UDC: 636.165.8

IMPROVEMENT OF THE METHOD OF BOTANICAL IDENTIFICATION OF HONEY

DOI: https://doi.org/10.15673/fst.v14i4.1895

L. Adamchuk, PhD, Associate Professor
Department of Standardization and Certification
of Agricultural Products
National University of Life and Environmental Sciences of Ukraine
Heroiv Oborony Str.15, b. 3, Kyiv, Ukraine, 03041

Article history

Received 26.05.2020
Reviewed 22.06.2020
Revised 23.09.2020
Approved 08.12.2020

Correspondence:

L.O. Adamchuk
E-mail: leonora.adamchuk@gmail.com

Cite as Vancuver style citation

Adamchuk LO. Improvement of the method of botanical identification of honey. Food science and technology. 2020;14(4):31-42. DOI: https://doi.org/10.15673/fst.v14i4.1895

Цитування згідно ДСТУ 8302:2015

Adamchuk L.O. Improvement of the method of botanical identification of honey // Food science and technology. 2020. Vol. 14, Issue 4. P. 31-42 DOI: https://doi.org/10.15673/fst.v14i4.1895

Copyright © 2015 by author and the journal "Food Science and Technology".
This work is licensed under the Creative Commons Attribution International License (CC BY).
http://creativecommons.org/licenses/by/4.0

Abstract. The pollen spectrum of honey reflects the regional crops, forest plantations, all the diversity of the plant species near the apiary. That is why the use, adaptation, and improvement of various methods of pollen analysis and interpretation of its results are topical questions in the countries which produce original varieties of honey on the international market. Ukraine is a leader in manufacturing and exporting honey in Europe and worldwide. Usually, Ukrainian honey is exported not as a finished food product, but as a raw material. This is because the pollen spectrum of Ukrainian honeys is provided with no description characterising their botanical and geographical origin, which, in turn, is because there are no pollen analysis methods implemented in production laboratories. The purpose of this study was improving the pollen analysis method to identify the botanical origin of Ukrainian honeys. The research was conducted in the laboratory of the Department of Standardisation and Certification of Agricultural Products in the National University of Life and Environmental Science of Ukraine, and in the production laboratory of Ascania-Pack Company. The analysis of harmonised methods of melissopalynology and standardised pollen detection methods has allowed developing a botanical method of honey identification adapted to be introduced in Ukrainian laboratories. The method suggested is a complex of individual procedures, operations, and techniques. It has been detailed how to prepare solution to fix and stain pollen preparations obtained from honey. The method of preparing a suspension of pollen grains from honey has been improved: extra dissolution of crystals and a lower speed of the centrifuge reduce the time of making the preparation and improve its quality. The method of obtaining preparations of pollen grains from honey has been adapted to harmonised international melissopalynological methods. New methods have been developed to count pollen grains in prepared monofloral and polyfloral honey samples. It has been detailed how to identify step-by-step the botanical origin of honey by pollen grains using international databases. The method has been implemented in the production laboratories of leading enterprises in Ukraine and Belarus.

Key words: pollen analysis method, pollen grain, monofloral honey, polyfloral honey, botanical identification, melissiopalynology.

Introduction. Formulation of the problem

Ukraine is a leader in manufacturing and exporting honey in Europe and worldwide. Usually, honey is exported not as a finished food product, but as a raw material. First of all, this is because the pollen spectrum of our honeys is provided with no description characterising their botanical and geographical origin. As far as export to EU countries is concerned, the current regulatory and technical documentation lacks characteristics for the types of monofloral Ukrainian honey. That is why they are sold as raw honey at a low purchase price, since there are no practically implemented methods of botanical identification of honey. Different countries use harmonised melissopalynological methods adapted to the technological possibilities of their own laboratories. So, it is necessary to take into account the regional differences in the percentage of dominant grains in monofloral honey varieties and different quantities of gametophytes of anemophilous plants. Improving the method of botanical identification of Ukrainian honeys and applying it in laboratory research will expand scientific knowledge in this field and have an economic effect on the product's export potential.

Analysis of recent research and publications

Pollen grains of plants are always present in raw honey produced by the commonly used technology. The pollen spectrum of honey reflects the regional

crops, forest plantations, all the diversity of the plant species near the apiary [1]. That is why the use, adaptation, and improvement of various methods of pollen analysis and interpretation of its results are topical questions in the countries which produce original varieties of honey on the international market.

Egypt exports honey made from Brazilian peppertree and cotton blossom honey. In 2016–2017, significant nonconformities were revealed during research of the pollen spectrum to confirm the botanical origin of Egyptian honey varieties and label them. Thus, 69% of cotton blossom honey (*Gossypium hirsutum* L.) was contaminated with pollen from maize, an anemophilous plant (*Zea mays* L.). 79% of pollen of melons (*Cucurbitaceae*) was found in sunflower honey, and 52% of eucalyptus pollen (*Eucalyptus* spp.) in honey from Brazilian peppertree (*Schinus terebinthifolia Raddi*). According to the results obtained, the authors are convinced that these honey varieties should be renamed maize, pumpkin, and eucalyptus honey. Scientists also point out that pollen grains of *Echium* spp. and *Trifolium alexandrinum* L. can be markers of geographical origin, as they are present in most of the honeys examined and are widespread in Egypt [2].

A. Mannapov, O. Legochkin, and A. Skachko adapted the pollen coefficient and Sawyer's method of botanical identification so that they could be used for honey produced in the Russian Federation. The method suggested consisted in comparing the botanical composition of honey obtained from the control hive and the data from Sawyer's pollen coefficient [3]. Besides, in the Russian Federation, for industrial purposes, there is an atlas of pollen grains created and used to identify them in honey [4].

V. Ivanova's works [5,6] present the results of comparative pollen analysis applied to white honey made in the Chuvash Republic and Bashkortostan. The nectar coefficient of the honey samples researched indicated the predominance of the lime tree, but the content of its pollen grains was less than 45%. This means that there are not enough pollen grains of this species in monofloral honeys. That is why for some honey varieties (lime, acacia), European Council Directive 2001/110 only allows a 20% content of pollen grains, which indicates that these varieties are monofloral. However, the author [5,6] concludes that these honeys cannot be classified as monofloral lime honey from the above-noted regions, but only as polyfloral. Today, in Bashkortostan, Kumanov's atlas of pollen grains is used [7].

In Turkey, pollen analysis of honey was used to study honey from different regions in Beypazarı, a district of Ankara Province. Nine samples of honey were established to be monofloral, others were classed as polyfloral. This method allowed identifying the pollen spectrum of 24 taxa, including 11 families and 13 genera of plants. The genus Astragalus (*Astragalus* spp.) was the predominant one. So, plants of this genus were recognised as the main source of nectar and pollen for honey in Turkey [8]. For the botanical identification of Turkish honeys, an atlas of honey plants was developed, indicating the geographical distribution of plants in different regions [9].

The pollen spectrum of Argentinian honeys was determined by the contents of honey sacs (organs in which bees transfer nectar to honeycomb for processing into honey) of honey bees (*Apis mellifera*). The polyflora used by bees in the western part of the Yungas sector in Jujuy (Argentina) was studied during the spring and summer of two consecutive production periods. A total of 46 pollen species belonging to 25 botanical taxa were identified. The predominant families, %: Euphorbiaceae (35.54), Fabaceae (26.27), Asteraceae (20.77), Vitaceae (16.14), and Myrtaceae (9.13). Less common were pollen grains of plants of the genera Eucalyptus, Eupatorium, Mimosa, Parapiptadenia excelsa, Sebastiania, Viguiera, Zanthoxylum, Cissus, and members of the families *Cactaceae* and *Euphorbiaceae* [10].

