STUDIA UBB CHEMIA, LVI, 3, 2011 (p. 157 - 163)

DETECTION OF SOME ROMANIAN HONEY TYPES ADULTERATION USING STABLE ISOTOPE METHODOLOGY

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ABSTRACT. 12 Romanian samples of honeys from different floral sources were analyzed for the determination of high fructose corn syrup (HFCS) adulteration of honey, by coupling an isotope ratio mass spectrometer to an elemental analyzer. Using the difference in stable carbon isotope ratio ($\delta^{13}C$) between honey and its protein fraction it is possible to evaluate the adulteration with small extent (minimum 7%). An obvious adulteration was observed for the multifloral honey, while for other 5 samples the negative difference indicated that these samples are not adulterated. For the other 6 samples, the positive difference indicates the absence of adulteration**.**

Keywords: *honey; adulteration; carbon isotope ratio; δ13C.*

INTRODUCTION

Among the numerous sectors affected by fraud, foods are not exempt and for the same reason: considerable financial gains. Beyond the harmful economic consequences for honest producers who comply with regulations, fraud in the food sector can have serious effects on public health, since certain added components may be toxic.

Honey is not produced in large quantities and is not a high added value product. Nevertheless, the product is not immune to fraud that involves the addition of syrups from a variety of sources (sugar cane, corn or beets). Fraud by spiking with syrups increased considerably in the 1970s as a result of massive production of these syrups. The market was flooded with low cost-price products of higher quality and whose oligosaccharide composition was close to those of native honey (case of totally invert sugars). The difficulty in detecting adulteration arises from the precise separation of these sugars with practically identical compositions [1]. For the detection of

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these adulterations a very precise method was developed by Doner and White in 1977 [2] based on the analysis of the stable carbon isotopic ratio analysis by mass spectrometry (SCIRA-MS).

Depending on their origin, sugars added are divided into two types: C3 and C4. Sucrose and the natural sugars of honey belong to the C3 type while cane sugar and sugars produced from the hydrolysis of maize starch are of the C4 type [3]. Maize and sugarcane metabolize by the Hatch-Slack or C4 metabolic pathway. The isotopic technique for the detection of adulterated honey is based on the natural differences in isotopic ratios between plants using C3 and C4 photosynthetic pathways. Generally, C4 plants, for example, corn, have $^{13}C/^{12}C$ isotopic ratios, referred to as $\delta^{13}C$ values, ranging between -8 and -13‰, whereas C3 plants, generally nectar-bearing plants, have values between -22 and -30‰ [4]. For the detection of adulterated honey (addition of C4 plant sugars such as cane sugar and corn syrup), an internal standard $^{13}C/^{12}C$ isotope ratio method was developed [5]. This procedure utilizes the $\delta^{13}C$ value of the protein extracted from the honey as an internal standard, the purity of the honey is then judged in comparison to the $\delta^{13}C$ of the corresponding whole honey [6-9]. The method currently used, allows the detection of 7-10% adulteration with cane sugar or maize syrups. Since bees produce all proteins in honey by reaction between enzymes and nectar, the isotope ratio of honey and its protein will have very close values if honey is pure [10]. Addition of C4 sugars will affect just the isotopic ratio of the honey, but not its protein composition.

In this work, as different sources of honey can have different $\delta^{13}C$ values, it was considered as appropriate to include four types of Romanian honey in this initial study to establish a baseline range of $\delta^{13}C$ values for Romanian honeys. For this purpose 12 honey samples from four different floral types were analyzed.

RESULTS AND DISCUSSION

From the 12 samples analyzed, 6 samples have had the difference between $\delta^{13}C$ protein and $\delta^{13}C$ honey positive, indicating the absence of adulteration. At five samples the negative values obtained, range -0.06 to -0.98, gives us the reason to calculate apparent adulteration, the values obtained being between 0.38 and 6.39%. Since these differences are below the internationally accepted threshold of - 1 ‰ (7 % adulteration), the samples are considered not adulterate. For sample number 5 the adulteration is higher than the bench-mark, the calculated value being 10.81%, this indicating the presence of added C4 sugars.

