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Determination of C4 sugars and invertase of multifloral honey samples from Bulgaria

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Abstract

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Honey is a natural product and it is famous for its tremendous therapeutic potential since ancient times. Also, honey is an excellent and widely used food. In this respect as a human food, honey should be pure and unadulterated. On the other hand, an increase in the demand for honey has resulted in adulteration by different sugar syrups. Therefore, the quality is important for consumer confidence. The data about C4 sugars in Bulgarian honey samples is not available. Thus, the results presented in this study could be considered as a first step for characterisation in respect of this parameter. The aim of the present study was to determine invertase and C4 sugars in honey samples. In the present study, 111 honey samples from Bulgaria were analyzed. Only 5 of them were adulterated with C4 sugars. No significant correlation was found between the invertase activity and the content of C4 sugars in all analyzed honey samples. We expect our report to be a starting point for wider investigations, particularly aimed at a deeper understanding of the quality and authenticity of bee honey.

Keywords: honey; C4 sugars; honey adulteration; honey quality; invertase activity; multifloral honey

Introduction

Honey is a popular natural product that is consumed as healthy food and applied in the treatment of a broad spectrum of diseases. It is a very complex product composed of major compounds including monosaccharides such as glucose and fructose and minor components such as amino acids, enzymes, vitamins and minerals (Celechovska and Vorlova, 2001; Carratu et al., 2011; Kirs et al., 2011; Escuredo et al., 2012). Based on its components, honey is highly esteemed by consumers as a naturally pure and healthy product.

However, many factors influence the basic properties of honey including the nectar-providing plant species, geographic area and harvesting conditions (Iglesias et al., 2012). The quality and the honey composition are also affected by many other factors, such as overfeeding of bees with sucrose, harvesting prior to maturity, and adulteration with sugar syrups. In this respect, the determination of the common quality parameters such as fructose, glucose, sucrose, hydroxymethylfurfural (HMF), proline content may give hints at possible manipulation. Indeed, they are usually not enough sensitive to detect some admixtures of foreign sugars to honey. The reason is that honey is showing large compositional variations depending on the botanical type and geographical origin. Due to the complex nature of honey, sometimes purity and quality are difficult for determination by using common methods such as physicochemical parameters. Therefore more specialized procedures need to be developed (Siddiqui et al., 2017).

Also according to Aries et al. (2016) a database for honey samples, sugar syrups and bee feeding products is needed.

The availability of authentic samples from the biobank will greatly facilitate the development process. To date, no universal method exists that is able to determine all the different types of honey adulterants with sufficient sensitivity and robustness. As a consequence, several complementary methods have to be applied in order to perform a reliable assessment of honey quality and authenticity.

Taking all this into consideration, invertase and C4 sugars are one of the good parameters for identification of honey quality.

Lichtenberg-Kraag (2014) analyzed the enzymes amylase and invertase in honey. Enzymatic activity is an indicator reflecting the process of converting nectar into honey during the ripening process. Even though the enzymes are usually added by the bees, some differences in enzyme activity were demonstrated depending on the botanical origin of the nectar.

On the other hand, honey could be adulterated by adding sugar syrups (Padovan et al., 2007). It should be evaluated whether honey has been adulterated with foreign sugars or whether honey was produced by intensive sugar feeding of the bees. The adulteration of honey by means of feeding bees with different sugar syrups during honey production or adding sugar syrups after production, causes serious problems for pure honey producers and consumers (Elflein and Raezke, 2008; Tosun, 2013). Generally, these syrups are sugar solution (sugar water in ratio 1:1), inverted sugar syrup, Isosweet. Sometimes beekeepers used synthetic enzyme invertase to produce inverted sugar syrup. If the concentration of invertase is too high in the inverted sugar syrup it could contaminate the honey. In this case the honey has high levels of invertase. Therefore the analytical methods applied for adulteration detection must be independent from these variability factors. It is well known that sugar cane and corn sugar syrups have unique isotopic 13C/12C signatures. Sugar cane and corn (maize) use the C4 photosynthetic cycle. In contrast, most honey is derived from the nectar from C3 plants (Cengiz et al., 2014). Thus, 13C/12C carbon stable isotope ratio mass spectrometric analysis (δ13C-IRMS) can be used to distinguish different natural or synthetic sugar sources according to their δ13C isotopic values. The IRMS analysis for honey is published as AOAC method. The purpose of this method is to detect sugars from C4 plants (sugar cane and corn) in honey. According to this procedure, honey is considered to be adulterated, if the C4 sugar percentage is ≥ 7%.

