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METHODS FOR DETERMINING THE BOTANICAL ORIGIN OF HONEY

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ABSTRACT

The demand for monofloral, original, and special (functional) kinds of honey, or those with geographical indication, is forecast. At the same time, there is a need to improve the methods for determining the botanical and geographical origin of honey. The purpose of the research was to select and apply a variety of techniques for identifying the botanical origin of honey for its correspondence to acacia species. Samples of honey from the Kyiv, Odesa, and Dnipro regions extracted in the spring and summer period were used in the research. Organoleptic, physicochemical, NMR spectrometry, and advanced melissopalynology methods were applied. The tests were carried out at the laboratories of the Department of Certification and Standardization of Agricultural Products, NULES, Ukraine; the Ukrainian Laboratory of Quality and Safety of Agricultural Products; and the Bruker BioSpin GmbH company (Germany). According to the research results, the requirements for acacia honey were met by the organoleptic method for samples B1 and B2; by the physicochemical method for A0 and A2; by NMR spectroscopy for not a single sample, all being assessed as polyfloral; and by pollen analysis for B1 and B2. The conducted studies confirm the need for a comprehensive approach to the identification of the botanical origin of honey for its conformity to acacia species. There is a need to review the physicochemical indicators for the compliance of honey with the acacia species obtained in Ukraine. After all, even the modern NMR spectrometry technique indicated that the specially fabricated sample that did not contain acacia pollen grains was acacia honey. Identification of the botanical origin of monofloral honey, in particular acacia, should be carried out in the following sequence: pollen analysis (by dominant pollen grains), safety (presence of antibiotics, pesticides), physicochemical parameters according to international requirements, organoleptic parameters.

Keywords: acacia honey; evaluation; method; NMR spectrometry

INTRODUCTION

According to **Global Industry Analysts Inc. USA (2016)**, the global honey market is projected to reach 2.4 million tonnes by 2022. **Carreck (2018)** believes that much of this growth is due to the demand for monofloral and special (functional) kinds of honey, or those that have a specific geographical region of origin. That is the relevance of our research, along with the improvement of the methods for determining the botanical and geographical origin of honey, methods of analysis and discrimination of individual varieties of product, its safety, and quality.

At the moment, the current regulatory and technical documentation in different countries of the world governing the safety and quality of honey is not harmonized. There are differences between European legislation and Codex Alimentarius standards. Also, different countries maintain up-to-date quality criteria that do not comply with the Codex or EU directives on honey. The necessity to establish national requirements for each country (harmonized with international ones) is mainly due to the lack of provisions regarding the physicochemical characteristics of monofloral honey and the declaration of its geographical origin **(Thrasyvoulou et al., 2018)**.

There are currently two documents in Ukraine governing the safety and quality of honey, **DSTU 4497:2005 (2007)** and **Nakaz MinAPK No. 330 (2019)**. Therefore, the search for methods that allow the main botanical varieties and geographical species of Ukrainian honey to be studied is a relevant area for analysis and subsequent research.

It has been established **(Ulloa et al., 2013; Lenhardt et al., 2015; Machado De-Melo et al., 2018; Maione, Barbosa and Barbosa, 2019)** that the organoleptic and physicochemical characteristics of honey vary depending on botanical and geographical origin, as well as climatic conditions, processing and storage. And other bee products as well **(Ivanišová et al., 2015)**.

It is relevant to use honey as a biomarker to collect environmental information, identify sources of environmental contamination and assess soil, water and air pollution **(Machado De-Melo et al., 2018)**. One of the first and most common methods of determining the botanical origin of honey is melissopalynology, that is, a microscopic analysis of the pollen contained in honey shows the nectar content of certain plants.

Maione, Barbosa and Barbosa (2019) consider that it is still used as one of the most accurate methods for qualitative or quantitative analysis of the content of pollen grains in honey. Along with this, there are several modern techniques. The botanical origin of honey is identified by PARAFAC (parallel factor) analysis of excitation emission matrix fluorescence spectra **(Lenhardt et al., 2015)**. Emissions of phenolic compounds and Maillard reaction products reveal the greatest difference among honey varieties of different botanical origin. Falsified honey samples with 100% sensitivity to variety compliance can be detected using the partial least squares discriminant analysis (PLSDA) classification model based on PARAFAC models. Among the honey samples tested, PLSDA identifies linden with a difference of 0.5%, acacia with 10%, and sunflower and polyfloral meadow honey with differences of about 20% compared to the results of pollen analysis. The disadvantage of the method is the large discrepancy between the results obtained even within the same honey variety, which indicates the need for its improvement.

Soares et al. (2015) proposed the use of DNA-based methods for the identification of botanical honey species. For this, five DNA extraction methods were used (NucleoSpin Plant Kit (methods A and B) and DNeasy Plant Mini Kit, as well as internal CTAB and Wizard-based methods). The results demonstrated that the Wizard method had the best performance in terms of DNA quality. The disadvantages of DNA-based methods are their cost and unsuitability to industrial conditions of use.

Today, the use of analytical methods is becoming widespread. Mainly, this concerns nuclear magnetic resonance (NMR) spectroscopy for the authentication of honey (botanical and geographical origin).