Regarding the honey authenticity related to the botanical origin, the variations of parameters are presented in Table 2. The lowest values were obtained for honeydew and the highest for tilia.

Carbon isotopic ratios of honey protein are also influenced by the climate, and can be used as a discriminating factor to determine the geographical origin of honey [11] in addition with the values of other isotopes [12,13]. Depending on the climate area, relatively large differences (1, 8‰) have been shown in samples from different European regions even for the same floral type of honey [13].

Table 1. Experimental data obtained for the analysis of honey and its protein

No.	Botanical origin	$\delta^{13}C_{protein}$	Average $\delta^{13}C_{protein}$	$\delta^{13}C_{honey}$	Average $\delta^{13}C_{honey}$	Difference $\delta^{13}C_{protein} - \delta^{13}C_{honey}$	Adulteration (%)
1	Honeydew	-24.97 -25.17	-25.07	-25.21 -25.23	-25.22	0.15	-
2	Acacia	-24.77 -24.75	-24.76	-24.35 -24.16	-24.26	-0.5	-
3	Honeydew	-25.42 -25.47	-25.45	-25.16 -25.09	-25.12	-0.33	-
4	Tilia	-24.54 -24.51	-24.53	-25.87 -25.83	-25.85	1.32	-
5	Multifloral	-25.83 -25.95	-25.89	-24.17 -24.10	-24.14	-1.75	10.8
6	Honeydew	-25.06 -25.05	-25.06	-26.50 -26.47	-26.48	1.42	-
7	Acacia	-24.86 -24.82	-24.84	-25.98 -25.97	-25.98	1.14	-
8	Tilia	-25.59 -25.74	-25.67	-25.60 -25.62	-25.61	-0.06	-
9	Honeydew	-25.16 -25.16	-25.16	-25.85 -25.89	-25.87	0.71	-
10	Honeydew	-25.77 -26.06	-25.91	-25.84 -25.74	-25.79	-0.12	-
11	Honeydew	-25.52 -25.32	-25.42	-25.80 -25.80	-25.80	0.38	-
12	Tilia	-24.93 -25.15	-25.04	-24.11 -24.01	-24.06	-0.98	-

We found $\delta^{13}C$ values for the acacia honey protein in the range of that from literature. The $\delta^{13}C$ values for acacia honey protein from France [1]

varies between -23.55 ‰ and -25.78 ‰, similar values being found for acacia honey protein from different regions of Europe [13]. In the case of honeydew protein the mean value of $\delta^{13}C$ obtained by us was -25,35 ‰, close to that from Spain, (-24.7‰)[14]. Also comparable values to that obtain by us were also found in two regions from Slovenia, -25.6‰ and -24.7‰ [12].

Table 2. Range of $\delta^{13}C$ for different botanical origin of honey

No.	Botanical origin	$\delta^{13}C_{protein}$		$\delta^{13}C_{honey}$	
		max.	min.	max.	min.
1	acacia	-24.76	-24.84	-24.26	-25.98
2	tilia	-24.53	-25.67	-24.06	-25.85
3	honeydew	-25.06	-25.91	-25.12	-26.48
4	multifloral	-25.83	-25.95	-24.10	-24.17

The negative differences in $\delta^{13}C$ values can be due to isotope effects that occur in the synthesis of protein material from different dietary constituents, differences in pollen content from various sources or from feeding C4 plant sugars syrups to bees at the start of the season to build up colony strength. It is known that $\delta^{13}C$ values of animals and their products are dependent [9,15,16] on their diet. Some differences are due to isotopic effects in the synthesis of the protein from dietary constituents, but these effects are reduced because usually the honey is collected from more than one colony, from different locations on a period of several weeks. At the beginning of spring, when bees are fed with sugar syrups, the protein produced by the bees may reflect the isotopic composition of the food, but the bee population being constantly renewed at 3-4 weeks and fed with honey collected previously, the influence of corn sugars to the $\delta^{13}C$ values of the proteins is diluted quickly [5].

The results, obtained for investigated honey samples, pointed out that the mean value of honey protein for our samples (-25,35‰) is very close to that found for samples from temperate climate areas (-25.4‰ in Limousin, France and -26.1‰ in Allgau, Germany [13]).

