Jorrit D.J. van den Berg[1]
Nicoletta D. Vermist[1]
Leslie Carlyle[1,2]
Michal Holčapek[3]
Jaap J. Boon[1]

[1]FOM Institute for Atomic and Molecular Physics, Unit for Macromolecular Mass Spectrometry, Kruislaan 407, 1098 SJ, Amsterdam, The Netherlands
[2]Canadian Conservation Institute, 1030 Innes Road, Ottawa, Ontario, K1A 0M5, Canada
[3]University of Pardubice, Department of Analytical Chemistry, Nám. Legií 565, 53210 Pardubice, Czech Republic

Effects of traditional processing methods of linseed oil on the composition of its triacylglycerols

Different oil processing methods were performed, which included washing with water and treatment with lead-based driers, with and without heating to different temperatures, giving a set of 7 oils to be investigated. The effects of the traditional processing methods of linseed oil on its triacylglycerol (TAG) composition were studied, using the following analytical methods: high performance size exclusion chromatography (HPSEC), Fourier transform infrared spectroscopy (FTIR), high-performance liquid chromatography-atmospheric pressure chemical ionisation-mass spectrometry (HPLC-APCI-MS), direct temperature resolved mass spectrometry (DTMS), matrix assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF-MS), and electrospray ionisation Fourier transform ion cyclotron resonance mass spectrometry (ESI-FTICR-MS). A decrease of the initial *cis*-double bonds and the formation of *trans*-double bonds upon heating of the oils was observed. Heating a lead and oil mixture to 150°C, or heating the oil alone to 300°C led to the highest degree of oxidation. A difference was observed for the oxidation patterns for oils with and without the addition of lead. Furthermore, levels of oxygen incorporation were higher when lead was added to the oil. High temperature treatment of the oils resulted in an increased average molecular weight. The changes in the initial conformation of the double bond systems observed with FTIR were supported by HPLC-APCI-MS measurements that showed the formation of a number of new isomeric TAGs in the heated oil compared to freshly pressed, untreated oil. Oligomerisation up to hexamers was observed with HPSEC, and MALDI-TOF-MS. The formation of oligomers up to trimers only, however, was observed with ESI-FTICR-MS. Incorporation of oxygen was mainly observed with MALDI-TOF-MS and ESI-FTICR-MS whereas with DTMS and FTIR hardly any evidence was found for this.

Key Words: Triacylglycerols; Linseed oil; Oxidation; Mass Spectrometry

Received: May 7, 2003; accepted: November 7, 2003

DOI 10.1002/jssc.200301610

Original Paper

1 Introduction

Drying oils are one of the oldest binders used in paints for both decorative and protective purposes. Today they are still in use as raw materials in modern coating systems such as alkyd paint or polyester resins. Traditionally, the oil was pressed from the seeds of plants like flax, walnut, or poppy. After removal of all unwanted material like foreign seeds or chaff by processes such as blowing with air or sieving, the seeds were ground to a fine meal and pressed to extract the oil. The oils obtained are mixtures of triesters of glycerol (also called triacylglycerols or TAGs) with a high content of double and triple unsaturated fatty acids (see **Table 1**). It is important to note that the number of different TAGs is much larger than the number of fatty acids. Theoretically, for the 5 main fatty acids 75 different TAGs can be obtained when positions 1 and 3 of the glycerol backbone are considered to be unequal. Aside from TAGs, the oil can contain other substances, some of which can have a marked effect on the (drying) properties. Free fatty acids are always present in a relative proportion of about 0.5 to 2%. Furthermore, about 0.1–0.2% water can be dissolved in the oils, without any effect on their clarity. Other substances present are phosphatides, of which lecithin is the best known, carotenoid pigments and sterols [1].

In principle, freshly pressed oil can be used for paint formulation although normally the water-soluble component or mucilage (phosphatides and other non-TAG matter), which is present after expression, is removed. Additional

Correspondence: Jorrit D.J. van den Berg, Netherlands Forensic Institute, Volmerlaan 17, 2288 GC Rijswijk, The Netherlands. Phone: +31 70 4135270. Fax: +31 70 4135458. E-mail: j.van.den.berg@nfi.minjus.nl.

Abbreviations: APCI, atmospheric pressure chemical ionisation; DAG, diacylglycerol; DCM, dichloromethane; DHB, 2,5-dihydroxybenzoic acid; DTMS, direct temperature resolved mass spectrometry; ECN, equivalent carbon number; ELSD, evaporative light scattering detector; FTICR, Fourier transform ion cyclotron resonance; MAG, monoacylglycerol; PDA, photodiode array; TAG, triacylglycerol.

Table 1. Fatty acid composition of freshly prepared linseed oil[a] [77].

Fatty acid[b), c)]	Freshly pressed oil (F) [d)]	% of total FA			
		Europe	Canada	Argentina	India
16:0 (palmitic acid)	5.9	4–6	5–6	4–5	9–10
18:3 (linolenic acid)	57.6	56–71	54–61	45–53	50–61
18:2 (linoleic acid)	16.4	12–18	14–16	15–24	13–15
18:1 (oleic acid)	17	10–22	19–20	19–21	10–21
18:0 (stearic acid)	3.1	2–3	3–4	5–6	7–8

a) The relative amounts largely depend on climate and the variety of *Linum usitatissimum* of the seeds [78].
b) 18:1 indicates the carbon chain length: number of double bonds.
c) Trace amounts of 16:1, 20:0, 20:1, and C22 fatty acids have also been reported.
d) Provided by Dr. Nimal Ratnayake, Nutrition Research Division, Health Canada Banting Research Centre, Ottawa, Canada.

processing is undertaken to give the paint specific properties desired by the painter. Traditionally, processing included the removal of mucilage by washing or settling, and various procedures to enhance drying, such as heating and/or treatment with driers (metallic compounds). Oils were also treated by sun or chemical bleaching to reduce their initial colour [2, 3].

Some of these processes lead to changes in the composition of the original highly unsaturated triacylglycerols (TAGs). Hydrolysis can occur giving rise to di- and monoacylglycerols (DAGs and MAGs, respectively) [4, 5]. Incorporation of oxygen into the unsaturated fatty acid strands leads to oxidised TAGs [6–8] and low molecular weight (volatile) breakdown products [9, 10]. The presence of driers during processing increases the rate of formation of active radicals due to the catalysed breakdown of hydroperoxides [11, 12]. As a result of the breakdown of the various oxidised products various radicals are formed that can recombine to form cross-linked material with an increased molecular weight. This cross-linked material will consist of the different oxidised and hydrolysed species [13–17].

As a result of heating during oil processing *trans* isomerisation of linoleic and linolenic fatty acids will occur leading to an increase in the number of isomeric TAGs and their oxidation products [18]. At the same time, heat-induced Diels-Alder addition reactions of fatty acids will result in intermolecular cross-linked TAGs, intramolecular coupled species [19–23] and cyclic FAs [24–28]. The latter two types of compounds will not lead to an increase of the average molecular weight of the oil since no cross-linking between TAGs is occurring.

1.1 Analytical techniques

In order to study the alterations of the oil due to processing a broad range of analytical techniques can be used, each with its own advantages and disadvantages. To elucidate the qualitative composition of a TAG mixture based on chromatographic retention times, it is necessary to synthesise a large variety of pure oxidised TAG standards. However, this still does not result in a definitive structure for the unknown compounds investigated [29]. These mixtures of oxygenated TAGs are difficult to analyse because of the presence of a large variety of homologs and stereoisomers, which overlap with each other and with analogues of unoxidised TAGs when chromatographic methods are used [30–32]. In theory it should be possible to structurally identify these overlapping species by a combination of chromatographic techniques and mass spectrometry or nuclear magnetic resonance. The established method for the separation of triacylglycerols (TAGs) is non-aqueous reversed-phase high-performance liquid chromatography (HPLC) with solvents like methanol-2-propanol-hexane [33], dichloromethane-acetonitrile [32, 34], dichloromethane-acetonitrile-propionitrile [35, 36], pure propionitrile [37], acetone or chloroform with acetonitrile [38], or methanol-2-propanol [39]. The retention of particular acylglycerol species increases with increasing equivalent carbon number (ECN) defined as ECN = CN-2 $\times$ DB, where CN is the carbon number in all acyl chains and DB is the number of double bonds. Under optimised chromatographic conditions, most of the TAGs with the same ECN value can also be separated according to the number of double bonds, in addition to the separation according to ECN. Four HPLC detection techniques are most frequently used for TAG detection:

a) UV detection at 200–205 nm
b) Evaporative light-scattering detection (ELSD)
c) Positive-ion atmospheric pressure chemical ionization (APCI)
d) Positive-ion electrospray ionization (ESI) mass spectrometric detection.

Mass spectrometric techniques provide structural information and good sensitivity. ESI mass spectra show only $[M + Na]^+$ or $[M + NH_4]^+$ ions [40] without the fragmentation

or adducts with other added cation like $[M + Li]^+$ [31]. Information on acyl chains can be obtained by tandem mass spectrometry (MS/MS) analysis [40] which opens up the possibility of determining double bond position(s), e.g. in case of MS/MS of $[M + Li]^+$ ions [31]. APCI yields both molecular weight and individual acyl moiety information, but unfortunately the ionisation efficiencies of individual TAGs and DAGs are dependent on the number of double bonds [33] (similarly to ESI [40]), which causes problems with direct determination of relative amounts of the acylglycerol species.

There are a number of other mass spectrometric techniques that could be applied to the analysis of (oxidised) TAGs as well. In order to be satisfactory, an ionisation method should be chosen that produces MS information on both the molecular weight as well as characteristic fragment ions to facilitate the identification of both the location and the nature of functional groups formed upon oxidation. Ionisation techniques such as (low voltage) electron ionisation [41] and (atmospheric pressure) chemical ionisation [31, 36, 42] are therefore thought to be more suitable than electrospray (ESI) [29, 43–45] unless MS/MS is performed. More recently, the coupling of matrix assisted laser desorption/ionisation (MALDI) or ESI with Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) has proven suitable for the analysis of oxygenated TAGs without prior chromatographic separation [46, 47]. However, this technique involves structural identification by MS/MS, which is time-consuming.

All of the aforementioned analytical techniques require that the analyte has to be brought into the gas phase, preferentially intact. This is more difficult to achieve for the higher molecular weight material that is formed upon cross-linking, especially since the polarity will also be high due to the presence of oxygen containing functional groups. Besides, the nature of the oxidation process in oils is such that a broad envelope of molecular masses will be formed with a lot of isomeric structures. Therefore, in order to study the properties of both the high- and low molecular weight material formed, the following techniques provide additional information: size exclusion chromatography (SEC) [28, 48, 49], Fourier transform infrared spectroscopy (FTIR) [50–52], and nuclear magnetic resonance (NMR) [53–57]. SEC gives an approximation of the molecular weight distribution of the sample. The ratio of the relative amounts of free fatty acids, triacylglycerols, oligomers, and higher molecular weight material, which is a result of processes such as oxidation and/or heat induced cross-linking and hydrolysis and/or degradation, can be determined. FTIR provides information on the presence of free and esterified fatty acids, specific functional groups, and the double bond conformation. NMR techniques provide information especially on the presence of specific functional groups. The advantage of FTIR and solid state NMR techniques is that the intact sample can be analysed and does not need to be dissolved. Most of the techniques previously described have been used to analyse the set of seven oils, except NMR.

1.2 Experimental design

Artists' oil recipes were recreated using historical recipes. Hand-ground oil paint was produced with authentic materials as described in historical sources [2, 3, 58] in order to investigate the effects of traditional oil processing on the linseed oil triacylglycerols and the physical properties of the paint. The processes that were monitored include water-washing, treatment of the oil with heat alone, and treatment with and without heat in the presence of lead(II) monoxide as drier. Chemical changes induced by these processing methods were evaluated relative to the freshly pressed linseed oil. The oils obtained were analysed by SEC, FTIR, MALDI-time-of-flight mass spectrometry (MALDI-TOF-MS), direct temperature resolved mass spectrometry (DTMS), and ESI-Fourier transform ion cyclotron resonance mass spectrometry (ESI-FTICRMS). The freshly pressed oil and a heated oil were also investigated using HPLC coupled to an atmospheric pressure chemical ionisation (APCI)-MS system.

