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Optimization of Microwave-Assisted Extraction of Curcumin from *Curcuma longa* L. (Turmeric) and Evaluation of Antioxidant Activity in Multi-Test Systems

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Abstract: Turmeric (*Curcuma longa* L.) is a medicinal plant, and its biological activities mainly arise from the main constituent, known as diferuloylmethane or curcumin. In the present paper, microwave-assisted extraction (MAE) was investigated for the recovery of curcumin from turmeric in comparison to conventional heat-assisted extraction (CHAE) technique. Various experimental conditions, such as solvent concentration (0-100%, v/v), MAE temperature (30-130 ^{o}C) and MAE time (0-20 min) were investigated to optimize the extraction of curcumin from turmeric. The identification and quantification of curcumin in extracts were performed by HPLC-DAD system. Antioxidant potential and radical scavenging abilities of microwave-assisted extract and conventional heat-assisted extract of turmeric (MAET and CHAET) were evaluated using different systems including total phenolic content (TPC), total antioxidant capacity (TAC), and radical scavenging activities. MAET and CHAET showed high antioxidant activity in all test systems, but the antioxidant properties of MAET were stronger than those of CHAET.

Keywords: *Curcuma longa* L. (turmeric); Curcumin; Microwave-assisted extraction; Total phenolic content; Total antioxidant capacity; Radical scavenging activity.

1. Introduction

Reactive oxygen species (ROS) and antioxidants in humans are maintained in balance in a normal physiological state, and excessive accumulation of ROS in certain conditions can cause oxidative damage to biological macromolecules (*i.e.*, lipids, proteins, DNA), leading to various diseases including cancer and coronary heart diseases [1]. Almost all organisms have established antioxidant defense systems, including antioxidative enzymes and food constituents, to remove or repair the damage on these macromolecules [2]. Exogenous dietary antioxidants, comprising fruits, vegetables

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and spices, have been shown to contain beneficial components, including phenolics with antioxidant properties [3].

Turmeric (*Curcuma longa* L.) as a perennial herb is the member of the Zingiberaceae (ginger) family. It has been known in many countries as an important dietary source in addition to its use in traditional medicine as an antiseptic, wound healing, and anti-inflammatory plant [4]. The main constituent of turmeric is curcumin: (1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, whereas diarylheptanoid curcuminoids, namely demethoxycurcumin and bisdemethoxycurcumin, are the secondary phytochemicals. Curcumin has been used as a colouring agent, flavoring substance and food preservative in the food industry where it imparts colour to sauces, mustards, soups, dairy and meat products, as well as in the cosmetic industry to brighten and clean the skin [5, 6]. Additionally, curcumin is used to dye wool and thread as a colourant in the textile industry [6, 7]. Recently, it has been shown that curcumin has anti-inflammatory, anti-cancer, antibacterial and antioxidant properties to minimize oxidative damage through free radical scavenging, and may potentially have chemotherapeutic properties [8, 9]. Its use as a general disease-preventive herbal medicine (especially in gastro-intestinal cancers) may be summarized to stem from various activities such as chemosensitizing, radiosensitizing and radioprotective, chemopreventive, chemotherapeutic, angiogenesis and metastasis inhibitive, and immunologic modulative activities [10].

Numerous methods are reported for extraction of antioxidant components from natural sources such as conventional heat-assisted extraction, soxhlet extraction, supercritical fluid extraction, ultrasonic-assisted extraction and microwave-assisted extraction (MAE). Among these, MAE is a relatively new method used for the rapid extraction of target compounds from natural sources. MAE proved to be considerably more effective and economical. In MAE, the target compounds migrate out from the matrix through the solvent while the solvent diffuses into the sample matrix and extracts the components by solubilization as in classical solvent extraction [11]. MAE has several benefits, to mention that it can be completed in minutes, polar or non-polar solvents can be used, and all extraction parameters can be controlled by a software-based system [12]. The extraction process shows further advantages, including higher recoveries, better reproducibility, requirement for considerably less extraction time and lower amounts of solvent consumption compared to conventional extraction [13, 14]. The system is designed to operate at elevated temperature monitored by a fiber optic temperature probe. For the best extraction of samples, the MAE parameters such as solvent composition, time and temperature should be optimized before analysis.

The main objectives of the present study were to optimize a rapid and effective microwave-assisted method for the extraction of curcumin from turmeric and to assess the antioxidant properties of MAET and CHAET with *in vitro* antioxidant assays for the first time. For the screening of antioxidant properties, the extracts were evaluated for their total phenolic content, total antioxidant capacity, and antioxidant activity. Antioxidant activity of sample extracts was described in terms of free radical scavenging, hydroxyl radical scavenging, hydrogen peroxide scavenging and superoxide anion radical scavenging activities. As a result, the effects of MAET on the recovery of antioxidant constituents were compared to those of CHAET to confirm the advantage of microwave-assisted method.

