Development of histological methods for detection of carrageenan in yoghurt

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Abstract. Currently, in the market of fermented milk products there are various types of adulteration, which, as known, result in the low nutritional and biological product value and can be potentially dangerous to human health. Yoghurt is often exposed to adulteration; it is in great demand at consumers. Our work objective was the development of histological methods for the detection of carrageenan in yoghurt. As a result of the studies conducted, a histological method was developed for the first time, which can help to detect to high precision the unacceptable additive carrageenan in yoghurt. That way, the developed method allows to detect the adulteration of yoghurt.

1. Applicability
The problem of fermented milk products identification is burning currently, since there is a significant increase in the range and variety of milk products due to adding plant-based components to their composition (vegetable oils, soy proteins, etc.) [1].

The purpose of adulteration is gaining illegal profit by reducing the production costs as a result of unauthorized replacement of high-quality biologically valuable raw materials with less valuable ones. Most modern methods of adulteration, one way or another, boil down to changes in technology, use of cheap raw materials and subsequent bringing of physicochemical parameters to the requirements established by regulatory documentation. Along with the deliberate misrepresentation of the consumer regarding the properties and origin of products, decrease in food and biological value, the production and sale of adulterated products also contribute to unethical competition in the food market, at which point bona fide manufacturers are at a disadvantage [2], [3].

Yoghurt is often exposed to adulteration. Yoghurt is a fermented milk product typified by a high content of dry fat-free milk substances, produced using a mixture of starter microorganisms - thermophilic lactic streptococci and Bulgarian lactobacillus. [4].

An important problem to identify yoghurt is the need to confirm the absence of plant components in it, in particular, carrageenan. It is a food additive E407, presenting a polymer that belongs to the group of polysaccharides. Its main property is the ability to swell, getting a flexible texture. [5].

Despite numerous positive characteristics, in some countries, carrageenans are not allowed to be included in infant food composition and a range of products intended for children. The potential
danger of carrageenan is associated with the presence of toxic ethylene oxide in its composition. In truth, this information is confirmed not in all countries and many inspection authorities estimate E407 as a completely safe substance. However, scientists believe that some types of thickener can provoke the development of serious digestive system diseases [6].

The research of American scientists has shown that there is a certain type of carrageenan (degraded carrageenan) that can lead to various stomach diseases, including colon cancer. There are also opinions that stabilizer E407 can cause various inflammatory processes in the body. This fact is so reliable and consistent that this additive is often used in scientific experiments to cause inflammation for the purpose of its further treatment. [7].

To detect the content of carrageenan in milk and milk products the method of gas chromatography is used. The disadvantages of this method are high labour intensity, low accuracy, long duration, high production cost.

However, we have developed a new method for determining the adulteration of yoghurt - histological. In particular, this method will allow determining to a high precision the presence of a foreign impurity carrageenan in the product.

Purpose and objectives of research. The purpose of this work is to develop a histological method for determining yoghurt adulteration.

In accordance with the set purpose, the following tasks were completed:

- to artificially adulterate yoghurt using carrageenan;
- to prepare histological sections of adulterated and native products;
- to compare and analyze histological sections of natural and adulterated yoghurt.

2. Material and method of analysis

As materials under study, 4 samples (2 control and 2 test) of pasteurized 3.2% fat content milk in quantity 100 ml were used.

The test milk sample was adulterated with carrageenan: 5 g of carrageenan was dissolved in 10 ml of distilled room temperature water. Everything was thoroughly mixed to a sticky consistency and added to milk.

Then 5 g of yoghurt starter was added to the control and test milk samples. The contents of the samples were mixed and placed in a thermostat for 8 hours at a temperature of 40°C.

Then the histological sections of control and test yoghurt samples were made.

3. Test results and discussion

4 samples of 3.2% fat content yoghurt 100 ml each (2 - control, 2 - test) were taken for the test. The control sample is a natural product. The test sample is adulterated with carrageenan.

Histological sections of the control and test yoghurt samples were stained with the Lugol's solution end Sudan III.

The results of the tests conducted are presented in figures 1-4.
From the data presented in figure 1, it is clear that natural yoghurt has no extraneous additives. In the field of view of the figure milk protein and fat are visible, which are typical for a natural milk product.

**Figure 1.** Histological section of natural yoghurt (control sample). Staining with the Lugol's solution. Magnification 100x.

**Figure 2.** Histological section of yoghurt adulterated with carrageenan (the second test sample). Staining with the Lugol's solution. Magnification 100x.
From the data presented in figure 2, we can see that, in addition to the natural product components (protein and fat), there is an extraneous impurity in yogurt in the form of a shapeless lump of brown color, which is carrageenan.

**Figure 3.** Histological section of natural yoghurt (control sample). Staining with the Sudan III.

From the data presented in figure 3, it is clear that natural yoghurt has no extraneous additives. In the field of view of the figure milk protein and fat are visible, which are typical for a natural milk product.

**Figure 4.** Histological section of yoghurt adulterated with carrageenan (the second test sample). Staining with the Sudan III. Magnification 100x.
From the data presented in figure 4, we can see that, in addition to the natural product components (protein and fat), there is an extraneous impurity in yogurt in the form of a shapeless lump of violet color, which is carrageenan.

4. Conclusion
In the process of producing yoghurt it is not permitted to use carrageenan. The raw materials used for producing the product must comply with the requirements in terms of safety [7, 8, 9].

Thus, according to the conducted tests, the histological method developed by us is able to detect high precision impurities in yogurt that are unacceptable at the production of this product, what will allow to identify unscrupulous producers of fermented milk products.

References
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