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Changes in the physicochemical and microbiological properties of probiotic-fermented low-fat yoghurt enriched with barley β-glucan during cold storage

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Abstract

This study aimed to investigate the quality attributes of probiotic-fermented low-fat yoghurt enriched with barley β-glucan (BβG) during cold storage (5 °C) for 21 days. Low-fat yoghurt formulation was based on substitution of fat in the skim milk (SM) with BβG (0.75 %, w/v). Four formulations of yoghurt were prepared. The control formulation (without the addition of BβG) was produced from full cream bovine milk and fermented by yoghurt starter (YS). The first treatment (YS) was produced from SM without BβG and fermented by YS. The second treatment was produced from SM with the addition of BβG and fermented by YS (YSBβG). The third treatment was produced from SM without BβG and fermented by *Bifidobacterium lactis* Bb-12, and *Lactobacillus acidophilus* LA-5 (PY). The fourth treatment was produced from SM with the addition of 0.75% BβG and fermented by *Bifidobacterium lactis*, and *L. acidophilus* (PYBβG). All samples were evaluated for their chemical composition, microbiological properties, the viability of probiotic microorganisms, sensory quality attributes during the storage period. The results indicated that addition of BβG improved the survival of probiotic bacteria and YS culture during storage period wherein the BβG-enriched yoghurt had high viable count. There were no significant differences ($p \leq 0.05$) between the treatments in the microbiological quality and chemical composition. On the other hand, the addition of BβG improved the formation of flavor compounds in yoghurt. The substitution of fat with BβG significantly ($p \leq 0.05$) enhanced sensory attributes of yoghurt, wherein BβG-enriched samples recorded high score and acceptability. The lactic bacteria count was 9×10^7 CFU mL^{-1}, and probiotic culture count was higher than 1×10^6 CFU mL^{-1}, which guarantees their effect and ability to survive in the digestive tract and spread in the intestine. It could be concluded that substitution of fat with BβG is a sufficient delivery truck of probiotic culture and BβG could be used safely in functional dairy products.

Key words: **dairy products, fat replacer, microbiological quality, sensory evaluation, *Bifidobacterium lactis, Lactobacillus acidophilus***

Introduction

There is an increased interest and popularity of functional foods due to the growing awareness and demand of consumers. Fortification of food products with β-glucan (βG) as a functional bioactive ingredient, is of great interest. β-glucans comprise a group of β-D-glucose polysaccharides naturally found in cell walls of cereals, yeast, bacteria, and fungi, with different properties dependent on the source (Gangopadhyay et al., 2015). Food and Drug Administration (FDA) has approved βG (3 g/day) to treat coronary heart disease (FDA, 2005). β-D-glucose (βDG) is poorly utilized in the human digestion tract and acts therefore as a non-caloric food which can be used in foods as a thickener, water retention, oil bending agent and an emulsion stabilizer (Satrapai et al., 2007; Santipanichwong and Suphantharika, 2009; Ferreira et al., 2010; Shen et al., 2010; Mahrous et al., 2014; Hassan et al., 2015). Some investigations have been focused on fortifying foods with βG including pasta or tea cakes (Aman et al., 2004), muffins (Tosh et al., 2010), bread (Moriarety et al., 2010), and beverages (Wood, 2007; Marsh et al., 2014).

Yoghurt consumption has been steadily increased over the past years. The per capita consumption of yoghurt is expected to rise until the year 2020 (Singh et al., 2012; Ahmed et al., 2017). There has been a great interest in the fortification of yoghurt with bioactive compounds to improve its nutritional value and benefits to health. Many studies have reported the influence of different ingredients like skim milk (SM) powder (Rohm and Schmidt, 1993), pectin, raspberry and blueberry concentrate (Fagan et al., 2006; Boycheva et al., 2011; Han et al., 2012), gum acacia (Fagan et al., 2006; Hassan et al., 2015), guar gum (Brennan and Tudorica, 2008), protein (Guzman-Gonzalez et al., 1999; Abdel-Haleem and Awad, 2015), gelatin (Fiszman et al., 1999; Soheil et al., 2010), κ-carrageenan (Xu et al., 1992), calcium (Aportela-Palacios et al., 2005; Coskun and Senoglu, 2011), inulin (Guven et al., 2005; Fagan et al., 2006; Brennan and Tudorica, 2008; Balthazar et al., 2015; Glibowski and Rybak, 2016), and fiber (Elsanhoty et al., 2009; Ozcan and Kurtuldu, 2014; Ibrahim and Khalifa, 2015; Sah et al., 2016) on the function properties of dairy products and probiotic yoghurt.

β-glucan (βG) use in food is interesting, especially in yoghurt. Yet, there are limited reports on the fortification of yoghurt with barely β-glucan (BβG). Being a widely spread food, low-fat yoghurt enriched with βG might be helpful for many people suffering from diseases. It is well known that the amount and the type of fat consumed are important to the etiology of several chronic diseases, such as obesity, cardiovascular diseases and cancer (Shen et al., 2010; Gangopadhyay et al., 2015). Sahan et al. (2008) studied the effects of adding βG to yoghurt but they used very low levels of βG (0.05 %) from a βG hydrocolloidal composite. Vasiljevic et al. (2007) studied the growth and metabolic activity of probiotic organisms in βG-enriched yoghurt and reported that the addition of oat βG resulted in improved probiotic viability and stability. Brennan and Tudorica (2008) found that βG (0.5%) addition improved serum retention and viscoelastic nature of yoghurt.