2. Materials and Methods

2.1. Reagents and Materials

The following chemical substances of analytical reagent grade were supplied from the corresponding sources: Neocuproine (Nc: 2,9-dimethyl-1,10-phenanthroline), curcumin (from turmeric), acetonitrile: Sigma (Steinheim, Germany); trolox (TR), Nafion® 115 perfluorinated membrane (thickness 0.005 in.): Aldrich (Steinheim, Germany); copper(II) chloride dihydrate, ammonium acetate (NH_4Ac), acetic acid, ethanol (EtOH), and methanol (MeOH): Merck (Darmstadt,

Germany). Turmeric was purchased from Ayfer Kaur Baharat ve Tabii Bitkiler (Spice Bazaar, Istanbul, Turkey). Microwave assisted extraction of turmeric was made using a Milestone ETHOS ONE microwave extraction system (Shelton, CT, USA). The absorption measurements were made using a Varian CARY Bio 100 UV-vis spectrophotometer (Mulgrave, Victoria, Australia). The optical thickness of the cuvettes was 1 mm for Nafion solid sensor measurements. A Waters Breeze™ 2 Model HPLC system (Milford, MA) equipped with a 1525 binary pump, a column thermostat, a 2998 photodiode array detector (Chelmsford, MA) and a Hamilton 25 mL syringe (Reno, NV) was used for chromatographic measurements. Data acquisition was accomplished using Empower PRO (Waters Associates, Milford, MA). The analyses were carried out using a reverse-phase ACE C18 column (4.6 mm x 250 mm, 5 mm particle size) (Milford, MA).

2.2. Preparation of solutions

For the TAC test of CUPRAC method [15], 10 mM $CuCl_2$ aqueous solution, 7.5 mM Nc ethanolic solution, 1 M ammonium acetate (NH_4Ac) aqueous solution were prepared. Trolox and curcumin were freshly prepared in EtOH at 1 mM and kept at +4 °C in a refrigerator prior to analysis.

2.3. Extraction procedures

Microwave-assisted treatment of turmeric was carried out in a series of twelve Teflon closed vessels with an automatic fiber optic temperature control terminal using a MAE system under different set of conditions with respect to time (0–20 min), temperature (30 and 130 ^{o}C) and solvent mixture (MeOH and EtOH in water). A power of 0-1500 W was applied to heat the materials inside the oven to the designated temperatures in a short time. In a typical run (*e.g.*, microwave heating at 80 °C for 2 min), 0.1 g turmeric sample was immersed in 20 mL of 80% MeOH. After that, the mixture was placed in the microwave oven under stirring, and the temperature was raised to 80 °C in 3 min and kept at 80 ^{o}C for 2 min. After extraction, the mixture was cooled to room temperature.

For CHAE technique, 0.25 g sample was taken and added to 50 mL of 50% MeOH, and this solution was decanted to a distillation flask into which a few pieces of boiling stone were added and refluxed at 80 °C for 1/2 h. The flask was cooled to room temperature under running tap water. The obtained methanolic extract was filtered through a filter paper, and then through 0.45 μm PTFE syringe filters (Whatman), and kept at +4 °C in a refrigerator prior to analysis.

2.4. HPLC analysis of curcumin

Curcumin concentration was determined using the HPLC method described in the study of Wichitnithad et al. (2009) [16]. An isocratic elution program was used in the reverse-phase HPLC analysis. Mobile phase consisted of acetonitrile:acetic acid (2% in double-distilled water) mixture (40:60, v/v) at a flow rate of 1.2 mL/min with a column temperature of 33 °C, a total run time of 30 min per sample, and a detection wavelength of 425 nm. Using the above working mode, the calibration curves in the form of linear equations of peak area *versus* concentration were constructed for curcumin.

