

Effect of Different Methods for Rice Bran Oil Extraction on Crude Oil %, Oil Quality and Bioactive Components of Some Rice Varieties

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ABSTRACT

Rice grains of ten rice varieties namely, Giza177, Giza 178, Sakha 101, Sakha 102, Sakha 104, Sakha 106, Giza 182, Yasmine, Sk2034(H1), and SK2046 (H2) were provided by Rice Research Program, Field Crop Research Institute, Agriculture Research Center, Sakha, Kafr El-Sheikh, Egypt during 2018 and 2019 growing seasons. The experiment was laid out as split – plot design in a randomized complete block design (RCBD) with three replicates. The main – plot was devoted to tested ten rice varieties while sub – plot devoted to three different methods for rice bran oil extraction (hexane, isopropanol and screw press). The results were recorded on (hexane, isopropanol and screw press) as recorded by SES 2008 , were revealed that, there are significant difference among rice cultivars for all the studied characters, except peroxide value and free fatty acids%. Comparison between means showed that, Giza 178 variety gave the highest values for crude oil%, total phenolic content, tocotrienols, tocopherols and gama oryzanol in both studied seasons. Furthermore, Yasmin rice cultivar showed the highest values for saponification number in both seasons. The highest iodine value was recorded with Sakha 104 and SK 2034 H1 varieties in 2018 and 2019 seasons, respectively. Extraction of rice bran oil by isopropanol showed the highest values for crude oil%, iodine value, total phenolic content, tocotrienols, tocopherols and gama oryzanol in 2018 and 2019 seasons. Interaction between rice cultivars and different methods for extraction of rice bran oil revealed that using Giza 178 variety and extraction of rice bran oil by isopropanol solvent gives the highest values of crude oil %, total phenolic content, tocotrienols ,tocopherols and gama oryzanol in 2018 and 2019 seasons. Correlation coefficient was highly significant and positive between crude oil and tocotrienols highly significant and positive correlation were found among total phenolic and each of tocotrienols, tocopherols and gama oryzanol ,indicated that selection for total phenolic components enhancement each of tocotrienols, tocopherols and gama oryzanol. Finally, using Giza 178 as a donor can improve bran oil content in hybrid rice program.

Keywords: Rice, bran, bran oil, extraction methods, cultivars, Tocotrienols, Tocopherols and total phenolic compounds.

INTRODUCTION

In Egypt, the cultivated area of rice is about 1.3 million feddan which annually produces 5.27 million tons paddy rice producing 0.5 million tons of rice bran that could produce approximately 0.07 million tons of crude oil every year (RRTC, 2020). Rice bran is the main by product from rice processing industry. During the whitening process, which applies friction to the grain surface, the bran is removed from the kernel and usually accounts for about 10% of raw rice weight (Bhatnagar *et al.* 2014). Rice bran oil (also known as rice bran extract) is extracted from the germ and inner husk of rice, commonly called rice bran. The rice bran contains 10–23% oil and (unlike oat bran) negligible amounts of water-soluble glucans and larger amounts of insoluble dietary fiber. It is notable for its high smoke point of 232 °C (450 °F) and its mild flavor, making it suitable for high temperature cooking methods such as stir frying and deep frying. It is popular cooking oil in several Asian countries, including Japan and China. Many people have replaced their usual cooking oils with rice bran oil due to its higher nutritional value. It is the by-product of rice processing, accounted for 5-8% in grain weight. Rice bran contains grease (12-24%), protein (12-15%), cellulose (9.8-10%), vitamins (VB1, VB2, VD) and minerals. Rice bran oil, contains the unsaturated fatty acids, accounted for 38-42% oleic acid and 32-35% linoleic acid. The linoleic acid is widely recognized as an essential fatty acid and decreased the blood cholesterol, prevents atherosclerosis and other health effects, so the rice bran oil is a kind of ideal edible vegetable oil (Wang, 2006; Marlene *et al.*, 2005). High efficiency and maximize extracted oil components from rice bran become research hotspots in oil industry (Ghosh, 2007 and Xing *et al.*, 2011). Rice bran oil is recognized as a type of healthy oil due to its content of palmitic (13.9–22.1 %), oleic (35.96–49.2 %), and linoleic acids (27.3–41.0 %) This oil also contains 95.6 % saponifiable lipids, such as glycolipids and phospholipids, and 4.2 % unsaponifiable lipids, such as tocopherols, tocotrienols, γ -oryzanol, sterols and caroteneoids. The tocopherol and tocotrienol content can be as high as 0.10–0.14 %, whereas the oryzanol