In Bulgaria botanically different honeys are produced, so it is evident that defining good, reliable and meaningful quality parameters for this important natural product is professionally challenging. Based on current knowledge about the quality of honey the aim of the present study is to determine the invertase and C4 sugars in multifloral honey samples from Bulgaria and to find a possible correlation between these two parameters.

Materials and Methods

A total of 111 multifloral honey samples were produced in different regions of Bulgaria. They were collected during 3 years. All samples were analyzed immediately after receiving at the Central Laboratory of Veterinary Control and Ecology.

The invertase activity was carried out using the method established by the European Honey Commission (Bogdanov et al., 1997). The C4 sugars were determined by 13C/12C carbon stable isotope ratio mass spectrometric analysis (δ13C-IRMS) according to Association of Official Analytical Chemistry (AOAC, 1998).

The statistical analysis was performed by using SPSS Statistical Package (version 21 for Windows). The concentrations were expressed as mean ± standard deviation, minimum and maximum values. Correlations were established using Pearson's correlation coefficient (r).

Results and Discussion

The adulteration of honey with sugar syrup has been a problem for a long time. Unscrupulous beekeepers could feed their bee families with such syrup or they could add it later after honey extraction.

In the present study a total of 111 multifloral honey samples from different regions of Bulgaria were analyze for C4 sugars and invertase. The results of the analyses were given separately for the honey samples without any content of C4 sugars and for the samples with C4 sugars below and above 7%. In 62 samples C4 sugars were not detected (Table 1). In this respect, more than 50% of all analyzed samples did not contain C4 sugars at all and they were from unadulterated sources.

Table 1
Invertase and negative C4 sugars in multifloral honey sample, (n = 62)

	Invertase, U/kg	C4 sugars, %
Ranges	29.40 – 166.50	negative
Mean±SD	73.55 ± 24.76	

The second parameter which was analyzed is invertase. It is accountable for the conversion of nectar to honey and serves as a sensitive indicator of honey quality. In particular, the invertase is the enzyme responsible for converting

sucrose to fructose and glucose which are the main sugars in honey.

It is well know that invertase activity determination is used as a parameter related to the freshness of honey. In this respect, invertase activity is one of the main criterions for the quality of honey (Bogdanov et al., 1997). Actually, it is also a more sensitive parameter than the determination of hydroxymethylfurural (HMF), (Vorlova and Pridal, 2002). According to Vorlova and Celechovska (2002) the enzyme participates in the biological value of honey. In the present study invertase varied in a very large range (Table 1). According to Oddo et al. (1999) the invertase activity in multifloral honey samples range from 50 to 200 U/kg. In this study the minimal value was under 50 U/kg (Table 1). The variability in invertase activity found in the all analyzed honey samples is connected with their origin. All samples are multifloral and the nectar collected from the honey bees is a mix from different flowers. Also the large ranges of this parameter was probably due to factors such as nectar collection period, physiological stage of the colony, abundance of nectar flow and its sugar content (Oddo et al., 1999).

Until now, honey was considered to be adulterated, if the C4 sugar percentage was ≥ 7%. For all 44 honey samples the C4 sugars are under 7% (Table 2), but they contain a small concentration of C4 sugars. Indeed, this means that they were not adulterated with C4 sugars. No significant correlation ($r = 0.064$, $p > 0.05$) was found between the invertase activity and the content of C4 sugars in these samples.

