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Generation of lipid peroxidation products in culinary oils and fats during episodes of thermal stressing: a high field ^{1}H NMR study

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Abstract The oxidative deterioration of glycerol-bound polyunsaturated fatty acids (PUFAs) in culinary oils and fats during episodes of heating associated with normal usage (30–90 min at 180°C) has been monitored by high field ^{1}H NMR spectroscopy. Thermal stressing of PUFA-rich culinary oils generated high levels of *n*-alkanals, *trans*-2-alkenals, alka-2,4-dienals and 4-hydroxy-*trans*-2-alkenals via decomposition of their conjugated hydroperoxydiene precursors, whereas only low concentrations of selected aldehydes were produced in oils with a low PUFA content, lard and dripping when subjected to the above heating episodes. Samples of repeatedly used, PUFA-rich culinary oils obtained from restaurants also contained high levels of each class of aldehyde. The dietary, physiological and toxicological ramifications of the results obtained are discussed.

Key words: Culinary oil and fat; Thermal stressing; Lipid peroxidation; Conjugated hydroperoxydiene; Aldehyde; ^{1}H NMR

1. Introduction

Many humans are continually exposed to oxidized oils and fats in the diet which arise from either shallow or deep fat frying processes, and the possibility that regular consumption of such materials may be deleterious to human health has attracted much interest [1]. The most important reaction involved in the oxidative deterioration of lipids therein is the thermally induced, radical-mediated autoxidation of polyunsaturated fatty acids (PUFAs), primarily generating conjugated hydroperoxydiene (CHPD) species. Such PUFA-derived CHPDs are particularly unstable at temperatures associated with standard frying practices (180°C), and are degraded to a wide variety of secondary peroxidation products, including a range of aldehydes (predominantly *n*-alkanals, *trans*-2-alkenals, alka-2,4-dienals, 4-hydroxy-*trans*-2-alkenals and malondialdehyde) which have the capacity to exert a range of toxicological effects in view of their high reactivity with critical biomolecules in vivo (e.g. endogenous thiols such as glutathione, free amino acids, proteins such as low-density lipoprotein, and DNA; reviewed in [2]).

In view of these observations, the detection and quantification of specific products arising from the oxidation of culinary oils and fats during standard frying practices is of paramount importance. Previous investigations of this nature have been hampered by a requirement for the concentrations of each component with putative toxicological properties to be individually monitored by labour-intensive, time-consuming laboratory methods [3], some of which lack specificity (e.g. spectrophotometric determination of thiobarbituric acid (TBA)-reactive substances [4]). However, the multicomponent analytical ability of high resolution nuclear magnetic resonance (NMR) spectroscopy now serves to overcome these problems. In this communication we report the application of high resolution ^{1}H NMR spectroscopy in determining the nature and levels of potentially toxic PUFA autoxidation products generated in culinary oils and fats when subjected to episodes of heating according to standard frying procedures.

2. Materials and methods

2.1. Thermal stressing of commercially available culinary oils and fats

Corn (maize) oil (containing 57% (w/w) polyunsaturates, 30% (w/w) monounsaturates and 13% (w/w) saturates), sunflower seed oil (64% (w/w) polyunsaturates, 22% (w/w) monounsaturates, 14% (w/w) saturates and an unspecified concentration of added α-tocopherol), soyabean oil (52% (w/w) polyunsaturates, 21% (w/w) monounsaturates and 13% (w/w) saturates), rapeseed oil (31% (w/w) polyunsaturates, 59% (w/w) monounsaturates and 6% (w/w) saturates), groundnut (peanut) oil (34% (w/w) polyunsaturates, 45% (w/w) monounsaturates and 21% (w/w) saturates), grapeseed oil (62% (w/w) polyunsaturates, 22% (w/w) monounsaturates and 11% (w/w) saturates) and olive oil (12% (w/w) polyunsaturates, 72% (w/w) monounsaturates and 12% (w/w) saturates) were commercially available samples purchased from local retail shops. Commonly utilised commercial brands of lard (containing 4–11% (w/w) polyunsaturates, an unspecified monounsaturate content and 45–57% (w/w) saturates), beef fat (i.e. dripping) and pure Indian butter ghee (containing 99.8% (w/w) butterfat and 0.01% ethyl butyrate) were similarly obtained. Coconut oil (containing 94% (w/w) saturates and an unspecified content of mono- and polyunsaturates) was obtained from Sigma Chemical Co. (Poole, Dorset, UK).

Samples of control (unheated) and repeatedly used culinary frying oils were kindly supplied by a fast-food/take-away establishment. Electronic integration of the *bis*-allylic-CH_2, ω-CH_3 and highly unsaturated fatty acid acyl chain terminal-CH_3 group proton resonances (δ = 2.76, 0.90 and 0.95 ppm, respectively) in 400 MHz ^{1}H NMR spectra of the above control (unheated) sample revealed that the polyunsaturate content of this material was 40 molar %, of which approximately one-quarter comprised highly unsaturated fatty acids, i.e. those with ≥3 unconjugated double bonds (predominantly linolenoylglycerol species).

Pentanal, hexanal, *trans*-2-heptenal, *trans*-2-octenal, *trans*-2-nonenal, *trans,trans*-nona-2,4-dienal and *trans,trans*-deca-2,4-dienal were purchased from Aldrich Chemical Co. (Gillingham, Dorset, UK). 2-Thiobarbituric acid and the ethyl ester of linoleic acid were obtained from Sigma, and deuterated NMR solvents (C^2HCl_3 and ^2H_2O) were purchased from Goss Scientific Ltd. (Great Baddow, Essex, UK).

Samples of each of the above culinary oils (20 g) and fats (10 g) were placed in glass vessels (details of which are given below) and heated at a temperature of 180°C on an electronically controlled hot-plate in the

