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EVALUATION OF NATURAL ANTIOXIDANTS EXTRACTED FROM RICE MILLING BY PRODUCTS

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ABSTRACT

This investigation was carried out to study the extraction of some natural phenolic compounds from by-products of the rice milling (defatted black rice bran, defatted white rice bran, hull black rice and hull white rice). The extracted phenolic compounds were tested as natural antioxidants compared with synthetic antioxidants (butylated hydroxytoluene (BHT)) using cotton seed oil after deep frying of potato for 25 hours.

The polyphenols were extracted from by-products of the rice milling using different solvents. The total polyphenolic compounds were identified by HPLC method. The oxidative rancidity of cotton seed oil containing different concentrations of extracted polyphenols (100, 200 and 400 ppm) was estimated by rancimate method. Another set of nutritional experiments were performed to elucidate the extent at which the polyphenols are safe for human being. Several solvents (methanol, ethanol and aceton) were used to extract the natural antioxidants from by-products of the rice milling (defatted black rice bran, defatted white rice bran, hull black rice and hull white rice). The results indicated that methanol was the best solvent for extracting polyphenolic compounds. However, amount of polyphenolic compounds were as follow: 1050.12, 890.15, 930.50 and 680.25 ppm from defatted black rice bran, defatted white rice bran, hull black rice and hull white rice; respectively. HPLC analysis of total polyphenols extracted from by-products of the rice milling indicated that ferulic acid was the major phenolic compound which identified of defatted black rice bran and defatted white rice bran followed by P-coumaric acid then vanillic acid. While, vanillic acid was the major phenolic compound that presented and identified in hull black rice and- hull white rice extracts followed by p-coumaric acids. The polyphenols extract at level of 400 ppm possessed superior antioxidant effect. Biological assay was conducted on male albino rats using phenolic compounds extract at level of 400 ppm (which consider as a superior concentration of antioxidant) had no effect on liver and kidney activities. In addition, histological examination of rats fed on oils treated with extracts of defatted black rice bran, defatted white rice bran at 400 ppm concentration had no alternations different than those observed in rats fed on control diets.

INTRODUCTION

Antioxidant compounds are gaining importance due to their dual role in the food industry as lipid stabilizers and in preventive medicine as suppressors of excessive oxidation that causes cancer and ageing (Devi *et al.,* 2007). Although synthetic compounds like butylate hydroxytoluene(BHT), tertiarybutylhydroquinone (TBHQ), butylated hydroxyanisole (BHA) and propyl gallate (PG) are widely used as antioxidants in the food industry, their toxicological aspects together with consumer preference for natural products have popularized the use of natural antioxidants (Moure *et al.,* 2001). Cereal grains, especially rice, contain special phenolic acids (such as ferulic acid, p-

coumaric and diferulate) that are not present in significant quantities in fruit and vegetables (Adom and Liu, 2002).

Rice is a rich source of many bioactive compounds including phenolic antioxidants that have the potential to reduce the risk of disease, such as inhibiting platelet aggregation (Daniel, *et al.,* 1999), reducing the risk of coronary heart disease and cancer (Martinez-Valverde, *et al.,* 2000), and preventing oxidative damage of lipid and low-density lipoproteins (Morton, *et al.,* 2000).

Defatted rice bran, the predominant by-product of RBO extraction is a good source of insoluble dietary fibre, protein, phytic acid, inositol and vitamin B (Hargrove, 1994). Though an aqueous ethanolic extract of defatted rice bran (DRB) was reported to have antioxidant activity in the active oxygen method (AOM) test, the extract was not characterized chemically or phytochemically (Shin, *et al.,* 1992).

In general, the rice grain has a hard husk protecting the kernel inside. After the husk is removed, the remaining product is called brown rice, including bran, germ and endosperm. The commercial rice-milling process leads to products with low-value fractions, such as husk and bran. Because rice husks are inedible, they are used in various non-food applications as low-value waste materials. However, rice husks offer the valuable nutritional advantage that they contain an antioxidant defence system to protect the rice seed from oxidative stress (Ramarathnam, *et al.*, 1988). In addition, Jeon *et al.*. (2006) reported that phenolic compounds, from methanolic extracts of rice husk, exhibit high antioxidant activity against scavengers of singlet oxygen and inhibit high hydrogen peroxide-induced damage to cellular deoxynucleic acid (DNA) in human lymphocytes.

Coloured rices are reported as potent sources of antioxidants and encouragements as viable sources of antioxidants for functional foods were made (Yawadio, *et al.,* 2007). Furthermore, anthocyanin pigments extracted from black rice have been reported to be highly effective in reducing cholesterol levels in the human body and an inhibitory effect of cell invasion on various cancer cells (Lee, *et al.,* 2008). Pigmented rice varieties have the potential to promote human health because they contain antioxidative compounds that have the ability to inhibit the formation or to reduce the concentration of reactive cell-damaging free radicals (Parrado *et al.,* 2003).

For this reasons, the present study was taken to evaluate the efficiency of the polyphenolic compounds extracted from different by-products of the rice milling (defatted black and white rice brans, hull black rice and hull white rice) as a new sources of natural antioxidants for inhibiting the rancidity of fried oils (cotton seed oil). Moreover, to evaluate the safety of extracted polyphenolic compounds compared with butylated hydroxyltoluene (BHT).

MATERIALS AND METHODS

Materials:

Black rice bran, white rice bran, hull Black rice, and hull white rice were obtained from the Rice Research and Training Center (RRTC), Sakha,

Kafr El-Sheikh Governorate, Egypt. Commercial antioxidant; butylated hydroxytoluene (BHT) was purchased from sigma chemical company.

Methods:

Preparation of samples:

The black rice bran and white rice bran (20g) were soaked in 100 ml n-hexane at room temperature for 48 hr, then filtered. This process was repeated three times using fresh solvent each time to extract most of the oils from the samples.

Extraction of polyphenolic compounds:

Polyphenolic compounds present in black rice bran , white rice bran , hull Black rice , and hull white rice were extracted by different solvents (methanol, ethanol and acetone) at 25°C with shaking in an incubator at 150 rpm for 16 h. The mixtures were centrifuged at 2500 rpm for 20 min and the supernatants were collected. The residues were re-extracted under the same conditions, and supernatants from both extractions were combined and concentrated to dryness using a rotary evaporator at 35°C. The dried extract was redissolved in 5 mL of 50% of each solvent and used as crude extracts (Butsat and Siriamornpun, 2010).

