



Changes in antioxidant activity and volatile compounds of functional yoghurt fortified with rice bran during storage

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ABSTRACT

The evaluation of yoghurt samples fortified with 1% (w/v) rice bran (RB) and follow up the changes during storage for two weeks at refrigerator was carried out. The evaluation was based on total free fatty acids, phenolic contents, antioxidants activity and volatile compounds. Yoghurt samples were analyzed at zero time and after 5,7 and 14 days. The results showed a significant increase in total phenolic content during storage until 7 days of storage and a decrease had occurred in both control and supplemented yoghurt samples. The fortification with RB at 1% showed a remarkable increase in phenolic content compared to control sample. The fortified sample exhibited nearly double concentration (35.9 mg/mL) of phenolic content compared to (17.8 mg/ml) in control sample at the end of storage. The antioxidant activity increased significantly ($P < 0.05$) during storage compared to zero time and in fortified sample with rice bran at 1% compared to control. A total of 19 volatile compounds were identified in control sample, only 18 were found in fortified yoghurt due to the absence of limonene. The major volatile compounds were, acetaldehyde, dimethyl sulfide, propanal and 2-propanol which represented 20.6%, 15.5%, 9.27% and 7.27%, respectively in fresh control sample while their corresponding values in fortified yoghurt were 21.52%, 16.4 3%, 9.18% and 6.18 % respectively. It could be concluded that fortification of yoghurt- milk with 1% rice bran succeed in produced functional product which had healthy properties beside its valuable nutritive value.

Keywords: Yoghurt, rice bran, storage, antioxidant activity, volatile compounds.

INTRODUCTION

Wastes of cereals or grains after milling process are considered valuable products and worthwhile byproducts [1]. Among different cereals byproducts; rice bran is a best and good source of protein, lipid, fiber and phytosterols. The bran layer of rice kernel contains high level of bioactive compounds such as γ -oryzanol, anthocyanins and phenolic compounds, which may reduce low-density lipoprotein cholesterol, improve lipid profiles and have anti-inflammatory and anti-oxidative activities that may help to fight against heart diseases and prevent diabetes [2]. Rice bran protein is superior to other cereal proteins because of its high-protein efficiency ratio, lysine content and hypoallergenic properties [3]. Rice bran is the best source of total lipids [4]. Phytosterols are also very important ingredient of rice bran; phytosterols prevent cholesterol absorption, plant sterols might protect certain types of cancer.

On the other side, rice bran oil contains very high concentrations of vitamins such as vitamin E, thiamine, and niacin. It is rich also in minerals such as aluminum, calcium, chlorine, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc [5].

Furthermore, presence of antioxidants also brightens prospects of rice bran utilization in food industry. So, rice bran can be evaluated as a potential food ingredient [6]. It has been used in food as full-fat, defatted bran, bran oil, and

protein concentrates. Recently, it is used in the production of baked foods, snacks, crackers, breads, cereals, pastries, pancakes, noodles, muffins, biscuits [1,4]

Yoghurt is accepted and delicious dairy product all over the world with a high nutritive value and positive effects on human health [7]. It is a fermented milk product which has been defined by medical, nutritionist and food scientist professionals as one of the “super foods” touted to enhance health, defy aging and impede the progression of changes that lead to hypertension; diabetes ,Alzheimer’s and cancer diseases [8].

Yoghurt flavour is formed by volatile components via the fermentation and/or thermal degradation of some milk constituents. The critical factor factors. The basic volatile organic compounds (VOC) participating in the formation of the flavor of typical yogurt are carbonyl compounds, such as acetaldehyde, acetone, 2-butanone, diacetyl, ethyl acetate, and ethanol [9].

Little work have been carried out on the fortification of yoghurt with rice bran; therefore the present study aimed to evaluate the effect of fortification of yoghurt-milk with rice bran (1% w/v) on the volatile flavour compounds, free fatty acids, total phenolic components and antioxidant materials as well as sensory properties of yoghurt samples during storage for two weeks at refrigerator.

