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Detection of adulterated honey produced by honeybee (*Apis mellifera* L.) colonies fed with different levels of commercial industrial sugar (C_3 and C_4 plants) syrups by the carbon isotope ratio analysis

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ABSTRACT

In the present study, one hundred pure and adulterated honey samples obtained from feeding honeybee colonies with different levels (5, 20 and 100 L/colony) of various commercial sugar syrups including High Fructose Corn Syrup 85 (HFCS-85), High Fructose Corn Syrup 55 (HFCS-55), Bee Feeding Syrup (BFS), Glucose Monohydrate Sugar (GMS) and Sucrose Sugar (SS) were evaluated in terms of the $\delta^{13}C$ value of honey and its protein, difference between the $\delta^{13}C$ value of protein and honey ($\Delta\delta^{13}C$), and C_4% sugar ratio. Sugar type, sugar level and the sugar type * sugar level interaction were found to be significant ($P < 0.001$) regarding the evaluated characteristics. Adulterations could not be detected in the 5 L/colony syrup level of all sugar types when the $\delta^{13}C$ value of honey, $\Delta\delta^{13}C$ (protein–honey), and C_4% sugar ratio were used as criteria according to the AOAC standards. However, it was possible to detect the adulteration by using the same criteria in the honeys taken from the 20 and 100 L/colony of HFCS-85 and the 100 L/colony of HFCS-55. Adulteration at low syrup level (20 L/colony) was more easily detected when the fructose content of HFCS syrup increased. As a result, the official methods (AOAC, 978.17, 1995; AOAC, 991.41, 1995; AOAC 998.12, 2005) and Internal Standard Carbon Isotope Ratio Analysis could not efficiently detect the indirect adulteration of honey obtained by feeding the bee colonies with the syrups produced from C_3 plants such as sugar beet (*Beta vulgaris*) and wheat (*Triticium vulgare*). For this reason, it is strongly needed to develop novel methods and standards that can detect the presence and the level of indirect adulterations.

1. Introduction

Honey, which is accepted as a functional food, is sold at high rates due to its excellent nutritional value. Honey should be unadulterated, hygienic and pure (Codex, 2001; Council Directive 2001/110/EC). However, this valuable product is vulnerable to various adulterations at each stage of production and processing (Anklam, 1998; Bogdanov et al., 2005; Ruiz-Matute, Weiss, Sammatora, Finely, & Sanz, 2010; White & Winters, 1989). The adulteration of honey can be done by direct addition of sucrose syrups that are produced from sugar beet or by the addition of industrial sugar (glucose and fructose) syrups obtained from starch by heat, enzyme or acid treatment (Martin, Macias, Sanchez, & Rivera, 1998; Padovan, De Jong, Rodrigues, & Marchini, 2003; Ruiz-Matute, Soria, Martinez-Castro, & Sanz, 2007) to the honey and by feeding the bee colonies with excessive amounts of these syrups during the main nectar period (Guler, Bakan, Nisbet, & Yavuz, 2007).

Direct adulteration creates unfairness not only for the consumers but also for the pure honey producers (Anklam, 1998; Bogdanov et al., 2005; Guler et al., 2007). This issue has been investigated by many scientists for many years (Cabanero, Recio, & Ruperez, 2006; Elflein & Raezke, 2008; Kerkvliet & Meijer, 2000; Padovan et al., 2003; White & Winters, 1989). In the beginning, the scientists concentrated on different physicochemical parameters like melissopalynological pattern, botanic origin (Kropf et al., 2010; Oddo et al., 2004). sensory analysis (Guler, Bek, & Kement, 2008) sugar profile, amino acid profile, enzyme activities (diastase, invertase), hydroxymethylfurfural and proline (Bogdanov et al., 2005; Cotte, Casabianca, Chardon, Leheritier, & Grenier-Loustalot 2003; Cotte et al., 2004; Guler et al., 2007; Kerkvliet & Meijer, 2000; Oddo et al., 2004; Padovan, Rodriquez, Leme, Jong David, & Marchini, 2007; Raezke & Elflein, 2007). Although each property has its own importance on the quality

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determination of the honey, their analyses are time consuming and not sensitive enough for determination of professional adulteration. To solve the problems discussed above, scientists concentrated on development of new analytical methods like stable carbon isotope ratio mass spectrometry analysis (Doner & White, 1977; White & Doner, 1978). The principle of this method is based on the differences between the $^{13}C/^{12}C$ ratio of C_4 originating from monocotyledonous species of cane sugar and corn, when compared to dicotyledon species (C_3 plants) (Bender, 1971; Elflein & Raezke, 2008; Padovan et al., 2003; Simsek, Bilsel, & Goren, 2012). The isotopic ratio of $^{13}C/^{12}C$ indicates the way of CO_2 fixation and utilization by plants. While the C_3 plants, which are the main nectar providing sources of the honeybees, utilize the Calvin and Benson cycle, the C_4 plants from, which invert sugar syrups are produced, use both the Calvin and Benson cycle and the Hatch–Slack cycle. It is known that the C_4 plants use more CO_2 than the C_3 plants. The $\delta^{13}C$ values of the C_3 and the C_4 plants show variability between $-23‰$ to $-28‰$ and $-9‰$ to $-15‰$, respectively (Cabanero et al., 2006; Ruiz-Matute et al., 2010). There is a general agreement that the difference between the $\delta^{13}C$ value of protein and honey ($\Delta\delta^{13}C$) should not be greater than 1‰, which, above this value, corresponds to 7% adulteration. In addition, the $\delta^{13}C$ value of honey should be more negative than $-23.5‰$ in order to be classified as pure (Cabanero et al., 2006; Padovan et al., 2003; Ruiz-Matute et al., 2010).

