

Influence of starch products on the vitality and activity of lactic acid bacteria in yogurt

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Abstract

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Introduction. Products of enzymatic hydrolysis of corn starch are promising multifunctional ingredients in yogurt, but their use requires studying their influence on the vitality and activity of lactic acid bacteria, as well on some physico-chemical characteristics of the product.

Materials and methods. Yoghurt with a fat content of 1% with maltodextrin, glucose, and glucose-fructose syrup in an amount of 9% in terms of dry matter were studied. The number of lactic acid bacteria cells was determined by plating on MRS solid nutrient medium, water activity was measured using a HygroLab 2 device, syneresis of clots was estimated by centrifugation, and active acidity – by the potentiometric method.

Results and discussion. The influence of starch product with different dextrose equivalents addition on the viability and activity of lactic acid bacteria *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* during fermentation and storage of yogurt has been studied. An increase of dextrose equivalent and monosaccharides content in starch products reduce the fermentation time of milk due to the increase of lactic acid bacteria activity. A slight decrease in water activity in the presence of glucose-fructose syrup in yogurt in an amount of 9% had virtually no effect on the milk fermentation process. The number of lactic acid bacteria increased during the first seven days of yogurt storage added with glucose-fructose syrup. On the 14th day of storage, the concentration of cells of *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* became almost the same in all yogurts due to almost complete consumption of carbon sources. When the storage of yogurt was extended to 28 days, the most stable content of lactic acid bacteria was found in yogurt added with maltodextrin due to its prebiotic properties. The increases of active acidity and syneresis in all yogurts were greatest in the first 8–14 days. Presence of dextrans in yogurt stabilizes its physical and chemical properties during storage.

Conclusions. Adding starch-containing products to yogurt allows to activate the activity of lactic acid bacteria and improve their survival during yogurt storage.

Introduction

Yogurt is one of the most popular fermented beverage characterized by exceptional taste, thick consistency and containing at least 10^7 CFU/ml of lactic acid bacteria beneficial to human health (Gómez-Gallego et al., 2018, Hadjimbei et al., 2013). Dessert yoghurts additionally contain food additives or fillers including sweeteners, of which sucrose, honey and fructose are most often used (Kang et al., 2019; Martínez et al., 2024; Prokisch et al., 2022).

Carbohydrates are consumed by lactic acid bacteria during the fermentation of milk mixtures (Ayivi et al., 2020; Yeboah et al., 2023;), therefore, when using sweeteners of a carbohydrate nature, it is relevant to study their effect on activity and vital functions of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* present in starter cultures.

There is limited information about the specific effects of carbohydrates of sweeteners on the activity of lactic acid bacteria in yogurt. Thus, Popa and co-authors (2011) did not find a significant difference between the effects of various sweeteners (honey, sucrose, glucose-fructose syrup, and inulin) on the activity and viability of lactic acid bacteria and bifidobacteria in bio-yogurt.

The highest activity of *Lactobacillus acidophilus* was observed in the presence of honey with different oligosaccharide contents (Popa et al., 2011). Addition of fructose and oligofructose in an amount of 2% in fermented milk drinks during their treatment with probiotic starter cultures of *Lactobacillus*, in particular *L. brevis* B1, improved their physicochemical, rheological, sensory and microbiological properties (Danylenko et al., 2022; Zielinska et al., 2021).

The presence of oligofructose and fructose ensured a higher amount of lactic acid bacteria in fermented milk drinks for 35 days during storage compared to control where no saccharides were added. Oligofructose turned out to be the most effective stimulator of bacterial growth. During storage, pH values decreased in all samples, which was most obvious between days 7 and 21 (Zielińska et al., 2021).

Along with traditional sweeteners, products of enzymatic hydrolysis of starch are widely used in dessert products (Eke-Ejiofor, 2015; Nikolić et al., 2023).

