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ORIGINAL PAPER

Effects of β-glucan extracted from *Saccharomyces cerevisiae* on the quality of bio-yoghurts: *in vitro* and *in vivo* evaluation

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Abstract

The present study aimed to extract β-glucan from *Saccharomyces cerevisiae* for use as a fortification at different ratios (0, 0.1, 0.5, and 1%) on the growth of three bio-yoghurt starters (*Lactobacillus acidophilus, Bifidobacterium bifidum* and *Streptococcus thermophilus*). The properties of bio-yoghurts were also studied, along with the effect of feeding bio-yoghurts on blood parameters and weight in animal subjects. Identification of β-glucan extracted from yeast was performed by FTIR and HPLC technique. The viability of probiotic bacteria in bio-yoghurt, containing 1% β-glucan during 14 days of storage at 5 °C, was evaluated along with investigation of pH, titratable acidity and syneresis. The results showed that the yield of β-glucan extracted from *Saccharomyces cerevisiae* ATCC 36,858 reached 3.35%. β-glucan had a water-holding and fat binding capacity of 224% and 80.34%, respectively. Bio-yoghurts fortified with 0.1% and 0.5% β-glucan were deemed acceptable by expert panels. In addition, bio-yoghurt with β-glucan groups outperformed the control group in terms of blood parameters ($P \leq 0.05$). The bio-yoghurt-treated mice showed higher levels of immunity than the control mice. After 45 days of feeding, the weight of the animals in all treatments increased, with the fourth treatment being superior at an average animal weight of 50.86 g. The proposed approach could be beneficial as a fat replacer in the preparation of bio-yoghurts and did not negatively influence a considerable number of acceptability scores. The results achieved open up the possibility to use β-glucan in the preparation of various health and therapeutic foods in the future as a prebiotic chemical candidate.

Keywords β-glucan · Probiotic bacteria · Prebiotic compound · Bio-yoghurts

Introduction

β-glucans are a polymer comprised of non-starch polysaccharides with glucose linked to glycosidic bonds at β-(1,3), (1,4) or (1,6). Their length and branching structure, though, can make a big difference. It is naturally found in the cell walls of yeasts, filamentous fungus, seaweed, and cereals with significant differing physicochemical properties dependent on the source (Marcotuli et al., 2020). β-glucan is one of the most important non-digestible polysaccharide fibers and among the many polysaccharides found in fungus, β-glucans are a major reason fungus are applied in cosmetics, food additives, and medicine (Nguyen et al. 2021). Moreover, β-glucan is a functional ingredient that provides numerous health benefits such as lowering blood cholesterol. In addition, it is anti-inflammatory, anti-cancer, produces fatty acids and supports immune function. So far, β-glucan has been employed in a number of health and nutritional applications in several countries, including the USA, Sweden, Finland and UK (Khan et a., 2018; Yuan et al. 2020).

In addition, according to current recommendations, the EFSA has suggested a 3 g per day minimum dose of

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β-glucan for the reduction of blood cholesterol levels (EFSA 2021). However, in the food industry, β-glucan is used for its various functional properties like thickening, emulsifying and stabilizing agent (Karp et al. 2019; Szpicer et al. 2018; Summo et al. 2020).

In the last few years, there has been increasing demand for a new range of food products, as consumption of high-fat products has decreased due to greater consumer awareness of the potentially harmful effects of fat on health (Di Vita et al. 2019). Fat intake is associated with high blood cholesterol and heart disease, so dietary fiber has been successfully used as a saturated fat replacement in many foods (Essa and Elsebaie 2018). Nowadays, yeast β-glucan is widely used in the food industry as a thickener, emulsifier, and low-calorie food ingredient such as low-fat yoghurt which causes beneficial changes in natural flora composition in the colon, allowing beneficial bacteria to become more prevalent in the body (Mehrinejad Choobari et al. 2021). However, previous studies have been performed to evaluate β-glucan as a substitute for fats in bio-yoghurts and as a functional bioactive prebiotic ingredient; the modification of gut microbiota has been linked to the relief of cognitive deficits by β-glucan and its hypoglycemic action (Kaur and Riar 2020; Wang et al. 2020; Almasi et al. 2021). Some commercial yogurt products have been recently reformulated to include live strains of *Lactobacillus acidophilus* and species of *Bifidobacterium* in addition to the conventional yogurt starter organisms. These products referred to as bio-yogurts have rapidly gained popularity on the world market (Lourens-Hattingh and Viljoen 2002). Thus, the present study aimed to evaluate the viability of probiotic bacteria and the sensorial and functional properties of bio-yoghurts fortified with β-glucan extracted from *Saccharomyces cerevisiae* (*S. cerevisiae*) *in vitro* and *in vivo*.

Materials and methods

Materials

Skim cow milk powder was purchased from a local market (Regilait, France), and raw cow's milk was purchased from the Agricultural Research Station, College of Agri-culture, University of Basrah. All chemicals used in this study were obtained from OXOID, Merck, BDH (England), and Difco (USA).

Yeasts strains

Six yeast strains were used to produce β-glucan. *S. cerevisiae* (Saf-instant 31,150), USA, *S. cerevisiae* Lallemand BRY-97, UK, *S. cerevisiae* Natu N-1212, China, *S. cerevisiae* Yuva-Maya YM-100, Turkey, were procured from the local market in Basrah City, Iraq. *S. cerevisiae* ATCC 36,858 and *S. boulardii* ATCC MYA¬796 were supplied by the Food Science Department, College of Agriculture, University of Basrah, Iraq.

