

Microwave-Assisted Extraction of Piperine from *Piper nigrum*

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A novel microwave-assisted technique (MAE) for the extraction of piperine from coarsely powdered black pepper (*Piper nigrum*) was studied. The parameters such as nature of the solvent, microwave energy input, and solid loading were optimized. The mechanism of the enhancement of extraction rates was investigated by microscopic studies of the irradiated material. Studies have revealed that dielectric heating of the polar cellular matrix resulted in remarkable swelling and coalescence of the oil cells and other constituents. The resulting pressure, built-up within the cell, breaks open the cell, releasing the constituents and providing easy access for solvent penetration and subsequent solubilization of piperine and other substances. Because the cell wall is mainly composed of slightly ionic cellulose, there is a marked increase in the dielectric heating rates. Rapid degradation of the cellulosic cell wall occurs, thereby further increasing the permeability of the wall toward solvent penetration. Selective extraction of piperine in nonpolar solvents to the extent of 94% with a purity of 85% has been achieved. The MAE procedure is simple, rapid, and reliable.

Introduction

Piperine (C₁₇H₁₉O₃N), one of the major constituents of black pepper (*Piper nigrum*), has received increasing attention in recent years, because of its medicinal properties and as bio-availability enhancer in formulations of several drugs.^{1,2} The methylenedioxyphenyl group of piperine also has insecticidal activity.³ Piperine shows a protective effect against radiation and therefore could be administered to cancer patients before radiotherapy.⁴

Conventionally extraction of piperine is carried out by solvent extraction using aliphatic and chlorinated hydrocarbons, for extended periods stretching over 16–24 h.⁵ The extraction of piperine into these organic solvents is, however, not selective, and the extract invariably contains resins and gums as major components. The oleoresin extract containing about 40–50% piperine can be used as such or needs to be processed further to recover 95–98% pure piperine particularly for pharmaceutical applications. Treatment with aqueous alkali solutions and crystallization from aqueous alcoholic solutions are the most common methods of purification of piperine from the extract which further reduces the yield of piperine.⁵

Piperine extraction has been reported using either dense or supercritical carbon dioxide or carbon dioxide + ethanol mixtures.^{6–8} The extract contains essential oils and piperine, of the same composition as that obtained by extraction using dichloromethane, and further solvent purification steps are still essential for obtaining piperine free from oleoresins.⁶ The supercritical fluid extraction overcomes the problems associated with volatile, toxic, and inflammable solvents, but the purity and yield of piperine are comparable to those obtained by organic solvent extraction.

In recent years, microwave radiation is being extensively investigated for solventless extraction of volatile organic chemicals from a wide variety of natural

materials.^{9–11} The volatile components are rapidly vaporized from the solid matrixes on absorption of microwave energy and then recovered by condensation. Microwave-assisted extraction (MAE) of the fungal metabolite ergosterol¹² and dry extraction of *Achillea millefolium* for its fragrant volatile oils,¹³ menthol from *Mentha piperita*¹⁴ and rapeseed oil from *Brassica napensis*,^{15,16} are some of the well-studied processes. MAE looks promising for those substances which do not undergo any thermal degradation because of localized heating.¹⁷

The heating by microwaves is because of interaction of the radiation with the dielectric field associated with polar molecules and ions. The microwave radiation interacts with dipoles of polar and polarizable materials. For nonpolar solvents, only atomic and electronic polarizations contribute to their dielectric absorption and heating rates by microwave irradiation are, therefore, poor. The concerted forces applied by the electric and magnetic components of the microwave radiation are rapidly changing in direction (2.4×10^9 /s) at a frequency of 2450 MHz, causing the heating of the polar molecules as these molecules try to orient themselves in the direction of the field.¹⁸ Because solids, semisolids, and liquids cannot respond instantaneously to the changing directions of the microwave field, the friction among the molecules manifests itself as heat.¹⁹ The thermal effect is almost instantaneous at the molecular level. This effect, however, is limited to a small area near the surface of the dielectric material because microwaves have a low penetration depth and are completely absorbed at the boundary. In large particles and agglomerates of small particles, this may lead to inhomogeneous distribution of heat. The heating of the rest of the material results from passive distribution of the heat along the temperature gradient from the outer areas to the center by conduction. It may be possible to adjust the wavelength of the microwave radiation so that the energy at the source itself is high and the depth of penetration is more. Usually in a microwave-absorbing medium the power of the microwave radiation falls almost exponentially over the distance.

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Extraction of piperine using microwave radiation has not been previously reported because piperine is not a volatile compound. In this study, we have considered a hybrid process of microwave radiation with conventional solvent extraction to recover piperine from *P. nigrum* fruits. Assessment of piperine from the cell structure is facilitated by exposure of the whole fruits to the microwave radiation, which influences the microstructure of the whole pepper fruits. Enhanced mass-transfer rates and the high selectivity for piperine extraction are the major observations of this work.

