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Enrichment of the low-fat yoghurt with oat β-glucan and EPS-producing *Bifidobacterium bifidum* improves its quality

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Abstract. The addition of β-glucan or EPS-producing bacteria is mainly used to improve the quality and the acceptability of low-fat yoghurt. The purpose of this study was to investigate the effect of adding β-glucan, EPS-producing *Bifidobacterium bifidum*, or both on physical properties, fermentation time, and organoleptic criteria of low-fat yoghurt, additionally to the viability of *L. bulgaricus* and *B. bifidum*. Two types of low-fat yoghurt (1.5% fat) were prepared, with the addition of standard oat β-glucan by 0.15% or without its addition. Each type of yoghurt mixture was inoculated with two kinds of starters: traditional and probiotic (*B. bifidum*) culture. The physicochemical properties, the count of viable bacterial starter culture, and the organoleptic evaluation for all yoghurt types were evaluated after storage 24 h at 4 °C. Moreover, the fermentation time was monitored. The incorporation of both β-glucan and EPS in yoghurt resulted in the highest viscosity (13.7 mPa.s) and WHC (55.94%), besides to the lowest syneresis (28.47%). The acidity and pH of the yoghurt samples were significantly affected ($p > 0.05$) by the β-glucan addition. The yoghurt type *Bifidobacterium* glucan had the shortest fermentation time (215 min), and the maximum viability of both *B. bifidum* (7.63 Log CFU g^{-1}) and *L. bulgaricus* (7.50 Log CFU g^{-1}). The β-glucan had a pronounced effect on the overall acceptability of yoghurt more than the EPS. In conclusion, enriching the low-fat yoghurt with oat β-glucan and EPS-producing *B. bifidum* is the highest effective method for improving the yoghurt's quality and the viability of probiotics.

Key words: acceptability, *B. bifidum*, low-fat, oat β-glucan, quality, viability, yoghurt.

INTRODUCTION

The low-fat yoghurt is one of the yoghurts variety produced from skim milk, which contains milkfat (0.5–2%) (Mbaeyi-Nwaoha & Iwezor-Godwin, 2015). Reducing the fat content in yoghurt performs defects in its viscosity and syneresis, therefore decreasing the quality and the acceptability of low-fat yoghurt (Tamime & Robinson, 1999). Katsiari & Voutsinas (1994) discussed the health disorders caused by excessive dietary fat intakes such as cardiovascular diseases, obesity, and certain forms of cancer and diabetes. In developed countries, consumers began to look for low-fat yoghurt in the market. However, the consumer's demand the low-fat yoghurt with the same quality of the full-fat yoghurt (Jirdehi et at., 2013). The producers began to assess different additives for improving the quality of low-fat yoghurts such as gelatin, *k*-carrageenan, inulin, pectin, dietary fibres, and other hydrocolloids (Sahan et al., 2008).

Recently, oat β-glucan is considered one of the effective hydrocolloid food additives. The β-glucan possesses different properties such as fat-replacer, stabilizer, and thickener. These properties aid in improving the water-holding capacity, viscosity, and body texture of low-fat yoghurt (Havrlentová et al., 2011; Ibrahim & Selezneva, 2017). Vasiljevic et al. (2007) studied the effect of oat β-glucan on the growth and the metabolic activity of probiotic bacteria in yoghurt; their study was concluded that the addition of β-glucan improves the probiotic viability and stability. The synbiotic effect of β-glucan (prebiotic) on protecting the viability of probiotics was explained by Ladjevardi et al. (2016). The producers and researchers were interested in the β-glucan because it was declared as a functional and bio-active food additive (El Khoury et al., 2012; Aboushanab et al., 2019; Dubrovskii et al., 2019). In 1997, the US Food and Drug Administration (FDA) approved a health claim for the use of oat-based foods for lowering the risk of heart disease and passed a unique ruling that allowed oat bran to be registered as the first cholesterol-reducing food at a dosage of 3 g β-glucan per day, with a recommendation of 0.75 g of β-glucan per serving (Guleria et al., 2015).