2.5. Determination of total antioxidant capacity (TAC)

TAC of turmeric extract was assessed according to the optical sensor-based CUPRAC method [15], which is based on the reduction of the CUPRAC chromogenic reagent (Cu(II)-Nc) to the Cu(I)–Nc chelate by antioxidants, *via* measurement of the light absorption of the cuprous chelate formed on the Nafion membrane at 450 nm. Nafion, a perfluorosulfonate ion exchange membrane having R-{-O-CF_2-$CF(CF_3)$-}$_x$-O-$(CF_2)_2SO_3H$ functional groups, was cut into 4.5 x 0.5 cm pieces and dipped into a

tube that contained 8.2 mL of CUPRAC reagent solution (2 mL of 10 mM $CuCl_2$, 2 mL of 1.0 M NH_4Ac, 2 mL of 7.5 mM Nc fresh solution, and 2.2 mL of distilled water), and agitated for 30 min in a rotator. The reagent−impregnated membrane (Nafion-Cu(II)-Nc) was taken out and immersed in a tube containing 8.2 mL of ethanolic antioxidant solutions. After agitating for 30 min, the absorbance of the colored membrane (Nafion-Cu(I)-Nc) was measured at 450 nm against a blank membrane prepared under identical conditions excluding analyte. TAC values of extracts were expressed using trolox equivalent (mmol TR/gram of sample) unit based on the calibration curve obtained with a trolox standard. The assays were carried out in triplicate and the results expressed as (mean values ± standard deviations).

2.6. Determination of total phenolic content (TPC)

Total phenolic content (TPC) of turmeric extract was determined using the Folin-Ciocalteu method [17]. The solutions used in the assay were prepared as follows: Lowry A: 2% aqueous Na_2CO_3 in 0.1 M NaOH; Lowry B: 0.5% $CuSO_4$ aqueous solution in 1% $NaKC_4H_4O_6$ solution; Lowry C: a mixture of 50 mL A and 1 mL B). The Folin–Ciocalteau reagent was diluted with H_2O at a volume ratio of 1:3 prior to use. Turmeric extract (x mL) was mixed with (2 − x) mL of distilled water and 2.5 mL of Lowry C solution in a test tube. Then, 0.25 mL of Folin–Ciocalteau reagent was added and mixed. After 30 min, the absorbance of the reaction solution was measured against blank at 750 nm. The result was converted to trolox equivalent (mmol TR/gram of sample) unit based on the calibration curve obtained with a trolox standard.

2.7. Determination of free radical scavenging (FRS) activity

The scavenging activity of turmeric extract on DPPH radicals was measured according to the method of Sánchez-Moreno et al. (1998) with minor modifications [18]. Briefly, turmeric extract (0.2 mL) was mixed with 2.8 mL of MeOH and 1 mL of 0.1 mM DPPH solution in a test tube. The tubes were stoppered, and after 30 min, the absorbance at 515 nm was recorded against a MeOH, where decolorization was a measure of DPPH free radical scavenging capacity. The free radical scavenging (FRS) activity was expressed as a percentage of DPPH decolorization using the equation:

FRS (%) = [$(A_{DPPH} - A_S) / A_{DPPH}$] x 100

where A_{DPPH} is the absorbance of DPPH solution without sample and A_S is the absorbance of the solution when the sample extract has been added at a particular level. The extract concentration providing 50% inhibition (EC_{50}) was calculated from the graph of FRS inhibition percentage against extract concentration.

2.8. Determination of hydroxyl radical scavenging (HRS) activity

The hydroxyl radicals (•OH) in aqueous media were generated through the Fenton system and spectrophotometrically determined −*via* hydroxylation of a probe− by the modified CUPRAC method [19]. To a test tube were added 1.5 mL of phosphate buffer (pH 7.0), 0.5 mL of 10 mM sodium salicylate, 0.25 mL of 20 mM Na_2–EDTA, 0.25 mL of 20 mM $FeCl_2$ solution, 18 mL H_2O, 0.2 mL turmeric extract, and 0.5 mL of 10 mM H_2O_2 rapidly in this order. The mixture in a total volume of 5 mL was incubated for 10 min in a water bath kept at 37 ^oC. After incubation, the reaction was stopped with adding 0.5 mL of 268 U/mL catalase solution, and mixed for 30 s. Final mixtures (0.5 mL of the incubation solution) were subjected to the HRS-CUPRAC method. The HRS activity (%) of turmeric extract was calculated using the equation:

HRS (%) = [$(A_0 - A) / A_0$] x 100

where A_0 and A are the CUPRAC absorbances of the system in the absence and presence of scavenger, respectively. The extract concentration providing 50% inhibition (EC_{50}) was calculated from the graph of HRS inhibition percentage against extract concentration.

