[Transaction]

Journal of the Korean Society
of Clothing and Textiles
Vol. 31, No. 12 (2007) p.1653~1661

Separation of Chromophoric Substance from Sappanwood under Different Extraction Conditions

Cheunsoon Ahn†

Dept. of Fashion & Industry, University of Incheon

염료 추출조건에 따른 소목의 색소성분 분리 거동

안 춘 순†

인천대학교 패션산업학과

(2007. 5. 17. 접수)

Abstract

The research aimed to establish the standard extraction procedure for examining brazilin, the major chromophoric substance of Sappanwood, using GC-MS with the ultimate goal of identifying the sappanwood dye in severely faded archaeological textiles. The amount of brazilin represented by the GC abundance was the largest when acetone was used as the extraction medium, followed by methanol. Shaking plate operated at room temperature was more effective than the waterbath shaker which was operated at 30°C. In both cases, the extraction method which incorporated one hour pre-soaking before the 12 hours of actual extraction resulted in a larger amount of brazilin detection than the extraction procedure without the one hour pre-soaking. In case of water extraction, pH 5 resulted in the most effective pH level for the extraction of brazilin. The best GC-MS parameter for detecting brazilin was to set the column temperature initially at 50°C, gradually increase to 210°C at a 23°C/min rate, finally increase to 305°C at 30°C/min rate, and hold for 14 minutes, and the MSD scan range at 75~400 m/z.

Key words: Sappanwood, Brazilin, GC-MS, Extraction, Dye identification; 소목, 브라질린, 가스크로마토그라피 질량분석, 추출, 염료 판정

I. Introduction

An archaeological textile renders itself as a source of both cultural and scientific understanding of the past. Due to its organic nature a textile recovered from the burial ground is often found with serious damages which is unrecoverable and as a result its cultural value is often underestimated. The most readily observed damage in an exhumed textile is color fading and in most cases the fading is severe enough to obscure the original color of the textile piece. This often presents the museum conservators the difficulties in textile conservation such as selecting the suitable washing and storage conditions for each textile piece. Moreover, the value of the textile piece as the primary document of the past is also limited considering that the badly faded textile cannot transport the full evidence of the past clothing culture. If such textile can be identified of its original color its value as the cultural heritage will be strengthened. However, dye identification even in an intact textile with its original color is a difficult task. The case is

†Corresponding author
E-mail: cssong@incheon.ac.kr
This work was supported by the University of Incheon Research Grant in 2006.

much more problematic with exhumed textiles which have turned tan to brown color due first to the fading of its original color and second to the staining from the burial ground.

Different research efforts were made for the identification of dye in archaeological textiles using various analytical instruments. In most cases the success of dye identification among the natural dyed textiles was largely dependent upon the presence of original color, which permitted the selection of a small number of comparable standard dyes. When the trace of original color is not present it is impossible to select a single or a manageable number of standard dye chemical for the comparative analysis. Moreover, when the dye fades the chemical structure of the chromophoric substance is no longer intact. And thus it is highly improbable to successfully carry out a parallel comparison between a 'fresh' standard dye chemical with the remains of dye in an exhumed textile. In order to cope with this problem, the author has developed a research method in which each possible dyestuff is analyzed separately in a simulated degradation condition, and the resulting data can then be used as part of a pool of standard dyes for the comparative analysis(Ahn & Obendorf, 2004; Ahn & Obendorf, 2007; Ahn, 2007).

In carrying out such degradation study of natural dyes, it is essential that each natural dyestuff is extracted and examined in a standardized method possible which can successfully detect the major chromophoric substances by the selected instrumental technique(Ahn & Obendorf, 2006). This research was to establish the standard extraction procedure for examining the major chromophoric substance in the heartwood of sappanwood using the gas chromatography mass spectrometry(GC-MS) with the ultimate goal of identifying the sappanwood dye in severely faded archaeological textiles using the same instrumental technique.