Brazil mainly exports eucalyptus honey. Using pollen analysis, the pollen spectrum was determined for honeys from the northwest of the state of Minas Gerais (Brazil). The pollen grains most common in the product obtained during the rainy season were of *Trema micrantha*, *Copaifera langsdorffii*, *Poaceae*, *Asteraceae*-2, *Cecropia* spp., and *Eupatorium* spp. In the honey obtained during the dry season, the most common were grains of *Acosmium dasycarpum*, *Cecropia* spp., and *Eupatorium* spp. Pollen grains of plants of the genus *Baccharis* spp. were detected in honey samples throughout all the research period, which may be indicative of the geographical origin of honey [1,11]. Along with that, in the state of Paraná (Brazil), studies of the pollen spectrum of honey revealed that it contained pollen of plants of the genera *Casearia*, *Eucalyptus*, *Galactia*, *Miconia*, *Morus*, *Myrcia*, *Syagrus*, and members of the family *Rubiaceae*. The pollen spectrum of the honey analysed was diverse. The predominant pollen was that of the families *Fabaceae*, *Asteraceae*, and *Myrtaceae*. They are considered the most effective honey plants for western honey bees (*Apis mellifera*) in this area [12].

Another group of studies on the topic considers botanical identification methods. Palynological analysis by means of light microscopy is the most used method to identify pollen in honey. Its advantages include the reliability of estimating the relative sizes and morphology of pollen grains of different taxa in the general pollen spectrum of honey. However, this method has several disadvantages: it is laborious, requires a lot of expertise and a database of standard samples (templates) of pollen grains from different regions. These disadvantages result in low taxonomic resolution for many plant taxa, which allows them to be identified only at the family level [13].

This leads to the search for new methods. They seldom become widely used, though. An example is

molecular detection of the botanical origin of pollen in bees' pollen load. This method is intended for studying the diversity of plant species visited by honey bees and, indirectly, the botanical origin of honey and its geographical traceability [14]. However, in our opinion, this method will prove ineffective in Ukraine, due to a lot of entomophilous pollen-bearing plants that do not produce nectar. From them, bees only collect pollen load, not honey, so the pollen spectrum of these products is significantly different.

In scientific laboratories, DNA identification methods are researched. However, they are not widely used in laboratories at enterprises. As a promising effective alternative to microscopic analysis, the scientists [15-17] suggest DNA metabarcoding using next-generation sequencing (NGS). Other authors [18] support the DNA barcoding method using the traditional Sanger sequencing. Nevertheless, this method is ineffective for identifying the mixed spectrum of pollen, as is most common in honeys. The DNA barcoding method requires isolating and sequencing individual pollen grains from mixtures or using methods of cloning, which is laborious and fraught with possible errors [19-20]. According to the results of Consortium for the Barcode of Life (CBOL) [21], an ideal DNA barcode should be regularly renovated with a pair of primers, provide bidirectional sequencing, and allow distinguishing most species. Among the different loci of the chloroplast genome, a group with rbcL, matK, rpoB, rpoC1, atpF – atpH, trnH-psb, and psbK – psbI, were suggested to evaluate plant products [22]. On the basis of the criteria of universality, quality of sequencing, and levels of species discrimination, each individual locus trnH – psbA, rbcL, and matK must correspomd to the plant DNA barcoding system, although none of them corresponds to the ideal DNA barcode marker [19]. That is why many researchers search for a universal barcode suitable to identify plant species, including other parts of the genome of non-chloroplasts (nuclear sites) [23]. However, at present, there are technical difficulties with these technologies, though they are improved regularly. The common ones include searching for methods of DNA extraction of homogenised mixed pollen samples (for example, NucleoSpin, GF-1 Plant, HigherPurity, and CTAB-PVP) [22]. These methods have become more widely used in analysing air mixtures of pollen for the study of allergens.

So, research by different groups of scientists aimed at identifying pollen grains of plants includes evaluation of the interaction between a plant and its polliniser, considers the pollen spectrum of honey for its botanical identification, monitors pollen sources in a particular region.

In Ukraine, botanical identification of honey is performed by the method of pollen analysis, which is described in paragraph 10.3 of DSTU 4497:2005 "Natural honey. Specification requirements". This method involves the use of acetic anhydride, acetic glacial acid, concentrated sulphuric acid, and fourfold centrifugation during sample preparation, which significantly complicates it. Determining the species composition of pollen grains involves counting them on the grid of a haemocytometer, and not in the microscope field of view. This increases the counting area and causes its error, because pollen grains smaller than 25 μm get into the grooves of the haemocytometer together with excessive liquid. Besides, the standardised method allows botanical determination of honey by counting not less than 200 grains, which contradicts the international harmonised method. Consequently, honey exporters have to re-analyse honey in foreign laboratories. Therefore, there is a need to simplify the method of pollen analysis and harmonise it with international ones.

Harmonised methods of melissopalynology [24] include initially estimating the proportional frequency of types of pollen grains, then identifying them, with their re-counting of 300, 500, and up to 1000. Further, the botanical and geographical origin of honey is determined. This method involves quantitative and qualitative determination, and interpretation of the results depends on the pollen spectrum of the dominant species in a particular region. Therefore, leading European laboratories have adapted these methods for their market segment. Thus, there is a need to adapt harmonised methods of melissopalynology to the study of the pollen spectrum of Ukrainian honeys.

The purpose of the work is to improve the method of pollen analysis to identify the botanical origin of Ukrainian honeys. To achieve this purpose, the **objectives** were set:

– to analyse theoretically and test practically harmonised methods of melissopalynology [24] and the standardised method according to DSTU 4497: 2005 "Natural honey. Specification requirements";

– to develop a method of botanical identification of honey adapted for practical implementation in the conditions of Ukrainian laboratories;

– to implement practically the improved method of pollen analysis for identification of the botanical origin of Ukrainian honeys in a production laboratory.

Research materials and methods

The materials used to test the methods were purchased from the company Khimlaborreaktyv (Ukraine). These were: a laboratory funnel Labexpert B-25-38 TC, a graduated low-form beaker Labexpert H-1-100 TC (GOST 25336-82); a measuring cylinder 1-50-2 Labexpert (GOST 1770-74); ashless tape filters (d=90mm, EKSIMKARGOTRADE, Ukraine): glass rods (L=220mm, d=5mm); a microscope slide SP-7101 with polished edges; a cover glass (24x24mm). The following reagents were purchased from Khimlaborreaktyv (Ukraine) and used in the work: glycerine pharm. p.a. (import, Germany); basic fuchsine for MBP (microbiological purposes) p.a.; edible gelatine bloom 200 (import, Germany) puriss.; phenol p.a (Lachema, Czech Republic).

The samples were prepared in the laboratory of Ascania-Pack Company. Its technical competence is certified according to the requirements of DSTU ISO/IEC 17025:2006. The equipment used to prepare the samples was: laboratory scales Scout SPX 223 (China); a laboratory centrifuge AFI-C300R-E for 50 ml test tubes (France); a multi-sample heated bath BB-10-MICROmed (China); a rotary mixer RM-1L/1S/M/L (ELmi ltd., Latvia). The botanical origin of honey was identified and the suitability of the method was checked in the scientific training laboratory of the Department of Standardization and Certification of Agricultural Products of the National University of Life and Environmental Sciences of Ukraine. The equipment used for the purpose was a digital microscope Sigeta Biogenic LED Trino Infinity (China), a microscope camera Sigeta M3CMOS 16000 (China), and the related software ToupView. The improved method of pollen analysis was implemented in the laboratory of Ascania-Pack Company, the largest honey exporter in Ukraine. For this, the above equipment was used, as well as a digital microscope Carl Zeiss Primo Star (Germany) and the related software.

