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Water activity and water content in Slovenian honeys

Helena Abramovič *, Mojca Jamnik, Lina Burkan, Milica Kač

Biotechnical Faculty, University of Ljubljana, SI-1111 Ljubljana, Slovenia

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Abstract

In the present study water activity (a_w) and water content (w) in 150 samples of Slovenian honeys were determined. The water content in honeydew honeys ranged from 13.4% to 18.0% and in flower honeys the water content was between 14.0% and 18.6%. The water activity in honeydew honeys varied from 0.483 to 0.591, and in flower honeys the water activity was in the range from 0.479 to 0.557. A statistically significant linear correlation between a_w and the water content of honeys was found. In honeydew honeys the water activity at the same water content was higher than in flower honeys. The change of physical state from crystallized to liquid has been found to influence the water activity. Crystallized samples showed a higher water activity compared to the corresponding value for the same honeys after liquefaction. The difference in water activity between liquefied and crystallized honeys is higher in flower honeys than in honeydew honeys.

Keywords: Honey; Water activity; Water content

1. Introduction

Water content is a very important quality parameter for practically every food product, as well as for its ingredients. It has a very important if not decisive influence on quality and especially on the shelf life of practically every material of biotic origin. Speaking of quality control often implies comments and discussion on the water content and it is important to be aware that it is much better and far more meaningful to speak of water activity (a_w) and not just of water content. Honey is no exception to this general rule.

Regulations dealing with food quality usually refer to water content. Official publications for honey declare that it should contain no more than 20% of water (Council Directive 2001/110/EC, 2002; Regulations about honey, 2004). There are some exceptions to the rule, but nevertheless the values are agreed upon (Regulations about honey, 2004). Though water content, at least at first glance, seems a more simple and better defined quantity compared to water activity, we should be aware of the fact that water activity is more related to quality problems (stability, viscosity and crystallization of honey). Additionally, one has to consider the fact that the official methods of measuring the water content are based on refractometric measurements (Regulations about honey, 2004) and as such often not directly applicable to crystallized honey. The advantage of being very well defined and very easily understandable made the water content the parameter of choice against water activity, which is linked to the so-called "disposable" or "available" water.

In various regions of Slovenia botanically different honeys of distinctly different origin are produced, so it is evident that defining good, reliable and meaningful quality parameters for this important natural product is professionally challenging.

Taking all this into consideration, the suggestion to replace the refractometric method by a simple measurement of water activity seems both logical as well as promising. Several approaches have been performed in this direction and as a result various models have been proposed to correlate water content to water activity (Cavia,

* Corresponding author.
E-mail address: helena.abramovic@bf.uni-lj.si (H. Abramovič).

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Fernández-Muiño, Huidobro, & Sancho, 2004; Chirife, Zamora, & Motto, 2006; Salamanca, Pérez, & Serra, 2001; Schroeder, Horn, & Pieper, 2005; Zamora, Chirife, & Roldán, 2006). The present work is – to the best of our knowledge – the first such attempt for Slovenian honeys and it deals also with some limitations concerning crystallization and the general applicability of such models.

The object of the following study was to determine water activity and water content in 150 samples of Slovenian honeys and create a statistically significant correlation between these two parameters. The influence of preheating of honey, i.e. the change of physical state from crystallized to liquid, on water content as well as on water activity has been studied and discussed in terms of sugar composition and different botanical origin.

2. Materials and methods

2.1. Materials

2.1.1. Honey

In this study 150 honey samples belonging to two different honey types were analysed. Among the investigated samples 75 were honeydew honeys (37 samples were produced in the year 2004, 38 samples in the year 2005) and 75 were flower honeys (38 samples were produced in the year 2004, 37 samples in the year 2005). Honey samples were obtained from bee keepers in four different geographical areas of Slovenia.

According to their physical state, i.e. texture, honey samples corresponded to one of the following types: clear liquid honey, solidly granulated or crystallized honey, partially crystallized honey, i.e. a mixture of liquid honey and crystallized honey.

2.1.2. Determination of water content

Water content was determined by measuring refractive index at room temperature with an ATAGO HHR-2N Atago refractometer (Atago Co., LTD), provided with a temperature correction scale to compensate when the sample temperature was other than 20 °C, according to Official Method 969.38 (AOAC, 1999). As refractive index determination is possible only in clear liquid honeys, the samples were preheated to liquefy in an oven at 45 °C in sealed containers and cooled down to room temperature. Water content was expressed as mass percentage. Determinations were done twice and the average was used. The standard deviation for each determination (as determined by tenfold replicates on representative samples) was less than 0.1% on the w/w scale, which means about 0.5% of the measured value.

