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GC-MS Analysis of Dyes Extracted from Turmeric

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Abstract: Standard extraction procedure for examining chromophoric substances of turmeric was investigated. Acetone and methanol were used as extracting solvents with different extraction procedures and pH levels. GC-MS analysis identified curcumene ***2*** (6.7 min), feruloylmethane ***3*** (8.3 min), coumaran ***4*** (6.09 min), vanillin ***5*** (6.2 min), and zingiberene ***6*** (10.5 min) as the major products. Curcumin ***1*** which has been known as the major chromophoric substance of turmeric was not detected in any samples. The maximum amount of curcumene ***2***, which was used as the fingerprint product for turmeric dye, was obtained by utilizing presoaking and decanting step with methanol prior to actual extraction step using a waterbath shaker (WMM). The highest relative abundance of curcumene ***2*** was detected in pH 6 sample followed by pH 5 indicating that the most appropriate pH level was in the range of pH 6-5.

Keywords: Turmeric, Curcumin, Curcumene, Dye extraction, GC-MS

Introduction

Recently, there has been growing interest in the conservation and characterization of textiles exhumed from different burial sites of Korea. Excavated textiles display various damages, and the extent and the type of damage have serious effect on their conservation procedures. The most readily observed damage in the exhumed textiles is color fading, and in many cases the fading is so severe that it is impossible to even guess the original color of the textile pieces. The loss of original hue in the exhumed textiles makes problems in documentation of the textile pieces as well as conservation procedures which are necessary for long-term museum storage. Hence, there is a strong need for the identification of original color or the dyestuff used in the heavily faded textiles of archaeological origin.

Various analytical approaches were used for the identification of natural dyestuff in museum textiles. In most cases the identification succeeded when the original color of the textile was still visible, and thus it was possible to carry out a comparative analysis with a standard dye chemical. However, without the hint of the original hue it is difficult to select one or a limited number of standard dyestuffs for the comparative analysis. An effective strategy for the problem is to analyze each possible dyestuff separately in a simulated degradation condition, the data which can then become part of a pool of standard dyes for the comparative analysis [1]. In doing so, it is imperative that each natural dyestuff is extracted and examined in a standardized method possible, which can extract and detect the major chromophoric substances. This research was to establish a standard extraction procedure for examining a major chromophoric substance in the rhizome of turmeric in order to identify turmeric dye in severely faded archaeological textiles.

Turmeric is the common name for *Curcuma longa L.* which is part of the ginger family, Zingiberaceae. The powered rhizome of turmeric is well known as the Indian spice curry and it also has been widely used in the past for dyeing cloth to yellow. Three major coloring substances were isolated from turmeric are curcumin ***1*** (or diferuloylmethane), demethoxycurcumin, and bisdemethoxycurcumin and these comprise about 3-5 % of the raw plant [2]. Among them curcumin is known as the most representative substance. Curcumin ***1*** (Figure 1) is an orange yellow volatile oil and it is classsified as the hydroxyketone type dye [3]. Curcumin ***1*** is readily soluble in organic solvents such as acetone and alcohol, but insoluble in water and ether [4]. Curcumin ***1*** is more widely used in the pharmaceutical application for its strong antioxidant property, which is attributable to its molecular structure having both phenolic and beta-diketone functional groups [5]. Poor colorfastness of fabrics dyed with turmeric toward light [6,7] or ozone exposure [8] is, in part, due to such molecular structure of curcumin.

Several research reports the extraction procedures of turmeric necessary for characterization and dyeing. The procedure is largely divided into water-based extraction [9-11] and solvent-

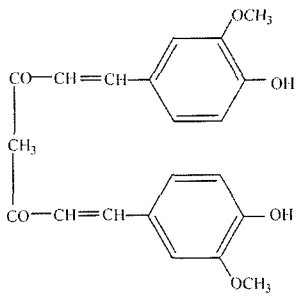

Figure 1. Structure of curcumin ***1***.

