Some Technical Aspects Affecting Rice Bran Stability for Functional Components Recovery

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ABSTRACT: In commercial rice production, brown rice is commonly produced by polishing, this produce rice bran, among other by-products. This investigation aimed to study the effect of stabilization methods on rice bran for functional components recovery. To achieve this goal lipase enzyme activity was inhibited by heating rice bran in microwave to stabilized rice bran at 850 W for 3 minutes, hot air at 150°C for 10 min and steaming at 130°C for 60 min. Then extracting crude oil by solvent and screw press. The defatted rice bran was used in making cookies and biscuits. Some bioactive components were determined. The obtained results of oil yield with microwave 850Wat 3 min was 19.97%. Peroxide value and free fatty acid were (6.33 meq/kg oil) and (2.88%) in microwave. Total phenolic content of rice bran oil was 18.62(mg gallic acid equivalent (GAE) /g oil). Gamma oryzanol was 15.37and 28.43g/100g for hexane and isopropanol extraction respectively. Tocopherols were 298.54 and 372.31(µg/g) for hexane and isopropanol extraction respectively. Tocotrienols were 492.62 and 595.76(µg/g) for hexane and isopropanol extraction respectively. Radical scavenging activity DPPH as (% inhibition) was 72.56 in microwave.
Key words: rice bran, brown rice, screw press, gamma oryzonol, tocopherols

INTRODUCTION

Rice is the stable food for the largest number of people on the earth and it is eaten by nearly half of the world's population (Maclean *et al*., 2002). Rice milling is main process on rice producing a head rice and other by-product. Rice bran, a by-product from rice milling, it is mostly used as animal feed because of quick hydrolysis of oil into free fatty acids. Biochemical instability of rice bran occurs immediately after milling. Free fatty acids formation has been reported as the time of storage extended without stabilization process done on the rice bran (lakkakula *et al*., 2004). Rice bran is rich in natural antioxidant, such as tocopherols, tocotrinols, oryzanoland phenolic compound. These compounds have shown their potential as antioxidant which protect from free radical damage (Nam *et al*., 2003). Rancidity of lipids in rice bran oil is the major problem for utilization of rice bran. The high fat content and enzymes lead to quality reduction of rice bran. The hydrolysis reaction turns triglycerides into glycerol and free fatty acids, which occurs soon after rice milling and is caused by the presence of lipase enzymes as catalyst. To prevent rice bran from becoming rancid, it must undergo a stabilization process or extraction of oil soon after the milling process, which are two effective methods for lipase enzyme inactivation and prohibition of rancidity of rice bran oil (Ju and Vali, 2005). Various stabilization methods, applied to protect rice bran and rice bran oil degradation (Zigoneanu *et al*., 2008). Steaming, (Juliano, 1985). pH lowering, (Amarasinghe *et al*., 2009). Heating is the most common methods to stabilize rice bran. Temperature above 110°C denaturate the enzyme responsible for lipid degradation in rice bran oil and did not have adverse effect on the

nutritional value of rice bran. Temperatures used for stabilization vary from 105^{o}C to 130^{o}C. the success of stabilization of rice bran and its oil depend on temperature. Duration of heat treatment, moisture content of treatment medium (Orthoefer, 2005). In Egypt, the cultivated area of rice was 0.809 million Hectare which annually produces 5.360 million tons rice seed producing 0.500 million tons rice bran (RRTC, 2014). The bran, being an important by-product, is mainly produced during rice milling operation amounts to 10% of the weight of rice grain. It is rich in protein 13-16% oil, 15-22% fiber, 6.20-14.40% ash, 8.00-17.75% vitamins and trace minerals (Baileys, 2006). In spite of rice bran contain highly amount bioactive nutritive compounds, rice bran oil utility is limited in Egypt. Moreover previous studies showed that rice bran oil had unsaturated fatty acids, about 77% of total fatty acids in rice bran oil (Tahira *et al*., 2007). The main unsaturated fatty acids in rice bran oil consisted of oleic acid and linoleic acid (Amarasinghe and Gangodavilage, 2004).

For the effective stabilization of rice bran, the treatment should sufficiently inactivate lipolytic enzymes but minimize the decomposition of bioactive components. However, study on the effect of stabilization treatments on the bioactive constituents in rice bran has not been performed. In this study, various heat treatments including dry-heating, microwave heating and autoclaving, were applied to rice bran and their effects on the storage stability as well as some of the lipophilic bioactive compounds in rice bran were investigated.

Therefore this investigation aimed to study the effect of stabilization methods on rice bran for functional components recovery.

MATERIALS AND METHODS

Materials

The newly harvested rice (*Oryza sativa)* produced in Egypt. 40 kilogram was obtained from Rice Research Training Center at Sakha- Kafer EL-Shikh. The selected variety used in this study was Sakha 103 cultivated in (2014) season.

