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ORIGINAL ARTICLE

Assessment of Acacia monofloral honey

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This work aims to formulate scientifically based criteria for assessing the quality of acacia monofloral honey. Honey sampling, analysis of organoleptic and physicochemical parameters were carried out according to DSTU 4497:2005 "Natural honey. Technical conditions". The identification of pollen grains of honey plants was carried out according to the atlas of pollen grains. We analyzed 50 acacia honey samples that came to the laboratory from different regions of Ukraine. We found that the content of dominant pollen from acacia ranged from 5 to 25%. In terms of pollen analysis, ten acacia honey samples belong to monofloral honey. We showed that the average value of proline in acacia honey ranged from 130 mg/kg to 195±53.9 mg/kg, electrical conductivity from 0.1±0.01 mS/cm to 0.2±04 mS/cm, the ratio of fructose to glucose should be at least 1.4. The necessity is justified concerning making changes to DSTU 4497:2005 "Natural honey. Technical conditions" and harmonization with international requirements for indicators of proline concentration and electrical conductivity in honey from acacia bee. The following criteria are proposed for assessing acacia monofloral honey quality: specific composition of pollen grains, proline content, conductivity indicators, and the ratio of fructose to glucose.
Keywords: acacia honey, diastase number, proline, electrical conductivity, pollen analysis.

Introduction

Bee honey is one of the most complex natural products; in its content, more than four hundred various components were found. Honey has excellent taste, diet, and nutritional properties. It correlates with nutritional and energy value with chocolate, cocoa, walnuts (Safiullin et al., 2010). It has a large number of enzymes. Organic acids improve appetite, enhance the secretory function of the gastric mucosa. Honey also has antibacterial, pharmacological, and immunobiological properties. The benefits of honey in human nutrition are undeniable (Elizarova, 2007).

Bees produce honey in natural and natural-anthropogenic landscapes; the finished product's quality substantially depends on their condition. The chemical composition of honey is not constant and depends on the source of nectar collection, the area where honey plants grow, the time of collection, honey maturity, bee breed, weather, and climate conditions. However, some honey compositions are characteristic and typical (Marghitas et al., 2010; Smanalieva, 2008). In recent years, the Institute staff has carried out a wide range of studies to assess the quality of honey of various botanical origin, produced in various Ukraine regions, and significant experimental material has been accumulated (Lazareva et al., 2017; Postoienko et al., 2017).

According to its botanical origin, natural bee honey is divided into floral, honeydew, and mixed (a natural mixture of floral and honeydew honey) (Directive, 2014; Codex Alimentarius Commission, 2001). Floral honey is formed as a result of processing the nectar of flowers by bees. It can be monofloral, i.e., from the nectar of one (or mainly one) plant species, and polyfloral (mixed), i.e., from the nectar of several plants (Bogdanov, 2009; Horn, 2007; DSTU 4497, 2005).

Monofloral honey is determined by the content of pollen grains of the main plant species, i.e., nectariferous ones: acacia, linden, sunflower, buckwheat, cotton, sainfoin, coriander (Kowalsk, 2013; Kulakov and Rusakova, 2002; Yarovaya and Savitskaya, 2001). Monofloral honey is in great demand in international markets and needs to improve methodological approaches to identify and assess its quality. The lack of optimized methodological approaches to identifying monofloral honey in Ukraine leads to the fact that cheap varieties of honey are considered valuable, for example, sunflower honey for linden one, melilot. Moreover, almost all outlets sell rare transparent acacia honey in winter. One can see on sale "raspberry" honey of brown color, "honeydew" honey with a pronounced aroma and taste of buckwheat, "acacia honey", which at best is sainfoin or honeycomb one. It is impossible not to say about the rich palette of "unique" honey collected from sea buckthorn, St. John's wort, sweet briar (Brovars'kyi et al., 2011).

Acacia honey is the most transparent and most expensive of all honey varieties (Czipa et al., 2019). Bees collect it from two acacia species: white (*Robinia pseudoacacia* L.) and yellow (*Caragana arborescens* L.). The color of acacia honey depends on the type of plant, which also affects some organoleptic indicators, such as aroma, but they are similar in biochemical parameters and medicinal properties (Aboud et al., 2011). Honey from white acacia in a liquid form is transparent, and upon crystallization, acquires white color. Honey from yellow acacia has a light yellow color with a slight greenish tint, and upon crystallization, it becomes white-yellow. Many honey varieties have a diastase number of 8 to 24 on a Shade or Gaute scale (Serrano et al., 2004; Huidobro et al., 1995). Acacia honey and citrus honey are usually low in enzymes (Presano Oddo 2004).

