### The symbiotic effect of oat $\beta$ -glucan enriching bio-low fat yogurt

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The properties of low-fat yogurt prepared with standardized milk (comprises fat – 2.5%, protein – 2.9%, and carbohydrates – 4.7%) and starter culture consisting of *Lactobacillus* and *Bifidobacterium spp.* with the addition of 86%  $\beta$ -glucan extract were studied. Two yogurt samples were prepared. In the control sample the extract, which contains the  $\beta$ -glucan, was not added, but the experimental sample was enriched by the  $\beta$ -glucan extract with a concentration of 0.15% from yogurt weight. The yogurt samples were stored at 4°C for 24 h. At the end of storage time, the symbiotic effect between the  $\beta$ -glucan and the bacterial starter culture was noticed. The total count of microorganisms (probiotics), compared with the control sample where the  $\beta$ -glucan was not added, was increased by 2·10<sup>7</sup> CFU/g of yogurt. The addition of  $\beta$ -glucan decreased the syneresis from 23.6% to 17.06%, increased the water-holding capacity from 44.1% to 49.6%. In addition, it was observed an increment in the viscosity of yogurt enriched with the  $\beta$ -glucan by 2.3 mPa·s. The experiments prove the structure-forming properties of  $\beta$ -glucan and its prospects in the production of low-fat yogurt with functional features.

**Keywords:** dairy products; low-fat yogurt; probiotics;  $\beta$ -glucan; symbiotic effect; structure-former; functional product. DOI: 10.17586/2310-1164-2019-12-4-111-116

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#### Влияние бета-глюкана из овса на свойства обезжиренного йогурта

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Исследованы свойства обезжиренного молочного йогурта, приготовленного из натурального молока (содержание жира – 2,5%, протеина – 2,9%, углеводов – 4,7%) и закваски, содержащей культуры *Lactobacillus и Bifidobacteriumspp.*, с добавлением экстракта86%  $\beta$ -глюкана. Было приготовлено два образца йогурта. В контрольный образец экстракт  $\beta$ -глюкана не вносился, в опытный образец экстракт  $\beta$ - глюкана вносили в количестве 0,15% от массы йогурта. Хранили образцы йогурта при температуре 4°C в течение 24 ч. По окончании хранения было отмечено, что  $\beta$ -глюкан положительно влияет на симбиотический эффект культур микроорганизмов. Общее количество микроорганизмов (пробиотиков) по сравнению с контрольным образцом увеличилось на 2·107 КОЕ/г йогурта. При добавлении  $\beta$ -глюкана в йогурт синерезис снизился с 23,6 до 17,06%, водоудерживающая способность увеличилась с 44,1 до 49,6%. Установлено, что при внесении  $\beta$ -глюкана, вязкость йогурта возрастает на 2,3 мПа·сек. Доказаны структурообразующие свойства  $\beta$ -глюкана и перспективность его применения при производстве обезжиренных йогуртов с функциональными свойствами.

**Ключевые слова:** кисломолочные продукты; обезжиренный йогурт; пробиотики; β-глюкан; симбиотический эффект; структурообразователь; функциональный продукт.

## Introduction

The oat  $\beta$ -glucan is a polysaccharide of glucose chain binding with  $\beta$  (1-3) and  $\beta$  (1-4) glucosidase linkage. It is considered to be one of the indigestible, water-soluble dietary fiber [1]. The health effect of oat  $\beta$ -glucan was noticed. It helps decreasing the risk of diabetes mellitus type II and the risk of cardiovascular diseases. The FDA recommended the oat  $\beta$ -glucan in a daily dose of 3 g/day for preventing the risk of CVD [2]. The application of oat  $\beta$ -glucan in different food products became wide and effective. The oat  $\beta$ -glucan has a potential effect on improving the quality of dairy and meat-based products. In dairy products, it can enhance their syneresis, viscosity, gel formation, and consistency. It acts as a fat replacer in low-fat dairy products and stabilizer [3].

The bio-low fat yogurt is a type of yogurt which is fermented using a probiotic bacterial. The probiotic bacteria are beneficial bacterial which enhance human health. They can produce exopolysaccharide (ESP) but in low quantities. The ESP is a polysaccharide that can improve the texture, viscosity, and the rheological charters of yogurt effectively [4].

