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Characterisation of aldehydic acids in used and unused frying oils

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Abstract

Aldehydic acids, expected to be present in auto-oxidised lipids as a result of decomposition of hydroperoxides by β-scission reactions, were analysed in unused and in frying oils used for two days. The oils studied were partially hydrogenated rapeseed/palm oil mixture, conventional sunflower oil and high oleic acid sunflower oil. The method involved saponification of lipids, elimination of the unsaponifiable matter, transmethylation and further fractionation of the methyl esters into polar and non-polar fractions by silica column chromatography. Both fractions were analysed by high-performance size-exclusion chromatography to determine the levels of non-polar dimers, polar dimers and oxidised monomers. The polar fraction was also analysed by gas chromatography and gas chromatography–mass spectrometry. Using this approach, the aldehydic acid methyl esters: methyl 8-oxooctanoate, methyl 9-oxononoate, methyl 10-oxo-8-decenoate, methyl 11-oxo-9-undecenoate and methyl 12-oxo-9-dodecenoate were characterised in used as well as unused frying oils.

Keywords: Oils, frying; Food analysis; Aldehydes; Fatty acids; Lipids

1. Introduction

Deep-fat frying is a popular way of cooking throughout the world and contributes markedly to our total caloric intake [1]. During the deep-fat frying process, the oil is continuously or intermittently subjected to high temperatures in the presence of air and food. A series of degradation reactions, including auto-oxidation, thermal oxidation, polymerisation, hydrolysis, cyclisation and fission, occur in the frying fat [1–3]. Lipid hydroperoxides, the primary products of fat oxidation during frying, readily degrade to a number of secondary oxidation products. Among them, two types of aldehydes are produced by β-scission [2,4] as shown in Fig. 1.

The volatile (short-chain) aldehydes have received much attention for their implications in rancid odour and flavour [5,6]. Aldehydes that remain bound to the parent lipid molecules (glycerol or cholesterol) through ester linkages are also produced along with the volatile aldehydes [7–9]. The term 'core aldehydes' was used as a generic name for these types of aldehydic esters [8]. The knowledge of the levels of the non-volatile aldehyde derivatives in the frying fat and fried products is of great importance because they are retained in the frying oil which is absorbed by the fried food and hence ingested by the consumer [10,11].

A number of studies have been carried out on the physiological effects of volatile aldehydes. 4-Hy-

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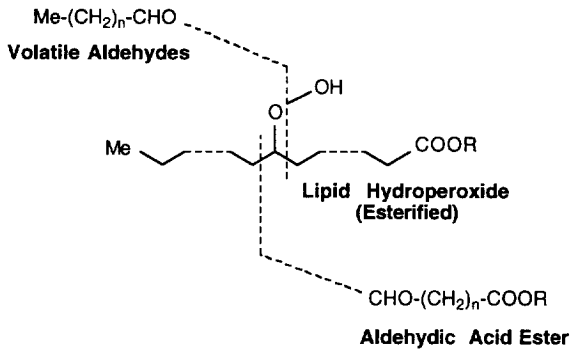

Fig. 1. Breakdown of hydroperoxide esters to volatile aldehydes and aldehydic acid esters by β-scission.

droxy nonenal, which has been extensively studied, was shown to be biologically active and to exhibit cytotoxic, hepatotoxic, mutagenic and genotoxic properties [12,13]. In general, aldehydes are also known to cross-link to proteins forming aldimines with free amino groups [4,14,15] and α,β-unsaturated aldehydes are particularly reactive towards sulfhydryl groups [16]. The group of esterified aldehydes, on the other hand, has rarely been investigated. In this context, it has been found that fractions containing low-molecular-mass compounds from auto-oxidised ^{14}C-labelled methyl linoleate or linoleic acid were easily absorbed and distributed in rat tissues [17–19]. Later, it was reported that 9-oxononanoic acid, the major aldehydic acid in oxidised lipids, could induce hepatic lipid peroxidation [20] and affect hepatic metabolism [21,22].

Regarding the analysis of aldehydic acids in oxidised lipids, Kuksis and his co-workers used a combination of TLC and HPLC–MS of dinitrophenyl hydrazone derivatives to provide qualitative data on the types of aldehydic acids present in intact lipid molecules [23,24]. However, those methods were highly complicated especially in the case of glycerol-bound aldehydic acids which may be present in hundreds of combinations with different fatty acids and/or their oxidation products. Therefore, by applying that methodology, it could be difficult to obtain quantitative data on the levels of these compounds in dietary lipids.

