Research article

Characterization of the evolution of free radicals and TALAs in linseed oil during heat treatment

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ABSTRACT

Various studies have demonstrated that employing ESR spin trapping to detect free radicals yields valuable insights into the vulnerability of bulk oils to oxidation. Consequently, this method can be employed to assess and compare the oxidative stability of different samples. This study was conducted to investigate the production and transformation of free radicals and trans isomers in linseed oil when subjected to different temperatures and durations of heating. These analyses revealed that the peak levels of free radicals PBN adducts were evident in linseed oil heated to 120 °C, while these levels decreased within 90 min and were absent at a higher temperature of 180 °C. Free radical PBN adducts were readily degraded at 180 °C. Levels of heat-induced trans isomers rose in linseed oil samples with rising temperatures but began to degrade at temperatures exceeding 240 °C partially. The content examination of these trans isomers revealed that the double bonds located at positions 9 and 15 exhibited a higher susceptibility to isomerization compared to the double bond at position 12. Furthermore, the values of k and E_a indicated that the synthesis of tri-trans-α-linolenic acid (TALAs) was more challenging compared to double-TALAs, and double-TALAs were more challenging than single-TALAs. This was because the tri-TALAs has a higher E_a value than the mono-TALAs and double-TALAs. The study has demonstrated that subjecting linseed oil to high-temperature heating leads to the production of free radicals and trans isomers. And PBN radical adduct is unstable at 180 °C and the double bonds at positions 9 and 15 could be isomerized more easily than that at position 12. These results indicated that controlling the formation of free radicals and single-TALAs isomers may be the key way to reduce the trans isomers of linolenic acid during cooking oil heating. In the follow-up study, we found that VE, VK3, ethyl caffeic acid and resveratrol had significant inhibitory effects on the formation of TALAs of linolenic acid, and the highest inhibitory rate of resveratrol with 5% addition could be reached to 30.86%. The above substances can be applied to the thermal processing of linseed oil to prevent the formation of TALAs.

1. Introduction

Linseed oil is commonly utilized as a culinary oil in regions of China such as Inner Mongolia, Gansu, and Ningxia. It has been documented to provide advantageous effects, such as the ability to prevent allergic reactions, maintain eyesight, avert cardiovascular disease, and reduce levels of blood lipids and cholesterol [1,2]. Linseed oil contains high levels of α-linolenic acid, which is a particularly important edible oil rich in UFAs. Linseed oil is rich in unsaturated fatty acids, especially α-linolenic acid, which accounts for 50–60% of linseed oil [3]. This is a particularly important edible oil rich in unsaturated fatty acids (UFAs).

The oil has advantageous qualities such as improving the capacity of blood to carry oxygen, relieving hypoxia associated with cerebrovascular or cardiovascular diseases, guarding against the occurrence of cardiovascular diseases, and boosting superoxide dismutase activity in humans [4,5].Nevertheless, these oils are characterized by numerous unsaturated double bonds and a diverse range of fatty acid types. The interconversion of fatty acid types, along with concurrent processes of isomerization, polymerization, and oxidation, further complicates the composition of these oil systems. Consequently, these complexities hinder the research emphasis on the properties and advantages of trans fatty acids (TFAs) [6]. TFAs have always been the focus of food safety issues; excessive intake of TFAs will lead to an increase in the incidence of cardiovascular disease. Linolenic acid forms TFAs through thermal isomerization [7,8].

The key factor influencing UFA thermal isomerization is temperature. At room temperature, the double bonds in UFAs stay stable, resulting in infrequent cis/trans isomerization [9]. However, at higher temperatures, TFAs can occur more easily [10]. Initial studies have shown that the conversion of cis/trans isomers of unsaturated fatty acids can occur by the rearrangement of double bonds, facilitated by the influence of sulfur-containing radicals (LS·), alkyl radicals (LOO·), and other free radicals [11]. Therefore, it is crucial to develop methods that can prevent these isomerization reactions by manipulating the formation and development of free radicals found in lipid samples, as well as studying the thermal isomerization processes.

Electron spin resonance (ESR) is utilized to detect radicals by evaluating initial oxidation events in oils and other food products [12–19]. ESR enables the direct tracking of unpaired electrons, offering in situ and non-destructive electron microscale details regarding orbits and atomic nuclei [20]. Free radicals are difficult to identify by ESR because of their short half-lives. However, this problem can be overcome by using a free radical scavenger. Numerous scavengers of free radicals have been developed, comprising nitroso compounds like 2- methyl -2- nitrosopropane (MNP), linear nitrones such as α-Phenyl-*tert*-butyl nitrone (PBN), and cyclic nitrones like 5,5-dimethyl-1-pyrroline-1-oxide (DMPO). Fig. 1 represents the capture response pathways that these three scavenger types mediate (Fig. 1A–C).

PBN exhibits powerful reactivity along with excellent lipophilic characteristics, making it a suitable instrument for monitoring oil oxidative stability [21]. PBN is frequently used as a means of quickly and readily quantifying free radical levels in a given oil system [22]. The lipid radical L· can form a relatively stable adduct of PBN by attacking the Cα atom next to the -N-O group in PBN. Lipid free radical formation in a system that is being heated can be monitored via ESR, and activities mediated mainly by LOO·, LO·, and L· include free radical chain reactions in lipid oxidation [17]. Studies of the production of free radicals and oxidation have the potential to provide mechanistic insights regarding trans isomer formation, given the abundance of basic fatty acids in linseed oil.

