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Safety and quality indicators of rapeseed and sunflower honey from different regions of Ukraine

Roman Kołacz*

Doctor of Veterinary Sciences, Professor
Nicolaus Copernicus University
87-100, 7 Gagarina Str., Torun, Poland
https://orcid.org/0000-0001-6439-7770

Olha Iakubchak

Doctor of Veterinary Sciences, Professor
National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str., Kyiv, Ukraine
https://orcid.org/0000-0002-9390-6578

Tatiana Taran

PhD in Veterinary Sciences, Associate Professor
National University of Life and Environmental Sciences of Ukraine
03041, Heroiv Oborony Str., 15, Kyiv, Ukraine
https://orcid.org/0000-0002-9370-8539

Julia Hryb

PhD in Veterinary Sciences
National University of Life and Environmental Sciences of Ukraine
03041, 15 Heroiv Oborony Str., Kyiv, Ukraine
https://orcid.org/0000-0003-0913-6927

Abstract. The relevance of the study lies in the growing demand for safe and high-quality bee products, primarily honey, and the non-admission of low-quality products that can harm the health of consumers in the Ukrainian and European Union markets. In Ukraine, sunflower and rapeseed honey are in the greatest demand in export potential. The purpose of the study is to determine the compliance of rapeseed and sunflower honey obtained in Ukraine with the requirements of

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*Corresponding author

national and European Food legislation. To achieve this purpose, organoleptic methods of natural honey research were used and the diastase number, acidity, content of pollen grains, mass fraction of water, mass fraction of reducing sugars, sucrose, and hydroxymethylfurfural were determined. Analysis of rapeseed honey for the presence of genetically modified pollen was conducted using the polymerase chain reaction method in real time. It was established that prototypes of sunflower honey from Vinnytsia, Odesa, and Kyiv regions met the requirements of the national standard and European Food legislation, without signs of fraud. Currently, the use of genetically modified organisms is subject to regulation. Samples of rapeseed honey taken from the Vinnytsia, Odesa, and Kyiv regions did not contain genetically modified deoxyribonucleic acid. In terms of physical and chemical parameters, rapeseed honey met the requirements of the national standard. Honey collected in the Kyiv and Vinnytsia regions had an average water content of more than 18.5%, which meets the requirements for first-grade honey according to the national standard. Honey collected in the Odesa region corresponded to the indicators of the top-grade honey, the water content in it averaged 17.7%. According to the main physical and chemical indicators, rapeseed honey collected in Ukraine also meets the requirements of European food legislation. In addition, rapeseed and sunflower honey from the Vinnytsia, Odesa, and Kyiv regions are natural and can be sold not only in Ukraine but also on the territory of the European Union and the World Trade Organisation

Keywords: flower pollen; control; legal regulations; fraud; genetically modified organisms

Introduction

Beekeeping is one of the most promising agro-industrial sectors in Ukraine. Among all bee products consumed by the population, the first place belongs to honey. Currently, Vinnytsia, Dnipro, Zhytomyr, Mykolaiv, Zaporizhzhia, Poltava, Donetsk, and Kirovohrad regions of Ukraine are the largest producers of natural honey. These regions produce about 70 percent of national natural honey exported to different countries (Vinichenko, 2021).

Natural honey is used in the food industry, pharmacological production, cosmetology, etc. The increase in honey consumption is influenced by the culture of health-improving nutrition, the demand for organic products, and the industry's focus on natural raw materials containing natural biologically active substances that positively affect the immune system (Samarghandian *et al.,* 2017), especially during a tough epidemic situation in the world. In the context of the pandemic, the honey market continues to develop actively, and exports to the European Union are increasing (Covaci *et al.,* 2023).

By origin, honey is divided into monofloral (obtained mainly from the nectar of one plant species) and polyfloral (obtained from different types of flower nectar). Monofloral honey is in better demand and more expensive than its polyfloral counterparts, which increases the need for an appropriate analysis method that could be used to determine its origin (Voyslavov *et al.,* 2021). A set of plant species identified by pollen analysis provides information about the local or regional origin of honey. A collection of pollen samples should be available to compare the morphology of pollen grains obtained directly from flowers with those obtained during honey sediment collection. The volume of this collection depends on the studied territory and the radius of action of the bees (Almeida-Muradian, 2020).

In Ukraine, the most extensive honey collection is provided by regions with large acreage

for sunflowers; sunflower honey accounts for the largest share of domestic production (Vinichenko, 2021). Sunflower honey is a valuable therapeutic and preventive product. This honey is characterised by a high content of glucose and fructose and a low content of sucrose. Among light varieties of honey, it is distinguished by a high enzymatic activity. In the European Union, sunflower honey is one of the most expensive varieties. In addition to sunflower honey, the most common monofloral varieties in Ukraine are rapeseed, buckwheat, linden, and acacia. Honey is used for various medical purposes, and recent studies confirmed its effectiveness in the treatment of various diseases due to its components and antibacterial, anti-inflammatory, antioxidant, and antiviral properties (Fakhlaei *et al.,* 2020). Doctors recommend including honey in the diet of schoolchildren and people living in ecologically unfavourable regions and in areas of high radioactive background. The therapeutic effect of honey consumption in diseases of the gastrointestinal tract, cardiovascular system, various metabolic disorders, etc. has been identified (Fakhlaei *et al.,* 2020). Notably, the physical and chemical properties of honey are considerably influenced by natural and climatic factors, industrial characteristics of a particular region, the state of agriculture, the content of radionuclides, and so on. Therefore, it is important to examine honey samples from different areas.

