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Original scientific article

# IMPACT OF YEAST SEDIMENT BETA-GLUCANS ON THE QUALITY INDICES OF YOGHURT

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The objective of the study was to investigate the potential application of beta-glucans obtained from yeast sediment resulting from the manufacture of local Viorica wine (2018 harvest). To determine the amount of beta-glucans in the yeast sediment, two were used: the calculation method and the laboratory method, obtaining similar quantities,  $29.92 \pm 0.47$  and  $28.17 \pm 0.32$  respectively. The beta-glucans obtained were incorporated in various concentrations (0.1%; 0.2%; 0.3%; 0.4% and 0.5%) in the yogurt obtained from skimmed milk. The effect of beta-glucan addition on the physicochemical properties of freshly prepared yogurts was investigated. The addition of beta-glucans positively influenced the formation of the gel relay resulting in a decrease in the fermentation time of yogurt. The final pH point of 4.5 was reached one hour earlier (in 4 hours) compared to the control sample (in 5 hours). The results showed that there are no significant changes in physicochemical properties (titratable acidity, pH, viscosity and syneresis). The results obtained report that beta-glucans can be used as a thickening agent for low-fat yogurts by shortening the fermentation period and not essentially changing the sensory characteristics. Experimental results showed that the glycemic index of yogurt samples with the addition of beta-glucans have similar values in the range of 28–30. Respectively, the yogurts under study are attributed to food category with low glycemic index.

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Научная статья

# ВЛИЯНИЕ БЕТА-ГЛЮКАНОВ ИЗ ДРОЖЖЕВОГО ОСАДКА НА ПОКАЗАТЕЛИ КАЧЕСТВА ЙОГУРТА

Кирсанова А. И.<sup>1</sup>, Боиштян А. В.<sup>1\*</sup>, Киселица Н.<sup>2</sup>, Симениук Р.<sup>1</sup><sup>1</sup> Технический университет Молдовы, Кишинев, Республика Молдова<sup>2</sup> Институт микробиологии и биотехнологии Академии Наук Республики Молдова, Кишинев, Республика Молдова**КЛЮЧЕВЫЕ СЛОВА:***ideal protein, amino acids, combinatorial problem, optimization criterion, plant raw materials, protein structure ranking***АННОТАЦИЯ**

Целью исследования было изучить возможности применения бета-глюканов, из дрожжевого осадка, полученных при производстве местного вина Виорика (урожай 2018 г.). Для определения количества бета-глюканов в дрожжевом осадке использовали два метода: метод расчета и лабораторный метод, получив значения  $29,92 \pm 0,47$  и  $28,17 \pm 0,32$  соответственно. Полученные бета-глюканы были введены в различных концентрациях (0,1%; 0,2%; 0,3%; 0,4% и 0,5%) в йогурт, полученный из обезжиренного молока. Было исследовано влияние добавления бета-глюканов на физико-химические свойства свежеприготовленного йогурта. Добавление бета-глюканов положительно повлияло на образование геля, что привело к уменьшению времени ферментации йогурта. Конечная точка pH 4,5 была достигнута на час раньше (за 4 часа) по сравнению с контрольным образцом (за 5 часов). Результаты показали незначительные изменения физико-химических свойств йогурта (титруемой кислотности, pH, вязкости и синерезиса). Полученные результаты показывают, что бета-глюканы можно использовать в качестве загустителя для обезжиренных йогуртов за счет сокращения периода ферментации и без существенного изменения органолептических характеристик. Результаты экспериментов показали значения гликемического индекса в диапазоне 28–30 для образцов йогурта с бета-глюканами. Соответственно, исследуемые йогурты относятся к категории пищевых продуктов с низким гликемическим индексом.

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## 1. Introduction

Interest in beta-glucans is outlined and argued by biological activities, including anti-cancer [1,2], anti-inflammatory [3] and immunomodulatory [4,5] properties. Due to the specific physical properties of beta-glucan, such as water solubility, viscosity and gelling, they are increasingly used in the food industry.