II. Experimental design

Sappanwood(Caesalpinia sappen L.) is a redwood of the pea family which is native to South India and cultivated throughout the Asian region. It was the

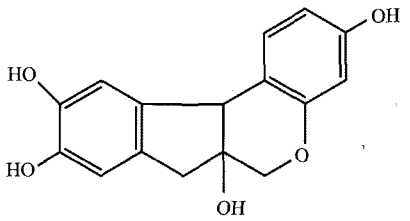


Fig. 1. Structure of brazilin.

source of valued type of red dye in the past and in the medicinal field it is acknowledged for its anti-bacterial and anti-coagulant properties. As a dye sappanwood dyes silk red with aluminum mordant, and dark purple with iron mordant(Nam, 1999). The major chromophoric substance of sappanwood is brazilin(7, 11b-dihydrobenz[b]indeno[1, 2-d]pyran-3, 6a, 9, 10 (6H)-tetrol, molecular weight 286.28)(Fig. 1). Brazilin is amber-yellow crystals which easily oxidizes to orange colored brazilein in air and light. It is soluble in water, more freely in alcohol or ether, and also in alkali hydroxide solutions(CambridgeSoft, 2001).

Extraction of sappanwood was carried out in different research efforts for various purposes. Kharbade and Agrawal(1985) extracted sappanwood with sodium hydrogen carbonate in water using waterbath at 80°C for 1~2 hours with the purpose of preparing standard sappanwood dye for the identification of red dye in old Indian textiles. Nam(1999) proposed that sappanwood should be extracted with distilled water, with the addition of small amount of vinegar, for 30 minutes at 100°C, repeat the procedure and mix the first and second extraction liquor for fabric dyeing. Nam suggested that the water should be adjusted to pH 5~6 for the extraction of deep red colored dye from the heartwood. Seo et al.(2005) extracted sliced sappanwood with deionized water at 90°C without the pH adjustment for the purpose of preparing the dye liquor. In another study, sappanwood was presoaked for 30 minutes before the actual extraction in order to eliminate pesticides or other extraneous matters(Cha & Kim, 1999). The extraction was carried out with distilled water at 100°C for 60 minutes, repeated the procedure three times, and then the extractions were mixed for fabric dyeing.

While the above studies used water as the extrac-

tion medium to prepare the dye liquor, other body of research used organic solvent to extract brazilin from sappanwood. Halpine(1996) extracted brazilwood, the European species of sappanwood, with ethanol to prepare brazilin standard for the purpose of identifying a number of natural pigments from museum objects. Xie et al.(2000) used methanol at 75°C to extract brazilin from sappanwood and investigated the medicinal property and effectiveness of brazilin. Methanol was used as the extraction medium for extracting brazilin from the 80-year old archival specimen of sappanwood(Oliveira et al., 2002) and for the extraction and isolation of the two other aromatic compounds related to brazilin from the heartwood of sappanwood(Fuke et al., 1985). Especially, Oliveira et al.(2002) extracted sappanwood with methanol at room temperature, repeating the procedure three time and then combining the liquor to maximize the detection of brazilin via instrumental analysis.

In view of the above literature, this research examined the efficiency of water and organic solvent, mainly methanol and acetone, in the extraction of brazilin from the heartwood of sappanwood(Fig. 2). When organic solvent was used as the extraction medium, the effectiveness of different extracting device- waterbath shaker and shaking plate- was also examined. When water was used as the extraction medium, the effectiveness of pre-soaking and different pH adjustments were also tested. The sample liquor obtained from different extraction procedures was analyzed using the GC-MS instrument in order to use the consistent method with the long-term project for identifying dye in badly faded archaeological textiles. Different GC-MS parameters were tested to identify the most efficient GC-MS method for detecting brazilin from the extracted samples.

