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Evaluation of factors affecting crystallization of disparate set of multi-flower honey samples

A. Grégrová*, V. Kružík, E. Vrácovská, A. Rajchl and H. Čížková

University of Chemistry and Technology, Prague, Faculty of Food and Biochemical Technology, Department of Food Preservation, Technická 5, 16628 Prague 6 – Dejvice, Czech Republic; *Correspondence: Adela.Gregrova@vscht.cz

Abstract. Honey crystallization is considered to be a natural process during its maturing and an indicator of natural honey composition. However, consumer evaluation of honey crystallization is usually negative. Crystallization depends on honey composition and it is influenced by methods and conditions of honey processing and storage (mechanical and thermal treatment). The aim of this work was to identify and evaluate general factors which can affect crystallization of blend multi-flower honeys (a disparate set of samples). The following qualitative parameters were determined: a content of 5-hydroxymethylfurfural, furfural, glucose and fructose, water and diastase activity, moisture and an absolute pollen count. A degree of honey sample crystallization was assessed by a sensory analysis. Effects of the various qualitative parameters on the crystallization degree were statistically evaluated. The honey crystallization degree was found to be a qualitative parameter positively correlated with the absolute pollen count. Using a multi-regression method (a cluster analysis) it was proven that the 5-hydroxymethylfurfural and moisture parameters were suitable characters with certain explanatory power to classify blend honey samples according to their crystallization degrees.

Key words: honey crystallization, multi-flower honey, qualitative parameter.

INTRODUCTION

Honey crystallization is a natural occurrence in honey maturing and in some respects it can be taken as an indicator of natural honey composition (Venir et al., 2010). Unfortunately partial or entire crystallization of commercial honeys is often considered a defect (by consumers), because liquid and transparent honeys are wrongly regarded as better quality honeys. Besides causing obvious changes in sensory, mainly visual, properties, honey crystallization can have another unsatisfactory effects, such as technological and processing problems (e.g. pouring), a loss of honey homogeneity, higher water activity related to honey fermentation due to microorganisms' development (Tosi et al., 2002).

Tendency to crystallization of honeys depends mainly on the following factors: chemical composition, a degree of supersaturation, viscosity, a fructose/glucose (F/G) ratio, moisture (M) and a dextrine content, water activity (a_w), micro-crystals and nucleation seeds (e.g. pollen grains) presence, age, storage temperature and thermal history (Bogdanov, 1993; Tosi et al., 2002; Tosi et al., 2004; Juszczak & Fortuna, 2006; Sudzina et al., 2009; Venir et al., 2010). Duration of an entire crystallization process

varies considerably for different honey types. In most honeys, crystallization begins within weeks or months at room temperature (Townsend, 1975; Assil et al., 1991).

Generally, blossom honeys crystallise faster due to a higher content of less soluble glucose (a content of approx. 40–50 g 100 g^{-1} in dry matter Gleiter et al., 2006; Laos et al., 2011) and pollen grains. On the contrary, crystallization proceeds more slowly in honeydew honeys, which contain less glucose (a content of approx. 30–35 g 100 g^{-1} in dry matter Gleiter et al., 2006) and more fructose, but could under some circumstances have a significantly higher content of crystal-forming melezitose and trehalose (Dobre et al., 2012).

Honey maturing may be impaired by exposure to high temperatures or by filtration (removal of pollen grains). Crystallization occurs more easily when honeys are disturbed (e.g. stirring, shaking and agitating) (Rybak-Chmielewska, 2004).

Crystallization can be controlled mainly by heating and proper temperature storage conditions. If honey is held at 40–71°C during processing (bottling), a crystallization degree is reduced. Crystals can be dissolved by mild heating of honey and quick heating at 60–71°C tends to dissolve crystals and expel incorporated air (which can also stimulate honey crystallization) (Townsend, 1975; Assil et al., 1991).