2 Materials and methods

2.1 Chemicals and reagents

Ammonium acetate (NH_4Ac) was purchased from Jansen Chimica, Geel, Belgium. Ethanol (EtOH), methanol (MeOH) (both p.a.), acetonitrile, and dichloromethane (DCM) (HPLC grade) were obtained from Merck, Darmstadt, Germany. Lead(II) oxide (99.9%), tristearoyl glycerol (approximately 99%), and 2,5-dihydroxybenzoic acid (DHB) are products of Sigma-Aldrich Chemie, Zwijndrecht, The Netherlands. Hexane (99.5%) was bought from Fluka Chemika, Buchs, Switzerland. Tetrahydrofuran (THF) was purchased from Biosolve Ltd., Valkenswaard, The Netherlands. Linseeds were supplied by MACOS BV, Swifterbant, The Netherlands. Umber (Umbra gebrannt Cyprisch bräunlich), lead white (Cremnitz lead white) and vine black (Rebschwarz reines Pflanzen) pigment were purchased from Kremer-Pigmente, Aichstetten, Germany. A second type of lead white pigment ($2PbCO_3 \cdot Pb(OH)_2$) was made according the traditional Dutch process by a private manufacturer, currently out of business.

2.1.1 Oil pressing

The oils were prepared by L. Carlyle. Flaxseed (Linseed) was first cleaned by blowing plant matter away with compressed air and shaking the seed through a metal sieve. The cleaned seeds were subsequently ground in an electric coffee grinder. The ground meal was then placed in a

custom-built stainless steel oil press[1]. Oil was expressed under a pressure of 200 to 350 kg/cm^2. The oil yield from the ground meal was approximately 22 to 23% [58].

2.1.2 Oil processing methods

Various historical recipes for oil processing were followed. Full details, including the recipes followed are included in a report by Carlyle [58]. Samples of the oils were stored in closed 3 mL vials, which had been flushed with nitrogen, in the dark at room temperature (18–20°C).

2.1.2.1 Linseed oil (F)

Freshly pressed oil was collected in a glass pipette and then transferred to clear glass bottles, which were kept stoppered until use. Oil was pressed regularly throughout the 14 weeks of the project, so that the fresh oil used for processing and paint making was never more than a maximum of a few weeks old.

2.1.2.2 Water-washed oil (W)

Following a recipe from the Handbook of Young Artists and Amateurs in Oil Painting [59], oil was mixed with two times its volume of water, and placed in a glass separation funnel. The oil and water were shaken together three times a day, five days a week, over a period of three weeks. The water was replaced with fresh water twice a week. At the end of three weeks, the water (plus water-soluble material which had separated from the oil) was poured off, and the oil filtered three times through cotton filter cloths.

2.1.2.3 Oil treated with drier at room temperature (D)

Lead(II) oxide was added to oil in a clear glass jar in the same ratio used for the lead-treated heated oil below (2:1 oil to drier *w/v*). Oil and drier were shaken together by hand, 3 times a day for one week. After two days of resting, the oil was decanted to a new container.

2.1.2.4 Heated oils (F150, F300, and D150)

Oil was heated in a round-bottom flask to an end temperature of 150°C and 300°C using a heating mantle. Maximum temperatures were reached after 15 min (150°C) and 57 min (300°C). A third oil sample was prepared by heating to 150°C in the same apparatus with the addition of the traditional drier, lead(II) oxide (litharge), in a ratio of 1 part drier to 2 parts oil, *w/v*. With drier added, the end temperature was reached in 18 min. During preparation of the oil treated with drier at elevated temperature (D150), a precipitate of drier and oil was formed in the container. This precipitate (D150 Precipitate) was separated from the oil for further analysis.

Observations on the paint rheology and colour were recorded during hand grinding of paint prepared with the oils, and during application to various substrates [58]. Paints were prepared with four pigments: two different lead whites, raw umber, and vegetable black. Paint samples are being monitored with a Minolta Spectrophotometer CM2002 for colour changes during alternating periods of dark storage (yellowing) and light exposure (bleaching). The formation of film-forming defects is being recorded with macro-photography. A selection of the paints has been subjected to both natural and artificial ageing and has been analysed for chemical changes. These findings, however, will not be discussed in this article.

2.2 Analytical methods

2.2.1 HPSEC

The formation of dimers and higher oligomers in each oil was monitored using HPSEC. Oil samples were dissolved in THF (10 mg/μL), centrifuged, and 10 μL was analysed on a Shimadzu HPSEC system, consisting of a SCL-10AD vp control panel, a LC-10AD vp pump, a DGU-14A degasser, a SIL-10AD vp autoinjector, a CTO-10AS column oven and a FRC-10A fraction collector (Shimadzu Benelux, 's-Hertogenbosch, The Netherlands). Separation was achieved on a PLGEL 5 μm 1000 Å column (300 × 7.5 mm) of Polymer Laboratories, Heerlen, The Netherlands. Three different detectors, connected in series, were used for detection: a SPD-10A vp UV/VIS detector operated at 240 nm, a RID-10A refractive index detector (both Shimadzu) and a Waters 996 photo diode array (PDA) detector (Waters Chromatography B.V., Etten Leur, The Netherlands), in combination with Class vp 5.03 (Shimadzu) and Millenium 32 (Waters) software, respectively. The system was operated at a temperature of 40°C with a flow rate of 1 mL/min. Calibration was performed with polystyrene standards (Polymer Laboratories, Heerlen, The Netherlands) with an average mass ranging from 580 to 370,000 in combination with tristearoyl glycerol (*m/z* 890).

2.2.2 FTIR

Initial chemical changes in the freshly prepared oils were investigated using a FTS-6000 Bio-Rad FTIR imaging system (Bio-Rad, Cambridge, MA, USA), consisting of a Michelson interferometer (Bio-Rad FTS-6000), an IR microscope (Bio-Rad UMA-500) and a MCT narrow band detector. A small droplet of oil was applied onto a Graseby Specac PN 2550 diamond cell (Graceby Specac, Orpington, Kent, UK) and analysed in transmission mode at a resolution of 4 cm^{-1}. Data were processed using Win-IR Pro 2.5 software of Bio-Rad.

[1] The oil press was custom designed by Hayo de Boer, The Netherlands Institute for Cultural Heritage, Amsterdam, who kindly lent it to this project. Further details on the oil press are included in a report by Carlyle [58].

2.2.3 HPLC/APCI-MS

Oil samples F and F300 were dissolved in acetonitrile – 2-propanol – hexane (2:2:1) yielding 3% solutions. HPLC separation subsequently was performed using a Waters 616 liquid chromatograph equipped with a Model 996 photodiode-array (PDA) detector and a Model 717 autosampler (all from Waters, Milford, MA, USA). The separation conditions were as follows: column NovaPack C18 (150 × 4.6 mm ID, Waters), flow rate of 1 mL/min, injection volume of 15 μL for fresh oil (F) and of 30 μL for heated oil (F300), UV detection at 205 nm, gradient of the mobile phase: 0 min – 90% acetonitrile/ethanol, 90 min – 18% acetonitrile/ethanol (i.e. 0.8%/min). The HPLC system was coupled to a VG Platform quadrupole mass analyser (Micromass, UK) using positive-ion atmospheric pressure chemical ionization (APCI). Mass spectra were obtained in the mass range $m/z = 35-1200$, with an ion source temperature of 100°C, APCI heater temperature 400°C and cone voltage of 20 V.

2.2.4 DTMS

For the analysis of the freshly processed material 0.5 μL oil was dissolved in 3 mL hexane. Aliquots of about 3 μL were applied to the analytical filament and dried in vacuo. The analyses were performed on a Jeol SX-102 double focussing mass spectrometer (B/E) (Jeol-Europe, Schiphol-Rijk, The Netherlands) using a direct insertion probe equipped with a Pt/Rh (9/1) filament (100 μm diameter). The probe filament was temperature programmed at a rate of 0.5 A/min to an end temperature of about 800°C. Compounds were ionised at 16 eV under electron ionisation conditions in an ionisation chamber kept at 180°C, mass analysed over the range m/z 20–1000, with a 3-s cycle time. Data was processed using a JEOL MP-7000 data system. A mass defect of 0 mu at 20 amu to 0.5 mu at 1000 amu was subtracted from decimal masses to give nominal masses.

2.2.5 MALDI-TOF-MS

All MALDI-TOF-MS spectra were obtained using a Bruker Biflex MALDI-TOF mass spectrometer (Bruker-Franzen Analytik GMBH, Bremen, Germany). The instrument used a nitrogen laser at 337 nm (OEM VSL-337i, Laser Science, Inc., Newton, MA, USA) attenuated to about 75% of its maximum power (250 μJ), with a 3 ns pulse width for desorption/ionisation. Positive MALDI spectra were obtained by averaging 50 individual spectra, recorded using delayed extraction, the reflector mode and an accelerating voltage of 19.6 kV, unless otherwise stated. Mellitin, isatin, and polyethylene glycol mixtures with an average molecular weight of 400 and 1000, respectively, were used for internal and external calibration. A saturated solution of 2,5-dihydroxybenzoic acid was used as matrix. An amount of 3–4 μL of this solution was mixed with 0.2–0.5 μL of a 100-fold diluted oil sample with DCM. Data were processed using XMASS 5.0 software (Bruker Daltonik GmbH, Bremen, Germany).

2.2.6 ESI-FTICR-MS

A modified APEX 7.0e FTICR-MS instrument (Bruker-Spectrospin, Fällanden, Switzerland) equipped with a 7 Tesla superconducting magnet and an in-house designed external ion source was used for ESI-FTICR-MS measurements of the oil samples. Details of instrumental parameters have been described before [60,61]. The ICR cell used was a home-built open cell. The fresh oils were dissolved in DCM:EtOH (7:3, *v/v*) (1/100 μL) containing 10 mM NH_4Ac. Ions were generated using an atmospheric pressure electrospray ionisation interface, maintained at 3–4 kV, and were subsequently trapped in the ICR cell at $\approx 5 \times 10^{-9}$ mbar. Obtained data were processed using XMASS 5.0 software (Bruker Daltonik GmbH, Bremen, Germany).

3 Results

3.1 Observations during preparation of the oils and paints

3.1.1 During oil processing

After the lead-treated oil was heated to 150°C (D150) it was decanted to a fresh glass jar. Approximately 15 days after preparation the oil appeared cloudy with a whitish-grey precipitate forming at the bottom of the jar. After a month there was a large white cloud in the oil, which, when shaken, became distributed throughout causing the oil to be uniformly cloudy. Prior to shaking, a sample of the whitish-grey precipitate was taken for analysis (D150 Precipitate).

3.1.2 During paint preparation

In some cases the oil processing method had a strong effect on the rheology of the paint produced during hand-grinding. For a full discussion of all the methods used and their effect on the paint see ref. [58], the results below are limited to the set of oils being reported on in this article. Two common terms to describe paint handling characteristics (rheology) are used to describe the general characteristics of the paint: short and long. Short paint has a buttery or smooth consistency, and long paint is more fluid[2)].

[2)] R. Gamblin, 2000, personal communication: The terms short and long (or length) are used to describe paint according to its behaviour when a knife is placed on the surface of a small pile of paint and drawn straight upwards. Paint sticking to the knife will be drawn out in "legs", if the legs break soon after raising the knife, the paint is short (having short legs), but if the paint can be drawn out in fine long strands before it breaks from the knife, then it has long legs. These characteristics are independent of viscosity and of other characteristics associated with its flow properties such as density and tack.

Table 2. Oil processing and paint rheology.

