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MOLECULAR ASPECTS OF THE CHEMICAL DRYING OF OIL PAINTS FORMULATED IN THE PERIOD 1890-1940

Pedro Alexandre Santos Leitão Caetano Alves

DOUTORAMENTO EM QUÍMICA (QUÍMICA)

2015

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Tese supervisionada pela Professora Doutora Maria Helena Ferreira da Silva Florêncio e pelo Professor Doutor Jaap J. Boon

ESPECIALMENTE ELABORADA PARA OBTER O GRAU DE

DOUTOR EM QUÍMICA (QUÍMICA)

2015

ACKNOWLEDGMENTS

This work would never been possible without the help, guidance, encouragement, and belief of so many people that it's hard not miss anyone who contributed in some way to the completion of this thesis.

First I have to acknowledge my supervisors: Prof. Helena for accepting me as her PhD student on a project she had no idea of, for mentoring me always and gave me the liberty to learn from others in the conservation field, and for all the valuable lessons in Life and Mass Spectrometry, as well as in Analytical sciences in general; and to Prof Jaap Boon who I met personally in 2006 at the end of the De Mayerne Programs and who believed that I could be trusted with a project that was not far from what he had built up in the previous Molart program, for building a project that is somehow a gap in our knowledge and giving me the best advice during all these years now: "don't try to reinvent the wheel" and "think critically".

Also for putting me in contact with world experts in conservation science all over, starting with Prof. Leslie Carlyle that was my "emeritus supervisor", and was my supervisor at Tate Britain Conservation Science, she taught me endless details of conservation, and later when we both moved back to Lisbon, the endless hours of fruitful discussion, conferences, paper reviewing, guidance and the "focus" word, "you'll have time for that at a Post-doc, Focus!" .

While at Tate with Leslie, I had the pleasure of being able to share the Lab and critical thinking with Joyce Townsend (thank you for the samples on the Camden town artists), Stephan Hackney and Bronwyn Ormsby (thank you ever since, and the Py-GC/MS analysis) who were pretty much the high standard reference on conservation science. Also to Jacob Thomas, for my research help, my first account of PhD finishing at a close, while I was pressing Oils.

But back home I had so many times the assistance and advice from my GEMAB group at FCUL, Prof. Carlos Borges ("we have a Book chapter!"), Prof. Filomena Duarte ("keep it simple") and Prof. Teresa Fernandez, as well as the younger members that put up with me on a daily basis Pedro Vaz (FTIR doubts), now PhD Paulo Madeira (I could never thank you enough for all that you taught me on MS, ion trap and FT-ICR, ESI or MALDI, thank you for being a friend "Daddy") and Rita, Tiago and Tânia, the "young team" for all the help the Lab, always believing in my work, for being such good colleagues and great lunch times.

While in Amsterdam I have to thank Klass Jan van der Berg, for accepting me at Netherlands Cultural Agency, and presenting me with a multidisciplinary and collaborative place aiming at building knowledge across science and art, and giving me access to the Technisches Mitteilungen fur Malerei whole archive. Also to the "basement people" Francesca Izzo (we will publish soon on Rodchenko samples), Beatriz and Alba Campo, for being such good people always with a smile.

In Lisbon I was also collaborating with José de Figueiredo Conservation and Restoration Lab, this is where I fell in love with conservation science, back in 2001, and cannot leave them out of these acknowledgments because they are the reason I chose these field of work, where I developed my Masters and have always supported me: Prof. António Candeias, Engª Isabel Ribeiro, Ana Mesquita e Carmo, Lília Esteves, Zé Oliveira, Zé Carlos Frade, Sara Valadas, Milene Gil and all the good people from the conservation departments.

I could not end this Thesis without my personal Friends, I'll just say *you know who you are, and a special thank you*. To Sandra for the final push till the End.

Aos meus Pais

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1 ARTIST'S OIL PAINT – CONTROVERSY & RESEARCH

ABSTRACT

This chapter reflects on the various perspectives of artist's oil paint uses, with a focus on the research on oil. Since the empirically based formulation introduced by artist's to the colourmen, to the science based developments in the raw materials, formulation, analysis and conservation science particularly.

1.1 INTRODUCTION

There are extensive studies and general citations on the subject of oil paint studies, and vegetable oils. The most widely used siccative oil is, by far, Linseed oil (*Linum usitatissimum*) only to be followed by Poppy (*Papaver somniferum*) and walnut oils (*Junglans Regia*). But it is thought that by the turn of the 19th to early 20th century

there were changes introduced in artist's oil paint formulation, mainly empirically, by *colourmen* which would affect Art works ever since.

The main aspect we are here dealing with is the introduction of less siccative oils, the semi-drying or even non-drying oils, in unknown quantities, which not only prolongs the shelf life of tube oil paints but imparts new characteristics to the paint.

Other possible consequences of these less drying formulations have been studied, in order to distinguish them from "normal" formulations, to understand the process of dripping paints, as a direct consequence of less drying starting materials.

1.2 A TECHNICAL DESCRIPTION OF OIL PAINT

Historically artist's oil Paint, as it has been used since early times, consists of a Binder, or Vehicle, and the pigment. These were ground together in a slab of Porfirus stone and collected with help of pallet knifes to be collected and stored in pig's bladders before the introduction of paint collapsible tubes.

The Pigments used are more or less described historically, ever since the first cave drawings to modern pigments, industrial and synthetic, inorganic and organic.

As for the Binders less is known or at least less is agreed on unanimously, and this is why we are here reflecting on this subject. Although it did not change as much as the pigments, it changed from artist to artist and workshop to workshop manufacturer and *colourmen*. In a simple way, the oil binding media consisted of drying or *siccative* oil, named this way for the ability to form a dry film with time.

Not so common, and the objective of this study, is the use of *semi-drying* or *nondrying* oils, or simply other types of oil, that would replace partly or entirely Linseed oil.

Drying oils are natural triacylglycerides (TAG's) containing high percentage of polyunsaturated fatty acids that give these oils the property of air-drying. These polyunsaturated fatty acids readily oxidise to form a three-dimensional network by the oxidative polymerization mechanism of drying oils (we will return to this point).

Most complex in the paint making is the addition of additives, and these could consist of a myriad of things, ranging from beeswax to resins, or fillers to driers of various sources, and these complex mixtures are extremely complicate analytical puzzles in chemistry.

We will focus on the Binding media and it's treatments for use in Oil Paint.

1.3 BRIEF HISTORY

Historically artist's oil Paint has been used since early times, it is known to been used since Egypt it's is already mention in Treatises since Vitruvius

Charles Eastlake did a good overview of past treatises, like Cennini [1], and presents a good summary in his "Materials for a History of Oil Painting" [2]. It would be exhaustive to present all the books related to this topic, like Field's "Chromathography"[3], Bouvier, Merimeé [4], Chevreul[5], Tingry, Muckley [6] [7]and others, but Leslie Carlyle has a brilliant text, The Artist's Assistant [8], which enumerates all of these to the 19th Century. There is also an extensive review on a

collection of texts in N.S Baer and N. Indictor "Linseed oil and related Materials: an annotated Bibliography" in three parts (Part I Antiquity to 1940; Part II from 1940 to 1960; Part III 1961-1972) Published by AATA)[9-11].

1.4 A VIEWPOINT ON SOME CRITICISM

The durability of a picture ought to be a matter of conscientious consideration with every painter. If he does not employ ordinary precaution in order to prevent or modify those changes which may take place in his works, sooner or later after they leave his hands, he is to a great extent responsible, if not culpable.

Preface to Muckley[6]

"*Of the three fixed-oils considered most proper for the purposes of painting, to wit, Poppy, Nut and Linseed Oil, the first named is that which since its introduction to the art has taken the lead of all the rest, and might be said almost to be the only oil now used, certainly with us, both the others, and especially the last, having in comparison but few favorers; though Nut Oil is that which the Italians adopted, while Linseed had the preference of the Dutch and Flemings.*"

Bouvier, Pierre Louis [12] *in Chapter IX p70*

"*Whether an oil might not be obtained of a drying quality and sufficient strength for oil painting which shall have the property of continuing permanently colorless remains for research. Yet, according to our present knowledge, it may be questioned whether oils do not uniformly change in color in proportion to their natural power of drying. But whether the oil of cotton, expressed from its greenish colored seed in the southern of the United States of North America, which is of a drying quality adapted to painting, be superior to other expressed oils in permanence of color etc we have not had an opportunity of trying*"

In *Field's "Chromathography", George Field, Windsor & Newton, 1864*[13]

The most important drying oils are those of linseed, poppy-seed, and walnut kernels; others are obtained from niger-seed, sunflower-seed, and hemp-seed. The first place is due to linseed oil.

In *A. Church's "The chemistry of Paint and Painting", 1890*[14].

Oils.—*The most commonly used oils are linseed and poppy oil. They are neither of them quick dryers, and are usually mixed with sugar of lead, manganese, etc., to hasten the drying. These have a tendency to affect the colors; but if one will have recourse to none but the pure oils, he must be patient with the drying of his picture. For this reason it would be well to use vehicles with the colors on the palette as little as possible—and that is against thin and smooth painting.*

Oil has the tendency to turn dark with time, thus turning the color dark also. The only way to reduce this tendency is to clarify the oil by long exposure to the sunlight. The early German painters used oil so clarified, and their pictures are the best preserved as to color of any that we have. But the drying is even slower with purified oil than with the ordinary oil.

It would be best, then, to use oil as little as may be in painting, and if you need a dryer, use it only as you actually need it in bad drying colors, and then very little of it.

In Parkhurst, d.b., "The painter in Oil" 1898 [7]

The adulteration of linseed oil with other oils may be recognised with more or less precision by means of several different tests. Most of these tests (oil of vitriol test, nitric acid test, etc.) produce reactions in which the oil and the acid acquire varied colours characteristic of different oils. These tests must be applied under exactly similar conditions of temperature, agitation, lapse of time, strength of acid, etc.; and even then, unless the experimenter is well-versed in the work, the indications obtained are sometimes perplexing and difficult to interpret. The amount of iodine absorbed by a given weight of linseed oil is also a measure of its drying power as shown in its capacity for absorbing oxygen. This 'iodine-value,' as it is called, is the amount of iodine absorbed from chloride of iodine in the presence of glacial acetic acid, by 100 grammes of oil. The iodine-value for linseed oil is somewhere near 200; the figures for walnut oil and poppy oil are always lower, while the semi-drying and the non-drying oils may not show half this value, and these are the oils likely to be used as adulterants. But such quantitative determinations can be properly performed only by the skilled chemist. There is another

In A Church's "The chemistry of Paint and Painting", 1890[14].

There is a need to correspond these descriptions with actual drying chemistry of the oils and oil paints on a molecular level, this is the main objective of this Thesis.

1.5 METHODS OF DETECTION AND CONTROVERSY

Preferred method of analysis of binding media has been GC-MS and, ever since the method by Mills (1960), there have been numerous developments for the same quantitation of individual Fatty Acids (FA) present in the samples from Paintings. It was thought that a fatty acid profile would be indicative of the type of oil used. Mostly based on the ratio of Palmitic (P) to Stearic (S) because these saturated FA were considered to be stable and their amounts would reflect the original ratio of the starting oil used for paint formulation. Based on this P/S ratio a classification (<1) was attributed to Linseed or(~5) to Poppy while Walnut would be in the middle (~3).

There are several attempts to modify this original method, to use new derivatisation methods, different extraction steps, and procedures for more complex systems; these will be dealt with in more detail in the 2nd chapter.

This method was first questioned when there was evidence of the formation of ghost images on framed paintings [15-17], so called because volatile Free Fatty Acids (FFA) were deposited on the inner side of a painting's display glass cage. Analysis of these accretions revealed that P and S FA's were leached out of the painting to deposit on the surface of the protective glass, this is to say we could no longer expect that the ratios based on the saturated FA's would reflect that they would stay unchanged in the ageing paint so, a new paradigm had to be built.

This new paradigm that we thought had to pass onto the characterization of all the chemical species involved as TAG's and their related species, oxidized or

hydrolysed into new species that had to be molecularly attributed in a developing paint. The history of the Fatty Acid profiles had to become a time-lapse video of TAG species that undergo molecular changes into new mapped species as they dry, age and mature into a dried oil paint system.

Further studies, revealed that not only unsaturated FA's were subject to Autoxidation, crosslinking and drying of the oil to a thin film, has it has been proved that saturated FA also change within the paint film.

1.6 Influence of research on Artist's Oil Paints

This work is an applied research on a field of interest, that needs to be dealt with as a dataset or database of Mass Spectra, that can contribute to the exact chemical knowledge of different formulations in oil paints as a base to attribute unknown samples of artist's paint to a specific characterized formulation.

Through the monitoring of historical accurate reconstructions of oil paint samples, from the raw material stages until it becomes a dried and maturing paint, these have to be understood on a molecular level to map the stage of drying of a given paint, and try to ascribe if it is, for instance, pure Linseed oil or a completely different formulation based on less drying oils.

We hope to introduce a manner of investigating the possible introduction of other types of oil into artist's oil paint formulation as an analytical method development.

1.7 AIMS AND OUTLINE OF CURRENTY STUDY

The critical review, in chapter 2, aims to elucidate all the previous and latest work related to the subject matter and in what way it brought light to this study. It is a thorough perspective in several subtopics from historical context with *Early studies of oil paint* ; *Common uses and primary sources* ; *History on the science of Oils and related subjects 1890 – 1940* ; *History on the science of Oils and related subjects after 1940* ; *Lipid Oxidation and autoxidation* ; *Detailed Studies (Molart/DeMayerne)* ; *Other Later Studies* to *Interconnection with other Paint Media* and *Model studies vs experiments on paintings*.

Chapter 3, The Raw Materials, offers a historical and chemical perspective on the latest known chemical characterization of all the different oils studied, and the historical and context in which they were mentioned in the historical sources.

Analytical Techniques, chapter 4, is a text guide of all the instrumental principles behind the advanced techniques herein used for unexperienced users. A small manual on the physical and chemical probes that reveals valuable chemical composition through a liquid sample of oil or paint.

Chapter 5, results and discussion, refer specifically to oil and paint analysis, respectively. They are both complementary and preview profiling of different FA's and TAG's profiles of specimens in samples based on Historically Accurate Paint Reconstructions. From their raw materials specification, and chemical characterization, to the development of new approaches of profiling different species, it's oxidation to drying and ageing particularly is presented by results based on molecular findings. As for the paints, an analysis of these formulations and time-based molecular evolution of the paint system is proposed.

Conclusions, in Chapter 5, are expected to reflect and conclude on the main results resulting from this study, and to mention these in the context of conservation

science and analytical chemistry. The main molecular changes occurring within the paint system as it dries, ageing of artist's oil paint, is a main goal.

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2 A REVIEW ON ARTIST'S OIL PAINT

ABSTRACT

This chapter is a perspective review of artist's oil paint research, with a focus on science based developments in physico-chemical analysis particularly within conservation science based research.

Some general introduction on the subject of oil paint studies and vegetable oils can be useful from several reviews and articles addressing oil science pertinent to artist's oil paint history of use and modern oil Paints [1, 2].

It reflects all the research that dealt directly with oil and oil paint from ancient times to rigorous technical description of drying mechanisms of different oils and oil mixtures. This research is intended for oil paint use, and to be used and processed for respective paints and paint technology. It is a thorough research and addresses the subject matter in terms of different points of view including some that are mentioned as references for historical purpose and context of discovery.

2.1 INTRODUCTION

Fatty acids, esterified to glycerol as triacylglycerides (TAGs), are the main constituents of oils and fats. The industrial exploitation of oils and fats, both for food and oleochemical products, is based on chemical modification of both the

carboxyl and unsaturated groups present in fatty acids (FA). Although the most reactive sites in FA are the carboxyl group and double bonds, methylenes adjacent to them are activated, increasing their reactivity. Only rarely do saturated chains show reactivity. Carboxyl groups and unsaturated centers usually react independently, but when in close proximity both may react through neighboring group participation. In these reactions, the reactivity of the carboxyl group can be influenced by the presence of a nearby double bond.

The coverage in this chapter is necessarily selective, focusing on aspects of fatty acid (FA), TriAcylGlycerides (TAG's) and lipid chemistry relevant to the analysis and industrial exploitation of oils and artist's oil paints. The emphasis is on fatty acids (FA) and triacylglycerols (TAG's) found in commodity oils and the reactions used in the paint and oleochemical industries. The practical application of this chemistry is dealt with, in detail, in other chapters. Current areas of research, either to improve existing processes or to develop new ones, are also covered, a common theme being the use of chemical (dryers) and enzyme catalysts. Recently, compounds of second-row transition metals rhodium and ruthenium and the oxides of rhenium and tungsten have attracted particular interest as catalysts for diverse reactions at double bonds. Recent interest in developing novel compounds by functionalizing the fatty acid chain is also mentioned. To date, few of these developments have found industrial use, but they suggest where future developments are likely. A number of recent reviews and books cover and expand the topics discussed here [1, 2].

We try to give a simple introduction to several subjects directly involved in the chemistry and historical uses of oils, the development of their analysis and development in their reaction chemistry. We focus in the autoxidation process leading to a mature film, as well as on the degradation processes leading to failure of

the film, leaching, metal soap formation, and dripping. We are trying to understand why this happens by yielding a chemistry answer to the conservation problems.

2.2 EARLY STUDIES OF OIL PAINT

Early descriptions of oil use in paint have been introduced in the 1st chapter to further investigate the oil paint system as a chemical entity in a process of molecular change with time from it's initial composition.

Discorides, or Theophilus, Cenninno Cenninni, Dossie or Eastlake, Merrifield or Church, Hurst or Parkhurst, are just a few of the beginners of the now emerging field of Lipidomics, or simple lipid/oil chemistry. These devoted men are the first who described the use, chemistry or analysis of oil or oil use in paint systems.

Giorgio Vasari (1511-1574) in his *Le vite de piu eccelenti pittori, scultori e architetti*, [Vasari G., 1568] declared that the technique of painting with oil was invented (or better was re-invented) in Europe around 1410 by the Flemish painter Jan van Eyck (1390-1441). The portrait of *Giovanni Arnolfini and his wife* (National Gallery, London, NG168), painted in 1434 by van Eyck, is considered one of the first and the best example of the new technique.

The Journal *Technische Mitteilungen für Malerei* was first published in 1884 by Adolf Keim, [van den Berg K. J., 2008]. It contains historically important technical data, provides examples of studies about oils and drying processes. Reviews and new texts are multidisciplinary discussions with chemists, artists, technicians, manufacturers and conservators. This opened ground for those other sources (ATSR presentation) such as Journal of the Oil and Fat Industries, which became Oil & Soap (which then became) the Journal of the American Oil Chemists Society

(JAOCS); Journal of the Oil and Color Chemists Association (JOCCA); or Farbe & Lack.

Specific Biliography in the paint instruction manuals from the Eighteenth Century are cited thoroughly in Leslie Carlyle's "The Artist's Assistant"[3]. Some other sources are cited in the extraordinary Annotated Bibliography, On Linseed Oil And Related Materials, covering from antiquity to 1972, By N. S. Baer and N. Indictor, published in AATA[4-6].

These are just some of the ever so valuable sources on the early stages of this painting technique we came to know as Oil Painting. These early records are accessed in the National Library (London), the Library of the National Gallery (London), and the library of the Courtauld Institute (London).

2.3 COMMON USE AND PRIMARY SOURCES

Some catalogs investigated gave an overview of produced and marketed oil colours by several *colourmen* [7-11]. Also the description of labnotes pertaining to the Windsor & Newton Archive at Hamilton Kerr Institute, in Cambridge, gave valuable insight on early formulation and source of raw materials. These labnotes are also valuable for oil preparation and processing methods intended to prepolimerise oil or release them from impurities, such as boiling, or washing, respectively, but also really important notes on the early use of driers in formulation, such as the first recipe for Manganoleates. [Leslie Carlyle, et al. 2012]

These resources will soon became available online, so as will be the Roberson's catalogue and archive now also at the Hamilton Kerr Institute (Cambridge UK).

Some of these recipes or processes will be addressed with more detail in the raw materials chapter, with some citations to frame early use and processing options.

2.4 HISTORY ON THE SCIENCE OF OILS AND RELATED SUBJECTS 1890 – 1940

Despite their impressive pedigree as important sources of energy, food and raw materials for human societies, the systematic study of plant or animal lipids did not begin until after the inception of the modern scientific revolution in the seventeenth century. One of the earliest landmarks in lipidology was the publication in 1666 of the "Hippocrates Chymicus" by the German alchemist, Otto Tachenius. In this book, Tachenius first suggested that fats contain an acidic substance – what we now call fatty acids (FA). He was also the first person to give a distinct definition of salt when he wrote that 'all salts are composed of two parts, of acid and alkali'. He further added that soap was the salt of an oily acid. Tachenius' statements were not accepted by the community of the time, and it was not until the French chemist Chevreul rediscovered the idea in 1816 through his laboratory work that Tachenius' definition of a salt was finally accepted [12].

The compositions of the major plant oils were gradually uncovered over the next few decades, with the suggestion that oleic acid and 'margaric acid' (a mixture of palmitic and stearic acids) were present as a mixture in vegetable oils [13]; the discovery of myristic acid in seeds of the Myristicaceae (Playfair, 1841[14, 15]); the isolation of lauric acid in the seeds of laurel, Laurus nobilis (Marsson, 1842 [16]); the preparation of linoleic acid from linseed (*Linum usitasissimum*) oil (Sacc,

1844[17]), although it was nearly a century before its structure was elucidated by Hilditch in 1939 as well as the isolation of first known hydroxy fatty acid, ricinoleic acid (18-1-OH), from castor oil [18]. A link with the principal membrane lipids of animals occurred when phospholipids, often called lecithins at the time, were also shown to be present in plant seeds (Töpler, H. (1861) *Landw. Vers. Sta.*, **3**, 85.), while lipases were first demonstrated in plant seeds by Muntz (Muntz (1871) *Ann. Chem. Liebigs.*, **22**, 472). The chemical structure of oleic acid, including the position of the double bond, was first described by Edmed (Edmed, F.G. (1898) the structure of oleic acid (in *Journal of Chemical Society*, **73**, 627) following an elegant series of oxidation steps. With this development, the stage was set for the systematic elucidation of the structures of each of the major plant fatty acids, although this task would take several more decades before it was finally achieved [19].

In 1816, the renowned French chemist Magendie found that dogs that were fed on a diet in which the only lipid component was olive oil did not live for more than a month [20]. Although olive oil contains about 7% linoleic acid, the high oleic/linoleic ratio would have led to marginal essential FA deficiency in these animals. This result, although not appreciated at the time, flagged up the nutritional importance of some classes of polyunsaturated fatty acids (which are lacking in olive oil) and of antioxidant vitamins, including the A and E complexes. The developments now recognized as significant were related in part to improved analytical procedures (chromatography and spectroscopy) and also to the growing appreciation of the biochemical significance of lipids one century later [21, 22].

With the benefit of hindsight, it is now possible to identify publications from the first half of the 20th century that contained ideas that became important in later decades and gave direction to many lipid studies. Why did these seminal ideas lie dormant for so long? [23].

Relatively little scientific work of note was done on plant lipids until the nineteenth century, when many important contributions were made by French and German chemists in particular. An early landmark occurred at the beginning of the nineteenth century when the Swiss chemist, Nicolas-Théodore de Saussure, demonstrated that linseed oil could condense with oxygen: this was an early hint of the existence of double bonds [24, 25].

Although the old masters had acquired extensive practical experience with the drying behavior of oils for paintings, exact scientific knowledge has been available only in fairly recent times, and even today not all processes and phenomena have been completely explained. For example, the chemical composition of poppy oil was not revealed until 1887 by the work of K. Hazura [26, 27]; yet Rubens is said to have used this material. The first reliable indices of the various characteristics of drying oils were introduced much later by Petruscheffski [28], Hesse, E. Täuber, and eventually through the comprehensive and thorough work of A. Eibner [28-36] or A.P.Laurie [37-39].

These were the first attempts of a science of oil paint, which would evolve slowly and would deliver some new insights, such as distinction between oils, other than linseed, through their analysis [40] relying on the hexabromide method.

The first two decades [41-65] of the 20th century devoted some interesting research with focus on the properties of oils and the intriguing process of drying. All sorts of chemical processing of these oils, were beginning to be understood by the methods of the time.

The drying process of unsaturated fatty acids FA used in painting is a complex sequence of physico-chemical reactions. It is almost certainly a combination of oxidation, isomerization, and polymerization, with oxygen, atmospheric water vapor and light playing key roles. As one would expect the different drying oils vary in their drying behavior from their diverse composition and so do the flexibility, color,

and permanence of the resulting compounds which form the film. The *oxins* formed in the paint layer are insoluble in the diluents used during painting. This is a very useful characteristic. Unfortunately, while the *oxins* age, they gradually form yellowish or brownish compounds which, to the painter's distress, may change or even disfigure his colours. The best *oxins* are therefore those that yellow as little as possible and yet have all the other desirable qualities.

There can be no question that linseed oil, provided it is pressed from high-quality seed and manufactured with care, dries to an exceptionally durable *linoxyn* film. However, *linoxyn* darkens notably during aging. Since Dürer's time, or even earlier, painters have been looking for other types of painting oils with better properties. The oils pressed from walnuts, poppy sees and sunflower seed proved to be most promising; Eibner, in particular, warned against the use of poppy oil, which, he said, seemed to gel rather than form a durable *oxin* film. Yet, since Rubens's time innumerable pictures have been painted with the poppy-oil colors without detrimental effect. The paint forms a complex physico-chemical system in which many of the dangers are rendered harmless. Although it can be proved that sunflower-seed oil, which closely resembles poppy oil, posses all the drawbacks of the latter, albeit the entire Russian production of artists' oil paints is based on this material, and the pictures painted with it are well preserved. Nevertheless, efforts to improve artits's painting oils are continuing.[1, 2].

Several references to new oils [61, 66-85] occurred during this research, and also some new references in the *Technisches Mitteilungen für Malerei* are still under revision at this time, too many to mention (possibly available online soon).

Attempts to describe autoxidation grew in time to several gradually more complex manners [73, 82, 86-126], most of them pioneering work from Long and his team [91, 96, 97, 101, 103, 104, 107, 108, 111, 113, 115, 121].

As an historical example an excellent article, by Dr. Kappelmeyer in the "Paint Oil and Chemical review", Jan., 1938, with regard to the chemistry of *Stand* oil production can be cited. Conclusions on the theory of polymerization are given as follows:

1. The usual type of polymerization observed with many organic compounds does not apply to drying oils under heat treatments where quite different conditions occur and prevail.
2. Formerly the theories held on the formation of four-ring systems have on further careful observation and study been found to be entirely unsupported by the facts as revealed, for example, in the chemical structure of cyclobutadiene or other cyclobutane derivatives.
3. The only satisfactory explanation as regards the polymerization of Stand oils, which will fall into line with the modern researches in this field of organic chemistry, is to be found in the possible dimerization of conjugate unsaturated systems similar to that of the diene synthesis.
4. On the basis of the diene synthesis, china wood oil and oititica oil can be polymerized, as these oils mainly consist of fatty acid triglycerides in which the carbon double bonds are present in a twofold conjugate system.
5. Such oils as linseed oil can only be polymerized after conjugate systems have been formed at a higher temperature by intermolecular displacement of the individual double bonds of linoleic and linolenic acids in accordance with the hypothesis by Schreiber.
6. Intermolecular re-arrangement requires little time hence thermal decomposition may take place as a side reaction in the case of such oils as Linseed and Perilla, and this may account for free fatty acids being formed in stand oils of this nature

7. From the experimental work so far carried out with respect to the polymerization of Stand oils one is led to the conclusion that the production of certain structural changes in the glycerides and fatty acids of a high molecular nature being formed which exhibit quite different colloidal properties

Dr. Kappelmeyer proposed a Diels-Alder diene synthesis as a basis for explaining the polymerization of vegetable oils, which is often referred to in the literature.

There are several references on this subject in JOCCA and, SOAP & OIL, and the other names for JAOCS, not to mention FARBE&LACK, but it was a too extensive study to review here.

A further landmark came in 1940 when T.P. Hilditch, in the United Kingdom, published the seminal and much reprinted volume entitled *The chemical constitution of natural fats* ([127, 128]) that could be a review of all previous work.

Topics such as essential fatty acids, eicosanoids, lipid biomembranes, lipids as bioactive compounds, and the importance of lipids as dietary components related to health and to disease, all became important [129].

2.5 HISTORY ON THE SCIENCE OF OILS AND RELATED SUBJECTS AFTER 1940

Meanwhile, in the early 1950s, A.J.P. Martin, who had jointly invented partition chromatography a decade earlier, teamed up with another young chemist, A.T.

James, in London. This duo presented a paper announcing the extension of partition chromatography to include a gas as the mobile phase at a Biochemical Society meeting in 1950. This marked the birth of gas-liquid chromatography or GLC, and the landmark paper describing the technique appeared 18 months later (James and Martin, 1952[130, 131]). Part of the impetus for developing GLC came from a colleague of James and Martin, who was keen to find an alternative to paper chromatography for the effective resolution of fatty acid mixtures. Since then, gas chromatography (GC) has developed rapidly, particularly during the 1960s, to provide both a preparative and an analytical tool for lipid, and especially fatty acid, analysis. The technique has now been applied in almost every area of analytical and biochemical research. Since the mid-1990s, hybrid techniques have been developed that have added a further dimension to GC analyses. Probably the most effective of these is the use a mass spectrometer as a detector rather than straightforward thermal conductivity or flame ionization detection systems. By employing a mass spectrometer in tandem with GC (called GC–MS), one can effectively do a two-dimensional separation and analysis of a lipid mixture.

Numerous additional physical and chemical techniques were applied to the analysis of plant lipids from the late 1950s and beyond[132]. Examples include the many forms of spectroscopy, such as ultraviolet, infrared, Raman and nuclear magnetic resonance; as well as, X-ray crystallography; hydrogenation; oxidation; and the various staining reagents that ranged from iodine vapor and fluorescein sprays to charring with concentrated mineral acids. Not all of these methods can be described here, but their use in the 1960s and 1970s has been comprehensively reviewed elsewhere (Hitchcock and Nichols, 1971[133]; Gurr and James, 1971[134]–1991; Gunstone, 1976[23, 135-138] [139, 140]).

The advent of lipid analysis through EI-MS was the pioneer of mechanistic studies of lipid TAG's and helped the identification of these classes as such, the best

protocols for molecular detection are firstly described in 1970's Methods in Enzymology Series [141].

Other techniques used for plant lipid analysis include the various forms of calorimetry, such as differential scanning calorimetry and differential thermal analysis that have been especially valuable in the study of the phase behavior of lipids, both in pure form and as mixtures *in situ*. There are several more recent manuals relating to the chemical analysis of lipids, which describe some of the contemporary techniques that are available (Christie, 1992–2003, 2003; Gunstone *et al.*, 1994; Grob and Barry, 1995; Baugh, 1997; McDonald and Mossoba, 1997; Hamilton, 1998) especially the recent volume for LC-MS [142].

Interesting references in the search for the composition and formulation of Oil paints, especially those used by artists, and also related Alkyds and other formulations with oils should be mentioned [126, 132, 143-189]. The cited bibliography gives a History behind the study of drying oils, it's use, and the first theories of the process of drying, as well as discovery or first analysis of different oils [126, 132, 143-189].

2.6 LIPID OXIDATION AND AUTOXIDATION

Lipid oxidation and autoxidation refers to the reaction of molecular oxygen with lipid molecules to add oxygen containing functional groups, they proceed through chain initiation, propagation and termination reactions. The oxidised molecules formed during oxidation are referred to as the primary oxidation products.

Secondary oxidation products are the molecules and fragments, which result from the decomposition or further reaction of the primary oxidation products.

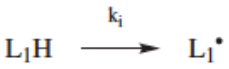
These are the most important reactions actually taking place during the production and processing of drying oils and they continue through the maturing and ageing of the oil paint to a dry film.

Oxidation of lipids has been studied extensively before, starting with the oxidation of free fatty acids (as their FAME's methyl esters) by the group of E. N. Frankell and further oxidized triacylglycerols (TAGOX's) [190, 191].

The classical kinetic scheme for the autoxidation of polyunsaturated FA (PUFA) was illustrated by Labuza (1971)[192], according to contemporary knowledge. On the basis of the original theory of Bolland [193-200], Bateman and co-workers at the British Rubber Producers' Research Association, it is accepted that autoxidation of PUFA occurs as a chain reaction that proceeds through three phases (Fig. 2.x), namely, (i) initiation, (ii) propagation, and (iii) termination. The classical lipid oxidation scheme proposed by these workers (Scheme 2.1) has been used since then to explain most of the observations and research findings, but remains unable to explain complex secondary phenomena and some of the details encountered in lipid oxidation studies (Chan 1987).

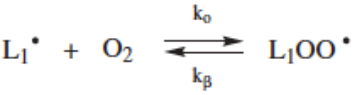
Classic Free Radical Chain Reaction Mechanism Of Lipid Oxidation

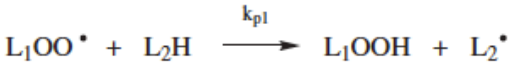
Initiation (*formation of ab initio lipid free radical*)

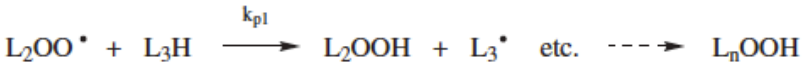


Propagation

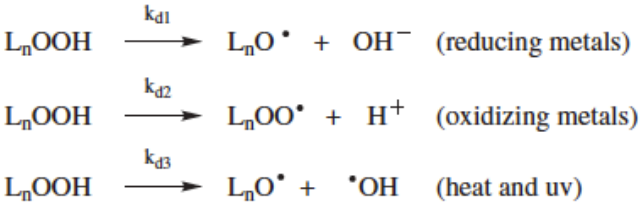
Free radical chain reaction established

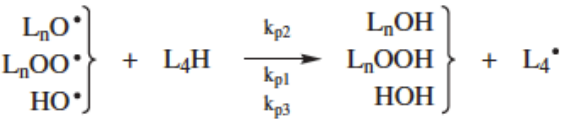


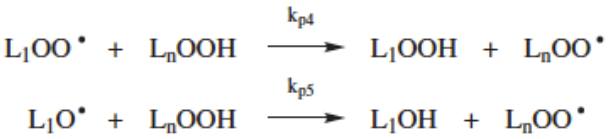


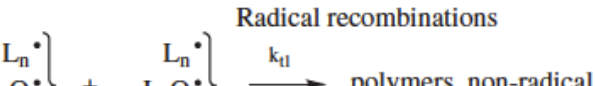


Free radical chain branching (*initiation of new chains*)

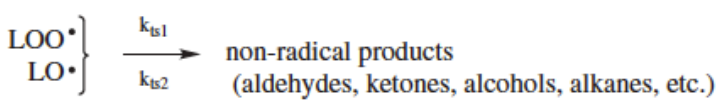








Termination (*formation of non-radical products*)



i - initiation; o - oxygenation; β - O_2 scission; p - propagation; d - dissociation; t - termination; ts - termination/scission

Part of the problem stems from considering lipid oxidation as precisely following classic free radical chain reactions. To be sure, lipids do oxidize by a radical chain mechanism, and they show initiation, propagation, and termination stages as are normally depicted (Scheme 2.1). However, the generalized reactions of the classic free radical chain reaction scheme are very much oversimplified and, because they do not portray the wide range of competing side reactions that contribute to the great complexities of lipid oxidation, they are often inconsistent with observed oxidation kinetics and product mixes.

The chemical mechanism of drying has been established as an oxidative radical chain reaction process, which has been summarized as follows:

1. A period of induction at the beginning of the reaction during which no visible change in physical or chemical properties in the oil is noticed; natural antioxidant compounds are consumed during this period.

2. The reaction becomes perceptible and oxygen uptake is considerable; discrete interaction of oxygen and olefins takes place followed by the formation of hydroperoxides.

3. Conjugation of double bonds occurs accompanied by isomerization of *cis* to *trans* unsaturation.

4. The hydroperoxides start to decompose to form a high free-radical concentration; the reaction becomes autocatalytic.

5. Polymerization and scission reactions begin and yield high molecular weight cross-linked products and low molecular weight carbonyl and hydroxyl compounds; carbon dioxide and water are also formed and are present in the volatile products of film formation.

It is now generally believed that the induction is slow at first but is autocatalytic and the rate increases steadily. The rate depends on the reaction conditions such as temperature, light, and traces of heavy metals or inhibitors in the oil.

To develop an integrated view of lipid oxidation we need to reconcile some common inconsistencies in proposed mechanisms, address some of the complexities that are important in directing downstream pathways and ultimate product mix. In doing so, attempts are made to bridge basic chemistry to applied lipid and oil paint chemistry. Old literature is cited liberally, despite current trends to ignore anything outside the previous two to five years, because the fundamental chemistry is still relevant, the early researchers in the field deserve recognition for their ground-breaking observations, and the information needs to be revisited to remind us of what already has been done to prevent "rediscovering the wheel."[201]

Given that drying oils are mixtures of complex compounds and that concurrent reactions occur simultaneously (such as polymerisation and degradation reactions), the mechanism of drying is thus quite complicated and not fully explainable with simple and uniform reaction schemes.

We try to summarize the important reactions in autoxidation applicable to drying oils, and siccative oils in particular, to help understand differences in product composition during curing and ageing.

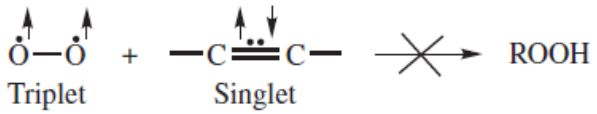
We have to stress the need to look beyond the classic radical chain reaction. Lipid oxidation mechanisms have been proposed based on kinetics, usually of oxygen consumption or appearance of specific products (e.g., LOOH) or carbonyls (e.g., malonaldehyde), assuming standard radical chain reaction sequences. However, when side reactions are ignored or reactions proceed by a pathway different from that being measured, erroneous conclusions can easily be drawn. The same argument holds for catalytic mechanisms.

Also, in light of the product and reaction pathway complexities presented in this review, kinetics of lipid oxidation will not be covered. That is not to say that kinetics are not important. However, kinetic analyses are always based on assumptions, and kinetic equations derived in different studies are often difficult to reconcile even in simple systems.

2.6.1 INITIATION

Initiation of lipid oxidation produces the *ab initio* lipid free radicals, L•. The initiation process is not well understood, nevertheless, it is not spontaneous.

Thermodynamically, oxygen can't react directly with double bonds because the spin states are different (addition to double bonds). Ground state oxygen is in a triplet state (two free electrons in separate orbitals have same spin direction, net positive angular momentum), whereas the double bond is in a singlet state (no unpaired electrons, paired electrons are in the same orbital and have opposite spin, no net angular momentum). Quantum mechanics requires that spin angular momentum be conserved in reactions, so triplets cannot invert (flip spins) to singlet states. Reaction then demands that the double bond be excited into a triplet state, which requires prohibitive amounts of energy (E_a = 35–65 kcal/mole). Thus, no direct reaction occurs.



To overcome this spin barrier, initiators or catalysts are required to start the lipid oxidation process by removing an electron from either the lipid or oxygen or by changing the electron spin of the oxygen.

The most common initiators are metals, light, heat, ozone, free radicals, lipoxygenase, heme or porphyrins.

Metals

It has been explained that the direct attack of oxygen on the double bond has low thermodynamic probability [202], and it has been considered that trace metal contaminants catalyze the initiation of autoxidation by producing free radicals through electron transfer. Alternative pathways are as follows, using cobalt as an example of a metal that can distinctly shift valence states in oxidation–reduction reactions:

1. Reduction activation of trace hydroperoxides in the system yields free radicals.

$Co^{2+}+ROOH \rightarrow Co^{3+} + OH^{-} + RO\bullet$

2. Direct reaction of a metal ion with oxygen:

$Co^{2+} + O_2 \rightarrow Co^{3+} + O_2^{-}\bullet$

The $O_2^{-}\bullet$ radical ion reacts readily with a proton to form the HO_2^{-} radical, which can initiate the chain reaction of oxidation.

3. Complex reaction of metal compounds with oxygen and subsequent formation of an HO_2^{-} radical.

$Co^{2+} + O_2^{-} \rightarrow Co^{3+} \ \bullet O_2$

$Co^{3+} \ \bullet O_2 + XH \rightarrow Co^{3+} + X^{-} + O_2^{-}\bullet$

4. Oxidation by electron transfer of the a-methylenic group by the metal ion.

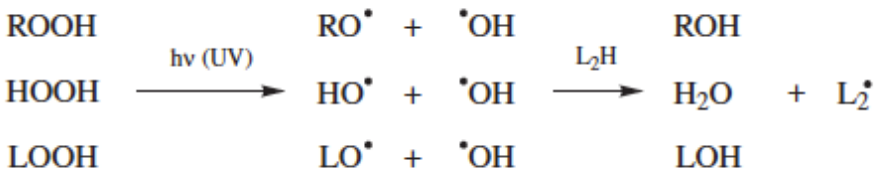
$Co^{3+} + RH \rightarrow Co^{3+} + H^{+} + R\bullet$

Redox-active metals are the initiators of perhaps greatest importance for lipid oxidation in oils, foods, and biological systems because they are ubiquitous and active in many forms, and trace quantities (<< micromolar) are sufficient for effective catalysis. Only metals undergoing one-electron transfers appear to be active catalysts; these include cobalt, iron, copper, manganese, magnesium, and vanadium. Many of the metal containing pigments drive this chain reaction, others slow it down, either way dryers were the primal factor in initiation for oil paints.

Light

Direct initiation of lipid oxidation by ultraviolet light, requires either direct deposition to sufficient energy to break covalent bonds or transformation of light energy to chemical energy that can catalyze the reaction. The activation energy E_a's for L-H and L-L scission reactions are higher than the corresponding bond energies (98.4 kCal/mol and 83.1 kCal/mol, respectively), and this photon energy is available only at wavelengths <254 nm .

The primary mechanism by which ultraviolet radiation initiates lipid oxidation is actually indirect, mediated through homolytic scission of any preformed hydroperoxides to generate the true initiators - LO•, HO• , and RO• - that abstract hydrogens from lipid molecules and form the *ab initio* L•.



The production of H_2O_2 during UV-irradiation in aqueous solutions should not be overlooked as another source of initiating radicals from ultraviolet light. H_2O_2 yields of 3.7 and 1.3 mmol per mole L and Ln, respectively, have been measured in solutions exposed to UV light [201], more than enough values for very active initiation of lipid autoxidation. This is rather relevant if we consider the procedure of water washing oils for artist's oil paint purposes.

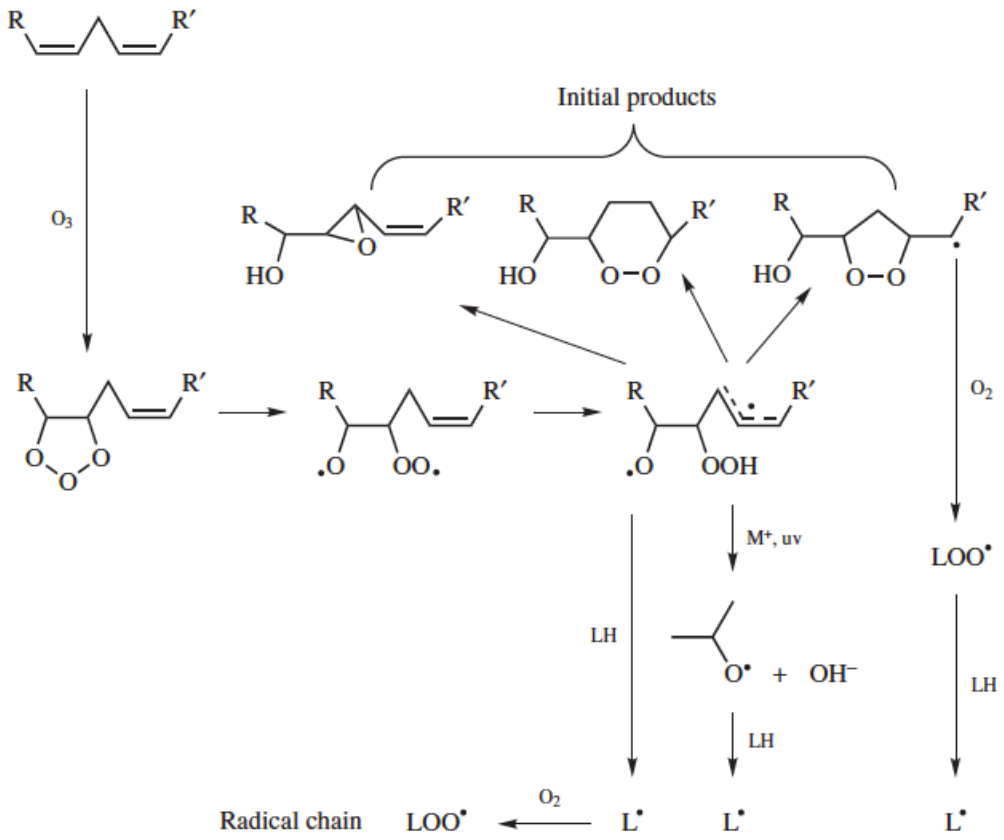
Heat

High temperatures (e.g., frying temperatures) have sufficient energy to break covalent C-C or C-H bonds in the acyl backbone to form a variety of lipid alkyl radicals [203], which then start the radical chains of oxidation. Moderate temperatures have lower energy, so act primarily by breaking O-O bonds in traces of ROOH or LOOH preformed by other reactions, particularly metals, lipoxygenase, or photosensitizers. The RO•, LO•, and •OH thus generated abstract hydrogens from neighboring lipids to form L• and initiate radical chains. As shown by the activation energies for the individual stages of lipid oxidation, LOOH decomposition and its subsequent contribution to propagation is the major catalytic effect of heat [204]. Effects of increased LOOH decomposition are amplified by increased rates of subsequent H abstractions by LO• and LOO•, which is reflected in the doubling of oxidation rate for every 10ºC rise in temperature.

