Electronic Supplementary Material (ESI) for Food & Function.

Characterisation of peroxidation products arising from culinary oils exposed to continuous and discontinuous thermal degradation processes

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Summary S1. Calculation of molar percentage of certain key acyl groups

The molar percentage of certain key acyl groups in the studied culinary fat and oils can be determined from the formulae outlined in equation (1) to (7).

Omega-3 acyl groups (%) = 100 × $[A_B / (A_B + A_A)]$ (1)

Oleic (or monounsaturated) acyl groups (%) = 100 × $[(A_E - 2\,A_G - A_H) / 3\,A_I]$ (2)

Linoleic acyl groups (%) = 100 × $(2\,A_G / 3\,A_I)$ (3)

Linolenic acyl groups (%) = 100 × $(2\,A_H / 3\,A_I)$ (4)

Polyunsaturated acyl groups (%) = Linoleic (%) + Linolenic (%) (5)

Unsaturated acyl groups (%) = Oleic (%) + Linoleic (%) + Linolenic (%) (6)

Saturated and modified acyl groups (%) = 100 × $[1 - (A_E / 3\,A_I)$ (7)

Iodine value = 10.54 + 13.39 × [100 × (A_K / Signal peaks ranging between A_A to A_K)] (8)

Where A_B is the area of signal B (methylic protons of ω-3 acyl groups), A_E is the area of signal E (*mono*-allylic protons of all unsaturated acyl groups), A_G is the area of signal G (*bis*-allylic protons of linoleic acyl groups), A_H is the area of signal H (*bis*-allylic protons of linolenic acyl groups) and A_I *is the area of signal I (protons of carbon atoms 1 and 3 of the glyceryl group) and* A_K *is the area of signal K (olefinic protons of all unsaturated acyl groups)* (Martínez-Yusta *et al.*, 2014; Nieva-Echevarría *et al.*, 2016).

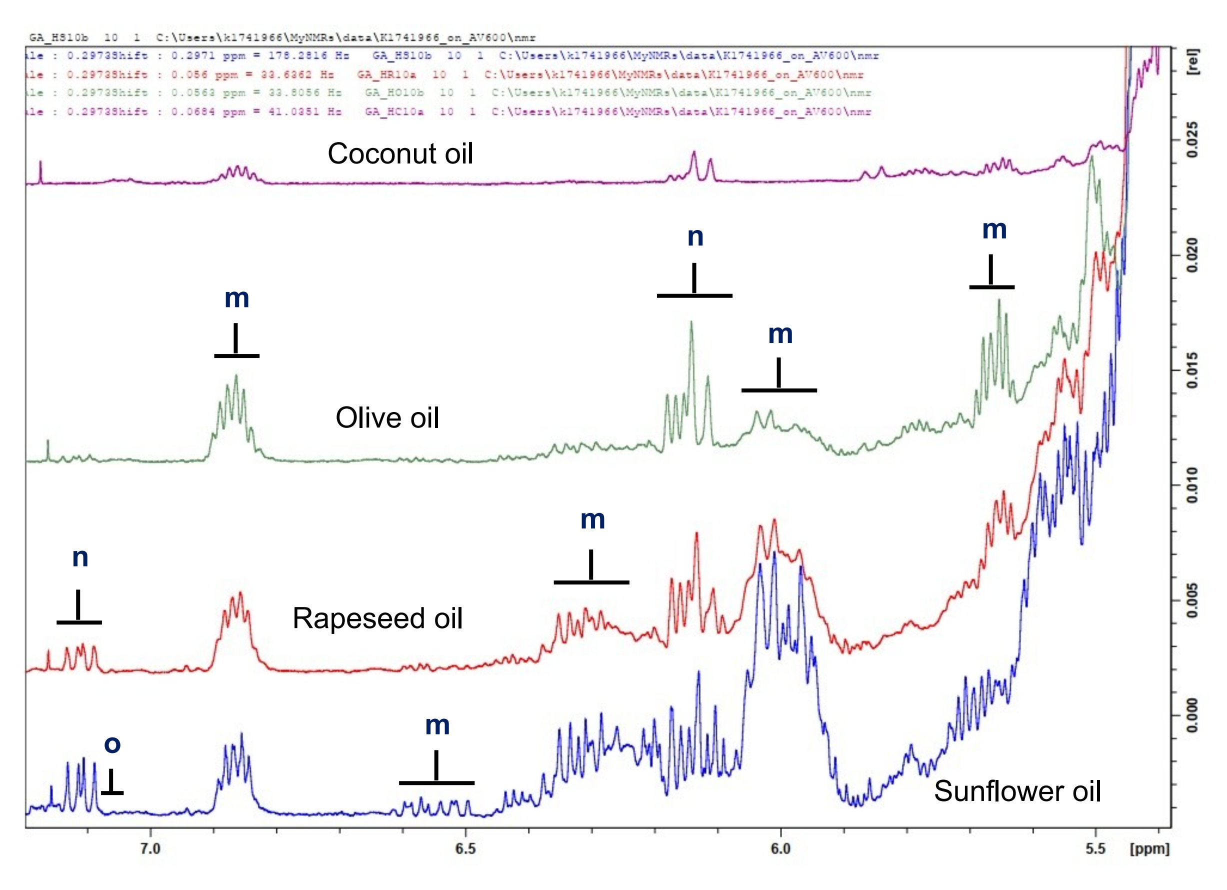

Fig. S1 ^{1}H NMR spectra of conjugated diene hydroperoxydienes and hydroxymonoenes (primary LOPs) present in the 5.4 – 7.1 ppm regions of culinary oils thermally stressed continuously at time 300 min.

Letters assigned to signals in Figure S1 correspond to those in Table 2.

