Improvements in monofloral honey quality control

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Abstract. The paper presents the results of determining the activity of the most important honey enzyme, invertase, depending on the impact of main technological factors, as well as identifying the minimum permissible enzyme content in raw and processed honey. The studies were carried out in the laboratory of the Federal Beekeeping Research Center. The invertase activity and diastatic number was determined by a photometric method. The honey samples were examined in five replicates. The enzyme activity was determined after 30 and 90 days of honey storage. Deep freezing of honey at a temperature of -18 °C ensured the best preservation of the invertase activity in monofloral honey that came from an assortment of plants. Pasteurization of natural honey reduced the activity of enzymes of honey that comes from different plants by an average of 71.9%. Mechanical treatment of honey reduced the invertase activity by an average of 54.7%. Based on a comparative analysis of invertase and diastatic number, invertase was found to reflect the degree of honey treatment in the best way. The research findings will result in a set of recommendations to enhance the technology for honey processing and quality control, with a subsequent introduction of this indicator in the state standard for natural honey.

1. Introduction
Preserving and improving the quality of agricultural products is an ongoing concern. Expert appraisal of products is one of the most important and complicated issue, due to an interindustry character. The quality of livestock products can be controlled, for which purpose there are some requirements governing scientifically based quality standards, means and methods of control to provide a reliable and fast determination of properties [1].

The concept of standard is a term that is derived from the English word and means a sample, a model. On that ground, standardization of natural honey implies the establishment of standards in order to streamline activities in a particular area, achieve a universal optimal norm, subject to safety and operating conditions [2].

Natural honey is one of the most expensive foods, which is why it has long been vulnerable to adulteration – both in the past and modern times. Determining the quality of both feed and food honey is a crucial point in veterinary and sanitary examination, as well as standardization of this product. The adulteration of natural honey is a deliberate action that is aimed at deceiving the consumer by changing the properties of bee honey, assigning it a special botanical origin or rare nutritional properties [3, 4]. So far, increasing amounts of adulterated honey on the Russian market has been addressed by many scientists. Based on the research of the Research Beekeeping Center and other
scientific laboratories, it was found that 75% of the selected and tested honey samples were poor-quality [5, 6]. As far back as 2018, Sergey Dankvert, the head of the Federal Service for Veterinary and Phytosanitary Surveillance, reported that a third of honey on the Russian market can be classified as adulterated. For 2019, the situation did not change greatly and the share of honey counterfeit for the indicated period amounted to 70%, though subject to the region and particular producer. Moreover, domestic honey is not competitive at the international level, which results from the presence of residual contaminants and other regulatory inconsistencies [7, 8].

Despite being a global problem, adulteration is successfully prevented in those countries where not only public and scientific but also government bodies are involved, with a reliable legal basis being elaborated [9].

2. Problem statement
In the Russian Federation, the quality of honey is regulated by GOST 19792 - 2017 Natural honey [10]. Product specifications stipulate basic regulatory physical and chemical quality indicators. The honey that does not pass a commodity examination and is sold in the markets is appraised by experts at meat and dairy and food control stations and in veterinary laboratories [11, 12].

Among other discrepancies in Russian and international regulatory requirements that more accurately characterize the preservation of the properties of honey, many countries have introduced an invertase enzyme activity indicator (U/g). However, there are no minimum requirements for this indicator abroad and its presence alone is determined [13]. Accordingly, due to the relevance of transforming regulatory requirements to address the issues that arise following the country’s accession to WTO membership and to ensure the production of relatively high quality products, it is imperative to improve the quality control methods for natural honey [14].

The invertase activity in honey is expressed in units of activity per gram (U/g), while the derivative, the invertase number – in grams of sucrose that is cleaved within an hour by the invertase contained in 100 g of honey (g/100 g). It is considered an indicator of the duration and storage conditions, the degree of heating [15].

3. Material and methods
An invertase activity index most accurately reflects the degree of its properties after primary and other types of processing. With this in view, the study aims to identify the impact of the main technological processes on the activity of the invertase enzyme in honey.

To achieve this goal, the following tasks were set:

- to produce honey samples for the planned study;
- to examine the prepared samples for compliance with the regulatory requirements of GOST 19792-2017 Natural honey. Technical specifications;
- to determine the botanical origin of the selected samples in accordance with GOST 31766-2012 Monofloral honey. Technical specifications;
- to ensure the storage of selected honey samples under negative and positive temperature conditions, to perform mechanical processing;
- to obtain experimental data on the effect of treatment regimes on honey, as compared with test results;
- to conduct a comparative analysis of the susceptibility of natural honey enzymes after treatments;
- to perform biometric processing of data obtained during the planned study.