Oil processing method[a)]	Percent oil by weight to produce 25 g. Dutch Process lead white	Observations: Handling quality while brushing[b)]
F	13.8%	Short
F150	20.6%	Short and sticky and dense
F300	13.8%	Long, sticky, stringy, not a viable paint
D	17.2%	Short and stiff
D150	13.8%	Long and sticky
W	19.3%	Short and very creamy (pleasant to work)

a) Oil code summary.
F = Freshly pressed oil (untreated) (Z in [58]).
F150 = Freshly pressed oil heated to 150°C. (ZH150 in [58]).
F300 = Freshly pressed oil heated to 300°C. (ZH300 in [58]).
D = Oil treated with drier at room temperature (A-2 in [58]).
D150 = Oil treated with drier while heating to 150°C. (AH2 in [58]).
W = Freshly pressed oil washed in water for 3 weeks. (X in [58]).
For a detailed explanation of codes see experimental section.

b) Definitions of terms are given in text

These terms were modified with additional adjectives, such as stringy, sticky, stiff, or dense (see **Table 2**).

The greatest effect on paint rheology was observed with lead white ground in oil heated to 300°C (F300, Table 2). The resulting paint was long, stringy, sticky, dense, and extremely difficult to manage during application. It also had very poor covering power [62]. When the oil was only heated to 150°C (F150), the paint was short, dense, and sticky, but much more manageable. Adding drier (lead(II) oxide) and heating to 150°C (D150) made the paint more fluid and manageable than when it was heated without drier, but it was still somewhat sticky. Lead white ground with untreated freshly pressed oil (F) was more buttery than paint made with water-washed oil (W), but the water-washed oil was the nicest of all to handle, it was creamy and pleasant to work, although slightly more viscous than lead white paint prepared with untreated oil.

With the lead white paints, the quantity of oil used did not correlate directly with paint rheology (see Table 2). For example the most fluid, that is "stringy" paint in the set, was that heated to 300°C (F300) yet the percent oil by weight was only 13.8% compared to the least fluid paints which took 17.2% (D) and 20.6% (F150). Paints made with umber and vegetable black pigments did not exhibit the range of difference between long and short experienced with the lead white. Although there were some differences according to the oil processing method, these paints generally tended to be short, none developed the fluidity and length seen in some of the lead whites. During grinding in the oil heated to 300°C (F300) and the lead-treated oil heated to 150°C (D150), the lead white pigment gave the impression that it was "dissolving" into the oil. This did not occur while grinding the umber and vegetable black.

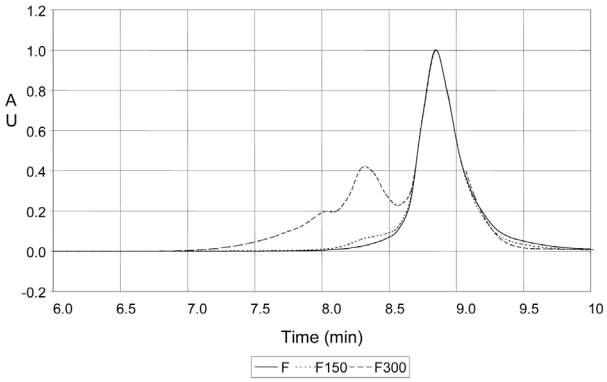


Figure 1. HPSEC chromatograms of oils F, F150, and F300.

3.2 HPSEC

HPSEC analysis was applied to obtain information on the formation of cross-linked materials in the processed oils and to monitor the degree of de-esterification and oxidation. The molecular weight distributions of the oils F, F150, and F300 are shown in **Figure 1**. The peak eluting at t_R = 8.7 min can be ascribed to intact (unsaturated) TAGs based on standards establishing the elution times of a saturated tristearoyl glycerol and a free stearic acid which were determined to be 8.7 and 9.5 min, respectively. The material detected at longer retention times suggests the formation of low molecular weight breakdown products resulting from oxidation or hydrolysis of the ester bonds. The latter leads to the evolution of free fatty acids, unless the fatty acid had already formed a cross-link prior to hydrolysis. Breakdown products can consist of diacylglycerols (DAGs), dimeric fatty acids, monoacylglycerols (MAGs) or free fatty acids. Another possibility is that material is retained on the column upon elution, which leads to

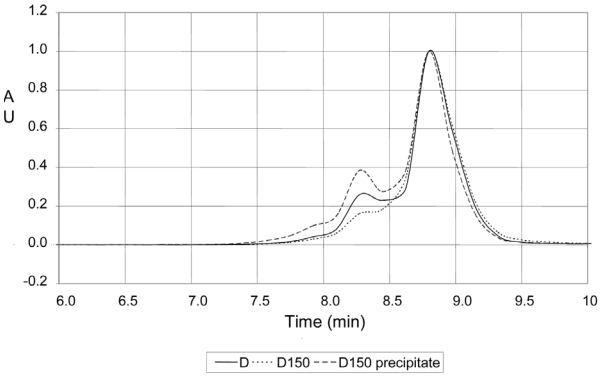


Figure 2. HPSEC chromatograms of oils D, D150, and the THF soluble part of D150 Precipitate.

a tail in the chromatogram. Peaks with a decreased retention time compared to the elution time of TAGs are ascribed to oligomerised TAGs or combinations of breakdown products with TAGs.

It is clear from a comparison of the molecular distributions that heating led largely to formation of higher molecular weight cross-linked TAGs (see Figure 1). The effect of heating to 150°C is less pronounced than heating to 300°C. Peaks eluting at 8.3 and 8.02 min are ascribed to dimeric and trimeric TAGs, respectively. This material can be formed by heat-induced Diels-Alder reactions [20, 22] or cross-linking upon autoxidation. An approximate molecular weight scale determined from calibration with the standard series (not shown) suggests that oligomers up to 6-mers (molecular weight ± 5400) are present in the oil heated to 300°C. Relatively low amounts of low molecular weight products were detected in both of the heated oils (F150 and F300).

Water washing of the oil did not lead to great alterations as a chromatogram similar to the fresh oil was obtained (results not depicted). The addition of litharge as drier, however, led to increased amounts of dimeric material as can be seen in **Figure 2** for oil D. This is probably due to lead-catalysed oxidation leading to higher amounts of free radicals, and subsequent cross-linking. The same phenomenon was observed for the heated mixture of drier and oil (D150).

The results of HPSEC analysis of the THF soluble fraction of the precipitate, depicted in Figure 2, indicate that the whitish-grey precipitate (D150 Precipitate) is enriched in high molecular weight material relative to the rest of the sample D150 and to the oil mixed with the drier alone (D). It is likely that it precipitated as the cross-linked material formed because its increased lipophobicity led to reduced solubility in the oil. The tendency of the cross-linked material to accumulate at the bottom of the jar means that oil in the uppermost part of the container will contain less cross-linked material than anticipated given the treatment carried out with heat and the drier.

Table 3. Absorption maximums of mono and dimeric TAG compounds (retention times 8.7 and 8.15 min, respectively) in processed oils[a] as determined with photodiode array detection.

Oil sample	Maximum (nm) (t_R = 8.7)	Maximum (nm) (t_R = 8.15)
F	219.5	–
F150	220.7	–
F300	234.8	234.8
D	234.8	237.1
D150	221.8	234.8
D150 Precipitate	237.1	237.1
W	233.6	–

[a] For an explanation of codes used see experimental section.

Inspection of the photodiode array detector data (**Table 3**) shows that there is a correlation between the extent of cross-linking and an increase in the maximum absorptivity wavelength. This was observed as early as 1926 by Stutz [63] who studied the light absorption of different commercial linseed oils. There is an increase by a few nm in the maximum wavelength observed in the monomeric material for the untreated oil F, and the heat-treated oils (F150 and F300) (Table 3), which indicates the formation of an absorbing conjugated double bond system. The value obtained for the oil heat-treated with drier (D150) is lower than the value for the oil treated with drier but without heat (D), and the highest value of all samples is found for the precipitate (D150 Precipitate), which confirms that the number of chromophores is highest in the higher molecular weight material. This is also reflected by the fact that the maximum wavelength is highest for the dimeric material in most oils. Interestingly, according to the data in Table 3, the maximum wavelength for the water washed oil has increased despite its unchanged molecular weight. The higher value for the maximum wavelength appears to indicate that water washing has some effect on the double bond system of the TAGs. *Cis-trans* isomerisation of the unsaturated fatty acids upon (phot)oxidation during the relatively long washing procedure may account for this since the oil was placed on the window-sill in a clear glass container.

3.3 FTIR

Analysis of the processed oils by FTIR provides information about the degree of oxidation and hydrolysis and changes in double bond conformation. A number of characteristic spectral features can be seen in the FTIR spectra of the oils F and F300 (see **Figure 3**). There are 4 distinctive peaks in the 3000 cm^{-1} region that can be attribu-

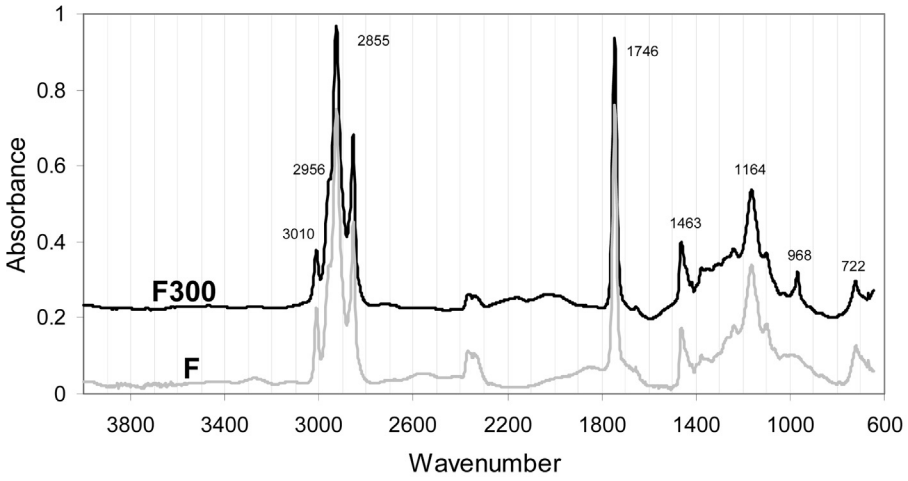


Figure 3. FTIR spectra of oils F and F300.

Table 4. Tentative absorption band assignments for the peaks observed in the processed oils.

Band (cm^{-1})	Assignment
3010	C–H stretching of aliphatic –CHCH=CH–
2956	C–H stretching of CH_3
2927	C–H stretching of CH_2
2855	C–H stretching of CH_2
1746	C=O stretching of ester
1652	C=C stretching of *cis* –CH=CH–
1463	C–H bending of CH_2, CH_3
1239	C–O stretching in ester
1164	C–O stretching
1101	C–O stretching
968	*trans* C–H out-of-plane deformation
722	*cis* C–H out-of-plane deformation

ted to C-H stretching vibrations, which appear at 3010, 2956, 2927, and 2855 cm^{-1}, respectively. An intense carbonyl band of the ester linkages of TAGs can be seen at 1746 cm^{-1} whereas no distinct signal indicative for the formation of free fatty acids is observed at 1700 cm^{-1}. Several bands from both the fatty acid chain and the carboxylic acid functional groups are seen in the region 1600–600 cm^{-1}. The assignments of the different bands are listed in **Table 4**. The spectra for both oils look very similar except for the absorption bands that are assigned to the presence of *cis* and *trans* unsaturations. A distinct band at 968 cm^{-1} emerges in the spectrum of the heat-treated oil F300, on top of a broader distribution ranging from 920–1080 cm^{-1} while the bands at 3010 and 722 cm^{-1} have decreased. This implies that non-conjugated *cis* olefinic bonds have been mostly transformed to non-conjugated *trans* double bonds, and in minor quantities to conjugated *trans* double bonds (a closer inspection of the FTIR spectra revealed new bands of low intensity at 988 and 947 cm^{-1}). The ratios of the areas of the bands at 3010 and (2927 + 2855) cm^{-1} have been determined for

Table 5. Ratios of the FTIR absorption bands area of *cis* CH=CH (3010 cm^{-1}) and CH_2 (2927+2855 cm^{-1}) in FTIR data of processed oils[a].

