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Microwave Assisted Extraction of Bioactive Compounds from Turmeric (*Curcuma longa)*

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Abstract Microwave-assisted extraction (MAE) was used to extract curcuminoids from turmeric (Curcuma longa). Several MAE conditions were tested, and the method proved to be superior to soxhlet extraction and maceration with regard to time expended. The antioxidant activities of MAE and soxhlet extraction determined from 1/IC50 by the DPPH radical assay were higher than synthetic antioxidant 2, 6-di-ter-butyl-4-methylphenol (BHT). MAE exhibited a range of antioxidant activities varying from 1.37-1.87 mM TEAC/g extract in ABTS assay while soxhlet extraction and maceration were 1.72 and 1.40 mM TEAC/g extract, respectively. The total phenolic contents of MAE ranged from 181.00 to 196.36 mg/g extract; expressed as gallic acid equivalent, while soxhlet extraction and maceration were 180.10 and 170.44 mg/g extract. The contents of curcumin, demethoxycurcumin and bisdemethoxycurcumin of extracts obtained from MAE were in range of 163.00-183.77, 91.73-99.48 and 46.36-53.35 mg/g extract, respectively which were higher than soxhlet extraction. Higher curcumin content may be major contributor to its higher antioxidant activity. The optimum condition of MAE was 900 watt for 1 min of exposure time as it provided higher bioactive compounds and antioxidant activity than conventional methods.

Keywords: Microwave-assisted extraction, Turmeric, Antioxidant activity, Total phenolic content, Curcuminoids content

Introduction

The rhizome of plant Curcuma longa, commonly called turmeric, is widely used as a coloring agent and spice in many food systems. The phenolic compounds of turmeric are name curcuminoids, which include mainly curcumin (diferuloyl methane), demethoxycurcumin, and bisdemethoxycurcumin (Revathy *et al*., 2011). Curcuminoids have a variety of

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phamaceutical properties including antioxidant (Singh *et al*., 2010), anticarcinogenesis, anti-inflammatory and antirheumatic activities (Goel *et al*., 2008). The application of polyphenols has recently attracted great interest in the functional food due to their benefit to human health. Therefore, many researchers are emphasizing on evaluation and characterization of plant constituents. It is generally accepted that food composition data is useful and can represent one of the key characteristics for screening the quality raw material. The detailed information on antioxidant potential including active compound content is also the important criterion. For plant extract, extraction procedure is the one of main parameters influencing on the quality of an extract (Tiwari *et al*., 2011). The most conventional method for extraction of curcumin is Soxhlet extraction (Mandal *et al*., 2008). The solvent is heated to reflux and percolates the solid material. The disadvantage is that the solute is rinsed at the boiling temperature of the solvent which may cause damage of thermolabile compounds.

Recently, microwave-assisted method has been used as technique for extracting valuable compounds from plant materials. It can be adaptable on a small or large scale (Zhang *et al*., 2011). This procedure operates cell bursting due to a sudden temperature increase inside cellular structures followed by leaching out of active compounds, thus leads to faster and efficient extraction (Mandal *et al*., 2008). Although some applications of microwave-assisted extraction (MAE) for phenolic compounds have been appeared in the literatures (Dandekar *et al*., 2001; Mandal *et al*., 2008), there are only a few in which interactive effect between microwave power and duration of exposure for curcuminoids extraction is available. Moreover, extraction condition required differs from case to case thus should be studied to select the optimum condition with time minimization and efficiency enhancement. Therefore, this study was conducted to examine the interactive effect of microwave power and duration of irradiation on yield, antioxidant capacity, total phenolic and curcuminoids content.

Materials and methods

Dried Turmeric Samples

Dried turmeric given by Northern Green Crop (NGC) Partnership Limited, Chiang Mai was used in this study. The sample was ground in a hammer mill with 1.0 mm sieve. The turmeric powder was kept in aluminum foil laminated polyethylene bags under vacuum packing.