Exopolysaccharides (EPS) are either homopolysaccharide polymers or heteropolysaccharides produced by plants, fungi, algae, and bacteria. Among the various EPSs producers are the lactic acid-producing bacteria (LAB) and probiotics (Sanalibaba & Cakmak, 2016). In food industries, the incorporation of *Bifidobacterium* in the process of fermented dairy products has gained special attention because of their ability to produce a huge quantity of EPS. The EPS produced by *Bifidobacterium spp.* are used as viscosifiers, stabilizers, emulsifiers, gelling agents, thickeners, and water-binding agents to modify the rheological properties and texture of fermented milk products. In current years, the EPS produced by *Bifidobacterium* can be considered as natural additives, which are preferred by some consumers over stabilizers of plant or animal origin (Audy et al., 2010; Lal et al., 2019).

The addition of EPS to food products increase their health benefits. Polak-Berecka et al. (2013) discussed the beneficial effects of EPS on human health, such as cholesterol-lowering ability, immunomodulating, antitumoral activities, and prebiotic effect. Moreover, the probiotics, producers of EPS, possess special properties that augment the health benefits of dairy products. These probiotic's properties are anti-pathogenicity, anti-diabetic, anti-inflammatory, anti-obesity, anti-cancer, anti-allergy, and angiogenic activities (Kerry et al., 2018).

The previous researches (Cartasev & Rudic, 2017; Elsanhoty & Ramadan, 2018) didn't determine the most effective food additive for enhancing the properties of low-fat yoghurt and increasing the viability of LAB and probiotic bacteria. Therefore, the objective of this research is to study the effect of oat β-glucan addition or EPS-producing *Bifidobacterium bifidum* utilization or both on the physical properties of low-fat yoghurt, its fermentation time, and its organoleptic criteria. Inspection of the potential effect of oat β-glucan and EPS on the viability of *L. bulgaricus* and *B. bifidum*.

MATERIALS AND METHODS

Materials

Low-fat standardized, homogenized, ultra-pasteurized milk was manufactured by the company 'House in the village' (LLC 'WBD,' Moscow, Russia) according to the Russian standard 'GOST 31450–2013' and was bought from commercial markets in Saint-Petersburg, Russia. The milk ingredients were mentioned on the package as the following: fat 1.5%, protein 3.0%, and carbohydrate 4.7%.

Food grade standard oat β-glucan extract was produced by the company Hangzhou Johncan Mushroom bio-technology Co., Ltd (Hangzhou, China). Its β-glucan content (86%) was indicated on the package.

Two types of lyophilized bacterial starter cultures named 'Yogurtel' were selected for yoghurt preparation. One of them is a traditional yoghurt starter culture '*Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*' and the other one is a probiotic starter culture containing '*Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Bifidobacterium bifidum*', both of them were produced by LLC Laktinal Company (Moscow, Russia).

Yogurt preparation

The processing of yoghurt explained by Aboushanab et al. (2018) began with milk mixture preparation, as presented in Fig. 1. Two types of milk mixtures were prepared, one with oat β-glucan addition by 0.15% and the other one without oat β-glucan. The addition of β-glucan must be gradually following by heating up to 60 °C with continuous mixing on a magnetic stirrer to aid the dissolving of β-glucan.

Figure 1. The technology of yoghurt milk preparation.

At the temperature of fermentation (40–42 °C), each type of milk mixture was inoculated by two types of starter cultures with a dose of 0.5 g L^{-1}. The first starter culture was the traditional culture, and the second one was the probiotic culture contains *B. bifidum*. The inoculation process was carried out under a sterile condition of microbiological laminar flow (BAVP-01-Laminar-S-1.2, Lamsystem Company, Russia).

After the inoculation, the milk mixture was appropriately mixed and incubated directly in a thermostable incubator (CLN32, POL-EKO lab, Poland) at temperature 40–42 °C for fermentation. The fermentation process was stopped at a specific pH 4.75, and the fermentation time was determined for each type of yoghurt, followed by sudden cooling at 4 °C.

