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Formic Acid and Microwave Assisted Extraction of Curcumin from Turmeric (*Curcuma longa* L.)

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ABSTRACT

Curcumin is a natural bioactive compound originated from the rhizomes of turmeric (*Curcuma longa* L.). This study was performed to investigate formic acid and microwave assisted extraction of curcumin from turmeric (*Curcuma longa* L.). In order to enhance the curcumin extraction, different parameters such as particle size, effect of pretreatment with water, radiation intensity and type of solvent were investigated. For analysis of curcumin content, two methods were developed. Spectrophotometric methods at the stage of optimization and high performance liquid chromatography (HPLC) for determination of the purity of curcumin were used. At particle size of 0.21mm and input power of 100W using acetone as organic solvent, the highest curcumin extraction yields were achieved. The results showed that water is a suitable modifier for the pretreatment of turmeric with microwave irradiation. For purification of curcumin in HPLC analysis, methanol and water were used as co-solvents. Maximum obtained curcumin purity was 82.4%.

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1. INTRODUCTION

The rhizomes of turmeric (*Curcuma longa* L.) contains an active organic compound known as curcumin which is used as flavor and food additive [1]. The chemical structure of curcumin was introduced in early 1910s by the German scientists Milobedzka and Lampe [2]. Curcumin as a natural polyphenolic compound has number of special features and pharmacological properties such as antioxidant, anti-flammatory, anti-carcinogenic, anti-parasitic, anti-mutagenic and anti-microbial characteristics [3-5].

Generally extraction of curcumin from turmeric has been carried out using different methods. Theses extraction techniques include soxhlet extraction, maceration, supercritical carbon dioxide extraction, digestion, ultra-sonic assisted extraction, pressurized hot water extraction, enzyme-assisted extraction, conventional solvent extraction and hot and cold percolation [6-12]. Extraction techniques have been enhanced with the aid of microwave as thermal pretreatment method.

Microwave-assisted extraction process is a suitable technique, having several advantages such as short extraction time, high extraction efficiency and low-energy requirement [13]. In microwave heating, there is a direct relation between heating mechanism and dielectric properties of the solvent [8]. When the dielectric constant of a solvent increases, the absorption of microwave energy also increases. Table 1 summarizes dielectric constants for several common solvents [14].

Application of microwave for extraction of active biological compounds such as phenolic compounds from rice grains [15], green tea polyphenols [16]

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withanolides from leaves of ashwagandha [17], solanesol from tobacco leaves [18], artemisinin from *Artemisia annua* L. [19], paclitaxel from *Taxus baccata* L. [20], tanshinones from *Salvia* miltiorrhiza Bunge [21] andginsenosides from ginseng root [22] has been reported in the literature. In addition, optimization of microwave conditions were investigated; in contrary the relation between mesh size, moisture, the dielectric constant of solvent on extracted curcumin and formic acid-assisted extraction never been mentioned.

The goal of present work was to study the effect of parameters such as mesh size, necessary microwave power to destruct cell wall, pretreatment of wet turmeric by microwave irradiation and suitable organic solvent for extraction of curcumin with the aid of microwave. A novel extraction method for the extraction of curcumin by organic acid was developed. In fact, polyphenolic compounds are more soluble in carboxylic acids such as acetic and formic acids; however, the high boiling points of these acids required long duration of heat exposure for the recovery of solvents and may cause curcumin to be denatured. For solving such problem, the solute was neutralized and curcumin formed solid phase in carboxylic acid. Finally, the slurry was precipitated and curcumin in solid phase was filtered.

2. MATERIAL AND METHODS

2. 1. Material The rhizomes of turmeric were supplied from a local market (Amol, Iran). The rhizome of turmeric was milled; then, turmeric powder was sieved using different mesh sizes from 10-80. The obtained particle sizes are stated in Table 2. The polyphenolic chemical formulation and structure of curcumin is illustrated in Figure 1.

Analytical grade organic solvents such as acetone, methanol, acetic acid, ethanol, curcumin with purity of 99% and sodium hydroxide were purchased from Merck (Darmstadt, Germany). Formic acid with purity of 98% was supplied by Riedel-deHaen (Seelze, Germany).

