

INFLUENCE OF GLUCOSE CHANGES ON WATER ACTIVITY IN SELECTED HONEYS

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Abstract. The paper presents an analysis of the influence of glucose on water activity in two selected honeys, in both liquid and crystallized states. Two honeys, rape and buckwheat, characterized by essential differences in their glucose and fructose content, were investigated. The relation between decrease of water activity in both types of honey and an added fraction of glucose monohydrate after liquefaction was determined. Making use of the NIR spectroscopy, values of the solid phase fraction in both honeys in their crystallized state and changes of water activity in the honeys after crystallization were analysed. It was found that the formation of crystallized glucose phase in honey is accompanied by surface interactions that lead to a drop in water activity. The resultant increase of water activity after crystallization, a_w^{cr} , was represented as the difference between the increase caused by the formation of the solid phase, a_w^s , and surface interactions a_w^A . The impact of the solid phase morphology on surface interactions was verified experimentally by analysing water activity after the introduction of two glucose monohydrate fractions of different crystal grain size into the honeys.

Keywords: honey, state of crystallization, water activity, glucose, monohydrate glucose

INTRODUCTION

Honey is a concentrated solution of monosaccharides, in which 60 to 85% of the product is composed of both fructose and glucose (Lazaridou *et al.* 2004). Generally, fructose is the dominant component and only in very few types of honey, such as rape and dandelion honeys, glucose fraction is greater than that of fructose (Cavia *et al.*, 2002). Practically, however, glucose is found in the state of supersaturation in all honey types and it crystallizes into a monohydrate during storage (Doner 1977, White 1978). It is commonly emphasized that the process of glucose crystallization changes honey structure which in its solid form is not readily

accepted by consumers (Cavia *et al.* 2002). There is another aspect of the process that has been underestimated, namely that the glucose crystallization process changes the way in which water is bound in the product resulting in an increase of water activity in honey, which in turn may lead to its fermentation (Iurlina and Fritz 2005, Sanz *et al.* 1994, Wojtacki 1989). Due to the fact that saccharide solutions bind much more water than when in the crystallized form, glucose crystallization in honey will cause an increase of water activity (Sikorski *et al.* 2002). A system is obtained in which the phase of crystallized glucose monohydrate forms a skeleton immersed in a diluted liquid solution dominated by fructose (Schley and Büskes-Schluz 1987). In liquid honeys, linear correlations between water content and water activity are generally observed (Bakier 2006a, Cherife *et al.* 2006, Gleiter *et al.* 2006). Rüeegg and Blanc, who investigated the increase of water activity caused by crystallization processes in honeys originating from various countries, showed that the increment ranged from about 0.012 to 0.12 at an average difference amounting to 0.027 (Rüeegg and Blanc 1981). Analogous results obtained for 49 Argentinean honey samples ranged between 0.014 and 0.056 and oscillated around the average value of 0.034 (Zamora and Chirife 2006). Recent research has shown that the increment of water activity after crystallization depends on the origin of honey and in polyfloral honeys the increment value amounts to about 0.04 and in honeydew to 0.02 (Gleiter *et al.* 2006). It is also emphasized, at the same time, that there is no correlation between the increase of water activity after crystallization and the water content in the product (Gleiter *et al.* 2006, Rüeegg and Blanc 1981). In the literature on the subject, however, there is no explanation of such considerable differences between the increments of water activity in various honeys after crystallization. Water activity in honey can also affect the course of honey crystallization itself (Tabouret 1979).