III. Materials and Methods

1. Materials

The heartwood of sappanwood was purchased from Korean traditional medicinal market. Methanol and acetone(both HPLC grade) were purchased from Mallinckrodt Baker(Paris, KY). Reagent grade H_2SO_4 and NaOH were purchased from EM Science(Darmstadt, NJ). Deionized distilled water by Corning Megapure System MP-1 was used throughout the

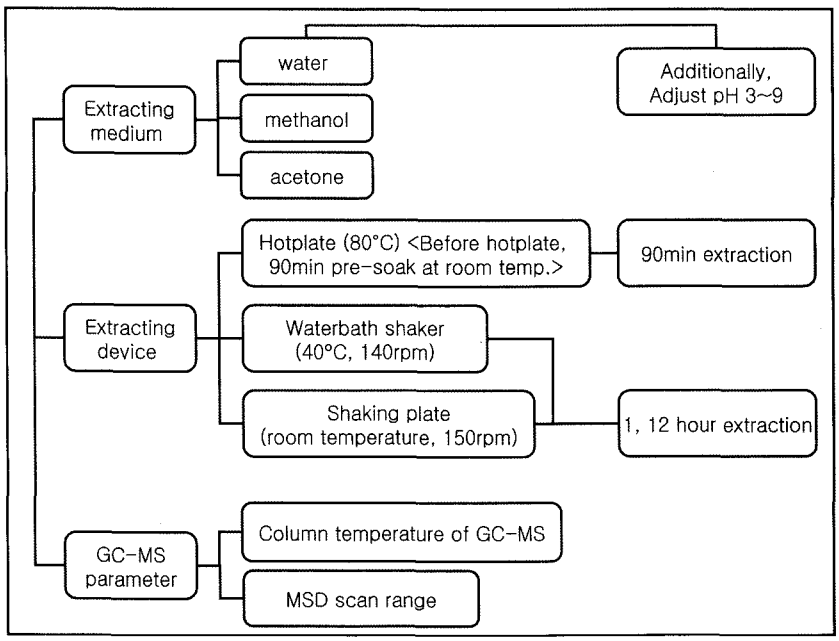


Fig. 2. Variations in extraction and analysis methods.

experiments. A 0.45μm glass fiber attached syringe filter(Alltech, Deerfield, IL) was used for filtering samples for GC-MS analysis.

2. Methods

Heartwood of sappanwood was washed thoroughly, dried, and powdered using a mill(Thomas Scientific Model 3383-L10). The effectiveness of the extracting device was tested as follows. Two Erlenmyer flasks were each prepared with 20g of ground sappanwood and 200ml methanol. One flask was extracted on a shaking plate(room temperature, 150 rpm) for 12 hours, and the other flask was extracted in a waterbath shaker(30, 140rpm) for 12 hours. In order to test the effectiveness of pre-soaking, 1g of sappanwood was mixed with 10ml methanol and extracted for 1 hour on the shaking plate(room temperature, 150rpm), and then the extract was filtered using a Buchner funnel. Fresh methanol was added to the flask and the extraction was carried out for 12 hours on the shaking plate. 1g sappanwood was mixed with 10ml acetone and extracted for 1 hour on the shaking plate. The liquor was filtered and fresh acetone was added to the flask, extraction taking place for 12 hours. 1g of new sappanwood was extracted with methanol on the shaking plate for 12 hours, and another 1g of sappanwood was extracted with acetone on the shaking plate for 12 hours. For the comparison of different pH levels, 6 Erlenmyer flasks with 5g of ground sapppanwood and 100ml water were prepared and placed at room temperature for 90 min. The liquor was filtered using the buchner funnel and then fresh 100ml water was added to each flask. The six flasks were adjusted to pH 3, 5, 6, 7, 8, 9 each and heated for 90 min at 80°C on the heating plate. Extraction samples were filtered for the GC-MS analysis using a 0.45mm, glass fiber enhanced syringe filter.

