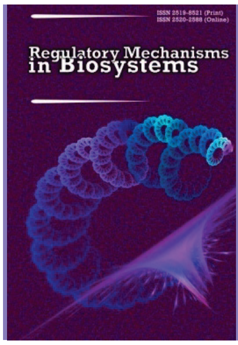

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Physicochemical quality indicators of honey: An evaluation in a Ukrainian socioecological gradient

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Physical and chemical quality indicators of 65 polyfloral honey samples from three administrative districts of Chernivtsi region in South Western Ukraine were studied. The chosen administrative districts were Putyla (representing the ‘Traditional villages’ stratum), Storozhynets (the ‘Intermediate’ stratum) and Khotyn (the ‘Intensive agriculture’ stratum), which reflect a steep gradient of social and ecological conditions such as land cover and land use, level of economic development, culture and demography. The quality of honey was determined in accordance with the requirements of the Ukrainian national standard and the EU Directive relating to honey (or Codex Alimentarius Honey Standard) by using the following indicators: reducing sugars and moisture content, diastase activity, free acidity, pH, electrical conductivity, hydroxymethylfurfural (HMF) and proline content. The profile of carbohydrates was analyzed, in particular glucose, fructose, sucrose, maltose, trehalose, melezitose, raffinose. The ratio of fructose to glucose (F/G) was determined. All tested samples complied with the international standards for the mass fraction of reducing sugars, diastase activity and hydroxymethylfurfural content. For physical and chemical parameters, the studied honey samples were of high quality. The content of the reducing sugars in the honey samples varied within the range of 66.0–97.6%. The fructose content ranged from 342 to 549 mg/g, and the glucose content variation ranged within 283–517 mg/g. The average fructose/glucose ratio was 1.2 for honey samples from the three studied districts. Besides fructose and glucose, some oligosaccharides, such as maltose, trehalose and melezitose, were found in the examined honey. Melezitose was detected in the honey samples from ‘Traditional village’ (21 samples) and ‘Intermediate’ (5 samples) districts. The total variability of HMF content in the studied 65 honey samples from apiaries in the three districts of Chernivtsi region ranged from 0.19 to 30.8 mg/kg. The minimum moisture content was found to be 16.2% (in the ‘Traditional village’ and ‘Intermediate’ strata), and 22.2% was the maximum (in the ‘Intermediate’ stratum). Our studies have shown that free acidity of the samples varied within the range 13.5 to 58.0 meq/kg. Proline content variability for the three studied geographical areas ranged from 82.3 to 1201.2 mg/kg. The studied samples of honey had a low pH level (~ 3.7), high content of proline (~ 513 mg/kg) and reducing sugars (~ 80%), which indicates its nutritional value and naturalness. Deviations from the honey standards in moisture content, acidity and electrical conductivity was revealed in 8% to 10% of all samples.

Keywords: polyfloral honey; private apiaries; honey quality; honey standards; Chernivtsi region; Ukraine.

Introduction

There is a growing world-wide demand for natural and healthy food products. As a product of nectar or honeydew processed by honey bees, honey is considered to be both a good food product and one that possesses health benefits. Nowadays, interest in honey is primarily associated with the search for natural ways to strengthen human immunity, which is especially important during the COVID-19 pandemic (Al-Hatamleh et al., 2020), and the promotion of honey as a sugar substitute (Iqbal et al., 2020). The market of beekeeping products is becoming more globalized and, as a result, competition for foreign markets is intensifying. Ukraine is one of the top five global producers and exporters of honey (Dankevych et al., 2018; Fedoriak et al., 2019). To maintain a high level of competitiveness, it is necessary to ensure that the quality of honey products meets both international and national standards. The chemical composition of polyfloral honey depends on many factors, primarily driven by regional climatic conditions, plant species and the corresponding pigments in the nectar (carotene, xanthophyll, phenolics, etc.), the honey harvest season, environmental factors and treatment methods applied by beekeepers (Halouzka et al., 2016; Pavlova et al., 2018). In order to examine a large range of variation in parameter values for the multiple factors affecting honey quality, Chernivtsi region in SW Ukraine was chosen as a case study. This region represents a steep gradient between mountainous remote areas with traditional village livelihoods, and lowlands with intensive farming, mainly rapeseed (*Brassica napus*), soybean (*Glycine max*), sunflower (*Helianthus annuus*) crops and apple orchards (Fedoriak et al., 2021). Honey bees, which are currently widespread on the territory of the Chernivtsi region, are mainly hybrids between two subspecies, *Apis mellifera carnica* and *A. m. macedonica* (Cherevatov et al., 2019; Cherevatov et al., 2020). Being the smallest region (8,100 km^2), it represents 1.3% of the total area of Ukraine. Despite its small size, the honey harvest was 942 tons in 2019. However, there is significant potential for beekeeping and high-quality honey production, due to the unique natural and climatic conditions. The peculiarity of the region is the length of the territory from west to east and the influence of the Carpathian mountain system on the climate. In general, the climate is quite mild and humid, but the complex terrain causes some differences – in the east it is more continental, in the mountains and foothills it is severer. This patchiness in the environmental conditions drives the wide diversity of nectar plants and hence diversification of honey varieties. Moreover, the landscapes function as coupled socioecological systems (Partelow, 2018) that have been dramatically changed by human activities. Therefore, the assessment of physical and chemical indicators of honey quality is an important condition for the development of beekeeping and the prospect of export opportunities in the region.

The aim of the study was to check compliance of honey from the apiaries located in the landscapes with different socioecological conditions with Ukrainian and international quality standards. We also aimed to find out whether there was a statistically significant difference between the physicochemical quality of honey between different socio-ecological conditions. This is an important study as future economic developments in the honey production depend on meeting national and European standards.