Ozone

Ozone adds directly to double bonds in fatty acids to form ozonides that decompose to lipid alkoxyl and peroxyl radicals that abstract hydrogen to initiate radical chains. In the process, internal rearrangements within the original lipid

molecule(s) yield hydroxy epoxides and hydroxy epidioxides with 1,3- and 1,4-cyclic hydroperoxides:



Ozone preferentially reacts with the most unsaturated fatty acids present[201].

Free Radicals

All the initiating processes generate some form of radical that ultimately reacts with lipids to produce the *ab initio* lipid radical that starts the autoxidation chain.

Radical	Half-life with Typical Substrate, 10^{-3} M, 37°C	Ave. rx Rate, k (L $mol^{-1}sec^{-1}$)	F
$HO^{\bullet}$	10^{-9} sec	10^{9}–10^{10}	
$RO^{\bullet}$	10^{-6} sec	10^{6}–10^{8}	
$ROO^{\bullet}$	10 sec	10^{1}–10^{3}	
$L^{\bullet}$	10^{-8} sec	10^{4}–10^{8}	
$AnOO^{\bullet}$	10^{-5} sec		
$O_2^{-\bullet}$		~1	
$HOO^{\bullet}$		10^{0}–10^{3}	

	18:1	18:2	18:3	20:4
$HO^{\bullet}$	$\sim 10^{9}$	9.0×10^{9}	7.3×10^{9}	$\sim 10^{10}$
Monomer		8.0×10^{9}	8.0×10^{9}	
Micellar		1.3×10^{9}	2.5×10^{9}	
Non-allylic H	4×10^{2}	3.4×10^{3}	7.0×10^{3}	1.0×10^{4}
$RO^{\bullet}$	3.3×10^{6}	8.8×10^{6}	1.3×10^{7}	2.0×10^{7}
t-$BuO^{\bullet}$	3.8×10^{6}	9.1×10^{6}	1.3×10^{7}	2.1×10^{7}
	(*trans*) 3.3×10^{6}	(*trans*) 8.8×10^{6}		
aqueous	6.8×10^{7}	1.3×10^{8}	1.6×10^{8}	1.8×10^{8}
$ROO^{\bullet}$	1.1	6×10^{1}	1.2×10^{2}	1.8×10^{2}
$O_2^{-\bullet}$	no rx	no rx	<1	<1
	(MLOOH) 7.4×10^{3}			
$HOO^{\bullet}$	no rx.	1.1×10^{3}	1.7×10^{3}	3.1×10^{3}
		$<3 \times 10^{2}$		
O_3 —CCl_4	6.4×10^{5}	6.9×10^{5}		
—aq SDS	9.5×10^{5}	1.1×10^{6}		
$SO_3^{-\bullet}$		1.8×10^{6}	2.8×10^{6}	3.9×10^{6}
$GS^{\bullet}$	$<2 \times 10^{6}$	8×10^{6}	1.9×10^{7}	3.1×10^{7}
$^1O_2$	0.74×10^{5}	1.3×10^{5}	1.9×10^{5}	2.4×10^{5}
$O^{-\bullet}$	7.5×10^{2}	9.7×10^{3}	1.2×10^{4}	1.9×10^{4}
$NO_2^{\bullet}$	1.2×10^{6}	6.2×10^{6}	6.6×10^{6}	

[a]Aqueous solution.
[b]H abstraction from unsaturated alkenes.

This table lists rate constants for a number of reactions important in initiation of lipid oxidation[201]. For the most part, the rate constants speak for themselves. Nevertheless, a few comments need to be added.

Not surprisingly, hydroxyl radicals have the fastest reaction rates with lipids. However, HO• are so strongly oxidizing that their reactions are also very nonspecific, and they attack lipids indiscriminately at all sites along acyl chains [205]. These radicals then "migrate" (by intramolecular abstraction) to the doubly allylic H's in dilute monomer solutions, or abstract H's from doubly allylic sites of neighboring lipids in concentrated solutions, yielding the dienyl radicals that, when oxygenated to LOO•, become the main chain carriers.

2. 6. 2 - HYDROGEN ABSTRACTION

Hydrogen abstraction by free radicals is generally quite specific, occurring preferentially at allylic hydrogen positions where the C-H bond energies are the lowest (see Table). The active sites are the allylic carbon adjacent to a double bond, especially those bis-allylic to two double bonds with one on each side, such as carbon number 11 in a 9,12-octadecadienoic (linoleic) acid and this is even more the case in three double bonds such as in Linolenic acid (with two bis-allylic sites for abstraction).

	E (kJ/mol)	E (kcal/mol)[a]	Relative Ease of H Abstraction[b]
H–CH=CH_2	431	105	
H–CH_2–CH_2–CH_3	419	99	
H–CH_2–CH=CH_2	356	85	
R–**H**CH–CH=CH–CH_2–CH_3	322	77	
R(CH_2=CH)–HC**H**–CH_2–	310	74	1
R–CH=CH–HC**H**–CH=CH–	272	65	62
ROOH	377	90	

The order of reactivity is doubly allylic H's between two double bonds > singly allylic H's next to double bonds >>H's α to the COOH group > H's on methylene groups further down the acyl chains. The one exception to this "rule" is the hydroxyl radical, HO•, which is so electrophilic and reactive that it abstracts H's

indiscriminately from all positions along the acyl chain. The radicals formed, either migrate to the acyl carbon with the weakest bonding, i.e. the allylic H's, or abstract allylic hydrogen from a neighboring lipid molecule.

Older literature always presents the initial radicals in equivalent resonant positions with equal probability of forming hydroperoxides. The reactivity of drying oils is based on the mesomeric stabilization of the radical intermediate: the unpaired electron is delocalized over several carbon atoms, and less energy is required to eliminate the proton as illustrated below. The three resonant positions for linoleic acid or its ester and the three hydroperoxides resulting from these, are shown in reaction below[206]. Comparable resonant structures have been published for oleate, linolenate, and arachidonate [201].

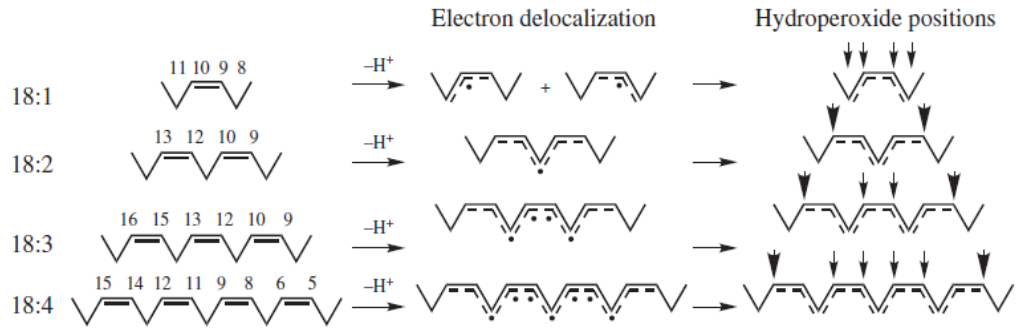


Figure 2-0-1 Hydrogen abstraction sites, delocalization by means of resonant structures of radical formed and correspondent locations of Hydroperoxide formation[201].

Translating this into observed behavior, isolated double bonds behave as if there were two separate resonant systems of equal probability, so oleic acid yields (C9 + C11) and (C8 + C10) hydroperoxides from the two resonance systems. In 1,4-diene systems, H abstraction occurs preferentially at the doubly allylic hydrogen between

the two double bonds, and the resonance system with the unpaired electron extends across both double bonds with electron density focused at the central carbon (11) and electron deficient positions at external carbons 9 and 13.

Data from Porter and colleagues [207-212] shows quite conclusively that both positional and geometric isomerism proceed through the delocalized allyl radical for oleate (see Map), or dienyl radical for linoleate and higher PUFAs (see Map), via alternating removal of the peroxyl oxygen by β-scission, migration of the free radical, and re-addition of the oxygen at a new carbon position or orientation. There can be interconversion of peroxyl position and orientation indefinitely as long as the radical is in the manifold. Once the peroxyl radical is protonated, it becomes fixed as the hydroperoxide, but can return to the manifold if the LOOH hydrogen is abstracted.

Hamilton reviewed[213] the proportion of each hydroperoxide isomer formed in his studies from simple methylated acids which complemented studies by Frankel[214, 215] later developed in review[190, 191].

Methyl Oleate PV= 72-123	8-OOH	26-28%
	9-OOH	22-28%
	10-OOH	22-24%
	11-OOH	26-28%
Methyl Linoleate PV= 72-123	9-OOH	48-53%
	13-OOH	48-53%
Methyl Linolenate V=134-1803	8-OOH	28-35%
	12-OOH	8-13%
	13-OOH	10-11%
	16-OOH	41-52%

Initiation Map of oxidation structures, derived from Bailey's[201].

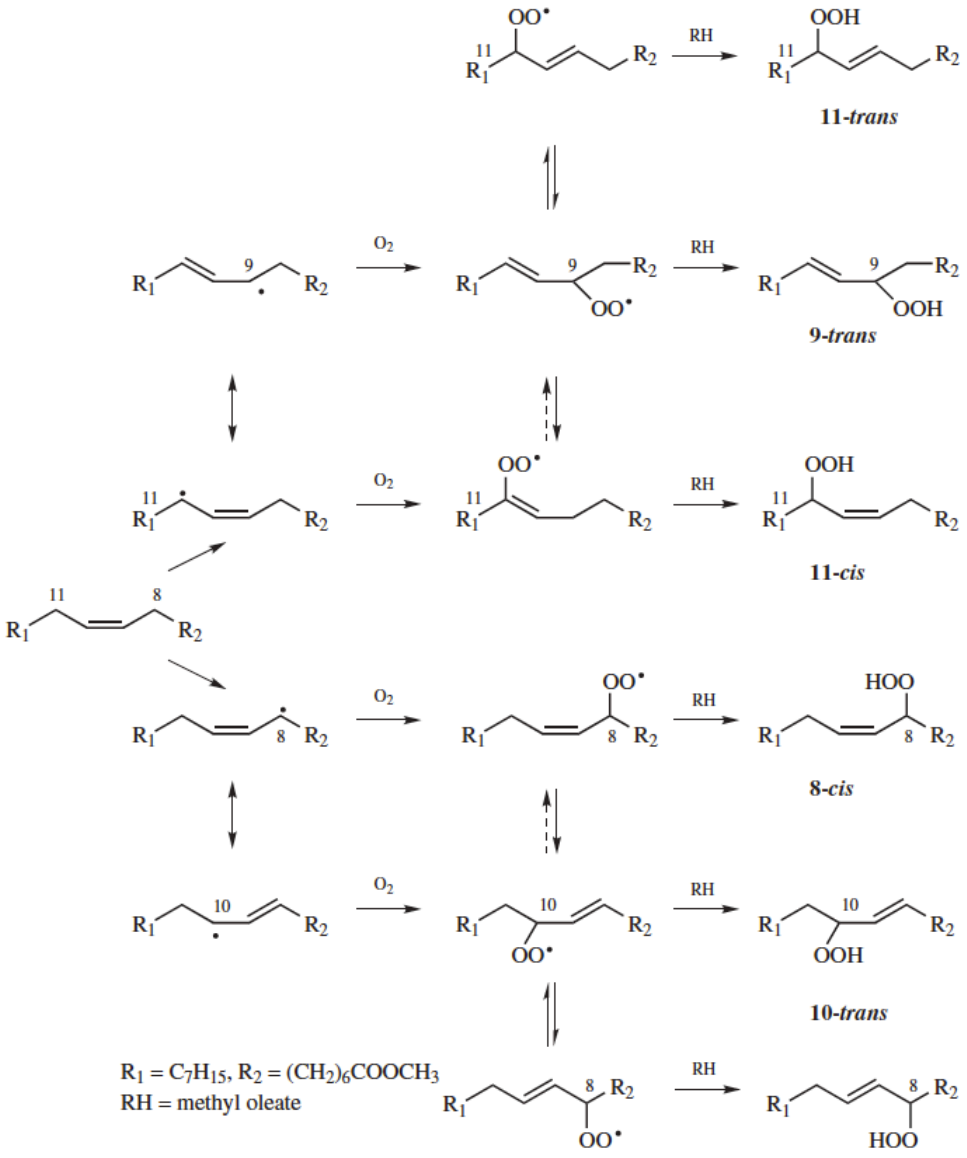


Figure 2-0-2 Isomerization and hydroperoxides in oleic acid

Lipid hydroperoxides were identified as autoxidation products of polyunsaturated fatty acids (PUFA) in the early work of Farmer and Bolland [193-200]. Farmer and his group developed the free radical theory of autoxidation, which involves an

attack of oxygen at the allylic position with the formation of unsaturated hydroperoxides. Although hydroperoxides are more stable than radical species, they are still weak oxidizing agents that decompose to peroxyl and alkoxyl radicals, leading to secondary oxidation products including aldehydes, ketones, alcohols, acids, and lactones (Benzie 1996).

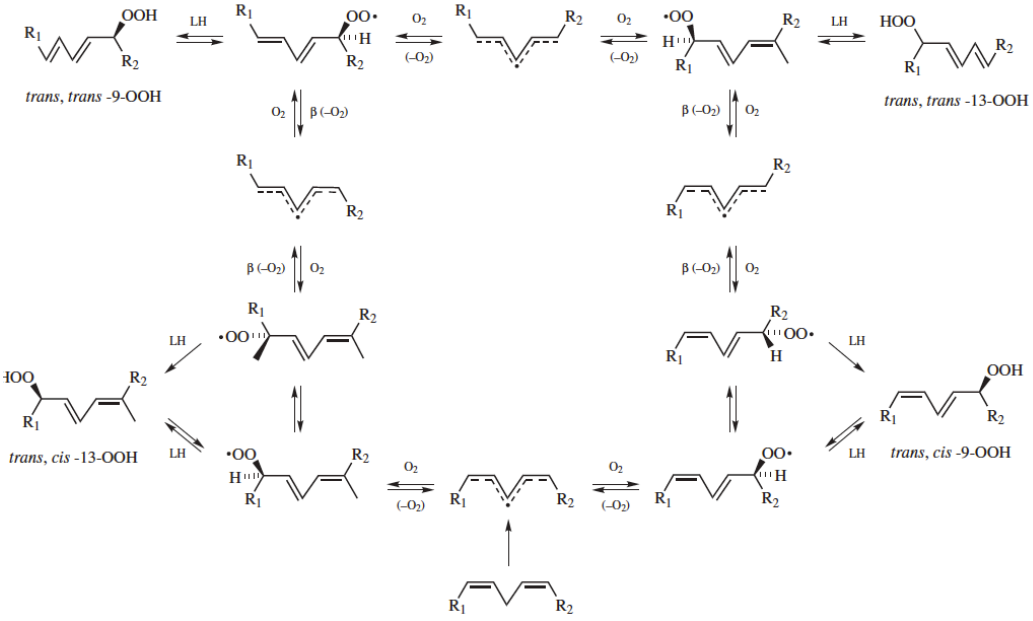


Figure 2-3 Isomerization and hydroperoxides for Linoleic acid

Isomerization

To explain different proportions of *trans,cis* and *trans,trans* isomers, Porter distinguishes thermodynamic and kinetic processes [216]. Kinetically, hydroperoxides will form whenever an abstractable hydrogen atom is available, but thermodynamically, the system equilibrium moves toward *trans,trans* isomers in the absence of good H donors, as in organic solvents. The observed isomer mix reflects the balance and competition between these two processes in a given system. When

good H donors are present, the *trans,cis* isomers kinetically form first. The H atoms can come from a protic solvent, an antioxidant, a cosubstrate, or the allylic hydrogens of the fatty acid chains themselves. For oleic and linoleic acids with only slightly bent chains, *trans,cis* formation is favored in oriented systems or at high concentrations that increase interchain contact. *Trans,trans* isomers are favored in dilute solutions, aprotic solvents, and at elevated temperatures in which there is less interchain contact and decreased H availability. With linolenic, arachidonic, and higher polyunsaturated fatty acids, the fatty acid chains bend back on each other, bringing double bonds and allylic hydrogens from opposite ends of the chain into proximity with the peroxyl radicals. When oxidized neat, higher PUFAs thus have an immediate internal H source and characteristically yield high proportions of *trans,cis* peroxides (kinetic products). However, when an H donor is lacking (e.g., low concentrations, aprotic solvent, elevated temperature), *trans,trans* cyclic hydroperoxides become dominant [207].

The *cis/trans* ratio changes with reaction system and with temperature. *Cis* isomers are enhanced by the presence of antioxidants such as tocopherol and by high concentrations of lipids, whereas *trans* isomers are enhanced by even mild heating, which reduces contact between lipid and potential H donors. Contrary to earlier reports, the *cis/trans* ratio does not vary with extent of oxidation unless reaction conditions are changing or H abstraction from LOOH is occurring, allowing LOO• to undergo *ß*-scission.

2.6.3. - PROPAGATION OR CHAIN PROPAGATION

Although there is quite general agreement on the mechanism of the chain propagation reaction, there is much less unanimity of opinion on the primary reaction to produce the radicals (indicated as R• above) responsible for the initiation of the chain reaction.

The classic free radical chain depicts propagation as proceeding directly and entirely by hydrogen abstraction. In reality, however, H abstraction by peroxyl radical LOO• is very slow (k = 36–62 L mol^{-1} sec^{-1}) (see table) and selective, abstracting only hydrogens with low bond energy (e.g., doubly allylic -CH_2-, thiols, phenols) [217]. Consequently, there is plenty of time for alternative reaction pathways to compete and change the direction of oxidation [218] yielding distinctly different products at different rates and having significant consequences to the ultimate mixture of products. Addition, cyclization, and scission reactions compete with H abstraction to reroute LO• and generate products and additional radical species.

Ultimately, radicals are always transferred between molecules by hydrogen abstractions, but the original LOO• may not be the propagating radical, and the product mix is much more complex than implied by the simple free radical chain. At least one of the reactions (e^- transfer) stops rather than propagates the radical chains.

Peroxyl radicals LOO• are the chain carriers in early stages of lipid oxidation. Competing reactions of LOO• include:

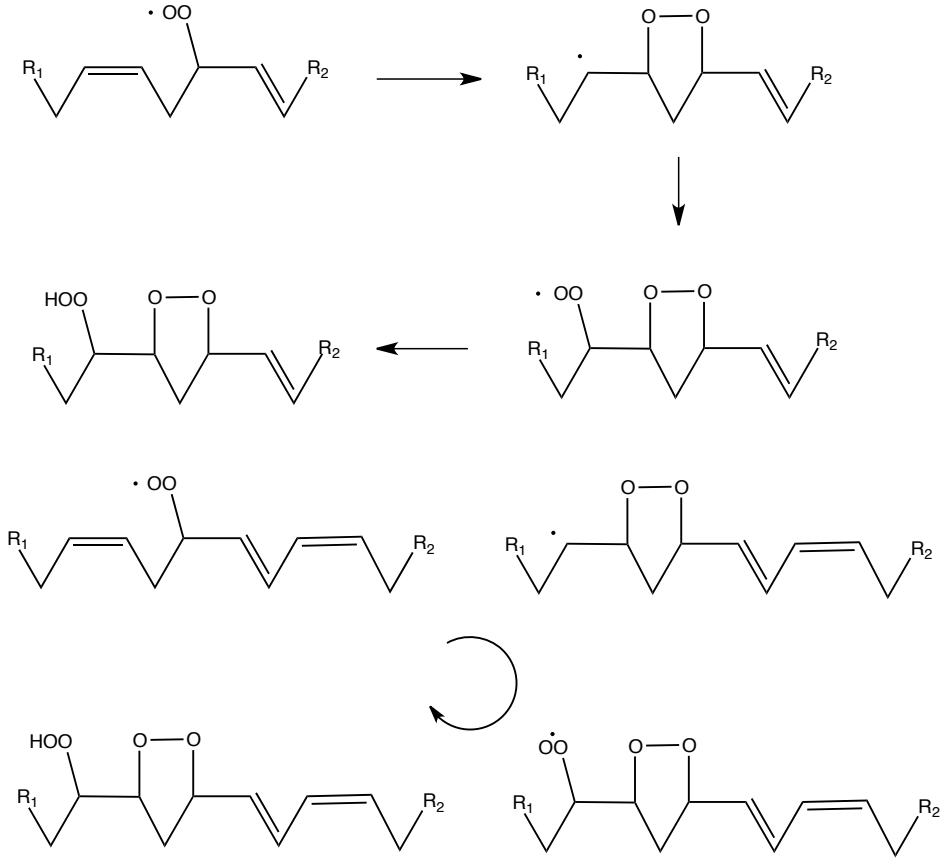
a. Atom or group transfer (H-abstraction)

Hydrogen abstraction is at the center of the classic free radical chain reaction scheme. Peroxyl radicals initially formed at any site on a FA pass the unpaired electron to adjacent lipid molecules by abstracting hydrogens from an allylic position or a hydroperoxide, this process repeats itself indefinitely until the chain is intercepted.

b. Rearrangement/cyclization of LOO

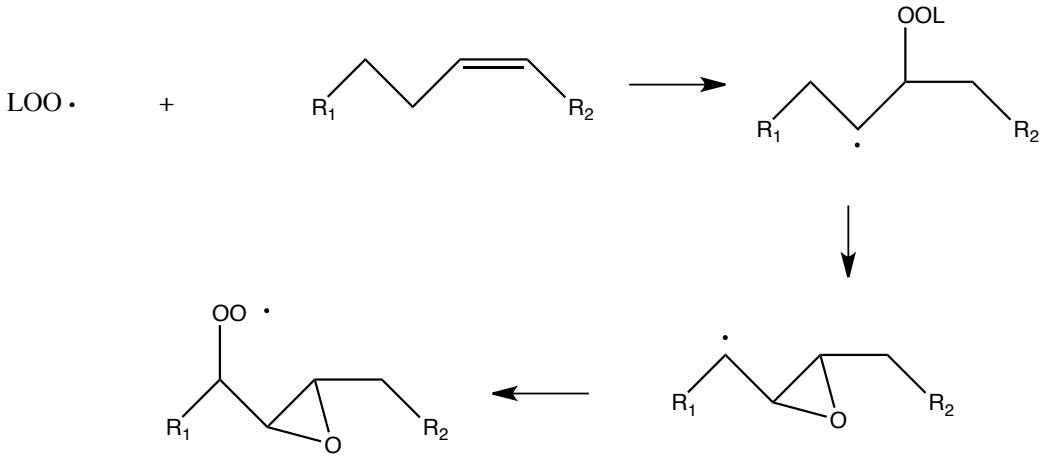
Cyclization requires a cis double bond homoallylic to a hydroperoxide.

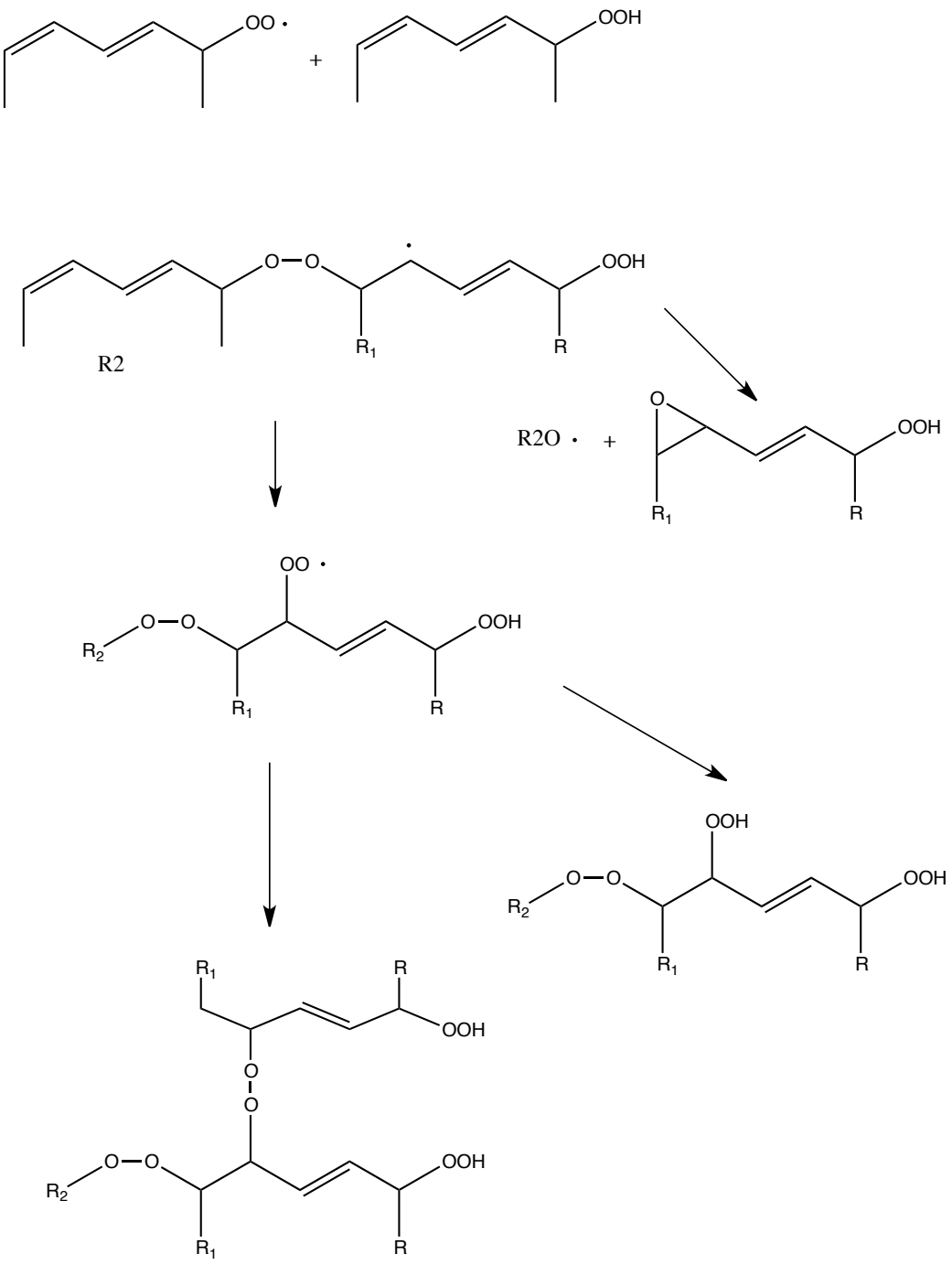
Here is an example from linoleic and linolenic acids:



c. Addition to double bonds (crosslinks, chain propagation)

The peroxyl radical addition becomes competitive when abstractable hydrogens are limited or when there is a double bond that is conjugated, terminal or 1,1 disubstituted.




OO ·
+
OOH
O—O
·
OOH
R1
R
R2
R2O ·
+
O
OOH
R1
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R2
R1
R
OOH
O—O
OOH
R2
R1
R
R1
R
OOH
O
O
O—O
OOH
R2
R1
R

d. Disproportionation

From tetroxides new alkoxyl radicals are formed rather than stable products.

e. β-scission (mediates isomerization)

Beta-scission of peroxyl radicals cleaves the C-O bond and releases O_2 leaving an alkyl radical behind.

f. Recombination

g. e^- Transfer (LOO• e^- LOO^-)

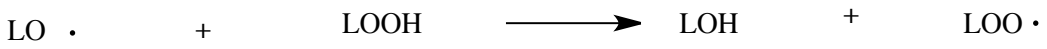
The first four reactions all contribute to chain propagation, although under different conditions. Disproportionation leads to branching and a shift in kinetics, and β-scission mediates isomerization, as was described in the previous section. Recombination (f) and electron transfer (g) terminate radical chains.

Alkoxyl (LO•) radicals are responsible for propagation of the radical chain during the very rapid oxidation that follows after the induction period ends. In the earliest stages of oxidation, LOO• cyclization and addition reactions can proceed before LOOH formation via H abstraction, but LO• can only be generated via LOOH decomposition, so their reactions become important as secondary events in oxidation. Nevertheless, because LO• react faster than LOO• by several orders of magnitude, LO• becomes dominant almost as soon as LOOH breaks down.

There are four major mechanisms for radical chain propagation by alkoxyl radicals. The mechanism dominating in a given system is determined largely by double bond structure, solvent conditions, and steric factors.

a. Hydrogen abstraction

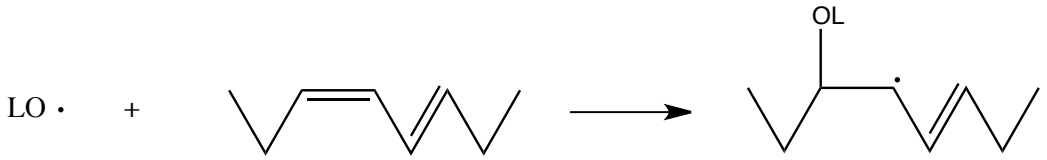
Alkoxyl radicals abstractions are very fast, but less selective; they abstract both allylic and bis-allylic hydrogens whereas peroxyl radicals abstract only the latter.



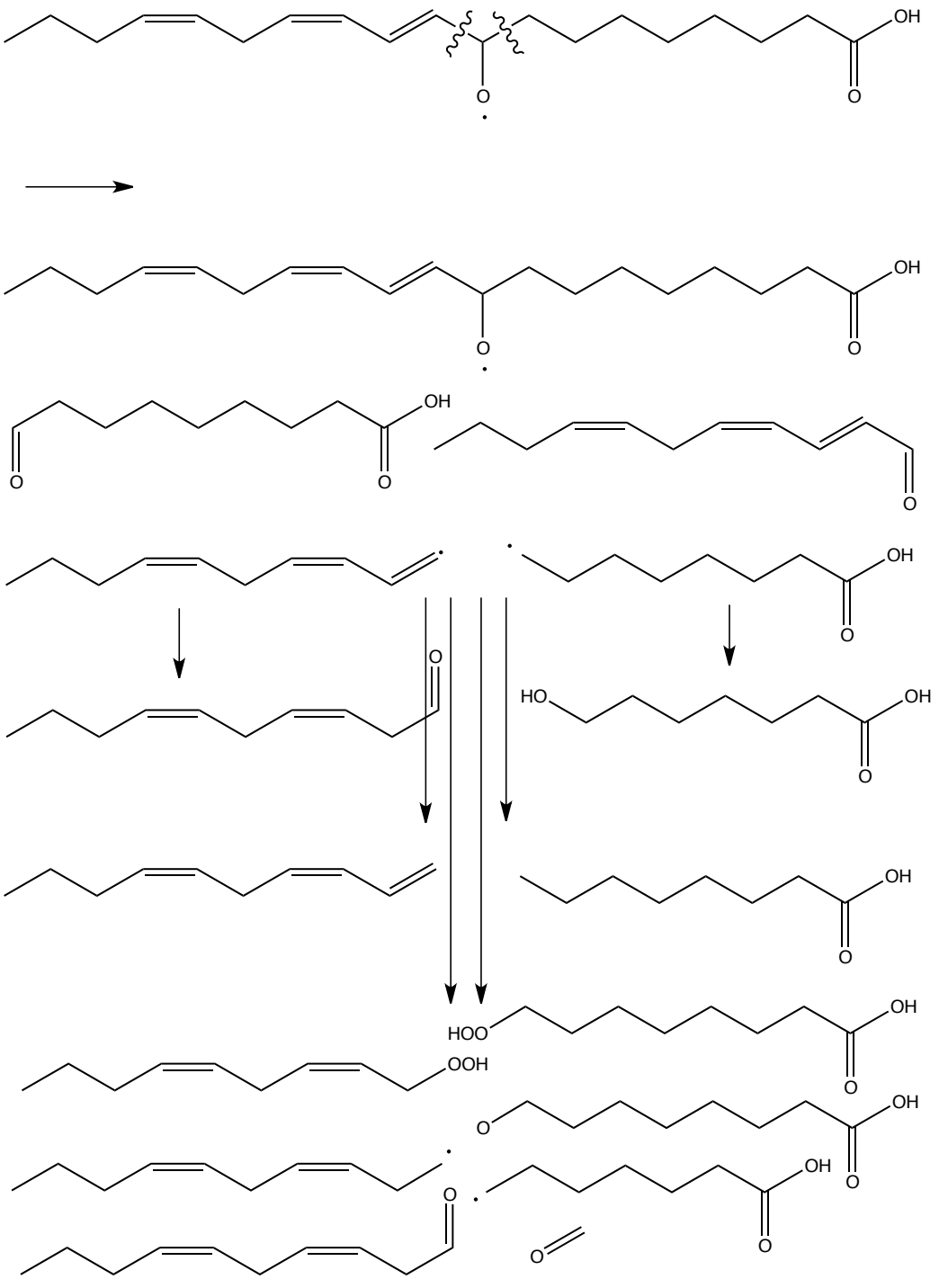
b. Rearrangements/cyclization

Cyclization of Alkoxyl radicals involves 1,2 addition to an adjacent double bond to form epoxides and epoxy-allylic radicals.

c. Addition



d. α-and β-Scission (fragmentation)



α and β Scission fragmentation

Propagation reactions of LOOH: mono- vs. bimolecular decomposition and chain branching

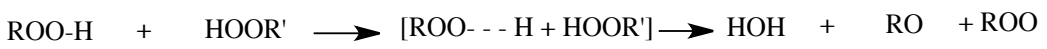
Ultimately, production of lipid hydroperoxides, even by indirect routes, becomes the major process driving the oxidation reaction forward. LOOH are the first stable products of lipid oxidation, accumulating in the absence of pro-oxidant heat, metals, hemes, ultraviolet light, peroxyl radicals, or antioxidant acids or nucleophiles. However, from a practical standpoint, one or more of these or other decomposing factors are nearly always present, so the low energy O-O and O-H bonds undergo a variety of scission reactions. Indeed a large proportion of LOO• and all of the LO• involved in propagation are not *ab initio* radicals, but derive from some form of LOOH decomposition.

As stated before redox-active metals break the O-O bond by electron transfer, hence LOOH decomposes heterolytically to generate radicals and ions. Reducing metals generate alkoxyl radicals (LO•) and hydroxide ions (OH^-), whereas oxidizing metals give peroxyl radicals (LOO•) and hydrogen ions (H^-).

Homolytic scission is much more effective in terms of lipid oxidation because two propagating radicals are released per hydroperoxide: LO• is more reactive and more selective than LOO•, and HO• is extremely reactive. HO• is rather unselective, abstracting hydrogen atoms all along the acyl chain, and it can also readily add to double bonds (still generating a radical).

Once hydroperoxides are formed, even in trace amounts, they can play a profound role in the autocatalysis[219]. Monomolecular decomposition yields two free radicals:

A bimolecular reaction, perhaps proceeding through intermediate hydrogen bonding, is more probable:



Either the monomolecular or the bimolecular decomposition serves to feed new radicals into the reaction to initiate the chain reaction of autoxidation. These radicals may further react through different paths. They may follow a radical chain mechanism or other well-known radical reactions, such as coupling or disproportionation.

The reactions may lead to the formation of dimers or polymers or may achieve cross-linking or chain branching, resulting in an insoluble, infusible film (i.e., drying). Apparently, the dominant reaction path depends on the temperature. At room temperature, mostly C-O-C bonds are produced, whereas C-C bonds are predominantly formed under baking conditions.

The free radicals may also undergo chain cleavage reactions. Low molecular weight by-products, such as water, carbon dioxide, aldehydes, ketones, and alcohols may be formed, which cause the odor and taste of the oils. For example, the strong odor of rancid soybean oil was shown to be caused by 2-pentylfuran found in oxidized oil in storage (23).

Chemically, the air-drying of a non-conjugated oil such as linseed is characterized by the adsorption of 12–16% by weight of oxygen.

TABLE 2. Lifetimes and Hydrogen Abstraction Rates of Various Radicals that Initiate Lipid Oxidation.

Radical	Half-life with Typical Substrate, 10^{-3} M, 37°C		Ave. rx Rate, k (L $mol^{-1}sec^{-1}$)		Reference
HO•	10^{-9} sec		10^9–10^{10}		191
RO•	10^{-6} sec		10^6–10^8		191
ROO•	10 sec		10^1–10^3		191
L•	10^{-8} sec		10^4–10^8		191
AnOO•	10^{-5} sec				192[a]
$O_2^{-\bullet}$			~1		193[b]
HOO•			10^0–10^3		194[b]
	18:1	18:2	18:3	20:4	
HO•	~10^9	9.0×10^9	7.3×10^9	~10^{10}	9,195
Monomer		8.0×10^9	8.0×10^9		196
Micellar		1.3×10^9	2.5×10^9		195
Non-allylic H	4×10^2	3.4×10^3	7.0×10^3	1.0×10^4	196
RO•	3.3×10^6	8.8×10^6	1.3×10^7	2.0×10^7	9
t-BuO•	3.8×10^6	9.1×10^6	1.3×10^7	2.1×10^7	197
	(*trans*) 3.3×10^6	(*trans*) 8.8×10^6			197
aqueous	6.8×10^7	1.3×10^8	1.6×10^8	1.8×10^8	198
ROO•	1.1	6×10^1	1.2×10^2	1.8×10^2	199–201
$O_2^{-\bullet}$	no rx	no rx	<1	<1	196, 200
	(MLOOH) 7.4×10^3				202
HOO•	no rx.	1.1×10^3	1.7×10^3	3.1×10^3	193
		$<3 \times 10^2$			200
O_3 —CCl_4	6.4×10^5	6.9×10^5			203
—aq SDS	9.5×10^5	1.1×10^6			203
$SO_3^{-\bullet}$		1.8×10^6	2.8×10^6	3.9×10^6	194
GS•	$<2 \times 10^6$	8×10^6	1.9×10^7	3.1×10^7	204
$^1O_2$	0.74×10^5	1.3×10^5	1.9×10^5	2.4×10^5	205
$O^{-\bullet}$	7.5×10^2	9.7×10^3	1.2×10^4	1.9×10^4	196
NO_2•	1.2×10^6	6.2×10^6	6.6×10^6		206

[a]Aqueous solution.
[b]H abstraction from unsaturated alkenes.

Figure 2-4 Cited From Bailey's industrial oil and fat products[201]

Recognizing conditions that shift chain propagation mechanisms in lipid oxidation:

a. Hydrogen abstraction from other fatty acid chains by LOO• and LO• is favored under conditions providing close contact between lipid chains without competition from other H sources—i.e., in aprotic environments such as neat lipids and the lipid interior of membranes, where lipid chains are closely associated. In solvents, H abstraction is favored at moderate lipid concentrations where enough substrate is present to supply hydrogen. However, at low lipid concentrations, cyclization or scission dominate, whereas at high concentrations, radical additions and recombinations become more important.

b. Hydrogen abstraction rates increase with solvent polarity and temperature— but under these conditions, accelerated propagation of lipid oxidation as in (a) must compete with H abstraction from solvent or other nonlipid sources and also with increased rates of scission.

c. Cyclization is favored when oxygen is limited and abstractable hydrogens are not available, i.e., in neat lipids, aprotic solvents, and low lipid concentrations. Cyclization is facilitated by polyunsaturation, radical formation at internal positions, and iron chlorides. As temperature increases, cyclization diminishes in importance as a propagation mechanism, because it is less affected by temperature than other propagation processes and because epi-dioxide peroxyl radicals have an increasing tendency to dimerise rather than abstract hydrogen.

d. Scission is favored over H-abstraction in polar protic solvents that provide the protons necessary to stabilize the scission products, but an excess of water shifts propagation to termination as protons for stabilization of secondary products are drawn from nonlipid sources and increased hydrolysis yields tertiary lipid oxidation

products. Scission also increases markedly with temperature as thermal energy facilitates bond rupture.

e. Propagation by addition is generally a minor reaction whenever hydrogen sources are readily available, but increases when abstractable hydrogens are limited in aprotic solvents, particularly when there is a conjugated double bond. Thus, addition becomes more important once oxidation chains are established. Addition also increases with lipid concentration, but under these conditions it also must compete with increased rates of H abstraction.

2.6.4. - TERMINATION

Termination is one of those not so clear terms used to imply that a process is coming to a close. In lipid oxidation, "termination" is an even fuzzier concept in that, from a practical standpoint, the lipid oxidation chains probably never fully stop. In addition, a specific radical may be terminated and form some product, but if this occurs by H abstraction or rearrangement, another radical is left behind so the chain reaction continues.

Free radicals terminate to form non-radical products by four major mechanisms:

a. Radical recombinations

b. A variety of cleavage reactions (when proton sources are present) to stabilize products

c. Co-oxidations of other molecules (radical transfer)

d. Eliminations

We now know that autoxidation is only one pathway by which oxygen and unsaturated acids interact and that we must also consider photo-oxidation with singlet oxygen and enzymatically induced oxidation. The latter occurs in both plants (mainly with C18 substrates to give jasmonates which are enzymatically oxidized from linolenic acid) and animals (mainly with C20 substrates to give eicosanoids). Though similar in that they all proceed through hydroperoxides, these oxidation reactions differ in detail.

2.6.5. -ANALYSIS OF LIPID OXIDATION

From the above, it is now clear that there is no fixed sequence of reaction pathways for lipid oxidation. Rather, the most active pathways probably change with the reaction system determined by the type and concentration of lipid, the solvent, phase distributions of catalysts, surface and interfaces, and numerous other factors. As a consequence, no standard assay will give a complete or accurate picture of the progress of lipid oxidation. Indeed, one of the difficulties in sorting out controlling factors is that so few lipid oxidation studies have analyzed products quantitatively as well as qualitatively, and even fewer have measured multiple classes of products simultaneously.

Several decades of detailed, painstaking product analysis, as discussed above, have now provided a reasonably clear picture of what kind of compounds are generated during lipid oxidation. But we still need coordinated quantitative analyses of all the classes of products to determine relative contributions of the various pathways

under specific reaction conditions. Such information would tremendously improve our ability to tailor oxidation analyses to individual systems as well as to design more effective antioxidant strategies.

Oxidation and other reactions taking place during storage/processing of vegetable oils can lead to deterioration of both their nutritional and sensory quality. There are also health risks associated with some of the originated products. High temperatures significantly enhance the rate and extent of lipids oxidation. The unsaturated fatty acids, both free and bound in triacylglycerols (TAGs), undergo free radical chain reactions in the initiation phase yielding the breakdown of highly unstable hydroperoxides. This leads to a variety of products, such as cyclic peroxides, epoxides, hydroxy-/oxoderivatives, aldehydes, hydrocarbons and/or polymers. In addition to TAGs, thermal processing also affects the levels of sterols, phenolics, lipophilic vitamins, and other minor components present in oils.

Lísa et al. developed a non-aqueous reversed-phase method using inverse gradient compensation for a CAD to determine the triacylglycerol composition of plant oils without external standard calibration [220, 221].

The retention in the Ag-HPLC is governed by the DB number. More DBs mean stronger interactions and therefore higher retention times. In practice, the situation is not simple because other factors have to be taken into account, such as the DB geometry (*cis* vs. *trans*), distances among individual DBs (from the conjugation to remote DBs), overall molecular structures, the type of stationary phase, the composition of mobile phase, the gradient slope, separation temperature, and the overall method.

In some early studies, oxidation product fractions were collected following preparative HPLC. Fractions were subjected to NMR analysis to identify functional groups, and then in some cases, gas chromatography–mass spectrometry (GC–MS) was applied after derivatization. In almost all studies, either the fatty acid methyl

esters (FAMEs) or the headspace volatiles, which result from FA decomposition, were studied by GC-MS.

Many excellent chapters and books have been written on lipid oxidation [222]. Studies of lipid oxidation have different perspectives by different authors: following early kinetics by oxygen uptake or hydroperoxides of TAG's production (LOOH), determining volatile products by gas chromatography (GC) or nonvolatile products by high-performance liquid chromatography (HPLC), or analyzing specific catalyst or antioxidant effects on oxidation. Oxidation mechanisms are then interpreted in the context of each study/author. There have been few attempts to integrate multiple approaches to lipid oxidation, and as a result, descriptions of lipid oxidation have been disparate and totally dependent on the individual aspect being studied. This can be quite confusing to anyone not deeply immersed in the field. It needs a wider context to provide an accurate overall picture of complex lipid oxidation reactions and quantitative analysis [139, 140, 201].

2.7 DETAILED STUDIES (MOLART/DEMAYERNE)

The NWO Priority Program MOLART - *Molecular Aspects of Ageing in Art* (1995 - 2001) - has been acting as a catalyst to bring together members of the research community on technical studies in art history, molecular sciences and conservation in the Netherlands. MOLART has been a recognizance program on molecular studies of art objects especially paintings. It has achieved an increased awareness of the potential of the molecular approach to understand complex phenomena caused by internal and external factors that change the quality of paintings. New molecular

level information was obtained that elucidates processes of chemical change in the natural products used as binding media, varnishes and in photo-chemically sensitive pigments. Historical studies of painting materials and painting methods revealed new information sources suggesting hitherto unknown paint material preparation techniques. MOLART has opened up the multidisciplinary field of molecular studies of paintings and their conservation[223].

