OFFICIAL vs. ADVANCED PHYSICO-CHEMICAL ANALYTICAL METHODS IN FOOD SCIENCE: CASE STUDY ON WATER CONTENT vs. WATER ACTIVITY IN HONEY

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Abstract
On the case study of Slovenian honey the classical refractometric method for water content determination (the official method) is compared to the method of determination of water activity (a practical but not official method). Special attention is given to calculation of one parameter to the other, to the uncertainties about using various models (calibration curves), to the demands about choosing representative samples and to the importance of dealing with a large enough and transparent data base. It gives the reasons for using water activity instead of water content as a quality parameter of honey. It also represents a rather summarized report on treatment of a large and well documented data base on Slovenian honeys, which gives a good quantitative argumentation for the use of the general model as well as (botanically) specific models in finding correlations between water content and water activity.
Key words: honeys, Slovenian honeys, water activity, water content, analytical methods

URADNE ALI MODERNE FIZIKALNO KEMIJSKE ANALIZNE METODE V ŽIVILSTVU: PRIMER VSEBNOSTI VODE IN AKTIVNOSTI VODE V MEDU

Izvleček
Na primeru analize slovenskega medu obravnava prispevek primerjavo klasične refraktometrične metode vsebnosti vode (uradna metoda) z metodo določanja aktivnosti vode (praktična, a neuradna metoda). Posebno pozorno govori o možnostih pretvorbe enega parametra v drugega: o negotovostih pri uporabi modelov (umeritvenih krivulj), o zahtevah pri izbiri reprezentančnih vzorcev in o pomembnosti dovolj obsežne in pregledne podatkovne baze. Utemeljuje smiselnost uporabe aktivnosti vode namesto vsebnosti vode kot parametra pri opisovanju kvalitete medu. Dodatno kratka predstavitev obdelave primerno velike in dobro dokumentirane podatkovne baze o vzorcih slovenskega medu

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kvantitativno utemelji uporabo tako splošnega kot tudi (sortno) specifičnih modelov za povezovanje vsebnosti vode in aktivnosti vode.
Ključne besede: med, slovenski med, vsebnost vode, aktivnost vode, analizne metode

1 INTRODUCTION

The official methods cited and recited in various regulations dealing with a great majority of products (and food is no exception to this general rule) often look obsolete, time consuming, not modern…. Practically for every one of them we have at least some if not a handful of alternatives, which give quicker and better results, which are more meaningful and often the procedures involved are much easier to perform. "Easier" meaning: asking for less time, less man power, not always less money though, but the results are more accurate, more reproducible…. Who has not heard of this chemical paradise?

But an official method has to be universally applicable. Everyone needing a certificate should be in a situation to get one, which means somehow also being able to pay for it.

Further on, the values which make a sample acceptable or not acceptable have to rely on a huge data base of previous measurements performed on representative samples (and such a data base is as a rule not available for recent methods).

And finally, if such a data base is not available, at least there should be an alternative solution, meaning that there should be a simple and straightforward way to calculate one values from the others and *vice versa*, which brings us back to the old and reliable calibration curve and to our case study, namely to water content vs. water activity in honey.

Water content is a very important quality parameter for honey as for practically every food product as well as for its ingredients. It is relevant for the quality as such and very explicitly also for the shelf life of every material of biotic origin. Quality control often implies dealing with water content (e. g.: honey should not contain more than 20 % of water) but it is important to be aware that it is far more meaningful to speak of water activity (a_w) and not of water content (w). Honey is no exception to this general rule, though also here the water activity concept is still a little strange and regarded as a (often not necessary) novelty. Though water content seems more simple and better defined quality compared to water activity, the latter is more related to quality problems (stability, viscosity and crystallization of honey). Additionally, one has to consider that the official methods of measuring water content are based on refractometric measurements (Regulations about honey, 2004) and as such often not directly applicable to crystallized honey. Water activity, the so called "disposable" or "available" water is professionally more to the point compared to water content, though the latter seems more practical and more comprehensible.

Replacing the refractometric method by a simple measurement of water activity seems both logical and easy, the first step being establishing a good correlation between the results of both methods and several models (in this case linear correlation between one and the other parameter) have been proposed for different groups of honey (Abramovič et al., 2008; Cavia et al., 2004; Chirife et al., 2006; Salamanca et al., 2001; Schroeder et al., 2005; Zamora et al., 2006). This presentation tests the model developed for Slovenian honey (Abramovič et al., 2008) against a new, much larger data set.