Determination of yoghurt physical criteria, pH and titratable acidity

All the yoghurt measurements were performed after keeping 24 h at 4 °C. The potential of yoghurt for syneresis was determined by weighing 25 g of yoghurt on a filter paper, let it drain for 2 h at 4 °C and weight the separated whey (Sahan et al., 2008; Kaur & Riar, 2019). The syneresis was expressed by %, using the following equation:

$$Syneresis\ \% = \frac{Weight\ of\ whey\ (g)}{Initial\ sample\ weight\ (g)} \times 100 \quad (1)$$

The water holding capacity (WHC) of yoghurt was proved by centrifuging 5 g yoghurt at 4,500 rpm at 10 °C for 30 min, preceded by weighting the separated whey (Sahan et al., 2008; Ladjevardi et al., 2016). The WHC was calculated by the subsequent formula:

$$WHC\ \% = [1 - \frac{W_t}{W_i}] \times 100 \quad (2)$$

where, W_t – weight of separated whey by g; W_i – weight of the initial sample by g.

The viscosity was measured by viscometer (Fungilab V100003 Alpha Series L, Fungilab Inc., USA) with AISI 316 stainless steel spindle R3 by rotation speed 100 rpm and expressed by mPa·s. Measurements were made for 1 min at 15 °C (Mårtensson et al., 2001).

The pH analysis was performed by a digital pH-meter (Titrino plus 848, Metrohn, Swiss) supplied by electrode and thermometer, at 4 °C.

Lactic acid, described as titratable acidity, was estimated by titrating 1 g yoghurt mixed with 9 mL of distilled water and few drops of phenolphthalein (0.10%) as an indicator against 0.1 M NaOH till the appearance of the pink colour (Zainoldin & Baba, 2009; Nikoofar et al., 2013; Skripleva & Arseneva, 2015). The acidity was calculated by the following formula:

$$Acidity\ °T = \frac{10 \times V_{NaOH} \times 90 \times 0.1}{W} \quad (3)$$

where 10 – dilution factor; V_{NaOH} – the volume of NaOH used to neutralize the lactic acid, 0.1 – molar concentration of NaOH; W – the weight of the initial sample by g, 90 – is the molecular weight of lactic acid.

Determination of culture viability

The viability determination of bacterial culture (*Lactobacillus bulgarigus and Bifidobacterium bifidum*) was initiated by diluting 1 mL of yoghurt samples in sterile peptone water (0.10%) using ten-fold serial dilution method. The samples were cultivated and enumerated from a suitable dilution using a pour plate method on MRS agar medium for *L. bulgarigus* (Ibrahim & Carr, 2006) and on MRS-bile agar medium (contains 0.15% bile salt) for *B. bifidum* (MRS-agar, Oxoid Ltd, Hampshire, UK, and Bile salts, Sigma, Reyde, USA) (Sohrabvandi et al., 2012). Both agar media were autoclaved at 121 °C for 15 min with atmospheric pressure 1 bar (15 psi). All the samples were incubated at 37 °C for 72 h under the aerobic condition of Memmert incubator (IN30, Memmert GmbH + Co., Germany) for *L. bulgarigus* and under the anaerobic condition of CO_2 incubator (MCO-18AC, Sanyo, Panasonic, Japan) for *B. bifidum.* The count number of viable bacteria was calculated in term of CFU g^{-1} of yoghurt, meanwhile studied and analyzed in form of Log CFU g^{-1} (Mortazavian et al., 2007).

Organoleptic evaluation

The acceptance of yoghurts was evaluated by 10 trained panel members from professors and PhD students in the department of food biotechnology, their age varies from 25 till 60 years old. The yoghurts were evaluated for their colour, wheying-off, flavour (aroma and taste), consistency (firmness and texture) and overall acceptability according to the scoring scale (0–10), where 0 is the lowest quality and 10 is the highest quality. The yoghurt samples were served to panellist cold at 4 °C by random order after explanation of aims and procedure of evaluation (Dinkçi et al., 2015).