Table 2
Invertase and C4 sugars under 7% in multifloral honey sample, (n = 44)

	Invertase, U/kg	C4 sugars, %
Ranges	43.70 – 134.60	0.40 – 6.80
Mean±SD	76.96 ± 21.95	2.07 ± 1.55

Adulteration above 7% was detected in 5 of the 111 multifloral honey samples from Bulgaria (Table 3). In such cases, the adulteration can be performed directly via the addition of commercial sugar syrups to the honey or indirectly via overfeeding honey bee colonies with C4 sugars. Our results on invertase content in the adulterated with C4 sugars multifloral honey samples are in the ranges 38.30-98.20 U/kg.

Table 3
Invertase and C4 sugars above 7% in multifloral honey sample, (n = 5)

Sample	C4 sugars, %	Invertase, U/kg
1	9.30	85.20
2	9.90	84.80
3	13.80	98.20
4	8.00	66.20
5	8.30	38.30
Mean±SD	9.86 ± 2.33	74.54 ± 23.25

According to the conducted study the minimal value for invertase activity was under 50 U/kg. Furthermore it is comparable to the value for unadulterated honey samples (Table 1 and 2). Again, no significant correlation ($r = 0.737$, $p > 0.05$) was found between the invertase activity and the content of C4 sugars in these 5 honey samples.

Fugure 1 and 2 presented the box plot diagrams of invertase and content of C4 sugars. For the content of C4 sugars sample 3 was outlier (13.80%). As can be seen from Figure 1 minimum and maximum values of invertase activity vary in a very large range. The median is 84.80 U/kg. For the content of C4 sugars the mean and median values are almost identical about 9% (Figure 2).

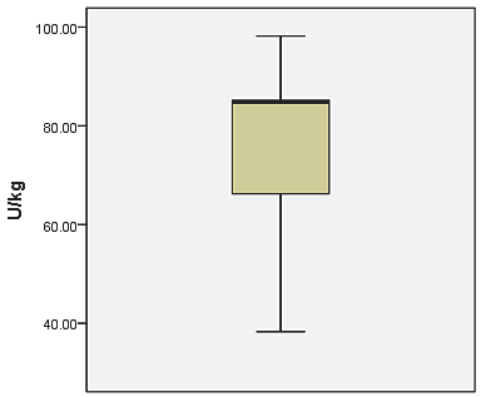

Fig. 1. Box plot of invertase for adulterated with C4 sugars multifloral honey samples (n = 5). Minimum, maximum and median values are shown

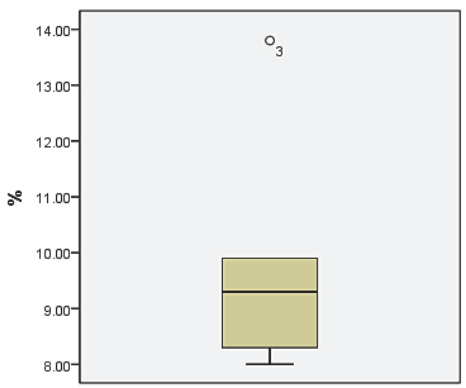

Fig. 2. Box plot of C4 sugars of adulterated multifloral honey samples (n = 5).
Minimum, maximum and median values are shown

Conclusions

The verification of the honey authenticity is a complex analytical task. The determination of the quality parameters such as invertase and C4 sugars are important.

Five of all the 111 analyzed multifloral honey samples were adulterated with C4 sugars. No significant correlations between the invertase activity and the content of C4 sugars were found for the adulterated honey samples. Although there have been many studies reporting the adulteration of honey, little has been documented regarding the Bulgarian multifloral honey. Therefore, this study was one of the first attempts to describe important honey quality parameters such as the content of C4 sugars in multifloral honey samples. We expect our report to be a starting point for wider investigations, particularly aimed at a deeper understanding of the quality and authenticity of bee honey.

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