

The role of enzymes in honey

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Abstract

This study focused on the analysis of diastase activity, hydroxymethylfurfural, moisture content and electrical conductivity in honey samples from stores in Brno. We collected and analysed 34 honey samples. We proceeded in accordance with the “Harmonised Methods of the International Honey Commission” (Bogdanov 2009). Three of the blossom honey samples were labelled “Czech Honey”. Of the parameters analysed, only diastase activity was found to be in noncompliance with the value of minimal diastase activity set forth by Decree No. 76/2003 Coll. and Council Directive 2001/110/EC. All the analysed samples met the hydroxymethylfurfural concentration, moisture content and electrical conductivity according to legislative values, except for one “Czech Honey” sample with a moisture content and hydroxymethylfurfural concentration exceeding the limit value according to Quality Standard for Czech Honey No. 1/1999.

Diastase activity, electrical conductivity, hydroxymethylfurfural, moisture content

Introduction

Honey contains a number of enzymes. Enzymes are minority components in honey. The level of their activity, which indicates the freshness of the honey, good manufacturing practice, quality and authenticity of the honey, is important. The enzymes present in honey can be divided into enzymes naturally occurring in honey and honey-foreign enzymes (Kundříková-Burešová and Bartošová 2018). The natural enzymes in honey are of plant origin (catalase and acid phosphatase) coming from plant nectar (Persano Oddo et al. 1999) or they come from the bees themselves being excreted by the bees' exocrine glands. The exocrine glands – salivary and hypopharyngeal – enrich nectar with invertase, glucose oxidase and diastase (Al-Sherif et al. 2017). The quantity of enzymes added to the nectar by the bees depends on a number of factors, including the phase of the bees' life, the strength of the bee colony, the nutrition of the bees and the temperature conditions (Persano Oddo et al. 1999). Honey-foreign enzymes do not occur naturally in honey and get into honey by means of the unauthorised addition of industrially produced syrups (rice, corn or wheat syrups). Honey-foreign enzymes include β -fructofuranosidase, β -amylase and γ -amylase (Kundříková-Burešová and Bartošová 2018). Despite the presence of a number of enzymes specific to honey, the European (Council Directive 2001/110/EC, as amended) and national (Decree No. 76/2003, as amended) legislation demands checks only on the activity of diastase. The botanical origin of the honey, the freshness of the honey, the storage conditions, the heating of honey and the addition of sugar syrups, all have an effect on the diastase activity in honey.

In view of the fact that honey is the most frequently faked foodstuff on the market, and taking into consideration the frequency with which diastase activity beneath the legal limit is determined by the Czech Agriculture and Food Inspection Authority (CAFIA), we set ourselves the aim of assessing the quality of the honeys currently available on the market and determining the diastase activity, the 5-hydroxymethylfurfural content, the water content and the electrical conductivity.

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Material and Methods

We subjected a total of 34 samples of honey, of which 28 samples of blossom honey and 6 samples of honeydew honey, to the analysis of selected physicochemical parameters. These samples of honey were collected in January 2018, primarily from retail chains in Brno, with the exception of 3 honeys obtained directly from a beekeeper at a specialised beekeeper's store in Brno. The analysed samples of honey came from the Czech Republic (10), the European Union (EU) and outside the EU (18), outside the EU (1), Italy (1), Turkey (1), Spain (1), Mexico (1) and Slovakia (1). Three of the samples of honey were labelled "Czech Honey". The samples of honey were stored at room temperature of up to +25 °C in their original consumer packaging. We determined the water content, the electrical conductivity, the 5-hydroxymethylfurfural (HMF) content and the diastase activity in all the samples of honey collected. During the determination of the water content, electrical conductivity and HMF content, we proceeded in accordance with the "Harmonised Methods of the International Honey Commission" (Bogdanov 2009). We determined the diastase activity spectrophotometrically with the use of a T-AMZHY standard substrate for the determination of diastase (α -amylase) activity in honey in the form of tablets supplied commercially by the company Megazyme Inc., Ire. In determining the diastase activity, we proceeded in accordance with the official methodology of the manufacturer "Diastase Activity (α -amylase) in Honey, Assay Procedure" (Megazyme International Ireland 2013).

Results and Discussion

We compared the values for diastase activity, HMF content, water content and electrical conductivity we measured (Table 1) with the limit values given in Decree no. 76/2003 Sb., as amended, and Council Directive 2001/110/EC, as amended. We also compared the values measured for honeys declared "Czech Honey" on the label with the limit values given in quality standard of the Czech Beekeepers' Association CZECH HONEY No. 1/1999.

