Ultrasound-Assisted Extraction of Phenolic Compounds From Brown Rice and Their Antioxidant Activities

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ABSTRACT

In this study, the phenolic compounds from brown rice from the Sung Yod area in Phatthalung province, southern Thailand were extracted by ultrasound-assisted extraction. The efficacy of the brown rice extract in stabilizing refined palm oil during accelerated storage was studied. It was found that the total phenolic content obtained by ultrasound-assisted extraction was 15.31% higher than the content obtained by conventional extraction. The optimal conditions using ultrasound-assisted extraction for the brown rice from Sung Yod Phatthalung were 60% volume per volume ethanol concentration, pH 6 and an extraction time of 25 min. The total phenolic acid and 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of the brown rice extract under these conditions were 1.30 mg ferulic acid equivalent per gram dry sample and 86.55%, respectively. The oxidation properties (peroxide value, PV and thiobarbituric acid reactive substances, TBARS) of the antioxidant from the brown rice extract in the refined palm oil were compared with other synthetic antioxidants—butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT)—at 60 °C. The data obtained indicated that the antioxidant activities of the brown rice extract was higher than those of the control and BHA but lower than for BHT. Moreover, the PV and TBARS values decreased with an increasing concentration of the brown rice extract in refined palm oil. The results revealed that the brown rice extract was a potential antioxidant for the stabilization of refined palm oil.

Keywords: phenolic compound, brown rice, antioxidant activity, refined palm oil, ultrasound-assisted extraction

INTRODUCTION

Rice (*Oryza sativa* L.) has been consumed for a long time in Asia, especially in China, Japan, Korea and many countries in Southeast Asia and recently, whole grain pigmented rice has been categorized as one of the potent functional foods since it contains high amounts of phenolic compound (Yawadio *et al*., 2007).

Phenolic compounds have become a research hotspot for their potential to reduce the risk of disease, such as by inhibiting platelet aggregation, reducing the risk of coronary heart disease and cancer, and preventing oxidative damage of lipids (Butsat and Siriamornpun, 2010). Most of the chemical additives previously used as antioxidants in the food industry were synthetic phenolic compounds, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate (PG). However, because of their probable toxicological effect together with the consumer preference for natural products, some

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synthetic antioxidants have been restricted by the imposition of a permitted dosage and subsequently, the use of natural products has become more and more popular (Soong and Barlow, 2004).

Cereal grains, especially rice, contain special phenolic acids (such as ferulic acid, *p*-coumaric acid and diferulate) that are not present in significant quantities in fruit and vegetables (Adom and Liu, 2002). Brown rice contains more nutritional components, such as dietary fiber, phytic acids, vitamins E and B and gamma aminobutyric acid (GABA), than ordinary milled rice; these biofunctional components exist mainly in the germ and bran layers which are removed by polishing or milling (Champagne *et al.*, 2004). Ferulic acid and *p*-coumaric acid have also been reported to be the major phenolic components in brown rice and exist in the form of free, soluble conjugated and insoluble bound compounds (Sawaddiwong *et al.*, 2008). However, there is no available literature on the efficient extraction of phenolic compounds from brown rice. In order to seek more environmentally friendly methods, decrease the solvent consumption, shorten the extraction time, increase the extraction yield and enhance the quality of extracts, various novel extraction techniques have been developed for the extraction of nutraceuticals from plants. For example, Tabaraki and Nateghi (2011) reported that ultrasound technology was used for the extraction of polyphenols and antioxidants from rice bran. The application of ultrasound-assisted extraction offers many advantages including the reduction of solvents, temperature, time and water consumption for extraction; for example, Wang *et al.* (2008) found that the optimum conditions for ultrasound-assisted extraction of phenolic compounds from wheat bran were: ethanol concentration, 64%; extraction temperature, 60 °C; and extraction time, 25 min. The experimental total phenolic content was 3.12 mg gallic acid equivalent per gram of wheat bran.

