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Carbon isotopic composition of Mexican honey

Edith Cienfuegos; Isabel Casar; Pedro Morales

Laboratorio de Espectrometría de Masas de Isótopos Estables[1], Instituto de Física, Universidad Nacional Autónoma de Mexico, Apartado Postal 20-364, 01000 Mexico

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SUMMARY

We characterized Mexican honey from the main production regions, using the internal stable carbon isotope ratio (ISIRA) of the honey and its protein. We found the average $\delta^{13}C$ given by White for American honey is very close to the corresponding value for Mexican honey, even though clear evidence that C_4 and CAM plants are visited by bees. We found adulteration of Mexican commercial samples using the criterium of –1.00 ‰ difference also given by White. Two pyrolysis methods were tested to optimize the accuracy of the carbon isotope ratio analysis of honey. We found that the quartz method showed smaller variation in the data due to a complete combustion of the honey. The honey protein was isolated and purified using the protein dialysis procedure recommended by the Association of Official Analytical Chemists. We found that the direct precipitation method without dialysis is not useful for samples which are adulterated by more than 50%.

Keywords: honey, protein, carbon isotope ratio, Mexico

[1]New address: Instituto de Geología, Universidad Nacional Autónoma de Mexico, Apartado Postal 70-296, 04510 Mexico

INTRODUCTION

Due to its tropical climate, water availability and wide variety of year-round flowering plants, Mexico's honey production amounts to nearly 50 000 tons per year from which almost 70% are exported to Europe and USA. This honey is produced in about 2.1 million bee colonies owned by nearly 45 000 beekeepers, from which only a two dozen are commercial honey packers and processors; approximately 1000 are professional beekeepers and the rest are farmers for which honey provides an economically important income and pollination for their crops, they are organized in co-operative production groups.

Great efforts have been made by the government and several apicultural associations in the main honey producing regions to regulate production, not only because in the international market unclassified honey attracts a lower price, but also to ensure the integrity of the production and to avoid adulteration with low-cost sweeteners such as high fructose corn syrup (HFCS) and cane sugar derivative products.

A number of parameters should be analysed to demonstrate honey adulteration. Among these parameters, stable isotope ratio analysis in honey has proven to be useful in detecting the addition of HFCS or cane sugar to honey, and it has been adopted as a method by the Association of Official Analytical Chemists (AOAC) (White & Doner, 1978).

It is well established that the $\delta^{13}C$ values of animal tissues and their products are dependent on the amount of $\delta^{13}C$ in their diet (Minson *et al.*, 1975; De Niro & Epstein, 1978). This means that, honey and its protein will reflect the carbon-13 content of the plants from which the bee collects the nectar and pollen. The plants follow three different photosynthetic fixation pathways (C_3, C_4 & CAM) which have differing amounts of carbon-13. Products of a C_3 or Calvin photosynthetic cycle have $\delta^{13}C$ values (the ratio between two stable isotopes of carbon is conventionally expressed as $\delta^{13}C$, ‰) that range from –22‰ to –35‰ with a mean value of –27.8‰ ± 1.5‰. Cane sugar, corn, sorghum and some other tropical plants exhibit the C_4 photosynthetic pathway discovered by Hatch-Slack (Bender, 1971; Krueger & Reesman,1982), with an average $\delta^{13}C$ value of –13.5‰ ± 1.5‰. Cactus and succulent plants exhibit the CAM (crassulacean acid metabolism) photosynthetic pathway, which shows a large variation in $\delta^{13}C$ since their values depend on which carboxylation enzyme is preferentially involved.

In 1989, White and Winters improved the sensitivity of the original method of using the $\delta^{13}C$ value of the honey (C_3 plants) to detect the presence of C_4 sugars using internal standard isotope ratio (ISIRA) by isolating the protein from the honey and comparing its carbon-13 content of the honey (when there is no adulteration the $\delta^{13}C$ of honey is the same as the $\delta^{13}C$ of the protein). This method permits an objective evaluation of possible adulteration. In 1992, White included the ISIRA method in a collaborative study and described in full the dialysis procedure used.

The aim of this paper is to use the ISIRA procedure to study the $\delta^{13}C$ of Mexican honey and its natural variations and to compare the results with the studies done by White, given that there are many arid and semiarid production regions in Mexico where some of the plants exhibit the C_4 and CAM pathway, which are not present in the temperate regions of North America where White performed his study. Special care will be taken to detect a C_4 label on the honey due to the presence of C_4 and CAM plants.

