Purity Assessments of Major Vegetable Oils Based on $\delta^{13}C$ Values of Individual Fatty Acids

Simon E. Woodbury[a], Richard P. Evershed[a,*], and J. Barry Rossell[b]

[a]School of Chemistry, University of Bristol, Bristol, BS8 1TS, United Kingdom, and [b]Leatherhead Food Research Association, Surrey, KT22 7RY, United Kingdom

ABSTRACT: The fatty acid compositions and $\delta^{13}C$ values of the major fatty acids of more than 150 vegetable oils were determined to provide a database of isotopic information for use in the authentication of commercial maize oil. After extraction of oils from seeds, nuts or kernels, and methylation, fatty acid compositions were determined by capillary gas chromatography. All compositions were within the ranges specified by the Codex Alimentarius. Gas chromatography combustion-isotope ratio mass spectrometry was employed to determine the $\delta^{13}C$ values of the major fatty acids of the oils. A large number of pure maize oils and potential adulterant oils from various parts of the world were studied to assess the sources of variability in $\delta^{13}C$ values. Such information is vital to establishing the compound specific isotope technique as a reliable means of assessing vegetable oil purity. Variability in $\delta^{13}C$ values was related to the geographical origin of the oil, year of harvest, and the particular variety of oil. This suggests that the ultimate $\delta^{13}C$ values of fatty acids are determined by a combination of environmental and genetic factors.
JAOCS 75, 371–379 (1998).

KEY WORDS: Authenticity, $\delta^{13}C$ values, gas chromatography (GC), gas chromatography combustion–isotope ratio mass spectrometry (GCC–IRMS), fatty acid methyl esters, fatty acids, stable carbon isotopes, vegetable oils.

The international production of maize oil is currently of the order of 1.5×10^6 tons annually, which represents approximately 2% of total world vegetable oil production (1). Because of its good shelf life, flavor stability and a healthful fatty acid profile, maize oil is a prime target for fraudulent adulteration with cheaper vegetable oil. This adulteration can constitute major economic fraud (3), and may have implications for public health (4). A recent report by the Ministry of Agriculture, Food and Fisheries (MAFF; London, United Kingdom) revealed that, of 291 edible oils available from retailers, approximately 7% contained in excess of 5% of another oil. Of the 79 maize oils analyzed, approximately 14% were adulterated similarly (5,6), confirming the necessity to develop new analytical techniques to combat this fraudulent activity.

*To whom correspondence should be addressed at School of Chemistry, Cantock's Close, University of Bristol, Bristol, BS8 1TS, United Kingdom. E-mail: R.P.Evershed@bris.ac.uk.

Numerous methods have been employed to verify the purity of edible oils (3,7–9), with many of these tests having been developed to reveal particular blends of oils (10–12). The purity of many oils can adequately be established from their fatty acid or triacylglycerol compositions, or from their sterol or tocopherol profiles. However, the detection of adulterant oils in maize oil presents a particular problem (2). The fatty acid composition of maize oil has a high natural variability, and blends of oils may readily be prepared with fatty acid compositions lying within the range expected for pure maize oils. The high sterol and tocopherol contents of maize oil also mask the presence of adulterant oils.

Adulterant oil has been detected in maize oil by measuring the stable carbon isotope ratios ($\delta^{13}C$ value) of whole oils (1–3). This technique exploits the differences in the ratio of $^{13}C/^{12}C$ imparted on chemically identical end-products by different biosynthetic pathways. Stable carbon isotope ratio measurements determine variations in the amount of ^{13}C present in compounds, which range from approximately 1.075 atom% in C_3 plants to approximately 1.100 atom% in C_4 plants. It is more convenient to express these variations in ^{13}C content relative to an international standard, using the δ-notation, which is expressed in units per mil (‰) (Equation 1). Thus:

$$\delta^{13}C(‰) = \{(R_{sample}/R_{standard}) - 1\} \times 10^3 \quad [1]$$

where $R = {}^{13}C/{}^{12}C$.

The international standard is a marine carbonate, Pee Dee Belemnite (PDB) (13,14), which has an accepted value of $R_{PDB} = 0.0112372 \pm 0.000009$. This represents an abundance of ^{13}C of 1.1112328 atom%. C_3 and C_4 plants have lower atom% of ^{13}C; hence, their $\delta^{13}C$ values are negative. Virtually all terrestrial plants, including all commercially available vegetable oils except maize oil, are C_3 plants. Some tropical grasses, including maize, complete the initial "Hatch-Slack" step *via* a four-carbon compound (15,16). These pathways discriminate against ^{13}C to different extents; hence, C_3 plants have $\delta^{13}C$ values of approximately –28%, whereas C_4 plants have $\delta^{13}C$ values of approximately –14% (16).

Previous work by Rossell (2,3) has shown that as little as 10% (w/w) of impurity can easily be established by stable carbon isotope analysis of whole vegetable oils. Woodbury

et al. (17) then showed that the detection limit of impurity could be further improved by the use of gas chromatography combustion–isotope ratio mass spectrometry (GCC–IRMS) (18–20), in which the $\delta^{13}C$ values of individual compounds are measured as they elute from the GC column. Preliminary investigations indicate that adulteration of maize oil at 5% (w/w) may be readily detected, which would increase the potential usefulness of the compound specific stable isotope technique (17). The technique, however, has been shown to be ineffective in distinguishing between commercially important C_3 vegetable oils (21).

Before the GCC–IRMS technique can be used routinely to assess the purity of maize oils, it is essential to assess the sources of natural variation in the $\delta^{13}C$ values of fatty acids of authentic maize oils and other commercially important oils. These data are required together with the fatty acid compositions of the oils, as determined by GC, to construct mixing curves that can be used to predict $\delta^{13}C$ values of the fatty acids of blends of oils and to identify the nature and proportion of an adulterant oil in a commercial oil (22). The results presented herein describe the construction of a database of $\delta^{13}C$ values and fatty acid compositions of 52 maize oils and 72 potential adulterant oils that represent most, if not all, of the major commercially important vegetable oils. Samples (29) of winter oilseed rape, which included seven different varieties covering five consecutive harvest years, also were studied.

