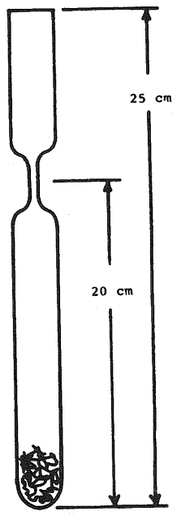


**Figure 992.08B. Construction of combustion tube.**

Heat sealed combustion tube in oven at 500°C for ≥1 h. (*Caution*: Considerable pressure is generated in combustion tubes at 500°C. Provide adequate protective shielding.) Let oven cool to room temperature and remove combustion tube.

Score flame-sealed nub with steel file, and insert nub end of tube into connecting hose of purification system. Evacuate connecting hose and open combustion tube by bending hose until scored nub breaks off. Pass combustion products through traps of dry ice–acetone and liquid $N_2$, evacuating any noncondensible gases. Warm liquid $N_2$ traps, and pass liberated $CO_2$ through pentane ice trap to recondense in flask cooled with liquid $N_2$.

### D. Determination

*See* **984.23E** (*see* 44.5.09).

### E. Calculations

*See* **984.23F** (*see* 44.5.09). Report results as $\delta\ ^{13}C$ ‰.

Reference: *J. AOAC Int.* **75**, 725(1992).

## 37.1.64

## AOAC Official Method 992.09
## Sugar-Beet-Derived Syrups in Frozen Concentrated Orange Juice
## $\delta^{18}O$ Measurements in Water

**Stable Isotope Ratio Mass Spectrometric Method**
**First Action 1992**
**Final Action 1997**

(Applicable to classification of frozen concentrated orange juice with $\delta^{18}O$ value <+8.9‰ as diluted with groundwater-prepared product.)

Results of the interlaboratory study supporting acceptance of the method:

100% and 90% orange juice, mean $\delta^{18}O$ value = 12.24‰: $s_r$ = 0.88; $s_R$ = 1.81; $RSD_r$ = 7.2%; $RSD_R$ = 14.8%

75% and 60% orange juice, mean $\delta^{18}O$ value = 6.73‰: $s_r$ = 0.30; $s_R$ = 1.76; $RSD_r$ = 4.5%; $RSD_R$ = 26.1%

### A. Principle

Test portion is equilibrated with $CO_2$ to achieve isotopic equilibrium between oxygen in test portion water and oxygen in $CO_2$. After equilibration, $CO_2$ is removed and purified, and $^{18}O/^{16}O$ is measured by isotope ratio mass spectrometer. Differences in $\delta^{18}O$ values for pure orange juice concentrate (63–67° Brix; mean $\delta^{18}O_{SMOW}$ = +14.28‰; SMOW is standard mean ocean water) and ground water used in preparation of sugar beet syrups (usual range of $\delta^{18}O_{SMOW}$ = −5 to −10‰) provide a measure of beet syrup in orange juice concentrate.

### B. Apparatus

(**a**) *Equilibration system.*—60 mL plastic syringes, 2.5 cm 22 gauge needles, 1.8 cm diameter steel balls, 40 cm diameter wheel to which 12 syringes can be attached and rotated at 10–12 rpm by electric motor.

(**b**) *Purification system.*—Vacuum-tight glass manifold including liquid $N_2$ traps, analyte collection bottle, and high vacuum source (Figure **992.09**).

(**c**) *Ratio mass spectrometer.*—Instrument designed or modified for isotope ratio measurement and capable of accuracy of 0.01% of abundance at mass 46.

### C. Reagents

(**a**) *Pressurized carbon dioxide.*—100%.

(**b**) *Reference water standard.*—Standard mean ocean water (SMOW).

(**c**) *Carbonate standards.*—(*1*) NIST RM 8544 NBS 19 (limestone). (*2*) NIST RM 8543 NBS 18 (carbonite), or equivalent.

(**d**) *Phosphoric acid.*—100%.

