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Oxidation kinetic studies of oils derived from unmodified and genetically modified vegetables using pressurized differential scanning calorimetry and nuclear magnetic resonance spectroscopy

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Abstract

Evaluation of oxidative stability of a series of vegetable oils and oils derived from genetically modified vegetables were carried out using pressure differential scanning calorimetry (PDSC). The purpose of including the genetically modified oils along with other oils were to illustrate the effect of high oleic and linoleic content on the thermal and oxidative behavior of these oils. Kinetic and thermodynamic parameters were computed and variation of results explained in terms of structural data derived from quantitative ^{1}H and ^{13}C NMR spectroscopy. For a variety of vegetable oil samples used in the study, log b (program rate of heating; i.e. 1, 5, 10, 15 and 20°C/min) was linearly related ($R^2 = 0.99$) to the reciprocal of absolute temperature corresponding to maximum oxidation rate (peak height temperature). From the resulting slope it was possible to compute activation energy (E_a) for oxidation reaction and various other kinetic parameters, e.g. rate constant (k), Arrhenius frequency factor (Z) and half-life period ($t_{1/2}$). The presence of C–C unsaturation in the fatty acid (FA) chain, their nature and relative abundance, affect thermal and oxidative stability of the oil and subsequently their kinetic and thermodynamic parameters. Quantitative analysis of the NMR spectra yielded various other structural parameters that were correlated with start (T_S) and onset (T_O) temperature of vegetable oil oxidation, and certain important kinetic parameters (E_a and k). This is a novel approach, where statistical models were developed as a predictive tool for quick assessment of oxidative and thermodynamic data. The correlations developed have an adjusted R^2 of 0.922 and higher using 3 or 4 NMR derived predictor variables. These correlations revealed that in addition to nature and abundance of C=C, relative abundance of other structural parameters (e.g. bis-allylic methylene group, allylic-CH_2, α-CH_2 to C=O, etc.) influence oxidation and kinetic data. Published by Elsevier Science B.V.

Keywords: PDSC; NMR; Vegetable oil; Oxidation; Kinetics; Statistical correlation

1. Introduction

It is a well-known fact that when vegetable oils are exposed to oxidizing environment they undergo oxidative degradation. Oxidation is the single most important reaction of oils resulting in increased acid-

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ity, corrosion, viscosity and volatility when used as lubricant base oils. The triacylglycerol structure form the backbone of mostly available vegetable oils and these are associated with different fatty acid (FA) chains. It is therefore a complex association of different FA molecules attached to a single triglycerol structure that constitute a vegetable oil matrix. The presence of unsaturation in the triacylglycerol molecule due to C=C from oleic, linoleic and linolenic acid moieties, functions as the active sites for various oxidation reactions. Saturated FAs have relatively high oxidation stability [1], which decreases with increasing unsaturation in the molecule. Oxidation usually takes place through a radical initiated chain mechanism [2] involving: initiation ($RH \rightarrow R^{\bullet}$, $R^{\bullet} + O_2 \rightarrow RO_2^{\bullet}$); propagation ($RO_2^{\bullet} + RH \rightarrow RO_2H + R^{\bullet}$, $R^{\bullet} + O_2 \rightarrow RO_2^{\bullet}$); branching ($RO_2H \rightarrow RO^{\bullet} + {}^{\bullet}OH$, $RO^{\bullet} + RH + O_2 \rightarrow ROH + RO_2^{\bullet}$, ${}^{\bullet}OH + RH + O_2 \rightarrow H_2O + RO_2^{\bullet}$); chain stopping inhibition ($InH + RO_2^{\bullet} \rightarrow In^{\bullet} + RO_2H$); peroxide decomposition ($RO_2H \rightarrow$ inert products). The free radicals generated during the initiation stage react with O_2 to form peroxy free radicals and hydroperoxides [3]. During this period, O_2 is consumed in a zero-order process [4], apparently leading to intermediates that are not too well characterized, prior to the formation of peroxides [3]. The latter undergoes further reaction to form alcohols, ketones, aldehydes, carboxylic acids [5], leading to rancidity and toxicity [6], thereby accelerating the oil degradation process [7,8]. These compounds have molecular weights that are similar to vegetable oils and therefore remain in solution. As the oxidation proceeds, the oxygenated compounds polymerize to form viscous material that, at a particular point, becomes oil insoluble leading to oil thickening and deposits.

The extent of oxidation and formation of oxidation products are further complicated by the amount of unsaturation, structural differences in the various triacylglycerol molecules and presence of antioxidants. All these factors, together or individually can change the specific compounds formed and the rates of their formation [9]. In addition to unsaturation in the molecule, oxidative degradation and kinetics of oxidation is also influenced by methylene chain length, bis-allylic methylene groups, etc. The cumulative effect of various structural parameters in the triacylglycerol molecule makes oxidation a highly complex process and no simple kinetic model alone would hold good for such systems.

