ELSEVIER

Journal of Chromatography A, 805 (1998) 249–257

JOURNAL OF CHROMATOGRAPHY A

$\delta^{13}C$ analyses of vegetable oil fatty acid components, determined by gas chromatography–combustion–isotope ratio mass spectrometry, after saponification or regiospecific hydrolysis

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

[a]*School of Chemistry, Cantock's Close, University of Bristol, Bristol BS8 1TS, UK*

[b]*Leatherhead Food Research Association, Randalls Road, Leatherhead, Surrey KT22 7RY, UK*

Received 23 October 1997; received in revised form 29 December 1997; accepted 29 December 1997

Abstract

The $\delta^{13}C$ values of the major fatty acids of several different commercially important vegetable oils were measured by gas chromatography–combustion–isotope ratio mass spectrometry. The $\delta^{13}C$ values obtained were found to fall into two distinct groups, representing the C_3 and C_4 plants classes from which the oils were derived. The $\delta^{13}C$ values of the oils were measured by continuous flow elemental isotope ratio mass spectrometry and were found to be similar to their fatty acids, with slight differences between individual fatty acids. Investigations were then made into the influence on the $\delta^{13}C$ values of fatty acids of the position occupied on the glycerol backbone. Pancreatic lipase was employed to selectively hydrolyse fatty acids from the 1- and 3-positions with the progress of the reaction being followed by high-temperature gas chromatography in order to determine the optimum incubation time. The 2-monoacylglycerols were then isolated by thin-layer chromatography and fatty acid methyl esters prepared. The $\delta^{13}C$ values obtained indicate that fatty acids from any position on the glycerol backbone are isotopically identical. Thus, whilst quantification of fatty acid composition at the 2-position and measurement of $\delta^{13}C$ values of oils and their major fatty acids are useful criteria in edible oil purity assessment, measurement of $\delta^{13}C$ values of fatty acids from the 2-position does not assist with oil purity assignments.

Keywords: Combustion; Oils, vegetable; Food analysis; Isotope ratio mass spectrometry; Fatty acids; Triacylglycerols

1. Introduction

$\delta^{13}C$ values [1] are now widely used as indicators of authenticity for flavours [2,3], beverages [4,5], and foods [6–8]. The authenticity of vegetable oils is of continuing concern, and a recent report by the Ministry of Agriculture, Food and Fisheries (MAFF), UK, revealed that of 291 edible oils available from retailers, approximately 7% contained in excess of 5% (w/w) of another oil [9]. More worryingly, of the 79 maize oils analysed, approximately 14% contained more than 5% (w/w) adulterant oil. It is, therefore, imperative that new analytical techniques are developed to combat such fraudulent activities.

Numerous techniques are available to the analyst to assist with the authentication of edible oils, the most commonly used criterion being the fatty acid composition [10]. It is not, however, always possible to expose adulteration using this technique because

*Corresponding author.

0021-9673/98/$19.00
PII S0021-9673(97)01304-6

of the natural variability inherent in oil fatty acid compositions and because blends of oils, closely mimicking authentic oils in fatty acid composition, may be prepared [10]. A refinement of this fatty acid profiling approach involves determination of the fatty acid composition at the 2-position of the triacylglycerol (Fig. 1), since the fatty acid composition at this position is known to differ from the overall fatty acid composition. During the biosynthesis of triacylglycerols, acyl groups are transferred from acyl-coenzyme A to glycerol-3-phosphate by two distinct enzymes, specific for the 1- and 2-positions. The enzyme involved with the transfer of acyl groups to the 1-position (acyl-CoA: glycerol phosphate 1-O-acyl transferase) exhibits marked specificity for saturated acyl-CoA thiolesters whereas the second enzyme (acyl-CoA: 1-acyl glycerol phosphate 2-O-acyl transferase) shows specificity towards mono- and dienoic fatty acyl-CoA thiolesters. The final step transfers a fatty acid from acyl-CoA to the diacylglycerol, and the diacylglycerol transferase responsible for the catalysis of this step has a wide fatty acid specificity [11].

Fatty acid profiling at the 2-position relies on the regiospecific cleavage of fatty acids from the 1- and 3-positions of the glycerol backbone by pancreatic lipase, leaving 2-monoacylglycerols. The 2-monoacylglycerols are recovered by thin-layer chromatography (TLC) and fatty acid methyl esters prepared. This method is currently used in the authentication of edible oils, particularly olive oil [12], and is described in the International Standards Organization Method ISO 6800.