Siddiqui et al. (2017) consider NMR spectroscopy to be sufficiently powerful and accurate, and therefore suitable for creating prints for honey of different origin for their further comparison. In Romania, studies on stable isotopes, selected as representative discrimination parameters of different botanical or geographical origin of honey, have been conducted using isotope ratio mass spectrometry (IRMS) and site-specific natural isotope fractionation measured by nuclear magnetic resonance (SNIF-NMR) methods. The authors **(Dinca et al., 2015)** confirmed the high efficiency of the method but emphasized the need to create an informative base for prints of honey of different botanical origin.

Gok et al. (2015) established the predictive ability of Fourier transform infrared (FTIR) spectroscopy and chemometrics to determine the botanical composition of honey. **Cozzolino, Corbella and Smyth (2011)** proposed spectroscopic methods in the infrared (IR) wavelength range of the electromagnetic spectrum to evaluate and control the composition of honey. The use of IR spectroscopic technologies has its place in the determination of the botanical origin of honey by comparing the spectra of the mid-infrared (MIR) spectrum.

Etzold and Lichtenberg-Kraag (2008) used the most common types of honey for calibration, and classification models were obtained by calibration of principal components analysis (PCA) and properly graded FTIR concerning the physicochemical and sensory properties of the selected samples.

To improve honey discrimination, **Corvucci et al. (2015)** proposed the use of FT-micromanaging spectrography and multivariate analysis. At the same time, successful results using the PCA model have also been achieved by other authors. This method is currently being refined and enhanced.

Modern sensory methods include the use of an electronic nose (e-nose) in multivariate analysis and selection of sensors to detect the botanical origin and determine honey quality. **Huang et al. (2015)** first implemented three sensor selection algorithms, namely, uninformation variable elimination (UVE), successive projections algorithm (SPA) and competitive adaptive reweighted sampling (CARS), which were applied to analyse the e-nose fingerprints of honey. Three different sampling modes were tested for the classification of monofloral honey: static headspace sampling (SHS), solid-phase microextraction (SPME) and inside needle dynamic extraction (INDEX). The last two showed better ability to remove volatile components. In subsequent experiments, preference was given to the SPME sampling mode, which proved to be more accurate.

Ampuero, Bogdanov and Bosset (2004) found a positive correlation between e-nose and pollen analysis of honey. Discriminant function analysis (DFA) was also conducted using an e-nose based on a mass spectrometer when the DFA diagram indicated a significant separation of honey odours from other odour sources **(Hong et al., 2011)**. Thus, the e-nose method can be used to identify honey but needs further refinement.

Ulloa et al. (2013) investigated a method for determining the botanical origin of honey using sensory synthesis of impedance electronic language (e-language) and optical spectroscopy (UV-Vis-NIR), namely, PCA and cluster analysis (CA).

In 2016, the mathematical method for identifying honey was first described. **Gan et al. (2016)** considered the use of PLSDA, a support vector model (SVM), and an interval partial least squares model (iPLS). The results showed that the spectra and sensors classified the botanical origin of honey quickly and accurately, and the overall accuracy for the calibration and forecasting set was 100% for e-nose and electronic tongue (ET) analysis using the SVM model and near-infrared (NIR) and MIR analysis using the iPLS model. At the same time, overall accuracy for calibration and forecasting sets was above 96% in PLSDA NIR, MIR and ET models. The results showed that ET is more suitable for detecting botanical falsification of honey. However, there is a need to create calibration and forecasting sets for each sample of honey produced in the world. This makes it impossible to use this method in practice.

Son et al. (2019) investigated whether the zymography of nectar chitinases is a potential marker for determining or validating the botanical origin of honey. However,

zymography is the first examination of the activity of nectar enzyme in honey. This method is also being refined.

Chekryga, Niczievskaya and Borodaj (2019) have proposed a method for determining the botanical origin of honey, which is to use a natural drop of a honey sample without pre-treatment. According to the authors, the advantage of the proposed method over others is that when using it there is no deformation of pollen grains, which are in a natural state, including their spatial location. However, the proposed method casts doubt on the quantitative evaluation of pollen grains and the reliability of the analysis results due to the uneven arrangement of grains of different weights in the thickness of honey.

Sensory data obtained from ET and e-nose histograms of honey colour show a high discriminatory ability to determine the origin of honey. Therefore, many authors **(Machado De-Melo et al., 2018; Pascual-Maté et al., 2018)** believe that PCA, discriminant analysis (DA) and CA are the best methods for performing the experimental and predictive method for determining the origin of honey. PCA and DA continue to be favoured due to their ease of application and interpretation, while machine learning algorithms are more complex for modelling and the use of classifiers. Nevertheless, the use of both machine learning algorithms and PCA-DA models have achieved excellent results for discrimination of the origin of honey. Finally, a common trend is the use of hybrid methods that combine multivariate analysis of data and methods **(Peng Kek et al., 2017; Machado De-Melo et al., 2018; Pascual-Maté et al., 2018)**.

