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Identification and quantitation of lipids in Atlantic mackerel (*Scomber scombrus*), wild and farmed Atlantic salmon (*Salmo salar*), and salmon feed by GC-MS

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Chemistry and biotechnology

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Acknowledgements

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Abstract

The main objective of this study was to elucidate and quantitate the fatty acid (FA) profiles of mackerel (*Scomber scombrus*), wild and farmed salmon (*Salmo salar*), and salmon feed. Due to the increasing proportions of vegetable oils in salmon feed, it was of interest to evaluate its effects on the FA profile of farmed salmon. To determine how much the feed affects the FAs in farmed salmon, it was of interest to compare the concentrations of the important n-3 FAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in wild salmon and farmed salmon. It was also of interest to look at the FA profile of another fatty wild fish, mackerel, to compare it to the salmon. The fish were evaluated from a health perspective by discussing the contents of n-3 and n-6 FAs, saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs). The nutritional quality indices; atherogenicity index, thrombogenicity index, as well as the n-6/n-3 ratio were also subsequently discussed.

Total FA profile in fish and feed was found using a gas chromatograph coupled with a single-quadrupole mass spectrometer. The method of extraction and derivatisation of the lipids had already been established and included extraction of the lipids with solvents, and further derivatisation to fatty acid methyl esters. The fish lipids were subsequently fractioned by off-line solid-phase extraction to neutral lipids, free fatty acids, and polar lipids. The lipid content was found to be 3.1 ± 1.5%, 2.14 ± 0.32%, and 8.97 ± 0.63% of muscle in respectively mackerel, wild salmon, and farmed salmon. A total of 37, 36, 35, and 34 FAs were found in respectively mackerel, wild salmon, farmed salmon, and salmon feed adding up to 39 unique FAs. The content of n-3- and n-6 FAs were greatest in farmed salmon as a result of the feed composition. The contents of SFAs, MUFAs, and PUFAs in mackerel were respectively 33.1, 35.3, and 31.6%, while 15.0, 55.4, and 29.6%, respectively in farmed salmon, and 26.3, 47.4, and 26.3%, respectively in wild salmon. Both wild salmon and farmed salmon contained approximately the same amount of EPA and DHA with 520 and 523 mg/100 g fish muscle, respectively. The mackerel, however, was significantly richer in EPA and DHA (1,004 mg/100g fish muscle) compared to the salmons. The results suggested that substituting a diet of farmed salmon with either wild salmon or mackerel might prove more nutritionally favourable.

Sammendrag

Hovedmålet med denne studien var å identifisere og kvantifisere fettsyreprofiler av makrell (*Scomber scombrus*), vill- og oppdrettslaks (*Salmo salar*) og laksefôr. På grunn av de økende andelene av vegetabilske oljer i laksefôr, var det av interesse å evaluere effekten på oppdrettslaksens fettsyreprofil. For å bestemme hvor mye fôret påvirker fettsyrene hos oppdrettslaks, var det av interesse å sammenligne konsentrasjonene av den viktige n-3 fettsyrene eikosapentaensyre (EPA) og dokosaheksaensyre (DHA) hos villaks og oppdrettslaks. Det var også interessant å se på fettsyreprofilen til en annen fet villfisk, makrell, for å sammenligne den med laksen. Fiskene ble evaluert fra et helsemessig perspektiv ved å diskutere innholdet i n-3 og n-6 fettsyrer, mettede fettsyrer (SFA), enumettede fettsyrer (MUFA) og flerumettede fettsyrer (PUFA). Næringsmessige kvalitetsindekser; atherogenisitetsindeks, trombogenisitetsindeks og n-6/n-3-forholdet ble også deretter diskutert.

Total fettysreprofil i fisk og fôr ble funnet ved bruk av en gasskromatograf kombinert med et singel-kvadrupol massespektrometer. Metoden for ekstraksjon og derivatisering av lipidene var allerede etablert og inkluderte ekstraksjon av lipidene med løsningsmidler, og videre derivatisering til fettsyremetylestere. Fiskelipidene ble deretter fraksjonert ved off-line fastfaseekstraksjon til nøytrale lipider, frie fettsyrer og polare lipider. Lipidinnholdet ble funnet å være 3,1 ± 1,5%, 2,14 ± 0,32% og 8,97 ± 0,63% av muskelen til henholdsvis makrell, villaks og oppdrettslaks. Det ble funnet 37, 36, 35 og 34 fettsyrer i henholdsvis makrell, villaks, oppdrettslaks og laksefôr, og utgjorde totalt 39 unike fettsyrer. Innholdet av n-3- og n-6 fettsyrer var størst hos oppdrettslaks som et resultat av fôrsammensetningen. Innholdet av SFA, MUFA og PUFA i makrell var henholdsvis 33,1, 35,3 og 31,6%, mens henholdsvis 15,0, 55,4 og 29,6% i oppdrettslaks og henholdsvis 26,3, 47,4 og 26,3% i villaks. Både villaks og oppdrettslaks inneholdt omtrent samme mengde EPA og DHA med henholdsvis 520 og 523 mg/100 g fiskemuskel. Makrellen hadde et betydelig rikere innhold av EPA og DHA (1 004 mg/100 g fiskemuskel) sammenlignet med laksene. Resultatene antydet at det å erstatte dieten av oppdrettslaks med enten villaks eller makrell kan vise seg å være mer ernæringsmessig gunstig.

Abbreviations

AI	Atherogenicity index
ALA	α-linolenic acid
CHD	Coronary heart disease
CVD	Cardiovascular disease
DC	Direct current
DHA	Docosahexaenoic acid
EFA	Essential fatty acid
EFSA	European Food Safety Authority
EI	Electron ionisation
EPA	Eicosapentaenoic acid
FA	Fatty acid
FAME	Fatty acid methyl ester
FFA	Free fatty acid
GC	Gas chromatography
IS	Internal standard
LA	Linoleic acid
LOD	Limit of detection
LOQ	Limit of quantification
m/z	Mass/charge
ME	Methyl ester
MS	Mass spectrometry
MUFA	Monounsaturated fatty acid
NIFES	Norwegian Institute of Nutrition and Seafood Research
NL	Neutral lipid
OA	Oleic acid
PL	Polar lipid
PUFA	Polyunsaturated fatty acid
RIC	Reconstructed ion chromatogram
RF	Radio frequency
rpm	Revolutions per minute
RRF	Relative response factor
SFA	Saturated fatty acid
S/N	Signal to noise ratio
SPE	Solid-phase extraction
TAG	Triacylglyceride
TI	Thrombogenicity index
TIC	Total ion current

1. General introduction

Fish has been, and continues to be, an important nutrition source for humans. From an early age we are told that fish is healthy for us due to the marine n-3 fatty acids (FAs). Both the Atlantic mackerel (*Scomber scombrus*) and Atlantic salmon (*Salmo salar*) have been considered to be great sources of these n-3 FAs as well as polyunsaturated fatty acids (PUFA), which are highly valued for their benefits on human health (Lundbye et al., 2017; Guizani & Moujahed, 2015). In Norway, farmed Atlantic salmon has become an export-article of great importance. The Atlantic salmon lives in the Atlantic Ocean and adjoining rivers. There are farms located across the entire Norwegian west coast as illustrated in Figure 1.1.

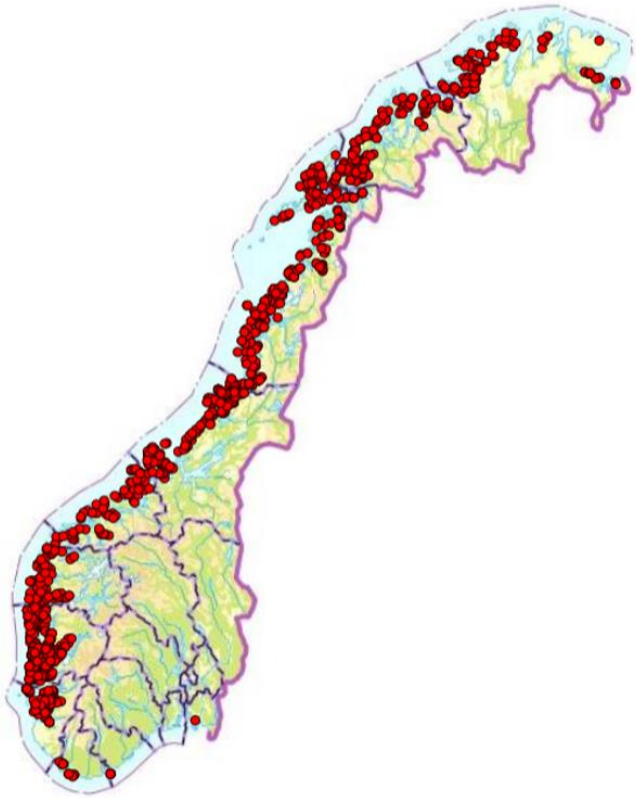


Figure 1.1: *A map over all the aquaculture farms utilised for Atlantic salmon in Norway (2019). The map is constructed with the directorate of fisheries' own mapping solutions (Directorate of fisheries, 2020a).*

Around 40 years ago, a breeding program was initiated and wild salmons from different rivers in Norway were collected and selectively bred to promote favourable traits, such as growth rate and survivability in captivity (Skogheim, 2018). Today, the fish have adapted to a different environment than their wild counterpart. Several studies state that escapees from farms leads

to genetic interference in the wild salmon population (Diserud et al., 2019; Glover et al., 2011). In the recent years, there has been a significant increase in escapees from these farms. The directorate of fisheries (2020b) estimates that around 290,000 farmed salmons escaped in 2019. In Norway, the wild salmon population is threatened, and over the years, it has been halved. This is due to the genetic mixing from escaped farmed salmons, over-fishing, acidic rivers, parasites, sickness, etc. (Grefsrud et al., 2018; Skogheim, 2018). However, measures are taken to secure the survival of the population in the Norwegian rivers. Today there are approximately 400 salmon rivers in Norway, and about 500,000 salmons return to these rivers every year to spawn (Anon, 2019).

Apart from the Atlantic salmon, the Atlantic mackerel is one of the most important and valuable fish populations in Norway. In 2017 the export of mackerel was worth over 4 billion Norwegian kroners (Marine research institute, 2020). The Atlantic mackerel is a small pelagic fish with units distributed in both European and African waters, spanning from Morocco to northern Norway, in the Baltic sea, the Mediterranean, etc (Iversen, 2002). The spawning season for the mackerel is from February to July. Shortly after spawning, the southern and western units will migrate to the Norwegian Sea and North Sea to feed, where they generally will remain until August/September (Iversen, 2004). The diet of the Atlantic mackerel mainly consists of copepods (Óskarsson et al., 2016). The Atlantic mackerel is well known for its high fat content and seems to be among the species with the highest content of long chained PUFAs (Ackman, 1990).

The PUFAs found in fish oils can be divided into two families, the n-3 and n-6, which exhibits different biological effects (James et al., 2000). Compared to the wild salmon, the farmed salmon consist of more n-3 FAs, but also significantly more n-6 FAs. The abundance of n-6 FAs compared to n-3 FAs have resulted in a high n-6/n-3 ratio. A low ratio is desirable in order to reduce the risk of cardiovascular diseases (CVDs) (Simopoulos, 2002). There has been a lot of debate around which is the optimal ratio is and according to Simopoulos (2002) and Yang et al. (2016) it is around 1 – 5/1. However, the importance of this ratio is debated, and the FAO does not give any specific recommendations (FAO, 2010). According to Simopoulos (2002) does the Western population receive more n-6 FAs than recommended through the diet and need to incorporate more n-3 to lower the n-6/n-3 ratio. Examples of everyday products containing n-6 are grain products, nuts and plant oils. These are products that most people receive daily.