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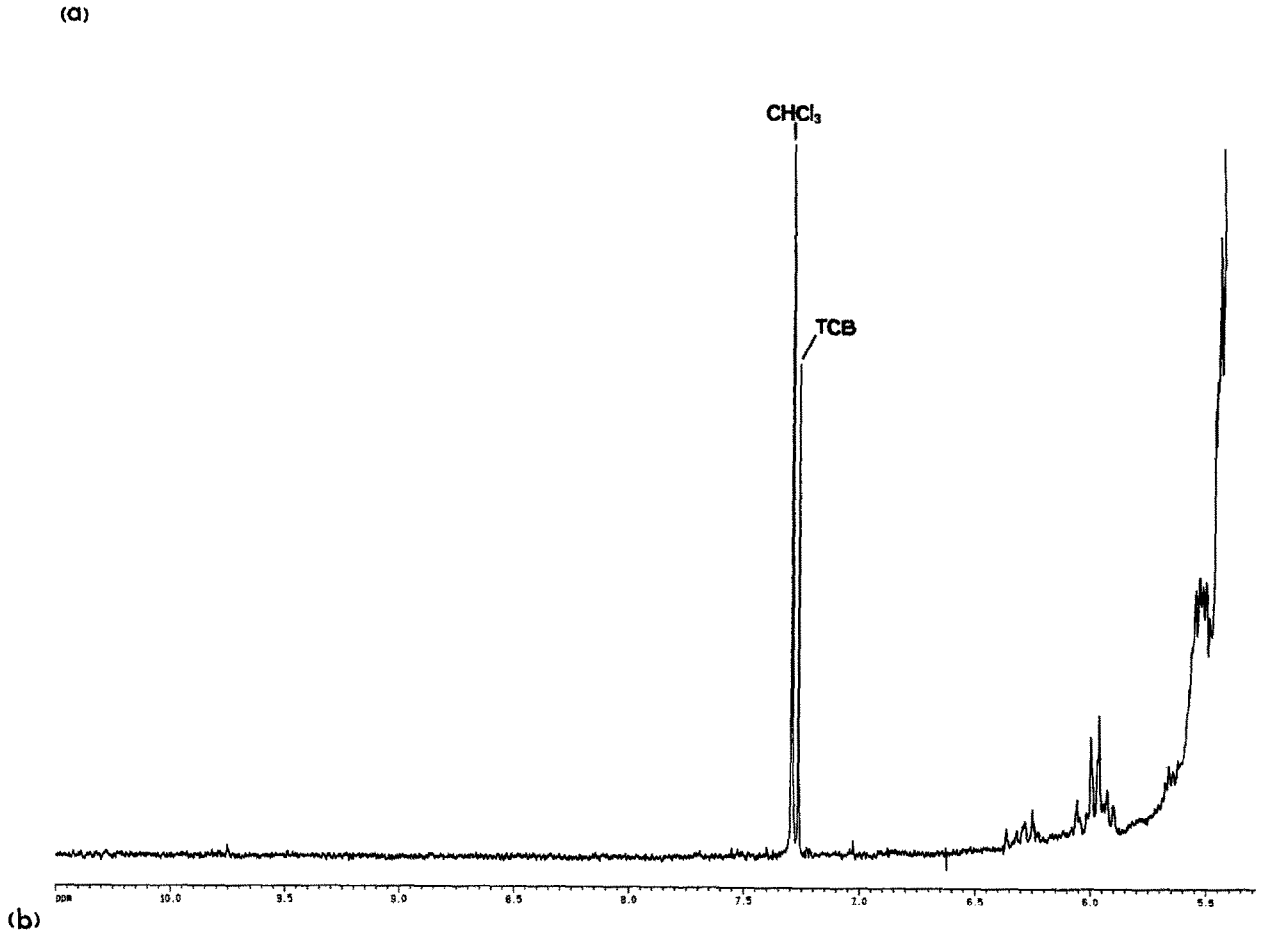
(a)
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Fig. 1a and 1b.

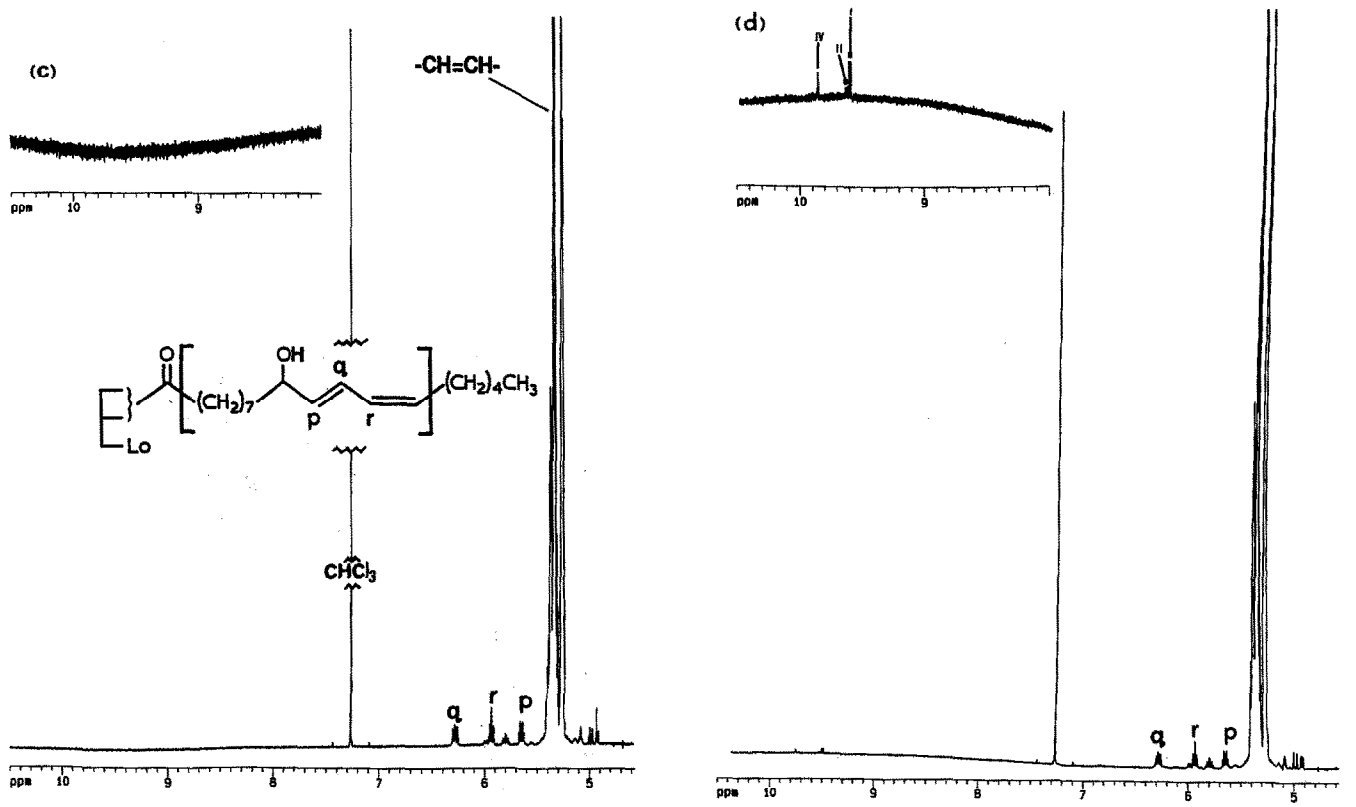


Fig. 1c and d.

presence of atmospheric O_2 for periods of up to 90 min. Aliquots (1.0 ml) of the oils and fats were removed at time-points of 30, 60 and 90 min, and then cooled to ambient temperature prior to storage, as described below, and ^{1}H NMR measurements. Selected culinary oils and fats were subjected to a further 30 min heating episode at an elevated temperature (250°C). The temperature of these samples was continuously maintained at 180 ± 3°C (or 250 ± 5°C, where appropriate) throughout the heating process using a calibrated thermometer.