The De Mayerne (2002-2007) program had the ambition to further develop and extend the knowledge and insights on molecular and paint technical structure of paintings obtained in the MOLART-project. The mission of the De Mayerne program was to establish a strong cross-disciplinary central research program on technical studies in art history and molecular conservation studies of art objects in the Netherlands. It intends to stimulate the participation of the natural sciences in the analysis and solution of art technical and conservation problems in paintings and related art objects. The research program intended to give a better insight in studio practices of painters, in ageing processes in works of art and the effects of conservation practices, which is of great importance for the preservation of our cultural heritage. Special attention was given to the multidisciplinarity of the projects and their application in art history, art chemistry and in conservation practice [224-231].

For the context of this thesis, the work on Linseed oil analytical studies are of notable importance, since it reviewed the basis of analytical approaches to oil paint analysis [232] as it has developed and resumed the model for the drying, maturing and ageing of linseed oil paint during autoxidation and beyond. It introduced TMAH derivatisation in GC-MS studies, and was one of the first to apply LC-MS to the time-based analysis of curing, drying and maturing of linseed oil paint. But also the solubility and leachable extracts of paint during cleaning of paintings is highly important and introduced new analytical verified procedures for extractable

components [232]. Not to discredit the complementary work of van der Weerd [229, 233, 234] using FTIR microscopy on the same subjects that is very relevant in this subject matter as he first monitored the first stages of drying of linseed oil from 0 to 90 hours, and also the best way to identify metal soaps.

The first volume of the Molart series explains the analytical chemical approach with unprecedented and thorough results on varnishes that have been used in painting. With an applied science based view of diterpenoids and triterpenoids, that can be too complicated for conservators, but a breakthrough for conservation science, a standard for others ahead to follow [228-230, 235, 236].

Pioneer work in this field by Van den Brink[236] stated: "MALDI-FTMS spectrum of a control sample of unaged egg between m/z 870 and 950. Two important clusters of peaks are observed, viz. m/z 875-885, and m/z 905-915. The peaks in these clusters are identified as sodium cationised C55, and C57 triglycerides respectively. The first cluster shows four fold to singly unsaturated triglycerides in high abundance. The peak at m/z 879 for instance is identified as a triply unsaturated triglyceride. The degree of unsaturation in the second cluster is higher, viz, six to two double bonds. The MALDI-FTMS spectrum of an identical sample that has been exposed to high light intensities for 16 days in comparison with that of unexposed sample shows that the relative intensities of the unsaturated triglycerides have decreased and that new clusters of peaks appeared at m/z 893-900, m/z 909-915, and m/z 922-932. The recurring mass difference of 15.995 between the peaks appearing and those depleted indicates that insertion of oxygen to the triglycerides has occurred. Insertion of up to four oxygen atoms is observed. Furthermore, the peak ratios in the clusters of the C55 and C57 triglycerides change upon ageing. Triglycerides with a high degree of unsaturation show lower relative intensities than those with a low degree of unsaturation. This suggests that oxygenation occurs as an increasing function of the degree of unsaturation."

These investigations, lipidomics, and Schilling's work [237-240], led us to aim at a better understanding of TAG oxidation processes in other oils than Linseed, which Jorrit van den Berg beautifully put into synthesis in his thesis and papers[232] complemented with Van der Weerd's FTIR analysis [229, 233, 234]. This project "molecular changes in drying processes of artist's oil paints formulated 1890-1940's" was built with all of these personal contributions in mind trying to build bridges at frontiers.

A talk in Portugal (*The 4th biennial meeting of the IPCR, 24th-25th November 2005, Lisbon, Portugal)* by Ester Ferreira gave us a full understanding of a cross section analysis [241], in a complete analytical way. The understanding of Metal soaps [242-245], it's formation and degradation were also a new topic, reviewed by Keune [224] and developed in her later work on cross section analysis through SIMS [246-248], such as are now dripping paints, a fertile subject to be fully understood. And the several darkening phenomena [249] that Van Loon also put into her thesis [230] or the grounds of Van Gogh studied by SIMS and nano SIMS [250] in the work of Marino.

A brilliant work by Keune *et al.*[251] was a collaborative one and made clear which techniques, or derivatization techniques, were useful to follow which forms - free, ester and metal bound FA – were in the TAG and TAGOX mixture in an oil paint.

The outcome of these extraordinary endeavors are mostly reflected in the series of PhD thesis of all the people involved [224, 225, 228-232, 235, 252, 253] in these projects not to mention all the papers that were submitted and peer-reviewed. It is a wealth of knowledge unlike any in this specific context. These studies were unique in the conservation science framework, and were able to open new perspectives with further studies of metal soap formation and mobility [248, 254-256], as well as trying to elucidate the mechanisms behind the "Paint diseases". These mechanisms are leaching, read lead formation, increased transparency, exudation, protrusion

formation, ghost images in framed canvases, mineralization inside metal soap aggregates, chemical reactivity and mobilization of materials inside, between and underneath the ground and paint layers. Besides, these studies contribute to the application of state of the art analytical instrumentation applied to art.

Ionomers were first mentioned on the thesis work of Corkery (1997) and in a paper [257-260] where he described metal soaps as liquid crystals, more typical for Lead soaps. The free reactive monocarboxilic acids were left as a "mobile phase" while diacids coordenated in an intrinsic network were described as "stationary phase". Yet we have to consider different stages in a maturing paint, like after the Hydrolysis stage. The MOLART Program has introduced these ideas in a model for metal soaps for paint, after first description by Boon et al in 1997 [261].

The advent of aluminium stearates [262] as additives in modern oils was a disadvantage because of the hygroscopic behavior that yield hydroxy derivatives [263] and the stearates were held responsible for water sensitive paints especially the water sensitivity of 40-50 year old paint due to micelles formed [264]. Nowadays, these can be probed through Al-NMR.

Up until now, studies are emerging to better elucidate the molecular changes in an oil paint system, for example, to find out which TAG's are not participating in crosslinking and it's relation to softening of the paint system or drip formation in paints who still pose problems for conservation and display of these paintings[265-267].

In the last decades, more importance has been attributed to the knowledge of Art technology source research within modern and contemporary materials in Art. This knowledge and understanding of how modern technologies and their developments had influence on the materials, for art making, their formulations and

transformations through ageing and maturing of the paint. Moreover, a conservation issue has arisen, modern art showed a bigger need for conservation-restoration treatments than it was expected. In particular, unvarnished oil paintings seem to be quite fragile and very sensitive to cleaning treatments. The cleaning of oil paint films can have several negative effects if it is not carefully carried out as Ken Sutherland verified [253, 268]. One of the main risks is leaching, that is to remove soluble material from the film leading to changes in both optical and mechanical properties. Since the low-molecular weight components are easily extracted (they function as plasticizers in oil films) [178], their removal could lower the elasticity of the film. Loss of material may also result in a matte appearance. During cleaning operations a slight swelling of an oil film due to the use of solvents is largely reversible. Differently, excessive swelling leads to disruption of the oil matrix and oil-pigment bonds [269]. Cleaning agents that affect pigments or even oil-pigment bonds can have similar swelling. This is especially true for polar solvents or aqueous mixtures [270]. Non-volatile solvents or reagents, for example resins soaps, might leave residues, which fill in voids and darken the oil film, providing a saturated appearance. Cleaning operations are however very delicate and they should be well designed after knowing the materials which constitute both the artistic layers and the dirt layers.

Among the first studies on contemporary paintings that is worth citing, are those of Willem De Kooning's works of art [271].

Of fundamental importance in the cleaning of contemporary paintings is the *Water sensitive oil Project*, part of 20th Century oil paints Project" carried out by the Rijksdienst voor Cultureel Erfgoed/Netherlands Cultural Heritage Agency (formerly ICN-Instituut Collectie Nederland) in collaboration with the Courtauld Institute of Art (London), the Getty Conservation Institute (GCI, LA) and the Tate.

This project investigates the cause of water sensitivity in well-bound manufactured oil paints used by artists from the late 19th century and the 20th century [Wijnberg L., 2007; Burnstock A., 2007; van den Berg K. J., 2009; Tempest H., 2010].

The water-and solvent sensitivity problem has been identified in a number of paintings and an international phenomenological survey of conservators suggests the problem is prevalent in works made in the 1950s and 1960s. Passages of soluble paint present particular problems for surface cleaning and display of these paintings, in particular works that are composed of flat planes of colour. Efflorescence and surface desaturation are other effects that have been noted in relation to the sensitive paints.

The project combined investigation of the phenomena and contextualization of the problem through investigation of case studies, and experimental replication of the effects seen in paintings in contemporary and modern manufactured oil paints. Some of the water sensitive paintings investigated are works of art from artists such as Karel Appel, Jasper Johns, Willem De Kooning, Paula Rego, etc.

The latest ICOM-CC Triennial conference in Melbourne brought to us, at least, five papers directly related with this investigations. Maybe the newest approach to the drying and autoxidation reactions from a kinetic point of view is the best example. A polymeric approach is used, which means that the network is described in terms of its *topology:* the connectivity between the cross-links of the network and the numbers of "units" between them. Like in a branched polymer molecule or polymer network, the monomer units of the network are the TAGs, esters of glycerol with long-chain fatty acids with one or more unsaturated bonds. They may become connected by cross-links that are formed by hydrogen abstraction or by addition reactions at the unsaturated bonds. Thus, at the start of the curing process, two fatty acids of two different non-cross-linked TAGs may become cross-linked by "consuming" the unsaturations and a network of two units is created. Subsequently,

at a remaining double bond, a third TAG may become connected, and so on [256]. Although the modeling of the autoxidation is straightforward, experimental values are based only on peroxide value PV and Oxygen uptake by the network; this model is worth following with quantitative values of TAG species and TAGOX for a more detailed picture of the drying mechanism.

By the same group of authors another paper tries to summarize new characterization information on synthetized metal soaps related to oil paint degradation [255] for a more accurate interpretation to understand this phenomena on a molecular level.

Also more related to the present thesis, is the work on slow drying oils (less siccative) as additives in modern oil paints through GC-MS analysis [272]. We hope we can relate to this work and compare diacid formation as an end result of ageing.

A collaborative work on 16th century and early 17th century English portraits gives a multi-analytical approach on the composition of paints that enables conservation retouching identification [273].

Another new perspective on the developmental stages in the life of a paint is the work of Bronken [267] which reflects a new approach to Softening and dripping paints which relate directly to our study of less siccative paints and the chemical difference associated with variations in the condition of the paint. The authors have proposed that a fraction of the oxidized oil binding medium phase separates from the paint due to a lack of anchoring points for the increasing amount of polar fatty acids resulting from the oil oxidation process [267].

2.8 OTHER LATER STUDIES

Although an array of methods has been developed to assess the extent of lipids oxidation, none of them has the potential to characterize all the oxidative changes of lipids in foods, much less in paint. Besides "classical" methods (some of them are internationally validated standards, which provide various indexes, such as a peroxide value (PV) or a *p*-anisidine value (*p*-AnV)), a number of selective instrumental methods are described herein. Various analytical techniques have been employed for oxo-lipids in their isolation and identification but also in describing their functionality.

Regarding all different classes of oxo-lipids GC-MS is specifically used for isoprostanes and other low molecular weight oxo-lipids, although it requires derivatization of solutes. In contrast, LC in combination with on-line-MS has proven to be well suited for analysis of intact oxo-lipids without (or minimal) derivatization.

The analysis of non-volatile oxidation products is typically performed by high performance liquid chromatography (HPLC) coupled to either ultraviolet detection (UV) [274] or, at present, more commonly by mass spectrometric detection (MS) [275, 276]. Volatile markers are the most widely monitored by gas chromatography-mass spectrometry (GC-MS) with either head-space or solid phase microextraction (SPME) pre-concentration step [277]. The preferred technique for the analysis of TAGs polymers, which are widely used as quality markers for frying oils, is high performance-size exclusion chromatography with refractometric detection (HP-SEC-RID) [278]. Relationship with oil paint is also valued in the work of Mallegol [279-282] and previewed by Porter[141].

In the recent decade, procedures such as matrix assisted laser desorption/ionization mass spectrometry (MALDI-MS) ([283, 284] , nuclear magnetic resonance (NMR) ([285]), head-space mass spectrometry (HS-MS) [286], and some other direct MS-based methods [287] (and also comparing sensory tests with electronic nose and GC-MS [288]) have been employed in this area.

Recently, direct MS analysis has been greatly simplified with the development of a number of new ambient desorption/ionization techniques [289], namely direct analysis in real time (DART) [290] and desorption electrospray ionization (DESI) [291, 292] being the most widespread. As demonstrated in previous studies, DART-MS is a powerful tool for analysis of TAGs and other lipids [293, 294] and[277].

Exceptional contributions have been made by the group Holcapek and Lísa [220, 221, 295-302] exploring LC-MS of TAG's in plant oils, in a number of different instrumental and experimental procedures, since early TAG profiles, to recent TAG's regio-isomer differentiation. These investigations enlightened our knowledge not only the basic procedures firstly with APCI ionization[302], response factors and comparison with other analytical methods (GC-FID, LC-ELSD, LC-DAD) [220] but also distinguished and compared NARP-LC-MS to novel SI-LC-MS , thought to overcome identification of number and position of double bond(s). Another contribution was the statistical evaluation by means of principle component analysis (PCA) of TAG's composition in 93 oil samples from 60 varieties of plants composed by 355 different TAG's [221], which is very valuable information.

The major challenge in analysis of DAGs and TAGs is the total number of molecular species present within any given oil, which results in multiple components appearing at the same molecular weight or truly isobaric compounds [220, 296, 300, 302]. Thus, the measurement of only the molecular ion species by MS, either the ammonium adduct ion or the alkali attachment ion [303];[304],

would be insufficient to uniquely identifying each component. Qualitative analysis can be carried out even when faced with complex mixtures of these neutral lipids if one employs tandem MS in three steps (MS^3) [304]. However, quantitative analysis is confounded by the isobaric nature of multiple species, and several strategies have been developed through which changes in molecular species can be assessed [305].

Notwithstanding, the following of lipid oxidation with respect to oil paint maturing, ageing and degradation has not yet been reviewed, and presents itself as a new approach to better understand the intricate oxidation processes involved in paint maturing with the premises of lipid oxidation in a more general sense.

Analysis of TAGs has increased in recent years and the advancement has been driven by the development of analytical technologies. Here we address oil composition with the help of analytical chemical techniques for determination of TAG and fatty acid profiles (FAPs) of vegetable oils using matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF/MS), HPLC-MSMS ($LC\text{-}MS^n$ on a triple quadrupole; or neutral loss scanning with or without LC; FT-ICR-MS high resolution Profiles of TAG's and FAP's) and gas chromatography–mass spectrometry (GC-MS). Considering the importance of TAG in its native form, rather than FAPs, special emphasis has been given to the TAG fingerprinting analyses of intact oils. MALDI-TOF/MS [306-308] also enabled calculation of the main fatty acids and their compositions in a simple manner from the TAG profiles; the results are found to be very similar to the prevailing methods of derivatization using GC-MS. Wiesman and Chapagain [306] also depict the potential of MALDI-TOF/MS as an easy, fast, and reliable technique to characterize the TAG and FAPs in vegetable oils.

Also Virgin Olive Oil (VOO) authenticity studies can bring a side view of the not so very different approach on TAG composition [309] although differentiation studies are based on minor Phenolic compounds.

It is well documented that TAGs are mainly affected by genetic factors delivered by the various oil crops and varieties [310]; however, the environment, agrotechnology, extraction process, and other factors may also contribute to the quality of the oil, and are associated with TAGs [311]. Another possible source of variability of the TAG composition in a specific vegetable oil is adulteration of the product – replacing high-cost ingredients with lower grade and cheaper constituents.

Since fatty acids do not present alone in oils, there is an increasing interest in their arrangement within the oil, namely – the TAG's profile. It is suggested that the TAG structure, and not only its fatty acid profile (FAP), is of special importance regarding its physiological effect [312]. Many studies concerning the metabolism of lipids and their effect within the human body emphasize the importance of the TAG structure–activity relationship. The molecular composition of a TAG mixture is typically very complex due to the combination of a variety of fatty acids, differing in their chain length, degree of unsaturation, and distribution between the *sn*-1, *sn*-2, and *sn*-3 position of glycerol backbone. Therefore, analytical methods enabling determination of both the FAP and TAG composition are greatly valued. Considering the importance of the TAG structure of oil in its native form, rather than only FAPs special emphasis has been given to the TAG fingerprinting of vegetable oils [306, 313].

Non-enzymatic methods for such structural analyses of lipids have been reviewed by Kuksis [314] as well as the analysis of oxidation within Glycerol esters [315].

Mass spectrometry is now an indispensable tool for lipid analysis and is arguably the driving force in the renaissance of lipid research. In its various forms, mass spectrometry is uniquely capable of resolving the extensive compositional and structural diversity of lipids in biological systems. Furthermore, it provides the ability to accurately quantify molecular-level changes in lipid species associated with changes in metabolism and environment [316].

The changes in neutral glycerolipids are now active fields of research to better understand these quantitatively more than qualitatively [305, 317].

The primary oxidation products are triacylglycerols (TAG) containing unsaturated fatty acyl hydroperoxides formed by a chain process occurring through free radical intermediates. The Rancimat method is currently the golden standard for measuring the oxidative stability of oils and fats. In one report, easy ambient sonic-spray ionization mass spectrometry (EASI-MS) is demonstrated to function as a direct (no pre-separation or sample preparation steps), fast and accurate method to monitor oils and fats oxidation, providing detailed overviews of the most immediate TAG hydroperoxide products[289].

Photooxidative oxidation can now be prevented by the use of hindered amine light stabilizers (HALS) which consume radical species, such as alkoxyl and peroxyl, in a process called Denisov cycle [318].

Despite their compositional complexity, TAG's comprise a large number of isobaric species that cannot be distinguished by conventional low-resolution mass spectrometry and therefore in-depth MS/MS tandem analysis was required for their accurate quantification. Recently, the progress in high-resolution mass spectrometry is changing the concept of lipidome characterization. Because exact masses of isobaric species belonging to different lipid classes are not necessarily identical, they can now be distinguished and directly quantified in total lipid extracts. By streamlining and simplifying the molecular characterization of lipidomes, high resolution mass spectrometry has developed into a generic tool for cell biology and molecular medicine [319] that can greatly improve our knowledge of oil paints.

The recent developments in new core-shell stationary phases for RP-HPLC can provide an optimized method for the analysis of TAG species. It is applied to some of the oils in this study and is a good source for review and comparison [320]. As

one method of choice (HPLC-ESI-MS) for the analysis of TAGs composition and evaluation of auto-oxidation and oxidation products of camellia seed oil [321] can also give a method of following oxidation.

Software has been developed for avoiding the laborious and time-consuming data treatment for HPLC-MS-MS data of lipid samples, one of which is described by Cvacka[322]. Named *TriglyAPCI* it allows interpreting spectra of single compounds, mixtures, or incomplete spectra lacking one of the diagnostic ions. The fragment intensities are used to distinguish regio-isomers. But an efficient chromatographic separation of TGs is shown to be crucial for correct spectra interpretation and avoiding false positive results[322]. The whole Bioinformatics developments applied to lipid science has a great role nowadays like the LipidMaps initiative as depicted in several book chapters[323, 324].

2.9 INTERCONNECTION WITH OTHER PAINT MEDIA

A lot of research has been done in Alkyd paints because they serve as a simple model and can be subjected to ageing test quite easy[325].

The drying process of Alkyds includes: (1) a solvent evaporation step (physical drying) and (2) autooxidative drying (chemical drying), which includes an induction period, oxygen uptake, peroxide formation, and peroxide decomposition. Radical reactions produce a crosslinked polymer network [326]and unsaturated fatty acid side chains are mainly responsible for autooxidation reactions. The reaction is initiated by hydrogen abstraction of the doubly-activated methylene group. The resulting radical R• reacts with O_2, leading to hydroperoxide species (ROOH).

Hydroperoxides decompose in a metal-catalyzed reaction to alkoxyl (RO•) and peroxyl radicals (ROO•). The radicals recombine and produce a three-dimensional polymer network, which is responsible for curing the paint film [327], [328], [329], [330] ,[331, 332], [333], [334],and [279, 280, 282].

On exposure of films of oil or alkyd media to artificial weathering conditions, there is a loss in weight, an increase in the oxygen content, in the carbonyl absorption in the infra-red spectra and in the amount of cross-linked polymer. Aldehydes, formic acid, carbon dioxide and water are evolved from the film, and acids of high oxygen content extracted by the water. Under exposure to shorter wavelength ultraviolet irradiation the phthalic acid of the alkyd resin is destroyed. [335]

W. J. Muizebelt and co-workers did brilliant work on the model of alkyds, and the autoxidation reaction with air oxygen. Since the crosslinking reactions are hard to follow in an actual coating system, they use model compounds. These are (m)ethyl esters of unsaturated fatty acids (linoleic and ricinoic) which contain the reactive substructures responsible for crosslinking of an alkyd. In this work, the oligomeric mixture resulting from crosslinking was studied with various mass spectrometric (MS) techniques. Consistent results were obtained with all MS ionization techniques: oligomers yielded signals consisting of groups of peaks 16 mass units apart, pointing to a series of oxygenated homologues. Differences were encountered, however, in the number of oligomers that could be seen with the various techniques. From the detailed ESI-MS and DCI-MS spectra, a difference in the crosslinking mechanism between conjugated and non-conjugated fatty acids becomes apparent (radical addition to the double bond or recombination of radicals, respectively). Also, using a mixture of the linoleic and ricinoic esters, mutual reaction between these two types of fatty acids can be demonstrated [336, 337].

The group of Maria Perla Colombini recently published new Alkyd studies based on the influence of organic and inorganic pigments in the drying mechanism, they concluded however, that the chemical drying of the paint, via auto-oxidation, is almost independent of the pigment and only depends on the alkyd content[338], more detailed studies followed [339, 340] that permitted to establish and optimize the identification of TAG's in complex archaeological organic residues[341] and vegetable oils [320].

Mixtures like Megilps, additions like carnauba wax, and other mediums will not be covered here but should be present as possibilities in medium identification or attribution.

2.10 MODEL STUDIES VS EXPERIMENTS ON PAINTINGS

In this subject area most references for the use of model substances are directly interconnected with the model substance, like ethyl linoleate or other paint media, like alkyds, that can act as model.

These studies have been mentioned before such as the work of Muizebelt [330, 336, 337, 342], and previous work of Mallegol and co-workers[279-282].

Several studies on the drying mechanism of Oil or oil Paints are mostly based on "model studies". As an example, the first studies were based on methyl linoleate to follow the allílic carbon, the formation of it's radical, the subsequent stabilization

from the adjacent double bonds and all the other possible reactions from a complex mixture of Tag's in an oil "paint media".

These studies all aim at a better knowledge of the formation of a cross-linked network via oxidative polymerization. Essentially, all of the polyunsaturated fatty acid groups in the triglycerides disappear within a few years. This was only achieved by the "simple" studies accomplished with "model" molecules, and consequently, the oil finally dries, forming at first a very elastic skin, called *linoxyn*. This is actually a rigid material, but, because of its content of nondrying constituents which act as plasticisers (such as liquid and/or semi-liquid fractions of saturated triglycerides), the polymeric matrix acquires a particular flexibility. If we could not have access to simple Model molecules that already form too many isomers it would be almost impossible to achieve slowly an approximation to the very complex mixtures formed within the molecular drying of oil paints. After the formation of dicarboxylic acids and the evaporation of volatile products (aldehydes, ketons, etc.), the *linoxyn* skin becomes very fragile and brittle with the resulting cracks and further powdery consistency. This happens because of the rupture in the polymeric network with hydrolysis (most of the paints will be subjected to this within 50 years time) and the formation of hydrophilic compounds (such as carboxylates and hydroxylates), which helps water penetration into the matrix, initially hydrophobic and apolar. Complexation with metals and soap formation as a means to stabilize free acids in the network, have only recently been an important topic in art analysis as well as fluidizing paints and it's origin [266].

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3 THE RAW MATERIALS - OILS

ABSTRACT

This chapter reviews oils research as raw materials particularly for artist's oil paint, with a focus on science based developments in this area, and historical context relevant to the oils used in Artist's Oil Paint Formulation. Chemical proprieties are presented whenever previously established by several methods to better characterize the starting materials.

3.1 INTRODUCTION

Vegetable oils have been used since early times as raw materials in various forms. They are mostly energy stores for flora. Here we address them in a singular way from seed to oil, as unprocessed oil to be used and processed for oil paint.

Oil fruit yielding pulp oils are quickly processed after harvesting in close vicinity to their place of origin. Therefore, only oil seeds are stored. It is very important to lower the water content of seeds to a degree that stops all biological and enzymatic

activity. The formation of free fatty acid (FFA) is a way to measure the influence of the water content on the biological activity.

Despite good drying and good storage conditions, the aging of seeds continues, so the storage time can't be unlimited. During aging, positive effects for oil processing such as decreasing enzyme activity go hand in hand with negative effects such as a tendency toward peroxidation. Measuring a seed's ability to germinate is a good method of estimating the stress seeds are exposed to during storage. The two are inversely proportional. Several papers exist that correlate germination ability with storage duration, storage temperature and relative moisture.

There is the commercial aspect, namely that seeds that are well stored and transported, have fewer FFA and are less oxidized, thus yielding higher prices. There is also the quality aspect. Damage to the oil can't be totally removed during processing, and in any case, such oils require much more severe treatment. It is recommended that seeds be well ventilated if stored for a long period of time. A temperature range of 4-1°C helps because it dramatically slows down all biological aging processes; furthermore insects can no longer tolerate these living conditions.

In contrast to oil fruit, oilseeds are less sensitive toward spoilage because they are much more stable mechanically and have a much lower water content. It is therefore much better to store the seeds than it is to store the oil because the seeds are equipped by nature with protective mechanisms and protective substances. These mechanisms have to ensure that the energy reserve of the seed, namely, the fat, can survive the time till germination and that may be very long. Even seeds of well-known plants that are not at all connected with oils and fat production contain triglycerides, sometimes in surprising amounts.

Most commodity oils contain fatty acids with chain lengths between C16 and C22, with C18 fatty acids dominating in most plant oils. Compilations of the fatty acid composition of oils and fats and less-common fatty acids are available. The fatty acid "profiles" (FAP's) became the identity card of each oil [1-8] but recently there is also interest in TAG's profiles [9].

When the drying characteristics of oils were relied upon as the sole (or major) way to obtain a dried varnish-based coating film, oils belonging to the linolenic or conjugated acid groups, such as linseed, perilla, tung, oiticica, and highly unsaturated *winterized* fish oils, were of the prime interest to coating formulators. Since the 1950s, with the advent of synthetic resins, particularly, alkyd resins, it has become possible to make considerable use of oils with poorer drying characteristics. Semidrying oils such as soybean oil, safflower oil, and sunflower seed oil have become viable as raw materials for making "chemically drying" paints. Nondrying oils, such as coconut oil, are also used in coating materials. However, their function is primarily that of a plasticizer rather than of an active component for air drying. In addition, castor oil, a non-drying oil, has been converted chemically by dehydration to give dehydrated castor oil with excellent drying property.

The reader may recall that pigments containing lead, manganese, or cobalt, as well as certain other compounds such as Prussian blue, act as catalysts that accelerate the drying of oils. Since great differences in drying rates of the various colors are considered undesirable in a painting and may lead to dangerous internal tension, colormen attempt to equalize the drying time as best as possible. Pigments known to be fast drying are therefore ground in the slow drying poppy oil, while those that dry slowly are prepared with the faster drying and hardening linseed oil.

Several treatments and pre-treatments are described in handbooks for artists [10-12] document that these refining operations are necessary to achieve good quality oil

paint: all the materials which could show negative aspects in the oil production and/or in drying processes, must be eliminated or at least reduced drastically. Different treatments and refining methods in oil preparation lead to three main categories of oils:

1. *Raw* or *cold-pressed* oils: in which the procedure consists of warming up slightly the crude oil and then let it rest in order to allow mucilaginous material to settle as sediment. The oil obtained after purification has a pale color and used as highest-quality medium for oil paint. The film ages properly and maintains a rather good flexibility.

2. Oils heated in presence of oxygen: the oil is *thickened* or *bodied* through oxidation by blowing air into the oil or heated with driers and thickened. This method leads to the creation of a more viscous and heavy oil medium.

3. Oils heated in absence of oxygen or *stand* oil: this is the name given to a drying oil which is refined by heating it at 300°C under anaerobic conditions. This causes the oil to pre-polymerize, making it effectively thicker.

3.2 SOURCES OF OILS AND ADULTERATION

Before the nineteenth century, many of the products on sale were not what they seemed or claimed to be. There was early adulteration and this undoubtedly includes oils. However, the early methods of proving the authenticity of oils were somewhat limited. Olive oil could be tested to differentiate it from lard oil or a

mixture with lard oil by cooling or by the reaction of a solution of mercury in nitric acid on the sample [13]. This was of particular importance to Jewish consumers. Specific gravity was also often used. Tallow could be tested for greases by examination and smelling of the released fatty acids (Anon, 1856). In England a select committee (Postgate, 1885) was told that, amongst the many non-oil items, cod liver oil was often diluted with bland oils, lard with mutton fat and butter with lard. These statements were later confirmed while it was also known that cod liver oil was often partially substituted by other fish oils (Anon 1856). These cases might still pose a problem, both in occurrence and detection, even today[13].

The importance of oils and fats in the economy, together with the expansion of possible uses, meant that chemists were amassing large quantities of data on the properties of oils and fats, both edible and non-edible. A textbook which includes many of these determined properties and developments was Lewkowitsch (1895, 1904[14]), much later replaced by determination of individual FA in oils and fat compiled by Hilditch and Williams (1964[15, 16]). By 1904, Lewkowitsch already contained over 1.000 pages on the processing, properties and methods of analysis of over 220 different oils from acorn oil, through purging nut and sod oils to yellow acacia oil.

Modern methods of authentication begun with the advent of GC which was able to separate the methyl esters of (short chain) FA [17, 18]. Recent methods of analysis for fatty acids (AOCS, 1990) are now one of the most frequent methods used in the analysis of fats. Apart from total fatty acid determinations, it became possible, after reaction with pancreatic lipase, to determine the average fatty acid composition at the 2-position of a fat (Christie, 1986) and thus detect inter-esterification.

HPLC was found to be useful in many authenticity determinations, either for the same or different components as those detected by GC. Triglycerides were the most immediate application. Now that the major components of commercial fats can be

completely separated by HPLC, the patterns of components can be analysed to detect adulteration through TAG analysis and enantiomeric separation of isobaric species. TAG's profiles aid in identification, apart from the pattern of components, the presence of any significant level of trilinolein in olive or other oils relatively low in linoleic acid content can show the presence of more unsaturated oils such as in soya, sunflower (normal high linoleic type) or cottonseed at low levels [19]. Almost all the work in authentication of oils is applied for olive oil [20] [21] or other expensive specialty edible oils.

The one thing lacking in work on authenticity is a good database of ranges of analysis values for oils. Fatty acid composition is well covered and expected ranges of sterols and tocopherol levels are also available, at least for the major oils (*Codex Alimentarius*, 1997; FOSFA, 1994; AOAC, 1997; [9]).

Tocopherols (and tocotrienols) in an oil can also be useful, though the absolute, and in some cases the relative, levels of each can be affected by age and refining. The tocopherol profile is, however, usually only of use as confirmation, together with other analyses on unsaponifiable matter[22].

Many other analyses can be useful. As has already been stated, triglyceride analysis can determine trilinolein in oils where it should not be present, but for triglyceride and most other analyses there is little information available at present as to the natural range in the oils, and so many conclusions can only be tentative[13]. Notwithstanding we believe that TAG profiling with enantiomeric separation and quantitation is the best way to fully characterize the oils in study and their degradation phenomena throughout time, so that other people can have free access to these parameters in order to trace back origin or mixtures of oils used in paint formulation.

The process of testing for authenticity of these oils should be approached in the same way as for hazelnut, walnut, macadamia, almond, apricot, pumpkin, poppy-

seed and rice bran oils, i.e. fatty acid profile, sterols, tocopherols and triglyceride composition. However, there is little generally available published material on the ranges of values to be expected from different sources.

Also oils from genetically modified plants can be analyzed through the polimerase chain reaction (PCR) amplification methodology and gel electrophoresis or other techniques for specimen identification through search in a protein database.

3.3 OIL QUANTIFICATION

Many of the tests described involve physical properties such as refractive index, viscosity or melting point of the fat, of the fatty acids or of the lead salts of the fatty acids. However, there were also many chemical tests such as Reichert, Polenske, iodine, acid, saponification and acetyl values. These all gave information as to the composition of the fat, some information as to fatty acid composition, others as to other non-glyceride components of the fat. Although the iodine value is a measure of the double bonds present in the unsaturated fatty acids in the fat, this and other chemical characteristics are now obtainable in more detail from FA profiling or TAG profiling.

Modern methods of authentication began with the development of chromatography. The relatively straightforward determination of total fatty acid composition in effect could have replaced many of the other tests previously carried

out on oils. Nevertheless, TAG's profiling methods are being implemented on a larger scale now.

Three GC analyses now used in authentication, largely for olive and oils that which should not be refined or solvent extracted, are the determination of waxes, aliphatic alcohols, triterpene alcohols (uvaol and erythrodiol), and stigmastadiene and other sterol-dehydration products (EEC, 1991). These analyses are used not to detect adulteration with other oils, but for detection of solvent-extracted or refined oils. However, it is possible that solvent extracted oils, wax, aliphatic alcohol and terpene alcohol compositions could prove useful in differentiating or detecting different oils, and this is relevant for oil use in paint.

The best approach in checking authenticity in any of the bulk oils is by carrying out a series of analyses quantifying FA and TAG species and comparing the results to those listed in the definitive literature (*Codex Alimentarius*, 1997; FOSFA, 1994 ; AOCS, 1997; AOCS, 2013).

Specific literature has been reviewed critically in the former chapter, although there is scarce detailed data with enantiomeric separation of TAG's.

3.4 Fatty acid Profiles vs. TAG Profiling

Oils tend to have different FA profiles with different times of harvest and, in particular, with respect to compositions varying with geographical source or cultivar. The advent of LC-MS gave the opportunity to map the TAG's, DAG's and

MAG's and free FA present within a given oil, which improved our knowledge on the actual composition of the oil before being broken down to it's constituent FA's by hydrolysis and methylation for any GC-MS analysis or Py-GC-MS.

There are several comprehensive texts on properties of different Oils and fats [1-9]. We'll try to sum up the properties of the oils investigated here, mostly drying and semi-drying oils.

This subject has also been reviewed in the previous chapter. In this chapter previous studies are mentioned, whenever possible, with the known profiles of each oil thought to be used in oil paint formulation.

3.5 Previous Studies, Profiles, Source profiling and Proprieties of Oils used in Oil Paint Formulation 1890-1940

This section is an illustration of previous studies related to oils, from their harvesting, processing and proprieties for use in oil paints. Several approaches combine to give a general view of application of oils in oil paint formulation, and the respective chemical characterization, namely their FA profiles and their respective TAG profiles, whenever possible. The intention is to contribute to a better knowledge of the starting materials used from the turn of the century, and their chemical characterization, which until now has been sort of a knowledge gap.

During the 1st and 2nd world wars when normal trade was largely paralyzed, numerous attempts were made to find suitable substitutes for the traditional drying oils for industrial coatings. Oils from cottonseed, from the seeds of spruce, pine, and stone pine were tried. Hempseed oil, olive oil, and even peanut oil were modified in order to induce fats drying with little yellowing. Although experts were able to score partial success, serious shortcomings in the new products often became apparent after several years. During years of normal trade attention turned once more to traditional painting oils, and it was concentrated on the perfecting cultivation and processing techniques in order to improve the quality. The only innovation for the western world in this respect is sunflower oil, which still needs to be improved for our purposes by the selection of more suitable seeds.

Seed from different cultivars, or even just different origins, have a considerable difference in the amount of oil contained in the seeds. Speaking generally, the coldest place in which the crop will grow and thrive produces the best oil. Conditions prevailing during the ripening of the seed play an important part, and have marked effect on the iodine value. For example, low temperatures produce high iodine values and vice-versa.

3.5.1 Linseed Oil

"*For painting one should use cold pressed oil. It dries quickly but goes yellow in time and easily turns rancid. It forms a layer of solid paint which has less tendency to crack than that made with nut or poppy oil.*"

Linseed oil is the most universally and important oil employed in the manufacture of oil paints and varnishes, and it is obtained from the seeds of the flax plant (*Linum usitatissimum*). In fact the name *Linum* originated from the Celtic word Lin or "thread", and the name *usitatissimum* is latin for "most useful"[23]. Flax is supposed to originate in the east and the first Egyptians may have introduced the cultivation of flax or their immediate successors may have obtained it from Asia before the time of the Phoenician colonies in Greece and Egypt about the period of the 14th dynasty, besides it is most probable that the Phoenicians introduced flax into Europe. In a pyramid, linseeds have been discovered from the period around 3500 B.C. Linseed came to Europe around the first century and from there was taken to America by the first settlers.

Flax is an annual plant; the seeds are flattened elliptical ovoid's, and pointed at the lower ends; smooth, shining, and of a brownish shade. They are 3 to 4 mm long, 2 to 3mm wide and ½ mm thick. The seeds are produced in a 10-seeded globular capsule, which frequently remains closed up to the time of maturity, but in some varieties opens suddenly. Under the shell is a hard layer of endosperm, surrounding the embryo. This layer is fairly thin and the oil is principally derived from the oval heart shaped seed leaves (cotyledons), which it encloses.

Different varieties of flax (*Linum usitatissimum*) are grown for fiber and for oil. Russia is the only country in Europe from which both flax fiber and oil are produced on a commercial scale. Linseed oil is well known as one of the most unsaturated vegetable oils, resulting from its high level of linolenic acid (50–60%, Table see end of chapter). As a consequence of this, it oxidizes and polymerizes very readily and is used in paints, varnishes, and inks, in the production of linoleum, and as a sealant for concrete. These uses diminished with the appearance of alternative petroleum-based products [24], but the natural oil is coming back into favor mainly for environmental reasons.

Linseed contains from 30 to 35% of oil, and cold pressing yields are lower about 20-25% of oil, of pale yellow color and a pleasant taste and smell. When hot pressed the yield of the oil is about 28%, amber in color, and slightly acrid in taste. Fresh seed is never pressed because the oil produced is turbid and sticky, so that is the custom to store the seed from three to six months before pressing.

Different kinds of flax seed differ somewhat in composition. The oil obtained from the Riga seed will be different to that of the Abyssinian Yellow, the latter having a lower percentage of oleic acid and higher amount of linolenic acid.

With recognition of the importance of n-3 acids (the so-called omega 3) in the human diet, the oil and seed - under the name of flaxseed - are being used increasingly in food products both for humans (cereals and breads) and for animals. This is independent of the growing use of linola oil (solin) discussed below.

Using chemical mutation, plant breeders in Australia developed a variety of linseed with a low level of linolenic acid (2%) and a high level of linoleic acid. This is called Linola®™ and is a linoleic-rich oil alike sunflower.

The contribution of linolenic acid in flaxseed oil shows a wide variability range and was affected by the growing conditions. As an example, flax varieties grown in Western Canada, average from 495 samples analyzed, contained 5% palmitic acid (16:0), 3% stearic acid (18:0), 17% oleic acid (18:1), 15% linoleic acid (18:2), and 59% linolenic acid (18:3) [25]. Although similar varieties were grown in North Dakota, the 11 cultivars assessed showed the following fatty acid composition: 5–6% of 16:0, 3–6% of 18:0, 19–29% of 18:1, 14–18% of 18:2, and 45–52% of 18:3.

Table 3.5-1 Linseed Oil TAG's composition as in Bailey's industrial oil and fat products [25]

in Flaxseed Oil (17).

Triacyglycerols[1]	Contribution (%)
PLnLn	7.6
PLLn	6.7
PLL	1.5
POL	1.6
LnLnLn	20.9
LLnLn	13.8
LLLn	3.7
OLnLn	8.4
LLL	0.9
OLLn	5.3
OLL	0.9
SLLn	1.1
OOL	3.4
OOLn	7.3
POLn	4.0
SLnLn	3.2
POL	1.6
PLL	1.5
OOO	3.3

[1]Abbreviations of fatty acid: P—palmitic; Ln—linolenic; L—linoleic; O—oleic; S—stearic.

As discussed above, triacyglycerols are the main components of vegetable oils and the composition of flax acylglycerols is presented in Table 1.

TABLE 2. Composition of Flaxseed and Major Oils (6, 10, Przybylski Unpublished Data).

Component	Flax	Linola™	Canola	Soybean	Sunflower
Fatty Acids (%)					
C16:0	5.3	6.1	3.8	11.2	6.0
C18:0	3.3	3.8	1.7	4.1	4.0
C18:1	17.9	15.5	58.2	24.3	16.5
C18:2	14.7	71.3	20.1	54.6	72.4
C18:3	58.7	2.0	9.6	8.3	0.5
SFA	9.0	10.0	6.2	15.6	11.2
MUFA	18.1	17.1	64.2	23.4	16.7
PUFA	72.9	72.9	29.6	61.0	72.1
Tocopherols (ppm)					
Alpha	20	15	272	116	613
Gamma	200	200	423	737	19
Delta	7	5	—	275	—
Plastochromanol-8	120	110	75	—	—
Total	347	330	770	1128	632
Phytosterols (%)					
Brassicasterol	1	1	14	—	—
Campesterol	27	23	28	18	7
Stigmasterol	8	4	1	15	7
β-Sitosterol	50	54	52	54	58
Δ^5-Avenasterol	10	18	5	2	4
Total sterols (g/kg)	2.3	2.2	6.9	2.6	3.1

Abbreviations: Fatty Acids: SFA—saturated; MUFA—monounsaturated; PUFA—polyunsaturated; Plastochromanol-8–derivative of gamma tocotrienol with longer side chain.

Sterols or phytosterols are present in flax oils at a level lower than those in many vegetable oils, 2.3 mg/g in flaxseed oil versus 4.1 to 6.9 mg/g in other oils (Table 2 above). The composition of sterols was similar to other oils, where beta-sitosterol was the main component followed by campesterol and Δ^5-avenasterol. Brassicasterol was found in trace amounts in flax oil. This phytosterol is characteristic to plants from the Brassica family and often is used as a marker for oil adulteration (Table 2).

Cold-drawn linseed oil has lately become popular again as cooking oil and should be of pale yellow color and have a mild pleasant flavor; hot pressed oil tastes acrid and

looks golden or brownish-yellow and is not good enough for use in paint. As mentioned above, the palest oil comes from flax white flowers. The seed cold-pressed at the relative low pressure of 588 pound per square inch (0,29 atm) yields approximately one third of the amount of oil obtainable by the more common hot pressing. Even with the most careful cold pressing, the passage of some albuminoid mucilage into the oil can't be avoided. Any good commercial product will have undergone several refining processes and have passed through a number of filters in order to eliminate all foreign matter. But even refined, first-grade linseed oil is not quite good enough for artists.

The value of chemical bleaching is still being debated, most writers recommend the ancient method of bleaching the oil in the sun in uncorked bottles filled to within two inches from the top, but this is rarely done today. Edible linseed oil, bought directly from the manufacturer should be purified again by shaking it vigorously with heated baryte powder. Once the solid have settled the oil should be crystal clear. Linseed oil should not be judged by its yellow color alone. Some relatively pale varieties may discolor white pigments, which are ground in them, to a greater extent than slightly more yellowish varieties of lower tinting power. The well-known artist's color manufacturers are supplied with choice quality oil, which is not generally available. Even the best linseed oil has a tendency to yellow, especially when ground with white lead, which has a strong catalytic action. Attempts to improve this medium are therefore continuing, and in certain cases other oils are used. A sample of good linseed oil spread thinly on glass should dry free of tackiness five days at 20ºC, but when ground with pigment the drying time is shortened considerably, depending on the characteristics of the pigment. Any given list of pigments should mention both drying activity and oil absorption characteristics for each pigment. The fresher the oil, the more pigment it can absorb

and still remains brushable. Good, buttery oil paint should contain as little oil as possible.

Beginners grinding their own color are more likely to include an undesirable excess of oil than would experienced paint manufacturers, a number of recipes can be found on most of the ancient manuals and books on oil painting, some of them are compiled in for example [26, 27].

Raw and Refined Linseed Oil

The chief sources of Linseed oil, as mentioned in historical records, came from America, Canada, Russia, India and Argentina. Baltic oil is obtained from seeds grown in Northern Russia, and is shipped from the port of Riga, and other Baltic centers. It possesses excellent drying properties. A smaller amount comes from Odessa in Southern Russia. La Plata seed comes from Argentina and is grown around the River Plate district. India furnishes Calcutta seed, but the oil is not of such a high quality as that from Russia [28].