At the first research stage, the harmonised melissopalynological methods [24] and the standardised method of pollen analysis (DSTU 4497:2005 "Natural honey. Specification requirements") were used. The second stage, consisted in developing an improved pollen analysis method to identify the botanical origin of honey and introducing it into production. The research was conducted during the years 2018–2020. To hone the method, 11 samples of honey were used in 2018 (polyfloral flower honey – 4; honeydew honey – 1; monofloral: acacia – 1, sunflower – 1, buckwheat – 1), 19 samples in 2019 (polyfloral flower honey – 9, monofloral: sunflower – 3, buckwheat – 1, amorpha – 1, phacelia – 1, bird's foot trefoil – 1, goldenrod – 1), 27 samples in 2020 (polyfloral flower honey – 16, polyfloral with a predominance of blueberry grains – 1, echinacea – 1, monofloral: sunflower – 5, buckwheat – 1, rapeseed – 1, lime – 1). The pollen grains in the prepared samples were calculated in duplicate. The honey samples were obtained from different regions of Ukraine.

Preparation of the solution to fix pollen specimens.

Preparation of glycerol gelatine: in a reagent jar with a screw cap, 10% of gelatine, 30% of glycerol, and 60% of distilled water were mixed, depending on the container volume. The accuracy of weighing was 0.1g. The solution was stirred to a uniform consistency, and, if necessary, filtered through a sieve with the mesh size 0.5–1 mm. The solution was stored at 0 to +5°C, in pure form or stained with ethanol fuchsine solution. The pure form of the solution was used to fix specimens from monofloral honey samples.

Preparation of ethanol fuchsine solution: for pollen analysis, it is best to use weak solution of ethanol fuchsine. It allows staining pollen grains with different intensity and makes them easier to identify. To prepare fuchsine solution, 100ml of 96° ethanol and 0.04g of basic crystalline fuchsine were mixed in a 200ml beaker and stirred with a glass stick until evenly coloured. The solution was stored in a dark glass reagent jar with a screw cap with a capacity of 250ml.

Preparation of stained glycerol gelatine with phenol for fixation. One part of chemically pure gelatine was dissolved in six parts of distilled water for 2 hours. Seven parts of chemically pure glycerol were added to the gelatine solution, and it was weighed. Then, per every 100g of the mixture obtained, 0.5g of crystalline phenol was added. Repeatedly stirred, it was kept in a heated bath for 10-15 minutes until a uniform mixture without flakes was formed. If necessary, it was filtered through a sieve with the mesh size 0.5-1mm. The ethanol fuchsine solution was added to the heated glycerol gelatine until it acquired a rich crimson colour (0.5ml of fuchsine solution per 10ml of glycerol gelatine).

Preparation of a specimen to be studied:

1. Coloured glycerol gelatine with fuchsine was heated to 40°C in a heated bath or in a heating chamber and left there until used.

2. The cover glass was placed on the clean dry microscope slide and contoured with a moisture-proof marker pen, thus highlighting the area (22×22mm) for the suspension to be applied. In this way, the required number of microscope slides was prepared. The slide was turned over to avoid staining the pollen grains suspension with the paint from the marker pen or washing off the edges of the square contour.

3. Before collecting the pre-prepared suspension of pollen grains from the centrifuge tube, it was carefully mixed by drawing it up, dispensing, and stirring it with a disposable tip of a laboratory dispenser (100 μl) or a disposable Pasteur pipette.

4. Using a laboratory dispenser (or a disposable pipette), all the suspension was transferred to the selected area of the slide and evenly distributed to cover all of it. Care was taken to avoid the formation of pollen grain clots visible to the naked eye. In this way, all the suspensions prepared were applied on individual microscope slides. It is important that an individual disposable tip of the laboratory dispenser should be used for each honey sample, and that first the capped test tube with the suspension and then the glass slide should be labelled with a code containing information about the honey sample to be tested.

5. The microscope slides with the suspension were placed in a heating chamber and dried at up to 40-45°C for 10-20min (different evaporation time for different amounts of liquid).

6. Preheated stained glycerol gelatine with fuchsine was applied diagonally (crosswise) on a clean dry cover glass. Then slowly, so that no air remained, the cover glass was placed onto the contoured area of the slide with the dried suspension within. Thus, the

suspensions of pollen grains and other prepared samples were fixed.

7. To allow the glycerol gelatine to spread evenly and the pollen grains to swell and get stained, the preparations were first left in the laboratory for 10 minutes, then transferred to the heating chamber and kept for 5 minutes at 40-45°C. After that, the pollen grain preparations were ready for identification.

Then, these specimens were examined or stored so that the surfaces of different microscope slides, especially on the cover glass side, did not come into contact. If necessary, for long-term storage, the slides were wrapped in parchment paper and stored in tightly closed containers for up to five years at 0 to +5°C.

Results of the research and their discussion

Theoretical analysis and practical testing of the harmonised melissopalynological methods [24] and of the standardised method (DSTU 4497:2005 "Natural honey. Specification requirements") have allowed developing an improved method of pollen analysis to identify the botanical origin of Ukrainian honeys.

The improved method includes several stages: the technique for preparing solution to fix pollen specimens; preparing a suspension of pollen grains from honey; the method of preparing a specimen for testing; the method of counting pollen grains in preparations from a monofloral honey sample; the method of counting pollen grains in preparations from a polyfloral honey sample; identifying the botanical origin of honey.

Particular attention should be paid to *the technique for preparing solution to fix a pollen specimen*. Our tests have shown that wrong concentrations lead to short-term storage of finished specimens with pollen grains, the latter being stained with ethanol fuchsine unevenly or indistinctly. For this stage of the method, it has been determined that excessive saturation of the fixing solution with ethanol fuchsine results in darkening of the pollen grains in a specimen, which makes it impossible to identify them by the exinal pattern and complicates botanical determination within one genus of plants. The method of preparing solution for fixation is described in the section *Materials and Methods*.

*Improving the procedure of preparing a suspension of pollen grains from honey***.** For pollen analysis of honey, a suspension of pollen grains contained in it was prepared. To this end, honey was twice dissolved in distilled water at 20–26°C and centrifuged for 5–10 min at 1000–2000 rpm.

Stages of preparing pollen grain suspension:

1. In a 50ml conical centrifuge tube, 10g of honey were weighed with an accuracy of 0.1g. If there were several samples, the scales were calibrated with the test tube stand placed on them. Honey was added to one of the test tubes, calibration was repeated, then another weighed sample was placed in the next test tube.

2. The tubes with honey were placed in the heated bath with the temperature 45–60°C to dissolve honey crystals completely. Honey can look liquid, but contain crystals, which make the results of pollen analysis less accurate because of contamination of the tested area.

3. 20ml of distilled water with the temperature 20–26°C was added to each tube, and the honey was dissolved with a glass rod. It is important to use a clean dry rod for each honey sample. The tubes were again placed in the heated bath at 45-60°C for 5-10 minutes.

4. The tubes were capped and transferred to the centrifuge. The solution was centrifuged for 10 min at 1000–2000 rpm. The supernatant liquid was drained, and 20ml of distilled water were added to each tube again. For this, disposable Pasteur pipettes were used, with the stream of water being directed into the narrowed bottom of the tube. Residual honey crystals were dissolved by drawing up and dispensing the solution and stirring it with the Pasteur pipette. It is important to use an individual disposable pipette for each honey sample.

5. The tubes were centrifuged again for 5–10min at 1000–2000rpm.

6. The supernatant liquid was drained, except for the last drop, which was absorbed with bibulous paper at the angle 45°. Then the suspension is ready.