Since the nature and size (capacity) of the glassware employed to heat the above materials was found to exert a major influence on the concentrations of thermally induced autoxidation products generated (a consequence of differing effective surface areas of the culinary oils or fats, i.e. the amount of these materials exposed to atmospheric O_2 during periods of thermal stressing), the same size and type of vessel was utilised to heat the same class of culinary oil or fat for the purpose of comparative, quantitative ^{1}H NMR evaluations. The glass vessels employed for each series of heating episodes consisted of 50 ml volume beakers (ghee, sample surface area 12.20 cm^2), 100 ml volume beakers (rapeseed oil, sunflower seed oil, grapeseed oil, olive oil, lard and dripping, sample surface area 18.41 cm^2), 100 ml volume conical flasks (corn and groundnut oils, sample surface area 24.64 cm^2), 13.5 mm diameter sample tubes (soyabean oil, sample surface area 1.43 cm^2), and 10 mm diameter sample tubes (ethyl linoleate, sample surface area 0.79 cm^2).

Samples were stored in 2 ml capacity glass sample tubes in the dark at ambient temperature for durations of 48–2,112 h. As expected, the storage time under these conditions was also found to influence the concentrations of ^{1}H NMR-detectable, PUFA-derived peroxidation products in thermally stressed culinary oils and fats, and hence each series of samples examined were stored for exactly the same time periods prior to ^{1}H NMR analysis. For each group of samples, the period of storage time was recorded and is specified in the figure legends (see section 3).

Samples of the model PUFA compound ethyl linoleate (2.0 g) were heated and stored in a similar manner prior to ^{1}H NMR analysis.

2.2. Proton NMR measurements

Proton (^{1}H) NMR measurements on the above samples were conducted on Bruker AMX-600 (University of London Intercollegiate Research Services (ULIRS), Queen Mary and Westfield College Facility, University of London, UK) or Bruker AMX-400 (ULIRS, King's College Facility, University of London, UK) spectrometers operating at frequencies of 600.13 and 400.13 MHz, respectively, and a probe temperature of 298 K. Typically, a 0.30 ml aliquot of each culinary oil or di-unsaturated fatty acid alkyl ester was diluted to a volume of 0.90 ml with deuterated chloroform (C^2HCl_3) which provided a field frequency lock, and these samples were then placed in 5-mm diameter NMR tubes. For culinary fats (lard, dripping and ghee), accurately weighed samples (ca. 50 mg) were directly dissolved in 0.60 ml of C^2HCl_3, the solutions thoroughly rotamixed and then transferred to 5-mm diameter NMR tubes. For experiments which involved the determination of NMR-detectable autoxidation products, samples were treated with 1,3,5-trichlorobenzene (final concentration 1.10×10^{-3} mol·dm^{-3}) which served as an internal quantitative NMR standard (δ = 7.227 ppm in C^2HCl_3 solution).

Further 0.30 ml aliquots of control and heated samples of ethyl linoleate were also diluted to a final volume of 0.90 ml with C^2HCl_3 and then treated with 0.05 or 0.03 ml vols. of ^2H_2O. The mixtures were thoroughly rotamixed, equilibrated at ambient temperature for a period of 45 min, centrifuged and the predominant, lower C^2HCl_3 phase removed for ^{1}H NMR analysis as described above.

Typical pulsing conditions were: 128 or 256 free induction decays (FIDS) using 32,768 or 65,536 data points, 72° pulses, a relaxation delay of 2.00 s and an acquisition time of 1.28 s. The spectral width was 7.246 Hz. Two-dimensional shift-correlated ^{1}H NMR (COSY) spectra were acquired on both the 400 and 600 MHz facilities (thermally stressed grapeseed oil and ethyl linoleate samples, respectively) using the standard sequence of Aue et al. [5], with 2,048 data points in the t_2 dimension, 512 increments of t_1, a relaxation delay of 2.00 s, and 48 transients. Exponential line-broadening functions of 0.20 Hz were routinely employed for purposes of processing.

