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Ohmic heating assisted extraction improves the concentrations of phytochemicals in rice bran oil and unsaponifiable matter

[1*]Moongngarm, A., [2]Loypimai, P., [3]Fitriati, A. and [1]Moontree, T.

[1]Department of Food Technology and Nutrition, Faculty of Technology, Mahasarakham University, Mahasarakham 44150, Thailand
[2]Division of Food Science and Technology, Faculty of Science and Technology, Bansomdejchaopraya Rajabhat University, Bangkok 10600, Thailand
[3]Food Science and Technology Department, Faculty of Agriculture, University of Jenderal Soedirman, Purwokerto 53123, Central Java, Indonesia

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Abstract

The impact of ohmic heating-assisted solvent extraction (OHM-AE) of oil and unsaponifiable matter (USM) from rice bran was investigated for different concentrations of tocols and γ-oryzanol. Rice bran samples with moisture contents (MC) of 30% and 40% were ohmically-treated with three levels of electric field strength (E) at 100, 150 and 200 V/cm prior to extraction of rice bran oil and USM. Oil and USM prepared by conventional methods (CM) were included as controls. OHM-AE increased the concentration of phytochemicals and antioxidant activity of both rice bran oil and USM. The highest concentrations of γ-tocopherol, γ-tocotrienol, γ-oryzanol and total phenolic content in the oil and USM were observed in the bran with 30% MC (extracted by OHM-AE at 150 and 200 V/cm), and the bran with 40% MC (extracted at 100, 150, and 200 V/cm). Similarly, OHM-AE under these conditions yielded higher oil recovery with stronger antioxidant activity than the CM method. This suggested that OHM-AE could be an effective method to extract rice bran, offering both increased oil yield and high concentration of phytochemicals.

Introduction

Rice bran is an inexpensive source of healthy edible oils, containing valuable bioactive compounds including tocols (tocopherols and tocotrienols), and γ-oryzanol (Loypimai *et al*., 2009; 2015). Epidemiological studies reported that tocols have shown protective effect on certain types of cancer, cardiovascular diseases and other chronic illnesses (Shahidi and de Camargo, 2016). Similarly, γ-oryzanol, a mixture of triterpene alcohols and phytosterols esterified with ferulic acid and unique to rice bran, has biological and physiological abilities such as serum cholesterol reduction, and anti-oxidative and anti-carcinogenic properties which attenuate allergic inflammations (Lerma-García *et al*., 2009; Farhoosh *et al*., 2011; Nagasaka *et al*., 2011). Tocols are mostly distributed in unsaponifiable matter (USM). The content and composition of USM for rice bran oil were determined at 0.96% phytosterols, 0.51% 4-methyl sterols, 0.52% triterpene alcohols, and 0.61% nonpolar components by Gopala Krishna *et al*. (2003). Thus, USM is considered a rich source of valuable bioactive compounds. However, the content of bioactive compounds in USM mainly depends on the quality of the raw rice bran material. One critical problem regarding the handling of raw rice bran is the rapid deterioration of fat due to enzymatic hydrolysis by lipase, lipoxygenase and lipid oxidation. As a result, the bran contains high free fatty acids (FFAs) and has an undesirable flavour (Loypimai *et al*., 2009; Patil *et al*., 2016). Hence, improved procedures are required to avoid fat deterioration and degradation of valuable bioactive compounds in the rice bran prior to the extraction process. Steaming is generally used to destroy enzymes and, therefore, stabilise the rice bran (Juliano, 1985; Loypimai *et al*., 2009). However, this process involves heat transfer by conduction and convection (Loypimai *et al*., 2009), resulting in the breakdown of thermally sensitive compounds in the bran. Therefore, a rice bran extraction method offering high oil yield while maintaining bioactive substances needs to be developed.

