Kasetsart J. (Nat. Sci.) 48 : 778 - 789 (2014)

Pigmented Rice Hull Extracts: Extraction of Phenolic Compounds and Their Antioxidant Activity in Oil-in-Water Emulsion

Nopparat Cheetangdee

ABSTRACT

Rice hull, an under-utilized agricultural waste, is always employed for low value purposes such as fuel. Hull of pigmented rice (*Oryza sativa* L., cultivars Sangyod) was used to produce rice hull extracts, and their antioxidant activities were observed. Initially, the effective conditions to prepare rice hull extracts were established by comparing enzyme- and ethanol-aided extraction methods. The extractor solvents—deionized water with Viscozyme-L (0.5 and 1% by weight of rice hull) at pH 6.25 and ethanol (concentrations of 50, 65, and 75%, volume per volume)—were mixed with rice hull (ratio of rice hull to extractor solvent was 1:10, weight per volume). The mixtures were heated at 50 °C for 0–5 hr. The extractor solvent and heating time influenced the total phenolic content (TPC) and antioxidant activities of the extracts. Generally, the TPC increased with increased extraction time. Using enzyme, a higher enzyme concentration effected an increase in the TPC and the antioxidant activities of the extracts. Ethanolic extraction was more efficient at recovering phenolic compounds than the enzyme-aided means. The extracts exhibited antioxidant activities through both primary and secondary mechanisms and their antioxidant abilities coincidentally increased with the TPC. Extraction using 65% ethanol for 2 hr could provide rice hull phenolic extracts (RHPE) with the highest TPC and antioxidant activities as indicated by radical inhibition effects, the reducing power and the ferrous chelating ability ($P < 0.05$). RHPE effected oxidative stability of soybean emulsions in different ways, depending on the applied concentration. At concentration levels of 0.5 and 1% (w/v), RHPE successfully improved the oxidative stability of the emulsions. However, a comparable oxidative stability with the control sample (emulsion without RHPE) was observed when RHPE was added at a level of 1.5% (w/v).

Keywords: pigmented rice hull, ethanolic extraction, enzymatic extraction, oil-in-water emulsion, phenolic compounds, antioxidant activity

INTRODUCTION

Lipid oxidation—one of important chemical deterioration processes in food containing lipids—can result in inferior product quality from both nutritional and sensorial aspects (McClements and Decker, 2000; Shih and Daigle, 2003; Shahidi and Zhong, 2011). Antioxidants are widely used to retard lipid oxidation and nowadays, there is growing interest in employing natural antioxidants because of concerns about the health hazards of synthetic agents (Shahidi *et al*., 1992). Phenolic compounds can be potently employed as natural antioxidants by exhibiting antioxidant activities through various mechanisms, such as free radical scavenging, singlet oxygen quenching, pro-oxidant metal ions chelating and pro-oxidative enzyme inhibition, (Škerget *et al*., 2005; Djeridane

Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University, Songkhla 90112, Thailand.
E-mail: nopparat.ch@psu.ac.th

Received date : 01/04/14 Accepted date : 11/09/14

et al., 2006; Dudonné *et al*., 2009; Shahidi and Zhong, 2011).

In the past, the recovery of phenolics from various plants has been extensively studied (Rice-Evans *et al*., 1996; Chen *et al*., 1999; Burda and Oleszek 2001; Exarchou *et al.,* 2002; Javanmardi *et al*., 2003; Lee *et al.,* 2003; Miliauskas *et al.,* 2004; Škerget *et al*., 2005; Djeridane *et al.,* 2006; Dudonné *et al*., 2009; Butsat and Siriamornpun, 2010). Diverse phenolic genres have been recovered from different plant species using different extraction conditions, resulting in dissimilar antioxidant activities of the extracts (Exarchou *et al*., 2002; Miliauskas *et al.,* 2004; Djeridane *et al.,* 2006; Dudonné *et al*., 2009). In plant cellular matrices, most phenolics are bound with composited polysaccharides in the cell wall structure (Renger and Steinhart, 2000). The use of carbohydrases enhanced cell wall degradation and thereby facilitated the liberation of phenolic compounds (Landbo and Meyer, 2001; Barzana *et al.,* 2002; Li *et al*., 2006; Zheng *et al*., 2009). The current work observed the effectiveness of carbohydrase to prepare rice hull phenolic extracts, by comparison with the solvent-aided extraction method.

Rice hull, an inedible portion generated as a by-product from the rice milling process, is always used in low value applications (Butsat and Siriamornpun, 2010). Most phenolics (approximately 42–50% of the total phenolics in rice grain) accumulate in the hull (Butsat and Siriamornpun, 2010). Shih and Daigle (2003) reported that methanolic rice hull extracts could exhibit antioxidant activity as potent as butylated hydroxytoluene in ground beef. The present work used the red pigmented Sangyod rice, a typical rice variety planted in southern Thailand. It has been suggested that the presence of pigments was correlated to the phenolic content and thereby, the antioxidant capacity of plant extracts (Chung *et al.,* 2000). Antioxidant activity has been reported in cell-based assay of the extracts from endosperm of the Sangyod rice (Srisawat *et al*., 2010); however, the antioxidant activities of extracts from its hull have not been elucidated.

The objectives of the present work were to establish the effective conditions for the preparation of rice hull extracts and to observe their influence on the oxidative stability of an oil-in-water (O/W) emulsion. Emulsion was selected as the studied model, because it is generally found as a composition in various food products, such as in milk, infant formula, and beverages (McClements, 2005). An emulsion is an oxidative-sensitive system due to the presence of large interfacial areas and possesses an appreciably different oxidation mechanism to a homogeneous model, such as bulk oil (McClements and Decker, 2000; Waraho *et al.,* 2011). Improvements to the oxidative stability of an emulsion may useful for further application in food processing.

