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EFFECT OF HEAT TREATMENT ON CHEMICAL AND SENSORY PROPERTIES OF HONEYS

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Abstract

Honey has been widely used since the ancient times. Due to its high nutritional value and its high price honey has become a target of adulteration. Several methods are applied for committing frauds. For some beekeepers and distributors it is a common practice to heat honey for elimination of crystallization, which is generally not desired by consumers. Nevertheless, during heat treatment the chemical composition changes, leading to a decrease in the amount of some components like vitamins, antioxidant components and enzymes, while some unwanted compounds like hydroxymethyl-furfural (HMF) are formed. HMF is also produced during long time storage at room temperature, but heating promotes this process. In our research the effect of heat treatment was studied on different chemical, physical and sensory properties of honeys. Linden, acacia, multiflora and sunflower honeys were examined. Three temperature levels - 40 °C, 50 °C, 60 °C- and three time periods – 30, 60, 120 minutes - were applied for the heat treatment. For tracking of the changes colour measurement, near infrared spectroscopy and electronic tongue methods were used. Evaluation of data was performed by uni- and multivariate statistical methods. Samples treated even at 40 °C showed significant differences compared to the control, mainly in ET and NIR results. Furthermore, the effect of the different time periods was also found significant. Results confirmed even heat treatment at 40 °C is detectable with the acquired rapid methods. higher heat treatment cause obvious sensory changes but the applied quick methods are able to trace the effect of minimal heat treatment on the quality of honey .

Keywords: honey, heat treatment, physicochemical properties

Összefoglalás

A méz ősidők óta használt szer, fontos piaci termék. A benne lévő értékes összetevők és magas ára miatt hamisítások egyre gyakoribb célpontja. Előfordul, hogy egyes termelők és forgalmazók felmelegítik a mézet, kiküszöbölve ezzel a kristályosodási folyamatot, amely a fogyasztók szemében általában nem kívánatos. A melegítés során azonban a beltartalmi paraméterek megváltoznak, egyes anyagok mennyisége csökkenni kezd (vitaminok, antioxidáns vegyületek, enzimek), míg egyes nem kívánatos vegyületek mennyisége megnőhet. Ilyen vegyület a hidroximetil-furfurol (HMF), amely hosszabb tárolás során szobahőmérsékleten is képződik, ám a

melegítés hatására keletkezése felgyorsul. Kutatásunkban a melegítés hatását vizsgáltuk a méz különböző kémiai, fizikai és érzékszervi tulajdonságaira hárs -, akác-, vegyes és napraforgómézek esetén. A hőkezelés tekintetében három hőmérsékleti szintet - 40 °C, 50 °C és 60 °C-, valamint három időtartamot – 30, 60 és 120 perc- határoztunk meg. A hőközlés alatt bekövetkező változások nyomon követésére szín, közeli infravörös spekroszkópia és elektronikus nyelv módszereket alkalmaztunk. Az adatelemzést különböző egy- és több változós statisztikai módszerekkel végeztük. Az eredmények értékelése során szignifikáns különbségeket kaptunk a kontrollhoz képest már a 40 °C-os hőkezelés során is, valamint az időtartam is befolyásoló szereppel bírt. Következtetésképpen elmondható, hogy már viszonylag kis hőközlés is kimutatható az alkalmazott gyorsmódszerekkel. Nagyobb hőkezelés esetén egyértelmű érzékszervi változás történt, mely azt mutatja, hogy a hőkezelés már az általunk alkalmazott mértékben is kimutathatóan megváltoztatja a méz minőségét.
Kulcsszavak: méz, hőkezelés, fiziko-kémiai tulajdonságok