Total phenolic compounds of the extract was determined spectrophotometrically using Folin-ciocalteau reagent according to the method described by Bonoli *et al.* (2004). The phenolic acids content was estimated by using a standard curve prepared with gallic acid.

Qualitative and Quantitative determination of phenolic compounds by HPLC:

For HPLC analysis, each residue of extracted phenolic compounds was dissolved in 5 ml methanol (HPLC) and then passed through a 0.45 lm filter. A 20 ll aliquot of sample solution was fractionated using a Shimadzu HPLC system equipped with a diode array detector on a250 mm _ 4.6 mm i.d., 5 lm, Inertsil C18 analytical column. The mobile phase consisted of purified water with phosphoric acid pH 2.58 (solvent A) and acetonitrile (solvent B) at a flow rate of 0.8 ml/min. Column temperature was set at 38˚C. Hydroxybenzoic acid compounds were detected at a wavelength of 280 nm and hydroxycinnamic acid compounds at 325 nm. Phenolic compounds in the samples were identified by comparing their relative retention times and UV spectra with authentic compounds and were detected using an external standard method (Evangelisti *et al.,* 1997).

Measurement of stability:

The oxidative stability of oils was estimated according to the method described by the AOCS (1993) by rancimate method using 679 rancimate (Metrohm, Herisav, Switzerland) at 100^{o}C with air flow rate at 20 L/hr.

Antioxidative assay of phenolic extracts:

The antioxidative activities of polyphenolic extracts were assayed by addition of each extract (at 400 ppm level of its polyphenols content) and BHT 200 ppm to cotton seeds oil free from antioxidants.

Frying performance:

Cottonseed oil (3 kg) heated in a domestic fryer (Model 7122 A, Tefal Super 500 deluxe, France) to 185 $\pm$ 5^{o}C. Potato tubers were first washed with tap water then manually peeled, cut into 5.0 x 0.7 x 0.7 cm pieces using

mechanical cutter (type chef, La Minerva, Italy) and submerged in tap water until frying. After draining off excess water, 200 gm of them were placed in a wire basket and deep fried in the tested cotton seed oils as follows:

1. Free from antioxidants, (control).
2. Containing (200 ppm) butylated hydroxytoluene (BHT).
3. Containing (400 ppm) polyphenolic compounds extracted from rice milling by-products by methanol.

The frying process was daily repeated for five hours. The heating frying cycle was continued for five days. At the end of each frying period, the oils were filtered through muslin to remove the remaining fried particles, allowed to cool overnight at room temperature. Two hundred ml of the filtered oils were taken and preserved in dark glass bottles with stoppers in a refrigerator until analyzed.

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

Thiobarbituric acid (TBA): Values were determined according to Sidwell *et al.* (1990). The concentration of malonaldehyde in oil samples were calculated by using standard curve. Absorbance was read at 532 nm against distilled water.

Biological assay:

Animals and experimental design:

Thirty five Albino rats, with average weight (119-121gm) were housed individually in cages with screen bottoms and fed on a basal diet (Table A) for 7 days under healthy condition. Rats were given free access to food and water throughout the experimental period of 6 weeks.

Table (A): Composition of basal diet (as reported by AOAC 2000).

Ingredient	%
Sucrose	33.9
Starch	33.9
Casein	15
Cottonseed oil	10
Cellulose fibers	2
Choline chloride	0.2
Salt mixture	4
Vitamin mixture	1

The animals were weight every week. Although fed intake was closely monitored, an exact record of feed spillage was impossible to make due to the rats constant digging and scattering of the food. At the end of the experiment, weight gain and food efficiency ratio (calculated as gm of weight gain/gm of foods intake) were calculated for each group of rats. After adaptation, rats were randomly divided into 7 groups (each of 5 rats) as shown in Table B. All cotton seed oils that used in frying potato for 25 hours without and after adding of different antioxidants (BHT 200ppm or natural extracted antioxidants at 400 ppm concentration), they were used instead of cotton seed oil of basal diet for preparation of experimental diets.

Table (B): Experimental design:

Dietary groups	Frying period of cotton seed oil used in diets (hours)	Concentration of antioxidant added to used oil	Source of antioxidants
G_1 control	0	-	-
G_2	25	-	-
G_3	25	200 ppm	BHT
G_4	25	400 ppm	Defatted black rice bran
G_5	25	400 ppm	Defatted white rice bran
G_6	25	400 ppm	Hull black rice
G_7	25	400 ppm	Hull white rice

Blood sampling:

In all mentioned groups (Table B), blood samples were taken from rats at the end of the experiment. The blood samples were collected after 12 hours fasting from Vein plexus eye, put into dry clean centrifuge tubes and left to colt. The blood was centrifuged for 10 minutes at 3000 rpm to separate the serum, which was carefully aspirated and transferred into clean quite plastic tubes and kept frozen at -18 $\pm$ 2°C until biochemical analysis **(**El-Khamissy, 2005).

Collection of organs:

At the end of experiment, all rats were scarified, the abdomen was opened, and the organs were separated by carefully dissection, cleaned from the adhesive matter and washed with running water, then weighted. Organs were kept in formalin solution (10% v/v) for the histopathological examination. The relative weight of the organs was calculated using the following equation:

$$\text{Relative weight} = \frac{\text{Organ weight}}{\text{Animal weight}} \times 100$$

Biochemical analysis:

Serum lipids including triglycerides, total cholesterol, high density lipoprotein cholesterol (HDL-C) concentrations were measured by enzymic-colorimetric procedures using commercial available kits. Triglycerides was carried out according to the method of Fossati and Prancipe (1982). Total cholesterol (TC) was determined following the method of Richmond (1973). High-density lipoprotine cholesterol (HDL-C) was performed using precipitating reagent according to the method described by Richmond (1973). Low-density lipoprotein cholesterol concentration was calculated as the difference between total cholesterol and HDL-cholesterol according to the method of Freiedwald *et al.* (1972). Glutamic pyruvic transaminase (GPT) or alanine amino transferase (ALT) and glutamic-oxaloacetic transaminase (GOT) or aspartate aminotransferase (AST) activities were determined according to the method described by Varley *et al.* (1980).

Histopathological examination:

After postmortem examination, tissue specimens were collected from the heart, liver, and kidneys of various animal groups. Specimens were fixed in 10% neutral buffered formaline and left up to 24 hrs, then washed in tap

water for about 12 hours. The washed samples were dehydrated by using ascending concentration of ethyl alcohol starting with 70% and ending with absolute alcohol. The samples were passed through the routine paraffin embedding technique after which paraffin blocks were done. Paraffin sections of 3-5 microns thick were prepared by microtom of paraffin blocks, then stained with hematoxylin and eosin (Culling, 1983) and subjected to the light microscopy for the histopathologic studies.

