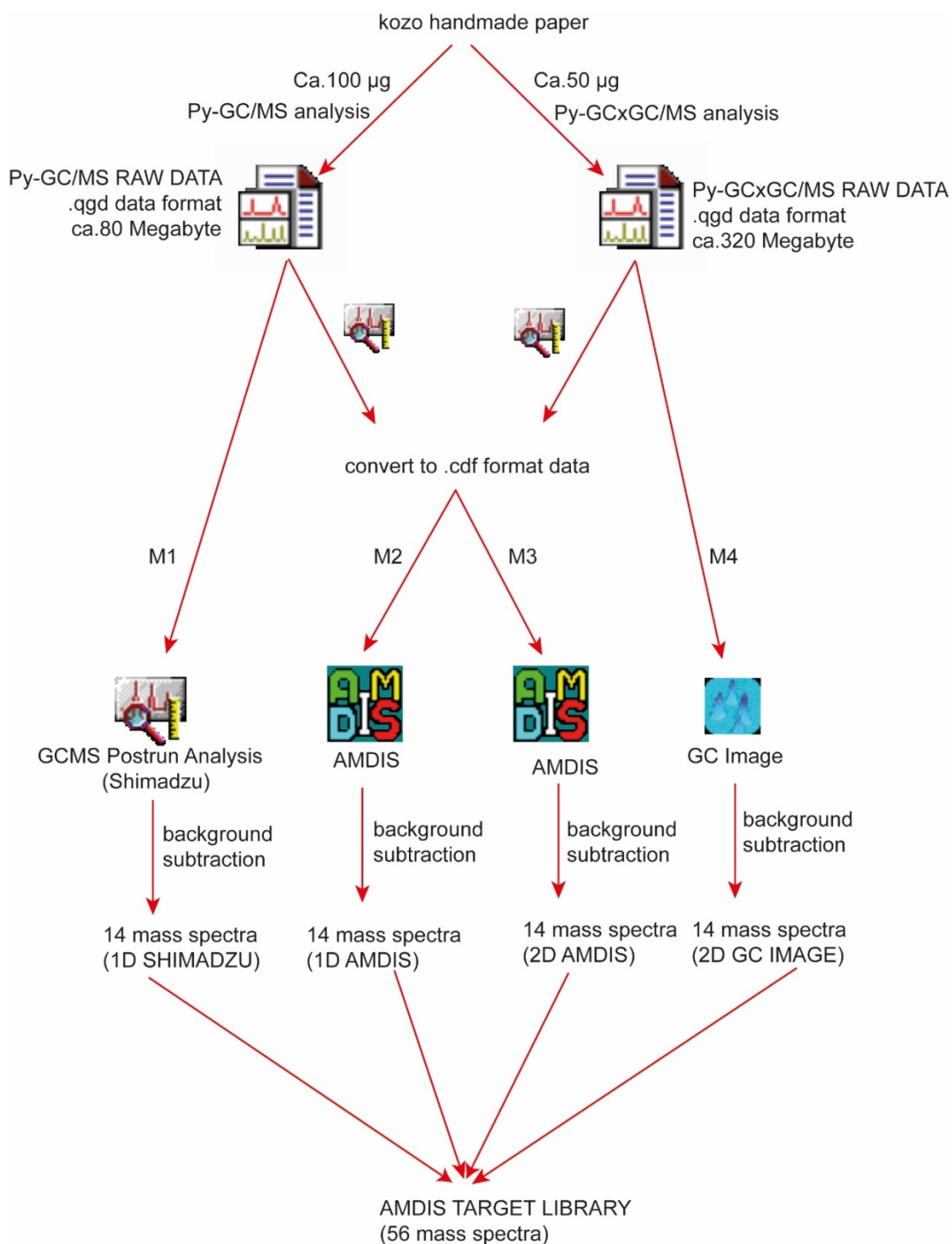


Figure S4. Target library of mass spectra collected from the procedure schematically reported in Figure S3 (the mass spectra are labeled in the format of 'Fig.S4-numbering-compound name-data source-treatment software' in accordance to the procedure described in Figure S3)

