Water-soluble vitamins in honeys of various botanical origin and their change during storage of honey

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Abstract. The richest chemical composition of honey determines its extremely high biological activity. The composition and concentration of bioactive compounds of honey largely depend on its botanical origin, i.e. they are determined by nectar and the pollen chemical composition of the nectariferous plant, on the soil on which they grow, as well as on climate and weather conditions. Along with enzymes and minerals, vitamins are found in honey in small but measurable amounts. Vitamins of honey are mainly represented by B-group vitamins. The content of vitamins in honey varies according to its botanical origin. The following types of honey were used for the study: acacia, sunflower, chestnut, linden, buckwheat. The quantitative determination of water-soluble B-group vitamins (thiamine, riboflavin, pyridoxine, pantothenic acid, nicotinic acid) in honey of various botanical origin was carried out in the accredited laboratory of the direction of chemical and biological research of beekeeping products of the Federal Research Center of Beekeeping by the method of capillary electrophoresis. It was revealed that chestnut and buckwheat honey present the highest levels of water-soluble vitamins content; that confirms the prospects of their use as a source of vitamins. Vitamins are unstable compounds that can be easily destroyed by various factors. Under the impact of daylight, the studied water-soluble vitamins contained in honey are destroyed during storage. The dynamics of changes in the vitamins content was traced during the storage of honey for 12 months in a dark place and in the light. Riboflavin and nicotinic acid, which is significantly reduced, is characterized by a higher sensitivity to the action of daylight during storage of honey.

1. Introduction
Sources of vitamins for humans are various food products of plant and animal origin. Human organism needs vitamins in very small quantities (from several μg to tens of mg), so they are minor components of food [1]. B-group vitamins are the most important bioactive compounds. Their high biological activity is based on participation in the construction of enzyme systems as coenzymes - low molecular weight non-protein substances that form a complex with enzymes that directly regulate biochemical reactions [2]. The whole complex of B-group vitamins provides the human body with the normal functioning of the nervous system and is responsible for energy metabolism.

Honey bees process the collected nectar to produce honey. Honey is a complex mixture, consisting mainly of carbohydrates, enzymes, amino acids and some macro and microelements. In relatively small but measurable amounts, honey contains water-soluble vitamins, the sources of which are nectar and, to a greater extent, plant pollen.

The chemical composition of nectar and pollen varies significantly depending on the type of plant,
on their organs and tissues biochemical characteristics and many other factors. The presence of vitamins in honey increases its value as a food product [3]. Studies conducted in different countries show that regular honey consumption increases the body's resistance to various diseases. When determining the quality of honey, the content of vitamins in honey is not taken into account and is not standardized, but popular scientific publications often use the vitamin value of honey and products based on it. Information on vitamins content in honey is very contradictory. The vitamin content in honey varies according to its botanical origin. Vitamins are unstable compounds that are easily destroyed under the influence of various factors, therefore, improper storage of honey can lead to a change in the content of important biological compounds in it, and thereby reduce the nutritional value of the product.

Various methods are used in our country to identify and quantify vitamins in various products. So the most widespread ones are titrimetric, spectrophotometric, polarographic, fluorimetric, photoelectrocolorimetric methods [4, 5]. The widely used chromatographic methods are accurate but complicated by the use of expensive equipment. Today, capillary electrophoresis is one of the modern and most promising methods of analysis, which is increasingly being used in various areas of analytical chemistry. Capillary electrophoresis is highly selective and sensitive method [6].

2. Materials and methods

The following types of honey were used for the research: acacia, sunflower, chestnut and linden, buckwheat. The composition of some B-group vitamins (B1 (thiamine chloride), B2 (riboflavin), B3 (pantothenic acid), B5 (nicotinic acid and nicotinamide), B6 (pyridoxine), B9 (folic acid) in the investigated types of honey was studied. The dynamics of changes in vitamins was also traced during storage of buckwheat honey for 12 months at room temperature (20-25 °C) in a dark place and in the light.

