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Evaluation of honeys in some quality indicators obtained from different plant species and locations

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The publication aimed to evaluate 40 samples of honey obtained from beekeepers from different types of plants (monofloral – *Aesculus hippocastanum*, *Brassica napus*, *Fagopyrum esculentum*, *Helianthus annuus*, *Phacelia tanacetifolia*, *Robinia pseudoacacia*, *Sinapis alba*, *Tillia* spp., and multiflorous) and different locations (Donetsk, Zhytomyr, Kyiv, Kharkiv, Kherson and Ivano-Frankivsk) in Ukraine in selected quality indicators. We determined significant differences between the samples in all honey quality indicators. In the collection of honey samples, we determined Moisture in the range of 15.20–23.20%, diastase 1.10–22.26 °Goethe, hydroxymethylfurfural 1.50–29.00 mg.kg^{-1}, sugar content 66.51–98.87%, sucrose 0.23–9.61% and proline content 119.29–334.31 mg.kg^{-1}. The quality indicators of the evaluated honey samples were determined within the limits according to the established criteria of *Codex Alimentarius* and EU legislation. We determined a water content of more than 20% in three honey samples. We determined lower HMF values than 3 in 4 honey samples and higher HMF values than 1 in 3 honey samples. We determined a lower proline content than 180 mg.kg^{-1} in 16 honey samples. We determined higher values of sucrose content than 5 g.100 g^{-1} in 5 evaluated honey samples. The results confirmed significant differences between the evaluated honey samples. Some samples of evaluated honey did not reach the required criteria for honey quality.

Keywords: honey, locality, moisture, diastase, hydroxymethylfurfural, sugar, sucrose, proline

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Introduction

China is the world's largest exporter of honey, with total exports of 128,330 tons in 2016. They are followed by Argentina (81,183 tons), Ukraine (54,442 tons), Vietnam (42,224 tons), India (35,793 tons), Mexico (29,098), Spain (26,874 tons), Germany (25,325 tons), Brazil (24,203 tons), and Belgium (20,816 tons) (Raezke et al., 2018).

Honey is mainly composed of water (15–20%) and two sugars (dextrose and levulose), with the presence of small amounts of at least 22 other more complex sugars (80–85%, w/w). Honey is mainly composed of sugar components, especially fructose and glucose, followed by sucrose and maltose (Kamal and Klein, 2011). Sugar in honey is responsible for the viscosity, and the hygroscopic and granulation characteristics of the honey. However, the sugar content of the honey depends on the botanical and geographical regions (Tafere, 2021).

Honey has also been reported to contain an intricate mixture of nitrogenous compounds, lactone, proteins, antibiotic-rich inhibine, enzymes, phenol antioxidants, aroma compounds, amino and organic acids, gluconic acid, phenolic acids, flavonoids, minerals, vitamins, 5-hydroxymethylfurfural (HMF) and other phytochemicals (Gheldof et al., 2002). Honey composition varies depending on its floral, geographical and entomological sources (Anklam, 1998; Gheldof et al., 2002; Tafere, 2021).

The honey composition, colour, aroma, and flavour depend mainly on the plant species and geographical regions involved in its production, and are also affected by processing, manipulation, packaging, and storage time (Tornuk et al., 2013; Escuredo et al., 2014; Karabagias et al., 2014; Tafere, 2021; Rajindran et al., 2022).

The main quality parameters of honey are diastase activity, the concentration of proline and electrical conductivity, as well as the content of free acid, hydroxymethylfurfural (HMF) and sucrose (Thrasyvoulou et al., 2018; Council EU, 2001).

Proline is the predominant free amino acid of honey, and it is a measure of the level of total amino acids (Truzzi et al., 2014). The proline content of honey is measured as a criterion for estimating the quality (Bogdanov, 2002) and the antioxidant activity of the honey (Meda et al., 2005; Saxena and Gautam, 2010) and it may be used also for characterization based on botanical origin (Bogdanov et al., 2004).

There are more than 180 substances in honey, but it is so distinctive and helpful primarily due to the presence of enzymes, which were brought by bees during the nectar processing (Aljohar et al., 2018). Activated enzymes are very sensitive to high temperatures and will lose their activity when they exceed a certain temperature. However, concentrated honey will go through high temperatures in the process of concentration, leading to the inactivation of a large number of active substances. Therefore, it is necessary to explore the effects of different heating conditions on the activity of enzymes in the honey.

Apart from several other components, honey also contains enzymes which are responsible for converting nectar and honeydew to honey. ln honey there are α-glucosidase (invertase), α- and β-amylase (diastase), glucose oxidase, catalase and acid phosphatase. The enzyme activity in honey has been widely studied for many years (Persano Oddo et al., 1999; Bonvehi et al., 2000; Vorlová and Přidal, 2002; Belay et al., 2017).

Honey contains multiple enzymes at low concentrations, the most prominent of which are diastase, invertase (α-glucosidase), glucose-oxidase, catalase, and acid phosphatase (Sak-Bosnar and Sakač, 2012). As one of the most important enzymes, diastase (α- and β-amylase) not only enriches the nutritional and therapeutic function of honey but is also taken as an important index to evaluate honey qualities. The diastase activity is usually expressed in Schade units (Kedzierska-Matysek et al., 2016), also known as the diastase number (DN), which is defined as the amount of enzyme that will convert 0.01 g of starch to the prescribed end-point in 1h at 40 °C under the conditions of the test. According to the Honey Quality and International Regulatory Standards, the diastase activity must not be less than or equal to 8, determined after processing and blending for all retail honey, and the activity must not be less than 3 for honey with naturally low enzyme content (Huang et al., 2019).