Acacia honey may not crystallize for a long time (from one to two to three years) at room temperature. In principle, it can be attributed to rare types of honey. Since Ukraine does not have an approved method for determining monofloral honey, its solution is essential and relevant at present.
This work aims to substantiate the criteria for assessing the quality of monofloral acacia honey.

Materials and Methods

Fifty samples of acacia honey from different regions of Ukraine for the laboratory methods of research were used.
In the study of acacia honey samples, the mass fraction of water, diastase activity, and the mass fraction of reducing sugars and sucrose, hydroxymethylfurfural (HMF) content, proline content, electrical conductivity, the definition of the species composition of pollen grains were determined. Investigations of honey of organoleptic and physicochemical indicators were carried out according to the normative documents specified in State Standard of Ukraine (DSTU) 4497: 2005 "Natural honey. Technical requirements" (DSTU 4497, 2005).
The refractive index used a refractometer previously tested on distilled water to determine the mass fraction of water in pure honey. The method is based on the change of refraction (diffraction) by the honey solution of light rays, depending on the content of dry residue. The more solids, the higher is the refractive index.
Diastase (amylase) is a glycoprotein with a molecular weight of 24,000 to 25,000 Da and has a-amylolytic properties (Bergner et al., 1975). Determination of diastase number is based on the ability of this enzyme to split up starch into amylodextrins. Quantitative determination of diastase enzyme activity was developed by F. Gothe (1914). This method is still used today. Units of Gothe denote the amount of 1% starch solution was split up by diastase (amylase) contained in 1 g of honey (calculated on dry substances) for one hour at 40°C to a substance which is not painted in blue color.
Determination of mass fraction of reducing sugars and saccharose was carried out according to the normative documents specified in DSTU 4497: 2005 "Honey natural. Specifications" (DSTU 4497, 2005). Following this normative document's requirements, the standard solution of invert sugar and the construction of the calibration graph was prepared. The optical density of parallel samples was measured using a photo electro colorimeter at a wavelength of 440 nm against water in a dish with 10 mm thickness.
The determination of HMF is based on obtaining coloration in the case of HMF reaction with barbituric acid and para toluidine.
Determination of Proline content is an essential criterion for honey's natural origin and Proline amino acid presence. The method is based on the spectrophotometric determination of the stained complex of proline with ninhydrin. The electrical conductivity of honey of different botanical origins was investigated by a conductometric method developed by the Commission at the International Beekeepers Organization "Apimondia".
The obtained data were processed statistically and mathematically using methods of variational statistics using the program "Microsoft Excel 15.0 with the calculation of arithmetic mean (M), standard error (m) (Mazur, 1997). The determination of the species composition of pollen grains was carried out by the microscopic method. The identification of pollen grains of nectariferous plants was carried out using an atlas (Karpovych et al., 2015; Burmistrov and Nikitina, 1990).

Results

The results of the organoleptic assessment of acacia honey quality are given in Table 1.

Table 1. Organoleptic evaluation of acacia honey samples from different regions of Ukraine.

Total samples	Compliance with acacia honey, %		
	By color	By consistency	By taste
50 (100%)	80	88	40

Organoleptic studies found that 40 samples of "acacia honey" out of the 50 ones correspond to acacia honey's typical characteristics by color. By consistency, 44 samples (6 samples with signs of varying degrees of crystallization) can be classified as liquid acacia honey. By taste, 20 samples are classified as acacia honey. According to organoleptic properties, the results obtained show the correspondence of studied product to the typical characteristics (from 40 to 88%). This justifies the possibility of their use as criteria for assessing the monofloral acacia honey.
The results of the study of specific physicochemical parameters of acacia honey are shown in table 2.

Table 2. Specific physicochemical characteristics of acacia honey.

