Stable Isotope Fractionation in Fruit Juice Concentrates: Application to the Authentication of Grape and Orange Products

Yunianta,[†] Ben-Li Zhang,[†] Michèle Lees,[‡] and Gérard J. Martin*,[†]

Université de Nantes, URA-CNRS 472, 2 Rue de la Houssinière, 44072 Nantes Cedex 03, France, and Centre Européen d'Analyse Isotopique Spécifique, Site de la Géraudière, CP 4005, 44073 Nantes Cedex 03, France

The thermokinetic fractionation coefficients of ^{2}H and ^{18}O in water of orange and grape juices were determined in experiments modeling the low-temperature evaporation processes carried out by agroindustries to concentrate fruit juices. These results can be used to refine the isotopic methods developed to control the adulteration of orange juices by sucrose and the concentration of musts to enrich wines. From the determination of the Brix value and the δ^2H and δ^{18}O isotope ratios of the remaining water of an orange concentrate, it is possible to infer the initial values of δ^2H and δ^{18}O in the pure juice. Knowledge of these parameters enables the detection level of sugar addition in the concentrate to be lowered. Conversely, by modeling the isotope fractionation processes that take place during must concentration, it is possible to use the δ^2H and δ^{18}O isotope ratios of wine water to improve the detection of wine enrichment by both sucrose addition and must concentration.

Keywords: *IRMS; deuterium; oxygen 18; water elimination; sugar addition*

(1) INTRODUCTION

Grape and orange are common fruits and probably the most widely consumed in the world. For both economical and technical reasons, trade in the juice from these fruits often involves juice concentrate. Some of the main citrus production areas, for example, are situated a long way from the countries in which the main consumers of citrus juices live. In this case, it is more economical to remove the water from the juice at the production site, transport the juice as a concentrate, and redilute the product in the consumer country. The distinction between a pure orange juice and a juice made from concentrate, however, must be clear in the interest both of the consumer and of providing a level playing field for producers and traders.

The problem of analytically characterizing fruit juices by using stable isotope tracers has been studied in some detail by Brause et al. (1984) and reviewed recently (Martin and Martin, 1995).

A further problem encountered in this area of fruit juice authentication is in the detection of undeclared sugar added to a juice or a juice made from concentrate, either in the form of beet (a C_3 source) or cane (a C_4 source) sucrose or as a solution of inverted beet syrup (HFBS). ^{13}C isotope ratio mass spectrometry (IRMS) (Doner and Bills, 1981) and ^{2}H-NMR spectroscopy (Martin and Martin, 1995) are exploited to determine the addition of C_4, C_3, or C_3+C_4 sugars to an orange juice or a concentrate. These methods involve a comparison of the isotopic values of an unknown juice to those representative of authentic samples. Since the isotope ratios of a juice are influenced to a certain extent by climatic and environmental factors, it is recommended to use as references for detecting and quantifying possible adulterations the extreme values encountered for authentic juices of the main production areas.

The quantity of added exogenous sugar thus calculated is then a default value.

When the geographical origin of the suspect concentrate is known, either from product labeling or from analytical data such as trace element composition (McHard et al., 1979; Nikdel et al., 1988) or amino acid profiles (Wallrauch and Faethe, 1988; Fang, 1988), it is possible to select from the data base the isotopic values corresponding to the correct production area. This referencing provides a more accurate assessment of the quantity of sugar added.

An alternative method for confirming or even identifying the origin of a concentrate may consist in studying the stable isotope composition of the remaining water in the concentrate. This work will present the theoretical principles and the practical aspects of this type of analysis.

Grape juice concentration is a specific concern of wine-producing countries, particularly in the European Union (EU). Although not strictly of the same nature as in the orange juice industry, adulterations arising from the improper use of concentrated grape juices or musts can be detected using methods similar to those applied to orange concentrates. In fact, the methods used to produce concentrated musts (CM) or rectified concentrated musts (RCM) resemble quite closely those used for orange juice concentration. In the case of RCM, the natural juices are first diluted and then rectified using cationic and anionic resin columns, which eliminate most inorganic and organic constituents with the exception of glucose, fructose, and sucrose. The CM or RCM may then be incorporated into the fermenting must to enrich the resulting wine. Although this kind of grape juice concentration is generally carried out in large scale installations similar to those used in the orange juice industry, it can also be done on a smaller scale in individual vineyards.

The enrichment of wines is strictly regulated in the EU (EU Regulations, 1987), and the tolerances permitted depend on the region, the year of production, and the nature of the wine produced. For example, all types of enrichment are prohibited in Mediterranean countries

---

* Author to whom correspondence should be addressed.
† Université de Nantes.
‡ Centre Européen d'Analyse Isotopique Specifique.

and for typical wines such as "naturally sweet wines" in France or those with a particular quality label such as the "mit Prädikat" wines from Germany. In other cases, specific allowances have been defined in terms of both the degree of enrichment permitted and the type of enrichment process used, since it is possible to enrich a wine either by adding cane or beet sugar to the must or by concentrating the must before fermentation. The regulations stipulate that both processes are mutually exclusive.

From the isotopic point of view the problem of must concentration is similar to that of citrus juice concentration. The modeling of the concentration process will also be applied to the detection of wine enrichment. More generally, it will be examined to which extent the determination of the stable isotope content of the remaining water in the concentrates can improve the authentication methods.