Ballabio et al. (2018) performed a comparative evaluation of methods for determining the botanical origin of honey. Thus, IR, NIR, Raman spectroscopy, PTR-ToF-MS and e-nose methods were applied to samples of common botanical varieties of honey. The best results were obtained with the synthesis of the NIR method and Raman spectroscopy, as well as PTR-ToF-MS. The accuracy of the final model was 99% on the test specimens and 100% on the calibration.

In Ukraine, due to a lack of the necessary equipment, only various pollen analysis techniques are used to identify the origin of honey. Therefore, the purpose of our work was to select and apply a variety of techniques to identify the botanical origin of honey for its correspondence to acacia variety.

Scientific hypothesis

According to many scientists, modern methods of researching honey can replace the classical methods of determining its botanical origin, such as pollen analysis and organoleptic evaluation. In studies, we expect to refute the view that NMR spectroscopy allows determination of the falsification of acacia honey without the use of melissopalynological research, provided that the geographical origin of the honey is not known.

As a result, one of the samples was falsified in a sophisticated manner and NMR spectroscopy did not reveal this. Also, two specimens were identified as acacia monofloral honey by pollen and organoleptic analyses. Instead, NMR spectroscopy showed that one of these samples did not meet the requirements for acacia honey by physicochemical parameters.

MATERIAL AND METHODOLOGY

Biological material

The research used honey samples obtained from the Kyiv, Odesa and Dnipro regions obtained in the spring and summer that could be realized on the market as acacia. Honey from bees was collected in March – May 2018. The samples were stored in glass containers at +15 to +20 °C away from sunlight until the start of the research in August 2018.

Honey samples

Sample A0 was obtained from nectar mixed with sugar syrup by bees (stored in the fall), which they processed into honey, put in cells and sealed; the sample was obtained from bees in the form of honey in March 2018 (Kyiv region). A1 – honey centrifuged from bee honeycombs in May 2018 (Kyiv region); A2 – in June 2018 (Kyiv region); B1 – in May 2018 (Odesa region); B2 – May 2018 (Dnipro region). Acacia bloom in Ukraine is due in May.

Organoleptic analysis

The analysis was conducted at the laboratories of the Department of Certification and Standardization of Agricultural Products, National University of Life and Environmental Sciences (NULES) of Ukraine, with the use of the methodology and requirements specified according to **DSTU 4497:2005 (2007)**.

Physicochemical analysis

The analysis was conducted at the Ukrainian Laboratory of Quality and Safety of Agricultural Products.

Chemicals

All chemicals were of analytical grade and were purchased from LLC "NVP"ALFARUS" (UA).

Mass fraction of water

The mass fraction of water was determined on an LR-01 laboratory refractometer (Maselli Misure s.p.a., Italy) using a standardized technique according to **DSTU 4497:2005 (2007)**.

Hydroxymethylfurfural, diastasis and proline

Hydroxymethylfurfural, diastasis and proline were investigated with a KFC-3 photocalorimeter (Russia) using standardized methods according to **DSTU 4497:2005 (2007)**. All the techniques have been pre-elaborated and described in detail for acacia honey of different geographical origin **(Adamchuk, Suchenko and Akulonok, 2019)**.

NMR spectrometry

The analysis was carried out at the laboratories of Bruker BioSpin GmbH **(Bruker, 2020)** (Germany) using Avance Neo and Benchtop NMR Fourier 80 devices (Germany) and a technique which allows the acquisition of a fingerprint to confirm the authenticity of the product or reveal adulteration by addition of sweetener **(Schievano et al., 2020)**. All NMR samples were prepared by dissolving ~240 mg honey in phosphate buffer solution (KH_2PO_4 in

D_2O), adjusting the honey (mg)/buffer (mL) ratio to exactly 240 mg.mL^{-1}. The pD was carefully adjusted from 133 to 4.40.

Botanical origin

Botanical origin was determined according to the adapted harmonized methods of melissopalynology **(Von Der Ohe et al., 2004)** using a Sigeta Biogenic Led Trino Infinity microscope (China) with 400× and 2000× magnification based in the laboratories of the Department of Certification and Standardization of Agricultural Products, NULES of Ukraine with the use of **DSTU 4497:2005 (2007)**. Identification of plants was carried out according to the methodology and experience of the team of the international network AgroBio*Net* **(Brindza et al., 2018)**.

Statistical analysis

Basic statistical analysis was carried out using SAS programming packages (SAS System V9.2). Correlation coefficients were calculated by CORR analysis **(SAS, 2009)**.

RESULTS AND DISCUSSION

In organoleptic research on honey for compliance with acacia variety requirements, evaluations were made by colour, taste, aroma, consistency, crystallization and the presence of signs of fermentation and mechanical impurities. The results of the organoleptic research are shown in Table 1.

According to the results of the organoleptic evaluation, only samples B1 and B2 met the requirements for acacia honey. Sample A0 had the lowest compliance (14%).

Among the physicochemical indicators were those that indicate the naturalness and enzymatic activity of honey – diastase number, and proline and hydroxymethylfurfural content. Also, they have values that are different from other honey varieties. The results are shown in Table 2.