2.9. Determination of hydrogen peroxide scavenging (HPS) activity

The ability of turmeric extract to scavenge hydrogen peroxide was determined according to the method of Özyürek et al. (2010) [20]. To a test tube were added 0.7 mL of phosphate buffer (pH 7.4), 0.4 mL of 1 mM H_2O_2, 0.4 mL of 0.1 mM $CuCl_2.2H_2O$ in this order (H_2O_2 incubation solution, used as reference). To the other two test tubes were added 0.5 mL of phosphate buffer (pH 7.4), 0.4 mL of 1.0 mM H_2O_2, 0.2 mL turmeric extract, and 0.4 mL of 0.1 mM $CuCl_2.2H_2O$ solution rapidly in this order (named as scavenger solutions-I and II). The mixtures in a total volume of 1.5 mL were incubated for 30 min in a water bath kept at 37 ^oC. At the end of this period, to both reference and scavenger solution-I was added 0.4 mL H_2O, and to scavenger solution-II was added 0.4 mL of 268 U/mL catalase solution, and mixed for 30 s. Final mixtures (1.0 mL of the incubation solution) were subjected to the HPS-CUPRAC method. The HPS activity (%) of turmeric extract was calculated using the equation:

$$HPS\ (\%) = [\ (A_0 - (A_1 - A_2))\ /\ A_0\ ] \times 100$$

where A_0 is the CUPRAC absorbance of reference H_2O_2 incubation solution, A_1 and A_2 are the CUPRAC absorbances of scavenger solutions-I and -II, respectively. The extract concentration providing 50% inhibition (EC_{50}) was calculated from the graph of HPS inhibition percentage against extract concentration.

2.10. Determination of superoxide anion radical scavenging (SARS) activity

The superoxide anion radicals ($O_2^{\bullet -}$) were generated *in vitro* in a non-enzymatic system (PMS-NADH) and determined spectrophotometrically by nitroblue tetrazolium (NBT) reduction method described by Yu et al. (2005) [21]. To a test tube were added 2.3 mL DMSO, 0.2 mL of turmeric extract, 2 mL of 468 μM NADH, 1 mL of 300 μM NBT, in this order. The reaction was started by adding 1 mL of 60 μM PMS solution to the incubation mixture. The mixture in a total volume of 6.5 mL was incubated for 5 min in a water bath kept at 25 ^oC, and the absorbance was read at 560 nm against DMSO. Decreased absorbance of the incubation reaction mixture indicated increased superoxide anion radical scavenging activity. The SARS (%) of turmeric extract was calculated using the equation:

$$SARS\ (\%) = [\ (A_0 - A)\ /\ A_0\ ] \times 100$$

where A_o and A are the absorbances of the incubation reaction mixture in the absence and presence of scavenger, respectively. EC_{50} values of extracts were calculated from the graph of SARS inhibition percentage against extract concentration.

2.11. Statistical analysis

Descriptive statistical analyses were performed using Excel software (Microsoft Office 2007) for calculating the means and the standard error of the mean. The assays were carried out in triplicate and the results expressed as mean value ± standard deviation (SD).

3. Results and Discussion

Curcumin is a major component of turmeric, being responsible for its biological activities [22-24]. Hence, this paper aimed to optimize the conditions for the preparation of 80% methanolic extract using microwave energy. Also the antioxidant and radical scavenging activities were evaluated and

compared with the results of the conventional method of extraction. Recently, MAE has been accepted as an alternative to conventional extraction techniques for the extraction of target components from plant samples [14]. Curcuminoids are present in oleoresin cells of the turmeric rhizome, and are exposed to solvent attack by breaking of the cell walls upon MW irradiation. MAE is also a good alternative to conventional solvent extraction because isolation of curcuminoids from the organic solvent by conventional recovery techniques such as lead acetate precipitation and alkaline extraction is not suitable for food and pharmaceutical industries due to toxicity and stability problems, respectively. MAE conditions were optimized for curcumin extracted from turmeric having a great variety of pharmacological activities.

3.1. Effect of solvent on MAE efficiency

To examine variations in extraction efficiency according to the solvent used, MAE was performed with acetone, chloroform, EtOH, MeOH, and methylene chloride. Figure 1 (a and b) shows that MeOH can be used to obtain higher extraction of curcumin than using EtOH and water respectively. In addition, Figure 1 (a and b) shows that the extraction of curcumin from turmeric was greatly influenced by the solvent concentration. If water was added to MeOH, the MeOH-water (80:20, v/v) solution gave the highest extraction of curcumin among other solvent mixtures tested. When the water concentration was increased in the solvent mixtures, curcumin extraction was decreased (Figure 1 (a)). TAC of the extract was decreased with increasing water concentration in solvent mixtures because of the hydrophobicity of curcumin as the major component of turmeric (Figure 1 (b)). So, 80% (v/v) MeOH concentration in water was the optimal choice in the following experiments. Although curcumin (diferuloylmethane) is a hydrophobic polyphenol having low polarity and low water-solubility [25], it may show an affinity to both polar and non-polar solvents through hydrogen-bonding interactions. Curcumin has a strong ability to form H-bonds with solvents like water and alcohols (MeOH, EtOH, *etc*.) in neutral and deprotonated form as acceptor, whereas the possibility of H-bonding in acetonitrile, dioxane, DMSO *etc*. cannot be avoided in the neutral/protonated form of curcumin as donor [26]. It is an established fact that MAE extraction efficiency of curcuminoids increases with the solubility of these compounds in the extracting solvent, which emphasizes the importance of the polarity of solvent and its additional interactions (*e.g*., H-bonds) with the solutes.

