Ultrasonic Assisted Extraction of Natural Pigments from Rhizomes of Curcuma Longa L.

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ABSTRACT

Ultrasound-assisted extraction (UAE) was evaluated as a simpler and more effective alternative to conventional extraction methods for the isolation of curcuminoids from turmeric plant rhizomes. The turmeric samples were extracted under indirect sonication in an ultrasonic bath, and compared with the conventional methods. It was found that the yield of ultrasound-assisted extraction was approximately three times higher than the traditional method. Taguchi experimental design was employed to study the effect of pH, solvent composition and extraction time on the yield of extracted curcuminoids from turmeric. The optimum combination was determined as a solvent composition ethanol/water 70:30 (V: V), pH of 3 and an ultrasound-assisted extraction time of 15 min. The extracts were analyzed using high performance liquid chromatography (HPLC).

1. Introduction

Turmeric (Curcuma Longa L.) is a natural coloring agent, and it has been found to be a rich source of phenolic compounds, namely, curcuminoids [1], curcumin (C), demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC) (Figure 1). It is widely used for the coloring of food and textile. Curcuminoids are known to have broad spectrum of biological activities and safety in foods or pharmaceuticals. Curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] is known for its antioxidant [2], anti-inflammatory, anti-parasitic, anti-allergic, anti-microbial, anti-mutagenic and anticancer properties [3]. The potential use of curcumin in the prevention of cancer and treatment of infection with human immunodeficiency virus (HIV) is attracted more attention [3]. Demethoxycurcumin and bisdemethoxy-curcumin showed strong antioxidant activity as efficient as curcumin [4].

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Isolation of organic compounds from plant extracts is of great importance; mainly because they can be used as an excellent source of phytotherapics. A variety of methods for extraction of plant material have been reported [5]. The traditional solvent extraction technique for plants materials are mostly based on solvent type. These techniques require long extraction times and have low efficiency. Moreover, many natural products are thermally unstable and may be degrading during thermal extraction [6,7].

In recent years, ultrasonic-assisted extraction (UAE) as a novel technique for extraction of plant tissues has gained increasing attention [8-10]. UAE have been found to enhanced extraction efficiency and extraction time. In addition, UAE can be carried out at lower temperatures which avoid thermal damage. It has been suggested that improvement of solvent extraction from material by ultrasound is due to mainly the mechanical effects of acoustic cavitations, which enhances mass transfer and solvent penetration into the plant material by disrupting the cell walls [11,12]. In order to achieve high extraction efficiency for a specific plant matrix, the optimization of ultrasound operational parameters (solvent, polarity, time, pH, etc.) is most important.

Even though the effect of ultrasound have been studied for over hundred of herbal species, to the authors’ knowledge, its effect on the UAE of this important plant, turmeric, is rare (if any) in scientific literature and also no study has been reported on optimization of UAE of curcumin.

In this paper, ultrasound effects on curcuminoids extraction from turmeric rhizomes are compared with the conventional methods. Influence of experimental parameters such as pH, time and solvent on pigment extraction from *Curcuma longa* was evaluated by Taguchi experimental design [13] in order to find an optimal use of ultrasound technique for curcuminoids extraction. The extracts were analyzed using HPLC.

2. Experimental

2.1. Plant materials

The commercial turmeric roots (from local market) were washed, dried and powdered to an average size of 20 µm before experiment and stored in dark sealed glass bottles to protect from humidity and light.
2.2. Chemicals

For chromatographic analysis, HPLC-grade methanol, supplied by Merck and ultra pure water of 18 MΩ/cm resistively, purified with a Milli-Q system (Milipore, Bedford, MA) were used. Mixture of H₃PO₄ / KH₂PO₄ was used for preparation of pH standard buffer solution and all other reagents of analytical grade were obtained from Merck. Curcumin analytical grade was purchased from Riedel. For HPLC analysis, a curcumin stock solution was prepared by dissolving 25 mg curcumin in 25 ml methanol.

