International Journal of
Food Science & Technology
www.ifst.org

International Journal of Food Science and Technology 2012, **47**, 24–31

Original article

Optimisation of the microwave-assisted extraction process for six phenolic compounds in *Agaricus blazei murrill*

Zuofa Zhang, Guoying Lv, Huijuan Pan & Leifa Fan*

Institute of Horticulture, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, China

(Received 18 March 2011; Accepted in revised form 14 August 2011)

Summary An efficient microwave-assisted extraction (MAE) technique was employed in the extraction of phenolic compounds from *Agaricus blazei murrill,* and phenolic compounds were quantified by High-performance liquid chromatography (HPLC). The MAE procedure was optimised, validated and compared with other conventional extraction techniques. MAE gave the best result because of the highest extraction efficiency within the shortest extraction time. The optimal conditions of MAE were 60% ethanol, ratio of solid/liquid 1:30, temperature 110 °C, irradiation power 500 W and three extraction cycles, each 5 min. This is the first report on combining MAE with HPLC for the extraction and quantification of phenolic compounds in *A. blazei murrill*. The developed MAE method provided a good alternative for the extraction of phenolic compounds in *A. blazei murrill* as well as other materials.

Keywords *Agaricus blazei murrill*, HPLC, microwave-assisted extraction, phenolic compounds.

Introduction

It has been reported that oxygen free radicals and other reactive oxygen species can cause oxidative injury to living organisms and thus play an important role in many lifestyle-related diseases such as arthritis, atherosclerosis, emphysema and cancer (Halliwell, 1991; Kehrer, 1993; Jacob, 1994). Therefore, the search of new product with antioxidative properties is very active domain of research. In recent years, synthetic antioxidants were reported to have the adverse effects such as toxicity and carcinogenicity (Williams *et al.*, 1999), and this situation has forced scientists to search for new natural antioxidants from herbs or the other materials.

Phenolic compounds are among the most potent and therapeutically useful bioactive substances, providing health benefits associated with reduced risk of chronic and degenerative disease (Luximon-Ramma *et al.*, 2005; Soobrattee *et al.*, 2005). Many of their biological effects have been attributed to free radical scavenging and antioxidant activity (Middleton *et al.*, 2000). The use of mushroom extracts as antioxidants is becoming increasing popular (Mau *et al.*, 2002; Lo & Cheung, 2005; Elmastas *et al.*, 2007), and the antioxidant properties were correlated with different antioxidant components such as total phenolics, carotenoids and ascorbic acid (Barros *et al.*, 2008).

The conventional liquid–solid extraction techniques, such as heat reflux extraction (HRE), ultrasonic extraction (UE) and maceration extraction (ME), are discommodious, laborious, time-consuming and require large volumes of toxic organic solvents. So increasing attention is paid to the development of more efficient extraction methods for the rapid extraction of active compounds from materials. Microwave-assisted extraction (MAE) has been accepted as a potential and powerful alternative to conventional extraction techniques in the extraction of organic compounds from materials. In respect of MAE, this technique has been applied in the development of methods for the extraction of organic compounds from matrices of soils (Serrano & Gallego, 2006), sediments (Morales *et al.*, 2005), seed (Prados-Rosales *et al.*, 2003) and foods (Robards, 2003; Wang & Weller, 2006). These studies show that these compounds are extracted more effectively when the energy provided by microwave is employed.

There were many reports about the extraction of phenolic compounds with the method of MAE from different materials (Ballard *et al.*, 2010; Liu *et al.*, 2010; Pérez-Serradilla & Castro, 2011), and the phenolic compounds stability was also investigated (Liazid *et al.*, 2007). The objectives of this study were to evaluate the performance of MAE for six phenolic compounds in

*Correspondent: Fax: +86 571 8640 4319;
e-mail: fanleifa2009@126.com

doi:10.1111/j.1365-2621.2011.02802.x

Agaricus blazei murrill and to optimise the MAE operating parameters. The parameters including ethanol concentration, ratio of solid/liquid, extraction time, extraction temperature, irradiation power and extraction cycles were studied in details. The phenolic compounds in *A. blazei murrill* were directly quantified by HPLC. The microwaving treatment on nutritional characteristics and antioxidant activity of *A. blazei Murril* has been reported (Sun *et al.*, 2011). However, to the best of our knowledge, this is the first report on combining MAE with HPLC for phenolic compounds in this mushroom, and the extraction efficiency of MAE was compared with conventional extraction techniques based on studying the extraction kinetics.

