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Determination of the diastase activity in honeys

Oznaczanie aktywności diastatycznej miodów

Abstract

The diastatic number in five samples of honey has been determined by two methods: according to guidelines included in Polish standard PN-88/A-77626 and the Phadebas method. Research shows that the storage of honeys did not have a significant impact on lowering of diastatic number. However, long-term storage of honey was characterized a reduction of the diastase number. The correlation between the two applied methods was very strong ($r = 0.921$).

Keywords: diastase number, honey

Streszczenie

Oznaczono liczbę diastazowa w pięciu miodach metodą rekomendowaną w normie PN-88/A-77626 i według metody Phadebas. Badania wskazują, że warunki przechowywania miodów nie miały większego wpływu na obniżenie liczy diastazowej. Jednak przechowywanie miodów przez dłuższy czas powoduje obniżenie wartości liczby diastazowej. Korelacja pomiędzy dwoma stosowanymi metodami była bardzo silna ($r = 0{,}921$).

Słowa kluczowe: liczba diastazowa, miód

1. Introduction

Honey is known as a traditional high-grade and health-promoting natural food product. The antibacterial and anti-inflammatory properties as well as antioxidant activities have led to the application of honey in medicine [1, 2].

Honey, like every food product intended for sale, should have a certain quality determined by the applicable legislation [3]. The appropriate organoleptic and chemical parameters are provided only with proper preparation, processing and storage of honey. Any strange tastes, odours or food ingredients cannot be present in honey marketed for human consumption. Honey also must not have begun to ferment or have been heated in the way that destroyed or significantly inactivated the natural enzymes. The main quality parameters of honey are: diastase activity, concentration of proline and electrical conductivity, as well as the content of free acid, hydroxymethylfurfural (HMF) and sucrose [3].

Diastase (α-amylase) is one of the predominant enzymes in honey, next to invertase and glucose oxidase, which is added to honey by the bee during the collection and ripening of flower nectar [4]. One unit of diastase activity is defined as that amount of α-amylase, which will convert 0.01 gram of starch to the prescribed end-point in one hour at 40 °C. The results are expressed in Schade units per gram of honey and termed Diastase Number (DN) [5].

By the end of 2002, in Poland, DN was determined based on the guidelines contained in Polish standard PN-88/A-77626 [6], which recommended the Schade procedure for the measurement of diastase activity [7]. Currently, Regulation of the Minister of Agriculture and Rural Development of January 14th, 2009 (Dz. U. No. 17, pos. 94) [8] is valid in Poland. This regulation is in accordance with the EU Honey Directive 2001/110/EC and the guidelines of the International Honey Commission (IHC) [9] formed in 1990 in order to establish a new standard for honey. IHC recommended Phadebas assays as the official analysis methods for the determination of diastase activity in honey.

In the PN-88/A-77626 method, the diastatic activity of the honey is determined based on a distribution of the starch solution by α-amylase. The Phadebas method uses a synthetic biochemical substrate known as the *Phadebas tablets,* which are a water-insoluble, cross-linked starch polymer dyed in blue. The tablets are hydrolyzed by α-amylase to water-soluble form and the absorbance of the blue solution is a function of the diastase activity. The method is based on measurement of the absorbance of the solution at 620 nm against distilled water [10].

The EU Honey Directive 2001/110/EC states that the honey marketed for consumption must meet the following criteria for DN: no less than 8 Schade units for most of honey and no less than 3 for honeys with low natural enzyme content e.g. citrus honeys [3].

The aim of the study was to control the diastatic activity of several varieties of honeys obtained from different sources: directly from beekeepers and purchased in a store. The honey samples were stored under various conditions to assess the effects of temperature and time of storage on the DN value. In the presented studies, the two methods were applied and compared. The diastase activity was measured according to the method recommended by Polish standard PN-88/A-77626 and according to the Phadebas test recommended by the valid Regulation of the Minister of Agriculture and Rural Development of January 14th, 2009 (Dz. U. No. 17, pos. 94).

2. Materials and methods

Samples of several varieties of honeys from different sources and stored under different conditions comprised the research materials. Characteristics of the samples are summarized in Table 1.

Table 1. Characteristic of analysed honey samples

Sample	Type of honey	Origin	Condition and time of storage
H1	Multiflorous	Non-commercial; from southern of Poland	Stored for 2 month at 4 °C
H2			Stored for 2 months at room temperature
H3	Multiflorous	Commercial; from EU countries and non-EU	Stored on a store shelf (lack of information about the time), and then in the cupboard for 4 years
H4	Buckwheat	Commercial; from EU countries and non-EU	Stored on a store shelf (lack of information about the time), and then in the cupboard for 2 years
H5	Honeydew	Commercial; lack of information about the origin	Stored on a store shelf (lack of information about the time), and then in the cupboard for 2 years

The diastase activity expressed by DN was measured according to Polish standard PN-88/A-77626 method (Method A) and the Phadebas method (Method B). DN determination was performed in three repetitions for each method.

