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Effects of ethanol concentration, extraction time and ultrasound-assisted extraction on the recovery of curcuminoid from turmeric (*Curcuma longa* L.) rhizome

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Abstract

Curcuminoids are a group of compounds extracted from turmeric rhizomes which have many medical advantages and health benefits. Extraction of curcuminoids from the raw material is the crucial step for using these active compounds. This research was conducted to optimize the conditions for curcuminoid extraction from turmeric powder using ethanol as an extracting solvent by conventional methods and using ultrasound-assisted extraction techniques. The results indicated that the highest total curcuminoid content (TCC) of 13.3145 mg curcumin/g turmeric powder (DW) was obtained using ultrasound-assisted extraction in 80% ethanol for 15 min. HPLC analysis showed that the extract was composed of curcumin, demethoxycurcumin and bisdemethoxycurcumin as 47.8762, 40.2941 and 15.2412 mg/g turmeric powder (DW), respectively.

***Keywords**: curcuminoids, curcumin, ethanol extraction, HPLC, turmeric powder, ultrasound-assisted extraction*

1. Introduction

The word "curcuminoid" indicates a group of compounds such as curcumin, demethoxycurcumin, and bisdemethoxycurcumin and cyclic curcumin. Of these, curcumin is the major component, and cyclic curcumin is the minor component (Priyadarsini, 2014). Turmeric (*Curcuma longa* L.) has been found to be a rich source of curcuminoids. Curcumin, which is the major constituent of curcuminoids is reported to be a natural antioxidant (Jayaprakasha, Jaganmohan, & Sakariah, 2005). Curcumin possesses many pharmacological properties such as antimicrobial activities against oral bacteria (Mohammed & Habil, 2015), anti-inflammatory, anti-carcinogenic and anti-cancer effects and particularly against colorectal cancer (Jurenka, 2009; Onsurathum, Pinlaor, & Boonmars, 2012; Prabha & Raj, 2016). Yodkeeree, Chaiwangyena, Garbisab, and Limtrakula (2009) indicated that curcuminoids showed anti-angiogenic properties and the ability to modulate the multidrug-resistance gene and alter protein function. The biological effects of curcumin are summarized in Figure 1.

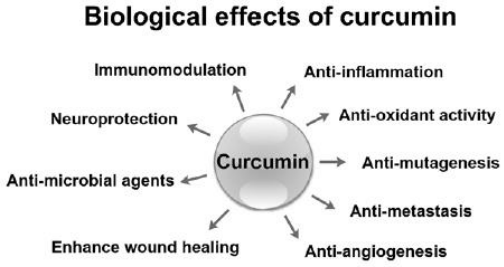


Figure 1 Biological effects of curcumin [Source: Onsurathum et al. (2012)]

However curcumin has well pharmacological properties, the therapeutic application of curcumin has been impeded by its shortcomings such as low aqueous solubility in acidic and physiological pH and its degradability in alkaline conditions (Xu et.al., 2016). Curcuminoids are also widely used in food processing as a natural colorant in dairy products, fats, oils and fat emulsions, edible ices, fruit and vegetable products, confectionery, cereal products, bakery wares, meat and meat products, fish and fish products, eggs and

eggs products, spices, soups, sauces and protein products (Surojanametakul, Satmalee, Saengprakai, Siliwan, & Wattanasiritham, 2010). Jayaprakasha et al. (2005) also reported that curcuminoids could be used in foods to extend their shelf life and increase the nutritional value for consumers. Naidu, Shyamala, Manjunatha, Sulochanamma, and Srinivas (2009) stated that curcuminoids from turmeric samples from different areas of India contained curcumin, demethoxycurcumin, and bisdemethoxycurcumin in ranges of 3-6.25%, 1.62-3.5%, and 1.12-3.07%, respectively. The chemical structure of curcuminoid components are shown in Figure 2.