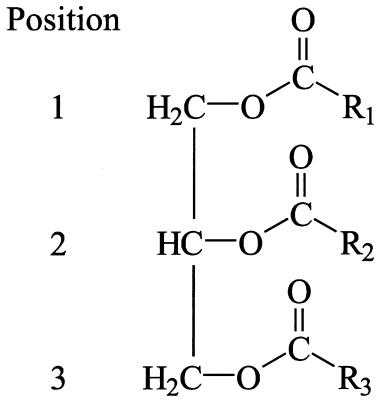

Fig. 1. General structure of triacylglycerols, indicating the 1-, 2- and 3-positions. For a C_{48} triacylglycerol (e.g., tripalmitin, in which $R_1=R_2=R_3=C_{15}H_{31}$) more than 94% of the carbon present resides in the fatty acids.

Maize oil is unique amongst commercially available edible oils in that it is derived from a C_4 plant, whilst all other commercially available edible oils are from C_3 plants. These plant classes differ in the mechanism by which carbon from the primary source, atmospheric carbon dioxide, is assimilated. Since the C_4 and C_3 pathways discriminate against CO_2 containing ^{13}C to different extents [7,13] different amounts of ^{13}C are found in chemically identical products of plant metabolism. The amount of ^{13}C present is expressed as a ratio to the amount of ^{12}C present, relative to an international standard, using the δ notation. This notation has units of per mil (‰). Thus:

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

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

The original international standard was a marine carbonate, Pee Dee Belemnite (PDB) [1] which had an accepted value of $R_{PDB} = 0.0112372 \pm 0.000009$. Supplies of PDB are now exhausted and the current international standard is a carbonate called NBS19. However, for ease of comparison with previous work in this field it was decided to present results versus the old standard, PDB. C_3 and C_4 plants have a lower atom % of ^{13}C than the PDB, hence, their $\delta^{13}C$ values are negative. Typically, C_3 plants have $\delta^{13}C$ values of approximately -28‰ whereas C_4 plants have $\delta^{13}C$ values of approximately -14‰ [13].

Whilst the $\delta^{13}C$ values of oils from C_3 and C_4 plants are firmly established and are in use as purity criteria for the authentication of maize oils [14], few studies have dealt with the $\delta^{13}C$ values of the individual components of oils [6,15–17]. The variabilities observed in $\delta^{13}C$ values of the major fatty acids because of plant type, geographic origin and year of harvest have been measured [17], however, this paper represents the first study in which the $\delta^{13}C$ values of fatty acids from different positions on the glycerol backbone are compared. The fatty acids were obtained by regiospecific hydrolysis of the triacylglycerols by pancreatic lipase. The duration of the incubation period was optimized by following the

extent of hydrolysis by high-temperature gas chromatography (HT-GC) of the incubate after periods of incubation ranging from 0 to 30 min. Following preparation of fatty acid methyl esters [6], fatty acid compositions of the oils were obtained and compared with the fatty acid compositions of the 2-positions. $\delta^{13}C$ values were then obtained by gas chromatography–combustion–isotope ratio mass spectrometry (GC–C–IR-MS) and compared with the $\delta^{13}C$ values of fatty acids from the 2-positions.

2. Experimental

2.1. Authentic vegetable oils

To ensure that only authentic, single seed oils were used in this study, oilseeds were obtained from seed growers and extracted at the laboratories of the Leatherhead Food Research Association. The extraction procedure used (ISO 659) was analogous to industrial oil extraction. Briefly, oilseeds were prepared by manual removal of the admixture. No cooking or pre-pressing was performed. After grinding, samples were extracted for 4 h with light petroleum ether (40–60°C boiling range, Fisons, UK). The partly defatted meal was then desolventised, reground and extracted for a further 2 h, the extracts passing 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.

2.2. Continuous flow elemental isotope ratio ($^{13}C/^{12}C$) mass spectrometry

To determine the $\delta^{13}C$ values of the oils, approximately 2 mg of each was weighed into tin foil capsules (EA Scientific, UK) by dripping from a clean needle. Capsules were then folded repeatedly to remove air and loaded into the carousel of the elemental analyser. Continuous flow elemental IR-MS was performed using a CE Instruments NC2500 elemental analyser fitted with an AS200 carousel, interfaced to a Finnigan MAT Delta S mass spectrometer. Oxygen for combustion was swept from a 10-ml loop into the furnace by helium carrier gas. Separation of gaseous CO_2 from N_2 was achieved using a packed column of 3 m length (Porapak Q) for N/C determination. Standardization of runs was achieved by direct injection of two portions of CO_2 of measured $\delta^{13}C$ value [$\delta^{13}C$ (CO_2) = −31.80‰] into the ionization source of the mass spectrometer. Performance was checked by comparison with $\delta^{13}C$ values of oils determined at other laboratories and using sucrose from the National Institute of Standards (RM 8542, sucrose anu, $\delta^{13}C$ = −10.47±0.13‰).