Figure S4-1-S1-1D-Shimadzu

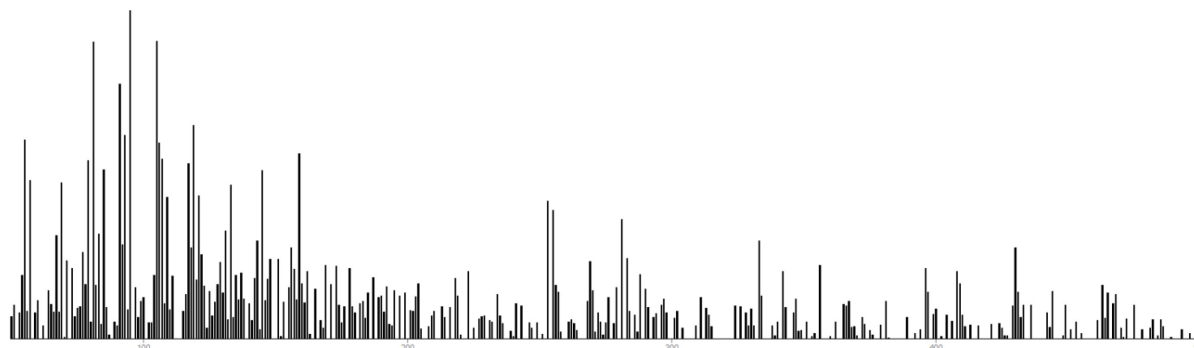


Figure S4-2-S1-1D-AMDIS

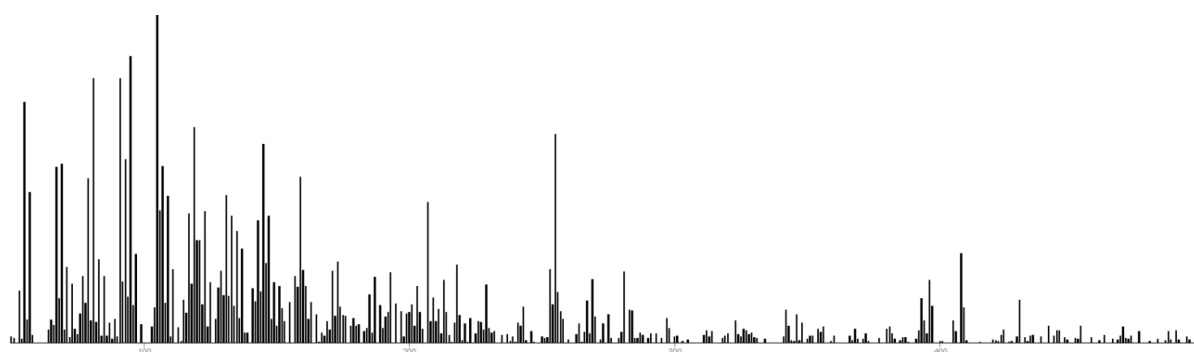


Figure S4-3-S1-2D-AMDIS

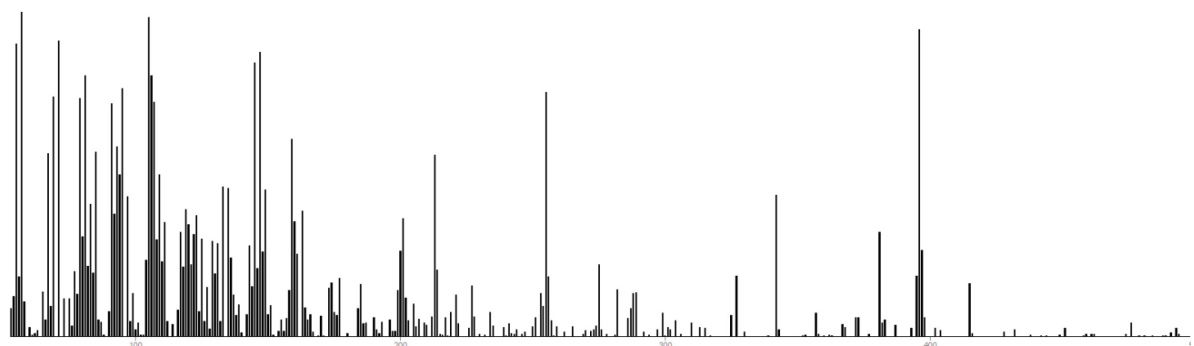


Figure S4-4-S1-2D-GC Image

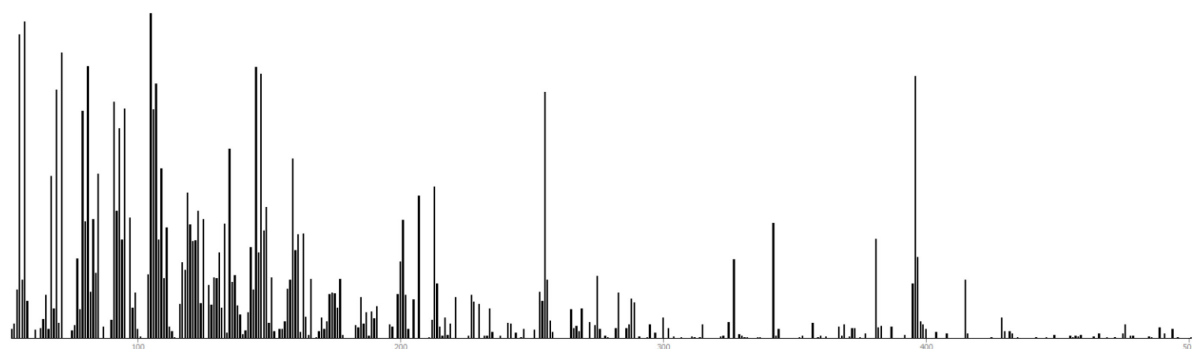


Figure S4-5-S2-1D-Shimadzu

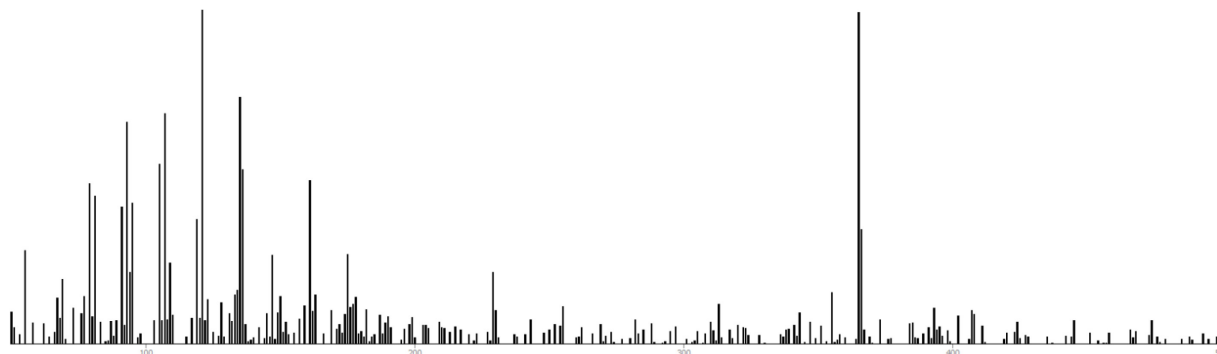


Figure S4-6-S2-1D-AMDIS

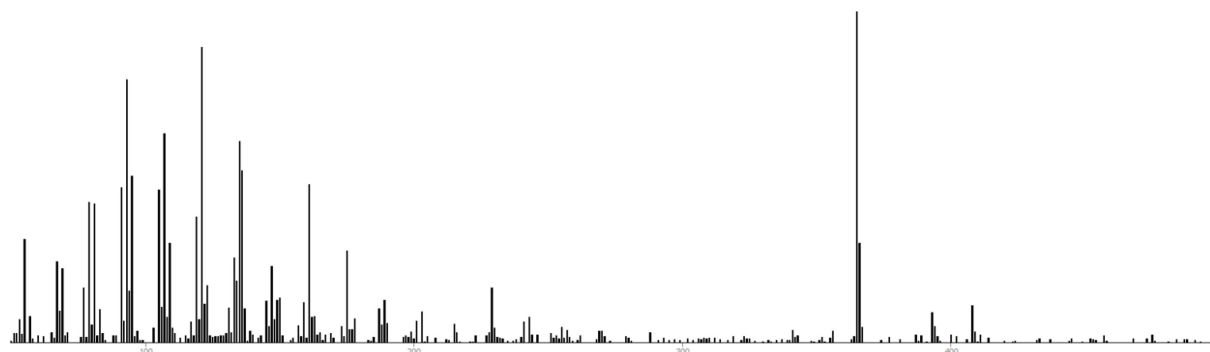


Figure S4-7-S2-2D-AMDIS

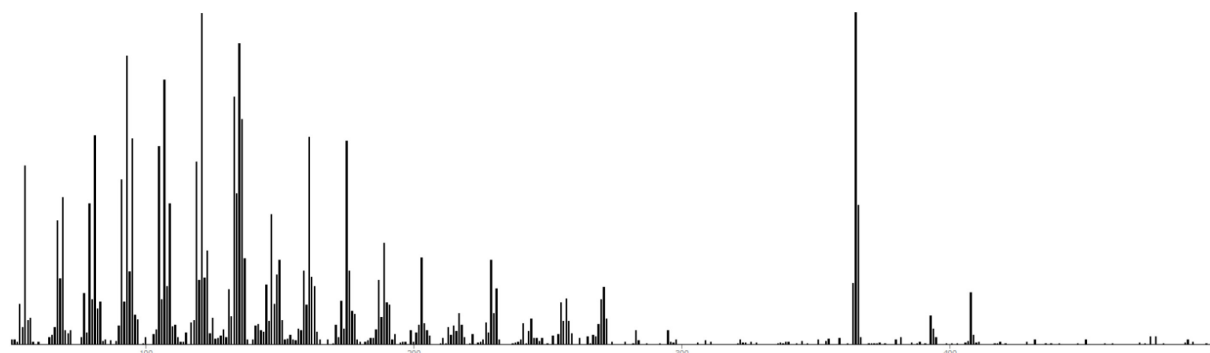


Figure S4-8-S2-2D-GC Image

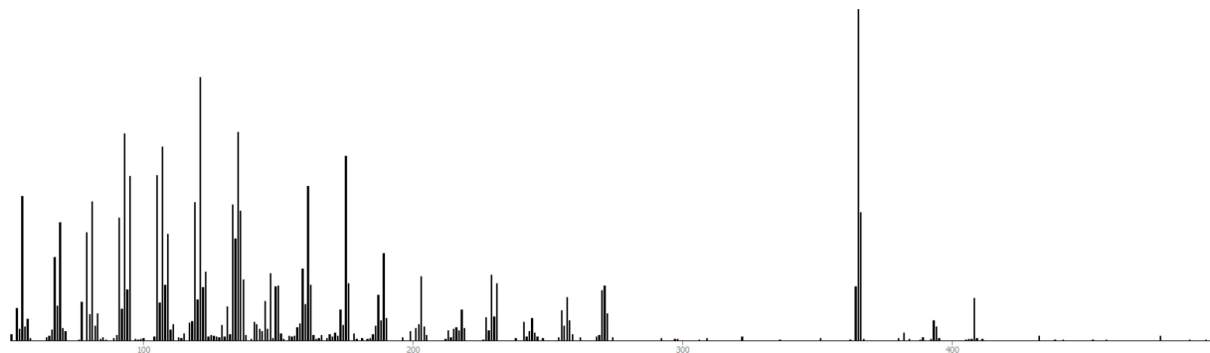


Figure S4-9-S3-1D- Shimadzu

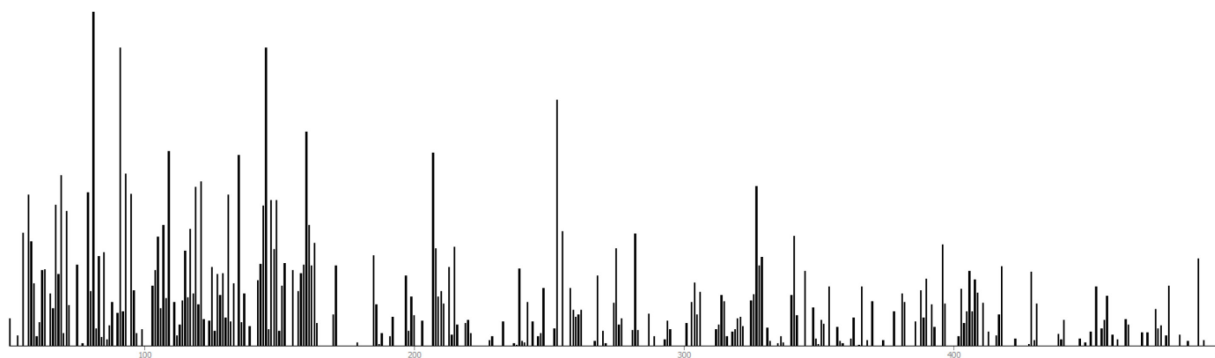


Figure S4-10-S3-1D-AMDIS

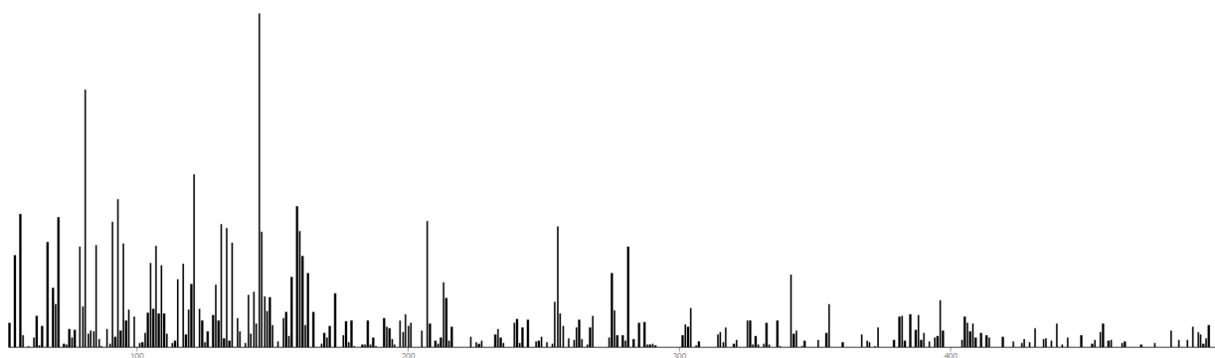


Figure S4-11-S3-2D-AMDIS

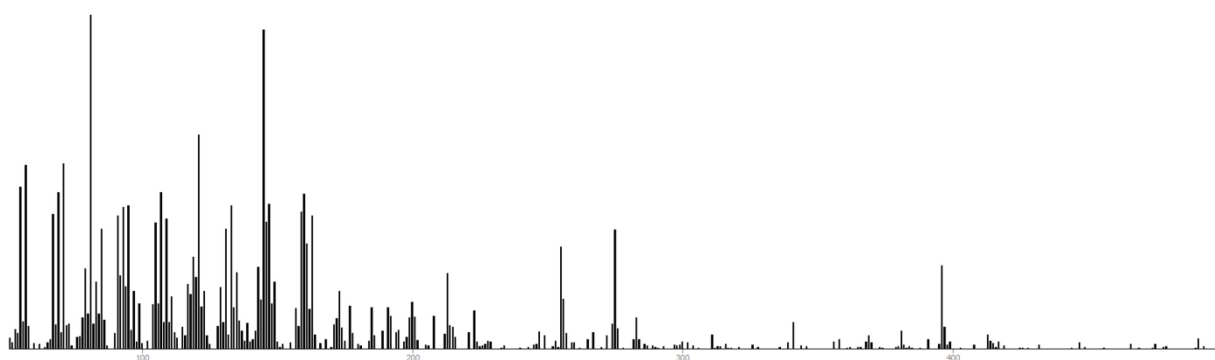


Figure S4-12-S3-2D-GC Image

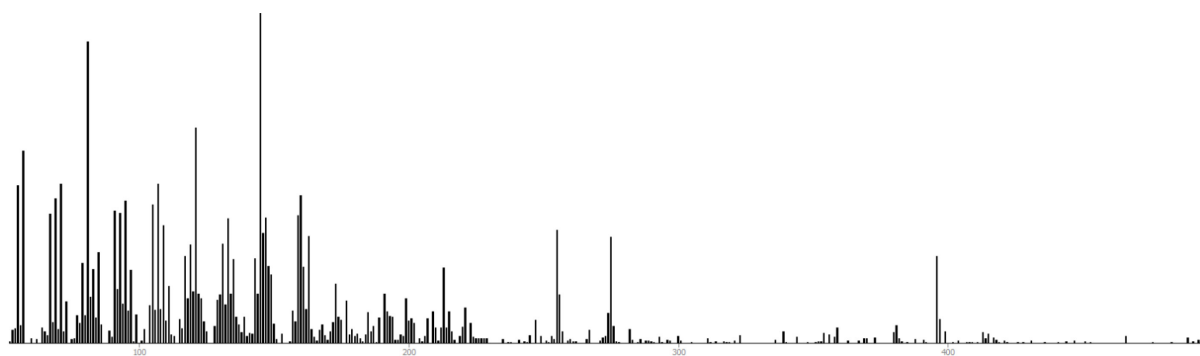


Figure S4-13-S4-1D- Shimadzu

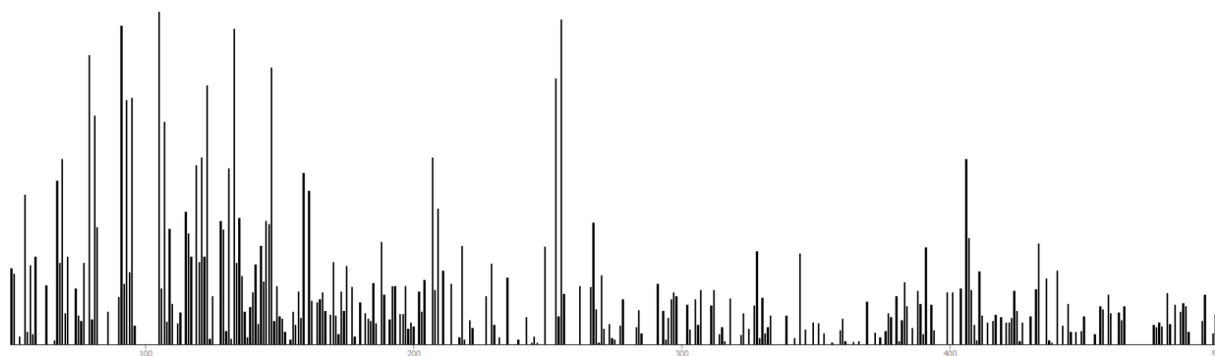


Figure S4-14-S4-1D-AMDIS

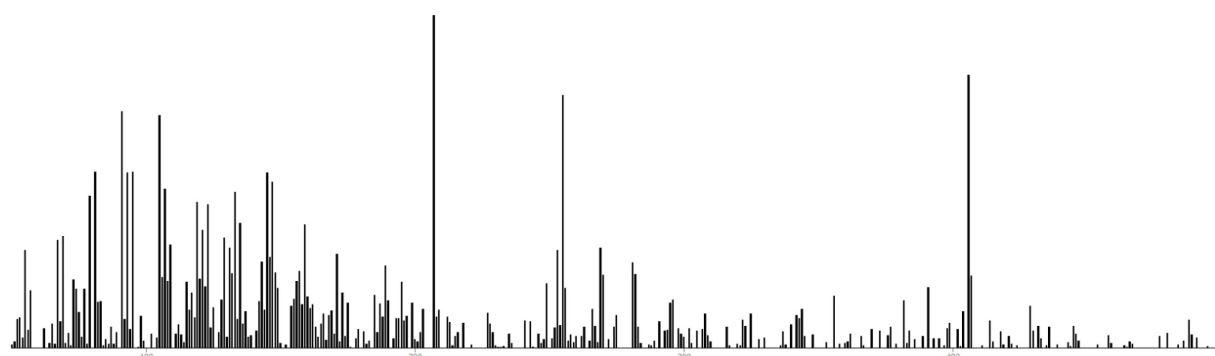


Figure S4-15-S4-2D-AMDIS

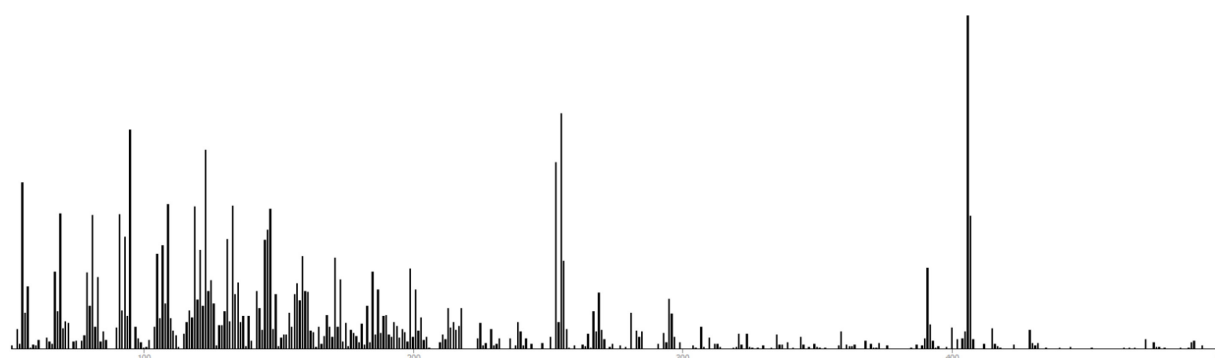


Figure S4-16-S4-2D-GC Image

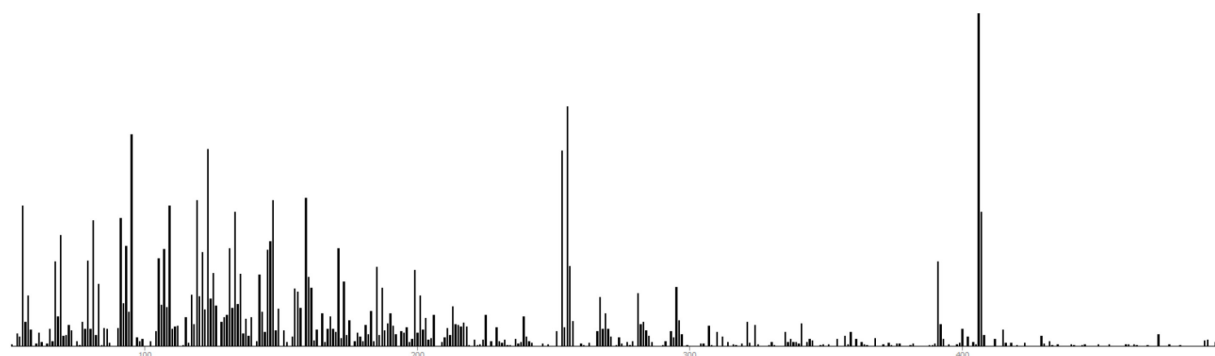


Figure S4-17-S5-1D- Shimadzu

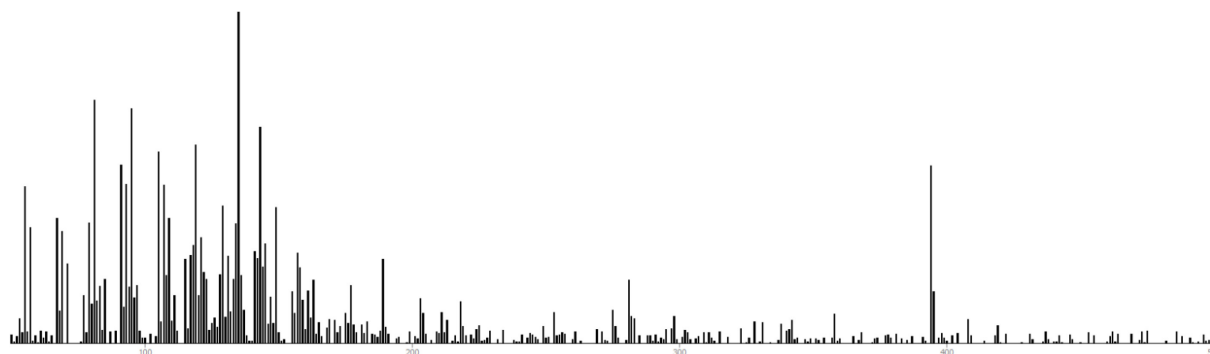


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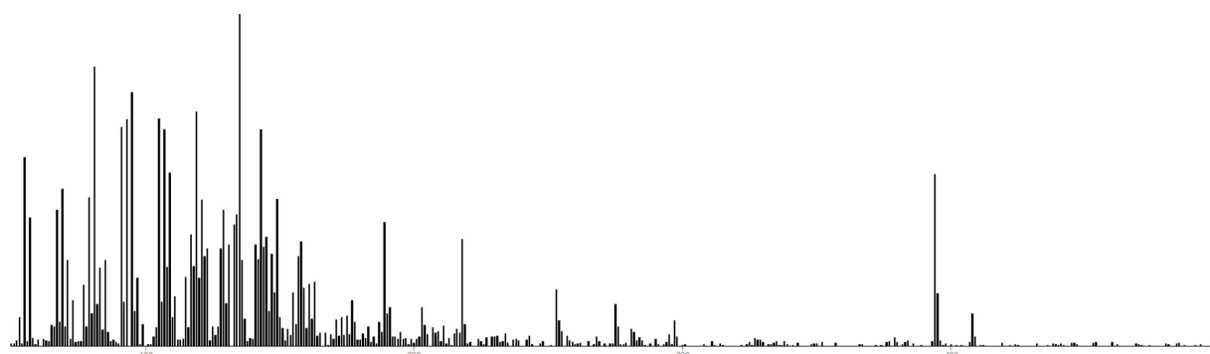


Figure S4-19-S5-2D-AMDIS

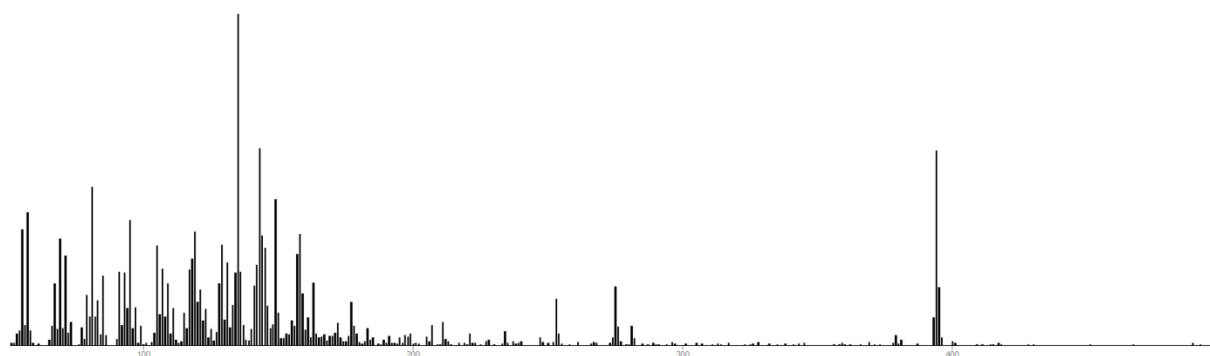


Figure S4-20-S5-2D-GC Image

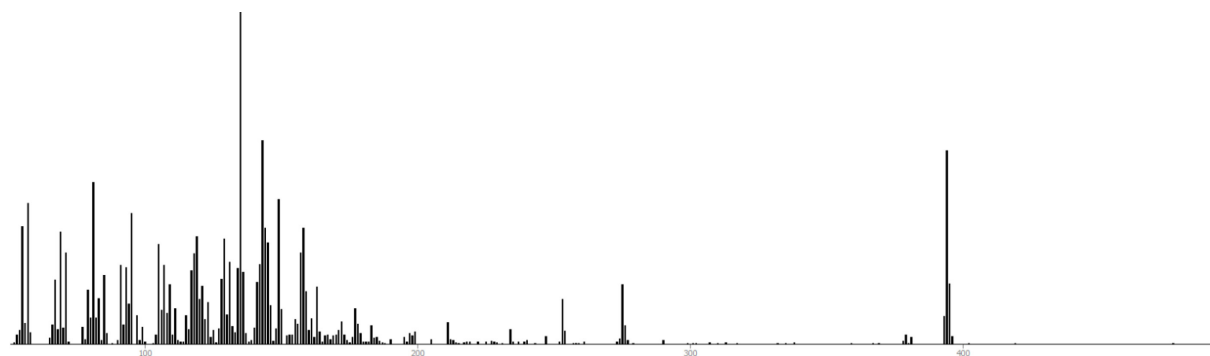


Figure S4-21-S6-1D-Shimadzu

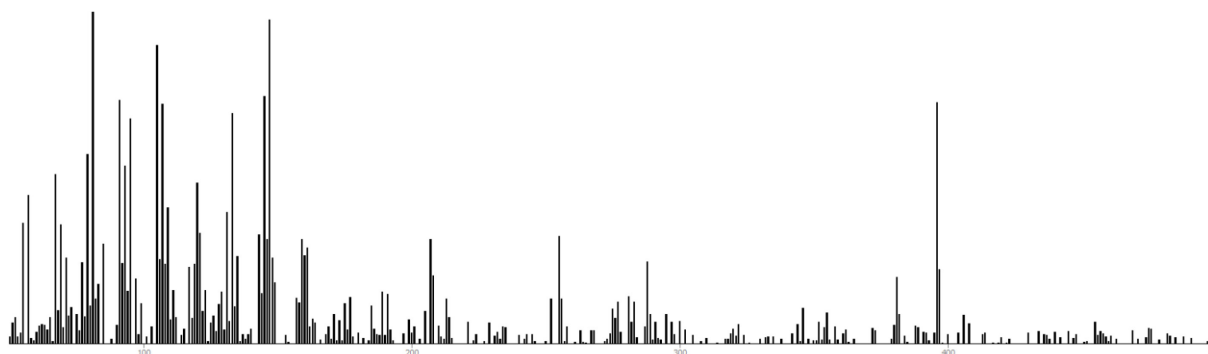


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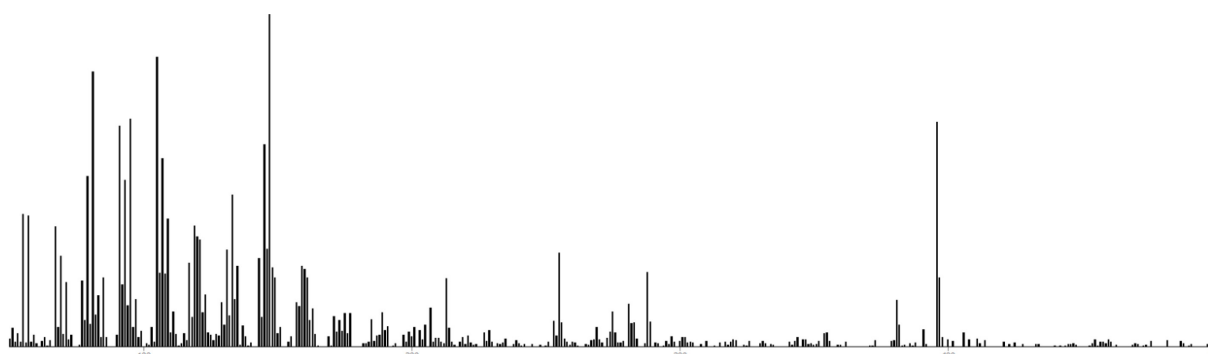


Figure S4-23-S6-2D-AMDIS

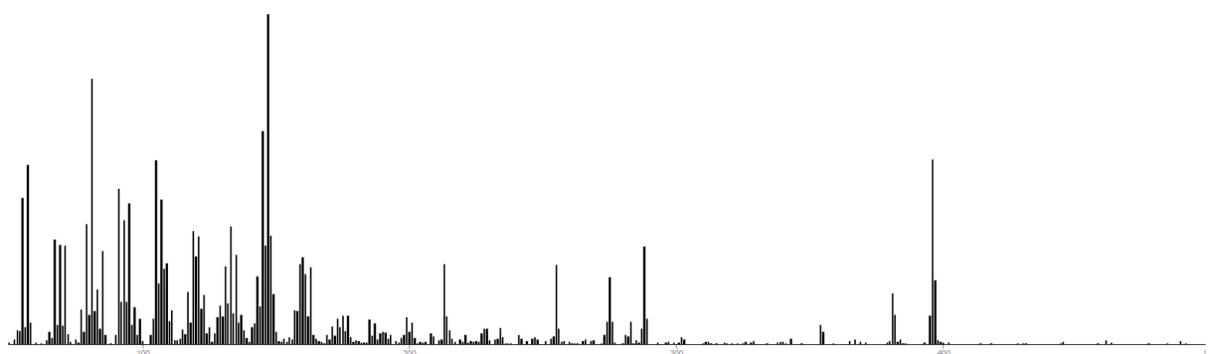


Figure S4-24-S6-2D-GC Image

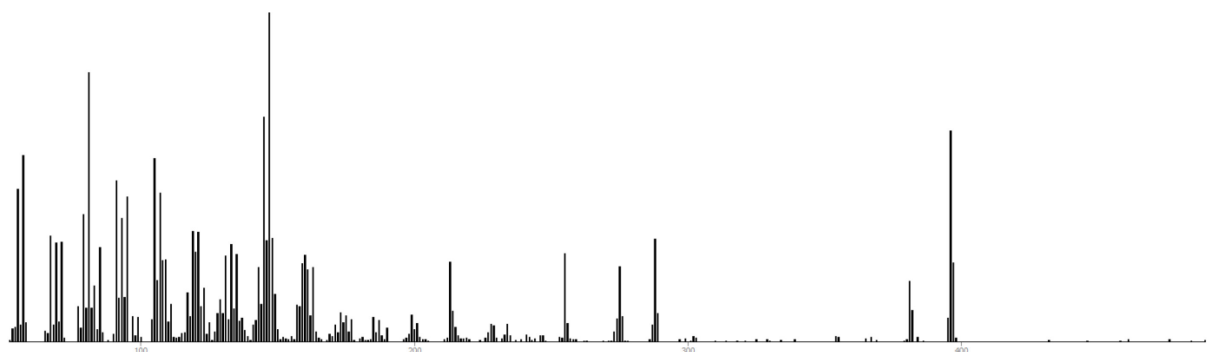


Figure S4-25-S7-1D-Shimadzu

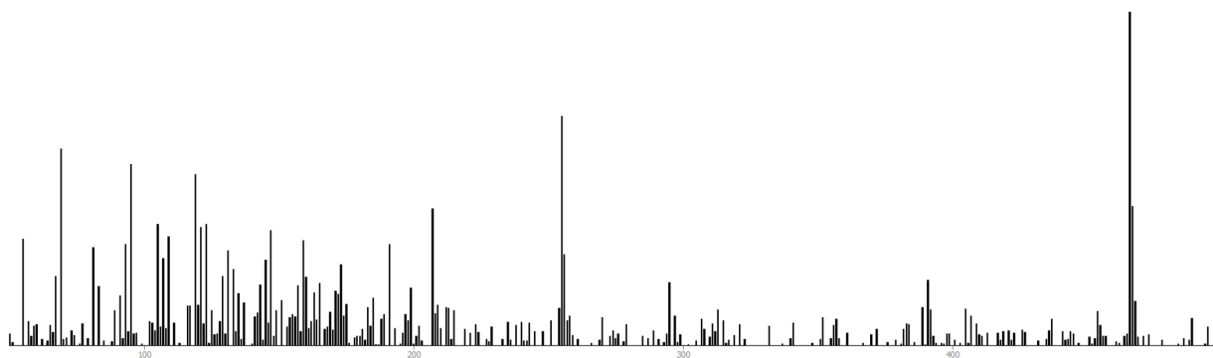


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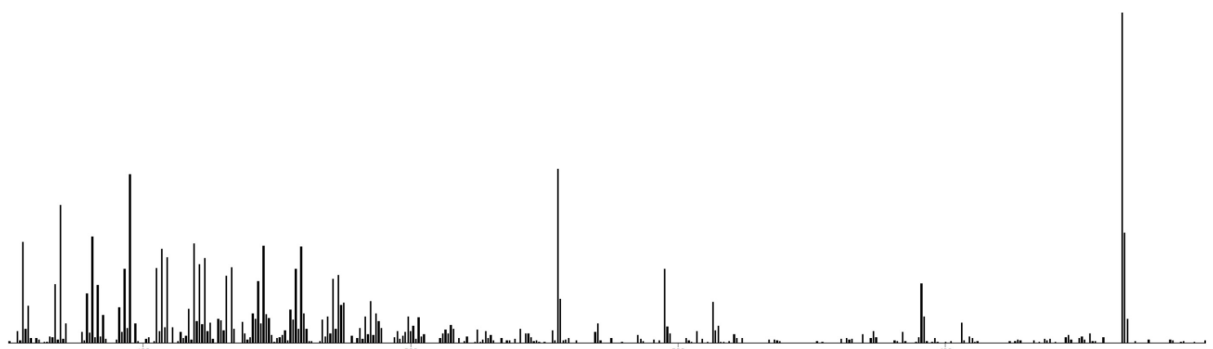


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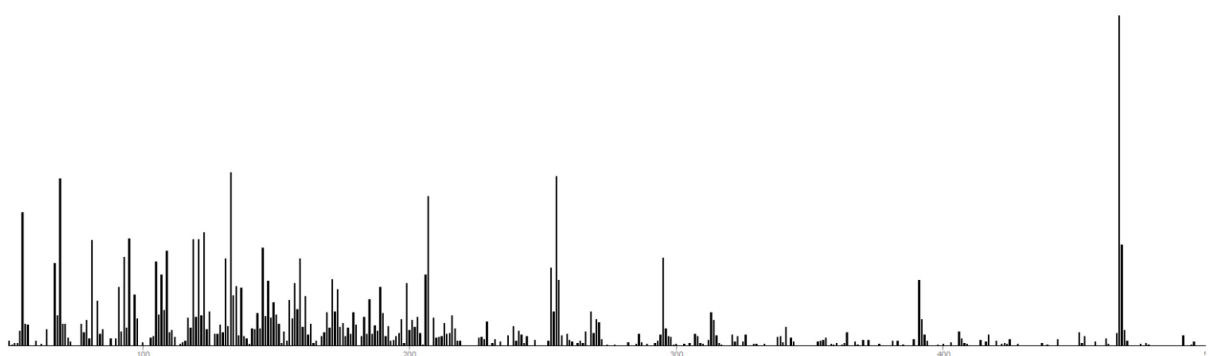


Figure S4-28-S7-2D-GC Image

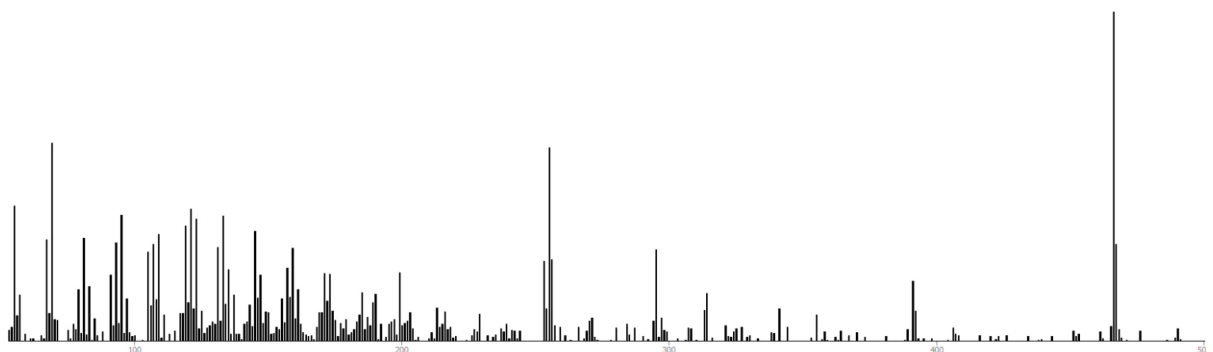


Figure S4-29-T1-1D-Shimadzu

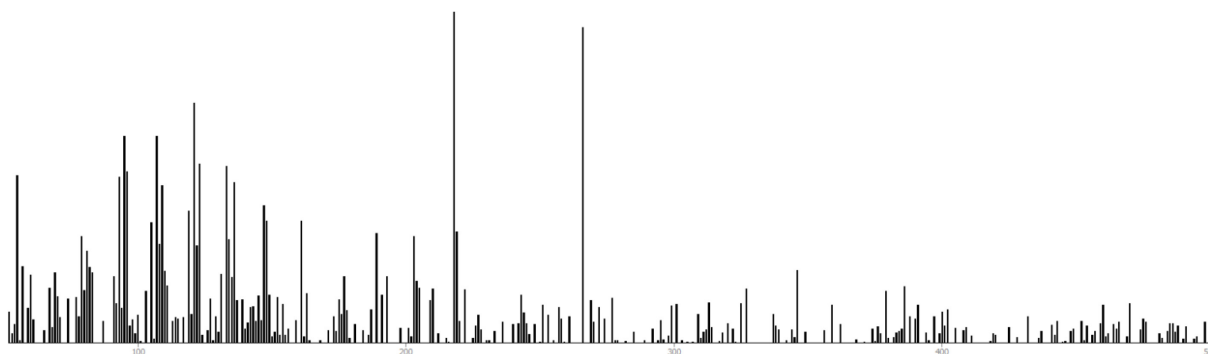


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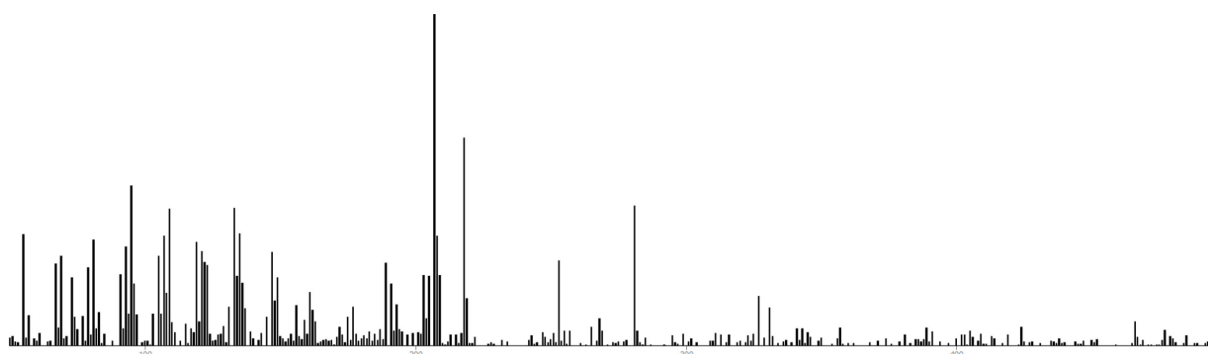


Figure S4-31-T1-2D-AMDIS

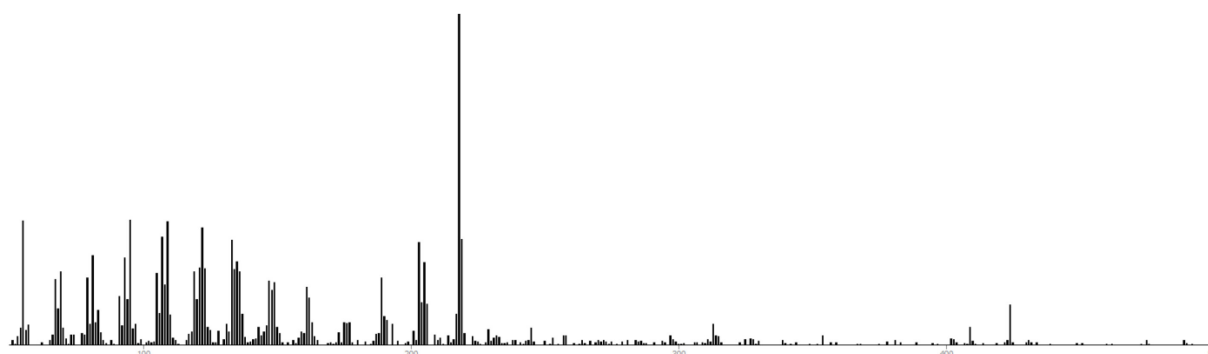


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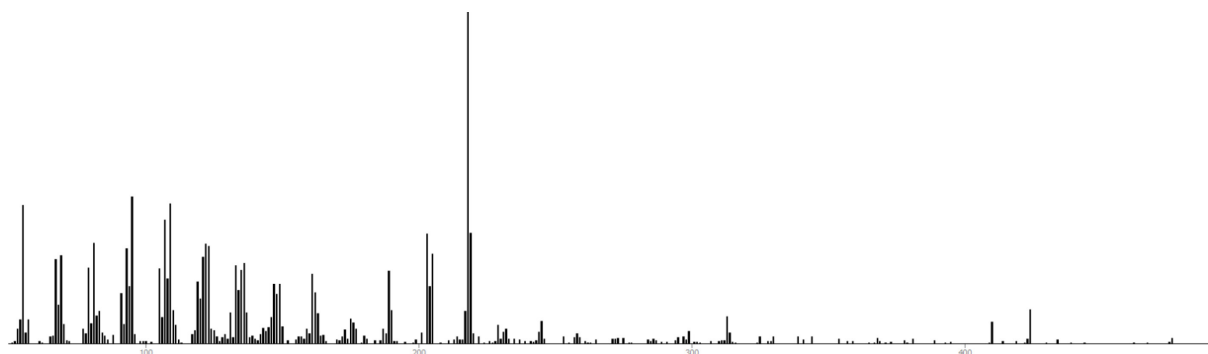