Both Atlantic salmon and mackerel are rich in the important marine n-3 fatty acids (FAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Although the human body can synthesise both EPA and DHA, it is incapable of synthesising enough of either and they must be incorporated into the diet. Alpha linolenic acid (ALA), however, is an n-3 FA the human body is unable to synthesise. ALA is therefore referred to as an essential fatty acid (EFA). Furthermore, ALA also act as a precursor to EPA and DHA. Linoleic acid (LA) and is the n-6 EFA most commonly found in plant-based materials. These EFAs are precursors to a range of metabolites including prostaglandins and long-chain PUFAs, and thus needed to be supplied in the diet (Dewick, 2009, pp. 49-51).

There has been reported a decreased concentration of n-3 FAs in farmed salmon compared to the level in previous years (Aas et al., 2019). Due to the scarcity and increasing price of marine oils, the feed that previously consisted of 90% fish meal and fish oils have been reduced to 25%, while the rest has been substituted with plant-based ingredients (Aas et al., 2019; Sprague et al., 2016). This substitution enabled a growth of 5.8% per annum in aquaculture production without a considerable increase in fish meal and fish oil consumption (Hamilton et al., 2020). In recent years in Norway, the proportion of plant-based ingredients like plant oil and plant protein in the feed have increased. Recently, up to 2/3 of the lipid fraction in salmon feed is of rapeseed oil origin. In Norway today, the feed consists of 70% plant-based ingredients as opposed to 60% in 2012 (Aas et al., 2019; Mørkøre et al., 2014). In contrast, the diet of wild salmon is based on small fish and crustaceans. Hence the feed provided to farmed salmon differs from the natural diet of wild salmon (Renkawitz & Sheehan, 2011). This has ultimately altered the FA profile of farmed salmon and resulted in an approximate 50 % reduction in the proportion of n-3, and an increase in proportion of n-6 FAs (FAO, 2018; Sissener, 2018; Sprague et al., 2016). The FA composition in salmon fillets have been shown to reflect that of the feed, possibly due to their limited ability to elongate and desaturate FAs (Sissener, 2018; Torstensen et al., 2005). This decrease in n-3 FAs in fish feed can potentially have negative effects on both the fish health and the consumer (Rosenlund et al., 2016). In Norway there are several feed producers, and they have a close follow-up program. The Norwegian Institute of Nutrition and Seafood Research (NIFES), in cooperation with the Norwegian Food Safety Authority, have made a program for monitoring the fish feed. The salmon feeds are controlled annually; in case of any levels of undesirable substances that exceeding the limits, the Food Safety Authority gets notified (Sele et al., 2019).

2. Aims of this study

The overall aim of this work was to study the current fatty acid levels, by elucidating and quantitating the FA compositions in the muscles of Atlantic salmon *S. salar*, and Atlantic mackerel *S. scombrus*, using an in-house designed and validated analytical method for derivatised lipids by GC-MS.

The partial objectives were:

- Obtaining the complete FA profiles of the fish by using solvent extraction to retrieve the lipids, derivatisation of the extracted lipids into FAMEs, and subsequent analysis by GC-MS.
- Fractioning of the fish lipids by off-line SPE into three fractions: neutral lipids, free fatty acids, and polar lipids, with subsequent quantitation of each class after analysis by GC-MS.
- Obtaining the complete FA profile of farmed salmon feed, evaluating the similarities between the FAs in the salmon and its feed.
- Determine the nutritional quality indices; atherogenicity index and thrombogenicity index by using empirical formulas, as well as the n-6/n-3 ratio.

3. Theory

3.1. Lipids

With no exact definition, lipids can be described in many ways (Akoh & Min, 2008, p. 1). Most definitions state that lipids comprise a diverse class of natural products with a shared characteristic of being soluble in non-polar, organic solvents such as chloroform, hexane, and ethers (Christie, 2010, p. 4). Others also add the insolubility in polar solvents, such as water (Gurr & James, 1971, p. 1; Kates, 1986, p. 1). These characteristics are due to the varying length of the hydrocarbon chains. As a class, lipids display a wide diversity in both structure and biological functions (Vance & Vance, 2002, p. 1). Lipids encompass compounds as fatty acids and their derivatives (mono-, di-, and triacylglycerides, and phospholipids), vitamins, waxes and sterols (Christie, 2010, p. 4). Biologically, lipids function as energy storage in living organisms in the form of triacylglycerides, and as signals for biological processes. Furthermore, due to their amphiphilic nature, phospholipids play a critical role in the formation of cell membranes by forming bilayers (Nelson & Cox, 2006, pp. 343, 348, 357).

3.1.1. Fatty acids

As Nelson & Cox (2006, p. 343) states, FAs are carboxylic acids with hydrocarbon chains ranging from 4 to 36 carbons long. However, the most common chain lengths are in the range from 12-22 carbon atoms for saturated chains, and 16-22 carbon atoms for unsaturated chains. Even though an even numbers of carbon atoms are the norm, uneven numbers of carbon atoms are found in nature (Rustan & Drevon, 2005). A FA whose chain predominantly consists of single bonds is called a saturated fatty acid (SFA). Unsaturation refers to the presence of double bonds within the hydrocarbon chain. Should only one double bond be present, the FA is said to be a monounsaturated fatty acid (MUFA), though if two or more double bonds are present the FA are referred to as a polyunsaturated fatty acid (PUFA). With the introduction of double bonds, geometric configurations such as *cis* and *trans* arise, where *cis* configuration is the most common of the two (Rustan & Drevon, 2005). Amongst the PUFAs, we find the n-3 and n-6 FAs. An illustration of a n-3 and a n-6 PUFA is given in Figure 3.1

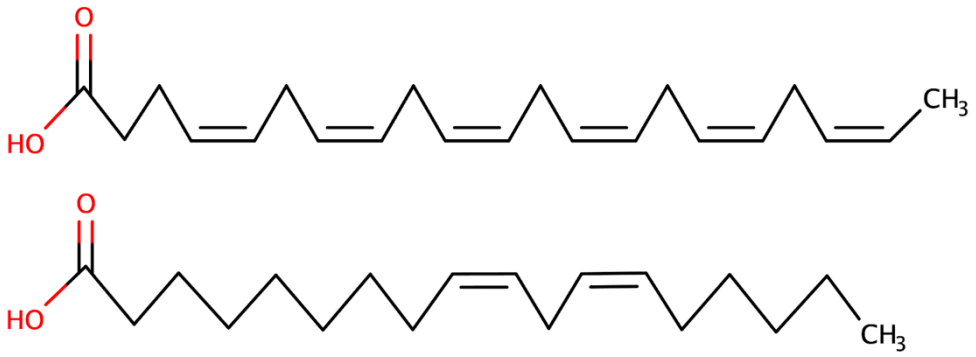


Figure 3.1: *The structure of the n-3 FA docosahexaenoic acid (DHA) (top), and the structure of the n-6 FA linoleic acid (LA) (bottom).*

3.1.2. Nomenclature of fatty acids

For a long time, trivial names for FAs have been used. These were often based on the Latin names of the botanical or zoological species they first were isolated from (Akoh & Min, 2002, p. 5). By the time IUPAC introduced a systematic nomenclature for FAs in 1979, the trivial names had become so established that they are still used in literature today, almost interchangeably with the IUPAC systematic names. Whereas trivial names do not contain structural information, the IUPAC system includes information on the number of carbon atoms present in the alkyl chain. If double bonds are present, the IUPAC also provide information on the position and configuration of the double bonds relative to the carboxylic acid terminus (Christie, 2010, p. 7). Due to the amount of information provided by the IUPAC system, certain FAs, especially PUFAs, get very long names. The shorthand designation also provides structural information, with the length of the alkyl chain, and the total number of double bonds. However, the shorthand designation does not pinpoint the positions of all the double bonds, only the location of the one closest to the methyl terminus of the alkyl chain. This is usually assigned the symbol “n” or the Greek letter “ω” (Devle, 2013). Furthermore, to specify the configuration of the double bonds it is recommended to add the *cis*/*trans*-configuration as a 't' for *trans* or 'c' for *cis* (Scrimegour & Harwood, 2007). An overview of the nomenclatures of some selected FAs found in fish, with their respective trivial name and shorthand designation are given in Table 3.1.

Table 3.1: The systematic name based on IUPAC nomenclature, trivial name, and shorthand designation of some common FAs found in fish.

IUPAC nomenclature	Trivial name	Shorthand designation
Octadecanoic acid	Stearic	C18:0
cis-9-Octadecenoic acid	Oleic acid	C18:1n-9c
all-*cis*-9,12-Octadecenoic acid	Linoleic acid	C18:2n-6c
all-*cis*-9,12,15-Octadecenoic acid	α-Linolenic acid	C18:3n-3c
all-*cis*-5,8,11,14,17-Eicosapentaenoic acid	EPA	C20:5n-3c
all-*cis*-4,7,10,13,16,19-Docosahexaenoic acid	DHA	C22:6n-3c

3.1.3. Acylglycerides

FAs are most abundantly found in nature as triacylglycerides (TAGs) and are less commonly found in their original carboxylic acid state as free fatty acids (FFAs). The structure of a TAG consists of three FAs connected to a glycerol unit through ester linkage, making it a neutral lipid (NL). TAGs are termed either simple, if all the FAs are identical, or mixed if two or more FAs are different. The mixed TAGs are the most abundant. The general structure of a TAG is illustrated in Figure 3.2 As previously stated in section 3.1, TAGs exhibit biological importance as energy storage, and are most commonly referred to as fats and oils depending on their state in room temperature (Dewick, 2009, p. 43). Additionally, monoacylglycerides (MAGs) and diacylglycerides (DAGs) are also part of this group of lipids, where MAGs and DAGs consist of one and two FAs, respectively, through ester linkages. The level of saturation affects the physical properties of FAs in room temperature. While TAGs containing SFAs appear as solid (commonly known as fats), the triglycerides containing PUFAs will appear as a liquid (commonly known as oils). This is largely due to the “kinks” in the molecular structure introduced by the double bonds, which in turn inhibits the PUFAs to align in a crystalline way and thus reducing the melting point (Hart et al., 2011, p. 442; Rustan & Drevon, 2005). Fats are usually found in animal products, whereas oils usually are found in vegetable products. However, a good proportion of the FAs in fish are unsaturated, thus appearing as an oil.

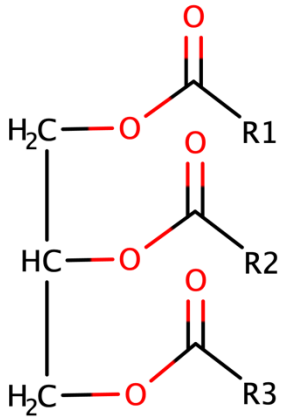


Figure 3.2: *The general structure of a triacylglyceride.*

3.1.4. Phospholipids

Phospholipids comprise of a glycerol-3-phosphate unit connected with two FAs through ester linkage. This group is also called glycerophospholipids. With the non-polar FAs and the polar phosphate group, the phospholipid experience amphiphilic properties (Nelson & Cox, 2006, p. 348). And due to this can form spherical bilayers, as earlier stated in section 3.1. Phospholipids are an important group of polar lipids (PL) due to the negatively charged phosphate group. Another important class of membrane-lipids are the sphingolipids. These two groups of phospholipids are illustrated in Figure 3.3. Unlike the phospholipids, they do not comprise of a glycerol unit. Instead, they comprise of a long-chained FA, a long-chained amino alcohol, called sphingosine, and a polar head (Nelson & Cox, 2006, p. 352).