The degree of starch hydrolysis is expressed by the dextrose equivalent value, which indicates the number of dextrose molecules released as a result of starch hydrolysis. The dextrose equivalent value can range from 0 for starch to 100 for glucose (Hidayat et al., 2015). Dextrins in starch products with medium and low dextrose equivalent effectively bind water, thicken milk drinks and ice cream mixtures, reduce the perception of sweetness, serve as a source of solids, and also imitate fat (Bass et al., 2017; Rayhani et al., 2008). Dextrins with the same dextrose equivalent may differ in physicochemical characteristics, in particular viscosity, sweetness, solubility, and bioavailability (Sun et al., 2020), which requires studying the functional and technological properties of each type of starch product in food.

Research of the dextrin use in yogurt are limited. Thus, it was shown that the addition of wheat dextrin in an amount of 15 g had a positive effect on the fermentation process, as well as viscosity, acidity, syneresis and overall acceptability of the product (Peerkhan et al., 2021). But the work did not study the peculiarities of the fermentation process and the microbiological characteristics of yogurt with dextrins.

It is known that monosaccharides (glucose and fructose) formed during the hydrolysis of starch affect fermentation processes (McGregor et al., 1987; Parker et al.,

2010), act as sweeteners, and reduce water activity, which can affect shelf life of food products (Jia et al., 2017). However, they do not exhibit structuring ability.

Despite their low cost, availability on the market and technological advantages, starch products are still used quite limitedly in dairy industry (Polischuk et al., 2019; Zargaraan et al., 2016). At the same time, glucose-galactose syrup (Mosquera-Martínez et al., 2023) and chemically modified starches (Cui et al., 2014; Schmidt et al., 2007) are more widely used in yogurt production. Meanwhile, functional waxy corn starches (CLARIA® Elite, Plus, Essential Starch), obtained from Tate & Lyle, (USA) in quantities of 1.88 and 2.35 (by nominal phase volume) demonstrated a consistent texture accompanied by the fullest taste.

The phase volume was calculated based on the effective starch concentration (c) in the continuous phase and the effective swelling volume after treatment (q). It has been found that yoghurts containing starch with a low effective volume of starch swelling (30 ml/g) and a phase volume slightly above the dense packing point (1.41) have the most preferable sensory properties (Wong et al., 2020). The combined effect of lactic acid bacteria producing exopolysaccharides with a known structure and starch (0.75%) on the rheological and physical properties of yoghurts was also studied (Verni et al., 2019). It has been found that the addition of starch to yogurt increases the importance of the rheological and physical properties of all blended yogurts, which probably occurs through repulsion between proteins and polysaccharides that contributes to thermodynamic incompatibility. But the microbiological indicators of yogurt with exopolysaccharides and starch were also not studied by the authors.

Based on a set of physicochemical indicators of yogurt samples with starch products, the feasibility of using molasses with an average dextrose equivalent in its composition was proven (Ivashchenko and Polishchuk, 2023). Molasses added into yogurt served as a thickener, stabilizer and sweetener. At the same time, the influence of starch destruction products on microbiological properties and physico-chemical characteristics of yogurt has not been studied.

Thus, chemically modified starches in yogurt perform only the function of structuring and moisture binding, which affects the consistency of the fermented milk clot and prevents syneresis. At the same time, starch hydrolysis products, as multifunctional ingredients, can thicken and stabilize the structure (at medium and low dextrose equivalent), sweeten dairy products with varying intensity depending on the depth of starch hydrolysis, and be a source of solid substances. In turn, their influence on the activity and viability of lactic acid bacteria has been practically not studied.

Therefore, the aim of the presented research was to study the influence of starch products with different dextrose equivalents on the microbiological and physicochemical characteristics of yogurt.

Materials and methods

Materials

The following products of enzymatic hydrolysis of corn starch were selected for the study: dry maltodextrin MD-10 (dextrose equivalent 10); dry glucose syrup GS-42 (dextrose equivalent 40-42); glucose-fructose syrup GFS-42 (dextrose equivalent 98).

The carbohydrate composition of the hydrolysis products of corn starch is given in Table 1.