Materials and methods

Materials and chemicals

Agaricus blazei murrill was purchased from local market in Hangzhou, China. Dried mushrooms were powdered into a homogeneous size by a disintegrator and then sieved (20–40 mesh). Gallic acid, protocatechuic acid, catechin, syringic acid, myricetin and quercetin were purchased from Sigma chemical company (St Louis, MO, USA), acetonitrile and glacial acetic acid were HPLC grade, and water was supplied by a Milli-Q water purifier system from Milipore (Bedford, MA, USA).

HPLC conditions

The HPLC system consisted of a Nuer 2500 series HPLC system with a photodiode array detector and Clarity Work Station. Chromatographic separation was performed on a Sepax C_{18} column (250 × 4.6 mm i.d., 5 μm). The absorbance of each sample solution was measured at 280 nm. The mobile phase was distilled water with 2% acetic acid (solvent A) and acetonitrile (solvent B). The gradients were 0 min, 94%A; 0–2 min, 90%A; 2–25 min, 75%A; 25–45 min, 10%A; 45–47 min, 0%A; 47–55 min, 0%A; and 55–60 min, 92%A. Run time was 60 min using a flow rate of 0.8 mL min^{-1}. The standards and all solvents used were of HPLC grade, and the HPLC chromatogram of extracts of *A. blazei murrill* is shown in Fig. 1.

The quantifications of phenolic compounds were performed by the internal standard method. The calibration curves of gallic acid, protocatechuic acid, catechin, syringic acid, myricetin and quercetin showed good linearity over the ranges of 0.8–89.0, 0.4–55.0, 11.6–128.0, 0.2–78.0, 0.8–135.0 and 0.6–88.9 μg mL^{-1}, respectively. The regression equations were $Y = 40.696X + 2.4139$ ($R^2 = 0.9932$, $n = 6$), $Y = 139.87 - 12.865$ ($R^2 = 0.9985$, $n = 6$), $Y = 12.445X + 0.896$ ($R^2 = 0.9876$, $n = 6$), $Y = 23.179X + 1.864$ ($R^2 = 0.9952$, $n = 6$), $Y = 102.84X - 36.27$ ($R^2 = 0.9912$, $n = 6$) and $Y = 8.97X + 0.36$ ($R^2 = 0.9927$, $n = 6$), respectively. Where Y is the peak area of analyte and X is the concentration of analyte (μg mL^{-1}). The LODs (S/N = 3) and LOQs (S/N = 10) for the analytes were < 10 and 30 ng, respectively.

Microwave-assisted extraction

Microwave-assisted extraction was carried out in a MARS-II (1000 W, 2450 MHz) microwave accelerated reaction system from SINEO Microwave Chemistry Technology (Shanghai, China). The materials (1.0 g) were accurately weighted and introduced in 100-mL PFTE (CEM) extraction vessels, and appropriate amount of solvent was added. Then, the container was lidded, and a temperature sensor would be inserted into the container to measure and control the internal temperature, which allows the technical parameters of instrumentation used to prevent overheating. The extraction process was performed under different conditions. After extraction, the vessels were left for 30 min to cool down below 35 °C. The effects of ethanol concentration, ratio of solid/liquid, irradiation time, extraction temperature, irradiation power and extrac-

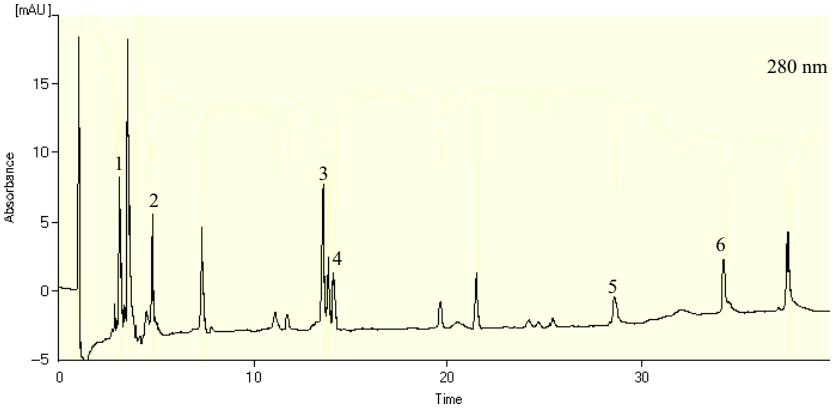

Figure 1 HPLC chromatograms of extracts of *Agaricus blazei murrill*. 1, gallic acid; 2, protocatechuic acid; 3, catechin; 4, syringic acid; 5, myricetin; 6, quercetin.

tion cycles on the extraction yields of six phenolic compounds were investigated.

Heat reflux extraction

The materials (1.0 g) were weighted and added into a round-bottom flask with 30 mL of ethanol–water (60:40, v/v) solution; the flask was placed into a water bath and connected with the condenser and then allowed to reflux at 90 °C for three 1-h cycles.