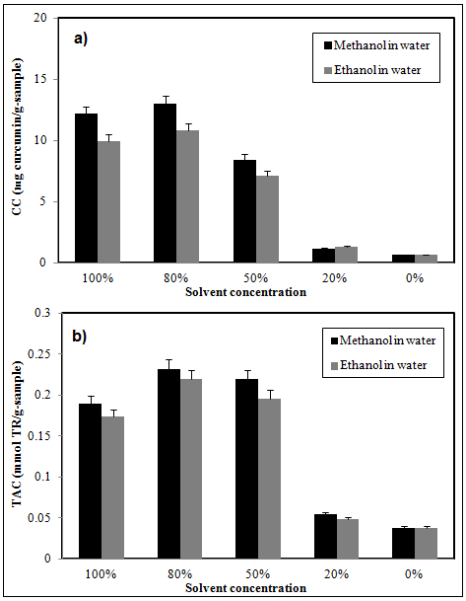

Figure 1. The effect of solvents used on the extraction of (a) curcumin and (b) antioxidant compounds (*N*=3).

3.2. Effect of temperature on MAE efficiency

Figure 2 shows the effect of MAE temperature on the extraction of curcumin from turmeric. The results indicated that curcumin concentration (CC) of extract was increased with the increase of MAE temperature. If MAE temperature was higher than 80 ^{o}C (as the local maximum of curcumin yield), CC of extract was decreased. On the other hand, the TAC of extract reached the highest level at 80 ^{o}C and stabilized. Unlike CC, an increase of temperature from 80 to 130 ^{o}C of MAE did not induce a decrease in the TAC of extract. According to these results, curcumin can be assumed to be degraded step by step at temperatures higher than 80 ^{o}C, and other phenolic antioxidants such as vanillin and ferulic acid were formed as degradation products of curcumin [27, 28] so as to maintain the TAC level at the saturation value reached at 80 ^{o}C. So, an optimal MAE temperature of 80 ^{o}C was used in the following experiments for extraction of curcumin from turmeric.

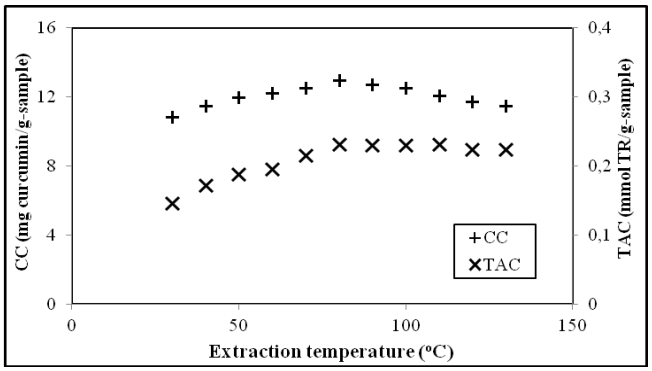

Figure 2. The effect of MAE temperature on the extraction of curcumin and antioxidant compounds (*N*=3).

3.3. Effect of time on MAE efficiency

Figure 3 shows the effect of time on the MAE of curcumin. The results indicated that the extraction of curcumin was increased with an increase in time. MAE reached a high level in 5 min and did not change significantly after this point. So, a best MAE time of 5 min was used in the following experiments.

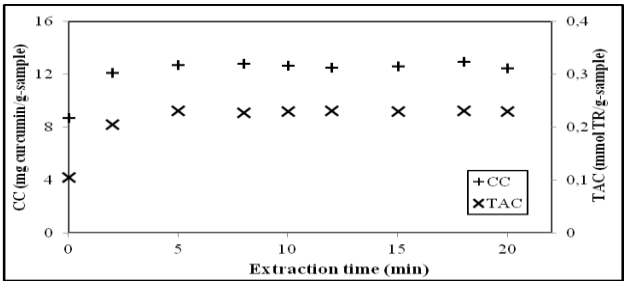

Figure 3. The effect of MAE time on the extraction of curcumin and antioxidant compounds (*N*=3).