2.3. Conventional methods

2.3.1. Solvent extraction

Curcuminoids were extracted sequentially as described in [14]. One gram of turmeric powder (20µm size) was soaked in 10 ml hexane overnight and the extract was filtered. The residue was extracted with 4×5 ml hexane for 15 minutes (total volume was 20 ml) and the filtrates were collected. Similarly the residue was extracted with 70% ethanol. The recovery of total curcuminoids was determined by UV-Vis spectrophotometer. The ethanol was evaporated to dryness and the extract stored for injection to HPLC. The experiment was performed in triplicate. The solvent extraction yield was about 4.43%.

2.3.2. Maceration

The dried powder (3 g) was extracted with 70% ethanol (30ml) on a shaker with 210 rpm at room temperature for 2 days. The extract was filtered through Whatman no. 1 filter paper. Other portions of the solvent were added to the solids and the extraction was repeated until the extractant was colorless. The extracts were combined and filtered. The filtrates were concentrated under reduced pressure at 50 °C using a rotary evaporator. The crude extract was then heated on a boiling water bath until constant weight was obtained and stored for injection to HPLC. The experiment was repeated three times. The total curcuminoids yield was 12.39%.

2.3.3. Soxhlet extraction

The extract was prepared by extracting 3 g of the turmeric powder with 70% ethanol (30 ml) using a soxhlet apparatus until the extractant was colorless. The extract was filtered and the filtrate was concentrated and evaporated under the same condition as described before and stored for injection to HPLC. The experiment was performed in triplicate. The total yield was 11.65%.

2.4. Sonication extraction

An ultrasonic cleaning bath (Sonorex, 35 KHz) and a double jacket cell were used for extraction procedure. The cell was kept at constant temperature (25°C) by circulating water from a controllable thermostat bath through the jacket, to avoid rising temperature caused by ultrasonic exposure. The following samples were filtered, washed with 15 ml solvent, evaporated to dryness using a rotary evaporator and diluted for injection to HPLC. In order to obtain total curcumurinoids, after certain dilution, the optical density of the samples at 420 nm was measured by using a Shimadzu Multi spec-1501 photo diode array spectrophotometer.

2.4.1. The effect of solvent composition on the ultrasound-assisted extraction of curcuminoids

In order to study the solvent effect on the ultrasound—assisted extraction of curcuminoids and determine the optimization levels of solvent compositions, 0.1 g of powdered turmeric were extracted in an ultrasonic bath, with 10 ml solvent with different compositions (70-96% Vol / Vol % ethanol), for 10 minute at pH 3. The results are shown in Figure 3.

2.4.2. The effect of ultrasound-assisted extraction time on the extraction of curcuminoids

In order to study the ultrasound effect comparing to the conventional method and determine the optimum levels of ultrasound-assisted extraction time, an accurately weighted amount of powdered turmeric (0.1g) were extracted in a ultrasonic bath, with 10 ml of 70% ethanol for 5 to 60 minutes at pH 3. Figure 4 shows the effect of time on extraction of curcuminoids.

2.5. HPLC analysis of curcuminoids

The HPLC system consisted of Waters liquid chromatograph (Milford, MA) equipped with a 600E multi solvent delivery system, an in-line degasser, a manual injector with 5 µl loop (Rheodyne 7125), and Waters 2487 dual wavelength absorbance detector. Millennium 32 software was used for controlling the analytical system and for data processing. Separations were carried out on a Novapak® C₁₈, 60 Å (Dublin, Ireland), 4 mm, 150×3.9 mm i.d. and analytical column
fitted with a Novapak® C<sub>18</sub>, 60 Å, 5 mm, 20×3.9 mm i.d. guard column. The HPLC conditions were based on ref. [15] which gave satisfactory resolution of the three curcuminoids CM, DMCM and BDMCM (Figure 2). The elution was carried out with gradient solvent systems with a flow rate of 1.0 ml/min at an ambient temperature. The mobile phase consisted of methanol (I), 2% acetic acid/water (II), and acetonitrile (III). Quantitative levels of curcuminoids were determined using the above solvents programmed linearly from 45 to 65% acetonitrile in (II) for 0-15 min. then the gradient changed back from 65 to 45% acetonitrile in (II) for 15-20 min with a constant amount of 5% (I). The calibration curves and the qualitative evaluations were carried out at 420 nm. Quantification was performed by comparing chromatographic peak area with those of external standard at the concentration range of 5-100 mg/ml (average of three runs for standard and samples).