Experimental Section

Whole pepper berries were obtained from M/s. Cancor India Ltd., Cochin, India. *P. nigrum* fruits are sessile globose drupes 4–6 mm in diameter with a pulpy pericarp. They are spherical and have a deeply and coarsely reticulated wrinkled dark brown surface. First air-dried *P. nigrum* fruits were pulverized in a high-speed mixer. The particles were then mechanically sieved into batches of different sizes. The particles of mesh size no. 6 were selected for all of the experiments unless otherwise stated. The microwave extraction experiments were performed in an IFB domestic microwave oven (model Neutron) operating at 2.45 GHz with a radiation output of 750 W. Conventional Soxhlet extraction of pepper was separately carried out using petroleum ether for 16 h in order to completely determine the piperine present in the raw material. The total piperine content was then estimated to be 5.1% using high-performance thin-layer chromatography (HPTLC).

The extraction studies were conducted by two methods.

(i) In the first technique, the raw material (2.0 g) was evenly spread on a glass tray with an average thickness of 2–3 mm. The tray was then placed in the microwave oven cavity of 25 dm³ capacity. The particles were subjected to microwave radiation for a prespecified time, then taken out of the oven, and suspended in petroleum ether. The time of exposure to microwave radiation was maintained constant at 2 min. Extraction experiments were carried out in a fully baffled 0.1 dm³ borosilicate cylindrical glass reactor (9 cm height and 7.0 cm internal diameter) equipped with a six-bladed turbine impeller having a diameter of 2 cm and four baffles. This entire assembly was kept in a constant-temperature bath. The samples of the solution were withdrawn at 15 min intervals and analyzed for the piperine content. The speed of agitation in the solvent extraction step was also kept constant at 1100 rpm to ensure rapid extraction and to eliminate external mass-transfer resistance, if any. It was independently verified that the speed of agitation has no effect on the rate of extraction beyond this rpm.

(ii) For the second technique, the microwave oven was modified. The modified setup (Figure 1) contained a glass vessel (15 cm height and 2 cm internal diameter) within the oven cavity, with a provision for continuous nitrogen sparging to maintain an inert atmosphere inside the vessel. The gas was sparged into the suspension of the raw material in an organic solvent, and the flow rate was maintained just enough to keep the particles in suspension. No additional hole was bored into the cavity. The holes present in the roof of the cavity and the provision present in the oven for insertion of thermal probe were used to pass the polypropylene tubes to carry the vapors formed from the extraction

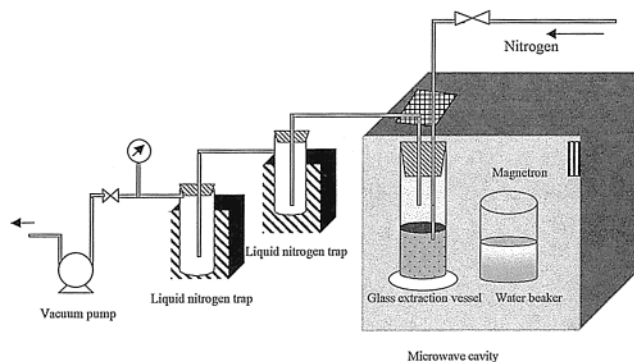


Figure 1. Modified setup of the microwave oven.

vessel. The effluent gas was then passed through two liquid-nitrogen traps, outside the oven cavity, to trap any volatile material including the organic solvent vaporized from the extraction vessel during the microwave treatment. In most cases, the condensate inside the nitrogen traps contained no piperine but about 1% volatiles. In most cases, however, especially with polar solvents such as ethanol, a significant amount of organic solvent (up to 20% of the initial charge) was recovered along with traces of volatile oils from black pepper. In the case of nonpolar solvents and less polar solvents such as heptane and petroleum ether, the solvent was recovered to the extent of 2.0–3.0% of the initial charge. The sample solutions from the extraction vessel were removed after a predetermined time to estimate the rate of extraction and the purity of piperine in the extract.

A 0.25 dm³ beaker containing 0.2 dm³ water was kept in the same microwave cavity along with the extraction vessel to avoid excessive heating of the sample. The extraction vessel and the beaker containing water were kept at the same positions in all of the experiments.

Analytical Procedure

The analysis of the extract phase was performed by HPTLC on 10 cm × 20 cm plates precoated with silica gel 60 F₂₅₄ (Merck, FRG 5715). The samples (2 μL) were applied to the plates using Linomat IV according to the data pair technique. A toluene–ethyl acetate (70:30, v/v) mixture was used as the eluent without chamber saturation. The chromatogram was developed to a distance of 9 cm. For the compound quantitations, calibration curves were plotted using standard piperine (purity 99.9%) as the external standard. The chromatograms were evaluated on the basis of peak areas measured at λ = 343 nm using a CAMAG HPTLC unit equipped with a TLC Scanner III densitometer operated with CATS software (version 3.04).