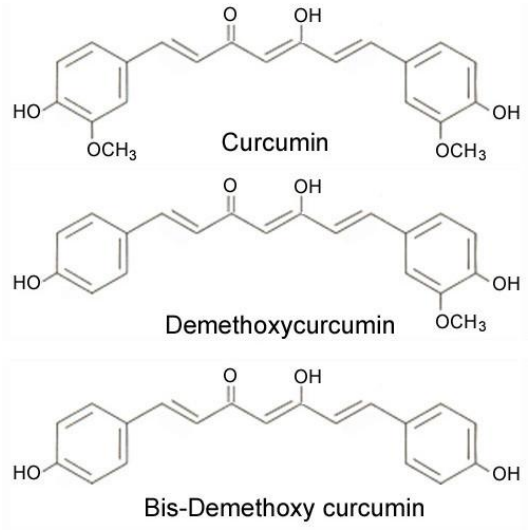


Figure 2 Chemical structures of curcuminoids: curcumin, demethoxycurcumin and bisdemethoxycurcumin [Source: Hastati et al. (2015)]

According to curcuminoid extraction from the dried root of turmeric rhizome, the most conventional method has been solvent extraction. The suitable solvents which meet extractability and regulatory criteria are isopropanol, ethyl acetate, acetone, carbon dioxide (has potential for using as a substitute for chlorinated solvents), methanol, ethanol and hexane (FAO, 2004). The solvent extraction method has usually been done by shaking or Soxhlet extraction with heating times ranging up to 12 h. The Soxhlet extraction is a time consuming, laborious process that use bulk amounts of organic solvents. As the heating process continues for long hours, this approach possibly involves high risk of thermal decomposition of target molecules (Kulkarni, Maske, Budre, & Mahajan, 2012). In the recent years, the ultrasonication technique has been widely used for active compound extraction. Sonication is the act of applying sound energy to agitate particles in a sample. Ultrasonic frequencies (> 20 kHz) are usually used, leading to the process being known as ultrasonication (Bendicho & Lavilla, 2000). The use of sonication significantly improves the extraction of organic compounds contained within the body of plants and seeds (Dolatowski, Stadnik, & Stasiak, 2007). The acoustic cavitation phenomenon which is generated by passing ultrasound waves through a solvent system caused higher efficiency of this technique. This phenomenon permits better penetration of the solvent into the sample, increasing the release of the solutes from the matrix to the solvent (Morelli & Prado, 2012). Rouhani, Alizadeh, Salimi, and Haji-Ghasemi (2009) also reported that ultrasound-assisted extraction was a simpler and more effective alternative to conventional extraction methods for the isolation of curcuminoids from turmeric plant rhizomes, with an extraction yield approximately three times higher than the traditional method.

2. Objectives

This research was aimed to optimize the curcumin recovery from turmeric rhizome by investigating the effects of ethanol concentration and extraction time including using the ultrasonication technique to assist extraction.

3. Materials and method

3.1 Materials

Small, dried pieces of *Curcuma longa* L. rhizomes were obtained from New Concept Product Co.,Ltd, Thailand. Curcumin, demethoxycurcumin and bisdemethoxycurcumin were purchased from Sigma-Aldrich Company. Ethanol as well as other chemicals and reagents were analytical/HPLC grade.

3.2 Preparation of turmeric powder

Dried pieces of turmeric rhizomes were ground in a Waring blender and passed through a number 60^{th} sieve. The fine turmeric powder (8-10% moisture content controlled) was packed in an aluminum foil bag, sealed and kept in a desiccator until used.

3.3 Experimental design

In this study, the effects of extraction parameters (ethanol concentration, extraction time and use of ultrasonication assisted extraction) on

extracting curcuminoids from turmeric powders were determined based on a single factor experiment. After obtaining the optimum conditions for curcumin extraction, the turmeric extract was subjected to component analysis (curcumin, demethoxycurcumin and bisdemethoxycurcumin) by HPLC.

(1) One gram of turmeric powder was accurately weighed into 125 ml Erlenmeyer flask and 30 ml of ethanol at different concentrations (70, 80, 85 and 95%) were added. The mixtures were placed on a shaker at 110 rpm for a fixed extraction time (3 h) at room temperature, and then filtered through Whatman No.4 filter paper. The total curcuminoid content (TCC) in the filtrate was determined using a spectrophotometer following the methods of Martins et al. (2013) with a modification using a standard curve of analytical-grade curcumin. Concentrations of 0, 0.3, 0.4, 0.5, 0.6 and 0.7 µg/ml of curcumin in 80% ethanol were measured at a wavelength of 427 nm by a UV-Vis spectrophotometer (Thermo Spectronic Genesys 20 Spectrophotometer). The TCC was expressed in mg curcumin/g dry weight of turmeric powder. The optimal ethanol concentration was selected based on the TCC in the filtrate.