Ultrasonic extraction

The materials (1.0 g) were weighted and put into a conical flask, 30 mL of ethanol–water (60:40, v/v) solution was added to it, and this was then extracted in an ultrasonic bath (Kunshan Ultrasonic Instrument Co. Ltd., Kunshan, China) with the power of 100 W at 25 °C for three 40 min cycles.

Maceration extraction

The materials (1.0 g) were accurately weighted and added into a flask with 30 mL of ethanol–water (60:40, v/v) solution and macerated at 25 °C for three 12-h cycles.

After the extraction step, the filtered solutions were concentrated to dryness in a rotary evaporator device (RE-52aa; Shanghai Huxi Instrument Co., Shanghai, China) under vacuum at 55 °C. The obtained dry extracts were diluted in methanol (2 mL). The sample was further 500-fold diluted and centrifuged at 11125 ***g*** for 10 min. The supernatant was filtered through a 0.45-μm nylon membrane and then injected into HPLC for analysis. Three replicate injections were analysed to determine the extraction yields of phenolic compound with the mean peak area.

The extraction kinetics of tested phenolic compounds in *A. blazei murrill* with different method

The extraction kinetics could monitor the extraction efficiency at different extraction times. In this study, the extraction conditions for MAE were 60% ethanol, ratio of solid/liquid 1:30, temperature 110 °C, irradiation power 500 W and the tested phenolic compounds were determined at 1, 3, 5, 10, 15 and 20 min; For UE, the extraction conditions were 60% ethanol, ratio of solid/liquid 1:30, temperature 25 °C and the tested phenolic compounds were determined at 10, 20, 30, 40, 60 and 90 min; For HRE, the extraction conditions were 60% ethanol, ratio of solid/liquid 1:30, temperature 90 °C and the tested phenolic compounds were determined at 5, 10, 30, 50, 80 and 120 min; and For ME, the extraction conditions were 60% ethanol, ratio of solid/liquid 1:30, temperature 25 °C and the tested phenolic compounds were determined at 3, 6, 9, 12, 24 and 48 h.

The effect of microwaves and heat on the tested phenolic compounds degradation

The samples of gallic acid, protocatechuic acid, catechin, syringic acid, myricetin and quercetin at concentration of 0.05 g L^{-1} were prepared in 60% ethanol. A volume of 20 μL was injected into HPLC system as a control. To study the effect of microwaves and heat on the tested phenolic compounds degradation, 50 mL of sample was placed into the Teflon vessels of the SINEO microwave extraction system and was kept at five different temperatures: 80, 90, 100, 120 and 150 °C. The experiments were carried out in triplicate. The samples were processed, collected and analysed as previously described in 'microwave-assisted extraction'.

Statistical analysis

All tests were conducted in triplicate. Data were reported as mean ± SD. Analysis of variance and significant differences among means were tested by one-way ANOVA using SPSS software (version11.5 for Windows; SPSS Inc., Chicago, IL, USA).

Results and discussion

Optimisation of MAE procedure

The factors concerning MAE include ethanol concentration, ratio of solid/liquid, extraction temperature, irradiation power and extraction cycles. The influence of each factor was studied by single-factor experiments. All assays were conducted in triplicate.

Effect of ethanol concentration

The selection of the most suitable solvent for extracting the analytes of interest from the sample matrix is a fundamental step in developing any extraction method. Based on our preliminary experiments, in this study, mixtures of ethanol–water were tested. The rest of the variables employed were solid/liquid ratio 1:20 (g mL^{-1}), three extraction cycles, each 10 min, extraction temperature 80 °C and irradiation power 400 W.

From Fig. 2a, it can be observed that the yields of phenolic compounds were greatly influenced by the ethanol concentration. The yields of the six tested compounds increased sharply with the increase of ethanol concentration up to 60%. When the ethanol concentration was over 70%, the extraction yields decreased. From these results, it is clear that the addition of some amount of water improved the