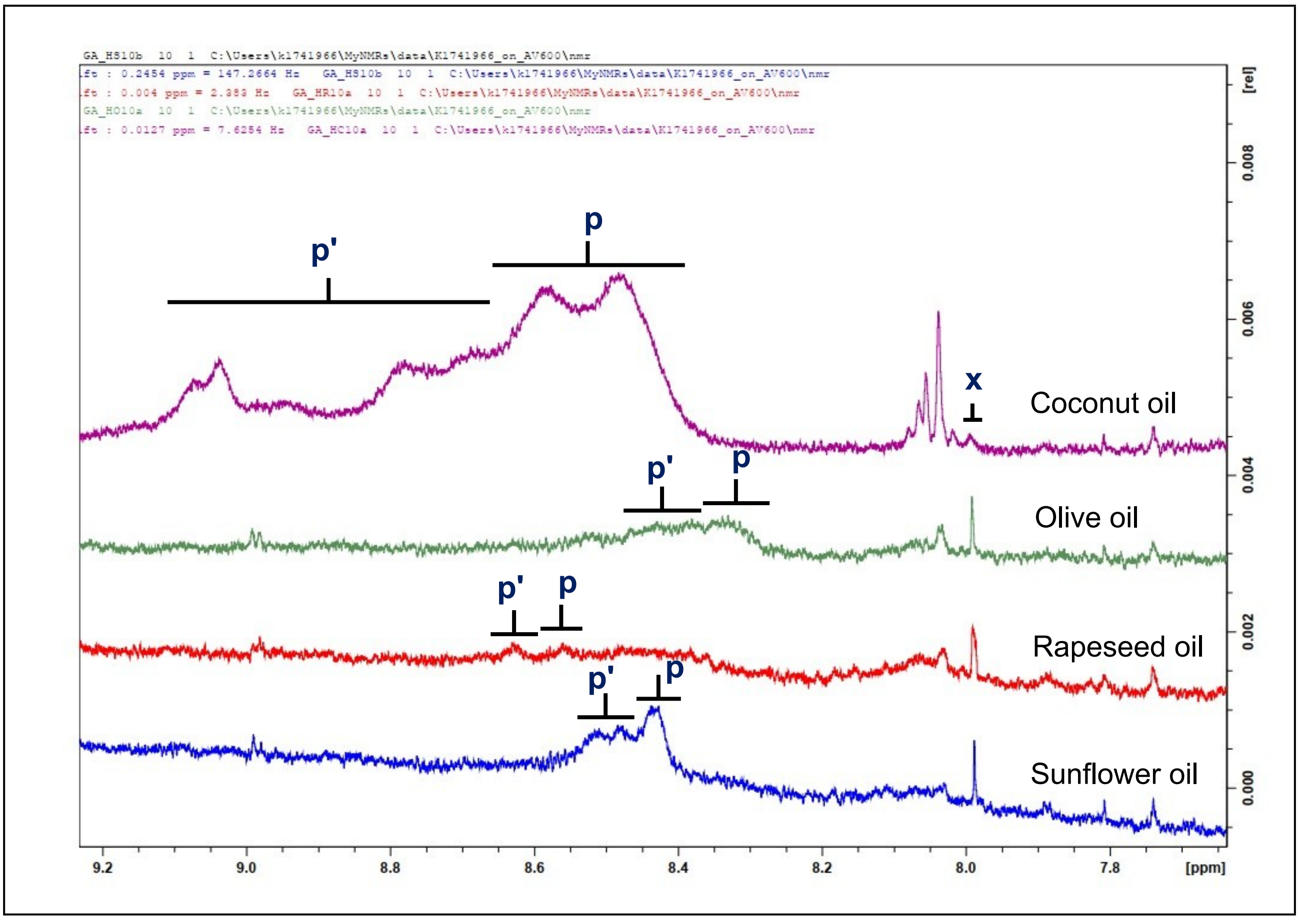

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Fig. S2 ^{1}H NMR spectra of a hydroperoxide group (primary LOPs) and methanoic acid present in the 7.5 – 9.2 ppm regions of sunflower oil thermally stressed continuously throughout a 300 min period.

Signal p is a designated OOH– (*E*,*E*) hydroperoxides group.

Signal p' is a designated OOH– (*Z*,*E*) hydroperoxides group.

Letter assignments correspond to those in Table 2.

