Influence of Stabilization Methods on Rice Bran Oil of Some Egyptian Rice Cultivars

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ABSTRACT

Newly harvested certified seeds in 2015 and 2016 growing seasons of five rice cultivars namely, Hybrid 1, Sakha 106, Giza 178, Giza 182 and Yasmine were provided by Rice Research Program, Sakha, Kafr El-Sheikh, Field Crop Research Institute, Agriculture Research Center, Egypt. A split plot design with three replicates was used. The main plots were devoted to rice cultivars and the sub plots were occupied by stabilization methods (unstabilized, stabilization by microwave, stabilization by hot air and stabilization by steaming). The results showed significant differences between cultivars for most studied characters. Yasmine rice cultivar gave the highest values for crude protein, crude fiber and Vitamin E contents, while Giza 178 rice cultivar showed the highest values for carbohydrates, crude oil and total phenolic contents in both seasons of study. In addition, stabilization by microwave showed superior values for crude oil, iodine value, total phenolic contents and vitamin E contents. Interaction between cultivars and stabilization methods indicated that using Giza 178 rice cultivar with microwave stabilization gave highest crude oil percentage.

Keywords: Rice, bran, bran oil, stabilization methods, cultivars, vitamin E, total phenolics

INTRODUCTION

In Egypt, the cultivated area of rice is about 1.3 million feddan which annually produces 5.36 million tons paddy rice producing 0.5 million tons of rice bran that could produce approximately 0.1 million ton of crude oil every year (RRTC, 2015).

Rice bran (RB), a natural byproduct, or co-product, obtained from the milling process of rice, is the external layer of rice grain. It contains seed coat layers and germ with few amounts of broken endosperm. The bran, being an important by-product, is mainly produced during rice milling operation and it's about 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 (Pourali *et al.*, 2009 and Elizabeth, 2011). Rice bran is a rich source of nutrients, bioactive compounds and a good source of fat, Since rice bran contains a high fat content (15-25%), rapid hydrolytic rancidity of crude fat catalyzed by lipase enzyme occurs immediately after milling process and yields free fatty acids (FFA) and glycerol, resulting in a drastic quality reduction and unsuitability of rice bran for human consumption (Lakkakula *et al.*, 2004).

Rice bran contains high nutritional components as well as phytochemicals such as Vitamin E (Tocols) or (tocopherols and tocotrienols) and gamma- oryzanol fraction that reportedly may have positive effects on human health These bioactive compounds have antioxidant activities and various beneficial effects on blood pressure, lipid profiles, glucose metabolism, and coronary management. Stabilized rice bran is sold as a health food in supermarkets or added by food manufacturers as an ingredient in foods such as crisp breads, cookies and breakfast cereals (Shirakawa *et al*., 2006 and Abd El Bary, 2012). Inactivation of lipase enzyme and inhibition of FFA formation must be carried out immediately after milling. Various methods, applied to stabilize rice bran, have been reported such as hot air drying, steaming, fluidization, refrigeration, chemical stabilization , microwave heating, and ohmic heating (Loypimai *et al.,* 2009).Significant increase in total phenolic compounds in rice bran oil depends on stabilization method where it was 13.38 mg GAE/g rice bran oil for unstabilized rice bran, 15.46 mg GAE/g rice bran oil for steaming stabilization, 17.93 mg GAE/g rice bran oil at hot air stabilization and 18.62 mg GAE/g rice bran oil for microwave stabilization. The

highest crude oil yield extraction was obtained by using microwave stabilization and was the best methods of stabilization as it gave the highest stability of rice bran oil (Marei, 2016). Rice bran oil (RBO) contains oleic (36-38%), linoleic (35-38%) and α-linolenic (1.8-2.4%) acids as unsaturated fatty acids and palmitic (21-25%) and stearic (2.7 - 3.0%) acids as saturated fatty acids (Gopala *et al*., 2005; Rubalya *et al*., 2010).