The functionality of  $\beta$ -glucan was increased by the symbiotic relationship between the oat  $\beta$ -glucan (prebiotic), and the probiotic. The symbiotic effect of  $\beta$ -glucan enhances the viability of probiotics and increases ESP production [5]. The incorporation of oat  $\beta$ -glucan in bio-low fat yogurt increases its nutritive value and functionality.

The main probiotic bacteria used in fermented dairy products are the Lactobacillus acidophilus, Lactobacillus casei, and the Bifidobacterium species. Enriching the low-fat yogurt with oat  $\beta$ -glucan can affect its quality and the viability of the probiotics bacteria (*L. acidophilus, L. casei and B. species*) used in yogurt fermentation. The principal aim of this work is to investigate the effect of oat  $\beta$ -glucan on the quality of bio-low fat yogurt and to study the symbiotic effect of oat  $\beta$ -glucan on the viability of probiotic bacteria. Work on the study of the effect of beta-glucans on lactic acid bacteria in the production of low-fat yogurt has not previously been carried out.

## Materials and methods

#### Materials

Low-fat standardized, homogenized, ultra-pasteurized milk was manufactured by Unimilk company (Smolensk, Russia) according to the Russian State Standard 31450-2013. It was bought from commercial markets in St. Petersburg, Russia. The milk ingredients were mentioned on the package as the following: fat - 2.5%, protein - 2.9%, and carbohydrate - 4.7%.

Food grade standard oat  $\beta$ -glucan extract was purchased from the internet pharmacy (Sochi) and produced by Hangzhou Johncan Mushroom Bio-Technology Ltd (Hangzhou, China). Its  $\beta$ -glucan content (86% w/w) was indicated on the packet.

Lyophilized probiotic bacterial starter culture named "Biolact Yogurtel" was selected for yogurt preparation. The bacterial composition of the starter culture is *Lactobacillus acidophilus, L. Fermentum, L. Plantarum, L. casei, Streptococcus thermophilus, Bifidobacterium bifidum, B. breve, and B. infantis.* It was produced by Laktinal Company (LLC "Laktinal Company, Moscow, Russia), and its optimum storage conditions are at relative humidity not more than 85% and temperature not more than 25°C or 8°C for 12 or 18 months respectively.

#### Experimental methods

#### 1. Production of bio-yogurt

The bio-yogurt was prepared by heating the standardized milk to  $30^{\circ}$ C by adding the standard  $\beta$ -glucan gradually by a concentration of 0.15% (w/v) and mixing properly on amagnetic stirrer (RCT basic, IKA, Germany). The dissolving of  $\beta$ -glucan was enhanced by heating up the milk mixture to  $60^{\circ}$ C with continuous mixing at 600 rpm for 20 min. The milk mixture was pasteurized at  $95^{\circ}$ C for 5 min with stirring and subsequently was cooled to fermentation temperature  $40-42^{\circ}$ C [1].

The milk mixture was inoculated by a commercial probiotic starter culture with a dose of 0.5 g/l milk. The inoculation process was carried out under a sterile condition of microbiological laminar flow (BAVP-01-Laminar-S-1.2, Lamsystem Company, Russia).

The milk was fermented in a thermostable incubator (CLN32, POL-EKO lab, Poland) at 40–42°C until it reached the pH 4.75 and suddenly was cooled to 4°C after the fermentation time was determined.

The control bio-yogurt sample was prepared using the previous method without the addition of standard  $\beta$ -glucan.

### 2. Bio-yogurt analyses

2.1.Determination of bio-yogurt physical criteria, pH and titratable acidity

All the bio-yogurt measurements were investigated after keeping 24 h at 4°C. The potential of bio-yogurt for syneresis was determined by weighing 25 g of yogurt on a filter paper, let it drain for 2 h at 4°C and weight the separated whey [2]. The syneresis was expressed by %, using the following equation

 $Syneresis\% = \frac{Weght \ of \ whey}{Initial \ sample \ weight(g)} \cdot 100.$ 

The water holding capacity (WHC) of bio-yogurt was proved by centrifuging 5 g yogurt at 4500 rpm at 10°C for 30 min, preceded by weighting the separated whey [3]. The WHC was calculated by the subsequent formula

$$WHC\% = [1 - \frac{Wt}{W_1}] \cdot 100,$$

where,  $W_t$  – weight of separated whey by g;

 $W_l$  – weight of the initial sample by g.

