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THE QUALITATIVE CHARACTERIZATION OF POLYFLORAL HONEY AND THE INFLUENCE OF THERMAL PROCESSING ON HYDROXYMETHYLFURFURAL CONTENT

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Abstract

Due to the low pH, water activity and high sugar content, honey is one of the healthiest food products. Few biological hazards are associated with the product, due to its composition. The hydroxymethylfurfural (HMF) content is recognized as a parameter that affects the freshness of honey. The content tends to increase during processing or due to long storage. The aim of this paper is to assess the physico-chemical quality of polyfloral honey from Alba County, Romania. The research was carried out on a sample of honey whose initial HMF values was 2.1 mg HMF/kg honey. The simulation of honey processing was carried out at temperatures between 30 and 100°C, and the treatment time varied between 30 and 300 min. The statistical correlation between the thermal treatment applied to the honey and time, calculated from the results obtained, represents an argument for a unique direction of hydroxymethylfurfural content.

Key words: *honey bee, HMF, processing, HACCP, statistics.*

INTRODUCTION

Honey, a natural traditional sweetener, is considered a functional food, and many studies have demonstrated its beneficial health properties (Rasad et al., 2018). Honey is used in the food industry, cosmetics, medicine due to its nutritional, therapeutic and dietary quality. Honey is obtained by processing flower nectar or plant manna (Mărghitaş, 2008).

Honey contains water, glucose, fructose, sucrose, dextrin, vitamins, minerals and small amounts of trace elements and proteins (Eteraf-Oskouei & Najafi, 2013). Honey is a unique natural product that can be consumed as such or added to products. It does not require major processing or preservation (Bratu, 1998). Quality control is considered as a mark of the food industry. This is a prerequisite for the product competitiveness. Regarding the taste, colour, flavour, or consistency, the smallest exception will lower the standard (Trienekens & Zuurbier, 2008).

The content of hydroxymethylfurfural (HMF) represents its ability to indicate the lack of freshness of the honey or its adulteration (Shapla et al., 2018). Legislative standards state that commercial honey intended for human consumption must not have more than 40 mg of HMF per kilogram of honey (CAC, 2001; CD, 2001).

In managing the risks associated with the consumption of honey, all stages of production must be taken into account. The HACCP (Hazard Analysis Critical Control Point) system, although not mandatory, comes for the purpose of quality and safety control in the honey industry as well, being a preferred tool to ensure that consumers are offered safe food (Formato et al., 2011).

The HACCP methodology outlines seven principles, which include conducting hazard analysis, evaluating Critical Control Points (CCPs), establishing critical limits, implementting a monitoring system, defining corrective actions, creating verification procedures, and maintaining comprehensive documentation

(CAC, 1999). In HACCP programs, "control points" (CP's) will also be identified. Control points play a vital role in a food processor's extensive product control system and can complement HACCP by helping in the delivery of safe food products with consistently high quality to consumers (Humber, 1992).

The article describes a systematic approach based on qualitative characterization of polyfloral honey and the influence of thermal processing on hydroxymethylfurfural content, including through risk assessment within the HACCP system. Analysis of 11 samples of honey from the main areas of Alba County, Romania, provided basic data for HMF content of honey. A sample from Alba Iulia area containing 2.1 mg/kg HMF was the subject of a thermal treatment simulation at different temperatures, in time.

MATERIALS AND METHODS

Polyflower honey samples from different areas of Alba County, Romania (Table 1), have been tested to study their qualitative properties.

Table 1. The origin and geographical area of the polyflower honey samples

Sample	The origin area of honey	Areal
S1	Alba Iulia	Plain
S2	Abrud	Mountainous
S3	Aiud	Plain
S4	Baia de Arieș	Mountainous
S5	Blaj	Hilly
S6	Câmpeni	Mountainous
S7	Cugir	Hilly
S8	Ocna Mureș	Hilly
S9	Sebeș	Plain
S10	Teiuș	Hilly
S11	Zlatna	Sub-mountainous

The samples were obtained from different beekeepers. Each sample was collected in a sterile container and kept in a dark place at 2-8°C until it was analysed.

The following physico-chemical parameters were analyzed: pH water activity, moisture (water content), HMF, glucose and fructose content (SR 784-3, 2009; AOAC, 1995).

pH value: an aqueous solution of 10% honey was prepared. pH was measured with a PH-meter PHT 810 (1340-5810) (EBRO, Germany) (SR 784-3, 2009; AOAC, 1995).

The *water activity (a_w)* was determined with the Aquaspector apparatus AQS-2-TC (Nagy Messsysteme GmbH, Gäufelden, Germany) (SR 784-3, 2009; AOAC, 1995).

The *water content (moisture)* of honey was determined by refractive index (RI) at 20°C using an ABBE AR 2008 refractometer (Kruss Scientific GMBH, Hamburg, Germany). The water content (%) was set with the help of standard tables according to the refractive indices.