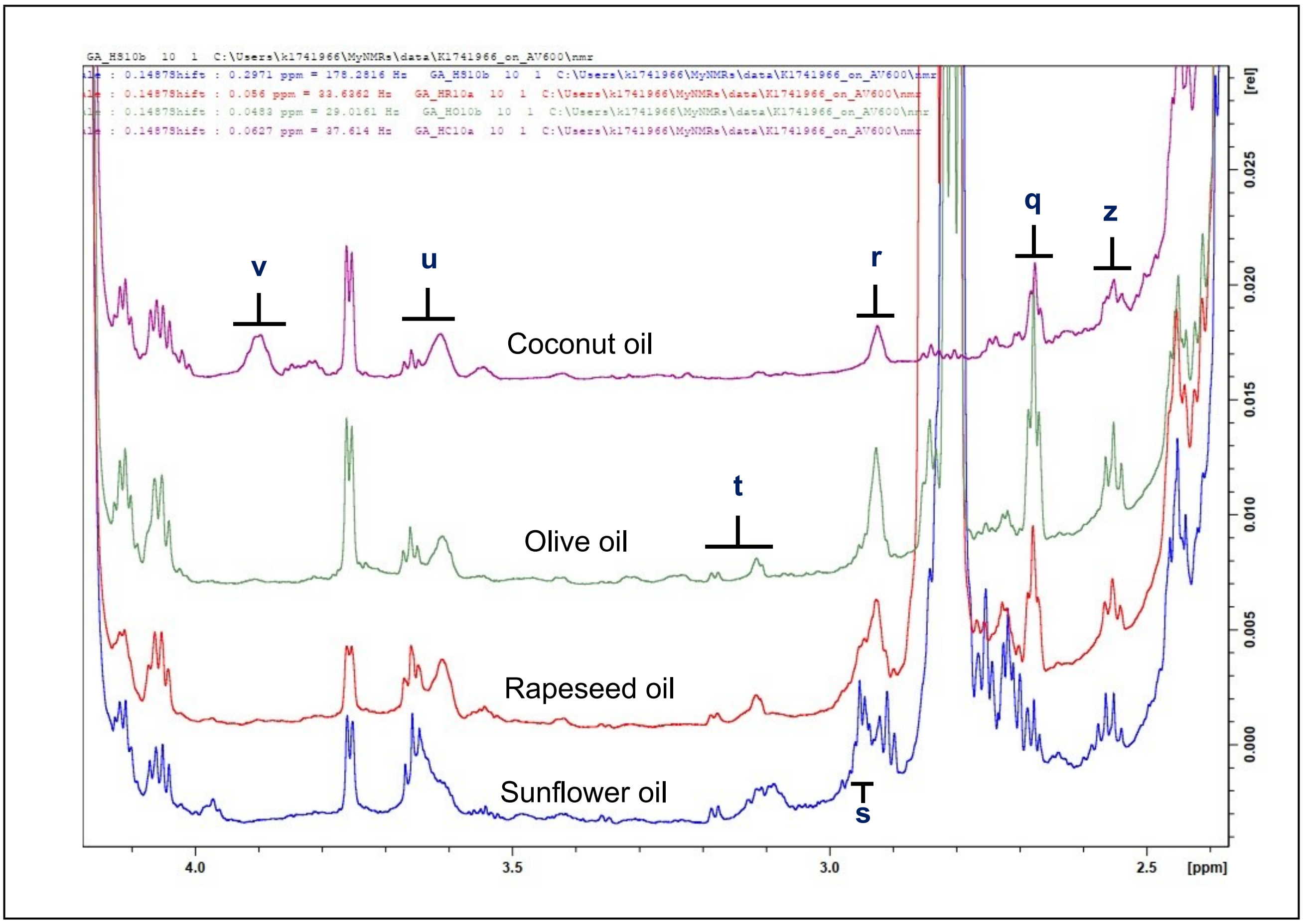

Fig. S3. ^{1}H NMR spectra of epoxides (secondary LOPs) and primary alcohols present in the 2.4 – 4.2 ppm regions of culinary oils thermally stressed continuously at time 300 min.

Letters assigned to signals in Figure S3 correspond to those in Table 3.

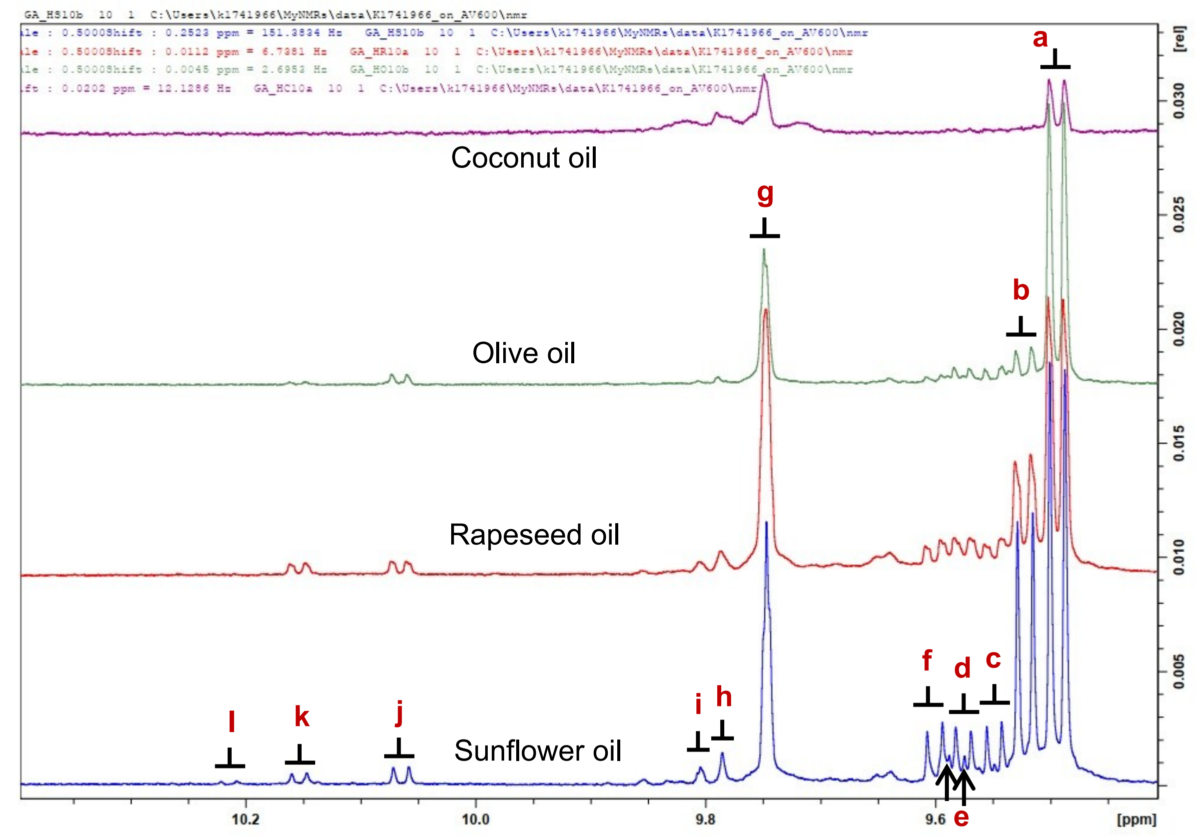

Fig. S4 ^{1}H NMR spectra of aldehydes (secondary LOPs) present in the 9.3 – 10.4 ppm regions of culinary oils thermally stressed continuously at time 300 min.

Letters assigned to signals in Figure S4 correspond to those in Table 4.

Table S1 Comparisons of the FA acyl groups of culinary oils between time 0 (unheated) and 120 min. (thermally-stressed) according to exposure to a continuous heating, and discontinuous heating and cooling systems

Acyl group (Molar %)	Unheated culinary fat and oil at 0 min				Thermally-stressed culinary fat and oil at 120 min							
	Coconut oil	Olive oil	Rapeseed oil	Sunflower oil	Coconut oil		Olive oil		Rapeseed oil		Sunflower oil	
					Cont.	Discont.	Cont.	Discont.	Cont.	Discont.	Cont.	Discont.
SFA	95.90±0.35	14.79±1.03	7.32±0.50	11.86±0.20	96.51±0.40	97.14±0.02	17.31±0.93	20.20±1.01	10.20±0.36	11.67±1.50	16.44±0.54	15.72±1.38
ω-3	-	-	10.09±0.14	-	-	-	-	-	8.90±0.29	8.83±0.27	-	-
MUFA (O)	3.42±0.34	76.21±1.00	63.42±0.44	31.21±0.09	3.10±0.14	2.55±0.01	74.88±0.64	72.91±0.60	64.35±0.27	62.93±0.39	30.62±0.26	32.32±0.43
L	0.68±0.04	8.23±0.12	21.06±0.80	56.92±0.13	0.39±0.26	0.32±0.02	7.19±0.25	6.53±0.39	17.98±0.20	18.99±0.87	52.94±0.69	51.96±1.76
Ln	-	0.77±0.08	8.21±1.29	-	-	-	0.62±0.04	0.35±0.04	7.47±0.25	6.41±0.28	-	-
PUFA (L+Ln)	0.68±0.04*	9.00±0.06	29.27±0.84	56.92±0.13*	0.39±0.26*	0.32±0.02*	7.81±0.29	6.88±0.42	25.45±0.14	25.40±1.11	52.94±0.69*	51.96±1.76*
UFA (O+L+Ln)	4.10±0.35*	85.21±1.03	92.68±0.50	88.14±0.20*	3.49±0.40*	2.86±0.02*	82.69±0.93	79.80±1.01	89.80±0.36	88.33±1.50	83.56±0.54*	84.28±1.38*
IV	15.04±0.25	80.59±0.08	106.80±0.33	123.74±0.49	13.57±0.49	12.54±0.07	77.14±1.23	75.76±1.15	100.30±1.33	100.52±2.05	114.46±0.70	110.72±2.07