One of the main problems for consumers is food fraud, since not only does the quality of food decrease, but also negative consequences for human health are possible. Food testing and counterfeit toxicology are essential to ensure that consumers are protected from fraud (Jaafar *et al.,* 2020). According to Codex Alimentarius, consumers have the right to receive truthful information about the food they consume (Codex Alimentarius…, 2019). It also notes that honey should not contain any artificially added ingredients, foreign tastes or aromas that may appear during processing and storage. It is also forbidden to heat or process honey to such an extent that its composition changes and its quality worsens.

The purpose of this study is to check the quality and safety indicators of honey varieties from different regions of Ukraine and establish their compliance with the main criteria of European and national food legislation.

Literature Review

The quality and safety of honey can be influenced by various factors, such as the period of honey collection, the terrain, the landscape of the territory, technological methods of keeping bees, feeding bees with sucrose, pumping unripe honey, unsanitary storage conditions, adding inverted sugar, etc.

To determine the safety and quality of the product, organoleptic and physico-chemical studies are conducted. In addition, an important criterion is the presence of genetically modified components in honey. Scientific data indicate that three genetically modified crops – soy, corn, and rapeseed – are most common in Ukraine (Kushnir, 2021).

It should be noted that currently, the legislation of Ukraine regulates the use of genetically modified organisms (Law of Ukraine…, 2012). A prerequisite for using such organisms in an open system is their identification. It is forbidden to release genetically modified organisms into the environment without their state registration, with the exception of testing. The legislation prohibits industrial production and introduction into circulation of genetically modified organisms, including products manufactured with their use, before official registration. Currently, not a single variety of agricultural plants is included in the state register of genetically modified organisms. Research on the impact of biotechnology products on human health and the environment still

continues. In the European Union countries, great attention is paid to food quality and biological safety of food products and a very cautious attitude towards genetically modified organisms (Regulation (EU)..., 2003; Bruetschy, 2019). Therefore, it is forbidden to sell honey without conducting an analysis of the content of genetically modified pollen. Among genetically modified plants, only rapeseed belongs to honey crops. The development of rapeseed crops is of great economic importance for Ukraine. The presence of a genetically modified rapeseed crop was recorded in the country (Oblap *et al.,* 2018). However, for the successful cultivation of this crop, various pesticides are used, which can affect bee products.

Measures are being actively implemented at the local and international levels to detect fraud (Fakhlaei *et al.,* 2020). High-quality and safe food products are the key to a healthy nation and an important link in the social and ecological well-being of the country.

Considering the economic and social conditions and the future development of beekeeping in Ukraine, it is necessary to analyse the world and European requirements for different varieties of honey. The issue of biological safety is important; therefore, in addition to the established organoleptic and physico-chemical studies of honey, including the determination of the characteristics of the pollen composition, it is planned to conduct studies on the content of genetically modified organisms and identify the competitiveness of the domestic product on the world market. The scientific originality of this study lies in the fact that for the first time, approaches to the method of pollen analysis are scientifically justified, which allows confirming the naturalness of national honey and its variety. In addition, the presented technique can be used to create an atlas of pollen of Ukraine to establish not only the variety of honey but also its geographical origin.

Materials and Methods

To conduct the study, samples of rapeseed and sunflower honey were selected, which were obtained from apiaries of different regions of Ukraine: Vinnytsia, Odesa, and Kyiv. The study was conducted during 2019-2021. A total of 60 honey samples were received for the study, indicating the date and place of selection. Organoleptic and physico-chemical parameters were determined according to the methods of the current national standard of Ukraine (National standard of Ukraine..., 2005).

The organoleptic parameters of honey and its pollen analysis were conducted based on the Department of veterinary hygiene after A. K. Skorokhodko of Life and Environmental Sciences of Ukraine 3 research groups were formed to examine sunflower honey. Experimental samples of honey were characterised by the presence of the main nectar source, which was a sunflower. Accordingly, they had similar organoleptic characteristics. Experimental samples of honey differed by the region of their collection. Considering the natural and climatic factors that affect the quality of honey, samples were taken from three regions of Ukraine for the study. The first experimental group contained 10 prototypes of honey from the Kyiv region, the second experimental group – 10 prototypes of sunflower honey from the Vinnytsia region, and the third experimental group – 10 prototypes from the Odesa region. Accordingly, three research groups were established to examine the sensory and physical-chemical parameters of rapeseed honey, with 10 samples in each group. The first experimental group included samples of rapeseed honey from the Kyiv region, the second experimental group included samples from the Vinnytsia region, and the third experimental group included samples of rapeseed honey from the Odesa region.

Determination of organoleptic parameters, namely: colour, crystallisation, and the presence

of signs of fermentation was performed visually in daylight in a laboratory transparent glass; taste – by tasting several grams of honey; two honey tastings were conducted sequentially. To determine the aroma, a sample of 30 g was taken, added to a weighing bottle, tightly closed with a lid, and heated in a water bath for 10 min, to a temperature of 45°C. After that, the lid of the weighing bottle was opened, and the aroma was examined by slowly inhaling the air. The consistency was determined at 20°C, the spatula was dipped in honey, raised and observed for the nature of its flow. The presence of mechanical impurities was examined by completely dissolving 50 g of honey in 50 mL of warm distilled water. The resulting solution was carefully poured into a laboratory glass cylinder and the degree of contamination of the product was identified. In the presence of mechanical impurities, they are found at the bottom of the cylinder or on the surface of the solution, depending on the relative density. To determine the quality, safety of the product, and the botanical composition of honey, laboratory tests are mandatory.

