Changes in the triacylglycerol content of flaxseeds during development using liquid chromatography-atmospheric pressure photoionization-mass spectrometry (LC-APPI-MS)

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Changes in the composition of triacylglycerols (TAGs) of three varieties of flaxseeds (H52, O116 and P129) during development were investigated using non-aqueous reversed phase liquid chromatography coupled to atmospheric pressure photoionization-mass spectrometry (LC-APPI-MS). 17 species of triacylglycerols were found and their fatty acyl composition was confirmed by fragment ions in the mass spectra. The major forms observed were LnLnLn, LnLLn, LnOLn, LLLn, LnLnP, OOLn and OLLn. The highest change in these compounds occurred in the early development period for most TAGs. The most active period of triacylglycerol synthesis was between 7 and 35 days after flowering (DAF). LnLnLn exhibited the most significant increase during flaxseed development. At maturity, similar TAG profiles were found for the three samples.

Key words: Flaxseeds, seed development, triacylglycerols (TAGs), APPI, mass spectrometry.

INTRODUCTION

The changes in the lipid composition of flaxseeds which take place during ripening exert a strong influence on the final flaxseed oil quality. Flaxseed oils, which are primarily triacylglycerols (TAGs), serve as an important source of fatty acids in the human diet in some regions. They can provide fatty acids essential for the biosynthesis of long-chain polyunsaturated fatty acids which are in turn important for the synthesis of cell membranes in the human body (Czaplicki et al., 2009). Since flaxseed TAGs are complex mixtures, sophisticated analytical techniques are required in order to monitor the changes in TAG profiles occurring during seed development. Separation of TAG mixtures has been achieved using many different chromatographic tech-niques including gas chromatography, supercritical fluid chromatography and high-performance liquid chromatography (LC) (Laakso, 1996). Man LC separations of TAG species have been described including the use of silver-ion columns, which separate by degree of unsaturation and cis/trans isomerism (Dobson et al., 1995), as well as non-aqueous reversed-phase (Lipp, 1995) and normal-phase chromatography (Adlof et al., 2002). Reversed phase LC is the methodology most commonly employed since separation according to both chain length and degree of fatty acid unsaturation can be achieved, resulting in resolution of many individual TAG molecules. However, due to the complexity of TAG mixtures, LC analysis of TAGs is better achieved by coupling to mass spectrometry (MS), which both acts as a universal detector for lipids and provides structural information crucial to confirm TAG composition (Harrabi et al., 2008) and potentially substitution pattern (Byrdwell 2005). Most LC/MS interfaces commonly used employ atmospheric pressure ionization (API) techniques such as electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) (Byrdwell, 2005). Atmospheric pressure photoionization (APPI) is an alternative technique demonstrated (Cai and Syage, 2006) to have some...
advantages for the analysis of TAGs relative to ESI or APCI (Cai et al., 2007). In addition to any sensitivity differences, APPI is particularly well-suited to non-aqueous systems whereas the other techniques require either post-column addition of polar solvent (ESI) or suffer from instability due to the need for a discharge needle in a high-organic environment (APCI).

Although, the TAG compositions of different varieties of flaxseeds have been studied at maturity (Holcapek et al., 2003; Tuberoso et al., 2007; Lisa et al., 2007), the evolution of their TAG content during maturation is not well-known. Thus, the objective of this work was to determine the triglyceride composition of three varieties (H52, O116 and P129) of flaxseed at different stages of maturation using liquid chromatography–atmospheric pressure photoionization–mass spectrometry (LC-APPI-MS).

MATERIALS AND METHODS

Chemicals and reagents

HPLC-grade chloroform, acetone, acetonitrile, toluene and isopropanol were purchased from Fisher Scientific Company (Ottawa, Ontario, Canada; Ottawa Admin. Center 112 Colonnade Road Nepean, Ontario K2E 7L6 Canada). Trinonadecanoic acid was obtained from Nu-chek Prep, Inc (Elysian, MN, USA; P.O. Box 295 Elysian MN 56028 U.S.A).

Plant material

Three varieties of flaxseeds, H52, O116 and P129, were obtained from Institut National Recherche Agronomie Tunis (INRAT), Tunisia. The varieties of flaxseed (Linum usitatissimum L.) were grown in restricted zones (15 x 3 m) on the Agronomy farm of the INRAT from the middle of November 2006 until the end of June 2007. Each sample was collected at intervals after a number of days of flowering (DAF) ranging from 7 to 56 DAF. Moisture and seeds weights were determined by weighing 100 seeds before and after drying to constant weight in a vacuum oven at 80°C for 72 h.