TABLE 1
The Origins and Numbers of Oilseeds Investigated

Maize oils (C_4 plants)			Other oils (C_3 plants)	
Origin	Region[a]	Number	Type	Number
Argentina	SAM	16	Babassu	3
Australia	AUS	6	Cocoa butter	9
Brazil	SAM	2	Coconut	2
"Europe"[b]	EUR	1	Copra	2
France	EUR	4	Cottonseed	3
Germany	EUR	3	Peanut	7
Greece	EUR	2	Illipe	3
Italy	EUR	2	Olive	1
New Zealand	AUS	1	Palm kernel	5
Nigeria	AFR	2	Blue poppy	1
Spain	EUR	1	Safflower	7
Turkey	ASA	3	Sesame	7
US	NAM	8	Shea	5
Zimbabwe	AFR	1	Soybean	6
			Sunflower	6
			Rapeseed[c]	5 + 29

[a]Seeds were classified by origin according to continental region as SAM (South American), NAM (North American), EUR (European), AUS (Australasian), AFR (African), or ASA (Asian).
[b]Information accompanying this sample specified origin as "Europe."
[c]Rapeseed samples included five oilseeds from around the world, and 29 oilseeds grown at the same location in Scotland.

EXPERIMENTAL PROCEDURES

Authentic oilseeds, nuts, kernels and maize germs, representing all commercially available edible oils, were obtained from suppliers worldwide. Samples (29) of winter oilseed rape were obtained from the Scottish Agricultural College, United Kingdom. The majority of samples were accompanied by details of origin and year of harvest. In total, 52 maize (C_4) samples were obtained, including samples from all major maize-producing nations, along with 72 nonmaize (C_3) samples. Table 1 summarizes the samples obtained.

Oilseeds were prepared by manual removal of the admixture. No cooking or prepressing was performed. After grinding in a suitable mill, samples of seed were extracted for 4 h with petroleum ether (40 to 60°C boiling range). The partly defatted meal was reground and extracted for a further 2 h, and the extracts were passed into the same flasks as previously. Solvent was initially removed into a collecting vessel, with any residual solvent being removed under vacuum at 60°C.

To determine the $\delta^{13}C$ values of the individual fatty acids of the oils, the triacylglycerols were saponified and subsequently acidified to yield fatty acids, which were then derivatized to fatty acid methyl esters (FAME). No kinetic isotope effect is associated with this derivatization because the reaction is rapid and quantitative with regard to the carbonyl (23). Briefly, 2 mL of 0.5 M methanolic sodium hydroxide was added to 100 μL of each oil in screw-capped test tubes and heated (70°C; 15 min) with occasional shaking. Each sample was then allowed to cool to room temperature (*ca.* 25°C) and acidified to pH 3 with 1 M aqueous hydrochloric acid. Fatty acids were extracted with three portions of hexane (2 mL each) and combined. Of the fatty acid solution, 250 μL was taken and blown to dryness under nitrogen gas in a screw-capped test tube, and 100 μL of BF_3/MeOH (Aldrich, Gillingham, United Kingdom) was added. Methylation was performed by heating the screw-capped test tube (70°C; 45 min). After cooling, 2 mL of double-distilled water was added, and the FAME were extracted into 2 mL diethyl ether. The diethyl ether was removed under nitrogen gas, and the FAME were dissolved in 2 mL hexane for analysis by GC and GCC–IRMS. Selected samples were studied by GC–MS to confirm the identities of the FAME.

GC analysis of the FAME was performed in a Hewlett-Packard 5890 Series II gas chromatograph (Hoofddorp, The Netherlands) as soon as possible after their preparation. A 25 m × 0.32 mm internal diameter BPX 70 (70% cyanopropyl equivalent) coated capillary column with 0.25 μm film thickness (SGE, Victoria, Australia) was employed with on-column injection and flame ionization detection. The temperature program was as follows: hold temperature at 40°C for 2 min, then increase to 200°C at 4°C per min, and hold at this temperature for 10 min. The carrier gas was helium, and data were collected with Hewlett-Packard Chemstation software. Identification of the FAME was achieved by comparison with gas-chromatographic data of similar vegetable oil FAME and through mass spectral analysis.

GCC–IRMS was performed with a Finnigan MAT Delta S mass spectrometer (Finnigan MAT GmbH, Bremen, Germany), coupled to a Varian GC (Varian Associates, Inc., Walnut Creek, CA) *via* a Pt/CuO combustion interface, which was

maintained at 850°C. Removal of water after combustion was facilitated by Nafion tubing (Perma Pure Products Inc., Toms River, NJ), and standardization of runs was achieved with six portions of CO_2 gas of known $\delta^{13}C$ value ($\delta^{13}C_{(CO_2)} = -31.80‰$), injected directly into the ion source of the mass spectrometer. Capillary column and temperature program details were exactly as for GC analysis of the FAME. Data were collected and processed with Finnigan MAT Isobase software.

To determine $\delta^{13}C$ values of the fatty acids from their corresponding FAME, a correction for the carbon atoms incorporated on derivatization had to be made. This was achieved by using the mass balance equation of Jones *et al.* (24) and assuming that there is no isotopic fractionation associated with the derivatization procedure. Hence,

$$\text{for } C_{16:0} \text{ fatty acid } 16.D_{FFA} = 17.D_{FAME} - X \quad [2]$$

$$\text{for } C_{18:1} \text{ and } C_{18:2}\ 18.D_{FFA} = 19.D_{FAME} - X \quad [3]$$

where $D_{FFA} = \delta^{13}C$ value of the free fatty acid, $D_{FAME} = \delta^{13}C$ value of the FAME, and $X = \delta^{13}C$ value of the derivatizing carbon (–29.0‰).

RESULTS AND DISCUSSION

The aim of this study was to produce a database of isotopic information for use in the detection of vegetable oil adulteration. As has been reported elsewhere (17,22), mixing curves may be produced from fatty acid compositions and $\delta^{13}C$ values to reveal adulteration of maize oil. This study was performed to assess the natural variability in these fatty acid compositions and $\delta^{13}C$ values in pure vegetable oils of known origin, which are essential in the accurate assignment of authentic and adulterated oils.

