Effect of Linoleic Acid Position in Triacylglycerols on their Oxidative Stability

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Four synthetically produced triacylglycerols containing linoleic and palmitic acid in different known positions were used to determine the effect of fatty acid position on their relative rates of autoxidation. The triacylglycerols were sn-1,3-dipalmitoyl-sn-2-linoleoyl (PLP); sn-1,2(2,3)-dipalmitoyl-sn-1(3)-monolinoleoyl (PPL); sn-2-palmitoyl-sn-1,3-dilinoleoyl (LPL); and sn-1(3)-palmitoyl-sn-1,2(2,3)-dilinoleoyl (PLL) glycerols. Oxidation was evaluated both by peroxide value, and reversed phase HPLC with ultraviolet absorbance detection at 235 nm for conjugated diene of triacylglycerol's oxidized linoleic acid. Triacylglycerols were oxidized at 60 °C in the dark. PLL had lower oxidative stability than LPL. This effect may be due to easier interactions between adjacent linoleic acid of PLL compared to nonadjacent linoleic acid of LPL during autoxidation. PLP had lower oxidative stability than PPL. Reversed phase HPLC analysis showed that at a low oxidation level, PLP and PPL produced monohydroperoxide and LPL and PLL produced both mono- and bishydroperoxides. These results are further evidence that fatty acid position on triacylglycerols plays a role in lipid oxidation.

Introduction

It has become increasingly clear that the position of fatty acids (FA) on the glycerol moiety of triacylglycerols (TAG) has an influence on chemical reactions, such as oxidation, and also biological metabolism (1-3). Several papers have reported results of investigations concerning the effect of FA position in TAG on oxidative stability. Raghuveer and Hammond (4) and Wada and Koizumi (5) found that increased unsaturated FA on carbon 2 increased TAG stability. Conversely, Zalewski and Gaddis (6) found that increase in unsaturated FA on carbon 2 decreased TAG oxidative stability. Other workers, e.g. Park et al. (7), found no effect on oxidative stability with regard to position of unsaturated FA in TAG. These inconsistent results may be due to lack of standardized, sensitive experimental designs and oxidative stability tests (8, 9).

We have recently reported the effect of FA position in the TAG on the oxidation of purified vegetable oil TAG, i.e. soybean oil (SBO), in the dark at 60 °C (10) and in fluorescent light at 25 °C (11); canola oil (CNO) (12); and blends and interesterified blends of SBO and palm olein (13). We determined a positive statistical correlation (R) of reduced oxidative stability with increased linoleic (L) acid concentration at carbon 2 of the TAG.

In addition, we have previously reported on the oxidative stability of individual, pure synthetic or model TAG containing L and linolenic acid (Ln) on known positions in the TAG (14, 15). It was determined that LnLnL oxidized faster than LnLLn and that LLLn oxidized slightly faster than LLLn. Competitive interactions between L and Ln in the TAG during autoxidation was proposed for this apparent stereospecific effect on TAG oxidative stability (16). Greater interaction between Ln residues than between Ln and L residues was postulated for the lower oxidative stability of LnLnL compared to LnLLn. The slightly lower oxidative stability of LLnL compared to LLLn was attributed to stronger interaction between Ln and L residues than between two L residues.

Frankel suggested that oxidative stability experiments be designed around the Schaal oven test which gives quick results that correlate well with bulk oil deterioration or shelf-life (8). He also suggested more than one oxidative stability test be used. It was found that initial oxidation products could be detected and quantified by peroxide value and ultraviolet spectrophotometry (8). We have employed Frankel's suggested methodology and report additional investigations concerning the effect of L position on oxidative stability TAG containing palmitic (P) and L acid.

Materials and Methods

Materials
PPL, LPL, PLL and PLP were synthesized by Awl and co-workers from dihydroxacetone and benzylidene precursors and acylation by the appropriate FA (17).
The isomeric purity of these TAG was 97 to 99%. Before oxidation, the TAG were purified by solid phase extraction chromatography to remove any oxidation products to give PV = 0 meq/kg by a previously published procedure (12). Solvents were purchased as high performance liquid chromatography (HPLC) grade and used without further purification.

Methods
Oxidation. TAG were oxidized (1.0 g) in duplicate with stirring in the dark under oxygen at 60 °C for 144 h. Oxidation progress was monitored by periodically analysing 10 mg samples for PV and 5 mg samples (in triplicate) for oxidation products (TAG-OX) by RP-HPLC-UV.