Rice bran oil (RBO) is generally considered to be one of the high quality vegetable oils in terms of its cooking attributes, shelf life, fatty acid composition and stability at higher temperatures. RBO enhances the taste and flavour of food items while reducing the absorption of oil during frying. Rice bran oil (RBO) is used in the manufacture of food products such as mayonnaise and salad dressings. In addition, RBO improves skin tone and delays wrinkle formation, helps hair protection against premature graying and strengthen hair roots when topically applied. Therefore, RBO is used as an ingredient in many cosmetics (Arab, *et al*., 2011). Rice bran oil has better oxidative stability than its competitive products such as soybean oil or cotton seed oil because of its high tocopherols and low linolenic acid content (Patel and Naik, 2004). The main drawback of rice bran is a fast oxidation reaction due to the high content of unsaturated fatty acid in its oil content. This is due primarily to the presence of endogenous enzyme lipase which caused the pro-oxidative mechanisms of oxidation leading to hydrolytic rancidity on the oil content that hydrolyze the ester bounds of triacylglycerol, releasing fatty acids and glycerol and forming of hydroperoxides (Yoshida *et al*., 2011). Rice bran oil contains tocotrienols and squalene that have powerful anti-cancer and anti-ageing properties (Sierra *et al*., 2005). Taking advantage of the valuable fact that micronutrient levels are so adequate in rice bran oil, it was considered that value of any other edible oil could be remarkably increased by addition of even small amounts of rice bran oil (Adhikari, 2002). The present investigation aimed to determine effect of stabilization methods on chemical composition of rice bran, stability and bioactive components of rice bran oil in some Egyptian rice cultivars.

MATERIALS AND METHODS

Newly harvested certified seeds in 2015 and 2016 growing seasons of five rice cultivars namely, Hybrid 1, Sakha 106, Giza 178, Giza 182 and Yasmine were provided by Rice Research Program, Field Crop Research Institute, Agriculture Research Center, Sakha, Kafr El-Sheikh, Egypt. A split plot design with three replicates was used. The main plots were devoted to rice cultivars and the sub plots were occupied by stabilization methods (unstabilized, stabilization by microwave, stabilization by hot air and stabilization by steaming).

Preparation of rice bran:

The paddy rice samples were cleaned by Dockage Tester Machine (Carter Day CO, model number XT3, USA) to remove the dust, foreign matter, mud balls, and immature green grains automatically. Drying by hot air was performed using rotary dryer Schule, Germany, thirty 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 packed in polyethylene bags. Bags were sealed (double sealing), then they were stored at -18^{0}C until used.

The percentage of bran for each cultivar was as follows: 9.25%, 9.63%, 9.81%, 9.52% and 8.89% for Hybrid 1, Sakha 106, Giza 178, Giza 182 and Jasmine, respectively.

Stabilization of rice bran:

The bran was stabilized according to the optimum methods explained by (Thanonkaew *et al*., 2012): Method (1): Heating rice bran in microwave at 850 W for 3 minutes. Method (2): Heating the bran with hot air at 150^{0}C for 10 min. Method (3): Heating the bran with steaming at 130^{0}C for 60 min. After cooling, bran was stored in polyethylene bags until used

Analytical methods:

1- Proximate chemical composition: Proximate composition of raw and stabilized rice bran including crude oil, crude protein, crude fiber and ash were carried out according to the AOAC (1990). Carbohydrates were calculated by difference.

2- Crude oil: Crude rice bran oil was extracted by cold extraction technique using n-hexane. The solvent was evaporated under reduced pressure. Percentage of free fatty acids (as oleic acid), iodine and peroxide values were determined according to AOAC (1990).

3- Total phenolic content**:** Total phenolics of rice bran oil were extracted by methanol according to the method described by Steel *et al.* (2005). The total phenolics was determined colorimetrically using Folin–Ciocalteu's reagent method as described by Singleton *et al.* (1999)

4- Determination of vitamin E: Rice bran (1g) was extracted. Prior to HPLC analysis, the extracts were filtered through a 0.45 mm syringe filter. Tocopherol and tocotrienol was determined, 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 used. Detection was operated at 292 nm. 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 tocopherol and tocotrienol were detected at 292 nm. Chromatograms were recorded, and peak areas were used to calculate the content of tocopherol and tocotrienol, against the standard curve of standards.

Analysis of variance and correlation were carried out according to Gomez and Gomez (1984) using SAS program, version 8. Means were compared using least significant differences (LSD) at 0.05 level of probability.