Physical and Chemical Analysis

Proximate analysis of dried turmeric was determined using the standard AOAC methods (AOAC, 2000), No. 955.04 for protein content, No. 905.02 for fat content, No. 945.46 for ash measurement, and No. 990.19 for moisture content. Water activity was determined using an AquaLab Water Activity Meter (Decagon, USA). The color of powder was measured in L* a* b* system by colorimeter Konica Minolta (CR-400 Series, Japan).

Microwave Assisted Extraction

The turmeric powder was extracted with microwave assisted extraction. Soxhlet extraction was also employed for comparison. The ground material (5g) mixed with 100 ml ethanol (95%) (Merck) was placed in the center of microwave oven (TOSHIBA, Model ER-300C(S), Power Max 900 W, frequency 2.45 x 109 Hz) with different duration of exposure: 1, 3 and 5 min and with different powers: 600W and 900W. The extraction was carried out in cycles of 30 s of irradiation and a cooling time of 10 min to reach a temperature of 25°C in order to avoid solvent boiling. The experiment was designed using a 2x3 Factorial in completely randomized design and each treatment was done in triplicate. For soxhlet extraction, ground material (5 g) was placed in a soxhlet apparatus and extracted with 100 ml of ethanol (95%) for 3 h. Solvent was evaporated under vacuum using a Büchi Rotavapor R-200 at 40°C. The evaporated extracts were kept in air-tight amber bottles after flushing with nitrogen gas for 30 s and stored at 4°C until they were analyzed.

Maceration extraction

The extraction method was modified from earlier study (Maisuthisakul *et al*., 2007). The ground sample (5g) was soaked with 100 ml of 95% (v/v) ethanol for 4.5 h at room temperature. The extracts were filtered through Whatman No. 4 paper, concentrated under vacuum (Büchi Rotavapor R-200) at 40°C. The evaporated extracts were thick and viscous materials and were kept in air-tight amber bottles after flushing with nitrogen gas for 30 s and stored at 4°C until they were analyzed.

Soxhlet extraction

A hundred milliliter of 95% (v/v) ethanol was added to 5 g of ground sample in a round bottom flask. The mixture was stirred carefully and then refluxed using Soxhlet apparatus at 80°C for 3 h. Solvent was evaporated under vacuum at 40°C. The evaporated extracts were kept in air-tight amber bottles after flushing with nitrogen gas for 30 s and stored at 4°C until they were analyzed.

Yield of extraction

The yield of all evaporated dried extracts based on dry weight basis was calculated from Eq. (1) shown below:

$$\text{Yield } (\%) = (W_1 \times 100)/W_2 \quad (1)$$

where W_1 was the weight of extract after evaporation of ethanol and W_2 was the dry weight of the turmeric powder.

Total phenolic compounds analysis

Total phenols were analyzed using the method that reported earlier with some modifications (Kähkönen *et al*., 1999). Each evaporated thick and viscous extract (0.0100 g) was diluted with 25 ml methanol. The extract solution (200 µl) was transferred into a test tube and then mixed thoroughly with 1 ml of Folin-Ciocalteu reagent (Loba chemie, India). After 3 min incubation, 0.8 ml of 7.5% (w/v) sodium carbonate was added. The mixtures were agitated with a vortex mixer, and then allowed to stand for 30 min in the dark. The absorbance of plant extracts was measured at 765 nm by a spectrophotometer (Thermo Scientific Genesys 10 UV scan, U.S.A.). Quantification of the total phenolics was performed using the linear regression equation of the gallic acid (Sigma-Aldrich Chemic GmbH, Buchs, Spain) standard curve, and expressed as gallic acid equivalents (GAE).

Antioxidant activity assays

Determination of DPPH radical-scavenging activity

Free radical scavenging activity of the extracts was determined using the stable free radical 2,2-Diphenyl-picrylhydrazyl (DPPH) (Sigma-aldrich, U.S.A.) method (Mandal *et al*., 2008). Three milliliters of extract solution (5-20 µg/ml) was added to 1 ml of DPPH radical solution (0.1 mM in methanol). The mixture was agitated with a vortex mixer and allowed to stand in the dark at

room temperature for 30 min. The decrease in absorbance at 517 nm was measured using spectrophotometer (Thermo Scientific Genesys 10 UV scan, U.S.A.). The percentage of DPPH radical scavenging activity was calculated according to Eq. (2) shown below:

DPPH radical scavenging activity (%) = $[A_0 – (A_1 – A_s)]/A_0$ x 100 (2)

where A_0 was the absorbance of control solution (DPPH without sample), A1 was the absorbance of the extract in DPPH solution and As, which was used for error correction arising from unequal color of the extract solutions, was the absorbance of the extract solution without DPPH.