Diastase activity and a 5-HMF (5-hydroxymethylfurfural) content are used as quality indicators of freshness or indicator of extensive heating of honey during its processing with limits given by the EU Honey Directive (Subramanian et al., 2007).

Tosi et al. (2004) determined the effect of high-temperature short time heating of honey on the following qualitative parameters related to honey quality and crystallization process: a 5-HMF content, diastase activity and crystallization starting time. Crystallization onset was proved to be delayed by 4 to 9 weeks for honeys treated by high-temperature short-time treatment at 80°C for 60 s in the transient stage and 30 s in the isothermal stage. 5-HMF and DN modifications recorded after the heat treatment were for example: a) the 5-HMF content increased to 7.9 mg kg^{-1} and the DN decreased to 14.4 in case of honey with a higher initial content of 5-HMF (7.5 mg kg^{-1}) and DN (14.6); b) both values stayed the same in case of honey with a lower initial content of 5-HMF (5.0 mg kg^{-1}) and DN (9.0) (Tosi et al., 2004).

Crystallization is also affected by presence and a number of crystallization centres, mainly pollen grains. Therefore, qualitative and quantitative melissopalynological analyses (Holdaway, 2004) of honeys mainly used to determine botanical and geographical origin of honeys can be employed (Louveaux et al., 1978; Song et al., 2012). Pollen grains but also plant and animal admixtures, e.g. spores and hyphae of fungi, algae, yeasts, chitin fragments, hairs, insects etc., can be removed by filtration (Přidal, 2003). Filtered honey has a significantly lower tendency to crystallise. Filtration also removes small crystals of glucose and contaminants that trigger crystallization process.

As was already mentioned, honey crystallization can be influenced by many factors and changes can be predicted to a certain extent only in the case of monoflower honeys. Since in the Czech Republic mainly blend honeys are processed, the aim of our study is to verify whether it is possible to identify general factors affecting a disparate set of multi-flower honey samples. The effects of various parameters on honey crystallization have been statistically processed.

MATERIALS AND METHODS

Honey samples

The total of 16 samples of different honeys were analysed including blend multi-flower honeys (European (EU) and Czech honeys; 2012) (Table 1). Samples no. 1–10 were sampled at different degrees of crystallization; they came from different batches with known thermal history (preheating at about 35°C and pasteurisation (75°C, 5 min) was the same for all the samples and varied only with time delays in a tank at 40°C), and were supplied directly by manufacturers. A set of samples no. 11–16 of different origin and with unknown thermal history was purchased in the Czech market.

Table 1. Analysed samples

Sample Number	Heating Period in a Tank	Manufacturer/Vendor	Origin
1	0 min	Medokomerc, CZ	EU
2	0 min	Medokomerc, CZ	EU
3	0 min	Medokomerc, CZ	EU
4	0 min	Medokomerc, CZ	EU
5	0 min	Medokomerc, CZ	EU
6	360 min	Medokomerc, CZ	EU
7	360 min	Medokomerc, CZ	EU
8	180 min	Medokomerc, CZ	EU
9	240 min	Medokomerc, CZ	EU
10	420 min	Medokomerc, CZ	EU
11	unknown	JSG med, CZ	EU
12	unknown	Kaufland, CZ	EU
13	unknown	Local Czech Beekeeper	CZ
14	unknown	Medokomerc, CZ	EU
15	unknown	Product Bohemia, CZ	CZ
16	unknown	IS import-export, SK	EU

Determination of qualitative parameters

The following physico-chemical, qualitative parameters were determined: a content of 5-hydroxymethylfurfural, furfural, glucose and fructose, diastase activity, water activity, moisture, presence and a number of crystallization centres (an absolute pollen count).

With the exception of water activity (Chirife et al., 2006) all the other analytical parameters were determined according to the harmonized methods for the analysis of honey (Bogdanov, 2009).