*Corresponding author.
Email: anuchitac@yahoo.co.th

Ohmic heating (OHM) has been shown to be an effective method for stabilising tocols, γ-oryzanol, and anthocyanins in rice bran (Loypimai *et al*., 2009; 2015) and successfully applied in conjunction with the extraction processes. Electricity applied during OHM breaks down the cell membranes, allowing solute diffusion (electro-osmosis effect) resulting in high yields of oil and bioactive compounds (Dons *et al*., 2010; Nair *et al*., 2014; Loypimai *et al*., 2015). OHM-assisted solvent extraction (OHM-AE) increased polyphenol yields from red grape pomace and tomato by-products (El Darra *et al*., 2013; Coelho *et al*., 2017), anthocyanins, tocols and γ-oryzanol from black rice bran (Loypimai *et al*., 2015), rice bran oil from rice bran (Lakkakula *et al*., 2004; Loypimai *et al*., 2015), food-grade pigments from the microalgae *Chlorella vulgaris* (Fraccola *et al*., 2016), essential oils from *Mentha piperita* and lavender (Gavahian *et al*., 2015; Gavahian and Chu, 2018), lipid from microalga Chlorella spp. (Yodsuwan *et al*., 2018), Gac aril powder (Aamir and Jittanit, 2017) and plant tissues (Gavahian *et al*., 2018). In the study of Lakkakula *et al*. (2004), OHM was used to stabilise rice bran and showed that the extraction yield of rice bran oil was improved; however, no information about the concentration of bioactive compounds extracted into the oil was documented. In addition, the bioactive compounds in rice bran oil are mainly unsaponifiable matters (USM); therefore, if USM is high in bioactive compounds, it could be a good source of functional ingredient. The present work was conducted to confirm that apart from increasing oil yield, OHM could also be applied to assist in the extraction of rice bran to improve the phytochemicals in oil and USM. The objectives of the present work were therefore to apply OHM to assist in increasing the extraction yields of oil and USM from rice bran, and to investigate the influence of OHM on the amount of bioactive compounds obtained and antioxidant activity.

Materials and methods

Materials and chemicals

Rice bran (*Oryza sativa* L. CV. RD 6) sample was collected from a rice milling factory in Bueng Kan Province, Thailand. The rice bran sample was automatically sifted using a vibratory sieve shaker (Retsch™, AS 200 series, USA) equipped with a sieve size of 750 µm (20 mesh) to separate husks, broken rice and other foreign fractions (Loypimai *et al*., 2009). The bran sample was packed into a polyethylene bag and stored at -20°C in a freezer (Natural, model NFT-4155/5.5Q, Thailand). The bran sample was analysed for the initial moisture content (AOAC, 2000) prior to analyses.

All chemicals and reagents were of analytical grade. Standard γ-oryzanol was purchased from Tsuno Food Industrial Co., Ltd. (Wakayama, Japan). Standard tocols (α-, δ-, γ-tocopherols and γ-tocotrienol), gallic acid and Folin-Ciocalteu's reagent were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Methanol (HPLC grade), acetonitrile, hexane, n-butanol and ethanol were purchased from BHD (Poole, Dorset, UK).

Sample preparation

Ohmic heating of rice bran

The rice bran was ohmically heated prior to extracting with solvent to obtain rice bran oil (OHM-AE), and then the oil was used to prepare rice bran USM. The equipment used and the procedures followed were as reported in our previous studies (Loypimai *et al*., 2009; 2015) with slight modifications. Briefly, 200 g rice bran sample was moistened to 30% or 40% (wet basis) moisture content (MC) by adding 58 or 101 mL of deionised water, respectively. Subsequently, 180 g moistened rice bran was ohmically-heated to 105°C for 1 min, using three levels of field strength (100, 150, and 200 V/cm) (Loypimai *et al*., 2015). During ohmic heating of the rice bran, the voltage, current, and temperatures were continuously recorded using a data logger (Digicon, DP-74SD). Following heating, the sample was cooled to room temperature and later used in extraction process.

Extraction of rice bran oil

The ohmically-treated samples were Soxhlet-extracted using n-hexane following AOAC (2000). Briefly, 50 g ohmically-treated sample was added into thimble paper cones and then placed in the Soxhlet extractor for 2 h, with each experiment performed in triplicate. The quantity of crude oil obtained was weighed and used for unsaponifiable matter preparation. The oil yield results were presented as percentage recovery (Loypimai *et al*., 2015):

$$\text{Oil recovery (\%)} = \frac{\text{Extracted rice bran oil (g)}}{\text{Mass of rice bran (g) dry basis}} \times 100 \quad \text{(Eq. 1)}$$

Control sample

Unheated rice bran (raw rice bran) was also extracted to obtain rice bran oil under the same conditions without ohmic heating (AOAC, 2000), and served as control.

