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Angiotensin-converting enzyme (ACE-1) inhibitory and antioxidant activities of probiotic yogurt enriched with rice bran

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Abstract

Angiotensin converting enzyme inhibitory (ACE-I) peptides make up the majority of the bioactive peptides created during yogurt production. In the current study, the influences of rice bran (RB) fortification at two different levels (1 and 2%) on several quality aspects of probiotic set yogurt were assessed during 14 days of cold storage. Probiotic yogurts were analyzed and contrasted with plain yogurt in terms of their in vitro ACE-I inhibitory activity, proteolysis, and antioxidant capability, as well as various physicochemical and sensory evaluations. The TS, TSS, syneresis, apparent viscosity, and water holding capacity values of yoghurt samples were positively impacted by RB addition up to 2% concentration. All yogurt batches had higher antioxidant, O-phthalaldehyde and ACE-inhibitory activity up until day 7, but they started to decline at day 14. The maximum activity was seen in yogurt with 2% RB during 7 days of storage, while control yogurt had the lowest antioxidant and ACE-inhibitory activity at zero time ($p < 0.05$). The panelists gave all RB-fortified yogurts lower ratings than they gave plain yoghurt. The viability of S. thermophilus, B. lactis BB-12 and L. acidophilus LA-5 was improved by the addition of various concentrations of RB. To produce dairy products with increased antioxidant and improved anti-ACE properties, rice bran may be employed to change the microbial fermentation of milk.

Keywords ACE- inhibitory activity, total phenolic content, antioxidant activity, proteolysis and rice bran

1. Introduction

The utilization of probiotic organisms [*Lactobacillus acidophilus* and *Bifidobacteria*] in food, especially dairy products, has garnered considerable interest in recent years. One of the fermented milk products that is widely consumed globally is yogurt, which contains probiotic bacteria, along with other dairy products like yogurt drinks. Due to its beneficial features for health, such as preventing allergies, immune system activation, treating diarrhea, restoring intestinal microbiata and lowering cholesterol, one popular functional food is probiotic yoghurt. Additionally, different probiotic bacteria strains that are utilized to make yogurt may help to produce bioactive peptides that have anticancer, antihypertensive and antioxidant properties [1]. ABT cultures which have *Lactobacillus acidophilus*, *Bifidobacteria* and *Streptococcus thermophilus* as main fermenting organisms are generally used in the production of probiotic yogurt. As well as this, *L. acidophilus*, *Lactobacillus casei* and *Lactobacillus paracasei* have been studied extensively in the previous studies of probiotic yogurt [1]. The least 10^6 CFU/mL of probiotic bacteria is the concentration of live cells that is most frequently advised for probiotic products [2,3]. Relying on the peptides created through fermentation and cold storage, probiotic yogurt may have enhanced health benefits. Given that the prevalence of hypertension is rising rapidly, recently, peptides that could really reduce blood pressure among hypertensive people have garnered a lot of attention [4]. These peptides' mechanism of action is based on the suppression of the ACE-1, but it cannot be ruled out that their activity may also involve a variety of other complex pathways that could have additional positive impacts on consumer health [5]. The enzyme ACE is especially necessary for the rennin-angiotensin system, which adjusts the balance of salt and water and blood pressure in the body [5]. Angiotensin I is hydrolyzed by ACE into a potent vasoconstrictor (angiotensin II), which results in an increase in blood pressure and degradation to a larger extent than

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necessary of bradykinin, which has vasodilatory activity [6].
Prebiotics were not digestible food ingredients and have positive effects on host health due to their characteristics that are responsible for activation or reproduction of probiotic bacteria in colon [7,8]. Numerous studies have been conducted to determine the effects of enriching milk with prebiotic substances to encourage the probiotics growth. The best way to fortify milk with prebiotics is to add fiber-rich foods like nuts, grains and fruits to it [3,9-12]. Foods containing both prebiotics and probiotics are referred to as synbiotic foods [13]. In addition to synbiotic is the synergy between prebiotic and probiotic [14]. The most common way that probiotic bacteria are applied is through dairy products, and yoghurt has been successfully marketed and modified to appeal to the target consumers. For a modulated production of yogurt, several way may be applied such as prebiotics addition or probiotic culture selection [15]. There are several researches were concerned about the synbiotic fermented milk [16,17].
The outer layer of rice, known as rice bran (RB), is a byproduct of the milling process [18]. Depending on the source and milling methods, rice bran's chemical make-up differs. It contains 25 to 45% carbohydrate, 12 to 25% oil 10 to 16% protein, 6 to 10% ash 6 to 15% crude fiber [19]. Traditionally, it has been used mostly in animal feeds, but in recent years, its use in human food and nutrition has increased dramatically [20]. More focus has been placed on rice bran polysaccharides (RBPSs) among these beneficial RB components because of their many biological advantages, such as their anti-inflammatory, antioxidant and anti-tumor properties, as well as the enhancement of immune function and diarrhea symptoms [20,21]. Therefore, the current study aimed to examine the effects of combining rice bran with milk on yogurt with respect to (1) yogurt acidification, (2) sensory and physicochemical properties, (3) antioxidant activity, (4) milk protein hydrolysis and (5) ACE-1 inhibition.

2. Materials and Methods:

2.1. Materials:

Raw cow milk with 12% total solids, 3.0% fat, 3.0% protein, and a pH of 6.70, as well as rice bran, were bought at local markets in Mansoura City, Egypt. The ABT-2 culture consisted of *Bifidobacteria lactis* BB-12, *Streptococcus thermophilus* ST-20 and *L. acidophilus* LA-5 and was obtained by Chr. Hansen, Egypt.

