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MICROWAVE ASSISTED EXTRACTION OF CURCUMINOIDS FROM *CURCUMA LONGA*

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ABSTRACT

A novel microwave assisted extraction (MAE) technique was investigated for selective and rapid extraction of curcuminoids from *Curcuma longa* (turmeric) into organic solvents. The degree of extraction and purity of curcuminoids were dependent upon the selected solvent and exposure time to microwaves. The extraction process was optimized using acetone at 20% power level (PL) giving 60% extraction of curcuminoids with 75% purity within 1 min. The dielectric heating of cellular matrix resulted in vaporization of volatile components, increasing internal pressure of the cell leading to remarkable swelling and finally rupturing of the cells. The degradation of cellulosic cell wall at higher temperature when subjected to microwave also increased permeability of solvents into the bio-matrix.

Key Words: Curcuminoids; *Curcuma longa*; Turmeric; Microwave assisted extraction

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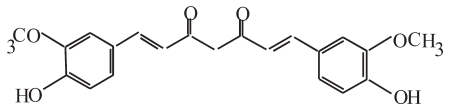
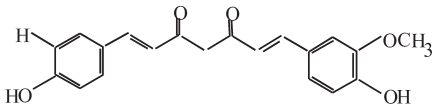
INTRODUCTION

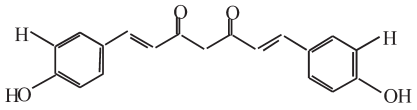
Curcumin ($C_{21}H_{20}O_6$) and two other related compounds, viz. demethoxy curcumin and bis-(demethoxy) curcumin, together known as curcuminoids are the active phytochemicals present in *Curcuma longa* species commonly known as turmeric. Curcuminoids are extensively used as food-coloring agent, natural antioxidant, and spice condiment. Recently, they have gained popularity for medicinal purposes, such as anticoagulative (1) and potent anticancer agent (2). Curcuminoids also have potential as anti-inflammatory, antibacterial, antifungal, antiparasitic, and antimutagenic agents (3) and as modest inhibitors of HIV-1 and HIV-2 proteases (4).



Curcumin



de-methoxy curcumin



Bis-demethoxy curcumin

In general, turmeric consists of about 13% (w/w) moisture, 69% (w/w) carbohydrates, 5% (w/w) fixed oils, 6% (w/w) volatile oils, 5% (w/w) proteins, and around 1–6% yellow pigment curcuminoids (5). In the process of extraction, the solvent has to reach the inside of the cells where these constituents are located to dissolve them.

Organic polar solvents are often used to isolate curcuminoids from *Curcuma* species. Defatting of the raw material before the extraction and post treatment after the extraction to recover the curcuminoids are necessary to get better purity, since frequently the purity of the extract is as low as 20% (6). The extraction of curcuminoids has been reported using ethanol–water (7), ethyl

acetate (8), acetone, (9) and benzene (10). These conventional organic solvent-based methods of extraction are labor intensive and time consuming, as the extraction needs to be conducted over several hours. The curcuminoids can be recovered from the extract by precipitation using $Pb(OAc)_2$, but such products cannot be recommended in the pharmaceutical and food industries due to the toxic nature of lead (11). Curcuminoids, being phenolic compounds, can be extracted with aqueous alkaline solutions too (12). However, they are unstable in alkaline conditions and the degradation rate rapidly increases with an increase in pH above 7.5, with a half-life of only 30 min at pH 10.2 (13).

In search of a rapid and efficient process for extraction of curcuminoids, we have investigated microwave-assisted extraction of curcuminoids from *C. longa* species. Microwaves are electromagnetic radiation of wavelength 0.1–10 cm. This region shows a phenomenon of microwave heating of a polar dielectric material, having either permanent or induced dipoles. When a dielectric material is placed in a microwave field, its molecules try to align themselves by the applied electric field either by distortion of the distribution of electron cloud within the molecule or by physical rotation of the molecular dipoles. If a molecule is able to align itself every time with the external field then there is no dielectric heating and the molecule acts as a conductor. If the external field reversal is very fast and the molecule is not able to even start to realign itself, then it acts as an insulator without dielectric heating. When the reversing speed of external field is such that molecule is stopped from rotating and is dragged back due to the field reversal it can cause dielectric heating. The dielectric heating affects polar molecules irrespective of their positions inside or outside of the material within the penetration depth of microwave radiation.

Microwave energy offers numerous potential processing advantages over the conventional heating methods to provide a rapid and volumetric heating to an absorbing medium (14). Use of microwave radiation is well known in organic synthesis. Many solvent-less synthesis using microwave irradiation showed enhanced reaction rates, greater selectivity, and the experimental ease to manipulation (15,16). For most materials, in particular biological tissues, the maximum penetration of the electromagnetic energy occurs in the microwave range (17). Various solvent-less extractions have been reported for volatile oils and fragrances, in the recent past, by exposure to microwave radiation (18,19). The vapors of volatile essential oils are rapidly generated by microwave radiation and are drawn into the vapor space. The material is then condensed outside the microwave cavity. The microwave-assisted extraction is very rapid and produces an expanded range of fragrances.

The extraction of nonvolatile components such as glycosides into organic solvents has been claimed to be accelerated by microwaves (20,21). The study of the impact of high electric field pulses on plant membranes has showed increased permeability and disintegration of cell walls (22). While working on structure of

carrot cells and potato cells, Alex et al. (23) had observed remarkable swelling of the cells because of the increased internal pressure. The pressure pushes the cell wall from inside, stretching, and ultimately rupturing it.

