Australian Journal of Basic and Applied Sciences, 3(4): 3642-3652, 2009
ISSN 1991-8178

Effects of Ohmic Heating on Lipase Activity, Bioactive Compounds and Antioxidant Activity of Rice Bran

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Abstract: The study aimed to investigate the effect of stabilisation using ohmic heating (OMH) on lipase activity, bioactive compounds and antioxidant activity of rice bran. The moisture content of rice bran samples was adjusted to 20, 30 and 40 %. Alternative current electricity (50Hz), with three levels of electrical field strengths (EFS) of 75,150 and 225 Vcm^{-1} were applied, in order to stabilise the rice bran. Lipase activity, free fatty acid (FFA), and thiobarbituric acid reactive substances (TBARS) of stabilised rice bran during storage at 4°C were investigated. The influence of OMH on the concentration of phenolic compound, α-tocopherol, γ-oryzanol and the antioxidant activity of samples were also evaluated. The results indicated that OMH was an effective method for stabilisation of rice bran with moisture addition. According to this study, the EFS at 150-225 Vcm^{-1} and MC of rice bran 30-40 % were the optimum conditions to retard the increasing of FFA content, lipase activity and lipid oxidation during storage. Moreover, the stabilised rice bran using OMH yielded the highest levels of phenolic compound, α-tocopherol, γ-oryzanol, and antioxidant activity.

Key words: Ohmic heating, rice bran, lipase activity, antioxidant activity

INTRODUCTION

Rice bran is the outer layer of the rice kernel and a by product of the rice milling process. Several studies have reported that rice bran is an excellent source of nutrients and bioactive compounds including proteins, vitamins, dietary fibers, tocopherols, tocotrienols and γ-oryzanol (Qureshi *et al.*, 2002; Ryynanen *et al.*, 2004; Ausman *et al.*, 2005; Minhajuddin *et al.*, 2005; Imsanguan *et al.*, 2008). Rice bran is also a satisfactory source of fat with a range between 12-20% (Marshall and Wadsworth, 1994). Since rice bran contains a high fat content, rapid deterioration of crude fat by lipase immediately occurs, following the milling process and yields free fatty acids (FFA) and glycerol. The fat hydrolysis causes the rice bran unsuitable for human consumption and lowers the oil yield (Lai *et al.*, 2005). Therefore, immediately after the milling process, inactivation of lipase and inhibition of the formation of FFA are necessary. The process that produces stable rice bran through the deactivating of enzymes which inhibit the deterioration of rice bran is called stabilisation. Various stabilisation methods, applied to protect rice ban oil degradation, have been reported such as steaming (Juliano, 1985; Azrina *et al.,* 2008), extrusion (Kim *et al.*, 1987), microwave heating (Lakkakula *et al.*, 2004); (Ramezanzadeh *et al.*, 2000) and ohmic heating (Lakkakula *et al.*, 2004).

Ohmic heating or Joule heating is a food processing method in which an alternating electrical current is passed through a food sample. Ohmic heating is distinguished from other electrical heating method by the presence of electrodes contained in the food; the frequency and the waveform of the electric field impose between the electrodes. Most foods contain ionic species such as salts and acids, therefore, electric current can be made to pass through food and generate heat inside it (FDA, 2000). The production of an inside-out heating pattern makes ohmic heating much faster than conventional outside-in heating. With proper formulation, electrical energy can be converted to heat uniformly in food matrices. The efficiency of OMH is dependent on the conductive nature of the food to be processed (Zoltai and Swearingen, 1996). As a result, OMH provides rapid and uniform heating and a high quality product with minimal changes of structure, nutrition, or organoleptic. Moreover, the use of OMH for food processing is cleaner and more environmentally friendly. Potential applications for OMH include blanching, evaporation, dehydration, fermentation, and extraction.

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Several studies have investigated a number of aspects for the application of OMH within the food industry, for example, its potential to increase dye diffusion in beet (Halden *et al.,* 1990), extraction of fruit juices (Lima and Sastry, 1999; Wang and Sastry, 2000) and the enhancement of the drying process of sweet potato (Zong and Lima, 2003). Lakkakula *et al.,* (2004) reported that ohmic heating was an alternative method for stabilisation of rice bran because it increased the oil yield of rice bran to a maximum of 92%, whilst only 53% of oil was extracted from the control samples. There have not been any investigations undertaken on the effect of ohmic heating on the stability of lipase, the level of phytochemicals and antioxidant activity of rice bran. Therefore this study was carried out to investigate the effect of ohmic heating on lipase activity, to evaluate the effect of ohmic heating on phytochemicals and antioxidant activity rice bran obtain from different ohmic heating conditions.

MATERIALS AND METHODS

Chemicals:

Standard α-tocopherol was purchased from Sigma-Aldrich Chemical Co., (St. Louis, Mo, USA). HPLC grade methanol, acetonitrile, hexane, ethyl acetate and ethanol were purchased from BHD (Poole, UK). Gamma oryzanol standard was purchased from Tsuno Food Industrial Co., Ltd. (Wakayama, Japan) Gallic acid, 1,1-diphenyl-2- picrylhydrazyl (DPPH) , bythylatedhydroxyanisole (BHA) and buthylatedhydroxytoluene (BHT) were obtained from Fluka Chemical (Buchi, Switzerland). All chemicals and reagents were analytical grade.