Results and Discussion

Our first attempt was to investigate the effect of microwave radiation on the pepper structure in the first step, and subsequently the extraction of piperine from pepper into petroleum ether was studied in the second step. Figure 2 compares the efficiency of petroleum ether (bp 60–80 °C) for extraction of piperine from microwave-treated pepper and untreated pepper. The extraction efficiency is based on the net amount of piperine and not on the basis of the total raw material charged into the extractor because the piperine content in the raw material varies from source to source. The most striking feature between two extraction curves is the difference

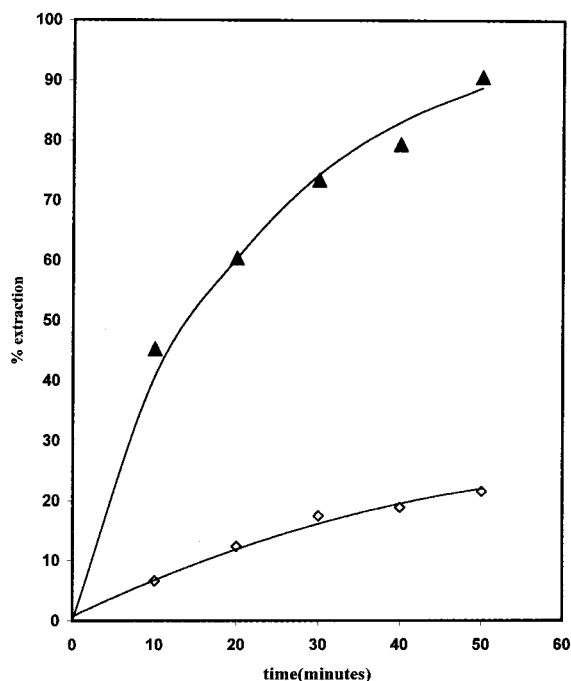


Figure 2. Extraction of piperine using petroleum ether with time before and after exposure to microwave treatment. Microwave exposure time: 120 s. Particle size: no. 6 mesh. Power level: 150 W. Solvent: petroleum ether (◇, untreated; ▲, microwave treated).

in the extents of extraction in the same time period, and the other is the rates of extraction. The nonirradiated pepper gave very poor recovery of piperine, about 20% in 1 h of intense agitation. The initial extraction seems to be from exposed piperine crystals from pulverized pepper, but in the later stages, the rate of extraction is slow. An enhanced recovery of piperine was observed from the pepper sample irradiated with microwaves for 2 min, almost reaching 95%, during the same time of extraction. The rate of extraction is also fast with a nonlinear behavior with time (Figure 2).

The percentage of extraction was fitted in a first-order process equation:

$$\% E = 100(1 - e^{-kt}) \quad (1)$$

The extraction rate constant (k) expresses the ease of extraction. The rate constant k increased from $4.237 \times 10^{-3} \text{ min}^{-1}$ for nonirradiated samples to 0.0415 min^{-1} for irradiated samples. The increased rate constant is about an order of magnitude higher than that for the nonirradiated extraction. An 80% increase in the extraction of piperine indicates the rapid extraction after microwave treatment as compared to 20% extraction without subjecting pepper to microwave irradiation before extraction.

The increased rates of extraction of piperine from irradiated samples are apparently because of facilitated access of the solvent to the piperine situated in the innermost parts of the cellular structure, and this enhancement is achieved because of cell breakage on exposure to microwave radiation. The cell wall in *P. nigrum* fruits is made up of cellulose crystallites, which are present in an ordered manner. The cell wall as a whole is inhomogeneous because it shows two phases upon swelling with water.²⁰ The pericarp of the pepper fruits consists of epicarp, mesocarp, and endocarp. Many of the pericarp cells are packed with small crystals, which are highly birefringent under crossed nicols. The

crystals are packed in an irregular manner within the parenchyma cells. Although not fully proven, it appears that these are crystals of piperine which make up 4–5% of the fruit weight.²⁰

The treatment of the dry pepper samples by microwave radiation leads to the hydrolysis of the cellulosic cell wall. The presence of polar $-\text{CH}_2\text{OH}$ and $\text{C}-\text{OH}$ groups in the glucopyranose residues gives relaxation times in the range of 170–300 ps.²¹ The cellulosic cell wall has a large molecular mass and significant hydrogen bonding within itself.²¹ Their localized fluctuations under microwave influence lead to an enhanced heating of the cellulosic material, which breaks down from its ordered structure to form different glucans.²² Because of dielectric heating of the polar components within the cell, temperatures to the order of 300 °C are reached and rapid hydrolysis of the cellulosic ether linkages occurs, which weakens the cell wall structure.²³

Microwave-dried products have been reported to show a higher porosity, generated because of the internal vapor formation from the volatile components.²⁴ When microwave energy is applied to raw material, the volatile compounds first vaporize within the cell. The intracellular material including cellular water and oil globules coalesces and creates a huge pressure on the cell membrane due to the vapor accumulation inside the cellular structure.^{25,26} The coalesced bubbles nucleate and expand, causing structural changes in the cell from within. The coalesced material is pushed toward the cell wall, and the pressure buildup inside the cell eventually overcomes the mechanical strength of the cell and the structure explodes.^{27,28} The organic solvent can now rapidly diffuse into the more open structure and dissolves piperine. The breakup of the cell wall removes the major resistance to mass transfer to and from the cell structure. This results in the increased mass-transfer rates of dissolved substances.