Statistical analysis:

Most of the received data were analyzed statistically using the analysis of variance and the means were further tested using the least significant difference test (LSD) as outlined by Steell and Torrie (1980).

RESULTS AND DISCUSSION

Phenolics are antioxidants, and there is a general belief that the phenolics present in plant food contribute in the prevention of the oxidative damage that is implicated in a range of diseases, including cancer, cardiovascular diseases and aging (Scalbert *et al.*, 2005). Total polyphenols extracted from defatted black rice bran, defatted white rice bran, hull black rice and hull white rice are given in Table (1). The data indicate that methanol was the best solvent for extracting polyphenols from by-products of the rice milling. High, amounts of extracted polyphenolic compounds by methanol from defatted black rice bran, defatted white rice bran, hull black rice and hull white rice were 5.4, 3.6, 2.7 and 1.9 (mg/g), respectively, comparing with other solvents. These results are agreement with those obtained by Devi and Arumughan, (2007); Devi *et al.* (2007), Lai, *et al.* (2009). Butsat, and Siriamornpun, 2010) and Araba, *et al.* (2011). On the other hand, the data in Table (1) indicate that defatted black rice bran contained the highest amounts of polyphenolic compounds with all using solvents comparing with other different by-products of the rice milling.

Table (1): Extraction of polyphenols from by-products of the rice milling with different solvents.

Extraction solvent	Total polyphenols (mg/g)			
	Defatted black rice bran	Defatted white rice bran	Hull black rice	Hull white rice
Methanol	5.4 a	3.6 a	2.7 a	1.9 a
Ethanol	4.0 b	2.8 b	2.1 b	1.5 b
Acetone	2.6 c	2.1 c	1.2 c	0.9 c

Each value was an average of three determinations.
Values followed by the same letter in column are not significantly different at P < 0.05.

Effect of polyphenolic extracts and BHT on the oxidative stability of cotton seed oil:

The polyphenolic compounds extracted from by-products of the rice milling were added to cotton seed oil at levels of 100, 200 and 400 ppm and 200 ppm of BHT. The stability of cotton seed oil as the induction period was measured at 100^{o}C by rancimat method. Results are presented in Table (2). It is clear that the addition of phenolic compounds extracted from by-products

of the rice milling increase the stability of cotton seed oil at all levels. Compared to control, induction periods of cotton seed oil were increased to 9.40, 8.90, 8.40, and 8.20 hrs. with 200 ppm of methanolic extracts of defatted black rice bran, defatted white rice bran, hull black rice and hull white rice; respectively. While, it was increased to 8.3 hr. by addition of BHT at 200 ppm concentration. These compounds are considered to be beneficial to health since; they act as antioxidants in the body by inhibiting lipid peroxidation scavenging, free radical and displaying antimutagenic properties. Similar results were obtained by Gutfinger (1981) and Foster (1997), who reported that a high polyphenol content was associated with high resistance of oxidation.

The percentage of antioxidant activity (A.A.) was calculated and data also presented in Table (2). Hence, it could be noticed that defatted black rice bran extract had the highest antioxidant activity among the investigated extracts followed by defatted white rice bran, hull black rice then hull white rice extract which had the lowest antioxidant activity. The antioxidant activity of the phenolic compounds had been also investigated by Nam *et al.* (2006), who reported that pigmented of rice extracts scavenged superoxide anions more effectively than hydroxyl radical. Furthermore, Pyo *et al.* (2004) found that a positive linear correlation (R = 0.943) was demonstrated between radical scavenging activity and total phenolic content of each extract.

Table (2): Effect of polyphenoilc extracts and BHT on the oxidative stability of cotton seed oil.

Source of antioxidants	Antioxidant concentration			Antioxidant concentration		
	100 ppm	200 pm	400 ppm	100 ppm	200 pm	400 ppm
	Induction period (hr)			Percentage antioxidant activity (A.A%)*		
Cotton seed oil (control)	4.2			0		
BHT	-	8.3	-	-	0.98	-
Defatted black rice bran	8.1.	9.40	10.60	0.93	1.24	1.52
Defatted white rice bran	7.80	8.90	9.40	0..86	1.12	1.24
Hull black rice	7.20	8.40	8.90	0.71	1.00	1.12
Hull black rice	7.0	8.20	8.80	0.67	0.95	1.10

$$\text{A.A. \%} = \frac{\text{Induction period of sample - induction period of control}}{\text{Induction period of control}} \times 100$$

In addition, all rice fractions (except for milled rice) showed the ability to scavenge the DPPH_ radical at a rate higher than BHT (0.2 mg/ml) (Liyana-Pathirana and Shahidi, 2007).

Fractionation and identification of polyphenols extracted from by-products of the rice milling:

The aforementioned set of experiments relevant to the antioxidant efficiency of the total polyphenolic extracts (defatted black rice bran, defatted white rice bran, hull black rice and hull white rice) and demonstrated that the total polyphenols compounds possessed remarkable antioxidant activity. Therefore, it is quite necessary to identify the phenolic compounds of total polyphenol extracts.

Table (3): Fractions of polyphenols (µ g / g) of defatted black rice bran, defatted white rice bran, hull black rice and hull white rice.

Samples / Polyphenolic compounds	Defatted black rice bran	Defatted white rice bran	Hull black rice	Hull white rice
	polyphenols fractions (µ g / g)			
Gallic acid	1.80	-	1.0	-
Protocatechuic acid	6.30	4.0	3.50	2.0
P-hydroxybenzoic acid	6.0	2.9	9.0	7.0
Chlorogenic acid	3.10	2.0	4.80	5.0
Vanillic acid	14.50	8.0	26.10	19.0
Caffeic acid	5.50	3.0	4.10	4.0
Syringic acid	2.3	1.0	1.90	1.10
P-coumaric acid	17.20	15.0	24.10	16.0
Ferulic acid	35.30	27.0	18.20	9.0
Sinapic acid	7.0	5.0	1.20	-

High performance liquid chromatography (HPLC) was used for the qualitative and quantitative determination of total polyphenols. The results (Table 3) indicte that, ferulic acid was the major phenolic compound identified in defatted black rice bran and defatted white rice bran extracts followed by P-coumaric acid then vanillic acid. While, vanillic acid was the major phenolic compounds presented and identified in hull black rice and hull white rice extracts followed by p-coumaric acids. These results are consistent with findings of Adom and Liu, (2002) and Butsat and Siriamornpun (2010). On the other hand, ferulic acid is the major component found in bran rice makes sense if it is linked to arabinoxylan in cell walls of the aleurone layers (McKeehen, *et al.*, 1999*).*

