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Cover letter

Dear Editor,

Herein I am submitting the revised version of the manuscript "Novel application of liquid chromatography/mass spectrometry for the characterization of drying oils in art: elucidation on the composition of original paint materials used by Edvard Munch (1863-1944)" by Jacopo La Nasa, Marco Zanaboni, Daniele Uldank, Ilaria Degano, Francesca Modugno, Hartmut Kutzke, Biljana Topalova-Casadiego, Eva Storevik Tveit and Maria Perla Colombini.
We retain that the revision has improved the paper, thanks to the suggestions of the referees.

Modern oil paints differ from those classically used in antiquity in their chemical and compositional features, due to the presence of a wide range of raw materials and additives. Recent developments in conservation science have highlighted the need to increase the available knowledge of compositional features of modern paint materials, and to develop analytical methods suitable for their characterization.

In this paper we report for the first time the characterization of a set of original paint materials used by Edvard Munch (1916-1944), and of a paint sample from an artwork by the same artist.
We used a multi-analytical approach based on the integration of three different mass spectrometric techniques: pyrolysis- gas chromatography-mass spectrometry (Py-GC/MS), gas chromatography-mass spectrometry (GC/MS), and high performance liquid chromatography coupled through an electrospray ionization source to a tandem quadrupole – time of flight mass spectrometer (HPLC-ESI-Q-ToF).
This work represents the first application of HPLC-ESI-Q-ToF for the characterization of triglycerides in a 100 years old oil paint.

In particular:

- TAGs profiling obtained by HPLC-ESI-Q-ToF permitted to characterize the oxidized triglycerides, and allowed us to identify the botanical origin of the oil used in the production of the paint tubes;

- the application of liquid chromatography allowed us to integrate data deriving from the fatty acid profiling, commonly used for the characterization of lipid materials in a paint samples, but poorly informative in determining the type of oils. Our results highlight the potentialities of liquid chromatography for the characterization of the chemical composition of lipid paint samples.

The application of this approach will contribute to address dating, authenticity and conservation issues relative to modern and contemporary artworks.

The manuscript is unpublished and has not been submitted for publication elsewhere. We sincerely hope that the paper is now suitable for publication in Analytica Chimica Acta.

Best regards

Dear Authors,
Below please find the comments of the reviewers.
As pointed out by the reviewers, there is some confusion with the numbering of the Figures. Please check ALL numbers of Figures in the text and in the Figure legends.
Furthermore, the last Figure in the pdf file (incorrectly called Figure 1) shows two chromatograms a and b, but the text of the figure legend does not clearly say what a and what b might be.

Figures numbering in the "Figures" file was corrected and the legend of Fig.8 was amended accordingly to the suggestion. The numbering in the main text was also checked and corrected where errors occurred. The authors apologize for the confusion.

Reviewer #1: This paper discusses the characterisation of lipid components in artists' paints by identification of the triacylglycerols (TAGs) using HPLC-ESI-QTOF. This technique has a large potential to overcome the cumbersome interpretation of fatty acid ratio's, the traditional way oil paints are characterised, certainly in modern and contemporary paints. A small number of labs are currently working on this innovative technique, and this paper is probably the first to apply this technology on historical samples. Overall, the manuscript is well written in a concise and scientific manner, although some parts need to be elaborated or clarified. Publication in Analytica Chimica Acta, albeit after minor revisions, is in my opinion worthwhile.

Remarks:

1. Page 10, line 27: if you are referring to the standard sample cups from Frontier Labs: despite the premium price, these are made of deactivated stainless steel. See: http://www.frontier-lab.com/wp/wp-content/uploads/2013/11/cup_select.pdf. There are also glass and glass/quartz versions available, but never heard about platinum cups. If you should happen to use platinum cups, please specify why you chose those and where you acquire them.

We used stainless steel cups. Platinum cups was a mistake. The text was corrected.

2. Page 17, line 56: LLO is said to be present in walnut and linseed oil. This is in contrast with the data in reference [30], written by some of the authors of this manuscript, which states that LLO is not present in linseed oil. Please correct this.

Corrected.

3. Page 18, line 5: OOP is said to be present in linseed oil, but not in walnut. According to ref [30], it is present in both oils. Please correct this.

Corrected. The sentence "[...] and OOP (m/z 881.776, $[M+Na]^+$), present in both raw materials" was added.

4. Page 18, line 10: the P/S ratio of paint tube 19 was quite high (3.8). For a linseed oil and walnut oil mixture one would expect a much lower P/S ratio somewhere in between the values for linseed and walnut oil (1.0 - 2.5). How do you correlate the findings of the TAG-analysis with the GC/MS-analysis? (In other words, how do you explain the high P/S ratio for a linseed/walnut oil mixture?)

A possible explanations is that the chemical composition of modern paint tubes is complex, and the paint tubes contain, in addition to natural oils, other additives such as metal soaps or surfactants. The proposed analytical approach does not allow the differentiation between fatty acids deriving from the hydrolysis process of TAGS and other oleochemicals, making the quantitative analysis results affected by the different amount of these additives. On the other side, the application of the HPLC-MS analysis allows the identification of the botanical origin of the oil showing how the P/S ratio is not trustable for this aim.
We modified the conclusions adding a comment related to this issue: "these results highlight the limited validity of P/S ratio only for the identification of the botanical origin of an oil: the presence of metal soaps or other minor sources of fatty acids could lead to erroneous interpretations of the origin of the material".

5. Page 18, lines 12-37: please elaborate this section. It is not clear to the reader why and how you identified paint tubes 11, 12, 15, 27, 30 as a mixture of linseed and walnut oils. As I understand it, the characterisation here is harder due to the complete oxidation of these samples. This could indeed suggest a drying oil, but nothing more (specific) in my opinion. If you are able to prove that these samples are indeed a mixture of linseed and walnut oils in a similar fashion as you did for sample 19, please elaborate this for at least one of the samples (e.g. sample 11) and/or add a table with the identified TAGs and their relative abundances. On the other hand, if you only assume the same drying oil mixture based on the results of sample 19, but you cannot prove this due to the high level of oxidation, I would strongly recommend you to significantly weaken your conclusion.

The paragraph was re-arranged and a more convincing explanation was inserted. Conclusions were smoothed according to the suggestion of the reviewer.

6. Page 19, line 7: OOS and OLS are said to be characteristic markers of linseed oil, while according to ref [30], these are not even present in linseed oil. Please correct this.

The whole paragraph was amended.

7. Page 19, line 9: ref [30] mentions other TAGs, that are apparently not identified in paint sample 13. These TAGs include POP and LLnP, which have relative abundancies of resp. 10% and 14% in [30]. Is there an explanation why these TAGs are missing in sample 13?

LLnP is readily oxidised (its oxidation product is one of the main peaks in the chromatograms of the more oxidised paint tubes: see discussion on pag. 18). We reckon this is likely to be the reason why it is never detected in the samples. With regard to POP, it was detected in some of the chromatograms, but below detection limits.

8. Page 19, line46: Just a thought: what TAGs do you expect in an egg yolk sample? Can you readily identify egg yolk using TAG-analysis?

Egg yolk can be detected by the characterization of the triglycerides profile as reported in reference [39]. However its unambiguous identification in a paint sample can not be performed only on the basis of the HPLC-MS analysis results: in this study, in order to exclude the presence of egg, Py-GC/MS analysis were performed.
We improved the discussion by adding the following paragraph: "Even if these triglycerides can also be related to the presence of egg yolk, the Py-GC/MS analyses allowed us to exclude the use of this material in the paint tube formulations" (pag. 20)

9. Page 20, lines 36 and 46: I think this should be figure 8a and 8b (not 7).

The text was corrected.

10. Page 20, line 49: "which may be related to the presence of linseed oil as raw material" --> it is not clear on what this assumption is based. See also remark 5: please elaborate the TAG analysis as you did for sample 19, or at least supply the identified TAGs and relative abundancies to permit critical readers to do the interpretation themselves. If this kind of interpretation is not possible due to the highly oxidised state - this is not clearly mentioned in the text -, then please clarify what indicates linseed oil. As I understand it, every drying oil oxidises. The only "reason" to specify linseed oil seems to me its low P/S ratio, capable of lowering the P/S ratio of a linseed/palm oil mixture to 2.5. This however seems a rather weak presumption.

We modified the whole section about discussion of results, in order to answer also to #5. We reckon that now the critical interpretation of the origin of the observed oxidised TAGs is clearer to the reader. We explained why the presence of linseed and walnut oil was hypothesized, and that it is an assumption based on the hypothesized formation pathways of oxidised TAGs.

11. Page 21, line 12: "univocally" --> unambiguously? You might also want to reconsider/alleviate this rather bold statement due to my concerns expressed in remarks 5 and 10. (Also on page 22 line 1)

The discussion of the results has been improved. Nonetheless, the conclusions were alleviated. The expression "univocally identify" has been substituted with "hypothesize", and we added some sentences which better describe the difficulti in drawing univocal conclusions, such as “, the presence of a third, minor source of TAGs might not be excluded, even if highly unlikely.”

12. References section: the whole section needs some attention. Please review the whole part carefully for formal issues. Some examples:
* Most book and conference references are lacking some spacers (commas and spaces). e.g. ref [4]: "J. Wiley & Sons2009". Also in references [15], [22], [29], [36] and maybe others.
* Please use journal abbreviations consequently. Sometimes abbreviations are used, but in many cases not.
* In some references, the family names are abbreviated. In my opinion this is bad practice, but please verify this with the journal policy. This is the case in refs [18] and [19]: J.D.J. van den Berg and K.J. van den Berg, and in [30]: J. La Nasa (the main author of this paper!).
* Ref [15]: what or who is meant with "VV.AA."?

Bibliography was thoroughly revised and the references corrected, according to the journal policy and the reviewer suggestions.

13. Figure 2b: please supply a scale on the X axis (time)

Figure 2b was modified as requested.

14. Figure 3b: please supply a scale on the X axis (time)

Figure 3b was modified as requested.

15. Figure 7: please correct the figure numeration (not "Figure 6")

Numbering was corrected.

16. Figure 8: please correct the figure numeration (not "Figure 1")

Numbering was corrected.

Reviewer #2: The manuscript is an interesting work about the use and comparison of three different types of mass spectrometry techniques (Py-GC/MS,GC/MS,HPLC/MS) as applied to the study of the chemical composition of the binder contained in some paint tubes used by Edvard Munch and to compare those data with those obtained with the same techniques on the painting "New Rays" by Edvard Munch. The authors especially showed the potential of HPLC/MS by determining the nature of the oil used as binder for the paint tubes and the novelty of this technique applied to the painting "New Rays", Edvard Munch. The obtained data could be of great interest for a broad range of scientists such as analytical chemists, conservation scientists, conservators, restorers, etc. Therefore, the manuscript could be considered to be well suitable for being published in the journal with minor review.

1- In this work several paint tubes from Munch's atelier as well as a 100 year paint sample form the painting "New Rays" of Edvard Munch were subject of a multi-analytical approach based on mass spectrometry. Based on the GC/MS results Table 2 shows the P/S (palmitic vs stearic) A/P (azelaic vs palmitic acid), and oleic vs stearic acid (O/S) parameters for each set of paint tubes and the paint sample. According to those data it is stated that the different oxidation states of the two sets of paint tubes were due to the different extents of exposure to air and light of the paint tubes without considering that the type of pigment used for the formulation of the paint highly influence the fatty acid profile and consequently the calculated parameters. Therefore, in order to better understand the calculated data in Table 2 the authors should describe in a more detailed way the selected paint tubes and the paint sample e.g. type of color and other information found on the labelling of the tube if present. These additional information should be added in the section 2.1.

We added the information found on the labels of the paint tubes in section 2.1. We also added a paragraph in section 3.1 discussing the possible influence of pigments on the oxidation of the oil media (pag.17)

minor remarks:

2- Please specify in the section 2.3.1 how much sample and HMDS was used for the Py-GC/MS analysis

The information were added.

3- Page 10 - Line 51: "10 °C/min to 280 °C, 300 °C for 2 min, 15 °C/min to 300 °C". Modify to "10 °C/min to 280 °C, 15 °C/min to 300 °C, 300 °C for 2 min".

The text was corrected.

4- Page 15 - Line 7 to 20: "The Py-GC/MS and GC/MS chromatograms of a representative sample of a Winsor & Newton and a Richard Wurm paint are reported in Figures 2 and 3, respectively. The results of the GC/MS quantitative analyses are reported in Table 2. The relative abundances of the identified TAG species in two representative samples of the paint tubes and the sample from the sketch are also reported in Table 3 and their distribution is reported as normalized histograms in Figure 4".
This part is not necessary since the results and the figures are better described and discussed in the next 3 sections.

These paragraph were eliminated, accordingly to the suggestion.

5- Page 15 - Line 43 to 46: "The chromatographic profile is characterized by the presence of monocarboxylic and dicarboxylic fatty acids: suberic, azelaic, sebacic, palmitic, oleic and stearic acids". For a better comprehension of that sentence and since you first mention monocarboxylic and then dicarboxylic acid,

it would be better to change the order to ...."palimitic, oleic, stearic, and suberic, azelaic, sebacic, respectively".

Corrected accordingly to the suggestion.

6- Page 16 - Line 22: "...between 0.5 and 3.9%...". According to the table 2 those values should be between 0.5 and 1.6%. Please check and correct the values.

The reviewer is right. The data were double checked and the text was corrected.

7- Caption of Table 3: The number of the samples 13 and 19 do not correspond to the numbering in the table 3 as well as to the text in the section 3.2 (pages 17-19). Please check the correct number of the samples.

We corrected the text.

8- There are two Figures 1 and two Figures 6. Please check and correct the numbering of the figures as well as into the text.

Done. Double checked both in the Figures file and in the text. We apologize for the confusion.

9- Page 20 - Line 22: "...whit dehydroabietic acid..." . Correct whit to with.

Corrected.

10- Page 20 - Line 51: "This results highlight...: Correct this to these.

Corrected.

Reviewer #3: The paper shows a very interesting work concerning the characterization of the triglyceride profiles in a modern paint sample using an analytical procedure based on HPLC-ESI-Q-ToF. In this study, mass spectrometry technique has been used to investigate the triacylglycerols (TAGs) content of nine paint tubes used by Edward Munch. It has been deeply and carefully studied and the aim of this contribution is clearly explained. Thus, I recommended publication after some minor revision.

In my opinion, despite the fact that there is a good INTRODUCTION the study of Tsakalof et al [1] and Manzano et al [2] should be included in the manuscript. Tsakalof demonstrates the pitfalls in the use of ratio P/S to characterize the drying oils used in painting. Very often the ratio of the corresponding chromatographic peak areas is used without finding its linearity. Tsakalof demonstrates that if the instrument response is not linear to the palmitic and stearic acids concentrations, the P/S ratio becomes dependent on sample dilution, and thus different dilutions of the same can give different P/S ratios. Since ratio P/S has been calculated to comparing the results with the HPLC-ESI-QToF analyses to check the characterization of drying oils, the linearity of the instrument must be discuss in the manuscript. This point should been taking into consideration before publishing (page 16, line 24).

The point raised by the reviewer is very important and we are aware of the work of Tsakalof and Manzano. We added their works to the reference list of the paper. In our laboratory the linearity of calibration curves is checked on a regular base and our quantitative analysis are performed at concentrations within the linearity range. P/S ratios were evaluated based on absolute quantities and not peak areas. The issue is addressed in the manuscript, page 13: "The GC/MS quantitative analysis for all the paint samples was performed using calibration curves calculated on the basis selected ion monitoring (SIM) chromatograms". We added also the information that the significant chemical parameters were calculated from the GC/MS data thanks to calibration curves (page 16, line 24 and following).

My general comments are as follows.
- In paragraph 2.4.2. High Performance Liquid Chromatography Samples preparation.
It should be include a reference about this step. It has been optimized the extraction procedure? Thus, this should be indicated in the text.

The references were added.