Oil sample	CH_2/*cis* CH=CH
F	18.90
F150	20.14
F300	30.31
D	19.69
D150	20.69
W	19.06

[a] For an explanation of codes used see experimental section.

the different oils and are depicted in **Table 5**. It is thought that during oxidation and/or cross-linking the number of CH_2 groups initially stays intact whereas the relative amount of *cis* double bonds in the oil will decrease. It should be noted at this point that upon advanced oxidation a broadening and a lowering of the bands at 2927 and 2855 cm^{-1} is expected [64]. It can be seen in Table 5 that the relative amounts of *cis* C=C bonds decreases upon heating (compare F vs. F150 and F300). There is very little difference between the unheated lead-treated oil D relative to the untreated oil F, indicating that fewer double bonds are involved in the initial cross-linking process when the sample is not heated. Upon heating in the presence of a drier (D150), the amount of consumed double bonds is slightly increased, in accordance with the observation for oil F150. The findings for D and F suggest that formation of oligomeric material is mainly due to radical-radical recombinations in the autoxidation process, in contrast to the more polymeric F300, which showed a much larger relative decrease in *cis* double bonds. This suggests that Diels-Alder condensation takes place when the oil is heated as high as 300°C. The water-washed oil only shows a slight increase in the ratio of the bands at 3010 and (2927, 2855) cm^{-1} compared to the untreated oil F,

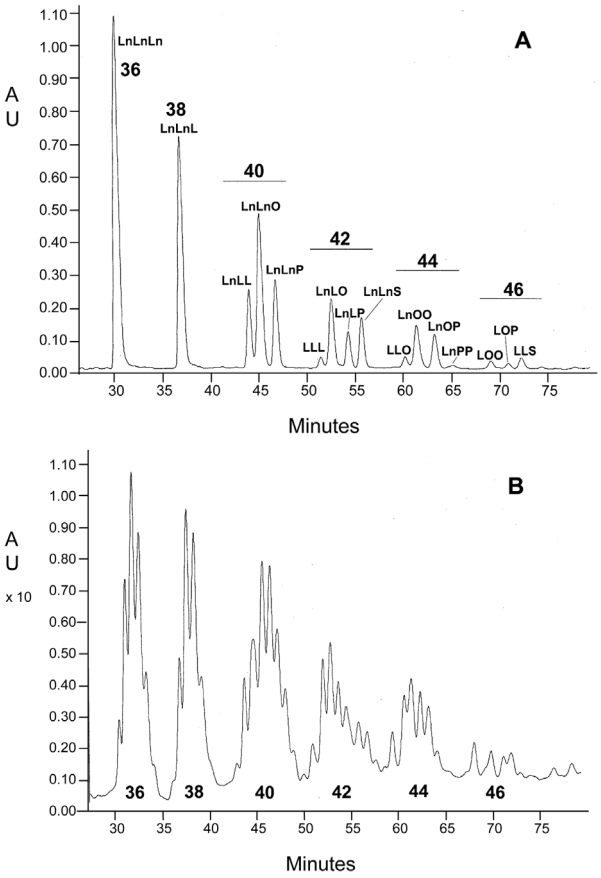


Figure 4. HPLC chromatograms obtained with UV detection at 205 nm of (a) fresh linseed oil (F), and (b) heated linseed oil (F300). HPLC conditions as in Experimental. Numbers correspond to equivalent carbon number (ECN).

which is in contrast to the large change in its light absorption observed with PDA detection (HPSEC).

3.4 HPLC APCI-MS

Changes in the processed oils due to oxidation and heat-induced isomerisation of double bonds can be investigated by HPLC-APCI-MS. Only the untreated freshly pressed oil F and the heated F300 were analysed in this study. The comparison of the chromatographic records with UV detection at 205 nm of fresh linseed oil (**Figure 4.a**) and of heated linseed oil (**Figure 4.b**) is shown. Only the part of chromatogram with TAGs is selected, because the greatest differences between these samples can be observed in this region. In the case of fresh oil, the identification of particular peaks is easy and unambiguous on the basis of their positive-ion APCI mass spectra [33]. The molecular weights can be determined from protonated molecules $[M+H]^+$ and adducts with ammonium $[M+NH_4]^+$ or sodium ions $[M+Na]^+$. The characteristic fragment ions $[M+H-RCOOH]^+$ and $[RCO]^+$ enable the identification of all acyl chains, as indicated in Figure 4.a. The

Table 6. Equivalent carbon numbers (ECNs) and *m/z* values of characteristic ions of identified triacylglycerols (TAGs) by HPLC/APCI-MS (for better clarity, the decimal places are not shown in the table. They are approximately XXX.7 for $[M+H]^+$, XXX.5 for $[M+H-RCOOH]^+$, and XXX.3 for $[RCO]^+$).

TAGs [a]	ECNs	$[M+H]^+$	$[M+H-RCOOH]^+$	$[RCO]^+$
LnLnLn	36	873	595	335
LnLnL	38	875	595, 597	335, 337
LnLL	40	877	595, 597	335, 337
LnLnO	40	877	595, 601	335, 339
LnLnP	40	851	573, 595	313, 335
LLL	42	879	599	337
LnLO	42	879	597, 599, 601	335, 337, 339
LnLP	42	853	573, 575, 597	313, 335, 337
LnLnS	42	879	595, 601	335, 341
LLO	44	881	599, 601	337, 339
LnOO	44	881	599, 603	335, 339
LnOP	44	855	573, 577, 599	313, 335, 339
LnPP	44	829	551, 573	313, 335
LOO	46	883	601, 603	337, 339
LOP	46	857	575, 577, 601	313, 337, 339
LLS	46	883	599, 603	337, 341

[a] TAG fatty acids: P = palmitic, S = stearic, O = oleic, L = linoleic and Ln = linolenic acid.

m/z values of observed ions are listed in **Table 6**. The identification of individual peaks in heated linseed oil is much more difficult due to the co-elution of chromatographic peaks. Another problem is the significantly decreased relative response despite doubled injection volume (30 μL), because the total signal is divided among many peaks and the resulting response of individual peaks is therefore reduced. A decrease of UV-absorbing groups at 205 nm is another possibility, which explains diminished signal intensity. The highest UV response is 1.1 AU for unheated oil in comparison to 0.11 AU for heated oil (see Figure 4.a/b).

APCI mass spectra are identical for "single" peaks in the chromatogram of fresh oil (Figure 4.a) and for corresponding "multiplet" peaks with the same ECN in the chromatogram of heated oil (Figure 4.b), which confirms that the molecular weights and also masses of acyl chains are identical. If the total ion current is compared with the reconstructed ion currents of characteristic TAG ions (see Table 6) for LnLnLn in the time window 28–35 min for heated oil, the same time profiles are obtained and no additional ions are found in heated oil. Therefore new TAGs in heated oil can differ due to the *cis*/*trans* isomerisation, the migration of double bonds, or the formation of cyclic fatty acids. These possibilities can be expected, as indicated by results obtained with FTIR and HPSEC.

The UV spectra in the range 195–500 nm were obtained for all chromatographic peaks using photo-diode array detection. The UV spectra of all peaks in the chromato-

gram of fresh oil (Figure 4.a) are identical with only one absorption maximum at 203 nm, as can be expected for TAGs. If the UV spectra of the group of peaks with the same ECN (Figure 4.b) is averaged, then a second minor band is observed in the region 225–255 nm for all groups of peaks. Attention was focused on five explicit peaks with ECN = 36 (notation: No. 36a – the first peak with t_R = 30.2 min, No. 36e – the last peak with t_R = 33.0 min). If the UV spectra are obtained only from the narrow time window at the top of peaks, then the maxima of the secondary UV bands can be determined: No. 36a – 254 nm, No. 36b – 252 nm, No. 36c – no secondary maximum, No. 36d – 233 nm, and No. 36e – 231 nm. This clearly confirms the presence of compounds with conjugated double bonds in peaks referred as 36a, 36b, 36d and 36e, while the peak 36c probably corresponds to LnLnLn. A similar behaviour can be observed for the peak with ECN = 38, where the following UV maximums were determined (peak notation as above): No. 38a – 247 nm, Nos. 38b and 38c – no significant maximum, No. 38d – 232 nm. This approach cannot be applied to other peaks, because more than one compound corresponds to other ECN values. This result clearly confirms that double bond migration occurs to some extent upon heating. Whether *cis*/*trans* isomerisation occurs cannot be deduced from the presented HPLC/MS data.

Wide and overlapping peaks with low abundances are observed in the time window 10–26 min (not shown) with characteristic ions differing by 16 *m/z* units (e.g. 871.7 and 887.7, 873.7 and 889.7, 875.7 and 891.7), which suggests the presence of oxidised TAGs. Each TAG can in principle lead to a large number of oxidised isomers differing in the position of oxidation, the number of oxygen atoms incorporated, and degree of *cis*/*trans* isomerisation. The compounds will have very similar but not identical chromatographic behaviour, which causes extensive peak splitting and broadening and makes the interpretation difficult. In the same way as the non-oxidised TAGs, a decrease of sensitivity is observed due to splitting of the total signal among many peaks. There are two possible solutions: First, MS analysis without HPLC which would give better sensitivity, but unfortunately compounds with the same molecular weights and different structures cannot be distinguished. The second option is optimisation of the separation conditions [31], but this is time-consuming and complete separation of all positional isomers of oxidation products has not been achieved so far.

HPLC/APCI-MS analysis of autoxidation products (incubation for 1–21 days at 50–60°C in the dark) [31] of pure standards of triolein, trilinolein, or trilinolenin enabled the identification of main oxidation products, e.g. mono- and bishydroperoxides, mono- and diepoxides, hydroxy TAGs, epidioxides and hydroperoxide epidioxides. The retention of identified oxidation products is in agreement with our findings. Two things should be pointed out: groups of broad and multiplet peaks of particular classes of oxidation products are observed (see Figure 1 in discussed paper [31]), APCI mass spectra of particular compound classes yield many fragment- and adduct ions, which reduces the *S/N* ratio. Compared to the oxidation of one pure TAG, the number of possible oxidation products in vegetable oil is much higher and this prevents a reasonable interpretation of the HPLC/MS data on the oxidation products in our study.

Small amounts of diacylglycerols (DAGs) were positively identified in the front part of the chromatogram (not shown): 1,3-LnLn (t_R = 6.46 min), 1,2-LnLn (6.87 min), 1,3-LnL (8.78 min), and 1,2-LnL (9.49 min). Cross-linked products of TAGs cannot be studied with the current HPLC/MS method, because such species are retained on the chromatographic column under the HPLC conditions used.

3.5 DTMS

When a complex mixture of materials is heated in the ion source, as is the case with DTMS, initially volatile, lower molecular weight material will evaporate and at higher temperature less volatile oxidised, i.e. more polar, and/or high molecular weight material will be brought into the gas phase. In cases where the sample cannot be evaporated pyrolysis of the organic material will take place by thermally assisted bond breaking. This will lead to a separation in a relatively short time period of 2 min of the different materials present in the sample. In order to detect the gaseous compounds by mass spectrometry electron ionisation is applied which makes it possible to quickly determine the presence of free fatty acids, oxidation products, and cross-linked material in processed oils. The analysis of TAGs using direct insertion mass spectrometry is well known [65–68] but the 70 eV electron ionisation most frequently utilised causes extensive fragmentation and reduced intensities of molecular ions [41]. Therefore, 16 eV EI ionisation was applied in this study, which leads to less fragmentation and an increased abundance of the molecular ions. Furthermore, in the case of DTMS the sample is introduced into the ion source on a Pt/Rh (9/1) filament, which is resistively heated, instead of a fixed temperature that has been used in direct insertion mass spectrometric analysis of oils.

