

**Figure 3.** Chromatogram of volatile sulfur compounds from Finnish beer; DC-200 column: (6) methyl ethyl sulfide (internal standard), (9) ethyl thiolacetate, other peaks as in Figure 1.

sponding values for  $\text{Me}_2\text{S}_2$  were less than 0.1 and 0.4  $\mu\text{g}/\text{L}$  (Tables II and III). The Hungarian wines differed from the others in that their  $\text{Me}_2\text{S}_2$  content was clearly higher. The average contents of methyl thiolacetate were, respectively, 6.7 and 11  $\mu\text{g}/\text{L}$  in white and red wines. The content of carbon disulfide varied between a trace and 10  $\mu\text{g}/\text{L}$ . Carbon disulfide has previously been tentatively identified in raw grain spirit (Ronkainen et al., 1973). The separation of  $\text{Me}_2\text{S}$  and  $\text{CS}_2$  was difficult and accomplished readily only on nonpolar columns.

It is clear that methyl thiolacetate is a normal metabolite formed by the action of yeast on methionine. Methyl and ethyl thiolacetates are commonly present in beers and wines and, together with other volatile sulfur compounds, can be accurately determined routinely by concentrating

the volatiles on a porous polymer trap before GC analysis. The content of thiols in beers and wines is normally very low, but the apparently ubiquitous thiolacetates form a potential source of thiols in these beverages.

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## Isotopic Composition of Carbon in Apple Juice

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The variability of the  $^{13}\text{C}/^{12}\text{C}$  ratio in pure apple juices has been measured to determine base line values for a method to detect the undeclared addition of 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  $\delta^{13}\text{C}$  (ppt) value for all samples is -25.4 (ppt), and the coefficient of variation is 4.88%. There are no significant variations in  $\delta^{13}\text{C}$  values with regard to apple variety or geographical origin of the apples, and the uniformity of the data suggests that it can be used to detect the adulteration of apple juice.

Plants use either the Calvin ( $\text{C}_3$ ) or Hatch-Slack ( $\text{C}_4$ ) pathway for photosynthetic carbon dioxide fixation, as revealed by differences in both leaf anatomy and  $^{13}\text{C}/^{12}\text{C}$  ratios in their organic carbon. Plants using the  $\text{C}_3$  cycle produce 3-phosphoglycerate as the first product of pho-

tosynthesis, while in plants using the  $\text{C}_4$  cycle, oxalacetate, malate, and aspartate are the initial products. All plants are slightly lighter in  $^{13}\text{C}$  than is the carbon dioxide of the atmosphere and  $\text{C}_3$  plants discriminate against  $^{13}\text{C}$  to a greater extent than do  $\text{C}_4$  plants, as established by Bender (1971) and Smith and Epstein (1971).

Recently, practical uses have resulted from the finding that the  $^{13}\text{C}/^{12}\text{C}$  ratio of a plant derived material reflects the photosynthetic pathway operating in the plant from which it was derived. Cane ( $\text{C}_4$  plant) sugar sucrose can be distinguished from beet ( $\text{C}_3$  plant) sugar sucrose by  $^{13}\text{C}/^{12}\text{C}$  analysis (Smith and Epstein, 1971), a distinction not possible chemically. Also, the undeclared addition of cane sugar to maple (*Acer saccharum*,  $\text{C}_3$  plant) sirup

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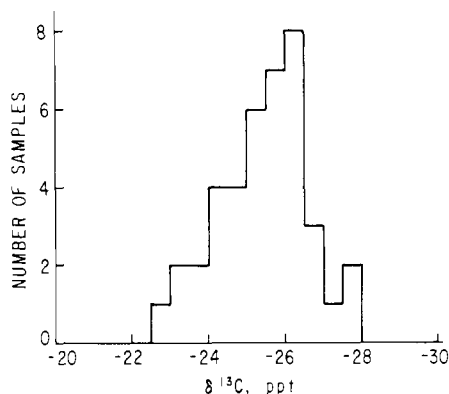


Figure 1. Distribution of  $\delta^{13}\text{C}$  (ppt) values among apple juices.

(Hillaire-Marcel et al., 1977) and corn ( $\text{C}_4$  plant) derived sirups to honey [all floral sources are  $\text{C}_3$  plants; Doner and White, (1977)] can be detected (White and Doner, 1978).

More recently,  $^{13}\text{C}/^{12}\text{C}$  analysis had been used to distinguish between vanillin from vanilla beans and that produced synthetically (Hoffman and Salb, 1979). Since  $^{13}\text{C}/^{12}\text{C}$  ratios of organic carbon from animals reflect their diets, protein from corn-fed animals can be distinguished from soy protein ( $\text{C}_3$  plant source) by the isotopic method (Gaffney et al., 1979).