Calcutta oil has a pale straw colour. Specific Gravity 0.932 iodine value 180 to 185. La Plata oil, straw coloured, specific gravity 0.931, iodine value of 170 to 175. Bombay oil straw coloured, specific gravity 0.932, iodine value of 180 to 185. Baltic oil, brownish-green in colour, specific gravity 0.936, iodine value of 190 to 195. Canadian, straw coloured, specific gravity 0.935, iodine value of 170 to 175.[28]

When freshly pressed linseed oil is raised to a temperature of 500ºF (260ºC). What is called the "break" is coagulated, and is separated from the oil, though prior to the

heating the same oil might have appeared quite bright and clear. The break is gelatinous in nature, varying in color from light brown to a dark brown, the latter color frequently occurring in samples from India.

When the oil cools down this bulky gelatinous matter settles in the bottom of the vessel, and after separation and drying it usually amounts to from 0.15 to 0.25 per cent. If left to re-dissolve in the oil, which the material will do to a greater or less extent, the result would be very objectionable, and in the past has often been the cause of drying troubles. To get rid of the break, it is often necessary to heat up the oil twice. There are now several brands of linseed oil, which are alkali refined, and are entirely free from break; such oils are largely used by varnish makers, and in the manufacture of *Stand Oils*. What is known as refined oil has always been treated to some kind of refining process before use. There are several methods of refining employed[28]:

1 Removing albuminous and suspended matters
2 Reducing the color, in other words bleaching
3 Reducing or neutralizing the free fatty acids
4 Removing taste and deodorizing

The refined oil, chiefly produced for use in the paint trade, carries out an acid refining process and an acid-refined oil usually has a pale color, and an acidity from 3 to 6 per cent.

The acid method of refining consists in adding sulphuric acid up to 2 per cent of the raw oil, which is carried out in Lead-lined tanks fitted with agitators by which the oil is kept vigorously stirred, while the acid is slowly added. The acid attacks the coloring matter, turning the oil green. Later the albuminous matters are attacked,

and this along with charred matters settle to the bottom of the tank, as a dark colored mass of *foots* containing a large amount of phosphates. After all the *foots* have been deposited, the oil is run into another tank where it is first steamed and then washed with water to remove as much acid as possible. On a large scale one would proceed as follows[28]:

At the end of a specified period which varies between 1 ½ h to 2 h, the oil is passed to the lower vat, steam is then turned on, being at a considerable pressure, which causes good agitation and is carried on for about 6 to 8 hours, sometimes longer. The condensed steam (water) assists in eliminating the acid. After steaming the whole is allowed to rest for about two days, during which time the charred matter, foots, water and acid settle out, while the oil floats on the top. In some plants after the acid agitation, the whole of the albuminous matter and char are allowed to settle before transferring to the lower vat, for steaming. The oil is allowed to remain at rest for about five to six days, so that as complete precipitation of extraneous matters shall be assured after which the suspended matter which should by this time have settled to the bottom of the tank and the water is drawn off and the oil pumped to tanks after resting a few days is filter pressed and passed on to permanent storage.

Oils refined by the acid-process have a low surface tension and therefore good wetting properties. Such acid refined oils are much used in preparing white lead paste, when the water in the white lead is under a process of pugging replaced by the oil. The acid refined oil usually gives nice pale oil, and apart from refining is an excellent bleaching process. Accordingly, bleaching consists not in the removal of the dark green coloring matter chlorophyll but in its change to a light yellow coloring matter, Xanthophyll, under the acid treatment[28].

Oils used by paint and varnish makers are usually if not universally alkali refined.[29-39]

Boiled Linseed Oil

It has been known from a great many years that linseed oil when heated to 500ºF (260ºC), for a few hours possesses the property of absorbing oxygen, and if certain salts known as dryers are added, this propriety is further increased, the resulting oil being known to the paint and varnish traders as *Boiled oil*. Three qualities were produced, Viz., *pale bodied oil*, *single bodied oil*, and *double bodied oil*. In the old days, the usual method employed was to heat the oil by fire in an oval shaped iron boilers of about 400 gallons capacity (1514,16 liters). Later wrought iron boiler plate was used to build the boiling tanks. The fires for heating the boilers were placed outside the building containing the tanks, for less risk of damage by fire. Should *frothing* occur, the fire had to be frequently withdrawn[28].

The *frothing* is due to small quantities of mucilage in the oil. Further moisture may be held responsible for as the active agent that often causes the formation of free acid. Linseed oil begins to boil quietly at about 500ºF (260ºC). The oil should in any case be heated gradually, and to arrive at a state of quiet ebullition at least two hours should be taken. After the oil has been boiling for about 30 min, the dryers are added, and further additions are made from time to time[28].

The dryers should never be added all at once, otherwise violent action may result which would be very difficult to control. Moreover by adding the dryers little by little the combination between the dryers and oil is more complete, and the boiling under better control[28].

Blown Linseed Oil

Blown linseed oil is an air-oxidized oil. It differs from raw and boiled linseed oils, on account of it being compatible with either of these oils. Blown oils contain an alcohol-soluble portion and an insoluble portion, which will mix with raw linseed oil. The miscibility varies with temperature[28]. [Crebert, 1938, Fette und Seifen]

Stand Oil

The introduction of *Stand Oil* belongs to Holland, and it is presumed to have been produced in the first instance by a Dutch painter who found out that when linseed oil was heated for some hours at a temperature of 300ºC (572ºF). It gained in viscosity, and the paints made with this thickened oil had a good gloss and excellent weather resistance. This product was called "*Standolie*" hence the name "*Stand Oil*"[28].

Relevant information From the W&N Archive, on oils and it's preparation: [1]

[1]

The Winsor & Newton Archive Database is a unique primary resource, now available in a Researchers' Edition. It comprises a computer-based indexing system with digitalized page-images of 85 handwritten books (15,003 pages) detailing manufacturing practices and recipes for 19th century artists' materials[40].

A review of all the recipes relating to oil, revealed that a variety of processing methods were being used but were not always reflected in the names of the much more limited product range found in the company's retail catalogues (compare Tables 1 & 2). Furthermore recipe names were not always indicative of the precise nature of the end product: while recipes for refining raw oils were called variously, '*Purifying Linseed Oil'* or *'Clarified Oil,'* and do not generally call for driers. One recipe for *'Purified Linseed Oil'* (Book 06 page P009) has a note dated 1833 that "Mr Hopkins" suggests "adding a portion of Litharge," and later on manganese is associated with purifying oils (see below).

Oils boiled alone at various temperatures were called '*Boiled Oil'*, but this latter name could also refer to recipes including driers. For example '*Boiled Oil*' throughout 1834 to 1836 (book HS), could include white copperas (Zinc sulphate), litharge (Lead monoxide); burnt or raw umber, and lead acetate. Furthermore boiling with lead acetate and litharge sometimes included shellac and lead white or white rosin.

[1] For the on-line index see, http://www-hki.fitzmuseum.cam.ac.uk/archives/wn/search.php. Current Researchers' Edition hosts are: The Rijksbureau voor Kunsthistorische Documentatie (RKD), The Hague, Netherlands; in the UK: The Hamilton Kerr Institute, University of Cambridge; The Conservation and Technology Department, Courtauld Institute of Art, University of London; Department of Conservation, Tate Britain, London, and in Portugal: the Department of Conservation and Restoration, Faculty of Sciences and Technology, New University of Lisbon.

In 1890 oils treated with manganese begin to appear and are variously referred to as '*Drying Linseed Oil*' and '*Drying Poppy Oil*' although this product appeared as '*Manganesed Oil*' in W&N catalogues (Table 1). In the archive, manganese treated oil appeared regularly from this time on (the last batch found in the database was dated 1926). The earliest recipe was dated 1st December 1890 and appears in book DR, page P001 entitled "*Drying Linseed Oil*" (see transcript below). However there were earlier references to the use of manganese: a note accompanying the c1846-7 recipe for "*Purifying Oil*" (Book 09, page P029, line 1) states: "Mr Ives says that oxide of Manganese and Sulphuric acid, are used to purify Linseed oil..." And there is a quote from the Chemical Gazette Nov 15th 1856 detailing a "Prof. Wagner's" experiences with protoborate of manganese (according to this note Prof. Wagner realised that the oxide or hydrated oxide of manganese "answers as well" (Book 19, page P007, line 1). A study of all the manganese-oil recipes shows that the company was experimenting to find the best results. At first they used "May & Baker's" linoleate of manganese (see transcript), then they prepared their own (referred to as '*Manganoleate*'). The manganoleate recipes show the proportion of the ingredients being altered with notes comparing results until they found a recipe, which was then adopted quite consistently.

Tables listed below are a review of all the oils mentioned in the W&N Archive [40].

These tables serve as an example of how the introduction of new formulations were introduced or discontinued in their catalogues.

Oils listed in W&N catalogues (from Carlyle 2001, 337–338 and Carlyle unpublished database)

Product Name	Catalogue First Appearance	Previous Catalogue Seen
Linseed Oil /Purified Linseed Oil	c. 1835	c. 1835 earliest to date
Poppy Oil	c. 1835	c. 1835 earliest to date
Nut Oil	c. 1835	c. 1835 earliest to date
Pale Drying Oil	c. 1835	c. 1835 earliest to date
Strong Drying Oil	c. 1835	c. 1835 earliest to date
Fat Oil	c. 1835	c. 1835 earliest to date
Manganesed Linseed Oil	1892	1889
Manganesed Poppy Oil	1892	1889
Purified Walnut Oil [in tubes]	c. 1897	1892
Oil Vehicle No.1, 1A, 1B [5]	c. 1897	1892
Oil Vehicle No.2, 2A, 2B [5]	c. 1897	1892
Oil Vehicle No.3, 3A, 3B [5]	c. 1897 (1900 catalogue states special orders only)	1892

W&N Archive Database, recipe names for oil preparations

Recipe Name	Date Range for Recipes
"Purifying Linseed Oil"	1833–1846
"Boiled Oil"	1834–1836
"Pale Oil"	1843
"Clarified Linseed Oil"	1844–1850
"Linseed Oil"	1844–1846
"Fat Oil"	1844–1855
"Drying Oil"	1843–1856
"Rudd's Pale drying Oil"	1843–1844
"Pale Drying Oil"	1848–1958
"Pale Prepared Oil"	1850
"Extra Boiled Oil"	1850
"Strong Drying Oil"	1853–1854
"Clarified Poppy Oil"	c. 1862–1882
"Drying Linseed Oil" [with manganese]	1890–1926
"Drying Poppy Oil" [with manganese]	1890–1926
Thickened Walnut Oil	1896
"Thickened Linseed Oil"	1896
"Thickened Poppy Oil"	1896
"Special Oil vehicles"	1898
"Oil Vehicle No.1, 1A, 1B"	1896–1902
"Oil Vehicle No.2, 2A, 2B"	1896–1901
"Oil Vehicle No.3, 3A, 3B"	1897–1898

Transcript Book DR, Page P001

Drying Linseed Oil

1st December 1890

20 Gallons Linseed Oil 5123 :

heated in new steam pan at 212º F

for 2 or 3 hrs with the cover off :

Then suspended in it, tied up in

coarse muslin-

1lb 4 ozs Manganoleate (May & Baker 29/11/90)

Put the cover on and only took off

to stir twice a day.

Kept the temperature at 212º F for

8 days and then left to cool.

Dec 10th. Drew off into air tight tank.

[note overleaf]

This batch has the number 713

having been first entered on that page

in the Old Varnish Book No 3.

This recipe demonstrates a very significant discovery: the issue of scale and duration in relation to oil processing. Winsor & Newton regularly prepared batches of 20 to 70 gallons (91 to 318 litres), and as seen above, batches could be kept at 212°F (100°C) continuously for up to eight days. This information is very valuable for evaluating the chemistry of commercially available drying oils and has obvious implications for extrapolating from commercial conditions to small-scale experiments.

Throughout the archival evidence of quality control is found in the system of tracking different batches of materials. As seen in the "*Drying Linseed Oil*" recipe above, in the first line the linseed oil is referred to by batch number which refers to its full preparation details and the supplier, thus allowing W&N to track this information throughout a variety of recipes where the material was subsequently used.

Another significant discovery was the instruction in the 1901-02 recipe for '*No.2 Poppy Oil*' (Book DR page P122A, line 1) to "add 1 quart Fatty acid", this appears to be the first clearly documented use of fatty acid as a deliberate addition to an artists' oil binder.

Investigation of oil processing recipes reveals that there could be a range of recipes for a 'single' product (e.g. boiled oil) and points to significant issues associated with scale and duration for oil preparation, which had not been anticipated in previous work[40].

3.5.2 Poppyseed Oil

"Poppy oil is whiter than any other Fixed oil used by the painter. For this reason it was very popular as a vehicle with the Dutch seventeenth century painters, and in France as a Medium for grinding light and cool pigments, such as delicate blues, for it does not tarnish as much as Linseed Oil."[26, 27]

"*Poppy Oil. For painting this oil is extracted from poppyseeds, and is the least viscous and cleanest of all oils. Although it takes very long time to dry, it has the advantage of not easily turning rancid. It is excellent in emulsions with glues for tempera. This oil is hot-pressed and all mucilage eliminated. It is discoloured by colours with a lead base.*"

poppy oil to be a less rapidly drying oil than linseed. Wolffen, in 1640, stated that poppy oil dries *throughout* in four or five days, while linseed oil forms a pellicle upon the *surface*. Joseph Petitot, writing from Geneva under date January 14, 1644, stated that umber is a siccative for

poppy oil. Poppy oil was introduced into painting in the beginning of the seventeenth century, after linseed and nut oil. Later on in the same century the Dutch painters acquired greater confidence in this more slowly drying oil, employing it not only in the painting process, but also for grinding their pigments, especially whites, blues, and pale tints.

In A H Church's "*The chemistry of Paint and Painting*", 1890, pp.42

Speaking of the juice expressed from the seed the black poppy *Dioscorides* observes that "*it easily diluted forms an emulsion with water, that when exposed to the sun the oil becomes separated from the mucilage and then burns in lamps with a very clear flame"*. Thus whether applied to any other use than that here indicated or not it is evident that poppy oil was known to the ancients.

Poppy (*Papaver somniferium*) grows in Persia, Asia Minor, Egypt, India, Russia, and France. It contains 40–70% of a semi-drying oil used by artists and as an edible oil, it's yield varies from 15 to 25%.

The chemical composition of poppy seed oil differs considerably from that of Linseed oil. It never becomes quite as hard when dry, but this fact is not always apparent when the oil is pigmented. Its most important advantages are that it yellows less than Linseed oil and maintains its consistency better when ground into thick, buttery oil paint. A small excess of linseed oil is more likely to make the paint too liquid – "*Long*" or runny - than is an excess of poppy oil. The best consistency is achieved with freshly pressed oil. The brush marks, a feature valued by the impressionists, are retained clearly by this type of paint. "*Short*" oil color is, of course, easier to apply with the palette knife.

A drying test on glass slides with poppy-seed oil may dry in ten to twenty days. The production of poppy-seed oil requires even more care than that of linseed oil. The white poppy yields the best oil, and the first run, at the beginning of the pressing, it is almost colorless, with a faint greenish yellow tinge, provided, of course, that it is cold-drawn. Because of the smallness of the poppy seed, it's separation from the seeds of weeds is much more difficult than with linseed. The drying time of commercial poppy-seed oil can therefore vary quite considerably. The fresh oil has a

very faint, nutty flavor. One occasionally finds poppy oil that is darker than linseed oil and yet has all the characteristics of the former, with the exception that it may dry a little faster. This has usually been pressed from the seed of colored poppies[41].

Eibner's objection to poppy oil was based on his observations during experiments in which he found that dried test samples resoftened after some time. Yet this is still to be verified experimentally and chemically monitored. Nevertheless, poppy-oil colors should never be used on fat, non-absorbent grounds.

The chief constituents of the oil are linoleic and linolenic acids, with a considerable amount of oleic acid. Rich in linoleic acid (72%), it also contains palmitic (10%), oleic (11%), and linolenic acids (5%) (79, 164).

Recently, there have been studies with the profiles of poppy seed oil and their possible adulteration with cheaper oils [42-44].

3.5.3 Walnut Oil

"*This is cold pressed oil from well dried nuts. It dries more slowly than linseed oil but has the advantage of not turning yellow in time so easily. It is specially suitable for use with the lakes.*"

"*Leonardo* recommends that when preparing nut oil the skin of the kernel should be removed since *'this skin separates from the oil and floats to the surface of the painting causing changes' … 'Choose the best nuts, shell them and put them to soak in clear water in a glass jar changing the water whenever you see it becoming clouded. It may be necessary to do this up to seven or eight times. After a time the nuts disintegrate when stirred and form a milky pap. Place this in*

dishes in the open air and you will see the oil float to the surface. To collect it in a perfect and clean condition, twist pieces of cotton wool and place one end in the oil letting the other end hang over the edge of a plate about two inches below the level of the oil in the plate. Little by little the oil will rise through the cotton wool and will drip into the vessel. All oils are naturally clear and it is the manner of extraction which may change them.'."

Codex Atlanticus, *Folio* 4/9 (*verso*)

Walnut (*Juglans regia*). The oil obtained from the seeds of common Walnut generally known as "*Nut Oil*" it is produced mainly in France, Italy and Germany. Used by artist's colourmen, walnut oil is an unsaturated oil with chief constituents containing both linoleic (50–60%) and linolenic acids (13–15%) isolinolenic, lauric and, myristic acids. It is also rich in tocopherols (1500 mg/kg of oil). It is used as a gourmet oil in Japan, France, and other countries. A paper gives the detailed composition (fatty acids, triacylglycerols, sterols, and tocopherols) of oil extracted with hexane and with supercritical carbon dioxide[45]. The main FA was linoleic acid (56.5%), followed by oleic acid (21.2%) and linolenic acid (13.2%). The main TAG was LLL (linoleic, linoleic, linoleic) (24.4%), followed by OLL (oleic, linoleic, linoleic) (19.6%) and LLLn (linoleic, linoleic, linolenic) (18.4%). The main component of sterols was β-sitosterol (85.16%), followed by campesterol (5.06%).

At present, walnut oil is fashionable in the gourmet kitchen. However, the amount is very small and it will remain a specialty oil in a narrow market segment. Walnut oil is produced by cold press extraction of walnut kernels. It is rich in unsaturated fatty acids, which comprise **>**90**%** of the oil.

In painting technique the term *nut oil* refers to a cold-pressed oil made from air dried walnuts only. In it's composition, as well as in his properties, particularly in

color, walnut oil resembles poppy oil rather than linseed oil. It yellows only slightly but dries faster than poppy oil and being less viscous, can absorb more pigment [28].

Following a suggestion by Weimar professor Fritz Fleischer, the firm of Gunther Wagner produced a range of artists' colors of extremely high quality called *Zetfarbe*. These were walnut-oil colors containing a high proportion of pigment, yet ones that could be strongly diluted without any risk. It would be pointless to enumerate the many advantages of this valuable artists' paint, since it is, unfortunately, no longer available. The manufacturers explain that since World War II walnut oil of the necessary high quality can no longer be obtained. It has practically disappeared from art-supply stores .

3.5.4 Perilla Oil

Parilla oil requires refining before being used for varnish manufacture, and on heating bleaches considerably. Stand oils can be made in a similar manner to linseed and when blown with air yields a pale oil possessing very rapid drying properties, due to the taking up of oxygen rapidly. Parilla is an Oil which has a considerable future as regards to the paint and varnish industries. Due to the fact that much of the oil expressed in Japan has been produced in numerous small mills considerable variations in quality have occurred in the past and all samples should be carefully examined[28].

This Oil is obtained from the seeds of the Perilla plant species, *Perilla frutescens* , also known as Wild Sesame, of which there are several and varieties but the chief sources of the Oil are obtained from *Parilla acymoides*, and *Parilla nankinensis* (Linn). Both black and white seeded plants are on the market. The Perilla plant grows in Manchuria, Japan, Northern India, in cold and high altitudes up to 4.000 feet(1219.2 meters), and is used in Japan as a lacquer oil [28].

The oil is extracted by pressure, and the average yield is about 40%, varying from 35 to 45 per cent. The expressed oil is pale in color and frequently as a green tinge, and an ethereal smell, which is considerably increased on heating. The oil dries rapidly and as very high iodine number. The oil breaks when heated to 600ºF. (315.5ºC), considerable "*break*" being produced in most cases and like linseed oil this must be removed before the use in paints and varnishes[28].

The composition and positional distribution of fatty acid on the glycerol backbone; thus, the stereospecific analysis of fatty acids in the triacylglycerol was considered important to be able to use the lipid for both industrial and dietary purposes[46].

The chemical composition of this oil is a linolenic-rich oil (57–64%) used as a drying oil. It also contains oleic (13–15%) and linoleic acids (14–18%) and comes mainly from Korea or India (nowadays) [47]. The amount of tocopherols in Perilla oil is higher compared with flax oil, and a similar contribution of gamma-tocopherol, above 90%, was observed. Shin and Kim [48] analyzed Perilla oil for lipid composition and established that it contained more than 90% triacylglycerols, 4% glycolipids, and 2% of phospholipids and lower amounts of sterol esters, hydrocarbons, free fatty acids and partial glycerides.

The total lipid content of Perilla seed was 45.6% (average of triplicate) on a dry weight basis. The major fatty acids of the Perilla oil were identified as linolenic (53%), oleic (20.9%), linoleic (15.4%), palmitic (7.3%) and stearic (2.5%) acids, in decreasing order (see above Table) while myristic acid was detected as a minor

component that could not be quantified. The total lipid composition obtained was similar to the results shown in previous reports [46].

Perilla oil has been used as a drying oil in paints, varnishes, linoleum, printing ink, lacquers, and for protective waterproof coatings on cloth. It has also been used for cooking and as fuel. The meal produced after oil extraction is often used as an animal feed ingredient. Recent descriptions of this oil come from Korea and India.

3.5.5 Hempseed Oil

"*The hemp plant (Cannabis Sativa) yields a roundish greenish-grey seed, very familiar to the lovers of canaries, from which, on expression an oil is obtained that is used for painting. (…) Hempseed oil when fresh as a greenish-yellow tint, but on keeping it slowly turns to a brownish-yellow; it's odour and taste are rather unpleasant. (…) In this country hempseed oil is rarely used as a paint oil, it's price being against it; still it has been mixed with linseed oil, and it's difficult to obtain the latter free from it, owing to the Russian linseed growers mixing hempseed with the linseed. In Russia and other places where hempseed is grown, the oil is used rather largely for painting.*"[41]

The oil obtained from the seeds of *Cannabis Sativa* is produced in India, Germany, France, Italy, Belgium, North America, Turkey, and Japan. The yield of oil varies from 15 to 25%. Hempseed is a drying oil giving rather a soft film[28]. In England it is rarely used as a paint oil still it has been mixed with linseed, and is difficult to find the latter free form of it due to the Russian linseed growers mixing hempseed with the linseed. In Russia, and other places were hempseed is grown, the oil is used rather largely for painting[28].

The oil is at first greenish or brownish-yellow, deepening with exposure to the air; the flavour is disagreeable, and the odour is mild. It has a sp. gr. of 0-9252 at 59° F . ; it thickens at 5° F . , and solidifies at - 13° to - 18° F . ; it dissolves in 30 parts of cold alcohol and any proportion of boiling; it saponifies with difficulty, forming a soft soap, but less soft than that from linseed oil. It is inferior for the painter's purposes.

Hempseed oil has an interesting fatty acid composition the constituents being linoleic, linolenic, iso-linolenic , oleic, palmitic and stearic acids. One report gives the following values: palmitic (4–9%), stearic (2–4%), oleic (8–15%), linoleic (53–60%), (n-3) linolenic (15–25%), (n-6) linolenic (0–5%), and stearidonic acid (0–3%).

3.5.6 Safflower Oil

Safflower seed oil is a minor oil obtained from the seed of *Carthamus tinctorius L*, has a long history of cultivation. Some would class it as the world's most ancient crop. In the first century A.D., Pliny wrote that safflower oil, called *oleum cinicium*, was used as a milder substitute for castor oil, and Pedanius Dioscorides, in *De Materia Medica* (the leading Western pharmacological text for 16 centuries). Grown particularly in India as a source of a valuable red-yellow or orange dye (Chartamin). Annual production of seed varies between 600,000 and 800,000 kg. Safflower was already well known to the old Egyptians. It was identified in 1887 by Schweinfurth

as one of the holy gifts to the mummy of pharaoh Amenophis I (1600 B.C.). The importance of safflower is illustrated by the fact that only the Pharaoh himself had the right to grow and market safflower. In Europe, safflower was first mentioned by the german philosopher, Albertus Magnus, around the year 1200. Safflower oil has been used in India as a drying Oil for years. Safflower oil exhibits the highest level of linoleic fatty acid of any commercially available oil [49]. This high level, combined with an absence of linolenic fatty acid, is what has made safflower oil attractive to consumers, initially as a quick-drying oil that could produce films that would not yellow with age and, more recently, as an edible oil with the highest available level of polyunsaturation[50].

Drying tests on glass slips concluded that the best dryer when these tests were undertaken (1950's), was cobalt resinate 1 per cent, causing the oil to drying in 21 hours. The cobalt acetate film dried in 28 hours, dryers of manganese and lead were much slower. If cobalt dryers are properly incorporated, they do not cause the oil to darken any more than using a pale refined linseed oil [28].

Normally safflower lends itself admirably to bleaching. Safflower oil lends itself to making a good boiled oil. When making stand oils with safflower the best results are obtained when using a Kestner isoelectric plant which results in very pale and low acidity stand oils which are a great advantage when making white enamels with a basic pigment like zinc oxide[28].

Normally it is a linoleic-rich oil (75% linoleic acid) with LLL (47%), LLO (19%), and LLS (18%) as the major triacylglycerols.

Much of the safflower processed in India in the past was crushed by a mortar-and pestle-like device called a *ghani*. Seed was cleaned by hand and then introduced into a *chakki*. This machine, which consisted of two horizontal stone wheels, one of which was turned by a blindfolded bullock, partially dehulled the cleaned seed passing between the stones. Hand winnowing and sieving next removed the hulls

from the seed kernels. The meals were pressed into balls after the addition of about 6% water. About 15 kg of the "balled" kernels were introduced into the *ghani*, an inverted conical mortar into which a heavy pole was placed. The pole was held to the side of the mortar by heavy weights and dragged around the perimeter by a team of *oxen*. A small amount of heated oil was added, and crushing then proceeded for 45 min, after which the oil was allowed to drain out through a small hole. A *ghani* could process about 100–120 kg of seed per day.

Later in the 20th century this semi-drying oil was more used in the paint industry as raw material in paint formulation [51].

3.5.7 Other drying or semi-drying oils used in oil paint formulation

Besides the three drying oils already described we may name that expressed from nigerseed, Guizotea oleifera. It is occasionally employed in grinding artist's colours as a substitute for Linseed and poppy oil. Tea-seed and camellia-seed oils, and the oils extracted in Japan from the seeds of Perilla ocymoides and from the kernels of Torreya nucifera are not of sufficient importance to demand description.

Church, 1890, "The Chemistry of Paints and Painting" pp.43

Sunflower seed oil is obtained from *Helianthus annus* and grows mainly in the USSR, Argentina, Western and Eastern Europe, China, and the United States as it is native of the great plains region from Nebraska to Northern Mexico. one of the most ancient oilseed species in North America, belongs to the family *Compositae* (*Asteraceae*) and the genus *Helianthus*. Cultivation of sunflower dates from times earlier than 3000 B.C., as indicated by archeological evidence obtained in sites once inhabited by the Hopi indians, in the north of Arizona.

There are approximately 50 varieties of sunflowers today, all of which descent from the North American wildflower *Helianthus annus roderalis*. Seeds used for oil are small, rounder and have thinner shells. It has a slightly sweetish taste and lacks the typical nutty flavor of darker oils.

In Russia there seems to be three principal varieties: one having the largest seeds and said to give more oil; one with smallish black seeds; and an intermediate form of striped seeds (both for eating and oil extraction). The mammoth Russian sunflower has heads 15 to 20 inches (38.1 to 50.8 cm) in diameter, and produces seeds about half an inch long. Russia has long been the classical country for production of sunflower oil and this semi-drying oil is the traditional medium in Russian oil paint.

Extraction by cold-pressing yields an oil of pale color which is chiefly used for edible processes after which it's hot pressed to give a darker oil which is used in paint and varnish. Experiments, from a paint point of view, were undertaken with basic pigments such as zinc oxide, Russian seed on the average contains 18-25% of oil; while Hungarian seeds have from 28-30%.

When the seeds are cold-pressed the oil is very pale and practically tasteless. The oil normally contains 60–75% of linoleic acid, >90% of oleic and linoleic acids combined, and virtually no linolenic acid. Its major triacylglycerols are typically LLL (14%), LLO (39%), LLS (14%), LOO (19%), LOS (11%), and other (3%).

As expected from its high linoleic acid content, the main triacylglycerol is trilinolein (36.3%), followed by oleo-dilinolein (29.1%); triolein being practically nonexistent (0.6%). Thus, the percentage of triacylglycerols (TAG) with four or more double bonds is higher than 80%.

Linoleates were found the best dryers in conjunction with sunflower oil. In preparing boiled oils the usual methods can be employed as regards this oil, as in the case of linseed, and a pale oil can be produced by the following method[28]:

One hundred pounds of the raw oil are heated for twenty hours at a temperature of 150ºF. (ºC.), using 1.5 per cent. of lead linoleate and 1.5 per cent. of manganese linoleate, the dryers having been previously dissolved in a portion of the oil at 300ºF. (ºC.). Air is blown into the heated oil, thoughout the period. By this means a pale oil will be produced which will dry in twenty-seven hours. When a darker oil is desired a temperature of 230-240ºF. (ºC.)can be employed and the lead and manganese increased to 8lbs. respectively.

Until two decades ago, the fatty acid composition of vegetable oils was closely related with their origin. The fatty acid profile of sunflower oil was thus defined within natural variation ranges. Current practices, however, are widely based on the production of oilseed of modified fatty acid composition. Several methods have been developed to this end.

Tung oil or China wood oil (*Aleurites fordii*)(*Olæococca vernica*) comes mainly from China, which explains its alternative name of China wood oil. It has been used there for hundreds of years, but its method of application in our modern paint and

varnish production industry may be said to date from the time when rosin-tung oil combination first began to be investigated and appreciated. The seeds from which the oil is obtained are derived from the tree *Aleurites fordii*, while another variety of aleurites known as *Aleurites Montana* which grows in the southern provinces of China, yields approximately 10 per cent. of the China wood oil produced. The large green apple like fruit ripens in August and September, and contains five to seven nut like oily seeds with fairly hard thick shells. The seeds yield 35 per cent of their weight in oil. The fruit of this tree that is 12 m *(A. fordii)* or 20 m *(A. montana)* in height contain 3-5 seeds. The seeds have a hard shell and contain -50% fat in their kernels. The tree can stand winter temperatures down to -15°C; the yield, however, suffers at or below -6°C. *A. monfuna* can be cultivated to altitudes of 1800 m, but to produce fruit, it requires 470 h above 7°C at least. The nuts are manually or mechanically harvested. Best yields reported are -3000 kgha. The shells of the seeds are used to loosen up the soil. The expeller cake contains 25**%** protein; it cannot be used for feeding because it also contains poisonous by-products and is therefore returned to the soil as fertilizer[28].

The oil when of good quality is pale amber in color, somewhat dull in appearance but lacking that brightness which is seen in rape or cottonseed oil. It has a peculiar and characteristic nutty odor and unpleasant taste. It is characterized by the presence of a conjugated triene acid (alpha-eleostearic, 9c11t13t-18:3, 69%). The oil dries more quickly than linseed with its nonconjugated triene acid, but oxidized tung oil contains less oxygen (5%) than does oxidized linseed oil (12%). Put another way, tung oil hardens at a lower level of oxygen-uptake than linseed oil.

This oil is exported mainly from China (30–40,000 tons) and is imported mainly by Japan, South Korea, Taiwan, and the United States (each 6000–7000 tons). Starting in 1993, attempts have been made to develop this crop in Mississippi. It is planned to have 15,000 acres planted by 2006 producing 30,000 tons of oil [52]).

Japanese wood oil, is a different oil, being obtained from the seeds of *Eleaococca Vernicia*.

Oititica Oil (*Licania rigida*) (Benth) This oil is obtained from the nuts of the tree known as Benth growing in a wide area in Northern Brazil in the states of Ceara, Piauhi, Parahyba, Rio Grande do Norte, and in the southern part of Pernambuco[28].

Oititica oil has been used by the natives of Brazil for a long time but the use of the oil for paint and varnish has only come forward to the outside world in recent years. Formerly the oil was prepared in a very primitive way, the seed being crushed, thrown in a cauldron of boiling water, and the oil, which rose to the surface skimmed off. Under modern factory conditions the oil is either extracted by pressure or treated with solvents such as trichloroethylene or a mixture of alcohol and benzene. Oil produced by the anglo-american press is usually lighter in color than oil recovered by extraction and does not "body" as quickly as the latter oil[28].

As regards drying properties Oititica oil is very similar to china wood oil being only slightly inferior in this respect. It shows less tendency to skin than china wood oil nor is it prone to crinkling and frosting.

Oititica oil can be made into *Stand* oils just like china wood oil, linseed or other drying oils, and blends of Oititica and Linseed are excellent. When mixed with Linseed oil in the proportion of 120 Linseed to 60 and heated to 280ºC., the oil should be kept at this temperature until the viscosity is about 40 poises[28].

Cottonseed oil was once the major vegetable oil in competition with the more widely used animal fats. The cotton plant (*Gossypium spp.*) is grown for its fiber with the oil being a byproduct and representing only about 11–12% of the gross value of the product.

The oil is consumed mainly in the country of origin with only limited exports/imports. Cottonseed oil is unusual among commodity vegetable oils in that it contains a relatively high level of palmitic acid (27%) along with oleic (18%) and linoleic acids (51%). Linolenic acid is virtually absent. Low levels of malvalic and sterculic acids (cyclopropene acids ~1%) are removed during refining. Gossypol present in the crude oil gives it a strong yellow color, and it's toxic to feedstock.

Castor Oil (*Ricinus comunnis*) the oil is obtained from the seeds of the Castor plant, which chiefly grows in India, and Central America. The seeds contain up to 50% of oil which is cold pressed and then hot-pressed. The castor bean consists of a capsule containing dark brown seeds that are 9-20 mm long, 6-15 mm wide and 4.5-9 mm thick. The seeds have a kernel that accounts for -25% of the seed weight. The thousand-seed weight is -650 g, the bulk weight -400 g/L. The seeds contain lipolytic enzymes that act so vigorously that slight damage to the seed leads to enormous fat splitting. Varieties that do not burst are harvested from the bush after drying; others must be gathered before drying.

The resulting cake is poisonous to cattle but can be used as manure. The chief constituent is ricinoleic triglyceride that contains only one double bond which is why this is a non-drying oil. Dehydration of castor oil and of castor acids gives products enriched in diene acids, some with conjugated unsaturation. The oil after

conversion can be heated up to 250-260ºC and bodied, which it does in a uniform manner, and is then quite soluble in white spirit or other hydrocarbon solvents, and can be used in connection to paints and varnishes. These products are valuable alternatives to drying oils such as Tung oil.

Sesame oil comes from the plant *Sesamum indicum*. This is grown mainly in India and China and in Myanmar (Burma), Sudan, Mexico, and Egypt with a total annual production of oil of 0.8 million tons. The seed has 40–60% of oil with almost equal levels of oleic (range 35–54, average 46%) and linoleic acids (range 39–59,average 46%).

Ground-Nut Oil. - The ground-nut or pea-nut (*Arachis hypogæa*) is very widely cultivated in the tropics for the sake of its oily seeds. In Java, the oil is extracted by drying the seeds in the sun and then subjecting them to pressure. In European mills, the nuts are first cleaned, then decorticated and winnowed, by which the kernels are left perfectly clean. These are crushed like any other oil seed, and put into bags, which are introduced into cold presses; the expressed oil is refined by passing through filter-bags. The residual cake is ground very fine, and pressed under 3 tons to the inch, in the presence of steam-heat; this affords a second quantity of oil, inferior in quality to the cold pressed. The usual product is 1 gal. of oil from 1 bushel of nuts by the cold process, besides the extra yield by the hot pressing.

In France, where the oil is largely prepared, three expressions are adopted, as with some sorts of gingelly: the first gives about 18 per cent, of superfine oil, fit for alimentary purposes; the second, after moistening with cold water, affords 6 per cent, of a fine oil, suitable for lighting; the third, after treating with hot water, yields 6 per cent, of *rabat*, or oil applicable only to soap-making. In India, the total mean yield is 37 per cent, at Pondicherry, and 43 in Madras.

The cold-pressed oil is almost colorless, of agreeable faint odor, and bland olive-like flavor. The best has a sp. gr. of about 0-918, or 0-9163 at 59° F . ; it becomes turbid at 37.5° F., concretes at 26.5°-25° F., and hardens at 19.5° F . By exposure it changes very slowly, but thickens with time, and assumes a rancid odor and flavor.

It is not a good oil for paint.

In George Terry, 1893, "*Pigments Paint and Painting (a practical book for practical men)*", E & F N Spon, London, Spon & Chamberlain, New York

Candlenut oil comes from the nuts of a tree with several names Lumbang, Kemiri, or Kukui, (*Aleurites moluccana Aleurites lumbag*). This is a tropical tree whose nuts contain a very unsaturated oil. This oil from the seeds of several species gives a good drying oil similar to linseed with chemical constitution similar to Tung oil. It is found in West Indies Brazil and Florida (USA).

It's FA composition is: 16:0 (6–8%), 18:0 (2–3%), 18:1 (17–25%), 18:2 (38–45%), and 18:3 (25–30%). Its iodine value, however, is not as high as that of linseed oil. It is used for cosmetic purposes and has been recommended for the treatment of burns.

Camelina or Gold of Pleasure (*Camelina sativa*) is also called false flax. *Camelina sativa (L.)* Crantz., is a plant from the Brassicaceae family, known as false flax, linseed dodder, and Gold-of-Pleasure, originated in the Mediterranean area and Central Asia (61). Seeds are small (0.7 mm _ 1.5 mm), pale yellow-brown, oblong, rough, and with a ridged surface. Camelina is listed as being adapted to the flax-growing region on the Prairies, in Europe, and other countries. It is primarily a minor weed in flax, which does not have seed dormancy.

Camelina oil has a unique fatty acid pattern and is characterized by a linolenic acid (C18:3) content ranging from 30% to 40%, eicosenic acid (C20:1) content of around 15%, and less than 4% erucic acid (21). The fatty acids in camelina oil are primarily unsaturated, with only about 12% being saturated (Table 4). About 54% of the fatty acids are polyunsaturated, primarily linoleic (18:2) and linolenic (18:3), and 34% are monounsaturated, primarily oleic (18:1) and eicosenoic (20:1).

In addition to its interesting fatty acid composition, this plant attracts attention because it grows well with lower inputs of fertilizers and pesticides than traditional crops, like rapeseed and linseed. The seed yield is in the range 1.5–3.0 t/ha, and the oil content is between 36% and 47%. The oil has an unusual fatty acid composition. It contains significant levels of oleic acid (10–20%), linoleic acid (16–24%), linolenic acid (30–40%), and C20 and C22 acids, especially 20:1 (15–23%).[53]

Marigold (*Calendula officinalis*). Interest in this seed oil is based on the fact that it contains significant levels (53–62%) of calendic acid along with linoleic acid (28–34%). Calendic acid (8t,10t,12c-18:3) is a conjugated trienoic acid, and this makes the oil an effective drying agent. Its alkyl esters can be used as a reactive diluent in alkyd paints replacing volatile organic compounds. The crop is being studied particularly in Europe.

Niger seed (*Guizotia Abyssinica*). This oil is obtained from the seeds of *Guizotia Abyssinica* and is of semi-drying character, occurring in Abissinia, East Africa and the East and West Indies[54]. It is referred to, even in the 19th century .

Fish Oils

A description of the oils of almost all marine-born animals was given by Hilditch and Williams (1964[15, 16]); a survey of the present usage of marine oils is offered by Opstved *et al.* (1990), but there was evidence long before [55, 56].

Certain fish oils have been known for a very long time to possess drying properties, and much research has been done especially when linseed and other vegetable drying oils have risen in price necessitating the substitution either wholly or in part the use of such oils as Menhaden or Pilchard especially in the manufacture of Paints and varnishes. Menhaden oil comes from the Menhaden, a fish living in the waters of the North Atlantic, and the Pilchard oil from a small fish somewhat similar to

sardine which is caught in large quantities in off the western coast of Canada. In the drying and semi-drying fish oils oxygen is absorbed and if all fish oils contained large amounts of the glycerides of clupanodonic acid (docosapentaenoic acid C22:5) they would dry almost equally to china wood oil, because of the great unsaturation of this acid. Unfortunately fish oils only contain about 12-15% of such acid and a relatively large amount of saturated acids, hence the slow drying of these oils. In the past not knowing the constitution of these oils and relying on iodine value, very disappointing results have been obtained. With regard to Pilchard oil, the raw oil has a greenish brown color, it is an highly unsaturated oil, and absorbs oxygen readily.

Table 3-3 Iodine values and average number of double bonds present in fish oils [25].

Average Number (n) of Double Bonds per Fatty Acid Molecule in Fish Oils

Fish	$C_{14:n}$	$C_{16:n}$	$C_{18:n}$	$C_{20:n}$	$C_{22:n}$	$C_{24:n}$	Iodine value
Herring	~1.0	1.2–1.7	1.5–2.2	2.2–3.5	3.8–5.5	1.8–2.0	125–160
Pilchard	—	~1.0	1.5–1.7	2.0–2.2	4.0–4.5	4.3–5.5	160–190
Sardines	—	~1.0	1.5–1.9	2.2–2.6	2.3–2.7	—	160–190
Menhaden	~1.0	1.5–1.7	1.6–2.0	4.0–5.0	3.8–5.0	—	155–195
Salmon	—	~1.0	2.6–2.8	4.2–4.7	6.4–6.8	—	140–165

Raw fish oils, that is to say untreated oil should never be used. Before they can be employed in the manufacture of paints, the albuminous matter must be removed by refrigerating, sometimes called "wintering". If very pale oils are required, the heating should be done in an inert atmosphere or in vacuum. This prevents oxidation and removes volatile components. Fish oils are also *Bodied* by heating to say 150ºC and *Blowing*, high temperatures should be avoided.

In using Menhaden oil it was found that cobalt was the best dryer and both cobalt linoleate at 0.1 per cent and cobalt resinate at 0.25 per cent gave good drying results, substituting cobalt by manganese linoleate at 0.25 per cent gave slow drying and

films were not as hard as with cobalt. Zinc linoleate did not appear to influence the drying or film hardness.

3.6 REFERENCE CHEMICAL COMPOSITION TABLE

Table Identified TAG's in reference oils as in [57] with corrected relative abundances.

TAGs	Tung oil	Linseed oil	Walnut oil	Poppyseed oil	Safflower oil	Sunflower oil	Castor oil	Olive oil	Almond oil	Soybean oil
LnLnLn	–	15	1	–	–	–	–	–	–	0.8
EEE	52	–	–	–	–	–	–	–	–	–
LnLnL	–	12.5	5.8	–	1.2	0.9	–	–	–	1.9
EEL	15	–	–	–	–	–	–	–	–	–
LnLnP	–	7.2	1.3	–	–	–	–	–	–	–
ELL	19.8	–	–	–	–	–	–	–	–	–
LnLL	–	14.4	14.8	1.2	1	0.8	–	–	–	7.2
PoLnL	3.9	–	–	–	–	–	–	–	–	–
MLL	–	–	–	–	–	–	–	–	–	0.6
LLnP	–	14.4	5.4	–	1	0.8	–	–	–	–
PoLL	2.2	–	–	–	–	–	–	–	–	–
LLL	3.4	5.4	20.5	28.5	26.9	20.3	–	–	13.6	16.9
LLP	–	–	11.5	19.2	21.2	16.7	–	3.1	7	16.9
PLnP	–	2.7	–	–	–	–	–	–	–	–
OLnP	–	–	3	–	–	–	–	–	–	–
GLL	1.6	–	–	–	–	–	–	–	–	–

TAGs	Tung oil	Linseed oil	Walnut oil	Poppyseed oil	Safflower oil	Sunflower oil	Castor oil	Olive oil	Almond oil	Soybean oil
LLO	1	–	–	18.1	19.4	–	–	8.2	–	21.3
PLP	–	10.8	–	–	–	–	–	–	18.6	1.9
LOP	–	–	11.4	7.1	4.7	20.6	–	14.5	10.9	–
MLO	–	1.6	–	–	–	–	–	–	–	–
OOL	0.9	–	16.3	10.2	4.8	5.5	–	–	24.2	15.7
OOP	–	2	3	3.3	2	16.8	–	25.1	6.9	–
POP	–	10.2	–	–	–	–	–	4.5	–	7.2
OOO	–	–	–	7.1	4.4	4.8	–	29.5	11.4	1.3
OLS	–	–	4.1	–	–	–	–	–	–	–
ALL	–	1.6	0.3	2.8	2.2	2.7	–	–	–	–
ErLO	–	–	–	–	–	–	–	–	–	1.9
OSP	–	0.4	–	–	–	1.7	–	2.7	–	0.7
OOS	0.1	–	1.2	2	2.3	–	–	9	6.3	4.4
ALO	–	1.6	–	–	2.1	–	–	–	–	–
BLL	–	–	–	0.1	2.4	1.7	–	–	0.8	–
BLO	–	–	–	0.1	2.1	1.6	–	–	–	–
AOO	–	–	–	–	–	1.8	–	2.5	–	–
LiLLn	–	–	–	0.1	1.7	–	–	3.1	–	0.4
LiLL	–	–	–	0.1	–	1.6	–	–	–	0.7
BOO	–	–	–	0.1	–	1.3	–	–	–	–
RnRnRn	–	–	–	–	–	–	49.5	–	–	–
RnRnL	–	–	–	–	–	–	20.2	–	–	–
RnRnO	–	–	–	–	–	–	17.1	–	–	–
RnRnS	–	–	–	–	–	–	10.2	–	–	–
RnLL	–	–	–	–	–	–	0.3	–	–	–
RnLO	–	–	–	–	–	–	2.6	–	–	–
RnLS	–	–	–	–	–	–	0.1	–	–	–

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4 - ANALYTICAL TECHNIQUES

ABSTRACT

This chapter reviews oils research science analytical techniques, physical principles and methodological procedures of the former, used as methods to further research on the Chemistry based developments in Molecular drying and ageing particularly of Oil Paint.

