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EFFECT OF SOME STABILIZATION METHODS ON NUTRITIONAL COMPOSITION OF RICE BRAN

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ABSTRACT: Rice bran (RB), a byproduct of rice milling industry and constitutes around 10% of the total weight of rough rice. It is primarily composed of aleurone, pericarp, subaleurone layer and germ. Rice bran is a rich source of vitamins, minerals, essential fatty acids, dietary fibers and other sterols. The main objective of this study is to describe the nutritional composition of rice bran that has been stabilized by either microwave or dry heat process which can be utilized as a functional food ingredient. The study also evaluated the influences of stabilization of rice bran in terms of free fatty acid and peroxide values. The results of this study suggested that dry heating and microwave heating were effective methods for stabilization of rice bran with lowering content of free fatty acids (FFA) and peroxide value (PV). These heating methods also provided higher contents of total phenolic compounds with increasing the antioxidant activities. However, it was suggested that microwave heating is not suitable to be used in small and medium scales in rural areas. But dry heating is a very efficient method for stabilization and could be applicable for small and medium scale operations in rural areas.

Key words: Rice bran, nutritional composition, stabilization methods, free fatty acids, enzyme inactivation.

INTRODUCTION

Rice (*Oryza sativa* L.) is an important cereal crop and a staple food for more than half of the world's population **(Wani *et al*., 2012)**. Rice processing involves various steps like cleaning, hulling and post hulling processing (whitening, polishing and grading). Processing of rice or milling produces several streams of materials such as husk, milled rice, and bran. The percentage of rice by-products is dependent upon several factors, such as the milling rate and type of rice. An ideal milling process will yield 20% husk, 8-12% bran depending on the milling degree and 68-72% milled rice or white rice, depending on the variety **(Norhaizan *et al.*, 2013)**. Rice bran, a "little known" food is highly nutritious and delivers a powerhouse of health supporting nutrients which is either thrown away or used for low-level animal feed and not to be efficiently utilized for human consumption **(Quereshi *et al*., 2000)**.

It is composed of pericarp, aleurone and subaleurone layers, portion of the germ, and small portion of the starchy endosperm. Rice bran is a rich source of oil, protein, fiber and micronutrients. Rice bran proteins have a high nutritional value and are hypoallergenic in nature **(Fabian and Ju, 2011)**. It is also rich in vitamins, minerals, and other nutrients having promising health-related benefits. Rice bran is rich in antioxidant compounds like polyphenols, vitamin E, tocotrienols and carotenoids that help prevent the oxidative damage to DNA and other body tissues **(Jariwalla, 2001)**. Many studies reported that rice bran has cholesterol lowering properties, cardiovascular health benefits and anti-tumor activity **(Tuncel *et al*., 2014)**. Rice

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bran has enormous applications in food industries for increasing the nutritional quality of processed foods. Rice bran being high in fiber content and in view of its therapeutic potential, its addition can contribute to the development of value-added foods or functional foods that currently are in high demand **(Hu *et al.*, 1996)**.

During the milling process, the bran layers are removed from the endosperm, the individual cells of rice bran are ruptured and the lipids come into contact with highly reactive lipases. These enzymes are endogenously present in the bran and initiate hydrolytic deterioration of kernel oil **(Ramezanzadeh *et al.*, 1999)**. Although being an excellent nutrient source, rice bran is not suitable for human consumption due to the rancidity caused by presence of lipases. While removing bran layers from the endosperm during milling, the individual cells are disrupted and lipase enzymes come into contact with fat causing hydrolysis to (FFA) and glycerol **(Malekian *et al.*, 2000)**. However, stabilization, an enzyme inactivation process, is widely employed to extend the shelf life of rice bran, enabling incorporation of rice bran back into our diet.