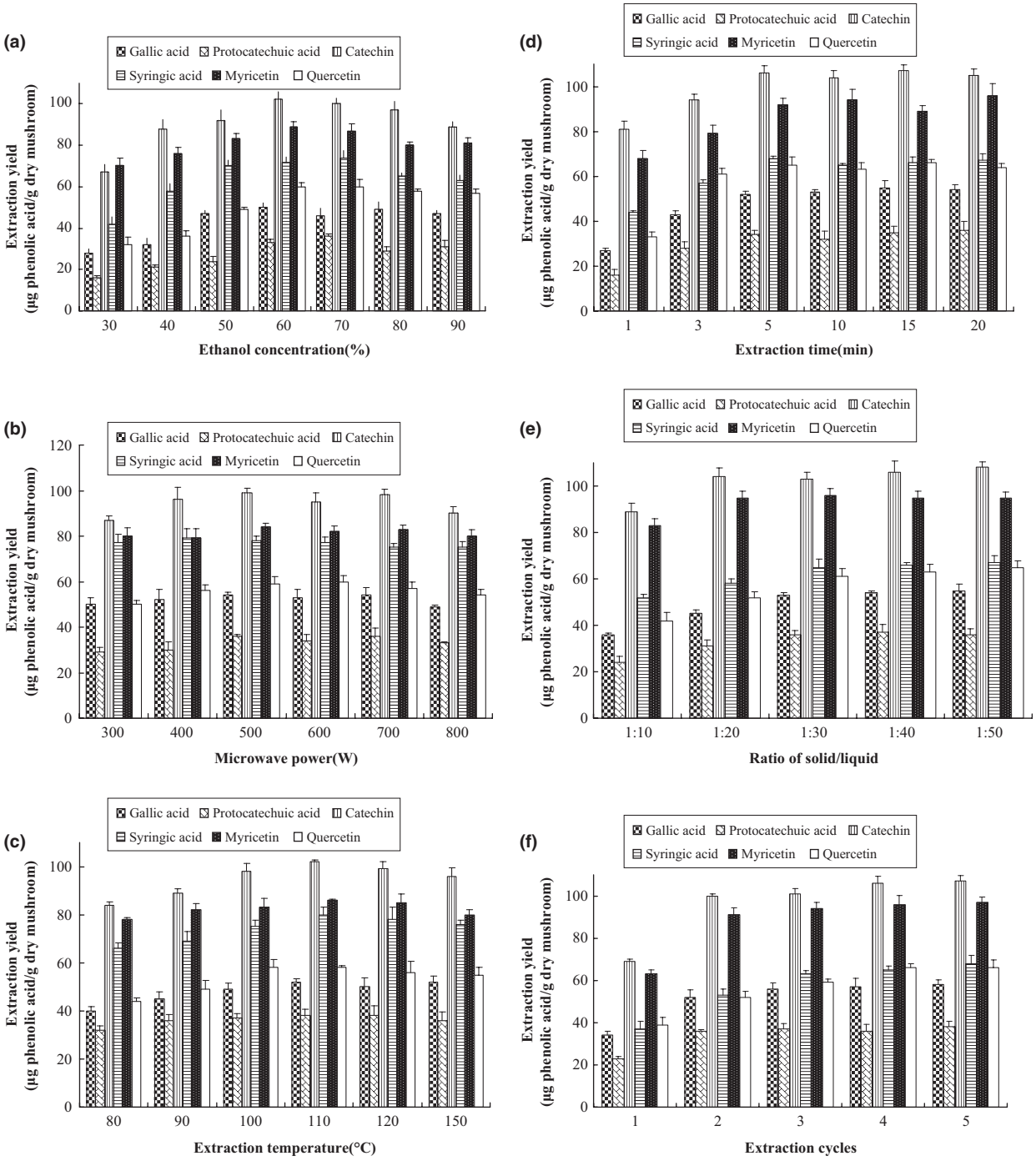

Figure 2 Effect of different extraction parameters on the yields of six phenolic compounds in single-factor experiments. Values are expressed as mean ± SD of three determinations.

extraction efficiency. The possible reason for the increased efficiency might be due to the increase in swelling of materials by water, which increased the contact surface area between the plant matrix and the solvent (Rostagno *et al.*, 2003). Therefore, 60% ethanol solution was used in the following experiments.

Effect of irradiation power

Microwave irradiation energy disrupts hydrogen bonds, because of microwave-induced dipole rotation of molecules and migration of dissolved ions. To evaluate the effect of irradiation power on MAE, the different microwave irradiation powers were controlled, e.g. 300, 400, 500, 600, 700 and 800 W. The rest of the extraction conditions were 60% ethanol as extraction solvent, solid/liquid ratio 1:20 (g mL^{-1}) and three extraction cycles, each 10 min under 80 °C extraction temperature. As shown in Fig. 2b, high microwave irradiation power enhanced the yields of phenolic compounds when the power was lower than 500 W. However, when the power was higher than 700 W, the yields declined. These data suggest that applying a higher irradiation power for a short time maybe the most effective way to extract phenolic compounds from *A. blazei murrill*. However, a higher irradiation power may lead to the thermal degradation of the phenolic compounds. Hence, 500 W was chosen as the appropriate microwave irradiation power.

Effect of extraction temperature

Extraction temperature is a factor that must be investigated to increase the effectiveness of extraction of analytes employing MAE. Generally, higher extraction temperature is profitable for the extraction and reduces the reaction time. To examine the performance of different extraction temperatures on the yields of phenolic compounds during MAE, an amount of 1.0 g materials was extracted with 60% ethanol solution for 10 min and a solid/liquid ratio of 1:20 (g mL^{-1}) under 500 W irradiation power at different temperatures, repeated three cycles.