According to the conducted studies, in terms of the mass fraction of water, hydroxymethylfurfural and diastasis number, all tested honey samples met the requirements. The highest proline content was found in the falsified sample A0 – 483.7 ±0.36 mg.kg^{-1}, which is 76% and 54% higher than the other samples from Kyiv region, which did not correlate with acacia varieties by organoleptic indicators; and 71% higher than the honey samples that corresponded to acacia species in organoleptic indicators (B1 and B2).

In general, according to the physicochemical parameters investigated, samples A0 and A2 corresponded to the requirements of **DSTU 4497:2005 (2007)** for acacia honey. At the same time, according to the requirements of another current regulatory document, which is used today for the circulation of the product in the country and its export, all the research samples met the established criteria.

Thus, according to the results of honey studies, which were carried out by standardized methods, the data obtained differed by organoleptic and physicochemical parameters in accordance with the acacia variety. By the latter, two samples, one of which was a pre-prepared falsification (A0), fully met the requirements for acacia honey compliance. This speaks about the need to review complex methods of evaluating honey to identify it as an acacia variety.

Table 1 Organoleptic honey research.

No.	Indicator		Honey sample				
	Name	Characteristic	A0	A1	A2	B1	B2
1.	Color	Colorless, light yellow, transparent	–	–	–	+	+
2.	Crystallization	Absent	–	+	+	+	+
3.	Signs of fermentation	Prohibited	–	–	–	+	+
4.	Taste	Sweet, delicate, without any foreign flavors	–	–	–	+	+
5.	Aroma	Very weak, no odours	–	–	–	+	+
6.	Consistency (liquid)	A small amount of honey is left on the spatula, which quickly drains into small drops	–	+	–	+	+
7.	Mechanical impurities	Prohibited	+	+	+	+	+
Compliance with acacia honey variety, %			14	43	29	100	100

Note: (+) – meets the requirements for acacia honey; (–) – does not meet the requirements for acacia honey.

Table 2 Physicochemical honey research (n = 2).

Sample	Mass fraction of water, no more than, %		HMF, no more than, mg.kg^{-1}		Diastasis, no less than, Goethe units		Proline, no less than, mg.kg^{-1}	Compliance with the DSTU requirements for acacia honey, %
Standard^{D}	**18.5^{HG}**	**21^{FG}**	**10**	**25**	**15**	**5**	**200**	
Standard^{N}	**20**		**40**		**3**		**100**	
A0	16.6		7.9 ±0.19		20.3 ±0.11		483.7 ±0.36	100
A1	15.0		7.7 ±0.00		19.1 ±0.16		118.2 ±0.73	75
A2	16.2		7.4 ±0.19		31.0 ±0.05		220.5 ±0.36	100
B1	16.6		2.2 ±0.10		10.0 ±0.05		141.2 ±0.73	75
B2	16.8		1.6 ±0.10		9.6 ±0.10		139.2 ±0.10	75

Note: D –standard defined in **DSTU 4497:2005**; N –standard defined in **Nakaz MinAPK No. 330** dated 19.06.2019; HG – highest grade; FG – first grade; HMF – hydroxymethylfurfural.

Table 3 NMR spectroscopy of sugars in honey, g.100g^{-1}.

No.	Indicator	A0	A1	A2	B1	B2
1.	Glucose + fructose	74.1	74.4	73.8	75.6	78.2
2.	Fructose/glucose	1.34	1.54	1.38	1.68	1.30
3.	Fructose	42.4	45.1	42.7	47.4	44.2
4.	Glucose	31.7	29.3	31.1	28.2	33.9
5.	Saccharose	0.7	2.5	1.1	3.5	0.6
6.	Turanose	1.7	2.7	2.4	2.9	1.9
7.	Maltose	2.4	2.7	2.6	3.3	2.2
8.	Raffinose	0.2	0.3	0.2	0.2	0.1

Table 4 Honey-Profiling^{TM} profile for sugars according to NMR spectroscopy.

Indicator	A0	A1	A2	B1	B2
Glucose + fructose	62.8 86.2	62.8 86.2	62.8 86.2	62.8 86.2	62.8 86.2
Fructose/glucose	0.88 1.58	0.88 1.58	0.88 1.58	0.88 1.58	0.88 1.58
Fructose	32.9 45.4	32.9 45.4	32.9 45.4	32.9 45.4	32.9 45.4
Glucose	25.6 43.9	25.6 43.9	25.6 43.9	25.6 43.9	25.6 43.9
Saccharose	<0.5 2.9	<0.5 2.9	<0.5 2.9	<0.5 2.9	<0.5 2.9
Turanose	0.4 3.0	0.4 3.0	0.4 3.0	0.4 3.0	0.4 3.0
Maltose	<0.5 3.6	<0.5 3.6	<0.5 3.6	<0.5 3.6	<0.5 3.6
Melezitose	<1.0 1.1	<1.0 1.1	<1.0 1.1	<1.0 1.1	<1.0 1.1
Maltotriose	<1.0 g.100g^{-1} in reference dataset	<1.0 g.100g^{-1} in reference dataset	<1.0 g.100g^{-1} in reference dataset	<1.0 g.100g^{-1} in reference dataset	<1.0 g.100g^{-1} in reference dataset
Gentiobiose	<0.3 0.4	<0.3 0.4	<0.3 0.4	<0.3 0.4	<0.3 0.4
Raffinose	<0.1 0.4	<0.1 0.4	<0.1 0.4	<0.1 0.4	<0.1 0.4
Mannose	<0.05 0.23	<0.05 0.23	<0.05 0.23	<0.05 0.23	<0.05 0.23