Changes in peroxide value (PV) of cotton seed oil during deep frying:

The data presented in Table (4) illustrate that, PV of cotton seed oil was increased by increasing the frying hours. On the other hand, PV of oils treated with phenolic extracts of defatted black rice bran, defatted white rice bran, hull black rice, hull white rice, BHT and control were increased to 19.40, 19.60, 19.80, 19.90, 19.80 and 26.30 meq/kg oil for 25 hours, respectively. Defatted black rice bran phenolic extract had the highest activity in decreasing the thermal oxidation. These results are consistent with findings of Kiyomi and Yasuko (1995) and Yanping *et al.* (1999), who reported that lipid peroxides were significantly reduced by the addition of antioxidants in processed foods and oil.

Table (4): Effect of polyphenolic extracts and BHT on peroxide value (PV) during the deep frying of cotton seed oil.

Treatments / Frying time (h)	Control*	BHT 200 ppm	Antioxidant extracted from by-products in the rice milling at 400 ppm conc.			
			Defatted black rice bran	Defatted white rice bran	Hull black rice	Hull white rice
	Peroxide value (meq/kg oil)					
0	7.0 f	7.0 f	7.0 e	7.0 f	7.0 f	7.0 f
5	12.5 e	10.5 e	10.3d	10.4e	10.3 e	10.5 e
10	15.6 d	11.9 d	12.6 c	11.9 d	12.1 d	11.9 d
15	18.4c	14.2 c	13.8 c	14.0 c	14.4c	14.3 c
20	19.3 b	16.9 b	16.9 b	16.8 b	17.1 b	16.9 b
25	26.3 a	19.8 a	19.4 a	19.6 a	19.8a	19.9 a

Each value was an average of three determinations.
Values followed by the same letter in column are not significantly different at P < 0.05.
*** Free from antioxidants.**

Changes in thiobarbituric acid (TBA) value:

The TBA value has been widely used for measuring oxidative rancidity in meat, its sensitive test for the decomposition products of highly unsaturated fatty acids, which do not appear in peroxide determination (Moawad, 1995). Thiobarbituric acid (TBA) of fresh and fried cotton seed oil was determined and the results are presented in Table (5). The rates of TBA values were rapidly increased with increasing the frying period of control. Comparing to heated control (8.0 mg malonaldhyde/kg oil) for 25 hours frying, all antioxidant extracts and BHT decreased the TBA value to 3.1, 3.4, 3.9, 4.2 and 4.05 mg malonaldhyde/kg oil, when defatted black rice bran, defatted white rice bran, hull black rice, hull white rice at 400 ppm concentration and BHT at 200 ppm concentration were used, respectively. Generally, Table (5) also cleared that, defatted black rice bran extract was more effective as antioxidant than those of the other studied by-products extracts and BHT. Similar results were obtained by Devi *et al.,* (2007), who showed that the DRB extracts were stable at high temperatures and therefore capable of protecting soybean oil against oxidation even at elevated temperatures.

Table (5): Effect of polyphenolic extracts and BHT on thiobarbituric acid valve (T.B.A) during the deep frying of cotton seed oil.

Treatments / Frying time (h)	Control*	BHT 200 ppm	Antioxidant extracted from by-products of the rice milling at 400 ppm conc.			
			Defatted black rice bran	Defatted white rice bran	Hull black rice	Hull white rice
	TBA (mg malonaldhyde/kg oil)					
0	0.71 e	0.71 de	0.71d	0.71d e	0.70d e	0.70 d
5	1.89 d	0.61 e	0.66 d	0.62 e	0.60 e	0.61 d
10	2.85 c	.80 d	0.75 d	0.80 d	0.83 d	0.75 d
15	3.9 b	1.80 c	1.60 c	1.75 c	1.85 c	1.9 c
20	7.9 a	2.92 b	2.01 b	2.8 b	3.1 b	3.3 b
25	8.0 a	4.05 a	3.1 a	3.4 a	3.9 a	4.2 a

Each value was an average of three determinations.
Values followed by the same letter in column are not significantly different at P < 0.05.
*** Free from antioxidants.**

Biological assay:

Data in Table (6) show the effect of feeding on oil treated with some natural antioxidants extracted from the rice milling by-products and BHT on food intake, body weight gain and food efficiency ratio (FER) of experimental rats. The results indicate that, the mean values of initial body weight of all rat groups were nearly the same. It was ranged from 117.30 to 119.40 gm; the rats fed on control diet had a higher final body weight. Also the results showed that, addition of oils treated with different natural antioxidants to the diet did not cause further increase in final body weight of rats compared to those caused by feeding diets containing untreated oil (control). Appeared also from the same results that rats fed on untreated oil (control) had greater, body weight gain, food intake and FER. The results also reveal that, values of the body weight gain, food intake and FER were in the same range for all dietary groups, which fed on diets containing different natural extracted antioxidants. Furthermore, Most *et al.* (2005) suggested that the body weight gain of the experimental rats depends on the content of protein and fat of their diets.

Table (6): Body weight gain, food intake and food efficiency ratio (FER) of experimental rats fed on oils treated with antioxidant compared to control.

Dietary groups	Initial weight gm	Final weight gm	Body weight gain		Food intake gm	Food efficiency ratio (FER)
			gm	%		
G_1	118.40 a	131.19 a	12.79a	10.80	408.50 a	0.0313 a
G_2	117.30 a	108.66 d	- 8.64	-	386.4 d	-
G_3	118.15 a	121.35 c	3.20 b	2.71	381.8 e	0.0084 b
G_4	118.20 a	121.36 c	3.16 b	2.67	401.65 b	0.0079 c
G_5	119.40 a	122.41b	3.01b	2.52	391.41c	0.0077c
G_6	119.40 a	122.20 b	2.80 c	2.35	367.13 f	0.0076 c
G_7	118.35 a	121.08 c	2.73 c	2.31	390.20 c	0.0071 d

Each value was an average of five determination.
Values followed by the same letter in column are not significantly different at P < 0.05.
G_1, G_2, G_3 ….etc. as mentioned in Table (B).