A variety of methods are available for stabilization of rice bran. The process of stabilization aimed at destruction or inhibition of lipase enzyme activity. This methods included dry or moist heating, microwave heating, ohmic heating, extrusion, gamma irradiation, refrigeration and acidification **(Ramarathnam *et al.*, 1989 ; Amarasinghe *et al.*, 2009)** have been conducted to improve its utilization in food. The most practical and inexpensive method to deactivate lipase in fresh rice bran is heat treatment as reported by **Randall *et al.* (1985)**. Heat treatment is the most common method to stabilize rice bran. High temperatures above 120°C denature the enzyme responsible for lipid degradation in RBO without destroying the nutritional value of the rice bran. Temperatures used for stabilization vary from 100 to 140°C **(Orthoefer, 2005)**.

Therefore this work was undertaken to study the effect of stabilization methods on nutritional composition of rice bran and some chemical characteristics of extracted oils.

MATERIALS AND METHODS

Materials

Samples of rice bran powder were obtained from Egyptian rice (Sakha 101), a popular short grain Japonica cultivar for consumption in the Egypt. Bran powder was obtained by milling rice grain in a local grinding mill in Sharkiya Governorate, Egypt during the season of 2014 and followed by sieving to separate grains from rice bran at 35 mesh. Rice bran was stored in polyethylene bags in freezer at 0°C to control the increase of rice bran free fatty acid.

Methods

Rice bran stabilization

Untreated rice bran (Un-RB)

Rice bran without any stabilization treatment was used as control.

Microwave heating of rice bran (MW-RB)

The method proposed by **Ramezanzadeh *et al.* (2000)** was slightly modified and used as follows: The moisture content was adjusted to 21% by adding deionized water prior to microwave stabilization. The microwave chamber of microwave oven (Model SM-2320MS Smart 230V-50HZ) The sample was (150 g) placed in microwaveable plastic container pre- heated at 100 % power for 2 min to establish a constant initial temperature and effective heating. Then the sample was heated at medium power for 3 min. Before being packaged using polyethylene zipper top bag, sample was allowed to cool at room temperature.

Dry-heating of rice bran (DH-RB)

A portion of rice bran (500 g) was transferred into shallow pans and spread uniformly in a layer of about 0.5 cm thickness. The pans were then placed in an electric oven and for dry heat rice bran was placed uniformally in 0.5 cm layer in electric oven by heating at 105°C for 10 min **(Yokochi, 1974)**. Before being packaged using polyethylene zipper top bag, sample was allowed to cool at room temperature.

Proximate chemical analyses of rice bran samples

Moisture, crude protein, crude fat, crude fiber and ash were determined due to the methods recommended in **AOAC (2005)**. Total carbohydrate was determined by difference.

Available carbohydrates were calculated by subtracting crude fiber from total carbohydrates.

The total energy value of the food formulation was calculated according to the method of **Mahgoub (1999)** using the following formula:

Total energy (kcal/100 g)=[(available carbohydrates (%) ×4.1) + (protein (%) ×4.1)+(fat (%) ×9.3)]

Minerals (Fe, Ca, Mg, Mn, and Zn) were determined after wet digestion by using Atomic Absorption Spectrophotometer according to **AACC (2000)**.

Vitamin B complex (thiamine, riboflavin) were determined by High performance Liquid Chromatography (HPLC) as described by **AOAC (2005)**.

Oil extraction

A weight of dry full fat rice bran was soaked in n-hexane (B.P 60-80°C) for 24 hr., at room temperature, then the obtained solution was filtered and solvent was removed by rotary evaporator according to **Kahlon *et al.* (1992)**. The crude oil has a dark greenish colour.

Free fatty acids (FFA)

FFA content of rice bran samples was determined using a standard titration method **(AOCS, 1989).**

Peroxide value (PV)

Peroxide value was determined by potassium iodide method according to **Leonard *et al.* (1987)**.