Rice bran sample Preparation

The paddy rice samples were cleaned by Dockage Tester Machine (Carter Day CO, style number XT3, USA) to remove the dust foreign matter, mud balls, and immature green automatically. Drying by hot air using rotary dryer Schule, Germany), 30 kilogram were used to produce brown rice using a lab scale testing husker (SATAKE model THU35 A, Tokyo, Japan) then milled by a lab scale Miller (SATAKE model TMI40, Tokyo, Japan). Rice bran was collected, sieved through a 20-mesh sieve, and backed in polyethylene bag. Bags were sealed (double sealing), these it was stored in freazed - 15^{o}C until uses. Polyethylene bag of unstabilized and stabilized rice bran were storaged at ambient temperature for 64 days.

Chemicals and reagents

Hydrochloric acid, isopropanol, sodium hydroxide, sodium sulphate anhydrous, cuprum sulphate, selenium dioxide, sulphuric acid, boric acid, isopropanol, hexane, ethyl acetate, Follin Ciocalteu's phenol reagent, sodium carbonate, ethanol, acetone, acetic acid, acetonitrile (HPLC grade) and methanol (HPLC grade) were purchased from Merck (Germany) and El Gomhouria Company for Trading Chemicals and drugs, whilst standard (γ-oryzanol and α-tocopherols) were obtained from Sigma (St. Louis, USA) and gallic acid was from Acros Organic (NJ, USA).

Stabilization procedure of rice bran

The bran was stabilized to inhibit the lipase enzyme activity through three methods:-

Method (1) Heating the rice bran in microwave at 850 W for 3 minutes then after cooling bran was stored in polyethylene bag until uses according to Thanonkaew *et al*. (2012).

Method (2) Heating the bran with Hot air at 150^{o}C for 10 min according to (Thanonkaew *et al*., 2012).

Method (3) Heating the bran with Steaming at 130^{o}C for 60 min according to (Thanonkaew *et al*., 2012).

Physical properties of white rice

1. Grain dimensions:

The average of length, width and thickness of 20 full length grains of rice were determined by Grain Shape Tester micrometer (model MK-100, Tokyo, Japan) as described by Nanda *et al*. (1976).

2. Grain shape:

Grain shape was estimated using the average of length and width of 20 full grains of rice using the procedure of Khush *et al*. (1979).

3. Grain index:

Grain index (g) was performed using weight of 1000 grains of rice samples as described by Khush *et al*. (1979).

4. Bulk density:

Bulk density (g/L) of rice samples was determined according to the method of Myklestad *et al*. (1978).

5. Whiteness degree% determination:

The percent of whiteness degree as assessed by whiteness meter (Kett Electric Laboratory C-300-3, Tokyo, Japan), with calibration plate made from calcium chloride (85.4$\pm$0.1), as described by USDA (1980) U.S. standard for milled rice.

Moisture content determination

The moisture content was determined according to AOAC (1990), using the infra-red moisture meter Model Satake MO 1780.

Crude protein determination

Crude protein was determined by a semi micro Kjeldahl method according to AOAC (1990).

Crude fat determination

Samples from stabilized rice bran (2g) were extracted with hexane in Soxhlet apparatus for 16-18 h. The solvent was evaporated from the extract under reduced pressure (AOAC1990).

Starch content determination

Starch content was determined according to Egan *et al*. (1981) by the general polarimetric method using Carl Zeiss, plarimeter (type Vtrna, Germany). The method included two determination. In the first, the sample was treated with warm diluted hydrochloric acid. After clarification and filtering, the optical rotation of the solution was read by polarimeter. In second, the sample was extracted with 40% ethanol. After acidifying the filtrate with hydrochloric acid, it was clarified and filtered and optical rotation was measured. The starch percentage was calculated as follows:

$$\text{Percentage of starch} = \frac{2000\,(p - p^{-})}{[\alpha]\, D^{20c}}$$

Where:

P: Total rotation in degree.

p^{-}: Rotation in degree given by substances soluble in 40% ethanol.

$[\alpha]\, D^{20c}$: Specific rotation of pure starch, the value for this factor is + 185.9^{o}, according to Egan *et al*. (1981)

Ash and minerals content determination

A known weight of bran sample (2g) was ignited in a muffle furnace at 550^{o}C according to (AOAC, 1990). Dry ash was used for determination of minerals the digested solution using atomic absorption FMD3 Zeiss according to AOAC (1990).

Crude Fiber determination

Two (grams) of the sample was mixed with sulphuric acid (200 ml, 1.25%, w/v). The mixture was boiled under a reflux condenser for 30 min, filtered through a gooch crucible then thoroughly washed with hot distilled water .The residue were boiled with aqueous sodium hydroxide solution (200ml, 1.25%) for 30 min, then filtered through a gooch crucible as described before. The residues was washed with hot water, then with ethyl alcohol and acetone and dried at 110^{o}C to constant weight. Ash content was subtracted from the dry weight of treated material to give the fiber content (AOAC, 1990).

Peroxide Value determination

The peroxide value was determined according to the method described in AOCS (1989).

Lipase activity

Lipase activity of stabilized and unstabilized rice bran was determined according to Attia *et al*. (1996).

Oil Extraction from rice bran samples

1. By solvent extraction:

Samples were extracted with hexane in Soxhlet apparatus for 16-18 h. The solvent was evaporated from the extract under reduced pressure according to AOAC (1990),

2. By Screw press extraction:

Rice bran was extracted by screw press extraction according to Amarasinghe and Gangodavilage (2004).