Statistical analysis

All the experiments were carried out in triplicate. The mean and the standard deviation (± SD) of all the results were calculated using the Origin 61 program. The ANOVA-one-way analysis was performed to the results mean using JASP program, version 0.11.10, by comparing the yoghurt types by paring and with establishing the significant difference at ($p \leq 0.05$). The mean of results and their SD were presented graphically and in tables using Excel 2013 program. As the bacterial counts do not have normal distribution, these data were transformed to logarithmic scale.

RESULTS AND DISCUSSION

Physicochemical properties and fermentation time of yoghurt

The yoghurt properties were described for the 4 types, yoghurt with traditional culture and without β-glucan (TW), yoghurt with *B. bifidum* and without β-glucan (BW), yoghurt with traditional culture and β-glucan (TG), and yoghurt with *B. bifidum* and β-glucan (BG).

The maximum decrease of syneresis (28.47 ± 1.03%) and the maximum increase of WHC (55.94 ± 1.74%) were in yoghurt prepared with *B. bifidum* and β-glucan (Fig. 2). The β-glucan addition led to a significantly different ($p < 0.05$) decrease in the syneresis of low-fat yoghurt and to a significantly different ($p < 0.05$) increase in its WHC more than the addition of *B. bifidum* by 7.76% and 7.86% respectively (Fig. 2).

This strong effect of β-glucan proves its ability to bind with the whey of yoghurt and prevent its weeping on the surface (Kaur & Riar, 2019). Cartasev & Rudic (2017) confirmed that the *B. bifidum* is one of the lactic acid-producing exopolysaccharides (EPS) which affect significantly ($p < 0.05$) the syneresis and the WHC of low-fat yoghurt but it is less effective than β-glucan addition, explained by Ladjevardi et al. (2016).

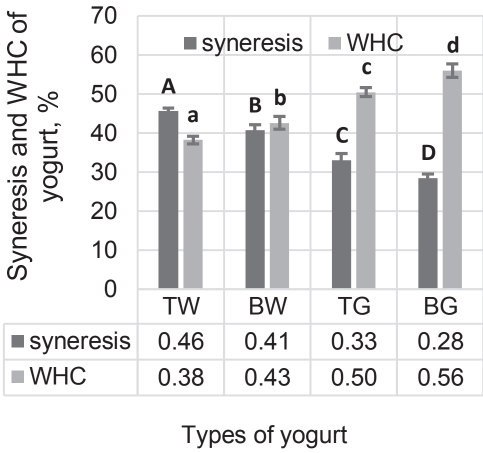

Figure 2. The effect of β-glucan and *B. bifidum* addition on the syneresis and the water holding capacity (WHC) of low-fat yoghurt. The columns are the mean of three observations and the black vertical bars are the standard deviations. The different large letters (A, B, C, D) and the different small ones (a, b, c, d) represent the significant ($p < 0.05$) difference in syneresis and WHC between the different types of yoghurt, respectively.

The definition of syneresis means the shrinkage of the gel, which then causes whey separation, and the WHC means the amount of whey bind to the yoghurt curd (Bahrami et al., 2013). These definitions prove the inverse correlation between the results of syneresis and the WHC in yoghurts.

The viscosity of low-fat yoghurt improved with the addition of oat β-glucan and with the presence of *B. bifidum* producing EPS. The addition of both β-glucan and exopolysaccharides to the low- fat yoghurt affected significantly ($p < 0.05$) to its viscosity. The viscosity of low-fat yoghurt increased by 3.8 mPa.s after the β-glucan addition only and by 1.6 mPa.s after the addition of *B. bifidum* producing EPS (Fig. 3, A). This observation agrees with Vasiljevic et al. (2007), who explained the hydrocolloid effect of β-glucan and its interaction with the curd of low-fat yoghurt. Furthermore, the β-glucan has the properties of stabilizer and fat replacer, which aid in increasing the viscosity of yoghurt (Jirdehi et al., 2013). It was reported that the presence of EPS produced by *B. bifidum* helps in enhancing the viscosity of control low-fat yoghurt (TW) (El-Sayed, 2005). The symbiotic relationship between the β-glucan and *B. bifidum* was discussed by Arena et al. (2017). This relationship aids in increasing the production of EPS and in further enhancing the viscosity of low-fat yoghurt.