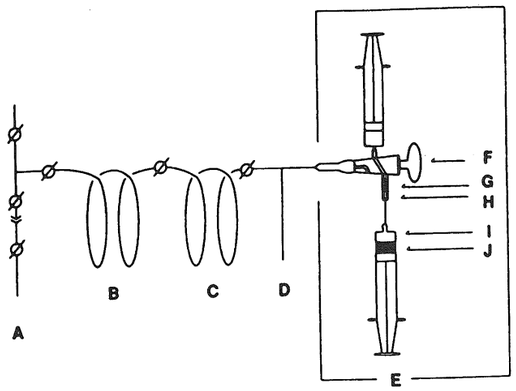


**Figure 992.09. Schematic diagram of vacuum line for collection and purification of $CO_2$: (A) sample bottle for collection of $CO_2$; (B) multitrap 2; (C) multitrap 1; (D) cold finger; (E) sample inlet, illustrated in detail; (F) 3-way stopcock; (G) capillary glass tubing; (H) silicone rubber septum; (I) equilibrated $CO_2$ in gaseous phase; (J) water sample.**





### D. Isolation of Analyte

Place steel ball in barrel of syringe, pour 30 mL test portion into barrel. Insert plunger and expel as much air as possible from syringe. Attach 2.5 cm 22 gauge needle. Use rubber septum attached to pressurized reservoir of pure $CO_2$ to add 20 mL $CO_2$. Remove syringe from septum and seal needle by piercing a No. 000 rubber stopper. Attach syringe to wheel and rotate in vertical plance at 10–12 rpm for ≥2 h. Maintain air (and test portion) temperature at 25° ± 0.5°C. Insert needle into septum on preparation line (*see* Figure **992.09**). Transfer $CO_2$ from syringe into preparation line; then move $CO_2$ into evacuated portion of preparation line up to first stopcock and cool finger with liquid nitrogen. Pump away any air through multitrap 1, also cooled in liquid nitrogen. Water vapor is retained in multitraps 1 and 2, which are cooled with dry ice–acetone slurry. For each test portion set, reference water standard (SMOW) should be analyzed 2 times. Analyze a standard before and after test samples.

### E. Determination

Calibrate reference gas of mass spectrometer using at least 2 carbonate standards. React carbonate standards with 100% $H_3PO_4$ at 25° ± 0.1°C. Make any necessary corrections due to instrumental error, such as zero enrichment, peak tailing, or gas mixing. Correct for $^{17}O$ contribution to mass 45.

### F. Calculations

Calculate $\delta^{13}C$ and $\delta^{18}O$ from:

$$\delta^{H}E\ (‰) = [(^{H}E/^{L}E_{\text{test portion}})/(^{H}E/^{L}E_{\text{standard}}) - 1] \times 1000$$

where $^{H}E$ and $^{L}E$ are heavy and light isotopic species of element E, respectively. A frozen orange juice concentrate with $\delta^{18}O$ value of +15‰ would have an $^{18}O/^{16}O$ ratio 15‰ greater than $^{18}O/^{16}O$ ratio in SMOW, and be "heavy" in $^{18}O$ relative to the standard.

Convert analytical data obtained relative to reference gas to PDB scale using the equation:

$$\delta_{(x-\text{PDB})} = \delta_{(x-\text{B})} + \delta_{(\text{B}-\text{PDB})} + [(\delta_{(x-\text{B})})(\delta_{(\text{B}-\text{PDB})})10^{-3}]$$

where (*x* − B) and (*x* − PDB) refer to analysis of test portion (*x*) relative to standard (B) and to PDB, and (B − PDB) is analysis of standard (B) relative to PDB, all δ values expressed in ‰ (standard B defined as carbon dioxide source used, derived from standard limestone, graphite, or crude oil).

Convert $\delta^{18}O_{PDB}$ values to $\delta^{18}O_{SMOW}$ by adding −0.26‰.

Correct test values by average deviation from expected value found for water standards by adding or subtracting appropriate ‰ value. A $\delta^{18}O$ value <+8.9‰ for frozen concentrated orange juice indicates the presence of groundwater-prepared product (typically sugar-beet-derived syrups).

Reference: *J. AOAC Int.* **75**, 1107(1992).

## 37.1.65

## AOAC Official Method 995.17
## Beet Sugar in Fruit Juices

**Site Specific Natural Isotope Fractionation–Nuclear Magnetic Resonance (SNIF–NMR®) Method**
**First Action 1995**
**Final Action 1998**

(Applicable to detection of 10–100% beet sugar in single strength juice [from squeezed fruits and from concentrate] and in juice concentrate.)