Free fatty acid% determination

Rice bran oil was determined according to AOAC (1990).

Iodine value determination

Samples were determined in rice bran oil according to the AOAC (1990).

Antioxidant Activity by DPPH (1,1-diphenyl-2-picrylhydrazyl) (% inhibition) determination

Rice bran and rice bran oil were determined according to Butsat and Siriamornpun (2010) with some modifications. The percent inhibition activity was calculated as: [(Ao - Ae)/Ao] * 100.

(Ao = absorbance without extract; Ae = absorbance with extract).

Defatted rice bran preparation

Twenty grams of stabilized rice bran was extracted with hexane and isopropanol according to Chen and Bergman (2005) and Proctor *et al*., (1995) with some modifications. The extraction method described by Proctor *et al*. (1995) was used. Fresh rice bran (20 g) and stabilized rice bran were mixed with 200 mL hexane or isopropanol and mixed with a vortex stirrer for 1, 2, 5, 10,15,20,25 and 30 min. The solvent/oil miscella was centrifuged for 5 min at 1,700 rpm and filtered, and the solvent was evaporated under nitrogen. The amount of extracted oil was then measured to choose the appropriate time for oil removal.

Total phenolic content determination in stabilized rice bran oil extracted with different solvents

The procedures for extraction of free and conjugated phenolic compounds were adapted based on the methods described by de Mira *et al.*(2009) with slight modifications, by varying the sample amount and repeating the extraction steps two times.

Tocopherol, tocotrienol and *γ- Oryzanol* determination

Rice bran (1g) was extracted. 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 C18 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 µL. 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 α-tocopherol, against the standard curve of standards.

Rice bran oil fatty acid determination

Gas chromatograph (GC-14B, Shimadzu, Kyoto, Japan) with a flame ionisation detector (FID) and a fused silica capillary column measuring 30 m * 0.25 mm * 0.25 mm DB-225 (50% cyanopropyl methyl and 50% methyl phenyl silicone, J&W Scientific, Folsom, CA, USA) was used. The injector and detector were both maintained at 250^{o}C. Nitrogen, at a rate of 1.0 mL min^{-}1, was used as the carrier gas. The oils obtained from the whole and milled rice grains by the continuous extraction using the AACC method 30-20 were used. The derivatisation of the fatty acids was performed according to the method of Zambiazi *et al.* (2007); briefly, samples of 45 mg of oil were weighed in test tubes with lids, and 1 mL of petroleum ether and 12 mL of 0.5 M HCl in methanol were added. The tubes were vortexed and heated at 65 8C for 1 h. Then, 5 mL of isooctane and 6 mL of distilled water were added, and the tubes were shaken. The upper layer was partially transferred to a 1.5 mL flask, from which 1.5 mL was taken and injected into the gas chromatograph with a 1:50 split ratio. The initial column temperature of 100 ^{o}C was maintained for 0.5 min and was then brought up to 150 ^{o}C at a rate of 8 8C min^{-}1. After 0.5 min at 150 ^{o}C, the temperature was increased to 180 ^{o}C at a rate of 1.5 ^{o}C min^{-}1. The column was held at 180 ^{o}C for 5 min and was increased to a final temperature of 220 ^{o}C at a rate of 2 ^{o}C minm^{-}1. The temperature was maintained for 6 more min for a total analysis time of 58 min. The identification of free fatty acids was performed in accordance to the retention time of the chromatographic patterns (myristic, palmitic, stearic, oleic, linoleic, arachdonic and erucic acids, all obtained from Sigma–Aldrich Co., USA). The Class-GC10 software (Shimadzu, Kyoto, Japan) was used to acquire and process the GC data.

RESULTS AND DISCUSSION

In commercial rice production, brown rice is commonly produced by polishing, that produce rice bran, among other by-products. Rice bran is underutilized in many countries of the world. In view of its potential nutritional value and the potential for recovering valuable oil and protein for functional in gradients for human diet, Fresh paddy, weighing approximately 30 kg and containing approximately 18% moisture, was subjected to dryness : by hot air using rotary dryer at 35, 40, and 45^{o}C, moisture contents of paddy rice were reduced to 13.8–14%. Dried paddy rice were subjected to husk, obtain the brown rice 81.62% and husk 18.38% then brown rice was milled at different times.
The obtained data were represented in Table (1). It was clear rice bran% was increased from 6.1 to 11.3% with the increasing of milling time.

Table (1). Effect of milling time of brown rice on rice bran content % and white rice % and white rice characteristics.

Property	Milling time		
	30 sec.	60 sec.	90 sec.
Rice bran content%	6.10	9.20	11.30
White rice %	75.52	72.42	70.32
Head rice %	72.62	68.54	65.92
Broken rice %	2.90	3.88	4.40
Characteristics of white rice			
Whiteness degree%	35.60	39.20	41.50
Grain index (g/1000 kernels)	19.95	19.33	18.63
Bulk density (g/L)	728	731	736
Dimensions:			
Length(mm)	5.15	5.02	4.97
Width(mm)	2.85	2.81	2.78
Thickness(mm	2.00	1.98	1.97
Shape(L/W)	1.81	1.79	1.78

These increasing of rice bran could be due some aleurone layer and endosperm cells were separated with rice bran according to elongation of milling period.

