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A ^{1}H NMR-Linked PCR Modelling Strategy for Tracking the Fatty Acid Sources of Aldehydic Lipid Oxidation Products in Culinary Oils Exposed to Simulated Shallow-Frying Episodes

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***Abstract*—**Objectives/Hypotheses: The adverse health effect potential of dietary lipid oxidation products (LOPs) has evoked much clinical interest. Therefore, we employed a ^{1}H NMR-linked Principal Component Regression (PCR) chemometrics modelling strategy to explore relationships between data matrices comprising (1) aldehydic LOP concentrations generated in culinary oils/fats when exposed to laboratory-simulated shallow frying practices, and (2) the prior saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acid (PUFA) contents of such frying media (FM), together with their heating time-points at a standard frying temperature (180 ^{o}C). Methods: Corn, sunflower, extra virgin olive, rapeseed, linseed, canola, coconut and MUFA-rich algae frying oils, together with butter and lard, were heated according to laboratory-simulated shallow-frying episodes at 180 ^{o}C, and FM samples were collected at time-points of 0, 5, 10, 20, 30, 60, and 90 min. (n = 6 replicates per sample). Aldehydes were determined by ^{1}H NMR analysis (Bruker AV 400 MHz spectrometer). The first (dependent output variable) PCR data matrix comprised aldehyde concentration scores vectors (PC1* and PC2*), whilst the second (predictor) one incorporated those from the fatty acid content/heating time variables (PC1-PC4) and their first-order interactions. Results: Structurally complex ***t**rans,trans*- and *cis,trans*-alka-2,4-dienals, 4,5-epxy-*trans*-2-alkenals and 4-hydroxy-/4-hydroperoxy-*trans*-2-alkenals (group I aldehydes predominantly arising from PUFA peroxidation) strongly and positively loaded on PC1*, whereas *n*-alkanals and *trans*-2-alkenals (group II aldehydes derived from both MUFA and PUFA hydroperoxides) strongly and positively loaded on PC2*. PCR analysis of these scores vectors (SVs) demonstrated that PCs 1 (positively-loaded linoleoylglycerols and [linoleoylglycerol]:[SFA] content ratio), 2 (positively-loaded oleoylglycerols and negatively-loaded SFAs), 3 (positively-loaded linolenoylglycerols and [PUFA]:[SFA] content ratios), and 4 (exclusively orthogonal sampling time-points) all powerfully contributed to aldehydic PC1* SVs (p 10^{-3} to < 10^{-9}), as did all PC1-3 x PC4 interaction ones (p 10^{-5} to < 10^{-9}). PC2* was also markedly dependent on all the above PC SVs (PC2 > PC1 and PC3), and the interactions of PC1 and PC2 with PC4 (p < 10^{-9} in each case), but not the PC3 x PC4 contribution. Conclusions: NMR-linked PCR analysis is a valuable strategy for (1) modelling the generation of aldehydic LOPs in heated cooking oils and other FM, and (2) tracking their unsaturated fatty acid (UFA) triacylglycerol sources therein.

***Keywords*—**Frying oils, frying episodes, lipid oxidation products, cytotoxic/genotoxic aldehydes, chemometrics, principal component regression, NMR Analysis.

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I. Introduction

A high proportion of humans are continually exposed to oxidised 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 evoked much interest [1]-[3]. The most important reaction involved in the oxidative degradation of lipids is the peroxidation of UFAs, particularly polyunsaturated fatty acids (PUFAs), a process which occurs during the heating of culinary oils according to high temperature frying practices. PUFAs are particularly susceptible to oxidative damage by virtue of the facile abstraction of one of their *bis*-allylic methylene group hydrogen atoms on exposure to heat, light, or radical species of sufficient reactivity, a process augmented by the low bond dissociation energy of their methylene group C-H bonds [1]-[4]. Following this, one major reaction pathway sequence for the resonance-stabilised carbon-centred pentadienyl lipid radical arising therefrom involves its interaction with molecular oxygen to produce a lipid peroxyl radical which, in turn, can abstract a hydrogen atom from a molecularly-localised, adjacent PUFA to form a conjugated lipid hydroperoxydiene (CHPD) and a further pentadienyl lipid radical species. This process is repeated many times and represents an autocatalytic, self-perpetuating chain reaction. However, CHPDs are unstable at standard frying temperatures (*ca*. 180 °C) and are degraded to a wide variety of secondary peroxidation products, which include saturated and unsaturated aldehydes, di- and epoxyaldehydes, lactones, furans, ketones, oxo- and hydroxy-acids, together with saturated and unsaturated hydrocarbons [4], [5]. Moreover, during the later stages of the peroxidation process, a series of polymerisation reactions occur. MUFAs, however, are much more resistant to lipid peroxidation, and hence lower levels of selected aldehydes are produced from the fragmentation of MUFA-derived hydroperoxymonoene (HPM) precursors, and generally only during prolonged exposure to high-temperature frying episodes or their continual reuse for this purpose. Correspondingly, saturated fatty acids (SFAs), predominantly stearic and palmitic acids in vegetable oils, are virtually completely resistant to thermally-induced peroxidative

degradation. Therefore, the relative rates and extents of primary lipid hydroperoxide production from acylglycerol fatty acid (FA) sources available in culinary oils are in the order PUFAs > MUFAs >>> SFAs [4]. Moreover, the rates and extents of the fragmentation of these lipid hydroperoxides to toxic secondary aldehydic LOPs also strongly correspond to this FA order [4], [5]. As expected, linolenoylglycerol species with 3 -CH=CH- double bond functions oxidise more rapidly than linoleoylglycerols which contain only 2 of these [4], [5].

Previously reported ^{1}H [6], [7], and two-dimensional ^{1}H-^{1}H and ^{1}H-^{13}C [8] NMR investigations of thermally-stressed, PUFA-containing culinary oils have revealed the generation of very high levels of extremely toxic α,β-unsaturated aldehydes [*trans*-2-alkenals, and *trans,trans*- and *cis,trans*-alka-2,4-dienals, amongst a range of other classes], and saturated *n*-alkanals, along with their HPM, CHPD and hydroxydiene precursors in UFA-rich culinary oils during standard frying/cooking practices. Indeed, samples of these repeatedly-utilised frying oils collected from fast-food retail outlets and restaurants have confirmed the production of such high total ($> 10^{-2}$ mol.kg^{-1}) concentrations of these classes of secondary LOPs during conventional, frequently-employed frying episodes. Indeed, further experiments conducted have shown that the total aldehyde levels generated are critically dependent on the oil PUFA content, with >70% of these being of the more highly toxic α,β-unsaturated classes [6]-[8]. Such results have been replicated and confirmed by a large number of research laboratories worldwide (most notably [9]). Further applications of this technique include the detection of cytotoxic and genotoxic 4-hydroperoxy-, 4-hydroxy- and 4,5-epoxy-*trans*-2-alkenals in PUFA (including linolenoylgycerol)-rich culinary oils when exposed to high temperature heating episodes in the presence of atmospheric O_2 [10], [11].

More recently, the availability of potentially health-threatening levels of cytotoxic and genotoxic *trans*-2-alkenals, *trans,trans*-alka-2,4-dienals and *n*-alkanals in fried potato chip (French fry) samples collected from a variety of fast-food retail outlets has been demonstrated (10-25 ppm for each class of ^{1}H NMR-detectable aldehyde monitored [12], [13]). This dietary-available source of toxic aldehydes is largely derived from the penetration of thermally-oxidised frying oils into this food product during deep-frying practices.

Therefore, in this investigation, we have employed a ^{1}H NMR-linked PCR chemometrics modelling approach to explore relationships between data matrices consisting of (1) aldehydic LOP concentrations generated in culinary oils/fats when thermally-stressed according to standard frying practices, and (2) the prior SFA, MUFA and PUFA contents (and [linoleoylglycerol]:[SFA] content ratios) of such frying media, together with the duration of their exposure to heating according to laboratory-simulated shallow-frying episodes. This study was performed in order to provide a valuable and reliable means of predicting the identities and concentrations of ^{1}H NMR-detectable, secondary aldehydic LOPs generated from the oleoyl-, linoleoyl- and linolenoylglycerol (and inversely SFA) contents of frying oil products when exposed to laboratory-simulated shallow-frying episodes at 180°C. The time-dependence of each class of aldehyde formed in this system was simultaneously evaluated using this PCR and further multivariate analysis modelling systems.