- In page 14, line 7, HPLC-ESI-Q-ToF must be corrected.

Done

- In page 15, line 49, the absence of polyunsaturated fatty acids for sample 19 is noted. However the observation of chromatogram, in Fig 2b, allows observing a peak of linoleic acid in this sample. In my opinion, this is one expected results. This might be justified by the use of alkyd paints since beginning 1900. According Ploeger [3], alkyd resins reduce the crosslinks for film formation which justifies remaining linoleic acid in the sample.

The peak of linoleic acid was under the quantitation limit and was then not considered in the results discussion. The presence of an alkyd resins was excluded because the GC/MS and HPLC-MS analysis results do not show the presence of typical molecular markers characteristic of such resin: aromatic components.

- Page 17, line 7, a reference should be provided.

References are provided at the end of the whole paragraph [4, 20, 35].

- Page 18, line 8, a reference should be provided. It should be include the reasons that allow confirming an oils mixture (linseed and walnut oils). It is possible to characterize a mixture of drying oils from the TAGs? If this is so, it should be clarified in the manuscript and must be highlighted by the authors.
- Page 19, line 9, for the same reasons a date should be provided. Conclusions no sufficiently argued are provided. Thus, this should be indicated in the text.

It is possible to characterize a mixture of drying oils from the TAGs if TAGS specific of a certain oil are detected. Given that the number of TAGs is much higher than the number of fatty acids, thus the profile of TAGs is much more specific than that of fatty acids. A drawback in applications to paint samples is that unsaturated TAGs survive in very low amount after curing and ageing.
Following also the suggestions of reviewer1, we modified the whole section about discussion of results, explaining how the detected oxidised TAGs can be related to walnut and linseed oil.

- The last figure must be corrected: Figure 7.

Done.

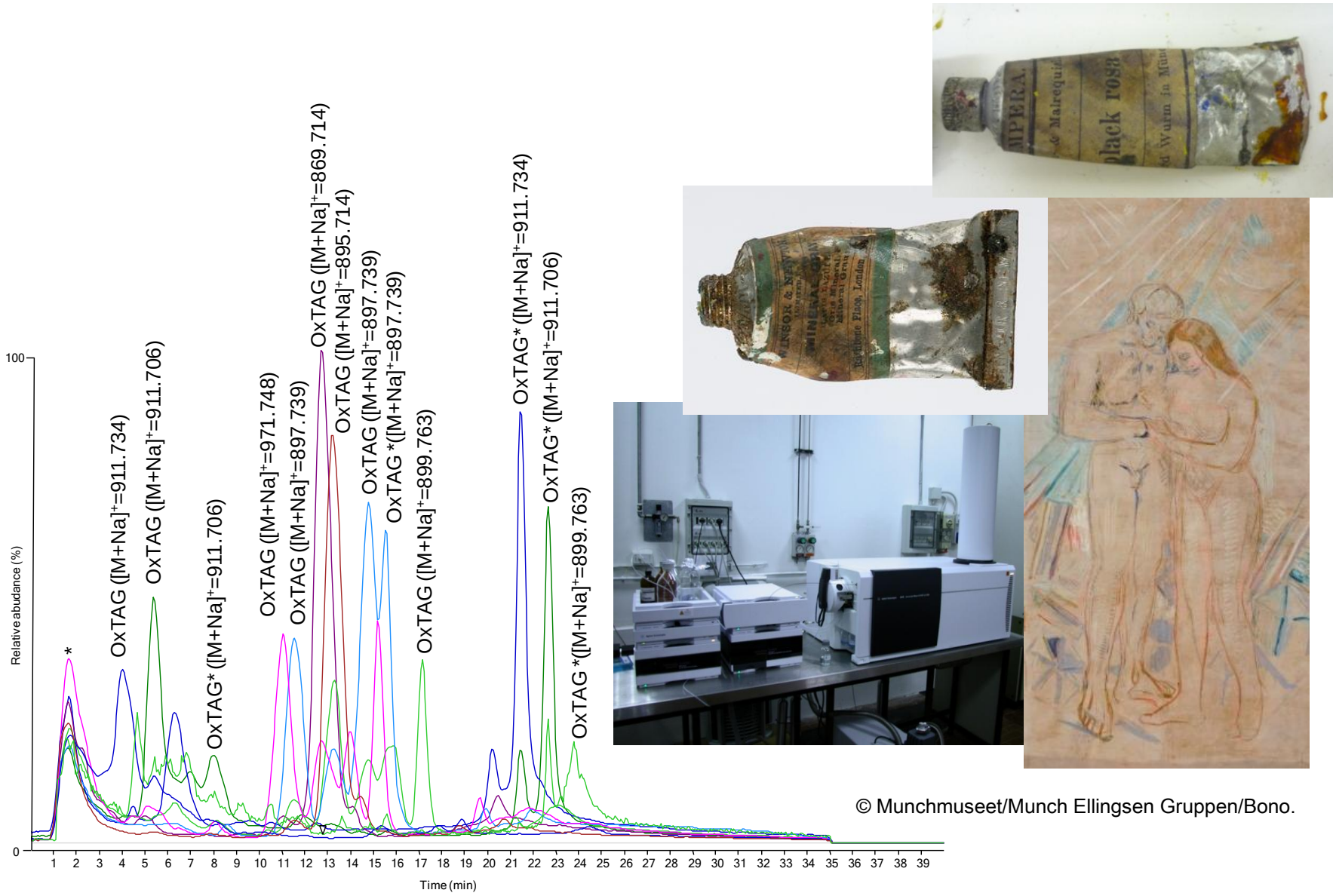
In my opinion, the contribution of the manuscript is clear. Nevertheless, in order to make a comparison with ratio P/S from GC-MS data, the authors must be to make an effort in order to corroborate the linearity of palmitic and stearic fatty acid.

The point raised by the reviewer was already addressed in the manuscript, page 13:
"The GC/MS quantitative analysis for all the paint samples was performed using calibration curves calculated on the basis selected ion monitoring (SIM) chromatograms". This implies that linearity was checked and that P/S ratios were evaluated based on absolute quantities and not peak areas. We indeed did not check the linearity of the ratio – we quantitated them separately and then calculated the ratio on the absolute quantities.

On the other hand, the interesting hypothesis about the use of palm oil should be better reasoned.

The hypothesis is better discussed in this version of the paper. Section 3.2 has been rearranged and a paragraph has been added motivating the identification of palm oil.
"Palm oil is the only plant oil to contain a relevant amounts of PPP. For this reason, even if the TAGs profile of fresh palm oil contains a larger variety of TAGs than what observed in the analysis of Richard Wurm sample 13, PPP in such high amounts can be considered a marker for palm oil. The differences between the observed triglyceride profile and the profile reported for fresh palm oil may be due to a pretreatment of the raw material before use. Palm oil diffused in Europe as an industrial material in the second half of 19th century after the exploitation of African colonials territories, and was included in the formulations of paints and soaps."

***Graphical Abstract**



Highlights

- We characterize artist's studio paint tubes (1916-1944)
- We present the first application of TAGs profiling to a 100 years old paint sample
- We apply a multi-analytical approach based on chromatography and mass spectrometry
- We obtain unambiguous conclusions on the composition of original paint materials

Novel application of liquid chromatography/mass spectrometry for the characterization of drying oils in art: elucidation on the composition of original paint materials used by Edvard Munch (1863-1944)

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Abstract

Modern oil paints, introduced at the beginning of the 20th century, differ from those classically used in antiquity in their chemical and compositional features. The main ingredients were still traditional drying oils, often used in mixtures with less expensive oils and added with several classes of additives. Consequently, detailed lipid profiling, together with the study of lipid degradation processes, is essential for the knowledge and the conservation of paint materials used in modern and contemporary art

A multi-analytical approach based on mass spectrometry was used for the study of original paint materials from Munch's atelier, owned by at the Munch Museum in Oslo. The results obtained in the analysis of paint tubes were compared with those obtained by characterizing a paint sample collected from one of the artist's sketches for the decoration of the Festival Hall of the University of Oslo (1909-1916).

Py-GC/MS was used as screening method to evaluate the presence of lipid, proteic or polysaccaridic materials. GC/MS after hydrolysis and derivatization allowed us to determine the fatty acid profile of the paint tubes, and to evaluate the molecular changes associated to curing and ageing. The determination of the fatty acid profile is not conclusive for the characterization of complex mixtures of lipid materials, thus the characterization of the triglyceride profiles was performed using an analytical procedure based on HPLC-ESI-Q-ToF.

This paper describes the first application of HPLC-ESI-Q-ToF for the characterization of the triglyceride profile in a modern paint sample, showing the potentialities of liquid chromatography in the field of lipid characterization in modern paint materials. Moreover, our results highlighted that the application of this approach can contribute to address dating, authenticity and conservation issues relative to modern and contemporary artworks.

Keywords

High performance liquid chromatography-Electrospray ionization-quadrupole-time of flight mass spectrometry; gas chromatography/mass spectrometry; modern paint; triglycerides; fatty acids; Edvard Munch

1. Introduction

The chemical characterization of micro samples of paint materials collected from artworks is often necessary for assessing the painting techniques used by artists and for the correct conservation and restoration of paintings. Moreover, the possibility to catalogue and investigate original paint materials used by an artist, recovered as atelier or studio materials with the aim to assess suppliers, type of paint, chemical composition and degradation processes, is an invaluable tool to complement the knowledge of an artist's technique, and to better preserve his artistic production. In selected cases, the comparison between atelier and artworks materials can also be exploited to address dating and authenticity issues.

In this study we use a multi-analytical approach based on different mass spectrometric analytical techniques to investigate the content of nine paint tubes used by Edvard Munch (1863-1944), a Norwegian painter who played a main role in 19th century Symbolism and in early 20th century Expressionism. Six of the tubes are oil paint from Winsor & Newton and three more tubes are from Richard Wurm, labelled as Tempera. The tubes are part of a corpus of atelier materials used in the last years of Munch's life, donated to the Munch Museum in Oslo (Norway) after his death, along with all his artworks and belongings [1].

The chemical and compositional features of modern oil paints are different from those of classical oil paint used in antiquity. The main ingredients of the first commercial paint tubes, introduced at the beginning of the 20th century, were traditional drying oils, as linseed or walnut oil, often used in mixtures with less expensive oils as castor or safflower, added with new classes of additives such as surfactants, metal soaps and dispersing agents [2-4]. Drying oils are characterized by the presence of triacylglycerols (TAGs) with a high number of unsaturated fatty acids (FAs) in the acyl chains, such as oleic, linoleic and linolenic acids. An oil is defined as "drying" when the percentage of double bonds in

the FAs in the acyl chains is higher than 65% (w/w): such composition confers to the oil the ability to chemically dry, forming a solid film upon exposure to air and light [2-5].

The identification of the lipid materials and possible additives in a paint layer, the study of the ageing processes undergone by modern oils used as binders, and the influence of the formulation on the ageing pathways and on the efficacy of conservation treatments are fundamental issues for the conservation of paintings, and represent a challenge for the analytical chemist. Consequently, lipid characterization, together with the study of lipid degradation processes, is an important research area in conservation science.

Different analytical methods have been applied for this purpose in the last decades. Infrared spectroscopy (FTIR), analytical pyrolysis coupled with gas chromatography/mass spectrometry (Py-GC/MS) and gas chromatography/mass spectrometry following a wet sample treatment are today the most common approaches for the characterization of lipid material. Moreover, the application of reversed phase high pressure liquid chromatography for the separation of complex mixtures of triglycerides in oils is gaining importance recently [6-12].

FTIR spectroscopy can be used as a fast non-invasive and non-destructive analytical technique that allows the detection of lipid materials, but in most cases not the identification of the botanical origin of aged oils [13, 14].

The coupling of analytical pyrolysis with GC/MS (Py-GC/MS) is a fast destructive analytical technique that allows the chemical characterization of lipid materials without any sample pretreatment. Nonetheless, the intrinsic non-quantitative nature of the technique does not allow the unambiguous identification of the raw source of the oil, especially in the case of mixtures. Thus, the interpretation of the pyrograms is a critical step, especially for aged materials, and requires not only a wide database of reference specimens, but also experience in interpreting the effect of the matrix. The simultaneous occurrence of different organic materials, the relative abundance of one material with respect to the others, the

presence of inorganic materials, sample morphology, and several other factors can contribute in different ways to the resulting pyrograms [15-17].

One of the most diffused analytical procedures for the identification of the botanical origin of the oil involves the use of a gas chromatographic/mass spectrometer system (GC/MS) to perform qualitative and quantitative analysis of the fatty acid profile of the lipid material after hydrolysis of ester bonds in surviving triglycerides. The literature reports a huge number of applications of GC/MS to lipid analysis in cultural heritage, entailing different types of sample pre-treatments and derivatization procedures [4, 18-20]. Moreover, different statistical treatments of the GC/MS experimental data have been proposed for the identification of the raw source of the oil. In particular, the palmitic to stearic acid ratio (P/S) is currently used to differentiate between linseed, walnut and poppyseed oils. This ratio was proposed to compare paint samples with reference materials since saturated acylic chains are supposed to be less subject to physical-chemical reactions during treatment and curing then unsaturated ones. Moreover, palmitic and stearic acids have a similar chemical reactivity, so their ratio has been hypothesized to be relatively unaltered during ageing [3, 21-23]. Nevertheless, recent studies have shown that the P/S ratio varies in time due to the different evaporation ratio of the two acids [24, 25]. Moreover, this parameter is affected by environmental contamination (both palmitic and stearic acids are known laboratory contaminants) or by the presence of other lipid sources (e.g. natural waxes, animal fat, or egg yolk), and it is generally unreliable in the case of oil mixtures [26, 27].

In this work, preliminary Py-GC/MS analysis using thermal assisted derivatization with a silanizing agent were performed on samples from Munch's paint tubes, in order to characterize the gross chemical composition of the paint, and to evaluate the presence of macromolecular species without any sample pretreatment. Secondly, we applied a validated GC/MS method to determine the fatty acid profile after a sample treatment

entailing fast microwave-assisted saponification. This was followed by a derivatization step with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA). GC/MS analysis enabled us to obtain quantitative information on the fatty acid content and on the presence of oxidation products of acylic chains [3, 28-30]. Finally, we propose to overcome the limitations deriving from the mere use of the P/S ratio to characterize oil paint samples by integrating the data on the fatty acid content with the characterization of the entire TAGs profile of the lipid material. HPLC-ESI-Q-ToF was applied for the first time to determine the triglyceride profile of the lipid fraction in a paint sample, allowing the identification of the oils used for the production of the different paint tubes.

We applied the same analytical approach used for the tubes to a paint sample from a Edvard Munch's artwork, "New Rays". The artwork is a sketch on canvas belonging to the Munch Museum, created by the artist in connection with the painted decorations for the Festival Hall (*Aula*) of the University of Oslo (Figure 1). The sketch is painted on a thick linen canvas with a white ground, and is mainly painted in one and two layers wet in wet. The Aula decorations are renowned as Munch's greatest decoration project, considering the area to decorate, the size of the paintings, and the time and effort he put into his preparing sketches, completed for the University of Oslo in 1916 [31]. The analytical results obtained on the artwork were compared to those obtained for the characterization of the paint in the atelier tubes.

2. Materials and methods

2.1 Paint samples

Nine samples from paint tubes from the atelier of Edvard Munch, used in the last period of his life, when his atelier was in Ekely (1916-1944), were analyzed. It is not to be excluded that some paint materials bought before this dates. Amongst the 9 samples, 6 were from tubes produced by Winsor & Newton (samples 11, 12, 15, 19, 27~~, 30~~), and 3 were "tempera" tubes produced by Richard Wurm (samples 7, 13, 14). The Winsor & Newton paint tubes were labeled as "Cremnitz White" (11), "Zinc White" (12), "Vermillion Pale" (15), "Mineral Grey" (19), and "Vermillion" (27). The Richard Wurm tempera tubes were labeled as "Cobalt" (7), "Organic Krapplack" (13), and "Ultramarine" (14).