Chemical shifts were referenced to tetramethylsilane (δ = 0.00 ppm,

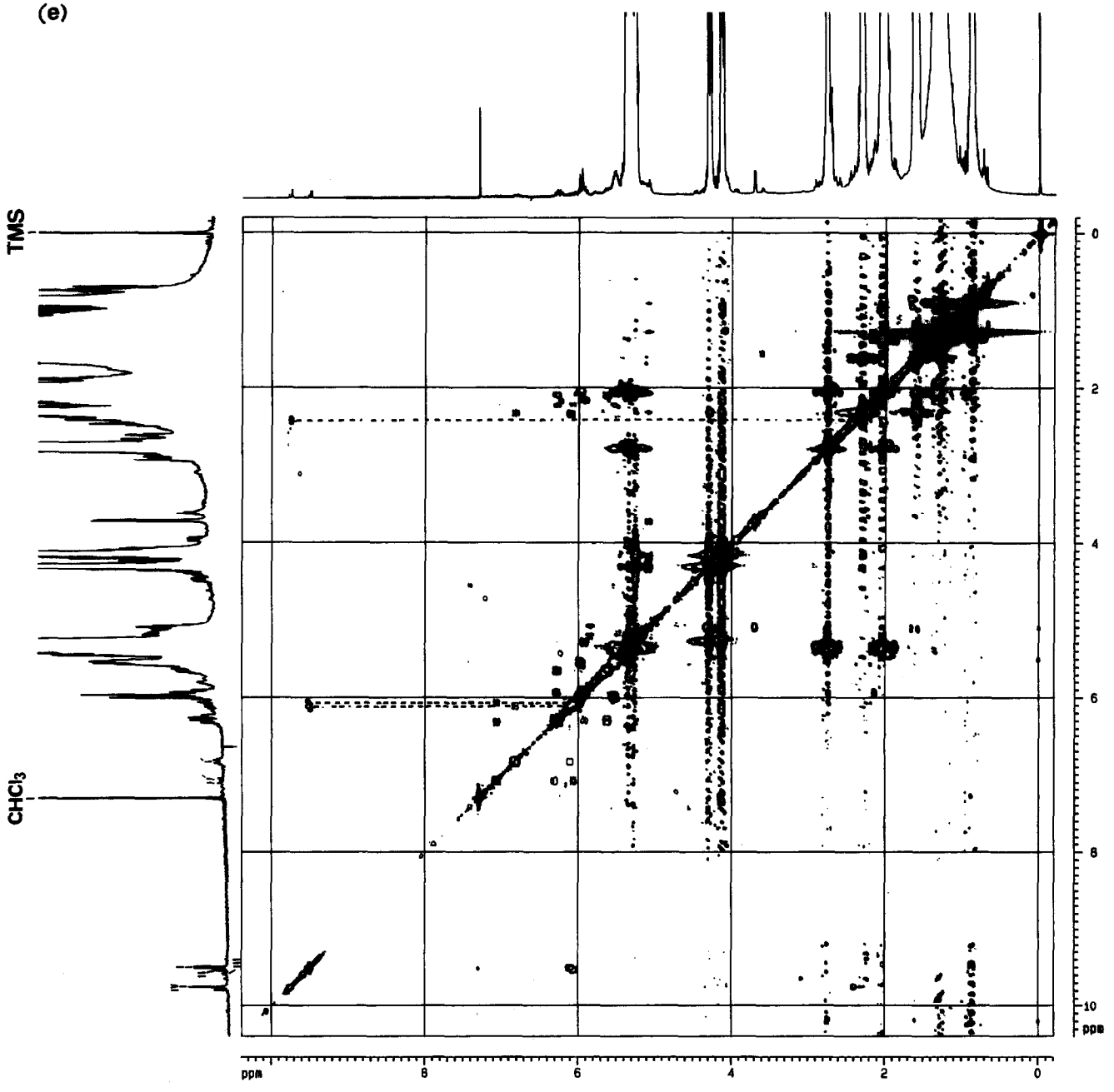


Fig. 1. Partial (5.30–10.50 ppm regions of) 400 MHz ^{1}H NMR spectra of a commercially available sample of corn (maize) oil acquired (a) before and (b) after heating at a temperature of 180°C in the presence of atmospheric O_2 for a period of 90 min in the manner described in section 2. A sample removed at the 90 min time-point was stored in the dark at ambient temperature (22°C) for 72 h prior to ^{1}H NMR analysis (the unheated control sample was stored in the same manner for an equivalent length of time). The partial, and expanded 8.00–10.50 ppm regions of 600 MHz ^{1}H NMR spectra of ghee obtained before and after heating at a temperature of 180°C for a 90 min period in the presence of atmospheric O_2 (section 2) are shown in (c) and (d), respectively. These samples were stored in the dark at ambient temperature for a period of 48 h prior to the acquisition of their ^{1}H NMR spectra. (e) 600 MHz two-dimensional COSY ^{1}H NMR spectrum of a commercially available sample of grapeseed oil subjected to a 90 min episode of heating at 180°C, followed by a further 30 min at an elevated temperature (250°C), in the presence of atmospheric O_2 (this material was stored in the dark at ambient temperature for 1,200 h prior to ^{1}H NMR analysis). This spectrum reveals clear connectivities between the aldehydic proton (-CHO) resonances and those located upfield, i.e. the 9.48 ppm doublet and vinylic proton multiplets centred at 6.85 (dt) and 6.10 ppm (dd) (*trans*-2-alkenals); the 9.52 ppm doublet and the vinylic proton doublet of doublets located at 7.07 and 6.04 ppm (alka-2,4-dienals); the 9.74 ppm triplet and a multiplet centred at 2.44 ppm (*n*-alkanals). Clear connectivities between multiplet resonances of the complex conjugated diene spectral pattern are also notable, together with data regarding the linkages of several of these signals to multiplets located further upfield. Typical spectra are shown. Abbreviations: I, II, III and IV, aldehydic group (-CHO) protons of *trans*-2-alkenals, alka-2,4-dienals (presumably *trans,trans*-isomers), 4-hydroxy-*trans*-2-alkenals and *n*-alkenals, respectively; I′, II′ and III′, vinylic proton resonances of *trans*-2-alkenals, alka-2,4-dienals and 4-hydroxy-*trans*-2-alkenals, respectively; a, b, c, d and e, vinylic proton resonances of the conjugated diene systems of 13- and/or 9-hydroperoxy-substituted octadecadienoylglycerol adducts (the *cis*-9,*trans*-11 and *trans*-10,*cis*-12 isomers, respectively) as denoted for the latter in (b); x and y,

internal) and/or residual chloroform (δ = 7.262 ppm). Resonances present in spectra of culinary oils and fats were assigned by a consideration of chemical shift values, coupling patterns and coupling constants. The molecular nature of particular classes of aldehydes detectable in spectra of thermally stressed culinary oils was confirmed by standard additions of authentic, commercially available compounds (*n*-alkanals, *trans*-2-alkenals and alka-2,4-dienals). The relative intensities of selected signals were determined by electronic integration, and the concentrations of aldehydes present were computed by comparing their resonance areas with that of the added 1,3,5-trichlorobenzene.

2.3. Selective estimation of the bi-functional aldehyde malondialdehyde (MDA) in culinary oil samples

A 0.75 ml aliquot of a 1.00 $g \cdot dm^{-3}$ solution of 2-thiobarbituric acid in double-distilled water was added to 2.00 ml vols. of control and thermally stressed culinary oil samples, the mixture thoroughly rotamixed, centrifuged (20 min, 500 × *g*), the lower (aqueous) phase removed and then heated at a temperature of 96°C for 20 min. A 0.30 ml aliquot of the resulting pink-coloured solution was then diluted to a final volume of 4.00 ml with double-distilled water prior to spectrophotometric analysis. Zero-order and corresponding second-derivative electronic absorption spectra of these samples were obtained on a Kontron Uvikon 860 spectrophotometer (scan rate 120 nm/min), and MDA concentrations were determined using an ε_{532} value of 1.56×10^5 $M^{-1} \cdot cm^{-1}$ for the 2:1 TBA:MDA adduct [6].

3. Results

Thermal stressing of PUFA-rich culinary oils in the above manner generated a series of new resonances in their ^{1}H NMR spectra characteristic of (i) CHPDs (conjugated diene olefinic proton multiplets in the 5.4–6.7 ppm chemical shift range, broad hydroperoxide -OOH group proton singlets located at 8.5–8.9 ppm, and a -CH(OOH)- proton multiplet centred at 4.35 ppm), i.e. *cis,trans*- and *trans,trans*-isomers of 9- and 13-hydroperoxy-octadecadienoyglycerol species [7–9]; (ii) α,β-unsaturated aldehydes (prominent doublets located at 9.48, 9.52 and 9.63 ppm (j = 8.2 Hz in each case) corresponding to *trans*-2-alkenals, alka-2,4-dienals and 4-hydroxy-*trans*-2-alkenals, respectively [10]); (iii) saturated aldehydes (triplet at 9.74 ppm (^3j = 1.7 Hz [10]); Fig. 1a and b). Two-dimensional COSY ^{1}H NMR spectra of heated culinary oil samples greatly facilitated distinction between the four classes of aldehyde detectable (Fig. 1e). The 9.48 ppm signal is presumably largely attributable to a mixture of *trans*-2-heptenal and *trans*-2-octenal, and that at 9.74 ppm to hexanal, since these adducts are the predominant *trans*-2-alkenals and *n*-alkanals, respectively, generated from the autoxidation of linoleate [11]. The total concentrations of both saturated and α,β-unsaturated aldehydes generated in thermally stressed culinary oils (1–20 and 2–30 × 10^{-3} $mol \cdot kg^{-1}$, respectively) increased approximately linearly with increasing time of heating (data not shown).