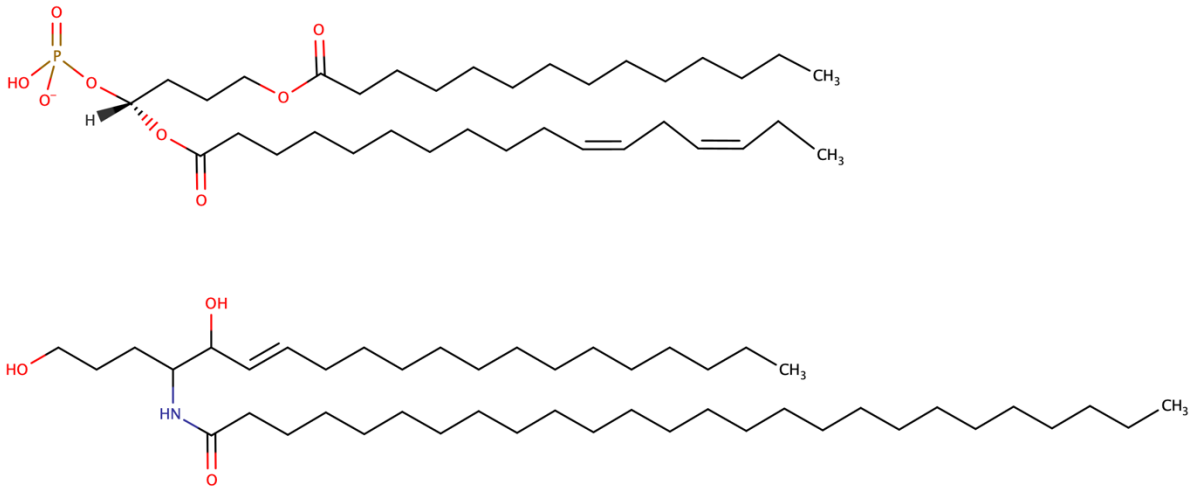


Figure 3.3: *The structure of phosphatidic acid, a glycerophospholipid (top), and the structure of ceramide, a sphingolipid (bottom).*

3.1.5. Fatty acids and human health

Throughout the years, many studies have been conducted to establish the importance of FA on human health. By far the most extensively studied are the n-3 PUFAs, which play a key role in human growth and development (Simopoulos, 1991). For example, the n-3 DHA is essential for early brain development, as well as in the maintenance of normal neural functions. Both the brain and the nervous system contain a significant amount of DHA (Horrocks & Yeo, 1999; Ruxton et al., 2004). Furthermore, EPA and DHA are also known to exhibit key roles in membrane functions, immunology and inflammation, as well prostaglandin metabolism (Simopoulos, 1991). Several diseases and disorders have been linked to deficiencies of DHA and n-3 PUFAs. Namely, cardiovascular disease, attention deficit hyperactivity disorder, unipolar depression and cystic fibrosis, among others (Horrocks & Yeo, 1999).

As previously stated in section 1, LA and ALA were presented as EFAs, which must be acquired through the diet due to the inability of the human body to biosynthesise them (Dewick, 2009, p. 49). This is largely due to the lack of enzymes that can introduce double bonds before sixth terminal carbon atom (Christie, 2010, p. 8; Simopoulos, 1991). LA is an n-6 FA and the precursor to arachidonic acid, while ALA is an n-3 FA and the precursor to both EPA and DHA. LA and ALA are desaturated and elongated to metabolise into arachidonic acid, and EPA and DHA, respectively (Dewick, 2009, p. 50). Additionally, Bourre et al. (1993) linked the removal of dietary ALA to an overall DHA deficiency, while Mantzioris et al. (1994) showed that a diet rich in ALA and low contents of LA elevated the EPA content in tissue. LA is found in most plant seeds, while ALA is mostly found in the chloroplast of green leafy vegetables seeds (Christie, 2010, p. 8; Simopoulos, 1991). However, EPA and DHA are most commonly found in fish oils.

Some MUFAs also exhibit beneficial effects on human health, e.g. oleic acid (OA; C18:1n-9c), a commonly occurring fatty acid in vegetable fats and oils, reportedly facilitates wound healing (Sales-Campos et al., 2013). Furthermore, exhibiting potential beneficial effects in patients suffering from type II diabetes by reversing the inhibitory effects in insulin production (Vassiliou et al., 2009). MUFAs along with the n-6 and n-3 PUFAs, generally exhibit an anti-atherogenic by inhibiting the aggregation of plaque. MUFAs and PUFAs also exhibit an anti-thrombogenic effect (Ulbricht & Southgate, 1991). Some MUFAs, however, are also associated with adverse health effects. Erucic acid (C22:1n-9c), a commonly found in

rapeseed, is reported to pose a health risk to children under the age of 10 (Knutsen et al., 2016). SFAs, however, are associated with disadvantageous health effects. For decades, recommendations for reducing dietary SFAs have been a cornerstone in reducing the risk of CVD and coronary heart disease (CHD) (Liu et al., 2017). The SFAs C12:0, C14:0 and C16:0 have been reported to be detrimental to human health by being proatherogenic which favours the adhesion of lipids to the circulatory system cells. Additionally, C14:0, C16:0, and C18:0 have been reported to exhibit a thrombogenic activity by accelerating thrombus formation (Ulbricht & Southgate, 1991). An overwhelming amount of studies have been conducted linking the substitution of SFAs with MUFAs and PUFAs to a decreased risk of CVD (Hooper et al., 2015; Kris-Etherton & Krauss, 2020; Siri-Tarino et al., 2015). For example, a study conducted by Hooper et al. (2015) showed a reduction of dietary SFA led to a 17% reduction in the risk of CVD. However, this is a debated topic, and newer research indicated no significant association between intake of SFAs and CVDs (Krauss & Kris-Etherton, 2020; Zhu et al., 2019).

The atherogenic index (AI) and thrombogenic index (TI) are two frequently employed indices for estimating the nutritional quality of lipids. The AI and TI show the potential to stimulate platelet aggregation (Matos et al., 2019). The AI indicates the relationship between the sum of the proatherogenic SFAs and the anti-atherogenic unsaturated FAs, whereas the TI indicates the relationship between the prothrombogenic and the anti-thrombogenic FAs (Ulbricht & Southgate, 1991). These indices are strongly associated with disease prevention and are claimed to promote health (Cherifi et al., 2018; Rhee et al., 2017).

The diet of pre-agricultural humans generally consisted of lean meat, fish, green leafy vegetables, fruits, berries, and honey. These foods helped shape the modern humans' genetic nutritional requirements. With the relatively recent addition of cereal grains as staple food, the human diet continues to move further away from the foods we are genetically predisposed for (Cordain, 1999; Simopoulos, 2006). This has negatively impacted the n-6/n-3 ratio. Humans originally evolved having a n-6/n-3 ratio of ~1/1, whereas the modern Western diets have a ratio of 15–17/1 (Simopoulos, 2002). A high imbalance in the n-6/n-3 ratio has been linked to many chronic diseases, including CHD and CVD. This is due to the tendency of n-6 FAs to be pro-inflammatory, whereas intake of marine n-3 FAs such as EPA and DHA blunts this effect (Simopoulos, 2008). To obtain a more optimal n-6/n-3 ratio of around 1–4/1, nutritionists therefore emphasise adding fish rich in n-3 FAs into Western diets (Simopoulos, 2002).

3.2. Solid-phase extraction

In a chemical analysis, the sample preparation is often the most time-consuming step. Solid-phase extraction (SPE) is today considered the most popular sample preparation method employed in organic analytical chemistry (Smith, 2015). It is very versatile and can be used for removing impurities from a sample, and separate analytes in a mix. SPE became popular when prepacked, disposable cartridges were introduced in 1978, but the name was not coined until four years later in 1982 (Miller, 2005, p. 405). The basic principle utilised by SPE is based on the analyte's affinity to either the stationary phase or the mobile phase. The solid phase acts as a sorbent for the analytes, and a vast array of sorbents are commercially available. Even though it is the type of analytes that dictates the choice of sorbent material, *n*-akylsilica has for many years been employed as the universal SPE sorbent (Hennion, 1999).

With SPE, the column is usually washed/pre-conditioned with an appropriate solvent, prior to sample application. This step is necessary to "activate" the sorbent, ensuring reproducible retention of the analytes (Mitra, 2003, p. 109). The sample is then applied, and depending on the sorbent material, the analytes are retained through either adsorption on the surface, or penetration of the of the outer layer of the molecules (Simpson, 2000, p. 3). A rinsing step is performed to remove the undesired matrix components from the sorbent. To recover the analytes, a solvent the analytes have a greater affinity to than the sorbent material must be introduced. Thus, leaving possible interfering compounds in the column (Mitra, 2003, p.109). A simple schematic illustration of the four steps is presented in Figure 3.4. By employing different solvents as mobile phases, SPE can be used to separate different classes of lipids into different fractions.

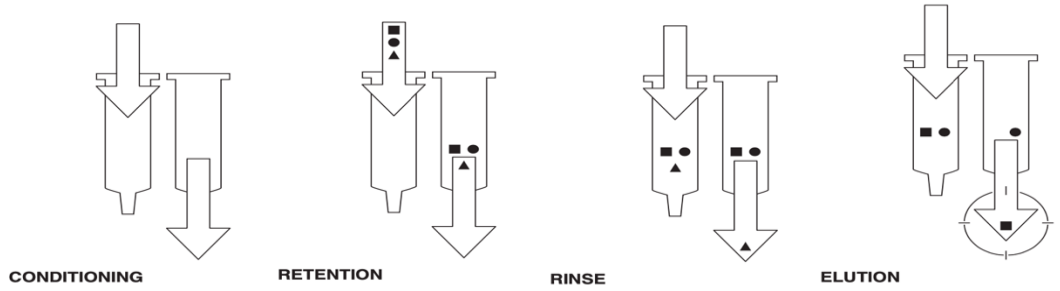


Figure 3.4: *The four basic steps for SPE: 1) The conditioning of the sorbent. 2) Loading of the sample. The analyte is adsorbed. 3) Rinsing away the interferents. 4) Elution of the analyte and undesired components retained (Mitra, 2003, p.109).*

3.3. Separation

The analytes in a complex sample mixture must be separated into their constituent parts to allow for identification and quantitation. When talking about separation you cannot avoid talking about chromatography. Chromatography has become the premier technique for separation and analyses. The principles of chromatography are based on the components' difference in affinity to a stationary and a mobile phase. The bigger the difference, the easier the separation. A complex sample mixture is injected onto a chromatographic column. The mixture is carried through the column with a mobile phase, either a gas, liquid, or a supercritical fluid, before the components elute from the column after a certain time. The stationary phase is often a viscous liquid that is either coated onto solid particles or the column wall itself (Miller, 2005, pp. 39, 43).