The following products are well known as sources of beta-glucans, such as: rice, oats, beans, lentils, peas and others [6]. The content of beta-glucans varies in the range from 0.4 to 10% [7]. In recent years, microorganisms, especially yeasts, capable of synthesizing a complex of bioactive substances, including beta-glucans, which have an important role in the vital activity

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of living organisms [8] and their obtaining from the point of view of economically, it is advantageous [9].

Based on the increased interest and the wide possibility of use, new sources of beta-glucans are in high demand today. Particular attention is paid to agri-food waste and by-products. In this context, the yeast sediment resulting from the production of wine can be valuable sources of beta-glucans [10]. At the same time, the wine industry has an essential role in the economy of our country, being one of the strategic branches, including:

- ❑ the Republic of Moldova is in the list of the top 10 wine producing and exporting countries in the world;
- ❑ wine exports represent 6.2% of the country's export revenues; At present, about 112 thousand ha of vineyards are registered, planted with over 50 varieties of grapes for wine (another 15 thousand hectares will bear fruit in two years);
- ❑ in the field of grape processing, manufacturing, storage and wholesale of wines, there are 187 enterprises, of which 22 also produce distillates (strong alcoholic beverages);
- ❑ the annual production is about 20.0 million decalitres (daL) of wine raw material and about 900 thousand daL / y of distillates;
- ❑ every fourth person from the rural area works in this sector – over 150 thousand people; exports to 68 countries around the world – Moldovan wine accounts for 2% of world wine volume.

It is worth mentioning that in 2019–94652 hl of yeast sediment waste from the manufacture of wines was reported by enterprises registered in the Wine Register of the Republic of Moldova [11].

### Use of beta-glucans

Food production is a competitive industry and the manufacturer is always looking to develop new ingredients to reduce the cost of raw materials. In addition, consumers have become interested in “natural” and healthy foods, preferring foods with low cholesterol, calories and fat, but high in dietary fiber. beta-glucan has demonstrable advantages in improving the physical properties of food as a thickening and water retaining agent, it is

also a good emulsifying stabilizer and fat substitute [12]. In addition, beta-glucan is nutritionally dysfunctional in the human digestive tract and therefore functions as a non-caloric food [13]. Information about its functional and health-beneficial properties can lead to the development of new food applications.

It should be noted that on 25 April 2016, Leiber GmbH submitted an application to the competent authority of Ireland to increase the use and levels of use of beta-glucans in yeast as a novel food ingredient. In particular, the company requested that the use of yeast beta-glucans be extended to other categories and types of food and that the maximum daily doses for the use of yeast beta glucans be increased for food categories already authorized by Implementing Decision 2011/762 / EU. Regulation (EU) no. Regulation (EC) No 609/2013 of the European Parliament and of the Council lays down general requirements for the composition and information of foods for infants and young children, foods for special medical purposes and substitutes for a total diet for weight control. Those acts also project the beta-glucans in yeast. Therefore, beta-glucans should be authorized without prejudice to the provisions of those acts and any other applicable legislation in parallel with Regulation (EC) No 1234/2007. 258/97 (Table 1).

Research into the possibility of using beta-glucans as a supplement has grown in recent years. Thus, beta-blockers are used as a thickening agent for low-fat or low-fat yogurts [15,16], as a stabilizer for mayonnaise [17], as a supplement in meat products [18] and other uses are considered. in the food industry (for example: bakery and pastry products) [19].

