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DETECTION OF HEAT TREATMENT OF HONEY WITH NEAR INFRARED SPECTROSCOPY

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Abstract

Heat treatment of honey is usually applied due to crystallized form is not preferred by the consumers and makes handling harder to producers and beekeepers. Our aim is to find a method that can detect heat treatment of honey even at lower levels. In the study honeys were heated at 40°C, 60°C, 80°C, 100°C for one, two, three and four hours. Moisture, pH, electrical conductivity, HMF, color were determined, and spectra of honeys were recorded with NIRS. Results showed that NIRS could distinguish the honeys heated at 40°C while HMF was able to detect higher than 60°C treatment at significant level.

Keywords

honey, NIR, chemometrics, HMF, heat treatment

Introduction

Honey is produced by honeybees (Apis mellifera) from nectar, sap of plants and secretion of insects. Owing to the botanical origin, honeys are differing in their physico-chemical properties including sugar composition. Honeys mainly consist of moisture and sugar, but also contains minerals, vitamins, enzymes, aroma components, organic acids and antioxidants [1]. According to the legislations and standards moisture content of honey should be under 20%, with some exceptions (Calluna honeys should be under 23%) [2], [3]. In honey different monosaccharides and disaccharides are present, and the two main sugars in honey are glucose and fructose. The ratio of these two sugars is characteristic for unifloral honeys from different botanical origin and have an important role in the crystallization of honeys. This crystallization process also depends on the storage time and temperature, moisture content and presence of particles such as pollen and beeswax [4]. Honeys with higher relative glucose content crystallizing quicker, as glucose is less soluble in water than fructose [5]. Honeys can be divided into three categories based on the speed of crystallization: quick such as sunflower (Helianthus anuus) canola, (Brassica napus), moderate like linden (Tilia spp.) or bastard indigo (Amorpha fruticosa) and slow crystallization for instance acacia (Robinia presudoacacia) chestnut (Castanea sativa) or honeydew [6] Notwithstanding crystallization is a natural process, beekeepers, producers and consumers do not prefer crystallized honeys, owing to handling, processing and organoleptic properties are not beneficial. This lower acceptance of crystallized honeys led to application of heat treatment on honeys. According to the Hungarian legislations, the core temperature of the honeys must be between 5°C and 40°C during processing and storage, meaning that heating up to 40°C temperature is allowed [7] However, in practice heat treatment at higher temperature is sometimes applied, because liquefaction of honey at 40°C takes too long [5]. However, heat treatment at higher temperature and/or longer time influence negatively the quality of honey thus treatment above 50°C is considered as adulteration [8]. In practice detection of heat treatment and freshness of honey is examined by the determination of hydroxymethyl-furfural (HMF) content, invertase and diastase activity. The problem of using HMF content as indicator of heat treatment that it increases significantly only at higher temperature (above 60°C) or longer time of heat

treatment. Therefore, minimal heat treatment such as treatment at 40°C or 50°C for instance for two hours cannot be detected using the determination of HMF content only, in spite of changes in crystal composition, aromatic profile and antioxidant profile can be significant [9], and determination of HMF takes time and need chemicals. The determination of the aforementioned enzyme activities are more applicable to check the freshness of honey, due to at long time storage they also can decrease [10]. Therefore, there is a need to find quick, reliable and easy to use method more sensitive for the detection of heat treatment. Near infrared spectroscopy could be a good solution to fulfill these requirements, because its application does not need sample preparation and it is quick, furthermore, in previous studies NIRS was successfully used in determination of authenticity of honey [11]. Our objective was to check the applicability of NIRS for the detection of heat treatment of honey. Further aim was to develop NIRS based method for rapid determination of heat treatment of honey which could support authorities work in the long term.

Materials and methods

Honeys and sample preparation

Acacia (RP), sunflower (HA) and bastard indigo (AF) honeys were collected from a beekeeper in three bottles each that served as parallel samples in our experiments. Honeys were heated for one, two, three and four hours at 40°C, 60°C, 80°C, 100°C, resulting in 153 sample including control (unheated) honey for the three different types. In the code system the first two letters are for the type of honey, second numbers are the temperature levels, and third numbers for the time interval, for instance AF-040-120.

Quality indicators

Moisture, pH and electrical conductivity of the tested honey samples were determined according to the IHC (International Honey Commission) method book [12]. In the case of moisture two replicates, and in the case of pH and conductivity three replicates were obtained for each 153 samples.

Color

CIE L*a*b* tristimulus coordinate system was used for the determination of color of the honey samples in transmittance arrangement using ColorLite sph850 spectrophotometer (ColorLite GmbH, Germany). Each parameter was determined in tree consecutives per each sample, however results of crystallized honeys were not evaluated due to the crystals can affect the transmission of the light.