Diastase (α-amylase) is one of the predominant enzymes in honey, next to invertase and glucose oxidase, which is added to honey by the bee during the collection and ripening of flower nectar (Persano Oddo et al., 1990). One unit of diastase activity is defined as that amount of α-amylase, which will convert 0.01 gram of starch to the prescribed end-point in one hour at 40 °C. The results are expressed in Schade units per gram of honey and termed Diastase Number (DN) (Bogdanov, 2002).

An organic compound known as 5-hydroxymethylfurfural (HMF) is formed from

reducing sugars in honey and various processed foods in acidic environments when they are heated through the Maillard reaction. In addition to processing, storage conditions affect the formation of HMF, and HMF has become a suitable indicator of honey quality. HMF is easily absorbed from food through the gastrointestinal tract and, upon being metabolized into different derivatives, is excreted *via* urine. In addition to exerting detrimental effects (mutagenic, genotoxic, organotoxic and enzyme inhibitory), HMF, which is converted to a non-excretable, genotoxic compound called 5-sulfoxymethylfurfural, is beneficial to human health by providing antioxidative, anti-allergic, anti-inflammatory, anti-hypoxic, anti-sickling, and anti-hyperuricemic effects. Therefore, HMF is a neo-forming contaminant that draws great attention from scientists (Shapla et al., 2018).

Beekeeping has been widely promoted in many countries as a major contributor to rural development. Honey is a sweet and viscous liquid which has sweetness due to the presence of monosaccharides. The major constituents of honey are sugars, water, proteins, enzymes, acids and minerals, while the major causes of quality deterioration include heating at high temperatures, high moisture content, adulteration, poor packaging and poor storage conditions.

The present work is a study of the moisture, natural occurrence of sugar content, hydroxymethylfurfural (HMF), proline and diastase in different honey samples from various regions of Ukraine.

Material and methodology

Origin of honey

The pollen analysis for identification of the botanical origin of Ukrainian kinds of honey 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. They were analysed 40 samples of various kinds of honey (monofloral – *Aesculus hippocastanum*, *Brassica napus*, *Fagopyrum esculentum*, *Helianthus annuus*, *Phacelia tanacetifolia*, *Robinia pseudoacacia*, *Sinapis alba*, *Tillia* spp., and multiflorous) (Table 1).

Table 1 Basic information about evaluated honey samples

№	Origin	Region	№	Origin	Region
S01	spring grasses	Donetsk	**S21**	*Helianthus annuus*	Zhytomyr
S02	*Robinia pseudoacacia*	Donetsk	**S22**	*Helianthus annuus*	Zhytomyr
S03	multiflorous	Donetsk	**S23**	*Helianthus annuus*	Zhytomyr
S04	multiflorous	Donetsk	**S24**	*Helianthus annuus*	Zhytomyr
S05	*Helianthus annuus*	Donetsk	**S25**	*Helianthus annuus*	Zhytomyr
S06	*Robinia pseudoacacia* (commercial honey)	Ukraine	**S26**	*Helianthus annuus*	Zhytomyr
S07	*Robinia pseudoacacia* (commercial honey)	Ukraine	**S27**	multiflorous	Zhytomyr
S08	*Brassica napus*	Kyiv	**S28**	multiflorous	Zhytomyr
S09	*Robinia pseudoacacia*	Kyiv	**S29**	multiflorous	Zhytomyr
S10	*Tillia* spp.	Kyiv	**S30**	*Helianthus annuus*	Kharkiv
S11	*Robinia pseudoacacia*, *Tillia* spp., *Phacelia tanacetifolia*	Kyiv	**S31**	*Helianthus annuus*	Kharkiv
S12	*Aesculus hippocastanum*, *Robinia pseudoacacia*, *Phacelia tanacetifolia*	Zhytomyr	**S32**	*Helianthus annuus*	Kharkiv
S13	*Fagopyrum esculentum*, *Sinapis alba*, *Phacelia tanacetifolia*, *Robinia pseudoacacia*	Kyiv	**S33**	*Helianthus annuus*	Kharkiv
S14	*Tillia* spp.	Kyiv	**S34**	multiflorous (eco honey)	Ivano-Frankivsk
S15	Orchard, Forest grasses, *Robinia pseudoacacia*	Kyiv	**S35**	multiflorous (eco honey)	Ivano-Frankivsk
S16	*Robinia pseudoacacia*, *Phacelia tanacetifolia*, *Tillia* spp.	Zhytomyr	**S36**	multiflorous (eco honey)	Ivano-Frankivsk
S17	*Robinia pseudoacacia* (commercial honey)	Ukraine	**S37**	*Brassica napus*	Zhytomyr
S18	*Robinia pseudoacacia* (commercial honey)	Ukraine	**S38**	*Brassica napus*	Zhytomyr
S19	*Robinia pseudoacacia* (commercial honey)	Ukraine	**S39**	*Echium vulgare*	Kherson
S20	medicinal herbs	Kyiv	**S40**	medicinal herbs	Kyiv

Physico-chemical 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).

Methods

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, diastase activity, proline, the proportion of invert sugars and sucrose were investigated with a KFC-3 photo calorimeter (UA) using standardized methods according to DSTU 4497:2005 (2007).

