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Oxygen and Hydrogen Isotopes in Fruit and Vegetable Juices[1]

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ABSTRACT

$^{18}O/^{16}O$ ratios from the juices of a number of fruits and vegetables were measured and found to be isotopically more enriched than the water in which they grew. Fast-growing high-water-content vegetables exhibited less enrichment than slower growing fruits such as apples, pears, and plums. $^{18}O/^{16}O$ measurements were also made on the water from various sections of several plants, and the enrichment was found to occur in the following order: leaves > fruit > stem ≥ ground water.

D/H and $^{18}O/^{16}O$ measurements were made on a series of grape juice samples and, when plotted against each other, gave a slope of 3.9, indicating that the physical process causing this enrichment was probably evaporation, *i.e.* evapotranspiration.

It is known that the water in the leaves of plants can become enriched in oxygen and hydrogen isotopes due to the process of evapotranspiration (6, 9, 11); however, little work in comparison has been done on the isotope ratios of water in fruit.

Orange juice was studied by Bricout (1) who found that the water oxygen-18 and deuterium content of the juice was greater than that of the water in which the plant grew. Oranges from two different geographical regions with differing water supplies were also studied. The difference between the $^{18}O/^{16}O$ ratio of the water and that of the juice was determined and, in both cases, this was found to be different, indicating that this enrichment was not constant for all areas.

Oranges were studied in another work by Bricout *et al.* (3) in which the water D/H and $^{18}O/^{16}O$ ratios of fruit from 17 different geographical regions were measured and compared with the meteoric water line (7). The origin of the line was taken as the environmental water in which the oranges grew. In all cases, the orange juice ratios fell below the meteoric line on another line with a slope of four. This observation was explained in this and another work by Bricout (1), in terms of evapotranspiration. Similar results have also been expressed in other publications (4, 5).

The slope of four obtained by Bricout was disputed by Epstein *et al.* (8), who found a plot of D/H *versus* $^{18}O/^{16}O$ from leaves to have a slope of 2.5, *i.e.* the standard evapotranspiration line. Epstein *et al.* suggested that partial equilibration between the atmospheric water and Bricout's fruit juice samples could have occurred, countering some of the kinetic isotope effects of evaporative transpiration, hence, changing the slope of the D/H, $^{18}O/^{16}O$ plot.

Vegetables, in addition to fruits, have also been studied. Lesaint *et al.* (10) studied the isotope ratios of the water from the leaves, sap, and fruit of tomatoes and maize, the results of which were plotted on a D/H, $^{18}O/^{16}O$ graph. The sap and leaf water values fell on an evaporation line (slope of three); however, the 'fruit' water was below this. Daily effects as well as that of air humidity were also noted.

A comprehensive study of 24 grape juices was carried out by Bricout (2) to measure geographical effects as well as variations between different grape types. It was found that differing varieties from the same area had similar D/H and $^{18}O/^{16}O$ values. The geographical effect was, however, much larger and considerable differences were noted between different areas.

It was, therefore, the aim of this work to measure the D/H and $^{18}O/^{16}O$ ratios of a number of fruits and vegetables and to compare the results with those already reported in the literature.

Table I. *$\delta^{18}O$ Measurements of the Water in a Variety of Fruits and Vegetables*

Fruit	$\delta^{18}O_{SMOW}$‰	Vegetable	$\delta^{18}O_{SMOW}$‰
Pomegranate	−3.5	Potato	−4.9
Blackberry	+2.7	Kumera (sweet potato)	−3.7
Apple	−0.4	Zucchini	−3.7
Pear	−0.3	Cucumber	−3.5
Orange	−1.9	Marrow	−2.2
Lemon	−2.5	Tomato (ripe)	−3.1
Grapefruit	+1.8	Tomato (unripe)	−2.4
Plum	0.0	Watermelon	−4.9
Nectarine	−0.4		
Peach	−1.2		
Passionfruit	−3.4		
Guava	−3.9	Local water	−7.5[a]
Strawberry	−0.5		
Mandarin	−2.9		
Tamarillo	−2.0		
Fejoa	−5.6		
Rosehip	−3.5		
Kiwifruit	−3.4		

