

ADULTERATION TESTING OF CHINESE HONEY

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As an interested party, I submit the following material to the International Trade Commission to provide background information on the question of adulteration of honey as received from mainland China over the past few years.

Honeydata Corporation was established in 1987 to provide analytical services to the honey industry, particularly, but not limited to, the detection of adulteration with cheaper sugar products. The analytical methods used are those developed at USDA by the writer and later refined to increase sensitivity.

The Stable Carbon Isotope Ratio method (SIRA)¹ * resulting from the USDA research detects the presence of corn and/or cane sugars in honey. It is based on the difference in the ratios of two carbon isotopes in plants, resulting from the differences between honey plants and cane and corn plants in their systems of photosynthetic assimilation of carbon. This is expressed as the value $\delta^{13}\text{C}$. The considerable variation in $\delta^{13}\text{C}$ values that were found for different honey types made it necessary to apply a confirmatory test² in cases where low-level (ca. 15-20%) adulteration was indicated by the SIRA test. These analytical procedures were collaboratively tested and accepted as official by the Association of Official Analytical Chemists in 1978, qualifying them for legal acceptance.

In 1989 the procedure was improved to greatly reduce uncertainty and eliminate the confirmatory test by using the protein (contained in all honey) as an "internal standard" for each sample, thus eliminating the use of average values of all honey for comparison with a that of a given sample value. In this procedure (ISIRA)³ the floral source of the honey, which influences the SIRA value, is immaterial; the purity of any sample is measured by the difference between SIRA values of the honey and the protein isolated from the same sample. A statistical tolerance equivalent to about 7% "added material" is allowed. This procedure was collaboratively tested and accepted by the AOAC International.⁴

Beginning in late 1990, samples described as Chinese honey were being received for testing; in 1991 87 samples of a total of 143 from 8 different customers were found to be from 10% to over 60% adulterated, The experience of one packer is illustrated by the accompanying chart. It shows that at the start of such testing (late 1990), most of the Chinese honey contained 10 - 25% added

* Numbers refer to literature cited at end of brief.

material. By August 1991, when the Chinese became aware that this packer would accept only honey with SIRA values more negative than $-23.4^{0}/_{00}$, the purity of the Chinese honey offered was largely acceptable. It is worth noting that even at present (September-November 1993) this packer rejected 23% of the Chinese honey offered because it failed the test. Additionally, another 23% was rejected because of pesticide residues.

The response of the Chinese authorities and some of those importing Chinese honey to the US was a worldwide disinformation campaign asserting that Chinese honey is different in this respect (SIRA) from that from the rest of the world, and therefore the SIRA standards should not be applied to them, and in any event, certification by Chinese Government that analysis by methods of the Codex Alimentarius proved that the offending honey was genuine. Inquiries about the purity of Chinese honey were received by Honeydata from packers and scientists in the US, Great Britain, Germany, and Japan. A typical response of mine (to a German honey packer) is given below.

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Thank you for your letter requesting information on the internal standard isotope ratio (ISIRA) method for honey analysis. Many of the questions therein have also been raised, particularly in connection with Chinese honey, by importers and packers here. I will attempt to deal with them below. The ISIRA method has now been accepted "official first action" by the Association of Official Analytical Chemists following an extensive collaborative study among nine laboratories in the US, Canada, and Germany; (the German collaborator was Dr. Josef Lipp, Gesellschaft für Strahlen- und Umweltforschung mbH, IngolstädterLandsstrasse 1, D-8042, Neuherberg). This study is now awaiting publication. Since that time I have modified the purification process for the protein to reduce the amount of time and effort required. The modification involves dialysis before precipitation. It is in the process of being added to the manuscript before publication.

Your questions:

Q. Is it true that only US honeys were used to establish the ISIRA method?

The original research on the method was done with US honeys because it is important to be certain of the authenticity of the samples. It might be possible to arrange testing of honeys from other countries if assurance of their purity could be obtained.