Milk fat plays an important role in the texture, flavor and color development of dairy products. Fat reduction can cause some defects in yoghurt and non-fat ice creams such as lack of flavor, weak body and poor texture (Huyghebaert et al., 1996). Although the manufacture of low-or non-fat dairy products was carried out for many years, the use of fat replacers in the manufacture of dairy products is still novel. Fat replacers, which decrease the calorific value of food, can be used to solve some physical and organoleptic problems originating from low-fat levels in the final products. Huyghebaert et al. (1996) indicated that fat replacers consist of mixtures of lipid originated fat substitutes, protein or carbohydrate originated fat mimetic, or their combinations.

To the best of our knowledge, there is no information about the addition of BβG as a fat substitute on the quality attributes of low fat yoghurt. The hypothesis of this work was that substitution of fat with BβG could deliver a truck of probiotic culture and BβG could be utilized safely in functional dairy products. The goal of this investigation was to study the quality attributes of probiotic-fermented low-fat yoghurt enriched with BβG during cold storage at 5 °C.

Materials and methods

Materials

Spray-dried SM powder (Type low heat, grade A) was obtained from the local market (Cairo, Egypt). Hoodlum hull-less barley was obtained from the Institute of Field Crop Research (Agricultural Research Centre, Giza, Egypt).

Extraction of barley β-glucan (BβG)

BβG was extracted from barely flour according to Benito-Román et al. (2011) with some modifications. Hull-less barley flour (HBF) was weighted in an Erlenmeyer flask, then water was added where liquid to solid ratio was 10:1 (v/w) and pH of the water was 6. Erlenmeyer flask was transferred to a water bath and incubated for 3 h at 55 °C. Then the flour was suspended at a high stirring rate. After the extraction, the separated mass was centrifuged at 5500 rpm (4 °C) for 10 min. βG was extracted from the clear supernatant after adjusting the concentration with ethyl alcohol (30 %) and keeping the mixture overnight at 5 °C wherein βG was precipitated. The precipitate was freeze-dried using freeze-drier (Christ BETA 2-16, Osterode/Harz, Germany). The final product was packed in a plastic container and keep at -20 °C.

Bacterial strains and culture preparation

Yoghurt cultures *Streptococcus salivarius* subsp. *thermophilus*, and *L. delbrueckii* sub sp. *bulgaricus* were obtained from the Egyptian Microbial Culture Collection (EMCC, Cairo Microbiological Resources Centre, Faculty of Agriculture, Ain Shams University, Egypt). *Bifidobacterium lactis*, and *L. acidophilus* LA-5 (freeze-dried red-set) were obtained from Chr. Hansen laboratories (Copenhagen, Denmark). Strains were cultured on deMan, Rogosa and Sharpe (MRS, Difico Laboratories) agar plates. Anaerobic strains were kept in an anaerobic jar (Anaerogen, Oxoid). Fully grown colonies were stored on plates at 4 °C with sub-culturing monthly. For long-term conservation of strains, spore or cell suspensions were kept in cryvials at -80 °C with 90 % glycerol as cryoprotectant. Lactobacilli were cultivated in MRS broth and bifidobacteria were grown in MRS broth with some supplementation with cysteine and incubated for 24 h at the suitable growth temperature. An appropriate volume of culture was used to inoculate 150 mL cultures and incubated for 24 h at 37 °C in an anaerobic jar (Anaerogen, Oxoid). The working culture was prepared by adding a few milligrams the subculture to 100 mL of previously reconstituted and sterile (121 °C/2 min) SM with 10 % total solids. This mixture was incubated at 42 °C until the onset of gelatin. Two mL of culture from this passage were transferred into 100 mL of sterile SM at 42 °C, and once again, the culture was incubated until a gel had formed. This second culture was used for the propagation of a bulk culture (1 L) for inoculation of the different treatments. Bulk cultures were prepared 24 h before the production of yoghurt.

Preparation of yoghurt

Preliminary studies were carried out to select the suitable amount of the freeze-dried BβG that can be used in the production of low fat set-style yoghurt. Different amounts (0.25, 0.5, 0.75 and 1 %) of BβG were incorporated in the yoghurt formulation as a fat replacer. The yoghurt samples were sensory evaluated and the results indicated that the 0.75 % BβG had the highest score. Based on this primary study, 0.75 % BβG was used in the production of low-fat yoghurt in our investigation.

Fresh raw cow's milk was obtained from the Dinah Company (Giza, Egypt) and the yoghurt was produced as a control sample according to Singh et al. (2012). To adjust the solid content in the yoghurt milk to 14 % (w/w), SM powder was used. The control treatment was produced from full cream milk without the addition of BβG and fermented by a yoghurt starter (YS). Four replicate trials were conducted in the manufacture of yoghurt with low-fat milk (0.1 %) and whole fat milk (3.1 %) as a control. SM was prepared by separating whole milk using a cream separator (MCSE500, China) at 40 °C. Experimental groups were divided into four portions. The first portion was pasteurized at 80 °C for 15 min and cooled to 47 °C, inoculated with 3 % (v/v) *Streptococcus thermophilus,* and *Lactobacillus delbrueckii* sub sp. *bulgaricus* (treatment I). The second portion was pasteurized at 80 °C for 15 min and cooled while SM was blended with 0.75 % (w/v) BβG, and the mixtures was homogenized with an Ultra Turrax blender (IKA, Merck, Germany) at 14000 rpm until all