The viscosity was measured by Fungilab 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 [4]. The pH analysis was performed by a digital pH-meter (Titrino plus 848, Metrohn, Swiss) supplied by electrode and thermometer. Lactic acid, described as acidity, was estimated by titrating 1 g yogurt 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 pink color [5]. The acidity was calculated by the following formula

$$Acidity\% = \frac{10xV_{\text{NaOH}} \cdot 0.009 \cdot 0.1}{W} \cdot 100,$$

where, 10 – dilution factor;

 $V_{\text{NaOH}}$  – the volume of NaOH used to neutralize the lactic acid;

0.1 – normality of NaOH;

W – the weight of the initial sample by g.

2.2. Count of viable probiotic bacteria

The viable probiotic bacterial count (Lactobacillus acidophilus, L. casei, and Bifidobacterium spp.) was initiated by diluting 1 ml of yogurt 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-bile agar medium (MRS-agar, Oxoid Ltd, Hampshire, UK, and Bile salts, Sigma, Reyde, USA).The MRS-bile agar medium was autoclaved at 121°C for 15 min after the addition of bile salt by a concentration of 0.15%. All the samples were incubated at 37°C for 72 h under the anaerobic condition of CO2 incubator (MCO-18AC, Sanyo, Panasonic, Japan) for the total probiotic count and under the aerobic condition of Memmert incubator (IN30, Memmert GmbH+ Co., Germany) for Lactobacillus acidophilus, L. casei count. The count of Bifidobacterium spp. was calculated as the difference between the total probiotic count and the Lactobacillus spp. count. The numbers of each viable probiotic bacteria were expressed in terms of CFU/g of yogurt [6, 7].

2.3. Sensory evaluation

The acceptance of bio-yogurt was evaluated by 10 expert panel members from professors and graduate students in the department of food biotechnology according to scoring scale (0-5) for its appearance (color and syneresis), flavor (aroma and taste), consistency (firmness and texture) and overall acceptability. The bio-yogurt samples were served to panelist cold at 4°C by random order.

2.4. Statistical analysis

All the experiments and its analysis were carried out in triplicate. The mean and the standard deviation of all the results were calculated using Origin 61 program. The ANOVA - one-way analysis was performed by the results mean using the Origin 61 and Excel 2013 programs with established significance difference ( $p \le 0.05$ ). The graphical representations were performed using the same programs.

#### **Results and discussion**

#### 1. The physical criteria of yogurt

The syneresis and water holding capacity (WHC) of bio-yogurt are reflecting the binding ability of curd to whey and preventing its separation. The consistency and texture of bio-yogurt are affected by its syneresis and WHC [3]. Effect of oat  $\beta$ -glucan addition on these criteria was presented in the results of syneresis and WHC of control bio-yogurt (C.S.) and bio-yogurt with 0.15% β-glucan (B.G.S.) (figure 1).



Figure 1. The syneresis and WHC of C.S. and B.G.S. clarified the effect of oat  $\beta$ -glucan addition

Results of syneresis and WHC, presented in Fig. 1, were (23.6 ± 1.55%; 44.1 ± 1.41%) and (17.06 ± 1.60%; 49.6 ± 1.2%) for the C.S. and B.G.S. respectively. The study of results demonstrated a significant difference (p < 0.05) in syneresis and WHC of the C.S. and B.C.S. This difference was explained by the effect of oat on whey as stabilizer and binder which help in decreasing the whey separation [8]. Furthermore, the symbiotic effect between the oat  $\beta$ -glucan and the probiotic bacteria aids in duplicating the production of probiotic exopolysaccharides (EPS), which enhances the syneresis and WHC of bio-low fat yogurt [9]. A strong inverse correlation (*R*= -0.98) was detected between syneresis decrement and WHC improvement, which is affected by the  $\beta$ -glucan addition and the EPS duplication [1].

The influence of adding the oat  $\beta$ -glucan by 0.15% on the viscosity, pH, acidity, and the fermentation time of bio-low fat yogurt is shown in Table 1.