To further investigate the mechanism of the rapid extraction rates, sections of whole *P. nigrum* fruits were studied by microscopy, before and after microwave irradiation, and images were scanned by an Image Pro Plus Analyzer. The scanned images are shown in Figure 3a–f. Figure 3a shows intact thin-walled parenchymatous and rectangular stone cells. The cell walls are made up of ordered cellulosic crystallites in the form of elongated polyhedrons. These cell walls can swell when soaked in water, showing a distinct inhomogeneity of two phases. The cell wall returns to its original dimensions on drying. Figure 3b shows ruptured cell walls on exposure to the microwave radiation. The cells also appear to be expanded after exposure to the radiation. Parts c and d of Figure 3 respectively show the oil cells before exposure to the microwave radiation and coalescence of the cellular constituents within the oil globules, which are then pushed toward the wall after exposure to radiation. Parts e and f of Figure 3 depicting the epidermal pericarp layer and palisadic layers of the pepper fruit sections, before and after exposure to microwave radiation, respectively show a substantial swelling and breakup of the cells.

Because the presence of polar components augments the effect of microwave radiation, a similar set of experiments was conducted with water-soaked whole *P. nigrum* fruits. Figure 4 compares the extraction of piperine from whole pepper fruits after soaking in water for different periods of time, followed by microwave radiation exposure for 120 s and then extraction using

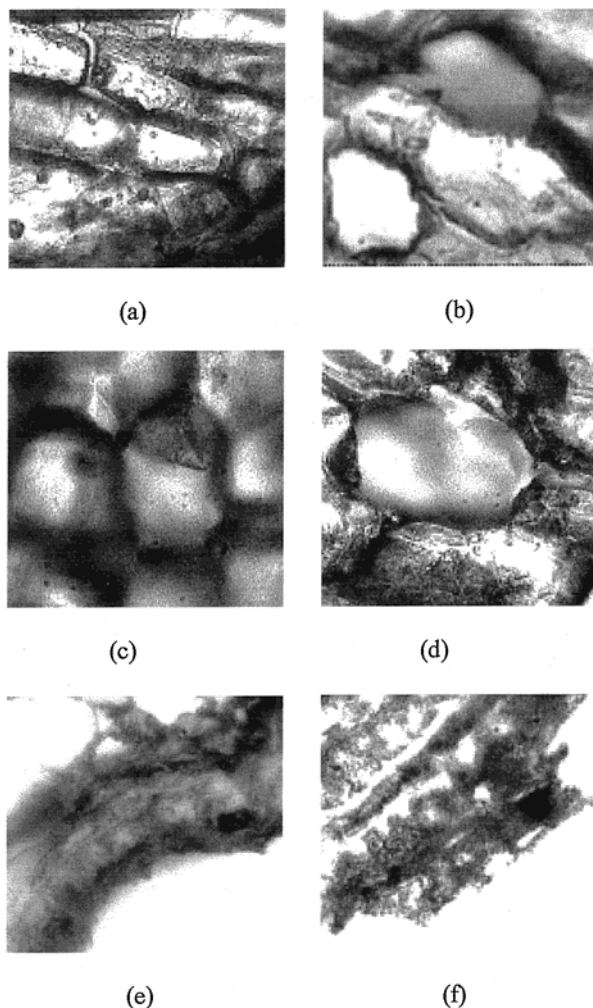


Figure 3. Structural changes in microwave-irradiated *P. nigrum* fruits. (a) Intact section of *P. nigrum*. (b) Section of *P. nigrum* after exposure to microwave radiation. (c) Intact section of *P. nigrum* showing oil cells. (d) Section of *P. nigrum* showing expanded oil cells after exposure to microwave radiation. (e) Section of *P. nigrum* showing the intact epidermal layers before exposure to microwave radiation. (f) Section of *P. nigrum* showing the expanded epidermal layers after exposure to microwave radiation.

petroleum ether. The percentage of water in the whole fruits increased from 28% in 1 h to 45% after soaking for 4 h. The water content was determined by taking the difference in the weight of the dry pepper samples and the soaked samples. The extraction rate constant also increased with the percentage of water absorbed by pepper. The rate constant with the dry pepper fruits is $1.77 \times 10^{-3} \text{ min}^{-1}$, which increased to $2.44 \times 10^{-3} \text{ min}^{-1}$ after soaking in water for just 1 h. Within 4 h of soaking, the rate constant value increased to $1.01 \times 10^{-2} \text{ min}^{-1}$ (Figure 4). There is a sixfold increase in the extraction rate after soaking pepper fruits in water for 4 h. The samples, which were soaked for a longer time and have a higher percentage of water, showed very high rates of extraction as compared to those soaked for shorter periods. The increase in the extraction efficiency is strongly dependent on the hydration of raw pepper (Figure 5).