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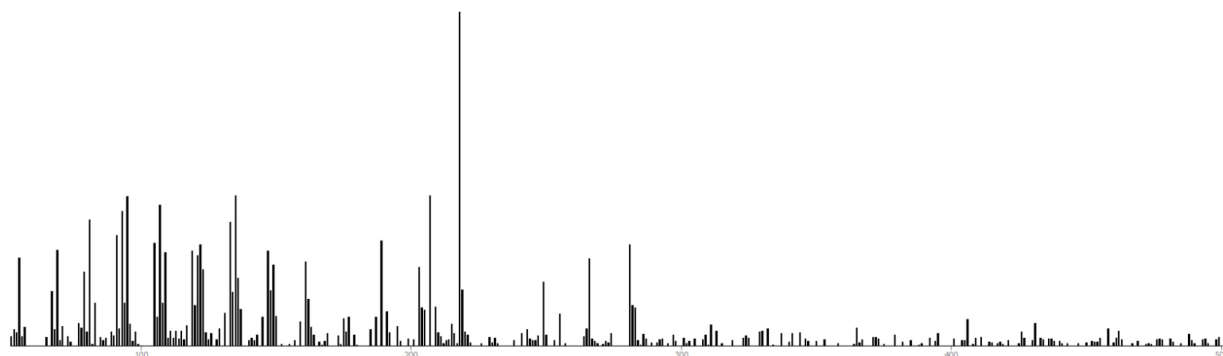


Figure S4-34-T2-1D-AMDIS

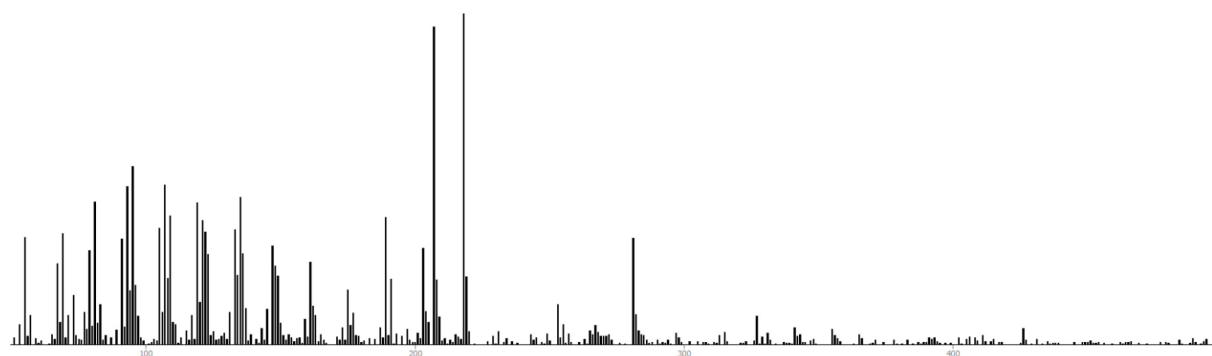


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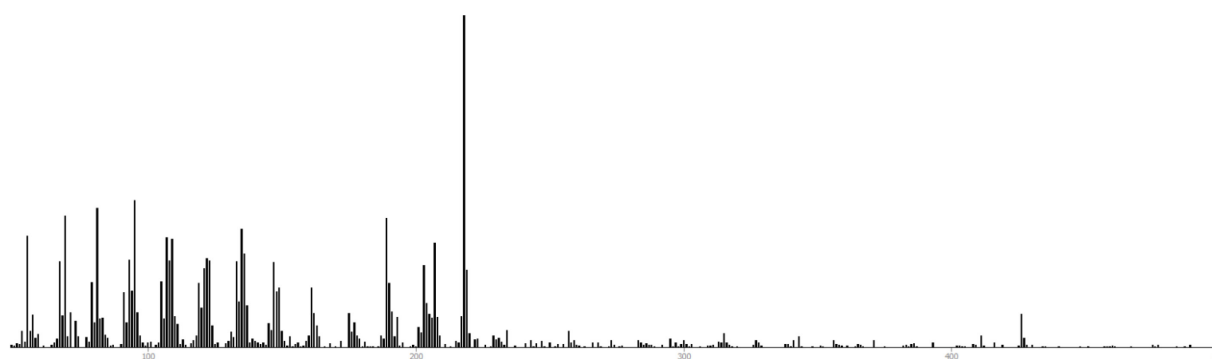


Figure S4-36-T2-2D-GC Image

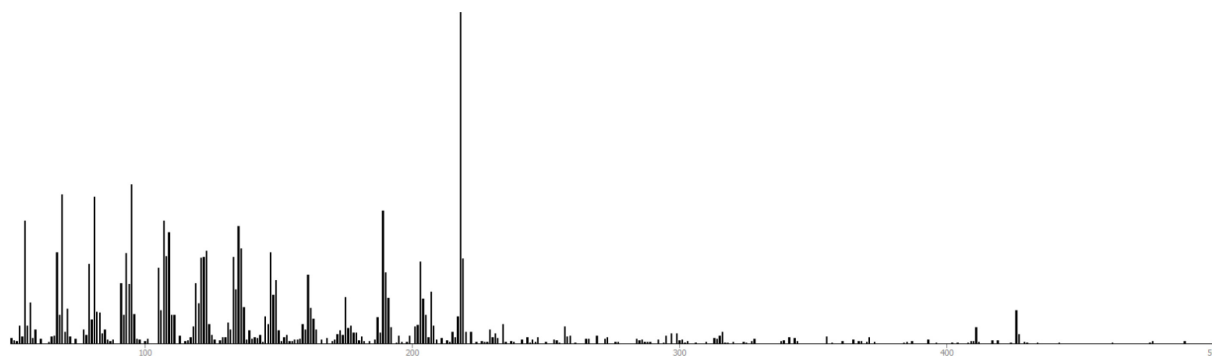


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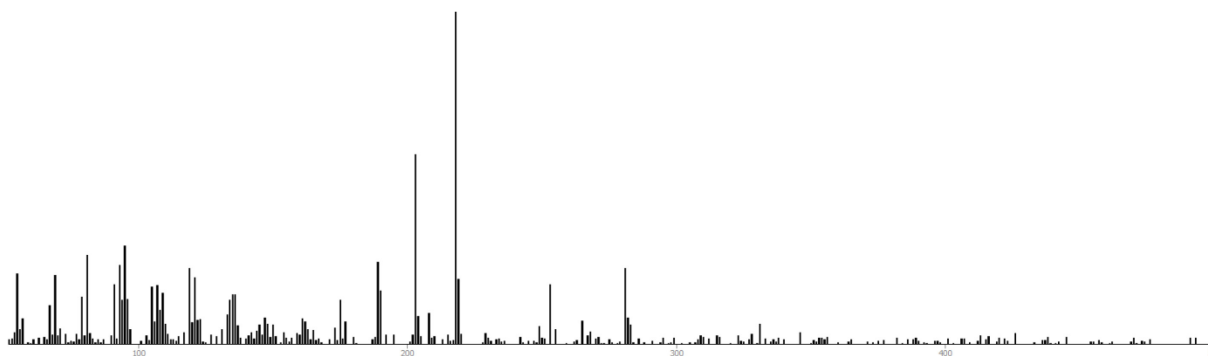


Figure S4-38-T3-1D-AMDIS

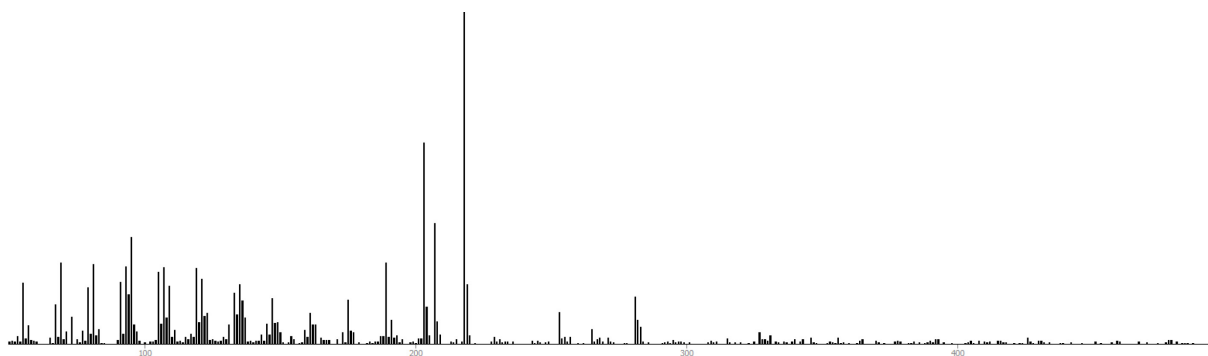


Figure S4-39-T3-2D-AMDIS

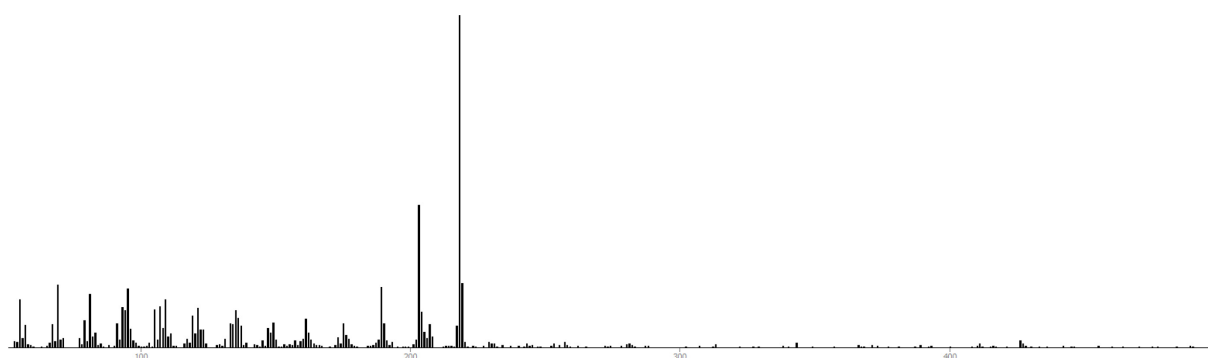


Figure S4-40-T3-2D-GC IMAGE

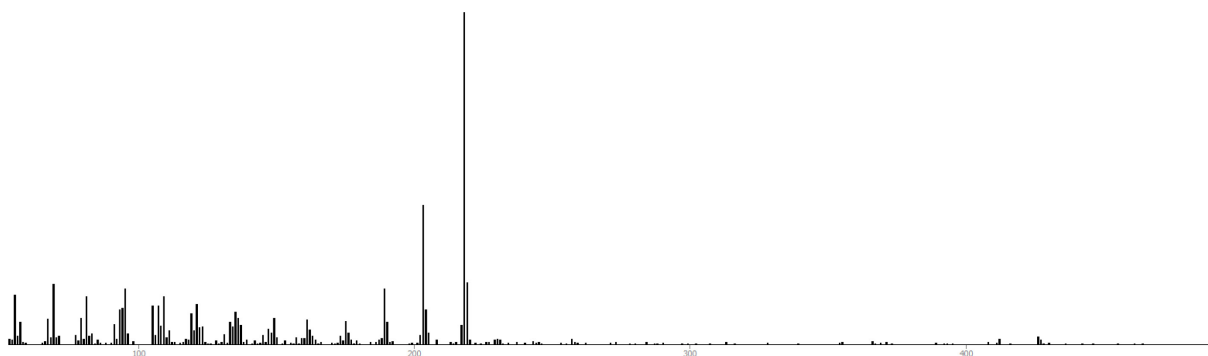


Figure S4-41-T4-1D-Shimadzu

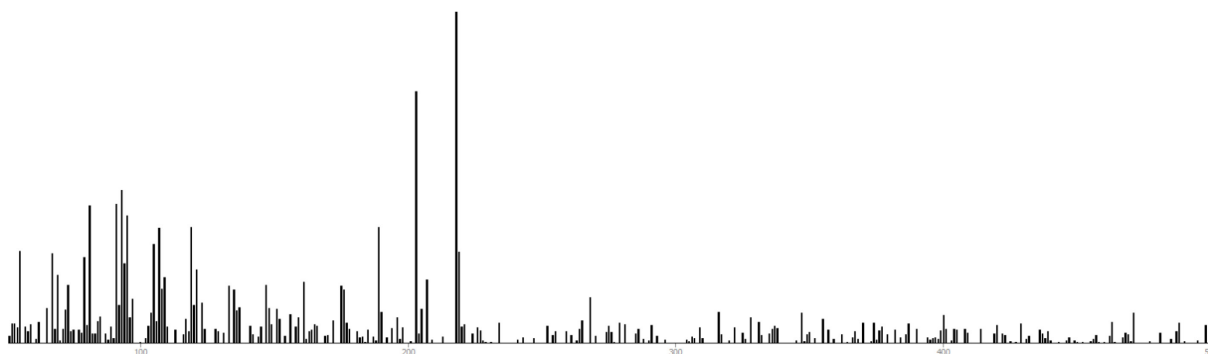


Figure S4-42-T4-1D-AMDIS

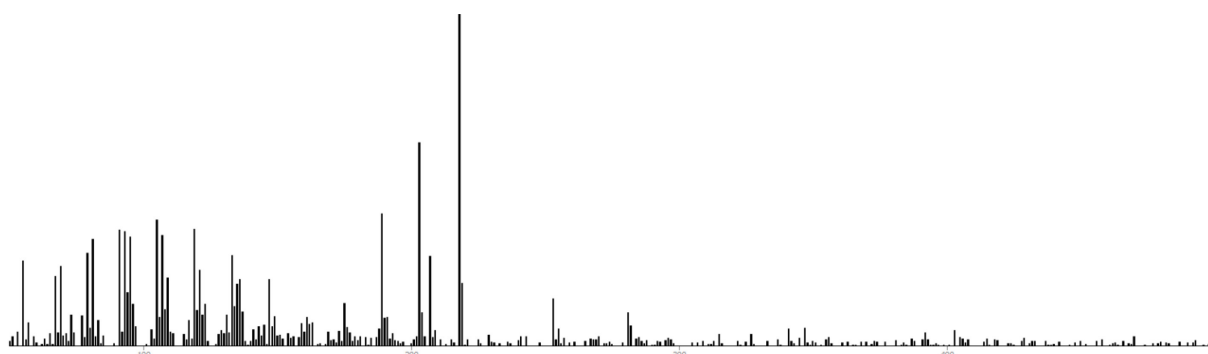


Figure S4-43-T4-2D-AMDIS

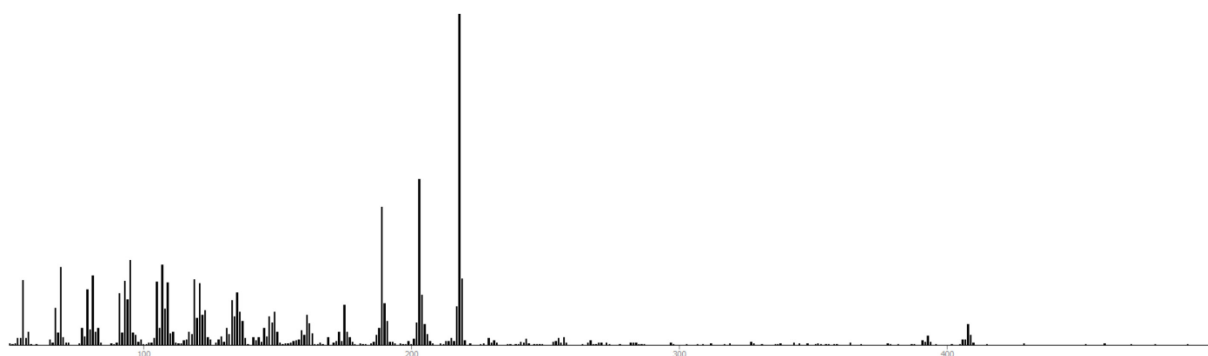


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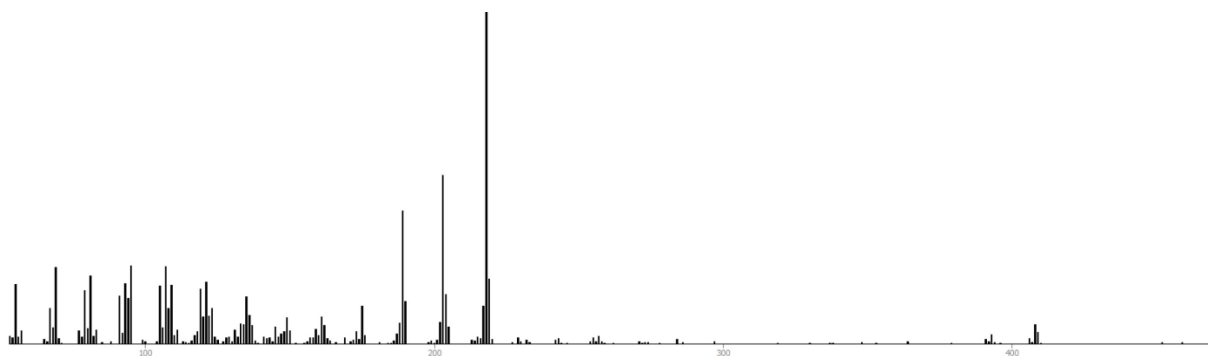


Figure S4-45-T5-1D-Shimadzu

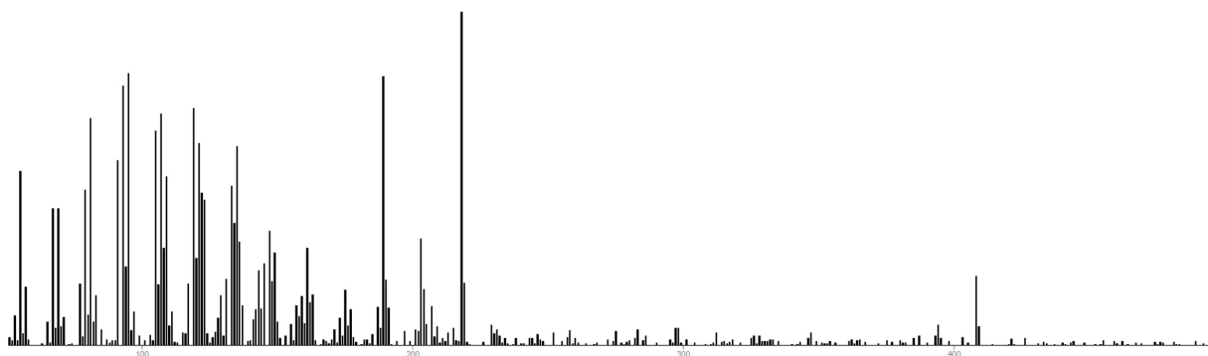


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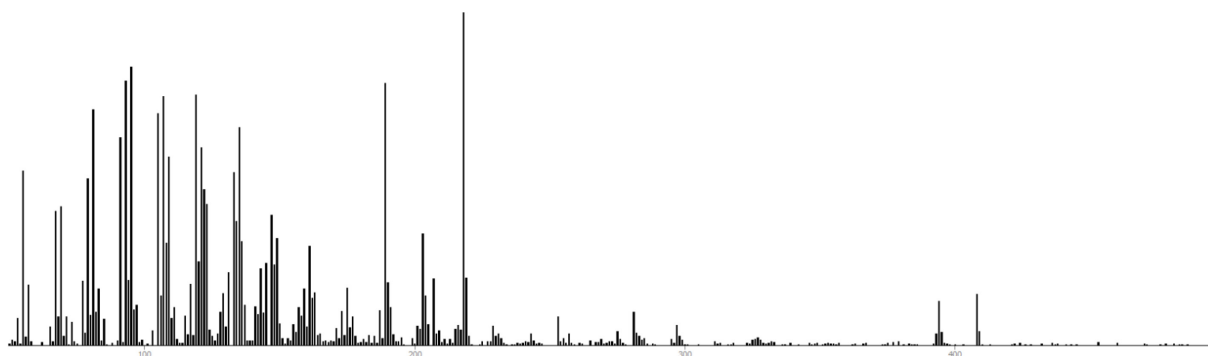


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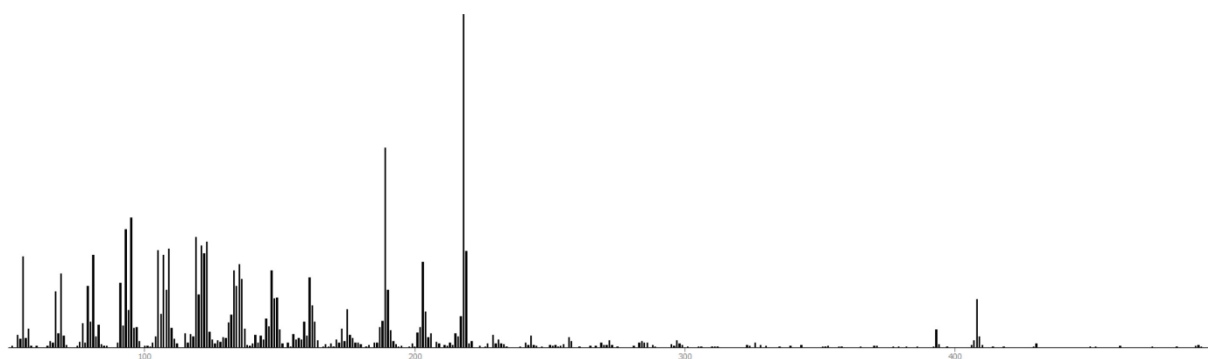


Figure S4-48-T5-2D-GC Image

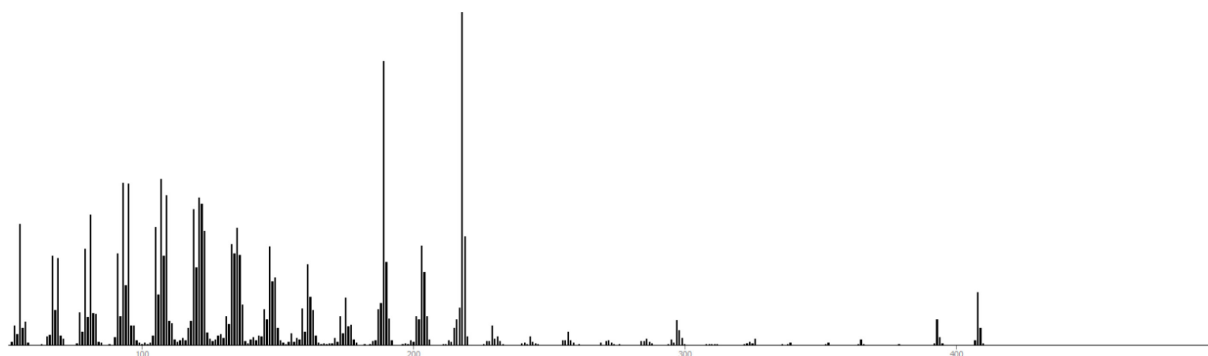


Figure S4-49-T6-1D-Shimadzu

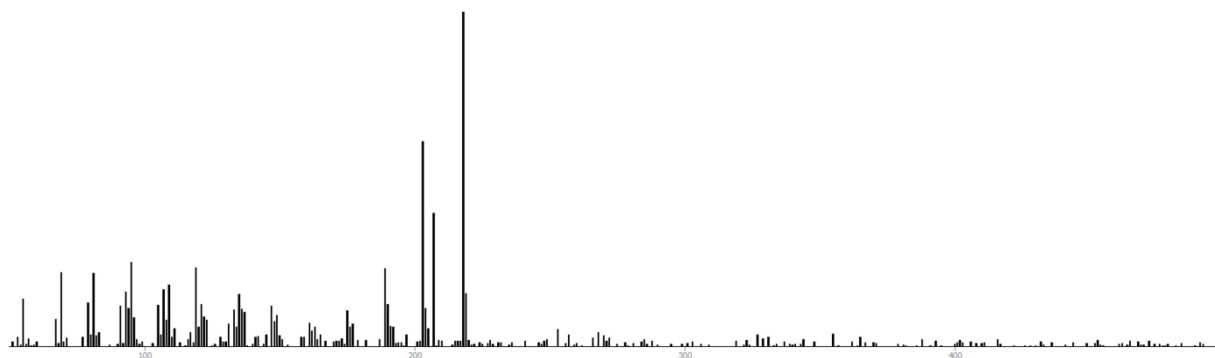


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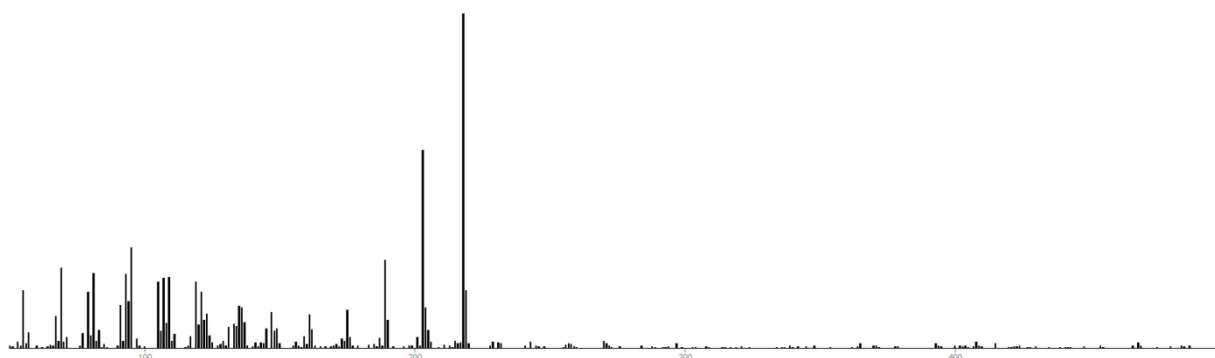