Unlike the way of preparing the suspension in the standardised method, we suggest reducing the weight of a honey sample (from 20 to 10g), dissolving the crystals both before and after honey is diluted with distilled water (instead of single dilution), and reducing the centrifugation time and speed from 3000 to 1000–2000rpm. All this makes it unnecessary to fix pollen grains further on using acetic anhydride, glacial acetic, and concentrated sulphuric acids, but their shape and structure remain unchanged. Another stage also excluded is merging the contents of several centrifuge tubes into one. Thus loss of part of the pollen grains and identification errors have become impossible. In general, preparing a pollen grain suspension now takes less time due to double centrifugation and changing its duration from 15 to 10 minutes.

The improved method of preparing suspension of pollen grains from honey differs from the harmonised international methods [24], as it involves the crystal dissolution stage due to regional features of Ukrainian honeys (fast crystallisation, a lot of dextrins). Other test parameters (sample weight, centrifuge velocity, distillate temperature) are included in the method we suggest, which allows using it when exporting honey.

Based on the results of testing the suggested improved method of preparing suspension of pollen grains from honey, we have drawn the following conclusions. The suspension can be stored in tightly closed tubes at 0 to +5°C for no longer than one month. Transferring the suspension from centrifuge test tubes to other tubes should be avoided so as not to lose part of the pollen grains (which can affect the results of the analysis). For longer storage, the specimen was

prepared from pollen grain suspension on a microscope slide. Properly prepared suspension should be clear, without clots and honey crystals. However, whether it is so or not can already be seen from the specimens. It is especially difficult to prepare suspensions of dark honeys (buckwheat, honeydew). They require additional centrifugation, with stages 4 and 5 repeated until a satisfactory result is obtained.

The method of preparing specimens for research has been adapted to the harmonised international methods [24]. The main difference from how it is done by the standardised method is the use of microscope slides (instead of a haemocytometer) and clearly defined research techniques and parameters (temperature, time). Preparation of the specimen by the adapted method is based on transferring the suspension of pollen grains onto a microscope slide, fixing, and staining it. All work should be performed in disposable gloves, changing them with each individual honey sample. The stages of preparing the suspension for research by the adapted method are given in *Materials and Methods*.

The results of testing the suggested method showed that it was necessary to monitor how a specimen dried. In the first seconds after evaporation of the liquid, the specimens are taken out for staining. If the wet spot glistens and is visible even after drying, it may indicate insufficient dissolution of sugars in the suspension. This can result from a comparatively high content of sugars (especially sucrose) in the honey under study. These specimens are reprepared using additional dissolution of honey crystals after dilution and re-centrifugation. While developing the adapted method, it was found that when glycerol gelatine (solution for fixation) was drawn up slowly, no air bubbles were formed in it, so this should be done but once, carefully, using a laboratory dispenser or a Pasteur pipette. On the other hand, if glycerol gelatine is applied very slowly, it will congeal too early and spread on the slide unevenly. This can result in uneven staining of pollen grains of one plant species, thus making it difficult to identify them.

The method of counting pollen grains in specimens from a monofloral honey sample developed by us is based on studying the dominant botanical species and confirming or refuting its monoflorality.

Before examining the specimens, the microscope was adjusted to our eyes. A data table was prepared for primary records of the identification results. For the study of monofloral honeys, we recommend that it should have the following arrangement (Table 1).

The additional data column was used to register excessive amounts of yeast and other inclusions of biological origin (OI), which indicate contamination of honey; the presence of destroyed grains that cannot be determined; the presence of mechanical impurities of natural (wax, bee hairs) and non-natural origin (starch grains, dust).

To identify honey as monofloral, the specimen was first examined under the magnification x100-200. The presence of the pollen grains declared as dominant (PG^1), and then of all the others (PG^2, PG^3) was visually determined. On detecting them, the magnification was increased to x400 for further study and calculation of individual species (Fig.1m, 2m). To determine the botanical origin of pollen grains, the magnification was changed to x1000 or x2000 if this was possible, and if it was not, the origin was established at lower magnification. Also, to facilitate identification within a plant genus (for example, with different species of clover or cornflowers), special software was used to measure the size of pollen grains.

A specimen was studied starting from the upper left edge and moving down, making sure that the first field of view of the microscope did not overlap the other one. In each field of the microscope, all determinable grains and inclusions were counted, except for those at the right and the lower limits of the visual field to prevent counting them twice.

Table 1 – Results of honey pollen analysis, sample no. ___ of __/__/20__.

No. of the microscope field	PG^1	PG^2	PG^3	OI	Additional data
1					
2					
3					
…					
n					
Total PG by type					

Note. PG^1 is the type of pollen grains for which the honey is declared as monofloral, under the magnification x100 – x200; PG^2 are all pollen grains of entomophilous nectar-producing plants found in the specimen except for PG^1; PG^3 are pollen grains of anemophilous (other) and entomophilous plants that do not produce nectar; OI are other inclusions, such as spores, fungi, yeast, algae.

On reaching the lower edge of the selected area of the microscope slide, the examination went on in the field of the microscope, moving to the right through one field and then upwards. On reaching the upper edge of the selected area of the slide, the examination went on through one field to the right and then moving down.