A content of 5-HMF and furfural was determined in a filtered, aqueous honey solution using HPLC equipped with UV detection (Thermostated Column Compartment TCC-100: Dionex, Germany; HPLC Pump Dionex P680; HPLC Dionex Summit ASI-100 Automated Sample Injector; UltiMate 3000, Photodiode Array Detector: Dionex, Germany).

A content of sugars (glucose and fructose) was analysed (after filtration of the solution) by HPLC with RI detection (Thermostated Column Compartment TCC-100: Dionex, Germany; HPLC Pump Dionex P680; HPLC Dionex Summit ASI-100 Automated Sample Injector; UltiMate 3000, RI Detector: Shodex RI – 101, Japan).

Diastase activity expressed as a diastase number (DN) was analysed using Phadebas tablets by a photometric method (Phadebas Amylase Test: Magle AB, Sweden) using Spectrofotometer Genesys 20 (Thermo Spectronic, USA) and water activity was determined using an electronic dew-point water activity meter, Aqua Lab Series 3 (Decagon Devices, USA).

Moisture was analysed by automatic digital refractometry, Refractometer RFM 340 (Bellingham + Stanley, United Kingdom). The water content was determined from the refractic index of the honey.

A quantitative melissopalynological analysis was performed using the modified procedure described in Přidal (2003). Pollen grains were determined microscopically and expressed as an absolute pollen count (APC, i.e. a number of pollen grains per 10 g of honey). Honey (10 g) were dissolved and diluted in distilled water; solution was centrifuged in a cuvette at 3 000 rpm (3 times for 5 min). The sediment was filtered through a microbiological filter and after drying it was illuminated by cedar oil. Pollen grains were counted in 60 view fields (at 1,000x magnification). A Digital Laboratory Microscope was used, Model DMBA-310 (Motic Deutschland GmbH, Germany).

Sensory evaluation

Samples were also sensorially assessed. Sensory evaluation was performed by 10 panellists from the Department of Food Preservation (University of Chemistry and Technology, Prague) according to Piana et al. (2004). To evaluate crystallization, a 10-point scale was used (1 = liquid; 10 = entirely crystallised).

Statistical analysis

The results were presented as mean values of three repetitions for each analysed qualitative parameter. A degree of linear relationship between the parameters was studied using the Pearson correlation matrix. A cluster analysis was performed to group the samples according to the studied variables. All statistical analyses were carried out using STATISTICA 10.0 (StatSoft, USA) software.

RESULTS AND DISCUSSION

The set of honey samples at different levels of crystallization (all samples were analysed approx. 18 month after production) and with different thermal history were analysed. The results for all determined qualitative parameters are given in Table 2.

Table 2. Comparison of the measured data

Sample number	Moisture (M; %)	Water Activity (a_w)	5-HMF (mg kg^{-1})	Furfural (mg kg^{-1})	Glucose (g 100 g^{-1})	Fructose (g 100 g^{-1})	Diastase Activity (DN)	F/G	G/M	Absolute Pollen Count (APC × 10^4)	Crystallization Degree
1	17.51	0.596	25.06	0.00	28.80	38.30	11.64	1.33	1.64	11.56	9
2	17.44	0.594	31.70	2.72	33.20	41.60	10.79	1.25	1.90	10.50	9
3	17.52	0.605	29.90	2.65	29.60	40.40	10.93	1.36	1.69	10.18	9
4	17.75	0.571	20.10	2.66	32.40	45.40	10.80	1.40	1.83	8.43	2
5	17.71	0.574	45.40	2.40	31.10	43.80	10.55	1.41	1.75	6.96	1
6	17.10	0.576	26.96	2.71	28.60	38.70	12.20	1.35	1.67	10.10	3
7	17.88	0.577	36.18	2.54	31.00	39.70	10.56	1.28	1.78	8.10	2
8	17.82	0.579	22.70	2.87	31.50	39.30	9.75	1.25	1.77	9.67	5
9	17.64	0.576	34.48	2.70	29.30	42.20	11.66	1.44	1.66	9.90	5
10	17.81	0.574	43.70	2.48	30.00	42.60	10.57	1.42	1.68	7.43	2
11	16.55	0.597	46.96	4.00	29.56	38.58	10.57	1.31	1.79	10.57	8
12	17.90	0.639	45.00	2.60	22.20	40.03	9.50	1.43	1.15	7.40	2
13	17.18	0.577	19.90	0.00	23.59	24.09	10.80	1.02	1.69	5.97	3
14	17.62	0.639	32.93	3.18	31.51	39.03	12.20	1.24	1.79	10.97	10
15	16.14	0.594	32.60	3.39	24.38	36.96	9.70	1.52	1.51	9.13	5
16	17.69	0.617	36.35	2.75	28.66	42.25	10.50	1.47	1.62	10.39	s5

