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The effect on the quality of bee honey when feeding the bees on inverted with confectionary invertase sugar syrup

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Abstract

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The study was carried out on the effects of using sugar syrup inverted with commercially available confectionery invertase as a supplement bee feed on the quality of bee honey. The results revealed an increased level of absorption; an increased level of HMF (2-hydroxymethyl-2-fufuraldehide), as well as high levels of Cu. The blue colour, which was revealed when adding Carraz I ($K_4Fe(CN)_6.3H_2O$) and Carraz II ($Zn(CH_3COO)_2.2H_2O$) solutions was associated with high levels of Fe, contained in the beet sugar syrup, inverted with confectionery invertase.

Keywords: supplementing bee feed; honey bees; sugar syrup; confectionery invertase; bee honey

Introduction

Different types of bee honey adulteration present a major problem, threatening the image of bee honey – this presumably most natural of all food substances, given that it is the product of plant carbohydrate sources, naturally processed by bees. Over the years different bee honey adulteration detection methods have been developed. The most reliable, modern methods, though require the use of specialized equipment. Some authors recommend the application of FTIR infrared spectroscopy for detecting bee honey adulteration with sugar beet sugar (Sivakesava & Irudayaraj, 2001).

According to Bogdanov (2006), environmental bee honey contamination is possible via the raw products that bees consume as a source for honey production (plant flower nectar; honeydew; pollen; sweet plant juices). Air, soil and plant contaminants contained in these raw products are then transferred by the bees into the honey they produce.

Another substantial issue, in this respect, is the practice of supplementing bee colony feed with sugar syrup (in 1:1 ratio), as well as with commercially available confectionery invertase inverted sugar syrup. Lately, incidences of attempts to alter the quality of bee honey by adding commercially available sugar syrup, inverted by the use of confectionery invertase have become greater in number.

In 2020, the first results of studies on the effects of this practice on the water and HMF (2-hydroxymethyl-2-fufuraldehide) content of bee honey were published (Dinkov, 2020).

This article focuses on providing additional data on the absorption, the specific optical rotation and the content of Cu and Fe in bee honey produced by bee colonies supplementary fed on the beet sugar syrup, inverted with commercially available confectionery invertase.

Materials and Methods

The experiment on the effects of introducing supplementary bee feed to bee colonies was carried out in June 2019, in

a private apiary on the territory of the municipality of Stara Zagora. Two groups with two bee hives in each were formed, one of them was marked as the experimental group, while the other one was the control group. The bee colonies which took part in the experiment were installed in Danant Blatt 10-frame bee hives. The bee colonies had previously been equalized applying the strength analogue method (2.5 kg bees per bee colony). The bee colonies in the control group were put in natural conditions, without introducing supplement feed, while the experimental group was given sugar syrup inverted with confectionery invertase. We considered it unnecessary to have a third group, supplementary fed on non-inverted sugar syrup (in 1:1 ratio) because of the widely available data on the effects of that type of supplementary feed on the quality of the thus produced bee honey (Dinkov et al., 2003).

Supplementary feed was introduced for 10 days in June 2019, as the daily dose was 500 ml per bee colony. The proper time for taking bee honey samples at the end of the experiment was visually determined with regard to the extent of honeycomb capping, as at least 2/3 of all honeycombs had to be properly capped, so that ultimately mature bee honey could be obtained. At the end of the experiment, 5 capped honey frames were taken from the store box of each bee hive (10 frames per group), which were after that uncapped, centrifuged in a honey extractor and filtered, so that average samples of bee honey from each group could be obtained.

The beet sugar used for preparing the sugar syrup used in the experiment was bought from a regular store in Bulgaria, produced by AGRANA Zucker GmbH, Austria (https://www.zahira.bg/producti/).