3.3.1. Gas chromatography

In this study, gas chromatography (GC) was employed to separate the analytes. GC is one of the most utilised methods for qualitative and quantitative analysis (Skoog et al., 2014, p. 887). A general illustration of a GC is shown in Figure 3.5. The sample, containing the analytes, are vaporised upon injection and carried through the column by an inert gas as the mobile phase. Several gases can be utilised but the most common one is helium. Separation is achieved due to the interactions between the compounds and the stationary phase. These interactions directly affect the time of elution of the specific compound (Miller, 2005, pp. 149-150, 43-44).

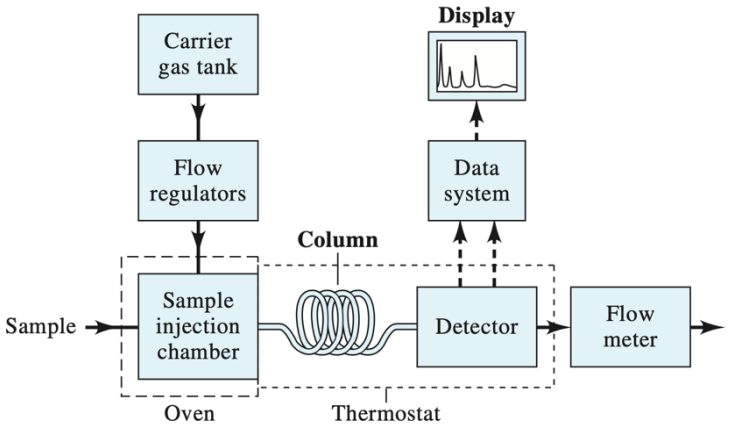


Figure 3.5: *A simple schematic diagram of a gas chromatograph, GC (Skoog et al., 2014, p. 888).*

One of the most commonly employed injectors is the split/splitless injector. The split injection technique is the easiest, simplest and oldest of the two. Typical injection volume is 1 µL. In a split-injector, a predetermined fraction of the sample, usually 0.1 – 10% enters the column, while the rest of the sample pass out through the purge valve. By opening or closing the split valve, the amount of sample introduced to the column can easily be controlled. In splitless mode, the split valve is initially closed, and all of the sample enters the column. Due to the increased amount of sample being introduced to the column, the sensitivity increase, and thus suited for trance analysis. However, it is more time consuming (Miller, 2005, pp. 150, 152).

The columns in GC are divided into two general types, the packed- and the capillary columns (Skoog et al., 2014, p. 890). As the name suggests, the packed columns contain small particles of what is either the stationary phase itself or coated with it. This type generally provides lower resolution compared to capillary columns (Eder, 1995). It is generally agreed that capillary columns are superior for most GC separations. Due to their superior performance and flexibility, the fused silica capillary columns have become the most popular type (Miller, 2005, pp. 154-157).

The most commonly employed detectors for the GC are the flame ionisation detector (FID) and the mass spectrometer (MS) detector. MS detectors offer several benefits compared to that of FID. While FID solely relies on the comparison of retention times between an analyte and its respective reference standard, the MS offer the ability to obtain spectrometric data such as molecular mass and structural information. Furthermore, the MS detectors are significantly more sensitive than their FID counterparts (Devle, 2013; Dodds et al., 2005).

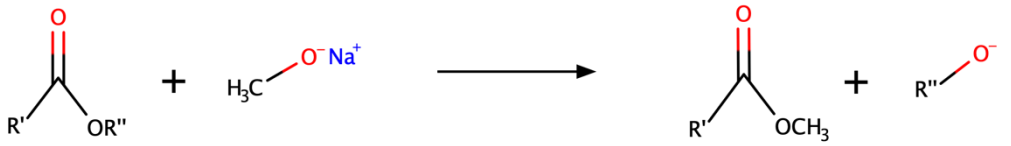
3.3.2. Transmethylation procedure

Prior to analysis by GC, the FAs are usually derivatised into fatty acid methyl esters (FAME). This is due to their initial, limited volatility (Devle, 2013). The most widespread method for acylglycerols is transmethylation by sodium methoxide. This is largely due to the rapid transmethylation rate where the glycerol unit is replaced through methanolysis, but also due to the method's mild conditions which prevents any undesirable reactions to occur, such as isomerisation of double bonds in MUFAs and PUFAs (Christie, 1993). Sodium methoxide is prepared by dissolving metallic sodium in methanol, where it changes the oxidation state. The reaction is shown in equation 3.1

(Eq. 3.1) $$2Na + 2CH_3OH \rightarrow 2CH_3ONa + H_2$$

The acylglycerols are completely trans-methylated in a matter of minutes at room temperature (Eder, 1995). And the chemical reaction is shown by equation 3.2.

(Eq. 3.2)



To produce FAMEs from FFAs, a methanolic solution containing an acid catalyst, boron-trifluoride, can be utilised. This method, developed by Morrison & Smith (1964), results in quantitative yields and very few undesirable reactions taking place. Additional heating is required for the complete reaction to take place (Morrison & Smith, 1964). The general reaction of esterification of an FFA by this method is shown in equation 3.3.

(Eq. 3.3)

3.4. Mass spectrometry

The detector utilised in this study was a mass spectrometer (MS). As illustrated in Figure 3.6, the MS is composed of an ion source, a mass filter, and a detector. In simple terms, an MS is an instrument that is kept under low pressures (10^{-5} – 10^{-8} torr) which produce ions from atoms and molecules, separates them based on their mass to charge ratio (*m/z*), and then detects them. A combination of gas chromatography and mass spectrometry is commonly referred to as GC-MS (Skoog et al., 2014, pp. 804, 895).

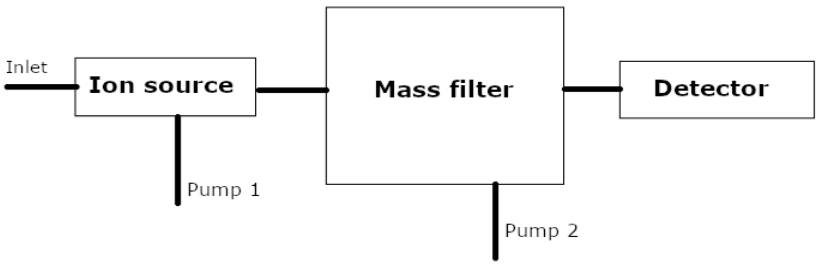


Figure 3.6: *A simple schematic diagram of a mass spectrometer.*

3.4.1. Ionisation source: electron ionisation

In an electron ionisation (EI) source, electrons are emitted from a heated filament at 70 eV, accelerated towards an anode, and collide with vaporised analyte molecules from the injected sample. Thus, causing the ionisation of the analyte by loss of an electron. Approximately 10 eV is required to ionise most organic molecules and the excess energy leads to extensive fragmentation, making EI a hard ionisation technique. As a result, the molecular ion is not always found. By employing two magnets on either side of the EI source, the distance travelled by the electrons are increased. The magnets are forcing the electrons into a helical path, further increasing the probability of collision with analyte molecules. When ions are formed, they are ejected from the ion source due to the high difference in potentials (Hoffmann & Stroobant, 2007, pp. 15-17). An illustration of an EI source is shown in Figure 3.7.

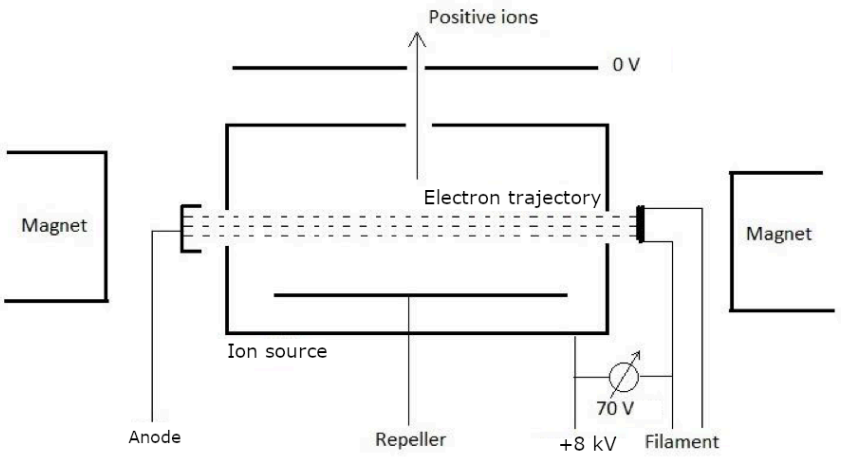


Figure 3.7: *Schematic diagram of an electron ionisation source.*

3.4.2. Mass filter: single quadrupole

The MS utilised in this study was equipped with a single quadrupole mass filter. The quadrupole analyser is a device which uses the stability of the trajectories in oscillating electric fields to separate ions according to their *m/z* ratios. A quadrupole consists of four parallel and, ideally, hyperbolical rods, where the two opposite rods have the same sign (+/-) potential. Connected to the rods are direct currents (DC) which alternates with radio frequency (RF). Combined DC and RF potentials on the rods can be set to filter out anything but the selected *m/z* ratio (Hoffmann & Stroobant, 2007, pp. 88-91). A simplistic illustration of a quadrupole is given in Figure 3.8.

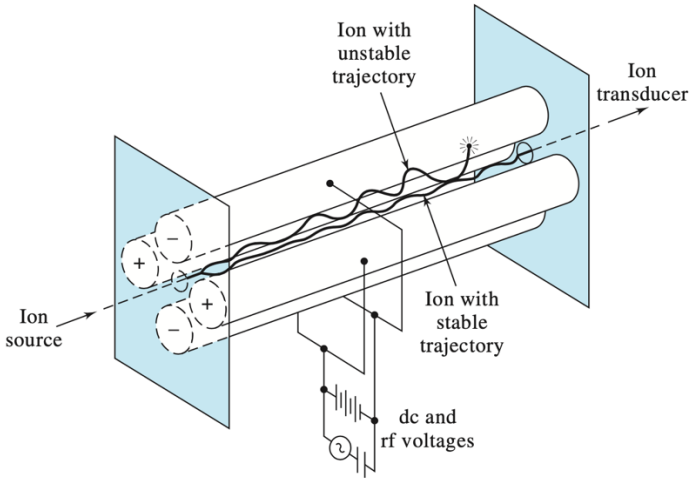


Figure 3.8: *Illustration of a single quadrupole mass filter (Skoog et al., 2014, p. 807).*

3.4.3. Detector: electron multiplier

The most widely used detector in MS is the electron multiplier. As shown in Figure 3.9, an ion from the mass analyser strikes the first dynode, called a conversion dynode, causing an emission of several secondary particles. These particles then accelerate and strike the next dynode held at a lower potential and are converted to secondary electrons. Due to the successive decreasing potentials of the dynodes, the electrons are accelerated towards the next dynode in the series. They strike the next dynode causing the emission of more electrons. This process continues as the secondary electrons travel towards the ground potential. Thus, creating a cascade of electrons, creating an amplified electron current (Hoffmann & Stroobant, 2007, pp 177-178).