Starter Culture Preparation

Lactobacillus acidophilus (LA-5), *Bifidobacterium bifidum* (Bb-12) and *Streptococcus ther-mophilus* (ST) (Chr. Hansen Holding A/S, Denmark) were activated in reconstituted skim milk (12%) and incubated at 37 °C for until full coagulation. This process was repeated in triplicate (Niamah et al. 2017).

Methods

Extraction of β-glucan

All of the related strains of yeast were grown on Yeast Extract Glucose Peptone Broth medium after inoculation with 10% (log 6.4 CFU/ml) w/v yeast cells and incubated in a shaking incubator at 150 rpm at 30 °C for 48 h (Al-Sahlany et al. 2020). Cells (biomass) were obtained after centrifugation at 5000 rpm, and the mass was washed with distilled water twice and then dried at 40 °C. β-glucan was extracted by adding 200 ml of 1% sodium hydroxide to 4 g of yeast biomass and then centrifuged in order to get rid of the supernatant. The precipitate was suspended in sodium hydroxide and the pH was adjusted to 7. Next, 30 ml of 0.5 N of HCl was added and left for an hour after which centrifugation was carried out, the precipitate was washed with distilled water, and 20 ml of ethanol was added and left for 12 h. Finally, the mixture was centrifuged at 5000 rpm for 20 min, and the residue was lyophilized by a freeze dryer (CHRIST-Germany) in order to obtain β-glucan (Al-Jumaiee et al. 2019).

β-glucan Properties

The yield of extracted β-glucans was calculated as follows: Crude β-glucan yield (%) = Weight of crude β-glucan/Weight of yeast cell powder ×100 (Du et al. 2014). The Water Holding Capacity (WHC) of β-glucan was measured according to the methods described by Wong and Cheung (2005). Briefly, 20 mL of distilled water was added to a centrifuge tube containing β-glucan (0.2 g) and vigorously mixed in a vortex mixer for 15 min. The tubes were centrifuged at 5000 rpm for 30 min., the supernatant was carefully discarded, and the amount of water held in the hydrated sample was determined by heating the pre-weighed pellet in a hot air oven for 2 h at 120 ˚C. The fat holding capacity (FHC)

was determined in triplicate, using the method applied by Lin et al. (1974). A volume of 10 ml of soy oil was added into a centrifuge tube containing β-glucan (0.2 g), and the mixtures were placed at room temperature, ambient conditions for 1 h and agitated in a vortex every 15 min. After centrifugation at 5000 rpm for 20 min., the supernatant was discarded and the residue was weighed. The fat absorption was obtained from the amount of soy oil bound to 1 g of dry sample. Infrared spectra of the extracted β-glucan and commercial β-glucan (Shaanxi Sciphar Hi-Tech Industry Co., Ltd., China) samples were recorded with an FT-IR spectrophotometer (Jasco, Japan) in the range of 4000 – 400 cm^{-1} using the KBr-disk method (Sun et al. 2019). The extracted β-glucan and commercial β-glucan were HPLC-analyzed using column Luna 5 µm C18.The mobile phase was acetonitrile at a flow rate of 0.5 mL/min. The injection volume for both the sample and the reference solution was 10 µL. The pH level was raised to 3.5. The detection was made using UV light with a wavelength of 305 nm.

Preparation of non-fat bio-yoghurt

The bio-yoghurt was made from skim milk with a solids content of 14%. The skimmed milk recovered and divided into two parts, the first was left without any treatment and used as a control sample, while the second was divided into three parts, which β-glucan was added at 0.1, 0.5 and 1%. Then, all bio-yoghurt samples were distributed in plastic cups by 50 g and pasteurized at 80 °C for half an hour and cooled to 24 °C. The plastic cups were inoculated with 2% of the probiotic starter culture and then closed and incubated at 42 °C until the pH reached 4.8. Fermentation was stopped, and the samples were stored for 14 days at a temperature of 4 °C (Hamid and Doosh 2021).

Culture viability determination

The bacterial viability starter was determined according to the methods of Niamah, Al-Sahlany & Al-Manhel (2016). The pour plate method was used to estimate the total bacterial count during storage after 1, 7 and 14 days. MRS-sorbitol, MRS-NNPL and M17 were used to enumerate viable cells of La-5, Bb-12 and ST, respectively.

Determination of pH and titratable acidity

pH values were determined by using a digital pH-meter (HANNA-Romana) at 25˚C; titratable acidity was estimated according to Al-Manhel and Niamah (2017).

Evaluation of syneresis

Syneresis evaluation was determined during storage times according to the methods of Niamah, Al-Sahlany & Al-Manhel (2016). Briefly, bio-yoghurt samples (50 g) were centrifuged at 3000 rpm for 10 min, after which the supernatant was weighed, and the percentage of syneresis was calculated according to the following equation: Syneresis (%) = (W/S) x100, where W is the weight of supernatant, and S is a sample weight.

Sensory analysis

The sensory evaluation of the bio-yoghurt samples was conducted by five trained panelist members from the Department of Food Science at the University of Basrah according to the method of Sahan et al. (2008), which includes a 0-5-point scale for consistency, appearance (color, syneresis), flavor and aroma.

Animals

For the *in vivo* stage of this study, thirty BALB/c mice (10 weeks old, weighing 25–30 g), were obtained from the College of Medicine, University of Basrah. The mice were fed a concentrated diet consisting of (30% wheat, 20% barley, 20% corn, 10% animal protein, 10% animal fat, 9% milk powder, and 1% salt) and divided into five groups of six mice each (TC, T1, T2, T3 and T4). The concentrated diet was provided to group TC, while group T1 received the diet plus 1 gm/day of bio-yogurt containing log 9.2 CFU/mL of probiotic bacteria. Groups T2, T3, T4 were given one gram of bio-yogurt containing log 9.2 CFU/mL of probiotic bacteria with 0.1, 0.5 and 1% of β-glucan. The regimens described above were provided every day for 45 days.