2.2. Analyses of the rice bran's chemical composition and minerals:

The chemical composition analysis of rice bran was determined using the methods of the Association of Official Analytical Chemists, comprising the dry matter content, crude protein content, crude fat content, mineral content and crude fiber content [22]. In a nutshell, method 962.09 was used to calculate the crude fiber content, the ash content was calculated using method 942.05, and the moisture content was estimated using method 934.01. The protein content was calculated using the Kjeldahl method, which was computed as nitrogen x 6.25 (method 2001.11). The amount of crude fat was assessed by Soxhlet extraction. utilizing a flame atomic absorption spectrophotometer Agilent 240 FS (Agilent Technologies, USA), levels of minerals were measured after the instrument was calibrated with the appropriate standard solutions in accordance with the official procedure established by the Association of Official Analytical Chemists.

2.3. Polysaccharides extraction:

The rice bran powdered was pre-extracted by Soxhlet system for 48 h with acetone, followed by extraction with methanol for 48h, the supernatant was eliminated. The precipitate was dried at 50 °C for 72h. the residue was added to hot distilled water (50-60 °C) at 1:10 g/ml for 1h and subsequently was centrifugated for 20 min at 10. 000, supernatant was kept, and three further extractions were performed on the precipitate to extract any remaining polysaccharides. The mixed supernatants were centrifugated for 20 min at 12.000g, the pellet was removes and the combined supernatants were added to ethanol (final concentration 80% (v/v)) and the precipitate was dried at 50 °C for 24 h and dissolved in appropriate distilled water [23].

2.4. Investigation of rice bran's monosaccharide content:

The analysis of monosaccharide composition of crude polysaccharide of rice bran was determined with slight modification according to [24]. Briefly, 1 g rice bran polysaccharide were added to 100 ml trifluoro acetic acid (4 M), followed by heated at 80 °C for 12 h and subsequently filtered by syringe filter (0.45 µm). HPLC (Agilent, USA) supplied with quaternary pump, refractive index (RI detector) operated at 40 °C and column (300 mm x 7.8 mm operated at 80 °C) used phenomenex ® Rezex RCM-Monosaccharide. The separation process is carried out using isocratic elution by flow rate 0.6 ml/min with HPLC grade water.

2.5. Functional yogurt production:

To produce functional yogurt, fresh raw cow milk was used. First, milk was separated into three experimental lots, each of which contained 1 and 2% RB powder with the exception of plain yogurt. Each mixture was heated for 15 minutes at 94 °C before being quickly cooled to 40 ± 1 °C in chilled water for the 0.03% (w/w) addition rate of the freeze-dried ABT-2 culture. All experimental yogurts were divided among 100 mL sterile plastic containers, which were then sealed and

kept at 4 °C after being incubated at 40 ± 1°C until a pH of 4.4–4.5 was obtained.

2.6. The procedure for making yogurt water extracts:

2.5 mL of sterile deionized water were mixed with 10 g of yogurt samples. The yoghurts' pH was measured, and then they were acidified with HCl to pH 4.0. (0.1 M). The acidified mixtures were then centrifuged (4500g, 12 min, 5 °C) after being heated in a water bath for 15 min at 45 °C. NaOH (0.1 M) was added to the supernatant to bring its pH up to 7.0. The neutralized supernatants were centrifuged again for 12 minutes at 5 °C (4500 g), and until it was required for analysis, the supernatant was gathered and kept at 20 °C in a freezer [9].

2.7. Determination of total titratable acid (TTA) and pH:

A titration with 0.1 N NaOH was used to calculate TTA. 9 mL of dH2O and 1 mL of sample were blended. As a pH indicator, A few drops of phenolphthalein at 0.1% were applied. The mixture was then continually stirred while being titrated with 0.1 N NaOH until a steady pink color emerged. The following formula was used to determine how much acid was created during fermentation [25]. Before determining pH, Initially, water was used to homogenize the yoghurt (1: 9 ratio). Using a digital pH meter (Hanna Hi2210), The homogenized sample's pH was measured. [26].

Lactic acid percentage = V NaOH x 0.009 x 0.1 N x dilution factor (10) x 100…. (1)

2.8. Physicochemical analysis of yogurt samples:

The method of AOAC [27] was used to measure the total solid and titratable acidity of yogurts, and a refractometer was used to determine the total soluble solids content. Yogurt samples' pH levels were measured using the technique of Metin [28]. Using a chroma meter (Konica Minolta, Inc., Osaka, Japan), the a (redness), b (yellowness), and L (lightness; 0 = black and 100 = white) values of yoghurt samples were determined [29]. Yogurt syneresis was found in accordance with Farooq and Haque [30]. A test tube containing about 10 g of yogurt was centrifuged at 5000 rpm for 20 min at 4 C to determine the water holding capacity (WHC), which was then expressed as (clear supernatant/initial weight) X 10 [31]. he apparent viscosity of the samples was measured using a viscometer (Brookfield Rotational Viscometer). Peroxide value (PV) analysis was performed using the acetic acid-chloroform method (AOCS Cd 8-53) and the findings were represented as milliequivalent peroxide/kg product [32].

2.9. Sensory evaluation of yogurt samples:

A previously described technique by Darwish, Qiu, Taher, Zaki, Abou-Zeid, Dawood, Shalabi, Khojah and Elawady [9] was slightly modified to quantify the sensory evaluation of yoghurt samples. In a nutshell, all yoghurt samples underwent a consumer approval test (hedonic sensory assessment) after being kept cold for an overnight period, seven days, and fifteen days. The majority of the untrained panelists were Mansoura University staff members and students, all of whom consumed yoghurt on a regular basis. The ratings were given on a 9-point hedonic scale, with extreme like on the right receiving a score of 9, neither like nor dislike on the center receiving a score of 5, and severe dislike on the left receiving a score of 1. The consumer panelists evaluated the body and texture, sourness, appearance, odor, taste and overall acceptance of the products. To avoid bias, the panelists were not given any information regarding the different sample types.

