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Analytical Methods

Microwave-assisted extraction of chlorogenic acids from green coffee beans

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ABSTRACT

Microwave-assisted extraction (MAE) has been considered as a potential alternative to conventional solvent extraction for the isolation of phenolic compounds from plants. Aqueous and alcoholic extracts of green coffee bean obtained by MAE were quantitatively analysed for total yield of extracts, chlorogenic acids, caffeine and total polyphenol content. The extracts were also evaluated for radical-scavenging activity, using 1,1-diphenyl-β-picrylhydrazyl radical. Under optimum conditions of time (5 min), temperature (50 °C) and wattage (800 W), the maximum chlorogenic acids and caffeine could be extracted with water as solvent. The extracts contained chlorogenic acids and caffeine in the ranges of 31–62% and 22–40%, respectively. The yields of MAE under optimum conditions were higher than those from the conventional solvent extraction at 5 min and 50 °C and the extracts showed radical-scavenging activity of >75%, even at the concentration of 25 ppm. The MAE process can thus be predicted and controlled for industrial application.

1. Introduction

Chlorogenic acid (5-*O*-caffeoyl-quinic acid), an ester of caffeic acid with quinic acid, has received considerable attention for its potential biological effects and wide distribution in nature (Clifford, 1999). It is one of the most abundant polyphenols in the human diet with coffee, fruits and vegetables as its major sources. Flowers and buds of *Lonicera japonica* Thunb, and the leaves of *Eucommia ulmodies*, which are rich in chlorogenic acids, are used in Chinese medicine. In the conventional method, chlorogenic acid is extracted from dried green coffee powder by refluxing with solvent, followed by filtration. However, this technique is time- as well as energy-consuming and laborious. Development of a new technique for the separation and purification of chlorogenic acid is thus of essential importance for the potential application and use of chlorogenic acid.

Microwave-assisted extraction (MAE) is a process that uses microwave energy, along with solvent, to extract target compounds from various matrices. The highly localised temperature and pressure can cause selective migration of target compounds from the material at a faster rate, thus providing enriched extracts compared to conventional extracts. MAE was used for extraction of interesting components from a wide variety of sample matrices and also as a promising alternative sample preparation technique for a number of applications (Gao, Song, & Liu, 2006; Rostagno, Palma, & Barroso, 2007; Zhang & Xu, 2007; Zhou & Liu, 2006). Compared to conventional methods, MAE can considerably reduce both extraction time and solvent consumption. An efficient microwave-assisted extraction (MAE) technique has been developed to recover chlorogenic acid from flower buds of *L. japonica* Thunb. The yield of chlorogenic acids rapidly reached 6.14% within 5 min under the optimal MAE conditions, with 50% ethanol as extraction solvent at 60 °C extraction temperature (Zhanga, Yang, & Liua, 2008). The MAE showed obvious advantages in terms of short duration and high efficiency to recover chlorogenic acid from raw plant materials in comparison with conventional heat-reflux extraction. The mechanism of the enhanced extraction by microwave assistance was studied by observing cell destruction of plant material after MAE treatment, using scanning electron microscopy. The enhanced extraction was related partly to a greater extent of cell rupture of the plant materials, and this was observed by scanning electron microscopy. The plant materials were significantly destroyed due to cell rupture during the MAE treatment. It is in this context that conditions for microwave-assisted extraction of green coffee beans were optimised, and the quality of the extracts was monitored through determination of the contents of chlorogenic acids, caffeine and total polyphenols, as well as evaluating the radical-scavenging activity. A comparative analysis between MAE and conventional heat reflux methods was also undertaken.

2. Materials and methods

2.1. Chemicals and reagents

Referenced standards, such as caffeine, chlorogenic acid, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH·), butylated hydroxyanisole

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(BHA), were purchased from Sigma Chemical Co., St. Louis, MO. Gallic acid and Folin Ciocalteu's (FC) phenol reagent were purchased from Loba Chemie and Merck, Mumbai, India, respectively. Anhydrous sodium carbonate (99.5%, LR) was procured from Ranbaxy, New Delhi. Solvents, such as hexane, methanol and other chemicals, were procured from Qualigens Fine Chemicals, Mumbai. All the chemicals were of analytical grade.