It is not always successful to analyze a sample without preliminary isolation of the determined components from the natural matrix. Natural matrices are the most complex objects of analytical chemistry [7]. Techniques for preparing a sample of a specimen for analysis are specific and in each case depend on their type and a given specimen. In most cases, sample preparation processes consist in the separation of the determined components from the matrix or, conversely, interfering substances from the analyzed medium in such a way that the maximum effect is achieved. In most cases, it is sample preparation that is a criterion for the quality of the obtained analytical data. During the work, the method of preparing honey samples for the determination of B-group vitamins was worked out, the extraction conditions (extractant composition, time) of water-soluble vitamins from the studied honey samples were selected. The preparation of a honey sample using an ultrasonic homogenizer turned out to be a more promising way, since in this case the highest results were obtained in the extraction of B-group vitamins from the studied honey samples.

The vitamins content was determined in aqueous solutions of honey in accordance with the regulatory documentation in force [8]. A weigh of honey was dissolved in double-distilled water, stirred on an ultrasonic homogenizer, and an extraction solution consisting of a solution of sodium tetraborate and a solution of sodium sulfite was added. The resulting solution was stirred and then centrifuged. The resulting extract from a sample of honey was used to determine vitamins.

Electrophoretic separation was carried out on «Agilent» capillary electrophoresis systems, on a quartz capillary with an effective length of 56 cm, with an inner diameter of 50 microns, a working buffer with a pH of 9.3, hydrodynamic injection - 50 mbar, the working voltage applied to the electrodes was + 20 kV, temperature - + 35 °C. Vitamins were detected at a wavelength of 215 nm with a bandwidth of 20 nm.

The essence of the method lies in the migration and separation of charged analyzed components under the action of an applied electric field. The capillary is flushed with a leading electrolyte. Next, a test tube with the same auxiliary solution (electrolyte) is set at the outlet to the operating position, and a test tube with a sample is placed at the inlet and introduced. After the voltage is applied to the
ends of the capillary, the components of the investigated mixture begin to move at different speeds, depending primarily on the charge and mass, and, accordingly, reach the detection zone at different times. We get a peak sequence - electrophoregrams. The qualitative characteristic of a substance is the time of migration, and the quantitative characteristic is the peak height or area which is proportional to the concentration of the substance. The results of the obtained measurements (electrophoretic data) are processed by the installed calculation program.

The botanical origin of honey is established by the identification of pollen grains of a certain type of nectariferous plant [9]. These studies are aimed at a integrated study of the composition, properties and nutritional value of honey.

3. Results
The content and concentration of honey bioactive compounds are largely determined by the chemical composition of nectariferous plants nectar and pollen. The characteristics of honey are presented in table 1. These plants are among the most widespread nectariferous plants in the central part of Russia.

Table 1. Botanical characteristics of the investigated honeys.

| Name and place of obtaining honey | The frequency of occurrence of pollen grains, % |
|----------------------------------|-----------------------------------------------|
| Buckwheat honey Ryazan region, Skopinsky district | Sowing buckwheat (*Fagopyrum esculentum*) - 40.5; Common melilot (*Melilotus officinalis*) – 3.5 |
| Flower honey with a predominance of Chestnut pollen grains Krasnodar Territory, Adler District | European chestnut (*Castanea sativa*) — 57.6; Norway maple (*Acer platanoides*) – 8.7 |
| Linden honey Primorsky Territory, Kirovsky district, p. Shmakovka | Manchurian linden (*T. mandshurica*) — 41.3; Crowned sawwort (*Serratula coronata*) – 8.4 |
| Sunflower honey Rostov region, village Krasny Sulin | Sunflower (*Helianthus*) - 46.8; Mustard (*Sinapis*) - 3.1; Rapeseed (*Brassica napus*) – 6.8 |
| Flower honey with a predominance of white acacia pollen grains Krasnodar Territory, Sochi District | Black locust (false acacia) (*Robinia pseudoacacia*) – 34.5; Dewberry (*Rubus fruticosus*) – 7.4 |

The results of determination of vitamins in honey are presented in table 2. Five types of honey were selected for the study: linden, acacia, buckwheat and chestnut and sunflower. Vitamins B_1 (thiamine) and pantothenic acid were not found in these honey samples.

