High-resolution 1H NMR analysis of continuous and discontinuous thermo-oxidative susceptibility of culinary oils during frying at 180°**C**

High-resolution ^{1}H NMR analysis of continuous and discontinuous thermo-oxidative susceptibility of culinary oils during frying at 180 °C

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Abstract

Lipid oxidations products (LOPs) are reactive mutagenic and carcinogenic species known to be generated in thermally stressed culinary oils. Mapping the evolution of LOPs in culinary oils exposed to standard frying practices − both continuous and discontinuous thermo-oxidation − at 180 °C is vital to our understanding of these processes, and to the development of scientific solutions for their effective suppression. Modifications in the chemical compositions of the thermo-oxidised oils were analysed using a high-resolution proton nuclear magnetic resonance (^{1}H NMR) technique. Research findings acquired showed that polyunsaturated fatty acid (PUFA)-rich culinary oils were the most susceptible to thermo-oxidation. Consistently, coconut oil, which has a very high saturated fatty acid (SFA) content, was highly resistant to the thermo-oxidative methods employed. Furthermore, continuous thermo-oxidation produced greater substantive changes in the oils evaluated than discontinuous episodes. Indeed, for 120 min thermo-oxidation durations, both continuous and discontinuous methods exerted a unique impact on the contents and levels of aldehydic LOPs formed in the oils. This report exposes daily used culinary oils to thermo-oxidation, and therefore, it permits assessments of their peroxidative susceptibilities. It also serves as a reminder to the scientific community to investigate approaches for suppressing toxic LOPs generation in culinary oils exposed to these processes, most notably those involving their reuse.

Keywords: Continuous thermo-oxidation, Culinary oil, Discontinuous thermo-oxidation, Frying temperature, α,β-unsaturated aldehydes (α,β-UAs)

1. Introduction

1.1. Background

Highly polyunsaturated fatty acid (PUFA)-rich culinary oils have long been considered as functional foods and nutraceuticals [1]. Indeed, these beneficial health effects have previously been supported by a broad spectrum of research investigations. These include the roles of PUFAs in certain key cardio-protective biochemical pathways through their anti-arrhythmic, anti-atherogenic, anti-inflammatory, anti-thrombotic, and hypolipidemic activities *in vivo*. These effects may reduce the risks of cardiovascular diseases, osteoporosis, diabetes, and ultimately carcinogenesis at the cellular level [1]. Consequently, food scientists and nutritionists continue to recommend PUFA-rich culinary oils as important components of a healthy diet [2]. Moreover, consumption of essential (such as linoleic and linolenic acids) fatty acids (FAs)-rich culinary oils and omega-3, and omega-6 FAs supplements have been increasing [3,4].

Notwithstanding, the susceptibility of PUFA-rich culinary oils to oxidation at room and storage temperatures [5], along with variable cooking temperatures of 70 and 100 °C [6], 180 °C [7−12], 160 °C [13], 170 °C [14], 190 °C [15−17], and 250 °C [7,18] will

clearly negate any possible health benefits offered by PUFA-rich culinary oils in view of. Even so, lipid oxidation products (LOPs), which are chemical oxidation and fragmentation products derived from the oxidation of monounsaturated fatty acid (MUFA)-rich and PUFA-rich culinary oils, have been shown to also be detectable in unheated culinary oils [9,10,19], albeit at much lower levels than those found in the thermally stressed products. Whilst being cytotoxic and genotoxic, evidence of passive transfer of LOPs from thermo-oxidised culinary oils into commercially-available fried foods have also been reported by [2,8,9,20,21].

LOPs comprise a group of chemically-reactive agents, either primary, secondary or tertiary, the most toxic secondary fragmentation products comprising α,β-unsaturated aldehydes (α,β-UAs) which are electrophilic alkylating agents. Primarily, conjugated hydroperoxydienes (CHPDs) and unconjugated hydroperoxymonoenes (HPMs) are formed from free radical reactions involving the chemical interaction of lipidic carbon-centred radicals with dioxygen under catalysis [1,22]. [22] explained that the facile abstraction of hydrogen atoms (H•) from the bis-allylic methylene group of linoleoylglycerols may arise from their exposure to light or radical species of sufficient reactivity, and these processes lead to oxidative damage and corresponding LOPs formation. This process is known to be facilitated by the low bond dissociation energy that exist between the C–H bonds of the methylene group [22]. LOPs have been implicated as one of the major underlying factors in disease ailments such as atherogenicity, mutagenicity and carcinogenicity, congenital malformations, gastropathy, hypersensitivity, inflammation, and teratogenicity [23]. This report focusses on thermo-oxidation of culinary oils in the presence of oxygen at 180 °C, most notably for oils and fats which are less commonly employed as frying media, with each product tested undergoing both continuous and discontinuous frying episodes.

As part of the creation of a directory for the thermo-oxidative dynamics of several culinary oils of varying unsaturation degree, the current study is built on previous investigations conducted on the continuous and discontinuous thermo-oxidation of coconut, olive, rapeseed, and sunflower oils by [9]; which was the first scientific report on this subject, and also subsequently on ghee, groundnut, extra virgin olive, macadamia, sesame, corn, and walnut oils [12]. According to both studies, susceptibilities of the culinary oils to thermo-oxidation, as manifested by chemical changes to triacylglycerol acyl chains and the evolution of LOPs following a

300 min (continuous) and 480 min (discontinuous) thermo-oxidation duration, were in order of PUFA-rich sunflower > MUFA-rich olive and rapeseed > saturated fatty acid (SFA)-rich coconut oils [9], and PUFA-rich sesame, corn, walnut > MUFA-rich groundnut, extra virgin olive, macadamia > SFA-rich ghee oils [12]. However, at the end of 120 min thermo-oxidation duration, the discontinuous episodes led to higher levels of aldehydic LOPs than those reported for continuous thermo-oxidation in both studies [9,12].

On reconsideration of the scientific conclusions drawn by [9,12] for the extensive varying degrees of unsaturation status of the culinary oils studied, this report aims to investigate the chemical changes to the acyl chains of fatty acids, along with the resultant evolution of LOPs in continuous and discontinuous thermo-oxidised coconut, rice bran, rapeseed, cold press rapeseed, grape seed, and hemp seed oils. Furthermore, the research questions originally outlined in [9] have also now been addressed in the current study by the use of culinary oils that were not investigated by [9,12]. It should also be noted that the coconut and rapeseed oil brands that were reported by [9] were different to those investigated in this study.

2. Materials and methods

2.1. Culinary oil samples

Six culinary oils of varying unsaturation degree were procured from a local supermarket in London, United Kingdom. These were coconut, rice bran, rapeseed, cold press rapeseed, grape seed, and hemp seed oils. These products were stored under dark room storage, and under the same ambient temperature conditions, for not more than 72 h prior to analysis. The percentage compositions of the key FA groups of the culinary oils obtained from their product labels was compared with data derived from the ^{1}H NMR measurements and is presented in Table 1. Accordingly, coconut oil was classified as SFA-rich oil. Rice bran, rapeseed, and cold press rapeseed oils were classified as MUFA-rich oils. Moreover, grape seed and hemp seed oils were classified as PUFA-rich products (Table 1).

It should also be noted that on the product labels of the procured oils, coconut oil was advertised as a cold press, organic, virgin oil. Rice bran and rapeseed oils were described to be 100% pure culinary oils. Furthermore, rice bran oil was extra-cold filtered, and cold press rapeseed oil was triple filtered. Hemp seed oil was described on its product

Table 1. A comparison between product label-specified (% w/w) and ¹H NMR-determined contents of FA groups (molar %), and IVs of culinary oils.

Culinary oil	Product label (% w/w)								¹H NMR-derived (molar %)							
	SFA	MUFA		PUFA			UFA	IVᵃ	SFA	MUFA		PUFA			UFA	IVᵃ
		ω-3	O	L	Ln	L+Ln	O+L+Ln			ω-3	O	L	Ln	L+Ln	O+L+Ln	
Coconut oil	87.00	—	—	—	—	—	—	—	96.32 ± 0.12	—	3.16 ± 0.13	0.52 ± 0.13	—	0.52 ± 0.01ᵃ	3.68 ± 0.12ᵃ	15.08 ± 0.06
Rice bran oil	22.83	—	39.00	—	—	34.78	73.78	—	25.06 ± 0.27	4.80 ± 0.23	40.75 ± 0.21	32.62 ± 0.11	1.57 ± 0.09	34.19 ± 0.05	74.94 ± 0.27	90.17 ± 0.05
Rapeseed oil	8.00	—	—	—	—	—	—	—	8.40 ± 0.10	10.65 ± 0.18	61.95 ± 0.13	20.78 ± 0.07	8.87 ± 0.10	29.65 ± 0.05	91.60 ± 0.10	107.08 ± 0.45
Cold press rapeseed oil	6.60	—	59.3	—	—	29.30	88.60	—	8.00 ± 0.46	10.38 ± 0.61	63.89 ± 0.61	18.94 ± 0.33	9.17 ± 0.18	28.11 ± 0.15	92.00 ± 0.46	106.89 ± 0.10
Grape seed oil	11.20	—	—	54.00	—	—	—	—	15.34 ± 0.13	—	19.15 ± 0.86	65.51 ± 0.74	—	65.51 ± 0.74ᵃ	84.66 ± 0.13ᵃ	125.39 ± 0.36
Hemp seed oil+	9.40	18.6	12.70	—	—	77.90	90.60	—	12.40 ± 0.16	15.74 ± 0.05	15.24 ± 0.23	56.04 ± 0.21	16.32 ± 0.02	72.36 ± 0.19	87.60 ± 0.16	144.69 ± 0.14

ᵃ Indicates an absence of Ln. +ω-6 content is 57.7% (w/w) according to the product label. ᵃIV is expressed in IV units. Abbreviations: SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; UFA, unsaturated fatty acid; ω-3, omega-3 fatty acid; O, oleic acid; L, linoleic acid; Ln, linolenic acid; IV, iodine value. The values derived from ¹H NMR data are presented as mean ± standard deviation molar % (FA) and mean ± standard deviation unit of IV for oils.

label to be derived from a cold press process, and rich in 18.6% omega-3 (ω-3) and 57.7% omega-6 (ω-6) FAs. Also noted on the product labels was the presence of some naturally occurring compounds in the oils. Rice bran oil was stipulated to contain 1750 mg natural plant sterols equivalents, 915 mg y-oryzanol, and 1090 mg combined plant sterols and stanols. Rice bran oil also contained 12 mg vitamin E per 500 mL oil. Considering the 12 mg per day legal daily Nutrient Reference Value (NRV) of this nutrient, rice bran oil therefore constituted 100% of the NRV for vitamin E.

2.2. Thermal stressing of culinary oil samples

Thermal stressing of culinary oil products was conducted in the presence of atmospheric O_2 at 180 °C using standard heating or cooking practices. In practice, the same brand of 100 mL clean and dry beakers and electronically controlled hot-plate (230 V, 50 Hz, 750 W; Stuart heat-stir (UK), Model SB162) were used for thermo-oxidation experiments. A ± 0.1 g accuracy electronic balance (Mettler (UK), Model AT261) was used to measure the exact amount of culinary oil to be thermally stressed. This was necessary for comparative, quantitative ¹H NMR evaluations [7,9]. As a precaution, all glassware was dried under nitrogen (N_2) prior to their use. Culinary oil bottles were completely sealed after discharging the amount of oil required for thermo-oxidation experiments.

2.3. Continuous thermo-oxidation

Continuous thermo-oxidation of culinary oils was performed according to the protocol primarily described by [9]; and with a minor modification as described in [12]. A 20 g mass of culinary oil was thermally stressed at 180 °C for 300 min. Aliquots (0.30 mL) of oils were sampled hourly for ¹H NMR measurement. As a precaution, samples were rapidly cooled on ice in order to suppress further heat-mediated oxidising actions towards unsaturated fatty acids (UFAs). There was no oil replenishment in this study, and continuous thermo-oxidation episodes were replicated 3-fold for each culinary oil investigated [9,12].