Material and methods

Chernivtsi region is made up of three major physiographic units: mountain, foothill and lowland. All three zones differ in natural conditions, vegetation, agricultural land composition, levels of economic development, culture and demographic indicators, forming a steep social and ecological gradient (Fedoriak et al., 2021). The most prominent difference among the major physiographic units refers to the intensity of agriculture. We chose one administrative district for each physiographic unit, representing the 'Traditional village' stratum, the 'Intermediate' stratum, and the 'Intensive agriculture' stratum (Fig. 1). The 'Traditional village' stratum (Putyla district) is located in the Eastern Carpathian Mountains. This area is dominated by traditionally practised subsistence farming including both growing crops and tending livestock. The main land cover classes are coniferous (spruce, stone pine) and mixed forests, and natural meadows located above the tree line. The 'Intermediate' stratum (Storozhynets district) is situated further to the east in the Carpathian Mountain foothills and combines features of the neighbouring two strata. Agricultural production and forestry are both major kinds of land use here while subsistence farming persists in the large villages beside modern agriculture with diverse crops. The 'Intensive agriculture' stratum (Khotyn district) in the east is represented mainly by agricultural land managed by big international agricultural businesses and private orchards. The proportion of crop land cover varied from 17% in 'Traditional village' stratum to 79% 'Intensive agriculture' stratum (Fedoriak et al., 2021).

Sixty-five polyfloral honey samples were received directly from beekeepers from three districts of Chernivtsi region (26 samples were collected in the 'Traditional village' stratum, 17 in the 'Intermediate' stratum and 22 in the 'Intensive agriculture' stratum). The samples were stored at 16 degrees C under dark conditions.

A comprehensive analysis of the honey was performed. This analysis included normalized quality indicators, pH and profile of carbohydrates. Honey quality assessment was performed by means of physicochemical methods of analysis. The mass fraction of reducing sugars and moisture content, diastase activity, acidity, electrical conductivity, hydroxymethylfurfural (HMF) and proline content were determined by conventional methods in accordance with the Ukrainian National Standards (national standard).

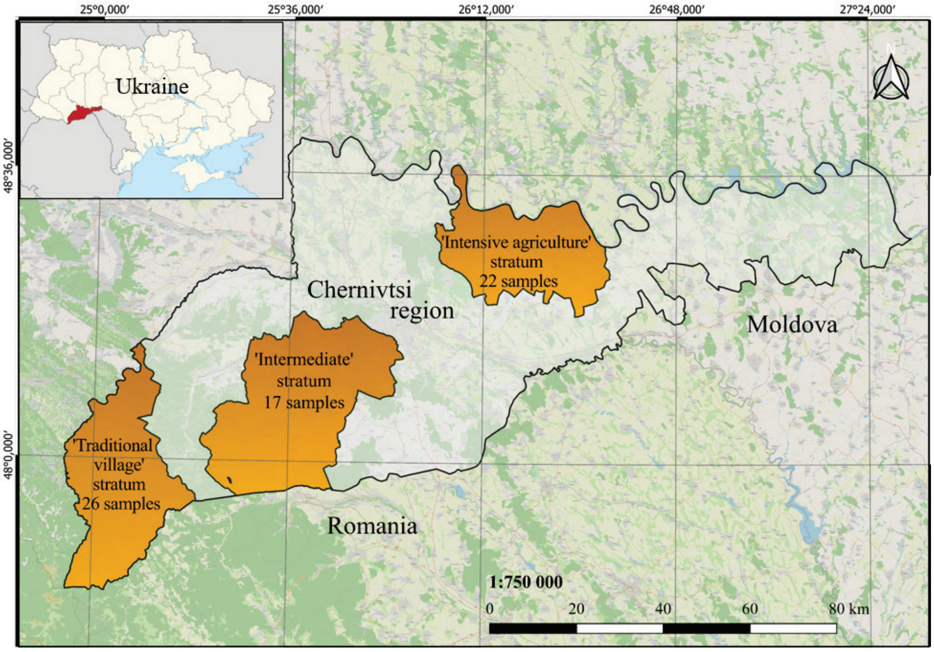

Fig. 1. Location of the three strata on the map of Chernivtsi region

Moisture content was determined refractometrically. A sample of honey was placed in a test tube, heated in a water bath to 60 degrees until complete dissolution of the crystals. Then the tube was cooled to room temperature, a drop of honey was applied onto the refractometer prism (type RHB 90 ATC, China, 2019) and the refractive index was measured, which was converted into a mass fraction of water in honey.

The content of reducing sugars was determined spectrophotometrically.

Preparation of honey solution: 2 g of the honey sample was dissolved in 20–30 ml of distilled water and the water was added up to 100 mL (solution A). Distilled water was added to 10 mL of solution A to reach 100 mL (solution B).

Determination of the mass fraction of reducing sugars: 20 mL of 1% potassium hexacyanoferrate (III) solution, 5 mL of 2.5 mol/dm^3 sodium hydroxide solution and 10 mL of solution B were added together to a 100 mL conical flask. The mixture was heated to boiling, then boiled for 1 minute and immediately cooled to room temperature. The optical density of the solution was measured on a Cary 60 UV-Vis Spectrophotometer, (USA, 2017) in a 10 mm cuvette at 440 nm wavelength against distilled water. The amount of reducing sugars (mg) was found by interpolation of optical density value of the samples with a calibration curve. The mass fraction of reducing sugars per the dry matter was calculated as relation of the amount of reducing sugars taken according to the calibration curve (mg) to the honey sample mass (g). The carbohydrate profile was determined by the method of high-performance liquid chromatography coupled with Corona Veo RS detector (USA, 2017) by the scientists of the Faculty of Science of Palacký University Olomouc (The Czech Republic). In brief: the isocratic elution was performed on the column Luna Omega Sugar 3 um 100Å, 150 x 3.0 mm at 30 °C and 1 mL/min of 80%

acetonitrile. The Corona Veo RS detector was tuned to 35 °C and frequency 25 Hz. The run time was 7 mins.

Diastase activity was determined spectrophotometrically and results were presented in Goethe units.