The present report establishes the uniformity of  $^{13}\text{C}/^{12}\text{C}$  ratios of apple ( $\text{C}_3$  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  $\text{CO}_2$  were frozen, excess oxygen was removed, and  $\text{CO}_2$  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  $^{12}\text{C}^{16}\text{O}^{17}\text{O}$  to the mass 45 signal. The  $^{13}\text{C}/^{12}\text{C}$  ratios were determined by comparison of the  $^{13}\text{C}^{16}\text{O}_2$  and  $^{12}\text{C}^{16}\text{O}_2$  ion beams and are expressed as  $\delta^{13}\text{C}$  values, where

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

and reference standard is  $\text{CO}_2$  produced from Peedee belemnite (PDB) calcium carbonate. A  $\delta^{13}\text{C}$  value of -25 (ppt) means that the  $^{13}\text{C}/^{12}\text{C}$  ratio of the sample is 2.5%

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

variety	no. of samples	$\delta^{13}\text{C}$ (ppt)		SD	CV, %
		range	mean		
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

less than that of the PDB standard ( $^{13}\text{C}/^{12}\text{C}_{\text{PDB}} = 0.011237$ ). This amounts to 281 less  $^{13}\text{C}$  atoms per million. Reproducibility for the  $\delta^{13}\text{C}$  determinations is 0.3 (ppt) or better.

#### RESULTS AND DISCUSSION

The  $\delta^{13}\text{C}$  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  $\delta^{13}\text{C}$  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  $\delta^{13}\text{C}$  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  $\delta^{13}\text{C}$  values of many land plants. Craig (1953) suggested that local environmental effects obscure the tendency for plants of the same species to possess identical  $\delta^{13}\text{C}$  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  $\delta^{13}\text{C}$  measurements for apple juice largely represent the carbohydrates, which constitute about 97% of the apple juice solids (Agriculture Handbook No. 8, 1963). The ranges of  $\delta^{13}\text{C}$  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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## Cucurbit Seed Coat Composition

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Seed coats of domestic and xerophytic feral species of *Cucurbita* were investigated to determine composition and potential value as a ruminant feed source. These cucurbit seed coats had a 15-24% crude protein content which is considerably higher than that usually associated with seed coats. Acid detergent fiber and lignin ranges were 63-71 and 24-33%, respectively. Gross energies were in the range of 4600 to 5000 kcal/kg. Calculated cell wall digestibilities were found to be similar to those of other seed coats used for ruminant roughage.

The purpose of this work was to investigate the general composition of seed coats from several species of the genus *Cucurbita*. The genus contains 27 species which includes squashes, pumpkins, and gourds (Whitaker and Bemis, 1975). These species are native to the American continents and have adapted to many climatic conditions since their origin in the tropical regions of south central Mexico (Bemis and Whitaker, 1969). There are five domesticated extant species which are grown mainly for local markets or home consumption and thus have negligible international impact (Whitaker, 1968).

Three domesticated and two wild *Cucurbita* species were selected for this study. The three domesticated species have been described by Whitaker (1968). *Cucurbita pepo* and *C. maxima* are temperate annuals, while *C. ficifolia* is a tropical, high altitude perennial. *C. foetidissima* and *C. digitata*, two xerophytic perennial species which have been described by Whitaker and Bemis (1975), show considerable promise for domestication (Bemis et al., 1978). *C. foetidissima* ranges extensively through dry regions of the western United States and northern Mexico. The characteristics and composition of this plant have been described by Berry et al. (1976) and Bemis et al. (1978). *C. digitata* is an extreme xerophyte limited to hot, desert regions of southwestern United States and adjacent Mexico.

The recognition of cucurbit seeds as a potential food source will grow as the worldwide requirement for edible oil and protein increases. These seeds contain approximately 30% oil and 30% protein, and the yield per hectare is comparable to other commercially grown oilseeds (Jacks et al., 1972). During the processing of oilseeds, large quantities of residual seed coats are produced. They

Table I. Physical Properties of *Cucurbita* sp. Seeds

	length × width, mm	seed wt per 100 seeds, g	seed coat in seed, %
<i>C. foetidissima</i>	9 × 5	4.0	33.0
<i>C. digitata</i>	9 × 5	5.0	45.0
<i>C. pepo</i>	15 × 9	10.0	21.0
<i>C. maxima</i>	18 × 11	22.0	20.0
<i>C. ficifolia</i>	17 × 12	20.0	25.0

constitute 8-45% of the whole seed weight and are often considered byproduct wastes. If cucurbit seeds become edible oil sources, waste disposal regulations and process economics would make their possible utilization as a ruminant feed of considerable interest.

## PROCEDURE

Seeds from *C. foetidissima*, *C. digitata*, and *C. ficifolia* were obtained from the University of Arizona. Seeds from *C. pepo* (Gray Zucchini) and *C. maxima* (Green Hubbard) were obtained from the Asgrow Seed Co. Physical properties were determined in triplicate sets of 10-100 seeds. Seed coats were separated from embryos by hand and ground in a Wiley mill to -40 mesh.

Proximate composition of the seed coats was obtained by the following established methods: (a) dry matter by vacuum oven drying at 110 °C overnight, (b) lipid content using 2:1 (v/v) chloroform/methanol by Soxhlet extraction, (c) protein content by the Kjeldahl method, (d) ash content by use of an electric muffle furnace at 600 °C, (e) neutral detergent fiber (NDF) by the method of Southgate (1976), (f) acid detergent fiber (ADF) and acid detergent lignin (ADL) by the Klason method of Van Soest (1963).

Estimated cell wall digestibility was calculated by the method of Goering and Van Soest (1970), and gross energy (GE) was determined by oxygen bomb calorimetry.

Additional analytical properties were determined by the following standard methods: (a) free sugars by extraction with 80% ethanol, (b) water-soluble polysaccharide content

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