Journal of Food Health and Bioenvironmental Science
Journal homepage : http://jfhb.dusit.ac.th/

Comparison of Extraction Solvents and Techniques Used for The Assay of Free and Bound Phenolic Acids from Rice Samples

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Article info

Article history:
Received 05 April 2018
Revised 11 June 2018
Accepted 19 June 2018

Keywords:
Extraction Method, Phenolic Acids, Rice

Abstract

The extraction and alkaline hydrolysis methods of free and bound phenolic acids were studied on Jasmine (Jasmine 85), Basmati (Basmati 370-1) and brown rice (Jasmine 85) (*Oryza satia L.*). Free phenolic acids in three rice samples were extracted by seven different solvent systems: 100% and 80% (v/v) methanol, 100 % and 80% (v/v) ethanol, 100% (v/v) water, 80% (v/v) acetone, and 35:35:30 % (v/v/v) of methanol:acetone:water with five different extraction techniques (shaker, regular ultrasonic (RUAE), high energy ultrasonic (HUAE), microwave (MAE) and pressurized liquid extraction (PLE)). The phenolic extracted contents were analyzed by the ferric reducing antioxidant power (FRAP) assay. Moreover, three different alkaline hydrolysis techniques: ultrasonic (UAAH), microwave (MAAH), and pressurized liquid assisted alkaline hydrolysis extraction (PAAH) were used for extracting bound phenolic acids and analyzed by liquid chromatography-mass spectrometry. Maximum extraction yields of total phenolic acids were obtained from 35:35:30 % (v/v/v) of methanol/acetone/water as solvent system with PLE technique while bound phenolic acids were obtained by MAAH technique. In addition, MAAH technique provides the highest extraction yields of bound phenolic acids. PLE and MAAH techniques were suitable for free and bound phenolic acids extraction from rice samples, respectively.

Introduction

Rice (*Oryza satia L.*) is an important cereal crop throughout the world. There is significant interest in rice due to the association of health and rice consumption in the past few decades (Zhai et al., 2001). Rice contains many chemical compositions. A large number of bioactive phytochemicals such as flavonoids and

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phenolic acids were found in rice samples (Frei & Becker, 2004). For the prevention of a wide range of health problems associated with cardiovascular disease, osteoporosis, and cancer, phenolic acids enriched extracts have been evaluated as an alternative solution to chemical medicine (Koodkaew & Limpichotikul, 2017; Nakornriab & Krasaetep, 2018; Nukit Meeprathom et al., 2018; Martin, et al., 2011). Rice is a good natural source of phenolic acids and a major food staple for more than half of the world's population especially in Asia. The global annual consumption of rice has increased from 421 to 453 million tons during the past five years (United State Department of Agriculture, 2010). Phenolic acids can be accounted in two subgroups according to their structures. The first subgroup is the hydroxybenzoic acid such as gallic acid vanillic acid and syringic acids. The second subgroup is the hydroxycinnamic acid such as caffeic acid, cinnamic acid and sinapic acids (Robbins, 2003). Ferulic acid and p-coumaric acid were found as the major phenolic acids in rice (Yu et al., 2012). 62% of total phenolic acids are found as free form or bound form, and are generally linked to cell wall structural components such as cellulose, lignin and proteins via ester bonds (Bonoli et al., 2004).

According to the wide variation in chemical and physical properties of phenolic acids, development of extraction procedure for phenolic acids has been challenging. Many researchers have proposed various extraction and alkaline hydrolysis methods for extraction of phenolic acids in rice samples. Summary of extraction procedures used for phenolic acids as free form and bound form in rice samples are presented in Table 1 and Table 2, respectively. Free phenolic acids in rice samples can be extracted by various techniques such as shaker, stirring, ultrasonic assisted extraction (UAE), microwave assisted extraction (MAE), and pressurized liquid extraction (PLE). In addition, various types of extraction solvent and its composition were used for extraction of free phenolic acids in rice samples such as methanol, ethanol, water and acetone with difference in the proportion of water.