Introduction

Honey has been widely used as a sweetener and a medical product. It is made by honeybees (*Apis mellifera*) from the nectar of flowers, sap of plant parts and also from the juicy material secreted by sucking insects. Honey is rich in nutritive materials, such as vitamins, minerals and antioxidants. Another important feature of honeys is the colour and they also have specific aromas and taste. These attributes depend on the origin of honey. Due to its high quality and valuable properties, honey has an increasing popularity among consumers. On the other hand, honey production gets more and more difficult due to the decrease in honeybee population caused by different diseases, environmental pollution and changes in climatic conditions. These factors determine an acceleration of the trends leading to honey adulteration. There are several methods used during the fraud of honeys. The two main types are the direct and indirect adulteration, but fermentation of honeys and colouring with ammonium-sulfite caramel are also possible methods *(Zábrodská and Vorlová*, 2014). Heating of honeys is also an adulteration of the original quality which remains unrevealed in many cases.It is known that producers and beekeepers heat honeys during processing, applying mainly two methods: heating in air-ventilated drying chambers and in hot water bath (*Turkmen et al.*, 2006). Heating is able to eliminate the often unwanted crystals, decrease viscosity and make honey handling easier for consumers and producers (*Turkmen et al*., 2006).On the other hand, according to *Zábrodská and Vorlová* (2014), long term heating above 50 °C cause changes in honey composition and it has an undesired effect on honey quality. In Hungary, honey heating is regulated by the legislation, as given in Codex Alimentarius Hungarius: heating and storage of special honeys must be between 5 – 40 °C and the core temperature during honey processing must not exceed 40 °C (*Magyar Élelmiszerkönyv*, 2009). Quality of honeys is also regulated in the Codex according to the relevant EU directive *(Czipa*, 2010; *Magyar Élelmiszerkönyv*, 2002).

During heating of honeys at different temperatures and periods several changes can be detected in colour and chemical composition. Amount of natural antioxidants, vitamins and enzymes could decrease, while some unwanted compounds such as hydroxymethyl-furfural (HMF) are formed. HMF is also produced during long time storage at room temperature, but heating promotes this process. In Hungary and in the European Union, the HMF content of honeys must not be higher than 40 mg/kg (*Magyar Élelmiszerkönyv,* 2002). According to the literature, detection

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of heat treatment in honey can be measured by HMF content and diastase activity changes (*Zábrodská and Vorlová*, 2014).

An Argentinian research group tested the diastase activity and HMF content changes in heated honeys. They found that diastase activity already decreased at the lowest heating level applied (60 °C-120 s) (*Tosi et al.*, 2004). In their other study they observed that HMF content didn't exceed the 40 mg/kg acceptable limit even when heating at 140 °C for 60 minutes (*Tosi et al.*, 2008). In another study, researchers found that HMF content was significantly higher at 60 °C or above this (*Kesić et al.*, 2017).

Hungarian researchers studied colour change during storing and heating process of honeys. They found that during 75°C and 90 °C heating for 5 five hours the L*a*b* parameters of honey changed significantly (*Csóka et al.*, 2014).

Our aim was therefore, to study the applicability of rapid methods in detection of short-time, low temperature heating processes.

Materials and methods

Sampling

Samples of linden, multiflora, sunflower and acacia honeys were analysed. The samples were acquired from beekeepers. The producers did not heated honeys previously.

Three bottles of each of the four honey types were used for the experiments. Samples were heated at 40°C, 50°C, and 60°C for 30, 60 and 120 minutes respectively in a hot water bath and then cooled down to room temperature (25 °C). The heat-treated honey samples were tested against the untreated (control) samples (three replicate per each honey type). Experiments were performed using three replicate samples from the three independent bottles, this resulted in 30 bottles in total, each filled with 40 g of honey per each honey type. Samples are summarized in

Table *1*.

Table 1: **Numbers of sample sizes per heat treatment levels for botanical groups**

Heat treatment levels		**Acacia**	**Linden**	**Multiflora**	**Sunflower**	**Total**
40 °C	30 min	3	3	3	3	12
	60 min	3	3	3	3	12
	120 min	3	3	3	3	12
50 °C	30 min	3	3	3	3	12
	60 min	3	3	3	3	12
	120 min	3	3	3	3	12
60 °C	30 min	3	3	3	3	12
	60 min	3	3	3	3	12
	120 min	3	3	3	3	12
Control	Control	3	3	3	3	12
Total		30	30	30	30	**120**

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Methods

Physicochemical parameters

The most commonly used physicochemical quality indicators of honeys like total soluble dry matter content, pH and electrical conductivity are rapid and were applied in several studies and honey monitoring programs *(Al-Ghamdi et al.*, 2017; *El Sohaimy et al.*, 2015; *Kesić et al.*, 2017). These parameters were determined according to the International Honey Commission methods (*Bogdanov*, 2002), testing the three replicate honey samples (three data points per treatment level).