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content ranges from approximately 0.9–2.9 %. Rice bran oil (RBO) is one of the best sources of tocopherols and oryzanol. Tocopherols (tocopherols and tocotrienols), a family of vitamin E-active substances, are widely used plant-based ingredients in the food, cosmetics and pharmaceutical industries (Bramley *et al.*, 2000; Abidi 2003). Studies suggested that tocotrienols are more efficient antioxidant, anti-cancer agent and inhibitor of cholesterol synthesis than the tocopherols (Singh *et al.*, 2013). Gamma-oryzanol is one of the major components of RBO, and it is a mixture of several ferulate esters of triterpene alcohols and plant sterols (Friedman, 2013). Oryzanol is a well known antioxidant compound and is linked with decreasing serum and plasma cholesterol, decreasing platelet aggregation, and cholesterol absorption. Moreover oryzanol has been used in the treatment of hyperlipidemia, and disorders of menopause (Patel & Naik 2004). Rice bran oil is unique among edible oils due to its rich source of nutritionally important phytochemicals such as oryzanol, tocotrienols, tocopherols, etc. γ – Oryzanol is one of the components of rice bran oil which have the potential to be used in pharmaceuticals, cosmeceutical and nutraceutical. It is present in rice bran oil at a level of 1-2% where it serves as natural antioxidant. Rice bran oil is a rich source of natural antioxidants which can be used as free radical scavengers. It contains about 0.1–0.14% vitamin E components. The rice bran oil antioxidants are very efficient in reducing low density lipoprotein and total serum cholesterol. The present investigation aimed to determine effect of different methods of rice bran oil extraction on crude oil %, its quality and bioactive components in some Egyptian rice cultivars.

MATERIALS AND METHODS

Rice grains of ten rice varieties namely, Giza177, Giza 178, Sakha 101, Sakha 102, Sakha 104, Sakha, 106 Giza 182 ,Yasmine, Sk2034(H1),and SK2046 (H2) were provided by Rice Research Program, Field Crops Research Institute, Agriculture Research Center, Sakha, Kafr El-Sheikh, Egypt during 2018 and 2019 growing seasons. The experiment was laid out as split – plot design in a randomized complete block design (RCBD) with three replicates. The main – plot was devoted to tested ten rice varieties while, sub – plot devoted to three different methods for rice bran oil extraction (hexane, isopropanol and screw press). Bran percentages of each cultivar were: 9.31, 9.65, 8.77, 9.51, 9.11, 9.46, 9.40, 8.75, 9.18 and 9.05, respectively. The bran was stabilized by heating rice bran in microwave at 850 W for 3 minutes according to the optimum methods explained by (Thanonkaew *et al.*, 2012).

Analytical methods:

- 1- Oil extraction from rice bran samples:
 - 1.1 Solvent extraction: rice bran oil was extracted by cold extraction technique using hexane and isopropanol according to (Chen and Bergman 2005).
 - 1.2 Screw press extraction: rice bran oil was extracted by screw press according to (Amarasinghe and Gangodavilage 2004).
- 2- Percentage of free fatty acids (as oleic acid), iodine, saponification number 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) and 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 were 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 μ m) (Phenomenex, USA). Gradient elution was then applied. Mobile phases A, B, and C were methanol, water and ethanol, 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 μ L. 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 simple correlation coefficient were carried out and computed 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 DISCUSSIONS

1- Effect of rice cultivars:

Performance of the studied rice cultivars is presented in Tables 1 and 2.

Data revealed that there were significant differences between rice cultivars for all studied characteristics, except peroxide value and free fatty acids% in the two seasons. Comparison among means performance showed that variety Giza 178 recorded the highest values of crude oil% (15.22 and 15.07 %), Total phenolic content (20.78 and 20.58 mg /GAE/g oil), Tocotrienols (531.34 and 532.31 mg/g), Tocopherols (329.96 and 331.32 mg/g) and gama oryzanol (21.31 and 21.42 mg/g) during the two seasons, respectively. Furthermore, Yasmin rice cultivar showed the highest values of saponification no. (188.33 and 188.03) in the first and second seasons, respectively. Moreover, the highest values of iodine (90.87 and 90.86) were recorded with Sakha 104 variety and SK 2034 H1 (90.86) in 2018 and 2019 seasons, respectively. Variation between rice cultivars might be due to their genetic background (Abd El Bary 2012 and Abd El Salam 2017).