3. Instrumentation

GC-MS analysis was carried out on the Hewlett-Packard 6890 Plus Series Gas Chromatograph coupled to the Agilent Technologies 5973N Mass Selective Detector system(GC-MSD). Front inlet was kept at splitless mode with initial temperature at 250°C. Separation of the compounds was carried out with Hewlett Packard 190915-433 capillary column(30m ×250 i.d., thickness 0.25μm). Based on the literature (Ahn & Obendorf, 2003; Candan et al., 2003; Fabbri et al., 2000; Hiserodt et al., 1996), the column temperature and the MSD scan range was adjusted to the five different instrumental parameters as displayed on <Table 1>. The assignment of possible degradation products was based on the match with standard mass spectrum available in the GC-MSD library database(Agilent Technologies, 2000).

IV. Results and Discussion

The GC-MS chromatogram of dye extracted from sappanwood after 12 hours of extraction in methanol is shown in <Fig. 3>. Four major peaks were detected repeatedly in different GC-MS trials around 6 min and 13 min retention times and the result is

Table 1. Five different parameters for GC-MS analysis of sappanwood extract

Parameter / Method	Temperature control within GC capillary column								MSD scan	
	initial temp. (°C)	rate of temp. Increase (°C/min)	final temp. (°C)	holding time (min)	rate of temp. increase (°C/min)	final temp. (°C)	final holding time (min)	run time (min)	low mass (m/z)	high mass (m/z)
Method 1	50	23	210	0	30	305	14	24.12	75	400
Method 2	50	10	210	0	-	-	-	16	120	400
Method 3	50	3	150	10	10	250	0	53.33	75	400
Method 4	35	15	305	0	-	-	-	18	70	770
Method 5	50	23	210	0	30	305	8	18.12	50	350