3.4. Comparative evaluation of antioxidant and antiradical activities of MAET and CHAET

Turmeric was extracted employing MAE and CHAE techniques, and both extracts obtained were analyzed for their antioxidant properties using different assays (*i.e.*, TPC, TAC, FRS, HRS, HPS, and SARS activities). Curcumin was the main component responsible for antioxidant activity as determined by HPLC assay.

HPLC protocol was standardized for the separation of curcumin, and the results indicated that curcumin yielded a peak at 22.3 min (λ=425 nm). The typical HPLC chromatograms of standard curcumin, curcumin after CHAE and MAE (along with other curcuminoids, such as demethoxycurcumin and bisdemethoxycurcumin) are shown in Figure 4(a-c). The linear calibration equation of curcumin as chromatographic peak area *versus* concentration drawn at the wavelength of 425 nm was: $y = 3.4 \times 10^{10}c - 63797$ ($r = 0.9999$). As the parametric symbols used in the calibration equations, *y* stands for peak area, c curcumin molar concentration, and *r* linear correlation coefficient. The individual curcumin content of turmeric was determined with the help of this HPLC calibration curve. The CHAET and MAET showed 10.08 and 13.21 mg/g-sample CC, respectively. Recovery percentage of curcumin from turmeric was found higher by using MAE in preference to CHAE technique.

In the light of literature, plant phenolics are highly effective free radical scavengers and antioxidant activity of natural foods is derived largely from phenolics. Therefore, there should be a close correlation between the phenolic content and antioxidant activity [29, 30]. The Folin-Ciocalteu method is not an antioxidant assay in the strict sense but a replaceable assay for measuring the quantity of phenolic compounds [31]. TPC of CHAET and MAET were 0.27 ± 0.01 and 0.35 ± 0.03 mmol TR/g-sample dry weight, respectively.

The optical sensor-based CUPRAC method was used for evaluating the TACs of turmeric extracts. This method utilized Cu(II)-Nc complex as the chromogenic oxidizing reagent which is immobilized on a Nafion membrane. As shown in Table 1, TAC of CHAET and MAET were found as 0.16 ± 0.01 and 0.23 ± 0.02 mmol TR/g-sample, respectively, expressed as trolox equivalents. TPC values of turmeric extracts were found higher than TAC, because the molybdato-phospho-tungstate heteropoly acid reagent of Folin-Ciocalteu method had an indefinite but much higher redox potential (compared to that of the CUPRAC method) in alkaline medium where most phenolic compounds are deprotonated and open to oxidative attack, and thus Folin-Ciocalteu measured all phenolic species nonselectively [32].

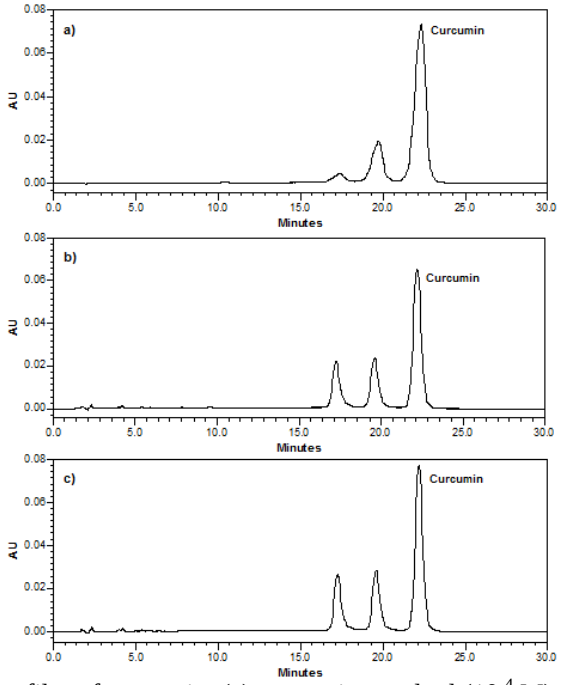

Figure 4. Chromatographic profiles of curcumin: (a) curcumin standard (10^{-4} M); (b) curcumin in CHAET; (c) curcumin in MAET.

Table 1. Comparison of TPC (mmol TR/g-sample) and TAC (mmol TR/g-sample) values of CHAET and MAET.

Methanolic Extract	TPC (mmol TR/g-sample)	TAC (mmol TR/g-sample)
CHAET	0.27 ±0.01	0.16 ±0.01
MAET	0.35 ±0.03	0.23 ±0.02

Samples were analyzed in triplicate.