For the determination of *HMF in honey*, 10 g of honey was dissolved in approximately 25 mL of distillate water and transferred to a 50 mL volumetric flask; 2 mL of honey solution and 5 mL of p-toluidine (Sigma-Aldrich Production GmbH, Switzerland) were placed in two test tubes. In one tube 1 mL of distilled water was added (reference solution) and in the other 1 mL of barbituric acid (Kanto Chemical Co. Inc.) solution 0.5% (sample solution). The absorbance was read in 1 cm cuvettes at 550 nm with a Lambda 20 UV VIS Spectrophotometer (Perkin Elmer, Waltham, MA, USA). The HMF content was determined by the external standard method (p 99%, Sigma-Aldrich, Milan, Italy) and by using the proposed formula for the method.

Glucose: for a quantity of 20 ml of solution (obtained from 1 g of honey in 100 ml of distilled water), 50 ml of sodium bicarbonate solution (Sigma-Aldrich Production GmbH), 50 ml of sodium carbonate solution (Sigma-Aldrich Production GmbH) and 20 ml of iodine solution (Sigma-Aldrich Production GmbH) were added. The mixture was kept closed in the dark for 2 hours, after which 12 ml of sulfuric acid solution (Sigma-Aldrich Production GmbH) was added and titrated with thiosulfate solution (Sigma-Aldrich Production GmbH). In parallel, a sample was prepared using the same volume of water instead of the 20 mL honey solution.

$$\text{Glucose } (\%) = [9.005 \cdot (V-V1) \cdot 5]/10$$

where: V - the volume of the iodine solution that was consumed in the honey sample, V1 - the volume of the iodine solution that was consumed in the control sample (Marghitaș, 2008; Popescu & Meica, 1997).

Fructose: in 20 ml of solution (obtained from 3 g of honey in 200 ml of distilled water) were added 2.5 ml of NaOH 4N and 8 ml of iodine solution (Sigma-Aldrich Production GmbH). Then 6 ml of sulfuric acid (Sigma-Aldrich

Production GmbH) and sodium sulfite (Sigma-Aldrich Production GmbH) were added until a straw-yellow colour was obtained. 0.5 ml of starch solution (Sigma-Aldrich Production GmbH) was added and continued with sodium sulfite 2% (Sigma-Aldrich Production GmbH) until the solution became colourless. The solution was neutralized with 0.1N NaOH in the presence of phenolphthalein (Sigma-Aldrich Production GmbH) (Marghitaș, 2008; Popescu & Meica, 1997).

RESULTS AND DISCUSSIONS

The quality of a bee's honey can also be identified by the association of various physico-chemical properties. From this point of view, parameters such as: pH, water activity, moisture, fructose, glucose and HMF content and others are evaluated and monitored to appreciate the quality of honey. Table 2 shows the physico-chemical results of the honey samples collected from the towns of Alba County.

Table 2. The values of the physico-chemical parameters for the polyfloral bee honey samples from Alba County

Parameter / Source/Area	pH	a_w	Fructose, %	Glucoze, %	Moisture, %	HMF, mg/kg
Alba Iulia	3.70±01	0.541±0.018	38.4±1.0	28.7±0.6	16.7±0.7	2.1±0.3
Abrud	3.66±02	0.602±0.033	40.8±1.2	29.2±0.8	15.9±0.9	7.2±1.0
Aiud	3.92±04	0.574±0.012	41.2±0.9	27.0±1.2	16.4±0.7	11.0±1.1
Baia de Arieș	3.68±03	0.559±0.027	39.9±1.5	29.8±1.6	16.5±1.1	4.7±0.4
Blaj	3.74±01	0.580±0.039	38.3±1.8	30.4±1.3	16.2±1.2	6.5±0.9
Câmpeni	3.76±02	0.567±0.034	40.5±1.3	27.9±1.1	15.3±0.7	5.2±0.6
Cugir	3.63±03	0.522±0.046	44.4±1.7	28.6±0.9	15.2±0.6	8.0±0.5
Ocna Mureș	4.08±04	0.503±0.025	41.3±2.1	29.9±2.0	17.0±1.5	12.1±1.3
Sebeș	3.81±01	0.567±0.029	38.2±0.4	27.8±1.4	15.8±0.6	7.4±0.17
Teiuș	3.79±02	0.548±0.010	39.5±0.9	27.6±1.0	15.7±0.8	4.9±0.4
Zlatna	3.95±05	0.545±0.017	39.0±0.6	29.1±0.7	16.1±1.2	6.6±0.8

The physico-chemical parameters meet the standards of the current regulations - EU Honey Directive (CD, 2001). The pH values align with the well-established characteristic of honey was naturally acidic. The typical pH range of honey is 3.2-4.5 (Dilnawaz et al., 1995; Hamid & Saeed, 1991; Stenson et al., 1960; White et al., 1963). In the samples collected from Alba County, the lowest value recorded was from the Cugir area with a value of 3.63, and the highest, of 4.08, in the Ocna Mures area.