Extraction of unsaponifiable matter

The USM were obtained by extracting the rice bran oil using the method of Lozano *et al*. (1993) with slight modifications. Briefly, the rice bran oil samples (5.0 g) were saponified by addition of 50 mL 1.0 M ethanolic potassium hydroxide (KOH) in a capped flask, and placed in an oven for 1 h at 95°C. The mixture was then immediately cooled in an ice bath before adding with 100 mL distilled water. The solution was extracted twice with 100 mL diethyl ether. The upper organic layers were combined and washed twice with 75 mL distilled water before separated by filtration, dissolving with chloroform and evaporation to dryness under vacuum in a rotary evaporator at 45°C. USM obtained was weighed and expressed as oil weight percentage.

High performance liquid chromatography analysis for tocols and γ-oryzanol

The tocol and γ-oryzanol contents in the crude rice bran oil and USM obtained from different extraction processes were measured using a Shimadzu LC-20AD series HPLC system (Shimadzu, Japan) equipped with an automatic injector, a column oven and a photodiode array detector based on the method of Gimeno *et al*. (2001) with slight modifications. The oil (100 mg) or USM (10 mg) sample was added to 2.0 mL *n*-hexane and mixed with a vortex mixer for 1 min to achieve complete homogeneity. The extract obtained was separated using Phenomenex Luna C_{18} column (4 µm, 150 × 4.6 mm, Phenomenex Inc., USA). The operating conditions were: injection volume, 20 µL; column temperature, 45°C; flow rate, 1.0 mL/min. The mobile phase used consisted of 95% methanol in water (solution A) and *n*-butanol (solution B). The elution scheme was: 0-12 min, 0-3%B; 12-15 min, 3-5%B; 15-25 min, 0%B. The detection wavelength for α-, δ-, γ-tocopherols, and γ-tocotrienol was set at 292 nm, whereas γ-oryzanol was set at 325 nm (Loypimai *et al*., 2009). The results of the test sample obtained were compared with retention times of reference standards.

Determination of antioxidant activity

Extracted oils and USMs were treated with methanol and hexane prior to analysis following Janu *et al*. (2014) and Loypimai *et al*. (2015). Briefly, 1.0 g extracted (oil/USM) sample was dissolved in 5 mL mixed solvent (methanol:hexane, 3:2) and sonicated using a Vibra cell sonicator (130 W, 20 kHz, Vibra cell, model CV334, USA) for 5 min. Methanolic extracts were evaporated in a rotary evaporator and the residue was dissolved in 2 mL methanol and stored at -20°C. All extractions were performed in triplicate and each was analysed for antioxidant activity using three different assays based on diverse food system mechanisms.

DPPH assay

The antioxidant activity assay using DPPH radical scavenging was evaluated following Dasgupta and De (2004). The percentage of inhibition activity was calculated as $[(A_{DPPH} - A_{extract}) / A_{DPPH}] \times 100$, where A_{DPPH} = absorbance of the DPPH solution, and Aextract = absorbance of the solution containing the extract at a particular level. Results were expressed as the sample concentration that provided 50% of inhibition activity (IC_{50}). The IC_{50} value was calculated by plotting inhibition activity percentage against concentration.

FRAP assay

FRAP assay of the extracted sample, as the reduction of Fe^{3+}-TPTZ to blue coloured Fe^{2+}-TPTZ, was determined following Benzie and Strain (1996) with slight modifications. The coloured product reading (ferrous tripyridyltriazine complex) was measured at 539 nm with results expressed in µM $FeSO_4$ equivalent per gram (g) of sample.

Total antioxidant capacity assay

The total antioxidant capacity assay was determined following Dasgupta and De (2004), and expressed as the number of equivalents of synthetic vitamin C standard.

Total phenolic content assay

The total phenolic content was determined by the reaction of the extract to Folin-Ciocalteu reagent following Iqbal *et al*. (2005), with results expressed as gallic acid equivalent (mg GAE) per gram (g) of sample.

Data analysis

The data were subjected to analysis of variance (ANOVA), based on one-way ANOVA, to analyse significant differences between treatments, followed by Duncan's Multiple Range Test using SPSS software (version 16.0). Differences were considered significant at $p < 0.05$.

Results and discussion

The raw bran sample showed low electrical conductivity since it contained low MC (9.85%) but high-fat content (13.3%). Our previous study (Loypimai *et al*., 2009) found that sufficient amount of deionised water was needed to increase the

Table 1. Tocols of rice bran oil and its unsaponifiable matter (USM) obtained from the bran prepared using different extraction processes on a dry weight basis of rice bran.