Peroxide value (PV). PV was determined by the colorimetric thiocyanate method (18).

TAG-OX analysis. The RP-HPLC column was a Vydac ODS, 5 μm, 0.46 x 25 cm (The Separations Group, Hesperia, CA); mobile phase was acetonitrile/methylene chloride/methanol (90:5:5, v/v/v) at a flow rate 0.8 mL/min; ultraviolet (UV) detector at 235 nm for conjugated diene functionality of oxidized L with sensitivity set at 0.4 absorbance units; sample size was 10 μL of a solution of 52 mg TAG-OX per 100 mL methylene chloride. Formation of oxidation products of LLP, LPL, PLL and PLP was monitored. Non-TAG-OX or unreacted TAG was removed from the HPLC column by stripping with methylene chloride between analyses. TAG-OX were identified using collected HPLC fractions. The solvent was removed by evaporation using a nitrogen gas stream. Intact TAG-OX was identified by proton nuclear magnetic resonance (H-NMR) and by capillary gas chromatography with flame ionization detection (GC) of the silylated, sodium borohydride transmethylated TAG-OX.

TAG-OX characterization by H-NMR. TAG-OX isolated by RP-HPLC were characterized by proton (H-NMR) nuclear magnetic resonance. NMR spectra were obtained at 400.13 MHz on a Brucker ARX Spectrometer (Bruker, Inc., Billerica, MA); mobile phase was acetonitrile/methylene chloride/methanol (90:5:5, v/v/v) at a flow rate 0.8 mL/min; ultraviolet (UV) detector at 235 nm for conjugated diene functionality of oxidized L with sensitivity set at 0.4 absorbance units; sample size was 10 μL of a solution of 52 mg TAG-OX per 100 mL methylene chloride. Formation of oxidation products of LLP, LPL, PLL and PLP was monitored. Non-TAG-OX or unreacted TAG was removed from the HPLC column by stripping with methylene chloride between analyses. TAG-OX were identified using collected HPLC fractions. The solvent was removed by evaporation using a nitrogen gas stream. Intact TAG-OX was identified by proton nuclear magnetic resonance (H-NMR) and by capillary gas chromatography with flame ionization detection (GC) of the silylated, sodium borohydride transmethylated TAG-OX.

TAG-OX characterization by GC. TAG hydroperoxides were reduced with sodium borohydride and then the TAG hydroxide was transmethylated with 0.5 mol/L KOH in methanol. The resultant hydroxyl methyl linoleate was silylated and then analysed along with the resultant unoxidized methyl ester by capillary GC (12). Capillary GC conditions were: SP2380 30 m x 0.25 mm i.d. column with a 0.20 m film (Supelco, Inc., Bellefonte, PA), temperature-programmed from 180 °C, after a 12 min hold, then at 5 °C/min to 220 °C with a 20 min hold at that temperature. Injector and detector temperatures were 240 °C and 280 °C, respectively. Helium inlet pressure was 15 psi. The ratio of oxidized L to unoxidized methyl esters was determined with L monohydroperoxide confirmed by a ratio of 0.5 and bishydroperoxide confirmed by a ratio of 2.0. Hydroxyl methyl and methyl esters were identified by GC retention with respect to standard compounds.

Data precision. Oxidations were performed in duplicate. HPLC analyses and peroxide value determinations were made in triplicate. The relative standard deviations averaged ± 5%.

Results and Discussion
Results of PV determinations for the autoxidation of the TAG pairs PLP and PPL, and PLL and LPL are presented in Table 1. These results showed firstly that, as expected, the diilnoleic-monosaturated acid containing TAG oxidized much faster than the monolnoleic-disaturated FA TAG. Secondly, for the TAG pair PLP and PPL, PLP began to oxidize slightly faster than PPL by 144 h with respect to experimental precision. The oxidative stability parameter (10, 12) or the slope of the linear regression plot or rate of PV change with oxidation time obtained by plotting peroxide values vs. oxidation time showed that PLP (0.084 meq/(kg.h)) had less oxidative stability than PPL (0.071 meq/(kg.h)). At the experimental peroxide range considered the oxidation curves were linear with linear regression coefficients (R) greater than 0.995 (10, 12). The PV oxidative stability parameter has a coefficient of variation of 5% or less with respect to a standard SBO (10). The TAG PPL occurs in SBO at 0.9 to 2.9% concentration (11). Obviously, more important to SBO oxidation are major TAG species such as PLL (LPL) which occur in SBO at 7.5 to 17.9% (11). PV data for the TAG pair PLL and LPL (Table 1) show that PLL oxidized faster than LPL during the entire peroxide range considered. The oxidative stability parameter, peroxide value change per h, was much greater for PLL (4.929 meq/(kg.h)) than for LPL (1.444 meq/(kg.h)). Thus, PLL with two adjacent linoleic acids had lower stability than LPL with the L separated by a saturated fatty acid.