Experiments procedures were conducted in conformance with the international guidelines for the care and use of laboratory animals elaborated by the National Academy of Sciences and published by the National Institutes of Health (The Guide for the Care and Use of Laboratory Animals, 2016).

Blood analyses

All mice were fasted and sacrificed humanely after 45 days of feeding (excepting one week of acclimation). An automatic hematological analyzer (CEE-DYN EM, France) was used to determine the red blood cell count (RBC), white blood cell count (WBC), hemoglobin (HB), platelet count (PLT), neutrophils, lymphocytes, monocytes, and eosinophils (Evivie et al. 2019). Total cholesterol (TC),

triglycerides (TG) and total protein were measured using a Biolabo company kit (France).

Animal weight

The animals' weights were monitored for 45 days. The formula daily weight gain (DWG) = (final weight-initial weight)/day was used to compute the daily weight increase.

Statistical analysis

A factorial experiment with the complete randomized design was used for analyzing data by SPSS software, version 21. The least-square design (L.S.D.) was used to compare among the means, and the treatments were performed in triplicate.

Results and discussion

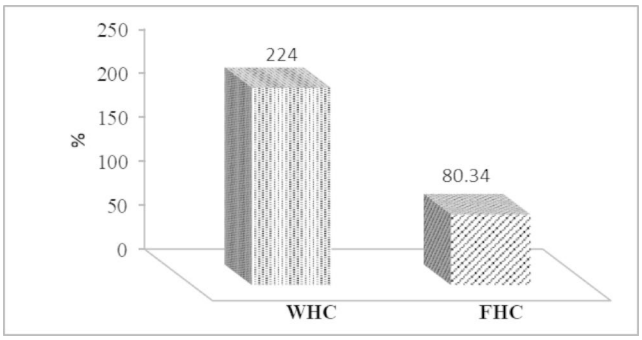

Fig. 2 Water and fat holding capacity of β-glucan extracted from *S. cerevisiae* ATCC 36,858

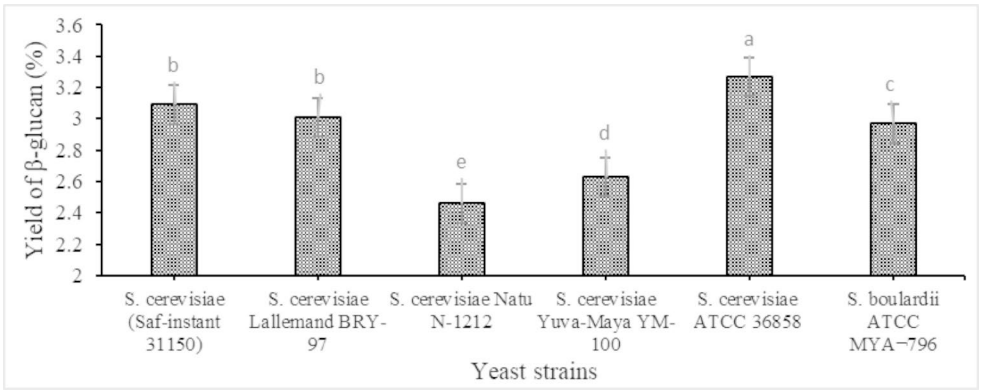

Fig. 1 The yield of β-glucan extracted from yeast strains, a-d Different superscripts in a column were significantly different ($p \leq 0.05$). ±SD: standard deviation