Q. If so, can these standards apply to all kinds of honey-qualities of the whole world?

We have applied the ISIRA test to commercial honeys from China, Argentina, Mexico, Spain as well as the US. We have also analyzed hundreds of honeys from around the world with the ordinary isotope ratio method; the distribution of their values is quite similar to that we found for the US honeys. The ISIRA method is normally applied only to honeys found less negative than $-23.5^0/_{00}$; very few of our analyses of overseas honeys over several years fell into this range (e. g. only 3 of 47 Argentine, 3 of 35 Australia, 0 of 14 Mexico). However, see the paper "Adulteration Testing of Southwestern Desert Honeys" for data on Mexican (and US) mesquite honeys. These honeys were questioned by US authorities earlier because their values appeared too far from the averages. The ISIRA test showed them to be genuine. However, of 126 known Chinese honeys analyzed by isotope ratio this year, only 33 were more negative than $-23.5^0/_{00}$. The protein values of all of those tested by ISIRA were more negative than -23.5 . We found $\delta^{13}C$ values of Chinese honeys (including "Acacia") to be in the normal range in 1979, 1980, but in 1985 alone a shipment of Chinese honey was found to have values in the range -20.2 to $-21.5^0/_{00}$, similar to this year.

It is noteworthy that in the original isotope ratio work (Jour. AOAC (1978) 61: 746-750) 35 of the 119 honeys analyzed were imported from 13 countries, including 2 from China (see Jour. Apicultural Res. (1978) 17: 94-99). Their $\delta^{13}C$ values were very similar to US honeys. None of them had values that would require ISIRA testing. I sincerely feel that the ranges and procedures we have established can apply to all honeys, independent of geographic or plant origin. There are of course honeys that are produced from a few CAM plants (acacia, citrus, and mesquite are not CAM plants). For CAM plants, the $\delta^{13}C$ values of the protein will be similar to those of the honeys if they are pure.

Q. After studying carefully your method description, it seems to be that obviously the scientists have problems with acacia honeys to find explanations why the values of $\delta^{13}C$ differ so much (between honey and protein). Please comment on this point.

Re "Acacia" honeys. The Chinese and Hungarian (and other) "acacia" are not true *Acacia* species, but rather from *Robinia*

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pseudoacacia, the locust, a totally different plant. The few values in the world literature for Robinia show $\delta^{13}\text{C}$ values to be in the -23.1 to -23.9‰ range. (see #30 in Table 4). This difference from the -25.4‰ average is about the same as for citrus (see Jour. AOAC (1983) 66: 1-3) and the values are not nearly as positive as the so-called Chinese "acacia" honey we have analyzed this year. All of the citrus, locust, and mesquite honeys we have tested by ISIRA have had only small differences between values for honey and protein, indicating purity.

Q. The same seems to be true with mesquite honeys, as it is mentioned in your description. Is it possible that natural aspects can influence the $\delta^{13}\text{C}$ value results? For example, such causes as floral origin of CAM plants like acacia, citrus, etc. honeys compared with C-4 plants?

Regarding mesquite honey, see above and also "Adulteration Testing of Southwestern Desert Honeys", American Bee Journal (1991) 131: 123-126. I will send you a copy with the copy of this letter. Note in the article that all of the 3 so-called Arizona catsclaw (*Acacia*) honeys (#64-66 in the 1989 paper) were later found to contain major amounts of mesquite pollen, accounting for the less-negative values. All however had similar values for the protein indicating their purity. While it is well-known that CAM plants may have much less negative values, none are significant sources of honey; in any event the biology and mechanism of honey-gathering and ripening would produce honey protein $\delta^{13}\text{C}$ values similar to those of the honey produced. Generally, climate and latitude do have minor effects on $\delta^{13}\text{C}$ values, but here again the protein values would be similarly affected, since they arise from nectar and pollen ingested by the bees.

Q. Is it possible that the $\delta^{13}\text{C}$ results depend on the condition as to how the honey was harvested, i. e. maybe there could be differences between the $\delta^{13}\text{C}$ results of ripe and unripe honey?