DN = diastase activity expressed as diastase number; APC = number of pollen grains per 10 g of honey; crystallization degree: 10 point sensory scale from 1 = liquid to 10 = entirely crystallised; all samples were analysed approx. 18 month after production.

Crystallization degree varied considerably and ranged from 1 (liquid) to 10 (entirely crystallised) points (Fig. 1). Given the nature of our study qualitative assessment of the crystallization process was based on sensory evaluation. Because of obvious differences between samples sensory analysis was chosen as an appropriate and sufficient method and there was no need to apply strict quantitative methods for this purpose, e.g. microscopy and calorimetry (Mazzobre et al., 2003; Venir et al., 2010).

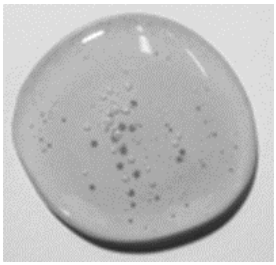
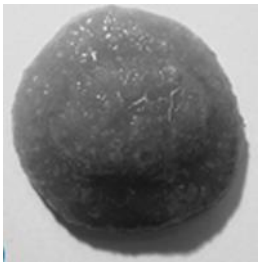
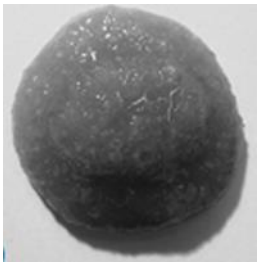
a)  b)  c) 

Figure 1. Honeys in different stages of crystallization (Panasonix Lumix DMC-FZ38; at 4x magnification; 5 g of blend multi-flower honeys on white anti-reflective surface) (a) natural liquid honey – crystallization degree 1, sample no. 5; b) partially crystallized honey – crystallization degree 5, sample no. 8; c) entirely crystallized honey – crystallization degree 10, sample no. 14).

For all samples, the moisture content, water activity, diastase activity, ratios of F/G and G/M were very balanced.

The content of 5-HMF (from 19.9 mg kg^{-1} to 47.0 mg kg^{-1}) and furfural (from not detected levels to 4.0 mg kg^{-1}) could point to heating or improper long-term storage of a sample. The diastase activity (indicating heating and/or improper storage) expressed as a DN ranged from 9.5 to 12.2. Quality of samples no. 1–10 heated in a tank at 40°C for varying periods of time was not negatively influenced by these temperature and storage conditions. Only in 2 samples, i.e. sample no. 5 and 10, the content of 5-HMF was slightly higher than the 40 mg kg^{-1} requirement (European Parliament and Council Directive, 2001), which could be caused by the raw material.

The glucose content was determined to range from 22.2 g 100 g^{-1} to 33.2 g 100 g^{-1} and the fructose content ranged from 24.1 g 100 g^{-1} to 45.4 g 100 g^{-1}. Botanical origin of multi-flower honey samples and the associated sugar composition influence honey crystallization (Escuredo et al., 2014). Honeys with a high glucose content and a low F/G ratio crystallised more rapidly (rape and sunflower based honeys). Honeys with a higher F/G ratio (more than 1.4) crystallised generally more slowly, e.g. bramble, chestnut, eucalyptus, heather, acacia and honeydew honeys (Escuredo et al., 2014); from the analyzed samples it was fulfilled by no. 5, 9, 10, 12, 15 and 16 with crystallization degree ranged from 1 to 5 points.