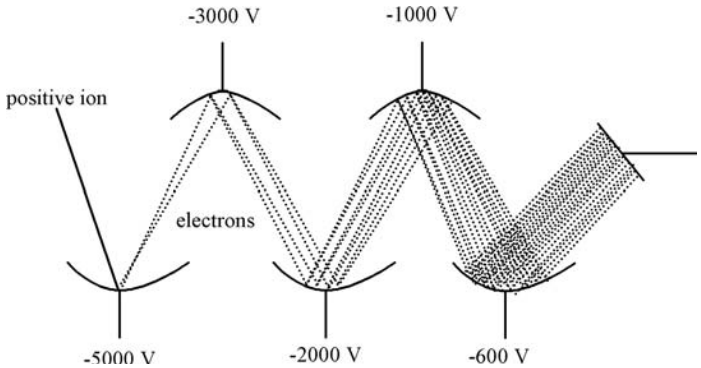


Figure 3.9: *Schematic diagram of an electron multiplier, where the first dynode is the conversion dynode (Hoffmann & Stroobant, 2007, p. 178).*

3.5. Quantitative analysis

The intensity of the signal of the sample to be analysed is compared to a component of reference, called an internal standard (IS). This method eliminates several sources of error (Hoffmann & Stroobant, 2007, p. 266). It is of upmost importance that the internal standard has as identical chemical and physical properties as the analyte as possible. Furthermore, the sample must not contain the IS naturally and it must exist in pure form (Miller, 2005, p. 303). An IS is added to the sample as early as possible in the analysis, and in the same order of magnitude as the analytes.

4. Methodology

4.1. Chemicals and equipment

Chemicals, internal standards and laboratory equipment used in this study are listed in Table 4.1 and 4.2 respectively. Also, the computer software employed for obtaining analytical data was Chromeleon v7.2.8 (Thermo Fisher Scientific, Waltham, MA, USA).

Table 4.1: Chemicals and internal standards used in this study.

Product	Manufacturer	Quality	CAS-number
Acetic acid	VWR Chemicals, France	100%	64-19-7
Boron trifluoride-methanol	Sigma Chemicals, Switzerland		373-57-9
C7:0	Larodan AB, Malmö, Sweden	>99%	111-14-8
C9:0	Larodan AB, Malmö, Sweden	>99%	112-05-0
C14:0 (13-methyl)	Larodan AB, Malmö, Sweden	>98%	2485-71-4
C14:0 (12-methyl)	Larodan AB, Malmö, Sweden	>98%	5502-94-3
C16:1n-9c	Larodan AB, Malmö, Sweden	>98%	2416-19-5
C16:1n-5c	Larodan AB, Malmö, Sweden	>98%	2416-20-8
C16:2n-4c	Larodan AB, Malmö, Sweden	>98%	5070-03-1
C18:1n-12c	Larodan AB, Malmö, Sweden	>99%	593-39-5
C18:1n-7c	Larodan AB, Malmö, Sweden	>99%	506-17-2
C18:1n-5c	Larodan AB, Malmö, Sweden	>98%	13126-39-1
C18:4n-3c	Larodan AB, Malmö, Sweden	>97%	20290-75-9
C19:0 FFA	Larodan AB, Malmö, Sweden	>99+%	10-1900-13
C19:0 PL	Larodan AB, Malmö, Sweden	99%	37-1900-11
C19:0 NL/TAG	Larodan AB, Malmö, Sweden	99%	33-1900-13
C20:1n-11c	Larodan AB, Malmö, Sweden	>98%	29204-02-2
C20:4n-3c	Larodan AB, Malmö, Sweden	>98%	24880-40-8
C21:5n-3c	Larodan AB, Malmö, Sweden	>98%	24257-10-1
C22:5n-3c	Larodan AB, Malmö, Sweden	>99%	108698-02-8
Chloroform	VWR Chemicals, France	100.0%	67-66-3
Diethyl ether	Sigma-Aldrich, Poland	≥99.8%	60-29-7
Helium	AGA, Norway	6.0	7740-59-7
n-Heptane	Acros Organics, Belgium	99+%	142-82-5
Isopropanol	VWR Chemicals, France	100,0%	67-63-0
Methanol	VWR Chemicals, Poland	99.9%	67-56-1
Nitrogen	AGA, Norway	5.0	7727-37-9
Sodium (s)	Merck, Darmstadt, Germany	Purum	
Sodium chloride	VWR Chemials, Belgium	99.9%	7647-14-5
Supelco 37 component FAME mix	Sigma-Aldrich, WY, USA	CRM	

Table 4.2: Laboratory equipment used in this study.

Instrumentation	Name	Manufacturer	Specifications
Automatic pipette	Finnpipette® F2	Thermo Scientific™	100-1000 µL
Automatic pipette	Finnpipette®	Thermo Scientific™	1-5 mL
Centrifuge	Avanti™ centrifuge J-25	Beckman Coulter™	
Culture tubes	Screwthread tubes	DURAN®	GL14
Evaporators	Pierce Reacti-Vap™ III	Thermo Scientific™	
Gas Chromatograph	Trace™ 1310	Thermo Scientific™	
GC cap	Aluminium cap	VWR international	11 mm
GC vial	Crimp vial	VWR International	1.5 mL 32x11.6 mm
Hamilton syringe	Microliter™ Syringes	Hamilton®	10, 50, 100, and 500 µL
Heating block	Dri-Block DB-3	Techne, Cambride	
Mass Spectrometer	ISQ™ QD	Thermo Scientific™	Single quadrupole
Micro weight	CP2P Sartorius	VWR International	
Milli-Q water	Automatic Sanitisation Module	Merck Millipore	230 V
Orbital Shaker	PSU 10-i	Biosan	
SPE-columns	Discovery DSC-NH2	Supelco/Sigma-Aldrich	500 mg, 3 mL
	Bond Elut, NH2	Agilent Technologies	500 mg, 3 mL
SPE-lid		Gilson	For 3 mL columns
Screw-capped tubes	Cellstar® Tubes	Greiner Bio-One	50 mL, 30x115 mm
Table-top centrifuge	EBA 20	Hettich®	
Vacuum controller	V-855	Büchi	
Vacuum evaporator	Syncore® Polyvap	Büchi	
Vacuum pump	V-700	Büchi	
Vortex-mixer	Yellowline TTS 2	IKA®-Werke	
Water bath	No 1004	GFL	
Weight	Extend Sartorius	VWR International	

4.1.1. Internal standards

A total of three different ISs of C19:0 were used for quantitation of FAMEs. These three were C19:0 for TAG, FFA, and PL. The volumes and concentrations of the added IS are displayed in **appendix I**. They were made separately by dissolving the appropriate IS of C19:0 in chloroform. The C19:0 TAG was prepared with a concentration of 10 mg/mL, while the C19:0 FFA, and PL to a concentration of both 10 and 1 mg/mL. The IS solutions were stored in GC-vials at -20 ℃ until use. The TAG IS proved to be particularly challenging to resolve when thawed. The vials were heated to room temperature and subsequently shaken to ensure a homogenous mixture.

4.2. Fish and salmon feed

The farmed Atlantic salmons (n = 3) and Atlantic mackerels (n = 3) were purchased fresh from "Son brygge og fiskebutikk", in Son, Norway. Both the farmed salmon and the feed came from the farm Vikenco AS located in Aukra, Norway. The feed was of the type "Rapid HF 1000 HQ 50A" and was produced on November 17th by EWOS AS, Scotland. The mackerels were caught in the sea outside of Hvaler, Norway. The wild salmons (n = 3) were acquired from Finnmarkfisk AS and were caught with salmon traps in Namsenfjorden, outside of Namsen, Norway. The wild salmons were frozen fresh at -20 ℃ since June 2019.

4.3. Sample preparation

The farmed salmons were filleted, deboned, and deskinned. The subcutaneous fat was removed so only the fish muscle remained. Figure 4.1 show a diagram of the muscles in both a salmon fillet (a) and cutlet (b). From the farmed salmon, both red and white muscles were sampled from all over the fillet as indicated by the blue rectangles in Figure 4.1a. The flesh was cut into smaller pieces and homogenised using a stave mixer. This was done separately for every fish. The resulting muscle mass was stored in blue-capped tubes in darkness at -20 ℃. The mackerel was sampled using the same method of approach. However, the entire fillets were sampled due to their small sizes. The wild salmons came in the form as cutlets, but the same procedure for acquiring the muscle mass was used, however, half of every cutlet in their respective packs were sampled as indicated in Figure 4.1b. The feed delivered as pellets. The pellets were grinded into a homogenous mixture using a mortar. To keep the feed as fresh as possible, the pellets were grinded prior to the lipid extraction.

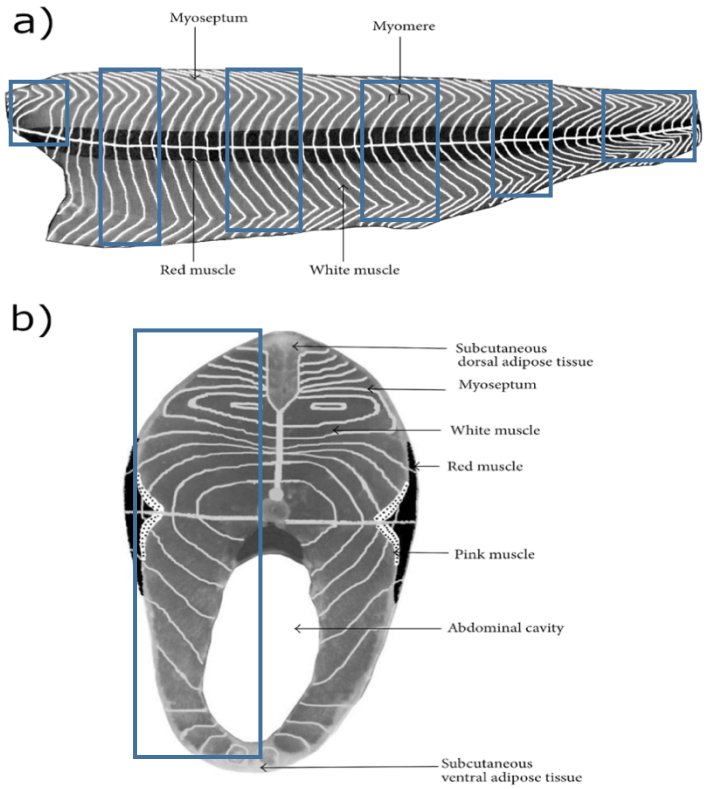


Figure 4.1: A diagram of salmon fillet in longitudinal section (a) presenting the W-shape of myomere and the two muscle types, and the cross section of a salmon cutlet (b). The blue rectangles indicate where the samples were sampled. Adapted from Listrat et al. (2016).

4.4. Total lipid content in fish muscle

To extract the lipids, Folch's method was employed. Folch et al. (1957) introduced a simple method for isolating the total lipid content from biological matrices. This is done by a liquid-liquid extraction by exposing animal tissue to a 2:1 chloroform methanol (v/v) mixture (Folch's solution), as well as a water/saline solution. The combination of a polar and non-polar solvents are necessary to extract neutral lipids as well as polar lipids from the sample tissue (Devle, 2013).

