Influence of Dietary Fiber Addition on the Properties of Probiotic Yogurt

T. Ozcan and O. Kurtuldu

Abstract—**In this study, the effects of using dietary fiber barley and oat β-glucan as a prebiotic on the viability of *Bifidobacterium bifidum* in probiotic yoghurt and properties of yogurt during storage were investigated. The survival of *B. bifidum* was within biotherapeutic level (> 7 log cfu/g) as a result of the prebiotic effect of barley and oat based β-glucan. The addition of β-glucan to yogurt significantly affected physicochemical properties including pH, titratable acidity (LA %), whey seperation, color (*L**, *a**, *b**) and sensorial properties of yogurts. In conclusion, β-glucan can be used on the development of cereal-based functional dairy products with sufficient viability and acceptable sensory characteristics.**

Index Terms—**Yoghurt, probiotic, dietary fiber, β-glucan.**

I. Introduction

Yogurt is a functional dairy product known for its therapeutic, nutritional, and probiotic effects. It is produced by fermentation of milk with the thermophilic homofermentative lactic acid bacteria *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* [1]. During recent years, an increasing interest has developed in foods that contribute to a positive effect on health beyond their nutritional value. Among these functional foods, much attention has been focused on probiotic products and food containing dietary fiber [2]–[4].

Probiotics can be defined as living microorganisms that have proved beneficial effects on health of the host and that improve the intestinal microbial balance [5], [6]. Benefitial effects of probiotics include improving the gut microbial balance, stimulation of the immune system, reduction of blood cholesterol level, and reduction in the incidence of cancer, cardiovascular diseases, diarrhea and osteoporosis [7]–[11].

One of the approaches to increase the number of probiotic bacteria in the intestinal microbiota is including prebiotics in food systems, which are non-digestible dietary fiber components, mainly carbohydrates [12]–[14].

Dietary fibre is naturally present in cereals, vegetables, fruits, and nuts. Based on their simulated intestinal solubility, dietary fibres are either classified as soluble or insoluble fibre. Soluble fibres include pectins, beta-glucans, galactomanan gums, and a large range of nondigestible oligosaccharides including inulin; insoluble fibres include lignin, cellulose, and hemicelluloses [15]–[17].

Foods rich in fibre components have high volume with low energy density, and should promote satiation and satiety, and play a role in the control of energy balance. These foods have the capacity of binding bile acids and metabolites of cholesterol that play an important role in digestion and absorption of lipids in the small intestine, lowering blood cholesterol, regulating blood glucose levels for diabetes management, producing short chain fatty acids and promoting the growth of beneficial gut microflora (i.e. as a prebiotic) [18]–[23]. Due to beneficiary health effects the recommended daily intake of fiber is about 38 g for men and 25 g for women [24].

Fiber can be used for improvement of some functional properties such as texture, water holding capacity, oil holding capacity, emulsification and/or gel formation, bulking agent in reduced-sugar applications, and shelf-life of processed foods [25]–[27].

Among the different rich in fibre-foods, cereals are one of the main sources of dietary fibres. Cereal grains, especially oat and barley, are rich in watersoluble fibers. β-glucans are unbranched polysaccharides that compose of (1-4) and (1-3) linked β-d-glucopyranosyl units in varying proportions, anda re the major component of cell wall material in oats (3–7%) and barley (5–11%), however, only present in small amounts in wheat (1%). β-glucans decrease, absorption and reabsorption of cholesterol and bile acids, delay digestion of lipids and glucose, lower the glycemic response and decreas the risk of heart disease [28]–[30]. The use of β-glucan on the properties of yogurt have been demonstrated by Vasiljevic *et al.* [31]; Sahan *et al.* [32]; Rosburg *et al.* [33]; in dairy gels by Sharafbafi [34] and cheese by Konuklar *et al.* [35] and Volikakis *et al.* [36].

Dairy products are not a good source of fiber, however, can provide an alternative vehicle for the development of fiber enriched foods. Information concerning the effects of cereal based products on the growth of probiotic microorganisms is very limited. Therefore, the objectives of this study were to investigate the addition of *Bifidobacterium bifidum* and a synbiotic combination (*B. Bifidum* with either oat-based or barley-based β-glucan) on viability of probiotic bacteria and the physiochemical characteristics of probiotic yogurt.