Moisture, and hence a crystallization degree, should be closely linked to water activity (a_w) (Tosi et al., 2004). In accordance with literature (Tosi et al., 2004) the water activity was determined in the range from 0.571 to 0.639 (a_w). Water activity increases with a moisture increase (Tosi et al., 2004). Moisture was determined in the range from 16.1% to 17.9% (M).

Most of the samples met the legislative requirements (European Parliament and Council Directive, 2001) for followed physico-chemical parameters except for samples no. 5 (5-HMF: 45.4 mg $kg^{-1;}$, limit max. 40), 10 (5-HMF: 43.7 mg kg^{-1}), 11 (5-HMF: 47.0 mg kg^{-1}), 12 (5-HMF: 45.0 mg kg^{-1}) and 13 (G+F: 47.7 weight %, limit min. 60).In accordance with the literature (Oddo & Piro, 2004; Song et al., 2012), the results of the quantitative melissopalynological analysis, i.e. the absolute pollen count (the number of pollen grains per 10 g of honey sample), varied significantly from $6.0 \cdot 10^4$ to $11.6 \cdot 10^4$. In the study by Song et al. (2012) 4 out of the 19 analysed samples (Chinese monoflower honeys) contained from $2.0 \cdot 10^4$ to $10.0 \cdot 10^4$ of pollen grains and 2 samples contained $> 10.0 \cdot 10^4$ of pollen grains (an absolute pollen count). Pollen grains from, for example, rape, black locust, sunflower and dandelion were identified in the samples; these plants are typical for Central and Eastern European countries (Oddo & Piro, 2004) (Fig. 2). Oddo & Piro (2004) determined mean absolute pollen counts for rape ($7.6 \cdot 10^4$), black locust ($0.9 \cdot 10^4$), sunflower ($1.9 \cdot 10^4$) and dandelion ($3.4 \cdot 10^4$) honeys.