Extraction of phenolic compounds

Phenolic compounds were extracted from rice bran samples twice using methanol 80% at a ratio of 1:20 (*W/V*). Each time, the mixture was shaked by a mechanical shaker (150 rpm) at room temperature for 16 hr. After centrifugation at 4000 rpm for 5 min, the supernatants obtained from each time were combined and concentrated to dryness by a rotary evaporator at 35°C. The dried methanol extract was dissolved in 5 ml of methanol 50% and used as crude extracts according to the method described by **Nara *et al.* (2006)**.

Determination of total phenolic content

Total phenolics in extracts were determined with the Folin- Ciocalteu reagent. Gallic acid was used as a standard and the total phenolics were expressed as mg/g gallic acid equivalents (GAE) according to **Maurya and Singh (2010)**.

Determination of antioxidant activity with radical scavenging method

Radical scavenging ability, using the stable radical, 2, 2-diphenyl- 1-picrylhydrazyl DPPH was performed **(Brand-Williams *et al.*, 1995)**.

Statistical Analysis

The data collected were subjected to analysis of variance (ANOVA) using the SAS program **(SAS, 2011)**. Means were separated using **Duncan (1958)** were performed to determine if differences among treatment means were significant at 95% confidence level ($P<0.05$).

RESULTS AND DISCUSSION

Proximate Chemical Composition of Rice Bran Samples Subjected to Different Stabilization Procedures

Proximate composition of raw and heat stabilized rice bran is shown in Table 1. It can be inferred that the moisture content of rice bran samples varied from 4.43 to 11.67%. In general, the unstabilized rice bran had maximum moisture content (11.67%) compared to stabilized rice bran samples. Stabilization definitely reduced the moisture content of the rice bran, out of the different techniques used for rice bran stabilization, Microwave stabilization was found to be effective in reducing the moisture content (4.43%). The present results are in accordance with those of **Ramezanzadeh *et al.* (2000), Da Silva *et al.* (2006) and Thanonkaew *et al.* (2012)**. They reported that moisture content depending on the processing, temperature and time. Results from the same Table showed that ash content of un-stabilized and stabilized rice bran of the current study (7.16 to 7.93%) respectively. Regarding

Table 1. Effect of stabilization methods on proximate chemical composition of stabilized rice bran

Treatment	Moisture (%)	Crude protein (%)	Fat (%)	Crude fiber (%)	Ash (%)	Carbohydrate (%)	Energy (Kcal/100g)
(Un-RB)	11.67 a	14.17 b	16.26 c	8.86 c	7.16 b	50.71 b	417.59 c
(DH-RB)	5.83 b	14.80 a	17.50 b	10.54 b	7.93 a	54.02 a	444.11 b
(MW-RB)	4.43 c	15.06 a	18.08 a	13.31 a	7.43 b	54.58 a	453.71 a

Values followed by different letters within same columns are significantly different at P = 0.05 with Duncan's multiple range test. Untreated rice bran (Un-RB), Dry-heating (DH-RB) and Microwave heating (MW-RB)

the carbohydrate content of rice bran samples varied from (50.71 to 54.58), it was maximum in microwave stabilized rice bran (54.58%). Apparent also from the same Table that, un-stabilized (Un-RB) and stabilized rice bran contain (16.26 to 18.08%) crud fat, respectively. Rice bran contains 12%-23% crude fat, depending on whether it is short-, medium-, or long-grain, locality, and variety of rice **(Barber and Benedito de Barber, 1980)**. Furthermore, the effect of stabilized either dry or moist heat had the same effect, that increased ether extract in rice bran. The augmentation of oil extractability could be related to the ability of heat to turn the fat in the cells coalesce into oil droplets and bracks down cell structure, thereby improving the speed and extent of oil extraction, or to degradation of lipoprotein these results are in agreement with those reported by **(Rizk *et al.,* 1994; Maud *et al.,* 2000).** As previously reported by other authors, different stabilization techniques may reduce enzymatic activity to a greater or lesser degree, resulting in increased or decreased oil extraction **(Lakkakula *et al.,* 2004)**.