On the other hand, yogurt is a healthy food product, widely consumed worldwide. Its popularity has made it possible to use it as a basis in the production of probiotic preparations. Based on the above, the beta-glucans in the residual yeasts from wine-making can be used as a supplement to obtain novel foods. The results of the research presented in this article had the following decisive arguments:

- ❑ the yeasts resulting from the production of wines can serve as a valuable source of beta glucans, at the same time they are accessible and in sufficient quantities;

Table 1

Authorized uses for beta-glucans in yeast (*Saccharomyces cerevisiae*) [14]

Nr d/o	Food category	Maximum level of beta-glucans in yeast
1	Food supplements as defined in Directive 2002/46 / EC, with the exception of food supplements for infants and young children	1,275 g / day for children older than 12 years and the adult population in general 0.675 g / day for children under 12 years of age
2	Substitutes for a total weight control diet as defined in Regulation (EU) No 1095/2010. 609/2013	1,275 g / day
3	Beverages based on fruit juices and / or vegetable juices including concentrated and dehydrated juices	1,3 g/kg
4	Fruit flavored beverages	0,8 g/kg
5	Powdered preparation for cocoa-based beverages	38,3 g/kg (in powder form)
6	Cereal bars	6 g/kg
7	Breakfast cereals	15,3 g/kg
8	Breakfast cereals made from whole grains and high in fiber	1,5 g/kg
9	„Cookies”	2,2 g/kg
10	„Crackers”	6,7 g/kg
11	Milk based beverages	3,8 g/kg
12	Fermented dairy products	3,8 g/kg
13	Other drinks	0,8 g/kg (ready to drink)
14	Dehydrated milk / Milk powder	25,5 g/kg
15	Soups and mixes for soups	0,9 g/kg (ready for consumption) 1,8 g/kg (condensed) 6,3 g/kg (in powder form)
16	Chocolate and sweets	4 g/kg
17	Protein bars and protein powder	19,1 g/kg
18	Jam, marmalade and other spreads	11,3 g/kg

□ taking into account the properties of beta-glucans to form gel, to change the viscosity of products, etc., we consider it appropriate to add them to yogurt — a healthy food product, widely consumed, whose popularity has made it possible to use it as a basis in the production of probiotics.

The experimental study presented in this article reports on the influence of beta-glucans addition on the evolution of some physico-chemical parameters during milk fermentation.

## 2. Materials and methods

The yeast sediment obtained after the manufacture of Viorica wine (fruit of 2018) from the Department of Oenology and Chemistry of the Faculty of Food Technology of the Technical University of Moldova was used to obtain beta-glucans.

The determination of beta-glucan content was performed after a simplified process for extraction from yeasts [20], which consists of the following steps:

- homogenization of cellular biomass at 15000 rpm for 10 minutes;
- separation of cell walls by centrifugation at 3500 rpm for 10 minutes;
- treating the cell walls with 1N NaOH in a ratio of 1: 5, heating at 90 °C for 1 hour;
- treatment with 0.5N acetic acid (1: 5 ratio) at a temperature of 75±5 °C for 1 hour;
- centrifugation at 3500 rpm for 10 minutes at room temperature;
- washing the beta-glucan sediment twice with distilled water;
- drying of beta-glucans at 50±5 °C.

The determination of the total nitrogen, fat and ash content was performed using official AOAC methods [21]. The protein content was calculated by multiplying the total nitrogen by 6.25. The fat was determined by the Soxhlet extraction method. The ash was determined by incinerating the dry samples at 600 °C. Total carbohydrate was determined by the hydrolysis procedure [22]. Glucose was determined by the hydrolysis method and then measured with a glucose oxidase kit (Glucose-PAP method, Germany). Glycogen was determined using the enzyme amyloglucosidase from Sigma, St. Louis, MO, USA [23] and then measured with the glucose oxidase set. The beta-glucans content was calculated by subtracting the glucose obtained from glycogen from total glucose. The results are reported on a dry matter basis.

The yogurt was obtained in laboratory conditions, and for the preparation of samples used: skimmed cow's milk powder (GOST 10970–87), starter culture YO-MIX 207 LYO containing *Streptococcus thermophilus*, *Lactobacillus delbrueckii ssp. Bulgaricus*, *Lactobacillus acid Bifidobacterium lactis* (VITAL PROBIOTIC YOGHURT), Danisco, Denmark and beta-glucan obtained from

residual yeasts after fermentation of the wine in a concentration of 0; 0,1%; 0,2%; 0,3%; 0,4% and 0,5%.