2-Effect of different methods for rice bran oil extraction:

Data in Tables (1, 2) showed that the effect of different methods of extraction for rice bran oil extraction showed significant differences for most studied characteristics, except peroxide value and free fatty acids % in both studied seasons. Extraction of rice bran oil by isopropanol showed the highest values of crude oil% (19.55 and 19.39 %), iodine value (89.84 and 90.10), total phenolic content (22 and 21.75 mg GAE/g oil), tocotrienols (590.68 and 590.47 mg/g), tocopherols (362.83 and 361.71 m/g) and gama oryzanol (27.87 and 27.72 mg/g) in 2018 and 2019 seasons, respectively. However, the highest values of saponification number (203.74 and 203.64) were noticed with extraction of rice bran oil by hexane in both seasons, respectively. Peroxide value and free fatty acids didn't significantly affected with rice varieties in both seasons, due to extraction with hexane or isopropanol were done by cold extraction procedure which are similar to screw press procedure. These results were in agreement with Marei (2016). Furthermore, Extraction by isopropanol showed highest values for most studied characters due to increase in

polarity. These results were in harmony with those obtained by Marei (2016).

3- Interaction between rice cultivars and different methods for extraction of rice bran oil:

Data in Tables (3 and 4) showed significant differences for interaction between rice cultivars and different methods for extraction of rice bran oil in both studied seasons.

Data revealed that using Giza 178 rice cultivar with extraction of rice bran oil by isopropanol solvent recorded highest values for crude oil % (20.25 and 20.12 %), total phenolic content (22.87 and 22.66 mg GAE/ g oil), tocotrienols (595.94 and 596.38 mg/g), tocopherols (375.38 and 380.12 mg/g) and gama oryzanol (28.93 and 28.76 mg/g) in 2018 and 2019 seasons, respectively. On the other side, Sakha 106 variety combined with hexane method recorded the highest value for gama oryzanol (mg/g), while, the Giza 182 with hexane method recorded the lowest value for total phenolic content (mg GAE/g oil). Egyptian Yasmin with using Screw press method recorded the lowest value for crude oil %, Egyptian hybrid Sakha 2034H1 with using hexane method recorded the highest value for tocotrienols. Finally, Egyptian Hybrid H2 combined hexane method recorded the lowest value for tocopherols. From these results, it could be concluded that, to get highly quantity and quality of oil extraction of rice bran to different rice varieties, Isopropanol method should be used Zhu, *et al* (2010) and Rather, *et al* (2016).

Correlation coefficient

The data presented in Table (5) showed highly significant correlation among tocotrienols and each of crude oil% (0.47) and total phenolic content (0.882), while, tocopherols had highly significant and positive correlated with each of total phenolic content (0.943) and tocotrienols (0.981). Moreover, the highly significant and positive relationship was found between gama oryzanol (mg/g) and each of total phenolic content (0.958), tocotrienols (0.971) and tocopherols (0.985). These results indicated that selection for total phenolic content will enhancement the tocotrienols, tocopherols and gama oryzanol through the rice breeding program. Similar results were obtained by Verma *et al.*, (2015).

Table 3. Crude oil%, total phenolic content (mg GAE / g oil) and tocotrienols (mg / g) as affected by the interaction between rice cultivars and different extraction methods of rice bran oil in 2018 and 2019 seasons and combined data.