II. Materials and Methods

A. Culinary Oil/Frying Media Samples Investigated

All culinary oils and alternative frying media were purchased from UK or USA retail stores. Each product was de-identified in the laboratory by transferring to coded but unlabelled storage containers. The specified SFA, MUFA and PUFA% (w/w) contents of these oils were 11, 28, and 61% for sunflower oil (two products of the same composition were tested); 15, 22, and 62% for corn oil; 7, 64, and 29% for canola oil; 13, 77, and 10% for extra-virgin olive oil; 7, 61, and 32% for rapeseed oil (product 1); 7, 62 and 31% for rapeseed oil (product 2); 7, 58 and 31% for a product described as 'vegetable oil' (which is presumably rapeseed oil from a consideration of its FA content); 11, 22 and 67% for linseed oil; 91, 7 and 2% for coconut oil; 52, 21 and 4% (w/w) for butter; 41, 48 and 8% for lard; and 4, 92, and 4% (w/w) respectively for a MUFA-rich algae oil (MARO-1). A second MUFA-rich algae oil investigated (MARO-2) had a content of 4, 93, and 3% (w/w) SFAs, MUFAs, and PUFAs, respectively.

The molar percentage of omega-3 FAs in the canola, linseed and 2 x rapeseed oil products tested was estimated by an ^{1}H NMR method which involved expression of the intensity (I) of the intelligently-selected bucket region for the omega-3 FA chain terminal-CH_3 function resonance (triplet, $\delta = 0.97$ ppm) to that of the total FA chain terminal-CH_3 signals [i.e. $I_{0.97}/(I_{0.90} + I_{0.97})$], the $\delta = 0.90$ ppm one representing that for all non-omega-3 FAs. The molar % omega-3 FA (predominantly linolenic acid) contents of these oils was found to be 9.9, 10.3, 10.6, and 53.1 molar % for rapeseed oils 1 and 2, canola, and linseed oils, respectively.

B. Experiments Involving the Exposure of Culinary Oils to Laboratory-Simulated Shallow-Frying Episodes

All of the above culinary oils/frying media were heated according to laboratory-simulated shallow-frying practices; 6 x 6.00 ml volume of each oil, or 6 x 6.00 g masses of lard, butter and coconut oil, were heated in rigorously pre-cleansed and dried 250 ml volume glass beaker vessels at 180°C for periods of 0-90 min. Samples of these products were collected at heating time-points of 0, 5, 10, 20, 30, 60, and 90 min. (n = 6 replicates per time-point).

C. ^{1}H NMR Analysis

Aldehyde concentrations in control and thermally-stressed culinary oils were determined by ^{1}H NMR analysis (Bruker AV 400 MHz spectrometer, Leicester School of Pharmacy facility) operating at a frequency of 399.94 MHz and a probe temperature of 298 K.

A 0.20 ml aliquot of each oil sample was diluted to a final volume of 0.70 ml with deuterated chloroform ($C^{2}HCl_3$) containing 3.67 x 10^{-3} mol.L^{-1} tetramethylsilane (TMS), the former providing a field frequency lock, the latter as an

internal chemical shift reference (δ = 0.00 ppm). Added 1,3,5-trichlorobenzene (final concentration 1.00 x 10^{-2} mol./L) served as an internal quantitative ^{1}H NMR standard. These solutions were then placed in 5-mm diameter NMR tubes. Typical pulsing conditions were: 128 or 256 free induction decays (FIDs) using 65,536 data points and a 4.5 s pulse repetition rate, the latter to allow full spin-lattice (T_1) relaxation of protons in the samples investigated. Resonances present in each spectrum were routinely assigned by evaluations of chemical shifts, coupling patterns and coupling constants. One- and two-dimensional correlation (COSY) and total correlation (TOCSY) spectra were obtained to confirm ^{1}H NMR assignments as previously described [6]-[8].

Culinary oil/frying media FA contents were confirmed by a modification of the ^{1}H NMR methods described in [9], [14].

D. Preprocessing of ^{1}H NMR Spectral Profiles: Determinations of Aldehydic LOP Class Concentrations in Culinary Oils, and Their Lower Limits of Detection and Quantification (LLOD and LLOQ Respectively)

The aldehydic LOP-CHO function resonance region of the spectral profiles acquired (i.e. those within the 9.00-10.20 ppm spectral range) were preprocessed by the application of a separate macro for the 'intelligent bucketing' processing sub-routine. These procedures were conducted using the ACD/Labs Spectrus Processor 2014 software package (ACD/Labs, Toronto, Ontario, Canada M5C 1T4), and this generated a culinary oil dataset matrix consisting of bucket variables (intelligently-selected buckets, abbreviated as ISBs) corresponding to the -CHO function resonances of a range of aldehyde classes, specifically *trans*-2-alkenals (*d*, δ = 9.475-9.515 ppm), *trans,trans*- and *cis,trans*-alka-2,4-dienals (both *d*, δ = 9.515-9.535 and 9.585-9.620 ppm respectively), 4,5-epoxy-*trans*-2-alkenals (*d*, δ = 9.540-9.560 ppm), 4-hydroxy-/4-hydroperoxy-*trans*-2-alkenals (both *d*, δ = 9.560-9.590 ppm), *n*-alkanals (*t*, δ = 9.740-9.765 ppm), and *cis*-2-alkenals (*d*, δ = 10.050-10.080 ppm), the latter previously first assigned in [13]. Prior to commencing this intelligent bucketing process, all spectra were examined visually for any inherent distortions and manually corrected, if required. The electronic intensities of resonances corresponding to each of the above -CHO resonance ISBs were normalised to that of added TCB.

'Between-frying cycle' sample coefficients of variation for all aldehyde class determinations made on the n = 6 replicated thermal stressing episodes ranged from 4.0-10.9% for all oils investigated, whereas those for repeat determinations made on the same oil sample were ≤ 2.5%.

Lower limits of detection and quantification (LLOD and LLOQ respectively) values for the typical *trans*-2-alkenal and *n*-alkanal LOPs (*trans*-2-octenal and *n*-hexanal respectively) were determined via the performance of serial dilutions of these analytes, and a consideration of the 3σ and 10σ signal-to-noise approaches using the *MNova* signal-to-noise ratio (SNR) software module. These LLOD and LLOQ values were 48 and 160 μmol.kg^{-1} respectively for *trans*-2-alkenals (equivalent to 15 and 51 μmol./mol. FA respectively); 42 and 140 μmol.kg^{-1} for *trans,trans*-alka-2,4-dienals (equivalent to 14 and 45 μmol./mol. FA respectively); and 25 and 83 μmol.kg^{-1} for *n*-alkanals (equivalent to 6 and 20 μmol./mol. FA respectively) for the 1/3 (v/v) diluted oil samples prepared. As expected, these values improved approximately 2-fold with lower C^2HCl_3 dilution levels of such oils (i.e. 2/3 rather than 1/3 dilutions), and also with greater numbers of ^{1}H NMR scans acquired (i.e. 1,024 or 2,048).

Calibration curves for typical *trans*-2-alkenals and *n*-alkanals (0-500 μmol.L^{-1} and 0.10-60.00 mmol.L^{-1}) were linear, with R^2 values ≥ 0.996 for neat C^2HCl_3 solutions, and ≥ 0.985 for aldehyde-'spiked' C^2HCl_3-diluted oil media prepared as described above.

The intrinsic peroxidative susceptibility indices (PSIs) were computed from the FA compositions of all oils tested as previously described [15], i.e. PSI = [0.025(% monoenoic FA)] + [1.00(% dienoic FA)] + [2.00(% trienoic FA)] + [4.00(% tetraenoic FA)] + [6.00(% pentaenoic FA)] + [8.00(% hexaenoic FA)]. However, for all vegetable- and algae-derived oils evaluated here, contributions to the PSI from tetraenoic, pentaenoic and hexaenoic FA sources were negligible.

E. Chemometrics Model Development and PCR Analysis

Datasets (mmol. aldehyde/kg oil) were analysed after autoscaling (involving subtraction of the column variable mean value, followed by division by its standard deviation so that all variables incorporated had unit variance).

Initially, principal component analysis (PCA) was performed on the two datasets, i.e. (1) the FA % composition indices, including the [linoleoylglycerol]:[SFA] molar ratio (input variables), together with heating time-point (0-90 min.); and (2) the aldehyde classification concentration output variables (Table I) in order to primarily generate orthogonal (uncorrelated) principal components (PCs) from each of these for the purpose of PCR analysis (orthomax rotation coupled with Kaiser normalisation was applied for this purpose).