The ability of free radicals to promote isomerization is well documented [6], with thermal processing inducing the isomerization of UFAs to yield more stable TFAs [23–26].This method allows for the evaluation of early-stage lipid oxidation related to various diets [27,28]. Li et al. [29] proposed that when α-linolenic acid is heated at high temperatures, it can produce single-TALAs, double-TALAs,
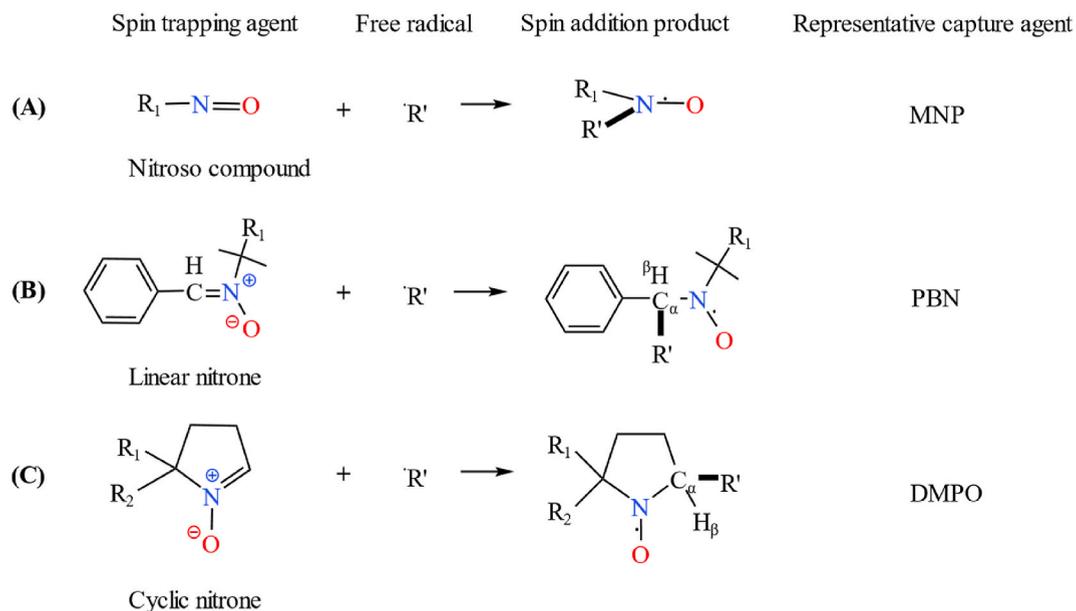
Fig. 1. The capture reaction processes of nitroso compounds(A), linear nitrone(B) and cyclic nitrone(C).

and tri-TALAs. This conclusion was reached based on their investigation of the thermal isomerization of oleic and linolenic acid. Specifically, they found that C18:3–15t; 9t,12t,15c, and C18:3-9c,12t,15t were favored at temperatures exceeding 220 °C, while C18:3-9t,12c,15c was favored at temperatures exceeding 180 °C.

In this study, ESR and GC-FID techniques were employed to observe the production of free radicals and the kinetics of formation of TALAs while heating linseed oil. These kinetic studies may provide insight into methods for controlling unwanted isomerization reactions during the heating of edible oils.

2. Materials and methods

2.1. Materials

Linseed oil, PBN, methanol, and boron trifluoride (14% in methanol) were obtained from Macklin. Mixtures of α-linolenic acid methyl esters (9c,12c,15c; 9c,12c,15t; 9t,12c,15c; 9c,12t,15c; 9t,12c,15t; 9t,12t,15c; 9c,12t,15t; 9t,12t,15t) and n-hexane were from Sigma-Aldrich.

2.2. ESR spectroscopy

An ESR analysis was conducted using a Bruker micro ESR spectrometer (Bruker, Germany) at a temperature of 25 °C, which is the standard room temperature. In brief, PBN samples (200 μL, 150 mmol/L in xylene) were mixed with 0.5 g linseed oil samples before being subjected to heat treatment. The samples were then heated to temperatures of 60, 90, 120, 150, and 180 °C, with corresponding heating durations of 30, 60, 90, 120, 150, 180, 210, and 240 min, respectively. To better explore changes in PBN free radical adducts and the reason why PBN free radical adduct disappeared at 180 °C, linseed oil was subjected to heating at temperatures of 90 °C and 180 °C for 120 min. Subsequently, the oil samples were transported to temperatures of 180 °C and 90 °C, respectively, for further analysis.

Individual samples of linseed oil were initially transferred into nuclear magnetic tubes (XWE-5mm-7–50, Chongqing Synthware Glass, CQ) and heated for appropriate amounts of time in an oil bath pot. ESR instrument settings were as follows: center field = 3460G, scan range = 100G, microwave power = 60 mW, mod coil amplitude = 3G, number of scans = 5, and number of points = 4440. Data collection was performed using the μESR Control Software and subsequently analyzed using the Origin 2021 program.

2.3. Linseed oil thermal processing

1.00 g of linseed oil was poured into individual 4 mL glass ampoule bottles. The bottles were then sealed using a propane-oxygen flame, leaving air in the headspace. After sealing, these oil samples were heated using an air-drying oven for 4, 8, 12, 24, 36, 48, 72, and 96 h at temperatures of 180, 210, 220, 230, and 240 °C (±0.1 °C).

2.4. Fatty acid methyl ester preparation

Fatty acid methyl ester preparation was performed as per the Chinese national standard GB 5009.168-2016. Briefly, samples of linseed oil (50 ± 5 mg) were dried in a 19 × 90 mm PTEF-lined screw cap pressure bottle. Then, 2 mL of sodium hydroxide (2% in methanol) was added to this bottle, and the cap was closed tightly, followed by incubation in a 70 °C water bath until the linseed oil was fully dissolved. Subsequently, a volume of 2 mL of boron trifluoride (14% concentration in methanol) was introduced into the container, and the samples were subjected to additional heating for 5 min. After cooling, the samples were then subjected to the addition of 2 mL of isooctane into the bottle, followed by agitation for 2 min. Then, 1 mL of a saturated NaCl solution was added to the bottle, and the supernatant fraction was passed through a 0.45 μm organic filter prior to transfer into a gas-phase vial. Fatty acid analyses were performed using 1 μL of the supernatant fraction.

2.5. GC-FID analyses

Linolenic acid isomers present in linseed oil samples were analyzed via GC-FID [27,28]. A GC instrument (TRACE3000, Thermo Fisher Inc.) equipped with an ionic liquid SLB-IL111 column (200 m × 0.25mm × 0.2 mm) and a flame ionization detector (FID, Thermo Fisher Inc.) was used for these analyses, with helium (99.999%) serving as a carrier gas at a 1.0 mL/min flow rate. Column temperature settings were as follows: 60 °C for 5 min, followed by an increase to 150 °C at 15 °C/min, 150 °C for 10 min, an increase to 170 °C at a 2 °C/min, 170°Cfor 15 min, an increase to 180 °C at 1 °C/min, 180 °C for 28 min, and a final increase to 185 °C at 0.2 °C/min followed by incubation at 185 °C for 10 min. The temperature of the injector was set to 240 °C, the volume of injection was 1 μL, and a split ratio of 1:60 was employed. The identification and quantification of linolenic acid isomers were performed by comparing their retention durations to those of commercially available standards. The amounts of linolenic acid isomers were reported as g/100 g of linseed oil.

