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The Crystallization Behaviour of Estonian Honeys

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Abstract. The feasibility of water activity and viscosity measurement was studied to characterize the isothermal crystallization of Estonian honeys. In parallel, samples were observed by light microscopy. The most important phenomenon for crystallization is the fructose/glucose ratio in favour of glucose. The increase in water activity and viscosity was noticed during crystallization. Polarized light microscopy was more sensitive than water activity or viscosity for determining the crystallization time.

Key words: Honey crystallization, microscopy, sugars, viscosity, water activity

INTRODUCTION

Honey is a highly concentrated solution of sugars, mostly glucose and fructose with small amounts of various more complex sugars. Many other components including amino acids, proteins, minerals, enzymes and vitamins also occur in honey.

Over time, liquid honey tends to crystallize. As most honeys are supersaturated solutions of glucose, this sugar may precipitate out spontaneously in the form of glucose monohydrate, and the solution then reverts to the more stable saturated state (Zamora et al., 2006).

Crystallization of honey can be a serious problem for processing since it affects honey flow during extraction, pumping, settling, filtration, mixing and bottling. In industrial processing, crystallised honey is heated and this process induces the formation of hydroxylmethylfurfural (HMF). HMF is a major quality factor of honey and is an indicator of freshness and exposure to heating. The colour, flavour and aroma of honey may be also altered by heat treatments (Krell, 1996).

As crystallization is usually undesirable in liquid honey, controlled crystallization can be used to produce a creamed honey. This type of honey has a large number of very small crystals, not perceived by the palate (Hartel, 2001).

There have been many published works to predict the tendency of a honey to crystallize (Manikis & Thrasivoulou, 2001; Bakier, 2007; Conforti et al., 2006; Lazaridou et al., 2004; Rüegg & Blanc, 1981). Most of the reports, however, indicate that all of the predictive methods for crystallization are not sufficiently accurate; thus, further investigation is needed.

The purpose of the present work is to measure the sugar content, water activity and viscosity of several honeys from Estonia, and to follow the water activity and viscosity shift due to crystallization. Also, the crystallization kinetics is measured by polarized light microscopy.

MATERIALS AND METHODS

Eleven (11) honey samples were collected directly from beekeepers in July 2010 from different areas of Estonia. Liquid honey samples were stored up to 84 days at controlled temperature (18 ± 2ºC) in airtight glass containers allowing spontaneous nucleation and crystal growth to occur.

Melissopalynological analysis was carried out according to the method described by Louveaux et al. (1978), using the non-acetolytic method. The pollen counts were expressed as percentages after counting 500–600 pollen grains. A binocular light-microscope Olympus CX21 (Japan) with 40 × 15 magnification was used.

Water activity was measured at 20°C using an electronic dew-point water activity meter, Aqualab Series 3 model CX (Decagon Devices, USA). For each determination three replicates were obtained and the average reported.

Polarized light microscopy was used to determine the isothermal crystallization kinetics. Images were acquired using Nikon eclipse E200 light microscope (Nikon, Japan) with polarizer filters. To prevent the water transfer, the junction of microscope glass and cover glass was sealed with waterproof silicone. Images were taken with magnification of 4 × 10.

The viscosity of honey was measured at room temperature by using viscometer RI:2:M/H (Rheology International LTP, Ireland), spindle ASTM7, speed 30 rpm, time 30 s.

The sugar content was determined by high-performance liquid chromatography (HPLC) (Waters, USA), equipped with Alliance Separations Module (Waters 2695, Milford, MA, USA), Aminex HPX-87H 300 × 7.8 mm column (BioRad, Philadelphia, PA, USA) and Refractive Index Detector (Waters 2414, Milford, MA, USA). 0.4 g of honey was dissolved in 50 ml Milli-Q water. The sample was filtered through a 0.2 μm Millipore filter and additional 10-fold dilution was made with HPLC eluent (0,005M H_2SO_4). The injection volumes of the samples were 20 μl, with a flow rate of 0.6 ml min^{-1}, isocratic. The HPLC sample peaks were identified by comparing the retention times obtained from standards. Triplicate injections were performed and average peak areas were used for the peak quantification.