2.4. Discontinuous thermo-oxidation

The 480 min discontinuous thermo-oxidation period instigated for culinary oils was based on the method initially described by [9] and modified somewhat by [12]. This employed 20 g of culinary oil for the discontinuous thermo-oxidation study. Aliquots of 0.30 mL of thermo-oxidised culinary oil

samples were rapidly cooled on ice (sampled culinary oils withdrawn were not replenished). Discontinuous thermo-oxidation episodes were repeated with n = 3 replicates [9,12].

2.5. ^{1}H NMR measurements

^{1}H NMR analysis of the FA compositions and LOP concentrations in thermally stressed culinary oils were monitored on a Bruker Avance III 600 MHz (TXI) spectrometer (Kingston University, London) operating at 600.13 MHz frequency. The operating and acquisitional parameters of the spectrometer were as reported by [9].

The preparation of 0.30 mL volumes of sampled oils for ^{1}H NMR measurements was based on the technique primarily described by [7]; with a minor modification described by [9]. Sampled culinary oils were prepared and placed into a 5-mm diameter NMR tube (Norrell HT, GPE Scientific). The internal standard used for the quantification of NMR-detectable LOPs in thermally stressed oils was 1,3,5-tribromobenzene (TBB). This preparation was based on the protocol by [9]. Chemical shift (δ values) of all ^{1}H NMR detectable resonances detectable were referenced to tetramethylsilane (TMS) (δ = 0.000 ppm), residual C^2HCl_3 (δ = 7.283 ppm) and/or TBB (δ = 7.537 ppm) [9].

2.6. Analysis of ^{1}H NMR spectra

Quantification of all ^{1}H NMR-detectable agents was conducted according to the principle that the area of the signal resonances generated on the ^{1}H NMR spectrum is directly proportional to the number of ^{1}H giving rise to them [24]. Primarily, ^{1}H signal intensities of all minor compounds found were expressed relative to that of the TBB quantitative internal standard.

2.6.1. Analysis of major compounds and IVs

Major compounds identified and characterized were all ^{1}H NMR-distinguishable FAs. The molar % of these FA classes were evaluated from their corresponding signal resonances in the ^{1}H NMR spectral profiles of culinary oils. Olefinic protons of acyl groups detectable in the ^{1}H NMR spectra of each oil were evaluated and expressed as IVs. The quantification of acyl groups and IVs was based on the equations proposed by [24,25]. Deductions of FA compositions were computed in three replicates, and the results are presented as mean ± standard deviation (molar % for acyl groups and IV units) [9].

2.6.2. Analysis of minor compounds

The signal resonances of all minor compounds were assigned according to the scientific report by [24,26]. The assignment of sn-1,2-diglyecerides was based on the ^{1}H and ^{13}C NMR spectra, as well as Total Correlation Spectroscopy (TOCSY) data on encapsulated marine oil supplements presented by [27]. The identification of minor natural compounds in culinary oils was necessary for the characterisation of the oils [10−12,21].

2.6.3. Analysis of LOPs

The signal resonances of LOPs detected in the ^{1}H NMR spectra of culinary oils were assigned based on previously available literature [8−12,21,24, 28−30]. Amongst all the different classes of LOPs identified and characterised, only aldehydic LOPs were quantitatively expressed.

To quantify aldehydes, the integral values of their respective resonances on the δ-scale of the ^{1}H NMR spectra were computed relative to the resonances of TBB. This was achieved by integrating the resonances of TBB at δ = 7.537 ppm and calibrating it to 3, which is the representation of its total number of protons on its chemical structure (Fig. 1). This calibrated value of TBB then served as the baseline for the integration of all the resonances of aldehydes. The concentrations of aldehydes were then evaluated by multiplying the integral values of each individual aldehyde by 1.1 mM concentration of TBB prepared in the 0.6 mL oil-TBB-C^2HCl_3 mixture in the 5 mm diameter NMR tube. The final concentrations of aldehydes were, therefore, computed in three replicates, and these were presented as mean ± standard deviation mM of aldehydes presented in mM. The mathematical computations of aldehydes was adapted from [7] and then modified by [9]. It has also been used in publications by [10−12,21].
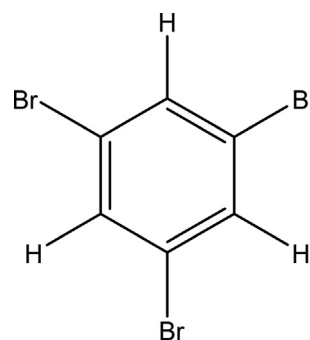

Fig. 1. The chemical structure of 1,3,5-tribromobenzene (TBB) depicting its three number of protons.

2.7. Statistical analysis of experimental data

For each aldehyde determined, the univariate experimental design for this study was classified as a 3-factor analysis-of-variance (ANOVA) system with different types of Culinary Oils (O_i), Sampling Time-Points (T_j) and Simulated Heating Episode Classifications (S_k) representing fixed qualitative explanatory effects at 10, 6, and 2 levels respectively (the Between-Time-Points effect was included as a qualitative rather than a quantitative variable in view of the non-linearity of relationships between all aldehyde concentrations and heating times at 180 °C; this is a major requirement assumption for the analysis-of-covariance (ANCOVA)). Also incorporated in the model were the three first-order Oil Type × Time-Point (OT_{ij}), Oil Type × Heating Episode Classification (OS_{ik}) and Time-Point × Heating Episode Classification (TS_{jk}) interaction effects. The mathematical model for this experimental design is shown in Equation (1), where y_{ijkl}, μ and e_{ijkl} represent output variable observations (individual aldehyde concentrations), the mean value in the absence of all contributory sources of variation, and fundamental error respectively. This univariate ANOVA analysis was performed for each of the 11 aldehyde analytes identified and determined individually.

$$y_{ijkl} = \mu + O_i + T_j + S_k + OT_{ij} + OS_{ik} + TS_{jk} + e_{ijkl} \qquad (1)$$

A similar experimental design and analysis was conducted on the oil FA content parameters (including IVs), although in this case there were only 8 output variables for consideration. All the above-mentioned descriptions were run using XLSTAT2016 (Addinsoft, Paris, France).

3. Results and discussion

3.1. Characterisation of the ^{1}H NMR spectra of unheated culinary oils

3.1.1. Major compounds

The FA profiles of major acyl groups of unheated culinary oils is shown in Fig. 2, and description of the assignments of the bulk resonances, which includes their chemical shift values, multiplicities, and characteristic functional groups of the FA acyl chains are provided in the Supplementary Information Table S.1 (https://www.jfda-online.com/cgi/editor.cgi?article=3439&window=additional_files&context=journal). Assignments of these ^{1}H NMR signals were made according to [24,31].

Minor compounds detectable in the culinary oils examined, e.g., sterols and stanols, etc., are documented and discussed in the Supplementary Information section SI.1.2 (https://www.jfda-online.com/cgi/editor.cgi?article=3439&window=additional_files&context=journal).

Signal A, located at 0.783−0.846 ppm, is a triplet signal ascribable to the overlapping resonances of the terminal methyl group protons ($-CH_3$) of all saturated, oleic, and linoleic FA acyl groups (Fig. 2(i)). Expanding signal A revealed the dominant signal, which was a composite oleoylglycerol and saturated FA acylglycerol signals ($O + S$), in SFA-rich coconut, and MUFA-rich rice bran, rapeseed, and cold press rapeseed oils (Fig. 2(ii)). However, the $O + S$ resonance was lower than that of linoleoylglycerols (Ls) in PUFA-rich grape seed and hemp seed oils (Fig. 2(ii)).

The bulk signal resonance of signal B, which is a triplet and located at $\delta = 0.883−0.927$ ppm, is ascribable to the terminal acyl chain−CH_3 group of ω-3 acyl groups (Fig. 2(ii)). The key chemical shift difference between signals A and B lies in the proximity of their terminal −CH_3 acyl groups to the double bond of their acyl chains. Signal B was indeed prominent in rapeseed, cold press rapeseed, and hemp seed oils, an observation reflecting their relatively high linolenoylglycerol contents. Signal B was ^{1}H NMR-undetectable in coconut and grape seed oils (Fig. 2(ii)), as expected.

Signal C is a triplet centred at $\delta = 1.119−1.344$ ppm, which is ascribable to the bulk methylene group protons ($-(CH_2)n-$) of acyl chains (Fig. 2(i)). Amongst the six culinary oils analysed, higher intensities of composite linoleoylglycerol and oleoylglycerol signals ($L + O$) were observed with a simultaneous lowered intensity of the saturated FA acylglycerol signal (S) in rapeseed, cold press rapeseed, grape seed, and hemp seed oils (Fig. 2(ii)). However, coconut and rice bran oils were characterized by a higher intensity S and lower intensity $L + O$ resonances. The intensity of the oleoylglycerol signal (O), which was ^{1}H NMR-undetectable in coconut oil, was the lowest in rice bran, grape seed, and hemp seed oils. In contrast, signal O was higher than that of S, but lower than that of $L + O$ in rapeseed and cold press rapeseed oils (Fig. 2(ii)).

The β-methylenic group protons ($-OCO-CH_2-CH_2-$) of all acyl groups are responsible for signal D. This resonance is a definitive multiplet and is positioned at $\delta = 1.491−1.596$ ppm (Fig. 2(i)).

Signal E is attributable to the *mono*-allylic protons β-to −CH=CH− units ($-CH_2-CH=CH-$) of all acyl groups (Fig. 2(i)). When expanded, signal E, located at $\delta = 1.896−2.041$ ppm, is a multiplet that
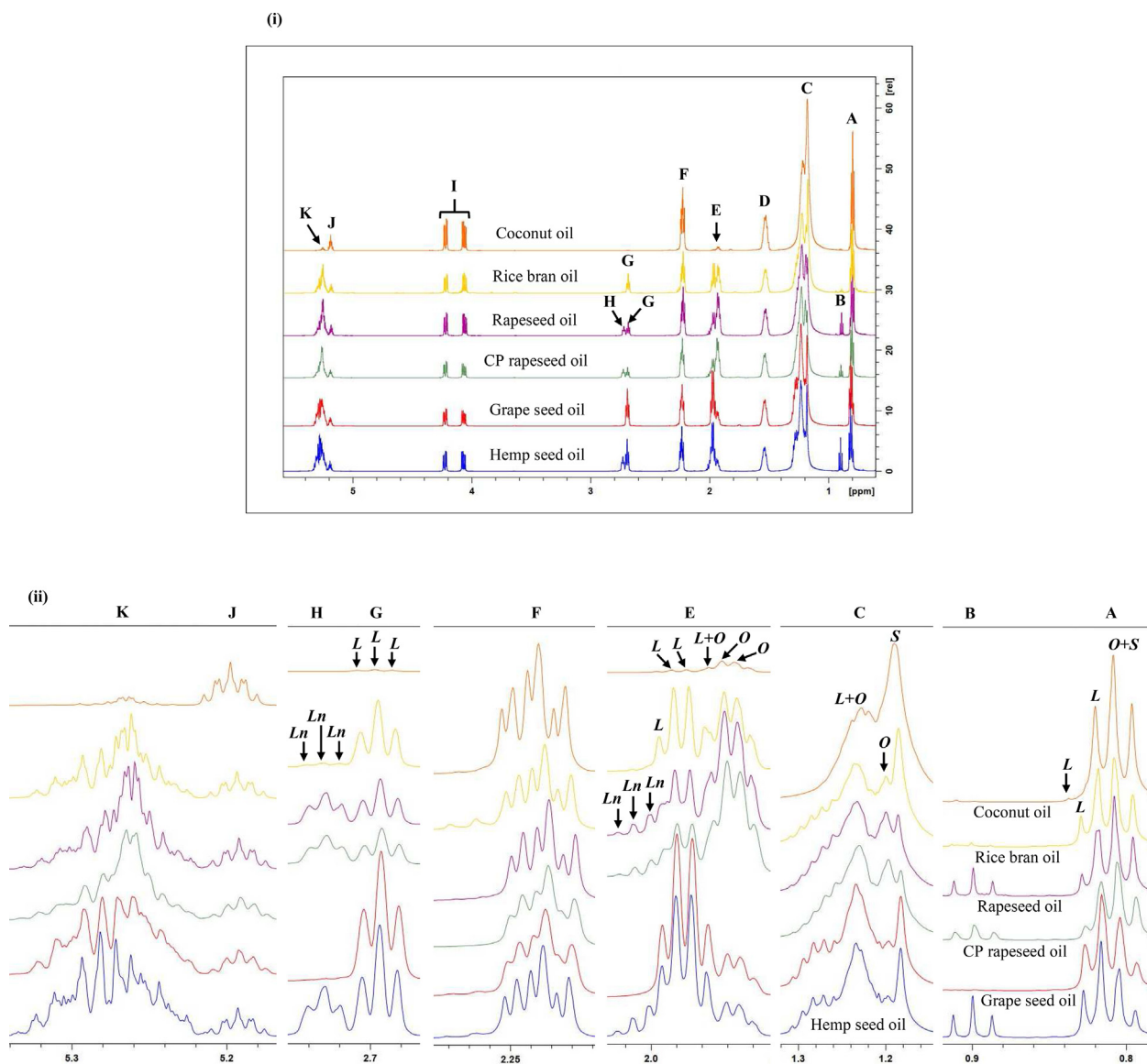
Fig. 2. ^{1}H NMR spectra showing major acylglycerol FA chain resonances present within the 0.0–5.4 ppm regions of unheated culinary oils. Letter assignments of resonances correspond to those provided in the Supplementary Information Table S.1.^{1}H NMR spectra showing expanded regions of resonances A, B, C, E, F, G, H, J, and K present within the 0.0–5.4 ppm regions of unheated culinary oils. Abbreviations: S, saturated fatty acid acylglycerol signal; O, oleoylglycerol signal; L, linoleoylglycerol signal; Ln, linolenoylglycerol signal; O + S, composite oleoylglycerol and saturated fatty acid acylglycerol signal; L + O, composite linoleoylglycerol and oleoylglycerol fatty acid acylglycerol signal; CP, cold press. Letter assignments of resonances correspond to those provided in the Supplementary Information Table S.1.