2.6. Kinetics of isomerization reactions

Equation (1) was used to calculate the amount of isomer formation. The activation energy (E_a) for these heat-induced reactions was

determined by calculating the reaction rate' *k*' at a minimum of three different temperature levels. In order to calculate the E$_a$ for isomerization reactions, Equation (2) was utilized:

$$C_{isomer} = k \bullet t \tag{1}$$

$$\ln k = -\frac{E_a}{R} \bullet \frac{1}{T} + c \tag{2}$$

Where Cisomer = isomer content (g/100g), *k* = the reaction rate, E$_a$ = activation energy (kJ), t = the reaction time(h), R = 8.314J/mol·K, T = degree kelvin(K), c = intercept of the fitting equation.

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Studies of the TALAs kinetics of heated linseed oils were based on both the consumption of linolenic acid and the formation of linolenic acid isomers. The concentrations of linolenic acid and TALAs were measured at regular time intervals during heating, and results were displayed in Arrhenius plots. In order to select the most suitable model for the experimental data, the resulting plots were fitted using zero-, first-, and second-order kinetics. The fit that yielded the highest R2 value was considered reflective of the proper kinetic model for the process. The plots were utilized to determine the respective rate constants for each reaction. To further verify the order of each reaction, residuals were calculated for each reaction step on the Arrhenius plots, and the order of the reaction step was confirmed by comparing the residual values.

2.7. Statistical analysis

Identification of the fatty acid peak in linseed oil samples was accomplished by comparing retention periods and mass spectra with reference compounds. Samples were measured three times, and SPSS 26.0 was used to analyze the data using the least-squares difference approach. The means ± standard deviation of the data were reported, with a significance level of *P* < 0.05.
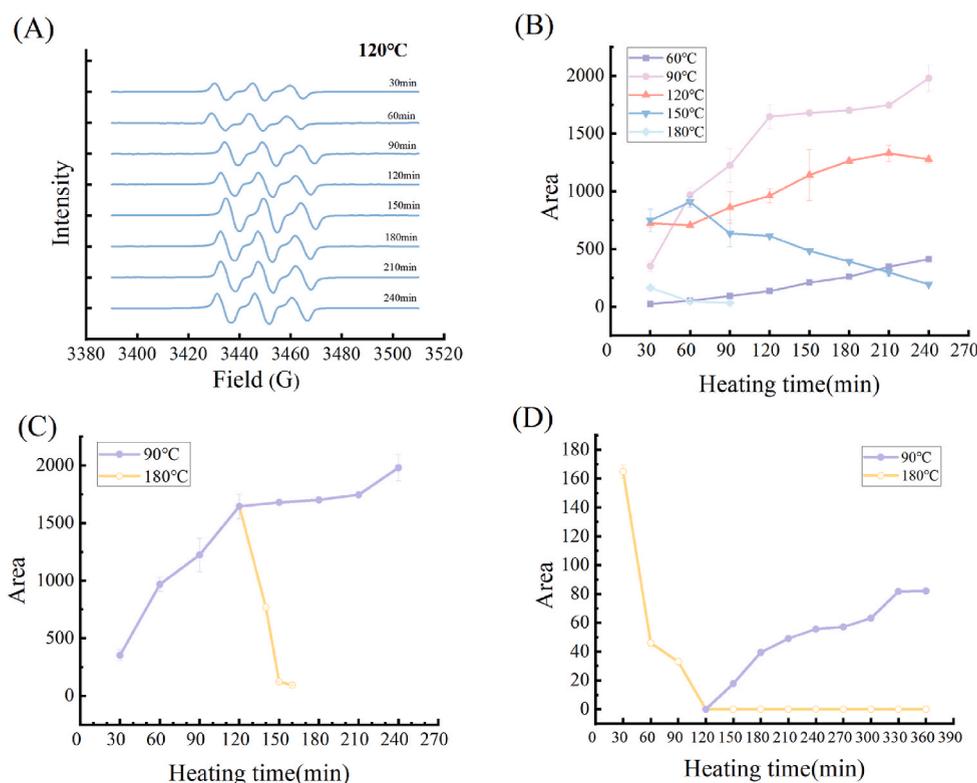
Fig. 2. (A). ESR spectra of PBN-free radical adducts of heated linseed oil at 120 °C. (B). The total area of spins trapped by PBN at different temperatures and times. (C). The changes of linseed oil heated 120min at 90 °C, which was transferred to 180 °C. (D). The changes of linseed oil heated 120min at 180 °C, which was transferred to 90 °C.

3. Results and discussion

3.1. The evolution of free radicals in linseed oil samples throughout heating

Fig. 2A displays the spectrum responses of linseed oil at 120 °C over varied durations of time. Fig. 2B shows the total spin regions trapped by PBN during heating at different temperatures for varying durations of time. A split triple peak corresponding to PBN free radical adducts was detected that was distinct from typical split 6 peaks [30,31]. This could be attributed to the different experimental heating conditions employed for the oil samples in this investigation. Previous research has shown that samples subjected to in situ heating had 6 separate peaks, while samples that were not subjected to in situ heating displayed a triple peak that was split triple peak [22,32]. [1]H splitting was also not clearly evident owing to the line-widening effects resulting from limited rotational mobility (Velasco, Andersen, & Skibsted, 2020).

The total spin area values rose from 60 to 90 °C, reaching peak levels at 90 °C as a consequence of more rapid trapping with rising temperatures, while oxidation-induced free radical generation was less robust at lower temperatures. Upon heating the samples to a temperature of 150 °C, the overall area of free radical spin first increased but subsequently decreased after reaching its maximum point. This observation likely indicates that the removal of these accumulated radicals exceeded their further capture, as oxygen was used within the system [33]. Consequently, a higher rate of oxygen consumption led to a faster depletion of spin adducts in comparison to additional spin trapping. The entire area of free radical spin dropped at a temperature of 180 °C after 90 min and subsequently became undetectable. This could be the outcome of collisions between recently formed free radicals and existing PBN free radical adducts, resulting in final free radical reactions [22,32]. The diminished response reliability observed at 180 °C may be attributed to the higher susceptibility of PBN to degradation at hot temperatures [34].

Further experiments were conducted to describe changes in PBN free radical lipid adducts more accurately. Subjecting linseed oil to a temperature of 90 °C for 120 min (Fig. 2C) resulted in the production of significant amounts of PBN free radical adducts. Subsequently, upon exposure to a temperature of 180 °C, the presence of these free radical adducts in the samples significantly diminished for 40 min, suggesting that at this heightened temperature, PBN adducts are swiftly exhausted, even if initially abundant. In contrast, if linseed oil samples were initially heated for 120 min at 180 °C prior to transfer to the environment of 90 °C (Fig. 2D), high levels of PBN free radical adduct generation were not evident, potentially owing to the rapid degradation of PBN at higher temperatures [34]. This could also be attributed to the increased production of aldehydes, ketones, and oxidation products at elevated temperatures [35].