Statistical analyses

Basic statistical analyses – the minimal and maximal values of the traits, arithmetic means, and coefficient of variation (CV, %) were performed using PAST 2.17. Results of the morphometric analysis were determined by mean ± standard deviation (SD) and statistical significance was estimated. The level of variability was determined by Stehlíková (1998). Hierarchical cluster analyses of similarity between plants were computed by the Bray-Curtis similarity index and were performed using PAST 2.17. All the observed traits were shown in graphic form.

Criteria for evaluating the quality of honey

By evaluating honey samples, we respected the honey quality criteria according to European directive 2001/110/EC and revised the *Codex* standard for honey. The *Codex* standard for honey adopted by the *Codex Alimentarius* Commission in 1981, revised in 1987 and 2001, has voluntary application and serves in many cases as a basis for national legislation (*Codex Alimentarius*, 2001b). The European Council followed the recommendations of *Codex* and issued Directive 2001/110/EC (EC, 2001), amended 2014/63/EU that laid down the production and trading parameters of honey within the Member States of the EU (EU, 2014).

Results and discussion

Natural honey is sticky, a viscous solution containing about 15–19% water, 80–85% carbohydrates (mainly glucose and fructose), 0.1–0.4% proteins, 0.2% ash and minor amounts of amino acids, enzymes, vitamins and other substances such as phenolic antioxidants (Buba et al., 2013; Kek et al., 2017; Živkov Baloš et al., 2019).

The total variability for the evaluated honey quality indicators is presented in Table 3 in the form of coefficients of variation. The results show that a low degree of variability was determined only for moisture (10.17%) and sugar content (12.31%). We noted a high degree of variability in the determined diastasis values (32.93%). We determined a very high degree of variability in HMF (52.80%). We determined an extremely high degree of variability in the sucrose content, up to 97.86%.

Moisture content

The water content (moisture) in honey depends on the production season, floral source, abundance of nectar flow, soil, ventilation of the beehive, colony strength, and meteorological conditions in the areas of honey production, primarily air humidity (Sousa et al., 2016; Lazarević et al., 2017). An important factor that could affect the water content is honey maturation and harvest time (Živkov Baloš et al., 2019).

The water content of honey (water-in-honey) is the quality aspect that determines the ability of honey to remain fresh and to avoid spoilage by yeast fermentation. Raw honey can have a water-in-honey content of less than 14% and the lower the water content the higher the perceived value of the honey. It is internationally recognized that good quality honey should be processed at less than 20% water content. Low water content is desirable because honey may begin to ferment and lose its fresh quality if the water-in-honey is greater than 20% (Tafere, 2021).

In the analysed honey samples (Tables 2 and 4) we found a moisture content in the range of 15.2 (S16 and S28 Multiflorous – Zhytomyr) – 23.2% (S31 Sunflower – Kharkiv).

Živkov Baloš et al. (2019) determined water content in different honey samples produced in regions of Serbia in the range from 15.2 ±0.8% (honeydew) to 18.9 ±1.8% (linden honey). Thrasyvoulou (1986) determined moisture 16.3–18.1% in blossom honeys and 15.3–18.3% in honeydew honeys.

All evaluated honey samples in the publications meet EU legislative criteria according to European directive 2011 and 2014 and revised *Codex* standard for honey (*Codex*, 2001; *Alimentarius*, 2001b).

Table 2 Comparison of honeys in some quality indicators obtained from different plant species and different locations in Ukraine