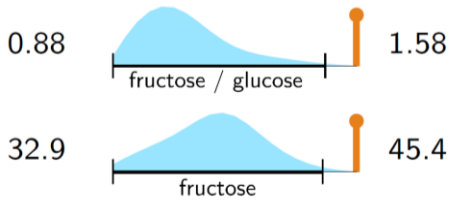

Figure 1 Discrepancy between the honey profile of sample B1 and the reference NMR database.

The next step was the evaluation of honey samples using a modern NMR spectroscopy technique that is widely used in internationally accredited laboratories. Its core consists of the complex analysis of physicochemical parameters, on the basis of which the profile of the honey is programmatically constructed and its botanical origin determined. NMR spectroscopy of honey made it possible to analyse 12 indicators for sugar, including the ratio of fructose to glucose (Table 3).

According to our data, the ratio of fructose to glucose (FG) ranged from 1.30 to 1.68. According to studies of the acacia honey profile by other scientists **(Marghitas et al., 2010)**, it is known that the FG index ranges from 1.43 to 1.57 (the content of acacia pollen grains in honey was in the range from 20.0% to 30.2%). According to other data **(Oddo et al., 2004)**, the highest average FG value for acacia honey from Europe is 1.61 (715 samples tested). At the same time, honey from Ukraine is accepted for export as acacia if FG is more than 1.5.

According to **Marghitas et al. (2010)** and **Schievano et al. (2020)**, sucrose and maltose content may also indicate botanical origin. Thus, for acacia honey, **Marghitas et al. (2010)** found that the sucrose content in acacia varieties ranged from 0.59 to 2.50 g.100g^{-1}, and the maltose content from 2.31 to 3.07 g.100g^{-1}. In addition, **Nakaz MinAPK No. 330** allows a sucrose content of not more than 10 g.100g^{-1}, and maltose is not specified. Thus, for sucrose, the honey we studied met the requirements for acacia variety compliance. The presence of sugars other than those listed in Table 3 was investigated, but their quantitative values were beyond the limit of determination. Among these were melezitose (limit of determination – 1 g.100g^{-1}), maltotriose (1 g.100g^{-1}), gentiobiose (0.3 g.100g^{-1}) and mannose (0.05 g.100g^{-1}).

The Honey-Profiling^{TM} profile was built from the set of indicators using software for NMR spectroscopy (Table 4).

Honey-Profiling^{TM} indicates a deviation from the norm for certain indicators. The profile of honey sample B1 contains red marks, which indicates the need for a more detailed analysis of individual indicators for this honey sample or re-analysis with NMR spectrometry (Figure 1).

If a negative result is obtained again, such samples are considered falsified. **Schievano et al. (2020)** recommend further CSSF-TOCSY experiments for refining the analysis to detect the sugar profile of honey and its falsification with sugar. According to their latest results, the level of fructose in the honey of European origin ranges from 36.7 to 49.4 g.100g^{-1}, which coincides with our results. NMR spectroscopy allows analysis of the acid composition of honey (Table 5).

Table 5 Acid NMR spectroscopy, mg.kg^{-1}.

		Basic criteria				
No.	**Organic acids:**	**A0**	**A1**	**A2**	**B1**	**B2**
1.	Citric acid	79	71	61	72	58
	Amino acids:	**A0**	**A1**	**A2**	**B1**	**B2**
2.	Alanine	11	7	8	<LOQ	8
3.	Proline	560	318	694	248	307
4.	Valine	11		<LOQ		
	Additional parameters of fermentation, processing and origin					
5.	Acetic acid	14	18	15	11	<LOQ
6.	Ethanol	21	6		<LOQ	
7.	Lactic acid	41	13	55	<LOQ	11
8.	Formic acid	25	27	50	10	21
9.	Pyruvic acid	15	<LOQ	21	<LOQ	
10.	Succinic acid	17	7	21	5	7

Note: <LOQ – below the limit of quantification.