MATERIALS AND METHODS

Materials

The hull of rice (*Oryza sativa* L, cultivar Sangyod) was obtained from a rice milling community enterprise (Phattalung, Thailand). The hull was pulverized and passed through a 40-mesh sieve before storing in a polyethylene bag at 4 °C. Refined soybean oil without exogenous antioxidants was provided by Lamsoon Co., Ltd. (Bangkok, Thailand). Viscozyme-L from *Aspergillus* sp. (density of 1.2 $g.mL^{-1}$), Folin Ciocalteu's phenol reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), and thiobarbituric acid (TBA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Trichloroacetic acid was obtained from Carlo Erba Reagenti (Rodano, Italy). $K_2S_2O_8$, $NaHPO_4.2H_2O$, $NaH_2PO_4.2H_2O$, and NaOH were the products of QREC (Auckland, New Zealand). HCl, isooctane, 2-propanol, methanol, and 1-butanol were bought from J.T. Baker (Deventer, the Netherlands). All chemicals were of analytical grade.

Preparation of rice hull extracts

Solvent-aided extraction: Ethanol-aided extraction was carried out according to the procedure of Butsat and Siriamornpun (2010) with some modifications. The hull was mixed in a homogenizer (Ultra Turrax T25, Ika, Germany) with ethanol (50, 65, and 75%, volume per volume; v/v) at a ratio of 1 to 10 (weight per volume; w/v) at 11,000 rpm for 2 min. The extraction was performed in a water bath (Memmart, Germany) at a controlled temperature of 45 ± 2 °C for 0–5 hr. After filtration, the supernatant was collected, freeze dried and kept at 4 °C.

Enzymatic-aided extraction: Enzymatic-aided extraction was conducted following the method of Li *et al*. (2006) with a minor modification. The hull was homogenized with deionized water (pH 6.25) at a ratio of 1 to 10 (w/v). Viscozyme-L (0.5 and 1% by weight of rice hull) was added. The mixture was heated at 45±2 °C for 0–5 hr. Then the enzyme activity was terminated by elevating the temperature to 90 °C for 5 min before immediate cooling in an ice bath. The mixture was filtered, freeze dried and stored at 4 °C.

Determination of total phenolic content

The total phenolic content (TPC) of the rice hull extracts was quantified using Folin-Ciocalteu calorimetry according to the method of Javanmardi *et al.* (2003). A sample of 50 µL of the extracts (0.5%, w/v) was mixed with 2.5 mL of Folin-Ciocalteu's phenol reagent (10 times dilution) and 2 mL of Na_2CO_3 (7.5%). After incubation at 45 °C for 15 min., absorbance at 765 nm was observed (UV-1700; Shimadzu Corp.; Kyoto, Japan). The TPC was estimated using gallic acid as a standard and reported as milligrams of gallic acid equivalent per gram dried weight (mg GAE.g^{-1}, dw) of sample.

Antioxidant activities of rice hull extracts

Rice hull extracts were dissolved in ethanol to obtain a concentration of 0.5% (w/v), before subjecting to analyses.

2,2-Diphenylpicrylhydrazyl radical inhibition effect: The DPPH radical inhibition effect was observed following the method of Xie *et al*. (2008). Rice hull extract solution (0.5 mL) was added with DPPH solution (2.5 µg.mL^{-1}, 2.5 mL) and incubated at room temperature for 30 min in the dark. The control was prepared in the same manner using an equal volume of ethanol instead of the extract solution. The DPPH radical inhibition effect was determined by a decrease in the absorbance at 517 nm and calculated using Equation 1:

$$\% \text{ inhibition} = [(A_{control} - A_{sample})/A_{control}] \times 100 \quad (1)$$

2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) radical inhibition effect: The ABTS radical inhibition ability was measured by the method of Thaipong *et al.* (2006). The $ABTS^{\bullet+}$ radical was generated by mixing equal volumes of ABTS (7.4 mM) and $K_2S_2O_8$ (2.6 mM) at ambient temperature for 12–16 hr in the dark. The $ABTS^{\bullet+}$ solution was then diluted with methanol to produce an absorbance (734 nm) of 1.1 ± 0.02. A sample of rice hull extract solution (150 µL) was mixed with the diluted $ABTS^{\bullet+}$ solution (2,850 µL) and incubated at ambient temperature for 2 hr in the dark. The absorbance at 734 nm was recorded. The control was prepared in the same manner using an equal volume of ethanol instead of the sample solution. The ABTS radical scavenging activity was quantified using Equation 1.

Reducing power:

The reducing power was estimated, according to the procedure described by Xie *et al*. (2008). Rice hull extract solution (2.5 mL) was mixed with $K_2Fe(CN)_6$ (1%, 2.5 mL) and heated at 50 °C for 20 min. The mixture was added with trichloroacetic acid (10%, 2.5 mL) and centrifuged (CR 22GIII, high-speed, refrigerated centrifuge; Hitachi; Tokyo, Japan) at 2,500×*g* for 5 min.. The supernatant (2.5 mL) was mixed with $FeCl_3$ (0.1%, 2.5 mL), and incubated at room temperature for 10 min. The absorbance at 700 nm was observed, with

a higher absorbance indicating better reducing power.

Fe^{2+} chelating activity: The ability to chelate Fe^{2+} was examined following the method of Xie *et al*. (2008) with a slight modification. Rice hull extract solution (1 mL) was mixed with $FeCl_2$ (2 mM, 50µL) and distilled water (1.85 mL), before centrifugation at 2,500×*g* for 5 min. The supernatant (0.5 mL) was mixed with Ferrozine solution (5 mM, 100 µL). The control was prepared in the same manner using an equal volume of ethanol instead of the sample solution. The absorbance at 562 nm was observed, and the Fe^{2+} chelating activity was estimated using Equation 1.