2.1.3. Determination of water activity

The water activity was determined at 25 °C using a CX–1 chilled-mirror dew-point water activity meter, Campbell Scientific, Ltd. (CX–1 water activity system: Instruction manual: Version 1/3.88, 1988). The equipment was calibrated with saturated salt solutions ($K_2Cr_2O_7$, NaCl, NH_4NO_3). Saturated salt solutions gave the declared a_w values that were obtained from Robinson and Stokes (1959). For each determination two or three replicates were obtained and the average was used. The standard deviation for each a_w determination of honey samples was less than 0.001, as determined by tenfold replicates on representative samples.

2.1.4. Determination of glucose, fructose and saccharose contents

Glucose, fructose and saccharose were determined by the use of high pressure liquid chromatography (HPLC) with refractive index detector according to Bugner and Feinberg (1992) and Official Method 977.20 (AOAC, 1999). The contents of sugars were expressed as mass percentage.

3. Results and discussion

Water content determination based on refractive index measurement is possible only in clear liquid honeys. Since the honey samples used in our study were of different physical form (liquid, crystallized or granulated) they were preheated to liquefy before measurement. We have assumed that no water loss occurred during preheating. To support this assumption, the water content determined in unheated clear liquid honey samples was compared to the water content determined in the same honeys after they have been preheated. In unheated honeys the mean value of water content was 15.9%, while in preheated honeys the mean value of water content was 15.7%. The distribution of water content for unheated honeys is comparable to that of preheated liquid honeys (histograms not shown). The result of the χ^2 test was $\chi^2_{calculated} = 4.3$. That is considerably below 9.49 which is the tabled value for χ^2 at $p = 0.05$ (Samuels & Witmer, 1999).

In Fig. 1 the water activity of flower and honeydew honeys of different water content is presented. All honey samples investigated were preheated before a_w measurements

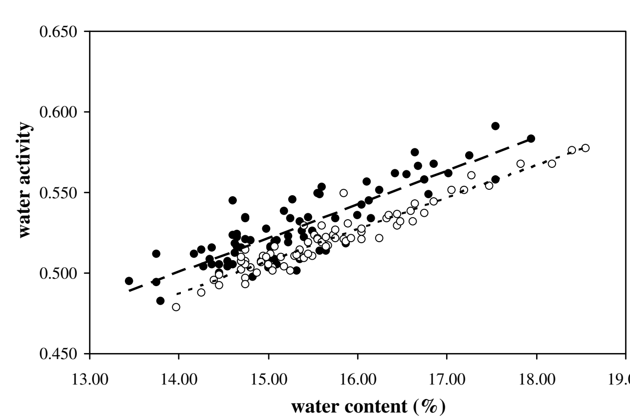

Fig. 1. Dependence of water activity of flower and honeydew honeys on water content (○, flower honeys; ●, honeydew honeys).

and water content determination, regardless of their physical state (liquid or crystallized). The water content in honeydew honeys ($N = 75$) ranged from 13.4% to 18.0% and in flower honeys ($N = 75$) the water content was in the range from 14.0% to 18.6%. These values showed that the investigated honeys complied with the European regulations (Council Directive 2001/110/EC of 20 December 2001 relating to honey., 2002) and national regulations on honey (Regulations about honey, 2004).

The crucial factor that determines enzyme activity and the growth as well as survival of micro-organisms in honey, is water activity. Water activity in honeydew honeys was from 0.483 to 0.591, with a mean value of 0.528. In flower honeys the water activity was in the range from 0.479 to 0.557, the mean value was 0.521. These values agree with previously reported data for water activity (Cavia et al., 2004; Gleiter, Horn, & Isengard, 2006; Zamora & Chirife, 2006). Since osmophilic yeasts are only able to grow above minimal water activity of 0.6, the honeys under investigation should be regarded as safe with respect to fermentation.