Extraction process	α-Tocopherol (µg/g)		γ-Tocopherol (µg/g)		δ-Tocopherol (µg/g)		γ-Tocotrienol (µg/g)	
	Oil	USM	Oil	USM	Oil	USM	Oil	USM
CM	10.1 ± 1.34^{c}	3273.3 ± 35.7d	33.6 ± 2.48^{c}	389.5 ± 22.8^{b}	ND	ND	66.9 ± 6.54^{c}	620.1 ± 40.5^{c}
OHM-AE (30% MC, E100)	33.1 ± 2.08^{b}	3920.3 ± 32.6^{bc}	53.1 ± 2.17^{b}	691.4 ± 31.4^{a}	23.9 ± 2.41^{a}	24.9 ± 4.74^{a}	109.1 ± 10.1^{b}	1155.4 ± 51.3^{b}
OHM-AE (30% MC, E150)	35.9 ± 1.74^{b}	4011.5 ± 84.9^{b}	60.8 ± 3.72^{a}	683.7 ± 22.3^{a}	25.4 ± 2.49^{a}	24.6 ± 3.45^{a}	125.2 ± 1.23^{a}	1272.2 ± 47.6^{a}
OHM-AE (30% MC, E200)	38.5 ± 2.67^{a}	4146.8 ± 60.2^{ab}	60.1 ± 6.67^{a}	672.5 ± 34.6^{a}	26.3 ± 3.29^{a}	25.0 ± 7.75^{a}	129.3 ± 2.24^{a}	1299.3 ± 38.9^{a}
OHM-AE (40% MC, E100)	37.5 ± 3.62^{a}	4235.6 ± 88.7^{a}	61.2 ± 1.22^{a}	683.4 ± 44.6^{a}	25.9 ± 5.14^{a}	24.5 ± 8.75^{a}	128.5 ± 3.28^{a}	1291.4 ± 44.^{a}
OHM-AE (40% MC, E150)	40.7 ± 6.25^{a}	4251.3 ± 98.4^{a}	60.7 ± 2.56^{a}	693.7 ± 33.2^{a}	26.1 ± 5.63^{a}	25.2 ± 7.63^{a}	128.9 ± 4.35^{a}	1296.7 ± 13.8^{a}
OHM-AE (40% MC, E200)	41.6 ± 3.53^{a}	4296.7 ± 53.2^{a}	60.9 ± 2.73^{a}	679.8 ± 39.1^{a}	26.2 ± 3.68^{a}	24.4 ± 9.04^{a}	129.2 ± 3.61 ^{a}	1288.8 ± 62.3^{a}

CM = conventional method; OHM = ohmic heating-assisted solvent extraction; MC = moisture content (% wet basis); E = electric field strength (V/cm); ND = not detected. Data are means ± SD of triplicate samples (n = 3). Means with different superscripts in the same columns differed significantly ($p < 0.05$).

electrical conductivity and allow the passage of electrical current during ohmic heating. Therefore, in the present work, the MC of the bran was adjusted to 30% and 40% (wb) prior to OHM-AE (Loypimai *et al*., 2009; 2015). Extraction yields of oil and USM were analysed; and bioactive compounds and antioxidant activity of the extracted oil and USM were determined and compared with CM samples.

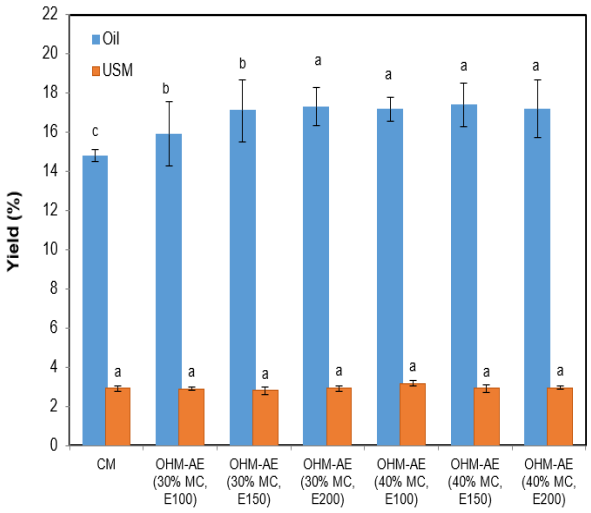

Figure 1. Yields of oil and unsaponifiable matter (USM) obtained from bran prepared by different extraction processes on a dry weight basis. Data are means ± SD of triplicate samples (n = 3). CM = conventional extraction; OHM-AE = ohmic heating-assisted solvent extraction; MC = moisture content (% wet basis); E = electric field strength (V/cm).