(2) THEORY

The simplest model to describe must concentration is provided by evaporation from a water pool. This process follows a kinetic or a thermodynamic behavior or a mixture of both behaviors according to the amount of humidity, h (0–100%), which is present at the evaporating surface. This model can be improved by taking into account the presence of salts or sugars, which are associated with water activity coefficients generally smaller than unity. Moreover, to further refine the model, it could be considered that the evaporation of a flowing liquid or of droplets raining down from the top of an evaporator is somewhat different from that of a large water surface. The turbulences that exist at the interface of the liquid and vapor layers are likely to induce some changes in the isotopic fractionation factor.

In the evaporative model, the effect of juice concentration on the hydrogen and oxygen isotope ratios, R_C, of water remaining in the concentrate may be related to the molar fraction of the residual water by

$$R_C/R_I = N_C/N_I^{\beta_{eff}-1} \qquad (1)$$

where R_I and N_I denote the isotope ratio and the mole number of water in the initial product and N_C is the mole number of remaining water in the course of the concentration process.

This Rayleigh-type equation may, in fact, apply to a mixture of thermodynamic and kinetic evaporation processes, providing that the effective coefficient, β_{eff}, is given the appropriate physical significance. When the vapor is in thermodynamic equilibrium with the evaporating water, β_{eff} is equal to the equilibrium constant of the isotopic exchange reaction:

$$HOH_{vap} + HOH^*_{liq} \rightleftharpoons HOH^*_{vap} + HOH_{liq} \ (K_e) \quad (2)$$

$$K_e = \frac{(HOH)_{liq}(HOH)^*_{vap}}{(HOH)^*_{liq}(HOH)_{vap}} = \frac{R_V}{R_L} \qquad (3)$$

R_V and R_L are the isotope ratios of the vapor and liquid phases, respectively, and (*) stands for HOD or $H^{18}OH$.

In this case K_e is equal to α, the thermodynamic fractionation factor.

Szapiro and Steckel (1967) and Van Hook (1972), for instance, have studied the vapor pressure of water to determine α, whereas Borowitz (1962) and Majoube

(1971) have obtained the fractionation coefficient by static or dynamic distillation procedures.

According to Majoube (1971), the thermodynamic fractionation factor α for hydrogen and oxygen is temperature dependent

$$\alpha_D = 1.276 \exp(-95.39/T) \quad \text{and}$$
$$\alpha_O = 1.0157 \exp(-7.430/T) \quad (4a)$$

and at 20 °C α is equal to

$$\alpha_D = 0.9215 \quad \text{and} \quad \alpha_O = 0.9903 \qquad (4b)$$

At higher temperature, the fractionation factor increases, and near the boiling point of water it is equal to

$$\alpha_D = 0.9881 \quad \text{and} \quad \alpha_O = 0.9957$$

The overall problem of the liquid–vapor equilibrium isotope effect has been thoroughly reviewed by Biegeleisen et al. (1973), and we have shown that the SNIF-NMR method can be applied to the simultaneous determination of the thermodynamic or kinetic fractionation factors associated with the different isotopomers of a given molecule (Moussa et al., 1990).

When the evaporation proceeds under nonequilibrium conditions, i.e. when the humidity of the atmosphere layer at the boundary of the water surface is much lower than 100%, the phenomenon is governed by the kinetic fractionation factor, $\beta\dagger$, which is significantly smaller than α and introduces a larger enrichment of the residual water. According to Mook (1987), $\beta\dagger$ at 20 °C is equal to

$$\beta_D\dagger = 0.9123 \quad \text{and} \quad \beta_O\dagger = 0.9824$$

However, all other factors being kept constant, the humidity above a water surface, for a given pressure, increases when the activity of the solution is lower than unity, and the kinetic behavior tends toward thermodynamic equilibrium when the salt or sugar concentration of the evaporating solution reaches the saturation point. For concentrations up to 400 g/L of glucose or sucrose in water, the solution activity remains higher than 0.95 and its influence on the isotopic fractionation is negligible (Fontes and Gonfiantini, 1967; Sofer and Gat, 1975; Friedman et al., 1976).

To check the validity of the Rayleigh-type model to evaluate the isotopic fractionation of the remaining water during the evaporation process of a juice or a must, we have carried out specific experiments on pure water and on grape or orange juices.

In the second part of this work, the model is applied to the characterization of orange concentrates and of wines which were either concentrated or enriched by sucrose addition or both.

(3) MATERIALS AND METHODS

(a) Concentration Model: Estimation of the ^2H and ^{18}O Fractionation Factors. Water extraction was simulated using a rotary evaporator linked to a vacuum pump (5 ± 2 × 10^{-2} mbar). A double liquid nitrogen trap was fitted between the evaporator and the pump. The temperature of the room was carefully recorded.

The samples under investigation were Nantes tap water (NTW) and grape juices (GJ) and orange juices (OJ) which were obtained from fruit or were commercially available (Tropicana Pure Premium, 100% pure, not from concentrate).

