Potato starch as a component increasing the antioxidant potential of yogurt

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Abstract. Potato starches treated with Amylosubtilin at a low dose have a higher DPPH-scavenging activity than native starch. The use of such partially hydrolyzed starches in non-fat yogurt production does not degrade the chemical characteristics and promotes the accumulation of exopolysaccharides in food. Yogurt with the addition of AM-starch has higher reduction properties and radical-binding activity even after 28 days of storage. The investigated protective effect increases the attractiveness of such products for people caring for their health.

Key words: antioxidant, potato, starch, yogurt

1. Introduction
Fermented milk drinks are one of the most popular drinks in many countries of the world. It can be made of milk of different origins and using different bacterial starter cultures. Many producers reduce the fat content in milk for such products. Reducing the fat content in mass consumption products is aimed at improving the diet of a large group of people, which is especially important in connection with the tendency to increase the calorific value of the food diet of the average person in developed countries.

However, fat-free or low-fat fermented milk products have texture defects, which causes consumers to refuse to buy such products. The formation of elastic gel is due to the solubilization of calcium phosphate during fermentation of milk [1]. Available fat balls in yogurt act as structural promoters of the protein network formed by squashing. The texture is one of the most important properties that determine the quality of yogurt and customer satisfaction [2]. The reduction or elimination of fat from raw milk strongly affects the physical and texture properties, namely reduces the viscosity and density of the product [3]. There are several ways to eliminate these deficiencies: by using fat substitutes, additional milk ingredients or carbohydrates, as well as by controlling the conditions of production, such as heating temperature [4]. Along with these methods can be used thickeners such as starch. It is widely used in yogurt production to increase viscosity and hardness, improve taste, reduce syneresis, and make the body of yogurt more attractive [5-6].

On the other hand, yoghurts are products with a high antioxidant, anticarcinogenic potential and reduce the risk of metabolic changes [7]. It is important to preserve these useful properties of the product during its transportation, storage for shelf life. The purpose of this work was to evaluate the contribution of enzyme-modified starches to the formation of the antioxidant potential of yogurt, as well as its preservation during 4 weeks of storage.

2. Materials and Methods
2.1. Preparation of non-fat yogurt
The starches used in this work have been described by us earlier [8]. Starches were added to milk (0.05 % fat, Valio, Russia) at a concentration of 1 % and subjected to heating, the mixture was stirred for uniform distribution of starch. Analysis of the physicochemical properties of milk was tested on a Klever-2M quality analyzer, according to the method recommended by the manufacturer. *Lactobacillus delbrueckii subsp. bulgaricus*, and *Streptococcus thermophilus* were used as the yogurt starter. Overnight yoghurt culture was grown on low-fat milk at 37°C for 16 hours. Milk without starch was used as a control, starch was added to the test samples at a concentration of 2% (w/w), the milk was heated to gelatinize the starch. After cooling the milky-starch mixture, the starting culture of yogurt was added to it (5% v/v). Milk was fermented at 40 °C, and stabilized at 6 °C for 16 h.

2.2. *Physicochemical analysis*

Analysis of protein, whey protein solids contents have been described by us earlier [9].

2.3. *Determination of the total amount of polysaccharides*

Isolation of exopolysaccharides and polysaccharides (total amount of polysaccharides) and their determination were carried out as described by Feldmanel et al. [10] with modifications. 10-15 g of the sample was placed in a laboratory flask and boiled in a water bath at 100 °C for 30 minutes. After cooling, the samples were centrifuged at 4000 rpm for 30 minutes and 0.7 ml of 85% trichloroacetic acid was added to 4 ml of the sample. Samples were cooled to 4 °C and centrifuged again at 8000 rpm for 10 minutes. The deposition of EPS (1 ml) from the samples was performed using cold ethanol (-20 °C, 3 ml). The samples were kept in the refrigerator for 48 h and then centrifuged (4 °C, 8000 rpm, for 10 min), the precipitate was re-dissolved in distilled water (the volume is equal to the sample volumes), and the EPS was determined.