Oxidative stability of oil-in-water emulsion

Preparation of emulsions containing rice hull phenolic extract: Rice hull phenolic extract **(**RHPE) was dissolved in ethanol to obtain a concentration of 5% (w/v). An oil-in-water (O/W) emulsion was produced by homogenizing Tween 20 dissolved in 10 mM phosphate buffer pH 7, soybean oil, and RHPE solution at 19,000 rpm for 2 min in an ice bath. The emulsions contained an oil fraction of 0.1, Tween 20 of 0.5% and RHPE at different concentrations (0, 0.5, 1, and 1.5% w/v). NaN_3 (0.02%) was applied to the emulsions as an antimicrobial agent. The emulsions were kept in a screw-capped bottle at 37 °C in the dark. Lipid oxidation was observed by the monitoring the peroxide value (PV) and the thiobarbituric reactive substances (TBARS) content over a period of 15 days.

Peroxide value determination: The method was modified from Hu et al., (2004). The emulsion (0.3 mL) was vigorously mixed with a mixture of isooctane:propanol (3:1, 1.5 mL) and centrifuged at 4000×*g* for 2 min. The solvent phase (200 µL) was reacted with a mixture of methanol:1-butanol (2:1, 2.8 mL), ammonium thiocyanate (3.97 M, 15 µL) and ferrous iron solution containing 0.132 M $BaCl_2$ and 0.144 M $FeSO_4.7H_2O$ (15 µL). The mixture was incubated at room temperature for 20 min. The absorbance at 510 nm was then recorded. The PV was quantified using cumene hydroperoxide as a standard and reported as milligrams of hydroperoxide equivalent per liter of sample.

Thiobarbituric reactive substances determination: The method used followed Aewsiri et al., (2009). The emulsion (1 mL) was mixed with thiobarbituric acid (TBA) solution consisting of 0.375% TBA, 15% trichloroacetic acid, and 0.25 N HCl (5 mL). The mixture was heated at 100 °C for 30 min before measuring the absorbance at 532 nm. The TBARS content was determined using malonaldehyde (MDA) as a standard and reported as milligrams of MDA equivalent per liter of sample.

Statistical analysis

The experiments were run in triplicate and mean values ± SD were determined. Statistical analysis was conducted using analysis of variance and applying Duncan's multiple range tests (SPSS for Windows; SPSS Inc.; Chicago, IL, USA) with significance tested at the 95% confidence level.

RESULTS AND DISCUSSION

Preparation of rice hull extracts

Initially, the effective conditions to extract phenolic compounds from rice hull were investigated. Figure 1 shows the TPC of the extracts prepared by enzyme- and ethanol-aided extraction at various times. Increasing the duration time increased the TPC of the extracts, which was in good accord with other work (Landbo and Meyer 2001; Durling *et al.,* 2007). Using enzyme produced a higher TPC with increased enzyme concentration. Ethanol-aided extraction clearly produced extracts with a higher TPC than did the enzyme-aided means, and the highest TPC of approximately 1.2 mg GAE.g^{-1}, dw was found for 65% ethanolic extraction for 2 hr ($P < 0.05$). The effectiveness of polar solvents to recover phenolics from various plants has been reported (Miliauskas

et al., 2004; Durling *et al.,* 2007; Dudonné *et al.,* 2009). The polarity of solvents played a crucial role in determining the yield and identity of the extracted phenolics. With less polar solvent, more lipophilic phenolic compounds are extracted, and *vice versa* (Bauman *et al*., 1999). With hydroalcoholic extraction, the increased ethanol content promoted the liberation of lipophilic phenolic species (Durling *et al.,* 2007). Dominant phenolic compounds found in the hulls of different rice varieties have been reported involving vanillic acid and *p*-coumaric acid for Khao Dawk Mali 105 rice (Butsat and Siriamornpun, 2010), *p*-coumaric acid, *o*-methoxycinnamic acid, and *N*-indolyl acetate for Japonica rice (Lee *et al.,* 2003), as well as cinnamic acid and benzoic derivatives for wild rice (*Zizania aquatic* L.; Asamaria *et al*. 1996). The composite phenolic compounds in plants show great diversity depending on the plant species, growth area and environment, as well as the extraction conditions, as for example with the solvent type, concentration and extraction time (Lee *et al*., 2003; Butsat and Siriamornpun, 2010).

In the present work, the inferior ability of enzyme-aided extraction was suggested by the lower TPC of the extracts. Generally, carbohydrases could enhance the bioactive compound extraction from the plant cells by facilitating the cell wall degradation (Landbo and Meyer, 2001; Barzana *et al.,* 2002; Li *et al.,* 2006). However, by degrading the cell wall, carbohydrases also facilitated the release of other components, such as proteins (Landbo and Meyer, 2001; Li *et al.*, 2006). These residues might interact with phenolics, and this could lead to lower phenolic recovering efficiency (Li *et al*., 2006). According to the manufacturer's datasheet, Viscozyme-L is a cocktail enzyme consisting of arabinase, cellulase, β-glucosidase, hemicellulase, and xylanase (Anon, 1991). β-Glucosidase promoted the degradation of some phenolic species, such as anthocyanin, and led to a lower TPC of black current pomace extracts (Landbo and Meyer, 2001).

