ELSEVIER

Chemical Engineering and Processing 42 (2003) 129–133

Chemical Engineering and Processing

www.elsevier.com/locate/cep

Microwave-assisted extraction of tea polyphenols and tea caffeine from green tea leaves

Xuejun Pan, Guoguang Niu, Huizhou Liu *

Young Scientist Laboratory of Separation Science and Engineering, State Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing, 100080, People's Republic of China

Received 17 October 2001; received in revised form 20 March 2002; accepted 20 March 2002

Abstract

A microwave-assisted extraction (MAE) method is presented for the extraction of tea polyphenols (TP) and tea caffeine from green tea leaves. Various experimental conditions, such as ethanol concentration (0–100%, v/v), MAE time (0.5–8 min), liquid/solid ratio (10:1–25:1 ml g^{-1}), pre-leaching time (0–90 min) before MAE and different solvents for the MAE procedure were investigated to optimize the extraction. The extraction of tea polyphenols and tea caffeine with MAE for 4 min (30 and 4%) were higher than those of extraction at room temperature for 20 h, ultrasonic extraction for 90 min and heat reflux extraction for 45 min (28 and 3.6%), respectively. From the points of extraction time, the extraction efficiency and the percentages of tea polyphenols or tea caffeine in extracts, MAE was more effective than the conventional extraction methods studied.

Keywords: Polyphenols; Caffeine; Green tea leaves; Extraction; Microwave-assisted

1. Introduction

Green tea leaves contain tea polyphenols, tea caffeine, amino acids, saponins, tannins, etc., with about 10–30% (w/w) polyphenols and 2–4% (w/w) caffeine. Tea polyphenols include catechines, flavanols, flavanones, phenolic acids, glycosides and the aglycons of plant pigments. They are soluble in water, ethanol, methanol, acetone etc. Tea polyphenols isolated from green tea leaves, are natural antioxidant [1], and have a scavenging effect on active oxygen radical [2]. Tea polyphenols have important applications in food industry and medicine for daily use. Tea polyphenols have a stronger anti-oxidative activity than butylated hydroxyanisole, butylated hydroxytoluene and DL-α-tocopherol; and the toxicity of tea polyphenols is lower than butylated hydroxyanisole, butylated hydroxytoluene and DL-α-tocopherol [3].

Microwave digestion of matrices for their eventual elemental analysis has been routinely used for several years [4]. Recently, microwave-assisted extraction (MAE) has been used for the extraction of biologically active compounds, such as extraction of essential oils from the leaves of rosemary and peppermint [5], extraction of taxanes from *Taxus* biomass [6], extraction of ergosterol and total fatty acids from fungal hyphae and spores, mushrooms, filtered air, artificially contaminated corn, naturally contaminated grain dust, and soil [7] and the extraction of azadirachtin-related limonoids from neem seed kernel [8].

Extraction of tea polyphenols and tea caffeine from green tea leaves has been reported [9–11]. However, no report has been done on the use of MAE for the extraction of tea polyphenols and tea caffeine from green tea leaves. The purpose of this work was to develop a MAE method and evaluate MAE and conventional extraction methods for the extraction of tea polyphenols and tea caffeine from green tea leaves.

* Corresponding author. Tel.: +86-10-6255-5005; fax: +86-10-6255-4264.
E-mail address: hzliu@home.ipe.ac.cn (H. Liu).

0255-2701/02/$ - see front matter
PII: S0255-2701(02)00037-5

2. Materials and methods

2.1. Plant materials and chemicals

Whole green tea leaves (*Thea sinensis* L.) (producing area: Hangzhou, China) were purchased from a local market.

Ethanol, methanol and acetone used in the experimental work were all of analytical reagent grade chemicals. Caffeine and all other chemicals for analysis of tea polyphenols and tea caffeine used were all of analytical reagent grade chemicals.

2.2. Microwave-assisted extraction

A household microwave oven (full power 700 W, National, Japan) was modified in our laboratory [12]. Green tea leaves were mixed with an appropriate solvent. The suspensions were irradiated with microwaves as follows: 45 s power on (heating to the desired temperature about 85–90 °C) and 10 s power off and then 3 s power on (for heating) and 10 s power off (for cooling) and so on to the pre-set extraction time. Superboiling of the solution did not occur.