2.2. Plant material

Robusta cherry green coffee beans were procured from the local market of Mysore, Karnataka, India. Coffee beans were ground and sieved (<720 μ), using a hammer mill (CMC-CM; Cadmach Machinery Private Limited, Ahmedabad, India). The ground coffee was packed in low-density polyethylene pouches and preserved for further analysis.

2.3. Extraction methodologies

2.3.1. Microwave-assisted extraction (MAE)

The ground coffee sample was defatted with hexane (1:6; w/v) for 8 h in a Soxhlet extraction system. Defatted coffee powder was extracted with different solvents, such as water, methanol and ethanol, using a Microwave lab station [(Model: STARTS configuration with control terminal 260, Milestone, Italy). Built in focused IR sensor and magnetic stirrer; Magnetron: SN: 133613; Frequency – 50 Hz] in a closed system under different sets of conditions of temperature, wattage and time. The slurry obtained was filtered to yield a clear extract that was used for quantitative analysis.

2.3.2. Conventional extraction

The ground coffee was defatted and extracted with water in a hot water bath at a temperature of 50 °C for 5 min. The slurry was filtered to get a clear extract that was used for quantitative analysis.

2.4. Analytical methodologies

2.4.1. Extraction yield

Yield of extracts was determined by evaporating a known quantity of the extract to dryness, using a rotary evaporator at 50 °C under reduced pressure, from which the yield was calculated.

2.4.2. Chlorogenic acids

The level of chlorogenic acids of the extracts was estimated by the UV spectrophotometric method. Extracts were treated with lead acetate and the absorbance was measured before and after treatment at 325 nm (AOAC, 2000). Chlorogenic acid content was calculated from standard curve.

2.4.3. Caffeine

The filtrates from the extractions were extracted with chloroform (1:4) and the combined extracts were dried and the absorbance was measured at 275 nm in a spectrophotometer (Cintra 10, GBC, Dandenong, Australia). The quantity of caffeine was calculated from a standard graph, prepared using a reference standard (AOAC, 2000).

2.4.4. Total polyphenols

Total polyphenols (TPP) content of the extracts was determined by using FC reagent. The samples (0.1 ml) were mixed with FC reagent (0.5 ml) and saturated sodium carbonate solution (7.5 ml). The sample solution was made up to 10 ml with distilled water and the absorbance was measured at 765 nm. Total polyphenol content was expressed as gallic acid equivalents (Swain & Hillis, 1959).

2.4.5. Radical-scavenging activity

The RSA of different extracts was evaluated according to the procedure described by Blois (1958) with slight modifications (Abdille, Singh, Jayaprakasha, & Jena, 2005). Sample solution (1 ml) at various concentrations (25, 50, 100 and 200 ppm), and butylated hydroxy anisole (BHA), were mixed with 0.1 mM methanolic solution (4 ml) of DPPH and allowed to stand at 27 °C for 20 min. The control was prepared, as above, without any extract and methanol was used for baseline correction. The optical density (OD) of the samples was measured at 517 nm. The RSA was expressed as the inhibition percentage and was calculated using the formula:

$$\%\ \text{RSA} = 100 \times \frac{(\text{control OD} - \text{sample OD})}{\text{control OD}}$$

2.4.6. Statistical analysis

All the analyses were carried out in triplicate and the results were provided as mean values with standard deviation. The obtained data were subjected to statistical analysis and the means, compared by Duncan's New Multiple Range test ($p \leqslant 0.05$), are presented.