On the other hand head rice % was decreased with increasing the milling time. Broken rice % was increased also by increasing the milling time. The obtained results were in agreement with the results obtained by Kasturi (2010) who studied the impact of milling on 9% milling degree found approximate bran yield 9 g/100g brown rice. In the same time whiteness degree of white rice was increased from 35.6% to 41.5% that could be due that thickness of layer removed by milling was increased by increasing the milling time. On the other hand grain index and length, width and thickness of white rice grain had a slightly decreased.

White rice milled at deferent time was subjected to proximate composition analysis; the obtained data was presented in Table (2).

Table (2). Proximate composition of white rice milled at deferent time.

Property	Milling time		
	30 sec.	60 sec.	90 sec.
Crude protein%	7.98	7.33	6.99
Crud fat%	0.89	0.84	0.67
Starch%	73.40	76.30	79.10
Crud fiber%	0.33	0.29	0.21
Ash%	1.17	1.03	0.98
Non-starch polysaccharides%	16.23	14.21	12.05
Moisture content%	13.80	13.80	13.80

The effect of milling time analysis was pronounced as by increasing milling time both of crude protein%, crude fat%, crud fiber%, ash% and non-starch polysaccharides% were decreased while starch% was increased in white rice. This dryness could be due to the compounds concentrated in outer layer of grain. The data obtained were in agreement with the results obtained by Monks *et al.* (2013) who studied the effects of the degrees of milling between 8% and 14% on ash and fat content in rice where ever ash content was decreased up to milling 12%.
Rice bran produced during different time of milling was subjected to proximate composition determination. The data obtained are represented in Table (3).

Table (3).Rice bran content and its proximate composition at different milling time (% on dry basis).

Property	Rice bran		
	Milling time		
	30 sec.	60 sec.	90 sec.
Rice bran content%*	6.1	9.2	11.3
Crude protein%	15.6	14.3	13.9
Crud fat%	19.8	19.1	18.2
Starch%	14.9	15.7	16.1
Crud fiber%	8.9	8.7	8.1
Ash%	7.9	7.5	7.2
Non-starch polysaccharides%	32.9	34.7	36.5
Moisture content%	13.4	13.5	13.4

* Rice bran content was calculated on paddy rice weight.

It was clear that highest amount of crude protein, crud fat, crud fiber and starch% were obtained at 30 sec. milling time. On other hand all these properties had slightly decreased at 60 sec. and further decreased at 90 sec.

That could be due to rice bran produced at 30 sec. of milling time contains a large amount of the bran layers only, without any endosperm material. The obtained results are in agreement with the results obtained by Kasturi (2010) who studied the impact of milling on total lipids in rice bran of Cheniere and Francis varieties and he found total lipids of rice bran milled at various degrees of milling ranged from 20.5 to 27.0%.

Stabilized defatted rice bran obtained extraction by hexane was subjected to a proximate composition determination. Proximate composition of stabilized defatted rice bran of different milling time of rice is shown in Table (4).

Table (4). Proximate composition of stabilized* defatted rice bran at different time of rice milling (% on dry basis).

Property	Defatted rice bran		
	30 sec.	60 sec.	90 sec.
Crude protein%	19.2	16.6	15.9
Crud fat%	1.8	1.7	1.5
Starch%	14.8	15.1	16.2
Crud fiber%	10.5	10.2	9.5
Ash%	16.3	15.9	14.5
Non-starch polysaccharides%	37.4	40.5	42.4
Moisture content%	13.3	13.6	13.2

*Rice bran stabilized by microwave

The highest crude fat% at 30 sec., while the lowest was at 90 sec, the highest crude protein % was obtained at 30 sec. and the lowest was at 90 sec.
The obtained results were in agreement with the results obtained by Webber *et al.* (2014) who studied Heat-Stabilized Defatted Rice Bran as an Alternative Growth Medium for *Saccharomyces cerevisiae* and found crude protein, crude lipid, starch and ash% were 19.1, 1.8, 14.9 and 16.4% respectively.

The results indicated that the highest crud protein % in defatted rice bran were obtained at 30 sec. of milling time. The reason for highest crud protein content at 30 sec. of milling time, could be due to that bran produced from 30 sec.of milling time (reasonably well-milled rice) of the brown rice contains a large part of the bran layers only, without any endosperm material.
Milling time of 60 sec. was chosen as optimum rice bran content for using in this.

Table (5) shows effect of heat stabilization at different temperature and time on lipase activity (enzyme unit /g dry mater) of rice bran.

Table (5). Effect of heat stabilization at different temperature and time on Lipase activity(enzyme unit /g dry mater) of rice bran .

Temperature °C (Oven hot air)	Time		
	10 min	20 min	30 min
	Lipase activity (Unit/g dry mater)		
140	8.9	0.59	0.31
150	0.00	0.00	0.00
160	0.00	0.00	0.00

It was evident that lipase was completely inactivated after 10 min at 150°C while it was inactivated completely after 10 min at 160°C. It was clear that the higher temperature the little time of inactivation. Inactivation by hot air at 150°C for 10 min was chosen as heat stabilization of rice bran. The obtained results were in agreement with the results obtained by Thanonkaew *et al.* (2012) who studied effect of stabilization of rice bran by domestic heating on mechanical extraction yield and found 150 $\pm$2°C, 10 min. Effect of stabilized rice bran with steaming stabilization at 110, 130, and 150°C for different time temperature on lipase activity of rice bran was represented in Table (6).