Three grams of homogenous muscle mass was transferred to 100 mL Erlenmeyer flasks, and added 60 mL of Folch's solution. Glass stoppers were placed on top of the beakers, with subsequent shaking on an orbital shaker (Biosan PSU-10i, Riga, Latvia) at 390 rpm for 30 minutes. The contents of the Erlenmeyer flasks were transferred to separatory funnels and added 12 mL of a 0.9% NaCl in Milli-Q water solution. Chloroform was used to wash the flasks for any lipid residues. The separatory funnels were shaken vigorously until satisfactory separation of the two phases were achieved, and the lower organic phases were transferred to

120 mL Büchi reagent tubes. Two additional liquid-liquid extractions were carried out with 10 mL chloroform and collected in the same reagent tubes. The gathered organic phases were dried using a vacuum evaporator system (Büchi, Syncore® Polyvap equipped with a V-700 vacuum pump and a V-855 vacuum controller) at 40 °C, 100 rpm, and an air pressure at 207 mbar. When most of the solvent had evaporated, the contents were transferred to pre-weighed culture tubes (DURAN®, GL14). The complete removal of solvent was carried out by inserting the tubes in heating blocks at 40 °C under pure nitrogen. The dry residues were weighed to calculate the total lipid content of the fish.

4.5. Complete fatty acid profile of fish

4.5.1. Extraction of lipids

Similarly, to the section above, the lipids was extracted using Folch's method. Several tests were performed to find the correct amount of IS. Different volumes of internal standard were added to allow quantitation of the compounds in the chromatogram. The volumes of IS used are listed in Table 4.3.

The homogenous fish mass was thawed, and 0.5 g was transferred to a 50 mL screw cap tube (Greiner Bio-One, Cellstar® Tubes) as quadruplicates. IS and 10 mL of Folch's solution were added and shaken at 390 rpm for 20 minutes using an orbital shaker. Then, 2 mL of 0.9% NaCl in Milli-Q water solution was added and shaken using a vortex mixer (IKA®-Werke, Yellowstone TTS-2). The two phases were then separated by centrifugation (Beckman Coulter™, Avanti™ J-25 equipped with a JA-12 fixed-angle rotor), at 2000 rpm for 5 minutes. The upper aqueous phases were discarded, and the lower organic phases were transferred to test tubes. The complete removal of solvent was carried out by inserting the samples in heating blocks at 40 °C under pure nitrogen.

Table 4.3: The amount of internal standard of trinonadecanoin (C19:0 TAG) utilised in lipid extraction of the fish samples.

Series 1	100 µL IS for the mackerel
	100 µL IS for the wild salmon
	200 µL IS for the farmed salmon
Series 2	10 µL IS for the mackerel
	10 µL IS for the wild salmon
	50 µL IS for the farmed salmon

4.5.2. Derivatisation of lipids

For FAME formation, a combined method for transesterification and esterification was employed. The dry lipid residues were dissolved in 1 mL of n-heptane and transferred to culture tubes. A sodium methoxide solution was prepared by dissolving metallic sodium, supplied by Merck (Darmstadt, Germany), in methanol to a final concentration of 5 mg/mL. To each culture tube, 1 mL the sodium methoxide, was added and the samples were shaken for 30 minutes at 390 rpm using an orbital shaker. After shaking, 1 mL of BF_3-methanol (14% BF_3 in methanol) was added and the tubes were heated in a water bath at 80 ℃ for 20 minutes. The tubes were then cooled to room temperature and the two phases were separated by centrifugation (Hettich®, EBA 20) at 2000 rpm for 5 minutes. The upper heptane phases were transferred to GC-vials and then diluted with n-heptane. Both the mackerel and the wild salmon samples were diluted 1:10, while the farmed salmon samples were diluted 1:50. The samples were stored in darkness at -20 ℃ until analysis with GC-MS.

4.6. Complete fatty acid profile of salmon feed

The complete FA profile for the salmon feed was found by the same method as the fish samples. The salmon feed was homogenised, and 0.5 g was transferred to a screw cap tube as quadruplicates and added 200 µL of C19:0 TAG IS in the first series, and 50 µL in a second series. The samples were diluted 1:100 with n-heptane. The samples were stored in darkness at -20 ℃ until analysis with GC-MS.

4.7. Separation of lipid classes by solid-phase extraction

For separation and quantitation of lipid classes, three different ISs were added. The different ISs and the added volumes are given in Table 4.4. The extracted lipids were resolved in 1 mL of chloroform and transferred to GC-vials. Blank samples of pure chloroform were also prepared. The samples were stored in darkness at -20 ℃ until fractioning.

Table 4.4: The three IS of C19:0 used in the extraction of lipid classes. Two different volumes of internal standard were added for the neutral lipids, and two different concentrations of FFA and PL IS was utilised.

	IS	Concentration [mg/mL]	Volume added [µL]		
			Mackerel	Wild salmon	Farmed salmon
NL	Trinonadecanoin	10	100 / 10	100 / 10	200 / 20
FFA	Nonadecanoic acid	10 / 1	10	10	50
PL	1,2-dinonadecanoyl-sn-glycero-3-phosphatidylcholine	10 / 1	25	25	15

The fractioning of the lipid classes was done by a SPE-robot (Gilson, GX-274 ASPEC™, Middleton, WI, USA). The lipids were fractioned into classes according to a pre-made program called: "NL-FFA-PL", where the NLs elute first, followed by the FFAs, and lastly, the PLs. Prior to use, the system was thoroughly rinsed with isopropanol. The prepacked columns (series 1: Discovery DSC-NH2, series 2: Bond-Elut NH2) were conditioned using 7.5 mL heptane, prior to the transfer of the samples (500 µL). NLs were eluted by 5.0 mL of chloroform, FFAs by 5.0 mL of a 98:2 diethyl ether and acetic acid (v/v) solution, and PLs by 5.0 mL of methanol. The flow rate was set to 1.0 mL/min.

4.7.1. Preparation of FAMEs from neutral and polar lipids

The NLs and PLs were individually transferred from SPE tubes to culture tubes and heated to 40 °C under pure nitrogen until dryness. The lipids were resolved in 2 mL n-heptane and added 1.5 mL of sodium methoxide (5.0 mg/mL). The samples were horizontally shaken at 390 rpm for 30 minutes and placed in vertical position for 30 minutes to separate the two phases. The heptane phases were transferred to GC vials, and stored in darkness at -20 °C until analysis with GC-MS. The NL fractions of farmed salmon were diluted 1:10 with n-heptane.

4.7.2. Preparation of FAMEs from free fatty acids

The FFAs were transferred from SPE tubes to culture tubes and heated to 40 °C under pure nitrogen until dryness. When dry, 1 mL of BF_3-methanol (14%) was added and heated in a water bath at 80 °C for 5 minutes. The samples were cooled down to room temperature, added 2 mL of n-heptane, and shaken using a vortex mixer. The samples were left for a couple of minutes in vertical position, before the heptane phases were transferred to GC vials. The vials were stored in darkness at -20 °C until analysis with GC-MS.

4.8. Analysis of fatty acids by GC-MS

The samples were analysed on a GC-MS (GC: Thermo Fisher Scientific, TRACE™ 1310, Waltham, MA, USA; MS: Thermo Fisher Scientific, ISQ™ QD, Waltham, MA, USA). The GC was equipped with a Rtx®-2330 column from Restek which was 60 m long, had an inside diameter of 0.25 mm and a film thickness of 0.2 µm. Helium was employed as carrier gas at a constant flow of 1.0 mL/min. The temperature program utilised was 110 minutes long and the specifics are shown in Figure 4.2.

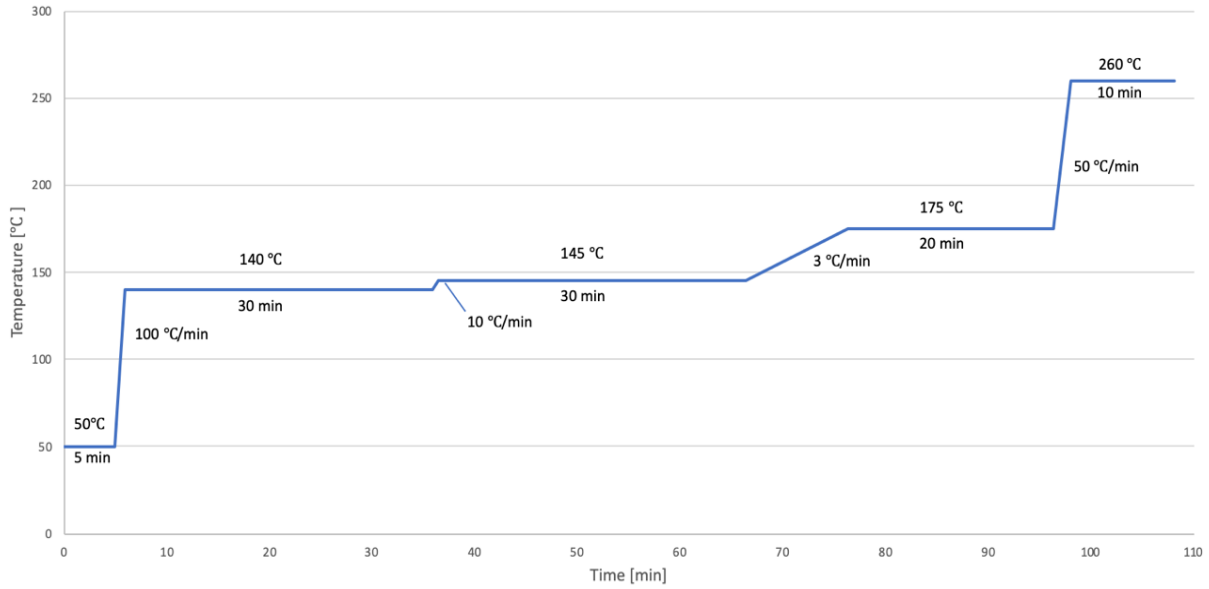


Figure 4.2: *Temperature program utilised on the GC-MS to separate the FAMEs.*

An injector with split ratio 1:10 was used. The mass spectrometer employed had an EI ionisation source which produced electrons with 70 eV. The chosen mass range was *m/z* 50 – 600. The mass filter was a single quadrupole and the detector was an electron multiplier.

4.9. Obtaining relative response factors

The relative response factors (RRFs) used for the quantitation of FAMEs were obtained by using the same method of approach as the previous work of Devle et al. (2009), with minor deviations. Four concentrations of 150, 300, 600, and 1200 mg/mL of the Supelco 37 component FAME Mix were prepared by diluting it with n-heptane. To the standard mixtures, stock solutions of C7:0, C9:0, and C19:0 FAME were added to give concentrations of 5, 10, 25 and 50 mg/mL each. Two injection replicates of each sample were analysed in full scan mode. By dividing the slope of the regression line of the individual FAMEs by the slope of the

regression line of the IS, the individual RRFs of the FAMEs were calculated. The internal standards chosen were not found in the biological samples. Undecanoic acid methyl ester (C11:0) was used as IS for the short- and medium-chain FAs (C4:0–C15:1n-5c). Nonadecanoic acid methyl ester (C19:0) was used as IS for the medium to long-chain FAs C16:0 – C24:1n-9c.

4.10. Identification and quantitation of FAMEs

The FAMEs were identified by two separate methods. Firstly, the FAMEs were identified by comparing the retention times to reference standards and secondly, by NIST library search (NIST 08, Gaithersburg, MD, USA). The software used for obtaining the data was Chromeleon 7.2.8 (Thermo Scientific™, USA). RRFs were obtained as previously described in section 4.9, and the concentration of the FAMEs were calculated using equation 4.1.

(Eq. 4.1) $$[FAME] = \frac{A_{FAME} * [IS]}{A_{IS} * RRF}$$

RRF is the relative response factor for the different FAMEs. [FAME] and [IS] is the concentration of the FAME and IS, respectively. The concentrations of the internal standards are given in **appendix I**. A_{FAME} and A_{IS} are the peak area of the FA and the IS, respectively.