*See* Table **995.17A** for results of the interlaboratory study supporting acceptance of the method.

### A. Principle

Using site specific ratios measured by nuclear magnetic resonance (NMR), it has been shown that repartition of deuterium within sites of an organic molecule may deviate strongly from statistical distribution. This behavior is generally informative about botanical origin, biosynthesis pathway, and sometimes geographical origin of the molecule. In particular, fermentation

**Table 995.17A. Interlaboratory study results for determination of beet sugar in fruit juices by SNIF–NMR method**

| Juice | Sugar added, % | Mean $(D/H)_I$[a] | $s_r$ | $s_R$ | $RSD_r$, % | $RSD_R$, % | r[b] | R[c] |
|---|---|---|---|---|---|---|---|---|
| Orange, single strength | —[d] | 104.51 | 0.22 | 0.29 | 0.21 | 0.28 | 0.61 | 0.81 |
| | 17[d] | 102.35 | 0.19 | 0.24 | 0.19 | 0.23 | 0.53 | 0.67 |
| | 25 and 28[e] | 101.25 | 0.22 | 0.33 | 0.22 | 0.32 | 0.62 | 0.92 |
| | | 101.02 | | | | | | |
| Orange, concentrated | —[d] | 101.27 | 0.23 | 0.36 | 0.23 | 0.35 | 0.64 | 1.00 |
| | 37 and 40[e] | 97.88 | 0.25 | 0.37 | 0.25 | 0.37 | 0.69 | 1.02 |
| | | 97.44 | | | | | | |
| Grape, single strength | —[d] | 102.18 | 0.21 | 0.21 | 0.21 | 0.21 | 0.59 | 0.59 |
| Grapefruit, concentrated | —[f] | 102.51 | 0.20 | 0.32 | 0.19 | 0.32 | 0.56 | 0.91 |
| Apple, single strength | —[d] | 99.00 | 0.20 | 0.28 | 0.21 | 0.29 | 0.57 | 0.79 |

[a] $(D/H)_I$ = Ratio of deuterium to hydrogen atoms on the methyl site of ethanol; HorRat not applicable.
[b] $r = 2.8 \times s_r$.
[c] $R = 2.8 \times s_R$.
[d] Blind duplicates.
[e] Youden pair.
[f] Known duplicates.

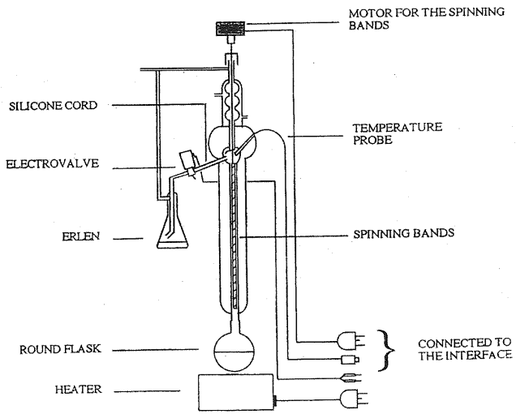


**Figure 995.17A. Distillation apparatus for extraction of ethanol.**

ethanols constitute reliable probe for characterizing precursor sugars and are used for determining addition of beet, beet invert, beet medium invert sugar, or similar sugars in fruit juices. Deuterium contained in sugars and water of juice will be redistributed after fermentation in molecules I ($CH_2DCH_2OH$), II ($CH_3CHDOH$), III ($CH_3CH_2OD$), and IV (HOD) of fermented juice. $(D/H)_I$ isotope ratio associated with molecule I is used for identifying added sugar. $(D/H)_I$ mainly characterizes vegetable species which biosynthetized sugar and to lesser extent geographical location of place of harvest (small variations are due to differences in $H_2O$ used during photosynthesis). Addition of beet sugar decreases $(D/H)_I$; addition of cane or corn sugar increases $(D/H)_I$.

### B. Apparatus

(**a**) *Abbe refractometer.*—Optional.

(**b**) *Computerized system for monitoring fermentation.*—Optional.

(**c**) *Steam distillation system.*—For quantitative separation of ethanol from alcoholic product or beverage; used in determination of alcohol content.