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based extraction [12,13]. When extracting turmeric in deionized water, studies found that boiling near 100 °C is necessary for obtaining the highest maximum absorbance in spectrophotometric analysis [9,10]. Similarly, Nam [11] recommended boiling turmeric and other dye plants in deionized warter for 15 minutes in an effort to standardize dye procedure for various natural dyestuffs. Hwang *et al.* [12] extracted turmeric in methanol at 30 °C for 6 hours for preparing the concentrated dye. And, some studies prepared the dye liquor by mixing the filtrate after extracting the same turmeric batch two or three times [12,14]. For characterization and purification of curcumin extracted from turmeric, Kim and Kim [13] extracted ground turmeric in methanol and separated curcumin with ethyl ether. In our previous research on madder, we found that the amount of alizarin in the extraction liquor can be maximized by adopting the 90 minutes-presoaking/decanting step before the actual extraction with fresh water at pH 3 [15]. During our preliminary investigation on turmeric applying both water-based and solvent-based extraction, we learned that neither curcumin ***1*** nor related products were detected by water-based extraction. And thus, this study focuses on the solvent-based extraction with differing pH levels and differing extraction steps to find the most effective procedure for extracting and detecting curcumin ***1*** or related products from turmeric dye.

Materials and Methods

Materials

Dried rhizomes of turmeric were purchased from Korean traditional medicinal market. Methanol (HPLC grade) was purchased from Mallinckrodt Baker (Paris, KY). Reagent grade H_2SO_4 and NaOH were purchased from EM Science (Darmstadt, NJ). Deionized distilled water was used throughout the experiments. A 0.45 μm glass fiber attached syringe filter (Alltech, Deerfield, IL) was used for filtering samples for GC-MS analysis.

Methods

Dry rhizomes of turmeric were thoroughly washed and powdered before use. In order to find the most effective extraction procedure for detecting major chromophoric substance by GC-MS analysis, several trials of different extraction procedures were followed. The procedures are outlined in Table 1 with the procedure identification label specified. 20 g of powdered turmeric was extracted with 200 m*l* methanol using two different shaking devices- a shaker board and a waterbath shaker. Shaker board was set at 150 rpm and the extraction was carried out at room temperature with flasks covered to block light. Waberbath shaker was set at 140 rpm, 30 °C, also covered to block light. In both shakers, turmeric was initially extracted for 1 h and the second extraction was followed with fresh 200 m*l* methanol, 12 h for the shaker board and 6 hrs for the waterbath shaker. The filtered turmeric of waterbath shaker was re-extracted with fresh 200 m*l* methanol for 12 h. The extractions were evaporated to dry on a hotplate, the residue was dissolved with methanol for the GC-MS analysis. These procedures were labeled 'SMM' for the shaker board extraction, and 'WMM' for the waterbath extraction.

Table 1. Extraction and GC-MS sample preparation procedures

Extracting device	Extracting solvent	Extraction steps	GC solvent	Procedure label
Shaking plate	Methanol	2-step	Methanol	SMM
	Acetone	1-step	Methanol	SAM
	Acetone	1-step	Acetone	SAA
Waterbath shaker	Methanol	3-step	Methanol	WMM
	Methanol	1-step	Methanol	WMM

A new extraction was carried out with fresh mixture of turmeric and methanol for 12 h in the waterbath shaker, following the WMM procedure. This liquor was divided into 6 vials and the pH of each vial was adjusted to pH 4, 5, 6, 7, 8, and 9. The extractions were evaporated to dry on the hotplate (80 °C) and the residue was dissolved with HPLC grade methanol, filtered for the GC-MS analysis. Two other extractions were carried out with 20 g of powdered turmeric and 200ml acetone using the shaker board. The extractions were evaporated to dry on the hotplate. One extraction was dissolved with acetone for the GC-MS analysis, and the procedure was labeled 'SAA'. The other was dissolved with methanol for GC-MS analyses, and this procedure was labeled 'SAM'.