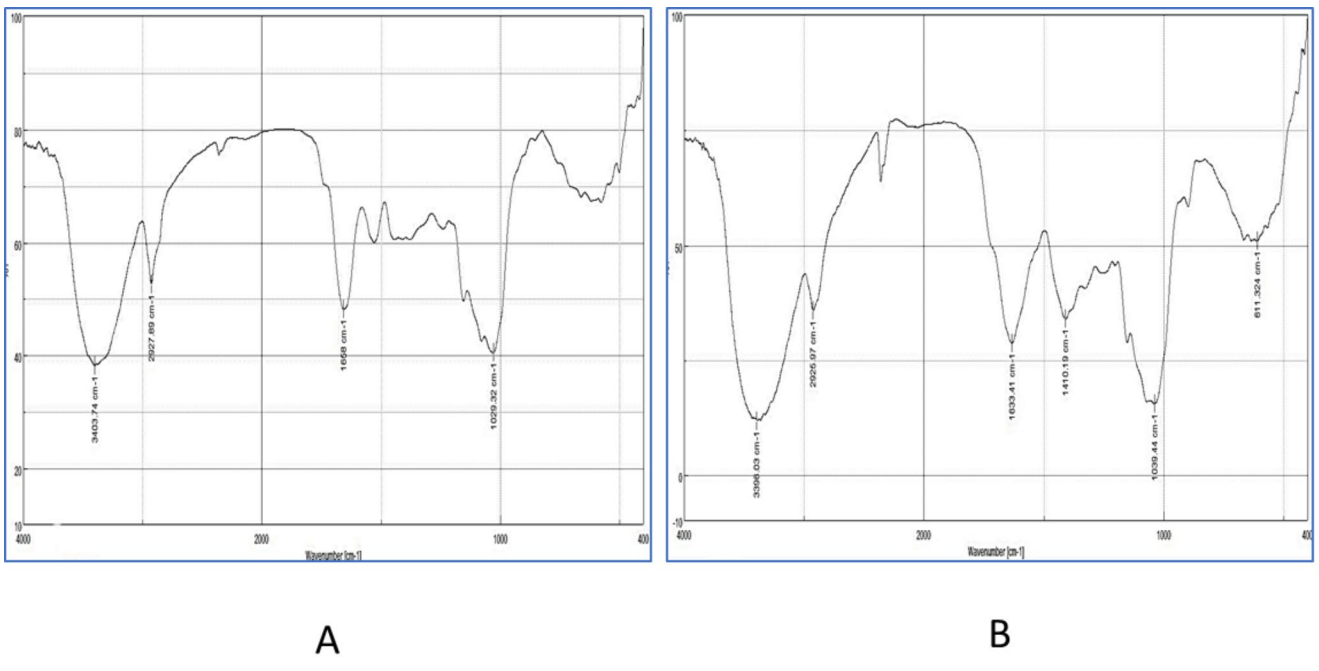

Fig. 3 Infrared spectra of β-glucan extracted from *S. cerevisiae* ATCC 36,858, A: β-glucan standard and B: extracted β-glucan from yeast

β-glucan Properties

The results showed that the yield of β-glucan from *S. cerevisiae* ATCC 36,858 was 3.35%, while the yield of β-glucan from *S. cerevisiae* Natu N-1212 was 2.46% (Fig. 1). This result was in agreement with that of the previous study in which the β-glucan from *S. cerevisiae* yield ranged from 0.34 to 7.35% (Many and Vizhi 2014; Asare 2015) found that the yield of β-glucan from *S. cerevisiae* was 2.9 to 10.3%. Such a contrast in results could be attributed to the difference in methods and conditions of the extraction, and may be due to the difference in the yeast strain.

Figure 2 shows that the water holding capacity (WHC) of β-glucan was 224%, while the fat holding capacity of β-glucan was 80.34%. This higher WHC was attributed to the extracted β-glucan and pentosan content as well as high concentrations of hydroxyl that can form hydrogen bonds with water (Nguyen et al. 2002). The current findings are also consistent with previous findings in which the water holding capacity of the β-glucan ranged between 3.14 and 4.52 g/g (Ahmad et al. 2010).

Identification of β-glucan extracted

The IR region of 4000-400 cm^{-1} provides information about fundamental vibrations. The IR spectra of commercial and extracted β-glucan are shown in Fig. 3a-b.

Carbohydrates (β-glucan) can be identified and extracted by peaks at wave numbers of 1039.44 cm^{-1}, 1029.32 cm^{-1} (C-O bond from the alcohol group), 2927.89 cm^{-1}, 2925.97 cm^{-1} (C–H stretch), 3396.04 cm^{-1} and 3403.74 cm^{-1} (-OH stretch) (Ahmad et al. 2016; Adabi et al. 2011). The presence of polysaccharides in the present study is in agreement with Fernando et al. (2017), who indicated that it can be depicted by the occurrence of absorption bands in region 1000–1200 cm^{-1}. This is due to the stretching of (CC) (C-O) groups and represents the presence of glucopyranose. It is also important to note that the spectra showed that the bands at 1633 cm^{-1} in the spectrum were due to the stretching of CN and NH groups of proteins and are an indicator of amide I and amide II. The absorption in the region of 1410 and 3396 cm^{-1} was due to the stretching of the hydroxyl group of water. Small peaks at 2925 cm^{-1} show the presence of small amounts of lipids in all absorption spectra (Mahmoud Amer et al. 2021).

The structural similarity of the extracted β-glucan with the β-glucan standard was verified by HPLC analysis. As shown in Fig. 4B, the analysis revealed one peak 5.70 min of liquid samples of β-glucan extracted from *S. cerevisiae* ATCC 36,858, which represents the purity of the yeast-extracted β-glucan. In Fig. 4 A, such a peak had the same retention duration as the β-glucan standard. The significant peak of β-glucan extracted from *S. cerevisiae* agrees with Ibrahim (2014), which also demonstrated one peak of β-glucan extracted from *S. cerevisiae* in HPLC analysis.

Culture viability determination

After one day of fermentation, the viability of bacteria in bio-yoghurts made without β-glucan was log 7.95, 8.22, and

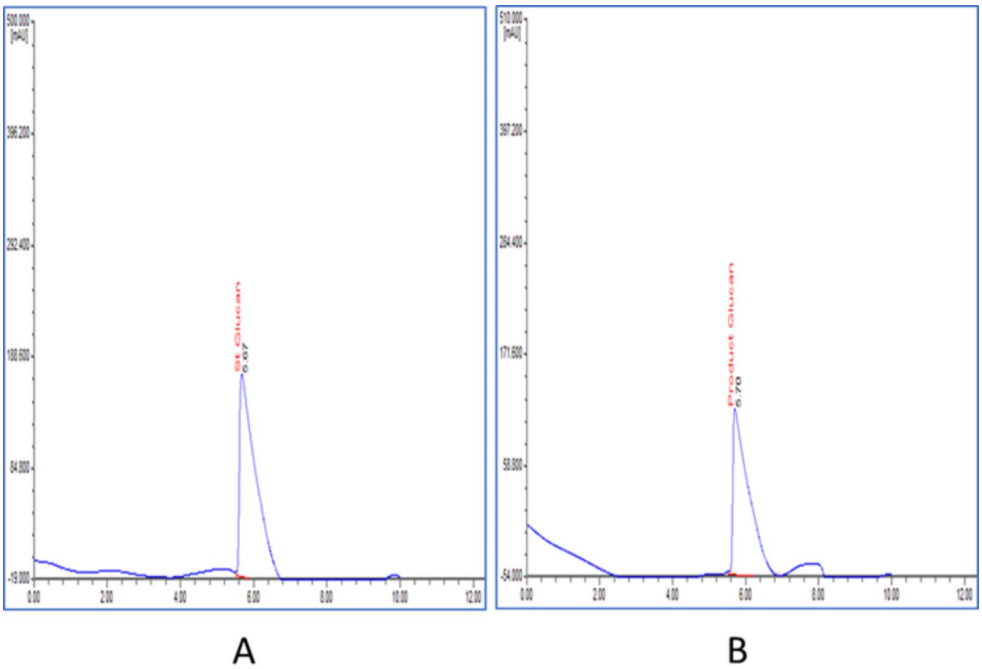

Fig. 4 HPLC analysis of β-glucan extracted from *S. cerevisiae* ATCC 36,858, A: β-glucan standard and B: extracted β-glucan from yeast