(2) Turmeric powder was extracted using the optimal ethanol concentration as determined in the first step but varying extraction times as 0.5, 1, 3 and 5 h respectively.

(3) Turmeric powder was extracted using the optimal ethanol concentration as determined in the first step with the exception of using ultrasound-assisted extraction in ultrasonic cleaning bath (SD-D400H, 400 W, 40 KHz) for 10, 15, 30, 45 and 60 min respectively.

(4) Turmeric powder was extracted using the optimal ethanol concentration as determined in the first step but using ultrasound-assisted extraction at 15 and 30 min in combination with 3 h shaking.

3.4 Analysis of curcumin, demethoxycurcumin and bisdemethoxycurcumin in the extracted solvent

The analysis of the 3 main components of curcuminoids in the extracted solvent from optimized conditions obtained from the experimental data were performed using high performance liquid chromatography (Column : EC 150/4.6 NUCLEODUR 100-5 C18ec, 250 L× 4.6 mm, 5 µm). The mobile phase consisted of acetonitrile: 5% acetic acid (50:50). The eluent flowed isocratically at a flow rate of 1 ml/min. The Photo Diode Array detector was adjusted at 427 nm with the injection volume of 20 µl. Standard curcumin, demethoxycurcumin and bisdemethoxycurcumin were dissolved in methanol. The sample solutions were diluted with methanol and filtered through 0.45 µm membrane filter before injection into HPLC.

3.5 Statistical analysis

Experimental results were reported as mean ± standard deviation of triplicate of assays. Statistical analysis was performed using SPSS software (SPSS Ver. 22). A one-way analysis of variance (ANOVA) with a Duncan New Multiple Range Test was used to determine the statistical significance ($p < 0.05$).

4. Results and discussion

4.1 Evaluation of ethanol concentration and extraction time

Ethanol was selected as an extraction solvent in this study because it is safer and less toxic as compared to other organic solvents. The experiment was carried out at room temperature to avoid decomposition of the active compounds. Table 1 shows the effect of ethanol concentration on TCC of turmeric powder extract.

Table 1 Effect of ethanol concentration on total curcuminoid content

Ethanol Concentration (%v/v)	TCC (mg curcumin/g DW)
70	11.1557 ± 0.2983^{c}
80	12.3242 ± 0.0440^{a}
85	11.7104 ± 0.0720^{b}
95	10.3469 ± 0.2914^{d}

[a-d]Mean value ± standard deviation of means with different letters indicate statistical difference ($p<0.05$)

As observed in Table 1, increasing the ethanol concentration up to 80% had significant effects on raising TCC but increasing concentration more than 80% significantly decreased TCC. This result could be due to the similar polarity of 80% ethanol and curcuminoid compounds under the principle of solvent extraction as "like dissolves like". Zhan et al. (2011) also reported that the extraction rate of curcumin increased when the ethanol concentration increased from 40% to 80% but decreased after the ethanol concentration was more than 80%. According to the effects of extraction time as shown in Table 2, the highest TCC was obtained by shaking turmeric powder in 80% ethanol for 3 h. After this point, the TCC decreased

because the prolonged extraction time lead to the breakdown of curcuminoid structures (Sogi, Sharma, Oberoi, & Wani, 2010).

Table 2 Effect of extraction times on total curcuminoid content

Time (h.)	TCC (mg curcumin/g DW)
0.5	12.6713 ± 0.7684^{ab}
1	12.1832 ± 0.0447^{bc}
3	13.0434 ± 0.0972^{a}
5	11.6436 ± 0.5105^{c}

[a-c]Mean value ± standard deviation of means with different letters indicate statistical difference ($p<0.05$)

4.2 Evaluation of ultrasound-assisted extraction

In this study, ultrasound-assisted extraction was investigated to increase the curcuminoid extraction efficiency. The sonication time was varied from 10-60 min and the results (Table 3) indicated that sonication times of 15-30 min produced the highest TCC. After 30 min of sonication, the extraction yield decreased. Wang et al. (1997) found that the ultrasound power is able to degrade compounds for long period of time.