The enrichment of low-fat yoghurt with oat β-glucan had a significant difference ($p < 0.05$) on its pH and titratable acidity after storage 24 h at 4 °C. The pH and the titratable acidity changed from (4.73 ± 0.012 and 94.3 ± 0.78, respectively) in TW yoghurt to (4.66 ± 0.012 and 102 ± 1.10, respectively) in TG yoghurt (Fig. 3, B, C). In contrast, the presence of ESP produced by *B. bifidum* in low-fat yoghurt affected non-significantly ($p > 0.05$) on its pH and titratable acidity after storage 24 h at 4 °C. The results of Fig. 3, B, C illustrate the changes of the pH and the acidity of low-fat yoghurt from (4.73 ± 0.012 and 94.3 ± 0.78, respectively) in TW yoghurt to (4.71 ± 0.015 and 95.7 ± 1.15, respectively) in BW yoghurt. The BG yoghurt had the highest decrease in pH (4.61 ± 0.015) and the maximum increase in the titratable acidity (105.1 ± 1.11; Fig. 3, B, C).

The increase of the acidity in low-fat yoghurt enriched with β-glucan could be due to the β-glucan effect in increasing the production of acetic and propionic acids during fermentation ((Kaur & Riar, 2019). The same changes of pH and titratable acidity in yoghurt containing β-glucan were observed by Dello Staffolo et al. (2004) compared to the pH and the titratable acidity results of control yoghurt. Ladjevardi et al. (2016) explained the symbiotic effect of prebiotic (β-glucan) in the presence of *Bifidobacterium* had a significant effect ($p < 0.05$) on decreasing the pH and increasing the acidity of low-fat yoghurt. The only presence of EPS-producing *B. bifidum* in low-fat yoghurt had a minimum effect on its pH and acidity (El-Sayed, 2005). The same evidence was indicated by Vinderola et al. (2000), who observed no significant changes in the pH of probiotic yoghurt made with *B. bifidum*.

The addition of β-glucan to low-fat yoghurt affected significantly ($p < 0.05$) on its fermentation time, in contrast to the presence of EPS-producing *B. bifidum*, which had no significant effect ($p > 0.05$). The BG yoghurt had the shortest fermentation time (215 ± 8.89 min; Fig. 3, D). The insertion of β-glucan and EPS together in the medium helped in shortening the fermentation time by 34 min, comparing to TW yoghurt (Fig. 3, D). Singh et al. (2012) explained this shortening by the prebiotic effect of β-glucan in accelerating the initial onset of yoghurt formation. The study of Schmidt et al. (2016) concluded that the presence of EPS-producing *B. bifidum* produced by the lactic acid bacteria in yoghurt doesn't affect its fermentation rate.