HMF content determination

HMF content of honey samples was determined according to Winkler-method, in three consecutives for each heat treatment level [12].

Near infrared spectroscopy

Transflectance spectra of the honey samples were recorded between 900 and 1700 nm with the NIRScanNano (Texas Instruments, Dallas, Texas, USA) handheld spectrometer. The layer thickness of the samples was 0.4 mm in the cuvette. Five consecutive spectra were recorded for each sample three times resulting in 765 spectra per honey type.

Statistical evaluation of the data

Results of quality indicators and color were analyzed with descriptive statistics, two-way ANOVA test was performed on the HMF results, followed by Tukey-pair wised comparison for the determination of significant changes between temperature and time levels. Results of NIR data between 950nm and 1630nm were pre-treated with Savitzky-Golay smoothing and multiplicative scatter correction. Principal component analysis was used to detect the patterns, while linear discriminant analysis models were built for the classification of temperature and time interval levels separately. LDA models were validated with three-fold cross validation. Data evaluation was performed in Microsoft Excel and R-project 3.5.2 using aquaP2 package.

Results and discussion

Results of the quality parameters and color measurement

Moisture content of the bastard indigo samples were between 16.33% and 17.27%, that suits to the EU legislation (<20%), such as the results of acacia and sunflower honeys. Moisture content - if honeys are heated in glass holders with a closed cup, - and electrical conductivity do not show clear trends. In the case of the results of pH an increasing tendency can be seen with the increase of the applied heat treatment for bastard indigo honeys, especially in the case of honeys that were treated at 100°C. Similar tendency was observed for the other two types of honey. Electrical conductivity of the AF honeys was between 537 µS/cm and 547 µS/cm, RP honeys between 108 µS/cm and 113 µS/cm and HA honeys between 378 µS/cm and 400 µS/cm. These results

also fit to the EU legislation for blossom honeys (<800 µS/cm) (Table 1.) [3].
Results of color showed similar tendency for all the three tested honey types. An increasing L* was followed by a decreasing lightness of the samples. The a* parameter increased with the elevation of the treatment temperature and applied time and similar tendency was obtained for b* resulting in more yellow intensity (Table 1.). The increasing of L* and b* in the beginning can be explained by the change of the structure of microcrystals, while the decreasing L*, (darkening of the honey) and more red intensity can be explained by the browning as a result of Maillard reaction [13]. Results of ANOVA test using color data showed that temperature, time and interaction of these two factors has a significant effect on the changes of L*, a*, and b* parameters ($p<0.001$) as a consequence of heat treatment in the case of AF honeys. For all of the parameters significant parameters were found between the three temperature and four time interval groups as a result of the pair-wised comparison ($p<0.05$).

Table 1. Results of the quality indicators and color for bastard indigo honeys

Treatment level	Moisture %	pH	Electrical conductivity µS/cm	L*	a*	b*
AF_cont_cont	16.4±0.17	4.35±0.02	536.56±2.3			
AF_040C_060M	16.33±0.26	4.36±0.01	541.22±3.42			
AF_040C_120M	16.51±0.36	4.36±0.01	542.22±5.72			
AF_040C_180M	16.98±0.04	4.39±0	546.56±4			
AF_040C_240M	16.8±0.21	4.38±0.01	540.67±1.5			
AF_060C_060M	17.04±0.1	4.34±0.02	541.67±6.24	71.26±0.77	5.89±0.12	57.92±1.08
AF_060C_120M	17.1±0.1	4.42±0.04	537.89±1.76	77.43±0.16	6.4±0.03	61.61±0.11
AF_060C_180M	17±0.27	4.44±0.01	539.44±5	77.5±0.26	6.32±0.03	62.35±0.38
AF_060C_240M	17.11±0.15	4.45±0.01	538±3.57	77±0.5	6.44±0.13	62.21±0.37
AF_080C_060M	16.93±0.19	4.43±0.01	545.22±2.17	76.02±0.6	6.52±0.1	61.57±0.79
AF_080C_120M	16.84±0.26	4.48±0.01	540.22±0.44	74.74±0.89	7.2±0.26	63.52±0.8
AF_080C_180M	17.04±0.24	4.5±0.01	538.11±1.76	75.61±0.57	8.35±0.15	66.56±0.16
AF_080C_240M	17.27±0.2	4.51±0	543.33±1.66	74.67±0.15	9.33±0.14	69.13±0.21
AF_100C_060M	17.02±0.12	4.51±0.01	542.89±2.09	73.59±0.57	8.26±0.16	65.02±0.36
AF_100C_120M	17.27±0.13	4.55±0.04	542.44±1.67	66.86±0.65	16.89±0.24	78.68±0.42
AF_100C_180M	16.93±0.22	4.56±0.01	541.89±2.03	58.83±0.21	24.85±0.27	81.45±0.23
AF_100C_240M	16.84±0.3	4.56±0.01	538.44±7.42	49.91±0.23	32.87±0.23	77.18±0.39