2. 2. Method

2. 2. 1. The Effect of Moisture on the Extraction of Curcumin 0.2g turmeric with different mesh sizes of 10, 30, 45, 70 and 80 was dried in an oven at 70°C till constant weight was achieved. The dried sample, 2.9 mg of turmeric powder, was weighted and dissolved in 10 ml methanol; in order to remove suspended particles the solution was filtered. The clear solution was analyzed for absorbance by spectrophotometer (JENWAY 6320D, UK) at wavelength of 420 nm. The analysis of wet and dry samples for curcumin contents was conducted.

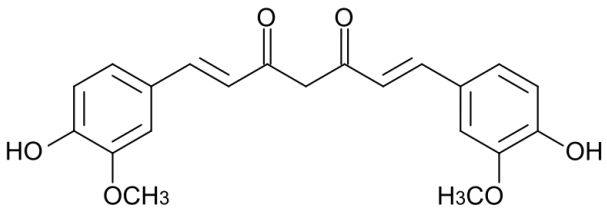

Figure 1. The chemical structure of curcumin

TABLE 1. Dielectric constants for solvents usually used in microwave-assisted extraction [14]

Solvent	Dielectric constant
Acetone	20.7
Methanol	32.6
Ethanol	24.3
Ethyl acetate	6.02
2-Propanol	19.9
Acetonitrile	37.5
Hexane	1.89
Water	78.3

TABLE 2. The particle size of turmeric in different mesh sizes

Particle size (mesh)	(mm)
10	2.00
30	0.595
45	0.354
70	0.210
80	0.177

2. 2. 2. Pretreatment of Turmeric with Microwave using Soaked and Dried Samples

2. 2. 2. 1. Effect of Soaking 0.2 g turmeric sample with different mesh sizes of 10, 30, 45, 70 and 80 was used for pretreatment. A commercial microwave (Model: SAMSUNG, Max power: 900W) was used for the extraction purpose. The samples were mixed with 0.5ml of water and treated under microwave irradiation in an intermittent way, i.e. irradiation-cooling-irradiation and power input of 100W. The irradiation time was kept for 2 min and then cooled off for 2 min. It was taken to cool the sample between irradiations. After irradiation, samples were dried for the removal of water. In another experiment, 0.2 g turmeric with different mesh sizes of 10, 30, 45, 70 and 80 without primary soaking was irradiated at the same condition. After irradiation, samples were analyzed for curcumin content

by spectrophotometry method. A concentration of 290mg/l of turmeric in methanol was prepared and its curcumin content was analyzed using spectrophotometric method at wavelength of 420 nm (the same as previous section).

2. 2. 2. 2. Optimization of Power of Microwave 0.2 g turmeric with mesh size of 70 was mixed with 0.5ml of water. The sample was treated under microwave irradiation in an intermittent way, i.e. irradiation-cooling-irradiation and power input 100, 300 and 900W with fixed irradiation and cooling time of 2 min. After irradiation, samples were dried at 70° C and analyzed for curcumin content by spectrophotometric method at a concentration of 290mg/l of turmeric in methanol.

2. 2. 3. Extraction with the Aid of Microwave

2. 2. 3. 1. Appropriate Solvent for Extraction of Curcumin 0.5 g of the pretreated powder at optimized condition was suspended in 30ml of different solvents such as ethanol, acetone, methanol, acetic acid and water. The suspension was radiated in microwave at power input of 100 W with exposure duration of 2min. The samples were then filtered and analyzed for their curcumin content.

2. 2. 3. 2. Optimization of Power 0.2 g of the pretreated powder was suspended in 30ml of acetone. The suspension was radiated in an intermittent way, i.e. irradiation-cooling-irradiation at different input powers of 100, 180 and 300W. The irradiation exposure time was fixed for 2 min.

2. 2. 3. 4. Purification of Sample A 0.13 g of acetone extracted sample was dissolved in 20 ml of methanol and then 30 ml of water as co-solvent was added. The solution was cooled in refrigerator at 4°C overnight. After crystallization, sample was filtered for analysis of curcumin using HPLC method.

2. 2. 4. Formic Acid-assisted Extraction A 4 g of irradiated powder was dissolved in 40ml of formic acid. The suspension was filtered and the filtrate was neutralized with 1.2 N sodium hydroxide solutions. After neutralization, the precipitated curcumin was collected. For the removal of undesired residues, the precipitate was washed with methanol. The solution was again filtered and then the solvent was vaporized.