2.3. $\delta^{13}C$ values of fatty acids

Triacylglycerols were subjected to saponification and the liberated fatty acids were subsequently methylated. $\delta^{13}C$ values of the derived fatty acid methyl esters (FAMEs) were measured, and corrected for the contribution of the derivatizing carbon. Briefly, approximately 100 μl of oil was taken and put into a screw-capped test tube. To this was added 2 ml of methanolic sodium hydroxide (0.5 *M*) and the mixture heated (70°C, 15 min) with occasional shaking. Each sample was allowed to cool to room temperature (ca. 25°C) and acidified to pH 3 with 1 *M* hydrochloric acid. Fatty acids were extracted with three portions of hexane (2 ml each, Rathburn, UK) and combined. Two hundred and fifty μl of the fatty acid solution was taken and blown to dryness under nitrogen gas in a fresh tube and 100 μl of 14% boron trifluoride–methanol complex (Aldrich, UK) 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 FAMEs extracted into 2 ml diethyl ether (Rathburn). The diethyl ether was removed under nitrogen gas and the FAMEs dissolved in 2 ml hexane for analysis.

FAMEs were identified by GC–MS using a Carlo Erba Mega Series gas chromatograph interfaced to a Finnigan MAT 4500 mass spectrometer. Chromatographic conditions were as follows: a 25 m×0.32 mm I.D. BPX 70 (70% cyanopropyl equivalent) coated capillary column with 0.25 μm film thickness (SGE, Australia) was used with on-column injection. The temperature program was as follows: hold temperature at 40°C for 2 min then increase to 200°C at 4°C/min and hold at this temperature for 10 min. The carrier gas was helium (BOC, UK). The mass

spectrometer was operated in electron ionization mode and scans were performed across the mass range m/z 50 to 650 at a frequency of one scan per second. Data were collected using an INCOS data system and processed using the Interactive Chemical Information Software (ICIS) package.

The $\delta^{13}C$ values of FAMEs were measured using GC–C–IR-MS. A Finnigan MAT Delta S mass spectrometer was coupled to a Varian GC via a Pt/CuO combustion furnace maintained at 850°C. Removal of water after combustion was facilitated using Nafion tubing and standardization of runs was achieved with six portions of carbon dioxide gas of measured $\delta^{13}C$ value [$\delta^{13}C\ (CO_2) = -31.80$‰], injected directly into the ionization source of the mass spectrometer. The between-run precision of this instrument is specified by Finnigan MAT as ±0.2‰. Injection onto the GC column was made manually, or using a Finnigan MAT A200S autosampler, via a septum programmable injector. Temperature programme and capillary column details were exactly as for the GC–MS analysis of the FAMEs. Data were collected and processed using Finnigan MAT Isobase software.

2.4. Regiospecific lipase hydrolysis

Pancreatic lipase (EC 3.1.1.3) was used to selectively hydrolyse fatty acids from the 1- and 3-positions of the triacylglycerols, leaving intact 2-monoacylglycerols. The procedure used was based on ISO 6800 with minor modifications. Briefly, the free acidity of the oils to be studied was determined, according to ISO 660. Since the free acidities of the oils were all found to be below 3% (w/w) no neutralization was required. Oils were purified on small alumina columns by pouring solutions containing 0.2 g oil per 1 ml hexane (Rathburn) through activated alumina packed into Pasteur pipettes. The columns were washed with three portions of 1 ml hexane and the eluates were collected and combined. Solvent was removed under a stream of nitrogen and oils were hydrolysed as soon as possible after purification. Approximately 100 μl of oil was placed in a screw-capped test tube with 2 ml of buffer solution [tris(hydroxymethyl)methylamine, 1 *M*, adjusted to pH 8 with 6 *M* hydrochloric acid], 0.5 ml of sodium cholate (1 mg/ml) and 0.2 ml of calcium chloride solution (220 mg/ml). This was brought to 40±0.5°C and 20 mg of pancreatic lipase (EC 3.1.1.3, Type II, crude, from porcine pancreas, Sigma, UK) was added. After mixing vigorously for 5 s, the tube was shaken in the water bath for exactly 10 min (0 to 20 min when assessing the activity of the pancreatic lipase), then removed from the water bath and vortex mixed for exactly 2 min. One ml of hydrochloric acid (6 *M*) and 1 ml of diethyl ether were then added and the tube shaken vigorously. After centrifugation (1200 *g*, 5 min) the organic phase was transferred to a vial and the extraction repeated.