GC-MS analysis was conducted on the Hewlett-Packard GC 6890 Series coupled to the Agilent Technologies 5973N MSD system. Operating conditions were as follows: Front inlet was kept at splitless mode with initial temperature at 250 °C. Products were separated on a Hewlett Packard 190915-433 capillary column (30 m × 250 μm i.d., 0.25 μm nominal film thickness) programmed from 50 °C to 305 °C, column flow 1.3 m*l*/min at initial temperature. Initial temperature at MSD was 310 °C. Mass spectra were recorded at scan range 80-250 *m/z*, and the threshold for the scan mode was 200. Assignment of possible degradation products was based on the match with standard mass spectrum available in the GC-MS library database [16].

Results and Discussion

Dye extracted from turmeric exhibited several noticeable peaks and numerous small peaks in the wide retention time range (Figure 2). Four peaks were detected repeatedly in different GC-MS trials around 6 min and 8 min retention times. They were identified as curcumene ***2*** (6.7 min), feruloylmethane ***3*** (8.3 min), coumaran ***4*** (6.09 min), and vanillin ***5*** (6.2 min) as they matched the ion fragmentation patterns of the reference peaks in the NIST MS library [16] (Table 2). In the

pH adjusted samples another noticeable peak was detected, and this product was identified as zingiberene ***6*** (10.5 min) according to the reference peaks in the NIST MS library [16] (Table 2). Curcumin ***1*** which has been known as the major chromophoric substance of turmeric was not detected in any samples.

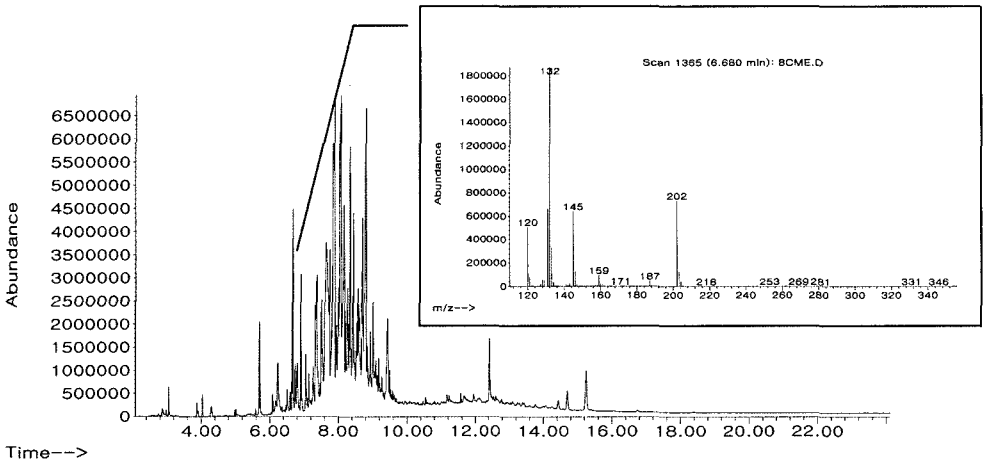
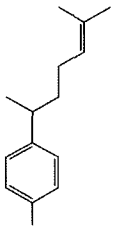
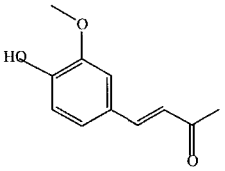
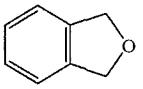
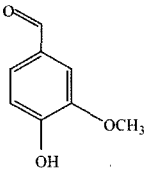
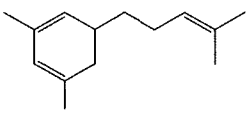


Figure 2. GC chromatogram of turmeric extraction according to WMM procedure (inlet: mass spectrum of curcumene ***2***).