FRS activity has a great importance due to the deleterious role of free radicals in foods and biological systems [33]. DPPH• as a stable free radical can accept an electron or hydrogen atom to become a stable diamagnetic molecule [34]. One of the most common methods to evaluate antioxidant activity of specific compounds or extracts is the DPPH-FRS activity assay which relies on the decrease of DPPH• absorbance at 515 nm induced by antioxidants. MAET (Figure 5a) and CHAET (Figure 5b) exhibited marked DPPH radical scavenging activity in a concentration-dependent manner. The phenolic group of curcumin was considered to be essential for its DPPH free radical scavenging activity, and the presence of methoxy group further increased this activity [35]. As seen in Table 2, the EC_{50} values (the concentration required to inhibit free radicals by 50%) of CHAET and MAET were found 1.97 ± 0.03 and 1.44 ± 0.08 mg/mL, respectively. These results demonstrate that MAET had a higher FRS activity than CHAET.

Table 2. EC_{50} values (mg/mL) of CHAET and MAET in the evaluation of antioxidant activity assays.

Methanolic Extract	FRS Activity (EC_{50}[a])	HRS Activity (EC_{50}[a])	HPS Activity (EC_{50}[a])	SARS Activity (EC_{50}[a])
CHAET	1.97 ±0.03	1.40 ±0.02	2.48 ±0.05	2.12 ±0.09
MAET	1.44 ±0.08	1.16 ±0.03	2.28 ±0.11	1.66 ±0.05

Samples were analyzed in triplicate.
[a] EC_{50} (mg/mL): Effective concentration at which 50% of radicals are scavenged.

Hydroxyl radical (•OH) is the most reactive free radical that can be formed from superoxide anion and hydrogen peroxide, in the presence of metal ions [36]. Because of the strongest oxidative activity of •OH, HRS activity is very important for evaluating the antioxidant activity of a food extract. In the present study, the HRS-CUPRAC method was used for determining the HRS activity of turmeric extract. Salicylate probe was used for detecting •OH generated by the reaction of iron(II)–EDTA complex with H_2O_2. The produced hydroxyl radicals attack both scavengers and the salicylate probe that are incubated in solution for 10 min. at 37°C. Added scavengers compete with salicylate for the •OH produced, and diminish chromophore formation from Cu(II)–Nc. The reaction was stopped by adding catalase after the incubation period. With the aid of this reaction, a kinetic approach was adopted to assess the HRS properties of turmeric extract. Figure 5a and Figure 5b illustrate that both of turmeric extracts showed strong HRS activities. The scavenging effect of extract on the •OH increased with the increase in concentration of sample. The EC_{50} of MAET was 1.16 ± 0.03 mg/mL, compared to the EC_{50} of CHAET as 1.40 ± 0.02 mg/mL (Table 2). So, the hydroxyl scavenging ability of MAET was higher than that of CHAET.

Hydrogen peroxide is thought to be the major precursor of highly reactive free radicals, and it has been reported to induce apoptosis in cells of the central nervous system [37]. The ability of both extracts of turmeric to scavenge H_2O_2 was determined according to the method of Özyürek et al. (2010) [20]. HPS activity has usually been determined by following the rate of H_2O_2 consumption in an incubation system (H_2O_2+scavenger) using the classical UV-method at 230 nm. Since some polyphenols have strong absorption in the UV-region, their HPS activity was determined without interference by directly measuring the concentration of H_2O_2 using the HPS-CUPRAC method at 450 nm in the presence of trace Cu(II) salt as catalyst. A concentration-dependent assay was carried out with MAET (Figure 5a) and CHAET (Figure 5b). Figure 5a and Figure 5b illustrate that turmeric extracts possessed significant scavenging activity on H_2O_2, their scavenging effect was increased with increasing concentration. The HPS activity of MAET (EC_{50}: 2.28 ±0.11 mg/mL) was higher than that of CHAET (EC_{50}: 2.48 ±0.05 mg/mL).