Table (6). Effect of steaming stabilization with different time and temperature on lipase activity (enzyme unit /g dry mater) of rice bran .

Temperature °C (steaming)	Time		
	45 min	60 min	90 min
	Lipase activity (Unit/g dry mater)		
110	10.4	0.35	0.29
130	8.8	0.00	0.00
150	0.00	0.00	0.00

Lipase activity was completely inactivated by steaming rice bran at 130°C for 60 min., therefore, this temperature degree of 130°C for 60 min was chosen. The obtained results were in agreement with the results obtained by Thanonkaew *et al.* (2012) who studied effect of stabilization of rice bran by domestic heating on mechanical extraction yield and found 130 $\pm$2°C, 60 min. Effect of stabilized rice bran with microwave stabilization of different time at 850 W on lipase activity of rice bran was represented in Table (7).

Table (7). Effect of *microwave stabilization with different time at 850 W on lipase activity (enzyme unit /g dry mater) in rice bran.

Time	2:30 min	3 min	3:30 min
Lipase activity (Unit/g dry mater)	0.54	0.00	0.00

* microwave at 850 W for 2:30, 3, and 3:30 minutes then after cooling bran was stored in plastic bags.

Lipase activity was completely inactivated in rice bran by microwave at 850 W for 3 min., therefore, this treatment was chosen. The obtained results were in agreement with the results obtained by Thanonkaew *et al.* (2012) who studied effect of stabilization of rice bran by domestic heating on mechanical extraction yield and found 150 $\pm$2^{o}C, 3 min and power 800 w.

Effect of stabilization methods on oil yield extracted from rice bran

Effect of stabilized with heating rice bran at 150^{o}C for 10 min, steaming rice bran at 130^{o}C for 60 min and microwave rice bran at 850(W) for 3min. on oil yield by hexane extracted was represented in Table (8).

Table (8). Effect of stabilization methods of rice bran on extracted oil yield.

Stabilization methods	Oil yield%*	increasing yield%
Unstabilized rice bran (control)	17.65	0.00
Hot air 150 ^{o}C, 10 min	19.51	10.54
Steaming 130 ^{o}C, 60 min	17.83	1.02
Microwave 850 W, 3 min	19.79	12.12

* Extracted by hexane.

It was clear that stabilization methods affected the oil yield as it increased from 17.65% in case of unstabilized rice bran to 19.79% in case of microwave and in case of at hot air at 150^{o}C and 10 min 19.51%.These increasing was about 10.54% in case of hot air stabilized rice bran. This increasing in oil yield after could be due to the effect of temperature on protein as it was denaturated and stabilized facilitates the oil separation. These results were in agreement with the results obtained by Tao *et al.* (1993) who studied microwave heating on rice bran stabilization and he found that oil yield was increased up to 19%. Table (9) shows the effect of stabilized rice bran with different methods on stability of crude rice bran oil.

Table (9). Effect of different stabilization methods on crude rice bran oil stability.

Stabilization methods	Lipase activity (Unit/g dry mater)	Crude rice bran oil stability		
		Peroxide value (meq/kg oil)	Free fatty acids% as oleic acid	Iodine value
Unstabilized rice bran (control)	7.31	9.87	4.95	90.1
Hot air 150^{o}C, 10 min	0.00	6.77	2.89	92.8
Steaming 130^{o}C, 60 min	0.00	6.85	2.97	92.0
Microwave 850 W, 3 min	0.00	6.33	2.88	92.9

Free fatty acids were dropped from 4.95 % in case of unstabilized rice bran to 2.89, 2.97 and 2.88% in case of hot air, steaming and microwave respectively. In the same respect peroxide value was decreased from 9.87 to 6.85, 6.77 and 6.33 for unstabilized, stabilized with steaming, hot air and microwave respectively.

Iodine value as the measure of unsaturated degree in oil, also was increased from 90.1 to 92.8, 92.0 and 92.9 in unstabilized, stabilized with steam, hot air and microwave methods respectively. These results were in agreement with the results obtained by Amarasinghe *et al.* (2009) who studied the effect of stabilization methods on rice bran oil stability, he found the free fatty acid was 2.8 for stabilized rice bran oil. These results were in agreement with the results obtained by Tiwari *et al.* (2012) who studied storage stability of refined rice bran oil using common packaging material; he found the peroxide value was 6.69 for stabilized rice bran oil. Effect of stabilized rice bran with microwave at 850 W for 3 min and heating by oven at 60^{o}C temperature for 48 hr. on the peroxide value for crude rice bran oil, was represented in Table (10)

Table (10). Effect of oven test at 60^{o}C on unstabilized and stabilized rice bran oil for 48 hr.