Bhandari and Bareyre made use of measurements of water activity changes to determine the amount of crystalline glucose in glucose mixture (Bhandari and Bareyre 2003). They showed that after liquefaction of the crystalline phase added to saturated glucose water solution, there was a linear correlation between the drop of water activity and the increase of the mass fraction of the solid phase in the form of glucose monohydrate. The final conclusion of the paper is that the changes of water activity can be used to determine the amount of the solid phase that forms the crystalline suspension (Bhandari and Bareyre 2003). It should be noted, however, that their reasoning shows signs of some simplification as it fails to take into account the surface interactions that occur between the crystallized phase and the solution. The results obtained by Bhandari and Bareyre by introducing an external glucose monohydrate of strictly defined mass and the resultant drop of water activity after the liquefaction of the monohydrate by heating need not mean that there is an equally good reverse phenomenon, i.e. that there is a proportional

increase of water activity after the granulation of a definite amount of the solid phase from the solution. This follows from the fact that in honey suspensions in crystallized states there also exist surface reactions. The crystalline mass fraction is comparable to the water content. Glucose crystals are characterized by very small dimensions and, as a result, they possess very large contact surfaces. In one cubic millimetre of granulated honey it is possible to find as many as several hundred thousands of crystals whose thickness amounts to several hundred of nanometers (Bakier 2003).

Reports concerning the amount of solid phase in crystallized honey are relatively rare. This is due, among other things, to difficulties related to the determination of the concentration value of glucose saturation in honey (Tabouret, 1979). Research work carried out by this author also showed that glucose-fructose interactions have a significant influence on the characteristics of the crystalline structure *in statu nascendi* (Bakier 2006c). Mass fraction of the crystallized phase in honey has been estimated to be about 15% (Schley and Büskes-Schluz 1987). After the crystallization process, honey is characterized by considerable differences in the morphology of its crystalline structure. Depending on the origin and the conditions of the crystallization process, the same type of honey can be found both in larger grain size or fine grain form (Cavia *et al.* 2002).

The investigations presented in this paper were concerned with the influence of glucose on water activity in both liquid and granulated honey. The thesis adopted was that the increase of water activity caused by the crystallization process is the sum of the increment of the parameter resulting from the formation of a definite amount of crystallized phase and the drop of water activity resulting from the surface interactions between the solid and liquid phases. The dependence between the drop of water activity after liquefaction of the glucose monohydrate added to two different types of honey characterized by different glucose and fructose content was empirically verified. An attempt was also made to determine the influence of the added crystallized phase and its morphology on the water activity in the investigated honeys.

MATERIALS AND RESEARCH METHODOLOGY

In the investigations two types of honey showing substantial differences in their chemical composition were used. One of them was rape honey characterized by a high glucose content whereas the other, buckwheat honey, was rich in fructose. The difference in their chemical composition can be seen in their values of water activity. The average glucose content in the rape honey amounted to $G = 40.74\%$ and that of fructose to $F = 37.90\%$ (Devillers *et al.* 2004). In the buckwheat honey the values were $G = 29.5\%$ and $F = 35.3\%$ (Crone 1975). The

honeys investigated were in granulated state. The first step of the investigations was to determine water activity and water content in both honeys. Next, the honeys were liquefied by heating at 65°C. After that they were cooled and used to prepare samples. Ten different samples of each type of honey were prepared. Each sample contained different amounts of crystallized glucose monohydrate added to the honey. The samples were composed of 50 g of liquid honey containing varying amounts of glucose monohydrate ranging from 0 (the first sample) to 10 g (the last sample). The honey samples were then liquefied by heating and mixing at 65°C. Later the samples were cooled, and after 24 hours the water activity in the samples was determined. The measurements were carried out at controlled temperature of 25°C using AQUA LAB CX-2. The measurements of each sample were repeated three times. The measurements of water content in honey were made by the refractometric method by measuring light refraction index with an Abbe-Refraktometer (Zeiss, Jena).

Further, the influence of the crystallized phase on changes of water activity in two-phase honey was investigated. The glucose monohydrate produced by Sigma-Aldrich Laborchemikalien GmbH Germany was separated into two fractions by sifting it through a sieve with 0.25 mm mesh. Automatic measurements of the geometric dimensions of the crystals were carried out using crystal photographs taken in the condition of shearing interferometry by Biolar PI microinterferometer and an image analysis computer program *analySIS* (SIS 2003). The geometric analysis of crystal populations was based on crystal size distribution according to the maximum crystal diameter using both Excel and Statistica software (Statistica 2002). Water activity was measured not later than 20 minutes after the introduction of the crystal and mixing the liquid honey with weighed mass of one of the two fractions. The photographs taken by Biolar PI microinterferometer were also used to present the crystalline structure of the investigated honeys.