II. Materials and Methods

A. Materials and Methods

1) Inoculum and yogurt production

Starter cultures were prepared for inoculum using the method described by Ozcan *et al.* [37]. Lyophilized cultures (Chr. Hansen's Laboratorium, Denmark) of *Bifidobacterium bifidum* Bb-12 and yogurt culture, *Streptococcus thermophilus* + *Lactobacillus delbrueckii* subsp. *bulgaricus*

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cultures were propagated in flasks of autoclaved reconstituted skim milk (10.70% total solids). To facilitate the activation of *B. bifidum* Bb-12, 0.05% L-Cys - HCl was added to diminish the oxidation-reduction potential of the medium. To stimulate the growth, 2% glucose and 1 % yeast extract were added. *B. bifidum* Bb-12 cultures were incubated at 37±1 ℃ for 23 h under anaerobic conditions by the Anaerobic System Anaerogen (Oxoid), whereas yoğurt culture was incubated at the 42±1 ℃ for 3 h under aerobic conditions, respectively. The necessary inoculum, to give approximately 8 or 9.0 $\log_{10}$ cfu g^{-1} in yogurt after inoculation, was calculated.

Skim milk powder was reconstituted in distilled water at 10.70% (wt/v) to yield reconstituted skim milk of the same overall composition as the raw skim milk and oat (Functional Foods Research Unit, National Center for Agricultural Utilization Research, IL/ USA*)* and barley based β-glucan (Naturex, France) at a level of 0,1% as a prebiotic were added to yogurt mixes. The yogurt mixes were then heat-treated at 90°C for 10 min prior to inoculation. Milks, used in production of yoghurt, were inoculated with 3% yogurt starter culture (C -control) and *B. bifidum* Bb-12 (B -*B. bifidum*, OB -oat based β-glucan, BB-barley based β-glucan) as probiotic culture, and were incubated in 42°C and 37°C, respectively to reach 10^7-10^8 cfu ml^{-1}, until the final pH value reached 4.8 and 4.6. The yogurts, produced in three replicates, were kept at room temperature (20°C) for 30 min., stored at 4±1 ℃ and assessed during 28 days of storage.

2) Enumeration of microorganisms

Probiotic bacteria were enumerated at the beginning and at the end of the fermentation, and on 1, 7, 14, 21 and 28 days of storage. MRS specific lactic agars (Fluka, Germany) were used to enumerate viable cells of *B. bifidum*. The plates were incubated at 37°C for 72 h under anaerobic conditions [38]. Cell counts were expressed in logarithm per gram of product ($\log_{10}$ cfu g^{-1}), being the geometrical mean of at least three plates. Viability proportion index (VPI) of probiotic microorganisms was calculated as following [39]:

$$\text{VPI} = \text{Final cell population } (\log_{10} \text{ cfu g}^{-1}) / \text{initial cell population } (\log_{10} \text{ cfu g}^{-1})$$

3) Physicochemical and sensory analysis

In yoghurt samples physicochemical parameters as pH, titritable acidity (LA%) [40], whey separation [41], color values (L^*,a^*,b^*) [42], and sensory parameters as appearance, texture, odor, color, taste and total acceptability values [43], were recorded throughout the storage of 28 days. The data from each experiment were analyzed by analysis of variance (ANOVA) using SAS software [44] and the differences observed among the treatments were determined by the LSD test at $p < 0.01$.

III. Results and Discussion

Table I shows the viability and viability proportion index (VPI) of *B. bifidum* in probiotic yogurt samples at the end of fermentation. The VPI of probiotic yogurt fortified with β-glucan (0.98) was significantly higher than yogurts B and OB ($p < 0.01$).