Indicators	Area of production, regions			
	Mykolaiv (n=6)	Cherkasy (n=18)	Chernihiv (n=18)	Kyiv (n=8)
Mass fraction of water,%	16.7±0.4	17.3±0.9	17.1±0.3	18.1±1.1
Mass fraction of renewable sugars,%	78±0.4	85.3±3.4	82.1±3.1	81.6±0.6
Mass fraction of sucrose, %	2.9±0.1	2.9±0.1	3.2±0.6	2.9±0.2
Diastase number, Gothe units	12.2±2.8	10.8±0.6	11.5±0.5	12.1±2.0
Electrical conductivity, mS/cm	0.2±0.02	0.1±0.01	0.2±0.04	0.1±0.02

The data obtained show the compliance of all parameters with the requirements of national DSTU 4497:2005. However, it should be noted that four samples of honey from acacia in the Mykolaiv region out of 6 ones have an increased diastase number, which is not typical for this kind of product. Four honey samples (from the Kyiv region), out of 8, have a diastase number above 12 Gothe units.

These samples also have increased electrical conductivity of honey, which indicates the participation of other honey plants in forming this type of honey.
It is known that the essential criteria for assessing the quality of honey are indicators characterizing its naturalness, such as the ratio of glucose to fructose and the content of proline (Lazareva and Postoienko, 2017; Postoienko and Lazareva, 2017). We found that for monofloral acacia honey, the ratio of fructose to glucose should be at least 1.40, the proline content varies in the range of 130.0±8.0-195±53.9 mg/kg. It was also found that the content of pollen grains of the dominant plant (acacia grains) was in the range from 11 to 25% (Table 3).

Table 3. Naturalness criteria (proline, glucose to fructose ratio) and the botanical origin of acacia honey.

Indicators	Area of production, regions			
	Mykolaiv	Cherkasy	Chernihiv	Kyiv
Proline, mg/kg	183.1±12.0	176.2±35.3	195±53.9	130.0±8.0
Glucose to fructose ratio	1.5±0.04	1.5±0.1	1.5±0.1	1.4±0.1
% pollen grains of acacia	11±3.0	25±3.0	15±3.0	16±4.0

Table 4 shows the pollen analysis results concerning acacia honey of different territorial origins. In samples from the Chernihiv region, the dominant pollen content from acacia ranged from 11 to 17%. There were pollen grains of amorpha from 7 to 15%, of gleditsia to 13%, clover, dead-nettle, fruit trees, willow, sweet briar, and elder. According to samples from the Cherkasy region, the content of pollen grains in acacia honey was from 14 to 21%. In addition to acacia grains, 11% of willow pollen grains, 9% Gleditsia, 4% pea, up to 9% fruit trees, clover from 9 to 15%, cruciferous from 3 to 9 were present. Analysis of pollen grains content is an essential and necessary method for establishing the botanical origin of honey.

Table 4. The results of pollen analysis of honey from acacia of different territorial origin.

Type of honey, the region of Ukraine	Pollen grains of the dominant plant,%	Pollen grains of other plants,%	Notation
Cherkasy, n=18	14–21	Up to 5% cruciferous	Monofloral crystallizes quickly
Kyiv, n=8	10-18	Up to 7% cruciferous	Crystallizes quickly
Chernihiv, n=18	11–17	up to 15% of different fruit trees	Monofloral
Mykolaiv, n=6	9–13	87–91	polyphlore