RESULTS AND DISCUSSION

Melissopalynological analysis showed that most of the analyzed honeys were polyfloral (73%) and the most occurring pollens were cruciferous (mainly rape *[Brassica napus]*), leguminous (mainly melilot *[Melilotus officinalis]* and white clover *[Trifolium repens]*), rosacean (mainly raspberry *[Rubus idaeus]*), and salicaceous (mainly willow [*Salix])* (Table 1). Usually honey is considered as unifloral when the relative frequency of the pollen of that *taxon* exceeds 45%. However, because of the numerous over- or under-represented pollen types, the pollen percentages can greatly

vary among unifloral honeys. Rape honeys are considered unifloral as their pollen is over-represented and should be over 60% (Von Der Ohe et al., 2004). The raspberry honeys, where the pollen amount was over 45%, were considered as unifloral according to Bryant & Jones (2001). Taking the pollen types into account, four (4) of all the 11 analyzed honeys could be considered as unifloral - raspberry (no. 1) and rape (no. 2, 3, 4). The polyfloral honey is typical to Estonia as there are few large mono cultural crops field: as a result, wildflower and forest honeys are most the common varieties in Estonia.

Table 1. Pollen types of honey samples (%).

	Honey samples										
Pollen type	1	2	3	4	5	6	7	8	9	10	11
Aceraceae											
Acer spp.	+			1	4	3	1	+	3	3	
Betulaceae	1	+		+		1	+		1	2	
Boraginaceae											
Echium vulgare		+			+				2		
Compositae											
Centaurea cyanus		+					+				
Taraxacum officinale					1		+	1	1	+	
Cruciferae											
Brassica napus s.l.	11	**60**	**77**	**76**	51	50	43	29	2	16	37
Fabaceae											
Galega officinalis		2	5			2	+	17			3
Hippocastanaceae											
Aesculus hippocastanum			+		1		+		+		
Hydrophyllaceae											
Phacelia tanacetifolia										2	
Leguminosae											
Melilotus officinalis s.l., *Trifolium repens* s.l.	4	5	10	2	5	10	4	27	18	27	15
Trifolium pratense s.l.		1	5	+	+	5	+	+		+	3
Rhamnaceae											
Frangula alnus		1		2				+		1	
Rosaceae											
Rubus idaeus s.l.	**79**	17	2	8	7	17	14	23	36	17	40
Filipendula ulmaria		+	+			+			+	2	
Salicaceae											
Salix spp.	5	9	+	6	27	7	34	2	15	24	
Umbelliferae	+	1	+	+	1	+	+	+	2	1	

+ Minor pollen (< 1%)

The time required for the honey to crystallize depends mostly on the ratio of fructose to glucose (F/G) (Gleiter et al., 2006). Higher glucose content has stronger effect on increasing the crystallization rate. In Fig. 1 the linear relationship between F/G and the crystallization time observed by polarized light microscopy ($R^2 = 0.8458$) is presented. Rape honeys (samples no 2, 3, 4) have low F/G ratio (0.95 ± 0.05) and the first crystals were observed almost immediately after harvesting (Fig. 2). The

crystallization continued showing the most dramatic growth during first week of storage. The F/G of polyfloral honeys was higher (1.03 ± 0.07) and the crystallization process was slower compared with rape honey (Fig. 2). The polyfloral honey samples where rape pollen was dominating also showed a fast crystallization rate (samples no 5, 6, 7). Raspberry honey is considered having medium crystallization rate (Manikis & Thrasivoulou, 2001) that was also shown in this research (sample no 1). Three polyfloral honey samples (no 9, 10, 11) had slow crystallization rates compared with others. These samples had high F/G ratio (> 1.05) but no common pollen type that connects them.

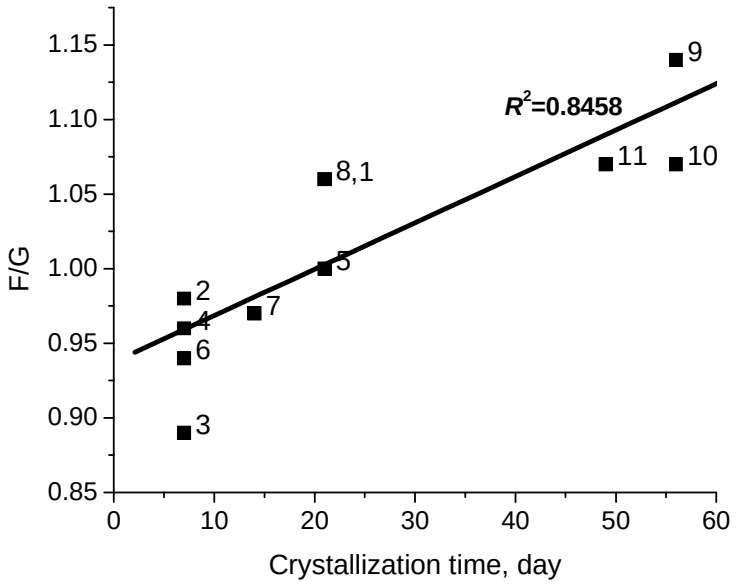

Figure 1. The influence of the ratio of fructose to glucose (F/G) on the crystallization time of honey samples.