To recover piperine from the cellular structure, intact or fragmented, the solvent should have access to the sites where piperine is located. An intact cell structure restricts accessibility to piperine, while microwave-treated cells should have a more open fragmented

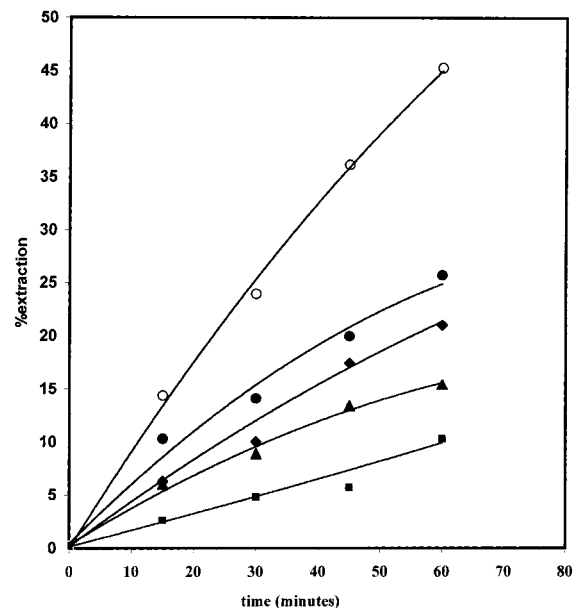


Figure 4. Extraction of piperine from whole pepper fruits with time before and after soaking in water and exposure to microwave radiation. Size of particle: no. 6 mesh. rpm: 110. Solvent: petroleum ether. Power level: 150 W (■, 0 h; ▲, 1 h; ◆, 2 h; ●, 3 h; ○, 4 h).

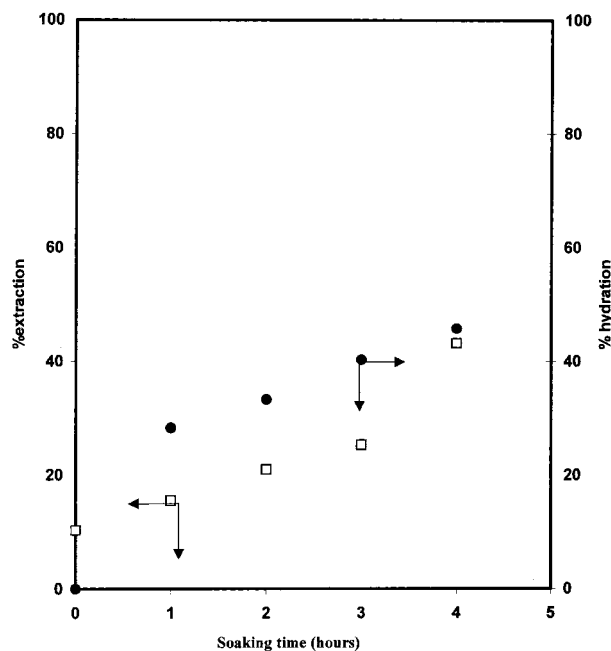


Figure 5. Variation of % extraction of piperine and % hydration with soaking time. Microwave exposure time: 2.0 min. Power level: 150 W. Solvent: petroleum ether (□, % piperine; ●, % hydration).

structure. The enhanced extraction of piperine in organic solvents should be because of the increased diffusion of the solvent into the cell structure followed by solubilization of piperine.

The dielectric permittivity of biopolymers increases with the degree of hydration and, therefore, leads to a rapid dielectric heating.²⁹ Water within the pepper cell forms a tightly bound primary monolayer, adsorbed on the protein and lipid molecules, causing a larger dielectric loss and thus increasing the rate of dielectric heating.³⁰ The heat thus generated is conducted rapidly via protons and other ionic species that are able to

migrate around the surface of the hydrated protein and lipid molecules.³¹ Though the tensile strength of the cell wall is very high (82.7 MPa)³² and cellulose has a considerable number of hydrogen bonds, which accounts for its strength, such hydrogen bonds are unstable above 250 °C and are rapidly hydrolyzed. Above 300 °C weakening of these bonds facilitates the rupture of the cell wall.²¹ The cellulose itself is an ionic conductor and can conduct rapidly dielectric heat generated within itself or the cell.³³ The fact that the samples soaked for longer periods in water showed enhanced extraction rates indicates that the polar components within the cellular matrix possibly attain very high temperatures at least above the limit of the stable cellulose structure which hydrolyze the cellulosic cell wall rapidly.

The extraction of pepper by a conventional Soxhlet extraction procedure, without any microwave treatment, yielded 43% oleoresins, containing 2.2% piperine in 120 h.

To increase the extraction efficiency and improve the recovery of piperine from pepper, further experiments were carried out in the microwave cavity by combining irradiation and extraction steps in a single unit. The effect of various operating parameters such as nature of the solvent, microwave radiation power, and percentage of solid loading on the extraction of piperine from pepper was examined.

Selection of the Solvent. Various solvents, ranging from polar to nonpolar, were studied for the extraction of piperine from *P. nigrum*. The choice of solvent for the MAE of piperine is dictated by the solubility of piperine in the selected solvent and its dielectric properties. Polar and polarizable solvents, which have a permanent dipole moment or an induced dipole, have relaxation processes in the microwave region and, therefore, absorb microwave radiation. Nonpolar solvents and poorly polarizable solvents, without any dipole moment, have no relaxation processes in the microwave region and are, therefore, transparent to microwaves. The capability, the extent /depth of penetration, and the efficiency of heating by microwaves depends on the polar nature and the dielectric property of the material. The power of microwave radiation drops exponentially while propagating through a loose material. The residual power at any point depends on the input power, the attenuation constant of the material, and the depth of penetration.