The determination of the titratable acidity was performed by titration and consists in neutralizing the acidic substances in milk with 0.1N NaOH (KOH) solution. By convention the titratable acidity is expressed in grams of lactic acid /100g product.

Determination of lactic acid was calculated from the relation: where: V — volume of 0.1 N NaOH solution used for titration, ml; m — mass of the analyzed sample, g; 0.9 — lactic acid conversion factor. The determination of the active acidity was performed at pH-meter pH-150MA.

The glycemic index of the tested samples was determined in vivo by monitoring the blood glucose level of the experiment participants until and after the consumption of the researched food products, according to ISO 26642: 2010 [24].

The statistical processing of the results was performed computerized using Excel, with the calculation of standard errors for the relative and average values of the significance threshold  $p \leq 0.05$ .

## 3. Results and discussion

### 3.1 Physico-chemical characteristics of yeast sediment

From the physico-chemical characteristics of the yeast sediment obtained after the manufacture of Viorica wine we notice that the carbohydrate content is  $49.90 \pm 0.51$ , the raw protein is  $32.59 \pm 0.51$ , and the ash is  $9.21 \pm 0.37$ . The beta-glucan content was determined by two methods: the calculation method ( $29.92 \pm 0.47$ ) and the laboratory method ( $28.17 \pm 0.32$ ). Thus, in the Figure 2 we notice that the number of beta-glucans is quite close and does not show an essential difference between the two methods.

Next, beta glucans were incorporated into the milk mixture intended for making yogurt. The characteristics of the mixtures are presented below in Table 3.

### 3.2 Evolution of titratable acidity and active acidity during yogurt fermentation

The structure and rheological properties of fermented dairy products are influenced by a number of factors: milk quality, pH, nature of bacterial cultures, temperature, mechanical processing, fermentation time, presence of additives, etc.

At the same time, the titratable acidity and the pH of yogurt with the addition of beta-glucans have a great sensory importance. The evolution of titratable acidity and pH is correlated with the intensity of lactic fermentation. Thermostatization of the samples under study was performed at a temperature of + 42 °C. The evolution of titratable acidity expressed in% lactic acid in yogurt samples with the addition of beta-glucans obtained from residual yeasts from wine production is shown in the Figure 1.

Table 2

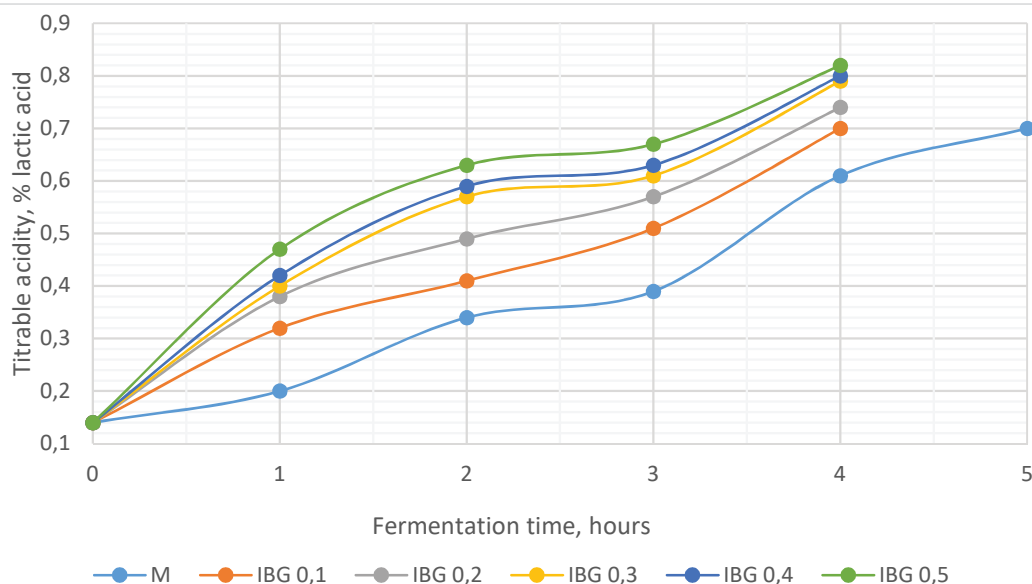
Physico-chemical characteristics of yeast sediment (mean ± SD)