In Fig. 1 we can see that a linear relationship exists between a_w and water content. In general, in honeydew honeys water activity at the same water content is higher than in flower honeys. The same observation has been found in an investigation performed by Gleiter et al. (2006). The water activity is mainly determined by the presence of soluble chemical species. In honey, sugars represent the largest portion of the latter. Among them fructose and glucose are predominant (Zamora et al., 2006). Small amounts of disaccharides (mainly saccharose and maltose) and oligosaccharides are also present (Mendes, Brojo Proenca, Ferreira, & Ferreira, 1998). The water activity is largely determined by the presence of monosaccharides while disaccharides have a smaller influence on water activity (Zamora et al., 2006). The contribution of higher saccharides and other compounds is not significant. According to data for sugar composition from literature sources (Mateo & Bosch-Reig, 1998) the honeydew honeys contain less monosaccharides than flower honeys. The same was confirmed in the present investigation where in representative honeydew honeys ($N = 8$) and flower honeys ($N = 8$) the contents of glucose, fructose and saccharose have been determined. The content of glucose in honeydew honeys was 22–29% and in flower honeys 24–32%, while the amount of fructose in honeydew honeys was in the range 29–42% and in flower honeys 36–44%. Saccharose in honeydew honeys as well as in flower honeys amounted to 1–2%.

To calculate water activity (a_w) from the water content (w) several investigators have proposed the following relation:

$$a_w = a + b \cdot w \quad (1)$$

where a and b are the regression parameters. The values of parameters a and b with the coefficient of correlation (r) which is a measure of the goodness of fit, obtained by linear regression analysis are presented in Table 1. For all honeys ($N = 150$) investigated in this study the values of the parameters were: $a = 0.23 \pm 0.02$, $b = 0.019 \pm 0.001$ and r was found to be 0.843.

Table 1
Comparison between correlations for Slovenian honeys and those found by Ruegg and Blanc (1981), Salamanca et al. (2001), Cavia et al. (2004), Beckh et al. (2004) and Chirife et al. (2006)

Source of data	a[a]	b[a]	r[a]
Ruegg and Blanc (1981)	0.271	0.0177	0.901
Salamanca et al. (2001)	0.248	0.0175	0.973
Cavia et al. (2004)	0.267	0.0196	0.891
Beckh et al. (2004)	0.342	0.014	0.727
Chirife et al. (2006)	0.267	0.0177	0.984
Present study	0.23 ± 0.02	0.019 ± 0.001	0.843

[a] Values given as in references cited, regardless of their uncertainty.

The correlation between a_w and the water content of the investigated honeys was compared with those found by other researchers (Beckh, Wessel, & Lüllmann, 2004; Cavia et al., 2004; Chirife et al., 2006; Ruegg & Blanc, 1981; Salamanca et al., 2001) who determined water content in honey by measurement of refractive index. All of them obtained a linear dependence of a_w on water content and proposed Eq. (1). Table 1 summarizes the values of the parameters in Eq. (1) obtained in these investigations. As we can see in Table 1 the regression parameters are slightly different. The reasons for these differences could be attributed to differences in sugar composition and to various techniques used for a_w determination.

Also the influence of preheating on water activity was investigated. The distribution of a_w values for unheated liquid honeys is comparable to that for preheated liquid honeys (histograms not shown). This was also confirmed by the χ^2 test: $\chi^2_{\text{calculated}} = 2.8$ which is considerably below 9.49 (tabled value at $p = 0.05$). But on contrary, for crystallized honeys the distribution of a_w values is shifted to lower values after preheating. The results of χ^2 test were: $\chi^2_{\text{calculated}} = 65.1$, which is considerably above 9.49 (tabled value at $p = 0.05$). Considering these observations we can conclude that not preheating *per se* but the change of physical state from crystallized to liquid influences the change of a_w. Ruegg and Blanc (1981), Zamora and Chirife (2006) and Schroeder et al. (2005) studied the change in water activity due to liquefaction in honeys and confirmed a decrease in water activity of honeys in liquefied samples as compared to the crystallized ones. Considering that glucose is less water soluble than fructose, honey's glucose is the main cause for honey crystallization. In liquid honey all five –OH groups of glucose interact with water molecules. During the crystallization process of honey, glucose crystallizes by forming glucose monohydrate and other water molecules are set free. Furthermore, this crystallization lowers the solute concentration in the liquid phase and thus increases the water activity.

In Fig. 2 the dependence of a_w for unheated crystallized flower honeys on water content in comparison to a_w of liquefied flower honeys ($N = 41$) is presented. As expected crystallized samples show higher water activities than

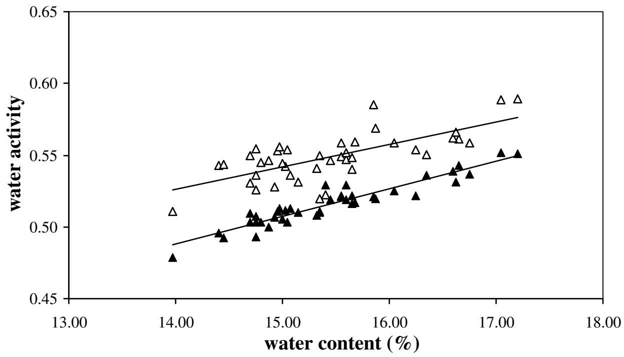

Fig. 2. Dependence of water activity for unheated crystallized flower honeys on water content compared with water activity of preheated crystallized flower honeys (△ crystallized; ▲ liquefied).