This paper describes microwave assisted extraction (MAE) of curcuminoids from *C. longa* rhizomes. We have demonstrated rapid extraction of *nonvolatile* curcuminoids after exposure to microwave radiation or with simultaneous microwave radiation into an organic solvent. The MAE can also be used as an efficient analytical sample preparation tool for natural products as extraction time is reduced to a few minutes as against the conventional soxhlet extraction, which requires several hours. The mechanism of enhanced extraction rates for curcuminoids is also discussed.

EXPERIMENTAL METHODS

Dry *C. longa* rhizomes obtained from M/s. Cancor Flavours and Extracts Ltd., Cochin (India), were pulverized and the coarse powder was separated by mechanical sieving. Particles of mean size around 3.5 mm were selected for the extraction studies. A domestic microwave oven from IFB (Model Neutron, Power Max 750 watts, Frequency 2450 MHz) (M/s IFB Industries, Ltd., Mumbai, India) was used for the extraction.

The extraction was conducted by two methods. In the first technique, dry irradiation of raw material was done by placing the raw material as a single layer of thickness of about 4 mm in a dish of 10 cm diameter in the microwave cavity. After irradiation for a pre-specified time, the raw material was suspended in acetone in a fully baffled cylindrical vessel of internal diameter 7 cm and height 9 cm, equipped with a four-blade turbine impeller of 2 cm diameter. The suspension density 5% (w/v) was kept constant for all the runs. The stirring speed in the solvent extraction step was kept constant at 1200 rpm to eliminate the effect of external mass transfer resistance. The samples were drawn every 10 min for the determination of the extracted curcuminoids.

In the second technique, a modified set-up (Fig. 1) was used for the extraction. A glass vessel of diameter 3 cm and length 12 cm was used with a provision for sparging nitrogen gas to maintain an inert atmosphere inside the vessel. The gas was sparged into the suspension of raw material in an organic solvent at a flow rate just enough to keep the particles in suspension. The exit gas was then passed through two liquid nitrogen traps, outside the microwave oven cavity to condense volatile material, if any, including the organic solvent, vaporized from the extraction vessel. In all the experiments, the condensate collected inside the traps contained no curcuminoids. In most cases, however, especially with polar solvents, a significant amount of the organic solvent (up to 20%) was recovered along with turmeric volatile oils (maximum around 20% v/v of the collected condensate). The

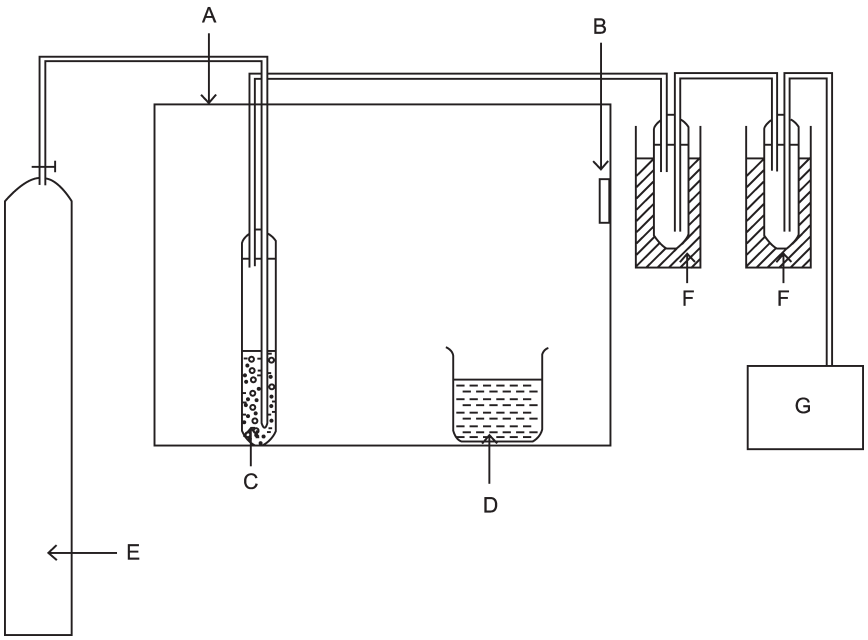


Figure 1. Modified setup of microwave assisted extraction. A, Microwave cavity; B, magnetron source; C, solvent + raw material; D, water; E, nitrogen cylinder; F, liquid nitrogen trap; G, vacuum pump.

suspension density was varied from 1% to 5% (w/v). The irradiation was done for pre-specified time and samples were withdrawn for analysis at different time intervals. The sample solutions were analyzed to estimate the rate of extraction and purity of curcuminoids in the extract.

Thin sections of dry *C. longa* rhizome were observed, before and after the microwave irradiation, under microscope of magnification 40 × and the images were scanned using *Image Pro Plus Analyser.*

The percent extraction is based on the curcuminoids present in the raw material, since the amount of curcuminoids in raw material varies from source to source. The total curcuminoids content in the raw material was determined separately by soxhlet extraction with acetone. The raw material was deffated using petroleum ether (boiling point 40–60°C fraction) prior to extraction of curcuminoids for 16 hr. The curcuminoids extraction was conducted for 10 hr/day for 6 days using 30 gm of the deffated raw material and about 200 mL of acetone. Everyday the extract was removed and fresh solvent was added. The percent curcuminoids present in the raw material was determined to be 5.8% (w/w).