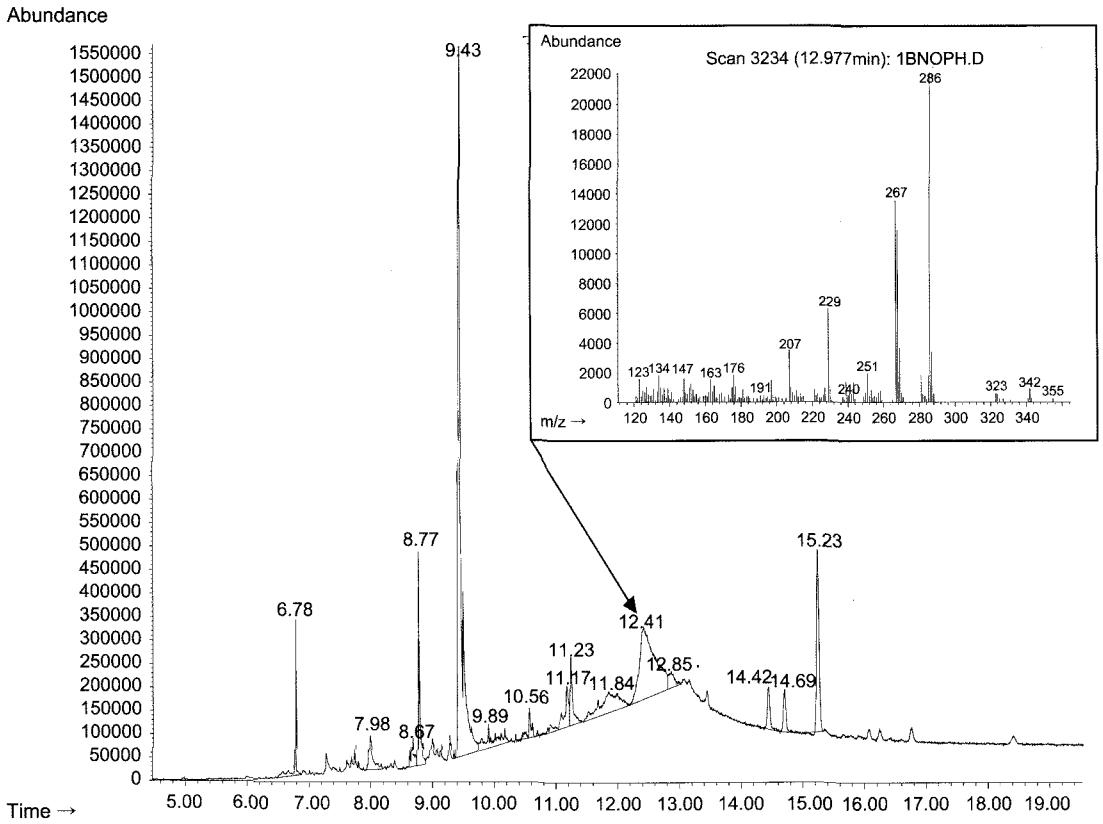


Fig. 3. GC-MS chromatogram of sappanwood showing brazilin peak at 12.41 min, mass spectrum of brazilin in the inlet.

shown in <Table 2>. Among these, the peak around 12.4~12.9 minutes in different GC-MS trials was identified as brazilin according to the peak's mass spectrum which exhibited the closest match with the ion fragmentation pattern of the reference peak of brazilin in the NIST MS library(Agilent Technologies, 2000). Three other peaks were assigned as phenol, 2, 4-bis(1, 1-dimethylethyl)-(6.7 min), hexadecanoic acid(8.7 min), and octadecanoic acid(9.4 min). For the comparison of the effectiveness of different extraction conditions, the amount of brazilin in each extraction sample was determined by the GC abundance of the brazilin peak detected in 12.4~12.9 retention time range.

<Table 3> shows the effectiveness of water, methanol, and acetone as the extraction medium for detecting brazilin via GC-MS analysis. The amount of brazilin as represented by the GC abundance was the highest in acetone and the lowest in water. Methanol resulted in more than twice the amount of brazilin detection than water, and furthermore acetone almost doubled the amount of brazilin detection than methanol. This result not only indicates that brazilin is better extracted from sappanwood by organic solvents than water, but it also implies that there will be higher amount of brazilin extraction when organic solvent with less polarity is used as the extraction medium. Between the two organic solvents used in this study, methanol with the OH bond is more polar than acetone with the C=O bond, former having the dielectric constant 33, and the latter 20.7(Newton). While the present result is contradictory to the methods of past research which extracted sappanwood primarily for dyeing fabrics(Cha & Kim, 1999; Nam, 1999; Seo et al., 2005), it is consistent with past research which focused on the extraction of sappanwood for the purpose of examining the characteris-

Table 2. Product identification of the four major peaks detected from the GC-MS analysis of the dye extracted from sappanwood

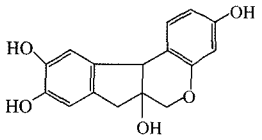
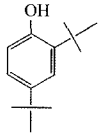
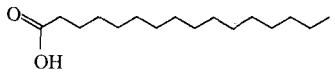
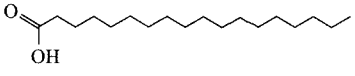
Retention time (min)	Assigned product	Chemical structure	Major ion fragments (*m/z*)	Relative abundance library	Relative abundance experimental
12.4~12.9	brazilin		286 267 268	100 54.4 47.6	100 61.2 52.3
6.7	phenol, 2, 4-bis (1, 1-dimethylethyl)-		191 192 206	100 14.9 16.4	100 14.8 15.6
8.7	hexadecanoic acid		256 129 213	100 75.0 42.1	97.3 100 70.7
9.4	octadecanoic acid		284 129 185	100 36.9 16.9	100 93.9 54.4

Table 3. Amount of brazilin detected when sappanwood was extracted with different extraction liquor

Brazilin amount \ Liquor type	water	methanol	acetone
GC Abundance	9030072	20365267	40988737
Relative percentile(%)	100	225.52	453.91

Table 4. Amount of brazilin in methanol extract using different shaking device

Brazilin amount \ Extracting device(time)	Shaking plate (12 hr)	Waterbath shaker (12 hr)
GC Abundance	44995540	20365267
Relative percentile(%)	100	45.26

tics of extracted brazilin(Fuke et al., 1985; Oliveira et al., 2002; Xie et al., 2000). Although it was observed that acetone extracted the largest amount of brazilin from sappanwood, one problem concerning acetone is that it would be difficult to handle the sappanwood extract of acetone with little lose of extraction liquor since acetone can easily evaporate at room temperature. In view of such problem, it is suggested that methanol is both effective and efficient in the extraction of brazilin from the heartwood of sappanwood.