The samples were obtained by squeezing the paint tubes in small vials and were partially dried at the surface. The material for the analysis was obtained sampling the bulk of the paint.

The analytical procedure was also applied to a paint sample (Woll M 946; P3) collected by the museum conservators from "New Rays", one of the sketches prepared by Munch for the decoration of the Festival Hall Aula of Oslo University (1909–1916), shown in Figure 1.

2.2 Materials and reagents

For the Py-GC/MS analysis, 1,1,1,3,3,3-hexamethyldisilazane (HMDS) was purchased from Sigma-Aldrich (U.S.).

The solvents used for the GC/MS analysis were diethyl ether, n-hexane and isooctane (HPLC grade; Sigma-Aldrich).

N,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethyl-chlorosilane used for fatty acid derivatization was purchased from Sigma-Aldrich. Fatty acid solutions were prepared in acetone, and contained lauric (4.10 µg/g), suberic (4.27 µg/g),

azelaic(3.95 µg/g), myristic (4.11 µg/g), sebacic (3.85 µg/g), palmitic acid (4.39 µg/g), oleic acid (6.32 µg/g) and stearic (6.62 µg/g) acids. All standard solutions were used to derive calibration curves. The acids were purchased from Sigma-Aldrich, purity > 99%. Tridecanoic acid (purity 99%; Sigma-Aldrich) solution in *iso*-octane, 139.91 µg/g, was used as internal standard for derivatization; hexadecane (purity 99%; Sigma-Aldrich), solution in *iso*-octane,142.00 µg/g, was used as internal standard for injection.

The solvents used for the HPLC analyses were: *iso*-propanol, *n*-hexane, chloroform and methanol (HPLC/MS grade; Fluka, U.S.).

2.3 Instruments and methods

2.3.1 Pyrolysis-Gas Chromatography/Mass Spectrometry

Analyses were performed using a multi-shot pyrolyzer EGA/PY-3030D (Frontier Lab, Japan) coupled with a 6890N gas chromatography system with a split/splitless injection port and combined with a 5973 mass selective single quadrupole mass spectrometer (Agilent Technologies, U.S.).

The samples (in the range 0.1-0.6 mg) were placed ~~in platinum~~ deactivated stainless steel sample cups on glass wool. The samples were added with 2.0 µL of HMDS and placed on top of the pyrolyzer at room temperature, and then quickly introduced in the pyrolysis chamber. Pyrolysis conditions were as follows: pyrolysis chamber temperature 550 °C, interface 280 °C. The GC injector temperature was 280 °C. The GC injection port operated in split mode and the best analytical results were obtained with a split ratio of 1:10. The chromatographic separation of pyrolysis products was performed on a fused silica capillary column HP-5MS (5% diphenyl-95% dimethyl-polysiloxane, 30 m x 0.25 mm i.d., 0.25 µm film thickness, J&W Scientific, Agilent Technologies), preceded by 2 m of deactivated fused silica pre-column with internal diameter of 0.32 mm. The chromatographic conditions for the analysis were: 32 °C for 10 min, 10 °C/min to 280 °C, ~~15 °C/min to 300 °C,~~ 300 °C

for 2 min 15 °C/min to 300 °C. The helium (purity 99.9995%) gas flow was set in constant flow mode at 1.2 mL/min.

MS parameters: electron impact ionization (EI, 70 eV) in positive mode; ion source temperature 230 °C; scan range 50-700 m/z; interface temperature 280 °C. Perfluorotributylamine (PFTBA) was used for mass spectrometer tuning. MSD ChemStation (Agilent Technologies) software was used for data analysis and peak assignment was based on the comparison with libraries of mass spectra (NIST 1.7, WILEY275).

2.3.2 Gas Chromatography/Mass Spectrometry

GC/MS instrumentation consisted of an Agilent Technologies 6890N Gas Chromatograph with a split/splitless injection port and coupled with a 5973 mass selective single quadrupole mass spectrometer.

Samples were injected in splitless mode at 280 °C. GC separation was performed on a fused silica capillary column HP-5MS (5% diphenyl-95% dimethyl-polysiloxane, 30 m x 0.25 mm i.d., 0.25 µm film thickness, J&W Scientific, Agilent Technologies). Chromatographic conditions were [28, 29]: initial temperature 80°C, 2 min isothermal, 10 °C/min up to 200 °C, 4 min isothermal for the separation of unsaturated C_{18} fatty acids and their isomers, 6 °C/min up to 280 °C, 40 minutes isothermal. The helium (purity 99.9995%) gas flow was set in constant flow mode at 1.2 mL/min. MS parameters: electron impact ionization (EI, 70 eV) in positive mode; ion source temperature 230 °C; scan range 50-700 m/z; interface temperature 280 °C. The injection volume was 2 µL.

Perfluorotributylamine (PFTBA) was used for mass spectrometer tuning.

Peak assignment was based on the comparison with libraries of mass spectra (NIST 1.7, WILEY275).

2.3.3 High Performance Liquid Chromatography/Mass Spectrometry

HPLC-ESI-Q-ToF analyses were carried out using a 1200 Infinity HPLC, coupled with a Quadrupole-Time of Flight tandem mass spectrometer 6530 Infinity Q-ToF detector by a Jet Stream ESI interface (Agilent Technologies).

The HPLC conditions were: Poroshell 120 EC-C18 column (3.0 mm x 5.0 mm, 2.7 µm particle size) with a Zorbax eclipse plus C-18 guard column (4.6 mm x 12.5 mm, 5 µm particle size); a flow rate of 0.3 mL/min, an injection volume of 1 µL and a column temperature of 45 °C. Separation was achieved using a gradient of methanol (eluent A) and *iso*-propanol (eluent B). The elution gradient was programmed as follows: 90% A for 5 minutes, followed by a linear gradient to 90% B in 25 min, then held for 5 min. Re-equilibration time for each analysis was 10 min.

The ESI operating conditions were: drying gas (N_2, purity >98%): 350 °C and 10 L/min; capillary voltage 4.5 KV; nebulizer gas 35 psig; sheath gas (N_2, purity >98%): 375 °C and 11 L/min. High resolution MS and MS/MS spectra were acquired in positive mode in the range 100-1700 m/z. The fragmentor was kept at 200 V, nozzle voltage 1000 V, skimmer 65 V, octapole RF 750 V. For the MS/MS experiments, voltages in the range 30-100 V in the collision cell were tested for Collision Induced Dissociation (CID), to obtain information on fragmentation pathways of selected analytes. The MS/MS spectra presented in the text were obtained at 50 V. The collision gas was nitrogen (purity 99.999%). The data were collected by auto MS/MS acquisition with an MS scan rate of 1.03 spectra/sec and an MS/MS scan rate of 1.05 spectra/sec; only one precursor was acquired per cycle (relative threshold 0.010%). The mass axis was calibrated daily using the Agilent tuning mix HP0321 (Agilent Technologies). MassHunter® Workstation Software (B.04.00) was used to carry out mass spectrometer control, data acquisition, and data analysis.

The structures of individual glycerides were identified by interpreting their tandem mass spectra and the identification of the oils was obtained by comparison with a set of

reference oils characterized in the same conditions [32]. The characterization of the oxidized triglycerides is reported in Table 1.

2.4 Sample treatment

2.4.1 Gas chromatography/mass spectrometry samples preparation

For the GC/MS analyses ~1 mg of each sample was subjected to saponification assisted by Milestone (U.S.) microwaves Ethos One (power 200 W) with 300 µL of KOH_{ETOH} 10% wt at 80 °C for 60 min. In order to maximize the extraction yields, two solvents were used for liquid/liquid extraction: the neutral compounds were extracted with *n*-hexane; the residual solution was acidified with hydrochloric acid (6 *M*) and then the acidic compounds were extracted with diethyl ether (400 µL, three times). The two extracts (neutral + acid fraction) were combined in order to analyze them in a single chromatographic run, evaporated to dryness under nitrogen stream and subjected to derivatization with 20 µL of *N*,*O*-bistrimethylsilyl-trifluoroacetamide , 150 µL of *iso*-octane and 5 µL of tridecanoic acid solution at 60 °C for 30 min. 5 µL of hexadecane solution were added just before injection [3, 28-30].

2.4.2 High performance liquid chromatography samples preparation

For the HPLC/MS analyses, 0.1-0.8 mg of each paint sample were subjected to extraction assisted by Milestone microwaves Ethos One (power 600 W) with 300 µL of a chloroform-hexane (3:2) mixture at 80 °C for 25 min. The solvents for the extraction and the best microwave conditions were adapted from [33, 34].

The extracts were dried under a nitrogen stream, diluted with 600 µL of elution mixture and filtered on a 0.45 µm PTFE filter (Grace Davison Discovery Sciences, U.S.) just before injection.

2.5 Quantitative analysis

The GC/MS quantitative analysis for all the paint samples was performed using calibration curves calculated on the basis selected ion monitoring (SIM) chromatograms. The selected ions for the SIM acquisition were: lauric acid m/z 117;257, suberic acid m/z 169;303, azelaic acid m/z 149;317, myristic acid m/z 117;285, sebacic acid m/z 149;331, palmitic acid m/z 117;3131, oleic acid m/z 117;339, stearic acid m/z 117;341).

For the HPLC-ESI-Q-ToF analysis, relative abundances were calculated on the basis of extract ion chromatograms, considering the isotopic distribution of the molecular species, and normalized to 100% for each chromatogram.

3. Results and discussion

This section reports on and discusses the results obtained by Py-GC/MS, GC/MS and HPLC-ESI-Q-ToF techniques for the analysis of Winsor & Newton and Richard Wurm paint tubes samples and for the sample from Munch's sketch. ~~The Py-GC/MS and GC/MS chromatograms of a representative sample of a Winsor & Newton and a Richard Wurm paint are reported in Figures 2 and 3, respectively. The results of the GC/MS quantitative analyses are reported in Table 2. The relative abundances of the identified TAG species in two representative samples of the paint tubes and the sample from the sketch are also reported in Table 3 and their distribution is reported as normalized histograms in Figure 4.~~

3.1 Gas chromatographic analyses

The Py-GC/MS chromatogram of sample 19 from a Winsor & Newton paint tube is reported in Figure 2a. The pyrolysis profile is characterized by the presence of the molecular markers of a drying oil: fatty acids and dicarboxyilic acids with a relevant amount of nonandioic acid (azelaic acid). The Py-GC/MS chromatograms of all the samples from Winsor & Newton paint tubes are characterized by a very similar profile to that presented in the figure.

Figure 2b shows the GC/MS chromatogram obtained for sample 19. The chromatographic profile is characterized by the presence of monocarboxylic and dicarboxylic fatty acids: palmitic, oleic, stearic, and suberic, azelaic, sebacic~~, palmitic, oleic and stearic~~ acids, respectively. The presence of dicarboxylic acids with 8, 9 and 10 carbon atoms, and the absence of polyunsaturated fatty acids is characteristic of an oxidized lipid binder, as dicarboxylic acids are formed during curing and ageing as oxidation products of polyunsaturated acylic chains [3]. Intermediate oxidation products are also observed, as hydroxy-acids with 18 carbon atoms in the acyl chain.

The samples from the five other Winsor & Newton tubes have the same qualitative composition, but are characterized by a variable amount of dicarboxylic acids and of oleic acid (C18:1). The total percentage content of dicarboxylic acids (ΣD%) is in the range 19.0-69.8 %, highlighting that the degree of oxidation and curing is different among the investigated samples.

Table 2 reports some significant chemical parameters calculated from the GC/MS data, thanks to calibration curves: palmitic vs. stearic acid (P/S), azelaic vs. palmitic acid (A/P), and oleic vs. stearic acid (O/S), compared with literature data relative to reference oils used as raw materials in modern oil media [4, 20, 35, 36]. The A/P parameter for Winsor & Newton samples ranges between 0.5 and 1.9, compatibly with an partially dried oil paint [4].

The ratio between palmitic and stearic acid (P/S) also shows a relevant variability in the six Winsor & Newton samples, ranging from 1.1 to 3.6. This range overlaps with the intervals reported to be characteristic of several raw materials used in the formulation of modern and contemporary oil paints.

The Py-GC/MS chromatogram of the Richard Wurm paint sample 13 is reported in Figure 3a. Even if the paint tube was labeled as “tempera”, no protein markers are observed in the pyrogram, which is characterized by the presence of a relevant amount of dicarboxylic acids, markers of a drying oil. The three Richard Wurm paint samples were characterized by similar pyrolytic profiles.

The gas chromatographic profiles of Richard Wurm paint tubes, obtained after saponification and derivatization, are characterized by palmitic, oleic, and stearic acids as the most abundant monocarboxilic acids, and by the presence of suberic, azelaic and sebacic dicarboxylic acids. The GC/MS chromatogram of sample 13 is shown in Figure 3b, and the quantitative parameters for each sample are reported in Table 2.

The Richard Wurm paint tubes were characterized by a degree of oxidation/curing comparable to that of Winsor & Newton paint tubes, with an A/P ratio in the range 0.5-1.9. The P/S ratio is in the range 4.5-5, highlighting the presence in the formulation of an oil with a relatively high palmitic acid content. The traditional drying oils used by artists before the diffusion of industrial paint materials, such as linseed oil, walnut oil and poppyseed oil, all have a P/S reported ratio usually below 4.0. Cotton oil and palm oil are characterized, on the other side, by an high content of palmitic acid [4, 20, 35].

The different oxidation states displayed by the fatty acid profiles in both the sets of paint samples can be explained with the fact that the paints in the tubes have undergone different extents of exposition to air and light, depending on the time of use of the tube and the amount of residual paint inside it. This may lead to the observed variability in the composition, especially for what concerns the amount of dicarboxylic acids and of residual oleic acid. In addition, it is well known that the composition of pigments has also an influence on the oxidation of oil paints, and in particular some cations contained in inorganic pigments can catalyze reactions with oxygen. These phenomena have likely played a role in producing the observed differences in the A/P values between paints from the same manufacturer. As an example, the Richard Wurm sample 13 , which contains an organic lake (label ""Organic Krapplack"), was characterized by an A/P ratio of 0.5, notably lower than that of Richard Wurm samples 7 and 14, which contain inorganic pigments. On the other side, it is not possible to highlight evident clear correlation between the degree of oxidation and the pigment composition for the samples of the Winsor & Newton group, and it cannot be excluded that binders and additives in tubes from the same trademark have different formulations.

We can conclude that the high variability of the fatty acid profiles in all the analyzed paint tubes and the possible presence of mixtures of different oils does not allow the

identification of the botanical origin of the oils used for the production of the paint tubes on the basis of the fatty acid profile.

3.2 HPLC-ESI-Q/ToF of triglycerides

In order to identify the lipid source used for the production of the Winsor & Newton paint tubes, HPLC/MS analysis of their triglyceride content was performed. The relative abundances of TAGs are shown in Figure 4 and reported in Table 3. The HPLC/MS extract ion chromatograms of the Winsor & Newton sample 19 are shown in Figure 5 ~~the relative abundances of TAGs are reported in Table 3~~ an example.

The triglyceride profile of sample 19 features the presence of OLS (m/z 907.776, $[M+Na]^+$), which is a specific marker of walnut oil [32]. Moreover, LnLnP (m/z 873.704, $[M+Na]^+$) was detected, which is typical of linseed or walnut oil [32]. The simultaneous presence of these two oils account for the qualitative TAG composition of sample 19: ~~of~~ LnLnLn (m/z 895.669, $[M+Na]^+$), LnLnL (m/z 897.690, $[M+Na]^+$), ~~LnLnP (m/z 873.704, $[M+Na]^+$),~~ LnLL (m/z 899.705, $[M+Na]^+$), OOP (m/z 881.776, $[M+Na]^+$), and LLL (m/z 901.718, $[M+Na]^+$) ~~and LLO (m/z 903.744, $[M+Na]^+$). All these TAGs~~ are present in both walnut and linseed oils [32].