ingredients were dissolved in milk. The homogenate was inoculated with 3 % (v/v) *Streptococcus thermophilus,* and *Lactobacillius dulbrueekii* subsp. *bulgaricus* (treatment II). The third portion was pasteurized at 80 °C for 15 min and cooled to 47 °C, inoculated with *Bifidobacterium lactis* Bb-12*, Lactobacillus acidophilus* LA-5 probiotic YS (treatment III). The forth portion was pasteurized at 80 °C for 15 min and cooled while SM was blended with 0.75 % BβG, and the mixtures was homogenized until all ingredients were dissolved in the milk. The homogenate was inoculated with 3 % *Bifidobacterium lactis,* and *L. acidophilus* probiotic YS (treatment IV). Yoghurt samples were dispersed into plastic cups (150 g), and incubated at 43 °C. All samples were kept at room temperature (21 °C) for 30 min then stored at 5 °C for 21 days. Samples were withdrawn after 4, 7, 15, and 21 days of storage for analysis.

Microbiological analysis

The total bacterial count (TBC) was determined according to Houghtby et al. (1992). Yeasts, molds and coliforms were enumerated according to the standard procedure (Marshall, 1992). Yoghurt cups were wiped from the outside with 70 % ethanol and their contents were thoroughly mixed with a sterile spatula. A composite subsample was prepared by transferring yoghurt into a sterile 250 mL Erlenmeyer flask that contained sterile phosphate buffer and blended with warm buffer (40 °C) until a homogeneous mixture was obtained. TBC and coliform were determined by the pour plate technique, while yeasts and molds were enumerated by the spread plate technique. TBC was determined using plate count agar and incubation was for 48 h at 32 °C. Coliform was enumerated on violet red bile agar after incubation at 37 °C for 24 h. Yeasts and molds were enumerated on plate count agar that contained 0.01 % chloramphenicol and 0.01 % chlortetracycline hydrochloride and incubated at 25 °C for 5 days, and at 5 °C for 10 days, respectively.

Streptococcus thermophilus was enumerated using of M17 agar according to Ravula and Shah, (1998), *L. delbrueckii* subsp. *bulgaricus* was enumerated according to Van de Casteele et al. (2006) using of MRS agar.

Enumeration and viability of *Bifidobacterium lactis* and *L. acidophilus. Bifidobacterium lactis*, and *L. acidophilus* were determined in MRS-OG mixture solution of 0.02 % Oxgall and 0.03 % Gentamince according to Lim et al. (1995). Plates were incubated an aerobically at 37 °C for 48 h. *L. acidophilus* was determined using lactobacillus selective agar plus 0.2 Oxgell (LBSO) (Gilliland and Walker, 1990). The plates were incubated at 37 °C for 4 days.

Chemical analysis

Total solid, protein, fat, lactose and ash content of yoghurt were determined according to AOAC (2000). Total titratable acidity (TTA) determined by titrating 10 g of sample with 0.1 N NaOH using phenolphthalein indicator. pH values of yoghurt samples were recorded using digital pH meter (model SA 720, Orion, USA). Acetaldehyde was determined according to Lees and Jago (1969) using Conway micro-diffusion-Semicarbazide method. Acetaldehyde reacts with the semicarbazide to form semicarbazone which has an absorption peak at 224 nm. The earlier procedure was followed to determine diacetyl content at 270 nm as described by Lees and Jago (1976). Organic acids were determined using high performance liquid chromatography (HPLC). HPLC, from Waters Associates equipped with 600E multi-solvent delivery system and millennium chromatography workstation, was used. The determination was carried out at wave length 210 nm, the flow rate of 1.5 mL min^{-1} and ambient temperature, Altec column (250x4.6 mm) with mobile phase 0.001 % H_2SO_4, were applied. The sample (3 g) was mixed with 7 mL of buffer mobile phase, homogenized (vortex for 1 min), extracted for 1 h and centrifuged at 7000xg for 5 min. The supernatant was filtered through 0.45 µm membrane filter (Sartorious SM 11606) then 20 µL were injected with 25 µL Hamilton syringe (Hamilton Co., Reno, NV). HPLC grade reagents were used as standards (Sigma, St. Louis, MO, USA). Twenty microliters of each membrane filtered (0.22 µm) sample were injected using M6k manual injector at ambient temperature equilibrated with mobile phase at flow rate of 1.5 mL min^{-1}. The absorbed organic acids (formic, pyruvic, lactic, acetic and citric) were eluted isocratically using the mobile phase 0.001 H_2SO_4 (1 L MQ water added to 1 mL sulfuric acid). Five organic acid standards were dissolved in HPLC water with known concentrations and 20 µL were injected under the same conditions. Organic

acids were quantified by comparison of peak areas of authentic samples with those of the corresponding organic acid standard solution using the millennium Data System Program. All analyses of yoghurt samples were done in triplicates.

Sensory evaluation

All yoghurt samples were stored at 0, 4, 7, 15 and 21 days at 5 ˚C and evaluated for flavor, texture, appearance and overall acceptability by 20 of staff members from Dairy Technology Department (Agricultural Research Center) and Institute of Genetic Engineering and Biotechnology (Agricultural Research Center) who are familiar with yoghurt sensory characteristics. A nine-point hedonic scale (Stone and Sidel, 1985) was utilized in this study (9 = like extremely, 5 = neither like nor dislike, and 1 = extreme dislike).