The objective of this paper was to design an analytical procedure to detect the presence of aldehydic acids in used frying oils with low alteration levels. This is an essential preliminary step to the further quantification of these compounds in dietary lipids expected to contain them in low concentrations. The methodology involved cleavage of the aldehydic acids from glycerol molecules by transmethylation, their concentration in a fraction devoid of unaltered fatty acids by silica column chromatography and then their identification by gas chromatography–mass spectrometry (GC–MS).

2. Experimental

2.1. Samples

The samples analysed in this study were three industrial frying oils: (i) a mixture of partially hydrogenated rapeseed/palm oil (RP), (ii) sunflower oil (SO) and (iii) high oleic sunflower oil (HOSO), analysed before and after 2 days of industrial frying of french fries. Details on the industrial frying process, including characteristics of the fryers and production scheme, were published elsewhere [25].

2.2. Chemicals and reagents

Silica gel 60 for column chromatography (particle size 0.063–0.100 mm) was from Merck (Darmstadt, Germany). The standard fatty acid methyl esters (FAMEs): methyl 8-eicosenoate (20:1Δ^8) and methyl oleate (18:1Δ^9), purchased from (Nu-Chek-Prep., Elysian, MN, USA), were used to prepare the two standard aldehydic acid methyl esters, methyl 8-oxo-octanoate and methyl 9-oxo-nonanoate, respectively using the osmium tetroxide–periodate method essentially as described by Kamido et al. [26]. Osmium tetroxide (OsO_4) and periodic acid (H_5IO_6) were purchased from Sigma (St. Louis, MO, USA). All other solvents and chemicals (of reagent grade or better quality) were obtained from local suppliers and were used without further purification.

2.3. Determination of fatty acid composition

Initial oils were methylated with 0.01 *M* NaOH in dry MeOH and then with 14% BF_3 in MeOH. FAMEs were analysed by gas–liquid chromatography (Varian 3400, Palo Alto, CA, USA) using a fused-silica capillary column (50 m×0.22 mm I.D.)

coated with BPX 70 (70% cyanopropylsiloxane, SGE, Austin, TX, USA). Temperature was set at 160°C for 15 min, then programmed to 220°C at a rate of 4°C min^{-1}. The fatty acid composition of these initial oils is given in Table 1.

2.4. Determination of total polar compounds

The percentages of the total polar compounds in unused and used frying oils were determined by the IUPAC standard method [27]. Briefly, starting from 1 g of oil, a non-polar fraction, containing unoxidised triglycerides, is eluted with hexane–diethyl ether (90:10) and then a second fraction, comprised of total polar compounds, is eluted with diethyl ether.

2.5. Separation of the polar and non-polar methyl esters by adsorption chromatography

For these analyses, 5-g oil samples were used. First, unsaponifiable matter was removed [28]. Briefly, samples were saponified using an ethanolic alkaline hydroxide solution and the unsaponifiable matter was extracted with diethyl ether. After acidification of the soap solution with 1 *M* HCl, fatty acids were extracted from the aqueous solution by repeated diethyl ether extractions. Following evaporation of diethyl ether, samples were dried under nitrogen to constant weight. Next, samples were methylated according to Metcalfe and Schmitz [29]. Methyl esters were then separated by silica column chromatography (30 g silica) into two fractions of different polarity, using 250 ml of *n*-hexane–diethyl ether (88:12, v/v) to elute first the non-polar fraction, followed by 150 ml of diethyl ether to obtain the polar fraction. Efficiency of the separation by adsorption chromatography was checked by TLC using hexane–diethyl ether–acetic acid (80:20:1, v/v/v) for development of plates and exposure to iodine vapour to reveal the spots. After evaporation of solvents, both fractions were weighed and then dissolved in diisopropyl ether until further analysis.