EXPERIMENTAL SECTION

- Buffalo’s milk samples were obtained from local market, Giza. Egypt; its composition was: TS was 16.5% and Fat was 6.5 %.
- Yoghurt starter culture was obtained from Dairy Microbiology Lab. National Research Centre. Egypt.
- Fresh Rice grains (Sakha 103) was obtained from Rice Research Department, Field Research Institute, Agric. Res. Center, Giza, Egypt.

Preparation of Rice bran

Samples were sieved through a 20-mesh sieve. They ground to obtain very fine powder and mixed homogenously then stored under freezing until used.

Preparation of yoghurt samples

Yoghurt samples were traditionally manufactured as mentioned latter by [9].

Analytical methods

Moisture, crude fiber, ash, protein and fat of raw materials were determined according to [10]. Total carbohydrates were calculated by difference. Total phenolic contents of yoghurt sample were determined -during storage- by an assay described by [12]. Absorbance at 725 nm was converted to total phenolic compounds expressed as mg gallic acid equivalent, (GAE)/mL) using a regression of known concentrations of gallic acid (Sigma-Aldrich, Germany

Flavonoids contents were determined using AlCl₃ method [11]; and expressed as catechine equivalents (mg CAT/g dry weight).

Determination of free fatty acids of yoghurt sample

Total free fatty acids of yoghurt samples were measured -during storage- as described by AOCS method Ca 5a-40 [10].

The percentage FFA as oleic acid was calculated as follows:

$$\text{FFA (\%)} = \frac{\text{NaOH (mL)} \times \text{N} \times 28.2}{\text{Mass (g)}}$$

Where: N = normality of NaOH and mass (g) refers to the mass of sample used.

Estimation of antioxidant activity

Antioxidant activity was determined using DPPH free radical-scavenging assay as reported by [13]. The antioxidant activity of tested samples was calculated as an inhibitory effect (%) of the DPPH radical formation as follows:

$$\text{Inhibition \%} = \frac{A_{517}(\text{control}) - A_{517}(\text{sample})}{A_{517}(\text{control})} \times 100$$

Volatile compounds analysis of yoghurt sample

Volatile flavor compounds of selected yoghurt samples were evaluated during cold storage as mention bellow:

Extraction of volatile compounds

The extraction was carried out as mentioned by [14] using headspace technique.

Gas chromatography-Mass spectrometry

The analysis was performed on an HP 5890 apparatus (Hewlett-Packard, Palo Alto, CA) equipped with a split/split less injector and an HP 5970 mass selective detector. The detection was realized by full-scan mode in the mass range from 39-400. A fused-silica capillary column, DB-Wax, 60 m, 0.32 mm i.d., 0.5 μ m film thickness (J&W Scientific, Folsom, CA), was used with helium carrier 1 mL/min. The column was held at 40 °C and the temperature increased at 3 °Cmin⁻¹ to 120 °C and at 7°C min⁻¹ to 220 °C.

Volatile compounds identification

Volatiles were identified by the combination of NIST-98 GC-MS spectrum library and the comparison of retention time under the same operating conditions [15].

Statistical analyses

All experiments were performed at least in triplicate and the results are presented as mean \pm SD (standard deviation). Statistical analysis was carried out using SPSS.16. One-way analysis of variance (ANOVA) and least significant difference (LSD) was performed to determine any significant difference among various treatments and also were used to compare between means. Significant level was set at $P \leq 0.05$.

RESULTS AND DISCUSSION*Proximate Composition of rice bran*

Table (1) reveals the contents of protein, fat, ash, crude fiber and total carbohydrates contents in the rice bran (RB).