The extraction data were fitted into a first order kinetic equation to estimate extraction rate constant (k), the reciprocal of which represents the characteristic time of extraction, i.e., a higher value of "k" should correspond to higher rate of extraction.

$$\%\text{Extraction} = b(1 - e^{-kt}) \quad (1)$$

where t = time, k = extraction rate constant, time^{-1} b = maximum extraction achieved at the specified conditions.

ANALYTICAL METHOD

The curcuminoid extracts were analyzed with high performance thin layer chromatography (HPTLC) using $10 \times 10\,cm^2$ silica gel 60 F_{254} plates from E. Merck (Darmastadt, Germany). The extracts were applied as 5 mM bands using Desaga Applicator AS-30 (Heidelberg, Germany). The separation was performed using chloroform–ethanol (95%)–glacial acetic acid (94:5:1, v/v) in a Thin Layer Chromatography (TLC) chamber previously saturated for 15 min at room temperature of 30°C. The plates were developed to a distance of 7 cm and then dried in air. The plates were scanned using Desaga Densitomer CD60 at wavelength 423 nm.

RESULTS AND DISCUSSION

Figure 2 shows extraction of curcuminoids after exposure to microwave radiation as compared to unirradiated raw material. The turmeric powder irradiated for 2 and 4 min with microwave showed marginally higher extraction of curcuminoids in 60 min by acetone. The percent extraction also increased from 64 to 75% under identical conditions. Further increase in the irradiation time, however, did not increase the rates further. The extraction in both the cases seemed to follow a first order dependence on time. The data were fitted into Eq. (1) and extraction rate constants were estimated. An increase in the extraction rate constant was observed with the irradiated raw material from that with nonirradiated sample.

Cellulose, which mainly constitutes the cell wall, is an ionic conductor with a large molecular mass and requires long relaxation time (24,25). The presence of hydrogen bonding and $-CH_2OH$ groups on the glucopyranose residues, however, can give relaxation times of 170–270 ps due to localized rotations of the $-CH_2OH$ groups (26). The dry microwave irradiation can hydrolyze ether linkages in cellulose and convert it into soluble fractions within 1 or 2 min (27). The higher temperatures attained by the cell wall enhance degradation of cellulose reducing it's mechanical strength. This disruption should increase the

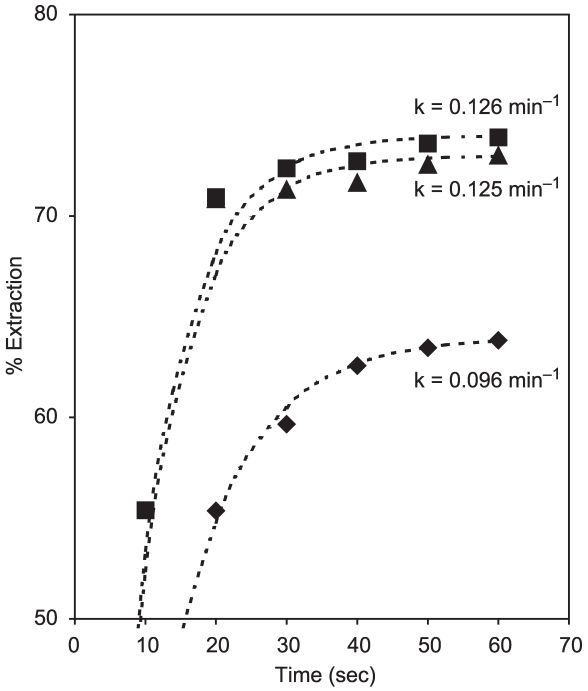
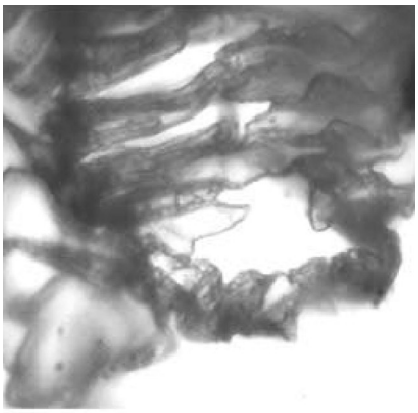


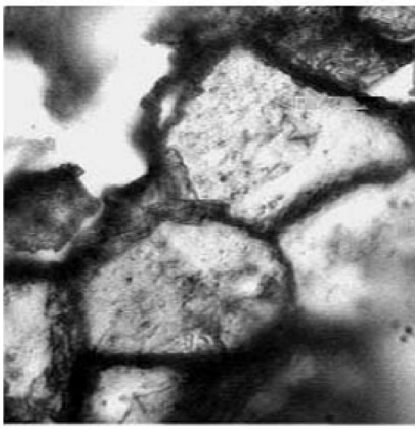
Figure 2. Effect of microwave irradiation of dry raw material on extraction. ◆: 0 Min; ▲: 2 min; ■: 4 min.

rate of extraction and the efficiency of extraction in given time. On the other hand for nonirradiated raw material, the cells were intact. Thus, the extraction rate was slow.