Time (hr)	Unstabilized rice bran oil	Stabilized rice bran oil
	Peroxide value (meq/kg oil)	
0	9.87	6.33
2	9.92	6.36
4	9.98	6.39
6	10.03	6.42
8	10.07	6.44
10	10.12	6.46
12	10.18	6.48
14	10.23	6.50
16	10.27	6.52
18	10.32	6.54
20	10.37	6.56
22	10.42	6.58
24	10.47	6.61
26	10.53	6.63
28	10.57	6.66
30	10.63	6.68
32	10.69	6.70
34	10.75	6.73
36	10.81	6.76
38	10.87	6.79
40	10.93	6.82
42	10.99	6.85
44	11.05	6.88
46	11.11	6.91
48	11.18	6.96

*Heating by oven at 60^{o}C temperature for 48 hr.

It was clear that peroxide value (meq/kg) for crude rice bran oil increased by increasing the period storage time, for example it was 6.33 at zero time of storage and increased up to 6.96 at 48 hr. The obtained results were agreement with the results obtained by Amarasinghe *et al.* (2009), who studied the effect of stabilization method on aqueous extraction of rice bran oil, and he found that peroxide value of crude rice bran oil was 6.06 meq/kg. Effect of deferent stabilization methods of rice bran on free fatty acids for crude rice bran oil stability during storage at ambient temperature for 64 days, was represented in Table (11).

Table (11). Effect of stabilization methods of rice bran on crude rice bran oil stability during storage at ambient temperature for 64 days.

Time (days)	Unstabilized rice bran	Hot air stabilized	Steaming stabilized	Microwave stabilized
	Free fatty acids% as oleic acid			
0	4.95	2.88	2.95	2.89
8	5.04	3.07	3.34	2.09
16	5.14	3.25	3.44	3.26
24	5.90	3.44	3.73	3.45
32	6.58	3.70	4.13	3.72
40	6.19	4.07	4.52	4.09
48	6.58	4.34	4.92	4. 37
56	6.86	4.72	5.31	4.75
64	7.24	5.13	5.71	5.16

It was clear that free fatty acid% for crude rice bran oil increased by increasing the period storage time, for example it was 4.95, 2.88, 2.95 and 2.89 at zero time of storage for unstabilized, stabilized with hot air, steaming, and microwave respectively, and increased up to 7.24, 5.13, 5.71 and 5.16% at 64 days for unstabilized, stabilized with hot air, steaming, and microwave respectively. The obtained results were in agreement with the results obtained by Amarasinghe and Gangodavilage (2004), who studied rice bran oil extraction in Sri Lanka for process equipment design, and he found that free fatty acid was 2.8% at zero time of storage for stabilized with hot air and steaming. Effect of stabilized rice bran with heating at 150^{o}C for 10 min., steaming at 130^{o}C for 60 min., and microwave at 850 W for 3 min on total phenolic content of rice bran, was represented in Table (12).

Table (12). Effect of different stabilization methods on total phenolic content in rice bran.

Stabilization methods	Total phenolic (mg GAE*/g rice bran)
Unstabilized rice bran (control)	3.62
Hot air 150 ^{o}C, 10 min	5.33
Steaming 130 ^{o}C, 60 min	4.01
Microwave 850 W, 3 min	5.69

* GAE= gallic acid equivalent

Total phenolic content were increase for stabilized rice bran could be due to inactivate or inhibit the oxidative enzymes. Total phenolic content were increase using microwave stabilized was 5.69 mg GAE/g rice bran. On the other hand total phenolic content was decreased up 3.62 mg GAE/g with unstabilized rice bran.

The obtained results were in agreement with Wiriyawattana and Suwonsichon (2014) who studied the effects of autoclave heating and microwave heating on stability and antioxidant activity of riceberry bran and found that total phenolic content by microwave heating was 5.66. Effect of stabilized rice bran with heating at 150^{o}C for 10 min., steaming at 130^{o}C for 60 min., and microwave at 850 W for 3 min. on antioxidant activity of rice bran, was represented in Table (13).

Table (13). Effect of different stabilization on antioxidant activity of rice bran

Sample (rice bran)	DPPH (% inhibition)
Unstabilized rice bran (control)	30.93
Hot air 150 ^{o}C, 10 min	37.25
Steaming 130 ^{o}C, 60 min	35.17
Microwave 850 W, 3 min	37.44

Antioxidant activity (DPPH) of rice bran was increase using microwave stabilized was 37.44. On the its was decreased up 30.93 with unstabilized rice bran. The obtained results were in agreement with Sirikul *et al.* (2009), who studied proximate composition, bioactive compound and antioxidant activity of rice bran and defatted rice bran from organic rice and conventional rice and found that DPPH for rice bran conventional was 24.96%.

Effect of stabilized rice bran with heating at 150^{o}C for 10 min., steaming at 130^{o}C for 60 min., and microwave at 850 W for 3 min on total phenolic content of rice bran oil was represented in Table (14).

Table (14). Effect of different stabilization methods on total phenolic content of rice bran oil.