Except MAE, other extraction methods are traditional in the references, such as heat reflux extraction and Soxhlet extraction [13], and ultrasonic extraction [14]. There is no microwave energy radiation by magnetic stirring for 0–90 min pre-leaching at room temperature and then MAE for 4 min.

2.3. Colorimetric analysis of tea polyphenols [15]

One milliliter extraction solution (obtained with different volume of extraction solvent and appropriate mass of leaves described in different experiments), 4 ml water and 5 ml ferrous tartrate tetrahydrate solution (1 g ferrous sulfate and 5 g potassium sodium tartrate tetrahydrate were dissolved in 1000 ml water in 1000-ml volumetric flask) were mixed with 15 ml buffer solution (0.067 mol l^{-1} pH 7.5 potassium phosphate) in 25-ml capacity bottle. It takes several minutes for color to develop.

With blank solution (prepared with solvent before used in extraction according to the procedure as mentioned above for analysis of tea polyphenols) as reference solution, absorbance (A) at Vis 540 nm with a 10 mm quartz cell was used to calculate the extraction of tea polyphenols according to Eq. (1) [15]. The errors were controlled to less than 0.5% through duplicated experiments and analysis.

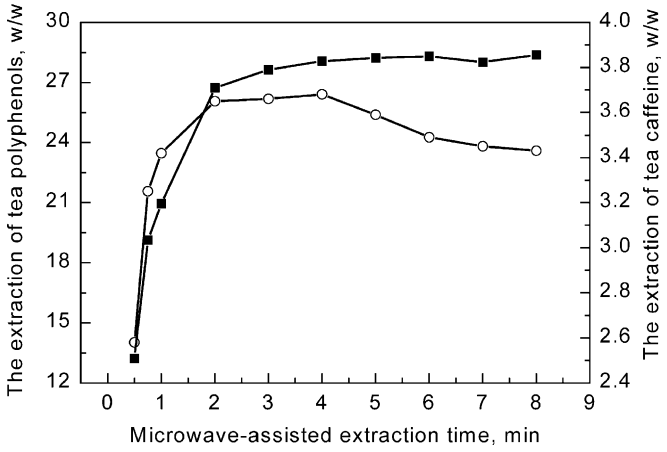


Fig. 2. Effect of MAE time on the extraction of polyphenols and caffeine Solvent: ethanol/water (1:1 v/v) 100 ml; whole green tea leaves: 5 g; liquid/solid ratio: 20:1 ml g^{-1} (■) the extraction of tea polyphenols; (○) the extraction of tea caffeine.

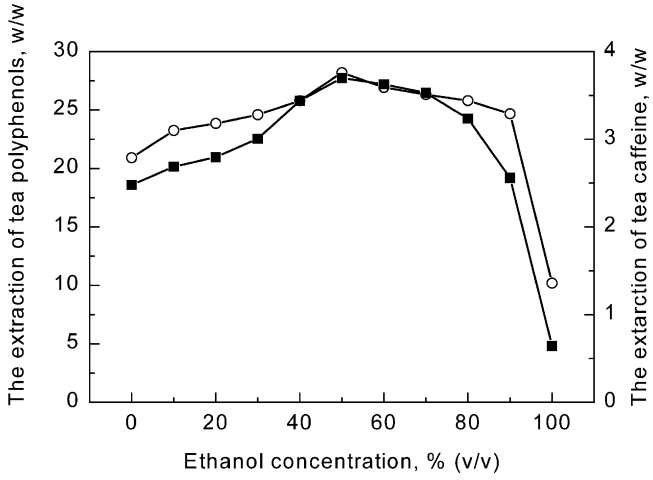


Fig. 1. Effect of ethanol concentration in water on the extraction of polyphenols and caffeine Solvent: 100 ml; whole green tea leaves: 5 g; MAE for 5 min; liquid/solid ratio: 20:1 ml g^{-1} (■) the extraction of tea polyphenols; (○) the extraction of tea caffeine.