Additionally, the crystalline mass fraction was obtained by NIR spectroscopy. The results of earlier investigations made it possible to determine that the absorbance of water suspensions of crystalline glucose at the wave number of $\nu = 7500 \text{ cm}^{-1}$ is directly proportional to the mass fraction of glucose monohydrate (Bakier 2005). Analogous relations can be also seen between the absorbance of crystallized honey and the crystallized phase contained in the honey (Bakier 2006d). This makes it possible to perform a comparatively accurate measurement of the crystallized phase content in the investigated honeys.

MEASUREMENT RESULTS AND THEIR ANALYSIS

Table 1 presents the parameters of the honeys used in the investigations. In spite of the fact that the buckwheat honey contained less water than the rape honey, it showed higher values of water activity both in its liquid and crystallized

state. In effect, the increment of water activity in the buckwheat honey after crystallization is considerably higher than in the rape honey.

Figure 1 shows the measurement results of water activity depending on the mass fraction of the glucose monohydrate added to the solution. The measurements were concluded after the liquefaction of the crystal fractions by heating the honey and the added crystals at 65°C. In either honey there was a linear drop of water activity correlated with increase of the mass fraction of the glucose monohydrate added to the honey. The regression equations obtained are linear in nature and are characterized by high determination coefficient. The straight line for the buckwheat honey lies above that for rape honey.

Table 1. Investigated honey parameters

No.	Type of honey	Water content	Water activity in crystallized state	Water activity after liquefaction	Increment of water activity after crystallization
		W (%)	a_w^{cr}	a_w^f	$\Delta a_w = a_w^{cr} - a_w^f$
1	Rape honey	19.6	0.607	0.578	0.029
2	Buckwheat honey	19.2	0.629	0.582	0.047

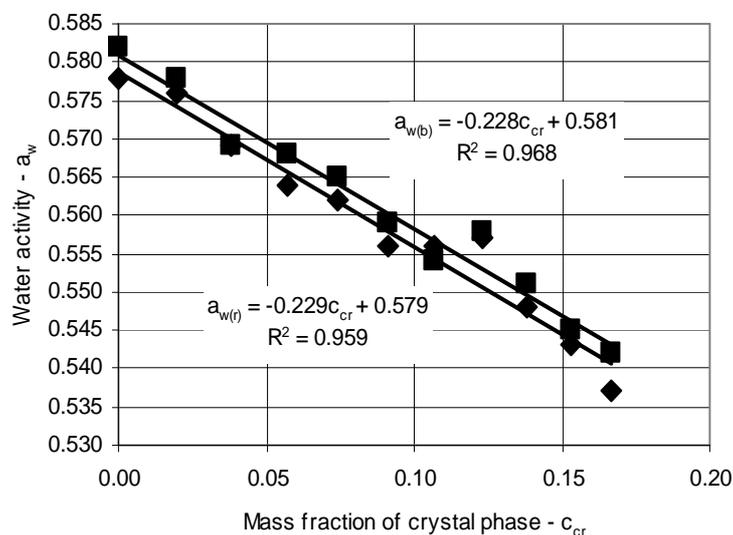


Fig. 1. Dependence of water activity on the mass fraction of the glucose monohydrate crystals additions after their liquefaction by heating: ♦ - rape honey, ■ - buckwheat honey