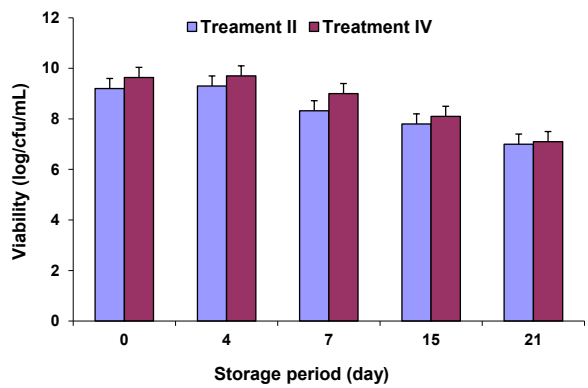
Statistical analysis

Statistical analysis was performed by running Student t-test using Stat view 512 software (1986). Chi-square was performed to compare between the controls and experimental yoghurt. Significant effects were declared $p<0.05$.

Results and discussion

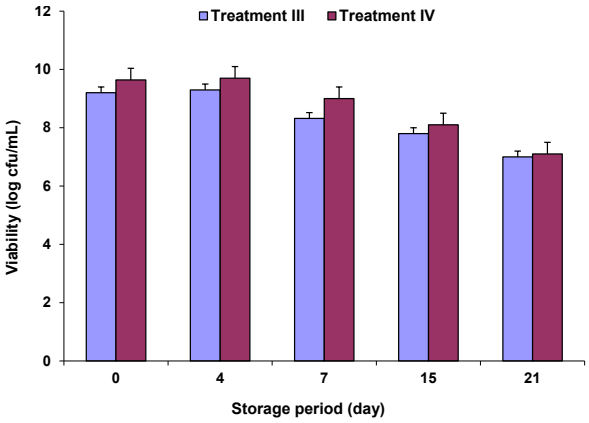
Microbiological properties

The viability and survival of probiotic bacteria are important parameters for assessing the product quality. Figure 1 presents the effect of adding BβG on the viability of *Bifidobacterium lactis* in yoghurt during 21 days of storage at 5 °C. The results showed that there was an increase in the log count of *Bifidobacterium lactis* in treatments with *Bifidobacterium lactis* and *L. acidophilus* enriched with BβG, reaching the highest count after 4 days of storage. Thereafter, a gradual decrease in the count was observed for all treatments during the storage period. The bifidobacterial counts remained above the therapeutic level of 10^7 CFU/g over the 21 days of storage for BβG treatments. However, even though *Bifidobacterium lactis* survival was time-dependent, average counts remained considerably above the therapeutic threshold, indicating that time dependency was not of practical significance. The results are in full concord with the results obtained by Vasiljevic et al. (2007); Elsanhoty et al. (2009); Arena et al. (2014) who attributed the decrease in the viability of *Bifidobacterium lactis* to their sensitivity towards low pH arising mainly from the high concentrate of lactic and acetic acids (Saccaro et al., 2011). Moreover, probiotic yoghurt with BβG had higher viable counts compared to other treatments. The present results are also in complete harmony with those obtained by other workers who demonstrated that addition of BβG enhanced the growth and survival of probiotic bacteria (Vasiljevic et al., 2007; Elsanhoty et al., 2009). The obtained results are also in agreement with Mousa and Abd El-Gawad (2007) who reported that the incorporation of synbiotics (Dairy-Lo and 0.1 Dairy Loid) improved the growth and survival of probiotic bacteria in labneh. Generally, there were better growth and survival of probiotic bacteria in the products supplemented with BβG. A further support is obtained from similar findings of other researchers who reported that the Bifidobacterium are relatively sensitive to low pH. The results are also in agreement with Chou and Hou (2002); Laine et al. (2003) and Rosburg et al. (2010) who reported that the Bifidobacterium can grow and reduce the pH of the oat-based medium. It could be concluded that addition of BβG had a protective effect on bifidobacteria in yoghurt when stressed by low-temperature storage.



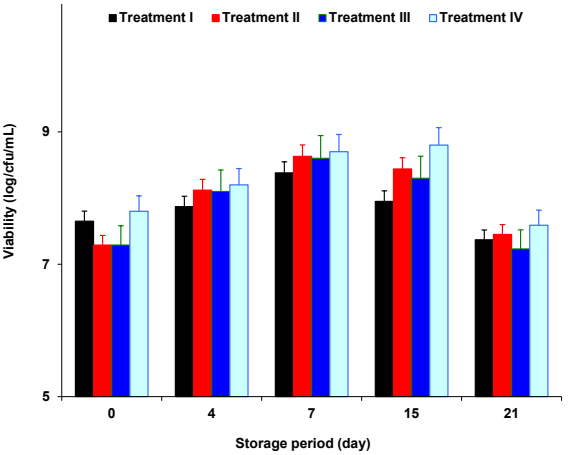
Treatment II =Yoghurt starter with 0.75 % BβG (YSBβG)
Treatment IV= Probiotic yoghurt starter with 0.75 % BβG (PYSBβG)

FIGURE 1. Effect of adding BβG on the viability (log/cfu/mL) of *Bifidobacterium lactis* in yoghurt during storage



Treatment III= Probiotic yoghurt starter (PYS)
Treatment IV= Probiotic yoghurt starter with 0.75 % BβG (PYSBβG)

FIGURE 2. Effect of adding BβG on the viability (log/cfu/mL) of *L. acidophilus* in yoghurt during storage



Treatment I =Yoghurt starter (YS)
Treatment II =Yoghurt starter with 0.75 % BβG (YSBβG)
Treatment III= Probiotic yoghurt starter (PYS)
Treatment IV= Probiotic yoghurt starter with 0.75 % BβG (PYSBβG)