4.11. Nutritional quality indices of the lipids

To estimate the nutritional quality of the lipids, two separate indices were to be calculated as well as the n-6/n-3 ratio. The AI and TI were calculated by using equation 4.2 and 4.3, respectively, according to Ulbricht and Southgate (1991).

(Eq. 4.2) $$AI = \frac{[C12{:}0 + (4 * C14{:}0) + C16{:}0]}{(\sum MUFAs + \sum n{-}6 + \sum n{-}3)}$$

(Eq. 4.3) $$TI = \frac{[C14{:}0 + C16{:}0 + C18{:}0]}{[(0.5 * \sum MUFAs) + (0.5 * \sum n{-}6 + (3 * \sum n{-}3) + (\frac{\sum n{-}3}{\sum n{-}6}))]}$$

4.12. Determining LOD and LOQ

The Chromeleon 7.2.8 software has a function that calculates the signal to noise ratio (S/N) automatically. This was utilised to determine the limit of detection (LOD) and the limit of quantitation (LOQ). A series of eight concentrations; 1.5, 5, 10, 15, 20, 50, 100, and 150 µg/mL of the Supelco 37 component FAME mix diluted with n-heptane was prepared and analysed in full scan mode with three injection replicates to determine which concentration yielded a S/N ratio of 3.0 and 10 for LOD and LOQ, respectively. The LOD and LOQ was only determined for four FAs of the FAME mix existing with the same amounts, namely C10:0, C18:0, C18:1n-9c, and C20:0 FAME.

5. Key results and discussion

The complete FA profiles of Atlantic mackerel, wild and farmed Atlantic salmon, and salmon feed were to be elucidated and quantitated, with the additional fractioning of the fish lipids into three fractions. These aims were the basis for **paper 1**, however, exclusively for the Atlantic salmon and feed. GC-MS was chosen as the analytical instrument in this study due to the potentially low concentrations of the FAs present in the samples. The reference standards used for the identification process are listed in **appendix II**.

The LOD and LOQ could not be determined successfully by the method given in section 4.12. This might be due to a ground fault of the building containing the GC-MS, causing inconsistent voltages. Far from ideal, an extrapolation of the data was performed to obtain the LOD and LOQ. The four FAMEs selected were considered to be representative for the FAMEs in the Supelco 37 Component FAME mix. The LOD and LOQ for e.g. C10:0 would resemble the ones of C14:0 and lower, and the LOD and LOQ for the C18:1n-9c would resemble the ones of the unsaturated FAMEs. With extrapolation in mind, the results showed a LOD in the ng/mL range from 37.1 – 866.5 ng/mL, and LOQ in the µg/mL range from 0.14 – 1.95 µg/mL. Both selected ion monitoring and reconstructed ion chromatogram offer better sensitivity and specificity by scanning pre-determined ions and, if utilised, would provide lower LOD and LOQ (Devle et al. 2009; Hoffmann & Stroobant, 2007, p. 229). The FAMEs with their respective LOD and LOQ can be found in **appendix III.**

The RRF-values used for the quantitation of FAMEs were obtained through the procedure described in section 4.10 and are given in **appendix IV**. To test the robustness of the method, the procedure was performed by two different personnel on different dates, months apart. Thus, the solvents used in the preparation of the concentration series were from different bottles. The individually determined RRF-values and the mean value are also given in **appendix IV**. Apart from a six FAMEs showing significant variation between different series, most FAMEs (34) do not and thus, the method is considered robust. The FAME C4:0 displayed the most significant variation with the values 0.69 and 0.33 for series 1 and 2, respectively. C4:0 is highly volatile and some might have evaporated causing uncertainty in the C4:0 content. Additionally, differences might have occurred due to the automatic integration function of the Chromeleon software. The integration sometimes stops prematurely and thus fail to integrate the entire peak. The chromatograms were checked to ensure all replicates had been integrated

equally, however differences might have occurred between the different series. Although time consuming, manual integration would offer a better control over that all the FAMEs have been integrated similarly. The FAMEs C4:0, C6:0 and C7:0, displayed significantly lower values (0.51, 0.61 and 0.65, respectively) than the rest. This is most likely due to the high volatility of the compounds. The mean values exist in the range 0.51 – 1.23, however all but the three short-chained FAMEs listed above existed in the range 0.87 – 1.23. The RRF-values were expected to show an increased deviation from 1.00 with the difference in chain length compared to the reference standard. C15:1n-5c – C16:0 was chosen as the turn point between the IS used, this was to keep the IS as closely related to the analyte FAMEs as possible. The values derived from the C11:0 IS displayed a general trend of getting larger with the chain length compared to the reference standard, however, the values derived from C19:0 IS did not show this same trend as clearly.

FAMEs not represented by the Supelco 37 Component FAME mix had to be assigned reasonable RRF-values. The MUFAs C18:1n-7c and C20:1n-7c, for example, were assigned the same values as C18:1n-9c and C20:1n-9c, respectively. The branched fatty acids, however, were all assigned the same values as their straight-chained counterparts. E.g. 13-methyltetradecanoic acid (C14:0 (13-methyl)) was assigned the same RRF-value as C15:0. Furthermore, the PUFAs C16:2n-6c, C20:4n-3c, and C21:5n-3c, for example, were assigned the same values as C16:1n-7c, C20:3n-3c, and C20:5n-3c, respectively. Considering the fact that these FAMEs represent but a small fraction of the total lipid content of the fishes, the increased degree of inaccuracy in quantitated concentrations attained by manually assigning RRF-values is considered negligible.

The method of using a GC coupled with a single quadrupole MS detector resulted in a satisfactory separation, and subsequent quantitation, of all FAs in Atlantic mackerel, wild and farmed Atlantic salmon, and salmon feed. For the routine analysis of Atlantic mackerel and Atlantic salmon, full scan acquisition was considered the most suitable. Due to the ability to identify FAMEs through spectral information and library searches, e.g. in NIST 08, it offers a major advantage over single ion monitoring. Full scan acquisition yields a plot of the total ion current (TIC), where the peaks are plotted as the relative intensity of the acquired mass signals against time. The TIC yields what is considered a conventional chromatogram diagram (Hübschmann, 2015).

5.1. Determination of total lipid content in fish muscle

The lipid content in the different fish species are given in Table 5.1

Table 5.1: Total lipid content in Atlantic mackerel (n = 3), wild (n = 3) and farmed Atlantic salmon (n = 3) given as percentages of fish muscle (wet weight) with standard deviation (SD).

	Lipid content [%] ± SD
Atlantic mackerel	3.1 ± 1.5
Wild Atlantic salmon	2.14 ± 0.32
Farmed Atlantic salmon	8.97 ± 0.63

The wild and farmed Atlantic salmon had a lipid content of 2.14 ± 0.32% and 8.97 ± 0.63%, respectively. These results confirm the observations of Jensen et al. (2012) and Lundbye et al. (2017) that the lipid content is significantly higher in farmed salmon. However, based in previously published literature (Jensen et al., 2012; Lundebye et al., 2017), the expected lipid content in wild salmon and farmed salmon is 6 – 8% and 12 – 14%, respectively. The results from this study for both wild and farmed salmon are therefore lower than expected. The primary reason for deviating results is believed to come from differences in samples procedure. Both Jensen et al., (2012) and Lundebye et al., (2017) sampled the salmon following the Norwegian Quality Cut, where only the flesh cut between the dorsal and adipose fin, and down to the gut is sampled. Furthermore, the subcutaneous fat is not removed. Our study focused on determining the lipid content in fish muscle and deemed it appropriate to remove the subcutaneous fat and sample cuts from all of the fish to get a representative muscle sample. Other possible reason could be slim fish or individual differences. A more representative result could have been obtained by increasing the sample parallels from several different fishes. Additionally, the wild salmon had been frozen since June 2019 and albeit being frozen fresh and stored in the freezer, some of the FAs may have been oxidised, or otherwise decomposed (Dawson et al., 2018). Furthermore, the fat cells might break due to freezing resulting in loss of some acylglycerides from the muscles. Thus, the results could have been more comparable if both farmed salmon and wild salmon had been fresh. However, most commercially available fish products have been frozen at some point, so these results might offer the most relevant picture of the nutritional values.

The Atlantic mackerel, however, showed a lipid content of 3.1 ± 1.5%. Based on previously published literature the lipid content of Atlantic mackerel caught in the summer season are expected to be approximately 4.5%, thus making the observed results somewhat lower than expected (Ackman & Eaton, 1971; Guizani & Moujahed, 2015). The lipid content has been shown to vary according to both geographical origin and season (Guizani & Moujahed, 2015; Romotowska et al., 2016). The lower than expected lipid content might be explained the same way as with the salmon, a difference in sampling procedure. Furthermore, the lipid content was rather uncertain as indicated by the larger standard deviation of 1.5 was observed in the lipid content compared to the values from the salmon. Apart from possible biological factors as age and sex, it might also originate from a poor removal of the subcutaneous fat. Considering that the mackerel were relatively small, and the skin removal posed a challenge, a varying amount of subcutaneous fat might have been sampled.

By assuming that a dinner portion of fish fillet is 200 g, one would receive 6.2 g of fat from mackerel, 4.3 g of fat from wild salmon and 17.9 g of fat from farmed salmon. Thus, farmed salmon consumption results in a significantly higher fat intake.

5.2. Complete fatty acid profile in fish

A total of 37, 36, 35, and 34 FAs were found in respectively mackerel, wild salmon, farmed salmon, and salmon feed adding up to 39 unique FAs. An overview of all FAs and their respective concentrations found in the different fishes and salmon feed is given in Table 5.2 along with the quantitative differences found in SFAs, MUFAs, and PUFAs. The proportions of the FAs along with the calculated n-6/n-3 ratio, AI, and TI are given in Table 5.3. The shortest fatty acid identified was C12:0, while C24:1n-9c was the longest. All the unsaturated FAs found exhibited a cis configuration. In order to compare the concentration of FAs in each fish, the peak areas from the chromatograms were used to calculate mg FA/100 g fish muscle. An overview of all FAs found in mackerel, wild salmon, farmed salmon, and salmon feed with their associated retention times, match factors and concentrations with standard deviations is listed in **appendix V**, **VI**, **VII**, and **VII**, respectively.

Table 5.2: Overview over every FA found in Atlantic mackerel (n = 3), wild (n = 3) and farmed Atlantic salmon (n = 3), and salmon feed with concentration and standard deviation. Concentrations are given in mg FA/100 g fish muscle.