Subsequently, PCR analysis was performed by a multiple linear regression (MLR) analysis model incorporating the sample scores vectors (SVs) obtained from the above PCA strategy. This approach involved regressing the aldehyde concentration output (dependent) variable SVs for two major PCs obtained (PC1* and PC2*) individually against four major PCs derived from the above input (independent) variable dataset (PC1-PC4, where PC1-3 represent linear combinations of the culinary oil/frying medium lipid composition variables, and PC4 that exclusively attributable to the time variable). In view of variable lag times for aldehyde generation, and the differing time-dependencies/rates of these processes 'between-frying oil/media', a total of three first-order PC1-3 x PC4 interaction SV variables were also incorporated into the model system (i.e. PC1 x PC4, PC2 x PC4 and PC3 x PC4) in order to ascertain their statistical significance and potential contributory roles in predicting aldehyde level output variable PC* SV values, together with the individual PC1-4 ones. The statistical significance of each predictor variable (PC1-PC4, and PC1-3 x 4 first-order interaction effects) was determined via analysis-of-variance (ANOVA) testing of PCR regression coefficients.

Therefore, the first (dependent output) PCR data matrix comprised aldehyde concentration scores vectors (PC1* and PC2*), whilst the second (predictor) one involved those arising from the FA content/heating time variables (PC1-PC4), together with their first-order PC1-3 x heating time-point (PC4) interactions.

An additional model applied involved a partial least-squares regression (PLS-R) strategy which employed the individual triacylglycerol FA composition and sampling time-point predictor variables (autoscaled) to successfully predict the PC1* and PC2* aldehyde concentration output variable SV values, i.e. it represented a mixed PCR/PLS-R analysis approach. The significance of PLS-R components arising from these predictor variables was tested via the determination of Q^2 and R^2Y model monitoring parameters.

Further PLS-R models were focused on an analysis of all the seven sampling collection time-points of the laboratory-simulated shallow frying episodes employed for nine of the frying media/oils tested, specifically canola, corn, sunflower, MARO-1, linseed, rapeseed, EVOO and coconut oils, and butter. This approach was employed in order to provide support for studies focused on predictions of the primary CHPD or HPM LOP precursors, and thenceforth the original unsaturation status of frying media FA sources, of simple and/or structurally-complex classes of aldehydic LOPs.

A further aspect of the study involved a relatively simple PCA model which examined only the 90 min. shallow-frying simulation heating time-points for five of the frying oils evaluated (canola, corn, extra-virgin olive, MARO-1 and sunflower oils) in order to determine the efficacy of this technique to distinguish between the patterns and levels of aldehydic LOPs generated within these frying media at this final time-point on the basis of their PSI values, which ranged from 7.40 (MARO-1) to 63.87 (corn oil).

Further investigations, which were targeted on determining the influence of culinary oil/frying media PSI values on the nature and concentrations of aldehydic LOPs produced, involved a univariate multiple regression strategy, in which individual aldehydic LOPs were the dependent variables, and PSI values and heating time-points served as predictor (independent) variables, along with their PSI x heating time-point interaction effects. The mathematical model for these analyses is given in (1), in which y_{ijk} represents the (univariate) ISB dependent variable integration values observed for each aldehyde classification, P_i and T_j the PSI and heating time-point sources of variation respectively (both fixed effects), PT_{ij} the PSI x time-point interaction effect, e_{ijk} fundamental (residual) error, and μ the aldehyde classification's overall population mean values in the absence of any significant, influential sources of variation.

$$y_{ijk} = \mu + P_i + T_j + PT_{ij} + e_{ijk} \quad (1)$$

All univariate and multivariate data analysis, i.e. PCA, PLS-R, PCR, and also canonical correlation analysis (CCorA), was performed using *XLSTAT2016* software modules, as was univariate analysis performed by an analysis-of-covariance (ANCOVA) model, with 'between-frying media', 'between-sampling (heating) time-points' and the first-order frying media x sampling time-point interaction effect considered as (fixed) sources of variation.

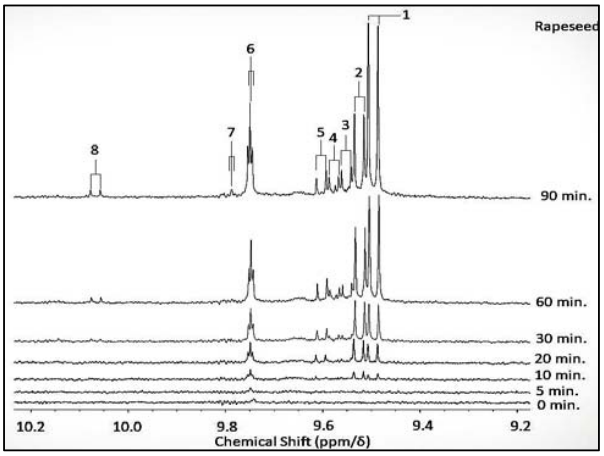


Fig. 1 Expanded aldehydic-C<u>H</u>O proton (9.15-10.25 ppm) regions of 400 MHz ^{1}H NMR spectra of rapeseed oil exposed to heating episodes at 180 ^{o}C for periods of 0, 5, 10, 20, 30, 60 and 90 min. according to laboratory-simulated shallow frying practices (Section II). Typical spectra are shown. Abbreviations: -C<u>H</u>O group resonances of 1, *trans*-2-alkenals; 2, *trans*,*trans*-alka-2,4-dienals; 3, 4,5-epoxy-*trans*-2-alkenals; 4, combined 4-hydroxy and 4-hydroperoxy-*trans*-2-alkenals; 5, *cis*,*trans*-alka-2,4-dienals; 6, *n*-alkanals; 7, low-molecular-mass short-chain *n*-alkanals, particularly propanal and *n*-butanal from the peroxidation of linilenoylglycerols; and 8, *cis*-2-alkenals

III. Results

Fig. 1 shows the time-dependent generation of the aldehydic-C<u>H</u>O function resonances of a series of up to eight aldehydes generated in a typical frying oil when exposed to a continuous laboratory-simulated shallow frying episode at 180^{o}C for 0-90 min. (results acquired for a rapeseed oil product are displayed). Clearly, these signals, which are assignable to a series of both saturated and unsaturated aldehyde classes, increase with increasing heating exposure time (assignments for these signals were confirmed via the acquisition of corresponding one- and two-dimensional ^{1}H-^{1}H COSY and TOCSY spectra for each heated oil, in addition to standard addition 'spiking' experiments performed with standard authentic aldehyde solutions in C^2HCl_3). All resonances visible were doublets, with the exception of signals 6 and 7, which are triplets (j = 1.70 and 1.76 Hz respectively). Spectra of all the PUFA-rich cooking oils also contained signals assignable to aldehydic LOP precursors, specifically *cis*,*trans*- *and trans*,*trans*-CHPDs (conjugated diene vinylic proton multiplets centred in the 5.40-6.60 and 5.40-6.30 ppm spectral regions respectively, together with added ^2H_2O-removable broad -OO<u>H</u> function resonances located within the δ = 8.20-8.80 ppm chemical shift range), and *cis*,*trans*-conjugated hydroxydienes (δ = 5.40-6.50 ppm region), as previously reported (data not shown) [6]-[8]. These broad -OO<u>H</u>

resonances were also observed in spectra acquired on thermally-stressed MUFA-rich oils such as extra-virgin olive oil, but in these cases their molecular sources are presumably predominantly HPMs.

Plots of mean±SEM total concentrations of each class of aldehyde determined by ^{1}H NMR analysis are shown in Fig. 2 for the highest concentration aldehydes detectable, specifically *trans*-2-alkenals, *trans,trans*-alka-2,4-dienals and *n*-alkanals. These data clearly confirm major, statistically-significant mean aldehyde concentration differences between the culinary oils/frying media tested at the great majority of time-points investigated ($p < 10^{-8}$, ANCOVA), which substantially increased with increasing durations of thermal stressing episodes according to our laboratory-simulated shallow frying episodes (also $p < 10^{-8}$, ANCOVA).

