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Utilization of Fermented Yeast Rice by the Fungus *Monascus ruber* AUMC 4066 as Food Coloring Agents

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Abstract

The red fermented rice has higher nutritive value and the function of building up body. The red rice contains abundant protein, fats, vitamins, trace elements and coarse fiber. Red yeast rice fermented by *Monascus ruber* 4066 was used for the study. Yogurts with *Streptococcus thermophilus* and *Bifidobacterium bifidum* were prepared using cow's milk supplemented with red yeast rice flour (RYRF) at 1%, 2%, 3% (wt/wt) and stored at 5°C to 14 days. All preparations of RYRF yogurts showed higher titratable acidity, viscosity, water holding capacity (WHC), hardness, adhesiveness and cohesiveness compared to that of plain yogurts throughout the storage period. The count of *B. bifidum* in RYRF yogurts (> 7 log cfu. g^{-1}) was more than that of plain yogurts (> 6 log cfu. g^{-1}) at the end of storage period, probably due to prebiotic effect of RYRF. There were significant differences among the yogurt samples in relation to their organoleptic properties. The current results recommend addition of 3% RYRF to yoghurt that enhanced physicochemical, organoleptic and textural properties and the viability of *B. bifidum* in bio-yoghurt as functional food.

Keywords: *Monascus ruber*; Yoghurt; *S. thermophilus*; *B. bifidum*; Functional food; Red yeast rice

Introduction

Yoghurt is one of the most popular fermented milk products worldwide [1] and is becoming more and more popular in Egypt. *Bifidobacteria* has been associated with health-promoting effects; there has been an increasing in incorporating this microbial group into dairy and dairy foods or supplementing dairy foods. The ultimate intent of this strategy is to provide the gastrointestinal tract of humans with viable populations of bifidobacteria. Food coloring is very important for commercial food production especially children food. Therefore, there is a need to other alternative sources of natural food colorants like microorganisms, some pigments have been produced from *Monascus* [2]. Production of pigments from *Monascus* depends on the kind of metabolites, yield, colour degree, stability, and safety, as well as on the strains, substrates and cultivation conditions [3-6]. *Monascus* fermented rice has been used as a natural food colorant, food preservative and as functional food because of the monacolin K (antihypercholesterolemic agents), γ-aminobutyric acid (GABA) (hypotensive agent), and dimerumic acid (antioxidant) found [7]. Bioactivity metabolites for *Monascus* spp. included aroma and flavor compound, antibacterial, anticancer, anti-cardiovascular disease, anticholesterols and antioxidant, human health supporting health care and immune enhancer metabolites [5,8]. Traditionally fermented red yeast rice proved to contain many active constituents such as compounds resembling statins, unsaturated fatty acid, sterols and B-complex vitamins [9]. Chen et al. [10] revealed that ethanolic extracts of two *M. ruber* strains cultivated on different media and analyzed by TLC and GC/MS were free of citrinin because the gene which responsible for citrinin biosynthesis has not been found in *M. ruber*. Therefore, the red pigments of *M. ruber* strains can be safe for biotechnological applications [11]. Colored yoghurt using the red pigment from *Monascus ruber* may have health-promoting constituents. This study investigated the effect of functional properties of red yeast rice *Monascus ruber* at different concentration of 1%, 2% and 3% (w/w) on the organoleptic, physicochemical and textural properties, and *B. bifidum* viability in yogurt samples during the storage period 14 days at 5°C.

Materials and Methods

Monascus strains

A strain of *M. ruber* AUMC 4066 (CBS 109.07) kindly obtained from Assiut University Mycological Centre Collection, Assiut, Egypt, was maintained on rice agar slant containing (in g/L): rice powder 50, KH_2PO_4 2.5, $NaNO_3$ 3$MgSO_4$. $7H_2O$. 0.5 and distilled water 1L, pH to 6 [4,11], the medium was dispensed in tubes and was then autoclaved at 121°C for 15 min. The fungus was incubated at 26°C for 10 days under static conditions, to the fully speculated agar slants, 10 ml of sterile distilled water were added and the spores were scraped under aseptic conditions. The obtained spore suspension was used as inoculum.

