Oxygen Isotope Ratios of Juice Water in Australian Oranges and Concentrates

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Australian orange juices from major growing regions have been surveyed over a 5 year period with a view to establishing a database of $^{18}O/^{16}O$ isotope ratios against which retail samples can be tested for authenticity. The $^{18}O/^{16}O$ ratios were found to follow a consistent pattern that had both a cyclic seasonal and a regional influence. Oxygen delta values ranged from a summer maximum of >+15‰ for oranges from inland regions to a winter minimum of ~+1‰ for oranges grown in coastal areas. However, over a shorter time period, the range of values was markedly less than this. Concentrated orange juices, pulpwashes, and peel extracts, as well as other citrus types, were also tested. The effect of some industry practices that have an effect on $^{18}O/^{16}O$ ratios was also investigated.

Keywords: *Authenticity; orange juice; isotopic analysis; $^{18}O/^{16}O$ ratios*

INTRODUCTION

In Australia, as in many other countries, there is a requirement for a truthful ingredient statement on the label of foodstuffs put up for retail sale. Fresh orange juice is a popular beverage and sells at a premium price compared to juice made from reconstituted concentrate. It ranges from very sweet navel orange juice in midwinter to a more acidic Valencia orange juice during summer. The cost advantage or seasonal unavailability of fresh juice may provide some manufacturers with the incentive to extend fresh juice with reconstituted concentrate or to water down high-solids fresh orange juice to the industry-accepted minimum strength of 10 °Brix. Both of these practices are infringements on the consumer's right to truth in labeling and are contrary to the Australian Citrus Industry's voluntary code of practice.

Water addition to natural fruit juices is determined by measuring the oxygen $^{18}O/^{16}O$ isotope ratio of the water in the juice. An isotopic enrichment occurs during fruit development, so that ^{18}O levels in natural juice water are significantly higher than in the groundwater or irrigation water that is taken up through the plant's root system or used by converters to reconstitute concentrate. This enrichment is generally linked to the loss of the lighter ^{16}O isotopes during leaf evapotranspiration. On the universally used delta scale, rain water is negative compared to the Standard Mean Ocean Water (SMOW) zero reference point, whereas most fruit juices, particularly orange juices, are significantly more positive. Studies appear in the literature about the application of isotopic fractionation to authenticity testing in oranges and apples (Bricout, 1971, 1973; Bricout et al., 1972; Nissenbaum et al., 1974; Krueger, 1988; Pupin et. al., 1998), grapes and wine (Dunbar, 1982a−c; Tardaguila et al., 1997), and other plants (Bricout, 1978).

Most of these authors refer to the physical factors that influence $^{18}O/^{16}O$ values in fruit juices (and their fermentation products). These are ultimately connected to the climatic conditions of the growing area, with average rainfall, temperature, and humidity all having an input into the regional isotopic profile. It has been reported that fruit grown in hot, dry climates has more isotopic enrichment than fruit grown in cooler, wetter countries. It follows from this that a "water map" of $^{18}O/^{16}O$ values can be constructed to check regional conformity. Such a map has already been prepared for major European wine-making regions to check wine appellation claims (Versini et al., 1995; Guillou, 1997).

$^{18}O/^{16}O$ values have also been applied to authenticity testing of concentrates (Brause et al., 1984; Doner et al., 1992; AOAC, 1994) to detect the addition of beet sugar syrups prepared using tap water as the solvent. The isotopic content of the residual water in concentrated orange juices is significantly heavier than in source juice, obeying an exponential law that can be approximately linearized (Martin and Martin, 1995).

This paper presents the results of an extended survey of ^{18}O levels in fresh orange juices of Australian origin, with a view to the production of a local water map for the major production areas, against which retail samples can be compared. It also presents some results for Australian orange juice concentrates, Australian peel extract concentrates, Brazilian pulpwashes, and other citrus varieties and examines the effects of long-term storage of fruit and frozen storage of fresh juice on $^{18}O/^{16}O$ isotope ratios.