7.13 CFU/g for La-5, Bb-12, and ST, respectively, and the numbers of cell viability decreased to log 6.54, 7.02, and 7 CFU/g, respectively, under storage conditions at 4˚C for 14 days. The viability of probiotic bacteria remained between 107 and 108 CFU/g, especially in bio-yoghurt fortified with β-glucan, which reached log 7.73, 8.33, and 7.17 CFU/g, for La-5, Bb-12, and ST, respectively, after 14 days at 5˚C (Table 1).

This result is attributed to the importance of prebiotics such as Arabic Gum, mannan, and another oligosaccharide that improved bacteria survival above 107 CFU/g (Karlton-Senaye et al. 2015). However, high acidity and low temperature negatively affected growth conditions and thus affected the viability of probiotic bacteria such as *Lactobacillus acidophilus* and *Bifidobacterium bifidum* (Shah et al. 1995; Ibrahim and Carr 2006). The results were not in agreement with Vasiljevic et al. (2007) who studied the effect of adding β-glucan extracted from oats and barley on the probiotic bacteria (*Bifidobacterium animalis* subsp. *lactis*) used in the manufacture of bio-yoghurts. There was no significant effect at the beginning of fermentation on the number of bacteria, but there was a change in the amount of lactose. Lee et al. (2020) indicated that the addition of β-glucooligosaccharides extracted from β -glucan had an effect on the survival and anti-bacterial activity of lactic acid bacteria. These results were in agreement with Elsanhoty and Ramadan (2018) and Mohammadian et al. (2019) who reported an increase in probiotic bacteria growth in bio-yoghurts fortified with β-glucan.

Physicochemical analysis

As noted in Table 2, storage and ratios of β-glucan had significant effects ($P < 0.05$) on the pH of bio-yoghurts. On the other hand, the pH of the yoghurt samples decreased during storage with the addition of β-glucan and ranged from 4.62 to 4.24 at a 1% ratio for 14 days, which was lower than the pH of the bio-yoghurt without β-glucan. This is because β-glucan as a prebiotic is a more suitable medium for fermentation of lactose into lactic acid and increasing the number of bacteria (Raikos et al. 2018).

This result agrees with numerous studies that re-ported a significant decrease in pH of 4.5 with an increase in the fermentation period of the bio-yoghurt containing β-glucan at a concentration of 0.2%, after which there was no significant difference between the control treatment and other treatments (Vanegas-Azuero and Gutiérrez 2018). The total acidity value of the control bio-yoghurt without β-glucan extract was 0.74% after fermentation, and, as shown in Table 2, storage and β-glucan ratios had significant effects ($P < 0.05$) on the total acidity of the bio-yoghurt. Increased total acidity values were observed in the bio-yoghurt samples with increased storage time. However, little difference was observed between the bio-yoghurt samples that contained β-glucan at 0.1 and 0.5% compared to the control sample, which was 0.74% and reached 0.87, 0.88 and 0.90 for the control and bio-yoghurt samples that contained β-glucan, respectively. The increase in total acidity was attributed to the activity of probiotic bacteria cultures during storage in the presence of β-glucan, which caused the increased production of organic acid during fermentation, increasing the total acidity of bio-yoghurt fortified with β-glucan as mentioned in previous studies Kaur and Riar (2020) who added

Table 1 Effects of β-glucan at different ratios on the viability of probiotic bacteria (log CFU/g) in bio-yoghurt samples during (0, 7 and 14) day

Strains		La-5			Bb-12			ST	
β-glucan (%)	0	7	14	0	7	14	0	7	14
0	$7.95^{a} \pm 0.13$	$7.11^{a} \pm 0.11$	$6.54^{a} \pm 0.16$	$8.22^{a} \pm 0.31$	$8.11^{a} \pm 0.34$	$7.02^{a} \pm 0.21$	$7.13^{a} \pm 0.01$	$7.11^{a} \pm 0.33$	$7.00^{a} \pm 0.12$
0.1	$8.22^{b} \pm 0.10$	$7.50^{b} \pm 0.05$	$6.78^{a} \pm 0.14$	$8.62^{b} \pm 0.11$	$8.50^{b} \pm 0.18$	$7.78^{b} \pm 0.32$	$7.15^{a} \pm 0.10$	$7.15^{a} \pm 0.13$	$7.10^{a} \pm 0.15$
0.5	$8.55^{b} \pm 0.22$	$7.80^{b} \pm 0.13$	$6.96^{b} \pm 0.11$	$8.85^{b} \pm 0.35$	$8.65^{b} \pm 0.30$	$8.16^{c} \pm 0.30$	$7.22^{a} \pm 0.17$	$7.18^{a} \pm 0.15$	$7.16^{a} \pm 0.22$
1	$8.91^{c} \pm 0.36$	$8.20^{c} \pm 0.19$	$7.73^{c} \pm 0.22$	$9.11^{c} \pm 0.15$	$9.20^{c} \pm 0.28$	$8.33^{c} \pm 0.26$	$7.25^{a} \pm 0.21$	$7.20^{a} \pm 0.22$	$7.17^{a} \pm 0.23$