[a] With small seasonal variations

MATERIALS AND METHODS

Oxygen Isotope Measurements of Fruits and Vegetables. The $^{18}O/^{16}O$ ratios of the fruit and vegetable juices were measured by directly equilibrating 20 ml of CO_2 with 10 ml of the filtered vegetable or fruit juice at 20°C for 48 h. The equilibrated CO_2 was then removed and purified of water and other trace contaminants by fractional distillation before being admitted into the mass spectrometer. $^{18}O/^{16}O$ ratios are expressed in the δ-notation where:

$$\delta^{18}0 = \left[\frac{(^{18}O/^{16}O)_{sample}}{(^{18}O/^{16}O)_{SMOW}} - 1\right] \times 1{,}000‰$$

and $(^{18}O/^{16}O)_{SMOW}$ is the ratio of the international standard

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Table II. $\delta^{18}O$ *Analysis of Water in Various Sections of Watermelon, Tomato, and Grape Plants*

Plant	Section	$\delta^{18}O_{SMOW}$‰
Watermelon	Fruit	−4.9
	Stem	−6.7
	Leaves	+1.2
Tomato	Fruit (ripe)	−3.1
	Fruit (unripe)	−2.4
	Stem	−3.1
	Leaves	No sample
Grape	Fruit	−2.4
	Leaves	+1.5
	Main stem[a]	−6.6
	Leaf petiole	−3.7
	Ground water	−7.0

[a] A sap sample was extracted from the main stem by cutting a main branch near the ground and collecting the fluid released over a 2-h period.

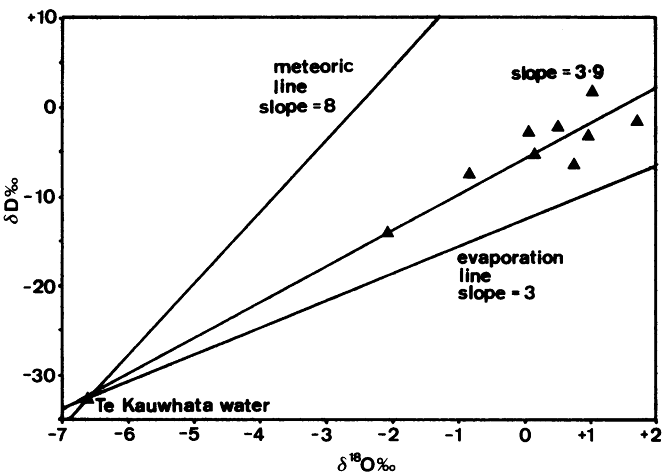

FIG. 1. δD *versus* δ^{18}O plot for a series of grape juices.

SMOW (Standard Mean Ocean Water).

Hydrogen Isotope Measurements of Fruit and Vegetable Juices. The D/H ratios of the vegetable/fruit juices were measured by converting the water from the juice to hydrogen via an uranium furnace at 600°C. A sample size of approximately 5 μl was used.

The D/H ratios are also expressed in the δ-notation where:

$$\delta D = \left[\frac{(^2H/^1H)_{sample}}{(^2H/^1H)_{SMOW}} - 1\right] \times 1{,}000‰$$

and $(^2H/^1H)_{SMOW}$ is the $^2H/^1H$ ratio of the international standard SMOW.

δD values were replicated to within ±0.5‰ (1σ), whereas δ^{18}O values could be replicated to ±0.05‰ (1σ).

RESULTS

Vegetable and Fruit Juice δ^{18}O Measurements. The first samples to be measured were a series of vegetable and fruit juices. This was done to determine the ^{18}O enrichments possible in the water of the fruit from a wide range of differing plants.

As can be seen from the results of Table I, the water in all of the fruits and vegetables was more enriched in ^{18}O than the water in which the plants grew. Direct comparison between different fruits (or vegetables) can not be made because they were all harvested at different times of the year, so they would have been growing under different climatic conditions. One general trend that can be noted, however, is that vegetables (*i.e.* zucchini [*Cucurbita pepo* var giromontiina], cucumber [*Cucumis sativus*], and watermelon [*Citrullus lanatus*]) do not exhibit as much ^{18}O enrichment as do the slower growing fruits such as apples, pears, and plums.

δ^{18}O Measurements of the Water of Different Sections of a Plant. Water was removed from various sections of a tomato, watermelon, and grape plant. In all cases (except that of the fruit), the plant material was finely chopped and then the water removed by freeze drying (Table II).

From the results, it can be seen that, from both the watermelon and the grape plant, the leaf water had the highest δ^{18}O values (tomato plant, no sample). This is in agreement with other published work (6, 9, 11). Because such a large amount of evapotranspiration occurs at the leaves of a plant, they are expected to be the site of highest isotopic enrichment.

