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Physichochemical Characteristics Of Honey Produced In Different Districts Of Albania

[1]**Elona Shahu, Ederina Ninga**
Food Safety and Veterinary Institute
Tirana, Albania
elona_shahu@yahoo.com

Fatjon Hoxha, Vlash Mara
Faculty of Biotechnology and Food,
Agricultural University of Tirana
Tirane, Albania

***Abstract*—The aim of this study, it was evaluated the quality of honey produced in 12-districts of Albania, as beekeeping is an activity spread throughout Albania. Thanks to the collaboration with Albanian Beekeepers Association (ABA) and the inspectors from official control authorities in all districts; a total number of 60-samples (5-samples per each district) were collected during three years of study 2016-2018. The samples were harvested in different seasons (spring and summer), with the aim to include and represent the honey produced in Albania; which are the predominant periods of the year when honey is produced, due to the bee plants flowering time. The quality parameters analyzed were the physicochemical parameters such as: water content, pH, hydroxymethylfurfural (HMF), free, lactone and total acidity, the dominant sugars in honey (fructose, glucose and sucrose), and total phenolic content (TPC). Based on the results and using factor analysis to explore possible influences of the districts on physicochemical parameters used in routine analysis, it was possible to differentiate honeys produced in the districts of Vlore, Gjirokaster, Kukes and Korce.**

Keywords—Honey, Physico-chemical Parameters, Quality Control, International Standards.

I. INTRODUCTION

Honey is made by bees, which collect the nectar from flowers and is called blossom honey, or collect the secretions of plants, or excretions of plant sucking insects on plants, known as honeydew [1]. After that, bees keep the nectar/honeydew in the stomach sac, and transform it through the addition of enzymes by bee glands, and deposit, dehydrate and store in the hive to let honey to ripen and mature [2, 3]. The chemical composition of honey is a mixture of sugars, where fructose (39.44%) and glucose (28.15%) are the main carbohydrates, followed by sucrose and maltose in low quantity, and other oligosaccharides in trace amounts. Also, the main component is water (17.90%), and other minor substances in trace amounts such as: organic acids, minerals, vitamins, flavonoids etc[4].This wide range composition is influenced by the floral origin, geographical origin, climate conditions, soil composition, season of the harvest, harvesting and extraction methods, processing and storage of honey.

It is well known that honey with the same botanical origin, produced in different areas shows differences in its composition, thus affected by geographic origin[5]. This is the same for other food products. Albania, being a Mediterranean country, the geographical position impacts its Mediterranean climate, which is characterized by a wet and soft winter and a hot and dry summer[6].

Albania is characterized by a rich diversity of ecolological systems including coastal zones, estuaries and lagoons, lakes and wetlands, grasslands, middle-low altitude coppice forests, high altitude forests, alpine vegetation and glacial areas. There are known around 3,250 species of plants, 165 families and more than 900 genera. Medicinal plants and non-timber forest products have been appreciated and used in Albania's culture and traditional ceremonies [7].

In literature, is reported that around 200 plants in Albania may be used by bees to collect nectar/honeydew and pollen [8], and the major bee flora is typical for Mediterranean flora [9].

Existing such biodiversity in climate characteristics and the richness of plant species grown, the aim of the study was to evaluate the quality of honey produced in 12-districts of Albania, and eventual influence of geographical origin on physicochemical quality and biological activity parameters of honey.

II. MATERIAL AND METHODS

The physicochemical analysis were carried out according to IHC Harmonized Methods[10].

Sugar Analysis

Chemicals and materials

The pH of each sample was measured using a solution containing 10 g of honey in 75 mL of CO2 free distilled water. After the pH determination the samples were used for determination of acidity by titrimetric methods (samples were titrated using sodium hydroxide to obtain the free acidity. Excess of sodium hydroxide (0.05 N) was added to hydrolyzed any lactose present and send immediately back to titration with HCl (0.05 N), hydrochloric acid. The total acidity was then calculated as the sum of free acidity and lactone acidity)

The moisture of honey samples was determined based on the refractometric method using a Abbe refractometer at 20º C after the homogenization and equilibrium. The determination of ash was performed

in an electric furnace by weighting five grams of each sample in a platinum ash dish. For the determination of electrical conductivity 20 gram of honey were accurately weighted and diluted with 100 ml of CO2-free distilled water.