Stable Isotope Fractionation in Fruit Juice Concentrates

J. Agric. Food Chem., Vol. 43, No. 9, 1995 **2413**

Table 1. Isotope Ratios Measured in Model Experiments Carried Out To Determine the Effective Isotope Fractionation Factor, β_{eff}, of the Evaporation Process of a Fruit Juice[a]

pure water ($t = 18$ °C)				sucrose solution 1 °$B = 10.65$, $t = 16$ °C				sucrose solution 2 °$B = 32.40$, $t = 16$ °C			
X_C	(D/H) (ppm)	δD	δ^{18}O	X_C	(D/H) (ppm)	δD	δ^{18}O	X_C	(D/H) (ppm)	δD	δ^{18}O
1.0000	149.90	−37.6	−6.2	1.0000	148.90	−44.0	−6.8	1.0000	148.20	−48.5	−7.2
0.9105	150.99	−30.6	−5.3	0.8922	149.90	−37.6	−5.7	0.8861	148.90	−44.0	−6.3
0.8365	151.90	−24.8	−4.5	0.7672	151.90	−24.8	−4.3	0.7980	150.40	−34.4	−5.4
0.7767	153.10	−17.1	−3.7	0.6417	153.50	−14.5	−2.5	0.7084	152.30	−22.2	−4.5
0.6751	154.70	−6.8	−2.3	0.5252	155.90	0.9	−0.6	0.5979	154.00	−11.3	−3.6
0.5990	156.10	2.2	−1.1	0.4327	158.30	16.3	1.7	0.5015	154.80	−6.2	−3.3
0.5132	158.21	15.7	0.6	0.3436	162.50	43.3	3.7	0.3041	160.20	28.5	/
0.4362	160.40	29.8	2.3	0.2744	164.30	54.8	6.1				
0.3793	161.80	38.8	3.7								

orange juice 1, °$B = 10.8$, $t = 15.5$ °C				orange juice 2, °$B = 12.7$, $t = 15.0$ °C				grape juice, °$B = 16.5$, $t = 18.0$ °C			
X_C	(D/H) (ppm)	δD	δ^{18}O	X_C	(D/H) (ppm)	δD	δ^{18}O	X_C	(D/H) (ppm)	δD	δ^{18}O
1.0000	157.70	12.5	5.5	1.0000	157.30	9.9	5.2	1.0000	155.40	−2.3	1.5
0.8971	158.10	15.0	6.3	0.8410	158.80	19.5	6.8	0.9361	155.81	0.3	2.2
0.7830	159.50	24.0	7.9	0.7536	160.20	28.5	7.9	0.8555	156.50	4.8	3.2
0.6910	160.50	30.4	9.2	0.5834	163.80	51.6	10.7	0.7814	157.60	11.8	4.3
0.5545	163.40	49.0	11.2	0.4374	165.50	62.5	12.8	0.7025	158.50	17.6	5.5
0.4714	165.60	63.2	13.1	0.2714	172.40	106.8	13.1	0.6215	160.11	27.9	7.0
0.3695	169.20	86.3	15.3	0.1229	181.20	163.3	17.3	0.5371	162.01	40.1	8.7
0.2623	172.70	108.8	18.6					0.4291	164.70	57.4	11.7

[a] X_C is the molar fraction of the remaining water in the concentrate, t the evaporation temperature, and °B the Brix value of the juice.

Increasing quantities of water were extracted from both NTW and OJ/GJ at a given temperature. After each stage, the concentration rate was determined and the isotope ratios of the remaining water, (D/H)$_w$ and (^{18}O/^{16}O)$_w$, were measured by mass spectrometry according to well-defined protocols (Martin et al., 1988).

For each concentration experiment, i, the masses of the juice remaining in the evaporator, m_i^J, and of the water evaporated, m_i^E, were carefully determined to a precision of 0.01 g and the internal and external temperatures of the evaporator were measured to within 0.5 °C. An aliquot, carefully weighed, of the remaining juice, a_i, was removed at each of the concentration steps for subsequent isotopic analyses. The concentration of sugars was also determined by means of the refractive Brix index. The cumulative molar fraction of the evaporated water was obtained by summing the individual molar fractions of water evaporated at each step and correcting for the aliquots taken off. The corrected evaporated contribution at step n, C_n, is given by

$$C_n = \frac{1}{m_0}\left[a_1\left(1 - \frac{m_2^E}{m_2^J}\right)\left(1 - \frac{m_3^E}{m_3^J}\right)\cdots\left(1 - \frac{m_{n-1}^E}{m_{n-1}^J}\right)\frac{m_n^E}{m_n^J} + \right.$$
$$a_2\left(1 - \frac{m_3^E}{m_3^J}\right)\cdots\left(1 - \frac{m_{n-1}^E}{m_{n-1}^J}\right)\frac{m_n^E}{m_n^J}\cdots + $$
$$\left. a_{n-2}\left(1 - \frac{m_{n-1}^E}{m_{n-1}^J}\right)\frac{m_n^E}{m_n^J} + a_{n-1}\frac{m_n^E}{m_n^J}\right] \quad (5)$$

where m_0 is the mass of water existing in the natural juice before concentration.

The molar fraction of the remaining water, $X_C = N_C/N_I$, intervening in eq 1, is given by

$$X_C = 1 - \sum_{i=1}^{n} C_i \quad (6)$$

The precision on the determination of the relative concentration of water is of the order of 0.001.

The results of the isotopic measurements are expressed in δ (parts per million) units according to

$$\delta(\text{‰}) = (R/R_{\text{ref}} - 1)1000 \quad (7)$$

where ref denotes a sample of Standard Mean Ocean Water

obtained from the International Atomic Energy Agency in Vienna (V.SMOW). The isotope ratios of this reference are $R_{\text{ref}}(^2\text{H}) = 155.76$ ppm and $R_{\text{ref}}(^{18}\text{O}) = 2005.2$ ppm (Gonfiantini, 1978). The standard deviations of repeatability for ^{2}H and ^{18}O determinations are 1.2‰, and 0.1‰, respectively (Koziet et al., 1995).