Results in Table (7) show the effect of feeding on oils treated with some natural antioxidants extracted from rice milling by-products and BHT on the relative organs weight (liver, kidney, spleen and heart) of experimental rats. It is apparent from Table (7) that the relative liver weight of rats fed on diets containing oil free from antioxidants (G_2) and natural antioxidant extracted from defatted white rice bran (G_5) was relatively higher than that of the all treatments. The liver weight ranged between 3.30 to 2.50 gm at the end of the feeding period (6 weeks). Concerning liver, spleen and heart weights, the percentage showed high significant changes in the relative weight of all treatments. Similar results were obtained by Zawistowski, *et al.* (2009), who showed that the liver of rats fed with experimental diets containing 3% Black Rice Fraction (BRF), cholesterol and cholic acid weighed significantly than counterparts not fed with the BRF.

Table (7): Relative organ weight (liver, kidney, spleen and heart) of experimental rats, fed on oils treated with antioxidants extracted from by-products of the rice milling and BHT .

Organs / Dietary groups	Liver		Kidneys		Spleen		Heart		Final body weight
	gm	R.W.G.*	gm	R.W.G.*	gm	R.W.G.*	gm	R.W.G.*	
G_1	3.03 b	2.31	0.93 a	0.71	0.40 a	0..30	0.48 ab	0.37	131.19 a
G_2	3.05 b	2.80	0.85 a	0.78	0.34 b	0.31	0.42c	0.39	108.66 d
G_3	3.21 ab	2.65	0.83 a	0.68	0.27 c	0.22	0.46 b	0.38	121.35 c
G_4	2.96 c	2.44	0.82 a	0.68	0.23 d	0.19	0.47 ab	0.39	121.36 c
G_5	3.30 a	2.70	0.95 a	0.78	0.27 c	0.22	0.49 ab	0..40	122.41 b
G_6	2.94 c	2.40	0.87 a	0.71	0.28 c	0.30	0.51 a	0.42	122.20 b
G_7	2.50 d	2.06	0.80 a	0.66	0.24 d	0.20	0.44 c	0.36	121.08 c

Each value was an average of five determinations.
Values followed by the same letter in column are not significantly different at P < 0.05.
*** Relative weight gain (R.W.G) =** $\frac{\text{Organ weight}}{\text{Animal weight}}$ **x 100, G_1, G_2, G_3 ….etc. as mentioned in Table (B).**

Data presented in Table (8) show the effect of feeding the rats on oils treated with some natural antioxidants extracted from rice milling by-products and BHT on serum lipid profile. From these results, it could be concluded that, rats fed on diet containing antioxidant extracted from defatted black rice bran (G_4) had a lower serum total cholesterol (TC) and total triglycerides compared with all other treatments at the end of the feeding period (6 weeks). Similar results were reported by Kim,(2005); Nam *et al.,* (2006) and Zigoneanu *et al.,* (2007), they reported that pigmented rice varieties with high antioxidative activities provide a source of antioxidants and they are very efficient in reducing low density lipoprotein cholesterol and total serum cholesterol. These findings are important diagnostic feature, since it is known that the changes in HDL cholesterol accompanied by total cholesterol levels serve as an indicator of risk to coronary heart disease (Martinez-Valverde *et al.,* 2000). It is well documented that the increase in HDL-cholesterol level leads to a decrease in the risk ratio (total cholesterol/HDL-cholesterol).

Table (8): Serum lipid profile fed on fried cotton of experimental rats fed on treated with antioxidants extracted from by-products of the rice milling and BHT.

Dietary groups	Total cholesterol mg/dL	HDLC mg/dL	LDL-C mg/dL	TC/HDL-C ratio	Total triglycerides mg/dL
G_1 (control)	99.51 f	51.81 f	47.70 e	1.92c	85.65 f
G_2	125.80 a	48.50 g	77.30 a	2.59 a	121.60 a
G_3	112.21 b	55.67 e	56.54 b	2.01 b	97.20 b
G_4	106.45 e	63.75 a	42.70 g	1.75 e	91.60 e
G_5	107.35 d	61.42 b	45.93 f	1.72 e	93.30 d
G_6	110.72 c	58.80 c	51.92 d	1.88 d	97.50 b
G_7	112.85 b	57.30 d	55.55 c	1.97 bc	95.55 c

Each value was an average of five determinations.
Values followed by the same letter in column are not significantly different at P < 0.05.
G_1, G_2, G_3 ….etc. as mentioned in Table (B).

Data in Table (9) show the effect of feeding on fried cotton seed oil treated with antioxidants extracted from rice milling by-products and BHT on liver function. From these results, show high significant in GPT and GOT activity values of rats fed on the different experimental diets for sex weeks. The GPT values were ranged between 21.3 (IU/L) for the control group and 31.3 (IU/L) for the group (2) diet on fried cotton seed oil for 25 hours. While values of GOT ranged between 49.7 (IU/L) for the control group and 58.6 (IU/L) for the group (2) fed on fried oil free from antioxidants.

Table (9): GOT and GPT (IU/L) rats fed on fried cotton seed oil treated with antioxidants extracted from by-products of the rice milling and BHT.

Dietary groups	GOT(IU/L)	GPT(IU/L)
G_1 (control)	49.7 f	21.3 e
G_2	58.6 a	31.3 a
G_3	53.7 c	26.3 c
G_4	51.5 e	25.4 d
G_5	52.9 d	26.6 c
G_6	54.1 b	27.6 b
G_7	53.9 c	27.3 b

Each value was an average of five determinations.
Values followed by the same letter in column are not significantly different at P < 0.05.
G_1, G_2, G_3 ….etc. as mentioned in Table (B).

Interpretations of the changing enzyme levels could only be made if the normal range of enzymes activities in serum are be known. In healthy humans, the concentration of cellular enzymes in the extracellular fluids are fairly low, ranging between 5-30 mu/ml (Foster, 1980 and Louz, 1997). Thus, as the results in Table (9) which indicated that, value measured for GPT activities in rats fed on different experimental diets were within the reference values in humans, and reflecting no volume of cellular damage. These results agree with those obtained by Abd El-Rahim *et al*. (1995) and Abd El-Salam and Abd El-Megeid (1998).

Histological examination:

The influence of BHT (200 ppm), total polyphenols (400 ppm) extracted from defatted black rice bran, defatted white rice bran, hull black rice and hull white rice on the heart, liver and kidney tissues of male albino rats was studied and the detected histopathological alterations were showed in Table (10) and Figures (1 - 10) of the examined organs in various treatments.

Table (10): Histopathlogical changes in heart, liver and kidney of rats fed on different experimental diets for 6 weeks.