Extraction yields of oil and USM

Figure 1 shows the yields of oil and USM obtained from bran prepared by different extraction processes. Statistical analysis indicated that extraction methods significantly affected percentage oil yield ($p < 0.05$), whereas no significant effect was observed between extraction methods regarding USM yield. Oil extracted by OHM-AE with 30% MC (E = 150 and 200 V/cm) and 40% MC (E = 100, 150 and 200 V/cm) showed the highest yield (ranging from 17.1 ± 1.57 to 17.4 ± 1.12%), followed by oil extracted by OHM-AE at 30% MC and E at 100 V/cm (15.9 ± 1.64%) and CM (14.8 ± 0.29%), respectively. Maximum yields of oil from rice bran using OHM-AE concurred with results reported by Lakkakula *et al*. (2004) who used OHM to increase the total percentage of lipids (92%) as compared to bran samples without OHM (53%). Loypimai *et al*. (2015) documented that bran treated by OHM provided a higher yield of extracted oil than raw bran. The yield of USM obtained in the present work was higher than reported by Afinisha Deepam *et al*. (2007), who found that USM was 52.80 mg/g in crude oil. This may be due to an electric field produced during OHM (appropriate MC) that induced electroporation and increased the permeability of cell walls and membranes, thereby enhancing oil extractability. Electric energy applied during OHM could also be responsible for the breakdown of the rice bran cell membrane (Nair *et al*., 2014). Moreover, the heat transfer during OHM was faster and smoother than the steam treatment which only used conduction and convection to generate heat inside the food materials (Goullieux and Pain, 2005).

The OHM-AE process creates heat by transforming internal energy from electric to thermal within the bran (especially the aleurone cells and surrounding surface area) and rapidly penetrates throughout the bran resulting in an increase in oil yield (Loypimai *et al*., 2015).

Table 2. γ-oryzanol of rice bran oil and its unsaponifiable matter (USM) obtained from the bran prepared by different extraction processes on a dry weight basis.

Extraction process	γ- Oryzanol (mg/g)	
	Oil	USM
CM	0.55 ± 0.08^{c}	19.6 ± 2.58^{c}
OHM-AE (30% MC, E100)	1.31 ± 0.03^{ab}	52.4 ± 8.90^{b}
OHM-AE (30% MC, E150)	1.34 ± 0.02^{ab}	69.3 ± 3.39^{ab}
OHM-AE (30% MC, E200)	1.36 ± 0.01^{ab}	78.5 ± 9.11^{ab}
OHM-AE (40% MC, E100)	1.39 ± 0.04^{a}	84.3 ± 13.7^{a}
OHM-AE (40% MC, E150)	1.41 ± 0.06^{a}	86.8 ± 21.3^{a}
OHM-AE (40% MC, E200)	1.39 ± 0.02^{a}	79.5 ± 16.6^{a}

CM = conventional method; OHM = ohmic heating-assisted solvent extraction; MC = moisture content (% wet basis); E = electric field strength (V/cm); ND = not detected. Data are means ± SD of triplicate samples (n = 3). Means with different superscripts in the same columns differed significantly ($p < 0.05$).

Tocols and γ-oryzanol

The tocol contents as α-tocopherol, δ-tocopherol, γ-tocopherol and γ-tocotrienol of extracted oil and USM are presented in Table 1. Different extraction methods significantly impacted ($p < 0.05$) on concentrations of α-tocopherol, γ-tocopherol and γ-tocotrienol. Oil and USM prepared by OHM-AE at 30% MC (E = 200 V/cm) and 40% MC (all levels of E) recorded the highest amounts of α-tocopherol (37.5 - 41.6 μg/g and 4,146.8 - 4,296.7 μg/g), γ-tocopherol (60.1 - 61.2 μg/g and 672.5 - 693.7 μg/g) and γ-tocotrienol (128.5 - 129.2 μg/g and 1,288.8 - 1,299.3 μg/g), respectively. However, no significant difference was observed among extraction methods regarding δ-tocopherol content. Likewise, highest γ-oryzanol content was found in oil or USM obtained from OHM-AE at 30% MC (E = 150 and 200 V/cm) and 40% MC (E = 100, 150 and 200 V/cm) (Table 2) with the values ranged from 1.34 - 1.41 mg/g in the oil, and 69.3 - 86.8 mg/g in the USM. Values for tocopherols also concurred with Pestana-Bauer *et al*. (2012) who found that tocopherol contents in rice bran oil were 0.49 (δ- tocopherol), 9.73 (β and γ tocopherol), 16.1 (α- tocopherol) mg/100 g. The concentration of γ-oryzanol in rice bran oil obtained is also in agreement with Iqbal *et al*. (2005) who reported that levels of γ-oryzanol in rice bran oil from five varieties (Pakistan) ranged between 0.511 and 0.789 mg/g.