Discussion

A wide range of studies has been carried out to assess the quality of acacia honey produced in different Ukraine regions. Significant experimental material has been accumulated that makes it possible to substantiate the criteria for assessing acacia honey quality, which qualifies it as "acacia monofloral honey". Evaluation of honey quality should be comprehensive and carried out sequentially in several stages (steps).
At the first stage (first step), organoleptic indicators should be determined to release properties specific for monofloral honey from acacia: consistency, color, taste. Even though the organoleptic indicators are entirely subjective and require specific skills and experience, even with their use, it is already possible to carry out a qualitative assessment for the monoflority of acacia honey. The next step in assessing acacia monofloral honey is the use of methods characterizing the physicochemical properties, quantitative indicators of which emphasize the specificity of acacia monofloral honey (diastase, electrical conductivity, reducing sugars, sucrose).
Our studies have shown that monofloral acacia honey had low diastase activity (10.8±0.6 Gothe units) and low electrical conductivity (0.1±0.01 mS/cm). Electrical conductivity refers to the indicators that make it possible to conclude honey's origin and distinguish honeydew from nectar (Tikhonova et al., 2013; Acqarone et al., 2007; Tsevegmid, 2005). Acacia honey had the lowest values for electrical conductivity, which coincides with Lazarevic et al. (2012). The critical point is that in DSTU 4497:2005 "Natural honey. Technical conditions," the value of electrical conductivity should begin with 0.2 units. We have found that acacia honey's electrical conductivity does not meet national requirements, but they comply with EU standards. This justifies the national regulatory framework's harmonization with world requirements for this indicator (Codex Alimentarius Commission, 2001).
The third stage of the evaluation of acacia monofloral honey that we propose is determining criteria whose quantitative indicators characterize its naturalness (proline, glucose to fructose ratio) and botanical origin in terms of pollen composition.
Proline is an amino acid that is one of the most important components of honey. The proline content is used as a criterion for this product's naturalness and maturity (Tsevegmid, 2006). However, today, there are no uniform requirements for the quantitative parameters of the product's proline content. According to the German Union of Beekeepers' agreement, the proline content in honey should be at least 180 mg/kg, and for enzymatically weak honey (rape, acacia, phacelia), this indicator may be lower (Horn, 2007). It should be noted that according to the requirements of DSTU 4497:2005 "Natural honey. Technical conditions," the proline content in acacia honey should be at least 200 mg/kg. The data obtained show that all the parameters analyzed comply with the requirements of DSTU 4497:2005 "Natural honey. Technical conditions" except for the values of the proline content. We found that the average value of the proline content in acacia honey was 189.0±9.2, less than indicated in the national regulatory document. This justifies the introduction of appropriate changes to the national standards. However, this indicator complies with existing EU requirements.
Depending on the melliferous plant's origin, the content of glucose and fructose in honey varies and affects its properties. The crystallization of honey depends on their ratio: honey with a large amount of fructose remains liquid longer, and honey saturated with glucose crystallizes very quickly. That is why acacia honey, in which the concentration of fructose significantly exceeds the glucose percentage, remains in a liquid state for a long time. The ratio of fructose to glucose in different honey types is diverse and ranges from 0.52 to 1.60. The value of this ratio depends on the chemical composition of nectar and the characteristics of invertase

activity. The lower this ratio (, the more glucose is in honey), the more likely honey (under different conditions) has to crystallize faster. Our data indicate that the ratio of fructose to glucose in acacia honey should be at least 1.40.
In the honey market, traders are interested in monofloral varieties of honey, botanical uniformity, and quality, confirmed by pollen analysis. To establish a botanical type of honey, the content of flower pollen of a given plant in relation to the total mass of pollen must not be lower: for acacia (from white acacia), lavender 10%; from sage 20%; heather, buckwheat, clover, linden, alfalfa, rape, citrus 30%; sunflower 35%; chestnut, sainfoin, cotton 45%.
Table 5 shows the quantitative indicators of physicochemical parameters that we proposed based on numerous studies, according to which acacia honey can be attributed to monofloral acacia honey.

Table 5. Indicators of physicochemical properties of acacia honey, proposed by us to determine its monoflority.

Indicator name	The characteristic and value of the indicator for acacia honey
Species composition of pollen grains,%, not less	10.0
Mass fraction of water,%, no more	17.0
Mass fraction of reducing sugars (to anhydrous substances),%, not less	80.0
Mass fraction of sucrose (to anhydrous substance),%, no more	10
Diastase number (to anhydrous substance), Gothe units, not less	5.0
The content of proline, mg per 1 kg, no less	180.0
Electrical conductivity, mS/cm	0.125-0.193
Glucose to fructose ratio	1.4

Thus, the proposed 3-stage in-depth analysis of acacia honey quality indicators allows us to identify the qualitative and quantitative characteristics typical for this kind of honey, giving the basis to classify it as monofloral.

Conclusions

The quality assessment of monofloral acacia honey should be comprehensive and carried out sequentially in several stages.
We established that acacia honey from different Ukraine regions differs in organoleptic, physicochemical characteristics, and pollen composition of both the dominant plant and the content of pollen grains from other plants.
According to the pollen analysis index, we registered that dominant pollen's content from acacia should not be lower than 10%. With a lower content of dominant pollen, typical signs of acacia honey change, and it acquires polyphlore honey properties. According to this criterion, the studied honey samples do not meet the national state standard requirements for monofloral honey, but they comply with the Codex Alimentarius requirements.
We proved that acacia honey has low diastase activity, which should be (to an anhydrous substance) at least 5 Gothe units.

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