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Characterization of Organic Art Materials by GC-MS

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ACKNOWLEDGMENT

This master project was a collaboration between the Faculty of Chemistry, Biotechnology and Food Science (KBM) at the Norwegian University of Life Sciences (NMBU), Oslo Metropolitan University (OsloMet) and Munch Museet in Oslo. The practical work was conducted at OsloMet from January 2018 to March 2019.

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SAMMENDRAG

Bakgrunn: Ved konservering av gamle malerier er sammensetning av bindemiddelet i malingen viktig å ha kjennskap til. Noen konserveringsteknikker kan ha skadelig virkning på maleriene dersom feil teknikk benyttes til feil type maling. Munch Museet er i besittelse av en stor kolleksjon malerier og kunstmateriell som har behov for kategorisering og konservering. De har derfor et ønske om å etablere metoder for å kunne kategorisere objektene i deres kolleksjon.

Hensikt: Dette prosjektet utgjorde den første fasen i identifikasjon av bindemidlene i olje- og proteinbasert maling. Hensikten var å opprette en spektradatabase basert på analyser av rene bindemidler, hvor datagrunnlaget dannes uten påvirkning fra andre elementer i malingen. For å gjøre dette måtte det først opprettes en analysemetode som kan differensiere mellom forskjellige bindemidler. Munch Museet bistod med bindemiddelprøver fra deres kolleksjon for metodeutviklingen i dette prosjektet. Disse prøvene inkluderte rene oljer og tørkede proteiner som benyttes i maling. Identifiserte olje- og proteinbaserte malingstubeprøver fra Munchs atelier ble også gitt som kontrollprøver.

Metoder: Det ble utviklet en analysemetode for oljer og en for proteiner, begge ved bruk av gasskromatografi (GC) og massespektrometri (MS). GC-MS ble sett på som egnet, da dette produsere to sett med kvalitative data. Mikrobølgeassistert syrehydrolyse, som bryter ned oljer til fettsyrer og proteiner til aminosyrer, ble valgt som første trinn i prøveopparbeidingsprosessen. Deretter ble fettsyrene og proteinene derivatisert før separate analyser ved GC-MS. Derivatiseringsproduktene var fettsyre metylestere og silanerte aminosyrer. Den relative fordelingen av derivater ble brukt som markører for å skille mellom ulike oljeprøver og ulike proteinprøver.

Resultater: Analysen av de rene oljeprøvene ga god kromatografisk separasjon, hvor ingen prøver falt under kvalitetskriteriene som var satt i metoden. Videre viste metoden seg å være svært selektiv for opparbeiding av fettsyrer i rene prøver og malingsprøver. Analysemetodens evne til å differensiere mellom de rene oljeprøvene var imidlertid dårlig. Sammenligning av de rene prøvene og malingsprøvene viste også at andre forbindelser i malingen påvirket den relative fordelingen av fettsyrer. Metoden oppnådde imidlertid en nedre kvantifiseringsgrense på 1,6 og 2,8 mg for henholdsvis rene oljer og maling.

De rene, proteinbaserte prøvene oppnådde unike aminosyrederivatfordelinger, og metoden hadde en nedre kvantifiseringsgrense på 1,6 mg. Metoden viste seg derimot uegnet for de proteinbaserte malingsprøvene, da det ikke ble detektert tilstedeværelse av aminosyrederivater ved bruk av metoden i disse prøvene.

Konklusjon: Analysemetoden for oljer var god nok til å detektere fettsyrer fra rene prøver og malingsprøver, men må utbedres dersom den skal kunne brukes pålitelig til identifikasjon. Metoden for proteinbaserte prøver var god nok til identifikasjon og deteksjon av rene prøver, men må forbedres for å kunne benyttes på malingsprøver.

ABSTRACT

Background: In conservation of old paintings, knowing the composition of the paint binder is of great importance. Some conservation techniques can be damaging to the paintings when it is applied to the wrong kind of paint. The Munch Museum is in possession of a large collection of paintings and art materiel in need of categorization and conservation. They therefore seek a method to help further categorize the objects in their collection.

Aim: This project constituted the first phase in identification of binders in oil- and protein-based paint. The purpose was to establish a spectra library of pure binders without interference of components that may be found in paints. To obtain this, we first sought to establish a method capable of detecting and differentiating the different binders. To this end, the Munch Museum provided samples of pure binders from their collection, including oil- and protein-based binders. They also provided oil- and protein-based paint samples from Munch's art studio to serve as control samples.

Methods: Different methods for oil- and protein-based samples, using gas chromatography (GC) and mass spectrometry (MS), were developed. GC-MS was considered suitable, as it produced two sets of qualitative data. Microwave assisted acid digestion was used to break down the oils into fatty acids, and the proteins into amino acids. The fatty acids were derived by methylation, and the amino acids were derived by silylation. Both the methylated fatty acids and the silylated amino acids were analyzed separately by GC-MS. The relative distribution of derivatives was used as markers to differentiate between different types of oil-based samples and different types of protein-based samples.

Results: The GC analysis of pure oil samples yielded satisfactory separation of the fatty acid derivatives and none of the samples fell short of the quality criteria imposed by the method. The method also proved to be quite selective in the preparation of fatty acids found in the pure oil samples and the oil-based paint samples. The method did however not differentiate well between the fatty acid derivate ratios in the pure oil samples. By comparing the pure samples with the analyses of the paint samples, a deviation in the fatty acids ratios in the paints was observed. This could indicate the other components in the paint had an effect on the fatty acid ratio. The method did however achieve a lower limit of quantification of 1.6 and 2.8 mg for pure oil samples and paint samples, respectively. The analysis of the protein-based samples achieved to differentiate between every protein sample analyzed and reached a lower limit of quantification of 1.6 mg. The method was however inadequate to detect any amino acid derivatives in the paint samples.

Conclusion: The analysis method developed for oils were good enough to detect signals of pure samples and paint samples, but must be improved to be able to properly differentiate between samples. The method used on the proteins was good enough for detection and differentiation of pure samples, but needs improvement in order to be used on paints.

TABLE OF CONTENTS

ACKNOWLEDGMENT I
SAMMENDRAG II
ABSTRACT III
LIST OF TABLES VI
LIST OF FIGURES VII
ABBREVIATIONS VIII

1 INTRODUCTION 1
1.1 THE EDVARD MUNCH COLLECTION OF REFERENCE PAINTING MATERIALS AT THE MUNCH MUSEET IN OSLO 1
1.2 THE RELEVANCE OF BINDING MEDIA ANALYSES IN THE ART AND CONSERVATION FIELD 2
1.3 BINDING MEDIA 3
1.3.1 Drying oils 3
1.3.2 Protein-based binders 9
1.4 ESTABLISHED METHODS 11
1.4.1 University of Pisa – methods for analysis of binders 11
1.4.2 FAME analysis 14
1.5 STUDY OBJECTIVES 15

2 METHODS 16
2.1 THEORY ON ANALYTICAL METHODS 16
2.1.1 Microwave assisted acid digestion 16
2.1.2 Derivatization 17
2.1.3 Chromatography 19
2.1.4 Mass spectrometry (MS) 25
2.2 EXPERIMENTAL 29
2.2.1 Typologies of samples 29
2.2.2 Different sample preparation methods based on binder type 33
2.2.3 Sample preparation 34
2.2.4 Analyses performed on samples 36

3 RESULTS 39
3.1 LIPIDS 39
3.1.1 Resolution 39
3.1.2 Signal-to-noise ratios 41
3.1.3 Fatty acid ratios 41
3.1.4 Sample grouping according to oil type 42
3.1.5 Ion source optimization 43
3.2 PROTEINS 43
3.2.1 Resolution 44
3.2.2 Signal-to-noise ratios 46
3.2.3 Amino acid ratios and ingroup comparisons 49
3.2.4 Comparison between groups 57

4 DISCUSSION 59
4.1 THE FAME METHOD AND ANALYSIS 59
4.1.1 Sample preparation 59
4.1.2 Analysis 60
4.2 PROTEIN BASED BINDERS 64
4.2.1 Sample preparation in relation to analysis results 64
4.2.2 Analysis 65

5 CONCLUSION 67

REFERENCES 68

APPENDIX 71
Appendix A – Software, instruments and materials 71
Appendix B – Chromatogram peak tables for oils 74
Appendix C – Mass spectra 77
Appendix D – Chromatogram peak tables for amino acids 79

LIST OF TABLES

Table 1.1 Common fatty acids in drying oil lipids 5
Table 1.2 Monoisotopic mass of amino acids 10
Table 1.3 Content of palmitic and stearic acid in different oil types 12
Table 1.4 Relative weights of amino acids in different tempera paints 13
Table 2.1 List of liquid oils 30
Table 2.2 List of dried protein-based samples 31
Table 2.3 List of paint samples 32
Table 2.4 Sample weights of dried samples 34
Table 2.5 Temperature program for the acid assisted hydrolysis. 35
Table 2.6 Temperature program for analysis of silylated amino acids. 37
Table 2.7 GC and MS settings for analysis of silylated amino acids. 37
Table 2.8 GC and MS settings for analysis of fatty acid methyl esters. 38
Table 3.1 C16:0-ME to C18:0-ME ratio in liquid oil samples 41
Table 3.2 C16:0-ME to C18:0-ME ratio in dried oil samples 42
Table 3.3 C16:0-ME to C18:0-ME ratio in oil-based paint samples 42
Table 3.4 Heat optimization of ion source 43
Table 3.5 Retention time for amino acid standards 44
Table 3.6 S/N for egg-based samples 46
Table 3.7 S/N for fish-based samples 47
Table 3.8 S/N for animal glues 48
Table 3.9 Signal-to-noise ratio casein in borax 49
Table 3.10 Leucine/serine ratio in egg-based samples 51
Table 3.11 P-values for egg-based samples 51
Table 3.12 Glycine/alanine ratio in fish-based samples 53
Table 3.13 P-values for fish-based samples 53
Table 3.14 Glycine/alanine ratio in animal glues 55
Table 3.15 P-values for animal glues 55
Table 3.16 Animal glues vs. fish-based samples 57
Table 3.17 P-values animal glues vs. fish-based samples 58
Table 4.1 Result comparisons 62

LIST OF FIGURES

Figure 1.1 General structure of drying oil lipids 4
Figure 1.2 Common fatty acids in drying oil lipids 4
Figure 1.3 Polymerization of fatty acids 6
Figure 1.4 Extraction of fatty acids 8
Figure 1.5 Structure of ovalbumin 9
Figure 1.6 Degradation of amino acid derivatives 13
Figure 2.1 Hydrolysis of a lipid with the assistance of heat and pressure 16
Figure 2.2 Formation and hydrolysis of a peptide bond 16
Figure 2.3 Basic saponification in methanol 17
Figure 2.4 Esterification of fatty acid 17
Figure 2.5 Structure of BSTFA 18
Figure 2.6 Silylation reaction 18
Figure 2.7 Signal peak and resolution 23
Figure 2.8 Diagram of a gas chromatograph 25
Figure 2.9 Mass spectrum of air 26
Figure 2.10 Cross section of an electron ionization source 28
Figure 2.11 Quadrupole mass filter 29
Figure 2.12 Liquid oils 30
Figure 2.13 Flow chart of the different sample preparation methods. 33
Figure 3.1 Chromatogram for oil-based samples 40
Figure 3.2 Chromatogram for protein-based samples 45
Figure 3.3 Amino acid derivatives in egg-based samples 50
Figure 3.4 Amino acid derivatives in fish-based samples 52
Figure 3.5 Amino acid derivatives in animal glues 54
Figure 3.6 Amino acid derivatives in casein in borax 56

ABBREVIATIONS

BSTFA	N, O-bis(trimethylsilyl)trifluoroacetamide
GC	Gas chromatography
LOD	Limit of detection
LOQ	Limit of quantification
m/z	Mass-to-charge ratio
MS	Mass spectrometry
MTBSTFA	N-tert-Butyldimethylsilyl-N-methyltrifluoroacetamide
S/N	Signal-to-noise ratio
SIM	Single ion monitoring
TIC	Total ion count
TMS	Trimethylsilyl

1 INTRODUCTION

1.1 THE EDVARD MUNCH COLLECTION OF REFERENCE PAINTING MATERIALS AT THE MUNCH MUSEET IN OSLO

Edvard Munch (1863-1944) was a prolific Norwegian painter, perhaps best known for the iconic painting "Scream". Munch practiced painting, printmaking, drawing and on occasion sculpturing. Munch experimented with a variety of different art materials and techniques, often without any real knowledge of the properties they held or how they behaved. An example of this is his tendency to paint outdoors in his open-air studio at Ekeli, exposing the paintings to outdoor conditions (Sandu, 2018). The legacy of Munch contains more than 1000 paintings, almost 18 000 prints of over 700 different motifs. There is also 7 700 drawings and watercolors and 13 sculptures. This collection now resides at the Munch Museet in Oslo, where it is displayed, stored and preserved (Sandu, 2018).

The Munch Museet is also in possession of correspondence containing information about how Munch used canvases, paint tubes and other materials. On several occasions he wrote about the painting materials he bought and used, such as the text was written about the creation of the Aula paintings «*The paintings are painted…on the world's most durable canvas - huge canvases that are no longer available. They have been painted with the excellent Winsor & Newton colours*» (Sandu, 2018).

The tube collection alone contains around 900 tubes, representing 31 different brands by 20 different manufacturers: Winsor & Newton, Morin & Janet (Ambor paint manufacturers), Devoe & Rainolds, Schminke, Arnbak, Talens, LeFranc, Le Franc & Bourgeois, Rowney, Gunther Wagner, Herman Neisch, Schoenfeldt, etc. This collection has not been extensively studied until recently.

In order to understand the interactions of the paints and materials in the artworks these tubes need to be investigated further. Manufacturers have been known to adulterate the composition of their products, by adding cheaper materials, which could lead to further degradation of the

artworks (Art Technological Source & Symposium; Public Paintings by Edvard et al., 2015). The study of the paint tube is also of great interest to cross link the findings in the tubes to Munch's artworks. In cases where different binders have been mixed, a library of pure reference materials could be of great value (Sandu, 2018).

The identification process can also be challenging due to some paints may have a very low quantity of binder (less than 20-25 %) in the paint. The degradation and aging process also adds to the challenge (Sandu, 2018).

1.2 THE RELEVANCE OF BINDING MEDIA ANALYSES IN THE ART AND CONSERVATION FIELD

The proper identification of the binding media in a work of art is of great importance for both conservators and art historians as it gives information on the techniques used by the artists (Hurt & Ocon). This does not simply create a better understanding of the materiality of the artwork under study, but it also generates greater understanding of what artistic techniques were available to artists in a given region at a given time in history. This information is of great importance as it can help date artworks, and possibly reveal its place of origin, if additional markers of provenance can be identified (Hurt & Ocon).

With knowledge of the organic binding media, it is also possible to verify the authenticity of artworks. As artworks are often highly valued collectibles, forgeries are often a problem in the art market. In 2004 "Landschaft mit Pferden" was sold to a German collector, later revealed to be a fake. The fake is believed to have been created by a German forgery ring responsible for at least 30 other forgeries to a total value of approximately $49 million (Zeveloff & Weiss, 2015).

For the conservators, knowledge of the type of binder used in a painting is necessary in order to understand its degradation mechanisms and to provide the best treatment possible for the preservation of the work. When treating a painting on canvas, there are four materials or layers that must be considered: the canvas itself (as a support), the ground layers, the paint layers (comprised of pigment, binder, filler and other additives), and the surface coating known as varnish. All these layers and their constituent materials must be considered when deciding what

kind of conservation treatment the painting can undergo (Brajer, 2009; Insall et al., 2017). All of them have their own physical and chemical limitations with regards to different conservation materials and methods. A poor choice in conservation method or material can in some cases lead to irreversible damage or permanent alteration of the painting in question (Tveit & Ferrer, 2016).

1.3 BINDING MEDIA

Binders are the organic media holding all the other components of the paint together, rendering it applicable over a surface or support (canvas, cardboard, paper, silk, wall etc.). Humankind has experimented with binders for up to 40 millennia (Aubert et al., 2018; Hurt & Ocon). Early man is recorded to have used various plant extracts and animal fats to attach pigments to cave walls. Through the ages the methods became more refined, leading to the diversification of the materials from different sources: plants secretions (gum Arabic, cherry tree gum), drying oils, glues from animal tissues and other animal products (beeswax, milk and eggs) (Hurt & Ocon). Each of this binding media has its own physical and chemical properties, aesthetic appearance, specific degradation, and ageing patterns (Schellmann, 2007; Winsor-Newton, u.d.). A short description of the main features of each of these two main groups, drying oils and protein-based binders, is given below.

1.3.1 Drying oils

Drying oils are obtained from seeds of plants as poppy flowers, linen and walnuts. The oils are mainly constituted of triglycerides, which are esters derived from glycerol and three fatty acids. The triglyceride stem remains a constant, but the three fatty acid branches can consist of various fatty acids as shown in figure 1.1 (Thompson, 2018). A list of some common fatty acids in drying oils are shown in figure 1.2.

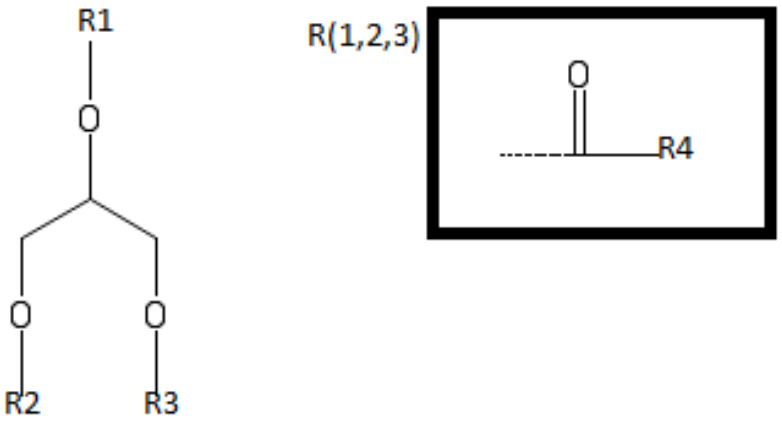

Figure 1.1 General structure of drying oil lipids
Shows the triglyceride stem on the left and the fatty acid branches on the right. R1, R2 and R3 can form ester bonds to the fatty acids forming a bond through the stippled line. R4 represents a carbon chain, some examples of which can be seen in figure 1.2. The structures are based on the descriptions provided by (Thompson, 2018).

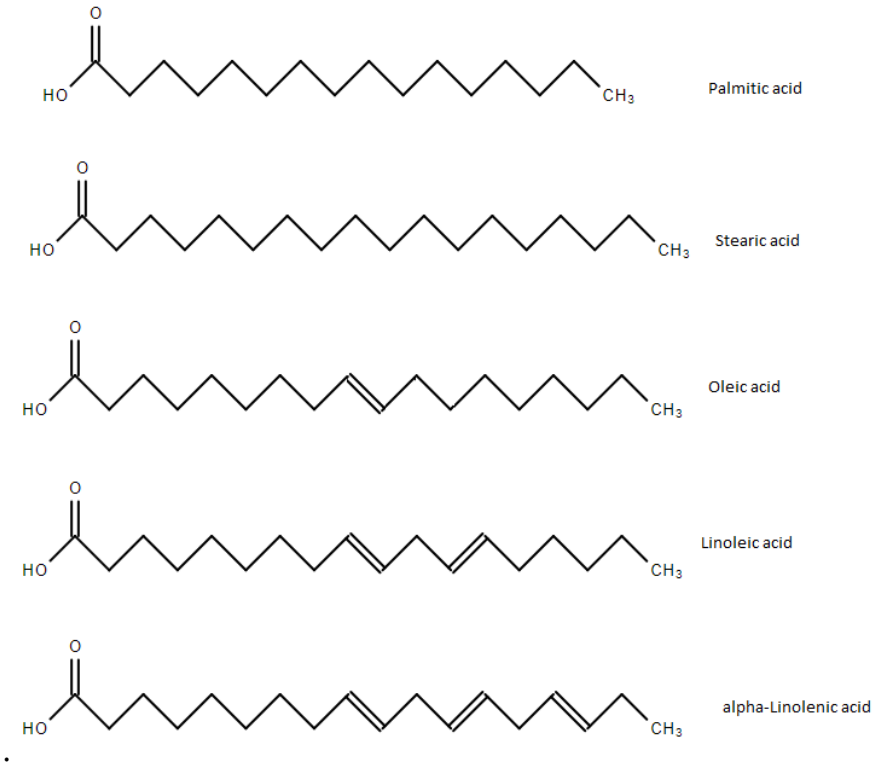

Figure 1.2 Common fatty acids in drying oil lipids
Shows four types of fatty acids often found as parts of drying oil lipids. From top to bottom: palmitic acid, stearic acid, oleic acid, linoleic acid and alpha-linolenic acid. See table 1.1 for addition information about these structures. The fatty acids chosen for this figure were based on the fatty acids analyzed by Colombini et al. (1999).

The fatty acid branches can comprise of various fatty acids (Thompson, 2018) and are often referred to by the number of chained carbon atoms to number of double bond ratio in the fatty acid (C:D) (IUPAC, u.d.). A selection of fatty acids is given in table 1.1.

Table 1.1 Common fatty acids in drying oil lipids
Shows the fatty acids found in figure 1.1 with monoisotopic molecular mass (ChemSpider, u.d.) and carbon-to-double bond ratio (C:D) (IUPAC, u.d.). The fatty acids depicted are the same as studied in other art conservation literature (Colombini et al., 1999).

Fatty acid name	C:D	Monoisotopic molecular mass (Da)
Palmitic acid	C16:0	256.240234
Stearic Acid	C18:0	284.271515
Oleic acid	C18:1	282.25589
Linoleic acid	C18:2	280.240234

As the triglyceride stem only has room for three fatty acids, a drying oil will be comprised of different triglycerides with different variations of fatty acids attached to them. The distribution of fatty acids in a given drying oil will however remain relatively constant (not accounting for degradation and variation in genotype) (Bayrak et al., 2010; Colombini et al., 1999; Lukey, 2001).

The differences in content of saturated and unsaturated fatty acids in the lipids are part of what distinguishes different drying oils from each other (Colombini et al., 1999). There can be some variations in the distribution of fatty acids in some plant-based oils based on the genotype of the plant in question. Literature reports that a variation in fatty acid composition in linseed oil was proven to be 4.07-7.02 % of total unsaturated fatty acid content for C16:0, and 3.21-6.70 % of the total unsaturated fatty acid content for C18:0, for the various genotypes analyzed (Bayrak et al., 2010). Variations like this should, with a high probability, be expected to occur in other plants used in the production of drying oils.

Function

Drying oils function as binders because of the unsaturated fatty acids in the lipids. These fatty acids contain double bonds that are susceptible to oxidation. This occurs when the non-conjugated double bonds in the triglycerides react with oxygen, forming hydro peroxides. These hydro peroxides decompose, forming oxy-radicals. This may lead to inter and intra cross linking between lipids (Lukey, 2001), by the formation of ether bonds as seen in figure 1.3.