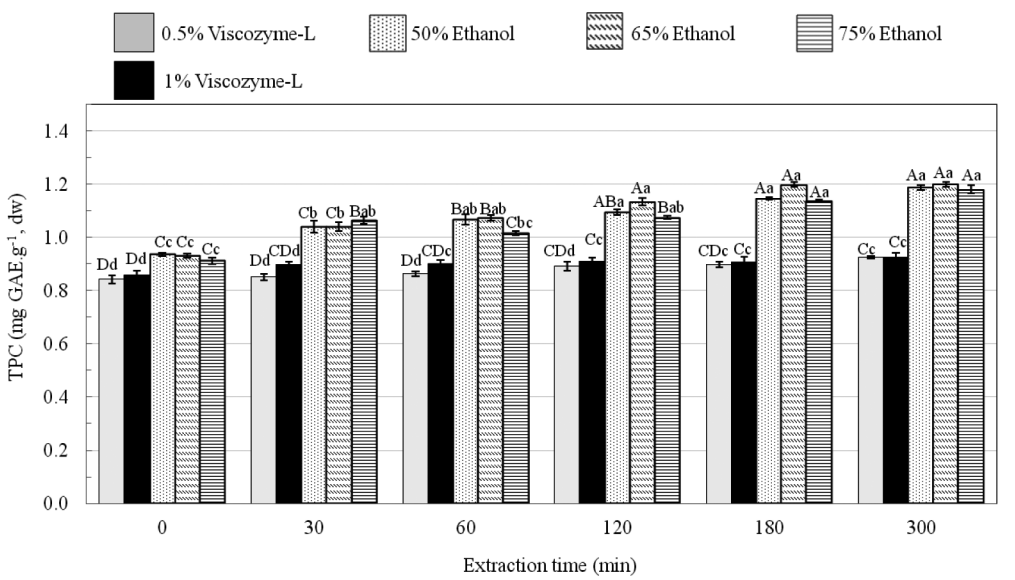

Figure 1 Total phenolic contents (TPC), measured in milligrams of gallic acid equivalent per gram dried weight (mg GAE.g^{-1}, dw), of rice hull extracts prepared by using Viscozyme-L (0.5 and 1%) and ethanol (50, 65, and 75%) at various extraction times. Mean values ± SD error bars (n = 3) are shown. Columns with the same capital letters (with regard to extractor solvent) or small letters (with regard to extraction time) are not significantly different at $P > 0.05$.

In using enzymes, various factors must be considered such as the type and concentration of the enzyme, the temperature and the extraction duration (Landbo and Meyer, 2001; Li *et al.,* 2006; Barzana *et al.,* 2002). Therefore, the effective recovery of phenolics from rice hull using Viscozyme-L requires the further investigation of other factors such as the mixing ratio of the plant materials and extractor solvent, the enzyme concentration and its use in combination with other enzymes.

Antioxidant activities of rice hull extracts

The antioxidant activities of rice hull extracts were examined through DPPH (Figure 2) and ABTS radical inhibition effects (Figure 3), reducing power (Figure 4) and Fe^{2+} chelating ability (Figure 5). Ethanol-aided means clearly provided extracts with a greater DPPH radical inhibition effect than those derived by enzyme-aided extraction ($P < 0.05$). The DPPH radical inhibition effect increased with extraction time. Similar behavior with DPPH assay was found for the ABTS radical inhibition effect. These results implied that rice hull extracts could act as an electron donor to stabilize active free radicals (Klompong *et al*., 2007). Better reducing power was found for the ethanolic extracts than for the enzyme-treated ones, as suggested by the higher Abs_{700} values ($P < 0.05$). With efficient reducing power, the extracts could quench free radicals and this led to the termination of the oxidative chain reaction (Duh *et al*., 1999). Generally, enzyme-aided extraction produced better radical scavenging effects and reducing power when the enzyme was applied at higher concentrations. The results from Figures 2–4 imply that the antioxidant activities of the extracts was through a primary mechanism. Next, the Fe^{2+} chelating ability of the extracts was observed. Ethanol provided the extracts with better ferrous-ion chelating efficiency than those derived by enzyme-aided means. Transition metal ions, such as Fe^{2+}, Cu^{2+} and Co^{2+}, could enhance lipid oxidation through a Fenton reaction, so lipid oxidation might be retarded by limiting free metal ion availability (Gordon *et al*., 2001). This result implied antioxidant activities of the extracts via a secondary mechanism.

The extraction conditions greatly influence the antioxidant properties of plant extracts (Pekkarinen *et al*., 1999; Exarchou *et al*., 2002; Miliauskas *et al*., 2004; Djeridane *et al.,* 2006; Dudonné *et al.,* 2009). In the present work, the antioxidant activities of the extracts were generally increased with increased TPC: The linear relationship between TPC and antioxidant activity showed high correlation factors of 0.9247, 0.9855 and 0.9323 for DPPH, ABTS and reducing assays, respectively (see insets of Figures 2–4). This suggested the importance of the phenolic content on the antioxidant activities of the extracts, which was in accord with previous reports (Exarchou *et al*., 2002; Lee *et al*., 2003; Miliauskas *et al.,* 2004; Djeridane *et al.,* 2006; Dudonné *et al.,* 2009; Butsat and Siriamornpun, 2010). With their structures containing aromatic rings and hydroxyl groups, phenolics could exhibit antioxidant activities via a redox property by forming resonance-stabilized phenoxyl radicals to make them a reducing agent, hydrogen-donor, metal chelator and oxygen quencher (Bors and Michel, 2002). *p*-Coumaric acid, the dominant phenolic compound present in the hull of *O. sativa* L (Butsat and Siriamornpun, 2010), could donate protons effectively as suggested by its potent DPPH and ABTS radical scavenging activities (Rice-Evans *et al.,* 1996). However, with respect to the metal chelating effect of rice hull extracts, there was a rather low correlation factor of 0.5704 (see inset of Figure 5). This might have been due to the impaired metal-ion chelating ability of the derived phenolics genres. The chemical structures of phenolic compounds are strongly related to their antioxidant activities (Chen *et al.,* 1999; Burda and Oleszek 2001; Škerget *et al.,* 2005). Elucidation of their antioxidant properties will require the further identification of the phenolic species of the extracts.