Types of yogurt	Viscosity, mPa·s	pH	Acidity, %	Fermentation time, h
C.S.	$10.2 \pm 0.9$	$4.7 \pm 0.02$	$1.06 \pm 0.01$	3.5
B.G.S.	$12.5 \pm 1.04$	$4.68 \pm 0.02$	$1.07 \pm 0.02$	3.0

Table 1. Effect of oat Beta-glucan addition by 0.15% on the viscosity, pH, acidity and the fermentation time of bio-low fat yogurt

The addition of oat  $\beta$ -glucan affected significantly (p < 0.05) on the viscosity of bio-low fat yogurt by increasing it 2.3 mPa·s. This increment is explained by the effect of oat  $\beta$ -glucan as stabilizer and thickener [10]. In addition, the symbiotic effect of oat  $\beta$ -glucan increased the production of EPS, which improves the viscosity of bio-low fat yogurt [11].

The pH and the titratable acidity varied between 4.7–4.68 and 1.06–1.07% respectively. These observations showed non-significant difference (p > 0.05) between the pH and the acidity content of C.S. and B.G.S. after keeping 24 h at 4°C, which proves that the addition of oat  $\beta$ -glucan cannot change the yogurt pH and acidity significantly [1&2]. Shortening end the in fermentation time of C.S. and B.G.S. by 30 min was also noticed, which can be the real cause of oat  $\beta$ -glucan as a prebiotic and additional nutrient for the probiotic bacteria. The oat  $\beta$ -glucan can accelerate the fermentation process and be used as an effective nutrient of the probiotic at the time of lactose depletion [10].

## 2. Viability of probiotic bacteria

The probiotic count was estimated as total probiotic count, *Lactobacillus spp.* count (*L. acidophilus and casei*) and *Bifidobacterium spp.* (*B. bifidum, breve,* and *infantis*). The different probiotic count of the C.S. and B.G.S. was expressed as probiotic count x  $10^7$  CFU/g and presented in figure 2.



Figure 2. The viable probiotic count in C.S. and B.G.S. The difference between the probiotic count in the C.S. and the B.G.S. manifests the symbiotic effect of  $\beta$ -glucan

The  $\beta$ -glucan addition had a significant effect (p < 0.05) on promoting the total viability of probiotic by 2·10<sup>7</sup> CFU/g yogurt, *Lactobacillus spp.* by 0.82 ·10<sup>7</sup> CFU/g yogurt and *Bifidobacterium spp.* 1.19·10<sup>7</sup> CFU/g yogurt. The previous researchers detected the role of  $\beta$ -glucan as prebiotic, which can explain its effect on the enhancement of the viability of probiotic and stimulating the production of their EPS [12&13]. The relationship between the prebiotics and he probiotics called symbiosis, in which the probiotics get benefits from the prebiotics as additional nutrients for boosting their viability [12&14].

### 3. Sensory evaluation of bio-low fat yogurt

The different sensory properties of bio-low fat yogurt were evaluated and presented in figure 3 using a scoring scale 0-5 to assess the effect of  $\beta$ -glucan addition.



Figure 3. Effect of  $\beta$ -glucan addition on the sensory properties of bio-low fat yogurt

The addition of oat  $\beta$ -glucan had a significant effect (p < 0.05) on the appearance (syneresis) and consistency of yogurt, which is reflected by improvement in the overall acceptability. These observations agree with previous researches that demonstrated no changes in the flavor of yogurt enriched with  $\beta$ -glucan; in contrast, the firmness and the appearance of yogurt with  $\beta$ -glucan were enhanced [2&12].

# Conclusion

The  $\beta$ -glucan has a remarkable effect on the bio-low fat yogurt quality. The syneresis and the water holding capacity of yogurt had significant improvement from (23.6%; 44.1%) to (17.06%; 49.6%) by the impact of  $\beta$ -glucan as a stabilizer. The investigation of yogurt viscosity showed a considerable increase by 2.3 mPa·s. The pH and the titratable acidity had not critical changes during the storage for 24 h. The symbiotic effect between the oat  $\beta$ -glucan (prebiotic) and the probiotics was noticed by shortening the fermentation time by 30 min and by promoting the viability of probiotic bacteria by a 2·10<sup>7</sup> CFU/g with stimulating the production of their EPS. The overall acceptability of the bio-low fat yogurt was enhanced by the oat  $\beta$ -glucan enrichment, especially in the appearance and the consistency of the yogurt. These observations clarify the vitality of the oat beta-glucan in increasing the beneficial value of the bio-low fat yogurt and its quality.

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