*Indicates that the linolenic acid acyl group signal was not $^{1+H\ \text{NMR-detectable}}$.

Abbreviations: *SFA, saturated acyl groups; MUFA, monounsaturated acyl groups; PUFA, polyunsaturated acyl groups; UFA, unsaturated acyl groups; ω-3, omega-3 acyl groups; O, Oleic acyl groups; L, linoleic acyl groups; Ln, linolenic acyl groups; IV; Cont., Continuous heating; Discont., Discontinuous heating and cooling.*

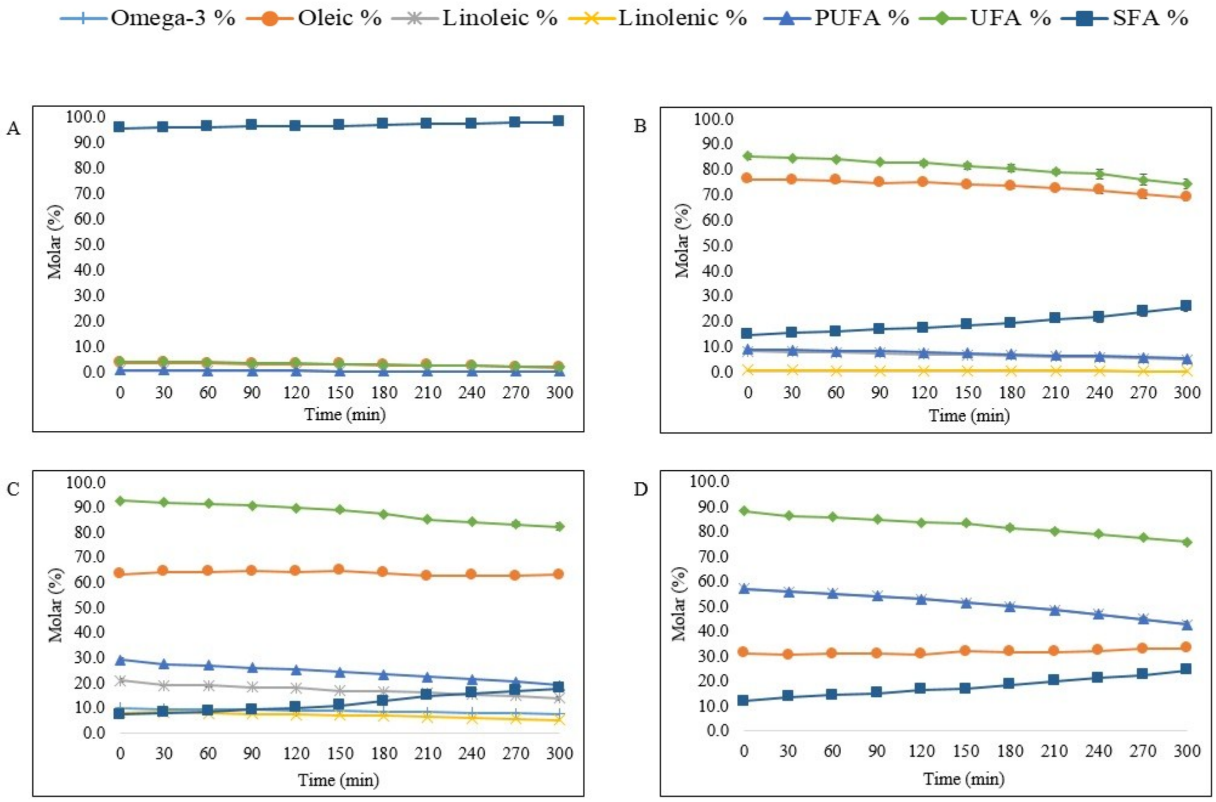

Fig. S5 Changes in percentage acyl groups of thermally-stressed coconut fat (A), olive oil (B), rapeseed oil (C) and sunflower oil (D) subjected to a continuous heating.