The following materials and reagents were used to conduct pollen analysis of honey: laboratory microscope "Granum" (China), Goryaev chamber "Sklopryad" (Ukraine); an electric centrifuge of the company "Dastan" (Kyrgyzstan) with a rotation speed of up to 5000 rpm, laboratory scales of the II accuracy class of the company "Radwag" (Poland); centrifuge tubes; water bath; laboratory mercury thermometer up to 100°C; a set of chemical glasses with a volume of 100 mL from the companies "Olis" and "Sklopryad" (Ukraine); rectified ethyl alcohol with a mass fraction of 96%; basic crystalline fuchsin, alcohol solution with a mass fraction of 10%; distilled water; acetic anhydride; glacial acetic acid; concentrated sulfuric acid, density 1.84 g/cm^3 (Ukraine, China).

To initiate the preparation for pollen analysis, a laboratory glass was utilized to weigh 20 g of honey with an accuracy of 0.01 g. Subsequently, 40 mL of distilled water was added to the measured honey. The resulting mixture was then placed in a water bath set at a temperature of 45°C. The solution was carefully heated in the water bath until the honey was completely dissolved. The resulting solution was poured into test tubes and centrifuged for 15 min at a speed of 3,000 rpm. After that, the upper layer was drained from each test tube, and 2 mL of distilled water was added to the sediment, mixed, and all solutions were poured into one test tube and centrifuged. Then the solution was drained, and 3 ml of a mixture of sulfuric acid and acetic anhydride in a ratio of 1:9 was added to the precipitate, carefully drop by drop to avoid splashing. Further, the contents of the test tube were mixed and placed in a water bath at a temperature of 80°C for two minutes. The test tube was again centrifuged for 15 min at a speed of 3,000 rpm. The sediment was washed with acetic acid (glacial), and then three times with distilled water until the vinegar smell disappeared. The resulting liquid was drained from the sediment after each washing and centrifugation for 15 min.

The sediment from the test tube was carefully poured onto filter paper to remove water. 0.1 mL of distilled water was added to the sediment, mixed, and a drop of the solution was taken and placed in a Goryaev chamber for further counting of pollen grains and determination of species composition. A suspension of pollen grains was applied to both grids of Goryaev's chamber, and a cover glass was applied so that excess liquid was removed into the grooves. At least 200 pollen grains were counted under a microscope at high magnification (X900) and their species composition was determined. The number of pollen grains of each species was calculated by the formula:

$$X = 100\ x\ a/b,$$

where *X* is the number of pollen grains of each species, %; *a* – the calculated number of pollen grains of each species, pcs; *b* – total number of counted pollen grains, pcs.

5 prototypes of sunflower and rapeseed honey were selected from each experimental group. During microscopy, the size of the pollen grain, surface features, shape, colouration, and the presence of apertures were determined. These apertures are the sites through which the pollen grains, from which the microsporangia later emerge, are released. They are characterised by the thinning of the enzyme and the formation of an intine composed of pectin cork. The latter were not clearly visualised during light microscopy. During the examination of the pollen surface, attention was paid to the presence of spikes, wrinkles, grooves, hooks, humps, etc. For better visualisation of pollen grains, the depth of field of the microscope was periodically changed. The shape of pollen grains depends on the ratio of the length of the polar axis to the equatorial diameter. Spherical, elliptical, oval, oval-oblong, triangular, hexagonal shapes of pollen grains are distinguished. During the research, pollen was distinguished by its colour as golden, yellow, dark yellow, brown, light yellow, yellow-green, grey-green. Using the gradation specified in the order on approval of requirements for honey, the botanical origin of honey was established, namely, for monofloral sunflower and rapeseed honey, the content of pollen grains of one plant species must be at least 30% (Order of the Ministry..., 2019).

An important indicator for determining the maturity and ability to store honey for a long time is the mass fraction of water. All samples with signs of crystallisation were heated in a water bath until the crystals were completely dissolved, after which a drop of honey was applied to the prism of the RL 2 refractometer (Poland). The mass fraction of the water content was found by the refractive index.

Examination of honey to establish the diastase number, hydroxymethylfurfural content, mass fraction of reducible sugars and sucrose, acidity was conducted in the Ukrainian Laboratory of Quality and Safety of Agricultural Products, Chabany, Kyiv region The sucrose content characterises the maturity of honey and is an indicator of botanical origin. An increased content may indicate fraud or insufficient maturity of honey. Determination of enzymatic activity serves as a reliable method for assessing the quality and naturalness of honey. In the countries of the European Union and according to national requirements, it is customary to characterise the enzymatic activity of honey by determining the diastase number in Goethe units. The acidity of honey was determined by the titrimetric method, and milliequivalents of sodium hydroxide solution with a concentration of 0.1 mol/dm^3 per 1 kg of honey were calculated. The content of hydroxymethylfurfural, expressed in mg per 1 kg of honey, is an indicator of the quality and safety of the product during heating.

Considering the content of pollen grains in natural honey, research was conducted on the range of genetically modified organisms in experimental samples of rapeseed honey by the polymerase chain reaction method in real-time (PRC Real-Time) on the ICycler IQ5 "Bio-Rad" amplifier (France). The research was conducted in the laboratory of molecular genetic research of the research centre for product testing of the state enterprise "Ukrmetrteststandart", which is accredited by the National Accreditation Agency of Ukraine in accordance with the requirements DSTU ISO/IEC 17025 (DSTU EN ISO..., 2019).

The grade of honey was determined in accordance with the requirements of the current national standard DSTU 4497:2005 (2005). "Natural honey. Technical specifications", the Council Directive on Honey (Council Directive 2001/110 / EC..., 2001), the criteria of the European Union (Codex Alimentarius..., 2019).