When assessing the activity of the lipase, a portion of the extract was taken, blown down under nitrogen gas and derivatized with bis(trimethylsilyl)trifluoroacetamide (BSTFA) (80°C, 1 h) and assessed by HT-GC as follows. The derivatized mixture of fatty acids, mono-, di- and triacylglycerols was blown down under nitrogen gas, dissolved in hexane and chromatographed using a Hewlett-Packard 5890 Series II gas chromatograph, fitted with an on-column injector and a DB1-HT (J&W Scientific) capillary column of 15 m×0.32 mm I.D. and 0.1 μm film thickness. The carrier gas was hydrogen, and the temperature program as follows: hold temperature at 50°C for 2 min, then increase at 10°C/min to 350°C and hold for 10 min. Fatty acids, mono-, di- and triacylglycerols were identified using the GC–MS instrument described above fitted with the DB1-HT capillary column and temperature program described here.

The 2-monoacylglycerols were isolated by TLC. Silica plates (LK6f, silica gel, 60 Å, 20×20 cm, 250 μm layer, Whatman, UK) were activated in a drying oven at 103±2°C for 1 h and allowed to cool in a desiccator before use. Using a TLC spreader, 0.5 ml of the combined extracts was applied in a line across the silica plate and the solvent allowed to evaporate. The plate was developed with hexane–diethyl ether–formic acid (70:30:1) until the solvent front reached a point 10 mm from the upper edge of the plate. The plate was then dried with a stream of nitrogen gas and visualised with iodine vapour. The band closest to the origin ($R_F = 0.1$) was identified as the monoacylglycerol band and scraped into a large vial. 2-Monoacylglycerols were then recovered by extracting the silica with diethyl ether. The diethyl

ether was removed with a stream of nitrogen gas and methyl esters were prepared by direct methylation of the 2-monoacylglycerols as described above.

2.5. Fatty acid compositions

The fatty acid compositions of the oils and the corresponding 2-position fatty acid compositions were obtained by GC of the fatty acid methyl esters. A Hewlett-Packard 5890 Series II gas chromatograph, fitted with an on-column injector and a BPX70 (SGE) capillary column of 25 m×0.32 mm I.D. and 0.25 μm film thickness was employed. The temperature program was as follows: hold temperature at 40°C for 2 min then increase to 200°C at 4°C/min and hold at this temperature for 10 min. The carrier gas was helium. Fatty acid compositions were expressed as percentage of the total fatty acids present, with no correction for detector response.

3. Results and discussion

The aim of this study was to assess whether differences exist between the $\delta^{13}C$ values of oils, their fatty acids and, specifically, fatty acids from the 2-position of the glycerol backbone. Since vegetable oils consist almost entirely of triacylglycerol molecules (Fig. 1), no appreciable difference was anticipated between the $\delta^{13}C$ values of the oils and their component fatty acids. Fatty acids located at the 2-position of the triacylglycerol backbone, however, are known to have particular metabolic significance [18,19] and the possibility existed that these fatty acids might display different $\delta^{13}C$ values from their counterparts located at the 1- and 3-positions.

3.1. Use of high-temperature gas chromatography to optimise hydrolysis conditions

Hydrolysis of fatty acids from the 2-position followed ISO 6800 with minor modifications as described above. Fig. 2 shows high-temperature gas chromatograms of the lipase digest, after derivatization, for digestions of 0, 2, 5, 10 and 20 min duration. The sequential hydrolysis of triacylglycerols is clearly indicated by the decrease of the triacylglycerol components and by the increase and