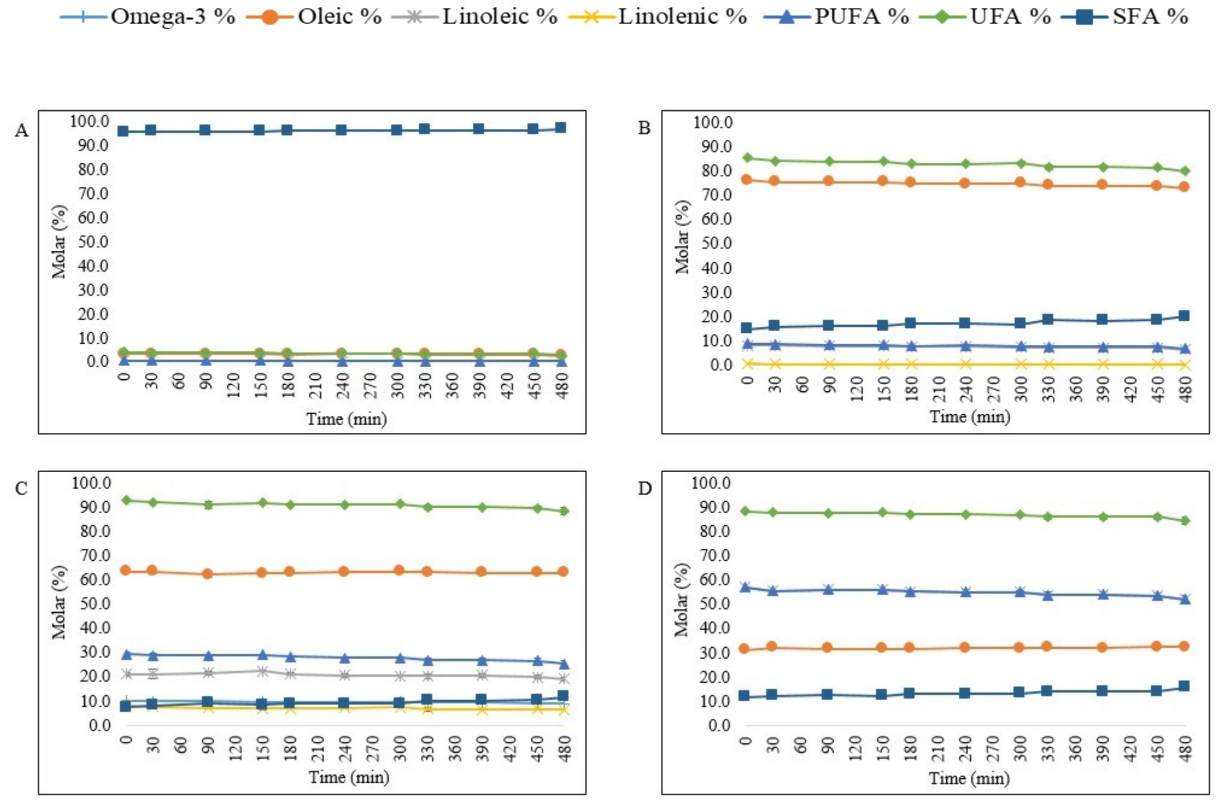

Fig. S6 Changes in percentage acyl groups of thermally-stressed coconut fat (A), olive oil (B), rapeseed oil (C) and sunflower oil (D) subjected to a discontinuous heating.

Summary S2. General observations for LOPs

As reviewed by Martínez-Yusta et al. (2014), LOPs, as directly observed in the ^{1}H NMR spectra may be quantified in two ways. The first being the absolute method that is in relation to a standard compound initially added to the lipid-reaction mixture during the 1H NMR analysis and the second being in relation to a major functional group, which could be triglyceride protons (signal I) or methylic protons (signal A) The latter serves the advantage of being faster and efficient (Guillén and Goicoechea, 2009). However, in this study, the first method was employed and the standard used was 1,3,5-tribromobenzene (TBB). Following that, all LOPs were quantified using equation (8).

$$\text{LOPs (mM)} = \text{Signal peak} \times \text{Concentration of TBB} \tag{8}$$

The signal peak in equation (8) represent any of the signals a to k. The concentration of TBB is 1.1 mM.

At constant temperature of 180°C, the amounts of LOPs generated increase proportionately with time. Also, the degree of unsaturation had a great influence on the evolution of LOPs (Grootveld et al., 2014). By implications, thermally-stressed SFArich coconut fat yielded only three types of LOPs namely; (E)-2-alkenals (signal a), n-alkanals (signal f) and 4-oxo-alkanals (signal g). The concentrations of these LOPs were all lower in comparison to those of the oils. There were striking similarities between olive oil and rapeseed oil since both oils are MUFA-rich oils (Table 1) and therefore, share a similar degree thermo-oxidation resistibility characteristics. Sunflower oil yielded the largest proportion of LOPs over a 300 min heating period This included the doublet ‘signal k’ which was absent in coconut fat, as well as olive and rapeseed oil.

Table S2 A comparison of LOPs generated in culinary oils between time 0 (unheated) and 120 min. (thermally-stressed) time-points according to continuous heating and discontinuous heating/cooling protocols

LOPs (mM)	Unheated culinary fat and oil at 0 min				Thermally-stressed culinary fat and oil at 120 min							
	Coconut fat	Olive oil	Rapeseed oil	Sunflower oil	Coconut fat		Olive oil		Rapeseed oil		Sunflower oil	
					Cont.	Discont.	Cont.	Discont.	Cont.	Discont.	Cont.	Discont.
(*E*)-2-alkenals	-	0.09±0.01	-	0.15±0.00	0.24±0.09	0.43±0.02	0.93±0.37	2.32±0.37	1.26±0.16	1.62±0.23	1.73±0.65	1.65±0.30
(*E*,*E*)-2,4-alkadienals	-	0.02±0.00	-	-	-	-	0.28±0.09	0.52±0.11	1.09±0.03	1.40±0.34	1.81±0.62	1.60±0.47
4,5-epoxy-(*E*)-alkenals	-	-	-	-	-	-	0.09±0.03	0.19±0.06	0.25±0.02	0.19±0.09	0.31±0.13	0.31±0.08
4-hydroxy-(*E*)-2-alkenals	-	-	-	-	-	-	0.09±0.03	0.20±0.06	0.31±0.06	0.31±0.09	0.35±0.11	0.36±0.12
4-hydroperoxy-(*E*)-2-alkenals	-	-	-	-	-	-	0.04±0.02	-	0.19±0.03	-	0.22±0.06	-
(*Z*,*E*)-2,4-alkadienals	-	-	-	-	-	-	0.07±0.01	0.12±0.04	0.37±0.03	0.49±0.10	0.46±0.16	0.42±0.13
n-alkanals	-	0.09±0.00	-	0.15±0.01	0.39±0.10	0.72±0.02	0.63±0.20	1.66±0.33	1.05±0.09	1.52±0.47	1.35±0.39	1.46±0.48
4-oxo-alkanals	-	-	-	-	0.09±0.08	0.12±0.04	0.03±0.01	0.10±0.03	0.05±0.02	0.13±0.05	0.10±0.03	0.12±0.04
n-alkanals (low mwt)	-	-	-	-	-	-	0.03±0.01	0.06±0.02	0.09±0.01	0.16±0.04	0.18±0.05	0.21±0.05
(*Z*)-2-alkenals	-	-	-	-	-	-	0.04±0.01	0.07±0.01	0.05±0.02	0.03±0.00	0.13±0.07	0.03±0.01
Signal K	-	-	-	-	-	-	0.02±0.01	-	0.13±0.09	-	0.18±0.07	0.04±0.01
Signal L	-	-	-	-	-	-	-	-	-	-	0.80±0.04	-