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Figure 1. Principal orange-growing areas in Australia: 1, Riverina-Murrumbidgee Irrigation Area (MIA); 2, Sunraysia (SUN); 3, Riverland (RIV); 4, Chittering River (WA); 5, Burnett District (QLD); 6, NSW Coastal (COAST).

MATERIALS AND METHODS

Nature of the Orange Juice Samples. The Australian fresh orange juice survey covered two programs during the years 1993−1997. Samples were either of navel or Valencia type. Sampling was conducted by Department of Agriculture field inspectors who collected batches of whole fruit weighing ∼5 kg. In the first program (1993−1994) 96 samples were taken randomly from stock submitted to the major processors at Leeton, Mildura, and Berri at fairly regular time intervals to obtain a continuous record of seasonal variation. In the second program (305 samples taken between 1994 and 1997), the geographical base was enlarged to include all of the Australian growing regions except the Northern Territory. Locations included the New South Wales Riverina-Murrumbidgee Irrigation Area (MIA: Stanbridge, Hillston, Lake Wyangang, Tharbogang, Leeton, Griffith, Beelbangera, Yanco, and Hanwood), Victorian Sunraysia region (SUN: Curlwaa, Dareton, Mildura, Redcliffe, Irymple, and Monak), South Australian Riverland (RIV: Cooltong, Paringa, Renmark, Berri, Loxton, Waikerie, Ramco, and Cadell), Western Australia (WA: Gin Gin and Chittering), and Queensland Burnett region (QLD: Mundubberah, Gayndah). Samples from New South Wales coastal regions (COAST: Gosford, Macleay Valley, and Clarence River area) were gathered by the principal author. Figure 1 shows the locations of these production areas. Australia's total orange production is variable, ranging, for example, from 582095 tonnes in 1994 down to 442077 tonnes in 1996 (juice and table fruit), with the three major areas (MIA, ∼30%: SUN, ∼20%, and RIV, ∼40%) producing the majority of the total yield (McLennan, 1998). QLD and COAST production is mainly consumed locally and does not significantly enter into the mainstream of juice manufacture.

The fresh orange samples were shipped to the laboratory and squeezed within 4 days of picking. Juice was extracted with a domestic reamer (either hand or electrical), strained to remove seeds, and stored in labeled 2 L polyethylene bottles in a freezer at −20 °C until analysis.

The Australian concentrates (prime orange juice concentrates and water-soluble peel extracts) were sampled during the first program from regional processing plants at the same time as the fresh oranges were selected. Concentrated juice samples (prime) were produced by means of falling film evaporators such as APV, Wigand, or Alfa-Laval types. Generally, the navel concentrates were produced at ∼57 °Brix and Valencia concentrates at ∼63 °Brix, because of their lower pectin contents. Peel extracts were produced from pectinase-degraded orange peels that had then been centrifuged to remove solid material, ion-exchanged to remove bitter ingredients, and then evaporatively concentrated in a similar manner to the primes. These necessarily contain some added tap water during manufacture. The Brazilian pulpwash samples (water-extracted soluble orange solids) were supplied by Schutzgemeinschaft fur der Fruchtsaft-Industrie (SGF). All concentrates were stored in a frozen state at −20 °C after receipt.

Prior to analysis, samples were brought to room temperature and thoroughly mixed to eliminate any isotopic fractionation that may have occurred during thawing.

Determination of Oxygen $^{18}O/^{16}O$ Ratios. Isotope ratios on prepared samples were determined by isotope ratio mass spectrometry (IRMS) using a Finnigan-MAT 252 following well-established methods. Results were expressed using the familiar delta notation

$$\delta E\ (‰) = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000$$

where E is the ^{18}O isotope, R is the heavy/light isotopic ratio, and the standard was SMOW as defined by Craig (1961).