2.10. O-Phthalaldehyde (OPA) method:

The peptide content of yogurt water extract was examined using the spectrophotometric OPA-based technique [33]. Prepared ingredients included 0.95 g of sodium tetraborate, 25 mL of dH_2O, and 20 g/100 mL (wt/wt) sodium dodecyl sulphate. 100 mL of -mercaptoethanol were added after dissolving 40 mg of OPA in 1 mL of methanol. In a volumetric flask, 25 mL of sodium tetraborate solution, 20 g/100 mL (wt/wt) SDS, and OPA mixture were combined. The volume was then brought up to 50 mL by adding sterile dH_2O. 30 mL of yogurt water extract were put into a plastic cuvette along with 1 mL of OPA reagent. After two minutes, a reading at 340 nm was recorded. A_{340} was converted to a standard curve based on tryptone.

2.11. ACE-inhibitory activity analysis:

According to Elbermawi, Darwish, El-Awady, Zaki, Qiu and Samra [10], with a small adjustment, the inhibitory activity was evaluated spectrophotometrically. The process relies on ACE cleaving hipuryl-histdylleucine (HHL) to hippuric acid. HHL (3.8 mM) was diluted in 200 µL of 0.1 M borate buffer, which contains 0.3 M NaCl and has a pH of 8.3, and then 35 µL of the yogurt water extract was added. The combination was then incubated for 10 min at 37 °C. 20µ L of ACE solution (0.1 U/mL in borate buffer) was added to start the reaction, which was then left to run for 30 min at 37 °C. The reaction was then put into a 250 µL solution of 1 M HCl to put an end to it. Hippuric acid was obtained by mixing 1.5 mL of ethyl acetate vigorously for 30 seconds and then centrifuging the mixture at 1200 g for 10 minutes. The solvent was then removed by placing 1 mL of the supernatant (ethyl acetate) in boiling water for 30 minutes in a fresh tube. To test absorbance at 228 nm, the residual hippuric acid residue was dissolved in 1 mL of distilled water. Distilled water served as a

control. Each sample was examined three times. Lyophilized aqueous extract (whey) produced from plain yogurt was utilized as a negative control whereas captopril (0.008 mg/mL) was used as a positive control. The formula for calculating ACE inhibitory activity was as follows:

ACE inhibition (%) = 1-[(A-C)/(B-D)] X 100…… (2)

wherein A represents absorbance with the sample, ACE and HHL; B represents absorbance with ACE and HHL without sample; C represents absorbance with the sample and HHL; and D represents absorbance with HHL without ACE and sample.

2.12. Enumeration of viable bacteria:

In yogurt samples taken at various points during storage, viable bacterial counts were counted. Different selective media were employed to count the microorganisms. By pour plating 1 mL of each dilution in M-17 agar (Oxoid) and incubating under aerobic circumstances at 39 ± 1 °C for 48 hours, the viability of *S. thermophilus* was evaluated [34]. MRS-NNLP agar, which includes paramycin sulphate (200 mg/L), lithium chloride (3000 mg/L), neomycin sulphate (100 mg/L), and nalidixic acid (150 mg/L), was used to count *B. lactis* BB-12. Just before pouring, filter sterilized NNLP (NNLP; Sigma Chemical Co.) was added to the autoclaved MRS foundation. To reduce the medium's oxidation-reduction potential and promote the development of anaerobic *bifidobacteria*, 0.05% final concentration of filter-sterilized L-cysteine-HCl was also added at the same time to the medium. Triplicate inoculated plates were anaerobically incubated at 37°C for 72 hours [35]. *L. acidophilus* LA-5 viable counts were counted on BL-MRS (agar enriched with 1.5 g/l bile salts) [36]. They underwent anaerobic incubation for 72 hours at 37 C. The counts of viable bacteria expressed as log CFU/mL.

2.13. Statistical analysis:

Every test was run in triplicate. The total titratable acid (TTA), pH, physicochemical parameters, sensory properties, OPA, TPC, antioxidant, and ACE-1inhibitory activities were all evaluated using an ANOVA test with a significance level of $p < 0.05$. The information was displayed as an average and standard deviation. The Duncan's multiple range tests were used to identify significant deviations between values. All statistical tests in the current study were evaluated using SPSS Statistics program.

3. Results and discussion:

3.1. Proximate and mineral composition of rice bran

The chemical composition of rice bran is shown in Table (1). Rice bran contains 16.15 ± 0.85% fat, 12.74 ± 0.87% protein, 8.23 ± 0.72% ash and 6.63 ± 0.80% fiber. Potassium was the most prevalent mineral in the rice bran sample's mineral makeup (2.03 ± 0.11%), followed by phosphorus and magnesium (1.23 ± 0.1 and 0.67 ± 0.09%, respectively), while minerals detected in the minor percentages were calcium, manganese, iron, zinc, sodium and copper (390.33 ± 2.1, 158.733 ± 2.4, 71.43 ± 0.76, 50.87 ± 1.6, 19.73 ± 1.74 and 2.42 ± 0.55 mg/Kg, respectively)

Table (1). The proximate composition and mineral content of rice bran

Parameter	Value
Proximate composition (%)	
Dry matter	91.15 ± 0.61
Ash	8.23 ± 0.72
Crude fiber	6.36 ± 0.79
Crude protein	12.74 ± 0.87
Crude fat	16.15 ± 0.85
Mineral content	
K (g/100g)	2.03 ± 0.11
P (g/100g)	1.23 ± 0.09
Mg (g/100g)	0.67 ± 0.08
Ca (mg/Kg)	390.3 ± 2.1
Fe (mg/Kg)	71.43 ± 0.76
Mn (mg/Kg)	158.73 ± 2.39
Zn (mg/Kg)	50.87 ± 1.6
Na (mg/Kg)	19.73 ± 1.74
Cu (mg/Kg)	2.42 ± 0.55