Results in Table 1 show that the protein content of the unstabilized rice bran was 14.80%. The protein content of the stabilized rice bran varied from 14.17 to 15.06%. The highest value (15.06 %) present in Microwave stabilized rice bran while the lowest value (14.17%) was for untreated rice bran. These results are in line with **Anderson and Guraya (2001)**. The crude fiber content ranged from 8.86 to 13.31%. High crude fiber was found in Microwave stabilized rice bran (13.31 %) and low value is present in unstabilized rice bran (8.86 %) these results are in a harmony with the findings of **Saima *et al.* (2009)**. Energy significantly increased, the increase was higher in case of Microwave stabilized rice bran (453.72%). The result notices that heat treatments increase the proximate chemical composition of rice bran. These findings are found to be similar to those reported by **(Abd El-Hady (2013), Mohammed *et al.* (2014) and Sangle *et al.* (2016).**

Minerals Content of Un-stabilization and Stabilization Rice Bran

Rice bran is a good source of minerals which are present in varied amounts as shown in Table 2. The mineral composition of rice grain depends considerably on the availability of soil nutrients during crop growth and generally present in higher levels in the bran layer of rice kernel **(Juliano and Bechtel, 1985)**. Mineral contents of rice bran for un-stabilized (Un-RB) and stabilized (microwave and dry heat) are given in Table 2. Results revealed that magnesium content was the highest element among all of the determined mineral contents. Whereas, stabilization process caused an increasing in most mineral contents of rice bran samples. **Saunders (1990)** and **Luh *et al.* (1991)** reported that the content of minerals in rice bran reflects the rice variety, degree of milling and growing environment. Composition of rice bran varies with the rice type, climatic conditions and rice processing methods **(Grist, 1985)**. It is well known that mineral content is strongly influenced by cultivation conditions, including soil structure condition and fertilization, as well as by rice processing **(Walter *et al.*, 2008)**. Furthermore, **Simone Aparecida dos *et al.* (2012)** indicated that different stabilization techniques may also alter rice bran mineral content.

Table 2. Effect of stabilization methods on mineral content of rice bran (mg/100g)

Treatment	Fe	Zn	Ca	Mn	Mg
(Un-RB)	1.04 c	4.5 a	46.45 c	9.34 b	667.69 c
(DH-RB)	7.35 b	2.5 b	59.75 a	9.96a	739.62 a
(MW-RB)	10.22 a	1.78 c	52.50 b	9.83 a	728.33 b

Values followed by different letters within same columns are significantly different at P = 0.05 with Duncan's multiple range test. Untreated rice bran (Un-RB), Dry-heating (DH-RB) and Microwave heating (MW-RB)

In addition, iron levels varied within ranges 1.04 to 10.22 mg/100 g rice bran sample. Microwave rice bran had significantly higher iron content than other unstabilized and dry heat stabilized rice bran. The levels of calcium in the bran ranged from 46.45 to 59.75 mg/100g rice bran sample. The highest calcium content was observed in dry heat rice bran (59.75mg/100g). The manganese content of rice bran samples was 9.34 to 9.96 mg/100g. The highest manganese content was observed in dry heat rice bran (9.96 mg/100g). The magnesium content of rice bran samples was 667.69 to739.62 mg/100g. The highest magnesium content was observed in dry heat rice bran which valued 739.62 mg/100g .The zinc levels varied from 1.78 to 4.5 mg/100 g rice bran sample. Unstabilized rice bran had significantly higher zinc content (4.5 mg/100g) than both dry heat stabilized rice bran and microwave rice bran. The obtained results agree partially with those of **Rosniyana *et al.* (2012) and Abd El-Galeel and El-Bana (2012)**.