The curcuminoids are present in oleoresin cells, in the central cylinder covered by rectangular cork cells, in the turmeric rhizome. The cork cells and oleoresin cells were separately observed for the effect of microwave radiation, as these cells are important in the extraction of curcuminoids. In the nonirradiated sections the cork cells are tangentially oblong, rectangular in shape, and perfectly packed (Fig. 3a), and the oleoresin cells show the strongly colored pigment (Fig. 3b). After microwave irradiation, packing of the cork cells was disturbed and the cells were swollen (Fig. 4a). The oleoresin cells were also swollen, sometimes with broken cell walls (Fig. 4b) with the pigment scattered near the broken part of cell wall and outside. The disturbed cork cells and the broken oleoresin cells make curcuminoids more easily accessible to the solvent and thus a prior microwave irradiation helps in increasing extraction rates of curcuminoids.



(a)



(b)

Figure 3. (a) Cork cells before microwave irradiation. (b) Oleoresin cells before microwave irradiation.

Effect of Water Soaking of Raw Material

Since polar compounds are affected the most by microwave radiation, experiments were conducted with raw material soaked in water for different periods of time. The water soaked particles of raw materials were then dried using tissue paper before extraction. The percent of water uptake was determined by weight gain over the soaking period. The soaking time less than 4 hr gave the

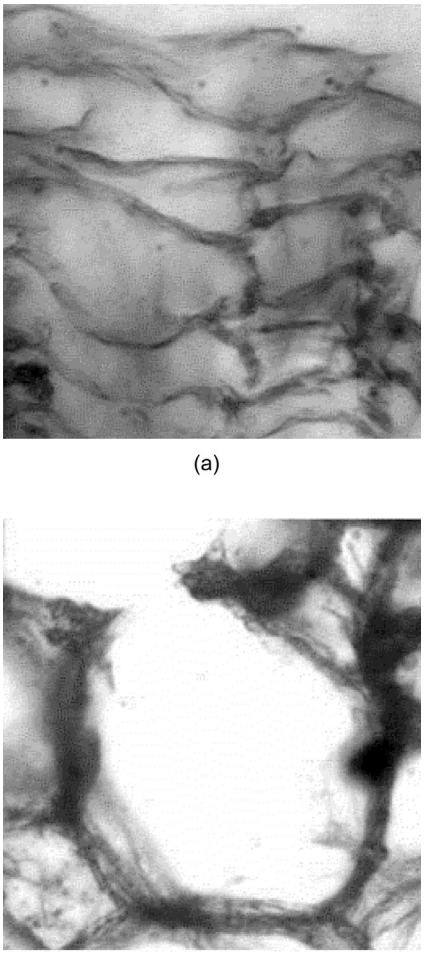


Figure 4. (a) Cork cells after microwave irradiation. (b) Oleoresin cells after microwave irradiation.

water uptake only up to 30% (w/w). The water uptake increased sharply after 4 hr and reached to 49% (w/w) after 6 hr. It further increased to 54, 61, and 65% (w/w) after 12, 18 and 24 hr, respectively. Figure 5 shows the effect of water present in the soaked raw material, when extraction was conducted with simultaneous microwave irradiation using the second technique. A very sharp increase in the extraction rate of the curcuminoids was observed in 45 sec, if the raw material

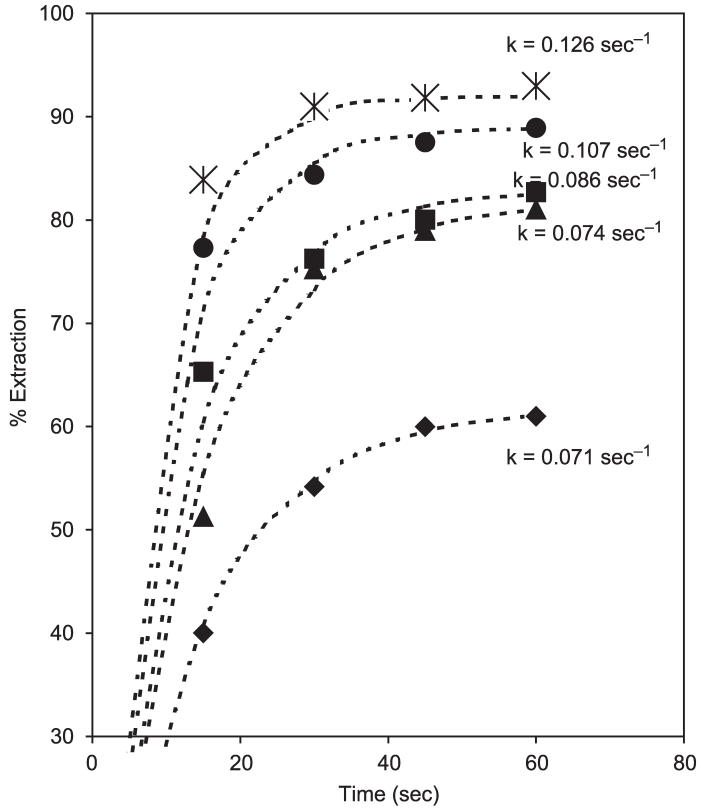


Figure 5. Effect of water soaking by raw material on microwave assisted extraction (solvent: acetone, power level: 20%, solid loading: 1% w/v). ◆: 0 Hr; ▲: 6 hr;: ■: 12 hr; ●: 8 hr; ✶; 24 hr.

was soaked in water for 12 hr. The extraction of curcuminoids reached around 90% during the same time of the extraction if the particles were soaked for 24 hr in water. An increase in the extraction rate constant was observed with the increase in percent hydration.