Many factors have been identified that influence the extraction efficacy, such as extraction methods, solvent type, solvent concentration, extraction temperature and extraction time (Pinelo *et al.*, 2005; Banik and Pandey, 2007; Silva *et al.*, 2007). Therefore, the aims of the current research were to evaluate the optimum ultrasound-assisted extraction conditions for brown rice extracts and to investigate the antioxidant activity among the brown rice extract and other synthetic phenolic compounds in refined palm oil during accelerated storage.

MATERIALS AND METHODS

Materials

Rice samples and preparation

Rice from Sung Yod Phatthalung (one of the recommended areas for rice cultivation in southern Thailand) was obtained from the Phatthalung Rice Research Center. These grains were milled to separate the husk from the brown rice. Then, the brown rice was ground and passed through a 0.25 mm sieve screen. The samples were analyzed for their proximate compositions according to Association of Official Agricultural Chemists (2000).

Chemicals and reagents

BHA and BHT were obtained from the Sigma Chemical Co., Bangkok Thailand. Ethanol and Folin-Ciocalteu's phenol reagent was obtained from Merck Bangkok, Thailand Ferulic acid, 2,2-diphenyl-1- picrylhydrazyl (DPPH) and 2-thiobarbituric acid were purchased from Fluka Analyticals, Bangkok, Thailand All chemicals and reagents were of analytical reagent grade. The refined edible palm oil used in this study was purchased from a local market in 2.5 L containers and was sold as being free of any synthetic antioxidant.

Methods

Ultrasound-assisted extraction and conventional extraction of phenolic compound

An amount of 5 g of two samples of brown rice (in triplicate) was accurately weighed

into separate 100 ml Erlenmeyer flasks and 100 mL of 60% volume per volume (v/v) ethanol was added. The first sample, which was from ultrasound-assisted extraction, was transferred to an ultrasonic bath (120 W, 45 kHz) at 30 °C for 25 min. The second sample, which was from conventional extraction, was stirred at a speed of 150 rpm, at 30 °C for 25 min. After the extraction, the extracts were filtered through Whatman number 1 filter paper and each solution was collected in a conical flask. The total phenolic contents of the brown rice extracts were determined using Folin-Ciocalteu reagent (Singleton *et al.*, 1999).

Selection of extraction solvent

An amount of 5 g of brown rice (in triplicate) was extracted with 100 ml of ethanol, isopropanol, acetone and hexane, respectively, in separate Erlenmeyer flasks and kept for sonication at 30 °C. After 25 min, the supernatant and the sediment were separated by vacuum filtration. The total phenolic contents of the brown rice extracts were determined using Folin-Ciocalteu reagent (Singleton *et al.*, 1999).

Effect of ethanol concentration on extraction of total phenolic compounds

An ethanol-water mixture was used as the extraction solvent and the phenolic compounds were extracted from the brown rice using ethanol concentrations at 40, 50, 60, 70 and 80% v/v. Then, 5 g of each sample (in triplicate) was macerated with the extracting solvents (100 mL) and sonicated for 25 min at pH 6.0 and a temperature of extraction of 30 °C. The extracts were filtered under vacuum and the filtrates were used for the determination of the total phenolic contents using Folin-Ciocalteu reagent (Singleton *et al.*, 1999).

Effect of pH on extraction of total phenolic compounds

An amount of 5 g of brown rice (in triplicate) was macerated with 60% v/v ethanol (100 mL) and sonicated at pH levels of 2, 4, 6 and 8 for 25 min and a temperature of extraction of 30 °C. The extracts were filtered under vacuum and the filtrates were used for the determination of the total phenolic contents using Folin-Ciocalteu reagent (Singleton *et al.*, 1999).

Effect of extraction time on extraction of total phenolic compounds

An amount of 5 g of brown rice (in triplicate) was macerated with 60% v/v ethanol (100 mL) and sonicated for 5, 15, 25, 35, 45 and 60 min, at pH 6.0 and a temperature of extraction of 30 °C. The extracts were filtered under vacuum and the filtrates were used for the determination of the total phenolic contents using Folin-Ciocalteu reagent (Singleton *et al.*, 1999).