Average±Standard deviation

Results of HMF determination

Results of the HMF test for all of the honey types showed that extreme increase in HMF content can be reached by heating honey at 100°C. In the case of AF honeys the limit established by EU Commission and Codex Alimentarius (40 mg/kg) was reached only in honey samples heated at 100°C for at least 2 hours (Fig. 1.). Similar results were obtained for RP and HA honey types, with the exception that HA honeys also showed higher than 40 mg/kg HMF content in the case of honey heated at 80°C for 4 hours, which can be explained by the higher glucose content of this honey type. ANOVA test showed that time, temperature and their interaction have significant effect ($p<0.05$) on the formation of HMF during heat

treatment. There was no significant difference observed in HMF content between honey samples heated at 40°C and 60°C compared to control for all the honey types. In the case of AF, only honeys heated at 100°C showed significant difference comparing control honey. This results is in accordance with results of previously published works [9].

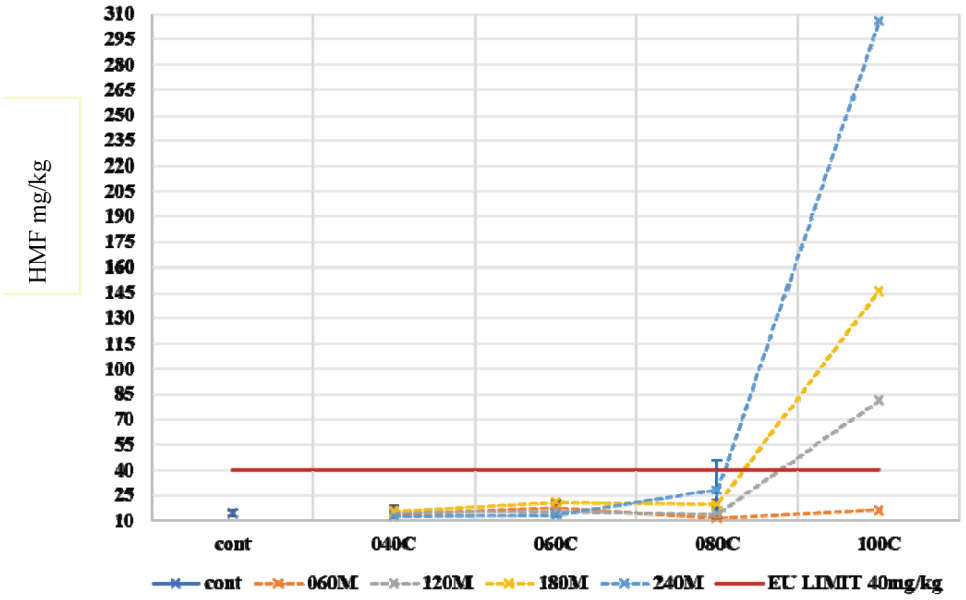

Figure 1. Results of HMF (Average and SD) content of bastard indigo honeys n= 51

Results of NIRS

PCA result of NIR spectra of AF honeys showed clear separation tendency of the tested samples according to the applied temperature through PC1 that describes the 95.52% of the total variance. The classification model built to discriminate based on the applied temperature showed the same phenomena (Fig.2).

LDA model provided average recognition and prediction abilities 77.93% and 77.26%, respectively. Control samples were classified correctly. This might be explained by the changes in phase and chemical structure as a consequence of heat treatment. Honeys heated at 40°C showed 94.45% correct classification during both training and cross-validation, misclassification was found belonging to the 60°C and 80°C. Honeys heated at higher levels showed lower than 70% correct classification for training and cross-validation, misclassifications were found belonging to other groups. Detailed results can be seen in Table 2.

Table 2 Classification table of the LDA model built for AF honeys to discriminate between temperature levels

%	Original→ Predicted↓	control	040C	060C	080C	100C
Training 77.93%	**control**	**100**	0	0	0	0
	040C	0	**94.45**	0	0	0
	060C	0	3.61	**65.64**	24.17	8.24
	080C	0	1.94	27.65	**61.39**	23.58
	100C	0	0	6.7	14.44	**68.18**
Cross validation 77.26%	**control**	**100**	0	0	0	0
	040C	0	**94.45**	0	0	0
	060C	0	3.88	**63.69**	23.88	8.52
	080C	0	1.67	29.05	**61.12**	24.43
	100C	0	0	7.26	15	**67.05**

The reason of the overlapping between the groups treated at higher temperature (60°C, 80°C, 100°C) may be due to the effect of the time interval which is also influencing the compositional changes of honey, however the average recognition and prediction abilities of the time intervals were 50.30% and 50.19%, respectively, for the whole data set of AF. The non-heated honey was classified correctly also in this model, while the other groups showed misclassification. These results show, that applied temperature has a higher effect for the absorbance spectra of the honeys. Similar results were obtained for HA honeys, while did not show clear separation in the case of RP honeys, that can be because of its chemical composition and that phase change is not as strong as in the case of the two other types.