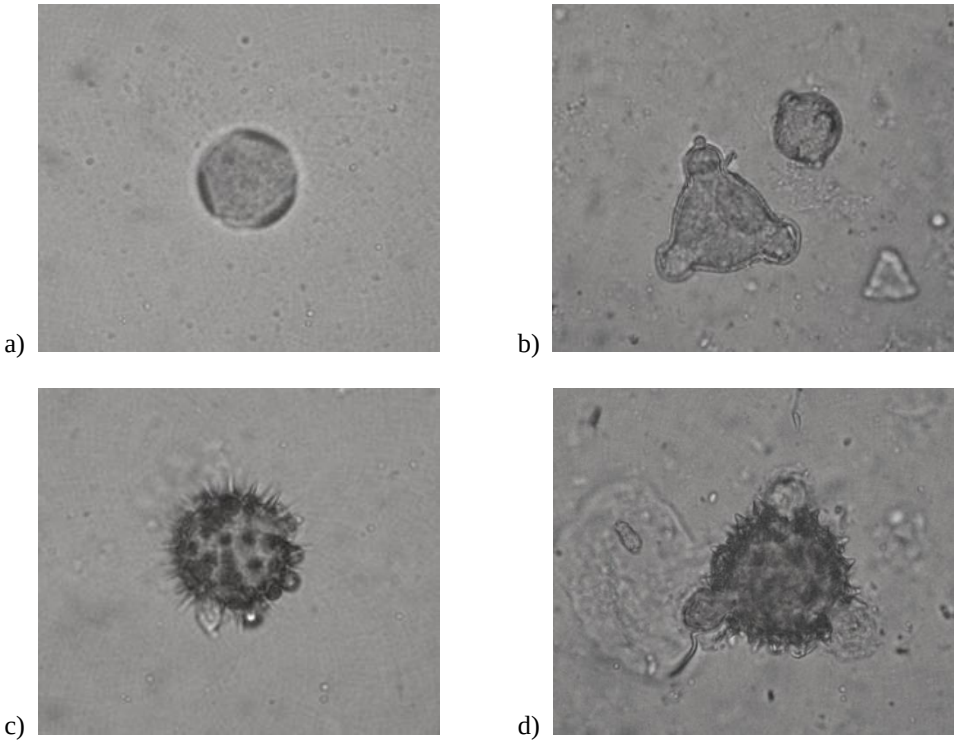


Figure 2. Examples of the found pollen grains (microscopy; at 1,000x magnification) (a) rape; b) black locust; c) sunflower; d) dandelion).

The effects of various parameters on honey crystallization were statistically evaluated. A correlation matrix and a cluster analysis were applied to evaluate relationships between the qualitative parameters and the properties of honey samples (Table 3; Figs 3, 4).

Table 3. Correlation matrix

	Moisture	a_w	5-HMF	Furfural	Glucose	Fructose	DN	Fructose/ Glucose	Glucose/ Moisture	APC	Crystallization
Moisture	**1.00**										
a_w	0.04	**1.00**									
5-HMF	0.00	0.28	**1.00**								
Furfural	– 0.21	0.20	0.48	**1.00**							
Glucose	0.35	– 0.29	– 0.08	0.28	**1.00**						
Fructose	0.40	0.01	0.37	0.54	0.59	**1.00**					
DN	0.05	– 0.04	– 0.27	– 0.20	0.35	– 0.01	**1.00**				
Fructose/Glucose	– 0.05	0.10	0.45	0.47	– 0.05	0.71	– 0.22	**1.00**			
Glucose/Moisture	0.02	– 0.48	– 0.30	0.07	0.83	0.11	0.40	– 0.41	**1.00**		
APC	–0.18	0.33	– 0.13	0.26	0.39	0.28	0.45	0.15	0.25	**1.00**	
Crystallization	– 0.25	0.47	– 0.14	0.07	0.25	– 0.05	0.37	– 0.20	0.28	**0.82***	**1.00**

DN = diastase activity expressed as diastase number; APC = number of pollen grains per 10 g of honey; * the critical limit for correlation coefficient r in the case of the 16 samples (confidence level $\alpha = 0.05$) = 0.497.

The results of the crystallization degree evaluation were correlated with the other determined qualitative parameters (Table 3). In accordance with the literature (Escuredo et al., 2014), a statistically significant correlation (α = 0.05) was demonstrated only in the case of the absolute pollen count (r = 0.82; critical value for correlation coefficient: 0.497) for the total disparate set of honey samples (no. 1–16). On the contrary, in the case of the samples with known thermal history and from the same producer (no. 1–10), a statistically significant correlation (α = 0.05) was also demonstrated between the crystallization degree and the water activity (r = 0.92; critical value for correlation coefficient: 0.632). In accordance with the literature (Gleiter et al., 2006; Zamora and Chirife, 2006), it was proven that a_w of crystallised honeys was higher than a_w of liquid (re-dissolved) honeys, because a_w increase during crystallization process is mainly related to glucose crystallization.