Stabilization methods	Total phenolic content (mg GAE* /g oil)	Increasing percentage of total phenolic content
Unstabilized rice bran (control)	13.38	0%
Hot air 150 ^{o}C, 10 min	17.93	34%
Steaming 130 ^{o}C, 60 min	15.46	15.55%
Microwave 850 W, 3 min	18.62	39.16%

* GAE= gallic acid equivalent

This increasing total phenolic content could be due to inactivate or inhibit phenol oxidaze enzyme.Total phenolic content increased by microwave 850 W was 18.62 mg FAE/g oil, 3 min, while total phenolic content decreased by unstabilized rice bran was 13.38 mg GAE /g oil. The obtained results were in agreement with Siger *et al.* (2008) who studied the content and antioxidant activity of phenolic compounds in cold-pressed plant oils, and found that 14.4 mg CAE/1000 g oil in rice bran oil. Effect of stabilized rice bran with microwave at 850 W for 3 min and heating at 150^{o}C for 10 min on fatty acid composition of rice bran oil was represented in Table (15).

Table (15). Effect of stabilization methods on fatty acid composition (%) of rice bran oil.

Fatty acid	Unstabilized rice bran oil (control)	stabilized rice bran oil	
		Microwave 850 W, 3 min	Hot air 1 20 ^{o}C, 20 min
14:0	0.40	0.42	0.41
16:0	14.45	14.57	14.59
18:0	1.02	2.08	2.11
18:1	44.98	44.52	44.53
18:2	37.31	36.55	36.56
18:3	0.98	0.91	0.88

Fatty acid composition (%) non significant on effect of stabilization rice bran at 850 W for 3 min and heating rice bran at 150^{o}C for 10 min.The obtained results were in agreement with Marlene *et al.* (2005) who studied rice bran oil, not fiber, lowers cholesterol in humans and found that14:0, 16:0, 18:0,18:1, 18:2 and18:3 (g/100 g) were 0.40, 14.60, 2.09, 44.51. 36.59. and 0.87. Effect of some extracted methods with hexane, isopropanol and screw press and extracted on oil yield, was represented in Table (16).

Table (16). Effect of some extracted methods at different time on *oil yield.

Extraction Time (min)	Hexane extracted oil weight (% of bran)	Isopropanol extracted oil weight (% of bran)	Screw press oil weight (% of bran)
1	14.68	14.60	1.80
2	14.86	14.84	2.05
5	15.91	15.89	2.75
10	16.79	16.70	3.47
15	17.84	17.81	4.24
20	18.87	18.85	4.89
25	19.78	19.79	5.92
30	19.79	19.80	5.96

* Stabilized rice bran with microwave rice bran at 850 W for 3 min.

Oil yield increasing with solvents extracted (hexane or isopropanol) were 19.79% and 19.79%, while decreasing oil yield with screw press was 5.96%. The obtained results were in agreement with Proctor and Bowen (1996) who studied the ambient-temperature extraction of rice bran oil with hexane and isopropanol 1 and found that hexane extracted was 15.2 1% and Isopropanol extracted was 16.28% Sayasoonthorn *et al.* (2012) who studied optimum operating setting, oil extraction level and press cake and found that maximum oil from screw press was 4.17%.

Stabilized rice bran with microwave at 850 W for 3 min and extracted with hexane and isopropanol. And its effect on quality of rice bran oil was represented in Table (17).

Table (17). Effect of stabilized rice bran on quality of rice bran oil extracted by different methods.

Solvent extraction	Free fatty acid (%)	Peroxide value (meq/kg oil)
Hexane	2.88	6.33
Isopropanol	2.74	6.27
Screw press	2.79	6.30

Free fatty acid (%) and peroxide value (mlq/kg oil) were 2.88 and 6.33, while the lowest value was 2.74 and 6.27, respectively. Free fatty acid% and peroxide value (meq/kg oil) non significant on quality of rice bran oil extracted by different methods. The obtained results were in agreement with Proctor and Bowen (1996) who studied the ambient-temperature extraction of rice bran oil with hexane and isopropanol and found that Free fatty acid for hexane extracted was 2.58 % and for Isopropanol extracted was 2.4 1%. Effect of stabilized rice bran with microwave rice bran at 850 W for 3 min and extracted with hexane and isopropanol on bioactive components of rice bran oil extracted by different methods, was represented in Table (18).

Table (18). Effect of *stabilized rice bran oil extracted by different methods on bioactive components.

Bioactive component	Unstabilized rice bran oil	Oil rice bran		
		Hexane	Isopropanol	Screw press
Total phenolic content (mg FAE/g oil)	13.38	18.62	22.54	20.43
Gamma oryzanol (g/100g)	14.29	15.37	28.43	19.37
Tocopherols (µg/g)	296.38	298.54	372.31	314.43
Tocotrienols (µg/g)	490.41	492.62	595.76	501.64

*Microwave at 850 W for 3 minutes

These increasing of total phenolic content, gamma oryzanol, tocopherols and tocotrienols at samples were extracted by isopropanol could be due to increase in polarity.Total phenolic content, gamma oryzanol, tocopherols and tocotrienols were increase using Isopropanol forextraction 22.54 mg FAE/g oil, 28.43 g/100 g, 372.31 µg/g, and 595.76 µg/g, while the lowest values were 18.62 mg FAE/g oil, 15.37g/100 g, 298.54 µg/g, and 492.62 µg/g, respectively.
The obtained results were in agreement with Marlene *et al.* (2005) who studied the rice bran oil, not fiber, lowers cholesterol in humans and found that rice bran oil extracted with hexane α-Tocopherol, γ-Tocopherol, α –Tocotrienol, γ –Tocotrienol (µg /g) were 180.0, 38.0, 218.0, 59.0 and Oryzanol (mg/g) was 15.8.