The above results make it possible to state categorically that an increase of the mass fraction of the liquefied glucose monohydrate is accompanied by a linear drop of water activity of the solution after its liquefaction. Since the liquefied crystals have an identical composition to the crystal phase *in statu nascendi*, it is possible to draw an analogical conclusion to that formulated by Bhandari and Bareyre who stated that there was an analogous increase of water activity which accompanied the formation of a definite crystal mass fraction in the honey crystallization process (Bhandari and Bareyre, 2003). However, this situation could be true only if there were no surface interactions between the honey crystals and the solution. Assuming the dependence to be valid and that the dependence between the drop of glucose concentration in the liquid phase determines the increase of water activity according to the equations presented in Fig. 1, then we can write as follows:

- for the rape honey
$$- a_w^{cr} = a_w^s = a_w^f + 0.229 \cdot \Delta c_{cr}'; \quad (1)$$

- for the buckwheat honey
$$- a_w^{cr} = a_w^s = a_w^f + 0.228 \cdot \Delta c_{cr}'. \quad (2)$$

Making use of the water activity values obtained for the investigated honeys in both crystallized and liquid states (Tab. 1), the concentration of the crystallized phase expressed by the mass fraction can be presented in the following form:

- for the rape honey
$$\Delta c_{cr}' = \frac{\Delta a_w}{0.229} = \frac{a_w^{cr} - a_w^f}{0.229} = \frac{0.029}{0.229} = 0.127 \quad (3)$$

- for the buckwheat honey
$$\Delta c_{cr}' = \frac{\Delta a_w}{0.228} = \frac{a_w^{cr} - a_w^f}{0.228} = \frac{0.047}{0.228} = 0.207 \quad (4)$$

Expressing the mass content of the solid phase in percentages we have 12.69% and 20.65% for the rape and buckwheat honey, respectively. The above values of mass content in the crystallized phase are both surprising and unacceptable. This follows from analysis of the chemical composition of honey. In the buckwheat honey the glucose mass content amounts merely to 29.5% whereas the fructose mass content reaches 35.3% (Crone 1975). It seems logical that product of higher glucose content should crystallize more monohydrate. Independent measurements of the solid phase content in the honeys, carried out by NIR spectroscopy, showed that the percentage content of the solid phase in the rape honey was 29.5% and the buckwheat honey 28.0% (Bakier, 2006d). It seems that the results obtained by NIR spectroscopy are more reliable and show that the amount of the solid phase determined by water activity measurements were considerably underestimated. In particular, the most

outstanding result was recorded for the rape honey where the difference was as high as 16.91%. It would be most interesting to explain, however, why the determination of the amount of the solid phase by measuring water activity in the solid state gives such low values for the solid phase. In an attempt to solve this problem a thesis was postulated that the morphology of the honey crystalline structure considerably affects the way in which water is bound in the product and also, as a result, affects water activity itself.