4.1 INTRODUCTION

The analysis of vegetable oils has drawn attention since early times. Yet, the development of analytical techniques used for this purpose has changed entirely with the passing of time, with the multiple improvements in the analytical Instruments and also with new methods applied to these particular specimens of Lipids.

This chapter is devoted mainly to the theory behind each method used in our studies and their particular advantages for each analytical instrumental method.

4.2 INFRARED SPECTROSCOPY AND MICROSPECTROSCOPY

Infrared spectroscopy is based on the interaction of electromagnetic radiation with matter, namely the Infrared part of the electromagnetic spectrum. In Paint analysis it is very helpful in assigning both binding media and pigment constituents that have infrared active moieties.

Particularly, if you couple a FTIR instrument with an Infrared microscope, the ability to monitor Infrared bands on tiny microsamples of art works with Microspectrometry is now a standard method to both assign and monitor changes in moieties pertaining both to different oil or other binding media, just as it is a recognized method to assign different pigments especially in the NIR region.

If you compress a sample in a diamond anvil cell [1] you are able to achieve good spectra. Most samples, but not all, might be embedded in a polyester resin[2] and the microtoming a cross-section might be possible.

There are innumerous handbooks on the theory behind FTIR and Microspectroscopy, (review on master thesis) but we cannot left aside the Monograph on Getty Books by Michelle Derrick [3] which is an essential read and is such a fundamental work especially if you're debating issues on conservation of art works, after a chapter in practical guide to infrared microspectroscopy [4]. Notable mention is also Jaap van der Weerd's work [5, 6] on the MolArt series, addressing not only microscopic Imaging of embedded Paint cross-sections but also FTIR studies of the effect of pigments in the ageing of oil, and one of the first to address chemical changes in old master paintings, such as dissolution, metal soap formation, protrusions, efflorescence , and remineralisation processes. Other researchers also pushed this forward.

4.3 MASS SPECTROMETRY

The first and most important question that arises in someone's head is: What is mass spectrometry?

> *The basic principle of mass spectrometry (MS) is to generate ions from either inorganic or organic compounds by any suitable method, to separate these ions by their mass-to-charge ratio (m/z) and to detect them qualitatively and quantitatively by their respective m/z and abundance. The analyte may be ionized thermally, by electric fields or by impacting energetic electrons, ions or photons. The ... ions can be single ionized atoms, clusters, molecules or their fragments or associates. Ion separation is effected by static or dynamic electric or magnetic fields.[7]*

Although this definition dates back to 1968, when mass spectrometry was at its childhood, it is still valid. Nevertheless, two additions should be made. These concern the fact that besides electrons, (atomic) ions or photons, energetic neutral atoms and heavy cluster ions can also be used to ionize the analyte. Secondly, ion separation by *m/z* can occur in field free regions, as effectively demonstrated by the time-of-flight analyser, provided the ions possess a well-defined kinetic energy at the entrance of the flight path.

From the 1950s to the present, mass spectrometry has evolved tremendously. The pioneering mass spectrometrist had a home-built naked instrument, typically a

magnetic sector instrument with electron ionisation. At the present there are too many types of ion sources, analysers, and detector systems all built in a conveniently designed instrument that it's hard to recognize from the outside. Mass spectrometry (MS) is probably the most versatile and comprehensive analytical technique currently available in the chemists and biochemists' arsenal. This analytical technique measures precisely the molecular masses of individual compounds by converting them into charged ions and analysing them in what is called a mass analyser. This is the simplest, but somewhat reductionist, definition of mass spectrometry. The days of the simple determination of the m/z ratio of an organic compound are over, today mass spectrometry can be used to determine molecular structures, to study reaction dynamics and ion chemistry, provides thermochemical and physical properties such as ionisation and appearance energies, reaction enthalpies, proton and ion affinities, gas-phase acidities, and so on. Mass spectrometry is so versatile that even several areas of physics, pharmaceutical sciences, archaeology, forensic and environmental sciences, just to state a few, have benefited from the advances in this instrumental technique.

In the recent publication "A History of European Mass Spectrometry" edited by Prof. Keith R Jennings, [8] one that has no idea of the first spectrometers and how they evolved can go through a personal history with many of those directly involved in their success, from developments to applications, a step by step ride through all the milestones of Mass Spectrometry, and a complete and unabridged text for anyone interested in this subject, here is just a summary of some main events:

The history of mass spectrometry starts in 1898 with the work of Wien, who demonstrated that canal rays could be deflected by passing them through superimposed parallel electric and magnetic fields. Nevertheless, its birth can be credited to Sir J. J. Thomson, Cavendish Laboratory of the University of Cambridge through his work on the analysis of negatively and positively charged cathode rays

with a parabola mass spectrograph, the great grandfather of the modern mass spectrometers. [9]; [10] In the next two decades, the developments of mass spectrometry continued by renowned physicists like Aston, [11] Dempster, [12] Bainbridge, [13, 14] and Nier. [15, 16]

In the 1940s, chemists recognised the great potential of mass spectrometry as an analytical tool, and applied it to monitor petroleum refinement processes. The first commercial mass spectrometer became available in 1943 through the Consolidated Engineering Corporation. The principles of time-of-flight (TOF) and ion cyclotron resonance (ICR) were introduced in 1946 and 1949, respectively [17]; [18].

Applications to organic chemistry started to appear in the 1950s and exploded during the1960s and 1970s. Double-focusing high-resolution mass spectrometers, which became available in the early 1950s, paved the way for accurate mass measurements. The quadrupole mass analyser and the ion trap were described by Wolfgang Paul and coworkers in 1953. [19] The development of gas chromatography/mass spectrometry (GC/MS) in the 1960s marked the beginning of the analysis of seemingly complex mixtures by mass spectrometry. [20, 21] The 1960s also witnessed the development of tandem mass spectrometry and collision-induced decomposition, [22] being a breakthrough in structural and quantitative analysis, as well as in the development of soft ionisation techniques such as chemical ionisation [23].

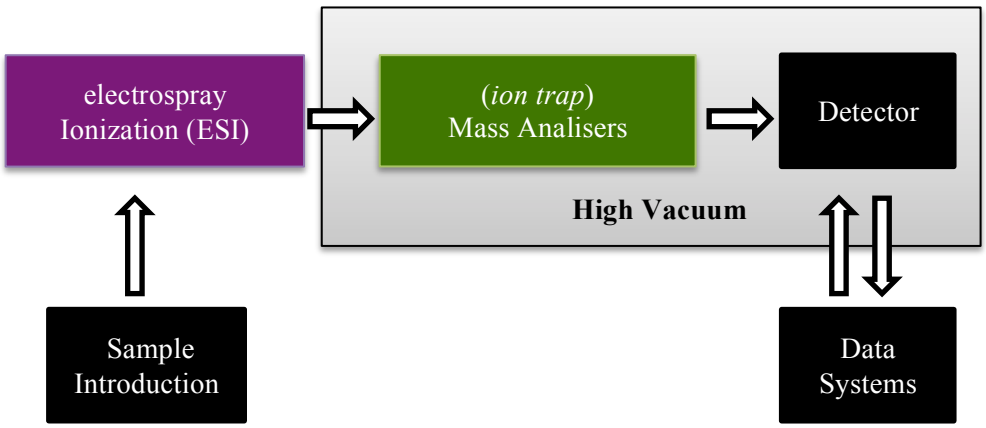
By the 1960s, mass spectrometry had become a standard analytical tool in the analysis of organic compounds. Its application to the biosciences, however, was lacking due to the inexistence of suitable methods to ionise fragile and non-volatile compounds of biological origin. During the 1980s the range of applications in the field of the biosciences increased "exponentially" with the development of softer ionisation methods. These included fast atom bombardment (FAB) in 1981, [24] electrospray ionisation (ESI) in 1984-1988, [25] and matrix-assisted laser

desorption/ionization (MALDI) in 1988 [26]. With the development of the last two methods, ESI and MALDI, the upper mass range was extended beyond 100 kDa and had an enormous impact on the use of mass spectrometry in biology and life sciences. This impact was recognised in 2002 when John Fenn (for his work on ESI) and Koichi Tanaka (for demonstrating that high molecular mass proteins could be ionised using matrix assisted laser desorption) were awarded with the Nobel Prize in Chemistry.

Concurrent with the development of ionisation methods, several innovations in mass analyser technology, such as the introduction of high-field and superfast magnets, as well as the improvements in the TOF and Fourier transform ion cyclotron resonance (FTICR) enhanced the sensitivity and the upper mass range. The new millennium brought us two new types of ion traps, the orbitrap which was invented by Makarov [27] and the linear quadrupole ion trap (LIT) which was developed by Hager. [28].

The coupling of high-performance liquid chromatography (HPLC) with mass spectrometry was first demonstrated in the 1970s [29]; nevertheless, it was with the development and commercialisation of atmospheric pressure ionisation sources (ESI, APCI) that for the first time the combination of liquid chromatography and mass spectrometry entered the realm of routine analysis. [30-33]

Figure 4.4.3-1. Diagram of the major components common to a typical modern mass spectrometers.



Generally, a mass spectrometer is composed of five components (Fig. 4.1): inlet system, ion source, mass analyser, ion detector, and data system.

Samples are introduced in the mass spectrometer and transferred into the gas phase through the inlet system that could be at atmospheric pressure or under vacuum. In the ion source, the gas-phase analytes are ionised and transferred into the mass analyser where they are separated according to their mass-to-charge ratios (m/z). Ion detection can be accomplished by electron multiplier systems that enable m/z and abundance to be measured and displayed by means of an electric signal perceived by the data system, which also controls the equipment. All mass spectrometers are equipped with a vacuum system in order to maintain the low pressure (high vacuum) required for operation. This high vacuum is necessary to allow ions to reach the detector without undergoing collisions with other gaseous molecules. In fact, collisions would produce a deviation of the trajectory and the ion would lose its charge against the walls of the instrument. On the other hand, a relatively high pressure environment could facilitate the occurrence of ion-molecule reactions that would increase the complexity of the spectrum. In some experiments the pressure in the source region or in a part of the mass spectrometer is intentionally increased to study ion-molecule reactions or to perform collision-induced dissociations. The high vacuum is maintained using mechanical pumps in conjunction with turbomolecular, diffusion or cryogenic pumps. The mechanical pumps allow a vacuum of about 10^{-3} torr to be obtained.

Once this vacuum is achieved the operation of the remainder of the vacuum system allows a vacuum as high as 10^{-10} torr to be reached.

4.4 IONIZATION SOURCES - ESI-MS

One of the crucial steps in mass spectrometry is the formation and transfer of ions from a sample to the gas-phase. This can be done by a variety of available ionization techniques. In order to make a correct choice, it is necessary to take into account the internal energy transferred during the ionization process and the physico-chemical properties of the analyte.[34] Some ionization techniques are very energetic and cause extensive fragmentation. Other techniques are softer and only produce ions of the molecular species. Electron ionization, chemical ionization and field ionization are only suitable for gas-phase ionization and thus their use is limited to compounds sufficiently volatile and thermally stable. There is, however, a large number of compounds that are either thermally labile or do not have sufficient vapour pressure. For these compounds to be analysed, they must be directly extracted from the condensed phase to the gas-phase. [34]

There are three groups of methods for the formation of gas-phase ions: [35]

i - Volatile materials are generally ionized by interaction of their vapours with electrons (Electron Ionization - EI), with ions (Chemical Ionization - CI), or with strong electric fields (Field Ionization - FI);

ii - Nonvolatile and thermally labile materials can be desorbed into the gas-phase via bombardment with fast atoms (Fast Atom Bombardment - FAB), ions (Secondary Ion Mass Spectrometry - SIMS and Liquid SIMS), laser photons (Matrix Assisted Laser Desorption/Ionization - MALDI) or electrosprayed solvent (DESI) [36];

iii - Liquid solutions of the analyte may directly be converted to gas-phase ions via spray techniques (Electrospray Ionization - ESI, Atmospheric Pressure Chemical Ionization - APCI, Atmospheric Pressure Photoionization - APPI).

The next subsection will address Electrospray Ionization.

The electrical atomization of liquids was first observed by Georg Mathias Bose in 1745. In 1882 Lord Rayleigh determined an instability criterion for the charged liquid droplets. Between 1914 and 1920, Zeleny [37-39] studied the droplet shape as a function of the applied voltage and established a criterion for the instability of an electrified liquid at the end of a capillary tube.[40, 41]

The development of electrospray by Fenn and co-workers [25, 42] was largely ignored by the mass spectrometry community until, before a small audience at the 1988 ASMS meeting, they showed that multiply charged ions could be obtained from proteins, allowing their molecular weight to be determined in instruments for which the mass range was limited to as low as 2000 Da. At the beginning, ESI was considered to be an ionization source dedicated to protein analysis; nevertheless its use was extended not only to other polymers and biopolymers, but also to the analysis of small polar molecules. In fact, one of the first papers published by Fenn and co-workers was indeed about the use of electrospray with small molecules.[43]

ESI principles and biological applications have been extensively reviewed in the literature [44-46], and several books either on this subject or that addressed this topic appeared over the years.[7, 29, 34, 40, 47-49]

There are three major steps in the production of gas-phase ions by electrospray:

i - Production of charged droplets at the electrospray capillary tip (The Electrophoretic Mechanism);

ii - Shrinkage of the charged droplets by solvent evaporation and repeated droplet disintegrations (fissions), leading to very small highly charged droplets;

iii - The actual mechanism by which gas-phase ions are produced from very small and highly charged droplets.

Production of charged droplets at the electrospray capillary tip (The Electrophoretic Mechanism)

As shown in the schematic representation of the charged droplet formation (Figure 4.2) a voltage, *Vc*, of 2-3kV is applied to the metal capillary, usually located at 1-3 cm from the counter electrode (in ESIMS this counter electrode has an orifice leading to the mass spectrometric sampling system). Because the capillary tip is very narrow, the electric field, *Ec*, at the capillary tip is very high ($Ec \approx 10^6$ V/m).

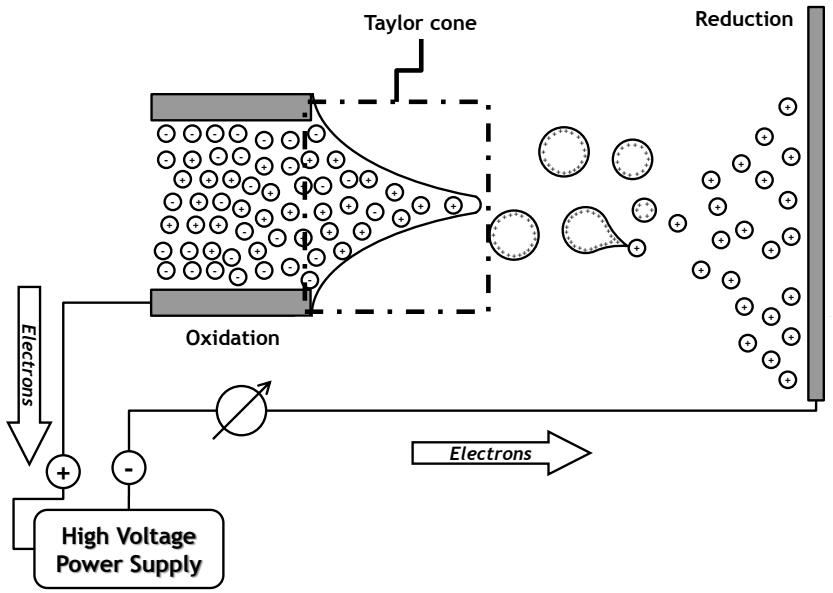


Figure 4. 2. Schematic representation of the electrospray events occurring at atmospheric pressure.

When the capillary of radius r_c is located at a distance d from the planar counter electrode, the magnitude of E_c for a given potential V_c can be estimated using the approximate relationship:[50]

$$E_c = \frac{2V_c}{r_c ln\left(\frac{4d}{r_c}\right)} \qquad \text{(Equation 4.1)}$$

Where V_c is the applied potential, r_c is the capillary outer radius and d is the distance from the capillary tip to the counter-electrode. E_c, is proportional to V_c. E_c is essentially inversely proportional to r_c and it decreases slowly with the electrode separation d due to the logarithmic dependence on d [51, 52].

The typical solution used in ESI-MS consists of a polar solvent in which the analyte is soluble. Because ESI-MS is a very sensitive method, very low concentrations, of analyte can be used. Methanol or methanol-water, acetonitrile or acetonitrile-water are often used as solvent, at electrolyte concentrations as low as 10^{-7} M. Other solvents such as toluene (that have a very low solubility for electrolytes) can also be used [52].

The imposed field, E_c, will also partially penetrate the liquid at the capillary tip. When the capillary is the positive electrode (positive ion mode) some positive ions will drift toward the liquid surface and some negative ions will drift away from it until the imposed field inside the liquid is removed by charge redistribution. The accumulated positive charge at the surface leads to its destabilization since the positive ions are drawn down-field but cannot escape from the liquid. The surface is then drawn down-field in such a way that a liquid cone is formed. This is called the Taylor cone, named after Sir Geofrey Taylor who was one of the first to investigate the conditions under which a stable liquid cone can exist with the competing forces of an electric field and the surface tension of the liquid [53].

At a sufficiently high field, E_c, the cone is not stable and a liquid filament with a diameter of a few micrometers and a surface rich in positive ions, is emitted from the Taylor cone tip. At some distance downstream, the liquid filament becomes unstable and forms separate droplets which are charged with an excess of positive electrolyte ions, the cone jet mode (Figure 4a). The described cone jet mode is one of the possible and the best characterized modes in electrospray literature. The length of the unbroken liquid filament decreases if the field E_c is increased. At higher fields, a multispray condition is reached in which the central cone disappears and droplet emission occurs from a crown of four to six short liquid tips formed at the rim of the capillary (Figure 4b-c) [54, 55]. The most commonly observed spray modes have been defined by Jaworek [56], however, many of these exhibit pulsating characteristics which translate into a periodical variation of the droplet characteristics [57, 58]. Furthermore the different spray modes generate droplets with different size and charge distribution [59-61]. Nevertheless the most effective spray mode for producing droplets suitable for ESI-MS is the cone jet spray mode [52, 53] in which a stable non-pulsating Taylor cone is formed [59, 62, 63].

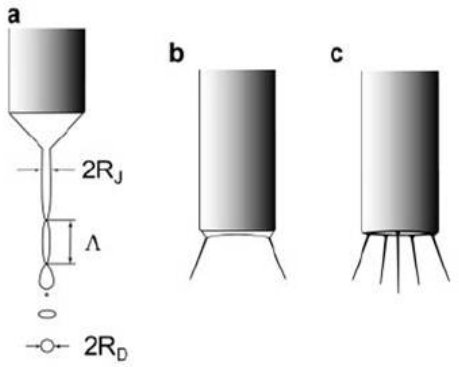


Figure 4. 3. Different forms of electrospray at the tip of the capillary: a) Cone jet mode (the tip of the cone is extended into a liquid jet, R_j= jet radius, R_D= droplet radius); b) and c) multi-jet modes (multiple jet modes are observed when voltage is increased from *a* to *b* to *c*).

Due to an excess of positive electrolyte ions at the surface of the cone and cone jet, the droplets are positively charged. These charged droplets drift downfield through the air towards the opposing electrode. Solvent evaporation at constant charge leads to droplet shrinkage and an increase of the electric field normal to the surface of the droplets. At a given radius the repulsion between the charges overcomes the surface tension of the droplet which causes a coulomb fission of the droplet (coulomb explosion) [52]. This droplet fission occurs via formation of a cone and cone jet that split into a number of small progeny droplets in a process that bears close resemblance with the cone jet formation at the capillary tip. [64] Further evaporation of the parent droplet leads to repeated fissions and these progeny droplets can also evaporate and undergo fissions. Very small charged droplets result, that lead ultimately to gas-phase ions, by processes that will be described in the following sections.

The ion separation mechanism which is called the electrophoretic mechanism is also most plausible on energetic grounds. Electrical double layers are already formed at low fields in electrolyte solutions. The resulting positive-negative ion redistribution reduces or completely removes the imposed field and therefore suppresses other forms of ionization such as field ionization which requires very high electric fields [51, 52]. Another way to show that electrospray results from electrophoretic charging is to deionize the solvent. Experiments involving deionized methanol and the addition of SF_6 to the atmospheric gas (in order to prevent electric discharges) showed an intermittent electrospray current attributed to the absence of electrolytes to sustain a stable electrospray operation [55].

If the charge separation is electrophoretic, at a steady state the positive droplet emission will continuously carry off the positive ions. Considering the requirements for charge balance in such a continuous electric current device, and that only electrons can flow through the metal wire supplying the electric potential

to the electrodes (Figure 4) it is clear that the electrospray process should involve an electrochemical conversion of ions to electrons. As such, the electrospray source can be viewed as a special type of electrolytic cell in which part of the ion transport does not occur through an uninterrupted solution, but as charged droplets and later as gas-phase ions [51, 52]. Thus, in the positive ion mode where positively charged droplets and later positive gas-phase ions are the charge carriers, a conventional electrochemical oxidation reaction should be occurring at the liquid/metal interface of the spray capillary. This reaction supplies the extra positive ions to the solution that prevent the build-up of a charge imbalance.

Shrinkage of the charged droplets by solvent evaporation and repeated droplet disintegrations (fissions)

As the solvent evaporates from charged droplets, usually with assistance of resistive heating, the size of the droplets becomes smaller while the charge that they carry remains constant.[65] The assumption that the charge remains constant is reasonable since the emission of ions from solution to the gas-phase is a highly endoergic process (Equation 4.2) [52]; furthermore it has been shown in the literature that droplets within the micrometer range or larger do not emit gas-phase ions.[60]

$$Na^{+}_{(aq)} \rightarrow Na^{+}_{(g)}$$
$$-\Delta G^{0}_{sol} = 410\ kJ\ mol^{-1} \qquad \text{(Equation 4.2)}$$
$$-\Delta H^{0}_{sol} = 444\ kJ\ mol^{-1}$$

This results in increasing electrostatic stress near the surface of a given droplet. When the force of electrostatic repulsion between like charges becomes equal to the surface tension holding the droplet together, the so-called "Rayleigh

stability limit" has been reached as defined by the Rayleigh equation (Equation 4.3) [52, 65, 66] where Q_R is the excess charge on the droplet of radius R, γ is the surface tension and ε_0 is the permittivity of vacuum.

$$Q_R = 8\pi(\varepsilon_0 \gamma R^3)^{1/2} \quad \text{(Equation 4.3)}$$

Just prior to reaching the Rayleigh stability limit, droplets undergo what is referred to as "Coulomb fission", a process which leads to the production of smaller progeny droplets (Figure 4.4.4). According to the literature, droplets with sizes in the 1 μm range, undergo fission at approximately 80% of the Rayleigh limit [60, 67]. The droplet will then shrink again, due to solvent evaporation, at constant charge until, once again, near the Rayleigh stability limit, fission occurs. The droplet does not split evenly into two smaller droplets of approximately equal mass and charge. Charged droplets are not static entities, but rather they may distort from spherical into oblate or prolate shapes[51, 60]. Shape irregularities of these types stimulate disruptions in which a stream of much smaller droplets is emitted in what was originally termed "uneven fission" [67] and nowadays is known as "droplet-jet fission" [51, 66]. Figure 4.4 shows two shadowgraphs in which this disruption is clearly seen. This disruption pattern is similar to the disruption at the tip of the Taylor cone. The emitted stream of progeny droplets carries off only about 2% of the mass of the parent droplet, but about 15% of the parent's charge. The progeny droplets, which are quite monodisperse, have a radius of about 1/10 of the parent's radius [60].

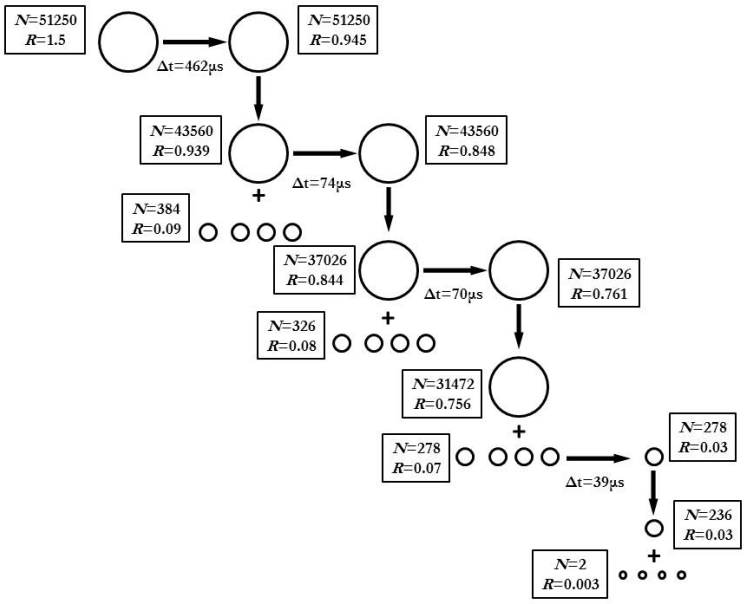


Figure 4.4 Schematic representation of the time history of parent and progeny droplets, N = number of elementary charges on the droplet, R = radius of the droplet in μ m (image taken from reference [51]).

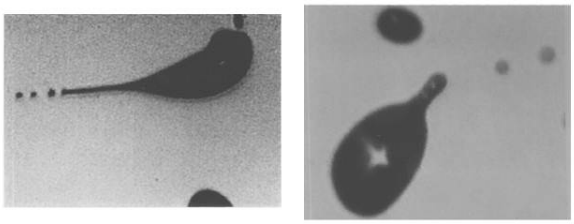


Figure 4.4 Flash shadowgraphs showing droplets undergoing Coulomb fission (images taken from reference [65]).

The charge-to-mass ratio is thus increased in the progeny droplets relative to the parent droplet from which they have been produce, but the overall repulsive force of like charges near the surface is attenuated because the charge is spread over a larger total surface area [65]. The droplet-jet fission process may repeat itself a

second, and perhaps a third, time upon further shrinkage of the offspring droplets [65].

Formation of gas-phase ions from charged droplets: ion evaporation model (IEM) and charge residue model (CRM)

There are mainly two possible mechanisms for the production of gas-phase ions in electrospray: the charge residue model (CRM) proposed by Dole et al.[68] and the ion evaporation model (IEM) proposed by Iribarne and Thomson.[69, 70] Both these models are feasible, although under different conditions. The ion evaporation model typically prevails for relatively small ions (m/z < 3300),[71, 72] whereas the charge residue model seems to be valid for larger multiply charges species[73]. Nevertheless, it can be considered that these models describe the two extremes of the same general process. These will be described in the following sections.

Ion Evaporation Model

Iribarne and Thomson, who worked with small ionic analytes such as Na^+ and Cl^-, proposed the ion evaporation mechanism (IEM).[69, 70] The model predicts that direct ion emission from the droplets will occur after their radius shrink to less than 10 nm. Hence, the ion evaporation process replaces Coulomb fission as a way to remove charge from the droplet (Figure 4.4) [52] .

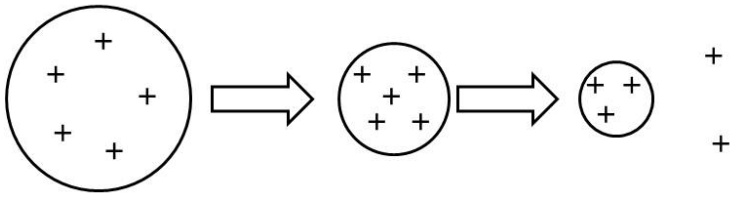


Figure 4.4. Gas-phase ion formation via ion evaporation model.

This model is supported by experimental [69] and theoretical [70] results.

Recently, molecular dynamics simulations helped in the understanding of the formation of gas-phase ions from charged droplets [74, 75]. Vertes and co-workers simulated the behaviour of analyte ions in water nanodroplets and concluded that the analyte ions evaporated from the nanodroplets (close to the Rayleigh limit) with a solvation shell of approximately 10 water molecules [74].

Charge Residue Model

The charge residue mechanism was proposed by Dole,[68] who was then interested in the determination of the molecular masses of polystyrenes. For such macromolecules, Dole assumed that some of the droplets formed would contain one analyte molecule, as well as the ionic charges at the surface. Solvent evaporation from these droplets will lead to a gas-phase ion which charge has its origins from the surface charges of the parent droplet (Figure) [52].

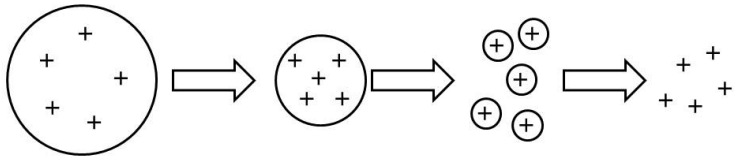


Figure 4.7 Formation of gas-phase ions via charge residue model.

Up to now, only small ions were considered and, an unequivocal decision, as to whether the gas-phase ions were produced by ion evaporation, or the charged-residue mechanism could not be attained. In fact, many of the observed results in mass spectrometric experiments can be explained by both mechanisms.

The status of the ion evaporation and charge residue theories for macroions (particularly the analytically important large polyprotonated and polydeprotonated

proteins and nucleic acids) is different. The ion evaporation theory (see previous section) was derived as a model for small ions, but the extension of its equations to macroions is available. A qualitative extension of the ion evaporation model which deals with multiply charged macroions has been proposed by Fenn.[76]

When dealing with macromolecules, proteins for example, one must take into account their large size and their behaviour regarding the solvent. With the exception of the locations that carry polar and charged residues, the protein is on the whole solvophobic in the typical polar solvents used. This solvophobic behaviour, together with the repulsion of the other charges on the surface, assists the escape of the protein and its proton charges from the droplet. Nevertheless, this process cannot be expected to be fast, and may not compete with the rapid evaporation of solvent from the droplet, which may lead to Rayleigh fission or even to the formation of a charged residue.

The charged-residue theory does not require any significant modifications for macromolecules, and becomes the more natural mechanism for these entities. If the charged-residue model holds, it would statistically be expected to observe multimers as a result of more than one protein molecule being present in the final droplet. Richard Smith and co-workers [77] observed multiply charged monomers as well as low intensity dimmers and trimers. Smith et al. found an empirical correlation (Equation 4.4) between the molecular mass (M) and the average observed charge state (Z_{av}) of ions formed from starburst dendrimers (these are multi-branched alkyl-amine polymers with relatively rigid structures and close to spherical form, i.e. they resemble globular proteins), where a and b are constants and b=0.53 led to the best fit [52].

$$\boldsymbol{Z_{av} = aM^b} \qquad \text{(Equation 4.4)}$$

A similar relationship was found by Standing and co-workers where the value of b was between 0.52 and 0.55 [52].

Using literature data, Fernandez de la Mora showed that (Equation 4.4) holds and that it could be derived from the Charge Residue Mechanism[73]. This variation of the CRM received a wide acceptance. Samalikova and Grandori questioned the validity of the model [78-80], but their conclusions were, however, questioned by Nesatyy and Suter[81].

The Charge Residue Mechanism allowed quantitative predictions of the protein charge state in the gas-phase using a simple empirical correlation between charge state and protein mass. Recently, Gross and co-workers[82] suggested a modification of the CRM in which CRM is preceded by IEM.

Regardless of which method formed the ions, after they are formed from the charged droplets, they move in the potential field toward the entrance capillary, and some of the ions become entrained in the flow of gas that enters the mass spectrometer. A countercurrent gas, or "curtain gas," surrounds the inlet capillary in most modern instrument designs. This helps to reduce the amount of buffer allowed into the heated capillary and helps to break up solvent clusters. After passing down the heated capillary, the spray exits the rear of the heated capillary, where it undergoes supersonic free jet expansion in an area of partial vacuum (<1 torr;). In this region, ions are focused by a tube lens onto a skimmer that samples ions off-center from the cone of spray. Once past the skimmer, ions are kept in their trajectories by ion guides. These ion guides may be plate lenses or multipoles (or a combination of these). After these ion-focusing elements, ions travel into the high vacuum region to the mass analyzer.

4.5. MASS ANALYSERS

There are numerous types of mass analysers; nevertheless, the main purpose is the same for all: to separate ions according to their mass-to-charge (m/z) ratios.

The mass analysers can be divided into:

- Sector instruments: a combination of magnetic and electrostatic sectors separates the ions according to their masses and focus the ion beam in terms of kinetic energy;
- Quadrupoles and Ion traps: a radiofrequency electric field allow the separation of the ions according to their m/z ratios;
- Time-of-Flight (TOF): the ions are accelerated and the time necessary to travel a specific distance (the length of the flight tube) can be used to determine the m/z ratio.
- Ion Cyclotron Resonance (ICR): a high magnetic field induces in the ions cyclotron motion and the frequency of this motion can be related to their m/z ratios.
- Linear Ion Trap (LIT) & Orbitrap: these are the newest members of the trap family,

The next three subsections will address Quadrupole Ion Trap (QIT), Triple Quadrupole (TQ) and Fourier Transform Ion Cyclotron Resonance (FTICR).

4.5.1 QUADRUPOLE ION TRAP

Unlike beam instruments, mass separation in quadrupole ion traps is achieved by storing ions in the trapping space and by manipulating their motion in time, rather than in space. This task is accomplished with an oscillating electric field created within the boundaries of the trap.

The development of the quadrupole ion trap can be divided into three ages: mass-selective detection[83-85], mass-selective storage[86, 87] and mass-selective axial ejection[88].

The second age, mass-selective storage, took place during the late 1960s to the early 1980s. The third age is called mass-selective axial ejection and its development started in 1979 Finnigan Corporation (nowadays Thermo Scientific)this mode of operation that led to the dramatically increase of interest in ion trap instruments.

The quadrupole ion trap (Figure 4.8) consists of two hyperbolic electrodes serving as end caps and a doughnut-shaped ring electrode [7].

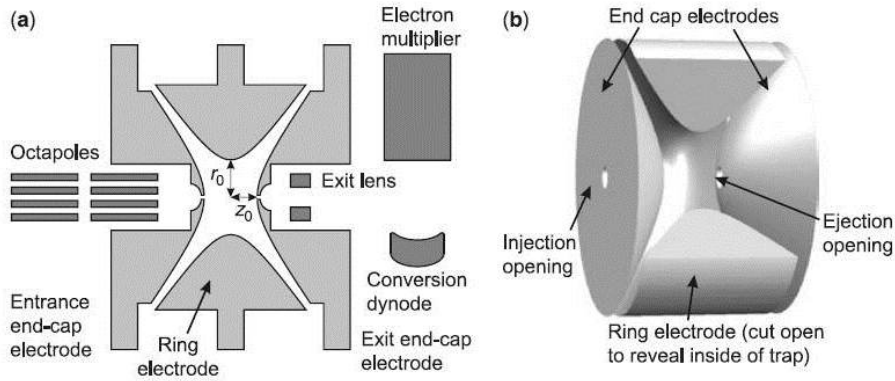


Figure 4.8 (a) Cross-section of a quadrupole ion trap; (b) three-dimensional perspective view of a quadrupole ion trap. (Image taken from reference [89])

The three-dimensional quadrupole field is created by applying a potential to the ring electrode and maintaining the end-cap electrodes at ground potential[29, 90].

The working principle of the quadrupole ion trap is based on creating stable trajectories for ions of a certain m/z or m/z range while removing unwanted ions by colliding them with the walls, or by axial ejection from the trap due their unstable trajectories.

For the ion trap, the electric field has to be considered in three dimensions. The complete derivation and solving of the Mathieu type equations that describe the motion of the ions within the ion trap is outside the scope of this thesis and can easily be found in the literature.[7, 34, 87, 90, 91] The trapping parameters, or Mathieu parameters, a_z (Equation) and q_z (Equation) are used as coordinates of the stability regions and can be used to construct the stability diagram of the quadrupole ion trap (Figure). In these equations $\omega = 2\pi f$ where f is the fundamental RF frequency of the trap (≈1 MHz).

$$\boldsymbol{a_z = -2a_r = -\frac{16eU}{m_i r_0^2 \omega^2}} \qquad \text{(Equation 4.5)}$$

$$\boldsymbol{q_z = -2q_r = -\frac{8eV}{m_i r_0^2 \omega^2}} \qquad \text{(Equation 4.6)}$$

For an ion to remain stored within the volume of the trap it has to be simultaneously stable in the *r* and z directions (circled regions A and B in Figure a). The region A, the closest to the origin, shown in expanded form in Figure b, is of great importance for the operation of the ion trap. Region B, on the other hand, remains to be explored. In Figure b, the $\beta_z=1$ stability boundary intersects the q_z axis at $q_z=0.908$; this working point is that of the ion of lowest m/z ratio that can be stored in the ion trap for a given set of instrumental conditions; *i.e.* the low-mass cutoff.

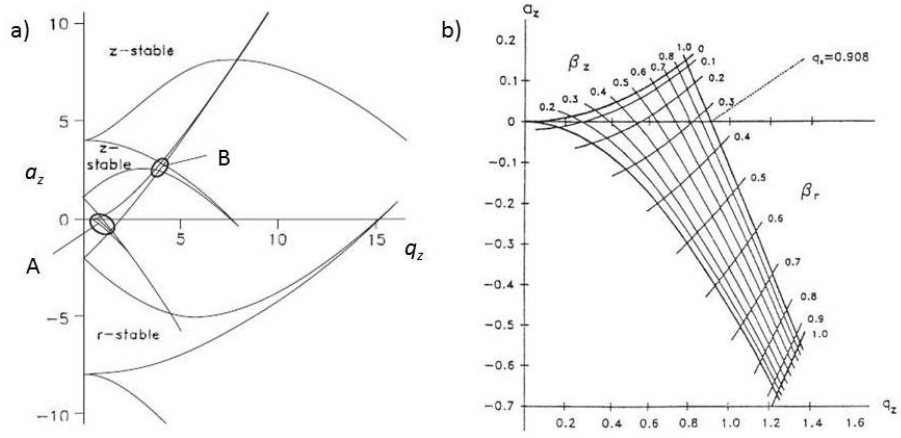


Figure 4.9: a) Stability diagram in (a_z, q_z) space for the quadrupole ion trap in the *r*- and z-directions (A and B are regions of simultaneous stability); b) expansion of region of simultaneous stability closest to the origin (region A) for the quadrupole ion trap in the *r*- and z-directions. (Image taken from reference [29])

A three-dimensional representation of an ion trajectory in the ion trap is depicted in Figure and shows the general appearance of a Lissajous curve or figure-of-eight composed of two fundamental frequency components.

A complete description of the quadrupole ion trap theory is available in the literature, for example, the book entitled *Quadrupole Ion Trap Mass Spectrometry* by

Raymond E. March and John F. J. Todd covers all theoretical aspects of the ion trap.[87]

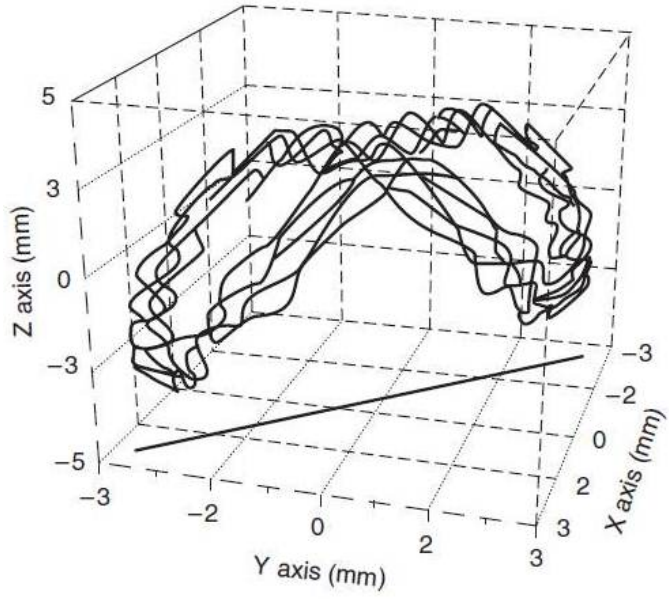


Figure 4.10 Trajectory of a trapped ion of m/z 105. The projection onto the *x-y* plane illustrates planar motion in three-dimensional space. (Taken from reference [87])

4.5.2 TRIPLE QUADRUPOLE

As the name states, triple quadrupoles normally consist of several quadrupoles in tandem. I will not describe in detail the quadrupole analyser as it is well described in

any Mass Spectrometry manuals and it gave origin to the quadrupole ion trap as previously described.

A quadrupole consists of four parallel metal rods. Each opposing rod pair is connected together electrically, and a radio frequency (RF) voltage is applied between one pair of rods and the other. A direct current voltage is then superimposed on the RF voltage. Ions travel down the quadrupole between the rods. Only ions of a certain mass-to-charge ratio (m/z) will reach the detector for a given ratio of voltages: other ions have unstable trajectories and will collide with the rods. This permits selection of an ion with a particular m/z or allows the operator to scan for a range of m/z-values by continuously varying the applied voltage.

Pioneering work in MS/MS has been achieved in some part due to the development of the triple quadrupole mass spectrometer by Yost and Henke [92]. At present, the triple quadrupole is the most widely used tandem mass spectrometer. It is a linear assembly of three quadrupoles as shown in Fig.4.11. Only the first and the third quadrupoles are mass analysers, being operated with the combination of both r.f. and d.c. potentials necessary for mass selection. The second quadrupole, the central one, has a fixed r.f. voltage only. Thus, ions of every mass can pass this quadrupole, which is used as a collision cell with ion focusing properties[92].

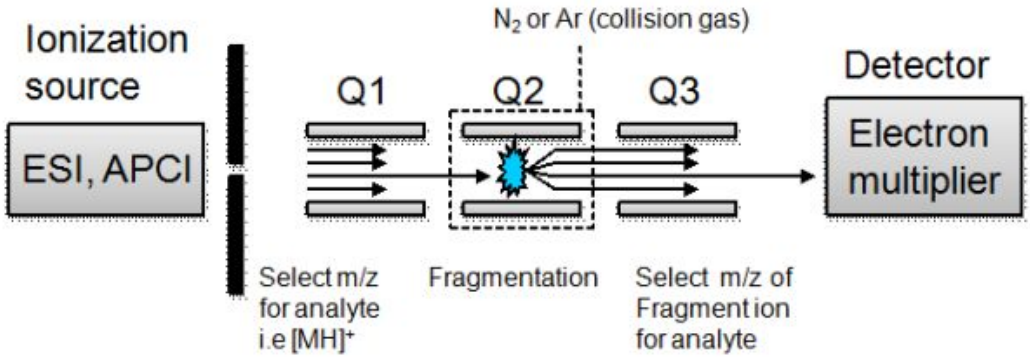


Figure 4.11. Triple quadrupoles normally consist of several quadrupoles in tandem.

In this analysers there are normal types of quadrupoles, symbolized Q1 or Q2, and there are RF-only quadrupoles, q2 , which consists normally the central collision cell if a collision gas is introduced into this q2 at a pressure that enables an ion entering the q to undergo collisions.

This alignment of two mass filters in succession with a collision quadrupole in between, allows to elucidate the structure of the ionised sample molecules. Four main modes can be performed as follows [93].

Product ion scan

- This is the most common tandem mass spectrometric experiment, the first quadrupole Q_1 is set to select an ion of a known a given m/z value, which is fragmented in q_2-the collision cell, typically filled with helium, argon or xenon. The third quadrupole Q_3 is then set to scan the entire m/z range, giving information on the sizes of the fragments made. From the ion fragmentation information, a product ion spectrum, formerly known as a daughter ion spectrum, is obtained and the structure of the original parent ion can be deduced. This experiment is commonly performed to identify transitions used for quantification by tandem MS.

Precursor ion scan

- A certain product ion is selected in Q_3, and the precursor masses are scanned in Q_1. This experiment is selective for ions having a particular functional group (e.g., a phenyl group) released by the fragmentation in q_2.

Neutral loss scan

- Both Q_1 and Q_3 are scanned together, but with a constant mass offset. This allows the selective recognition of all ions which, by fragmentation in q_2, lead to the loss of a given neutral fragment (e.g., H_2O, NH_3). Similar to the precursor ion scan, this technique is also useful in the selective identification

of closely related class of compounds in a mixture. This scan allows the selective recognition of all ions which, by fragmentation, lead to the loss of a given neutral fragment.