Table 1 Peroxide value analyses to monitor triacylglycerol oxidationa

<table>
<thead>
<tr>
<th>Oxidation time (h)</th>
<th>PLP</th>
<th>PPL</th>
<th>PLL</th>
<th>LPL</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>2.0</td>
<td>2.2</td>
<td>35.0</td>
<td>11.3</td>
</tr>
<tr>
<td>48</td>
<td>3.6</td>
<td>3.6</td>
<td>55.5</td>
<td>19.0</td>
</tr>
<tr>
<td>72</td>
<td>5.1</td>
<td>5.3</td>
<td>75.0</td>
<td>26.8</td>
</tr>
<tr>
<td>96</td>
<td>6.8</td>
<td>5.8</td>
<td>95.5</td>
<td>35.9</td>
</tr>
<tr>
<td>120</td>
<td>9.8</td>
<td>9.2</td>
<td>115.4</td>
<td>44.8</td>
</tr>
<tr>
<td>144</td>
<td>12.6</td>
<td>10.4</td>
<td>135.5</td>
<td>57.8</td>
</tr>
</tbody>
</table>

aData determined by colorimetric procedure for peroxide value monitoring of triacylglycerol oxidation process (see experimental section).

bMean of three determinations with relative standard deviation average ±5%. P = palmitic acid; L = linoleic acid.
oxidation curves were linear with linear regression \((R)\) greater than 0.995. Evaluation of vegetable oil oxidation and stability is better accomplished with at least two oxidation state analysis techniques which measure oil oxidation changes during conditions normal to oil deterioration \((8)\). The initial oxidation products, hydroperoxides, can be measured by PV determination and also by RP-HPLC-UV \((8)\). In our previous research \((11-13)\) an oxidative stability parameter was developed, which was obtained by linear regression of the plot of formation of total hydroperoxides, as UV absorbance \((at 235 \text{ nm})\) detector area counts, vs. oxidation time \((h)\). Good linear relationships \((R > 0.976)\) were determined for total hydroperoxide formation with oxidation time, determined by RP-HPLC-UV, and PV with oxidation time. Similar \(R\) for RP-HPLC-UV and PV were obtained in the previous study on synthetic \(L\) and \(Ln\) TAG \((14)\).

RP-HPLC-UV measured monohydroperoxides formed from PLP, PPL, PLL, and LPL. Also measured were the bishydroperoxides of PLL and LPL. The PLP and PLL monohydroperoxides showed complex peaks, which had RP-HPLC elution times between 59 to 68, and 57 to 67 min, respectively. The PLL and LPL monohydroperoxides also gave complex peaks, which eluted between 40 to 60, and 50 to 62 min, respectively. The bishydroperoxides eluted between 15 to 20, and 21 to 22 min, respectively. Complex RP-HPLC-UV peaks have been observed previously for other synthetic TAG \((14)\) and for SBO and CNO TAG \((10, 11)\) and CNO TAG \((12)\) and SBO–palmolein TAG products \((13)\).

It is definitely observed that for TAG-OX quantities within experimental error, PLP oxidized much faster than LPL. Further linear regression analysis of plots of the data with oxidation time showed TAG-OX oxidative stability parameters of \(24.5 \times 10^3\) area counts/h for PLP and \(5.79 \times 10^3\) area counts/h for LPL. This indicated that PLL is considerably less oxidatively stable than LPL. These TAG-OX results were consistent with PV for PLP and PLL. The PV data showed that the position of \(L\) in the TAG pairs PLP and PPL has a slight influence on their autoxidation. For these TAG, \(L\) on TAG carbon 2 slightly reduced the TAG oxidative stability under oxidation conditions pertinent to oil deterioration \((8)\). This is consistent with our previous studies on SBO \((10, 11)\) and CNO TAG \((12)\) and SBO–palmolein TAG products \((13)\).