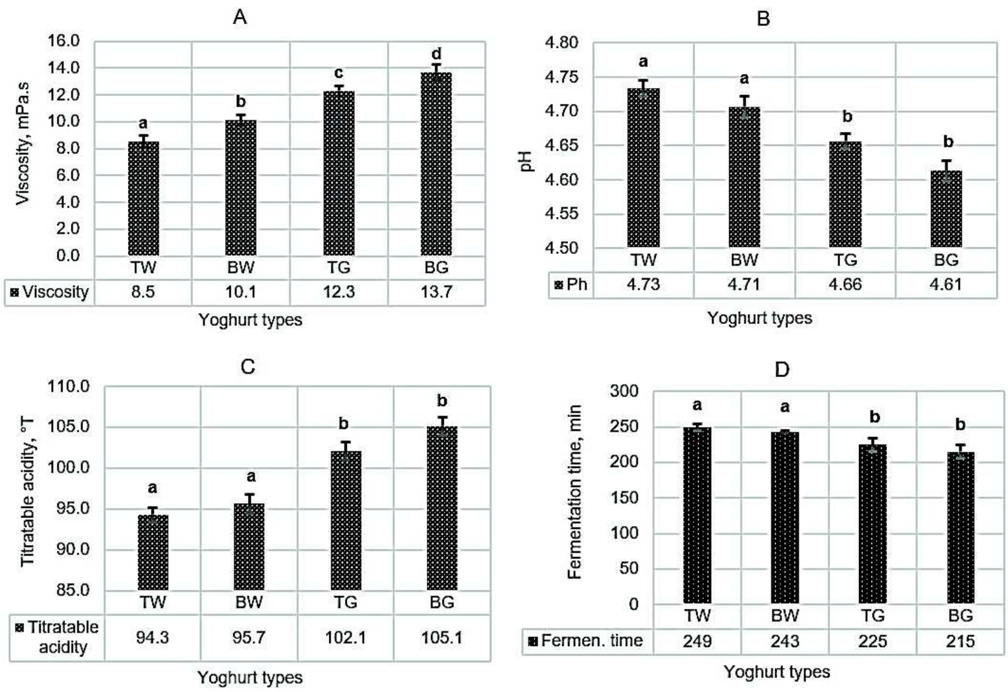

Figure 3. Changes in A. viscosity; B. titratable acidity; C. pH and D. fermentation time of low-fat yoghurt with the addition of the β-glucan and the *B. bifidum* producing EPS. The columns are the mean of three observations and the black vertical bars are the standard deviations. The different letters (a, b, c, d) represent the significance ($p < 0.05$) difference of each character in the different types of yoghurt.

Bacterial viability

As mentioned previously that the oat β-glucan influenced the fermentation time of yoghurt by its shortening, but consequently, it affected the count of viable bacteria (*L. bulgaricus* and *B. bifidum*).

The BG yoghurt had the highest count for the L. bulgaricus (7.50 ± 0.006 Log CFU g^{-1}) and for the B. bifidum (7.63 ± 0.005 Log CFU g^{-1}) comparing to their count in TW and BW yoghurts (Fig. 4). This increment manifests the significant effect ($p < 0.05$) of β-glucan addition on the culture viability. It was explained that the addition of β-glucan protects the Bifidobacterium and the Lactobacillus from the stress conditions such as the low-temperature and the high acidity

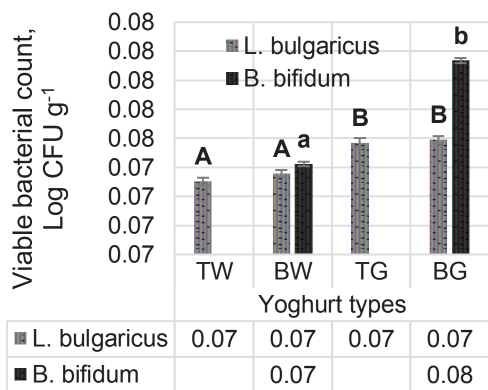

Figure 4. The changes in bacterial culture count with the addition of β-glucan and EPS produced by B. bifidum. The different large letter (A, B) and different small letters (a, b) represent the significant ($p < 0.05$) difference in the viability of L. bulgarigus and B. bifidum respectively, in the different types of yoghurt.

(Rosburg et al., 2010). The β-glucan improved the viability of *B. bifidum* more than the viability of *L. bulgaricus* by 0.12 Log CFU g^{-1} (Fig. 4). The reason for this increment is the synbiotic effect of the prebiotics (β-glucan) on the probiotics, which encourages the growth and the survival of probiotic bacteria (Elsanhoty & Ramadan, 2018).

The production of EPS-producing *B. bifidum* by the *B. bifidum* had no significant difference ($p > 0.05$) on the count of viable *L. bulgaricus*. The previous research works described the restricted effect of EPS on protecting the bacterial starter culture from the harsh external environmental condition during the storage of yoghurt (Kumar Singha, 2012; Cartasev & Rudic, 2017).