3. Results and discussion

3.1. Selection of the solvent

The solvents, namely ethanol, methanol and water, were selected from earlier studies (Ramalakshmi, Rahath Kubra, and Jagan Mohan Rao, 2008) to extract chlorogenic acids from green coffee beans under the MAE conditions of 5 min, 800 W and 50 °C. The extraction efficiency is presented in Table 1. The yield of the extraction was maximum (18.1%) when water was used as a solvent compared to the other solvents, which were in the range of 8.95–9.05%. The reason could be that the dielectric constant and polarity of water are higher than the alcohol, which helps in the absorption of microwaves. Extractability of the solvent mainly depends on the solubility of the compounds in the solvent system, the mass transfer kinetics of the product and the strength of the solute/matrix interactions.

3.2. Effect of duration of MAE radiation

The quantities of chlorogenic acids in the aqueous extracts obtained at the power of 800 W at different time intervals are presented in Table 1. The yield of the extract was found to be in the range of 17.1–17.5%. The MAE of 5 min resulted in a greater extraction of chlorogenic acids and caffeine than did the extraction of 2 min. However, increasing time to more than 5 min did not yield better extraction. Hence, 5 min was selected for further experiments. Pan, Niu, and Liu (2003) reported that MAE reached maximum extraction of caffeine and polyphenols after 4 min from tea leaves.

3.3. Effect of MAE temperature on extraction efficiency

Temperature of the extraction method is also very important for the isolation of bioactive compounds from the plant materials. The experimental results of different temperatures (30, 50, 70 and 90 °C) are given in Table 1. The total extraction efficiency is maximum at the temperature of 50 °C. Also, the amounts of chlorogenic acid and caffeine of microwave-assisted extracts were maximum at the same temperature. There is not much difference in the total yield or the quality characteristics of the extracts when the temperature of the extraction was further increased from 50 °C, which

Table 1
Yields of MAE extracts, chlorogenic acid and caffeine.

Exp set	Variable parameter	Constant parameter	Variables	% Yield of MAE extracts	% Yield chlorogenic acids	% Yield[P] caffeine
1	Solvent	Time (5 min) Temperature (50 °C) Wattage (800 W)	Methanol	9.05 ± 0.07[a]	5.6 ± 0.14[b] (61.87)	3.44 ± 0.07[b] (38.10)
			Ethanol	8.95 ± 0.07[a]	4.95 ± 0.07[a] (55.31)	3.05 ± 0.07[a] (34.08)
			Water	18.1 ± 0.14[b]	8.4 ± 0.28[c] (46.41)	7.25 ± 0.07[c] (40.06)
2	Aqueous extract					
2a	Time	Temperature (50 °C) Wattage (800 W)	2 min	17.2 ± 0.07[a]	6.05 ± 0.07[a] (35.28)	3.75 ± 0.07[a] (21.87)
			5 min	17.5 ± 0.14[b]	6.75 ± 0.07[c] (38.57)	4.6 ± 0.14[c] (26.29)
			10 min	17.1 ± 0.07[a]	6.35 ± 0.07[b] (37.24)	4.4 ± 0.14[b] (25.81)
2b	Temperature	Time (5 min) Wattage (800 W)	30 °C	17.6 ± 0.07[b]	6.65 ± 0.07[b] (37.89)	6.68 ± 0.07[b] (38.06)
			50 °C	18.1 ± 0.07[c]	8.1 ± 0.04[d] (44.88)	7.25 ± 0.07[b] (40.17)
			70 °C	17.6 ± 0.14[b]	6.75 ± 0.07[c] (38.35)	4.75 ± 0.07[a] (26.99)
			90 °C	16.8 ± 0.07[a]	6.35 ± 0.07[a] (37.91)	4.65 ± 0.07[a] (27.76)
2c	Wattage	Time (5 min) Temperature (50 °C)	400 W	15.4 ± 0.14[a]	4.7 ± 0.14[a] (30.52)	3.7 ± 0.14[a] (24.03)
			600 W	17.8 ± 0.07[b]	5.8 ± 0.14[b] (32.68)	4.25 ± 0.07[b] (23.94)
			800 W	18.2 ± 0.14[c]	8.4 ± 0.28[c] (46.13)	7.25 ± 0.07[c] (39.81)

Values are means ± SD of triplicates; values are compared within the experimental sets of 1,2a, 2b, 2c. Values having different superscripts are significantly ($p < 0.05$) different.
The values in parentheses are the percentages of the compound in the respective extract.
[P] Includes related compounds, which show absorption at 275 nm.

showed that the extraction temperature was optimum at 50 °C and this was chosen for the further experiments.

