STUDY REGARDING THE QUALITY OF HONEY - CHANGES IN HYDROXYMETHYLFURFURALDEHYDE (HMF) CONTENT OF THE HONEY FROM TRANSYLVANIA (ROMANIA)

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Abstract
Honey is the sweet viscous substance elaborated by the honey bee from the nectar of floral plants. It is produced in almost every country of the world and is a very important energy food, used as an ingredient in hundreds of manufactured foods, mainly in cereal based products, for sweetness, flavor, color, caramelization and viscosity.
Sugars represent the largest portion of honey composition (95-99% of the honey solids). Fructose and glucose are the most abundant sugars in honey, but others are usually mentioned, namely, saccharose, maltose, melibiose and trealose. Its composition depends highly on the types of flowers used by the bees as well as regional and climatic conditions.
Adulteration of honey is possible, so its quality must be controlled analytically with the aim of guaranteeing the genuinity and preserve consumer from commercial speculations.
Detection of honey adulteration is difficult and depends on the composition of the honey or on the presence of a specific component in the adulterant, like HMF (5-hydroxymethylfurfuraldehyde).
The presence of HMF in honey could be due to the partial decomposition of the endogenous or exogenous fructose or to the honey overheating. Therefore, the HMF content is essential to evaluate the quality of honey to the current legislation.
Both the Codex Alimentarius Commission (Alinorm 01/25, 2000) and the European Union (Directive 110/2001) established that its concentration in honey usually should not exceed 80 or 40 mg/kg, respectively.
The present paper focuses on the determination of sugars (fructose and glucose) and HMF from different types of honey from Transylvania using UV-VIS spectrophotometry.
The experimental results showed that some of investigated samples have high soluble solids, reducing sugars, but low moisture content and HMF indicating a good quality of the honey. In other samples, the too high HMF indicates their adulteration with invert sugar.

Keywords: honey, HMF, natural invert sugar and sucrose, water activity; acidity, HACCP

1. AIMS AND BACKGROUND

Honey is the sweetest natural food product obtained by honey bees (*Apis mellifera L*), who use "raw matter" provided by plants directly, and also, to a smaller extent, they use elements from other sources [1-5].A basic raw matter from plants is flower or extra-flower nectar, to which some other elements can be added such as the excretions of insects (aphids and other plant-feeding insects), resulting in a product known as honeydew honey. According to *Codex Alimentarius* [3-6], the nectar from flowers or from other sources is rich in sugars that bees process, enrich with the substance they produce and turn into specific products that they then store in wax combs as honey. Due to its physical-chemical proprieties honey has a large nutritive and medicinal value: high content in glucose and fructose easily assimilated; protects the digestive tract and favours the absorption of iron in the intestine; helps assimilate calcium and magnesium; antianemic effect and increase the haemoglobin in blood; antibacterial and cicatrising role [7].According to STAS 784/3-1989, the main physical and chemical characteristics of honey found in Romanian stores are presented in table no. 1.

Table 1. Physical, chemical and microscopic characteristics of honey

Water, % max.	20
Acidity, ml NaOH sol.1N/100g max.	4
Reducing sugar, expressed as invert sugar, %, min.	70

Hydrolysable sugar, expressed as sucrose, %, max	5
Ash, %, max.	0.5
Hydroxymethylfurfuraldehyde (HMF), mg / 100 g max.	1.5 *
Colour index, mm. (Pfund scale)	max.18
Water activity [8]	0.562 - 0.740

*Honey found in jars for sale allows for an HMF content of max. 4 mg per 100g.

Bee honey is a food product which is susceptible of adulteration. Honey adulteration is caused by human factor in order to market a greater quantity and to obtain undue benefitsis. Honey bee can bee mix with different products: sugar, invert sugar, glucose syrup, iso-syrups, treacle, flour, starch, gelatine, glue, pectin, etc. [9].

In general, honey authenticity analysis has to evaluate if the physical-chemical and sensor parameters fall within the limits set by the current legislation; it also has to identify any changes or alterations found in the product, to reveal fraud and especially to identify originality.