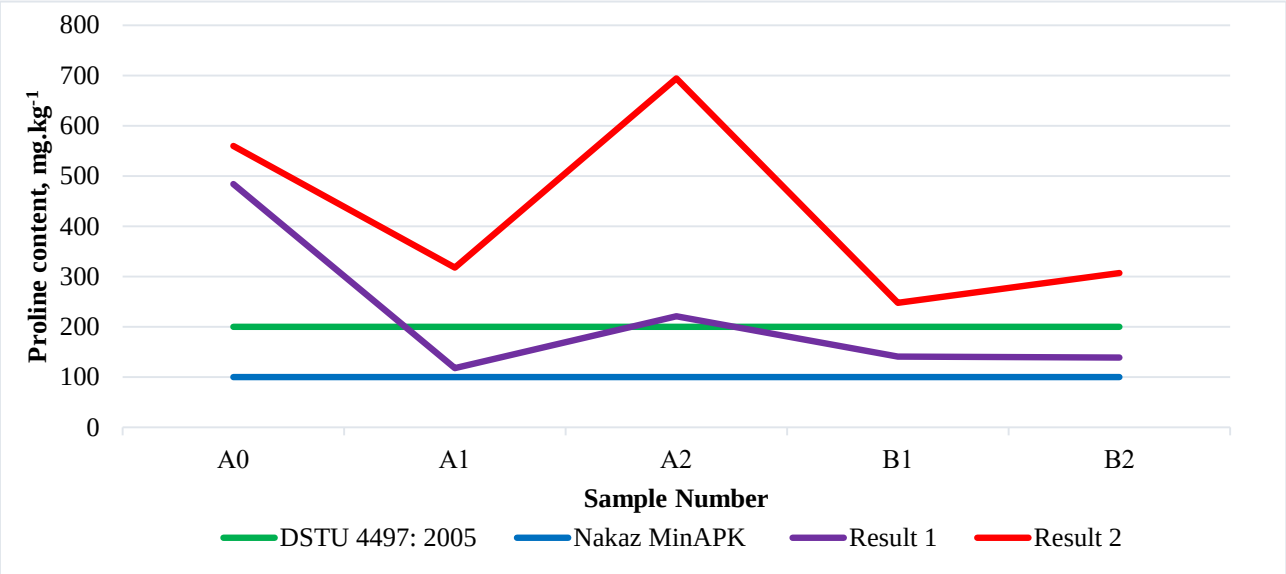

Figure 2 Proline in the investigated honey samples, mg.kg^{-1}: result 1 – by the method standardized according to **DSTU 4497:2005** "Natural honey. Specifications"; result 2 – by NMR spectrometry.

Among organic acids, citric acid was found in the range from 58 to 79 mg.kg^{-1}; malic (limit of detection – 100 mg.kg^{-1}) and quinic acids (300 mg.kg^{-1}) were beyond the limit of quantification qualification. High proline content was characteristic of all samples tested. The highest was found in sample A2 – 694 mg.kg^{-1}. In general, the results from proline studies differed from those obtained using standardized techniques. Some of the amino acids were beyond quantification, namely aspartic acid (detection limit – 150 mg.kg^{-1}), glutamine (200 mg.kg^{-1}), leucine (40 mg.kg^{-1}), tyrosine (50 mg.kg^{-1}) and phenylalanine (100 mg.kg^{-1}).

A comparison of the proline indicator results obtained according to the requirements of **DSTU 4497:2005 (2007)** and the criteria for the honey composition according to **Nakaz MinAPK No. 330 (2019)** is shown in Figure 2.

In addition, special substance markers are used to identify honey from individual geographical regions. For example, for manuka honey (New Zealand), it is methylglyoxal. The markers in the test specimens were beyond quantification, namely 3-phenyl lactic acid (quantification limit – 300 mg.kg^{-1}), dihydroxyacetone (20 mg.kg^{-1}), kynuric acid (60 mg.kg^{-1}), methylglyoxal (30 mg.kg^{-1}) and shikimic acid (80 mg.kg^{-1}).

Thus, NMR spectrometry makes it impossible to determine honey as regional (geographical definition of honey from Ukraine).

Also, additional indicators were used to control the parameters of fermentation, processing and origin (Table 5). Among these, acetic acid was detected in four test specimens, ranging from 11 to 14 mg.kg^{-1}; ethanol in two – from 6 to 21 mg.kg^{-1}; lactic acid in four – from 11 to 55 mg.kg^{-1}; formic acid in all samples in the range from 10 to 50 mg.kg^{-1}; pyruvic acid in two – from 15 to 20 mg.kg^{-1}; and succinic acid in all samples – from 5 to 21 mg.kg^{-1}. Other substances were beyond detection, namely 2,3-butanediol (detection limit – 20 mg.kg^{-1}), 5-hydroxymethylfurfural (5 mg.kg^{-1}), acetoin (20 mg.kg^{-1}) and fumaric acid (5 mg.kg^{-1}). All of them give an opportunity to evaluate the safety and quality of honey in terms of physicochemical composition, but do not give an understanding of its botanical origin.