Rice Bran Preparation:

Rice bran (*Oryza sativa* L. CV. RD-6 samples were purchased from rice milling factory in Mahasarakham province. Freshly milled rice bran was immediately passed through a 20 mesh sieve to remove broken rice and husks. The initial moisture content of rice bran was determined (AOAC, 2002) before subjecting to stabilisation.

Rice Bran Stabilisation Using Ohmic Heating:

The moisture content of the samples was adjusted to three different levels: 20, 30 and 40 % by adding deionized water (Ramezanzadeh *et al.,* 2000) and then they were heated by ohmic heating. This stabilisation process of rice bran was finished within 12 h. The ohmic heating unit was a lab-scale ohmic heater equipped with titanium electrodes and enclosed on a Teflon tee. The 180g of rice bran sample was placed between the electrodes. An alternating current of 50 Hz with three different levels of electrical field strengths (EFS) (75, 150, and 225 Vcm^{-1}) was applied. A 1.5 KW capacity dielectric heater operating at 13.56 MHz was used. Two electrodes, size 40x30 cm, were connected to the dielectric heater. The bottom electrode was made from 20 gauge aluminum sheet, whilst the top electrode was made from 20 gauge aluminum wire mesh, which facilitated the escape of moisture from the sample through the top electrode during heating. A sample holder, size 14.4x14.4xl.5 cm, was fabricated out of 3 mm thick Teflon sheet. Teflon was used since it has a very low dielectric loss factor of 0.08 (Sreenarayanan and Chattopadhyay, 1986).) and hence it absorbs practically no energy from a radio frequency electric field. The top electrode of the dielectric heater was placed above the sample holder on Teflon spacers, leaving an air gap of 0.5 cm above the surface of the sample. The average electric field intensity applied was 0.5 K Vcm^{-1}. The voltage, current and temperature were continuously measured by using a data logger. The sample was removed from the heater after it reached the maximum temperature and it was cooled to room temperature before placing in the sample bag (polyethylene). The rice bran which was stabilised by steaming method at 105°C for 15 min (Juliano, 1985) and raw rice bran served as control. The stabilised samples were stored at -20°C upon analysis.

Determination of Effect of OMH on Rice Bran Characteristics:

The stabilised rice bran and raw rice bran were stored at 4°C. FFA, TBARS, and lipase activity measurement was made after storage for 0, 7, 14 and 21 days.

Determination of Lipase Activity:

Sample Extraction:

The sample extraction was undertaken by following the method reported by Prabhu *et al.,* (1999) with some modifications: rice bran (5g) was defatted by stirring for 30 min with 30 mL of n-hexane. The defatted bran was allowed to air-dry for 1 h, so that practically all hexane was removed and then 30 mL of 0.5mM $CaCl_2$ in 50mM phosphate buffer pH 7.0 was added. The defatted bran was stirred at 10ºC for 30 min. The

suspension was then centrifuged at 3000 rpm for 15 min at 4ºC. The supernatants were collected as the crude lipase extract and applied for lipase activity measurement.

Enzyme Assay:

Lipase activity was measured against *p*-nitrophenyl-butyrate (*p*-NPB) and *p*-nitrophenyl-laulate (*p*-NPL) substrates. The assay was accomplished by following the method described by Hatzinikolaou *et al.,* (1999). Solution A: 40 mg of *p*-NPB or *p*-NPL were dissolved in 12 mL isopropanol solution. Solution B: 0.4g Triton X-100 and 0.1g gum Arabic were dissolved in 90 mL of 0.1M potassium phosphate buffer (pH 7.0). The substrate solutions were prepared by dropwise addition of 0.3 mL solution A (*p*-NPB) or 0.2 mL solution A (*p*-NPL) into 3.0 mL solution B under intense mixing (vortex). These emulsions were stable for 1 h at room temperature. For the *p*-NPB substrate system, 0.1 mL of crude lipase extract was added to 3.3 mL of substrate solution and the reaction rate after incubating at 35°C for 5 min was measured by spectrophotometer at 410 nm. For the *p*-NPL substrate system, 0.1 mL of crude lipase extract was added to 3.2 mL of the corresponding substrate solution and the mixture was incubated in shaking water-bath at 35°C for 20 min. The reaction was terminated by boiling for 5 min following centrifugation 6000 rpm for 10 min the absorbance of the clear supernatant was measured at 410 nm. In both cases, a sample without enzyme taken the same treatment was used as a blank. One unit (U) of enzyme activity was defined as the amount of enzyme required for the liberation of 1 µmol p-NP per minute under the assay conditions.