3.2. The initial α-linolenic acid composition in linseed oil samples

The 8 linolenic acid methyl ester isomers with mixed standards peaks were used for qualitative and quantitative analyses based on peak area and retention time values. The spectrum studies provide information on the initial composition of linolenic acid in linseed oil samples, as shown in Table 1. Total linolenic acid methyl ester levels in linseed oil samples were approximately 66.4343g/100g, with the three mono-TALAs (including C18:3-9c,12c,15t, C18:3-9t,12c,15c, and C18:3-9c,12t,15c) accounting for 1.4936% of total linolenic acids, while double-TALAs (including C18:3-9c,12t,15t, C18:3-9t,12t,15c, and C18:3-9t,12c,15t) accounted for just 0.0269%.

3.3. The evolution of linolenic acid isomers throughout thermal treatment

Samples of linseed oil were able to undergo *trans*-isomerization upon heating. After heated linseed oil was methylated, linolenic acid trans isomers were submitted to qualitative and quantitative GC-based analysis to examine how different linolenic acid isomers evolved over time and at different temperatures (Fig. 3). When linseed oil samples were heated at 180–250 °C, *cis*-linolenic acid isomer levels initially trended downwards (Fig. 3 G), *trans*-linolenic acid isomer levels initially trended upwards with increasing temperature and time values before ultimately being degraded to varying extents. C18:3:9c, 12t, 15t was not produced in the whole heating system, which showed that C18:3:9c, 12t, 15t needs to cross a higher energy barrier when isomerization was carried out.

C18:3:9t,12c,15t, C18:3:9t,12c,15c, C18:3:9c,12t,15c, and C18:3:9c,12c,15t were detected in all heating intervals at temperatures from 180 to 250 °C (Fig. 3A–D). The prolongation of heating time from 180 to 210 °C was associated with an upward trend in the amount of C18:3:9t,12c,15c, with respective final C18:3:9t,12c,15c contents of 3.0430 g/100g and 14.3616 g/100g at 180 °C and 210 °C. At 220 °C and 230 °C, peak levels (17.7893, 16.8856 g/100g) were evident at 72 h, whereas by 96 h, these levels had

Table 1

Mixed calibration curve of α -linolenic acid isomers and the content of α -linolenic acid isomers in raw linseed oil.

Fatty acids	Standard curve (x (mg/mL), y (peak areas))	R^2	Linseed oil concentration (g/100g)
C18:3:9t,12c,15c	y = 1.74686x	0.98877	0.6198 ± 0.0202
C18:3:9c,12t,15c	y = 1.69609x	0.9947	0.0770 ± 0.0016
C18:3:9c,12c,15t	y = 1.98691x	0.99532	0.7968 ± 0.0250
C18:3:9c,12t,15t	y = 1.95655x	0.99508	
C18:3:9t,12c,15t	y = 2.20370x	0.99295	0.0269 ± 0.0036
C18:3:9t,12t,15c	y = 2.56459x	0.994	
C18:3:9t,12t,15t	y = 2.29917x	0.99432	
C18:3:9c,12c,15c	y = 2.32490x	0.99405	64.9138 ± 1.5083
Total linolenic acid			66.4343

(A) C18:3-9t,12c,15c
(B) C18:3-9c,12t,15c
(C) C18:3-9c,12c,15t
(D) C18:3-9t,12c,15t
(E) C18:3-9t,12t,15c
(F) C18:3-9t,12t,15t
(G) C18:3-9c,12c,15c
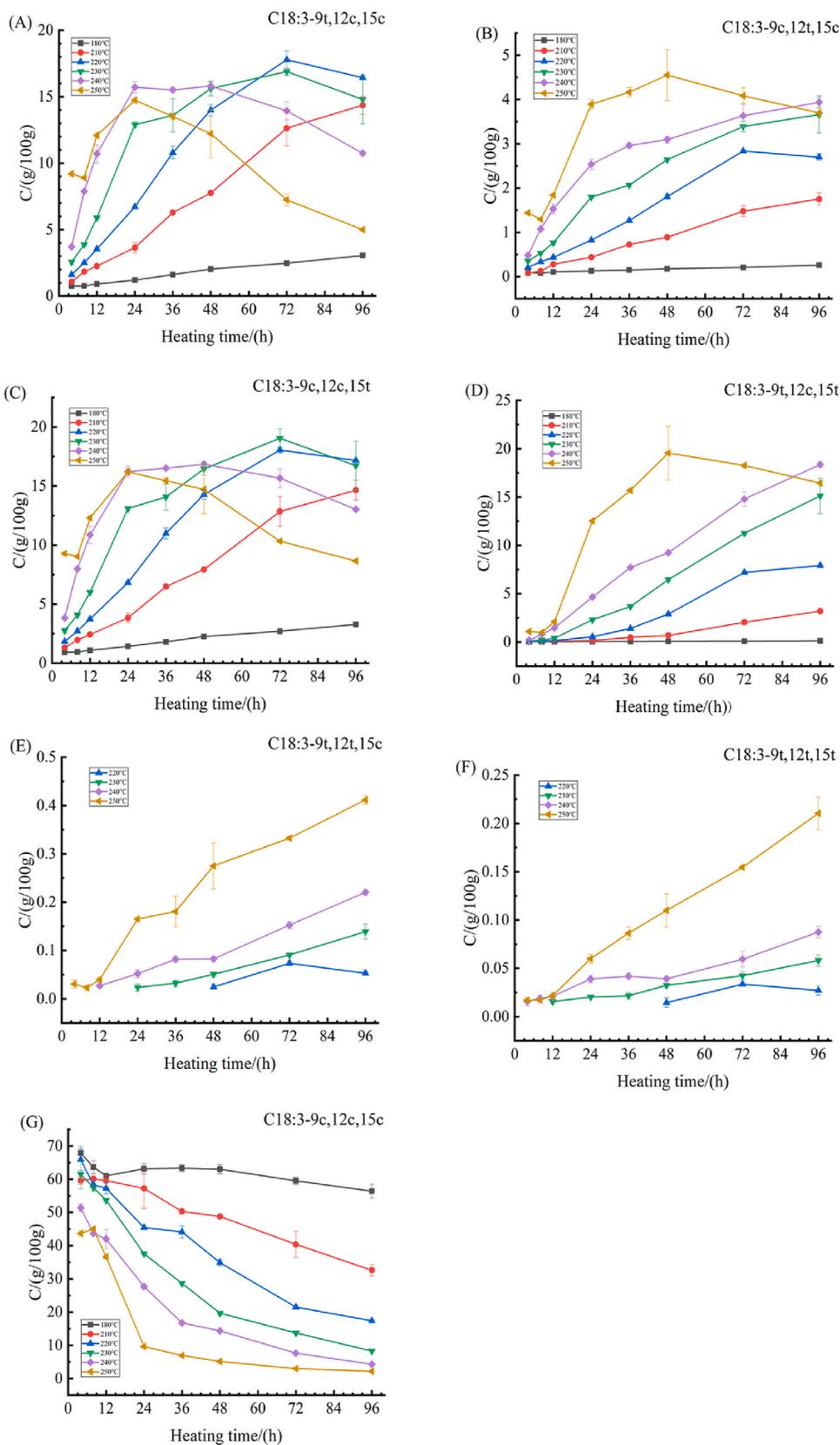
(caption on next page)