Selected reaction monitoring (SRM) / Multiple reaction monitoring (MRM)

- Both Q_1 and Q_3 are set to a selected mass, allowing only a distinct fragment ion from a certain precursor ion to be detected. SRM is a very selective analysis mode, which can increase sensitivity. If Q_1 and/or Q_3 is set to more than a single mass, this configuration is called multiple reaction monitoring

4.5.3 FT-ICR[1]

The theory of cyclotron resonance was developed in the 1930s by Lawrence (1951 Nobel Prize in Physics). Lawrence built the first cyclotron accelerator to study the fundamental properties of the atom. Subsequently, Penning devised the first trap for charged particles by using a combination of static electric and magnetic fields to confine electrons [94]. In the 1950s the principle of ion cyclotron resonance was first incorporated into a mass spectrometer, called the omegatron, by Sommer and co-workers, who successfully applied the concept of cyclotron resonance to

[1] Adapted from: Paulo J. Amorim Madeira, Pedro A. Alves and Carlos M. Borges, Chapter 2- High resolution Mass Spectrometry Using FTICR and *Orbitrap* Instruments, *In* Fourier Transform Materials Analysis, edited by Salih Mohammed Salih, 2012, Intech

2All of the equations will be presented in S.I. units. To convert, for example, m/q to *m/z* the reader should take into account that $q = z \times e$,

determine the charge-to-mass ratio of the proton [95]. Major improvements in ICR awaited McIver's introduction of the trapped ion cell. Unlike the conventional drift cell, the trapped ion cell allowed for ion formation, manipulation and detection to occur within the same volume in space. The trapped ion cell differed from previous ICR cell designs by the inclusion of "trapping" electrodes. By applying small voltages to these electrodes, McIver was able to trap ions for 1-2 ms (approximately 100 times that of the drift cell). These advantages led to a much greater dynamic range, sensitivity and mass resolution. More importantly, the extended trapping capability of the McIver cell was a prerequisite for the FTICR detection technique invented by Comisarow and Marshall later that decade. In the second half of the 1970s, Comisarow and Marshall adapted Fourier transform methods to ICR spectrometry and built the first FTICR-MS instrument [96, 97]. Since then, FTICR-MS has matured into a state-of-the-art high-resolution mass spectrometry instrument for the analysis of a wide variety of compounds (biological or not).

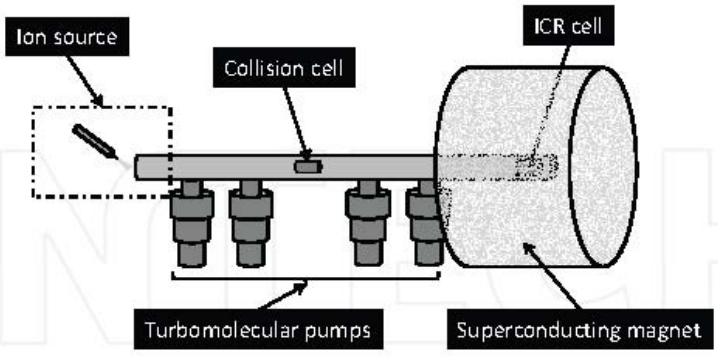


Figure 4.12 Schematic representation of an FTICR mass spectrometer. Note that not all components are present in this scheme, for example, the ion optics is not presented, nor the rotary vacuum pumps that are needed for the proper functioning of the turbomolecular pumps

All FTICR-MS systems have in common five main components: a magnet (nowadays usually a superconducting magnet); analyser cell (placed in the strong

magnetic field created by the magnet); ultra-high vacuum system, and ion source (Fig. 4.12); and a sophisticated data system (many of the components in the data system are similar to those used in NMR).

In this section, we shall not discuss the magnet, vacuum and data systems, and focus on the ICR cell, which is the heart of the FTICR-MS instrument. It is here that the ions are stored, mass analysed and detected.

Inside the FTICR cell an ion has three natural motions: the cyclotron, the trapping and the magnetron motions. The nature of each motion will be briefly explained in the following paragraphs.

Many fundamental aspects of FTICR can be understood from very simple idealised models:

- Ion cyclotron frequency, radius, velocity and energy, as a function of ion mass, ion charge and magnetic field strength, follow directly from the motion of an ion in a spatially uniform static magnetic field.

- Ion cyclotron motion may be rendered coherent (and thus observable) by the application of a spatially uniform RF electric field (excitation) at the same frequency as the ion cyclotron frequency. The ICR signal results from induction of an oscillating "image" charge on two conductive infinitely extended opposed parallel electrodes. A frequency-domain spectrum is obtained by Fourier transformation of the digitised ICR signal.

- Confinement of ions by application of a three-dimensional axial quadrupolar DC electric field shifts the ion cyclotron frequency, whereas excitation and detection remain essentially linear, but with a reduced proportionality constant.

- Collisions broaden the ICR signal in a simple way, and actually make it possible to cool and compress an ion packet for improved detection.

• Although FTICR-MS has been coupled to virtually every type of ion source, most ion sources work best outside the magnet. Thus, several methods have been developed to guide the externally generated ions into the ion trap inside a high-field magnet.

• The above features may be combined in various experimental "event sequences" to perform tandem-in-time mass spectrometry (MS/MS or MS^n).

Cyclotron motion

An ion moving in the presence of a uniform electric and magnetic fields, E and B, is subjected to a Lorentz force given by equation 4.7, where *m*, *q* and *v* are the mass, charge and velocity.

$$F = ma = m\frac{dv}{dt} = qE + qvB \qquad \text{(Equation 4.7)}$$

Let us now consider only the presence of the magnetic field, B. If the ion maintains constant speed (i.e. no collisions), then the magnetic field bends the ion path into a circle of radius *r*, the cyclotron motion (Fig. 4.13).

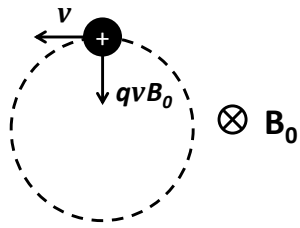


Figure 4.13: Ion cyclotron motion for a positive or negative ion due to the presence of a magnetic field (B) perpendicular to the plane of the paper.

The angular acceleration and angular velocity, ω, are defined by equations 4.8 and 4.9.

$$\frac{dv}{dt} = \frac{{v_{xy}}^2}{r} \qquad \text{(Equation 4.8)}$$

$$\omega = \frac{v_{xy}}{r} \qquad \text{(Equation 4.9)}$$

Substituting these terms in equation 4.7 we obtain[2]

$$\omega c = \frac{qB}{m} \qquad \text{(Equation 4.10)}$$

Equation 4.10 is the celebrated "cyclotron" equation that relates the cyclotron frequency, ω_c, with the mass and charge of the ion. It is clear that for a given m/z all ions have the same ICR frequency independent of their initial velocity; hence, energy focussing is not required to precisely determine the m/z ratio of an ion.

Several useful conclusions arise from the cyclotron equation (eq. 4.10). Considering an m/z range of 10 to 100 000, the frequencies lie between a few kHz and a few MHz (Fig. 4.14), which is a very convenient range for commercially available electronics.

2All of the equations will be presented in S.I. units. To convert, for example, m/q to *m/z* the reader should take into account that $q = z \times e$, where z is in multiples of elementary charge (e = 1.602×10-19 C) and m is in atomic mass units ($1u$ = 1.660×10-27 kg).

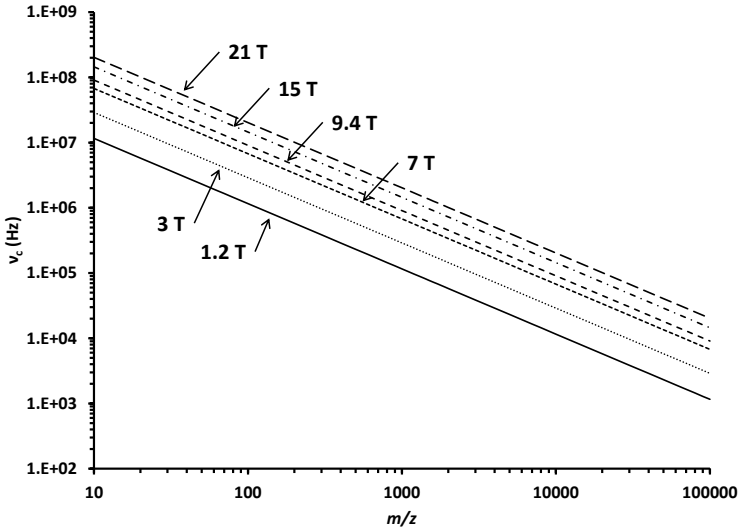


Figure 4.14. ICR orbital frequency (for ions in the m/z range between 10 and 100 000) as a function of m/z at 1.2 T, 3 T, 7 T, 9.4 T, 15 T (highest magnetic field strength commercially available) and 21 T (highest magnetic field strength magnet under development at the National High Magnetic Field Laboratory in the USA).

The first derivative of the cyclotron equation with respect to m gives equation 4.11, which allows us to conclude that frequency resolution and mass resolution are essentially the same (apart from the minus sign).

$$\frac{d\omega c}{dm} = -\frac{qB}{m2} = -\frac{\omega c}{m} \Leftrightarrow \frac{\omega c}{d\omega c} = -\frac{m}{dm} \qquad \text{(Equation 4.11)}$$

Finally, considering that the average kinetic energy of an ion with velocity v_{xy} in equilibrium with its surroundings at a temperature T (in K) is given by equation 4.12 (where k is the Boltzmann's constant) and that the ion cyclotron orbital radius, r, is given by equation 4.13 (derived from equation 4.9).

$$\frac{1}{2}m\langle {v_{xy}}^2\rangle \cong kT \Leftrightarrow v_{xy} = \sqrt{2kT/m} \qquad \text{(Equation 4.12)}$$

$$r = \frac{mvxy}{qB_0} \qquad \text{(Equation 4.13)}$$

Substituting v_{xy} in equation 4.13 we obtain an expression that relates the ion cyclotron orbital radius as a function of m/z ratio (equation 4.14).

$$r = \frac{1}{qB_0}\sqrt{2mkT} \qquad \text{(Equation 4.14)}$$

Considering T=298 K and various magnetic field strengths, we can construct a graphical representation of r as a function of m/z (Fig. 4.15). This representation allows us to conclude that even large ions are confined by the magnetic field to a small orbital radius. For example, a "modest" superconducting magnet of 3 T confines an ion with m/z 2000 to an orbit with a radius smaller than 0.5 mm.

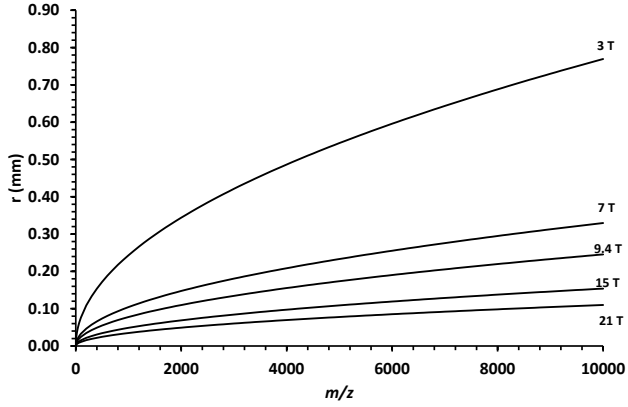


Figure 4.15. ICR orbital radius, *r*, vs. m/z ratio at 298K and 3 T, 7 T, 9.4 T, 15 T and 21 T

It is also possible to relate the kinetic energy of a trapped ion with its orbital radius by rearranging equation 4.13 (equation 4.15). A graphical representation of the ion's kinetic energy as a function of the orbital radius, depicted in Fig. 4.16 for a singly charged ion at m/z 400, reveals that ions can be heated to high kinetic energies even in a relatively small container.

$$E_k = \frac{r^2 q^2 {B_0}^2}{2m} \qquad \text{(Equation 4.15)}$$

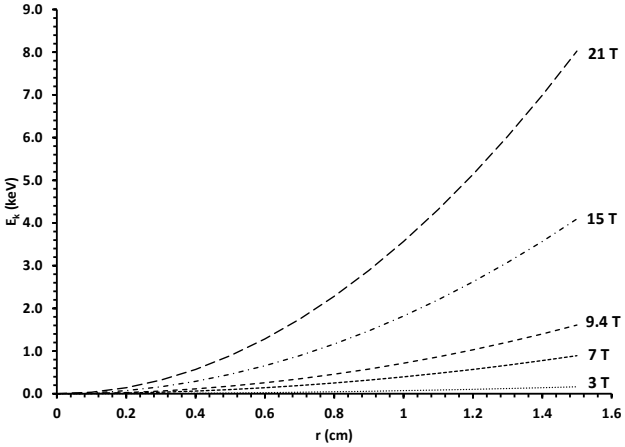


Figure 4.16. Ion kinetic energy as a function of the ICR orbital radius (singly charged ion at m/z 400).

Trapping motion

The static magnetic field effectively confines ions in the xy plane; nevertheless, ions are still free to escape along the z-axis. To prevent this, a small electrostatic potential (equation 10), usually ≈ 1 V, is applied to the trapping electrodes (positioned at $z=\pm d/2$ from the center of the ion trap).[98]

$$V(z) \cong \frac{V_t}{2} + \frac{k'z^2}{2} \qquad \text{(Equation 4.16)}$$

where k' is a constant.

The trapping electric field can be obtained by the negative gradient with respect to z of the electrostatic potential (equation 4.17).

$$E(z) = -\frac{dV(z)}{dz} = -k'z \qquad \text{(Equation 4.17)}$$

This electric field subjects the trapped ion to a force, *F(z)*, given by equation 4.18

$$F(z) = -\frac{d^2z}{dz^2} = -qk'z \qquad \text{(Equation 4.18)}$$

Equation 4.18 resembles the harmonic oscillator equation; hence, ions trapped in a quadratic *z*-potential must oscillate back and forth along the *z*-direction at a natural trapping frequency, the so-called trapping motion (or trapping oscillation). The trapping frequency, ω_T, and the *k'* constant are defined by equations 4.19 and 4.20, respectively.

$$\omega_T = \sqrt{\frac{k'q}{m}} \qquad \text{(Equation 4.19)}$$

$$k' = \frac{4V_T}{d^2} \qquad \text{(Equation4.20)}$$

Substituting *k'* in equation 4.19 we obtain

$$\omega_T = \frac{2}{d}\sqrt{\frac{qV_T}{m}} \qquad \text{(Equation4.21)}$$

In general, the trapping frequency is much smaller than the ICR frequency so it is not detected.

Magnetron motion

The combination of the magnetic field and the radial component of the electric field created by the trapping potential induce a third motion: the magnetron rotation. The magnetron frequency (equation 4.22), ω_m, is independent of both the mass and charge of the ion. Nevertheless, it is proportional to the trapping voltage (V_T) and the cell geometry factor (α) and inversely proportional to the cell edge length (a) and the magnetic field strength (B)[98].

$$\omega_m = \frac{2\alpha V_T}{a^2 B} \qquad \text{(Equation4.22)}$$

Cyclotron + Trapping + Magnetron Motions

The three natural ion motions (cyclotron rotation, magnetron rotation and trapping oscillation) are depicted in Fig. 4.17. As mentioned earlier, the magnetron and trapping frequencies are usually much smaller than the cyclotron frequency and generally are not detected. Nevertheless, several reviews on the subject of ion trajectories inside the ICR cell and their influence on the cyclotron frequency were published over the years, e.g. the one by Vartanian *et al.* [98].

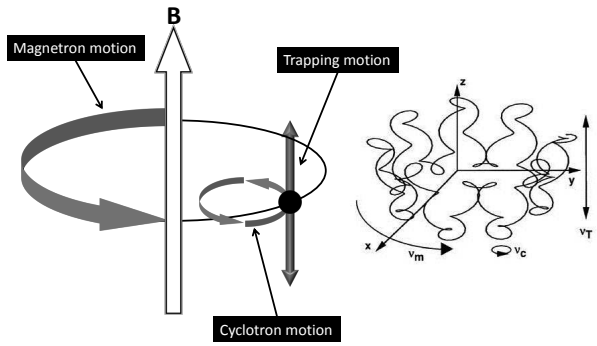


Figure 4.17 Schematic representation of the three natural motions of an ion confined in an ICR cell and the resulting ion trajectory shape.

Excitation and detection of an ICR signal

Ion cyclotron motion does not by itself generate an observable electric signal. When the ion packets enter the ICR cell, their ion cyclotron orbits are centred on the z-axis (i.e. they are too small to be detected) and must be made spatially coherent by moving them away from the centre of the cell. For that purpose excitation is needed and this is achieved by applying a spatially uniform electric field oscillating at or near the cyclotron frequency of ions of a particular m/z range.

Excitation is used in three ways in FTICR-MS:

- To accelerate ions coherently to a larger (and thus detectable) orbital radius – Fig. 4.18 a);
- To increase ion kinetic energy above the threshold for ion dissociation and/or ion-molecule reaction – Fig. 4.18 b);
- To accelerate ions to a cyclotron radius larger than the radius of the ion trap, so that ions are removed from the instrument – Fig. 4.18 c).

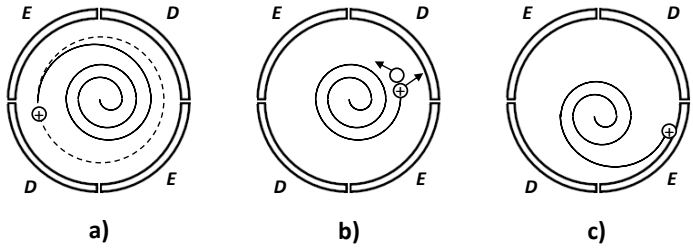


Figure 4.18 Ion cyclotron excitation uses: a) acceleration of ions to larger orbital radius for detection; b) acceleration of ions to induce ion dissociation or ion-molecule reactions; c) removal of unwanted ions from the ICR cell. (*E* – Excitation electrode, *D* – Detection electrode)

In this chapter we will focus on the detection; the other two ways of using excitation (to increase the ion's kinetic energy and to remove undesired ions from the instrument) will not be addressed in this chapter. Nevertheless, more information can be obtained in a review by Marshall and co-workers.[99]

Spatial coherence is created by applying an oscillating resonant, $\omega = \omega_c$, phase-coherent electric field excitation *E(t)* (equation 4.23).

$$E(t) = E_0 \cos \omega t \, j \qquad \text{(Equation4.23)}$$

This linearly-polarised electric field can be decomposed into two counter-rotating components, $E(t)=E_L(t)+E_R(t)$ (equations 4.24 and 4.25).

$$E_L(t) = \frac{E_0}{2} \cos \omega t \, \boldsymbol{j} + \frac{E_0}{2} \sin \omega t \, \boldsymbol{i} \qquad \text{(Equation4.24)}$$

$$E_R(t) = \frac{E_0}{2}\cos\omega t\,\boldsymbol{j} - \frac{E_0}{2}\sin\omega t\,\boldsymbol{i} \quad \text{(Equation4.25)}$$

With the excitation the ion speeds up and its radius increases linearly with time and the rate of power absorption is given by equation 4.26. [99]

$$A(t_{excite}) = \frac{{E_0}^2 q^2 t_{excite}}{4m} \qquad \text{(Equation4.26)}$$

The integration of equation 4.26 from $t=0$ to $t=t_{excite}$ yields the total energy absorbed during the excitation period and assuming a complete conversion into kinetic energy we obtain

$$\frac{m{\omega_c}^2 r^2}{2} = \int_0^{t_{excite}} A(t)\,dt = \frac{{E_0}^2 q^2 (t_{excite})^2}{8m} \qquad \text{(Equation4.27)}$$

Substituting the cyclotron equation (equation 4.10) in equation 4.27 we obtain an expression that relates the radius with the excitation electric field and the excitation time.

$$r = \frac{E_0 t_{excite}}{2B_0} \qquad \text{(Equation4.28)}$$

An interesting conclusion arises from equation 4.28 in that the orbital radius of the excited ions is independent of the m/z ratio, which means that ions of different m/z ratios can be excited to the same ICR orbital radius.

The detection of the ions occurs as the ion packets pass two detector plates. As the ion packets have past these plates, charge moves within the detection circuit to counteract the proximity of the ions. The potential change (voltage) between the detection plates can be measured as a function of time and it is from here that the raw data is obtained. It should be noted that the ions repeatedly pass the detector plates for the duration of the acquisition, as non-destructive detection is employed. The magnitude of the signal is proportional to the total charge and to the proximity of the ions to the detection plates (orbital radius), and is independent of magnetic field strength. The raw data will represent the detections of all the ions at the same time, with their different cyclotron frequencies. It is therefore necessary to extract data concerning the different ion packets. This is done through the usage of a mathematical procedure known as Fourier transform (FT) where frequency information is obtained from time-domain data. Fig. 4.19 illustrates the process of obtaining a mass spectrum from the time-domain data through Fourier transform, conversion to m/z and calibration.

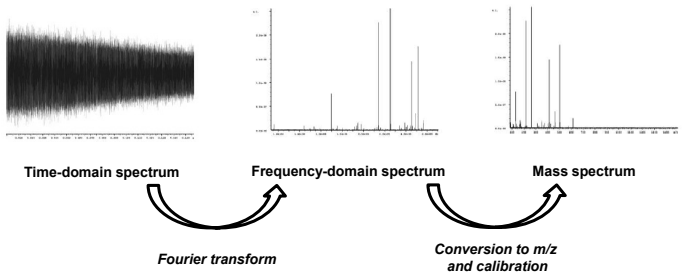


Figure 4.19. Illustration of the processing of raw data. A Fourier transform is performed on time-domain data to convert it to the frequency-domain, and the resulting spectrum is then calibrated to m/z values.

Unlike other mass spectrometers (e.g. sector instruments, time-of-flight, quadrupole) where mass analysis and ion detection are spatially separated events, in FTICR all analytical steps are made on the same spatial place but separated in time.

Fig. 4.20 shows a typical sequence of events for a tandem mass spectrometry experiment performed in a FTICR mass spectrometer. Before ion introduction, the ICR cell is emptied with a quench pulse. After the ions have been introduced into the cell a significant amount of time is required for the ion selection, dissociation, excitation, detection, time-domain data storage and Fourier transformation events before the next experiment (i.e. sequence) is started. The time involved in the events that follow ion introduction, greatly depends on the instrument used and on the type of experiment (for example, the acquisition of a normal full scan mass spectrum will take less time than other mass spectra since the ion selection and dissociation steps will not be performed) [100].

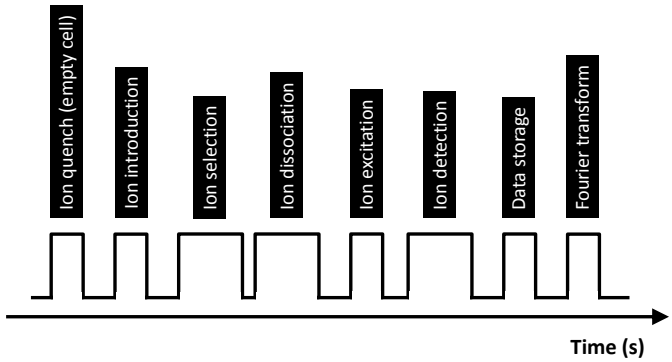


Figure 4.20. Example for a tandem mass spectrometry sequence performed in a FTICR mass spectrometer. The sequence shows the order of the different time-separated analytical steps.

For further information, in the case of FTICR mass spectrometry several reviews [101, 102] and books are available [103-106].

4.6 LC-MS

The coupling of high-performance liquid chromatography (HPLC) with mass spectrometry was first demonstrated in the 1970s [29], nevertheless, it was with the development and commercialization of atmospheric pressure ionization sources (ESI, APCI) that for the first time the combination of liquid chromatography and mass spectrometry entered the realm of routine analysis.[30-33]

There has been an increasing level of interest in liquid chromatography/mass spectrometry (LC/MS) for lipid analysis as the number of commercially available instruments having atmospheric pressure ionization (API) interfaces has increased. As prices on LC/MS instruments having API sources have come down, the number of instruments installed in labs worldwide has burgeoned. Articles in the literature have now described extensive use of electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) for analysis of most classes of lipids. The benefits of these API sources for lipid analysis are becoming widely recognized[107].

While ESI-MS has found its greatest use in the burgeoning field of proteomics, its commercial availability, affordability, and ease of use as an LC detector have caused it to be applied to almost every class of molecules. Some molecules are particularly amenable to ESI-MS because they are either already ionic (such as phospholipids) or they contain easily ionizable functional groups (such as proteins). Other classes of molecules are neutral, have little functional group diversity, and do not readily form protonated molecules under normal ESI conditions. These classes of molecules require the use of an ionic reagent (electrolyte/buffer) that can produce adduct ions. Triacylglycerols are one such class of neutral molecules that are not

readily ionized without the use of a reagent buffer to promote adduct formation. The special steps that must be taken for TAG analysis by ESI-MS will be described below, omitting the discussion of the electrospray process.

As mentioned, TAG's are not readily ionized under ESI-MS conditions and sometimes suffer ion supression. The references cited in the introductory section had a common characteristic: each one used an ionic additive to form an adduct with TAG molecules. Typically, these were cations in the form of ammonium or an alkali metal (Na or Li), usually provided as acetates. The nature of the cation affected the ability of the adduct to fragment and produce beneficial MS/MS spectra.

As discussed before, Duffin *et al.* (13) were the first to apply ESI-MS to neutral lipid analysis. It had already been demonstrated that ESI-MS could be effective for TAG analysis, and that the data obtained by ESI-MS was valuable and complementary to the data obtained by APCI-MS. And as mentioned previously, ESI-MS of neutral lipids requires an ionic additive, or reagent, of some type. Ionic adducts are formed between the neutral analyte and the ionic additive, in the ESI source.

The mass spectra obtained by ESI-MS typically exhibit pseudomolecular ions (adduct ions formed with the ionic additive) as base peaks, if minimal up-front CID voltage is applied. The identity of the adduct determines whether or not MS/MS can be performed. $[M+Na]^+$ adducts were shown to be more reproducible and sensitive than $[M+NH_4]^+$ adducts, but the $[M+Na]^+$ yielded poor MS/MS spectra.

When applied to triacylglycerols, the separation is in ascending order of the total number of carbon atoms in the aliphatic chains of the three fatty acids, with a double bond in any acyl groups reducing the retention time to roughly that of a component with two fewer carbon atoms in total. Because of the dual nature of the separation process, the order of elution of components is less easy to follow intuitively than with silver ion chromatography.

The relative retention time of a given component has been defined in terms of an 'equivalent carbon number' (ECN) or 'partition number' value, defined as the actual number of carbon atoms in the aliphatic residues (CN) less twice the number of double bonds (n) per molecule (the carbons of the glycerol moiety are not counted for this purpose), i.e.

$$ECN = CN - 2n$$

Two components having the same ECN value are said to be 'critical pairs'. For example, triacylglycerol species containing the fatty acid combinations 16:0-16:0-16:0, 16:0-16:0-18:1, 16:0-18:1-18:1 and 18:1-18:1-18:1 have the same ECN value and tend to elute close together. However, with modern equipment, all of these four components should be separable.

The group of Colombini has a remarkable work establish and optimize the identification of TAG's in complex archaeological organic residues[108] and vegetable oils [109] with new core-shell stationary phases this endeavor for vegetable oils is quite a source for comparison of initial oil composition results.

The advent of LC-MS application to Lipids had enormous impact. Especially it is quite hard to trace Oxolipids or TAGOX. Sjovall *et al.* [110] used HPLC with detection by ESI-MS to determine the elution factors of numerous di-nitro-phenil hydrazine (DNPH) derivatives of core aldehydes, as well as nonderivatized monomeric TAGOX, such as hydroperoxides, epoxides and hydroxides. Later, Sjovall *et al.* [111, 112] applied their HPLC/ESI-MS approach to identification of the oxidation products formed by TAG standards after oxidation by *t*- butyl hydroperoxide (TBHP). These and other applications of ESI-MS to TAGOX analysis are discussed in the thorough treatment of the subject by Kuksis (and co-workers) [107, 113, 114]. One on-line resource which is a wide review of application to TAG's including extensive recent bibliography is Byrdwell's home page [115].

One example is found for camellia oil that can be a unique example on following oxidation in vegetable oils [116] (HPLC‑ESI-MS) was used for the analysis of TAGs composition and evaluation of auto-oxidation and oxidation products of the seed oil products were epoxy hydroperoxides, epoxy epidioxides, and epoxides.

We tried to give a brief description of some LC-MS methods applied to vegetable oils in the critical review (see chapter 2), but there seems not to have been reported these type of oil oxidation mechanisms, by HPLC or LC-MS/MS, at least not applied to artist's oil paint but it's common to recur to GC-MS or Py-GC-MS.

4.7 GC-MS

Gas chromatography GC is probably the most widely used method for traditional artist's medium analysis especially when coupled to MS and it is now present in almost any lab adjacent to a Museum. Early accounts of oil paint analysis by Mills [117, 118] and Schilling[119-122] provided the basis for it's wide application nowadays.

With the introduction of modern materials some of these labs turned to Py-GC-MS because of high molecular weights of new materials and the inability to extract diagnostic compounds from the polymer matrix that can be broken down to volatile fragments through pyrolysis . Also new derivatization procedures[123] were applied to better follow what is happening inside a paint matrix.

Some recent applications of GC-MS and Py-GC-MS to modern oil based mediums are reviewed in a reference text book [124] .

Also recent work by Izzo [125] helped to review and define the problem in modern oil paints [126]from which it is followed the formulation and degradation of 20th century artists' oil paints [127].

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5 DISCUSSION & CONCLUSIONS

ABSTRACT

This chapter reviews oils research, with a focus on science based developments in Molecular drying and ageing. It presents data of complementary methods used in the characterization and analysis of oils as starting materials for historically accurate paint reconstructions. Providing the basics for further development of oil paint analysis with known starting materials, and the effects of its processing treatments.

5.1 INTRODUCTION

Chemical markers used in paint have been in general based in FA profile quantification, previously based on FAMES quantification by GC-MS. With this quantification we can then correlate data to further investigate how these FFA are linked in the oils TAG's as raw materials, and even in mixtures of these oils. The ESI procedure on negative ion mode, with previous hydrolysis, can provide the quantitative information on FA profile. This will enable us to better characterize the different oils newly introduced for the formulation of artist's oil paints (1890-1940), as raw materials. ESI-MS in positive ion mode with ammonium adducts will reveal TAG composition of the several oils investigated and also possible oxidation and polymerization of the oils during drying and ageing.

The evidence of paint ageing can be observed by several techniques. The Oil standards, with known historically accurate production process, can be analysed from the start of the process of drying, by determining the chemical composition of the initial oil and monitoring its oxidation products/drying products/hydrolysis products, throughout maturing and ageing.

Nowadays, with the availability of high standard analytical technology, it is possible with spectrometric techniques to identify or quantitatively describe the "state" of drying, This study can contribute to the chemical justification of the different analysis methods for oil analysis within the framework of study of artist paints.

Oil chemical analysis: The analytical approach…

from profiling to identifying Target Chemical markers.

The composition of vegetable oils is rather complex. Drying oils suitable for oil paints is a subclass of vegetable oils with a high concentration of linoleic and linolenic fatty acids. The interest is not only to determine the constituent triglycerides but also to find out how the oil composition changes as a result of cleanup treatments, heat treatments and oxidation/cross linking during drying. These possible changes have been mentioned in a previous chapter (see chapter 2).

For this purpose we have to establish a prediction method based on a known quantity of at least 50% L (Linoleic) and Ln (Linolenic) (at least 50% of bisallylic H abstraction, essential for the paint to dry to a stable network)[Muizebelt]

The analytical strategy chosen is mass spectrometric profiling – mostly direct electrospray MS- followed by target "hyphenated" analytical techniques such as LC-MS and MSMS. The chapter will first present data on the composition of the oils after electrospray MS in positive and negative ion modes using an ion trap mass spectrometer. The nominal mass spectra in the mass range of m/z 800 to 1000 will be tentatively identified. The spectra of the oils and their various treatments will also be compared using principal component analysis distribution maps and feature spectra that can support and explain the spatial distribution will be presented. The LCMS will at first be used as a profiling method using the mass chromatograms of the pseudomolecular ion peaks of the triacylglycerides to evaluate the number of isomers in the oils. Previous studies have already shown that the number of structural isomers increases strongly upon heating [1]. This second profiling method should give therefore a rapid insight into the complexity and even increased complexity due to treatments intended to modify the oils as known from literature from traditional paint making (see chapter 1,2 and 3).

The target analysis approach consists of accurate mass measurement in direct electrospray mode using an FT-ICR-instrument and LCMSMS on specific ions deemed to be of particular diagnostic value. The accurate mass values will support the tentative identifications list composed on the basis of nominal mass work with the ITMS. The LCMSMS work will provide a deeper insight in the structural changes due to the treatments. The latter approach can also provide more insight into the positional distribution and nature of the fatty acyl moieties. Data from target analytical work will be presented after the profiling data chapters.

These procedures will support the establishment of the (physico-chemical) molecular structure of the ageing different phases of the many paints and processes studied. Therefore, a possible reaction map for the different phases of the molecular chemical mechanism of drying, maturing and ageing, of oil paints, is presented.

5.2 RESULTS & DISCUSSION

The results presented encompass oil identification of main constituents based on results of ESI MS spectra both from ITMS or FT-ICR-MS.

Target analysis of marker species is presented later based on LC-MSMS, and ESI-FT-ICR-MS or MSMS.

5.3 OIL IDENTIFICATION

We aim to compare the relative distribution of the same compounds in our oil profiles. These are a mixture of (isomeric) TAG's with different degree of unsaturation and number of carbon atoms. To get a first idea of the differences, we narrow the m/z values of these profiles in order to selectively monitor TAG's, DAG's, MAG's and FA spectral regions in their respective m/z values (See Fig5.2.1-1). This distribution can also be expressed and analysed by PCA.

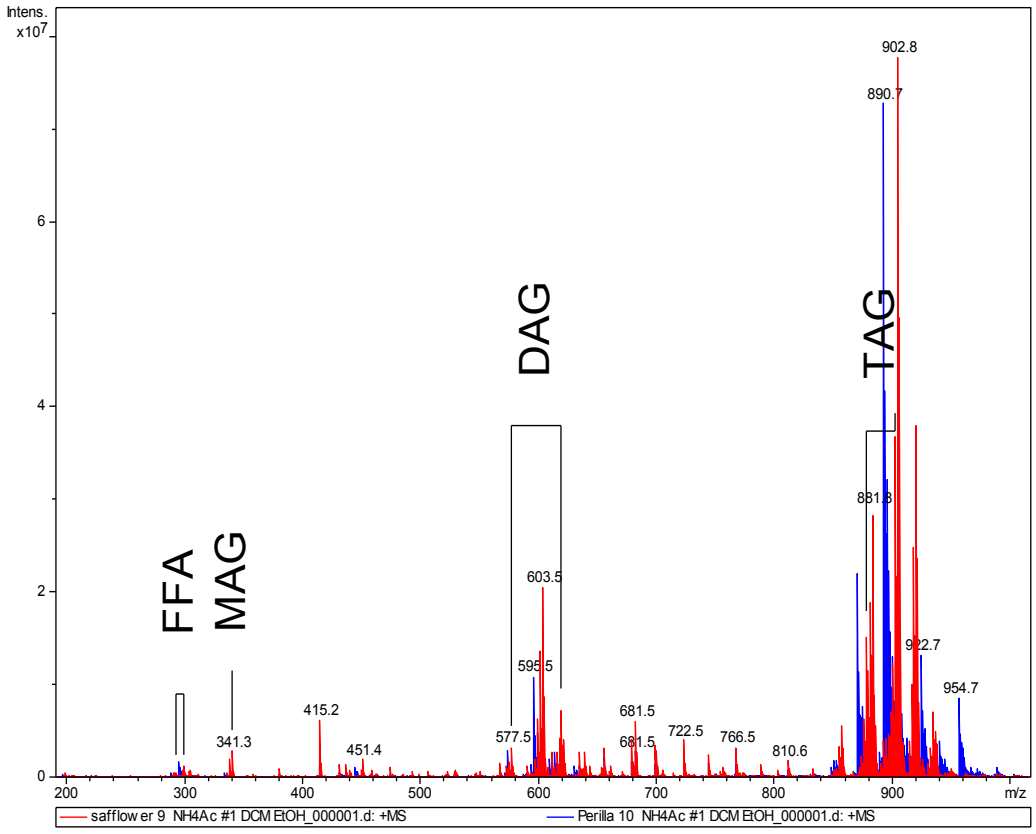


Figure 5.3-1 High-resolution positive ion mode mass spectra of Safflower oil(red) and Perilla (blue).

For simplifying purposes we focus firstly on TAG profiles and comment on these for each oil and a comparison of the differences between them. But it is relevant for characterization purposes to also focus in different m/z value ranges; mostly DAG's are detected with a loss of one FA around 600. In between these values we will try to identify several scission products mostly related with loss of C9 fragments as a consequence of autoxidation of the most siccative oils. The area around 300 reflects the presence of MAG's and the FA as smaller pieces of this intricate puzzle.

The tentative attribution of TAG's will be based on the data presented in Table1.

Table 1 Triacylglyceride composition and possible adducts for Ms identification.

TAG	CN:DB	ECN	MW	M+H	M+NH4
PPLn	53:3	44	828.720691	829.727967	846.754516
PPL	53:2	46	830.736341	831.743617	848.770166
PPO	53:1	48	832.751991	833.759267	850.785816
PLnLn	55:6	40	850.705041	851.712317	868.738866
PLLn	55:5	42	852.720691	853.727967	870.754516
PLL/OLnP	55:4	44	854.736341	855.743617	872.770166
POL/PSLn	55:3	46	856.751991	857.759267	874.785816
PSL/POO	55:2	48	858.767641	859.774917	876.801467
PSO	55:1	50	860.783291	861.790568	878.817117
LnLnLn	57:9	36	872.689391	873.696668	890.723217
LnLnL	57:8	38	874.705041	875.712318	892.738867
LnLnO/LnLL	57:7	40	876.720691	877.727968	894.754517
LnLO/LnLnS/LLL	57:6	42	878.736341	879.743618	896.770167
LnOO/LnLS/OLL	57:5	44	880.751991	881.759268	898.785817
SOLn/LOO/SLL	57:4	46	882.767641	883.774918	900.801467
SOL/OOO/SSLn	57:3	48	884.783291	885.790568	902.817117
SOO/SSL	57:2	50	886.798941	887.806218	904.832767
SSO	57:1	52	888.814591	889.821868	906.848417

5.3.1 POSITIVE ESIMS SPECTRA OF THE OILS AND TREATED OILS: FIG A-Z

FA profile given by GC-MS and/or negative ion mode ESI-MS, complemented by ESI-MS obtained in iontrap and FT-ICR-MS will enable to reveal how the FA are bound in TAG's. It may also reveal molecular changes characteristic of each complex mixture of oil, be it more or less siccative, based on features that can be identified in the mass spectra.

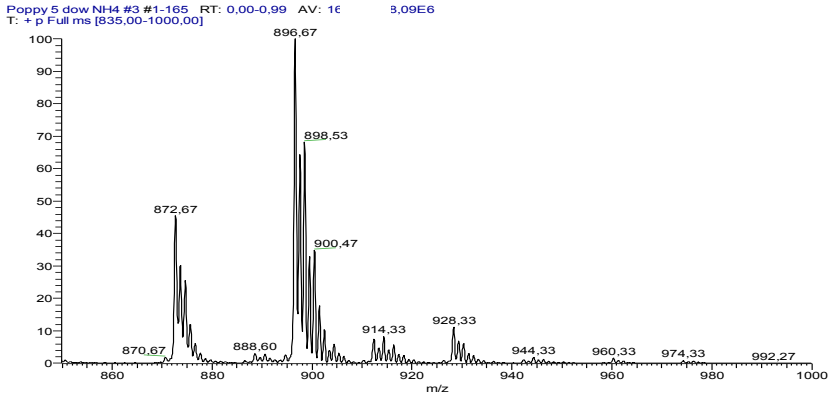


Figure 5.3-2 Positive ion mode spectra (ESI-iontrap-MS) of Safflower oil

Positive ion mode ESI-MS TAG profiles are more conclusive than a mere FA profile given by GC-MS or negative ion mode ESI-MS, because they improve description of the actual species that are present in the oil. Although these profiles are complimentary as we can molecularly build TAG species based on the

constituent FA, we are unable to describe the TAG composition with only FA profiles.

One simple example, in order to get an idea of what we can see in the a spectra, of a Triglyceride Mix standard is shown below (Fig. 5.3 -3) albeit seeing sodiated adducts we can attribute the various TAG's identified in the spectra.

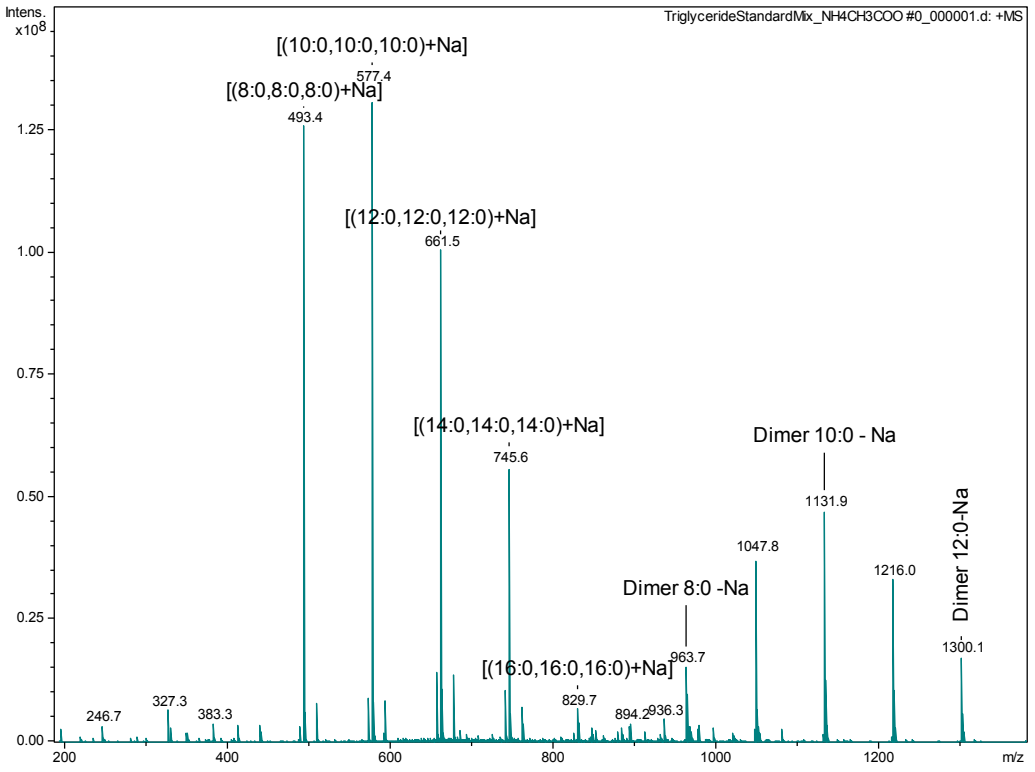


Figure 5.3-3 High resolution mass psectra of a triglyceride Mixture of Tricaprin, Tricaprylin, Trilaurin, Trimyristin, and Tripalmitin.

TAG profiles are able to monitor TAG's species, oxidized or not, cross-linked or not, without the need for previous hydrolysis to it's FA before analysis.

Not only different vegetable sources can contribute with different profiles, of more or less siccative oils, but also time of harvest and cultivar can influence the relative abundances of different TAG's.

Due to the somewhat softer nature of the electrospray ionization method, compared to MALDI, less fragmentation of the TAG's is expected, detecting ammonium cationised molecular ions of intact TAG's and trace amounts of DAG's. Under the experimental conditions used, TAGs formed only ammonium adducts without fragmentation, as observed elsewhere [2].

If we compare the TAG's profile in positive ion mode we have a straightforward understanding of the degree of unsaturation of the various oils in study.

For safflower we can identify a semi-drying oil that has a lower degree of unsaturation, as compared, for example, with Perilla that is known for it's good drying proprieties. In practice, we can identify two clusters of triglycerides TAG 55:x and TAG 57:x (where x corresponds to the number of double bonds). In each cluster the difference in relative abundance of more saturated vs unsaturated species, reveal a less siccative oil vs more siccative oil, respectively (see Fig 5.3-4).

Specifically in the spectra of Safflower and Perilla (see Fig 5.3-4) we are able to attribute siccative differences as we recognize most intense TAG's for Perilla 57:9 (890 m/z) and for Safflower 57:3 (902 m/z). The intensities of unsaturated TAG's in the mass spectra reveal the drying or non-drying nature of the oils. The clusters observed represent the mixture of different TAG's with same carbon number but different number of double bonds.