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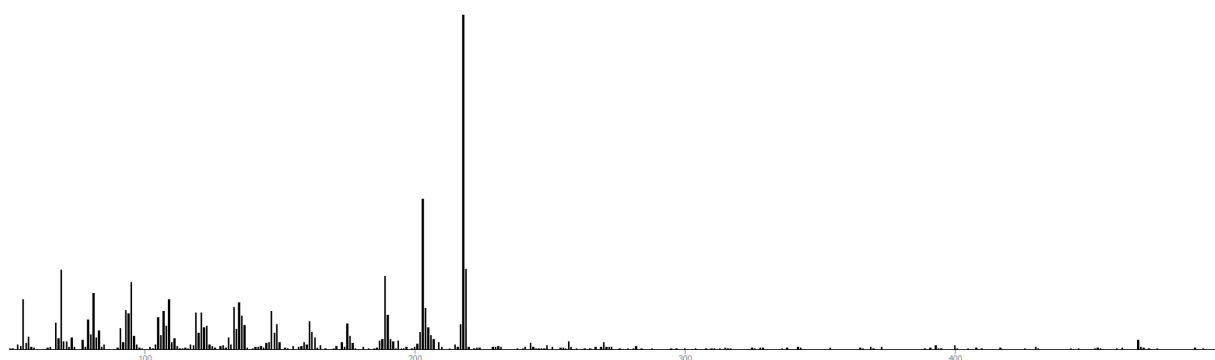


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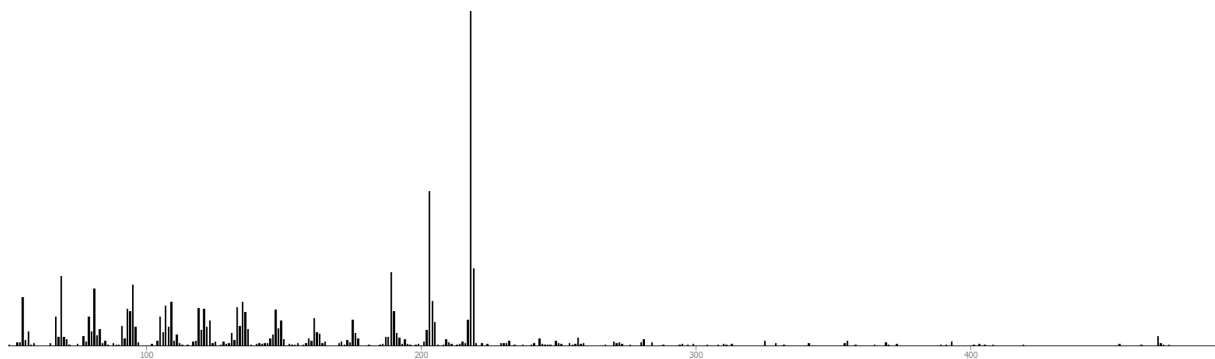


Figure S4-53-T7-1D-Shimadzu

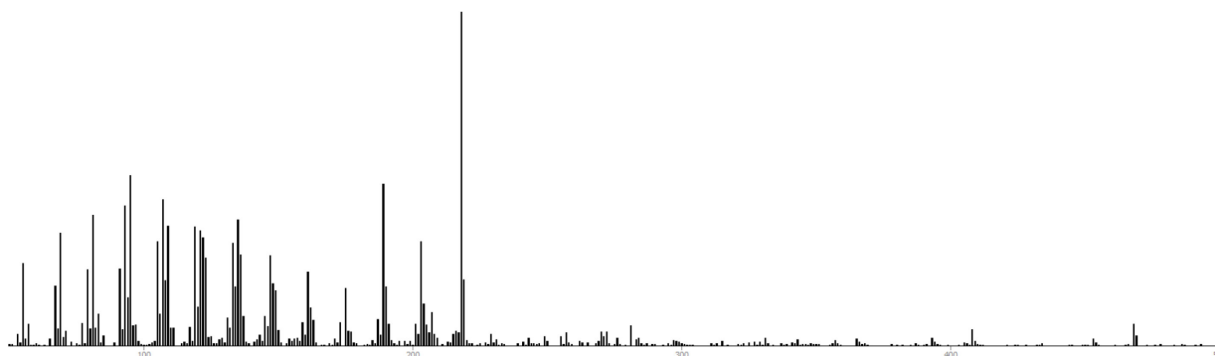


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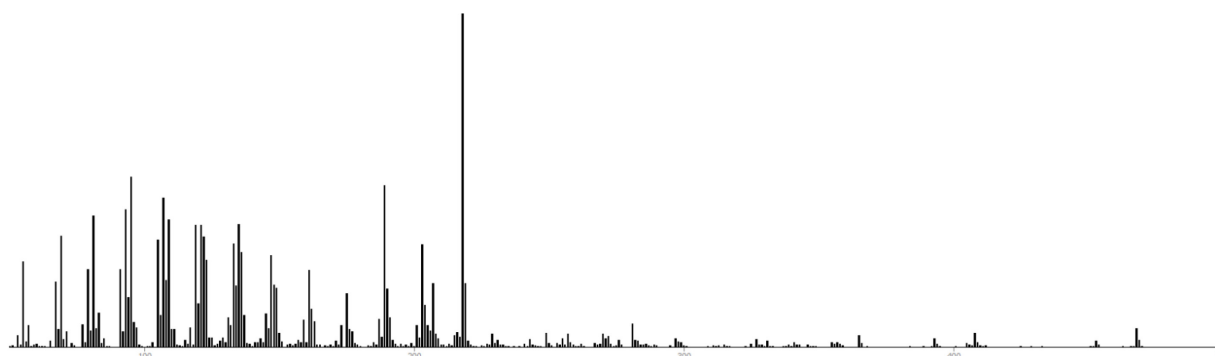


Figure S4-55-T7-2D-AMDIS

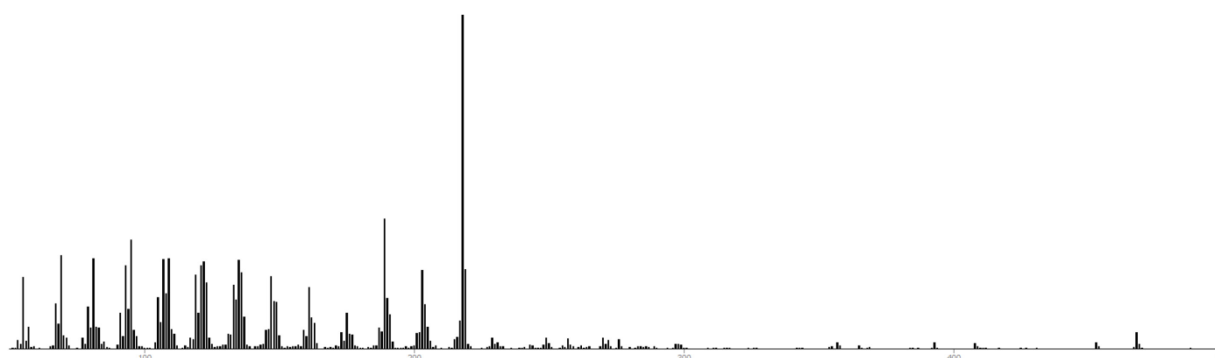


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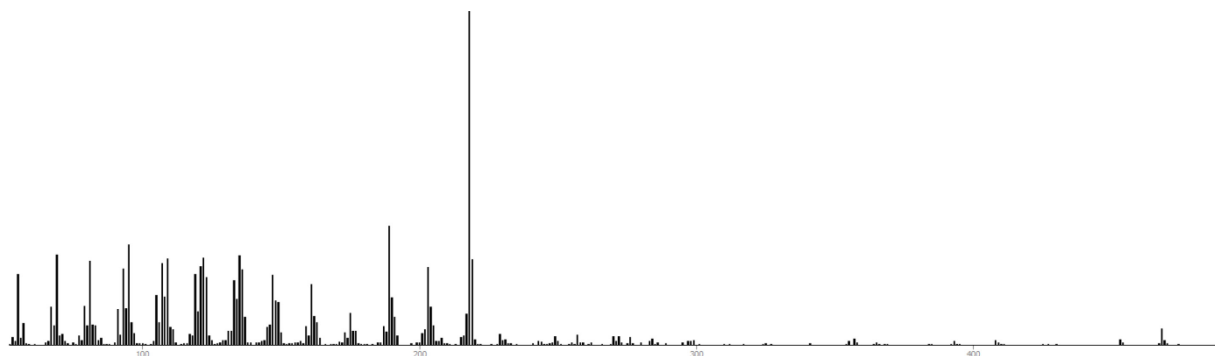
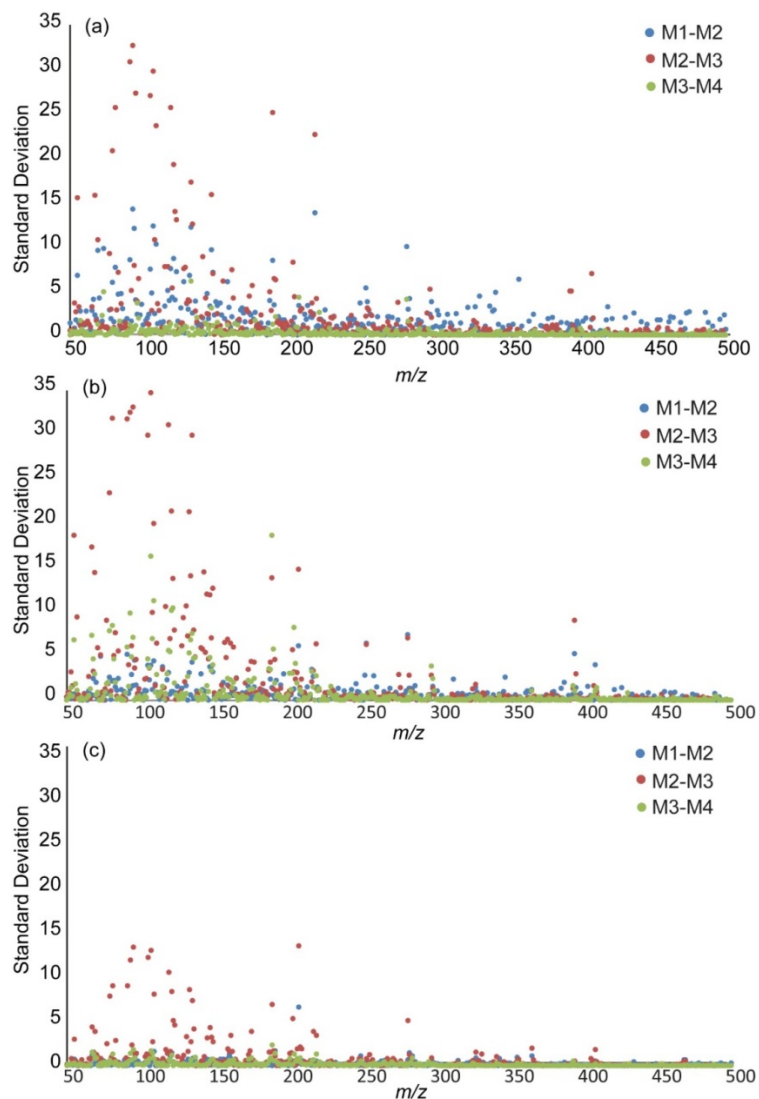


Figure S5. The standard deviation of mass spectra (base-peak normalized to 100) between M1-M2, M2-M3 and M3-M4 for compound S5 (a), T5 (b) and T7 (c). (M1, M2, M3 and M4 correspond to Figure S3)



Characterization and materiality of modern Korean handmade paper (*Hanji*) for conservation purposes

Abstract: a set of more than three hundred modern Korean handmade papers were investigated in this study through samples collected in the sample book *Hanji, Korean handmade paper*. It contains samples in great numbers with detailed information on the papermaking conditions together with data resulting from mechanical and optical tests undergone. This work first analysed these data using methods such as regression and principal components analysis followed by a direct fiber observation using a digital microscope. It provides the advantage not necessary to take the materials from the paper and it allows to exam all parts of a sample, thus being beneficial when dealing with cultural heritage artefacts in the conservation process. As the results, correlation is shown between fiber orientation and papermaking technique (*Gadumtteugi* vs *Heulimtteugi*). Fiber identification revealed the characteristic features of *Hanji* made of *Dak* fibers with the non-sampling method. In parallel, pyrolysis-gas chromatography/mass spectrometry is performed for the first time on *Hanji* samples with 18 pyrolysis product from lignin identified (H/G/S type) as well as the pattern of terpenic fingerprint compounds that constitute biomarkers for *Hanji* with the ions m/z 218, 203, 189 being characteristic for identification. This multi-analytical study reveals and highlights the principal characteristics of *Hanji* that can be beneficial for the identification of fiber origin, making method, treatment as well as provides comprehensive and overall information for conservation purposes.

Keywords: Korean handmade papers; *Hanji*, papermaking methods, PCA, fiber identification, pyrolysis-gas chromatography/mass spectrometry

Introduction

The Korean peninsula was among the first places to acquire the papermaking technology from China. Traditionally, Korean handmade paper, commonly referred as *Hanji*, was a unique symbol of culture in Korean peninsula. It is a soft and durable paper which has prestigious reputation in commercial trade with China in Goryeo period (918 AD–1392 AD) and was treasured as the munbangsawu (four friends of scholar's studio) (Yum, 2008). Traditional *Hanji* is permanent and its life span can exceed 1000 years when held in proper conditions (Choi *et al.*, 2012, Jeong *et al.*, 2014, Jeong *et al.*, 2014). *Hanji* has long been used for conservation in Republic of Korea while recently it has drawn attention from paper conservators in Western countries for its conservation applications and professional links such as Group 130° was established (Paolo & Poggi, 2015).

In the process of paper conservation, information for paper (such as grams per square metre, color, thickness, endurance etc.) is important reference for conservators. A comprehensive knowledge about the kind of paper, relevant producing technique and physical properties is a key point for paper conservators in selecting and using the conservation and restoration materials. Although the characteristics of *Hanji* varies with type, quality, date of fibre extraction and place of manufacture in conservation practice (Paolo & Poggi, 2015), no study has been reported to unfurl the details. Moreover, most attempt to characterize *Hanji* from designed experiment were usually with limited samples that cannot reveal a general cognition of modern *Hanji*.

Due to the efforts of prof. Hyoung-Jin Kim (Kookmin University, Seoul), a four-volume sample book *Hanji, Korean handmade paper* was published in 2010 (Fig.1A). This book

provides more than three hundred *Hanji* samples from 26 paper mills across South Korea. It includes valuable information not only on the papermaking process but also provides results of property measurements (mechanical, optical and pH etc.) performed on these handmade paper samples (Fig.1B and Fig.1C). This book forms a coherent whole and thus can be considered as a corpus reflecting the modern Korean handmade papers: a well edited reference sources for conservators as well as other practitioner that use *Hanji*. Nevertheless, only 50 copies had been distributed world widely that largely restrict its accessibility as the function for a handy reference. Moreover, the data provided by this sample book has to be processed in order to better define the general characteristics of modern *Hanji*. In fact, the statistical analysis is a tool for depicting the overall information from large data, for instance, the statistical analysis of 493 fiber analysis results of ancient handmade papers in China between 1966 and 2016 revealed a very clear temporal change in fiber sources utilization in the history of papermaking in China (Li, 2018) and a systematic characterisation of 178 Chinese papers revealed the detailed information that differed from contemporary western papers from 19th and 20th century (Brown *et al.*, 2017).

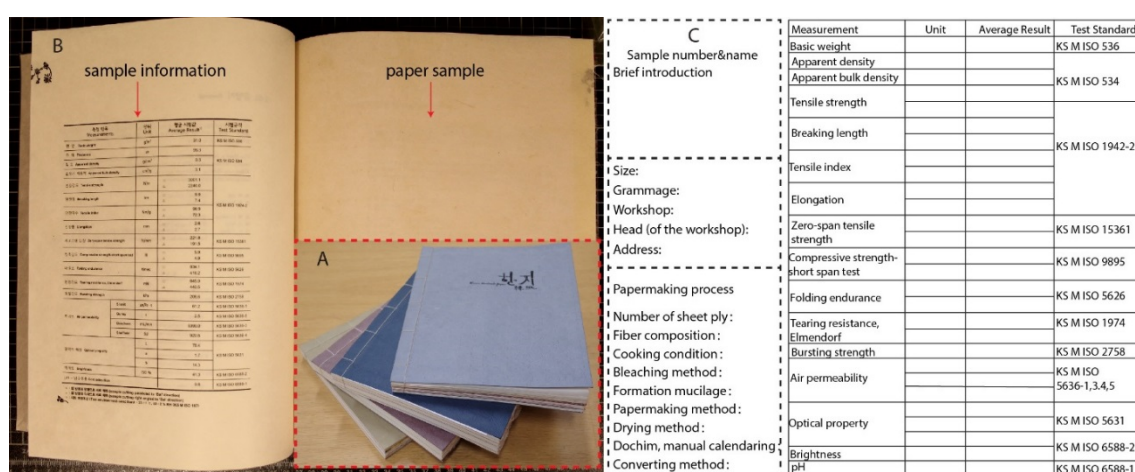


Figure 1. The four-volume of sample book *Hanji, Korean handmade paper* (A) provides paper samples with making information and property measurements data (B). (C) is an example of the information included.

For the purpose of unfurling the information of modern *Hanji* from a large data set (usually rare in handmade paper studies) as well as make it wider and easier access for conservators, systematical and statistical methods such as regression and principal components analysis (PCA) were employed to the large set data to explore the main features of *Hanji*. Moreover, Additional investigation such as fiber analysis and chemical composition of the samples has to be studied since it was not provided. To acquire this information, digital microscopy was used at low magnification (50x) for fiber orientation and at higher magnification (500x, 1000x) for fiber identification. This technique is non-invasive and allows direct examination of all parts of the whole sheet, in contrast to the stain method that is traditionally used.

In conservation practice, chemistry has contributed to paper conservation which identified the paper component and studied the mechanism of paper's aging and degradation as well as providing various testing methods (Carter, 1996a, 1996b; Daniels, 1996). These methods usually require large quantity of samples with comparative complex sample preparation process. So, an easy sampling preparation, quick analysis process and reliable data interpretation method for the characterization of conservation materials has to be defined.

In this study, a novel analytical method based on Py-GC/MS was applied as a quick way to acquire *Hanji*'s chemical profiles. Typical lignin markers of phenolic compounds with p-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) aromatic moieties, and terpenic compounds with the characteristic ions of m/z at 218, 203, 189 was identified and described in the pyrolysis of *Hanji*. The composition and percentages of monomeric aromatic compounds depends on the plant source and pretreatment method and thus can be implications for *Hanji*'s discolouration (Daniels 1996) as well as paper quality and plant species identification (Pérez *et al.*, 2002; Das *et al.*, 2018). The terpenic compounds can be a quick fiber identification reference of papermaking fibers from the the *Moraceae* family as revealed in the current studies.

Materials and methods

Materials

The materials used in this study consist of 309 *Hanji* samples contained in the four-volume sample book *Hanji, Korean handmade paper* edited by prof. Hyoung-Jin Kim (Kookmin University, Seoul) and published in 2010 (Fig.1) (Kim, 2010). 50 copies have been distributed worldwide to libraries and conservation research institutions.

Data analysis

The data about papermaking methods, pH, mechanical property (grammage, thickness, tensile strength, folding endurance) and color information of 309 handmade papers in the sample book was filled up into an excel sheet in the Microsoft Excel (2013) and then analyzed using Microsoft Excel (2013) and the Unscrambler X (Version 10.4) software from CAMO Software AS.

Microscopic observation

Microscopic analysis of *Hanji* samples were performed by a digital microscope (VHX-1000, Keyence, Japan). The samples were placed directly onto the microscope platform. For fiber orientation examination, light sources in horizontal and vertical directions were utilized in light reflection mode as described elsewhere (Masuda, 2010). For the fiber identification, the observation was conducted in reflection mode with halogen lamp as light source. Polarized light microscopy was utilized to identify the papermaking fibers under both Crossed Nicol and Open Nicol conditions.

Py-GC/MS Analysis

Py-GC/MS analyses were performed using a vertical micro-furnace-type pyrolyzer PY-2020iD (Frontier Lab, Fukushima, Japan) directly connected to the injection port of a Shimadzu QP2010 gas chromatograph linked with a Shimadzu QP2010Plus quadrupole mass spectrometer (Shimadzu, Champs-sur-Marne, France). The sample was placed in a stainless steel sample cup. The paper samples were weighted with an XP2U Ultra Micro Balance (Mettler Toledo, Viroflay, France). Typical quantities of paper used in this work were ca. 100 µg weighted with the microbalance. The sample cup was placed on top of the pyrolyzer at

near ambient temperature. The sample cup was introduced into the furnace at 500 °C, and then the temperature program of the gas chromatograph oven was started. The Py-GC interface was held at 320 °C. Chromatographic separation was carried out on an Frontier Lab DB5-ms 5% phenyl–95% dimethyl polysiloxane fused silica capillary column (30 m length, 0.25 mm inner diameter and coated with a 0.25 µm film thickness). The oven temperature was initially held 3 min at 40 °C, and then ramped at 5 °C min⁻¹ to 325 °C, where it was held for 10 min. The total duration of GC analysis was 70 min. The helium carrier gas Alphagaz 1 (Air Liquide, France) was used in the linear velocity mode (1 mL min⁻¹). The injector was held at 280°C and used in split mode (1:10 of the total flow). The mass spectrometer was operated at 5000 u.s⁻¹, with a scan range from 50 to 500 u, using electron ionization at 70 eV. The interface was kept at 300 °C and the MS source at 200 °C. The mass spectra of the pyrolysis products were recorded using the average spectrum +average and subtract spectrum modes in the Shimadzu GCMSsolution software. Identifications were achieved on the basis of EI mass spectra by interpretation of the main fragmentations and using the NIST MS library (2011).

Results and discussions

Overview and features of modern Hanji

As composed in the sample book, almost all the modern Korean handmade papers were made of *Dak* (Korean name for paper mulberry) fibers. During the process of making *Hanji*, plant ash was traditionally utilized to create a mild alkali cooking solution. However, nowadays only 13% *Hanji* are still cooked with plant ash while most are treated with sodium carbonate or sodium hydroxide which permits shorter treatment time. The sheet formation of *Hanji* involves two main techniques: *Heulimtteugi* (*Oebal*) and *Gadumtteugi* (*Ssangbal*) which differs in the mould shape and formation process (Cheon *et al.*, 1999, Kim *et al.*, 2004). 212 out of 309 samples (69%) were made by *Gadumtteugi* making method while 97 samples (31%) by *Heulimtteugi* which illustrated that the tradition of *Heulimtteugi* is less practiced since it requires intensive labor and virtuosity.

The pH of *Hanji* ranged from 5.3 to 9.8 with 98.3% possessing pH value above 6 and only 1.7% (5 samples) with the pH value between 5~6 (Fig.2A). 83.3% can be considered as non-acidic papers with pH above 7. This characteristic feature is linked with the treatment process with plant ash (or sodium carbonate, sodium hydroxide) which is different from machine made papers. It is noted that the dying process can greatly affect the pH of *Hanji* as for the samples having pH value under 6, four out of five were dyed papers.