2.6. Taguchi experimental design

It has been recognized that ultrasound power has great potential for applications in a wide variety of industrial processes as it offers potential cost saving in time, chemicals, energy and reduces effluent [16,17]. In this context, applying power of ultrasound in plant extracts interest and optimization of ultrasound operational parameters is of main importance in order to achieve high extraction efficiency. Taguchi-based optimization technique is a unique and powerful optimization discipline that allows optimization with minimum number of experiments [13]. Thus by this method, it is possible to reduce the time and cost of experimental investigations and improve the performance characteristics.

Figure 2: HPLC chromatograms of bisdemethoxycurcumin (1), demethoxycurcumin (2), and curcumin (3).
Figure 3: Effect of ethanol composition on the UAE. Extraction condition: 0.1 g sample in 10 ml solvent; extraction time, 10 min.

Figure 4: Effect of time on the UAE. Extraction condition: 0.1 g sample in 10 ml; solvent composition: 70% ethanol.
Orthogonal array, \( L_9 \), which denotes three parameters each with three levels, was chosen. Each experiment was repeated three times under the same conditions at different times to observe the effects of noise sources in the extraction process. All the results at each step of the design are expressed as the mean of three experiments. Mean value of these replications is the response of this treatment. Table 1 represents the selected orthogonal array for this study [13]. A, B and C are effective factors and E is for determination of error. Factors and their levels used in the present study are illustrated in Table 2. The yield of extraction was considered as Taguchi array response, obtained from HPLC analysis. The importance of each factor is studied by signal to noise (S/N) ratio considering both mean and variance at the same time. After conducting the experiments, the results were converted into signal-to-noise (S/N) ratio data.

The S/N ratio analysis was performed by computing the signal-to-noise ratio for each level of process parameters. Usually, there are three categories of performance characteristics in the analysis of the S/N ratio: that is, the lower, the better; the higher, the better; and the nominal, the better. For a typical experiment, a larger S/N ratio corresponds to better quality characteristics, and the optimal level of the process parameters is the level with the greatest S/N ratio.

### Table 1: Orthogonal array \( L_9 \) of Taguchi.

<table>
<thead>
<tr>
<th>Run</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
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<tr>
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</tr>
<tr>
<td>4</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
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<td>3</td>
<td>1</td>
<td>2</td>
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<td>7</td>
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<td>3</td>
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<td>3</td>
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<tr>
<td>9</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

This is the foundation for the determination of the optimum level for each factor. Since the current study takes the percentage extraction of curcumin (w/w) as the quality characteristics, the higher-the-better criterion was applied when evaluating the S/N ratios of the various extraction parameters. The larger S/N ratio reflects the more relative effect of this factor and given by:

\[
\frac{S}{N} = -\log\left\{\frac{1}{n}\sum_{i=1}^{n}1/y_i^2\right\} \quad (1)
\]

\[
\Delta = (S/N)_{\text{max}} - (S/N)_{\text{min}} \quad (2)
\]

Where \( y \) is response, \( n \) and \( \Delta \) are number of experiments and the difference of maximum and minimum of signal to noise ratio, respectively. Then, results were subject to the analysis of variance.

3. Results and discussion

3.1. Sonication extraction

3.1.1. The effect of solvent composition on extraction of cucuminoids

Since ethanol is extensively used in the extraction of plant materials [5], non toxic and economic, the different mixtures of ethanol-water were considered as solvent for studying the solvent composition effect. As can be seen in Figure 3, the extraction efficiency increases with the ethanol percentage as expected. It is obvious that the extraction efficiency increases with ethanol concentration up to 70%, after which it starts to decrease, which is probably due to the effect of solvent polarity and ultrasonic cavitations properties.