Lipid extraction

The total lipids were extracted by the method of Folch et al. (1957) modified by Bligh and Dyer (1959). Seeds (2.5 g DW) were washed with boiling water for 5 min to denature the phospholipases (Douce, 1964) and then crushed in a mortar with a mixture of CHCl3-MeOH (2:1, v/v). The boiling water (5 ml) was added and the homogenate was centrifuged at 3000 g for 15 min. The lower chloroformic phase containing the total lipids was kept and dried in a rotary evaporator at 40°C. A 50 mg sample of the extracted lipid was fully dissolved in 5 ml dichloromethane. To 100 µl of this solution in an LC vial, 50 µl of a 1 mg/ml solution of the trinonadecanoic acid internal standard and 850 µl of dichloromethane was added.

HPLC-APPI-MS

LC/MS experiments were performed using an Agilent LC system (Agilent Technologies; Palo Alto, CA- 2660 Matheson Boulevard East, Mississauga ON, L4W 5M2) coupled to an Applied Biosystem MDS Sciex QSTAR Elite Q-TOF MS with a photoionization ion source. The TAG extracts were separated using an Ascentis™ C18 LC column (15 cm x 2.1 mm, 3 µm) with C18 guard cartridge (Supelco). The mobile phase consisted of A, acetonitrile and B, isopropanol. A solvent gradient program was run from 30 to 90% B in 25 min. Subsequently, the LC system was programmed to return its initial solvent composition over 0.1 min, followed by a 5 min re-equilibration prior to next injection. The injection volume was 5 µl and flow rate was 200 µl/min. The entire effluent from the column was directly transferred to the MS through photoionization interface.

MS analysis of the analytes was performed using APPI in the positive ion mode. Toluene was used as dopant delivered at flow rate of 20 µl/min by an isocratic pump (Agilent Technologies; Palo Alto, CA- 2660 Matheson Boulevard East, Mississauga ON, L4W 5M2). Analyst QS 2.0 software was used for data acquisition and analysis. Nitrogen was used as curtain gas, nebulizing gas, drying as and lamp gas (4 L/min). The mass range recorded was from 50 to 1300 amu. The other optimal instrumental conditions were as follows: photoionization voltage 1300V; curtain gas setting 25; gas 1 setting 20 and gas 2 setting 70; declustering potential (DP), 35V; focusing potential (FP), 150; second declustering potential (DP2), 15V; and ion source temperature: 400°C.

Estimations of TAG concentrations were achieved using the relative peak areas of each TAG compared to the trinonadecanoic acid tri-C19:0 internal standard and assuming equivalent response factors. Although, this method is only semi-quantitative, likely overestimating highly unsaturated components relative to saturates, it does allow the estimation of relative TAG concentrations and the evolution of individual TAG species over the course of seed ripening to be evaluated. As examples of the results, the total ion current (TIC) chromatogram of flaxseed oil is given in Figure 1 and the APPI mass spectrum of OLLn taken from an LC/MS run is given in Figure 2. The latter shows that the fragment ions present confirm the fatty acyl groups present.

Statistical analysis

Statistical analysis was performed using the Proc ANOVA in SAS (software version 8). For each oil sample, three determinations were performed.

RESULTS AND DISCUSSION

Physical characteristics of flaxseed

The flaxseed colour was adopted as a visual ripening criterion. In fact, the seeds turn from green to green-brown and finally to brown when completely ripe (Herchi et al., 2009). As shown in Table 1, the seed weight (100 seeds) at 7 DAF (0.2 g) was the lowest because of seed immaturity. Seeds increased progressively in weight (100 seeds) as maturity progressed to obtain a maximum of 1 g at ripe seed (56 DAF). These results are similar to those of Kaliangile and Grabe (1988) who signalled that there is a strong positive correlation between maturity stage and seed dry matter due to the accumulation of organic matter in the ripe seeds.