It is internationally accepted that adulteration with C4 sugars is not confirmed unless the negative difference between the honey and protein $\delta^{13}C$ values is equal to or more negative than -1 ‰. When the differences between $\delta^{13}C$ values are positive, they indicate the absence of adulteration with C4 sugars, being considered as zero.

CONCLUSIONS

In this work 12 Romanian honeys from different floral sources and their corresponding protein extracts were analyzed in order to determine the presence of adulteration. The detection of honey adulteration with HFCS was realized by coupling an isotope ratio mass spectrometer to an elemental analyzer. Using the difference in stable carbon isotope ratio between honey and its protein fraction it is possible to evaluate the adulteration of honey with small amounts (minimum 7%).

The results of the 12 analyzed samples with different geographical origin (acacia, tilia, honeydew, multifloral) showed an obvious adulteration for the multifloral honey, while for other 5 samples a negative difference was observed between $\delta^{13}C$ protein si $\delta^{13}C$ honey, but lower than the internationally accepted value of -1‰ , indicating that for this samples no C4 sugars were added. For the other 6 samples the differences between the $\delta^{13}C$ values of the honeys and their corresponding protein extracts were positive, indicating the absence of adulteration. Also, the obtained results denoted (indicated) that the mean value of honey protein of our samples correspond to that obtained for samples from temperate climate areas.

EXPERIMENTAL SECTION

The detection of sugar adulteration of honey can be realized by measuring the difference between the isotope ratio value of the whole honey sample and that of its separately prepared protein fraction. The procedure consists in diluting a known amount of honey in distilled water adding sodium tungstate solution and sulphuric acid to precipitate proteins. This solution is immersed in an 80ºC water bath until visible flocculates form. After the proteins are separated and dried. To measure the whole honey, the honey samples are placed in an oven for drying [17-18].

The analyzed CO_2 was obtained by a microcombustion technique. The samples, whole honey and its protein fractions, were loaded into Sn capsules and moved into the quartz tube of an elemental analyzer (NA1500 NCS, Carlo Erba Instruments) where the samples were combusted in a stream of oxygen at 1020ºC. The quartz tube was filled with tungstic oxide on alumina and elemental Cu where the oxygen excess was reduced. On the PORAPAQ column the resulting mixture of combustion gases were separated (due to the different times of retention of the gases on the column). The CO_2 obtained was trapped in an ampoule at liquid nitrogen temperature. The water resulting from the combustion reaction was absorbed by a $MgClO_4$ trap. CO_2 was purified and before connecting the ampoule containing the sample to the mass spectrometer a gas pressure adjustment was made [19].

In this work we used the on-line technique, which is called EA-IRMS, i.e. Elemental Analyzer coupled with Isotope Ratio Mass Spectrometry.

This method makes it possible to eliminate the nuisance of off-line purifying procedures and gives an easy, fast, comfortable and more precise way to measure our samples. This solution allows the measurement the $\delta^{13}C$ value of original honey samples as low as 10 gram. However we needed only 2 mg of prepared (flocculated and/or dried) samples to measure in.

^{13}C to ^{12}C ratio was measured in a Delta Plus type mass spectrometer developed by Thermo Finnigan, coupled with Carlo Erba Elemental Analyzer. The spectrometer has a dual inlet system and triple ion collector (the ion signals are measured simultaneously). The carbon stable isotope ratio analysis values are reported with respect to the VPDB international standard. However the laboratory utilizes our own working standard gas in daily operations.

The apparent sugar content might be calculated as follows:

$$Adulteration[\%] = \left[\frac{\delta^{13}C_{protein} - \delta^{13}C_{honey}}{\delta^{13}C_{protein} - (-9.7)} \right] \times 100$$

where the $\delta^{13}C$ value of -9.7 ‰ is derived from the mean value for the high fructose corn syrup.

To establish the reproducibility of the technique 2 replicates of each honey samples were analyzed.

ACKNOWLEDGMENTS

Financial support for this work was provided by the PN II Program, Contract No. 52-1040/ 2008 (IZOTRAS).

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