The optimum conditions (ethanol concentration, pH and extraction time) were selected according to the maximum values of the total phenolic content using Folin-Ciocalteu reagent (Singleton *et al.*, 1999). Moreover, the DPPH radical scavenging activity of the brown rice extract under these conditions was determined according to the method reported by Brand-Williams *et al.* (1995).

Accelerated oxidation study

The brown rice extracts (0.02, 0.1, and 0.5% weight by weight, w/w), BHT and BHA (0.02% w/w) were added into the refined palm oil while stirring to ensure complete dissolution. All samples were kept in an oven at 60 °C for 12 d. The measurements of the concentration of the primary (peroxide value, PV) and secondary products (thiobarbituric acid reactive substances, TBARS) contents from the oxidation of the oil samples were determined at 3 d intervals over the 12 d of the storage period.

All determinations were carried out in triplicate and data were reported as mean ± SD.

RESULTS AND DISCUSSION

Proximate composition

The proximate composition of the Sung Yod brown rice variety from Phatthalung province is presented in Table 1. The results showed that the content of moisture, lipid, dietary fiber, ash and protein was 10.54 ± 0.10, 2.62 ± 0.04, 4.25 ±

Table 1 Proximate composition (%) of brown rice varieties.

Component	Chiang Phatthalung[a]	Sung Yod Phatthalung[b]	Sung Yod Phatthalung[c]
Moisture	13.47±0.24	9.28±0.06	10.54±0.10
Lipid	2.58±0.04	2.67±0.06	2.62±0.04
Dietary fiber	2.60±0.02	4.51±0.16	4.25±0.01
Ash	1.30±0.01	1.42±0.12	1.43±0.21
Protein	6.12±0.09	10.36±0.04	10.12±0.16

Values are means of triplicate determination ± SD.
[a] Sawaddiwong *et al*. (2008). [b] Sompong *et al*. (2011). [c] The current work**.**

0.01, 1.43 ± 0.21 and $10.12 \pm 0.16\%$, respectively, which agreed with the results from Sawaddiwong *et al*. (2008) and Sompong *et al*. (2011). Protein influences the nutritional quality of rice (Sompong *et al*., 2011). In this study the protein content was appreciably high (greater than 7%) and thus the brown rice is of interest for inclusion in food products. The total carbohydrate content was higher than 70%, and thus it is considered as a good source of energy (Sompong *et al*., 2011).

Effect of extraction methods on total phenolic content

The total phenolic content of the brown rice extracts obtained by the two extraction methods was determined by Folin-Ciocalteu assay and the results of this colorimetric method were expressed as milligrams of ferulic acid equivalent per gram dry sample (mg FAE per gram DW). A conventional extraction method was compared to the ultrasound-assisted extraction using the same conditions. The crude phenolic compounds present in the brown rice extracted by conventional and ultrasound-assisted extraction method were 1.11 ± 0.09 and 1.28 ± 0.05 mg FAE per gram DW, respectively. Thus, the phenolic content obtained by the ultrasound-assisted extraction method was higher than the conventional extraction method by 15.31%. Pingret *et al*. (2012) reported that ultrasound-assisted extraction of the total phenolic content from apple pomace after 40 min was 30% higher than the content obtained by conventional extraction.

Effect of extraction solvent on total phenolic compounds of brown rice

In this study, the efficiency was compared of ethanol, isopropanol, acetone and hexane on the extraction of the total phenolic contents as ferulic acid equivalent (FAE) from brown rice. Substantial differences in the total phenolic content were observed among the various solvent extracts as shown in Figure 1. The ethanol extracts contained the highest content of total phenolics, followed by the isopropanol, acetone and hexane extracts. These results were similar to those achieved by Wang *et al*. (2008). In addition, ethanol is less toxic and can be easily recovered by reduced pressure distillation (Wang *et al*., 2008). Therefore, ethanol was used as the extraction solvent in the following study.