Grammage is another intrinsic physical characteristic of paper and is defined based on its weight in grams per square meter (GSM). It can be seen from Fig.2B that more than half of *Hanji* samples are with grammage between 20~40 GSM while 24.3% are between 40~60 GSM. Only 5.8% have grammage more than 80 GSM. The majority of *Hanji* is of low grammage (the average value is 44.4 GSM). As for the thickness, the majority (40.8%) are in the range of 100~150 µm (Fig.2C) while the papermakers can also produce thin papers that can be used for paintings and calligraphy to have unique effect. Several *Hanji* presents this character with 11 samples having thickness less than 50 µm with 32.5 µm as the minimum value (thin, soft and transparent papers). The correlation of grammage and thickness is calculated and shown in Fig.2D. It shows that *Hanji* having the grammage ranging from 5.6~90 GSM and thickness ranging from 32.5~300 µm were conglomerated and can be classified into four main groups with the first two groups taking the majority (blue square and red dot in Fig.2D). The density of paper ρ is calculated as the grammage w divided by the thickness t (Equation 1) and it is influenced by the type of fibre and all paper technological

operations such as fibre separation, beating, pressing, drying and calendering. Many constructional materials have homogeneous structures while for paper the density varies between the plant species. The accuracy of the *Hanji* density characteristic determination is consequently dependent on the thickness measurement which is difficult to measure precisely from a rough and compressible material such as a single paper. However, the density for *Hanji* can be easily calculated from the correlation of grammage and thickness: as calculated from Equation 1, the grammage equals to density multiplied by thickness (Equation 2) which is the slope of the correlation. As revealed from the correlation analysis (Fig.2D), the density of *Hanji* is 308 kg/m³ with variations depends on fiber furnish or *Hanji* making conditions. The linear regression with a coefficient of determination value of 0.87 shows a well matched correlation. From the F-test, the Significance F is 2.87 E-13 which indicate significance correlation. The T-test for the intercept (P-value 1.46E-13) and the variable (grammage, P-value 2.87E-13) also indicate their significance in the modeling. (15 samples which are thick paper board, crumpled or hollow papers, were not taken for regression analysis).

$$\rho = w/t \text{ (kg/m}^3\text{)}$$

$$\text{Equation 1}$$

$$w = \rho t \text{ (g/m}^2\text{)}$$

$$\text{Equation 2}$$

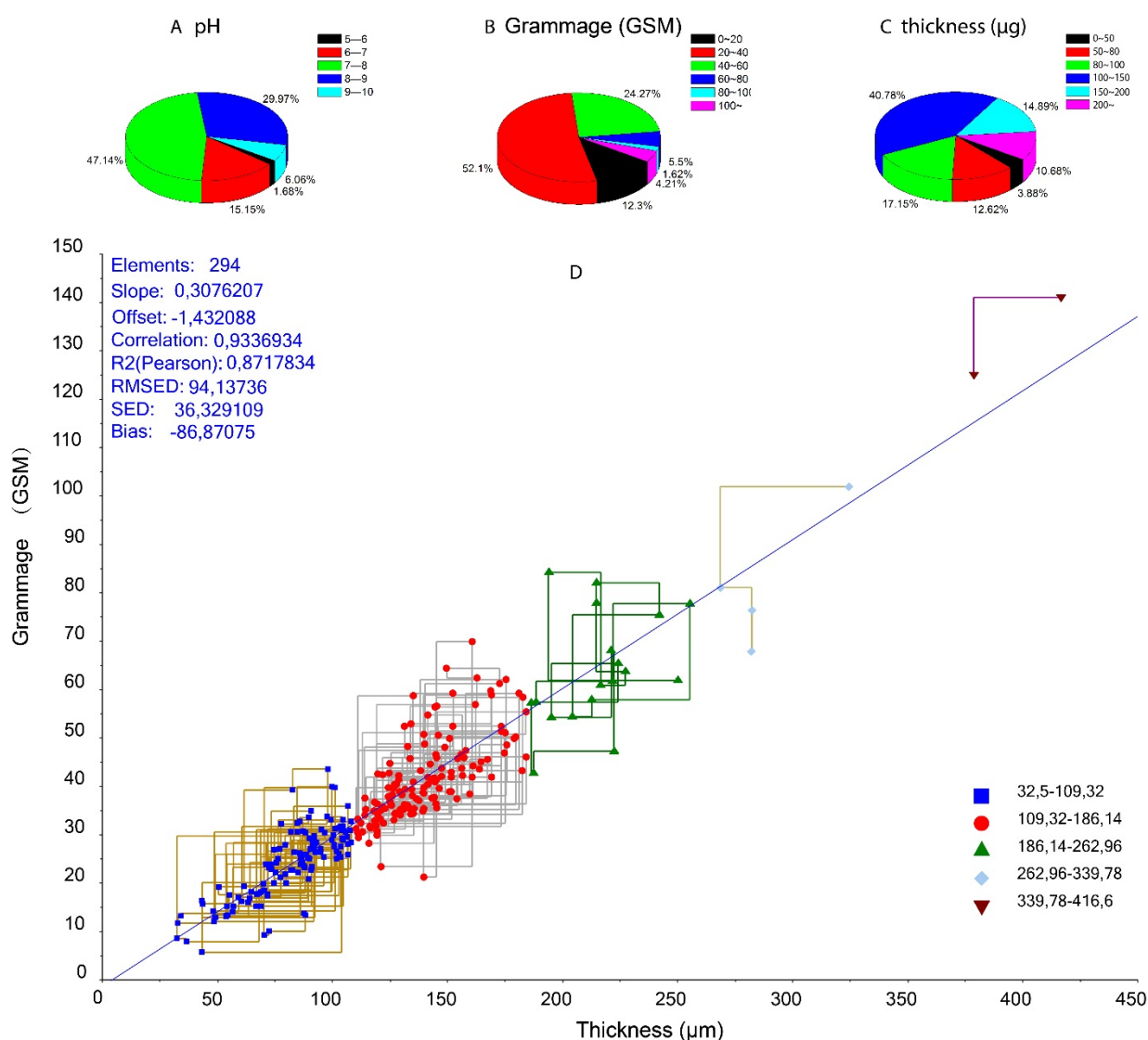


Figure 2. The pH (A), grammage (B) and thickness (C) of *Hanji* samples. (D) presents the correlation between the grammage and thickness.

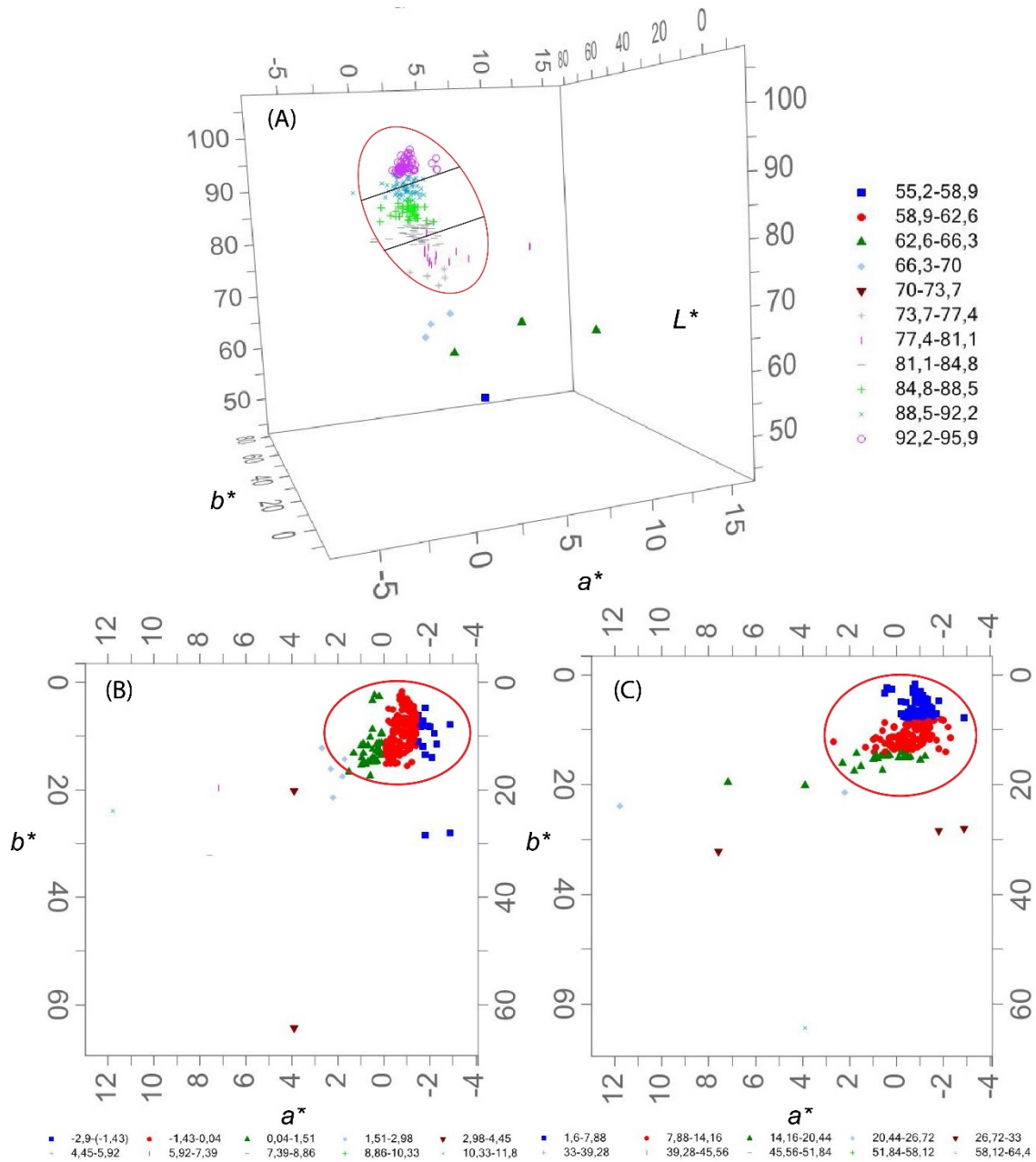


Figure 3. The L^* a^* b^* color space of un-dyed *Hanji* paper (A). (B) and (C) are projections in a^* and b^* space.

227 samples are un-dyed which may be a presentation of the nature color for *Hanji*. The L^* , a^* , b^* color space of the un-dyed *Hanji* papers is shown in Fig.3. It can be seen that the lightness range from 69.9 to 95.9 with an average value of 88.2. The color represent of modern *Hanji* can be classified into groups with L^* value ranging from low, medium and high (fig.3A). As for a^* and b^* coordinate, all the papers are located on the $-a^*+b^*$ and $+a^*+b^*$ space with $-a^*+b^*$ space having the majority (fig.3B and fig.3C). With the b^* ranging from 0 to 20, the yellowness is the main characteristic. The majority of the b^* value clustered within 1.6-7.88 and 7.88-14.16 with small samples having values ranging to 20. For coordinate a^* , the majority clustered in the range -1.43 to 0.04 with another two group (-2.9 to -1.43, 0.04 to 1.51). This indicates that most *Hanji* represent more greenness than redness in color representation. Samples with larger values were also present in Fig.3. They all went through processes such as mix with colored clay, black bark. To summarize, the un-dyed *Hanji*

present the color of greenness-yellowness while little take the color of redness-yellowness. They were within a color gamut of L^* (69.9-95.9), a^* ((-3)-3), b^* (0-20). The color gamut of un-dyed *Hanji* paper can be a quick reference for conservators to check if the target document is within the color gamut of un-dyed *Hanji* as to select proper *Hanji*. It also contributes to paper color database when analyzing papers from different times and origins (Sakamoto & Okada, 2013, 2015).

Influence of papermaking technique on *Hanji*'s property

The mechanical property of *Hanji* with different making method was analyzed. It can be seen from Fig.4A that the tensile strength are mostly located in the range of 352.8~3000 N/m (grain-direction) and 183.1~2000 N/m (cross-directions) (grain and cross directions are machine papermaking terms used in standard testing method, they are used here for pointing handmade paper's directions). In detail, it is interesting to note that the majority of *Hanji* (75.7%) hold the property that the tensile strength for grain-direction is higher than cross-direction with the reverse ones mostly being *Heulimtteugi Hanji*. The *Heulimtteugi Hanji* (94 samples) showed a proportion of 50% with the ratio tensile strength grain-direction/cross-direction greater than 1. However, the *Gadumtteugi Hanji* (188 samples) comparatively showed a proportion of 95% with the ratio tensile strength grain-direction/cross-direction greater than 1. The result indicated that the alignment of the fiber direction of *Heulimtteugi Hanji* present more diversity than those of *Gadumtteugi Hanji* that has more consistency in fiber alignment in one direction.

The folding endurance varies from several (minimum 1.2 times) to thousand times (maximum 1681.1 times) (Fig.4B). 60% of the samples have the folding endurance between 1.2~500 times. As can be seen from their dispersion, almost all the *Gadumtteugi Hanji* were distributed under the diagonal line in Fig.4B which means the ratio of folding endurance grain-direction/cross-direction is higher than 1. With 56.7% above the diagonal line and 44.3% under, the *Heulimtteugi Hanji* presented quite different profiles. The size of the difference is further explored by plotting the ratio of folding endurance grain-direction/cross-direction (Fig.4C). It can be seen that the ratio is more diverse for *Gadumtteugi Hanji* while, comparatively, the ratio for *Heulimtteugi Hanji* is more concentrated within 0-2.7 (with 6 samples in exception). Fig.4C indicate that *Heulimtteugi* tend to produce more even dispersed fiber orientation which reflected by its physical properties. The result showed that the *Heulimtteugi Hanji* presents folding endurance more even than *Gadumtteugi Hanji* which can be one consideration when choosing *Hanji* for applications requiring frequent folding in both directions.

To further investigate the statistical characteristic of the data profile, PCA was performed with tensile strength and folding endurance data values. In view of data disperse range and to derive information from the raw data, the values were put into PCA analysis without normalization. As can be seen from Fig.5, the *Gadumtteugi* and *Heulimtteugi Hanji* were clustered into two parts with *Heulimtteugi Hanji* locate in the positive PC2 coordinate while *Gadumtteugi Hanji* situated in the negative PC2 coordinate (with part in the positive coordinate). Mapping with the tensile strength value (grain direction), within each type, they can be classified into three groups with the tensile strength ranging from low, medium and high (Fig.5A). While mapping with the cross direction value (Fig.5B), *Heulimtteugi Hanji* can still be classified into three groups meanwhile the *Gadumtteugi Hanji* is divided either low or high types. Mapping with the folding endurance value (grain direction) (Fig.5C), the low and medium value were clustered together while mapping with cross direction value showed that *Gadumtteugi Hanji* has weak values in the cross direction folding endurance (Fig.5D). The PCA result showed well group information for handmade papers made with different sheet

formation process and can be a statistical tool to evaluate different type and different properties of *Hanji*. PCA analysis focus on a general trend, while, some samples that don't have well cluster position was observed and this maybe an indication that the mechanical property was further influenced by other factors such as the cutting length of fibers (Lim & Choi, 2008), fiber layers (Seo & Kim, 2011), dochim (Jeon, 2002), and fillers (Lee *et al.*, 2015) as revealed in previous studies.

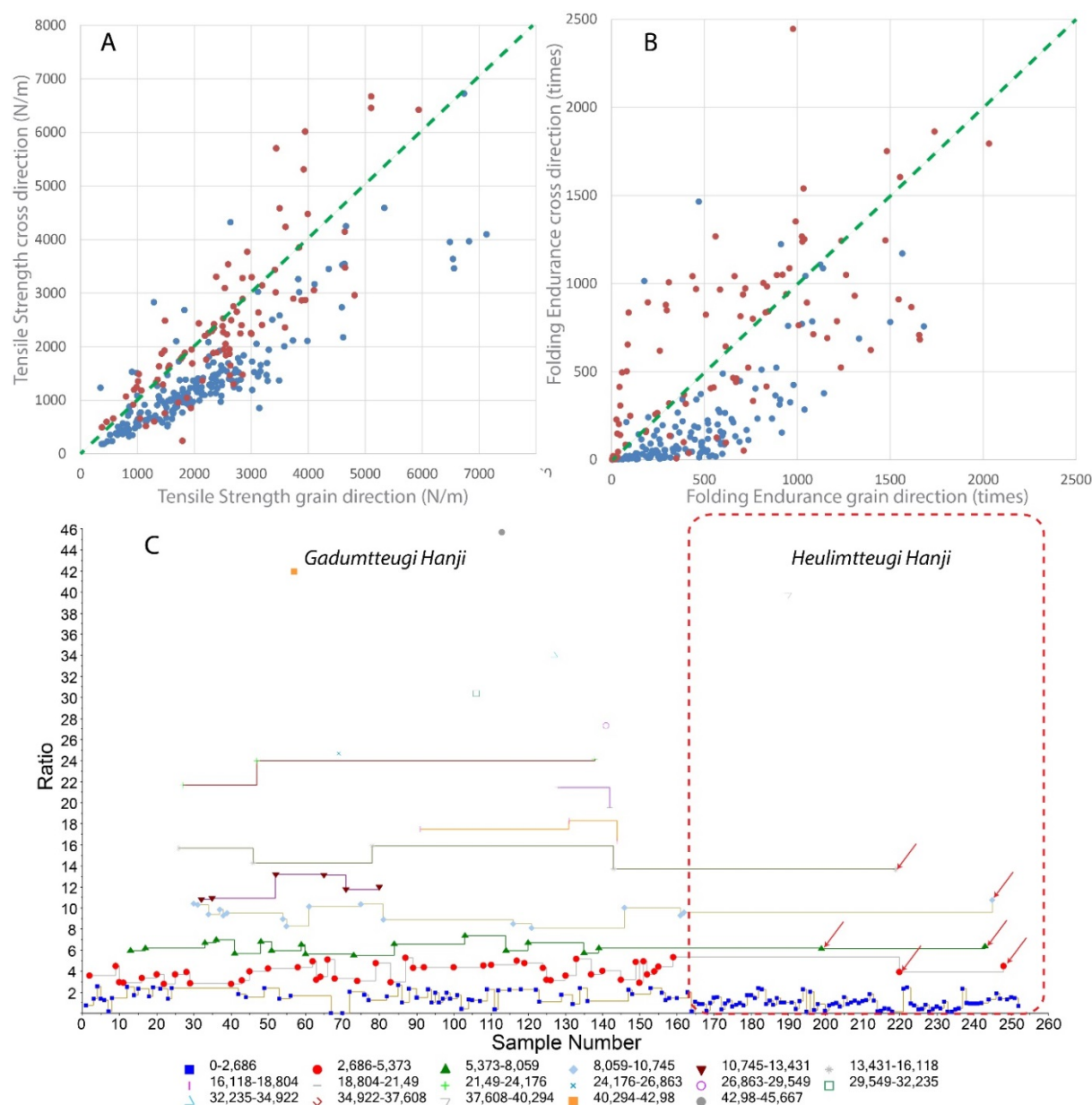


Figure 4. The tensile strength (A) and folding endurance (B) of modern *Hanji*. In (A) and (B), red dot is Heulimtteugi and blue dot is Gadumtteugi Hanji. (C) is the ratio of grain-direction/cross-direction of folding endurance of Gadumtteugi and Heulimtteugi *Hanji*.

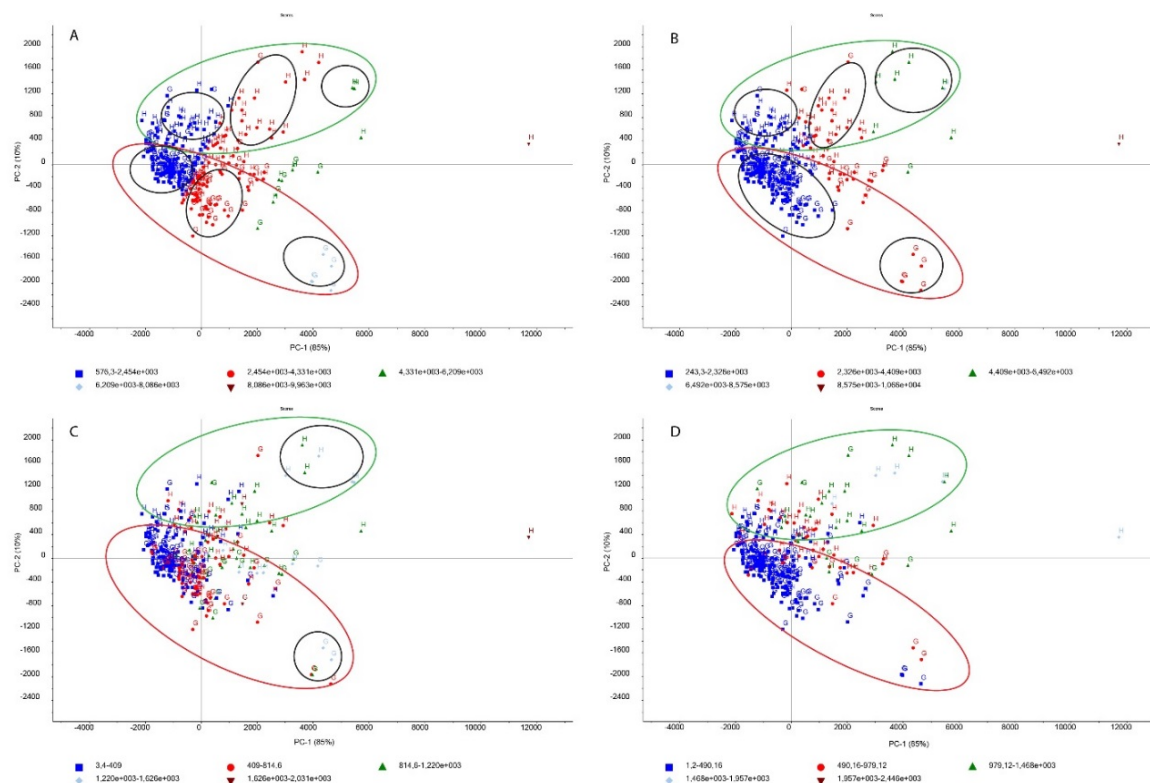


Figure 5. Scores plot in PCA analysis of the mechanical property of *Hanji* with mappings of tensile strength value in grain-direction (A) and cross-direction (B), folding endurance value in grain-direction (C) and cross-direction (D).

By comparison, *Heulimtteugi Hanji* shows profiles of more evenness in considering the mechanical values in both directions. The detailed profile of mechanical property for different types of *Hanji* (Fig.4, Fig.5) is interesting especially for choosing *Hanji* for different application purposes (archives, restorations etc.) (Zhang, 2013, 2016). For instance, priority consideration could be considered to *Heulimtteugi* making method when the application requires repeatedly folding and rough treatment in both directions while book spine which requires one direction frequent folding may choose designed ones in *Gadumtteugi Hanji*. The PCA analysis affirmed the subjective cognition but providing more detailed profiles with the trends statistically depicted which is only possible with great number of samples available.

Microscopic characterization of Hanji

The fiber orientation is a factor of dispersion of fiber directions that can be reflected by its physical properties as was discussed above. This point indicates that fiber orientation information has clues tracing back to the papermaking methods or papermaker's movements when making papers (Masuda, 2010). The properties reflecting the traces associated with different making method can be applicable for museum samples to explore a non-invasive method. For such purpose, a direct microscopic analysis and characterization is explored for the characterization of surface fiber orientation with an examination of all the *Hanji* samples.

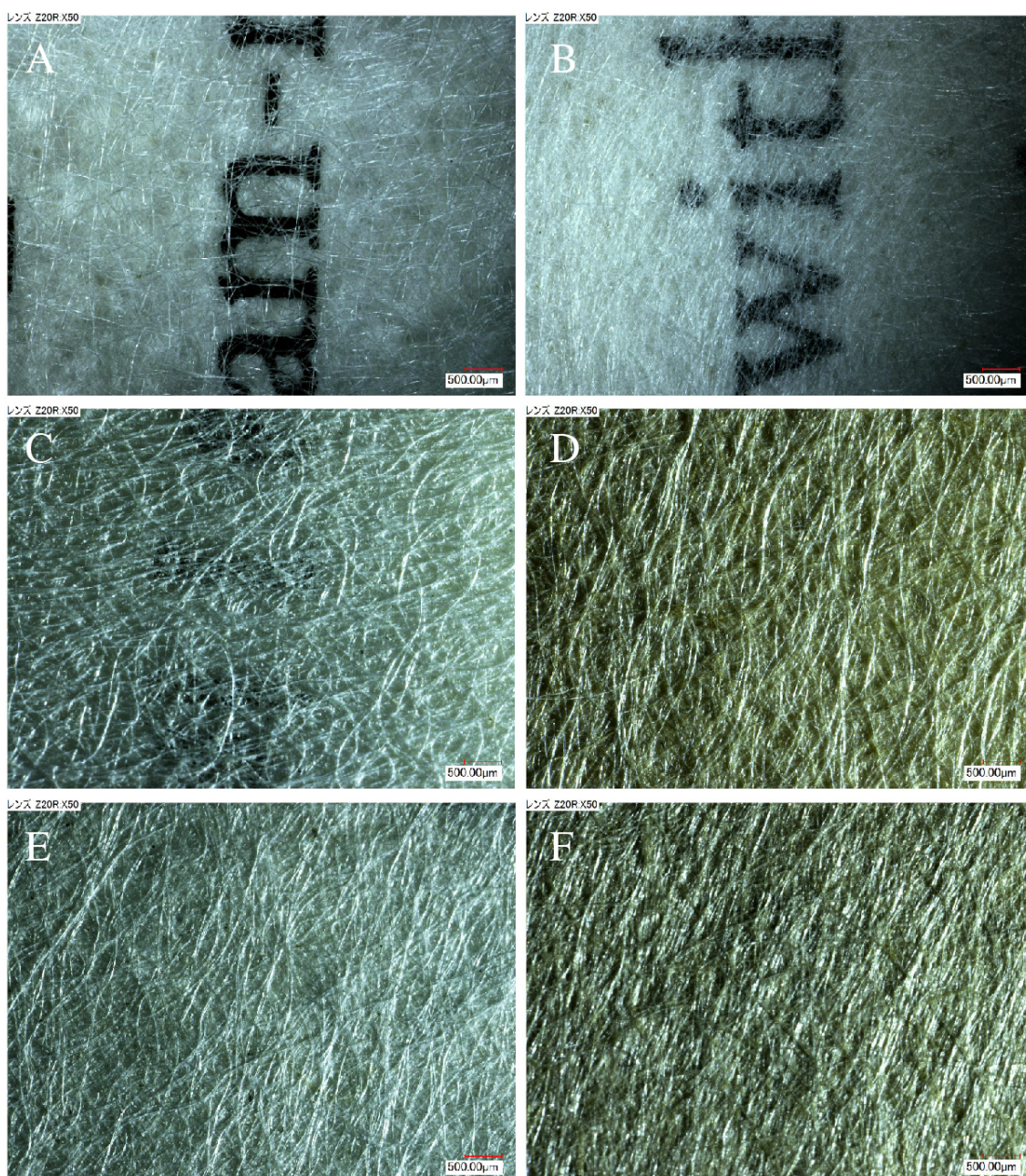


Figure 6. The microscopy observation of fiber orientations. (A), (C) and (E) are examples of Heulimtteugi *Hanji*; (B), (D) and (F) are examples of Gadumtteugi *Hanji*. (C) and (E), (D) and (F) are the recto and verso side within one paper.