Quantification of total amount of polysaccharides. 5% solution of phenol in water (dissolve 5 g of fresh phenol in distilled H₂O and fill up to 100 ml in a flask) and 1 mg / ml glucose (dissolve 250 mg glucose in dH₂O and fill up to 250 ml in a flask) solutions were prepared. Glucose solutions prepared in different proportions in 6 test tubes were used to obtain a calibration line. 400 μl of the sample was placed in a glass tube and 400 μl of a 5% solution of phenol in water was added. For control, 400 μl of distilled water + 400 μl of a 5% solution of phenol in water were used. After that, 2 ml of concentrated sulfuric acid was sharply added to the solution in vitro. Samples were incubated for 10 minutes, then stirred and left for 10 minutes at 30 °C. Samples at 490 nm in quartz cuvettes were measured and compared with a control sample. The amount of EPS (mg) was calculated using a glucose calibration curve.

2.4. *Evaluation of antioxidant scavenging capacity by 2,2-di-phenyl-1-picrylhydrazyl (DPPH) assay*

Antioxidant scavenging capacity was analyzed according to the procedure described by [11]. Samples of yogurt were previously diluted Lertittikul 10 times, after which 1 ml diluted samples or starch solution (2 or 5 %) were used for analysis.

2.5. *Reduction power*

The reduction power assay was carried out following the procedure described by Lertittikul et al. [12].

3. *Results and discussion*

The reducing power of AM-0.5 and AM-1 starch gels was higher than that of native potato starch (Fig.1A), the activity of other samples was comparable to native starch. The ability to binding of free radicals of all enzyme-modified starches was higher than native starch (Fig.1B).
In the next stage, starches were used as an ingredient in nonfat yogurts. The addition of modified or native starches did not affect the amount of protein. However, the amount of protein transferred to whey in case of using enzyme-modified starches increased (Tabl.1).

**Table 1.** Chemical properties of yogurts.

| Samples   | Whey protein, % | Protein, % | Dry matter, % |
|-----------|----------------|------------|---------------|
|           | 1 day          | 28 days    | 1 day         | 28 days    | 1 day         | 28 days    |
| Control   | 2.85±0.21      | 3.02±0.09  | 4.15±0.09     | 4.13±0.12  | 9.68±0.31     | 9.82±0.21  |
| Native    | 3.06±0.11      | 3.10±0.08  | 4.09±0.11     | 4.02±0.16  | 10.72±0.20    | 10.13±0.11 |
| AM-0.05   | 3.30±0.13      | 3.26±0.11  | 4.21±0.12     | 4.18±0.12  | 11.14±0.25    | 10.40±0.29 |
| AM-0.1    | 3.17±0.14      | 3.20±0.12  | 4.26±0.08     | 4.10±0.11  | 11.09±0.41    | 10.16±0.23 |
| AM-0.5    | 3.15±0.12      | 3.25±0.08  | 4.18±0.11     | 4.09±0.09  | 10.82±0.23    | 10.12±0.17 |
| AM-1      | 3.10±0.11      | 3.16±0.21  | 4.21±0.13     | 3.92±0.12  | 10.78±0.24    | 9.82±0.33  |

The amount of EPS was two or more times higher in enzyme-modified starch samples compared to native starch samples. (Fig.2). Probably the presence of partially hydrolyzed starch in yogurts stimulates the production of EPS. It is also possible that lactobacteria use enzyme-modified starch as an added nutrient source.

**Figure 2.** Total amount starch and EPS of yogurt samples produced by addition of potato starches.

Enzyme-modified starch samples had a higher level of reduction power than native starch samples, and especially when compared to a control sample (Fig 3A). DPPH-scavenging activity was 65-68%, 8-10% higher than the control and native starch sample (Fig. 3B). Moreover, DPPH-scavenging activity was higher in samples with enzyme-modified starch even after 28 days of storage.
Figure 3. Reduction power (A) and DPPH-scavenging activity (B) of yogurt produced by addition of potato native or fermented modified starches.

This data show that the addition of partially hydrolyzed potato starch to skim milk and the subsequent fermentation of the yoghurt starter bacteria leads to the accumulation of EPS and improves the antioxidant properties of the dairy product.

The addition of partially hydrolyzed starches creates conditions that improve antioxidant properties in skimmed yogurts. Although the primary function of starches is to stabilize and adjust the texture of skimmed fermented milk products, the investigated protective effect increases the attractiveness of such products for people caring for their health.

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Acknowledgments
The reported study was funded by RFBR, project number 20-016-00025.