In the insert of **Figure 5.a**, the total ion current (TIC) is depicted for oil F. It can be seen that most of the sample evaporates within a relatively small time domain, pointing towards a low degree of polymerisation. According to the TIC, free fatty acids, MAGs, and DAGs are almost absent. These materials are expected to evaporate at lower temperature [69]. The mass spectrum (Figure 5.a) of the summed scans 23–25 (see insert Figure 5.a) shows intact molecular ions (*m/z* 848–884), fragments due to

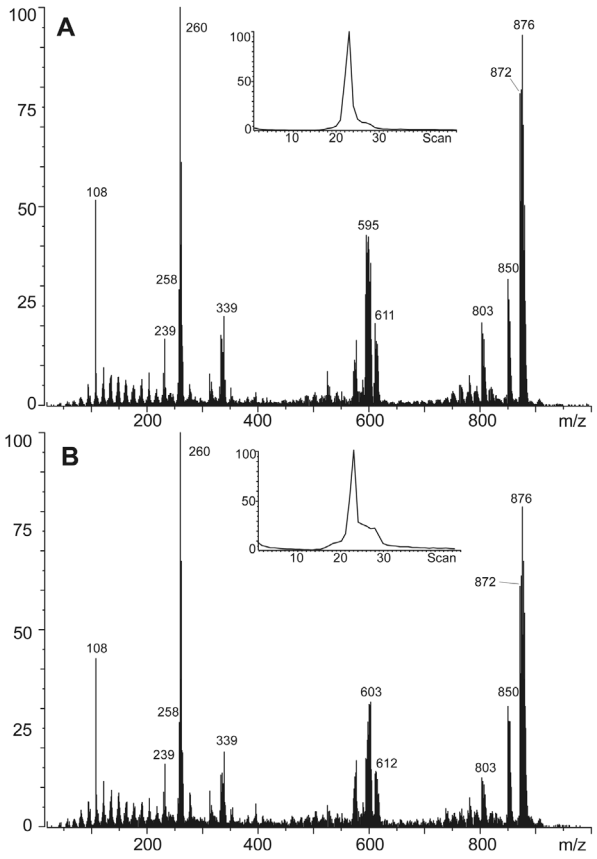


Figure 5. DTMS mass spectrum of (a) oil F, and (b) oil F300 (scan 23–25). Insert: Total Ion Chromatogram (TIC).

the loss of one or two intact (un)saturated fatty acids (*m/z* 570–620 and 310–344, respectively) and the acylium ions of saturated C16 and (un)saturated C18 fatty acids (*m/z* 239, 258–267). Fragment ions at *m/z* 258 are ascribed to a triple unsaturated acylium ion that has lost two additional hydrogens [41]. The most important molecular ions and fragments are assigned in **Table 7**. Closer inspection of the mass region just above the molecular ions reveals two small clusters with an increased mass of 16 and 32, respectively, pointing to incorporation of 1, respectively 2, additional oxygen atoms.

On first inspection, the mass spectrum of summed scans 23–25 of the heated oil F300 (**Figure 5.b**) looks very similar to the previous spectrum. However, when the lower masses of each cluster are examined it is immediately clear that heating has resulted in the disappearance of the highly unsaturated molecules relative to the less unsaturated species. This phenomenon confirms that upon heating and/or oxidation TAGs with double bonds are consumed to form oligomeric material, which is also suggested by the TIC (see insert) which shows that there is relatively more material detectable at higher temperature indicative of the more polar and/or polymeric nature of the material. Intact molecular ions of cross-linked TAGs are not detectable with the settings used.

Table 7. Most important (fragment) ions of TAGs observed upon 16 eV direct temperature resolved mass spectrometry analysis of linseed oil.

Mass(es)	(Fragment) Ion	Composition
872–888	$M^{+\cdot}$	$C_{57}H_{110-2n}O_6$ (n = 1,2,3,...9)[a]
850–860	$M^{+\cdot}$	$C_{55}H_{106-2n}O_6$ (n = 1,2,3,...6)
805–819	$[M\text{-}C_5H_9]^+$	$C_{52}H_{101-2n}O_6$ (n = 1,2,3,...8)
783–791	$[M\text{-}C_5H_9]^+$	$C_{50}H_{97-2n}O_6$ (n = 1,2,3,...5)
613, 614,...,619	$[M+O\text{-}RCOO(H)]^{+}$[b]	$C_{39}H_{74-2n}O_5$ (n = 1,2,3,4)
594, 596,...,604	$[M\text{-}RCOOH]^{+\cdot}$	$C_{39}H_{74-2n}O_4$ (n = 1,2,3,...6)
595, 597,...,605	$[M\text{-}RCOO]^+$	$C_{39}H_{75-2n}O_4$ (n = 1,2,3,...6)
572, 574,...,578	$[M\text{-}RCOOH]^{+\cdot}$	$C_{37}H_{70-2n}O_4$ (n = 0,1,2,3)
573, 575,...,579	$[M\text{-}RCOO]^+$	$C_{37}H_{71-2n}O_4$ (n = 0,1,2,3)
331, 335,...,341	$[RCO+74]^+$	$C_{21}H_{41-2n}O_3$ (n = 0,1,2,3)
313	$[RCO+74]^+$	$C_{19}H_{37}O_3$
259, 261, ..., 267	$[RCO]^+$	$C_{18}H_{35-2n}O$ (n = 0,1,2,3,4)
258, 260, 262, 264	$[RCO\text{-}H]^{+\cdot}$	$C_{18}H_{34-2n}O$ (n = 1,2,3,4)
230, 232, 234	$[R\text{-}H]^{+\cdot}$	$C_{17}H_{35-2n}$ (n = 1,2,3)
108	$[R\text{-}C_9H17]^+$	C_8H_{12}

[a] n denotes the number of double bonds present.
[b] R denotes the (unsaturated) carbon chain attached to the carboxylic acid.

The quantity of the different TAGs present in the oils was determined using the relative intensity of the molecular ion peaks. The contribution of the second isotope peak, which has an intensity of 20% relative to its molecular ion for the all C18 TAG, was taken into account. Our analytical results on the composition of the fresh untreated oil F are in reasonable agreement with direct insertion measurement values reported for untreated linseed oil in the literature [67]. Relative changes between the oils as a result of different processing methods can be detected easily (see **Table 8**) although absolute quantification is not achieved in this way because of differences in ionisation efficiency, fragmentation behaviour upon electron ionisation, and susceptibility towards oligomerisation and/or oxidation of the TAGs. Heating leads to a relative decrease of TAGs with triple unsaturated fatty acids (*m/z* 872–876) and therefore a relative increase of TAGs without linolenic acid groups. This is most clearly seen for oil sample F300. The addition of lead drier to the oil leads to a small decrease in the relative number of TAGs with the more highly unsaturated fatty acids. This becomes evident when comparing sample F and D. The presence of the drier enhances oxidation and as a consequence the decrease of more reactive triple unsaturated species will be faster [70]. The composition of the precipitated material, D150 Precipitate, was clearly different from that of the oil above (D150) and a strong reduction of the triple unsaturated fatty acids is observed. The trends observed in Table 8 point to Diels-Alder type reactions that lead to oligomerisation, although loss of highly unsaturated fatty acids by oxidation cannot be excluded. Part of the oxi-

Table 8. Percentages of different TAGs present in the processed oils, as determined by DTMS[a].

TAG MW	F [b]	F150	F300	D	D150	D150 Precip.	W	TAG Name [c], [d]
828	0.52	0.54	0.84	0.56	0.62	0.70	0.71	PPO
830	0.29	0.33	0.65	0.36	0.39	0.13	0.28	PPL
832	0.31	0.33	0.41	0.12	0.22	0.16	0.24	PPLn
850	7.88	7.86	7.93	7.67	7.98	8.24	8.81	PLnLn
852	5.06	5.08	5.42	4.97	5.49	5.68	5.37	PLLn
854	3.95	3.96	5.63	4.68	4.62	5.41	4.88	PLL
856	0.76	0.89	1.19	0.80	1.17	1.19	1.06	POL/PSLn
858	0.33	0.26	0.46	0.31	0.25	0.37	0.32	PSL
860	0.21	0.18	0.20	0.18	0.17	0.15	0.15	PSO
872	19.69	19.36	16.02	19.42	18.42	16.03	19.00	LnLnLn
874	15.99	15.76	13.54	15.43	15.12	14.13	14.96	LnLnL
876	19.40	19.08	18.05	18.76	18.54	17.77	18.17	LnLnO/LnLL
878	12.99	12.74	13.47	12.94	12.55	13.49	12.39	LnLO/LnLnS
880	9.05	9.23	10.72	9.21	9.84	10.76	9.45	LnOO/LnLS/OOO
882	2.51	2.56	3.68	3.31	3.59	3.84	3.25	SOLn/LOO/SLL
884	0.62	0.67	1.04	0.79	0.73	1.08	0.65	SOL/OOO/SSLn
886	0.09	0.05	0.21	0.21	0.20	0.36	0.14	SOO/SSL
888	0.10	0.11	0.55	0.23	0.07	0.47	0.15	SSO

[a] See Materials and Methods section for DTMS conditions.
[b] See experimental section for explanation of codes.
[c] TAG fatty acids: P = palmitic, S = stearic, O = oleic, L = linoleic and Ln = linolenic acid.
[d] Different isomers are possible, e. g. 886

dised species, especially the hydroperoxides, are not stable and will fragment upon electron ionisation, which makes quantification of the oxidation process by DTMS difficult without prior derivatisation.

3.6 MALDI-TOF-MS

MALDI-TOF-MS was applied to study the oxidation and formation of oligomeric material in more detail. Analysis of the oils with MALDI-TOF-MS in both the linear and reflectron mode confirm that intact molecular TAG ions, cross-linked and/or oxidised products thereof, and minor amounts of low molecular weight materials are present in all of the oils studied. A typical example of an analysis in both the linear and reflectron mode is given for oil F300 in **Figure 6** and **Figure 7**, respectively. Besides the two clusters of intact sodiated TAGs with 55 and 57 carbon atoms, respectively, small amounts of protonated diacylglycerol (DAG) fragment ions are observed for all the oil samples at *m/z* 573–579 and 595–605 [71, 72]. Only samples F300 and to a lesser extent D150 Precipitate showed significantly higher signals for these fragments, although identical laser power was used. In addition, trace amounts of intact sodiated DAGs are observed ranging

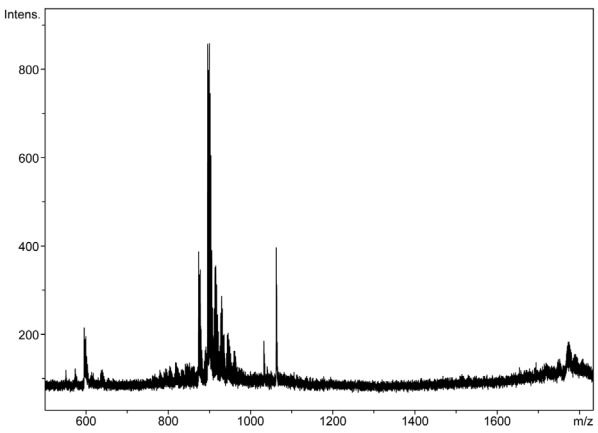


Figure 6. MALDI-TOF-MS spectrum of oil F300 (Reflectron mode).

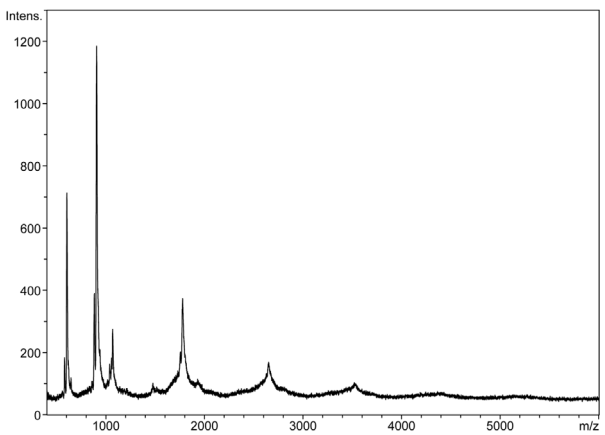


Figure 7. MALDI-TOF-MS spectrum of oil F300 (Linear mode).

Table 9. Overview of MALDI-TOF-MS measurements of the processed oils.

Sample	Maximum number of oxygen atoms[a)] incorporated into TAGs	Maximum number of cross-linked TAGs[b)]
F	1	1
F150	2	2
F300	4	6
D	4	3
D150	4	2
D150 Precipitate	6	4
W	4	2

[a)] As obtained with reflectron mode measurements.
[b)] As obtained with linear mode measurements.

from *m/z* 635 to 643, in all samples except for the untreated oil. This indicates that hydrolysis is not a major process occurring during the treatment of the oils, which is in accordance with the results obtained with the other analytical techniques previously described.