(b) Enrichment of Juices by Concentration and Addition of Sucrose. Approximately 3 kg of commercially obtained grapes from three different origins (A, B, and C) were pressed to give four separate samples of grape must: 1, reference sample (no sugar added and no concentration); 2, concentrated (20%); 3, with added sucrose (34 g/L); and 4, concentrated (10%) with added sucrose (17 g/L).

Each sample was then fermented following the standard EU procedure of 1990 (EU Regulation, 1990) until no sugar remained. The alcohol was distilled and the relevant parameters were measured by ^{2}H-NMR and mass spectrometry according to dedicated procedures (Martin et al., 1988).

(4) RESULTS AND DISCUSSION

(a) Isotopic Fractionation Resulting from Concentration. Several experiments have been conducted to model fruit juice concentration as described in section 3a. The isotope ratios of the remaining water have been measured as a function of its molar fraction, X_C. The deuterium contents are expressed in Table 1 as both D/H ratios and δD values, and the oxygen isotope parameters are given in δ^{18}O units. The δ values can be converted to isotope ratios by means of eq 7. The effective fractionation factor β_{eff} is computed from a nonlinear regression of the model (eq 1).

$$Y_{\text{obs}} = aX^b \quad (8)$$

Y_{obs} and X are, respectively, the isotope ratio R_c and the molar fraction ($X_C = N_C/N_I$) of the remaining water, a represents the isotope ratio of the unconcentrated juice (R_I), and b is equal to $\beta_{\text{eff}} - 1$.

Note that a linear regression of the logarithmic form of eq 8 gives slightly different results for β_{eff} and that the forced intercept of the nonlinear model

$$Y_{\text{obs}}/a = X^b \quad (9)$$

does not improve significantly the results.

Table 2. Values of the Isotopic Fractionation Factor β_{eff} Computed from the Concentration Experiments Described in Table 1[a]

product	isotope	R_I(obs) (ppm)	R_I(calcd) (ppm)	β_{eff}	MSD (ppm)
pure water	^2H	149.9	149.9	0.9188	0.124
	^{18}O	1992.8	1992.6	0.9897	0.010
sucrose solution 1	^2H	148.9	148.5	0.9171	0.439
	^{18}O	1991.6	1991.3	0.9895	0.246
sucrose solution 2	^2H	148.2	148.1	0.9219	0.392
	^{18}O	1990.8	1991.3	0.9933	0.579
orange juice 1	^2H	157.7	156.9	0.9241	0.492
	^{18}O	2016.2	2016.1	0.9897	0.237
orange juice 2	^2H	157.3	157.0	0.9250	0.490
	^{18}O	2015.6	2017.9	0.9940	1.962
grape juice	^2H	155.4	155.0	0.9298	0.237
	^{18}O	2008.2	2007.9	0.9881	0.199

[a] R_I is the isotope ratio of water in the starting juice either measured experimentally or derived from the regression performed according to eq 8. MSD is the mean standard deviation between the observed and predicted values.

The results of the computations given in Table 2 may be summarized as

$$(\text{at } 18 \text{ °C}) \ \beta_{eff}(^2H) = 0.923 \ (0.003) \quad (10a)$$

$$(\text{at } 18 \text{ °C}) \ \beta_{eff}(^{18}O) = 0.991 \ (0.002) \quad (10b)$$

The values of the ^2H and ^{18}O fractionation factors are consistent with the data quoted in section 2. The standard deviation of the six different determinations of β_{eff} is of the order of 2×10^{-3} and has the same magnitude as that which can be computed from the experimental errors on R and X: $dR = 0.2$ ppm (^2H) or $0.2‰$ (^{18}O) and $dX = 0.001$.

The ratio $(1 - \beta_{eff}(D))/(1 - \beta_{eff}(O))$ is of nearly the same value for pure water (7.9) and for the juices (9.1), whatever the sugar concentration of the starting products. This indicates that fruit juices with Brix values up to 20 behave, as far as isotopic fractionation is concerned, as pure water and follow the true evaporation line (Craig et al., 1963). The influence of the evaporation temperature is negligible between 15 and 25 °C for concentration grades lower than 30% but may be significant, although the change is smaller than 3×10^{-3} in relative value, for concentrated juices. Consequently, if the evaporation process is conducted near 20 °C, the isotopic enrichment of the remaining water of a concentrated juice may be estimated from the diagrams of Figure 1 when the concentration rate is known. Conversely, the concentration degree of a juice may be estimated from the determination of its isotopic content.

This fractionation model will now be applied to the two complementary situations of fruit juice concentration and wine enrichment.

(b) Quantification of Added Sugar in Orange Concentrates. The determination of the addition of beet sucrose in orange juices or concentrates is efficiently carried out by ^2H-NMR spectroscopy (SNIF-NMR method) after transformation of the fermentable sugars into ethanol (Martin et al., 1986; Martin and Martin, 1995).