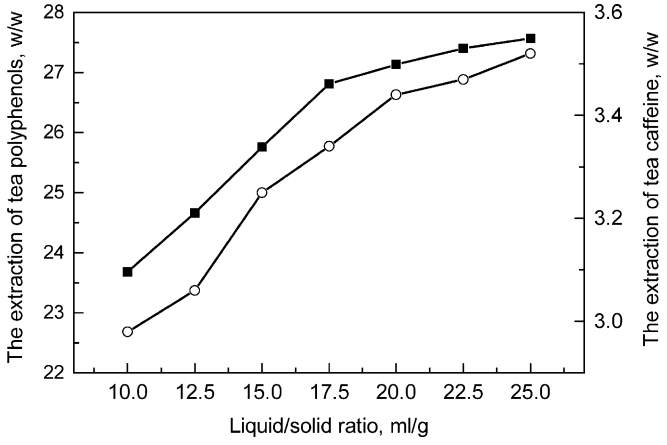


Fig. 3. Effect of liquid/solid ratio on the extraction of polyphenols and caffeine Solvent: ethanol/water (1:1 v/v) 100 ml; MAE for 4 min (■) the extraction of tea polyphenols; (○) the extraction of tea caffeine.

The extraction of tea polyphenols (%, w/w)

$$= 2A \times 1.957 \times \frac{L_1}{L_2 M} \times 100\% \quad (1)$$

where, L_1, total volume of extraction solution, ml; L_2, volume of extraction solution used for colorimetric analysis, ml; M, mass of tea leaves, mg; A, absorbance at Vis 540 nm; 1.957, when absorbance was 0.5 at Vis 540 nm with a 10 mm quartz cell, the concentration of tea polyphenols was 1.957 mg ml^{-1}.

2.4. Colorimetric analysis of tea caffeine [16]

Twenty milliliters of extraction solution (obtained with different volume of extraction solvent and appropriate mass of leaves described in different experiments), 10 ml hydrochloric acid (0.01 mol l^{-1}) and 2 ml lead acetate basic solution (50 g $Pb(CH_3COO)_2$ $Pb(OH)_2$ were mixed in 100 ml water and then were collected to stand at least for 12 h) were mixed with 218 ml water in a 250-ml volumetric flask. The mixed solution was stand for 1 h and then was filtered. After that, 50 ml filtered solution and 0.2 ml sulfuric acid (H_2SO_4) solution (4.5 mol l^{-1}) were mixed with 49.8 ml water in a 100-ml volumetric flask. The mixed solution was stand for 30 min and then was filtered. With blank solution (prepared with solvent before used in extraction according to the procedure for analysis of tea caffeine) as reference solution, the filtered solution was measured at UV 274 nm with a 10 mm quartz cell.

A good linear ranging from 0 to 0.02 mg ml^{-1} caffeine (analytical reagent grade chemicals caffeine as standard) was found. Regression equation (at UV 274 nm) and correlation coefficient were $Abs = 50.13C + 0.0002$ and $R = 0.9996$ ($n = 9$) respectively. Calculation of the concentration of caffeine from the calibrated regression equation according to absorbance at UV 274 nm and it was used to calculate the extraction efficiency of tea caffeine according to the following Eq. (2) [16]. The errors were controlled to less than 0.2% by duplicated experiments and analysis.

The extraction of tea caffeine (%, w/w)

$$= \frac{C \times L_1 \times 250/20 \times 100/50}{M} \times 100\% \quad (2)$$

where C, the concentration of caffeine calculated from the calibrated regression equation based on the absorbance at UV 274 nm, mg ml^{-1}; L_1, total volume of extraction solution, ml; M, mass of green tea leaves, mg.

In the present work, the percentage of tea polyphenols or tea caffeine in extracts was defined as following Eq. (3):

The percentage of tea polyphenols or tea caffeine in extracts (%, w/w)

$$= \frac{\text{Mass of tea polphenols or tea caffeine in extracts}}{\text{Mass of extracts}} \times 100\% \quad (3)$$

3. Results and discussion

3.1. The effect of ethanol concentration on the extraction of polyphenols and caffeine

Fig. 1 shows that the extraction of polyphenols and caffeine in green tea leaves was greatly influenced by the ethanol concentration in water. When the ethanol volume percentage in the solvent was lower than 50% (v/v), the extraction was increased with the increase of ethanol concentration. When the ethanol volume percentage in the solvent was higher than 50% (v/v), the extraction was decreased with the further increase of ethanol concentration. So 50% (v/v) ethanol concentration in water was used in the following experiments.