Several bench top oxidation tests are available primarily as screening tool for oxidative stability of vegetable oils. Evaluation of oxidation is complex and a fully acceptable protocol is yet to emerge. Estimation of peroxide content (peroxide value, PV) can be used as an index of oxidation if the peroxides formed are stable and do not decompose after formation, which in most cases are not true. The activation energy for the formation of peroxide is 146–272 kJ/mol [10] and that of decomposition of lipid peroxide is 84–184.5 kJ/mol, suggesting peroxides are less stable than lipids [11]. Two other methods for measuring the oxidative stability are the active oxygen method (AOM) [12] and the Rancimat method [13,14]. In the former method, test oil is heated to 100°C and the oxidation is followed by measuring the PV of heated sample at regular time interval until PV = 100 meq/kg is reached, which gives the AOM endpoint. A large amount of sample, numerous analysis and critical control of airflow is required. Samples that form unstable peroxides, PV = 100 meq/kg may never be reached and such measurements have no meaning. In the AOM method consumption of O_2 may also be a measure for induction period. The Rancimat method is based on the fact that the volatile acids formed during oxidation [15,16] can be used as automated endpoint detection. Gordon and Murshi [17] have shown good correlation of Rancimat method at 100°C with oil stability as measured by peroxide development during storage at 20°C. In another study, Jebe et al. [18] have pointed out the advantages of Rancimat method at higher temperature. In the Sylvester test [19], the sample is heated to 100°C in a closed vessel and the pressure decreases due to O_2 consumption is monitored. Oxidograph [19] is an automated version of this method and induction period is determined from the sudden decrease in the O_2 pressure.

Oxidative status of oil can also be obtained by integrating the light curve during the chemiluminescence reaction [20]. The method is highly sensitive for the measurement of lipid oxidation. Matthäus et al. [21] have described a linear correlation ($R^2 = 0.99$) between iodimetric peroxide determination [22] and chemiluminescence method. Another official method to measure induction period is oil stability index (OSI)

[23]. The OSI values generally correspond well with AOM values if PV is 100 meq/kg or greater [24]. The method is automated and much easier as compared to AOM. However lengthy experimental time, large errors associated with small changes in O_2/air flow rate [25] and inability to differentiate between small changes in vegetable oil matrix are its main disadvantages.

In this paper a combination of PDSC with quantitative NMR spectroscopy has been used to study the thermal and oxidative stability of oils derived from unmodified and genetically modified vegetables. Thermal analysis such as PDSC methods are popular for the determination of oxidative stabilities of vegetable oils [26–28]. The PDSC analysis was carried out using a programmable heating rate mode with a constant flow of dry O_2 inside the pressure chamber. The inverse of maximum heat flow temperature (K) of all the oils exhibits Arrhenius behavior [29–31] [log of heating rate (b) varies linearly with reciprocal of temperature (K)]. The resulting slope was used to calculate the activation energy (E_a) and other kinetic parameters associated with the samples. The differences observed in the various kinetic and thermodynamic data for the various oils were explained in terms of the structural parameters derived from quantitative ^1H and ^{13}C NMR spectroscopy. Multi-component correlation models were developed using NMR derived predictor variables for thermal and kinetic parameters of vegetable oil oxidation. The results show the effect of differences in the structural parameters of the oil's FA chain in determining the relative oxidation behavior.

2. Experimental

2.1. Materials

The vegetable oils used were cottonseed, corn, canola (local grocery store), sunflower (Liberty Vegetable Oil), safflower (Liberty Vegetable Oil), soybean oil (Pioneer High Bred International) and the genetically modified vegetable oils were high oleic sunflower (International Flora Tech, Gilbert, AZ), high oleic safflower and high linoleic safflower oil (Oil Seeds International). The oils were used as received without any further purification. Table 1 presents the fatty acid composition of the oils by gas chromatographic method (AACC method 58-18, 1993) while Table 2 presents their iodine value (AOCS method, Cd 1-25, 1993) acidic value (AOCS method, Ca 5a-40, 1993) and peroxide values (AOCS method, Cd 8-53, 1993).

2.2. PDSC procedures

All the experiments were done using TA Instruments DSC 2910 thermal analyzer from TA Instruments (New Castle, USA) attached to a computer for data recording and further processing. This instrument has a maximum sensitivity of 5 mV/cm and temperature sensitivity of 0.2 mV/cm, which makes it fairly accurate in terms of data reproducibility. Nominally 1.5 mg of sample was placed in a hermetically sealed aluminum pan with a pinhole lid for interaction of the sample with the reactant gas (oxygen). The sample amount has significant impact on the shape and repro-