2.6. High-performance size-exclusion chromatography of the polar and non-polar fractions from the oils and lipid extracts

The polar and non-polar fractions obtained from the previous step were dissolved in diisopropyl ether to a concentration of ca. 15 mg ml^{-1} for analysis by high-performance size-exclusion chromatography (HPSEC). The system consisted of a Waters 510 pump with a 10-μl sample loop (Waters, Milford, MA, USA), an HP 1037 A refractive index detector and an HP 3392 integrator (Hewlett–Packard, Avondale, PA, USA). The separation was performed on two 100- and 500-Å Ultrastyragel columns (25 cm × 0.77 cm I.D.) packed with porous, highly cross-linked styrene–divinylbenzene copolymer (film thickness 10 μm, Waters) connected in series, with tetrahydrofuran (1 ml min^{-1}) as the mobile phase. Quantitation of each lipid class was based on the peak areas (assuming equal detector response) and on the gravimetric determination of the fractions previously obtained by adsorption chromatography. Details on calibration and reproducibility data can be found in an earlier publication [30].

Table 1
The fatty acid composition of the original oils used for frying

Sample[a]	Fatty acid composition (%, w/w)[b]									
	14:0	16:0	18:0	*t*18:1	*c*18:1	18:2	18:3	20:0	22:0	Others
RP 0	0.5	18.0	12.1	28.2	35.2	4.6	0.4	0.5	0.2	0.3
SO 0	0.1	6.7	4.8	n.d.	21.3	65.6	0.2	0.3	0.7	0.3
HOSO 0	0.1	4.3	3.4	n.d.	75.2	15.7	0.1	0.2	0.6	0.4

[a] The samples were industrial frying oils: a mixture of partially hydrogenated rapeseed/palm oil (RP), sunflower oil (SO) and high oleic sunflower oil (HOSO) analyzed before use.

[b] Fatty acids: 14:0 (myristic), 16:0 (palmitic), 18:0 (stearic), *t*18:1 (*trans*-oleic), *c*18:1 (*cis*-oleic), 18:2 (linoleic), 18:3 (linolenic), 20:0 (arachidic) and 24:0 (lignoceric). All samples also contained <0.1–0.2% of 16:1 (palmitoleic), 20:0 (eicosanoic) and 22:0 (behenic) given in a sum as others.

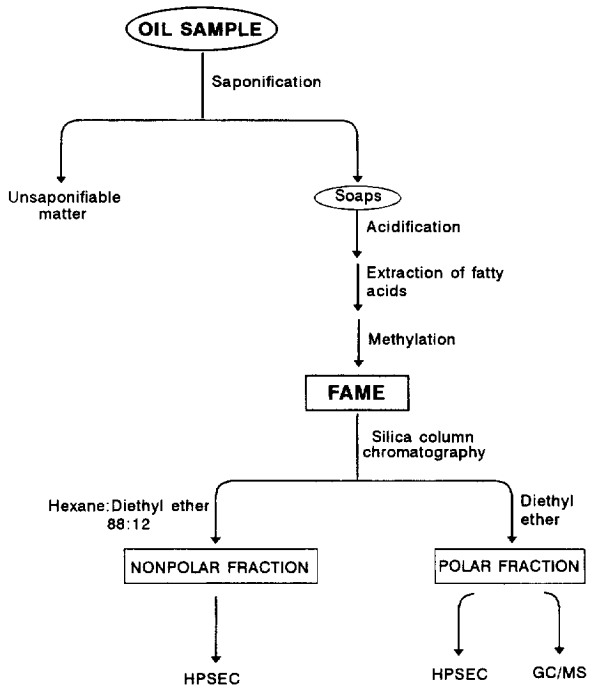

Fig. 2. Analytical scheme including steps followed for analyses of altered FAMEs (by HPSEC) and aldehydic acids (by GC–MS).

2.7. GC and GC–MS of the polar fractions

The polar fractions obtained from silica column chromatography were concentrated to 100 μl and injected into a medium-polarity DB-Wax capillary column: 30 m×0.25 mm I.D., film thickness 0.25 μm (J&W Scientific, Folsom, CA, USA) installed in an HP 5880 gas chromatograph operated under the following temperature programme: 90°C (2 min); 4°C min^{-1}; 240°C (40 min). The samples were introduced to the column via a split injector (split ratio 1:30) at a temperature of 275°C. The temperature of the flame ionisation detector was 275°C and the flow-rate of nitrogen, used as the carrier gas, was 2 ml min^{-1}.

GC–MS analyses were performed using the same injector temperature and a DB-Wax capillary column (30 m×0.25 mm I.D., film thickness 0.25 μm) in an HP 5890 gas chromatograph operated under the same temperature programme as above. The gas chromatograph was coupled to an AEI-VG 30/70 mass spectrometer (VG Analytical, Manchester, UK). Electron ionisation (EI) mass spectra (m/z 40–240) were obtained at a scan rate of 10 scans per min, a 70 eV ionisation energy and a 250°C interface temperature. The complete analytical scheme followed is presented in Fig. 2.