HMF content and antioxidant capacity

HMF content and antioxidant capacity of the linden honey samples were determined using the method after Winkler (*Bogdanov*, 2002). HMF measurement and ABTS assay (*Re et al.*, 1999) were performed using three replicate measurements for each parallel sample (nine data points for each treatment level). Results of the ABTS assay are reported as µmol ascorbic acid equivalents (ASE)/100 g honey.

Colour measurement

Colorimetric measurements were done in CIE L*a*b* tristimuli coordinate system by ColorLite sph850 spectrophotometer (ColorLite GmbH, Germany). In case of acacia samples all the heat treatment levels were analysed, while in case of the other three honey types only samples treated at 50°C or 60 °C were used for analysis to ensure the absence of crystals.

Electronic tongue

Electronic tongue (ET) measurements were done by an αAstree electronic tongue (*AlphaM.O.S.*, 2003) which was designed to recognize and analyse the dissolved compounds in liquid samples. The ET consists of a sensor array with seven potentiometric CHEMFET (chemically modified field effect transistor) sensors developed for food applications and an Ag/AgCl reference electrode. During the measurement, the potential difference is recorded between the reference electrode and the individual working electrodes, which depends on the chemical composition of the sample providing a unique fingerprint of the tested liquid. Honey samples were diluted using 10.0 g of honey dissolved in 100 ml distilled water. The three replicate honey samples were tested in repeated measurements resulting in 12 repetitions for all the treatment levels in total.

Near infrared spectroscopy

Near infrared (NIR) spectra of the honey samples were collected by a MetriNIR spectrometer in three consecutive measurements per each replicate sample, resulting thus nine scans for each heat treatment level (2nm spectral step in the 780-1700 nm spectral interval). Reflectance cells with 0.4 mm layer thickness were used during the data acquisition.

Statistical evaluation of data

Statistical evaluation of the univariate parameters was performed with two-way ANOVA, then Tukey test was applied for pair-wise comparison. NIR results were analyzed with principal component analysis (PCA), while ET results with PCA and linear discriminant analysis (LDA). R-project and Microsoft Excel 2013 were applied for data evaluation.

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Results

Results of physicochemical parameters

Results of physicochemical parameters measured on homogenized crystal-free honey samples can be seen in *Table 2*. Highest electrical conductivity and pH was measured in linden honeys. Acacia honey had the lowest electrical conductivity and the highest total soluble dry matter content.

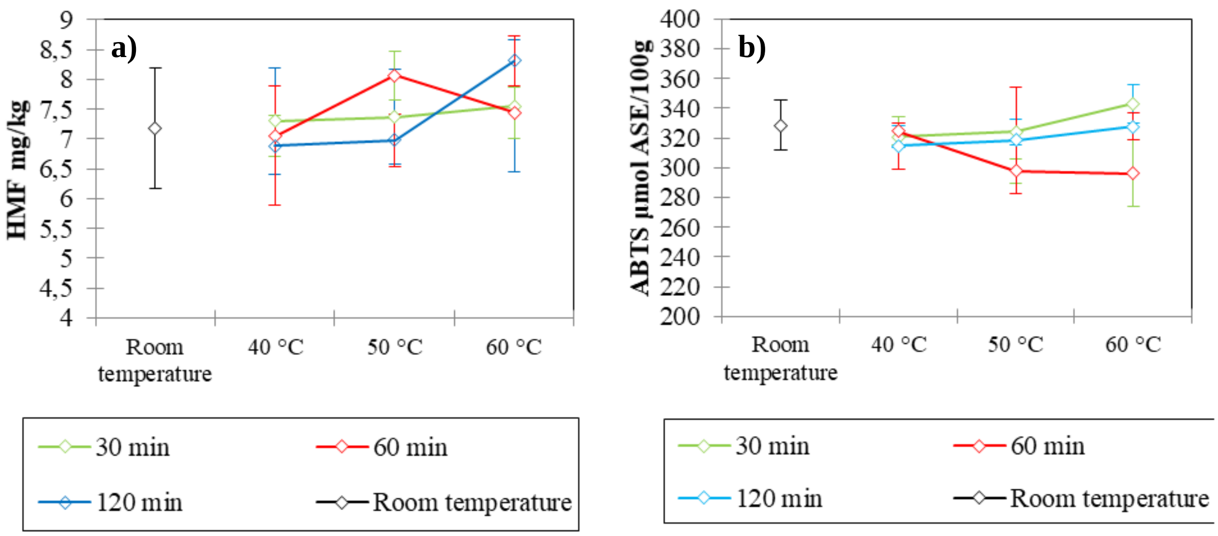
Table 2: **Results of honey physicochemical parameters by botanical groups for homogenized honeys**