The percentage of scavenging activity obtained was subsequently plotted against the extract solution concentration. The half maximal inhibitory concentration (IC_{50}) was then calculated from the equation analyzed from the logarithmic regression curve between extract concentration (µg/ml) and scavenging activity (Dajanta *et al*., 2010).

$ABTS^{\bullet+}$ decolorization assay

Antioxidant activity of extracts was measured using 2, 2′-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS) (Fluka, Germany) according to the earlier reported method with some modifications (Surveswaran *et al*., 2007). The ABTS radical cation ($ABTS^{\bullet+}$) solution was prepared by reaction of 7 mM ABTS and 2.45 mM potassium persulphate. The mixture was allowed to stand in the dark at room temperature for 12–16 h before use. The $ABTS^{\bullet+}$ solution was then diluted with ethanol to obtain an absorbance of 0.700 + 0.005 at 734 nm. $ABTS^{\bullet+}$ solution (3.9 ml) was added to 0.1 ml of extract solution (100 µg/ml) and mixed thoroughly. The reaction mixture was allowed to stand at room temperature for 6 min and the absorbance at 734 nm was immediately recorded (Thermo Scientific Genesys 10 UV scan, U.S.A.). A standard curve was performed using Trolox standard solution (various concentrations ranging from 0 – 500 µM). The absorbance of the reaction samples was compared to that of the Trolox standard. The results were expressed in term of Trolox equivalent antioxidant capacity (TEAC), expressed as mmol Trolox equivalents per 100 g dry weight of turmeric powder.

Curcuminoids analysis

Preparation of samples

Turmeric extracts (0.0100 g) were dissolved with 10 ml of methanol (RCI Labscan, Thailand). The extract solution was then diluted to obtain a 250 µg/ml concentration and filtered through a 0.45 µm filter (MS® Syringe filter, U.S.A.) prior to HPLC analysis. The sample was separately extracted in triplicate.

Analysis of curcuminoids pigment

Reversed-phase high performance liquid chromatography (RP-HPLC) analysis was conducted on an Agilent HPLC System (1200 series, Boeblingen, Germany), consisting of a binary pump and a diode-array detector (DAD) and equipped with a 250 x 4.6 mm i.d., 5 µm Restek C_{18} column (Restek, Bellefonte, USA). The chromatographic conditions followed a previously reported method (Rodr ́guez *et al*., 2004). The mobile phase was composed of 1% (v/v) acetic acid in filtered MilliQ water (solvent A) and acetonitrile (solvent B). The injection volume was 20 µl and the components were eluted using the following solvent gradient: 0 - 20 min, 40% solvent B; 20 – 32 min, 60% solvent B; 32 – 38 min, 100% solvent B; and from 38 – 40 min re-equilibrium back to 40% solvent B with the flow rate of 1 ml/min. The signals were observed at 425 nm. The mixture of curcumin (80%), demethoxycurcumin (17%) and bis-demethoxycurcumin (3%) (Sigma, U.S.A.) was prepared as standard solutions. The curcuminoid pigments in samples were identified and quantified on the basis of external standard curves (linearity of $R^2 > 0.98$).

Scanning electron microscopy

The structural changes of dried turmeric treated by microwave assisted extraction and soxhlet extraction were examined using scanning electron microscopy (SEM) (JEOL JSM 5901 LV). The materials were collected and air-dried after extraction. They were fixed on aluminum stubs with adhesive tape and covered by gold using a sputter coater. All samples were examined under high vacuum conditions at a voltage of 10.0 kV (50 µm, 500 × magnification).

