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Research Article
Properties of Full Fat Rice Bran and Yoghurt Fortified with it

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Abstract
Objective: Properties of Egyptian variety of Full Fat Rice Bran Powder (FFRBP) and the resultant yoghurt fortified with it were evaluated. **Materials and Methods:** Physical, chemical and functional properties of FFRBP (Sakha 103) were determined. **Results:** Gross chemical composition of FFRBP was 9.12, 9.15, 9.15, 42.13 and 38.03% for proteins, lipids, ash, crud fibers and carbohydrates, respectively. The total phenol compounds content in FFRBP was 3.48 mg g^{-1} as gallic, while flavonoids value was 1.68 mg g^{-1} as catachin and carotenoids ratio was 1.98 mg g^{-1} as Beta Carotene Equivalents (BCE). The water-holding capacity and oil-holding capacity were 3.2 g g^{-1}, 2.5 mL g^{-1}, respectively. Emulsifying activity and emulsion stability were also estimated. Buffalo's milk was fortified with FFRBP at rate of 0, 1, 3 or 5% to create 4 yoghurt treatments and were stored at 5 ± 2°C for 7 days. The FFRBP had no significant effect on pH value of obtained yoghurt compared with control sample (0% FFRBP). However, viscosity and redness degree of the fortified samples were increased. The whiteness degree of yoghurt was decreased as a percentage of FFRBP increased. Fortification of buffalo's milk with 1% w/v of FFRBP gave the best acceptability and excellent preference by panels. **Conclusion:** Rice bran is a cheap and valuable healthy by-product, which available from milling Egyptian factories. Fortification the yoghurt-milk with 1.0% FFRBP was succeeding in preparing acceptable yoghurt-product which had good physical properties beside its nutritious ingredients. In future, it can be applied these results at industrial scale.

Key words: Rice bran, yoghurt, flavonoids, antioxidants, emulsifying activity, emulsion stability, viscosity

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

A functional dairy food is a food given an additional function by adding new ingredients or more of existing ingredients. Recently, there has been an increasing tendency for development of functional dairy foods and their application in consumers' diets. Yoghurt is one of the most important groups of fermented dairy products containing enough count of live microorganisms and influencing host health[1,2]. As a proportion of the daily value, a serving of yogurt is a rich source of vitamin B_{12} and riboflavin with moderate content of protein, phosphorus and selenium. Yoghurt can be commercially produced with substantial variety in composition, flavors and additives.

Rice bran is a by-product of milling factories despite having nutritious ingredients and therapeutic action[3,4]. It considered a good source of fiber and bioactive components[5]. Rice bran is the best source of total lipids and phytosterols. Its oil contains very high concentrations of cycloartenol and 2.4-methylenecycloartanol, which made up over 40% of the total phytosterols. Rice bran is also rich in vitamins, such as vitamin E, thiamine, niacin, as well as minerals like aluminum, calcium, chlorine, iron, magnesium, manganese, phosphorus, potassium, sodium and zinc. On the other side, presence of antioxidants also brightens prospects of rice bran utilization for humans as functional ingredient[3].

Many studies have been done to using of rice bran in food industry[6-8]. Utilization of rice bran or its fractions in dairy products was rarely. Alonso[9] prepared frozen yoghurt fortified with nano-emulsion contained purple rice bran oil. El-Shibiny *et al.*[10] used rice bran in preparing a functional processed cheese. While, Kumari *et al.*[11] developed of rice incorporated symbiotic yoghurt with low retro gradation properties. Therefore, the aim of this study was planned to prepared set yoghurt fortified with Egyptian variety of Full Fat Rice Bran (FFRB) and evaluates its properties to produced valuable product.

MATERIALS AND METHODS

Materials: Fresh buffalo's milk samples were obtained from the herd of Faculty of Agriculture, Cairo University, Egypt. Yoghurt starter culture (*S. salivaricussup* sp., *thermophiles* and *L. delbruechiisup* sp., *bulgaricus*) was obtained Chr., Hansens Laboratory, Copenhagen, Denmark. Fresh rice grains (Sakha 103) was obtained from Rice Research Department, Field Research Institute, Agriculture Research Centre, Giza, Egypt. All chemicals and reagents were fine and analytical grade which were obtained from Sigma/Aldrich (St., Louis, MO, USA).