3. Results and discussion

3.1. Determination of total polar compounds

The percentages of total polar compounds in the oils as analysed by the IUPAC method as well as the distribution of altered fatty acid methyl esters in the oils as analysed by adsorption chromatography–HPSEC are shown in Table 2. Regarding total polar compounds, the method most widely used to evaluate frying oils, the increase was very low after two days of continuous frying in agreement with the short turnover period during the frying process [25].

Table 2
Total polar compounds (%, w/w) and altered fatty acids (mg/g oil) as analysed by adsorption chromatography–HPSEC

Sample[a]	Total polar compounds (%, w/w)	Distribution of altered fatty acids (mg g^{-1} oil)			
		Non-polar dimers	Polar dimers	Oxidized monomers	Total altered fatty acids
RP 0	4.1	2.8	1.0	7.0	10.8
RP 2	7.5	4.1	5.0	9.8	19.9
SO 0	3.1	4.7	2.3	10.6	16.6
SO 2	7.4	6.8	8.4	11.0	26.2
HOSO 0	3.3	3.1	0.7	9.1	12.9
HOSO 2	7.4	5.0	5.3	10.2	20.5

[a] The samples were industrial frying oils: a mixture of partially hydrogenated rapeseed/palm oil (RP), sunflower oil (SO) and high oleic sunflower oil (HOSO) analysed either as unused oils (O) or after 2 days (2) of frying french fries.

3.2. Separation of the polar and non-polar methyl esters by adsorption chromatography–HPSEC

Typical HPSEC chromatograms are shown in Fig. 3, for the non-polar (A) and polar (B) fractions obtained by adsorption chromatography. The chromatogram of the non-polar fraction is predominated by a major peak of unaltered fatty acids (15.12 min) and a small peak (13.55 min) of non-polar dimers linked through C–C bridges and without any extra oxygen in the fatty acid molecules. In the polar fraction, on the other hand, the major peak at 14.46 min corresponds to oxidised fatty acid monomers, a complex mixture of monomeric fatty acids with at least one oxygenated function, e.g., epoxy, keto or hydroxy. The second major peak at 13.57 min includes oxidised dimers obtained at high temperatures and mostly linked through C–O–C and C–C bridges [31–33]. In the latter structure, one of the two fatty acyls involved in the dimer formation possesses an extra oxygenated function. The third, small peak (15.24 min) includes short-chain oxidation products among which the aldehydic acids of interest are found, as judged by injecting the two standards methyl 8-oxooctanoate and methyl 9-oxononanoate.

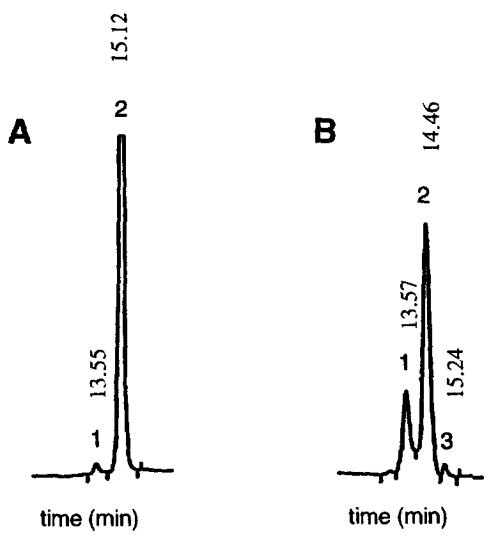

Fig. 3. Significant parts of the HPSEC chromatograms from used frying HOSO displaying changes in refractive index detector response with time for (A) the non-polar fractions and (B) the polar fractions of the samples analysed. Peaks: (A1) non-polar dimers, (A2) non-altered fatty acids, (B1) oxidised dimers, (B2) oxidised fatty acid monomers and (B3) short-chain oxidation products including aldehydic acids (For HPSEC conditions see Section 2.6).