Results and discussion

1- Effect of rice cultivars:

Performance of the studied rice cultivars, is presented in Tables (1, 2, 3 and 4). Data revealed that there were significant differences between rice cultivars for all studied characters except for ash %, peroxide value and free fatty acids %. Comparison between means showed that rice cultivar Yasmine gave the highest values for crude protein (14.72 and 14.30 %), crude fiber (8.45 and 8.87 %) and Vitamin E (Tocols) (754.9 and 782.1 µg/g). while, it showed the lowest values (47.75 and 47.83 %) for carbohydrates in 2015 and 2016 seasons, respectively. Furthermore, Giza 178 rice cultivar showed the highest values for crude oil (20.55 and 20.80 %), carbohydrates (48.88 and 49.65 %) and total phenolic contents (16.31 and 16.64 mg GAE/g oil) while, it showed the lowest values for crude protein (13.89 and 13.12 %) and crude fiber (8.25 and 8.03 %) in 2015 and 2016 seasons, respectively. Moreover, the highest values for iodine (92.23 and 92.65) was recorded with Hybrid 1 rice cultivar while, it showed the lowest values for vitamin E (738.5 and 715.2 µg/g) in 2015 and 2016 seasons, respectively. Sakha 106 gave the lowest values (19.92 and 20.06 %) for crude oil and iodine value (90.61 and 90.92 %) in 2015 and 2016 seasons, respectively. Variation between rice cultivars might be due to genetic factors (Abd EL Bary, 2012)

2- Effect of stabilization methods:

Data in Tables (1, 2, 3 and 4) showed that using different stabilization methods significantly affected most studied characters except for ash %. Unstabilized method showed the highest

values for crude protein (14.59 and 14.08 %), carbohydrates (49.95 and 50.38 %), crude fiber (8.60 and 8.75 %), peroxide value (9.37 and 9.89 meq/kg oil) and free fatty acids (4.71 and 4.92 %) while, it showed the lowest values for crude oil (18.33 and 18.42 %), iodine value (90.26 and 90.65), total phenolic content (13.26 and 13.48 mg GAE/g oil) and vitamin E (704.5 and 711.6 µg/g) in 2015 and 2016 seasons, respectively. This is might be due to the fast oxidation reaction of rice bran due to the high content of unsaturated fatty acid in its oil content. This is due primarily to the presence of endogenous enzyme lipase which caused the pro-oxidative mechanisms of oxidation leading to hydrolytic rancidity of the oil content that hydrolyze the ester bounds of triacylglycerol, releasing fatty acids and glycerol and forming of hydroperoxides (Yoshida *et al.,* 2011). Furthermore, stabilization using microwave showed superiority values for crude oil (21.10 and 21.31 %), iodine value (92.18 and 92.61), total phenolic content (18.50 and 18.26 mg GAE/g oil) and vitamin E (780 and 785.5 µg/g) while, it recorded the lowest values for carbohydrates (47.73 and 47.91 %), peroxide value (6.31 and 6.10 meq/Kg oil) and free fatty acids (2.70 and 2.85 %) in 2015 and 2016 seasons, respectively. The increase in crude oil % from (18.33 and 18.42 %) in case of unstabilized rice bran to (21.10 and 21.31 %) in case of microwave could be attributed to the effect of temperature on protein as it was denaturated and facilitate oil separation. These results were in agreement with Marei (2016). Moreover, decreased values of peroxide, free fatty acids and increased values of bioactive components (total phenolic contents and vitamin E) by using microwave method might be due to that heat could penetrate and effectively destroy lipase enzyme and combined with the prevention effect of rice bran to retard the development of oxidation products in rice bran so oil can be stored for long time due its high stability. These results were in harmony with those reported by (Abd El Bary 2012 and Marei 2016).

Table (1): Influence of stabilization methods on proximate chemical composition of rice bran for five rice cultivars grown during 2015 season. (On dry weight basis)

Treatments	Crude oil %	Crude protein %	Carbohydrates %	Crude fiber %	Ash %
C- (Cultivars)					
Hybrid 1	20.12	14.41	48.84	8.27	8.36
Sakha 106	19.92	14.39	48.85	8.38	8.46
Giza 178	20.55	13.80	48.88	8.25	8.52
Giza 181	20.37	14.46	48.26	8.35	8.55
Yasmine	20.45	14.80	47.75	8.45	8.53
$L.S.D._{0.05}$	0.096	0.150	0.028	0.058	n.s
(M) – Methods of stabilization					
Unstabilized	18.33	14.59	49.95	8.60	8.52
Microwave	21.10	14.34	47.73	8.34	8.47
Hot air	20.98	14.30	48.09	8.19	8.43
Steaming	20.70	14.25	48.29	8.23	8.52
$L.S.D._{0.05}$	0.220	0.045	0.326	0.080	n.s
Interaction					
C x M	**	n.s	n.s	n.s	n.s