MATERIALS AND METHODS

Sampling

Certified honey samples were taken from the main honey producing States of Campeche, and Yucatan, as well as from other states with arid and semiarid ecological settings. Special sampling was made in areas located near cane sugar plantations, where the honey harvested might show a C_4 or CAM label.

Certified honey was collected directly by the Campeche University from small communities of honey

TABLE 1. Comparison of pyrolysis methods.

Quartz at 900°C for 2 hr and 650°C for 1 h			Pyrex 550°C for 2 h		
Sample	***n***	**$\delta^{13}C_{PDB}$(‰) ± σ**	**Sample**	***n***	**$\delta^{13}C_{PDB}$(‰) ± σ**
RM 8539 NBS-22	10	–29.72 ± 0.02	RM 8539 NBS-22	10	–29.78 ± 0.08
Honey	10	–26.15 ± 0.02	Honey	10	–25.98 ± 0.14
RM 8540 PEF1	10	–31.82 ± 0.02			
RM 8542 Sucrose. ANU	10	–10.39 ± 0.01			

n = number of samples

TABLE 2. $\delta^{13}C_{PDB}$ (‰) on mixtures of cane sugar.

Cane sugar weight %	$\delta^{13}C_{PDB}$(‰) honey	$\delta^{13}C_{PDB}$(‰) protein dialysis-precipitation	Adulteration, %[1]	$\delta^{13}C_{PDB}$(‰) protein direct-precipitation	Adulteration, %[1]
0	–26.15	–25.79	0.00	–25.67	0.00
25	–21.89	–25.79	26.97	–25.54	25.69
50	–18.19	–25.70	52.26	–25.44	51.38
75	–14.65	–25.72	76.93	–23.12	71.84
100	–11.33		100.00		100.00
		–25.75 ± 0.04		–24.94 ± 1.06	

[1]To calculate adulteration %, we used equation 1 with the following values: $\delta^{13}C$ of the honey = –26.15 ‰ and the $\delta^{13}C$ of the cane sugar adulterant = –11.33 ‰

TABLE 3. $\delta^{13}C_{PDB}$ (‰) on mixtures of high fructose corn syrup (HFCS).

HFCS weight %	$\delta^{13}C_{PDB}$(‰) honey	$\delta^{13}C_{PDB}$(‰) protein dialysis-precipitation	Adulteration, %[1]	$\delta^{13}C_{PDB}$(‰) protein direct-precipitation	Adulteration, %[1]
0	–26.15	–25.79	0.00	–25.67	0.00
25	–22.39	–25.79	21.99	–25.54	20.71
50	–18.54	–25.79	48.90	–25.46	45.74
75	–14.58	–25.78	72.49	–23.16	66.87
100	–10.33		100.00		100.00
		–25.79 ± 0.004		–24.96 ± 1.04	

[1]To calculate adulteration %, we used equation 1 with the following values: $\delta^{13}C$ of the honey = –26.15 ‰ and the $\delta^{13}C$ of the HFCS adulterant = –10.33 ‰

farms in the state of Campeche and Yucatan (the largest honey producers with approximately 30% of the Mexican honey production). Sixty-three certified samples were collected as well as leaves from 42 nectar producing plants flowering during the whole year. The ecological setting of Campeche and Yucatan is a tropical environment with many species of flowering plants. The carbon-13 cycle to which these plants belong has not been determined for many of them. Among these are the flowering varieties which produce honey during the year of this area, three of them are mostly unifloral Dzidzilché (*Gymnopodium antigonoides*), Tajonal (*Viguiera helianthoides*) and Xtabentum (*Rivea Corymbosa*).

Also, for other regions, the sampling was performed by beekeepers from different Mexican States. Other samples were commercial brands of honey bought directly from stores.

Pollen and honey samples were taken from the Yautepec-Jojutla valley in the state of Morelos. In this valley

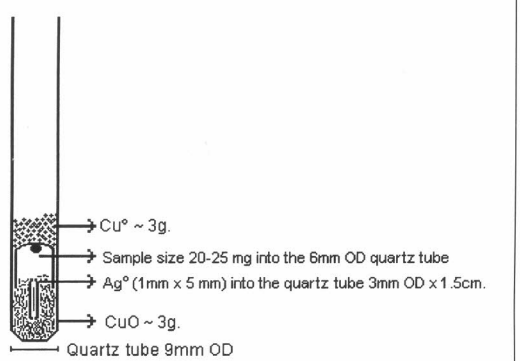

FIG. 1. Tube assembly.

TABLE 4. $\delta^{13}C_{PDB}$ of certified honeys and their protein fractions from the Yucatan peninsula.