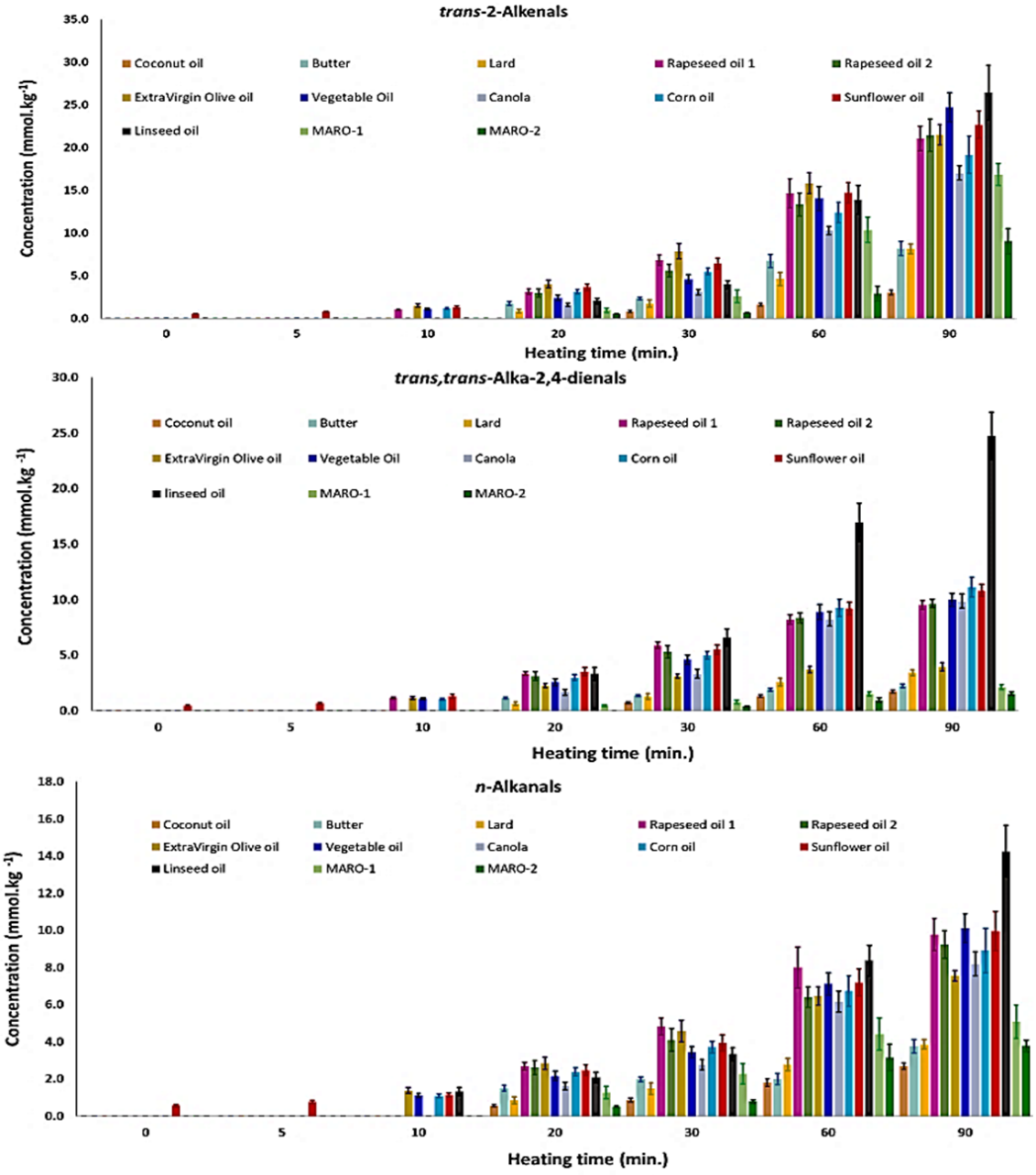


Fig. 2 Bar diagram plots of the mean±SEM ^{1}H NMR-determined concentrations of *trans*-2-alkenals (top), *trans,trans*-alka-2,4-dienals (middle) and *n*-alkanals (bottom) in mmol.kg^{-1} units in a wide series of culinary oils, butter and lard samples when exposed to laboratory-simulated shallow-frying episodes for periods of 0, 5, 10, 20, 30 60 and 90 min. A colour key is provided for each frying medium (frying oils, butter or lard) tested in this manner. Abbreviations: MARO-1 and -2 (light- and dark-green colour codes respectively) represent MUFA (oleoylglycerol)-rich algae frying oils

As expected, the concentrations of saturated and α,β-unsaturated aldehydes monitored in our ^{1}H NMR profiles increased with PUFA content of the frying oil/frying medium tested, and >70% of these were of the more highly toxic α,β-unsaturated classes, including *cis*- and *trans*-2-alkenals [(*E*)-2-alkenals], *trans,trans*- and *cis,trans*-alka-2,4-dienals [(*E*,*E*)- and (*Z*,*E*)-2,4-alkadienals respectively], along with 4-hydroperoxy-/4-hydroxy-, and 4,5-epoxy-*trans*-2-alkenals [the latter three all substituted (*E*)-2-alkenal derivatives] (Fig. 1). The markedly lower concentrations of both total saturated and α,β-unsaturated aldehydes generated in the MUFA-rich algae oils, SFA-rich coconut oils, and butter, proportionately reflects the higher SFA and/or MUFA, and lower PUFA contents of these frying media. Moreover, the substantially lower levels of *trans,trans*-alka-2,4-dienals generated in MUFA-rich oils, such as extra-virgin olive and MUFA-rich algae oils, when exposed to these laboratory-simulated frying episodes, are attributable to their low or very low PUFA contents [12], [13].

The production of *cis*-2-alkenals in oils when heated according to our protocol was found to be highly time-delayed, i.e. a prolonged lag period of at least 20-30 min. preceded the generation of this class of aldehyde (as shown in Fig. 1).

Primary PCA performed on the two separate datasets, i.e. (1) that comprising fatty acid composition indices [specifically the (w/w) %'s of total SFAs, MUFAs (predominantly oleoylglycerols), linoleoylglycerols, linolenoylglycerols, and the [linoleoylglycerol]:[SFA] content ratio, together with the laboratory-simulated shallow-frying episode time-point (0-90 min.); and (2) that containing the concentrations of seven different ^{1}H NMR-distinguishable aldehyde classes (Fig. 1), i.e. those of ^{1}H NMR-determined *trans*-2-alkenals, *cis*-2-alkenals, *trans,trans*- and *cis,trans*-alka-2,4-dienals, 4,5-epoxy-*trans*-2-alkenals, combined 4-hydroxy-/4-hydroperoxy-*trans*-2-alkenals (overlapping signals within the same ISB), and *n*-alkanals.

Firstly, for the lipid composition/index variable dataset, linoleoylglycerol content and [linoleoylglycerol]:[SFA] content ratio strongly and positively loaded on PC1; MUFA and SFA contents positively and negatively, respectively, loaded strongly on PC2; and linolenoylglycerol and [linoleoylglycerol]:[SFA] content ratio strongly and positively loaded on PC3 [Table I.A]. The SFA content variable also negatively contributed towards PC1 and PC3, but less so than on PC2 (i.e. loadings scores vectors of -0.535 and -0.422 respectively *versus* -0.734 for PC2).

Secondly, PCA performed on the aldehyde classification dataset revealed that *trans,trans*- and *cis,trans*-alka-2,4-dienals, 4,5-epoxy-*trans*-2-alkenals and 4-hydroxy-/4-hydroperoxy-*trans*-2-alkenals (designated as group I aldehydes predominantly arising from PUFA peroxidation), strongly and positively loaded on PC1*, whereas *n*-alkanals, and *cis*- and *trans*-2-alkenals (designated as group II aldehydes derived from both MUFAs and PUFAs, those from the former's HPM source appearing only after an extended time-lag period) strongly and positively loaded on PC2* [Table I.B].