Table 1. Approximate Chemical composition of rice bran

Analysis	Rice bran
Protein %	9.12
Fat %	9.15
Ash %	9.15
Crude fiber %	42.13
Carbohydrates %	38.03
Total phenolic (mg Gallic acid/g)	3.48
Total flavonoids mg Catechine/g	1.68
DPPH IC ₅₀ (mg)	1.65

IC₅₀: concentration of the compound required to scavenge the DPPH radical by 50%.

The RB was contained 9.12%, 9.15%, 9.15%, 42.13%, and 38.03% for protein, fat, ash, crude fibre, and total carbohydrates, respectively. These results are in agreement with those reported by [16-17]. Previous studies reported that rice bran is a rich source of fiber and considerably high ash and fat content [18].

The results in Table (1) showed that the content of RB from total phenolic content as gallic acid equivalent (GAE) was 3.48 mg/ g, which reflected that the RB is a rich source of phenolic content. The same trend was observed in total flavonoids as catechine equivalent (CT) in RB, where it was 1.68 mg CT/g. These results are in agreement with those reported by [19-20].

Antioxidant compounds in food play an important role as a health-protecting factor. Natural phenolic compounds exert their beneficial health effects mainly through their antioxidant activity [21]. These compounds are capable of reducing oxygen concentration, intercepting singlet oxygen, preventing 1st chain initiation by scavenging initial free radicals such as hydroxyl radicals, binding metal ion catalysts, decomposing primary products of oxidation to non-radical species and breaking chains to prevent continued hydrogen abstraction from substances. Radical scavenging (DPPH) action is known to be one of the mechanisms for measuring antioxidant activity. Table (1) reflected the antioxidant activity against the DPPH was 1.65mg/g

The results obtained from the determination of total phenolic content in control and supplemented samples during storage were displayed in Table (2). The results showed a significant increase in total phenolic content during storage until 7 days of storage and a decrease had occurred in both control and supplemented yogurt samples.

Table 2. Change of total phenolic (TPH) compounds and free fatty acids (FFA) in control and fortified yoghurt during 14-days storage at refrigerator

Treatment	Control				RB 1%			
	Zero time	5	7	14	Zero time	5	7	14
(TPH) mg/mL	18.91±0.025	19.3±0.12	21.4±0.27	17.8±0.82	29.64±0.03	33.8±0.76	38.5±0.19	35.9±0.91
FFA (%)	4.16±0.11	3.18±0.42	4.17±0.95	4.24±0.53	4.85±0.18	4.72±0.46	4.63±0.18	4.95±0.23

The fortification with RB at 1% showed a remarkable increase in phenolic content compared to control sample. The fortified sample exhibited nearly double concentration 35.9 mg/mL compared to 17.8 mg/mL in control sample at the end of storage. The phenolic compound play an important role in the sensory evaluation and dietary properties of food products. Therefore, phenolic content had a significant attention in the field of functional foods due to their antioxidant activity [22].

The changes in free fatty acids in studied yoghurt sample during the 14-days of storage are presented in Table (2). The obtained data showed that there is no statistically significant change ($P < 0.05$) in the fortified yoghurt with RB at 1% compared with control sample, indicating that there was no significant lipolysis during the storage period. Normally lipolysis tends to cause negative changes in yogurt due to the combination of factors such as low pH, low storage temperature and relatively short shelf life [23].

The fortification of yoghurt with RB at 1% showed an increase in FFA, which exhibited 4.85%, compared to 4.16% in control sample Table 3 at zero time. Both treatments showed increase in FFA from 5 day of storage to the end of storage, these results in good agreement with [24-25].

The antioxidant activity of yoghurt samples

The antioxidant activity increased significantly ($P < 0.05$) during storage compared with the zero time and in fortified sample with rice bran at 1% compared to control sample (Fig. 1). The findings confirm what has been found in previous studies by [26], which highlighted a close correlation between antioxidant activity and polyphenol content. The observed increase had occurred, reach maximum value at 7 days of storage, and then decrease at the end of storage.