Abbreviations: *Cont., Continuous heating; Discont., Discontinuous heating and cooling; mwt, molecular weight. Both signal K and L are unidentified.*

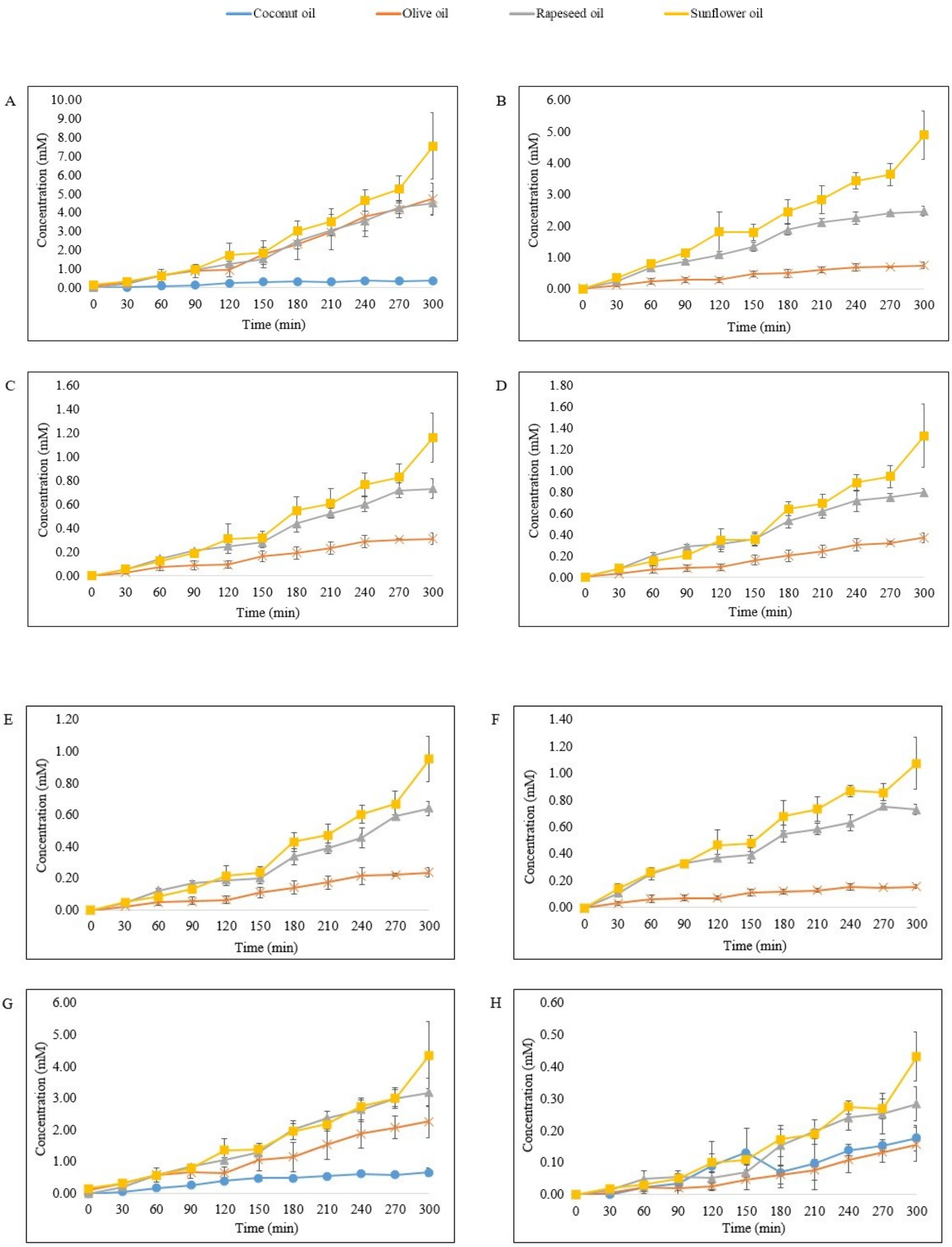

Fig. S7 Evolution of (*E*)-2-alkenals (A); (*E*,*E*)-2,4-alkadienals (B); 4,5-epoxy-(*E*)-alkenals (C); 4-hydroxy-(*E*)-2-alkenals (D); 4-Hydroperoxy-(*E*)-2-alkenals (E); (*Z*,*E*)-2,4-alkadienals (F); *n*-alkanals (G); 4-oxo-alkanals (H); *n*-alkanals (low mwt) (I); (*Z*)-2-Alkenals; (J); Unidentified unsaturated aldehyde (K and L); of thermally-stressed culinary oils subjected to a continuous heating.