No literature values were available for leaf petiole ^{18}O data. (Note: in this case the leaf petiole implies the thin connecting stem between the leaf and the main stem). However, it seems likely that the water in this section of the plant would have an intermediate value between that found in the leaves and that found in the main stem. This was in fact observed.

The ^{18}O value of the water in the fruit was heavier than that found in the stem in two out of the three cases. For ripe tomatoes, the $^{18}O/^{16}O$ ratio was the same as the stem water, whereas for unripe fruit it was heavier. The reasons for this at the moment are unknown.

The ^{18}O content of the water in the stem of the grape plant was found to be similar to that of the groundwater in which the plant was growing. The water was sampled from a bore and not directly from around the roots of the plant; therefore, it may not be representative of the water actually present at the root surface. If this was so, then the passage of water into this plant may have been without isotopic fractionation as has previously been reported (6, 9, 11).

D/H and $^{18}O/^{16}O$ Measurements of a Series of Grape Juices. As previously stated, it has been found that evaporation causes isotopic enrichment in the fruit of plants. We therefore decided to measure the D/H and $^{18}O/^{16}O$ ratios of some grape juices grown under New Zealand climatic conditions and compare the results obtained with those already reported in the literature.

As can be seen from Figure 1, a straight line can be drawn through the points, the relationship being:

$$\delta D_{SMOW} = 3.9(\delta^{18}O_{SMOW}) - 6.1$$

Also drawn on the figure are the meteoric water line and an evaporation line. The experimental line for grape juice lies between these two with a slope of 3.9 indicating that the physical process causing isotopic enrichment of the water in grapes is probably evaporation. This is in agreement with the results obtained by Bricout for orange juices, where a slope of 4 was obtained (1). It is still possible that in grapes partial equilibration between atmospheric water and the fruit juice occurs, thus providing an explanation for the fact that the points do not lie on an evapotranspiration line with a slope of 2.5 as found by Epstein *et al.* (8).

DISCUSSION

Water in a plant can become enriched in deuterium and ^{18}O by the process of evapotranspiration. This enrichment has been shown to be highest in the leaves, but with the enrichment also occurring in the fruit. The water in fast-growing high-water-content vegetables, *e.g.* watermelon, zucchini, and cucumber, was found to be less enriched than that in the slower growing fruits such as apples, pears, and plums.

A D/H, $^{18}O/^{16}O$ plot of the water from a number of grape juice samples had a slope of 3.9, indicating that the physical process causing this enrichment was evapotranspiration.

A possible application of this work could be in the detection of added water to commercial vegetable and fruit juice preparations.

LITERATURE CITED

1. Bricout J 1973 Composition en isotopes stables de l'eau des jus de fruits. Ann Fals Exp Chim 66: 195–202
2. Bricout J 1978 Recherches sur le fractionnement des isotopes stables de l'hydrogène et l'oxygène dans quelques végétaux. Rev Cytol Biol végét-Bot 1: 133–209
3. Bricout J, JC Fontes, L Merlivat 1972 Sur la composition en isotopes stables de l'eau des jus d'orange. CR Acad Sci Paris Série D 274: 1803–1806
4. Bricout J, L Merlivat 1971 Sur la tenur en deutérium des jus d'orange. CR Acad Sci Paris Série D 273: 1021–1023
5. Bricout J, Y Mouaze 1971 Analytical criterion for distinguishing between natural orange juice and diluted concentrate. Fruits 26: 775–788
6. Dongmann G, H Nürnberg, H Förstel, K Wagener 1974 Enrichment of water (oxygen-18) in the leaves of transpiring plants. Radiat Environ Biophys 11: 41–52
7. Epstein S, T Mayeda 1953 Variation of oxygen content of waters from natural sources. Geochim Cosmochim Acta 4: 213–214
8. Epstein S, P Thompson, CJ Yapp 1977 Oxygen and hydrogen isotopic ratios in plant cellulose. Science 198: 1209–1215
9. Farris F, BR Strain 1978 The effects of water-stress on leaf (oxygen-18)-water enrichment. Radiat Environ Biophys 15: 167–202
10. Lesaint C, L Merlivat, J Bricout, JC Fontes, R Gautheret 1974 Sur la composition en isotopes stables de l'eau de la tomate et du mais. CR Acad Sci Paris Serie D 278: 2925–2930
11. Zundel G, W Miekeley, B Grisi, H Förstel 1978 The ^{18}O-water enrichment in the leaf water of tropical trees: comparison of species from the tropical rain forest and the semi-arid regions in Brazil. Radiat Environ Biophys 15: 203–212