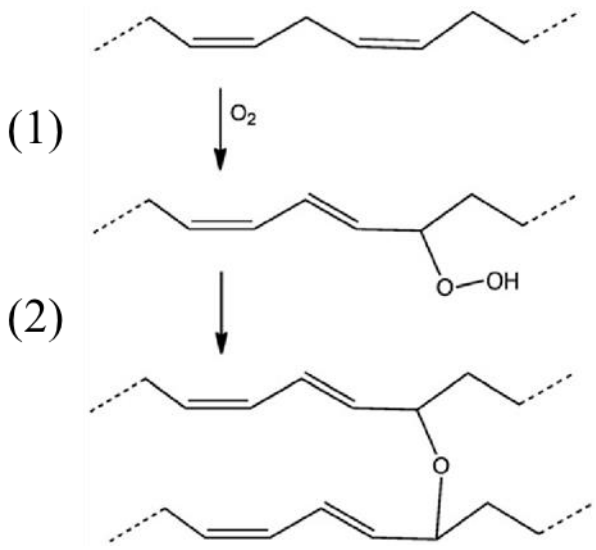

Figure 1.3 Polymerization of fatty acids
Shows the polymerization of two fatty acids by means of oxidation (Jadhav et al., 2013). (1) A non-conjugated fatty acid reacts with oxygen forming a peroxide by moving the double bond. (2) The peroxide can then react with another fatty acid by the elimination of H2O resulting in ether bond formation.

This polymerization process can alternatively result in peroxide and carbon-carbon bonds as well as ether bonds. To speed the rate of polymerization of non-conjugated drying oils, catalysts are sometimes used. These may consist of octanoates and naphthenates of cobalt, manganese, lead, and zirconium. These compounds are believed to act as catalysts for the formation of hydro peroxides in the fatty acids (Lukey, 2001).

The polymerization of conjugated fatty acids is also possible; these are generally faster reactions and formed by free radical chain growth (Lukey, 2001). However, as the fatty acids relevant for this study are mainly non-conjugated, this reaction will not be explored further.

The polymerization of the fatty acids in the lipids ideally form a continuous chain of lipids forming a large latticework, within which the pigment and other components are held in place. This superstructure is reliant on the direct bonds between the fatty acids, and the triglycerides holding the fatty acids together. It is the breaking of these bonds that in large lead to the degradation of drying oils (Lukey, 2001).

Degradation

The degradation of drying oils happens in two stages. The first is a part of the natural function of the binder, the uptake of oxygen. During this first stage, oxidation occurs leading to an uptake of mass in the form of oxygen. This may lead to an increase in weight of up to 14 % of the initial weight of the drying oil. After this initial stage, the oil will begin to lose weight in the form of small volatile compounds diffusing out of the dried oil (Tumosa & Meckleburg, 2013).

Tumosa & Meckleburg (2013) used linseed oil as a benchmark, stating that any oil with a lower linoleic acid content than linseed oil may have a greater weight loss than that gained in the initial stage. This weight loss may lead to channels and cavities in the dried oil, which can lead to pooling of solvent and even the collapse of the three-dimensional structure of the polymer framework.

Another degradation process affecting the three-dimensional structure of drying oils is hydrolysis. The ester bonds between the triglyceride stem and the fatty acids are susceptible to hydrolysis. Should these bonds be hydrolyzed, much of the structural strength described previously is lost. This is especially true for pants that are high in oleic acid as these fatty acids only have one double bond, thus are only able to polymerize with one other fatty acid without relying on the triglyceride stem. The hydrolysis of these bonds also completely frees palmitic and stearic acid. This may, in some cases, lead to these two fatty acids pooling in certain areas of the drying oil (Tumosa & Meckleburg, 2013). An example of this can be seen in figure 1.4.

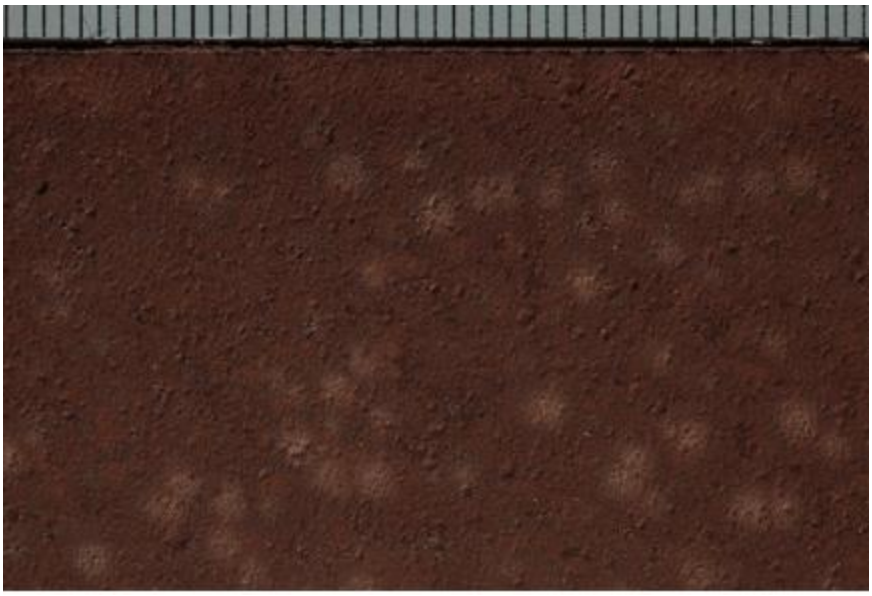

Figure 1.4 Extraction of fatty acids
Shows the extraction of fatty acids (yellowed dots) on a film of burnt umber paint (Tumosa & Meckleburg, 2013). The scale at the top is given in millimeters

Furthermore, after the breaking of the ester bonds, a carboxylic acid group is freed. This leads to the increase in acidity in the drying oils. When in a paint, this increase in acidity can lead to reactions with substrates and pigments. These reactions can lead to the further creation of acids by oxidation. A common example of this kind of reaction occurs at C9 if a double bond is present at this point in the chain. This leads to the formation of azelaic acid. If stored at relatively high temperatures (70-80 °C), the freed palmitic and stearic acids can evaporate, leading to further loss of mass and stability in the binder. With large enough loss of mass, shrinkage and cracking may occur (Tumosa & Meckleburg, 2013).

1.3.2 Protein-based binders

Protein-based binders are proteins that are added to paint or used as adhesives. Proteins have been used in this way by humankind for millennia due to their ability to adhere to surfaces and each other when treated in specific ways (Aubert et al., 2018; Schellmann, 2007). This chapter will explore the properties, function and degradation of proteins as binders in paints.

Properties of proteins

Protein-based binders consist of aggregated proteins, large biomolecules that consist of amino acids held together by peptide bonds (polypeptide chains). The structure of a protein in its natural state is determined by the sequence of amino acids it is made up of.

The protein-based binders analyzed in this study are animal glues (fish and mammalian), egg and casein from milk. All are made up of several different proteins. They do however share many of the same basic mechanisms as binders. They all consist of proteins that are denatured, by heat or solvent (Gossett et al., 1984). An example of a protein found inn egg white (ovalbumin) is given in figure 1.5. A selection of the amino acids found in protein-based binders relevant to this study is given in table 1.2.

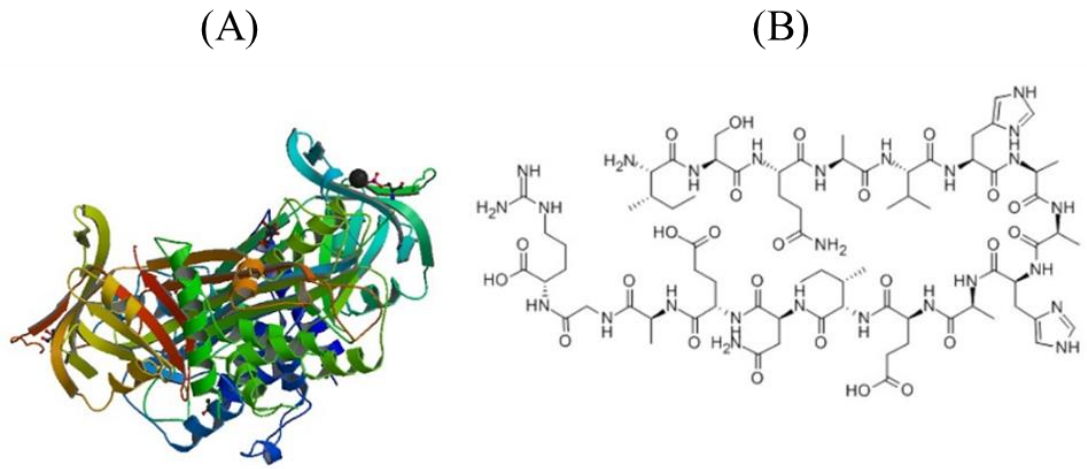

Figure 1.5 Structure of ovalbumin
Shows (A) the three-dimensional structure (Foresight-Biosciences, u.d.) and (B) the molecular structure (APExBIO, u.d.) of ovalbumin.

Table 1.2 Monoisotopic mass of amino acids
Shows the monoisotopic mass of 12 amino acids commonly found in proteins in protein-based binders (ChemSpider, u.d.).

Amino Acid	Monoisotopic mass (Da)
Alanine	89.047676
Glycine	75.032028
Valine	117.078979
Leucine	131.094635
Isoleucine	131.094635
Glycine	75.032028
Serine	105.042595
Threonine	119.058243
Aspartic Acid	133.037506
Hydroxyproline	131.058243
Glutamic Acid	147.053162
Phenyl Alanine	165.078979

Function as binders

Denaturation breaks the intramolecular bonds in the protein, while leaving the peptide bonds intact. Primarily hydrogen bonds are broken this way, and the rate of denaturation is determined by the degree of breakage of these non-peptide bonds.

Denaturing a protein can be done in several ways. Heat is a common method, but altering other factors in the protein environment is also possible. This can include methods such as altering the pH value or the polarity of their surroundings (Haurowitz & Koshland, 2019).

When denatured, the three-dimensional structure is unfolded from its usually compressed state, leaving long chains of amino acids. By removing the source of denaturation, the protein can either re-assume its natural shape or aggregate with other proteins. The latter is the reason why proteins can be used as glues or binders (Schellmann, 2007).

Degradation

As with oil paints, protein-based paints are also subject to degradation. As an example, rabbit glue may be subject to degradation due to UV light, heat, dirt, insects, mould and interactions with certain pigments (Down, 2012; Schellmann, 2007). UV light especially has an ability to break hydrogen bonds, thus weakening the cohesion of the binder (Down, 2012).

1.4 ESTABLISHED METHODS

The methods used in this Master thesis drew inspiration mainly from two sources. Two paint sample analyses conducted at the University of Pisa (Colombini, 1998, 1999), and a master thesis analyzing the content of fatty acids (Bekken, 2013).

1.4.1 University of Pisa – methods for analysis of binders

Two papers relevant to this study have been published by Colombini et al. on the topic of analyzing binders in paint. One paper describes their analysis of both protein-based binders and drying oils (Colombini et al., 1999). The other contains the method of extraction and derivatization of amino acids (Colombini et al., 1998).

Methods

Drying oils

The method used by Colombini et al. in the 1999 paper, starts by breaking down the samples through microwave assisted acid digestion to free the fatty acids in the oils, and then derivatizing the fatty acids with a reagent named N-tert-Butyldimethylsilyl-N-methyltrifluoroacetamide (MTBSTFA) to make them compatible with GC-MS. This paper produced some results on the fatty acid content of different types of paint that were used in this study. An excerpt of these findings are provided in table 1.3. The C16:0-to-C18:0 ratios in table 1.3 displays a slight increase in fatty acid ratio for linseed oil and poppy oil with the addition of white lead pigment. Walnut oil, oil from egg and the mix of egg and linseed oil shows a slight decrease in amino acid ratios. The table also provides a 2-9 %, relative standard deviation, so the changes in the relative masses could be a consequence of this.

Table 1.3 Content of palmitic and stearic acid in different oil types
Shows the content of Palmitic and stearic acid in linseed oil, walnut oil, poppy oil, egg, a mix of linseed oil and egg and the previously mentions oils when mixed with white lead pigment (Colombini et al., 1999). The numbers given for palmitic and stearic acid are percentages of the total of fatty acids present in the samples. The palmitic to stearic acid ratio was not originally a part of the Pisa paper (calculated for the purposes of this study).

Oil type	Palmitic Acid (%)	Stearic Acid (%)	Palmitic acid/Stearic Acid ratio
Linseed oil	20.4	14.9	1.4
Walnut oil	22.5	9.6	2.3
Poppy oil	26.4	7.1	3.7
Egg	34.0	11.2	3.0
Linseed oil + egg (1:1 mix)	27.6	12.3	2.2
Linseed oil + white lead pigment	21.7	13.7	1.5
Walnut oil + white lead pigment	21.0	9.9	2.1
Poppy oil + white lead pigment	26.2	6.7	3.9
Egg + white lead pigment	33.0	12.5	2.6
Linseed + egg + white lead pigment	27.4	13.7	2.0

Protein based binders

The method employed by Colombini et al. to prepare and analyze the samples containing protein-based binders is described in the 1998 paper.

In their method, they make use of the same process of microwave assisted acid digestion and derivatization reagent as the 1999 paper. This paper reports the stability of the MTBSTFA derivates over time (figure 1.6). This paper also produced some other results relevant to this study, namely the amino acid ratios found in various reference tempera paints with different protein-based binders (table 1.4).

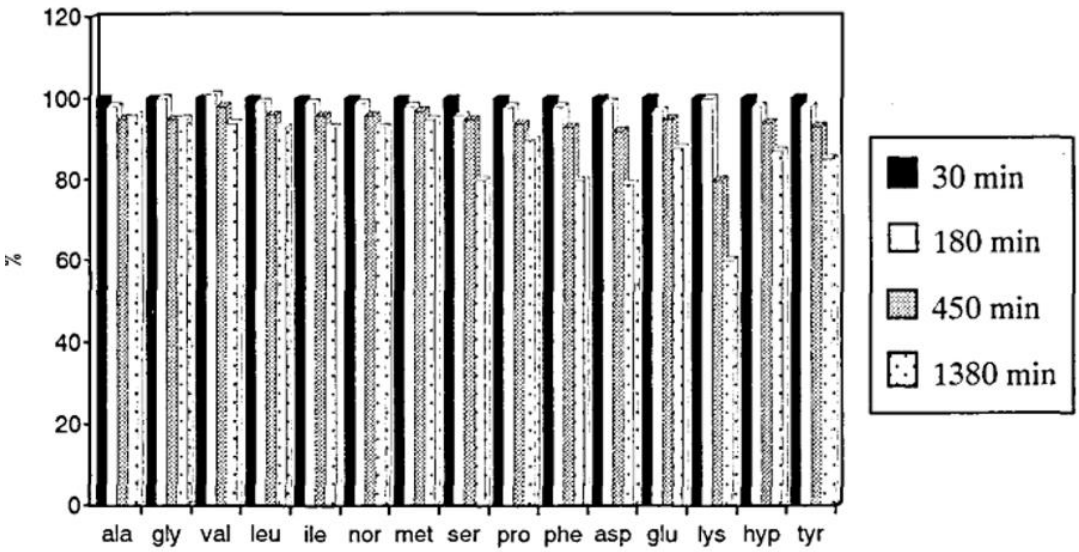

Figure 1.6 Degradation of amino acid derivatives
Shows the degradation of the amino acid derivatives used by the University of Pisa (Colombini et al., 1998). The graph illustrates the measured relative weight of alanine (ala), glycine (gly), valine (val), leucine (leu), isoleucine (ile), norvaline (nor), methionine (met), serine (ser), proline (pro), phenyl alanine (phe), aspartic acid (asp), glutamic acid (glu), lysine (lys), hydroxy proline (hyp) and tyrosine (tyr) after 30 (100 %), 180, 450 and 1380 minutes of storage.

Table 1.4 Relative weights of amino acids in different tempera paints
Shows the measured relative weight of the amino acids in six types of tempera paint (based on egg, milk, animal glue, animal glue + egg and animal glue + milk) (Colombini et al., 1998). The weight is relative to the weight of the sample analyzed. The amino acids analyzed were alanine (ala), glycine (gly), valine (val), leucine (leu), isoleucine (ile), norvaline (nor), methionine (met), serine (ser), proline (pro), phenyl alanine (phe), aspartic acid (asp), glutamic acid (glu), lysine (lys), hydroxy proline (hyp) and tyrosine (tyr).

Tempera	*Egg*	*Milk*	*Animal glue*	*Glue + egg (1:1)*	*Glue + milk (1:1)*
ala	11	5	14	13	12
gly	6	3	38	22	27
val	13	12	3	8	7
leu	18	16	5	10	9
ile	11	10	2	5	5
met	5	3	1	2	2
ser	11	3	3	7	2
pro	3	4	2	4	4
phe	8	9	3	7	6
asp	4	8	8	6	6
glu	7	22	14	11	16
lys	—	—	—	—	—
hyp	0	0	6	3	3
tyr	3	4	1	2	2

When compared to analyses done on paintings from the 13-17 century, some observations were made on the aging process of the amino acid ratios (Colombini et al., 1998):

- The content of proline was found to decrease over time.
- The ratios of Leu/Ala, Val/Ala and Ala/Phe remained unchanged when comparing the old paints to the references.
- Glu and Asp decreased by a large margin in egg-based binders, and to a slight degree for Ser.
- Pro/Leu was the only ratio in animal glue that showed a high decrease over time. This ratio was decreased by a factor of five.
- Casein binders showed no significant changes.
- It was difficult to get consistent results when analyzing lysine.

1.4.2 FAME analysis

As an alternative to MTBSTFA derivatization, hydrolysis followed by methylation can be performed. The details of this derivatization will be given in chapter 2.1.2. This technique produces fatty acid methyl esters (FAME), which are compounds well suited to GC-MS analysis. In a master project conducted by Bekken, this derivatization technique was used in the analysis of fatty acids found in human lipids (Bekken, 2013). The methods used in her study were employed to analyze phospholipids found in human serum. As lipids are not the primary content of serum and the sample size taken was 800 µl, the method was deemed to be a good starting point for the work in this study. Namely, analyses that could work without copious amounts of analytes.

In the 2013 study by Bekken, the derivatization was preformed using an acid (HCl) in a water-free methanolic environment to saponify the lipids. This step also served as the acid catalyzed esterification of the fatty acids. The pH in the samples were adjusted with sodium bicarbonate. The fatty acid methyl esters were then extracted with n-hexane and ready for analysis.
The 2013 study performed the GC-MS analysis on a BPX70 column suited for separation of volatile polar compounds (TRAJAN, u.d.).

The same study also remarked that the ideal injector temperature for such an analysis was 280 °C, as to avoid discrimination of the heavier FAMEs.

1.5 STUDY OBJECTIVES

In the conservation of works of art, knowing the composition of the paint is of great importance. One such component is the binder used in the paint. Establishing a reliable method of analysis of binders is a long process, the first step of which is to establish a reference library of spectra for different types of binders for use in further research. With this aim in mind, this study will attempt to establish a library of chromatographic and mass spectra of binders in their pure form. This is to avoid interference of any component in actual paint that could alter the results.

To establish a reference library of spectra, one must first have a reliable method to generate the spectral data. The reliability is evaluated by the methods ability to create accurate and reproducible spectral data. Any spectral data obtained through such a method should ideally be unique to the analyzed sample in question, so it may be distinguished from data obtained from the analysis of a different binder.

As art conservators prefers their methods to as un-invasive as possible, establishing a method capable of analyzing as small sample as possible is desirable. Determining the lower limit of detection for the analytical method is therefore an important goal of this study. As a secondary objective, this study will also test the method on a set of paints with known binders, as a test of accuracy for the method, and to evaluate whether the method can indeed be used on paint samples.

The goals of this study can be summarized as follows:

I. To establish one or more analytical methods to identify the binders in different paints.

II. To evaluate the uniqueness of the spectra generated by the methods developed.

III. To determine a lower limit of detection for the method.

IV. To test the viability of the methods developed on real paint samples.

V. To compare any results of real paint samples to the spectra generated by the analysis of raw/pure binders.

2 METHODS

2.1 THEORY ON ANALYTICAL METHODS

2.1.1 Microwave assisted acid digestion

The hydrolysis performed in this study serves as both a precursor to the later derivatization and as a means of breaking apart paints and dried binders. This method makes use of the laws of reaction kinetics to accelerate the process of hydrolysis, by the presence of acid, heat and pressure (LibreTexts, 2019). The hydrolysis process for lipids and proteins are shown in figure 2.1 and figure 2.2, respectively. Microwave assisted acid digestion is thus a versatile tool when hydrolyzing lipids and peptides and is used in the sample preparation of established paint analyses (Colombini et al., 1999; LibreTexts, 2019; Schoolbag, n.d.).

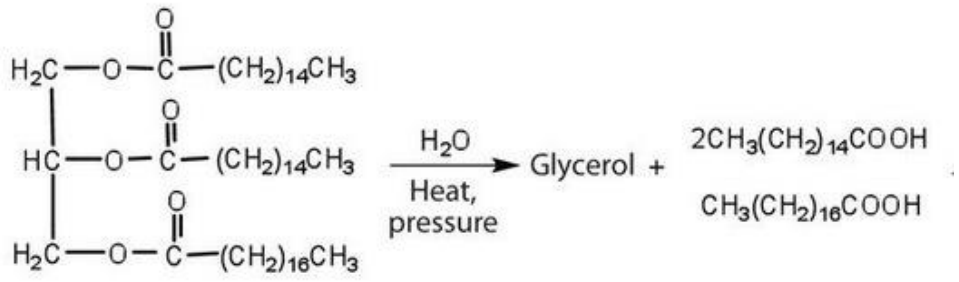

Figure 2.1 Hydrolysis of a lipid with the assistance of heat and pressure (LibreTexts, 2019*).*

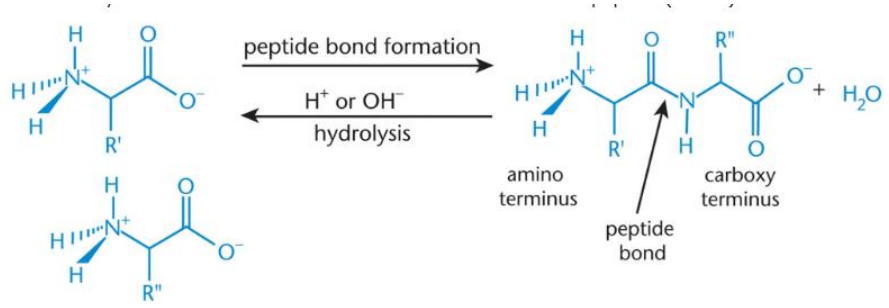

Figure 2.2 Formation and hydrolysis of a peptide bond (Schoolbag, n.d.). The hydrolysis can be done the presence of either an acid or a base.

2.1.2 Derivatization

Derivatization is a method of altering an analyte before analysis. When the analyte is to be analyzed by GC, there are two common reasons for derivatization. The first one is to increase the volatility of the compound, making analysis of non-volatile analytes possible. The second is reactivity. There are several active groups that can react with the stationary phase of a GC column. For instance, WAX columns are susceptible to hydrolysis if exposed to proton donors, thus such groups should be altered to avoid degradation of the stationary phase (AGILENT, n.d.). This study utilizes three such methods of derivatization, namely saponification, esterification and silylation.

Saponification and esterification of fatty acids

Saponification is the process of breaking an ester bond by hydrolysis. This can be done with the assistance of an acid or a base. In this study, saponification was done with NaOH in methanol forming fatty acid anions (LibreTexts, 2014). The reaction equation for this process is given in figure 2.3.

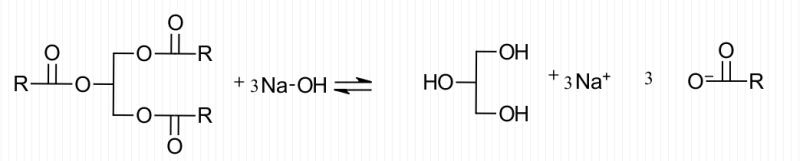

Figure 2.3 Basic saponification in methanol. R denotes a random carbon chain. The figure is based in illustrations found at LibreTexts (2014).