^{1}H NMR analysis also demonstrated the thermally induced consumption of glycerol-bound PUFAs present (i.e. selective reductions in the intensities of *mono*- and *bis*-allylic-CH_2- group resonances located at 2.06 and 2.76 ppm, respectively, and that of the olefinic protons at 5.38 ppm).

Subjection of commercially available samples of ghee to episodes of heating at 180°C in the above manner also generated a series of aldehydes (*n*-alkanals, *trans*-2-alkenals and alka-2,4-dienals; Fig. 1c and d). However, the 5.4–6.7 ppm (conjugated diene) regions of the 600 MHz ^{1}H NMR spectra of control (unheated) and thermally stressed samples contained reso-

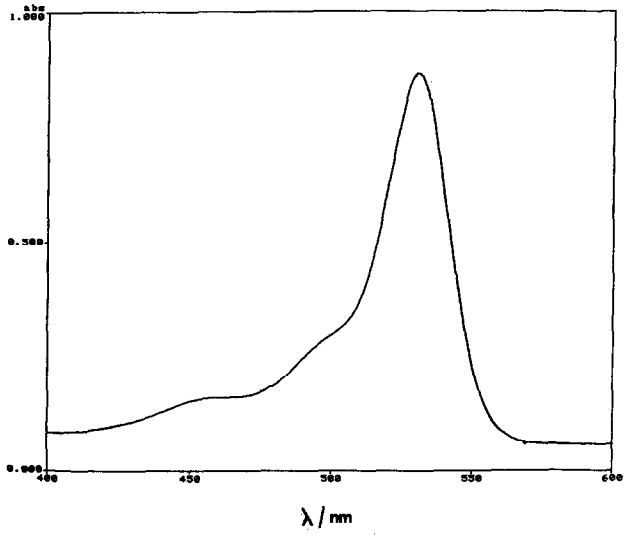


Fig. 2. Electronic absorption spectrum of the product arising from the reaction of malondialdehyde (MDA) present in an aqueous extract of a thermally stressed soyabean oil sample with 2-thiobarbituric acid (TBA) (section 2), i.e. the 2:1 TBA:MDA adduct. The culinary oil was heated at a temperature of 180°C in the presence of atmospheric O_2 for a 90 min period prior to analysis. A typical spectrum is shown.

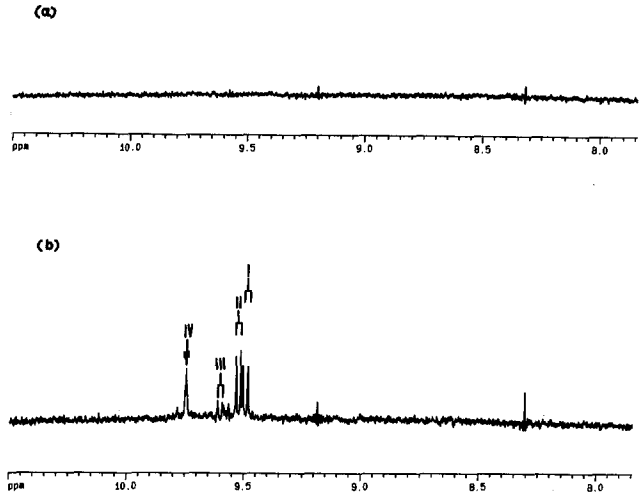
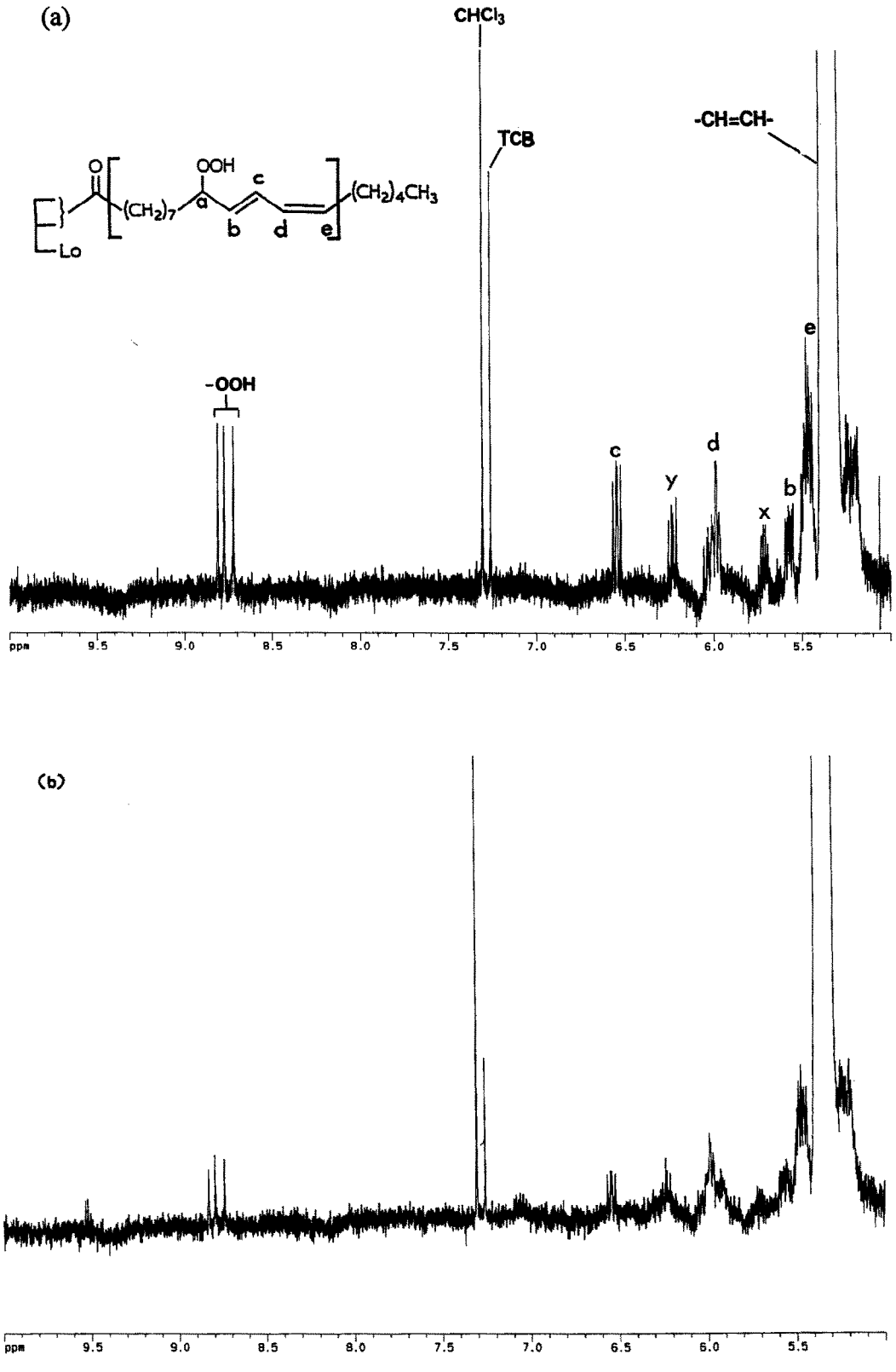