3.4. Effect of microwave power on extraction

Experiments were performed to determine the effect of microwave power on the extraction efficiency and the results are given in Table 1. Microwave powers of 400, 600 and 800 W were attempted for the extraction at the temperature of 50 °C for 5 min and the quality parameters were analysed. The extraction of chlorogenic acids and caffeine increased with the increase in microwave power up to 800 W. The experiments could not be carried out beyond 800 W, due to the limitation with the equipment. Pan et al. (2003) conducted experiments of MAE for polyphenols and caffeine at the microwave power of 700 W from green tea leaves. Chen and Spiro (1995) reported microwave-assisted extraction of rosemary leaves and found that the concentration of extracts increased with increase in microwave power and the induction stage appeared. However, Chemat, Amar, Lagha, and Esveld (2005) confirmed that there was not much difference in the extraction yield when the microwave power was increased from 50 to 150 W during the extraction of caraway seeds. The difference may due to the nature of the plant material and the components to be extracted.

3.5. Yield of chlorogenic acids

The percentage yields of chlorogenic acids in the aqueous and solvent extracts obtained at different MAE parameters/conditions are presented in Table 1. The yield was found to be in the range of 4.7–8.4% in aqueous extractions, whereas the range was 4.9–5.6% in alcohols. The yield increased up to 5 min and no further increase was observed beyond 5 min. The yield of chlorogenic acids increased with increase in wattage up to 800 W. The experiments could not be carried out beyond 800 W, due to limitation with the equipment. Extraction yield increased, along with temperature, up to 50 °C but decreased at higher temperatures, which could be due to degradation. The highest yield of chlorogenic acids (8.4 ± 0.28%) was obtained under MAE conditions of 5 min, 800 W and 50 °C, using water as the solvent of extraction. Further, the extracts were rich in chlorogenic acids. The percentage of the chlorogenic acids was in the range of 32.7–61.9. Although the yield of methanol extract is low, when compared to water extract, it contained the maximum chlorogenic acid (61.9%).

3.6. Yield of caffeine

A similar trend, as for chlorogenic acids, was observed in the case of caffeine. The maximum yield of caffeine was obtained under MAE conditions of 5 min, 800 W and 50 °C in aqueous extraction. The percentage extraction yields of caffeine at different MAE parameters are shown in Table 1. Further, the extracts were also rich in caffeine, next to chlorogenic acids. The percentage of the caffeine was in the range 21.9–40.1%.

3.7. Total polyphenols (TPP) of extracts

Total polyphenol content in microwave-assisted extracts was measured in terms of gallic acid equivalents. During the estimation, Folin Ciocalteu's reagent was added to the extract. In the alkaline medium, it reacts with polyphenols and forms complexes for which absorbance was measured at 760 nm. The various polyphenols that are present in the coffee are caffeic acid, protocatechuic acid, gallic acid, quinic acid, pyrogallol, apart from chlorogenic acids (Varnam & Sutherland, 1994). Polyphenol contents in extracts, under different MAE conditions using different solvents, are presented in Table 2. TPP was present in the range of 12–24 mg gallic acid equivalents/g in aqueous extractions, whereas the range was 10–17 mg gallic acid equivalents/g in alcoholic extracts. TPP content in extract increased, along with time up to 5 min. However, TPP decreased slightly, along with increase in wattage and temperature. This may be due to degradation of other phenolics at higher wattage and temperatures. Also the TPP content was found to be higher in methanol extract than in ethanol extract, indicating more solubility of polyphenols in methanol than ethanol.