Aqueous honey solution was prepared by dissolving 5 g of honey in 10 mL distilled water, then added to a 50 mL volumetric flask and made up to the mark with distilled water. 14 mL of the mixed reagent (8 parts of 0.25% starch solution; 5 parts of acetate buffer pH = 5; 1 part of 0.1 mol/dm^3 sodium chloride) were poured into three test tubes. The tubes were closed with stoppers and placed in a water bath at 40 degrees. Then 1 mL of honey solution was poured into two test tubes, and 1 mL of distilled water was added to the third tube (control experiment). The content of the tubes was thoroughly mixed and boiled in a water bath for 15 min at 40 degrees. Then the samples were quickly cooled to 20 degrees using ice. 2 mL of mixed reagent from the test tubes was mixed with 40 mL distilled water, 1 mL of 0.25% iodine solution in three volumetric 50 mL flasks. The content of the flasks was made up to the mark with distilled water, thoroughly mixed, and kept in a water bath for 10 min at 20 degrees.

The optical density of the samples was measured on a Cary 60 UV-Vis Spectrophotometer (USA, 2017), 10 mm cuvette, wavelength 590 nm. The diastase activity of honey was defined as the amount of enzyme that can convert 0.01 g of starch in 1 hour at 40 °C under test conditions. The results were presented in Goethe (or Shade) units per 1 g of dry matter.

Free acidity was determined using a titration method with a solution of sodium hydroxide pH 8.3. A honey sample 10.0 ± 0.01 g was diluted with distilled water (75 mL) and titrated with a 0.1 mol/dm^3 NaOH solution.

Electrical conductivity was measured by a digital benchtop multiparameter instrument, type PC 52+ DHS XS instruments (Italy, 2019) in a 20% (w/v) honey solution diluted with distilled water.

Hydroxymethylfurfural (HMF) content was determined by the Winkler spectrophotometric method on a Cary 60 UV-Vis Spectrophotometer (USA, 2017). It is based on the interaction of HMF, para-toluidine and barbituric acid with the formation of a red-coloured complex. The optical density of solution was measured at a wavelength 550 nm with fixation of the maximum optical density value for 2–6 min since the time of adding barbituric acid. The results were presented in mg HMF/kg honey.

Content of proline was determined spectrophotometrically by measuring optical density of the proline with a ninhydrin coloured complex at a wavelength 510 nm. 5.0 ± 0.01 g of the honey sample was diluted in 100 mL distilled water. The content of proline (P), mg/kg was determined as relation of honey solution optical density multiplied by dilution factor to optical density of the standard solution of proline (0.0008 g/25 mL of solution).

Hydrogen index was registered in a honey solution by a digital benchtop multiparameter instrument, type PC 52+ DHS XS instruments (Italy, 2019) using pH electrodes constantly stirring on a magnetic stirrer.

The obtained experimental results were compared with the norms of the national standard DSTU 4497:2005 and the EU Directive relating to honey and Codex Alimentarius Honey Standard. The Directive 2001/110/EC and Codex Alimentarius Honey Standard state the general regulations regarding the composition and content of various types of honey. According to the Ukrainian standard, honey is divided into two basic classes i.e. Extra Class and First Class (Table 1).

Table 1
Comparison of characteristics of physical and chemical indicators of honey quality in accordance with various normative documents

Quality Indicators	Ukrainian National Standard DSTU 4497:2005		International Standards (The EU Directive 2001/110/EC; Codex Alimentarius Honey Standard)
	extra class	the first class	
Moisture content, %	< 18.5	< 21.0	< 20
Reducing sugars content, %	> 80.0	> 70.0	> 60
Diastase activity, Goethe units	> 15.0	> 10.0	schade units, > 8
HMF content (mg/kg), not more than	< 10.0	< 25.0	< 40
Free acidity, milliequivalents NaOH, 0.1 mol/dm^3 per 1000 g	40.0	50.0	< 50 milliequivalents of acid per 1000 g
Proline content, mg/kg	> 300	> 300	is not rationed
Electrical Conductivity, mS/cm	0.2–1.0	0.2–1.5	< 0.8

Statistical analysis of the results was performed using the software R Studio, Version R 4.1.2 (USA, 2022). Normality of data distribution was verified with the Shapiro-Wilks test. All variables failed to meet parametric test assumptions. Then variables were tested by methods of nonparametric statistics (Kruskal-Wallis ANOVA) for non-normal distributed data. Post hoc comparisons were conducted using Kruskal-Wallis and pairwise Wilcoxon rank sum tests with a Bonferroni adjustment.

Results

Twelve physicochemical indicators of honey quality were analyzed in the sixty-five polyfloral honey samples collected from three strata of Chernivtsi region. The obtained results on reducing sugars' content, account of fructose, glucose, and fructose/glucose ratio in the examined honey samples are displayed in Figure 2.

The content (%) of the reducing sugars in the honey samples varied within the range of 66.0% to 97.6% (Fig. 2). It showed a significant difference between the 'Intensive agriculture' and 'Intermediate' strata of Chernivtsi region. The mean of reducing sugars content for 'Traditional village' stratum was found to be 79.2% ranging from 67.3% to 90.4%. The variation in the 'Intermediate' stratum extended between 66.0% and 89.2% with mean 76.6%. In the 'Intensive agriculture' it varied from 72.2% to 97.7% with a mean of 82.8%.

The studied honey samples had the following sugar profile: fructose, glucose, sucrose, maltose, trehalose and melezitose. The investigated samples of honey did not differ significantly in the content of glucose. The fructose content varied from 342 to 549 mg/g, and the glucose content variation ranged within 283 to 517 mg/g (Fig. 2). A higher average content of fructose and glucose are predominant in the honey samples from 'Intensive agriculture' apiaries (458 and 393 mg/g, respectively) and 'Traditional village' apiaries (438 and 375 mg/g) (Fig. 2). All the studied samples of honey displayed higher fructose content than glucose content. The average fructose/glucose ratio was 1.2 for honey samples from the three studied districts. The minimum ratio of monosaccharides was noted in the samples of honey from 'Intermediate' (1.05 to 1.30) and it increased in the samples from 'Intensive agriculture' (1.05–1.41) and 'Traditional village' (1.09–1.47) districts.