3.2. The effect of MAE time on the extraction of polyphenols and caffeine

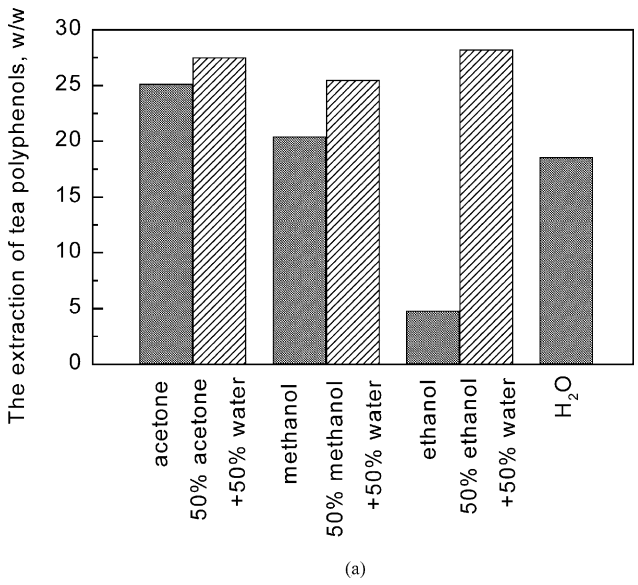
Fig. 2 shows the effect of MAE time on the extraction of polyphenols and caffeine. The results indicate that the extraction of polyphenols and caffeine was increased with the increase of MAE time. MAE reached a high point in 4 min. If MAE time was longer than 4 min, the extraction of caffeine was decreased with the increase of time. So MAE time for 4 min was used in the following experiments.

3.3. The effect of liquid/solid ratio on the extraction of polyphenols and caffeine

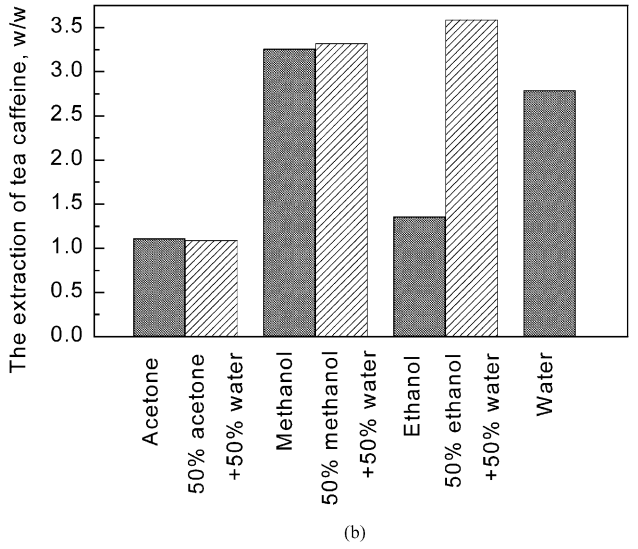
Fig. 3 shows that the extraction of polyphenols and caffeine was increased with the increase of liquid/solid ratio. If the extraction was carried out under high liquid/solid ratio, the concentration of tea polyphenols and tea caffeine in extraction solution was low. The liquid/solid ratio of 20:1 (ml g^{-1}) was sufficient to reach the high extraction, and it was used afterwards.

3.4. The effect of different solvents on the extraction of polyphenols and caffeine

Fig. 4(a) shows that acetone can be used to obtain higher extraction of polyphenols than using methanol, water and ethanol respectively. The methanol can give



(a)



(b)

Fig. 4. The effect of solvents used on the extraction of polyphenols and caffeine Solvent: 100 ml; whole green tea leaves: 5 g; MAE for 4 min; liquid/solid ratio: 20:1 ml g^{-1}.

higher extraction of caffeine than using water, ethanol and acetone respectively (Fig. 4(b)). If water was added to ethanol, the ethanol/water (1:1 v/v) solution gave higher extraction of polyphenols and caffeine than the other solvents tested. As the ethanol is non-toxic and can be mixed with water in different ratio, so it is chosen to extract polyphenols and caffeine from green tea leaves.

