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Separation and Identification of Curcuminoids from Asian Turmeric (*Curcuma longa* L.) Using RP-HPLC and LC-MS

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In this study, three major curcuminoids in turmeric, including curcumin, demethoxycurcumin and bismethoxycurcumin, were efficiently extracted by optimizing the extraction conditions and were simultaneously identified using a fast and reliable HPLC-UV-MS method and thin layer chromatography. The analysis was performed with a C_{18} column and the UV wavelengths were fixed at 425 and 254 nm. The total extraction yields of curcumin and demethoxycurcumin increased with the extraction time from 1 to 4 h, but scarcely increased after 4 h. LC-MS following thin layer chromatography analysis provided efficient molecular weight information for the two major curcuminoids in the turmeric extracts.

Key Words: Curcuminoids, Turmeric, HPLC, LC-MS, Thin layer chromatography.

INTRODUCTION

Curcuma species have been used as traditional medicines because of their various pharmacological activities including wound healing, promoting digestion, anticancer, antioxidant, hepatoprotective, *etc*.[1,2]. The yellow colour that is characteristic of the turmeric rhizome is due to the presence of 4-8 % of volatile and 3-5 % of curcuminoids[3,4]. The curcuminoids such as curcumin, demethoxycurcumin and bismethoxycurcumin are the major diaryl heptanoids in turmeric and have been shown to contribute to many of the medicinal properties of this species, such as antiinflammatory, cancer prevention and anti HIV activities[3,5].

The identification and relative amounts of component in turmeric oil have been determined by LC-MS, GC and GC-MS[6]. In particular, techniques previously used UV-VIS spectrophotometry, thin-layer chromatography (TLC), HPLC, capillary electrophoresis and gas chromatography (GC) and could easily detect accidentals[4]. And simple, rapid and reliable TLC, RP-HPLC and LC-MS methods have been applied for qualitative and quantitative analysis of the major components of curcuminoids in turmeric and demethoxycurcumin (DMC) (*Curcuma longa* L.).

EXPERIMENTAL

Asian turmeric (*Curcuma longa* L.) was acquired from the KIOM, Center for Herbal Medicine Improvement Research Principal Research Scientist Center, Korea in May, 2011. Standard samples were prepared by dissolving 2 mg of the standard chemical (curcumin, Sigma-Aldrich, China) in 4 mL of methanol and adjusting the concentration to 500 ppm. HPLC-grade methanol (MeOH) and acetic acid (AA) were purchased from J.T. Baker (USA). TLC plates were purchased from Sorbent Technologies and Membrane filters were purchased from Waters, Milford, MA, USA.

Solvent extraction: The standardized *Curcuma longa* L. consisted of dried samples without particles (sieving < 30 μm) and moisture content of 10.30 %. 5 g for each extraction) of powdered *Curcuma longa* L. was used for extraction using a solvent composition (200 mL for each extraction) of increasing polarity by the dipping method for 4 h at 25 ºC. Each extract was filtered, concentrated under vacuum using a rotavapor and refrigerated for 24 h. This was repeated twice and the average value was applied. Each sample was filtered through a 0.2 μm membrane filter prior to HPLC analysis.

TLC, HPLC and LC-MS analysis: The experiments were performed with a Dionex HPLC system equipped with an ultimate 3000 pump, ultimate diode array detector (DAD), 10 μL sample injector loop (Dionex, ID × L 0.18 mm × 550 mm, USA) and Chromeleon data acquisition system (Dionex version 7.0.1.272). The standard curcumin was confirmed by LC-MS analysis (Varian: 500-MS IT Mass Spectrometer). The chromatographic columns were obtained as Knauer RP-column

(250 mm × 3.0 mm, 5 µm, C_{18}, with precolumn Eurospher 100-7, Germany). The injection volume was 10 µL and the flow rate of the mobile phase was 1.0 mL/min. The UV spectra were recorded between 200 and 500 nm for peak characterization and the detections wavelength were set at 425 and 254 nm. UV spectra were measured with a UV-1600 visible spectrophotometer. The mobile phase solvents were A (water/acetic acid = 99.9/0.1, v/v) and solvent B (acetonitrile/acetic acid = 99.9/0.1, v/v). The run time was 0.5 h and an isocratic method was applied (solvent A 40 % and B 60 %). The analyses were performed by a silica XHL TLC plates glass (5 cm × 10 cm, 250 µm, Sorbent Tech., USA) developed with a mobile phase composed of chloroform-methanol (97:3, v/v) and stained with a 10 % H_2SO_4 reagent to detect the curcuminoids.