Sample (yeast sediment)	Carbo hydrate (as glucose)	Beta-Glucans (by calculation)	Crude protein (TN×6.25)	Ash	Fat
Win Viorica	49,90±0,51	29,92±0,47	32,59±0,51	9,21 ±0,37	1,32±0,51

Table 3

Characteristic of the milk mixture intended for making yoghurt

Indices	M Control	IBG 0,1	IBG 0,2	IBG 0,3	IBG 0,4	IBG 0,5
beta-glucan content, %	0	0,1	0,2	0,3	0,4	0,5
Total dry matter, %	11,00	12,36	12,77	13,52	14,01	14,50
Titratable acidity, % lactic acid	0,14	0,14	0,14	0,14	0,14	0,14
pH	6,23±0,10	6,24±0,20	6,30±0,10	6,32±0,15	6,35±0,11	6,35±0,10



**Figure 1.** Evolution of titratable acidity (expressed in% lactic acid) during the fermentation period in yogurt samples with the addition of beta-glucans

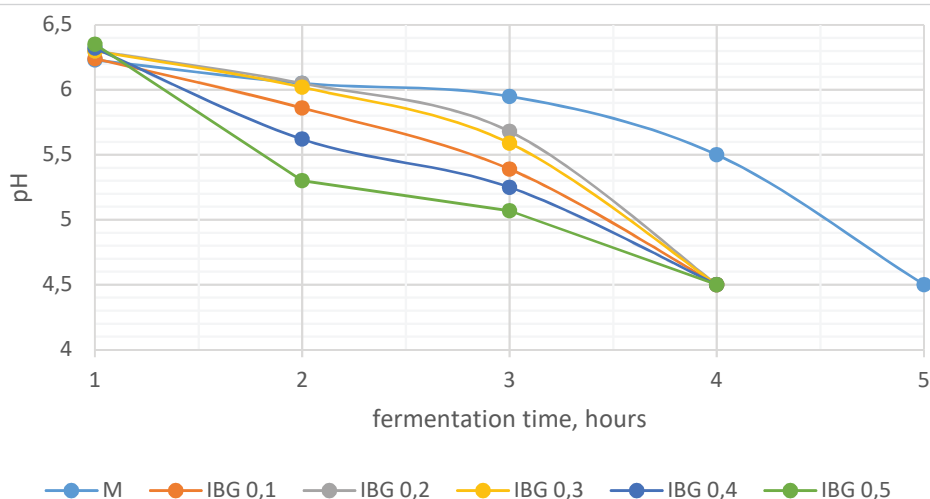
During fermentation the reducing carbohydrates are fermented quickly as a result, lactic acid accumulates. This process promotes increased acidity. Lactose degradation begins after the addition of the starter culture and continues during the thermostating of the samples. It should be noted that in yogurt for sale the minimum acidity must be 0.6% lactic acid. This important moment is stipulated in the acts of the International Dairy Federation, and is also found in the Technical Regulations of the Republic of Moldova „Lapte și produse lactate” (Milk and Dairy Products). In the yogurt samples under study, at the end of the thermostating period, the recommended value is found in the recommended range. We mention that in the yogurt samples with beta-glucans, with the increase of the addition amount, the acidification of the milk proceeds faster than in the control sample, which leads to the decrease of the coagulation time. Thus, the end of coagulation in the case of yogurt samples with 0.2%, 0.3%, 0.4% and 1.5% of beta-glucans was set at 4 hours, and in the control sample and yogurt sample with 0, 1% beta-glucans — after 5 hours of fermentation in the Figure 2.