The research results were processed statistically using Microsoft Excel. Tukey test was used, which is a modified student criterion. Indices a, b indicate values that significantly differed in the same row of the table ($P < 0.05$), P – critical significance level, which is 0.05 (or 5%) and is acceptable for most biological studies.

Results and Discussion

It was established that the colour of all experimental samples of sunflower honey was amber. Bee honey shows a significant variety of aromatic shades, depending on the type of honey plants from which the nectar was collected, the conditions and shelf life, the degree of subsequent temperature treatment, and so on. The aroma of honey was pleasant and pronounced. The taste of sunflower honey is characteristic, pleasant, and has a somewhat irritating effect on the oral mucosa, there are no foreign tastes. The sweet-tart taste of honey is due to the concentration and type of sugar. The consistency of the samples in the first, second, and third groups is dense, the crystallisation is fine-grained, and the presence of crystallisation does not worsen the nutritional qualities and medicinal properties of honey. Careful studies of honey samples and their aqueous solutions did not reveal any signs of fermentation or mechanical impurities. Thus, according to organoleptic parameters, all samples of sunflower honey meet the requirements of the national standard.

The colour of rapeseed honey in the experimental samples of the first, second, and third groups was milky with a yellowish tinge. The aroma of experimental honey samples is pleasant, weak, and without foreign odours, the taste is characteristic, pleasant, somewhat irritates the oral mucosa, and no foreign flavours were detected. The consistency of samples of the first, second, and third groups of honey is viscous, crystallisation is fine-grained. During the examination of samples of rapeseed honey and their aqueous solutions, no signs of fermentation and mechanical impurities were found. Thus, according to organoleptic parameters, all prototypes of rapeseed honey meet the current requirements.

It should be noted that laboratory studies are characterised by greater objectivity in determining the honey variety compared to organoleptic parameters. The method of assessing the botanical origin of honey based on the analysis of pollen grains is essential and helps to increase the economic efficiency of the production of this product (Muhati & Warui, 2022) since monofloral honey is more valued in the world market due to its good quality and original sensory characteristics (Manolova *et al.* , 2021). In addition, the content of pollen grains serves as one of the additional methods for establishing food fraud (Pospiech *et al.,* 2021).

Natural honey must contain flower pollen, the species and quantitative composition of which depends on the species ratio of honey plants. Bee pollen contains more than 250 biologically active substances, such as amino acids, carbohydrates, lipids, enzymes and coenzymes, vitamins, and also has antioxidant activity, which is explained by the presence of phenolic acids and flavonoids (Tutun *et al.,* 2021). The largest proportion of pollen found in honey comes from the main nectar sources. Among agricultural crops, sunflower, rapeseed, buckwheat, melons, etc. are distinguished. Honey is considered monofloral in terms of a certain pollen content from one plant species, but up to 10-20 types of pollen from other plants are found in natural honey during research. The absence of pollen clearly indicates the presence of fraud. The presence of characteristic structural elements of the aperture – furrows, pores, various shapes, colour, and size, allow determining the botanical affiliation of pollen grains (Pospiech *et al.,* 2021).

According to the appearance of pollen grains contained in experimental samples of

honey, its botanical origin was established. Specially developed bases of pollen grains of plants are used to identify bee products in international practice. The territory of Ukraine is characterized by the presence of a variety of vegetation of the Carpathians, steppes, forest-steppe and Polissia, which complicates the evaluation of honey, especially in the absence of a Ukrainian pollen grain base. Moreover, products obtained from different natural and climatic zones are unique in terms of the content of minerals, vitamins, amino acids, and other useful components. The examination of these properties of honey, bee bread, and bee pollen, including their botanical origin, will create a domestic informative base.

A microscopic view of the pollen grains contained in the honey samples is shown in Fig. 1.

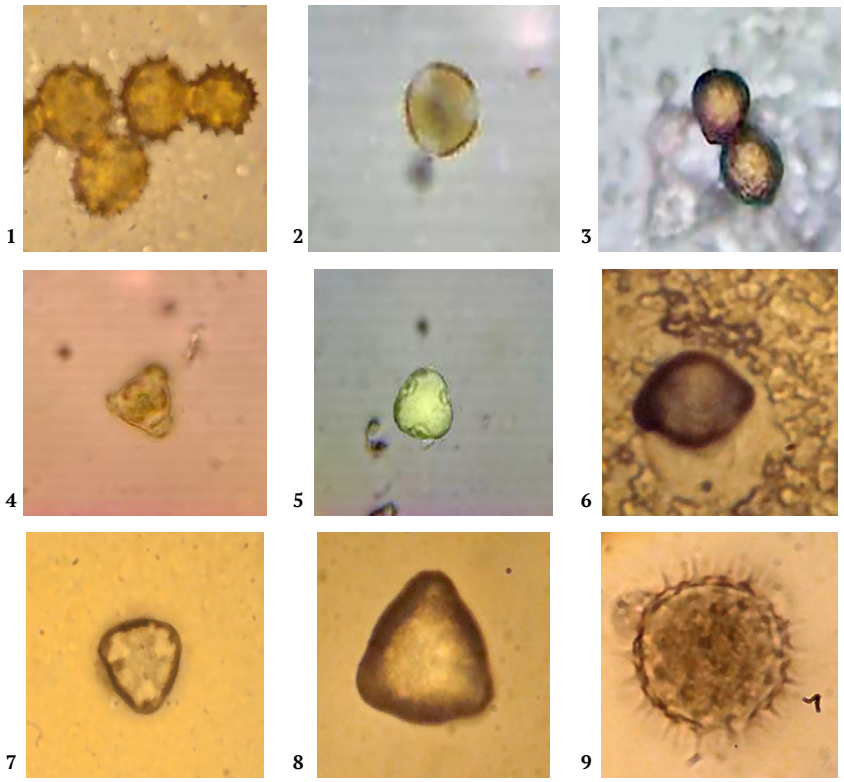

Figure 1. Morphology of pollen grains of various plant species in honey samples

Note: *1 – Common sunflower (Helianthu sannuus); 2 – Rapeseed (Brassica napus); 3 – Buckwheat (Fagopyrum esculentum); 4 – Black locust (Robinia pseudoacacia); 5 – Little-leaf linden (Tilia cordata); 6 – Red clover (Trifolium pretense); 7 – Sour cherry (Cerasus vulgaris); 8 – Apple (Malus domestica); 9 – Field sow thistle (Sonchus arvensis)*