№	Moisture/water, %	Diastase, °Goethe	HMF, mg.kg^{-1}	Sugars, %	Sucrose, %	Proline, mg.kg^{-1}
S01	17.2 ±0.00	7.62 ±0.15	10.8 ±0.10	98.15 ±0.16	1.17 ±0.21	245.28 ±0.95
S02	15.8 ±0.00	9.11 ±0.19	10.1 ±0.10	95.95 ±0.10	0.73 ±0.10	132.69 ±0.95
S03	16.2 ±0.10	22.26 ±0.10	15.8 ±0.10	89.08 ±0.05	0.84 ±0.11	280.13 ±0.95
S04	17.0 ±0.10	10.15 ±0.05	2.3 ±0.19	88.15 ±0.10	2.13 ±0.11	237.24 ±0.80
S05	17.0 ±0.10	7.52 ±0.50	12.4 ±0.10	99.09 ±0.05	0.32 ±0.00	119.29 ±0.85
S06	17.1±0.00	12.11 ±0.15	10.5 ±0.10	92.44 ±0.09	1.04 ±0.05	298.89±0.95
S07	16.5 ±0.10	14.01 ±0.10	11.3 ±0.10	95.72±0.09	1.03 ±0.04	290.85 ±0.95
S08	17.8 ±0.10	9.45 ±0.05	10.4 ±0.19	95.69 ±0.00	1.05 ±0.05	207.75 ±0.95
S09	16.8 ±0.10	1.10 ±0.10	11.4 ±0.10	92.70 ±0.04	9.61 ±0.00	247.96 ±0.95
S10	21.0 ±0.10	12.75 ±0.00	4.2 ±0.19	88.45 ±0.14	8.89 ±0.09	148.77 ±0.85
S11	21.2 ±0.00	12.71 ±0.15	15.8 ±0.10	103.28 ±0.05	1.33 ±0.00	296.21 ±0.95
S12	18.4 ±0.00	10.12 ±0.05	11.0 ±0.00	86.27 ±0.05	7.37 ±0.14	264.04 ±0.95
S13	18.6 ±0.10	8.56 ±0.50	15.1 ±0.19	94.65 ±0.05	1.42 ±0.05	260.52 ±1.06
S14	18.6 ±0.10	12.47 ±0.05	12.0 ±0.00	96.54 ±0.04	0.80 ±0.00	176.19 ±1.06
S15	15.3 ±0.00	14.25 ±0.10	5.8 ±0.10	93.44 ±0.04	1.32 ±0.00	334.31 ±0.00
S16	15.2 ±0.00	12.13 ±0.00	14.1 ±0.10	90.95 ±0.04	0.84 ±0.04	224.38 ±1.06
S17	16.8 ±0.10	11.09 ±0.00	10.3 ±0.00	67.76 ±0.05	1.58 ±0.00	209.32 ±1.06
S18	17.0 ±0.00	8.65 ±0.15	11.4 ±0.10	77.03 ±0.05	1.48 ±0.11	254.49 ±1.06
S19	17.2 ±0.10	9.98 ±0.10	12.3 ±0.10	86.24 ±0.05	0.23 ±0.00	298.16 ±0.00
S20	18.2 ±0.10	11.36 ±0.05	13.6 ±0.19	81.44±0.00	2.42 ±0.05	169.38 ±0.00
S21	18.1 ±0.10	14.01 ±0.05	29.0 ±0.19	73.24 ±0.05	1.18 ±0.11	170.38 ±2.72
S22	17.0 ±0.10	9.84 ±0.05	23.0 ±0.19	87.94 ±0.05	1.91 ±0.00	275.25 ±1.36
S23	16.5 ±0.00	5.14 ±0.10	12.6 ±0.10	78.15 ±0.05	2.58 ±0.05	300.27 ±0.00
S24	18.8 ±0.00	12.75 ±0.00	2.6 ±0.10	72.35 ±0.05	0.65 ±0.11	216.13 ±1.48
S25	21.2 ±0.10	4.79 ±0.10	6.6 ±0.10	109.08 ±0.06	1.07 ±0.06	199.34 ±1.48
S26	20.8 ±0.10	11.20 ±0.15	6.9 ±0.19	98.55 ±0.11	1.51 ±0.06	178.36 ±1.48
S27	17.7 ±0.00	11.27 ±0.00	1.5 ±0.00	97.74 ±0.05	2.41 ±0.14	187.08 ±1.39
S28	15.2 ±0.10	11.07 ±0.10	6.0 ±0.10	78.18 ±0.21	6.25 ±0.10	218.58 ±1.39
S29	19.6 ±0.10	14.45 ±0.15	7.4 ±0.10	91.25 ±0.11	2.25 ±0.05	191.02 ±1.38
S30	18.1 ±0.10	12.56 ±0.00	3.1 ±0.19	68.91 ±0.16	0.64 ±0.11	303.63 ±1.40
S31	23.2 ±0.00	12.44 ±0.05	10.4 ±0.19	73.53 ±0.06	3.38 ±0.06	240.12 ±1.40
S32	18.8 ±0.00	10.44 ±0.05	10.1 ±0.10	78.63 ±0.05	2.38 ±0.11	174.64 ±0.00
S33	19.3 ±0.10	11.28 ±0.00	9.5 ±0.0	81.08 ±0.05	2.40 ±0.00	175.64 ±2.81
S34	18.8 ±0.10	8.60 ±0.10	8.0 ±0.10	66.51 ±0.05	1.84 ±0.00	283.78 ±1.40
S35	21.4 ±0.00	20.60 ±0.15	6.7 ±0.00	76.09 ±0.06	1.01 ±0.11	277.33 ±0.00
S36	20.0 ±0.00	7.88 ±0.10	8.8 ±0.00	81.02 ±0.05	3.79 ±0.05	183.11±1.26
S37	18.1 ±0.10	11.25 ±0.05	7.6 ±0.10	81.71 ±0.05	1.29 ±0.00	168.89 ±1.26
S38	18.8 ±0.00	12.31±0.00	6.5 ±0.10	87.94 ±0.05	7.47 ±0.00	151.11 ±1.26
S39	17.4 ±0.10	14.48 ±0.05	6.0 ±0.10	92.88 ±0.00	2.02 ±0.00	169.89 ±1.26
S40	17.3 ±0.10	11.10 ±0.05	5.8 ±0.10	69.34 ±0.05	2.66 ±0.00	152.89 ±0.00

Notes: HMF – hydroxymethylfurfural

Table 3 Basic statistical characteristic of the variability of evaluated honey samples

Indicator	Moisture, %	Diastase, °Goethe	HMF, mg.kg^{-1}	Sugars, %	Sucrose, %	Proline, mg.kg^{-1}
Min	15.20	1.10	1.50	66.51	0.23	119.29
Max	23.20	22.26	29.00	109.08	9.61	334.31
x̄	18.13	11.12	9.97	86.43	2.36	222.83
s	1.84	3.66	5.26	10.64	2.31	56.43
V, %	10.17	32.93	52.80	12.31	97.86	25.32

Notes: HMF – hydroxymethylfurfural (mg.kg^{-1}); min, max – minimal and maximal measured values; x̄ – arithmetic mean; s – standard deviation; V – coefficient of variation (%)