HMF determination

10 g of honey was weighted and dissolved in 50 mL volumetric flask with of ultra-pure water, and the solution was filtered through a 0.45 µm filter before analysis. Hydroxymethylfurfural (HMF) was determined in a clear, filtered, aqueous honey solution using reverse phase HPLC equipped with UV detection. The absorbance was measured at 285 nm. Mobile phase was water-methanol (90+10 by volume), both HPLC quality.

Total phenolic content (TPC) was determined with Folin- Ciocalteubased on the work of Singleton with some minor modifications[11]. Briefly, 0.3 ml of the sample extract and 6 ml deionized water were mixed with 0.5 ml of 10% Folin- Ciocalteu reagent and the solution was incubating 6 min at room temperature. Then, 3 ml of 7.5% sodium carbonate were added. After 30 min at 40°C, absorbance was measured at 765 nm. As a standard was used gallic acid, concentration 50-250 µl/ml. Blank was prepared by mixing water and reagent. Results were expressed as mg gallic acid per kilogram honey.

Glucose, fructose andsucrose were purchased from Tokyo Chemical Industry, TCI (Europe, Belgium). All chemicals used whose purity were of analytical purity grade. Ultra-pure water (MicroPure water purification system, 0.055 µS/cm, TKA, Thermo Fisher Scientific, Niederelbert, Germany) was used to prepare standard solutions and blanks. Syringe filters (13 mm, PTFE membrane 0.45 µm) were purchased from Supelco (Bellefonte, PA). Filter paper (Whatman No. 1) was supplied by Merck (Darmstadt, Germany).

High-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC/PAD)

0.3 g of honey was weighted and dissolved in 1000 mL of ultra-pure water, and the solution was filtered through a 0.45 µm filter before analysis. Chromatographic experiments were performed using DIONEX ICS 3000 DP liquid chromatography system (Dionex, Sunnyvale, CA, USA)equipped with a quaternary gradient pump (Dionex). The carbohydrates were separated on a Carbo Pac®PA100 pellicular anion-exchange column (4×250 mm, particle size - 8.5 µm, pore size – microporous, <10 Å, (Dionex) at 30 °C. The mobile phase consisted of the following linear gradient (flow rate, 0.7 mL/min): 0–5 min, 15% A, 85% C; 5.0–5.1 min, 15% A, 2% B, 83% C; 5.1–12.0 min, 15% A, 2% B, 83% C; 12.0–12.1 min, 15% A, 4% B, 81% C; 12.1–20.0 min 15% A, 4% B, 81% C; 20.0–20.1 min 20% A, 20% B 60% C; 20.1–30.0 min 20% A, 20% B 60% C; where A was 600mM sodium hydroxide, B – 500mM sodium acetate and C was ultrapure water. Before the analyses, the system was preconditioned with 15% A, 85% C, for 15 min. Each sample (25 µL) was injected with an ICS AS-DV 50 autosampler (Dionex). The electrochemical detector consisted of gold as the working and Ag/AgCl as the reference electrode.

III. Results and Discussion

In Table 1 are shown the results of the characteristics of honeys produced in all the districts of Albania. All the parameters analyzed, except HMF are in accordance with the threshold limits established in honey standard [2, 3]. The water contents of honeys ranged from 14.74% to 19.94% (max. 20%). High water content influences the shelf-life of honey, due to the increased activity of yeasts, which are responsible for initiating the fermentation process and deterioration of honey. Honey produced in the districts of Vlora and Gjirokastra, show low water content, respectively 15.86% and 14.74%.Related to ash and electrical conductivity (EC), the values are typical for honey obtained from nectar. In literature, values of EC under 0.5 mS/cm are reported for blossom honey [12]. Also, EC serves in routine analysis to differentiate some monofloral honeys [13]. pH values in the range 3.66-4.42, confirming the blossom origin of honey samples[14].