The presence of LOP (m/z 879.731, $[M+Na]^+$), LLO (m/z 903.744, $[M+Na]^+$), OOL (m/z 905.760, $[M+Na]^+$), ~~OLS (m/z 907.776, $[M+Na]^+$)~~ and OOS (m/z 909.830, $[M+Na]^+$) can be ascribed to ~~specific markers of~~ walnut oil, ~~and of OOP (m/z 881.776, $[M+Na]^+$), and~~ while that of OSP (m/z 883.788, $[M+Na]^+$) to ~~characteristic of~~ linseed oil.

By a semi-quantitative point of view, the high relative abundance of LnLnL and LnLL in the sample are consistent with the high content of both linseed and walnut oils in terms of these TAGs. The relatively high amount of OLS in the sample's extract is harder to rationalize. Nonetheless, summarizing, although the simultaneous presence of other oils in minor amounts cannot be ruled out, TAGs profiling strongly suggests ~~, indicates~~ that the

lipid material used in the preparation of paint tube 19 is a mixture containing ~~both~~ linseed and walnut oils.

The TAGs profiles of the other samples from Winsor & Newton paint tubes, namely 11, 12, 15 and 27 ~~and 30~~, are characterized by the presence of hydroxylated acylglycerols deriving from the oxidation of triglycerides containing unsaturated acyl substituents, while not oxidized TAGs are absent in these paint tubes. The triglyceride profiles of this group of samples are very similar each other and, as an example, the extract ion chromatograms obtained for paint tube 11 are reported in Figure 6.

The oxidized TAGs identified in these five samples are characterized by the presence of a number of residual double bonds compatible with the oxidation of triglycerides containing 3 double bonds in the acyl chain. ~~The presence of these oxidized TAGs can be related to the presence of oxidized linseed and walnut oils, which were identified in the less oxidized sample 19.~~

The three main oxidized TAGs visible in Figure 6 are characterized by the raw formulas $C_{53}H_{100}O_7$ (m/z 869.7), $C_{55}H_{100}O_7$ (m/z 895.7), and $C_{55}H_{100}O_8$ (m/z 911.7). The study of their CID (see Table 1) allowed us to identify them as $C18:2_{1OH}$, P, P; $C18:2_{1OH}$, O, P; and $C18:2_{1OH}$, $C18:1_{1OH}$, P. Considering hydration of the double bonds as one of the typical ageing processes undergone by TAGs, the structure of the original acyl chains can be deduced. Thus, these oxidized TAGs originated from the three TAGs LnPP, LnOP, and LnLP (no exact determination of the sn position was performed at this stage of investigation):. LnPP is specific for linseed oils while LnOP of walnut oil. LnLP is typical of both linseed and walnut oils. On the basis of this interpretation of the origin of the oxidised TAGs, we hypothesise that the also the Winsor & Newton paint in tubes 11, 12, 15 and 27 was produced using linseed and walnut oils, as that in sample 19, even if in these samples original TAGs related to these oils could not be detected.

The HPLC/MS characterization of the triglyceride composition was also performed for the extract of the samples from the Richard Wurm paint tubes. The HPLC extract ion chromatograms of Richard Wurm paint sample 13 are reported in Figure 7 and the TAGs relative abundances in Table 3.

The HPLC profile is characterized by the presence of PPP (m/z 829.716, $[M+Na]^+$), PPS (m/z 857.729, $[M+Na]^+$) and PSS (m/z 885.871, $[M+Na]^+$) as main triglycerides. These TAGs are typical of palm oil [33, 37, 38]. Even if these triglycerides can be related to the presence of egg yolk, the Py-GC/MS analyses allowed us to exclude the presence of egg proteins and the use of this material in the paint tube formulations.

Palm oil is the only plant oil to contain a relevant amounts of PPP. For this reason, even if the TAGs profile of fresh palm oil contains a larger variety of TAGs than what observed in the analysis of Richard Wurm sample 13, PPP in such high amounts can be considered a marker for palm oil. The differences between the observed triglyceride profile and the profile reported for fresh palm oil may be due to a pretreatment of the raw material before use. Palm oil diffused in Europe as an industrial material in the second half of 19th century after the exploitation of African colonials territories, and was included in the formulations of paints and soaps.

The analysis also reveals the presence of lower amounts of LnLnLn (m/z 895.669, $[M+Na]^+$), LnLnL (m/z 897.690, $[M+Na]^+$), LnLnP (m/z 873.704, $[M+Na]^+$), LnLL (m/z 899.705, $[M+Na]^+$), PLnP (m/z 853.719, $[M+Na]^+$), OOP (m/z 881.770, $[M+Na]^+$), OSP (m/z 883.788, $[M+Na]^+$), suggesting the presence of linseed oil and OOS (m/z 909.830, $[M+Na]^+$), and OLS (m/z 907.776, $[M+Na]^+$) that could derive from the possible presence of a minor source of triglycerides. ~~characteristic TAGs of linseed oil.~~

The TAG profiles show that Richard Wurm paint tube 13 contains a lipid mixture composed by linseed and palm oils: the high amount of the markers of palm oil with respect to those of linseed oil observed in the chromatograms in Figure 7 can be due to

the original recipe of the paint material or can be related to the nature of the TAGs in palm oil. Being PPP, PPS and PSS saturated TAGs, they did not took part in the reticulation processes or in the photo-oxidation processes significantly, and were thus relatively more available for extraction with respect to the highly unsaturated markers of linseed oil. The presence of palm oil was also ascertained in paint tubes samples 7 and 14, that were characterized by similar triglycerides compositions. The presence of palm oil, characterized by an high amount of triglycerides containing palmitic acid, accounts for the high P/S values observed in the GC/MS analysis of fatty acids for the Richard Wurm paint tubes.

Due to the non siccative nature of palm oil, its exploitation in a paint material can only be explained taking into account that the triglycerides and fatty acid composition of palm oil and egg yolk, typically used for the preparation of tempera, are similar and characterized by the presence of triglycerides with an high amount of saturated TAGs [39]. Thus, we can hypothesize that the producer used a combination of linseed and palm oils in order to prepare a commercial material characterized by similar proprieties to those of egg yolk. This hypothesis also accounts for the “tempera” label on the Richard Wurm tubes.

3.3 Analysis of the Edvard Munch’s sketch

The proposed combined analytical approach was applied to a paint sample from the painted sketch by Edvard Munch *New Rays*, owned by the Munch Museum (Oslo).

The preliminary Py-GC/MS analysis of the sample showed presence of the markers of a drying oil (fatty acids and dicarboxylic acids) while highlighted the absence of proteins.

The GC/MS quantitative analysis results are reported in Tables 2 and 3. The qualitative profile of the paint sample is similar to those of the paint tubes samples, being characterized by the presence of suberic, azelaic, sebacic, palmitic, oleic and stearic acids as main fatty acids. The sample also contains traces of terpenoid compounds with

abietadiene skeleton, whith dehydroabietic acid as most abundant. These compounds are known molecular markers of a *Pinaceae* resin [40].

The A/P ratio (1.3) and the total amount of dicarboxylic acids (49.7%) observed in this sample are characteristic of an oxidized drying oil, and the P/S ratio is 2.5. To better characterize the lipid materials used by the artist in his sketch, HPLC analysis was performed. The chromatographic profile obtained for the unoxidized triglycerides is reported in Figure ~~7a~~8a, and the resulting TAGs distribution in Table 3. The main triglycerides are PPP (m/z 829.716, $[M+Na]^+$), PPS (m/z 857.729, $[M+Na]^+$) and PSS (m/z 885.871, $[M+Na]^+$), characteristic of palm oil. The lipid material in the sample is in an advanced oxidation state, as demonstrated by the presence of oxidized triglycerides featuring inclusion of oxygen in the acylic chains (Figure ~~7b~~8b), which may be related to the presence of linseed oil as raw material.

~~This~~ These results highlight a perfect match between the composition of the paint sample from *New Rays* and the content of the Richard Wurm paint tubes from Munch's atelier.

4. Conclusions

The new analytical approach based on the integration of the HPLC-ESI-Q-ToF analysis of the triglyceride fraction proved to improve the reliability of the characterization of an oil binder with respect to the classical approaches based on Py-GC/MS and GC/MS.

The gas chromatographic methods allowed us to assess the presence of a lipid material in the paint samples. The use of liquid chromatography coupled with mass spectrometry allowed us to clarify the ~~nature~~ composition of the oil components and to ~~univocally identify~~ hypothesise the botanical origin of the lipid materials contained in the paint tubes. In details:

- the Py-GC/MS analysis of the Winsor & Newton paint tubes allowed us to identify the presence of a drying oil. The lipid materials sampled from these paint tubes were

characterized by different oxidation states, and the GC/MS quantitative analysis showed a P/S ratio in the range 1.1-3.6. The HPLC analysis allowed us to characterize the lipid materials as a mixture which contains walnut and linseed oils (the presence of other minor additives shall in principle not be excluded). These results highlight the limited validity of P/S ratio for the identification of the botanical origin of an oil: the presence of metal soaps or other minor sources of fatty acids could lead to erroneous interpretations of the origin of the material;

- the Py-GC/MS analysis of the Richard Wurm “tempera” paint tubes showed the presence of the molecular markers of a drying oil, while the molecular markers characteristic of proteins were absent. The quantitative GC/MS analysis yielded a P/S ratio in the range 4.5-5.0, which is higher than the typical values of traditional drying oils (linseed, walnut or poppyseed oils). The HPLC-MS chromatograms were characterized by the presence of markers of palm and linseed oils. The presence of palm oil, characterized by an high amount of palmitic acid, accounts for the high P/S ratio determined by GC/MS. The high similarities of the triglyceride composition of palm oil and the binding medium based on egg yolk suggests that the producer used a combination of linseed and palm oil in order to obtain a paint material with physical proprieties similar to those of a egg based tempera. Also in this case, the presence of a third, minor source of TAGs might not be excluded, even if highly unlikely.

The application of an innovative analytical procedure allowed us to overcome the limitations related to the use of the fatty acid profile for the identification of a lipid material, and to unambiguously identify the botanical origin of the lipid materials used for the production of the paint tubes.

The analytical approach was successfully applied for the characterization of a paint sample collected from the sketch *New Rays*, proving the efficiency of the combined analytical approach in the characterization of lipid materials in case-studies. In the paint

sample, the characteristic triglycerides of linseed oil and palm oil were detected. The analytical results allowed us to relate the original paint materials of the artist's atelier with those applied in his artwork. The final results of this study clearly highlights that this new approach is a powerful tool for solving attribution problems.

In conclusion, the application of HPLC-MS lipid profiling represents a step forward in the analysis of lipid materials in paint samples, overcoming the issues related to use of the classic analytical approaches in the field of cultural heritage.

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Table

Table 1 – Observed oxidized TAGs in the HPLC-ESI-Q-ToF chromatograms of the oxidize pain Winsor & Newton paint tubes. Acyl substituent abbreviations: $C_{\text{n° of carbon atoms: n° of unsaturation, n° of OH}}$; O: oleyl ($C_{18:1}$); S: stearyl ($C_{18:0}$); P: palmityl ($C_{16:0}$).

TAGs	**MS^1**		**MS^2**	
Assigned structure	*Precursor ion*	*Formula*	*Product ions and fragmentation pattern*	
$C_{18:1,2OH}$ $C_{18:1,1OH}$ $C_{18:1,1OH}$	*971.7*	$[C_{57}H_{104}O_{10}+Na]^+$	971.7 857.7 673.7 657.7 337.2 321.2 135.1	$[M+Na]^+$ $[M-C_7H_{13}O+Na]^+$ $[M-C_{18}H_{33}O_3+Na]^+$ $[M-C_{18}H_{33}O_4+Na]^+$ $[C_{18}H_{33}O_4+Na]^+$ $[C_{18}H_{33}O_3+Na]^+$ $[C_7H_{13}O+Na]^+$
$C_{18:2,1OH}$ $C_{18:1,1OH}$ P	*911.7*	$[C_{55}H_{100}O_8+Na]^+$	*913.7* *655.5* *615.5* *613.5* *321.2*	$[M+Na]^+$ $[M-C_{16}H_{31}O_2+Na]^+$ $[M-C_{18}H_{31}O_3+Na]^+$ $[M-C_{18}H_{33}O_3+Na]^+$ $[C_{18}H_{33}O_3+Na]^+$
$C_{18:2,1OH}$ O P	*895.7*	$[C_{55}H_{100}O_7+Na]^+$	*895.7* *639.5* *613.5* *599.5* *577.5* *319.2*	$[M+Na]^+$ $[M-C_{16}H_{31}O_2+Na]^+$ $[M-C_{18}H_{33}O_2+Na]^+$ $[M-C_{18}H_{31}O_3+Na]^+$ $[M-C_{18}H_{31}O_3]^+$ $[C_{18}H_{31}O_3+Na]^+$
$C_{18:1,1OH}$ O P	*897.7*	$[C_{55}H_{102}O_7+Na]^+$	*897.7* *641.5* *615.5* *600.5* *577.5* *321.2*	$[M+Na]^+$ $[M-C_{16}H_{31}O_2+Na]^+$ $[M-C_{18}H_{33}O_2+Na]^+$ $[M-C_{18}H_{33}O_3+Na]^+$ $[M-C_{18}H_{33}O_3]^+$ $[C_{18}H_{33}O_3+Na]^+$
$C_{18:2,1OH}$ S P	*897.7*	$[C_{55}H_{102}O_7+Na]^+$	*897.7* *641.5* *613.5* *599.5* *579.5* *319.2*	$[M+Na]^+$ $[M-C_{16}H_{31}O_2+Na]^+$ $[M-C_{18}H_{35}O_2+Na]^+$ $[M-C_{18}H_{31}O_3+Na]^+$ $[M-C_{18}H_{31}O_3]^+$ $[C_{18}H_{31}O_3+Na]^+$
S $C_{18:1,1OH}$ P	*899.7*	$[C_{55}H_{104}O_7+Na]^+$	*899.7* *643.5* *615.5* *601.5* *579.5* *321.2*	$[M+Na]^+$ $[M-C_{16}H_{31}O_2+Na]^+$ $[M-C_{18}H_{35}O_2+Na]^+$ $[M-C_{18}H_{33}O_3+Na]^+$ $[M-C_{18}H_{33}O_3]^+$ $[C_{18}H_{33}O_3+Na]^+$
$C_{18:2,1OH}$ P P	*869.7*	$[C_{53}H_{100}O_7+Na]^+$	*869.7* *613.5* *573.5* *551.5* *319.2*	$[M+Na]^+$ $[M-C_{16}H_{31}O_2+Na]^+$ $[M-C_{18}H_{33}O_3+Na]^+$ $[M-C_{18}H_{33}O_3]^+$ $[C_{18}H_{31}O_3+Na]^+$

Table 2 – Characteristic parameters calculated from the fatty acid profile of Winsor & Newton and Richard Wurm paint tubes samples based on GC/MS: palmitic vs. stearic acid (P/S), azelaic vs. palmitic acid (A/P), and oleic vs. stearic acid (O/S). The paint tubes data are compared to those reported in literature for reference oils used as ingredients of modern oil paints [5, 20, 35, 36]. The parameters are compared to the one from the Munch's sketch *New Rays*.