Sucrose was only detected in two samples of honey from the 'Intermediate' strata with a content of 5.3 and 11.2 mg/g. The sugar profile that we have been able to determine displayed an absence of raffinose in all samples of honey. Besides fructose, glucose and sucrose, some oligosaccharides, such as maltose, trehalose and melezitose, were reported to be found in the honey. Some samples of honey contained maltose in small quantities. Its content varied from 0.2 to 31.3 mg/g, in 14 samples of honey from the 'Traditional village' stratum, in the 11 samples from the 'Intermediate' stratum the range was 0.2 to 12.7 mg/g and 4 samples from the 'Intensive agriculture' stratum contained 1.9 to 25.0 mg/g of maltose respectively (Table 2). Trehalose was only identified in two samples from the 'Intermediate' strata and one from the 'Traditional village'. Its content was 5.2, 7.2 and 3.9 mg/g, respectively.

Table 2
The fraction of honey samples in each studied stratum where oligosaccharides were detected

Strata	% of honey samples			
	sucrose	maltose	trehalose	melezitose
'Traditional village', *n* = 26	–	53.8	3.8	80.8
'Intermediate', *n* = 17	11.8	64.7	11.8	29.4
'Intensive agriculture', *n* =22	–	18.2	–	–

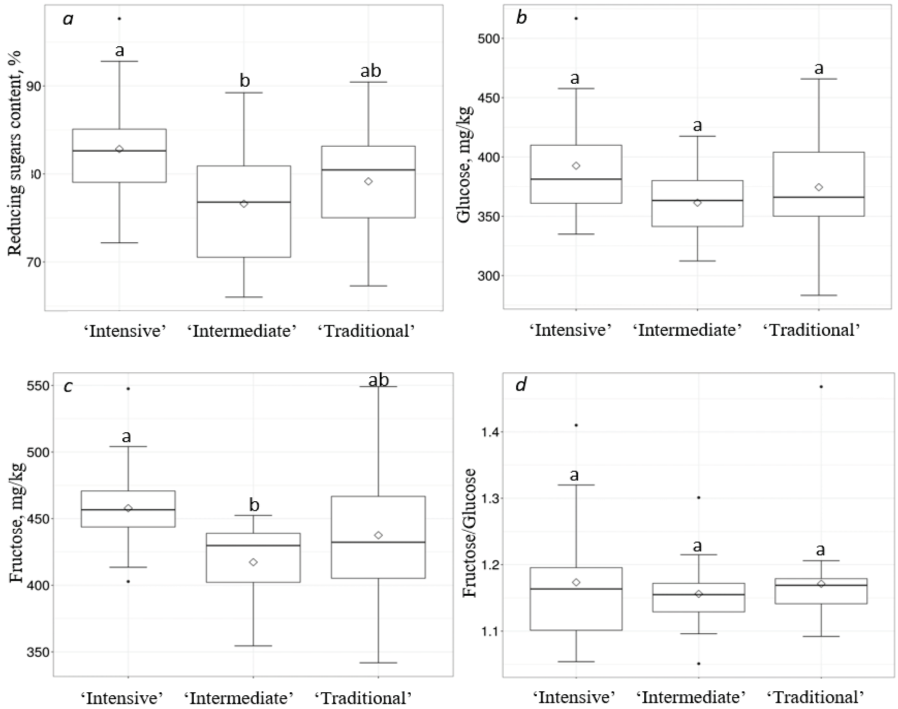

Fig. 2. Reducing sugars 'content (*a*), glucose (*b*), fructose (*c*) and fructose/glucose ratio (*d*) in the study samples from the three strata of Chernivtsi region ('Intensive' n = 22; 'Intermediate' n = 17; 'Traditional' n = 26; each sample measured in triplicate): the rectangle box indicates interquartile range (25%–75%); upper and lower whiskers – maximum and minimum values; horizontal line inside the box – median; diamond – mean; dots – outliers; different letters – significant differences (P < 0.05) between groups of variables according to the results of the Kruskal-Wallis test and pairwise Wilcoxon rank sum test with a Bonferroni adjustment