(**d**) *Electronic densitometer.*

(**e**) *Preparatory distillation system.*—For separation of ethanol (*see* Figure **995.17A**); with manual Cadiot column and spinning band (Teflon moving part) or computerized distillation system, electric heating mantle with voltage regulator, 1 L round-bottom flask with ground glass neck joint, 125 mL conical flasks with ground glass joints, and 125 and 60 mL glass bottles with plastic stoppers.

Performance characteristics: Preparatory distillation system must be capable of isolating ≥96% ethanol present in fermented products of 3–20% (v/v) alcoholic content. Alcoholic grade of distillate must be ≥90% (w/w) to guarantee that isotopic fractionation on distillate is <0.2‰ (parts per 1000) for $^{13}C$ and 0.2 ppm (parts per million) for $(D/H)_I$.

(**f**) *Karl Fischer titrator.*—Optional.

(**g**) *Nuclear magnetic resonance (NMR) instrument.*—NMR spectrometer fitted with specific "deuterium" probe tuned to characteristic frequency $n_0$ of field $B_0$ (e.g., for $B_0$ = 9.4T, $n_0$ = 61.4 MHz) with proton decoupling channel ($B_2$) and field-frequency stabilization channel (lock) at fluorine frequency; automatic sample changer (optional); appropriate data processing software; and 10 mm diameter high precision NMR "sample" tubes.

Performance characteristics: Resolution measured on spectrum, transformed without apodization (e.g., exponential multiplication, LB = 0; *see* Figure **995.17B**) and expressed by half-width of methyl and methylene signals of ethanol and methyl signal of tetramethylurea internal standard must be <0.5 Hz. Sensitivity, measured with exponential multiplying factor (LB = 2; *see* Figure **995.17C**) must be ≥150 for methyl signal of ethanol of alcoholic strength 95% (v/v) (≥93.5% [w/w]). The relative standard deviation of $(D/H)_I$ calculated from signal heights obtained in 10 repetitions of the spectrum must be <0.35%.

(**h**) *System for preparation of test samples for NMR.*—Optional.

(**i**) *Fermentation vessel.*—1.5 L capacity, fitted with device that prevents air entry while allowing $CO_2$ escape. There must be no loss of ethanol during fermentation, and essentially all fermentable sugars (>98%) should be fermented. [*Note:* Items (**a**), (**c**), (**d**), and (**f**) may be replaced by any appropriate system (e.g., gas chromatography, pycnometer, etc.) for alcoholic grade measurement and by liquid chromatography for sugar content determination. Alcoholic grade of fermented juice (3–12% alcohol, w/w) must be measured with absolute precision of 0.05% alcohol (w/w) and alcoholic grade of distillate (90–96%, w/w) must be measured with absolute precision of 0.1% alcohol (w/w).]

### C. Reagents

(**a**) *Active dry yeast.*—*Saccharomyces bayanus cerevisiae*, or equivalent.

(**b**) *Karl Fischer reagent.*—Available commercially or prepare as follows: Dissolve 133 g I in 425 mL dry pyridine in dry glass-stoppered bottle. Add 425 mL dry methanol or ethylene glycol monomethyl ether (preferred). Cool to <4°C in ice bath and bubble in 102–105 g $SO_2$. Mix well and let stand 12 h. Reagent is reasonably stable, but restandardize daily with sodium tartrate·$2H_2O$ (1 mg sodium tartrate·$2H_2O$ = 0.150 mg $H_2O$). Alternatively, standardize with weighed $H_2O$ in methyl alcohol as follows: Transfer accurately weighed amount (ca 50 mg) $H_2O$ to titration vessel and titrate with Karl Fischer reagent to electronic end point. Calculate $C$ = mg $H_2O$/mL reagent.

(**c**) *Tetramethylurea (TMU) internal standard.*—Certified reference standard with known, monitored isotope ratio $(D/H)_{st}$; used for determination of natural deuterium isotope content; available from Institute for Reference Materials and Measurements (IRMM; Retieseweg, B-2440 Geel, Belgium).

(**d**) *Hexafluorobenzene ($C_6F_6$).*—Used as field-frequency stabilization substance (lock).