Table 1
The fatty acid composition of the vegetable oils by GC analysis

Vegetable oil	Palmitic	Stearic	Oleic	Linoleic	Linolenic
Cotton seed oil	22	–	25	50	3
Corn oil	7	–	47	42	4
Safflower oil	6	4	20	70	–
High oleic safflower oil	5	3	80	12	–
High linoleic safflower oil	3	2	10	85	–
High oleic sunflower oil	5	–	90	5	–
Soybean oil	16	–	23	53	8
Sunflower oil	6	–	35	55	4

Table 2
Typical chemical properties of the vegetable oils

Vegetable oil	Iodine value	Free fatty acid	Peroxide value
Cotton seed oil	109.14	0.33	34.74
Corn oil	119.85	0.20	26.60
Canola	99.05	0.22	22.35
Safflower oil	135.21	0.21	32.02
High oleic safflower oil	83.61	0.31	27.82
High linoleic safflower oil	121.17	0.00	32.23
High oleic sunflower oil	80.80	0.10	23.88
Soybean oil	116.98	0.22	27.35
Sunflower oil	124.77	0.30	39.11

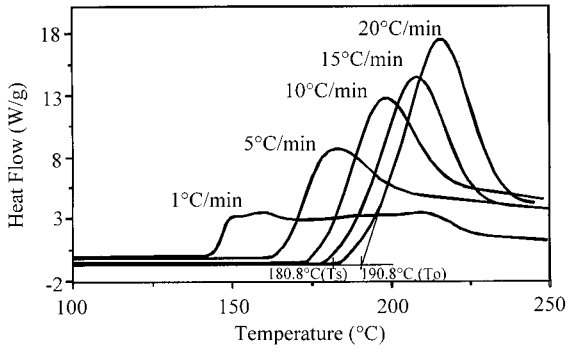

Fig. 1. PDSC exothermic plots for high oleic sunflower oil at different heating rates.

ducibility of DSC exotherm. An optimum weight in the range 1.0–1.5 mg was found to yield consistent results. A film thickness of less than 1 mm was required to ensure proper oil–O_2 interaction and eliminate any discrepancy in the result due to oxygen diffusion limitations [32,33]. The module was first temperature calibrated using the melting point of indium metal (156.6°C) at 10°C/min heating rate and later at 1, 5, 15 and 20°C/min to be used in the study. For kinetic studies, the system was equilibrated at 35°C and heated using above heating rates. Oxygen gas (dry, 99% pure, obtained commercially) was pressurized in the module at a constant pressure of 3450 kPa and maintained throughout the length of the experiment. These conditions maintain maximum contact with the sample and eliminate any limitation due to oxygen diffusion in the oil medium. However, extreme care should be taken to maintain the pressure of the reactant gas (O_2) constant. The inverse of peak height temperature corresponding to maximum oxidation from the exotherm was plotted against log of heating rate (b). Using linear regression method and subsequent data computation, various kinetic parameters were obtained. Fig. 1 presents the calorimetric curves (in scanning mode) of a typical vegetable oil at different heating rates. The figure shows the start temperature (T_S) at 180.84°C and onset temperature (T_O) representing temperature of rapid oxidation at 190.8°C (only at 20°C/min) for high oleic sunflower oil. T_O be defined as the temperature when rapid increase in the rate of oxidation is observed in the system. This temperature is obtained from extrapolating the tangent drawn on the steepest slope of reaction exotherm. A high T_O would suggest a high oxidative stability of the vegetable oil matrix. The oxidation start temperature (T_S) is the temperature during which primary oxidation products begin to form in the vegetable oil matrix [32]. It is also when loss of small molecular fragments due to evaporation are observed. Lower T_S and T_O indicates thermally unstable matrix.

2.3. NMR experiments

Quantitative NMR spectroscopy has proved to be a potential technique for the structural characterization of mineral [33–35] and vegetable base oils and their various genetically modified versions [36,37]. All the spectra were recorded in Fourier Transform mode on an AMX 400 MHz BRUKER machine. The proton spectra were obtained at an observing frequency of 400 MHz using a 5 mm dual probe. Sample solution (15%, w/v) was prepared in deuterated chloroform (99.8% $CDCl_3$) with tetramethyl silane (TMS) as an internal standard. A pulse delay (D1) of 1 s, acquisition time (AQ) 6.8 s and pulse width (P1) 8.5 μs was used for 16 repetitive scans (NS). For quantitative ^{13}C NMR measurements, sample solution (30%, w/v) was prepared in $CDCl_3$ (containing 1% tetramethylsilane) without any spin-lattice relaxation agent. All the experiments were performed in inverse gated decoupling mode at an observing frequency of 100 MHz and D1 = 40 s, AQ = 1.24 s, P1 = 7 μs was used. Optimally 2 K repetitive scans were taken for good signal-to-noise ratio (Fig. 2). Selective ^{13}C NMR spectra were obtained in the olefin and carbonyl region of

