Development of an express method for the quantitative assessment of the contamination of wheat flour with Bac. spores. subtilis

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Abstract. The causative agent of potato bread disease (Bacillus subtilis, ssp. Mesentericus) develops in the crumb of wheat bread. To prevent potato disease of bread, it is necessary to control raw materials and finished products in order to identify their microbiological contamination. Various methods can be used to determine the presence of bacteria that cause potato bread disease. These methods are usually subdivided into four groups: 1) bacteriological; 2) technological; 3) biochemical and 4) physical. Within the framework of all four groups of methods, there is no single method for analyzing the detection of pathogens of potato disease in bread, which would be easily reproduced in any laboratory and would have a correct assessment of the results.At the Department of Nutrition Technology of the Ural State University of Economics, an express method for the colorimetric determination of the contamination of wheat flour with Bac spores has been developed. subtilis by the content of erythrodextrins in it, which give a reddish-brown color when interacting with iodine solution. The advantages of the improved method for determining the potato disease of bread include: speed and rapidity; the possibility of both qualitative and quantitative determination of Bac. subtilis in flour to predict the occurrence of potato bread disease in wheat flour bread.

1 Introduction

The causative agents of potato disease are spore-forming bacteria belonging to the subspecies Bac. subtilis and Bac. mesentericus (which, according to the new classification, are combined into a single species Bacillus subtilis) are practically ubiquitous in nature - soil, air, plants. Bacteria of this species die during baking of bread at temperature heating, but leave viable spores that can survive at high temperatures (over 250 C) [1-10].

During the research, it was determined that at the moment there is no low-cost express method for determining the degree of infection of wheat flour and bread from it with potato disease without preparing test baked goods and incubating baked bread for at least 36 hours. There is also a method developed by V.P. Medvedev and colleagues at GosNIIKhP [11], which allows to determine the presence of spore bacteria Bac. subtilis in grain raw materials and finished products. The method is based on the proteolytic (gelatinase) activity of spore bacteria. It allows for 6 hours identifying the activity of spore bacteria that cause potato bread

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disease. The results of this express method indicate the qualitative indicators of bacteria in proteolytic terms, do not take into account the activity of α-amylases of spore-forming bacteria that cause dextrinization of starch in grain raw materials, are not informative in terms of quantitative assessment of contamination and also often present a distorted picture of the real degree of contamination of raw materials.

The most convenient and accurate method today is the instrumental viscometric method for determining the contamination of grain with pathogens of potato disease in bread, developed by the All-Russian Research Institute of Agriculture of the Russian Agricultural Academy [12]. However, this method is more applicable to grain than wheat flour bread. The disadvantage of this method can be considered the need to use the device PChP-3, equipped with a water bath to create favorable conditions for the action of the enzyme α-amylase (37 C). Without observing the temperature regime of the incubation medium, the analysis results will be unreliable.

This fact required the development of an improved method for determining the contamination of wheat flour, as well as a descriptive point scale for the degree of contamination of bread made from wheat flour.

When developing a method for assessing the contamination of wheat flour with spores of potato bacillus, two conditions were set: rapidity (the ability to obtain a result within no more than 6 hours) and the reliability of the results, the possibility of both qualitative and quantitative registration of contamination.

2 Materials and methods

The technique includes several stages.

The first stage involves obtaining a calibration graph for the content of erythrodextrins in contaminated wheat flour.

To construct a calibration graph for the content of erythrodextrins in wheat flour, an extract from potato slices was prepared according to the method of EZ Tepper [13]. The potato tubers were finely chopped, placed in a flask with 100 ml of sterile tap water, and shaken for 2 minutes. With a sterile hook (prepared from a conventional wire needle), the potatoes were removed from the flask and transferred sequentially into the second, third, fourth, fifth, sixth and seventh flasks, also containing 100 ml of sterile tap water. In each flask the potato was washed for 2 minutes.

