

"HARD JOB"

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## DEVELOPMENT AND USE OF THE INTERNAL STANDARD CARBON ISOTOPE RATIO METHOD FOR C-4 SUGARS IN HONEY

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The original isotope ratio method for detecting C-4 (corn and cane) sugars in honey was qualified by AOAC in 1977. The wide range of values found for pure honeys made it necessary to use a statistical approach in setting limits for pure honey. A range of  $-23.5\text{‰}$  to  $-21.5\text{‰}$  was set for questionable samples; honeys more negative than  $-23.5\text{‰}$  were considered pure and samples less negative than  $-21.5\text{‰}$  were considered adulterated without further testing. Those between these two limits would require further testing by the TLC test. This test has several shortcomings: it requires visual judgement of colors and intensities, does not include cane sugar, and is somewhat vulnerable to newer improved high-fructose corn syrups. The ISCIRA (internal standard) test was developed in the late 1980's and accepted by AOAC in 1991 to eliminate the ambiguous TLC test, though it was realized (and so stated in the AOAC method description) that it is valid for honeys of any isotope ratio value.

This method eliminates the uncertainties inherent in the earlier procedure. It uses the protein isolated from the honey, which represents the isotopic composition of the honey while it is in the beehive, as a standard with which to compare the isotope ratio of the questioned sample. The ISCIRA index (formerly called the difference value) is obtained by subtracting the  $\delta^{13}\text{C}$  of the honey from that of the protein. If this value is more negative than  $-1\text{‰}$ , the sample is unequivocally adulterated. The amount of added sugar can be estimated from the index: each  $-1\text{‰}$  is equivalent to 7% added sugar. This method is an official method of AOAC International (991.41).

I have prepared an extensive review of 20 years of isotope ratio testing of honey, together with Ken Winters, Coastal Science Laboratories, a British expert on honey quality control, and a professor from the Technical University of Munich, Germany. This effort is in response to the inundation of adulterated Chinese honey received since 1990 in the US, Germany, and England.

We have examined ISCIRA analyses of 224 pure honeys from the US, England, Germany, Italy, Mexico, and Spain, and 282 samples of Chinese honey imported into the first three and Canada.

The Chinese have contended for years that their honey is different from all others and should not be expected to have the same isotope ratio ranges. However, we have compared the isotope ratio values of the protein from Chinese honey and that of the 224 pure honeys from the five countries noted above. There is no difference in the distribution of their values. However,

when the values for the Chinese honeys and the 224 known pure honeys are compared, there is a vast difference in the less negative (more adulterated) direction. The Chinese have excellent isotope ratio spectrometers and have published a paper accepting the reliability of the internal standard procedure<sup>1</sup>. The original isotope ratio method (978.17) alone is incapable of detecting adulteration of honeys with  $\delta^{13}\text{C}$  values more negative than  $-23.5\text{‰}$ ; such honeys have been considered pure. The older method is thus vulnerable to the addition of C-4 sugars in amounts that do not increase the  $\delta^{13}\text{C}$  value above  $-23.5\text{‰}$ . Although this is not a problem with the ISCIRA method, since the protein value obtained thereby reflects the isotope ratio of the original honey before adulteration, in reality since the isotope ratio of the honey is done first, there appears to be an unfortunate tendency not to continue and determine the value for the protein if that of the honey is below  $-23.5\text{‰}$ . By selecting a honey with rather negative  $\delta^{13}\text{C}$  value (say  $-25.5\text{‰}$ ) up to 10% C-4 sugar can be added and not detected without ISCIRA testing. Although we have been aware of this possibility, only recently have we seen examples of this practice in commercial trade.

We have obtained internal standard isotope ratio values for a group of 118 honeys, domestic and imported into the US, all but 16 tested in 1996 and 1997. We selected 76 that had protein  $\delta^{13}\text{C}$  values (and hence values for the original honey before adulteration) equal to or more negative than  $-23.5\text{‰}$ . Of these 35 (46%) were found adulterated up to 25%. Of the 42 honeys between  $-23.4$  and  $-23.0\text{‰}$ , 66% were adulterated.

As Associate Referee for Honey, I am now attempting to clarify this situation in the method description to eliminate this ambiguity. The cooperation of the FDA in requiring complete internal standard isotope ratio testing for all honey samples within their jurisdiction is vital to the economic health of the honey industry and the crop pollination which it provides.

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<sup>1</sup> Mass Spectroscopy of Carbon Isotopes in Honey and its Proteins, by Cao Yaching, Zhou Keyu, and Sun Guohuang, Nanjing Soil Institute, Academia Sinica, Published in *Shipin Keuxel Beijing*: (1993): 70-73 (Chem Absts. 119, 13727m).