The evolution of pH is correlated with the intensity of lactose fermentation and the increase of titratable acidity, but it is also influenced by the buffer substances found in beta -glucans.

### 3.3 Viscosity and syneresis

The viscosity value of the yogurt samples varies from 1210 mPa·s in the case of yogurt without added beta-glucans to 11598 mPa·s in the case of the IBM 0.5 yogurt sample containing 0.5% beta-glucans. The addition of beta-glucans causes a slight increase in viscosity which does not differ significantly from the viscosity level of the yogurt control sample.

Syneresis is a complex physical and biochemical phenomenon, and is a thermodynamic property of gels that consists in reducing the volume of the gel, caused by the expulsion of a quantity of whey with the aging of yogurt. The intensity and depth of the yoghurt syneresis depends largely on the internal surface of the solid phase, the porosity (spaces occupied by whey) and the permeability of the gel [25]. The porosity of the gel depends on the size and association of the solid elements of the gel, and the permeability is dependent on the size of the solid elements, the shape and size of the pores. In clots formed by lactic acidification the pores have a micellar character [26]. In this case during the syneresis the clot contracts relatively slowly (absence of forces likely to create contracting forces), the porosity decreases continuously, but the permeability remains relatively high throughout the process due to the fact that the network consists of demineralized caseins. Supplementing yogurt with beta-glucans



**Figure 2.** Evolution of pH during the fermentation period in yogurt samples with the addition of beta-glucans



changes both the structure of the network and the permeability of the curd and can affect the syneresis process. We notice that with the increase of the added amount of beta -glucans the syneresis of yogurt is slightly decreasing from 12.01 to 10.11 according to the Table 4.

Table 4

Synthesis, apparent viscosity of beta-glucan fortified yoghurts (mean $\pm$ SD)		
	Viscosity, mPa $\cdot$ s	Syneresis, %
M	1210 $\pm$ 0,3 <sup>1</sup>	12,01 $\pm$ 0,4 <sup>1</sup>
IBG 0,1	1542 $\pm$ 0,4 <sup>1</sup>	11, 86 $\pm$ 0,3 <sup>1</sup>
IBG 0,2	1558 $\pm$ 0,3 <sup>1</sup>	11, 74 $\pm$ 0,2 <sup>1</sup>
IBG 0,3	1566 $\pm$ 0,3 <sup>1</sup>	11,62 $\pm$ 0,3 <sup>1</sup>
IBM 0,4	1585 $\pm$ 0,2 <sup>1</sup>	10,09 $\pm$ 0,5 <sup>1</sup>
IBM 0,5	1598 $\pm$ 0,5 <sup>1</sup>	10,11 $\pm$ 0,4 <sup>1</sup>

<sup>1</sup> Means within a column with different superscripts are significantly different ( $P < 0.05$ ).

Therefore, the addition of beta -glucans improves both the structure of the network and the permeability of the curd and reduces the process of synergism — moments appreciated in the production process in the dairy industry.

### 3.4 Sensory characteristics of yogurt

All yogurt samples were analyzed according to the main indices of organoleptic characteristics [27]: appearance, color and consistency, smell, shows in the Table 5.

It should be noted that the sensory characteristics of yogurt samples with the addition of beta-glucans in different amounts do not influence the smell of yogurts obtained. At the same time, the external appearance and consistency are changed in essentially: the consistency of the curd becomes firmer but still remains without gas bubbles, with poor elimination of whey.

### 3.5 Glycemic index

Reliable glycemic index tables compiled from the scientific literature are essential for improving the quality of research, examining the relationship between glycemic index, glycemic load and health. The glycemic index has proven to be a more useful nutritional concept than the chemical classification of carbohydrates, allowing new perspectives on the relationship between the physiological effects of carbohydrate-rich foods and health. Several studies have shown that chronic consumption of a diet with a high glycemic load is associated with an increased risk of developing type 2 diabetes, cardiovascular disease and certain types of cancer [28].

The experimental results showed that the glycemic index of the studied yogurts is in the range 28–30 (Figure 3) and are quite close.