Typically, raw honey contains around 16-18% moisture. For Alba County, the range obtained for the water content of honey was 15.2-17%. However, it's internationally recognized that high-quality honey should ideally have a water content of less than 20% (Singh & Singh, 2018). The water activity (a_w) of honey typically falls within the range of 0.5 to 0.65 (Chen, 2019; Abramovic et al., 2008; Cavia et al., 2004; Chirife et al., 2006).

Honey is predominantly composed of carbohydrates, making up approximately 80% of its content, with the majority being glucose and fructose (Pauliuc et al., 2022). The fructose content in honey typically ranges from 21% to 43%, and the fructose-to-glucose ratio varies from 0.4 to 1.6 or even higher (Bobiș et al., 2018). The values recorded for the glucose and fructose content for Alba County, Romania fall within those stipulated in specialized literature.

Typically, freshly extracted honey maintains hydroxymethylfurfural (HMF) levels below 10 mg/kg. Elevated levels beyond this threshold may suggest overheating during the extraction process. Additionally, the HMF content in honey following processing and/or blending should not exceed 40 mg/kg (CAC, 2001).

HACCP serves as a method for identifying hazards and implementing control measures that prioritize prevention over primarily relying on testing end-products (Dahiya et al., 2009). In order to achieve the HACCP plan related to the bee honey production process, it is absolutely necessary to approach the detailed technological flow diagram first, for the honey under study and which will have a specific character, customized for each company. The flow diagram allows better identification of contamination points or paths throughout the production chain, which helps to more easily establish measures to

prevent physical, chemical and microbiological contamination (Bevilacqua et al., 2023).
Figure 1 shows the technological stages of bee honey conditioning, received from beekeepers in collection-packaging centers.

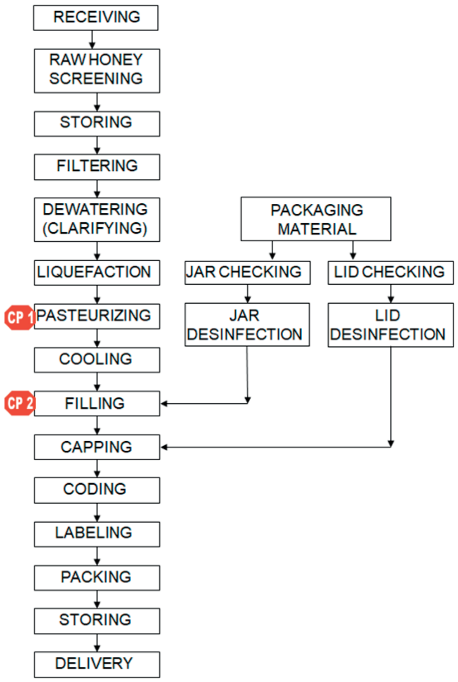

Figure 1. Control points that can lead to the increase of HMF content in process flow diagram of honey processing

HACCP is a management system where food safety is addressed throughout the technological flow through the analysis and control of biological, chemical and physical hazards. The HACCP plan is implemented to identify critical control points and control points (Formato et al., 2011).
The HACCP plan is implemented to identify critical control points (CCP's) and control points (CP's), the last one being used for quality specifications (CQP). A CCP indicates a high food safety risk (likely to occur) and a CP indicates a low food safety risk (not likely to occur). CQP implies identifying the quality of products, tools, and other aspects used in food processing. In the case of bee honey processing, the increase in the HMF value is considered a quality problem (Vica et al., 2009).
The problem, with the exception of the identification of potential fraudulent tendencies of falsification of honey, can be identified by analyzing certain degradation products (metabolites), such as hydroxymethylfurfuralor 5-hydroxymethyl-2-furaldehyde ($C_6H_6O_3$) (HMF) (Gregorc et al., 2020). The stages identified as points of attention (control) from the point of view of HMF content are presented in Table 3.
Following the analysis and evaluation of the dangers that may appear in all the technological stages of bee honey conditioning, received from the beekeepers, it was identified the need to include in the HACCP plan pasteurization and filling of jars, as points of attention (control) from the point of view of growth of the HMF content of honey.
In order to determine the variation of the HMF content of polyflora bee honey for 300 min, the evolution of the parameter was followed at 5 different temperatures between 30°C and 100°C.