Determination of Free Fatty Acid (FFA):

The FFA value was determined by AOCS official method (Ca 5a-40 procedure) (AOCS, 2004). FFA was calculated as oleic acid and expressed as percentage of the total lipids

Determination of Thiobarbituric Acid Reactive Substances (TBARS):

The extent of lipid oxidation was monitored by the formation of TBARS. The TBARS value was determined every seven day of storage by following the method reported by Mielnik *et al.,* (2003) with some modifications 5g of rice bran samples with 30 mL of 7.5% aqueous solution thichloroacetic acid (TCA) was sonicated for 20 min using sonicator (Vibracell VC 130, Sonics, USA). The slurry was centrifuged at 3600 rpm for 15 min. The supernatant (5.0 mL) of upper layer was collected and mixed with 5.0 mL of 20 mM aqueous thiobarbituric acid (TBA) in a test tube. The samples were incubated at 100 ºC for 35 min in a water-bath and cooled for 10 min in cold water. Absorbance was measured at 532 nm by spectrophotometer (Spectronic Genesys 5, USA) against a blank containing 5.0 mL distilled water and 5.0 mL TBA reagent. The results expressed as milligram malondialdehyde per kilograms of rice bran were calculated from the standard curve of 1, 1, 3, 3-tetraethyoxypropane (TEP).

Determination of Bioactive Compounds:

Determination of Total Phenolic Content (*Iqbal *et al.,* 2005*):

The reaction was initiated by mixing 0.2 mL of appropriate diluted rice bran extract, 0.8 mL of freshly prepared diluted Folin-Ciocaltue reagent and 2 mL of 7.5% sodium carbonate. The volume of the resulting mixture was adjusted to 7 mL by deionized water and placed in the dark for 2 h to ensure completion of the reaction. The absorbance of the resulting blue-colored mixture was measured at 765 nm by spectrophotometer (Shimazu, Japan). Gallic acid was used as the calibration standard and the results were calculated as gallic acid equivalent (mg) per gram (g) of bran.

Determination of α-Tocopherol and γ- Oryzanol* (Chen and Bergman, 2005; Azrina *et al.*, 2008)*:

Rice bran (1g) was extracted by following the method reported by (Azrina *et al.*, 2008). Prior to HPLC analysis, the extracts were filtered through a 0.45 mm syringe filter. An analysis of γ- oryzanol and α-tocopherol was performed, using the reversed phase high performance liquid chromatography (RP-HPLC), according to the method reported by Chen and Bergman (2005), with some modifications. The Shimadzu HPLC system (model L-6200A), equipped with a Photo diode array detector (Shimadzu, Japan) and a computer system, was applied. Detection was operated at 292 and 325 nm, simultaneously. The spectra, from 250 to 600 nm, were recorded for all peaks. The extracted samples were injected through a guard-column and separated on a C_{18} column (4.60 x 150mm, 4 µm) (Phenomenex, USA). Gradient elution was then applied. Mobile phases A, B, and C were methanol, water and buthanol, respectively. The gradient was as follows: 0-12 min 92% A, 4% B and 4% C: 12-25 min linear gradient, from 4% B to 3 % B and 4% C to 5 % C, with flow rate of 1.5 mL /min and injection volume of 20 mL. The α-tocopherol was detected at 292 nm and γ-oryzanol

was detected at 325 nm. Chromatograms were recorded and peak areas were used to calculate the content of γ-oryzanol and a-tocopherol, against the standard curve of standards.

Determination of Antioxidant Activity:

6.1 Extraction of Rice Bran:

Finely ground bran (5.0g) was extracted in 80% methanol (25 mL) by placing the mixture in a sonicator for 10 minutes. The mixture was filtered and residue was then subjected to the same procedure twice. The residue was extracted with 0.15 mol/L hydrochloric acid and the extracts were combined and filtered through filter paper, evaporated to dryness under reduced pressure at 45ºC by a rotary evaporator (Buchi, Switzerland) and used for antioxidant activity analysis.

Antioxidant Activity:

Antioxidant activity was evaluated using three different methods:

DPPH Radical Scavenging Activity:

To evaluate the free radical scavenging activity, the extract was allowed to react with a stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) by following the method described by Dasgupta and De (2004). The methanolic extract (sample extract, 0.1 mL) was added to 3 mL of the 0.004% methanol solution of DPPH. Absorbance at 517 nm was measured after 30 min and the percent inhibition activity was calculated as $(A_C\text{-}A_S) / A_C) \times 100$ (A_C = Absorbance without extract; A_S = Absorbance with extract). The results were expressed as the concentration of test compound to inhibit 50% of free radical (IC_{50}).

Lipid Peroxidation Assay:

Lipid peroxidation was used to measure the lipid peroxide formed in egg yolk homogenate as lipid-rich media ((Dasgupta and De, 2004). Egg yolk homogenate (0.5 mL of 10% v/v) and 0.1 mL of extract were added to a test tube and made up to 1.0 mL with distilled water. Subsequently 0.05 mL of 0.07M FeSO4 was added to induce lipid peroxidation and the mixture was incubated at room temperature for 30 min. The 1.5 mL of 20% acetic acid (pH 3.5) and 1.5 mL of 0.8% (w/w) thiobarbituric acid in 1.1% sodium dodecyl sulphate were combined. The resulting mixture was vortexed and heated at 95°C for 60 min. After cooling, 5.0 mL of butanol were added to each tube and centrifuged at 3000 rpm for 10 min. The absorbance of supernatant was measured at 532 nm. Inhibition of lipid peroxidation was expressed as IC_{50} values.

