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Measurements of Oxygen-18/Oxygen-16 Stable Isotope Ratio in Citrus Juice: A Comparison of Preparation Methods

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The $^{18}O/^{16}O$ ratios of the water of a number of citrus juices were measured by utilizing two preparation methods (i.e., distillation and charcoal treatment). The charcoal preparation method not only was proven to be a rapid method that circumvented the need for distillation but also was more accurate and reliable in accounting for the real isotopic ratio of $^{18}O/^{16}O$ in citrus juices. Data obtained by utilizing the charcoal preparation method were ca. 0.5‰ higher than those obtained by distillation. This discrepancy was related to incomplete water distillation. The superior accuracy obtained with the charcoal method indicates that it should be possible to utilize the isotope ratio for the detection of citrus juice adulteration.

In recent years the study of stable isotopes in food products revealed the possibiity of applying new and advanced methods to detect adulteration. For instance, the isotope ratio of $^{13}C/^{12}C$ was utilized to detect adulteration in honey (Doner and White, 1977; Doner et al., 1979; White and Doner, 1978a,b; Ziegler et al., 1977), apple juice (Doner and Phillips, 1981; Doner et al., 1980), whisky (Simpkins and Rigby, 1982), fruit juice concentrates (Parker, 1982), wine (Dunbar, 1982a), grape juice (Dunbar, 1982b), and citrus juice (Doner and Bills, 1981; Nissenbaum et al., 1974).

Bricout (1973) differentiated between a natural fruit juice and a dilute concentrate, by measuring the isotopic composition of their water. On the basis of measuring the stable isotopes deuterium and ^{18}O, he concluded that isotopic analysis allowed a very confident distinction between natural fruit juice and reconstituted juice. According to Nissenbaum et al. (1974), the technique is based on the fact that the rainwater or irrigation water, when transported from the roots to the fruit, is fractionated, probably by evapotranspiration, in such a way that the light isotopes (hydrogen and ^{16}O) are lost preferentially to the heavy isotopes (deuterium and ^{18}O).

Variations in the heavy isotope ^{18}O content and the ratio $^{18}O/^{16}O$ in a number of marine water and freshwater samples in different places in the world were reviewed by Epstein and Mayeda (1953). In Israel the ^{18}O values for rain- and groundwater were reported to be –5‰ and –4.5 to –5.5‰, respectively, and for deuterium –25‰ in a rainwater and –15 to –25‰ in groundwater (Gat and Dansgaard, 1972).

Nissenbaum et al. (1974) reported that the deuterium content of citrus juice was too widespread to be applicable in detecting addition of water to citrus juice. Hence, deuterium measurement was precluded from being utilized as a routine detection method for adulteration.

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A rapid technique for sample preparation that circumvents the need for distillation required normally in isotopes determination was proposed by Nissenbaum and Feld (1980). They also indicated that a major drawback in applying isotopic techniques on a routine basis is the need to distill the sample prior to analysis. Furthermore, the low-temperature vacuum distillation used (Bricout, 1973) is a tedious and time-consuming procedure.

This research was undertaken with the aim of reviewing the determination of $^{18}O/^{16}O$ and to furnish a rapid, easy-to-use, accurate, and reliable method to be utilized in the routine preparation and determination of stable isotopes in citrus juice.

MATERIALS AND METHODS

Samples of natural citrus juice (orange and grapefruit) and reconstituted citrus juice were blended (in order to obtain a broad range of isotopic composition) and prepared for the analysis of $^{18}O/^{16}O$ by adapting two methods: vacuum distillation (Bricout, 1971) and charcoal treatment (Nissenbaum and Feld, 1980).