comprises a combination of linolenoylglycerol (Ln), L, O, and L + O resonances (Fig. 2(ii)). The intensity of the Ln resonance was in decreasing order of magnitude: hemp seed > rapeseed > cold press rapeseed > rice bran oils (Fig. 2(ii)). Ln was completely ^{1}H NMR-undetectable in coconut and grape seed oils (Fig. 2(ii)), as expected. Amongst all oils explored here, coconut oil was characterised by the lowest intensities of the L, O, and L + O signals (Fig. 2(ii)). Rapeseed and cold press rapeseed oils, and grape seed and hemp seed oils, were

distinguished by the highest and lowest intensities of O, respectively (Fig. 2(ii)). Clearly, the degree of unsaturation of the oils contributed to the variations of intensities of L, O, and L + O in the culinary oils (Fig. 2(ii)), as expected.

Signal F is attributable to the −OCO−CH$_2$− α-methylene group protons of all acyl groups (Fig. 2(i)). As a series of closely superimposing triplets, this resonance's intensity was strikingly similar in rice bran, rapeseed, and hemp seed oils, and in cold press rapeseed and grape seed oils

(Fig. 2(ii)). The highest relative intensity of signal F was that observed in coconut oil (Fig. 2(ii)) − the reason being that coconut oil has high levels of medium-chain FAs and much lower levels of long-chain FAs.

Signals G and H are doublet of doublet (apparent triplet) signals attributable to *bis*-allylic protons (−HC−CH_2−CH−) of diunsaturated ω-6 and triunsaturated ω-3 acyl groups of linoleoyl- and linolenoylglycerols, respectively (Fig. 2(i), Supplementary Information Table S.1 (https://www.jfda-online.com/cgi/editor.cgi?article=3439&window=additional_files&context=journal)). Signal G at δ = 2.667−2.718 ppm is vital for quantifying the molar % of linoleic acyl groups (Fig. 2(ii)). Moreover, signal H, located at δ = 2.718−2.769 ppm, is important for the quantification of linolenic acid acyl chains (Fig. 2(ii)). As expected, signal H was ^{1}H NMR-undetectable in coconut and grape seed oils (Fig. 2(ii)). The degree of unsaturation of the culinary oils is reflected by the intensities of the *L* and *Ln* signals combined (Fig. 2(ii)). Furthermore, the intensities of signals G and H, reflecting *L* and *Ln* oil contents respectively, are critical for evaluating the relative susceptibilities of culinary oils to thermo-oxidation.

Signal I is attributable to the protons on carbon atoms 1 and 3 of the glycerol backbone functions of triacylglycerols (−CH_2OCOR). Characterised as an ABX coupling pattern when considered with the adjacent 2-glycerol backbone position −CH− proton of these species (signal J), this signal is located at δ = 4.032−4.266 ppm (Fig. 2(i)). Amongst all culinary oils investigated, the relative intensity of signal I was highest in coconut oil and lowest in cold press rapeseed oil (Fig. 2(i)), the former being attributable to the abundance of medium-rather than long-chain FAs in this oil's triacylglycerol adducts.

As noted above, signal J is ascribable to position 2 of the glycerol backbone proton (>CHOCOR) and is the X component of the ABX coupling pattern of this system multiplet, δ = 5.161−5.219 ppm) in the ^{1}H NMR spectra of oils (Fig. 2(i)). Similar to signal I (Fig. 2(i)), when spectrally expanded, the intensity of the signal resonance of signal J was highest in coconut oil and lowest in cold press rapeseed oil (Fig. 2(ii)).

The overall unsaturation degree of a culinary oil could also be determined from the resonance intensity of signal K. Signal K is a multiplet assignable to the olefinic functions (−CH=CH−) of a series of unsaturated acyl chains. Indeed, variations in the unsaturation degree of culinary oils produced substantial differences in the intensities of the multiplet pattern(s) of this signal, and overall, its intensity was

directly proportional the oils degree of unsaturation, and correspondingly, IVs. For example, amongst the culinary oils analysed in this report, SFA-rich coconut oil was characterised by having the lowest intensity of signal K (Fig. 2(ii)), as expected. However, signal K of PUFA-rich hemp seed oil was of more resonances and much higher intensity.

For illustration purposes, myristic (first), ω-6 linoleic (middle), and ω-3 linolenic (third) FAs arbitrary assigned and esterified to a TAG backbone have their respective protons well labelled (Fig. 3) and matched with the characterisation in Fig. 2. This configuration also includes a free oleic FA ester (Fig. 3). The proton nuclei attached to the alphabet-labelled carbon atoms (Fig. 3) can be traced from the resonances of the major acyl groups located in the ^{1}H NMR spectra of the oils (Fig. 2). This analogy concurs with the illustration portrayed for butyric acid/ester acyl groups identified and characterised in ghee [12].

3.2. Oxidation products derived from oil MUFAs

3.2.1. Primary LOPs

The continuous and discontinuous thermo-oxidation of culinary oils at 180 °C resulted in the formation of primary LOPs, which in this report, were CHPDs and HPMs. These are described and discussed in the Supplementary Information section SI.1.3.1 (https://www.jfda-online.com/cgi/editor.cgi?article=3439&window=additional_files&context=journal).

3.2.2. Secondary LOPs

Secondary LOPs detected and identified in the thermo-oxidised culinary oils comprised epoxy-FAs, primary alcohols, formic acid and aldehydes (both saturated and α,β-unsaturated), amongst many other classes of degradation products. Secondary LOPs are derived from the fragmentation of primary hydroperoxide-containing LOPs, which have been implicated to be less stable than secondary LOPs. Albeit, since they are more stable than their hydroperoxide precursors, and are also absorbed from the gut into the systemic circulation, secondary LOPs may be considered to be more damaging to healthy human cells [8,23].

3.2.2.1. Epoxy-FAs and primary alcohols.
All ^{1}H NMR-detectable epoxy-FAs and primary alcohols present in the thermo-oxidized culinary oils are described and discussed in the Supplementary Information section SI.1.3.2.1 (https://www.jfda-online.com/cgi/
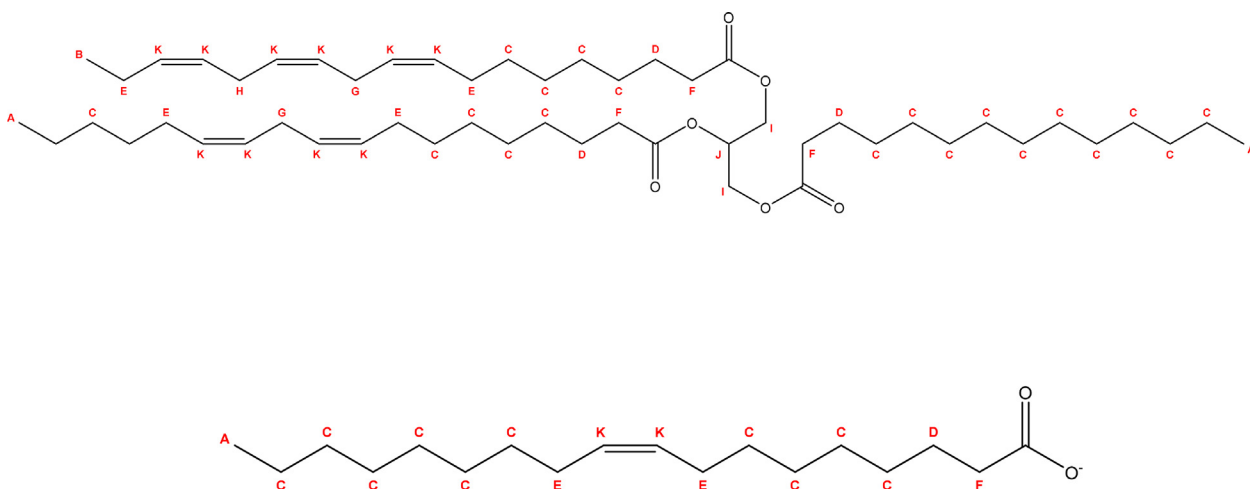
Fig. 3. An open structure of free oleic FA/ester, as well as myristic (first), ω-6 linoleic (middle), and ω-3 linolenic (third) FAs indiscriminately esterified to a TAG backbone.

3.2.2.2. Aldehydes. The evolution of aldehydic LOPs was largely dependent on the thermo-oxidation duration of the culinary oils (Fig. 4(i)). Indeed, the signal intensities of aldehydic LOPs increased with prolonged thermo-oxidation duration of culinary oils, with a constant temperature of 180 °C being maintained. This was observed in all thermo-oxidised culinary oils, and is typically demonstrated in

Fig. 4(i) by hemp seed oil which was thermos-oxidised for a duration of 300 min.