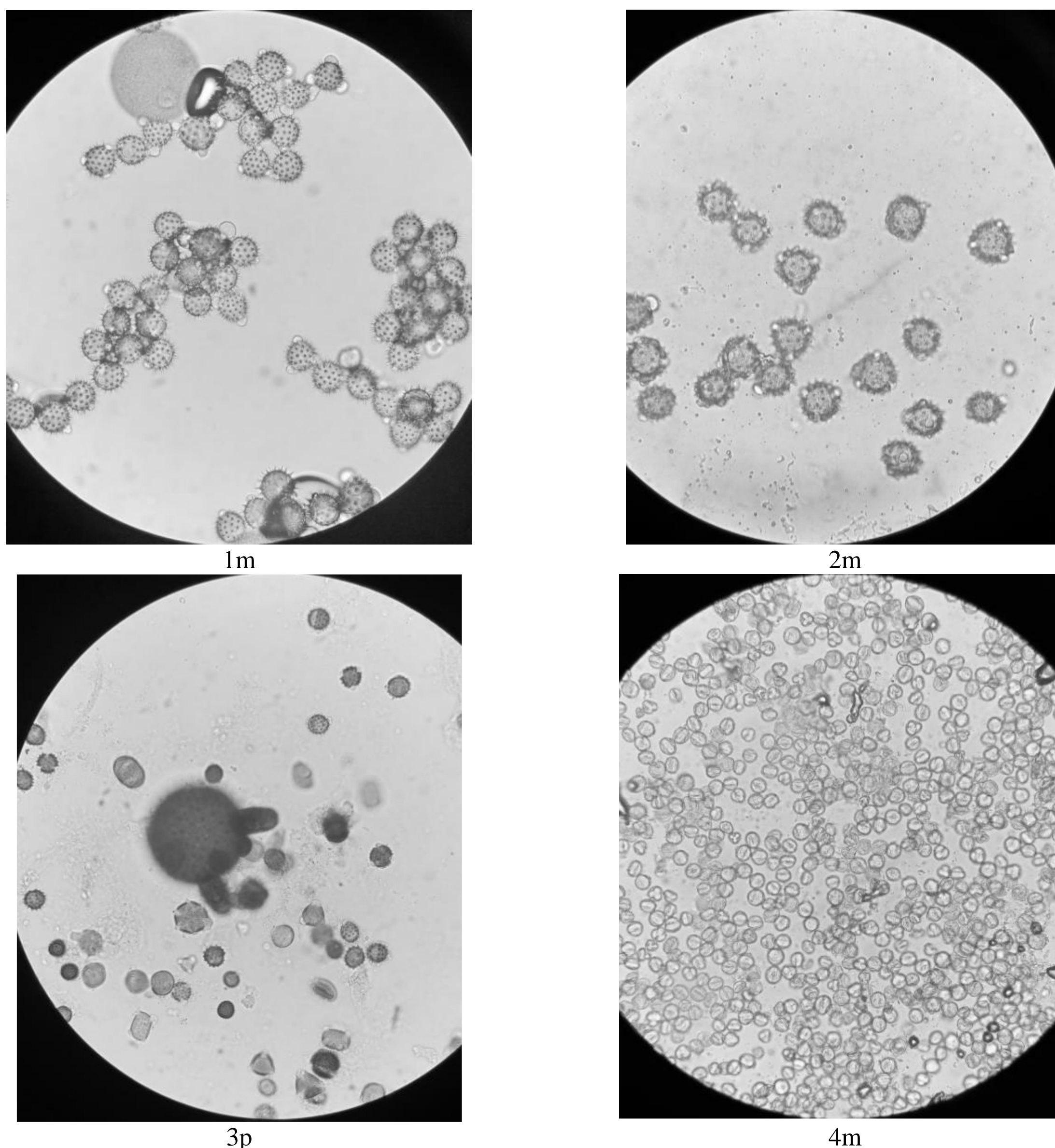

Fig. 1. Specimens for studying the pollen spectra from monofloral and polyfloral honeys:
1m – monofloral sunflower, x400; 2m – monofloral thistle, x400;
3p – polyfloral meadow, x400; 4m – monofloral clover, x200.

Pollen grains were counted according to this scheme until the PG^1 count reached 300 pollen grains for the acacia and lime honeys (*Robinia* spp. and *Tilia* spp. as underrepresented species) or 600 for all other honeys.

The total amount of all the elements researched (PG^1+PG^2+PG^3+OI) should range from 500 to 1000 units. The monoflorality of honey by the dominant pollen grains was determined as follows: $(PG^1/(PG^1+PG^2)) \times 100$, where PG^1 is the dominant pollen grain type by which a honey is declared to be monofloral, and PG^2 are all other pollen grains of entomophilous nectar-producing plants. The specimens of monofloral honeys are shown in the figure. If $PG^1 \geq 30\%$ (and for acacia and lime 20%), honey is considered monofloral by this type of pollen grains. If while examining a specimen, a lot of pollen grains of a PG^2 type are detected, this honey is treated in the further research and calculations as polyfloral.

Unlike the formula for calculating the species composition of pollen grains given in DSTU 4497:2005 “Natural honey. Specification requirements”, our method of counting pollen grains in specimens from monofloral honey samples is more accurate. It allows the researcher to focus on the dominant species, which saves time that could be spent on counting other ones. Besides, our method allows taking into account some quantity of pollen grains of anemophilous plants that could get from the air, as well as honeydew elements (fungi, algae).

The method of calculating pollen grains in specimens from polyfloral honey samples developed by us includes the two basic stages: 1) general pollen

analysis of polyfloral honey by families and 2) complete pollen analysis of honey.

General pollen analysis of polyfloral honey by families. Before studying a specimen, the microscope was adjusted to our eyes. First, a data table was prepared for primary records of the results of identification by families (Table 2). To identify of pollen grains more easily, one should know which period of the beekeeping season the honey originates from. In the early spring, the prevailing plant species are those from the sage family (Lamiaceae) and rose family (Rosaceae), later in the spring, it is the cabbage family (Brassicaceae), in the early summer, it is the bean family (Fabaceae), and in the late summer, it is the aster family (Asteraceae).

The additional data column was used to record information of the same kind as when researching monofloral honey specimens. A preparation was first examined under the magnification x100–200, and the present pollen grains from plants of individual families were visually determined. In this way, it was determined how many columns Table 2 should have. After all families were included in the table, the grains were calculated under the magnification x400 (Fig.: 3p). This magnification is enough to determine which plant family the pollen grains belong to.

One starts calculating from the upper left corner, gradually descending along a diagonal line towards the right corner (moving in stairlike angles: down, to the right – calculating – down, to the right – calculating). This allows covering all the variety of pollen grains over the entire area of the specimen as quickly as possible. Besides, the calculation method used for monofloral honey specimens is applicable here, too.

As it can be seen from the figure, the saturation of a specimen with pollen grains is different, which can also be due to a honey's varietal characteristics. When developing the method, it was found that it was better to form Table 2 immediately using MS Excel with formulae incorporated into it for calculating the total number of pollen grains (total PG by families). The SUM function can be used to this end.

This allows seeing the total of pollen grains calculated. On reaching the lower edge of the selected area of the microscope slide, the examination went on in the field of the microscope, moving to the right and up through one field and then moving up along a diagonal line to the upper left corner. It is important that one step should be equal to one field of view of the microscope. This makes it impossible to calculate the same grains twice. On reaching the upper edge of the selected area of the slide, the examination went on through one field to the right and then moving down. The calculation continued until the total of all the elements under study was at least 1000 (usually, it is about 10 diagonals).

The botanical composition of honey was determined as a percentage of pollen grains of each family to their total number. We recommend using Table 3 formed by means of MS Excel with integrated formulae listed in the table.

To study the pollen composition of honey in detail, its *complete pollen analysis* was performed. To this end, pollen grains were identified within each family by genera or species under the magnification x1000 or x2000, and special software was used that allowed measuring the size of pollen grains and examining the features of their exine. Complete pollen analysis was used for honeys with geographical indication.

If honeydew elements occurred in a specimen, they were calculated individually, and their number was determined as a percentage. According to the harmonised method [24], a honey is classed as honeydew if its specimen contains at least 50% of honeydew elements, and if there are more than 30% of them, a honey is termed honeydew-floral.

The suggested methods of calculating pollen grains comply with the current Ukrainian legislation on determining the origin of monofloral and polyfloral honeys (Directive No. 330 of 19.06.2019 "Approval of the requirements to honey") and are in accordance with the technical parameters of international harmonised melissopalinological methods.

Table 2 – Results of honey pollen analysis by family, sample no. ___ of __/__/20__

No. of the microscope field	PG^1 *Aster*	PG^2 *Bras*	PG^3 *Fab*	PG^4 *Lam*	PG^5 *Ros*	PG^n	PG Pol	OI	Additional data
1									
2									
3									
…									
n									
Total PG by family									

Note. PG^1 Aster are pollen grains of plant species and genera belonging to the aster family (Asteraceae); PG^2 Bras are pollen grains of plant species and genera belonging to the cabbage family (Brassicaceae); PG^3 Fab are pollen grains of plant species and genera belonging to the bean family (Fabaceae), PG^4 Lam are pollen grains of plant species and genera belonging to the sage family (Lamiaceae); PG^5 Ros are pollen grains of plant species and genera belonging to the family of roses (Rosaceae), PG^n are pollen grains of plant species and genera belonging to another plant family (there can be several of them); PG Pol are pollen grains of anemophilous (other) and entomophilous plants that do not produce nectar; OI are other inclusions, such as spores, fungi, yeast, algae.