Overall, as expected, the concentration of the compounds increased with oil saponification. Interestingly, USM obtained from OHM-AE showed higher tocol contents (tocopherols and tocotrienols) and γ-oryzanol, and concentration of γ-oryzanol presented a two-fold increase when compared with CM. This was due to the breakdown of cell membranes as the bran was heated. Electricity applied under OHM at appropriate MC levels enhanced solvent extraction and release of intracellular bioactive compounds into the oil. This finding concurred with our previous studies (Loypimai *et al*., 2009; 2015) that rice bran stabilised using OHM gave higher yields of α-tocopherol, γ-oryzanol, and phenolic compounds and black bran colorant powder than rice bran stabilised by steaming and untreated rice bran. Loypimai *et al*. (2015; 2016) determined OHM assisted solvent extraction as an effective method for preparation of black rice bran colorant powder containing high concentration of bioactive compounds (anthocyanins, tocols and γ-oryzanol). In addition, pre-treatment using pulsed OHM induced a high degree of cell membrane damage of grape pomace and improved extraction acceleration of total polyphenols (El Darra *et al*., 2013).

Total phenolic content (TPC)

In addition to major bioactive substances as tocols and γ-oryzanol, TPC was determined in both oil and USM with results shown in Figure 2. Extraction processes significantly affected ($p < 0.05$) the TPC of both oil and USM. Oil and USM obtained from bran extracted with OHM-AE yielded higher TPC (ranging from 0.42 - 0.48 μg GAE/g oil and 0.41 - 0.47 μg GAE/g USM, respectively) than bran extracted by CM (0.33 ± 0.01 μg GAE/g oil and 0.29 ± 0.02 μg GAE./g USM, respectively). The extractability of phenolic compounds might be increased after rice bran was ohmically-treated. Total polyphenol content showed a positive relationship to the yield of extracted oil which was similar to Rombaut *et al*. (2015) who found a positive correlation between oil yield and polyphenol content. Alternatively, this might be due to a rapid deterioration of raw rice bran containing high fat content after milling, caused by the actions of lipase (hydrolysis) and lipoxygenase (lipid oxidation). Reactions of the bran can induce a decrease in polyphenol antioxidant activity resulting in low polyphenol content in the oil and USM. In

Table 3. Antioxidant activities of rice bran oil and its unsaponifiable matter (USM) obtained from the bran extracted using different extraction processes.

Extraction process	DPPH (IC_{50}, mg/g)		FRAP (mM $FeSO_4$/g)		Total antioxidant capacity (mg VCE/g)	
	Oil	USM	Oil	USM	Oil	USM
CM	138.7 ± 5.62^{a}	25.8 ± 2.51^{c}	0.019 ± 0.001d	0.14 ± 0.02^{c}	7.09 ± 1.44^{c}	65.1 ± 3.34^{c}
OHM-AE (30% MC, E100)	93.3 ± 2.16^{b}	18.2 ± 1.36^{b}	0.042 ± 0.007^{c}	0.42 ± 0.04^{b}	28.1 ± 3.11^{a}	74.8 ± 5.22^{a}
OHM-AE (30% MC, E150)	77.5 ± 2.62^{c}	17.8 ± 1.81^{ab}	0.047 ± 0.003^{c}	0.51 ± 0.11^{ab}	28.8 ± 2.18^{a}	77.7 ± 8.92^{a}
OHM-AE (30% MC, E200)	75.3 ± 5.07^{c}	17.4 ± 1.61^{ab}	0.056 ± 0.005^{b}	0.56 ± 0.05^{ab}	29.3 ± 2.69^{a}	79.4 ± 4.05^{a}
OHM-AE (40% MC, E100)	73.4 ± 1.79^{c}	16.6 ± 1.74^{a}	0.062 ± 0.003^{ab}	0.58 ± 0.13^{ab}	29.2 ± 1.61^{a}	78.5 ± 7.51^{a}
OHM-AE (40% MC, E150)	73.2 ± 2.32^{c}	17.3 ± 0.77^{ab}	0.063 ± 0.004^{ab}	0.63 ± 0.14^{a}	29.8 ± 2.97^{a}	78.3 ± 1.20^{a}
OHM-AE (40% MC, E200)	74.1 ± 1.99^{c}	16.8 ± 0.59^{ab}	0.065 ± 0.006^{a}	0.60 ± 0.11^{a}	28.6 ± 1.16^{a}	81.2 ± 2.43^{a}