Cultivars	Methods of rice bran oil extraction	Crude oil (%)			Total phenolic content (mg GAE / g oil)			Tocotrienols (mg / g)		
		2018	2019	combined	2018	2019	combined	2018	2019	combined
Giza 177	Hexane	19.03	18.89	18.96	18.37	18.20	18.29	490.82	488.38	489.60
	Isopropanol	19.60	19.45	19.53	22.58	22.25	22.42	592.36	590.20	591.28
	Screw press	5.20	5.05	5.13	20.33	20.12	20.23	501.22	500.37	500.80
Giza 178	Hexane	19.57	19.41	19.49	18.75	18.58	18.67	494.81	495.72	495.27
	Isopropanol	20.25	20.12	20.19	22.87	22.66	22.77	595.94	596.38	596.16
	Screw press	5.85	5.69	5.77	20.72	20.51	20.62	503.26	504.82	504.04
Sakha 101	Hexane	18.25	18.16	18.21	18.26	18.11	18.19	489.31	488.39	488.85
	Isopropanol	19.03	18.88	18.96	22.30	22.15	22.23	590.42	588.60	589.51
	Screw press	4.56	4.41	4.49	20.25	20.06	20.16	500.31	498.72	499.52
Sakha 102	Hexane	19.41	19.28	19.35	18.14	17.91	18.03	488.32	485.20	486.76
	Isopropanol	20.12	19.96	20.04	22.35	22.12	22.24	591.35	588.11	589.73
	Screw press	5.66	5.49	5.58	20.07	19.76	19.92	498.26	497.14	497.70
Sakha 104	Hexane	18.62	18.50	18.56	17.88	17.50	17.69	489.57	487.26	488.42
	Isopropanol	19.35	19.21	19.28	21.93	21.66	21.80	588.20	590.37	589.29
	Screw press	4.87	4.62	4.75	19.65	19.42	19.54	497.51	498.66	498.09
Sakha 106	Hexane	19.28	19.11	19.20	17.60	17.40	17.50	490.31	488.45	489.38
	Isopropanol	19.83	19.65	19.74	21.76	21.43	21.60	589.11	587.27	588.19
	Screw press	5.42	5.21	5.32	19.82	19.56	19.69	498.37	496.20	497.29
Giza 182	Hexane	19.14	18.83	18.99	17.42	17.28	17.35	488.20	490.15	489.18
	Isopropanol	19.75	19.60	19.68	21.35	21.12	21.24	590.66	592.36	591.51
	Screw press	5.33	5.17	5.25	19.57	19.29	19.43	496.70	498.49	497.60
Yasmine	Hexane	18.11	17.90	18.01	18.26	18.13	18.20	487.11	490.56	488.84
	Isopropanol	18.85	18.67	18.76	22.12	21.87	22.00	591.72	590.13	590.93
	Screw press	4.36	4.20	4.28	20.14	19.76	19.95	490.55	488.32	489.44
Hybrid 1	Hexane	18.86	18.55	18.71	17.86	17.59	17.73	486.50	483.27	484.89
	Isopropanol	19.47	19.31	19.39	21.50	21.33	21.42	588.36	590.10	589.23
	Screw press	5.06	4.91	4.99	19.85	19.62	19.74	497.82	495.17	496.50
Hybrid 2	Hexane	18.48	18.3	18.39	17.44	17.30	17.37	485.32	488.36	486.84
	Isopropanol	19.21	19.02	19.12	21.25	20.90	21.08	588.72	591.20	589.96
	Screw press	4.70	4.52	4.61	19.34	19.10	19.22	498.20	496.48	497.34
L.S.D		0.01	0.02	0.01	0.02	0.01	0.02	1.31	1.88	1.59

Table 4. Tocopherols (mg / g) and gama oryzanol (mg / g) as affected by the interaction between rice cultivars and different extraction methods of rice bran oil in 2018 and 2019 seasons and combined data.

Cultivars	Methods of rice bran oil extraction	Tocopherols (mg / g)			Gama oryzanol (mg / g)		
		2018	2019	combined	2018	2019	combined
Giza 177	Hexane	295.37	293.21	294.29	14.81	14.50	14.66
	Isopropanol	370.21	366.80	368.51	27.90	27.63	27.77
	Screw press	312.45	305.62	309.04	19.22	19.07	19.15
Giza 178	Hexane	298.89	296.38	297.64	15.31	15.59	15.45
	Isopropanol	375.38	380.12	377.75	28.93	28.76	28.85
	Screw press	315.62	317.45	316.54	19.68	19.92	19.80
Sakha 101	Hexane	293.72	290.50	292.11	15.11	14.88	15.00
	Isopropanol	371.86	368.35	370.11	28.13	27.82	27.98
	Screw press	307.21	302.82	305.02	19.25	19.02	19.14
Sakha 102	Hexane	290.65	287.30	288.98	14.81	14.60	14.71
	Isopropanol	365.92	361.47	363.70	27.90	27.55	27.73
	Screw press	305.81	301.28	303.55	19.03	18.76	18.90
Sakha 104	Hexane	296.65	290.82	293.74	14.66	14.91	14.79
	Isopropanol	360.82	356.13	358.48	27.50	27.33	27.42
	Screw press	310.53	304.19	307.36	18.62	18.43	18.53
Sakha 106	Hexane	293.70	290.33	292.02	14.33	14.56	14.45
	Isopropanol	356.12	359.20	357.66	27.31	27.57	27.44
	Screw press	304.80	300.89	302.85	18.90	18.51	18.71
Giza 182	Hexane	288.54	285.60	287.07	15.05	14.46	14.76
	Isopropanol	350.85	354.75	352.80	28.11	27.74	27.93
	Screw press	302.57	307.23	304.90	18.42	18.57	18.50
Yasmine	Hexane	294.42	291.41	292.92	15.13	14.82	14.98
	Isopropanol	356.80	360.21	358.51	28.42	28.13	28.28
	Screw press	309.75	302.75	306.25	19.36	19.11	19.24
Hybrid 1	Hexane	291.66	288.79	290.23	14.78	14.55	14.67
	Isopropanol	362.73	357.82	360.28	27.36	27.21	27.29
	Screw press	311.55	307.46	309.51	18.92	18.50	18.71
Hybrid 2	Hexane	287.89	280.43	284.16	14.61	14.36	14.49
	Isopropanol	357.62	352.29	354.96	27.12	27.43	27.28
	Screw press	302.93	300.31	301.62	18.60	18.31	18.46
L.S.D		1.16	1.56	1.36	0.04	0.02	0.03