<Table 4> shows the GC-MS result of methanol extract of sappanwood using waterbath shaker and shaking plate. When both samples were extracted for 12 hours, the amount of brazilin represented by the GC abundance was higher in the methanol extract which used the shaking plate than that which used the waterbath shaker. The amount of brazilin more than doubled with the used of the shaking plate. The result indicates that brazilin is better extracted from sappanwood with the shaking plate operated at room temperature than with the waterbath shaker operated at 30°C. The result of the present investigation supports the method utilized by Oliveira et al.(2002), while it contradicts with Xie et al.(2000) who extracted sappanwood with methanol at 75°C.

Based on the above result on the shaking plate, the effect of pre-soaking prior to the actual extraction was examined of both methanol and acetone extrac-

tion using the shaking plate(Table 5). In case of methanol extraction, 12 hours of extraction after one hour pre-soaking prior to the 12 hours of actual extraction resulted in a higher amount of brazilin detection than the 12 hours of extraction without the one hour pre-soaking. However, in case of acetone, 12 hours of extraction without the one pre-soaking before the actual extraction resulted in a higher amount of brazilin than the 12 hours extraction with before pre-soaking step. In case of both methanol and acetone, brazilin was detected by the 1 hour pre-soaking only, but the amount was much lower than that of the actual 12 hours of extraction. Again, acetone seems to have a better effect as the extraction medium than methanol. However, due to the high volatility of acetone, it is suggested that methanol is a more suitable extraction solvent for sappanwood, and furthermore the extraction procedure adopting one hour pre-soaking before the actual extraction step would give the best result. Mixing of the two methanolic extracts- the extract from one hour pre-soaking and the extract from 12 hours of extraction- will maximize the amount of brazilin detection. The above data supports Nam(1999), Cha and Kim(1999) or Oliveira et al.(2002) who prepared either the dye solution or analytical extract by mixing the liquor obtained by repeated extraction steps.

<Table 6> shows the effect of different pH adjustment and also the effect of Method 1 and Method 5 GC parameters on the extraction of brazilin. When their GC abundance was compared, pH 5 resulted in the most effective pH level for the extraction of brazilin from the heartwood of sappanwood. Between the five different GC parameters Method 1 and Method 5 exhibited the best detection of brazilin, and between the two methods Method 1, which was operated by setting the column temperature initially at 50°C, gradually increased to 210°C at a 23°C/min rate, finally increased to 305°C at 30°C/min rate, and held for 14 minutes<Table 1>, exhibited more than 2~9 times of brazilin detection than Method 5. In both cases, pH 5 was the most effective pH level.

Considering the present result, it is highly recommended that when sappanwood is extracted in water for dyeing a fabric, the extraction liquor should be adjusted to pH 5 condition for the best dyeing result. The result of the present study is consistent with Nam(1999) who suggested that water extracting sappanwood should be adjusted to pH 5~6 for the extraction of deep red colored dye. It is expected that the deepest red color of the sappanwood dye is obtained when large amount of brazilin is extracted from the heartwood. The comparison of five different GC-MS parameters on their effectiveness of brazilin detection has an important implication for the GC-MS analysis of dye identification. In order to ensure

Table 5. Examination of the effect of pre-soaking prior to the actual extraction(Shaking plate was used)