3.8. Radical-scavenging activity (RSA) of extracts

Free radical-scavenging activity of the extracts was tested using the DPPH· model system and the results are presented in Table 2. The principle involved in this method is that the antioxidants react with the stable free radical, i.e. 1,1-diphenyl-2-picrylhydrazyl (deep violet colour) and convert it to 1,1-diphenyl-2-picrylhydrazine with discoloration. The degree of discoloration indicates the scavenging potential of the extracts. RSA in extract was measured in terms of percentage inhibition of DPPH free radicals. Extracts at different concentration, ranging from 25 to 200 ppm were taken and RSA evaluated. The RSA of water extracts was high-

Table 2
Polyphenols content and radical-scavenging activity (RSA) of extracts from MAE.

Exp set	Variable parameter	Variable	TPP (mg/g)[z]	% RSA at different concentrations			
				25 ppm	50 ppm	100 ppm	200 ppm
1	Solvent[y]	Methanol	1.5 ± 0.14[b] (16.57)	45.6 ± 0.07[b]	61.7 ± 0.14[b]	81.7 ± 0.00[b]	83.5 ± 0.21[b]
		Ethanol	0.95 ± 0.07[a] (10.61)	38.5 ± 0.14[a]	56.4 ± 0.14[a]	75.6 ± 0.00[a]	81.4 ± 0.14[a]
		Water	3.1 ± 0.14[c] (17.13)	80.3 ± 0.21[c]	82.6 ± 0.00[c]	83.2 ± 0.14[c]	85.8 ± 0.07[c]
2	For aqueous extract						
2a	Time[x]	2 min	2.95 ± 0.07[a] (17.20)	77.2 ± 0.07[a]	79.5 ± 0.14[a]	82.1 ± 0.14[a]	84.2 ± 0.21[b]
		5 min	3.45 ± 0.07[c] (19.71)	78.6 ± 0.21[c]	82.1 ± 0.14[b]	83.7 ± 0.07[b]	85.2 ± 0.21[a]
		10 min	3.1 ± 0.14[b] (18.18)	77.8 ± 0.14[b]	82.2 ± 0.14[b]	82.1 ± 0.14[a]	84.8 ± 0.07[c]
2b	Temp.[w]	30 °C	4.2 ± 0.14[d] (23.93)	78.5 ± 0.00[c]	81.2 ± 0.14[c]	81.9 ± 0.14[b]	81.9 ± 0.07[b]
		50 °C	3.85 ± 0.07[c] (21.33)	79.6 ± 0.14[d]	82.4 ± 0.21[d]	83.9 ± 0.21[d]	83.9 ± 0.14[d]
		70 °C	3.6 ± 0.14[b] (20.45)	77.6 ± 0.14[b]	80.1 ± 0.40[b]	82.3 ± 0.14[c]	82.3 ± 0.07[c]
		90 °C	2.85 ± 0.07[a] (17.01)	75.1 ± 0.14[a]	78.8 ± 0.07[a]	80.2 ± 0.28[a]	80.2 ± 0.21[a]
2c	Wattage[v]	400 W	3.5 ± 0.14[c] (22.73)	78.4 ± 0.21[a]	81.6 ± 0.21[b]	83.0 ± 0.14[a]	84.3 ± 0.21[a]
		600 W	2.6 ± 0.14[b] (14.65)	78.7 ± 0.14[b]	82.0 ± 0.07[a]	84.1 ± 0.14[b]	84.6 ± 0.21[b]
		800 W	2.2 ± 0.14[a] (12.08)	81.4 ± 0.21[c]	83.1 ± 0.00[c]	84.2 ± 0.14[b]	86.7 ± 0.07[c]

Values are means ± SD of triplicates; values are compared within the experimental sets of 1,2a, 2b, 2c. Values having different superscripts are significantly ($p < 0.05$) different.
[z] As gallic acid equivalents; the values in parentheses are TPP contents in the respective extract.
Constant parameters:
[y] Temperature – 50 °C; Time – 5 min; Wattage – 800 W.
[x] Temperature – 50 °C, Wattage – 800 W.
[w] Wattage – 800 W, Time – 5 min.
[v] Temperature – 50 °C, Time – 5 min.