The observed enhancement of extraction of organic compounds by ultrasound is due to phenomenon of cavitations produced in the solvent by the passage of an
ultrasonic wave [7]. As ultrasound passes through a liquid, the expansion cycles exert negative pressure on the liquid and create cavities or micro-bubbles in the liquid. This occurs when the negative pressure exceeds the local tensile strength of the liquid, which varies with the surface tension, vapor pressure and viscosity of the liquid. These bubbles will absorb the energy from the sound waves and grow during the expansion cycles and recompress during the compression cycle. The increase in pressure and temperature caused by the compression leads to the collapse of the bubbles, which causes shock wave that passes through the solvent, enhancing the mass transfer within the plant materials.

The intensity of ultrasonic cavitations in the solvent mixture was affected by the surface tension, viscosity and medium vapor pressure [7]. Ultrasonication in low vapor pressure liquid produces few cavitation bubbles. High vapor pressure liquid is not very effective, more bubbles are created, but they collapse with less intensity due to a smaller internal/external pressure differential. As the pressure of liquid viscosity, acoustic cavitations occurs more easily in the liquid with low viscosity because the ultrasonic intensity applied could more easily exceed the molecular forces of the liquid. Furthermore, the liquid with low viscosity has low density and high diffusivity, and can easily diffuse into the pores of the plant materials [9-12]. Surface tension of liquid also influences cavitation effects. Liquid having small surface tension requires lower energy to produce cavitations bubbles. The values of viscosity, surface tension and vapor pressure for used solvents are: 0.89 cP, 72.8 mN/cm and 23.8 mmHg for water and 1.2 cP, 23.7 mN/cm and 59.02 mmHg for ethanol respectively. As can be seen, viscosity and vapor pressure of ethanol is higher than water but surface tension of water is larger. Thus, for a water-ethanol mixed solvent under ultrasonic irradiation a nonlinear behavior with increasing the percent of pure solvent concentrations is not surprising.

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. This was probably due to the relative polarity and the decrease in effective swelling of plant materials. Furthermore, the presence of water lowers the mixture viscosity, thus mass transfer improves. At higher water contents, the product recovery decreases due to the increase of the mixture polarity which is not favorable for extraction of lipophilic curcuminoids.

It seems that extraction efficiency in water-ethanol mixed solvent is affected by several phenomena. The results are reported in [18].

3.1.2. The effect of ultrasound-assisted extraction time on the extraction of curcuminoids

The effect of extraction time is shown in Figure 4. As expected, the curcuminoids extraction was increased by increasing the extraction time. When the extraction time is longer than 15 min, the extraction yield decreases with increasing the time. It was found that the ultrasound power is able to degrade compounds for long period of time. This effect is related to ultrasound power, stability of compounds and the medium [19].

3.1.3. The effect of pH

It has been found that curcuminoids decompose rapidly at neutral-basic pH conditions [19-20]. Degradation kinetics of curcumin under various pH conditions and the stability of curcumin in various buffers have been reported [19]. Study on a series of pH conditions ranging from 3 to 10 have shown that decomposition is pH-dependent and occurred faster at neutral-basic pH conditions. Degradation rate constants in 0.1 buffer solution of phosphate and phosphate-citrate at pH 3.6, 7.2 and 8 were 5.842, 3.541, 73.75 and 656.65 respectively [19]. About 90% of Curcumin decomposes in 0.1 phosphate buffer medium, (pH 7.2 at 37 °C) within 30 min. High stability of curcuminoids in acidic pH may be contributed to the conjugated diene structure. It was found that the stability of curcuminoids was strongly improved by lowering the pH. Various investigations have shown that the pH range for UAE optimization is set between 3-6 [19, 20].

3.1.4. Optimization of the sonication condition

The collected data of L₉ orthogonal array were analyzed by WinRobust software (version 1.01) to evaluate the effect of each parameter on the optimization criteria. Table 3 shows the results of calculated S/N data for the extraction based on L₉ matrix design. To use the S/N ratio for the optimal extraction performances, S/N calculation was performed to maximize extraction of curcuminoids.
Table 3: Curcuminoid recoveries at different conditions from the L₉ design using ultrasonic irradiation.