Total Antioxidant Capacity Assay:

Antioxidant activity was determined by total antioxidant capacity (Dasgupta and De, 2004). 0.3 mL of the extract was mixed with 3.0 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated at 95°C for 90 min. After the mixture had cooled at room temperature, the absorbance was measured at 695 nm against a blank. The antioxidant activity is expressed as the number of equivalents of ascorbic acid, gallic acid, tocopherols and butylates hydroxytoluene.

Statistical Analysis:

The means and standard deviations of FFA content, TBARS values, lipase activity and antioxidant activity were reported in triplicate determinations for each sample. Two-way analysis of variance (ANOVA) in completely randomize design (CRD) using 3×3 factorial was used to test significant differences of moisture content and the levels of electrical field strengths. Multiple comparison tests were performed by using Duncan multiple range tests to determine the significant difference between treatments. Statistical significance was declared at $P < 0.05$.

RESULTS AND DISCUSSION

Electrical Resistance and Conductivity of Rice Bran:

Rice bran samples with different levels of moisture content were subjected to measure the electrical resistance and conductivity. The results showed that rice bran samples with higher moisture content had less electrical resistance whereas the electrical conductivity was high in higher moisture content samples as indicated in Table 1. This demonstrates that electrical conductivity is a function of food components. Ionic components and moisture mobility increase electrical conductivity, whilst lipids and fats decrease it (Bengston *et al.,* 2006).

Table 1: The electrical resistance and conductivity of rice bran with different moisture contents.

Moisture content(%) of rice bran	Electrical resistance(ohm)	Electrical conductivity($S \cdot m^{-1}$)
8.10*	19.00 ± 0.04	0.21 ± 0. 003
20	10.40 ± 0.01	0.39 ± 0. 003
30	0.42 ± 0.01	0.97 ± 0.003
40	0.16 ± 0.01	5.4 ± 0.025

*Moisture content of rice bran before adding with water
Values are means of triplicate samples

Effect of Applying OMH on Temperature Changes of Rice Bran:

The fresh bran was placed in the OMH unit and the temperature changes were tracked by a data locker placed in the core of the unit. The rice bran sample with the higher moisture content indicated a higher of temperature (Fig.1). The rice bran sample, with 40% moisture content and electric field strength of 225 volt/cm, showed the most rapid change in temperature and the highest temperature of 124.3°C. The results were similar to those in a study by Yongsawatdigul *et al,.* (1995); Lakkakula, *et al.,* (2004). However, the increase of temperature was dependent upon several factors such as the physical property of the food, chamber area and distance between electrodes (Castro *et al*., 2004). The increase in temperature was related to that of the electric field strength, linearly, as shown in Fig. 1 and Table 1. The electrical conductivity was linearly correlated with temperature when the electrical field was sufficiently high (at least 60 VCm^{-1}) (Bengston *et al.,* 2006)

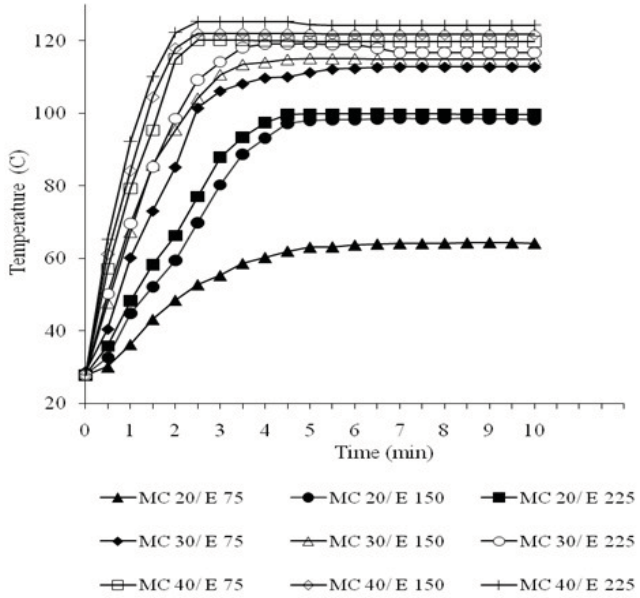

Fig. 1: The temperature changes of rice bran affected by different conditions of OMH (MC20/E75-MC40/E225 = Rice bran with moisture content of 20, 30 and 40% combined with electrical field strength of 75, 150 and 225 Vcm-1; Values are means of triplicate samples)

The Effect of Different Stabilisation Conditions on Lipase Activity:

Lipase is an endogenous of rice bran and it is highly effect on rice bran shelf life, because there is decomposition of lipids in the bran into free fatty acid by this enzyme. In order to measure the rice bran lipase activity, the substrate specificity, p-Nitrophenyl-Butyrate (p-NPB) and p-Nitrophenyl-Laulate (p-NPL) were used. The results are shown in Fig 2 and 3, respectively. It is clear that the raw rice bran indicats the highest lipase activity. The results were supported by Icier *et al.,* (2006) who used ohmic heating (50 Vcm^{-1}, 54 s) to obtain the effective peroxidase inactivation of pea puree. (Castro *et al.*, 2004) showed that the electric field has a significantly effect on the lipoxygenase (LOX) inactivation with approximately 5 times lower *D* values than that of conventional heating (water bath). However, they also found that some enzyme such as pectinase and β-galactosidase (β-GAL) were not affected by the presence of the electric field. The presence of an electric field can influence biochemical reactions by changing molecular spacing and increasing interchain reactions. Moreover, the presence of the electric field may remove the metallic prosthetic groups present in the enzymes LOX and PPO thus causing the enhancement of activity loss (Castro *et al.*, 2004).

