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Quality Evaluation of Commercialised Honey in Tirana Using Physicochemical Analysis

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Abstract

In this paper, a total of five market samples and one sample from a credible beekeeper were analyzed to evaluate the quality of honey in Albania. The physicochemical parameters analyzed included: moisture, pH, free acidity, ash content, insoluble matter and 5-hydroxymethylfurfural (5-HMF). The results obtained were compared with Albanian Standard of Honey (*Directive No. 21, Date 25.11.2010*). Based on the results it was observed that values of pH, free acidity, ash content and insoluble matter were within the limits for all samples analyzed. Only sample **MS4** exceeded the moisture level of 20% set in the standard. Four out of five market samples (MS): **MS2**, **MS3**, **MS4** and **MS5** recorded an amount of HMF between 59.65 - 285.75 mg/kg (maximum limit in the standard is 40 mg/kg), compared with sample **B1** (beekeeper's sample), where the HMF amount was very low, 1.047 mg/kg. High values of HMF indicate that honey had undergone excessive heat treatment during processing and/or inadequate storing conditions, which can affect honey quality. HMF parameter is very important because it evaluates honey freshness and quality.

Keywords: Honey, Physicochemical, Quality, HMF, Standard

1. Introduction

The most important primary product of beekeeping is honey, and the history of its use is parallel to the history of mankind. In every culture, evidence of honey uses can be found as a food source but also as a symbol employed in religious, magic and therapeutic ceremonies [11].

Over the last years, honey production and consumption in Albania has increased steadily. The consumer request for natural products has increased the consciousness of people and beekeeping is transformed from a simple passion to a familiar business, where it is likely to obtain significant profits. Honey production from the year 2000 to 2016 has increased by 265%, with an estimated production for 2016 at 3,923 tonnes, and the number of beehives for the same period has increased by 298%, with a total number in 2016 at 302,962 beehives [7].

Honey is a natural and healthy product, predominantly composed of sugars and other constituents such as enzymes, amino acids, organic acids, carotenoids, vitamins, minerals, and aromatic substances [12,14,19]. It is rich in flavonoids and phenolic acids that exhibit a wide range of biological effects and acts as natural antioxidants [6]. According to Albanian standard of honey (*Regulation No. 123, Date 30.03.2007*), which in 2007 was adopted from EU Regulation 2001/110/EC, and it was updated in 2010 (*Directive No. 21, Date 25.11.2010*) [13], honey is defined:

"*as the natural sweet substance produced by Apis mellifera bees from the nectar of plants or from secretions of living parts of plants or excretions of plant-sucking insects on the living parts of plants,*

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which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in honeycombs to ripen and mature" [17].

The composition of honey changes during processing or prolonged periods of storage where different process occurs involving heat treatment, oxidation, fermentation, and as such modifying honey composition. For instance the formation of 5-Hydroxymethylfurfural (5-HMF), which is a product of decomposition of sugars and/or Maillard reaction when honey is pasteurized or stored for a long period [6]. Honey quality is associated with sensorial characteristics, physicochemical analysis and microbiological criteria [8]. Generally, consumers are unaware of the quality of honey they buy. That being the case, it is necessary to control the quality of commercial honey, due to related cases of adulteration [9]. People think that honey is a raw product, extracted from honeycomb, which can be consumed directly, but most of the honey available in the market is processed like other food products. Honey is pasteurized to destroy microorganisms (generally yeasts) and to delay crystallization, which enable the shelf-life of the product to be extended and make it pleasant to consumers, since they like honey to be in a liquid state. The study was designed to evaluate the quality of commercial honey available in the market for purposes of comparison with raw honey coming from a credible beekeeper. Just as important is to see if it complies with Honey Standard. Also, it would help gather necessary data for honey consumers. The quality of honey is generally evaluated by physicochemical analysis of its constituents and it has two principal purposes, to verify, first, its authenticity i.e. to uncover potentially frauds such as adulteration with artificial honey (sugar syrups) etc, and to determine its quality in respect to the needs of the processor and the market [11], because when it comes to honey marketing it is important to guarantee consumers that they get what they are paying for. *Codex Alimentarius Commission* (**CODEX STAN 12-1981**) has internationally defined the composition limits of the natural product and it also gives indication of the officially approved analytical methods.