To ensure the authenticity of the oils used in this study, oils were extracted from their respective oilseeds, nuts, kernels, or germs. The procedure used was analogous to industrial solvent extraction procedures and was based on the international standard method for determination of oil content of oilseeds (ISO 659; Reference 25), though quantitative aspects were omitted for brevity. Fatty acid compositions and $\delta^{13}C$ values of the major fatty acids of a total of 153 oils were determined. This enabled the assessment of the influence of the following factors on the ultimate $\delta^{13}C$ values of fatty acids from the many different oils (26–28): (i) plant class (C_3 or C_4); (ii) hemisphere of origin; (iii) continent of origin, and (iv) variety of plant species.

The fatty acid compositions of the samples used in this study were expressed as percentage of total fatty acids by assuming similar flame ionization detector responses for all components. Typical chromatograms of the FAME of maize, groundnut, cocoa butter, coconut, palm kernel, and sesame seed oil are shown in Figure 1, together with the $\delta^{13}C$ values of the major fatty acids (mean of three determinations) of each oil. Fatty compositions of the maize and C_3 oils are summarized in Table 2 and are within the ranges for authentic oils

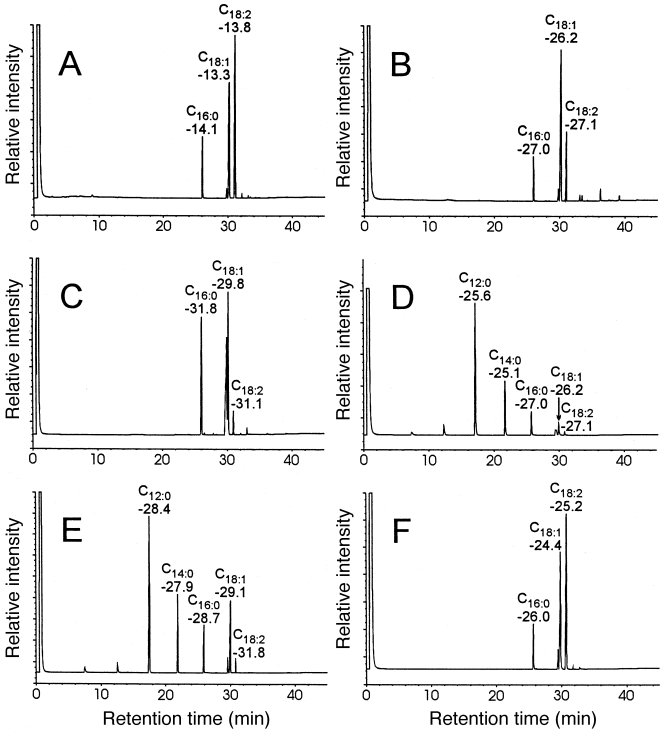

FIG. 1. Chromatograms for fatty acid methyl esters of six oils from this study, together with $\delta^{13}C$ values of the major fatty acids, corrected for the contribution of the derivatizing carbon (means of three determinations). A: maize, B: groundnut, C: cocoa, D: coconut, E: palm kernel, F: sesame.

specified in the Codex Alimentarius (29). Fatty acid compositions of vegetable oils have been studied extensively elsewhere (3,8) and will not be discussed further here.

The $\delta^{13}C$ values of the major fatty acids $C_{16:0}$, $C_{18:1}$, and $C_{18:2}$ were calculated from $\delta^{13}C$ values of the corresponding FAME as described above and are summarized in Table 3. The $\delta^{13}C$ values fell into two distinct groups (30), representing the C_4 (maize) and C_3 oils (16). Figure 2 shows typical output from the GCC–IRMS instrument for one of the pure maize oils. The partial mass chromatogram (Fig. 2B) displays baseline resolution of components, an essential requirement for the accurate determination of $\delta^{13}C$ values (Fig. 2A). Figure 3 shows frequency plots of the $\delta^{13}C$ values of each major fatty acid. Differences in the modes of CO_2 fixation employed by the two plant classes and their differing discriminations against ^{13}C cause these differences in $\delta^{13}C$ values of chemically identical end products, and this phenomenon has been studied extensively elsewhere (16).

The $\delta^{13}C$ values of the maize oils studied here displayed a bimodal distribution according to hemisphere of origin (Fig. 4). The same distribution was observed within the $\delta^{13}C$ values of all three major fatty acids. Such a distribution has been observed previously for $\delta^{13}C$ values of whole vegetable oils (2) and may be used, in conjunction with documentation that confirms the origin of the oil, to reduce the range of $\delta^{13}C$ values representing authentic oils. No such bimodal distribu-

TABLE 2
Variation in the Fatty Acid Compositions of the Vegetable Oils Studied

	Fatty acid composition (%) of vegetable oil							
Fatty acid	Maize	Babassu	Cocoa	Coconut	Copra	Cottonseed	Groundnut	Illipe
$C_{8:0}$		2.5–1.8		2.2–2.5	1.4			
$C_{10:0}$		4.3–3.8		5.5–5.6	4.0–4.6			
$C_{12:0}$		43.5–44.4	0.1	51.6–51.9	47.7			0.2–0.8
$C_{14:0}$		17.6–18.6	0.1–0.2	19.7–20.0	19.7–22.0	0.6–0.8		0.1–0.3
$C_{16:0}$	6.7–17.6	9.8–10.6	24.3–25.4	9.6	10.7–11.2	21.3–24.3	8.8–11.5	16.8–20.0
$C_{18:0}$	1.6–3.3	3.7–4.0	35.0–38.7	2.6–2.8	3.4	2.2–3.0	2.5–4.3	45.7–46.2
$C_{18:1}$	22.4–40.1	14.7–15.7	31.6–35.1	6.6	7.7–9.3	15.0–20.8	39.2–61.3	30.7–33.1
$C_{18:2}$	38.4–61.7	2.1–2.4	2.5–3.2	1.4–1.8	1.9	52.3–56.4	16.0–37.1	0.9–1.2
$C_{18:3}$	0.5–1.2		0.2–0.6			0.1–0.4	0.1	0.1–0.2
$C_{20:0}$	0.4–0.8	0.1	1.0–1.3			0.2	1.4–1.8	1.2–2.0
$C_{20:1}$	0.2–0.4	0.1	0.1		0.1	0.1	1.1–1.7	0.1
$C_{22:0}$	0.1–0.4		0.2–0.3		0.1	2.7–4.2	0.1	0.1
$C_{22:1}$	0.1–1.0	0.0	0.0		0.2			
$C_{24:0}$	0.2–0.4		0.1			0.1	1.2–2.0	0.1