Thus, according to international tables and recommendations in the field we can say that yogurts with added beta-glucans are assigned to the category of foods with low glycemic index and can be recommended in diets that reduce the risk of type 2 diabetes, cardiovascular disease and others [29].

## 4. Conclusions

Changing the technological properties of dairy products, such as yogurt, has gained considerable interest in the development of new products. The addition of beta -glucans accelerates the fermentation and coagulation process and reduces the duration of yogurt fermentation. The evolution of the pH in the fermentation of yogurt with the addition of beta -glucans is correlated with the intensity of lactose fermentation and the increase in titratable acidity, but it is also influenced by the buffers that are formed during manufacture.

Beta -glucans from yeast sediment obtained from the manufacture of local Viorica wine (harvest year 2018) are technologically promising ingredients. In addition, these natural ingredients attract attention due to their health-promoting properties. At the same time, it is necessary to perform microscopy by scanning the beta -glucan powders and studying the textural features of yogurt with the addition of beta -glucans.

Table 5

Sensory characteristics of yogurts with the addition of beta-glucans							
Nr. ord.	Characteristics	Yogurt Samples	M	IBG 0,1	IBG 0,2	IBG 0,3	IBG 0,4
1	Exterior appearance and consistency		The curd with the right consistency, without gas bubbles, with poor removal of whey	Firm curd, gas bubble station and whey removal			The curd with a firm consistency, without gas bubbles, with poor whey elimination
2	Smell		Specific flavor of yogurt, with specific characteristics of lactic fermentation				
3	Taste		Pleasant sour, specific flavor of yogurt	Pleasant sour, specific flavor of yogurt		Pleasant sour, weak yogurt flavor, weak gulceag	

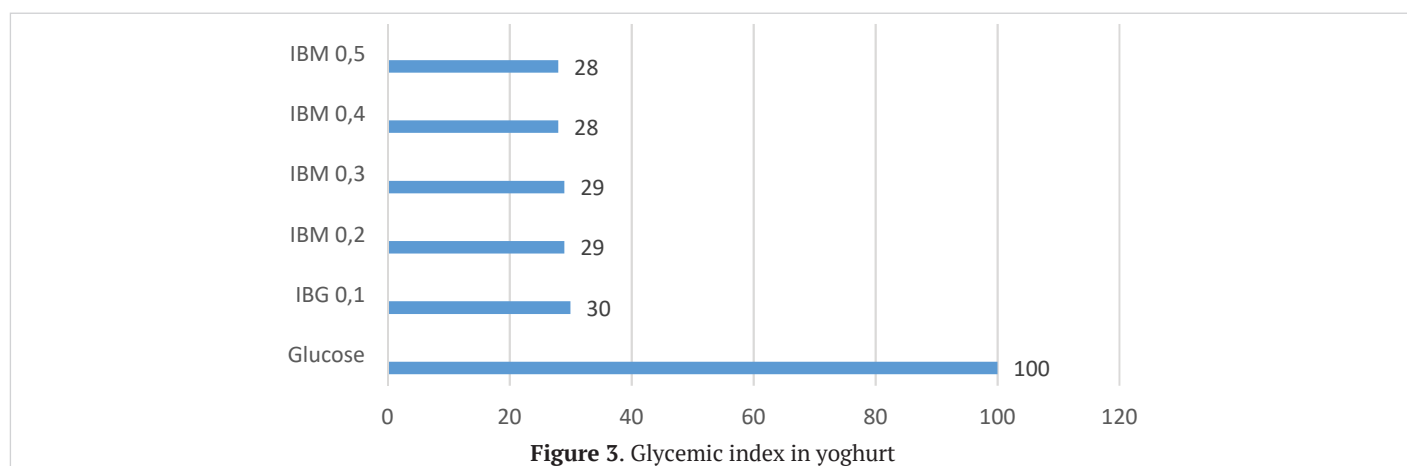


Figure 3. Glycemic index in yogurt

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