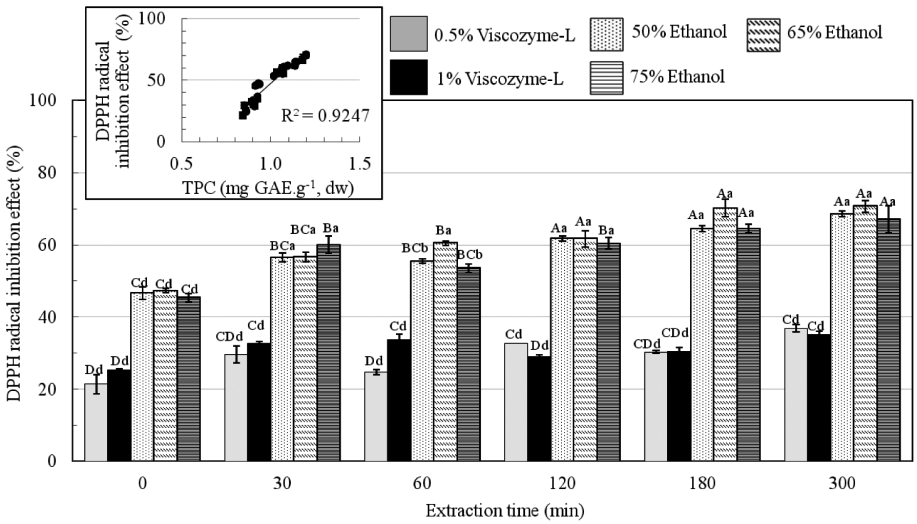

Figure 2 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical inhibition effect of rice hull extracts prepared using Viscozyme-L (0.5 and 1%) and ethanol (50, 65, and 75%) at various extraction times. The inset shows the correlation (R^2 = Correlation coefficient) between total phenolic content (TPC) measured in milligrams of gallic acid equivalent per gram dried weight (mg GAE.g^{-1}, dw) and the DPPH radical inhibition effect of rice hull extracts. Mean values ± SD (n = 3) are shown. Columns with the same capital letters (with regard to extractor solvent) or small letters (with regard to extraction time) are not significantly different at $P > 0.05$.

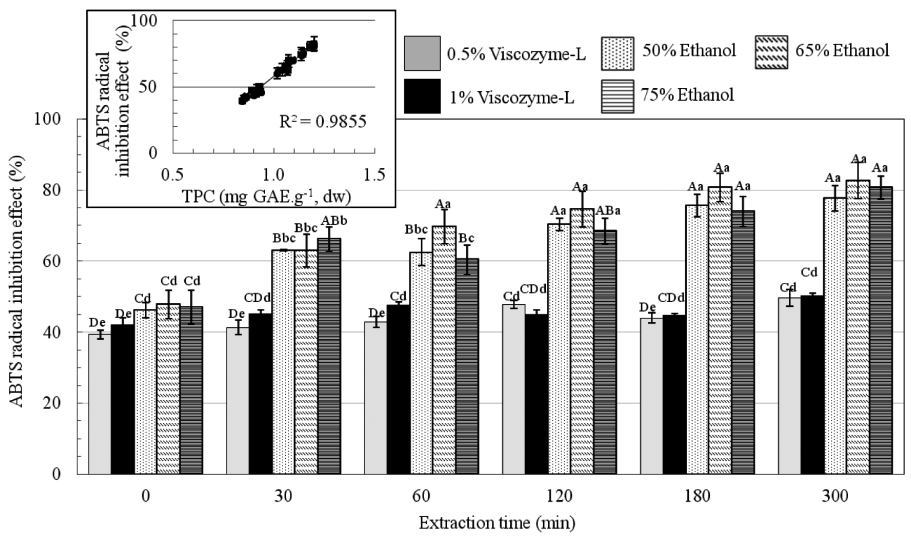

Figure 3 2,2′-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) radical inhibition effect of rice hull extracts prepared using Viscozyme-L (0.5 and 1%) and ethanol (50, 65, and 75%) at various extraction times. The inset shows the correlation (R^2 = Correlation coefficient) between total phenolic content (TPC) measured in milligrams of gallic acid equivalent per gram dried weight (mg GAE.g^{-1}, dw) and ABTS radical inhibition effect of rice hull extracts. Mean values ± SD (n = 3) are shown. Columns with the same capital letters (with regard to extractor solvent) or small letters (with regard to extraction time) are not significantly different at $P > 0.05$.

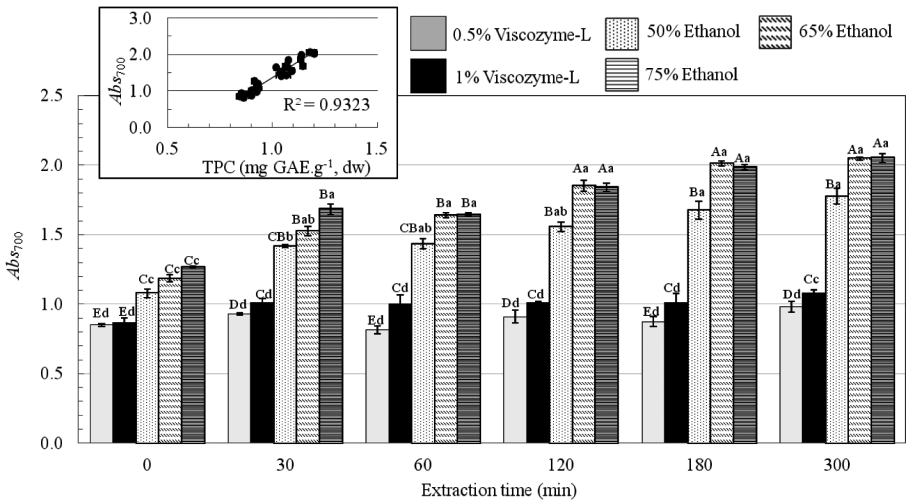

Figure 4 Reducing power (Abs_{700}) of rice hull extracts prepared by the aid of Viscozyme-L (0.5 and 1%) and ethanol (50, 65, and 75%) at various extraction times. The inset shows the correlation (R^2 = Correlation coefficient) between total phenolic content (TPC) measured in milligrams of gallic acid equivalent per gram dried weight (mg GAE.g^{-1}, dw) and reducing power of rice hull extracts. Mean values ± SD (n=3) are shown. Columns with the same capital letters (with regard to extractor solvent) or small letters (with regard to extraction time) are not significantly different at $P > 0.05$.