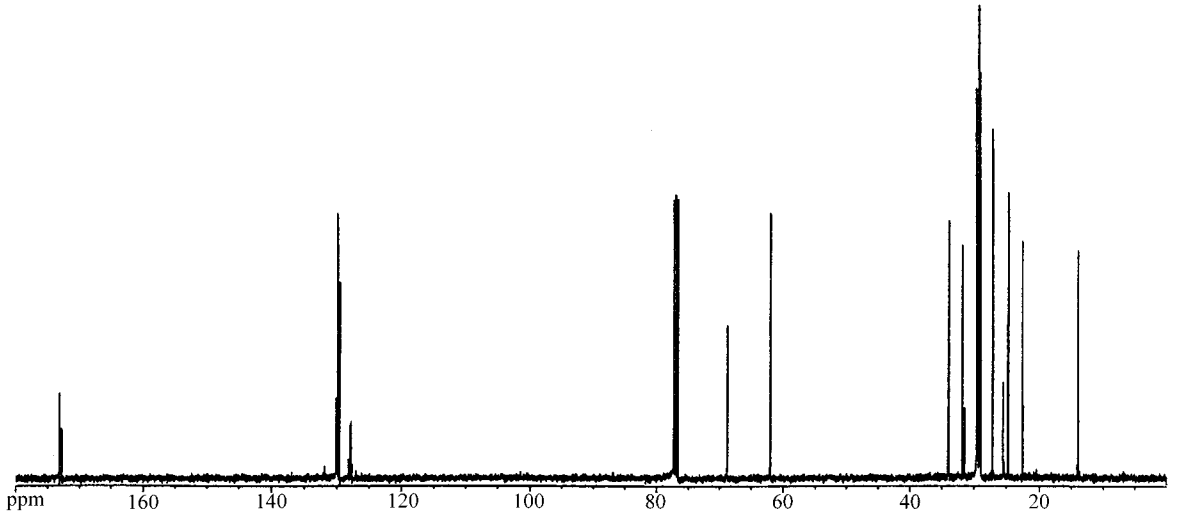

Fig. 2. Quantitative ^{13}C NMR spectra of high oleic sunflower oil showing the distribution of all the carbon types.

the vegetable oils using D1 = 20 s, P1 = 7 μs, AQ = 21.496 s and NS = 256 in inverse gated decoupling mode. This resulted in better resolution of the complex olefin carbons and helps identifying the *cis*- and *trans*-protons through correlation spectroscopy (HMQC and COSY-45). Using accurate 90° proton decoupler pulse calibration with 10% ethyl benzene in $CDCl_3$, DEPT-135 experiments [38] were carried out to identify various CH_n $(n = 0-3)$ environments in the triacylglycerol molecule. A combination of these above procedures helped in recognizing and computing the various structural parameters of vegetable oils.

2.4. Statistical method

A professional version of Minitab® 12 (MINITAB, State College, PA) was used for the current study. The program uses a best subsets regression method for the various predictor variables derived from quantitative NMR measurement. The components of the predictor variables were selected on the basis of their independent and collective influence on the various kinetic and thermal properties (i.e. T_S, T_O, E_a, etc.) of vegetable oil oxidation. The p values were calculated and t-tests were done to identify the variables that imparted maximum influence on these properties with probability factor exceeding 95%.

3. Results and discussion

3.1. Analysis of kinetic data

All the PDSC experiments were carried out using a hermetically sealed pinhole aluminum pans. It has also been observed that under a steady O_2 pressure of 3450 KPa, the size of the pinhole does not make a significant difference on the position of peak maximum. However, when using an open pan (SFI type) at high pressure, there is a slight variation in the position of the corresponding peak maximum when compared to pinhole pans. The high O_2 pressure allows rapid equilibration of the reactant gas inside the pan and thus any inconsistency due to access rate of oxygen and egress rate of volatile degradation product is effectively eliminated.

As the sample was heated (Fig. 1) under constant O_2 pressure, the peak maximum shifts to a higher temperature. The maximum heat flow temperature observed at each b is converted to absolute value (K = 0°C + 273.2°C) and its inverse was plotted against log b. As the experiments are performed in large excess of O_2, the consumption of O_2 during the oxidation process can be neglected. This would also mean that under this condition the reaction rate is independent of the oxygen concentration and the reaction can be assumed to be first order as long as

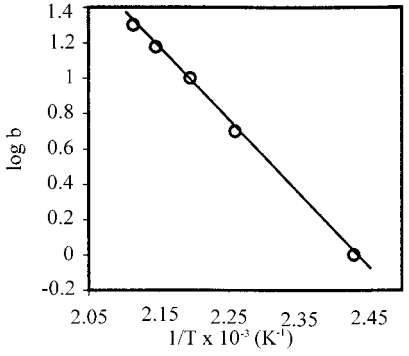

Fig. 3. Plot of log b versus inverse of peak height temperature for high oleic sunflower oil.