Sugars content

Honey is a sweet, thick, supersaturated sugar solution produced by honey bees (*Apis mellifera*) from plant nectars, plant secretion and excretions of plant-suckling insects of the living parts of plants (*Codex Alimentarius*, 2001a). It is one of the known natural sources of sweetness and energy for man. Honey is composed mainly of disaccharides which contain two monosaccharides, glucose and fructose, with a percentage of water and other groups of substances (Kamal and Klein, 2011). Small quantities of other sugars are also present, in the form of other disaccharides, trisaccharides and oligosaccharides which are formed during the ripening and storage effects of bee enzymes and acids of honey (Ball, 2007). Chemical compositions of honey differ depending on the plant species on which the bees forage, the climatic conditions, and other factors (Buba et al., 2013). The very concentrated solution of several sugars produces the characteristic physical properties of honey like high viscosity, high density, graduation tendencies, tendency to absorb water from the atmosphere and immunity from some types of spoilage.

More than 95% of the honey solids are carbohydrates, with monosaccharides (fructose and glucose) predominating. The presence of monosaccharides (fructose, glucose), disaccharides (e.g. maltose, sucrose, isomaltose,), and oligosaccharides (e.g. erlose, melezitose, raffinose) in most abundantly produced and, on the other hand, also in very specific honeys is documented (Cote et al., 2003; De La Fuente et al., 2006; Ouchemoukh et al., 2010; Pacholczyk-Sienicka et al., 2022).

Sugars represent the largest portion of honey composition (i.e., more than 95% of the honey solids); the monosaccharides fructose and glucose are the most abundant while small amounts of disaccharides (maltose and sucrose) are also present; other disaccharides and higher sugars (trisaccharides and oligosaccharides) are also present in quite small quantities. Due to the high content of monosaccharides (fructose and glucose) and relatively low moisture content, the water activity of honey is usually, but not always, below 0.60 which is enough to inhibit the growth of osmotolerant yeasts (Zamora and Chirife, 2004; Chirife et al., 2006).

Generally, honey is rich in glucose and fructose and the percentage of sucrose in honey should be lower, which is less than 5% (*Codex Alimentarius*, 2001a). However, it is assumed that green honey contains higher sucrose content compared to glucose and fructose level (Rajindran et al., 2022).

In the evaluated honey collections, we determined the sugar content in the range from 66.51% (S34 Multiflorous – Ivano-Frankivsk) to 98.78% (S25 Sunflower – Zhytomyr). Bandeira et al. (2018) determined in the honey collection the content of sugars in the range of 62.87–91.56%.

Sucrose content

The general provision for sucrose content is less than 5% with the exception listed for both *Codex* and Directive. From these exceptions, only *Eucalyptus*, *Robinia*, *Citrus* and *Lavandula* are listed as important for honey production and can be found predominantly in honey. The sucrose content of honey from *Eucalyptus* generally is less than 4.2% (Persano Oddo and Piro, 2004) while honey from dandelion (*Taraxacum officinale*) may occasionally have sucrose of more than 5%.

Directive 2001/110 EU declares the following Compositional criteria for honey.

Sucrose content:

- in general, not more than 5 g.100 g^{-1};
- false acacia (*Robinia pseudoacacia*), alfalfa (*Medicago sativa*), Menzies Banksia (*Banksia menziesii*), French honeysuckle (*Hedysarum*), red gum (*Eucalyptus camadulensis*), leatherwood (*Eucryphia lucida*, *Eucryphia milliganii*), *Citrus* spp. not more than 10 g.100 g^{-1};
- lavender (*Lavandula* spp.), borage (*Borago officinalis*) not more than 15 g.100 g^{-1}.

Sucrose reached a value in our honey samples (Tables 2 and 4) the contents were in the range of 0.23 (S19 *Robinia pseudoacacia* (commercial honey) – Ukraine to 9.61 (S09 *Robinia pseudoacacia* – Kyiv). We determined higher values of sucrose content than 5 g.100 g^{-1} in 5 evaluated honey samples, namely S09 (*Robinia pseudoacacia* – Kyiv), S10 (*Tillia* spp. – Kyiv), S12 (Multiflorous – Zhytomyr), S28 (Multiflorous – Zhytomyr) and S38 (*Brassica napus* – Zhytomyr).

Thrasyvoulou (1986) determined sucrose in the interval 1.5–4.2 g.100 g^{-1} in blossom honeys and 5.6–7.2 g.100 g^{-1} in honeydew honeys.

Tarapatskyy et al. (2021) studied botanical origin of Polish honey based on physicochemical properties and bioactive components. Authors determined sucrose content in linden (3.22–5.08 g.100 g^{-1}), buckwheat (0.35–0.67 g.100 g^{-1}), honeydew (5.17–10.46 g.100 g^{-1}), and multifloral honey (4.02–6.78 g.100 g^{-1}).

Hydroxymethylfurfural (HMF) and diastase

HMF is a breakdown product of sugars, produced when honey is heated, and diastase is an enzyme that is inactivated by heating. The levels of these two constituents also change during storage. Because the rate of HMF formation and diastase inactivation during storage or heating varies in different honeys, and also because there is a large variation in amounts of them in fresh, unprocessed honeys, doubts have arisen about the validity of their use as evidence of overheating (Schade et al., 1958).

Directive 2001/110 EU (EC, 2001) declares the following Compositional criteria for honey.