Oxygen $^{18}O/^{16}O$ ratios were determined on carbon dioxide after equilibration with juice water according to the isotopic exchange reaction (Epstein and Mayeda, 1953):

$$C^{16}O_2 + H_2{}^{18}O \rightleftarrows C^{16}O^{18}O + H_2{}^{16}O$$

To carry out the isotopic exchange, centrifuged samples (10 mL) were equilibrated with 25 mL of carbon dioxide plus air (∼1:1) in 50 mL disposable plastic syringes (Yoshida and Mizutani, 1986) for 2 h at 25 °C in an air-conditioned room, and after appropriate cryogenic purification $\delta^{18}O$ was determined relative to Pee Dee Belemnite (PDB) (Craig, 1957). A control juice of known isotopic composition was run with each batch of samples. This control was either a juice having a known $\delta^{18}O$ value relative to SMOW determined by interlaboratory comparison studies conducted by the European Commission of Standardisation CEN/TC 174 (Koziet et al., 1995), against two international water reference standards provided by the International Atomic Energy Agency (IAEA), or another juice analyzed by an external laboratory to IAEA water standards. Results for the samples were then adjusted to ‰ SMOW by adding or subtracting the appropriate correction required to align measured $\delta^{18}O_{PDB}$ of the control with consensus $\delta^{18}O_{SMOW}$. A number of the samples were also reanalyzed against water standards by several external laboratories as an interlaboratory check.

RESULTS

$^{18}O/^{16}O$ Ratios of Fresh Orange Juices. The data produced from the samples surveyed in this investigation show the extent to which there is a strong seasonal and regional influence on $^{18}O/^{16}O$ ratios in the juice in fresh Australian oranges. A cyclic seasonal pattern of elevated summer values alternating to much lower winter readings occurred during every year that was surveyed. This is illustrated in the plots in Figure 2, which compares results of an entire year (1993) of sampling from neighboring MIA and SUN regions, and data in Table 1, which cover the major juice production areas in Australia (MIA, SUN, and RIV) over a 5 year period (1993−1997). The seasonal influence is also apparent in Figure 3, which graphs data from the second survey and includes the QLD, WA, and COAST growing regions. The $^{18}O/^{16}O$ ratios fluctuated over a small range within each time frame, probably because of variations in the way citrus orchards were managed and differences in factors such as the age and rootstocks

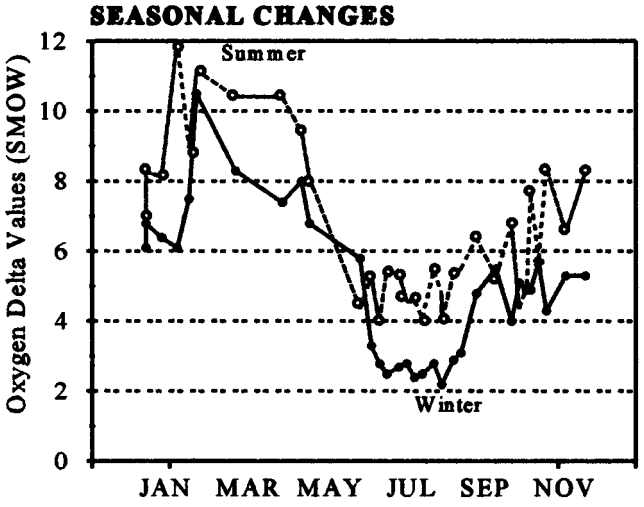

Figure 2. Comparison of seasonal variation in $^{18}O/^{16}O$ ratios for orange juice from Riverina-MIA (●) and SUN (○) areas during 1993.

Table 1. $^{18}O/^{16}O$ Ratios of Orange Juices from Principal Growing Regions (MIA, SUN, and RIV) over a 5 Year Period

month	minimum value (‰)	maximum value (‰)	average value (‰)	no. of samples
January	5.4	15.2	10.2	37
February	7.5	15.1	10.4	12
March	8.3	16.3	12.2	24
April	7.2	14.5	10.9	11
May	8.0	9.4	8.5	3
June	4.4	6.8	5.9	4
July	1.0	7.5	4.2	41
August	1.1	6.1	3.6	29
September	1.6	6.4	4.1	15
October	2.6	9.6	6.2	21
November	4.3	11.5	7.8	20
December	3.8	11.9	8.0	14

of the trees themselves. The minimum values during this second sampling period were somewhat higher than found in the first survey.