	Fatty acid composition (%) of vegetable oil								
Fatty acid	Olive	Palm kernel	Blue poppy	Rape	Safflower	Soybean	Sesame	Sunflower	Shea
$C_{8:0}$		1.7–2.3							
$C_{10:0}$		2.5–3.0							
$C_{12:0}$		42.9–48.6			0.1	0.4		0.1	0.0–0.1
$C_{14:0}$		16.4–17.4		0.0–0.1	0.1–0.2	0.1–0.3		0.0–0.1	0.0–0.1
$C_{16:0}$	8.7	8.7–10.3	9.8	4.5–5.0	5.5–7.3	10.2–11.5	8.0–9.8	5.5–9.8	3.1–3.7
$C_{18:0}$	3.8	2.6–3.3	2.2	0.1–1.8	2.1–2.8	3.7–4.1	4.9–5.9	3.6–6.9	44.3–52.3
$C_{18:1}$	77.5	15.1–18.3	14.7	59.7–54.3	10.6–12.8	16.7–28.9	37.3–41.1	18.0–41.4	34.8–43.8
$C_{18:2}$	6.7	1.7–2.6	72.0	18.6–21.0	75.0–79.5	48.3–57.1	42.9–47.0	45.4–67.5	5.9–6.3
$C_{18:3}$	0.4		0.5	7.3–9.2	0.1	4.5–9.5	0.3–0.4	0.1	0.1–0.3
$C_{20:0}$	0.5	0.1–0.2	0.1	0.6	0.2–0.4	0.3–0.4	0.2–0.6	0.2–0.5	1.3–1.5
$C_{20:1}$	0.4	0.2		1.3–1.5	0.2–0.3	0.1–0.3	0.1–0.2	0.2–0.3	0.3
$C_{22:0}$	0.1			0.3–0.4	0.1–0.2	0.4–0.6	0.0–0.2	0.7–1.1	0.1
$C_{22:1}$	0.4	0.3		0.1–0.4			0.2		0.2
$C_{24:0}$	0.1	0.0–0.1		0.1	0.1	0.1–0.2	0.1	0.2–0.4	0.1

tion was observed in the C_3 oils. However, by considering different oil types together, differences in $\delta^{13}C$ values may have been masked. In addition, none of the C_3 oils was sampled sufficiently extensively to reveal differences according to hemisphere of origin.

Differences in $\delta^{13}C$ values of maize oils from different hemispheres are also observed when mean $\delta^{13}C$ values are calculated for maize oils from each continent. In particular, $\delta^{13}C$ values of fatty acids from North American corn oils are depleted by 0.5 to 1.0‰ relative to their South American

TABLE 3
A Summary of $\delta^{13}C$ Values of the Major Fatty Acids of the Vegetable Oils Studied

		$\delta^{13}C$ (‰)					
Origin of oil	Number	Range ($C_{16:0}$)	Mean ($C_{16:0}$)	Range ($C_{18:1}$)	Mean ($C_{18:1}$)	Range ($C_{18:2}$)	Mean ($C_{18:2}$)
Maize (C_4)							
Africa	3	–14.7 to –16.0	–15.4	–13.6 to –14.7	–14.1	–14.4 to –15.9	–15.0
Asia	10	–16.3 to –17.4	–16.9	–14.9 to –16.5	–15.9	–14.8 to –16.8	–16.1
Australasia	7	–15.2 to –17.4	–15.9	–14.1 to –17.1	–15.1	–14.4 to –17.1	–15.4
Europe	6	–15.2 to –17.4	–16.5	–14.4 to –16.4	–15.4	–14.1 to –16.5	–15.5
North America	8	–14.1 to –16.7	–15.6	–13.3 to –16.1	–15.0	–13.9 to –16.3	–15.3
South America	18	–13.8 to –16.2	–15.0	–12.9 to –16.1	–14.0	–13.3 to –16.6	–14.6
Other (C_3)							
Africa	19	–26.6 to –32.4	–29.1	–25.9 to –30.6	–28.3	–25.4 to –32.7	–29.0
Asia	16	–26.0 to –32.7	–29.2	–24.4 to –30.7	–27.9	–25.2 to –32.3	–29.0
Australasia	7	–25.6 to –31.2	–27.8	–25.7 to –30.0	–27.1	–24.2 to –31.0	–27.9
Europe	9	–27.2 to –29.8	–28.7	–25.2 to –28.5	–27.7	–27.3 to –29.3	–28.1
North America	7	–26.5 to –29.7	–28.8	–25.9 to –28.6	–27.5	–27.6 to –29.6	–28.8
South America	8	–24.7 to –32.1	–28.3	–25.4 to –31.0	–27.9	–27.5 to –31.3	–28.8