TAG composition at fully ripe seeds

The TAG composition of three flaxseed oils at maturity (56 DAF), expressed as a percentage of total TAG peak
17 unsaturated species of TAG were detected during the maturation of flaxseed. In each case, the proposed TAG structure was confirmed by the molecular weight and fragment ion information seen in the APPI mass spectra, as shown in Figure 2 for OLLn where the fragment ions arise from loss of O, L and Ln where Ln represents linolenic acid; L, linoleic acid; O, oleic acid; P, palmitic acid; and S, stearic acid. Hence, there is high confidence in the assignments although, no regiospecific analysis was performed and the structures given are not meant to imply a particular fatty acyl arrangement on the glycerol backbone. The main TAGs found in flaxseed oil with peak area percentages of 6 or more were LnLnLn, LnLLn, LnOLn, LLLn, LnLnP, OOLn,
### Table 1. Physical characteristics of flaxseed during maturation.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Day after Flowering (DAF)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7</td>
</tr>
<tr>
<td>Seed colour</td>
<td>Green</td>
</tr>
<tr>
<td>100 seeds dry weight (g/100 seeds)*</td>
<td>0.2±0.05</td>
</tr>
<tr>
<td>Moisture content (%)*</td>
<td>70±1.41</td>
</tr>
</tbody>
</table>

*a average of three determinations of three varieties (H52, O116 and P129). Location : Institut National Recherche Agronomie Tunis (INRAT); Year: 2006 to 2007; Period: 7 DAF to 56 DAF (maturity).

### Table 2. Triacylglycerols (TAGs) identified in flaxseed oil at maturity listed according to their molecular weight, fragment ions confirming TAG identity, retention times (tR), Partition number (PN), percentage of relative peak areas (mean and standard deviation of a triplicate analyses) and comparison with literature results using other analytical methods.

<table>
<thead>
<tr>
<th>m/z</th>
<th>TAG</th>
<th>Observed fragment ion (m/z)</th>
<th>tR (min) LC/APPI-MS</th>
<th>PN H52 LC/APPI-MS (area %)</th>
<th>PN O116 LC/APPI-MS (area %)</th>
<th>PN P129 LC/APPI-MS (area %)</th>
<th>Lisa et al, 2007 LC/CAD (%)</th>
<th>Tuberoso et al., 2007 LC/UV, 207nm (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>873</td>
<td>LnLnLn</td>
<td>595</td>
<td>9.61</td>
<td>36</td>
<td>29.1±1.6b</td>
<td>29.8±0.9b</td>
<td>36</td>
<td>29.1±0.9a</td>
</tr>
<tr>
<td>875</td>
<td>LnLln</td>
<td>595,597</td>
<td>10.93</td>
<td>38</td>
<td>16.7±0.5a</td>
<td>16.6±0.3a</td>
<td>16.4±0.5a</td>
<td>13.4</td>
</tr>
<tr>
<td>877</td>
<td>Llln</td>
<td>597,599</td>
<td>12.22</td>
<td>40</td>
<td>7.2±0.7a</td>
<td>7.3±0.3a</td>
<td>7.7±1.5b</td>
<td>4.1</td>
</tr>
<tr>
<td>877</td>
<td>LnOln</td>
<td>595,599</td>
<td>12.49</td>
<td>40</td>
<td>14.4±1.0a</td>
<td>14.6±1.2a</td>
<td>15.3±0.5b</td>
<td>14.5</td>
</tr>
<tr>
<td>851</td>
<td>LnLnP</td>
<td>573,595</td>
<td>12.93</td>
<td>40</td>
<td>7.2±0.2a</td>
<td>7.4±1.0a</td>
<td>6.1±0.9b</td>
<td>5.8</td>
</tr>
<tr>
<td>879</td>
<td>Olln</td>
<td>597,599,601</td>
<td>13.87</td>
<td>42</td>
<td>6.4±0.2a</td>
<td>5.9±0.1b</td>
<td>7.0±0.6c</td>
<td>7.7</td>
</tr>
<tr>
<td>853</td>
<td>LnLP</td>
<td>575,597</td>
<td>14.42</td>
<td>42</td>
<td>4.8±0.5a</td>
<td>4.7±0.3a</td>
<td>5.7±0.2b</td>
<td>7.5+SLnLn</td>
</tr>
<tr>
<td>881</td>
<td>OOLn</td>
<td>599,603</td>
<td>15.51</td>
<td>44</td>
<td>6.8±0.3a</td>
<td>5.6±0.3b</td>
<td>6.4±0.3a</td>
<td>7.4</td>
</tr>
<tr>
<td>855</td>
<td>LnOP</td>
<td>573,577,599</td>
<td>15.91</td>
<td>44</td>
<td>2.8±0.4a</td>
<td>3.0±0.4a</td>
<td>2.9±0.4a</td>
<td>3.2</td>
</tr>
<tr>
<td>829</td>
<td>PlnP</td>
<td>573</td>
<td>16.27</td>
<td>44</td>
<td>0.02±0.01a</td>
<td>0.01±0.01b</td>
<td>0.02±0.01a</td>
<td>0.3</td>
</tr>
<tr>
<td>883</td>
<td>OLO</td>
<td>601,603</td>
<td>16.84</td>
<td>46</td>
<td>0.45±0.1a</td>
<td>0.60±0.1b</td>
<td>0.55±0.1b</td>
<td>2.2</td>
</tr>
<tr>
<td>883</td>
<td>Soln</td>
<td>599,601,605</td>
<td>17.34</td>
<td>46</td>
<td>1.6±0.1a</td>
<td>2.2±0.1b</td>
<td>2.1±0.1b</td>
<td>-</td>
</tr>
<tr>
<td>857</td>
<td>OLP</td>
<td>575,601</td>
<td>17.79</td>
<td>46</td>
<td>0.12±0.02a</td>
<td>0.25±0.05b</td>
<td>0.12±0.02a</td>
<td>3.5+SOLn+BLnL</td>
</tr>
<tr>
<td>885</td>
<td>OOO</td>
<td>603</td>
<td>18.34</td>
<td>48</td>
<td>1.3±0.1a</td>
<td>0.87±0.05b</td>
<td>1.3±0.1a</td>
<td>2</td>
</tr>
<tr>
<td>859</td>
<td>OOP</td>
<td>577,603</td>
<td>18.79</td>
<td>48</td>
<td>0.64±0.02a</td>
<td>0.60±0.01b</td>
<td>0.74±0.04b</td>
<td>0.9</td>
</tr>
<tr>
<td>885</td>
<td>SlLn</td>
<td>601</td>
<td>19.21</td>
<td>48</td>
<td>0.22±0.01a</td>
<td>0.20±0.01a</td>
<td>0.12±0.02a</td>
<td>&lt;0.05±</td>
</tr>
<tr>
<td>887</td>
<td>SSO</td>
<td>603,605</td>
<td>20.19</td>
<td>50</td>
<td>0.43±0.01a</td>
<td>0.39±0.02b</td>
<td>0.58±0.07b</td>
<td>0.7</td>
</tr>
</tbody>
</table>