Table 3 – Botanical composition of honey by family, sample no. ___ of__/__/20__

Element to be counted	Number of elements, pcs.	Number of elements, %
PG^1 *Aster*	X^1	$= (X^1 \times 100) / X^{total}$
PG^2 *Bras*	X^2	$= (X^2 \times 100) / X^{total}$
PG^3 *Fab*	X^3	$= (X^3 \times 100) / X^{total}$
PG^4 *Lam*	X^4	$= (X^4 \times 100) / X^{total}$
PG^5 *Ros*	X^5	$= (X^5 \times 100) / X^{total}$
PG^n	X^n	$= (X^n \times 100) / X^{total}$
Honeydew elements	X^7	$= (X^7 \times 100) / X^{total}$
Total number	$= SUM(X^1 : X^7) = X^{total}$	100

Identification of the botanical origin of honey is the final stage of pollen analysis. It should be noted that the method of pollen analysis of honey cannot be used properly without good knowledge of plant species and of their Latin names. Primarily, it is necessary to study the biological diversity of plant species that form the flora of the region from which honey originates. A variety of well-illustrated atlases and plant identification guides can help learn when plants bloom, how they are distributed geographically, what their pollination types are, whether they produce nectar, and how much of it if they do, etc.

To find a plant's correct botanical name, we recommended the resource The Plant List [25]. It allows checking whether the name of a plant in the guide (atlas) is actually in use, before searching through international databases for information on its pollen grains.

The name of plant species considered is typed in the search line of the resource The Plant List. The resource shows the result in a tabular form. The table contains columns for the name of the species, the status of this name, the scientific community's confidence in this name, a reference to a source (in most cases, it is the World Checklist of Selected Plant Families), date of validation. Accordingly, "accepted" means the established name of a species, "synonym" denotes a synonym for the established name, and "unresolved" means that the name is still being decided upon. Then, pollen grain types classified by plant family are studied along with the main sources of nectar for monofloral honeys. Usually, this knowledge comes with professional experience.

To identify pollen grains, we recommend using printed atlases (for example, *Атлас пыльцевых зёрен* by Karpovich et al., 2015 [4]) or different databases of pollen grains. On top: PalDat - Palynological Database, an online publication on recent pollen [26], PollenAtlas [27], PollenWarnDienst [28], Discover Life [29], The Global Pollen Project [30], The Open Platform for Pollen Identification, Science & Plants for Schools [31]. We have also developed a method for creating standard samples of pollen grains depository for their botanical identification in honey, which is confirmed by patents UA 144626 U [32] and UA 144627 U [33].

Approbation of results

The improved method of pollen analysis for identification of the botanical origin of Ukrainian honeys has been implemented and successfully used during the years 2019–2020 in a laboratory of Ascania-Pack Company. Since November 2019, the method has been used in the laboratory of the Honey Family Farm (Grodno, Belarus). The method was also tested during research of the pollen spectrum of acacia honey from different regions of Ukraine [34].

Conclusion

Studying the harmonised methods of melissopalinology and the standardised method of pollen analysis has allowed developing a method of botanical identification of honey adapted for practical implementation in Ukrainian laboratories.

The method suggested includes: detailed technique of preparing solution to fix pollen specimens; an improved method of preparing suspension of pollen grains from honey; a specimen preparation method adapted to international harmonised methods; new methods of counting pollen grains in specimens from monofloral and polyfloral honey samples; detailed step-by-step identification of the botanical origin of honey by pollen grains.

The method has been implemented in production laboratories of the leading enterprises in Ukraine and Belarus.

The direction for further research is creating databases of pollen spectra of polyfloral honeys from different regions of Ukraine and developing a system of their geographical labels, such as Polisian, Podilian, Slobidian, and other honey varieties.

Acknowledgements. The author Leonora Adamchuk thanks the International Visegrád Fund for scholarship ID #51910842 and research internships, during which the results and knowledge presented in this paper were obtained.

References:

1. Simeão CMG, Silveira FA, Sampaio IBM, Bastos EMAF. Pollen analysis of honey and pollen collected by Apis mellifera linnaeus, 1758 (Hymenoptera, Apidae), in a mixed environment of Eucalyptus plantation and native cerrado in Southeastern Brazil. Brazilian Journal of Biology. 2015:75(4);821-829. https://doi.org/10.1080/00173130903363550