Once separated from the glycerol stem, the fatty acids can be esterified with methanol in an acidic solution. This reaction should be performed in the absence of water, pushing the equilibrium towards fatty acid methyl esters (Britannica, 2016). -ME was added to the nomenclature for fatty acids in this study, to distinguish them from regular (non-derivatized) fatty acids. The reaction equation for this process is given in figure 2.4.

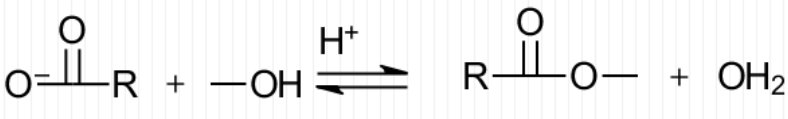

Figure 2.4 Esterification of fatty acid
Shows the esterification of fatty acids in an acidic methanol solution, forming fatty acid methyl esters. R denotes a carbon chain. The figure is based in illustrations found at LibreTexts (2014).

Sialylation

Silylation is a common method of derivatization for GC analysis. This derivatization method replaces an active hydrogen with an alkylsilyl group. The silylation reagent used in this study is BSTFA (N, O-bis(trimethylsilyl)trifluoroacetamide) (figure 2.5). The alkylsilyl group for this reagent is trimethylsilyl (TMS) (Sigma-Aldrich, n.d.). BSTFA reacts with the active proton in the -OH, -COOH, =NH, -NH_2 and -SH groups (Sigma-Aldrich, n.d.) as shown in figure 2.6.

Silylation of a compound, increases its volatility, reduces polarity, and increases its thermal stability. It also increases the molecular weight of the initial compound by 72 Da per TMS silylation per group. Silylated compounds are susceptible to hydrolysis by water. The reaction should thus be performed in an atmosphere devoid of H_2O(due to active protons) and stored in sealed containers (Sigma-Aldrich, n.d.).

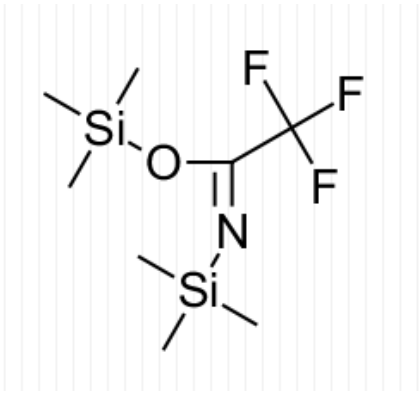

Figure 2.5 Structure of BSTFA (Sigma-Aldrich, n.d.).

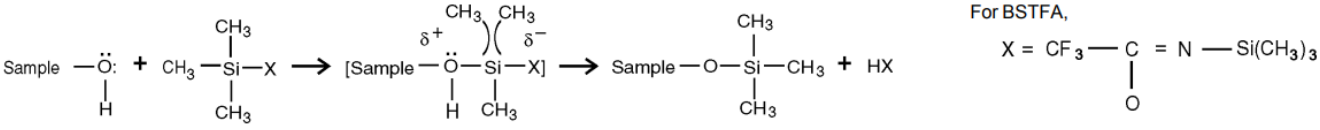

Figure 2.6 Silylation reaction
Shows the silylation reaction (Sigma-Aldrich, n.d.). The sample compound in this reaction has an active proton as part of a hydroxy group. X represents the leaving group of the BSTFA reagent.

2.1.3 Chromatography

Chromatography is a technique used to separate different types of molecules in a solution. This is done by injecting the solution into a column with two phases. One phase remains stationary (stationary phase), while the other moves continuously through the column (mobile phase).

The purpose of the mobile phase is to function as a carrier for anything entering the column, while the purpose of the stationary phase is to slow down the progress of the molecules entering it. The rate at which the stationary phase retains different types of molecules is the factor separating the molecules entering the column. The mechanisms of retention are physiochemical, but may otherwise varry greatly. These will be explored later in this chapter.

Given a solution containing two solutes (distinct chemicals) A and B, if solute A has a greater ability to be retained by the stationary phase than solute B, solute A will spend more time traversing the column. The factor at which a solute is retained in the stationary phase of the column is called the retention factor (Harris, 2010).

Retention factor

The retention factor *k* is a measurement of how much time a solute spends in the stationary phase (adjusted retention time, t'_r) divided by how much time it spends in the mobile phase (t_m) (Harris, 2010). This is calculated with equation 1.

$$k = \frac{t_r - t_m}{t_m} = \frac{t'_r}{t_m} \qquad \text{(Equation 1)}$$

k: the retention factor.

t_r: the time it takes the solute to pass through the column.

t_m: the time it takes the mobile phase to pass through the column.

t'_r: the amount of time the solute spends in the stationary phase.

Given a solute that takes twice the time it takes the mobile phase to pass through the column, it is logical that it will have spent half of its time in the stationary phase. This translates into moles of solute in the stationary phase divided by moles of solute in the mobile phase. This can also be expressed by using the a partition coefficient *K* (Harris, 2010). Both examples are given in equation 2.

$$k = \frac{c_s V_s}{c_m V_m} = K \frac{V_s}{V_m} \quad \text{(Equation 2)}$$

c_s: the concentration of the solute in the stationary phase.

V_s: the volume of the stationary phase.

c_m: the concentration of the mobile phase.

V_m: the volume of the mobile phase.

K: is the partition coefficient (c_s/c_m).

Equation 2 shows that two solutes with different physiochemical properties will have different retention factors when passing through the same column under the same conditions. The degree of chromatographic separation between two solutes is given by the ratio between their respective retention factors, or relative retention α. Relative retention can be expressed in three ways, as shown in equation 3 (Harris, 2010).

$$\alpha = \frac{t'_{r2}}{t'_{r1}} = \frac{k_2}{k_1} = \frac{K_2}{K_1} \quad \text{(Equation 3)}$$

k_1: the retention factor for the first of the two solutes to exit the column.

k_2: the retention factor for the second of the two solutes to exit the column.

t'_{r1}: the adjusted retention time for the first of the two solutes to exit the column.

t'_{r2}: the adjusted retention time for the second of the two solutes to exit the column.

K_1: the partition coefficient for the first of the two solutes to exit the column.

K_1: the partition coefficient for the first of the two solutes to exit the column.

α: the relative retention.

The relative retention between two solutes is a measurement of how much they will separate when passing through the same column, given the same conditions. The greater the relative retention, the more separation. This does not however, account for any diffusion or band widening effects that occurs within the column (Harris, 2010).

Diffusion

As a band of solute moves through the column, diffusion will occur. Ideally, the band should be infinitely thin at the entrance of the column, but it will have broadened as it exits. This is due to the random movement of molecules in the solute as it moves through the column. This random movement will move molecules from areas of high concentration to areas of low concentration. The concentration of the solute will remain highest at the center of the band, causing it to give the band a Gaussian shape in the detector (Harris, 2010).

Diffusion rate is given by the diffusion coefficient *D*. This is defined by the flux *J*, which is the movement of number of molecules per square meter per second. The diffusion coefficient is a negative value to account for the net flux from areas of high concentration to areas of low concentration. The definition of the diffusion coefficient is given in equation 4 (Harris, 2010).

$$J = -D\frac{dc}{dx} \qquad \text{(Equation 4)}$$

J: the number of molecules crossing each square meter per second (mol/m^2*s).

D: the diffusion coefficient.

The rate of diffusion is highly dependent on the type of solute and the mobile phase they move in. As a rule of thumb, the diffusion coefficient will be approximately 10^4 times lower in a liquid than in a gas. Larger molecules will also diffuse considerably slower than smaller ones. As an example, serum albumin diffuses 100 times slower than glycine in water (Harris, 2010).
What can be observed from equation 4 however, is that diffusion of a solute increases with time spent in the column (Harris, 2010).

There are different mechanisms causing diffusion and band widening in GC. Diffusion as a mechanism does create the need for better quality control than relative retention. This is done by calculating resolution (Harris, 2010).

Resolution

Because of the band broadening effect of diffusion, two solutes with similar but different retention factors can still overlap. This creates a need for a way to measure the quality of separation, hence resolution. Resolution is a product of the difference in retention time between two detected solute peaks, and their respective peak widths. This is generally done using the width at half the peak height, as this makes it easier to measure (Harris, 2010).

The equation for calculating resolution is given in equation 5 (Harris, 2010). Examples of different resolution values between signal peaks is given in figure 2.7. As observed in this figure, a resolution of 1.5 or above is preferred, as there is only an overlap of 0.13 % (LibreTexts, 2016).

$$Resolution = \frac{\Delta t_r}{w_{av}} = \frac{\Delta V_r}{w_{av}} = \frac{0{,}589\Delta t_r}{w_{\frac{1}{2}aw}} \qquad \text{(Equation 5)}$$

Δt_r: the difference in retention time between the two peaks measured in time.

ΔV_r: the difference in retention time between the two peaks measured in volume.

w_{av}: the average of the two peak widths.

$w_{1/2aw}$: the average of the two peak widths at half peak height.

0,589: a constant correcting for the use of half height.

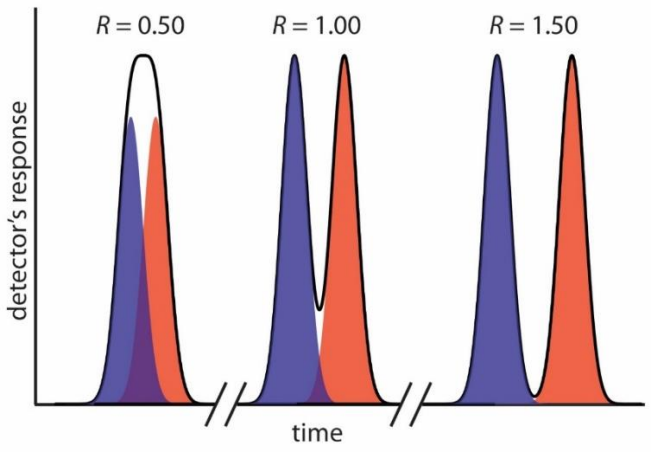

Figure 2.7 Signal peak and resolution
Shows three pairs of signal peaks with different resolutions (R) (LibreTexts, 2016). Blue denotes solute 1, red denotes solute 2 and purple denotes the overlap of solute 1 and 2.

Signal-to-noise ratio

The signal-to-noise ratio (S/N) is a measurement of the quality of a chromatographic peak. There are multiple ways of measuring S/N. One method used for calculating S/N, provided by the Chromelion software, is given in equation 6. The distance of the noise measurement from the base of the peak can be altered. In this study, the default setting for Chromelion 7.2.9 was used in all analyses.

The S/N is used as a quality measurement, defining the limit of quantification (LOQ) and the limit of detection (LOD). When the signal of a peak becomes lower than the limit set as the LOQ, it is too low for reliable quantification of the peak. Likewise, with the LOD, any peak signal falling below this threshold is considered too low for detection (Harris, 2010). LOD and LOQ is often set to 3 and 10, respectively, as this corresponds to relative standard deviation of 5 and 15 % (Dolan, 2006).

$$\frac{S}{N} = 2 * \frac{Peak\ Height}{Average\ noise\ height} \qquad \text{(Equation 6)}$$

Peak height: the height (measured in total ion count, TIC) of the peak in relation to the baseline.

Noise height: the height (TIC) of the noise from lowest to highest point within a set distance (minutes) from the base of the peak in each direction.

Gas chromatography (GC)

This type of chromatography follows the principles described in beginning of this chapter. The mobile phase in this system is comprised of a gas, high purity Helium (Harris, 2010). The type of stationary phase can vary, in this study two WCOT (Wall coated open tubularly) capillary columns were used. In this variation of the system, the solutes are separated by being transported through a long (several meters) thin capillary column, coated with a solid or liquid stationary phase. There are several factors that impact the separation of solutes in gas chromatography. The solutes affinity for the stationary phase in the column and the volatility of the solute. The affinity of the solute for the stationary phase is determined by the solutes ability to bond with or be absorbed by the stationary phase. The volatility is determined by the molecular mass of the solute, intermolecular bonds and temperature. The only one of these parameters that can be altered during a GC separation is the temperature. As the solutes only move through the column while in gas phase, the temperature in the column is one of key factors in successful GC separation. There are two main factors impacting the separation of solutes are volatility (a product of molecular size, intermolecular bonds and temperature) and the solutes affinity to the stationary phase of the column (either through solubility or adsorption strength) (Harris, 2010).

To this end, a gas chromatograph is constructed around an oven that houses the column. One side of the column is connected to a heated injection module, while the other end is connected to a detector. The injection module is also where the mobile phase enters the column. As with maintaining steady temperatures, a steady non-fluctuating gas flow is also paramount to be able to produce analyses with any degree of reproducibility. To maintain this the instrument needs a precise flow controller, assisted by mechanisms in the injector. A diagram of a gas chromatograph is given in figure 2.8.

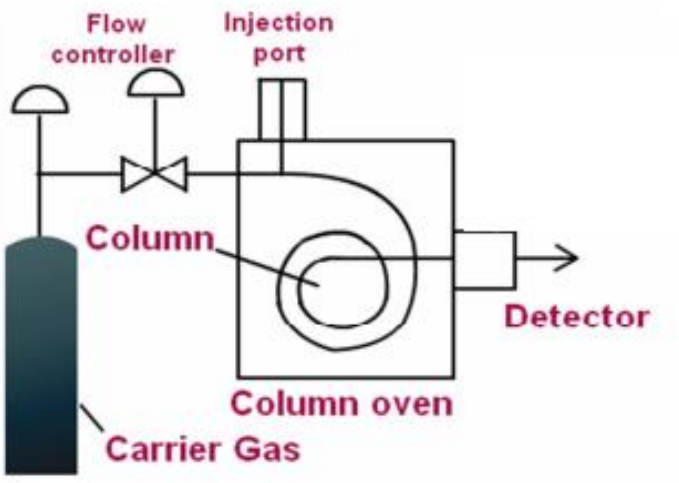

Figure 2.8 Diagram of a gas chromatograph (Bekken, 2013).

The injection technique used in this study is called split injection. With split injection, the sample is injected into a heated glass liner leading into the entrance of the column, the liner is heated to evaporate the sample injected. Warm carrier gas is also flowing through the liner to further assist in the rapid vaporization of the sample. The heat should be high enough to rapidly heat the sample homogenously without exceeding the temperature limits of the column. The glass liner also has two other outlets, a purge vent allowing gas to glow beneath the injection septum to avoid that space becoming a dead volume. There is also a slit valve to regulate compensate for the volume increase when the sample vaporizes, so the flow through the column remains constant (Harris, 2010).

2.1.4 Mass spectrometry (MS)

Mass spectrometers are qualitative and quantitative detectors capable of analyzing almost any atom or molecule carrying a positive or negative charge. These instruments are capable of separating molecules based on the relationship between their molecular weight and the charge held by the molecule. This is known as the mass-to-charge ratio (m/z), and is defined in equation 7 (Harris, 2010).

$$\frac{Molecular\ mass}{Charge\ held\ by\ molecule} = m/z \qquad \text{(Equation 7)}$$

Where:

The molecular mass is given in u or Da.

The charge is the formal charge of the molecule.

The secondary feature of mass spectrometers is to measure a signal intensity relative to the number of ions that reach the detector unit. The combination of these two features makes the mass spectrometer a powerful tool for analysis, capable of measuring the number of different ions present in an analyzed sample at the same time. This is represented in a mass spectrum (Harris, 2010). An example of a mass spectrum is provided in figure 2.9.

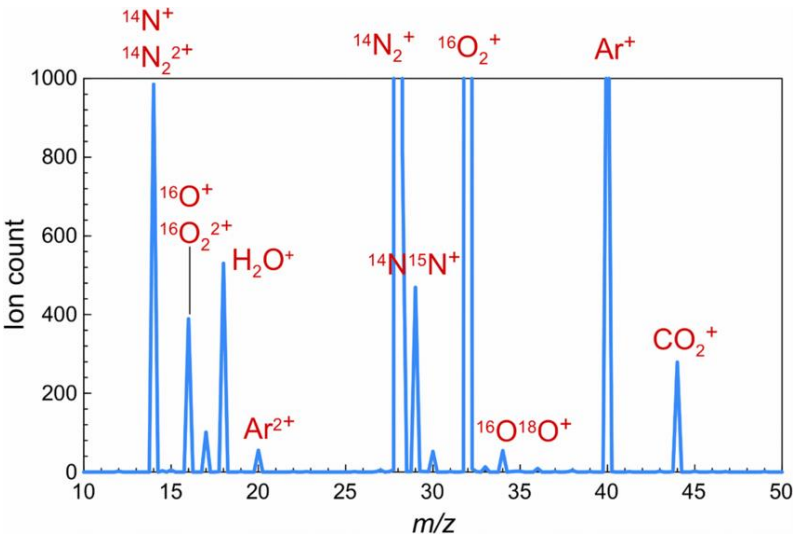

Figure 2.9 Mass spectrum of air
Shows the mass spectrum of air, with the mass-to-charge (m/z) on the x-axis and the ion count on the y-axis (Ohkubo et al., 2014).

The gasses analyzed in figure 2.9 shows the relationship between mass and charge. N^+ and $N_2{}^{2+}$ occupy the same spot on the x-axis, despite $N_2{}^{2+}$ having twice the mass. All the components analyzed in figure 2.9 were ionized through a technique known as electron ionization (EI, formerly known as electron impact). This technique will be further discussed in the next chapter.

The ionization takes place as the first step in three steps that make up a mass spectrometer analysis. After a sample enters the mass spectrometer it is ionized. Then the ions are sorted by m/z in a mass analyzer, and the sorted ions are sequetially sendt to the detection uint, yealding one signal per different m/z value pressent in the sample (Harris, 2010). The total of these m/z values, as well as their abundance, makes up the mass specrum as sprevoiusly seen in figure 2.9.

There are a multitude of different MS techniques differentiated in their mode of ionization and mass analyzer systems. A quadrupole mass analyzer was chosen for this study.

EI ionization

Electron ionization is an ionization technique where molecules that enter the ion source is subjected to a stream of electrons. The energy of the electrons is usually 70eV (Gates, 2014a). When an analyte enters the stream, an electron on the molecule may be ejected from its orbit. This results in an ionized molecule in the form of a radical (Gates, 2014a).

Given the high energy transferred to the molecule, the molecule will in most cases fragment to rid itself of excess energy. The two fragments will typically consist of a radical ion fragment and a non-ion fragment. Should the radical ion fragment still hold to much energy, it will fragment further causing two more fragments, repeating the process until a stabile fragment is formed. Given the somewhat random nature of the energy transferred to the molecule, ionizing several molecules at the same time will produce several different patterns of fragmentation. An ion repeller accelerates the ions formed in the ion source through a series of focusing lenses into the mass analyzer (Harris, 2010). A schematic showing this process is given in figure 2.10.

The amount of energy required to ionize different molecules vary. This means that analyzing an equal amount of two different molecules may produce two signals of unequal intensity. The degree to which the signal is decreased or increased is called the detector response factor. The impact of this factor may be eliminated in the final results by normalizing them against a sample of known quantity (Harris, 2010).

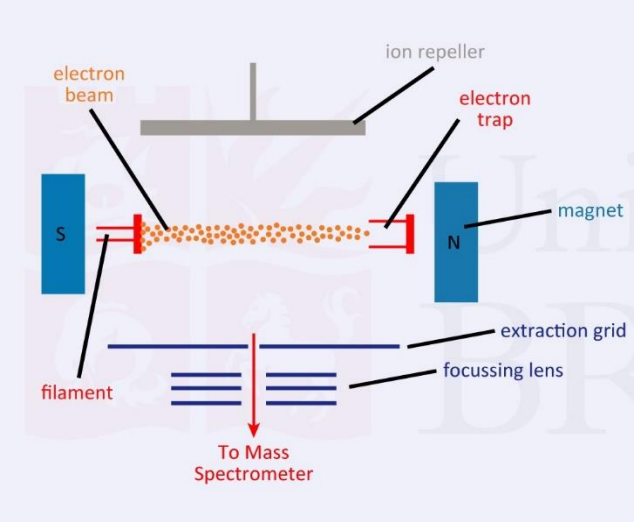

Figure 2.10 Cross section of an electron ionization source
Shows the cross section of an EI ion source (Gates, 2014a). The sample enters the center of the electron beam from the direction of the viewer. The electron beam is produced in a filament (left) and is attracted to the magnet (right), being caught in an electron trap. The repeller (top) accelerates the ions formed in the beam further into the mass spectrometer (bottom).

The quadrupole

The mass filter in a quadrupole consist of four magnetic rods arranged as seen in figure 2.11. An ion accelerated by the repeller in the ion source will pass between the four rods. The four rods are divided into two pairs. One pair maintains a magnetic field holding either positive or negative charge, the other pair of rods are connected to an alternating current. The rods connected to the alternating current maintains a magnetic field with a frequency in the radio frequency area. Both fields exert force on any ion passing through the quadrupole. The frequency of the alternating field resonates with specific m/z ratios. When this happens, the ion with a resonant m/z will oscillate to the frequency of the field and will pass through the quadrupole and enter the detection unit. Ions with non-resonant m/z ratios will have their course altered by the field and be thrown of their path and not reach the detection unit (Harris, 2010).

By cycling through field frequencies of the field, the quadrupole can analyze ions with different m/z ratios per second. Some ions are lost this way, as the frequency will be alternating, the path through the quadrupole will only be open for one m/z ratio at the time. By locking the frequency to a specific m/z ratio the number of ions with that m/z ratio passing through the quadrupole can increase hundredfold (Kyle, 2017).

Detector units used in quadrupoles measures the amount of ions, giving a signal proportional to the number of ions. When combined with GC, this signal is referred to as a total ion count (TIC) (Harris, 2010).

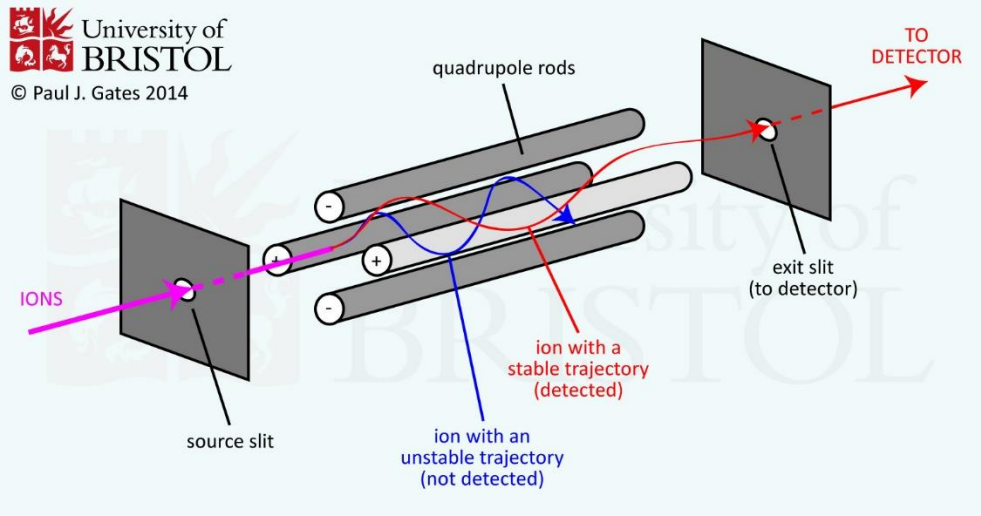

Figure 2.11 Quadrupole mass filter
Shows the arrangement of the four magnetic rods and the path of an ion from the ion source through the quadrupole to the detector (Gates, 2014b).