Fermentation media and cultivation

Commercial rice from those marketed at Assiut was used. The fungus was grown on natural rice medium. Aliquots of 200 gm rice moistened with 20% water in 500 ml Erlenmeyer flasks were autoclaved. Flask was inoculated with 10 ml spore suspension and incubated at 26°C for 10 days. Flasks containing cultures were put at 45°C till completes drying. The dried cultures were ground and used as food coloring agent.

Production of probiotic yogurts

Set and stirred probiotic yogurts were produced using a standard yogurt manufacturing procedures. Standardized milk (4% fat and 12% SNF) was homogenized at 60°C and then pasteurized at 95°C for 15 min.

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The milk was then cooled at 43°C and aseptically inoculated with 0.01% (w/w) of each strain of *L. acidophilus* and *B. bifidum*. Additionally, an equal mixture of the two strains with a ratio of 1:1 (0.01% w/w) was added to the milk. Bar-berry extract in different concentration of 4% and 5% (w/w) was further added to the sterile containers to produce set yogurt and then it was incubated at 37°C for 18 h. Besides, after the preliminary incubation, barberry extracts in concentration of 4% and 5% (w/w) were added to obtain stirred yogurt. All the cultured milk groups were then left for a final fermentation at 43°C. The fermentation was terminated when the acidity level reached a pH value of 4.7. Next, yogurt samples were cooled to 5°C and held at this temperature for one day. All the experimental samples were subjected to microbiological analysis during the 21st day of storage period.

Red yeast rice

Red yeast rice fermented by strain of *M. ruber*, AUMC 4066 from Assiut University Mycological Centre, Assiut, Egypt. Preparation of inoculum *Monascus ruber* strains were grown on rice slants at 26°C under static conditions.

Production of RYRF yoghurt

Yogurts were produced using a standard yogurt manufacturing procedures [12]. Red yeast rice flour at different concentration of 1%, 2% and 3% (w/w) was added to the sterile containers in cow milk (3% fat, 11.5% TS, 0.17%TA and 6.6 pH) and pasteurized in a water bath at 85°C for 30 min. The milk was then cooled at 43°C and aseptically inoculated with yogurt starter culture *Streptococcus thermophilus* and *B. bifidum* at a ratio of 1:1 and then it was incubated at 37°C for 3-4 hrs until reaching ~ 4.6 pH. All the cultured milk groups were then left for a final fermentation at 43 ± 1°C. The fermentation was terminated when the acidity level reached a pH value of 4.7. Next yogurt samples were cooled to 5°C and held at this temperature for one day. All the experimental samples were subjected to organoleptic, physiochemical, and textural properties as well as *B. bifidum* viability analysis during the 14 day of storage period.

Physicochemical properties

Total solid content, fat content, pH and titratable acidity of raw cow's milk were measured according to AOAC [13]. Moisture, crude protein, rude fat, crude fiber, ash and carbohydrate contents of the rice were analyzed according to AOAC [14]. The titratable acidity and pH of yogurt samples were also measured during the storage period. Water holding capacity (WHC) of red yeast rice flour yogurt was determined according to the method of AOAC [14], Isanga and Zhang [15] with slight modifications. Ten grams of yogurt was measured into an oven dried centrifuge tube and centrifuged at 4600 rpm for 30 min and the WHC was calculated as follows:

$$\text{WHC }(\%) = (1 - W_1/W_2) \times 100$$

Where,

W_1= Weight of whey after centrifugation.

W_2 = Initial weight of yogurt sample.

Apparent viscosity of red yeast rice flour yogurts was measured directly with Brookfield Digital Rheometer, model HA-DVIII ultra (Brookfield Engineering Laboratories INC). The concentrate was placed in a small sample adapter; HA-07 spindle was selected for the sample measurement. A thermostatic water bath provided with the instrument Machine (TMS-Pro) Food Technology Corporation, Sterling, Virginia, USA) was used to regulate the sample temperature. The rheological parameters and texture hardness for red yeast rice flour yogurts were measured.