Groups	Heart	Liver	Kidney
1	Normal	Normal	Normal
2	Haemorrhagic subendocardiely deg. myocardium	Kupffer cell proliferated	Hyperemic deg. Tubules
3	Normal	Fatty change	Hyperemic degenerated RT
4	Normal	Normal	Normal
5	Normal	Normal	Normal
6	Oedema in endocardium deg. myocardium	Normal	Normal
7	Normal	Normal	Hyperemic

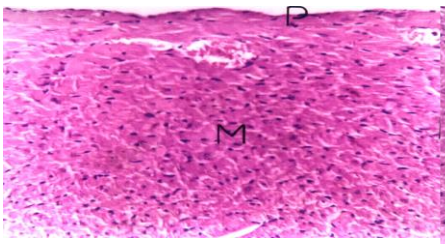

Fig. (1): Heart of rat in group (1) Showing the normal histological structure of the pericardium (P) and myocardium (M).

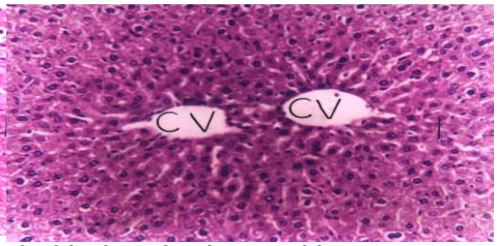

Fig. (2): Liver of rat in group (1) Showing the normal histological structure of the (cv) central veins and surrounding hepatocytes in cords.

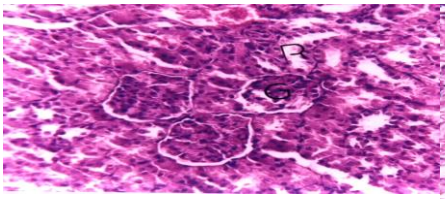

Fig. (3): Kidney of rat in group (1) Showing the normal histological structure of the glomeriuli (G) and renal tubules (R) in the cortical portion.

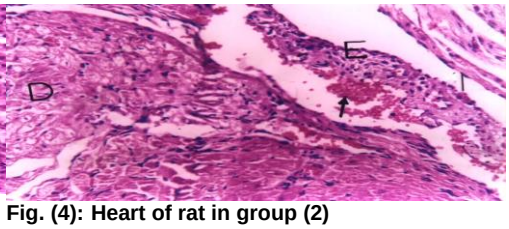

Fig. (4): Heart of rat in group (2) Showing subendocordial haemorrhage (arrow) with degeneration in the underlying myocardium (D).

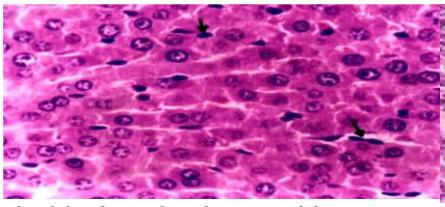

Fig. (5): Liver of rat in group (2) Showing diffuse proliferation of the kupffer cells (arrow) in between the hepatocytes.

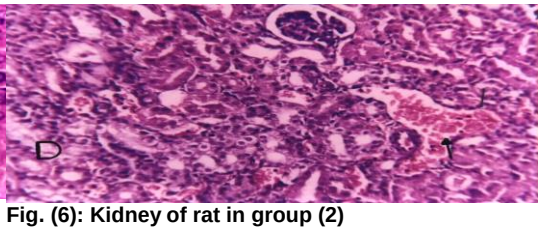

Fig. (6): Kidney of rat in group (2) Showing hyperemic blood vessels (arrow) in between the degenerated tubules (D).

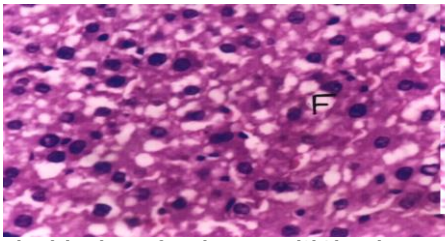

Fig. (7): Liver of rat in group (3)Showing fatty change in the cytoplasm of the hepatocytes in diffuse manner (F).

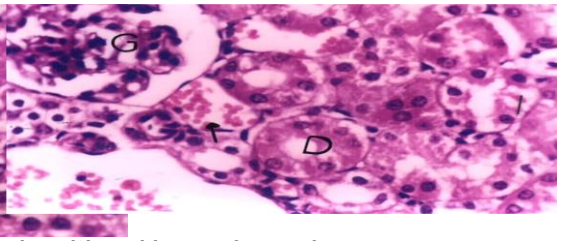

Fig. (8): Kidney of rat in group (3)Showing hyperemic glamerular tuft of the glomeruli (G) with degeneration in the epithelial cells linging the tubules (D).

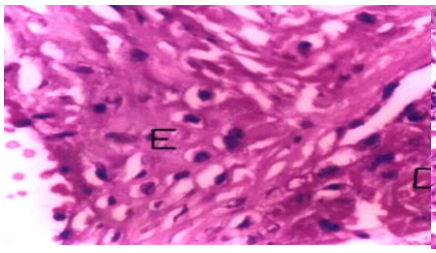

Fig. (9): Heart of rat in group (6) Showing oedema of the endocardium (E) with degenerated underlying myocardium (D).

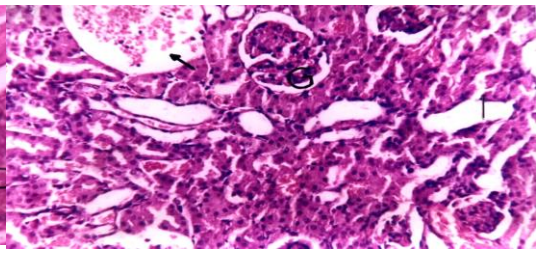

Fig. (10): Kidney of rat in group (7) Showing hyperemic in theblood vessels (arrow) and glameruli (G).

CONCLUSION

From the previous results, it could be concluded that, the polyphenols extracted from defatted black rice bran, defatted white rice bran, hull black rice and hull white rice could be added at levels of 200-400 ppm to increase the shelf life of oils and in the same time possessed no hazard effect on human being health.

REFERENCES

AOAC. (2000). Association of Official Agricultural Chemists. Official Methods of Analysis. 15th Ed., Washington D.C., USA.

Abd El-Rahim, E.A.; Abd El-Fatah, O.M. and Abd El-Lateef, B.M. (1995). Chemical and biological evaluation of barley grains and its milling and sieving fractions. J. Agric. Sci. Mansoura Univ., 20(45): 4224-4247.