2.2 EXPERIMENTAL

The following methods was conducted during 2018 at Oslo Metropolitan University (former Oslo and Akershus University College of Applied Sciences). Lists of software, instruments and materials are given in appendix A.

2.2.1 Typologies of samples

Most of the samples analyzed in this study were provided by the Conservation Department at Munch museum in Oslo. They can be included in four categories: analytical standards, liquid oils, dried samples and paint samples. The analytical standards consist of a fatty acid methyl ester standard solution, and 11 amino acids. The analytical standards were the only ones not provided by the Munch museum and can be found in appendix A table A.6.

Liquid oil samples

Seven liquid oil samples were provided by the Munch Museum. The liquid oils are all pure commercial sold oils used in fine art, they are of unknown age and some are of unknown origin. A list of the seven liquid oils is provided in table 2.1. A photo of the seven oils is provided in figure 2.12.

Table 2.1 List of liquid oils
Shows the seven liquid oils received from the Munch museum.

Liquid oil sample	Name of producer and/or place of origin	Approximate date
Oxidized linseed oil	AB WILH.BECKER, Stockholm	*unknown*
Poppy oil, refined oil for painting	Kremer, Germany	Bottle opened during 2014
Bleached linseed oil	Italy (*unknown producer*)	More than 10 years old
Stand oil	Kremer, Germany	*unknown*
Walnut oil for tempera	Kremer, Germany	Bottle opened during 2014
Poppy oil for oil painting	Maimeri, Italy	More than 10 years old
Linseed stand oil for oil colors	Rowney, England	*unknown*

Figure 2.12 Liquid oils
Shows the seven oils provided by the munch museum in sample vails placed in front of their packaging. The photo was taken at the Munch Museum at Tøyen. (source: private photo).

Dried samples

Twenty-five dried samples were provided by the Munch museum. Twelve of these were used in this study. The protein-based binders were chosen with representing different characteristics while still being able to group them by protein source. The two dried oil samples were chosen as linseed oil and poppy oil were the two only died oil types available. All dried samples cam on glass microscopy plates after storage at the Munch museum since their year of preparation. The selected oils can be found in table 2.2.

Table 2.2 List of dried protein-based samples
Show the dried protein-based samples selected for analysis in this study, their reference name for bookkeeping purposes, the amount of pure binder diluted in water to make the sample and the year the sample was prepared.

Sample Name	Reference name	Dilution factor (%)	Year of preparation
Entire egg	EG	undiluted	2010
Egg yolk	gemma d'uovo	undiluted	2009
Egg white foam	clara d'uovo	undiluted	2012
Fish glue	none	15	2010
Fishleim	FL	*unknown*	2010
Animal glue	3CA	15	2009
Animal glue in pearls	none	16	*unknown*
Rabbit glue in pearls	2CA	10	*unknown*
Casein in borax	none	undiluted	2012
Bleached linseed oil	LO (made from the bleached linseed oil in table 2.2.A)	undiluted	2009
Poppy oil	poppy oil	undiluted	2009

Paint samples

Among 14 paint samples, taken from tubes found in Edvard Munch's painting studio 8 were selected for analysis. These paints had previously been analyzed by analysts from the Getty Conservation Institute and served at control samples for the method used in this study. The eight paints selected were chosen due to their organic and inorganic phase, making sure to have at least one blue and one red paint with in the respective categories of binding media: drying oils and tempera. A list of these samples can be found in table 2.3.

Table 2.3 List of paint samples

Shows the eight paints selected for analysis in this study. The list includes the name, code for bookkeeping purposes, tube label, brand, paint type given on the label and the organic and inorganic phase discovered by the Getty Conservation Institute. The organic phase column also includes the method used to identify the compounds found.

Name	Code	Label color - tube	Brand	Label	Country	Organic phase	Inorganic phase
Wurzel-krapplack dunkel nr. 6	MØR 1.1	wurzel-krapplack dunkel nr. 6	Mussini ölfarben Schmincke	oil	Germany	Oil, Beeswax (GC-MS), Oil, Resin (FTIR)	Phosphates, Sulfates, Carbonates, Alluminium oxides, Metal soaps
Laque de garence palé	AMR 7.1	laque de garence palé	Ambor	oil	France	Oil (Poppy) (GC-MS), Oil, Resin (Dammar) (FTIR)	Phosphates, Sulfates, Alluminium oxides, Silicates (traces), Carbonates, Metal Soaps.
Blau de cobalt Célesre	AMB 3.1	blau de cobalt Célesre	Ambor	oil	France	Oil (Poppy), Beeswax (Traces) (GC-MS, Oil,Gum (FTIR)	Metal Soaps (Zinc Stearate), Aluminium Oxides
Blau turquoise	AMB 5.2	blau turquoise	Ambor	oil	France	Oil (Lineseed), Beeswax (GC-MS), Oil, Gum (FTIR)	Sulfates, Silicates, Metal Soaps (Zn stearates), Zn white
Vermillion clair	BXF R 1.1	vermillion clair	Blockx Fils	oil	Belgium	Resin, Oil (FTIR)	Silicates, Sulfates, Phosphates
Ultramarin dunkel	HNB 2.2	ultramarin dunkel	Herman Neisch & Co	tempera	Germany	Egg, Gum (GC-MS), Oil, Egg, Resin (FTIR)	Carbonates, Titanium oxides, Sulfates
Krapplack	HNR 1.1	krapplack	Herman Neisch & Co	tempera	Germany	Egg (GC-MS), Egg, Resin (FTIR)	Phosphates, Sulfates, Carbonates, Alluminium oxides
Bleu de cobalt	LFB 1.2	bleu de cobalt	Le Franc	oil	France	Egg, Beeswax (GC-MS), Resin, Egg (FTIR)	Sulfates (Ba), Metal soaps

2.2.2 Different sample preparation methods based on binder type

After the acid hydrolysis of the sample, two different methods were used to further preparation based on the content of the sample. The organic phase containing the fatty acids was processed through a method of saponification and esterification. The amino acids in the aqueous phase was derivatized with BSTFA. Finally, all samples were analyzed by GC-MS. The process for each of these methods is represented in a flow chart in figure 2.13.

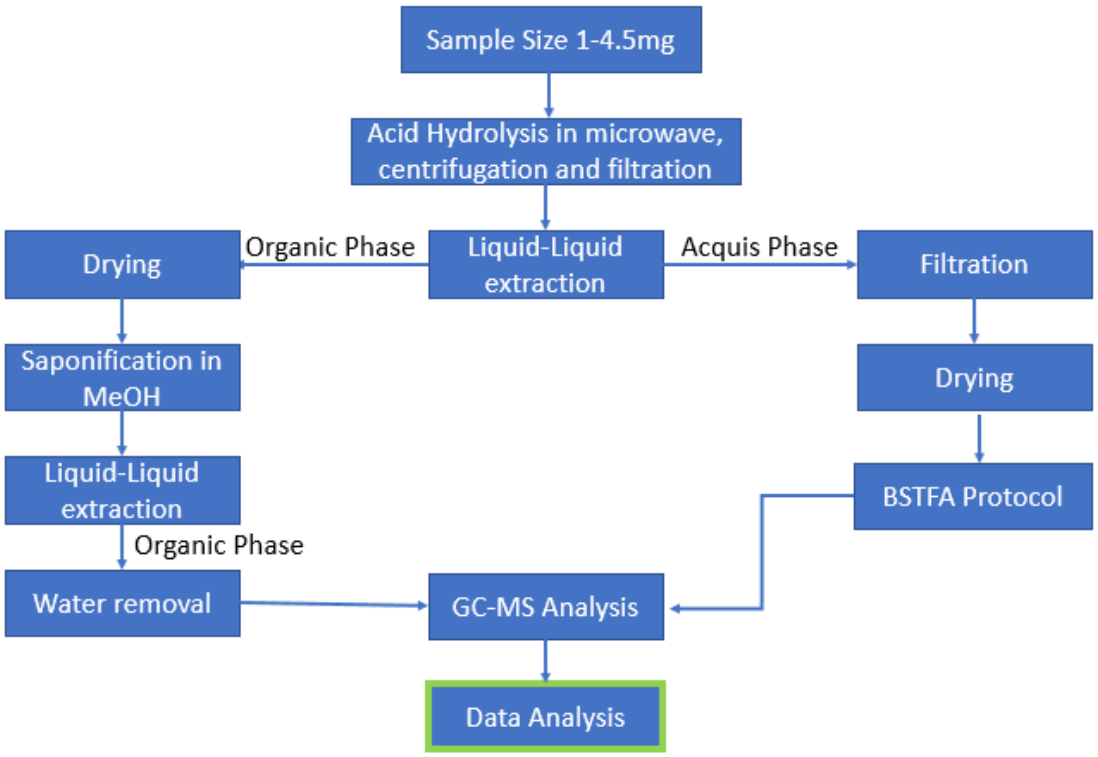

Figure 2.13 Flow chart of the different sample preparation methods.

2.2.3 Sample preparation

1.5-4.5 mg of dried samples were weighed on an analytical scale and placed in a reactor tube belonging to a microwave reactor. Al list of weights for each sample used in this study is given in table 2.4. The liquid oil samples were not weighed as it was determined that their sticky nature made weighing them accurately difficult. The liquid oils were added in drops far exceeding the weight limit of the paint samples. Corrections to this higher content of analytes in the liquid oil samples were made at later stages in the analysis. The amount of liquid oil used was 2-3 drops per oil type.

Table 2.4 Sample weights of dried samples
Shows the measured weight of each dry sample analyzed in this study. *Note that some of the reference standards exceed the weight limit imposed on the paint samples. This was justified as the reference standards were not used to indicate limit of detection.

Sample type	Sample ID	Weight (mg)
Dried oils	Dried linseed oil	2.8
	Dried poppy oil	1.6
Oil based paints	HSB 1.1	3.0
	AMB 5.2	3.8
	RWB 1.1	1.9
	AMB 3.1	1.9
	BXFR 4.1	3.5
	AMR 7.1	3.5
	MØR 1.1	2.8
Dried protein samples	Rabbit glue in pearls	3.5
	Animal glue	1.6
	Animal glue in pearls	4.0
	Fish glue	5.2
	Fishleim*	7.8
	Entire egg*	14.9
	Egg white foam	1.9
	Egg yolk	4.7
	Casein in borax*	11.6
Protein based paints	HNB 2.2	3.1
	HNR 1.1	2.9
	LFB 1.2	4.1

Microwave assisted digestion

5 ml of 6 M hydrochloric acid solution, prepared from concentrated hydrochloric acid and milli-Q water was added to the reaction tube. The tube was then shaken gently. The reaction tube was then placed in the StarSYNTH microwave oven and run through the program described in table 2.5. The solution from the acid digestion was then extracted with 3 times 5 ml of n-Pentane. The supernatant was transferred to a 50 ml measuring flask and left to evaporate at room temperature for three days. The aqueous phase left in the reactor tube was diluted with 5 ml deionized water.

Table 2.5 Temperature program for the acid assisted hydrolysis.

Time (min)	Power (W)	Temperature (°C)
10	250	100
30	500	100
15 (Ventilation)	0	Room temperature

Amino acid sample preparation for GC-MS analysis

After microwave assisted digestion, the derivatization process by silylation of the amino acids was commenced. 1 ml of the diluted aqueous phase from the microwave assisted acid digestion was transferred to a 1.5 ml PCR tube using a sole-use syringe filtered through a syringe filter as the syringe content was ejected. The filter was pre-wetted with the same aqueous phase. This PCR tube was then placed in a CentriVAP concentrator connected to a vacuum unit for 180 minutes at 75 °C.

A mixture of 100 µl of pyridine, 1.2 µl tertbutyl amine and 20 µl of BSTFA was then added to the tube in a nitrogenous atmosphere. The solution was transferred to a GC sample tube, and left to react in the pre-heated column oven of a GC for 30 minutes at 70 °C. The amino acid standards were also prepared using this derivatization method (no drying step was necessary since standards were provided in powder form).

Fatty acid sample preparation for GC-MS analysis

After microwave assisted digestion, the derivatization process by saponification and esterification of the fatty acids was commenced. Three solutions were prepared in advance of the derivatization. A 0,5 M methanolic sodium hydroxide solution made from sodium hydroxide pellets and HPLC grade methanol, a 7 % methanolic sulfuric acid solution made from concentrated sulfuric acid and HPLC grade methanol and a saturated sodium chloride solution made from sodium chloride and deionized water.

8 ml of 0,5 M the methanolic sodium hydroxide solution was added to the measuring flask containing the dried residue of the organic phase from the liquid-liquid extraction. The flask was heated in hot tap water for 5 minutes and periodically gently stirred. The flask was then cooled to room temperature. 10 ml of the 7 % methanolic sulfuric acid solution was then added and the flask was heated in hot tap water for 5 minutes and periodically gently stirred. The flask was then cooled to room temperature. 5 ml of n-pentane from Merck was then added to the flask and the flask was filled to the 50 ml mark by adding the saturated NaCl solution. The organic phase was added to a GC sample vail and the aqueous phase was discarded. A small amount of sodium sulfate was then added to the sample vail. The organic phase was then transferred to a new vail carefully as to not transfer any of the sodium sulfate. The pentane solution was then analyzed on the GC-MS.

2.2.4 Analyses performed on samples

GC-MS analysis of silylated amino acids

The solution containing the silylated amino acids were analyzed using a Thermo scientific GC-Trace 1310 with a Tri plus autosampler and an ISQ QD Single quadropol detector. The column used on the gas chromatograph was a 30 m HP-5MS column. All samples were analyzed in technical triplicates.

The data was processed with Chromelion version 7.2.9. The temperature program for this analysis is given in table 2.6. The other settings for the analysis of the silylated amino acids are given in table 2.7.

Table 2.6 Temperature program for analysis of silylated amino acids.

Time (min)	Temperature increment (°C/min)	Temperature (°C)	Hold time (min)
0	0	100	2
2	12	100	0
8.667	40	180	0
12.667	0	260	2
14.667	0	260	0

Table 2.7 GC and MS settings for analysis of silylated amino acids.

Instrument	Parameter	Value
GC	Gas Flow (ml/min)	1
	Split ratio (-)	20
	Injection volume (µl)	2
	Injector temperature (°C)	295
MS	Transfer line temperature (°C)	260
	Ion source temperature (°C)	250
	Mass filter (m/z)	85-350
	Analysis start (min)	2.1
	Analysis end (min)	13

GC-MS analysis of fatty acid methyl esters

The solution containing the FAMEs were analyzed using a GC-Trace 1310 with a Tri plus auto-sampler and an ISQ QD Single quadrupole detector. The column used on the gas chromatograph was a 30m DB-WAX. All samples were analyzed in technical triplicates. FAME standard RM1 was analyzed by the same method (no preparation steps necessary).

The data was processed with Chromelion version 7.2.9. The analysis of the FAMEs was run on a 15 minutes isothermal 200 °C program. The other settings for this analysis can be found in table 2.8.

Table 2.8 GC and MS settings for analysis of fatty acid methyl esters.
*The split ratio for the paint and dried samples was 1:20. **The injection volume for the paint and dried samples was 3 µl.

Instrument	Parameter	Value
GC	Gas Flow (ml/min)	1
	Split ratio (-)	1:20-1:160*
	Injection volume (µl)	1-3**
	Injector temperature (°C)	280
MS	Transfer line temperature (°C)	200
	Ion source temperature (°C)	295
	Mass filter (m/z)	40-350
	Analysis start (min)	2
	Analysis end (min)	15

3 RESULTS

3.1 LIPIDS

The drying oils were analyzed by the measuring the signal strength of stearic acid methyl ester (C18:0-ME) and palmitic acid methyl ester (C16:0-ME) in each sample.

The retention time for these two analytes varied to some degree, due to the GC column degrading to some extent over time. The identification for these two analytes was carried out by running analyses of a FAME standard at several points in time to confirm the shifts in retention time. This information is found in appendix B tables B.1 and table B.2. The final analyses were however not able to run the FAME standard due to heat issues in the room causing the pentane in the sample to evaporate rather than be drawn into the injection needle. The column was subject to some degree of degradation due to leaks, causing some drift in the retention times necessitating a different means of comparing the FAME standard retention times to the sample retention times. A comparison of the mass spectra of C16:0-ME and C18:0-ME were done on a previously analyzed FAME standard and the first injection of "Poppy oil, Maimeri". The two sets of mass spectra can be found in appendix C. Having identified the peaks in the poppy oil, the retention time of the other injections were compared to this sample as a retention time standard.

Each sample was quality controlled by measuring resolution and the signal-to-noise ratio.

3.1.1 Resolution

All samples analyzed were found to pass the requirement of having a resolution greater than 1.5 for both C18:0-ME and C16:0-ME. All resolution values for FAMEs are found in appendix B. The chromatogram for the first injection of a selection of oil-based samples (bleached linseed oil, linseed stand oil (Rowney), dried linseed oil and AMB 5.2 (linseed oil-based paint)) is given in figure 3.1.

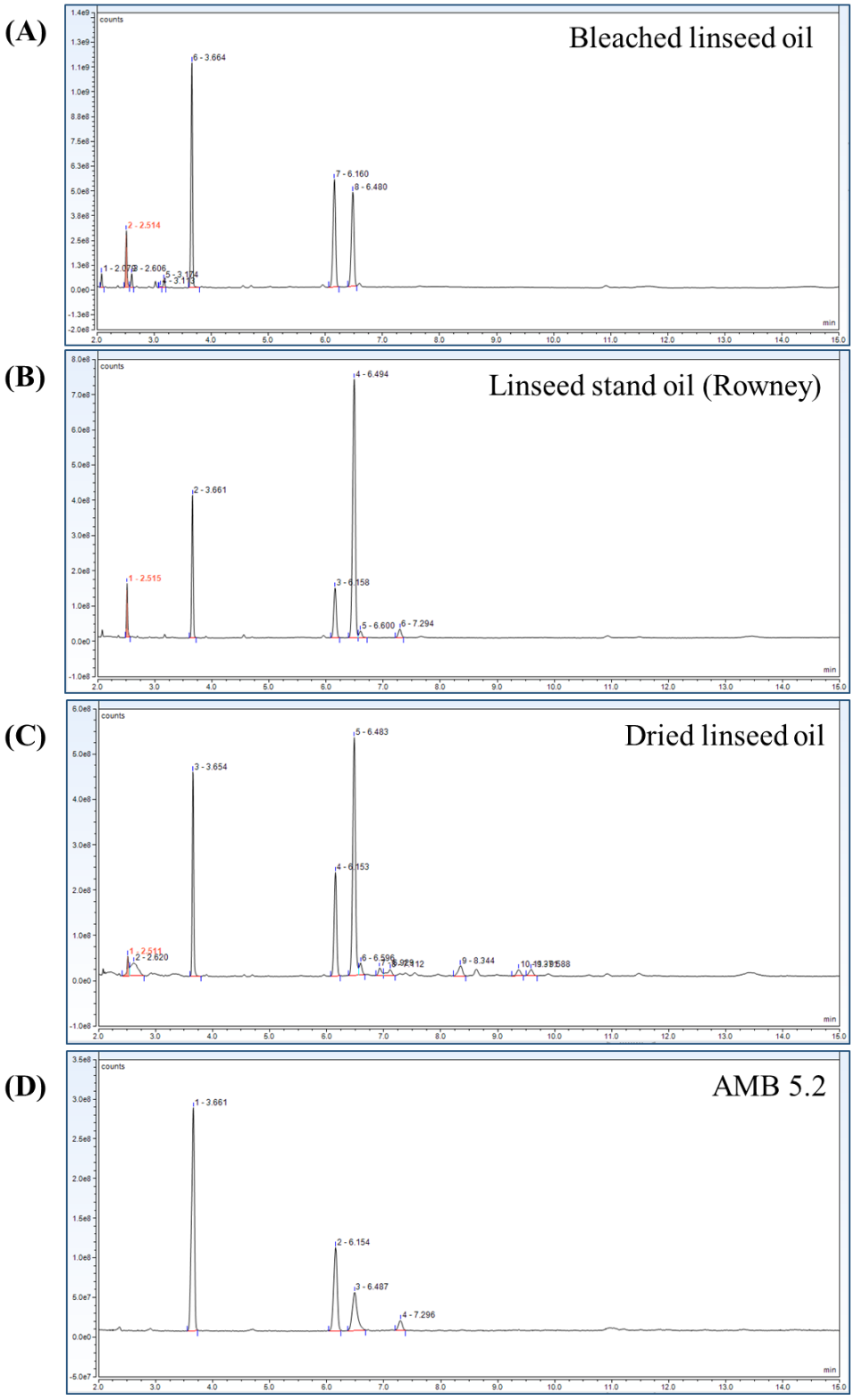

Figure 3.1 Chromatogram for oil-based samples

Shows the chromatogram from the first injection of a selection of oil-based samples: (A) bleached linseed oil, (B) linseed stand oil (Rowney), (C) dried linseed oil and (D) AMB 5.2 (linseed oil-based paint). The x-axis represents the retention time (min) and the y-axis represents the signal strength (TIC).

3.1.2 Signal-to-noise ratios

All samples analyzed were found to have a S/N of 10 or above for both C18:0-ME and C16:0-ME., except for AMB 3.1. This sample had only two successful injections, both failing the criteria for quantification of stearic acid methyl ester. A list of all S/N for FAMEs can be found in appendix B.

3.1.3 Fatty acid ratios

The relative ratio between C16:0-ME and C18:0-ME was found by dividing the signal for C16:0-ME by the signal of C18:0-ME. This ratio was individually calculated for each injection of each sample.

Liquid oil samples

The average ratio of C16:0-ME to C18:0-ME in each liquid oil sample are given in table 3.1.

Table 3.1 C16:0-ME to C18:0-ME ratio in liquid oil samples
Shows the average C16:0-ME to C18:0-ME ratio for each of the liquid oil samples. The average ratios were calculated as an average of three technical replicates for each sample. Variation in the triplicates of each sample is shown by standard deviation. All oils analyzed had a relative standard deviation of 1-6 %, except for the walnut oil, which had a relative standard deviation of 33 %.

Liquid oil sample	C16:0-ME/C18:0-ME ratio	Standard deviation
Oxidized Linseed oil	1.23	0.023
Poppy Oil Kremer	1.71	0.043
Bleached Linseed oil	1.24	0.012
Stand oil Kremer	1.59	0.081
Walnut oil Kremer	1.83	0.61
Poppy Oil Maimeri	1.41	0.048
Linseed stand oil Rowney	1.20	0.0093

Dried oil samples

The average ratio of C16:0-ME to C18:0-ME in each dried oil sample are given in table 3.2.

Table 3.2 C16:0-ME to C18:0-ME ratio in dried oil samples
Shows the average C16:0-ME to C18:0-ME ratio for each of the dried oil samples. The average ratios were calculated as an average of three technical replicates for each sample. Variation in the triplicates of each sample is shown by standard deviation. The two samples had an average relative standard deviation of 2 %.

Dried Oil sample	C16:0-ME/C18:0-ME ratio	Standard deviation
Dried poppy oil	1.41	0.029
Dried linseed oil	1.16	0.19

Oil-based paint samples

The average ratio of C16:0-ME to C18:0-ME in each oil-based paint sample are given in table 3.3.