Method A is based on the distribution of the starch by α-amylase. The first step of the research was to prepare the starch solution. Anhydrous starch was dissolved in water and the suspension was rapidly brought to the boil point. The flask was stirred constantly and boiled gently for approx. 3 minutes. The solution was immediately transferred to a 100 mL volumetric flask, cooled down rapidly to room temperature and made up to volume with water. The clear blue solution was made on the day of use.

Iodine solution was prepared by dissolving of 10.0 g of twice-sublimated iodine and 20.0 g of potassium iodide in 40 mL of water. The solution was diluted to 500 mL and kept in a closed dark bottle.

Ten grams of honey was dissolved in 25 mL of distilled water saturated with toluene and titrated with sodium hydroxide solution (0.05 mol/L). Phenolophtalein was used as an indicator in the titrations. After titration, the solution was transferred to a 100 mL volumetric flask and made up to volume with water.

In the next step, the following were added to 12 polypropylene tubes: the solution of honey, water, acetic acid, sodium chloride and 1% starch solution in the amounts indicated in Table 2 [6]. Test tubes were placed in a water bath at 45–50 °C and incubated for one hour. The tubes were then cooled and 1–2 drop of 0.05 mol/L of iodine solution was added to each one. The change of colours of the solutions was observed immediately.

Table 2. DN values in the prepared samples

Sample no.	Honey solution [mL]	Water [mL]	Acetic acid [mL]	Sodium chloride [mL]	Starch solution [mL]	DN
1	10.0	4.0	0.5	0.5	1.0	1.0
2	10.0	2.5	0.5	0.5	2.5	2.5
3	10.0	0.0	0.5	0.5	5.0	5.0
4	7.7	2.3	0.5	0.5	5.0	6.5
5	6.0	4.0	0.5	0.5	5.0	8.3
6	4.6	5.4	0.5	0.5	5.0	10.9
7	3.6	6.4	0.5	0.5	5.0	13.9
8	2.8	7.2	0.5	0.5	5.0	17.9
9	2.1	7.9	0.5	0.5	5.0	23.8
10	1.7	8.3	0.5	0.5	5.0	29.4
11	1.3	8.7	0.5	0.5	5.0	38.5
12	1.0	9.0	0.5	0.5	5.0	50.0

Diastase activity in the honeys was also determined using Phadebas tablets (Phadebas Amylase Test, Magle AB, Sweden) by a photometric method (Method B) using Shimadzu UV-160A spectrophotometer. Absorbance at 620 nm of the analysed solutions was directly proportional to the diastatic activity of the honey samples.

The first step of Method B was to prepare acetate buffer by dissolving 13.6 g of sodium acetate trihydrate in water. The pH of the solution was adjusted to 5.2 with glacial acetic acid. The solution was diluted to 1L with distilled water and stored in a glass bottle.

One gram of honey was weighed, quantitatively transferred to a 100 mL volumetric flask and made up to volume with acetate buffer. Five millilitres of the sample was transferred to the test tube and placed in a water bath at 40 °C. At the same time, under the same conditions, the blank (5 mL of acetate buffer) was heated in a water bath at 40 °C. After 15 minutes, 1 Phadebas tablet was added to the two solutions, stirred (approx. 10 sec.) and placed back into the water bath at 40 °C. After exactly 30 minutes, 1 mL of sodium hydroxide solution was added for interrupt the enzyme reaction.

In the next step, the solutions were centrifuged (5 minutes; 1500 rpm) and the absorbance was measured at 620 nm against distilled water as the reference sample.

The DN, repeatability and reproducibility were calculated based on the models specified in the instruction for Phadebas method [10]

3. Results and discussion

In Method A, a colour change were observed. The purple colour indicated a complete hydrolysis of starch. Based on the data contained in Table 2, the appropriate value of DN was chosen. Fig. 1 shows an example image illustrating the experiment by Method A for sample

H3. In tubes 5 and 6, incomplete hydrolysis of starch is observed. The reaction is complete only in tube 7. The DN for the solution in tube 7 is 13.9 Schade units.

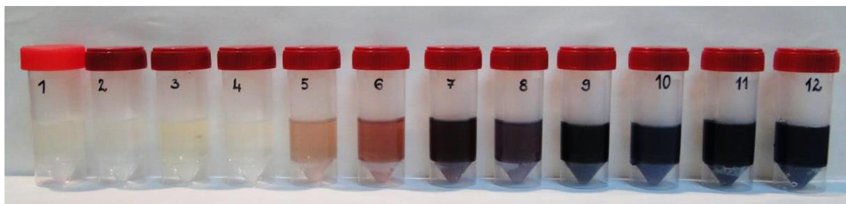

Fig. 1. The picture of honey sample 3 prepared by Method A

Diastase activity determined using Phadebas tablets is proportional to the absorbance at 620 nm.

The repeatability (r) and reproducibility (R) determined by Method B were calculated based on equations specified by the Phadebas producer [10]:

$$r = 0.02 + 0.03 \cdot \Delta A_{620}$$
$$R = 0.04 + 0.32 \cdot \Delta A_{620}$$

0.02, 0.03, 0.04 and 0.32 means constant to account for the relationship between the diastatic activities of honey, ΔA_{620} is the difference in absorption of the test solution of honey and the blank.