Effect of Stabilization Method on Vitamin B Complex Content (mg/kg) of Rice Bran

Rice bran is a rich source of many nutrients, especially vitamins B. **Saunders (1985)** reported that the bran–germ–polish component contains 78% of the rice kernel thiamine, 47% of the riboflavin. The variation of vitamin content reflects the degree of polishing to get the different fraction of the rice bran **(Hammond, 1994)**. Vitamin contents of rice bran are shown in Table 3 which is present in varied amounts of B complex vitamin particularly B1 and B2. The stabilized rice bran (dry-heating and microwave heating) contain the lowest contents of vitamin B1and B2 which were (29.0 and 28.8%) and (2.69 and 2.37%), respectively compared to unstabilized rice bran (29.7%) and (2.72%) for vitamin B1and B2 respectively. The obtained results agree partially with those of **(Rosniyana *et al.* (2009) and Mohammed *et al.* (2014)**.

Effect of Stabilization Method on Total Phenolic Content (mg/g) and Antioxidant Activity (%) of Rice Bran

Total phenolic contents (TPC) and antioxidant activity (AA) as DPPH of methanolic extract are shown in Table 4. Results indicated that TPC content ranged from 7.40 to 9.25 (mg/g). The highest TPC (9.25) was observed in microwave stabilized rice bran, otherwise AA ranged from 66.43 to 70.45%. The highest AA content was observed in microwave stabilized rice bran (70.45%). These results agreed with **Aleksander *et al.* (2008)** findings. **Amonrat (2012)** suggested that heating methods also provided higher contents of total phenolic compounds and flavonoid also increased the antioxidant activities. Roasting increased the total phenolic content (TPC) **(Neel and Fereidoon, 2011)**. The concentration of total phenolics in rice bran has been positively correlated with their antioxidants activity (**Stratil *et al.*, 2007; Muntana and Prasong, 2010; Laokuldilok *et al.*, 2011**). Several studies have been made concerning the relationship between the phenolic structure and antioxidant activity **(Faure *et al.*, 1990; Cuvelier *et al.*, 1992; Tsushida *et al.*, 1994)**.

Effect of Stabilization Method on the Free Fatty Acid (FFA) and Peroxide Value (PV) of Rice Bran

The obtained results are shown in Table 5. Rice bran oil normally contains 1.5 -2% FFA right

Table 3. Effect of stabilization methods on Vitamin B complex content of rice bran (mg/kg)

Treatment	Vitamin B1 (thiamine) (mg/kg)	Vitamin B2 (riboflavin) (mg/kg)
(Un-RB)	29.7 a	2.72 a
(DH-RB)	29 a	2.69 a
(MW-RB)	28.8 a	2.37 b

Values followed by different letters within same columns are significantly different at P = 0.05 with Duncan's multiple range test. Untreated rice bran (Un-RB), Dry-heating (DH-RB) and Microwave heating (MW-RB)

Table 4. Effect of stabilization methods on total phenolic content (mg/g) and antioxidant activity (%) of rice bran

Treatment	Total phenolic Content (TPC) (mg/g)	Antioxidant activity (AA) (%)
(Un-RB)	7.40 c	66.43 b
(DH-RB)	8.52 b	67.71 b
(MW-RB)	9.25 a	70.45 a

Values followed by different letters within same columns are significantly different at P = 0.05 with Duncan's multiple range test. Untreated rice bran (Un-RB), Dry-heating (DH-RB) and Microwave heating (MW-RB)

Table 5. Effect of stabilization method on the free fatty acid (FFA) and peroxide value contents (PV) of rice bran

Treatment	FAA (%)	PV (meq/kg oil)
(Un-RB)	1.96 a	8.73 a
(DH-RB)	1.53 b	7.50 b
(MW-RB)	1.35 c	6.86 c