Diastase activity and hydroxymethylfurfural content (HMF) determined after processing and blending:

a) Diastase activity (schade scale):
   - in general, except baker's honey not less than 8;
   - honeys with low natural enzyme content (e.g., citrus honeys) and HMF content of not more than 15 mg.kg^{-1}, not less than 3 mg.kg^{-1}.

b) HMF
   - in general, except baker's honey, not more than 40 mg.kg^{-1} (subject to the provisions of (a), second indent);
   - honeys of declared origin from regions with tropical climates and blends of these honeys.

In our honey samples (Tables 2 and 4) the diastase content observed values from 1.10 (S9 *Robinia pseudoacacia* – Kyiv) to 22.26 (S3 Multiflorous – Donetsk) °Goethe. In the evaluated honey collection, we determined lower values as provided for by the Criteria for samples S01 (Spring grasses – Donetsk); S05 (*Helianthus annuus* – Donetsk), S09 (*Robinia pseudoacacia* – Kyiv), S23 (*Helianthus annuus* – Zhytomyr), S25 (*Helianthus annuus* – Zhytomyr) and S36 (Multiflorous – eco honey – Ivano-Frankivsk).

Hydroxymethylfurfural (HMF) reached the value in our samples (Table 2–4), the results were in the range of 1.5 (S27 Multiflorous – Zhytomyr) to 29.0 (S21 *Helianthus annuus* – Zhytomyr). In the evaluated honey collection, we recorded lower HMF values than 3 in samples S04 (Multiflorous – Donetsk), S24 (*Helianthus annuus* – Zhytomyr), S27 (Multiflorous – Zhytomyr) and S30 (*Helianthus annuus* – Kharkiv). We determined higher

Table 4 Honey samples from the evaluated collection with high and low values of the evaluated characters

Indicator/sequence of sample		High values (V) / № of sample (S)					Low values (V)/№ of sample (S)				
		1st	2nd	3rd	4th	5th	36th	37th	38th	39th	40th
Moisture, %	V	23.2	21.4	21.2	21.2	21.0	16.2	15.8	15.3	15.2	15.2
	S	31	35	25	11	10	3	2	15	28	16
Diastase, °Goethe	V	22.26	20.60	14.48	14.45	14.25	7.62	7.52	5.14	4.79	1.10
	S	3	35	39	29	15	1	5	23	25	9
HMF, mg.kg^{-1}	V	29.0	23.0	15.8	15.8	15.1	4.2	3.1	2.6	2.3	1.5
	S	21	22	11	3	13	10	30	24	4	27
Sugars, %	V	98.87	97.28	99.09	98.55	98.15	72.35	69.34	68.91	67.76	66.51
	S	25	11	5	26	1	24	40	30	17	34
Sucrose, %	V	9.61	8.89	7.47	7.37	6.25	0.73	0.65	0.64	0.32	0.23
	S	9	10	38	12	28	2	24	30	5	19
Proline, mg.kg^{-1}	V	334.31	303.63	300.27	298.89	298.16	152.89	151.11	148.77	132.69	119.29
	S	15	30	23	6	19	40	38	10	2	5

Note: S – № of sample; V – sample value in evaluated indicator; HMF – hydroxymethylfurfural

HMF values than 15 in samples S11 (Multiflorous – Kyiv), S21 (*Helianthus annuus* – Zhytomyr) and S22 (*Helianthus annuus* – Zhytomyr).

Thrasyvoulou (1986) determined diastase in the interval 27.0–60.0 DU in blossom honeys and 26.7–32.0 DU in honeydew honeys, content of HMF was not find in both honeys.

Vorlová and Přidal (2002) studied invertase and diastase activity, IN/DN ratio and HMF in fresh (floral, honeydew, compound) honeys and determined values ranged from 0.8–20.4 (IN), 11.2–30.3 (DN), 0.05–0.91 (IN/DN ratio) and 0.00–15.40 mg.kg^{-1} HMF for floral honey, 4.0–25.9 (IN), 15.9–40.3 (DN), 0.20–0.85 (IN/DN ratio) and 1.40–10.30 mg.kg^{-1} HMF for compound honey and 10.8–24.6 (IN), 13.6–45.4 (DN), 0.54–1.44 (IN/DN ratio) and 0.00–11.30 mg.kg^{-1} HMF for honeydew honey. The relation of both enzymes is expressed by the correlation r = 0.7492, p <0.01).

Tosi et al. (2008) examined treated honey samples. DN decrease from 25.8 to 8.1 after 1200 s at 90 °C heating and HMF with an initial concentration of 5.8 increased to 32.4 mg.kg^{-1} but did not reach the 60 mg.kg^{-1} limit.

Four of the most abundant honey types produced in Croatia (black locust, sage, chestnut, and honeydew honey) are characterised according to the protein and proline content and enzyme activities (Flanjak et al., 2016). The characterisation was done to determine specificities and contribute to the characterisation of unifloral honeys. Dark honey types (honeydew and chestnut honey) had a higher proline content (493.7 ±223.3 and 699.0 ±142.9 mg.1,000 g^{-1}, respectively), diastase (21.7 ±8.4 and 25.8 ±5.9 DN, respectively), and invertase (176.1 ±48.9 and 155.2 ±39.7 U.kg^{-1}, respectively) than sage and black locust honey (346.3 ±139.3 and 157.0 ±21.5 mg.1,000 g^{-1}; 19.9 ±6.8 and 11.2 ±2.1 DN; 94.7 ±52.1 and 52.1 ±20.7 U.kg^{-1}, respectively). Honeydew honey, otherwise known to possess high proline content (493.7 ±223.3 mg.1,000 g^{-1}) and enzyme activity, had a low protein content (59.4 ±21.8 mg.100 g^{-1}) comparable to black locust honey (30.4 ±7.9 mg.100 g^{-1}).