Table 1

Carbohydrate composition of maltodextrin, dry glucose syrup and glucose-fructose syrup

Content	MD-10	GS-42	GFS-42
Dry matter (DM)%, not less	95	95	70
Glucose, % DM	1.0	16	54
Fructose, % DM	-	-	42
Maltose, % DM	3.4	15	2
Maltotrioses, % DM	5.0	16	1
Dextrins, % DM	90.6	53	1

Preparation of yogurt samples

Yogurt samples were prepared from normalized milk to obtain a finished product with a fat content of 1%. According to changes to the Standard of Identity for Yogurt developed by the Food and Drug Administration (FDA, 2023), 1% fat yogurt can be classified as low-fat (0.29 to 1.76% fat).

The control yogurt contained 4% skim milk powder and 5% sucrose. The total content of dry matter of milk powder and sucrose (9%) in the test samples was replaced with an equivalent dry matter content of starch products.

The specified composition of yogurt was previously justified based on the results of an analysis of quality indicators of the studied samples with varying contents of starch hydrolysis products (Ivashchenko and Polishchuk, 2023). The balance of dry matter content in the sample with liquid glucose-fructose syrup was maintained by adding an equivalent content of skimmed milk powder.

The abbreviations for the yoghurt samples are given below:

- Control (contains 5% sucrose and 4% skimmed milk powder);
- Sample 1 (contains 9% dry maltodextrin MD-10);
- Sample 2 (contains 9% dry glucose syrup GS-42);
- Sample 3 (contains 9% dry matter of GFS glucose-fructose syrup).

Yogurt samples were prepared in volumes of 200 ml. Starch products and other recipe ingredients were added to milk normalized for fat content before pasteurization with stirring using a Daihan HS-50 A laboratory mixer for 2 minutes at a speed of 200 rpm.

The samples were filtered and pasteurized at a temperature of $87 \pm 2^\circ\text{C}$ for 2-3 minutes. The pasteurized mixtures were cooled to a temperature of $41 \pm 1^\circ\text{C}$ and inoculated with 10^7 CFU in 1 ml of pure cultures until titrated acidity values did not exceed pH 4.8. For fermentation of milk mixtures, a starter was used to produce yogurt containing lactic acid bacteria *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*. Before fermentation, the dry lyophilized starter was activated in part of the thermally treated milk for 2 hours at a temperature of $41 \pm 1^\circ\text{C}$.

During fermentation, the dynamics of changes in the active acidity of yogurt samples was studied. After fermentation, yogurt samples were cooled to a temperature of $4 \pm 2^\circ\text{C}$ and the water activity and concentration of lactic acid bacteria cells were determined. During storage in a refrigerator for up to 28 days at a temperature of $4 \pm 2^\circ\text{C}$, the degree of syneresis, active acidity and concentration of lactic acid bacteria cells were determined in yogurt samples.

Methods

Active acidity was measured potentiometrically using a laboratory pH/MV/ISE/Temp ADWA AD1200 ATC meter.

Water activity (A_w) was determined using a HygroLab 2 device (Rotronic, Switzerland), with an accuracy of ± 0.001 A_w units. The water activity indicator was expressed from 0.00 to 1.00 A_w (0-100% rh). The measurement was carried out at a temperature of 20 °C. Before measurement, the device was calibrated against a special humidity standard (95% HR).

The number of lactic acid bacteria cells in yogurt was determined by serial tenfold dilution with sterile saline (0.85% NaCl) followed by plating on MRS (De Man–Rogosa–Sharpe) solid nutrient medium (CondaLab, Spain). To create anaerobic conditions, inoculated Petri dishes were filled with an additional layer of MRS agar, cooled to 45°C. Colony-forming units (CFU) were counted after anaerobic incubation at 37°C for 48 hours.

Syneresis of milk-protein yogurt clots was determined by centrifugation. To do this, 25 ml of yogurt, after mixing in a calibrated test tube, was centrifuged using a laboratory centrifuge Sigma 2-6E (Germany) for 20 minutes at 1000 rpm and a temperature of 20°C, and the volume of separated whey was measured, which was expressed by in ml per 100 g of product (Polischuk et al., 2020). The measurements were carried out during storage of yogurt samples after 0, 7, 14, 28 days.