The studies were carried out in the laboratory under the Federal Beekeeping Research Center. The invertase enzyme activity was determined by the photometric method according to GOST 34232-2017 Honey. Methods for determining the activity of sucrose (invertase), diastatic number, insoluble matter. Experimental honey samples in five replicates were placed in 250 ml glass containers and stored in conditions of below-zero temperature -18 ºC. The enzyme activity was determined after 30 and 90 days of storage.
Some of the prepared samples was subjected to heating in a standard pasteurization mode of 75 °C (for 5 minutes), followed by storage at 15-16 °C. The invertase and diastase enzyme activity was determined immediately after processing, after 30 and 90 days. Some samples were machined for 30 minutes using a FIRST AUSTRIA FA-5259-3 dough mixer with a power of 700 W and a rotation speed of 1000 rpm, and sequentially stored at 15-16 °C. The invertase activity was determined immediately, after 30 and 90 days. The test honey samples were stored in five replicates, in identical containers, under controlled conditions at 15-16 °C. The enzyme activity was coupled with the study of the samples.

4. Results and discussion

Based on the pollen analysis, the target honey is considered monofloral and belongs to the following types of entomophilous plants: false acacia (67%), chestnut (78%), rape (58%), buckwheat (68%), sunflower (79%), and linden (59%).

The study of the invertase activity in the above monofloral honey varieties resulted in the fact that the enzyme activity in the honey that came from acacia was 33.5 ± 0.34 U/g, chestnut – 69.8 ± 0.28 U/g, rape – 39.9 ± 0.21 U/g, buckwheat – 65.5 ± 0.17 U/g, sunflower – 75.5 ± 0.19 U/g, and linden – 59.0 ± 0.31 U/g.

All prepared honey samples met the requirements of the state standard for physical and chemical indicators according to GOST 19792-2017. Natural honey. Technical specifications.

The results of determining the invertase activity in honey after being stored under freezing conditions at -18 °C are presented in Table 1.

| Common name   | Control | After 30 days experiment | After 90 days experiment |
|---------------|---------|--------------------------|--------------------------|
| False acacia  | 33.5±0.43 | 27.4±0.31                | 31.5±0.27                |
| Chestnut      | 69.8±0.28 | 56.5±0.42                | 64.3±0.34                |
| Rape          | 39.9±0.21 | 28.7±0.38                | 37.0±0.31                |
| Buckwheat     | 65.5±0.17 | 57.6±0.64                | 62.4±0.43                |
| Sunflower     | 75.5±0.19 | 67.8±0.54                | 70.4±0.42                |
| Linden        | 59.0±0.31 | 50.1±0.41                | 55.1±0.38                |

The above results indicate that deep freezing of raw honey contributes to a better preservation of the invertase activity, as compared with it being stored under control conditions (15-16 °C).

The decreased enzyme activity is uniform in both test and experimental samples. The greatest decrease in activity under freezing conditions after 90 days occurred in a rape sample and amounted to an average of 20.8% in relation to the source result, while the lowest decrease was in sunflower honey – 9.5% in relation to the corresponding test result. In all test honey samples, after 30 days the activity decreased on average by 17.2 ± 2.59% (range of fluctuations 10.2-28.1%), after 90 days – on average by 29.5 ± 3.96% (range of fluctuations 20.1-43.3%). The value of the invertase activity index in the samples after 30 days decreased on average by 6.5 ± 0.46% (with fluctuation range of 4.7-7.9%). The difference with the parallel benchmarks did not exceed 8.3 U/g. A decreased activity in the test samples after 90 days averaged 13.7 ± 1.69% (with fluctuation range of 9.5–20.8%), the difference with the corresponding test indices did not exceed 10.6 U/g.

The results of the honey invertase activity after being pasteurized are presented in Table 2.
Table 2. Invertase activity (U/g) after pasteurization treatment of honey at 75 °C for 5 minutes, (M±m)

| Common name   | Control     | After pasteurization | After 30 days | After 90 days |
|---------------|-------------|----------------------|--------------|--------------|
|               |             | control              | experiment   | control      | experiment   |
| False acacia  | 33.5±0.43   | 30.0±0.47            | 27.4±0.31    | 19.4±0.28    | 19.0±0.61    |
| Chestnut      | 69.8±0.28   | 64.5±0.31            | 56.5±0.42    | 31.5±0.53    | 49.5±0.53    |
| Rape          | 39.9±0.21   | 33.7±0.51            | 28.7±0.38    | 20.3±0.47    | 24.2±0.43    |
| Buckwheat     | 65.5±0.17   | 60.1±0.49            | 57.6±0.64    | 43.4±0.27    | 50.3±0.46    |
| Sunflower     | 75.5±0.19   | 71.2±0.41            | 67.8±0.54    | 55.3±0.48    | 60.3±0.38    |
| Linden        | 59.0±0.31   | 53.1±0.57            | 50.1±0.41    | 24.5±0.38    | 46.0±0.39    |

The above results indicate that the honey pasteurized at 75 °C for 5 minutes and subsequently stored has a reduced invertase activity, as compared with it being stored under control conditions (15-16 °C).