State of origin	Plant	$\delta^{13}C_{PDB}$(‰) honey	$\delta^{13}C_{PDB}$(‰) protein	Difference protein-honey	Apparent corn/cane content %[1]
Campeche	Xtabentun[2]	–26.36	–26.27	0.09	
Campeche	Xtabentun,Tajonal	–26.14	–26.11	0.03	
Campeche	Tajonal	–26.05	–25.49	0.56	
Campeche	Tzalam[5],Dzidzilcé[3]	–25.03	–24.83	0.20	
Campeche	Box-Katsim	–25.82	–26.20	–0.38	2.30
Campeche	Solen-Ak,Sak-Ak	–26.39	–26.09	0.30	
Campeche	Tajonal[4]	–26.35	–25.78	0.57	
Campeche	Dzidzilché, Habín[6]	–24.44	–24.46	–0.02	0.14
Campeche	Dzidzilché, Habín	–24.38	–24.67	–0.29	1.94
Campeche	Dzidzilché, Habín	–24.36	–24.48	–0.12	0.81
Campeche	Dzidzilché, Habín	–24.26	–24.23	0.03	
Campeche	Dzidzilché, Habín	–24.33	–24.42	–0.09	0.61
Campeche	Dzidzilché Tajonal	–25.26	–24.99	0.27	
Campeche	Dzidzilché, Tajonal	–25.48	–25.12	0.36	
Campeche	Dzidzilché, Tajonal	–26.00	–25.75	0.25	
Campeche	Dzidzilché, Tajonal	–26.08	–25.98	0.10	
Campeche	Dzidzilché, Tajonal	–26.12	–25.95	0.17	
Campeche	Dzidzilché, Tajonal	–26.23	–25.47	0.76	
Campeche	Dzidzilché, Tajonal	–26.03	–25.68	0.35	
Campeche	Dzidzilché, Tajonal	–26.26	–25.98	0.28	
Campeche	Dzidzilché, Tajonal	–26.35	–25.76	0.59	
Campeche	Dzidzilché, Tajonal	–25.93	–25.48	0.45	
Campeche	Dzidzilché, Tajonal	–25.86	–25.17	0.69	
Campeche	Dzidzilché, Tajonal	–26.26	–25.62	0.64	
Campeche	Dzidzilché, Tajonal	–25.99	–25.48	0.51	
Campeche	Dzidzilché, Tajonal	–25.79	–25.29	0.50	
Campeche	Dzidzilché, Tajonal	–25.93	–25.20	0.73	
Campeche	Dzidzilché, Tajonal	–26.15	–26.20	–0.05	0.30
Campeche	Dzidzilché, Tajonal	–25.44	–24.92	0.52	
Campeche	Dzidzilché, Tajonal	–25.84	–25.09	0.75	
Campeche	Dzidzilché, Tajonal	–25.41	–24.94	0.47	
Campeche	Dzidzilché, Tajonal	–24.89	–24.76	0.13	
Yucatán	Thousand flowers	–24.90	–24.94	–0.04	0.26
Yucatán	Thousand flowers	–25.66	–25.82	–0.16	0.99
Yucatán	Thousand flowers	–26.20	–26.07	0.13	

State of origin	Plant	$\delta^{13}C_{PDB}$(‰) honey	$\delta^{13}C_{PDB}$(‰) protein	Difference protein-honey	Apparent corn/cane content %[1]
Yucatán	Thousand flowers, Tajonal	–26.39	–26.45	–0.06	0.36
Yucatán	Thousand flowers,Tajonal	–25.21	–25.77	–0.56	3.48
Yucatán	Tajonal	–25.91	–25.06	0.85	
Yucatán	Tajonal	–25.66	–25.10	0.56	
Yucatán	Tajonal	–26.00	–25.14	0.86	
Yucatán	Tajonal	–25..75	–26.35	–0.60	3.60
Yucatán	Tajonal	–25.64	–24.92	0.72	
Yucatán	Tajonal	–25.88	–25.48	0.40	
Yucatán	Tajonal	–25.96	–26.45	–0.49	2.93
Yucatán	Tajonal	–25.38	–25.03	0.35	
Campeche	Dzidzilché, Tajonal	–25.63	–26.01	–0.38	2.33
Campeche	Dzidzilché, Tajonal	–25.53	–24.93	0.60	
Campeche	Dzidzilché, Tajonal	–25.49	–25.00	0.48	
Campeche	Dzidzilché, Tajonal	–25.87	–25.11	0.76	
Campeche	Dzidzilché, Tajonal	–25.59	–25.37	0.22	
Campeche	Dzidzilché, Tajonal	–25.60	–25.09	0.51	
Campeche	Dzidzilché, Tajonal	–25.54	–25.18	0.36	
Campeche	Dzidzilché, Tajonal	–25.43	–24.08	1.35	
Campeche	Dzidzilché, Tajonal	–25.42	–25.05	0.37	
Campeche	Dzidzilché, Tajonal	–25.45	–25.63	–0.18	1.13
Campeche	Dzidzilché, Tajonal	–25.57	–25.79	–0.22	1.37
Campeche	Dzidzilché, Tajonal	–25.44	–25.12	0.32	
Campeche	Dzidzilché, Tajonal	–25.95	–25.73	0.22	
Yucatán	Thousand flowers	–24.74	–25.35	–0.61	3.90
Yucatán	Tajonal	–24.63	–24.71	–0.08	0.53
Nayarit		–26.15	–25.79	0.36	
Ags.	Orange[7]	–22.56	–22.55	0.01	
Veracruz	Orange	–23.47	–22.55	0.92	
	Average ±σ	–25.55 ± 0.72	–25.79 ± 0.74		