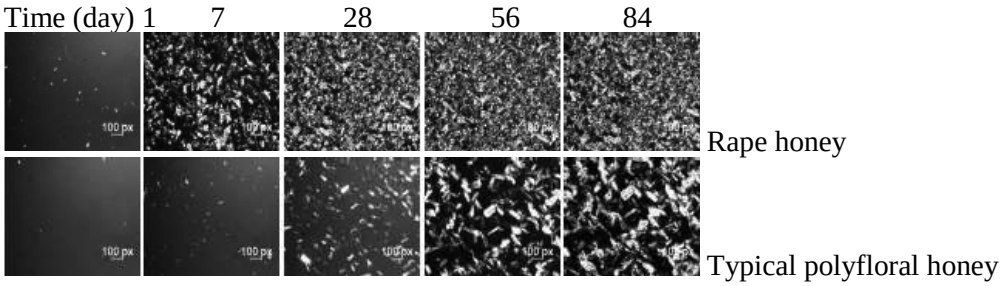

Figure 2. The formation of crystals in honey samples during storage.

The water activity of honey is mainly determined by the molal concentration of soluble chemical species; thus, water activity in honey results mainly from the concentration in the water of the monosaccharides fructose and glucose, and to a lesser extent, to some disaccharides such as sucrose, maltose/isomaltose (Chirife et al., 2006).

During crystallization, glucose starts to crystallise first. Fructose has a higher solubility and stays in solution for a longer time. All five hydroxyl groups of glucose interact with water molecules. After crystallization glucose is found as glucose monohydrate; each glucose molecule fixes only one molecule of water. Therefore, less water is fixed in the crystallised state. The content of free water is higher and in accordance with the water activity (Gleiter et al., 2006).

In Fig. 3 the water activity change due to crystallization is shown in the case of rape (*Brassica napus*) honey and polyfloral honey. The water activity of rape honey increased with time, reaching a plateau after 56 days of storage and indicating the complete crystallization of glucose. During crystallization the increase in honey viscosity was also observed (Fig. 3). The viscosity increased with time and would be the result of a structure formed by crystals in honey samples.

The water activity of polyfloral honey changed slightly during storage (Fig 3). The viscosity increased slowly with time. After 84 days of storage the polyfloral honeys were still flowing, having viscosity of 1,100 Pa*s.

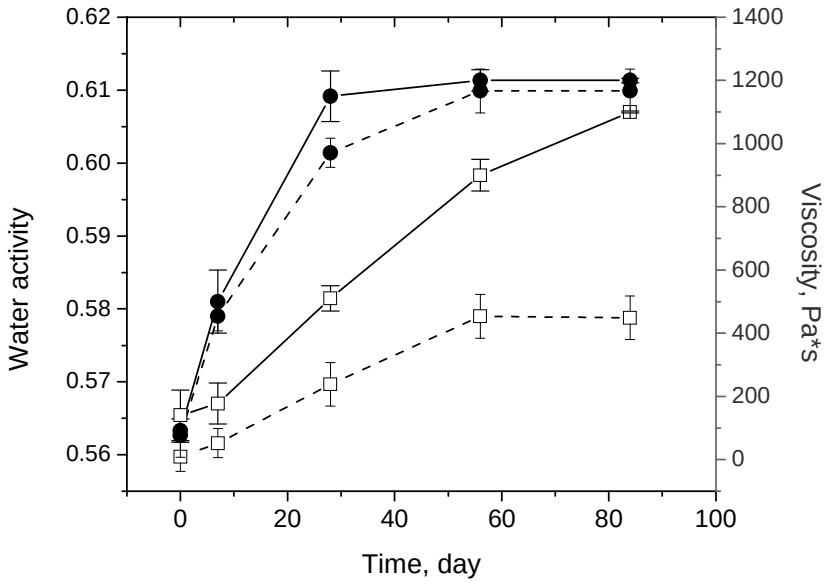

Figure 3. The change of water activity (---) and viscosity (—) during storage of rape (*Brassica napus*) (●) and polyfloral (□) honey samples.

CONCLUSIONS

Estonian honeys are mostly polyfloral. Most of the honeys will crystallize during storage. The most important phenomenon for crystallization is the fructose/glucose ratio in favour of glucose. The crystallization of glucose releases water and the water activity increases. During crystallization the viscosity of the honey increases as a structure is formed by crystals.

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