Sunflower pollen was found in common sunflower honey (Fig. 1.1). The pollen grain is medium-sized, spherical in shape, has a golden colour and a spiky surface. The percentage of sunflower pollen grains in it averaged 52% (Table 1).

Rapeseed honey was found to contain mainly rapeseed pollen (Fig. 1.2) – pollen grain of medium size, yellow colour, rounded shape, with a reticulated surface. The percentage of rapeseed pollen grains in the product averaged 68% (Table 2).

Table 1. Pollen grain content in sunflower honey (%), x ± SD, n = 5

Plant type	Experimental group 1, Kyiv region, Ukraine	Experimental group 2, Vinnytsya region	Experimental group 3, Odesa region
Common sunflower	51.6 ± 4.9	49.2 ± 6.5	55.6 ± 42
Buckwheat	17.0 ± 3.0	19.4 ± 2.3	17.6 ± 26
Field sow thistle	11.0 ± 4.1	9.4 ± 5.5	10.4 ± 4.0
Red clover	11.2 ± 3.1	9.2 ± 3.9	8.8 ± 4.3
Cornflower	3.6 ± 1.8	4.6 ± 2.1	3.4 ± 1.5
Little-leaf linden	2.4 ± 1.5	4.8 ± 1.6	2.4 ± 1.7
Other types of plants	3.2 ± 1.8	3.4 ± 1.7	1.8 ± 0.9

Table 2. Pollen grain content in rapeseed honey (%), x ± SD, n = 5

Plant type	Experimental group 1, Kyiv region, Ukraine	Experimental group 2, Vinnytsya region	Experimental group 3, Odesa region
Rapeseed	66.4 ± 4.8	68.2 ± 3.6	68.8 ± 5.4
Black locust	12.4 ± 3.7	11.2 ± 2.5	11.8 ± 3.4
Sour cherry	7.6 ± 3.8	9.8 ± 2.8	7.4 ± 4.1
Apple	10.8 ± 4.3	8.2 ± 3.0	9.8 ± 3.8
Other types of plants	2.8 ± 1.5	2.6 ± 1.8	2.2 ± 1.3

During microscopy of experimental samples of honey, pollen grains of other plant species were found that differed in morphological characteristics, namely: buckwheat, black locust (Robinia vulgaris), field sow thistle, red clover, sour cherry, apple, blue cornflower, linden, etc. Pollen grains of buckwheat were found (Fig. 1.3), which are characterised by an average size, rounded shape, dark yellow colour, and a reticulated surface. Black locust (Fig. 1.4) is characterised by a triangular pollen grain shape, yellow colour, medium size, and fine-spotted surface. Little-leaf linden pollen (Fig. 1.5) was identified by the triangular-flattened shape of the pollen grain, which has a yellow-green colour, medium size, and fine-speckled surface. The pollen grain of red clover (Fig. 1.6) is ellipsoid, brown in colour, medium-sized, with a fine-speckled surface. In sour cherries (Fig. 1.7), the pollen grain is triangular in shape, medium in size, light yellow in colour, and has a wavy ornament on the surface. For the apple (Fig. 1.8), the triangular shape of a pollen grain, yellow colour, small-speckled surface, and medium size are characteristic. The pollen grain of field sow thistle (Fig. 1.9) is characterised by a porous surface with long spikes, rounded shape, dark yellow colour, and medium size. Pollen analysis of honey gave a positive result: pollen grains were found in all experimental samples, which is a sign of its naturalness.

An important indicator of the quality of natural honey is its water content. According to the Council Directive on honey (Council Directive 2001/110/EC..., 2001), the water content of honey should be no more than 20% (the exception is heather honey). This is an important physical and chemical parameter for the shelf life of honey. Usually, the water content in honey ranges from 13 to 25%, while the average value is about 17%. According to scientific data, the water content in mature honey ranges from 14 to 21% and is one of the essential quality indicators that affects not only product storage, but also changes in its constituent components (Almeida-Muradian *et al.,* 2020). An increase

in humidity can be observed in unripe honey, when adulterated with water or when liquid sugar syrup is added. In addition, such honey has a higher probability of fermentation during storage (Almeida-Muradian *et al.,* 2020). It should be noted that the water content may change during long-term storage, which is associated with chemical and enzymatic processes that occur immediately after pumping honey from the honeycomb. The results of studies of the physical and chemical parameters of sunflower honey are presented in Table 3.