Statistical analysis of the experiment results was carried out using Statistica 6.0, Microsoft Office Excel 2007 and Mathcad. Data were expressed as mean \pm standard deviation to define three measurements.

Results and discussion

The content of lactic acid bacteria *Streptococcus thermophilus* and *Lactobacillus delbrueckii* spp. *bulgaricus* in freshly prepared yogurt samples is shown in Figure 1.

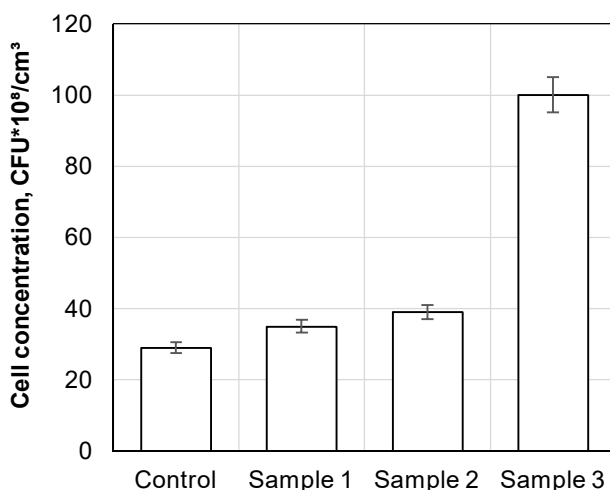


Figure 1. Concentration of lactic acid bacteria cells in fresh yogurt

According to Figure 1, the positive effect of all starch hydrolysis products on the activity of lactic acid bacteria is obvious. It should be noted the significant role of glucose-fructose syrup in activating the vital activity of lactic acid bacteria cells in the composition of yogurt starter due to its higher bioavailability for bacteria compared to other starch products.

By the level of additive influence on the lactic acid bacteria activity, they can be placed in the following sequence: GFS > GS-42 > MD-10. Thus, the content of bacterial cells in the sample with glucose-fructose syrup was 2.8 times higher than in yogurt with maltodextrin and 2.5 times higher than in yogurt with glucose syrup.

Fructose consists 42% of dry matter in glucose-fructose syrup, the positive effect of which on lactic acid bacteria is well known (Zielińska et al., 2021).

Mono-, di- and trisaccharides in the composition of GFS and CS-42 are bioavailable carbon sources and promote the active development of lactic acid bacteria during milk fermentation, in contrast to indigestible high-molecular prebiotics, such as fructooligosaccharides and galactooligosaccharides activating beneficial microbiota in the human intestine (Davani-Davari et al., 2019).

The prebiotic properties of maltodextrin were shown by Bisar et al. (2019). In this study, maltodextrin moderately activated lactic acid bacteria *S. thermophilus* and *L. delbrueckii* spp. *bulgaricus* during milk fermentation.

The effect of GS-42 on milk fermentation was close to that of the yogurt with MD-10, which is explained by the rather high content of higher sugars in them – 53% and 90.6%, respectively.

The concentration of cells of lactic acid microorganisms in yogurt samples (Figure 1) is correlated with data on changes in active acidity during fermentation of milk mixtures (Table 2).

Table 2

Changes in the active acidity of milk mixtures with starch products during fermentation*

Yogurt	Duration of fermentation, hours						
	0	1	2	3	4	5	6
Control	6.57 ±0.21	6.10 ±0.14	5.60 ±0.19	5.02 ±0.15	4.72 ±0.19	4.68 ±0.15	4.55 ±0.16
Sample1	6.54 ±0.20	6.42 ±0.18	6.05 ±0.20	5.70 ±0.18	5.14 ±0.19	4.75 ±0.18	4.63 ±0.16
Sample 2	6.40 ±0.20	6.27 ±0.17	5.71 ±0.17	5.10 ±0.20	4.80 ±0.15	4.55 ±0.14	4.51 ±0.13
Sample 3	6.23 ±0.17	5.73 ±0.16	5.17 ±0.16	4.77 ±0.18	4.45 ±0.16	4.38 ±0.14	4.30 ±0.15

* pH values of 4.8 and below are highlighted with a dark background, which is a criterion for completing the milk fermentation process.