CM = conventional method; OHM = ohmic heating-assisted solvent extraction; MC = moisture content (% wet basis); E = electric field strength (V/cm); ND = not detected. Data are means ± SD of triplicate samples (n = 3). Means with different superscripts in the same columns differed significantly ($p < 0.05$).

addition, ohmic heating with moisture addition at 30 - 40% inhibited these enzymes and no subsequent temperature rise occurred (Loypimai *et al*., 2009). Lakkakula *et al*. (2004) successfully applied OHM treatment as an effective method for the stabilisation of rice bran, resulting in high oil content in bran and higher oil yield. Nevertheless, no significant difference in TPC was observed between the oil and USM obtained.

Antioxidant activities

Three established methods including DPPH radical scavenging (IC_{50}), ferric reducing/antioxidant power (FRAP), and total antioxidant capacity (TAC) were used to determine the antioxidant activity of oil and USM extracts obtained from different extraction processes. Oil and USM obtained from the different oil extraction processes significantly affected ($p < 0.05$) IC_{50}, FRAP and TAC (Table 3). Oil and USM obtained from OHM-AE at 30% MC (E = 150 and 200 V/cm) and 40% MC (E = 100, 150, and 200 V/cm) yielded significantly lowest values ($p < 0.05$) of IC_{50} (73.2 - 77.5 mg/g oil and 16.6 - 17.8 mg/g USM, respectively), and highest TAC (28.1 - 29.8 mg VCE/g oil and 74.8 - 81.2 mg VCE/g USM, respectively) ($p < 0.05$). The highest value of FRAP assay was recorded from oil extracted by OHM-AE at 40% MC (all levels of E), and USM prepared by OHM-AE at 30% MC (E = 150 and 200 V/cm) and 40% MC (E = 100, 150, and 200 V/cm). This may be due to the electroporation effect of OHM (see earlier discussion), which increased the solvent extractability and release of antioxidative substances such as tocopherols, tocotrienols and γ-oryzanol into the oil. However, the antioxidant activity increased when oil was saponified to become USM. Similar results were reported by Gopala Krishna *et al*. (2003) who found that components of unsaponifiable matter obtained from chemically refined rice bran oil included phytosterols (0.96%), 4-methyl sterols (0.51%), triterpene alcohols (0.52%) and nonpolar components (0.61%) that mostly showed antioxidant activity. Ohmically-extracted Zenyan essential oils were effective as natural antioxidants to reduce the stable free radical DPPH (IC_{50} = 25 μg/mL) in mayonnaise (Gavahian *et al*., 2013). By contrast, higher phenol contents did not correspond to higher antioxidant capacity in wines (Galanakis *et al*., 2015).

Conclusion

In the present work, the application of ohmic heating-assisted solvent extraction resulted in increased oil yield, improved concentration of phytochemicals including γ-tocopherol, γ-tocotrienol, γ-oryzanol and total phenolic content, and increased antioxidant capacity in oil and USM. Extraction methods did not significantly influence the yield of USM. Interestingly, highest concentrations of γ-tocopherol, γ-tocotrienol, γ-oryzanol and total phenolic content in oil and USM were observed in bran extracted by OHM-AE at 30% MC (E = 150 and 200 V/cm) and 40% MC (E = 100, 150, and 200 V/cm). In addition, OHM-AE under these conditions yielded higher oil recovery and gave stronger antioxidant activity in all experiments as compared to bran extracted by CM. This suggests that OHM-assisted solvent extraction could be an effective process to prepare functional USM from rice bran, offering both increased oil yield and high concentration of bioactive compounds.

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