Table 3
Comparative analyses between conventional heat reflux extraction and MAE.

Exp. Set	Method of Extraction	Parameters	%Yield	
			Caffeine[P]	Chlorogenic acids
I	Conventional heat reflux method of extraction	Time (5 min) Temperature (50 °C) Sample: solvent (1:4)	3.05 ± 0.21[a]	3.95 ± 0.21[a]
II	Microwave-assisted extraction (MAE)	Time (5 min) Temperature (50 °C) Wattage (800 W) Sample: solvent (1:4)	7.25 ± 0.07[b]	8.4 ± 0.28[b]

Values are means ± SD of triplicates; values having different superscripts are significantly ($p < 0.05$) different.
[P] Includes related compounds, which show absorption at 275 nm.

er than that of alcohol extracts. It was found to be in the range of 75–85%, even at 25 ppm concentration, for water extracts. The RSA values of ethanol and methanol extracts were 38% and 45%, respectively at 25 ppm concentration. The RSA of alcohol extracts increased with concentration and reached approximately 80% at 100 ppm concentration.

3.9. Comparative analysis of MAE and conventional extracts

The optimum MAE extraction conditions of time, temperature and wattage were determined to be 5 min, 50 °C and 800 W. The comparative analysis of extraction efficiency of chlorogenic acids and caffeine, between the conventional heat reflux method and MAE under optimal conditions, is presented in Table 3. The yields in MAE were shown to be higher for water than alcoholic solvents, namely ethanol and methanol. This may be due to the difference in dielectric constants of the respective solvents. It is clearly evident from the comparative data (Table 3) that MAE shows significantly higher and better extraction yields than does the conventional heat reflux method, for both chlorogenic acids and caffeine under optimal extraction conditions.

4. Conclusions

Microwave-assisted extraction has been considered as a potential alternative to traditional solid–liquid extraction for the isolation of phenolic compounds, such as chlorogenic acids, from plants. The present study has focussed on microwave-assisted extraction of chlorogenic acid, caffeine and total polyphenols from green robusta cherry coffee. Aqueous and alcoholic (ethanol and methanol) extracts of green coffee bean, obtained by microwave-assisted extraction (MAE), were quantitatively analysed for chlorogenic acids, total yield of extracts, caffeine and total polyphenol content. The extracts were also evaluated for radical-scavenging activity (1,1-diphenyl-2-picrylhydrazyl radical). It was found that MAE at a temperature and wattage of 50 °C and 800 W, respectively, for the extraction period of 5 min, resulted in maximum yield of the above compounds. Under the optimum conditions of time (5 min), temperature (50 °C) and wattage (800 W), maximum chlorogenic acids (8.40%) and caffeine (7.25%) could be extracted and these concentrations were higher than those after the heat reflux extraction at 5 min and 50 °C, respectively. The extracts contained chlorogenic acids in the range of 32–62% and caffeine in the range of 22–40%. MAE was compared with conventional methods of extraction and was efficient and reduced both time and solvent consumption. Also the extracts showed radical-scavenging activity of >75%, even at the concentration of 25 ppm. Microwaves are fast and reliable and the efficiency of extraction of phenolic compounds was improved in comparison with that of the heat reflux method. Water, a microwave-transparent solvent, proved to be the best choice to extract phenolic compounds from defatted and ground robusta cherry coffee when microwaves were used. This may be attributed to the better absorption of microwave energy, which increases temperature inside the plant cells, resulting in the breaking of cell walls and releasing compounds into the sur-

rounding solvent. The MAE process can be predicted and controlled for industrial application with suitable arrangements for separation of active compounds. Hence, this method can be used selectively to prepare chlorogenic acid-rich conserves from coffee beans for use in functional foods.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.foodchem.2011.06.057.

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