the oxidation initiation rate is constant (initiation; $RH \rightarrow R^{\bullet}$, $R^{\bullet} + O_2 \rightarrow RO_2^{\bullet}$). Under this condition the concentration of $[R^{\bullet}] \ll [RO_2^{\bullet}]$. In a separate study, comparison of the peak areas or enthalpies for aged (ΔH_a) and fresh (ΔH_f) peanut oil confirms a first-order reaction for oxidation process [31]. The slope of the line $[\delta \log b/\delta(1/T)]$ was generated from the log b versus inverse temperature plot (Fig. 3) by linear regression technique using a Minitab® statistical software. A high coefficient of correlation ($R^2 = 0.98$) was obtained for each of the vegetable oils used in the study. The data points used in the correlation are average of triplicate experiments under identical condition.

Assuming a first-order reaction for the system during oxidation, the kinetic parameters can be calculated based on maximum heat flow temperature and program rate relationships [29–31,33]. Using the slope of the line thus generated for each oil, individual activation energy (E_a) was computed using Ozawa–Flynn–Wall equation:

$$E_a = -2.19R\frac{\delta \log b}{\delta(1/T)}$$

where R is the gas constant (8.314 J/(K mol)) and b the heating rate (°C/min) used. Since the reaction followed first-order kinetics, other kinetic parameters could be obtained from E_a for each vegetable oil. Arrhenius pre-exponential factor (Z) was calculated from E_a for individual oils at different b using:

$$Z = \frac{bE_a\, e^{E/RT}}{RT^2}$$

Similarly, the temperature dependence of specific rate constant (k) can be described by Arrhenius equation as $k = Z\, e^{-E_a/RT}$ and half-life period as $t_{1/2} = 0.693\, k^{-1}$. Table 3 presents the calculated kinetic data of the vegetable oils obtained at $b = 10°C/min$. It was observed that E_a for most of the oils studied varied in the range 63–89 kJ/mol, resulting in subsequent variation in the Z, k and $t_{1/2}$ values.

The activation energy is influenced by the degree of poly-unsaturation present in the vegetable oils. It is generally observed that a high poly-unsaturation (linoleic and linolenic acid content) would lower and high oleic content in the FA chain would increase the E_a for oxidation. Similarly percent increase in the saturated-CH_2 carbons in the FA chain would improve the resistance to initial thermal breakdown. The activation energy requirement for such system is considerably high. This would result in delaying the onset of initial oxidation process (T_S) where bond scission takes place to form primary oxidation products. The percentage of

Table 3
Calculated values of kinetic parameters of the vegetable oils; $b = 10°C/min$

Vegetable oil	E_a (kJ/mol)	T (K)	E/RT	k (min^{-1})	ln Z	$t_{1/2}$ (min)	T_S (°C)	T_O (°C)
Cotton seed oil	63.30	477.4	17.02	0.37	16.04	1.87	139.5	149.92
Corn oil	77.78	464.3	20.15	0.43	19.31	1.61	162.6	176.95
Canola	88.45	454.0	23.43	0.51	22.76	1.36	119.8	143.04
Safflower oil	75.27	455.6	19.87	0.44	19.04	1.57	147.4	166.35
High oleic safflower oil	88.70	471.8	22.62	0.48	21.88	1.44	172.0	177.76
High linoleic safflower oil	73.51	456.9	19.35	0.42	18.48	1.65	152.4	166.29
High oleic sunflower oil	86.57	471.6	22.08	0.47	21.32	1.47	169.6	177.04
Soybean oil	79.66	464.5	20.63	0.44	19.81	1.57	161.3	178.22
Sunflower oil	63.81	446.2	17.21	0.38	16.25	1.82	129.8	145.42

Table 4
Quantitative ^{13}C NMR derived structural parameters of vegetable base oils[a]

Vegetable oil	Percentage of olefin carbons	Percentage of saturated-CH_2 carbons	Percentage of bis-allylic-CH_2 carbons	Percentage of ω-2 carbons of saturated, mono and *n*-6 polyunsaturated acids	Percentage of 3-terminal methyl carbons
Cotton seed oil	8.05	38.31	7.99	5.04	5.41
Corn oil	9.63	36.34	7.76	4.89	6.16
Safflower oil	9.14	32.38	9.44	5.05	5.36
High oleic safflower oil	9.66	43.05	5.63	4.26	5.50
High linoleic safflower oil	9.48	32.41	10.17	5.34	4.58
High oleic sunflower oil	9.86	41.90	6.05	4.61	6.15
Soybean oil	9.12	36.55	10.84	5.06	5.66
Sunflower oil	8.95	34.94	9.65	6.02	5.62

[a] NMR data reported is the average value of three independent experiments. The repeatability of the data is ±0.2.

oleic content (Table 1) and percentage of saturated-CH_2 carbon (Table 4) of the different vegetable oils explains the observed trends in various kinetic parameters (Table 3) to a certain extent. However, the presence and abundance of poly-unsaturation and saturated methylene chain length of the FAs do not conclusively explain the relative variation of E_a and other kinetic data among the vegetable oils. It was observed that several other structural parameters of the FA chain influenced susceptibility to thermal and oxidative degradation in varying extent. These parameters can be obtained through extensive analysis of quantitative NMR spectral data.