As confirmed in Fig. 2c, with increasing extraction temperature from 80 to 110 °C, the extraction yields of six phenolic compounds increased rapidly and reached its maximum at 110 °C. Above 110 °C, the yields of phenolic compounds increased slowly and even decreased. Increasing temperatures may also cause an opening of the cell matrix, decreasing solvent viscosity and increasing diffusivity; as a result, the efficiency of extraction increased (Camel, 2000; Pan *et al.*, 2000). However, high temperature may cause the decomposition of active compounds. So, 110 °C was chosen as the optimum temperature for extraction.

Effect of extraction time

It is necessary to select a proper extraction time to guarantee the completion of the extraction. Studies were carried out at different times, e.g. 1, 5, 10, 15, 20 and 25 min. The rest of the extraction conditions were 60% ethanol as extraction solvent, solid/liquid ratio 1:20 (g mL^{-1}), extraction temperature 110 °C, irradiation power 500 W and this process were repeated three cycles. As shown in Fig. 2d, the yields of phenolic compounds increased quickly from 1 to 5 min and reached its maximum at 5 min. Then, the extraction yields slightly decreased with the extension of the extraction time. This may be due to the decomposition of phenolic compounds at long irradiation time. Thus, 10 min was considered as the appropriate extraction time.

Effect of ratio of solid/liquid

In investigating the influence of ratio of solid/liquid on yields of phenolic compounds, several tests were performed at different ratios of solid/liquid. The rest of the variables employed were 60% ethanol as extraction solvent, three cycles, each 5 min, extraction temperature 110 °C and irradiation power 500 W. It was exhibited in Fig. 2e that the yields of six phenolic compounds increased with decreasing ratios of solid/liquid. In the tested range of 1:30 to 1:50 (g mL^{-1}), there was no significant difference. If the extraction was carried out under high liquid/solid ratio, the concentration of phenolic compounds in extraction solution was low. It meant that more energy and time were needed to condense the extraction solution in later separation and purification process. Hence, a value of 1:30 (g mL^{-1}) was considered as the optimal ratio of solid/liquid for the MAE process.

Effect of extraction cycles

The effect of successive extractions of the residue, i.e. extraction cycles, was investigated in this experiment. The rest of the variables employed were 60% ethanol as

Table 1 Recoveries for microwave-assisted extraction (MAE) at 60, 80, 100, 120 and 150 °C of six phenolic compound standards[a]

	60 °C	80 °C	100 °C	120 °C	150 °C
Gallic acid	98.5 ± 3.2	98.9 ± 5.2	104.1 ± 5.9	95.3 ± 6.2	90.5 ± 2.2
Protocatechuic acid	96.6 ± 5.6	103.5 ± 3.9	98.7 ± 2.8	99.6 ± 0.7	90.7 ± 6.1
Catechin	101.4 ± 2.8	96.4 ± 4.6	99.2 ± 1.2	102.1 ± 3.8	106.5 ± 5.9
Syringic acid	95.1 ± 6.1	101.2 ± 3.2	102.1 ± 3.5	93.4 ± 6.4	88.5 ± 2.6
Myricetin	98.7 ± 2.9	100.6 ± 1.8	100.2 ± 0.8	72.8 ± 3.8	0
Quercetin	96.8 ± 4.4	94.6 ± 2.4	98.5 ± 1.9	97.4 ± 5.2	94.6 ± 3.1

[a]Mean ± SD for recoveries relative to the reference.