2 MATERIALS AND METHODS

2.1 Honey samples included in this study

398 honey samples of Slovenian origin were analysed, they originated from all over Slovenia and were collected and kept on the Biotechnical faculty in Ljubljana for various research purposes. The group included 81 samples originating in 2007, 282 in 2006, 33 were older and two originated from an unknown year. They were all kept in tightly closed plastic containers at room temperature, the analyses took place in 2008. Each honey sample belonged to one of the following three categories: clear liquid honey, solidly granulated or crystallized honey or partially crystallized honey (i. e. a mixture of the first two categories).

2.2 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 ºC. This analysis is according to Official Method 969.38 (AOAC, 1999). Good refractive index determination is possible only in clear liquid honeys, so the samples were preheated in an oven in sealed containers at 45 ºC to liquefy and then cooled down to room temperature. The assumption that no water loss occurred during preheating was verified in our earlier study (Abramovič et al., 2008). The standard deviation for each determination was less than 0.1 % on the w/w scale, which means about 0.5 % of the measured value (determined by tenfold replicates on representative samples).

2.3 Determination of water activity

The water activity was determined at 25 ºC using a CX-1 chille-mirror dew point water activity meter, Campell scientific, Ltd. (CX-1 water activity system: Instruction manual: Version 1/3.88, 1988). Calibration was done according to the manual (Abramovič et al., 2008). The standard deviation for each water activity determination of honey samples was less than 0.001 (determined by tenfold replicates on representative samples).

3 RESULTS AND DISCUSSION

Figure 1 shows the linear correlation between water activity and water content for 363 representative samples of Slovenian honey (Nježič, 2008). This representative group consists of 81 samples of the year 2007 and 282 samples of the year 2006. The least squares method gave the following equation as the best possible model:

$$a_w = (0{,}23 \pm 0{,}01) + (0{,}019 \pm 0{,}001) \cdot w \qquad R = 0{,}831 \qquad (1)$$

The equation obtained (eq. 1) is identical to equation 2 which was obtained in the same way for 150 Slovenian honey samples (75 for the year 2004 and 75 for the year 2005) in 2006 (Abramovič et al., 2008), namely:

$$a_w = (0{,}23 \pm 0{,}02) + (0{,}019 \pm 0{,}001) \cdot w \qquad R = 0{,}843 \qquad (2)$$

This confirms the minimal influence of the crop year as well as the fact that the samples are really representative.
Further on 23 three subgroups of the Slovenian honey samples under investigation were formed and for each of them a linear fit such as equations 1 and 2 was calculated (Nježič, 2008). Influences of botanical origin of honey, its geographical origin as well as that of the crop year were observed. Some of these equations are given below. Without going into details, which is beyond the scope of this publication, we can conclude that the impact of the botanical origin of the honey is most pronounced, that of the geographical origin is somewhat smaller, while that of the crop year is of no importance. Some figures, though, especially when we are talking of groups of samples of very different sizes or those containing considerable portions of some botanically special honeys (the impact of the botanical origin was mentioned above!) may suggest some additional differences. For the considerations discussed here, one has to emphasize, that the group used to make the model (i. e. to establish the correlation) should, generally speaking, first be large enough and second: match the samples for which it will be used as much as possible. To test the robustness of the model a minor number (about 10 % of the total) of results obtained in our previous studies (Abramovič et al., 2008) was also considered. Table 1 gives 17 of these 23 groups to illustrate some important concepts of the correlations mentioned.