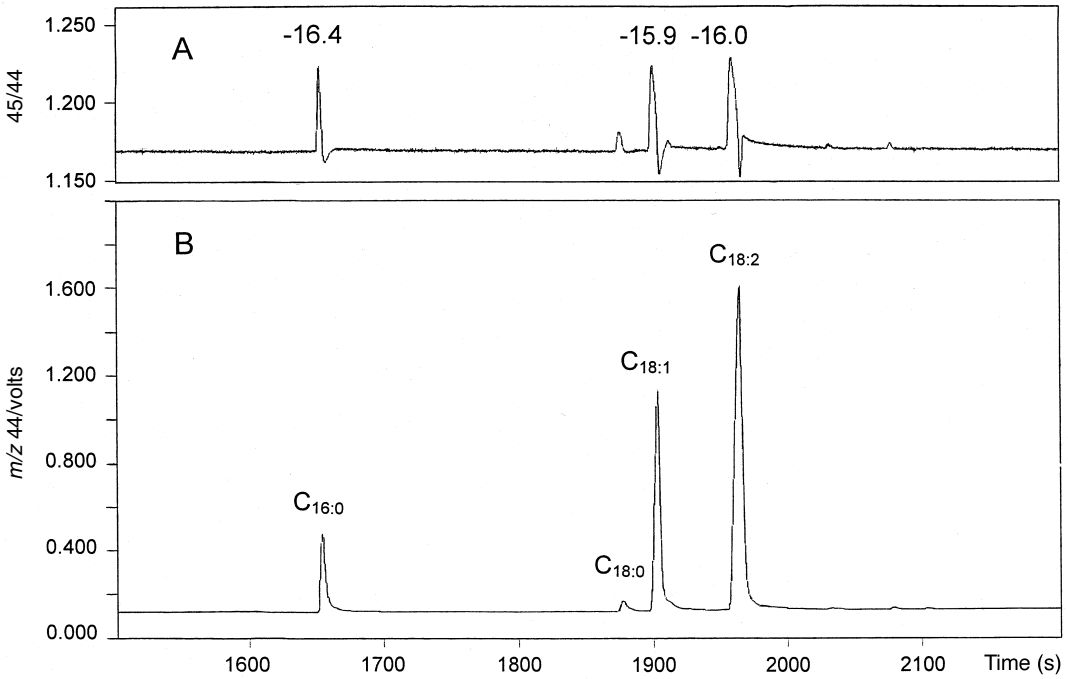

FIG. 2. Output from the gas chromatography combustion–isotope ratio mass spectrometry (GCC–IRMS) instrument for fatty acid methyl esters from a pure maize oil. (A) The instantaneous ratio of ions *m/z* 45 to *m/z* 44, with $\delta^{13}C$ values of the fatty acids, corrected for the contribution of the derivatizing carbon, and (B) the instantaneous intensity of the *m/z* 44 ion current, analogous to the output from the flame ionization detection (FID) of a GC. Peaks were identified by comparison with GC traces of fatty acid methyl esters (FAME) of authentic oils and by GC–MS analysis.

counterparts, and European corn oils display differences of similar magnitude relative to their African counterparts. While these similar differences are observed for European and African C_3 oils, no such differences are observed for North and South American C_3 oils. As mentioned above, this is probably a reflection of the great diversity of C_3 oils considered in this study. A better comparison would require a large number of samples of one particular oil grown at numerous sites around the world, as was done for the maize oils in this study.

The reason for differences in the $\delta^{13}C$ values of the maize oils grown in Northern and Southern Hemispheres is not immediately obvious. Because analogous varieties were drawn from the two hemispheres, the isotopic differences clearly do not result from genetically predetermined physiological, metabolic, or biochemical differences between different varieties. A more likely explanation lies in the $\delta^{13}C$ value of the primary carbon source, namely the atmospheric CO_2 fixed by the plants during their growth and lipid biosynthesis. Differences arise in the $\delta^{13}C$ value of atmospheric carbon dioxide for a variety of reasons: (i) fossil fuel effects (31,32); (ii) seasonal variations in CO_2 concentrations due to plant and soil respiration rates (32,33); and (iii) oceanic reservoir effects (32–34). A north–south gradient in $\delta^{13}C$ values of CO_2 of 0.2‰ in January 1980 was reported by Mook *et al.* (32), the more negative $\delta^{13}C$ values of CO_2 from the Northern Hemisphere being ascribed to the predominance of fossil fuel combustion in the Northern Hemisphere. A similar gradient is observed for maize oils from the Northern and Southern Hemispheres, giving a difference in mean $\delta^{13}C$ values of $C_{18:1}$ fatty acids of 0.6‰. Seasonal variations in $\delta^{13}C$ values of atmospheric CO_2 have also been observed (32,33), and these variations are of greatest magnitude in the Northern Hemisphere. This may account for the greater difference seen between mean maize $\delta^{13}C$ values as compared with mean atmospheric CO_2 $\delta^{13}C$ values. Because growth occurs mainly during the months when atmospheric CO_2 is most depleted in ^{13}C differences between $\delta^{13}C$ values of the metabolic products of maize from Northern and Southern hemispheres are increased. Further variations in $\delta^{13}C$ values may be attributed to the availability of soil moisture (35). Less negative $\delta^{13}C$ values are associated with the reduced physiological availability of soil moisture (36), and this is reflected in the mean $\delta^{13}C$ values of maize oils from South America and Africa when compared with their North American and European counterparts (Table 3). It was beyond the scope of this study to carry out $\delta^{13}C$ measurements of the atmospheric CO_2 or to determine soil moisture content throughout the growing season at the sites that yielded the seeds.

Figure 5 is a plot of the $\delta^{13}C$ values of the major fatty acids ($C_{16:0}$, $C_{18:1}$, and $C_{18:2}$) of seven strains of winter oilseed rape harvested in five consecutive years. The order-