Abd El-Salam, S.M.M. and Abd El-Megeid, A.A. (1998). Biological studies on some legumes as hypoglycemic and hypocholeterolemic agents. Home Economics. Fifth Scientific Conference, 5-6 May 1-21, Egypt.

Adom, K. K.,and Liu, R. H. (2002). Antioxidant activity of grains. J. of Agric and Food Chem, 50, 6182–6187.

AOCS (1993). Official Methods and Recommended Practices of the American Oil chemists Society, 4th ed. D. Firestone, AOCS, Champaign.

Araba, F., Alemzadehb, i. and Maghsoudib, V. (2011). Determination of antioxidant component and activity of rice bran Extract . Chemistry and Chemical Engineering ,4 , 120 -128 .

Bonoli, M., Verardo, V., Marconi, E., and Caboni, M. F. (2004). Antioxidant phenols in barley (Hordeum vulgare L.) flour: comparative spectrophotometric study among extraction methods of free and bound phenolic compounds. J. of Agric. and Food Chem.,52, 5195-5200.

Butsat, S. and Siriamornpun S. (2010) . Antioxidant capacities and phenolic compounds of the husk, bran and endosperm of Thai rice. Food Chemistry 119, 606–613

Culling, C.F. (1983). Handbook of Histopathological and Histochemical Techniques 3rd Ed., Butterworth , London.

Daniel, O.; Meirer, M.S.; Schlatter, J. and Frischknecht, P. (1999). Selected phenolic compounds in cultivates plants: Ecologic function, health implications and modulation by pesticides. Environ. Health Perspect. 107, 109-114.

Devi, R. and Arumughan, C. (2007). Phytochemical characterization of defatted rice bran and optimization of a process for their extraction and enrichment. Bioresource Technology, 98, 3037–3043.

Devi, R.; Jayalekshmy, A. and Arumughan, C. (2007). Antiradical efficacy of phytochemical extracts from defatted rice bran in the bulk oil system. Food Chem., 104, 658-664.

El-Khamissy, A. (2005). Studies on Biological Effects of Some Diabetes Foods. Ph.D. Thesis, Dept. Hom. Economics Faculty of Specific Education, Tanta Univ.

Evangelisti, F.; Zunin, P.; Tisconia, E.; Petacdchi, R.; Drava, G. and Lanteri, S. (1997). Stability to Oxidation of Virgin Olive Oils as Related to Olive Conditions: Study of Polar compounds by Chemometric Methods. J .A .O .C .S. 74(8): 1017-1022.

Fossati, P. and Prancipe, L. (1982). Triglycerides determination after enzymatic hydrolysis. Clin. Chem., 28: 2077.

Foster, R.L. (1980). The nature of enzymology. Room Helm, London, pp. 276-312.

Foster, S. (1997). Grape seed extract. Health Food Business, 43(4): 42-43.

Freiedwald, W.T.; Levy, R.I. and Fredrickson, D.S. (1972). Estimation of the concentration of low-density lipoprotein separate by three different methods. Clin. Chem., 18: 499-502.

Gutfinger, T. (1981). Polyphenols in olive oils. J.A.O.C.S., 58, 966.

Hargrove, K. L. (1994). Processing and utilization of rice bran in the United States. In W. E. Marshall and J. I. Wadsworth (Eds.), Rice Science and Technology (pp. 381–404). New York: Marcel Dekker Inc.

Jeon, K. I., Park, E. J., Park, H. R., Jeon, Y. J., Cha, S. H., and Lee, S. C. (2006). Antioxidant activity of far-infrared radiated rice hull extracts on reactive oxygen species scavenging and oxidative DNA damage in human lymphocytes. J. of Medicine and Food, 9, 42–48.

Kim, J.S. (2005). E vitamin fraction in rice bran inhibits auto oxidation of cholesterol and linoleic acid in emulsified system during incubation. J. Food Sci., 70, 286-291.

Kiyomi, K. and Yasuko, S. (1995). Formation of lipid peroxides in processed foods in storage and the inhibitory effects of vitamin A and Vitamin E in lipid peroxidation. Kassigeku Kenkyu, 41, 91-96.

Lai ,P. ,Li b,K. , Lu c, S and Chen H. (2009). Phytochemicals and antioxidant properties of solvent extracts from Japonica rice bran . Food Chem.,117 , 538–544

Lee, J.-C., Kim, J.-D., Hsieh, F.-H., and Eun, J.-B. (2008). Production of black rice cake using ground black rice and medium-grain brown rice. Inter. J. of Food Sci. and Tech., 43, 1078–1082.

Leonard, W.A.; Woods, A.E. and Wells, M.K. (1987). Food Composition and Analysis. Publ. by Van Nastrand Rinhold Com. New York. pp. 255-258.

Liyana-Pathirana, C., and Shahidi, F. (2007). Antioxidant and free radical scavenging activities of whole wheat and milling fractions. Food Chem., 101,1151– 1157.

Louz, S.L.L.(1997). Biochemical Study on Soybean Products. M.Sc. Thesis, Dept. of Biochemistry, Fac. of Agric., Cairo Univ., Cairo, Egypt.

Martinez-Valverde, I.; Periago, M.J. and Ros, G. (2000). Nutritional importance of phenolic compounds in the diet. Arch. Latinoam. Nutr. 50, 5-18.

McKeehen, J. D., Busch, R. H., and Fulcher, R. G. (1999). Evaluation of wheat (Triticum aestivum L.) phenolic acids during grain development and contribution of Fusarium resistance. J.of Agric. and Food Chem., 47, 1476–1482.

Moawad, R.K. (1995). Effect of pre-treatment on Ouality Attributes and Nutritive Value of Frozen Beef and Chicken Meats. Ph.D. Thesis, Dept. of Biochemistry, Fac. of Agric., Cairo Univ., Cairo, Egypt.

Morton, L. W., Abu-Amsha, C., Puddey, I. B., and Croft, K. D. (2000).Chemistry and biological effects of dietary phenolic compounds: Relevance to cardiovascular diseases. Critical and Experimental Pharmacology and Physiology, 27, 152–159.

Most, M.; Tulley, R.; Morales, S. and Lefever, M. (2005**).** Rice bran oil, not fiber, lowers cholesterol in humans. Am. J. Clin. Nutr. 81: 64-68.

Moure, A.; Cruz, J.M.;l Franco, D.; Domingez, J.M.; Sineiro, J.; Dominguez, H.; Nunez, M.J. and Parajo, J.C. (2001). Natural antioxidants from residual sources. Food Chem., 72, 145-171.