The results show that glucose-fructose syrup, due to its high content of monosaccharides, accelerates fermentation by about 1 hour; glucose syrup has virtually no effect on the activity of lactic acid bacteria, and maltodextrin even somewhat prolongs the achievement of proper acidity. This could be explained by the different ratio between monosaccharides, oligosaccharides and dextrans in the products of starch hydrolysis, which exhibit prebiotic properties differently and bind free water (Gänzle et al., 2012).

Changes of the concentration of lactic acid bacteria cells in yogurt samples with starch products of varying degrees of hydrolysis during storage up to 28 days is shown in Figure 2.

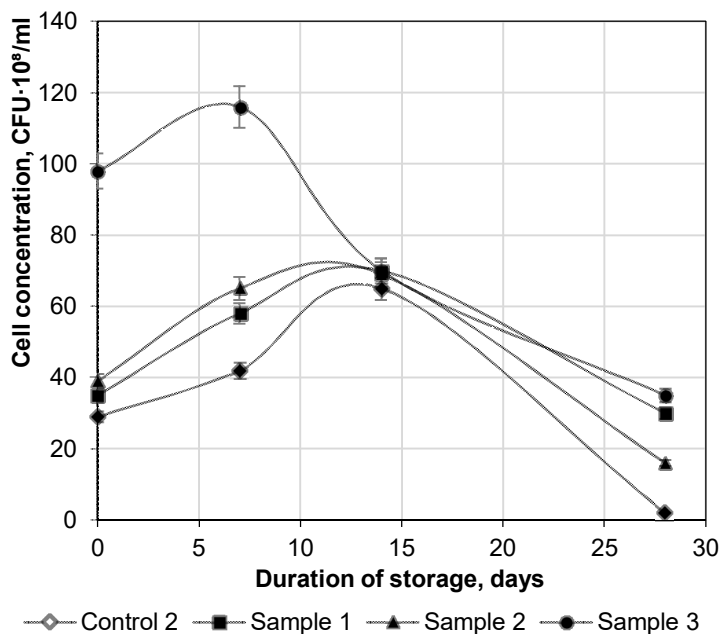


Figure 2. Concentration of lactic acid bacteria cells during storage of yogurt samples with starch products

During storage of yogurt, the increase in the concentration of microbial cells actively continued in sample 3 with glucose-fructose syrup in the first 7 days, after which, on the 14th day, their minor dieback was observed. Samples 1 and 2 showed a similar pattern of changes in the concentration of microbial cells, which maximally increased on the 14th day of storage as the limit for the suitability of yogurt for consumption.

In the control yogurt, the content of bacterial cells also increased on the 14th day, followed by their significant decrease on the 28th day of storage. It should be noted that the concentration of lactic acid bacteria on the 14th day of storage becomes almost the same for all yoghurts, which is due to the almost complete consumption of available carbon sources by the bacteria. Maltodextrin (sample 1) had the greatest positive effect on the viability of lactic acid bacteria during long-term storage of yogurt, which to some extent contributed to the stabilization of the quality of the product. The detected effect coincides with the data of Batawy et al. (2018).

Figure 3 shows the dynamics of active acidity of yogurt samples during storage for up to 28 days at a temperature of 4 ± 1 °C.

According to Figure 3, in all samples during storage there was a decrease in active acidity, which is especially typical during the period from the beginning of storage from 2-4 days to 16-20 days. Sample 1 with maltodextrin had the most stable acidity indicators. At the same time, the acidity of sample 3 with glucose-fructose syrup demonstrated high activity of lactic acid bacteria, even when yogurt was stored at low positive temperatures, especially in the period from 2 to 16 days. The recommended active acidity value for yogurt is usually set at 4.5 and below (Weerathilake et al., 2014). But from January 1, 2024, in accordance to the decision of the Food and Drug Administration (FDA), the maximum pH value of yogurt is set to 4.6 and below.