Table (2): Influence of stabilization methods on proximate chemical composition of rice bran for five rice cultivars grown during 2016 season. (On dry weight basis)

Treatments	Crude oil %	Crude protein %	Carbohydrates %	Crude fiber %	Ash %
C- (Cultivars)					
Hybrid 1	20.29	14.08	49.25	8.11	8.27
Sakha 106	20.06	13.75	49.32	8.50	8.37
Giza 178	20.80	13.12	49.65	8.03	8.40
Giza 181	20.58	13.95	48.58	8.59	8.30
Yasmine	20.54	14.30	47.83	8.87	8.46
$L.S.D._{0.05}$	0.037	0.175	0.052	0.076	n.s
(M) – Methods of stabilization					
Unstabilized	18.42	14.20	50.38	8.75	8.25
Microwave	21.31	13.94	47.91	8.50	8.34
Hot air	21.09	13.76	48.45	8.33	8.37
Steaming	20.92	13.45	49.06	8.10	8.47
$L.S.D._{0.05}$	0.141	0.156	0.421	0.152	n.s
Interaction					
C x M	**	n.s	n.s	n.s	n.s

Table (3): Influence of stabilization methods on crude oil stability and bioactive components of rice bran oil for five rice cultivars grown during 2015 season.

Treatments	Iodine value	Peroxide value (meq/Kg oil)	Free fatty acids (%)	Total phenolic content (mg GAE/g oil)	Vitamin E (Tocols) (µg/g oil)
C- (Cultivars)					
Hybrid 1	92.23	7.18	3.17	16.07	738.5
Sakha 106	90.61	7.17	3.36	16.10	743.4
Giza 178	91.29	7.35	3.33	16.31	750.4
Giza 181	91.50	7.28	3.21	16.20	751.5
Yasmine	90.91	7.38	3.30	16.22	754.9
$L.S.D._{0.05}$	0.198	n.s	n.s	0.085	3.097
(M) – Methods of stabilization					
Unstabilized	90.26	9.37	4.71	13.26	704.5
Microwave	92.18	6.31	2.70	18.50	780.0
Hot air	91.74	6.63	2.80	17.58	772.8
Steaming	91.05	6.78	2.89	15.39	733.7
$L.S.D._{0.05}$	0.238	0.148	0.097	0.100	4.027
Interaction					

C x M	n.s	n.s	n.s	*	n.s

Table (4): Influence of stabilization methods on crude oil stability and bioactive components of rice bran oil for five rice cultivars grown during 2016 season.

Treatments	Iodine value	Peroxide value (meq/Kg oil)	Free fatty acids (%)	Total phenolic content (mg GAE/g oil)	Vitamin E (Tocols) (µg/g oil)
C- (Cultivars)					
Hybrid 1	92.65	7.29	3.35	16.34	715.2
Sakha 106	90.92	7.35	3.50	16.18	728.5
Giza 178	91.66	7.42	3.42	16.64	763.4
Giza 181	91.87	7.34	3.36	16.53	769.8
Yasmine	91.32	7.46	3.45	16.39	782.1
$L.S.D._{0.05}$	0.174	n.s	n.s	0.042	4.881
(M) – Methods of stabilization					
Unstabilized	90.65	9.89	4.92	13.48	711.6
Microwave	92.61	6.10	2.85	18.26	785.5
Hot air	92.10	6.74	2.93	17.96	760.6
Steaming	91.44	6.88	2.98	15.94	750.3
$L.S.D._{0.05}$	0.173	0.122	0.066	0.211	6.524
Interaction					
C x M	n.s	n.s	n.s	*	n.s

3- Interaction between rice cultivars and different stabilization methods:

Data in Table (5) showed significant differences for the interaction between rice cultivars and different stabilization methods for crude oil and total phenolic contents. Data revealed that using Giza 178 rice cultivar and microwave stabilization gave highest values for crude oil (21.71 and 21.87 %) while, the highest total phenolic contents, which are considered one of bioactive components (18.75 and 18.60 mg GAE/g oil) was recorded for Yasmine rice cultivar and microwave stabilization in 2015 and 2016 seasons, respectively.