Table 3.3 C16:0-ME to C18:0-ME ratio in oil-based paint samples
Shows the average C16:0-ME to C18:0-ME ratio for each of the oil-based paint samples. The average ratios were calculated as an average of three technical replicates for each sample. Variation in the triplicates of each sample is shown by standard deviation. The paints in this table had an average relative standard deviation of 5 %.

Paint sample	C16:0-ME/C18:0-ME ratio	Standard deviation
AMB 5.2	2.19	0.0034
AMR 7.1	2.87	0.080
MØR 1.1	1.46	0.13

3.1.4 Sample grouping according to oil type

The results of the palmitic acid methyl ester/stearic acid methyl ester ratios were divided into four groups determined by their content. Linseed oils, poppy oils, miscellaneous and paints, with the goal of determining the content of the paints. The linseed oils had a ratio ranging from 1.15 to 1.23. The poppy oils ranged from 1.41 to 1.70. The miscellaneous oils had ratios of 1.81 and 1.51 respectively. These values and their standard deviations made determining the content of the paints statistically infeasible, but the paint sample MØR 1.1 fell within the interval for poppy

oils. The AMB 5.2, AMR 7.1, and RWB 1.1 all fell outside the intervals for both poppy and linseed oils and bore no resemblance to the two miscellaneous oils.

3.1.5 Ion source optimization

The four temperatures tested as a parameter in the ion source (285, 290, 295 and 300 °C), produced the TIC values shown in table 3.4. The final temperature chosen was the one with high TIC value for both C16:0-ME and C18:0-ME. This was deemed to be at 295 °C, as the TIC of C16:0-ME appeared to be falling past this temperature. At this temperature C18:0-ME was also at it's second highest in this test.

Table 3.4 Heat optimization of ion source
Shows the results of the heat optimization of the ion source at 285, 290, 295 and 300 °C. The measured TIC of C16:0-ME and C18:0-ME, showed the highest TIC values (bold font) at 295°C and 300°C.

Temperature (°C)	C16:0-ME (TIC)	C18:0-ME (TIC)
285	1749720.733	4170049.647
290	1570552.24	3911857.004
295	**1765413.938**	4200560.43
300	1590590.572	**4336282.141**

3.2 PROTEINS

The results from the analyses of the different protein-based binders are all given by the measurement of the most abundant BSTFA derivate of the amino acids given in table 3.5. The signal from each amino acid was evaluated according to retention time, resolution and S/N. The final amino acid ratio was measured by using only signals passing the three mentioned quality parameters.

Table 3.5 Retention time for amino acid standards
Shows the amino acids selected to determine the relative amino acid ratio for the protein-based binders and paints. The degree of derivatization (number of TMS-groups) and retention times are also given.

Amino acid standard	Retention time (min)
Alanine 2TMS	3.645
Glycine 2TMS	3.866
Valine 2TMS	5.022
Leucine 2TMS	5.751
Isoleucine 2TMS	5.958
Glycine 3TMS	6.127
Serine 3TMS	6.719
Threonine 3TMS	7.032
Aspartic Acid 3TMS	8.505
Hydroxyproline 3TMS	8.624
Glutamic Acid 3TMS	9.392
Phenyl Alanine 2TMS	9.501

3.2.1 Resolution

Due to the resolution limit of 1.5, defined in chapter 2.1.3, three signal peaks in three different injections in three different samples were discarded due to potential inaccuracies in measured areas (hydroxyproline 3TMS for third injection of egg yolk, phenyl alanine 2TMS for third injection of animal glue and hydroxyproline 3TMS for third injection of casein in borax). All other signal peaks in every other injection for all the samples had resolution values above 1.5. All resolution values for protein-based samples are given in appendix B.The chromatogram for the first injection of a selection of protein-based samples (entire egg, fish glue, animal glue and casein in borax) is given in figure 3.2.

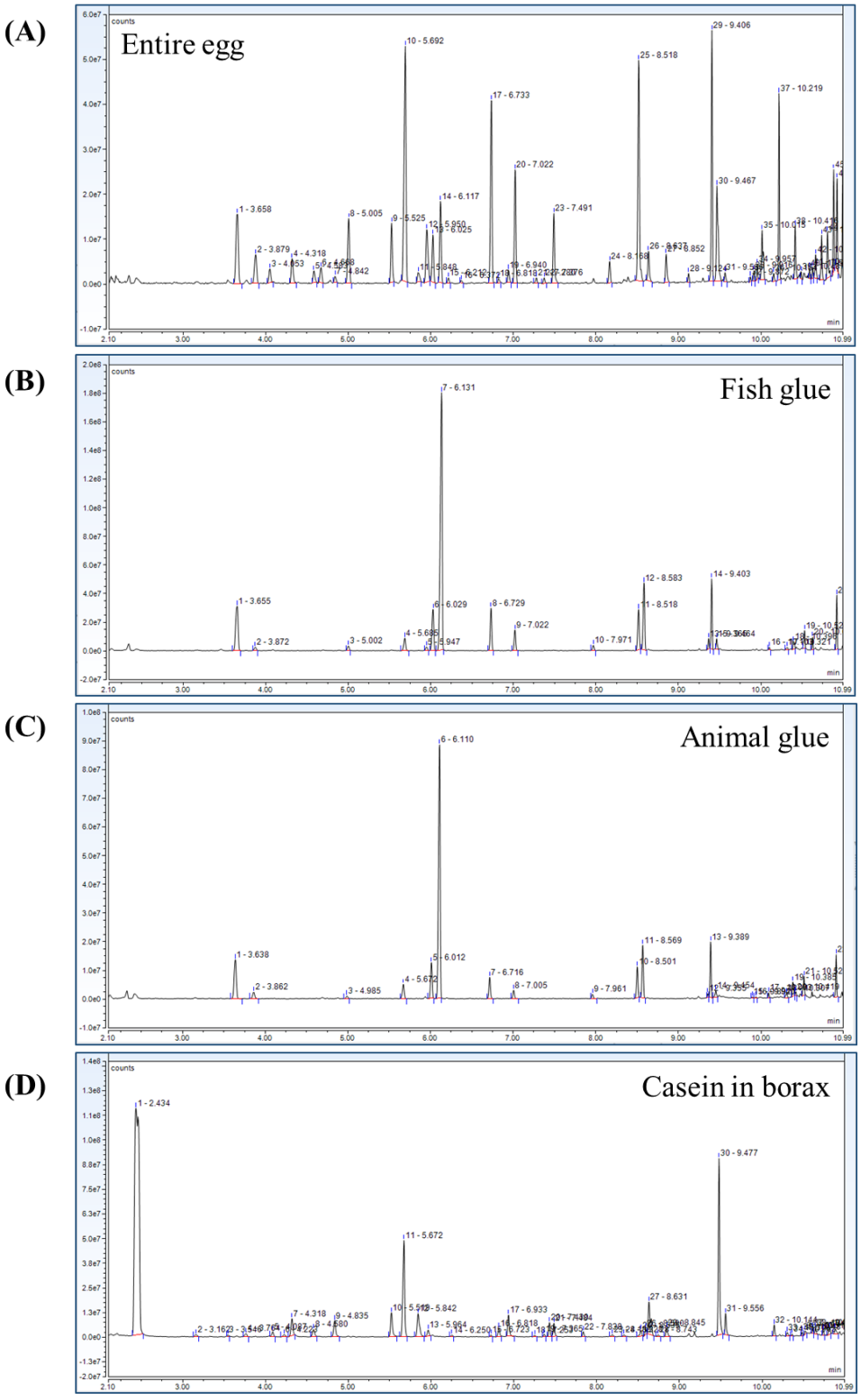

Figure 3.2 Chromatogram for protein-based samples
Shows the chromatogram from the first injection of a selection of protein-based samples: (A) entire egg, (B) fish glue, (C) animal glue and (D) casein in borax. The x-axis represents the retention time (min) and the y-axis represents the signal strength (TIC).

3.2.2 Signal-to-noise ratios

The lower LOQ was set to 10 and the lower LOD was set to 5. Any signal falling under the lower LOQ was excluded from the final amino acid ratio of the sample.

Egg-based samples

The entire egg samples achieved a S/N above 10 for all the 11 amino acid derivates, in all three injections. The egg yolk sample achieved a S/N above 10 for 10 out of 11 amino acid derivates, in all three injections. Hydroxyproline 3TMS was consistently below the limit of detection in all injections and was therefore not included in the amino acid ratio.The egg foam sample preformed similarly to the egg yolk sample. All amino acid derivates passed the limit of detection criteria however, no peak was observed at the retention time associated with hydroxyproline 3TMS.

The average S/N for each amino acid derivate, in each egg-based sample, is given in table 3.6.

Table 3.6 S/N for egg-based samples
Shows the average signal-to-noise ratio for each amino acid derivate for each type of egg-based sample. No peak was detected at the retention time associated with hydroxyproline in the egg white foam sample.

Amino Acid Derivate	Entire Egg	Egg Yolk	Egg white Foam
	S/N	S/N	S/N
Alanine 2TMS	77.4	45.4	31.3
Valine 2TMS	67.0	22.6	19.3
Leucine 2TMS	143.9	391.7	84.6
Isoleucine 2TMS	73.5	52.7	18.5
Glycine 3TMS	132.3	70.4	40.5
Serine 3TMS	138.8	95.6	75.2
Threonine 3TMS	71.6	88.8	50.6
Aspartic Acid 3TMS	127.6	221.3	122.4
Hydroxyproline 3TMS	37.5	3.8	No peak detected
Glutamic Acid 3TMS	103.4	107.0	101.0
Phenyl Alanine 2TMS	37.1	30.0	29.7

Fish-based samples

Both fish-based samples had low S/N for valine 2TMS and isoleucine 2TMS.
For the fish glue, valine 2TMS passed S/N threshold of 10 in 2 out of three injections. On the third injection, valine 2TMS had a signal to noise ratio of 9.9. This was judged to be close enough to 10 not to discard the signal for the purpose of determining amino acid ratio. Furthermore, the signal for isoleucine 2TMS was below the lower LOQ in all three injections and were thus not included in the amino acid ratio.

For the fishleim sample, isoleucine 2TMS was below the lower LOQ in all three injections. One injection had a S/N below 10, one had a S/N below the lower LOD and the third had no observable signal at the retention time in question. Isoleucine 2TMS was therefore not include in the amino acid ratio for this sample. The signal in one injection was also below the lower LOQ for valine 2TMS, thus this signal was discarded in the amino acid ratio.

The average S/N for each amino acid derivate, in each fish-based sample is given in table 3.7.

Table 3.7 S/N for fish-based samples
Shows the average signal-to-noise ratio for each amino acid derivate for each type of fish-based sample.

Amino Acid Derivate	Fish glue	Fishleim
	S/N	S/N
Alanine 2TMS	52.9	85.4
Valine 2TMS	10.5	9.9
Leucine 2TMS	31.3	73.3
Isoleucine 2TMS	7.1	4.9
Glycine 3TMS	464.3	578.9
Serine 3TMS	153.1	247.9
Threonine 3TMS	76.5	66.4
Aspartic Acid 3TMS	53.2	99.5
Hydroxyproline 3TMS	90.2	92.9
Glutamic Acid 3TMS	73.6	437.0
Phenyl Alanine 2TMS	12.0	29.2

Animal glues

For the rabbit glue in pearls sample, all injections passed the criteria for lower LOQ quantification for all amino acid derivatives. Animal glue (3CA) had no observable peaks for isoleucine 2TMS, valine 2TMS was below lower LOD in all three injections, thus excluding both amino acid derivatives from the amino acid ratio. Phenyl alanine 2TMS only had one injection produce a peak with a S/N ratio above the lower LOQ. Animal glue in pearls also had no observable signal for isoleucine 2TMS, yet one of the injections had valine pass the lower LOD criteria.

The average S/N for each amino acid derivate, in each type of animal glue is given in table 3.8.

Table 3.8 S/N for animal glues
Shows the average signal-to-noise ratio for each amino acid derivate for each type of animal glue. No peak was detected at the retention time associated with isoleucine in animal glue (3CA) and animal glue in pearls.

Amino Acid Derivate	Rabbit glue in pearls	Animal Glue (3CA)	Animal glue in pearls
	S/N	S/N	S/N
Alanine 2MTS	81.4	60.4	144.1
Valine 2 TMS	16.4	3.8	8.9
Leucine 2 TMS	68.1	14.7	41.9
Isoleucine 2TMS	15.5	No peak detected	No peak detected
Glycine 3 TMS	512.2	336.7	371.3
Serine 3TMS	106.0	57.9	104.5
Threonine 3TMS	61.1	23.5	39.8
Aspartic Acid 3TMS	30.4	51.8	64.5
Hydroxyproline 3TMS	68.5	85.2	96.7
Glutamic Acid 3TMS	99.9	62.0	202.9
Phenyl Alanine 2TMS	21.1	12.2	22.0

Casein in borax

Only 6 amino acid derivatives were observed in the casein in borax sample. Out of the six, serine 3TMS did not pass the lower LOQ criteria. Glutamic acid 3TMS did not pass the lower LOD criteria in one injection, but the other two injections passed. The average S/N for each amino acid derivate in the casein in borax are given in table 3.9.

Table 3.9 Signal-to-noise ratio casein in borax
Shows the average signal-to-noise ratios for all amino acid derivates observed in the casein in borax sample.

Amino Acid Derivate	Casein in borax
	S/N
Serine 3TMS	7.7
Threonine 3TMS	46.3
Aspartic Acid 3TMS	13.4
Hydroxyproline 3TMS	67.4
Glutamic Acid 3TMS	9.4
Phenyl Alanine 2 TMS	240.0

3.2.3 Amino acid ratios and ingroup comparisons

The ratio for each amino acid derivate was then calculated for each injection. The average ratio (%) was then used to compare the different samples in each group, and the differences between the groups.

Egg-based samples

The amino acid derivate ratios for each sample in the egg-based sample group are shown in figure 3.3.

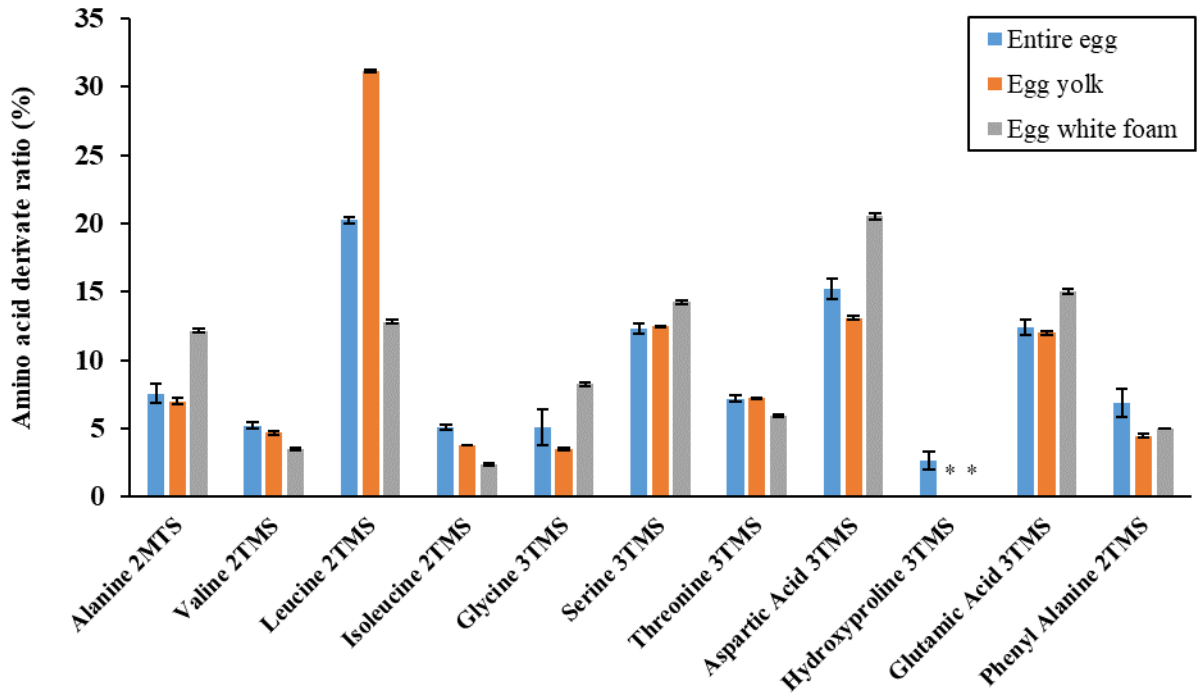

Figure 3.3 Amino acid derivatives in egg-based samples
Shows the average ratios (%) of amino acid derivatives in the egg-based samples (entire egg, egg yolk, and egg white foam), based on three injections of each sample. The error bar represents the standard deviation between the three injections. * Amino acid derivate not detected in the sample.

The three samples in the group (entire egg, egg yolk and egg white foam) was statistically differentiated by the ratio between leucine 2TMS and serine 3TMS. This ratio was calculated for each injection and used to determine whether the samples were significantly different by means of a Student's t-test. The ratios are given in table 3.10. The tests were performed at 95 % confidence, assuming normal distribution and equal variance unless otherwise stated. The results from these tests, given in table 3.11, shows that all the samples in the egg-based sample group were statistically different form each other (p-value < 0.05).

Table 3.10 Leucine/serine ratio in egg-based samples
Shows the average leucine 2TMS to serine 3TMS ratio for each type of egg-based sample. The average ratios were calculated as an average of three technical replicates for each sample. Variation in the triplicates of each sample is shown by standard deviation.

Egg-based sample	Leucine 2TMS/ Serine 3TMS ratio	Standard deviation
Entire egg	1.65	0.07
Egg yolk	2.49	0.02
Egg white foam	0.90	0.007

Table 3.11 P-values for egg-based samples
Shows p-values from comparisons of the average leucine 2TMS to serine 3TMS ratio for different types of egg-based samples. P-values were calculated by Student's t-test at 95 % confidence, assuming normal distribution. P-value<0.05 was considered significant (bold font). * The p-value was calculated assuming unequal variance, as these samples did not pass the F-test for variance (at 95 % confidence). The other comparisons was done assuming equal variance since F-test confirmed equal variances for those groups.

Comparison			P-value
Entire egg	vs.	Egg yolk	**4.3E-05**
Entire egg	vs.	Egg white foam	**0.0032***
Egg yolk	vs.	Egg white foam	**1.9E-08**

Fish-based samples

The amino acid derivate ratios for each sample in the fish-based sample group are shown in figure 3.4.

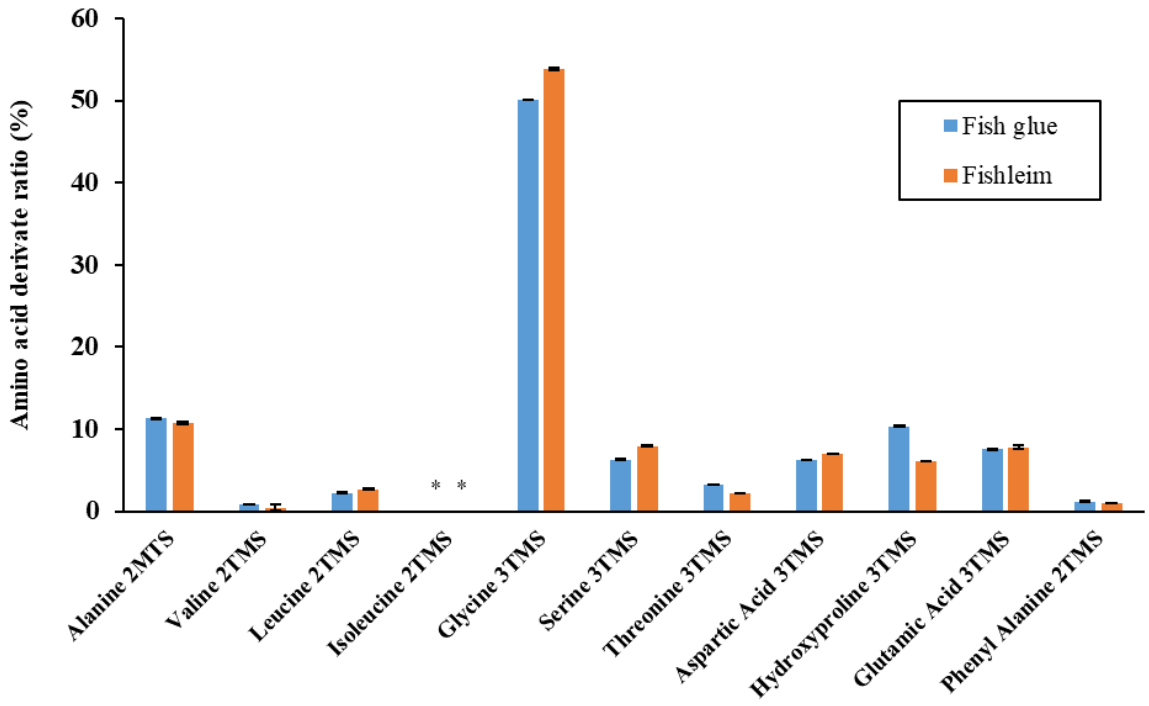

Figure 3.4 Amino acid derivatives in fish-based samples
Shows the average ratios (%) of amino acid derivatives in fish-based samples (fish glue and fishleim), based on three injections of each sample. The error bar represents the standard deviation between the three injections.
* Amino acid derivate not detected in the sample.

The two samples in the group (fish glue and fishleim) was statistically differentiated by the ratio between glycine 3TMS and alanine 2TMS. This ratio was calculated for each injection and used to determine whether the samples were significantly different by means of a Student's t-test. These ratios are given in table 3.12. The t-test was performed at 95 % confidence, assuming normal distribution, and equal variance. The result from this test, given in table 3.13, shows that fish glue and fishleim were statistically different form each other (p-value < 0.05).

Table 3.12 Glycine/alanine ratio in fish-based samples
Shows the average glycine 3TMS to alanine 2TMS ratio for fishleim and fish glue. The average ratios were calculated as an average of three technical replicates for each sample. Variation in the triplicates of each sample is shown by standard deviation.

Fish-based sample	Glycine 3TMS/ Alanine 2TMS ratio	Standard Deviation
Fish glue	4.44	0.04
Fishleim	5.01	0.05

Table 3.13 P-values for fish-based samples
Shows p-value from comparisons of the average glycine 3TMS to alanine 2TMS ratio for fishleim and fish glue. P-value was calculated by Student's t-test at 95 % confidence, assuming normal distribution and equal variance (confirmed by F-test at 95 % confidence). P-value<0.05 was considered significant (bold font).

Comparison			P-value
Fishleim	vs.	Fish glue	**0.00010**

Animal glues

The amino acid derivate ratios for each sample in the animal glue group are shown in figure 3.5.