Also, the evolution of the different types of LOPs, and of course their concentrations, varied amongst all thermally stressed culinary oils investigated. This is largely attributable to variations in the unsaturation degree of the culinary oils. Interestingly, there were only three types of aldehydic LOPs, specifically (E)-2-alkenals (signal a), n-alkanals (signal g) and 4-oxo-n-alkanals (signal h) were ^{1}H NMR detectable in continuous thermo-oxidised SFA-rich coconut oil (Fig. 4(ii)). This varied slightly during discontinuous thermo-oxidation episodes, since as traces of two additional α,β-UAs i.e., (E,E)-2,4-

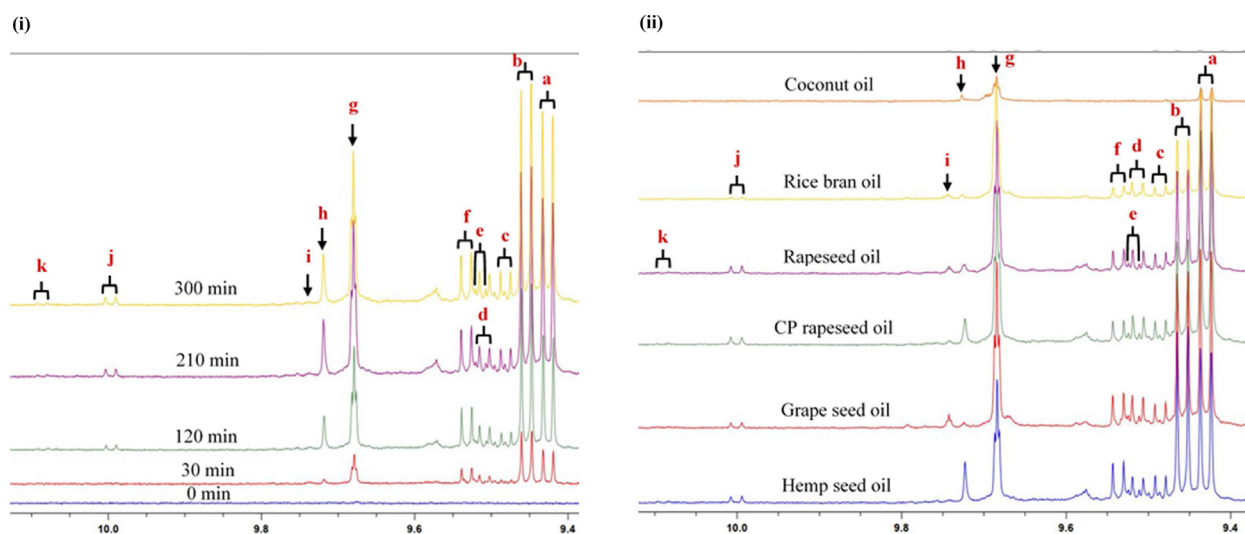Fig. 4. ^{1}H NMR spectra showing expanded 9.4–10.2 ppm regions of (i) hemp seed oil thermally stressed at increasing time-points and (ii) all culinary oils thermally stressed at 300 min. These spectra contain resonances attributable to aldehydes (secondary LOPs). Abbreviation: CP, cold press. Letter assignments of resonances correspond to those provided in the Supplementary Information Table S.5.

alkadienals (signal b) and 4,5-epoxy-(*E*)-alkenals (signal c), were also identified in addition to the above aldehydic LOPs in this frying medium during the 330—480 min stages of this process. As expected, only very low levels of such aldehydes were generated in this SFA-oil on exposure to both types of thermal stressing episode.

This study supported the findings on the thermo-oxidation of coconut oil as [9,11] characterised (*E*)-2-alkenals, *n*-alkanals, and 4-oxo-*n*-alkanals as the only three aldehydic LOPs detected in a differently branded coconut oil thermally stressed in continuous (with and without partial substitutions) and discontinuous fashions, and under similar experimental conditions. SFA-rich culinary oils are, of course, extremely resistant to peroxidation [[2,9]]. Consequently, SFA-laden oils such as coconut oil and animal fat (lard) generate little or no LOPs when subjected to laboratory-simulated high temperature frying practices, or when used as domestic and/or commercial frying media [[9,32]].

The signal intensities of the aldehydic LOPs identified in the present study's thermo-oxidised coconut oil could also be compared to those of the 10 aldehydic LOPs (signal a to j) identified in thermo-oxidised SFA-rich ghee [[12]]. Furthermore, it was reported that (*E*)-2-alkenals, (*E,E*)-2,4-alka-dienals, and *n*-alkanals were the major aldehydic LOPs determined in both continuous and discontinuous thermo-oxidised ghee [[12]] when compared to (*E*)-2-alkenals, *n*-alkanals, and 4-oxo-*n*-alkanals in both continuous and discontinuous thermo-oxidised coconut oil, in this report and those of [9,11]. This comparison of aldehydic LOPs in thermally stressed coconut oil of different brands, and ghee, serves as a testament to how resistant SFA-rich culinary oils are to thermo-oxidation, and therefore are potentially safer culinary oils to use for frying processes.

As profiled in Fig. 4(ii), all MUFA- and PUFA-rich culinary oils were susceptible to thermo-oxidation; however, the evolution of aldehydic LOPs found to be much greater in the PUFA-rich culinary oils than they were in MUFA-rich products. It was noteworthy that the identification of signal k has previously been identified as an alkenal species based on the two-dimensional $^1H-^1H$ total correlation spectroscopy (TOCSY) analysis by [12]. This LOP was detected in all MUFA- and PUFA-rich culinary oils, but not so in rice bran oil (Fig. 4(ii)).

3.3. Culinary oil FA compositions and IVs

During the 180 °C thermo-oxidation of the culinary oils, MUFAs and PUFAs are degraded in the presence of atmospheric oxygen, and this process gives rise to the production of saturated (and modified) fatty acids (S(+M)FAs) and LOP fragmentation products. Changes in the acyl groups of the thermo-oxidised culinary oils were more profound during continuous thermo-oxidation episodes than they were during the discontinuous ones (Figs. 5 and 6). This is ascribable to the 120 min regular cooling durations that eliminate the catalysis of heat and cut between the 30 min thermo-oxidation durations involved in the discontinuous thermo-oxidation process (Fig. 6).

The molar %'s of linoleic, and ω-3 and linolenic FAs (present in rice bran, rapeseed, cold-pressed rapeseed, and hemp seed oils), as well as PUFAs and UFAs, decreased throughout the 300 min thermo-oxidation duration of continuous heating. Simultaneously, S(+M)FAs of the continuous thermo-oxidised culinary oil increased in approximately equivalent proportions as UFAs decreased (Fig. 5). As an exception, smaller changes in ω-3 FAs, i.e., 4.80 ± 0.23 M % (unheated) to 4.29 ± 0.30 (at 300 min of continuous thermal stressing episode), and 4.33 ± 0.18 M % (at 480 min of discontinuous thermal stressing episode) were reported in thermo-oxidised rice bran oil (Figs. 5 and 6). In contrast, oleic FAs decreased in continuous thermo-oxidised SFA-rich coconut oil, and became stabilised in MUFA-rich rice bran, rapeseed, and cold press rapeseed oils (Fig. 5). Oleic FAs also increased throughout the 300 min continuous thermo-oxidation of grape and hemp seed oils (Fig. 5), and this is a consequence of the diminishing contents of PUFAs therein, i.e., PUFAs are peroxidatively degraded and hence removed from oils more rapidly than MUFAs are. In continuous thermo-oxidised coconut oil, however, oleic FAs decreased appreciably [9]. Nonetheless, the trends observed for changes in these MUFAs have been observed to differ somewhat amongst culinary oils of varying unsaturation degree when exposed to differential thermo-oxidisation episodes [9,11,12].

Increases observed in the oleic FAs of PUFA-rich oils stem from the thermo-oxidation of these more unsaturated and peroxidatively-susceptible FAs [9,12]. However, in situations where there are only very low levels of PUFAs present, MUFAs will also appreciably decrease in concentration as observed in continuous thermo-oxidised coconut oil (Fig. 5). Hence, the continuous thermo-oxidation of MUFAs will then ultimately lead to default increases in the contents of S(+M)FAs [9,12]. As expected, MUFA-rich culinary oils were indeed more resistant to thermo-oxidation processes than PUFA-rich culinary oils [9,12].

Coconut oil

Rice bran oil

Rapeseed oil

Cold press rapeseed oil

Grape seed oil

Hemp seed oil

Iodine value

Fig. 5. Modifications in FA contents and IVs of thermally stressed culinary oils exposed to a continuous thermo-oxidation episode at 180 °C for 480 min. These parameters are presented as mean ± standard deviation molar % (FA) and unit (IV), respectively, for each oil investigated.
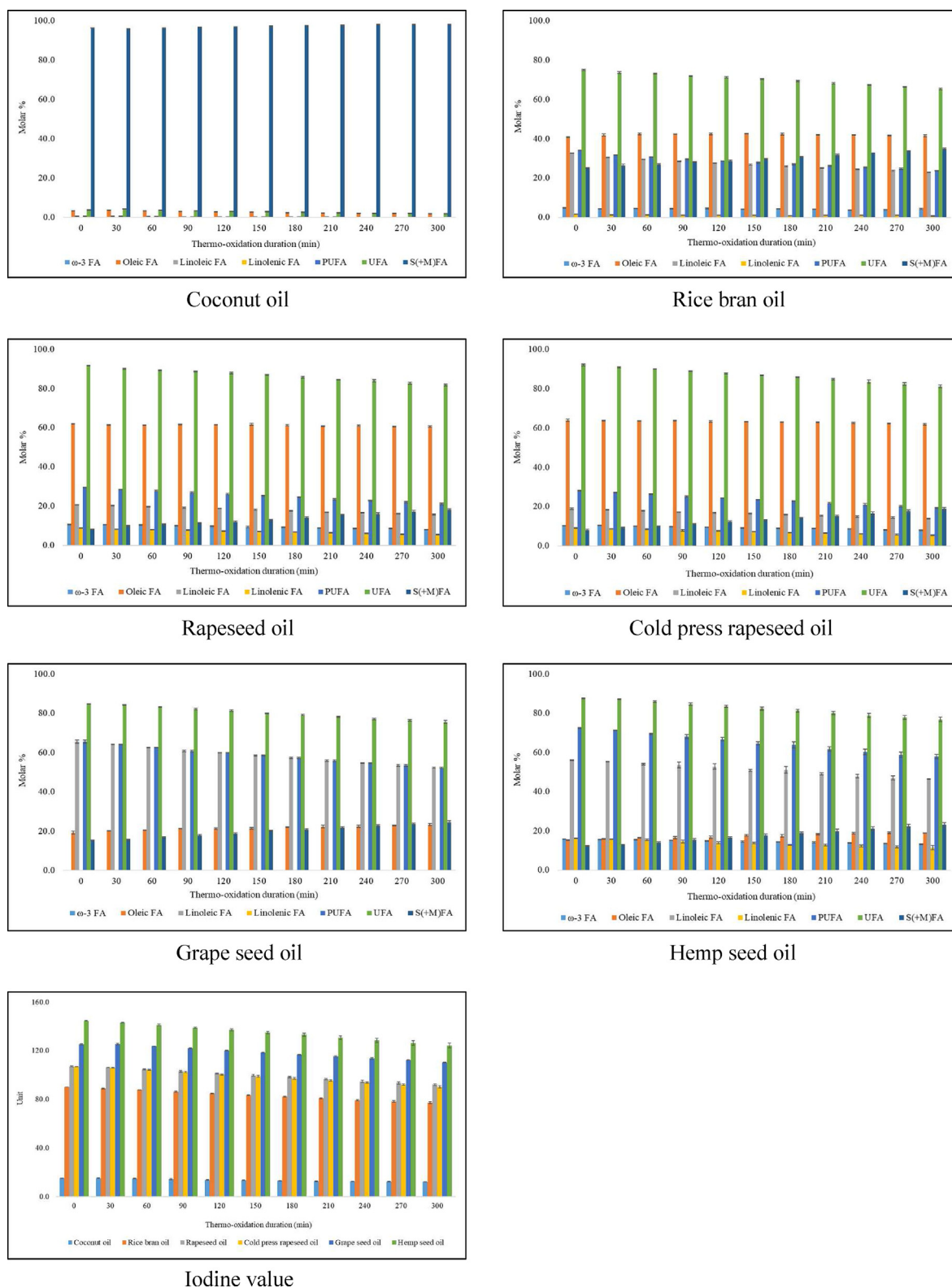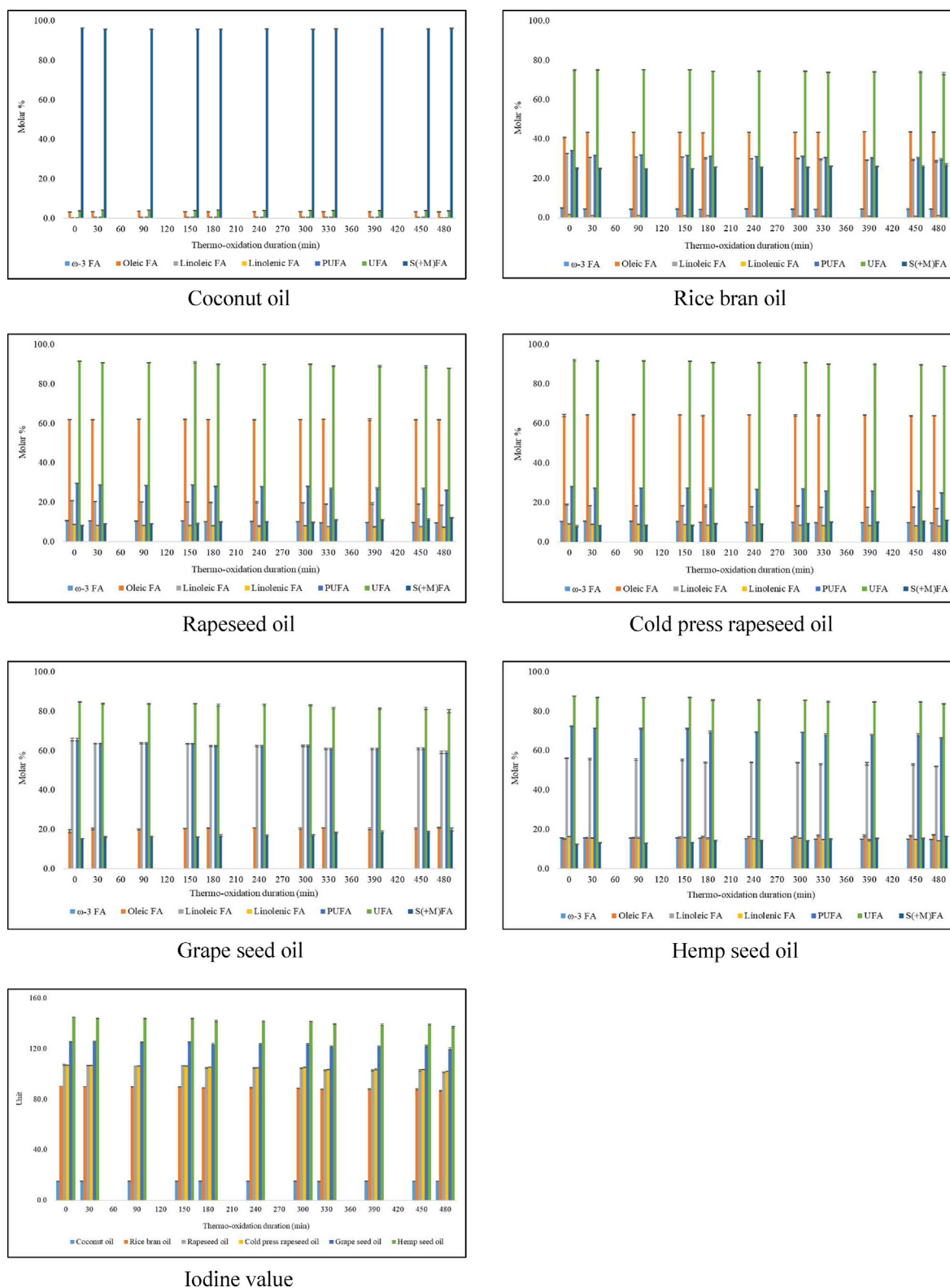
Fig. 6. Modifications in FA contents and IVs of thermally stressed culinary oils exposed to a discontinuous thermo-oxidation episode at 180 °C for 480 min. These parameters are presented as mean ± standard deviation molar % (FA) and unit (IV), respectively, for each oil investigated.