The regional influence can also be seen in Figures 2 and 3. For example, Figure 2 shows that there was a small but consistent difference throughout the passage of a year for MIA and SUN growing regions, which are situated only a few hundred kilometers from each other. Figure 3 shows that this regional variation also occurred Australia-wide, with spring and summer (September−April) figures for Valencia oranges being higher for RIV and SUN than for MIA and areas in Western Australia. On the other hand, winter values (mostly navel but with some Valencia) were higher for fruit from Queensland and Western Australia than from other regions. The lowest winter and summer values were for NSW coastal fruit.

$^{18}O/^{16}O$ Ratios of Concentrates. ^{18}O values were determined on concentrates during the first year of the survey only, principally because of less concentrate production by the local industry after that time. The results for Valencia oranges (produced at ~65 °Brix and predominantly during October−March) were considerably higher than those reported by Brause for this type of concentrate (Brause, 1984), with a mean value of +17.1‰ and standard deviation (SD) of 1.85 for 69 samples from the RIV and SUN areas. The values for 22 samples of navel juice from the same areas had a mean of +14.2‰ and SD of 2.3. The lower values for navel oranges were considered to be due to the essentially winter production of this type of fruit (lower initial $^{18}O/^{16}O$ values) and a practice of concentrating it to only 57 °Brix to prevent pectin-induced gelation, which tends to occur if more water is extracted. The distribution of values for prime juices is shown in Figure 4.

The ^{18}O values for pulpwash (26 samples) and peel extracts (29 Valencia and 13 navel samples) cover a lower range of values than found for prime concentrates, reflecting the use of exogenous water in their manufacture. The distribution of values for these juice byproducts is shown in Figure 5.

Effects of Long-Term Storage of Oranges and of Freezing Fresh Orange Juice. It has been reported (Dunbar, 1982a,b) that changes in $^{18}O/^{16}O$ isotopic ratios occur in picked grapes as a result of water loss through the skin and in the must after partial freezing. A similar physical effect for both of these physical processes should occur for other fruits including citrus.

Oranges are not always squeezed immediately after picking but might be traded interstate or held over at the extraction plant during times of overproduction. The time lapse between picking and processing could extend to several weeks. An experiment was conducted to assess the effects of long-term storage on ^{18}O values. The oranges used were freshly picked from a single tree and then numbered and stored in an open container in the laboratory. Six oranges were randomly taken from the pooled collection at regular intervals and weight loss, °Brix, and oxygen delta value on the combined extract from all six of them were determined. The results for each set of oranges are shown in Table 2. These show a slow increase in oxygen delta value in the water remaining in the fruit. Because laboratory conditions are less variable than would normally be encountered in industry and evaporation rates depend on air flow, humidity, and temperature, the trends that the data show are indicative only.

Orange juice may also be "slush frozen" and held in storage. Small amounts of ice separate from the juice during this process. The occurrence is similar to the first stage of commercial freeze concentration, when ice crystals are continuously removed from the juice as it is progressively chilled (Chin, 1993). An isotopic fractionation is known to occur in water during freezing and thawing. A model experiment to assess the effects of ice removal was conducted by thawing a frozen juice without thorough remixing. A 2 L plastic bottle of frozen Valencia juice was allowed to equilibrate to room temperature, so that the unmelted ice floated to the top and concentrated juice remained as a lower layer. Successive fractions from the top down were sampled for determination of °Brix and oxygen $^{18}O/^{16}O$ ratios. Results are summarized in Table 3. These show a trend similar to that reported by Dunbar (1982a) of a small increase in $^{18}O/^{16}O$ ratios for ice and a decrease in $^{18}O/^{16}O$ ratios for freeze-concentrated juice compared to the original juice. The results show that in freeze concentration processes, the remaining juice is depleted in the heavy isotope, whereas during an evaporation process it is enriched. The isotopic fractionation factor is 3 times smaller for the freeze process of pure water than for evaporation under a vacuum (Majoube, 1971), and our results indicate that it is decreased for sugar solutions. It follows that in slush-frozen juice incomplete remixing of the ice layer, which has a slightly higher $^{18}O/^{16}O$ ratio, will lead to a commensurate decrease in ^{18}O in the