Methods

Preparation of Rice bran powder: Rice grains were sieved through a 20-mesh sieve to remove broken pieces of rice and husks. Then, it was mixed homogenously and stored within tight poly ethylene bags and kept in a deep freeze until used. Rice bran was ground to obtain very fine powder to increase hydration capacity and remove grittiness by decreasing mean particle size and producing a desirable mono modal size distribution. The average composition were 9.12, 9.15, 9.15, 42.13 and 38.03% for protein, lipids, ash, crude fiber and carbohydrates, respectively according to the first article[12].

Preparation of set yoghurt: Buffalo's milk (2 L) was divided into four equal portions. One part had no Full Fat Rice Bran Powder (FFRBP) served as a control. The latter portions were supplemented individually with FFRBP at the rate of 1, 3 and 5% (w/v) to create three treatments. All individual portions were heated to 85°C/10 min and cooled to 42°C. All yoghurt-milk were inculcated with 3.0% of mixed starter culture (1:1), dispensed into plastic cups (150 mL) and incubated at 42°C until a uniform coagulation was formed. The yoghurt samples were stored at 5±2°C and analyzed fresh and after 7 days of storage. Three replicates were done from each treatment. Analytical evaluations had been done only on yoghurt samples fortified with 1.0% (w/v) FFRBP according to the pre-sensory evaluation of all treatments which indicated that it is the best treatments.

Analytical methods

Color determination: Color parameters of full fat rice bran as well as yoghurt samples were determined using a spectro-colorimeter (Tristimulus color machine) with the CIE lab color scale (Hunter, Lab Scan XE-Reston VA, USA) in the reflection mode. Color was expressed in terms of L, a and b according to Sapers and Douglas Jr.[13]. Where: L represents darkness from black to white (100), a represents color ranging from red (+) to green (-) and b represents color ranging from yellow (+) to blue (-).

Water and oil-holding capacity: Water-Holding Capacity (WHC) and Oil-Holding Capacity (OHC) of FFRBP were determined according the method of Chau *et al.*[14]. Briefly, 1 g of rice bran was weighed and then stirred into 10 mL distilled water or corn oil for 1 min in a vortex (Thermolyne vortexer). These fibrous suspensions were centrifuged at 2200×g for 30 min and supernatant volume measured. Water-holding capacity was expressed as gram of water held per gram of sample and oil-holding capacity as gram of oil held per gram of fiber.

Emulsifying activity and emulsion stability: Emulsifying activity and emulsion stability were evaluated according the procedure of Chau *et al.*[14]. Briefly, 100 mL of 2 g/100 mL fibrous suspension were homogenized using a Caframo RZ-1 homogenizer at 2000 rpm for 2 min. Then, 100 mL of corn oil (Mazola, CPI international) were added to each sample and homogenized for 1 min. The emulsions were centrifuged in 15 mL graduated centrifuge tubes at 1200×g for 5 min and emulsion volume measured. Emulsifying activity was expressed as the milliliter of the emulsified layer volume of the 100 mL entire layer in the centrifuge tube. Emulsion stability was determined by heating the prepared emulsions to 80± °C for 30 min, cooling them to room temperature and centrifuging at 1200×g for 5 min. Emulsion stability was expressed as milliliter of the remaining emulsified layer volume of 100 mL the original emulsion volume.

Total phenolic and flavonoids contents: Total phenolic contents of rice bran samples were determined using the method of Folin-Ciocalteu[15]. Results were expressed as gallic acid equivalent (mg GAE g^{-1} dry weight). Flavonoids contents were determined using $AlCl_3$ method and expressed as catechin equivalents (mg CAT g^{-1} dry weight).

Antioxidant activity determination of FFRBP

DPPH radical scavenging activity: Antioxidant activity was determined using DPPH radical-scavenging assay as reported by Grzegorczyk *et al.*[16]. Various concentrations of ethanol and ethanol extracts of tested samples (50, 100, 150 and 200 µg mL^{-1}) were added to 4 mL of 0.1 mM DPPH solution in methanol and the reaction mixture was shaken vigorously. After incubation for 30 min at room temperature the absorbance was recorded at 517 nm. The TBHQ used as a reference in the same concentration range as the test extract. A control solution, without a tested compound was prepared in the same manner as the assay mixture. All the analyses were done in triplicate. The degree of de-colorization indicates the radical-scavenging efficiency of the extract. The antioxidant activity of tested samples was calculated as an inhibitory effect (I%) of the DPPH radical formation as follows:

$$\text{Inhibition (\%)} = \frac{A517_{(control)} - A517_{(sample)}}{A517_{(control)}} \times 100$$