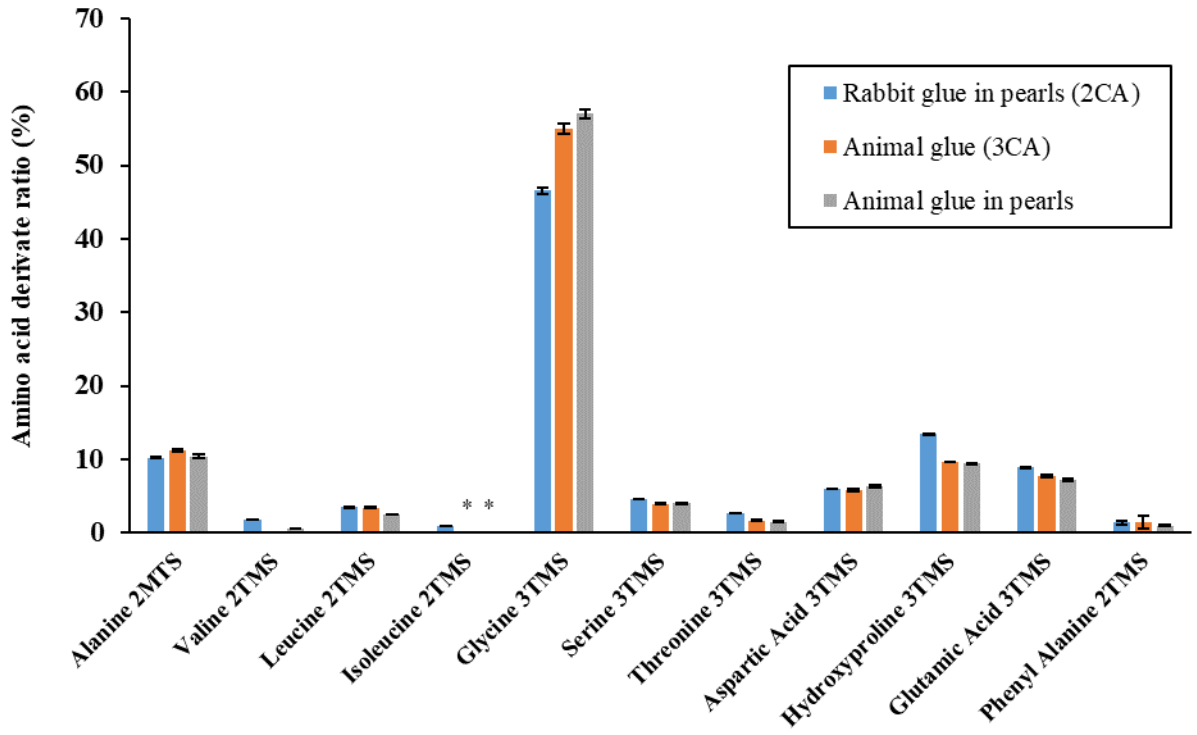

Figure 3.5 Amino acid derivatives in animal glues
Shows the average ratios (%) of amino acid derivatives in animal glue samples (rabbit glue in pearls (2CA), animal glue (3CA) and animal glue in pearls), based on three injections of each sample. The error bar represents the standard deviation between the three injections. * Amino acid derivate not detected in the sample.

The three samples in the group (rabbit glue in pearls (2CA), animal glue (3CA) and animal glue in pearls) was statistically differentiated by the ratio between glycine 3TMS and alanine 2TMS. This ratio was calculated for each injection and used to determine whether the samples were significantly different by means of a Student's t-test. These ratios are given in table 3.14. The t-tests were performed at 95 % confidence, assuming normal distribution and equal variance. The results from these tests, given in table 3.15, shows that the different types of animal glues were statistically different form each other (p-value < 0.05).

Table 3.14 Glycine/alanine ratio in animal glues
Shows the average glycine 3TMS to alanine 2TMS ratio for each type of animal glue. The average ratios were calculated as an average of three technical replicates for each sample. Variation in the triplicates of each sample is shown by standard deviation.

Animal-based binder	Glycine 3TMS/ Alanine 2TMS ratio	Standard Deviation
Rabbit glue in pearls (2CA)	4.546	0.007
Animal glue (3CA)	4.90	0.05
Animal glue in pearls	5.5	0.2

Table 3.15 P-values for animal glues
Shows p-values from comparisons of the average glycine 3TMS to alanine 2TMS ratio for different types of animal glue. P-values were calculated by Student's t-test at 95 % confidence, assuming normal distribution and equal variance (confirmed by F-test at 95 % confidence). P-value<0.05 was considered significant (bold font).

Comparison			P-value
Rabbit glue in pearls	vs.	Animal glue (3CA)	**0.00018**
Rabbit glue in pearls	vs.	Animal glue in pearls	**0.0015**
Animal glue (3CA)	vs.	Animal glue in pearls	**0.0088**

Casein in borax

The amino acid derivate ratios for the casein in borax sample are shown in figure 3.6.

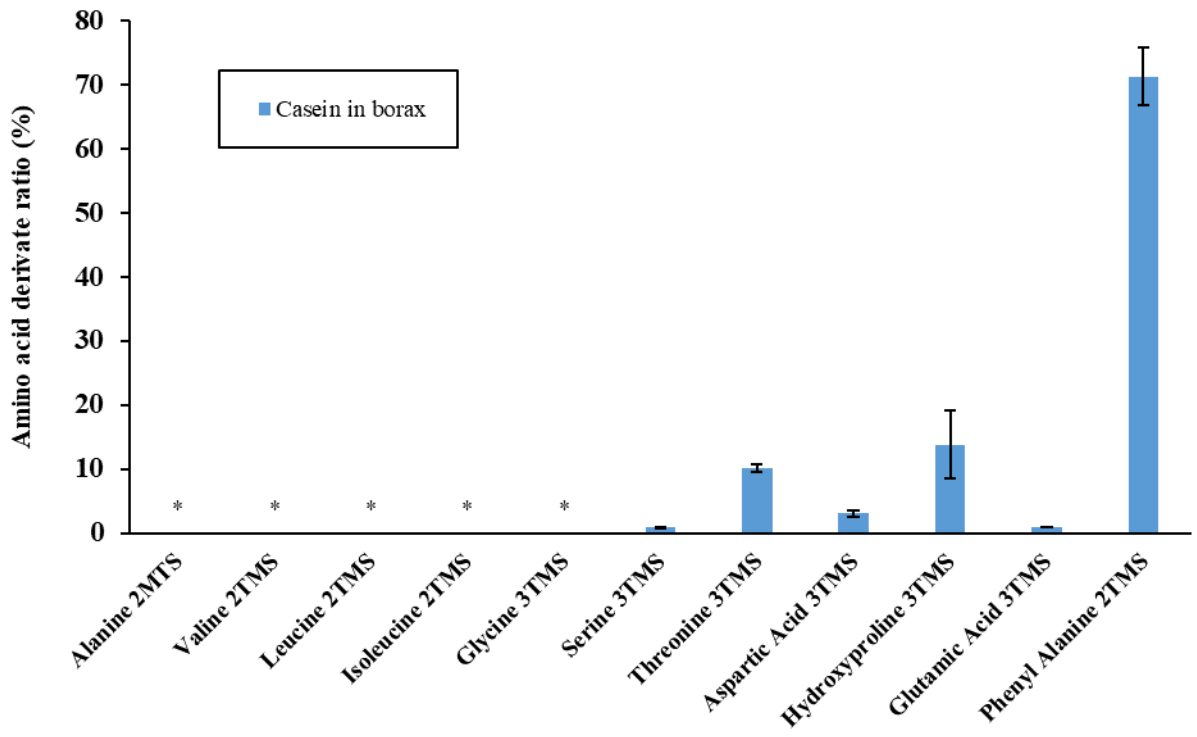

Figure 3.6 Amino acid derivatives in casein in borax
Shows the average ratios (%) of amino acid derivatives in casein in borax, based on three injections of the sample. The error bar represents the standard deviation between the three injections. * Amino acid derivate not detected in the sample.

The results from the casein sample in borax differed from the other protein-based sample groups in three ways. Firstly, a peak identified as boric acid by the MS was found during the first minutes of the analysis. This peak was completely saturating the MS detector. Secondly, the MS also identified a peak as a TMS derivate of lactic acid. Thirdly, none of the first five amino acid derivatives (based on retention time) were visible in this analysis. The mass spectra database used was Mainlib in the NIST Mass Spectral Library.

3.2.4 Comparison between groups

No statistical test was preformed to determine whether the egg-based group could be mistaken for any of the other protein-based group in this study. This was due to the significantly higher content of leucine 2TMS, observed to be approximately 5-10 times more abundant than in the animal glues and fish-based samples. The relative amount of glycine 3TMS was also observed to about 5-10 times lower than the animal glues and fish-based samples.

The casein in borax sample too, was omitted from any kind of statistical testing to determine whether it could be mistaken for any other sample or group. This was due to the lack of several amino acid derivatives, and the apparent presence of lactic acid TMS derivatives and boric acid.

Comparison of animal glue and fish-based samples

The animal glues and fish-based samples were compared by first calculating the average glycine/alanine and alanine/serine ratio for each group individually. The averages for each group are found in table 3.16.

Table 3.16 Animal glues vs. fish-based samples
Shows the averages and standard deviations for the glycine 3TMS to alanine 2TMS, and alanine 2TMS to serine 3TMS ratios, in both the animal glues and fish-based samples.

Sample type		Glycine 3TMS/ Alanine 2TMS ratio	Alanine 2TMS/ Serine 3TMS ratio
Animal glues	Average	4.98	2.56
	Standard deviation	0.431	0.276
Fish-based samples	Average	4.73	1.57
	Standard deviation	0.318	0.242

The ratios for each group (animal glues and fish-based samples) was then compared by two Student's t-tests. The tests were performed by using the measured values for each injection to achieve the highest possible standard deviation for each ratio in each group. This also increases the degrees of freedom. The results from the two tests can be found in table 3.17.

The p-value for glycine/alanine was above 0.05, thus there was no significant difference between the animal glues and fish glues when comparing this ratio. The p-value for alanine/serine was lower than 0.05, thus there was a significant difference between animal glues and fish-based samples when comparing the alanine/serine ratio.

Table 3.17 P-values animal glues vs. fish-based samples
Shows p-values from comparisons of average glycine 3TMS to alanine 2TMS ratio and alanine 2TMS to serine 3TMS ratios between animal- and fish-based binders. P-values were calculated by Student's t-test at 95 % confidence, assuming equal variance (confirmed by F-test at 95 % confidence). P-value<0.05 was considered significant (bold font).

Comparison			P-value	
			Glycine 3TMS/ Alanine 2TMS ratio	Alanine 2TMS/ Serine 3TMS ratio
Animal glues	vs.	Fish-based samples	0.24	**8.4E-06**

4 DISCUSSION

4.1 THE FAME METHOD AND ANALYSIS

Throughout this chapter, time constraints will be a common theme. This was due to some instrument malfunctions on GC-MS. These included some leaks and the replacement of a faulty ion detector. Some limitations were also placed on the versatility of the quadrupole of the GC-MS, as the software lacked the required license to run SIM analyses.

4.1.1 Sample preparation

The sample preparation was, as describes in chapter 2.2.3, a mix of the methods used by Colombini et al. (1998, 1999) and Bekken (2013), with some modifications made due to which chemicals and materials were available. Unlike the mentioned papers, there was no effort made to test sample recovery in the preparation method in this study. There are however several notes to be made when comparing the method used in this study, and the obtained results, to the method and results from Colombini et al. and Bekken.

The first note relates to the temperature of the microwave and the amount of HCl used for the microwave assisted acid digestion. These differences was mainly due to the limitations of the microwave oven use in this study. The seal for the reactor tubes could not handle temperatures above 100 °C, and the difference in amount of HCl solution used, was due to the size of the same reactor tubes. The combination of these two differences may have had an impact on how much of the fatty acids were extracted from the samples.

As described in chapter 2.2.1, heat and pressure accelerates the hydrolysis of lipids. By lessening these two parameters, the hydrolysis preformed in this study could have achieved a lesser effect than the one performed by Colombini et al. (1999). Recovery tests must be conducted to determine the impact of the change in temperature and HCl amount. The amount of organic solvent used to extract the fatty acids relative to the aqueous phase was however a lot higher (3:1 ratio versus 1:10). This could have led to a greater amount of fatty acids being extracted from the

aqueous phase, but needs confirmation by running recovery tests. The amount of organic solvent used would otherwise have little effect on the method, aside from drying time.

The paints had on occasion, as seen in the case of AMB 3.1, somewhat low signal strength (see appendix B table B.2). AMB 3.1 especially stands out as it had the lowest sample mass of any of the analyzed paints, at 1.9 mg (see chapter 2.2.3 table 2.4). This could be remedied in the sample preparation by concentrating the final pentane solution from 3 ml to 500 µl. This would increase the concentration of the sample by a factor of 6, thus increasing the signal, potentially by the same amount.

The derivatization method of oil-based samples established in this project remains quite similar to Bekken's method (2013), but with a reversal of steps, different acids, bases and organic solvents, the addition of a polar extraction step and shorter timeframe. Especially the shorter timeframe could have had a negative impact on the degree of derivatization, and thus the amount of FAMEs available to be analyzed.

4.1.2 Analysis

The GC-MS analysis performed in this study is quite different to the one performed by Bekken (2013). Due to fewer FAMEs in the samples, the analysis time was here shortened to 15 minutes, with the thermal limits of the column keeping the analysis from being even shorter. Although the two fatty acid methyl esters (C16:0-ME and C18:0-ME) had a retention time significantly shorter than 15 minutes, the analysis time was never shortened, kept at 15 minutes to allow for possible contaminants to leave the column before the subsequent analysis.

Resolution

The resolution values for the analyses of the fatty acid methyl esters all passed the 1.5 criteria. The lowest value recorded was at the first injection of AMB 5.2. The cause of this was the 0.333 min retention time space between stearic acid methyl ester and the peak following it. According to the FAME standard, this should be C18:1 methyl ester.

Signal-to-noise ratios

The S/N disqualified one peak from being quantified. This was stearic acid methyl ester in AMB 3.1. This was probably due to the size of the sample and possibly due to a smaller amount of binder being present in the sample in the first place. As it is preferable to not rely on increasing the size of the sample, other alternatives should be considered.

The lower LOW is now defined by MØR 1.1. This paint had a sample size of 2.8 mg and a S/N of 10.2 and 12.7 for both (only two) injections made. To be able to consume as little paint as possible, this sample size should be decreased by altering the sample preparation for this analysis. This could be done at the step after adding the saturated NaCl solution to the measuring flask as described in chapter 2.2.3. The full amount of pentane used to extract the FAMEs could be condensed to a smaller volume to increase the concentration of the sample. A split-less injection is another thing to consider, but this would necessitate that sample be dried end re-solved in a less volatile solvent.

C16:0-ME-to-C18:0-ME ratios

The goal of this study was to establish a library of chromatographic fingerprints to be able to identify binders in unknown paints. As all binders in all samples were known at least to some extent, the groupings were already made in the results chapter.

Although not a perfect comparison, Colombini et al. (1999) quantified the amount of fatty acids present in each sample rather that looking at the raw signal data of derivatives. The findings from this study still reflect the findings obtained by Colombi and colleagues to some degree. A comparison of these two studies can be found in table 4.1.

Table 4.1 Result comparisons

Shows the measured palmitic acid methyl ester/stearic acid methyl esters (signal/signal) ratio compared to their respective C16:0/C18:0 (w%/w%) ratio, and the measured results of the reference standards given in palmitic acid methyl ester/stearic acid methyl esters (signal/signal) and the metal content of these samples.

Oils and oils with white lead pigment			
Oil Type	**Measured results in this study (C16:0-ME/C18:0-ME) FAME ratio**		**Colombini et al. (1999) results (C16:0/C18:0) w%/w%**
Linseed Oils	Oxidized Linseed Oil	1.23	1.4
	Bleached Linseed Oil	1.24	
	Linseed stand Oil Rowney	1.2	
	Stand oil Kremer	1.59	
	Dried Linseed Oil	1.16	
Linseed Oil with lead white pigment	-		1.5
Poppy Oils	Poppy Kremer	1.71	3.7
	Poppy Oil Maimeri	1.41	
	Dried Poppy Oil	1.41	
Poppy oil with lead white pigment	-		3.9
Walnut Oil	Walnut Kremer	1.83	2.3
Walnut oil with lead white pigment	-		2.1
Paint samples in this study			
Name	**Measured results (C16:0/C18:0) FAME ratio**		**Metal Content**
AMB 5.2 (Linseed)	2.19		Metal Soaps (Zn stearates), Zn white
AMR 7.1 (Poppy)	2.87		Aluminum oxides, Metal Soaps.
MØR 1.1 (Unknown Oil)	1.46		Aluminum oxides, Metal soaps

The three groups, Linseed, poppy and walnut oil, each had some overlap. This made distinguishing them from each other infeasible. Without considering the paint samples, the only oil from this study that did not directly overlap with another group was walnut oil. Had more different types of samples been analyzed, this may not have been the case.

When comparing the liquid and dried oil samples from this study, to the poppy oil, walnut and linseed oil from the Colombini et al. paper (1999), two things were apparent. The C16:0/C18:0 ratios are much higher than the FAME ratios from this study. The difference in fatty acid ratio in the Colombini et al. paper is also greater between the different oil types. This would suggest that normalizing for the detector response factor helps differentiating between different oils.

The Colombini et al. paper (1999) also appears to suggest that white lead pigment increases the fatty acid ratios of linseed and poppy oil, which could be a reason why the analyzed paint samples from this study are higher than the dried and liquid samples. The only paint sample that is comparable to the liquid and dried oils samples is MØR 1.1, which has a FAME ratio close dried poppy oil and the poppy oil from Maimeri.

An idea for future studies would be to firstly quantify the fatty acids, thus correcting for the detector response factors. Secondly, to run analyses on dried samples containing some different metal ions in parallel to pure binders, to determine what impact these may have on the fatty acid ratios.

Mass spectra data

In the case when the FAME mass spectra had to be used to confirm the presence of C16:0-ME and C18:0-ME in the Poppy oil from Maimeri, this was possible due to the presence of identifying ions. Looking at the spectra in appendix C, the base peak is 74 m/z in the four spectra. They also share almost identical distributions in the peaks 43 m/z, 87 m/z and 143 m/z. What set the stearic acid methyl ester apart from the palmitic acid methyl ester, was the presence of their assumed molecular ions, 261 m/z and 299 m/z, respectively.

4.2 PROTEIN BASED BINDERS

4.2.1 Sample preparation in relation to analysis results

The sample preparation for this analysis was based on the sample preparation method used by Colombini et al. (1998). The only real difference lay in the derivatization reagent used and the microwave oven temperature. The sample sizes used for some of the reference standards were in few cases a bit larger than the 1-5mg used by Colombini, but even the samples in the lower end of the sizes used in this study produced results. This did not hold true for the paint samples. None of the paint samples produced any results that could be compared to the reference standards. This appears to indicate that the amounts of the various derivatized amino acids from the paint samples were too low for detection. There are a few steps in the sample preparation that may have led to this:

- The amount of proteinaceous binder in the paint samples were too low for analysis for the method to be performed as written in chapter 2.2. The data provided by Colombini et al. (1998) seems to suggest this, as the w/w % of per amino acid to the paint sample size often could be as low as 1 %.
- The BSTFA may have had some interactions with some chemicals in the paints, leading to improper derivatization of the amino acids.
- The lower temperature in the microwave failed to extract the amino acids from the paint samples due to some unforeseen mechanism in the paints strengthening the bonds in the paint.

The most probable scenario is the first one. However, this would also be the easiest to remedy in future analyses without increasing the sample size of the paints. The method used in this study has room to increase the amount of aqueous phase in the evaporation stage by a large margin.

The 1 ml aliquoted from the hydrolysate could potentially be increased to 10 ml. This would require the entire hydrolysate to be centrifuged and filtered, but would increase the amount of sample to be derivatized, potentially by 10 times. This would increase the drying time of the hydrolysate dramatically, as only 1 ml already takes 180 minutes.

A decrease in split ratio would not be advisable as going lower than 1:20 appeared to push the limits of what the GC could handle when tested. Adapting a different injection technique like a split-less injection could solve this problem.

4.2.2 Analysis

The main difference in analysis technique than the one used by Colombini et al. (1998) appears to be analysis duration. It is hard to compare them, as the column used by Colombini and colleagues appears to be have gone out of production with no available information on what composed its stationary phase. The results are also not possible to compare directly as Colombini et al. quantified the amount of amino acids and this study relied on the signal strength provided by the amino acid derivatives. The results of this part of the study will therefore be judged mainly on resolution, S/N, amino acid ratios and how the different groups of binders differed from each other.

Resolution

Only three peaks were discarded by criteria their resolution value, one of which also were discarded due to a too low S/N. These peaks had retention time in areas of notably lower resolution values (often 2.5 or lower). The retention times of these areas were at 6.0, 8.5 and 9.4 minutes. The different binders were not all as affected by this. Summarized:

- The fish-based samples had notably lower resolution values at all the mentioned retention times.
- The animal glues had notably lower resolution values at 8.5 and 9.5 minutes.
- The egg-based samples had low resolution values at 6 minutes.
- The casein sample had lower resolution values at 8.5 minutes. This one stands out also as having very few amino acid peaks in general, as seen in figure 3.2.

Should a protein-based paint contain compounds with retention times close to these values, based on the paint binder type, the peaks in that area in the chromatogram would probably have to be discarded. It could point to an issue with the temperature gradient chosen, that 8.5 minutes is an

area of low resolution in almost all the sample types. This suggests that the temperature gradient should be lowered to 11 °C/min or less from 12 °C/min, and by this probably resolve the other problem areas as well. It is also worth noting that all the three peaks discarded were at the third consecutive injection of the same sample. This could indicate that some of the solutes in the sample accumulated within the column. Although, this was not indicated by the blank run after the analysis. However, should accumulation of solutes prove to be the cause of the problem, increasing the length of the run or adding a high temperature gradient at the end of the program should help.

Signal-to-noise ratios

A few amino acid derivatives were disqualified for either quantification or detection due to low S/N. This may have due to an issue with the analysis method, but may also have been caused by issues with the detector. During the time period this analysis was performed, the MS produced small signal spikes across the spectrum from 40-500 m/z, particularly at the lower end of this spectrum. This led to increased noise in every analysis made. The issue was resolved when the detector unit was replaced later. Howerer, time constraints left no room for re-analyzing the samples. Table 1.6, provided by Colombini et al. (1998), indicated that the sample preparation would have to be done from the beginning, as the degradation of the derivatives would have been too great for an accurate measurement.

Amino acid ratios and group comparisons

All the statistical analyses made to group and to differentiate the samples analyzed in this study appeared to suggest the samples in each group (Egg based binders, Fish based binders, Animal glues and casein in borax) were statistically different to each other based on their amino acid ratios. The statistics also conclude that no group were statistically equal to any other group. There are however one limitation to this study, as it does not consider possible variation within each individual binder. It is not certain the result would be as conclusive if multiple different brands of each type of binder were analyzed. To counter this, due to the nature of proteins, it is also possible that the variation could be somewhat limited. This would need to be tested.

5 CONCLUSION

The method developed for detecting and differentiating protein-based binders was perhaps the greatest success of this study. The sample preparation method yielded results even when analyzing sample sizes as small as 1.6 mg (set as lower limit of detection). The analysis method separated every amino acid derivate chosen for evaluation, with no two peaks having a resolution value below 1.5. It was possible to differentiate both between and within the groups of animal glues, egg-based, fish-based and casein-based binders, as each sample analyzed had unique amino acid derivate ratios. The method developed in the analysis of protein-based binders however failed to produce any results when used to analyze protein-based paints. As such, no final evaluation of this method was possible according to the goals of this study.

The method developed for the analysis of the oil-based samples showed promising results, with regards to signal-to-noise ratio and resolution. The lower limit of detection was set to 1.6 mg for oil-based binders, and 2.8 mg for oil-based paints. The method did however have issues producing results that could be used to differentiate samples from each other based on content. The optimization of the ion source determined that 295 °C was ideal when analyzing palmitic acid methyl ester and stearic acid methyl ester.

In conclusion, the analysis method developed for oils were good enough to detect signals of pure samples and paint samples, but must be improved to be able to properly differentiate between samples. The method used on the proteins was good enough for detection and differentiation of pure samples, but needs improvement in order to be used on paints.

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APPENDIX

Appendix A – Software, instruments and materials

Table A.1 Software.