As is shown in Fig.6, the fiber orientation can be disperse in both directions (Fig.6A, Fig.6C) as well as strong orientation in one direction (Fig.6B, Fig.6D). The fiber orientation is clearly observable especially with thin and transparent *Hanji* samples. For instance, Fig.6A shows a thin Heulimtteugi *Hanji* sample and the intercross of fibers is clearly depicted that could be associated with the papermakers forwards-backwards-leftwards-rightwards movements in making the paper. Fig.6B shows a thin Gadumtteugi *Hanji* sample and the strong fiber alignment in one direction indicate this *Hanji* may be produced with a comparatively strong movement in one direction (such as forwards-backwards). Also, the fiber orientation in the recto and verso side within one paper can be different (Fig.6C, Fig.6E) or similar (Fig.6D, Fig.6F) as observed in both Heulimtteugi and Gadumtteugi *Hanji* samples.

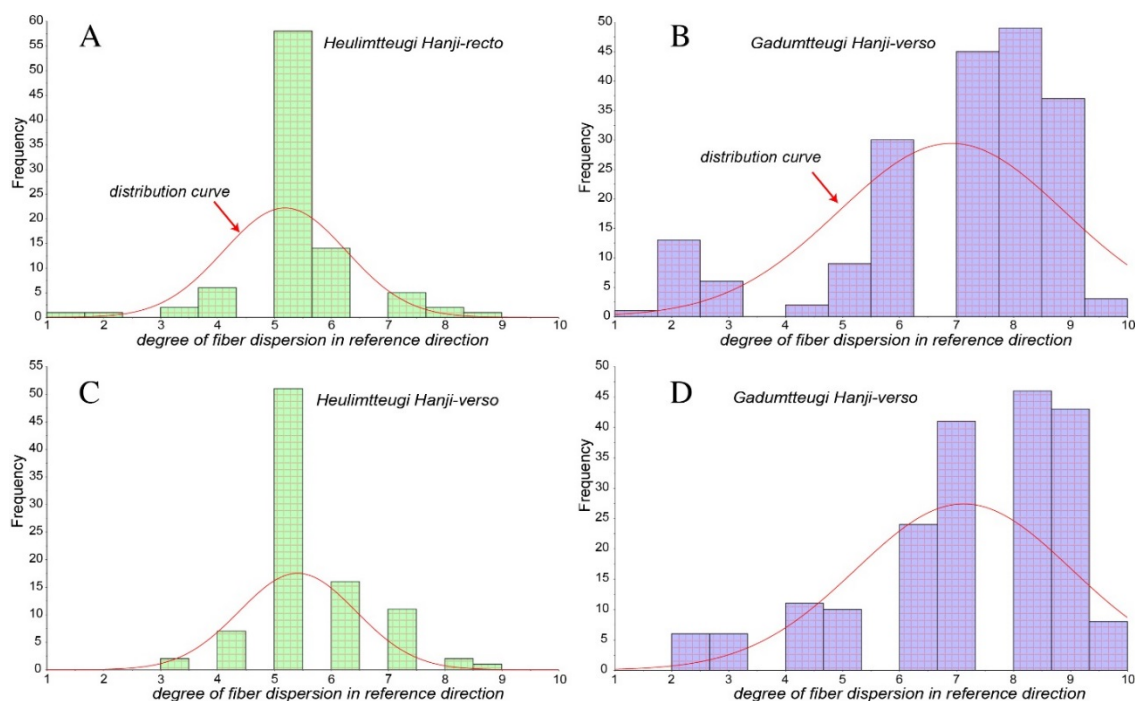


Figure 7. The histogram of the probability in occurrence of the fiber orientation. (A) and (C) are the frequency in recto and verso side of *Heulimtteugi Hanji*, (B) and (D) is the frequency in recto and verso side of *Gadumtteugi Hanji*.

The main features from the probability in occurrence in large quantities were measured. In the observation process, the vertical direction (vertical to the top-bottom direction of the sample book) was chosen as a reference direction. The degree of fiber dispersion in the vertical direction was observed and recorded as weak (weak in the vertical direction), medium (intercross in both direction) and strong (strong in the vertical direction). According to the observed disperse degree in the reference direction, quantification was applied to record from weak to strong with a corresponding number from 1 to 10 and then probability in occurrence was calculated (Fig.7). It shows that although both methods can produce papers with different fiber dispersion degree, their profile differs in probability of occurrence: the *Gadumtteugi* method has high probability to produce strong fiber orientation papers while *Heulimtteugi* method tend to produce papers with fiber orientation more evenly distributed as is indicated by the distribution curve which resembles a gaussian distribution (Fig.7A and Fig.7C). Also, within the same paper (the recto and verso side within one paper), the frequency of fiber orientation differs as was shown in Fig.7A, Fig.7C and Fig.7B, Fig.7D which indicated that the papermakers' actual movement in the sheet formation process affects the dispersion of fibers within both methods.

The result of the fiber orientation of modern Korean handmade papers shows different features compared to the investigated ancient Dunhuang documents that strong fiber orientation only on the writing side: of the 359 investigated samples, 86% have fiber orientation on recto side while only 6% have fiber orientation on verso side (Sakamoto, 2015). This difference traced back to their differences in the papermaking actions such as *Tamezuki* action (ancient time style) and *Nagashizuki* action (papermaking action arose around medieval and currently practiced, *Gadumtteugi* making method is similar to *Nagashizuki* action style) (Barrett & Lutz, 1983; Satoshi, 2006).

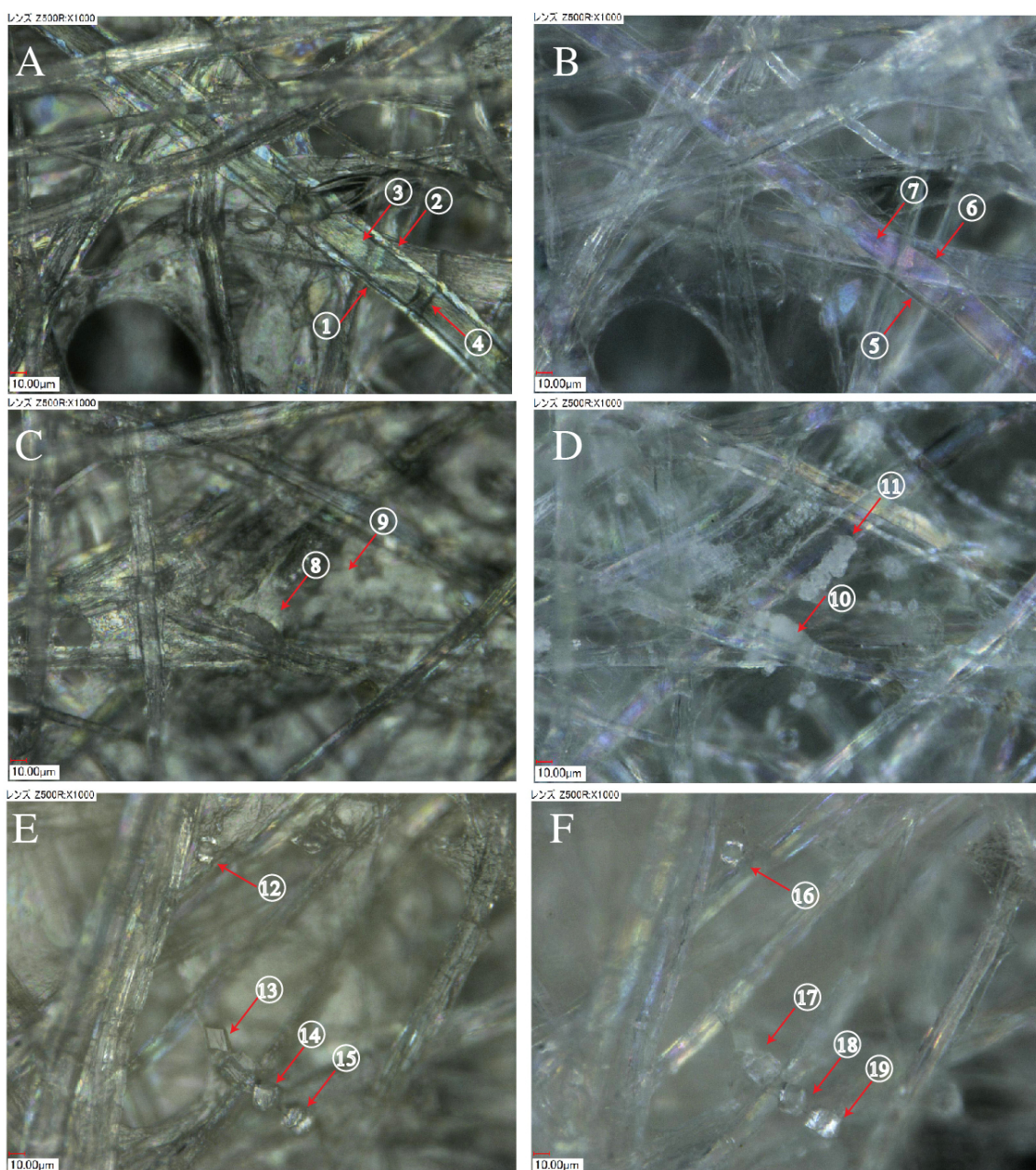


Figure 8. The direct microscopic observation of fibers from *Hanji* samples. (A), (C) and (E) were under Open Nicol conditions, (B), (D) and (F) were under Crossed Nicol conditions.

Due to the pioneer work of Dr. Julius Wiesner, the scientific analysis method was established for handmade paper fiber analysis through identification of the fiber materials with the principle of observing similarity in morphological features (Wiesner, 1864). From the early stage until now, most microscopic analysis of paper fibers rely on stain method to color and distinguish the morphological features for identification that requires micro-destructive of the tested samples (Kim & Choi, 2013; Shi & Li, 2013). The limitation of the stain method is the sampling process. Scanning Electron Microscope (SEM) is currently applied yet sampling and preparations are also required (Collett, 2007). It can only sample a small part of the analyzed samples and the fiber composition may not be representative for the entire sample. In this case, a protocol developed without sampling for a direct characterization of handmade paper fibers could be more useful in the field of conservation and archiving which can analysis the entire sample meanwhile meet the requirement for non-destructive analysis. The identification of

Dak fibers for making *Hanji* was examined by means of a digital microscopy to explore a quick identification and characterization without stain process.

Under the direct microscopic observation, the paper mulberry fibers were characterized to be covered with a translucent membrane which usually appears to be polychromatic under Open Nicol conditions (point 1, 2 in Fig.8A) while appears to be transparent under Crossed Nicol conditions (point 5, 6 in Fig.8B). The fiber shows longitudinal striations, cross-markings (point 3 in Fig.8A) and dislocation (point 4 in Fig.8A) on the surface. The dislocation and wrinkles may visually fade away when the fiber takes on polychromatic colors under Open Nicol conditions (point 7 in Fig.8A). Some cluster crystals (Calcium Oxalate) (point 8, 9 in Fig.8C, point 10, 11 in Fig.8D) and cubic crystals (point 12-15 in Fig.8E, point 16-19 in Fig.8F) were also observed. They are more easily detected under Crossed Nicols conditions than under Open Nicol conditions. Many cubic and cluster crystals are frequently observed in handmade mulberry papers that constitute features for their identification (with the exception of fibers that are thoroughly washed in the papermaking process). However, the morphological features could fade due to pulping, aging and contamination, and relying on microscopic observation for identification may need further material information for a mutual authentication such as their chemical composition and fingerprint compounds as is discussed below.

The Py-GC/MS characterization of *Hanji* fibers

Cellulose, hemicelluloses and lignin are the main components of the plant cell walls, which, in turn, are the main constituents of paper. Lignin is a polyphenol produced by oxidative condensation of three phenylpropanoid monomers, namely (a) p-coumaryl, (b) coniferyl and (c) sinapyl alcohols. The phenyl moieties of such compounds differ in the hydroxy and methoxy substituents and classified as p-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) units (Galletti & Bocchini 1995). For paper conservators, presence of lignin in a paper is usually disliked as the initial strength of the paper is relatively low and it contains many chromophores that interact with light causing the photoyellowing of paper (Carter, 1996b). Physical and chemical treatments evolved in modern papermaking can ensure complete removal of lignin while hand papermaking cannot. On the another hand, our previous investigations on Japanese handmade papers by pyrolysis coupling with both GC/MS and GCxGC/MS revealed it to be a quick and reliable method for the characterization of East Asian handmade paper materials in focusing on the terpenic biomarkers (Han *et al.*, 2016; Avataneo & Sablier 2017; Han *et al.*, 2017). With the previous study of Japanese papermaking materials, the pyrolysis chromatogram of *Hanji* paper could be properly sampled to enlarge the investigate scope to compare papermaking materials in other parts of East Asian regions.

The pyrolysis of *Hanji* samples made from *Dak* fibers resulted in numerous peaks generated from the thermal carbohydrate backbone degradation of which the peak of 1,6-anhydro- β -D-glucose (levoglucosan) takes the majority abundance (a characteristic feature of cellulosic materials). Thermal decomposition of cellulose is the result of two competing reactions as detailed elsewhere (Avataneo & Sablier, 2017). Within the same region, lignin markers of phenolic compounds of different types were identified that mainly composed of G and S moieties (Fig.9 and Table 1). It has been observed that handmade papers from different fiber origins present different lignin compound distributions (Table 1, Table 2 and Fig.9). For example, *Hanji* made from gampi (H1-05) and mitsumata (H1-06) present much higher lignin pyrolysis produces especially for compound 12 (demonstrated by m/z 180 in fig.9D), compound 13, 15, 19 (demonstrated by m/z 180 in fig.9D) and compound 14, 18 (demonstrated by m/z 196 in fig.9D) although sodium carbonate and sodium hypochlorite

have been applied for cooking and bleaching. The use of sodium hydroxide and sodium hypochlorite showed to reduce these compounds as compared from Hanji H1-08 with other samples (made of *Dak*) use different treatment. The use of terpenic and steroid compounds for paper characterization has been previously discussed (Avataneo & Sablier, 2017) and the lignin types can be potential marker for differentiation of handmade papers as well as for preservation implications as its types and percentage varies with different plants.

The detection of another group of biochemical marker compounds present in fiber plants that elutes in the later part of the chromatogram (Fig.9) has been used for their identification. It can be seen from the total ion chromatogram (TIC) that compound eluting in the plant marker region at a comparatively high temperature. The dominant structures obtained from the pyrolysis of *Hanji* identified in this region consist of triterpene of oleanane and ursane structures (Fig.9 and table 1). The identification detailed elsewhere (Avataneo & Sablier, 2017).

As can be seen from its extracted ion chromatograms (EIC) in Fig.9C, the distribution of the terpene compound with the selected ions (m/z 218, 203 and 189) constitute the main compounds in the plant marker region. Compared with Japanese handmade paper (*kozo*) previously studied, the distribution pattern of the terpenic compounds can be used as a quick fingerprint for identification of plant fibers from the *Moraceae* family. In fact, current research on *Hanji* and *kozo* paper revealed that the species in *Broussonetia* genus (*Broussonetia papyrifera* and *Broussonetia kazinoki*) are quite similar in chemical compositions and may further subject to a higher resolution analysis like a GCxGC separation for their discrimination.

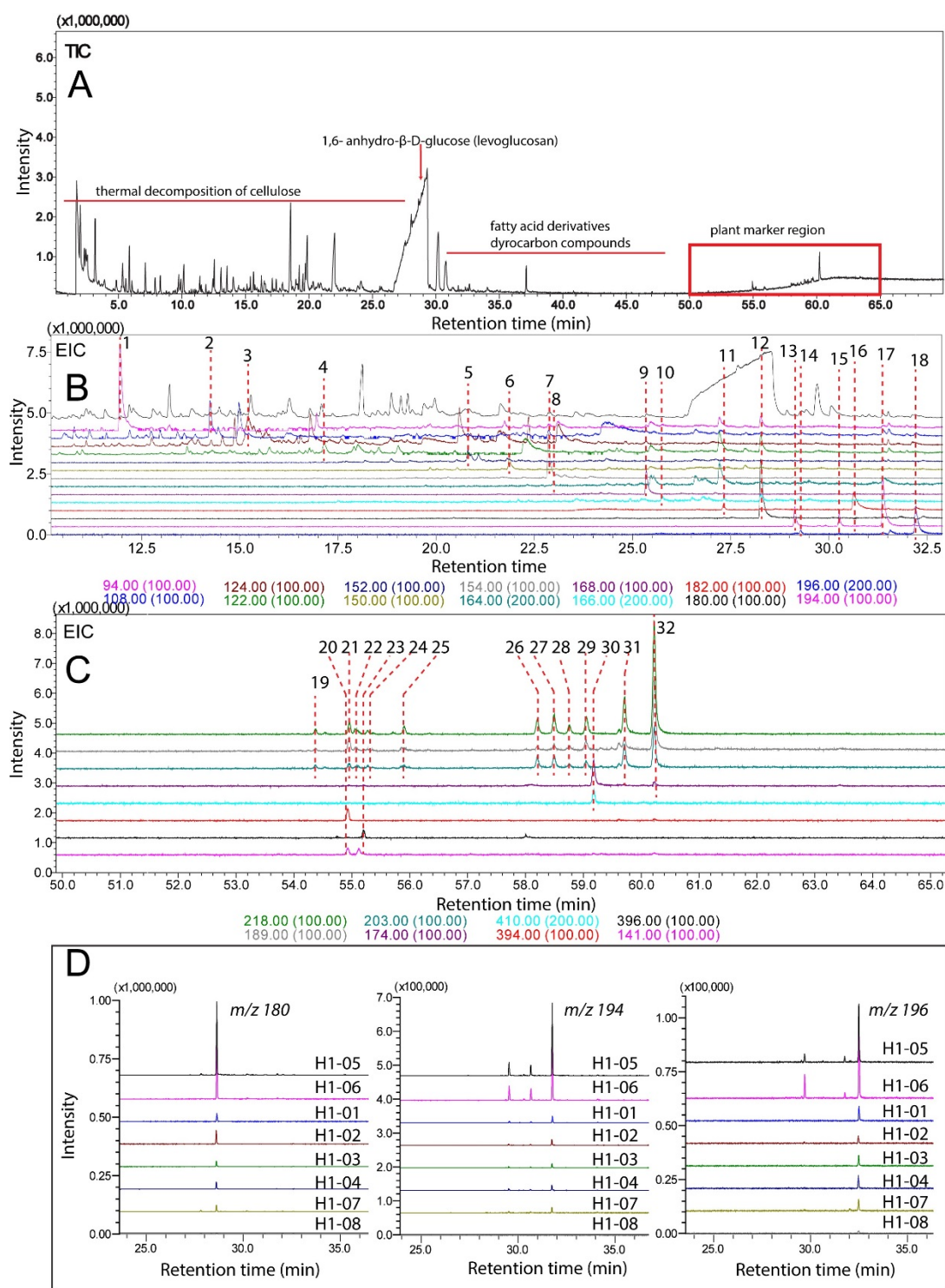


Figure 9. The TIC of *Hanji* sample (A) and the EIC chromatogram of pyrolysis products of lignin (B) and terpenic compounds distribution pattern (C).

Table 1. Assignment of Pyrolysis of *Hanji* plant markers.

No	Ret. time (min)	MW	main ions	Attribution	Type*
1	11.97	94	94, 66, 55, 58	Phenol	L-H
2	14.23	108	108, 107, 79, 85	2-Methylpheno	L-H
3	15.19	124	124, 109, 81, 95	Guaiacol	L-G
4	17.17	122	122, 107, 91, 77	2,6-Dimethylphenol	L-H
5	20.82	152	152, 137, 122, 128	4-Ethylguaiacol	L-G
6	21.86	150	150 135 107 77	4-Viny lguaiacol	L-G
7	22.86	154	154 139 111 93	2,6-Dimethoxyphenol	L-G
8	22.93	164	164 149 103 121	Eugenol	L-G
9	25.36	168	168 153 125 107	2,6-Dimethoxy-4-methyl-phenyl	L-S
10	25.77	166	166 137 122 94	Homovanillin	L-G
11	27.33	182	182 167 122 136	4-Ethyl-2,6-dimethoxypheno	L-S
12	28.28	180	180 165 137 91	2,6-Dimethoxy-4-vinylphenol	L-S
13	29.12	194	194 91 119 131	4-Allyl-2,6-dimethoxyphenol	L-S
14	29.22	196	196 167 110103	2,6-Dimethoxy-4propylpheno	L-S
15	30.26	194	194 179 151 119	cis-2,6-Dimethoxy-4 propenyl-phen	L-S
16	30.62	182	182 181 167 91	Syringaldehyde	L-S
17	31.38	194	194 179 119 91	trans-2,6-Dimethoxy-4-propenylphe	L-S
18	32.22	196	196 181 153 123	Acetosyringone	L-S
19	54.37	408	218, 203, 189, 119	Oleanane-2,12-diene	T-O
20	54.96	408	218, 189, 119, 95	Urs-2,12-diene	T-U
21	54.92	412	135, 143, 394, 81	Stigmasta-4,6-dien-3-ol	S
22	55.05	-	218, 189, 95, 119	Ursane structure NA	T-U
23	55.19	396	145, 81, 396, 275	stigmasta-3,5-diene NA	S
24	55.27	-	218, 189, 203, 95	Ursane structure NA	T-U
25	55.90	408	218, 107, 119, 105	Ursane structure NA	T-U
26	58.20	424	218, 203, 95, 119	Oleanane-12-en-3-one	T-O
27	58.49	426	218, 203, 189, 95	Oleanane-12-en-3-ol	T-O
28	58.75	424	218, 95, 109, 107	Urs-12-en-3-one	T-U
29	59.05	426	218, 95, 107, 121	Urs-12-en-3-ol	T-U
30	59.17	410	174, 410, 159, 161	Stigmasta-3,5-dien-7-one	S
31	59.70	468	218, 203, 189, 95	Oleanane-12-ene-3-acetate	T-O
32	60.22	468	218, 189, 95, 135	Urs-12-ene-3-acetate	T-U

*type: L: lignin, H: *p*-hydroxyphenyl, G: guaiacy, S: syringy, T: terpene, O: oleanane, U: ursane, S: Stigmasta.

Table 2. the fiber origin, making method of the test sample from H1-01 to H1-08.