Bound phenolic acids in rice samples can be

Table 1 Summary of common procedures used for extraction of free phenolic acids from rice samples

No.	Extraction procedure	Optimum solvent	Reference
1	Ten grams of samples were extracted by MAE at 2450 MHz for 3.5 min	100% methanol	Dar & Sharma, 2011
2	Two grams of samples were extracted by mechanical shaker for 1 hour at RT	80% methanol	Qiu et al., 2010
3	2.5 g of rice samples were extracted by PLE for 15 min at 1500 psi and RT	70% methanol	Vichapong et al., 2010
4	Two grams of samples were extracted by shaker for 10 min at RT	80% ethanol	Mira et al., 2009
5	0.5 g of rice samples were extracted by UAE at different extraction parameter (solvent, time, and temperature)	65% ethanol	Tabaraki & Nateghi, 2011
6	400 mg of samples were extracted by UAE with different extraction conditions (solvent, sample to solvent ratio, extraction time and temperature)	Water	Onofre & Hettiarachchy, 2007
7	Rice samples (210 μm) were extracted by shaking water bath at 32 ºC for 4 hour	70% acetone	Tananuwong & Tewaruth, 2010
8	0.3 g of rice samples were extracted by stirring extraction for 1 day at 4 ºC under nitrogen condition	7:7:6 v/v/v methanol:acetone : water	Zhou et al., 2004

extracted by using alkaline hydrolysis. Generally, the sample was treated with NaOH, and was then acidified by acid to liberate phenolic acids. The liberated phenolic acids were then extracted with diethyl ether. However, alkaline hydrolysis may lead to significant losses of phenolic acids and derivatives (Krygier et al., 1982). This problem can be solved by the addition of ascorbic acid (1%) and ethylenediamine tetraacetic acid (EDTA; 10mM) to prevent the degradation of phenolic acids during alkaline hydrolysis (Nardini et al., 2002). In addition, comparisons of the extraction efficiencies, sensitivity, hydrolysis time, and precision between ultrasonic assisted alkaline hydrolysis (UAAH) and conventional alkaline hydrolysis techniques were studied (Santos et al., 2011). UAAH provides better extraction efficiencies, sensitivity, hydrolysis time and precision. In this research, ultrasonic assisted alkaline hydrolysis (UAAH), microwave assisted alkaline hydrolysis (MAAH) and pressurized liquid assisted alkaline hydrolysis (PAAH) methods have been used for evaluation of bound and total phenolic acids from plant material.

In this research, the techniques used for extraction of phenolic acids from rice samples have been studied. For free phenolic acids, seven extractants (100 and 80% (v/v) methanol, 100 and 80% (v/v) ethanol, 100% (v/v) water, 80% (v/v) acetone, and 35:35:30 (v/v/v) methanol: acetone:water) have been studied for their extraction efficiencies. In addition, extraction efficiencies of different extraction techniques (shaker, regular ultrasonic (RUAE), High energy ultrasonic (HUAE), MAE and PLE) have been compared. Moreover, alkaline hydrolysis time and methods (UAAH, MAAH and PAAH) for determination of total phenolic acids in rice samples have been compared. Three varieties of rice samples (Jasmine, Basmati and Brown rice) were selected and analyzed by LC-MS analysis.

Materials and methods

1. Sample materials

Jasmine, Basmati and organic long grain brown rice samples were purchased from a local grocery store (Giant Supermarket in Beltsville, Maryland, USA). These grains were ground in a coffee grinder. The ground samples were kept under nitrogen at -60 ºC until analyzed.

2. Chemicals and reagents

HPLC grade of methanol, ethanol, ethyl acetate, and acetone were obtained from Fisher Chemicals (Fair Lawn, New Jersey, USA). Analytical grade of formic acid, sodium hydroxide and hydrochloric acid were obtained from Aldrich Chemical Company (Mikwaukee, Wisconsin, USA). Ascorbic acid, ethylenediamine

Table 2 Summary of common procedures used for extraction of bound phenolic acids from rice samples

No.	Extraction procedure	Optimum solvent	Reference
1	The residue from free phenolic acids extraction was hydrolyzed with 2M NaOH by shaker at RT under nitrogen atmosphere for 15 min	Ethyl acetate	Vichapong et al., 2010
2	The crude methanol extracted of rice samples were hydrolyzed with 4M NaOH by shaker for 4 hour	Ethyl acetate	Qiu et al., 2010
3	The residue from free phenolic acids extraction was hydrolyzed with 4M NaOH by stirring under nitrogen atmosphere for 4 hour at RT	Ethyl acetate	Tabaraki & Nateghi, 2011
4	Rice flour was direct hydrolyzed with 4M NaOH with stirring under nitrogen atmosphere for 4 hour at RT	Ethyl acetate	Zhou et al., 2004

tetraacetate, Trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid), ferric chloride and TPTZ (2,4,6,Tris (2-pyridyl)-S-Triazine) were purchased from Sigma (St. Louis, Missouri, USA). Ascorbic acid was purchased from J.T. Baker (New Jersy, USA) and EDTA was purchased from EMD Chemical INC. (Darmstadt, Germany). Deionized water was obtained by using a millipore Milli-Q purification cation system (Millipore Corp., New Bedford, Massachusetts, USA) Polyvinylidene difluoride (PVDF) syringe filters with pore size 0.45 µm were obtained from National Scientific Company (Duluth, Georgia, USA).