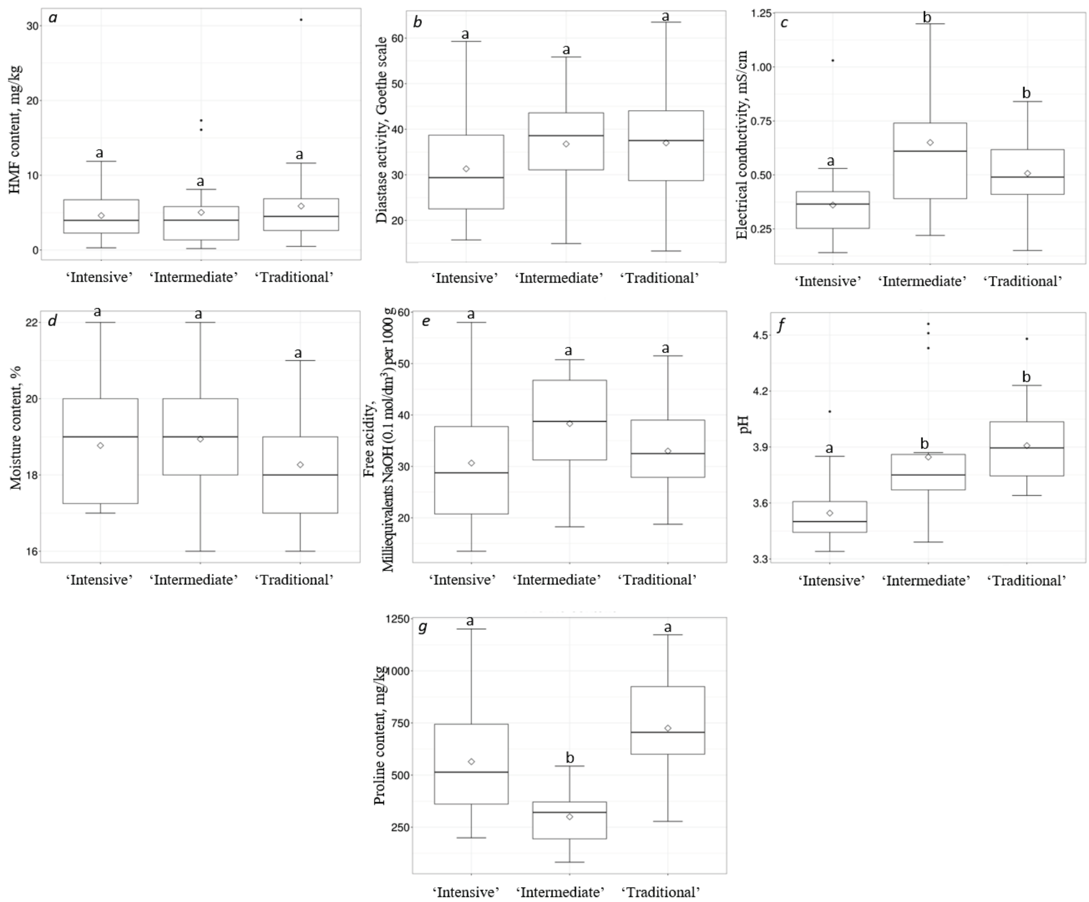

Fig. 3. Physicochemical indicators of honey quality: hydroxymethylfurfural content (*a*), diastase activity (*b*), electrical conductivity (*c*), moisture content (*d*), free acidity (*e*), pH (*f*) and proline content (*g*) in the study samples from the three strata of the Chernivtsi region ('Intensive' n = 22; 'Intermediate' n = 17; 'Traditional' n = 26; each sample measured in triplicate): the rectangle box indicates interquartile range (25%–75%); upper and lower whiskers – maximum and minimum values; horizontal line inside the box – median; diamond – mean; dots – outliers; different letters – significant differences (P < 0.05) between groups of variables according to the results of Kruskal-Wallis test and pairwise Wilcoxon rank sum test with a Bonferroni adjustment

Melezitose was detected in the honey samples from 'Traditional village' (21 samples) and 'Intermediate' (5 samples) districts (Table 2). The content of melezitose varied within a broad range, in particular 0.7–107.1 mg/g was registered in the samples from 'Traditional village' and 7.4 to 112.3 mg/g in the samples from 'Intensive agriculture'. Small amounts of trehalose were detected in only three samples from the 'Traditional village' (3.9 mg/g) and the 'Intensive agriculture' (5.2 and 7.2 mg/g) districts. Consequently, the presence of melezitose may indicate that some honeydew impurities are contained in the honey samples from 'Traditional village' and 'Intensive agriculture'.

The total variability of HMF content in the studied honey samples from 65 apiaries from the three districts of Chernivtsi regions ranges from 0.19 to 30.8 mg/kg (Fig. 3). The limits of HMF content fluctuation in samples of honey from the 'Traditional village' stratum (0.48 to 30.82) are higher compared to others (0.29–11.86 and 0.19–17.33 for the samples from the 'Intensive agriculture' and 'Intermediate' strata respectively), but differences were not significant.

Variability of diastase activity in honey samples from Chernivtsi region ranges from 13.9 to 63.5 Goethe units (Fig. 3). It should be noted that the maximum range (from 13.29 to 63.49 Goethe units) of diastase activity indicators was registered in the 'Traditional village' stratum. The average value of this indicator is also the highest in this area (37.00 Goethe units). No statistically significance in the diastase activity was found between strata.

Our studies have revealed significant variability in the electrical conductivity of honey samples (Fig. 3). Statistical differences were observed between the 'Intensive agriculture' stratum and the 'Traditional village' stratum and between the 'Intermediate' stratum and the 'Intensive agriculture' stratum according to Wilcoxon W-test, at $P < 0.05$ for electrical conductivity. It varied in range from 0.14 mS/cm in 'Intensive agriculture' to 1.2 mS/cm in 'Intermediate'.

The minimum moisture content was found to be 16.2% (in the 'Traditional village' and 'Intermediate' stratum), and 22.2% was the maximum (in the 'Intermediate' stratum).

Our studies have shown that free acidity of the samples varied within the range of 13.5 to 58.0 meq/kg (Fig. 3). The obtained results showed no statistical significance between strata.

According to the results of our studies, the pH of honey samples ranged from 3.34 to 4.56 (Fig. 3). The average values of the hydrogen index were 3.55, 3.85 and 3.91 for honey samples from the 'Intensive agriculture', the 'Intermediate' and the 'Traditional village' strata, respectively. A significantly higher concentration of hydrogen ions in the honey solutions from the 'Intensive agriculture' stratum was demonstrated in accordance with Wilcoxon W-test.

Proline content variability for the three studied geographical areas ranged from 82.3 to 1201.2 mg/kg (Fig. 3). The content of proline in the samples of honey from the 'Intermediate' stratum significantly differed from the 'Intensive agriculture' and 'Traditional village' strata in accordance with the Wilcoxon W-test. The highest average value (725.0 mg/kg) of proline content was observed in the samples from the 'Traditional village' stratum, which has the least anthropogenic effect compared to other districts we studied. The lowest average value (300.0 mg/kg) was found in the samples from the 'Intermediate' stratum.

Discussion

The largest part of the dry matter in honey consists of carbohydrates, represented by mono-, di- and trisaccharides. Glucose and fructose are the principal constituents of honey carbohydrates (da Silva et al., 2016). These components determine the basic properties of honey such as sweetness, nutritional value, granulation tendency, crystallization and hygroscopicity. The glucose and fructose content of honey are derived mainly from plant nectar; only a small amount is formed from sucrose and is accumulated in the process of its maturation under the influence of enzymes and organic acids contained in honey. The content of glucose and fructose is known as an indicator of honey naturalness (Kornienko et al., 2017). The sucrose content in natural honey is insignificant and may decrease in the course of storage due to the process of self-inversion.

In accordance with the requirements of international standards, in particular the European Directive 2001/110/EC and the standard of the Codex Alimentarius Commission, the share of reducing sugars in natural honey must be not less than 60%. At the same time, according to the national standard DSTU 4497:2005, the mass share of reducing sugars content should be at least 80% for Extra Class honey and at least 70% for First Class honey according with DSTU 4497:2005.