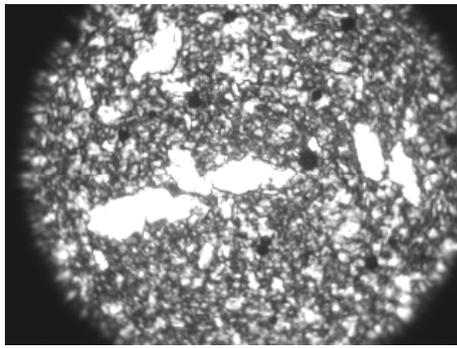


Photo. 1. Structure of rape honeys
magnification 110 x

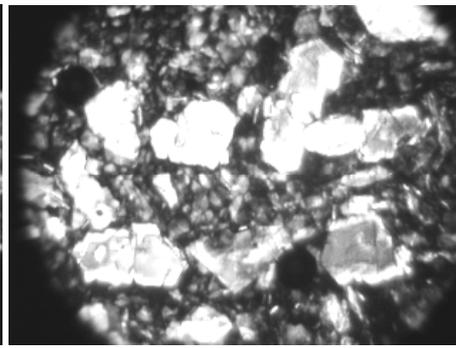


Photo. 2. Structure of buckwheat honeys
magnification 110 x

The investigated honeys show a very different crystalline structure. The morphology of rape honey grains is shown in Photo 1 and that of buckwheat grains in Photo 2. The particular crystals in the rape honey are very fine in their structure and their size is relatively uniform. As a result there is a very extensive contact area between the crystals and the liquid phase. The buckwheat honey, however, manifests coarse-grained structure dominated by large-size aggregates. The external surface of this type of structure is significantly smaller than in the rape honey. This phenomenon suggests that there exist stronger surface interactions between the crystallized and liquid phases in the rape honey, which considerably affects water activity in honey. Mathematical formalization of the proposed thesis will then state that the resultant water activity in the product after crystallization constitutes the difference between the activity resulting from the crystallization of the solid phase a_w^s and the decrease of the parameter caused by the surface interaction a_w^A :

$$a_w^{cr} = a_w^s - a_w^A. \quad (5)$$

For such an interpretation the dependencies (1) and (2) will not express the resultant water activity a_w^{cr} but an increment of water activity caused by the formation of the solid phase in the solution a_w^s . Assuming the mass content of the

solid phase determined by NIR spectroscopy to be equal to 29.6% in the rape honey, from dependence (1) we obtain $a_w^s = 0.646$. An analogous procedure carried out according to equation (2) for the buckwheat honey also gives the same value i.e. $a_w^s = 0.646$. Knowing the resultant increments of water activity of the analysed honeys in their crystallized states and making use of dependence (5), we can calculate the decrement of water activity caused by surface interactions:

- for the rape honey $a_w^A = 0.039$,
- for the buckwheat honey $a_w^A = 0.017$.

The results of the above analysis are analytical in nature and are conditioned by the data obtained from NIR spectroscopy measurements. The obtained values clearly show that the assumptions were correct, as we can observe a considerable influence of surface interactions between the solid and liquid phases on the increase of water activity in both rape and buckwheat honeys. The rape honey that is characterized by fine grain structure shows, as expected, much intensive surface interactions.

In order to conduct direct empirical verification of the effect of the interactions between the solid phase, i.e. glucose monohydrate crystals, and the liquid phase, i.e. the liquefied honey, an analysis was conducted to measure the changes of water activity after the introduction of the crystals into the honey. The glucose monohydrate crystals were separated into two distinct fractions using a sieve with mesh of 0.25 mm. The through grains (minus mesh) constituted the fine grained fraction whereas the plus mesh (the short) formed the coarse-grained fraction. The numerical distribution of the crystals according to the maximum diameter in the examined fractions for 100 randomly chosen crystals is shown in Figures 2 and 3. The influence of the crystal mass fraction on the changes of water activity is shown in Figures 4 and 5.

The results of water activity changes in the investigated honeys in the function of crystal mass fraction of both fractions could have been predicted except for the buckwheat honey. In this honey (Fig. 5) a decrease of water activity accompanied by the increase of crystal mass fraction can be observed. At the same time there is a greater drop of water activity after the introduction of finer grained fraction while there is a weaker decrease of the activity when coarser grain fractions are introduced. This can have a decisive influence on the water activity in honey crystal suspensions.

In the case of the rape honey water changes observed after the introduction of the solid phase seem to be quite surprising. After the introduction of fine-grained fraction there is an unexpected increase of water activity in all cases. An introduction of a small mass of coarse-grained fraction initially decreases water activity and above 5% increases water activity. However, such a behaviour of the rape honey can be explained in a relatively easy way by instant initiation of the crystallization process caused by the introduction of the crystalline phase.

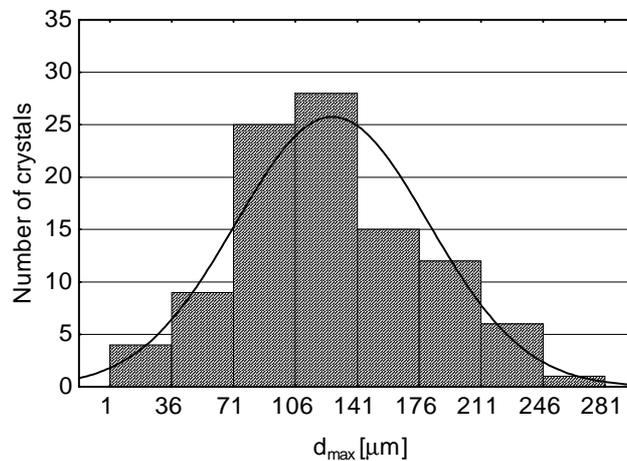


Fig. 2. Numerical distribution of fine-grained crystals according to the maximum diameter (minus mesh 0.25 mm)