Textural Properties

Texture profile analysis of plain and RYRF yoghurt samples (which shape was 2 × 2 × 2 cylindrical) was done using a Universal Testing apparatus equipped with 1000 N (250 lbf) load cell and connected to a computer programmed with Texture Pro™ texture analysis software (program, DEV TPA with 2sec holding time between cycles). A flat rod probe (49.95 mm in diameter) to uniaxial compressed samples with the following parameters conduction to 30% of their original height. Each sample was subjected to two subsequent cycles (bites) of compression-decompression. Data were collected and the texture profile parameters were calculated from DEV TPA texture analyzer and computer interface. Calculation described by Bourne [16] Szczesniak et al. [17] was used to obtain the texture profile parameters which included (hardness, cohesiveness, springiness, gumminess and chewiness).

Sensory properties

Stored RYRF yoghurt samples were assessed by 8 panelists using a sensory rating scale of 1 (poor) to 9 (excellent) for some sensory parameters such as the color, odor, texture, flavor, and overall acceptability, as described by Bodyfelt et al. [18]. All RYFR yogurt samples were presented to the panelists in the plastic jars (80 ml) and at 4°C to 6°C. The tasting panel was randomly selected among the staff members who very familiar with fermented dairy products and who were evaluated for sensory acuity and consistency.

Statistical analysis

The data from the organoleptic, physicochemical and textural properties and *B. bifidum* viability were analyzed using by one-way ANOVA using SPSS.16 statistical software. A p value <0.05 was considered statistically significant for all analysis.

Results and Discussion

The chemical composition of the rice for moisture, crude protein, crude fat, crude fiber, ash and carbohydrate were 11.00 ± 0.019%, 20.00 ± 0.02%, 0.80 ± 0.01%, 0.20 ± 0.03%, 0.60 ± 0.02% and 74.40 ± 0.01%, respectively.

Organoleptic properties of RYRF yoghurts

The effect of different RYRF on the organoleptic properties of yoghurt is given in Table 1. Significant differences were observed among all the sensory attributes of yoghurt (p<0.05) (Table 1). The 3% RYRF yoghurts had highest color, texture, flavor, odor and overall acceptability score in comparison to 1% and 2% RYRF yoghurts. The scores of color, texture, flavor, odor and overall acceptability were significantly (p>0.05) changed by the increase of RYRF concentration and the prolong storage time in 2 weeks at 5°C. However, all the organoleptic properties slightly decreased during the storage of RYRF yoghurts. The decline in organoleptic properties during storage might be related to the associated decrease in pH values [19-21].

Physicochemical characteristics of the red yoghurts

pH values and titratable acidity: The pH values and titratable acidity of the RYRF yoghurts were detected during storage for 14 days at 5°C. The results revealed that pH values of control, 1%, 2% and 3% of RYRF yoghurts significantly decreased (p<0.05), while the titratable acidity significantly increased (p<0.05) during the storage (Figure 1). The Decline of pH values and simultaneous increase in titratable acidity of the plain and RYRF yoghurts could be attributed to the starter culture's activity, such as post acidification by bacteria used during fermentation and storage period [22-24]. The fiber in rice might have improved the growth and activity of lactic acid bacteria in the

Concentration (RYRF)	Storage date (day)	Color	Texture	Flavor	Odor	Over all acceptability
1%	1	6.93 ± 0.49[a]	7.62 ± 0.58[a]	8.12 ± 0.58[a]	7.68 ± 0.37[a]	7.31 ± 0.59[a]
	7	6.68 ± 0.59[ab]	7.27 ± 0.66[ab]	7.68 ± 0.45[ab]	6.68 ± 0.59[b]	6.87 ± 0.51[ab]
	14	5.31 ± 0.65[b]	5.37 ± 1.02[b]	6.01 ± 0.66[a]	5.37 ± 0.83[c]	5.12 ± 0.35[b]
2%	1	8.25 ± 0.37[a]	8.25 ± 0.75[a]	8.64 ± 0.26[a]	8.68 ± 0.37[a]	8.56 ± 0.41[a]
	7	7.81 ± 0.25[b]	7.93 ± 0.03[b]	8.12 ± 0.59[b]	7.93 ± 0.49[b]	7.50 ± 0.37[ab]
	14	7.31 ± 0.37[c]	6.87 ± 0.51[c]	7.37 ± 0.35[c]	6.12 ± 0.79[c]	7.18 ± 0.59[b]
3%	1	9.25 ± 0.59[a]	9.25 ± 0.75[a]	9.75 ± 0.26[a]	8.68 ± 0.37[a]	9.56 ± 0.41[ab]
	7	8.62 ± 0.35[b]	8.68 ± 0.37[b]	8.68 ± 0.59[b]	7.93 ± 0.49[b]	8.43 ± 0.41[ab]
	14	7.50 ± 0.37[c]	7.50 ± 0.53[c]	7.37 ± 0.35[c]	6.12 ± 0.79[c]	7.50 ± 0.59[b]