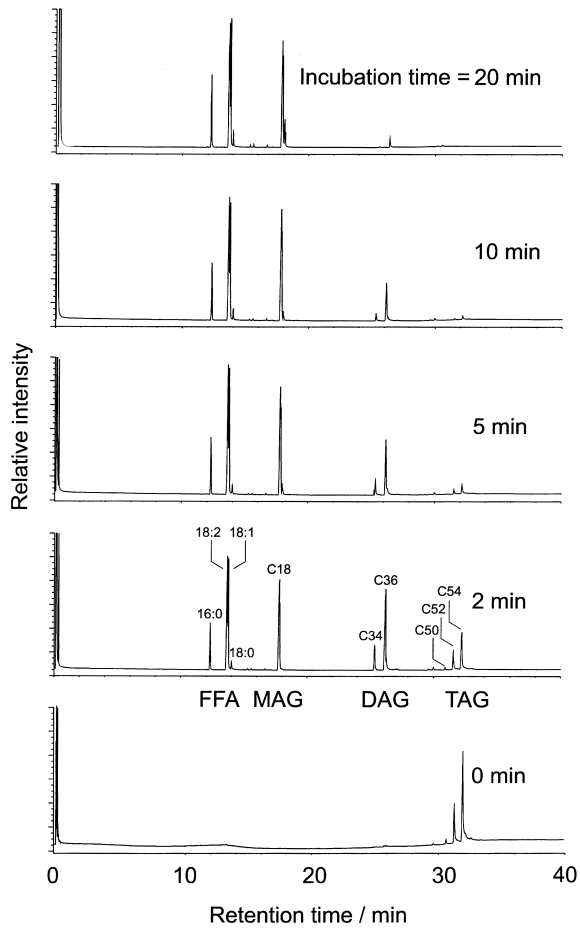

Fig. 2. High-temperature gas chromatograms indicating the course of hydrolysis of maize oil triacylglycerols (TAGs) by pancreatic lipase. Initial hydrolysis to 1,2- and 2,3-diacylglycerols (DAGs) and subsequent hydrolysis to 2-monoacylglycerols (MAGs) is indicated, with concomittant liberation of free fatty acids (FFAs). Major fatty acids (palmitic 16:0, stearic 18:0, oleic 18:1 and linoleic 18:2) are labelled, as are the monoacylglycerols of 18 carbon atoms (C_{18}), diacylglycerols of 34 and 36 carbon atoms (C_{34} and C_{36}) and triacylglycerols of 50, 52 and 54 carbon atoms (C_{50}, C_{52} and C_{54}). Acylglycerol lipids of differing unsaturation coelute on this column hence peaks may represent more than one mono-, di- or triacylglycerol component.

decrease of the abundance of the diacylglycerol components. The monoacylglycerol components have decreased in magnitude after 20 min of incubation and this may be attributed to migration of fatty acids from the 2-position, which is not hydrolysed, to the 1- and 3-positions, which are hydrolysed. Close inspection of Fig. 2 reveals a second monoacyl-

glycerol peak increasing in magnitude at prolonged incubation times. Mass spectral analysis indicates that this peak represents 1-monoacylglycerols of 18 carbon atoms, arising from transesterification during incubation. In Fig. 3, the relative amounts of each lipid class (free fatty acids, mono-, di- and triacylglycerols) are expressed as percentages of the total lipid present. The sequential hydrolysis of the triacylglycerols is, again, clearly indicated. An incubation time of 10 min gave optimal hydrolysis with minimal loss of 2-monoacylglycerols and this incubation time was subsequently adopted.

3.2. Fatty acid compositions of whole oils and at the 2-positions

Fatty acid compositions of the oils and the corresponding fatty acid compositions at the 2-position are summarised in Table 1. The fatty acid compositions reported here are typical for these oil types [10], the most abundant fatty acids in vegetable oils being palmitic ($C_{16:0}$), oleic ($C_{18:1}$) and linoleic ($C_{18:2}$) acids. Other fatty acids were quantified from the gas chromatograms but are omitted from Table 1 for clarity. The fatty acid compositions at the 2-position reflect the specificities of the enzymes involved in the biosynthesis of the triacylglycerols as discussed above. The abundance of saturated fatty acids at the 2-position is low, whilst the mono- and particularly the dienoic acids are present at elevated levels when compared with the parent oil [11].

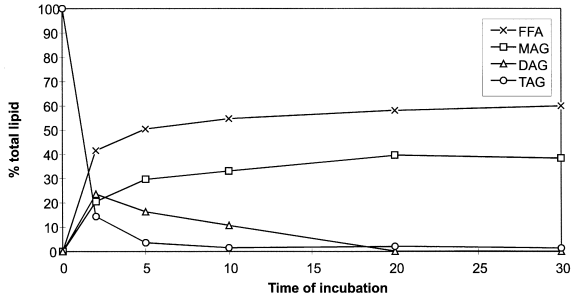

Fig. 3. Plot of the relative amounts of tri-, di- and monoacylglycerols (TAGs, DAGs and MAGs) and free fatty acids (FFAs) as enzymatic hydrolysis progresses. The slight decrease in monoacylglycerols and associated increase in free fatty acids at prolonged incubation times arises from migration of fatty acids from the 2-position to the 1- and 3-positions, where they are subsequently hydrolysed.