Fig. 3. Line plots of the contents of eight isomers of linolenic acid methyl ester in linseed oil heated at 180 °C,210 °C,220 °C,230 °C,240 °C,250 °C for 4h,8h,12h,24h,48h,72h,96h. Peaks: (A)C18:3-9trans,12trans,15trans; (B) C18:3-9trans,12trans,15cis; (C) C18:3-9trans,12cis,15trans; (D) C18:3–9cis,12cis,15trans; (E) C18:3–9 cis,12trans,15cis; (F) C18:3-9trans,12cis,15cis; (G) C18:3–9 cis,12 cis,15 cis.

respectively decreased to 16.4208 g/100g and 14.7940g/100g. At 240 °C and 250 °C, the highest levels (15.7081 and 14.7428 g/100g) were evident at 24 h, whereas by 96 h these levels had decreased to 10.7465 g/100g and 4.9947 g/100g, respectively (Fig. 3A). Upward trends in C18:3:9c,12t,15c levels were evident in the 180–240 °C range, with final levels of 0.2578, 1.7507, 2.6947, 3.6616, and 3.9354g/100g at the respective analyzed temperatures. In contrast, at 250 °C, the peak levels (4.5501 g/100g) were evident at 48 h before declining to a final concentration of 3.6966 g/100g (Fig. 3B).

Increasing trends in C18:3:9c,12c, 15t levels were evident with the prolongation of heating at 180 °C and 210 °C, with final levels of 3.2746 and 14.6510 g/100g, respectively. At 220 °C and 230 °C, these C18:3:9c,12c,15t peaked at 72 h (18.0435, 19.0339 g/100g) before ultimately declining to 17.1697 and 16.7164 g/100g, respectively. At 240 °C, peak levels of this isomer were evident after 48 h (16.8549 g/100g) before declining to a final concentration of 13.0225 g/100g. At 250 °C, peak levels were evident after 24 h (16.2027 g/100g) before declining to a final concentration of 8.6543 g/100g (Fig. 3C). Levels of C18:3:9t,12c,15t rose with the prolongation of heating at temperatures from 180 to 240 °C, with respective final concentrations of 0.1197, 3.1980, 7.9132, 15.1190 and 18.3555g/100g respectively. At 250 °C, the highest levels of this isomer were evident after 48h (19.5492g/100g) before declining to 16.4395g/100g at 96 h, potentially owing to high-temperature degradation (Fig. 3D).

C18:3:9t,12t,15c and C18:3:9t,12t,15t were not detectable at 180 °C or 210 °C (Fig. 3E-F), and they were only detectable after 48 h at 220 °C for 48h. At 230 °C, C18:3:9t,12t,15t were only detectable at 12 h, while C18:3:9t,12t,15c was detected at 24h. At 240 °C, C18:3:9t,12t,15t was detected at 4 h, whereas C18:3:9t,12t,15c was only evident at 12 h. At 250 °C, C18:3:9t,12t,15c and C18:3:9t,12t,15t were all detected at the start of heating, with C18:3:9t,12t,15c and C18:3:9t,12t,15t levels trending upward with the course of heating although their levels remained low even at the end of heating (Fig. 3E-F).

3.4. Kinetics for C18:3-9t,12c,15c, C18:3-9c,12t,15c, C18:3-9c,12c,15t isomers formation

Fig. 3A–C exhibits the plots showing the changes in concentration for C18:3-9t,12c,15c, C18:3-9c,12t,15c, and C18:3-9c,12c,15t during the heating of linseed oil. When the heating time was ≤24 h, a strong linear correlation was evident between the formation of C18:3-9t,12c,15c and C18:3-9c,12c,15t. Since there were no clear linear relationships observed between the formation of C18:3-9t,12c,15c, C18:3-9c,12t,15c, and C18:3-9c,12c,15t during the later stages of heating, kinetic studies were performed using the levels of these isomers that exhibited linear relationships.

Kinetic parameters for the formation of C18:3-9t,12c,15c, C18:3-9c,12t,15c, and C18:3-9c,12c,15t isomers are provided in Table 2. Fig. 4A–C displays Arrhenius plots, which graph the natural logarithm of the 'ln k' against the 1/T. These graphs demonstrate a notable and consistent linear relationship.

As shown in Table 2, the reaction rate k values for C18:3-9t,12c,15c at 180, 210, 220, 230, and 240 °C were 0.0502, 0.1520, 0.2797, 0.5371, and 0.6545 h^{-1}, respectively, while the k values for C18:3-9c,12t,15c at these same respective temperatures were 0.0037, 0.0185, 0.0376, 0.0550, 0.0644, and 0.0948 h^{-1}. In addition, the k values for C18:3-9c,12c,15t at 180, 210, 220, 230, and 240 °C were 0.0595, 0.1597, 0.2848, 0.5454, and 0.6756 h^{-1} (Table 2). These k values for C18:3-9c,12t,15c were significantly lower than those for C18:3-9t,12c,15c and C18:3-9c,12c,15t, indicating that levels of C18:3-9c,12t,15c were significantly lower than levels of C18:3-9t,12c,15c and C18:3-9c,12c,15t. The k values for all isomers exhibited a positive correlation with rising temperatures, suggesting that higher temperatures promoted the enhancement of these isomerization events.

The formation of C18:3-9t,12c,15c, C18:3-9c,12t,15c, and C18:3-9c,12c,15t exhibited zero-order kinetics when exposed to air at various temperatures. These findings align with the results reported by Li et al. in earlier investigations [36]. The reaction rate k values for C18:3-9t,12c,15c and C18:3-9c,12c,15t were similar within 24 h, as were their E_a values, suggesting that the formation of C18:3-9t, 12c,15c and C18:3-9c,12c,15t isomers in linseed oil samples was limited by a similar energy barrier during the heating process, further

Table 2
Kinetic parameters for the formation of α-linoleic acid isomers.