Due to several drawbacks detected in the aforementioned methods, the following revised preparation procedures were employed. (a) Distillation method: (1) A sample of about 5 g was frozen in one leg of a Ritenberg "trousers" (Figure 1) at ca. –60 °C by using an acetone/dry ice bath. (2) The Ritenberg trousers was attached and the system was evacuated by using an oil vacuum pump. When vacuum was established (10^{-2} mm Hg), the Ritenberg trousers was cut off from the vacuum system by using a high-vacuum stopcock. (3) The sample was then heated slowly from –60 to +50 °C, at a rate of 20 °C/h. The water vapor was condensed and collected in the other leg of the Ritenberg trousers by using an acetone/dry ice trap. (4) The distillation was ended when all water had evaporated and a constant weight was obtained (after ~24 h). (b) Charcoal treatment: (1) To exclude suspended particles, samples (ca. 10 g) were centrifuged for 10 min at 15 000 rpm, in closed cuvettes. (2) The supernatant was mixed with activated charcoal powder (washed with acid; BDH Chemicals, Ltd., England; activated at 105 °C in a vacuum oven overnight) for 60 min in a closed flask. (3) The slurry was centrifuged at 15 000 rpm for 10 min in closed cuvettes
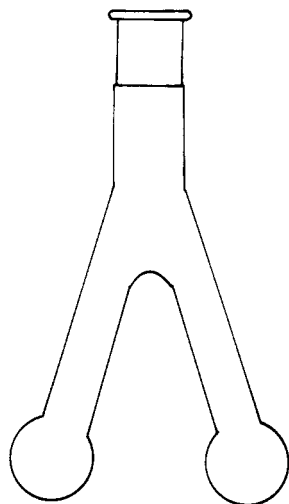
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Figure 1. Schematic description of Ritenberg "trousers".

(large errors could be incorporated if the cuvettes are not closed). (4) The supernatant was filtered through Whatman No. 2 filter paper. The funnel was covered with aluminum foil to prevent evaporation. For the mass spectrometric measurement, $\sim$5 mL was used. It is worth noting that special precaution was required to avoid any unaccounted evaporated water (e.g., in centrifugation and filtration) during sample preparation. For instance, up to 20% error may be expected if open cuvettes are used during centrifugation.

Determination of $\delta^{18}O$. The water drawn from the samples following the above preparation methods was analyzed for ^{18}O by using the regular procedure for water analyses (Bricout, 1973). This included equilibration of the samples with CO_2 (which served as the measurement gas) for 24 h at 25 °C. Analyses were done in duplicate. A mass spectrometer (Varian, Model Mat 250, West Germany) was used for the determination of $CO_2(46)/CO_2(44)$.

The results were expressed in the δ notation

$$\delta R \equiv \delta^{18}O, \text{‰} = \frac{R_{sample} - R_{standard}}{R_{standard}} \times 1000 \qquad (1)$$

where R_{sample} is the $^{18}O/^{16}O$ ratio of the sample, ppm and $R_{standard}$ is the standard ratio $^{18}O/^{16}O$ of SMOW (standard mean ocean water = 1993.4 ppm) as defined by Craig (1961).

Fractionation Effect. To elucidate the effect of fractionation during evaporation, a two-step distillation was used. Results of the first step (2 h) included the amount of water (W_1, g) and the stable isotope ratio (δR_1, ‰). The distillation was renewed for the second step following the technique outlined previously. This yielded the final values of water which was evaporated (W_2, g) and δR_2, ‰).

The results obtained by the two-step evaporation procedure were compared with those obtained by distillation of identical samples in one step (W, g; δR, ‰).

Statistical Analyses. Regression analysis was carried out by using computer-aided statistical programs BMDP1R, BMDP2R, and BMDP9R (Dixon, 1981).

RESULTS AND DISCUSSION

A comparison of the analytical results obtained from citrus juice and water samples analyzed by the conventional distillation technique, with those derived by the charcoal treatment, is given in Table I. These values indicated that results obtained with the two methods were in good agreement and gave almost identical values of

Table I. Comparison of ^{18}O Stable Isotopes (Mean ± Two Standard Deviations) Obtained by the Distillation and Charcoal Preparation Methods with Identical Samples[a]

| | | $\delta^{18}O$, ‰ | |
| | | distillation | charcoal |
sample	n	method	treatment
water	6	−4.29 ± 0.17	−4.18 ± 0.17
juice 1	5	−3.45 ± 0.36	−2.79 ± 0.24
juice 2	4		−2.85 ± 0.20
juice 3	5	0.33 ± 0.21	1.20 ± 0.16
juice 4	6		1.15 ± 0.19
juice 5	7		2.62 ± 0.11

[a] n = number of samples.