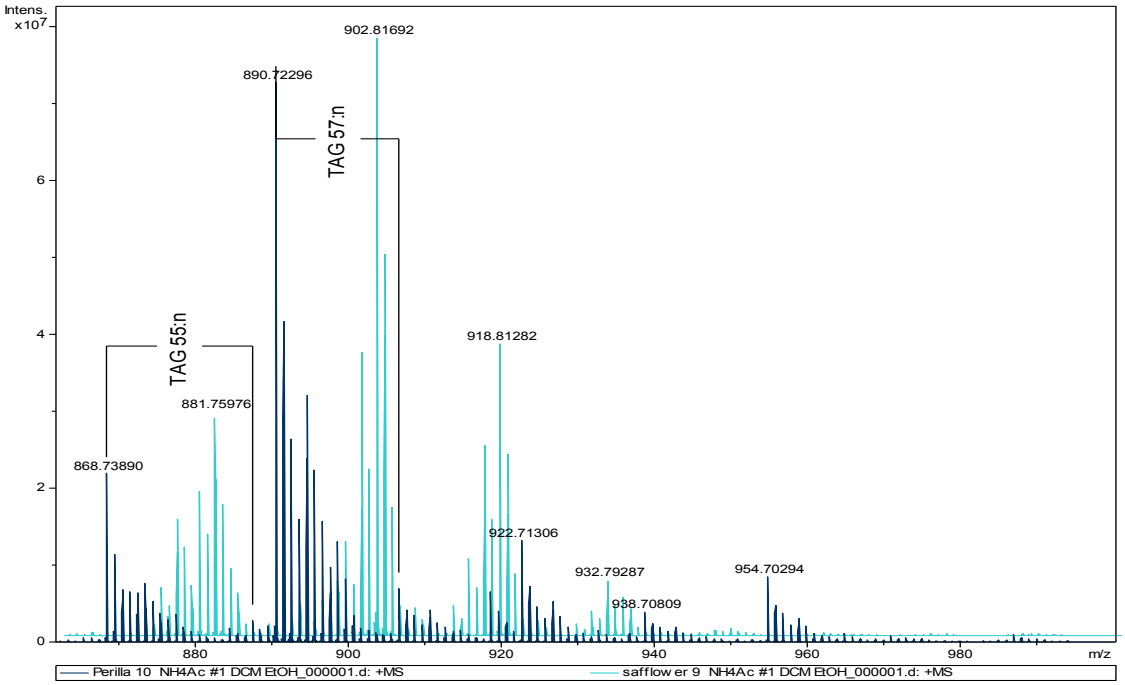


Figure 5.3-4 - MS positive ion spectra (ESI-FT-ICR-MS) of Safflower (dark blue - less drying) and Perilla (light blue - more drying) Oils.

As noticed from the spectra the most abundant peaks for the Safflower TAG's 57:n have 6 double bonds less than the most intense for Perilla. That is clear also in the TAG's with palmitic acid (55:0 for Safflower and 55:6 for Perilla, 881. 8 *m/z* and 868.7 *m/z*, respectively).

The DAG fragment ions are observed as protonated species and are formed from ammonium cationised TAG's by neutral loss of a FA plus ammonia. Observable DAG ions form also two clusters at 573.5 and 595.5 assigned to DAG 37:3 and DAG 39:6 the clusters representing a DAG with one C16 FA and one C18 FA with 3 or less unsaturations, and the other cluster, two C18 FA with 6 to 0 double bonds.

With the aim of separating different contributions of the spectra, in between the DAG's and TAG's regions of the mass spectra, we can spot peaks from TAG's species formed from scission around the C9 of one of the FA in their composition.

Also intact DAG's can be discriminated from fragments of oxygenated TAG species that can also be found on the basis of the cation. Furthermore, oxygenated TAG's which are present in larger amounts with time and treatments of the oils, can be detected with loss of a fatty acid. We exemplify this in the oil treatment sections but fragment ions from ESI-produced $[M + NH_4]^+$ produce neutral loss fragments with loss of ammonia.

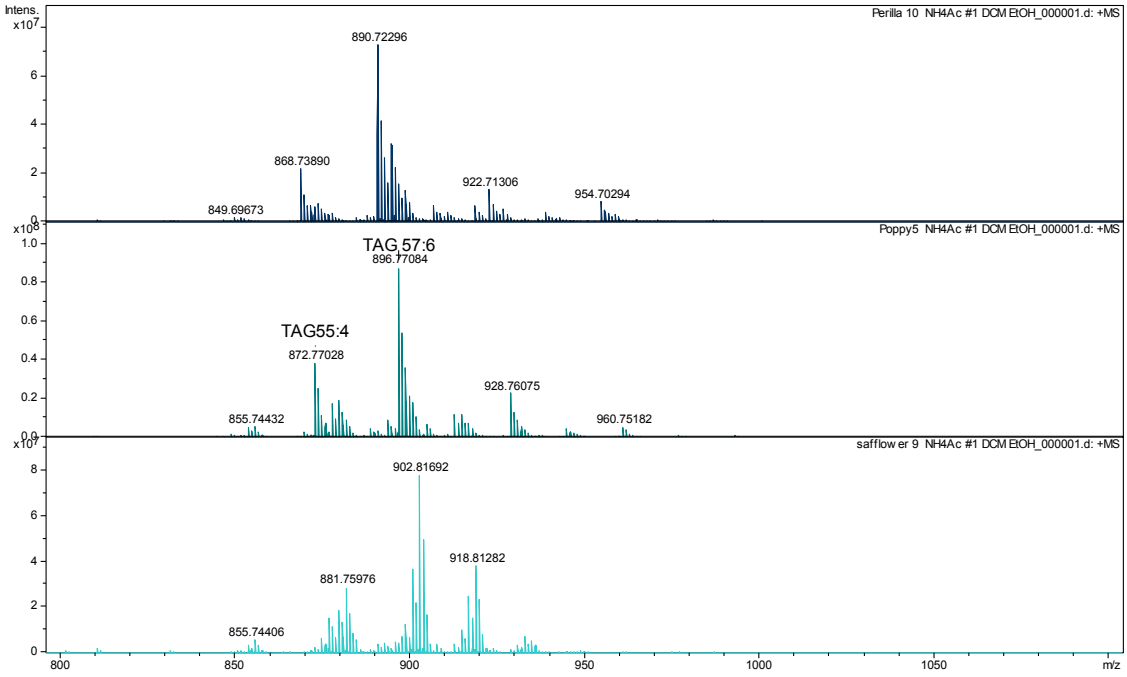


Figure 5.3-5 High resolution mass spectra of Perilla, Poppy and Safflower oils, from more siccative to less siccative oils.

ESI-produced $[M + NH_4]^+$ ions fragment producing three types of ions, $[M + NH_4 - R_nCOONH_4]^+$, $[R_nCO + 74]^+$ containing the glycerol backbone but minus one hydroxyl group, and R_nCO^+ ions, from which the carbon number and the degree of unsaturation of each acyl group are obtained [3]. The mechanism for the

formation of these ions had been described previously from labeling studies in which the hydrogen atoms on the glycerol backbone were replaced with deuterium atoms[4] fragmentation schemes for glycerolipids can be found in a recent review[5].

In addition, three series of ions are produced by charge-remote fragmentations (CRFs), and analysis of their patterns gives the position and the number of double bonds on the acyl groups. Information about the position of each acyl group on the glycerol backbone, however, is not provided by collision-activated dissociation of $[M + NH_4]^+$ ions [3], also in DAG fragments the peak intensities of $[M+NH4–RnCOOH–NH3]^+$ show regio-positional differences.

With this in mind a table of assignment can be made to interpret these losses by fragmentation.

As for other oils we can order them from more to less siccative based on the degree of unsaturation, that is, with mass differences of TAG's separated by approximately two mass units that correspond to another double bond on the FA chain of TAG's.

It can be quite easy to spot a less drying oil if it appears in greater intensities of Tag's on a slightly higher mass corresponding with less unsaturation. We can see this above (Fig 5.3-5) with the examples of Perilla, Poppy, and Safflower with peaks for TAG55:6 and TAG 57:9 for Perilla, but less drying TAG55:4 and TAG 57:6 for Poppy or even Safflower with TAG 55:0 and TAG 57:3. In simple terms, a shift into higher masses reflects less siccative oils with less unsaturation.

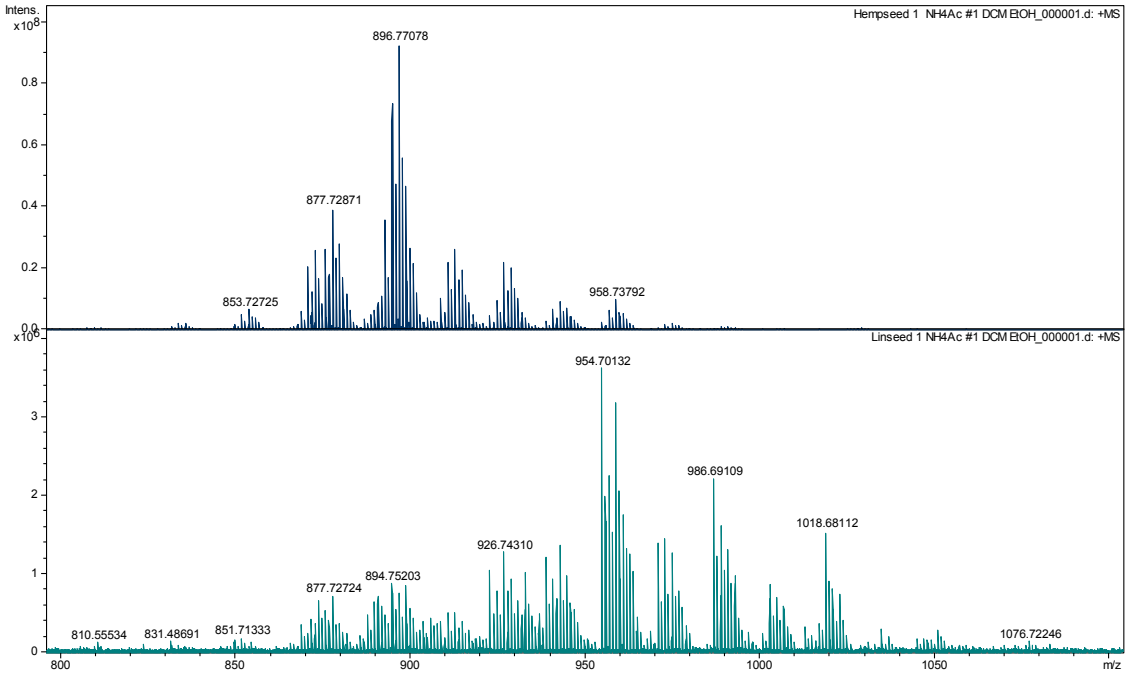


Figure 5.3-6. High resolution mass spectra of Hempseed and Linseed oil.

From the comparison of Hempseed oil with linseed we can follow different stages of drying observed from the clusters $[M+NH4]^+$ TAG55:x or TAG57:x and the replication of these clusters separated by a 16 or 32 amu mass difference. In hempseed we detect more 16 amu mass differences, and in Linseed we can follow further 32 amu mass differences, to prove that oxidation follows to a greater extent. This extent of the drying/oxidation reaction, that incorporate oxygen in the crosslinks of different TAG's or between different FA of different or same TAG's, is revealing of the extent of the oxidation process. A peroxide link could add a 32 amu mass difference, as in the linseed spectra we can detect this happening at least 4 times as a proof of cross-linking of the oil.

We should also have in mind, while observing the spectra, that the 15.99946 mass differences are not a difference of 8 double bonds, or odd chain FA (not present in

vegetable oils) containing TAG's, but oxidation products incorporating oxygen into the FA like a Hydroxyl function. These Oxidation products might be detected at a nominal mass of 14 e.g. epoxides will be detected at a 13.979 amu mass difference while CH2 has a 14.015 amu mass difference.

The oxidation observed here is from after the oils were extracted and is denoted how siccative oils can progress in incorporation of oxygen from mild or greater exposition to different ambient conditions. For the determination of Triglyceride profiles of the cold-pressed oils, these should be carefully preserved in nitrogen atmosphere in order to avoid unnecessary oxidation, as they are visibly and easily oxidized. As oil samples age it almost unavoidable that these gain some oxygen even if just by carefully preparing a sample, time and again.

Nevertheless this process is what we are trying to follow and characterize from the early ages of oil to paint, so sometimes accelerated ageing is recommended.

5.4 WATER WASHING AND HEAT TREATMENTS

As for the water washing procedure (see Apendix), before using the oil for paint purposes, it only confirms the words of the ancient recipes for this procedure. The mass spectra of oils after the treatment here denote a strong oxidation of the initial oils. Which is in accordance to what's expected for this procedure.

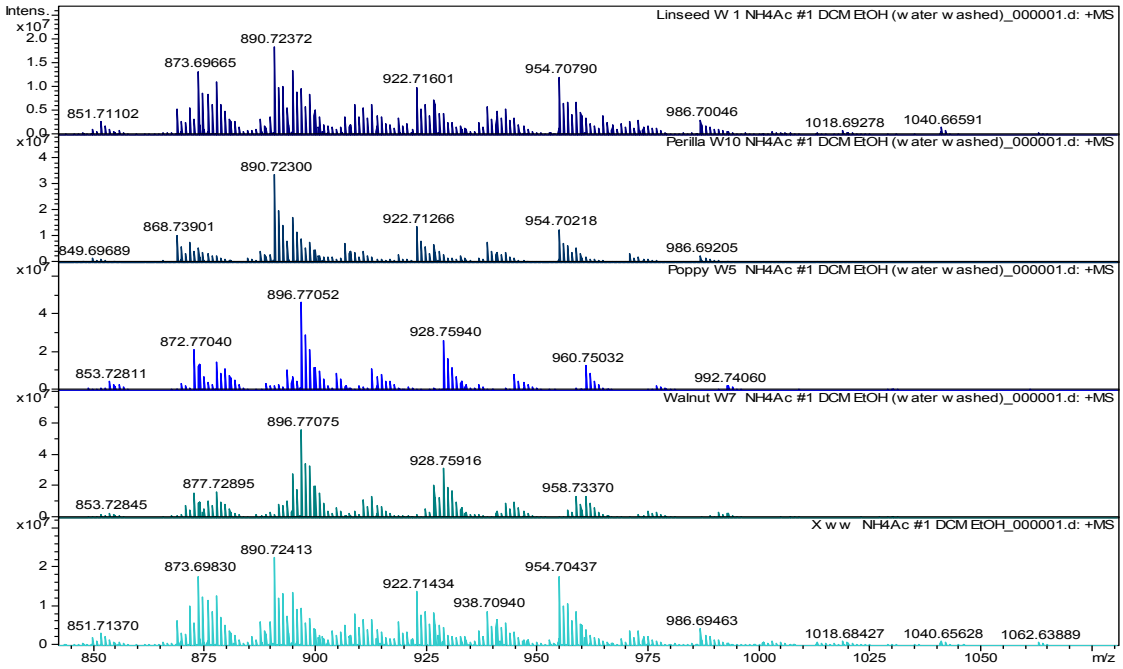


Figure 5.4-1 All Oils submitted to the water washing procedure, Linseed W1, Perilla W10, Poppy W5, Walnut W7, e X W W (Linseed1999)

In the mass spectra of water washed Linseed we can follow the inclusion of four 32 amu mass differences in cluster series 890.7 *m/z* to 922.7 *m/z*, 954.7 *m/z*, 986.7 *m/z*, until 1018.6 *m/z*, representing the formation of hydroperoxides. But these same features are also present in other strong drying oils like Perilla or the Linseed oil processed in 1999. Less drying oils like Poppy and Walnut present identical features, 32 amu differences, slightly shifted to higher masses that correspond to less unsaturated TAG's.

It is also denoted in DAG neutral fragment ions with the loss of ammonia, that there is the Oxygen inclusion, one example (see Fig 5.4 -2) would be epoxidation with the loss of two hydrogen atoms observable with a mass difference of 13.979 amu (instead of 14.071 for CH_2).

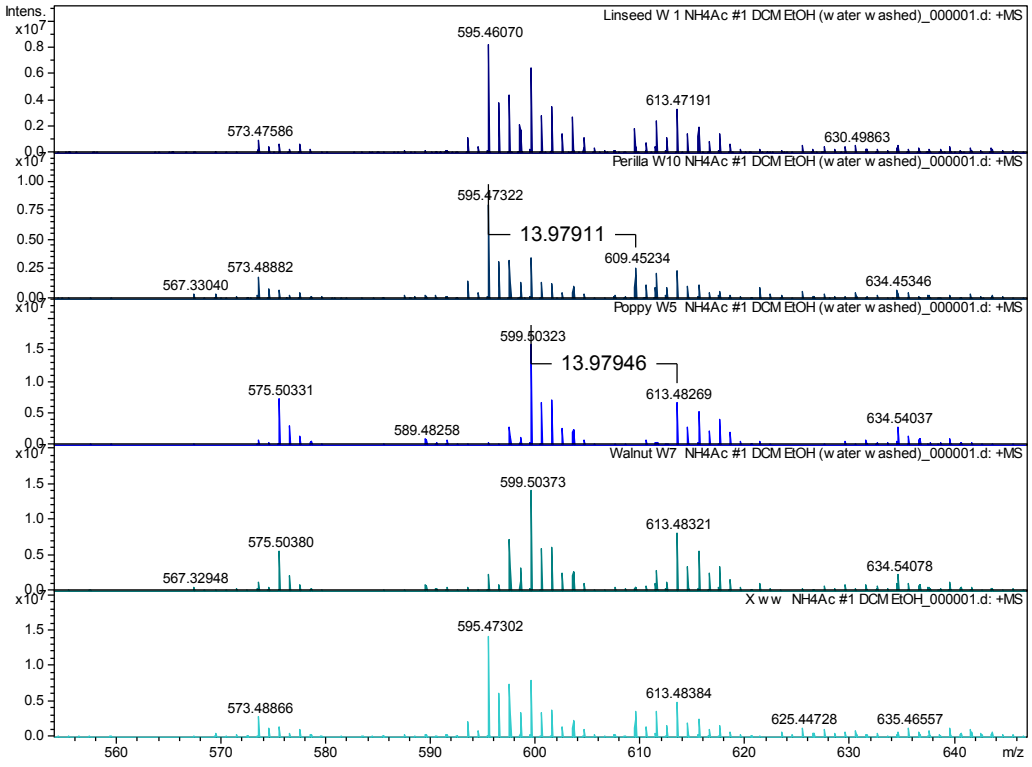


Figure 5.4-2 High resolution spectra of the same waterwashed oils in the DAG region of the spectra.

Even the less drying oils like Poppy and Walnut show the same mass differences in this spectra leading to same epoxidation shift than the more siccative Linseed and Perilla. Nevertheless we can spot multiple oxygen inclusion at a higher degree than heated oils (as we will see later) and this can sustain autoxidation mechanism of oxygen inclusion is favored under water washing conditions (See Fig 5.4-3), as it removes soluble anti-oxidants and might facilitate oxidation by the contact with oxygen through water stirring and exposing to air. It has an unprecedented inclusion of oxygen in the oil TAG structure and it will promote several points of conexion for later stages cross-linking. These first time analysed washing procedure should be more closely described with targeted approach to find marker compounds. Such presence of primary and secondary oxidation products chemically

verifies what species are present for the cross-linking mechanism of oil to a solid film, as in paint.

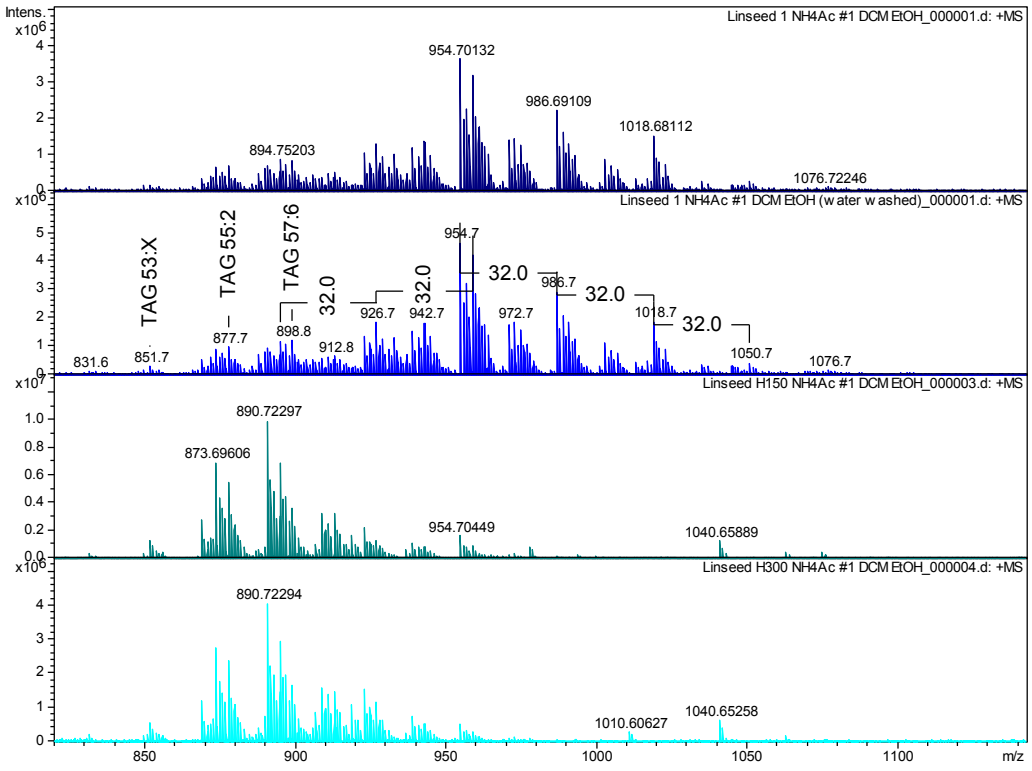


Figure 5.4-3 High resolution Spectra of Linsedd, cold-pressed, waterwashed, and heated to 150ºC and 300ºC.

As for spectra for the heat treatments, there is a feature present in all 3 spectra (from fig. 5.4 - 3) 16 amu mass differences, as perceptible in Linseed mostly the one heated up to 150ºC, the Linseed treated in 1999 [Z150], and to a lesser extent in Poppy oil. In aged oil from 1999, with the same heating to 150ºC the 32 amu mass differences also prevail up to 3 times. The fragmentation of TAG's as a result of oxidation in between the DAG and TAG regions is a characteristic of pre heated oils, present in very small amounts in the water washed oils.

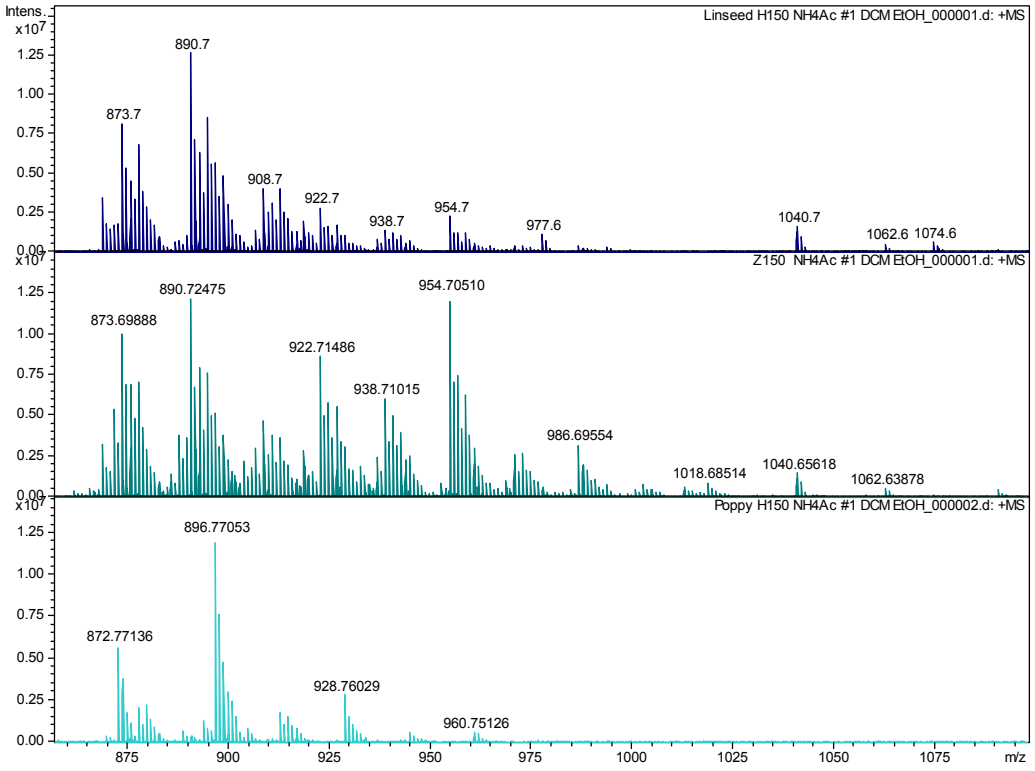


Figure 5.4-4 High resolution mass spectra of oils heated to 150ºC (Linseed, Linseed 1999, and Poppy)

For processed oils at 150ºC show evidence of the presence of oligomers at higher masses, besides a dimer at 1040.7 m/z for a DAG C16C18 (573) with a peroxide link (2xO 28) to DAG diC9 (415) and 1062.6 m/z for DAG C18C18 (595) with the same DAG diC9, there are other oligomer peaks at 2662.4 and 2709.2 for linseed and poppy, or 2617.1 and 2702.3 for linseed 1999, possibly attributed to combinations of these fragments by condensation to polymers of oxidized species e.g. the 2617 could be attributed to 2x C16C18DAG one C18C18 DAG and a TAG of 18:2/18:2/16:0 . Better explanation of these high masses would be found with MS^2 of isolated masses to find characteristic fragments.

The heating of the oils to up to 300ºC don't show a very different picture, as the oxidation is detected through similar mass differences as the previous treatment although to a lesser degree. Both Linseed and Poppy present mild incorporation of oxygen, a pattern in the spectra resulting from the heat is probably favoring condensation reactions of alkyl radicals formed. Muizebelt studied rates of C-C, C-O-C, and C-OO-C formed in alkyds by NMR, it would be a way to find how the cross-links are formed and predict more on those grounds [6-8].

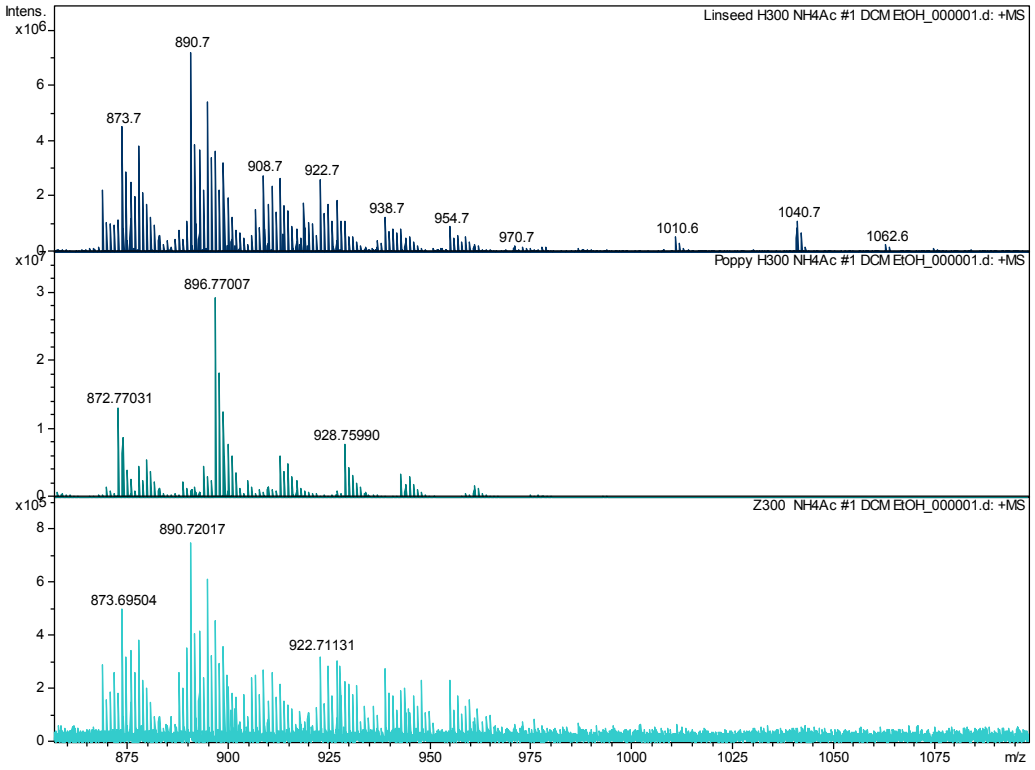


Figure 5.4-5 High resolution mass spectra of oils heated to 300ºC (Linseed, Poppy, and Linseed 1999)

From the comparison of the mass spectra we can detect some general features to the procedures applied for oil treatments of Linseed oil. Firstly, it can stand out

from comparison with unheated linseed (see previous section), that there's a certain degree of oxidation taking place. A mass difference of 32 amu is in accordance with the multiple inclusion of Oxygen, and this is perhaps more perceptible in the water washed oil but very distinctive in the heated oils. There's also 16 amu differences in these heated oils (m/z 922.7; m/z 938.7 and m/z 954.7) corresponding to the incorporation of Oxygen, while crosslinking, in the process of oxidation.

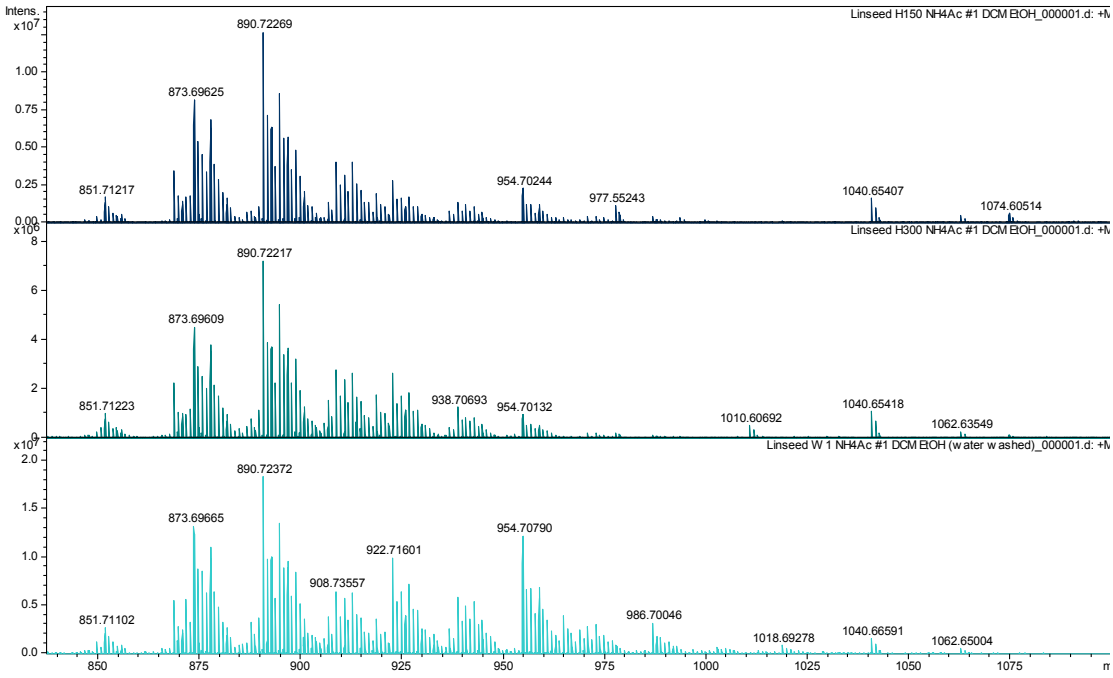


Figure 5.4-6. High-resolution spectra of Linseed oil heated to 150ºC; heated to 300ºC and water washed according to procedure.

Inserts of the spectra in between the TAG and DAG regions reveal some mass differences attributed to oxidation. We assume this is favored by the treatment of the oil procedures. There is evidence on a molecular level for TAG oxidative cleavage products, such as the loss of C9 moiety products from TAG 55 and TAG

57. In these spectra we can find TAGOX series which results from TAG's with azelaic acid (diC9 FA), as an example 766.5 m/z could be attributed to [diC9,C16,C18] [TAG 43:0+2O+NH_4-NH_3]$^+$ or 699,4 m/z attributed to [diC9, diC9, C18] [TAG 36:0+4O+NH_4-NH_3]$^+$. These are not ammonium adducts, as would be expected, justifiable if there is collisional fragmentation with the loss of NH_3. Another possibility is, for example, 810.5 m/z that could be attributed to [diC9,C18:0,C18:1+OH] [TAG 45:1+3O+NH_4-NH_3]$^+$

These peaks distribute in a series with 44 amu mass differences that can account, for example, a C18 chain instead of C16 (28 amu) with incorporation of O (16 amu) in total corresponding to C2H4O. Should these reflect chain breaking for condensation should be one possible explanation.

For illustration purposes m/z 810.5 would be [TAG 45:1+3O+NH_4-NH_3]$^+$. It seems these break down products in the oil are activated by heat.

The results obtained on the processed oils indicate that peroxide functions have been present mainly in the C9 and C10 positions (see Chapter 2). This is justifiable because C9 is the most common position of a double bond in the unexposed samples (cold pressed oils) because oleic acid, linoleic and linolenic all have a double bond present in the C9-position.

As observed before the main primary photo-oxidation products of Oleic acid are 10-hydroperoxide-9-trans-octadecenoate and 9-hydroperoxide-10-trans-octadecanoate, but still there is a wider range of possibilities in thermal oxidation as we detect in the heat treatments reconstructions.

Each processing of the oil reveals characteristic spectra that we can further evaluate by means of Principal Component Analysis in order to group the samples accordingly to each treatment process in a following paper .

Nevertheless, we can spot some molecular differences right away from the spectra. It can be proved directly from the intensities of oil spectra if they're either more or less siccative or whether they have more or less double bonds in the overall TAG's composition of each oil. From each spectrum we can also notice a lower intensity of TAG's with more double bonds (e.g. TAG 57:9) to be replaced with oxidized species spotted with a 16 amu or 32 amu additions in the mass spectra. Besides the already mentioned breakdown products at lower m/z values.

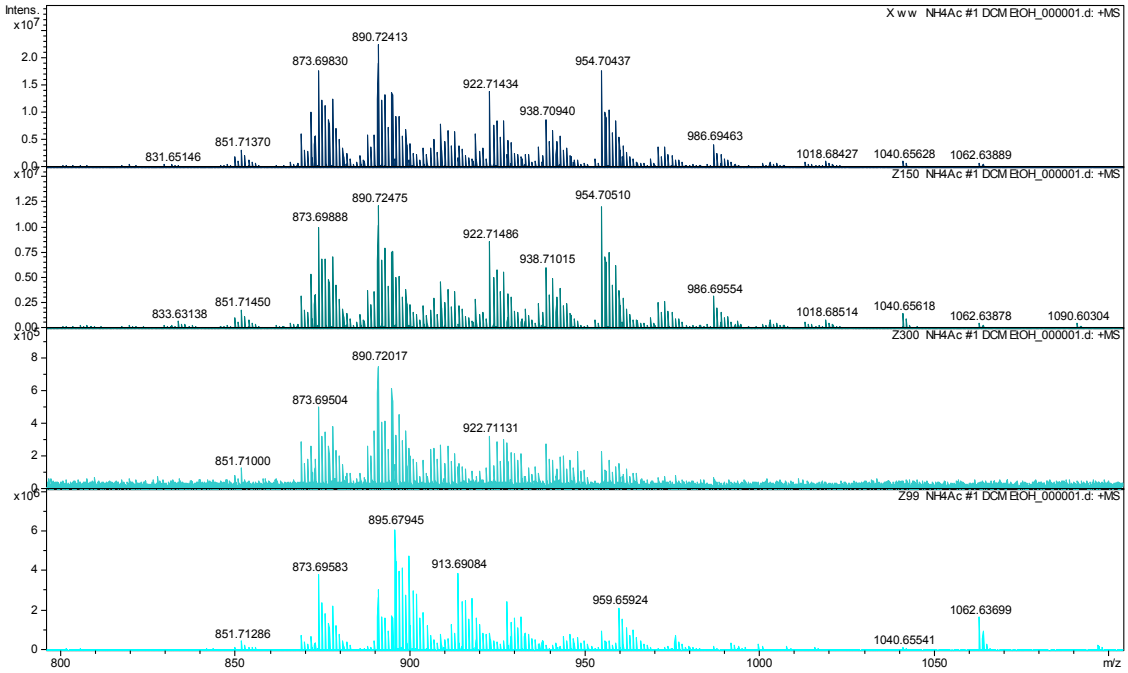


Figure 5.4-7. High resolution spectra of Linseed oil from 1999 water washed according to procedure, heated to 150ºC; heated to 300ºC and cold pressed.

In the particular case of water washing it can be observed that the number of Oxygens in oxidized TAG's are greater than the number of unsaturations (e.g. Tag 57:4+6O). Hence it must be concluded that functionalities such as hydroperoxides,

epidioxides, hydroperoxide-epidioxides, diols, and triols are present in the oxidized samples.

Hydroperoxides are very common as primary oxidation products and very often mentioned in the literature since they are well-known precursors in the product breakdown stream [9]. Diols and triols are said to occur as breakdown products of linoleic hydroperoxides [10]. Hydroperoxide epidioxides are only reported as oxidation progress breakdown of linolenate [11]. In highly siccative oils there is more probability of detecting these compounds than in non-drying oils that do not contain a significant relative amount of linolenic acid.

Addition reactions or condensation are also a source of higher mass compounds

Samples of linseed treated in 1999 present the same features as those from 2009, although slightly more oxidized. As a measure of the extent of oxidation water washed oils seem more oxidized in the TAG's region of the spectra, followed by those heated to 150ºC and heated to 300ºC, to what could be expected from higher temperatures, as it is said in chapter 2, that heat especially high temperatures, have sufficient energy to break covalent bonds C-C or C-H in the acyl backbone to form a variety of lipid alkyl radicals [12] which then start radical chains of oxidation. Moderate temperatures have lower energy, so act primarily by breaking O-O bonds in traces of ROOH or LOOH performed by other initiation. The RO•, LO• and •OH thus generated abstract hydrogens from neighboring lipids to form L• and initiate radical chains LOOH decomposition and it's contribution to propagation is the major catalytic effect of heat [13]. Effects of increased LOOH decomposition are amplified by increased rates of subsequent H abstractions by LO• and LOO• , which is reflected in the double oxidation rate for every 10ºC rise in temperature [14].

With this said, it is expected to se more C-C bonds throughout the process of radical condensation since there is the alkyl radicals to incorporate in the cross-linking to a polymerized oil. Compared with the untreated oil it is relevant to confirm that there are higher intensity ratios for DAG's and DAG's fragment ions in the heated oils. This indicates that fragmentation is occurring within the heat treatments. Hence, higher oxygen incorporation in the water washed samples is also verified.

From the results it can be clear that initial TAG's, with slight oxidation, do change upon processing methods, through different pathways following the autoxidation processes reviewed in chapter 2, it is hereby confirmed that water washing greatly enhances the hydroperoxide formation and inclusion of oxygen but without the oxidative breakdown characteristic of thermal processes.

Extracts can be trimethylsilylated (TMS)(see Appendix) in order to detect all free carboxylic and hydroxyl groups whereas ester bonds remain intact. This approach can be used to detect hydroxyl groups along the fatty chain as a consequence of oxidation maintaining the ester bonds of the TAG's as previously stated and confirmed in van den Brink's thesis. Also by this derivatization it is possible to verify oxidation products that are not derivatized, such as, epoxides, epidioxides or keto groups.

We have to follow the 73.1913 amu of TMS shift to higher masses in order to ascribe a hydroxyl or peroxyl group.

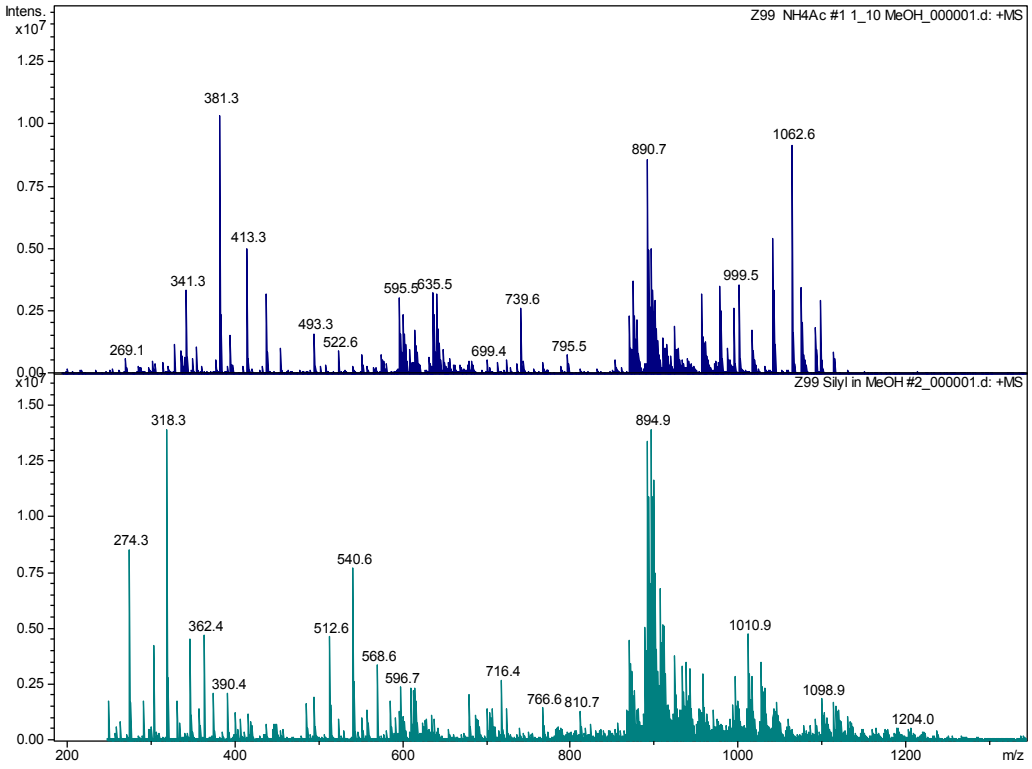


Figure 5.4-8 High resolution spectra for Linseed Oil and silylated sample

The shift from OH to OTMS ether equals a 72,2 amu mass difference and OOH to OOTMS ester has the same mass difference, we can however detect which fragment it comes from, and how much oxygen was incorporated. As an example the DAG fragments 595.5 *m/z* whith one TMS ester and 3O would be seen as 716.4 *m/z*, following the same principle 635.5 *m/z* would be seen at 766.6 *m/z*.

The TMS derivatives of TAG's also show at e.g. 1010.9 *m/z* TMS with 2 O and 1098.9 for 2TMS 3O of C55 Tag's 1204.0 3TMS plus 5O. If this was applied to the water washing procedure some newer conclusions could be made, unfortunately the silylation was not applied to these treated oils.

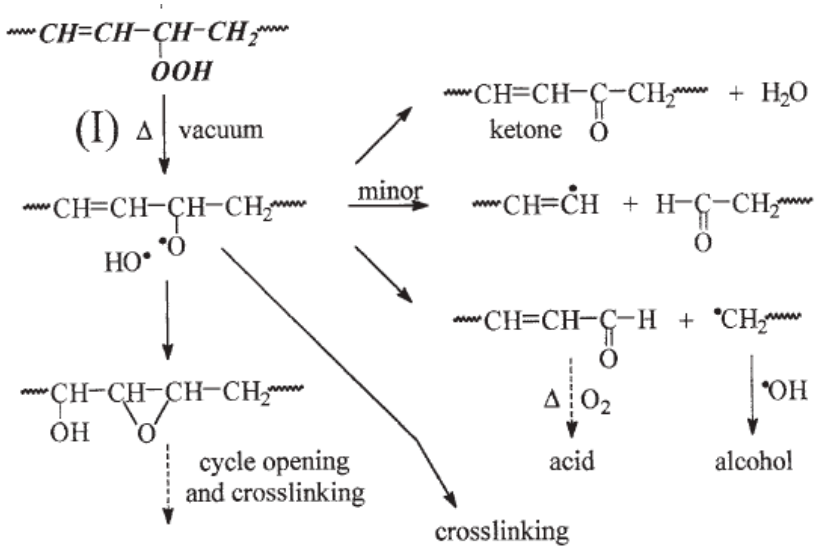
5.4.1 TENTATIVE IDENTIFICATION BASED ON NOMINAL MASS DATA: LIST.

To build on the chemistry of the mixture within each oil TAG profile, as for example, to see how different individual components (FA and di acids) are associated in MAG/DAG/TAG (take note fragment ions are not always ammoniated) we use the high resolution mass spectra to sum up the characteristic features within each oil and within each treatment.

Particular studies of model compounds of oil paints, either by FT-IR or MS related techniques, have been obtained by some research groups[6, 7, 15, 16] and these are in accordance with study of vegetable oils used in paint formulation.

In one of these studies, several oxidation products were identified. FTIR studied Alcohols identified by the nitrite band at 779 cm^{-1} after NO treatment. Subtraction of the initial IR spectrum of non-oxidized linseed oil from that of the oxidized linseed oil after SF4 treatment revealed the presence of a broad band between 1730 and 1670 cm^{-1}. This band indicates the formation of saturated and unsaturated ketones at 1720 and 1698 cm^{-1}. The band at 1772 cm^{-1} was attributed to the formation of γ-lactones and per esters (esters of per acids). Unsaturated and saturated acid fluorides identified after SF4 treatment at 1810 and 1843 cm^{-1}, respectively, revealed the presence of carboxylic acids[15].

As would be expected the homolytic decomposition of the ROOH generates an alkoxyl radical that can lead to an alkyl radical or vinyl radical by β-scission reactions. The secondary oxidation products formed by reaction I (Scheme below) consist of epoxides, ketones, alcohols, and aldehydes, which are later oxidized into free FA's or (di)acids[15] as expected.