Table 2 also shows the levels of non-polar dimers, polar dimers and oxidised monomers as analysed by adsorption chromatography–HPSEC. In agreement with the results from total polar compounds, the increase in total altered fatty acids (ca. 10 mg g^{-1}) was very low and was mainly due to the rise in content of dimeric compounds. As to the unused oils, the values obtained were normal for refined oils due to the presence of minor glyceridic compounds from crude oils which are not eliminated during refining, i.e. diglycerides or oxidized triglycerides, or to their formation during deodorization at high temperature (e.g. dimers). Given that the amounts of unsaponifiable compounds in the samples analysed were in the same magnitude as those of altered fatty acids, it became essential to eliminate the unsaponifiable matter to obtain reliable quantitative data. Quantitative data on the small peak in the polar fraction (15.24 min) including the short-chain aldehydic acids could not be obtained since the total peak areas corresponded to amounts <5 μg g^{-1}, levels significantly lower than those obtained for the rest of the classes.

3.3. GC separation of the polar fraction

A typical GC profile of the polar fractions analysed in this study is displayed in Fig. 4. Compounds eluting at retention times shorter than 35 min are of molecular mass lower than normal C_{18} fatty acids. Although formation of artifacts (e.g. hydroxymethoxy compounds originating from epoxy compounds) during transesterification cannot be excluded, no modification has been found in the case of aldehydic acid methyl esters as checked by comparing chromatographic profiles before and after simulating transmethylation of thermoxidised methyl linoleate (data not shown).

Methyl 9-oxononoate (23.39 min) was the major peak in all samples analysed. Interestingly, all samples also showed smaller peaks for methyl 8-oxooctanoate (20.57 min) and methyl 10-oxo-8-decenoate (30.07 min) as judged from co-chromatography of synthetic standards and/or from MS fragmentation patterns which will be discussed later. Frying oils

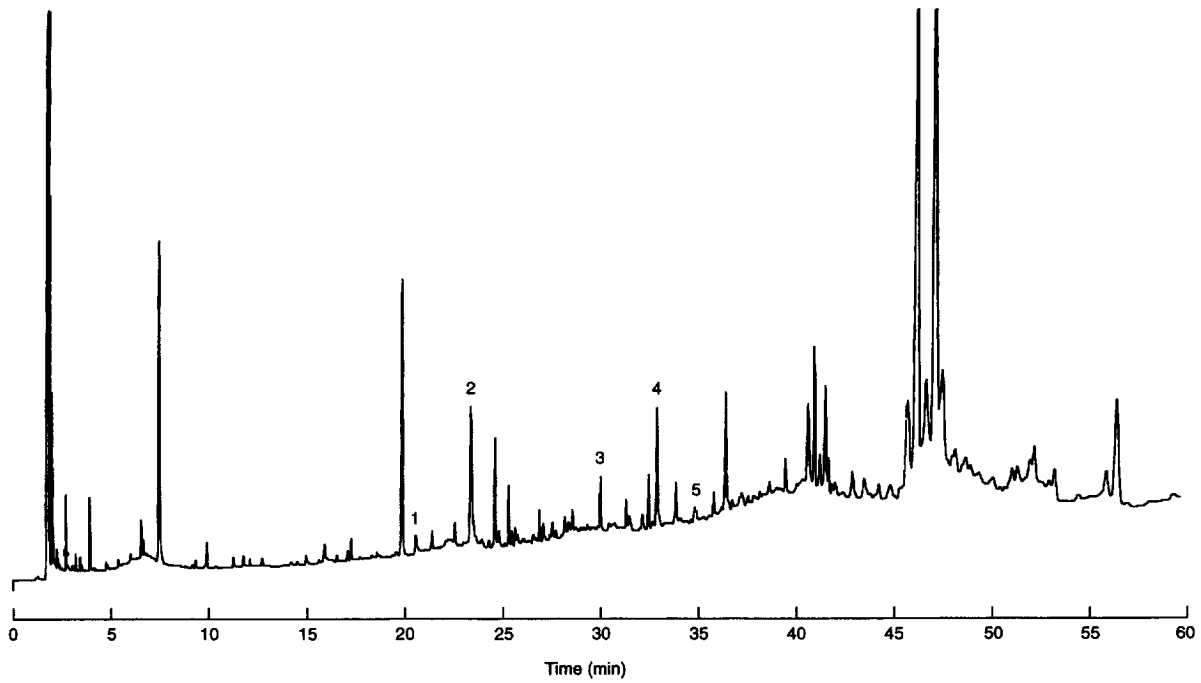

Fig. 4. Representative gas chromatogram of the polar fraction from used frying HOSO on the polar DB Wax column (90°C, 2 min, 4°C min^{-1}, 200°C). Peak assignments (1) methyl 8-oxooctanoate, 20.25 min; (2) methyl 9-oxononanoate, 23.05 min; (3) methyl 10-oxodecenoate, 29.73 min; (4) methyl 11-oxoundecenoate 32.23 min; and (5) methyl 12-oxododecenoate, 34.56 min. Peaks eluting between 40 and 60 min are due to oxidised C_{18} fatty acids.