Fig. 3. Expanded aldehydic (8.00–10.50 ppm) regions of the 400 MHz ^{1}H NMR spectra of (a) control (unheated) and (b) repeatedly utilised samples of culinary frying oils obtained from a fast-food/take-away establishment. Typical spectra are shown. After collection, the above samples were stored in the dark at ambient temperature for a 12 h period prior to analysis. Abbreviations as in Fig. 1.

←

Figure 1 (*continued*)

resonances assignable to the vinylic protons of *trans,trans*-conjugated hydroperoxydiene species; HOO-, broad hydroperoxy group proton signals of conjugated hydroperoxydienes; p, q and r, vinylic proton resonances of the conjugated diene systems of 13- and/or 9-hydroxy-substituted octadecadienoylglycerol adducts (the *cis*-9,*trans*-11 and *trans*-10,*cis*-12-isomers, respectively) as specified for the latter in (c); TMS, tetramethylsilane; $CHCl_3$, residual chloroform; TCB, 1,3,5-trichlorobenzene.


(a)
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-CH=CH-
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Fig. 4a and 4b.

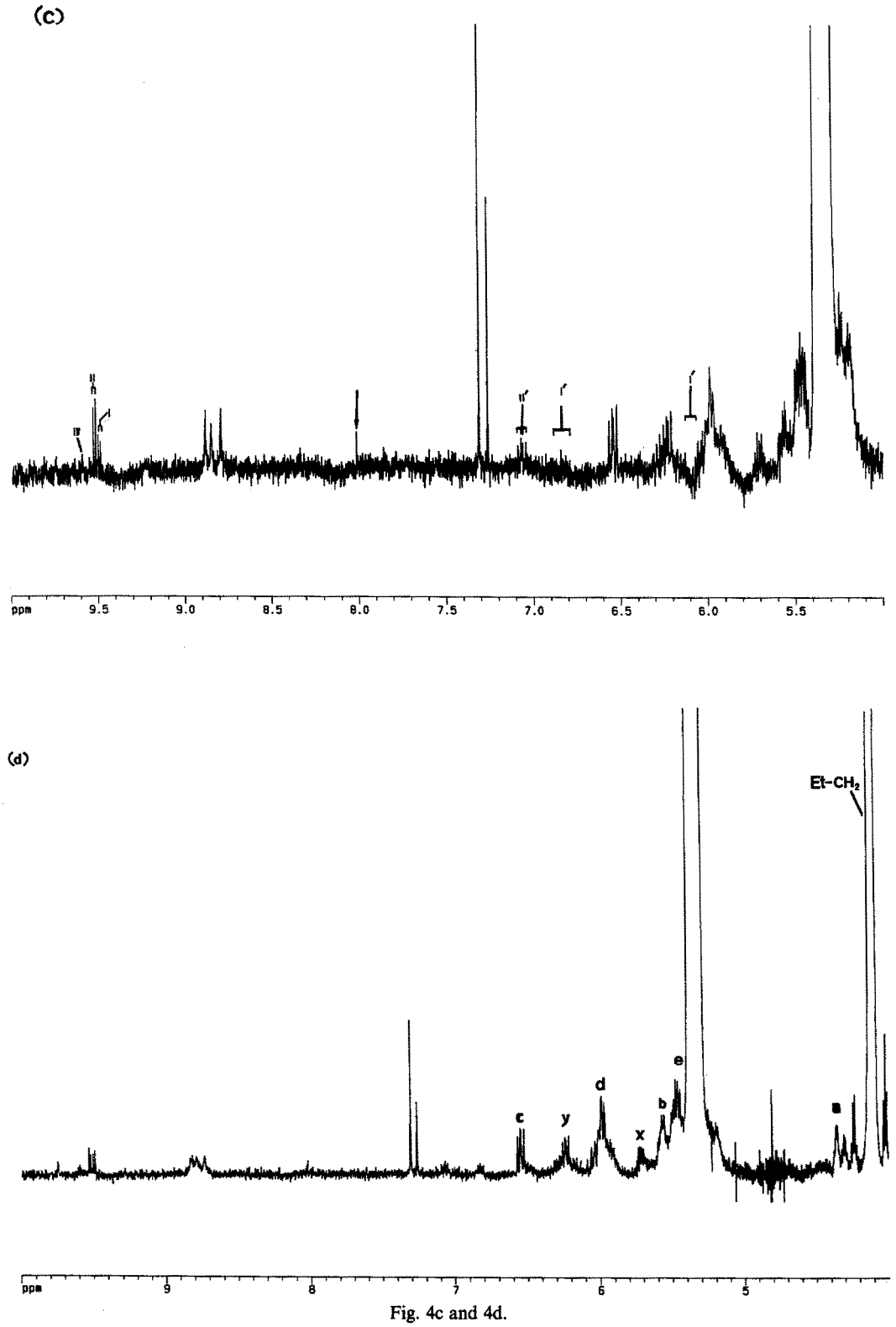

Fig. 4c and 4d.

(e)