No.	fiber composition	cooking conditio n	mucilag e	metho d	bleachin g	beatin g	pH	use
H1-01	100% <i>Dak</i>	<i>yukjae</i>	<i>Dakpul</i>	<i>G</i>	<i>Non</i>	<i>K</i>	8.7	conservation
H1-02	96%Dak+4%Anp i	<i>yukjae</i>	<i>Dakpul</i>	<i>H</i>	<i>Non</i>	<i>K</i>	9.8	conservation, art work
H1-03	96%Dak+4%Anp i	<i>yukjae</i>	<i>Dakpul</i>	<i>H</i>	<i>Non</i>	<i>K</i>	5.8	conservation
H1-04	96%Dak+4%Anp i	<i>yukjae</i>	<i>Dakpul</i>	<i>H</i>	<i>Non</i>	<i>K</i>	8.1	conservation
H1-05	100%Anpi	Na ₂ CO ₃	<i>Dakpul</i>	<i>G</i>	NaClO	<i>N</i>	7.4	printmaking
H1-06	100%Samjidak	Na ₂ CO ₃	<i>Dakpul</i>	<i>G</i>	<i>Non</i>	<i>N</i>	6.5	calligraphy
H1-07	100% <i>Dak</i>	Na ₂ CO ₃	<i>Dakpul</i>	<i>G</i>	NaClO	<i>K</i>	7.5	crafts
H1-08	100% <i>Dak</i>	NaOH	PAM	<i>G</i>	NaClO	<i>K</i>	5.8	paintings

Dak: paper mulberry, anpi: gampi, samjidak: mitsumata, yukjae: natural plant ash, dakpul: natural formation aid from Hibiscus manihot. PAM: Polyacrylamide, G: Gadumtteugi, H: Heullimtteugi, K: knife beater, N: niagara beater.

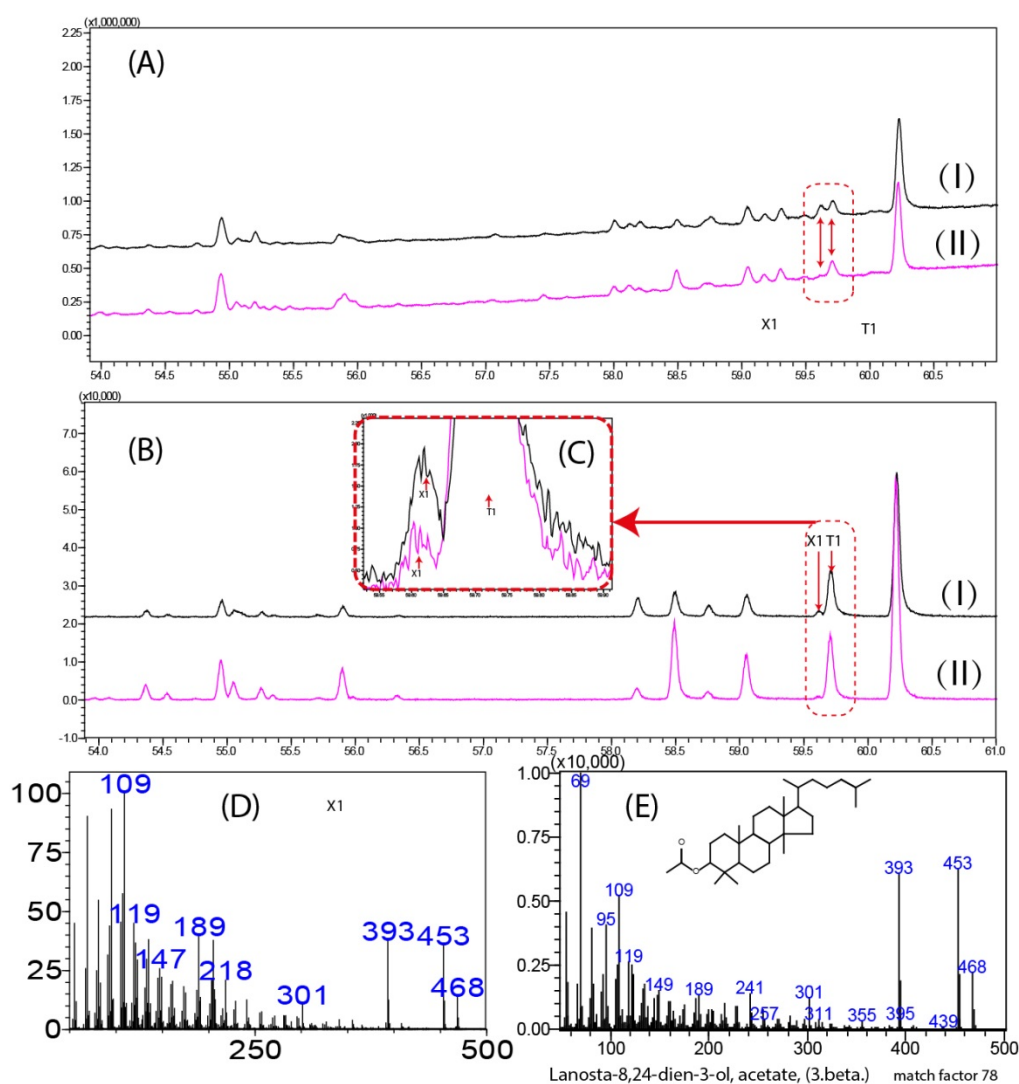


Figure 10. (A) is the TIC chromatogram while (B) is the EIC m/z at 218. In (A) and (B), (I) is hanji and (II) is washi. (C) is the enlarged demonstration of the region in (B), (D) is the mass spectra of compound X1 after background subtraction and (E) is the NIST library search results with high match factor.

Since hanji and washi are made from quite similar species, microscopic observation cannot distinguish paper fibres from these two areas. The similarity can also be observed from their similar compound distribution patterns in the ROI demonstrated in Fig.10A and Fig.10B.

Nevertheless, a difference of relative intensity ratio of compound X1 and compound 31 (31 in Table 1, refers as C31) has been noticed with hanji have relative higher ratio. As can be seen from the mass spectra and the NIST library search result, X1 could possibly be a sterol compound. Its structure was assigned to lanosta-8,24-dien-3-ol, acetate. The difference noticed in the chromatogram such as the difference of the relative intensity observed in the EIC of m/z at 218 (Fig.10B and Fig.10C), can be a way to indicate their change in composition.

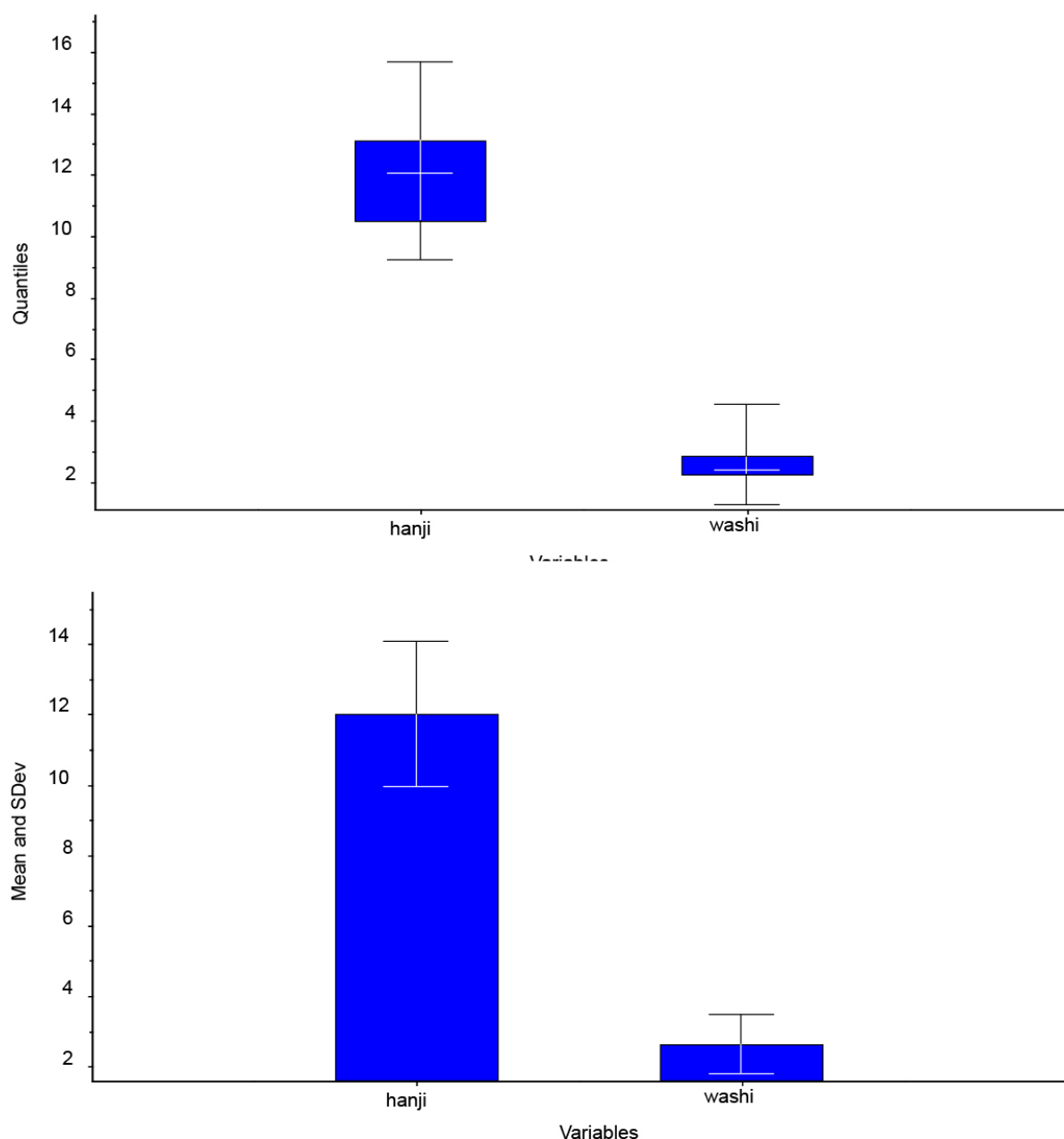


Figure 11. The ration of the EIC (m/z at 218) area of X1/C31 (in percentage). The data are from 12 test of hanji papers and 12 test of kozo papers that have signals of X1 and C31 at EIC m/z at 218 and respective retention time at 59.6 min and 59.7 min, respectively.

As can be observed from Fig.11, the X1/C31 ration (EIC area at m/z 218) was averaged to 12% for hanji while the average value for washi is 3%. As can be observed from the box plot, this ratio differs largely in hanji and washi which could be one information used for their differentiation. One point for the noticed ratio difference lies in that the m/z at 218 (compound X1) is in general a minor peak in the EIC plot and depending on the testing conditions, it is occasionally observed to be too low to integrate its area both in hanji and washi paper analysis. Under these conditions, this criterion cannot systematically be applied, but opens the way for the differentiation of handpaper of different origins in their papermaking processes.

Conclusion

A comprehensive study of Korean handmade papers gathered in a hanji sample book was conducted. The overview feature and the characteristics of the different papermaking method were revealed from large data sets of modern *Hanji*. It can be used for setting up database that will allow comparison with other handmade papers in East Asian regions and also provides referential information for *Hanji* as a conservation material. Direct digital microscopy showed to be a pertinent method not only for detecting the type of *Hanji* papermaking technique involved through the observation of the fiber orientation but also suitable for identifying fibers without sampling (unlike the commonly and widely used stain method and is appropriate for conservation and archiving practices). 18 markers from pyrolysis lignin were identified that mainly composed of G and S moieties and formed the base for characterization of *Hanji* with different pre-treatment in the following studies. The patterns of plant markers of a terpene origin are shown by Py-GC/MS in the chemical fingerprint of *Hanji* with the presence of m/z 218, 203, 189 used as a quick fingerprinting check for plant fibers from the *Moraceae* family.

In a general sense for paper conservation and archiving, this study pointed out what benefit can be derived from protocol well-defined samples as well as stressed a situation where these samples are rare and lacking in scientific research on handmade papers. For further studies, the evaluation of different *Hanji* with different pre-treatment for used in restoration processes (mechanical property change due to usage, aging, environmental influence, S/G ratios of lignin compounds etc.) is a subsequent research topic which may generate other practical results.

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Annex 46 Supplementary Information for the discussion in section 3.2

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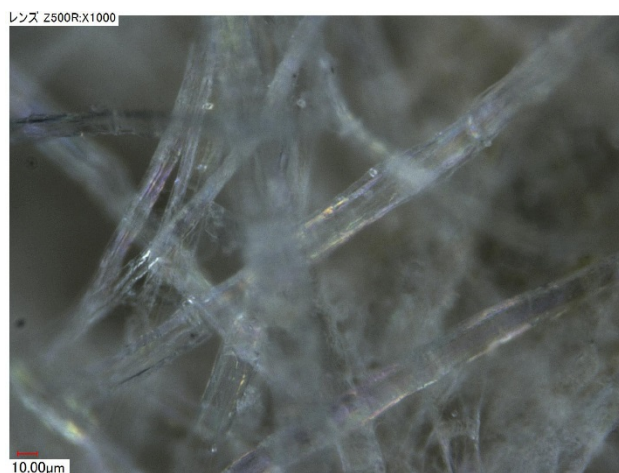
Figure S3. Score plot (A) and loading plot (B) resulting from the PCA treatment of peak areas as variables using un-normalized variables weighting as 1 in the ROI from the EIC (m/z 218) of the Moraceae family S1-S5 and S6.

Figure S4. Score plot obtained from the PCA of all groups of samples (un-normalized values set as 1/STD from the EICs of markers in the ROI). In Figure S4B, the number before ‘--’ corresponding to the numbering in Table 2 and the number after ‘--’ represents the chosen EIC (m/z).

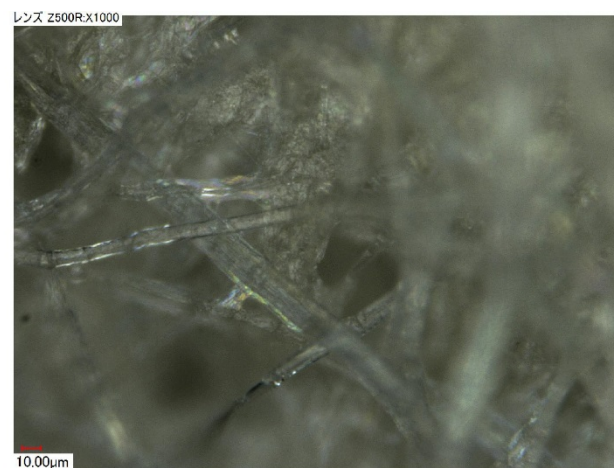
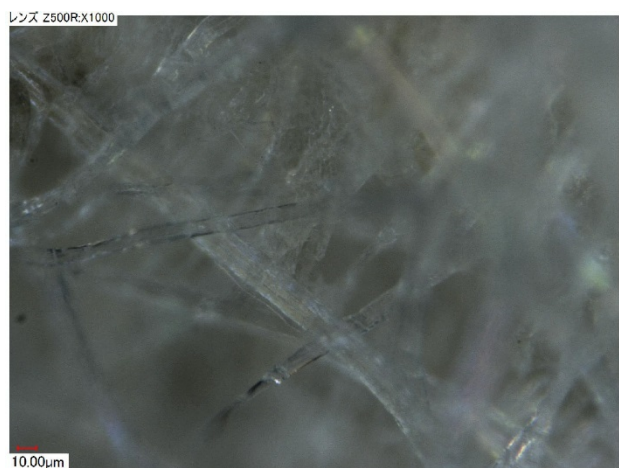
Fig.S5, The illustration of identification of hand papermaking fibers within the Moraceae family using Py-GC/MS and microscopic observation, for the microscopic features, refer to Fig.SX2. (This figure will put in the supporting information)

Figure S1. Morphological features of the investigated handmade paper fibers (S1-S6) under digital microscopic observation.

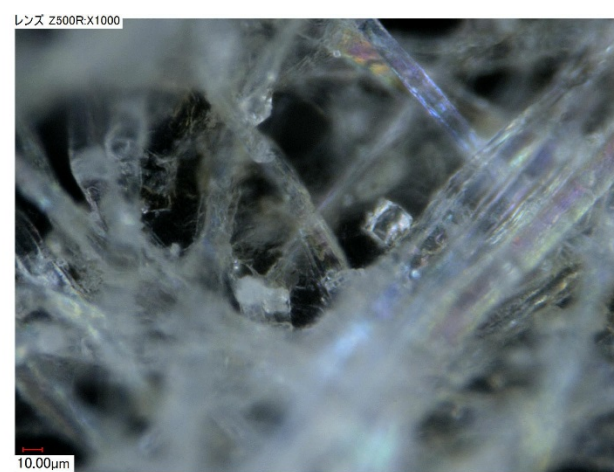
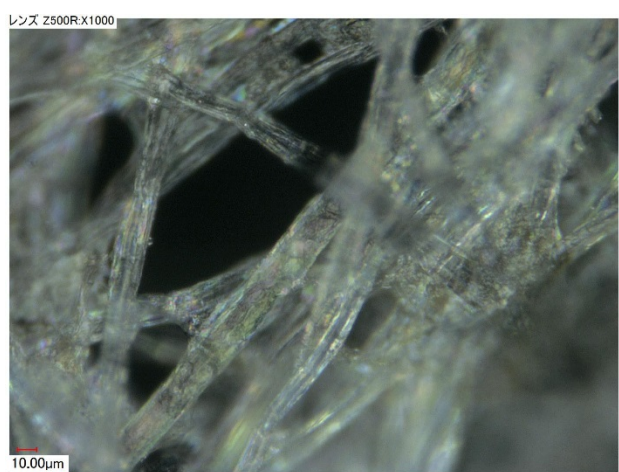
Sample S1



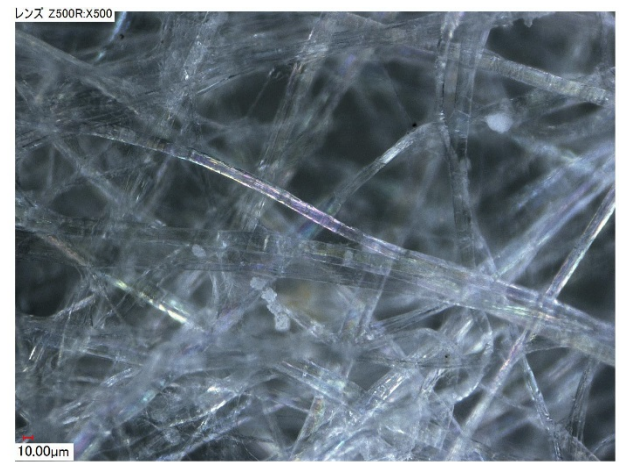
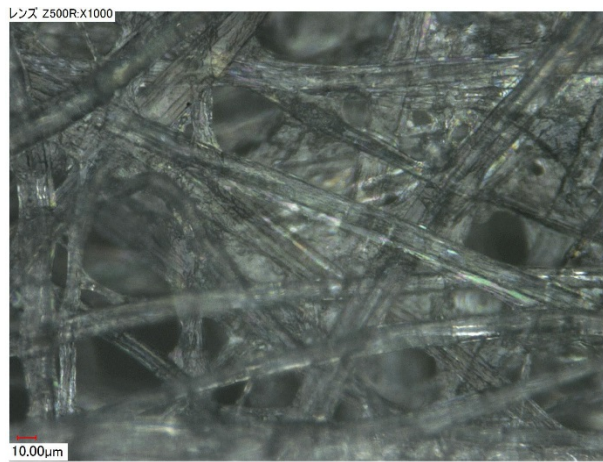
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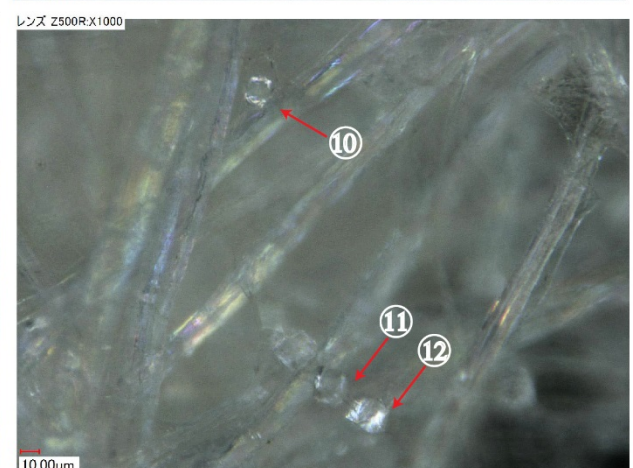
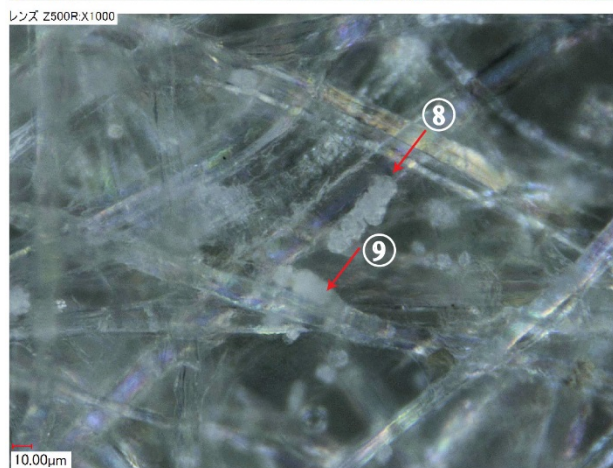
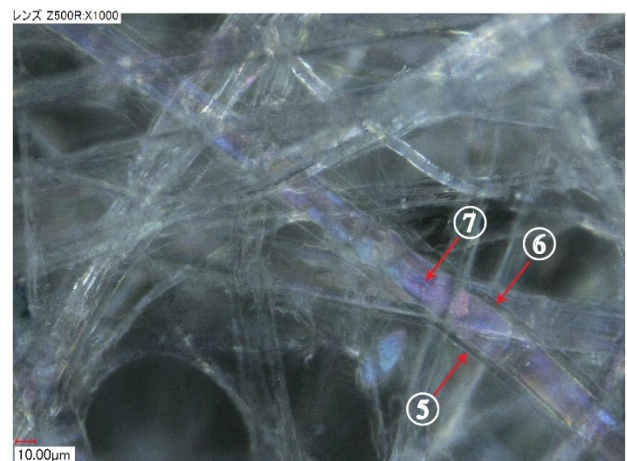
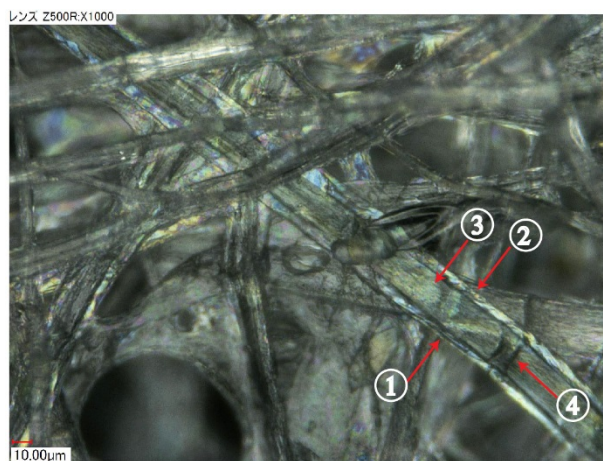
Sample S3



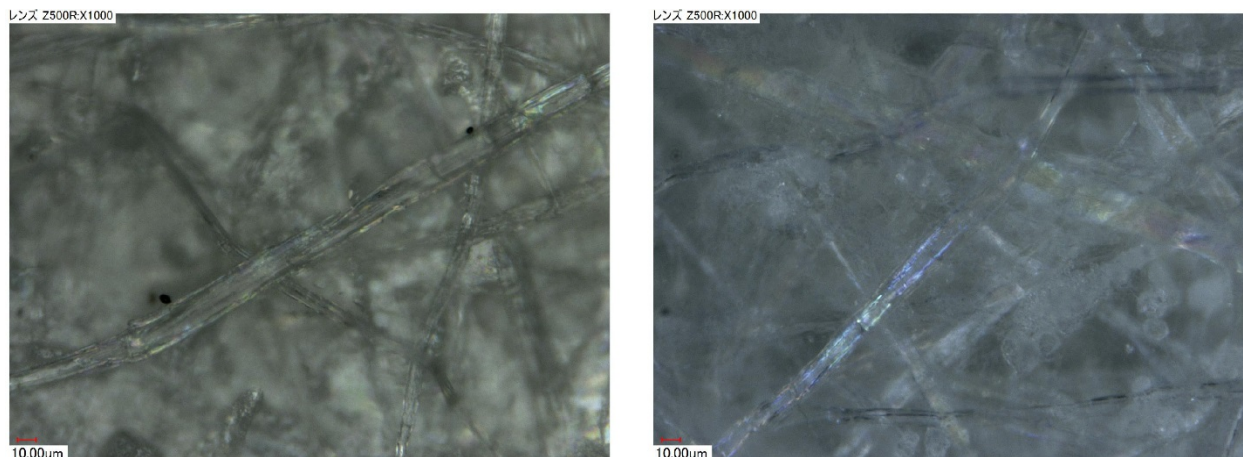
Sample S4



Sample S5



Sample S6



Short discussion of Fig.S1:

1. Test conditions

Microscopic analysis of Hanji samples were performed by a digital microscope (VHX-1000, Keyence, Japan). The samples were placed directly onto the microscope platform. For the fiber identification, the observation was conducted in reflection mode with halogen lamp as light source. Polarized light microscopy was utilized to identify the papermaking fibers under both Crossed Nicol and Open Nicol conditions.