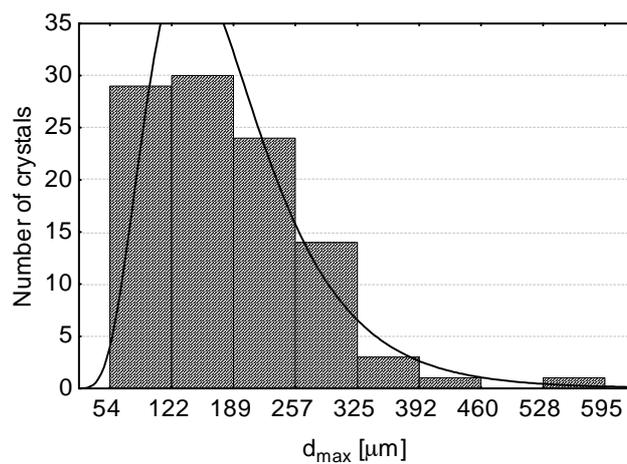


Fig. 3. Numerical distribution of coarse-grained crystals according to the maximum diameter (plus mesh 0.25 mm)

In honey processing technology this phenomenon is known as induced granulation and is used to start quick heterogeneous crystallization process (Bakier 2003, Cherife *et al.* 2006). For the rape honey, which shows a tendency for fast crystallization, the increase of water activity can be already noticed after 20 minutes since the introduction of crystals into the honey. Nevertheless it is still possible to observe differences in the interactions between finer and coarser grain

fractions. The finer crystals will start the crystallization process at considerably lower mass fraction than in the case of coarse-grained crystals.

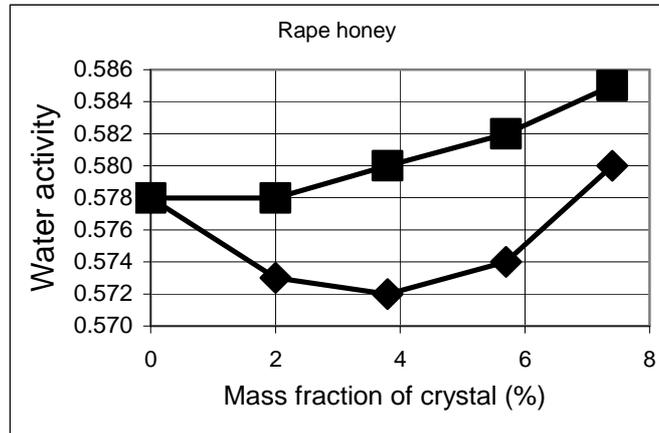


Fig. 4. Changes of water activity in rape honey after the introduction of: ■ – fine-grained crystals (minus mash), ◆ - coarse-grained crystals (plus mash)

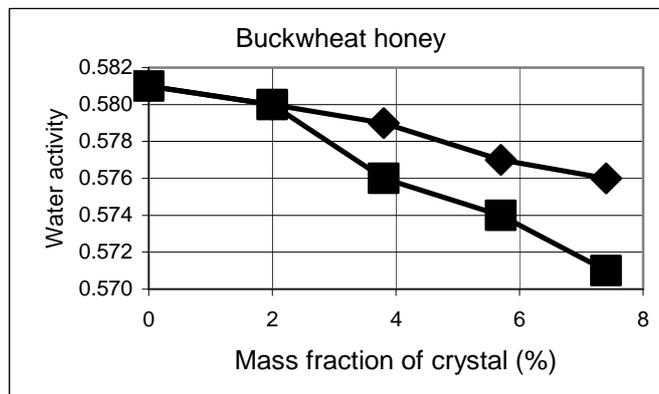


Fig. 5. Changes of water activity in buckwheat honey after the introduction of: ■ – fine-grained crystals (minus mash), ◆ - coarse-grained crystals (plus mash)