The extraction rate was very rapid with simultaneous microwave radiation as compared to conventional solvent extraction. The extraction is almost 60% in 1 min while the same percent extraction needs 1 hr by conventional extraction method. The absorbed water forms a tightly bound primary monolayer on proteins and lipid molecules within the cell (28). An increased dielectric permitivity of biopolymers with increasing hydration should lead to an enhanced

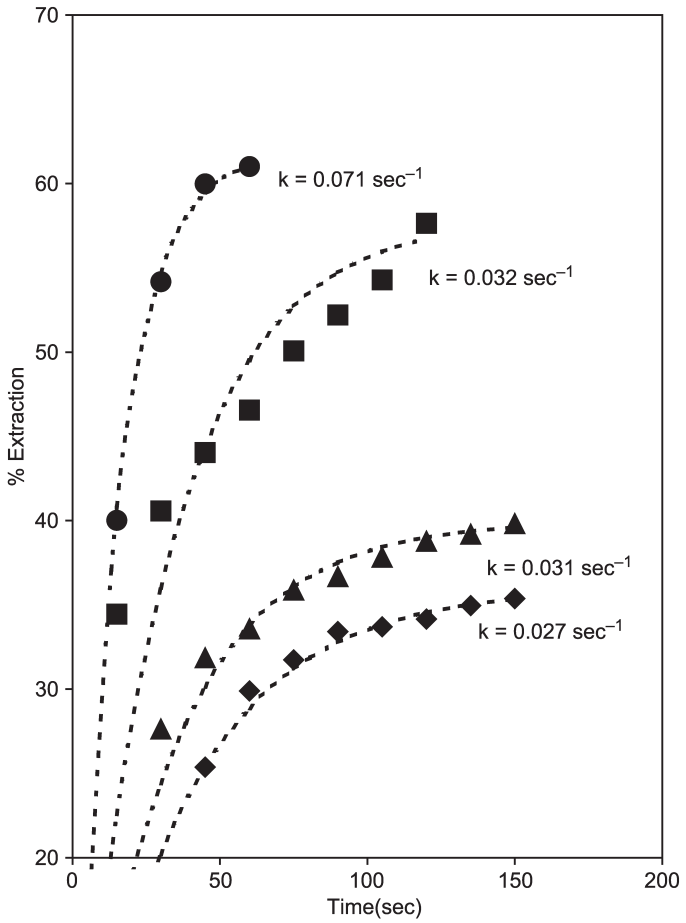


Figure 6. Microwave assisted extraction using different solvents (power level: 20%, solid loading: 1% w/v). ◆: DCE; ▲: IPA; ■: EtOH; ●: acetone.

dielectric heating within the cellulosic cell wall (29). The tensile strength of cellulosic cell wall is very high ($8.3 \times 10^6\,N/m^2$) because of a considerable number of hydrogen bonds which are stable only below 250°C (30). The absorbed water gets rapidly heated on exposure to microwave radiation and cellulose itself being ionic conductor, rapidly conducts this heat and consequently undergoes rapid hydrolysis (24,25). The intracellular polar compounds of the rhizomes, therefore, play an important role in the MAE.

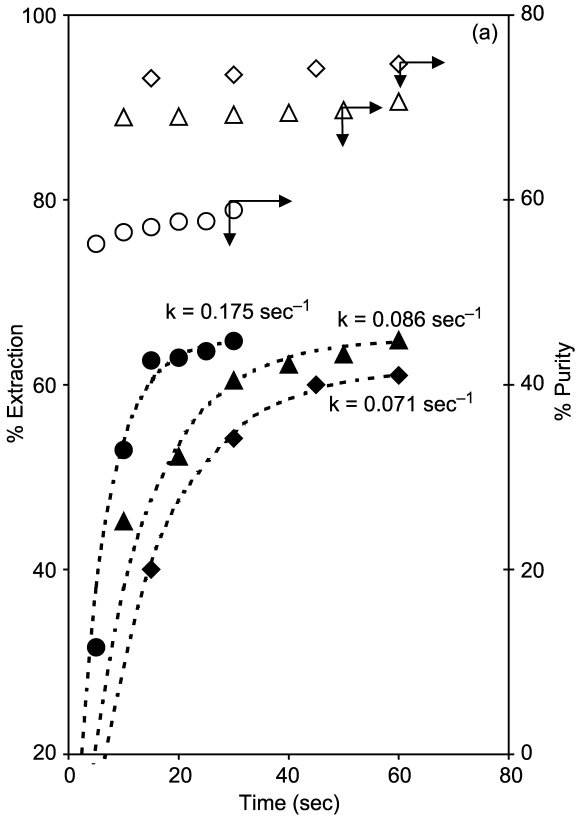


Figure 7. Effect of microwave power level (PL) on extraction (filled symbols) and purity (empty symbols). a. [Solid loading 1%] ◆: 20% PL; ▲: 40% PL; ●: 60% PL; b. [solid loading 2%] ◆: 20% PL; ▲: 40% PL; ●: 60% PL; c. [solid loading 5%] ◆: 20% PL; ▲: 40% PL; ●: 60% PL.