Figure 6 shows that piperine was extracted largely in the nonpolar solvents such as petroleum ether. The repeatability has been plotted as bars to indicate the variation in repeated experiments. The purity of the extracted piperine was also high, approximately 95% with petroleum ether. However, with more polar solvents such as dichloromethane and ethanol, piperine was extracted only to an extent of 75–80%, also with a lower purity of 72% and 60%, respectively. The extraction efficiency has been described on the basis of the actual content of piperine present in the raw material.

This increased rate of extraction and the selectivity with respect to piperine when nonpolar solvents are used for extraction could be due to the increased penetration depth (D_p) of the microwave radiation. It is defined as the depth into a material where the microwave power falls to half of its value at the surface. An approximate relationship for the D_p of microwaves is given as³⁴

$$D_p \propto \lambda_0(\epsilon'/\epsilon'')^{1/2} \quad (2)$$

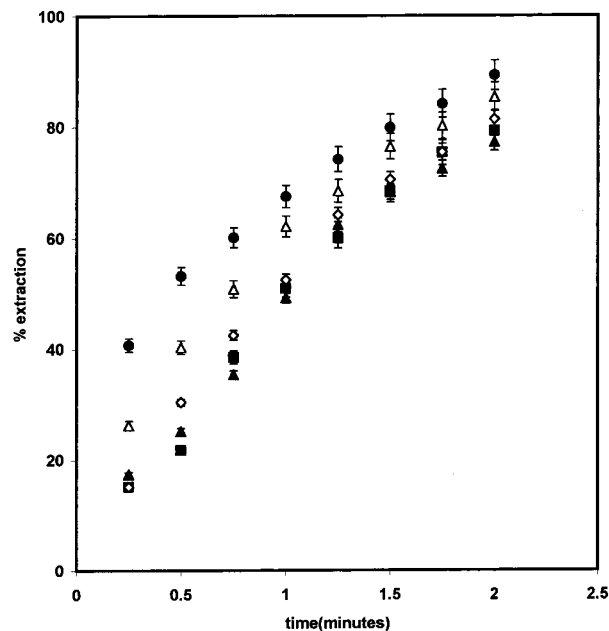


Figure 6. Extraction of piperine using different solvents with time. Power level: 150 W. Solvent: petroleum ether. Particle size: no. 6 mesh. Time of exposure: 2 min (□, ethanol; ▲, dichloromethane; ◇, toluene; △, heptane; ●, petroleum ether).

where λ_0 = free space wavelength of radiation (cm), ϵ' = dielectric constant (ability of the molecule to be polarized by the electric field), and ϵ'' = dielectric loss (the efficiency with which the electromagnetic radiation is converted into heat).

Polar solvents have a smaller penetration depth for microwave radiation because of the significant absorption of radiation by these solvents themselves. From the reported dielectric constants³⁴ and the dielectric losses,³⁴ the penetration depths of ethanol and dichloromethane are estimated to be 24.5 and 59.6 cm as compared to hexane and heptane, which have longer penetration depths of 1185.9 and 1210.8 cm, respectively. The extraction of piperine was nearly 80% with polar solvents, but the rate of extraction was initially slow and then increased rapidly. For a polar solvent, the total polarization effect is mainly dominated by dipolar polarization (α_d), resulting from the orientation of permanent dipoles by the electric field and the interfacial polarization (α_i), which occurs when there is a buildup of charges at the interface.³⁴ In the microwave range of frequencies, the time in which the field changes is the same as the response time of the dipoles. The time lag indicates that the polar solvents absorb energy from the field and are heated rapidly. In addition to the dielectric heating taking place within the cell, the polar solvents also contribute to the heating effects causing more damage to the cell wall and increase the rate of cell rupture. Lower selectivity was obtained in this case because of the extraction of oleoresins from the cellular matrix along with piperine because of the enhanced ability of the hot polar solvent to penetrate within the cell and dissolve the oleoresins also. For nonpolar solvents, only atomic polarizations (α_a) and electronic polarizations (α_e) are predominant. Atomic polarizations arise from the relative displacement of the nuclei to the unequal charge distribution within the molecule, and electronic polarization arises from the realignment of the electrons around the specific nuclei. Because the time scales for these polarizations are much faster than