FIGURE 3. Effect of adding of BβG on the total microbial counts (log/cfu/mL) in yoghurt during storage

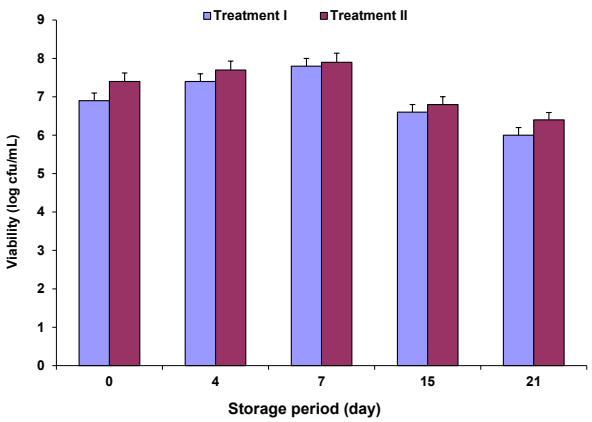
Data in Figure 2 present the effect of BβG enrichment on the viability of *L. acidophilus* in yoghurt during storage. The log count of treatment III which produced without BβG was nearly similar to the log count of treatment IV that formulated with adding BβG. There were no significant differences ($p<0.05$) between treatment III and treatment IV in the viability of *L. acidophilus*. The data indicated that there were gradual decreases in the count for all treatments during the storage period. *L. acidophilus* remained nearly the therapeutic level of 10^7 CFU/mL at the end of 21 days of storage in BβG-enriched samples. From the obtained data, it could be concluded that the addition of BβG improved the viability of *L. acidophilus*. Similar results were reported by other researchers concerning the viability and survival of *L. acidophilus* and other lactobacilli in oat mash (Charalampopoulos et al., 2002; Angelov et al., 2006; Akalin et al., 2012; Elsanhoty et al., 2009; Champagne et al., 2011). The growth in BβG substrate was also similar to those reported in oat substrates inoculated *L. plantarum* (Angelov et al., 2006; Mahrous et al., 2014). In addition, similar results were obtained by Phuapaiboon et al. (2013) who reported that the addition of pineapple to yoghurt enhanced the probiotic viability during 28 days of storage.

Figure 3 presents the total microbial counts in all yoghurt treatments during storage period at 5 °C. Results indicated that there was gradual increase by increasing the storage period reached the highest level after 7 days of storage, and then decreased. The total microbial count changes of a stirred yoghurt showed the same trend of *Bifidobacterium lactis* growth as shown in Figure 1, which represented most of the microflora found in yoghurt beside other microorganisms that resisted the heat treatment. In addition, the results showed that the addition of BβG to milk had no effect on the total microbial counts, since all treatments recorded total microbial counts similar to control. The highest total microbial count was recorded in control treatment. The total microbial count was slightly increased during the storage period. At the end of storage, yoghurt samples containing BβG had the highest total microbial counts. Similar results were obtained by Zare and Orsat (2012); Digbabul et al. (2014) and Mahrous et al. (2014) who found that the addition of probiotics to yoghurt has no changes in viable cell counts during storage for 28 days.

TABLE 1. Changes in yeasts and molds count (log 10) of yoghurt during storage

Treatment	Storage period (day)				
	0	4	7	15	21
Control	1.25	2.19	3.44	4.71	6.98
I - Yoghurt starter (YS)	1.24	2.21	3.55	4.87	6.2
II - Yoghurt starter with 0.75 % BβG (YSBβG)	1.44	2.3	3.71	4.81	7.83
III - Probiotic yoghurt starter (PYS)	1.63	2.11	3.45	4.91	6.91
IV - Probiotic yoghurt starter with 0.75 % BβG (PYSBβG)	1.71	2.33	3.97	5.1	8.97

All yoghurt samples contained coliforms <1.0 CFU/g during the storage period. In this work, all yoghurt samples showed a significant increase in yeast and mold counts whereas the initial yeast and mold count were high in BβG-enriched yoghurt (Table 1). Similar results were reported by Tamine and Robinson (2004) and Ifeanyi et al. (2013) who found that initial counts of yeast and mold were not more than 1 CFU/mL, but when storage time get longer the yeast and mold counts increased. Similar results were also obtained by Elsanhoty et al. (2009), Gohnamy et al. (2009), and Mahrous et al. (2014) who stated that the coliforms were not detected all over storage period in yoghurt at the beginning and at the end of the storage periods.



Treatment I =Yoghurt starter (YS)
Treatment II =Yoghurt starter with 0.75% BβG (YSBβG)

FIGURE 4. Effect of adding BβG on the viability (log/cfu/mL) of mixed yoghurt cultures (*S. thermophilus* and *L. bulgaricus*) in yoghurt during storage

Figure 4 shows the viability of yoghurt cultures *S. thermophilus* and *L. bulgaricus* in yoghurt containing bifidobacteria, *L. acidophilus* and BβG. The date in this figure indicated that there was an increase in the number of *S. thermophilus* and *L. bulgaricus* during storage period until reach the highest level then decreased after 7 days of storage. The mixed yoghurt cultures (*S. thermophilus* and *L. bulgaricus*) survived at a level of the level of 10^6 CFU/mL. The high survival of yoghurt cultures was consistent with studies indicating that *S. thermophilus* and *L. bulgaricus* strains survived well during cold storage at low pH (Dave and Shah, 1997; Saccaro et al., 2011). The data also suggest an effect of time on average cell counts wherein the cell count decrease slightly by decreasing of storage time. The obtained results agreed with Vasiljevic et al. (2007); Elsanhoty et al. (2009); Ashraf and Shah (2011); Arena et al. (2014) and Salmerón et al. (2015) who reported that the addition of prebiotics improved the survival of the probiotic bacteria and culture microorganisms due to high fermentability of BβG by microorganisms.