TABLE I: Viability of Probiotic Microorganisms and Their Relevant Viability Proportion Index in Different Treatments at the End of Fermentation

Yogurt Type	Initial population ($\log_{10}$ cfu g^{-1})	Final population ($\log_{10}$ cfu g^{-1})	VPI
B	9.33[a]	8.95[b]	0.96[b]
BB	9.42[a]	9.30[a]	0.98[a]
OB	9.10[b]	8.78[b]	0.96[b]

VPI, Viability proportion index.
B: yogurt containing *B. bifidum*, BB: yogurt containing *B. bifidum* and barley-based β-glucan, OB: yogurt containing *B. bifidum* and oat-based β-glucan

Fig. 1 shows the viability of *B. bifidum* in yogurt containing β-glucan during 28 days of cold storage per 7-day intervals. Results demonstrated that *Bifidobacterium* growth and viability were greatly enhanced by β-glucan supplementation, and B, BB and OB yogurts found to contain sufficient levels of probiotic bacteria to obtain the desired therapeutic impact after 28 days of storage; with final counts of 7.53, 7.76 and 7.47 $\log_{10}$ cfu g^{-1}, respectively. These results were in agreement with Vasiljevic *et al.* [31], Rosburg *et al.* [33], and Elsanhoty *et al.* [45], who reported an increase in probiotic bacteria growth in yogurts supplemented with β-glucan. The highest viable numbers of *B. bifidum* were observed in the yogurt made with barley-based β-glucan (BB) (Fig. 1).

Viable counts of *B. bifidum* at day 7 were significantly higher than the viable counts for the other days of storage. There was not significant difference between microbial counts at days 14, 21 and 28. The content of viable probiotic microorganisms decreased during storage, and the rate of this loss is dependent on the type of yogurt and whether a lactic starter culture was used or not [46]. Thus, in order to display the expected health benefits in the gastro-intestinal tract it has been suggested that fermented dairy products should contain probiotic bacteria at 10^7 cfu ml^{-1} at the time of consumption [47]. Due to the poor growth of bifidobacteria in milk, it is generally recommended that their inoculation level in fermented milk should be that of the desirable level of the probiotic culture in the final product. However, increased inoculum does not guarantee viability of bifidobacteria during fermentation and storage of fermented milk, which has been described as variable depending on the species and supplements added [48], [49].

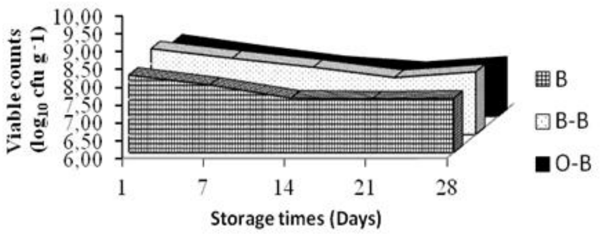

Fig. 1. Viability of *B.* bifidum in probiotic yogurt during storage.

C: control yogurt, B: yogurt containing *B. bifidum*, BB: yogurt containing *B. bifidum* and barley-based β-glucan, OB: yogurt containing yogurt containing *B. bifidum* and oat-based β-glucan.

Physicochemical properties of probiotic yogurt samples were shown in Table II. Significant differences ($p < 0.01$) were observed in physicochemical properties of yogurt samples made from barley and oat-based β-glucan containing *B. bifidum*. The titratable acidity of BB yogurt was higher than that of OB yoğurt. These results might be explained byvariations in survival of *B. bifidum* in these yogurts. Whey separation or syneresis is defined as the expulsion of whey from the network which then becomes visible as surface whey and negatively affects consumer perception of yogurt [50]. The supplemantation of β-glucan significantly decreased whey separation in all yoğurt samples; and this effect could be related to the gelling capacity of β-glucans and their high ability to cross-link gel network and elastic casein–protein–glucan matrix.

Sensory properties of yogurt have a large effect on consumer acceptability, and especially, color is the first sensory characteristic perceived by the consumer. For the probiotic yogurt, the sensory properties of the products are essential, once they should display similar sensorial acceptance and sensory attributes (texture, aroma, and flavor) of traditional yogurts [51]. Changes in color values (L^* (lightness), a^* (red–green axis), and b^* (yellow/blueness) of probiotic yogurt samples were shown in Table II. ($p < 0.01$). The L^* value for the control (C) yogurt were significantly higher than the L^* values for probiotic yogurt containing barley-based and oat-based β-glucan. The a^* and b^* values for all thetreatments were significantly different from each other (Table II).