Reaction			T/(°C)					
			180	210	220	230	240	250
C18:3:9t,12c,15c	C18:3:9t,12c,15c	k/(g/100g)hrs	0.0502	0.1520	0.2797	0.5371	0.6545	–
		Ea (kJ/mol)				86.04		
C18:3:9c,12t,15c	C18:3:9c,12t,15c	k/(g/100g)hrs	0.0037	0.0185	0.0376	0.0550	0.0644	0.0948
		Ea (kJ/mol)				92.68		
C18:3:9c,12c,15t	C18:3:9c,12c,15t	k/(g/100g)hrs	0.0595	0.1597	0.2848	0.5454	0.6756	–
		Ea (kJ/mol)				81.04		
C18:3:9t,12c,15t	C18:3:9t,12c,15t	k/(g/100g)hrs	0.0013	0.0143	0.0603	0.1345	0.1924	0.4073
		Ea (kJ/mol)				164.36		
C18:3:9t,12t,15c	C18:3:9t,12t,15c	k/(g/100g)hrs	–	–	0.0005	0.0011	0.0017	0.0057
		Ea (kJ/mol)				165.38		
C18:3:9t,12t,15t	C18:3:9t,12t,15t	k/(g/100g)hrs	–	–	0.0003	0.0006	0.0009	0.0022
		Ea (kJ/mol)				140.30		
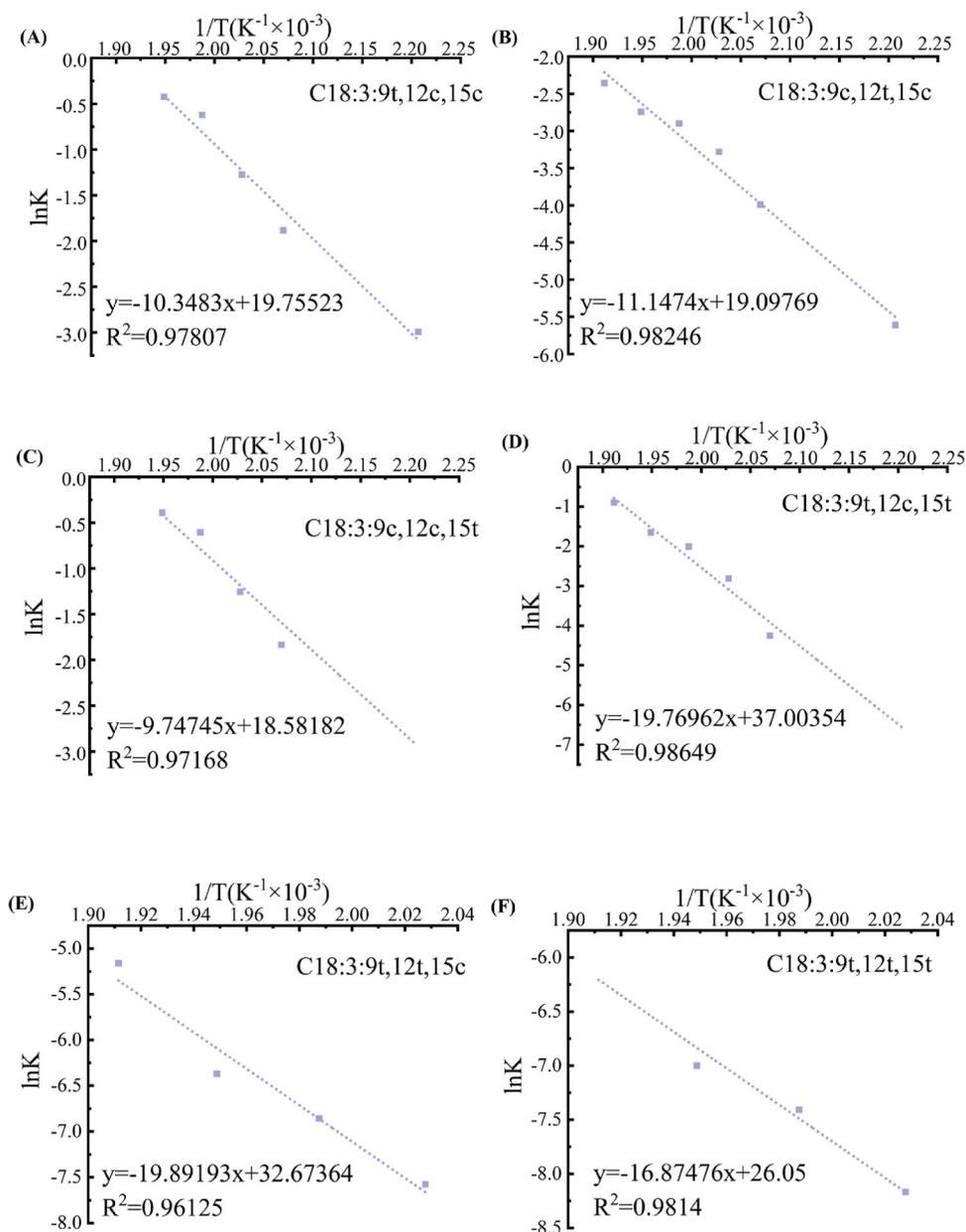
Fig. 4. The relationship between ln k and 1/T for the C18:3-9t,12c,15c(A), C18:3-9c,12t,15c(B), C18:3-9c,12c,15t(C), C18:3-9t,12c,15t(D), C18:3-9t,12t,15c(E) and C18:3-9t,12t,15t(F) isomers at different temperatures.

explaining why similar levels of C18:3-9t,12c,15c and C18:3-9c,12c,15t production were evident during the heating of linseed oil. Based on these results, it appears that the isomerization of the double bonds located at positions 9 and 15 could happen with a higher probability compared to the isomerization of the double bonds situated at position 12. This was due to the proximity of positions 12 and 15 to the structural chain's extremities [6,10].

3.5. The kinetics of C18:3-9t,12c,15t, C18:3-9t,12t,15c isomer formation

Additionally, the kinetics of double-TALA formation in linseed oil exposed to air during thermal treatment were investigated using GC. The concentrations of double-trans linolenic acid isomers, such as C18:3-9t,12c,15t and C18:3-9t,12t,15c, increased linearly as the heating time was extended at temperatures ≤240 °C. (Fig. 3D and E). At temperatures over 240 °C, the isomerization of C18:3-9t,12t, and 15c continued to occur linearly over time. However, this was not observed for C18:3-9t,12t, and 15t, possibly due to processes such as isomer polymerization, oxidation, hydrolysis, and decomposition that take place at extremely high temperatures. When

heating time remained $\leq$48 h, a clear linear correlation was observed between heating time and the formation of both C18:3-9t,12c,15t and C18:3-9t,12t,15c (Fig. 4D and E). C18:3-9t,12c,15t and C18:3-9t,12t,15c formation kinetics were thus analyzed in greater detail over a 48h heating period.