	Total soluble dry matter%	pH	Electrical conductivity µS/cm
Acacia (n=3)	82.83 (±0.06)	3.52 (±0.27)	127.00 (±1.73)
Sunflower (n=3)	79.20 (±0.12)	3.45 (±0.03)	362.67 (±1.00)
Multiflora (n=3)	78.43 (±0.06)	3.48 (±0.03)	433.33 (±4.16)
Linden (n=3)	80.30 (±0.10)	3.84 (±0.01)	552.67 (±2.31)

HMF content and antioxidant capacity results

Results of the change in HMF content and ABTS antioxidant capacity of linden honey after the heat treatment are shown in **Hiba! A hivatkozási forrás nem található.** a) and b), respectively. In case of HMF content temperature ($p<0.01$), in case of ABTS treatment time ($p<0.001$) and for both parameters interaction of treatment time and temperature ($p<0.01$) had significant effect on the results. According to the Tukey test, significant differences were found in the HMF content of honey samples treated at 40°C and 60 °C, but not at 50 °C. Only the 60-minutes treatment caused significantly higher ABTS compared to the other time intervals. Monotonic decrease of antioxidant capacity was detected only in case of 120 minutes treatment time. The lack of a progressive trend can be the consequence of the formation of new antioxidants during Maillard reaction (*Nicoli et al.*, 1999).

***Figure 1*: Means and standard deviations of a) HMF (n=90) and b) ABTS (n=90) of linden honey samples at different heat treatment levels**



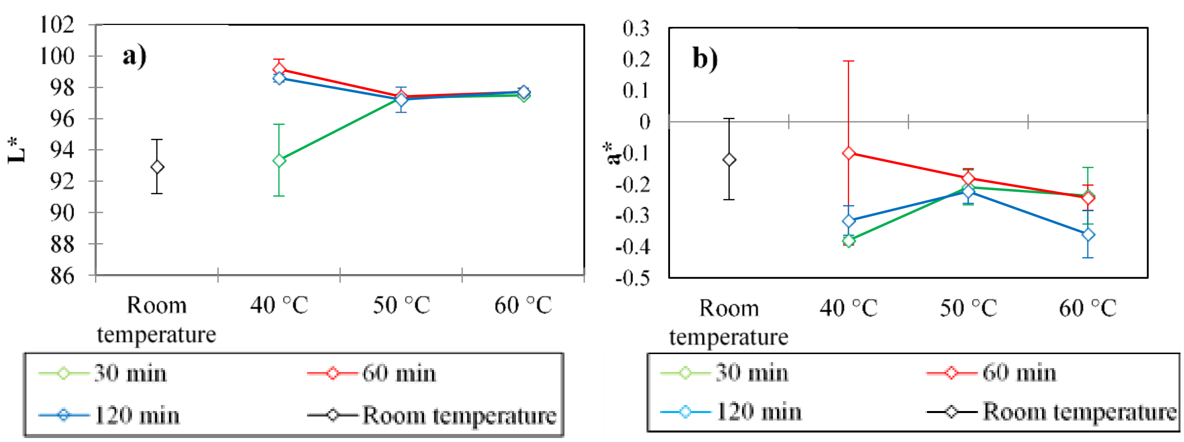
Results of colour measurement

Treatment time (p<0.001) and interaction of temperature and treatment time (p<0.001) had significant effect on L* parameter of acacia honey (

Figure **2** *a*). In case of parameter a* (red-green colour scale) (

Figure **2** *b*) only treatment time was found significant (p=0.042).