Values followed by different letters within same columns are significantly different at P = 0.05 with Duncan's multiple range test. Untreated rice bran (Un-RB), Dry-heating (DH-RB) and Microwave heating (MW-RB)

after milling. Less than 5% FFA is desirable in the crude oil for economic refining purposes **(Enochian *et al.*, 1980)**. FFA contents are the most important measure of rice bran stabilization. Rice bran with more than 5% FFA is considered to be unsuitable for human consumption **(Tao *et al.*, 1993; Malekian *et al.*, 2000)**. Rice bran contains lipases, primarily responsible for the hydrolysis of triglycerides into glycerol and free fatty acids; further oxidized by peroxidases, provoking bran's rancidity. In present study, increase in FFA was used as criterion of lipase activity. The highest FFA level was observed in unstabilized rice bran (1.96%) compared to dry heat stabilized rice bran (1.53%) and microwave stabilized rice bran (1.35%). Hence, stabilizing the bran just after milling can prevent oil deterioration. These results are in agreements with results reported by **Sangle *et al.* (2016)**. The variance of FFA content may be due to differences in rice cultivar, FFA determination method and/or milling and storage conditions. Heating in the presence of moisture is much more effective in permanently denaturing lipases **(Ramezanzadeh *et al.*, 2000)**. In the same Table, the PV content of untreated rice bran measured immediately after milling was found to be 8.73 meq/kg, decreased in dry heating to be 7.50 meq/kg and the FFA level in microwave heating was found to be 6.86 meq/kg. The results of this study suggested that dry heating and microwave heating were the most effective methods for stabilization of rice bran and lowering FFA and PV. These results are in agreements with those reported by **Amonrat *et al.* (2012)**.

Conclusion

Rice bran is a good source of large quantity of essential nutrients such as minerals, vitamins, fiber, amino acids and antioxidants. Considering the importance of rice bran, it can serve as an important raw material for the development of nutraceuticals and functional foods. Owing to numerous health benefits associated with the consumption of rice bran, the increase in the shelf life which achieved by stabilization with different techniques is promising good. This study can help predict the best method for stabilization to enhance the supplementation of rice bran in various food systems.

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تأثيـــر بعــض طــرق التثبيت علــــى التــركــيب الغــذائــــى لنخــالــــــة الأرز

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نخالة الأرز هى منتج ثانوى من ضرب الأرز وتمثل حوالى ١٠% تقريبا من الوزن الكلى للأرز الشعير (الخام) وتتكون من أغلفة القشرة الداخلية وطبقة الأليرون وجزء من الإندوسبرم، ونخالة الأرزهى مصدر غنى بالفيتامينات والمعادن والأحماض الدهنيه الأساسيه والألياف الغذائيه وغيرها من الأستيرولات، ومع ذلك فإن استخدامها محدود وهذا يرجع إلى وجود انزيم الليبيز والليبوكسيجينيز الذى يسبب التزنخ عند عملية ضرب الأرز، والهدف الرئيسى من هذا البحث هو بيان التركيب الغذائى لنخالة الأرز والتى تم معاملتها بعملية التثبيت بالحرارة عن طريق كلا من فرن الميكرويف والحرارة الجافة باستخدام الفرن الكهربى المنزلى والتى يمكن الاستفادة منها كعنصر غذائى وظيفى، ولقد قيمت الدراسة أيضا عمليتي التثبيت بالحرارة على النخالة لكلا من قيم الأحماض الدهنية الحرة وقيم البيروكسيد وأظهرت النتائج أن الحرارة الجافة والميكرويف من أكثر الطرق فاعليه لتثبيت نخالة الأرز مع انخفاض محتوى الأحماض الدهنيه ورقم البيروكسيد كما توفر هذه الطرق محتوى مرتفع من مركبات الفينول الكلية ومضادات الأكسدة، ولقد أوضحت الدراسة أن المعاملة الحرارية بالميكرويف غير مناسبة فى المناطق الريفية ولكن المعاملة الحرارية بالفرن هى طريقه فعاله لعملية التثبيت ويمكن تنفيذها فى المناطق الريفية.

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