No. of repeaters = 3, ^{a,b,c} Different superscripts in a column were significantly different ($p \leq 0.05$). ±SD: standard deviation

Table 2 Effects of β-glucan at different ratios on the physicochemical analysis of bio-yoghurt samples during (0, 7 and 14) day

Tests		pH		Total acidity (%)			Syneresis (%)		
β-glucan (%)	**1**	**7**	14	1	7	14	1	7	14
0	$4.70^{a} \pm 0.01$	$4.61^{a} \pm 0.01$	$4.41^{a} \pm 0.01$	$0.74^{a} \pm 0.04$	$0.81^{a} \pm 0.02$	$0.87^{a} \pm 0.04$	$6.63^{a} \pm 0.09$	$6.78^{a} \pm 0.04$	$7.09^{a} \pm 0.10$
0.1	$4.68^{a} \pm 0.04$	$4.63^{a} \pm 0.02$	$4.36^{b} \pm 0.01$	$0.76^{a} \pm 0.03$	$0.83^{a} \pm 0.01$	$0.88^{a} \pm 0.01$	$6.22^{a} \pm 0.04$	$6.28^{b} \pm 0.07$	$6.51^{b} \pm 0.05$
0.5	$4.66^{a} \pm 0.01$	$4.60^{a} \pm 0.02$	$4.32^{b} \pm 0.02$	$0.77^{a} \pm 0.04$	$0.84^{a} \pm 0.01$	$0.90^{b} \pm 0.10$	$5.87^{b} \pm 0.07$	$6.05^{c} \pm 0.06$	$6.27^{b} \pm 0.04$
1	$4.62^{b} \pm 0.09$	$4.58^{b} \pm 0.01$	$4.24^{c} \pm 0.01$	$0.80^{b} \pm 0.06$	$0.85^{b} \pm 0.01$	$0.93^{c} \pm 0.01$	$4.61^{c} \pm 0.01$	$4.70^{d} \pm 0.06$	$4.92^{c} \pm 0.05$

No. of repeaters = 3, ^{a,b,c} Different superscripts in a column were significantly different ($p \leq 0.05$). ±SD: standard deviation

the β-glucan at different ratio (0.5, 1, 1.5 and 2%) into the bio-yoghurt. The whey syneresis, viscosity, texture and sensory properties through storage time were improved.

One of the disadvantages or undesirable qualities of the bio-yoghurt is the syneresis (separation of whey) from the fermented dairy product. It was observed (Table 2) that there was a gradual significant decrease ($p < 0.05$) in the separation of the whey with an in-creased concentration of β-glucan compared to the control sample. However, storage neg-atively affected syneresis, as increased storage increased the process of separation of whey, while bio-yoghurts fortified with 1% β-glucan showed significantly improved ability to retain amounts of whey compared to the control samples. Similar observations were also reported by Raikos et al. (2018), who found that decreased separation of whey from bio-yoghurt with increased prebiotic added to the storage. In addition, Mejri et al. (2014) indicated that the addition of β-glucan reduced the syneresis of skimmed bio-yoghurt. Similarly, Singh et al. (2012) observed that the addition of β-glucan significantly decreased syneresis in all bio-yoghurt samples. The author attributed this to the ability of β-glucan to form transverse cross-linking of the casein, protein, and β-glucan gel network. However, β-glucan works to bind water in the three-dimensional network of the product. In other words, the hydration and network structure trapped the water molecules in this structure and prevented the water from escaping, thereby reducing the syneresis (Ng et al. 2017; Sharaf Eddin et al. 2021).

Sensory analysis

The sensory evaluation scores of bio-yoghurt samples are presented in Table 3. It was observed that the additive did not influence any of the consistency attributes of the bio-yoghurt fortified with β-glucan with full-fat bio-yoghurt. The general acceptability scores of the samples decreased during storage. The sensory evaluation scores were high by using 0.5% β-glucan compared to the control sample. This highlights the significance of using β-glucan as a fat replacer in bio-yoghurt. Vanegas-Azuero and Gutiérrez (2018) reported that low-fat dairy products fortified with β-glucan can mimic the capabilities, mouthfeel, and sensory properties of full-fat products. This result could also be related to the decrease in syneresis, resulting in a more homogeneous appearance than that of the control sample. However, increasing β-glucan resulted in a reduction in flavour and aroma acceptability as well as greater thickness of the bio-yoghurt samples during storage time. These results were similar to those of Huang et al. (2020) in which the best sensory characteristics of bio-yoghurt resulted from the addition of 0.10% polysaccharide extracts, indicating that a high concentration of fibers could negatively affect low-fat bio-yoghurt (Guven et al. 2005). Previous studies found the best results for sensory properties when β-glucan was added at 0.25% to the bio-yoghurt during storage time (Kılıç and Akpınar 2013; Khorshidian et al. 2018; Bahrami et al. 2013), indicated an improvement in the texture and

Table 3 Sensory evaluation of the bio-yoghurt containing β-glucan with different ratios at different storage times (0, 7 and 14) day