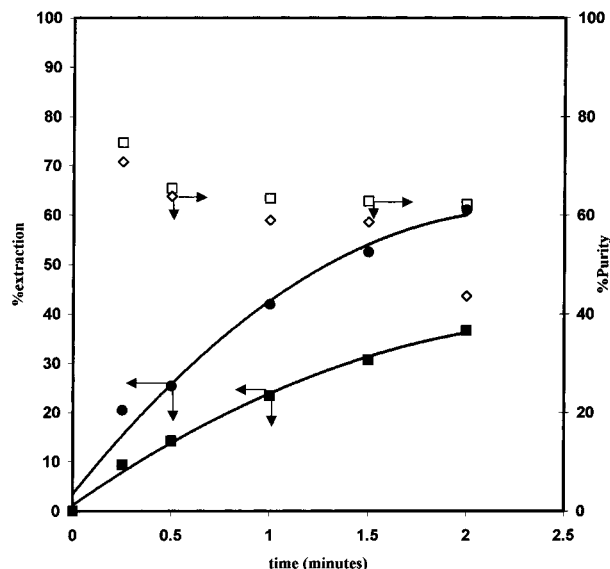


Figure 7. Extraction of piperine with time using mixed solvents. Power level: 150 W. Solvent: petroleum ether. Particle size: no. 6 mesh (●, petroleum ether/EtOH (3:1); ■, toluene/EtOH (3:2); ◇, petroleum ether/EtOH (% purity); □, toluene/EtOH (% purity)).

the microwave frequencies, these effects do not contribute significantly to solvent heating.³⁴

To minimize the solvent heating effects, petroleum ether can be used as a solvent for extraction of piperine. The polar compounds present within the *P. nigrum* cells only absorb the incident radiation. This results in selective extraction of piperine from the raw material into nonpolar solvents.

Extraction with Mixed Solvents. To tailor the microwave energy absorption in an initially nonpolar phase, the extraction was studied with mixtures of petroleum ether/ethanol (3:1) and toluene/ethanol (3:2). The selectivity of extraction was, however, reduced in these cases as compared to the pure nonpolar solvents (Figure 7). The addition of small amounts of a polar solvent with a large dielectric loss tangent usually leads to high heating rates for the mixtures of polar and nonpolar solvents.²² Nonpolar organic solvents have negligible dielectric loss and, therefore, do not couple efficiently with microwaves. The addition of a small amount of alcohols can lead to dramatic coupling effects.³⁴ The energy transfer between the polar molecules, which are coupled with microwaves and the nonpolar solvent, is rapid and, therefore, this provides an effective mechanism for increased cell rupture, leading to the extraction of unwanted oleoresins and thus reducing the selectivity of extraction. Because the selective extraction process requires the hydrophobic properties of nonpolar solvents, the use of these solvent mixtures did not provide any additional benefits.

Effect of Different Power Levels. Because petroleum ether was found to be the most suitable solvent for the extraction of piperine, further extraction studies were conducted with petroleum ether to study the effect of various power levels on the microwave-enhanced extraction. The initial studies at higher power levels of 300 and 450 W and longer irradiation times resulted in a significant solvent loss of 16–20%. At the power level settings of 150 W, the solvent loss reduced to 8% in 2 min. Figure 8 shows that even at a 150 W power level a considerable extraction of piperine occurred within 120 s. Hence, this was used to obtain a more efficient

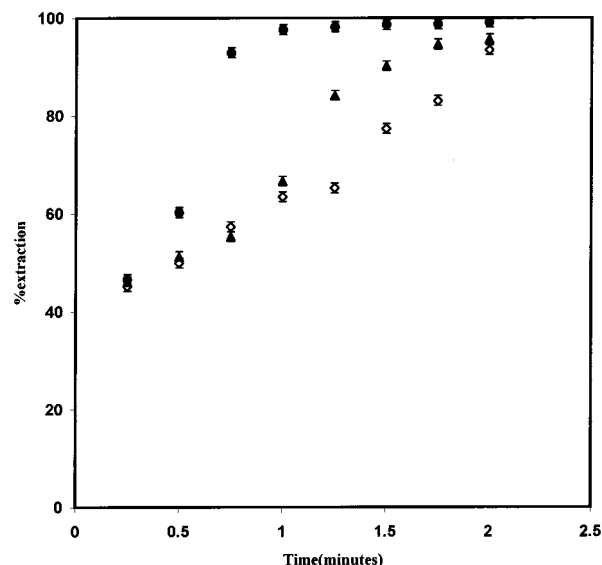


Figure 8. Extraction of piperine with time of microwave exposure at various power levels. Power level: 150 W. Solvent: petroleum ether. Particle size: no. 6 mesh (◇, power level of 150 W; ▲, power level of 300 W; ●, power level of 450 W).

Table 1. Extraction of Piperine as a Function of Solid Loading in Repeated Experiments^a

| % solid loading | % piperine extracted | % solid loading | % piperine extracted |
|-----------------|----------------------|-----------------|----------------------|
| 1 | 96.8 | 5 | 56.4 |
| 2 | 91.3 | 10 | 45.7 |
| 4 | 65.7 | 15 | 41.3 |
| | 95.4 | | 54.3 |
| | 91.2 | | 47.3 |
| | 64.2 | | 42.7 |

^a Power level: 2 W. Solvent: petroleum ether. Particle size: no. 6 mesh.

extraction of piperine. A steady rise in the extraction of piperine was observed at lower power levels with time.