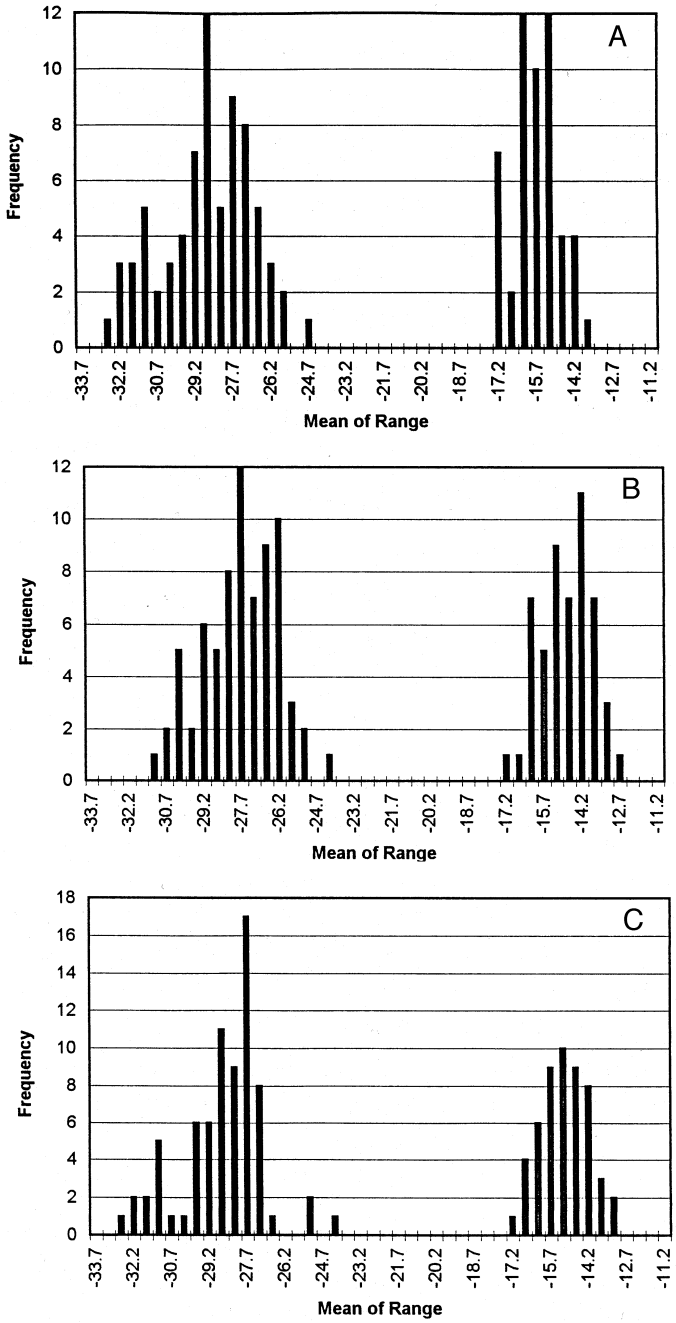

FIG. 3. Frequency plots of the $\delta^{13}C$ values of the major fatty acids from C_4 and C_3 vegetable oils. Ranges –11.0 to –11.4‰ through to –33.5 to –33.9‰ are shown. The $\delta^{13}C$ values of the two major plant classes are apparent for each fatty acid component. A, 16:0; B, 18:1; C, 18:2.

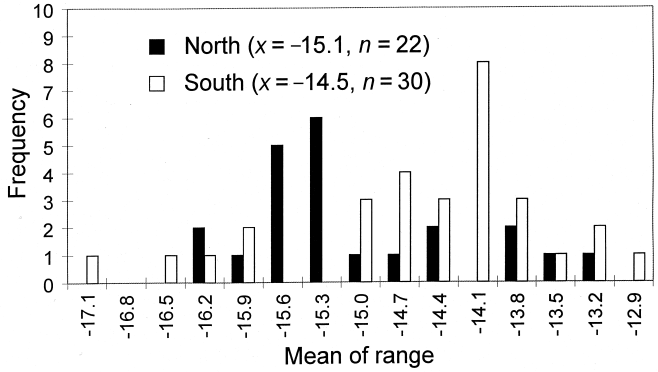

FIG. 4. A frequency plot of $\delta^{13}C$ values of the $C_{18:1}$ fatty acid of maize oils from the Northern and Southern Hemispheres shows a bimodal distribution. Ranges –17.6 to –17.4‰ through to –13.1 to –12.9‰ are shown.

ing of $\delta^{13}C$ values by strain is largely similar for each harvest year, suggesting that the ultimate $\delta^{13}C$ values of the major fatty acids are at least partly genetically predetermined. These differences are of the order of 1.0 to 1.5‰, much smaller than the *ca.* 15‰ difference between C_3 and C_4 plants. Notably, varieties Rocket and Samourai produce fatty acids that are most depleted in ^{13}C, while varieties Envoy and Idol show the smallest depletion. Seed from each harvest year may be assumed to have grown under identical conditions; thus variations in the $\delta^{13}C$ values from year to year may be ascribed to climatic variations between growing seasons. The 1991/1992 season displays the greatest depletion in ^{13}C, with broadly similar $\delta^{13}C$ values for the other harvest years studied. Rainfall patterns and differences in the number of hours of sunshine received during lipid biosynthesis are known to influence $\delta^{13}C$ values of the biosynthetic products by governing stomatal opening and the diffusion of CO_2 into the leaves of the plant (16).

In this paper, we have reported ranges of fatty acid compositions and $\delta^{13}C$ values of a large number of authentic oils from various sites around the world. Differences in $\delta^{13}C$ values were observed according to: (i) plant class (C_3 or C_4); (ii) hemisphere of origin; (iii) continent of origin; and (iv) variety of plant. The relative magnitudes of these differences are summarized in Figure 6. This is the first time that such a large number of $\delta^{13}C$ values of individual fatty acids from edible oils has been reported. Differences in the $\delta^{13}C$ values of fatty acids of oils grown at different locations and in different years were observed, these differences being partly genetic and partly environmental in origin. These data provide a basis for future biochemical studies of the fatty acids in plants based on their $\delta^{13}C$ values. Most importantly for authenticity studies, these data may be used to predict $\delta^{13}C$ values for the major fatty acids of maize oils of any origin that have suffered adulteration with any commercially available C_3 oils. The data presented herein provide an essential foundation for the development of the use of GCC–IRMS as a new analytical tool for the assessment of maize oil purity. The use of this database for the detection of adulteration will be the subject of a separate paper now in preparation.

ACKNOWLEDGMENTS

We thank the Natural Environment Research Council for support of mass spectrometry facilities through grants NERC GR3/7731 and F60/G6/36/01. Andrew Gledhill and Jim Carter are thanked for their expertise and assistance in the technique of GCC–IRMS. F.O.S.F.A.

