Ultrasonic-assisted extraction of polyphenols and chlorophyll contents from rice seed lings (*var. Sukhothai 1*)

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Abstract

Ultrasonic-assisted extraction presently is an interest technique for recovering the valuable compounds from the vegetal materials. This study was conducted for comparing a traditional extracting technique (shaking) to an ultrasonic-assisted extraction (28, 45, 100 kHz) on rice seedling (*var. Sukhothai 1*) sample. In combination, solvent effect (water and various concentrations of ethanol) under 50ºC 60 min extracting conditions were also investigated. Antioxidant properties, total polyphenol contents as well as chlorophyll contents in the extracts were analyzed. The extracts obtained by higher ethanol concentration are more desirable and that the ultrasonic technology can be potentially used in the extraction process to improve efficiency.

Keyword: rice seedling, polyphenols, antioxidant properties, chlorophyll, extraction

1. Introduction

Seedlings are young plants grown from seeds i.e. wheat, barley, oat, rye, or rice. During germination, these plants degrade any large compounds into smaller ones such as vitamins, minerals, dietary fiber, antioxidants, superoxide dismutase (SOD),fatty acids, amino acids, polyphenols, and chlorophyll (Hattori, 2002).The cereal seedlings are currently found to be excellent sources of vitamin K, folic acid, calcium and protein as well as good sources of vitamin C and vitamin B, which are beneficial to human health. Gallic acid, catechin, rutin and isoquercetinare common polyphenols found in the rice seedling (Phimphilai et al., 2013). As the rice seedlings are perishable, they have been preserved or extracted for maintaining or minimizing any losses of functional qualities (Phimphilai et al., 2012).A traditional extraction method, especially shaking, requires a long period of maceration and extraction (Carrera et al., 2012). Using other extracting-aid procedures has been recently reported.

Ultrasonic-assisted extraction is a methods utilized for recovering the valuable compounds from the vegetal materials (Vilkhu et al., 2008). In 2000, the U.S. Food and Drug Administration (USFDA) released a report on alternative food processing technologies in which several solvents, such as water and ethanol, have been used in several processes including

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shaking and ultrasonication to recover active compounds from many plant cells (USFDA, 2000). The naturally active compounds are valuable in food applications, food supplements including pharmaceutical products.

This research was aimed to compare efficacies of extraction conditions on total polyphenols, pigments or chlorophyll, as well as antioxidant activities of the particular Thai rice seedling extracts.

2. Materials and Methods

2.1 Chemicals and solvents

All chemicals used in this study were of analytical grade. Distilled water as well as ethanol solution with concentrations of 95%, 70% and 50% (v/v) were used as extraction solvents.

2.2 Raw materials

Rice seedlings (var. *Sukhothai* 1) were provided by Natural Rice Co., Ltd. (Sukhothai, Thailand). An optimum age of rice seedling is 12-15 days after germination. The seedlings were freshly cut and stored at -20°C under vacuum condition until studied. In preparation, the seedlings were thoroughly water-rinsed, drained and cut into pieces (0.5 -1.0 cm) prior to an extraction step.

2.3 Extraction methods

Three grams each of rice seedling was mixed with 50 mL of extracting solvent; water, 95% ethanol (v/v), 70% ethanol (v/v) and 50% ethanol (v/v), in Erlenmeyer flasks. All the extractions were carried out at 50°C for 60 min using a shaker bath at 120 rpm (*Zhang* et al., 2009) or an ultrasonic bath (Honda, Japan) with frequencies of 28 kHz, 45 kHz and 100 kHz. All sample extracts then were collected into small vials and stored under -20°C for 24 hours prior to analyses.

2.4 Analysis of antioxidant activity (AOA)

2.4.1 2,2'-azino-bis (3-ethylbenzothaiazoline-6-sulfonic acid) ($ABTS^{•+}$) assay

Radical scavenging activity determination was modified from the method described by Re and colleagues (1999). Sample solution was diluted before measured an absorbance at 730 nm using UV-VIS spectrophotometer (Perkin Elmer, Germany). Trolox solution was used to create a standard curve and the activity was reported as trolox equivalent antioxidant capacity (mg) in1 g dry sample (mg TEAC/1g DM).

2.4.2 Ferric reducing antioxidant power (FRAP) assay

Ferric reducing antioxidant power (FRAP) assay was conducted using the method described by Benzie and Strain (1996) with some modifications. The absorbance was measured at 593 nm and $FeSO_4$ solution was used to create a standard curve. A result was expressed as $FeSO_4$ equivalent (mg) in 1 g dry sample.

2.5 Total polyphenol content (TPC)

Total polyphenol content (TPC) was determined using the method described by Pinsirodom and Changnoi (2001) with some modifications. To create a standard curve, gallic acid solution was used and the absorbance was measured at 734 nm. Each result was reported as gallic acid equivalent (mg) in 1 g dry sample (mg GAE/1g DM).

2.6 Chlorophyll content

Chlorophyll contents were determined using the method described by AOAC Official Method 942.04 (2006). Acetone was firstly used in chlorophyll extraction and diethyl ether was applied to purify the sample. After adjusting to a certain volume, absorbances were measured at 642.5 and 660 nm. and reported as total chlorophyll contents.