Tocopherols (µg/g), Tocotrienols (µg/g) and Gamma oryzanol (mg/g) for stabilized rice bran were 298.54, 492.62 and 15.37, respectively. Some bioactive compounds content non significant effect on stabilization of rice bran.

The obtained results were in agreement with Marlene *et al.* (2005) who studied the rice bran oil, not fiber, lowers cholesterol in humans and found that α-Tocopherol, γ-Tocopherol, α –Tocotrienol, γ –Tocotrienol (µg /g) were 180.0, 38.0, 218.0, 59.0 and Oryzanol was 15.8 (mg/g).

Effect of stabilized rice bran with heating at 150^{o}C for 10 min, steaming at 130^{o}C for 60 min., and microwave at 850 W for 3 min and extracted with hexane on antioxidant activity of rice bran oil, was represented in Table (19).

Table (19). Effect of different treatments on antioxidant activity of rice bran oil.

Treatment	DPPH as (% inhibition)
Unstabilized rice bran oil (control)	65.84
Hot air 150^{o}C, 10 min	72.37
Steaming 130^{o}C, 60 min	70.29
Microwave 850 W, 3 min	72.56

Antioxidant activity (DPPH) of rice bran oil was increase using microwave stabilized was 72.56. On the its was decreased up 65.84 with unstabilized rice bran. The obtained results were in agreement with Daud *et al.* (2015) who studied the Antioxidant Properties of rice bran oil from different varieties extracted by solvent extraction methods and found that antioxidant activity of rice bran by microwave heating was 73.74 %. Effect of stabilized rice bran with microwave rice bran at 850 W for 3 min and hot air 150^{o}C, 10 min on minerals content of rice bran was represented in Table (20).

Table (20). Effect of stabilization methods on minerals content of rice bran.

Minerals content	Stabilized rice bran		
	Microwave	Hot air	Unstabilized rice bran
	mg/100g		
Calcium (Ca)	68.76	68.81	67.85
Iron (Fe)	3.60	3.66	3.70
Magnesium (Mg)	80.31	80.27	80.20
Phosphorus (P)	177.83	177.73	177.61
Potassium (K)	215.22	214.99	214.93
Sodium (Na)	439.84	439.71	439.62
Zinc (Zn) content	1.51	1.63	1.72
Copper (Cu)	0.18	0.18	0.19
Manganese (Mn)	1.58	1.59	1.61

Data was no significant effect of stabilization on minerals content of rice bran.The obtained results were in agreement with Abdel Bary (2012) who studied the qualitative and quantitative assessment for bran and bran oil of some Egyptian rice varieties and found that calcium, iron, magnesium and phosphorus were 69, 3.61, 80 and 178 mg/100g.

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الملخص العربى

تأثير بعض المعاملات التكنولوجية على ثبات رجيع الكون لاستعادة مكونات وظيفية

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لإنتاج الأرز تجاريا يتم تبييض الأرز البني عادة ، ويعد رجيع الكون من المنتجات الثانوية. و لذلك كان الهدف هو دراسة تأثير طرق تثبيت رجيع الكون لاستعادة مكونات وظيفية وتغذوية . ولتحقيق هذا الهدف تم تثبيط انزيم الليبيز عن طريق تسخين رجيع الكون في الميكروويف في ٨٥٠ وات لمدة ٣ دقائق، والهواء الساخن في ١٥٠ °م لمدة ١٠ دقائق وبخارالماء في ١٣٠°م لمدة ٦٠ دقيقة . ثم استخلاص الزيت الخام بواسطة المذيبات والعصير الميكانيكى . و تم استخدام رجيع الكون منزوع الدهن في صنع الكعك والبسكويت. تم تقدير بعض المكونات النشطة بيولوجيا. النتائج التي تم الحصول عليها من عائد الزيت مع الميكروويف ٨٥٠وات لمدة ٣دقائق كان ١٩.٩٧٪. وكانت قيمة البيروكسيد والأحماض الدهنية الحرة (٦,٣٣ مكافىء/ كيلوجرام زيت) و (٢.٨٨٪) في الميكروويف. بلغ إجمالي

المحتوى الفينولي من زيت رجيع الكون ١٨.٦٢ (ملجرام حامض الجاليك المكافىء / جرام زيت). جاما أوريزانول ١٥,٣٧ و ٢٨,٤٣ جم / ١٠٠جرام لمستخلص الهكسان والأيزوبروبانول على التوالي. التوكوفيرول ٢٩٨.٥٤ و٣٧٢.٣١ (ميكروجرام / جرام) لمستخلص الهكسان والأيزوبروبانول على التوالي. توكوترينول ٤٩٢.٦٢ و٥٩٥.٧٦ (ميكروجرام / جرام) لمستخلص الهكسان والأيزوبروبانول على التوالي. تثبيط نشاط الشوارد الحرة (DPPH) ثنائى فينيل بكريل هيدرازيل ٧٢.٥٦% في الميكروويف.