Results are mean of three values ± Standard error (SD)

3.2. Composition of monosaccharide:

Monosaccharide compositions of RBP was assessed by HPLC. The results indicated that glucose was presented as the essential monosaccharide of RBP and the percentage of glucose, galactose, arabinose, mannose and xylose was 34.44, 10.11, 3.15, 2.69 and 4.12% (Table 2). These results was contestant with other reports of Liu, Cao, Zhuang, Han, Guo, Xiong and Zhang [20], who showed that monosaccharides of RBP are composed of glucose, galactose, xylose, mannose and arabinose at a molar ratio of 32:6:5:2:4. This result also partly agreed with Zha, *et al.* [37], who found out three types of polysaccharides in hot aqueous extracts from rice bran and determine their compositions of monosaccharides according to GC and GC-MS analysis, PW1 was consisted of glucose, galactose, mannose, arabinose and ribose with molar percentage of 54.1%, 21.7%, 10.5%, 6.3% and 7.4% respectively, while the molar percentage of PW3 was 50.7%, 32.7%, 10.1%, 4.1% and 2.4%. Wang, *et al.* [38] also detected that the polysaccharide of rice bran was consisted of xylose, arabinose, galactose and glucose at molar ration of 2:4:4:1. The variation among the compositions of monosaccharide of RBP may be due to contradiction between raw materials, steps of purification and protocols of extraction.

Table (2): Monosaccharides composition of rice bran polysaccharides

Parameter	Value
Glucose	34.44 ± 0.70
Galactose	10.11 ± 0.55
Arabinose	3.15± 0.35
Mannose	2.70 ± 0.30
Xylose	4.12 ± 0.61

Results are mean of three values ± Standard error (SD)

3.3. Effects on rice brane on yogurt fermentation:

Initial pH measurements for all yogurts were similar (6.59-6.61; Fig. 1). During the 90–180-minute fermentation period, yogurts fortified with RB experienced pH lowering rates that were faster (0.47 ± 0.05 and 0.52± 0.03 pH unit/h for 1 and 2% RB, respectively) than control (0.34 ± 0.05 pH unit/h). As a consequence, yogurt fortified with 2% RB was the first to achieve pH 4.5 (298 min), followed by 1% RB and plain yogurts (327 and 400 min, respectively). Rice bran appeared to increase the yogurt bacteria's metabolic activity. The pH scale measures the amount of H^+ that is present as a result of lactic acid bacteria (LAB) producing organic acids. By using a typical starting culture, yogurt was fermented thanks to the symbiotic interactions of two bacterial groups. According to Sandine and Elliker [39], Formic acid and carbon dioxide, among other *S. thermophilus* metabolites, encourage the growth of certain *Lactobacillus* spp., whereas amino acids and short peptides produced by *Lactobacillus* spp. proteolytic activity promote the growth of *S. thermophilus* [40]. Yogurts' pH dropped to lower pH levels (4.2 - 4.4) after being refrigerated for 28 days, potentially because of the accumulation of acetic acid, acetaldehyde, formic acid, and lactic acid [41]. TTA is displayed in Fig. (2) and represents the proportion (%) of lactic acid that is found in yogurt during fermentation. Compared to pH changes represented by the "S" saped- curve, the increase in TTA for all yogurt samples was practically linear. At each stage of the incubation, the TTA for the yogurt samples fortified with various concentrations of RB was higher than that of plain yogurt. Between the 90th and 210th minute of fermentation, yogurt fortified with 2% of RB produced TTA at the greatest rate (0.213 ± 0.007), followed by yogurt with 1% of RB and plain (0.193± 0.004 and 0.1567 ± 0.003%/h, respectively). The total amount of hydrogen ions in the sample of yogurt, excluding those bonded to alkaline ions, is known as total titratable acidity. Thus, when evaluating the starter culture's capability for fermentation, TTA determination is more crucial [42]. According to Østlie, *et al.* [43], the production of lactic, formic, butyric, acetic, butyric, and citric acid in yogurt is linearly associated with the TTA accumulation [44]. Accordingly, differences in microbial population during fermentation can be linked to changes in yogurt's titratable acidity [45].

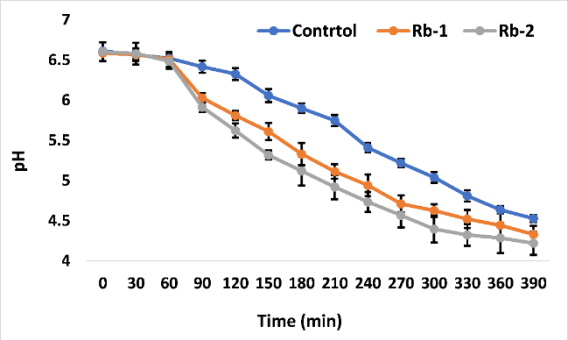

Fig. (1): pH levels of yogurts during the process of fermentation in the presence or absence of 1% and 2% of rice bran (RB) at 41 °C. Vertical bars represent the means of treatments ± standard deviations.

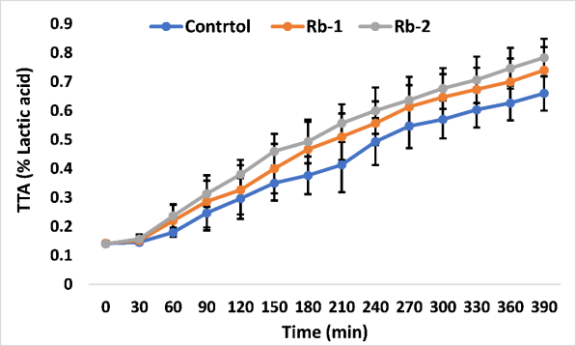

Fig. (2): Alterations in total titratable acid (% lactic acid) during yogurt fermentation at 41 C in the presence or absence of 1% and 2% rice bran (RB). Vertical bars represent the means of treatments ± standard deviations.