2.7 Statistical analysis

A completely randomized design (CRD) was applied to this experiment. The data were analyzed using ANOVA (SPSS version 16.0) and mean comparisons were conducted by Duncan’s new multiple range test. Any significant differences were defined at $p \leq 0.05$.

3. Results and Discussion

3.1 Antioxidant activities

Both extracting factors; method and solvent, significantly showed differences on antioxidant activities in rice seedling extracts where $ABTS\bullet^{+}$ and FRAP were observed (Table 1). Ultrasonication with all extracting solvents significantly facilitated the rice seedling antioxidant extraction ($ABTS\bullet^{+}$) comparing to the shaking method. However, water-soluble antioxidants with ferric-reducing ability were not much different among the extracting devices. Combination of 28kHz or 45kHz ultrasonication with 95% ethanol resulted in the highest antioxidation activities

(ABTS•+) of rice seedling extract with the activities equivalent to 31.60mg trolox per gram of the seedling (dry basis). However, lower antioxidant activities were found in all samples extracted with a 100kHz ultrasonic comparing to other lower frequencies. It has been stated in a literature that cavitation yield from ultrasonic decreases as frequency increases. The cavitation bubbles, therefore, tend to be smaller and less energetic resulting in a reduction yield (Kirpalani and McQuinn, 2006).

Table 1. Effect of extracting method and solvent on antioxidant properties of rice seedling extracts.

Antioxidantactivities (AOA)	Method	Solvent			
		Water	50%Ethanol	70%Ethanol	95%Ethanol
$ABTS^{\cdot +}$ (mgTrolox equiv. perg sample, db.)	Shaker	12.93±0.38^{dZ}	16.73±0.56^{cZ}	19.10±1.65^{bXY}	20.55±1.33^{aX}
	US 28kHz	22.38±0.25^{dW}	26.66±0.07^{bW}	24.01±0.04^{cW}	31.60±0.07^{aW}
	US 45kHz	19.41±0.06^{dX}	25.52±0.02^{bX}	21.42±0.04^{cWX}	28.68±0.02^{aW}
	US 100kHz	17.66±0.04^{dY}	23.82±0.09^{bY}	19.51±0.03^{cY}	26.22±0.10^{aX}
FRAP (mg$FeSO_4$ equiv. perg sample, db.)	Shaker	7.84 ±0.15^{dW}	11.92±1.26^{cY}	15.78±2.70^{bX}	23.06±1.08^{aY}
	US 28kHz	7.76 ±0.12^{dX}	19.78±0.47^{cW}	19.97±0.79^{bW}	27.77±0.71^{aX}
	US 45kHz	7.78 ±0.31^{dWX}	18.79±0.07^{bX}	13.60±0.11^{cY}	31.04±0.27^{aW}
	US 100kHz	6.96 ±0.44^{dY}	18.17±0.70^{cX}	21.03±0.51^{bW}	29.93±0.64^{aWX}

Data are expressed as mean ± SD(n=6).

$^{a-d}$ Values in each row with different letters are significantly different($p<0.05$).

$^{W-Z}$Values in each column of each analytical property with different letters are significantly different ($p<0.05$).

US = Ultrasonication.

From the study, it was revealed that antioxidant compounds in the rice seedling were both in hydrophilic and hydrophobic parts, but likely to have more composition in ethanol-soluble compounds. High concentration of ethanol (95%), therefore, could be used to obtain the highest antioxidant-activity extract from the particular rice seedling.

In 2013, Oliveira and colleagues reported similar results, which were expressed as passion fruit oil extract to solvent ratio, where ethanol was the most efficient solvent. However, the appropriate solvent was selected based upon the economic considerations. Type of solvent is one of the factors generally investigated during the extraction and ethanol was commonly used as a solvent to recover the plant compounds in a conventional method (Hayouni et al., 2007).

3.2 Total polyphenol contents(TPC)

Total polyphenols in the seedling extracts were reported as gallic acid equality (Table 2). Traditional shaking with ethanol combination showed slight increment in total polyphenol contents ($p<0.05$) of the extracts. However, application of ultrasonic wave with ethanol significantly increased amounts of the particular compounds. This study indicated that high concentration of ethanol (95%) could be used to achieve higher total polyphenol contents in the extract with 28kHz, 45kHz, or 100kHz ultrasonic-assisted devices. The ultrasonic frequencies used in this study showed smaller effects on the extracted compounds than that of the solvent factor.

Table 2. Effects of extracting method and solvent on total polyphenol contents (TPC) of rice seedling extracts.

Analyses	Method	Solvent			
		Water	50%Ethanol	70%Ethanol	95%Ethanol
Total polyphenol contents (mggallic acid equiv./g sample, db.)	Shaker	$6.17±0.04^{dW}$	$6.61±0.32^{cZ}$	$6.79±0.71^{bX}$	$6.96±0.08^{aX}$
	US 28kHz	$5.88±0.11^{dX}$	$8.82±0.09^{cY}$	$9.51±0.02^{bWX}$	$10.49±0.27^{aW}$
	US 45kHz	$5.22±0.04^{dY}$	$9.99±0.18^{aW}$	$6.98±0.10^{cX}$	$9.17±0.14^{bWX}$
	US 100kHz	$5.34±0.42^{dY}$	$9.30±0.09^{cX}$	$9.56±0.19^{bW}$	$9.00±0.11^{aW}$

Data are expressed as the mean±SD(n=6).