Linoleic FAs were significantly depleted in continuously thermo-oxidised coconut oil at the 240 min thermo-oxidation time-point (Fig. 5), despite their low ^{1}H NMR-determined starting (control) concentration of 0.52 $\pm$ 0.01 M % (Table 1). However, this level only decreased to 0.44 $\pm$ 0.07 M % of linoleic FAs at the 480 min thermo-oxidation duration of discontinuous thermo-oxidation (Fig. 6). According to [9]; oleic FAs in coconut oil depleted from 3.42 $\pm$ 0.34 M % at time 0 min (unheated) to 1.63 $\pm$ 0.55 M % at 300 min (continuous) and 2.55 $\pm$ 0.01 M % at 480 min (discontinuous). Moreover, the molar % distribution of oleic FAs in unheated coconut oil is also expected to influence the thermo-oxidation dynamics involved, as expected. Furthermore, the disparity between the two thermo-oxidation durations is presumably attributable to the cooling effect that was factored in the discontinuous thermo-oxidation episodes of the culinary oils.

Overall, the thermo-oxidation of total UFAs of 300 min period continuous thermo-oxidised culinary oils were in the order: cold press rapeseed (10.89 M %) > hemp seed (10.73 M %) > rapeseed (9.83 M %) > rice bran (9.73 M %) > grape seed (9.11 M %) > coconut oils (1.88 M %) (Fig. 5). The thermo-oxidation of total UFAs with a 480 min discontinuous thermo-oxidation episodes were in order of grape seed (4.62 M %) > hemp seed (4.01 M %) > rapeseed (3.79 M %) > cold press rapeseed (3.05 M %) > rice bran (1.78 M %) > coconut oils (0.49 M %) (Fig. 6). Of course, this also impacted the IVs of the thermally stressed culinary oils. Indeed, at the 300 min continuous thermo-oxidation duration, these values decreased in the order of hemp seed (20.21 unit) > cold press rapeseed (16.57 unit) > rapeseed (15.01 unit) > grape seed (15.00 unit) > rice bran (12.78 unit) > coconut oils (2.87 unit) (Fig. 5). For the 480 min discontinuous thermo-oxidation duration, IVs decreased in the order of hemp seed (7.65 unit) > rapeseed (5.85 unit) > grape seed (5.70 unit) > cold press rapeseed (4.99 unit) > rice bran (3.51 unit) > coconut oils (0.08 unit) (Fig. 6). For both thermo-oxidation processes, reductions in UFA levels and IVs of the studied culinary oils had the least impact on SFA-rich coconut oil, a conclusion also reached by [9,12].

This report re-affirms the conclusions that discontinuous thermo-oxidation episodes, in the long experimental run, led to the preservation of unsaturation degree more so than that observed during continuous thermo-oxidation of the oils [9,12]. The observed chemical modifications in acyl group contents of the culinary oils when subjected to thermal stressing episodes at high temperatures are essential to understand, forecast, and predicts the peroxidative susceptibilities of oils during thermo-oxidation processes [9,12]. Moreover, this knowledge is fundamental in establishing scientific measures necessary to targets and suppress drastic oxidation changes of the unsaturated acyl groups of highly susceptible oils.

To evaluate differences between the two thermo-oxidation episodes, the 120 min time-point of continuous thermo-oxidation was compared with the cumulative 120 min time-points (30 min $\times$ 4 thermally stressing episodes) of discontinuous thermo-oxidation (Fig. 7). This was to assess which thermo-oxidation strategies would give rise to the lowest level of thermo-oxidation. In most cases, the 120 min time-point of discontinuous thermo-oxidation resulted in a higher level of preservation of UFAs and IVs than that observed with continuous thermo-oxidation (Fig. 7). Indeed, all analysed triacylglycerol FAs (except S(+M)FAs) and IVs, were found to be lower for the 120 min discontinuous thermo-oxidation process involving grape seed oil than when this oil was exposed to a continuous thermo-oxidation episode (Fig. 7). However, overall, differences observed between 120 min continuous and discontinuous thermo-oxidation episodes of culinary oils were small (Fig. 7). These results are consistent with those found in previous reports [9,12].

3.4. Evolution of LOPs

Freshly purchased unheated culinary oils were found to contain aldehydic LOPs based on the resonances of these toxins in ^{1}H NMR spectra acquired on these oils (Fig. 8, Supplementary Information Figs. S.6 and S.7 (https://www.jfda-online.com/cgi/editor.cgi?article=3439&window=additional_files&context=journal)). The area under the signal intensities of (E)-2-alkenals, (E,E)-2,4-alkadienals, and n-alkanals were evaluated based on the integral value and concentration of the added TBB internal standard to be 0.02 $\pm$ 0.00, 0.02 $\pm$ 0.00, and 0.04 $\pm$ 0.00 mM, respectively, in rice bran oil, and 0.10 $\pm$ 0.01, 0.04 $\pm$ 0.00, and 0.14 $\pm$ 0.01 mM, respectively, in grape seed oil. Unheated rapeseed oil was also found to contain 0.08 $\pm$ 0.02 mM (E)-2-alkenals and 0.08 $\pm$ 0.01 mM n-alkanals. Furthermore, 0.04 $\pm$ 0.00 mM n-alkanals were quantified in unheated hemp seed oil (Fig. 8, Supplementary Information Figs. S.6 and S.7 (https://www.jfda-online.com/cgi/editor.cgi?article=3439&window=additional_files&context=journal)). Exposure of culinary oils to oxidative stress of various forms during extraction and processing conditions, as well
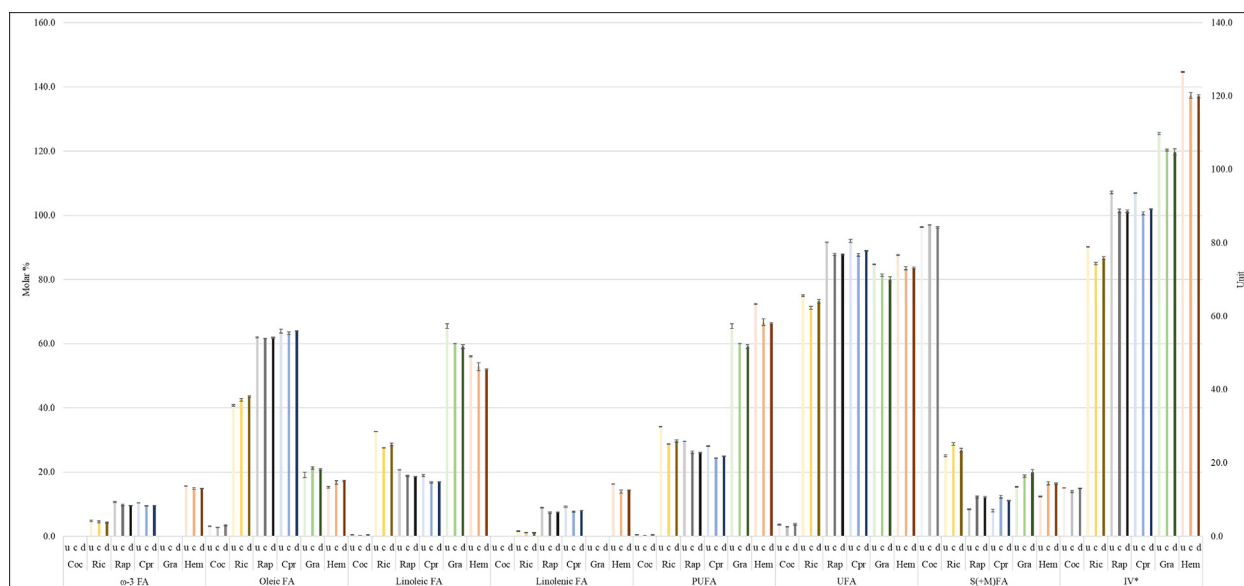
Fig. 7. Modifications in FA contents and IVs in unheated and thermally stressed culinary oils subjected to continuous and discontinuous thermo-oxidation episodes at 180 °C for a period of 120 min *IV is expressed in IV units. Abbreviations: u, unheated oil; c, continuous thermo-oxidation episode; d, discontinuous thermo-oxidation episode; Coc, coconut oil; Ric, rice bran oil; Rap, rapeseed oil; Cpr, cold press rapeseed oil; Gra, grape seed oil; Hem, hemp seed oil; ω-3 FA, omega-3 fatty acid; PUFA, polyunsaturated fatty acid; UFA, unsaturated fatty acid; S(+M)FA, saturated (and modified) fatty acid; IV, iodine value. The values are presented as mean ± standard deviation molar % (FA) and unit (IV) of oil tested.

as prolonged shelf storage of culinary oils following processing are two of the major reasons that could account for the detection of these low levels of aldehydic LOPs in unheated culinary oils [7,23,32].