3.4. Physicochemical properties of yogurt samples:

After 7 days, samples' physicochemical characteristics were evaluated, and the findings are displayed in Table (3). Yogurt containing 2% RB was shown to have the highest levels of total soluble solids (TSS) and total solids (TS), while plain yogurt had the lowest levels of these parameters (Table 3). According to Demirci, *et al.* [46], adding more rice bran to formulation of yogurt dramatically enhanced TS and TSS levels.

A key flaw in yogurt products, syneresis, is caused by an extreme lack of curd stability. As shown in Table (3), the rice bran-based yogurts showed lower syneresis levels than plain yogurt. This could be explained by the fact that RB-fortified yogurt may have improved water-holding properties due to the dietary fibers found in RB, including pectin, ß-glucan, arabinogalactan, hemicellulose, and galactooligosaccharide. Additionally, Hu and Yu [47] demonstrated that dietary fibers from RB, including hemicellulose and insoluble fibers, have a range of beneficial properties, such as a high swelling capacity and capacity for holding water, which allowed them to bind more water and have a harder texture. Furthermore, oat and barley ß-glucan significantly reduced separation of whey in yogurts containing *B. bifidum*, according to Ozcan and Kurtuldu [48].

Table (3) contains the samples' viscosity values. The set yogurt appeared to have less apparent viscosity after addition of RB. All of the RB added yogurts had viscosities that were lower than plain yogurt. This is most likely a result of stabilizing casein aggregates and covering caseins with polysaccharides like pectin and ß-glucan. These findings concur with those of Tseng and Zhao [32], who found that adding wine grape pomace as a prebiotic to yogurt decreased its perceived viscosity values. Additionally, El-Said, *et al.* [49] reported that decreasing viscosity values were achieved by increasing the concentration of pomegranate peel extracts. They linked these findings to how the extract affected the electrostatic aggregation of networks in yogurts. However, yogurts with inulin and peach dietary fiber added showed considerably greater apparent viscosities than plain yogurt [50,51].

The amount of additional rice bran caused an increase in peroxide levels (PV). At day 7, The PV for plain yogurt was the lowest (1.06 mequiv/kg), while the PV for 2% RB yogurt was the highest (1.96 mequiv/kg) (Table 3). This outcome was in line with observation Sanabria [52] that adding purple rice bran oil boosted the PV value of frozen yogurt.

The presence of RB had an impact on the L^* values ($p < 0.05$). RB-containing yogurt samples had lower L^* values than the control group. L^* values rapidly fell as RB addition increased (Table 3). Additionally, RB added samples had greater a^* and b^* values than plain yogurt. The yellowish tint of RB can be used to explain the rising b values (Table 3). The similar finding regarding when wheat bran was added to yoghurt, the L* value fell or the a* and b* values rose [46].

3.5. Sensory evaluation of yogurt samples:

The results that adding RB to yogurt samples had a negative impact on the sensory aspects in terms of overall preference, taste, sourness, body texture, odor and appearance (Fig. 3). The panelists gave all RB-fortified yogurts lower ratings than they gave plain yogurt. Increased rice bran content in this study may have decreased yogurt acceptance. In agreement with the present study, Demirci, Aktaş, Sözeri, Öztürk and Akın [46] found that yogurts with RB scored lower on the sensory scale than plain yogurt. Yogurts with more fiber had better body and texture, but their overall flavor quality decreased, according to Fernández-García, *et al.* [53]. Similar to this, Hashim, *et al.* [54] found that yogurt that has been enriched with date fiber at different concentrations and 1.5% wheat bran did not receive higher overall sensory scores or be more palatable. As seen in Fig. (3), the yogurt fortified with 1% RB demonstrated increased acceptance when compared to the other fortified yogurt. Lack of a ritual of adding cereal-based additions to yoghurt among the volunteers, even though most consumers are aware of the health advantages of these materials, may be a contributing factor to the comparatively low preference scores reported for RB enrichment.

Table (3): Physicochemical characteristics of yogurt samples

Parameters	Plain yogurt	1% RB	2% RB
Total solid (%)	12.64 ± 0.18 ^{c}	13.51 ± 0.17 ^{b}	14.10 ± 0.16 ^{a}
Total soluble solids (%)	10.08 ± 0.20 ^{c}	10.63 ± 0.09 ^{b}	11.20 ± 0.23 ^{a}
WHC (%)	38.35 ± 0.43 ^{c}	41.22 ± 0.31 ^{b}	43.68 ± 0.51 ^{a}
Syneresis (% v/w)	10.63 ± 0.09 ^{a}	9.75 ± 0.12 ^{b}	8.28 ± 0.27 ^{c}
Apparent viscosity (cP)	3177 ± 87.8 ^{a}	1966 ± 152.8 ^{b}	1620 ± 203 ^{b}
Peroxide (mequiv./Kg)	1.05 ± 0.03 ^{c}	1.27 ± 0.05 ^{b}	1.74± 0.07 ^{a}
L^*	89.23 ± 0.11 ^{a}	86.14 ± 0.29 ^{b}	83.33 ± 0.38 ^{c}
b^*	6.26 ± 0.12 ^{c}	10.20 ± 0.22 ^{b}	11.79 ± 0.32 ^{a}
a^*	-2.90 ± 0.15 ^{c}	-1.14 ± 0.10 ^{b}	-0.46 ± 0.12 ^{a}

Significant differences are denoted by lowercase letters in the same row ($p < 0.05$).