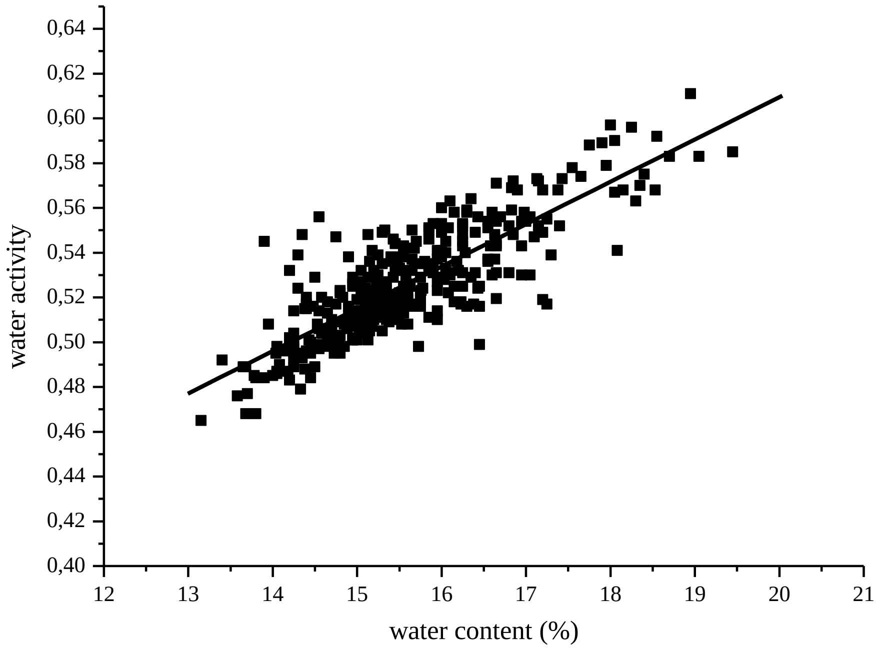

Figure 1: Water activity vs. water content for the 363 samples of Slovenian honey (years 2006 and 2007).
Slika 1: Odvisnost aktivnosti vode od vsebnosti vode za 363 vzorcev slovenskega medu letnikov 2006 in 2007.

Finally, let us consider some published models dealing with correlation between water activity and water content. They are presented in Table 2. It should be noticed that they were developed for honeys of very different botanical and geographical origin and that in the first eight models the coefficients in the equation are given without any errors of estimation. For some not even the number of samples included (N) is given, so comparison is anything but straightforward.

Table 1: Some representative linear fits between water content and water activity for various (sub)groups of the honey samples under investigation.
Preglednica 1: Nekatere reprezentativne linearne povezave med vsebnostjo vode in aktivnostjo vode za zanimive podskupine vzorcev medu.

Group analyzed	Equation of the linear fit $a_w = f(w)$	R	N
Slovenian honeys, 2006 and 2007[1]	$a_w = (0.23 \pm 0.01) + (0.019 \pm 0.001) \cdot w$	0.831	363
Slovenian honeys, 2006[1]	$a_w = (0.21 \pm 0.01) + (0.020 \pm 0.001) \cdot w$	0.880	282
Slovenian honeys, 2007[2]	$a_w = (0.30 \pm 0.03) + (0.014 \pm 0.002) \cdot w$	0.698	81
Slovenian honeys, 2007[3]	$a_w = (0.20 \pm 0.03) + (0.021 \pm 0.002) \cdot w$	0.802	54
Floral honeys, 2006	$a_w = (0.22 \pm 0.01) + (0.019 \pm 0.001) \cdot w$	0.958	81
Floral honeys 2007[4]	$a_w = (0.19 \pm 0.04) + (0.021 \pm 0.003) \cdot w$	0.933	12
Floral honeys, 2006 and 2007	$a_w = (0.22 \pm 0.01) + (0.020 \pm 0.001) \cdot w$	0.957	93
Honeydew honeys, 2006	$a_w = (0.18 \pm 0.02) + (0.023 \pm 0.001) \cdot w$	0.902	102
Honeydew honeys , 2007[5]	$a_w = (0.24 \pm 0.06) + (0.019 \pm 0.004) \cdot w$	0.749	20
Honeydew honeys, 2006 and 2007	$a_w = (0.18 \pm 0.02) + (0.023 \pm 0.001) \cdot w$	0.888	122
Chestnut honeys	$a_w = (0.13 \pm 0.03) + (0.025 \pm 0.002) \cdot w$	0.930	28
Lime honeys	$a_w = (0.19 \pm 0.01) + (0.022 \pm 0.001) \cdot w$	0.986	20
Oilseed rape honeys	$a_w = (0.24 \pm 0.04) + (0.017 \pm 0.003) \cdot w$	0.781	27
Honeys from Alpine macroregion	$a_w = (0.22 \pm 0.01) + (0.020 \pm 0.001) \cdot w$	0.904	144
Honeys from Dinaric macroregion	$a_w = (0.20 \pm 0.02) + (0.021 \pm 0.001) \cdot w$	0.808	138
Honeys from Panonic macroregion	$a_w = (0.24 \pm 0.02) + (0.017 \pm 0.001) \cdot w$	0.867	80
H. from Mediterranean macroregion	$a_w = (0.22 \pm 0.03) + (0.019 \pm 0.002) \cdot w$	0.894	29

[1]among the samples there are no oilseed rape honeys
[2]27 samples oilseed rape honeys included
[3]27 samples oilseed rape honeys excluded
[4]a very small group, the model gives good values for the interval used
[5]a small group, the model gives good values for the interval used