Table 1
The proportions of the major fatty acids (palmitic $C_{16:0}$, oleic $C_{18:1}$ and linoleic $C_{18:2}$), expressed as percentage of the total fatty acids as determined by GC of fatty acid methyl esters, are compared with the proportions of the major fatty acids at the 2-position of the triacylglycerols.

	Fatty acid composition (% of total fatty acids)					
	All positions			2-position		
Sample	$C_{16:0}$	$C_{18:1}$	$C_{18:2}$	$C_{16:0}$	$C_{18:1}$	$C_{18:2}$
Maize 12	13.7	36.1	44.4	6.3	36.5	54.9
Maize 39	14.6	29.0	50.4	1.3	28.0	69.7
Maize 48	14.6	35.8	41.9	2.7	41.8	53.3
Groundnut	9.9	48.5	28.7	3.6	35.0	60.1
Rapeseed	4.5	64.3	19.1	0.3	54.7	31.9
Soybean	10.4	20.3	54.8	0.5	19.0	72.7
Sunflower	6.0	25.8	62.9	0.3	25.0	74.7

3.3. $\delta^{13}C$ values of oils and individual fatty acids

Fatty acid $\delta^{13}C$ values were calculated from the $\delta^{13}C$ values of the fatty acid methyl esters as measured by GC–C–IR-MS. Correction for derivatizing carbon was made using the mass balance equation of Jones et al. [20]. Measurement of $\delta^{13}C$ values of oils and their major fatty acids [palmitic ($C_{16:0}$), oleic ($C_{18:1}$) and linoleic ($C_{18:2}$)] followed established procedures [6,7]. The $\delta^{13}C$ values of the oils, their major fatty acids and major fatty acids from the 2-position are summarised in Table 2. Each fatty acid $\delta^{13}C$ value represents the mean of at least three determinations, with typical standard deviations of circa 0.4‰. Each set of data from the GC–C–IR-MS instrument was manually inspected to ensure that peaks representing the elution of fatty acids from the GC column and combustion furnace were correctly assigned. Typical output from the GC–C–IR-MS instrument is presented in Fig. 4. The $\delta^{13}C$ values were seen to fall into two distinct groups, representing the maize (C_4) and other (C_3) oils. For each oil, $\delta^{13}C$ values of the oil, its component fatty acids and the fatty acids from the 2-position were similar although slight differences were observed between the $\delta^{13}C$ values of the different fatty acids. Palmitic acid ($C_{16:0}$) was generally observed to be depleted in ^{13}C relative to oleic ($C_{18:1}$) and linoleic ($C_{18:2}$) acid by approximately 1‰, a trend which has been observed previously [6]. In Table 3, the $\delta^{13}C$ values of the major fatty acids from all positions and from

Table 2
$\delta^{13}C$ values of oils, their major fatty acids and major fatty acids from the 2-position

	$\delta^{13}C$ values of oils and fatty acids (‰)						
		Fatty acids from all positions			Fatty acids from the 2-position		
Sample	Oil	$C_{16:0}$	$C_{18:1}$	$C_{18:2}$	$C_{16:0}$	$C_{18:1}$	$C_{18:2}$
Maize 12	−14.3	−14.6	−13.8	−13.9	−14.7	−13.5	−13.6
Maize 39	−14.8	−16.0	−16.1	−16.3	−15.6	−14.2	−14.9
Maize 48	−14.5	−16.8	−16.1	−16.6	−15.9	−14.4	−14.3
Groundnut	−26.8	−27.6	−27.2	−27.7	−29.5	−27.9	−28.7
Rapeseed	−28.1	−29.9	−29.0	−29.0	−27.9	−28.4	−28.7
Soybean	−30.8	−32.0	−31.6	−31.9	nd[a]	−30.2	−31.2
Sunflower	−28.6	−30.5	−29.5	−29.6	nd[a]	−29.2	−29.6

These $\delta^{13}C$ values are means from at least three determinations and have typical standard deviations of 0.4‰.
[a] nd = Not determined, since very minor peaks in the GC–C–IR-MS m/z 44 trace.

the 2-position were expressed relative to the $\delta^{13}C$ values of the oils to reveal differences between the $\delta^{13}C$ values of oils and their fatty acids. Whilst small differences (generally <2‰) were found between the oils and their component fatty acids, the only systematic variation observed was the depletion of palmitic acid, this depletion being independent of position occupied. Hence, there is no significant