Table 3 Effect of sonication time on total curcuminoid content

Time (min.)	TCC (mg curcumin/g DW)
10	12.1330 ± 0.2314^{b}
15	13.3145 ± 0.2830^{a}
30	13.5064 ± 0.2372^{a}
45	12.1801 ± 0.0428^{b}
60	11.7222 ± 0.1723^{c}

[a-c]Mean value ± standard deviation of means with different letters indicate statistical difference ($p<0.05$)

Therefore, a sonication time of 15 min was chosen as the extraction procedure in combination with shaking at 3 h (The highest TCC was obtained by shaking turmeric powder for 3 h.) to increase the efficiency of curcuminoids extraction shown in Table 4. However, the data indicated that ultrasound-assisted extraction was a simpler and more effective alternative to conventional extraction methods for the isolation of curcuminoids from turmeric plant rhizomes (Rouhani et al., 2009), and that it saves time from extraction. Ultrasound-assisted extraction in combination with shaking had little effect on TCC. Using economical point of view, ultrasound-assisted extraction using 80% ethanol as an extracting solvent for 15 min which giving the TCC content of 13.3145 mg/g (DW) was selected for curcuminoids extraction from turmeric powder. The experiment on curcuminoids extraction by ultrasound-assisted extraction (35 kHz) using 65-96% ethanol of Rouhani et al. (2009) indicated that the highest yield of curcuminoids from turmeric rhizomes was obtained when using 70% ethanol, pH of 3 and extraction time of 15 min. Usually, by varying the solvent polarity from water to ethanol, the extraction efficiency for lipophilic spices increases. At the same time, the product recovery decreases with decreasing water percentage. Another report by Zhan et al. (2011) showed that extraction of curcumin from turmeric powder with 80% ethanol (ratio of powder: solvent = 1:10) by ultrasound-assisted extraction (100 W, 1 h) provided a curcumin content of 12.4400 mg/g. Conversely, using ultrasound assisted-extraction of 3 g turmeric powder in 70% ethanol by an ultrasound sonication probe at 20 kHz, 300 W for 5 min gave the TCC of 2.7650 mg/g (Martin et al., 2013). These data clearly show that the performance of the sonication method can be affected by parameters such as extraction time including type of solvent and solvent concentrations.

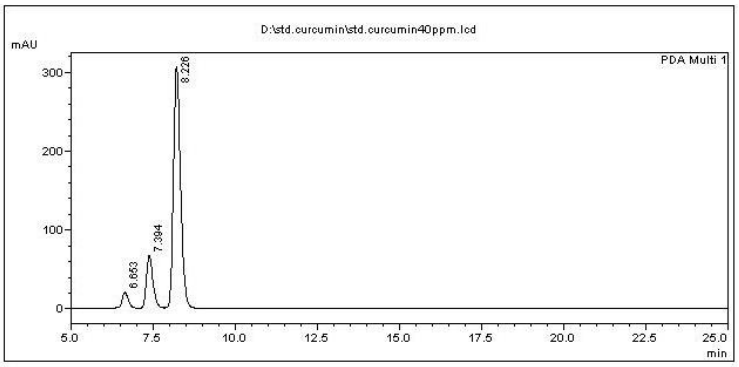
Table 4 Effect of ultrasound-assisted extraction in combination with shaking on total curcuminoid content

Extraction Method and Condition	TCC (mg curcumin/ g DW)
Ultrasonication 15 min	13.3145 ± 0.2830^{a}
Ultrasonication 15 min + shaking 3 h.	10.4438 ± 0.3240^{c}
Ultrasonication 30 min + shaking 3 h.	11.5794 ± 0.2249^{b}
shaking 3 h.+ ultrasonication 30 min	11.3809 ± 0.0432^{b}

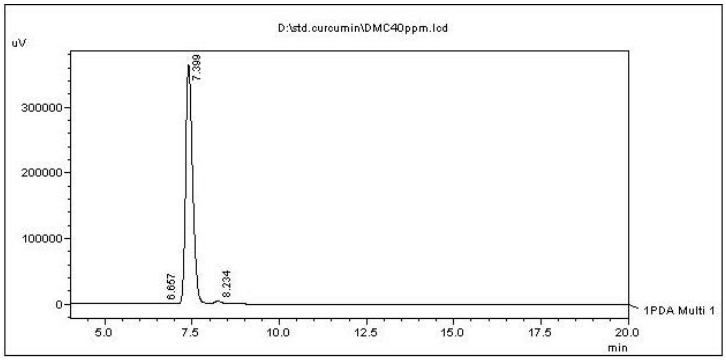
[a-c]Mean value ± standard deviation of means with different letters indicate statistical difference ($p<0.05$)