RESULTS AND DISCUSSION

In order to extract the curcuminoids from turmeric efficiently, the variables involved in this procedure were optimized, including the extraction solvent methanol, the extraction method (dipping) and the extraction time (1-7 h). The peaks of curcumin and demethoxycurcumin appeared at 3.42-3.44 min and 3.18-3.19 min, respectively (Table-1). The extraction yields of these two compounds increased with the extraction time from 1 h (6.48 %) to 4 h (7.86 %), but scarcely increased after 4 h. The HPLC analysis conditions for the best separation and quantification of curcumin, demethoxycurcumin and bisdemethoxycurcumin were successfully established by changing the solvent compositions of the mobile phase (Table-2). Here, increasing the amount of acetonitrile mobile phase B: 90-40 % quickly increased the retention time, but resolution decreased. On the other hand, increasing the water (mobile phase A: 60-10 %) and slowing the retention time decreased the UV sensitivity. Thus, the optimum mobile phase condition was acetonitrile B: 60 %. While using 100 % methanol for dipping (25 ºC), as shown in Fig. 1, the extraction rates of total turmeric yield and curcuminoids increased with extraction time from 1-7 h, with the highest extraction at 7 h, However, extraction efficiency showed a similar trend after 4 h (7.72 %, 0.386 g), 5 h (7.760 %, 0.388 g), 6 h (7.78 %, 0.389 g) and 7 h (7.86 %, 0.393 g), respectively. Therefore, the optimal extraction time was 4 h.

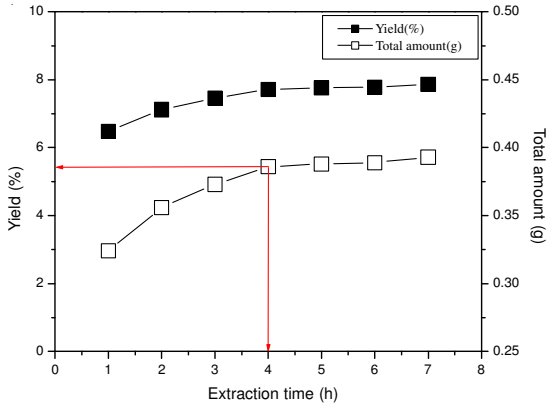


Fig. 1. Extraction yields of turmeric and curcumin with different extraction times

TABLE-1
EXTRACTION YIELDS WITH TURMERIC USING EXTRACTION SOLVENT OF 100 % MeOH

Extraction time (h)	Retention time t_R (min) # 1	Retention time t_R (min) # 2	Retention time t_R (min) # 3	Total amount (g)	Yield (%)
1	3.443	3.193	2.929	0.324	6.480
2	3.426	3.183	2.919	0.356	7.120
3	3.431	3.183	2.923	0.373	7.460
4	3.423	3.188	2.934	0.386	7.720
5	3.433	3.191	2.934	0.388	7.760
6	3.427	3.187	2.919	0.389	7.780
7	3.425	3.180	2.923	0.393	7.860

*Extraction method: dipping (25 ºC).

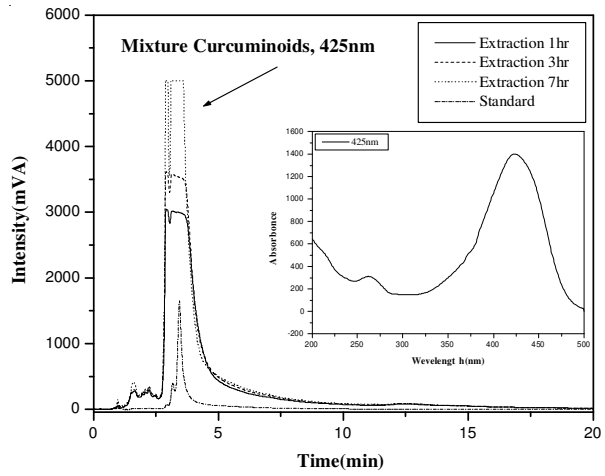
TABLE-2
EXTRACTION AMOUNTS FOR VARIABLE MOBILE PHASE COMPOSITIONS FOR CURCUMINOIDS FROM TURMERIC BY DIPPING METHOD