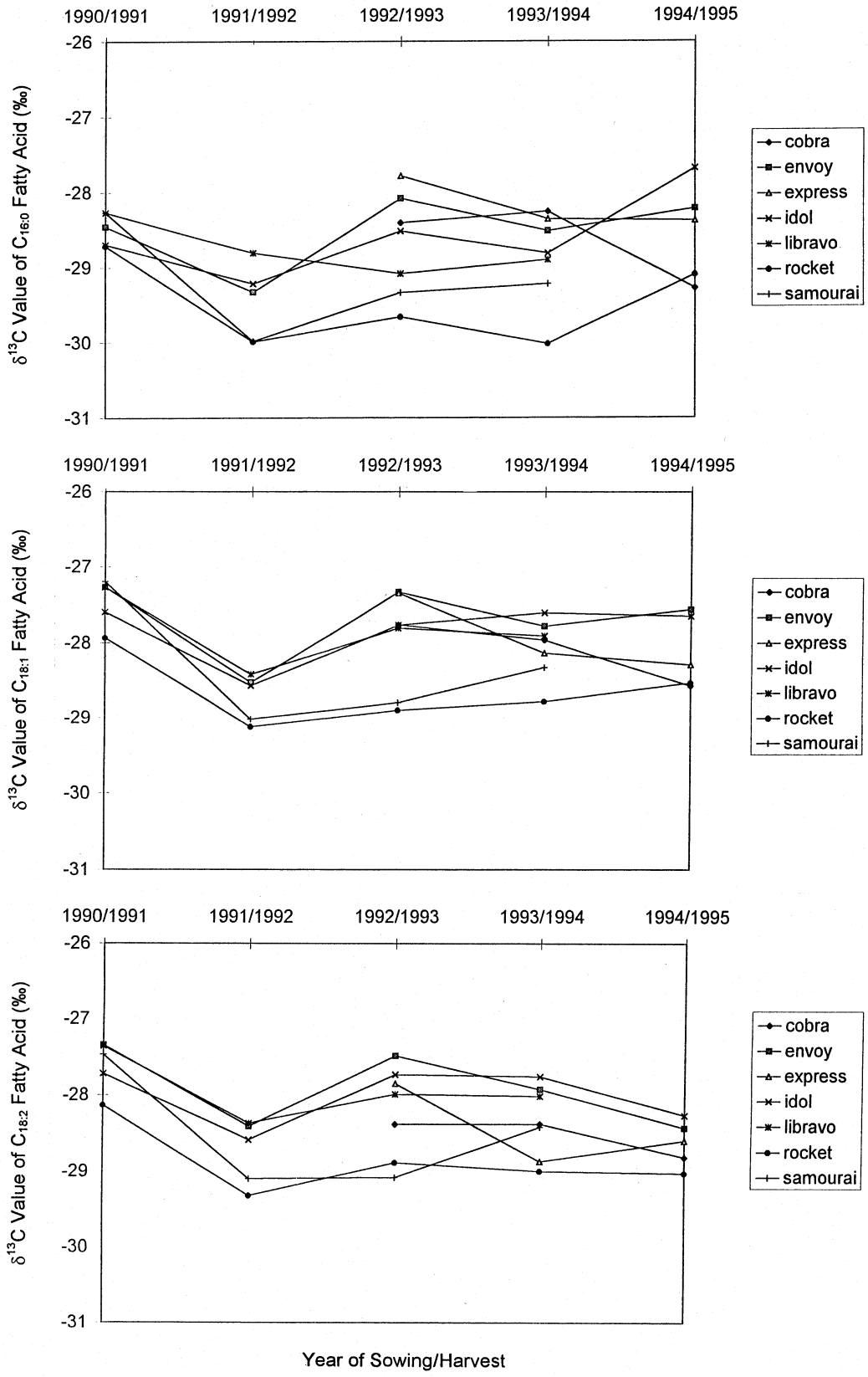

FIG. 5. Variations in the $\delta^{13}C$ values of the major fatty acids of seven different strains of winter oilseed rape, sown and harvested in five different years.

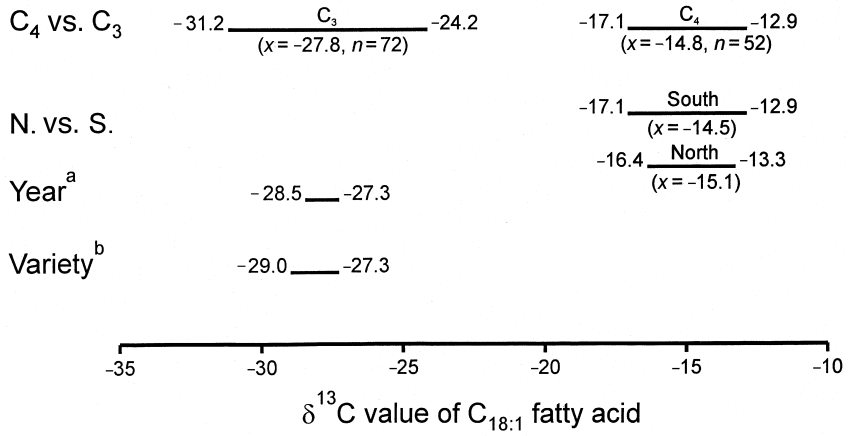

FIG. 6. A summary of the variations in $\delta^{13}C$ values of oleic acid due to plant type, origin, and year of harvest. [a]Year-to-year variability is shown for variety Idol (winter oilseed rape) grown in five consecutive years. [b]Variability between varieties for 1992/1993.

International is thanked for financial support, as are Unilever Research, Bedford, United Kingdom, and Nestec Ltd., Lausanne, Switzerland. Thanks are extended to the many suppliers of the oilseeds used in this study.