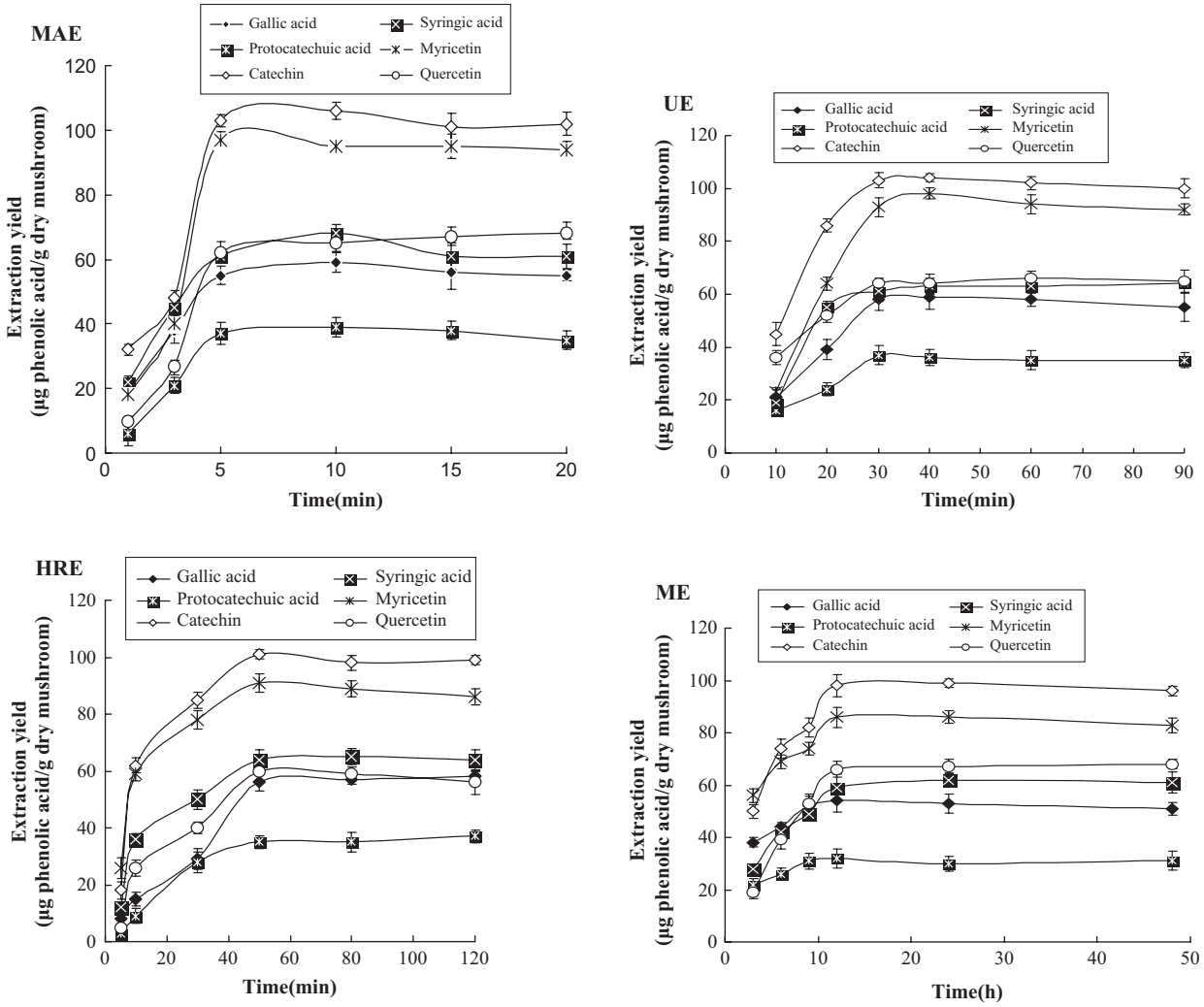

Figure 3 Extraction kinetics of six phenolic compounds in *Agaricus blazei murrill* by different extraction techniques. Values are expressed as mean ± SD of three determinations. MAE, Microwave-assisted extraction; HRE, Heat reflux extraction; UE, Ultrasonic extraction; ME, Maceration extraction.

extraction solvent, solid/liquid ratio 1:30 (g mL^{-1}), extraction for 10 min, extraction temperature 110 °C and microwave irradiation power 500W. The residue was taken back and re-extracted using fresh solvent each time. It can be seen from Fig. 2f that the yields of tested phenolic compounds increased slowly without significant differences among the yields except for catechin after three cycles. To save solvent, energy and time, three-cycle extraction is enough to release most of phenolic compounds into solvent.

The effect of microwaves and heat on the tested phenolic compounds degradation

Before proposing an extraction method in the determination of polyphenols, it is necessary to assure whether these compounds are stable under the extraction conditions. In this study, we evaluate the stability of phenolic compounds with the same method of extraction (500 W, 5 min) and with the same solvent (60% ethanol) at different temperatures: 60, 80, 100, 120 and 150 °C. All the extractions have been performed in triplicate, and the extracts have been analysed by HPLC.

As can be observed in Table 1, the recoveries of most of the tested phenolic compounds extracted are over 95% when the temperature was below 100 °C. As the temperature is increased, considerable degradation of the phenolic compounds is observed. For myricetin, the recovery was only 72.8% at 120 °C, when the temperature was up to 150 °C, no myricetin was found, and the same result was obtained by Liazid *et al.* (2007).

Table 2 Comparison of MAE and conventional extraction methods under the optimal conditions

Method	Yield (μg g^{-1})					
	Gallic acid	Protocatechuic acid	Catechin	Syringic acid	Myricetin	Quercetin
MAE	59.7	38.9	107.9	68.5	96.8	66.6
HRE	55.7	35.8	102.3	66.2	93.1	54.1
UE	57.8	36.9	106.7	65.3	94.8	62.6
ME	52.4	34.5	101.9	64.3	92.7	57.9

Values are expressed as mean of three determinations.
MAE, Microwave-assisted extraction; HRE, Heat reflux extraction; UE, Ultrasonic extraction; ME, Maceration extraction.