		Parameters			*Unsaturated fatty acids (%)*			
		P/S	***A/P***	***O/S***	***Oleic***	***Linoleic***	***Linolenic***	***Other***
Fresh Oils	*Castor*	1.0	-	7.0	7	3	-	*Ricinoleic* 87
	Coconut	3.2	-	1.9	29	9	-	-
	Cotton	7.7	-	8.3	25	45	-	-
	Linseed	1.5	-	4.7	19	24	47	-
	Oiticica	0.6	-	1.4	14	21	-	*Licanic* 48
	Palm	5.6	-	6.9	46	9	0.3	-
	Peanut	-	-	-	54	26	-	-
	Poppyseed	4.0	-	6.1	12	13	58	-
	Rapeseed	1.0	-	15	15	15	8	*Erucic* 60
	Safflower	2.3	-	2.6	18	69	4	-
	Soybean	2.8	-	2.1	23	54	8	-
	Sunflower	1.4	-	4.6	23	65	-	-
	Tall	-	-	-	46	41	3	*Rosin* 8
	Tung	1.0	-	2.5	5	3	-	*Eleostearic* 87
	Walnut	2.0	-	11.9	23	52	18	-
Paint Tubes	*W&N 11*	1.1	0.5	1.0	26	-	-	-
	W&N 12	2.5	0.7	0.5	9	-	-	-
	W&N 15	2.0	1.6	0.3	4	-	-	-
	W&N 19	3.6	0.8	0.3	4	-	-	-
	W&N 27	2.8	1.4	1.2	12	-	-	-
	RW 7	4.5	1.9	0.2	1	-	-	-
	RW 13	5.0	0.5	0.8	5	-	-	-
	RW 14	4.9	1.3	0.8	8	-	-	-
	New Rays	2.5	1.3	0.3	3	-	-	-

Table 3 – TAGs profiles (%) of the Winsor & Newton (sample 13) and Richard Wurm (sample 19) paint tubes acquired by HPLC-ESI-Q-ToF. Acyl substituent abbreviations; Ln: linolenyl ($C_{18:3}$); L: linoleyl ($C_{18:2}$); O: oleyl ($C_{18:1}$); S: stearyl ($C_{18:0}$); P: palmityl ($C_{16:0}$). The profiles of the paint tubes are compared to the one of the Munch's sketch.

	Relative abundances (%)		
TAGs	*Winsor & Newton (19)*	*Richard Wurm (13)*	*New Rays*
LnLnLn	7.1	3.9	-
LnLnL	9.5	4.8	-
LnLnP	7.1	4.9	-
LnLL	24.3	1.6	-
LLL	3.8	-	-
LLO	6.6	-	-
LOP	6.7	-	-
PPP	-	33.5	60.3
OOL	6.5	-	-
OOP	4.3	5.2	-
OLS	15.3	3.9	-
PLnP	-	2.0	-
PPS	-	22.5	23.7
OSP	7.1	3.1	-
OOS	1.3	3.9	-
PSS	-	10.6	15.9

Figure
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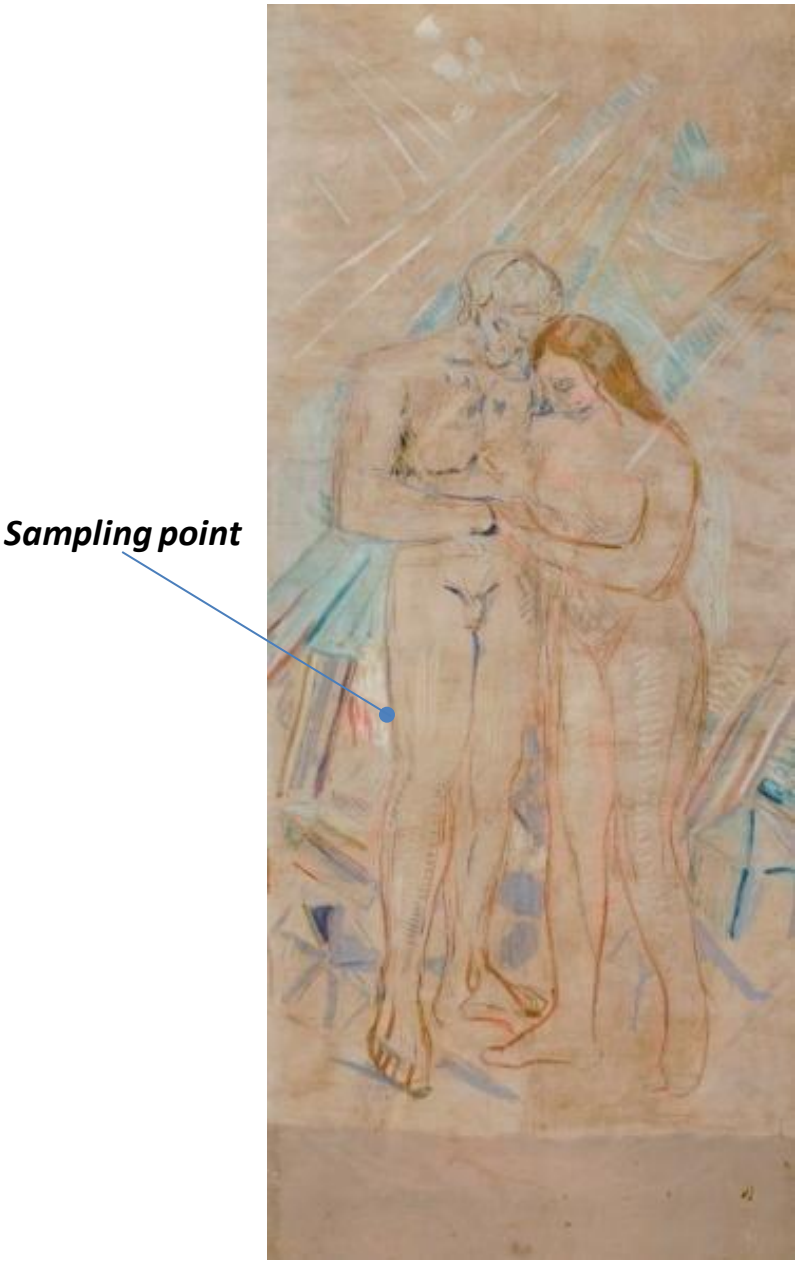

Figure 1 –Edvard Munch, New Rays (1909–1916) sampling point ©Munchmuseet/Munch Ellingsen Gruppen/Bono.

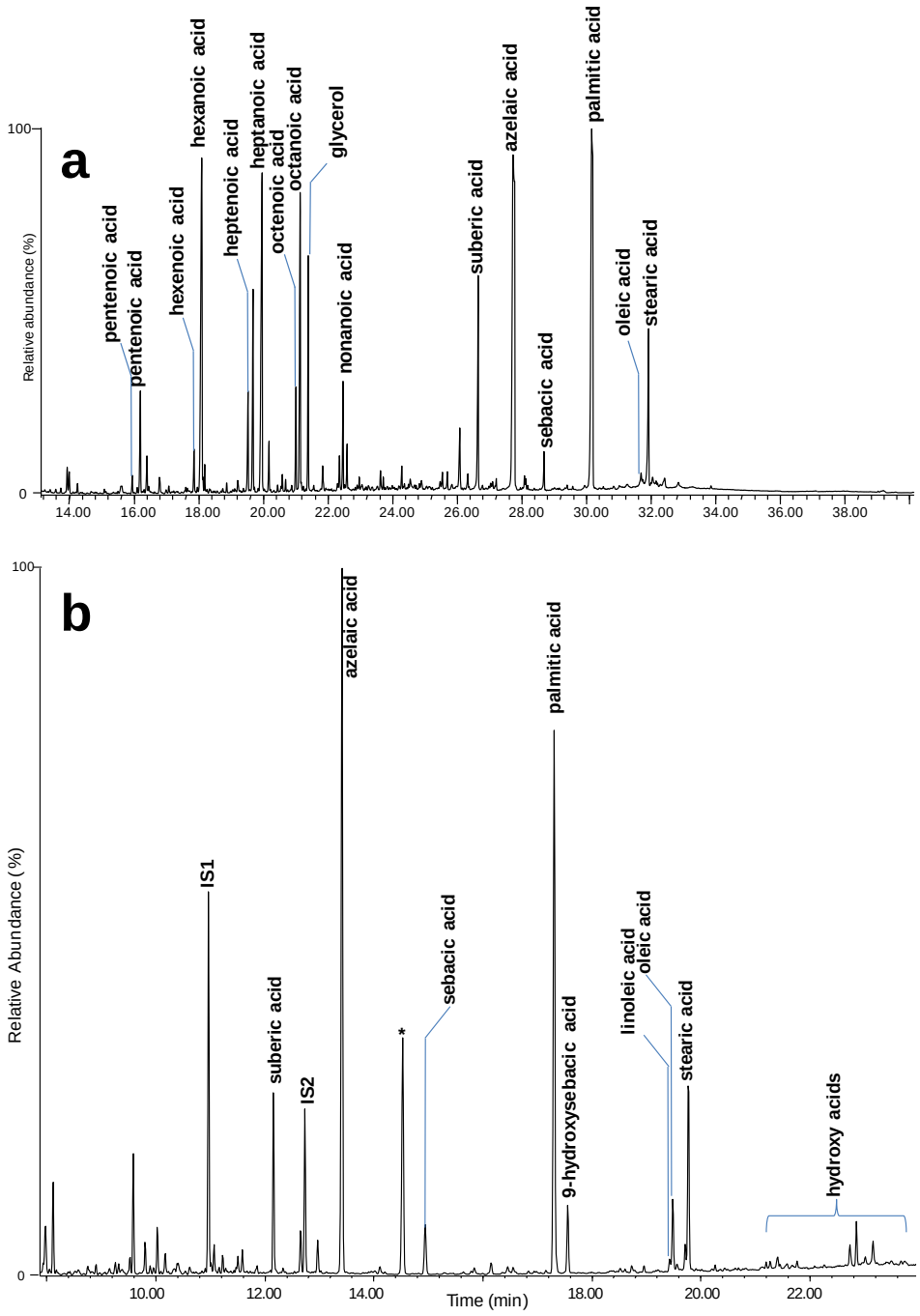

Figure 2 – Py-(HMDS)-GC/MS (a) and GC/MS (b) chromatograms of the Winsor & Newton paint sample 19: IS1: hexadecane, IS2: tridecanoic acid; *:contamination.

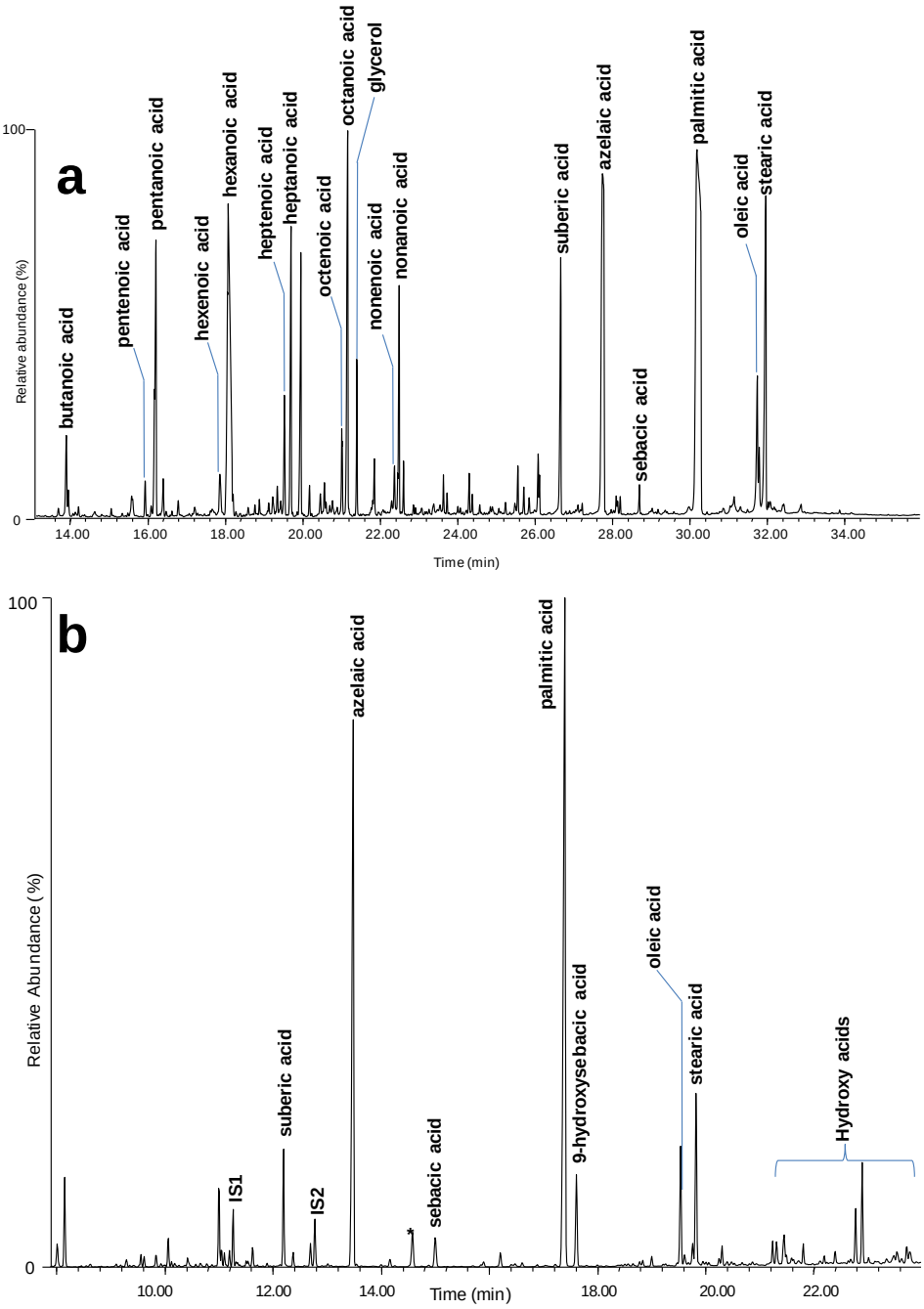

Figure 3 – Py-(HMDS)-GC/MS (a) and GC/MS (b) chromatograms of the Richard Wurm paint sample 13: IS1: hexadecane, IS2: tridecanoic acid; *:contamination.

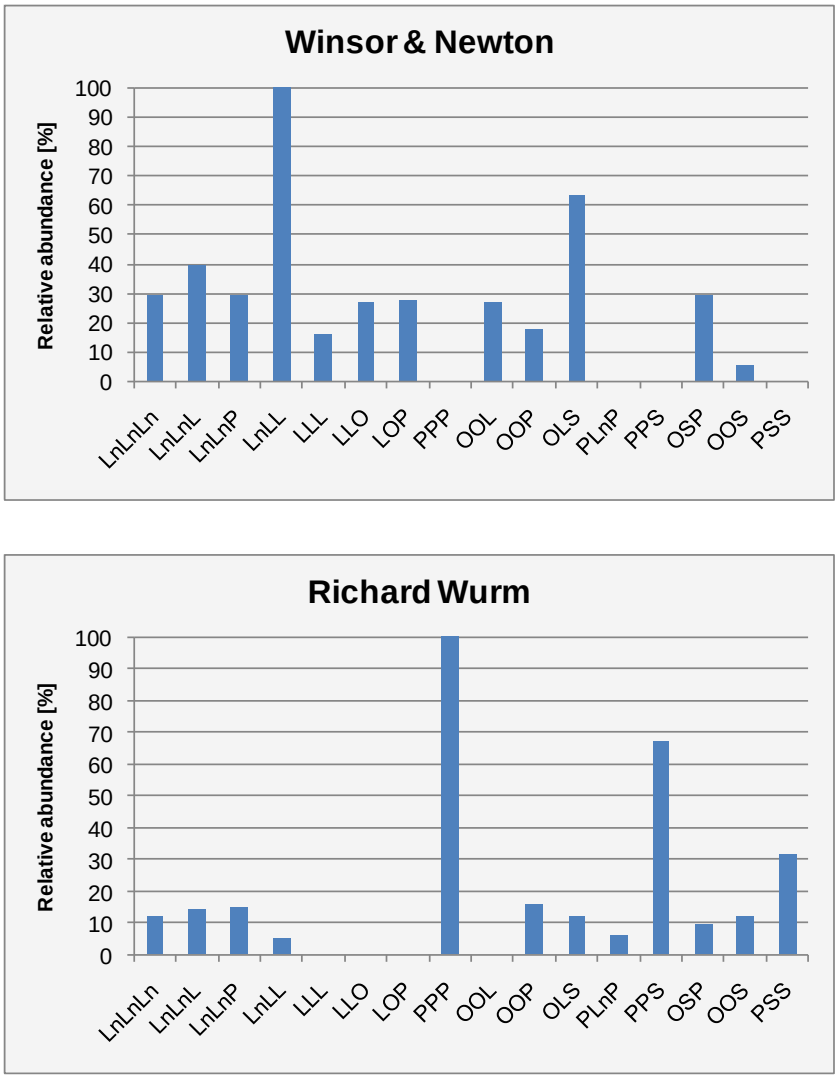

Figure 4 – TAGs distribution in the Winsor & Newton (sample 19) and the Richard Wurm (sample 13) paint tubes.