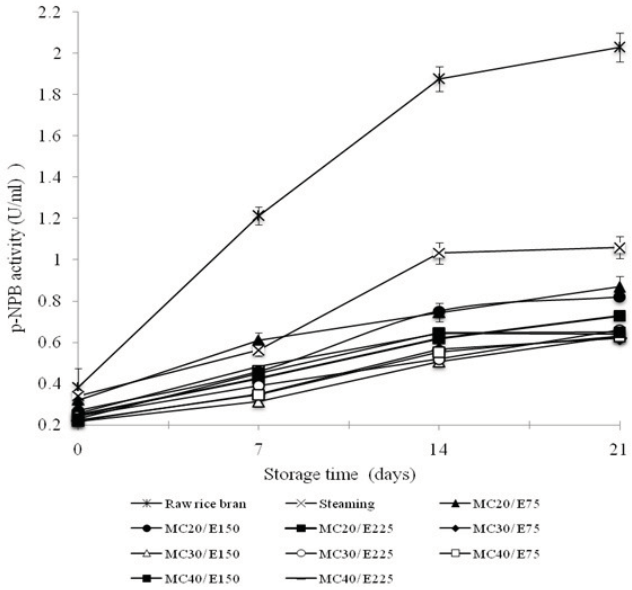

Fig. 2: Lipase activity of stabilised rice bran stored for 21 days at 4°C using p-NPB as a substrate(MC20/E75-MC40/E225 = Rice bran with moisture content of 20, 30 and 40% combined with electrical field strength of 75, 150 and 225 $Vcm^{-}1$; Values are means of triplicate samples)

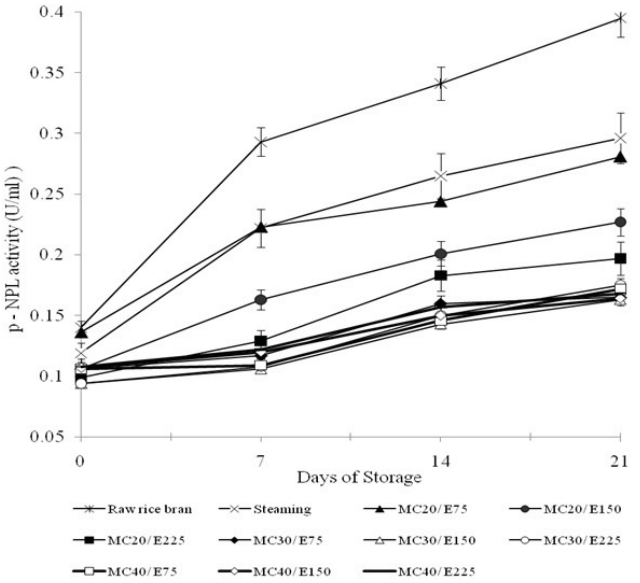

Fig. 3: Lipase activity of stabilised rice bran stored for 21 days at 4°C using p-NPL as a substrate (MC20/E75-MC40/E225 = Rice bran with moisture content of 20, 30 and 40% combined with electrical field strength of 75, 150 and 225 $Vcm^{-}1$; Values are means of triplicate samples)

Effect of OMH on the Change of Free Fatty Acid:

The influences of ohmic heating on bran stability as determined by the change in FFA are indicated in Fig. 4. The FFA concentration of raw rice bran was significantly higher than those of stabilised rice bran when storage time increased. The FFA content of raw rice bran was more than 10% after storage for 21 days whereas the FFA content of rice bran, stabilised at different conditions, slightly increased to approximately 3%. Therefore, the stabilised rice bran was still suitable for human consumption, according to (Tao *et al.*, 1993), but the bran with over 5% FFA is considered unsuitable for human consumption. The rate of FFA formation

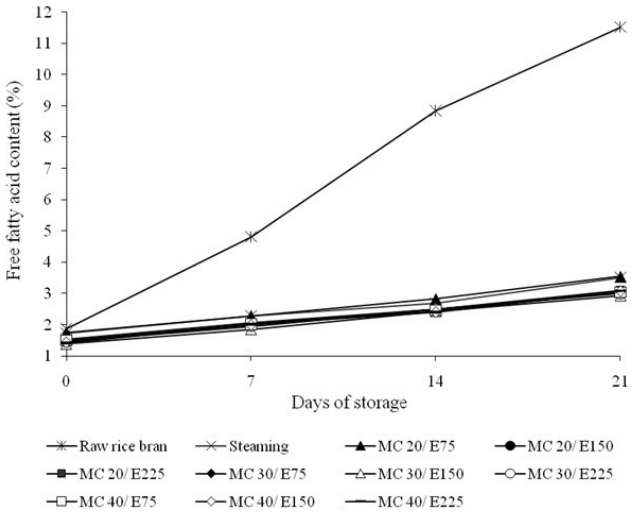

Fig. 4: Effect of ohmic heating on free fatty acid (FFA) content (%) of rice bran during storage at 4°C (MC20/E75-MC40/E225 = Rice bran with moisture content of 20, 30 and 40% combined with electrical field strength of 75, 150 and 225 Vcm-1; Values are means of triplicate samples)