(**e**) *Calibrating standard solutions.*—For calibrating NMR spectrometer; use 3 sealed standard NMR tubes containing TMU, $C_6F_6$, and ethanol certified standard reference materials (prepared from cane sugar or maize alcohol, grape alcohol, and beet alcohol with different standardized isotope concentrations). Available from IRMM.

### D. Fermentation

(*Note*: Accurately perform all weighing operations in **D**, **E**, and **G**. Record all weights of preparation flasks and masses of products





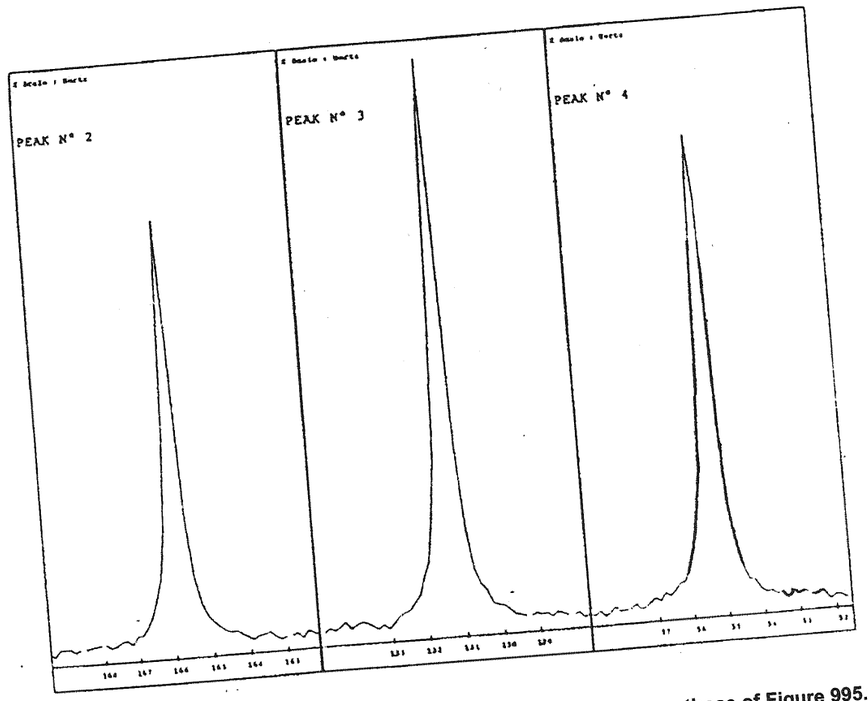


**Figure 995.17B. $^2H$ NMR spectrum of ethanol taken under the same conditions as those of Figure 995.17C, but without exponential multiplication (LB = 0).**

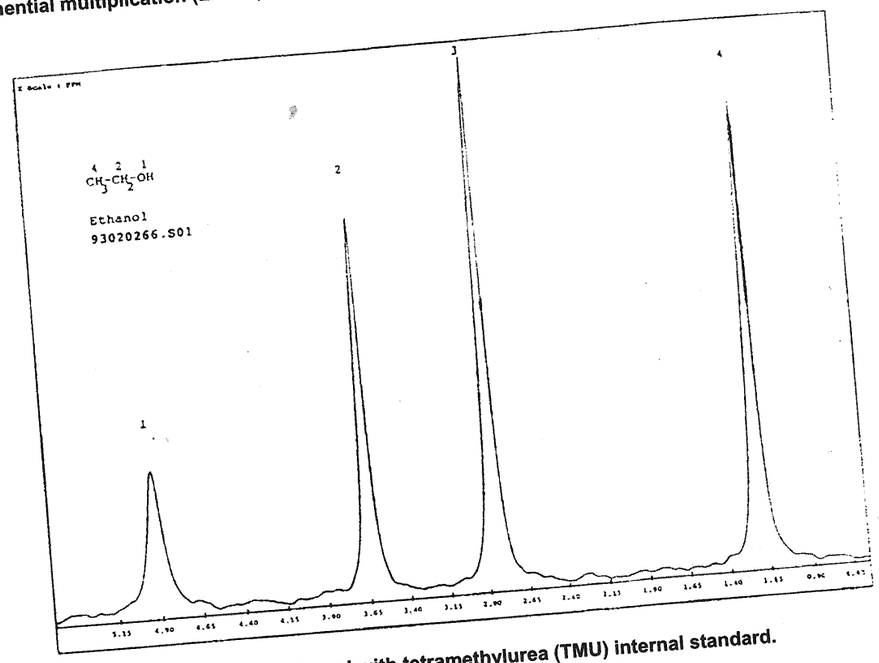


**Figure 995.17C. $^2H$ NMR spectrum of ethanol with tetramethylurea (TMU) internal standard.**



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placed into preparation flasks. Use average of duplicate weights in calculations.)