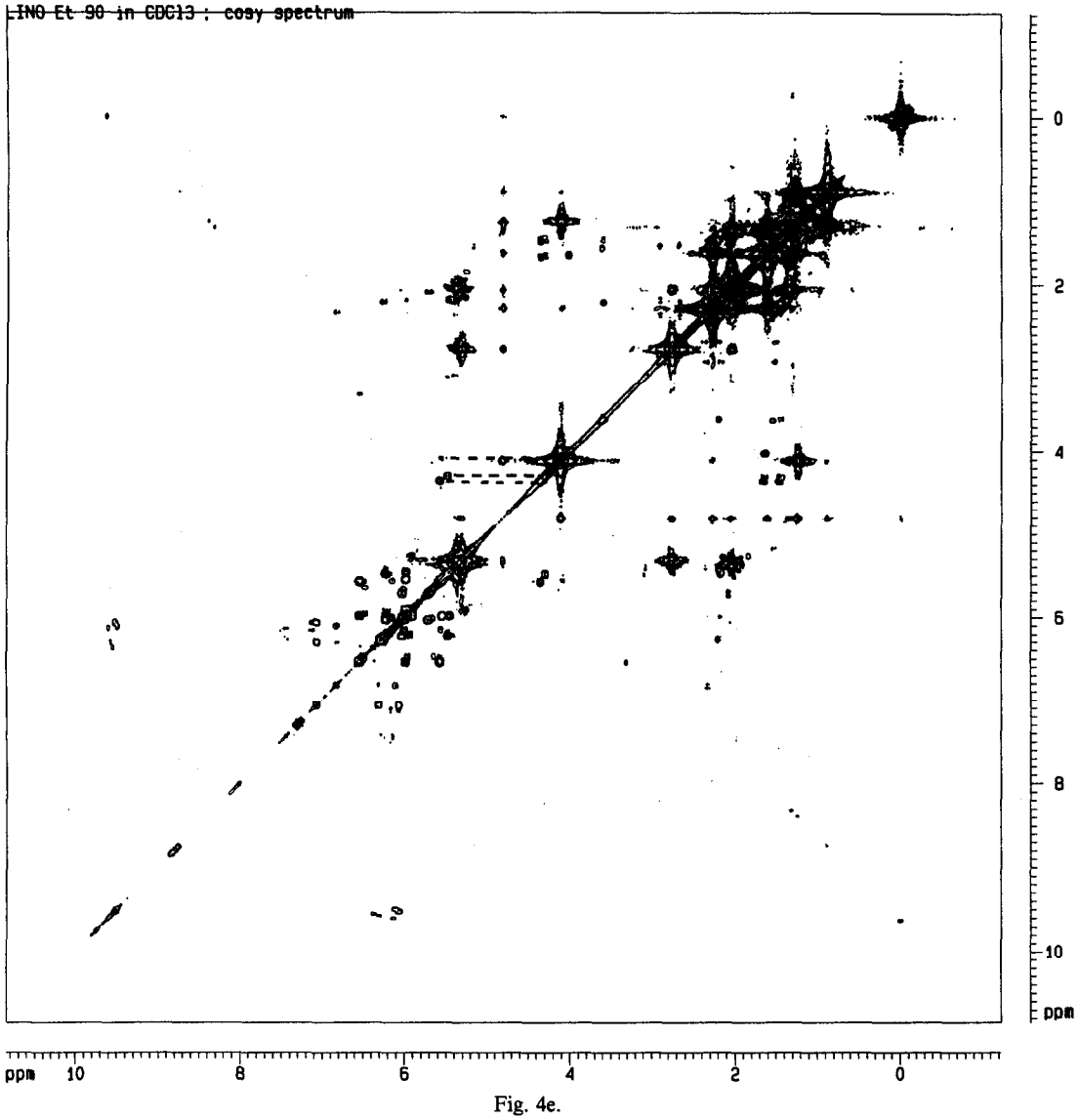

Fig. 4e.

nances assignable to conjugated hydroxydiene species [7,12] (i.e. multiplets centred at 6.28 (*dd*, j = 13.9, 10.1 Hz), 5.93 (*dd*, j = 11.0, 10.1 Hz) and 5.64 ppm, corresponding to the 11-, 10- and 12 position vinylic protons, respectively, of 13-hydroxy-*cis*-9,*trans*-11-octadecadienoyl glycerol) rather than CHPDs. Such conjugated hydroxydienes arise from the thermally induced fragmentation of their corresponding *cis,trans*-linoleate hydroperoxides (Eqs. 1 and 2).

$$\mathrm{LOOH} \rightarrow \mathrm{LO^{\bullet}} + {}^{\bullet}\mathrm{OH} \quad (1)$$

$$\mathrm{LO^{\bullet}} + \acute{\mathrm{L}}\mathrm{H} \rightarrow \mathrm{LOH} + \acute{\mathrm{L}}^{\bullet} \quad (2)$$

Visible absorption spectra of the product arising from the reactions of aqueous extracts of thermally stressed culinary oils with TBA (Fig. 2) were very similar to that of the adduct generated from the heating of an authentic, pure sample of MDA with this reagent at 95°C [13]. Indeed, the selectivity of this method was confirmed by second-derivative spectrophotometric analysis (i.e. no interfering absorption bands attributable to the chromophoric TBA adducts of alternative aldehydic species were detectable). Reaction of an aqueous extract derived from a typical sample of soyabean oil preheated at 180°C for a period of 90 min had an absorbance value at a wavelength of 532 nm, corresponding to an MDA concentration of 1.14×10^{-5} mol·kg^{-1}, assuming a 100% recovery of this bi-functional aldehyde into the lower, aqueous phase.

^{1}H NMR analysis revealed high levels of potentially toxic aldehydic fragments, together with a corresponding marked consumption of PUFAs in samples of repeatedly used frying oils obtained from fast-food/take-away establishments (Fig. 3). Commercially available olive oil, coconut oil, lard and beef fat (dripping) samples, however, generated only very low levels of specific aldehydes (i.e. those giving rise to the signals at 9.48