Software	Reference
Chromeleon 7 Chromatography Data System software	Thermo Scientific (2018). *Chromeleon Chromatography Data System software* (Version 7.2.9). Software. Available from: https://www.thermofisher.com/order/catalog/product/CHROMELEON7 (read 09.03.2019)
NIST 17 Mass Spectral Library	Scientific Instrument Services (2017). The NIST 17 Mass Spectral Library & Search Software (Version NIST 2017/2014/EPA/NIH). Software. Available from: https://www.sisweb.com/software/ms/nist.htm (read 13.03.2019)

Table A.2 Laboratory equipment

Product name	Supplier
GC-Trace 1310	Thermo fisher scientific
MS: MS-ISQ QD Single Quad Mass Spectrometer	Thermo fisher scientific
Autosampler: Tri Plus	Thermo fisher scientific
Microwave: StarSYNTH MA084 Labstation	Milestone
Centrifuge: CentriVAP Concentrator	Labconco
Vacum Unit: CVC 3000	Vaccubrand
Analytical Weight: ML104 Newclassic ML Analytical Balance	Mettler Toledo

Table A.3 Analysis columns.

Product name	Catalogue Number	Producer
GC Column 5inch cage DB-WAX 30m, 0.25mm, 0.25u	AG122-7032E	Agilent Technologies
GC Column HP-5MS 30m, 0.25mm, 0.25u	AG19091S-433	Agilent Technologies

Table A.4 Miscellaneous Laboratory Equipment.

Syringes	Maximum Volume	Product number	Supplier
SGE 1µ Syringe	1 µl	000500	SGE
SGE 5µ Syringe	5 µl	000803	SGE
Hamilton 1ml Syringe	1 ml	1001	Hamilton
Soft Ject Sole use syringe	1 ml	5010.200V0	Henke Sass Wolf
Syringe Filter	**Size/Filter Size**	**Product number**	**Supplier**
VWR Syringe Filter	25 mm / 0,45µm	28145-485	VWR

Table A.5 Chemicals and reagents.

Chemical Name	Purity grade	Article nr.	Cas nr.	Supplier
n-Pentane	Analytical Grade	1071772500	109-66-0	Merck
Methanol	HPLC Grade	494291	67-56-1	Merck
Sodium Hydroxide Pellets	≥98%	S8045	1310-73-2	Sigma Aldrich
Concentrated Sulfuric Acid	Reagent Grade	SS1118	7664-93-9	VWR
Pyridine	≥98%	83684.230	110-86-1	VWR
Tertbutyl amine	≥98%	B89205	75-64-9	Sigma Aldrich
BSTFA	GC Grade	3-3023	25561-30-2	Supelco
Sodium sulphate	≥ 98%	239313	7757-82-6	Sigma Aldrich

Table A.6 Analytical standards. All amino acids had a purity of 98 % or above. All methylated compounds are contained within the FAME Mix-RM1.

Amino Acid	Article nr.	Cas nr.	Supplier
D-Serine	S4250	312-84-5	Sigma Aldrich
L-Threonine	T8625	72-19-5	Sigma Aldrich
L-Valine	V0500	72-18-4	Sigma Aldrich
L-Isoleucine	12752	73-32-5	Sigma Aldrich
L-Aspartic Acid	A8949	56-84-8	Sigma Aldrich
L-Leucine	L8000	61-90-5	Sigma Aldrich
L-Glutamic Acid	49449	56-86-0	Fluka
L-Phenyl Alanine	78019	63-91-2	Sigma Aldrich
Hydroxy Proline	H54409	51-35-4	Sigma Aldrich
Glycine	104201	56-40-6	Merck
L-Alanine	A7627	56-41-7	Sigma Aldrich
Fame Mix-RM1	O7006	-	VWR
Methyl arachidate	-	1120-28-1	VWR
Methyl linoleate	-	112-63-0	VWR
Methyl linolenate	-	301-00-8	VWR
Methyl oleate	-	112-62-9	VWR
Methyl palmitate	-	112-39-0	VWR
Methyl stearate	-	112-61-8	VWR

Appendix B – Chromatogram peak tables for oils

Table B.1 Chromatogram peak table values for FAME standard, including identified fatty acids methyl esters, retention times, peak areas, signal-to-noise ratios (S/N) and resolutions. Only one injection was made.

Name	Retention Time (min)	Area (counts*min)	S/N	Resolution
Palmitic Acid Methyl Ester	3.501	33408127.5809	726.4	27.302
Stearic Acid Methyl Ester	5.667	25352451.1829	492.6	-

Table B.2 Chromatogram peak table values for different oil types, including identified fatty acids methyl esters, retention times, peak areas, signal-to-noise ratios (S/N) and resolutions. Each oil type was analyzed in technical triplicates (three injections) or duplicates (two injections). *: low S/N-ratio, signal discarded for use in quantification. *Table continues on next page*.

Oil type	Injection nr.	Name	Retention Time (min)	Area (counts*min)	S/N	Resolution
Oxidized Linseed Oil	1	Palmitic Acid Methyl Ester	3.681	47955669.6454	174.8	37.463
		Stearic Acid Methyl Ester	6.184	39431724.8443	91.8	3.854
	2	Palmitic Acid Methyl Ester	3.664	41295013.6325	146.9	38.018
		Stearic Acid Methyl Ester	6.167	32891979.4005	84.1	3.830
	3	Palmitic Acid Methyl Ester	3.674	53500864.8739	176.6	37.576
		Stearic Acid Methyl Ester	6.177	43931523.0545	88.6	3.716
Poppy Oil Kremer	1	Palmitic Acid Methyl Ester	3.671	45874906.4807	249	16.226
		Stearic Acid Methyl Ester	6.167	24864676.6763	154.3	3.955
	2	Palmitic Acid Methyl Ester	3.672	45867582.4205	248.6	16.730
		Stearic Acid Methyl Ester	6.168	25947123.4594	439.4	4.042
	3	Palmitic Acid Methyl Ester	3.664	46513915.3947	260.8	16.735
		Stearic Acid Methyl Ester	6.160	25970775.7768	281.3	4.038
Bleached Linseed Oil (Italy)	1	Palmitic Acid Methyl Ester	3.664	36736860.5637	389.7	37.964
		Stearic Acid Methyl Ester	6.160	27891285.1195	81.3	3.878
	2	Palmitic Acid Methyl Ester	3.664	37253387.7869	357.1	38.683
		Stearic Acid Methyl Ester	6.160	28159516.0775	71.8	3.952
	3	Palmitic Acid Methyl Ester	3.661	39981253.0586	377	38.571
		Stearic Acid Methyl Ester	6.157	30208757.2865	71.7	3.930
Stand Oil Kremer	1	Palmitic Acid Methyl Ester	3.691	143712463.4110	352.1	3.625
		Stearic Acid Methyl Ester	6.204	101799181.5426	381.1	-
	2	Palmitic Acid Methyl Ester	3.681	143305574.6005	370.1	3.686
		Stearic Acid Methyl Ester	6.201	99848135.5115	339.9	-
	3	Palmitic Acid Methyl Ester	3.691	153654294.5694	316.9	3.347
		Stearic Acid Methyl Ester	6.215	109905275.9049	321.6	-

Table B.2 *Continued*.

Oil type	Injection nr.	Name	Retention Time (min)	Area (counts*min)	S/N	Resolution
Walnut Oil Kremer	1	Palmitic Acid Methyl Ester	3.691	125623411.1017	317.9	3.152
		Stearic Acid Methyl Ester	6.171	22936776.5362	-	-
	2	Palmitic Acid Methyl Ester	3.691	125381374.1243	360.3	3.536
		Stearic Acid Methyl Ester	6.208	86339896.6008	323.5	-
	3	Palmitic Acid Methyl Ester	3.684	129146815.5981	338.2	3.468
		Stearic Acid Methyl Ester	6.208	89223443.7363	327.5	-
Poppy Oil Maimeri	1	Palmitic Acid Methyl Ester	3.664	33295466.2129	562.9	38.693
		Stearic Acid Methyl Ester	6.160	14190894.5852	57.2	3.927
	2	Palmitic Acid Methyl Ester	3.667	33225209.6829	453.5	38.872
		Stearic Acid Methyl Ester	6.164	14386446.9850	59.6	3.909
	3	Palmitic Acid Methyl Ester	3.665	34741715.6667	540.4	39.453
		Stearic Acid Methyl Ester	6.161	14871254.8359	59.7	3.980
Linseed Stand Oil Rowney	1	Palmitic Acid Methyl Ester	3.661	11844606.0181	67.1	39.303
		Stearic Acid Methyl Ester	6.158	7249140.1756	38.6	4.027
	2	Palmitic Acid Methyl Ester	3.667	42467076.5135	147.9	16.682
		Stearic Acid Methyl Ester	6.167	25004594.8497	671	3.975
	3	Palmitic Acid Methyl Ester	3.671	43318162.6872	156.1	16.562
		Stearic Acid Methyl Ester	6.170	25917617.2169	211.8	3.948
Dried Linseed Oil	1	Palmitic Acid Methyl Ester	3.654	13474374.5460	135.3	39.254
		Stearic Acid Methyl Ester	6.153	11729420.4734	105.3	3.913
	2	Palmitic Acid Methyl Ester	3.682	89992823.6075	290.4	4.167
		Stearic Acid Methyl Ester	6.195	76390218.1239	279.2	3.422
	3	Palmitic Acid Methyl Ester	3.664	43290303.4927	181.6	37.944
		Stearic Acid Methyl Ester	6.167	36179545.6579	108.4	3.904
Dried Poppy Oil	1	Palmitic Acid Methyl Ester	3.695	2419780.3637	50.6	25.233
		Stearic Acid Methyl Ester	6.205	1733306.7622	26.5	2.923
	2	Palmitic Acid Methyl Ester	3.712	2511442.2244	46.6	26.220
		Stearic Acid Methyl Ester	6.219	1802390.1888	29.7	2.874
	3	Palmitic Acid Methyl Ester	3.681	2810221.6266	63.9	26.127
		Stearic Acid Methyl Ester	6.191	1944231.7440	39.6	2.951
AMB 3.1	1	Palmitic Acid Methyl Ester	3.681	653850.9411	11.2	25.421
		Stearic Acid Methyl Ester	*6.177*	*394865.1538*	*9.5**	*3.630*
	2	Palmitic Acid Methyl Ester	3.674	586270.0124	11.4	26.184
		Stearic Acid Methyl Ester	*6.181*	*343108.5957*	*5.7**	-

Table B.2 *Continued*.

Oil type	Injection nr.	Name	Retention Time (min)	Area (counts*min)	S/N	Resolution
AMB 5.2	1	Palmitic Acid Methyl Ester	3.661	16480849.7561	153.7	23.879
		Stearic Acid Methyl Ester	6.154	7529170.1792	68.7	2.538
	2	Palmitic Acid Methyl Ester	3.671	16481525.9726	140.3	25.856
		Stearic Acid Methyl Ester	6.170	7509634.7947	176.3	2.688
	3	Palmitic Acid Methyl Ester	3.664	15841519.3758	140.3	25.709
		Stearic Acid Methyl Ester	6.164	7217185.8944	157.2	2.700
AMR 7.1	1	Palmitic Acid Methyl Ester	3.662	5272857.2167	54.6	27.099
		Stearic Acid Methyl Ester	6.162	1857461.4690	43.2	3.150
	2	Palmitic Acid Methyl Ester	3.667	7487086.5745	57.6	25.646
		Stearic Acid Methyl Ester	6.167	2526883.9109	41.4	3.094
	3	Palmitic Acid Methyl Ester	3.672	7453248.9530	57	25.057
		Stearic Acid Methyl Ester	6.172	2648661.5532	46.9	2.974
MØR 1.1	1	Palmitic Acid Methyl Ester	3.661	759214.0210	11.4	25.302
		Stearic Acid Methyl Ester	6.157	488169.2287	10.2	3.365
	2	Palmitic Acid Methyl Ester	3.664	790214.7260	17.5	30.384
		Stearic Acid Methyl Ester	6.167	573862.6658	12.7	3.890

Appendix C – Mass spectra

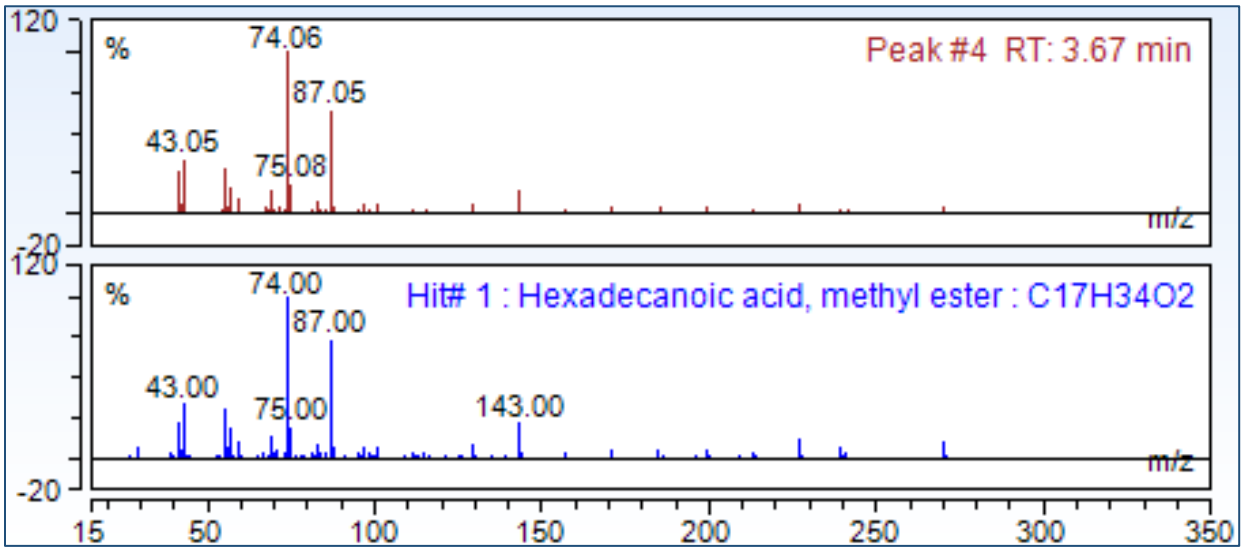


Figure C.1 Mass spectrum for C16:0 methyl ester (C17H34O2) from Poppy Oil Maimeri (red) compared to library spectrum of the same molecule (blue). Mass-to-charge ratios (m/z) is on the x-axis and the relative abundance (compared to base peak) on the y-axis. Red spectrum corresponds to the first injection of Poppy Oil Maimeri in appendix B table B.2.

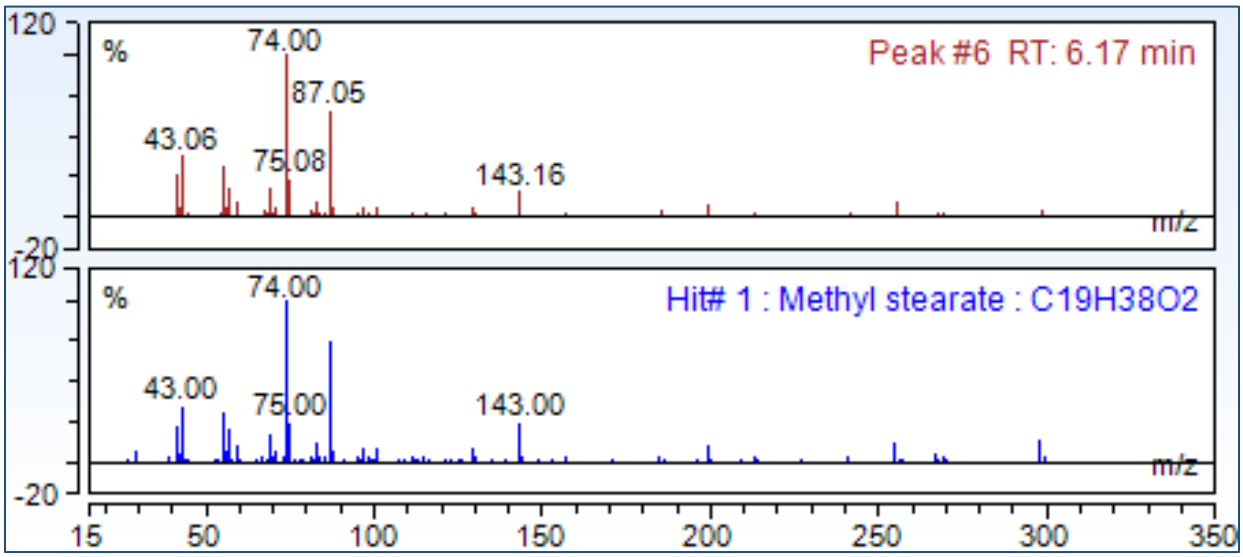


Figure C.2 Mass spectrum for C18:0 methyl ester (C19H38O2) from Poppy Oil Maimeri (red) compared to library spectrum of the same molecule (blue). Mass-to-charge ratios (m/z) is on the x-axis and the relative abundance (compared to base peak) on the y-axis. Red spectrum corresponds to the first injection of Poppy Oil Maimeri in appendix B table B.2.

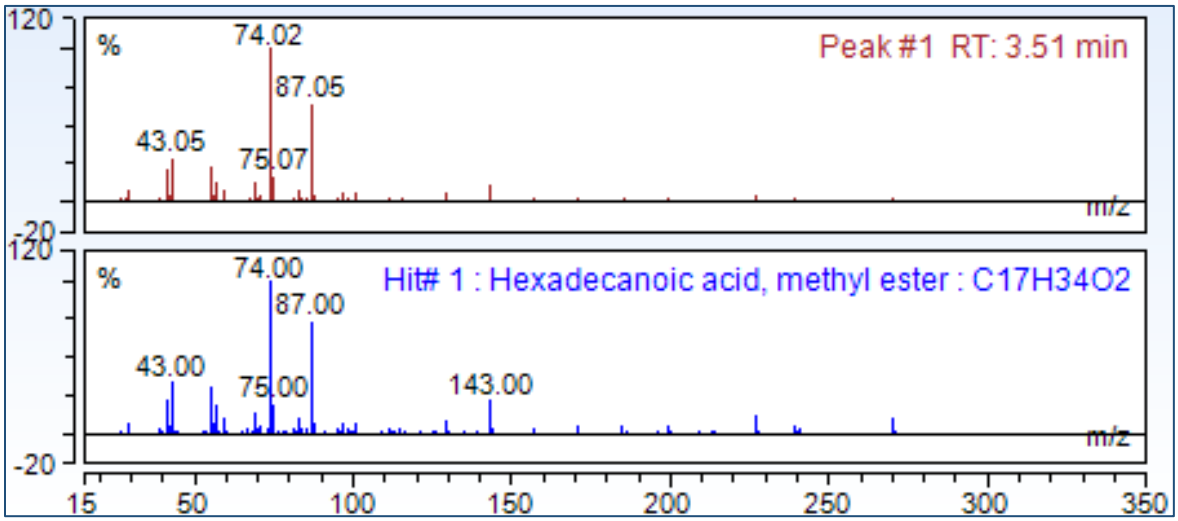


Figure C.3 Mass spectrum for C16:0 methyl ester (C17H34O2) in FAME standard (red) compared to library spectrum of the same molecule (blue). Mass-to-charge ratios (m/z) is on the x-axis and the relative abundance (compared to base peak) on the y-axis. Red spectrum corresponds to FAME standard in appendix B table B.1.

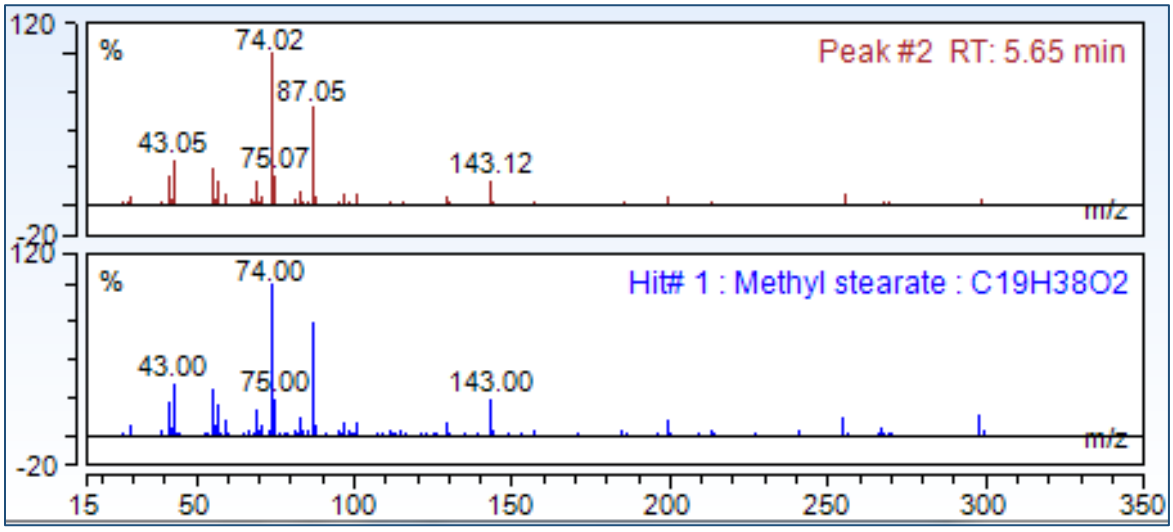


Figure C.4 Mass spectrum for C18:0 methyl ester (C19H38O2) in FAME standard (red) compared to library spectrum of the same molecule (blue). Mass-to-charge ratios (m/z) is on the x-axis and the relative abundance (compared to base peak) on the y-axis. Red spectrum corresponds to FAME standard in appendix B table B.1.

Appendix D – Chromatogram peak tables for amino acids

Table D.1 Egg yolk
Chromatogram peak table values for egg yolk, including identified amino acid derivatives, retention times, peak areas, signal-to-noise ratios (S/N) and resolutions. Sample was analyzed in technical triplicates (three injections). **: low S/N-ratio, signal discarded for use in identification and quantification.

Injection nr.	Name	Retention Time (min)	Area (counts·min)	S/N	Resolution
1	L-Alanine 2TMS	3.656	373790.4164	37	4.062
	L-Valine 2TMS	5.002	252843.0363	20.5	11.874
	Leucine 2TMS	5.689	1731896.134	148.6	6.442
	Isoleucine 2TMS	5.948	209886.9315	23.1	2.043
	Glycine 3TMS	6.111	190750.9419	79	2.816
	Serine 3TMS	6.73	689889.9059	114.5	6.178
	Threonine 3TMS	7.019	399438.6843	164.5	8.592
	Aspartic Acid 3TMS	8.512	735265.7657	217.4	3.901
	Hydroxyproline 3TMS	*8.628*	*27298.6737*	*4.2***	*18.282*
	Glutamic Acid 3TMS	9.4	671771.4565	140.1	2.3
	Phenyl Alanine 2TMS	9.461	254535.5328	40.5	18.384
2	L-Alanine 2TMS	3.645	386475.8374	53.3	4.07
	L-Valine 2TMS	4.995	258821.3504	24.4	12.59
	Leucine 2TMS	5.682	1701810.497	431	3.344
	Isoleucine 2TMS	5.94	208538.6049	76.8	2.066
	Glycine 3TMS	6.107	190996.8163	75.2	2.736
	Serine 3TMS	6.726	682371.4373	63.9	8.811
	Threonine 3TMS	7.015	392887.3584	38.2	7.65
	Aspartic Acid 3TMS	8.508	712334.4647	65.3	3.685
	Hydroxyproline 3TMS	*8.627*	*26303.1674*	*2.8***	*22.351*
	Glutamic Acid 3TMS	9.396	658395.2669	108.4	2.402
	Phenyl Alanine 2TMS	9.46	240640.9434	30.2	17.754
3	L-Alanine 2TMS	3.647	423765.9187	45.8	4.173
	L-Valine 2TMS	4.994	280328.4525	23	12.713
	Leucine 2TMS	5.681	1826225.989	595.5	3.211
	Isoleucine 2TMS	5.939	225398.9892	58.1	2.054
	Glycine 3TMS	6.106	210135.4253	57.1	2.855
	Serine 3TMS	6.725	738833.8065	108.3	5.84
	Threonine 3TMS	7.014	429173.0439	63.6	7.77
	Aspartic Acid 3TMS	8.511	765149.9458	381.2	3.694
	Hydroxyproline 3TMS	*8.626*	*25502.8542*	*4.4***	*1.006*
	Glutamic Acid 3TMS	9.398	695839.9436	72.6	2.211
	Phenyl Alanine 2TMS	9.46	263840.8494	19.4	13.492

Table D.2 Entire egg
Chromatogram peak table values for entire egg, including identified amino acid derivatives, retention times, peak areas, signal-to-noise ratios (S/N) and resolutions. Sample was analyzed in technical triplicates (three injections).