Incorporation of oxygen into the TAGs is seen for all oil samples and in the case of highest oxidised oils for the DAGs as well. Oxidised products were already present before the freshly pressed oils underwent further processing. Different numbers of oxygen atoms are built-in depending on the processing method as can be seen in **Table 9** and **Figure 8.a–e**. In these figures a partial mass spectrum is depicted of the mass range of the monomeric TAGs and the oxidised species of some of the oils analysed. Insertion of oxygen is observed, for instance, in *m/z* 896, $[LnLnLn+Na]^+$, which becomes *m/z* 912 (See Figure 8.c for the details).

It is clear from these results that a rather large number of different species is formed upon (thermal-)oxidation of the linseed oil. Dimers were observed in the mass spectra recorded in the reflectron mode for samples F300 and D150 Precipitate, next to the monomeric TAGs. The observed masses indicate that no oxygen is present in these dimers, which would imply they are formed by a Diels-Alder cyclisation reaction. Carbon-carbon links due to radical-radical combinations can be formed theoretically also but are less likely since closer inspection of these dimers reveals that no hydrogens seem to be consumed. The most unsaturated species that is detected contains an equivalent of 18 double bonds, i. e. 2×9. Theoretically it cannot be excluded that the "dimers" consist of non-covalently bound complexes that are formed during the MALDI analysis. The SEC results are more straightforward in this respect, and do confirm the presence of cross-linked material in the oils. Analysis of the oils using the linear mode shows (see Table 9) that most oils contain higher molecular weight material. The highest degree of cross-linking is observed in oil sample F300 (Figure 6), which contains traces of oligomeric TAGs up to hexamers. This is in agreement with the upper mass calculated from the HPSEC results. The HPSEC results of the remaining oils fit nicely with the linear MALDI-TOF-MS data.

3.7 ESI-FTICRMS

High resolution ESI-FTICRMS was also used in order to determine the exact elemental composition of the TAGs in the oil and, primarily, the oxidised compounds formed upon processing. Due to the softer nature of the electrospray ionisation technique compared to MALDI, less fragmentation of the TAGs is expected. **Figure 9** shows the ESI-FTICR mass spectrum of oil F150. Ammonium cationised molecular ions are observed of both intact TAGs and trace amounts of DAGs. The exact masses obtained for the different TAG species, including the error in mass assignment, and their identities are depicted in **Table 10**. As can be seen the observed masses agree well with the true values. The loss of fatty acids, giving rise to protonated fragment ions as was seen with MALDI-TOF-MS, is not observed here. In the TAG mass window three major clusters are detected: a cluster of TAGs that consists of two (unsaturated) C18 and one C16 fatty acyl chain, a cluster with only (unsaturated) C18 fatty acyl chains and their mono-oxygenated derivative, as determined by the mass increment of *m/z* 15.993. The exact mass of oxygen (^{16}O), 15.9949, agrees well with this mass difference in the third cluster. In total, the incorporation of 4 additional oxygen atoms is observed for the monomeric TAGs. In the higher mass region three clusters are clearly visible. In the mass window between 1475 and 1525, low intensity ammonium cationised molecular ions can be found (see also Table 10), which are ascribed to a combination of TAGs and DAGs (see insert A, Figure 9). No oxygen incorporation is observed. The two highest clusters in the dimer region (*m/z* 1710–1820, see insert B) are formed by the combination of two non-oxidised TAGs. Isolation of some of these dimeric ions, followed by collisionally induced dissociation (CID) using argon as collision gas, resulted in the formation of monomeric TAGs with a maximum number of 9 double bonds and a double bond distribution in accordance with the number of double bonds originally present in the dimer (results not shown). Although data on the monomeric TAGs showed the loss of one of the fatty acyl chains upon ester cleavage under the same MS/MS conditions, this was not observed for the dimeric material. These findings suggest that part of the dimers is not covalently bound but exists as a complex in the gas phase, formed during the ionisation process. Dimers with one or two oxygens and trimeric TAGs are observed as well (see insert of *m/z* 2590–2700, Figure 9.C). The signal of these clusters already starts to overlap and it is clear that upon further oxidation as the

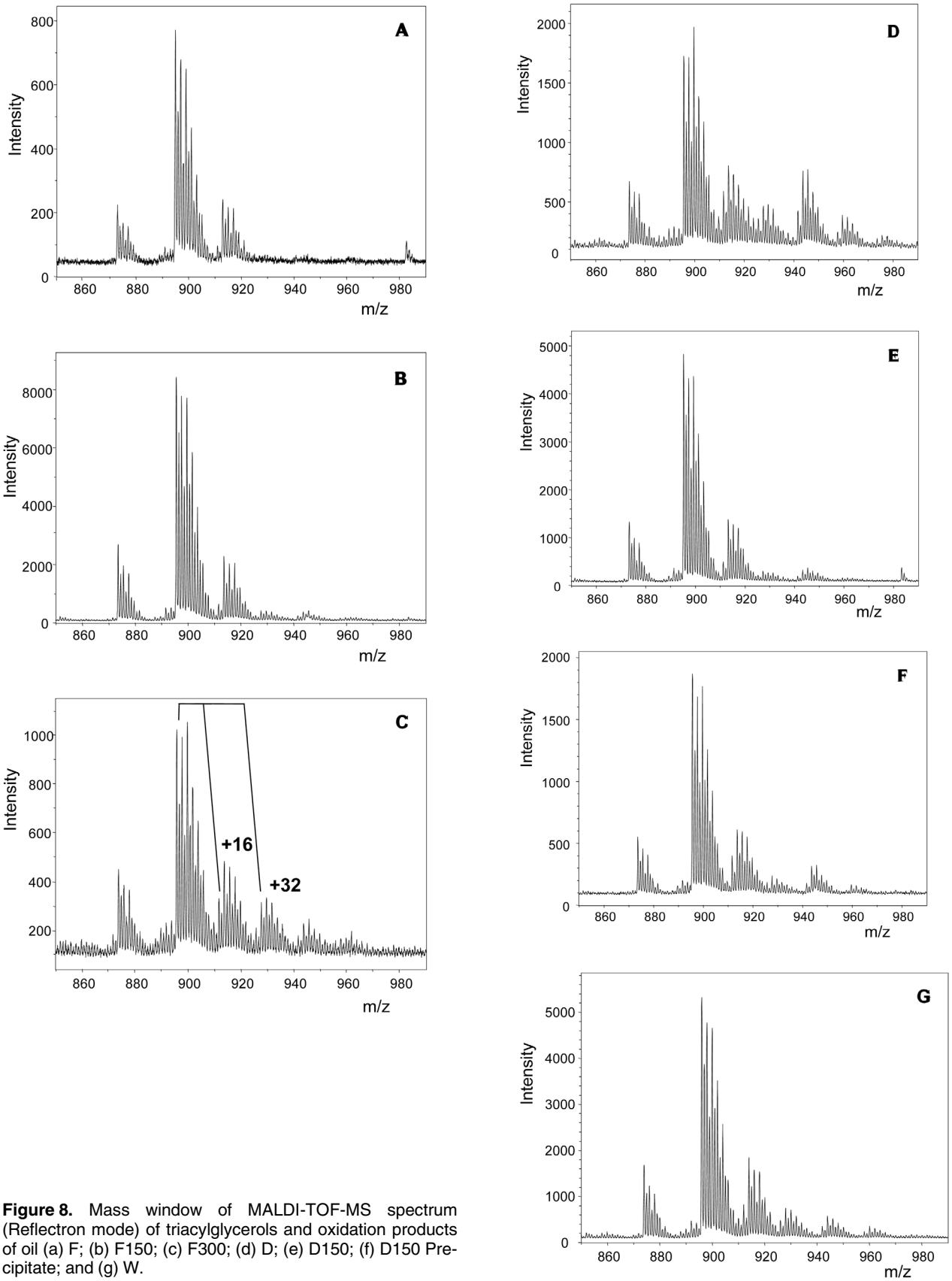


Figure 8. Mass window of MALDI-TOF-MS spectrum (Reflectron mode) of triacylglycerols and oxidation products of oil (a) F; (b) F150; (c) F300; (d) D; (e) D150; (f) D150 Precipitate; and (g) W.

Table 10. Masses observed by ESI-FTICR-MS analysis of oil sample F150.

Material	True MW	Measured MW	Error (ppm)
TAGs[a]			
LnLnLn	890.7238	890.7307	7.79
LLnLn	892.7394	892.7445	5.70
OLnLn/LLLn	894.7551	894.7607	6.30
OLLn/LLL/SLnLn	896.7707	896.7755	5.34
OOLn/OLL/SLLn	898.7864	898.7914	5.60
OOL/SLL/SOLn	900.8020	900.8060	4.42
OOO/SOL/SSLn	902.8177	902.8217	4.47
SOO/SSL	904.8333	904.8364	3.41
All C18 DAG-TAG Dimers			
$n = 14$ [b]	1505.2148	1505.2406	17.15
$n = 13$	1507.2304	1507.2588	18.82
$n = 12$	1509.2461	1509.2720	17.17
$n = 11$	1511.2617	1511.2815	13.08
$n = 10$	1513.2774	1513.3027	16.73
$n = 9$	1515.2930	1515.3132	13.31
$n = 8$	1517.3087	1517.3320	15.36
$n = 7$	1519.3243	1519.3435	12.61
All C18 TAG-TAG Dimers			
$n = 18$	1763.4132	1763.4532	22.7094
$n = 17$	1765.4288	1765.4657	20.8992
$n = 16$	1767.4445	1767.4751	17.3392
$n = 15$	1769.4601	1769.4893	16.4999
$n = 14$	1771.4758	1771.5059	17.0174
$n = 13$	1773.4914	1773.5214	16.9135
$n = 12$	1775.5071	1775.5378	17.3167
$n = 11$	1777.5227	1777.5547	18.0003
$n = 10$	1779.5384	1779.5687	17.0527
$n = 9$	1781.5540	1781.5883	19.2506
All C18 TAG-TAG Dimers with 1 oxygen			
$n = 15 + O$	1785.4550	1785.509	30.17774
$n = 14 + O$	1787.4707	1787.501	16.74489
$n = 13 + O$	1789.4863	1789.51	13.12164
$n = 12 + O$	1791.5020	1791.547	25.02425
$n = 11 + O$	1793.5176	1793.557	21.73438
$n = 10 + O$	1795.5333	1795.564	17.00386
All C18 TAG-TAG-TAG Trimers			
$n = 23$	2644.1651	2644.272	40.4120
$n = 22$	2646.1808	2646.276	35.8275
$n = 21$	2648.1964	2648.3	38.9533
$n = 20$	2650.2121	2650.282	26.4530
$n = 19$	2652.2277	2652.329	38.0269
$n = 18$	2654.2434	2654.337	35.1535

[a] P = palmitic acid, S = stearic acid, O = oleic acid, L = linoleic acid, Ln = linolenic acid.

[b] *n* denotes the total number of double bonds within the material.

oils dry a rather complex envelope of peaks will be obtained. High-resolution FTICR-MS will be beneficial in this case since small mass differences will exist between the different species. The ESI-FTICR-MS results obtained for all of the oils are summarised in **Table 11**. The results and trends are the same as the previously described MALDI data (Table 9) with, however, two exceptions: the number of cross-linked TAGs for F300 is 3, whereas 6 was observed with MALDI (in the linear mode). This may be due to the poorer detection sensitivity of FTICR-MS especially at higher mass. Furthermore, the degree of oxygenation of sample F150 as detected with ESI is somewhat higher. A closer comparison of the results obtained with both MALDI-TOF-MS and ESI-FTICR-MS shows for almost all samples a strong resemblance in the TAG mass window (see **Figure 10** for a comparison of two of the oils). There only seems to be a slightly better sensitivity for the oxidised species with ESI.