TABLE I. Mean±SD results of honey physicochemical analysis for all the districts in Albania

District		Moisture (%)	Ash(%)	EC(mS/cm)	pH	Free Ac.(meq/kg)	Lact Ac.(meq/kg)	Tot. Ac. (meq/kg)	HMF (mg/kg)	TPC (mg/kg)	Glucose (%)	Fructose (%)	Sucrose (%)	F+G	F/G	G/U
Diber	**Mean**	17.08	0.05	0.47	3.66	27.45	4.35	31.80	13.10	547.72	29.09	39.33	2.39	68.42	1.35	1.74
	SD	0.37	0.02	0.12	0.28	3.41	1.26	4.63	1.61	63.41	0.31	1.60	0.16	1.91	0.04	0.04
Durres	**Mean**	16.88	0.16	0.43	3.97	26.65	5.88	32.53	**44.55**	412.30	27.89	34.26	3.19	62.15	1.28	1.70
	SD	0.39	0.21	0.03	0.36	8.50	2.00	6.81	**34.84**	203.53	4.77	2.45	0.55	2.32	0.31	0.32
Elbasan	**Mean**	17.10	0.04	0.36	3.97	24.50	3.52	28.02	13.70	412.66	26.53	35.45	1.67	61.98	1.34	1.55
	SD	0.36	0.01	0.07	0.19	3.56	2.15	1.81	0.68	114.02	0.13	3.87	0.41	3.75	0.16	0.04
Shkoder	**Mean**	17.88	0.06	0.43	4.42	27.62	3.37	30.99	27.79	**613.76**	25.84	36.65	**5.62**	62.49	1.43	1.43
	SD	0.20	0.01	0.05	0.20	5.16	2.18	4.06	11.67	202.81	4.58	4.55	0.50	9.13	0.08	0.24
Lezhe	**Mean**	17.04	0.05	0.37	4.08	25.02	2.80	27.83	14.04	528.34	25.75	38.03	3.81	63.77	1.49	1.49
	SD	0.37	0.02	0.20	0.29	2.88	1.63	4.44	8.42	189.17	2.65	0.42	2.87	3.06	0.14	0.17
Kukes	**Mean**	16.28	0.07	0.55	3.96	34.45	10.55	45.00	25.72	569.16	30.35	42.85	3.44	73.20	1.42	1.87
	SD	0.31	0.01	0.05	0.33	2.91	2.51	1.85	19.26	175.71	1.80	1.70	0.81	3.50	0.03	0.06
Tirane	**Mean**	16.92	0.04	0.35	3.92	25.11	6.43	31.54	13.46	345.24	27.77	39.14	3.91	66.91	1.41	1.67
	SD	0.32	0.01	0.05	0.22	6.82	3.25	6.52	0.80	100.25	2.97	4.20	1.37	7.17	0.00	0.18
Vlore	**Mean**	**15.86**	0.04	0.37	4.13	**18.03**	4.45	22.48	10.78	**202.34**	26.36	35.93	1.61	62.28	1.37	1.69
	SD	0.54	0.01	0.09	0.11	10.61	1.08	11.64	0.06	39.65	0.32	0.20	0.29	0.12	0.03	0.00
Gjirokaster	**Mean**	**14.74**	0.04	0.34	3.90	23.86	11.49	35.35	16.18	383.36	27.23	39.49	3.94	66.72	1.46	1.87
	SD	0.40	0.01	0.04	0.08	5.60	1.50	4.64	4.07	121.64	2.11	0.85	1.62	2.96	0.09	0.15
Fier	**Mean**	17.00	0.04	0.35	3.84	27.94	3.35	31.29	22.50	295.58	27.60	34.27	1.06	61.87	1.24	1.60
	SD	0.34	0.01	0.04	0.13	7.47	1.23	7.91	0.57	90.62	0.04	0.26	0.20	0.30	0.01	0.03
Korce	**Mean**	19.94	0.05	0.44	3.67	37.00	5.05	42.05	22.80	522.44	27.91	44.30	4.15	72.20	1.59	1.35
	SD	0.57	0.02	0.15	0.02	3.13	1.35	3.53	10.52	75.97	1.37	1.48	1.73	0.11	0.13	0.06
Berat	**Mean**	18.58	0.04	0.39	3.85	26.15	2.60	28.75	12.09	275.04	30.29	39.25	2.88	69.53	1.30	1.62
	SD	0.35	0.02	0.10	0.11	7.46	2.78	8.07	0.38	16.36	0.49	1.66	1.12	1.17	0.08	0.02