Effect of Solvent

Different solvents of varying polarity were investigated for their efficacy for the extraction of curcuminoids. Dichloroethane (DCE), isopropyl alcohol (IPA), ethyl alcohol (EtOH) (95%), and acetone were used for the MAE of curcuminoids as they have medium polarity, fairly high boiling points, and good solubility for curcuminoids. Less polar and high boiling point solvents like toluene and petroleum ether were inefficient in extracting curcuminoids because of the low solubility of curcuminoids. Highly polar solvents, however, may not be

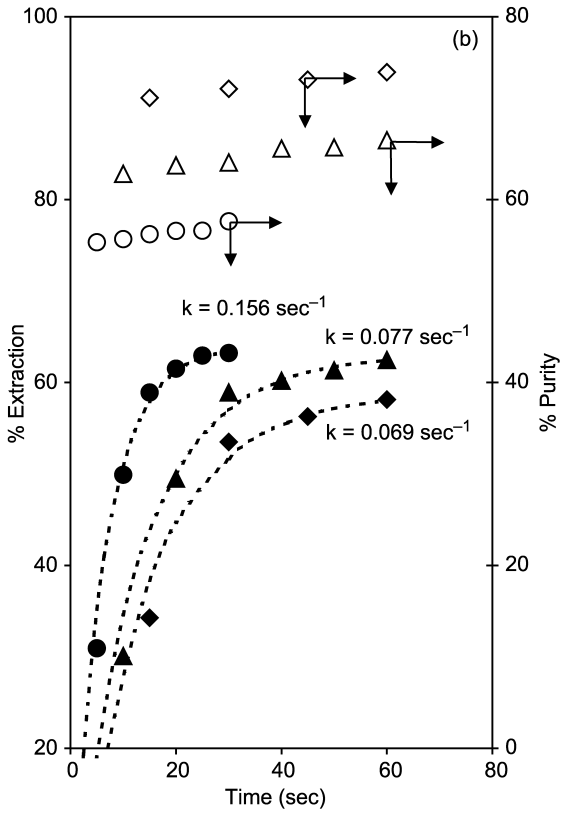


Figure 7. Continued.

(*continued*)

suitable for MAE as they themselves absorb a significant portion of the microwave radiation.

The temperature of the solvents was measured after 1 min of irradiation during the experiments and was found to be increasing steadily, and the increase was dependent on solvent polarity and power level (PL). The initial temperature of solvents was 30°C in all the experiments. Dichloroethane showed an increase of 5°C at 20% PL within a minute whereas IPA showed an increase of 6°C at the same PL. Ethyl alcohol also showed an increase of 6°C at 20% PL within a minute and acetone showed an increase of 8°C at the same PL within a minute of irradiation. When PL was increased further, the temperature increased to 10 and

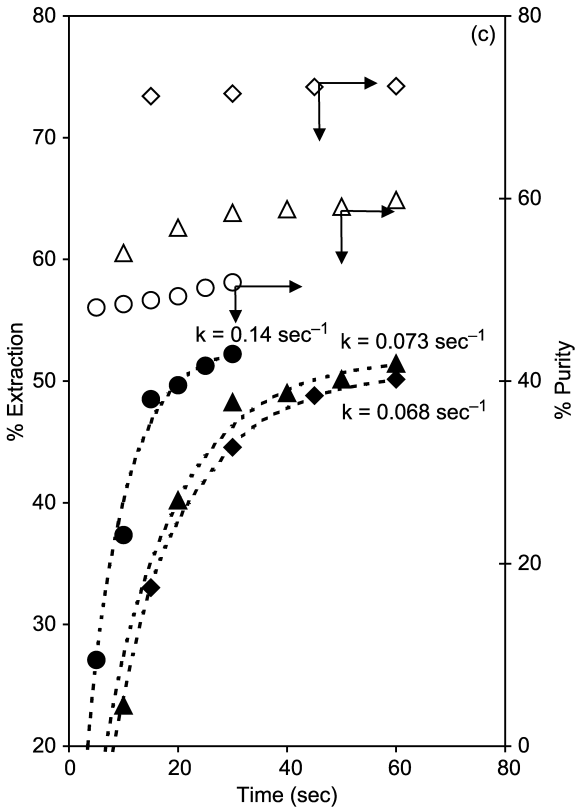


Figure 7. Continued.

12°C in case of acetone for 40 and 60% PL, respectively, whereas there was no effect on temperature of solid loading. In all the experiments the increase in temperature of water kept in the same microwave cavity was constant with respect to PL i.e., 10, 13, and 16°C within a minute at PL 20, 40, and 60%, respectively.

Figure 6 shows about 25–60% extraction of curcuminoids in 2 min from dry raw material. Amongst the solvents, acetone was found to be the best one, giving the highest extraction (around 60%) of curcuminoids in less than 1 min with 74% purity of curcuminoids. In the case of extraction from a natural complex matrix, the desired compound or the group of compounds is present in various cells in different parts of the raw material. In the process of leaching these compounds, the solvent has to reach and dissolve them. Solvent usually attacks

the cell wall of raw material and penetrates it to reach the compounds. It also dissolves various other impurities in the process. Thus for efficient extraction, solvent penetration through the cell wall should be fast and selectivity towards desired compound should be high.

The extraction rate constant and percent extraction were observed to increase with solvent polarity and solubility of curcuminoids. A solvent with higher polarity and higher solubility of curcuminoids helps in more efficient leaching of curcuminoids in the MAE.