Chemical composition

Table 2 presents the chemical composition of different yoghurt formulations. The total solids (TS) content of samples ranged from 11.98 % to 12.13 %, the protein content ranged from 3.00 % to 3.12 %. In general, the obtained values of chemical composition agree with values reported by Dublin-Green and Ibe (2005). The addition of BβG and usage of probiotic bacteria in the production of yoghurt had no significant effect on the levels of protein, TS, lactose, and ash in different formualtions. The obtained results were agreement with Gohnamy et al. (2009); Has-

sanein et al. (2008); Elsanhoty et al. (2009) and Mahrous et al. (2014) who reported that the addition of BβG and oat βG has no effect on the chemical composition of stirred yoghurt and labneh.

TABLE 2. Composition of different yoghurt samples

Treatment	Total solids (%)	Lipids (%)	Protein (%)	Lactose (%)	Ash (%)
Control	14.3±1.4	3.1±0.4	3.31±0.3	3.75±0.2	1.09±0.1
I -Yoghurt starter (YS)	12.3±1.2	ND	3.12±0.4	3.70±0.1	0.98±0.2
II -Yoghurt starter with 0.75% BβG (YSBβG)	12.11±1.6	ND	3.11±0.1	3.80±0.2	0.99±0.1
III- Probiotic yoghurt starter (PYS)	11.98±1.3	ND	3.00±0.2	3.6±0.2	0.98±0.0
IV- Probiotic yoghurt starter with 0.75% BβG (PYSBβG)	12.21±1.1	ND	3.11±0.3	3.9±0.3	1.01±0.1

ND= not determined

TABLE 3. Changes in pH of yoghurt during storage

Treatment	Storage period (day)				
	0	4	7	15	21
Control	4.21±0.4	4.09±0.3	3.94±0.7	3.88±0.5	3.80±0.4
I -Yoghurt starter (YS)	4.23±0.5	4.11±0.6	3.99±0.6	3.89±1.2	3.83±0.6
II -Yoghurt starter with 0.75% BβG (YSBβG)	4.31±0.3	4.02±0.5	3.88±0.7	3.81±0.9	3.75±0.7
III- Probiotic yoghurt starter (PYS)	4.50±0.9	4.44±0.4	4.33±0.8	4.29±0.7	3.99±0.3
IV- Probiotic yoghurt starter with 0.75% BβG (PYSBβG)	4.17±0.8	3.97±0.3	3.88±0.7	3.84±0.5	3.82±0.7

Data are presented as means ± SDM (*n*=3)

Data in Table 3 represented the changes of pH in yoghurt during storage period at 5 °C. Results indicated that the lowest value of pH was 4.17 and the highest value was 4.5. There was an increase in the pH values during storage periods to rich the values from 3.81 to 3.99. Data indicated that there was a little decrease in the pH values in the formulations contained BβG. The obtained results agreed with the results obtained by Gee et al. (2007) and Mahrous et al. (2014) who reported that addition of high concentrated BβG had no significant effect on the ability of the starter cultures to ferment. Our results indicated that pH of yoghurt supplemented with BβG tended to be lower than before addition. The obtained results were in disagreements with Sahan et al. (2008) who reported that the use of βG hydrocolloidal composite in the manufacture of low-fat yoghurt did not significantly influence pH, titratable acidity, acetaldehyde, volatile fatty acid and tyrosine levels at any storage time. Prasanna et al. (2013) and Sah et al. (2016) also observed similar pH changes during refrigerated storage of yoghurt-type products and probiotic yoghurt fortified with fiber-rich pineapple peel powder during cold storage.

TABLE 4. Changes in TTA of yoghurt samples during storage

Treatment	Storage period (day)				
	0	4	7	15	21
Control	0.76±0.1	0.81±0.2	0.85±0.1	0.92±0.2	1.2±0.4
I - Yoghurt starter (YS)	0.72±0.1	0.80±0.2	0.83±0.1	0.91±0.2	1.18±0.3
II - Yoghurt starter with 0.75 % BβG (YSBβG)	0.90±0.3	0.94±0.5	1.2±0.2	1.38±0.2	1.43±0.7
III - Probiotic yoghurt starter (PYS)	0.85±0.2	0.95±0.1	1.09±0.1	1.21±0.3	1.23±0.3
IV - Probiotic yoghurt starter with 0.75 % BβG (PYSBβG)	0.91±0.1	1.1±0.2	1.21±0.2	1.25±0.3	1.48±0.3

Data are presented as means ± SD (*n* = 3)

Total titratable acidity (TTA) changes in low fat yoghurt during cold storage are presented in Table 4. The results showed that TTA gradually increased throughout the storage period and recorded the highest level at the end of storage. The results indicated that BβG-enriched formulations contained higher TTA than other treatments, while control sample contained the lowest TTA during cold storage. These results confirmed the pH values (Table 3) and both *L. acidophilus* and *bifidobacteria* counts in Figures 1, 2 and 4. The increase in the lactic acid bacteria counts might be due to decreasing the pH values and increasing TTA. Similar results were obtained by Dello Staffolo et al. (2004); Elsanhoty et al. (2009); Akalın et al. (2012) and Mahrous et al. (2014). Our finding is in contrast with Vinderola et al. (2000) who observed no significant changes in pH of probiotic yoghurt made with *B. bifidum*. One reason for these differences could be the fact that the drop in pH is mainly due to the activity of the yoghurt starters rather than the bifidobacteria.