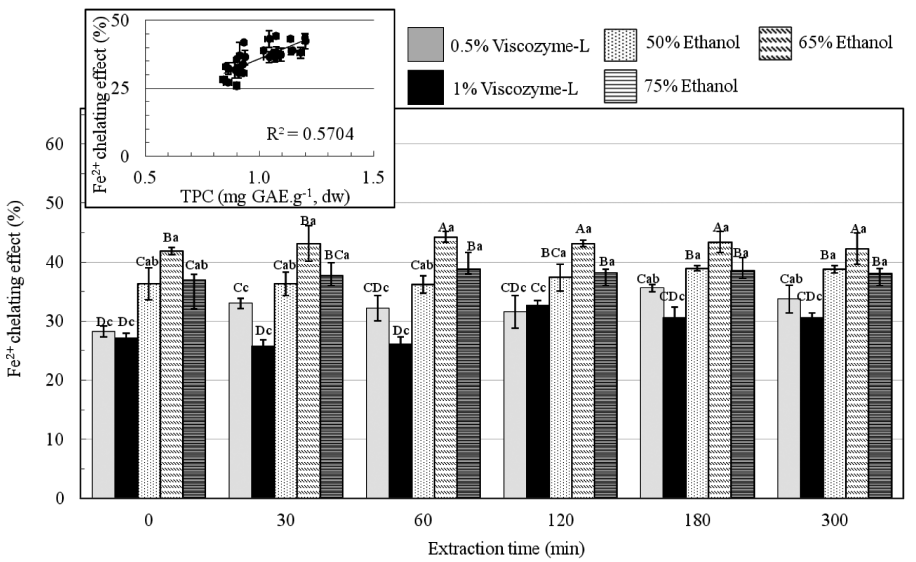

Figure 5 Fe^{2+} chelating effect of rice hull extracts prepared using Viscozyme-L (0.5 and 1%) and ethanol (50, 65, and 75%) at various extraction times. The inset shows the correlation (R^2 = Correlation coefficient) between total phenolic content (TPC) measured in milligrams of gallic acid equivalent per gram dried weight (mg GAE.g^{-1}, dw) and the Fe^{2+} chelating effect of rice hull extracts. Mean values ± SD (n = 3) are shown. Columns with the same capital letters (with regard to extractor solvent) or small letters (with regard to extraction time) are not significantly different at $P > 0.05$.

Overall, 65% ethanolic extraction for at least 2 hr was sufficient to provide the extract with the highest TPC and antioxidant activities ($P < 0.05$), as suggested by all observed assays. However, the extraction yield should be estimated in future work to clearly elucidate the recovering efficiency. This selected condition was performed to prepare rice hull phenolic extract (RHPE) for the following study.

Oxidative stability of oil-in-water emulsion containing rice hull phenolic extract

Soybean O/W emulsions containing RHPE at different concentrations were prepared and the development of the PV and the TBARS content was observed during a period of 15 days (Figure 6). RHPE effected oxidative stability of the emulsions, depending on the applied concentration. At concentration levels of 0.5 and 1%, RHPE successfully improved the oxidative stability, as implied by the clearly lower PV and TBARS content of the RHPE-added emulsions than for the control (emulsion without RHPE, $P < 0.05$). This could be ascribed to the antioxidant activities of RHPE as was shown in the previous results. The methanolic extracts of some plants, including olive, St. John's wort, hawthorn, oregano and laurel leaf, could improve the oxidative stability of Tween-20-based emulsions (Škerget *et al.,* 2005). The oxidative stability of sunflower oil emulsions was also successfully prolonged by tea and rosemary extracts (Roedig-Penman and

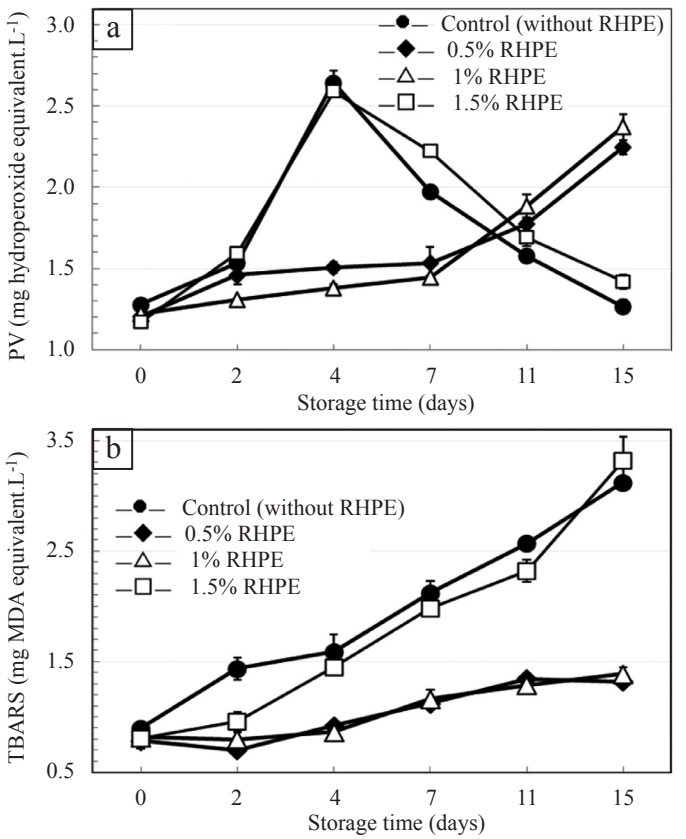

Figure 6 Storage time dependence on: (a) peroxide value (PV); and (b) thiobarbituric reactive substances (TBARs) content in the emulsions containing rice hull phenolic extract (RHPE) at various concentrations (0, 1, and 1.5%, w/v). The emulsions were stored at 37 °C in the dark for 15 days. Mean values ± SD ($n = 3$) are shown. MDA = Malonaldehyde.