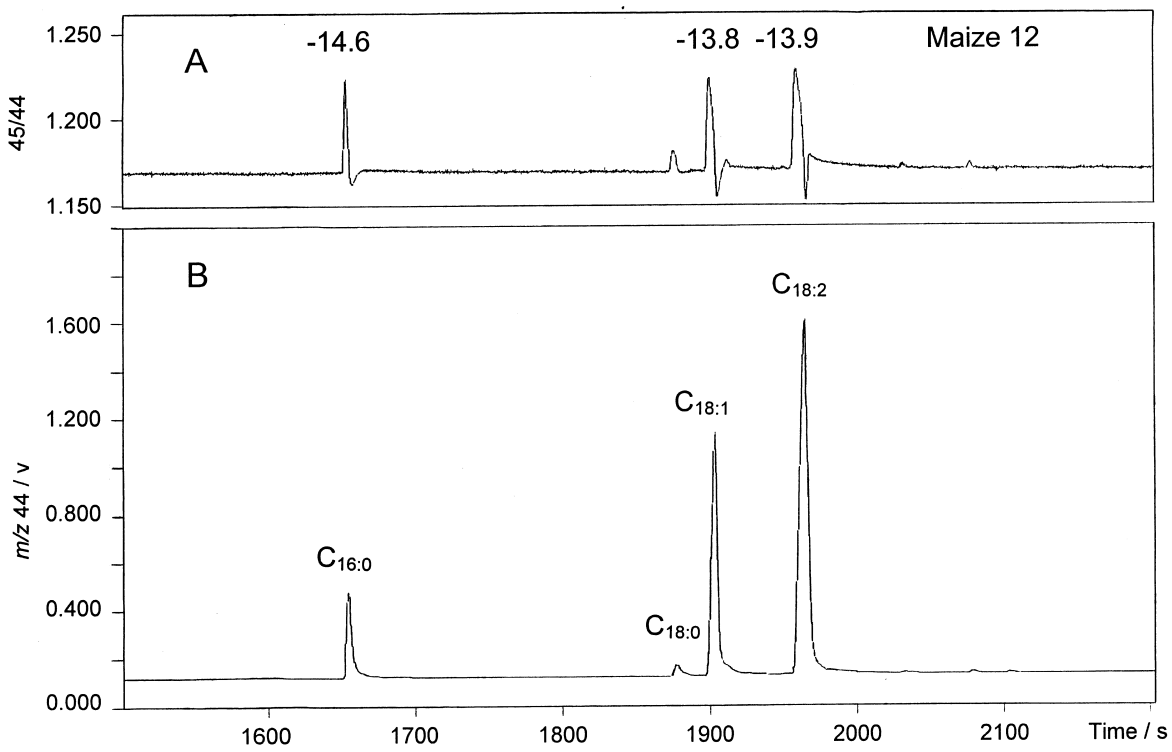

Fig. 4. Output from the GC–C–IR-MS instrument for fatty acid methyl esters from a pure maize oil showing (A) the instantaneous ratio of ions m/z 45 to m/z 44, with $\delta^{13}C$ values of the fatty acids, corrected for the contribution of the derivatizing carbon, and (B) the instantaneous intensity of the m/z 44 ion current, analogous to the output from the flame ionization detector of a GC system. Peaks were identified by comparison with GC traces of FAMEs of authentic oils and by GC–MS analysis.

Table 3
$\delta^{13}C$ values of the major fatty acids from all positions and from the 2-position are expressed relative to the $\delta^{13}C$ values of their parent oils

	$\delta^{13}C$ values of oils and fatty acids (‰)						
		All positions			2-position		
Sample	Oil	$C_{16:0}$	$C_{18:1}$	$C_{18:2}$	$C_{16:0}$	$C_{18:1}$	$C_{18:2}$
Maize 12	−14.3	−0.3	+0.5	+0.4	−0.4	+0.8	+0.7
Maize 39	−14.8	−1.2	−1.3	−1.5	−0.8	+0.6	−0.1
Maize 48	−14.5	−2.3	−1.6	−2.1	−1.4	+0.1	+0.2
Groundnut	−26.8	−0.8	−0.4	−0.9	−2.7	−1.1	−1.9
Rapeseed	−28.1	−1.8	−0.9	−0.9	+0.2	−0.3	−0.6
Soybean	−30.8	−1.2	−0.8	−1.1	nd[a]	+0.6	−0.4
Sunflower	−28.6	−1.9	−0.9	−1.0	nd[a]	−0.6	−1.0

[a] nd = Not determined.

difference between the $\delta^{13}C$ values of fatty acids in the 2-position, fatty acids from all positions and the oil itself.