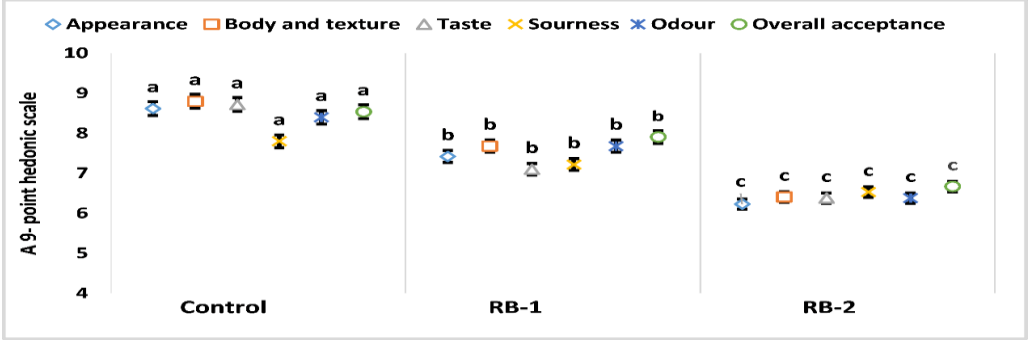

Fig. (3): Sensory scores of yogurt samples fortified with different concentration of RB compared with plain yogurt after storage period for 7 days at 5±2°C. Lowercase letters present a significant impact on the sensory assessment. Vertical bars represent the means of treatments ± standard deviations.

3.6. Total phenolic content:

Yogurt fortified with 2% of RB had the highest (TPC) content (30.23 ± 0.87 mgGEA/mL), followed by yogurt fortified with 1% of RB and plain yogurts (20.4 ± 0.85 and 11.17 ± 0.8 mgGEA/mL, respectively) (Fig. 4). During refrigerated storage, the TPC considerably ($p < 0.05$) rose for all yogurt samples, reaching maximum values of 38.4 ± 0.87, 28.17 ± 0.96, and 16.93 ± 0.70 mgGEA/mL in yogurts that were 2% RB, 1% RB, and plain yogurt, respectively (Fig. 4). Due to the absence of any plant extracts in plain yogurt, the TPC values in control represent phenolic substances linked to the breakdown of milk protein [55]. Tyrosine, an amino acid, is one example where a phenolic side chain has been proposed to contribute to the reading in TPC [56]. Another idea is that during fermentation and post-acidification, bacteria use phenolic acids like p-coumaric acid and ferulic to produce other phenolic acids like p-hydroxybenzoic acids and vanillic before the aromatic ring structure is broken down [57]. The presence of native phytochemical substances (such as flavonoids and phenolic compounds) in rice bran can be used to explain the higher TPC in yogurt supplemented with RB [58].

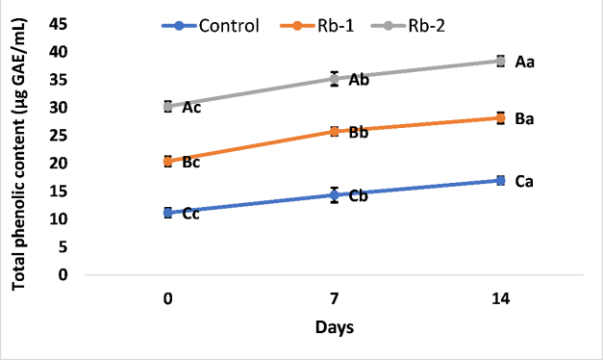

Fig. (4): TPC in yogurt supplemented with RB when stored in a refrigerator (4 °C) compared with control. Values are means ± SD after three replications of each experiment. Gallic acid equivalent (ugGAE)/mL units were used to express the total phenols. Significant changes in TPC between storage period conditions are indicated by lowercase letters ($p < 0.05$). Significant TPC changes across treatment are denoted by capital letters ($p < 0.05$).

3.7. DPPH scavenging activity of yogurt samples:

Following fermentation and when being stored, the antioxidant activity of all yogurt samples fortified with RB was higher ($p < 0.05$) than that of plain yogurt (Fig. 5). On day 7 of cold storage, each yogurt's ability to inhibit DPPH oxidation climbed to its greatest levels, which were 60.87 ± 0.91, 51.2 ± 1.4, and 28.27 ± 1.22% for RB-2, Rb-1 and plain yogurt, respectively. By day 14, the amount of inhibition had gradually decreased between 6.5 and 15.5% (Fig. 5). The specific phytochemical contents of rice bran and the results of microbial metabolic activities most likely contributed to the increased antioxidant activities in yogurt samples supplemented with RB than in plain yogurt [59]. Even at low temperatures, yoghurt bacteria are metabolically active, may be responsible for the maximum inhibition of DPPH inhibition following seven days of storage. Several of the antioxidant effects of polyphenols may have changed because of ongoing microbial development during chilled storage. Several of the antioxidant effects of polyphenols may have changed as a result of ongoing microbial development during chilled storage [57]. It is believed that increased breakdown of phenolic components with antioxidant properties is what caused the decline in antioxidant activity during yogurt preservation in the refrigerator [60] or increasing the interaction between polyphenol and milk protein [61]. To take advantage of the strong antioxidant activities beneficial for a preventive cardiovascular effect, yogurt consuming is highly advised within 7 days following yogurt production [62].