The content of sucrose addition, C, expressed in percent (w/w), is computed from

$$C \ (\%) = \left[\frac{(D/H)_I^{ref} - (D/H)_I^X}{(D/H)_I^{ref} - (D/H)_I^{beet}} \right] \times 100 \quad (11)$$
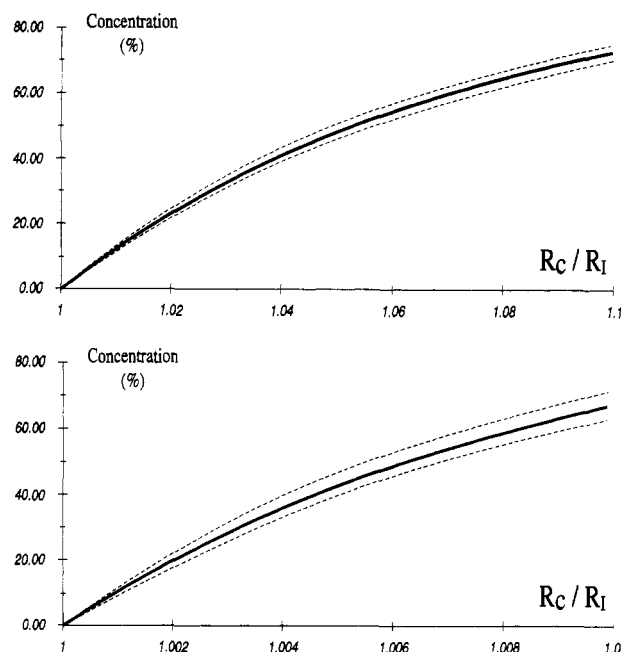

Figure 1. Theoretical curves representing the dependence of the neat isotope fractionation R_C/R_I, for deuterium (a, top) and for oxygen 18 (b, bottom), on the concentration degree of a juice. R_C and R_I are the isotope ratios of the starting juice and of the concentrate. The intermediate curve corresponds to a value of the effective isotope fractionation factor β_{eff} (D) = 0.925 or $\beta_{eff}(^{18}O)$ = 0.991, whereas the limit curves are associated with values of β_{eff} equal to 0.930 and 0.920 for D and to 0.992 and 0.990 for ^{18}O.

where $(D/H)_I$ is the isotope ratio of the methyl site of ethanol and X, ref, and beet refer to the unknown juice, the reference juice, and the beet sucrose added. Equation 11 requires knowledge of the $(D/H)_I^{ref}$ values of citrus juices from authenticated botanical and geographical origins. To this aim, a comprehensive data bank, EURODAT (trademark of Eurofins S.A. Nantes), has been built up and is maintained. However, for commercial concentrates, the geographical origin is not always known, or may be even falsified. When the analysis is carried out with a view to detect the adulteration of a concentrate by beet sucrose, the reference isotope ratio $(D/H)_I^{ref}$ is assumed to have the lowest value available in the data bank for the juice considered. This attitude minimizes the second-order risk, i.e., condemning as adulterated a natural product. As a consequence, adulterated concentrates have a greater chance than juices of being judged as natural.

In Figure 2, natural orange juices of a given year but from four different regions of production (South America and the Mediterranean basin) are represented in the plane of the two isotopic variables of the juice water $(D/H)_W^S$ and $\delta^{18}O_W^S$. The juices from these countries are typical examples, for the year considered, of products relatively rich (Israel, Brazil) and poor (Spain, Argentina) in ^2H and ^{18}O. Consider now an unknown concentrate characterized by the following values:

$$°B = 64.5 \quad C \ (g/L) = 845$$

$$(D/H)_W^X = 166.6 \text{ ppm} \quad \delta^{18}O_W^X = 12.8$$

We shall assume that this sample may be safely expected to have been produced in any of the four considered countries.

The concentrate is diluted to 11°B (116 g/L) and fermented in standard water. A value $(D/H)_I^X = 100.9$

Stable Isotope Fractionation in Fruit Juice Concentrates

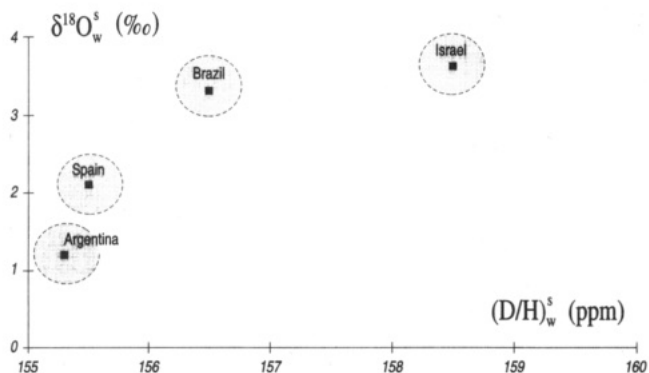*J. Agric. Food Chem.,* Vol. 43, No. 9, 1995 **2415**

Figure 2. Representation of orange juices from four typical regions of production in the plane of the two isotope ratios $(D/H)_W^S$ and $\delta^{18}O_W^S$ of the water of the juices.

Figure 3. Representation of wines from four typical regions of production in France, in the plane of the two isotope ratios $(D/H)_W^Q$ and δO_W^{18Q} of water (Q denotes water from the fermentation medium, whereas S denotes water from the juice). The isotopic data used to draw this diagram are extracted from the EURODAT data bank on wines built up in Nantes.

ppm is determined for the methyl isotope ratio of ethanol extracted from the fermentation medium. By retaining a value $(D/H)_I^{beet} = 92$ ppm for ethanol from beet sucrose and a limit value $(D/H)_I^{ref} = 102.1$ ppm for ethanols derived from orange juices typical of the considered regions (Figure 2), a value of 12% of added sucrose is computed from eq 11. This interpretation can be improved by exploiting the concentration model for inferring the isotopic parameters, R_I, of the juice used to prepare the concentrate. These parameters are expected to provide information on the origin of the concentrate and therefore enable a refined selection of the $(D/H)_I^{ref}$ value.