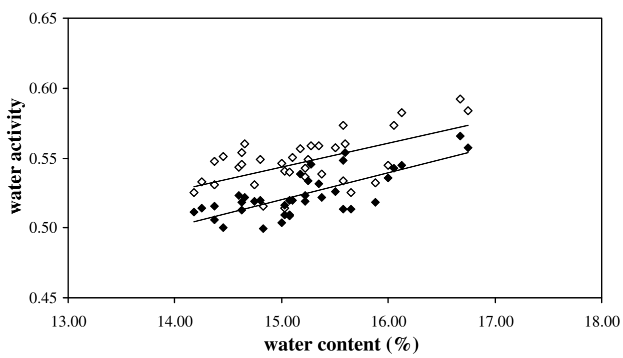

Fig. 3. Dependence of water activity for unheated crystallized honeydew honeys on water content compared with water activity of preheated crystallized honeydew honeys (◇ crystallized; ◆ liquefied).

liquefied flower honeys having the same water content. In unheated flower honeys the mean value of a_w amounted to 0.549, while in liquefied flower honeys the mean value of a_w was 0.516. In Fig. 3 water activity values for unheated crystallized honeydew honeys are compared to a_w of liquefied honeydew honeys ($N = 36$). It can be seen that, similar to flower honeys, water activities of crystallized honeydew samples (the mean value of a_w amounted to 0.547) are higher than those in liquefied honeys (the mean value of a_w was 0.524). Water content in this part of investigation was certainly measured after preheating. As this procedure leads to a slight water loss one could assume that this effect may contribute to the effects described in Figs. 2 and 3. The difference in a_w that should arise solely from water loss during preheating (the difference between the mean values for water content in unheated and heated honey being 0.2%, calculated as follows: $b \cdot \Delta w = 0.019 \quad (\%)^{-1} \cdot 0.2\% = 0.0038$) is much lower than the difference in a_w between preheated and unheated honey (this being 0.033 for flower or 0.023 for honeydew honey).

The difference in water activity between liquefied and unheated crystallized honeys is higher in flower honeys than in honeydew samples. Also Schroeder et al. (2005) observed that the increase of the water activity in flower honeys during crystallization is greater than in honeydew honeys. The influence of honey type (flower vs. honeydew honey) on the difference of water activity between crystallized honey samples and the same samples in liquid form can be explained by the different monosaccharides/oligosaccharides ratio and by the different content of glucose in honeydew and in flower honeys. In general, flower honeys contain more glucose, more monosaccharides and less oligosaccharides than honeydew honeys (Mateo & Bosch-Reig, 1998). Taking into consideration that the decrease in a_w is mainly the consequence of binding of water molecules to the molecules of glucose during liquefaction of crystallized honey, the effect should be most expressed in a system with a relatively higher amount of this monosaccharide.

4. Conclusions

A statistically significant linear correlation between water activity and water content of Slovenian honeys was found. In honeydew honeys water activity at the same water content was higher than in flower honeys. The change of physical state from crystallized to liquid has been found to influence the water activity. Crystallized samples showed higher water activity than liquefied honeys. The difference in water activity between liquefied and crystallized honeys was higher in flower honeys than in honeydew samples. During preheating of honeys only slight water loss occurred.

One could summarize that the water content/water activity relationship, i.e. the linear model to transform one into the other can be considered reliable, but for better accuracy as well as precision, care should be taken that the samples used in establishing the model match the samples analyzed in as many aspects as possible (type of honey, preparation of the sample, botanical characteristics, provenience and the like). Differences between the models can possibly be traced back to such inconsistencies.

Exchanging one parameter (the well established water content) by the other (the more simply obtainable water activity) looks promising, but a universally acceptable formula to compare "the old and the new" values is very probably not obtainable and will ultimately not bring much practical advantage. Considering water activity as an additional parameter seems to be a far better option. A very simple measurement on a relatively inexpensive and easily operated instrument gives valuable additional information about the quality of the sample.

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