Gordon, 1997). However, incorporating RHPE at 1.5% failed to improve the oxidative stability of the emulsions: The PV and TBARS content of the RHPE-added emulsion were not different from the control ($P > 0.05$). In an emulsion system, lipid oxidation occurs preferably at the oil-water interfaces (Chen *et al.,* 1999; McClements and Decker, 2000). The emulsifier plays an important role in the formation of interfacial films, which act as a barrier to protect oil drops from water-soluble pro-oxidants (Waraho *et al.,* 2011). At sufficiently high concentration, phenolic compounds could interact with the emulsifier and thereby alter the geometry of the interfacial films (Heinonen *et al.,* 1998; Pekkarinen *et al*., 1999). This might result in the inferior oxidative stability of the emulsion. By adding caffeic acid to Tween-20-based emulsions, partitioning of Tween 20 at the interfaces was changed, resulting in a negative effect on the oxidative stability of the emulsions (Pekkarinen *et al*., 1999). Gallic acid and delphinidin also adversely affected the oxidative stability of a liposome model (Heinonen *et al.,* 1998). The present study showed that RHPE could prolong the oxidative stability of the emulsions, depending on its concentration level. This suggested that the ability of RHPE to retard lipid oxidation was related to its own antioxidant ability as well as its affinity with other components in the system (Heinonen *et al*., 1998). To further elucidate this effect, other characteristics of the RHPE should be further investigated, for example solubility in the polar/non-polar phase and hydrophilicity.

CONCLUSION

The extraction of phenolic compounds from the hull of pigmented rice was conducted using ethanol- and enzyme-aided methods. The total phenolic content (TPC) of the extracts generally increased with increased extraction time. Increasing the enzyme concentration provided extracts with a higher TPC and better antioxidant activities as indicated by their free radical scavenging effects and reducing power. Ethanolic extraction was more efficient than the enzyme-aided means in the recovery of phenolic compounds. The rice hull extracts exhibited antioxidant properties through both primary and secondary mechanisms, and their antioxidant abilities tended to increase with the TPC level. In this work, 65% ethanolic extraction for 2 hr produced rice hull phenolic extracts (RHPE) with the highest TPC and antioxidant activities ($P < 0.05$). RHPE influenced the oxidative stability of soybean O/W emulsions, depending on the concentration level. Incorporating RHPE at levels of 0.5 and 1% successfully improved the oxidative stability as indicated by the lower PV and TBARS content of the RHPE-added emulsions than those observed for the control (emulsion without RHPE). However, at a concentration of 1.5%, the oxidative stability of the RHPE-added emulsion and the control sample was comparable.

ACKNOWLEDGEMENT

The author is grateful for financial support from the Halal Food Science Center, Prince of Songkla University (contract No. SAT-HC56-ST05).

LITERATURE CITED

Aewsiri, T., S. Benjakul, W. Visessanguan, J.B. Eun, W. Wierenga and H. Gruppen. 2009. Antioxidative activity and emulsifying properties of cuttlefish skin gelatin modified by oxidized phenolic compounds. **Food Chem.** 117: 60–68.

Anon. 1991. **Product sheet of Viscozyme-L.** Novo Nodisk A/S, Enzyme Process Division. Bagsvaerd, Denmark.

Asamaria, A., P.B. Addis, R.J. Epley and T.P. Krick. 1996. Wild rice hull antioxidants. **J. Agric. Food Chem.** 44: 126–130.

Barzana, E., D. Rubio, R.I. Santamaria, O. Garcia-Correa, F. Garcia, V.E. Ridaura Sanz and A.

López-Munguía. 2002. Enzyme-mediated solvent extraction of carotenoids from marigold flower (*Tagetes erecta*). **J. Agric. Food Chem.** 50: 4491–4496.

Bauman, D., M. Hadolin, A. Rizner-Hraš and Z. Knez. 1999. Supercritical fluid extraction of rosemary and sage antioxidants. **Acta Alimentaria** 28: 15–28.

Bors, W. and C. Michel. 2002. Chemistry of the antioxidant effect of polyphenols. **Ann. NY Acad. Sci.** 957: 57–69.

Burda, S. and W. Oleszek. 2001. Antioxidant and antiradical activities of flavonoids. **J. Agric. Food Chem.** 49: 2774–2779.

Butsat, S. and S. Siriamornpun. 2010. Antioxidant capacities and phenolic compounds of the husk, bran, and endosperm of Thai rice. **Food Chem.** 119: 606–613.

Chen, Z.Y., P.T. Chan, K.Y. Ho, K.P. Fung and J. Wang. 1999. Antioxidant activity of natural flavonoids is governed by number and location of their aromatic hydroxyl groups. **Chem. Phys. Lipids** 79: 157–163.

Chung, I., K.H. Kim, J.K. Ahn and J.O. Lee. 2000. Varietal variation in antioxidative activity of rice grain by DPPH and TBA methods. **Korean J. Crop Sci.** 45: 261–266.

Djeridane, A., M. Yousfi, B. Nadjemi, D. Boutassouna, P. Stocker and N. Vidal. 2006. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. **Food Chem.** 97: 654–660.

Dudonné, S., X. Vitrac, P. Coutiére, M. Woillez and J.M. Mérillion. 2009. Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays. **J. Agric. Food Chem.** 57: 1768–1774.

Duh, P.D., Y.Y. Tu and G.C. Yen. 1999. Antioxidant activity of water extract of Harng Jyur (*Chrtsanthemum morifolium* Ramat). **Lebensm-Wiss. U-Technol.** 32: 269–277.