<table>
<thead>
<tr>
<th>Method</th>
<th>Condition</th>
<th>Yield of extraction(%) w/w (total curcumnioids) in 0.1 gr. solid sample</th>
<th>Curcuminoid content (%w/w in each run)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taguchi</td>
<td>P pH</td>
<td>S %Ethanol T Time</td>
<td>C DMC BDMC</td>
</tr>
<tr>
<td>1</td>
<td>3 70</td>
<td>5</td>
<td>15.00 10.23 2.92 1.86</td>
</tr>
<tr>
<td>2</td>
<td>3 80</td>
<td>10</td>
<td>16.05 10.65 3.24 2.16</td>
</tr>
<tr>
<td>3</td>
<td>3 95</td>
<td>15</td>
<td>14.73 9.85 2.92 1.92</td>
</tr>
<tr>
<td>4</td>
<td>4 70</td>
<td>10</td>
<td>14.65 9.80 2.84 2.02</td>
</tr>
<tr>
<td>5</td>
<td>4 80</td>
<td>15</td>
<td>15.98 10.68 3.12 2.18</td>
</tr>
<tr>
<td>6</td>
<td>4 95</td>
<td>5</td>
<td>12.90 8.54 2.54 1.82</td>
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<tr>
<td>7</td>
<td>6 70</td>
<td>15</td>
<td>18.34 12.40 3.57 2.37</td>
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<tr>
<td>8</td>
<td>6 80</td>
<td>5</td>
<td>13.65 9.08 2.66 1.91</td>
</tr>
<tr>
<td>9</td>
<td>6 95</td>
<td>10</td>
<td>12.18 8.11 2.36 1.71</td>
</tr>
</tbody>
</table>

Table 4: Analysis of variance (ANOVA) for the UAE of curcuminoids.

<table>
<thead>
<tr>
<th>Variables</th>
<th>DF a</th>
<th>SS b</th>
<th>Variance</th>
<th>F c</th>
<th>P % d</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>2</td>
<td>4.88</td>
<td>2.44</td>
<td>5.3</td>
<td>51.15</td>
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<td>% ethanol</td>
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<td>3</td>
<td>1.5</td>
<td>3.3</td>
<td>31.45</td>
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<tr>
<td>Time</td>
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<td>0.74</td>
<td>0.37</td>
<td>0.8</td>
<td>0.08</td>
</tr>
<tr>
<td>Error</td>
<td>2</td>
<td>0.92</td>
<td>0.46</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>10.51</td>
<td>-</td>
<td>-</td>
<td>100</td>
</tr>
</tbody>
</table>

a Degree of freedom  
b Sum of squares  
c F-test for 95% confidence level  
d Percentage of contribution.

The results of variance analysis are given in Table 4. Statistical analysis of variance (ANOVA) was performed to see whether the process parameters are statistically significant. The F-value for each parameter indicates which parameter has a significant effect on the extraction and is simply a ratio of the squared deviation to the mean of the squared error. Usually, the larger F-value shows greater effect on the extraction value due to the change of the process parameter. Optimal combination of the process parameters can be predicted using ANOVA analysis and performance characteristics.

F-value for this condition with 95% confidence is 4.28 [13]. As seen in Table 4, only the pH values have meaningful effect on the extraction of curcumine within the working range. As mentioned previously, curcuminoïdes decompose rapidly at neutral-basic pH conditions. Therefore, to obtain higher extraction yield, it is important to extract them in acidic solution. It was also seen that solvent composition has no significant effect on extraction of curcuminoïds but has more meaningful effect than time of extraction.
The degrees of the influences of parameters on the performance characteristics are given at the graphs in Figure 5. The optimal level of a process parameter is the level with the highest S/N. To deduce the experimental conditions for the data given in this figure, consider Figure 5 with parameter t (reaction time). Now, let us try to determine the experimental conditions for the first data point. The t value for the point in level 1 is 5 min. Table 1 shows the experiments which t level (column t) is 1. Table 3 shows that for experiments 1, 6, 13 and 8 the column t is 1. The experimental conditions for the second data point, are those for which its column level is 2 (i.e. experiments nos. 2, 4 and 9), and so on. The numerical value of the maximum point in each graph marks the best value of that particular parameter.