*a Each value is the mean (standard deviation (SD) of a triplicate analyses performed on different samples. Means with different letters (a-c) within a row are significantly different at p ≤ 0.05. Location : Institut National Recherche Agronomie Tunis (INRAT); Year: 2006 to 2007; Period: 7 DAF to 56 DAF (maturity).
and OLLn. The same TAG species was observed from all three flaxseed varieties. All three also showed the highest values for LnLnLn (27 to 30%), LnLLn (16 to 17%), LnOLn (14 to 15%), LLLn (7 to 8%), LnLNp (6 to 8%), OOLn (5 to 7%) and OLLn (5 to 7%). The results (Table 2) are in broad agreement with those described in literature (Tuberoso et al., 2007; Lisa and Holcapeck, 2008); although, with some differences in relative intensity. However, it should be noted that the interpretations presented are all confirmed by mass spectrometry as described earlier. Based on TAG composition at maturity given in Table 2, the percentage of the major fatty acid species present were calculated and given in Table 3 for the three varieties of flaxseed oil. It is apparent that the fatty acid composition of the 3 varieties are not significantly different, based on this data (P ≤ 0.05). However, it should be noted that LC/MS results without response factor correction tend to overestimate the more unsaturated components which give rise to more abundant ions. Thus, all of the components containing 2 or 3 Ln groups are seen in higher abundance by LC/MS than literature values obtained using other LC detectors (Table 2). Consistent with this suggestion, approximate values for total saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids in mature seeds determined by gas chromatography with a flame ionisation detector (Herchi et al., 2011) were found to be ~13, 23 and 63% compared to ~6, 15 and 79% determined by LC/MS (Table 3). However, the objective of the present work was to identify the TAG compositions and how they change with seed maturity which is adequately achieved in the LC/MS data. Furthermore, this method is amenable to direct analysis of the small lipid amounts collected from immature seeds.