Conclusions

Results of the tests of quality indicators of honey samples treated at different temperature and for different time intervals showed that pH of honey changes significantly as a result of heating at 100°C, while change of moisture content - if honeys are heated in glass holders with a closed cup, - and electrical conductivity do not show clear trends. Results of color measurement showed increasing tendency of L* in the beginning and b*, as a results of microcrystal structure changes, while fastened Maillard reaction caused the clear darkening of honeys at high heat treatment levels (80°C and 100°C). HMF content of honeys did not change at an extreme level below 80°C 240 minutes heat treatment, below this level the limit set by EU… was not reached. However changes in composition and phase of the honey was proven by the result of NIRS at lower heat treatment levels. Control honeys were clearly distinguished from heated ones by their NIR spectra, which can be not only because of the chemical changes occurred during heating but also because of the phase change. It is important to know that liquid phase can be elongated with the heat treatment, but later on honeys going to recrystallize, but as a results of heat treatment generate changes in the crystal structure, after recrystallization more rough crystals will be formed, and crystallization defects also could appear. Our study shows that HMF method can only detect extreme high temperature treatment or extreme long heat treatment, because significant increase in HMF content was observed only above 60°C heat treatment while NIR method was sensitive enough to recognize the changes in the phase and composition of honey treated at 40°C heat treatment.

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References

[1] P. M. Da Silva, C. Gauche, L. V. Gonzaga, A. C. O. Costa, és R. Fett, „Honey: Chemical composition, stability and authenticity”, Food Chem., köt. 196, o. 309–323, ápr. 2016, doi: 10.1016/j.foodchem.2015.09.051.

[2] CODEX STAN 12-1981, CODEX STANDARD FOR HONEY. 2001.

[3] 2001/110/EC, COUNCIL DIRECTIVE 2001/110/EC of 20 December 2001 relating to honey, köt. L 10/47. 2001.

[4] R. A. Gleiter, H. Horn, és H. D. Isengard, „Influence of type and state of crystallisation on the water activity of honey”, in Food Chemistry, 2006, köt. 96, sz. 3, o. 441–445, doi: 10.1016/j.foodchem.2005.03.051.

[5] S. Bogdanov, „Liquefaction of honey”, Apiacta, köt. 28, sz. 1, o. 4–10, 1993.

[6] L. P. Oddo és R. Piro, „Main European unifloral honeys: descriptive sheets”, Apidologie, köt. 35, o. S38–S81, 2004, doi: 10.1051/apido.

[7] Codex Alimentarius Hungaricus, 2-100 számú irányelv Megkülönböztető jelöléssel ellátott mézfélék. 2009.

[8] B. Zábrodská és L. Vorlová, „Adulteration of honey and available methods for detection – a review”, Acta Vet. Brno, köt. 83, sz. 10, o. S85–S102, 2014, doi: 10.2754/avb201483S10S85.

[9] Z. Bodor és mtsai., „Effect of heat treatment on chemical and sensory properties of honeys”, Anim. Welf. Etológia És Tartástechnológia Anim. Welf. Ethol. Hous. Syst., köt. 13, sz. 2, o. 39–48, 2018, doi: 10.17205/SZIE.AWETH.2017.2.039.

[10] S. Bogdanov, K. Ruoff, és L. P. Oddo, „Physico-chemical methods for the characterisation of unifloral honeys: a review”, Apidologie, köt. 35, o. 4–17, 2004, doi: 10.1051/apido:2004047.

[11] D. Cozzolino, „7 - Near Infrared Spectroscopy and Food Authenticity”, in Woodhead Publishing Series in Food Science, Technology and Nutrition, M. Espiñeira és F. J. B. T.-A. in F. T. T. and T. Santaclara, Szerk. Woodhead Publishing, 2016, o. 119–136.
[12] S. Bogdanov, „Harmonised Methods of the International Honey Commission”, Int. Honey Comm. IHC, sz. 5, o. 1–62, jan. 2009, doi: 10.1007/s13398-014-0173-7.2.
[13] M. Kędzierska-Matysek, M. Florek, A. Wolanciuk, P. Skałecki, és A. Litwińczuk, „Characterisation of viscosity, colour, 5-hydroxymethylfurfural content and diastase activity in raw rape honey (Brassica napus) at different temperatures”, J. Food Sci. Technol., köt. 53, sz. 4, o. 2092–2098, ápr. 2016, doi: 10.1007/s13197-016-2194-z.