(**a**) *Fermentation of nonpreserved single strength juice made from squeezed fruit or from concentrate.*—Determine soluble solids content in the juice (% w/w) as in **932.12** (*see* 37.1.15). Into fermentation vessel, **B(i)**, place 0.6 L single strength juice at ca 12% soluble solids. Keep ratio of soluble solids (%) to volume (L) of 20 ± 2 by increasing or decreasing the volume if soluble solids differ from 12% by >1%. Add 0.3 g dry or reactivated yeast as follows: If deuterium isotope ratio of juice water is known, reactivate dry yeast 15 min before use in small amount of lukewarm nondistilled water adjusted at the same deuterium isotope ratio as juice water. If isotope ratio of juice is not known, use fresh dry yeast directly in juice. Install fermentation device to prevent air entry. Let juice ferment at ca 20°C until all sugars are converted to alcohol. Use optional system, **B(b)**, for monitoring fermentation, if desired. Centrifuge fermented liquid. Check for complete fermentation of fermentable sugars by measuring residual sugars using liquid chromatography or color reaction.

Determine alcoholic strength of supernate as follows: Place 20 mL supernate in round-bottom flask, dilute with distilled water to 200 mL (10-fold dilution), and steam distill, **B(c)**. Collect distillate and measure alcohol content by densimetry, **B(d)**. (The repeatability for 5% alcoholic solution is 0.05%.) Alternatively, determine alcoholic strength of fermented product by either pycnometric or gas chromatographic method.

(**b**) *Fermentation of juice concentrate.*—Determine content of soluble solids in juice concentrate (% w/w) as in **932.12** (*see* 37.1.15). Dilute concentrate in fermentation vessel, **B(i)**, with $H_2O$ to 0.6 L or slightly more to obtain ca 12% soluble solids in diluted product (for optimal fermentation speed, 12 ± 0.5% soluble solids is recommended). Add 0.3 g dry yeast and homogenize by shaking. Install fermentation device to prevent air entry. Let juice ferment at 20°C until all sugars are converted to alcohol. Centrifuge fermented liquid. Check for complete fermentation of fermentable sugars by measuring residue sugars using liquid chromatography or color reaction.

Determine alcoholic strength of supernate as in (**a**).

(**c**) *Fermentation of juices preserved with $SO_2$.*—Place 400 mL juice or concentrate rediluted to 12% soluble solids (w/w) $(D/H)_w^s$ into 2 L flask of rotary evaporator. Evaporate $H_2O$ at 40°C until residue is fully dry but not caramelized. Redilute to 12% with distilled water and then proceed as with nonpreserved single strength juices, (**a**).

Retain 50 mL single strength juice, rediluted concentrate, or $SO_2$-treated product for determination of isotope ratios $(D/H)_w^s$ [(D/H) of water before fermentation (i.e., test sample *s*)] and of $^{18}O/^{16}O$. Store retained portion at –20°C or stabilize it with NaF. Also retain 50 mL tap water (w) used for dilution to determine deuterium ratio.

### E. Distillation of Ethanol

Place 3 pumice stones (to prevent bubbling) into round-bottom distillation flask. Weigh flask ($w_0^B$). Place 400 mL homogeneous fermented juice (V) into flask and weigh again ($w_1^B$). Calculate weight of juice added to flask ($w^J = w_1^B - w_0^B$).

Circulate $H_2O$ in condenser of distillation apparatus, **B(e)**. Place 125 mL ground conical flask, previously weighed ($w_0^E$), to collect distillate. Attach round-bottom flask containing fermented juice to Cadiot column and heat contents to boiling. When boiling liquid is refluxing, switch on motor of spinning band, and wait 5 min to equilibrate.