ABTS equivalents: The ABTS (2,2 azinobis-3-ethyl benzo thiazoline-6- sulfonic acid, Trolox Equivalents (TE) assay was done according to the method of Arnao *et al.*[17] with some modifications. The stock solutions included 7.4 mM ABTS solution and 2.6 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in the dark. The solution was then diluted by mixing 1 mL ABTS-d+solution with 60 mL methanol to obtain an absorbance of 1.170.02 U at 734 nm using the spectrophotometer. Fresh ABTS-d+solution was prepared for each assay. Sample extracts (150 mL) were allowed to react with 2850 mL of the ABTS-d+solution for 2 h in a dark condition. Then the absorbance was taken at 734 nm using the spectrophotometer. The standard curve was linear between 25 and 600 mM trolox. Results are expressed in mM trolox equivalents (TE) g^{-1} fresh mass.

Ferric reducing power: The assay Ferric Reducing Ability of Plasma (FRAP) was done according to Benzie and Strain[18] with some modifications. The stock solutions included 300 mM acetate buffer (3.1 g $C_2H_3NaO_2{\cdot}3H_2O$ and 16 mL $C_2H_4O_2$), pH 3.6, 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCl and 20 mM $FeCl_3{\cdot}6H_2O$ solution. The fresh solution was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ solution and 2.5 mL $FeCl_3{\cdot}6H_2O$ solution and then warmed at 37±1 °C before using. Fruit extracts (150 mL) were allowed to react with 2850 mL of the FRAP solution for 30 min in the dark condition. Readings of the colored product (ferrous tri-pyridyl-triazine complex) were then taken at 593 nm. The standard curve was linear between 25 and 800 mM trolox. Results are expressed in mM TE g^{-1} fresh mass.

Analytical methods of yoghurt samples

pH value determination: The pH values of yoghurt samples were determined using laboratorial pH-meter with glass electrode.

Parent viscosity estimation: Viscosity of yoghurt samples was determined using a Brookfield Synchro-Lectric viscometer (Model LVT, Brookfield Engineering Inc., Stoughton, MA). Readings were taken at the speed of 3-50 sec^{-1} using spindle -04 at 5±1 °C for upward curve. Apparent viscosity was expressed as pascal (Pa sec).

Sensorial evaluation: Yoghurt samples were assessed for their appearance (25 points), body and texture (50 points), flavor (25 points) and all over acceptability (100 points) by 20 trained regular panels.

RESULTS AND DISCUSSION

Properties of full fat rice bran powder

Colour attributes of FFRBP: Colour attributes of Full Fat Rice Bran Powder (FFRBP) are shown in Table 1. The FFRBP characterized with low lightness, while high redness and yellowish. The colour values were 63.13, 6.46 and 22.95 for lightness, redness and yellowish, respectively. The high redness and low lightness could be attributed to the presence of high fibre (42.13%) and red to brown pigments which obviously present in the fibre. This result confirmed by Bhatty[19], who found Hull-less barley bran, which had the low dietary fibres (20.4%) had the high lightness value (L: 81.2).

Functional and physical properties of FFRBP: In Table 2, Water-Holding Capacity (WHC), Oil-Holding Capacity (OHC), emulsifying capacity and emulsifying stability were 3.2 g g^{-1}, 2.5 mL g^{-1}, 46 mL/100 mL and 96 mL/100 mL, respectively. Similar observations were found by Holloway and Greig[20] and Daou and Zhang[21]. However, the value of WHC of FFRBP was lower than that reported by Bhatty[19] for barley (3.7 mL g^{-1}) and oat bran (3.6 mL g^{-1}). It was showed that rice bran contains a high level of dietary fibers (β-glucan, pectin and gum). Also, the OHC of FFRBP was lower than that of barley bran (3.3 mL g^{-1}) but higher than that of oat bran (0.8 mL g^{-1}).

Total phenols, total flavonoids and total carotenoids of FFRBP: In Table 3, FFRBP had relatively high content of total phenols (3.48 mg gallic acid g^{-1}), 1.68 total flavonoids mg catechin g^{-1} and 1.89 total carotenoids (mg BCE g^{-1}) which improve the health properties and antioxidant activity of yoghurt. In addition, FFRBP characterized with presence of carotenoids, which well known for its antioxidant activity. These results are in agreement with that found by Al Okbi *et al.*[6].