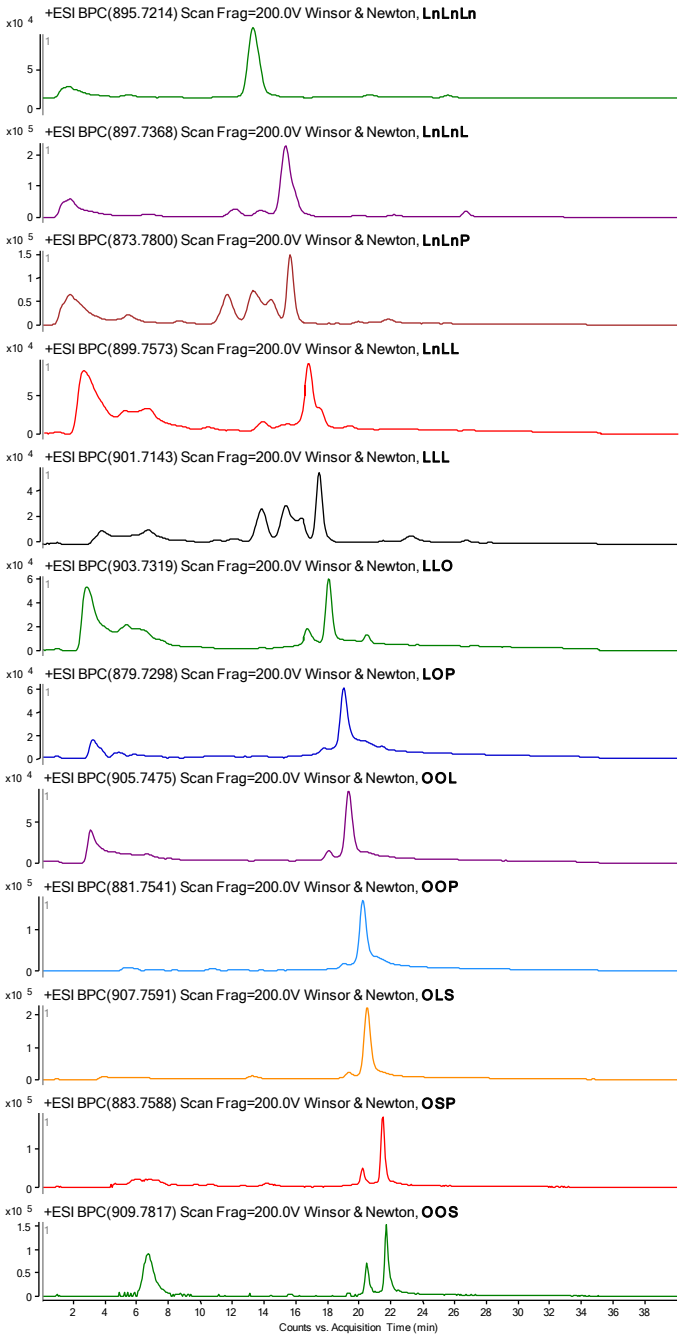

Figure 5 –HPLC-ESI-Q-ToF extract ion chromatograms referring to 12 identified TAGs species in the Winsor & Newton paint tube (sample 19).

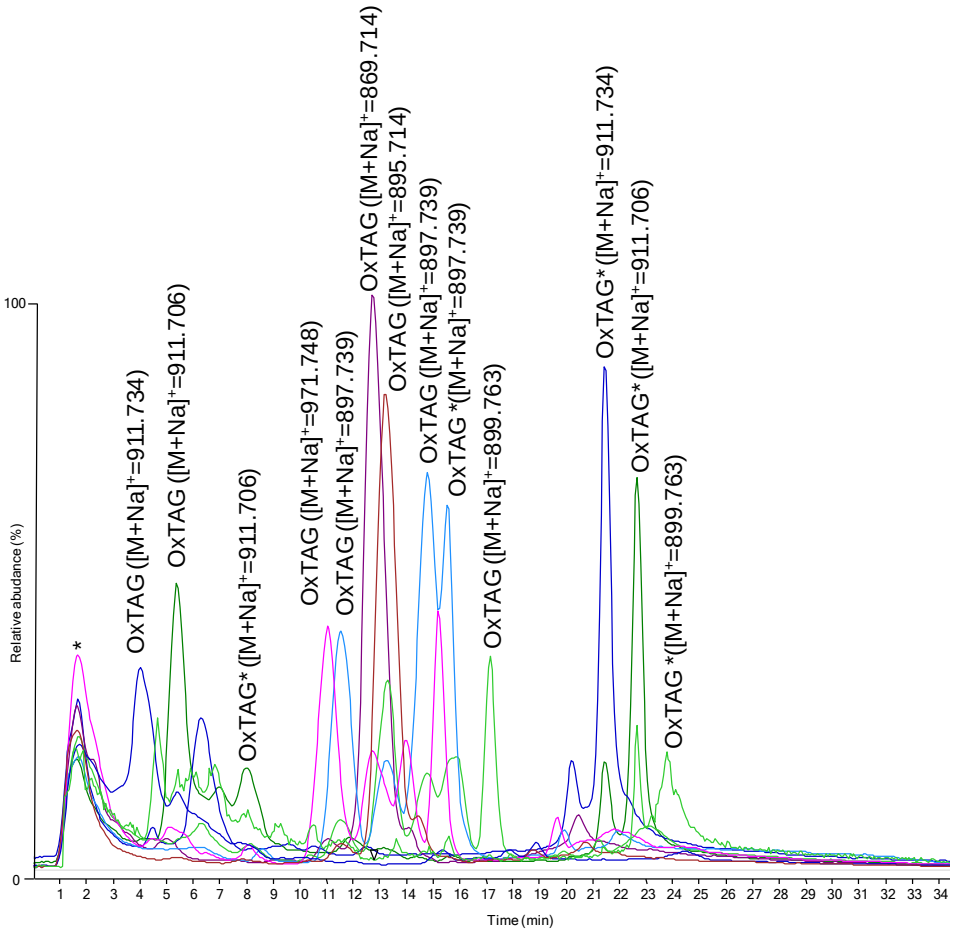

Figure 6 –HPLC-ESI-Q-ToF extract ion chromatograms referring to oxidized triglycerides species in the Winsor & Newton paint tube (sample 11).

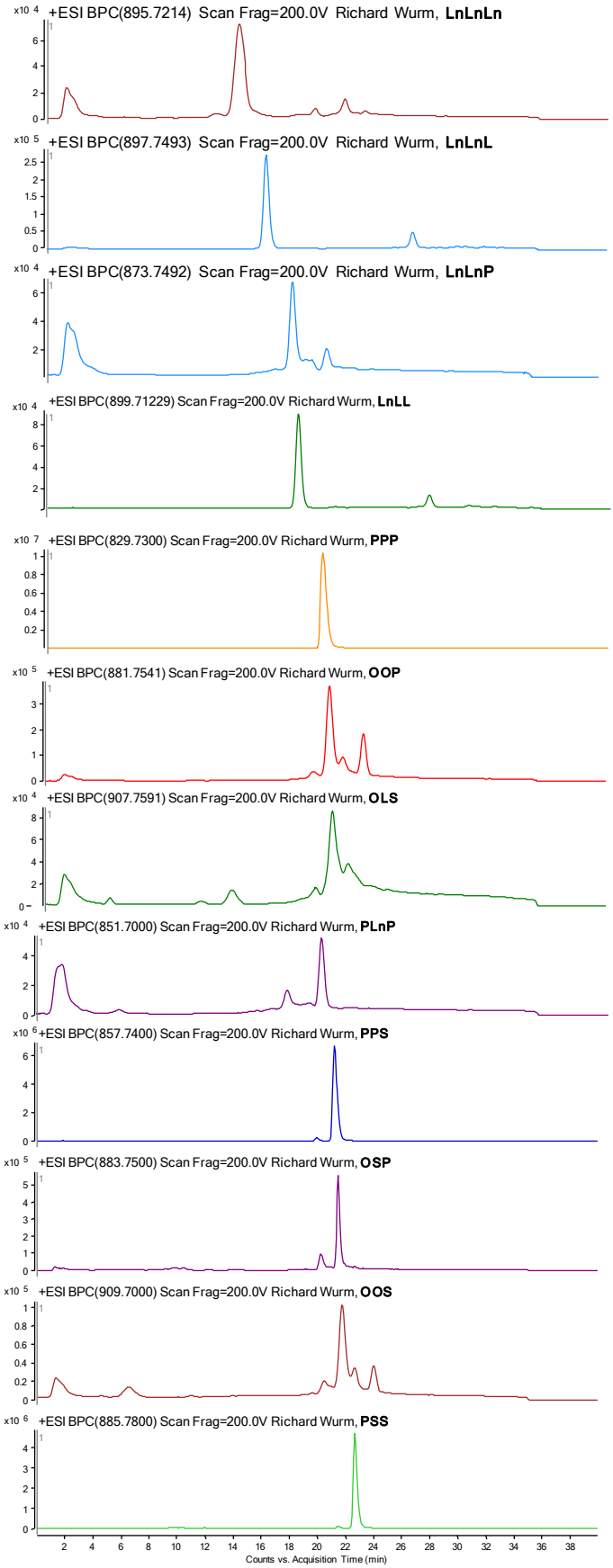

Figure 7 –HPLC-ESI-Q-ToF extract ion chromatograms referring to 12 identified TAGs species in the Richard Wurm (sample 13) paint tube.

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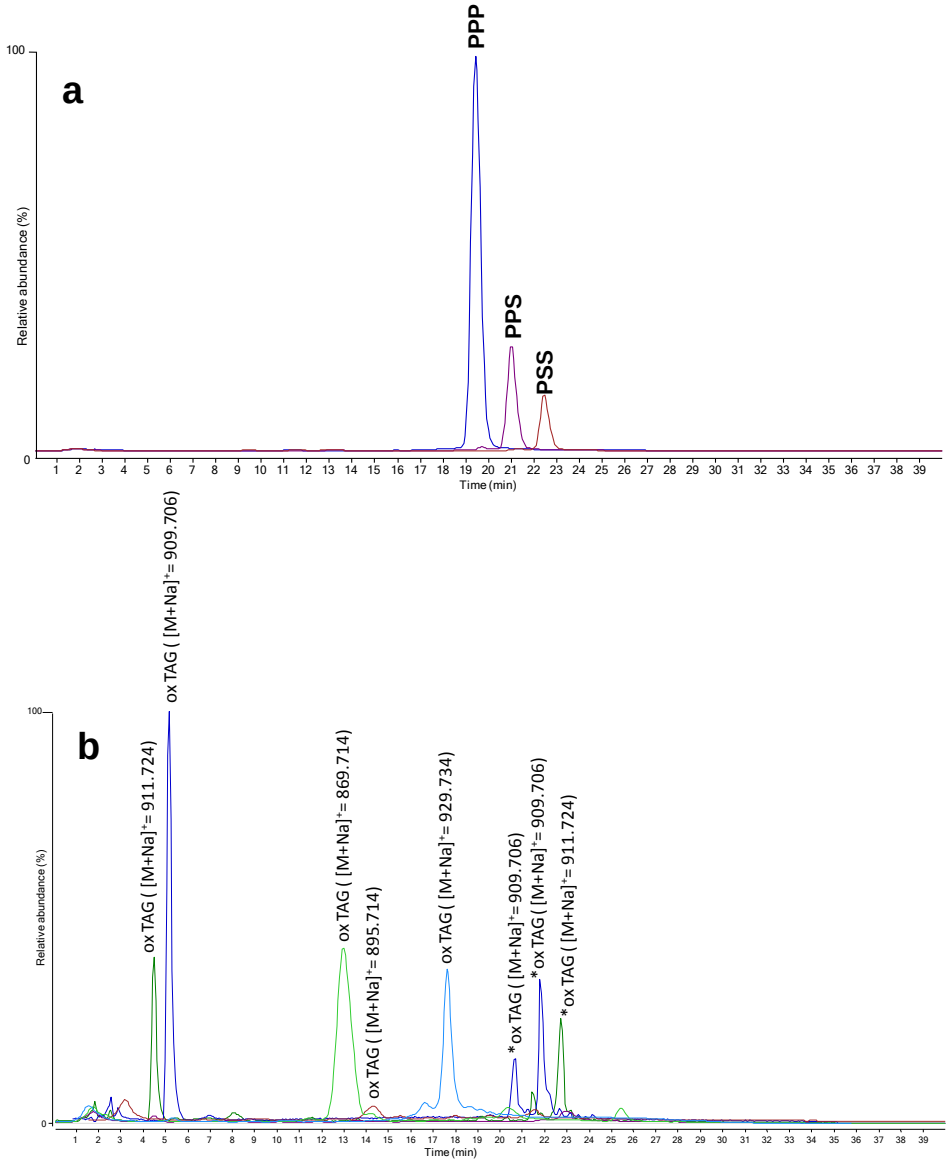

Figure 8 – HPLC-ESI-Q-ToF chromatogram of the Munch's sketch M912 paint sample; the figure was obtained by overlapping the extract ion chromatogram relative to the unoxidized TAGs species (a) and oxidized species (b).

Novel application of liquid chromatography/mass spectrometry for the characterization of drying oils in art: elucidation on the composition of original paint materials used by Edvard Munch (1863-1944)

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Abstract

Modern oil paints, introduced at the beginning of the 20th century, differ from those classically used in antiquity in their chemical and compositional features. The main ingredients were still traditional drying oils, often used in mixtures with less expensive oils and added with several classes of additives. Consequently, detailed lipid profiling, together with the study of lipid degradation processes, is essential for the knowledge and the conservation of paint materials used in modern and contemporary art

A multi-analytical approach based on mass spectrometry was used for the study of original paint materials from Munch's atelier, owned by at the Munch Museum in Oslo. The results obtained in the analysis of paint tubes were compared with those obtained by characterizing a paint sample collected from one of the artist's sketches for the decoration of the Festival Hall of the University of Oslo (1909-1916).

Py-GC/MS was used as screening method to evaluate the presence of lipid, proteic or polysaccaridic materials. GC/MS after hydrolysis and derivatization allowed us to determine the fatty acid profile of the paint tubes, and to evaluate the molecular changes associated to curing and ageing. The determination of the fatty acid profile is not conclusive for the characterization of complex mixtures of lipid materials, thus the characterization of the triglyceride profiles was performed using an analytical procedure based on HPLC-ESI-Q-ToF.

This paper describes the first application of HPLC-ESI-Q-ToF for the characterization of the triglyceride profile in a modern paint sample, showing the potentialities of liquid chromatography in the field of lipid characterization in modern paint materials. Moreover, our results highlighted that the application of this approach can contribute to address dating, authenticity and conservation issues relative to modern and contemporary artworks.

Keywords

High performance liquid chromatography-Electrospray ionization-quadrupole-time of flight mass spectrometry; gas chromatography/mass spectrometry; modern paint; triglycerides; fatty acids; Edvard Munch

1. Introduction

The chemical characterization of micro samples of paint materials collected from artworks is often necessary for assessing the painting techniques used by artists and for the correct conservation and restoration of paintings. Moreover, the possibility to catalogue and investigate original paint materials used by an artist, recovered as atelier or studio materials with the aim to assess suppliers, type of paint, chemical composition and degradation processes, is an invaluable tool to complement the knowledge of an artist's technique, and to better preserve his artistic production. In selected cases, the comparison between atelier and artworks materials can also be exploited to address dating and authenticity issues.