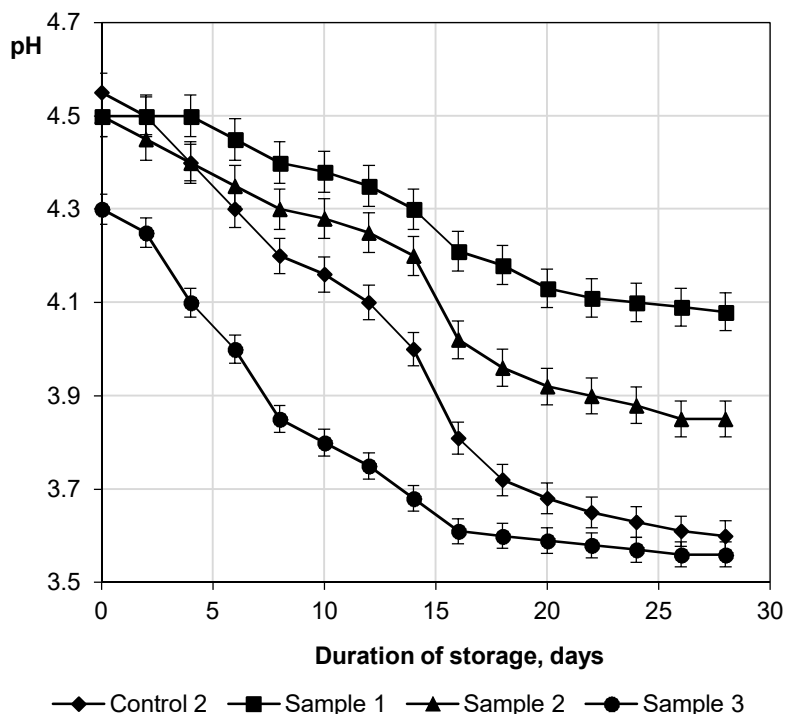


Figure 3. Changes in active acidity of yogurt samples with starch products during storage

As for the minimum pH value of yogurt, it is recommended to keep it at 4.0 to avoid the product having an overly sour taste (Deshwal et al., 2021). Following these requirements, the recommended pH range for yogurt is 4.0 to 4.6. According to the specified criterion, a sample of yogurt with GFS meets requirements for active acidity values only during the first 6 days of storage. The control yogurt meets requirements for active acidity within 14 days, and the sample with glucose syrup within 18 days. As for the sample with maltodextrin, its active acidity is quite stable throughout the entire storage period.

At the next stage of study, the degree of syneresis of yogurt with starch products during storage was studied. The results are shown in Table 3.

Table 3

Syneresis of yoghurt samples during storage

Yogurt	Time of storage, days							
	0	4	8	12	16	20	24	28
Control	2.0 ±0.11	3.5 ±0.18	5.3 ±0.20	5.8 ±0.21	6.4 ±0.19	6.6 ±0.22	6.8 ±0.23	6.9 ±0.24
Sample1	0.5 ±0.02	2.2 ±0.15	3.2 ±0.17	3.8 ±0.14	4.2 ±0.16	4.3 ±0.16	4.3 ±0.22	4.4 ±0.21
Sample 2	1.1 ±0.04	2.8 ±0.16	4.0 ±0.17	4.5 ±0.18	4.7 ±0.18	4.9 ±0.19	5.0 ±0.20	5.2 ±0.19
Sample 3	2.0 ±0.15	4.2 ±0.17	6.1 ±0.02	6.6 ±0.20	6.9 ±0.23	7.3 ±0.24	7.7 ±0.20	7.9 ±0.28

During storage, syneresis of all samples increased most in the first 8-12 days, after which this indicator somewhat stabilized. The control sample of yogurt without starch products and the sample of yogurt with glucose-fructose syrup retained moisture the worst.