The volatile compounds in yoghurt play an important role for the consumer acceptance. These volatile metabolites are known as major aroma compounds of yoghurt (Cheng, 2010). Acetaldehyde is the most important compound contributing to typical yoghurt aroma, which is mainly generated by threonine metabolism by yoghurt starters. The most important compounds produced by lactic starter cultures are acetaldehyde, diacetyl, acetone, acetic acid and lactic acid. The balance between them is thought to determine the yoghurt acceptability. In this study, acetaldehyde and diacetyl were determined. Data in Table 5 indicated that there were differences between the treatments ($p<0.05$) and slight impact of BβG on the levels of acetaldehyde. The values of acetaldehyde and diacetyl were arranged from 3.34 to 3.99 mg/kg for acetaldehyde and from 6.21 to 7.11 for diacetyl at the end of storage period. In our study, the levels of acetaldehyde and diacetyl were decreased with time of storage. Similar findings were observed in the manufacture of maize porridge with malted barley, yoghurt with barley flour, labneh with BβG and yoghurt with oat βG (Helland et al., 2004; Elsanhoty et al., 2009; Mahrous et al., 2014). The decrease in acetaldehyde opens a new way for their conversions to ethanol *via* the action of alcohol dehydrogenase (Tamime and Robinson, 2001). These results were in accordance with those reported by Serra et al. (2009) and Salmerón et al. (2015).

TABLE 5. Changes in acetaldehyde and diacetyl concentrations of yoghurt samples during storage

Treatment	Acetaldehyde				
	Storage period (day)				
	0	4	7	15	21
Control	5.61±0.1	5.39±0.1	4.31±0.4	4.12±0.1	3.91±0.1
I - Yoghurt starter (YS)	5.59±0.2	5.38±0.4	4.27±0.1	4.1±0.2	3.92±0.2
II - Yoghurt starter with 0.75 % BβG (YSBβG)	5.81±0.4	4.43±0.1	4.14±0.3	3.99±01	3.34±0.4
III - Probiotic yoghurt starter (PYS)	5.82±0.9	4.95±0.2	4.36±0.1	4.11±0.1	3.99±0.3
IV - Probiotic yoghurt starter with 0.75 % BβG (PYSBβG)	5.89±0.1	5.29±0.3	4.44±0.1	4.1±0.1	3.65±0.1

Treatment	Diacetyl				
	Storage period (day)				
	0	4	7	15	21
Control	11.65±1.9	10.93±1	9.91±1.1	8.41±0.8	7.12±0.5
I - Yoghurt starter (YS)	11.70±1.2	10.86±0.9	9.93±0.9	8.42±0.5	7.11±0.3
II - Yoghurt starter with 0.75 % BβG (YSBβG)	11.90±1.3	10.14±1	9.89±1.2	7.85±1.1	6.78±0.7
III - Probiotic yoghurt starter (PYS)	10.63±1.6	9.87±.1.1	8.83±0.9	7.10±1	6.21±1.1
IV - Probiotic yoghurt starter with 0.75 % BβG (PYSBβG)	11.36±1.7	10.39±1.1	8.99±1.1	8.2±0.8	6.85±0.6

The organic acids in fermented dairy foods are indicators of the metabolic activity of added bacterial cultures. These acids act as natural preservatives and contribute to the characteristic sensory properties. Data in Table 6 presents the organic acid's profile in different yoghurt treatments during storage. In fresh samples, lactic acid was detected and determined in all treatments but there was increase in lactic acid in the treatments I and II. This means that the addition of BβG supported the development of lactic acid. There was also an increase in the lactic acid and acetic acid production throughout the storage period. The production of pyruvic and formic acids was detected after 15 days of storage in all samples, but increased when BβG was added. The obtained results agreed with Adhikari, et al. (2002), Volikakis et al. (2004), Elsanhoty et al. (2009), Başyiğit Kılıç and Akpınar (2013) and Salmerón et al. (2015).

TABLE 6. Changes in levels of organics acids in yoghurt samples during storage

Treatment	Storage period (day)	Organic acid (mg/100 mL)			
		Lactic acid	Acetic acid	Formic acid	Pyruvic acid
I-YS	0	130.63	51.34	ND[a]	ND
II- YSBβG		149.11	56.67	ND	ND
III- PYS		160.12	53.81	ND	ND
IV- PYSBβG		161.32	71.13	ND	ND
I-YS	4	131.81	51.98	ND	ND
II- YSBβG		151.12	53.76	ND	ND
III- PYS		164.19	52.21	ND	ND
IV- PYSBβG		166.34	66.77	ND	ND
I-YS	7	135.23	52.85	ND	ND
II- YSBβG		156.16	55.54	ND	ND
III- PYS		166.56	54.71	ND	ND
IV- PYSBβG		169.15	63.18	ND	ND
I-YS	15	138.78	54.11	24.76	19.70
II- YSBβG		159.23	57.12	26.78	22.43
III- PYS		168.67	57.71	37.16	36.43
IV- PYSBβG		171.60	68.97	34.86	38.32
I-YS	21	148.78	52.12	32.86	29.76
II- YSBβG		164.98	51.41	34.84	28.98
III- PYS		177.97	53.52	42.64	40.29
IV- PYSBβG		188.98	62.66	44.24	46.53