3.5. The effect of pre-leaching time before MAE on the extraction of polyphenols and caffeine

Fig. 5 shows that the extraction of polyphenols and caffeine were influenced by pre-leaching time at room temperature before MAE for 4 min. If the pre-leaching

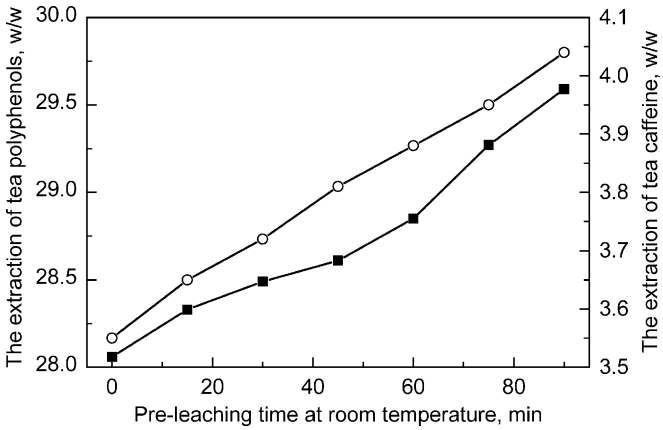


Fig. 5. Effect of pre-leaching time at room temperature on the extraction of polyphenols and caffeine Solvent: ethanol/water (1:1 v/v) 100 ml; whole green tea leaves: 5 g; liquid/solid ratio: 20:1 ml g^{-1}; MAE for 4 min; room temperature: about 20 °C (■) the extraction of tea polyphenols; (○) the extraction of tea caffeine.

time was 90 min, the extraction of polyphenols was increased from 28.06 to 29.59%, while the extraction of caffeine was increased from 3.55 to 4.04%. It is obvious that pre-leaching before the MAE is useful for improving the extraction of both polyphenols and caffeine.

3.6. Comparison of MAE and conventional extraction methods

Table 1 shows that the MAE for 4 min after pre-leaching for 90 min gave higher extraction of polyphenols and caffeine than the extraction at room temperature for 20 h, ultrasonic extraction for 90 min and heat reflux extraction of 45 min respectively. The results show that the time of heat reflux extraction, ultrasonic extraction and extraction at room temperature was respectively about 10, 20, 300-folds of time of extraction with MAE. MAE can greatly reduce the extraction time for the same extraction.

Table 2 shows that MAE also gives higher percentages of polyphenols and caffeine in extracts in comparison with those of ultrasonic extraction and heat reflux extraction respectively. The MAE can give the highest extraction selectivity.

4. Conclusion

Conditions for MAE of polyphenols and caffeine from green tea leaves have been studied. MAE has been shown to be an efficient method for extraction of polyphenols and caffeine from green tea leaves. Compared with the conventional extraction methods, the MAE procedure provided high extraction, high extraction selectivity, requiring short time, and less labor intensive.

Table 1
Comparison of the results of the extraction with MAE and conventional extraction methods

Extraction method	Extraction time	The extraction, w/w	
		Polyphenols	Caffeine
Extraction at room temperature	20 h	28	3.6
Ultrasonic extraction	90 min	28	3.6
Heat reflux extraction	45 min	28	3.6
MAE (after pre-leaching for 90 min at room temperature)	4 min	30	4

Solvent: ethanol/water (1:1 v/v) 100 ml; whole green tea leaves: 5 g; liquid/solid ratio: 20:1 ml g^{-1}; room temperature: 20 °C; Ultrasonic extraction: 20–40 °C; Heat reflux extraction at boiling point about 85 °C.