[a,b,c] Values in the same columns having different superscripts are significantly different (p<0.05).

Table 1: Organoleptic properties of RYRF yoghurts samples during 14 day at 5°C (mean ± standard deviation).

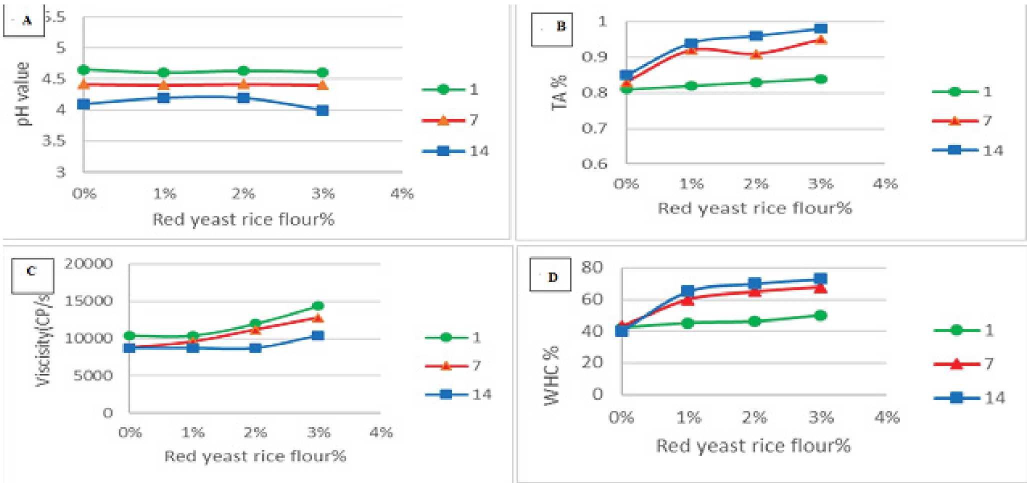

Figure 1: The changes of A: mean pH values and B: mean values of titratable acidity, C: mean apparent viscosity and mean, D: Water holding capacity (WHC) of the RYRF yoghurts at control 1%, 2% and 3% at during 14 days at 5°C respectively.

Concentration (RYRF)	Storage days	Hardness	Adhesiveness	Cohesivess	Springiness	Gumminess	Chewiness
Control 0%	1	0.7 ± 0.10[a]	0.176 ± 0.02[a]	0.25 ± 0.01[a]	13.69 ± 2.32[a]	0.20 ± 0.12[a]	3.05 ± 1.52[a]
	7	0.6 ± 0.20[b]	0.175 ± 0.01[ab]	0.67 ± 0.05[b]	13.75 ± 1.61[b]	0.30 ± 0.24[b]	4.45 ± 1.00[b]
	14	0.9 ± 0.20[c]	0.203 ± 0.03[b]	0.39 ± 0.03[c]	13.75 ± 1.25[b]	0.40 ± 0.12[c]	5.80 ± 2.00[c]
1%	1	0.7 ± 0.10[a]	0.244 ± 0.04[a]	0.28 ± 0.03[a]	13.65 ± 2.23[a]	0.25 ± 0.15[a]	3.15 ± 3.11[a]
	7	0.9 ± 3.43[b]	0.228 ± 0.04[b]	0.45 ± 0.01[b]	13.75 ± 1.65[b]	0.40 ± 0.34[b]	5.57 ± 1.85[b]
	14	1.0 ± 0.10[c]	0.294 ± 0.03[c]	0.49 ± 0.12[c]	13.75 ± 3.03[b]	0.50 ± 0.10[c]	6.99 ± 1.71[c]
2%	1	0.8 ± 0.20[a]	0.259 ± 0.02[a]	0.34 ± 0.03[a]	13.55 ± 1.05[a]	0.25 ± 0.21[a]	3.48 ± 2.13[a]
	7	0.9 ± 0.02[b]	0.258 ± 0.03[ab]	0.54 ± 0.05[b]	13.72 ± 1.13[b]	0.50 ± 0.34[b]	6.57 ± 1.52[b]
	14	1.0 ± 0.30[c]	0.294 ± 0.03[b]	0.47 ± 0.02[c]	13.75 ± 2.15[c]	0.50 ± 0.23[b]	6.71 ± 1.43[c]
3%	1	0.9 ± 0.30[a]	0.342 ± 0.06[a]	0.39 ± 0.06[a]	13.52 ± 1.12[a]	0.35 ± 0.11[a]	4.70 ± 2.34[a]
	7	1.0 ± 0.30[b]	0.486 ± 0.01[b]	0.63 ± 0.01[b]	13.75 ± 3.54[b]	0.50 ± 0.17[b]	6.00 ± 2.15[b]
	14	1.0 ± 0.20[ab]	0.350 ± 0.03[a]	0.48 ± 0.03[c]	13.75 ± 1.05[b]	0.70 ± 0.22[c]	9.88 ± 2.45[c]