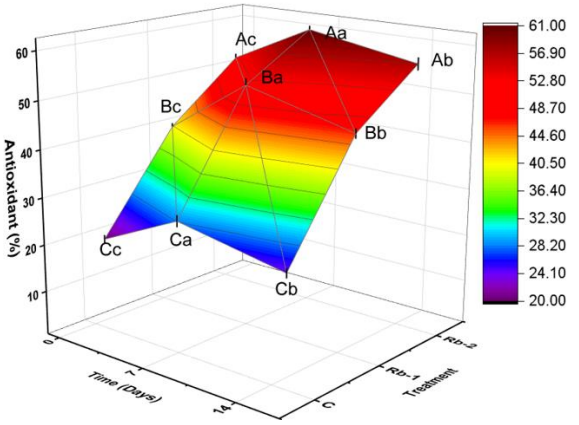

Fig. (5): Response surface plot for antioxidant ability of yogurt fortified with different concentration (1 and 2%) of rice bran and plain yogurt over 14 days of refrigeration (5°C) storage (% suppression of DPPH oxidation). Values are means ± SD from three separate experiments. Significant changes in antioxidant activity between storage period conditions are indicated by lowercase letters ($p < 0.05$). Significant changes in antioxidant activity across treatment are denoted by uppercase letters ($p < 0.05$).

3.8. Proteolysis evaluation by o-phthalaldehyde (OPA) method:

On the initial storage day, all yogurt samples that had been fortified with RB had OPA values that were higher (17.3–18.5 mg/mL) than those of control (15.33 mg/mL) (Fig. 6). Seven days after being stored, yogurt that had been fortified with RB at various concentrations showed an increase in proteolysis (52.6–96%), with the highest peptide concentrations

being seen at 2% of RB (36.2 ± 1.2 mg/mL) followed by 1% of RB (26.4 ± 1.02 mg/mL). The OPA levels in yogurt fortified with RB (23.63 ± 1.0 and 18.9 ± 0.62mg/mL for 2 and 1% of RB, respectively) were higher than those in plain yogurt (15.6 ± 1.06 mg/mL) on day 14 of storage. Since *S. thermophilus* and *L. acidophilus* are metabolically active even at 4 °C [5], the presence of different concentrations of rice bran may have increased the amount of proteolysis by these bacteria, as evidenced by a significant rise in OPA values in yogurt after the first seven days of chilled storage (Fig. 6). This might change protein breakdown and possibly increase the generation of bioactive peptides [63]. Over the following 14 days, the OPA value in plain yogurt during refrigeration remained largely stable, whereas it decreased in yogurt fortified with different concentration of RB. Proteolysis was first accelerated by both RB concentrations before being slowed down. One potential explanation for this is because the RB promotes cell metabolism growth, which raises the acidity [64]. As a result, the lactic acid generated had an impact on the yogurt proteins, causing denaturation and aiding hydrolysis. However, the hydrolysis resulted in the production of peptides and free amino acids, which reduced the acidity. Similar to this, once the bacteria started consuming the generated organic acids and peptides after using up the sugar resources, proteolysis decreased [65].

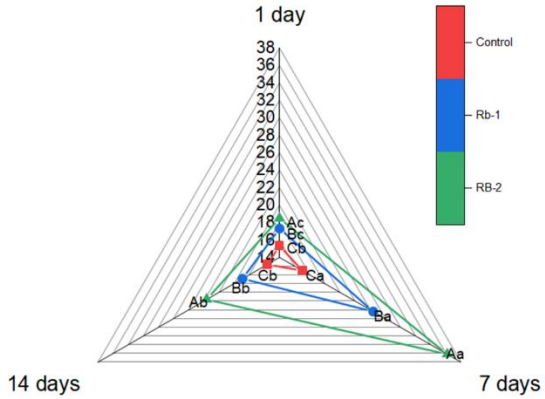

Fig. (6): O-phthalaldehyde (OPA) levels in plain and yogurt fortified with RB changed following fermentation (41 °C) and throughout 28 days of chilled storage (4 °C). Values are means ± SD from three separate experiments. Significant changes in OPA between storage time conditions are indicated by lowercase letters ($p < 0.05$). Significant variations in OPA between treatment doses are denoted by capital letters ($p < 0.05$).

3.9. ACE-inhibitory activity:

By attaching biomolecules like polyphenols, flavonoids, and bioactive peptides to the enzyme's active binding sites, biomolecules like ACE's enzymatic activity can be changed [66]. The ACE assay was used to assess the ACE-inhibitory activity of bioactive peptides created during the production of yogurt and refrigeration storage. The level of ACE inhibition peaked on day 7 of storage but subsequently decreased between 5.26 and 15.16 % on day 14 of storage (Fig. 7), corresponded with comparable reductions in OPA values. This indicates that during the first seven days of cold storage, the relatively less particular peptides created throughout fermentation have been further cleaved to smaller, more bioactive proteins, and that extensive proteolysis of these proteins during prolonged storage up to day 14 produced much smaller, less bioactive proteins. Amirdivani and Baba [25] found that produce fermented dairy products with stronger anti-ACE properties, the microbial fermentation of milk may be modified using peppermint, dill, and basil. At all storage times, yogurt samples fortified with RB at varying concentrations displayed greater anti-ACE activity than control ($p < 0.05$). The maximum ACE inhibition was seen in yogurt fortified with 2% of RB, followed by yogurt with 1% of RB. By attaching to ACE, biomolecules like phytochemicals can change the enzyme's activity [25]. According to Amirdivani and Baba [25], since the ACE activities were unaffected by the water extract of peppermint, dill, and basil, these herbs may have indirectly changed the degradation of milk proteins by influencing the metabolism and growth of the starter culture in yogurt.