The $\delta^{13}\text{C}$ values of the nectar and honey are fixed in the carbon atoms of the plant and are not changed by any subsequent treatment. With respect to ripe vs. unripe honey, loss of water cannot affect the values. However if a fermented syrup is added to an unripe honey, we have found that the yeast cells of the subsequently

ripened (natural or artificially) honey-sugar mixture will contaminate the protein preparation unless removed. We have done this and found that with the questioned Chinese honeys when the yeast cells are removed, the $\delta^{13}\text{C}$ values of the protein become about 0.8‰ more negative, equivalent to about 5.6% cane sugar.

Q. From the literature I am aware that in honeys protein values vary considerably. Furthermore, I think certain protein kinds get into the nectar through the bees. Owing to this reason, is it possible that on acacia honeys and eventually other honeys, the $\delta^{13}\text{C}$ value of protein shows a more negative figure compared with the $\delta^{13}\text{C}$ value of honey?

All studies of the origin of honey protein agree that nearly all of the protein is composed of the enzymes added by the bees, with a small amount from the plant. In any event, such plant-derived protein would have nearly the same $\delta^{13}\text{C}$ values as the nectar. You will note from the 1989 paper that the differences found for the 50 certified honeys are either positive or negative. Because of this we permit a difference of -1.0‰ before considering a honey to be impure. This is explained in the article.

Examination of SIRA data obtained at the time from Chinese honey would appear to support, in the absence of data from Chinese honey, the idea that such honey may have less negative SIRA values, but as shown in the Tables below, application of the ISIRA procedure to these same honeys completely disproves it.

ISOTOPE RATIO VALUES FOR HONEY (HONEYDATA)

Origin	No. Samples	SIRA VALUES			
		Average	Range	Outliers	
Australia	35	-24.4‰	-23.1‰ to -26.4‰	1	
Argentina	47	-25.5	-20.2 to -26.7	3	
Mexico	14	-24.6	-23.6 to -25.7	0	
China	33	-22.4	-21.5 to -23.0	33	

DATA FROM LITERATURE

Imports to US (1975) ¹	35	-25.8	-23.9 to	-27.4	
US domestic (1974-5) ¹	84	-25.2	-22.5	-27.4	
Both of above	119	-25.4	-22.0	-27.4	
United Kingdom ⁵	102	-25.5	-21.8	-27.0	
Imports to Germany ⁶	28	-25.1	-22.5	-26.9	1
US-ASCS (1987-89) ⁸	9217	-25.4	-22.0	-29.7	8

^a Private communication

The ISIRA procedure was applied to 50 of the US domestic honeys listed above during the development of the procedure. Later it was applied to the 33 Chinese honeys.

	ISOTOPE RATIO VALUES (‰)			
	Average Honey	Range	Average Protein	Range
50 US Honeys	-24.30	-22.0 to -25.7	-24.04	-20.6 to -25.8
33 Chinese honeys	-22.38	-21.5 to -23.3	-24.47	-21.5 to -23.3 ²
28 Imports to Germany ⁶	-25.1	-22.5 to -25.1	-25.09	-22.6 to -26.9

(One German sample, *Aloe*, (a CAM plant), had honey, -14.7‰ , protein -15.6‰
All of the Chinese samples above failed the ISIRA test.

As noted above, the Chinese explanation of the difference in SIRA values which we interpret as indicating the presence of added sugars is that these differences are caused by certain (largely unidentified) types of honey plants indigenous to China but unknown elsewhere, which have SIRA values outside of the ranges of honeys from the rest of the world, and that the isotope ratio procedures are of no validity.

This proposal is without merit because as seen above the use of ISIRA testing clearly shows the presence of non-honey sugars. It has been claimed that Chinese "Acacia" honey is responsible for these differing values, since the rather sparse data on SIRA values of true *Acacia* honey show values less negative than other types, in approximately the range found for Chinese honeys. While Chinese "Acacia" honey is a very desirable type, it must be pointed out that the honey labelled as "acacia" from China, Hungary, and elsewhere is not from the *Acacia* species, but rather from *Robinia pseudoacacia*, the "false acacia" or "falsche akazie" or black or honey locust. From the literature the average of three samples of such honey of European origin (confirmed by pollen analysis) was

-23.3⁰/₀₀. In our study establishing the internal standard isotope ratio method³ a value of -23.4⁰/₀₀ for this honey type from the U. S. and -23.9⁰/₀₀ for its protein was recorded.