Table 2  H-nuclear magnetic resonance \((\text{CDCl}_3)\) of triacylglycerol autoxidation products

<table>
<thead>
<tr>
<th>Oxidation producta</th>
<th>Shifts, p.p.m. ((\text{Multiplicity}^b, \text{number protons}, \text{assignment}^c))</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPL and PLP MonoOOH</td>
<td>7.90(brs, 1 OOH) 6.8–5.4(4m, 4, CH=CH=CH=CH) 5.22 ((m.1.\text{CHOOH})) 4.40–4.08 ((2m.5.\text{glycerol moiety protons})) 2.30 ((m.6.\text{CH}_2=C=O)) 2.05 ((m.2.\text{CH}_2=C=C)) 1.62 ((m.6.\text{CH}_2=\text{CO})) 1.25 ((\text{brs.} \text{(CH}_2)_n)) 0.90 ((m.9.\text{CH}_3))</td>
</tr>
<tr>
<td>LPL and PLL MonoOOH</td>
<td>7.90(brs, 1 OOH) 6.8–5.4(4m, 4, CH=CH=CH=CH) 5.35 ((m.4.\text{CH}=\text{CH})) 5.22 ((m.1.\text{CHOOH})) 4.40–4.08 ((2m.5.\text{glycerol moiety protons})) 2.30 ((m.6.\text{CH}_2=\text{CO})) 2.02 ((m.6.\text{CH}=\text{CH}=\text{CH})) 1.60 ((m.6.\text{CH}_2=\text{CO})) 1.28 ((\text{brs.} \text{(CH}_2)_n)) 0.89 ((m.9.\text{CH}_3))</td>
</tr>
<tr>
<td>LPL and PLL BisOOH</td>
<td>7.99(brs.2 OOH) 6.6–5.4(4m, 8, CH=CH=CH=CH) 5.25 ((m.2.\text{CHOOH})) 4.40–4.08 ((2m.5.\text{glycerol moiety protons})) 2.30 ((m.6.\text{CH}_2=\text{CO})) 2.04 ((m.4.\text{CH}_2=C=C)) 1.65 ((m.6.\text{CH}_2=\text{CO})) 1.30 ((\text{brs.} \text{(CH}_2)_n)) 0.85 ((m.9.\text{CH}_3))</td>
</tr>
</tbody>
</table>

\(a\) Oxidized TAG fatty acids: \(P = \text{palmitic}; L = \text{linoleic.}\)

\(b\) Multiplicity: s = singlet, m = multiplet.

\(c\) Proton assignment for hydroperoxy fatty acids of the triacylglycerol are based on chemical shifts reported previously \((14)\).
Table 3  Reversed phase high performance liquid chromatography with ultraviolet UV absorbance detection at 235 nm for conjugated diene of oxidized linoleic acid analysis of the autoxidation of synthetic triacylglycerols at 60 °C

<table>
<thead>
<tr>
<th>Oxidation time (h)</th>
<th>PLP</th>
<th>PPL</th>
<th>PLL</th>
<th>LPL</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>6.46×10⁴</td>
<td>4.13×10⁴</td>
<td>8.53×10⁴</td>
<td>5.00×10⁵</td>
</tr>
<tr>
<td>48</td>
<td>10.49</td>
<td>8.18</td>
<td>11.67</td>
<td>8.00</td>
</tr>
<tr>
<td>72</td>
<td>13.73</td>
<td>9.48</td>
<td>22.51</td>
<td>17.50</td>
</tr>
<tr>
<td>120</td>
<td>17.97</td>
<td>13.49</td>
<td>36.67</td>
<td>27.50</td>
</tr>
<tr>
<td>144</td>
<td>23.35</td>
<td>17.30</td>
<td>46.67</td>
<td>35.00</td>
</tr>
</tbody>
</table>

* Determined by RP-HPLC-UV with summation of UV detector area counts of mono- and bishydroperoxides formed during triacylglycerol oxidation time, h (see experimental section).

* Mean of three determinations with relative standard deviation average ±5%.

P = palmitic acid; L = linoleic acid.

(10, 14–16). Also these results, which indicated that PLL is less stable than LPL, were consistent with our previous oxidative stability studies of SBO and SBO-palmolein products where PLL is a major TAG (10, 11, 13).

Acknowledgement

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References


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