Volatile flavour compounds of yoghurt sample

The volatile compounds in control and fortified yoghurt samples are presented in Table (3). While, a total of 19 volatile compounds were identified in control sample, only 18 were found in fortified yoghurt due to the absence of limonene. The major volatile compounds were, acetaldehyde, dimethyl sulfide, propanal and 2-propanol which represented 20.6%, 15.5%, 9.27% and 7.27%, respectively in fresh control sample while their corresponding values for fortified yoghurt were 21.52%, 16.43%, 9.18% and 6.18% respectively. These results in agreement with the previous studies of [27-28].

A significant decrease had occurred in acetaldehyde during storage, which exhibited 17.3% after storage for 5 days at refrigerator compared to 20.6% in fresh control sample. The decrease in acetaldehyde concentration could be due to the alcohol dehydrogenase activity of yogurt starters; this enzyme converts acetaldehyde to ethyl alcohol during storage [29] and/or evaporation from the sample [30]. Limonene, which exhibited 1.55% in fresh control and decreased to 1.50% after 5 days of storage did not identified in fortified sample Table (3).

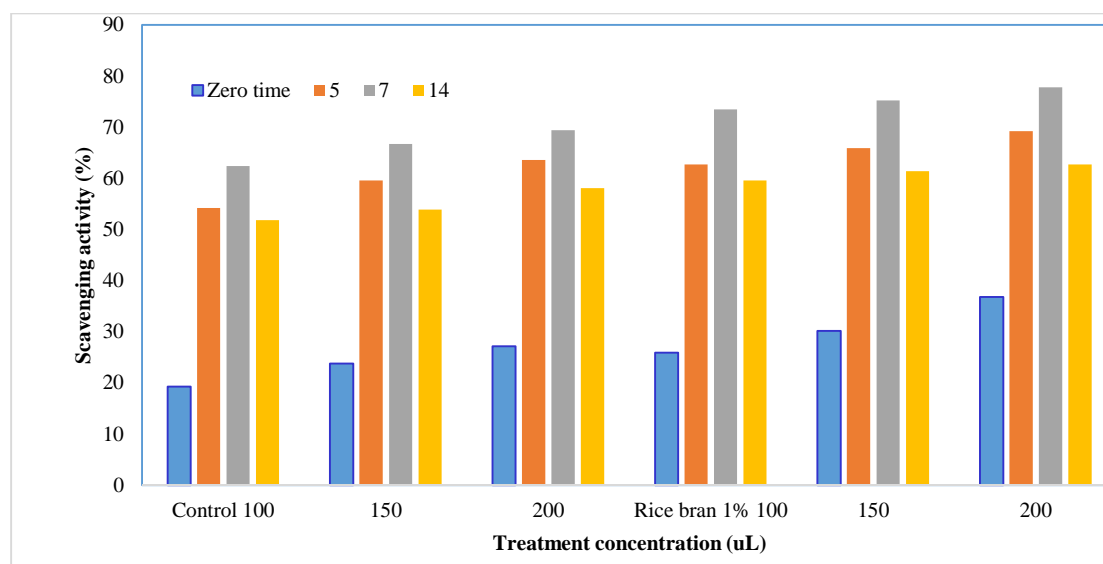


Fig. 1. Antioxidant activity of plain and fortified yoghurt with rice bran at 1% during storage for two weeks at refrigerator

Table 3. Changes in volatile flavor compounds in control and fortified yogurt with rice bran at 1% during storage