As shown in Table 2, the respective reaction rate *k* values for C18:3-9t,12c,15t at 180, 210, 220, 230, 240, and 250 °C were 0.0013, 0.0143, 0.0603, 0.1345, 0.1924, and 0.4073 h^{-1}, while the *k* values for C18:3-9t,12t,15c at 220, 230, 240, and 250 °C were 0.0013, 0.0143, 0.0603, 0.1345, 0.1924, and 0.4073 h^{-1}, respectively. The *k* values for C18:3-9t,12c,15t were lower than those for C18:3-9t,12t,15c, indicating that C18:3-9t,12c,15t content in these samples was lower than the C18:3-9t,12t,15c content. The E_a required for the formation of single-TALAs was generally lower compared to that for double-TALAs. This observation supports a model where thermal isomerization can more easily produce single-TALAs, while the formation of isomers with two *trans*-double bonds requires higher energy levels than those with only one *trans*-double bond.

3.6. Kinetics of C18:3-9t,12t,15t isomer formation

For tri-TALA isomers under sealed conditions, the relationship between ln *k* and heating temperature (1/T) was clearly visible, as shown in Fig. 4F. The corresponding reaction rate k values for C18:3-9t,12t, and 15t at 220, 230, 240, and 250 °C were 0.0003, 0.0006, 0.0009, and 0.0022 h^{-1}, as demonstrated in Table 2. A clear linear association following zero-order kinetics was thus evident with respect to the C18:3-9t,12t,15t thermal isomerization reaction. Furthermore, the energy required for the formation of single-TALAs tended to be lower than that of tri-TALAs, confirming the hypothesis that single-TALAs could be thermally isomerized more easily than isomers having three trans double bonds, which required higher energy. This was due to the fact that thermodynamics dominated the development of the tri-TALAs and that they were more stable than single-TALAs [36].

4. Conclusions

In conclusion, the current findings have verified that subjecting linseed oil to elevated temperatures leads to the formation of unbound reactive molecules and trans isomers. The study specifically examines the development of these reactive molecules and trans isomers at varying temperatures and over varied heating durations. These analyses demonstrated that peak free radical levels were evident in linseed oil samples heated at 90 °C, while free radical levels decreased within 90 min at 180 °C. Subsequent experimental results confirmed that free radical adducts of PBN were also readily degraded at 180 °C. In addition, the number of trans isomers in linseed oil increased as the temperature rose but started to degrade partially when the temperature was above 240 °C. The kinetic study suggests the values of k for all isomers increased as the temperatures rose, indicating that higher temperatures were favorable for promoting these isomerization events. The trans isomer content in these linseed oil samples and these *k* values for C18:3-9c,12t,15c were significantly lower than those for C18:3-9t,12c,15c and C18:3-9c,12c,15t, also further revealed that the double bonds at positions 9 and 15 were more susceptible to isomerization than the double bond at position 12. When heated above 240 °C, a linear relationship was not true for C18:3-9t,12c,15t, potentially owing to isomer polymerization, oxidation, hydrolysis, and decomposition at this extremely high temperature. Additionally, the E_a to form single-TALAs was generally lower than that of double- and tri-TALAs. This is consistent with a model in which single-TALAs can be produced more easily by thermal isomerization, whereas isomers containing two or three *trans*-double bonds require more energy to form than isomers containing a single *trans*-double bond. Furthermore, the precise mechanism of inhibition of free radicals and trans isomers, as well as the link between them, remain unresolved. Consequently, we shall continue our investigations to establish the foundation for the inhibition of heat-induced isomerization of oils and fats. These results indicated that controlling the formation of free radicals and single-TALAs isomers may be the key way to reduce the trans isomers of linolenic acid during cooking oil heating.

Data availability

Data will be made available on request.