3. Procedures and conditions for extraction of free phenolic acids

3.1 Extraction solvent effect

The method for comparison of extraction solvent effect was carried out using a SRV-160 ultrasonic bath (Advanced sonic processing system, Connecticut, USA). Aliquots of 100 mg of rice samples in 15 mL screw cap tube and 5 mL of extraction solvent was added to each tube. Extractions were carried out at ambient temperature for 30 min using seven different solvent conditions (100 and 80% (v/v) methanol, 100 and 80% (v/v) ethanol, 100% (v/v) water, 80% (v/v) acetone, and 35:35:30 (v/v/v) methanol: acetone: water). The extracts were centrifuged at 4000 rpm for 10 min and the supernatant was separated. Aliquots of rice extracts were adjusted to the final volume of 5 mL and filtered through a 0.45 µm PVDF syringe filter prior to test by FRAP assay.

3.2 Comparison of extraction techniques

Five different methods namely: automated wrist shaker (LAB-LINE instruments Inc., Illinois, USA), Regular ultrasonic assisted extraction (RUAE, 100 W; Branson Ultrasonic Corporation, Connecticut, USA), High energy ultrasonic assisted extractions (HUAE, 500 W; Advance Sonic Processing Systems, Connecticut, USA), Microwave assisted extraction (MAE; CEM North Carolina, USA) and Pressurized liquid extraction (PLE; Model ASE 200, Dionex Corporation, Sunnyvale, California, USA) were compared using the optimum extraction solvent as previous described.

In case of automated wrist shaker, RUAE and HUAE, 100 mg of ground rice sample powder was mixed with 5mL of extraction solvent. The mixture was extracted at ambient temperature for 30 min. The mixture was centrifuged at 4000 rpm for 10 min and the supernatant was separated by simple decantation. Aliquots of supernatant was adjusted to the final volume of 5 mL, filtered through a 0.45 µm PVDF syringe filter and filtered extracts were used for the assay of the phenolic acids by FRAP procedure. Four replicate extractions were carried out with each sample.

For microwave assisted extraction (MAE), 100 mg of sample and magnetic bar was placed in the 10 mL glass vial. The mixture was mixed with 5 mL of acetone:methanol:water, 35:35:30% (v/v/v) using discover microwave extractor. Extraction was carried out with a frequency of 2455 MHz and output power 725 W at different temperatures (40 ºC and 100 ºC) for 30 min. The mixture was centrifuged at 4000 rpm for 10 min and the supernatant was separated by simple decantation. Aliquots of supernatant was adjusted the final volume to 5 mL, filtered through a 0.45 µm PVDF syringe filter and filtered extracts were used for the assay of the phenolic acids by FRAP procedure. Four replicate extractions were carried out with each sample.

In case of pressurized liquid extraction (PLE), 100 mg of sample powders were placed in a 5 mL stainless steel extraction cell and extracted with PLE. The top and bottom of the extraction cell were placed by two circular cellulose filters (size 13.5 mm) in order to prevent suspended particle from entering the collection vial. Ottawa Sand was used to fill the cell at the remaining void volume. Both extraction cell and collection vials were appropriately arranged in the two designated carousels. Extraction condition was performed under 1000 psi at 40 ºC and 100 ºC, with 5 min pre-heat time, 5 min heat time, 30 min static extraction time, 10% flush, and 90 s purge time with one extraction cycle. The extracts were collected in 60 mL sample vials with Teflon coated rubber caps. Each extract was transferred to 5 mL volumetric flask and adjusted the total volume to 5 mL using same extraction solvent, filtered through a 0.45 µm PVDF syringe filter prior to analyze by FRAP assay.

4. The extraction of total phenolic acids (free phenolic acids and bound phenolic acids)

4.1 Comparison of alkaline hydrolysis time

250 mg of sample powder with 5 mL of hydrolysis solution was carried out using microwave assisted extraction technique (frequency of 2455 MHz and output power 725 W) at 56 ºC for 5, 15 and 30 min. The mixture was acidified by adding 1.65 mL of 6 N HCl. The hydrolyzed phenolic acids were extracted twice with ethyl acetate (5 mL each time). Samples were centrifuged

at 4000 rpm for 10 min and the upper ethyl acetate layer was transferred to a clean vial. The organic ethyl acetate layer was evaporated under a slow steam of nitrogen. The dried residue was re-dissolved in 1 mL methanol: water (80:20% (v/v) vortexed well and filtered through a 0.45 µm PVDF syringe filters. The filtered extracts were analyzed by high performance liquid chromatography with photodiode array detector and tandem mass spectrometry (LC-PDA-MS). All samples were hydrolyzed, extracted and analyzed in quadruplicate.