[1]To calculate adulteration %, we used equation 1 with the following value of the HFCS adulterant: $\delta^{13}C$ = -9.7 ‰, which was the value suggested by White and Winters in 1989
[2]Xtabentun (*Rivea corymbosa*)
[3]Dzidzilché or Tsitsilché (*Gymnopodium antigonoides*)
[4]Tajonal or Tah (*Viguiera helianthoides*)
[5]Tzalam (*Lysiloma bahamensis*)
[6]Habín (*Piscidia piscipula*)
[7]Orange. (*Citrus sinensis*)

Table 5. $\delta^{13}C_{PDB}$ of honey and their protein fractions from others Mexican States.

State of origin	$\delta^{13}C_{PDB}$ ‰ honey	$\delta^{13}C_{PDB}$ ‰ protein	Difference protein-honey	Apparent corn/cane content, %[1]
Guerrero*	–25.00	–24.76	0.24	
Guerrero*	–24.88	–23.98	0.90	
Guerrero*	–25.10	–24.90	0.20	
Guerrero*	–25.36	–25.56	–0.20	1.26
Guerrero■	–25.75	–25.82	–0.07	0.43
Sonora*	–23.06	–23.53	–0.47	3.40
Sonora*	–23.86	–24.28	–0.42	2.88
Sonora*	–24.35	–24.05	0.30	
Sonora*	–25.60	–24.68	0.92	
Sonora*	–25.02	–25.17	–0.15	0.97
Durango*	–25.27	–24.82	0.45	
Michoacán*	–26.14	–23.40	2.74	
Michoacán■	–24.94	–24.85	0.09	
Michoacán■	–25.38	–25.26	0.12	
Edo. de México*	–26.91	–24.98	1.93	
Edo.de México■	–25.78	–25.72	0.06	
Edo.de México■	–25.97	–25.41	0.56	
Edo. de México■	–25.94	–25.76	0.18	
Edo. de México■	–26.45	–26.43	0.02	
Tlaxcala*	–25.76	–25.32	0.44	
Tlaxcala*	–25.78	–26.02	–0.24	1.47
Veracruz*	–25.77	–25.35	0.42	
Veracruz■	–25.47	–26.34	–0.87	5.23
Veracruz■	–25.77	–26.03	–0.26	1.59
Veracruz■	–25.91	–25.14	0.77	
Veracruz■	–26.63	–25.79	0.84	
Chiapas*	–25.69	–25.86	–0.17	1.05
Yucatán■	–26.88	–25.94	0.94	
Aguascalientes■	–25.36	–24.73	0.63	
Aguascalientes■	–25.92	–25.78	0.14	
Aguascalientes■	–24.44	–25.35	–0.91	5.81
Jalisco*	–26.55	–26.22	0.33	
S.L.P.*	–24.18	–24.56	–0.38	2.56
D.F.■	–25.05	–25.72	–0.67	4.18
D.F.■	–25.33	–25.02	0.31	