Table 2
Comparison of the percentage of polyphenols and caffeine in extracts obtained with different extraction methods

Extraction methods	The extraction of polyphenols (%) (w/w)	The extraction of caffeine (%) (w/w)	The percentage of polyphenols in extracts (%) (w/w)	The percentage of caffeine in extracts (%) (w/w)
MAE	30	4	77	10
Ultrasonic extraction	28	3.6	75	10
Heat reflux extraction	28	3.6	72	9

Solvent: ethanol/water (1:1 v/v) 100 ml; whole green tea leaves: 5 g; liquid/solid ratio: 20:1 ml g^{-1}; MAE for 4 min after pre-leaching for 90 min at room temperature about 20 °C; ultrasonic extraction at 20–40 °C for 90 min; heat reflux extraction at boiling pointing point about 85 °C for 45 min.

MAE was suitable for fast extraction of polyphenols and caffeine from green tea leaves. Food and medicinal industries would be benefited from this emerging technology of MAE, which was rapid, safe and more eco-friendly than conventional extraction methods.

Acknowledgements

This work was supported by National Natural Science Foundation of China (No. 29836130).

References

[1] H. Tanizawa, S. Toda, Y. Sazuka, T. Taniyama, T. Hayashi, S. Arichi, Y. Takino, Natural antioxidants. I. Antioxidative components of tea leaf (*Thea sinensis L.*), Chem. Pharm. Bull. 32 (1984) 2011–2014.
[2] B.L. Zhao, X.J. Li, R.G. He, S.J. Cheng, W.J. Xin, Scavenging effect of extracts of green tea and natural antioxidants on active oxygen radicals, Cell Biophys. 14 (1989) 175–185.
[3] W.J. Chen, S.Q. Wan, Research progress on polyphenols of tea, Nat. Prod. Res. Dev. 6 (2) (1994) 74–80.
[4] H.M. Kingston, L.B. Jassie, Introduction to Microwave Sample Preparation: Theory and Practice, American Chemical Society, Washington DC, 1988.
[5] S.S. Chen, M. Spiro, Study of microwave extraction of essential oil constituents from plant materials, J. Microwave Power Electromagn. Energy 29 (1994) 231–241.
[6] M.J.I. Mattina, W.A.I. Berger, C.L. Denson, Microwave assisted extraction of taxanes from *Taxus* biomass, J. Agric. Food Chem. 45 (1997) 4691–4696.
[7] J.C. Young, Microwave-assisted extraction of the fungal metabolite ergosterol and total fatty acids, J. Agric. Food Chem. 43 (1995) 2904–2910.
[8] J. Dai, V.A. Yaylayan, G.S.V. Raghavan, J.R. Parè, Extraction and colorimetric determination of azadirachtin-related limonoids in neem seed kernel, J. Agric. Food Chem. 47 (1999) 3738–3742.
[9] Y.Z. Ge, H. Jin, New methods for extraction of tea polyphenols, Chin. Herbal Med. 25 (3) (1994) 124–125.
[10] J. Li, Y.S. Feng, A study on extraction of tea polyphenols with supercritical carbon dioxide, Nat. Prod. Res. Dev. 8 (3) (1996) 42–47.
[11] Q.H. Hu, M. Jiang, J.C. Zhu, Research on technology of extraction of tea caffeine and polyphenols, Nat. Prod. Res. Dev. 9 (2) (1997) 63–66.
[12] X.J. Pan, H.Z. Liu, G.H. Jia, Y.Y. Shu, Microwave-assisted extraction of glycyrrhizic acid from licorice root, Biochem. Eng. J. 5 (2000) 173–177.
[13] Y.R. Lu, Chinese Medicine Chemistry, Xueyuan Publishing House, Beijing, 1995, pp. 10–11.
[14] G.B. Lü, J. Liu, Determination of Glycyrrhizin in Radix Glycyrrhizae and its preparation by HPLC, Chin. J. Pharm. Anal. 8 (3) (1988) 137–139.
[15] M.G. Song, F.K. Yang, N.Y. He, R.Y. Fei, J.X. Wang, L.M. Wang, Determination of tea polyphenols content, China National Standard *GB8313-87*, 1987.
[16] M.J. Li, L. Zhong, J.H. Yan, K. Cheng, W.J. Guo, J.A. Wang, Determination of tea caffeine content, China National Standard *GB8312-87*, 1987.