In this study we use a multi-analytical approach based on different mass spectrometric analytical techniques to investigate the content of nine paint tubes used by Edvard Munch (1863-1944), a Norwegian painter who played a main role in 19th century Symbolism and in early 20th century Expressionism. Six of the tubes are oil paint from Winsor & Newton and three more tubes are from Richard Wurm, labelled as Tempera. The tubes are part of a corpus of atelier materials used in the last years of Munch's life, donated to the Munch Museum in Oslo (Norway) after his death, along with all his artworks and belongings [1].

The chemical and compositional features of modern oil paints are different from those of classical oil paint used in antiquity. The main ingredients of the first commercial paint tubes, introduced at the beginning of the 20th century, were traditional drying oils, as linseed or walnut oil, often used in mixtures with less expensive oils as castor or safflower, added with new classes of additives such as surfactants, metal soaps and dispersing agents [2-4]. Drying oils are characterized by the presence of triacylglycerols (TAGs) with a high number of unsaturated fatty acids (FAs) in the acyl chains, such as oleic, linoleic and linolenic acids. An oil is defined as "drying" when the percentage of double bonds in

the FAs in the acyl chains is higher than 65% (w/w): such composition confers to the oil the ability to chemically dry, forming a solid film upon exposure to air and light [2-5].

The identification of the lipid materials and possible additives in a paint layer, the study of the ageing processes undergone by modern oils used as binders, and the influence of the formulation on the ageing pathways and on the efficacy of conservation treatments are fundamental issues for the conservation of paintings, and represent a challenge for the analytical chemist. Consequently, lipid characterization, together with the study of lipid degradation processes, is an important research area in conservation science.

Different analytical methods have been applied for this purpose in the last decades. Infrared spectroscopy (FTIR), analytical pyrolysis coupled with gas chromatography/mass spectrometry (Py-GC/MS) and gas chromatography/mass spectrometry following a wet sample treatment are today the most common approaches for the characterization of lipid material. Moreover, the application of reversed phase high pressure liquid chromatography for the separation of complex mixtures of triglycerides in oils is gaining importance recently [6-12].

FTIR spectroscopy can be used as a fast non-invasive and non-destructive analytical technique that allows the detection of lipid materials, but in most cases not the identification of the botanical origin of aged oils [13, 14].

The coupling of analytical pyrolysis with GC/MS (Py-GC/MS) is a fast destructive analytical technique that allows the chemical characterization of lipid materials without any sample pretreatment. Nonetheless, the intrinsic non-quantitative nature of the technique does not allow the unambiguous identification of the raw source of the oil, especially in the case of mixtures. Thus, the interpretation of the pyrograms is a critical step, especially for aged materials, and requires not only a wide database of reference specimens, but also experience in interpreting the effect of the matrix. The simultaneous occurrence of different organic materials, the relative abundance of one material with respect to the others, the

presence of inorganic materials, sample morphology, and several other factors can contribute in different ways to the resulting pyrograms [15-17].

One of the most diffused analytical procedures for the identification of the botanical origin of the oil involves the use of a gas chromatographic/mass spectrometer system (GC/MS) to perform qualitative and quantitative analysis of the fatty acid profile of the lipid material after hydrolysis of ester bonds in surviving triglycerides. The literature reports a huge number of applications of GC/MS to lipid analysis in cultural heritage, entailing different types of sample pre-treatments and derivatization procedures [4, 18-20]. Moreover, different statistical treatments of the GC/MS experimental data have been proposed for the identification of the raw source of the oil. In particular, the palmitic to stearic acid ratio (P/S) is currently used to differentiate between linseed, walnut and poppyseed oils. This ratio was proposed to compare paint samples with reference materials since saturated acylic chains are supposed to be less subject to physical-chemical reactions during treatment and curing then unsaturated ones. Moreover, palmitic and stearic acids have a similar chemical reactivity, so their ratio has been hypothesized to be relatively unaltered during ageing [3, 21-23]. Nevertheless, recent studies have shown that the P/S ratio varies in time due to the different evaporation ratio of the two acids [24, 25]. Moreover, this parameter is affected by environmental contamination (both palmitic and stearic acids are known laboratory contaminants) or by the presence of other lipid sources (e.g. natural waxes, animal fat, or egg yolk), and it is generally unreliable in the case of oil mixtures [26, 27].

In this work, preliminary Py-GC/MS analysis using thermal assisted derivatization with a silanizing agent were performed on samples from Munch's paint tubes, in order to characterize the gross chemical composition of the paint, and to evaluate the presence of macromolecular species without any sample pretreatment. Secondly, we applied a validated GC/MS method to determine the fatty acid profile after a sample treatment

entailing fast microwave-assisted saponification. This was followed by a derivatization step with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA). GC/MS analysis enabled us to obtain quantitative information on the fatty acid content and on the presence of oxidation products of acylic chains [3, 28-30]. Finally, we propose to overcome the limitations deriving from the mere use of the P/S ratio to characterize oil paint samples by integrating the data on the fatty acid content with the characterization of the entire TAGs profile of the lipid material. HPLC-ESI-Q-ToF was applied for the first time to determine the triglyceride profile of the lipid fraction in a paint sample, allowing the identification of the oils used for the production of the different paint tubes.

We applied the same analytical approach used for the tubes to a paint sample from a Edvard Munch's artwork, "New Rays". The artwork is a sketch on canvas belonging to the Munch Museum, created by the artist in connection with the painted decorations for the Festival Hall (*Aula*) of the University of Oslo (Figure 1). The sketch is painted on a thick linen canvas with a white ground, and is mainly painted in one and two layers wet in wet. The Aula decorations are renowned as Munch's greatest decoration project, considering the area to decorate, the size of the paintings, and the time and effort he put into his preparing sketches, completed for the University of Oslo in 1916 [31]. The analytical results obtained on the artwork were compared to those obtained for the characterization of the paint in the atelier tubes.

2. Materials and methods

2.1 Paint samples

Nine samples from paint tubes from the atelier of Edvard Munch, used in the last period of his life, when his atelier was in Ekely (1916-1944), were analyzed. It is not to be excluded that some paint materials bought before this dates. Amongst the 9 samples, 6 were from tubes produced by Winsor & Newton (samples 11, 12, 15, 19, 27), and 3 were “tempera” tubes produced by Richard Wurm (samples 7, 13, 14). The Winsor & Newton paint tubes were labeled as “Cremnitz White” (11), “Zinc White” (12), “Vermillion Pale” (15), “Mineral Grey” (19), and “Vermillion” (27). The Richard Wurm tempera tubes were labeled as “Cobalt” (7), “Organic Krapplack” (13), and “Ultramarine” (14).

The samples were obtained by squeezing the paint tubes in small vials and were partially dried at the surface. The material for the analysis was obtained sampling the bulk of the paint.

The analytical procedure was also applied to a paint sample (Woll M 946; P3) collected by the museum conservators from “New Rays”, one of the sketches prepared by Munch for the decoration of the Festival Hall Aula of Oslo University (1909–1916), shown in Figure 1.

2.2 Materials and reagents

For the Py-GC/MS analysis, 1,1,1,3,3,3-hexamethyldisilazane (HMDS) was purchased from Sigma-Aldrich (U.S.).

The solvents used for the GC/MS analysis were diethyl ether, n-hexane and isooctane (HPLC grade; Sigma-Aldrich).

N,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethyl-chlorosilane used for fatty acid derivatization was purchased from Sigma-Aldrich. Fatty acid solutions were prepared in acetone, and contained lauric (4.10 µg/g), suberic (4.27 µg/g),

azelaic(3.95 µg/g), myristic (4.11 µg/g), sebacic (3.85 µg/g), palmitic acid (4.39 µg/g), oleic acid (6.32 µg/g) and stearic (6.62 µg/g) acids. All standard solutions were used to derive calibration curves. The acids were purchased from Sigma-Aldrich, purity > 99%. Tridecanoic acid (purity 99%; Sigma-Aldrich) solution in *iso*-octane, 139.91 µg/g, was used as internal standard for derivatization; hexadecane (purity 99%; Sigma-Aldrich), solution in *iso*-octane,142.00 µg/g, was used as internal standard for injection.

The solvents used for the HPLC analyses were: *iso*-propanol, *n*-hexane, chloroform and methanol (HPLC/MS grade; Fluka, U.S.).

2.3 Instruments and methods

2.3.1 Pyrolysis-Gas Chromatography/Mass Spectrometry

Analyses were performed using a multi-shot pyrolyzer EGA/PY-3030D (Frontier Lab, Japan) coupled with a 6890N gas chromatography system with a split/splitless injection port and combined with a 5973 mass selective single quadrupole mass spectrometer (Agilent Technologies, U.S.).

The samples (in the range 0.1-0.6 mg) were placed in deactivated stainless steel sample cups on glass wool. The samples were added with 2.0 µL of HMDS and placed on top of the pyrolyzer at room temperature, and then quickly introduced in the pyrolysis chamber. Pyrolysis conditions were as follows: pyrolysis chamber temperature 550 °C, interface 280 °C. The GC injector temperature was 280 °C. The GC injection port operated in split mode and the best analytical results were obtained with a split ratio of 1:10. The chromatographic separation of pyrolysis products was performed on a fused silica capillary column HP-5MS (5% diphenyl-95% dimethyl-polysiloxane, 30 m x 0.25 mm i.d., 0.25 µm film thickness, J&W Scientific, Agilent Technologies), preceded by 2 m of deactivated fused silica pre-column with internal diameter of 0.32 mm. The chromatographic conditions for the analysis were: 32 °C for 10 min, 10 °C/min to 280 °C, 15 °C/min to 300 °C, 300 °C

for 2 min. The helium (purity 99.9995%) gas flow was set in constant flow mode at 1.2 mL/min.

MS parameters: electron impact ionization (EI, 70 eV) in positive mode; ion source temperature 230 °C; scan range 50-700 m/z; interface temperature 280 °C. Perfluorotributylamine (PFTBA) was used for mass spectrometer tuning. MSD ChemStation (Agilent Technologies) software was used for data analysis and peak assignment was based on the comparison with libraries of mass spectra (NIST 1.7, WILEY275).

2.3.2 Gas Chromatography/Mass Spectrometry

GC/MS instrumentation consisted of an Agilent Technologies 6890N Gas Chromatograph with a split/splitless injection port and coupled with a 5973 mass selective single quadrupole mass spectrometer.

Samples were injected in splitless mode at 280 °C. GC separation was performed on a fused silica capillary column HP-5MS (5% diphenyl-95% dimethyl-polysiloxane, 30 m x 0.25 mm i.d., 0.25 µm film thickness, J&W Scientific, Agilent Technologies). Chromatographic conditions were [28, 29]: initial temperature 80°C, 2 min isothermal, 10 °C/min up to 200 °C, 4 min isothermal for the separation of unsaturated C_{18} fatty acids and their isomers, 6 °C/min up to 280 °C, 40 minutes isothermal. The helium (purity 99.9995%) gas flow was set in constant flow mode at 1.2 mL/min. MS parameters: electron impact ionization (EI, 70 eV) in positive mode; ion source temperature 230 °C; scan range 50-700 m/z; interface temperature 280 °C. The injection volume was 2 µL.

Perfluorotributylamine (PFTBA) was used for mass spectrometer tuning.

Peak assignment was based on the comparison with libraries of mass spectra (NIST 1.7, WILEY275).

2.3.3 High Performance Liquid Chromatography/Mass Spectrometry

HPLC-ESI-Q-ToF analyses were carried out using a 1200 Infinity HPLC, coupled with a Quadrupole-Time of Flight tandem mass spectrometer 6530 Infinity Q-ToF detector by a Jet Stream ESI interface (Agilent Technologies).

The HPLC conditions were: Poroshell 120 EC-C18 column (3.0 mm x 5.0 mm, 2.7 µm particle size) with a Zorbax eclipse plus C-18 guard column (4.6 mm x 12.5 mm, 5 µm particle size); a flow rate of 0.3 mL/min, an injection volume of 1 µL and a column temperature of 45 °C. Separation was achieved using a gradient of methanol (eluent A) and *iso*-propanol (eluent B). The elution gradient was programmed as follows: 90% A for 5 minutes, followed by a linear gradient to 90% B in 25 min, then held for 5 min. Re-equilibration time for each analysis was 10 min.

The ESI operating conditions were: drying gas (N_2, purity >98%): 350 °C and 10 L/min; capillary voltage 4.5 KV; nebulizer gas 35 psig; sheath gas (N_2, purity >98%): 375 °C and 11 L/min. High resolution MS and MS/MS spectra were acquired in positive mode in the range 100-1700 m/z. The fragmentor was kept at 200 V, nozzle voltage 1000 V, skimmer 65 V, octapole RF 750 V. For the MS/MS experiments, voltages in the range 30-100 V in the collision cell were tested for Collision Induced Dissociation (CID), to obtain information on fragmentation pathways of selected analytes. The MS/MS spectra presented in the text were obtained at 50 V. The collision gas was nitrogen (purity 99.999%). The data were collected by auto MS/MS acquisition with an MS scan rate of 1.03 spectra/sec and an MS/MS scan rate of 1.05 spectra/sec; only one precursor was acquired per cycle (relative threshold 0.010%). The mass axis was calibrated daily using the Agilent tuning mix HP0321 (Agilent Technologies). MassHunter® Workstation Software (B.04.00) was used to carry out mass spectrometer control, data acquisition, and data analysis.

The structures of individual glycerides were identified by interpreting their tandem mass spectra and the identification of the oils was obtained by comparison with a set of

reference oils characterized in the same conditions [32]. The characterization of the oxidized triglycerides is reported in Table 1.

2.4 Sample treatment

2.4.1 Gas chromatography/mass spectrometry samples preparation

For the GC/MS analyses ~1 mg of each sample was subjected to saponification assisted by Milestone (U.S.) microwaves Ethos One (power 200 W) with 300 µL of KOH_{ETOH} 10% wt at 80 °C for 60 min. In order to maximize the extraction yields, two solvents were used for liquid/liquid extraction: the neutral compounds were extracted with *n*-hexane; the residual solution was acidified with hydrochloric acid (6 *M*) and then the acidic compounds were extracted with diethyl ether (400 µL, three times). The two extracts (neutral + acid fraction) were combined in order to analyze them in a single chromatographic run, evaporated to dryness under nitrogen stream and subjected to derivatization with 20 µL of *N*,*O*-bistrimethylsilyl-trifluoroacetamide , 150 µL of *iso*-octane and 5 µL of tridecanoic acid solution at 60 °C for 30 min. 5 µL of hexadecane solution were added just before injection [3, 28-30].

2.4.2 High performance liquid chromatography samples preparation

For the HPLC/MS analyses, 0.1-0.8 mg of each paint sample were subjected to extraction assisted by Milestone microwaves Ethos One (power 600 W) with 300 µL of a chloroform-hexane (3:2) mixture at 80 °C for 25 min. The solvents for the extraction and the best microwave conditions were adapted from [33, 34].

The extracts were dried under a nitrogen stream, diluted with 600 µL of elution mixture and filtered on a 0.45 µm PTFE filter (Grace Davison Discovery Sciences, U.S.) just before injection.

2.5 Quantitative analysis

The GC/MS quantitative analysis for all the paint samples was performed using calibration curves calculated on the basis selected ion monitoring (SIM) chromatograms. The selected ions for the SIM acquisition were: lauric acid m/z 117;257, suberic acid m/z 169;303, azelaic acid m/z 149;317, myristic acid m/z 117;285, sebacic acid m/z 149;331, palmitic acid m/z 117;3131, oleic acid m/z 117;339, stearic acid m/z 117;341).

For the HPLC-ESI-Q-ToF analysis, relative abundances were calculated on the basis of extract ion chromatograms, considering the isotopic distribution of the molecular species, and normalized to 100% for each chromatogram.