State of origin	$\delta^{13}C_{PDB}$ ‰ honey	$\delta^{13}C_{PDB}$ ‰ protein	Difference protein-honey	Apparent corn/cane content, %[1]
D.F.■	–26.88	–26.86	0.02	
Puebla*	–26.48	–26.24	0.24	
Morelos*	–24.15	–24.33	–0.18	1.23
Morelos*	–26.81	–25.04	1.77	
Morelos*	–26.86	–26.27	0.59	
Morelos*	–25.03	–25.06	–0.03	0.20
Morelos■	–24.69	–24.45	0.24	
Morelos■	–24.76	–25.55	–0.79	4.98
Morelos■	–26.29	–26.15	0.14	
*	–25.82	–24.96	0.86	
*Orange	–24.85	–24.31	0.54	
*	–25.71	–23.15	2.56	
*	–27.54	–25.95	1.59	
*	–27.14	–23.50	3.64	
Average ±σ	–25.60 ± 0.91	–25.19 ± 0.85		
Campeche■	–23.45	–24.62	–1.17	7.84◆
Campeche■	–25.21	–26.44	–1.23	7.35◆
Campeche■	–23.46	–26.63	–3.17	18.72◆
D.F.*	–19.26	–20.53	–1.27	11.73◆
Aguascalientes■	–22.28	–23.68	–1.40	10.01◆
Edo. de México■	–24.38	–25.94	–1.56	9.61◆
Jalisco*	–24.71	–25.86	–1.15	7.11◆
Jalisco■	–18.71	–24.74	–6.03	40.09◆
Jalisco■	–18.09	–23.67	–5.58	39.94◆
Jalisco■	–18.14	–23.45	–5.31	38.62◆
Guanajuato■	–20.38	–23.77	–3.39	24.09◆
Guanajuato■	–20.32	–23.57	–3.25	23.43◆
Guanajuato■	–19.25	–23.86	–4.61	32.56◆
D.F.■	–25.07	–26.82	–1.75	10.22◆

[1] To calculate adulteration %, we used equation 1 with the following value of the HFCS adulterant: $\delta^{13}C$ = -9.7 ‰, which was the value suggested by White and Winters in 1989

* Honey samples from beekeepers

■ Honey samples from several commercial brands available at the stores

◆ Adulterated honey using White's (1989) criteria

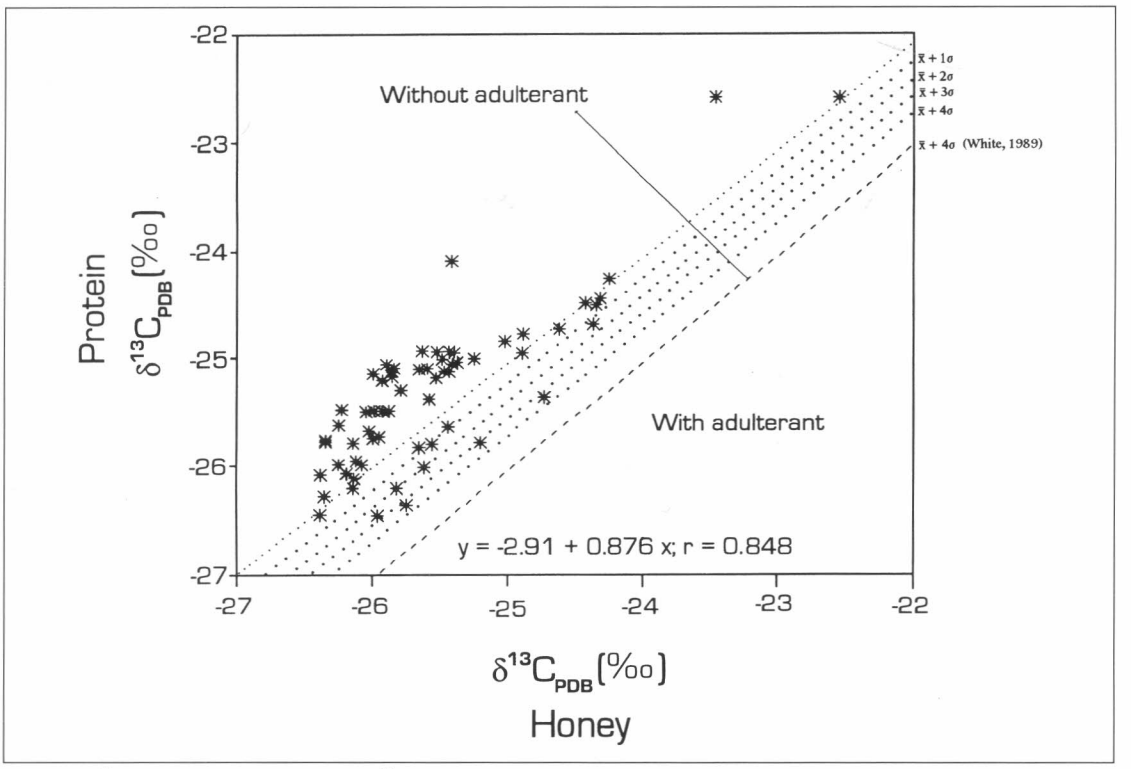

FIG. 2. $\delta^{13}C_{PDB}$ of honey *vs.* their $\delta^{13}C_{PDB}$ of the protein fraction from certified honey from Yucatan peninsula.