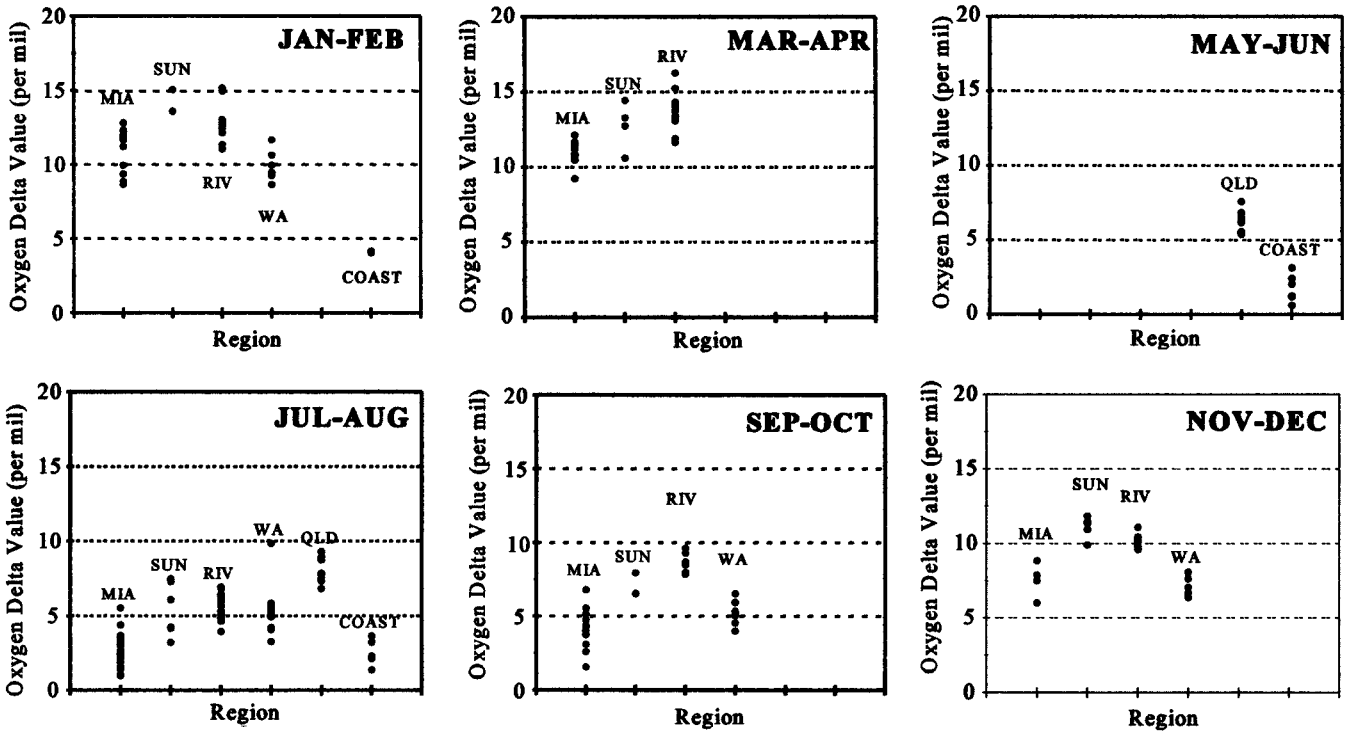

Figure 3. Range of $^{18}O/^{16}O$ ratios in orange juice over 4 years' production (1994−1997) from all major growing regions, presented in 2-month periods. See Figure 1 for key to abbreviations. Blanks in data occur when there was no production from the region during that period.