Table 3. Physical and chemical parameters of sunflower honey, x ± SD, n = 10

Plant type	Experimental group 1, Kyiv region, Ukraine	Experimental group 2, Vinnytsya region	Experimental group 3, Odesa region
Mass fraction of water, %	18.08 ± 0.49	18.32 ± 0.46	17.91 ± 0.38
Mass fraction of reducing sugars, %	86.95 ± 1.89	85.92 ± 2.03	84.19 ± 2.48
Mass fraction ofsucrose, %	1.55 ± 0.82	1.32 ± 0.64	1.86 ± 0.95
Diastase number, Goethe units	18.90 ± 3.03	21.54 ± 3.80	20.10 ± 3.65
Content of hydroxymethylfurfural (HMF), mg per 1 kg	6.29 ± 1.72	7.04 ± 1.00	5.59 ± 1.09
Acidity, milliequivalents of sodium hydroxide per 1 kg	15.40 ± 2.29	14.41 ± 2.54	13.59 ± 1.77

The composition of the dry residue of honey includes about 300 substances and ash elements. The main ones are carbohydrates, which make up 95-99% of dry matter, among which monosaccharides – glucose and fructose-predominate. Honey also contains 11 disaccharides and 12 polysaccharides. The content of carbohydrates varies widely, depending on the botanical origin of honey, the conditions of storage and processing of nectar by bees. Despite the variety of carbohydrates contained in honey, the content of glucose, fructose, and sucrose in the product is now used for its examination. A mixture of grape and fruit sugars, namely glucose and fructose, is commonly called inverted sugar since it is obtained from nectar as a result of the breakdown of sucrose in the bee's honey chamber and in the honeycombs under the action of the invertase enzyme. Accordingly, the higher the content of inverted sugars in honey, the better its quality.

The results of the research show that the average mass fraction of water in honey collected in the Kyiv, Vinnytsia, and Odesa regions was less than 18.5%, which meets the requirements for top-grade honey.

The percentage content of reducing sugars in the honey of all experimental groups was more than 80%, which also meets the requirements for a higher grade. Honey adulterated with saccharin, glycerin, starch, gelatin or sucrose, just like a product obtained from bees that were fed sugar syrup, contains less inverted sugars. According to the national standard, the mass fraction of sucrose in flower honey normally ranges from 1-6%. The specified value is obtained during the ripening of honey, due to the work of the enzyme invertase, which breaks down sucrose to simple sugars. After pumping honey for several months at room temperature, this process continues. According to Codex Alimentarius and the Council of the European

Union, sucrose concentrations should not exceed 5 g per 100 g of honey. If the sucrose content in honey is more than 5%, this indicates its immaturity or fraud (Aykas, 2023). According to the laboratory analysis of sunflower honey samples, the mass fraction of sucrose was not more than 3.5%, which meets the requirements of the national standard for a top-grade product.

It should be noted that enzymes are biological catalysts that accelerate decomposition and synthesis reactions. Natural honey includes the following active enzymes: alpha- and beta-amylase, glucose oxidase, diastase, catalase, invertase, phosphatase, peroxidase, etc. (Puscion-Jakubik *et al.,* 2020). The amount and composition of honey enzymes depend on its botanical and regional origin, the period of nectar collection, natural and climatic conditions, primary processing, and so on. One of the most valuable and studied is the enzyme diastase, which breaks down starch into amylodextrins (glucose and maltose). The activity of this enzyme is expressed in Goethe units. It was found that heating honey to a temperature above 40°C causes partial or complete inactivation of enzymes and heating to 60°C causes their destruction (Adamchuk *et al.,* 2019). Diastase activity is also an indicator of the naturalness of honey and, according to the requirements of the national standard, should be at least 10 Goethe units for first-grade honey and 15 units for premium honey.

According to the results of studies of the enzymatic activity of diastase, presented in Table 3, prototypes of sunflower honey meet the requirements of the top grade, which indicates the proper quality and naturalness of the product.

Current DSTU 4497: 2005 "Natural honey. Technical specifications" regulates the maximum hydroxymethylfurfural content for the top-grade honey – 10 mg/kg, and for the honey of the first grade – 25 mg/kg. Notably, hydroxymethylfurfural can be formed in honey in case of violation of storage conditions, when exposed to external high temperatures, during heating. The level of toxicity of hydroxymethylfurfural for humans has not been precisely determined, as it depends on the state of health. According to scientific data, an adult can take from 30 to 150 mg of hydroxymethylfurfural daily from various food sources and it is reported that the concentration of hydroxymethylfurfural in the human body decreases to zero within 24-48 hours. Even if one consumes up to 240 mg of hydroxymethylfurfural per day (Alaerjani *et al.,* 2022). Based on the results of the conducted studies, it was established that all samples of sunflower honey had a low content of hydroxymethylfurfural, which meets the requirements of the national standard, confirms the quality of this product, the absence of exposure to high temperatures on honey, and indicates the safety for human use.

The acidity of honey depends on the type of raw material, the season of its production, and the degree of its maturity. Honey contains organic (malic, lactic, citric, formic, tartaric, acetic, oxalic, etc.) acids that have an acidic reaction which prevents the development of microorganisms and extends the shelf life of the product (Dobrinas *et al.,*, 2022). According to the current requirements for the physical and chemical parameters of natural honey, the acidity of top-grade honey should not exceed 40 meq/kg, and for the honey of the first grade – no more than 50 meq/kg. It was established that all samples of sunflower honey selected from the Kyiv, Vinnytsia, and Odesa regions met the requirements of the current DSTU for a top-grade product. In addition, an assessment of the product's compliance with the criteria of the European Union (Codex Alimentarius..., 2019) was performed according to the main physico-chemical indicators, and it is necessary to note that all experimental samples of honey meet the requirements of

European food legislation, which indicates the competitiveness of Ukrainian sunflower honey.