β-glucan (%)	Storage days	Variables			
		Consistency (5 points)	Appearance (5 points)	Flavor (5 points)	Odor (5 points)
0	1	$4.4^{a} \pm 0.3$	$4.3^{a} \pm 0.2$	$4.8^{a} \pm 0.4$	$4.5^{a} \pm 0.2$
	7	$4.0^{b} \pm 0.1$	$4.2^{a} \pm 0.1$	$4.6^{b} \pm 0.2$	$4.3^{b} \pm 0.2$
	14	$3.8^{c} \pm 0.1$	$3.9^{b} \pm 0.3$	$4.2^{c} \pm 0.3$	$4.0^{c} \pm 0.1$
0.1	1	$4.5^{a} \pm 0.2$	$4.3^{a} \pm 0.1$	$4.5^{a} \pm 0.2$	$4.5^{a} \pm 0.5$
	7	$4.3^{a} \pm 0.2$	$4.1^{b} \pm 0.1$	$4.4^{a} \pm 0.3$	$4.2^{b} \pm 0.2$
	14	$4.1^{b} \pm 0.1$	$4.0^{c} \pm 0.3$	$4.1^{b} \pm 0.1$	$4.0^{c} \pm 0.1$
0.5	1	$4.8^{a} \pm 0.4$	$4.9^{a} \pm 0.3$	$4.6^{a} \pm 0.1$	$4.5^{a} \pm 0.1$
	7	$4.7^{a} \pm 0.3$	$4.7^{b} \pm 0.1$	$4.4^{b} \pm 0.2$	$4.4^{a} \pm 0.1$
	14	$4.5^{b} \pm 0.2$	$4.6^{b} \pm 0.1$	$4.3^{b} \pm 0.2$	$4.2^{b} \pm 0.3$
1	1	$4.8^{a} \pm 0.2$	$4.3^{a} \pm 0.1$	$3.9^{a} \pm 0.4$	$4.0^{a} \pm 0.3$
	7	$4.6^{b} \pm 0.2$	$4.2^{a} \pm 0.2$	$3.7^{b} \pm 0.2$	$3.9^{a} \pm 0.4$
	14	$4.5^{b} \pm 0.2$	$4.0^{b} \pm 0.1$	$3.6^{b} \pm 0.1$	$3.7^{b} \pm 0.1$

No. of repeaters = 3, ^{a,b,c} Different superscripts in a column were significantly different ($p \leq 0.05$). ±SD: standard deviation

Table 4 Blood parameters of animal subjects following oral administration of bio-yoghurt with or without β-glucan for 45 days

Parameters	control	T1	T2	T3	T4
RBC $\times 10^6/\mu L$	$3.5^{a} \pm 0.05$	$3.8^{a} \pm 0.02$	$3.6^{a} \pm 0.08$	$4.2^{b} \pm 0.05$	$4.5^{b} \pm 0.01$
WBC$\times 10^3/\mu L$	$6.2^{a} \pm 0.04$	$6.7^{b} \pm 0.09$	$6.7^{b} \pm 0.01$	$6.9^{b} \pm 0.03$	$7.2^{c} \pm 0.03$
HB(g/L)	$11.2^{a} \pm 0.15$	$12.4^{a} \pm 0.09$	$13.6^{b} \pm 0.22$	$15.0^{c} \pm 0.13$	$15.1^{c} \pm 0.26$
PLT$\times 10^3/\mu l$	$183.0^{a} \pm 0.80$	$188.0^{b} \pm 0.73$	$190.0^{b} \pm 0.98$	$222.0^{c} \pm 0.57$	$250.0^{d} \pm 0.91$
neutrophils (%)	$50.5^{a} \pm 0.50$	$61.6^{b} \pm 0.45$	$64.1^{c} \pm 0.66$	$65.1^{c} \pm 0.48$	$66.7^{c} \pm 0.52$
lymphocytes (%)	$25.0^{a} \pm 0.50$	$30.0^{b} \pm 0.55$	$33.0^{c} \pm 0.63$	$34.0^{c} \pm 0.82$	$38.0^{d} \pm 0.97$
Monocytes (%)	$4.1^{a} \pm 0.11$	$5.8^{a} \pm 0.08$	$5.2^{a} \pm 0.10$	$7.5^{b} \pm 0.12$	$8.1^{b} \pm 0.09$
Eosinophils (%)	$0.7^{a} \pm 0.02$	$1.9^{b} \pm 0.04$	$2.3^{c} \pm 0.12$	$2.5^{c} \pm 0.11$	$2.6^{c} \pm 0.15$
TC (mg/dl)	$110.7^{a} \pm 0.25$	$91.1^{b} \pm 0.31$	$88.0^{c} \pm 0.35$	$87.6^{c} \pm 0.40$	$83.3^{d} \pm 0.44$
TG (mg/dl)	$95.8^{a} \pm 0.37$	$77.9^{b} \pm 0.33$	$73.4^{b} \pm 0.51$	$72.1^{cb} \pm 0.83$	$70.6^{c} \pm 0.75$
TP (mg/dl)	$4.3^{a} \pm 0.72$	$5.3^{b} \pm 0.45$	$5.8^{b} \pm 0.33$	$6.5^{c} \pm 0.28$	$7.2^{d} \pm 0.65$

rheological properties of bio-yoghurt when using β-glucan and xanthan gum as a fat replacer at a ratio of 0.1–0.3%.

Blood criteria of animal subjects

Table 4 shows the blood parameters of animal subjects in the control sample, T1, T2, T3, and T4 groups. For the RBC test, for example, the control, T1, and T4 groups gave readings (reported as ×106/μL) of 3.5, 3.8, and 4.5, respectively. In the WBC test (103/μL), the control sample, T1, T2, T3 and T4 were 6.2, 6.7, 6.7, 6.9 and 7.2, respectively.

No. of repeaters = 3, [a,b,c] Different superscripts in a column were significantly different ($p \leq 0.05$). ±SD: standard deviation.