Mobile phase composition (%)		t_R (min) # 1	t_R (min) # 2	t_R (min) # 3	Peak area (mAU) # 1	Peak area (mAU) # 2	Peak area (mAU) # 3
Water (A)	Acetonitrile (B)						
60	40	22.657	20.120	18.013	442.718	90.363	13.847
50	50	6.967	6.300	5.720	557.333	102.930	17.179
40	60	3.323	3.057	2.823	628.805	102.874	21.488
30	70	2.197	2.063	1.933	618.578	97.281	19.580
20	80	1.757	1.627	–	615.375	117.335	–
10	90	1.417	–	–	669.980	–	–

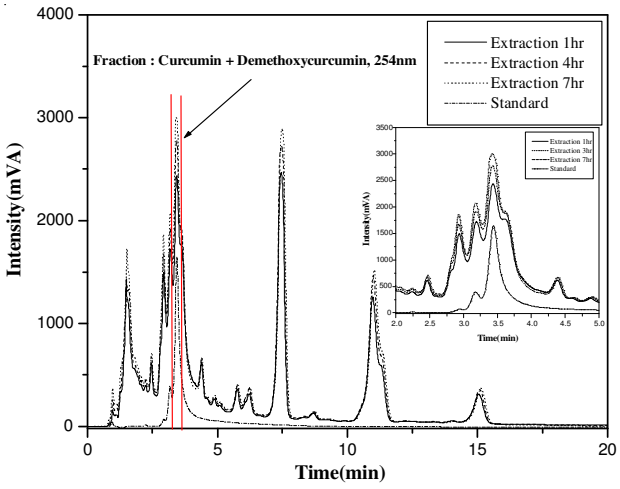
Water (A)	Acetonitrile (B)	Peak area (%) # 1	Peak area (%) # 2	Peak area (%) # 3
60	40	80.49	16.43	2.52
50	50	82.27	15.19	2.54
40	60	81.52	13.34	2.79
30	70	82.29	12.63	2.57
20	80	82.14	15.66	–
10	90	99.4	–	–

–: Not detected.

Fig. 2 shows the chromatograms for different extraction times (1, 3 and 7 h at 25 ºC). The dried turmeric (*Curcuma longa* L.) was pulverized and extracted by 100 % methanol using 200 mL of solvent for each extraction. The characterization of curcuminoids were achieved by comparing the HPLC retention times (t_R) and UV maximum absorptions of target peaks in the samples with those of standards (Fig. 3). The collected fraction (fraction time 3.3-3.6 min) was analyzed by thin layer chromatography (TLC) and the major components curcumin (fraction # 1) and demethoxycurcumin (fraction # 2) were isolated using preparative thin-layer chromatography (PTLC). From the results, high purity (+98 % by UV) curcumin and demethoxycurcumin were obtained. A simple twice development TLC method was developed and quantitative determinations of curcumin and demethoxycurcumin were also performed. Quantification was performed on the basis of linear calibration plots of the UV absorption peak area at 425 nm.

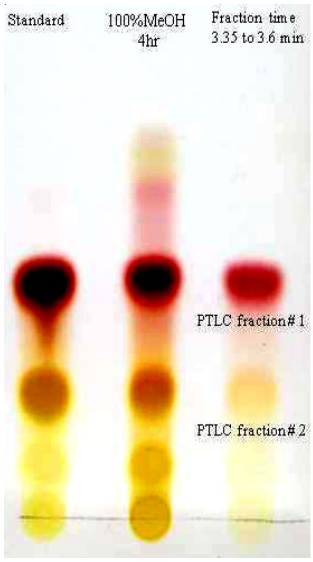


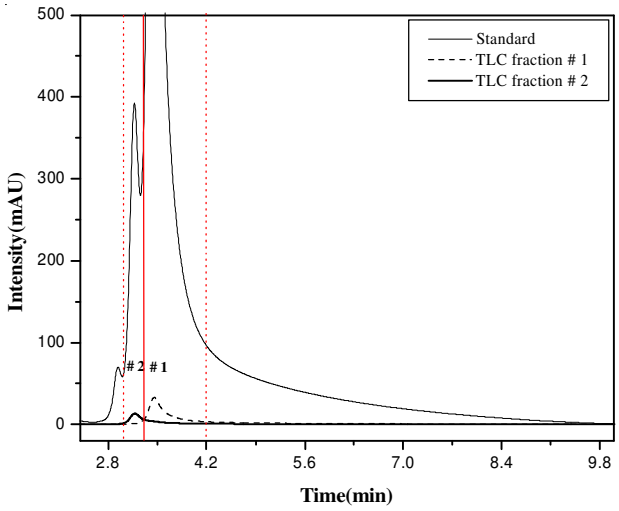
(a)