4.2 Comparison of alkaline hydrolysis techniques

For ultrasonic assisted alkaline hydrolysis (UAAH), 250 mg of sample powder was mixed with 5mL of a hydrolysis solution (containing 10 mM EDTA and 1% ascorbic acid in 2 N sodium hydroxide solution). The mixture was flushed with nitrogen gas and placed in a sonicator bath at 45 ºC for 30 min. The mixture was acidified and followed by the same steps as section 4.1

For microwave assisted alkaline hydrolysis (MAAH), 250 mg of sample powder was hydrolyzed by microwave assisted extraction (frequency of 2455 MHz and output power 725 W) with a 5mL of hydrolysis solution at 25 ºC and 100 ºC for 30 min. The mixture was acidified and followed by the same steps as section 4.1

For pressurized liquid assisted alkaline hydrolysis extraction (PAAH), 250 mg of sample powders were placed in 66 mL zirconium extraction cell. Two circular cellulose filters (size 30 mm, Dionex Corporation, California, USA) were placed at the top and bottom of the extraction cell in order to prevent suspended particle from entering the collection vial. The sample was hydrolyzed by 5mL of hydrolysis solution. The extraction condition was carried out at 1000 psi at 40 ºC and 100 ºC, with a 1 min pre-heat time, a 5 min heat time, a 5 min static extraction time, and 90 s purge time for each extraction cycle. A total of three extraction cycles were performed for each sample. The extracts were collected in 250 mL bottle with Teflon coated rubber caps. Each extract was acidified by adding 4 mL of 6 N HCl and added ethyl acetate (2 x 5 mL) for extracted phenolic acids. After shaking well, aliquots were transferred to a 250 mL separatory funnel and removed the bottom layer. The top layer in separatory funnel was centrifuged at 4000 rpm for 10 min. Combined supernatant was evaporated to dryness under steady stream of nitrogen. The residue was re-dissolved in 1 mL methanol: water (80:20% (v/v)). The extracted was filtered through a 0.45 µm PVDF syringe filters and analyzed by LC-MS.

5. Determination of free phenolic acids by FRAP assay

The FRAP assay was performed using a method described by Benzie & Strain (1996) with some modifications as described in previous publication (Luthria, 2012). A FRAP working solution was made by mixing TPTZ (10 mM in 40 mM HCl), acetated buffer and ferric chloride hexahydrate (20 mM) in a ratio of 1:10:1 (v/v/v). Each aliquot of 10 µL of sample extract and Trolox standard solution was mixed with 240 µL of FRAP working solution. The mixture was incubated for 10 min and measured the absorbance at 595 nm by Spectramax 384 Plus microplate reader from Molecular Devices (California, USA).

6. Separation and detection of phenolic acids by HPLC system

The analysis of phenolic acids was analyzed using an Agilent 1100 LC system. The system features a diode array and a mass spectrometer MSD (SL) detector (Agilent, Palo Alto, CA USA)equipped with an electron spray ionizer (ESI). Reversed phase C18 Luna column (Phenomenex, Lorance, CA, USA, 150 x 4.6 mm; particle size 5 µm), and preceding guard column (Phenomenex, 4 x 3.0 mm), were used to separate the phenolic acids. Solvent A and B were comprised of 0.1% (v/v) formic acid in water and methanol. The flow rate used was 1 ml/min. Linear gradient of 5% (B) to 30% (B) for 25 min, followed by 30% (B) for 35 min, gradient elution was then increased from 30% (B) to 100% (B) for 10 min and a linear mode was used as 100% (B) for 5 min. After 75 min, the mobile phase was returned to 5% (B) where it was held for 10 min for column equilibration. The drying gas was set at temperature 350 ºC, flow rate 13 L/min and a nebulizer pressure of 50 psi. Mass spectra were acquired in the positive and negative ion modes at 70 and 250 voltages. Mass scanning was set from 100 to 2000 mass units.

Results and discussion

The HPLC chromatograms and the identification of phenolic acids in Jasmine, Basmati and Brown rice are shown in Fig. 1 and Table 3.