Table 2. Major products detected from turmeric extraction and their GC-MS result

Retention time, minutes	Cpd no.	Product assignment[§]	Chemical structure	Major ion fragments (m/z)	Relative abundance library	Relative abundance experimental
6.68	***2***	Curcumene [benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-]		202 132 119	23.3 74.3 100	39.0 100
8.32	***3***	Feruloylmethane [3-buten-2-one, 4-(4-hydroxy-3-methoxyphenyl)-]		192 177 145	79.7 88.2 100	100 95.1 83.8
6.09	***4***	Coumaran [benzofuran, 2,3-dihydro-]		120 119 91	100 26.5 72.8	100 23.5 46.2
6.23	***5***	Vanillin [benzaldehyde, 2-hydroxy-3-methoxy-]		152 151 106	100 36.0 38.6	95.4 100
10.57	***6***	Zingiberene [1,3-cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-]		204 119 93	8.0 78.5 100	86.1 100

[§]Chemical name of the products in parentheses.

Studies on curcumin and turmeric either in the form of commercial powder or oil have shown that curcumin is highly unstable during the sample preparation and instrumental analyses, leading to a rapid formation of degradation products [17]. Such instability is largely due to the powerful antioxidant property of curcumin ***1***, which is well-known by its ability to scavenge reactive oxygen and nitrogen free radicals [5]. Curcumin ***1*** has a symmetrical diketone structure which has two o-methoxy phenolic OH groups attached to the symmetrical β-diketone structure. It has been proposed that the phenolic OH group or the CH_2 group in the β-diketone moiety is responsible for the free radical scavenging ability of curcumin ***1*** [18]. In relation to such theoretical background, a study using HPLC for analyzing commercial curcumin identified degradation products such as vanillin ***5***, feruloylmethane ***3***, and ferulic acid in addition to curcumin ***1*** itself [17]. On the other hand a GC-MS analysis on the rhizome of turmeric identified curcumene ***2*** and zingiberene ***6*** as its main constituents and no trace of curcumin ***1*** [19]. Similar result was observed in the investigation of turmeric oil in which curcumene ***2*** and zingiberene ***6*** were identified as part of the major constituents of turmeric oil [2,20]. In view of the previous investigation on curcumin, it is not surprising that curcumin ***1*** was not detected from our samples. It is highly probable that a rapid degradation of curcumin ***1*** occurred immediately after the sample injection into the GC port, and thus producing curcumene ***2***, zingiberene ***6***, and the products ***3***, ***4***, and ***5*** as the GC-MS fingerprints of turmeric dye. In our study, we utilized the GC-MS result on curcumene ***2*** as the signature of curcumin's presence in the extracted samples.

Table 3 shows the percent relative abundance of products ***2-5*** in the samples of different extraction procedures. The sample which showed the highest relative abundance of curcumene ***2*** was the second extraction liquor of the three-step waterbath extraction (WMM). Although the degradation products feruloylmethane ***3*** and vanillin ***5*** were also detected, their abundance was relatively low. Considering the first extraction only, the extractions using acetone appeared to be the most effective method (SAA and SAM), better result was obtained when acetone was used for the GC sample preparation as well (SAA). The amount of curcumene ***2*** detected in SAA sample was over 90 % of that of the second extraction liquor of WMM (100 %) with no apparent degradation products.

The data indicates that the maximum amount of curcumene ***2*** can be obtained by utilizing the presoaking and decanting step with methanol prior to the actual extraction step. This procedure would extract some of the miscellaneous substances which are inherent in the natural turmeric root and thus maximize the detection of the major chromophoric product. In addition, considering the amount of feruloylmethane ***3***-a colorless oily substance-in the first extract, it would be advisable to waste the first extract in order to optimize the color intensity of the turmeric dyebath. The result of the present study is similar to our previous research on madder, which also required a presoaking/decanting step with water to obtain the highest relative abundance of alizarin from the natural plant. According to the data, the amount of curcumene ***2*** can be increased by mixing the second and the third extraction liquor. Such data supports the past attempts in preparing the turmeric dyebath by mixing the filtrate after extracting the same turmeric batch two or three times [12,14]. In the present study, the sample extraction with acetone and usage of acetone for GC-MS sample preparation (SAA) seems to be the best alternative choice to the second extraction using the waterbath (WMM). However, since acetone can easily evaporate at room temperature, it would be difficult to handle the turmeric extract using acetone with little lose of extraction liquor.