2. Sofany A, Naggar YA, Naiem E, Giesy JP, Seif A. Authentication of the botanical and geographic origin of Egyptian honey using pollen analysis methods. Journal of Apicultural Research. 2020;59(5):946-955. https://doi.org/10.1080/00218839.2020.1720950
3. Mannapov AG, Legochkin OA, Skachko AS. Koefficient pylcevogo analiza pri ocenke botanicheskogo proishozhdeniya meda. Pchelovodstvo. 2017;6:55-59.
4. Karpovich IV, Drebezgina ES, Elovikova EA, Legotkina GI, Zubova EN, Kuzyaev RZ, et al. Atlas pylcevyh zeren. Ekaterinburg: Uralskij rabochij. 2015.
5. Ivanova VYu, Arestova IYu. Melissopalinologicheskij analiz meda. Science Time. 2015;16(4):302-311.
6. Ivanova VYu, Arestova IYu. Pylcevoj analiz meda s chastnoj paseki Yantikovskogo rajona (Chuvashiya). Privolzhskij nauchnyj vestnik. 2015;45(5-1):44-48.
7. Kurmanov RG, Ishbirdin AR. Pylcevoj atlas. Ufa: Izdatelstvo "Gilem". 2013.
8. Tümerdem Ç, Pehlivan S, Doğan C. Pollen analysis of honeys from beypazarı district of Ankara Province (Turkey). Mellifera. 2016;16(1):19-26. Available from: https://dergipark.org.tr/tr/download/issue-full-file/25682
9. Özhatay N, Koçyiğit M, Bona M. Istanbul's Honey Plants “Where there are flowers, there is honey” BAL-DER Healthy. Living through Bee Products Platform Association: 2017.
10. Mendez MV, Sanchez AC, Flores FF, Lupo LC. Recurso polinífero utilizado por Apis mellifera (Himenoptera: Apidae) en un área de bosque subtropical del noroeste de Argentina. Revista de Biología Tropical. 2018;66(3):1182-1196. https://doi.org/10.15517/rbt.v66i3.30856
11. Ramalho M, Guibu LS, Giannini TC, Kleinert-Giovannini A, Imperatriz-Fonseca VL. Characterization of some southern Brazilian honey and bee plants through pollen analysis. Journal of Apicultural Research. 1991;30(2):81-86. https://doi.org/10.1080/00218839.1991.11101238
12. Nascimento AS, Marchini LC, Carvalho LC, Araújo DFD, Silveira TA. Pollen spectrum of stingless bees honey (Hymenoptera: Apidae), Paraná State, Brazil. Journal of Entomology and Zoology Studies. 2015;3(2):290-296. https://doi.org/10.1080/00173134.2020.1733074
13. Bell KL, de Vere N, Keller A, Richardson RT, Gous A, Burgess KS, et al. Pollen DNA barcoding: current applications and future prospects. Genome. 2016;59(9):629-640. https://doi.org/10.1139/gen-2015-0200
14. Marquele-Oliveira F, Carrão DB, Souza RO, Baptista NU, Nascimento AP, Torres EC, et al. Fundamentals of Brazilian Honey Analysis: An Overview. In: Toledo VAA. (ed) Honey Analysis. In Tech. 2017;7:139-170. https://doi.org/10.5772/67279
15. Bell KL, Fowler J, Burgess KS, Dobbs EK, Gruenewald D, Lawley B, et al. Applying pollen DNA metabarcoding to the study of plant – Pollinator Interactions. Applications in Plant Sciences. 2017;5(6):1600124. https://doi.org/10.3732/apps.1600124
16. Haba E. Molecular detection of the botanical origin of pollen in honey bee-collected pellets: a comparison of methods. IPB Digital Library; 2019 [cited 2020 May 5]. Available from: http://hdl.handle.net/10198/19828
17. Richardson RT, Lin C-H, Sponsler DB, Quijia JO, Goodell K, Johnson RM. Application of its 2 metabarcoding to determine the provenance of pollen collected by honey bees in an agroecosystem. Applications in Plant Sciences. 2015;3(1):1400066. https://doi.org/10.3732/apps.1400066
18. Hawkins J, De Vere N, Griffith A, Ford CR, Allainguillaume J, Hegarty MJ, et al. Using DNA metabarcoding to identify the floral composition of honey: A new tool for investigating honey bee foraging preferences. PLoS ONE. 2015;10(8):e0134735. https://doi.org/10.1371/journal.pone.0134735
19. Hollingsworth ML, Graham SW, Little DP. Choosing and using a plant DNA barcode. PLoS ONE. 2011;6(5):e19254. https://doi.org/10.1371/journal.pone.0019254
20. Keller A, Danner N, Grimmer G, Ankenbrand M, von der Ohe K, von der Ohe W, et al. Evaluating multiplexed next-generation sequencing as a method in palynology for mixed pollen samples. Plant Biology. 2015;17(2):558–566. https://doi.org/10.1111/plb.12251
21. Hollingsworth PM, Forrest LL, Spouge JL, Hajibabaei M, Ratnasingham S, van der Bank M, et al. A DNA barcode for land plants. Proc. Natl. Acad. Sci. U.S.A. 2009;106(31):12794-12797. https://doi.org/10.1073/pnas.0905845106
22. Hollingsworth ML, Clark A, Forrest LL, Richardson J, Pennington RT, Long DG, et al. Selecting barcoding loci for plants: evaluation of seven candidate loci with species-level sampling in three divergent groups of land plants. Molecular Ecology Resources. 2009;9(2):439-457. https://doi.org/10.1111/j.1755-0998.2008.02439.x
23. Sickel W, Ankenbrand MJ, Grimmer G, Holzschuh A, Härtel S, Lanzen J, et al. Increased efficiency in identifying mixed pollen samples by metabarcoding with a dual-indexing approach. BMC Ecology. 2015;15(1):1-9. https://doi.org/10.1186/s12898-015-0051-y
24. Von Der Ohe W, Oddo LP, Piana ML, Morlot M, Martin P. Harmonized methods of melissopalynology. Apidologie. 2004;35(1):S18-S25. https://doi.org/10.1051/apido:2004050
25. The Plant List. A working list of all plant species. Version 1.1 [Internet]. Taxonomic Working Group of the World Flora Online [accessed 2013 Jan 1, cited 2020 May 20]. Available from: http://www.theplantlist.org/
26. PalDat – Palynological Databasa- an online publication on recent pollen. PolDad 3.3 [Internet]. Society for the Promotion of Palynological Research in Austria, Viena; 2000 [updated 2020, cited 2020 May 20] Available from: https://www.paldat.org/
27. PollenAtlas. [Internet]. Bologna, Italy: Council of Agricultural Research and Economics (CREA) Research Unit for Apicolture and Sericulture [accessed 2017, cited 2020 May 20] Available from: https://pollenatlas.net/
28. PollenWarnDienst [Internet]. Wien, Austria: Medical University of Vienna. [accessed 2016, cited 2020 Mar 10] Available from: https://www.pollenwarndienst.at/
29. Discover Life [Internet]. USA, International Center for Public Health and Environmental Reseach (PHER) [updated 2018 Jan 28, cited 2020 May 20] Available from: https://www.discoverlife.org
30. Martin AC, Harvey WJ. The Global Pollen Project: a new tool for pollen identification and the dissemination of physical reference collections. Methods Ecol Evol. 2017:8:892-897. https://doi.org/10.1111/2041-210X.12752.
31. Pollen Image Library. Science & Plants for Schools [Internet], 2011. Cambridge University Botanic Garden: Science & Plants for Schools [updated 2020, cited 2020 May 20]. Available from: http://www-saps.plantsci.cam.ac.uk/pollen/index.htm
32. Adamchuk L, Sukhenko V, inventors. Leonora Adamchuk, assignee. Method of obtaining model nutrient medium for depository of standard samples of pollen grains for their botanical identification in honey. Ukrainian Patent 144626. 2020 Oct 12.
33. Adamchuk L, Sukhenko V, inventors. Leonora Adamchuk, assignee. Method of creating standard samples of pollen grain depository for their botanical identification in honey. Ukrainian Patent 144627. 2020 Oct 12.
34. Adamchuk LO, Sukhenko VYu, Akulonok OI. Fermentatyvna aktyvnist akatsiievoho medu. Novitni tekhnolohii. 2019;3(10):52-61. https://doi.org/10.31180/2524-0102/2019.3.10.06

УДОСКОНАЛЕННЯ МЕТОДИКИ БОТАНІЧНОЇ ІДЕНТИФІКАЦІЇ МЕДУ

Л.О. Адамчук, кандидат сільськогосподарських наук, доцент, *E-mail*: leonora.adamchuk@gmail.com
Кафедра стандартизації та сертифікації сільськогосподарської продукції
Національний університет біоресурсів і природокористування України, вул. Героїв Оборони, 15,
м. Київ, Україна, 03041

Анотація. Пилковий спектр меду відображає регіональні сільськогосподарські культури, лісові насадження, різноманітність та видовий склад рослин наявних поблизу пасіки. Тому використання, адаптація і удосконалення різних методів пилкового аналізу та інтерпретації його результатів поширюється у країнах світу, які є виробниками оригінальних сортів меду на міжнародному ринку. Україна займає лідируючі позиції з виробництва та експорту меду у Європі та світі. Зазвичай на експорт мед іде, не як готовий харчовий продукт, а як сировина. Це пов'язано з відсутністю опису пилкового спектру українських медів, який характеризує їхнє ботанічне та географічне походження через відсутність впроваджених у виробничих лабораторіях методик пилкового аналізу. Метою роботи було удосконалення методики пилкового аналізу для ідентифікації ботанічного походження українських медів. Дослідження проводили на базі лабораторії кафедри стандартизації та сертифікації сільськогосподарської продукції Національного університету та біоресурсів природокористування України та виробничій лабораторії ТОВ «Асканія-Пак». На підставі аналізу гармонізованих методів мелісопалінології та стандартизованого методу пилкового аналізу розробили адаптовану до практичного впровадження в умовах українських лабораторій методику ботанічної ідентифікації меду. Запропонована методика включає сукупність окремих технік, методів та способів. Деталізовано техніку готування розчину для фіксації та фарбування пилкового препарату з меду. Удосконалено спосіб приготування суспензії пилкових зерен з меду, завдяки чому зменшуються витрати часу та покращується якість препарату внаслідок застосування додаткового розчинення кристалів та зменшення швидкості обертання центрифуги. Адаптований до гармонізованих міжнародних методик мелісопалінології спосіб приготування препарату пилкових зерен з меду. Розроблено нові методи підрахунку пилкових зерен у препаратах зі зразків монофлорного і поліфлорного медів. Наведено розгорнуту покрокову ідентифікацію ботанічного походження меду за пилковими зернами з використанням міжнародних дата-баз. Наведену методику впровадили у виробничих лабораторіях провідних підприємств України та Білорусі.

Ключові слова: метод пилкового аналізу, пилкове зерно, монофлорний мед, поліфлорний мед, ботанічна ідентифікація, мелісопалінологія.