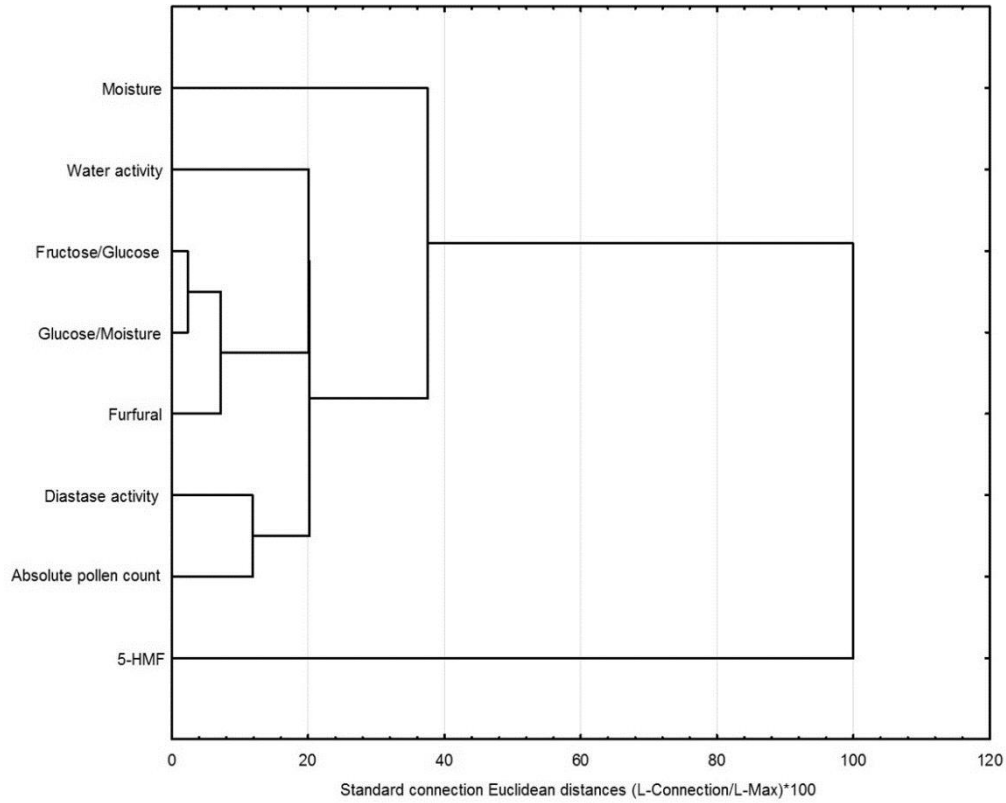


Figure 3. Dendrogram of 8 qualitative parameters for honey samples characterisation.

Since the direct linear correlation (the correlation matrix) was not very demonstrative due to the complexity of the honey crystallization process, a multivariate statistical technique (a cluster analysis) was applied to reveal deeper structures in the data set. This method assesses similarity of the objects based on a combination of all measured characters.

The cluster analysis was applied to prove the ability to distinguish between honeys of a different crystallization degree. For statistical evaluation, a data matrix of the 16 honey samples including the following qualitative parameters was used: moisture, water activity, ratios of G/F and G/M, a content of 5-HMF and furfural, diastase activity and an absolute pollen count (Tosi et al., 2004; Escuredo et al., 2014).

Fig. 3 shows a dendrogram of the characters (for the 8 qualitative parameters), which expresses their reciprocal similarity. Six similar characters (a sub-cluster for the F/G and G/M, furfural and water activity characters and a sub-cluster for the diastase activity and the absolute pollen count) create a large combined cluster (reading from the right to the left), to which a less similar moisture character is connected. The 5-HMF character is completely dissimilar to the others and it has been indicated as an outlying character.