3. Results and discussion

This section reports on and discusses the results obtained by Py-GC/MS, GC/MS and HPLC-ESI-Q-ToF techniques for the analysis of Winsor & Newton and Richard Wurm paint tubes samples and for the sample from Munch's sketch.

3.1 Gas chromatographic analyses

The Py-GC/MS chromatogram of sample 19 from a Winsor & Newton paint tube is reported in Figure 2a. The pyrolysis profile is characterized by the presence of the molecular markers of a drying oil: fatty acids and dicarboxyilic acids with a relevant amount of nonandioic acid (azelaic acid). The Py-GC/MS chromatograms of all the samples from Winsor & Newton paint tubes are characterized by a very similar profile to that presented in the figure.

Figure 2b shows the GC/MS chromatogram obtained for sample 19. The chromatographic profile is characterized by the presence of monocarboxylic and dicarboxylic fatty acids: palmitic, oleic, stearic, and suberic, azelaic, sebacic acids, respectively. The presence of dicarboxylic acids with 8, 9 and 10 carbon atoms, and the absence of polyunsaturated fatty acids is characteristic of an oxidized lipid binder, as dicarboxylic acids are formed during curing and ageing as oxidation products of polyunsaturated acylic chains [3]. Intermediate oxidation products are also observed, as hydroxy-acids with 18 carbon atoms in the acyl chain.

The samples from the five other Winsor & Newton tubes have the same qualitative composition, but are characterized by a variable amount of dicarboxylic acids and of oleic acid (C18:1). The total percentage content of dicarboxylic acids (ΣD%) is in the range 19.0-69.8 %, highlighting that the degree of oxidation and curing is different among the investigated samples.

Table 2 reports some significant chemical parameters calculated from the GC/MS data, thanks to calibration curves: palmitic vs. stearic acid (P/S), azelaic vs. palmitic acid (A/P),

and oleic vs. stearic acid (O/S), compared with literature data relative to reference oils used as raw materials in modern oil media [4, 20, 35, 36]. The A/P parameter for Winsor & Newton samples ranges between 0.5 and 1.9, compatibly with an partially dried oil paint [4].

The ratio between palmitic and stearic acid (P/S) also shows a relevant variability in the six Winsor & Newton samples, ranging from 1.1 to 3.6. This range overlaps with the intervals reported to be characteristic of several raw materials used in the formulation of modern and contemporary oil paints.

The Py-GC/MS chromatogram of the Richard Wurm paint sample 13 is reported in Figure 3a. Even if the paint tube was labeled as “tempera”, no protein markers are observed in the pyrogram, which is characterized by the presence of a relevant amount of dicarboxylic acids, markers of a drying oil. The three Richard Wurm paint samples were characterized by similar pyrolytic profiles.

The gas chromatographic profiles of Richard Wurm paint tubes, obtained after saponification and derivatization, are characterized by palmitic, oleic, and stearic acids as the most abundant monocarboxilic acids, and by the presence of suberic, azelaic and sebacic dicarboxylic acids. The GC/MS chromatogram of sample 13 is shown in Figure 3b, and the quantitative parameters for each sample are reported in Table 2.

The Richard Wurm paint tubes were characterized by a degree of oxidation/curing comparable to that of Winsor & Newton paint tubes, with an A/P ratio in the range 0.5-1.9. The P/S ratio is in the range 4.5-5, highlighting the presence in the formulation of an oil with a relatively high palmitic acid content. The traditional drying oils used by artists before the diffusion of industrial paint materials, such as linseed oil, walnut oil and poppyseed oil, all have a P/S reported ratio usually below 4.0. Cotton oil and palm oil are characterized, on the other side, by an high content of palmitic acid [4, 20, 35].

The different oxidation states displayed by the fatty acid profiles in both the sets of paint samples can be explained with the fact that the paints in the tubes have undergone different extents of exposition to air and light, depending on the time of use of the tube and the amount of residual paint inside it. This may lead to the observed variability in the composition, especially for what concerns the amount of dicarboxylic acids and of residual oleic acid. In addition, it is well known that the composition of pigments has also an influence on the oxidation of oil paints, and in particular some cations contained in inorganic pigments can catalyze reactions with oxygen. These phenomena have likely played a role in producing the observed differences in the A/P values between paints from the same manufacturer. As an example, the Richard Wurm sample 13 , which contains an organic lake (label ""Organic Krapplack"), was characterized by an A/P ratio of 0.5, notably lower than that of Richard Wurm samples 7 and 14, which contain inorganic pigments. On the other side, it is not possible to highlight evident clear correlation between the degree of oxidation and the pigment composition for the samples of the Winsor & Newton group, and it cannot be excluded that binders and additives in tubes from the same trademark have different formulations.

We can conclude that the high variability of the fatty acid profiles in all the analyzed paint tubes and the possible presence of mixtures of different oils does not allow the identification of the botanical origin of the oils used for the production of the paint tubes on the basis of the fatty acid profile.

3.2 HPLC-ESI-Q/ToF of triglycerides

In order to identify the lipid source used for the production of the Winsor & Newton paint tubes, HPLC/MS analysis of their triglyceride content was performed. The relative abundances of TAGs are shown in Figure 4 and reported in Table 3. The HPLC/MS

extract ion chromatograms of the Winsor & Newton sample 19 are shown in Figure 5 as an example.

The triglyceride profile of sample 19 features the presence of OLS (m/z 907.776, $[M+Na]^+$), which is a specific marker of walnut oil [32]. Moreover, LnLnP (m/z 873.704, $[M+Na]^+$) was detected, which is typical of linseed or walnut oil [32]. The simultaneous presence of these two oils account for the qualitative TAG composition of sample 19 : LnLnLn (m/z 895.669, $[M+Na]^+$), LnLnL (m/z 897.690, $[M+Na]^+$), LnLL (m/z 899.705, $[M+Na]^+$), OOP (m/z 881.776, $[M+Na]^+$), and LLL (m/z 901.718, $[M+Na]^+$) are present in both walnut and linseed oils [32].

The presence of LOP (m/z 879.731, $[M+Na]^+$), LLO (m/z 903.744, $[M+Na]^+$), OOL (m/z 905.760, $[M+Na]^+$), and OOS (m/z 909.830, $[M+Na]^+$) can be ascribed to walnut oil, while that of OSP (m/z 883.788, $[M+Na]^+$) to linseed oil.

By a semi-quantitative point of view, the high relative abundance of LnLnL and LnLL in the sample are consistent with the high content of both linseed and walnut oils in terms of these TAGs. The relatively high amount of OLS in the sample's extract is harder to rationalize. Nonetheless, summarizing, although the simultaneous presence of other oils in minor amounts cannot be ruled out, TAGs profiling strongly suggests that the lipid material used in the preparation of paint tube 19 is a mixture containing linseed and walnut oils.

The TAGs profiles of the other samples from Winsor & Newton paint tubes, namely 11, 12, 15 and 27, are characterized by the presence of hydroxylated acylglycerols deriving from the oxidation of triglycerides containing unsaturated acyl substituents, while not oxidized TAGs are absent in these paint tubes. The triglyceride profiles of this group of samples are very similar each other and, as an example, the extract ion chromatograms obtained for paint tube 11 are reported in Figure 6.

The oxidized TAGs identified in these five samples are characterized by the presence of a number of residual double bonds compatible with the oxidation of triglycerides containing 3 double bonds in the acyl chain.

The three main oxidized TAGs visible in Figure 6 are characterized by the raw formulas $C_{53}H_{100}O_7$ (m/z 869.7), $C_{55}H_{100}O_7$ (m/z 895.7), and $C_{55}H_{100}O_8$ (m/z 911.7). The study of their CID (see Table 1) allowed us to identify them as C18:2_{1OH}, P, P; C18:2_{1OH}, O, P; and C18:2_{1OH}, C18:1_{1OH}, P. Considering hydration of the double bonds as one of the typical ageing processes undergone by TAGs, the structure of the original acyl chains can be deduced. Thus, these oxidized TAGs originated from the three TAGs LnPP, LnOP, and LnLP (no exact determination of the sn position was performed at this stage of investigation):. LnPP is specific for linseed oils while LnOP of walnut oil. LnLP is typical of both linseed and walnut oils. On the basis of this interpretation of the origin of the oxidized TAGs, we hypothesise that the also the Winsor & Newton paint in tubes 11, 12, 15 and 27 was produced using linseed and walnut oils, as that in sample 19, even if in these samples original TAGs related to these oils could not be detected.

The HPLC/MS characterization of the triglyceride composition was also performed for the extract of the samples from the Richard Wurm paint tubes. The HPLC extract ion chromatograms of Richard Wurm paint sample 13 are reported in Figure 7 and the TAGs relative abundances in Table 3.

The HPLC profile is characterized by the presence of PPP (m/z 829.716, $[M+Na]^+$), PPS (m/z 857.729, $[M+Na]^+$) and PSS (m/z 885.871, $[M+Na]^+$) as main triglycerides. These TAGs are typical of palm oil [33, 37, 38]. Even if these triglycerides can be related to the presence of egg yolk, the Py-GC/MS analyses allowed us to exclude the presence of egg proteins and the use of this material in the paint tube formulations.

Palm oil is the only plant oil to contain a relevant amounts of PPP. For this reason, even if the TAGs profile of fresh palm oil contains a larger variety of TAGs than what observed in

the analysis of Richard Wurm sample 13, PPP in such high amounts can be considered a marker for palm oil. The differences between the observed triglyceride profile and the profile reported for fresh palm oil may be due to a pretreatment of the raw material before use. Palm oil diffused in Europe as an industrial material in the second half of 19th century after the exploitation of African colonials territories, and was included in the formulations of paints and soaps.

The analysis also reveals the presence of lower amounts of LnLnLn (m/z 895.669, $[M+Na]^+$), LnLnL (m/z 897.690, $[M+Na]^+$), LnLnP (m/z 873.704, $[M+Na]^+$), LnLL (m/z 899.705, $[M+Na]^+$), PLnP (m/z 853.719, $[M+Na]^+$), OOP (m/z 881.770, $[M+Na]^+$), OSP (m/z 883.788, $[M+Na]^+$), suggesting the presence of linseed oil and OOS (m/z 909.830, $[M+Na]^+$), and OLS (m/z 907.776, $[M+Na]^+$) that could derive from the possible presence of a minor source of triglycerides. The TAG profiles show that Richard Wurm paint tube 13 contains a lipid mixture composed by linseed and palm oils: the high amount of the markers of palm oil with respect to those of linseed oil observed in the chromatograms in Figure 7 can be due to the original recipe of the paint material or can be related to the nature of the TAGs in palm oil. Being PPP, PPS and PSS saturated TAGs, they did not took part in the reticulation processes or in the photo-oxidation processes significantly, and were thus relatively more available for extraction with respect to the highly unsaturated markers of linseed oil. The presence of palm oil was also ascertained in paint tubes samples 7 and 14, that were characterized by similar triglycerides compositions. The presence of palm oil, characterized by an high amount of triglycerides containing palmitic acid, accounts for the high P/S values observed in the GC/MS analysis of fatty acids for the Richard Wurm paint tubes.

Due to the non siccative nature of palm oil, its exploitation in a paint material can only be explained taking into account that the triglycerides and fatty acid composition of palm oil and egg yolk, typically used for the preparation of tempera, are similar and characterized

by the presence of triglycerides with an high amount of saturated TAGs [39]. Thus, we can hypothesize that the producer used a combination of linseed and palm oils in order to prepare a commercial material characterized by similar proprieties to those of egg yolk. This hypothesis also accounts for the “tempera” label on the Richard Wurm tubes.

3.3 Analysis of the Edvard Munch’s sketch

The proposed combined analytical approach was applied to a paint sample from the painted sketch by Edvard Munch *New Rays*, owned by the Munch Museum (Oslo).

The preliminary Py-GC/MS analysis of the sample showed presence of the markers of a drying oil (fatty acids and dicarboxylic acids) while highlighted the absence of proteins.

The GC/MS quantitative analysis results are reported in Tables 2 and 3. The qualitative profile of the paint sample is similar to those of the paint tubes samples, being characterized by the presence of suberic, azelaic, sebacic, palmitic, oleic and stearic acids as main fatty acids. The sample also contains traces of terpenoid compounds with abietadiene skeleton, with dehydroabietic acid as most abundant. These compounds are known molecular markers of a *Pinaceae* resin [40].

The A/P ratio (1.3) and the total amount of dicarboxylic acids (49.7%) observed in this sample are characteristic of an oxidized drying oil, and the P/S ratio is 2.5. To better characterize the lipid materials used by the artist in his sketch, HPLC analysis was performed. The chromatographic profile obtained for the unoxidized triglycerides is reported in Figure 8a, and the resulting TAGs distribution in Table 3. The main triglycerides are PPP (m/z 829.716, $[M+Na]^+$), PPS (m/z 857.729, $[M+Na]^+$) and PSS (m/z 885.871, $[M+Na]^+$), characteristic of palm oil. The lipid material in the sample is in an advanced oxidation state, as demonstrated by the presence of oxidized triglycerides featuring inclusion of oxygen in the acylic chains (Figure 8b), which may be related to the presence of linseed oil as raw material.

These results highlight a perfect match between the composition of the paint sample from *New Rays* and the content of the Richard Wurm paint tubes from Munch's atelier.

4. Conclusions

The new analytical approach based on the integration of the HPLC-ESI-Q-ToF analysis of the triglyceride fraction proved to improve the reliability of the characterization of an oil binder with respect to the classical approaches based on Py-GC/MS and GC/MS.

The gas chromatographic methods allowed us to assess the presence of a lipid material in the paint samples. The use of liquid chromatography coupled with mass spectrometry allowed us to clarify the composition of the oil components and to hypothesize the botanical origin of the lipid materials contained in the paint tubes. In details:

- the Py-GC/MS analysis of the Winsor & Newton paint tubes allowed us to identify the presence of a drying oil. The lipid materials sampled from these paint tubes were characterized by different oxidation states, and the GC/MS quantitative analysis showed a P/S ratio in the range 1.1-3.6. The HPLC analysis allowed us to characterize the lipid materials as a mixture which contains walnut and linseed oils (the presence of other minor additives shall in principle not be excluded). These results highlight the limited validity of P/S ratio for the identification of the botanical origin of an oil: the presence of metal soaps or other minor sources of fatty acids could lead to erroneous interpretations of the origin of the material;
- the Py-GC/MS analysis of the Richard Wurm "tempera" paint tubes showed the presence of the molecular markers of a drying oil, while the molecular markers characteristic of proteins were absent. The quantitative GC/MS analysis yielded a P/S ratio in the range 4.5-5.0, which is higher than the typical values of traditional drying oils (linseed, walnut or poppyseed oils). The HPLC-MS chromatograms were characterized by the presence of markers of palm and linseed oils. The presence of

palm oil, characterized by an high amount of palmitic acid, accounts for the high P/S ratio determined by GC/MS. The high similarities of the triglyceride composition of palm oil and the binding medium based on egg yolk suggests that the producer used a combination of linseed and palm oil in order to obtain a paint material with physical proprieties similar to those of a egg based tempera. Also in this case, the presence of a third, minor source of TAGs might not be excluded, even if highly unlikely.

The application of an innovative analytical procedure allowed us to overcome the limitations related to the use of the fatty acid profile for the identification of a lipid material, and to unambiguously identify the botanical origin of the lipid materials used for the production of the paint tubes.

The analytical approach was successfully applied for the characterization of a paint sample collected from the sketch *New Rays*, proving the efficiency of the combined analytical approach in the characterization of lipid materials in case-studies. In the paint sample, the characteristic triglycerides of linseed oil and palm oil were detected. The analytical results allowed us to relate the original paint materials of the artist's atelier with those applied in his artwork. The final results of this study clearly highlights that this new approach is a powerful tool for solving attribution problems.

In conclusion, the application of HPLC-MS lipid profiling represents a step forward in the analysis of lipid materials in paint samples, overcoming the issues related to use of the classic analytical approaches in the field of cultural heritage.

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