1. Effect of solvent and solvent composition on the free phenolic acids

Due to varied structure of free phenolic acids, creating a standard method for satisfactorily extracting all phenolic acids was challenging. Seven distinct extraction solvents

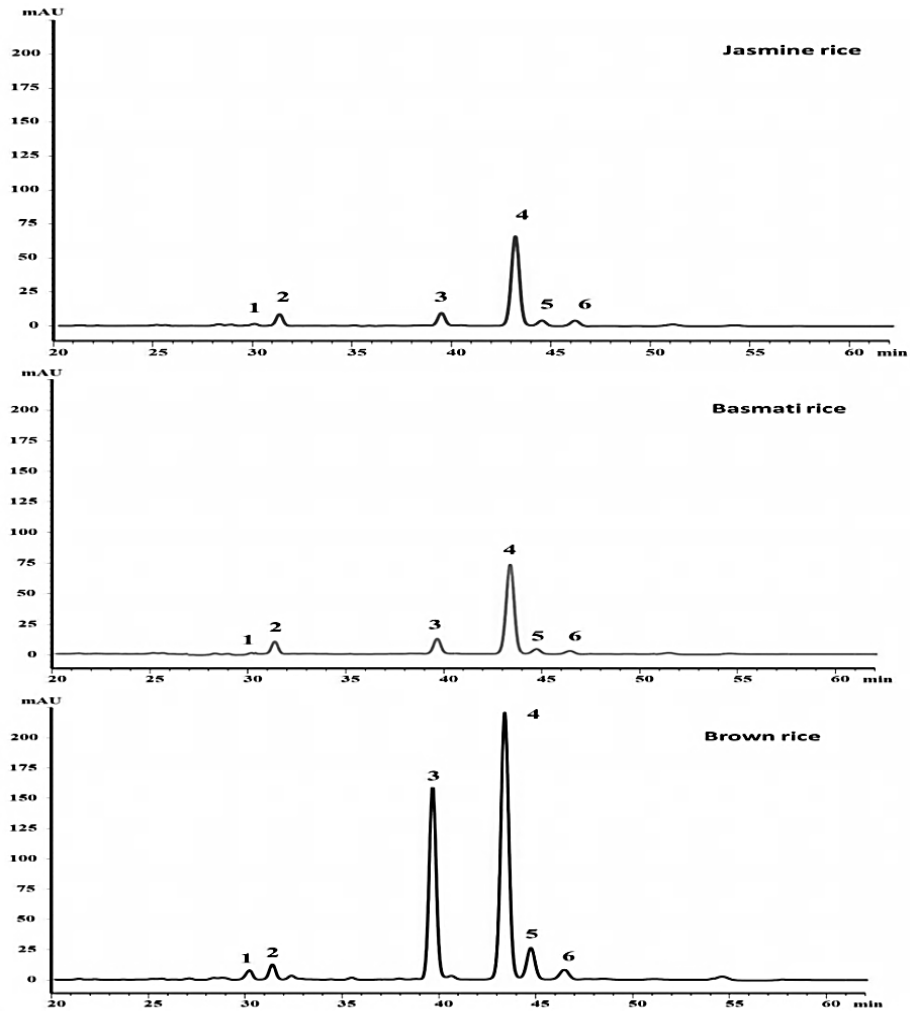

Fig. 1 The Chromatograms of phenolic acids in Jasmine, Basmati and Brown rice samples; 1) caffeic acid, 2) syringic acid, 3) p-coumaric acid, 4) ferulic acid, 5) sinapic acid, and 6) ferulic acid isomer

Table 3 Identification of phenolic acids from three rice samples by HPLC-PDA-MS

Phenolic acids	t_R (min)	λ_{max} (nm)	$[M+H]^+/[M+H]^-$
Caffeic acid	30.19	218, 242, 296, 324	181/179
Syringic acid	31.34	218, 274	199/197
p-Coumaric acid	39.68	228, 298, 310	165/163
Ferulic acid	43.41	216, 236, 296, 324	195/193
Sinapic acid	44.76	220, 236, 324	225/223
Ferulic acid isomer	46.50	216, 236, 318	195/193

(100 and 80% (v/v) methanol, 100 and 80% (v/v) ethanol, 100% (v/v) water, 80% (v/v) acetone and 35:35:30 (v/v/v) of methanol:acetone:water) were used to investigate the appropriate solvent for extracting free phenolic acids. HUAE was implemented in the evaluation of the extraction efficiency of free phenolic acids.

