Isotopic Composition of Carbon in Apple Juice

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The variability of the $^{13}C/^{12}C$ ratio in pure apple juices has been measured to determine base line values for a method to detect the undeclared addition off cane or corn sugars to apple juice. Forty apple juice samples representing 18 varieties of apples were analyzed by isotope ratio mass spectrometry. The mean $\delta^{13}C$ (ppt) value for all samples is −25.4 (ppt), and the coefficient of variation is 4.88%. There are no significant variations in $\delta^{13}C$ values with regard to apple variety or geographical origin of the apples, and the uniformity off the data suggests that it can be used to detect the adulteration of apple juice.

Plants use either the Calvin (C_3) or Hatch–Slack (C_4) pathway for photosynthetic carbon dioxide fixation, as revealed by differences in both leaf anatomy and $^{13}C/^{12}C$ ratios in their organic carbon. Plants using the C_3 cycle produce 3-phosphoglycerate as the first product of photosynthesis, while in plants using the C_4 cycle, oxalacetate, malate, and aspartate are the initial products. All plants are slightly lighter in ^{13}C than is the carbon dioxide of the atmosphere and C_3 plants discriminate against ^{13}C to a greater extent than do C_4 plants, as established by Bender (1971) and Smith and Epstein (1971).

Recently, practical uses have resulted from the finding that the $^{13}C/^{12}C$ ratio of a plant derived material reflects the photosynthetic pathway operating in the plant from which it was derived. Cane (C_4 plant) sugar sucrose can be distinguished from beet (C_3 plant) sugar sucrose by $^{13}C/^{12}C$ analysis (Smith and Epstein, 1971), a distinction not possible chemically. Also, the undeclared addition of cane sugar to maple (*Acer saccharum*, C_3 plant) sirup

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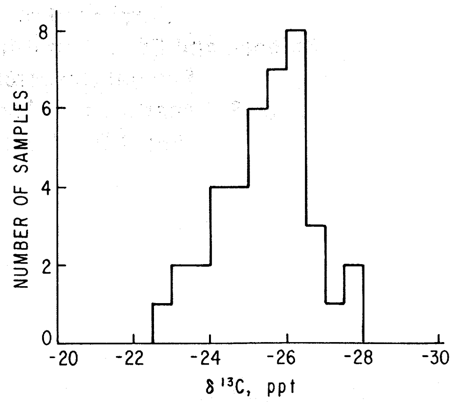

Figure 1. Distribution of $\delta^{13}C$ (ppt) values among apple juices.

(Hillaire-Marcel et al., 1977) and corn (C_4 plant) derived sirups to honey [all floral sources are C_3 plants; Doner and White, (1977)] can be detected (White and Doner, 1978).

More recently, $^{13}C/^{12}C$ analysis had been used to distinguish between vanillin from vanilla beans and that produced synthetically (Hoffman and Salb, 1979). Since $^{13}C/^{12}C$ ratios of organic carbon from animals reflect their diets, protein from corn-fed animals can be distinguished from soy protein (C_3 plant source) by the isotopic method (Gaffney et al., 1979).

The present report establishes the uniformity of $^{13}C/^{12}C$ ratios of apple (C_3 plant) juices prepared from the commercially most significant apple varieties. This information was required to provide base line data for a recently developed method to detect the undeclared addition of inexpensive corn or cane sweeteners to apple juice. There is recent evidence (Beaton and Gold, 1979) that such adulterated mixtures are being mislabeled as pure apple juices. A comprehensive report on the analysis of several hundred commercial apple juices and concentrates is in preparation (Krueger and Reesman, 1979) and confirms that such adulteration occurs.

EXPERIMENTAL SECTION

Fresh whole apples were obtained from growers, packers, or by local purchase. In most cases the exact proveniences of the apples were known. The apples were squeezed by hand in the laboratory, and the juice was passed through fine bolting cloth to produce a relatively pulp-free juice sample.