Table 3. Analysis and assessment of the dangers related to the increase of HMF corresponding to the identified CP

Stage	Hazard type	Hazard description	Stage role	Control	Limit	CP
Pasteurization	Chemical	Excessive heating may cause increased HMF content and reduced enzyme (diastase) activity. It negatively influences the taste, aroma and colour of honey (becoming more "browning").	The heat treatment reduces the moisture content of the honey, destroying the yeast cells and liquefying the crystals, also increasing its shelf life.	Monitoring the time and duration of honey pasteurization. Ensuring that the working process and storage environment do not exceed 60°C.	HMF <40 mg/kg	CP1
Jar filling	Chemical	The use of hot sterilized jars increases the temperature of the honey and the risk of increasing the HMF content and reduced enzyme (diastase) activity.	Keeping the product in sterile (risk-free) packaging to ensure its preservation and to be easy to transport.	Cool the jars to room temperature (about 15 min) before adding the honey.	HMF <40 mg/kg	CP2

In Table 4, the HMF values resulting from the treatment of polyfloral bee honey at different temperatures over time are presented comparatively.

Table 4. The values of HMF content obtained at different temperatures over time

Time [min] \ Temp. [°C]	30	50	70	80	100
0	2.1±0.7				
30	5.3±0.8	10.2±0.3	11.5±0.2	11.7±0.5	15.8±0.7
60	5.4±0.5	10.8±0.8	12.2±0.7	19.6±0.4	46.3±2.8
90	5.7±0.3	11.7±0.5	14.5±0.3	20.4±0.2	50.7±4.1
120	6.6±1.1	12.3±0.9	17.8±0.15	27.3±0.9	60.2±3.7
150	9.0±1.4	15.5±1.6	19.4±2.2	36.1±1.4	74.7±2.2
180	11.4±2.3	17.2±1.0	20.3±1.8	42.9±3.1	82.3±3.8
210	15.2±4.0	22.0±3.3	44.2±4.9	50.7±3.8	84.1±4.5
240	17.1±2.8	29.6±2.4	52.1±6.7	63.9±2.5	86.5±3.9
270	19.5±3.9	32.8±1.8	60.6±2.0	74.8±4.7	88.0±2.3
300	20.1±2.7	37.5±4.1	65.0±3.3	86.6±2.9	90.4±3.7

Table 4 presents the variation of HMF with the temperature at which the polyfloral honey was treated. The HMF value shows an increasing trend over time for all the temperatures to which the honey was subjected, starting from 2.1 mg/kg, respectively registering a value of 90.4 mg/kg for the honey subjected to 100°C for 300 min.

In chemistry, it's uncommon for a process to be solely affected by a single variable parameter. Instead, it's intriguing to observe how other parameters, through their fluctuations, notably impact the variations observed in the outcome (Gluck, 1971). Using MATLAB, experimental data regarding the time variation of the HMF content for poly-floral honey samples at different temperatures were processed and analyzed. Figure 2 presents both the experimental data and the surfaces generated by statistical Mathematical model. A correlation was established between the HMF content of honey in time (t) and the applied temperature (T) using a two-degree polynomial relation with two independent variables.

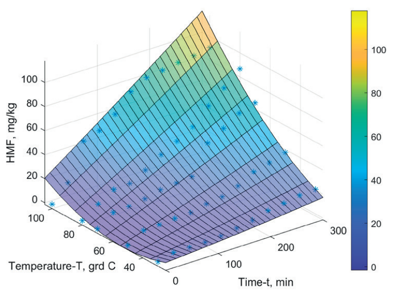

Figure 2. The variation of HMF content of polyfloral bee honey depending on the applied temperature and time

The second order regression equation for honey samples that correlates the variation of the HMF content with the time and the temperature has the following form:

$$HMF\ (mg/kg) = 21.2496 - 0.0585 \cdot t - 0.8637 \cdot T + 0.0035 \cdot t \cdot T + 3.6856 \cdot 10^{-5} \cdot t^2 + 0.0081 \cdot T^2$$

where: t - time, min; T - temperature, °C.

The accuracy of the correlation is confirmed by the adequacy indicators: the model accuracy indicator ($r^2 = 0.944$), the correlation coefficient ($r = 0.972$) and the standard deviation ($\sigma = 6.467$). The calculated correlation parameters validate a very good prediction capacity of the statistical mathematical model.

CONCLUSIONS

The physical properties and nutritional chemical composition of honey from Alba County, Romania were investigated to highlight the quality of the polyfloral honey from this region. The equation of the statistic model can approximate the variation in time of the HMF content at different temperatures. These represent a control and prediction tool for the appreciation of the product quality in time.

The system for food safety - HACCP acts through the forms of control, monitoring and verification, documents and pays more attention to prevention. In addition to the recording and monitoring systems of the critical control points, the attention points indicate a low food safety risk, but which will still generate honey quality problems. Thus, 2 stages were identified, the pasteurization and filling of the jars, which can lead to an increase in the HMF content, the control and the established limits.

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