[a,b,c] Values in the same columns having different superscripts are significantly different (p<0.05).

Table 2: Apparent viscosity of plain and RYRF yoghurts samples during 14 day at 5°C (mean ± standard deviation).

RYRF yoghurts (as prebiotic) that may also explain the higher titratable acidity values for RYRF yogurts compared to plain yogurt throughout the storage [25] (Tables 2 and 3).

Viscosity: The rheological analysis showed that the yoghurt with RYRF had a higher viscosity than control samples. The changes of the viscosity values of the RYRF yoghurts during 14 days' storage at 5°C are presented in Figure 1. The viscosity apparently decreased ($p<0.05$) at the storage prolonged of the RYRF yoghurts. Similar finding was obtained by Cueva and Aryana [26]. Yoghurt viscosity is due to casein-particle aggregation leading to gelation [27]. When pH values decreased, the mesh of j casein micelle of the milk would become larger, the decreased viscosity is probably because of less water being held by the casein micelle in yoghurts [26] (Table 3).

Water holding capacity (WHC): Higher acidity stimulates separation whey from yoghurt [28]. In the present study addition of RYRF to yoghurt resulted in higher acidity, less whey separation or higher WHC in RYRF yoghurt compared to plain yogurt was observed during the storage period. This may be probably due to high total solids [28] from yoghurts amended RYRF and case strengthened in the RYRF yogurt gel structure and less loose whey. Also rice components

	Storage days	RYRF Concentration %			
		Control	1%	2%	3%
pH	1	4.65 ± 0.0^{a}	4.60 ± 0.04^{a}	4.63 ± 0.01^{a}	4.4.61 ± 0.04^{a}
	7	4.42 ± 0.01^{b}	4.40 ± 0.02^{b}	4.42 ± 0.02^{b}	4.40 ± 0.03^{b}
	14	4.10 ± 0.02^{c}	4.20 ± 0.02^{c}	4.20 ± 0.03^{c}	4.00 ± 0.03^{c}
TA%	1	0.81 ± 0.24^{a}	0.82 ± 0.28^{a}	0.83 ± 0.12^{a}	0.84 ± 0.25^{a}
	7	0.83 ± 0.19^{b}	0.92 ± 0.15^{b}	0.91 ± 0.23^{b}	0.95 ± 0.34^{b}
	14	0.85 ± 0.21^{c}	0.94 ± 0.22^{c}	0.96 ± 0.27^{c}	0.98 ± 0.16^{c}
viscosity	1	10400 ± 6.13^{a}	10400 ± 5.24^{a}	12000 ± 5.32^{a}	14400 ± 6.24^{a}
	7	8800 ± 6.14^{b}	9600 ± 7.42^{b}	11200 ± 6.28^{b}	12800 ± 5.98^{b}
	14	8800 ± 7.25^{b}	8800 ± 4.78^{c}	8800 ± 4.13^{c}	10400 ± 4.59^{c}
WHC%	1	42 ± 0.02^{a}	45 ± 0.02^{a}	46 ± 0.03^{a}	50 ± 0.04^{a}
	7	43 ± 0.01^{b}	60 ± 0.05^{b}	65 ± 0.01^{b}	68 ± 0.04^{b}
	14	40 ± 0.01^{c}	65 ± 0.01^{c}	70 ± 0.02^{c}	73 ± 0.02^{c}

^{a,b,c} Values in the same columns having different superscripts are significantly different ($p<0.05$).