Effect of ethanol concentration on total phenolic compounds of brown rice

Ethanol-water mixtures were used as the extraction solvent in the study. The effects of the ethanol concentration in the extraction solvent on the content of the phenolics in the brown rice extracts are shown in Figure 2. When the ethanol concentration increased from 40% to 60% v/v, the total phenolic content of the extracts increased from 1.12 to 1.31 mg FAE per gram DW. When the ethanol concentration reached 70% v/v, the total phenolic content in the brown rice extracts decreased and at a concentration of 80% v/v, the total phenolic content was 1.03 mg FAE per gram DW. In general, the polarity of the

ethanol-water mixture increased continuously with the addition of water to the ethanol. More polar phenolic compounds may be extracted according to the "like dissolves like" principle (Tabaraki and Nateghi, 2011). Thus, it could be seen that more phenolics were extracted using 60% v/v ethanol than by using 80% v/v ethanol. A similar effect was reported in the extraction of phenolic antioxidants from peanut skins (Neptoe *et al*., 2005), wheat bran (Wang *et al*., 2008), brown rice (Sawaddiwong *et al*., 2008), and rice bran (Tabaraki and Nateghi, 2011). Therefore, 60% v/v ethanol was selected for further study to extract the phenolic compounds from the brown rice.

Effect of pH on extraction of total phenolic compounds

The influence of pH on the total phenolic compounds of the brown rice extracts was studied over a pH range from 2 to 8 with 60% v/v ethanol as the extraction solvent and an extraction time of 25 min. The effects of pH on the content of the phenolics in the brown rice extracts are shown in Figure 3. When the extraction pH increased from 2 to 6, the total phenolic content of extracts increased from 0.49 to 1.25 mg FAE per gram DW. When the pH reached 8, the total phenolic content in the brown rice decreased. Therefore, extraction of the phenolic compounds appeared to be pH dependent.
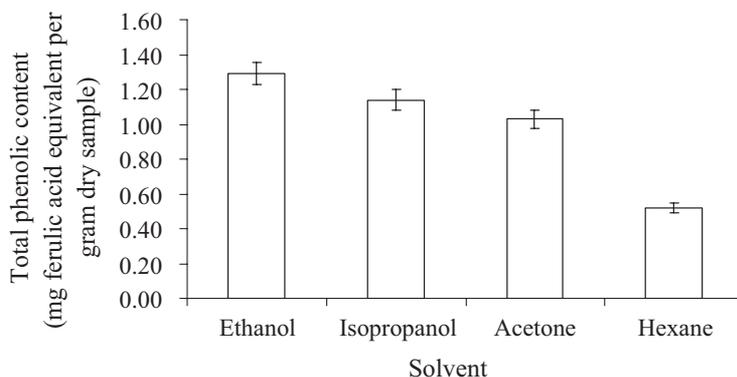

Figure 1 Content of total phenolic compounds of brown rice extracted using various solvents. The vertical bars represent the standard deviation (n = 3). Extraction conditions under ultrasound-assisted extraction: pH 6.0; extraction time 25 min; extraction temperature 30 °C.
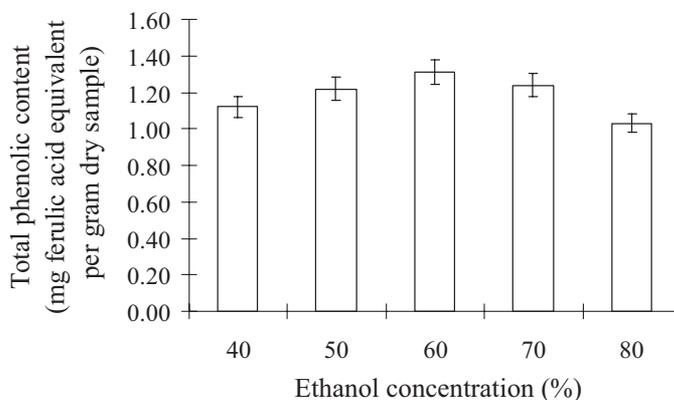

Figure 2 Effect of ethanol concentration on the total phenolic content from brown rice. The vertical bars represent the standard deviation (n = 3). Extraction conditions under ultrasound-assisted extraction: pH 6.0; extraction time 25 min; extraction temperature 30 °C.