Flaxseed oil has a unique fatty acid profile, rich in the essential ω-3 fatty acid α-linolenic acid. Hence, there are correspondingly large amounts of triacylglycerols containing high levels of polyunsaturated fatty acids (PUFA) including LnLNp, LnLLn, and LnOLn in flaxseed oil compared to other most other widely cultivated oilseeds (Tuberoso et al., 2007). The importance of linolenic acid in diet is well established (Tuberoso et al., 2007). The minor forms of TAG detected were LnOP, PLnP, OLO, SOLn, OLP, OOO, OOP, SLnS and SOO. Their levels range from 0.01 to 3%. The amount of PLnP was very small consistent with the low levels of palmitic acid in flaxseed oil. For comparison, the TAG composition of flaxseed is different from that of sunflower oil where LnLNp (2%) and LnLL (1%) were the minor components, and OLL (28%) and LLL (20%) were the major forms (Cunha and Oliveira, 2006). It has been suggested that these quantitative differences in the TAG composition of vegetable oils can be used as markers for their detection of oil adulteration (Christopoulos et al., 2004).

### Evolution of TAG levels during flaxseed development

The triacylglycerol contents of flaxseeds observed at different stages of their maturation are presented in Figure 3 for each of the varieties studied. An increase in the TAG content of the total lipid extract was observed during the development of flaxseed, in agreement with the results of Harrabi et al. (2008) who reported similar increases during seed development. In the early stages of development, the TAG content was very low, in the range of 10 to 11 mg/100 mg of lipid at 7 DAF. Given that the various glycerolipids, including TAGs and some phospholipids, may be derived from the same phosphatidate precursor and that the cells of immature seeds need the phospholipids for membrane construction, it is reasonable to assume that the low levels of TAG are a reflection of the higher synthesis of phospholipids during this period. TAG can be obtained from PLs especially from phosphatidylcholine (Stymne et al., 1987) as has been shown in the seeds of *Carthamus tinctorious* (Kesri and Mazliak, 1983) and rape (Perry et al., 1992), as well as in olive fruit (Del Cuvillo et al., 1995). The highest rate of TAG synthesis in flaxseed was observed from 7 to either 35 DAF (varieties H52, P129) or 42 DAF (varieties O116); thereafter, the total amount of TAG was relatively constant (P ≤ 0.05). According to Ohlrogge and Jaworski (1997), seeds have the potential for TAG biosynthesis, and therefore specific expression of diacylglycerol acyltransferase, the only specific enzyme to TAG biosynthesis, seems insufficient to explain abundant TAG biosynthesis in the seeds. It can be seen from Figure 3 that the variety O116 develops TAG more slowly, reaching peak levels about a week.

### Table 3. Fatty acid composition* of triacylglycerols in flaxseed oil at maturity estimated from LC/MS data.

<table>
<thead>
<tr>
<th>Variety</th>
<th>C16:0</th>
<th>C18:0</th>
<th>C18:1</th>
<th>C18:2</th>
<th>C18:3</th>
<th>ΣSFAa</th>
<th>ΣMUFAc</th>
<th>ΣPUFAd</th>
</tr>
</thead>
<tbody>
<tr>
<td>H52</td>
<td>5.2±0.7a</td>
<td>0.8±0.2b</td>
<td>15.2±1.3a</td>
<td>14.3±0.7a</td>
<td>64.5±4.5a</td>
<td>6±0.9b</td>
<td>15.2±1.3a</td>
<td>78.8±5.2a</td>
</tr>
<tr>
<td>O116</td>
<td>5.3±0.5a</td>
<td>1.0±0.2b</td>
<td>14.3±1.0b</td>
<td>14±1.5a</td>
<td>65.4±1.4b</td>
<td>6.3±2.5b</td>
<td>14.3±1.0b</td>
<td>79.4±2.9b</td>
</tr>
<tr>
<td>P129</td>
<td>5.2±1.0a</td>
<td>1.0±0.5b</td>
<td>16±1.4a</td>
<td>15±0.6b</td>
<td>62.8±2.0c</td>
<td>6.2±1.5b</td>
<td>16±1.4a</td>
<td>77.8±2.6c</td>
</tr>
</tbody>
</table>

a Each value is the mean (standard deviation (SD) of a triplicate analyses performed on different samples. Means with different letters (a-c) in the same column are significantly different at p ≤ 0.05. Fatty acids: C16:0 (palmitic), C18:0 (stearic), C18:1 (oleic), C18:2 (linoleic), C18:3 (linolenic); b Sum of major saturated fatty acids; c Sum of major monounsaturated fatty acids; d Sum of major polyunsaturated fatty acids. Location: Institut National Recherche Agronomie Tunis (INRAT); Year: 2006 to 2007; Period: 7 DAF (maturity).
later than varieties H52 and P129. However, the final TAG concentration (Figure 3) and composition (Tables 2 and 3) of all three varieties are similar.