The detection of aldehydic LOPs in freshly purchased, unheated culinary oils of varying unsaturation degree has been regularly reported. For example, this was originally specified in [7]. Moreover [9,11], reported 0.02−0.15 mM concentrations of (E)-2-alkenals, (E,E)-2,4-alkadienals, and n-alkanals in unheated olive and sunflower oils. Unheated sunflower oil, according to [10]; also contained 0.04−0.17 mM (E)-2-alkenals, (E,E)-2,4-alkadienals and (Z,E)-2,4-alkadienals, as well as n-alkanals. Furthermore, 0.02−0.13 mM (E)-2-alkenals, (E,E)-2,4-alkadienals, and n-alkanals were quantified in unheated ghee, groundnut, extra virgin olive, macadamia, sesame, corn, and walnut oils [[12]]. Although these are low concentrations, their presence, together with significant concentrations of epoxy-FAs and/or primary LOPs in these unheated frying media may present a potential health risk to human consumers, and therefore, their use for non-frying/cooking processes must be treated with some caution [[7,23,32]].

Notwithstanding, no aldehydic LOPs were detected in unheated coconut and cold press rapeseed oils (Fig. 8, Supplementary Information Figs. S.6 and S.7 (https://www.jfda-online.com/cgi/editor.cgi?article=3439&window=additional_files&context=journal)),

which concurs with the reports of [9,11]; since these oils were also found to be free of aldehydic LOPs. The extremely higher degree of saturation of coconut oil (96.32 ± 0.12 M %) (Table 1) may account for the oil's higher resistance to the formation of ^{1}H NMR detectable LOPs. On the other hand, a carefully controlled extraction technique and a well-driven logistic handling of cold press rapeseed oil may contribute to the oil being devoid of aldehydes. Furthermore, the naturally-occurring constituents with antioxidant potential, which happens to be present in cold press oils (otherwise stripped in the refined oil forms) may also ensure a stronger protective barrier to further boost the unheated oil's resistance to LOPs formation.

As noted above, a 300 min continuous thermo-oxidation episode generated more LOPs in the culinary oils than those found in with the 480 min discontinuous thermo-oxidation process (Supplementary Information Figs. S.6 and S.7 (https://www.jfda-online.com/cgi/editor.cgi?article=3439&window=additional_files&context=journal)). This is in concurrence with the degree of oxidation of acyl groups for the two thermo-oxidation systems employed and described in section 4.1. These research findings were similar to that of [9]. However [12], reported few exceptions, where for example (E,E)-2,4-alkadienals, (Z,E)-2,4-alkadienals, and (Z)-2-alkenals were higher at the 480 min time-point in discontinuously thermo-oxidized macadamia oil
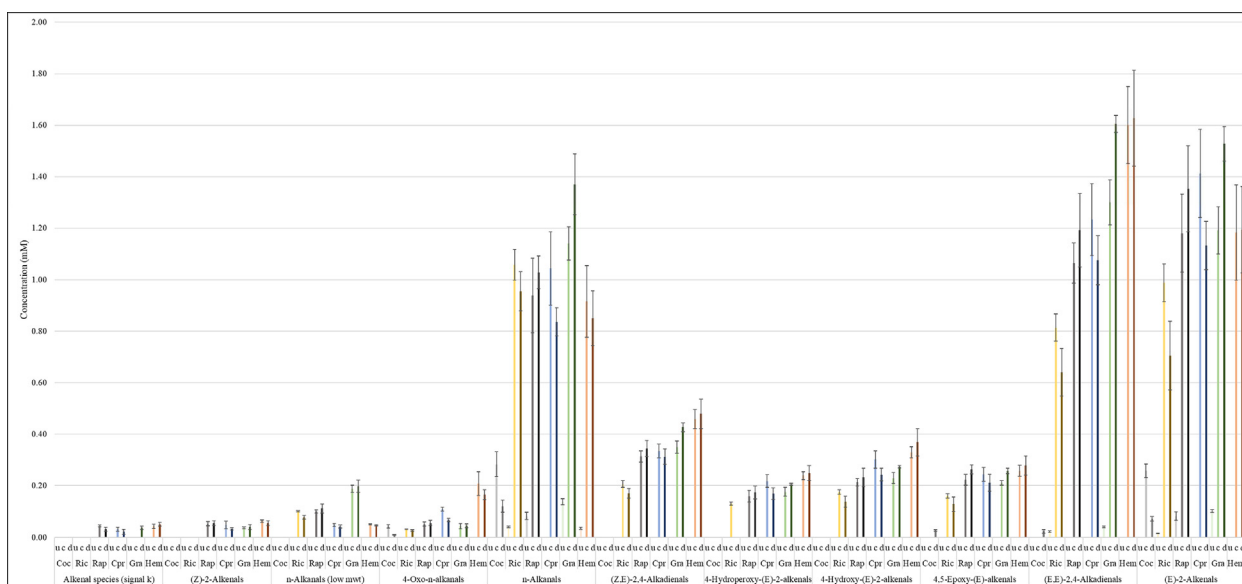
Fig. 8. Evolution of aldehydic LOPs in unheated and thermally stressed culinary oils exposed to either continuous or discontinuous thermo-oxidation episodes at 180 °C for 120 min. Abbreviations: u, unheated oil; c, continuous thermo-oxidation episode; d, discontinuous thermo-oxidation episode; Coc, coconut oil; Ric, rice bran oil; Rap, rapeseed oil; Cpr, cold press rapeseed oil; Gra, grape seed oil; Hem, hemp seed oil. The values are presented as mean ± standard deviation mM (mmol LOP per 1.0 L oil).

than they were at the 300 min period of continuous thermo-oxidation. In addition, they also reported that the levels of α,β-UAs and low-molecular-mass n-alkanals to be higher on completion of the thermo-oxidation duration of discontinuous thermo-oxidized extra virgin olive oil than they were when exposed to the continuous thermo-oxidation phase [12]. Remarkably, for both macadamia and extra virgin olive oils, (Z,E)-2,4-alkadienals were consistently higher throughout the discontinuous thermo-oxidation phase than they were during the continuous episodes [[12]]. They explained that during the cooling periods in the discontinuous thermo-oxidation phase, oxygen levels rejuvenate in the culinary oils. The dissolved oxygen contributes to the formation of LOPs in the oils and this is facilitated by heat despite the gradual continuous drop in temperature during the cooling of oils in the discontinuous thermo-oxidation phase [12].

The evolution of LOPs were also dependent on the degree of unsaturation of culinary oils [9,11,12], as expected. By implication, PUFA-rich grape seed and hemp seed oils generated more LOPs, regardless of the thermo-oxidation technique, than MUFA-rich rice bran, rapeseed, and cold press rapeseed oils, which of course also produced more LOPs than SFA-rich coconut oil (Supplementary Information Figs. S.6 and S.7 (https://www.jfda-online.com/cgi/editor.cgi?article=3439&window=additional_files&context=journal)). Indeed, such thermally stressed

SFA-rich oils generated very little or no LOPs; however, on a mole-for-mole equivalence scale, those rich in PUFAs always produced higher levels of LOPs [7,8,22,32]. This was also observed in culinary oils exposed to facilitated peroxidation processes when exposed to continuous and discontinuous thermo-oxidation episodes [9,12], in addition to partially-substituted continuous thermo-oxidation [11] and shallow frying episodes [33]. As an exception, the highest amounts of (E)-2-alkenals, amongst all the culinary oils explored here, were found in continuous thermo-oxidized cold press rapeseed oil (Supplementary Information Fig. S.6 (https://www.jfda-online.com/cgi/editor.cgi?article=3439&window=additional_files&context=journal)). Being a predominantly MUFA-rich oil, the oleic (63.89 ± 0.61 M %) and somewhat, linoleic FAs (18.94 ± 0.33 M %) (Table 1) of cold press rapeseed oil, in addition to (E,E)- and (Z,E)-2,4-alkadienals peroxidation products, may account for the higher distribution of (E)-2-alkenals in cold press rapeseed oil [[34]]. This, was however, not the case for (the refined) rapeseed oil despite having similar contents of oleic (61.95 ± 0.13 M %) and linoleic FAs (20.78 ± 0.07 M %) (Table 1).

Notably, the evolution of (E)-2-alkenals, (E,E)-2,4-alkadienals, 4,5-epoxy-(E)-alkenals and n-alkanals in discontinuous thermo-oxidized coconut oil only commenced at 330 min. 4-Oxo-n-alkanals, on the other hand, were detected in discontinuous thermo-

oxidized coconut oil at the 480 min time-point (Supplementary Information Figs. S.6 and S.7 (https://www.jfda-online.com/cgi/editor.cgi?article=3439&window=additional_files&context=journal)). In contrast, the evolution of (*E*)-2-alkenals and *n*-alkanals, as well as 4-oxo-*n*-alkanals in continuous thermo-oxidized coconut oil was found to be initiated at the 30 and 60 min time-points, respectively (Supplementary Information Fig. S.6 (https://www.jfda-online.com/cgi/editor.cgi?article=3439&window=additional_files&context=journal)). (*E*,*E*)-2,4-Alkadienals and 4,5-epoxy-(*E*)-alkenals were undetectable in continuously thermo-oxidized coconut oil (Supplementary Information Fig. S.6 (https://www.jfda-online.com/cgi/editor.cgi?article=3439&window=additional_files&context=journal)). However, as reported by [9]; the evolution of (*E*)-2-alkenals and *n*-alkanals in coconut oil was first identified at the 30 min sampling time-point. 4-Oxo-*n*-alkanals, on the other hand, was initially identified at 60 and 180 min in continuously and discontinuously thermo-oxidised coconut oil, respectively [[9]]. Of course, the high resistance of coconut oil to thermo-oxidation is largely attributable to its high SFA content, which comprises high levels of medium-chain triacylglycerols of which capric, caprylic, and lauric FAs are examples [35]. As triacylglycerols, these medium-chain FAs are readily metabolised *in vivo* leaving no residues, and there is evidence available that they also provide anti-inflammatory responses, and also improve cardiac function and fasting insulin levels. Moreover, they have been reported to also diminish sphingolipid, ceramide, and acylcarnitine concentrations in blood plasma [35−37].

4-Hydroperoxy-(*E*)-2-alkenals, which were detected throughout continuously thermally-stressed rice bran oil, was undetected in the discontinuous phase (Supplementary Information Figs. S.6 and S.7 (https://www.jfda-online.com/cgi/editor.cgi?article=3439&window=additional_files&context=journal)). (*Z*)-2-Alkenals, however, which were firstly identified at the 150 min time-point in continuously thermally stressed rice bran oil, was also completely undetectable in the discontinuous phase. Furthermore, 4-oxo-*n*-alkanals, which were detected throughout continuously thermo-oxidised rice bran oil, was only identified at the 480 min time-point when this oil was exposed to the discontinuous thermal stressing episode (Supplementary Information Figs. S.6 and S.7 (https://www.jfda-online.com/cgi/editor.cgi?article=3439&window=additional_files&context=journal)). The unidentified alkenal species (signal k) was undetectable in thermo-oxidised rice bran oil (Supplementary Information Figs. S.6 and S.7 (https://www.jfda-online.com/cgi/editor.cgi?article=3439&window=additional_files&context=journal)).

Respectively, 4-oxo-*n*-alkanals, (*Z*)-2-alkenals, and the unidentified alkenal species (signal k) were first detected in continuously thermo-oxidized rapeseed oil at the 60, 60, and 120 min sampling time-points (Supplementary Information Fig. S.6 (https://www.jfda-online.com/cgi/editor.cgi?article=3439&window=additional_files&context=journal)), as opposed to the 150, 240, and 390 min ones when thermally stressed according to the discontinuous process (Supplementary Information Fig. S.7 (https://www.jfda-online.com/cgi/editor.cgi?article=3439&window=additional_files&context=journal)). 4-Hydroperoxy-(*E*)-2-alkenals were also first identified in rapeseed oil at the 60 min continuous thermo-oxidation time-point (Supplementary Information Fig. S.6 (https://www.jfda-online.com/cgi/editor.cgi?article=3439&window=additional_files&context=journal)), rather than its first detection at 180 min for the discontinuous thermo-oxidation simulated frying process (Supplementary Information Fig. S.7 (https://www.jfda-online.com/cgi/editor.cgi?article=3439&window=additional_files&context=journal)).