Free acidity was in the range 18.03-37.00 meq/kg, lactone acidity 2.60-11.49 meq/kg and total acidity 22.48-45.00 meq/kg. Values of free acidity above 50 meq/kg (the maximum allowed), are indication that fermentation has occurred in honey. HMF is a parameter of honey freshness, because its content increases during processing and storage [15]. The range for HMF is between 10.78-44.55 mg/kg, resulting Durres district exceeding the maximum limit of 40 mg/kg established in honey standard. Total Phenolic Content (TPC) registered the minimum value for honeys produced in the district of Vlora (202.35 mg GAE/kg) and the maximum in honeys produced in Shkoder (613.76 mg GAE/kg). Glucose is in the range 25.75-30.35%, fructose in the range 34.26-44.30%; confirming the dominant sugar in honey, being fructose. Sucrose in honey must not exceed 5% (except some unifloral honeys, which are indicated in honey standard), and according to values in Table 1, they are in correspondence with the limit established, except for mean value of honeys in the district of Shkodra. The sum of fructose+glucose it is an important criteria, and is used to discriminate blossom honey and honeydew honey. In all the districts, the mean value is above 60g/100g of honey, classifying the samples as honey made from the nectar of flowers. The last two parameters indicated in Table 1; the ratio Fructose/Glucose and Glucose/Water are very important in predicting the crystallization phenomena in honey[16]. The range for F/G is between 1.24 and 1.59, and for G/W between 1.35 and 1.87.

Using only the physicochemical parameters in routine analysis, through the factor analysis, in Table 2 are shown the sorted factor loadings taking in consideration absolute values above 0.3 and Communalities.

TABLE II. SORTED ROTATED FACTOR LOADINGS AND COMMUNALITIES FOR SOME PHYSICOCHEMICAL QUALITY PARAMETERS IN ROUTINE ANALYSIS (VARIMAX ROTATION)

Variable	F1	F2	F3	F4	F5	F6	F7	Communality
Free acidity	0.977	0	0	0	0	0	0	1
Total acidity	0.942	0.303	0	0	0	0	0	1
Lactones acidity	0	0.942	0	0	0	0	0	1
pH	0	0	-0.990	0	0	0	0	1
EC	0	0	0	0.983	0	0	0	1
Ash	0	0	0	0	0.990	0	0	1
Moisture	0	0	0	0	0	-0.942	0	1
Variance	1.9179	1.0703	1.0071	1.0031	1.0014	1.0002	0	7
% Var	0.274	0.153	0.144	0.143	0.143	0.143	0	1

As it is shown in Table 2, in the first Factor which represents 27.4% of the data variability, the contribution is presented by free acidity and total acidity, which show a strong positive correlation (value above 0.9).

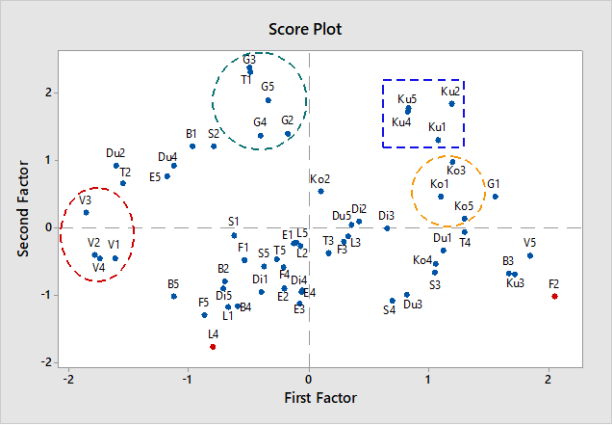

Fig 1: The score plot of data samples of all districts of Albania

The second factor F2 represents only 15.3% of the data variability, and the parameter which contributes to it and has a strong positive correlation with lactones acidity (value above 0.9). pH shows to have a strong negative correlation in the third factor F3. In general, to represent these data the first two factors are taken in consideration. In Fig. 1 is shown the score plot of data samples (60 samples in total; 5 samples per each district), and the samples are presented by the first letter of the districts (in the case of same first letter, also the second letter was took in consideration).

Based on the results, it was possible to distinguish honey samples (4 from 5 samples) produced in the districts of Vlora, Gjirokastra, Kukes and Korça (3 from 5 samples). Two samples (F2 and F4), are not grouped with other samples, considering that they represent extreme differences from the pool of data.

IV. CONLUSIONS

According to physicochemical analysis, in general all the parameters analyzed are within the values established in quality criteria in the honey standard. HMF shows values above the maximum limit, and is an indication of heat treatment during extraction. It is evident that physicochemical parameters used in routine analysis, can be used to differentiate honeys produced in some districts of Albania, and this is confirmed by factor analysis.

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