Table 1. Values of diastase activity, hydroxymethylfurfural content, water content and electrical conductivity by origin of honey

Parameter		Origin of honey		Type of honey	
		CR [n = 10]	not CR [n = 24]	Blossom	Honeydew
		Min – max		$\bar{x} \pm SD$	
Diastase activity	[DN]	5.2 – 16.4	5.5 – 22.4	11.0 \pm 3.81	16.9 \pm 6.77
$\bar{x} \pm SD$		9.2 \pm 3.04	13.2 \pm 5.10		
HMF	[mg·kg ⁻¹]	5.0 – 31.6	8.4 – 37.9	18.3 \pm 7.62	14.0 \pm 4.91
$\bar{x} \pm SD$	16.5 \pm 8.30	18.0 \pm 7.06			
Water	[%]	15.3 – 18.6	15.5 – 18.7	17.2 \pm 0.72	16.2 \pm 0.49
$\bar{x} \pm SD$		16.9 \pm 0.94	17.1 \pm 0.70		
El. Cond.	[mS·m ⁻¹]	B 14 – 55 HD 84	B 14 – 69 HD 82 – 151		
$\bar{x} \pm SD$		B 33 \pm 14.0 HD 84 \pm 0.1	B 34 \pm 13.0 HD 113 \pm 27.1	33 \pm 13.1	109 \pm 27.0

B – blossom honey, HD – honeydew honey, \bar{x} – average, SD – standard deviation, CR – Czech Republic, HMF – hydroxymethylfurfural

We determined a diastase activity of less than 8 DN in 7 samples of honey (21%) of the total number of analysed samples of honey (n = 34). In two cases, these were honeys originating in the Czech Republic, blossom honey with an activity of 6.1 \pm 0.18 DN and honeydew honey with an activity of 5.2 \pm 0.09 DN, purchased at a specialised beekeeping store. During the inspections CAFIA performed in 2017 when a total of 22 samples of

honey from the retail network were analyzed, it found an average diastase activity of 11.8 DN (Kundříková-Burešová and Bartošová, 2018), i.e. almost identical to the average diastase activity (12.0 ± 4.90 DN) we determined in our study. The CAFIA also states that it determined a diastase activity beneath the legal limit in 14% of analysed samples of honey (Kundříková-Burešová and Bartošová 2018).

All the samples of honey we analysed met the legislative limit for the content of HMF ($40 \text{ mg} \cdot \text{kg}^{-1}$). The quality standard of the Czech Beekeepers' Association CZECH HONEY requires "Czech Honey" to contain a maximum $20 \text{ mg} \cdot \text{kg}^{-1}$ of HMF. In one sample of "Czech Honey", declared as "Czech Meadow Blossom Honey" on the package, we found that this limit was exceeded by $6.6 \text{ mg} \cdot \text{kg}^{-1}$. The average value of HMF in honeys from the Czech Republic of $16.5 \pm 8.30 \text{ mg} \cdot \text{kg}^{-1}$ is comparable with the average content of HMF in honeys from outside the Czech Republic of $18.0 \pm 7.06 \text{ mg} \cdot \text{kg}^{-1}$. The CAFIA did not find any HMF values above the permitted level during its centrally organized inspection in honeys from the retail network, although the average HMF content of $11.5 \text{ mg} \cdot \text{kg}^{-1}$ in the samples it analysed (Kundříková-Burešová and Bartošová 2018) was lower than the value of $17.6 \pm 7.35 \text{ mg} \cdot \text{kg}^{-1}$ we found in our study. The HMF content, along with the diastase activity, represents an important indicator of the quality, freshness and authenticity of honey.

The legislative limit of 20% for the content of water was not exceeded in any of the honey samples. The CAFIA did not find the permitted content of water in honey being exceeded, either (Kundříková-Burešová and Bartošová 2018). The quality standard of the Czech Beekeepers' Association CZECH HONEY is stricter in terms of the content of water in honey and demands that "Czech Honey" has a maximum water content of 18%. We found this limit being exceeded in one sample in which a water content of $18.6 \pm 0.06\%$ was recorded. The average water content in honeys from the Czech Republic of $16.9 \pm 0.94\%$ is almost identical to the content of water in honeys not originating in the Czech Republic ($17.1 \pm 0.70\%$).

Electrical conductivity is one of the determinants for honeydew honey, for which a minimum conductivity of $80 \text{ mS} \cdot \text{m}^{-1}$ is required. The limit for electrical conductivity was met by all the samples of honey.

Conclusions

On the basis of our analysis we can conclude that the diastase activity is the most frequently violated qualitative parameter of honeys from retail chains because 21% of our analysed honeys failed to observe this parameter. This is in agreement with the finding of the inspection authority. A lower diastase activity may be the result of inappropriate heating during the liquefaction of honey, storage at a higher temperature or the illegal addition of sugar syrups.

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