Measurement of Carbon Isotope Ratios of Honey Samples from Turkey by EA-IRMS

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Abstract

The authenticity of bee products is specially important. The major concern about honey quality is to ensure that honey is authentic in respect to the legislative requirements. Recently, the analysis of stable isotopes by isotope ratio mass spectrometry coupled to elemental analyzer (EA-IRMS) has been developed for the authenticity proof of different foods, leading to determine the geographical origin of honey and for the detection of honey adulteration. In the present study, honey samples from different cities across Turkey were analyzed by EA-IRMS. The acceptable difference in $^{13}C/^{12}C$ values between honey and its associated protein extract is 1‰, showing that the honey is pure or not. All the samples were found to be pure as the differences were below 1‰. The $\delta^{13}C$ (‰) values were found to differ from city to city.

Keywords: Honey, authenticity, adulteration, carbon isotope ratio, EA-IRMS

1. Introduction

Honey consumption has increased during the past decade as consumers prefer natural and pure products with no additives or preservatives. Although the adulteration of honey is not injurious to health, problems of honey fraud negatively influence market growth by damaging consumer confidence (Cabanero et al., 2006). The authenticity of honey is important for a honey consumer, in agreement with its declared botanical and geographical origin. It is also of a great importance for the commercial part of honey production (Chudzinska and Baralkiewicz, 2010).

The isotope ratio of $^{13}C/^{12}C$ is expressed as $\delta^{13}C$ in the unit of ‰ (per mil), representing a deviation from an internal standard, Vienna Pee Dee Belemnite (VPDB) (Jacob et al., 2000). The formula of $\delta^{13}C$ and the degree of adulteration for honey are given as follows (Cabanero et al., 2006; Padovan et al., 2003):

$$\delta^{13}C\ (‰)=[\ (^{13}C/^{12}C_{\ sample})/(^{13}C/^{12}C_{\ standard})-1]\times 10^3$$

$$\%adulteration=[(\delta‰protein-\delta‰honey)/(\ \delta‰protein-\delta‰sweetener)]\times 100$$

Stable isotope ratio mass spectrometry (IRMS) was accepted as as an official method by Association of Official Analytical Chemistry for the detection of honey adulteration. It was introduced in the 1980s, which is based on the difference between $^{13}C/^{12}C$ ratio of C_4 originates from monocotyledonous species of sugar cane and corn, when compared to dicotyledons species (C_3 plants) (AOAC Official Method 998.12,2005; Padovan et al., 2003).

As the carbon isotope ratios of C_4 and C_3 plants in photosynthetic cycles are different, a natural honey is expected to have the characteristic properties of C_3 plants since bees collect their nectar from flowering plants of dicotyledons. The $^{13}C/^{12}C$ ratio, in the range of 1-99 in nature, reflects the photosynthetic cycles. In Calvin and Benson cycle (C_3), atmospheric CO_2 enters into the cycle through ribulose-1-5-diphosphate, forming a six-carbon intermediate, rapidly breaking into two molecules of 3-phosphoglycerate, then converting to carbohydrate by ribulose-1,5-diphosphate carboxylase. In Hatch-Slack cycle (C_4), atmospheric CO_2 enters into the cycle through phosphoenolpyruvate, forming oxalic acid, then converted to malic and aspartic acids. C_3 and C_4 cycles are observed in plants with Crassulacean Acid Metabolism (CAM). $^{13}C/^{12}C$ (δ) ratios of C_3 and C_4 change from -23 to -28 ‰ and -9 to -15 ‰, respectively, indicating that the absorption of CO_2 by C_4 plants during the photosynthesis is relatively more than C_3 plants (Cabareno et al., 2006; Ruiz-Matute et al., 2010).

As bees mainly produce honey from C_3 plants, honey samples having $\delta^{13}C$ less than -23.5 ‰ are under suspicion. The difference between stable carbon isotope ratios ($^{13}C/^{12}C$, ‰) of a honey sample and its protein fraction should not be more than 1 ‰. Comparison of the $^{13}C/^{12}C$ (δ) ratio of a honey sample and its ratio of a honey sample and extracted proteins enables us to suspect adulteration. The honey samples were considered adulterated when the δ in the honey sample compared to the protein was above 1‰ (Padovan et al., 2003;2007).

In this study, honey samples from beekeepers from different regions of Turkey were analyzed by EA-IRMS. $\delta^{13}C$ (‰) values of honey and its associated protein extracts were determined to indicate the geographical distribution of honey across Turkey and as well as honey adulteration.

2. Materials and methods

Honey samples were collected different regions of Turkey.

Protein was extracted from honey according to AOAC (2005) method 998.12. Sample (10-12 g) was filtered into a centrifuge tube of 50 ml and the residue was washed by distilled water (3×4=12 ml). 2 ml of freshly prepared sodium tungstate (10%) and 2 ml of H_2SO_4 (0.335M) were added into the centrifuge tube and vortexed. Then it was incubated in a water bath at 80ºC until a clear solution appeared. In case a clear solution did not appear, 2 ml of of H_2SO_4 (0.335M) was added to the solution. 30 ml of distilled water was added and centrifuged at 1500*g* for 15 min. Supernatant was discarded and the precipitate was separated by a spatula from the tube and was dried in an oven at 75ºC for 3 h.