Brazilin amount \ Extraction method		With pre-soak		Without pre-soak
		1 hr presoak	12 hr extraction	12 hr extraction
Methanol	GC Abundance	5877875	26464125	20365267
	Relative percentile(%)	100	450.23	346.47
Acetone	GC Abundance	6545721	27767720	40988737
	Relative percentile(%)	111.36	472.41	697.33

Table 6. Comparison of the amount of brazilin in a) different ph samples of water extraction and b) different GC parameters

	pH	pH 3	pH 5	pH 6	pH 7	pH 8	pH 9
GC Method 1	GC Abundance	4159545	9030072	4490846	8617563	0	1134256
	Relative percentile(%)	100	217.09	107.96	207.17	0	27.26
GC Method 5	GC Abundance	459067	3739614	695124	0	0	0
	Relative percentile(%)	11.03	89.90	16.71	0	0	0

the utmost condition for the detection of major chromophoric substance using the GC-MS, each dye should be analyzed with the specific instrumental parameter which is favorable to the detection of the chromophoric substance under investigation.

V. Conclusions

This research was part of a long-term project for establishing a data pool of the degradation products of natural dyes by GC-MS for the purpose of identifying dye from badly faded exhumed textiles using the same instrumental technique. Standardization of extracting major chromophoric substance from the natural dye materials was one of the crucial factors for the success of investigating the major chromophoric substance as well as their degradation products from the natural dye materials. The purpose of the present investigation was to standardize such method for sappanwood dye. Methanol was the most effective extraction medium, considering the amount of brazilin detection and also the ease of laboratory handling. The use of shaking plate and pre-soaking prior to the actual extraction step would enhance the amount of brazilin detection from the same heartwood material. The appropriate GC-MS parameter for analyzing the sappanwood extract was also established. The present findings quantitatively verifies the methanolic extraction procedure previously used in research dealing with the brazilin analysis. The method identified in this research could be used as the standard procedure for analyzing the sappanwood dye after treating it with the laboratory simulation of the burial degradation. Ultimately, it is hoped that the methods of extraction and analysis established in this research could identify the degradation products of brazilin so as to single out the usage of sappanwood dye from any severely faded archaeological textiles. As an additional effect, it is hoped that the result of the present investigation could also be used as the standardized extraction procedure for preparing the best dye liquor of sappanwood, since the largest amount of brazilin detection would result in the deepest sappanwood red color.

References

Agilent Technologies. (2000). National Institute of Standards and Technology 98 Mass Spectral Libraries. NIST 98, Rev. D.02.00.

Ahn, C. & Obendorf, S. K. (2003). Separation of chromophoric substance from madder plant under different extraction and analytical conditions. *Journal of the Korean Society of Clothing and Textiles, 27*(11), 1350–1357.

Ahn, C. & Obendorf, S. K. (2004). Dyes on archaeological textiles: Analyzing alizarin and its degradation products. *Textile Research Journal, 74*(11), 949–954.

Ahn, C. & Obendorf, S. K. (2006). GC-MS analysis of dyes extracted from turmeric. *Fibers and Polymers, 6*(2), 158–163.

Ahn, C. & Obendorf, S. K. (2007). GC-MS analysis of curcumin dye after selective degradation treatment. *Fibers and Polymers, 8*(3), 278–283.

Ahn, C. (2007). Analysis of the degradation products of turmeric using GC-MS. *Journal of the Korean Society of Clothing and Textiles, 31*(6), 859–868.

CambridgeSoft. (2001). *The merck index.* N.J.: Merck & Co.

Candan, F., Unlu, M., Tepe, B., Daferera, D., Polissiou, M., Sokmen, A., & Akpulat, H. A. (2003). Antioxidant and antimicrobial activity of the essential oil and methanol extracts of Achillea millefolium subsp. Millefolium Afan. (Asterceae). *Journal of Ethnopharmacology, 87*, 215-220.

Cha, O. S. & Kim, S. H. (1999). A study on the dyeability and physical properties of mordanted and natural-dyed fabrics. *Journal of the Korean Society of Clothing and Textiles, 23*(6), 788–799.