Calculated values of E_a and k (Table 3) do not follow the theoretical inverse relation (activation energy is directly proportional to inverse of rate constant). The possible reason for this deviation is the large difference in the entropy of complex molecular structures that participates in oxidation reaction. Oxidation is a very complex process leading to numerous oxidation products involving various intermediates [39]. These intermediate compounds have their own rate constant. The overall E_a is the cumulative effect of all the activation energies available in the system during the period of oxidation. These intermediate oxy-compounds undergo further reaction in presence of oxygen to form high molecular weight polymeric oil insoluble deposits. Similar observations have also been noticed during the oxidative behavior of mineral base oils [35].

Fig. 4 presents the variation of T_S and T_O as a function of different heating rate for a typical vegetable oil. For all the oils studied, the overall shape of the graphs remained the same. Both T_S and T_O showed rapid increase till 10°C/min heating rate with no significant change after 15°C/min heating rate. The plausible reason for this trend could be, during slow heating, primary oxidation products formed do not get a chance to escape the oil medium through evaporation. Instead they react with excess of O_2 and oil molecules to kick off rapid oxidation process. This process will trigger T_O close to T_S only in the neighborhood of 1°C/min. However, at higher heating rate, the primary oxidation products formed are rapidly lost to the surrounding gaseous phase before undergoing further reaction in the oil medium. T_O in this case was generally delayed and started at a much higher temperature than T_S. Similar observations have been noticed for all the oils.

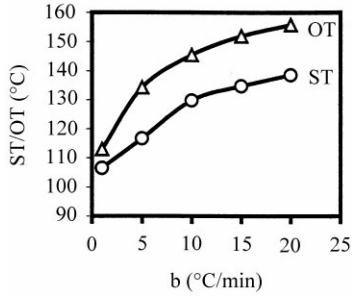

Fig. 4. Variation of start (○) and onset (△) temperature of oxidation with heating rate for sunflower oil.

3.2. Analysis of NMR data

High resolution NMR, especially ^{1}H and ^{13}C NMR, is being used increasingly as a potential tool to provide insight into the chemical structure of triacylglycerol molecules and the nature of unsaturation in the FA chain [40–42]. However, very few works report the use of NMR derived structural parameters to explain thermal, oxidative and kinetic behavior of vegetable oils. The quantitative nature of the NMR derived parameters is capable of differentiating vegetable oils in terms of structural differences in the hydrocarbon chain(s) of acyl moiety [43]. A typical ^{13}C NMR spectrum (Fig. 2) of a genetically modified high oleic sunflower oil shows the complexity of the saturated carbons in the spectral range 10–35 ppm. A combination of Distortionless Enhancement by Polarization Transfer (DEPT-135), Correlation Spectroscopy (COSY-45), Heteronuclear Multiple Quantum Coherence (HMQC) and inverse gated ^{13}C NMR experiments were required for correct assignment of the carbon signals.

In a triacylglycerol molecule, the carbonyl carbons are observed in 173.5–172.0 ppm and olefin carbons in 130.3–127.5 ppm range. These olefin carbons are from mono- and poly-unsaturation in the FA chain, mostly in the form of oleic and linoleic acids. The $-CH_2$ group α to >C=O is observed at 34.3–33.6 ppm; ω-3 carbons of saturated and unsaturated (mono and poly) FAs at 32.0–31.2 ppm; saturated-CH_2 groups in the FA chain at 30.0–28.5 ppm; *cis*-allylic carbons at 27.5–26.5 ppm; bis-allylic-CH_2 carbons at 26.0–25.0 ppm; three β-carbonyl-CH_2 carbons at 25.0–24.0 ppm; ω-2 carbons of saturated, mono and *n*-6 poly-unsaturated acids at 23.0–22.0 ppm and three terminal methyl carbons at 14.5–13.5 ppm range. Similarly, the major ^{1}H NMR signals were observed for olefin protons at 5.5–5.0 ppm; bis-allylic-C**H**$_2$ protons at 2.9–2.7 ppm; α-C**H**$_2$ proton to C=O at 2.35–2.25 ppm; allyl-C**H**$_2$ proton at 2.1–1.9 ppm and –C**H**$_2$ protons on saturated carbons at 1.40–1.15 ppm range. There are reports in the literature for the calculation of fatty acid composition based on NMR spectrum [36]. Most of the data reported show a lot of consistency between the compositional results of unsaturated fatty acids derived from quantitative NMR and GC analysis.