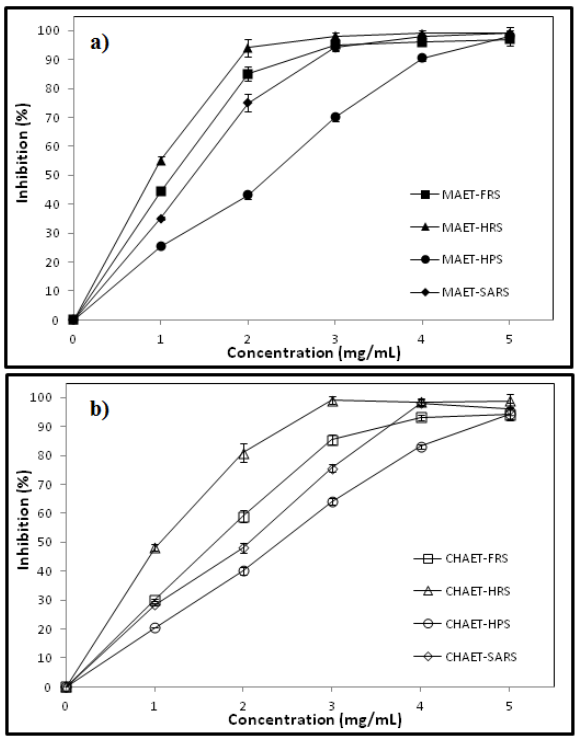

Figure 5. Radical scavenging activities (DPPH•, •OH, H_2O_2 and $O_2^{\bullet-}$) (a) MAET and (b) CHAET at different concentrations (1-5 mg/mL). Each value is expressed as mean ± standard deviation (*N*=3).

Superoxide anion radical ($O_2^{\bullet-}$) has gained great attention due to its important role in the progression of a number of human diseases and carcinogenesis. So it is important to eliminate excessive $O_2^{\bullet-}$ in vivo to prevent important diseases [38]. In this study, SARS activity of turmeric extract was evaluated according to the method of Yu et al. (2005) [21]. Superoxide anion ($O_2^{\bullet-}$) can be formed from dissolved oxygen by PMS–NADH coupling reaction, and $O_2^{\bullet-}$ reduces the yellow dye (NBT^{2+}) to produce a blue formazan, of which the absorbance value is measured at 560 nm. Antioxidants are able to inhibit formazan formation. The decrease of absorbance with antioxidants indicates the consumption of $O_2^{\bullet-}$ in the reaction mixture. Figure 5a and Fig. 5b illustrate that scavenging effects of turmeric extract on the $O_2^{\bullet-}$ radicals increased with increasing concentration. Extract of turmeric showed strong SARS activity. As seen in Table 2, MAET was found to have more SARS activity than CHAET, demonstrating EC_{50} values of 1.66 ± 0.05 and 2.12 ± 0.09 mg/mL, respectively.

The special antioxidative properties of curcumin may be analyzed in the light of structure-activity relationships. The bimolecular rate constant for curcumin in scavenging DPPH free radical (possibly to produce the phenoxy radical 1-e oxidation product) was found to be approximately 1800 times greater than that for its dimethoxy derivative; although the energetics to remove a hydrogen atom from both phenolic -OH and the -CH_2 group of the β-diketo structure were very close, the phenolic -OH was considered to be essential for both antioxidant activity and free radical kinetics, a fact further confirmed by density functional theory calculations [39]. Both antioxidant and anti-inflammatory actions of curcumin may be associated with the β-dicarbonylic system, which has conjugated double bonds as dienes [40]; structure-activity expectations suggest that a hydroxyl group at the *para*-position is most critical for the expression of biological activity [41]. The methoxy groups in *ortho*-position with respect to phenolic-OH groups, as well as the overall symmetry of the molecule with delocalized π-electron structure, may enhance electron transfer, making curcumin a stronger antioxidant.

3. Conclusion

The effects of two types of extraction methods (MAE and CHAE) on the recovery of curcumin from turmeric were studied. TAC and CC parameters were used to optimize the MAE conditions. MAE was previously optimized with respect to the level of the power source using acetone as solvent, showing a disadvantage of rapid heating and fast evaporation from the reaction vessel [42]. An efficient MAE process was developed for fast extraction of curcumin from turmeric using methanol and compared with CHAE for the first time. The curcumin content, TPC, TAC, FRS, HRS, HPS, and SARS activities in turmeric extracts were measured with the use of MAE and CHAE. The antioxidant capacity and radical scavenging activity measurements on turmeric exhibited that these species possess considerable antioxidant potential due to their curcumin contents. The various antioxidant mechanisms of turmeric extracts may be attributed to their strong scavenging action of free radicals and reactive species such as •OH, H_2O_2, and $O_2^{\bullet-}$. It may be suggested that curcumin (along with other curcuminoids) could be used in the safe preservation of food systems to increase the shelf-life, with further investigations for preventing possible prooxidant effects [43]. The results indicated that MAET possesses abundant phenolic content and exhibits excellent antioxidant activities when compared to CHAET. Finally, the antioxidant properties of MAET was superiour to those of CHAET which suggested that MAE technique was more beneficial than CHAE for the extractive recovery of antioxidant components with less extraction time and solvent consumption.

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