2. The microscopic feature (the following is explained with the example of sample S5 in Fig.S1)

Under the direct microscopic observation, the paper mulberry fibers were characterized to be covered with a translucent membrane which usually appears to be polychromatic under Open Nicol conditions (point 1, 2 in Fig.S1) while appears to be transparent under Crossed Nicol conditions (point 5, 6 in Fig.S1). The fiber shows longitudinal striations, cross-markings (point 3 in Fig.S1) and dislocation (point 4 in Fig.S1) on the surface. The dislocation and wrinkles may visually fade away when the fiber takes on polychromatic colors under Open Nicol conditions (point 7 in Fig.S1).

Some cluster crystals (Calcium Oxalate) (point 8, 9 in Fig.S1) and cubic crystals (point 16-19 in Fig.S1) were also observed. They are more easily detected under Crossed Nicols conditions than under Open Nicol conditions. Many cubic and cluster crystals are frequently observed in handmade mulberry papers that constitute features for their identification when compared with other plant fibers.

The similar morphological features has been observed within S1 to S6: under the microscopic observation, each morphology of paper, i.e. fibre and crystal morphology, made from moraceae families has similar feature. It gives us difficulty of classification among them.

Figure S2. Score plot (A) and loading plot (B) resulting from the PCA treatment of peak areas as variables using variables weighting as 1/STD in the ROI from the EICs of the Ma group handmade paper samples showing the PC1 vs PC2. S11-S14 are corresponding to Table 1. In Figure 5B, the number before ' - ' corresponding to the numbering in Table 2 and the number after ' - ' represents the chosen EIC (m/z).

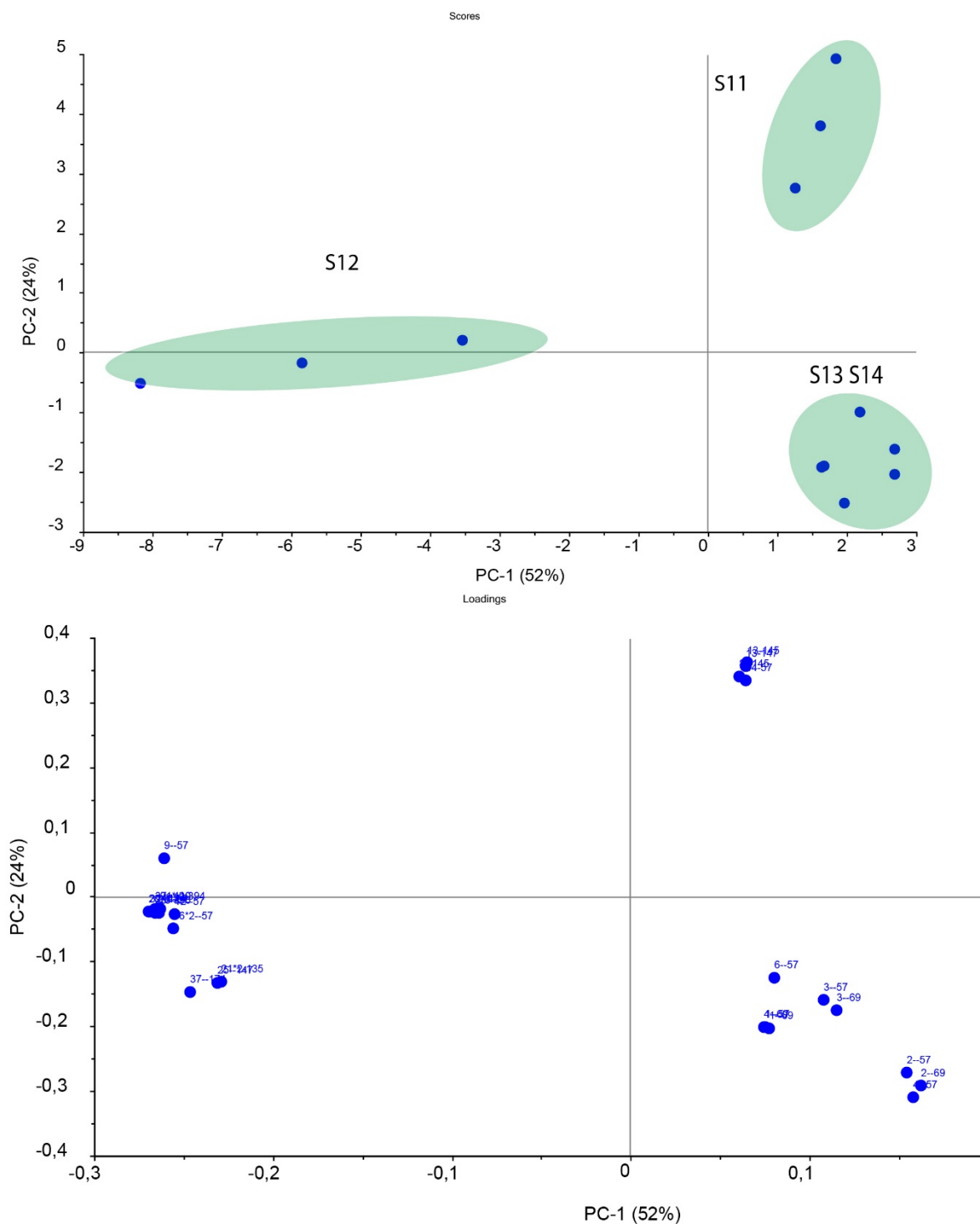


Figure S3. Score plot (A) and loading plot (B) resulting from the PCA treatment of peak areas as variables using un-normalized variables weighting as 1 in the ROI from the EIC (m/z 218) of the Moraceae family S1-S5 and S6.

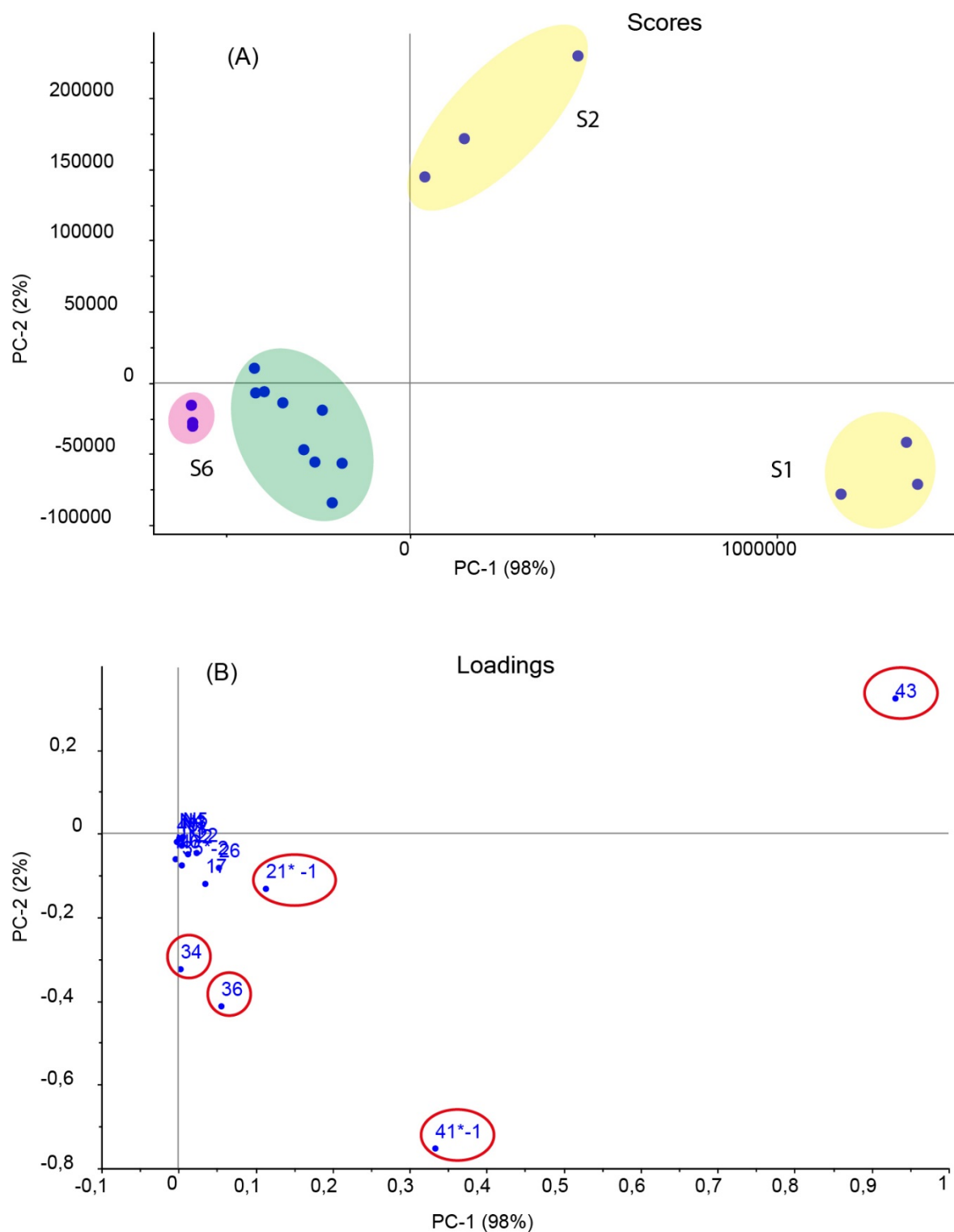


Figure S4. Score plot obtained from the PCA of all groups of samples (un-normalized values set as 1/STD from the EICs of markers in the ROI). In Figure S4B, the number before '--' corresponding to the numbering in Table 2 and the number after '--' represents the chosen EIC (m/z).

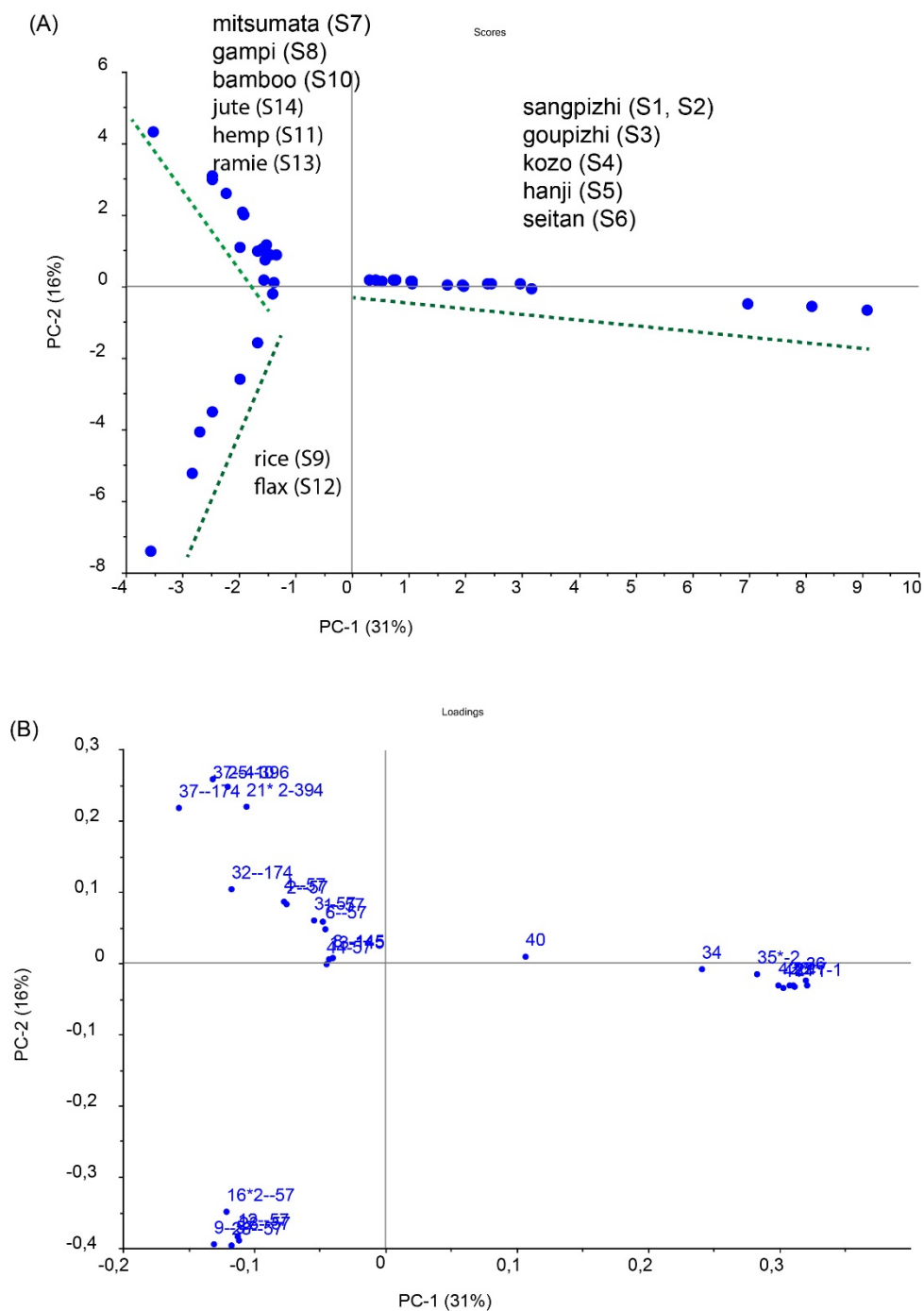
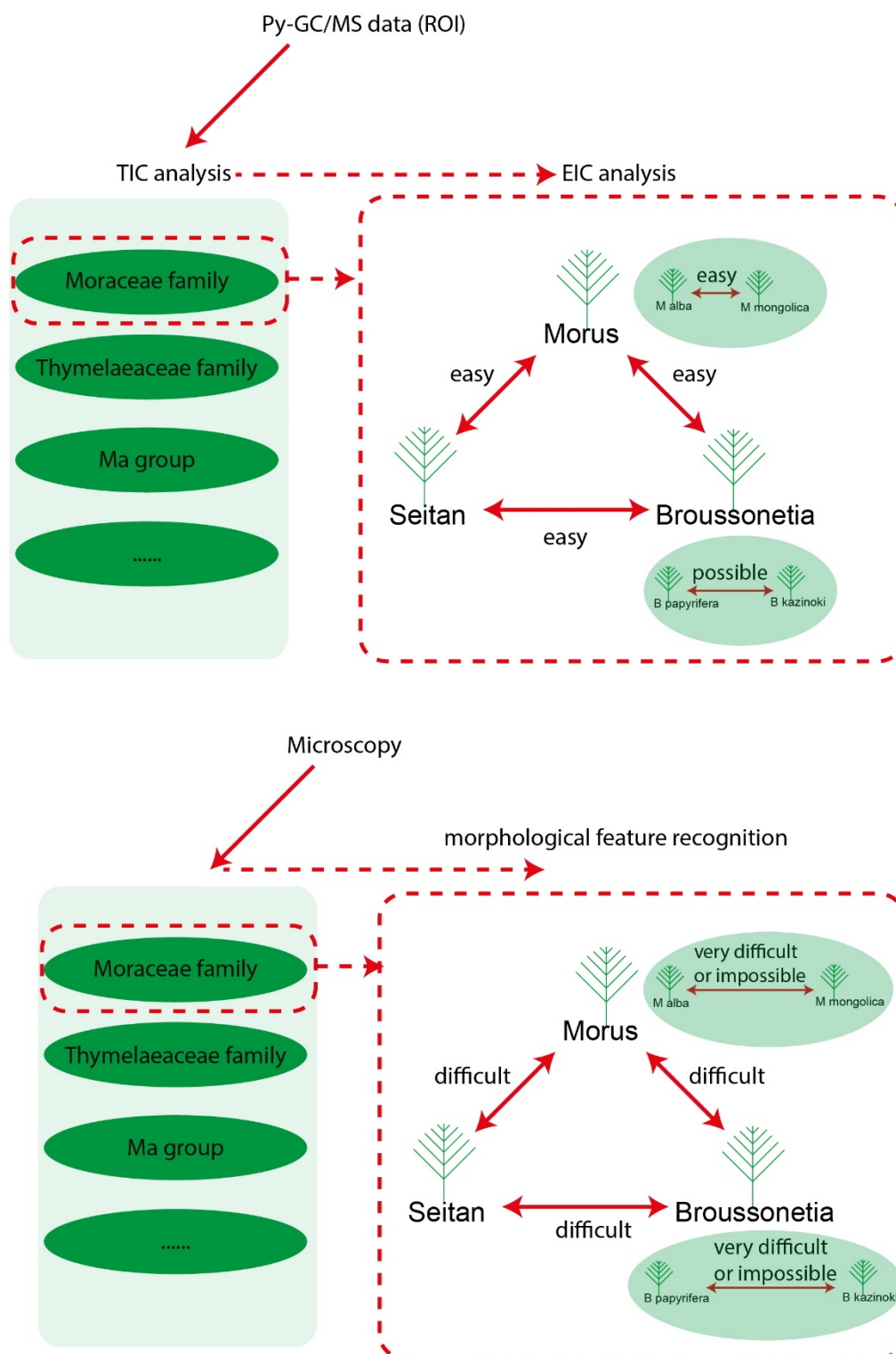


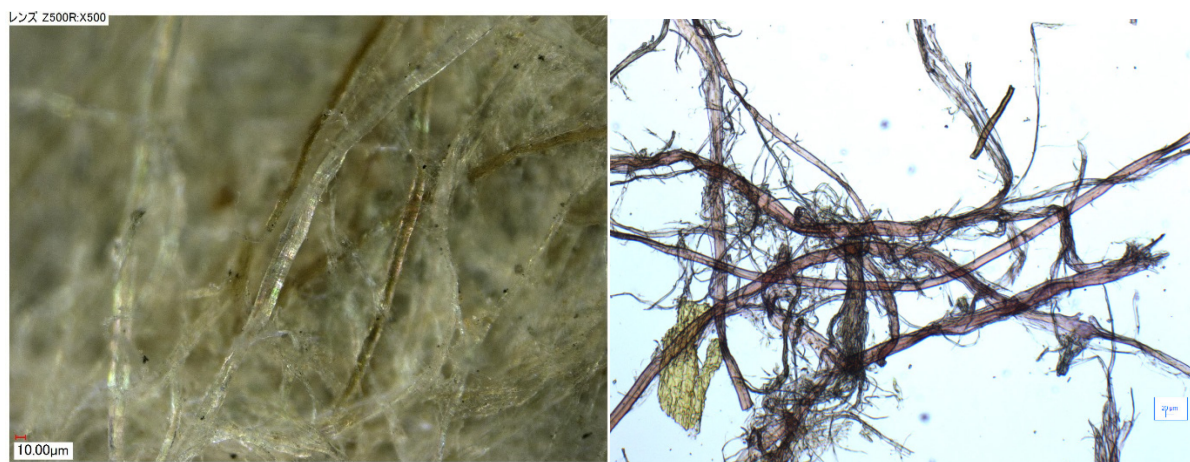
Fig.S5, The illustration of identification of hand papermaking fibers within the Moraceae family using Py-GC/MS and microscopic observation, for the microscopic features, refer to Fig.S1.



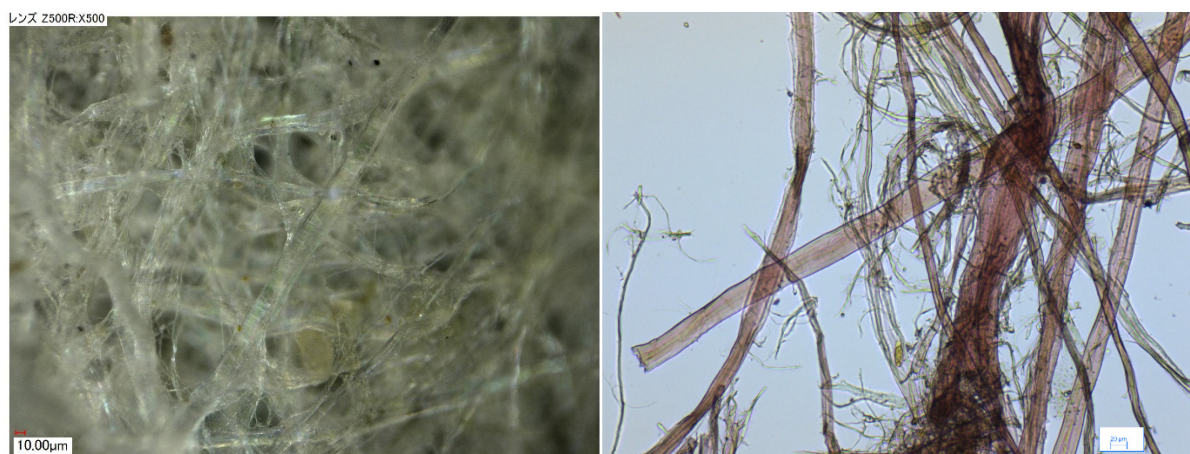
Annex 47 Supplementary Information for the discussion in section 3.4

The digital microscopic observation (conduct with Dr. Sakamoto Shouji, RyuKoku University) and the Graff C stain microscopic observatoin (conduct with Mr. Léon-Bavi Vilmont, Centre de recherche sur la conservation des collections)

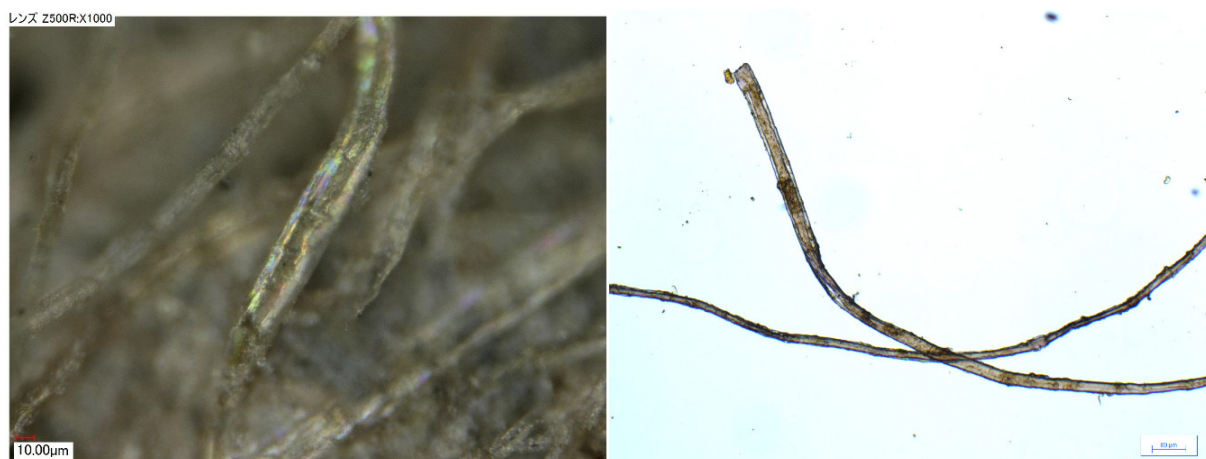
Sample A



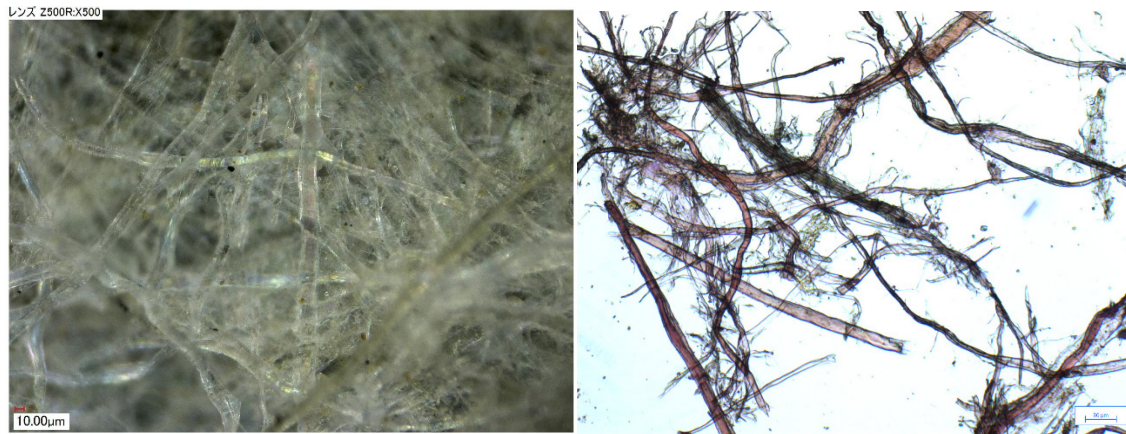
Sample B



Sample C



Sample D



Sample E