Table 3: The mean changes of the pH, titratable acidity % (TA), viscosity(CP/s) and WHC% of plan and RYRF yoghurts samples during 14 day at 5°C (mean ± standard deviation).

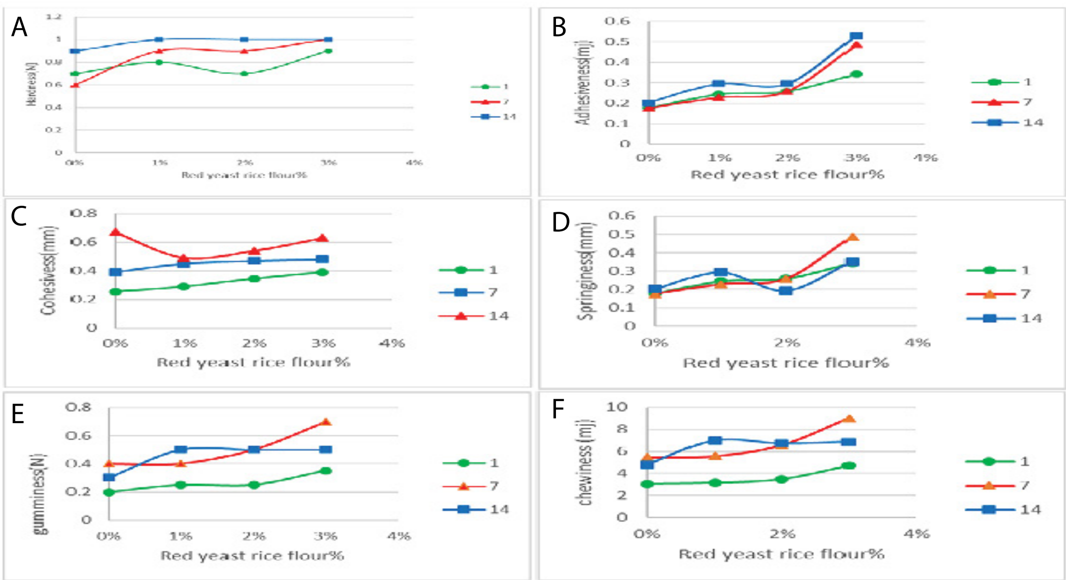

Figure 2: The changes of mean values of A: hardness, B: adhesiveness, C: cohesivess, D: springiness, E: gumminess and F: chewiness and of the RYRF yoghurts at control,1%, 2%, 3% at during 14 days at 5°C.

can enhance the texture, gelling, thickening and stabilizing food properties products [29]. The current results are also in agreement with the previous studies on inulin, orange, apple and wheat fibres [29-32]. Increase in WHC as the storage time progresses could also be due to reabsorption of whey back into the yogurt gel [33]. In contrast, Küçükçetin et al. [34] reported a decreasing trend in WHC of plain yogurt during 15 days of storage at 4°C.

Textural properties

Textural properties of RYRF yogurts have been defined by hardness, adhesiveness, cohesiveness, springiness, gumminess and chewiness [35]. The textural properties of RYRF yogurts were also analyzed during 14 days at 5°C and the results revealed significant differences ($p<0.05$) throughout the shelflife period, being not similar at 1 and 14 days for each type of RYRF yogurt. The texture profile differed with RYRF percentage and storage period. Hardness and adhesiveness has increased with the addition of 1%, 2% and 3% RYRF to yoghurt during the storage period. These results agree with those obtained by the previous works who reported an increase in hardness and adhesiveness of yogurts obtained with the 2% oat-maltodextrin during refrigerated storage for 21 days [36,37], and those obtained by Kasenkas [38] during storage of probiotic torba yogurt. The adhesiveness had a positive effect on the thickness of the yogurts, and was an important factor governing the stability of the products resulting in good mouth-feel and improving the texture characteristics and the stability of yogurts during storage [38]. The RYRF yogurts significantly ($p<0.05$) increased the cohesiveness and springiness of the yogurts during storage period (Figure 2). Cohesiveness and springiness are related to stronger gel structures, indicating greater structural integrity; may be due to the increase in charged groups on the amino acid groups-a function of whey protein denaturation [38]. Chewiness and gumminess were found to be affected positively by RYRF content in yoghurt samples.