Fabbri, D., Chiavari, G., & Ling, H. (2000). Analysis of anthraquinoid and indigoid dys used in ancient artistic works by thermally assisted hydrolysis and methylation in the presence of tetramethylammonium hydroxide. *J. Anal. Appl. Pyrolysis, 56*, 167–178.

Fuke, C., Yamahara, J., Shimokawa, T., Kinjo, J., Tomimatsu, T., & Nohara, T. (1985). Two aromatic compounds related to brazilin from Caesalpinia sappan. *Phytochemistry, 24*(10), 2403–2405.

Halpine, S. M. (1996). An improved dye and lake pigment analysis method for high-performance liquid chromatography andd diode-array detector. *Studies in Conservation, 41*, 76–94.

Hiserodt, R., Hartman, T. G., Ho, C. T., & Rosen, R. T. (1996). Characterization of powdered turmeric by liquid chromatography-mass spectrometry and gas chromatography-mass spectrometry. *Journal of Chromatography A., 740*, 51–63.

Kharbade, B. V. & Agrawal, O. P. (1985). Identification of

natural red dyes in old Indian textiles: Evaluation of thin-layer chromatographic system. *Journal of Chromatography, 347*, 447–454.

Nam, S. W. (1999). *Natural dyeing class*. Laboratory of Natural dyeing, Sungkeunkwan University, Suwon.

Newton, T. A. O=Chem Directory, Solvents. *The University of Southern Maine*. Retrieved April 20, 2007, from http://www.usm.maine.edu/~newton/Chy251_253/Lectures/Solvents/Solvents.html

Oliveira, L. F. C., Edwards, H. G. M., Velozo, E. S., & Nesbitt, M. (2002). Vibrational spectroscopic study of brazilin and brazilein, the main constituents of brazilwood from Brazil. *Vibrational Spectroscopy, 28*, 243–249.

Seo, H. S., Jeon, D. W., & Kim, J. J. (2005). Effect of aluminum potassium sulfate addition on the color change in Caesalpinia Sappan dyeing by rice straw ash solution. *Journal of the Korean Society of Clothing and Textiles, 29*(11), 1465–1474.

Xie, Y. W., Ming, D. S., Xu, H. X., Dong, H., & But, P. P. H. (2000). Vasorelaxing effects of Caesalpinia sappan involvement of endogenous nitric oxide. *Life Sciences, 67*, 1913–1918.

요 약

본 연구는 출토직물의 염료 성분 판정을 위한 장기적인 프로젝트의 일환으로서 천연염료 식물 중 우리나라는 물론 동서양 각지에서 과거에 염색식물로 널리 사용되었던 소목에 대하여 주 색소성분인 브라질린을 가스크로마토그라피 질량분석법(GC-MS)으로 검출할 수 있는 최적의 염료 추출 및 분석 조건을 확립하는데 그 목적을 두었다. 소목추출액에 대한 GC-MS 분석 결과 증류수보다는 메탄올이, 메탄올보다는 아세톤 추출이 브라질린 추출효과가 높았다. 추출기기 간에는 30°C로 진행된 waterbath shaker보다 상온에서 진행된 shaking plate가 브라질린 추출이 높았으며 두 기계 모두 1시간보다는 12시간 추출이 월등히 높은 추출량을 보였다. 증류수를 사용해 pH 3~9로 조절하여 브라질린의 추출효과를 조사한 결과 pH 5 조건에서 브라질린 검출량이 가장 많았다. GC-MS 분석 조건은 컬럼내 온도를 초기온도 50°C로 하고 온도증가율을 23°C/min로 하여 210°C까지 끌어 올리고 다시 온도증가율을 30°C/min로 하여 최종적으로 305°C로 가열한 후 14분 동안 holding하여 전체 분리시간을 24.12분으로 한 방법이 가장 효과적이었으며 MSD 스캔범위는 75~400m/z이 적합하였다.