also showed peaks for methyl 11-oxo-9-undecenoate (32.96 min) and methyl 12-oxo-9-dodecenoate (34.90 min). None of the samples analysed in this study have shown a peak for methyl 13-oxo-9,11-tridecadienoate but this peak has been detected in samples of high alteration levels [34]. Similarly, we could not characterise peaks for 10-oxodecanoate which was mentioned as a breakdown product from 11-hydroperoxy-9-octadecenoic acid [6,9].

3.4. Mass spectrometry of the aldehydic acids

Table 3 shows the main aldehydic acids expected

Table 3
List of the aldehydic acids expected as secondary oxidation products from the oxidation of oleic and linoleic acids

Fatty acid	Hydroperoxides[a]	Aldehydic acids[a]
Oleic acid	8-Hydoperoxy-9ζ-octadecenoic acid (8-HPOE)	8-Oxooctanoic acid (8:0)
(18:1 Δ^9 Acid)	9-Hydroperoxy-10ε-octadenenoic acid (9-HPOE)	9-Oxononanoic acid (9:0)
	10-Hydroperoxy-8ε-octadecenoic acid (10-HPOE)	10-Oxo-8ε-decenoic acid (10:1 Δ^8)
		9-Oxononanoic acid (9:0)
	11-Hydroperoxy-9ζ-octadenenoic acid (11-HPOE)	11-Oxo-9ζ-undecenoic acid (11:1 Δ^9)
Linoleic acid	9-Hydroperoxy-9ε,11ζ-octadecadienoic acid (9-HPOD)	9-Oxononanoic acid (9:0)
(18:2 $\Delta^{9,12}$ Acid)	13-Hydroperoxy-9ζ,11ε-octadecadienoic acid (13-HPOD)	13-Oxo-9ζ,11ε-tridecadienoic acid (13:2 $\Delta^{9,11}$)
		12-Oxo-9ζ-dodecenoic acid (12:1 Δ^9)

[a] ε = *trans* and ζ = *cis*.

as secondary oxidation products from the hydroperoxides of oleic and linoleic acids. These acids are: 8-oxononanoic acid (8:0), 9-oxononanoic acid (9:0), 10-oxodecanoic acid (10:0), 10-oxo-8ε-decenoic acid (10:1 Δ^8), 11-oxo-9ζ-undecenoic acid (11:1 Δ^9) and 13-oxo-9ζ,11ε-tridecadienoic acid (13:2 $\Delta^{9,11}$) (6,9). In addition, 10-oxodecanoic acid (10:0) and 12-oxo-9ζ-dodecenoic acid (12:1 Δ^9) can be formed