TABLE I.A
LOADINGS OF FRYING OIL/MEDIUM FA CONTENT/INDEX AND HEATING TIME-POINTS ON PCS1-4 (INPUT PREDICTOR VARIABLES) FOR THE PCR MODEL DEVELOPED. THE PCS ON WHICH THESE INPUT VARIABLES MOST STRONGLY LOAD UPON ARE INDICATED IN BOLD

Fatty Acid Index (Molar % or Content Ratio) or Heating Time (min.): PCR Input Variables	PC1	PC2	PC3	PC4
SFAs	-0.535	**-0.734**	-0.422	0.000
Oleoylglycerols (18:1)	-0.194	**0.966**	-0.164	0.000
Linoleoylglycerols (18:2)	**0.996**	-0.022	-0.041	0.000
Linolenoylglycerols (18:3)	-0.101	-0.038	**0.991**	0.000
[Linoleoylglycerol]:[SFA] Ratio	**0.700**	0.137	**0.682**	0.000
Heating time-point (min.)	0.000	0.000	0.000	**1.000**

TABLE I.B
LOADINGS OF ALDEHYDIC LOPS ON PCS 1* AND 2* (OUTPUT DEPENDENT VARIABLES) FOR THE PCR MODEL DEVELOPED. THE PCS ON WHICH THESE OUTPUT VARIABLES MOST STRONGLY LOAD UPON ARE INDICATED IN BOLD

[Aldehyde] (mmol.kg^{-1})	PC1*	PC2*
***trans*-2-Alkenals**	0.435	**0.886**
***trans,trans*-Alka-2,4-dienals**	**0.822**	0.555
4,5-Epoxy-*trans*--2-alkenals	**0.789**	0.603
4-Hydroxy/4-hydroperoxy-*trans*-2-alkenals	**0.801**	0.586
***cis,trans*-Alka-2,4-dienals**	**0.874**	0.477
***n*-Alkanals**	0.554	**0.813**
***cis*-2-Alkenals**	0.485	**0.835**

PCR analysis of the resulting SV values arising from both the lipid composition/heating time-point and aldehyde classification datasets (i.e. a final multiple regression model for the prediction of aldehyde-loading PC1* and PC2* SVs from PCs 1-4, and the featured first-order interaction effects, demonstrated that PCs 1 (predominantly positively-loaded linoleoylglycerol concentrations and [linoleoylglycerol]:[SFA] ratio), 2 (predominantly positively-loaded oleoylglycerol concentrations and negatively-loaded SFA ones), 3 (predominantly positively-loaded linolenoylglycerols and [linoleoylglycerol]:[SFA] ratio), and 4 (exclusively positively-loaded sampling time-points), all powerfully contributed to aldehydic PC1* SVs ($p = 2.07 \times 10^{-6}$, 1.70×10^{-8}, $< 10^{-9}$ and $< 10^{-9}$ respectively), as indeed did all PC1-3 x PC4 interaction sources of variation ($p < 10^{-9}$ for PC2 x PC4 and PC3 x PC4, and 1.01×10^{-3} for PC1 x PC4). These contributions were positive for all PUFA-associated PCs and their interactions with PC4, but negative for the PC2 and PC2 x PC4 contributions, as might be expected from the knowledge that the thermally-induced fragmentation of PC2-linked oleoylglycerol-derived HPM species do not generate di-unsaturated and more structurally-complex substituted aldehydes, which were found to strongly load on PC1*, and which only arise from PUFA CHPD precursors. The marked statistical significance of the PC1-3 x PC4 interaction effects observed is also expected in view of increasing lag phases for the peroxidation of linolenoyl-, linoleoyl- and oleoylglycerols, in that order. However, the strongest correlation of PC1* was that with PC3, i.e. positively-loaded linolenoylglycerol contents and [linoleoylglycerol]:[SFA] content ratios, and this is at least partially consistent with the former's more rapid

peroxidation rate than that of linoleoylglycerols, and also the abilities of CHPDs derived from PUFAs in general to generate more structurally-complex aldehydic fragmentation products than those arising from MUFA peroxidation.

Co-significant loadings of the [linoleoylglycerol]:[SFA] ratio SVs with those of linoleoylglycerol and linolenoylglycerols (PC1 and PC3 respectively) are, of course, also not unexpected. Similarly, co-significant negative loadings of SFA content SVs on PC2 with highly significant positive ones from those of oleoylglycerol contents are also predictable from the consideration that levels of aldehydic LOPs proportionately diminish with increasing culinary oil SFA content [6]-[8], [12], [13].

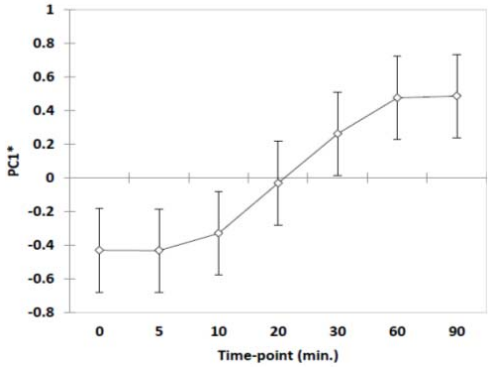
The PCR model developed also revealed that the PC2* output variable SVs were substantially positively dependent on two of the above PC SV values (specifically those of PC1 and PC2, with PC2's contribution being *ca.* twice that of PC1 (p = 4.23 x 10^{-9} and < 10^{-9} respectively), and both their interactions with heating time-points (as PC4 SVs, $p < 10^{-9}$ in each case), and most especially PC4 itself ($p < 10^{-9}$). However, PC3 SV values contributed towards PC2* negatively, albeit less effectively so (p = 6.85 x 10^{-6}); its interaction with PC4 (PC3 x PC4) was not statistically significant.

Models performed without the [linoleoylglycerol]:[SFA] ratio variable were found to be less effective than those performed with it, although reductions found in R^2 values obtained for prediction of the output variables PC1* and PC2* (0.846 and 0.773 respectively were obtained with this added ratio variable) were only marginal, i.e. ≤ 5%. However, we elected to retain this variable in the PCR model developed.

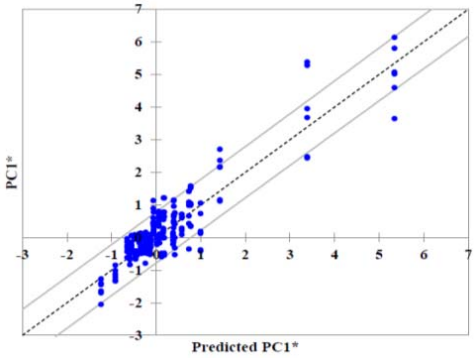
Notwithstanding, complete removal of the four PC1-3 x PC4 first-order interaction sources of variation from either of these models (specifically those with and without the added [linoleoylglycerol]:[SFA] ratio variable) gave rise to major decreases in these R^2 parameters, e.g., for the model with the above ratio included, R^2 values for PC1* and PC2* were reduced to 0.439 and 0.635 respectively. This is clearly ascribable to the differing lag periods and time-dependencies for the generation of each aldehydic contributor to their PC* SV output variables for each culinary oil/frying medium explored (Fig. 2).

Plots of PC1* and PC2* versus heating time-point at 180°C demonstrated mean autocatalytic peroxidation lag times of 10 and *ca.* 25 min. for the generation of aldehydes loading on these PCR output components respectively [Figs. 3 (a) and (c) respectively]. These observations are consistent with the susceptibility and resistivity of linoleoyl-/linolenoylglycerols and oleoylglycerols to thermo-oxidation, respectively. Indeed, the relative rates of peroxidation of oleic:linoleic:linolenic acids are 1:12:25 respectively [4]. There was a very good agreement between the actual and estimated PC1* and PC2* values from the PCR models developed [r = 0.92 and 0.88, Figs. 3 (b) and (d) respectively].

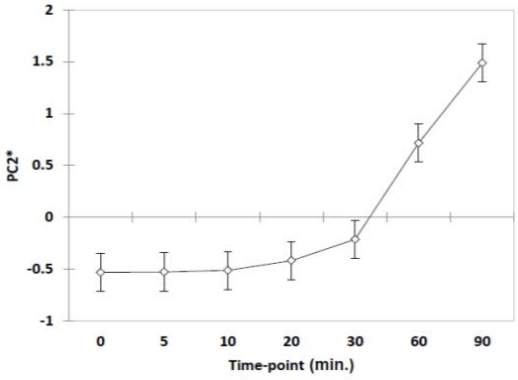
Fig. 4 compares plots of both PCR analysis-determined PC1* and PC2* SVs versus that of PC4 (the latter exclusively representing oil sampling time-point SVs) for PUFA-rich sunflower with that of MUFA-rich extra virgin olive oils. Clearly, these data confirm that the high MUFA (oleoylglycerol) content of extra virgin olive oil negatively contributed towards PC1* at the later sampling time-points, whereas both MUFAs and PUFAs positively contributed towards PC2*, as noted in Table I. These observations are fully consistent with the limited ability of the olive oil product to generate the di-unsaturated and structurally-substituted classes of aldehydes, which arise from PUFAs and not MUFAs in view of the very low content of the former acylglycerol species in this oil product [10% (w/w)]. However, for PUFA-rich sunflower oil (predominantly linoleoylglycerols), PC1* SV values increase with increasing heating time-point, as expected.