In different countries, more restrictive laws and regulations exist, which one must refer to if marketing is intended in these countries. Legal quality standards serve to protect the consumer and regulate trade import/exports between countries [11].

2. Material and Methods

The present study was carried out at the laboratory of Center of Food Research (**CFR**), Faculty of Biotechnology and Food, Agricultural University of Tirana, Albania. A total of six samples of polyfloral honeys were collected for purposes of this study, with five samples being collected in Tirana markets across different spots and one sample collected from a credible beekeeper from region of Skrapar (each sample weighing 0.5 kg). The samples were analyzed to determine the physicochemical parameters such as: moisture, pH, Free acidity, insoluble matter, ash content and 5-hydroxymethylfurfural (5-HMF) according to International Honey Commission Methods [2]. The analysis was performed in triplicate.

2.1 Physicochemical parameters

2.1.1 Moisture content

Moisture content of honey samples was determined at ambient temperature by using an Abbe 60/DR refractometer (Bellingham & Stanley Ltd, UK). The refractive index increases as the solid content increases. The measurement was performed on 100 g of honey at 20°C and a correction factor of 0.00023 was added per °C (temperature above 20°C) to obtain the corresponding percentage of moisture from refractive index by consulting Chataway table.

2.2.2 pH and Free Acidity

10 g of sample were dissolved in 75 ml of distilled water in a 250 ml beaker. The solution was stirred with magnetic stirrer and the pH electrode was immersed in the solution and the pH was recorded. The solution was titrated with 0.1M NaOH to pH 8.30.

Free acidity, expressed as milliequivalents acid/kg honey was calculated:

Free acidity = ml of 0.1M NaOH x 10 (1)

2.2.3 Ash content

5 g of sample were weighted into an ash dish and two drops of olive oil were added. The sample was burned and incinerated in muffle furnace at 550°C for 5 h. The ash was cooled in a desiccator to reach ambient temperature and after that was weighted.

2.2.4 Insoluble matter

20 g of honey were dissolved in 200ml beaker of water at about 80°C and mixed well. The prepared sample is first filtered through a dried and steady sintered funnel. The funnel was dried for an hour at a temperature of 135°C, then cooled in desiccator and measured with an

accuracy of 0.1 mg. The amount of matter insoluble was calculated according to the following formula:

$$\textbf{\% Insoluble Matter in g/100g} = \frac{m}{m_1} x100 \quad (2)$$

where:

m = mass of dried insoluble matter

m_1 = mass of honey taken

2.2.5 Hydroxymethylfurfural (HMF)

The HMF content was determined according to White based on Bogdanov methods of analysis [2]. Five grams of honey were dissolved in 25 ml of water, transferred quantitatively into a 50 ml volumetric flask, added by 0.5 ml of Carrez solution I and 0.5 ml of Carrez II and make up to 50 ml with water. The solution was filtered through paper rejecting the first 10 ml of the filtrate. Aliquots of 5 ml were put in two test tubes; 5 ml of distilled water were added to one tube (sample solution); 5 ml of sodium bisulphite solution 0.2% were added to the second (reference solution). The absorbance of the solutions at 284 and 336 nm was determined using a Biochrom Libra S22 UV/Vis Spectrophotometer.

The HMF content was calculated by the equation:

HMF (mg/kg) = (A_{284}-A_{336}) x 149.7 x (5 g of sample) (3)

where:

A_{284}: the absorbance at 284 nm

A_{336}: the absorbance at 336 nm

149.7: a factor calculated by the molecular weight of HMF and the mass of the sample.

3. Results and Discussion

The obtained results are shown in Table 1, where the Beekeeper sample was nominated with B1 and market samples were nominated with MS1-MS5. The mean value and standard deviation are presented for all the samples.