According to proper use instructions, 5 g of confectionery invertase enzyme were added to 50 kg of 60% sugar syrup, so that the process of hydrolysis could take place for 12 h, at a temperature of 55°C. In case the temperature is increased to 70°C, 90% enzyme activity is achieved in 4 h, which in fact were the parameters we applied when preparing the inverted sugar syrup we used in our experiment (Invertaza, 2020). The confectionery invertase inverted sugar syrup was found to have refractometrically determined water content of 25.65%, which was equal to the water content of traditionally used supplementary bee feed sugar syrup (in 1:1 ratio), containing 0.625 kg of non-inverted sugar and 0.625 l of water (Dinkov et al., 2003).

The results referring to the water content and HMF of the honey produced in the experiment were published in 2020 (Dinkov, 2020). The remaining results are first published in this article, due to COVID-19 restrictions and scientific activity constraints over the pandemic period. Additional study on the specific optical rotation (SOA) was carried out, as the index parameter was determined by using the Optech Polarymeter Model PL1LED: 24 hours after taking the samples, Carraz I ($K_4Fe(CN)_6.3H_2O$) and Carraz II ($Zn(CH_3COO)_2.2H_2O$) solutions were subsequently added to them and then filtered (Bogdanov et al., 1997).

On the 48th hour of the process of sample preparation for determining SOA, the samples were filtered through filtering paper and their absorption was measured by means of a spectrophotometer (Spectrophotometer SP-870 plus, Meterteh) at a wave length of 540 nm (which is the wave length used for determining the HMF of bee honey). We performed double measuring of each sample, on two consecutive days. The average results are presented in Table 1.

At the same time, the atomic absorption and the concentrations of Cu and Fe in the sugar syrup inverted with confectionery invertase, as well as in the bee honey produced by the bee colonies fed on that sugar syrup were measured and calculated by means of a calibration scale, using an atomic absorption spectrometer (AAC Perkin Elmer, ANALYST 800 AA SPEKTROMETER model), at the Science Research Laboratory in the Department of Agrarian Science at Trakia University, Stara Zagora, Bulgaria.

Results and Discussion

It was found that the degrees of absorption were lower (0.093) in the sugar syrup inverted with confectionery invertase, compared to the samples of honey, produced by the bee colonies supplementary fed on such sugar syrup (0.103) (Table 1).

Table 1. Absorption and specific optical rotation of bee honey, produced by bee colonies supplementary fed on sugar syrup inverted with confectionery invertase

Quality parameters	B	C
A	0.093	0.103
SOA $[\alpha]_D^{20}$	- 13.9	- 21.9

A – absorbtion
SOA – specific optical rotation in $[\alpha]_D^{20}$
B – sugar syrup inverted with confectionery invertase
C – bee honey, produced by bee colonies supplementary fed on sugar syrup inverted with confectionery invertase

Despite the higher negative values of SOA, measured in the sugar syrup inverted with confectionery invertase, they were quite similar to the ones measured in nectar types of bee honey (Dinkov, 2003). Based on these results, we came to the conclusion that by applying this indicator alone, bee honey adulteration of this type cannot be unequivocally identified in nectar types of bee hone (Table 1).

An important finding was made during the experiment – light blue colouring appeared when we added Carrez I solution to the sugar syrup inverted with confectionery invertase when preparing the samples for SOA analysis, which then turned more intensively dark blue in colour, after adding Carraz II solution (Figures 1 and 2).

When additional analysis of the Cu content in the sugar syrup inverted with confectionery invertase samples was made, we revealed that the level of concentration of this heavy metal was 0.42 mg.kg^{-1}, while the concentration of Cu in the honey produced by be colonies supplementary fed on such sugar syrup was almost 50% higher, reaching 0.62 mg.kg^{-1}. The concentration of Fe in the bee colonies was 0.43 mg.kg^{-1}, whereas in the sugar syrup inverted with confectionery invertase, which was used as supplementary bee feed, it was as high as 15.92 mg.kg^{-1}.