This could be explained by considering that the highest stability of phenolic compounds occurs in a weak acid solvent (pH 6) but phenolic compounds are unstable at other pH levels. A similar effect was reported using solvent at various pH levels in the extraction of phenolic compounds from black glutinous rice (Tananuwong and Tewaruth, 2010) and various yam cultivars (Chen *et al*., 2008) where the total phenolic contents were the highest at pH levels of 6.8 and 5, respectively.

Therefore, a pH of 6 was selected for further study to extract the phenolics from the brown rice.

Effect of extraction time on extraction of total phenolic compounds

The contents of the total phenolic compounds extracted from the brown rice at different times of sonication are presented in Figure 4. A substantial increase in the total
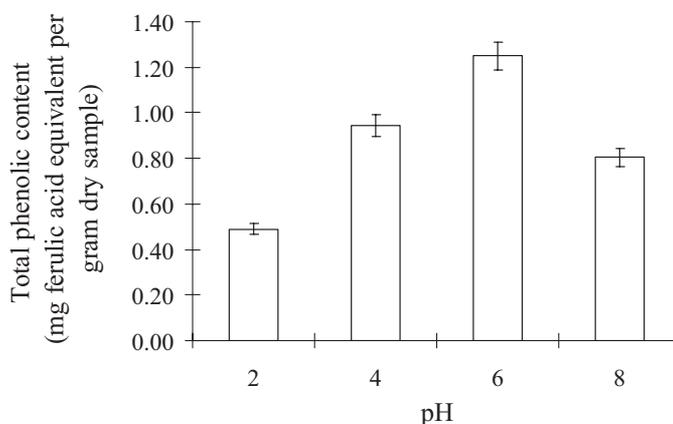

Figure 3 Effect of pH on the total phenolic content from brown rice. The vertical bars represent the standard deviation (n = 3). Extraction conditions under ultrasound-assisted extraction: ethanol concentration 60% volume per volume; extraction time 25 min; extraction temperature 30 °C.
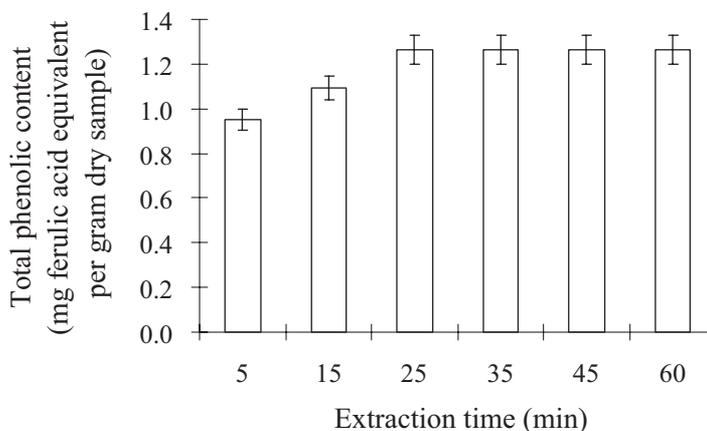

Figure 4 Effect of extraction time on total phenolic content from brown rice. The vertical bars represent the standard deviation (n = 3). Extraction conditions under ultrasound-assisted extraction: ethanol concentration 60% volume per volume; pH 6.0; extraction temperature 30 °C.

phenolic content was observed over the extraction time (5–60 min), and the total phenolic content reached a maximum of approximately 1.26 mg FAE per gram DW. Mass transfer controls the solvent extraction of any component from a plant matrix; when the solvent saturates the extracted compound, the concentration gradient becomes null and the phenomenon stops (Rodrigues *et al.*, 2008). In the ultrasound extraction of phenolic acid from the brown rice, the mass transfer stopped after 25 min and the process can then be interrupted.