Conclusion

The present study indicated that using Giza 178 rice cultivar with microwave stabilization gave highest values for crude oil percentages. Also, data revealed that Yasmine rice cultivar with microwave stabilization gave highest value for total phenolic compounds which are considered as one of bioactive components that have antioxidant activities and various beneficial effects on blood pressure, lipid profiles, glucose metabolism, and coronary management.

Table (5): Mean value for crude oil % and total phenolic contents as affected by the interaction between rice cultivars and stabilization methods in 2015 and 2016 seasons. (On dry weight basis)

Cultivars	Methods of stabilization	Crude oil %		Total phenolic contents (mg GAE/g oil)	
		2015	2016	2015	2016
Hybrid 1	Unstabilized	18.36	18.75	13.15	13.36
	Microwave	20.53	20.87	18.25	18.02
	Hot air	20.95	21.15	17.52	17.88
	Steaming	20.63	20.40	15.38	15.90
Sakha 106	Unstabilized	18.42	18.17	13.13	13.25
	Microwave	20.39	20.86	18.33	18.10
	Hot air	20.48	20.55	17.61	17.89
	Steaming	20.38	20.65	15.33	15.51
Giza 178	Unstabilized	18.31	18.55	13.43	13.70
	Microwave	21.71	21.87	18.62	18.35
	Hot air	21.48	21.46	17.79	18.26
	Steaming	20.69	21.30	15.39	16.25
Giza 181	Unstabilized	18.21	18.39	13.37	13.65
	Microwave	21.32	21.50	18.57	18.22
	Hot air	21.04	21.27	17.42	17.90
	Steaming	20.92	21.15	15.45	16.34
Yasmine	Unstabilized	18.43	18.25	13.24	13.43
	Microwave	21.34	21.47	18.75	18.60
	Hot air	21.17	21.33	17.54	17.85
	Steaming	20.87	21.11	15.37	15.68
L.S.D.$_{0.05}$		0.064	0.035	0.104	0.072

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الملخص العربي

تاثير طرق التثبيت علي زيت رجيع الكون لبعض أصناف الأرز المصرية

خالد مصطفى حمدى عبدالسلام

تم حصاد التقاوى المعتمدة في موسمي زراعة ٢٠١٥ و٢٠١٦ لخمسة أصناف أرز شعير وهي هجين ١، سخا ١٠٦، جيزة ١٧٨، جيزة ١٨٢ و ياسمين من برنامج بحوث الأرز، معهد بحوث المحاصيل الحقلية، مركز البحوث الزراعية، سخا، كفرالشيخ، مصر.و قد تم إستخدام تصميم القطع المنشقة بثلاث مكررات حيث وزعت أصناف الأرز في القطع الرئيسية بينما كانت طرق تثبيت رجيع الكون الاربع في القطع الفرعية (رجيع الكون غير مثبت، رجيع الكون مثبت بالميكروويف، رجيع الكون مثبت بالهواء الساخن و رجيع الكون مثبت باستخدام بخار الماء). و أوضحت النتائج وجود فروق معنوية بين الأصناف بالنسبة لمعظم الصفات المدروسة. وكانت أعلى القيم لنسبة البروتين الخام، نسبة الألياف الخام وفيتامين E لصنف الأرز ياسمين وكانت أعلى القيم لنسبة الكربوهيدرات، نسبة الزيت الخام، والمواد الفينولية الكلية لصنف الأرزجيزة ١٧٨ . وبالإضافة إلى ذلك، كانت الطريقة المثلى لتثبيت رجيع الكون هي التثبيت بالميكروويف الذي أدى إلى وجود زيادة في الزيت الخام،الرقم اليودي، والمواد الفينولية الكلية وفيتامين E. أوضحت التفاعلات بين الاصناف وطرق التثبيت أن إستخدام صنف الارز جيزة ١٧٨ و التثبيت بالميكروويف أدى الى زيادة فى نسبة الزيت الخام.