Table 1: Physicochemical analysis of honey samples

Parameter	B1 Mean ± SD	MS1 Mean ± SD	MS2 Mean ± SD	MS3 Mean ± SD	MS4 Mean ± SD	MS5 Mean ± SD
Moisture (%)	14.15 ± 0.64	16.40 ± 0.85	16.00 ± 0.28	17.80 ± 0.57	21.00 ± 0.57	16.60 ± 0.57
pH	3.21 ± 0.04	3.76 ± 0.09	3.51 ± 0.09	3.33 ± 0.057	3.06 ± 0.11	2.96 ± 0.042
Free Acidity (meq/kg)	29.00 ± 0.42	22.00 ± 0.21	11.00 ± 0.43	12.00 ± 0.85	17.00 ± 0.34	20.50 ± 0.64
Ash Content (%)	0.14±0.02	0.24±0.03	0.04±0.028	0.01±0.014	0.06±0.011	0.05±0.014
Insoluble Matter (%)	0.12±0.01	0.00±0.00	0.00±0.00	0.015±0.008	0.01±0.012	0.005±0.0012

Only sample **MS4** exceeded moisture limit of 20% set in the standard and other samples were within limit. The moisture must be under 18% for a long period of storage, because high water content can influence honey fermentation and it serves as a criterion of quality and honey shelf-life. Also, water content can affect crystallization and HMF formation. Relating to pH values, the samples exhibited values that are within the normal range for polyfloral honeys, with sample **MS1** having the highest value of pH 3.76. Microorganism's activity is inhibited in low pH values. Free acidity was within the limit established, less than 50 meq/kg. Sample **B1** had the highest value of 29 meq/kg and the values of market samples were within the range 11-22 meq/kg. Determination of free acidity is important because values above the limit show that honey has started the fermentation process, since honey yeasts transform sugars and alcohols into acids [4]. The degree of acidity depends not only on the total concentration of organic acids and the type present in honey, but also on mineral composition [15].

In Table 2 are shown the statistical results of parameters in all analyzed samples.

Usually, blossom honeys have low mineral content, within the range 0.02 - 0.3%, because they are produced from the nectar of flowers [12], as such in the standard, mineral content was set at <0.6%, until ash content was proposed to be replaced in routine analysis by electrical conductivity [1] and in 2001 revision of Codex Alimentarius Standard of honey (CODEX STAN 12-1981) and EU Directive related to honey (2001/110/EC), this change was adopted [5,17].

Table 2. Mean values and the covariance of the six samples analyzed.

	Mean	SD*	CV	Min.	Max.
Moisture	16.99	2.29	13.49	14.15	21.00
pH	3.30	0.29	8.95	2.96	3.76
Free Acidity	18.58	6.74	36.27	11.00	29.00
HMF	119.93	121.90	101.65	1.05	285.75
Ash Content	0.09	0.09	94.80	0.01	0.24
Insoluble Matter	0.02	0.05	187.61	0.00	0.12

*SD = Standard Deviation; CV = Coefficient of variance; Min. =Minimum value; Max. = Maximum value

As it is shown in Table 1, ash content was very low in samples **MS2, MS3, MS4** and **MS5** that show a percent under or equal to 0.06%, and samples **B1** and **MS1** show values at 0.14 and 0.24% respectively. In relation to insoluble matters, the percentage was zero in samples **MS1** and **MS2**, which is an indication of filtration of honey. This parameter is very important in order to detect honey impurities such as beeswax, dirt particles, honey comb debris etc. Sample **B1** showed the highest value of 0.12%. The limit in the standard is set not more than 0.1% in general and for pressed honey at ≤ 0.5%.