A decline in the enzyme activity during subsequent storage is accelerated in experimental samples compared to test ones. Following the heating, the activity most drastically decreased after 90 days in a false acacia sample and averaged 79.1% with respect to the initial control result, while the lowest decrease was with rape honey – 9.5% with respect to the corresponding control result.

The value of the invertase activity index in honey samples after 30 days decreased on average by 44.2 ± 5.02% (with fluctuation range of 26.8-58.5%). The difference with the parallel benchmarks did not exceed 25.6 U/g.

A decline in the experimental samples after 90 days averaged 71.9 ± 2.35% (with fluctuation range of 64.2-79.1%). The difference with the corresponding control indices did not exceed 37 U/g.

The results of the invertase activity after the mechanical treatment of honey are presented in Table 3.

Table 3. Invertase activity (U/g) after the mechanical treatment of honey (M±m)

| Common name   | Control     | After 30 days | After 90 days |
|---------------|-------------|--------------|--------------|
|               |             | control      | experiment   | control      | experiment   |
| False acacia  | 33.5±0.43   | 27.4±0.31    | 20.1±0.43    | 19.0±0.61    | 10.8±0.51    |
| Chestnut      | 69.8±0.28   | 56.5±0.42    | 43.3±0.27    | 49.5±0.53    | 31.2±0.37    |
| Rape          | 39.9±0.21   | 28.7±0.38    | 21.4±0.41    | 24.2±0.43    | 14.4±0.48    |
| Buckwheat     | 65.5±0.17   | 57.6±0.64    | 47.1±0.48    | 50.3±0.46    | 39.4±0.59    |
| Sunflower     | 75.5±0.19   | 67.8±0.54    | 56.4±0.39    | 60.3±0.38    | 35.5±0.31    |
| Linden        | 59.0±0.31   | 50.1±0.41    | 40.7±0.41    | 46.0±0.39    | 28.9±0.33    |

The above results indicate that the honey being whipped at 1000 rpm within 30 minutes and subsequently stored has a reduced invertase activity, as compared with it being stored under control conditions (15-16 °C).

A decline in the enzyme activity during subsequent storage was accelerated in experimental samples as compared to control ones. Following the whipping, the activity most drastically decreased after 90 days in a false acacia sample and averaged 67.8% with respect to the initial control result, while the buckwheat honey had the lowest decrease in this case – 39.9% with respect to the corresponding control result.

The value of the invertase activity index in honey samples after 30 days decreased on average by 34.8 ± 3.27% (with fluctuation range of 25.3-46.4%). The difference with the parallel benchmarks did not exceed 13.2 U/g.
A decrease in the activity in the experimental samples after 90 days averaged 54.7 ± 3.85% (with vibrational range of 39.9-67.8%). The difference with the corresponding control indices did not exceed 24.8 U/g. The results of comparing the effect of mechanical processing and pasteurization on the activity of the invertase enzyme and the diastatic number of honey are presented in Figure 1.

![Graph showing decline in activity and diastatic number after treatment](image)

**Figure 1.** Drawdown activity of honey enzyme group after treatment

The data presented above suggest that the diastatic number of natural honey is less susceptible to the effects of technological processing. Thus, following the mechanical treatment, the invertase activity in honey after 90 days decreased on average by 54.7 ± 3.58, and the diastatic number decreased just by 27.3%. Following the heating, the invertase activity after 90 days decreased by 71.9%, and a decline in the diastatic number did not exceed 19.9%. In this respect, the invertase enzyme is most susceptible and indicative of technological processing.

5. Conclusion

Deep freezing of honey at a temperature of -18 °C contributes to the best preservation of the invertase activity in monofloral honey that comes from different plants. A reduced activity in the experimental samples after 90 days averaged 13.7±1.69%. The difference between the control and experimental indicators did not exceed 10.6 U/g.

Pasteurization of natural honey at 75 °C for 5 minutes reduces the activity of honey enzymes of different botanical origin. In total, the invertase activity declines in 90 days after the heating up to 71.9±2.35% in relation to the control result.

Mechanical processing of natural honey at 1000 rpm within 30 minutes reduces the activity of honey enzymes of different botanical origin. In total, the invertase activity declines in 90 days after the heating up to 54.7 ± 3.58% in relation to the control index.

Comparing the effect of technological processing on natural honey enzymes suggests that the invertase enzyme best shows the degree of honey treatment, being an enzyme of a more sensitive class. Hence, it is advisable to recommend an invertase activity indicator to be introduced into the state standard for natural honey.

The findings will be used to develop draft recommendations on improving the technology for obtaining, processing and storing natural honey, and to provide a framework tailored to enhance the quality control methods for monofloral honey.

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