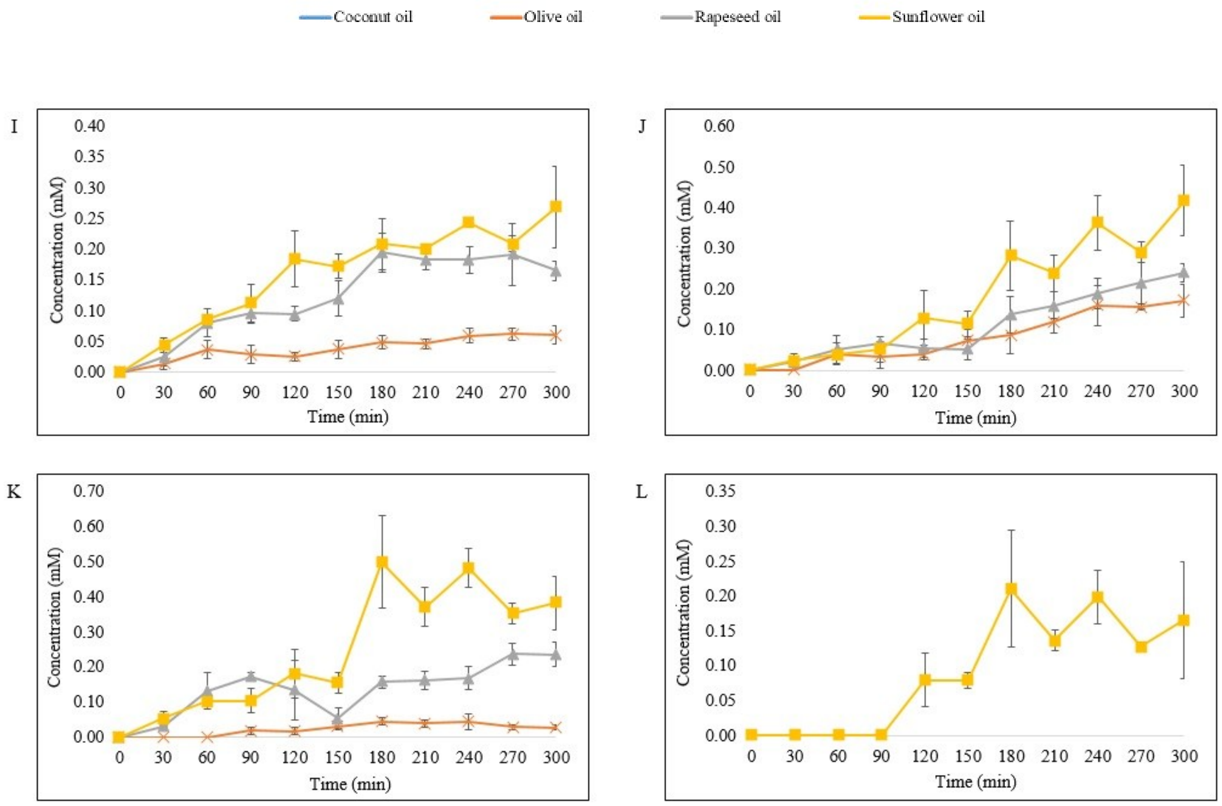

Fig. S7 (*Continued*) Evolution of (*E*)-2-alkenals (A); (*E*,*E*)-2,4-alkadienals (B); 4,5-epoxy-(*E*)-alkenals (C); 4-hydroxy-(*E*)-2-alkenals (D); 4-Hydroperoxy-(*E*)-2-alkenals (E); (*Z*,*E*)-2,4-alkadienals (F); *n*-alkanals (G); 4-oxo-alkanals (H); *n*-alkanals (low mwt) (I); (*Z*)-2-Alkenals; (J); Unidentified unsaturated aldehyde (K and L); of thermally-stressed culinary oils subjected to a continuous heating.

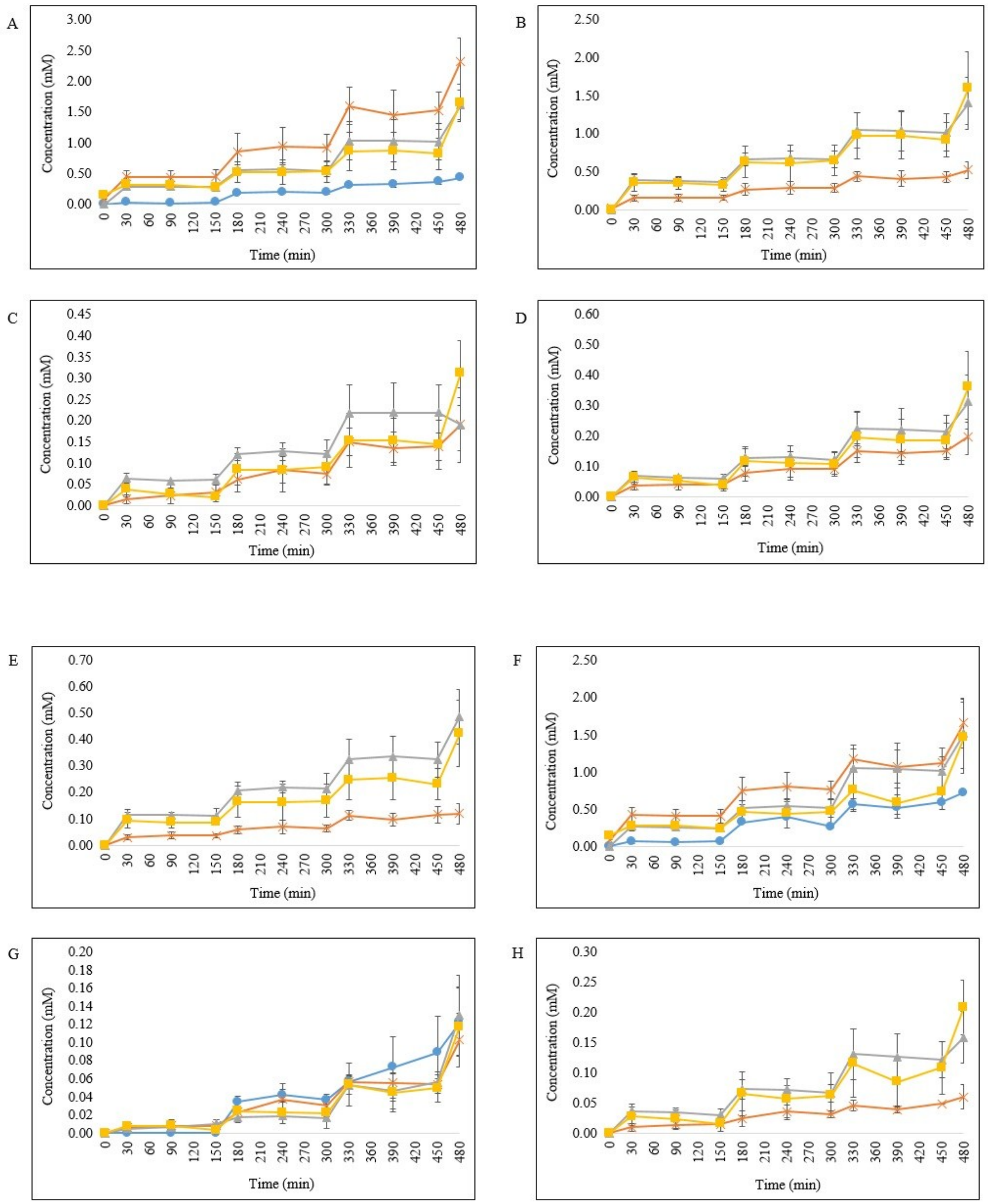

Fig. S8 Evolution of (*E*)-2-alkenals (A); (*E*,*E*)-2,4-alkadienals (B); 4,5-epoxy-(*E*)-alkenals (C); 4-hydroxy-(*E*)-2-alkenals (D); (*Z*,*E*)-2,4-alkadienals (E); *n*-alkanals (F); 4-oxo-alkanals (G); *n*-alkanals (low mwt) (H); (*Z*)-2-Alkenals; (I); Unidentified unsaturated aldehyde (J); of thermally-stressed culinary oils subjected to a discontinuous heating.