The basic criterion for identifying honey adulteration with chemically inverted sugar is the hydroxymethylfurfuraldehyde (HMF) content in honey. The presence of HMF in honey could be due to the partial decomposition of the endogenous or exogenous fructose or to the honey overheating.

As a general rule, natural honey has almost or no HMF content at all. The applicable standard limits the HMF content to a maximum of 1.5 mg/100g for honey sold in bulk, and to 4.0 mg/100g for the honey sold by the jar.

Concentrations of HMF in honey over 10 mg/100g lead to suspicions of added invert sugar, as large amounts of HMF are formed through sucrose inversion by heating and in the presence of organic acids.

Elevated concentrations of HMF in honey provide an indication of overheating, storage in poor conditions or age of the honey. Both the Codex Alimentarius Commission (Alinorm 01/25, 2000) and the European Union (Directive 110/2001) established that its concentration in honey usually should not exceed 8 or 4 mg/100 g, respectively.

Besides the elevated concentrations of HMF, an analysis of adulterated honey with artificial invert sugar indicates a high content of sucrose. Invert sugar presents an inferior value to that of natural honey.

Water activity is an important criterion for the evaluation and control of honey bee safety and quality, illustrated by its incorporation into FDA and USDA regulations, GMP and HACCP requirements, and in NSF International Draft Standard 75. [10]

The present paper focuses on the determination of HMF and sugars (natural invert sugar and sucrose) from different types of honey from Transylvania using UV-VIS spectrophotometry and Elser method, respectively.

2. EXPERIMENTAL

10 bulk liquid honey samples of known origin gathered from different areas of Transylvania county (Romania) during 2008 and presented in table no. 2 were used for analysis. Each honey sample was purchased in duplicate in a jar of 500 g.

Table 2. Honey samples for analysis

Sample	Commercial name	Source
S_1	Polyfloral honey	Blaj, Alba county
S_2	Polyfloral honey	Dej, Cluj county
S_3	Polyfloral honey	Alba Iulia, Alba county
S_4	Polyfloral honey	Teius, Alba county
S_5	Lime tree honey	Simeria, Hunedoara county
S_6	Lime tree honey	Alba Iulia, Alba county
S_7	Lime tree honey	Targu Mures, Mures county
S_8	Acacia honey	Alba Iulia, Alba county
S_9	Acacia honey	Saliste, Sibiu county
S_{10}	Acacia honey	Hateg, Hunedoara county

The main physical and chemical indicators that reflect bee honey quality were analysed: hydroxymethylfurfuraldehyde (HMF), sucrose, invert sugar, humidity, ash, acidity. For the duplicate samples, the values set by STAS

784/3-89 were taken under consideration [11-15].

HMF concentrations were determined by the Winkler method. 10g of honey were dissolved in 20 ml water and transferred to a 50 volumetric flask. 2 ml of the solution and 5.0 ml of p-toluidine solution were put in two different test tubes; to one tube was added 1 ml of distilled water (reference solution); to the second, 1 ml of barbituric acid solution 0.5% (sample solution). The absorbance of the solutions at 550 nm was determined using a UV–VIS spectrophotometer (model T80 PG Instruments, UK). The quantitative value of HMF was determined by the external standard method (p 99%, Sigma-Aldrich).

The HMF content expressed in mg for 100 g honey was calculated according to the following equation:

$$HMF\ mg/100g = \frac{E}{S} \cdot 19.2$$

where: E – absorbance value;
S – diameter of the flask (cm);
19.2 – the conversion factor for the absorbance in mg equivalent of HMF.

In order to determine *sucrose* and *invert sugar*, the Elser method was used, according to STAS 784/2-1989.

Moisture content was determined measuring the refractive indices at 20^0C by an ABBE refractometer and the corresponding moisture content (%) was calculated according to AOAC method.

Ash content was directly determined by calcination at the temperature of 525^0C, at a constant mass.