2. 2. 5. HPLC Analysis of Curcumin Purified samples were analyzed by HPLC using UV detector. The dissolved samples in methanol were injected at the flow rate of 1.0 ml/min at ambient temperature. The HPLC column for curcumin analysis was C18 (250×4.6mm), the mobile phase was acetonitrile-water (90 and 10%) and detector measured at wavelength of 420nm. Curcumin purity was calculated by Equation (1):

$$\text{Curcumin purity (\%)}=\frac{\text{curcumin content (g)}\times 100}{\text{extracted content (g)}} \quad (1)$$

2. 2. 6. FTIR Characterization A Fourier transform infrared (FTIR) spectrometer (WQF-510A, China) was used to obtain the FTIR spectra of curcumin; the detection wavelength was in the range of 400–4000 cm^{-1}.

3. RESULTS AND DISCUSSION

Figure 2 depicts the extracted curcumin concentration with respect to particle size. In this figure, concentrations of curcumin extracted from four different samples of turmeric including soaked, dried, with and without moisture were compared. As the particle size decreased from 2 to 0.21mm, the curcumin concentration increased. In contrary,when the particle size decreased from 0.21 to 0.177mm, the curcumin content decreased.

That was probably due to destruction of curcumin molecule in very fine size under heat and irradiation. Therefore, decreasing particle size to 0.21 mm was in favor of extraction process while particle size smaller than 0.21 mm may cause limitation. In fact, curcumin is a thermo sensitive molecule. For long duration of microwave irradiation more heat is generated. The main reason for reduced curcumin concentration extracted from very small particle size is the generated heat can cause curcumin degradation.

We applied microwave technique as part of pretreatment of turmeric which has distractive impact on turmeric plant cell wall. The sample soaked in water and irradiated under microwave had higher concentration of curcumin compared to dried irradiated sample. This concept was also pointed out by Wakte while who has extracted curcumin from turmeric with the microwave-assisted extraction [10].

This phenomenon is probably due to presence of water molecules which destroy the plant cell wall of turmeric. Besides that cellulosic materials deform due to high dielectric constant of water. Effect of moisture content on curcumin extraction is also shown in Figure 2. As it is illustrated, the moisture has a negative impact on curcumin extraction. Removal of moisture from turmeric increased curcumin content compared to sample with moisture. The presence of moisture may cause to decrease solubilizing capacity of solvent [7].

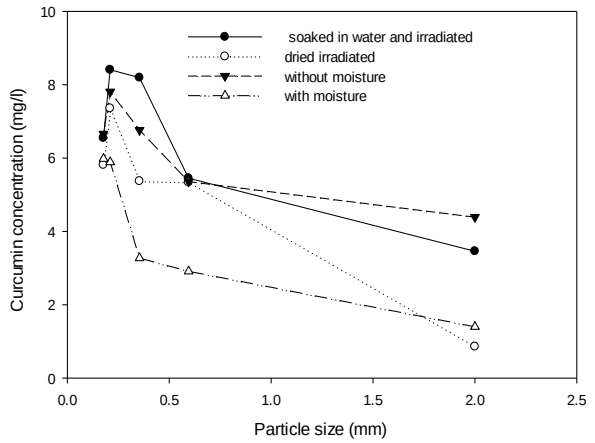

Figure 2. Effect of moisture and pretreatment of turmeric with microwave on curcumin concentration

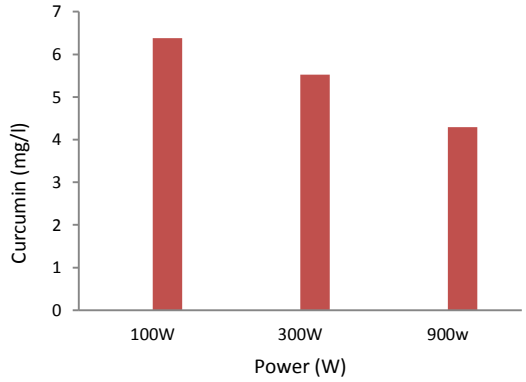

Figure 3. Effect of microwave power on extraction of curcumin from turmeric cell (irradiation exposure time 2 min and particle mesh sizeof 70)