was over 5 fold, in unstabilised rice bran, whilst that of stabilised rice bran was approximately 2 fold within 21 days under study condition (4ºC). The content of FFA was positively related to the activity of lipase, as indicated in Fig. 2 and 3. OHM could retard the forming of FFA compared with raw rice bran and steamed rice bran. However, there was no significant difference between the rice bran samples treated with different electric field strengths and moisture contents. These results are supported by (Lakkakula *et al.*, 2004), who showed that the FFA content in untreated bran stored at 4°C for 6 weeks increased from 3.96% to 18.03% whilst the FFA of ohmic heated bran samples (21% moisture content) , which were stored identically as the untreated bran increased from 3.25% to 5.54%. Sreenarayanan and Chattopadhyay (1986) obtained an increase in FFA from 4.2% to 6.2% during a six week cold storage period by using dielectric heating (0.5 kV/cm, 13.56 MHz) to treat rice bran with moisture content of 21%.

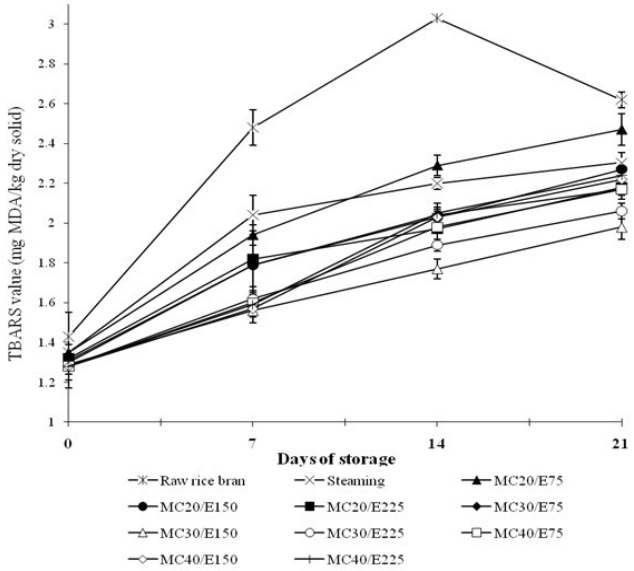

Fig. 5: The effect of ohmic heating on TBARS values of rice bran stabilised (malondialdehyde (mg)kg-1 of rice bran) (MC20/E75-MC40/E225 = Rice bran with moisture content of 20, 30 and 40% combined with electrical field strength of 75, 150 and 225 Vcm-1; Values are means of triplicate samples)

Effect of OMH on Thiobarbituric Acid Reactive Substances (TBARS):

The TBARS values for ohmically heated and steaming stabilised rice bran samples as compared to the raw rice bran are displayed in Fig.5. In the raw rice bran, the TBARS values showed the most rapid increased and reached their maximum at 14 days of storage. This could be due to (in the raw rice bran) lipase still being active and hydrolyses lipids in rice bran freeing fatty acids and thus being ready for oxidation. The values for TBARS, of raw rice bran, were increased up to 2 weeks of storage and then decreased. This may due to some TBARS is developed by the lipid oxidation to other oxidation products (Ryu and Cheigh, 1980). The lowest TBARS values were observed in the rice bran with a moisture content of 30% and being ohmically heated by an electric field strength at 150 Vcm^{-1} with the level of 1.98 mg malondialdehyde (MDA)kg^{-1} dry solid after 21days. This may be due to the fact that, in this condition, the heat could penetrate and effectively destroyed lipase and it combined with the prevention effect of rice bran to retard the development of oxidation product in rice bran. The stabilised rice bran can reduce lipid oxidation because it contains a number of antioxidants and this result could also indicate by the antioxidant results and bioactive compounds of rice bran as shown in next section of the paper. The antioxidant activity of compounds is often described by its ability to delay the onset of autoxidation by scavenging reactive oxygen species, or the ability to act as chain breaking antioxidants to inhibit the propagation phase of lipid autoxidation (Yuan *et al.,* 2005).

Effect of OMH on Total Phenolic Compounds, α-Tocopherol and γ-Oryzanol Content:

Results showed the total phenolic content ranging from 1.79 to 3.28 mg gallic acid equivalent g^{-1} of rice bran. The a-tocopherol and g-oryzanol content ranged from 2.17- 4.58 µg/g and 264.50 – 418.46 μgg^{-1}, respectively (Table 2). Stabilised rice bran with moisture content of 30-40 % and EFS of 75 - 225 Vcm^{-1} yielded significantly higher amount of phenolic compounds, a-tocopherol and g-oryzanol than those of rice bran at 20% moisture content and rice bran stabilised by heating. The total phenolic, a-tocopherol, and g-oryzanol content of the rice bran stabilised by OMH were comparable to those of rice bran reported by(Chotimarkorn *et al.*, 2008).