Comparing the data in Tables 3 and 4 indicate that high E_a is usually associated with high percentage of saturated-CH_2 carbons and low percentage of bis-allylic-CH_2 carbons (Table 4). An increase in the methylene carbons of the FA chains, without being interrupted by –C=C– bonds, increases the thermal and oxidative stability. It was also noticed that protons on bis-allylic methylene groups (Table 5) are highly susceptible to rapid radical attack and initiate oxidative degradation of the triacylglycerol molecule. This is mainly due to the resonance stabilization of the radical generated by the adjacent double bonds. Similarly, an increase in the percentage of olefin proton in the FA chain decreases the E_a for oxidation. These olefin protons are mainly from mono- (high oleic content) and poly-unsaturation (high linoleic and linolenic content) in the FA chains.

Conjugated olefin structures offer easy removal of $H^{\bullet}$ due to better resonance stabilization of the reactive

Table 5
Quantitative ^{1}H NMR derived structural parameters of vegetable base oils[a]

Vegetable oils	Percentage of olefin proton	Percentage of bis-allylic-C**H**$_2$ proton	Percentage of α-C**H**$_2$ to C=O	Percentage of allyl-C**H**$_2$	Percentage of –C**H**$_2$ on saturated carbons
Cotton seed oil	8.80	2.83	5.87	8.76	54.87
Corn oil	10.04	3.38	5.76	10.51	51.36
Canola	8.31	1.61	5.95	10.85	54.96
Safflower oil	11.15	4.25	6.04	10.86	48.35
High oleic safflower oil	7.18	0.75	5.80	10.75	57.03
High linoleic safflower oil	10.99	4.38	5.97	10.88	48.46
High oleic sunflower oil	6.90	0.62	5.63	10.55	57.81
Soybean oil	10.26	4.13	6.15	10.40	51.71
Sunflower oil	10.15	3.85	5.87	9.56	51.10

[a] NMR data reported is the average value of three independent experiments. The repeatability of the data is ±0.2.

parent radical thus generated in the FA chain. Thus, higher the mono-unsaturation in the genetically modified vegetable oil, the better the thermal/oxidative stability observed in terms of T_S and T_O. The poly-unsaturation in the FAs is associated with bis-allylic methylene protons and low percentage of saturated-CH_2 carbons (short methylene chain length) (Tables 4 and 5). However, it was also noticed that none of these factors independently account for the extent of thermal and oxidative degradation of vegetable oils (E_a, k, T_S, T_O), or follow a linear relationship with any of the NMR derived structural parameters. Apparently, several FA structural moieties influence these properties to a varying degree. The relative extent to which these structural parameters affect thermal/oxidative behavior can be obtained by multi-component statistical analysis of the data.

3.3. Statistical analysis of NMR and DSC data

There are many limitations of using single structural parameter to explain thermal and oxidative behavior of oils. Due to the presence of several intermediate steps and short-lived species during oxidative degradation, there exits no linear relationship between properties (e.g. T_O, T_S, E_a, k, etc.) and structure that can be used universally for all vegetable oils. The primary reason for this is that several structural parameters influence these properties to varying degree during vegetable oil oxidation. Using multi-component linear regression analysis, T_S of oxidation was found to correlate with several NMR derived structural parameters. The results of this statistical analysis yielded the following equation:

$$\text{Start temperature } (^\circ\text{C}) = 54.8 - 13.5A + 1.37B - 3.93C + 21.7D \quad (1)$$

where A is the percentage of olefin carbons; B the percentage of saturated-CH_2; C the percentage of bis-allylic-CH_2; D the percentage of ω-2 carbons of saturated, mono- and n-6 poly-unsaturated FA acids (generally linoleic acid in vegetable oils) [36]. The correlation is statistically quite satisfactory with adjusted coefficient of determination (adjusted R^2) being 0.96 and standard error of Y-estimate 3.07. Analyzing the p values and t-test on the predictor variables used in the above equation, A and D showed maximum influence on T_S with minor contribution from B and C. The correlation improved further from 0.92 to the present value on adding the contribution from B during T_S of oxidation. This factor suggests that with the increase in methylene carbons in the FA chain, the induction time for oxidation is further delayed. The experimental uncertainty for the determination of T_S is $\pm 2^\circ$C obtained from three independent experiments. The present model (Eq. (1)) holds good for determining T_S in the range 100–200°C.

Similarly, various structural parameters were found to affect T_O, and the results of the statistical analysis yielded the following equation:

$$\text{Onset temperature } (^\circ\text{C}) = 103 - 12.4A - 4.84C + 24.7D + 5.93E \quad (2)$$

where E is the terminal methyl carbons.