Extraction kinetics

In the extraction process, the extraction depends on both the extraction efficiency and the chemical change of the target compound. The extraction yield is defined as the amount of the target compound transferred into the extraction solvent. The changes in the chemical structure of target compounds in the extraction procedure can directly influence theirs extraction yield (Chen *et al*., 2008). The extraction kinetics of six phenolic compounds under four extraction methods is shown in Fig. 3.

In MAE, with an increase in extraction time, the extraction yields of phenolic compounds increased in the initial 5 min and then keep constant from 5 to 20 min except for syringic acid (slightly decreased). In HRE, the yields of phenolic compounds reached its maximum in 50 min and did not significantly change from 50 to 120 min. In UE, the yields of six phenolic compounds increased with the increase in extraction time up to 30 min and remain constant. As for ME, it possessed a poor capability for the extraction of phenolic compounds, no clear increase in the yields of phenolic compounds was observed with increasing extraction times.

Comparison of different extraction techniques

In this study, MAE, HRE, UE and ME techniques were compared for their extraction efficiency of six phenolic compounds from *A. blazei murrill* (Table 2). The extraction time of MAE, HRE, UE and ME were 10, 50, 30 min and 12 h, respectively. The extraction temperature of MAE and HRE was 110 and 90 °C, and the extraction temperature of UE and ME was room temperature. The extraction yields of phenolic compounds obtained using MAE and UE were higher than those using HRE and ME methods. In MAE, the target compounds can sufficiently absorb microwave energy and be quickly transferred into the extraction solvent, the extraction time was dramatically reduced, and the extraction efficiency was considerably increased. Therefore, MAE represented a simple and efficient method for the extraction of phenolic compounds from *A. blazei murrill*. In this study, the results were supported by some reports that MAE possesses many advantages compared with other methods for the extraction of compounds such as saving extraction time and solvent and having high extraction efficiency (Chee *et al*., 1996; Xiong *et al*., 1999).

Conclusions

An efficient MAE method was developed for the extraction of six phenolic compounds from *A. blazei murrill*. The target phenolic compounds were directly quantified by HPLC. To the best of our knowledge, this is the first report on combining MAE with HPLC for the extraction and quantification of phenolic compounds in *A. blazei murrill*. Compared with conventional extraction techniques, MAE process required less extraction time and provided higher extraction efficiency. The optimum MAE conditions were extracted with 60% ethanol solution, ratio of solid/liquid of 1:30, at an extraction temperature of 110 °C and three extraction cycles, each 5 min under irradiation power of 500 W. The results showed that MAE has an obvious predominance for the extraction of active compounds from mushroom, especially phenolic compounds. Therefore, we conclude that MAE has a good potential for the extraction of phenolic compounds from *A. blazei murrill* and can be referenced for the extraction of other compounds from mushrooms.

Acknowledgments

This research was supported by grants from Innovation Group Project in Edible Mushroom of Zhejiang Province (No. 2009R05A50B01). We acknowledge all staff for their valuable assistance in conducting this study.

References

Ballard, T.S., Mallikarjunan, P., Zhou, K. & O'Keefe, S. (2010). Microwave-assisted extraction of phenolic antioxidant compounds from peanut skins. *Food Chemistry*, **120**, 1185–1192.

Barros, L., Falcao, S., Baptista, P., Freire, C., Vilas-Boas, M. & Ferreira, I.C.F.R. (2008). Antioxidant activity of *Agaricus sp*.