Table 2. Vitamins content in honey of various botanical origin, mg / 100 g.

| Botanical name of honey | Vitamin name | nicotinamide | B_2 | B_6 | nicotinic acid | Bc |
|------------------------|--------------|--------------|-----|-----|----------------|----|
| Chestnut               |              | 0.38±0.021   | -   | 4.70±0.015 | 0.10±0.002 | 0.35±0.004 |
| Buckwheat             |              | 0.14±0.014   | 0.07±0.008 | 1.30±0.008 | 0.05±0.001 | 0.21±0.003 |
| Linden                 |              | 0.11±0.005   | 0.03±0.002 | 1.01±0.010 | 0.04±0.003 | 0.20±0.002 |
| Acacia                |              | 0.10±0.008   | 0.04±0.007 | 0.40±0.001 | - | 0.10±0.004 |
| Sunflower             |              | 0.12±0.016   | 0.03±0.005 | 0.30±0.007 | 0.04±0.003 | 0.10±0.005 |

The table shows that chestnut honey is the leader in vitamin B_6 content. This type of honey has the highest content of all detectable B-group vitamins, except for vitamin B_2. Also, chestnut honey is richer in nicotinamide. Buckwheat honey has a higher content of vitamin B_2 in comparison with honeys of other botanical origin. Sunflower and acacia honey have the lowest levels of vitamin B_6 and folic acid content. Vitamin B_1 exists in two forms - nicotinic acid and nicotinamide. Nicotinic acid is most abundant in linden honey, and this vitamin is not found at all in honey from white acacia. The
physiological and biochemical characteristics of different plant species can be the reason for the
differences in vitamins content.

Storing honey at room temperature (20-25 °C) causes a decrease in some important bioactive
substances. Vitamins are unstable compounds that can be easily destroyed by various factors.
Therefore, a study was carried out to assess the effect of storing honey at room temperature in a dark
place and in the light on the change in the content of water-soluble B-group vitamins in it. With the
method of capillary electrophoresis, a quantitative determination of water-soluble B vitamins was
carried out during the storage of honey for one year. Buckwheat honey was selected for the study.

It is noted that the studied water-soluble vitamins contained in honey are destroyed during storage
under the influence of daylight. Nicotinamide turned out to be unstable during storage of honey, its
decrease averaged 29.2% when stored in a dark place and 58.3% when stored in the light (Fig. 1).

**Figure 1.** Changes in the content of nicotinamide and riboflamine in honey during storage (a - content
of nicotinamide, mg / 100g, b - content of riboflamine, mg / 100g)

Vitamin B2 (riboflavin) is characterized by a higher sensitivity to light during storage of honey. The
decrease in riboflavin averaged 13.6% when stored in a dark place and 52.3% when stored in the light
(Fig. 1).

Studies of honey have also been carried out on the effect of daylight and storage in a dark place of
the vitamins of nicotinic and folic acids. Nicotinic acid is even more unstable during honey storage. Its
greatest decrease occurred during storage in the light. The decrease in nicotinic acid averaged 20.0%
of the original honey when stored in a dark place, and when stored in the light, the decrease in
nicotinic acid averaged 40.0% (Fig. 2).

**Figure 2.** Changes in nicotinic and folic acids in honey during storage (c - content of nicotinic acid,
mg / 100g, d - content of folic acid, mg / 100g)

Folic acid, like other studied vitamins, decreases when honey is stored under different conditions.
When storing honey in a dark place, the decrease in its amount averaged about 19.0% of the original
honey, and when storing honey in the light, the decrease in folic acid averaged 31.3% (Fig. 2)
Thus, storing honey at room temperature \((20 \pm 2^\circ \text{C})\) causes a decrease in the content of water-soluble vitamins. The decrease in B-group vitamins in daylight is more significant than when honey is stored in a dark place.

4. Conclusion
Honey of different botanical origin has a different content of certain water-soluble B-group vitamins. Chestnut and buckwheat honey have the highest levels of water-soluble vitamins. The content of vitamin \(B_2\) is approximately the same in all studied honeys, with the exception of buckwheat honey. Thiamine and pantothenic acid were not found in the investigated honeys. During the research, it was noted that during storage of honey there is a decrease in the content of water-soluble vitamins. The decrease in water-soluble B-group vitamins in honey in daylight is more significant than when it is stored in a dark place.

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