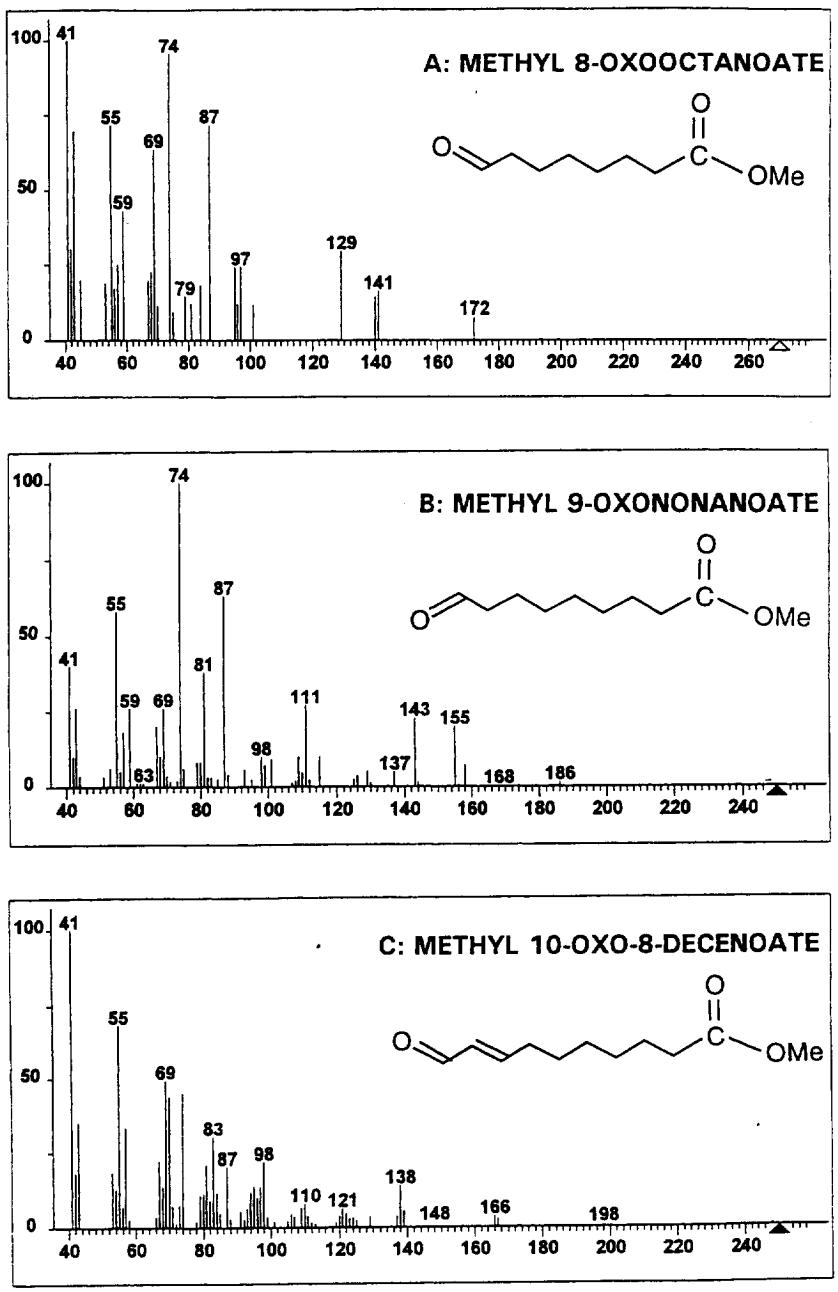

Fig. 5. Mass spectra of the methyl esters of the aldehydic acids identified.

through further reactions of vinyl radicals also produced from hydroperoxide scission [6,7,9].

Although mass spectra for methyl 9-oxo-nonanoate, methyl 10-oxo-8-decenoate and 12-oxo-dodecenoate were published before in papers dealing with different aspects related to lipid secondary oxidation products [35–37], no reference discussing mass spectra of aldehydic acids in frying oils is available. Knowledge about the mass spectra of these compounds is of great practical interest for the analysis of such complicated lipid oxidation fractions especially if techniques such as selective ion monitoring are to be used. For this purpose, the spectra of the aldehydic acid methyl esters analysed in this study are presented in Fig. 5 to provide a comprehensive mass spectral database. Fragments typical for methyl esters occur at *m/z* 87, 74 [CH_3O-$C(=OH^+)$-$CH_2^{\cdot}$] and 59 ($CH_3OC{=}O^+$) [38,39]. Although the other major peaks at *m/z* 41, 55 and 69 are generally characteristic of unsaturated compounds, they may also result from fragmentation at the carboxyl end (Fig. 6, reactions 1–3). In the spectra of methyl 10-oxo-8-decenoate and methyl 11-oxo-9-undecenoate, fragments resulting from the conjugated oxo/ene end are more prominent than those resulting from the methyl ester end. The addition of a double bond is known to increase the abundance of ions of the general formulae $C_nH^+_{2n-1}$ [*m/z* 69 ($C_5H_9^+$), 55 ($C_4H_7^+$) and 41 ($C_3H_5^+$)]. The ions at *m/z* 70 which are especially enhanced in these two compounds may have originated from the McLafferty rearrangement of a γ-hydrogen followed by β-cleavage (Fig. 6, reaction 4) and the ions at *m/z* 83 may result from reaction 5 (Fig. 6). The ion at *m/z* 70 in the spectrum of 12-oxo-9-dodecenoate may result by the same mechanism as above after