Durling, N.E., O.J. Catchpole, J.B. Grey, R.F. Webby, K.A. Mitchell, L.Y. Foo and N.B. Perry. 2007. Extraction of phenolics and essential oil from dried sage (*Salvia officinalis*) using ethanol-water mixtures. **Food Chem.** 101: 1417–1424.

Exarchou, V., N. Nenadis, M. Tsimidou, I.P. Gerothanassis, A. Troganis and D. Boskou. 2002. Antioxidant activities and phenolic composition of extracts from Greek oregano, Greek sage, and summer savory. **J. Agric. Food Chem.** 50: 5294–5299.

Gordon, M.H., F. Paiva-Martins and M. Almeida. 2001. Antioxidant activity of hydroxytyrosol acetate compared with that of other olive oil polyphenols. **J. Agric. Food Chem.** 49: 2480–2485.

Heinonen, M., D. Rein, M.T. Satué-Gracia, S.W. Huang, J.B. German and E.N. Frankel. 1998. Effect of protein on the antioxidant activity of phenolic compounds in a lecithin-liposome oxidation system. **J. Agric. Food Chem.** 46: 917–922.

Hu, M., D.J. McClements and E.A. Decker. 2004. Antioxidant activity of a proanthocyanidin-rich extract from grape seed in whey protein isolate stabilized algae oil-in-water emulsion. **J. Agric. Food Chem.** 52: 5272–5276.

Javanmardi, J., C. Stushnoff, E. Locke and J.M. Vivanco. 2003. Antioxidant activity and total phenolic content of Iranian *Ocimum* accessions. **Food Chem.** 83: 547–550.

Klompong, V., S. Benjakul, D. Kantachote and F. Shahidi. 2007. Antioxidative activity and functional properties of protein hydrolysate of yellow stripe trevally (*Selaroides leptolepis*) as influenced by the degree of hydrolysis and enzyme type. **Food Chem.** 102: 1317–1327.

Landbo, A.K. and A.S. Meyer. 2001. Enzyme-assisted extraction of antioxidative phenols from black currant juice press residues (*Ribes nigrum*). **J. Agric. Food Chem.** 49: 3169–3177.

Lee, S.C., J.H. Kim, S.M. Jeong, D.R. Kim, J.U. Ha and D.U. Ahn. 2003. Effect of far-

infrared radiation on the antioxidant activity of rice hulls. **J. Agric. Food Chem.** 51: 4400–4403.

Li, B.B., B. Smith and M. Hossian. 2006. Extraction of phenolics from citrus peels: II. Enzyme-assisted extraction method. **Separ. Purif. Technol.** 48: 189–196.

McClements, D.J. and E.A. Decker. 2000. Lipid oxidation in oil-in-water emulsions: Impact of molecular environment on chemical reactions in heterogeneous food systems. **J. Food Sci.** 65: 1270–1282.

McClements, D.J. 2005. **Food Emulsions: Principles, Practices, and Techniques.** 2nd ed. CRC Press. Boca Raton, FL, USA. 609 pp.

Miliauskas, G., P.R. Venskutonis, T.A. van Beek. 2004. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. **Food Chem.** 85: 231–237.

Pekkarinen, S.S., H. Stöckmann, K. Schwarz, M. Heinonen and A.I. Hopia. 1999. Antioxidant activity and partitioning of phenolic acids in bulk and emulsified methyl linoleate. **J. Agric. Food Chem.** 47: 3036–3043.

Renger, A. and H. Steinhart. 2000. Ferulic acid dehydrodimers as structural elements in cereal dietary fiber. **Eur. Food. Technol.** 211: 422–428.

Rice-Evans, C.A., N.J. Miller and G. Paganga. 1996. Structure antioxidant activity relationship of flavonoids and phenolic acids. **Free Radical Biol. Med.** 20: 933–956.

Roedig-Penman, A. and M.H. Gordon. 1997. Antioxidant properties of catechins and green tea extracts in model food emulsions. **J. Agric. Food Chem.** 45: 4267–4270.

Shahidi, F., P.K. Janitha and P.D. Wanasundara. 1992. Phenolic antioxidants. **Crit. Rev. Food Sci.** 32: 67–103.

Shahidi, F. and Y. Zhong. 2011. Revising the polar paradox theory: A critical overview. **J. Agric. Food Chem.** 59: 3499–3504.

Shih, F. and K.W. Daigle. 2003. Antioxidant properties of milled-rice co-products and their effects on lipid oxidation in ground beef. **J. Food Sci.** 68: 2672–2675.

Srisawat, U., W. Panunto, N. Kaendee, S. Tanuchit, A. Itharat, N. Lerdvuthisopon and P. Hansakul. 2010. Determination of phenolic compounds, flavonoids, and antioxidant activities in water extracts of Thai red and white rice cultivars. **J. Med. Assoc. Thailand** 7: 83–91.

Škerget, M., P. Kotnik, M. Hadolin, A.R. Hraš, M. Simonič and Ž. Knez. 2005. Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. **Food Chem.** 89: 191–198.

Thaipong, K., U. Boonprakob, K. Crosby, L. Cisneros-Zevallos and D.H. Byrne. 2006. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. **J. Food Compos. Anal.** 19: 669–675.

Waraho, T., D.J. McClements and E.A. Decker. 2011. Mechanisms of lipid oxidation in food dispersions. **Trends Food Sci. Technol.** 22: 3–13.

Xie, Z., J. Huang, X. Xu and Z. Jin. 2008. Antioxidant activity of peptides isolated from alfafa leaf protein hydrolysate. **Food Chem.** 111: 370–376.

Zheng, H., I. Hwang and S. Chung. 2009. Enhancing polyphenol extraction from unripe apples by carbohydrate-hydrolyzing enzymes. **J. Zhejiang Univ. Sci. B.** 10: 912–919.