Table 5. The relationship among the studied characters of some rice genotypes from the combined data during two seasons.

Traits	Crude oil %	Total phenolic content (mg GAE / g oil)	Tocotrienols (mg / g)	Tocopherols (mg / g)
Total phenolic content (mg GAE / g oil)	0.056			
Tocotrienols (mg / g)	0.466**	0.882**		
Tocopherols (mg / g)	0.348	0.943**	0.981**	
Gama oryzanol (mg / g)	0.247	0.958**	0.971**	0.985**

CONCLUSION

The present study indicated that using Giza 178 rice cultivar with extraction of rice bran oil by isopropanol method gave the highest values for crude oil % and bioactive components which have various beneficial effects on human health.

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الملخص العربي

تأثير الطرق المختلفة لاستخراج زيت رجيعة الأرز على نسبة الزيت الخام، جودة الزيت والمكونات النشطة بيولوجيا في بعض أصناف الأرز

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سحا ١٠٤ في موسم ٢٠١٨ بينما أعلى قيمة لليود في موسم ٢٠١٩ لصنف أرز هجين ١. وبالإضافة إلى ذلك، كانت الطريقة المثلى لاستخلاص زيت رجيعة الكون هي ايزوبروبانول الذي أدى إلى وجود زيادة في الزيت الخام، الرقم اليودي، والمواد الفينولية الكلية و tocotrienols, tocopherol و gama oryzanol. أوضحت التفاعلات بين أصناف الأرز وطرق استخلاص زيت رجيعة الأرز أن استخدام صنف الارز جيزة ١٧٨ واستخلاص زيت رجيعة الأرز بايزوبروبانول أعطى أعلى القيم لنسبة الزيت الخام والمواد الفينولية الكل tocotrienols, tocopherols و gama oryzanol في موسمي ٢٠١٨ و ٢٠١٩. أوضح معامل الارتباط وجود ارتباطا معنويا موجبا بين محتوى الزيت من المواد الفينولية ونسبة الزيت المستخلص كما أوضحت وجود علاقة معنوية موجبة بين المواد الفينولية بأنواعها المختلفة، لذلك فإذا تم الانتخاب للتركيز العالي للمواد الفينولية سوف يعمل علي زيادة تركيز الفينولات المتعدد ونسبة الزيت.

تم الحصول على تقاوى معتمدة تم حصادها في موسمي زراعة ٢٠١٨ و ٢٠١٩ لعشرة أصناف من الأرز الشعير وهي جيزة ١٧٧، جيزة ١٧٨، سحا ١٠١، سحا ١٠٢، سحا ١٠٤، سحا ١٠٦، جيزة ١٨٢، ياسمين، هجين ١ وهجين ٢ من برنامج بحوث الأرز، معهد بحوث المحاصيل الحقلية، مركز البحوث الزراعية، سحا، كفرالشيخ، مصر. وقد تم استخدام تصميم القطع المنشقة مرة واحدة بثلاث مكررات حيث وزعت أصناف الأرز في القطع الرئيسية بينما كانت طرق استخلاص زيت رجيعة الكون في القطع الفرعية (هكسان وايزوبروبانول والعصر الميكانيكي). وأوضحت النتائج وجود فروقا معنوية بين أصناف الأرز لجميع الصفات المدروسة باستثناء قيمة البيروكسيد ونسبة الأحماض الدهنية الحرة. وقد سجلت أعلى القيم لنسبة الزيت الخام، والمواد الفينولية الكلية tocotrienols, tocopherol و gama oryzanol لصنف الأرزجيزة ١٧٨ في كلا موسمي الدراسة. علاوة على ذلك، أظهر صنف ياسمين أعلى القيم لصفة التصبن في كلا الموسمين. بينما، تم تسجيل أعلى قيمة لليود مع صنف الأرز