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Determination of the Composition and Quality of Honey by the Method of High Effective Liquid Chromatography

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Abstract: *the article discusses a test method for the determination of honey sugars before and after inversion, to determine the quantitative content of monosaccharides, maltose and other di- and oligosaccharides in the composition of honey.*

Key words: honey, high performance liquid chromatography, monosaccharides, oligosaccharides, maltose, sucrose.

Introduction

The importance of honey can hardly be overestimated due to the fact that the substances included in its composition are of great importance in the food industry and medicine. Honey is a natural product and has been used by humanity throughout its history. But close attention to its composition and properties has appeared relatively recently.

It is now known that the main part of bee honey is carbohydrates (glucose, fructose, sucrose, maltose, etc.). Their total content reaches 80%. Glucose and fructose make up 80-90% of the sum of all saccharides in matured honey, sucrose - up to 5%. Water is about 18%, depending on the type and maturity of the honey. The remaining 2-3% are microelements and various polyphenolic compounds that provide medicinal and antioxidant properties of honey.

Main part

This method of analysis is indirect and does not allow determining the quantitative content of each of the monosaccharides. It also does not allow us to unambiguously determine the sucrose content, since honey may contain maltose and other di- and oligosaccharides. For example, when artificially adding edible sugar to bees' complementary foods, the content of oligosaccharides in honey increases, which can be harmful when people with diabetes mellitus eat such honey.

A similar situation is observed when determining the other components of honey. Thus, the method for determining 5-hydroxymethylfurfurap is based on its spectrophotometric analysis in the presence of barbituric acid and para-toluidine (Winkler's method). This method is so indirect, highly dependent on the quality of the reagents used, and does not have high reliability.

Taking into account the current situation in the field of methods for determining the composition of the quality of honey, the development of modern, simple: safe and highly efficient methods of sample preparation and analysis is urgent. The most promising in this direction are high-performance liquid chromatography methods. They allow you to efficiently and accurately determine the content of all the main components of honey, as well as unwanted substances such as antibiotics.

Material method

To achieve this goal, the following tasks had to be solved:

• Development, validation and certification of methods for determining the carbohydrate composition of honey by high-performance liquid chromatography.

• Development, validation and certification of a method for determining the content of 5-HMP in honey by HPLC.

• Development, validation and certification of a method for determining antibiotic residues in honey by HPLC.

For the first time, work was carried out to prepare a methodology for its introduction into GOST, including such stages as:

• Sample preparation

• Selection of chromatographic conditions

• Choice of column and sorbent

• Selection of the eluent and its composition

• Choice of chromatography mode (eluent flow rate, column temperature).

The inclusion of the validation stage allows you to determine the reliability of the analysis method, which is necessary for the introduction of the method into GOST.

Practical significance:

1. The developed methods, subject to their inclusion in GOST, can be used in various production laboratories and laboratories of regulatory organizations.

The following provisions are brought to the defense:

- the results of the development of a method for determining the carbohydrate composition of honey and its validation.

- the results of the development of a methodology for determining the content of 5-GM <in honey and its validation.
- results of the development of a method for the determination of chloramphenicol in honey and its validation.

2. To prepare standard and working solutions of the required concentration, the following were used:
- micropipettes with a volume of 100 and 1000 ml,
- pipette with a volume of 5 ml,
- bulk volumetric flasks with a volume of 25 ml,
- ultrasonic bath,
- acetonitrile for HPLC.
- analytical scales were used for weighing the samples,
- membrane filters with a diameter of 13 mm and a pore size of 0.45 microns were used to filter the prepared solutions.

Determination of the carbohydrate composition of honey

A solution of standards was prepared using an accurate sample. To ensure long-term storage, the standard solution was mixed with acetonitrile in a volume ratio of 1/1. Working solutions of standards were prepared by diluting a standard solution with a mixture of water - acetonitrile with a volume ratio of 1/1.

When analyzing honey samples, the solutions were prepared as follows: 1 g of the test honey (accurately weighed) was dissolved in 50 ml of water for HPLC.

The solution was stirred until the honey was completely dissolved in the ultrasonic bath at room temperature in the "stirring" mode. Then the solution was filtered through a membrane filter with a pore diameter of 0.45 microns. The filtrate was mixed with acetonitrile in a 1/1 volume ratio and analyzed by HPLC.

In the process of developing the methodology, the analysis conditions were determined:
- the working wavelength of 5-HMP was determined from the UV spectrum and was 284 nm;
- to increase the separation efficiency, a gradient elution mode was used:

0-7 minutes - water: acetonitrile (97: 3 vol.); 7-12 minutes - water: acetonitrile (0: 100 vol.); 12-17 minutes - water: acetonitrile (97: 3 vol.);
- the volumetric elution rate was 1.0 ml / min;
- injection volume 20 ml;
- the temperature of the column thermostat is 30 ° C;

The composition of the phase can be varied to achieve optimal separation.

In the process of developing the technique, the analysis conditions were determined: - the elution mode is isocratic, the composition of the mobile phase is acetonitrile / water in a volumetric ratio of 82/18 without mixing of the two; containers. This eliminated baseline fluctuation. The composition of the mobile phase can be varied to achieve complete separation of the glucose and fructose peaks.

Conclusion

When developing the method, the standard solution was prepared according to an accurate weighed portion. Working solutions - by diluting the standard solution immediately before analysis. The advantages and disadvantages of existing methods are critically examined and the limited possibilities for determining the qualitative and quantitative composition of honey are shown. As a result, the goal of the study was formulated.

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