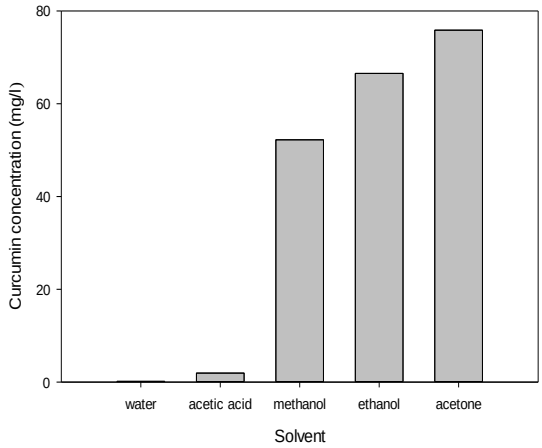

Figure 4. Effect of extraction solvent on curcumin concentration (power 100W, irradiation exposure time 2 min and particle mesh size 70)

Figure 3 represents the concentration of curcumin extracted from pretreated samples. According to this figure, appropriate power input for destruction of soaked powder with size of 0.21mm was 100W; the small particle size of mesh 70 and high dielectric constant of water caused an unsuitable devastation in turmeric. The obtained data show that when the power consumption increased by 9 folds, the concentration of curcumin decreased by 42.78%. That is most probably due to destruction of curcumin molecule for long exposure to microwave irradiation and additional power consumption. High microwave power caused longer time duration of wave exposure.

The selection of suitable organic solvent for curcumin extraction was based on the obtained experimental data illustrated in Figures 4 and additional information about physical properties of solvents as summarized in Table 3 [14]. The results show that extraction of curcumin was not dependent to dielectric constant of solvents. It means microwave-assisted extraction of curcumin was probably independent of dielectric constant. Polarity of organic solvents is another property of extractive solvents. The polarity index of organic solvents is close to each other except for water. Among organic solvents used for extraction of curcumin, acetone demonstrated maximum curcumin concentration. Although dielectric constant for acetone was less than dielectric constants of ethanol and methanol; however, concentration of curcumin extracted by acetone was higher than ethanol and methanol. This extraction power is most probably independent of dielectric constants. The polarity index of solvents may directly has positive impact on solvency of curcumin. It was found that dielectric constant is a suitable parameter for cell wall disruption not for curcumin extraction. Acetone was also used in other reports for extraction of curcumin from turmeric because it has a high solubilizing capacity for curcumin [8, 23, 24]. Acetic acid is not considered as a suitable solvent for extraction, because it has a high boiling point which takes long duration for the removal of solvent.

Figure 5 represents the effect of input power on curcumin extraction with acetone. As the obtained data show,with tripling the input power the concentration of curcumin decreased by 92.6% that is most probably due to destruction of curcumin molecule for longer exposure and additional power consumption. Such decrease in concentration could also be due to the low boiling point of acetone which could not tolerate high input power.

The purity of extracted curcumin using formic acid and microwave assisted extraction was measured based on analysis of samples by HPLC method. The percentages of curcumin using only formic acid and microwave assisted extractions along with the organic solvent were 45.1 and 82.4%, respectively. The mixture of water and methanol was a suitable solution for

purification step. Although extraction with formic acid was fast, but its purity efficiency was lower than purified curcumin from microwave-assisted extraction. That was probably due to destruction of curcumin in formic acid. It seems acetone might be a preferred solvent to formic acid for extraction of curcumin.

Figure 6 shows the FTIR spectrum of purified curcumin. The justifications of purified curcumin are free O-H group (3504 cm^{-1}), C=O and C-C (enol) (1450 −1630 cm^{-1}), C−H (methyl) (2963 cm^{-1}), C−H (aryl) (3121 cm^{-1}) and C−O−C (1000−1300 cm^{-1}) typically attributed to symmetric and asymmetric configurations of C−O−C chains [25].

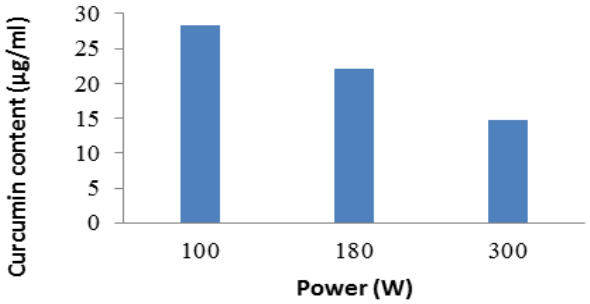

Figure 5. Effect of microwave power on curcumin extraction using acetone (irradiation exposure time 2 min and particle mesh size of 70)