Samples with maltodextrin and glucose syrup, containing moisture-binding higher sugars, demonstrated the least syneresis, which can be explained by the participation of high-molecular starch destruction products in additional cross-linking of the protein gel (Arab et al., 2023). Thus, the presence of high molecular weight residues of starch destruction improves the consumer properties of yogurt, including storage time.

The water activities in the yogurt samples are shown in Figure 4.

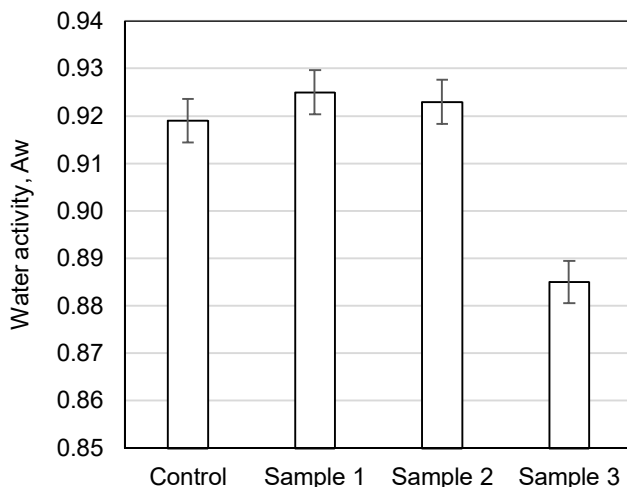


Figure 3. Water activity in the yogurt samples

Glucose-fructose syrup has the greatest influence on water activity in yogurt due to high concentration of glucose and fructose, 96% from total solids (Zuorro, 2021). As a result of a decrease in the content of monosaccharides in glucose syrup and maltodextrin, the activity of water in yogurt 1 and 2 increases slightly, but is almost no different from the activity of water in the control yogurt. So, starch products with low and medium dextrose equivalent affect water activity, similar to the combined effect of sugar and skimmed dry milk in the control yogurt. As for yogurt 3, a slight decrease in water activity to 0.855 did not significantly effect on the activity and vital activity of lactic acid bacteria during yogurt storage.

Thus, based on the results of the study, it can be concluded that the carbohydrate composition of starch products has a direct effect on the microbiological and physico-chemical parameters of yogurt, including throughout the entire shelf life. With an increase in the dextrose equivalent of starch products, their ability to accelerate the fermentation process of milk mixtures increases, which is accompanied by a more active accumulation of lactic acid bacteria cells in freshly made yogurt. At the same time, maltodextrin, due to its prebiotic properties, to some extent stabilizes the viability of lactic acid bacteria during long-term storage of yogurt.

The results of the study are of practical importance, since they allow to take into account the peculiarities of using starch products with different carbohydrate compositions.

Conclusions

1. The ability of starch products to influence water activity and the dynamics of milk fermentation by *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* depends on their carbohydrate composition. By increasing the dextrose equivalent, the monosaccharides in starch syrup, bioavailable to bacteria, reduce the fermentation time of milk in the production of yogurt. The effectiveness of this influence increases in the following sequence: maltodextrin → glucose syrup → glucose-fructose syrup. A slight decrease in water activity when the glucose-fructose syrup content in yogurt is 9% has virtually no effect on the dynamics of the milk fermentation process.
2. During storage of yogurt, there is an active increase in the concentration of bacterial cells in the presence of glucose-fructose syrup in the first 7 days, and on the 14th day their minor dieback was observed. On the 14th day of storage, the concentration of microbial cells *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus* becomes almost the same for all samples due to almost complete consumption of the available carbon sources. When yogurt storage is extended to 28 days, maltodextrin has the greatest effect on the viability of lactic acid bacteria due to its prebiotic properties.
3. Starch products with different dextrose equivalents can be used in yogurt, taking into account the specifics of their effect on the activity and viability of bacterial cells of the starter and the specified physico-chemical parameters of the finished product.
4. Further research will include studying the sensory characteristics and synergetic ability of milk-protein yogurt clots during storage

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