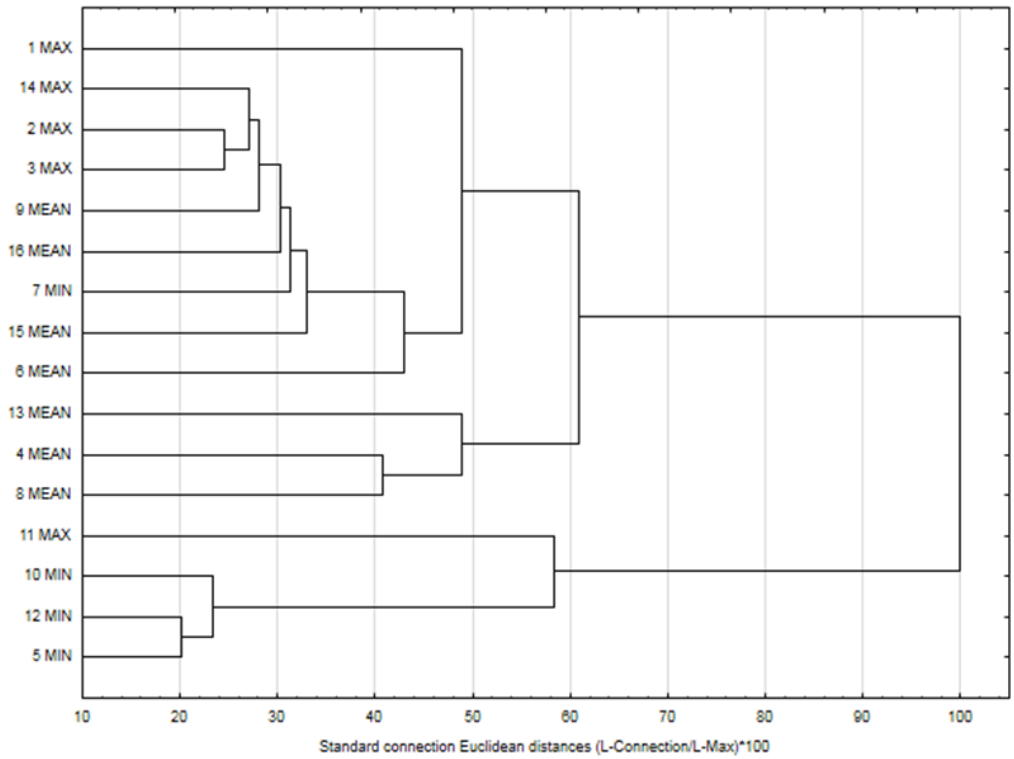


Figure 4. Dendrogram of the 16 honey samples classification (samples indication: maximal, mean and minimal crystallization degree).

A dendrogram of the objects (Fig. 4) allowed classifying the 16 honeys into several expectable clusters. Fig. 4 also shows the samples that were significantly different from the others. It is obvious (reading from the right to the left) that the samples are divided into 3 dominant clusters. The first cluster contains samples no. 1, 14, 2, 3, 9, 16, 7, 15 and 6. The second one contains honeys no. 13, 4 and 8; and the third one includes honeys no. 11, 10, 12 and 5. The first cluster contains maximally and mean crystallised honeys. This group of honeys is characterised by high water and diastase activity and a low

content of 5-HMF. This largest cluster includes both maximally and some mean crystallised honeys due to the similarity of the parameters analysed for these groups of samples. The second cluster of mean crystallised honeys is characterised by the lowest content of 5-HMF in its category. The third cluster is made up of minimally crystallised honeys that have the highest values for moisture and 5-HMF content; the values of absolute pollen count and diastase activity are the lowest. Because of high content of 5-HMF as a result of strong heating it should be emphasized that strongly heated honey crystallizes differently than fresh ones. Two atypical samples, i.e. no. 7 and 11, differ from the other samples in the group due to the following parameters (Fig. 4): F/G and absolute pollen count for sample no. 7; moisture and diastase activity for sample no. 11. Moisture and 5-HMF parameters could be considered as the characters with certain explanatory power to classify samples due to their different crystallization degree.

CONCLUSIONS

Due to complexity of honey crystallization, this process is influenced by a number of factors and conditions of processing and storage of honey. Contrary to the literature, only the absolute pollen count was demonstrated as a qualitative parameter positively correlated with the honey crystallization degree. Using a cluster analysis we found 2 more qualitative parameters, i.e. 5-HMF and moisture content, which influence crystallization degree of blend multi-flower honeys. It was found that most of the analysed multi-flower honey samples with the values of 5-HMF content around or higher than 40.0 mg kg^{-1}, moisture content > 17.7% and absolute pollen count < $9.0 \cdot 10^4$ stayed in a liquid state for approx. 18 months after production.

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