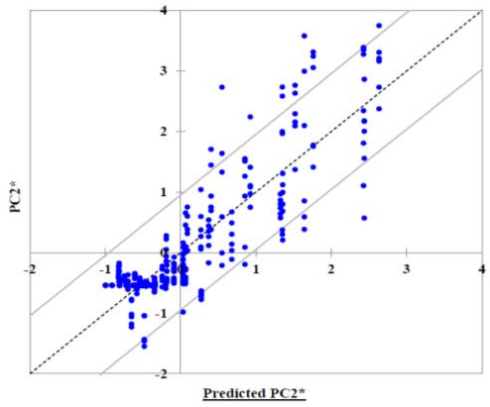
(a)



(b)



(c)



(d)

Fig. 3 (a) Time-point dependence of PC1* scores vectors, demonstrating an aldehyde generation lag-phase of only 5 min.; (b) Plot of observed versus predicted PC1* scores vectors for the PCR model developed (r = 0.92); (c) Time-point dependence of PC2* scores vectors, revealing an aldehyde generation lag-phase of *ca.* 25 min.; (d) Plot of observed versus predicted PC2* scores vectors for the PCR model developed (r = 0.88). The time-point scales in (a) and (c) have been ordered as shown for purposes of clarity

These results were confirmed by canonical correlation analysis (CCorA) of these two sets of PC* SVs (Fig. 5), which clearly demonstrated powerful correlations between the scores vectors of PC2* and PCs 2 and 4, whereas PC1* exhibited a marked dependence on PC3. Both PC1*'s and PC2*'s SVs were also found to be associated with PC1, but to a much lesser extent than they were with PCs 2-4.

An additional mixed PLS-R/PCR modelling strategy involving individual autoscaled % FA content and time variables as input predictor variables, and aldehydic LOP-loading PC1* and PC2* SV values as output ones, was also explored (Fig. 6). This model also confirmed that PC1* SVs were strongly correlated with oil % linolenoylglycerol content and [linoleoylglycerol]:[SFA] concentration ratio and their interactions with the heating time-point variable (Fig. 6). However, PC2* SVs were strongly dependent on % MUFA content, together with the interaction of this variable with heating time-point. The frying medium linoleoylglycerol content and its corresponding first-order interaction with time-point was also found to be related to PC2*, although to a lesser extent [Fig. 6 (b)]. As expected, SFA content, and also its interaction with heating time-point, were negatively correlated (i.e. anti-correlated) with both PC1* and PC2* SV output variables.

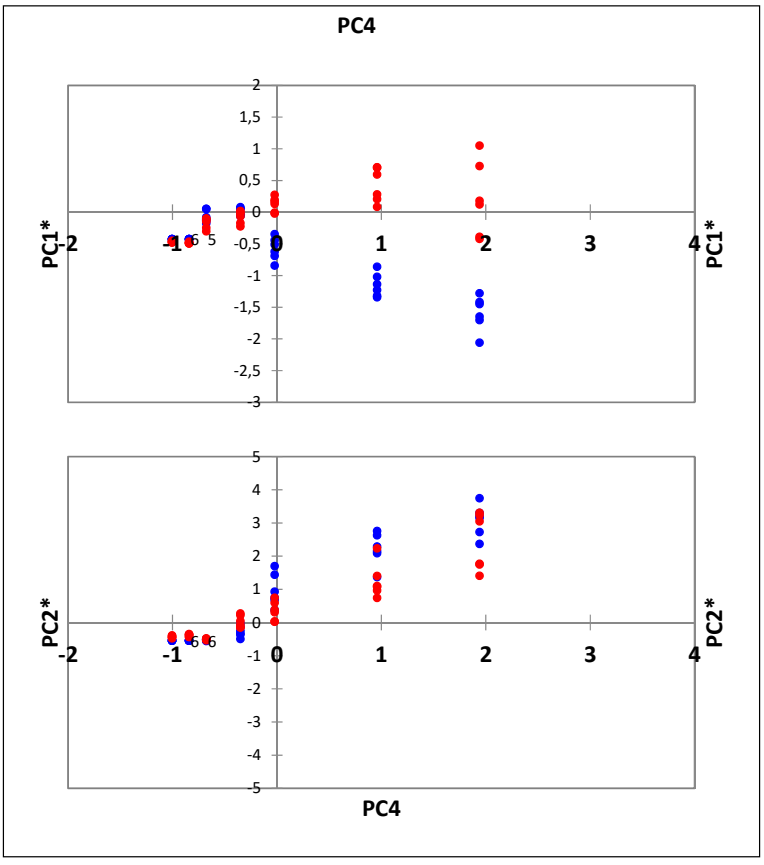


Fig. 4 Top and bottom, plots of PC1* and PC2* versus PC4 SVs respectively (the latter exclusively representing sampling time-point) for PUFA-rich sunflower (red) and MUFA-rich extra virgin olive oil (blue); all SVs were acquired from the PCR model developed

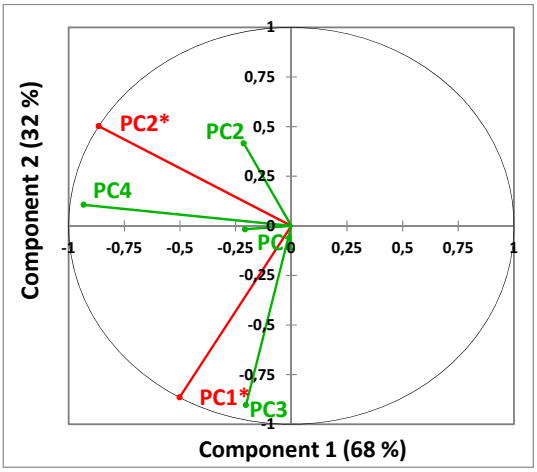
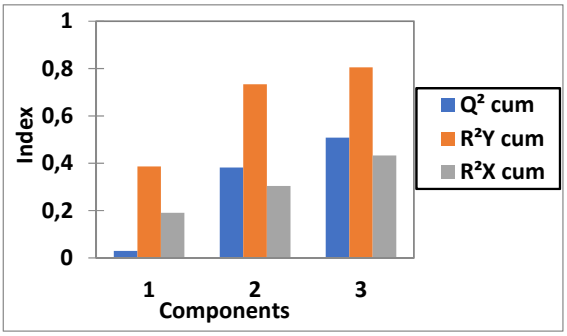
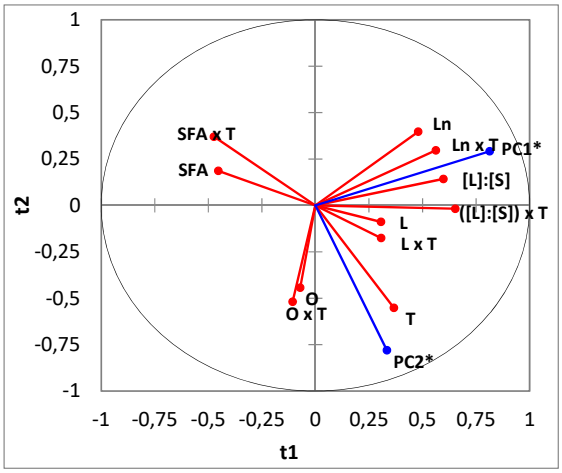


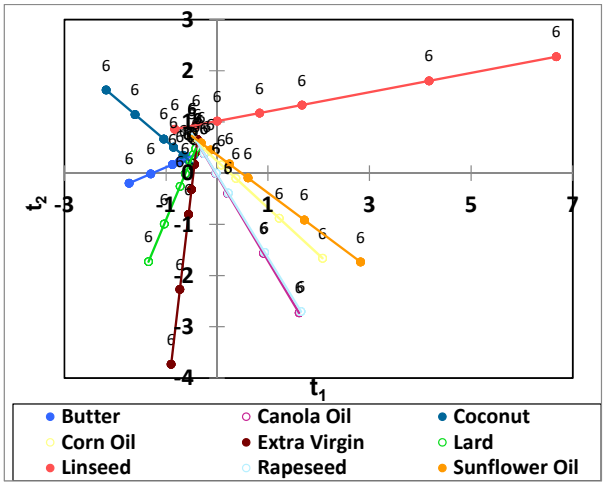
Fig. 5 Canonical Correlation Analysis (CCorA) plot of the contributions of culinary oil acylglycerol content and aldehydic LOP concentration SVs (PC1-4 and PC1*-PC2* respectively) towards CCorA components 1 and 2, which account for 68 and 32% of model variance respectively



(a)



(b)



(c)