4 Discussion

From the above results it is clear that the initial TAGs, which are already slightly oxidised, do change upon processing. Of the oil processing methods investigated two treatments had a very significant effect on the chemical properties of the oils: heating the fresh oil to an end-temperature of 300°C and heating a mixture of oil and drier (lead(II) oxide) to 150°C. Both of these oils were shown to contain constituents with a significantly increased molecular weight and number of incorporated oxygen atoms. The average molecular weights of the other oils investigated (heat treatment alone to 150°C (F150), lead and oil mixed at room temperature (D), and water washing (W)) were less affected by processing. According to the results reported here, it is also evident that hydrolysis is not an important process during the oil processing. The comparison between sample D150 and the precipitate D150 Precipitate, which showed higher molecular weight material concentrated in the precipitating material, is important in terms of sampling for analysis and in the preparation of historically accurate paint samples. It indicates that the oil constituents are not uniformly distributed throughout the oil. Therefore if the oil is not well stirred prior to use or sampling, false conclusions could be reached regarding the degree of chemical alteration, i.e. chemical drying in the mixture. This phenomenon also implies that during use, artists may be experiencing a difference in the management and drying characteristics of their paint according to the age of their oil and whether they poured from the top of the container or used the oil remaining at the bottom of the container.

Although reasonable amounts of cross-linked material were demonstrated by SEC in almost all of the oils, it was very difficult to identify this material with the three mass spectrometric techniques, DTMS, MALDI-TOF-MS, and ESI-FTICR-MS. MALDI analysis in the linear mode was most effective. With this technique it was shown that oligomers as high as hexamers were formed in oil heated to

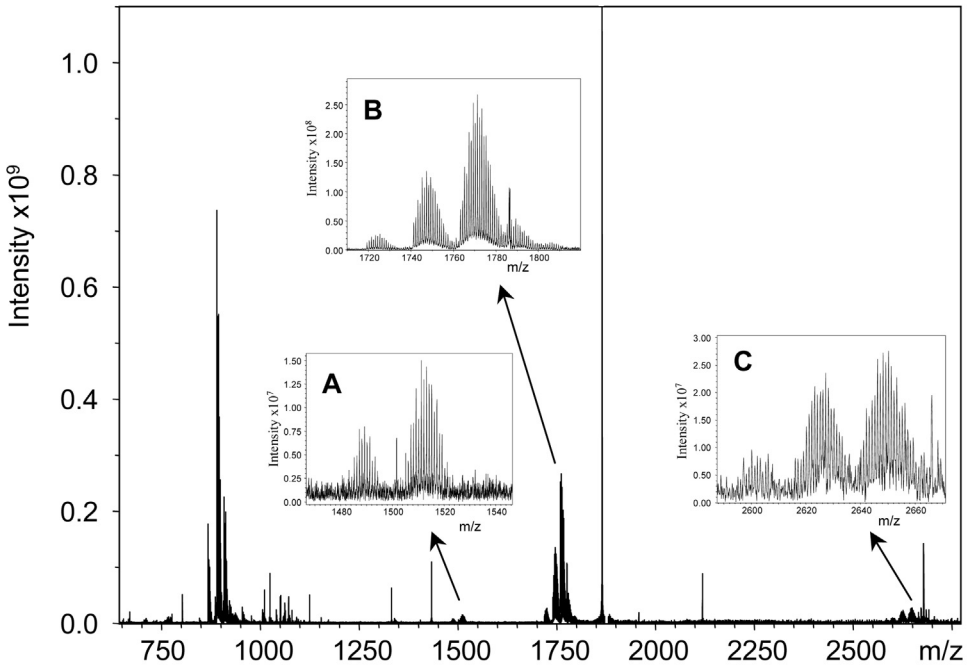


Figure 9. ESI-FTICR-MS spectrum of oil F150. Insert A. DAG-TAGs, B. TAG-TAGs, and C. TAG-TAG-TAGs

Table 11. Overview of ESI-FTICR-MS results of the processed oils.

Sample	Maximum number of incorporated oxygen atoms in TAGs	Dimers	Trimers	Maximum number of incorporated oxygen atoms in (TAG)2s
F	1	DAG-TAG, (TAG)2	(TAG)3	1
F150	4	DAG-TAG, (TAG)2	(TAG)3, DAG-(TAG)2, (DAG)2-TAG	2
F300	5	(DAG)2, DAG-TAG, (TAG)2	(TAG)3, DAG-(TAG)2	2
D	5	(TAG)2	(TAG)3	3
D150	4	(TAG)2	(TAG)3	2
D150 Precipitate	7	(TAG)2	(TAG)3	>7
W	4	(DAG-TAG), (TAG)2	(TAG)3, DAG-(TAG)2, (DAG)2-TAG	3

300°C (F300). This is in agreement with the SEC results. Surprisingly, upon mass spectrometric analysis the other, less cross-linked, oils showed only minor differences in their molecular weights despite their different SEC traces. Clustering of gaseous TAGs leading to dimers and trimers could partially cause this. The big advantage of the mass spectrometric techniques is that the incorporation of oxygen into the TAGs can be monitored easily. The benefit of ESI-FTICR-MS compared to MALDI in this process is its capability of exact mass determination. This is indispensable in the case of multiply oxygenated species formed upon ongoing oxidation of TAGs containing both C16 and (unsaturated) C18 fatty acids. Surprisingly, the major products of oxidation observed contain only 1 oxygen atom (see Figure 8–10) whereas a hydroperoxide, a 2 oxygen atom containing functional group, is expected to be formed based on the autoxidation theory [6, 7, 31, 73]. Comparing the ion distribution of the non-oxidised TAGs with that of the mono oxidised species indicates that in this case oxidation is also accompanied by the loss of a double bond, as if water is added. This has been observed in mass spectra of autoxidised ethyl linolenate [74, 75] and of certain oils [71] as well, although it has never been explained. In almost all oil samples analysed, however, species are detected which do have a mass increment of 32, in accordance with the formation of hydroperoxides. When comparing oils F and F300 on the one hand and oils D, D150 and the precipitate D150 Precipitate on the other, it can be seen immediately that the oxidation seems to follow a different path for the lead-containing oils. After the

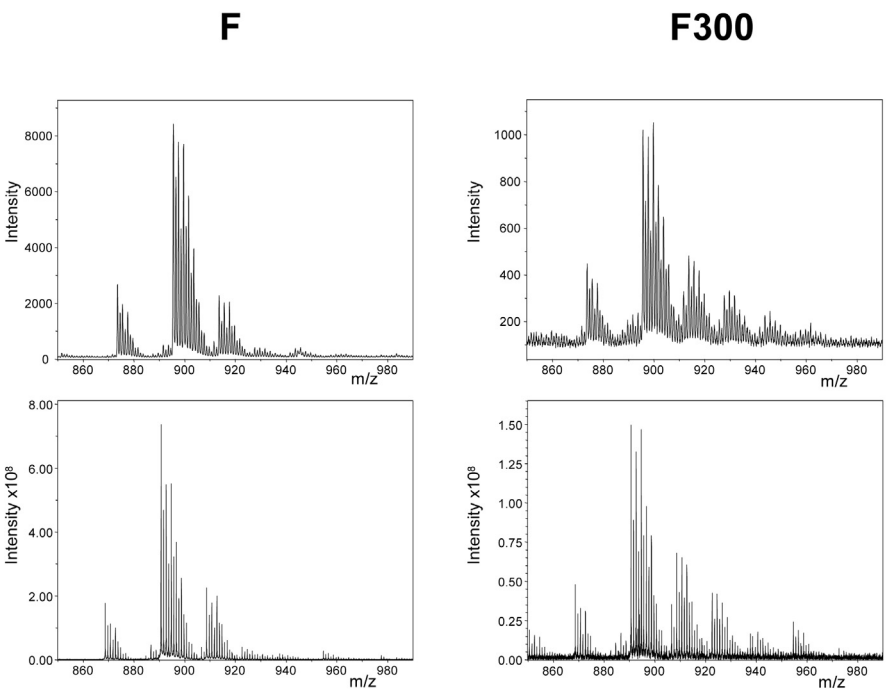
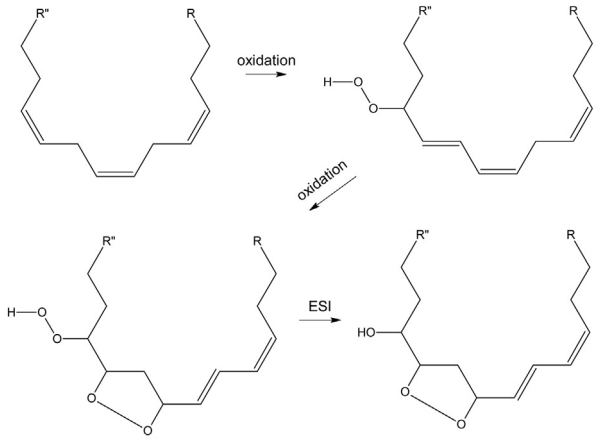


Figure 10. Comparison of MALDI and ESI results for TAGs and oxidation products of oils F and F300.

first oxygen is incorporated, there seems to be a preference for the simultaneous incorporation of two more oxygen atoms in the next step rather than one, as indicated by the higher intensity of triply oxygenated TAGs relative to doubly oxygenated species. Surprisingly, doubly oxygenated TAGs are present in lower amounts compared to the singly and triply oxygenated species. Autoxidation, as described in the literature, starts with the formation of hydroperoxides (ROOH), which would lead to a mass increase of 32 amu for the TAGs. A number of secondary oxidation products can then be formed: either a hydroperoxide attaches to previously unsubstituted fatty acyl chains or a second hydroperoxide adds to the fatty acyl chain already oxidised. In the latter case, an epidioxide is formed preferentially [31, 76] (See Scheme 1). This would lead to a total increase of 64 amu in the corresponding mass spectra. The thermal decomposition of the hydroperoxides, which can give rise to the radicals $RO^{\cdot}$ and $^{\cdot}OH$, is slow and rather complex [11]. However, the ions of (transition) metals are effective as secondary catalysts of the autoxidation. Initially they act as a one-electron donor to give $RO^{\bullet}$. This radical can then pick up hydrogen from RH to give ROH and $R^{\bullet}$. The epidioxides are thought to have a higher stability towards breakdown by metal ions, relative to the hydroperoxides, which will lead to an oxygen distribution similar to that observed for the lead-containing oils. The increase in oxidation observed for samples F150 and F300 is postulated to be caused by a synergy of both the higher amount of more reactive conjugated fatty acyl strands formed upon heating and the thermal breakdown of hydroperoxides. The fact that only monoxygenated species are seen in oil F suggests that



Scheme 1. Proposed mechanism for the formation of triple oxygenated species upon ESI-FTICR-MS analysis of oxidised TAGs.

the initially formed hydroperoxides are not stable under the conditions used.

DTMS analysis takes its place in between SEC and MALDI. Information can be obtained rapidly on the nature of the oil sample, based on polarity and molecular weight through the desorption profile, as well as its composition via the mass spectrum. The differences in the oils as can be observed with DTMS are mostly related to changes in the composition of the residual TAGs after processing.

For the oil samples investigated clear differences in the degree of oxygenation of the TAGs were observed with both ESI and MALDI analysis. In general the number of newly incorporated oxygen atoms correlates positively

with the average molecular weight of the oil. Oil sample D, with lead drier but not heat-treated, is a good example of this phenomenon. All other oils with high oxygen content have also been heated. The heating has been shown to lead to oligomerisation. In both the MALDI- and ESI-experiments rather large amounts of oxidised species were detected. However, with DTMS only a slight indication was found for oxidation products, whereas with FTIR not even a slight trace of hydroxy groups or hydroperoxides was observed. The reason for these differences in sensitivity are not entirely clear, but they may be due to higher ionisation efficiency of the more oxidised fractions in MALDI- and ESI-MS.

5 Conclusion

This study shows that the processing methods of freshly pressed linseed oil, as used by painters in the past, all have a clear effect on both the physical and the chemical properties of the linseed oil. This supports the observations made during paint preparation with lead white pigment and oils where there were significant differences in paint rheology according to the oil treatment. Two major chemical processes were observed for the oils: oxidation and oligomerisation. This is accompanied by a relative decrease of the percentage of the triple unsaturated fatty acids (linolenic acid), the most reactive component of the TAGs. High temperatures applied with or without the addition of a lead drier are shown to stimulate the observed changes. Litharge, when added to the oil as drier, clearly influenced the way autoxidation proceeds. Higher amounts of oxidised TAGs are found in these oils. The prepolymerisation by heating of the oil with a lead drier led to the formation of a precipitate on the bottom of the vial. This was enriched in oxidised polymeric material, compared to the upper part of the oil.