The main physical and chemical indicators were determined when studying samples of rapeseed honey collected in the Kyiv, Vinnytsia, and Odesa regions. It was established that the average value of the mass fraction of water in honey collected in the Odesa region was 17.73%. This corresponds to the indicators of top-grade honey. The mass fraction of water in the experimental samples of rapeseed honey of the Odesa region substantially differed from the indicators of honey obtained from the Kyiv and Vinnytsia regions, which is probably due to the natural and climatic conditions of storage at a higher average daily temperature. Rapeseed honey collected in the Kyiv and Vinnytsia regions showed an average mass fraction of water of more than 19.0%, which meets the requirements for a first-grade product and is 1.3% higher compared to honey obtained from the Odesa region (Table 4).

Table 4. Physical and chemical parameters of rapeseed honey, x ± SD, n = 10

Plant type	Experimental group 1, Kyiv region, Ukraine	Experimental group 2, Vinnytsya region	Experimental group 3, Odesa region
Mass fraction of water, %	19.50 ± 1.22[a]	19.00 ± 0.41[a]	17.73 ± 0.21[b]
Mass fraction of reducing sugars, %	89.20 ± 1.31	89.18 ± 0.84	91.07 ± 2.57
Mass fraction of sucrose, %	3.21 ± 0.19[a]	1.50 ± 0.24[b]	1.66 ± 0.90[b]
Diastase number, Goethe units	11.65 ± 1.18	12.12 ± 1.57	11.10 ± 1.42
Content of hydroxymethylfurfural (HMF), mg per 1 kg	1.74 ± 1.26	2.61 ± 1.53	1.59 ± 0.67
Acidity, milliequivalents of sodium hydroxide per 1 kg	13.6 ± 1.14	12.69 ± 0.32	13.5 ± 0.41

Note: *different superscript letters [a, b] indicate the values that considerably differed in the same row of the table (P < 0.05) based on the results of comparison using the Tukey test*

Honey with a high mass fraction of water can quickly deteriorate, so it is not subject to long-term storage.

The percentage of reducing sugars in all honey samples was at a high level, which confirms the quality of the product and meets the requirements of the top grade. According to laboratory analysis, the mass fraction of sucrose in experimental samples of rapeseed honey was below 3.5%, which indicates the maturity of honey. In the first experimental group, rapeseed honey from the Kyiv region contained 3.21% sucrose, which is on average 2 times higher than its content in the product from the Vinnytsia and Odesa regions. The result obtained may be due to the fact that honey has not fully passed the fermentation process provided by invertase. However, this type of honey meets the requirements: the maximum permissible sucrose content, according to the national standard, is 3.5% – for top-grade honey, 6% – for honey of the first grade.

The enzymatic activity of honey, which was determined by diastase activity, ranged from 10 to 14 Goethe units. The amount of hydroxymethylfurfural per 1 kg of honey was less than 10 mg, which indicates compliance with the storage conditions of the product and the absence of exposure to external high temperatures or heating.

It was found that the acidity of all samples of natural rapeseed honey selected from the Vinnytsia, Odesa, and Kyiv regions met the requirements for top-grade honey according to the current national standard. According to other physical and chemical indicators, namely, the mass fraction of water in the product samples of the Kyiv and Vinnytsia regions, rapeseed honey met the requirements of the national standard for first-grade honey. Honey collected in the Odesa region met the requirements for a top-grade product. The mass fraction of water, respectively, was below 18.5%, which is probably due to the weather conditions of the area and the storage of the product. Considerable differences in physical and chemical indicators of honey quality in national and international requirements include the following values: the content of reducing sugars according to the national standard is 10-20% higher than required by international legislation; the content of sucrose according to the requirements of DSTU 4497:2005 is 1.5% lower compared to Codex Alimentarius and Honey Directive 2001/110/EC (Council Directive..., 2001). The permissible content of hydroxymethylfurfural according to the national standard is up to 25 mg/kg, and in the EU and WTO countries (World Trade Organisation) – should not exceed 15 mg/kg. The content of the mass fraction of water must not exceed 20% according to the Honey Directive 2001/110/EC. Thus, rapeseed honey meets the requirements of Ukrainian and European food legislation.

In accordance with the standards of the European Union, quality and safety control of honey includes the establishment of organoleptic and basic physical and chemical parameters. In addition, the maximum permissible levels of residues of antibiotics, sulfonamide drugs, pesticides, heavy metals, radionuclides, and the content of pollen of genetically modified organisms are set.

Many countries have introduced state regulations on the use of genetically modified organisms and appropriate labelling of products made from raw materials of biotechnological origin. The following countries have partially or completely abandoned the use of genetically modified products: Austria, Germany, France, Great Britain, Spain, and others (Seifert, 2021). Rapeseed is one of the most common biotechnological crops. Therefore, it is important to conduct research on the presence of genetically modified rapeseed DNA in honey. It should be noted that seeds of both domestic varieties and hybrid seeds of this crop imported from abroad are used for sowing in the fields of Ukraine. The state register of plant varieties suitable for distribution in Ukraine contains 466 registered rapeseed hybrids and parental forms. Regarding the use of genetically modified rapeseed, the information is not officially confirmed (Shebanin *et al.,* 2021).

After testing prototypes of rapeseed honey from the Vinnytsia, Odesa, and Kyiv regions, it was found that there were no target sequences of the 35s promoter of the cauliflower mosaic virus *(CaMV)* and *NOS* (nopaline synthase) from *Agrobacterium tumefacient*. Thus, no genetically modified organisms were found in the experimental samples of rapeseed honey.

During organoleptic and physico-chemical examinations of sunflower and rapeseed honey, their compliance with the current national regulations was established, and the data obtained were compared with the criteria of the European Union and the requirements of Codex Alimentarius. In particular, discrepancies were found regarding the content of reducing sugars and sucrose, the permissible level of hydroxymethylfurfural, water content and, considering the regulations in different countries regarding the use of raw materials of biotechnological origin, rapeseed honey was examined for the content of genetically modified pollen.