Statistical analysis

Data was expressed as mean ± SD of triplicate observations. The data was also subjected to analysis of variance (Two - Way ANOVA) and Duncan’s

multiple range tests. The significant differences between mean were defined at $P < 0.05$.

Results and Discussion

Proximate composition of dried turmeric

The physical and chemical qualities of dried turmeric are shown in Table1. The color of turmeric powder was expressed in CIELAB, L* defines lightness, a* denotes the red/green value and b* the yellow/blue value. The color value of turmeric powder showed a neutral or grey direction. The +a* direction indicated redness and +b* movement represented a shift toward yellow.

The obtained turmeric powder exhibited moisture content of 8.62% on dry weight basis which was conformed to Thai industrial standard (TIS. 890-2532) limited to 10%. Moreover, water activity values was concordant with dried food specification (a_w<0.6). The protein and fiber contents of sample were slightly different from the earlier record (Kuttigounder *et al*., 2011). The variation depends on many factors such as water supply handling, fertilizer application, harvesting and storage management. The values of ash found in this study were in the range of Prevention of Food Adulteration Rules (PFA) ($\leq$9% by weight).

Effect of microwave assisted extraction

Type of solvent was one of important parameters for microwave assisted extraction (Zhang *et al*., 2011). The choice of solvent should be considered not only its affinity with the target compound but also its ability to absorb microwave energy. Many organic solvents were used to extract phenolic compounds such as methanol, ethanol, acetone and ethyl acetate (Proestos *et al*., 2008). However, the use of ethanol had more advantages than that of others, including higher extraction efficiency, environmental compatibility, lower toxicity and acceptability for food (Wu *et al*., 2012). In addition, the water content of the matrix allows more reproducible results (Kaufmann *et al*., 2002). For this study, 95% (v/v) ethanol was employed in the present microwave assisted extraction process.

The effect of microwave power

In this study, extraction at different microwave power *i.e.* 600 and 900 watt was investigated. This main factor had influence on total phenolic content, TEAC values and curcuminoids content as shown in Table2.

From the Table2, although the extraction at 600 W resulted in higher of total phenolic compounds (193.73 mg GAE/g extract), antioxidant activity in terms of TEAC values was lower than that of 900 W. In present study, no correlation could be found between total phenolic content and antioxidant activities.

However, high content of curcuminoids was responsible for higher antioxidant activity. The extraction at 900 W of microwave power which exhibited significantly higher curcuminoids content was found to have higher antioxidant activity analyzed by $ABTS^{\bullet+}$ radicals assay ($P \leq 0.05$).This result may be due to the contribution of curcumin, a principle polyphenol, to antioxidant activity of turmeric (Kumar *et al*., 2006).

Previous study reported relation between temperature and microwave power as high microwave power can bring up an extraction temperature of system (Chan *et al*., 2011). For this study, the microwave assisted extraction was done with 30 sec of cycle time in order to prevent a drastic boiling of solvent. The temperature of mixture was immediately measured after a cycle. It was found that extract temperatures were 65°C and 72°C for microwave irradiation at 600 W and 900 W, respectively.

Curcumin which exhibited the highest antioxidant activity among three main curcuminoids (Abas *et al*., 2006) is stable for heating up to 160°C (Zebib *et al*. 2010). Therefore, rising microwave power from 600 to 900 W can improve curcumin extraction. This result coincided with the earlier report which indicated that yield of active compound was improved by increasing the extraction temperature (Pan *et al*., 2010). However, the high microwave power might cause poor extraction yield due to degradation of thermal sensible compounds.

The effect of duration of exposure

In this study, the extraction was carried out for 1, 3 and 5 min in cycles of 30 sec of irradiation and a cooling time of 10 min. The results of this main effect show in Table3.

According to Table3, there were no significant differences observed for duration of microwave exposure (1, 3 and 5 min) in all test examined ($P > 0.05$) excepted for extraction yield. Exposure for 5 min showed a significant higher extraction yield ($P \leq 0.05$). All extracts displayed total phenolic content from

188.28 to 193.63 mg GAE/g extract, IC_{50} values from 6.49 to 6.73, EC_{50} values from 0.16 to 0.17 µg/µg DPPH and anti-radical power from 5.81 to 6.09. The antioxidant activities in terms of TEAC values were varied from 1.62 to 1.75 mM/g extract.