Volatile	RI ^a	Control		RB 1%		Odour Description	Identification method ^c
		Zero time	5 days	Zero time	5 days		
Acetaldehyde	711	20.6 ^b	17.3	21.52	20.17	Ethereal, fresh, green, pungent	MS, RI, ST
Dimethyl sulfide	759	15.5	9.86	16.43	16.09	lactone-like, sulfurous, cabbage	MS, RI
Propanal	803	9.27	7.52	9.18	8.16		MS, RI
2-Propanone	806	1.84	1.77	1.69	1.63	Sweet, fruity	MS, RI
Methyl acetate	835	3.92	2.83	4.91	4.82		MS, RI
Butanal	879	2.53	1.95	1.64	1.58		MS, RI, ST
Ethyl acetate	896	4.18	3.72	5.18	5.13	Solvent-like, fruity, pineapple	MS, RI
3-Methylbutanal	917	2.56	2.49	4.09	4.07		MS, RI, ST
Methanol	919	1.92	1.85	1.12	0.94		MS, RI, ST
2-Propanol	955	7.27	6.84	6.18	6.16		MS, RI, ST
Ethanol	958	4.83	5.79	5.64	5.71	Mild, ether	MS, RI, ST
2-Butanol	1051	1.84	0.83	0.42	0.30		MS, RI
Butyl acetate	1083	3.87	3.52	5.35	5.29		MS, RI
Dimethyl disulfide	1085	2.59	2.48	5.18	5.13	Boiled cabbage, cauliflower, garlic	MS, RI
Hexanal	1092	2.72	1.82	4.95	4.28		MS, RI, ST
1-Butanol	1183	0.93	0.73	1.07	0.75	Green, cut-grass	MS, RI
Limonene	1192	1.55	1.50	n.d	n.d		MS, RI, ST
3-Methyl-2-butenal	1228	3.91	2.78	3.13	3.19	Metallic, aldehydic, herbaceous	MS, RI, ST
Octanal	1315	4.74	4.19	4.54	4.48		MS, RI

^a: Retention index; ^b: Values are expressed as relative area percentage to the total identified volatile compounds. ^c: Compounds identified by GC-MS and/or by comparison of MS and RI of standard compound run under similar conditions, n.d: not detected

The decrease in limonene in the present study differ from the observations of [31] who found that limonene content in cow milk yoghurt increased with the increase of storage time. The reduction in volatile compounds in control sample during storage may be due to the reactions that resulted in the formation of or conversion to other compounds and the reactions were due to bacterial metabolic enzymes. In addition, the loss of flavour compounds may be due to volatilization [32-33].

Acetaldehyde is the most typical aroma compound of natural or plain yoghurt [34], being responsible for its fresh-fruity note. Acetaldehyde is the major volatile compounds in studied samples, it was 20.6% and 21.52% in control and fortified yogurt, respectively at zero time. After storage a significant decrease had occurred in control 17.3% compared to slight decrease in fortified sample 20.17% Table (3). The obtained results showed a reversible relationship between acetaldehyde and ethanol in both control and fortified yogurt. This relationship may be due to that acetaldehyde is mostly reduced to ethanol by alcohol dehydrogenase enzyme. Ethanol concentration increased from 4.83% at zero time to 5.79% after 5 days of storage in control sample. A similar trend was observed in fortified yogurt, which exhibited ethanol concentration of 5.64% at zero time and increased to 5.71% after storage. The obtained results in agreement with [35-36]. Hexanal an aldehyde (fruity note) was identified in studied control and fortified yoghurt samples and its concentration was lower in control yoghurt (2.72%) than the fortified sample (4.95%) at zero time. Hexanal is likely to be formed during α -oxidation of unsaturated fatty acids [37]. Among the

identified esters ethyl acetate (pineapple and fruity notes), methyl acetate were identified in investigated yoghurt samples. Ethyl acetate was detected in all yoghurts during storage period in high quantities. The highest mean value of ethyl acetate (5.18%) was found in fortified yoghurt at zero time whereas the lowest mean value (3.72%) was found in control yoghurt after storage (Table 3). Ethyl acetate has already been reported in yoghurt [38]. In general, addition of RB at 1% increase the concentration of identified volatile compounds and remarkable keep the concentration remained almost constant during storage.

CONCLUSION

It could be concluded that supplemented of yoghurt –milk by 1% rice bran (w/v) resulted in produced yoghurt sample with highly antioxidant activity and improvement of volatile compounds content.

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