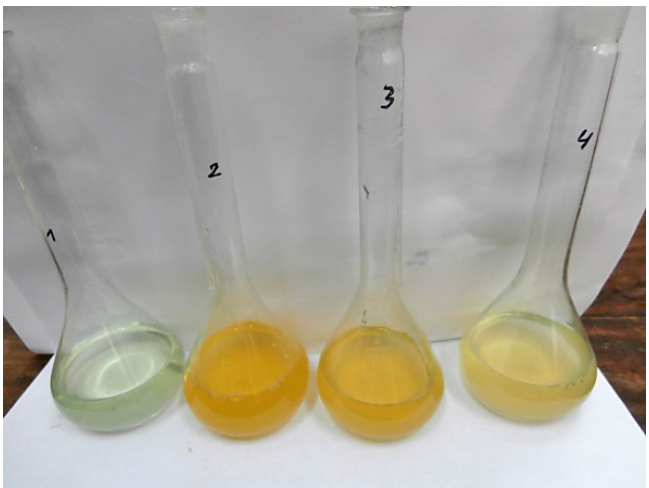

Fig. 1. Light bluish colouring of sample I: sugar syrup inverted with confectionery invertase, after adding Carraz I solution, in comparison with samples of bee honey

Fig. 2. More intensive dull blue colouring of sample I: sugar syrup inverted with confectionery invertase, after adding Carraz II solution, in comparison with samples of bee honey

In our initial studies, we revealed significant changes in HMF of the honey produced by bee colonies supplementary fed on sugar syrup inverted with confectionery invertase (Dinkov, 2020). Intensive supplementation of bee feed with sugar syrup obtained through hydrolysis with confectionery invertase does not lead to increased water content but results in unacceptably high levels of HMF (40.3 mg.kg^{-1} and 53.7 mg.kg^{-1}) (Dinkov, 2020), with regard to the respective Bulgarian legislation (The Bulgarian Ministry of Heath Order, 2002),

In our previous studies on the effects of supplementing bee feed with non-inverted sugar syrup (1:1) on the quality of bee honey, we had not found out increased levels of HMF. We neither found out any negative effects on the organoleptic indicators of the samples of bee honey, produced by bee colonies fed on such sugar syrup (Dinkov et al., 2003).

The higher pH indicator (pH=6), necessary for the hydrolysis of carbohydrates under the effect of bee invertase (Bogdanov et al., 1997) and respectively the lower pH level (pH=4.5), in combination with the recommended higher temperature (70°C), necessary for the process of hydrolysis to take place under the effect of confectionery invertase (Invertaza (invertase), 2020), in our opinion, are the main reasons explaining the higher levels of HMF in the sugar syrup inverted with confectionery invertase. Later on, when the bees fed on such sugar syrup process it in their stomachs, the chemical substance is transferred into the bee honey as well (Dinkov, 2020). These results have been validated by other authors as well, who also conclude that HMF is transferred into the bee honey produced by bee colonies supplementary fed on sugar syrup containing high levels of this chemical compound (White & Siciliano, 1980).

Certain differences in the concentration of Fe in the different types of bee honey have also been found in our country. The concentration of this element in Bulgarian honeydew honey was found to be 5.19 ± 0.06 mg.kg^{-1} (Dinkov & Mihaylova, 2009). Mladenov (1978) found substantially higher concentration of Fe in acacia honey – 11.05 mg.kg^{-1}. Dinkov et al. (2000) also studied the concentration of Fe in bee honey and they revealed almost identical levels of element in acacia honey (11.04-14.71 mg.kg^{-1}), but almost by half lower concentration in polyfloral types of bee honey – Fe (5.47-7.98 mg.kg^{-1}). Substantially lower average concentration of Fe (2.316 mg.kg^{-1}) have been found in Bulgarian

fennel honey (Parvanov et al., 2011).

The explanation of the blue colouring we observed during the process of preparation of the samples for SOA analysis (Bogdanov et al., 1997) is the reaction between potassium ferrocyanide ($K_4Fe(CN)_6.3\ H_2O$), contained in the Carrez I solution used, and Fe, resulting in the distinctive colour, which has also been validated by other authors (Kreshkov, 1976).