The occurrence of unimolecular or bimolecular decomposition of ROOH is not relevant as the main products are the same. Two types of reactions lead to alkoxyl radicals that are the species of interest in the determination of a mechanism.

Also review studies such as Byrdwell's[17] or Christie's Lipid Analysis[18-23] can help to design the best analytical approach to reveal the nature of the species present in the paint: after the loss of volatile species, oxidized species remain in the oils as secondary oxidation products (see chapter 2).

5.4.2 FATTY ACYL MOIETIES DERIVED FROM NEGATIVE ESIMS OF THE OILS AND TREATED OILS.

Negative ion mode ESI-MS on the same oils enables us to see Fatty acyl moieties. These mass spectra can be also more informative on Oxy compounds detectable in negative ion mode.

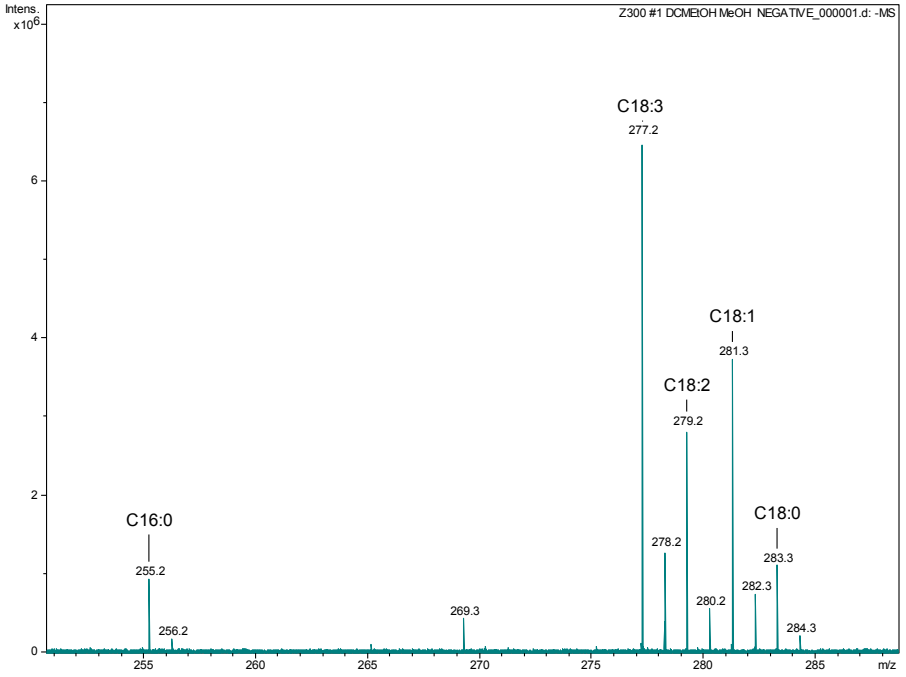


Figure 5.4-9 High resolution negative ion mode spectra of a 1999Linseed oil, heated to 300ºC.

In the negative ion mode we have proof of the constituent FAs and their relative abundance, since in the spectra we can identify the most common FA in the oil, as $[M-H]^-$ of Palmitic, Linolenic, Linoleic, Oleic and Stearic, respectively.

Nevertheless we should not relate intensities directly to the FA present because of different ionization efficiency of saturated vs unsaturated species, since the presence of double bonds tends to facilitate ionization. Consequently standard calibration should be considered.

Not much seems to have happened in this oil sample. It seems as it was a cold-pressed oil but it was heated to 300ºC although HART project oils were heated for shorter periods of time than the 2009 reconstructions. Maybe that's why it does not show many differences in the spectra.

The presence of special FA's such as ricinoleic acid would be indicative of the use of castor oil, while 11-eicosenoic (gondoic) acid and 13-docosenoic (erucic) acid are the biomarkers for rapeseed oil [24].

Palmitic/Stearic ratios alone cannot provide absolute proof of the kind of oil in modern formulations, since there are different contributions, which may interfere e.g. metal stearates, castor wax, semi drying oils, non-drying oils [25] also the contribution of other components should be regarded, and not just diacids formed.

Profiling studies PART B

5.5 LCMS mass chromatograms of the oils and treated oils.

The LCMS was at first used as a profiling method using the mass chromatograms of the pseudomolecular ion peaks of the triacylglycerides to evaluate the number of isomers in the oils. Previous studies have already shown that the number of structural isomers increases strongly upon heating ([26]). This second profiling method should therefore give a rapid insight into the complexity and increased complexity due to treatments intended to modify the oils as known from literature from traditional paint making [27-33].

Trying to develop a more detailed study of component TAG's and possible TAGOX LC-MS can give a Targeted complementary view, by separating and identifying Isomers of TAG isobaric species. We can also see how different oils reflect TAGOX composition and also later identify effects of treatments.

The target analysis approach consists of accurate mass measurement in direct electrospray mode using an FT-ICR-instrument and LCMSMS on specific ions deemed to be of particular diagnostic value. The accurate mass values support the tentative identifications list composed on the basis of nominal mass work with the ion trap MS. The LCMSMS work can provide a deeper insight in the structural changes due to the treatments. The latter approach, LCMSMS, can also provide

more insight into the positional distribution and nature of the fatty acyl moieties from TAG's within each oil.

Table 5.5-1 Peak identification in LC-MS experiments.

Peak	TAG	m/z
1	LnLnLn	890,7
2	LnLnL	892,7
3	LnLL / LnLnO	894,7
4	LnLnP	868,7
5	LnLnS / LnLO	896,7
6	LnLP	870,7
7	LnOO / LnSL	898,7
8	LLP / OLnP	872,7
9	LnSO/ LLS / LOO	900,8
10	OLP / SLnP	874,7
11	SOL / SSLn / OOO	902,8
12	OOP / SLP	876,8
13	SOO	904,8
14	SOP	878,8

Liquid chromatography (LC), LC-MS or LC- MSMS experiments, that confirm each component in the List (Table1) and subsequent changes, can also reveal the number of isomers for each extracted chromatogram of a given pseudo molecular ion. The appearance of oxidation products of TAG's (also known as TAGOX) and attribution of these molecular species are in agreement with the drying processes.

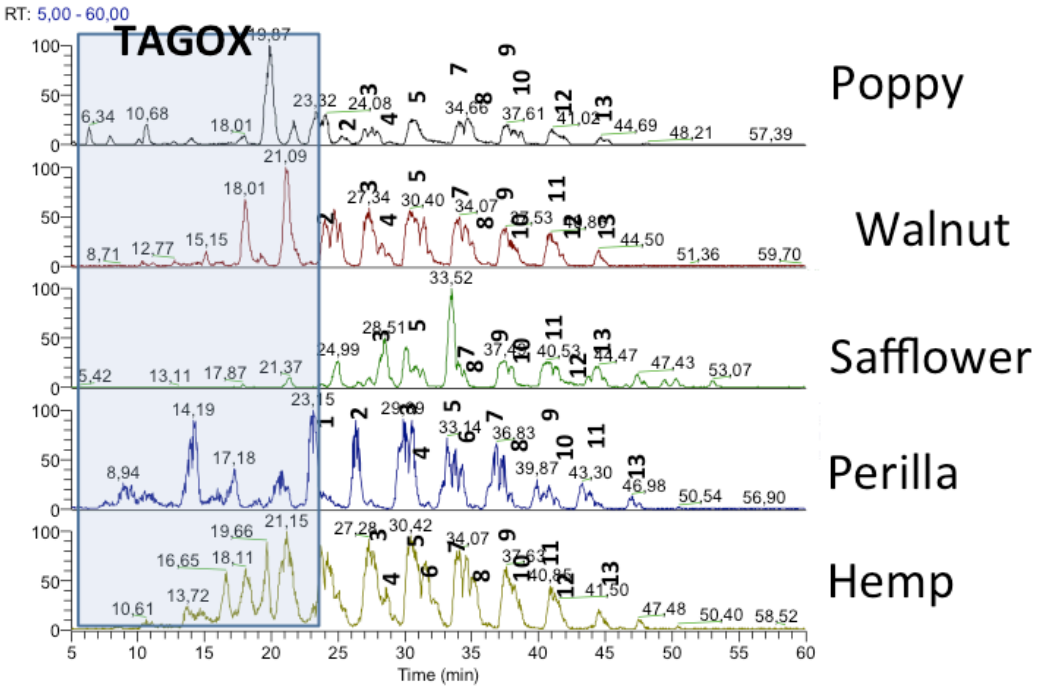


Figure 5.5-1 Chromatograms from different cold pressed oils.

As previously reported by Van der Berg et.al. with HPLC-APCI-MS of linseed oils, there's a conversion of "singlet" peaks in chromatogram fresh oil to "multiple" peaks in chromatogram of heated linseed oil. In this study this is observed when using HPLC-ESI-MS, since almost all the oils analyzed show these characteristic "multiple" peaks. Some exception can be found for samples of cold pressed

Linseed from 1999, which are already at some stage of drying at the point when they were analyzed, confirming the natural ageing process (not shown). Each oil show a very distinct profile and the way isobaric species can be separated in the extracted chromatograms is an advantage of this combined method with the high resolution spectra where only by MS^n we were able to attribute different isobaric species, here we can even do LC-MSMS to better separate these species.

Tentative proof of identification of isomers due to double bond position, migration, rearrangement (*cis/trans*) or the formation of cyclic FA's can be addressed. All of these justify the complex multiple peaks from all the oils, found in the chromatograms, to a different extent.

Presence of TAGOX and isomerisation, can be observed in MS^n such as in naturally aged Linseed oil and even to a greater extent in aged water washed oils. It is easily detected from the absence of the most unsaturated TAG's which react faster in order to produce TAGOX.

The degree of isomerization is well noted through the sequence of Tag's resolved through the chromatogram reflecting ECL (Equivalent Carbon Length) value of known compounds, and cis/trans isomers in multiple peaks. These can all be seen in the extracted chromatograms of Saffllower oil (Fig.5.5-2), which were assigned to the respective TAG species.

Even in the simpler chromatograms the amount of information that can be processed in order to better characterize the starting oils, and their composition, is very thorough and we can assign the different peaks to all of the isobaric species present, but it would be exhaustive to present all the data from each oil.

Differences in the isomerization through heat processes are very distinctive, and these cis/trans conformation could be verified easily by FTIR as it is evident in the extracted chromatograms for each oil.

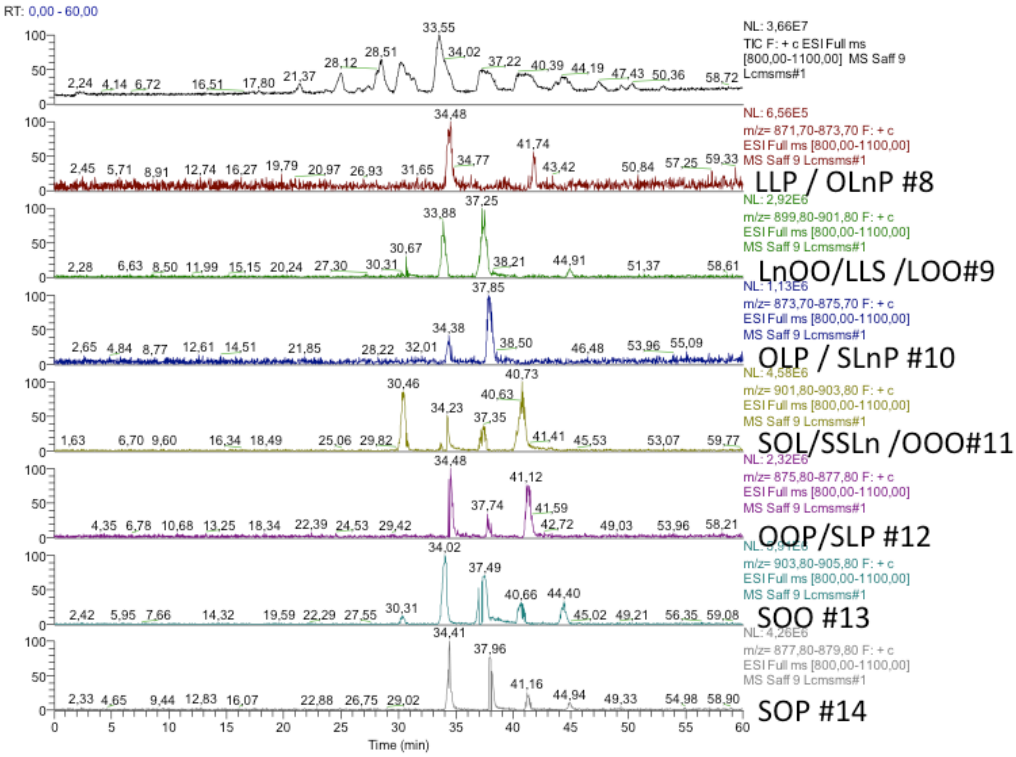


Figure 5.5-2 Extracted chromatograms of isobaric species from Safflower oil.

The oxidation products of pure standards, triolein, trilinolein and trilinolenin, enabled identification of main oxidation products as e.g. Mono- and Bis hydroperoxides, mono- and diepoxides, hydroxy Tag's, epidioxides and hydroperoxides epidioxides. Model studies also detected oligomers when the TAG standards were heated, up to tetramers[34].

Further work on target analysis would be complementary to attribute each TAGOX by m/z by high resolution MS, as it was referred in the oil treatments section, to confirm Id's or new unknowns, and LC-MS to tentatively identify isomers due to double bond rearrangement or conformation.

ESI-MS on a triple quadrupole instrument might add a closer look for monitoring, for example, the loss of the previously known FA with Neutral Loss Scanning (NLS) or selectively monitor known TAG's with Molecular Reaction Monitoring (MRM).

5.6 WATER WASHING AND HEAT TREATMENTS

The impact of water washing procedure is here undertaken as a prologue to heat treatments, because it's considered as a normal procedure in the preparation of oils for oil paint manufacture, as seen before from the spectra, and it has not been examined before in other studies.

The results here shown illustrate the extent of oxidation just by the water washing procedure, probably because of the removal of different species of anti-oxidants, namely tocopherols and other sterols. Removal of the "mucilage" further activates oxidation of the oil to a solid film by the autoxidation drying mechanism.

The contrast between the chromatograms of Safflower and 1999 Linseed are clear in showing the difference of water washing. The water washing procedure in the Linseed oil is affecting all the chromatogram and there is an appearance of a multitude of new species particularly in the first part of the chromatogram that are not present in Safflower as it's initial composition is much less reactive to oxygen.

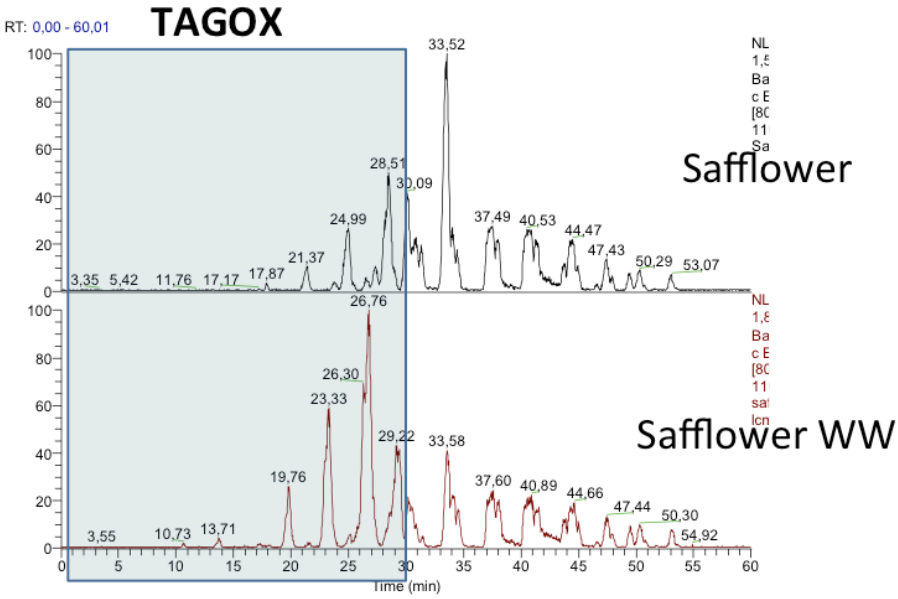


Figure 5.6-1 Mass chromatograms of Safflower oil and its water washed oil.

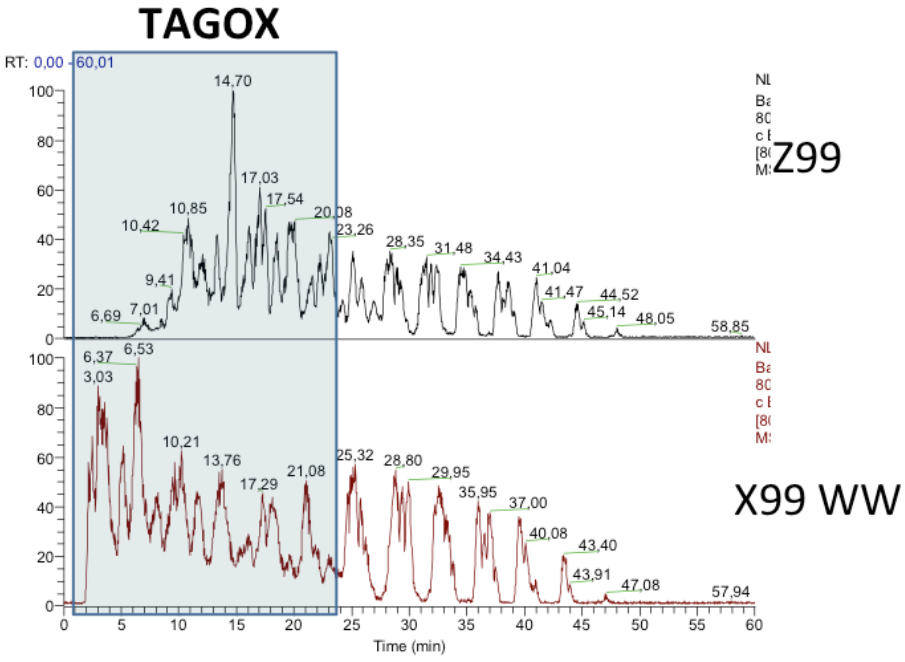


Figure 5.6-2 Mass chromatograms of linseed oil, before and after water washing.

From the HPLC-MS mass chromatograms of safflower oil versus linseed (1999 extraction) we take notice on the simple and straightforward presentation for the less drying oil, safflower, as opposed to Linseed, which has an altogether more complex view due to isomerization and oxidation products.

An identical tendency for broader peaks and less resolved multiple peaks is also associated with the water washing procedure as observed in the chromatograms (see Fig. 5.6 - 1/5.6 - 2).

The amount of LC-MS and LC-MSMS runs of all the oils in study, is an heavy weight database for their characterization, but it is more useful for targeted peaks as an approach is to answer questions that could not be verified only by MS or other techniques.

5.7 PAINT RECONSTRUCTIONS

By making reconstructions with historically appropriate materials, we can learn why materials were prepared in a certain way, what governed the artist's choice, and how such materials behave in application, thereby gaining direct insight into workshop practices[35].

Once recreated, the products of old recipes can function as reference samples for comparison with what we see and find through analyses, and to assist the interpretation of analytical results [36, 37].

Previously described HART project (Historicallly Accurate Oil Paint Reconstruction Techniques) samples were provided by Dr. Leslie Carlyle. These samples are aged naturally since 1999, so we can follow the early stages of the maturing of a paint system. Three white pigments were used in the manufacture of the paint mock-up films, Dutch stack process Lead white (D), Kremer Pigment Lead White (K) and Basic Lead White (B).

Here we aim at a better understanding of how the oils studied develop into a paint system and what to expect as oxidation products to recognize them in the light of their mass spectra. This endeavor is not quite as straightforward as for the oils alone but we have control samples quite well characterised. We aim at a first interpretation of main products of ageing in the paints subjected to analysis, as we already characterized the oils that serve as raw material to the reconstructions.

Recent overview of materials detected in a range of manufactured artists' oil paints[25, 38, 39] is quite more complex than these reconstructions.

It is expected that we encounter fragments of TAG's, with or without extensive oxidation, as the paint dries and alters it's composition.

We try to find a pattern recognizable from the starting oil mass spectra to the present paint reconstruction. Pigmented paint is clearly different than oil alone and we can detect the catalytic effect of white lead in such paints.

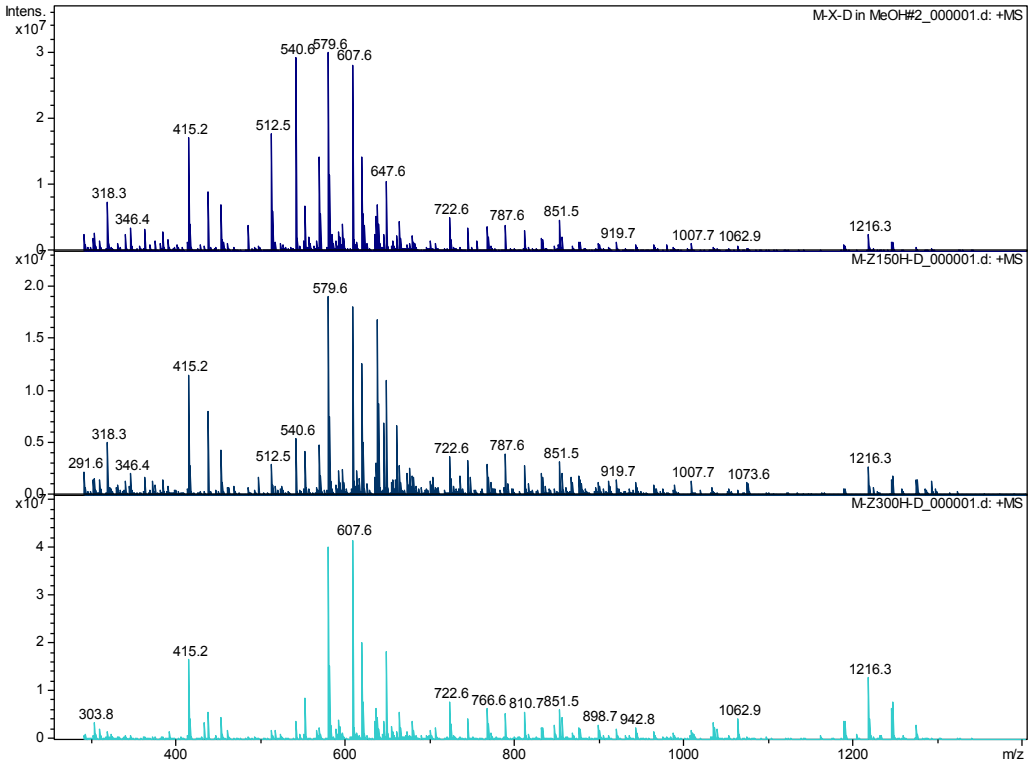


Figure 5.7-1 High resolution mass spectra of Paint extracts made with oil from Linseed 1999, after water washing (ZD), heating to 150ºC (Z150) and 300ºC (Z300), with Dutch stack process Lead white (D).

The 579.6 *m/z* peak in the mass spectra of the paint (Fig. 5.7 - 1), is most probably due to DAG fragments analogous to SP (stearoyl gliceryl palmitate) $[M\text{-}RCOOH]^+$. The 607.6 *m/z* is also most probably due to DAG fragments but analogous to SS (stearoyl gliceryl stearate) $[M\text{-}RCOOH]^+$. These are probably retained in the paint because they're the most saturated DAG's.

Starting from here we can further attribute several compounds to the corresponding peaks in the spectra. These attributions confirm the recognized DAG fragments in the spectra of the original oil used to make the respective paints.

The high intensity peak at 415.2 m/z is present in almost all paint samples and it is characteristic of a DAG fragment constituted by two C9 diacids formed as a result of extensive oxidation mainly at carbon at position 9 in the unsaturated FA carbon chain. The presence of DAG containing two azelaic acid moities is revealing of the drying nature of the initial oil TAG's. This finding is in accordance with previous GC-MS analysis of oil paints after hydrolysis and methylation in which both azelaic and suberic acids were found as markers of oxidation, and according to the MolArt model they would function as a stationary phase when forming metal soaps and act as a stabilizer for paint[40].

Also it is evident that the oxidation here is progressed because of the lower amounts detected of intact TAG's when compared with all the other oxidized species present. Relevant in this assumption is the presence of dimers in the higher masses regions, such as 1216.3 m/z could be from a dimer of a C18C18 DAG, or a dimer of 2 C16C18 DAG's with a peroxide link (+2O). Considering this interpretation the 1061.3 m/z can be assigned to a dimer of C16C18 DAG's. Combinations of these fragments are also seen in several spectra. This can confirm the relative degree of oxidation in the early stages of a paint to solid film.

Paints in solution with ethanol show a different profile that is reflected in the respective mass spectra. Probably, more polar compounds are extracted more easily.

Yet the spectra of these extracts in ethanol are significantly different and too complex to interpret. Only we might add that possibly more polar fractions are extracted.

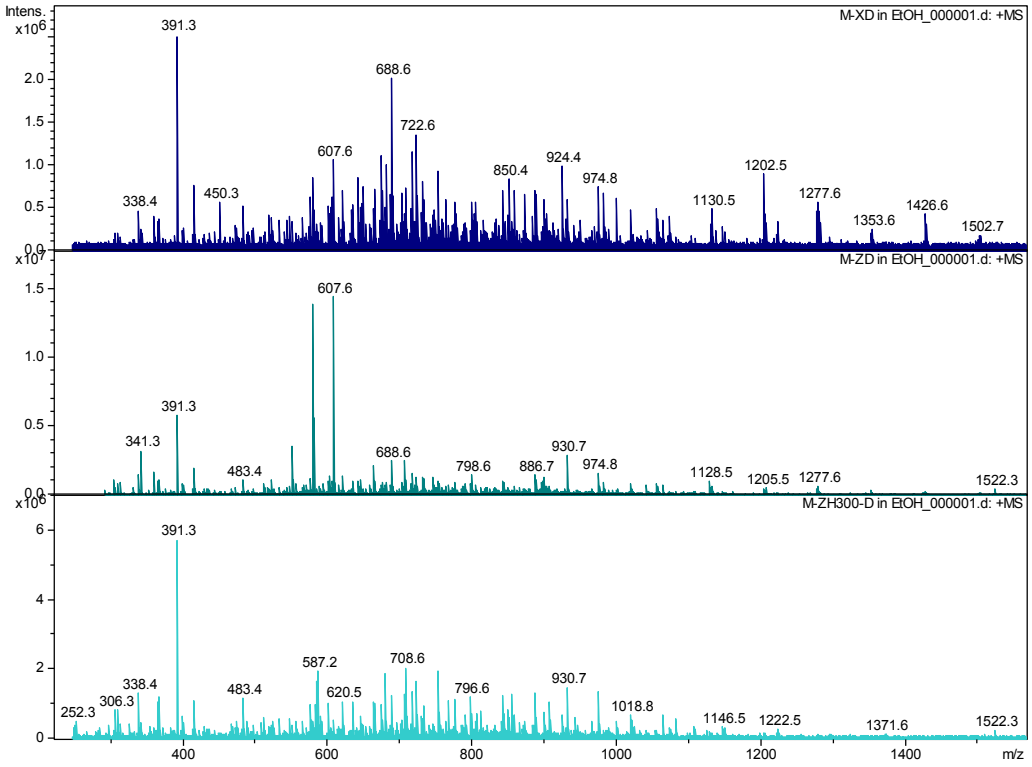


Table 5.7-1 High resolution mass spectra of Paints made with oil from Linseed 1999, after water washing (XD), heating to 150ºC (Z150) and 300ºC (Z300), with Dutch stack process Lead white (D). Paints extracted by Ethanol.

The use of Dryers was also a case study, in which we can detect some of the same features of the linseed paints. The peak from DAG with azelaic acid at 415.2 is present to an even greater extent, together with most intense peaks at 579.6 and 607.6 from DAG's with C16C18 and C18C18, respectively. As mentioned before these are saturated DAG's and support the notion of the unsaturated species reacting away in the radical chain course of Autoxidation.

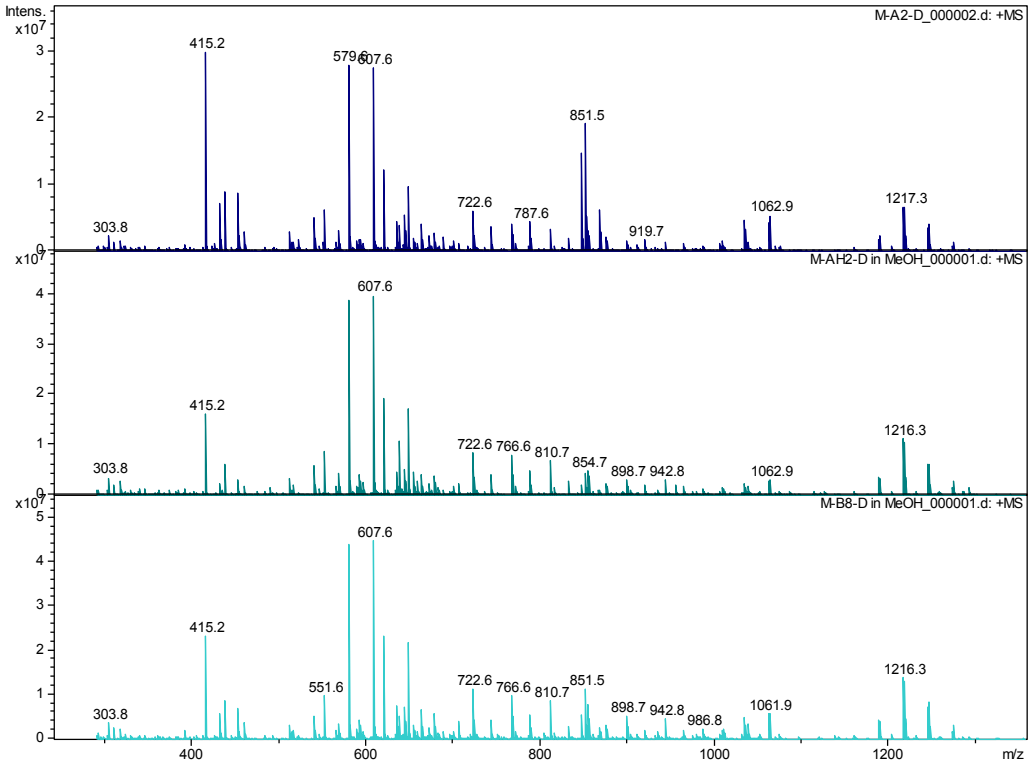


Figure 5.7-2 High resolution mass spectra of paint extracts from oils prepolymerised with dryers and Dutch stack process lead white.

In all oils prepolymerised with dryers and Dutch stack process lead white, the higher values at 1216.3 m/z could be from a dimer of a C18 TAG ether linked to a MAG with Palmitic acid, or a dimer of 2 C16C18 DAG's with a peroxide link (+2O). Considering this interpretation, m/z 1061 is attributed to C18TAG incorporate azelaic acid into a dimer

Possibly 2856.9 m/z could derive from two times 1216.3, explained before, plus 415.2 m/z from a di-C9diacid DAG fragment, ether linked to the rest (+14amu).

Higher masses (not shown) could reveal tetramers of mixed TAG's and diC9 DAG's incorporating Oxygen as seen in 2693.5 m/z, 2810.6 m/z, 2856.9 m/z and 3432.4 m/z.

Samples were collected from a case of tube paints belonging to Aleksander Rodchenko's grandson. A total of 8 samples were analyzed in order to understand the early stages of paint drying from commercial formulations.

The descriptions of manufacturer, origin and date (when possible) are mention to better classify each sample, the pigment and/or colour is mentioned whenever possible.

Rod1 Vassiliev Fréres(St. Petersbourg) - Blue (-1920)

Rod2 Hermann Neisch (east Germany) - Cremnitz white

Rod3 Polish Drakon (-1923x) oil Blue

Rod4 Winsor & Newton (through Germany)

Rod5 Le Franc and Bourgeois Cadmium Red

Rod6 Schoenfeine (Chrom……..Deep) green

Rod7 (Russia) Red

Rod9 Rubens Reine (Herrmann &Co - Chrome Oxide) green

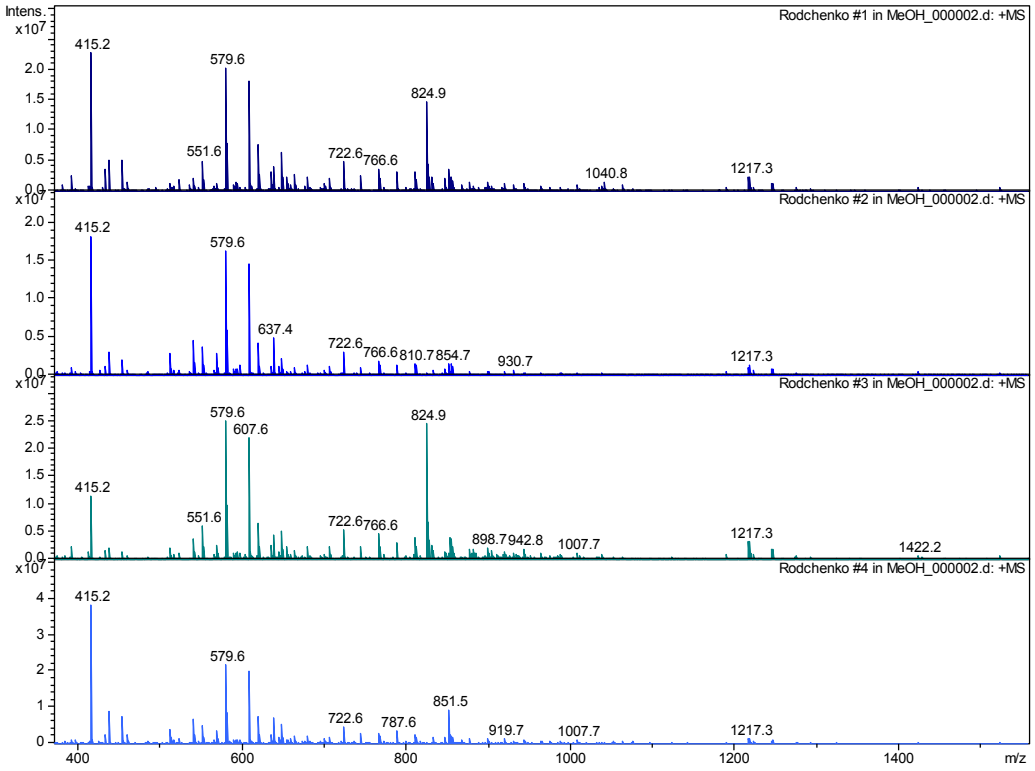


Figure 5.7-3 Oil tube paints from the Rodchenko palette (see sample descriptions)

All of the first four colours have a distinctive and very similar spectra with a high intensity of DAG with azelaic acid at 415.2 m/z, with most intense peaks at 579.6 and 607.6 m/z from DAG's with C16C18 and C18C18, respectively.

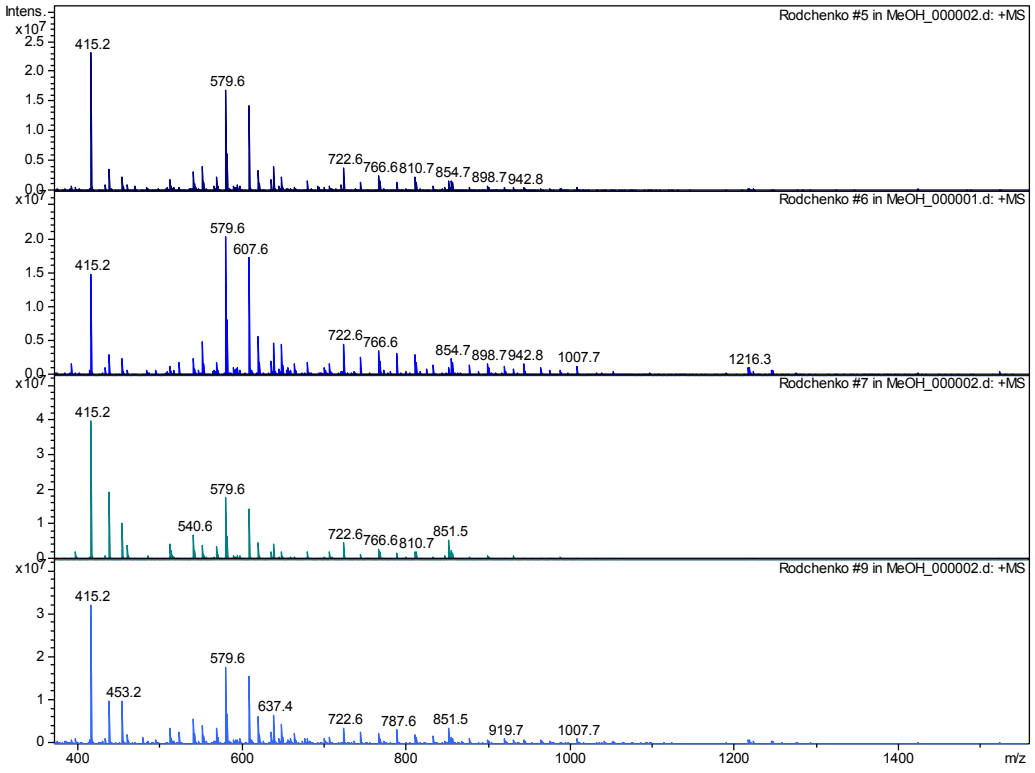


Figure 5.7-4 Oil tube paints from the Rodchenko palete (see sample descriptions)

These other 4 samples are also very similar in their features and also alike the ones presented before with main peaks at 415.2 m/z for oxidated species from azelaic acid and 579.6 and 607.6 from saturated DAG fragments left after oxidation.

The tube paints were sampled from the paint tubes and were collected in sample vials, they are reacting away and are now clearly not with the original composition as it seen in the spectra of the 8 samples.

5.8 GENERAL DISCUSSION

ESI-FTMS of the different oils was carried out by the addition of ammonium acetate to produce cationised ions. As a result, TAG's, DAG's and a complex mixture of oxidized compounds thereof could be identified from the spectra of the starting oils after, some natural ageing, water washing and the heat treatments.

In addition, because of the efficiency and relative stability of ion production, fragmentation processes of these complex mixtures could be recognized, in order to proceed in the attribution of different species in a sample.

The high mass resolution of FTMS data is also a valuable asset in order to correctly ascribe and interpret possible isobaric species, that was not feasible on an Ion trap instrument. An example is to be able to discriminate between TAG n:x (n C atoms and x unsaturations) and TAG (n-1):(x+1)+O (mass difference of 0.0364). It could erroneously be assumed that such peaks originate from TAG's containing fatty acyl groups with an odd number of carbons (not present in vegetable oils for instance) instead of being oxidized.

The mass accuracy of the FTMS data obtained on the several oil samples allows thorough characterization of these from starting materials to processed oils, ultimately to paints, and the altogether molecular changes can be monitored from differences thereof. This approach is relevant in conservation science in order to be able to "trace back" artist's paints to it's original formulations, as well as being important in the chemical description of the changes within time, besides the already mentioned processing of the starting oils and paints.

Another perspective is the wider analysis of different more or less siccative oils, and their relation to the effective chemistry happening from oil paint to a solid film and the possible problems arising from the use of non-drying oils. This type of study

was first introduced by van der Berg in the study of Linseed oil. It is within the aim of complementing this study that we introduce new oils for paint formulation and as references for new studies. It is intended to further explore disparities in relation to initial controlled historical formulations to the future of a paint system.

Improving the basic knowledge of the chemistry of all these oils serves as a starting point to be able to further extrapolate to possible oil mixtures.

To my knowledge the water washing procedure has not been subject to this type of analysis before. In this way it is a scientific development to relate the chemistry of these treatment with known historical recipes. Through the results shown it appears that the actual removal of *mucilage* favors the process of oxidation of the oil intended for paint purposes. Just as well as the shaking, stirring, renewal of water and contact to air, all have a role in the initiation of oxidation in this processing method.

From the comparison of these oils we are able to discriminate what is a rule of thumb for all of them but, more importantly, to discriminate characteristics of a specific oil and a given treatment. Trimethylsilyl derivatives were efficiently ionized in ESI and could help in this task assigning Hydroxide and hydroperoxide function.

The complete TAG profiles of the studied oils serves as a model of starting characteristics that should be present in an oil for oil paint, or to be able to question if these are suitable for paint use, Safflower should not be a good candidate for paint formulation. Although it is continuously used in cheaper formulations. Should we further investigate mixtures, questions like this have to be answered on the necessary profile needed for paint purposes to be sufficiently siccative admixtures should be formulated with more than 50% Linolenic acid and Linoleic.

The chromatograms of different oils show extensive oxidation and duplets in LC-MS extracted chromatograms indicate *cis* /*trans* isomerization, the latter confirmed in FTIR.

Charge remote fragmentations of the $[M+NH4]^+$ detected ions should be used to propose mechanisms from evidence peaks.

MS/MS table of Starting materials vs products (reveal chemical markers).

We can take a closer look with specific TAG's quantitation in Triple quadrupole through MRM of selected TAG's m/z and of NLS of specific FA's .

5.9 AIMS AND FUTURE STUDY

ESIMS at Amolf on standoils were performed by Prof. Jaap Boon and were replicated in Lisbon, both low and high resolution spectra were obtained. Stand oils are even more complex because of the heating process. They reveal quite a number of higher masses than TAGs. Work done by ESIMS on standoils or prepolymerised oils in general, and also paints, would be the probable next step for rigorous interpretation of chemical markers, as we can see in the discussion section.

Access how introduction of new oils in paint formulation affect the drying of oil paints means that the chemical markers of degradation should be understood in a wider context, and to justify chemical and morphological changes through the chemical markers of oxidation/ageing.

Another aim would be to further develop on how the influence of oil processing affects oil mixtures, from the results of the simple different oils studied.

These data will possibly be available through a database construction for oil paint characterization and shared through the Mass Spectrometry and Chromatography User's group (MaSC) of ICOM-CC.

Because of the transition of white lead white based pigments to Zinc white (and Titanium white) the study of the effects on the organic paint constituents would be taking this a little further. This taking place in that period, beginning of the 20th century, and the probable comparison with commercial paints around at that time, which are available although in remote collections, would also be a very valuable contribution.

The build-up of a database on reference oils and respective paints is intended for further generalized use. The goal would be to be part of the solution to the analysis of contemporary oil paints in selective samples from paintings kindly retrieved from several collections.

To study accelerated ageing of the oils and pigment in solution, with methanol as solvent, is under current process on Molart Ageing box. Based on these results, in a small scale study, there would be the possibility of following the PCA analysis on model oils throughout time. For example, a video or time lapse film of how the reconstructed spectra of all oils studied moves in graphical representation with time of ageing, and recording of how the vector time reflects on the other spectral differences attributed to molecular changes (vectors).

In order to better see differences from the spectra it would also help if we could relate ratios of different species accordingly to each paint sample. For example the ratio of diacids to DAG's as a measure of the stages of drying.

5.10 CONCLUSIONS

Although there is still a lot to improve in our chemical understanding of the drying processes of artist's oil paints, the path is delineated for more oil paints to be traced to the original oils or oil mixtures.

Combined mass spectrometry methods as ESI-MS (Iontrap, FT-ICR-MS, Triple quadrupole) or MALDI, coupled with LC-MS or GC-MS, but also FTIR and NMR, all contribute in some way to a "better view" on drying mechanisms and associated chemical changes. Each method with it's own specificity can give some contribution to the understanding of the drying processes, but it is the combination of all of these that permits a closer investigation of the chemical changes on the time frame of an oil to oil paint and from stringy film to solid layer.

The work described here can give a contribution to a better knowledge of how possibly an oil, as the starting material, develops into a complex mixture of cross-linked network of oxidized lipids. In particular ESI-MS and LC-MS work are able to give a small inside view on the complex process of oxidation and drying of paint.

The analysis revealed how important is the fact that the differences in the starting oils impart on the composition of the latter paint. For example, characteristic unsaturation of the TAG's profile in each oil has to contain at least 50% of Linoleic or linolenic FA's such as the most siccative Perilla, Linseed, but also walnut and Poppy, as for Safflower it could reveal it's semi-drying proprieties influence the drying of a paint as it ages to a solid film. It is now recognized that non-drying oils or mixtures could fail as a medium and reveal dripping problems *in extremis*[41].

Processing methods such as water washing and heat treatments are also main causes of change in the oils as they age and dry. These should be understood chemically in order to scientifically follow the changes that provide the oils suitable for paint use. We have found that the early stages of drying are critical in establishing of a good paint system that will last centuries, such as we now know paints with less drying oils are not stable paints.

Although the painting technique was established empirically, the effect on the actual chemistry of the oil of different processing methods can be related to lab notebooks and recipes consulted, and might also justify centuries old methods of oil paint making.

The advantage of the chemical knowledge obtained through the mapping of possible reactions in the "life" of an oil paint is still a fertile ground for more science based exploratory work or time based monitoring of the paint reconstructions at later dates.

Addressing possible mechanisms and attribution of oxidized species of these mediums is a complex world we may start to shed a light on. Establishing routes of preferential reactions or privileged decompositions as a consequence of processing methods on starting oils would be main future research objectives.

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