Both MALDI-TOF-MS and ESI-FTICR-MS are shown to be very useful for the analysis of oxygenated TAGs and their oligomers. Similar results for the composition of the TAGs and the degree of oxygenation were obtained with both techniques. FTIR, and also DTMS, are shown to be less useful for the characterisation of oxygenated TAG species. These techniques are more focussed on the bulk of the material whereas MALDI- and ESI seem to emphasise the oxidised TAGs.

References

[1] N.O.V. Sonntag, in *Bailey's industrial oil and fat products*, D. Swern (Editor). John Wiley & Sons, New York 1979, pp. 45–83.

[2] L.A. Carlyle, *Ph.D. Thesis*, London 1991.

[3] R. Keller, *Maltechnik* **1973**, *2*, 74–105.

[4] M.M. Paulose, S.S. Chang, *J. Am. Oil Chem. Soc.* **1973**, *50*, 147–154.

[5] A. Crossley, T.D. Heyes, B.J.F. Hudson, *J. Am. Oil Chem. Soc.* **1962**, *39*, 9–14.

[6] N.A. Porter, S.E. Caldwell, K.A. Mills, *Lipids* **1995**, *30*, 277–290.

[7] H.W.-S. Chan, in *Autoxidation of Unsaturated Lipids*, H.W.-S. Chan (Editor). Academic Press Inc. Ltd., London 1987, pp. 1–16.

[8] E.N. Frankel, W.E. Neff, K. Miyashita, *Lipids* **1990**, *25*, 33–39.

[9] R.A. Hancock, N.J. Leeves, *Progr. Org. Coatings* **1989**, *17*, 321–336.

[10] W. Grosch, in *Autoxidation of Unsaturated Lipids*, H.W.-S. Chan (Editor). Academic Press Inc. Ltd., London 1987, pp. 95–140.

[11] W.A. Waters, *J. Am. Oil Chem. Soc.* **1971**, *48*, 427–433.

[12] D.M. Miller, G.R. Buettner, S.D. Aust, *Free Radic. Biol. Chem.* **1990**, *8*, 95–108.

[13] A. Hopia, *Lebensm. Wiss. Technol.* **1993**, *26*, 563–567.

[14] K. Miyashita, K. Fujimoto, T. Kaneda, *Biol. Chem.* **1982**, *46*, 751–755.

[15] K. Miyashita, K. Fujimoto, T. Kaneda, *Agric. Biol. Chem.* **1982**, *46*, 2293–2297.

[16] T.G. Toschi, A. Costa, G. Lercker, *J. Am. Oil Chem. Soc.* **1997**, *74*, 387–391.

[17] H.W. Gardner, in *Autoxidation of Unsaturated Lipids*, H.W.-S. Chan (Editor). Academic Press Inc. Ltd., London 1987, pp. 51–90.

[18] J.C. Martin, M. Nour, F. Lavillonnière, J.L. Sébédio, *J. Am. Oil Chem. Soc.* **1998**, *75*, 1073–1078.

[19] J. Pokorny, M.K. Kundu, S. Pokorny, M. Bleha, J. Coupek, *Die Nahrung* **1976**, *20*, 157–163.

[20] C. Boelhouwer, J.T. Knegtel, M. Tels, *Fette, Seifen, Anstrichm.* **1967**, *69*, 432–436.

[21] K. Figge, *Chem. Phys. Lipids* **1971**, *6*, 164–182.

[22] J.C. Martin, M.C. Dobarganes, M. Nour, G. Marquez-Ruiz, W.W. Christie, F. Lavillonnière, J.L. Sébédio, *J. Am. Oil Chem. Soc.* **1998**, *75*, 1065–1071.

[23] J.L. Le Quere, J.L. Sébédio, R. Henry, F. Couderc, N. Demont, J.C. Promé, *J. Chromatogr.* **1991**, *562*, 659–672.

[24] J.L. Sebedio, J.L.L. Quere, O. Morin, J.M. Vatele, A. Grandgirard, *J. Amer. Oil Chem. Soc.* **1989**, *66*, 704–708.

[25] G. Dobson, W.W. Christie, E.Y. Brechany, J.L. Sébédio, J.L.L. Quere, *Lipids* **1995**, *75*, 171–182.

[26] W.W. Christie, E.Y. Brechany, J.L. Sébédio, J.L.L. Quere, *Lipids* **1993**, *66*, 143–153.

[27] G. Dobson, W.W. Christie, J.L. Sébédio, *J. Chromatogr.* **1996**, *723*, 349–354.

[28] J.C. Martin, F. Lavillonnière, M. Nour, J.L. Sébédio, *J. Am. Oil Chem. Soc.* **1998**, *75*, 1691–1697.

[29] O. Sjövall, A. Kuksis, L. Marai, J.J. Myher, *J. Am. Oil Chem. Soc.* **1997**, *32*, 1211–1217.

[30] L. Steenhorst-Slikkerveer, A. Louter, H.-G. Janssen, C. Bauer-Plank, *J. Am. Oil Chem. Soc.* **2000**, *77*, 837–845.

[31] W.E. Neff, W.G. Byrdwell, *J. Chromatogr. A* **1998**, *818*, 169–186.

[32] W.C. Byrdwell, W.E. Neff, *J. Chromatogr. A* **2001**, *905*, 85–102.

[33] M. Holčapek, P. Jandera, J. Fischer, B. Prokes, *J. Chromatogr. A.* **1999**, *858*, 13–31.

[34] S. Héron, E. Lesellier, A. Tchapla, *J. Liq. Chromatogr.* **1995**, *18*, 599–611.

[35] W.E. Neff, W.C. Byrdwell, *J. Liq. Chromatogr.* **1995**, *18*, 4165–4181.

[36] W.C. Byrdwell, E.A. Emken, W.E. Neff, R.O. Adlof, *Lipids* **1996**, *31*, 919–935.

[37] H.R. Mottram, S.E. Woodbury, R.P. Evershed, *Rapid Commun. Mass Spectrom.* **1997**, *11*, 1240–1252.

[38] A. Stolyhwo, H. Colin, G. Guiochon, *Anal. Chem.* **1985**, *57*, 1342–1354.

[39] J.-T. Lin, C.L. Woodruff, T.A. McKeon, *J. Chromatogr. A* **1997**, *782*, 41–48.

[40] K.L. Duffin, J.D. Henion, J.J. Shieh, *Anal. Chem.* **1991**, *63*, 1781–1788.

[41] W.M. Lauer, A.J. Aasen, G. Graff, R.T. Holman, *Lipids* **1973**, *5*, 861–877.

[42] T. Kusaka, S. Ishihara, M. Sakaida, A. Mifune, Y. Nakano, K. Tsuda, M. Ikeda, H. Nakano, *J. Chromatogr. A* **1996**, *730*, 1–7.

[43] A. Ravandi, A. Kuksis, J.J. Myher, L. Marai, *J. Biochem. Biophys. Methods* **1995**, *30*, 271–285.

[44] F.-F. Hsu, J. Turk, *J. Am. Soc. Mass Spectrom.* **1999**, *10*, 587–599.

[45] L. Steenhorst-Slikkerveer, A. Louter, H.G. Janssen, C. Bauer-Plank, *J. Am. Oil Chem. Soc.* **2000**, *77*, 837–845.

[46] O.F. Van den Brink, J.J. Boon, P.B. O'Connor, M.C. Duursma, R.M.A. Heeren, *J. Mass Spectrom.* **2001**, *36*, 479–492.

[47] O.F. Van den Brink, M.C. Duursma, R.M.A. Heeren, J.J. Boon, in E. Gelpi (Editor), 15th International Mass Spectrometry Conference, Barcelona 2000.

[48] G. Márquez-Ruíz, M.C. Pérez-Camino, M.C. Dobarganes, *J. Chromatogr.* **1990**, *514*, 37–44.

[49] A. Hopia, *Lebensm. Wiss. Technol.* **1993**, *26*, 568–571.

[50] R.J. Meilunas, J.G. Bentsen, A. Steinberg, *Stud. Conserv.* **1990**, *35*, 33–51.

[51] J.H. Hartshorn, *J. Coating Technol.* **1982**, *54*, 53–61.

[52] E.M. Salazar-Rojas, M.W. Urban, *Prog. Org. Coatings* **1989**, *16*, 371–386.

[53] G.L. Marshall, *Eur. Polym. J.* **1986**, *22*, 231–241.

[54] S. Husain, M. Kifayatullah, G.S.R. Sastry, N.P. Raju, *J. Am. Oil Chem. Soc.* **1993**, *70*, 1251–1254.

[55] M.S.F. Lie, J. Mustafa, *Lipids* **1997**, *32*, 1019–1034.

[56] W.E. Neff, M. El-Agaimy, *OCL Ol. Corps. Gras* **1996**, *3*, 71–74.

[57] C.J.L. Silwood, M. Grootveld, *Lipids* **1999**, *34*, 741–756.

[58] L. Carlyle, *Molart Fellowship: Historical reconstructions of artists's oil paint: an investigation of oil processing methods and the use of medium-modifiers*, Canadian Conservation Institute, Ottawa 2000.

[59] L. Osborn, *Handbook of Young Artists and Amateurs in Oil Painting*, Wiley and Putnam, New York 1845.

[60] R.M.A. Heeren, J.J. Boon, *Int. J. Mass Spectrom. Ion Proc.* **1996**, *157/8*, 391–403.

[61] S. Koster, M.C. Duursma, J.J. Boon, R. Heeren, *J. Am. Soc. Mass Spectrom.* **2000**, *11*, 536–543.

[62] R. Mayer, *A dictionary of art terms and techniques*, Barnes and Nobles Books, New York 1981.

[63] G.F.A. Stutz, *Ind. Eng. Chem.* **1926**, *18*, 1235.

[64] J. Mallégol, L. Gonon, J. Lemaire, J.-L. Gardette, *Polym. Degr. Stab.* **2001**, *72*, 191–197.

[65] R. Ryhage, E. Stenhagen, *J. Lipid Res.* **1960**, *1*, 361–391.

[66] M. Barber, T.O. Merren, W. Kelly, *Tetrahedron Letters* **1964**, *18*, 1063.

[67] R.A. Hites, *Anal. Chem.* **1970**, *42*, 1736–1740.

[68] A.J. Aasen, W.M. Lauer, R.T. Holman, *Lipids* **1973**, *5*, 869–878.

[69] J.J. Boon, S.L. Peulve, O.F. Van den Brink, M.C. Duursma, D. Rainford, in *Early Italian paintings techniques and analysis*, T. Bakkenist, R. Hoppenbrouwers, H. Dubois (Editors). Limburg Conservation Institute, Maastricht 1997, pp. 35–56.

[70] E. Ucciani, A. Debal, in *Oils & Fats Manual*, A. Karleskind, J.-P. Wolff (Editors). Intercept Ltd., Andover 1996, pp. 325–443.

[71] F.O. Ayorinde, B.E. Eribo, K.V. Balan, J.H. Johnson Jr., L.W. Wan, *Rapid Commun. Mass Spectrom.* **1999**, *13*, 937–942.

[72] W.J. Simonsick, C.W. Ross, *Int. J. Mass Spec. Ion Proces.* **1996**, *157/8*, 379–390.

[73] E.N. Frankel, in *Autoxidation in food and biological systems*, M.G. Simic, M. Karel (Editors). Plenum Press, New York 1980, pp. 141–177.

[74] W.J. Muizebelt, J.C. Hubert, R.A.M. Venderbosch, *Progr. Org. Coatings* **1994**, *24*, 263–279.

[75] W.J. Muizebelt, M.W.F. Nielen, *J. Mass Spectr.* **1996**, *31*, 545–554.

[76] E.N. Frankel, W.E. Neff, K. Miyashita, *Lipids* **1990**, *25*, 40–47.

[77] C. Sultana, in *Oils & Fats Manual*, A. Karleskind, J.-P. Wolff (Editors), Lavoisier Publishing, Paris 1996, p. 159.

[78] D. Swern, *Bailey's industrial oil and fat products*, 4th edition, D. Swern (Editor). John Wiley & Sons, New York 1979.