As it is expected, pH in level 1 (pH 3) has more effect than other levels. The mean effect of S/N for solvent composition decreases from level 1 to level 3. It seems that the optimum level for solvent composition is 70% of ethanol. Correspondingly, the optimum level for time is level 3 (15 min). Therefore, the optimum condition is P1, S1, and T3. Table 4 gives the results of ANOVA (Analysis of Variance). The F-value gives the values of F-test statistic, and P-value reflects the level of significant of each factor. It is obvious that the most effective factor is pH.

Once the optimal level of the design parameters has been selected, the final step is to predict and verify the improvement of the quality characteristic using the optimal level of design parameters. The predicted S/N ratio using the optimal level of the design parameters can be calculated as

$$[S/N]_{predicted} = [S/N]_m + \sum_{i=1}^{n} ([S/N]_i - [S/N]_m)$$

(3)

where $[S/N]_m$ is the total mean S/N ratio, $[S/N]_i$ is the mean S/N ratio at the optimal level, and the number of the main design parameters that affect the quality characteristic. To test the predicted results, confirmation experiments were performed twice at the same working conditions. Predicted S/N ratio calculated from Win Robust software is 14.93 ppm, and experimental recovery in optimum condition was 14.90 ppm. There is a good agreement between the predicted and experimental recovery. Consequently, recovery of curcuminoids in UAE can be improved through the Taguchi method approach. The results showed that maximum extraction, occurred in the conditions: pH (3), Solvent (70% ethanol), time of extraction (15 min).
### Table 5: Yield of curcuminoids extraction using different methods.

<table>
<thead>
<tr>
<th>Method</th>
<th>Condition</th>
<th>Temperature (ºC)</th>
<th>Yield of extraction (% w/w) (total curcuminoids in 0.1 gr. solid sample)</th>
<th>Curcuminoid content (% w/w in each method)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent extraction</td>
<td>Ethanol 70%</td>
<td>R</td>
<td>4.43</td>
<td>2.78 0.85 0.73</td>
</tr>
<tr>
<td>Soxhlet extraction</td>
<td>Ethanol 70%</td>
<td>80</td>
<td>11.65</td>
<td>6.64 1.43 3.58</td>
</tr>
<tr>
<td>Maceration</td>
<td>Ethanol 70%</td>
<td>R</td>
<td>12.39</td>
<td>6.70 1.15 4.53</td>
</tr>
<tr>
<td>Sonication extraction</td>
<td>Ethanol 70%</td>
<td>R</td>
<td>18.34</td>
<td>12.40 3.57 2.37</td>
</tr>
</tbody>
</table>

R: Room temperature (25ºC)  
DMC: Demethoxycurcumin  
BDMC: Bisdemethoxycurcumin

#### 3.2. Comparison of UAE with other conventional techniques

Comparison with the three conventional extraction techniques reveals that UAE can reach much higher yield within 15 min (Table 5). It showed that the major component of all extraction methods was curcumin and the ultrasonic extraction gave the highest yield of curcumin (12.40 %, w/w), demethoxycurcumin (3.57 %, w/w), bisdemethoxycurcumin (2.37 %, w/w) and total curcuminoids (18.34 %, w/w). Compared to other methods, ultrasonic extraction was simple, low cost in terms of solvent used and less time consumed. This method promotes better penetration of solvent into plant particles and uses low extraction temperature which affects the stability of active components.

#### 4. Conclusions

The utilization of ultrasound has proven to be a much simpler, faster and more effective method than the conventional extraction methods to extract organic compounds from plant matrix. It was shown that the ultrasound-assisted extraction of curcuminoids has about three time higher yield than the traditional extraction methods. The performance of the sonication method can be affected by parameters such as solvent composition, extraction time and pH. The Taguchi design has been successfully used to test the relative importance of medium components and environmental factors on extraction efficiency. The optimization of the variables in the sonication method shows that the optimized sonication condition was determined to be: pH 3, 70% ethanol: water mixture and 15 minute extraction. This method should be considered as an efficient method for extracting higher quality and quantity of curcuminoids from *Curcuma longa L.* rhizomes for pharmaceutical applications.
5. References


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