All the honey samples from private apiaries located on the territory of the three study districts of Chernivtsi region were found to comply with the international standards in terms of reducing sugars' content (Table 3).

Table 3
The percentage of honey samples which meet the requirements of Ukrainian and International Standards in the three sampled strata in Chernivtsi region

Indicators, %	'Traditional village'			'Intermediate'			'Intensive agriculture'		
	Ukrainian national standard		International standards the EU Directive 2001/110/EC; Codex Alimentarius	Ukrainian national standard		International standards the EU Directive 2001/110/EC; Codex Alimentarius	Ukrainian national standard		International standards the EU Directive 2001/110/EC; Codex Alimentarius
	extra class	the first class		extra class	the first class		extra class	the first class	
Moisture content	62.0	38.0	96.0	41.0	59.0	84.0	46.0	54.0	86.0
Reducing sugars content	53.8	38.5	100.0	35.3	52.9	100.0	72.7	27.3	100.0
Diastase activity	95.8	4.2	100.0	94.1	5.9	100.0	100.0	–	100.0
HMF content	88.5	7.7	100.0	88.2	11.8	100.0	90.9	9.1	100.0
Free acidity, milliequivalents NaOH	79.2	16.6	95.8	52.9	35.3	88.2	81.0	9.5	90.5
Electrical conductivity	100	–	96.2	82.4	17.6	82.4	95.5	4.5	95.5
Proline content	96.1		–	52.9		–	81.0		–

All the samples from the 'Intensive agriculture' stratum, 92.3% of samples from the 'Traditional village' stratum and 88.2% from the 'Intermediate' stratum met the requirements of the national standard. The vast majority of honey samples from the 'Traditional village' (53.8%) and 'Intensive agriculture' strata (72.7%) were classified as Extra Class honey, whilst the First-Class honey samples prevailed in the 'Intermediate' stratum (52.9%, Table 3).

The sweetness of honey depends on the concentration of constituent sugars and their origin. The sweetest honey has high fructose concentration. The content of glucose and fructose in the dry matter constitutes approximately 70–80% of all sugars contained in flower honey and 55–65% in honeydew honey (Kowalski et al., 2013). The content of invert sugars in flower honey samples from 18 different places in Bosnia and Herzegovina ranged from 64.8% to 85.0% (Prazina & Mahmutović, 2017). In high-quality honey, the glucose content is usually lower (about 30–35%) than fructose (about 35–40%). Some physical properties of honey depend on their ratio. The higher amount of glucose in honey leads to its faster crystallization, while the fructose content influences its taste, making it sweeter and more hygroscopic (da Silva et al., 2016).

The ratio of fructose and glucose (F/G) in the prevailing majority of cases exceeds 1.0 and this indicator can be used to identify monofloral honey. Data from different sources state that acacia and chestnut types of honey are rich in fructose (F/G 1.5–1.7), while oilseed rape and dandelion types of honey demonstrate a higher glucose content (Prazina & Mahmutović, 2017). Also, if we compare acacia honey to buckwheat and linden honey we see that higher glucose content compared to fructose was observed in the latter ones (Kowalski et al., 2013). As for the information on the ratio of fructose and glucose content in the honeydew honey, it is quite ambiguous. Thus, Primorac et al. (2009) noted a higher content of fructose rather than glucose (32.4:31.0%) in the honeydew honey samples from

Croatia, while the corresponding honey samples from Macedonia demonstrated the opposite results of 36.8% glucose and 33.6% fructose.

Apart from monosaccharides, flower honey contains a number of disaccharides, among which sucrose, maltose, trehalose and turanose are the main ones (Bogdanov et al., 2008). Their content ranges between 3.29–18.6% and oligosaccharides content is noted as 0.13–10.0% (Kowalski et al., 2013). The most common disaccharide is sucrose. According to the current European requirements for honey quality, the sucrose content in all types of flower honey is set at not more than 5 g/100 g, except for some monofloral types of honey (*Banskia*, *Citrus*, *Hedysarum*, *Medicago* and *Robinia*) with the sucrose content of up to 10 g/100 g and *Lavandula* honey containing up to 15 g/100 g of sucrose (EU Council, 2002; Codex Alimentarius Commission, 2001). The requirements of the Ukrainian national standard DSTU provide for a sucrose content of not more than 3.5% (for the Extra Class) and not more than 6% (for the First Class) (DSTU, 2007). The absence of sucrose displayed for the all examined samples of honey is probably a result of the correct maturation of honey (Kowalski et al., 2013).

Maltose disaccharide contributes to honey sweetness and its content can vary within the range of 2.8 to 7.5% (Kowalski et al., 2013). The presence of various trisaccharides such as melezitose, maltotriose, and raffinose is an indicator of the honeydew content in the samples (Bogdanov et al., 1999). The trisaccharide melezitose content that is usually not spotted in flower honey is contained in a significant proportion in honeydew honey. Melezitose is contained in honey produced by bees from honeydew of both deciduous and coniferous plants (Rybak-Chmielewska et al., 2013). Honeydew often gets into flower honey in various quantities. Numerous authors are looking for the set parameters by which honey can be quickly identified. Bogdanov & Gfeller (2006) used discriminant analysis to classify flower, honeydew and mixed honey types and it was noted that melezitose, as the only variable, had a high discriminant power of 96% for the classification of honey. Honeydew honey contains more oligosaccharides of melezitose and raffinose compared to flower honey. According to the EU Honey Standard, honeydew honey is produced by bees from honeydew (secretion), which is a sweet, transparent and viscous substance of animal origin (secreted by insects) and honeydew drops (juice from the leaves and stems of plants) (EU Council, 2001). Honeydew contains a more complex range of sugars than nectar therefore honeydew honey has a much lower content of disaccharides and more oligosaccharides than flower honey. This is closely related to the fact that honeydew contains enzymes (which are absent in nectar) secreted by the salivary glands and intestines of insects (Victorita et al., 2008).