The results obtained from these experiments showed that the optimum extraction conditions for extracting the total phenolic content from the brown rice extracts were obtained by ultrasonic-assisted extraction at 60% v/v ethanol concentration, pH 6.0 and 25 min extraction time which produced the maximum total phenolic content (1.30 mg FAE per gram DW) and DPPH radical scavenging activity (86.55%).

Accelerated oxidation studies

Effect of addition of antioxidants on peroxide value of refined palm oil

The peroxide value (PV) is often used as a measurement of the oxidative deterioration of oil, fat and fatty acids; hence, the efficacy of an antioxidant extract to prevent the onset of oxidation could be predicated by the rate of formation of these primary oxidation products. The effect of the addition of antioxidants on PV is shown in Figure 5. From the results obtained, the PV of refined palm oil samples with additives was in the range 9.19-14.54 milliequivalent $(mEq).kg^{-1}$ for stabilized samples after storage for up to 12 d, while the maximum PV value for the control (without antioxidant added) sample was 16.16 $mEq.kg^{-1}$. The addition of antioxidant decreased the PV of the refined palm oil. At all stages, the highest PV was observed for the control sample followed by BHA, brown rice extract and BHT at 0.02% w/w of an antioxidant extract, respectively. Brown rice, at all concentrations, controlled peroxide value appreciably, revealing a good antioxidant efficacy in the stabilization of the oil. A regular increase in the PV as a function of storage time was observed for all the samples at all intervals. Moreover, in Figure 5 shows that increasing the brown rice extract concentration
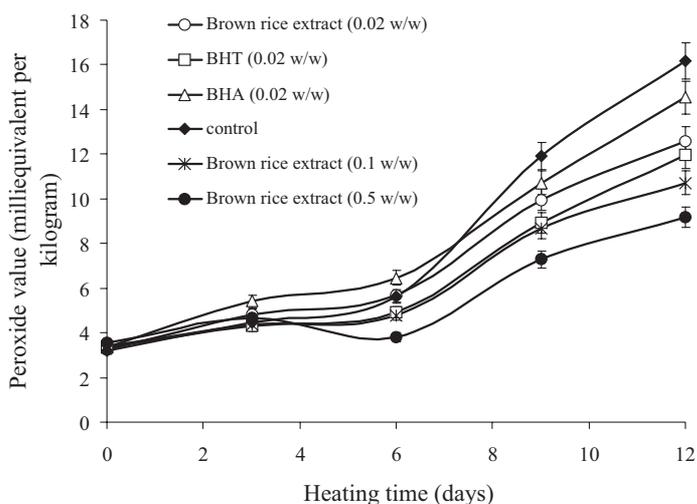

Figure 5 Effects on peroxide value of refined palm oil at 60 °C of addition of antioxidants—butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and brown rice extract—at different concentrations. (Error bars show SD.)

substantially reduced oil oxidation. Moreover, the maximum PV content of the refined palm oil with 0.5% w/w brown rice extract was 9.19 mEq.kg^{-1}, which was less than for the other extracts indicating that 0.5% w/w brown rice extract was the most effective antioxidant, as was reported by Iqbal and Bhanger (2007) who studied the stabilization of sunflower oil (SFO) by garlic extract during accelerated storage. In that study, the methanolic extract of garlic at three different concentrations of 250 (SFO-250), 500 (SFO-500) and 1,000 ppm (SFO-1,000) was added to preheated refined sunflower oil. It was reported that the PV of the SFO containing antioxidants was lower than that of the SFO without antioxidants. The highest PV was observed for the control sample followed by SFO-250, SFO-500, SFO-BHA, SFO-1,000 and SFO-BHT, respectively.