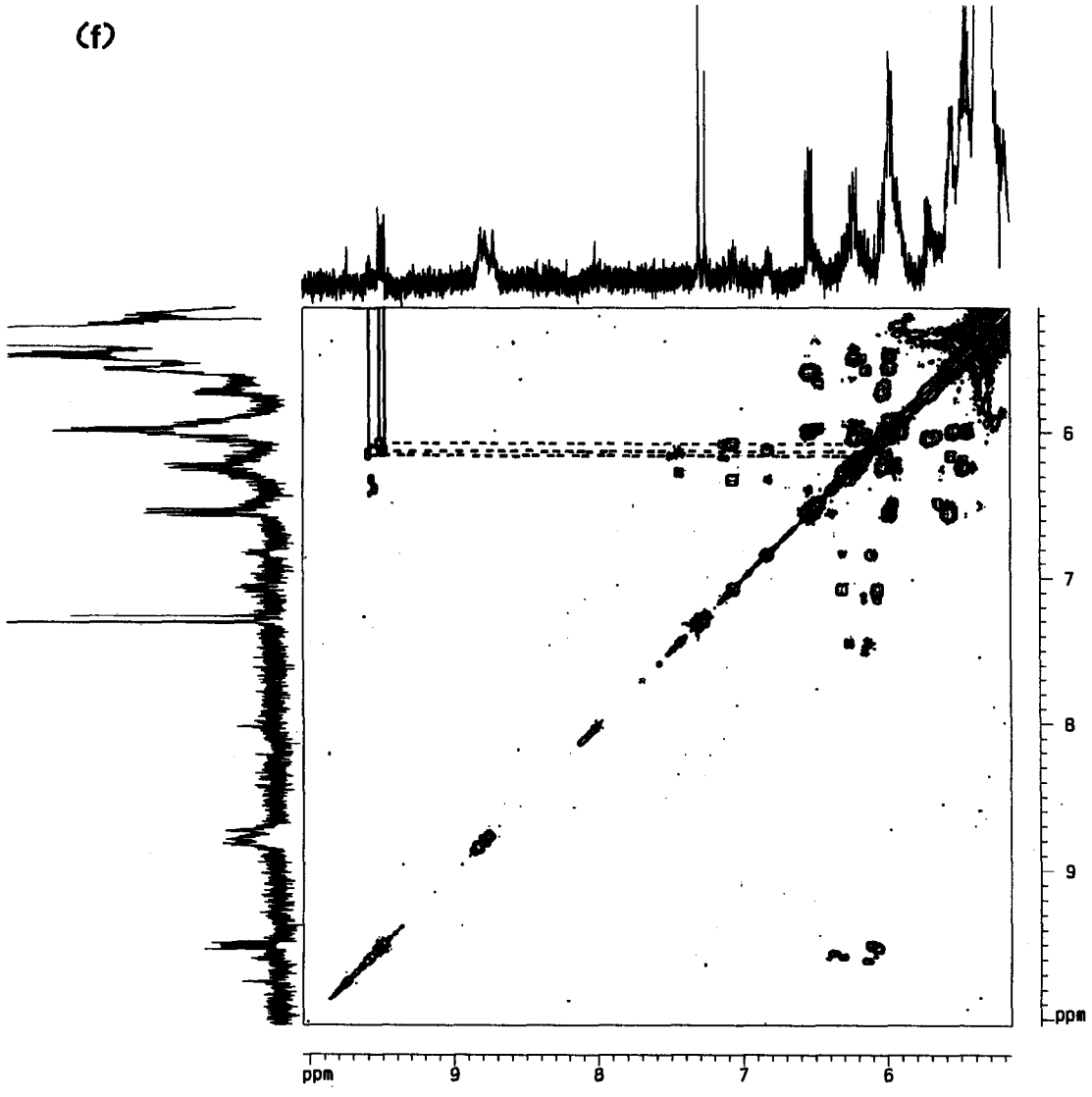

Fig. 4. Expanded 5.00–10.00 or 4.00–10.00 ppm regions of 600 MHz ^{1}H NMR spectra of a commercially available sample of ethyl linoleate acquired (a) before, and at (b) 30, (c) 60 and (d) 90 min after heating at a temperature of 180°C in the presence of atmospheric O_2. (e and f) complete, and expanded 5.15–10.05 ppm regions, respectively, of a 600 MHz two-dimensional COSY ^{1}H NMR spectrum of thermally stressed ethyl linoleate (i.e. heated at 180°C for a 90 min period as above). For the spectra shown in (e) and (f), clear connectivities between (i) the conjugated diene vinylic proton multiplets centred at 5.56, 5.53 and 5.60 ppm and those at 4.09, 4,27 and 4.34 ppm, respectively, and (ii) the aldehydic (-CHO) and vinylic proton resonances of α,β-unsaturated aldehydes (*trans*-2-alkenals, alka-2,4-dienals and 4-hydroxy-*trans*-2-alkenals) are indicated. A number of further aldehydic group proton doublet resonances are clearly resolvable from those located at 9.48, 9.52 and 9.63 ppm in the spectrum shown in (f). These additional aldehydic group signals may arise from further α,β-unsaturated aldehydes known to be generated from the oxidative degradation of PUFAs, e.g. 4-hydroxy-2,5-undecadienal and 4,5-dihydroxydecenal. Typical spectra are shown. Abbreviations as Fig. 1. The arrow in spectra (b–d) denotes a sharp singlet resonance (δ = 7.97 ppm) generated in spectra during episodes of heating at 180°C as described above.

(doublet) and 9.74 ppm (triplet)) when subjected to episodes of heating at 180°C in the manner described above (data not shown), presumably a reflection of the relatively low PUFA content of these materials.

Thermal stressing of the model PUFA compound, ethyl linoleate, generated a variety of aldehydic components (*n*-alkanals, *trans*-2-alkenals, alka-2,4-dienals and 4-hydroxy-*trans*-2-alkenals) arising from the decomposition of their CHPD precursors (Fig. 4). Such CHPDs were also detectable in corresponding 600 MHz ^{1}H NMR spectra of control (unheated) samples of this di-unsaturated fatty acid ester which were allowed to autoxidise in the dark for a 2,088 h period in the presence of atmospheric O_2 (Fig. 4a). The thermally induced generation of aldehydes in this sample was accompanied by a corresponding decrease in the intensities of the *cis,trans*- and *trans,trans*-CHPD signals (Fig. 4b–d). As expected, shaking of control and thermally stressed samples of ethyl linoleate with a small quantity of added ^2H_2O (see section 2) completely removed the broad 8.72, 8.775 and 8.81 ppm hydroperoxide group proton (-OOH) resonances from their ^{1}H NMR spectra.

Interestingly, two-dimensional COSY ^{1}H NMR spectra of ethyl linoleate heated at 180°C for a period of 90 min (Fig. 4e and f) contained a conjugated diene multiplet signal (δ = 5.56 ppm) that was clearly connected to one located at 4.09 ppm. These signals are attributable to the 12- (5.56 ppm) and 13-(4.09 ppm) position protons of the conjugated *cis,trans*-13-

hydroxydiene adduct generated from the thermally induced fragmentation of its corresponding CHPD, the latter resonance representing the methine proton of the hydroxy group-bearing carbon (a similar pattern of linked multiplet signals is expected for the *cis,trans*-9-hydroxydiene isomer). Two-dimensional ^{1}H NMR analysis of heated ethyl linoleate samples also revealed connectivities between the conjugated diene system vinylic proton resonances centred at 5.53 and 5.60 ppm (of greater intensity than the above 5.56 ppm multiplet) and those at 4.27 and 4.34 ppm, respectively. The lower (4.27, 4.34 ppm) and higher frequency (5.53, 5.60 ppm) signals are attributable to the single methine proton of the hydroperoxide-bearing carbon (e.g. proton a in Figs. 1 and 4) and the vinylic proton directly adjacent to (i.e. α-to) hydroperoxide group-substituted carbons (e.g. proton b in Figs. 1 and 4), respectively, of *cis,trans*-CHPD species.

4. Discussion

Multicomponent analysis of thermally stressed culinary oils and fats by high-field ^{1}H NMR spectroscopy provides much useful information regarding the chemical nature and levels of PUFA-derived autoxidation products present. Such information is a critical primary requirement for future investigations of the toxicological hazards putatively associated with the regular consumption of these materials [14–23].

The toxicological effects putatively exerted by aldehydes generated in culinary oils and fats during episodes of thermal stressing are, of course, critically dependent on the rate and extent of their in vivo absorption from the gut into the systemic circulation. Interestingly, previous investigations have indicated that such secondary PUFA autoxidation products are absorbed [24] (unlike their conjugated hydroperoxydiene precursors [25]), and further experiments to evaluate this are currently in progress.

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