mushroom by chemical, biochemical and electrochemical assays. *Food Chemistry*, **111**, 61–66.
Camel, V. (2000). Microwave-assisted solvent extraction of environmental samples. *Trends in Analytical Chemistry*, **19**, 229–248.
Chee, K.K., Wong, M.K. & Lee, H.K. (1996). Optimization of microwave-assisted solvent extraction of polycyclic aromatic hydrocarbons in marine sediments using a microwave extraction system with high-performance liquid chromatography -fluorescence detection and gas chromatography-mass spectrometry. *Journal of Chromatography A*, **723**, 259–271.
Chen, L.G., Jin, H.Y., Ding, L. *et al*. (2008). Dynamic microwave-assisted extraction of flavonoids from Herba Epimedii. *Separation and Purification Technology*, **59**, 50–57.
Elmastas, M., Isildak, O., Turkekul, I. & Temur, N. (2007). Determination of antioxidant activity and antioxidant compounds in wild edible mushrooms. *Journal of Food Composition and Analysis*, **20**, 337–345.
Halliwell, B. (1991). The biological toxicity of free radicals and other reactive oxygen species. In: *Free Radical and Food Additives*. Pp. 37–57. London: Taylor & Francis Ltd.
Jacob, R.A. (1994). Nutrition, health and antioxidants. *Inform*, **5**, 1271–1275.
Kehrer, J.P. (1993). Free-radicals as mediators of tissue-injury and disease. *Critical Reviews in Toxicology*, **23**, 21–48.
Liazid, A., Palma, M., Brigui, J. & Barroso, C.G. (2007). Investigation on phenolic compounds stability during microwave-assisted extraction. *Journal of Chromatography A*, **1140**, 29–34.
Liu, J.L., Yuan, J.F. & Zhang, Z.Q. (2010). Microwave-assisted extraction optimized with response surface methodology and antioxidant activity of polyphenols from hawthorn (*Crataegus pinnatitida Bge*.) fruit. *International Journal of Food Science and Technology*, **45**, 2400–2406.
Lo, K.M. & Cheung, P.C.K. (2005). Antioxidant activity of extracts from the fruiting bodies of *Agrocybe aegerita var. alba*. *Food Chemistry*, **89**, 533–539.
Luximon-Ramma, A., Bahorun, T., Crozier, A. *et al*. (2005). Characterization of the antioxidant functions of flavonoids and proanthocyanidins in Mauritian black teas. *Food Research International*, **38**, 357–367.
Mau, J.L., Lin, H.C. & Chen, C.C. (2002). Antioxidant properties of several medicinal mushrooms. *Journal of Agriculture and Food Chemistry*, **50**, 6072–6077.
Middleton, J.r., Kandaswami, C. & Theoharides, T.C. (2000). The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease and cancer. *Pharmacological Reviews*, **52**, 673–839.
Morales, S., Canosa, P., Rodriguez, I., Rubí, E. & Cela, R. (2005). Microwave assisted extraction followed by gas chromatography with tandem mass spectrometry for the determination of triclosan and two related chlorophenols in sludge and sediments. *Journal of Chromatography A*, **1082**, 128–135.
Pan, X.J., Liu, H.Z., Jia, G.H. & Shu, Y.Y. (2000). Microwave-assisted extraction of glycyrrhizic acid from licorice root. *Biochemical Engineering Journal*, **5**, 173–177.
Pérez-Serradilla, J.A. & Castro, M.D.L. (2011). Microwave-assisted extraction of phenolic compounds from wine lees and spray-drying of the extract. *Food Chemistry*, **124**, 1652–1659.
Prados-Rosales, R.C., Garcia, J.L.L. & Castro, M.D.L. (2003). Rapid analytical method for the determination of pesticide residues in sunflower seeds based on focused microwave-assisted Soxhlet extraction prior to gas chromatography-tandem mass spectrometry. *Journal of Chromatography A*, **993**, 121–129.
Robards, K. (2003). Strategies for the determination of bioactive phenols in plants, fruit and vegetables. *Journal of Chromatography A*, **1000**, 657–691.
Rostagno, M.A., Palma, M. & Barroso, C.G. (2003). Ultrasound-assisted extraction of soy isoflavones. *Journal of Chromatography A*, **1012**, 119–128.
Serrano, A. & Gallego, M. (2006). Continuous microwave-assisted extraction coupled on line with liquid-liquid extraction: determination of aliphatic hydrocarbons in soil and sediments. *Journal of Chromatography A*, **1104**, 323–330.
Soobrattee, M.A., Neergheen, V.S., Luximon-Ramma, A., Aruoma, O.I. & Bahorun, T. (2005). Phenolics as potential antioxidant therapeutic agents: mechanisms and actions. *Mutation Research*, **579**, 200–213.
Sun, L.P., Zhuang, Y.L. & Bai, X. (2011). Effects of boiling and microwaving treatments on nutritional characteristics and antioxidant activities of *Agaricus blazei Murril*. *International Journal of Food Science and Technology*, **46**, 1209–1215.
Wang, L.J. & Weller, C.L. (2006). Recent advances in extraction of nutraceuticals from plants. *Trends Food Science and Technology*, **17**, 300–312.
Williams, G.M., Latropoulos, M.J. & Whysner, J. (1999). Safety assessment to butylated hydroxyanisol and butylated hydroxytoluene as antioxidant food additives. *Food and Chemical Toxicology*, **37**, 1027–1038.
Xiong, G.H., Tang, B.Y., He, X.Q., Zhao, M.Q., Zhang, Z.P. & Zhang, Z.X. (1999). Comparison of microwave-assisted extraction of triazines from soils using water and organic solvents as the extractants. *Talanta*, **48**, 333–339.