From the Brix value of the concentrate the concentration degree of the water of the natural juice can be estimated to be about 60%. The ratios of the isotopic parameters of the concentrate and starting juice, R_C^X/R_I^X, corresponding to this concentration degree are then deduced from the curves of Figure 1. From the measured values of the R_C^X parameters of the concentrate quoted above, the following values of the R_I^X parameter of the starting juice are calculated:

$$(D/H)_W^S = 156.1 \, (\pm 0.4) \text{ ppm} \qquad \delta^{18}O_W^S = 3.2 \, (\pm 0.2)$$

Referring now to Figure 2 or to a more exhaustive data bank, it can be concluded that the concentrate was made from Brazilian oranges. The extreme value determined for the methyl isotope ratio $(D/H)_I^{ref}$ of ethanol obtained from Brazilian orange juices being equal to 103.8 ppm, a 24% addition of sucrose to the concentrate is computed from eq 11 instead of 12%.

(c) Determination of Grape Must Concentration. Values of the isotopic parameters of the water contained in concentrated and rectified musts CM and CRM (60–70 °Brix) produced in the south of France, Italy, and Spain range between 162 and 166 ppm and between 12 and 16‰ for $(D/H)_W^S$ and $\delta^{18}O_W^S$ respectively. Referred to the natural grape juices from the same countries, a 100 g/L concentration of the must leads to increases of 1.2 ppm for $(D/H)_W^S$ and 1.6‰ for $\delta^{18}O_W^S$, respectively. Consequently, when a concentrated must is fermented, the resulting wine water has also higher isotopic parameters than those of natural wines of the same country and vintage. As an example, the natural wines of four production areas in France, for the year 1991, are represented in Figure 3 in the plane of their water isotopic parameters. The regions are well differentiated at 95% confidence since the standard deviation for each group is of the order of 0.8 ppm and 1‰ for $(D/H)_W^S$ and
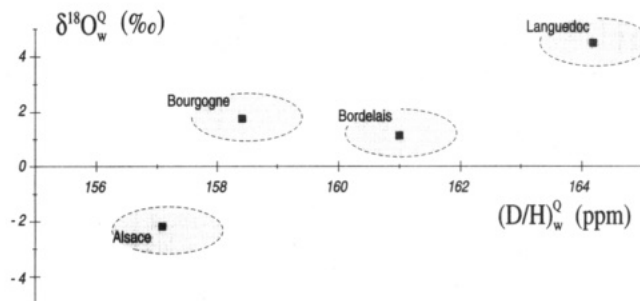
Table 3. Influence of Concentration and Enrichment of Grape Juices on the Isotopic Parameters of Ethanol and Water Extracted from the Corresponding Wines[a]

case	sample	concn (%)	beet sugar addition (g/L)	ethanol			water	
				$(D/H)_I$	$(D/H)_{II}$	$\delta^{13}C$	$(D/H)_W^Q$	δO_W^{18Q}
1	A	0	0	100.4	126.7	−27.4	160.1	0.5
2	A	20	0	100.9	130.3	−27.2	166.0	3.0
3	A	0	34	99.1	127.3	−27.4	161.2	0.5
4	A	10	17	99.6	129.0	−27.3	164.1	1.5
1	B	0	0	101.7	125.0	−24.8	160.9	2.3
2	B	20	0	102.4	127.8	−24.8	164.0	4.1
3	B	0	34	100.7	125.8	−25.1	161.2	2.1
4	B	10	17	101.5	126.6	−24.9	162.2	3.0
1	C	0	0	98.2	117.6	−26.4	157.1	0.5
2	C	20	0	99.4	121.6	−26.5	161.2	3.0
3	C	0	34	97.6	118.7	−26.3	157.0	0.5
4	C	10	17	97.9	122.1	−26.1	158.7	2.5

[a] $\delta^{13}C$ represents the overall ^{13}C content of ethanol. $(D/H)_W^Q$ (ppm) and δO_W^{18Q} are the isotopic parameters of the wine water. Case 1 corresponds to the fermentation of the natural juice. In case 2 the juice has been concentrated. In case 3 it has been enriched by addition of beet sugar, and in case 4 it has been both concentrated and enriched.

$\delta^{18}O_W^S$, respectively. To estimate the influence of must concentration and must enrichment on the isotopic parameters of the final wine, model experiments were carried out on three typical natural grape juices (Table 3). The natural juice is fermented in four situations: as it stands, after addition of sucrose, after concentration, and after both sucrose addition and concentration. The isotopic parameter of the methyl site of the wine ethanol, $(D/H)_I$, decreases in enriched wines as expected (Martin, 1990). With regard to the isotopic parameters of the wine water, it is observed that $(D/H)_W^Q$ and δO_W^{18Q} increase significantly with only a 10% concentration grade. The hydrogen and oxygen isotopic ratios exhibit similar behavior, and the agreement between the concentration grade computed from either $(D/H)_w$ or $\delta^{18}O_w$ is satisfactory as shown in Table 4.