Injection nr.	Name	Retention Time (min)	Area (counts·min)	S/N	Resolution
1	L-Alanine 2TMS	3.658	529929.704	36.3	4.114
	L-Valine 2TMS	5.005	371762.5748	104.3	13.599
	Leucine 2TMS	5.692	1450702.51	170.1	3.279
	Isoleucine 2TMS	5.95	360228.7655	107.8	1.746
	Glycine 3TMS	6.117	403004.4221	229.7	2.68
	Serine 3TMS	6.733	890740.0242	282.6	2.412
	Threonine 3TMS	7.022	534086.2971	108	8.324
	Aspartic Acid 3TMS	8.518	1098988.742	77.1	3.607
	Hydroxyproline 3TMS	8.637	139962.0624	35.7	6.745
	Glutamic Acid 3TMS	9.406	879187.2568	118	1.821
	Phenylalanine 2TMS	9.467	523756.9966	44.9	2.776
2	L-Alanine 2TMS	3.655	310831.3588	47.6	5.27
	L-Valine 2TMS	5.008	204588.0756	26.8	14.715
	Leucine 2TMS	5.692	751421.8152	91.4	6.552
	Isoleucine 2TMS	5.95	187128.9903	22	2.142
	Glycine 3TMS	6.121	136564.3297	17.4	18.43
	Serine 3TMS	6.733	473473.1792	59.7	9.019
	Threonine 3TMS	7.025	269948.1408	34.3	14.346
	L-Aspartic Acid 3TMS	8.518	597740.1355	93.1	4.478
	Hydroxyproline 3TMS	8.661	104144.5101	34.2	5.86
	Glutamic Acid 3TMS	9.403	485929.7824	69.2	2.32
	Phenylalanine 2TMS	9.464	215153.226	21.7	23.916
3	L-Alanine 2TMS	3.645	463177.2543	148.3	2.066
	L-Valine 2TMS	4.995	334224.3445	70	13.731
	Leucine 2TMS	5.682	1364811.62	170.2	3.121
	Isoleucine 2TMS	5.944	356930.8326	90.7	1.671
	Glycine 3TMS	6.11	403793.2409	149.7	2.864
	Serine 3TMS	6.726	789093.3135	74.1	5.788
	Threonine 3TMS	7.015	460131.1801	72.4	7.427
	Aspartic Acid 3TMS	8.511	960849.6277	212.5	2.342
	Hydroxyproline 3TMS	8.634	212396.8955	42.5	4.136
	Glutamic Acid 3TMS	9.399	792184.9925	123.1	1.772
	Phenylalanine 2TMS	9.46	507085.1016	44.7	2.805

Table D.3 Egg white foam

Chromatogram peak table values for egg white foam, including identified amino acid derivatives, retention times, peak areas, signal-to-noise ratios (S/N) and resolutions. Sample was analyzed in technical triplicates (three injections).

Injection nr.	Name	Retention Time (min)	Area (counts·min)	S/N	Resolution
1	L-Alanine 2TMS	3.651	329350.408	30.3	4.176
	L-Valine 2TMS	4.998	96998.6696	18.3	17.234
	Leucine 2TMS	5.682	352340.556	69.2	7.031
	Isoleucine 2TMS	5.94	65000.0649	20.8	2.073
	Glycine 3TMS	6.11	225608.6608	40.6	18.08
	Serine 3TMS	6.726	392924.1503	74.5	8.842
	Threonine 3TMS	7.015	159236.3446	53.6	46.883
	Aspartic Acid 3TMS	8.508	554327.994	83	23.908
	Hydroxyproline 3TMS	-	-	-	-
	Glutamic Acid 3TMS	9.396	413449.0659	141.9	2.641
	Phenyl Alanine 2TMS	9.46	136474.6532	40.1	19.97
2	L-Alanine 2TMS	3.638	344030.9416	29.7	4.151
	L-Valine 2TMS	4.988	100948.0409	19.4	17.022
	Leucine 2TMS	5.672	358692.6724	71.3	7.139
	Isoleucine 2TMS	5.937	64347.416	11.1	1.957
	Glycine 3TMS	6.104	227036.0001	40.6	17.9
	Serine 3TMS	6.716	394966.7623	74.9	8.708
	Threonine 3TMS	7.008	167432.5095	45.1	28.028
	Aspartic Acid 3TMS	8.501	572023.0174	152.9	30.385
	Hydroxyproline 3TMS	-	-	-	-
	Glutamic Acid 3TMS	9.392	419312.4567	63.9	2.37
	Phenyl Alanine 2TMS	9.454	138731.534	19.5	19.051
3	L-Alanine 2TMS	3.638	332245.8411	34	4.482
	L-Valine 2TMS	4.988	94903.3834	20.1	16.428
	Leucine 2TMS	5.672	349532.7457	113.3	7.122
	Isoleucine 2TMS	5.933	68610.437	23.5	2.091
	Glycine 3TMS	6.104	230506.051	40.4	18.414
	Serine 3TMS	6.719	390262.6245	76.3	8.932
	Threonine 3TMS	7.008	165775.722	53.1	27.288
	Aspartic Acid 3TMS	8.505	571826.3786	131.3	32.541
	Hydroxyproline 3TMS	-	-	-	-
	Glutamic Acid 3TMS	9.392	407563.4574	97.2	2.446
	Phenyl Alanine 2TMS	9.454	137493.3113	29.5	19.556

Table D.4 Rabbit glue in pearls

Chromatogram peak table values for rabbit glue in pearls, including identified amino acid derivatives, retention times, peak areas, signal-to-noise ratios (S/N) and resolutions. Sample was analyzed in technical triplicates (three injections).

Injection nr.	Name	Retention Time (min)	Area (counts·min)	S/N	Resolution
1	L-Alanine 2 TMS	3.638	779999.2821	85.2	4.69
	L-Valine 2TMS	4.995	136208.1284	15.3	17.728
	Leucine 2tms	5.678	258438.6676	67.3	7.061
	Isoleucine 2tms	5.944	70530.5459	21.5	1.941
	Glycine 3TMS	6.121	3548329.501	553.8	16.632
	Serine 3TMS	6.723	340483.291	107	8.92
	Threonine 3TMS	7.015	200928.5792	58.5	28.486
	Aspartic Acid 3TMS	8.511	446914.6149	31.5	2.006
	Hydroxyproline 3TMS	8.58	1011486.245	69.5	27.194
	Glutamic Acid 3TMS	9.399	658237.4402	38.2	2.556
	Phenylalanine 2TMS	9.46	92329.4039	11.6	40.527
2	L-Alanine 2 TMS	3.645	824032.707	79.1	4.467
	L-Valine 2TMS	4.998	146914.3026	17.2	17.904
	Leucine 2tms	5.682	282419.1819	74.1	6.916
	Isoleucine 2tms	5.944	75620.8963	10	2.08
	Glycine 3TMS	6.127	3739920.011	473.4	16.005
	Serine 3TMS	6.726	372871.5111	104.4	8.919
	Threonine 3TMS	7.018	210176.7322	63.5	29.191
	Aspartic Acid 3TMS	8.518	479402.5691	30.5	2.006
	Hydroxyproline 3TMS	8.586	1094383.095	68.8	17.461
	Glutamic Acid 3TMS	9.403	720770.1519	234.3	2.457
	Phenylalanine 2TMS	9.464	137457.9658	40.5	38.476
3	L-Alanine 2 TMS	3.641	803935.8131	80	4.333
	L-Valine 2TMS	4.995	142451.1409	16.6	17.581
	Leucine 2tms	5.678	285005.4959	62.8	6.841
	Isoleucine 2tms	5.94	67145.8429	14.9	1.993
	Glycine 3TMS	6.121	3658826.803	509.3	16.517
	Serine 3TMS	6.723	366933.4279	106.5	8.893
	Threonine 3TMS	7.015	216378.4896	61.3	28.597
	Aspartic Acid 3TMS	8.511	471250.9755	29.1	1.996
	Hydroxyproline 3TMS	8.58	1053655.649	67.2	28.019
	Glutamic Acid 3TMS	9.399	708177.0216	27.1	2.468
	Phenylalanine 2TMS	9.46	102803.2571	11.1	36.513

Table D.5 Animal glue

Chromatogram peak table values for animal glue, including identified amino acid derivatives, retention times, peak areas, signal-to-noise ratios (S/N) and resolutions. Sample was analyzed in technical triplicates (three injections). *: low S/N-ratio, signal discarded for use in quantification. **: low S/N-ratio, signal discarded for use in identification and quantification.

Injection nr.	Name	Retention Time (min)	Area (counts·min)	S/N	Resolution
1	L-Alanine 2TMS	3.638	426642.6325	68.5	4.613
	L-Valine 2TMS	*4.985*	*25659.0107*	*3.5***	*17.556*
	Leucine 2TMS	5.672	127747.6092	12.9	8.959
	Isoleucine 2tms	-	-	-	-
	Glycine 3TMS	6.11	2069818.738	442.5	17.43
	Serine 3TMS	6.716	152008.7231	58.8	8.731
	Threonine 3TMS	7.005	63929.4513	24.8	27.996
	Aspartic Acid 3TMS	8.501	215161.4824	47.7	2.165
	Hydroxyproline 3TMS	8.569	364385.661	81.3	30.019
	Glutamic Acid 3TMS	9.389	296049.2503	45.2	2.521
	Phenylalanine 2TMS	*9.454*	*51170.5531*	*5.5**	*18.049*
2	L-Alanine 2TMS	3.641	440906.1659	57.9	4.325
	L-Valine 2TMS	*4.995*	*27370.5686*	*4.1***	*17.236*
	Leucine 2TMS	5.679	134349.4657	14.2	8.669
	Isoleucine 2tms	-	-	-	-
	Glycine 3TMS	6.114	2165808.191	261.3	17.824
	Serine 3TMS	6.723	150695.9054	49.4	8.986
	Threonine 3TMS	7.012	64563.5109	21.1	27.678
	Aspartic Acid 3TMS	8.508	229542.1525	50.9	2.015
	Hydroxyproline 3TMS	8.573	375013.3538	81.4	25.42
	Glutamic Acid 3TMS	9.396	288900.0499	13.4	31.609
	Phenylalanine 2TMS	-	-	-	-
3	L-Alanine 2TMS	3.638	432880.6406	54.8	4.553
	L-Valine 2TMS	*4.991*	*27320.3065*	*3.9***	*16.366*
	Leucine 2TMS	5.675	128218.7491	17	8.835
	Isoleucine 2tms	-	-	-	-
	Glycine 3TMS	6.114	2138833.123	306.2	16.918
	Serine 3TMS	6.719	152978.7584	65.6	8.505
	Threonine 3TMS	7.008	60411.0529	24.5	30.398
	Aspartic Acid 3TMS	8.508	227547.8368	56.7	2.019
	Hydroxyproline 3TMS	8.573	377880.1626	92.9	23.197
	Glutamic Acid 3TMS	9.392	301249.9997	127.4	2.552
	Phenylalanine 2TMS	9.457	56798.2877	18.9	1.372

Table D.6 Animal glue in pearls

Chromatogram peak table values for animal glue in pearls, including identified amino acid derivatives, retention times, peak areas, signal-to-noise ratios (S/N) and resolutions. Sample was analyzed in technical triplicates (three injections). *: low S/N-ratio, signal discarded for use in quantification. **: low S/N-ratio, signal discarded for use in identification and quantification.

Injection nr.	Name	Retention Time (min)	Area (counts·min)	S/N	Resolution
1	L-Alanine 2TMS	3.656	1038555.134	145.6	4.126
	L-Valine 2TMS	*5.006*	*54090.9116*	*4.5***	*16.613*
	Leucine 2TMS	5.686	258227.099	13.3	8.241
	Isoleucine 2tms	-	-	-	-
	Glycine 3TMS	6.135	5962402.989	276.4	14.75
	Serine 3TMS	6.73	412446.7476	105.7	8.615
	Threonine 3TMS	7.019	158901.5997	39.3	27.667
	Aspartic Acid 3TMS	8.519	643225.8479	57.8	1.995
	Hydroxyproline 3TMS	8.587	963630.8559	86.3	27.52
	Glutamic Acid 3TMS	9.403	730502.9085	14.4	21.335
	Phenylalanine 2TMS	9.46	105503.475	21	1.68
2	L-Alanine 2TMS	3.648	1124984.602	145.8	4.183
	L-Valine 2TMS	*4.998*	*53946.9857*	*8.9**	*16.406*
	Leucine 2TMS	5.678	264690.2213	42.2	6.921
	Isoleucine 2tms	-	-	-	-
	Glycine 3TMS	6.131	6097024.146	447	14.82
	Serine 3TMS	6.726	422692.2893	90.9	8.759
	Threonine 3TMS	7.015	160891.2049	35.4	28.765
	Aspartic Acid 3TMS	8.515	698178.1374	68.7	1.852
	Hydroxyproline 3TMS	8.579	1011274.982	103.1	18.416
	Glutamic Acid 3TMS	9.399	793410.2053	264.5	2.399
	Phenylalanine 2TMS	9.46	89108.628	21.9	1.757
3	L-Alanine 2TMS	3.648	1161858.062	141	3.865
	L-Valine 2TMS	4.998	68922.5205	13.2	12.527
	Leucine 2TMS	5.678	273803.0362	70.3	6.316
	Isoleucine 2tms	-	-	-	-
	Glycine 3TMS	6.131	6204735.436	390.5	14.791
	Serine 3TMS	6.726	448279.8106	117	8.682
	Threonine 3TMS	7.015	168191.6267	44.6	13.586
	Aspartic Acid 3TMS	8.515	705265.6571	67	1.9
	Hydroxyproline 3TMS	8.579	1033096.399	100.7	17.089
	Glutamic Acid 3TMS	9.399	806063.9546	329.8	2.413
	Phenylalanine 2TMS	9.46	104488.4067	23	1.692

Table D.7 Fish glue
Chromatogram peak table values for fish glue, including identified amino acid derivatives, retention times, peak areas, signal-to-noise ratios (S/N) and resolutions. Sample was analyzed in technical triplicates (three injections). *: low S/N-ratio, signal discarded for use in quantification.

Injection nr.	Name	Retention Time (min)	Area (counts·min)	S/N	Resolution
1	L-Alanine 2TMS	3.655	1093379.263	52.5	4.039
	L-Valine 2TMS	5.002	76573.5175	10.4	17.035
	Leucine 2TMS	5.685	223089.862	30	6.338
	Isoleucine 2TMS	*5.947*	*66891.0269*	*7.8**	*1.945*
	Glycine 3TMS	6.131	4901836.971	538.1	15.372
	Serine 3TMS	6.729	620233.011	151.3	8.679
	Threonine 3TMS	7.022	310849.3086	73.4	27.946
	Aspartic Acid 3TMS	8.518	608616.9654	51.8	1.857
	Hydroxyproline 3TMS	8.583	1009358.971	86.4	27.083
	Glutamic Acid 3TMS	9.403	746736.5592	75.4	2.486
	Phenyl Alanine 2TMS	9.464	119197.2701	13	26.928
2	L-Alanine 2TMS	3.645	1132368.553	54.9	3.971
	L-Valine 2TMS	*4.995*	*88390.1426*	*9.9**	*16.839*
	Leucine 2TMS	5.675	229355.1925	30.9	6.851
	Isoleucine 2TMS	*5.94*	*62910.2183*	*5.5**	*1.972*
	Glycine 3TMS	6.124	5008284.359	360.4	15.48
	Serine 3TMS	6.723	625747.2678	161.3	8.697
	Threonine 3TMS	7.012	316965.9258	82.2	27.899
	Aspartic Acid 3TMS	8.511	625757.5553	53	1.927
	Hydroxyproline 3TMS	8.576	1032519.652	91.1	27.271
	Glutamic Acid 3TMS	9.396	748841.2039	68.7	2.463
	Phenyl Alanine 2TMS	9.457	117203.3673	11.3	18.759
3	L-Alanine 2TMS	3.645	1156096.818	51.3	4.035
	L-Valine 2TMS	4.995	88260.7869	11.2	17.203
	Leucine 2TMS	5.678	221905.5272	33	6.685
	Isoleucine 2TMS	*5.94*	*75976.2474*	*7.9**	*2.058*
	Glycine 3TMS	6.124	5094636.455	494.3	15.435
	Serine 3TMS	6.726	637632.4526	146.7	8.623
	Threonine 3TMS	7.015	328399.6619	74	27.157
	Aspartic Acid 3TMS	8.511	638232.8289	54.9	2.028
	Hydroxyproline 3TMS	8.579	1056203.927	93.1	27.763
	Glutamic Acid 3TMS	9.399	761268.9004	76.8	2.302
	Phenyl Alanine 2TMS	9.457	123230.3293	11.7	18.79

Table D.8 Fishleim

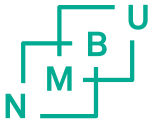
Chromatogram peak table values for fishleim, including identified amino acid derivatives, retention times, peak areas, signal-to-noise ratios (S/N) and resolutions. Sample was analyzed in technical triplicates (three injections). *: low S/N-ratio, signal discarded for use in quantification.

Injection nr.	Name	Retention Time (min)	Area (counts·min)	S/N	Resolution
1	L-Alanine 2TMS	3.648	1092342.087	76.3	4.099
	L-Valine 2TMS	*4.995*	*55519.0877*	*6.7**	*12.749*
	Leucine 2TMS	5.679	275960.1085	77.8	6.168
	Isoleucine 2TMS	*5.944*	*34166.4269*	*6**	*1.984*
	Glycine 3TMS	6.128	5535528.923	869.7	15.073
	Serine 3TMS	6.726	812973.2898	281.2	8.535
	Threonine 3TMS	7.012	231511.334	78.3	28.556
	Aspartic Acid 3TMS	8.512	715001.7866	96.1	1.944
	Hydroxyproline 3TMS	8.577	627376.4593	91	18.741
	Glutamic Acid 3TMS	9.4	809468.246	424.4	2.478
	Phenyl Alanine 2TMS	9.461	95220.6646	35.1	1.645
2	L-Alanine 2TMS	3.638	1115280.715	86	4.01
	L-Valine 2TMS	4.988	62776.6785	11.1	12.301
	Leucine 2TMS	5.672	281969.8285	84.7	5.786
	Isoleucine 2tms	-	-	-	-
	Glycine 3TMS	6.121	5581518.475	249	15.029
	Serine 3TMS	6.719	831226.6228	223.4	8.603
	Threonine 3TMS	7.008	231689.1875	63	28.567
	Aspartic Acid 3TMS	8.505	732850.2317	101	2.057
	Hydroxyproline 3TMS	8.573	637286.9019	94.9	19.376
	Glutamic Acid 3TMS	9.396	824911.0933	262	2.358
	Phenyl Alanine 2TMS	9.454	98045.1198	27.6	1.689
3	L-Alanine 2TMS	3.638	1145007.247	93.9	4.131
	L-Valine 2TMS	4.988	65859.0975	11.9	13.052
	Leucine 2TMS	5.668	276171.5981	57.3	5.919
	Isoleucine 2TMS	*5.937*	*32852.0191*	*3.7**	*1.833*
	Glycine 3TMS	6.121	5687367.285	618	15.016
	Serine 3TMS	6.719	831130.5459	239	8.615
	Threonine 3TMS	7.008	234993.8698	57.9	28.802
	Aspartic Acid 3TMS	8.505	729732.8774	101.4	1.976
	Hydroxyproline 3TMS	8.569	638598.7949	92.9	17.764
	Glutamic Acid 3TMS	9.392	792686.7868	624.7	2.28
	Phenyl Alanine 2TMS	9.45	96347.729	24.9	1.775

Table D.9 Casein in borax

Chromatogram peak table values for casein in borax, including identified amino acid derivatives, retention times, peak areas, signal-to-noise ratios (S/N) and resolutions. Sample was analyzed in technical triplicates (three injections). *: low S/N-ratio, signal discarded for use in quantification. **: low S/N-ratio, signal discarded for use in identification and quantification.

Injection nr.	Name	Retention Time (min)	Area (counts·min)	S/N	Resolution
1	L-Alanine 2TMS	-	-	-	-
	L-Valine 2TMS	-	-	-	-
	Leucine 2TMS	-	-	-	-
	Isoleucine 2TMS	-	-	-	-
	Glycine 3TMS	-	-	-	-
	Serine 3TMS	*6.723*	*19423.1421*	*7.9**	*2.727*
	Threonine 3TMS	6.933	247619.8237	46.3	9.051
	Aspartic Acid 3TMS	8.535	77717.6396	22	1.65
	Hydroxyproline 3TMS	8.631	515366.1786	117.4	3.171
	Glutamic Acid 3TMS	*9.39*	*21343.1172*	*1.2***	*3.011*
	Phenyl Alanine 2TMS	9.477	1716225.522	80	2.786
2	L-Alanine 2TMS	-	-	-	-
	L-Valine 2TMS	-	-	-	-
	Leucine 2TMS	-	-	-	-
	Isoleucine 2TMS	-	-	-	-
	Glycine 3TMS	-	-	-	-
	Serine 3TMS	*6.723*	*21989.7038*	*7.6**	*2.739*
	Threonine 3TMS	6.93	245960.1155	41	9.947
	Aspartic Acid 3TMS	8.532	85808.3684	10.4	1.73
	Hydroxyproline 3TMS	8.631	250076.9073	46.2	1.062
	Glutamic Acid 3TMS	9.393	22727.8755	15	3.255
	Phenyl Alanine 2TMS	9.478	1744886.575	307.7	2.848
3	L-Alanine 2TMS	-	-	-	-
	L-Valine 2TMS	-	-	-	-
	Leucine 2TMS	-	-	-	-
	Isoleucine 2TMS	-	-	-	-
	Glycine 3TMS	-	-	-	-
	Serine 3TMS	*6.723*	*20100.0685*	*7.5**	*2.93*
	Threonine 3TMS	6.937	266500.6039	51.6	8.858
	Aspartic Acid 3TMS	*8.536*	*67097.6837*	*7.9**	*1.729*
	Hydroxyproline 3TMS	8.634	277264.8805	38.5	0.961
	Glutamic Acid 3TMS	9.396	22981.9674	12	3.198
	Phenyl Alanine 2TMS	9.481	1870434.467	332.3	2.769



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