Список літератури:

1. Pollen analysis of honey and pollen collected by Apis mellifera linnaeus, 1758 (Hymenoptera, Apidae), in a mixed environment of Eucalyptus plantation and native cerrado in Southeastern Brazil / Simeao C.M.G. et al // Braz. J. Biol. 2015; Vol. 75, № 4. P. 821-829. https://doi.org/10.1590/1519-6984.23513
2. Authentication of the botanical and geographic origin of Egyptian honey using pollen analysis methods / Sofany A. et al / Journal of Apicultural Research. 2020. Vol.59, Issue 5. P. 946-955. https://doi.org/10.1080/00218839.2020.1720950
3. Маннапов А.Г., Легочкин О.А., Скачко А.С. Коэффициент пыльцевого анализа при оценке ботанического происхождения меда // Пчеловодство. 2017. № 6. С. 55-59.
4. Атлас пыльцевых зерен / Карпович И.В. и др. Екатеринбург: Уральский рабочий. 2015. 314 с.
5. Иванова В.Ю., Арестова И.Ю. Мелиссопалинологический анализ меда // Science Time. 2015. № 4, вип. 16. С. 302-311.
6. Иванова В.Ю., Арестова И.Ю. Пыльцевой анализ меда с частной пасеки Янтиковского района (Чувашия) // Приволжский научный вестник. 2015. № 5-1, вип. 45. С. 44-48.
7. Курманов Р.Г., Ишбирдин А.Р. Пыльцевой атлас. Уфа: Издательство "Гилем", 2013. 304 с.
8. Tümerdem Ç., Pehlivan S., Doğan C. Pollen analysis of honeys from beypazarı district of Ankara Province (Turkey) // Mellifera. 2016. Vol. 16, № 1. P. 19-26 URL: https://dergipark.org.tr/tr/download/issue-full-file/25682. (viewed 20.04.2020)
9. Özhatay N., Koçyiğit M., Bona M. Istanbul's Honey Plants "Where there are flowers, there is honey" BAL-DER Healthy: Living through Bee Products Platform Association. 2017. 264 p.
10. Recurso polinífero utilizado por Apis mellifera (Himenoptera: Apidae) en un área de bosque subtropical del noroeste de Argentina / Mendez M.V. et al. // Revista de Biología Tropical. 2018. Vol. 66, №. 3. P. 1182-1196. https://doi.org/10.15517/rbt.v66i3.30856
11. Characterization of some southern Brazilian honey and bee plants through pollen analysis / Ramalho M. et al. / Journal of Apicultural Research. 1991. Vol. 30, №. 2. P. 81-86. https://doi.org/10.1080/00218839.1991.11101238
12. Pollen spectrum of stingless bees honey (Hymenoptera: Apidae), Paraná State, Brazil / Nascimento A.S. et al. Journal of Entomology and Zoology Studies. 2015. Vol. 3, №. 2. P. 290-296. https://doi.org/10.1080/00173134.2020.1733074
13. Pollen DNA barcoding: current applications and future prospects / Bell K.L. et al. // Genome. 2016. Vol. 59, Issue 9. P. 629-640. https://doi.org/10.1139/gen-2015-0200
14. Fundamentals of Brazilian Honey Analysis: An Overview / Marquele-Oliveira F. et al // Honey Analysis. In Tech. 2017. № 7. P. 139-170. https://doi.org/10.5772/67279
15. Applying pollen DNA metabarcoding to the study of plant – Pollinator Interactions / Bell K.L. et al. // Applications in Plant Sciences. 2017. Vol. 5, №. 6. P. 1600124. https://doi.org/10.3732/apps.1600124
16. Haba E. Molecular detection of the botanical origin of pollen in honey bee-collected pellets: a comparison of methods. IPB Digital Library, 2019. 102 p. URL: : http://hdl.handle.net/10198/19828 (viewed 5.05.2020)
17. Application of ITS2 metabarcoding to determine the provenance of pollen collected by honey bees in an agroecosystem / Richardson R.T. et al // Applications in Plant Sciences. 2015. Vol. 3, №1. P. 1400066. https://doi.org/10.3732/apps.1400066
18. Using DNA metabarcoding to identify the floral composition of honey: A new tool for investigating honey bee foraging preferences / Hawkins J. et al // PLoS ONE. 2015. Vol. 10. №. 8. 20 p. https://doi.org/10.1371/journal.pone.0134735
19. Hollingsworth M.L., Graham S.W., Little D.P. Choosing and using a plant DNA barcode // PLoS ONE. 2011. Vol. 6, №. 5. 5 p. https://doi.org/10.1371/journal.pone.0019254
20. Evaluating multiplexed next-generation sequencing as a method in palynology for mixed pollen samples / Keller A. et al. // Plant Biology. 2015. Vol. 17, №2. P. 558-566. https://doi.org/10.1111/plb.12251

21. A DNA barcode for land plants / Hollingsworth P.M. et al // Proc. Natl. Acad. Sci. U.S.A. 2009. Vol. 106. №. 31. P. 12794-12797. https://doi.org/10.1073/pnas.0905845106. PMID:19666622
22. Selecting barcoding loci for plants: evaluation of seven candidate loci with species-level sampling in three divergent groups of land plants / Hollingsworth M.L. et al // Molecular Ecology Resources. 2009. Vol. 9, №. 2. P. 439-457. https://doi.org/10.1111/j.1755-0998.2008.02439.x
23. Increased efficiency in identifying mixed pollen samples by metabarcoding with a dual-indexing approach / Sickel W. et al // BMC Ecology. 2015. Vol. 15, №. 1. P. 1-9. https://doi.org/10.1186/s12898-015-0051-y
24. Harmonized methods of melissopalynology / Von Der Ohe W. et al / Apidologie. 2004. Vol.35, Suppl. 1. P. S18-S25. https://doi.org/10.1051/apido:2004050
25. The Plant List: [Web-site]. USA, 2013.URL: http://www.theplantlist.org (viewed: 20.05.2020)
26. PalDat – Palynological Database: [Web-site]. Wien, Austria, 2000. URL: https://www.paldat.org/ (viewed: 20.05.2020)
27. PollenAtlas: [Web-site]. Bolongna, Italy, 2017. URL: https://pollenatlas.net/ (viewed: 20.05.2020)
28. PollenWarnDienst: [Web-site]. Wien, Austria, 2016. URL: https://www.pollenwarndienst.at/ (viewed: 20.05.2020)
29. Discover Life: [Web-site]. USA, 2018. URL: https://www.discoverlife.org (viewed: 20.05.2020)
30. Martin A.C., Harvey W.J. The Global Pollen Project: a new tool for pollen identification and the dissemination of physical reference collections // Methods Ecol Evol. 2017. Vol.8. P.892-897. https://doi.org/10.1111/2041-210X.12752.
31. Pollen Image Library. Science & Plants for Schools: [Web-site]. Cambridge, 2011. URL: http://www-saps.plantsci.cam.ac.uk/pollen/index.htm (viewed: 20.05.2020)
32. Спосіб одержання модельного харчового середовища для депозитарію стандартних зразків пилкових зерен для їх ботанічної ідентифікації у меду: пат. на корисну модель 144626 Україна: МПК A23L 21/20 A23L 21/25 / Адамчук Л.О., Сухенко В.Ю.; власник Адамчук Л.О. № u202003330; заявл. 01.06.2020; опубл. 12.10.2020, Бюл. № 19.
33. Метод створення стандартних зразків депозитарію пилкових зерен для їх ботанічної ідентифікації у меду: пат. на корисну модель 144627 Україна: МПК A23L 21/20 A23L 21/25 / Адамчук Л.О., Сухенко В.Ю.; власник Адамчук Л.О. № u202003331; заявл. 01.06.2020; опубл. 12.10.2020, Бюл. № 19.
34. Адамчук Л.О., Сухенко В.Ю., Акульонок О.І. Ферментативна активність акацієвого меду // Новітні технології. 2019. Вип. 3(10). С. 52-61. https://doi.org/10.31180/2524-0102/2019.3.10.06