The percentage of lymphocytes in the above order was 25, 30, 33, 34 and 38, respectively. The general trend was that the performance of bio-yoghurt with β-glucan or bio-yoghurt without β-glucan treatments was superior to that of the control sample. After 45 days of administration, the probiotic bacteria in bio-yoghurt with or without β-glucan reduced the TC level in the blood of experiment animals, compared with the control sample. The results of the TC level in T1, T2, T3 and T4 were 91.1, 88.0, 87.6 and 83.3 (mg/dL), respectively, while the control sample was 110 mg/dL. The triglyceride content of the experiment animals' blood serum after 45 days of dosing was 77.9, 73.4, 72.1 and 70.6 for samples T1, T2, T3 and T4, respectively. The total protein (mg/dL) levels of T1, T2, T3 and T4 were 5.3, 5.8, 6.5 and 7.2 respectively. The results revealed a significant difference in the levels of RBC, WBC, HB and PLT for the probiotic and prebiotic samples compared to the control. The results of the current study agree with those of many other studies in which the β-glucan effect on immunomodulation of animals treated with β-glucan either orally or intraperitoneal resulted in improvements in the animals' immune systems El-Boshy et al. 2008; Salim et al. 2011. Triglycerides were lowered in all treatment groups, with the bio-yoghurt with 5% β-glucan (T4) group having the lowest level of triglycerides. This outcome was corroborated by the findings reported by Ranji et al. (2019), who found that *Lactobacillus acidophilus* and *Bifidobacterium bifidum* could dramatically reduce TC and TG levels in the blood. Kaddam et al. (2019) discovered prebiotic compounds such as acacia gum that decreases the levels of TC and TG, indicating that they may have a role in modifying the lipid profile of individuals with sickle cell disease and dyslipidemia. Total protein levels increased dramatically when probiotics and synbiotics (probiotic and prebiotic) were given orally. Dong et al. (2013) discovered that supplementing *Lactobacillus plantarum* increased TP levels and that proteolytic enzymes generated by this bacterium improved dietary protein digestion and absorption in animal subjects.

Weights of animals

The weight of animals in the four groups and the control sample increased during the experiment. The animal weight of the control sample was 28.11 g but had increased to 42.68 g after 45 days. T1, T2, T3, and T4 each weighed 27.95, 27.83, 28.05, and 27.91 g, respectively. Animal weights in the four groups were 44.36, 47.78, 47.99, and 50.86 g, respectively, at the end of the experiment (Fig. 5).

The ability of the probiotic bacteria and prebiotic compounds such as β-glucan to spread to the host gut and improve nutrient absorption through the secretion of digestive enzymes would help to explain why the weight of the

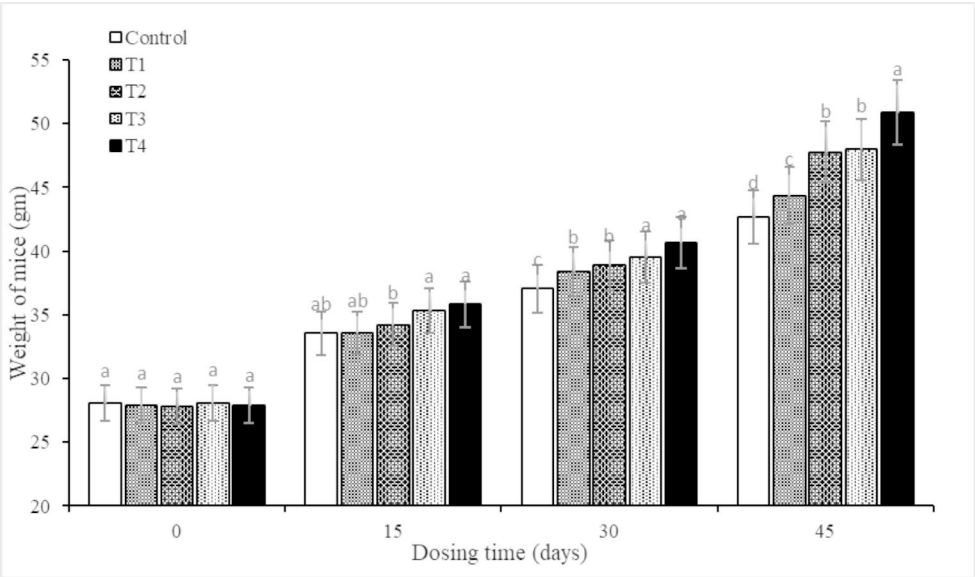

Fig. 5 Measurement of animal weight (g) after 45 dosing days of bio-yoghurt with 0, 0.1, 0.5 and 1% β-glucan treatments, a-d Different superscripts in a column were significantly different ($p \leq 0.05$). ±SD: standard deviation

animals increased in all treatments (Fuller and Gibson 1997). The results of this study were in agreement with those of Chundakkattumalayil et al. (2019), in which there was an increase in the weight of the animals after being fed a synbiotic diet containing *Lactobacillus plantarum* KX519413 and Acaci gum for 21 days.

Conclusions

In the current study, *S. cerevisiae* was used as an application to obtain β-glucan, which was observed as a prebiotic that improved the viability of bacteria, especially at ratios of 0.5 and 1% in bio-yoghurt during 14 days of cold storage. The bio-yoghurt was fortified and improved on some characteristics included increasing acidity and reduction of syneresis. Our findings suggest that this approach could also be beneficial as a fat replacer in the preparation of bio-yoghurt and did not negatively influence a considerable number of acceptability scores. This result opens up the possibility of the use of β-glucan in the preparation of various health and therapeutic foods in the future. Blood and serum biochemical parameters as well as weight improved in animals fed bio-yoghurt-treated diets. Moreover, in some cases, the animals that were fed bio-yoghurt with 1% β-glucan fared better than those fed bio-yoghurt alone. Thus, β-glucan could be considered to be a strong prebiotic chemical candidate with potential therapeutic uses.

Declarations

Declaration of competing interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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