Conclusions

The organoleptic studies and the results of pollen analysis confirmed the botanical origin of natural sunflower and rapeseed honey obtained from different regions of Ukraine: Kyiv, Vinnytsia, and Odesa. According to pollen analysis, prototypes of the product belong to monofloral honey – the content of dominant pollen was 52% in sunflower honey, 68% – in rapeseed. Sunflower honey meets the main physical and chemical national requirements for top-grade honey. The water content of sunflower honey averaged 18.1%, which meets the requirements of both the national standard and the requirements of the EU Council Directive relating to honey and Codex Alimentarius. The mass fraction of sucrose was not more than 3.5%, which corresponds to the top grade and meets the requirements of the national standard, Codex Alimentarius, and EU Council Directives (no higher than 5%). The average diastase number was 20.2 Goethe units, which considerably exceeds the requirements of Codex Alimentarius and the EU Council Directive (no higher than 8 Goethe units). Regarding the content of hydroxymethylfurfural, Codex Alimentarius and the EU Council Directive regulate no more than 40 mg/kg, which is notably higher than the obtained indicators, averaging 6.3 mg/kg. Therefore, according to the main indicators, sunflower honey meets not only national, but also international and European requirements for the quality and safety of the product, so it is in demand in many foreign countries. Rapeseed honey collected in the Kyiv and Vinnytsia regions, according to the requirements of the national standard and considering the water content in it, meets the requirements for a first-grade product, and honey from the Odesa region – top grade. Rapeseed honey produced in Ukraine also meets the main European and Codex Alimentarius requirements both in terms of organoleptic parameters and basic physical and chemical characteristics. Considering some differences in the regulations of the European Union and Codex Alimentarius on the quality and safety of natural honey, in particular, the moisture content and free acidity, and in the national standard – the water content and active acidity, it is necessary to harmonise methods for these indicators. Additionally, an examination of rapeseed honey for the presence of biotechnological pollen was conducted and the following conclusion was obtained: experimental samples of rapeseed honey do not contain genetically modified DNA. Rapeseed honey collected in the Kyiv, Vinnytsia, and Odesa regions has sufficient export potential. Comprehensive studies of sunflower and rapeseed honey have confirmed its naturalness and the absence of fraud in the product. Further studies should focus on pollen analysis and physico-chemical examination of flower and linden honey.

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None.

Conflict of Interest

None.

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Показники безпечності та якості ріпакового і соняшникового меду з різних областей України

Роман Колач

Доктор ветеринарних наук, професор
Університет Миколи Коперника,
87-100, вул. Гагаріна, 7, м. Торунь, Польща,
https://orcid.org/0000-0001-6439-7770

Ольга Миколаївна Якубчак

Доктор ветеринарних наук, професор
Національний університет біоресурсів і природокористування України,
03041, вул. Героїв Оборони, 15, м. Київ, Україна
https://orcid.org/0000-0002-9390-6578

Тетяна Володимирівна Таран

Кандидат ветеринарних наук, доцент
Національний університет біоресурсів і природокористування України,
03041, вул. Героїв Оборони, 15, м. Київ, Україна
https://orcid.org/0000-0002-9370-8539

Юлія Володимирівна Гриб

Кандидат ветеринарних наук
Національний університет біоресурсів і природокористування України,
03041, вул. Героїв Оборони, 15, м. Київ, Україна
https://orcid.org/0000-0003-0913-6927

Анотація. Актуальність дослідження зумовлена зростанням попиту на безпечні та якісні продукти бджільництва, насамперед меду, та недопущенням на вітчизняний та ринки Європейського Союзу неякісних продуктів, які можуть завдати шкоди здоров'ю споживачів. В Україні найбільшим попитом в експортному потенціалі користується соняшниковий та ріпаковий мед. Метою досліджень було визначення відповідності ріпакового та соняшникового меду, отриманого в Україні, вимогам національного та європейського харчового законодавства. Для вирішення поставленої мети були використані органолептичні методи дослідження меду натурального та проведено визначення діастазного числа, кислотності, вмісту пилкових зерен, масової частки води, масової частки відновлювальних цукрів і сахарози та гідроксиметилфурфуролу. Методом полімеразної ланцюгової реакції в реальному часі здійснено аналіз ріпакового меду на наявність генетично модифікованого пилку. Встановлено, що дослідні зразки соняшникового меду з Вінницької, Одеської та Київської областей відповідали вимогам національного стандарту та європейського харчового законодавства, без ознак фальсифікації. Нині підлягає регулюванню використання генетично модифікованих організмів. Зразки меду ріпакового, відібрані з Вінницької, Одеської та Київської областей, не містили генетично модифікованої дезоксирибонуклеїнової кислоти. За фізико-хімічними показниками

ріпаковий мед відповідав вимогам національного стандарту. Встановлено, що мед, зібраний в Київській та Вінницькій областях мав середній показник вмісту води – вище 18,5%, що відповідає вимогам до меду першого ґатунку згідно з національним стандартом. Мед, зібраний в Одеській області, відповідав показникам меду вищого ґатунку, вміст води в ньому в середньому становив 17,7%. За основними фізико-хімічними показниками ріпаковий мед, зібраний в Україні, також відповідає вимогам європейського харчового законодавства. Також ріпаковий і соняшниковий мед із Вінницької, Одеської та Київської областей є натуральними і можуть реалізовуватися не лише в Україні, а й на території Європейського Союзу та Світової Організації Торгівлі

Ключові слова: квітковий пилок; контроль; нормативно-правові акти; фальсифікація; генетично модифіковані організми