[a]ND= not determined

TABLE 7. Effect of adding BβG on organoleptic properties of yoghurt during storage

Treatments	Storage period (day)	Flavor (50)	Body and texture (40)	Appearance (10)	Total (100)
Control	0	42	36	9	87
	4	43	35	9	87
	7	43	35	9	87
	15	43	37	9	89
	21	44	37	8	89
I - Yoghurt starter (YS)	0	42	35	9	86
	4	42	35	9	86
	7	43	35	9	87
	15	43	37	9	89
	21	44	37	8	89
II - Yoghurt starter with 0.75 % BβG (YSBβG)	0	42	36	9	87
	4	43	37	9	89
	7	45	37	9	91
	15	43	36	9	88
	21	44	36	9	89
III - Probiotic yoghurt starter (PYS)	0	44	35	9	88
	4	44	35	9	88
	7	45	36	9	90
	15	45	37	9	91
	21	46	37	9	92
IV - Probiotic yoghurt starter with 0.75 % BβG (PYSBβG)	0	46	35	9	90
	4	44	36	9	89
	7	44	37	9	90
	15	43	37	9	89
	21	44	36	9	89

Sensory evaluation

Table 7 presents the effect of the addition of BβG on the sensory characteristics of yoghurt during storage periods. In general, the addition of BβG improved various characteristics of yoghurt in comparison to the control sample. The addition of BβG affected appearance, color, texture and overall preference, but no such effect was found for flavor. There were no differences in textural properties between treatments. The treatments with BβG had higher appearance scores than other treatments. Different yoghurt formulations had a good quality and strong curd integrity without any sign of shrinkage, disintegration into lumps, and syneresys at the end of storage period. Similar results were obtained by Tuinier et al. (2000), Elsanhoty et al. (2009), Mahrous et al. (2014), and Salmerón et al. (2015). It could be said that the low-fat yoghurt enriched with BβG had good flavor, taste, texture and appearance among all treatments of yoghurt.

Conclusions

Yoghurt consumption has been steadily increased over the past years. In the present study, yoghurt prepared with *Bifidobacterium lactis*, *L. acidophilus, Streptococcus thermophilus* and *L. delbrueckii* subsp. *bulgaricus* showed good compatibility with yoghurt starter culture. The addition of BβG enhanced the viability of the probiotic strains in the fermented products during cold storage. Yoghurt samples containing 0.75 % BβG were acceptable to expert panelists and had scores similar to the control yoghurt sample. It could be concluded that substitution of fat in yoghurt with BβG was a sufficient delivery truck of the probiotic culture and BβG could be safely used in functional dairy products.

Promjene fizikalno-kemijskih i mikrobioloških parametara tijekom skladištenja niskomasnog probiotičkog jogurta obogaćenog β-glukanima iz zobi

Sažetak

Cilj ovog rada bio je ispitati parametre kvalitete tijekom 21. dana hladnog skladištenja (5 °C) probiotičkog niskomasnog jogurta obogaćenog β-glukanima iz zobi (BβG). Niskomasni jogurt proizveden je iz obranog mlijeka u prahu (SM) s dodatkom BβG (0,75 %) kao zamjene za mast. Pripremljene su četiri različite serije jogurta. Kontrolni uzorak (bez dodatka BβG) pripremljen je od punomasnog kravljeg mlijeka koje je fermentirano klasičnom jogurtnom kulturom (YS). Prva serija jogurta proizvedena je od SM bez dodatka BβG koje je fermentirano jogurtnom kulturom YS. Druga serija jogurta proizvedena je od SM uz dodatak BβG koje je fermentirano jogurtnom kulturom YS (YSBβG). Treća serija jogurta proizvedena je od SM bez dodatka BβG koje je fermentirano probiotičkim sojevima *Bifidobacterium lactis* Bb-12 i *Lactobacillus acidophilus* LA-5 (PY). Četvrta serija jogurta proizvedena je od SM uz dodatak 0,75 % BβG koje je fermentirano probiotičkim sojevima *Bifidobacterium lactis*, i *L. acidophilus* (PYBβG). Svim uzorcima su tijekom perioda skladištenja određivani kemijski sastav, mikrobiološka kvaliteta, broj živih stanica probiotičkih bakterija i senzorska svojstva. Dobiveni rezultati su pokazali da je dodatak BβG poboljšao preživljavanje probiotičkih bakterija, kao i bakterija u sastavu jogurtne kulture. Nisu uočene značajnije razlike ($p \leq 0,05$) u mikrobiološkoj kvaliteti i kemijskom sastavu proizvedenih serija jogurta. Međutim, dodatak BβG poboljšao je tvorbu arome u jogurtima. Zamjena masti dodatkom BβG značajno ($p \leq 0,05$) je poboljšala senzorska svojstva jogurta koji su imali veće ocjene i prihvatljivost. Broj živih stanica bakterija mliječne kiseline kretao se oko 9×10^7 CFU mL^{-1}, dok je broj živih stanica probiotičkih bakterija bio iznad probiotičkog minimuma od 1×10^6 CFU mL^{-1}. Iz svega navedenog može se zaključiti da je zamjena mliječne masti s BβG uspješan način osiguravanja preživljavanja probiotičkih bakterija te se može sigurno primjenjivati u proizvodnji funkcionalnih mliječnih proizvoda.

Ključne riječi: **mliječni proizvodi, zamjena za mast, mikrobiološka kvaliteta, senzorska ocjena, *Bifidobacterium lactis, Lactobacillus acidophilus***

Conflict of interest statement

No conflicts of interest exist in this study.

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