4. Conclusions

This is the first study in which the $\delta^{13}C$ values of vegetable oils and their fatty acid components have been compared. The measurements made here indicate that the $\delta^{13}C$ values of oils and their fatty acids are the same as the $\delta^{13}C$ values of fatty acids in the 2-position, despite its metabolic significance and separate esterification system in triacylglycerol biosynthesis. These similarities arise because the kinetic isotope effect, which discriminates against ^{13}C, is greatest when the ^{13}C is contained in small molecules such as CO_2. Once primary fixation has occurred, further kinetic isotope effects are small and the isotope ratios of metabolic products remain almost constant [13]. This study was performed to assess whether differences could be detected between the $\delta^{13}C$ values of fatty acids in the 2-position and the $\delta^{13}C$ values of fatty acids from any position on the glycerol backbone. Such isotopic measurements could then be used as additional purity criteria to expose fraud in the vegetable oil trade. However, whilst quantification of fatty acid composition at the 2-position and measurement of $\delta^{13}C$ values of oils and their major fatty acids are useful criteria in edible oil purity assessment, measurement of $\delta^{13}C$ values of fatty acids from the 2-position does not assist with oil purity assignments. The application of HT-GC in this study clearly shows the value of this technique in following the progress of specific enzyme reactions [21]. A future application of this technique might be in animal studies, where essential fatty acids reflect the $\delta^{13}C$ values of dietary lipids whilst other fatty acids may be more indicative of the animal's metabolic condition and fatty acid turnover rate [11].

Acknowledgements

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 GC–C–IR-MS. F.O.S.F.A. International are thanked for financial support, as are Unilever Research, Bedford, UK, and Nestec Ltd., Lausanne, Switzerland. Thanks are extended to the suppliers of the oilseeds used in this study.

References

[1] H. Craig, Geochim. Cosmochim. Acta 12 (1957) 113–149.
[2] G. Lamprecht, F. Pichlmayer, E.R. Schmid, J. Agric. Food Chem. 42 (1994) 1722–1727.
[3] D. Juchelka, A. Mosandl, Pharmazie 51 (1996) 417–422.
[4] J. Bricout, J. Koziet, J. Agric. Food Chem. 35 (1987) 758–760.
[5] O. Bréas, F. Reniero, G. Serrini, Rapid Commun. Mass Spectrom. 8 (1994) 967–970.
[6] S.E. Woodbury, R.P. Evershed, R.E. Griffith, J.B. Rossell, P. Farnell, Anal. Chem. 67 (1995) 2685–2690.

[7] J.B. Rossell, Fat Sci. Technol. 96 (1994) 304–308.
[8] T. Yasui, J. Jap. Soc. Food Sci. Technol. 38 (1991) 864–870.
[9] MAFF Working Party on Food Authenticity, Authenticity of Single Seed Vegetable Oils, MAFF, UK, 1996.
[10] J.B. Rossell, Fat Sci. Technol. 93 (1991) 526–531.
[11] M.I. Gurr and J.L. Harwood, in Lipid Biochemistry, Chapman and Hall, London, 4th ed., 1991, Ch. 4.
[12] European Commission Regulation 2568, Off. J. Eur. Communities 34 (1991) L248/1.
[13] M.H. O'Leary, BioScience 38 (1988) 328–336.
[14] Report on the 15th Session of the Codex Committee on Fats and Oils, Paragraph 50.
[15] G. Bianchi, F. Angerosa, L. Camera, F. Reniero, C. Anglani, J. Agric. Food Chem. 41 (1993) 1936–1940.
[16] S. Kelly, I. Parker, M. Sharman, J. Dennis, I. Goodall, Food Chem. 59 (1997) 181–186.
[17] S.E. Woodbury, R.P. Evershed and J.B. Rossell, J. Am. Oil Chem. Soc., (1998) in press.
[18] J.C. Martin, P. Bougnoux, J.M. Antoine, M. Lanson, C. Couet, Lipids 28 (1993) 637–643.
[19] A. Zampelas, C.M. Williams, L.M. Morgan, J. Wright, T. Quinlan, Br. J. Nutr. 71 (1994) 401–410.
[20] D.M. Jones, J.F. Carter, G. Eglinton, E.J. Jumeau, C.S. Fenwick, Biol. Mass Spectrom. 20 (1991) 641–646.
[21] T.L. Bereuter, E. Lorbeer, J. Chromatogr. A 697 (1995) 469–474.