Moreover, the content of curcumin, demethoxycurcumin and bis-demethoxycurcumin accounted for 169.52 to 173.39 mg/g extract, 92.94 to 95.82 mg/g extract and 48.01 to 49.86 mg/g extract, respectively. It may be implied that number of cycles was not affected to active compounds in extracts. In order to avoid risk of thermal degradation, long extraction time can be reduced through extraction cycle (Chan *et al*., 2011).

The interactive effect between microwave power and duration of exposure

The significant differences were observed for interactive effect among irradiation power and duration of exposure in all test examined excepted for demethoxycurcumin content ($P \leq 0.05$) (Table4). The irradiation at 600 W of microwave power, extraction yields and antioxidant activities which measured by ABTS assay increased with the increasing of extraction period from 1 to 5 min. This phenomenon may be caused by the low rate of mass transfer at low temperatures, which would require more time for phenolic compounds to dissolve from the raw materials into the solution (Li *et al*., 2012). In contrast, at higher temperatures, dissolution of the phenolic compounds can reach the equilibrium in a shorter time.

The condition at 900 W for 1 min which was significantly reduced in extraction time showed higher content of total phenolic compounds and curcuminoids. Although the extraction condition at 600 W for 5 min provided a higher extraction yield, the antioxidant activities in terms of IC_{50} was lower than those of 900 W for 1 min. As comparison with soxhlet extraction and maceration method, the irradiation at 900 W for 1 min also showed higher content of total phenolic and curcuminoids. The power of free radical terminators in terms of IC_{50} values of extracts obtained from microwave assisted extraction were in the range of 6.28 to 7.42 µg/ml while that obtained from soxhlet extraction and maceration method were 6.39 and 15.96 µg/ml. In contrast, IC_{50} value of BHT, a synthetic antioxidant, reached 14.36 µg/ml. The scavenging abilities of microwave assisted extraction and soxhlet extraction were thus better than BHT.

Since the extract obtained from irradiation at 900 W for 1 min provided better results of antioxidant activities including total phenolic compounds and curcuminoids content than the others, it was thus considered as the optimum condition with significant reduction in extraction time when compared to

conventional approaches. This study was coincided with the earlier finding which suggested that a higher microwave temperature with a short extraction time are more effective in extracting phenolic compounds using microwave assisted extraction (Li *et al*., 2012).

Shrinkage structure of turmeric tissue

In order to provide evidence to clarify the mechanism of microwave assisted extraction of active compounds from turmeric, structural changes after extraction were observed by scanning electron microscopy as shown in Figure1.

Figure 1 shows the micrographs of the untreated dried turmeric sample, sample treated by microwave irradiation, soxhlet heating and maceration extraction, respectively. As shown in Figure1a, the structure of dried turmeric was generally still complete and compact. There was no destruction on cell walls. After microwave assisted extraction, the disintegration and some interstices were observed (Figure1b) which may due to sudden temperature rise and internal pressure increase (Yoshida *et al*., 2010). Microwave heats simultaneously and rapidly, which may cause the interior superheating in plant materials. The superheating results in the violent vaporization of polar liquor (*e.g*., water) inside plant tissues and cells, and the pressure of steam might rupture (even penetrate) cell walls and tissue surfaces (Zhang *et al*., 2013) The microwave assisted extraction mechanism is different from that of soxhlet extraction and maceration which depend on permeation and solubilization processes to bring the analytes out of cell (Kaufmann *et al*., 2002; Jyothi *et al*., 2010; Zhang *et al*., 2013).

The changes observed for soxhlet extraction (Figure1c) were not considerably different from those obtained by microwave assisted extraction; however, it showed that cells were more opened which could be attributed to long time heating. As seen in Figure1d, shallow ruptures took place on the surface of sample after maceration extraction.

Under optimum conditions, microwave irradiation showed higher antioxidant activity and drastic reduction in extraction time than those obtained by conventional extraction method which might cause decomposition of sensitive molecules as heating process continued for long hours.

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