Table 2: The effect of ohmic heating on bioactive compounds of rice bran (mgg^{-1})

Stabilisation condition	Bioactive compound		
	Total phenolic	α-tocopherol	γ-oryzanol
MC20/E75	1.94 ± 0.02^{e}	0.026 ± 0.003^{c}	3.44 ± 0.19^{c}
MC20/E150	2.15 ± 0.05^{d}	0.038 ± 0.0017^{b}	3.79 ± 0.20^{b}
MC20/E225	2.46 ± 0.02^{c}	0.040 ± 0.002^{b}	3.98 ± 0.22^{ab}
MC30/E75	2.94 ± 0.03^{b}	0.042 ± 0.0032^{ab}	4.07 ± 0.12^{a}
MC30/E150	3.28 ± 0.08^{a}	0.046 ± 0.002^{a}	4.18 ± 0.07^{a}
MC30/E225	3.26 ± 0.02^{a}	0.046 ± 0.003^{a}	4.18 ± 0.06^{a}
MC40/E75	3.25 ± 0.07^{a}	0.045 ± 0.002^{a}	4.15 ± 0.05^{a}
MC40/E150	3.22 ± 0.02^{a}	0.0448 ± 0.002^{a}	4.17 ± 0.04^{a}
MC40/E225	3.24 ± 0.04^{a}	0.045 ± 0.004^{a}	4.15 ± 0.03^{a}
Steaming	2.46 ± 0.13^{c}	0.025 ± 0.0013^{c}	3.82 ± 0.16^{b}
Raw bran	1.79 ± 0.16^{d}	0.018 ± 0.16^{d}	2.65 ± 0.27^{d}

Values are means of triplicate samples (on a dry weight basis)

$^{a-d}$ Means within a column with different numbers are different ($P \leq 0.05$)

MC stands for moisture content and E stand for electrical field strength (Vcm^{-1})

Effect of OMH on Antioxidant Activity:

DPPH Radical Scavenging Activity:

The antioxidant activity by DPPH radical scavenging method, as expressed by the concentration of the amount extract to inhibit 50% of stable free radical (IC_{50}), showed that the reference standards, ascorbic acid and BHT, were the strongest antioxidant (Fig. 6). The rice bran with moisture content of 20% (225 EFS), 30% and 40%, in combination with the electrical field strengths of 75,150, 225 Vcm,$^{-1}$ indicated the strongest antioxidant activity This suggests there exists an optimal moisture content for ohmic heating and the provision of a higher moisture content does not significantly impact on the activity of antioxidant.

2. Lipid Peroxidation Assay:

Lipid peroxidation of egg yolk lipids undergo rapid non-enzymatic peroxidation when incubated in the presence of ferrous sulphate (Dasgupta and De. 2004).The effect of ohmic heating on lipid peroxidation system are shown in Fig. 7. The results indicated that the rice bran with higher moisture content (30-40%) with higher EFS levels appeared to increase the oxidative stability expressed by the higher activity than rice bran stabilised by steaming and raw rice bran.

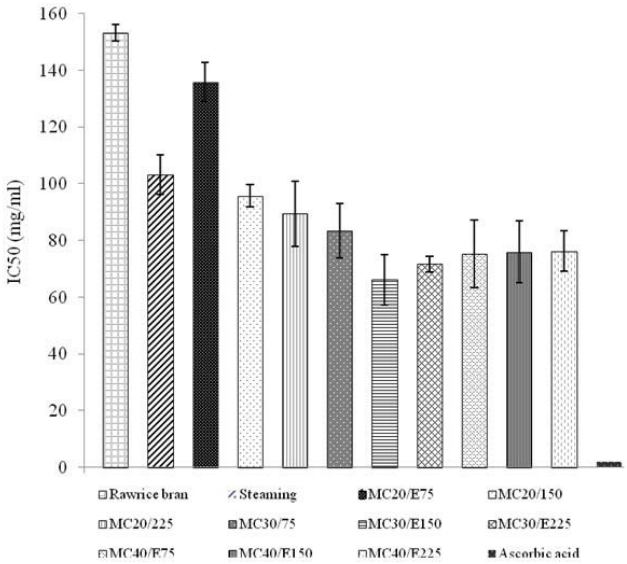

Fig. 6: DPPH radical scavenging activity (IC_{50}) of rice bran (mg/mL)IC_{50} = concentration of the extract to inhibit 50% of DPPH radical (MC20/E75-MC40/E225 = Rice bran with moisture content of 20, 30 and 40% combined with electrical field strength of 75, 150 and 225 Vcm^{-1}; Values are means of triplicate samples)