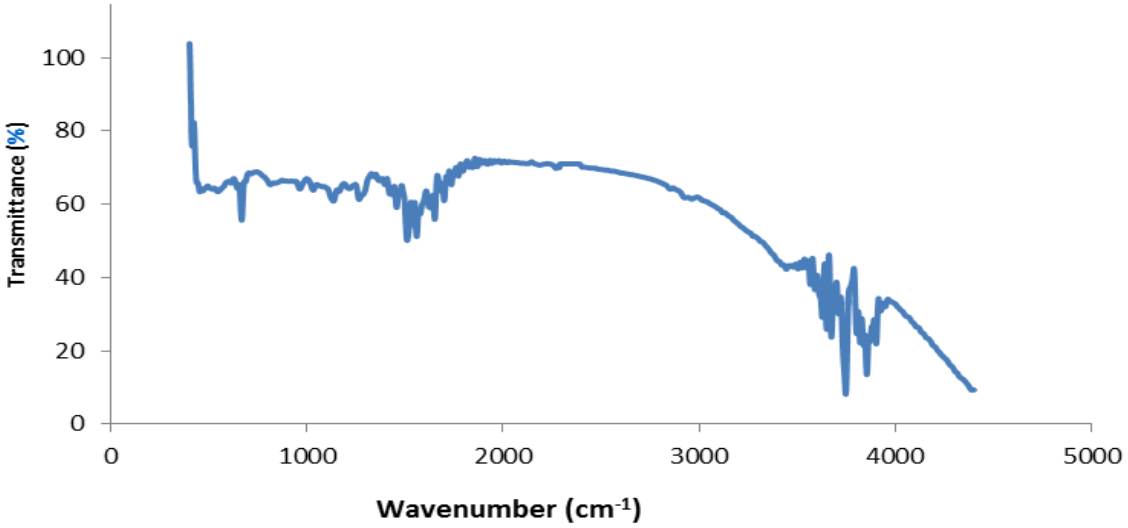

Figure 6. FTIR analysis for purified curcumin

TABLE 3. Physical properties of used solvents [14]

Solvent	Dielectric constant	Polarity[2] index	Boiling point (°C)
Methanol	32.6	5.1	65
Ethanol	24.3	5.2	78
Acetone	20.7	5.1	56
Water	78.3	9	100
Acetic acid	6.15[3]	6.2	117.9[4]

6. CONCLUSION

In this study, microwave-assisted extraction of curcumin from turmeric was examined under different conditions. Pretreatment of wet turmeric with microwave irradiation damaged the plant cell wall of turmeric and enhanced the production yield. The optimized conditions for curcumin extraction were particle size of 0.21mm and microwave input power of 100 W using acetone as suitable solvent. There was no specific relation between the dielectric constant and the concentration of extracted curcumin. It seems that curcumin extracted by microwave-assisted extraction was related to the polarity of organic solvent. As could be inferred from the results, water with high dielectric constant can be used to destroy the turmeric cell wall under microwave irradiation. Also purification of the extracted curcumin was performed using methanol and water which was an appropriate method to achieve high purity of 82.4% curcumin.

[2] http://img.docstoccdn.com/thumb/orig/159941026.png
[3] http://depts.washington.edu/eooptic/linkfiles/dielectric_chart%5B1%5D.pdf
[4] http://vpl.astro.washington.edu/spectra/c2h4o2.htm

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چکیده

کورکومین یک ماده طبیعی است که از ریزوم گیاه زردچوبه استخراج می‌شود. این مطالعه جهت بررسی استخراج کورکومین از ریزوم زردچوبه با کمک امواج مایکروویو و فرمیک اسید انجام شده است. به منظور افزایش میزان استخراج کورکومین، پارامترهای مختلفی مانند سایز ذره، تاثیر پیش تیمار زردچوبه با آب، شدت تشعشع و نوع حلال بررسی گردید. برای آنالیز میزان کورکومین، از دو روش متفاوت اسپکتروفوتومتری و کروماتوگرافی مایع با عملکرد بالا (HPLC) به ترتیب برای مراحل بهینه‌سازی و خالص‌سازی استفاده شد. بالاترین غلظت کورکومین زمانی به دست آمد که سایز ذره ۰/۲۱ میلی‌متر بود و از توان ۱۰۰ وات و حلال آلی استون برای استخراج استفاده شد. نتایج نشان داد که آب حلال مناسبی برای پیش تیمار زردچوبه با تشعشع مایکروویو است. برای خالص‌سازی کورکومین، حلال آب و متانول به کار گرفته شد. ماکزیمم خلوص کورکومین ۸۲/٤٪ به دست آمد.

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