***Figure 2:* Means and standard deviations of colour properties of acacia honey a) L* (n=30) and b) a* (n=30) at different heat treatment levels**



There was no significant change observed in parameter b* (yellow-blue colour scale).In case of sunflower honey samples treatment time, temperature and their interaction had significant effect on L* (lightness scale) and b* parameters (p<0.01). For a* only temperature was significant (p<0.05). Results of linden and multiflora honey types have been found similar, i.e. L* and b* parameters were significantly influenced by both temperature and treatment time (p<0.05), while parameter a* changed only with temperature (p<0.01). Results can be seen in *Table 3.*

In some cases an uncommon increase of L* parameter can be seen with higher heat treatment level (time and °C). It can be related to the morphological and micro-crystallization changes in honey composition *(Kędzierska-Matysek et al.*, 2016).

Table 3: **Colorimetric properties of honeys by heat treatment levels and botanical group**

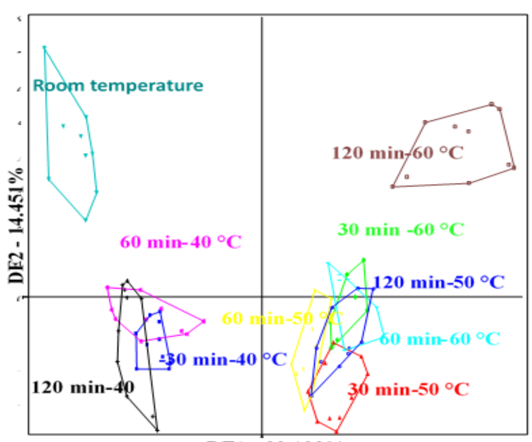
Botanical Group	**Colour**	**50°C**		**60°C**		
		60 min	**120 min**	**30 min**	**60 min**	**120 min**
	L*	54.9±3.2	56.7±12.2	83.6±1.3	87.1±0.2	84.2±3.2
	a*	-1.0±0.1	-1.1±0.1	-1.2±0.2	-1.5±0.1	-1.6±0.1
Linden	b*	26.0±1.0	26.7±4.0	35.8±0.6	37±0.1	36.4±0.8
	L*	73.8±6.1	73.9±1.9	85.5±0.6	85.6±0.3	84.2±0.8
	a*	1.9±0.2	1.8±0.1	2.3±0.1	2.3±0.0	2.4±0.4
Multiflora	b*	61.5±4.2	61.2±1.1	69.7±0.7	69.9±0.4	69.4±0.8
	L*	29.8±2.5	61.4±11.2	75.8±1.9	84±2.0	87.4±2.1
	a*	-0.2±0.1	0.0±0.1	-0.4±0.2	-0.2±0.2	-0.2±0.0
Sunflower	b*	30.3±1.6	48.2±6.6	56.1±1.1	61.5±0.7	63.9±1.7

Electronic tongue measurement results

Results of electronic tongue measurements showed good differentiation of the various temperature levels for each floral type, except acacia honey. In **Hiba! A hivatkozási forrás nem található.**. the differentiation of multiflora honeys is shown. The figure shows that treated honeys are in separate groups according to the temperature. DF1 presents 83.14% of the variance between groups while DF2 the 14.45% (summarized 97.59 %).

In case of linden and sunflower samples only separation of treated samples from untreated ones was observed.