Bac quantity. subtilis in the extract was determined using turbidity standards of 0.5; 1; 2; 3 on the McFarland scale, for which 0.5 was mixed; 0.1; 0.2; 0.3; 0.4 cm^3 suspension of barium chloride dihydrate and 99.5; 9.9; 9.8; 9.7; 9.6 cm^3 of sulfuric acid, respectively. The resulting suspension was poured into test tubes (5 ml), the volume of liquid was noted, hermetically sealed, and stored in a dark cabinet for no more than 3 months. At room temperature. Bac solutions were prepared in the same test tubes. subtilis extracted from potato slices with dilutions corresponding to turbidity standards of 0.5; 1; 2; 3 on the McFarland scale. At a wavelength of 600 nm solutions with a turbidity of 0.5; 1; 2; 3 on the McFarland scale have a transmittance of 74.3; 55.6; 35.6; 26.4; 21.5%, which corresponds to a bacterial concentration of $1.0 \cdot 108$; $3.0 \cdot 108$; $6.0 \cdot 108$; $9.0 \cdot 108$; $1.2 \cdot 109$, respectively.

Further, a control was prepared, for which a sample of healthy wheat flour weighing 5 g was quantitatively transferred into a volumetric flask and brought to 100 cm^3 with distilled water at a temperature of 30 ° C. Then the mixture was extracted with vigorous stirring (3000 $s - 1$) with a magnetic stirrer for 10 min, filtered, 5 cm^3 0.005 N was added to 5 cm^3 of the filtrate. iodine solution and determined the optical density on a FEK-56M at wavelengths of 660 and 530 nm, cuvette 5 mm. Samples for constructing a calibration scale were prepared in a similar way: Bac solutions were added instead of distilled water. subtilis, corresponding to a turbidity standard of 0.5; 1; 2; 3; 4.

3 Results and discussion

Bac. subtilis, releasing the amylolytic enzyme α-amylase, hydrolyze starch, and the hydrolysis proceeds in several stages:

1) dextrinization of starch. At this stage, the starch is broken down to dextrins with a sufficiently high molecular weight, as a result of which sticking of the crumb appears. Since α-amylase does not attack α-1,6-bonds, stable branched dextrins are formed during starch hydrolysis;

2) liquefaction. At this stage, an unpleasant specific smell of CPM appears. The second stage of hydrolysis is characterized by the release of proteolytic enzymes, which, by breaking down the protein, form decomposition products that give the bread a specific unpleasant smell. Dextrins are partially decomposed to tetra- and trimaltose;

3) saccharification - the appearance of stretching threads, an unpleasant specific smell. Tetra- and trimaltoses are very slowly hydrolyzed to di- and monosaccharides. The stage is characterized by a very low speed.

Most of the developed methods suggest the determination of potato disease of bread in already baked bread, which requires a significant investment of time for trial baking. However, wheat flour contaminated with Bac. subtilis and their spores are likely to provoke bread disease, the severity of which is determined by the number of spores. Therefore, the quantitative rapid determination of Bac. Subtilis in wheat flour can help establish the quality of flour and predict its potential for Bread Potato Disease.

When storing flour with a moisture content of not more than 14% Bac. Subtilis are usually inactivated, but when flour mash is prepared (by mixing wheat flour with warm water), they are activated and the first stage of flour hydrolysis to dextrins begins. Since hydrolysis to sugars is slower, an increase in the amount of dextrins in the flour mash can be observed during the first hour. This process usually takes place at a neutral pH of 6–7.

The proposed express method is based on determining the contamination of the flour of potato disease of bread by the content of erythrodextrins in it, which give a red-brown coloration when interacting with iodine solution after preparing flour mash from wheat flour with warm distilled water and its further extraction. According to the data of VA Marinchenko et al. [14], the maximum formation of erythrodextrins occurs between 10 and 20 minutes of starch hydrolysis, therefore, the time of hydrolysis before determining the optical density should not exceed 20 minutes. Since the hydrolysis to sugars does not have time to occur in a given period of time, they can be neglected. The generally accepted method for the determination of dextrins, based on their hydrolysis to glucose and its subsequent determination, cannot be applied in this case, since it implies the determination of all types of dextrins, and within the framework of this method, only the erythrodextrins that give red-brown staining with iodine must be detected in in contrast to achrodextrins and maltodextrins, which do not give staining, and amylodextrins, which give a blue color.

This method also cannot be applied to determine the potato disease of bread in wheat flour bread, since dextrins can also be formed during dough formation, fermentation, baking, but not under the influence of Bac. subtilis, but due to other physicochemical processes.