REFERENCES

1. Lee, K., P.N. Gillatt, and J.B. Rossell, Authenticity of Edible Vegetable Oils and Fats. Part XX: Determination of Maize Oil Purity by Stable Carbon Isotope Ratio Analysis, *LFRA Res. Rep. 719*, 1994, 61 pp.
2. Rossell, J.B., Stable Carbon Isotope Ratios in Establishing Maize Oil Purity, *Fat Sci. Technol. 96:*304–308 (1994).
3. Rossell, J.B., Purity Criteria in Edible Oils and Fats, *Ibid. 93:*526–531 (1991).
4. Slack, P.T., G.M. Wood, C.B. Mallinson, P.N. Gillatt, J.B. Rossell, W.J. Reid, and D.F. Lewis, Toxic Oil Disaster, *Nature 345:*583 (1990).
5. Ministry of Agriculture, Food and Fisheries, MAFF Single Seed Vegetable Oil Surveillance Exercise (1995) Food Surveillance Information Sheet Number 77 (pp. 1–4), Food Labelling and Standards Division, MAFF, Ergon House, c/o Nobel House, London.
6. Working Party on Food Authenticity, MAFF, Authenticity of Single Seed Vegetable Oils (1996).
7. Krishnamurthy, M.N., Updated Methods for Detection of Adulterants and Contaminants in Edible Oils and Fats: A Critical Evaluation, *J. Food Sci. Technol. 30:*231–238 (1993).
8. Rossell, J.B., B. King, and M.J. Downes, Composition of Oil, *J. Am. Chem. Soc. 62:*221–230 (1985).
9. Grob, K., A.M. Giuffre, U. Leuzzi, and B. Mincione, Recognition of Adulteration Oils by Direct Analysis of the Minor Components, *Fat Sci. Technol. 96:*286–290 (1994).
10. Amr, A.S., and A.I. Abu-Al-Rub, Evaluation of the Bellier Test in the Adulteration of Olive Oil with Vegetable Oils, *J. Sci. Food Agric. 61:*435–437 (1993).
11. Salivaras, E., and A.R. McCurdy, Detection of Olive Oil Adulteration with Canola Oil from Triacylglycerol Analysis by Reversed-Phase High-Performance Liquid Chromatography, *J. Am. Chem. Soc. 69:*935–938 (1992).
12. Wesley, I.J., F. Pacheco, and A.E.J. McGill, Identification of Adulterants in Olive Oils, *Ibid. 73:*515–518 (1996).
13. Craig, H., Isotopic Standards for Carbon and Oxygen and Correction Factors for Mass-Spectrometric Analysis of Carbon Dioxide, *Geochim. Cosmochim. Acta 12:*113–149 (1957).
14. Mohler, F.L., Reference Samples of Isotopic Abundance, *Science 122:*334–335 (1955).
15. Hatch, M.D., and C.R. Slack, Photosynthetic CO_2 Fixation Pathways, *Ann. Rev. Plant Phys. 21:*141–162 (1970).
16. O'Leary, M.H., Carbon Isotopes in Photosynthesis, *BioScience 38*:328–336 (1988).
17. Woodbury, S.E., R.P. Evershed, J.B. Rossell, R.E. Griffith, and P. Farnell, Detection of Vegetable Oil Adulteration Using Gas Chromatography Combustion/Isotope Ratio Mass Spectrometry, *Anal. Chem. 67:*2685–2690 (1995).
18. Matthews, D.E., and J.M. Hayes, Isotope-Ratio-Monitoring Gas Chromatography–Mass Spectrometry, *Ibid. 50:*1465–1473 (1978).
19. Brand, W.A., High Precision Isotope Ratio Monitoring Techniques in Mass Spectrometry, *J. Mass Spec. 31:*225–235 (1996).
20. Meier-Augenstein, W., The Chromatographic Side of Isotope Ratio Mass Spectrometry: Pitfalls and Answers, *LC GC Intl. 10:*17–25 (1997).
21. Kelly, S., I. Parker, M. Sharman, J. Dennis, and I. Goodall, Assessing the Authenticity of Single Seed Vegetable Oils Using Fatty Acid Stable Carbon Isotope Ratios ($^{13}C/^{12}C$), *Food Chem. 59:*181–186 (1997).
22. Woodbury, S.E., R.P. Evershed, J.B. Rossell, R.E. Griffith, and P. Farnell, Detection of Vegetable Oil Adulteration Using Gas Chromatography Combustion–Isotope Ratio Mass Spectrometry, Abstract of a talk at *Food Authenticity '96*. ISBN 0708405762, 1996.
23. Rieley, G., Derivatization of Organic Compounds Prior to Gas Chromatographic-Combustion-Isotope Ratio Mass Spectrometric Analysis: Identification of Isotope Fractionation Processes, *Analyst 119:*915–919 (1994).
24. Jones, D.M., J.F. Carter, G. Eglinton, E.J. Jumeau, and C.S. Fenwick, Determination of Delta 13-C Values of Sedimentary Straight Chain and Cyclic Alcohols by Gas Chromatography/Isotope Ratio Mass Spectrometry, *Bio. Mass Spec. 20:*641–646 (1991).
25. ISO 659, Oil Seeds–Determination of Oil Content, British Standards Institution, London.
26. Aparicio, R., L. Ferreiro, and V. Alonso, Effect of Climate on the Chemical Composition of Virgin Olive Oil, *Anal. Chim. Acta 292:*235–241 (1994).

27. Bianchi, G., F. Angerosa, L. Camera, F. Reniero, and C. Anglani, Stable Carbon Isotope Ratios ($^{13}C/^{12}C$) of Olive Oil Components, *J. Agric. Food Chem. 41:*1936–1940 (1993).
28. Dutta, P.C., S. Helmersson, E. Kebedu, G. Alemaw, and L.A. Appelqvist, Variation in Lipid Composition of Niger Seed (*Guizotia abyssinica* Cass.) Samples Collected from Different Regions in Ethiopia, *J. Am. Chem. Soc. 71:*839–843 (1994).
29. Codex Alimentarius Alinorm 87/17, Report of the 13th Session of the Codex Committee on Fats and Oils, Codex Alimentarius Commission, Rome.
30. Smith, B.N., and S. Epstein, Two Categories of $^{13}C/^{12}C$ Ratios for Higher Plants, *Plant Physiol. 47:*380–384 (1971).
31. Farmer, J.G., and M.S. Baxter, Atmospheric Carbon Dioxide Levels as Indicated by the Stable Carbon Isotope Record in Wood, *Nature 247:*273–275 (1974).
32. Mook, W.G., M. Koopmans, A.F. Carter, and C.D. Keeling, Seasonal, Latitudinal, and Secular Variations in the Abundance and Isotopic Ratios of Atmospheric Carbon Dioxide. 1. Results from Land Stations, *J. Geophys. Res. 88:*10915–10933 (1983).
33. Keeling, R.F., S.C. Piper, and M. Heimann, Global and Hemispheric CO_2 Sinks Deduced from Changes in Atmospheric O_2 Concentration, *Nature 381:*218–221 (1996).
34. Friedli, H., H. Lotscher, H. Oeschger, U. Siegenthaler, and B. Stauffer, Ice Core Record of the $^{13}C/^{12}C$ Ratio of Atmospheric CO_2 in the Past Two Centuries, *Ibid. 324:*237–238 (1986).
35. Smedley, M.P., T.E. Dawson, J.P. Comstock, L.A. Donovan, D.E. Sherrill, C.S. Cook, and J.R. Ehleringer, Seasonal Carbon Isotope Discrimination in a Grassland Community, *Oecologia Plantarum 85:*314–320 (1991).
36. Meinzer, F.C., P.W. Rundel, G. Goldstein, and M.R. Sharifi, Carbon Isotope Composition in Relation to Leaf Gas–Exchange and Environmental-Conditions in Hawaiian Meterosides-Polymorpha Populations, *Ibid. 91:*305–311 (1992).

[Received June 17, 1997; accepted September 3, 1997]