Kuc et al. (2017) studied the diastase activity of several varieties of honeys (multiflorous, honeydew and buckwheat) from different sources and stored under different conditions. Diastase activity (DN) determined by method, which is based on the distribution of the starch by α-amylase was in the range 10.9 (buckwheat and honeydew honey stored for 2 years) – 23.9 (multiflorous non-commercial from Poland, stored for 2 months at 4 °C) and results obtained by Phadebas method using UV-Spectrophotometer were in the interval 9.0 (multiflorous commercial from EU and non-EU, stored for 4 years) – 20.3 (multiflorous non-commercial from Poland, stored for 2 months at 4 °C).

The proline content

The values of proline content exceed the content of other amino acids. Its content is from 50 to 85% of the total amount of amino acids (Anklam, 1998; Hermosín et al., 2003). Proline content is a good marker of the botanical and geographical origin of honey (Costa et al., 1999). The higher content of proline is mainly found in sunflower honey, and the lower content is found in agave and eucalyptus honey. Based on the content of proline and phenylalanine, evidence of the addition of inverted syrup is possible (Singhal et al., 1997). The harmonized methods of the European Commission for honey include the spectrophotometric method for determining the proline content. A proline content lower than 180 mg.kg^{-1} may indicate the falsification of honey with the addition of sugar (Von Der Ohe et al., 1991).

The proline content in our honey samples (Tables 2 and 4) was in the range of 119.29 mg.kg^{-1} (S5 *Helianthus annuus* – Donetsk) to 334.31 mg.kg^{-1} (S15 Multiflorous – Kyiv). We determined a lower proline content than 180 mg.kg^{-1} in the following samples S01 (Spring grasses – Donetsk), S05 (*Helianthus annuus* – Donetsk), S10, S14 (*Tillia* spp. – Kyiv), S20 (Medicinal herbs – Kyiv), S21, S25, S26 (*Helianthus annuus* – Zhytomyr), S27 (Multiflorous – Zhytomyr), S32, S33 (*Helianthus annuus* – Kharkiv), S36 (Multiflorous – Eco honey – Ivano-Frankivsk), S37, S38 (*Brassica napus* – Zhytomyr), S39 (*Echium vulgare* – Kherson) and S40 (Medicinal herbs – Kyiv).

Janiszewska et al. (2012) investigated the free amino acids composition of 18 unifloral Polish honeys with different botanical origins (dominant buckwheat, raspberry, acacia, heather and goldenrod and honeydew honeys). Considerable variation in the total content of free amino acids ranging from 186.19 to 921.08 mg.kg^{-1} was stated. The dominant free amino acid in all types of honey was proline with the highest detected amount in one sample of heather honey 387.88 mg.kg^{-1}.

The proline content of Hungarian honey samples presented Czipa et al. (2012). The lowest proline concentration was measured in acacia honeys (252 ±38 mg.kg^{-1}) and the highest amount in coriander honey (2283 ±128 mg.kg^{-1}) and honeydew honey (1,089 ±137 mg.kg^{-1}). The rape, wild garlic, and asclepias honeys with values of 377 ±60 mg.kg^{-1},

476 ±27 mg.kg^{-1} and 485 ±114 mg.kg^{-1} of proline were following the acacia honeys. In the other honey types, the proline content is higher than 500 mg.kg^{-1} (linden, sunflower, chestnut, lavender). In flower honeys the proline content changed on a wide range because in these honeys the nectar and pollen ratios are very different.

Wen et al. (2017) studied and determined proline content in different floral origins (rapeseed, sunflower, buckwheat and *Codonopsis* honeys) from five different regions of China. The proline content varied among the four types of honeys, with the values decreasing in the order: buckwheat > *Codonopsis* > sunflower > rapeseed. The buckwheat honeys exhibited the highest proline content (average 610.16 mg.kg^{-1}) ($p < 0.05$), followed by *Codonopsis* honeys (494.49 mg.kg^{-1}), sunflower honeys (400.75 mg.kg^{-1}), and rapeseed honeys (201.61 mg.kg^{-1}).

In Beykaya's (2021) study, 60 honey samples (cotton, citrus, *Astragalus*, lavender, Jerusalem thorn, flower, cedarwood, pine, chestnut and *Nigella sativa*) were collected from different locations in Turkey and determined their physicochemical properties like hydroxymethylfurfural (HMF), proline, sugar content, invertase, diastase number, moisture, acidity, colour and electric conductivity (EC). The acid amounts of honeys ranged between 13.0–34.0 meq.kg^{-1} (*Astragalus* and *Nigella sativa*, respectively). The proline content of the honey samples used in this study varied between 300.0 ±11.8 and 881.7 ±42.6 mg.kg^{-1} (citrus and *Nigella sativa*, respectively) and the HMF content varied between 2.5 ±0.07 and 12.3 ±0.09 mg.kg^{-1} (cotton and cedarwood, respectively) according to honey types. Enzymes are one of the quality criteria for raw honey. The diastase number of honey samples was determined between 6.35 ±0.3 and 20.0 ±0.9 DN (citrus and *Nigella sativa*, respectively) and the amount of invertase enzyme ranged from 103.3 ±4.8 to 378.1 ±15.6 U.kg^{-1} (Jerusalem thorn and multiflorous honey).