Phenolic compounds of FFRBP: Table 4 illustrated the phenolic acid compounds content of FFRBP. The catechin, chyrsin, rosmarinic acid and protochatchnic acid contents were 64.63, 43.55, 41.30 and 20.19 (μg g^{-1}), respectively. It is clear that rice bran is rich in phenolic compounds which reflected the health benefit. Natural phenolic compounds exert their beneficial health effects mainly through their antioxidant activity[22], antimicrobial[23,24], anticancer[23], anti-inflammatory and anti-allergic[23]. These results are accordance with El-Shibiny *et al.*[10], who reported that rice bran was found to contain 841.33 mg total phenols/100 g and the following phenols were identified, catachin, chlorogenic acid, caffeic acid, vanillic acid, qumaric acid, ferulic acid, cinnamic acid and chyrisin.

Antioxidant activity of FFRBP: Table 5 reflects that the antioxidant activity of FFRBP. The DPPH radical, ABTS and

Table 1: Color attributes of full fat rice bran powder

Sample	L	a	b
Full fat rice bran powder	63.13	6.46	22.95

L: Darkness from black to white (100), a: Color ranging from red (+) to green (-), b: Color ranging from yellow (+) to blue (-)

Table 2: Physical properties of full fat rice bran powder

Sample	Water-holding capacity (g g^{-1})	Oil-holding capacity (mL g^{-1})	Emulsifying activity (mL/100 mL)	Emulsifying stability (mL/100 mL)
Full fat rice bran powder	3.20	2.50	460	96.0

Table 3: Total phenol compound, total flavonoids and total carotenoids of full fat rice bran sample

Sample	Total phenol (mg gallic acid g^{-1})	Total flavonoids (mg catechin g^{-1})	Total carotenoids (mg BCE g^{-1})
Full fat rice bran powder	3.48	1.68	1.89

BCE: Beta carotene equivalents

Table 4: Phenolic compounds contents of full fat rice bran powder

Compounds	Concentration (μg g^{-1})
Gallic acid	2.31
Protochatchnic acid	20.19
Gentisic acid	ND
Catechin	64.63
Chlorgenic acid	ND
Caffeic acid	ND
Syrngic acid	4.59
Vanillic acid	ND
Ferulic acid	4.61
Sinapic acid	ND
Coumarin	12.51
Rosmarinic acid	41.30
Cinnamic acid	0.91
Chyrsin	43.55

ND: Non-detected

FRAB were 1.65, 7.49 and 8.02, respectively. Obtained data reveled that FFRBP has highly antioxidant behavior. Antioxidant compounds in play an important role as a health-protecting agent. These compounds are capable of reducing oxygen concentration, intercepting singlet oxygen, preventing 1st chain initiation by scavenging initial free radicals[12]. Radical scavenging (DPPH) action or β-carotene is known to be one of the mechanisms for measuring antioxidant activity.

Properties of yoghurt fortified with full fat rice bran powder

pH value: Table 6 presented the pH values of yoghurt samples fortified with 1.0% FFRBP compared with control yoghurt during storage period at 5±2°C for 7 days. There was no big difference in the pH values of both yoghurt samples at day 1 and 7. These results reflect, FFRBP had no effect on the amount of lactic acid produced by the starter cultures. These results are not compatible with Schuier *et al.*[25], who reported that yoghurt samples containing probiotic and rice bran had significantly lower pH and higher acidity in comparison with the samples without any added bran.

Apparent viscosity: Viscosity of yoghurt samples during storage period at 5±2°C for 7 days is illustrated in Fig. 1. In general, the appearance viscosity of yoghurt samples was gradually decreased as the shear rate increased, which reflected that yoghurt samples show psuedoplastic behavior. On the first day of storage; the apparent viscosity of fortified sample (1.0% FFRBP) was higher than that of control yoghurt at shear rate <20 sec^{-1}. On the 7th day, the apparent viscosity was higher in fortified samples at different shear rates. The same trend was observed by Schuier *et al.*[25] in probiotic yoghurt fortified with rice bran. During storage period apparent viscosity increased in both yoghurt treatments, however, the increased rate was more pronounced in fortified yoghurt. These results could be attributed to the high crud fiber content in FFRBP[24], which hold more water and increase the viscosity. Also, the increase in viscosity confirmed the WHC of FFRBP (Table 2).

Colour attributes: Colour attributes of of yoghurt samples fortified with 1.0% FFRBP compared with control yoghurt during storage period at 5±2°C for 7 days are presented in Table 7. There are some differences between yoghurt fortified with 1.0% FFRBP and control yoghurt. The lightness and greenish degrees were lower in yoghurt fortified with 1.0% FFRBP than in control yoghurt. The yellowish degree was higher in yoghurt fortified with 1.0% FFRBP than in control yoghurt. The high redness as well as yellowish degrees and low lightness degree could be attributed to the presence of fibre and red to brown pigments which obviously present in the fibre. However, there were no much changes in the colour degrees during the storage period.