Jasmine, Basmati and Brown rice extracts were examined for antioxidant capacity by FRAP assay. The results presented in Fig. 2 showed that 35:35:30 (v/v/v) of methanol:acetone:water give the highest antioxidant capacity. A similar extraction trend was found in three rice samples (9.42±0.25, 9.05±0.31 and 60.64±2.64 μMol of Trolox equivalents/g from Jasmine, Basmati and

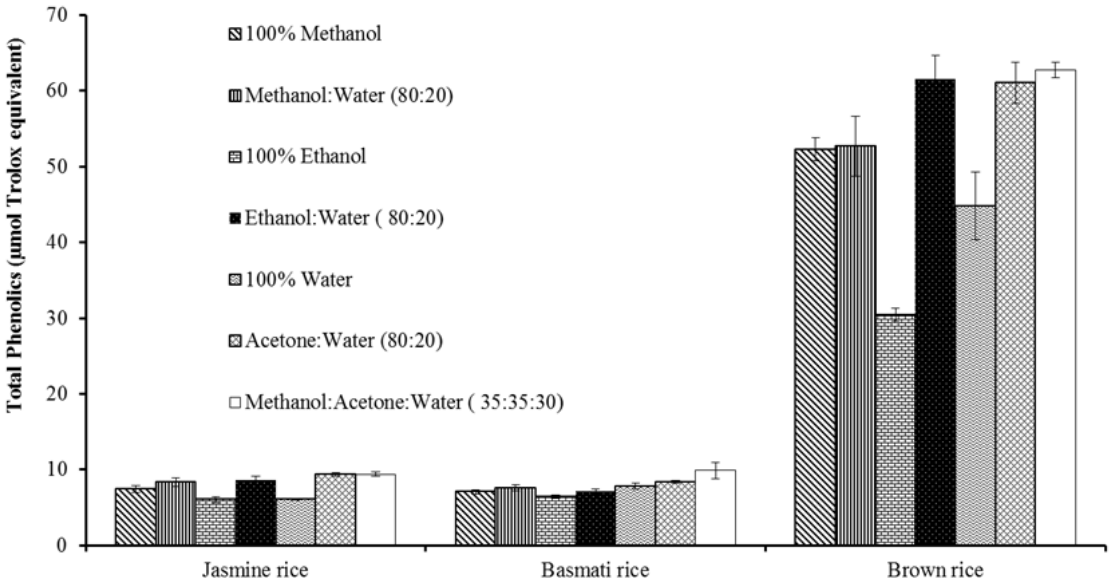

Fig. 2 Effect of the extraction solvent on free phenolic acids from three rice samples by using UAE

Brown rice, respectively). This mean that mixed solvent provide the highest free phenolic acids content. The results indicated that the polarity of mixed solvent, 35:35:30 (v/v/v) of methanol:acetone:water, is nearly that of phenolic acids. The result is similar to the findings of Zhou et al. (2004) thus, 35:35:30 ratio is the best composition solvent for extraction of free phenolic acids in rice samples, as shown in Fig. 2.

2. Extraction techniques efficiency on the free phenolic acids

Rice samples were extracted with the same solvent mixture (35:35:30% (v/v/v) of methanol:acetone:water) by five common extraction procedures: multi-wrist shaker, RUAE, HUAE, MAE (at 25 ºC and 100 ºC) and PLE (at 40 ºC and 100 ºC). As total volume of solvent used by PLE approximate 20 mL owing to the limitation of ASE instrumentation, yet comparisons were made with the extracts from PLE with the extracts from the other (5 mL) by using dilution factor calculation. For only MAE and PLE, the extraction temperature was also studied because both techniques can control the temperature during the extraction. The extraction efficiencies of various techniques were investigated in terms of the content of free phenolic acids.

The free phenolic acids of all sample extracts was determined. Fig. 3 indicates that PLE at 100 ºC provided a marginal increase in extraction efficiencies of free phenolic acids as compared with the others. PLE system used the high temperature and pressure in the extraction procedure, high temperature provides high solubility and high diffusion rate while high pressure keeps the solvent below its boiling point. The solvents penetrate through the solid samples at a much higher rate permitting a fast and efficient extraction at high pressures and high temperatures (Ju & Howard, 2003). From extraction trends with all three rice samples, PLE technique is the best choice for the extraction of free phenolic acids from rice samples.

In addition, brown rice showed the highest total phenolic content among the three rice samples due to brown rice contains different amount of phenolic acids as compared with the white rice (Hodzic et al., 2009; Vichapong et al., 2010).

3. Effect of hydrolysis techniques and time on the bound phenolic acid

In this study, three alkaline hydrolysis procedures for determination of bound phenolic acids from three rice samples were investigated. Jasmine, Basmati and brown rice were hydrolyzed by UAAH, MAAH and PAAH methods with hydrolysis solution (containing 2 N NaOH 10 mM EDTA and 1% ascorbic acid) at different times.