4.3 HPLC analysis of curcuminoid components

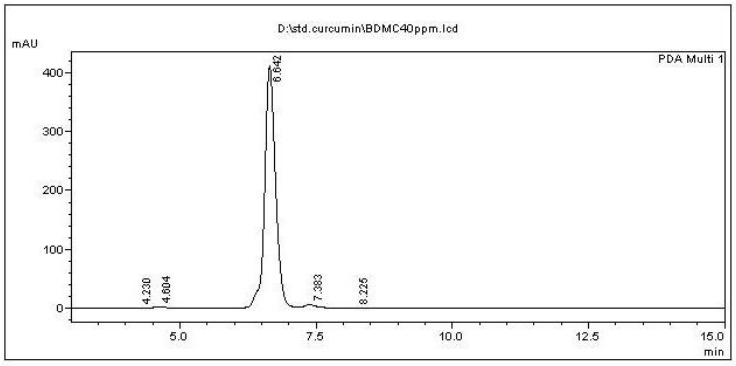
Curcuminoid extract obtained by ultrasound-assisted extraction using 80% ethanol as an extracting solvent for 15 min was analyzed by HPLC. The single peak of standard curcumin, demethoxycurcumin and bisdemethoxycurcumin are shown in Figure 3 with the retention time of 8.2260, 7.3990 and 6.6420 min respectively. The HPLC profiles of extracted curcuminoids using ultrasound-assisted extraction for 15 min are shown in Figure 4.



(a)



(b)



(c)

Figure 3 Chromatography profile of standard curcumin (a), demethoxycurcumin (b) and bisdemethoxycurcumin (c)

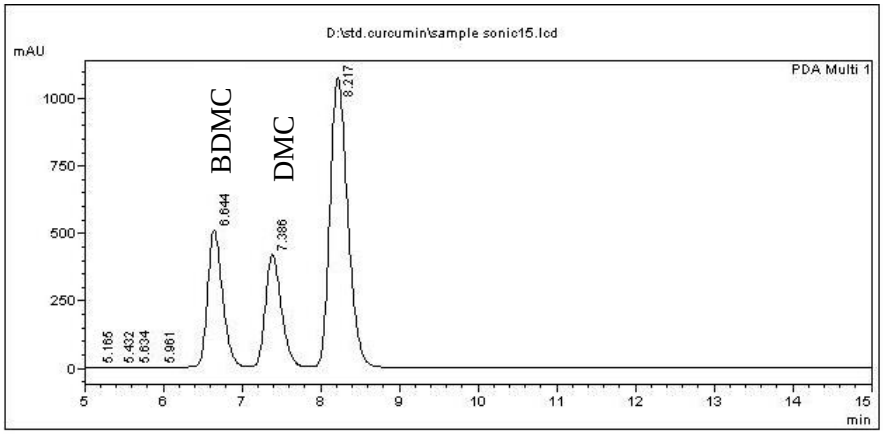


Figure 4 Chromatography profile of curcumin (C), demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC) in extracted solvent (ultrasound-assisted 80% ethanol extraction)

According to the chromatogram, curcumin was found to be the major compound followed by demethoxycurcumin and bisdemethoxycurcumin (Table 5). The study of curcuminoid content in different turmeric varieties of India extracted by ethanol of Taylor and McDowell (1992) indicated that curcumin, demethoxycurcumin and bisdemethoxycurcumin varied from 8.4-31.6 mg/g (DW), 4.8-17.5 mg/g (DW) and 2.2-8.9 mg/g (DW) respectively. The variation of curcuminoid content also depends on different extraction methods (Li et al., 2011).

Table 5 Curcumin, demethoxycurcumin and bisdemethoxycurcumin content in extracted solvent (ultrasound-assisted 80% ethanol extraction) analyzed by HPLC

Compounds	Content (mg/g DW)
Curcumin	47.8762
Demethoxycurcumin	40.2941
Bisdemethoxycurcumin	15.2412

5. Conclusion

The present study indicated that ultrasound-assisted extraction of curcuminoids from turmeric powder using 80% ethanol as an extraction solvent for 15 min could be used as an effective alternative for conventional solid liquid extraction. Extraction yields of curcuminoids were affected by ethanol concentration and extraction time. The curcuminoid components of the extract were mainly composed of curcumin. Bisdemethoxycurcumin content was slightly lower than curcumin and demethoxycurcumin was found in the lowest concentration of the extract.

6. Acknowledgements

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