Table 2: Some published models for correlation between water activity and water content for honey (Abramovič et al., 2008; Burkan, 2006; Cavia et al., 2004; Chirife et al., 2006; Zamora et al., 2006).
Preglednica 2: Pregled objavljenih regresijskih modelov povezave med aktivnostjo vode in vsebnostjo vode za med (Abramovič in sod., 2008; Burkan, 2006; Cavia in sod., 2004; Chirife in sod., 2006; Zamora in sod., 2006)

Equation of the linear fit	**R**	**Reference**	**Commentary**	**N**
$y = 0{,}014 \cdot x + 0{,}342$	0,727	Beckh *et al*., 2004	Samples of different botanical origin, partly crystallized	128
$y = 0{,}0177 \cdot x + 0{,}271$	0,901	Ruegg in Blanc, 1981	Liquid samples from different countries,	/
$y = 0{,}0177 \cdot x + 0{,}267$	0,985	Chirife *et al*., 2006	Liquid samples from Argentina	36
$y = 0{,}0175 \cdot x + 0{,}248$	0,973	Salamanca *et al*., 2001	Samples from Columbia	/
$y = 0{,}01955 \cdot x + 0{,}2674$	0,9438	Cavia *et al*., 2004	All the samples (see also the following three lines)	90
$y = 0{,}02147 \cdot x + 0{,}2393$	0,9872	Cavia *et al*., 2004	Non preheated samples, 1996, Burgos (continental climate)	30
$y = 0{,}02362 \cdot x + 0{,}2060$	0,9652	Cavia *et al*., 2004	Non preheated samples, 1998, Burgos (continental climate)	35
$y = 0{,}01476 \cdot x + 0{,}3375$	0,8933	Cavia *et al*., 2004	Non preheated samples, 1998 (oceanic climate)	25
$y = (0{,}019 \pm 0{,}002) \cdot x + (0{,}23 \pm 0{,}02)$	0,843	Abramovič in sod., 2008	Slovenian floral and honeydew honey, 2004 and 2005	150
$y = (0{,}021 \pm 0{,}002) \cdot x + (0{,}21 \pm 0{,}02)$	0,859	Burkan, 2006	Slovenian honeydew honey, 2004 and 2005	75
$y = (0{,}023 \pm 0{,}002) \cdot x + (0{,}18 \pm 0{,}03)$	0,870	Burkan, 2006	Slovenian honeydew honey, 2004	37
$y = (0{,}019 \pm 0{,}002) \cdot x + (0{,}23 \pm 0{,}03)$	0,869	Burkan, 2006	Slovenian honeydew honey, 2005	38
$y = (0{,}0199 \pm 0{,}0007) \cdot x + (0{,}21 \pm 0{,}01)$	0,956	Burkan, 2006	Slovenian floral honey, 2004 and 2005	75
$y = (0{,}020 \pm 0{,}001) \cdot x + (0{,}21 \pm 0{,}02)$	0,951	Burkan, 2006	Slovenian floral honey, 2004	38
$y = (0{,}021 \pm 0{,}001) \cdot x + (0{,}20 \pm 0{,}02)$	0,966	Burkan, 2006	Slovenian floral honey, 2005	37

Nadaljevanje preglednice s prejšnje strani.

Equation of the linear fit	R	Reference	Commentary	N
$y = (0{,}021 \pm 0{,}002) \cdot x + {} + (0{,}21 \pm 0{,}03)$	0,826	Burkan, 2006	Slovenian honey, 2004	75
$y = (0{,}018 \pm 0{,}001) \cdot x + {} + (0{,}24 \pm 0{,}02)$	0,873	Burkan, 2006	Slovenian honey, 2005	75

4 CONCLUSIONS

It can be supposed that impurities (e.g. plant particles) in honey as well as its colour influence the result of the refractometric water content measurement considerably. Therefore the results for some samples can be of questionable accuracy. The same is also true for (partly) crystallized samples, which can make the measurement impossible, though such crystallization may also escape unnoticed and in this way it becomes even more misleading. Floral, acacia and lime honey contain less impurities compared to honeydew and oilseed rape honey. The latter is also very prone to crystallization and some turbidity remains even after it has been preheated.
Further on we can summarize that:
Compared to water content water activity is easier to measure and more directly linked to the quality of honey.
Crystallization of honey leads to significantly increased values of water activity (because of the crystal formation more water becomes “disposable” or “available”; i. e. reaction prone and one can face quality problems sooner).
Correlation between water activity and water content is linear and significant, but the (dis)similarities between the samples used to make any model to predict water activity from water content and *vice versa* are crucial.
Water activity should become a standard quality parameter when describing honey.

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