The substantial differences between the levels of concentration of Fe in the sugar syrup inverted with confectionery invertase (15.92 $mg.kg^{-1}$) and in the bee honey produced by bee colonies supplementary fed on such sugar syrup (4.03 $mg.kg^{-1}$) show that the higher concentration in the supplementary feed does not necessarily lead to increased concentration in the thus produced honey. But in case of direct adulteration of bee honey with such inverted sugar syrup, the distinctive blue colouring related to the higher concentration of Fe in the syrup (Kreshkov, 1976) can be used as reasonable basis for further laboratory investigation aiming at revealing such bee honey adulteration (Figures 1 and 2).

According to available literature, the concentration of Cu in bee honey samples in Bulgaria have varied from 0.19 to 0.33 $mg.kg^{-1}$, as follows : 0,19 $mg.kg^{-1}$ (Mladenov, 1978); 0.29-0.33 $mg.kg^{-1}$ (Dinkov et al., 2000); 0.21 ± 0.01 $mg.kg^{-1}$ (Dinkov & Mihaylova, 2009). According to 2022 research on bee honey in Latvia, the concentration levels of Cu were much lover – 0.0999 $mg.kg^{-1}$ (Vaida et al., 2022).

The higher levels of Cu concentration (0.62 $mg.kg^{-1}$), which were found in bee honey produced by bee colonies supplementary fed on sugar syrup inverted with confectionery invertase can be explained by the accumulation of this element in the intestinal tract of bees, a fact that has been validated by other authors as well (Zhelyazkova et al., 2001). Due to prolonged intake of high amounts of Cu, contained in the inverted sugar syrup, apart from accumulation of this element in the intestinal tract of bees, it is possible for similarly increased levels of Cu to enter the hemolymphatic system of bees and thus their salivary gland secretion, which can then lead to increased concentration of Cu in the bee honey they produce (0.62 $mg.kg^{-1}$). The increased concentration levels of Cu in bee honey samples can also be associated with the specifics of bee honey production in the bee hive. The processed flower nectar is periodically put into honeycomb cells, which is also accompanied by a certain level of dehydration, which on its behalf leads to an increase of the level of Cu in the thus produced honey.

These results can also be used for further analysis of yet another negative effect of increased concentrations of Cu: the effect it has on the health state of honey bees, an issue raised by other authors as well (Roman, 2010). The relevance of the issue, as well as the need of closer monitoring has also been supported by the Opinion of the European Food Safety Authority (EFSA), 2018) on the need of lowering the present MRL on Cu concentrations of 5 $mg.kg^{-1}$ to 2 $mg.kg^{-1}$ in both sugar beet sugar and sugar cane sugar. Reference should be made that both types of sugar are commonly used for preparing sugar syrup for supplementing bee feed.

Conclusion

The use of sugar syrup inverted with confectionery invertase as supplementary bee feed is not recommended because it caused increased levels of HMF, which is associated with negative effects on the health state of honey bees (White & Siciliano, 1980), as well as on the quality of bee honey (Dinkov, 2020).

The absorption changes after adding Carrez I and Carrez II solutions, before determining SOA, in combination with the increased Cu concentrations revealed, as well as the bluish colouring, which is an indicator of increased Fe concentrations can be used as indirect indicators of possible direct honey bee adulteration with sugar syrup inverted with confectionery invertase or excessive bee feed supplementation with such syrup.

The high concentration of Cu in the sugar syrup inverted with confectionery invertase and subsequently in the bee honey produced by bee colonies supplementarily fed on such syrup suggests that Cu concentrations transfer from the sugar used. The safety of such honey as natural bee feed is thus compromised (Zhelyazkova et al., 2001). Further studies on Cu accumulation in humans, resulting from consumption of bee honey containing increased levels of Cu are necessary.

Collaboration

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