Table II. Stepwise Regression Analysis of the Results Obtained from Two-Step Distillation of Nineteen Samples of Orange Juice (for Notation, See the Text)

model no.	regression model	square multiple correlation, r^2
1	$\delta R = (1.159 \cdot \delta R_2 \cdot W_2 + 0.895)/W$	0.798
2	$\delta R = (1.008 \cdot \delta R_2 \cdot W_2 + 0.750 \cdot \delta R_1 \cdot W_1 + 0.364)/W$	0.905
3[a]	$\delta R = 1.030 \,(\delta R_1 \cdot W_1 + \delta R_2 \cdot W_2)/W$	0.996

[a] Regression through the origin.

stable isotope ratio $^{18}O/^{16}O$ when water was analyzed. Also, the standard deviation of both techniques was below 0.1 ‰ for $\delta^{18}O$, which is generally the precision of the determination (Nissenbaum and Feld, 1980). This agreement between the two techniques vanished when citrus sample determinations were compared. The results obtained with the distillation method were lower than those obtained with the charcoal method. This phenomenon repeated itself during 2 years of study and for more than 200 determinations.

This discrepancy between the results obtained with the two methods can be explained by studying the distillation process. It was logical to assume that during distillation the "light" isotopes were evaporated more readily than the "heavy" isotopes, causing fractionation. This fractionation may lead to large error if the distillation is not completed properly. To demonstrate this assumption, a two-step distillation experiment was designed (see Materials and Methods, Fractionation Effect).

Results of stepwise regression analysis on all the data points ($n = 19$) are given in Table II. On the basis of the multiple correlation coefficient derived, model 1 showed that the vital isotope information was in the second fraction. The first distillated fraction was only second in importance (model 2). Yet, a significant increase in the correlation coefficient was obtained when the two fractions were combined. This indicated that both fractions should be taken into consideration. Finally, model 3 showed that the results were completely additive when their relative weight and isotope ratios were considered. It was therefore concluded that in the distillation technique, unless all the water is evaporated, large errors can be incorporated.

During the distillation of the samples an increase of the viscosity is normaly observed. Hence, complete distillation of the water is not always possible. This is particularly problematic when the sample includes organic materials and dissolved solids, as in citrus juice. This finding has a special effect on the determination of the ^{18}O isotopes in concentrated citrus juice, as complete distillation of the concentrated products is difficult and sometimes impossible. This was proved by comparing the results obtained from the analyses of 12 samples of concentrated orange
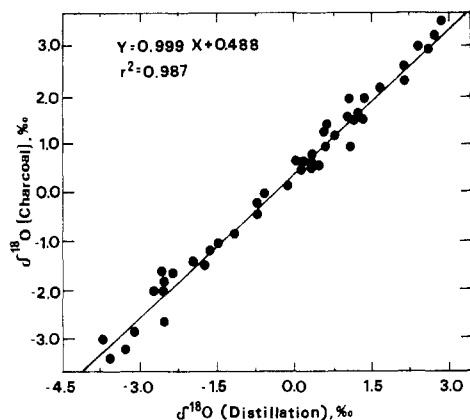
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Figure 2. Comparison of $\delta^{18}O$ derived by charcoal treatment and by the distillation method in citrus juice.

juice, which yielded the following range of $\delta^{18}O$: 5.21–10.73 and 6.42–9.38‰ for 30 and 60 °Brix, respectively. This wide range of results is related to the difficulty of evaporating all the water, as explained above.

It is worth noting that due to the fractionation phenomenon, utilization of the distilation method gives lower values of stable isotopes ratio. This fact is of great economic importance, as isotopic concentration has been utilized recently as a standard routine to establish adulteration of citrus juice (AFNOR, 1980).

The results obtained by the two preparation techniques were compared to derive the regression coefficients and to establish the relationship.

The data analyzed (44 samples) and the straight line derived by the least squares method are depicted in Figure 2. The regression analysis yielded the relation (using four digits)

$$Y = 0.9993X + 0.4876 \qquad r^2 = 0.987 \qquad (2)$$

where $Y = \delta^{18}O$ charcoal treatment, ‰, $X = \delta^{18}O$ distillation, ‰, and r = correlation coefficient.

Equation 2 indicates that both methods may be used to determine the $\delta^{18}O$ isotopic composition of citrus juice. However, the charcoal method provides a higher value, by ca. 0.5‰.

This difference may be related to difficulties with the distillation method, when complete evaporation of water

is not possible due to high viscosity of the concentrated medium. Furthermore, the organic compounds and dissolved solids may reduce the amount of free water.

Hence, the charcoal method not only proved to be a rapid procedure that circumvents the need for distillation but also is more accurate and reliable in accounting for the real isotopic ratio of $^{18}O/^{16}O$. The superior accuracy obtained with the charcoal method indicates that it should be possible to utilize the isotope ratio for the detction of citrus juice adulteration.

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