CRediT authorship contribution statement

Lu Huimin: Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Li Yongfu:** Writing – review & editing, Funding acquisition. **Qiu Ju:** Data curation.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Li Yongfu reports financial support was provided by Guizhou Provincial Basic Research Program (Natural Science). Li Yongfu reports a relationship with Guizhou Provincial Basic Research Program (Natural Science) that includes: funding grants. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] H. Zhao, C. Lu, Study on enrichment and purification technology of α-linolenic acid from Hovenia dulcis Thunb seed oil, Modern food science and technology 27 (10) (2011) 1225–1229.
[2] F.D. Anthony, P.K. Alex, P.B. Richard, Is docosahexaenoic acid synthesis from α-linolenic acid sufficient to supply the adult brain? Prog. Lipid Res. (2015) 54–66.
[3] Y. Zhou, J. Huang, Q. Jin, X. Wang, Comparison of lipid composition of cold-pressed linseed oil from different producing areas, China Grease 43 (9) (2018) 125–128.
[4] Y. Siqi, Z. Guofang, Q. Xiaoxi, et al., Improved Protective and Controlled Releasing Effect of Flaxseed Oil Microcapsules with Glycosylated Casein as Wall Materials, LWT, 2024, p. 191 115687.
[5] A. Yakdhane, S. Labidi, D. Chaabane, A. Tolnay, A. Nath, A. Koris, et al., Microencapsulation of flaxseed oil-state of art, Processes 9 (2) (2021) 295.
[6] L. Shao, H. Song, X. Li, J. Huang, Q. Jin, X. Wang, Correlations between trans isomers of α-linolenic acid and polar components in linseed oil during heating, Int. J. Food Sci. Technol. 55 (10) (2020) 3297–3305. No.10.
[7] W.B. Zhang, Z.J. Chen, R.J. Yang, X. Hua, W. Zhao, S.Y. Guan, Application of caseinate modified with Maillard reaction for improving physicochemical properties of high load flaxseed oil microcapsules, Article, Eur. J. Lipid Sci. Technol. 123 (5) (2021) 2000172.
[8] L. Manzhu, L. Tian, Q. Yang, et al., Mitigation mechanism of resveratrol on thermally induced trans-α-linolenic acid of trilinolenin, J].LWT (2023) 189.
[9] L. Changmo, Z. Yubin, L. Shuai, et al., Mechanism of formation of trans fatty acids under heating conditions in triolein, J. Agric. Food Chem. 61 (43) (2013) 10392–10397.
[10] F. Jiang, Q. Guo, Q. Li, et al., The kinetics of thermally induced isomerisation of trilinolenin, an improved gas chromatography study, Int. J. Food Sci. Technol. 52 (7) (2017) 1543–1551.
[11] R. Liu, X. Zheng, Z. Yin, Research progress on the application of natural antioxidants in vegetable oils and fats, Journal of Chongqing technology and business university (Natural Science Edition) 32 (10) (2015) 43–47.
[12] M.L. Andersen, L.H. Skibsted, Esr Spectroscopy for the Study of Oxidative Processes in Food and Beverages, Springer International Publishing. Modern Magnetic Resonance, 2018, pp. 1781–1794.
[13] H. Chen, P. Cao, B. Li, D. Sun, J. Li, Y. Liu, High sensitive and efficient detection of edible oils adulterated with used frying oil by electron spin resonance, Food Control 73 (2016) 540–545.
[14] J. Velasco, M.L. Andersen, L.H. Skibsted, Electron spin resonance spin trapping for analysis of lipid oxidation in oils: inhibiting effect of the spin trap alpha-phenyl-N-tert-butylnitrone on lipid oxidation, J. Agric. Food Chem. (5) (2005) 1328–1336.
[15] X. Feng, Y. Hua, C. Zhang, X. Kong, X. Li, Y. Chen, Effect of soaking conditions on the formation of lipid-derived free radicals in soymilk, Food Chem. 315 (8) (2020) 126237.
[16] B. Nikola, P. Simon, V. Hervé, EPR Spin-Trapping Study of Free Radical Intermediates in Polyalphaolefin Base Oil Autoxidation. Polymer Degradation and Stability, 2021.
[17] Y. Xie, S. Jiang, M. Li, Y. Guo, Y. Cheng, H. Qian, et al., Evaluation of the formation of lipid free radicals in the oxidation process of peanut oil, Lebensm. Wiss. Technol. 104 (2019) 24–29.
[18] H. Chen, P. Cao, B. Li, D. Sun, Y. Wang, J. Li, et al., Effect of water content on thermal oxidation of oleic acid investigated by a combination of EPR spectroscopy and SPME-GC-MS/MS, Food Chem. (2017) 1434–1441.
[19] H. Chen, Y. Wang, P. Cao, Y. Liu, Effect of temperature on thermal oxidation of palmitic acid studied by a combination of EPR spin trapping technique and SPME-GC-MS/MS, Food Chem. 234 (2017) 439–444.
[20] J. Su, J. Du, Electron Paramagnetic Resonance Spectroscopy-Principles and Applications, 2022.
[21] H. Chen, Y. Wang, P. Cao, Y. Liu, Thermal oxidation rate of oleic acid increased dramatically at 140 °C studied using electron spin resonance and GC-MS/MS, No.8, J. Am. Oil Chem. Soc. 96 (8) (2019) 937–944.
[22] M.K. Thomsen, D. Kristensen, L.H. Skibsted, Electron spin resonance spectroscopy for determination of the oxidative stability of food lipids, J. Am. Oil Chem. Soc. 77 (NO.7) (2000) 725–730.
[23] W. Tsuzuki, R. Nagata, R. Yunoki, T.N. Mitsutoshi Nakajima, Cis/trans-isomerization of triolein, trilinolein and trilinolenin induced by heat treatment, Food Chem. 108 (1) (2008) 75–80.
[24] A.A. Christy, Z. Xu, P.D.B. Harrington, Thermal degradation and isomerization kinetics of triolein studied by infrared spectrometry and gc-ms combined with chemometrics, Chem. Phys. Lipids 158 (1) (2009) 22–31.
[25] A.A. Christy, Thermally-induced isomerization of trilinolein and trilinoelaidin at 250°C: analysis of products by gas chromatography and infrared spectroscopy, Lipids 44 (12) (2009) 1105–1112.
[26] A.A. Christy, S.L. Arachchi, Thermally-induced cis-trans isomerization in trilinolein studied by infrared spectroscopy and gas chromatography, Advances in Natural and Applied Sciences 10 (2016) 168–176.
[27] Q. Guo, F. He, Q. Li, Z. Deng, J. Jin, Y. Ha, Reaction pathway mechanism of thermally induced isomerization of 9,12-linoleic acid triacylglycerol, J. Sci. Food Agric. 67 (2016) 255–264.
[28] S. Liu, Y. Zhu, N. Liu, D. Fan, M. Wang, Y. Zhao, Antioxidative properties and chemical changes of quercetin in fish oil: quercetin reacts with free fatty acids to form its ester derivatives, 3, J. Agric. Food Chem. 69 (3) (2021) 1057–1067.
[29] A. Li, Y. Ha, F. Wang, Q. Li, Determination of thermally induced trans-fatty acids in soybean oil by attenuated total reflectance Fourier transform infrared spectroscopy and gas chromatography analysis, 60(42), J. Agric. Food Chem. (42) (2012) 10709–10713.
[30] P.G. Mekarbane, B.J. Tabner, Esr spin-trap study of radicals present during the thermolysis of some di-tert-alkyl peroxides, Magn. Reson. Chem. 36 (11) (2015) 826–832.
[31] J. Yang, L. Qin, Y. Zhu, C. He, The regularity of heat-induced free radicals generation and transition of camellia oil, Food Res. Int. (2022) 111295.
[32] S. Jiang, Y. Xie, M. Li, Y. Guo, Y. Cheng, H. Qian, et al., Evaluation of the oxidative stability of edible oil by electron spin resonance spectroscopy, Food Chem. 309 (2020). Article 125714.
[33] J. Velasco, M.L. Andersen, L.H. Skibsted, ESR spin trapping for in situ detection of radicals involved in the early stages of lipid oxidation of dried microencapsulated oils, Food Chem. (Mar.30 Pt.2) (2020) 341.
[34] A. Silvagni, L. Franco, A. Bagno, F. Rastrelli, Thermoinduced lipid oxidation of a culinary oil: a kinetic study of the oxidation products by magnetic resonance spectroscopies, J. Phys. Chem. 114 (37) (2010) 10059–10065.
[35] I. Subotin, R. Druta, V. Popovici, E. Covaci, R. Sturza, Kinetic of forced oxidation of grape seeds, walnuts and corn germs oils in the presence of antioxidants, Food Nutr. Sci. (12) (2021) 1326–1339.
[36] Y. Li, Y. Yu, Q. Luo, Y. He, Z. Tian, Y. Zhao, Thermally-induced isomerization of linoleic acid and α-linolenic acid in rosa roxburghii tratt seed oil, Food Sci. Nutr. 9 (No.6) (2021) 2843–2852.