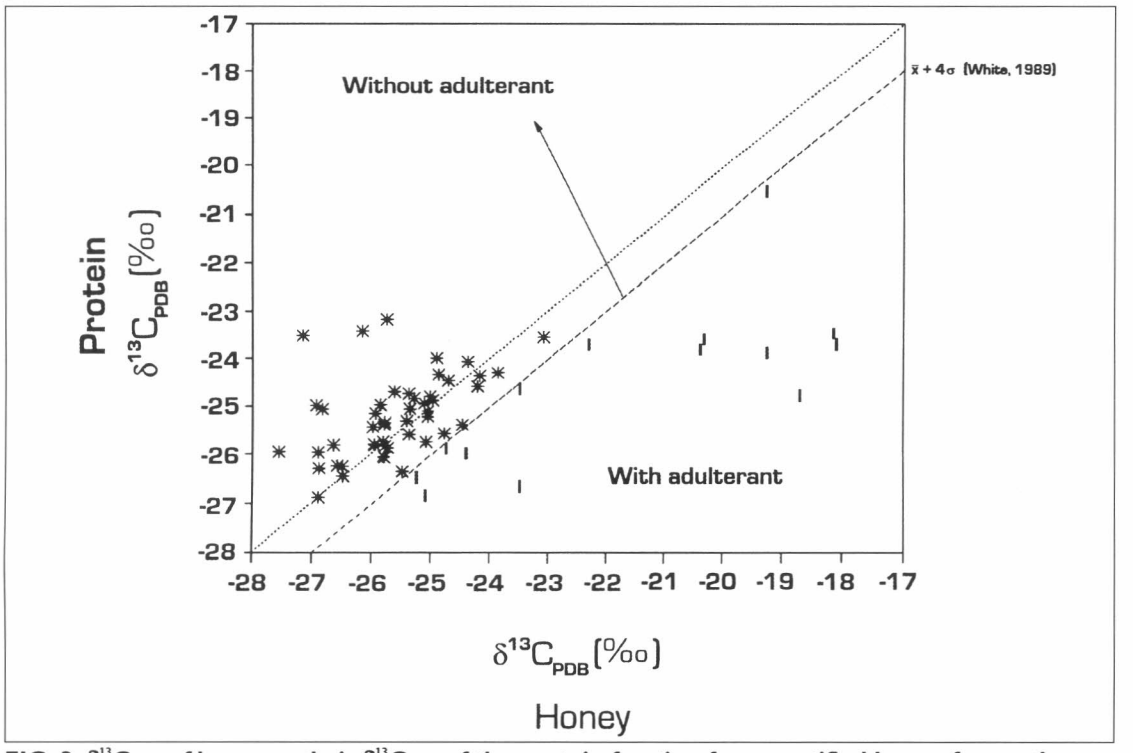

FIG. 3. $\delta^{13}C_{PDB}$ of honey *vs* their $\delta^{13}C_{PDB}$ of the protein fraction from certified honey from other Mexican states.

TABLE 6. $\delta^{13}C_{PDB}$ pollen and honey collected in beehives in Morelos.

	$\delta^{13}C_{PDB}$ (‰)
Honey	–25.38
Protein	–24.23
Brown pollen	–19.23
Orange pollen	–27.05
White pollen	–26.53
Yellow pollen	–14.56
Mixture pollen 1	–19.16
Mixture pollen 2	–20.29
Mixture pollen 3	–19.32

cane sugar and corn (*Zea mays*) are the sole harvest during the year, and during the cane's harvest the bees gather the sweet juices from the cut stalks.

Methodology

To obtain the carbon-13 content of the honey and its protein, we used our own modification of the Sofer's pyrolysis method. Below we describe briefly our procedure and compare it with the classical Sofer's pyrolysis method at 550°C.

When protein samples were needed we isolated the protein using the dialysis-precipitation method described by White (1992), then we do the pyrolysis of the sample.

Pyrolysis method

Since the classical combustion pyrex method at 550°C does not have an adequate reproducibility, a modification to the Sofer method (1980) was used. The modification includes the addition of activated metallic copper (Cu) to eliminate the NO_2 produced during the combustion of the protein (Mook & Jongsma, 1987). This method of quartz pyrolysis was tested against the classical pyrex method analysing 10 samples of NBS-22 (oil, RM 8539), 10 samples of PEF1 (polyethylene foil, RM 8540), 10 samples of sucrose ANU (sucrose, RM 8542) and 10 samples of our own laboratory honey standard (LHS). The quartz pyrolysis method is described below:

A quartz tube (9 mm outside diameter × 24 cm) prefilled with 3 g of cupric oxide (CuO) and a piece of silver foil (1 × 5 mm) placed in an open smaller quartz tube (3 mm outside diameter × 1.5 cm in length), to keep the silver from direct contact with the CuO, was cleaned at 600°C for 1h.