Hydroxymethylfurfural (HMF) is a cyclic aldehyde that is formed in an acidic environment when honey is heated and exposed to the Maillard reaction (a non-enzymatic formation of coloured melanoidins) from reducing sugars. However, the duration and storage conditions of honey may cause the formation of HMF. It was demonstrated by Shapla et al. (2018), that honey stored at low temperatures had a low content of HMF whilst honey stored at high and medium temperatures had a high content of HMF. The studies of Alias et al. (2018) revealed that HMF production increases proportionally to the increase of temperature and duration of heating. Thus, the content of HMF is a parameter that indicates the freshness of honey since it is usually either not registered or registered in small quantities in fresh honey. HMF tends to be formed faster from ketohexoses, such as fructose compared to glucose (Shapla et al., 2018).

According to the national standard, the HMF content in the "Extra Class" honey and the "First Class" honey must not exceed 10 and 25 mg/kg respectively. According to the International standards (Codex Alimentarius Commission, 2001; EU Council, 2002), the HMF content must be no more than 40 mg/kg. Thus, all samples of honey complied with the international quality standards (Table 3). It must also be stated that one sample from the 'Traditional village' stratum did not meet the national standard (not more than 25 mg/kg).

Honey diastase is an enzyme that is formed from flower nectar with the secretions of the honey bees salivary glands. However, it remains unclear why some honey types of different botanical origin have different diastase activity whereas some other types of honey (*Erica*, *Robinia*, *Rosmarinus*, *Erica*, *Taraxacum*, *Arbutus*, *Citrus*) demonstrate consistently low activity of this enzyme (3–5 Goethe units) (Thrasyvoulou et al., 2018). The different diastase activity is considered to be caused by a number of factors such as the period of nectar collection, the efficiency of nectar processing by honey bees, the age of the honey bees, the physiological state of the honey bee colony and others (da Silva et al., 2016; Gismondi et al., 2018).

The diastase activity measurement is used to assess the quality parameters of honey, in particular its freshness. The studies of Isopescu et al. (2014) have shown that diastase activity is an extremely variable indicator that depends on a number of uncontrolled external and internal factors. Consequently, its use as an indicator of the freshness of honey is extremely controversial.

Honey is often heated when it crystallizes to improve its texture, viscosity and product appearance. It is known that the loss of valuable properties of honey is proportional to temperature and heating time (Cozmuta et al., 2011). Control parameters such as the diastasis activity and HMF serve as indicators of intensity and time of honey heat treatment (Ramirez-Cervantes et al., 2000).

According to the Honey Quality Requirements of the EU Council Directive, the diastase activity must not be less than or equal to 8 Schade units, expressed as the diastase number (DN). DN in the Schade scale, which corresponds to the Goethe units number, is defined as 1 g of starch per 100 g of honey hydrolysed for 1 hour at the temperature 40 ºC.

DN exceeds the value of 25 Goethe units whereas HMF is either not registered or has low value in the freshly collected samples of honey. In the process of honey heating or long-term storage the diastase activity decreases and HMF, on the contrary, increases. If the diastasis number is less than 8 Goethe units or HMF is more than 40 mg/kg, the quality of honey is considered to be unsatisfactory and honey is classified as baking honey (Thrasyvoulou et al., 2018). The obtained results of diastase activity in all samples from the Chernivtsi region are consistent with the studies of other authors who also highlight the variability of diastase activity from 13.9 to 50 Goethe units (Isopescu et al., 2014).

We found (Table 3) that all of the studied honey samples complied with the national (not less than 10 Goethe units) and international (not less than 8 Goethe units) standards of quality. It should be noted that the maximum range of diastase activity indicators was registered in the 'Traditional village' stratum (Fig. 2). The average value of this indicator is also the highest in this area. In our opinion, this can be explained by the more diverse foraging resources for bees and the larger number of honey bees out in the meadows at this time period (Bálint et al., 2011). Moreover, it should be taken in consideration that this district undergoes less anthropogenic influence in comparison with other two strata.

Especially high levels of diastase activity are known for monofloral honey *Thymus* (Nousias et al., 2017). There are several species of *Thymus* widely represented in the 'Traditional village' stratum (Nachychko & Honcharenko, 2017). Therefore, this could be the reason that honey samples from this area have a higher average value of diastase activity compared to others. The highest value of this indicator (63.49 Goethe units) was also registered in these honey samples.

Electrical conductivity (EC) depends on the content of mineral salts, organic acids and proteins (Yücel & Sultanog, 2013) and it indicates the origin of honey (Karabagias et al., 2014). A value of ≤ 0.8 mSm/cm indicates the floral origin of honey, a bigger value of EC indicates that honey is of honeydew origin. Though there are some exceptions in particular, international standards state that such types of honey as *Persea americana* (avocado honey), *Polygonum aviculare* (knot weed honey), *Paliurus spina-christi* (Jerusalem thorn honey), *Gossypium* sp. (cotton honey), have electrical conductivity at value > 0.8 mS/cm. Our studies have revealed significant variability in the electrical conductivity of the honey samples. Although the average values of the study samples do not exceed the permissible limits stated in the national standard DSTU, there are samples of honey with high values of electrical conductivity that do not comply with the international standards (≤ 0.8 mS/cm). Most likely, these samples can be classified as honeydew honey or contain a special composition of mineral salts, organic acids and proteins that can cause high values of electrical conductivity (Yücel & Sultanog, 2013).

Moisture content in honey plays an important role in determining the general characteristics of honey and assessing its quality. Moisture content in honey depends on a number of factors: climatic conditions, flower composition, harvesting conditions etc. (Karabagias et al., 2014). Mature honey contains not more than 18% of moisture; international standards allow moisture content up to 20%, except for honey from heather (*Calluna vulgaris*) where moisture content level is allowed up to 23% (Thrasyvoulou et al., 2018). The higher the moisture content in honey, the greater is the probability of fermentation processes resulting in its colour and taste changes. Most samples of honey of different botanical origin have moisture content of about 18%. However, some monofloral types of honey (e.g. *Erica arborea*, *E. manipuliflora*, *E. verticillata*), clover honey (*Trifolium* spp.), *Arbutus unedo*, *Polygonum aviculare* naturally contain 20% of moisture (Thrasyvoulou et al., 2018). According to other studies, the moisture content in honey samples can range from 10.5% to 20.5% (Karabagias et al., 2014).