The purity of curcuminoids in extracts was observed around 70–75% in all the four solvents. However, the extraction rate was maximum in acetone. The heating rate of acetone was also high compared to other solvents. Thus, probably, relatively hot acetone attacks the cell wall more efficiently and reaches the curcuminoids rapidly. It can also dissolve curcuminoids more efficiently than the other three solvents. Moreover, acetone, being the best solvent for dissolving curcuminoids, showed a good selectivity towards curcuminoids. It thus gave higher extraction rates with a relatively high purity of curcuminoids.

The disadvantage of acetone as a solvent, however, is its rapid heating, which can lead to its relatively faster evaporation from the extraction vessel. The microwave irradiation time need to be reduced to avoid the solvent loss. Yet, overall efficiency of acetone was found the best for the extraction of curcuminoids.

Effect of Power Level

Three different PL were studied viz. 20, 40, and 60% to optimize the PL for efficient extraction of curcuminoids using acetone. Initial studies at higher PL viz. 80% or more resulted in almost 50% solvent loss within 120 sec. Figure 7a–c show that as PL was increased, percent extraction of curcuminoids also increased for a given time, but the purity of curcuminoids decreased. The solvent loss from extraction vessel at the same time increased substantially. At 20% PL, a 10% solvent loss was observed in 120 sec, whereas in the case of 40 and 60% PL it reached to 16 and 20%, respectively, in the same time. The extraction data were fitted into Eq. (1) and the estimated extraction rate constants are shown in Fig. 7a–c. An increase in the extraction rate constant was observed with the increase in PL as expected.

The domestic microwave oven used in this study operates on the principle of delivering power in cycles. When PL is set to 20% then the microwave delivers full power for 20% time and for remaining 80% of the cycle time it delivers no power. Thus, when PL was increased the overall power delivered was more. The increased PL of the microwave radiation gave longer exposure time and consequently enhanced dielectric heating. A remarkable swelling and coalescence of the cellular material was observed during microwave irradiation.

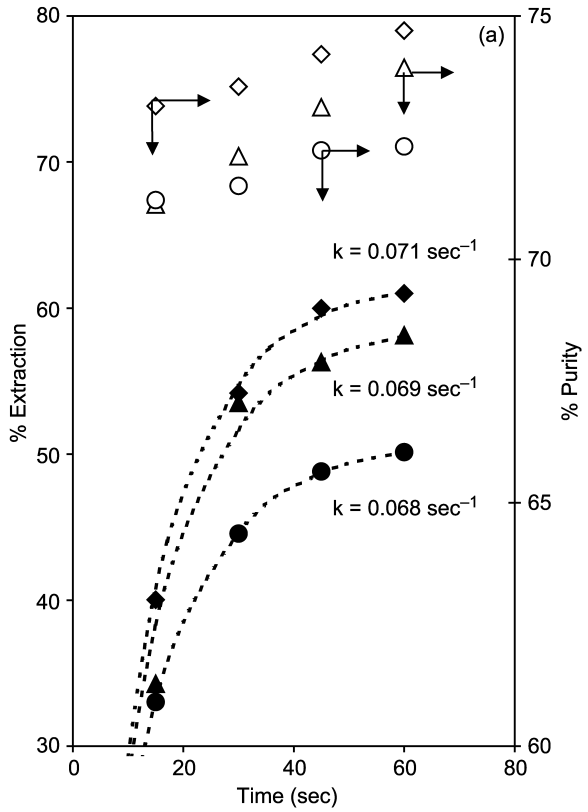


Figure 8. Effect of solid loading (SL) on extraction (filled symbols) and purity (empty symbols). a. [Power level 20%] ◆: 1% SL; ▲: 2% SL; ●: 5% SL; b. [power level 40%] ◆: 1% SL; ▲: 2% SL; ●: 5% SL; c. [power level 60%] ◆: 1% SL; ▲: 2% SL; ●: 5% SL.

The volatile and fixed oil globules on vaporization can increase the internal pressure beyond the stability of the cell wall. At same time, the protein structures agglomerate into larger particles. At low PL, this coalescence might take place over longer periods of time but at higher PL the rupture and confluencing of smaller lipid bodies into agglomerates occurs at an early stage of heating (31). This leads to a faster pressure built-up within the cell and faster softening of the cell wall. In addition, the increased PL can hydrolyze the cellulosic cell wall at a faster rate.

The solvent heating is also rapid and relatively hot solvent dissolves more curcuminoids as well as the impurities. Increased PL of microwave radiation thus

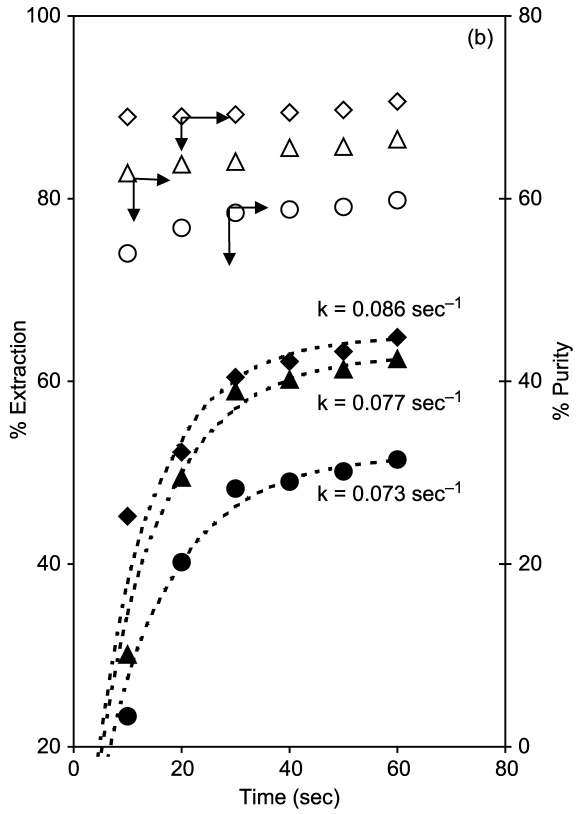


Figure 8. Continued.