The content of dextrins was determined by the formulas of MP Popov and EP Shanenko [15]:

- the content of erythrodextrins in the solution was calculated by the formulas:

$$C_{\text{A}} = 0,044D_{660} - 0,0123D_{530}; \quad (1)$$

$$C_{\text{D}} = 2D_{660} - 47,7C_{\text{A}}, \quad (2)$$

where CA - concentration of amylose in solution, mg / cm³; CD - concentration of dextrins in solution, mg / cm³; D660, D530 - optical density of the solution at wavelengths of 660 and 530 nm;
- then converted to dry matter:

$$D = \frac{2 \times C_D \times 100}{100 - W}, \quad \%, \tag{3}$$

where D is the mass fraction of dextrins in terms of dry matter,%; W - mass fraction of moisture in flour,%.
Table 1 shows data on the content of dextrins in flour.

Table 1. Content of erythrodextrins in contaminated flour

Concentration of bacteria Bac. subtilis in the sample, CFU / cm³	Content of erythrodextrins,%
1,0·108	0,431
3,0·108	0,684
6,0·108	0,814
9,0·108	1,016
1,2·109	1,177

Bac content dependence plot. subtilis in wheat flour depending on the content of erythrodextrins is shown in Figure 1.

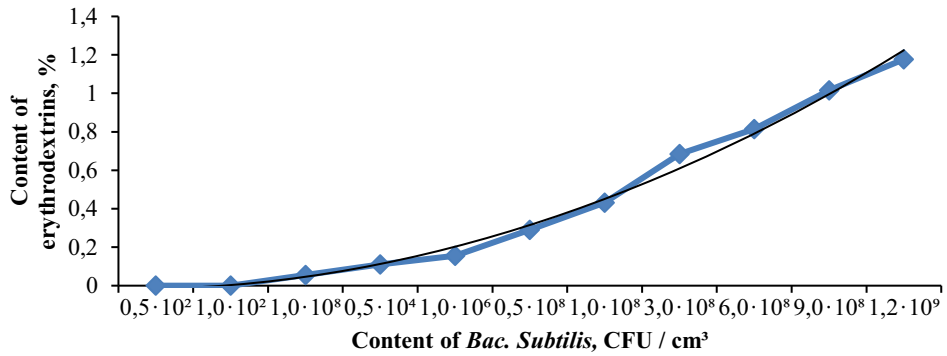

Fig. 1. Calibration graph of the Bac content. Subtilis in wheat flour depending on the content of erythrodextrins

When containing Bac. subtilis up to 2.0 · 10² flour can be considered healthy [11, 16].

The second stage involved determining the amount of Bac. Subtilis in contaminated wheat flour used for further research, as well as for commercial flour samples.

For the analysis, samples for determining the optical density / erythrodextrins content were prepared similarly to the control: a weighed portion of healthy wheat flour weighing 5 g was quantitatively transferred into a volumetric flask and brought to 100 cm³ with distilled water at a temperature of 30 ° C, the mixture was extracted with vigorous stirring for 10 min, filtered, to 5 cm³ of the filtrate poured 5 cm³ 0.005 N. iodine solution and determined the optical density on a FEK-56M at wavelengths of 660 and 530 nm, cuvette 5 mm. Then the content of erythrodextrins was calculated and the Bac content was determined by the calibration graph. subtilis.

4 Conclusion

This technique allows you to predict the period of occurrence of potato disease in baked bread.
A comparative assessment of the traditional and proposed methods for determining the potato disease of bread is presented in Table 2.

Table 2. Comparative assessment of methods for determining the potato disease of bread

Index	Test baking method (arbitration)	Method developed
Object of study	Test baking wheat flour bread	Wheat flour used for baking wheat flour bread
Sample preparation	2-3 hours	30 мин
The duration of the implementation of the method	From 24 to 72 hours	1 hour
quantitation	Absent	There is
Measurement accuracy indicators (precision),%	95	93
Relative error,%	No more than 5	No more than 7
Registered parameter	The thinning capacity of α amylase, the viscosity of the paste	The content of erythrodextrins in flour mash after its extraction

Thus, the proposed technique has a number of advantages and can be used for express detection of Bac. subtilis in contaminated wheat flour to predict the occurrence of potato bread disease.

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