**Table 995.17B. Typical number of scans per spectrum ($N_S$) used for various spectrometers**

| Spectrometer | $N_S$ for 10 mm probe |
|---|---|
| 7.05 T | 304 |
| 9.4 T | 200 |
| 11.7 T | 104 |

Collect boiling liquid between 78° and 78.2°C, with constant reflux ratio ca 0.9. (i.e., ca 20–30 mL). When temperature exceeds 78.5°C, discontinue collection for 5 min. After temperature returns to 78°C, start again collecting distillate until 78.5°C. Repeat this step until temperature, after discontinuing collection and operating within closed circuit, remains constant.

[*Note*: Complete distillation lasts ca 4 h. Generally 98–98.5% total alcohol in fermented juice is recovered from distillate, with strength 91–93% (w/w) (93–95%, v/v).]

Weigh conical flask containing distillate ($w_1^E$) and calculate exact weight of distillate ($w^D = w_1^E - w_0^E$).

Cool round-bottom flask containing residue and then weigh ($w_2^B$). Calculate weight of residue ($w^R = w_2^B - w_0^B$). Residue represents $H_2O$ in fermented product. Store 60 mL residue in 60 mL flask.

### F. Determination of Alcoholic Strength of Distillate

Determine $H_2O$ content ($m_w$; g) in distillate from **E** by Karl Fischer method using ca 0.25 mL distillate of exactly known mass (m).

Calculate alcoholic strength ($t_m^D$; %, [w/w]) of distillate as follows:

$$t_m^D = \frac{m - m_w}{m} \times 100$$

where m = mass of distillate used in Karl Fischer method.

Calculate weight losses of distillation ($w^L$) and yield of ethanol distillation (%) as follows:

$$\text{Weight losses } w^L = w^J - (w^D + w^R)$$

$$\text{Yield of distillation, \%} = 100 \times t_m^D \times w^D / (0.78924 \times V \times t^Q)$$

where V = volume of test portion, mL; $w^D$ = weight of distillate, g; and $t^Q$ = alcoholic strength of fermented juice, % v.

Yield of distillation should be 96%, otherwise isotope ratios of ethanol in distillate are modified due to significant isotopic fractionation during distillation.

Calculate relative weight losses ($Rw^L$; %) as follows:

$$Rw^L = 100\ (w^L/w^J)$$

$Rw^L$ >0.5% indicates abnormal losses during distillation step (e.g., leak in distillation system or error in weighing).

[*Note*: Conditions of high yield of ethanol distillation and alcoholic strength are strictly required to keep isotopic fractionation due to incomplete distribution below 0.2 µg/mL. These conditions can be achieved using optional computerized distillation system, **B(e)**.]



### G. Preparation of Alcohol Test Solution for NMR Measurement

Place 1.3 mL TMU internal standard solution into previously weighed 15 mL bottle. Weigh to the nearest 0.1 mg ($m_{st}$). Transfer 3.2 mL alcohol obtained in ethanol distillation **E**, into bottle and weigh again to the nearest 0.1 mg ($m_A$). Add 150 µL $C_6F_6$ and homogenize by shaking.

### H. Determination of Isotope Parameters

(**a**) *Calibration of spectrometer.*—Perform customary standardization for homogeneity and sensitivity of spectrometer according to manufacturer's specifications.

Use sealed tubes containing calibrating standard solutions, **C(e)**. Following procedure for recording deuterium NMR spectra, (**b**), check validity of standardization by determining isotope values of calibrating standard solutions. Compare results with given corresponding standard values provided by supplier. Standard deviation obtained on average of 10 repetitions of each spectrum must be <0.3 µg/mL for $(D/H)_I$. Average values of various isotopic parameters $(D/H)_I$ must be within the corresponding standard deviation of replicate given by supplier for those parameters for 3 calibrating standard solutions. Otherwise, repeat standardization and check of validity of standardization.