(*Z*)-2-Alkenals and the unidentified alkenal species (signal k) were both first identified in cold press rapeseed oil at the 90 min time-point during the continuous phase (Supplementary Information Fig. S.6 (https://www.jfda-online.com/cgi/editor.cgi?article=3439&window=additional_files&context=journal)), and 330 min when exposed to the discontinuous thermo-oxidation protocol (Supplementary Information Fig. S.7 (https://www.jfda-online.com/cgi/editor.cgi?article=3439&window=additional_files&context=journal)). The evolution of 4-oxo-*n*-alkanals, (*Z*)-2-alkenals, and unidentified alkenal species (signal k), respectively, commenced at 60, 120, and 150 min in continuously thermo-oxidised grape seed oil, and conversely, at 30, 480, and 480 min in the discontinuous process (Supplementary Information Figs. S.6 and S.7 (https://www.jfda-online.com/cgi/editor.cgi?article=3439&window=additional_files&context=journal)). In thermally stressed hemp seed oil, both (*Z*)-2-alkenals and unidentified alkenal species were all firstly observed at the 60 min continuous thermo-oxidation episode (Supplementary Information Fig. S.6 (https://www.jfda-online.com/cgi/editor.cgi?article=3439&window=additional_files&context=journal)), and conversely at 150 and 180 min, respectively, in the discontinuous phase (Supplementary Information Fig. S.7 (https://www.jfda-online.com/cgi/editor.cgi?article=3439&window=additional_files&context=journal)). These varying evolution times of aldehydic LOPs in continuously and discontinuously

heated frying oils were also highlighted by [9,12] for other culinary oils of varying unsaturation degrees.

Comparisons of aldehydic LOPs concentrations at the 120 min time-point for both continuous and discontinuous thermo-oxidation episodes are profiled in Fig. 8. At this time-point, all aldehydic LOPs were relatively lower in concentration in discontinuously thermo-oxidized coconut oil (except for (E,E)-2,4-alkadienals), rice bran oil (except for 4-oxo-n-alkanals), and cold press rapeseed oil than they were when exposed to the continuous frying episode simulation (Fig. 8). This was also the case for (Z,E)-2,4-alkadienals, n-alkanals, 4-oxo-n-alkanals, and (Z)-2-alkenals quantified in thermo-oxidised hemp seed oil (Fig. 8). Conversely, the concentrations of aldehydic LOPs found at the 120 min time-point for thermo-oxidized rapeseed (except for unidentified alkenal species (signal k)) and grape seed oils were found to be higher during the discontinuous phase than they were at the 120 min continuous thermo-oxidation process time-point (Fig. 8). Subsequently, the concentrations of 4-oxo-n-alkanals and low-molecular-mass n-alkanals were strikingly similar between the 120 min continuous and discontinuous thermo-oxidation time-points for grape seed oil (Fig. 8). Similar data have been reported in other thermo-oxidised culinary oils exposed to the same experimental conditions in related studies [[9,12]].

For example, [12]; reported higher levels of aldehydic LOPs in groundnut oil at the 120 min continuous thermo-oxidation episode time-point, which contrasts with the production of lower concentrations of aldehydic LOPs in ghee, extra virgin olive, macadamia, sesame, corn, and walnut oils when compared to those obtained at the corresponding time-point for discontinuous episodes. Moreover [9], also reported higher levels of low-molecular-mass n-alkanals, 4-oxo-n-alkanals, n-alkanals, and (E,E)-2,4-alkadienals in olive and rapeseed oils at 120 min discontinuous thermo-oxidation. Notwithstanding, no significant differences were reported in LOPs measured in sunflower oil between the two thermo-oxidation techniques at the 120 min time-point [9]. This level of resistance to thermo-oxidation was inherent in sunflower oil, since minor compounds with potential antioxidant activities may influence this inherent temperature-independent resistance to oxidation in this frying medium [[9]]. Furthermore, the rapid cooling (aided by ice) in continuous thermo-oxidation, and the non-rapid cooling to room temperature in discontinuous thermo-oxidation episodes, may also contribute to the disparities observed in the LOP concentrations observed for the two different frying methods.

The evolution of LOPs is perpetuated by the autoxidative degradation of acyl groups. The process is an autocatalytic, self-perpetuating chain reaction, mediated by oxygen and facilitated by one or more catalysts such as heat, oxidation-reduction-active metals, free radicals, light, and photosensitizing pigments [22,24,38]. Adapted from [39] and slightly modified, Fig. 9 illustrates a proposed mechanisms of LOPs formation in oils undergoing oxidation. A hydrogen atom is first abstracted from the acyl chain of a FA by a free radical (Fig. 9). This is facilitated by a low bond dissociation energy that exists between the carbon and hydrogen bonds [22]. Alternatively, other reaction cycles progress to and from the backbone of the reaction scheme. Epoxy radicals (epoxides−$^\bullet$) generated via cyclization of alkoxy radicals (LO$^\bullet$), for instance, interact with oxygen (O$_2$) to generate and feed more peroxyl radicals (LOO$^\bullet$) into the backbone of the reaction scheme (Fig. 9). Chemical reactions involving LOO$^\bullet$ also lead to the formation of epidioxides-$^\bullet$ and endoperoxides-$^\bullet$, which in the long run result in the formation of dimers. Alkene residues (−CH=CH−) generated from dimers−$^\bullet$ and/or epoxides−$^\bullet$ interact with LOO$^\bullet$ to produce epoxides−$^\bullet$, which further reacts with LO$^\bullet$ to produce alkanes, aldehydes, oxo compounds, and other scission radicals (Fig. 9). Furthermore, LO$^\bullet$, via recombination, cyclization, and chain scission processes, respectively, plays a vital role in the formation of peroxides and ketones, hydroxy- and hydroperoxy-epoxides, as well as alkanes, aldehydes, oxo compounds, and other scission radicals (Fig. 9).

During the oxidation of acyl groups at typically frying temperature ranges over a prolonged duration, dimerization and polymerisation of FAs also occurs. Polymers are produced by addition reactions of dimers-$^\bullet$ formed from the chemical reactions of LOO$^\bullet$ (Fig. 9). According to [40]; poly-formates, -esters, -ethers, and -hydroxy groups formed in a polymerisation reaction increase the viscosity of the oils undergoing oxidation. Moreover, poly-ethers harden cell membranes and clog blood vessels obstructing blood flow resulting in atherosclerotic plaque [40].

3.5. Statistical analysis of aldehydic LOP datasets

Results acquired from an analysis of the ANOVA model depicted in Equation (1) are listed in Table 2. Overall, the influence of all main effects was strikingly highly significant for all aldehydes monitored, p values obtained for these comparisons ranging
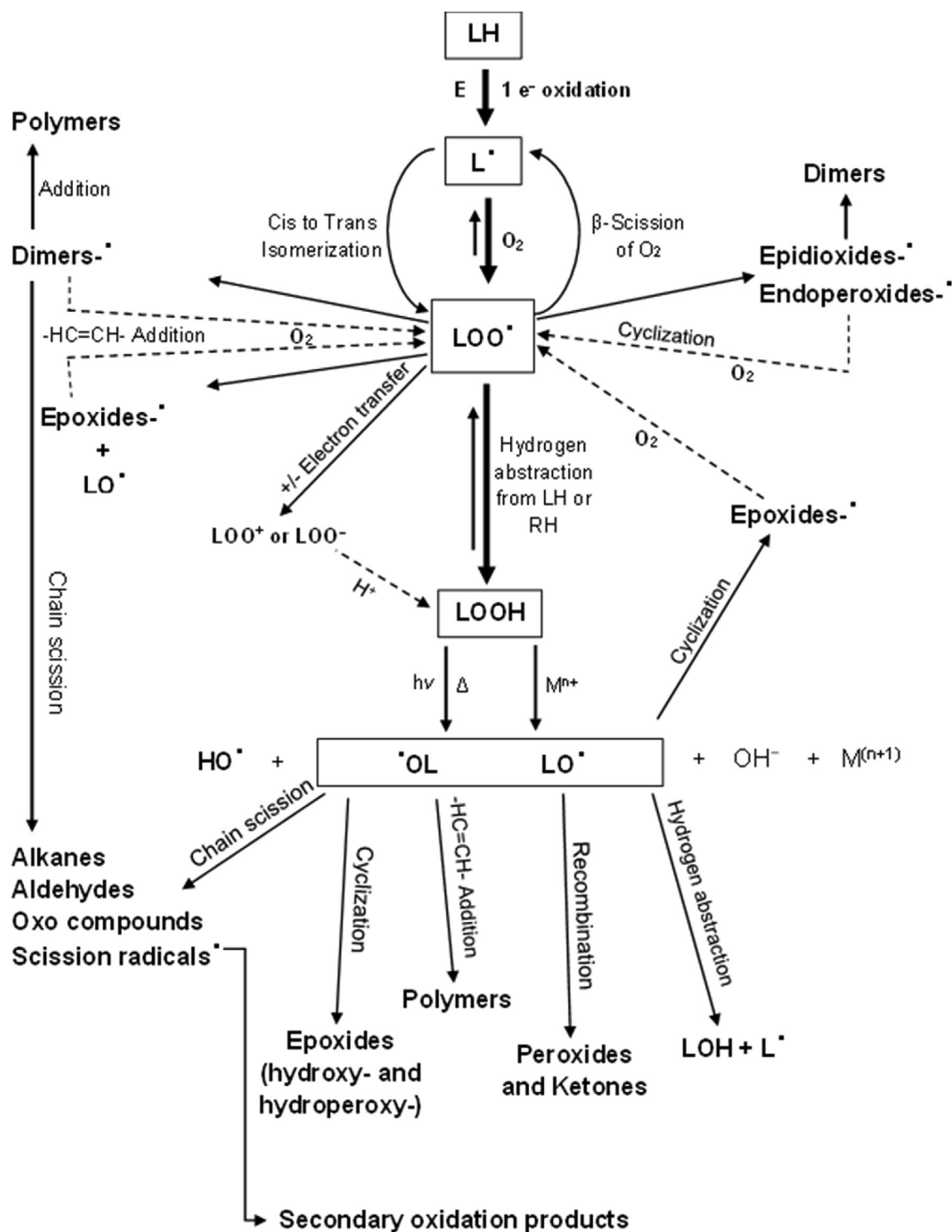
Fig. 9. A proposed integrated reaction scheme for the evolution of LOPs in oils. Adapted from [39] and slightly modified.

from 10^{-20} to 10^{-40}. As expected, for all aldehydes, the thermo-oxidation Time-Point and the Oil Type factors were markedly significant, results clearly consistent with the propagation of the lipid peroxidation process with increasing thermo-oxidation periods, and likewise with increasing oil unsaturated FA contents, most notably those of PUFAs. Likewise, the simulated Heating Episode simulation classification (i.e., continuous versus discontinuous processes) was also extremely statistically significant

Table 2. Statistical significance of all main sources of variation, and first-order interaction effects from ANOVA analysis of the aldehydic LOP dataset.