The hydrolysis time at 5, 15, and 30 min with MAAH

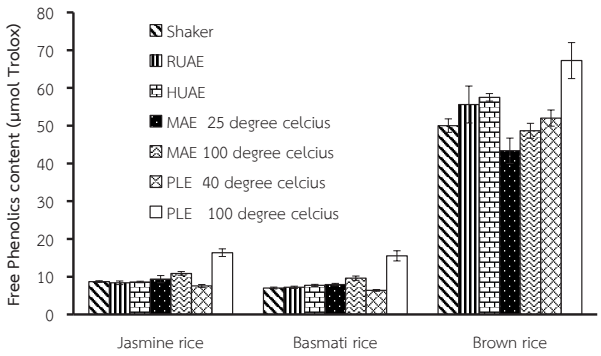

Fig. 3 Effect of the different extraction techniques on the content of free phenolic acids from three rice samples

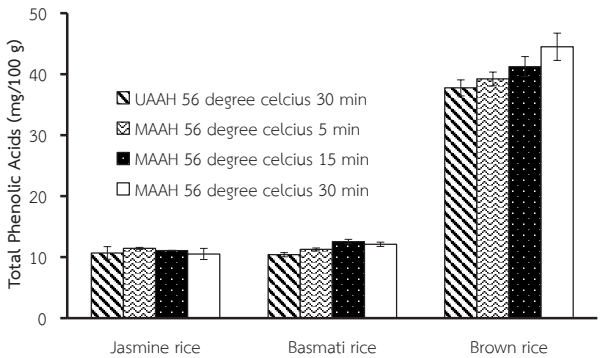

Fig. 4 Effect of hydrolysis technique and time on the total phenolic acids from three rice samples

at 56 ºC were compared with UAAH. The total phenolic acids at three different hydrolysis times were evaluated by LC-MS system. Fig. 4 indicates that the hydrolysis yields of three rice samples at 30 min gave the highest HPLC total phenolic acids, but had minor differences from 5 min. Moreover, when consider with the time consumption, hydrolysis time at 5 min can reduce the time of hydrolysis procedure by 3 times with good extraction efficiency. Thus, 5 min is suitable hydrolysis time and it is reasonable for reducing the time consumption during hydrolysis procedure.

4. Effect of techniques and temperature on the content of bound phenolic acids

The MAE and PLE are the extraction techniques which can control both pressure and temperature during the extraction procedure. Thus it is interesting to apply MAE and PLE instrument for assisting alkaline hydrolysis procedure. The influence of hydrolysis temperature on MAAH and classical PAAH were compared with UAAH. Three rice samples were hydrolyzed by MAAH and PAAH with hydrolysis solution at 56 and 100 ºC for 5 min. From Fig. 5, the maximum phenolic acids were achieved at 100 ºC. The breakage of bonds between various phenolic acids and the matrix causes higher phenolic acids at high temperature (Luthria et al., 2007).

Moreover, MAAH at 100 ºC show higher extraction yields than PAAH at 100 ºC and UAAH at 56 ºC with the similar trends of Jasmine, Basmati and brown rice samples. On the basis of the data in Fig. 5 and Table 4, it can be concluded that the MAAH at 100 ºC is the best techniques for extracting phenolics acids from rice samples.

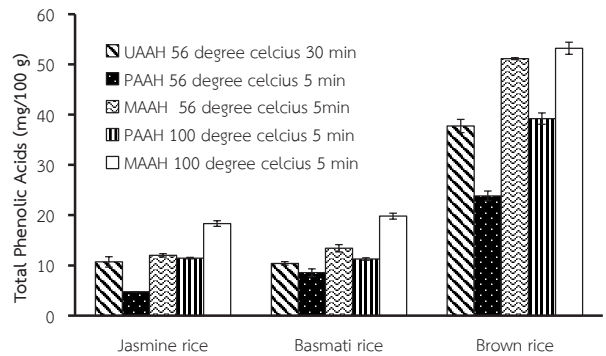

Fig. 5 Effect of hydrolysis techniques and temperature on the total phenolic acids from three rice samples

From LC-MS chromatogram in Fig. 1 and Table 4, brown rice shows the best source of phenolic acids, because it has higher amount of phenolic acids especially p-coumaric acid (8.92 ± 0.10mg/100) and ferulic acid (30.16 ± 0.67 mg/100 g).