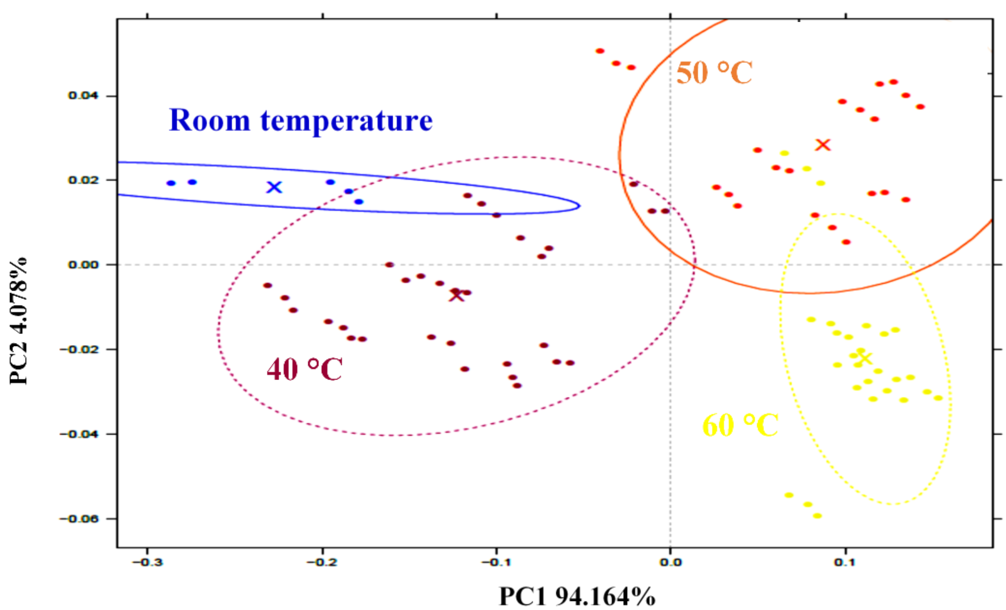
Figure 3: **Electronic tongue measurement results of multiflora honeys by heat treatment levels (n=95)**



Near infrared spectroscopy results

Results of NIR spectroscopy validated the electronic tongue results by also showing a good separation according to the temperature level in case of linden, multiflora and sunflower honey types (*Figure 4*). This figure shows that PC1 presenting 94.16% of the spectral variation shows the biggest difference among the non-treated sample (room temperature) and samples treated at 40, 50 or 60 °C. PC2 (4,078%) shows separation between samples treated at 50 and 60 °C and between room temperature and 40 °C treated honeys.

Figure 4. **PCA score plot of NIR results of sunflower honey samples calculated using the smoothed and MSC corrected spectra between 950-1630 nm (n=87)**



Result of linden honey also showed good separation between room temperature and other heat-treated samples based on PC1 (95.90%). In case of multiflora also a clear separation of room temperature honey was observed. However, there was no clear separation observed for acacia samples.

Discussion

In our study physicochemical parameters like pH, electrical conductivity and total soluble dry matter content were used for the characterization of honey types. Results showed that the applied minimal heat treatment had a significant effect on honey in every botanical group.

The lowest change levels were identified in acacia honeys, while in case of sunflower, linden and multiflora honeys similar results were obtained. The observed colour changes of honeys during heat treatment were similar with the results shown in the related literature: in this minimal heat treatment honeys got lighter and the yellowness also increased. It can be related to the crystallization and morphological changes of honeys during the process *(Kędzierska-Matysek et al*., 2016, *Kesić et al.*, 2017; *Tosi et al*., 2008).

The antioxidant capacity measurement and HMF content are not sensitive enough to recognize properly the minimal heat treatment. Electronic tongue and NIR measurements proved to be suitable to detect the changes occurring in heated honeys. In case of acacia honeys, we could only distinguish the 50 °C and 60 °C treatment levels, which can be explained by the lower aroma content of acacia honeys. However, in case of linden, multiflora and sunflower honeys already 40 °C heat treatment level could be detected, which means that such a low heating is capable of causing measurable changes in the quality of these honeys.

Conclusion

From the study, HMF and ABTS measurements were not sensitive enough to detect the applied minimal heat treatment. However, based on the results of colour, electronic tongue and NIR measurements, the differentiation was possible even between non-treated honey and honey treated at the lowest level (40 °C), especially in case of linden, multiflora and sunflower honeys. Thus the methods applied were effective in the detection of short-time, low temperature heating processes.

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