At maturity, the three varieties, H52, O116 and P129 had TAG concentrations of 87, 88 and 92 mg/100 mg of total lipid, respectively. A number of factors regulate TAG synthesis in seeds, including the amount of fatty acids produced in plastids (Bao and Ohlrogge, 1999) and the activities of the three major acyltransferases for the synthesis of TAGs (glycerol-3-phosphate acyltransferase, lysophosphatidate acyltransferase and diacylglycerol acyltransferase) (Tzen et al., 1993). Moreover, Carman and Kersting (2003) reported that regulation of phospholipid synthesis is interrelated with the metabolism of sphingolipids and TAGs. Furthermore, it is well known that storage phospholipids and TAGs share opposite accumulation patterns during seed maturation (Weber, 1969).

Change in individual TAG contents during flaxseed maturation

During flaxseed development, the major forms of TAG species (LnLnLn, LnLn, LLLn, LnOLn, LnLnP, OLLn and OOLn) represent 90% of the total TAG. The 10 species next in abundance to these (LnLP, LnOP, PLLn, OLO, SOLn, OLP, OOO, OOP, SLnS and SOO) were all present in low abundance, that is, the proportion of each was less than 4% of the total TAG concentration at maturity. Whereas, in corn oil, OLL, OOL, POL, LLL and PLL were the major species while LnLnLn and LnLnP were the minor species molecular (Harrabi et al., 2008). The changes in the proportion of the major species during maturation of P129 variety is shown in Figure 4.

Among the major species, LnLnLn exhibited the greatest increase during kernel development, from 3 to 25 mg/100 mg oil. Also, the amount of LnLnLn increased rapidly from 7 to 35 DAF. The proportions of OOLn and LnLnP increased and remained fairly stable during maturation. These two species appear to have been synthesized during the early stages of flaxseed development. The strong increase in LnOLn and LnLLn in turn reflect the increase in linoleic acid and oleic acid formed during maturation. Thus, it appears that LnLnLn, LnLnL, and LnOLn are the major species synthesized during the later stages of maturation. These results could be explained by the desaturation of linoleic acid to linolenic acid by Δ15-desaturase during seed development (Slabas et al., 2001). Moreover, these results suggest that during ripening of flaxseed, the linoleate desaturase activity increases, hence, permitting the accumulation of linolenic acid. In support of this, Bhatia and Sukhija (1970) reported that the percentage of linolenic acid increases (40 to 48%) during flaxseed maturation. Compared with LnLnLn and LnOLn, the rate of accumulation of LnLnP was less important. This may be because this latter form of TAG contains palmitic acid,
which was converted to stearic acid during fatty acid synthesis (Voelker and Kinney, 2001).

**Conclusion**

Triacylglycerol (TAG) compositions during flaxseed maturation were evaluated for the first time in three varieties of flaxseed (H52, O116 and P129) by LC/APPI-MS. TAG content increased during seed development up to around 35 DAF (variety H52, P129) or around 42 DAF (variety O116). The major TAG forms observed during development and at maturity and confirmed by APPI-MS were LnLnLn, LnLln, LLLn, LnOLn, LnLnP, OLLn and OOLn. In all varieties, LnLnLn exhibited the most significant increase during flaxseed maturation, followed by LnOLn. At maturity, similar TAG levels and TAG profiles were observed for all three varieties. This includes similar levels of the three most abundant TAGs, LnLnLn, LnOLn and LnLln. In addition to providing the molecular distribution of TAGs in flaxseed, the LC-APPI-MS technique also confirmed that linolenic is the major acid component of flaxseed oil. Thus, the technique can provide molecular TAG distributions in addition to estimating relative fatty acid compositions of vegetable oils.

**ACKNOWLEDGEMENT**

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