In order to determine *acidity*, the sample of honey diluted with water was titrated with sodium hydroxide using the phenolphthalein as indicator.

The *water activity* (a_w) was measured with Aquaspector AQS-2-TC.

3. RESULTS AND DISCUSSIONS

The results obtained in the ten honey samples investigated are presented in table no. 3.

Table 3. Physical –chemical parameters in the analysed natural honey

Sample	HMF [mg/100g]	Hydrolysable sugar -sucrose [%]	Reducing sugar- invert sugar [%]	Moisture content [%]	Ash content [%]	Acidity [^{0}A]	a_w
S_1	1.5	3.0	86	15.8	0.32	2.0	0.688
S_2	2.0	4.0	78	16.2	0.38	3.0	0.643
S_3	2.4	4.2	81	13.2	0.47	2.2	0.584
S_4	4.0	4.1	83	13.6	0.50	3.4	0.607
S_5	3.8	4.8	82	14.0	0.49	3.2	0.596
S_6	3.9	4.2	79	14.4	0.48	3.6	0.619
S_7	4.0	4.6	84	14.8	0.40	2.8	0.624
S_8	4.0	4.3	75	15.0	0.43	3.0	0.642
S_9	98	100	44	19.8	0.45	6.8	0.781
S_{10}	80	98	42	19.2	0.49	8.0	0.765

As tables 3 show, the sample S_1 was the only sample analysed which indicated high quality honey and thus, it was the only one which observed all the Codex Alimentarius norms.

Samples S_2-S_7 indicated a fairly good quality honey, which had not been adulterated with sugar syrup or sucrose.

On the contrary, samples S_9 and S_{10} showed quantities of HMF and sucrose which were about twenty times higher than the maximum allowed values. In the same samples, the content in invert sugar was about 60% lower than the minimum allowed value. The obtained results showed that samples S_9 and S_{10} had been adulterated by adding chemically inverted sugar syrup. These conclusions were confirmed by the results obtained for acidity determination, which in the case of samples S_9 and S_{10} exceeded 170% and 200%, respectively the limits set by the STAS and a_w values.

As regards water content in honey, all the analysed samples showed moisture values within the limits set by STAS 784/3 – 1989, which lead to the conclusion that none of the samples had been adulterated by water addition. Likewise, the ash content values fell within the set limits.

Water activity is an important critical control point for. These regulations and requirements

are based on the current FDA Food Code definition of potentially hazardous foods. Potentially hazardous foods are those that require temperature control because they support the rapid and progressive growth of pathogenic microorganisms.
Based on the conducted tests and risk analysis - hazards assessment that may occur during of natural honey processing, it was decided that HACCP plan should include the liquefying honey/heat treatment stage, like an important sources for hazards corresponding to "good beekeeping practice".
The plan for this stage is presented in table 4:

Table 4. Hazards analysis and evaluation during liquefying honey/heat treatment

Step	Liquefying honey, heat treatment
Type of hazard	Chemical
Hazard description	Overheating of honey while packing leads to reaching of too much HMF
Defect	Temperature, time, quick cooling
Importance/Gravity	Medium
Control measures	Temperature, time measuring
Measures	Use as industrial honey

The analysis of this plan leads to the idea that the liquefying/heat treatment of honey is a control point CP (AP – attention point) with a chemical risk for foodstuff safety, which requires a strict monitoring of temperature and time for this step.

4. CONCLUSIONS

The physical-chemical analysis of the ten different samples of natural honey from Transylvania showed that most of the investigated samples had low moisture, ash and HMF contents indicating a good quality of the honey.
In samples 9 and 10, the high values of the HMF and sucrose indicated their adulteration with invert sugar. None of the samples showed signs of water addition.
Water activity is a predictive indicator for control of microbial growth in honey. As the honey antioxidants molecules contain various free –OH groups, by forming hydrogen bonds, the amount of water available to micro-organisms is reduced, thus the water activity also decreases.

5. REFERENCES

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