Fig. 6 (a) Model quality as a function of the number of components for a mixed PCR/PLS-R analysis strategy involving the prediction of aldehyde-loading PC1* and PC2* SVs from autoscaled original lipid content and index variables (i.e. those unconverted to PC* SVs) and their interactions with the autoscaled orthogonal time-point variable; $Q^2 = 0.51$ and $R^2Y = 0.81$ for three PCs incorporated. (b) Correlations with *t* on the t_1 and t_2 axes for this mixed PCR/PLS-R model with frying oil acylglycerol fatty acid content/index and heating time-point variables (and the pre-selected first-order interactions of the acylglycerol content/index variables with time-point) as input variables (red), and PC1* and PC2* aldehydic LOP concentration-loaded SV output (blue) variables. Abbreviations: L, Ln, O and T, linoleoylglycerols, linolenoylglycerols, oleoylglycerols and heating time at 180°C (min.) respectively. [L]:[S] represents the molecular [linoleoylglycerol]:[SFA] ratio, and interactions of acylglycerol fatty acid index with the time variable are indicated as X x T, where X = acylglycerol fatty acid content or index. (c) Plots of model t_2 *versus* t_1 values for canola, coconut, corn, extra-virgin, linseed, rapeseed and sunflower oils, and butter and lard. Colour codes for each frying oil/media product are indicated [those for rapeseed (light blue) and canola (mauve) oils overlap substantially, and this is consistent with their very similar FA compositions). Datapoint numbers provided represent the number of replicates giving rise to each mean datapoint

Fig. 6 (c) displays a scores plot of t_2 *versus* t_1 for this mixed model analysis, and this clearly reveals that PUFA-rich oils all had positive t_1 scores, the magnitudes of which decrease with decreasing unsaturation status, i.e. in the order linseed > sunflower ~ corn > rapeseed ~ canola oils. Similarly, scores on t_2 for these oils were in the same order, with linseed oil having highly-positive, albeit heating time-dependent, values, whereas those for sunflower and corn, and rapeseed and canola oils, were increasingly lower. However, negative scores on t_1 were associated with the more thermo-oxidation resistant oils/ frying media, i.e. extra-virgin olive and coconut oils, butter and lard, with t_2 values increasing with decreasing unsaturation status (i.e. decreasing PSI value) of the products evaluated.

In an additional multivariate analysis model, PCA was performed only on the 90 min. sampling time-point dataset in

order to ascertain the ability of this approach to distinguish between sunflower, corn, canola, extra-virgin olive and MARO-1 oils with decreasing PSI values in that order (Fig. 7). This strategy was adopted to remove complications from the critical 'between-sampling-time-points' source of variation, and also to optimise the effectiveness of such a discriminatory model in the context of comparisons of the highest levels of aldehydic LOPs detectable, which are maximal at this final shallow frying simulation heating time-point for each oil assessed in this manner. For this analysis, *cis,trans*- and *trans,trans*-alka-2,4-dienals, 4,5-diepoxy-*trans*-2-alkenals, and 4-hydroy-/4-hydroperoxy-*trans*-2-alkenals all powerfully loaded on PC1* (loadings scores 0.85, 0.79, 0.84 and 0.73 respectively), whereas both *cis*- and *trans*-2-alkenals, and *n*-alkanals, all strongly loaded on PC2* (loadings scores 0.88, 0.82, and 0.81, respectively). These loadings scores' distribution between the two aldehydic LOP PC*s are fully consistent with those acquired on the full (0-90 min.) dataset, as outlined above. These data clearly revealed that it was possible to distinguish between each of the frying oils tested on the basis of their PSI values, i.e. highly significant clusterings for these products were observed. Indeed, PUFA-rich oils such as corn and sunflower oils (PSI values of 63.87 and 62.57 respectively) had positive PC1 SV values, but variable PC2 ones (approximately -3 to +2); canola oil with an intermediate PSI value (40.71) had virtually zero PC1 SV values, but its PC2 scores ranged from only -1.0 to +1.0. Moreover, the centroid value of the MUFA-rich extra-virgin olive oil product had PC1 and PC2 values of *ca.* -1.0 and +0.60, whereas that of the MARO-1 oil had a highly negative PC1 value, and a PC2 contribution which was close to zero. Variabilities in the individual sample contributions towards PC2 were lower for canola, extra-virgin olive and MARO-1 oils than those observed for PUFA-laden corn and sunflower oil products tested.

Finally, univariate multiple linear regression analysis of the autoscaled aldehyde concentration dataset according to the model specified in (1) found that, with the exception of *trans,trans*-alka-2,4-dienals and *cis*-2-alkenals, all aldehydic LOPs concentrations were very highly significantly influenced by heating time-point and also the first-order PSI x time-point interaction sources of variation ($p < 10^{-4}$), but not by the PSI value alone (Table II). This observation is again ascribable to variations in the autocatalytic lag times for and overall rates of aldehyde generation which are dependent on the relative contents of SFAs, oleoylglycerols, linoleoylglycerols and linolenoylglycerols in the culinary oils/frying media investigated. For *trans,trans*-alka-2,4-dienals, only the above interaction effect was found to be significant ($p < 10^{-4}$), whereas for *cis*-2-alkenals, the individual PSI and time-point sources of variation were highly statistically significant, but not the interaction one. Goodness-of-fit R^2 values for the abilities of these independent variables to predict aldehyde concentrations were strong for all aldehyde classes (R^2 = 0.73-0.91), with the exception of *cis*-2-alkenals, for which it was mediocre (R^2 = 0.54).

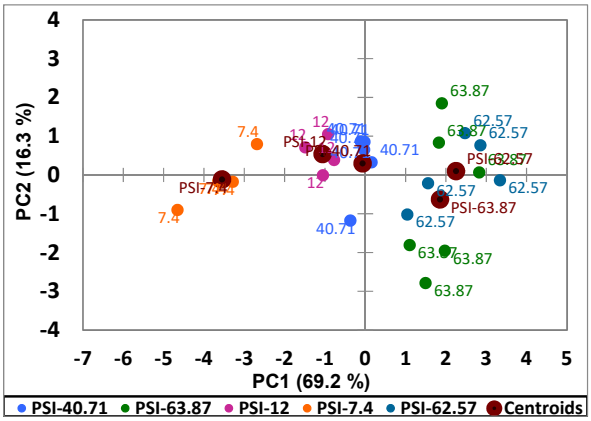


Fig. 7 PCA scores plot of PC2 *versus* PC1 for the aldehyde LOP product patterns determined in corn (green), sunflower (dark blue), canola (light blue), extra-virgin olive (purple) and MARO-I oils (orange) at the 90 min. time-points only. Datapoints are labelled with each oil's peroxidative susceptibility index (PSI) values, and the larger darker points represent oil product centroids. PCA was performed on an autoscaled aldehyde oil concentration dataset

TABLE II
STATISTICAL SIGNIFICANCE OF AND *P* VALUES FOR THE UNCORRELATED PSI AND HEATING TIME-POINT PREDICTOR VARIABLES, AND THAT OF THEIR FIRST-ORDER INTERACTION EFFECT, FOR A UNIVARIATE MULTIPLE REGRESSION MODEL INVOLVING AUTOSCALED CONCENTRATIONS FOR EACH CLASS OF ^{1}H NMR-DETECTABLE ALDEHYDIC LOP (1). ALSO LISTED IS THE GOODNESS-OF-FIT R^2 VALUE FOR EACH MULTIPLE REGRESSION MODEL DEVELOPED. ABBREVIATIONS: NS, NOT STATISTICALLY SIGNIFICANT

Aldehyde Class	PSI Value (P_i)	Time-Point (T_j) (min.)	P_i x T_j Interaction (PT_{ij})	R^2
***trans*-2-Alkenals**	ns	$<10^{-4}$	$<10^{-4}$	0.779
***trans,trans*-Alka-2,4-dienals**	ns	ns	$<10^{-4}$	0.816
4.5-Diepoxy-*trans*-2-alkenals	ns	$<10^{-4}$	$<10^{-4}$	0.762
4-Hydroxy-/Hydroperoxy-*trans*-2-alkenals	ns	$<10^{-4}$	$<10^{-4}$	0.913
***cis,trans*-Alka-2,4-dienals**	ns	$<10^{-4}$	$<10^{-4}$	0.843
***n*-Alkanals**	ns	$<10^{-4}$	$<10^{-4}$	0.732
***cis*-2-Alkenals**	1.15 x 10^{-4}	$<10^{-4}$	ns	0.538