Organoleptic characteristics of yoghurt

The ANOVA analysis showed statistically significant effect ($p = 0.0149$) of trial variants. The enrichment of low-fat yoghurt with the β-glucan (TG) or the EPS-producing *B. bifidum* (BW) or both (BG) improved significantly ($p < 0.05$) its wheying-off and its consistency compared to the TW yoghurt. The effect of β-glucan is stronger than the effect of EPS-producing *B. bifidum* on improving the yoghurt wheying-off and consistency (Table 1). Also Elsanhoty & Ramadan, (2018) found in their study, that the enrichment of low-fat yoghurt with β-glucan expressed the greatest changes in texture, appearance, and acceptability of yoghurt, and Han et al. (2016) that the preparation of yoghurt using EPS-producing LAB affects strongly its texture and body firmness.

In our study, the colour and the flavour of low-fat yoghurt were not significantly ($p > 0.05$) affected by the incorporation of β-glucan and EPS-producing *B. bifidum* into the low-fat yoghurt (Table 1). The similar results were reported by Patel et al. (2012), who noticed that polysaccharides and dietary fibres could improve the quality of yoghurt, while not affecting its colour, flavour, and odour.

Table 1. Effect of adding β-glucan and ESP-producing *B. bifidum* on the organoleptic analysis of low-fat yoghurt (mean ± SD)

Yoghurt types	Colour	Wheying-off	Flavour*	Consistency**	Overall acceptance
TW	$8.0^a \pm 0.21$	$5.0^a \pm 0.40$	$8.0^a \pm 0.31$	$4.7^a \pm 0.21$	$6.4^a \pm 0.21$
BW	$7.8^a \pm 0.15$	$6.3^b \pm 0.36$	$8.1^a \pm 0.23$	$5.9^b \pm 0.15$	$7.0^b \pm 0.15$
TG	$8.0^a \pm 0.20$	$8.5^c \pm 0.30$	$8.0^a \pm 0.15$	$7.9^c \pm 0.21$	$8.1^c \pm 0.17$
BG	$8.1^a \pm 0.10$	$9.2^d \pm 0.25$	$8.0^a \pm 0.10$	$9.0^d \pm 0.31$	$8.6^d \pm 0.15$

*Flavour includes aroma and taste of yoghurt. **Consistency includes firmness and texture of yoghurt. [a, b, c, d] Mean values in the same column with different letters are significantly ($p < 0.05$) different.

The yoghurt type *Bifidobacterium* glucan (BG) had the highest overall acceptance by evaluators. The overall acceptability of yoghurt was significantly ($p < 0.05$) affected by the β-glucan and the EPS-producing *B. bifidum* incorporation and the maximum acceptability (8.6 ± 0.15) was in BG yoghurt.

CONCLUSIONS

The results of the present work confirmed the effect of oat β-glucan and EPS-producing *B. bifidum* on enhancing the quality of low-fat yoghurt and the survival of probiotic bacteria. The incorporation of both β-glucan and EPS in BG low-fat yoghurt helped to maximize the viscosity and the water-holding capacity, besides to minimize the syneresis defect of yoghurt, compared to the control sample (TW). Fortification of yoghurt with oat β-glucan affected significantly ($p < 0.05$) its titratable acidity and pH after storage 24 h at 4 °C. In contrast, the production of EPS by the *B. bifidum* had no marked influence on the pH and the acidity of low-fat yoghurt. The yoghurt prepared with the oat β-glucan and EPS-producing *B. bifidum* showed the shortest fermentation time, compared to the other types of yoghurt. The survival of *L. bulgaricus* and *B. bifidum* modified considerably in the presence of oat β-glucan. The β-glucan had a prominent effect in increasing the viability of *B. bifidum* more than the viability of *L. bulgaricus*, which proves the synbiotic correlation between the prebiotics (β-glucan) and the probiotics (*B. bifidum*). The involvement of β-glucan into the low-fat yoghurt, which contains EPS-producing *B. bifidum*, boosted its overall acceptability. The addition of β-glucan had a pronounced effect on the wheying-off and the consistency of yoghurt more than the EPS. Therefore, enriching the low-fat yoghurt with oat β-glucan and EPS-producing *Bifidobacterium bifidum* is the highest effective method to improve the physical and the organoleptic properties of yoghurt, to enhance the viability of probiotics.

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