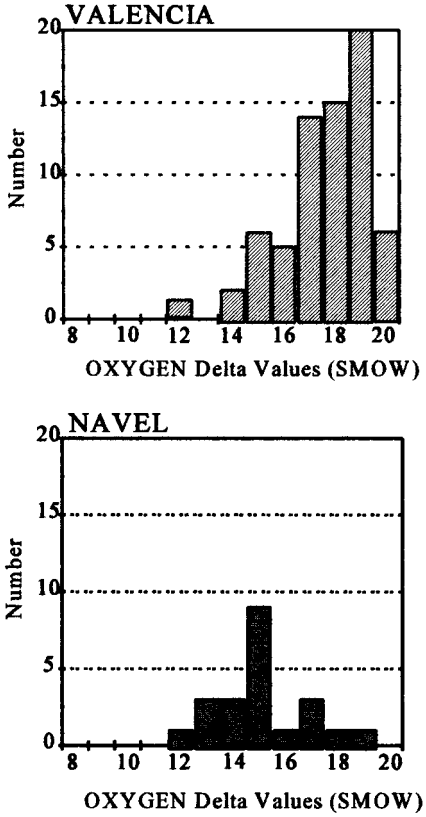

Figure 4. Distribution of $^{18}O/^{16}O$ ratios for Australian Valencia (top) and navel (bottom) concentrated orange juices.

unfrozen component of the remainder of the juice. Freeze-concentrated juice is manufactured by only a small section of the Australian industry.

$^{18}O/^{16}O$ Ratios of Other Citrus Varieties. $^{18}O/^{16}O$ ratios of other citrus varieties, such as lemon, grapefruit, mandarin, tangelo, Seville orange, and lime, which were grown coastally and collected during winter months, were generally comparable to oranges picked at the same time. Values are presented in Table 4. Mandarin (*Citrus reticulata*) may be added up to 10% to orange juice to enhance the color.

DISCUSSION

Seasonal and Regional Variations. As stated in the Introduction, isotopic enrichment in fruit juices is generally linked to leaf evapotranspiration. Isotopically enriched phloem water transports photosynthates to the developing fruit. Evaporation through the skin of the fruit has also been linked to isotopic enrichment in the case of grapes (Forstel, 1994). However, although this mechanism arguably makes a significant contribution to isotope levels in oranges, the steady decline in $^{18}O/^{16}O$ ratios experienced during April−June for late Valencia oranges studied in our first survey (see Figure 2) when compared to earlier summer values suggests that the fruit is not in a state of continuous isotopic enrichment, but rather that a dynamic two-way process may be occurring. It is known that under periods of stress the leaves will withdraw water from the fruit (xylem backflow), and indeed diurnal fluctuations in the volume of lemons were first reported >70 years ago [cited by Bartholomew and Sinclair (1951)]. This presents a scenario where water in the fruit is in a state of repeated exchange as it is withdrawn by the leaves during daytime temperature peaks and then replaced by stem water during late afternoon as the temperature drops. Because the vascular system of mature citrus fruit is confined mostly to the peel, the greatest cycling would be expected to occur from there, but it seems likely that the inner fruit is also involved in the process.

Given that evaporative effects are responsible for isotopic enrichment, current theory on leaf water $^{18}O/$