From the evaluated collection of honey samples, we noted repeatability in groups with extreme values (Table 4) sample S05 (*Helianthus annuus* – Donetsk) 4 times (1 High – Sugars/3Low – Diastase, Sucrose and Proline), S30 (*Helianthus annuus* – Kharkiv) 4 times (1High – Proline/3 Low – HMF, Sugars and Sucrose), S02 (*Robinia pseudoacacia* – Donetsk) 3 times (3 Low – Moisture, Sucrose and Proline),

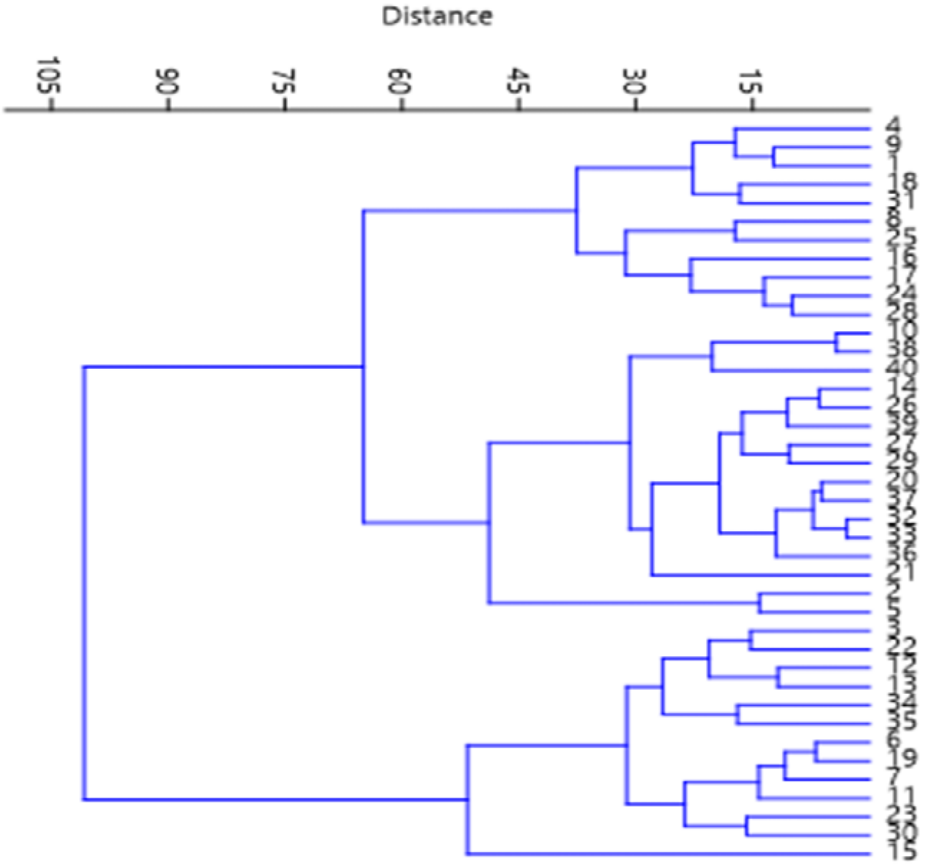

Figure 1 Cluster dendrogram of the relationships of evaluated honey samples of various origins according to some selected indicators

S10 (Multiflorous – Kyiv) 3 times (2 High – Moisture, Diastase, and 1 Low – Proline), S11 (*Tillia* spp. – Kyiv) 3 times (3 High – Moisture, HMF and Sugars) and S15 (Multiflorous – Kyiv) 3 times (3 Low – Moisture, Diastase, Proline).

Based on cluster analysis, the relationships of the tested honey samples of various origins and from different locations are graphically displayed on a dendrogram (Figure 1). The figure shows that the collection of honey was divided into 3 main clusters, which are similar in terms of their values and evaluated honey quality indicators.

Conclusion

In the presented study, 40 samples of honey obtained from beekeepers from different locations in Ukraine and different types of plants were evaluated. The collection of honey samples was evaluated in six basic indicators of honey quality – moisture, diastase, HMF, sugars, sucrose and content of proline. The obtained results expressly confirmed significant differences in each honey quality indicator as well as in the comprehensive evaluation of the samples. When comparing the results with the criteria of the standards of *Codex Alimentarius* and the EU legislation for honey, it was determined that some honey samples exceeded the specified limits. This means that not all honey samples reach the criteria for honey quality. Only 6 indicators were evaluated in the work, and such important indicators of honey quality as the content of heavy metals, the content of antibiotics, the content of residues after agro-pesticides and many others were not evaluated.Mandatory legislative control of the quality of honey for every beekeeper is not ensured in any country. Simultaneously, consumers can buy honey directly from beekeepers or supermarkets. However, it is generally known that even in supermarkets low-quality honey is provided to customers, as evidenced by the results of numerous inspections. This problem is very difficult to solve, especially if consumers buy honey directly from beekeepers. For this reason, it would be appropriate for individual countries to adopt laws for mandatory honey quality control for all beekeepers who ensure the sale of honey to consumers.

Ethical statements

This article does not contain any studies that would require an ethical statement.

Conflict of interest

None declared.

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