The concentration data were computed from eq 1. However, since the isotopic parameters which intervene in eq 1 are those of the starting medium, R_W^S, it is necessary to rearrange this equation introduce the isotope ratios of water after fermentation, R_W^Q. Indeed, when wines are analyzed, the isotope ratios of the grape juice to which eq 1 applies *stricto sensu* are no longer available. We have shown previously (Martin et al., 1986) that the isotopic parameters of the water medium before (S) and after (Q) fermentation are related by eqs 12

Table 4. Determination of the Concentration Grade (in Percent) of Three Natural Juices A–C from the Isotopic Parameters Measured on the Corresponding Wines[a]

concn (%)	A		B		C	
	a	b	a	b	a	b
theoretical	20	10	20	10	20	10
calcd from (D/H)$_W$	32	22	16	11	21	12
calcd from $\delta^{18}O_W$	24	11	18	7	24	20
mean value	28	16	17	9	22	16

[a] These results are those of the model experiments described in Table 3. a and b refer to cases 2 and 4 of Table 3, respectively.

$$(D/H)_W^Q = (D/H)_W^S + 0.45t \qquad (12a)$$

$$\delta^{18}O_W^Q \simeq \delta^{18}O_W^S \qquad (12b)$$

where t (%v/v) is the alcoholic grade of the final wine.

Taking these relationships into account, eq 1 can be transformed to express the concentration grade $\varrho = 1 - (N_C/N_I)$ by using either the hydrogen, $(D/H)_W^Q$, or the oxygen, $(O/O)_{,W}^{1816Q}$ parameters of the suspected wine (X) and of the corresponding unenriched wine (N):

$$\varrho_D = 1 - \exp\left\{\frac{1}{(\beta_D - 1)} \mathrm{Ln}\left(\frac{(D/H)_W^{Q(X)} - 0.45t^{(X)}}{(D/H)_W^{Q(N)} - 0.45t^{(N)}}\right)\right\} \qquad (13a)$$

$$\varrho_O = 1 - \exp\left\{\frac{1}{(\beta_D - 1)} \mathrm{Ln}\left(\frac{(^{18}O/^{16}O)_W^{Q(X)}}{(^{18}O/^{16}O)_W^{Q(N)}}\right)\right\} \qquad (13b)$$

For instance in the experiment involving case 2 sample B, of Table 3, the parameters used in eq 13a are the following $(D/H)_W^{Q(X)} = 164.0$ ppm, $(D/H)_W^{Q(N)} = 160.9$ ppm, $t^{(X)} = 12.6\%$, $t^{(N)} = 10.4\%$, and $\beta_{eff} = 0.925$. A concentration grade $\varrho_D = 16\%$ is computed (Table 4).

The concentration of the juice also has some influence on the isotopic parameters of the wine ethanol. Since the methyl, I, and methylene, II, hydrogens are partly derived from water (Martin et al., 1986, 1988), an increase in the isotope ratio, $(D/H)_W^S$, of the juice after water concentration results in a slight increase in the isotope ratios of the methyl $(D/H)_I^X$ and methylene $(D/H)_{II}^X$ sites of ethanol as compared to the corresponding parameters of the unconcentrated wine (N):

$$(D/H)_I^{(X)} =$$
$$(D/H)_I^{(N)} + 0.23((D/H)_W^{Q(X)} - (D/H)_W^{Q(N)}) \qquad (14a)$$

$$(D/H)_{II}^{(X)} =$$
$$(D/H)_{II}^{(N)} + 0.74((D/H)_W^{Q(X)} - (D/H)_W^{Q(N)}) \qquad (14b)$$

This behavior is verified in the results of Table 3. For the three samples considered, A, B, and C, the $(D/H)_I$ and $(D/H)_{II}$ values are significantly higher in case 2 compared to case 1. For instance, an increase of 2.8 ppm in the $(D/H)_{II}$ value of sample B is observed for a concentration grade of 20%, whereas a value of 2.3 ppm is predicted by eq 14b. The isotopic parameters of ethanol therefore provide additional criteria for detecting a concentration of the juice.

(5) CONCLUSION

Low-temperature evaporation of the water of fruit juice under thermodynamic or kinetic conditions induces significant isotopic fractionation of hydrogen and oxygen and the remaining water becomes enriched in the heavy isotopes. The isotope fractionation factors determined in orange and grape juices are very close to those determined for water. The Rayleigh equation, which relates the concentration rate to the isotope fractionation, may be applied in two complementary ways. In the case of orange concentrates suspected of containing sucrose, it is possible to infer the δ^2H and $\delta^{18}O$ values of the water of the natural juice before concentration from the measurement of δ^2H and $\delta^{18}O$ of the concentrate water and the Brix value of the concentrate. The isotopic contents of the water of the original juice thus calculated are good indicators for evaluating the $(D/H)_I$ and $\delta^{13}C$ parameters of the ethanols which would result from the pure orange sugars. These estimated parameters provide improved references for computing the amount of sucrose addition in concentrate. They enable the level of detection of this practice to be significantly lowered.

On the other hand, the Rayleigh equation may be exploited to evaluate the concentration rate if the isotope fractionation is known. This procedure was successfully applied to the detection of must concentration in a wine. The values of δ^2H and $\delta^{18}O$ of the wine water are compared to the values of the same parameters measured in pure grape musts or natural wines from the same region and year. Such reference values are now available in suitable data banks. The concentration grade is computed from the Rayleigh equation by using the appropriate values of the evaporative fractionation factors. Additional criteria are provided by the site-specific hydrogen isotope ratios of ethanol, which are also influenced by the concentration process.

Both applications demonstrate the usefulness of stable isotopes at natural abundance to trace back the origin of the raw materials and the nature of the transformation processes used in agroindustries.