(*continued*)

gives more extraction in lesser time but decreasing the purity of curcuminoids in the extract.

Since at 20% PL, the microwave radiation gave 60% extraction in 1 min with better purity of curcuminoids and the solvent loss from the extraction vessel was minimum, it was considered as the best PL.

Effect of Solid Loading

Three different solid loading were studied viz. 1, 2, and 5% (w/v) to estimate the effect of solid loading on extraction efficiency. Figure 8a–c show the

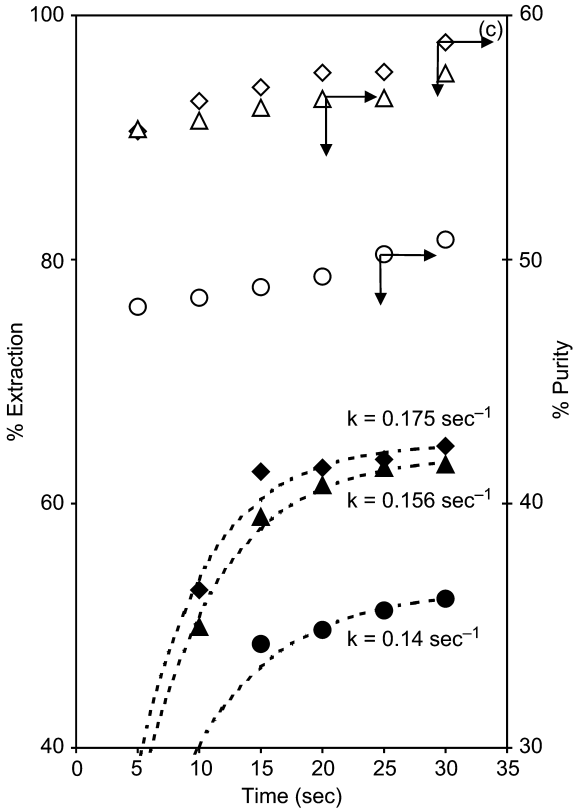


Figure 8. Continued.

effect of solid loading on extraction efficiency and purity of extraction. With the increased solid loading, particularly at 5% (w/v) solid loading, a decrease in percent extraction was observed. A decrease in the extraction rate constant was observed with the increase in solid loading for constant PL. The values of rate constant and maximum extraction of curcuminoids obtained under these conditions are reported in Table 1.

The incident microwave radiation per particle decreased with the increased solid loading at a given PL. This should give a relatively low dielectric heating effect, and thus a reduced effect of microwave radiation. The absorption of microwave radiation near the surface of vessel reduces the penetration depth of microwave radiation into the suspension (32). The raw material in the interior part of vessel, thus, will not be subjected to the same level of microwave radiation

Table 1. The Extraction Rate Constant and Maximum Extraction Achieved at Different Conditions

Dry Irradiation (min)	k (min^{-1})	b (%)
0	0.096	64
2	0.125	73
4	0.126	74

Water soaking (hr)	k (sec^{-})	b (%)
0	0.071	62
6	0.074	82
12	0.086	83
18	0.107	89
24	0.126	93

Solvent	k (sec^{-1})	b (%)
Dichloroethane	0.027	36
Isopropyl alcohol	0.031	40
Ethyl alcohol (95%)	0.032	58
Acetone	0.071	62

Effect of power level and solid loading		K (sec^{-1})			b (%)		
Power level	Solid loading (w/v)	1%	2%	5%	1%	2%	5%
20%		0.071	0.069	0.068	62	59	51
40%		0.086	0.077	0.073	65	63	52
60%		0.175	0.156	0.14	65	64	53

as that which is nearer the vessel surface. In suspended conditions, the time a particular particle experiences the microwave radiation should decrease on increasing solid loading.

In MAE of curcuminoids the rate of extraction is strongly dependent on solubility of curcuminoids in the solvent and the amount of power absorbed by turmeric raw material, which in turn is dependent on the polar contents of raw material. Also as incident microwave radiation per unit volume of extractor is constant for a particular PL, the effective absorption by raw material is inversely proportional to the suspension density of the raw material in the solvent. Detail calculations, however, are not possible due to nonavailability of reliable dielectric data for turmeric raw material. In addition, as composition of raw material changes according to season and area of cultivation, constants calculated may vary with the changes in raw material.

We believe this technique should be applicable to most natural products if they are nonvolatile and do not undergo any degradation on thermal treatment.

CONCLUSION

Microwave radiation directly affects the cells of natural raw material. The internal fast heating of cells leads to dielectric heating and coalescence of cellular matter, and ultimately the rupture of cell wall. These changes in cellular structure and solid matrix provide facilitated transport of the solvent into the solid structure and faster extraction of curcuminoids.

The extraction process was optimized at 20% PL giving 60% extraction of curcuminoids with 75% purity within 1 min. Compared to other classical extraction techniques, MAE gives an efficient and rapid extraction, which is less labor intensive.

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