Bifidobacterium bifidum viability

Live probiotics in yogurts confer health benefits to the host when

Storage days	RYRF Concentration			
	control	1%	2%	3%
1	8.1 ± 0.52[a]	8.2 ± 0.45[a]	8.2 ± 0.53[a]	8.3 ± 0.62[a]
7	7.9 ± 0.52[b]	8.9 ± 0.63[b]	8.8 ± 0.50[b]	9.0 ± 0.56[b]
14	6.1 ± 0.32[c]	7.0 ± 0.50[c]	7.3 ± 0.46[c]	7.0 ± 0.49[c]

[a,b,c] Values in the same columns having different superscripts are significantly different ($p<0.05$).

Table 4: Viability of *Bifidobacterium bifidum* (log CFU/g^{-1}) of plain and RYRF yoghurts samples during 14 day at 5°C (mean ± standard deviation).

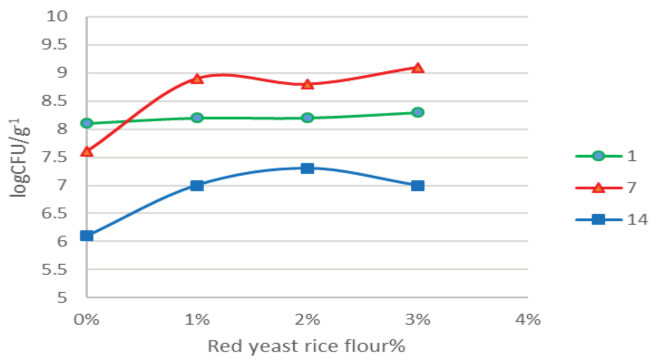

Figure 3: Viability of *Bifidobacterium bifidum* in RYRF at control, 1%, 2%, 3% at during 14 days at 5°C.

they are consumed in appropriate quantity. Accepted concentration of probiotic cultures 6-7 log cfu. g^{-1} of product at the time of consumption. The numbers of *B. bifidum* in bio-yoghurts were found to be (6-9 log cfu/g^{-1}) in studied samples which lies in the standards regulated by FAO/WHO [39,40] protocols, but usually not below 6 log cfu/g^{-1} (Table 4). Which makes bio-yoghurts to sustain their functional properties for entire time during refrigerated storage. Higher viable numbers of *B. bifidum* was observed in RYRF yogurt than in plain yogurt (Figure 3). *Bifidobacterium* spp. are known to produce exopolysaccharides [41]. Thus, exopolysaccharides production by *B. bifidum* might have been high in RYRF than in plain yogurts. This hypothesis is further supported by the higher survivability of bifidobacteria in RYRF yogurts compared to plain yogurt in the present study.

Conclusion

Supplementation of the RYRF to the yogurt enhanced physicochemical properties, textural properties, *B. bifidum* viability of yoghurt during storage for 14 days at 5°C. All concentration added RYRF to yoghurts showed higher titratable acidity, viscosity, WHC, hardness, adhesiveness and cohesivess compared to that of plain yogurts throughout the storage period. The numbers of *B. bifidum* in RYRF yoghurts (6-9 log cfu. g^{-1}) agreed with those standards and regulations of the previous studies but usually not below 10^6 cfu/g so RYRF yoghurts sustained their functional properties for entire time during storage. Addition of RYRF to yoghurt also exhibited stimulatory effect on the *B. bifidum* growth. In the case of RYRF the addition of prebiotics *B. bifidum* caused an increase in t viscosity and hardness values indicating viability of the process to obtain a commercial product.

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