$^{a-d}$ Values in each row with different letters are significantly different($p<0.05$).

$^{W-Z}$Values in each column of each analytical property with different letters are significantly different ($p<0.05$).

US = Ultrasonication

Among the studied factors, higher amplitude of ultrasound or ultrasonic could damage more cell wall, then releasing more antioxidants including phenolic compounds to the solvents (Hossain et al., 2012). Ultrasonic extraction could increase the solubility of target compounds as well as their diffusion rate and mass transfer of solvent while decreasing the viscosity and surface tension of solvent. Effect of different ethanol concentrations on plant extracts was previously examined (Spigno et al., 2007). In fact, ethanol could effectively recover tannins and bioactive compounds from raw plant materials, but solubility of these compounds was enhanced by a mixed solvent over a limited compositional range (Cacace and Mazza, 2003).

3.3 Chlorophyll contents

Results for chlorophyll analysis (Table 3) in the rice seedling extract showed that both extracting method and solvent have significant effects on their chlorophyll contents ($p<0.05$). Within each ultrasonic frequency, the stronger ethanol solvent applied, the higher chlorophyll contents were extracted. Maximum total chlorophyll contents were found in 95% ethanol with 100kHz ultrasonic-assisted extraction ($p<0.05$) at 50°C for 60 min extracting time.

Table 3. Effects of extracting method and solvent on chlorophyll contents of rice seedling extracts.

Analyses	Method	Solvent			
		Water	50%Ethanol	70%Ethanol	95%Ethanol
Chlorophyll (mg/100g)	Shaker	3.37 ± 0.39^{dX}	5.33 ± 0.14^{cY}	81.49 ± 5.96^{bZ}	197.30 ± 17.87^{aZ}
	US 28kHz	1.75 ± 0.46^{dY}	4.81 ± 0.22^{cY}	233.88 ± 1.31^{bW}	270.28 ± 4.68^{aX}
	US 45kHz	5.02 ± 0.53^{dW}	28.48 ± 1.12^{cW}	203.71 ± 11.16^{bX}	237.10 ± 4.04^{aY}
	US 100kHz	6.22 ± 2.10^{dW}	14.45 ± 0.62^{cX}	187.18 ± 0.35^{bY}	430.30 ± 1.64^{aW}

Data are expressed as the mean±SD(n=6).

$^{a-d}$ Values in each row with different letters are significantly different($p<0.05$).

$^{x-z}$ Values in each column with different letters are significantly different ($p<0.05$).

US = Ultrasonication

Ultrasonic waves promote the extraction through the interaction of waves with organic molecules that are presented in a particular sample. As a result, cell walls are broken and bioactive compounds are released (Oliveira et al., 2013). Besides, an appropriate solvent composition for bioactive recovery is the first critical step to be examined as differing solvents generally result in varying quality and quantity of extract compositions. In this study, water and different concentrations of ethanol were evaluated for the most suitable solvent composition for the rice seedling extractable compounds. Results indicated that the extraction efficiency, which is characterized by total polyphenol contents and chlorophyll contents, was increased with higher concentration of ethanol. Ethyl alcohol is a solvent with less polar than water. As a result, polyphenols and chlorophyll, of which non polar compounds mainly comprised, could be highly extracted from the rice seedling using the particular solvent. On the other hands, more hydroxyl group or more polar compound was more extractable in methanol and water than that in n-butanol and ethanol. Phillyrinin *Forsythia suspensa* was an example (Xia et al., 2011).

A combination of ultrasonic method and 95% ethanol showed the highest extraction efficiency among all examined conditions, suggesting that the ultrasonic vibration has either increased a number of ruptured cells and/or provided the faster access for a solvent to remove solutes from these cells. On the other hand the compounds are more accessible for an extraction solvent so that the external and internal mass diffusivities increase. Therefore, the extraction time and the ratio of solvent to sample were reduced dramatically for the same amount of extract (Chen et al., 2007).

Application of ultrasonication in ambient fluids is well known to cause cavitations in the samples, which were the same in plant materials (Chen et al., 2007). Cavitation refers to the formation, growth and violent collapse of micro-bubbles in the sonication liquid due to pressure fluctuations. Moreover, the type of solvent can play an important role in extracting antioxidant compounds and polyphenol from the complex samples.

4. Conclusion

Extraction method and solvent significantly affect the composition of rice seedling extract. Ethanol and water are effective for recovering antioxidant compounds and polyphenol, yielding extracts with high antioxidant activities as well as total polyphenol and chlorophyll contents. It can be concluded that the extracts obtained by higher ethanol concentration are more desirable and that the ultrasonic technology can be potentially used in the extraction process to improve efficiency. This study will be applied for the large-scale industrial processing and preparation of natural extracts rich in antioxidant compounds.

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