Stable Isotope Ratio Analysis (SIRA). The analyses of the carbon in the samples were conducted by Geochron Laboratories Division, Krueger Enterprises Inc., Cambridge, MA, on a Micromass 602D mass spectrometer with a dual-capillary inlet. Single-strength apple juices (about 12% solids) were combusted directly in purified oxygen at about 850 °C, and the gases were recirculated over CuO at 850 °C for 10 min; water and CO_2 were frozen, excess oxygen was removed, and CO_2 was transferred to a sample flask for analysis. Corrections applied to the measured differences included any zero enrichment in the capillary inlet system, valve mixing between sample and standard valves, tailing of major onto minor peak signals, and contribution of $^{12}C^{16}O^{17}O$ to the mass 45 signal. The $^{13}C/^{12}C$ ratios were determined by comparison of the $^{13}C^{16}O_2$ and $^{12}C^{16}O_2$ ion beams and are expressed as $\delta^{13}C$ values, where

$$\delta^{13}C \text{ (ppt)} = \left[\frac{(^{13}C/^{12}C) \text{ sample}}{(^{13}C/^{12}C) \text{ standard}} - 1 \right] \times 10^3$$

and reference standard is CO_2 produced from Peedee belemnite (PDB) calcium carbonate. A $\delta^{13}C$ value of −25 (ppt) means that the $^{13}C/^{12}C$ ratio of the sample is 2.5% less than that of the PDB standard ($^{13}C/^{12}C_{PDB}$ = 0.011237). This amounts to 281 less ^{13}C atoms per million. Reproducibility for the $\delta^{13}C$ determinations is 0.3 (ppt) or better.

Table I. $\delta^{13}C$ (ppt) for Apple Juices

variety	no. of samples	$\delta^{13}C$ (ppt) range	$\delta^{13}C$ (ppt) mean	SD	CV, %
Cortland	1		−26.1		
Davey	1		−23.9		
Golden Delicious	8	−23.9−−26.2	−25.0	0.818	3.27
Granny Smith	1		−23.0		
Green Gravin	1		−26.7		
Idared	1		−27.8		
Macoun	1		−25.1		
MacIntosh	11	−23.2−−27.9	−25.8	1.255	4.86
Newtown	1		−24.4		
Red Delicious	4	−22.5−−26.2	−24.8	1.640	6.61
Rhode Island Greening	1		−25.7		
Rome	2	−24.2−−27.3	−25.8		
Standard	1		−24.0		
Stark Splendor	1		−25.5		
Stayman	1		−26.2		
Winesap	1		−26.0		
Winter Banana	2	−24.6−−26.4	−25.5		
York	1		−25.7		
by state					
New York	13	−23.2−−27.9	−25.8	1.200	4.65
Virginia	7	−25.1−−27.3	−26.0	0.678	2.61
Washington	6	−24.0−−26.4	−24.9	1.107	4.45
all samples	40	−22.5−−27.9	−25.4	1.239	4.88

RESULTS AND DISCUSSION

The $\delta^{13}C$ values for 40 apple juice samples are summarized in Table I, and the distribution of values is given in Figure 1. For 26 of the samples, the geographical origin of the apples was known, and a tabulation by state is included.

The uniformity of the data suggests that there is no correlation between $\delta^{13}C$ value and apple variety. The range of 4.7 (ppt) for MacIntosh apples approaches the range found for all samples, 5.4 (ppt). Also, there is no apparent major geographical correlation, as all Virginia and Washington samples have $\delta^{13}C$ values within the range found for New York apple juices. These findings are in accord with the earlier report of Craig (1953), who found no geographical, species, or altitude effects on the $\delta^{13}C$ values of many land plants. Craig (1953) suggested that local environmental effects obscure the tendency for plants of the same species to possess identical $\delta^{13}C$ values.

The mean value for all apple juice samples was −25.4 (ppt), identical with that obtained previously for 119 honey samples (White and Doner, 1978). The coefficient of variation of just 4.88% may arise in part from the fact that the $\delta^{13}C$ measuements for apple juice largely represent the carbohydrates, which constitute about 97% of the apple juice solids (Agriculture Handbook No. 8, 1963). The ranges of $\delta^{13}C$ values for classes of compounds from single plants (Whelan et al., 1970) are wider than the range found for all the apple juice samples in this study.

The small variation in isotopic composition of carbon in pure apple juice assures that the isotopic method works well for the detection of corn or cane sugar adulteration in apple juice since corn and cane sugars have been shown to have a very different isotopic ratio (White and Doner, 1978; Hillaire-Marcel et al., 1977). A detailed collaborative study is now in progress to determine the precision and accuracy of stable isotope ratio analysis (SIRA) for this purpose.

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