All the investigated samples of honey met the criteria of the national standard DSTU for this parameter (Table 2). However, 4% of honey samples from the 'Traditional village' stratum, 14% from the 'Intensive agriculture' stratum and 16% from the 'Intermediate' stratum did not meet the international standards requirements.

Honey contains organic (about 0.3%) and inorganic (0.03%) acids, so it has an acidic environment. There are formic, acetic, lactic, amber, malic, grape, citric, pyruvic, gluconic and some other organic acids in its content. As for the inorganic acids, phosphoric and hydrochloric acids can be registered in honey. Acids can be found in honey in free and bound states and get there from nectar, honeydew, pollen grains, bee secretions. They can also be synthesized in the process of enzymatic decomposition and oxidation of sugars (da Silva et al., 2016).

Free acidity is a parameter that is associated with deterioration in honey quality and it is characterized by the presence of organic acids in equilibrium with lactone, internal esters and some inorganic ions such as phosphates, sulphates and chlorides (da Silva et al., 2016).

It is important to note that honey has natural acidity regardless of its geographical origin. The high value of free acidity indicates the enzymatic conversion of sugars into organic acids and it is an indicator of the honey freshness. Complex transformations underlying the process of storage are known (Acquarone et al., 2007). These transformations increase the content of free acids and correspondingly reduce the value of the hydrogen index. The changes occur more intensively after 12 months of honey storage.

The increase in acidity also occurs during the fermentation of honey. Honey sugars are converted into volatile acids ($C_2 – C_{12}$) by yeast. These volatile acids impair the organoleptic properties of honey, in particular its colour and taste (da Silva et al., 2016).

The exceedance of the free acidity index compared to the national standards for the First-Class honey and international standards was revealed in the 'Intermediate' stratum – 12% of all samples, in the 'Intensive agriculture' stratum – 9.5%, in 'Traditional village' stratum – 4%. The elevated values of free acidity indicate the fermentation of sugars into organic acids (Table 3).

The share of samples that corresponded to the Extra Class of honey according to the national standards DSTU ranged from 53% for the 'Intermediate' stratum to 79% and 81% for 'Traditional village' and the 'Intensive agriculture' stratum, respectively (Table 3). Significantly, high acidity of honey in the 'Intermediate' stratum indicates the fermentation of sugars into organic acids. This may be due to the peculiarities of the geographical region or the content of honeydew impurities that cause high content of organic acids.

The hydrogen index characterizes the activity or concentration of hydrogen ions in honey solutions. Although the pH limit is not currently set by the Regulatory Committees, the allowable values of hydrogen are 3.2–4.5 (da Silva et al., 2016). The obtained results showed correspondence of the honey pH in all the studied strata to the permissible level. The pH value of honey is closely related to the existence and activity of microorganisms. The optimal value for most organisms is from 7.2 to 7.4, therefore low pH level will prevent microbiological spoilage of honey (Ratiu et al., 2020). The pH value may be an indicator of fake honey. Thus, adding of high-fructose corn syrup to honey significantly increases the pH value.

Proline is a free amino acid that gets into honey from the nectar of flowers, pollen grains and is produced in large quantities by bees. The content of proline in natural honey is 45% to 85% of the total number of amino acids (Postoienko et al., 2019). Therefore, this indicator is used as a criterion for the naturalness and maturity of this product. If honey is collected being immature or containing sugar blend, the proline content will be very low. Proline content and diastasis activity are indicators that stand for the enzymatic activity of honey according to the current regulations and standards. By determining the proline content, it is easy to assess the quality of honey of varied botanical origin (Adamchuk et al., 2019). Moisture content affects the overall amount of proline (Lazareva & Postoienko, 2016).

In accordance with the requirements of the national standard DSTU 4497:2005, the content of proline must be not less than 300 mg/kg for all types of honey of the Extra Class and the First Class and not less than 200 mg/kg for acacia honey. Codex Alimentarius CODEX STAN12-1981 and Council Directive 2001/110/EC do not regulate the content of proline. However, according to the agreement of the German Beekeepers Association, the content of proline in natural honey must be not less than 180 mg/kg. High quality honey can contain up to 550 mg/kg of proline (Lazareva, 2015).

Although the average values of proline for the study samples of honey met the standards of the current national standard DSTU (300.3–725.0 mg/kg), there were samples found in each of the study districts that had a proline content < 300 mg/kg.

The content of proline differed in the samples of honey from the study districts according to the Wilcoxon W-test. Higher proline content was observed in the samples from the 'Traditional village' stratum, which experiences the lowest anthropogenic effect compared to the other districts we studied. The share of samples that do not comply with the national standard DSTU 4497:2005 increased in the following order: the 'Traditional village' stratum (3.9%) → the 'Intensive agriculture' stratum (9%) → the 'Intermediate' stratum (47.1%, Table 3).

Conclusions

For physical and chemical parameters, the study shows that the honey from the Chernivtsi region is of high quality. The share of reducing sugars is ~ 80%, which indicates its nutritional value. The study samples of honey have a low pH level (~ 3.7) and a high content of proline (~ 513 mg/kg). Separate samples of honey besides fructose and glucose also included oligosaccharides, such as maltose, trehalose and melezitose. No signs of sucrose were detected in most parts of analyzed honey, excluding two samples from the 'Intermediate' stratum. The samples of honey from apiaries in the 'Traditional village' and the 'Intensive agriculture' strata complied with international and national quality standards. This indicates their better quality in comparison with the samples from the 'Intermediate' stratum. A total of 8–10% of samples deviated from the norms of Ukrainian and international standards. Therefore, encouraging continuous and up to date monitoring of honey is relevant.

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The authors declare the absence of any conflict of interest.

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