LITERATURE CITED

Biegeleisen, J.; Lee, M.; Mandel, F. Equilibrium isotope effects. *Annu. Rev. Phys. Chem.* **1973**, *24*, 407–412.

Borowitz, J. L. The relative volatilities of the system $H_2^{17}O$–$H_2^{18}O$. *J. Phys. Chem.* **1962**, *66*, 1412–1414.

Brause, A. R.; Raterman, J. R.; Petrus, D. R.; Doner, L. W. Verification of authenticity of orange juice. *J. Assoc. Off. Anal. Chem.* **1984**, *67*, 535–539.

Craig, H.; Gordon, L. I.; Horibe, Y. Isotopic exchange effects in the evaporation of water I Low temperature experimental results. *J. Geophys. Res.* **1963**, *68*, 5079–5087.

Doner, L. W.; Bills, D. D. Stable carbon isotope ratios in orange juice. *J. Agric. Food Chem.* **1981**, *29*, 803–804.

EU Regulation. Common organization of the market related to wine and wine products. No. 822/87, March 16, 1987.

EU Regulation. Community analytical methods which can be applied in the wine sector. No. 2676/90, Sept 17, 1990.

Fang, T. T. Amino-acid pattern for analysis of fruit juice authenticity. In *Modern Methods of Plant Analysis, New Series, Vol. 8. Analysis of Nonalcoholic Beverages*; Linkens, H. F., Jackson, J. F., Eds.; Springer: Berlin, 1988.

Fontes, J. C.; Gonfiantini, R. Isotope behavior during the course of evaporation of two Sahara basins. *Earth Planet. Sci. Lett.* **1967**, *3*, 258–266.

Friedman, I.; Smith, G. I.; Hardcastle, K. G. Studies of quaternary saline lakes. II Isotopic and compositional

Stable Isotope Fractionation in Fruit Juice Concentrates

J. Agric. Food Chem., Vol. 43, No. 9, 1995 2417

changes during desiccation of the brines in owens Lake, California, 1969−1971. *Geochim. Cosmochim. Acta* **1976**, *40*, 501−511.

Gonfiantini, R. Standards for stable isotope measurements in natural compounds. *Nature* **1978**, *271*, 534−536.

Koziet, J.; Rossmann, A.; Martin, G. J.; Johnson, P. Determination of ^{18}O and ^{2}H content of waters of fruit and vegetable juice water. A European inter-laboratory comparison study. *Anal. Chim. Acta* **1995**, *302*, 29−37.

Majoube, M. Fractionation of ^{18}O and ^{2}H between water and vapor. *J. Chim. Phys.* **1971**, *68*, 625 and 1423−1436.

Martin, G. J. The chemistry of chaptalization. *Endeavour* **1990**, *14*, 137−143.

Martin, G. J.; Martin, G. G. NMR and MS stable isotope studies of fruit juice adulteration. In *Adulteration of Methods to Detect Fruit Juice Beverages*; Nagy, S., Wade, R. L., Eds.; Ag. Science: Auburndale, FL 1995; Chapter 1.

Martin, G. J.; Zhang, B. L.; Naulet, N.; Martin, M. L. Deuterium transfer in the bioconversion of glucose to ethanol studied by specific isotope labelling at the natural abundance level. *J. Am. Chem. Soc.* **1986**, *108*, 5116−5122.

Martin, G. J.; Guillou, C.; Martin, M. L.; Cabanis, M. T.; Tep, Y.; Aerny, J. Natural factors of isotope fractionation and the characterisation of wines. *J. Agric. Food Chem.* **1988**, *36*, 316−322.

McHard, J. A.; Faulk, S. J.; Wineforder, J. D. A comparison of trace element contents of Florida and Brazil orange juice. *J. Agric. Food Chem.* **1979**, *27*, 1326−1328.

Mook, W. G. Changes of $^{18}O/^{16}O$ and $^{2}H/^{1}H$ during evaporation of flowing surface water. *Isotopenpraxis* **1987**, *23*, 817−830.

Moussa, I.; Naulet, N.; Martin, M. L.; Martin, G. J. A site-specific and multi-element approach to the determination of liquid-vapour isotope fractionation parameters. The case of alcohols. *J. Phys. Chem.* **1990**, *94*, 8303−8309.

Nikdel, S.; Nagy, S.; Attaway, J. A. Trace metals: defining geographical origin and detecting adulteration of orange juice. In *Adulteration of Fruit Juice Beverages*; Nagy, S., Attaway, J. A., Rhodes, M. E., Eds.; Dekker: New York, 1988; Chapter 5.

Sofer, Z.; Gat, J. R. The isotope composition of evaporating brines: effect of the isotopic activity ratio in saline solutions. *Earth Planet. Sci. Lett.* **1975**, *26*, 171−186.

Szapiro, S.; Steckel, F. Physical properties of heavy oxygen water ii—vapor pressure. *Trans. Faraday Soc.* **1967**, *63*, 883−894.

Van Hook, A. W. Vapor pressure isotope effect in aqueous systems III. The vapor pressure of HOD. *J. Phys. Chem.* **1972**, *76*, 3040−3043.

Wallrauch, S.; Faethe, W. Amino acids: criteria for the evaluation of fruit juices. In *Adulteration of Fruit Juice Beverages*; Nagy, S., Attaway, J. A., Rhodes, H. E., Eds.; Dekker: New York, 1988; Chapter 2.