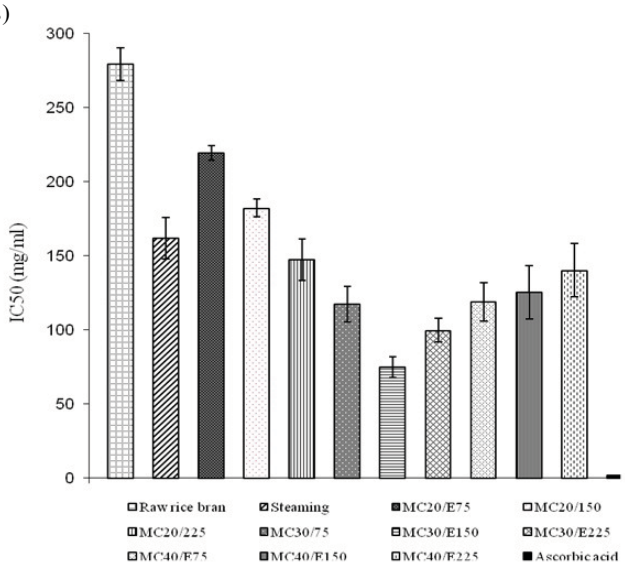

Fig. 7: Lipid peroxil radical scavenging activity (IC50) of rice bran (mg/mL) IC50 = concentration of the extract to inhibit 50% of free radical (MC20/E75-MC40/E225 = Rice bran with moisture content of 20, 30 and 40% combined with electrical field strength of 75, 150 and 225 Vcm-1; Values are means of triplicate samples)

3. The Total Antioxidant Capacity Assay:

The total antioxidant capacity of bran extract is expressed as the number of equivalent antioxidant standards (ascorbic acid, gallic acid, α-tocopherol and BHT). The total antioxidant capacity of stabilised rice bran samples as compared with that of raw rice bran is displayed in Table 3. The rice bran with moisture content of 30-40 % and EFS at 150-225 Vcm^{-1} indicated a greater antioxidant capacity in all antioxidant standard equivalents. These results were related to the concentration of phenolic compounds, a-tocopherol and g-oryzanol, which are antioxidant.

Table 3: Total antioxidant capacity of stabilised rice bran (ohmic heating, steaming) and raw rice bran (mg antioxidant standard equivalent /g rice bran).

Stabilisation condition	Total antioxidant activity			
	Ascorbic acid	Gallic acid	Tocopherol	BHT
MC20/E75	5.28 ± 0.05^{f}	1.53 ± 0.03 ^{f}	7.80 ± 0.08 ^{f}	5.07 ± 0.06^{f}
MC20/E150	5.81 ± 0.15^{e}	1.94 ± 0.09 ^{e}	8.67 ± 0.25 ^{e}	5.74 ± 0.18^{e}
MC20/E225	6.11 ± 0.11 ^{d}	2.18 ± 0.07 ^{c}	9.16 ± 0.18^{d}	6.12 ± 0.13^{d}
MC30/E75	7.05 ± 0.04 ^{c}	2.40 ± 0.03 ^{b}	10.53 ± 0.07 ^{c}	6.99 ± 0.05 ^{c}
MC30/E150	7.48 ± 0.08 ^{a}	2.71 ± 0.05 ^{a}	11.23 ± 0.12 ^{a}	7.52 ± 0.09 ^{a}
MC30/E225	7.29 ± 0.05 ^{b}	2.65 ± 0.03 ^{a}	10.95 ± 0.07 ^{b}	7.34 ± 0.06^{b}
MC40/E75	7.21 ± 0.11^{bc}	2.11 ± 0.07 ^{cd}	10.66 ± 0.17 ^{c}	6.93 ± 0.13 ^{c}
MC40/E150	7.19 ± 0.05^{bc}	2.09 ± 0.03 ^{cd}	10.63 ± 0.07^{c}	6.91 ± 0.05^{c}
MC40/E225	7.14 ± 0.04^{bc}	2.06 ± 0.02 ^{d}	10.54 ± 0.06^{c}	6.85 ± 0.04^{c}
Steaming	5.65 ± 0.07 ^{e}	2.04 ± 0.04 ^{d}	8.47 ± 0.11 ^{e}	5.67 ± 0.08 ^{e}
Raw bran	4.63 ± 0.15 ^{g}	1.42 ± 0.09 ^{f}	6.8 ± 50.24 ^{g}	4.49 ± 0.17 ^{g}

Values are means of triplicate samples (on a dry weight basis)

$^{a-g}$ Means within a column with different numbers are different ($P \leq 0.05$)

MC stands for moisture content and E stand for electrical field strength (Vcm^{-1})

Conclusions:

The results of this study suggested that ohmically heating rice bran with the addition of moisture is an effective stabilisation method. According to the studied conditions and the results, the optimum conditions to obtain a stabilised rice bran are through an adjustment of the moisture content of the rice bran from 30-40% and also the application of an electrical field strength between 150-225 Vcm^{-1}. The benefits were seen by an increase in the moisture content of the rice bran and EFS of alternating current to improve the stability and preserve the bioactive compounds. However, in this study, the ohmic heating unit was the lab scale. In order to develop this to a larger scale, an intensive study of many aspects is needed.

ACKNOWLEDGMENTS

Financial support provided by Mahasarakham University, Thailand and the Thailand Research Fund-Master Research Grants (TRF-MAG, 2008) is gratefully acknowledged. We acknowledge advice and consultation on the configuration of the ohmic heating unit by Tanongsak Moontree, Electrical Engineer and Lecturer in the Faculty of Technology, Mahasarakham University.

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