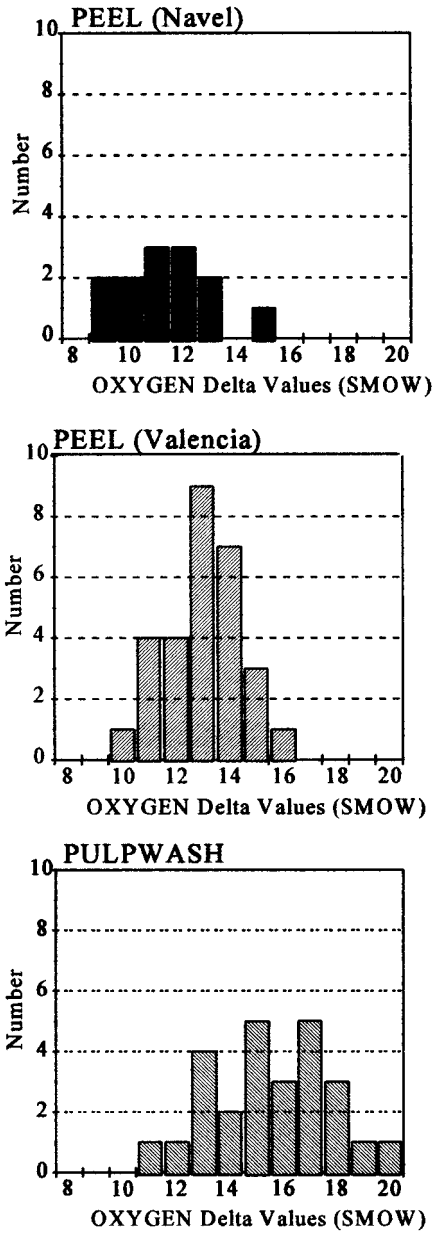

Figure 5. Distribution of $^{18}O/^{16}O$ ratios for concentrated Australian navel (top) and Valencia (middle) orange peel extracts and (bottom) imported pulpwashes.

Table 2. Changes in Fresh Navel Oranges during Prolonged Storage at Room Temperature

days lapsed after picking	weight loss (%)	°Brix of juice	$^{18}O/^{16}O$ ratio
1	nil	13.4	1.5
18	13.6 ±3.9	13.8	3.0
33	22.2 ±3.5	14.3	3.6
45	28.0 ±3.4	14.8	4.8
66	34.1 ±3.5	14.3	6.0

Table 3. Composition Gradient in Thawed Frozen Orange Juice without Remixing

fraction	°Brix	$^{18}O/^{16}O$ ratio
control	10.2	+4.3
top	3.8	+5.3
middle	8.9	+4.3
bottom	18.2	+3.2

^{16}O ratios and the effects of water stress provides an explanation for the seasonal and regional differences that are apparent in Figures 2 and 3. These are based on the Craig−Gordon model (Flanagan, 1993), which predicts that the isotopic composition of residual water is a function of three factors: (a) the isotopic composition of source water, (b) the isotopic composition of atmospheric water vapor, and (c) the ratio of air and leaf vapor pressure.

Table 4. Oxygen Delta Values of Other Winter Season Citrus Fruits of Australian Origin

type	$^{18}O/^{16}O$ ratio	type	$^{18}O/^{16}O$ ratio
lemon (Eureka)	+0.5	pumello	+2.6
grapefruit	+0.8	Seville orange	+4.8
mandarin	+2.2	Tahitian lime	+6.0
tangello	+4.6		

In the Australian context, the isotopic composition of source water is fairly predictable. Each of the regions Riverina, Sunraysia, and Riverland is heavily irrigated by water, which emanates from large dams located in the Snowy Mountains. At its outset this irrigation water has a uniform composition by virtue of its large mixed volume. It is released into the Murrumbidgee and Murray Rivers on demand and flows downriver through a series of storage reservoirs, diversion weirs, locks, and irrigation canals, before ultimately being drip-fed or sprayed onto the fruit orchards. There is a steady change in the isotopic composition of the river water during its progression downstream (a distance of some 2200 km from the Highlands catchment area westward to Lake Alexandrina) as it undergoes surface evaporation and also takes in some irrigation return. The overall effect is one of isotopic enrichment. Simpson and Herczeg (1991) report drops in $^{18}O/^{16}O$ levels in the river water of ∼5‰ over the length of the irrigation areas during summer and ∼2‰ during winter. Local storm and rain waters contribute only a small part of the total water intake of the land under cultivation, and their net effect is unlikely to be significantly different among inland regions.