(**b**) *Recording of deuterium NMR spectra of ethanol.*—Transfer alcohol test solution into 10 mm tube and place into probe. To obtain NMR spectra, maintain following conditions: constant probe temperature, 28°–29°C; acquisition time, 6.8 s for 1200 Hz spectral width (16K memory; i.e., ca 20 ppm at 61.4 MHz or 27 ppm at 46.1 MHz); 90° pulse (must be determined); delay time before acquisition must be the same as dwell time; quadrature detection, set offset O1 between OD and CHD signals of ethanol. Determine value of decoupling offset O2 from proton spectrum obtained through decoupling coil on the same tube. Good decoupling is achieved when O2 is set to the middle of frequency interval existing between $CH_3$ and $CH_2$ groups. Use broad band decoupling mode or composite decoupling mode for complete decoupling from proton. (*Note*: O1 and O2 represent frequency positions for observation and decoupling channels, respectively, on Bruker spectrometers.)

For each spectrum, perform number of scans per spectrum ($N_S$) sufficient to obtain signal-to-noise ratio as specified in **B(g)**. Repeat set of $N_S$ accumulations 10 times ($N_E$ [number of experiments, i.e., number of tubes analyzed in one run]). $N_S$ values depend on types of spectrometer and probe used. *See* Table **995.17B** for typical $N_S$ used for various spectrometers.

### I. Calculations

(**a**) *$(D/H)_I$ ratio.*—Calculate for each of 10 spectra (*see* Figure **995.17C** for NMR spectrum of ethanol), as follows:

$$(D/H)_I = 1.5866 \times T_I \times (m_{st}/m_A) \times [(D/H)_{st}/t_m^D]$$

where $T_I$ = [heights of signal I ($CH_2DCH_2OH$)]/[height of signal of TMU internal standard solution]; $(D/H)_{st}$ = isotope ratio of TMU internal standard solution provided by supplier.

Use of peak heights instead of peak area is valid under assumption that peak widths at half height are identical (*see* Figure **995.17B**). Each spectrum must be checked for this assumption.

Calculate average of 10 determinations and confidence interval for each of isotope parameters. Alternatively, for calculations and controls to be performed on-line, use software suitable for spectrometer computer.

(**b**) *Minimum amount of added sugar.*—To minimize second order risk (i.e., finding added sugar when there is none), calculate only minimum quantity of added sugar (percent total sugars) using the lowest $D/H_I$ ratio for authentic juices of the same origin $[(D/H)_I min]$.

[*Note:* Fermentation of juices made from concentrate does not take place in $H_2O$ having the same isotope concentration as natural juice. Therefore, for comparison, a normalization can be applied on $(D/H)_I$ and on $(D/H)_{II}$ [isotope ratio associated with molecule II ($CH_3CHDOH$)] to make them the same than if the juice had fermented in water having the same deuterium concentration as V.SMOW (Vienna Standard Mean Ocean Water) international reference.]

$(D/H)_I x$ = value measured on product to be analyzed after normalization for deuterium content of juice water.

If $(D/H)_I x > (D/H)_I min$, then no significant addition of beet sugar is detected. If $(D/H)_I x < (D/H)_I min$, calculate amount of added beet sugar (%) as follows:

$$\text{Beet sugar, \%} = 100 \times \frac{(D/H)_I min - (D/H)_I x}{(D/H)_I min - 92}$$

If added cane or corn sugar (percent C) was also detected with Carbon-13 analysis, $(D/H)_I x$ must be corrected for influence of cane or corn sugar (% $C_4$) as follows:

$$\text{Corrected } (D/H)_I x = (D/H)_I x - (\%\ C_4/100) \times [110 - (D/H)_I min]$$

*Note*: In practice, this calculation leads to significant underestimation of amount of added sugar, especially when mixture of $C_4$ and $C_3$ sugars has been used. In that latter case, bivariate statistical calculation at desired confidence level gives more accurate measurement of amount of added sugar. When value corresponding to raw material where sugar was added is known, it must be used in place of $(D/H)_I$ min to give better evaluation of percent added sugar.

Reference: *J. AOAC Int.* **79,** 917(1996).

## 37.1.66

## AOAC Official Method 999.05 Naringin and Neohesperidin in Orange Juice

### Liquid Chromatography Method First Action 1999

### A. Applicability

(The method determines 5–50 µg/g naringin and 5–50 µg/g neohesperidin in orange juice.)

*See* Table **999.05** for the results of the interlaboratory study supporting acceptance of the method.

### B. Principle

Orange juice is filtered to remove particulates, injected onto a column containing C18 phase where naringin and neohesperidin are separated from other components, and measured by UV absorbance at 280 nm.