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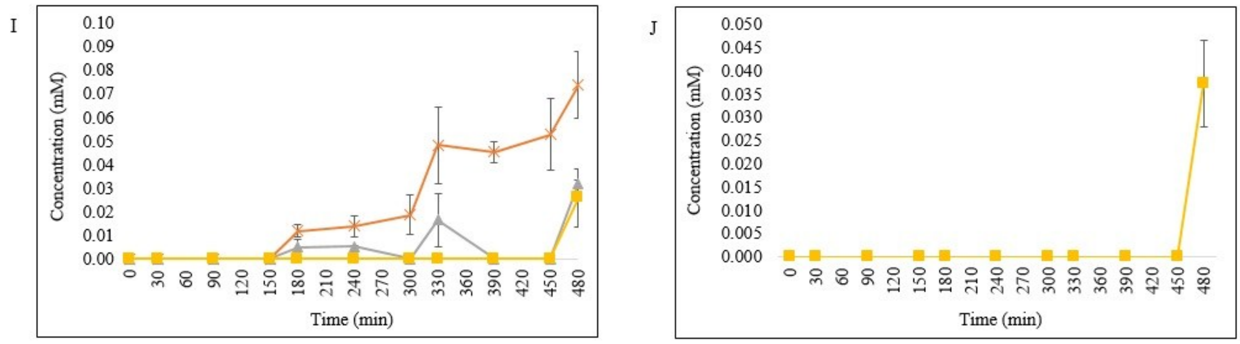

Fig. S8 Evolution of (*E*)-2-alkenals (A); (*E*,*E*)-2,4-alkadienals (B); 4,5-epoxy-(*E*)-alkenals (C); 4-hydroxy-(*E*)-2-alkenals (D); (*Z*,*E*)-2,4-alkadienals (E); *n*-alkanals (F); 4-oxo-alkanals (G); *n*-alkanals (low mwt) (H); (*Z*)-2-Alkenals; (I); Unidentified unsaturated aldehyde (J); of thermally-stressed culinary oils subjected to a discontinuous heating.

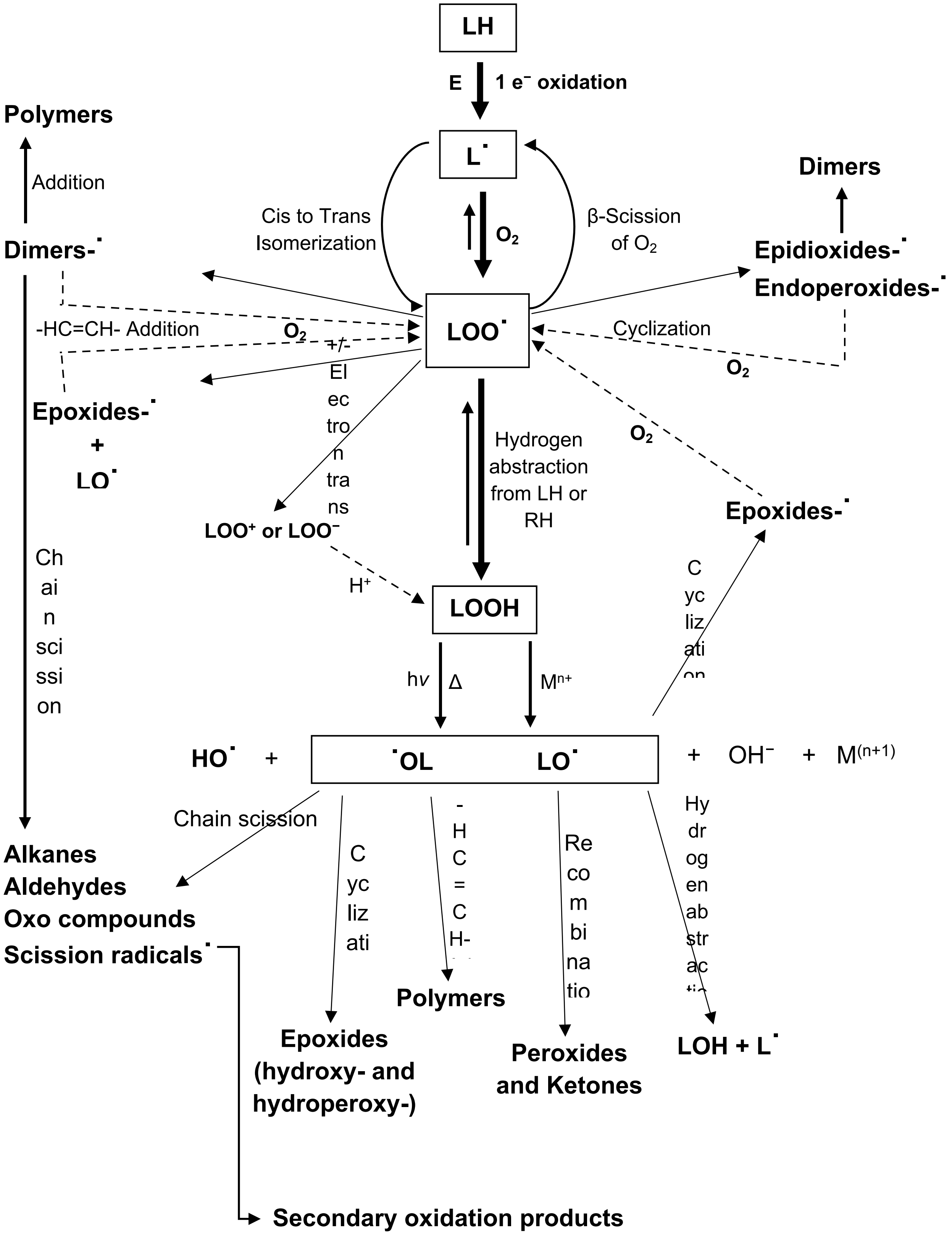

Fig. S9 A proposed integrated reaction scheme for lipid oxidation products. Adapted from Schaich, 2012 and slightly modified.