Conclusion

This study and other previously reported publications clearly illustrates that the extraction procedures and conditions have been essentially evaluated for accuracy and reproducible estimation of phenolic acids from different rice samples (Ban et al., 2010; Mitra & Brukh, 2003). In the present study, optimum total phenolic acids extracted from rice samples were obtained with 35:35:30 (v/v/v) methanol: acetone:water solvent using PLE. For bound phenolic acids, the best condition of hydrolysis procedure was performed by MAAH at 100 ºC. Both PLE and MAAH method for free phenolic and bound phenolic acids

Table 4 Quantification of phenolic acids from three varieties of rice samples by different extraction techniques

Samples	Extraction technique	Phenolic acid content (mg/100g ± SD)						
		Caffeic acid	Syringic acid	*p*-Coumaric acid	Ferulic acid	Sinapic acid	Ferulic acid isomer	Total phenolic acids
Jasmine rice	UAAH (56 ºC for 30 min)	0.15 ± 0.004	nd	0.58 ± 0.01	9.13 ± 0.97	0.34 ± 0.002	0.49 ± 0.06	10.69 ± 1.03
	PAAH (56 ºC for 5 min)	0.04 ± 0.001	nd	0.34 ± 0.002	4.22 ± 0.01	nd	0.17 ± 0.003	4.77 ± 0.007
	PAAH (100 ºC for 5 min)	0.15 ± 0.01	6.11 ± 0.61	0.37 ± 0.04	4.92 ± 0.29	nd	0.44 ± 0.01	12.00 ± 0.33
	MAAH (56 ºC for 5 min)	0.15 ± 0.004	nd	0.57 ± 0.002	9.73 ± 0.067	0.31 ± 0.01	0.68 ± 0.10	11.45 ± 0.15
	MAAH (100 ºC for 5 min)	0.14 ± 0.004	6.40 ± 0.29	0.65 ± 0.03	10.06 ± 0.24	0.41 ± 0.05	0.67 ± 0.06	18.33 ± 0.56
Basmati rice	UAAH (56 ºC for 30 min)	0.14 ± 0.01	nd	0.59 ± 0.02	9.22 ± 0.28	0.27 ± 0.02	0.17 ± 0.02	10.40 ± 0.34
	PAAH (56 ºC for 5 min)	0.05 ± 0.001	nd	0.65 ± 0.003	7.55 ± 0.70	nd	0.31 ± 0.05	8.55 ± 0.76
	PAAH (100 ºC for 5 min)	0.06 ± 0.002	3.02 ± 0.34	0.77 ± 0.05	8.91 ± 0.39	nd	0.65 ± 0.05	13.41 ± 0.73
	MAAH (56 ºC for 5 min)	0.16 ± 0.003	nd	0.67 ± 0.04	10.02 ± 0.16	0.38 ± 0.05	0.03 ± 0.004	11.26 ± 0.24
	MAAH (100 ºC for 5 min)	0.15 ± 0.003	7.45 ± 0.35	0.80 ± 0.03	10.62 ± 0.24	0.46 ± 0.04	0.32 ± 0.04	19.80 ± 0.59
Brown rice	UAAH (56 ºC for 30 min)	0.48 ± 0.02	nd	7.54 ± 0.23	25.35 ± 1.03	3.52 ± 0.10	0.86 ± 0.06	37.75 ± 1.32
	PAAH (56 ºC for 5 min)	0.11 ± 0.007	nd	6.26 ± 0.26	16.62 ± 0.67	nd	0.85 ± 0.03	23.84 ± 0.97
	PAAH (100 ºC for 5 min)	0.54 ± 0.03	11.06 ± 0.49	8.57 ± 0.08	26.15 ± 0.32	3.58 ± 0.06	1.25 ± 0.03	51.15 ± 0.19
	MAAH (56 ºC for 5 min)	0.49 ± 0.02	nd	7.70 ± 0.37	26.18 ± 0.66	3.37 ± 0.09	1.13 ± 0.01	39.22 ± 1.13
	MAAH (100 ºC for 5 min)	0.57 ± 0.02	7.93 ± 0.50	8.92 ± 0.10	30.16 ± 0.67	4.44 ± 0.14	1.19 ± 0.01	53.22 ± 1.20

Remark: nd is not detectable

extraction procedure can give the highest extraction efficiencies with fast and simple automatic methods. It is vital for accurate analysis to optimize the preparation procedures of the different classes of phenolic acids isolated from subject rice samples. This knowledge can apply to extracting a high amount of phenolic acids from rice to increase the antioxidant agent in healthy rice products or cosmetics.

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