Of the other regions, there is a mixture of dam water and artesian bore irrigation in Western Australia. Crops on the NSW coastal region and in Queensland are irrigated by rain water or storage water that is under regular replacement by fresh rain water. The Queensland region is situated at a latitude some 1500 km further northward than the Riverina, Sunraysia, and Riverland regions, and this must contribute to the higher delta values found for this area when regional comparisons are made. Precipitation becomes progressively richer in heavy isotopes as latitude decreases (Dansgaard, 1964).

Information on the other two components of the Craig−Gordon model is more difficult to ascertain, because it requires an in situ measurement of physical factors. However, disregarding localized aberrations that might occur within the leaf canopy, the components are closely linked to average daily temperature and relative humidity. Weather bureau data show a small but progressive increase in average temperature and decrease in relative humidity in weather stations along the Murray River as it progresses downstream.

Water stress is known to contribute to elevated $^{18}O/^{16}O$ levels in grapes. It has been reported that the greatest oxygen-isotope enrichment of grapevine must water occurs when the level of plant-available soil water is low or the root system is shallow (Tardaguila, 1997). Similar effects are likely in other fruits including oranges.

Relevance to Adulteration Testing. The use of the water map of regional and seasonal $^{18}O/^{16}O$ values for fresh Australian orange juice improves the sensitivity of oxygen isotope ratio analysis as a tool for detecting water addition, because it enables the regulator to focus on the narrower range of values associated with a particular season or region rather than testing juices against a blanket schedule of upper and lower limits. The overall span between summer inland and winter coastal $^{18}O/^{16}O$ levels in the juices tested in this survey is large (up to 15‰). Wholly reconstituted juice has, in our experience, a $\delta^{18}O/^{16}O$ value of ~ -2‰ and is therefore easily identified, but intermixing >50% of reconstituted concentrate with summer fruit juice would not decrease the $^{18}O/^{16}O$ value to a level lower than the database minimum for year-round production.

The manufacturing date of packaged fresh juice can be estimated from the use-by date on the label. Even after preservation with sorbate or benzoate salts, shelf life is limited to ~2 months. Testing against the minimum $^{18}O/^{16}O$ values for the month of manufacture (refer to Table 1) increases the likelihood of detecting tap water or reconstituted concentrate. This approach can be applied to >90% of the juice on sale in Australia, because it will have originated from the MIA−SUN−RIV regions. Of course, it would be necessary to verify that nonconformance had not originated from outside these main areas or that winter juice had not been held over in a slush-frozen state for long periods.

Straight watering is more likely to occur with navel than with Valencia orange juice because it generally has a high sugar content, with °Brix being 12−14. An appreciable amount of water could be added to navel juice without reducing the °Brix to <10. However, since tap water is ~−3.5‰ for Australian capital cities and can be lower in fresh rain water, its addition would lower $^{18}O/^{16}O$ levels of navel juice to below the database minimum of 1.0‰ if practiced in any reasonably large quantity. °Brix values for summer Valencia juice, which has a higher range of $^{18}O/^{16}O$ ratios, are closer to the industry minimum strength of 10 degrees, or even lower, so that straight water addition would be obvious from low sugar levels.

By comparison, the minimum MIA−SUN summer values for 1993 were 1−2‰ lower than for samples in later years (see Figures 2 and 3). There is a requirement for continual updating of the water map to monitor for changes that are likely to occur in future years in conjunction with long-term weather changes.

The effect of respiratory loss and water loss on $^{18}O/^{16}O$ levels from fruit held over before squeezing and changes caused by small separations of ice from juice held in a slush-frozen form are unlikely to be large enough to lead to false conclusions about watering.

Conclusions. The results of this investigation can be summarized as follows:

(1) Large changes in $^{18}O/^{16}O$ levels were observed in Australian orange juice within each year over a 5 year sampling period. These changes were predominantly linked to season. The geographical location of production also had an effect.

(2) The effectiveness of $^{18}O/^{16}O$ ratio analysis as a tool to detect water addition is improved by comparing $^{18}O/^{16}O$ levels of survey samples to monthly data for authentic juices.

(3) Small changes to $^{18}O/^{16}O$ ratios can occur as a result of storage. These are unlikely to be significant in the context of authenticity testing.

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