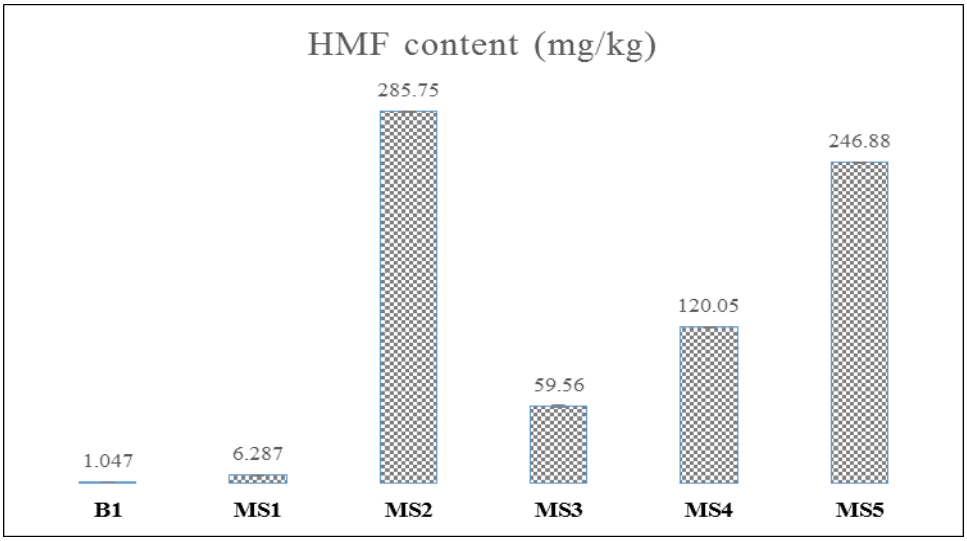

Figure 1. HMF content

Judging from HMF values from Figure 1, it is evident that market samples **MS2**, **MS3**, **MS4** and **MS5** exceed 40 mg/kg, which is the maximum content of HMF set in honey standard (honeys originating from tropical regions have a higher limit of 80 mg/kg). Sample **B1** showed a value of 1.047 mg/kg HMF, and it is evident that it is fresh honey, that has been harvested recently and has not undergone any heat treatment during extraction. Samples **MS2** and **MS5** had the highest values (respectively 285.75 and 246.88 mg/kg) and must be regarded as potentially adulterated, because levels of HMF above 200 mg/kg in honey are suggested to contain acid invert syrups [18]. 5-Hidroxymethylfurfural is a product of decomposition of sugars under heating and storing condition, and it depends on pH, moisture of honey. HMF increases during storage and heat treatment of honey. Also, metallic containers and floral type of honey affect HMF concentration [10,16]. It must be noted that only HMF cannot be used as an evidence of extensive heating or a prolonged period of storage and adulteration with invert syrup, without other indexes such as the presence of enzymes (diastase, invertase), organic acids and sugar analysis [6]. Therefore, the HMF content only gives an indication of excessive heating or inadequate storage conditions. High HMF content in honeys may also show the adulteration by adding invert syrup because this organic compound is produced by heating sucrose at high temperature in the presence of an acid to obtain invert syrup [3].

Actually, in Albania, the honey standard in force is *Directive No. 21, Date 25.11.2010*, although in routine control of honey quality methods of analysis are based on National Standard **SSH 376/1:1991**, and for honey characteristics, it is referred to **SSH 1435:1991**. Comparing Directive *No. 21, Date 25.11.2010* and **SSH 1435:1991,** in general, there exist differences in terms of expression and limits for parameters such as HMF, Free acidity and reducing sugars. It is imperative to use only one standard, *Directive No. 21, Date 25.11.2010*, which is in accordance with *Codex Alimentarius* standard of honey (CODEX STAN 12-1981, revised in 1987 and 2001) and EU Regulation 2001/110/EC [5,17]. Referring to the HMF limit set in SSH 1435:1991, it is lower (15 mg/kg), and related to free acidity, exists differences in unit expression and limit. Also, significant differences are found in reducing sugars. According to *Directive No. 21, Date 25.11.2010*, reducing sugars are not expressed as total, but as sum of fructose and glucose content, and the minimum limit is set according to origin: i) blossom honey or nectar honey, which is obtained from the nectar of plants, not less than 60 g/100g; ii) honeydew honey, which is obtained mainly from excretions of plant sucking insects (*Hemiptera*) on the living part of plants or secretions of living parts of plants, not less than 45 g/100g. The absence of harmonized standards related to composition criterion and methods of analysis often causes confusion when it comes in interpreting the results.

4. Conclusions

The results obtained show that honey available in the market, generally, is not within the limits of quality standard set in legislation (*Directive No. 21, Date 25.11.2010*). The most important constituent that gives an indication of honey aging and/or excessive heating during processing is HMF. Also, close attention should be paid to honey shelf-life set on the label, because honey that exceeds quality parameters should be designated as baker's honey. Also, it is an urgent need to adopt, harmonize and perform honey quality tests based on current legislation of honey standard and methods of analysis. The information obtained from this study might be useful for the unification of official standards by Albanian responsible authorities.

5. Acknowledgment

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