During the microwave treatment, membranes of oil droplets rupture and the oil accumulates near the cell wall.³⁵ The protein structures also agglomerate into larger particles. When the power level is low, this coalescence takes place over a longer period of time, but at higher power levels, the confluencing of smaller lipid bodies into larger agglomerates occurs at an early stage of heating, leading to a rapid cell rupture and an increased rate of extraction.³⁶ The temperature of the solvent, however, remained 30 °C at all power levels. However, the temperature of the 0.5 dm³ of water that was included in the oven chamber as a dummy load to absorb the excess microwave radiation increased from 32 to 71 °C within 2 min. This indicated that the intracellular water and the polar constituents within the *P. nigrum* cells are heated rapidly, enhancing extraction within that short period.

Effect of Solid Loading. The effect of solid loading on the extraction of piperine was studied with petroleum ether at the power level of 150 W. Table 1 shows that the extraction of piperine decreased as the solid loading of the suspension was increased. An increase in the particle concentration results in a stronger absorption of the microwave energy near the surface of the vessel normal to the incident radiation because more particles can encounter the radiation and most of the radiation will be absorbed near the surface of the vessel itself. The consequence of the shorter penetration depth is that the material in the interior of the irradiated sample will not be subjected to the same level of microwave radi-

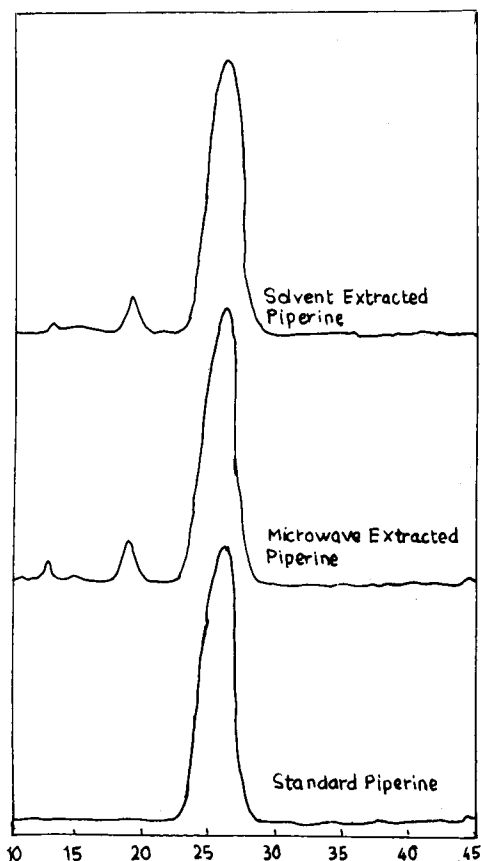


Figure 9. Comparative HPTLC chromatograms of extracted piperine.

tion and hence is affected to a lesser degree by the incident microwaves.³⁷ Lambert's law predicts an exponentially decaying absorption of energy as a function of depth into the sample.³⁸ Because of internal waves that are created within the microwave oven, a small variation in the sample thickness could make a large difference in the heating rates of the samples.³⁸ Thus, with increasing solid loading when the effective sample thickness increases, the particles situated in the interior region of the vessel are affected to a lesser degree, thus reducing the effective extraction of piperine. With increased solid loading, a longer time of exposure will be necessary to affect the same level of extraction.

The MAE might be applicable for the rapid extraction of piperine and provides an effective screening technique for the quality assessment of black pepper. For this extraction technique to prove to be a useful analytical tool, the parameters such as stability during extraction, precision, and limit of extraction have to be, however, evaluated. It can be seen from the representative chromatograms (Figure 9) that the samples are thermally stable and do not degrade during extraction because the chromatograms from solvent extraction and those obtained in the presence of microwaves are identical in nature. The precision of the extraction is defined as the expression of agreement between a series of extractions. The relative standard deviations between five sets of experiments were found to be 0.69%, indicating that this is a precise technique for extraction. The minimum limit of raw material giving a representative extraction profile is 0.1 g, which is an ideal weight to be used for quality screenings of pepper powder.

Conclusions

MAE can be considered as an effective technique for rapid and selective extraction of piperine. In comparison to conventional solvent extraction techniques, a "one-pot" microwave extraction technique provides higher extraction efficiencies, is less labor intensive, and is rapid. It can also be used as a quality control tool for the rapid screening of raw pepper.

Optimized conditions of microwave radiation power and solid loading were determined for MAE by using petroleum ether as a solvent. Rapid energy transfer is observed between the polar molecules, which couple with the microwaves and the nonpolar solvent, and this provides an effective mechanism for introducing nonpolar solvents as good extraction solvents. Cell rupture is facilitated by dielectric heating and coalescence of the constituents within the cellular matrix. Extraction of piperine from black pepper is, therefore, complete and rapid. Nonpolar solvents, though transparent to microwaves, can be used as effective solvents for the extraction of piperine from *P. nigrum*.

Nomenclature

λ_0 = free space wavelength of radiation, cm

ϵ' = dielectric constant (ethanol, 6.5; dichloromethane, 9.08; hexane, 1.89; heptane, 1.0035)

ϵ'' = dielectric loss (ethanol, 1.6; dichloromethane, 0.38; hexane, 2×10^{-4} ; heptane, 1.018×10^{-4})

% E = extraction of piperine, %

k = rate constant, min^{-1}

t = time, min

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