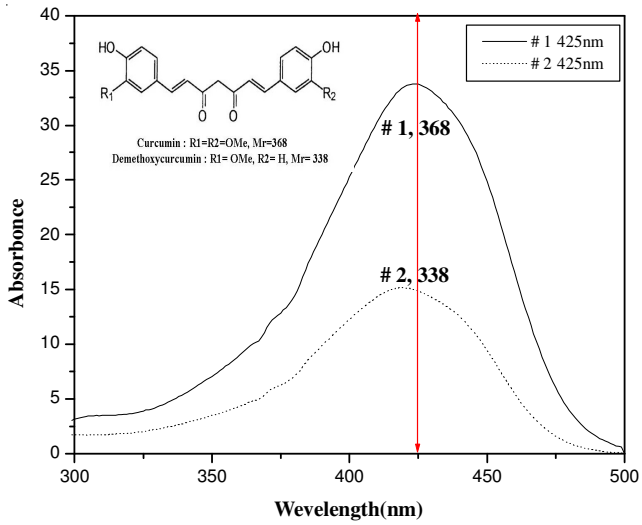
(b)

Fig. 2. Comparison of chromatograms for curcumin and demethoxycurcumin by different extraction times and UV wavelengths. (a) extraction time 1, 3, and 7 h, 425 nm and (b) extraction time 1, 3 and 7 h, 254 nm)





(a)



(b)

Fig. 3. Analysis of fractions collected (# 1 and # 2) from turmeric using PTLC and HPLC. (a) PTLC and HPLC analysis, (b) UV detector was fixed at 425 and 254 nm)

LC-MS analysis has been shown to be a powerful tool in metabolic profiling and metabolomics research. This technology can accurately determine the content of specific metabolites, even if these are found at low levels in plant samples[7].

One study using LC-MS to identify certain constituents of fresh turmeric has been reported[4,8]. Based on our simultaneous analysis of two major curcuminoids, the components detected by mass spectra were correlated from molecular mass information and relative response. Compounds # 1 and # 2 showed very intense protonated molecules $[M + H]^+$ at m/z 369.3 and 339.3, respectively (Fig. 4). Among them, curcumin and demethoxycurcumin were unambiguously identified, based on their UV spectra, mass spectra and retention times in comparison with the data of standard compounds.

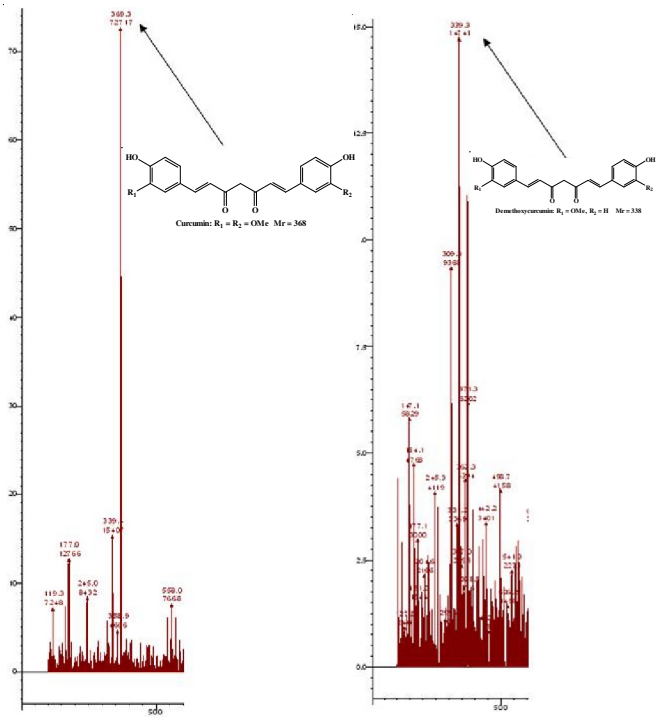


Fig. 4. Chromatograms of curcuminoids with 100 % MeOH extraction by LC-MS. (Shown Fig. 3 fractions # 1 and # 2)

Conclusion

The optimum operating conditions were experimentally determined to analyze three curcuminoids in the pretreatment extracts. The analysis was performed using a C_{18} column and the flow rate of the mobile phase and UV wavelengths were fixed at 1.0 mL/min and 425 and 254 nm, respectively. The total extraction yields of curcumin and demethoxycurcumin increased with extraction time from 1-4 h, but scarcely increased after 4 h. Moreover, a combination method of simple HPLC fractionation and TLC was developed for rapid qualitative and quantitative analysis of turmeric. The LC-MS data provide molecular weight information for the components in the turmeric extracts.

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