Table 4 shows the percent relative amount of products ***2-6*** in different pH adjustments, normalized to the relative abundance of curcumene ***2*** in the sample with its highest abundance. The highest relative abundance of curcumene ***2*** was shown at pH 6 followed by pH 5. The lowest relative abundance was examined in pH 9. Such data is consistent with the result of past research that curcumin is unstable in the basic pH condition and that its stability can be increased in a slightly acidic condition [17]. According to Wang *et al.* [17], the increased stability of curcumin in the acidic condition is due to its conjugated diene structure, which can be destructed when the proton is removed from the phenolic group in the neutral to basic pH. We confirmed such pH dependence of curcumin by visual detection as well as UV-VIS spectroscopy. While turmeric extraction in the acidic condition showed the typical yellow color, the color of turmeric extraction in neutral to basic condition was red. The stability of curcumin in the

Table 3. Relative abundance of products in methanol and acetone extractions with no pH control[§]

Extraction method / Product & retention time		Shaking plate (SMM)		Waterbath shaker (WMM)			Waterbath (WMM)	Plate (SAM)	Plate (SAA)
		1st	2nd	1st	2nd	3rd	1st	1st	1st
6.68 min	***2***	62.39	80.55	21.53	100	36.08	12.10	76.36	90.10
8.32 min	***3***	23.28	22.00	46.56	13.73		75.43		
6.09 min	***4***	3.49	6.86		40.62			12.45	
6.23 min	***5***	3.84	72.87	17.69	26.30	20.25	17.11	4.65	

[§]Percent area of peak in chromatograms normalized to curcumene ***2*** in the second extraction of the waterbath shaker (WMM) as 100.

Table 4. Relative abundance of six major products in different pH levels of turmeric extractions[§]

Retention time (minutes)	Cpd no.	pH 4	5	6	7	8	9
6.68	*2*	59.94	92.40	100	31.49	52.76	29.69
8.32	*3*	21.54			32.18	30.52	8.84
6.09	*4*	0.96			0.82	6.21	13.40
6.23	*5*					3.86	8.84
10.57	*6*		34.80	28.86	32.18	52.34	

[§]Percent area of peak in chromatogram normalized to curcumene *2* in pH 6 is 100.

slight acidic condition was further confirmed by the fact that the degradation products such as feruloylmethane *3*, coumaran *4*, and vanillin *5* were not observed in pH 6 and pH 5 samples. Based on the results, it is suggested that the most appropriate pH level for extracting turmeric with the aim of extracting and detecting the major chromophoric substance is in the range of pH 6 to pH 5, the best result was obtained with pH 6 in our investigation.

Conclusion

The purpose of this research was to establish standard extraction procedure for examining the chromophoric substance in the rhizome of turmeric with the ultimate goal of identifying turmeric dye in severely faded archaeological textiles. The result indicated that the maximum amount of curcumene *2* can be obtained by utilizing the presoaking and decanting step with methanol prior to the actual extraction step. And, the most appropriate pH level for extracting turmeric is in the range of 5 to 6.

Dye extracted from natural plants contains a number of different substances [21] some of which contribute to the color of the dye and others are the miscellaneous substances which not only affect the hue or the color intensity of the dye liquor but also hinder the detection of major chromophoric substance during the instrumental analysis. Therefore, in order to maximize the extraction and the detection of major chromophoric substance from the dye plant, different extraction methods should be carried out. In this research, we used the GC-MS analysis as our analytical tool for investigating the effectiveness of different extraction procedures. Considering the sensitivity of the GC-MS analysis, we believe that the extraction method suggested in this research may serve as the standard extraction procedure for other instrumental analysis of dye identification, including HPLC or TLC. And it is hoped that the result of the present research can serve as a step toward the dye identification of heavily faded archaeological textiles.

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