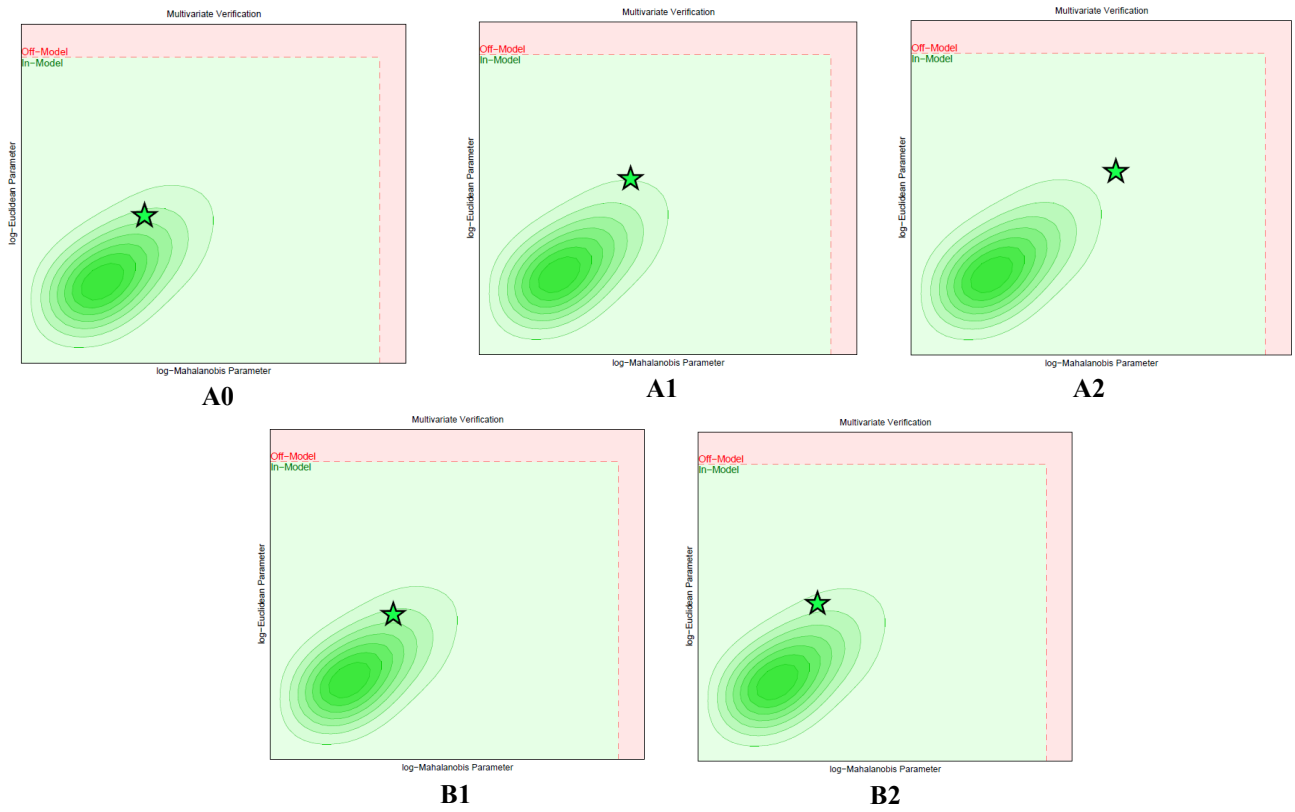

Figure 3 Multivariate verification models of investigated honey.

Upon completion of the test, software verification analysis was applied and graphical models of the studied honey samples were automatically constructed (Figure 3).

Displaying the results in the form of multivariate verification models gives us a visual understanding of how much a honey sample corresponds to the declared botanical origin by the complex of physicochemical parameters studied. Thus, samples A0 and B1 are closest to the centres of correspondence (intense green colour). For this, A0 is a falsification prepared in advance.

Schievano et al. (2020) claimed that no significant differences were found in the sugar profiles in a group of European (i.e. Italian and Eastern European) acacia honeys. This allowed them to assume the minor influence of different geographical origin on the sugar profile. However, in our opinion, the sugar profile should not change significantly if the honey is natural and not falsified with sugar.

At the same time, **Schievano et al. (2020)** found that the sugar profile of Chinese honey purchased on the Italian market did not match. They commented on the significant decrease in endogenous sugar, which they believe is the result of feeding bees with sugar syrups during the honey harvest period. This also confirms our previous conclusions regarding the confirmation of falsification.

However, in our sample A0, which was falsified using the method of feeding bees with sugar before winter, NMR spectrometry did not detect falsification.

Along with this, there are known methods **(del Campo et al., 2016; Spiteri et al., 2016)**, which allow the identification in honey of some species of plants, such as eucalyptus, heather, lavender, orange, thyme and rosemary. For this purpose, the authors used analysis of carboxylic acids (acetic, formic, lactic, malic and succinic acids), amino acids (alanine, phenylalanine, proline and tyrosine), carbohydrates (α- and β-glucose and fructose), ethanol and hydroxymethylfurfural.

Gerhardt et al. (2018) presented results where they were able to distinguish three varieties of honey, namely canola, acacia and honeydew honeys, with a predictive accuracy of 98.6% using additional HS-GC-IMS profiling.

Schievano et al. (2020) asserted that melissopalynological analysis and SCIRA also do not reveal any particular anomalies in Chinese acacia honey samples. This is probably due to new methods of falsification. According to our previous research **(Adamchuk, Suchenko and Akulonok, 2019)**, pollen analysis of honey using harmonized methods **(Von Der Ohe et al., 2004)**, which we adapted to the laboratory conditions of Ukraine, is reliable in the botanical identification of honey. Thus, in the 30 acacia honey samples tested, we found 20% to 30% *Robinia pseudoacacia* pollen grains. This indicates compliance with the current standards for acacia monofloral honey in Ukraine **(Nakaz MinAPK No. 330, 2019)**. In this case, the tested honey samples also met the requirements for diastasis and hydroxymethylfurfural.