Nam, S.H.; Choi, S.P.; Kang, M.G.; Koh, H.J.; Kozukue, N. and Friedman, M. (2006). Antioxidative activities of bran extracts from twenty one pigmented rice cultivars. Food Chem., 94. 613-620.

Parrado, J.; Miramontes, E.; Jover, M.; Marquez, J.C.; Angeles, M. and Terran, C. (2003). Prevention of brain protein and lipid oxidation elicited by water-soluble oryzanol enzymatic extract derived from rice bran. European J. of Natur., 42, 307-314.

Pyo, Y.; LE, T.; Logendra, L. and Rosen, R.T. (2004). Antioxidant activity and phenolic compounds of Swiss chard (*Beta vulgaris* subspecies cycla) extracts. Food Chem., 85, 19-26.

Ramarathnam, N., Osawa, T., Namiki, M., and Kawakishi, S. (1988). Chemical studies on novel rice hull antioxidants. 1. Isolation, fractionation, and partial characterization. J. of Agric. and Food Chem., 36, 732–737.

Richmond, N. (1973). Calorimetric method of determination of total cholesterol and high density lipoprotein cholesterol (HDLC). Clin. Chem. 19: 1350-1356.

Scalbert, A., Johnson, I.T.and Saltmarsh, M., (2005). Polyphenols: antioxidants and beyond. American J. of Clin. Nutr., 81, 215S–217S.

Shin, Z. I., Chang, Y. S., Kang, W.S., and Jung, S. U. (1992). Antioxidant extracted from the defatted rice bran. US Patent, 5,175,012.

Sidwell, C.G.; Salwin, H.; Benca, M. and Mitechell, Jr.; J.H. (1990). The use of thiobarbituric acid as a measure of fat oxidation. A.O.C.S., 3(12): 603-606.

Steell, R.G. and Torrie, J.H. (1980). Principles and procedures of statistics. 2nd Ed. pp 120. McGraw-Hill, New York, USA.

Varley, H.; Gewenlock, A. and Bell, M. (1980). Practical Clinical Biochemistry. Vol. 1, 5th ed. William Heinemen Medical Books, Ltd, London, pp. 741-897.

Yanping, W; Mourning, Z.; Yuhang, Z. and Zhying, P. (1999). Study on effect of different antioxidants on antioxidant properties of oils. Zhongguo Youzhi, 24, 37-39.

Yawadio, R., Tanimori, S., and Morita, N. (2007). Identification of phenolic compounds isolated from pigmented rices and their aldose reductase inhibitory activities. Food Chem., 101, 1616–1625.

Zawistowski, j. ; Kopec, A. and Kitts, D. (2009). Effects of a black rice extract (Oryza sativa L. indica) on cholesterol levels and plasma lipid parameters in Wistar Kyoto rats. J. of Functional Food, 1: 50-56.

Zigoneanu, I.G.; Williams, L.; Xu, Z. and Sabliov, CM. (2007). Determination of antioxidant components in rice bran oil extracted by microwave assisted method. Bio-resource Tech. 50, 320-325.

تقييم مضادات الأكسدة الطبيعية المستخلصة من ضرب وتبييض الأرز

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أجرى هذا البحث بهدف الحصول على بعض المركبات الفينولية المضادة للأكسدة الطبيعية المستخلصة من ضرب وتبييض الارز مثل رجيع الكون الأسود منزوع الدهن ورجيع الكون الابيض منزوع الدهن وقشور الارز الابيض وقشورالارز الاسود وإمكانية استخدامها كمضادات أكسدة طبيعية مقارنة بمضادات الأكسدة الصناعية BHT بإضافتها إلى زيت بذرة القطن أثناء تحمير البطاطس على فترات بمعدل ٥ ساعات يوميا لمدة تصل إلى ٢٥ ساعة. وتم فى هذه الدراسة استخلاص المركبات الفينولية العديدة من المخلفات الناتجة من ضرب وتبييض الارز السابقة بواسطة مذيبات مختلفة (ميثانول - ايثانول - اسيتون) بهدف أختيار أنسب المذيبات المستخدمة لهذا الغرض.

اجرى التعرف على بعض المركبات الفينولية بواسطة جهاز الكروماتوجرافيا السائلة (HPLC). كما تم دراسة ثبات المركبات المضادة للأكسدة للمستخلصات من مخلفات الزراعية الصناعية باستخدام جهاز الرانسيمات. وأجريت تجربة تغذية لمعرفة مدى تأثير المركبات الفينولية المستخلصة من المخلفات الزراعية الصناعية على وظائف الكبد والكلى لفئران التجارب. وأجرى تشريح لأنسجة القلب والكبد والكلى لفئران التجارب لمعرفة مدى تأثر هذه الأنسجة نتيجة إضافة المركبات الفينولية المستخلصة من المخلفات الزراعية الصناعية سابقة الذكر الى الزيت المضاف للوجبات المقدمه لها.

وقد أظهرت النتائج أن الميثانول كان أفضل مذيب لاستخلاص المواد المضادة للأكسدة حيث كانت الكمية المستخلصة من رجيع الكون الأسود منزوع الدهن ، رجيع الكون الابيض منزوع الدهن ، قشورالارز الاسود ، قشورالارزالابيض هى:

٥,٤- ٣,٦ - ٢,٧ - ١,٩ ميكروجرام كل جرام على التوالى وبناءا علية تم استخدام الميثانول لاستخلاص المواد المضادة للاكسدة ا للازمة لاستكمال باقي التجارب.

تفريد المركبات الفينولية العديدة بواسطة HPLC لمستخلصات المخلفات الناتجة من ضرب وتبييض الارز السابقة الذكر أظهر أنها تحتوى على (ferulic acid ويليه P-coumaric acid ثم vanillic acid) اساس المركبات الفينولية لرجيع الارز الاسود والابيض منزوع الدهن بينما vanillic acid هو أساس المركبات الفينولية لقشور الأرز الاسود والابيض ويليه P-coumaric acid .

عند إجراء تجربة تغذية الفئران على الوجبات المحتوية على زيت بذرة القطن المضاف له تركيز ٤٠٠ جزء فى المليون مضادات الأكسدة المستخلصة من ضرب وتبييض الارز و ٢٠٠ جزء فى المليون من بيتوليتيد هيدروكسى تولوين وتحليل سيرم الدم وفحص الأنسجة الداخلية وجد أن هذا التركيز هو الأمثل حيث لا يؤدى إلى أثار جانبية ضارة للفئران.

قام بتحكيم البحث

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