The sensory properties of yogurt samples were presented in Fig 2. It was noticed that there was a significance difference in sensory properties of yogurts ($p < 0.01$). Storage time significantly affected color, flavor and taste values of yogurts as depending on variations in microbial counts, pH and syneresis. Tudorica et al. [52] demonstrated that incorporation of β-glucans into low fat dairy products can make their mouthfeel, scoopability and sensory properties resemble those of full-fat products. Consequently, yogurt with oat-based β-glucan (OB) received higher scores for appearance, odor, color, taste, and overall acceptance than did yogurt containing barley-based β-glucan.

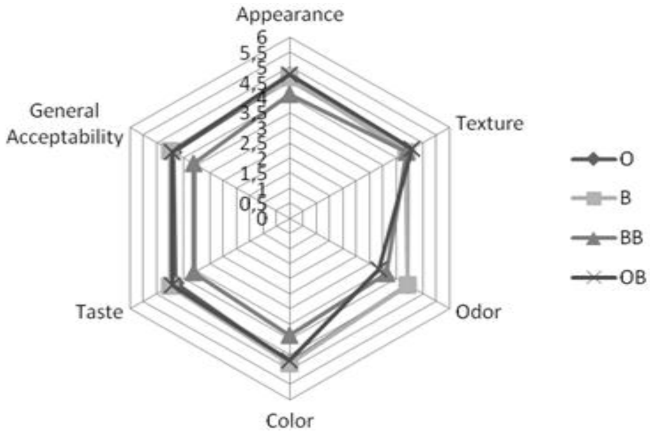

Fig. 2. Average sensory ratings of probiotic yogurts

C: control yogurt, B: yogurt containing *B. bifidum*, BB: yogurt containing *B. bifidum* and barley-based β-glucan, OB: yogurt containing yogurt containing *B. bifidum* and oat-based β-glucan.

TABLE II: THE CHANGES IN PHYSIOCHEMICAL PROPERTIES OF YOGURTS DURING STORAGE*

	N	pH	Titratable Acidity (%LA)	Whey Seperation (25 g mL^{-1})	Color values L^*	a^*	b^*
Yogurt Type							
C	10	4.05^{b}	1.14^{a}	7.78^{a}	98.30^{a}	-4.47^{a}	10.91^{b}
B	10	4.75^{a}	0.79^{bc}	7.01^{b}	97.45^{ab}	-4.04^{a}	11.02^{b}
BB	10	4.73^{a}	0.83^{b}	6.68^{b}	95.00^{c}	-5.43^{b}	13.88^{a}
OB	10	4.74^{a}	0.78^{c}	7.47^{a}	97.07^{b}	-4.16^{a}	11.24^{b}
Storage Time							
1	8	4.65^{a}	0.87 ^{ns}	8.01^{a}	94.45^{c}	-2.33^{a}	13.10^{a}
7	8	4.61^{b}	0.90 ^{ns}	7.38^{b}	97.92^{a}	-4.95^{b}	10.95^{c}
14	8	4.54^{c}	0.89 ^{ns}	7.04^{bc}	98.52^{a}	-5.10^{b}	11.28^{bc}
21	8	4.50^{d}	0.89 ^{ns}	6.71^{c}	97.98^{a}	-5.23^{b}	10.97^{c}
28	8	4.54^{c}	0.88 ^{ns}	7.04^{bc}	95.91^{b}	-5.01^{b}	12.52^{ab}

*Values presented are the means of three replicates trials
Significance level: significant at p<0.01 (**), different superscript letters on the same column indicate significant differences, ns: non significan
C: control yogurt, B: yogurt containing *B. bifidum*, BB: yogurt containing *B. bifidum* and barley-based β-glucan, OB: yogurt containing yogurt containing *B. bifidum* and oat-based β-glucan.

IV. CONCLUSION

Many studies have focused on the probiotics and prebiotics with health-promoting effects such as prevention of nutrition-related diseases. It is generally accepted that in order to appreciate the therapeutic effects, the probiotic foods should have a minimum concentration of > 10^7 $\log_{10}$ cfu viable cells per mL or g of product at the point of consumption. β-glucan, an alternative prebiotic, is also dietary fiber. Both fiber and probiotics are well known for their beneficial health effects, and together they may constitute a good source of functional foods. In conclusion, supplementation of β-glucan in yogurt was found to improve the viability and metabolic activity of *B. bifidum* by displaying a prebiotic effect and can be an alternative for development of cereal-based functional dairy products.

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