Effect addition of antioxidants on thiobarbituric acid reactive substances of refined palm oil

TBARS measures the formation of secondary oxidation products (such as aldehydes or carbonyls) which may contribute to the off-flavor of oxidized oils (Iqbal and Bhanger, 2007). TBARS for all the samples was determined for

up to 12 d of storage under accelerated conditions (Figure 6). From the results, the TBARS value of the refined palm oil samples with additives was in the range 1.89-2.65 mg malondialdehyde (MDA) per kilogram oil for the stabilized samples after storage for up to 12 d, while the maximum TBARS for the control (without antioxidant added) sample was 2.84 mg MDA per kilogram oil. The addition of antioxidant decreased the TBARS value of the refined palm oil. At all stages, the highest TBARS was observed for the control sample followed by BHA, brown rice extract and BHT at 0.02% w/w of an antioxidant extract, respectively. Moreover, in Figure 6, the TBARS values of the refined palm oil samples with additives were in the range of 1.89–2.65 mg MDA per kilogram oil for the stabilized samples and 2.84 mg MDA per kilogram oil for the control sample after 12 d of storage. The brown rice extract inhibited the formation of TBARS at all concentrations. Tananuwong and Tewaruth (2010) reported that the addition of dried black glutinous rice crude extract at 500 and 1,000 mg.kg^{-1} (oil weight basis) could retard an increase in TBARS. A higher concentration of the extract showed greater antioxidant activity.
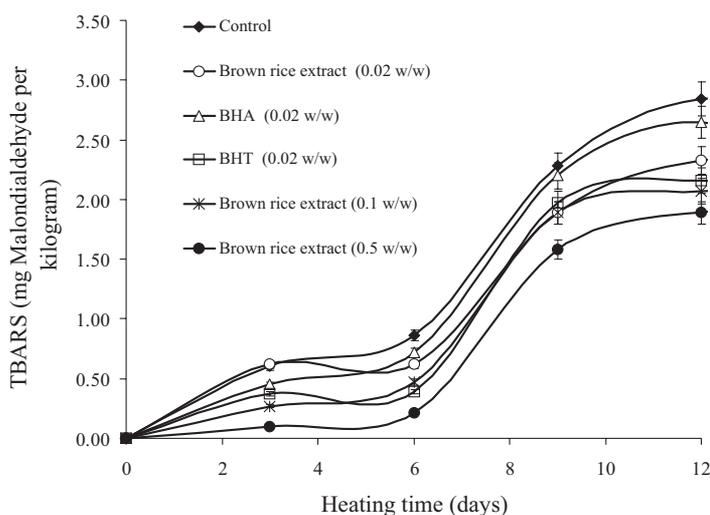

Figure 6 Effects on thiobarbituric acid reactive substances (TBARS) of refined palm oil at 60 °C of addition of antioxidants at different concentrations. (Error bars show SD.)

CONCLUSION

The results of this study indicated that an ultrasound-assisted extraction was more effective than the conventional extraction method in the extraction of phenolic acid from the brown rice. The total phenolic acid and DPPH radical scavenging activity of the brown rice extract under the optimal conditions (60% v/v ethanol concentration, pH 6 and extraction time 25 min) were 1.30 mg FAE per gram DW and 86.55%, respectively. The addition of 0.02, 0.1 and 0.5% w/w of butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and the brown rice extract to the refined palm oil resulted in the retardation of the oxidation of the oil when stored at 60 °C for 12 d. The extent of oxidation was shown by determination of the peroxide value (PV) and thiobarbituric acid reactive substances (TBARS). The antioxidant activities of the brown rice extract were higher than those of the control and BHA but lower than BHT. Moreover, the PV and TBARS values decreased with an increasing concentration of the brown rice extract in the palm oil.

ACKNOWLEDGEMENTS

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