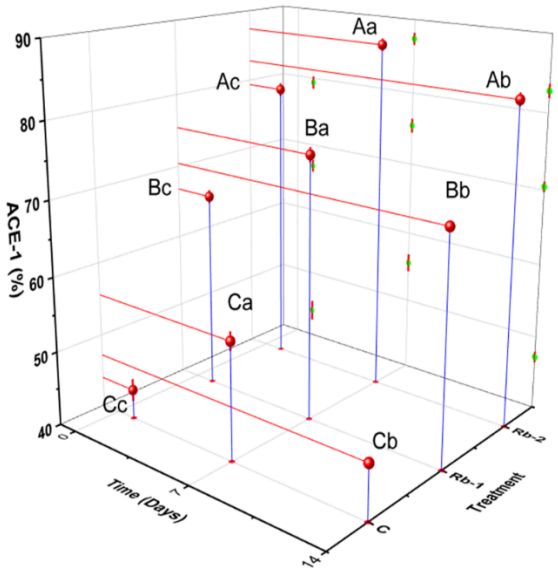

Fig. (7): Inhibition of the angiotensin-I converting enzyme (ACE-I) by a water extract of RB fortified yogurt stored at 4°C compared with control. Values are means ± SD from three separate experiments. Significant changes in ACE-1 between storage period conditions are indicated by lowercase letters ($p < 0.05$). Significant variations in ACE-1 between treatment concentrations are denoted by uppercase letters ($p < 0.05$).

3.10. Counts of viable microorganisms:

The changes in the *B. lactis* BB-12, *S. thermophilus* ST-20 and *L. acidophilus* LA-5 counts obtained in yogurt samples during storage are shown in Fig.8. Up to 14 days of storage, probiotic counts for *L. acidophilus* LA-5 and *B. lactis* BB-12 were typically greater than the minimal therapeutic threshold (10^6 CFU/ g). In all yogurts with or without RB, *S. thermophilus* levels ranged from 6.1to 9.28 log CFU/g. *S. thermophilus* counts in samples of RB-added yoghurt gradually declined between days 1 and 14 of storage. Our findings are consistent with those of Espírito Santo, *et al.* [67], who discovered a comparable decline in *S. thermophilus* counts in açai yoghurts co-fermented with *B. lactis* B104 between 1 and 14 days. The findings agreed with those of Saccaro, *et al.* [68] and Senaka Ranadheera, *et al.* [69], who noted a small decline in *S. thermophilus* during the storage of probiotic yoghurts with fruit juice and *L. acidophilus* LA5.

Throughout storage, the presence of RB with different concentrations had significant effect ($p < 0.05$) on the number of B. *lactis* BB-12 counts (Fig. 8). The yogurt with 2% RB and yogurt with plain yogurt had the lowest and greatest concentrations of *B. lactis* BB-12, respectively. At the end of the shelf life, the final counts of *B. lactis* BB-12 were 6.73 ± 0.06 and 7.27 ± 0.12, respectively. The count of *B. lactis* BB-12 in yoghurt fortified with 1% and 2% RB rose gradually up to 7 days of cold storage period. With regard to *L. acidophilus* LA5 counts, after d1 both 1 and 2 % RB fortified yogurt samples showed counts significantly higher than plain yogurt ($p < 0.05$). All microbial counts were negatively impacted by the longest shelf life (d 14), with *B. lactis* BB-12 showing by far the lowest values. A synergistic effect between different concentration of RB (1-2%) and probiotic strains *L. acidophilus* LA5 and *B. lactis* BB-12) is evident ($p < 0.05$). Similar to this, researchers who examined at various yogurt supplements noticed that *L. acidophilus* or *Bifidobacteria* levels rose [70,71]. Santos, *et al.* [72] had also studied the prebiotic flours used in dairy food processing and emphasized the possible prebiotic benefits of adding flour derived from fruits or other sources by encouraging the growth and increasing the viability of probiotics like *Lactobacillus* and *Bifidobacteria*.

4. Conclusion

The presence of rice bran modified the yogurt bacteria fermentation of milk leading to improved yogurt acidification, bioactive peptides formation and ACE inhibition. In the presence of 2% RB, followed by 1% RB, yogurt bacteria had the highest levels of proteolytic activity during fermentation and cold storage. Higher peptide synthesis during storage is well correlated with increased ACE inhibition. Fortification of RB resulted in an increase in pH, WHC, whereas a decrease syneresis of set yogurt. RB supplementation at different concentrations enhanced of total phenolic content and antioxidant activity of yoghurt samples. Unfortunately, acceptance of all RB-enriched yoghurts was lower than control. The probiotic yogurt's inclusion of rice bran had no negative influence on the bacterial counts. In contrast, it improved the probiotic strains' ability to survive. (*L. acidophilus* LA-5 and *B. lactis* BB-12) during cold storage. These RB fortified yogurts at different concentration, by virtue of the increased antioxidant activities and existence of bioactive peptides and may provide a novel range of yogurts with eligible multifunctional health benefits to consumers with hypertension.

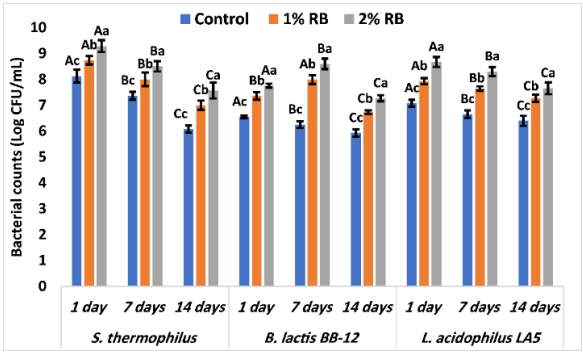

Fig. (8): Microbial changes of RB fortified yogurt and control during 14 days of cold storage. Vertical bars represent the means of treatments ± standard deviations. Significant changes in viable counts of bacteria between storage period conditions are indicated by uppercase letters ($p < 0.05$). Significant variations in microbial counts between treatments are denoted by lowercase letters ($p < 0.05$).

Data Availability Statement

All of the data is contained within the article and the Supplementary Materials.

Conflicts of Interest

The authors declare no conflict of interest.

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