For evaluation of the botanical origin of honey, the NMR spectrometry method proved to be ineffective for our samples. All honey was programmatically identified as polyfloral. Therefore, we applied the harmonized method of melissopalynology **(Von Der Ohe et al., 2004)**, which we adapted to the conditions of the laboratory at the Department of Certification and Standardization of Agricultural Products, NULES of Ukraine; and improved the way of counting and identifying pollen grains of different types of plants widespread in Ukraine.

The results of pollen analysis are shown in Table 6.

Table 6 Botanical origin of honey.

Sample	Pollen grains, %			
	Predominant ≥20%	**Secondary 10 – 20%**	**Minor ≤10%**	**Including ≤1%**
A0	-	Asteraceae *Barbarea vulgaris*	*Draba nemorosa* *Lamium album* *Trifolium repens* *Bistorta officinalis*	*Convolvulus arvensis* *Agrimonia eupatoria* Anemophilic species of plants
A1	-	*Robinia pseudoacacia* (17%) *Acer negundo* *Aesculus hippocastanum* *Ajuga reptans*	*Acer platanoides* *Acer tataricum* *Lamium album* *Salix* spp. *Tilia* spp. *Clinopodium vulgare* *Lycopus* spp.	Asteraceae Anemophilic species of plants
A2	-	*Robinia pseudoacacia* (16%) *Brassica napus* *Centaurea* spp.	*Ficaria verna* *Swida alba* *Ballota nigra* *Melilotus officinalis* *Tilia* spp. *Rosa canina* *Salix alba* *Catalpa bignonioides*	*Fragaria vesca* Fruit tree *Juglans* spp. Anemophilic species of plants
B1	*Robinia pseudoacacia* **(33%)**	Fabaceae *Acer* spp.	*Caragana arborescens* *Bunias orientalis* *Barbarea vulgaris* *Nasturtium officinale* *Brassica napus* *Salix* spp. *Lamium purpureum* *Primula* spp.	*Anemone* spp. Anemophilic species of plants
B2	*Robinia pseudoacacia* **(39%)**	*Gleditsia triacanthos* *Elaeagnus argentea*	*Salix* spp. *Vicia cracca* *Melilotus officinalis* *Quercus* spp. *Rhus hirta* *Lotus corniculatus* *Veratrum lobelianum* *Clinopodium vulgare* *Gagea* spp. *Allium ursinum* *Taraxacum officinale* *Cornus mas* *Geum rivale* *Ajuga reptans* *Scrophularia vernalis* *Tussilago farfara* *Amorpha fruticosa*	*Lamium purpureum* *Lamium maculatum* *Lamium galeobdolon* *Ribes* spp. Anemophilic species of plants

According to the results of the research, samples B1 and B2 correspond to the acacia variety by botanical origin. They contain 33% and 39% acacia (*Robinia pseudoacacia*) pollen grains, respectively. According to **Nakaz MinAPK No. 330 (2019)**, the only normative document regulating the criteria for monofloral honey varieties, for acacia honey, the *Robinia pseudoacacia* pollen grain content should be at least 20%.

Samples A1 and A2 also contained acacia grains, 17% and 16%, respectively. Along with this, sample A1 also contained a large amount of pollen from maple (*A. negundo*, *A. platanoides*, *A. tataricum*), horse-chestnut (*A. hippocastanum*) and bugleweed (*A. reptans*). Sample A2 contained a significant amount of pollen from rapeseed (*B. napus*) and several species of *Centaurea* spp., indicating the activation of flight activity of bees on field honey plants after the end of acacia flowering. The presence of willow (*Salix* spp.) pollen grains indicates a failure of adherence to acacia honey production technology, which is based on the use of additional cases of the 145-frame hive. Pollen from willow, which is an early pollen source, can only be present in the hive body.

The conducted studies confirm the need for a comprehensive approach to the identification of the

botanical origin of honey for its conformity to the acacia variety. There is a need to revise the physicochemical indicators regarding the conformity of honey obtained in Ukraine to the acacia variety. After all, even the modern NMR spectrometry technique indicated that sample A0, which did not contain acacia pollen grains and was specially fabricated, was acacia honey.

CONCLUSION

Identification of the botanical origin of monofloral kinds of honey, in particular acacia, should be carried out in the following sequence: pollen analysis (by dominant pollen grains), safety (presence of antibiotics, pesticides), physicochemical parameters according to international requirements, organoleptic parameters. The methods of determination and requirements for physicochemical indicators of honey require revision, improvement and harmonization.

In the future, the safety of acacia honey in terms of the content of contaminants that may be caused by anthropogenic environmental load requires analysis and research. Also, there is a necessity to find markers that will indicate the geographical origin of Ukrainian kinds of honey and thus protect them from falsification from other countries.

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