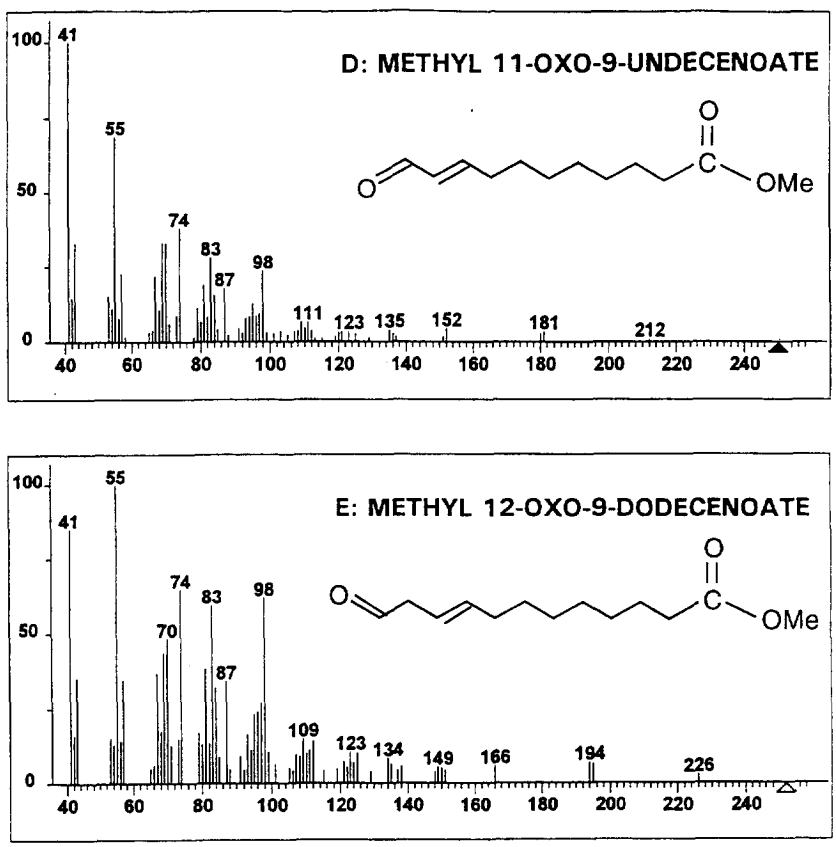

Fig. 5. (*continued*)

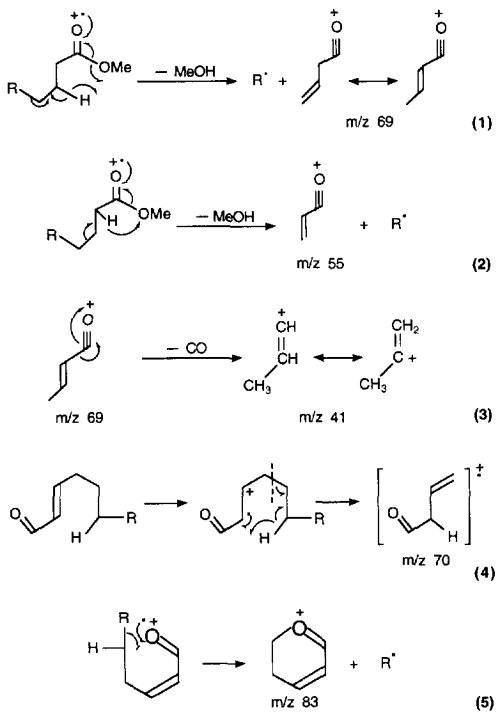

Fig. 6. Interpretation of some of the mass fragments shown in Fig. 5.

double bond migration which is a known feature for unsaturated compounds. The ion at m/z 83 can result from a similar reaction to that proposed above (Fig. 6, reaction 5) or by simple β-cleavage [38,39]. We were not able to explain the origin of the ion at m/z 98 observed in the spectra of methyl 9-oxononanoate, methyl 10-oxo-8-decenoate, methyl 11-oxo-9-undecenoate and methyl 12-oxo-9-dodecenoate.

4. Conclusions

Methyl 8-oxooctanoate and methyl 9-oxononoate were detected in all samples analysed in spite of the very low levels of polar fatty acids found. Efforts are now directed to quantitate them in samples with total polar compound levels close to 25%, the alteration limit for discarding used frying oils generally accepted in most European countries [40]. For analyses of these samples, elimination of unsaponifiable matter is not necessary and hence the analytical scheme (shown in Fig. 2) becomes simpler, i.e., starting directly from the obtention of fatty acid methyl esters.

Acknowledgments

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