The model (Eq. (2)) has a high coefficient of determination (adjusted $R^2 = 0.98$) and the error of Y-estimate is 2.0. T_O shows significant contribution from factors A and D. The effect of B was not found too significant to influence the T_O of oxidation. This was also confirmed by t-test and p values on the predictor variables used to generate the model. It appears that percentage of saturated-CH_2 carbons almost lose its influence on oxidative degradation beyond a particular temperature (in this case T_S). Saturated-CH_2 carbons are quite effective in delaying the oxidation process. However, when the temperature of the system increases and the molecules possess sufficient kinetic energy to cross the activation energy barrier, C–C saturated bond undergoes thermal cleavage and triggers rapid oxidative breakdown liberating heat. It was also observed that terminal-Me groups influence T_O, though marginally in vegetable oils. This was later confirmed from an ongoing separate study where branching sites were introduced in the FA chain resulting in improvement of oxidative behavior. The experimental uncertainty for determining T_O is $\pm 1^\circ$C (from three experiments) and the present equation is statistically valid within 140–190°C for determining T_O with high accuracy.

The quantitative data generated on the different vegetable oils using ^{1}H NMR spectroscopy were used as predictor variables to correlate E_a and k obtained from PDSC studies. Several structural moieties were identified that influenced these kinetic parameters

using the 'best subset regression' analysis technique. The correlation developed for E_a and k using ^{1}H NMR derived predictor variables are:

$$E_a\,(\mathrm{kJ/mol}) = 112 - 2.21(\%\,\text{olefin proton}) - 3.29(\%\,\text{allyl-C}\mathbf{H}_2\,\text{proton}) + 1.43(\%\,-\mathbf{CH}_2\,\text{on saturated carbons}) \quad (3)$$

$$k\,(\mathrm{min}^{-1}) = 0.0485(\%\,\text{allyl-C}\mathbf{H}_2\,\text{proton}) - 0.0228(\%\,\text{bis-allylic-C}\mathbf{H}_2\,\text{proton}) + 0.102(\%\,\alpha\text{-C}\mathbf{H}_2\,\text{proton to C=O}) - 0.602 \quad (4)$$

The adjusted coefficient of determination (R^2) for E_a and k is 0.99 and 0.92 while the error of *Y*-estimate are 0.18 and 0.01, respectively. The E_a was significantly affected by allylic-$\mathbf{CH}_2$ proton in the FA chain. In addition to this, unsaturation and chain length of saturated-CH_2 moieties in the triacylglycerol molecule influence E_a for vegetable oils (Eq. (3)). It may be pointed out that both types of quantitative NMR data (^{1}H and ^{13}C) could be used for correlation purposes. The structural parameters obtained from either method are mutually exclusive and correlate well with the thermal and kinetic data of vegetable oils. This is also evident from a close analysis of the ^{1}H and ^{13}C NMR results (Tables 4 and 5). Different structural parameters contribute in different magnitude to the total thermal and oxidative stability of a vegetable oil matrix. This also explains the nonconformity of theoretical relationship (E_a directly proportional to k^{-1}) between E_a and k, evident from the kinetic data on vegetable oil oxidation (Table 3). As the reaction products begin to build up during oxidation, they have their own kinetic rate constants [44]. It was observed that the speed of a particular reaction step is determined by its activation energy requirement and the result of competition among various kinetic rate constants. The final product distribution of the starting mixture is governed by kinetics of the system and the probability distribution. Allyl-$\mathbf{CH}_2$ and bis-allylic-$\mathbf{CH}_2$ protons influence the rate constant significantly with some contribution from –$\mathbf{CH}_2$ proton α to >C=O group (Eq. (4)). It is also interesting to note that chain length of the –$\mathbf{CH}_2$ protons in the FA do not have considerable influence on k as observed in case of E_a. Similar results were obtained from the kinetic studies of mineral base oils [33], where percentage of paraffin (*n*- and *iso*-) content and average chain length significantly influenced the E_a of oxidation. The results are valid in the range 63–89 kJ/mol and 0.3–0.55 (min^{-1}) for the calculation of E_a and k, respectively.

4. Conclusions

The complexity of vegetable oil oxidation is primarily due to the involvement of different structural parameters in the fatty acid chain. Different structural parameters participate in the reaction at different stages of oxidation. It is therefore not a good idea to measure the extent of oxidative degradation in terms of a single parameter, i.e. poly-unsaturation. We have noticed that allylic, bis-allylic protons, α-C$\mathbf{H}_2$ to C=O group, chain length of the saturated methylene groups, etc. have significant effect on the oxidation process at different stages. The ^{1}H and ^{13}C NMR derived structural information can be used to explain most of the thermal and kinetic behavior of unmodified and genetically modified vegetable oils. Statistical models developed on the start and onset temperature, and kinetic parameters like E_a and k, can be used as predictive tools for quick assessment of vegetable oil oxidation. The model has incorporated different NMR derived structural types of the vegetable oils that contributed to oxidation process. The coefficient of determination, *t*-test and *p*-values indicate that the models are statistically valid in the range specified yielding low error of *Y*-estimate in each case.

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