Indirect adulteration carried out by excessive supplementary feeding of the bee colonies in the main nectar flow period also creates injustice for both the consumers and the pure honey producers (Bogdanov et al., 2005; Guler et al., 2007; Oddo et al., 2004). The problems on detection of adulteration are also valid for indirectly adulterated honey. In addition, the latter issue has not been investigated in detail. Furthermore, it is not known how honeybees use different levels of these commercial sugar syrups. Also, it is not known which sugars are converted into which type and level of sugars in adulterated honey by honeybees. We do not have adequate knowledge as to whether indirect adulteration (overfeeding bee colonies with industrial sugar syrups) can be determined by using official methods (AOAC 978.17, 1995, AOAC 991.41, 1995; AOAC 998.12, 2005). There have been no published findings regarding the $\delta^{13}C$ value of honey and its protein, difference between the $\delta^{13}C$ value of protein and honey ($\Delta\delta^{13}C$), and C_4% sugar ratio in the indirectly adulterated honey. Furthermore, many scientists (Ozcan, Arslan, & Ceylan, 2006; Ruiz-Matute et al., 2010) have suggested that all these sugars should be tried with bee colonies in the field. Therefore, we decided to use different commercial industrial sugar syrups and levels as bee food during the main nectar flow period in order to answer all the questions discussed above.

Thus, the present study aimed to determine indirect adulteration in the honey produced by feeding honeybees with different levels (5, 20 and 100 L/colony) of various industrial sugars including HFCS-85 (*Zea mays*), HFCS-55 (*Zea mays*), glucose monohydrate (GMS, *Triticium vulgare*), sucrose (SS, *Beta vulgaris*) and bee food syrup (BFS) by using official methods (AOAC 978.17, 1995, AOAC 991.41, 1995, and AOAC 998.12, 2005). We investigated whether these values change with sugar types and levels, and whether indirect adulteration can be detected using the above criteria for different types and levels of industrial sugars.

2. Materials and methods

2.1. Materials

Honey samples were taken from the colonies in the Apicultural Research and Application Unit of the Agriculture Faculty of Ondokuzmayis University, Samsun, Turkey. Colonies were retained in Samsun (41.2°N, 36.20°E) in the Black Sea Region during the winter and spring, and in the vicinity of Gulacar Valley, near Gumushane (40.274°N, 39.29°E), during the nectar flow period (June, July and August). The main sources of nectar-producing plants in the valley were identified by the Department of Biology of the Faculty of Arts and Science, Ondokuzmayis University. The Gulacar valley is rich in nectar-producing plant species (Guler et al., 2007). The main plant resources are *Satureja thybra*, *Lamium album*, *Trifilium ambigum*, *Salvia forskahler*, *Astragalus microcehalus*, *Astragalus asciocalyx*, *Cichorium intybus*, *Thymus leucotrichus*, *Coronilla varia subsp. Varia*, *Salvia amasiaca*, *Melilotus officinalis*, *Medicago varia*, *Teucrium chamaedrys*, *Anchusa azurea*, *Galium verum* and *Veronica peduncularis*.

2.2. Sugar sources

Types, origins, compositions, forms, proportions and company's names of the industrial sugars used in the study are summarized in Table 1.

2.3. Methods

2.3.1. Maintenance and preparation of the colony

The colonies with two aged queen bees of the same genetic origin were used in the study. All of the environmental factors (frames covered with adult bees, frames covered with brood, foundation comb, drugs, transport) were standardized, and all of the maintenance and control procedures were performed by the same staffs.

The colonies were settled in empty beehives with bees, brood and honey frames. Standard bee-feeding methods were applied in the early spring (Guler, 2008; Sammataro & Avitabile, 1998). Twelve kilograms of sucrose syrup (1:1.5 w/w, water:sugar) were given to each colony in March and April to ensure the growth and strength of the forage worker bee population of colonies for the main nectar flow season. The honey and honeycomb frames of all colonies were taken at the end of May (Guler, 2008). After settling bees in the hives, cake and syrup were not further provided to the colonies. Transporting and shaking processes were done with standard beehives (Langstroth), and veterinary drugs were not used for any honeybee diseases. The processes were applied to all groups on the same day. Ten days after all these arrangements, colonies were transported (470 km) to another region (Gulacar Valley, Gumushane) in order to obtain rich and unpolluted nectar sources.

In the study, pure blossom honey (PBH), which was produced in the same environmental conditions by applying the shaking method (Guler, 2008), was used as the control. As with the other commercial industrial sugar groups, feeding, development and maintenance of the bee colonies were conducted up to a predetermined stage in the spring season, and after that point, the queen and worker bees together were shaken to the empty hives. After this process, no syrup, cake or chemicals were given to the colonies. Only comb foundations were given to the colonies. The honey produced in this study was regarded as a polifloral honey as there were many plant species in the region in which this study was conducted. In total, 130 colonies (ten colonies for the pure or control group and eight colonies for each of the three levels of five sugar syrup types; $10 + 5 * 3 * 8 = 130$) were used in the study.

2.3.2. Syrup levels and preparation

These industrial commercial sugars and levels were used for the first time as a bee food supplement during the main nectar flow period in this study. In all treatments, syrups were prepared daily, were mixed often, left for one day and finally were given to the colonies at 6:00–7:00 pm. Levels of 5, 20 and 100 L/colony of HFCS-85, HFCS-55, GMS, SS and BFS were used in the study. These levels were selected on the basis of syrup amounts that are used by