Honey samples of between 10 and 25 mg, which had been filtered to remove pollen and other particles, were weighed and loaded into another quartz tube (6 mm outside diameter × 1 cm), sealed at one end, and placed upside down in the combustion tube. Finally 3 g of Cu were added to the main tube (see fig.1) which was placed on a vacuum line and sealed after being evacuated to a pressure of 10^{-3} torr and then in a furnace where the temperature was increased slowly up to 900°C , maintained for 2 h, and then followed by 1 h at 650°C. After combustion, the tubes were once more placed on a vacuum line where the CO_2 produced was cryogenically cleaned through an ethanol-dry ice and liquid nitrogen cold trap. Non-condensable gases were pumped away and finally the purified CO_2 gas was analysed in a triple collector mass spectrometer (MAT 250) with an accuracy for the whole procedure of 0.02‰. All the analysis of the $^{13}C/^{12}C$ ratios are

TABLE 7. Rejection criteria for internal standard isotope ratio testing of honey for adulteration.

Limit	Probability of error	White, 1989	Honey table 4	Honey table 5
Number of samples:		50	63	49
$\bar{x}$		–0.13 ‰	–0.07 ‰	–0.12 ‰
σ		± 0.22 ‰	± 0.15 ‰	± 0.24 ‰
$\bar{x} + \sigma$	1 in 6	–0.35 ‰	–0.22 ‰	–0.36 ‰
$\bar{x} + 2\sigma$	1 in 44	–0.57 ‰	–0.38 ‰	–0.59 ‰
$\bar{x} + 3\sigma$	1 in 770	–0.79 ‰	–0.53 ‰	–0.83 ‰
$\bar{x} + 4\sigma$	1 in 25 000	–1.01 ‰	–0.69 ‰	–1.06 ‰

$\bar{x}$; mean negative difference. Protein value minus honey value
σ; standard deviation of the negative difference

reported as $\delta^{13}C$ in ‰ relative to the international PDB standard (CO_2 from carbonate shell of a Cretaceous mollusc, *Beleminitella americana* from the Pee Dee Formation in South Carolina, USA) (Craig, 1953).

Honey protein isolation and purification

We tested the direct-precipitation method (AOAC 991.41) against the dialysis-precipitation procedure, to test the extent at which both methods work with mixtures of honey with a high percentage of C_4 adulterate compound. To do so, we used prepared samples of cane sugar mixed with honey, HFCS mixed with honey and a pure honey sample (LHS). (The mixtures prepared contained approximately 0%, 25%, 50%, 75% and 100% wt/wt of each adulterant). To calculate the percentage of adulteration we used the formula described by White (1989).

(1)

$$\text{adulteration (\%)} = \frac{\delta^{13}_{PDB}(\text{protein}) - \delta^{13}_{PDB}(\text{honey})}{\delta^{13}_{PDB}(\text{protein}) - \delta^{13}_{PDB}(\text{adulterant})} \times 100$$

For all the honey samples of this study we followed the dialysis-precipitation procedure described by the Association of Official Analytical Chemists (White, 1992).

RESULTS

The comparison of the $\delta^{13}C$ of the honey pyrolysed by the two methods are shown in table 1. The results of evaluation of the isolated and purified honey protein of the artificial mixtures of honey and cane sugar and HFCS are showed in tables 2 and 3.

The Mexican state origin, the floral type and the $\delta^{13}C$ obtained for the honey and its protein are in table 4 and shown in figure 2.

The $\delta^{13}C$ of honey from other Mexican States are in table 5 and shown in figure. 3.

The $\delta^{13}C$ of pollen and honey collected in bee hives from Morelos state are shown in table 6.

Rejection criteria for internal standard isotope ratio testing of honey for adulteration are shown in table 7.