IV. DISCUSSION

Results acquired in this study clearly demonstrate that ^{1}H NMR-linked multivariate analysis with strategies involving PCR serve as valuable chemometrics approaches for (1) modelling the time-dependent generation of aldehydic LOPs in heated culinary oils during laboratory-simulated standard

shallow-frying processes, and (2) deduction of their parental fatty acid sources therein, and thenceforth their corresponding CHPD and/or HPM precursors generated. Indeed, we found that the PC2* SV PCR variable, which was predominantly powerfully loaded with the structurally-simpler *cis*- and *trans*-2-alkenal, and *n*-alkanal aldehydic LOPs, was strongly linked to PC's 2 and 4 (the former significantly positively and negatively loaded with culinary oil % MUFA and SFA contents respectively, the latter the completely orthogonal heating time at 180^{o}C PC4 SV variable), and these classes of aldehydes are the predominant ones generated from oleoylglycerol HPM sources, although PUFA-derived CHPDs also serve as major precursors of these secondary LOP toxin classes (trans-2-octenal and n-hexanal being major products) via the fragmentation of such hydroperoxide species [4], particularly at the high temperatures employed for frying purposes. Indeed, all of the significantly dependent PC1* aldehyde output variables (*trans,trans*- and *cis,trans*-alka-2,4-dienals, 4,5-epoxy-*trans*-2-alkenals and 4-hydroxy-/4-hydroperoxy-*trans*-2-alkenals, as noted above) exclusively arise from the thermally-mediated degradation of PUFA-derived hydroperoxides (CHPDs) and not oleoylglycerol-derived HPMs. However, the PC1* SV variable, which was strongly loaded with the more structurally- complex, di-unsaturated and substituted aldehydic LOPs (*trans,trans*-alka-2,4-dienals, and 4,5-epoxy-trans-2-alkenals derived therefrom, together with 4-hydroperoxy-/4-hydroxy-*trans*-2-alkenals), were found to be statistically correlated with the SVs of PC3, and to a lesser extent, those of PC1. This observation is consistent with the very strong loadings of both oil % linolenoylglycerol content and [linoleoylglycerol]:[SFA] ratio on PC3, since such aldehydes arise only from PUFAs available in such products. Indeed, the peroxidation of linolenoylglycerols gives rise to the generation of 4-hydroperoxy-*trans*-2-hexenal and thenceforth its 4-hydroxy-substituted reduction product [4], [5].

Although linseed oil is not employed as a frying oil in view of its high risk status, it was included in this study for reference purposes, i.e. in order to provide valuable molecular information on the pattern of aldehydic LOPs generated from a linolenoylglycerol-rich vegetable oil (linolenic acid content 53.1% for the oil product evaluated here).

Plots of PC1* *versus* PC4 SVs (the latter representing the exclusively autoscaled time-points) for MUFA- and PUFA-rich extra virgin olive and sunflower oils, respectively (Fig. 4), revealed that, subsequent to a lag phase, the former FA source contributed positively towards this output PC* at the low heating time-points, but negatively so at the higher ones, an observation arising from its high peroxidation-resistant oleoylglycerol content [>75% (w/w)]. However, a corresponding plot for sunflower oil showed a substantial increase in this parameter following a similar lag-phase. Moreover, PC2* was found to increase with PC4 SVs for both oils subsequent to a longer lag-phase.

Moreover, PC1* variable SVs appeared to be less dependent on the orthogonal PC4 time-point ones than those of PC2*, and this reflects the much greater autocatalytic lag periods involved in the peroxidation of MUFAs at standard high temperature frying temperatures (and therefore their greater demand for heat input energy) than those required by PUFAs (predominantly linoleoylglycerols in sunflower oil), i.e. this observation appears to reflect the greater susceptibility of PUFAs to peroxidation over that of MUFAs during these laboratory shallow-frying mimic experiments.

Overall, exposure of PUFA-laden culinary frying oils to shallow-frying episodes (laboratory-simulated or actual) generates very high concentrations of toxic aldehydic LOPs, which are likely to engender chronic human health threats, particularly in subjects consuming large quantities of fried foods, e.g. ≥ 4 fried meals per week [13]. Notwithstanding, the results presented in this study also clearly demonstrated that predominantly MUFA-rich, PUFA-deplete oils such as the algae frying oils explored here, and, to a lesser extent, extra-virgin olive oil, were particularly resistant to oxidation under the experimental conditions employed, i.e. much more so than PUFA-rich oils such as linseed, sunflower and corn oils. Indeed, only very low levels of toxic aldehydes were formed in the algae frying oils at commonly-utilised shallow-frying time-points of 5-20 min.

The adverse toxic effects of LOPs produced in culinary oils during typical frying practices have, to date, received little or no attention from the scientific community - this information has been available to researchers, nutritionists, frying oil producers and health authorities for more than 25 years [6], [7]. The multivariate modelling strategies developed here may serve to enhance our understanding of the potential deleterious health effects presented by aldehydes generated in frying media when exposed to such thermal stressing episodes. The most common aldehydes derived from the oxidation of linoleoylglcerols include acetaldehyde, pentanal, hexanal, *trans*-2-heptenal, *trans*-2-octenal and *trans*-2-nonenal, whereas those derived from oleoylglycerols are predominantly nonanal and *trans*-2-decenal. Additionally, more complex aldehydes such as 4,5-epoxy-*trans*-2-alkenals and *cis,trans*- and *trans,trans*-alka-2,4-dienals arise from the peroxidation of linoleoylglycerols.

The FA acylglycerol sources of such LOPs, and the generation of higher levels of more toxic aldehydic LOP species, are most readily predictable from (1) the mixed PCR/PLS-R model plots of t_2 versus t_1 [as shown in Fig. 6 (c)], and (2) PCA scores plots for oils with increasing PSI values. Indeed, the latter plots readily distinguish between different classes of culinary oils commonly employed for frying purposes, and also confirms that the risk status of their use for this purpose clearly increases with increasing PUFA content; i.e. those with PC1* values > 1 are of high risk (corn and sunflower oils), those with values *ca.* 0 are of intermediate risk (canola oil), whereas those with such values significantly < 0 (MARO-1 and extra-virgin olive oil) are of a relatively low risk status.

There is a considerable amount of supporting evidence available in the scientific literature which demonstrates that such LOPs, particularly aldehydes, exert very high levels of toxicity [12], [13]. Indeed, aldehydes and further LOPs are

much more toxic than *trans*-fatty acids, the potential adverse health effects of which have been much publicised. These agents act as potent toxins since they are extremely chemically-reactive. Indeed, they cause damage to important biomolecules such as DNA; indeed, α,β-unstaurated aldehydes have the ability to directly alkylate DNA base moieties [16].

In 1998, it was first demonstrated that typical aldehydes arising from the heating of culinary oils according to frying practices are absorbed from the gut into the systemic circulation *in vivo* [17], where they have access to and therefore exert damage to cells, tissues and essential organs.

Previously reported investigations have demonstrated that such aldehydes exert a very wide range of concentration-dependent cellular stresses. Indeed, their deleterious health effects include the induction and perpetuation of atherosclerosis and its cardiovascular disease sequelae [18]-[20]; potent mutagenic and carcinogenic properties [16], [21]-[24]; powerful pro-inflammatory effects at very low concentrations [25], [26]; teratogenic properties (embryo malformations during pregnancy [27]); gastropathic properties (peptic ulcers) following dietary ingestion [28]; neurotoxic actions [29]; and impaired vasorelaxation connected to the induction of significant systolic blood pressure rises [30].

V. Conclusions

Multivariate ^{1}H NMR-linked chemometrics analysis serves as a valuable strategy for (1) modelling the time-dependent generation of aldehydic LOPs in heated frying media during standard shallow-frying practices, and (2) the identification of their parent FA sources therein. These observations are of much relevance to the adverse health effects associated with the dietary consumption of such LOPs, and will support future investigations focused on the public health hazards presented by the human consumption of foods fried in LOP-containing, UFA-rich edible oils. In this manner, results acquired here may permit the future prediction of the risk status of ingested fried foods from the FA compositions of oils and other FA-containing matrices used for their frying; these oil aldehydic LOP sources are readily taken up by foods such as potato chips fried therein, and hence are available for human consumption [12], [13]. Such evaluations should be made along with more generalised ones regarding the status of frying practices and the acylglycerol frying media involved (for example, deep- *versus* shallow-frying processes, home domestic *versus* restaurant location, frying temperatures employed, oil reuse status and storage conditions prior to frying use, etc.).

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