Total $^{13}C/^{12}C$ of honey and protein samples were analyzed by Thermo- Flash EA 1112 HT elemental analyzer coupled via a Thermo ConFlo IV Interface to a Thermo DELTA V Plus isotope ratio mass spectrometer. 200 μg honey and protein sample were weighed in small tin capsules using a Sartorius CPA2P ultra microbalance and placed into the auto-sampler unit of elemental analyze (EA). Sucrose (IAEA-CH-6), Sucrose (Fluka),and Sucrose (Sigma) were used as reference standards for linear calibration curve. Samples and reference standards were measured three times.

3. Results and discussion

The honey samples were collected directly from beekeepers in Turkey (Table 1). The carbon isotope ratios of honey samples in Antalya were found to range from -24.420 to -24.980 ‰ and protein extracts from -24.950 to -24.924 ‰. While the highest stable isotope ratio was found to be -26.454 ‰ in the honey sample from Denizli, the lowest was -24.420‰ from Antalya. Samples from Diyarbakır showed -24.831 to -26.443 ‰ of $\delta^{13}C$ values for honey samples and -25.361 to -26.482 ‰ of $\delta^{13}C$ values for protein extracts. These values were acceptable; as bees harvest nectar from plants, which are C3 plants, this yields carbon isotope values averaging -25.3 ‰ while C4 plants (such as corn syrup) are significantly at around -10‰, as shown in Fig. 1.

Table 1. $\delta^{13}C$ values of honey samples and their proteins from different regions in Turkey

Locality of honey	Location number	Type of honey	$\delta^{13}C$ values for honey (‰)	$\delta^{13}C$ values for protein extracted from honey (‰)	Differences in $\delta^{13}C$ (‰) values (honey-protein)	Honey quality
Adapazarı	1		-25.125	-25.780	0.655	P
Ağrı	2	2a	-24.967	-25.296	0.329	P
		2b	-25.782	-25.911	0.129	P
		2c	-24.925	-24.905	0.020	P
		2d	-25.277	-25.229	0.048	P
Antalya	3	3a	-24.420	-24.950	0.530	P
		3b	-24.980	-24.924	0.056	P
Ardahan	4		-25.626	-25.791	0.165	P
Diyarbakır	5	5a	-26.443	-26.482	0.039	P
		5b	-24.831	-25.361	0.43	P
		5c	-26.08	-25.725	0.355	P
Denizli	6	6a	-25.670	-25.781	0.111	P
		6b	-26.454	-26.060	0.394	P
Ordu	7	7a	-25.117	-25.291	0.174	P
		7b	-26.155	-25.793	0.362	P
Sivas	8	8	-24.690	-24.693	0.003	P
Tunceli	9	9a	-24.596	-24.043	0.553	P
		9b	-25.438	-24.889	0.549	P
		9c	-25.462	-25.430	0.032	P
		9d	-25.261	-25.130	0.131	P
		9e	-25.373	-25.173	0.200	P

As seen in Fig. 1, if a pure honey has been adulterated with corn syrup, the isotope ratio will fall between these two values, proving adulteration (Anonymous,

2013). The range of values found for bee-produced honey was -21.96‰ to -30.47‰ for C3 plants and -11.82‰ to -19.00‰ for C4 plants (Padovan et al., 2003).

-25	-17.5	-10
100% honey	$\delta^{13}C$	100% corn syrup

Figure 1. A scheme representing $\delta^{13}C$ values (Anonymous, 2013)

The difference in $\delta^{13}C$ (‰) values was highest for the sample from Adapazarı (0.655). The samples from Ağrı, Ardahan, Denizli, Ordu and Tunceli showed around 0.1-0.2 of difference in $\delta^{13}C$ (‰) values. The finding for Ardahan was in agreement with Simsek et al.(2012), indicating 0.1-0.2 difference in $\delta^{13}C$ (‰) values. The authors found the highest difference (0.84‰) in Antalya.

For the same samples having very close values for honey and protein as observed in Ağrı (0.020;0.048), Tunceli (0.032) and Diyarbakır (0.039), honey is considered as pure. Bees produce all protein in honey by reactions between enzymes and the nectar, resulting in very close values for pure honey. The addition of corn or cane sugar syrups to pure honey will change its carbon isotope ratio composition, but not its protein composition (White and Winters, 1989; White, 1992). Tosun (2013) pointed out the difficulty in detecting adulteration of honey with C_3 sugar syrups since stable carbon isotope ratio analysis fails to detect the adulteration of honey with C_3 sugar syrups although it is useful in detecting the adulteration of honey with C_4 sugar syrups.

This study reports the $\delta^{13}C$ (‰) values of honey samples collected from beekeepers across Turkey. The $\delta^{13}C$ (‰) values were found to differ from city to city. The samples from beekeepers showed no sign of adulteration. The difference in $^{13}C/^{12}C$ between honey and its associated protein extract was lower than 1 ‰.

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