Table 5: Antioxidant activity of full fat rice bran powder

Sample	DPPH IC_{50} (mg)	ABTS (mM TE g^{-1})	FRAB (mM TE g^{-1})
Full fat rice bran powder	1.65	7.49	8.02

IC_{50}: Concentration (μg mL^{-1}) of the compound required to scavenge the DPPH radical by 50%, ABTS: 2,2 azinobis-3-ethyl benzo thiazoline-6-sulfonic acid, TE: Trolox equivalents, FRAB: Ferric reducing ability of plasma

Table 6: pH values of yoghurt samples fortified with 1.0% FFRBP compared with control yoghurt during storage period at 5±2°C for 7 days

Items	Storage period (days)	Control yoghurt	Fortified yoghurt
pH	1	4.66	4.68
	7	4.52	4.49

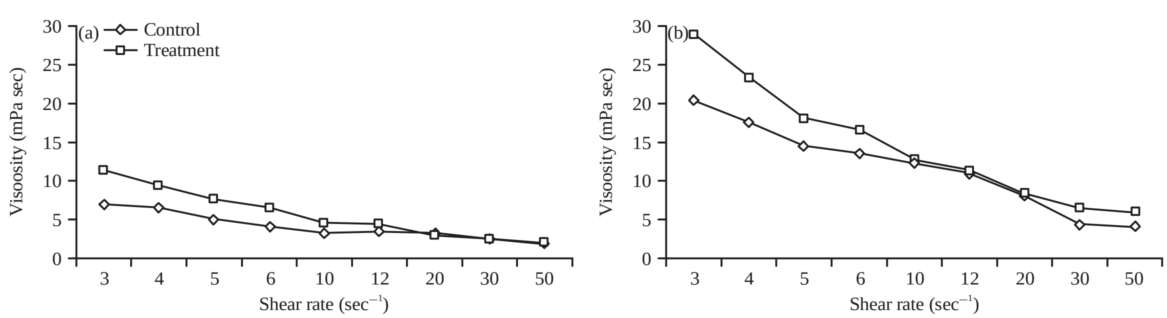

Fig. 1(a-b): Viscosity of yoghurt fortified with 1.0% FFRBP compared with control yoghurt during cold storage period at 5±2°C for 7 days

Table 7: Colour attributes of yoghurt samples fortified with 1.0% FFRBP compared with control yoghurt during storage period at 5±2°C for 7 days

Colour attributes	Storage period (days)	Control yoghurt	Yoghurt with 1.0% FFRBP
L	1	92.96	90.90
	7	93.15	90. 51
A	1	-2.25	-1.54
	7	-2.54	-1.73
B	1	9.96	11.09
	7	10.34	12.26

L: Darkness from black to white (100), a: Color ranging from red (+) to green (-), b: Color ranging from yellow (+) to blue (-)

Table 8: Sensory properties of yoghurt samples fortified with 1.0% FFRBP compared with control yoghurt during storage period at 5±2°C for 7 days

Colour attributes	Storage period (days)	Control yoghurt	Yoghurt with 1.0% FFRBP
Appearance	1	23.5	21.8
(25)	7	23.6	22.5
Flavor	1	23.6	24.6
(25)	7	23.8	26.0
Body and texture	1	47.2	47.8
(50)	7	45.8	48.1
Total scores	1	94.1	94.3
(100)	7	93.2	95.6

Sensorial properties: In Table 8, appearance scores of yoghurt fortified with 1.0% FFRBP was lower than that of control yoghurt. However, yoghurt fortified with 1.0% FFRBP had the highest scores in flavor, body and texture as well as total scores compared with control yoghurt, especially at 7 days. The high acceptability of yoghurt fortified with 1.0% FFRBP could be attributed to the rice bran, unlike other fibers, has sweet and palatable taste[26]. Inversely, Fernandez-Garcia and McGregor[27] showed that adding fiber to yoghurt would lead to decreasing the overall flavor and texture scores. However, Kumari *et al.*[11] found that the sensory properties of rice-incorporated yoghurts varied based on the rice varieties utilized.

CONCLUSION

Rice bran is a cheap and valuable healthy by-product, which available from milling Egyptian factories. Fortification the yoghurt-milk with 1.0% FFRBP was succeeding in preparing acceptable yoghurt-product which had good physical properties beside its nutritious ingredients.

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