Aldehyde	Chemical Shift Value (ppm)	Oil Type (O)	Time-point (min) (T)	Heating Episode Class (S)	O × T Interaction Effect	O × S Interaction Effect	T × S Interaction Effect
(E)-2-Alkenals	9.48	$<10^{-30}$	$<10^{-30}$	$<10^{-30}$	$<10^{-30}$	$<10^{-30}$	$<10^{-30}$
(E,E)-2,4-Alkadienals	9.52	$<10^{-30}$	$<10^{-30}$	$<10^{-30}$	$<10^{-30}$	$<10^{-30}$	$<10^{-30}$
4,5-Epoxy-(E)-2-alkenals	9.54	$<10^{-40}$	$<10^{-40}$	$<10^{-40}$	$<10^{-40}$	$<10^{-40}$	$<10^{-40}$
4-Hydroxy-(E)-2-alkenals	9.56	$<10^{-40}$	$<10^{-40}$	$<10^{-40}$	$<10^{-40}$	$<10^{-40}$	$<10^{-40}$
4-Hydroperoxy-(E)-2-alkenals	9.58	$<10^{-40}$	$<10^{-40}$	$<10^{-40}$	$<10^{-40}$	$<10^{-40}$	$<10^{-40}$
(Z,E)-2,4-Alkadienals	9.60	$<10^{-20}$	$<10^{-20}$	$<10^{-20}$	$<10^{-20}$	$<10^{-20}$	$<10^{-20}$
n-Alkanals	9.74	$<10^{-20}$	$<10^{-20}$	$<10^{-20}$	$<10^{-20}$	$<10^{-20}$	$<10^{-20}$
4-Oxo-n-alkanals	9.78	$<10^{-30}$	$<10^{-30}$	$<10^{-30}$	$<10^{-30}$	$<10^{-30}$	$<10^{-30}$
n-Alkanals (low mwt)	9.80	$<10^{-20}$	$<10^{-20}$	$<10^{-20}$	$<10^{-20}$	$<10^{-20}$	$<10^{-20}$
(Z)-2-Alkenals	10.06	$<10^{-40}$	$<10^{-40}$	$<10^{-40}$	$<10^{-40}$	$<10^{-40}$	$<10^{-40}$
Alkenal species (signal k)	10.15	$<10^{-20}$	$<10^{-20}$	$<10^{-20}$	$<10^{-20}$	$<10^{-20}$	$<10^{-20}$

Table 3. Statistical significance of all main sources of variation, and first-order interaction effects from ANOVA analysis of the culinary oil FA content dataset.

Acyl group	Oil Type (O)	Time-point (min) (T)	Heating Episode Class (S)	O × T Interaction Effect	O × S Interaction Effect	T × S Interaction Effect
ω-3 FA	$<10^{-30}$	$<10^{-30}$	$<10^{-30}$	$<10^{-30}$	$<10^{-30}$	$<10^{-30}$
Oleic FA	$<10^{-8}$	$<10^{-8}$	$<10^{-8}$	$<10^{-8}$	$<10^{-8}$	ns
Linoleic FA	$<10^{-50}$	$<10^{-50}$	$<10^{-50}$	$<10^{-50}$	$<10^{-50}$	$<10^{-50}$
Linolenic FA	$<10^{-20}$	$<10^{-20}$	$<10^{-20}$	$<10^{-20}$	$<10^{-20}$	$<10^{-20}$
PUFA total	$<10^{-50}$	$<10^{-50}$	$<10^{-50}$	$<10^{-50}$	$<10^{-50}$	$<10^{-50}$
UFA total	$<10^{-50}$	$<10^{-50}$	$<10^{-50}$	$<10^{-50}$	$<10^{-50}$	$<10^{-50}$
SFA total	$<10^{-50}$	$<10^{-50}$	$<10^{-50}$	$<10^{-50}$	$<10^{-50}$	$<10^{-50}$
IV	$<10^{-50}$	$<10^{-50}$	$<10^{-50}$	$<10^{-50}$	$<10^{-50}$	$<10^{-50}$

Abbreviations: ns, not significant.

for all aldehydes evaluated, with the continuous approach always yielding higher aldehyde levels.

For the first-order interaction effects investigated, all the first-order interaction effects tested were found to be significant for all aldehydic output variables considered. These interaction effects are, however, expected and correspond to the differential peroxidative responses of different types of oils to differing thermo-oxidation Time-Points, and also, the Simulated Heating Method selected.

A completely analogous ANOVA of the culinary oil FA content parameter dataset (Table 3) revealed that, with the exception of the oleoylglycerol content for the T × S interaction effect, all output variables and their first-order interaction effects were extremely significant, i.e., they all exert dominating effects on the thermo-oxidation of all culinary oils evaluated in this study.

A second experimental design model was employed for the dataset acquired from the thermo-oxidation of culinary oils according to either continuous or discontinuous frying episodes at 180 °C for a fixed period of 120 min (Equation (2)). For this simpler model, there was only one first-order interaction effect (Oil Type × Oil Heating Method) considered. Main factors were Type of Oil

(O_i) and Heating Method (S_j), with the only interaction effect represented by OS_{ij}.

$$y_{ijk} = \mu + O_i + S_j + OS_{ij} + e_{ijk} \qquad (2)$$

When applied to the analysis of data acquired according to this model, both factors and their first-order interaction effect were found to be substantially significant for:

i. all aldehydic LOPs analysed (Table 4) and

ii. All acylglycerol FA contents (Table 5)

This confirms their vital importance in the thermally-induced peroxidation process involved in experiments conducted.

This study is part of a bulky study presently undertaking by this research group to create a vast database for all culinary oils of varying unsaturation degrees in response to their susceptibility to the two most dominant frying techniques; continuous and discontinuous thermo-oxidation processes at a cooking temperature of 180 °C. The database created will represent a library of toxicity inventory for the studied culinary oils based on their susceptibility to continuous and discontinuous thermo-oxidation. A significant impact of this scientific knowledge would be the practical use of oils that are highly resistant to thermo-oxidation to blend and

Table 4. *Statistical significance of all main sources of variation, and first-order interaction effects from ANOVA analysis of the aldehydic LOP dataset for analysis according to the model described in Equation (2).*

Aldehyde	Oil Type (O)	Heating Episode (C or D)	Oil × Heating Episode Interaction
(E)-2-Alkenals	$<10^{-13}$	$<10^{-13}$	$<10^{-13}$
(E,E)-2,4-Alkadienals	$<10^{-17}$	$<10^{-17}$	$<10^{-17}$
4,5-Epoxy-(E)-2-alkenals	$<10^{-14}$	$<10^{-14}$	$<10^{-14}$
4-Hydroxy-(E)-2-alkenals	$<10^{-15}$	$<10^{-15}$	$<10^{-15}$
4-Hydroperoxy-(E)-2-alkenals	$<10^{-17}$	$<10^{-17}$	$<10^{-17}$
(Z,E)-2,4-Alkadienals	$<10^{-18}$	$<10^{-18}$	$<10^{-18}$
n-Alkanals	$<10^{-11}$	$<10^{-11}$	$<10^{-11}$
4-Oxo-n-alkanals	$<10^{-12}$	$<10^{-12}$	$<10^{-12}$
n-Alkanals (low mwt)	$<10^{-19}$	$<10^{-19}$	$<10^{-19}$
(Z)-2-Alkenals	$<10^{-11}$	$<10^{-11}$	$<10^{-11}$
Alkenal species (signal k)	$<10^{-13}$	$<10^{-13}$	$<10^{-13}$

suppress the toxicity of oils that are highly susceptible to thermo-oxidation at cooking temperatures. This knowledge will also provide the foundation to engineer oils that are more prone to thermo-oxidative susceptibility at cooking temperatures to conform to oils that are less prone, and therefore, safe to use at those cooking temperatures. In addition, this study will throw highlights on oils, whose toxicological properties may be more disturbing, to be target oils for further studies and scientific improvements as illustrated in the reports by [11,12]. Another scientific significant area of interest that this study may be useful to is the fast-food and restaurant establishments. By implication, the toxicological catalogue produced from this study will provide insights into choosing oils and frying techniques experimentally established to be safe for their long duration routine frying episodes. This was typically explored to a degree and established in the scientific report by [8,9,21,22]. Notwithstanding, since this bulky study is aimed at addressing all oil types, whose unsaturation degrees covers a broader range, this study would also apply to small scale users in selecting the most suitable frying technique and culinary oil for cooking.

4. Conclusions

This research advances extensive studies aimed to build a database for continuous and discontinuous thermo-oxidation of culinary oils of varying unsaturation degree. It also established that the thermo-oxidative susceptibility of culinary oils at a constant temperature of 180 °C are in order of PUFA- > MUFA- > SFA-rich culinary oils. This underscores the requirement to implement standard scientific techniques for human health benefits in order to improve the thermo-oxidative resistivity of PUFA- and MUFA-rich culinary oils. For the first time, this report uses high-resolution 600 MHz ^{1}H NMR technique to highlight the similarities and differences that exist between rapeseed and cold press rapeseed oils, and also, describes the impact of both continuous and discontinuous thermo-oxidation frying episodes on the peroxidative degradation of rice bran, cold press rapeseed, grape seed, and hemp seed oils. The similarities included the molar % proportions of FA groups and unit of IVs of the unheated oils (Table 1), as well as the resonances of the acyl groups obtained from the ^{1}H NMR spectra of rapeseed and cold press rapeseed oils (Fig. 2). The differences, on the other hand, between those oils were in relation to their thermo-oxidative dynamics. For instance, the UFA contents and IVs of rapeseed and cold press rapeseed oils illustrated that cold press rapeseed oil oxidised more at the 300 min time-point of continuous thermo-oxidation episodes than rapeseed oil. This was also evidently depicted in the concentrations of aldehydes quantified for both oils at the 300 min time-point of continuous thermo-oxidation. The opposite was also determined for FAs, IVs, and aldehydes at the 480 min time-point of discontinuous thermo-oxidation

Table 5. *Statistical significance of the two main sources of variation, and first-order interaction effects, from ANOVA analysis of the culinary oil FA content dataset analysed according to the model depicted in Equation (2).*

Acyl group	Oil Type (O)	Heating Episode (C or D)	Oil × Heating Episode Interaction
ω-3 FA	$<10^{-8}$	$<10^{-8}$	$<10^{-8}$
Oleic FA	$<10^{-8}$	$<10^{-8}$	$<10^{-8}$
Linoleic FA	$<10^{-13}$	$<10^{-13}$	$<10^{-13}$
Linolenic FA	$<10^{-14}$	$<10^{-14}$	$<10^{-14}$
PUFA Total	$<10^{-15}$	$<10^{-15}$	$<10^{-15}$
UFA Total	$<10^{-12}$	$<10^{-12}$	$<10^{-12}$
SFA Total	$<10^{-12}$	$<10^{-12}$	$<10^{-12}$
IV	$<10^{-14}$	$<10^{-14}$	$<10^{-14}$

phase. In addition, the unheated forms of both oils revealed the presence of (E)-2-alkenals and n-alkanals in rapeseed oil whereas cold press rapeseed oil was analysed to be devoid of any form of aldehydes. Overall, this research adds six more culinary oils — of which four are new entries — to the previous 11 classes studied. The results obtained from this study reaffirms the fact that lower levels, as well as types of aldehydic LOPs evolved in both continuously and discontinuously thermo-oxidised coconut oil. Furthermore, a 300 min continuous thermo-oxidation period gave rise to higher extents of peroxidative damage to oils than those observed at the 480 min time-point of the discontinuous thermo-oxidation process.

Author contributions

D.P.N. and M.G.: conceptualization and resources. A.L.G., D.P.N. and M.G.: methodology and validation. G.A.: formal analysis and investigation. G.A. and A.L.G.: data curation. A.L.G., M.G. and G.A.: writing—original draft preparation and visualization. D.P.N., M.G., G.A. and A.L.G.: writing—review and editing. M.G.: experimental design and statistical analysis. A.L.G. and D.P.N.: supervision and project administration. All authors have read and agreed to the published version of the manuscript.

Funding

Data availability statement

Raw data is available on request.

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgments

List of abbreviations

^{1}H NMR Proton nuclear magnetic resonance
ANCOVA Analysis-of-covariance
ANOVA Analysis-of-variance
C^2HCl_3 Deuterated chloroform
CHPDs Conjugated hydroperoxydienes
HPMs Hydroperoxymonoenes
IV Iodine value
L Linoleoylglycerols
L + O Composite linoleoylglycerol and oleoylglycerol signals
Ln Linolenoylglycerol
LOPs Lipid oxidations products
MUFA Monounsaturated fatty acid
O Oleoylglycerol signal
O + S Composite oleoylglycerol and saturated FA acylglycerol signals
PUFA Polyunsaturated fatty acid
S Saturated FA acylglycerol signal
S(+M)FA Saturated (and modified) fatty acid
SFA Saturated fatty acid
TAG Triacylglycerol
TBB 1,3,5-tribromobenzene
TMS Tetramethylsilane
TOCSY Total correlation spectroscopy
UFA Unsaturated fatty acids
UK United Kingdom
α,β-UAs α,β-unsaturated aldehydes

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