CONCLUSIONS

As a result of the analyses conducted, it is possible to conclude unequivocally that strong surface interactions between honey crystalline and liquid phases can be observed. In effect water is bound and there is a slower increase of water activity after the crystallization process than expected taking the amount of the solid phase produced. The surface interactions become the stronger the finer the crystalline

phase is, i.e. the more extensive is the contact area between the crystals and the solution. Making use of the NIR spectroscopy results, it was shown that the drop of water activity in the rape honey caused by surface interactions between the crystalline and liquid phases was $\Delta a_w^A = 0.039$ and was greater than the resultant increase of water activity following the crystallization - namely $\Delta a_w^{cr} = 0.029$. This is a very valuable item of information as it indicates that the increase of water activity caused by honey crystallization process may be considerably limited by controlling the process in such a way as to obtain fine-grained structures.

Direct measurements of the solid phase by measuring the increase of water activity after the crystallization show relatively big errors. The finer the crystallized structure of the honey, the greater the error is to be expected. In view of the above the approach proposed by Bhandari and Bareyre to determine the amount of crystallized phase in honey cannot be accepted (Bhandari and Bareyre 2003). Nevertheless it seems possible to determine the mass fraction of the solid phase in honey by measuring its water activity. It requires, however, taking into account the crystalline structure of the honey and, by the same token, the nature of surface interactions between the crystallized and liquid phases of honey. The results of the investigations presented in the paper should be considered only as an introduction to further analysis of the problem. In fact the results focus on the qualitative aspect of the observed dependencies but require further, more detailed quantitative analyses.

A relatively unexpected effect of the research appeared to be observation of the influence of grain size on the initiation of crystallization. Introduction of a finer grain fraction speeded up the crystallization process considerably, which was manifested by an increase of water activity. It seems that measurements of water activity of a given honey after the introduction of a standardized crystal will permit analysis of its crystallization propensity. However, it requires further investigations in the field in question.

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WPŁYW PRZEMIAN FAZOWYCH GLUKOZY NA ZMIANY AKTYWNOŚCI WODY W WYBRANYCH MIODACH

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Streszczenie. W pracy przedstawiono analizę wpływu udziału masowego glukozy na aktywność wody w dwóch wybranych miodach w stanie płynnym i skryształizowanym. Do badań zastosowano dwa miody odmianowe charakteryzujące się różną zawartością glukozy i fruktozy – rzepakowy i gryczany. Wyznaczono zależność pomiędzy spadkiem aktywności wody w tych miodach a dodatkiem masowym monohydratu glukozy po upłynnieniu. Posiłkując się wartościami udziału masowego fazy stałej w mediach w stanie skryształizowanym wyznaczonymi za pomocą spektroskopii w bliskiej podczerwieni, analizowano zmiany aktywności wody w miodzie po kryształizacji. Stwierdzono, że powstaniu fazy kryształicznej glukozy w miodzie towarzyszą oddziaływania powierzchniowe, które powodują obniżenie aktywności wody. Wypadkowy przyrost aktywności wody po kryształizacji – a_w^{cr} , przedstawiono w postaci różnicy pomiędzy przyrostem a_w^s – spowodowanym wydzieleniem fazy stałej i oddziaływaniami powierzchniowymi a_w^A . Wpływ morfologii fazy kryształicznej na oddziaływania powierzchniowe zweryfikowano doświadczalnie poprzez badanie zmiany aktywności wody po wprowadzeniu do miodu dwóch frakcji monohydratu glukozy różniących się wielkością kryształów.

Słowa kluczowe: miód, stan skryształizowany, aktywność wody, glukoza, monohydrat glukozy