The less negative values from the literature for citrus⁷ and mesquite⁸, and indeed true *Acacia* spp. have been cited by the Chinese as evidence for the validity of the less negative values for Chinese honeys. In fact, the ISIRA testing done on these honey types (US citrus⁷, mesquite⁸, black locust³, and catsclaw⁸ (a true *Acacia* plant) has proved them to be pure⁸, with little difference between honey and protein values, even though the SIRA values alone would disqualify them. The validity of the floral sources has been verified by pollen analysis.

It is worth mentioning here that a world-wide compendium of honey plants (Directory of Important World Honey Sources, International Bee Research Association, 1984) lists only five Asian *Acacia* honey sources, mostly tropical. It states that *Robinia pseudoacacia* grows in temperate Asia, and is a major honey source in China.

At the time of the testing of the Chinese honeys discussed here, it was learned that the Chinese had no knowledge of modern testing methods, relying instead on the methods of the Codex Alimentarius, which do not deal with honey adulteration. Since then we have provided them with information on the testing methods described here, and we have been advised that the highly expensive instrumentation for the SIRA and ISIRA testing has been acquired by the Chinese authorities.

One point must be considered in this respect: the disposition of honeys that have been rejected by those in this country who adequately test their purchases. The relatively small number of those testing compared with the number of packers, makes it obvious that in the earlier period discussed above, and probably at present, by far the largest part of the Chinese honey imported since 1990 has been used in the domestic markets without any concern for its purity. The packers who do test are at a considerable disadvantage financially since the cost of adequate testing is by no means small, and must be absorbed by the honest packers without any way to recover the cost of testing. It appears obvious that over the past few years, millions of pounds of adulterated Chinese honey has been disposed of in the US market.

The FDA, which might be considered responsible for testing imported honeys for purity, do not seem to have the funding to undertake adequate testing of

imported honey, probably because it is an economic violation, not one affecting public health, and therefore of low priority.

In conclusion, it is quite evident that the availability of cheap Chinese honey forces packers to use it in preference to domestic honey or to offer low prices for whatever US honey they buy; and any adulteration of the Chinese honey further increases profit to Chinese exporters and decreases income to US producers.

REFERENCES CITED

1. White, J. W., & Doner, L. W. (1978) Mass spectrometric detection of high-fructose corn sirup in honey by use of $^{13}\text{C}/^{12}\text{C}$ ratio: collaborative study. *J. Assn. Off. Anal. Chem.* 61: 746-750
2. Kushnir, I. (1979) Sensitive thin layer chromatographic detection of high fructose corn syrup and other adulterants in honey. *J. Assn. Off. Anal. Chem.* 62: 917-920
3. White, J. W. & Winters, K. (1989) Honey protein as internal standard for stable carbon isotope ratio detection of adulteration of honey. *J. Assn. Off. Anal. Chem.* 72: 907-911
4. White, J. W. (1992) Internal standard stable carbon isotope ratio method for determination of C-4 plant sugars in honey: Collaborative study, and evaluation of improved protein preparation procedure. *J. AOAC International* 75: 543-548
5. Burroughs, L. F., and Otlett, R. L. (1986) The proline content and stable carbon isotope ratio of genuine United Kingdom honey. *J. Assoc. Publ. Analysts* 24: 91-93
6. Rossmann, A., Lüllmann, C., and Schmidt, H-L. (1992) Massenspektrometrische Kohlenstoff-und Wasserstoff-Isotopen-Verhältnismessung zur Authentizitätsprüfung bei Honingen. *Z. Lebensm. Untersuch. Forsch.* 195: 307-311
7. White, J. W., & Robinson, F. A. (1983) $^{13}\text{C}/^{12}\text{C}$ ratios of citrus honeys and nectars and their regulatory significance. *J. Assn. Off. Anal. Chem.* 66: 1-3
8. White, J. W., Bryant, V. M., Jr., and Jones, J. G. (1991) Adulteration testing of southwestern desert honeys. *American Bee J.* 131: 123-126