The results of repeatability and reproducibility are summarized in Table 3.

Table 3. Summary of repeatability (*r*) and reproducibility (*R*) for Method B

Sample	*r*	*R*
H1	0.04	0.24
H2	0.04	0.21
H3	0.03	0.11
H4	0.03	0.12
H5	0.03	0.13

For Method B, the DN of the samples were calculated from linear regression of diastase number against ΔA_{620} yielded the following relation [10]:

$$DN = 28.2 \cdot \Delta A_{620} + 2.64$$

The results of DN calculated by Method A and Method B are summarized in Table 4. Correlation between DN obtained by the two methods is shown in Fig. 2.

Table 4. DN values obtained by the two used methods

Sample	DN by Method A	DN by Method B
H1	23.9	20.3
H2	17.9	17.6
H3	13.9	9.0
H4	10.9	9.4
H5	10.9	11.0

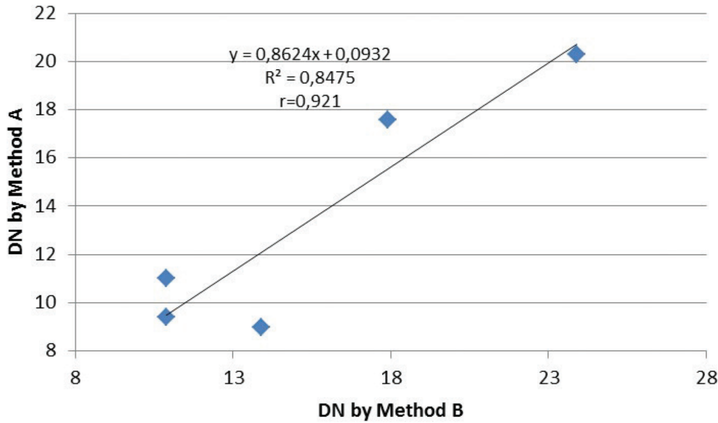

Fig. 2. Correlation between DN by Method A and Method B

The biggest difference between Method A and Method B occurred in the case of a sample of multiflorous honey H3. A possible explanation might be that the Phadebas test uses a defined substrate, whereas commercially available starch may vary considerably in quality. The differences in the two methods could also be explained by the lower precision of enzymatic methods. In the case of the other samples, the differences between the two methods were very small. Correlation between DN obtained by the two methods was very strong. The correlation coefficient (*r-Pearson*) for the methods is 0.921.

The DN values marked by Method B are very similar to the results of tests conducted in accordance with Method A. All tested samples were within regulatory requirements because the DN is greater than 8. The highest value activity of α-amylase has a non-commercial flower honey H1 derived directly from the beekeeper and stored at 4 °C. The same honey samples, but stored at room temperature, are characterized by slightly lower DN (17.6). This insignificant difference may indicate little effect of storage conditions on the value of DN in fresh honey. The lowest DN was marked for multiflorous honey H3. To determine the effect of storage conditions on diastatic activity of honeys, further studies are indicated.

4. Conclusions

Two different methods were used to determine the number of diastase in honeys: the method recommended in Polish standard PN-88/A-77626 and the Phadebas method. There is a strong correlation between the diastase activities determined by using those two methods.

References

[1] Al-Mamary M., Al-Meeri A., Al-Habori M., *Antioxidant activities and total phenolics of different types of honey,* Nutrition Research 22, 2002, 1041–1047.

[2] Mandal M.D., Mandal S., *Honey: Its medicinal property and antibacterial activity,* Asian Pacific Journal of Tropical Biomedicine 1, 2011, 154–160.

[3] Council Directive 2001/110/EC of 20 December 2001 relating to honey. Official Journal of the European Communities, http://www.ihc-platform.net/honeydirective2001.pdf (access: 23.05.2017).

[4] Persano Oddo L., Baldi E., Accorti M., *Diastatic Activity of Some Unifloral Honeys,* Apidologie, 21, 1990, 17–24.

[5] Diastase Activity (a-AMYLASE) in Honey Assay Procedure K-Amzhy 10/07. Megazyme International Ireland 2007, https://www.megazyme.com/docs/analytical-applications-downloads/amylase_in_honey_30602 (access: 23.05.2017).

[6] Polska norma PN-88/A-77626 Miód pszczeli, Wyd. Normal, Alfa, Warszawa 1988.

[7] Schade J. E., Marsh G.L., Eckert J. E., *Diastatic activity and hydroxymethylfurfural in honey and their usefulness in detecting heat adulteration,* Food Research 23, 1958, 446-463.

[8] Rozporządzenie Ministra Rolnictwa i Rozwoju Wsi z dnia 14 stycznia 2009 r. w sprawie metod analiz związanych z dokonywaniem oceny miodu (Dz. U. Nr 17 poz. 94).

[9] Harmonised methods of the International Honey Commission. Stefan Bogdanov, Bee Product Science, http://www.ihc-platform.net/ihcmethods2009.pdf (access: 23.05.2017).

[10] Honey Diastase Test. Instructions for use, Magle AB, Sweden 2010, www.phadebas.com (access: 23.05.2017).