DISCUSSION

To calibrate the pyrolysis method, we used NBS-22 (oil, RM8539); PEF1 (polyethylene foil, RM8540); sucrose ANU (sucrose, RM8542) which are reference materials distributed by NIST on the behalf of IAEA, and are intended for carbon isotope ratio calibration of organic materials, the $\delta^{13}C$ results are shown in table 1. For comparing of the two pyrolysis methods we chose for honey our laboratory honey standard, and NBS-22 for reference because it is a very uniform material. The standard deviation of the $\delta^{13}C$ for both the NBS-22 and our laboratory honey standard if they are analysed with the modified quartz method and the results compared to the standard deviation of the classical method, had not only a smaller standard deviation (table 1) but also greater reproducibility (0.02‰ over a period of one year). This may be due to the following facts: the combustion of honey at 550°C is probably incomplete since its chemical composition is quite complex, and also due to the presence of NO_2 derived from the nitrogen in several organic compounds in honey.

To compare how the isolated procedures work with high concentrations of the C_4 adulterants, the proteins of the samples were isolated by dialysis-precipitation procedure and by the direct-precipitation method (991.41). The tables 2 and 3 present the results for honey and its protein. For the artificial honey mixtures with different percentages of cane sugar and HFCS, we observed that the $\delta^{13}C$ of the protein using the dialysis-precipitation procedure did not change even at high levels of adulteration with C_4 plant sugars. However using method 991.41 we observed that for samples adulterated with more than 50%, the isolating procedure was not good enough to eliminate the C_4 plant sugar adulteration, that can be observed in the variability of $\delta^{13}C$ for the protein in the different mixtures samples.

Due to the economic importance of the Yucatan-Campeche region we made a study to correlate the $\delta^{13}C$ of 42 leaves from honey precursor plants (at this moment these are botanically unclassified) against honey from the region. The $\delta^{13}C$ average for leaves was –28.2‰ ± 1.2‰ with $\delta^{13}C$ range from –26.0‰ to –31.4‰, and the $\delta^{13}C$ of 63 certified honey from Campeche and Yucatan (table 4, fig 2) was –25.5‰ ± 0.7‰ with $\delta^{13}C$ range from –24.2‰ to –26.4‰, which implies not only that all the nectar-pollen producing plants are C_3, but also, that during a flowering season the honey produced has a $\delta^{13}C$ with a very narrow range.

Even though care was taken to find a C_4 or CAM label on the honey produced in Mexico (table 5, fig. 3), no clear evidence was found aside from a slightly more negative value for the $\delta^{13}C$ of the average Mexican honey = –25.6‰ + 0.9‰, compared to the average $\delta^{13}C$ of the honey harvested in the USA ($\delta^{13}C$ = –25.20‰; Doner & White, 1977) ($\delta^{13}C$ = –24.30‰; White, 1989).

To have a better understanding of the effects of expansion in size of fields of C_4 plants in the honey label, honey and pollen samples were collected from Yautepec-Jojutla valley in the State of Morelos. In this valley cane sugar and corn are the main crops, and during the sugar cane harvest the bees gather the sweet juices from the cut stalks and pollen from the corn. The results of $\delta^{13}C$ of the pollen and honey (table 6) show that the bees often visit and gather pollen not only from C_3 plants but also from C_4 plants. However, the honey maintains a $\delta^{13}C$ of C_3 plants' label. Although it is worth mentioning, that the $\delta^{13}C$ of their protein is more positive and the difference between protein and honey is 1.15‰. It is also interesting to note that in honey from the state of Morelos shows a positive difference

between the protein and the honey. Even though there is no proof, this might be due to the C_4 pollen collected by the bees for food (table 5, fig. 3). Some beekeepers maintain that the bees visit some plants for nectar and others for pollen. This may be the reason for seeing a more positive $\delta^{13}C$ value in the protein fraction.

With the results of the protein-honey values of the certified samples from Campeche-Yucatan statistics were done to determine the accepted value of the negative difference between $\delta^{13}C_{Protein} - \delta^{13}C_{Honey}$ (table 7). For this group of samples a negative average that included 4σ we got –0.69‰ which is small compared to the –1.01‰ proposed by White in 1989, probably due to the uniformity of the ecological system from which the samples of honey were taken. For the group of honey samples from other parts of Mexico (top part of table 5) the negative average that included 4σ have a value –1.06‰, which is very close to White's criteria.

Utilizing White's (1989) formula and criteria (–1‰ difference between the protein and the honey), we found that approximately 18% of the samples analysed from the group 'other Mexican States' (the adulterated honeys are listed on the bottom of table 5) show that they were adulterated.

We consider that all the criteria developed by White to characterize American honey are applicable to Mexican honey, without any restriction to evaluate adulteration by C_4 plants.

We are hoping that the use of this method for detecting adulteration will minimize the threat of loosing the Mexican honey export markets. To do so the authors have been in touch with the authorities to include ISIRA method in the Official Mexican Quality Standards for honey.

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