	Mean ± SD [mg/100 g]			
Fatty acid	Atlantic mackerel	Wild Atlantic salmon	Farmed Atlantic salmon	Salmon feed
C12:0	1.10 ± 0.43	1.38 ± 0.15	n.d.[b)]	n.d.
C13:0 (4,8,12-trimethyl) [a)]	2.78 ± 2.58	1.71 ± 0.12	n.d.	n.d.
C14:0	161.0 ± 76.1	93.7 ± 13.2	145.5 ± 35.0	653.1 ± 26.2
C14:0 (13-methyl)	4.64 ± 3.76	3.71 ± 0.53	2.71 ± 0.62	19.53 ± 1.69
C14:0 (12-methyl)	1.76 ± 1.53	2.33 ± 0.35	1.57 ± 0.33	8.04 ± 0.50
C15:0	12.7 ± 9.03	8.13 ± 1.42	7.94 ± 1.75	56.11 ± 3.32
C16:0	801 ± 317	475.7 ± 59.5	819 ±189	3,097.4 ± 91.5
C17:0	30.6 ± 21.2	11.67 ± 1.27	15.44 ± 3.77	101.22 ± 8.77
C18:0	279 ± 111	117.7 ± 15.4	250.4 ± 64.1	1,312 ± 38.9
C20:0	5.03 ± 2.60	2.22 ± 0.29	24.54 ± 6.68	146.34 ± 7.20
C22:0	2.05 ± 0.74	n.d.	7.81 ± 0.69	70.27 ± 4.88
C24:0	n.d.	n.d.	2.95 ± 0.42	35.71 ± 1.10
∑ SFAs	**1,302 ± 546**	**718.2 ± 92.3**	**1,278 ± 303**	**5,500 ± 184**
C16:1n-9c	5.46 ± 3.79	3.66 ± 0.83	9.63 ± 2.73	25.77 ± 2.45
C16:1n-7c	161.7 ± 81.3	174.5 ± 34.5	219.1 ± 56.1	935.8 ± 32.2
C16:1n-5c	4.32 ± 3.48	5.14 ± 0.73	n.d.	n.d.
C17:1n-7c	7.75 ± 3.97	6.95 ± 1.25	6.78 ± 2.02	27.50 ± 2.23
C18:1n-12c	5.56 ± 5.24	21.32 ± 7.26	10.25 ± 4.71	n.d.
C18:1n-9c	467 ± 202	467.7 ± 61.0	3,756 ± 943	12,427 ± 345
C18:1n-7c	142.4 ± 68.6	105.46 ± 5.12	256.3 ± 68.6	887.6 ± 26.8
C18:1n-5c	4.66 ± 3.76	6.12 ± 0.75	n.d.	n.d.
C20:1n-11c	13.9 ± 13.4	21.54 ± 3.89	11.88 ± 4.81	37.60 ± 2.67
C20:1n-9c	211 ± 135	219 ± 48.1	292.3 ± 93.6	583.9 ± 17.3
C20:1n-7c [a)]	6.05 ± 2.83	6.31 ± 2.28	7.47 ± 2.00	26.48 ± 1.54
C22:1n-9c	347 ± 235	241.5 ± 61.1	124.8 ± 61.9	352.5 ± 14.0
C24:1n-9c	14.6 ± 12.4	13.50 ± 1.05	30.3 ± 11.2	61.57 ± 1.87
∑ MUFAs	**1,391 ± 770**	**1,293 ± 228**	**4,725 ± 1,254**	**15,366 ± 446**
C16:2n-4c	3.71 ± 2.68	6.62 ± 2.17	11.58 ± 3.06	83.11 ± 5.77
C18:2n-6c (LA)	66.7 ± 28.7	22.94 ± 3.90	1179 ± 284	4157 ± 117
C18:3n-6c	n.d.	n.d.	5.54 ± 1.99	16.04 ± 1.32
C18:3n-3c (ALA)	20.9 ± 18.6	17.51 ± 2.34	482.9 ± 94.2	2,396.5 ± 70.5
C18:4n-3c	85.5 ± 51.8	23.58 ± 0.93	36.29 ± 8.55	167.5 ± 11.7
C20:2n-6c	8.10 ± 5.61	5.71 ± 1.63	71.5 ± 19.1	26.17 ± 2.38
C20:3n-6c	0.89 ± 0.61	1.54 ± 0.43	14.00 ± 3.59	62.00 ± 1.06
C20:3n-3c	4.03 ± 2.80	3.98 ± 1.15	29.27 ± 6.20	10.67 ± 0.29
C20:4n-6c	8.19 ± 3.65	7.17 ± 1.34	10.75 ± 2.50	10.01 ± 5.05
C20:4n-3c	13.0 ± 11.3	29.93 ± 4.56	50.7 ± 15.2	66.29 ± 3.92
C20:5n-3c (EPA)	269 ± 127	166.8 ± 30.8	186.7 ± 36.3	903.8 ± 30.2
C21:5n-3c	6.60 ± 5.36	8.24 ± 2.25	18.68 ± 4.29	55.20 ± 4.85
C22:5n-3c	22.5 ±12.6	70.18 ± 4.08	94.7 ± 20.3	160 ± 6.71
C22:6n-3c (DHA)	735 ± 332	353.2 ± 88.9	335.8 ± 68.7	1,016.1 ± 30.5
∑ PUFAs	**1,244 ± 602**	**717 ± 145**	**2,524 ± 568**	**9,134 ± 292**
Total	**3,937**	**2,729**	**8,531**	**30,000**
∑ n-6	83.9 ± 38.5	37.36 ± 7.30	1,281 ± 311	4,275 ± 127
∑ n-3	1,157 ± 561	673 ± 135	1,234 ± 254	4,777 ± 159

a) The FA is not confirmed by a standard, only by NIST library search. b) n.d. – not detected.

Table 5.3: Overview over every FA found in Atlantic mackerel (n = 3), wild (n = 3) and farmed Atlantic salmon (n = 3), and salmon feed given as proportions and standard deviation (SD). Proportions are given in percentages of lipid content.

	Mean ± SD [%]			
Fatty acid	Atlantic mackerel	Wild Atlantic salmon	Farmed Atlantic salmon	Salmon feed
C12:0	0.03 ± 0.01	0.05 ± 0.01	n.d.[b)]	n.d.
C13:0 (4,8,12-trimethyl) [a)]	0.07 ± 0.07	0.063 ± 0.004	n.d.	n.d.
C14:0	4.09 ± 1.93	3.43 ± 0.49	1.71 ± 0.42	2.18 ± 0.09
C14:0 (13-methyl)	0.12 ± 0.10	0.14 ± 0.02	0.03 ± 0.01	0.07 ± 0.01
C14:0 (12-methyl)	0.04 ± 0.04	0.09 ± 0.01	0.018 ± 0.004	0.027 ± 0.002
C15:0	0.32 ± 0.23	0.30 ± 0.05	0.09 ± 0.02	0.19 ± 0.01
C16:0	20.36 ± 8.07	17.43 ± 2.18	9.61 ± 2.22	10.32 ± 0.30
C17:0	0.78 ± 0.54	0.43 ± 0.05	0.18 ± 0.04	0.34 ± 0.03
C18:0	7.08 ± 2.82	4.31 ± 0.56	2.94 ± 0.75	4.37 ± 0.13
C20:0	0.13 ± 0.07	0.08 ± 0.01	0.29 ± 0.08	0.49 ± 0.02
C22:0	0.05 ± 0.02	n.d.	0.09 ± 0.01	0.23 ± 0.02
C24:0	n.d.	n.d.	0.035 ± 0.005	0.119 ± 0.004
∑ SFAs	**33.1 ± 13.9**	**26.32 ± 3.38**	**14.98 ± 3.55**	**18.33 ± 0.61**
C16:1n-9c	0.14 ± 0.10	0.13 ± 0.03	0.11 ± 0.03	0.09 ± 0.01
C16:1n-7c	4.11 ± 2.07	6.39 ± 1.26	2.57 ± 0.66	3.12 ± 0.11
C16:1n-5c	0.11 ± 0.09	0.19 ± 0.03	n.d.	n.d.
C17:1n-7c	0.20 ± 0.10	0.26 ± 0.05	0.08 ± 0.02	0.09 ± 0.01
C18:1n-12c	0.14 ± 0.13	0.78 ± 0.27	0.12 ± 0.06	n.d.
C18:1n-9c	11.85 ± 5.13	17.14 ± 2.24	44.0 ± 11.1	41.42 ± 1.15
C18:1n-7c	3.62 ± 1.74	3.86 ± 0.19	3.00 ± 0.80	2.96 ± 0.09
C18:1n-5c	0.12 ± 0.10	0.22 ± 0.03	n.d.	n.d.
C20:1n-11c	0.35 ± 0.34	0.79 ± 0.14	0.14 ± 0.06	0.13 ± 0.01
C20:1n-9c	5.36 ± 3.42	8.05 ± 1.76	3.43 ± 1.13	1.95 ± 0.06
C20:1n-7c [a)]	0.15 ± 0.07	0.23 ± 0.09	0.09 ± 0.02	0.09 ± 0.01
C22:1n-9c	8.81 ± 5.96	8.85 ± 2.24	1.46 ± 0.73	1.17 ± 0.05
C24:1n-9c	0.37 ± 0.32	0.50 ± 0.04	0.36 ± 0.13	0.21 ± 0.01
∑ MUFAs	**35.3 ± 19.6**	**47.40 ± 8.36**	**55.4 ± 14.7**	**51.22 ± 1.49**
C16:2n-4c	0.09 ± 0.07	0.24 ± 0.08	0.14 ± 0.04	0.28 ± 0.02
C18:2n-6c (LA)	1.70 ± 0.73	0.84 ± 0.14	13.83 ± 3.33	13.86 ± 0.39
C18:3n-6c	n.d.	n.d.	0.06 ± 0.02	0.053 ± 0.004
C18:3n-3c (ALA)	0.53 ± 0.47	0.64 ± 0.09	5.66 ± 1.10	7.99 ± 0.23
C18:4n-3c	2.17 ± 1.32	0.86 ± 0.03	0.43 ± 0.10	0.56 ± 0.04
C20:2n-6c	0.21 ± 0.14	0.21 ± 0.06	0.84 ± 0.22	0.09 ± 0.01
C20:3n-6c	0.02 ± 0.02	0.06 ± 0.02	0.16 ± 0.04	0.044 ± 0.004
C20:3n-3c	0.10 ± 0.07	0.15 ± 0.04	0.34 ± 0.07	0.036 ± 0.001
C20:4n-6c	0.21 ± 0.09	0.26 ± 0.05	0.13 ± 0.03	0.21 ± 0.02
C20:4n-3c	0.33 ± 0.29	1.10 ± 0.17	0.59 ± 0.18	0.22 ± 0.01
C20:5n-3c (EPA)	6.82 ± 3.21	6.11 ± 1.13	2.19 ± 0.43	3.01 ± 0.10
C21:5n-3c	0.17 ± 0.14	0.30 ± 0.08	0.22 ± 0.05	0.18 ± 0.02
C22:5n-3c	0.57 ± 0.32	2.57 ± 0.15	1.11 ± 0.24	0.54 ± 0.02
C22:6n-3c (DHA)	18.68 ± 8.44	12.94 ± 3.26	3.94 ± 0.81	3.39 ± 0.10
∑ PUFAs	**31.6 ± 15.3**	**26.29 ± 5.30**	**29.63 ± 6.66**	**30.45 ± 0.97**
∑ n-6	2.13 ± 0.98	1.37 ± 0.27	15.02 ± 3.65	14.25 ± 0.42
∑ n-3	29.4 ± 14.3	24.68 ± 4.95	14.48 ± 2.98	15.92 ± 0.53
n-6/n-3	0.07	0.06	1.04	0.89
AI	0.55	0.43	0.19	0.23
TI	0.29	0.22	0.18	0.21

a) The FA is not confirmed by a standard, only by NIST library search. b) n.d. – not detected.

5.3. Comparison of the fish fatty acid profiles

As the feeding regime is widely different for the mackerel, wild and farmed salmon, it was expected to be reflected in the FA profiles given in Figure 5.1.

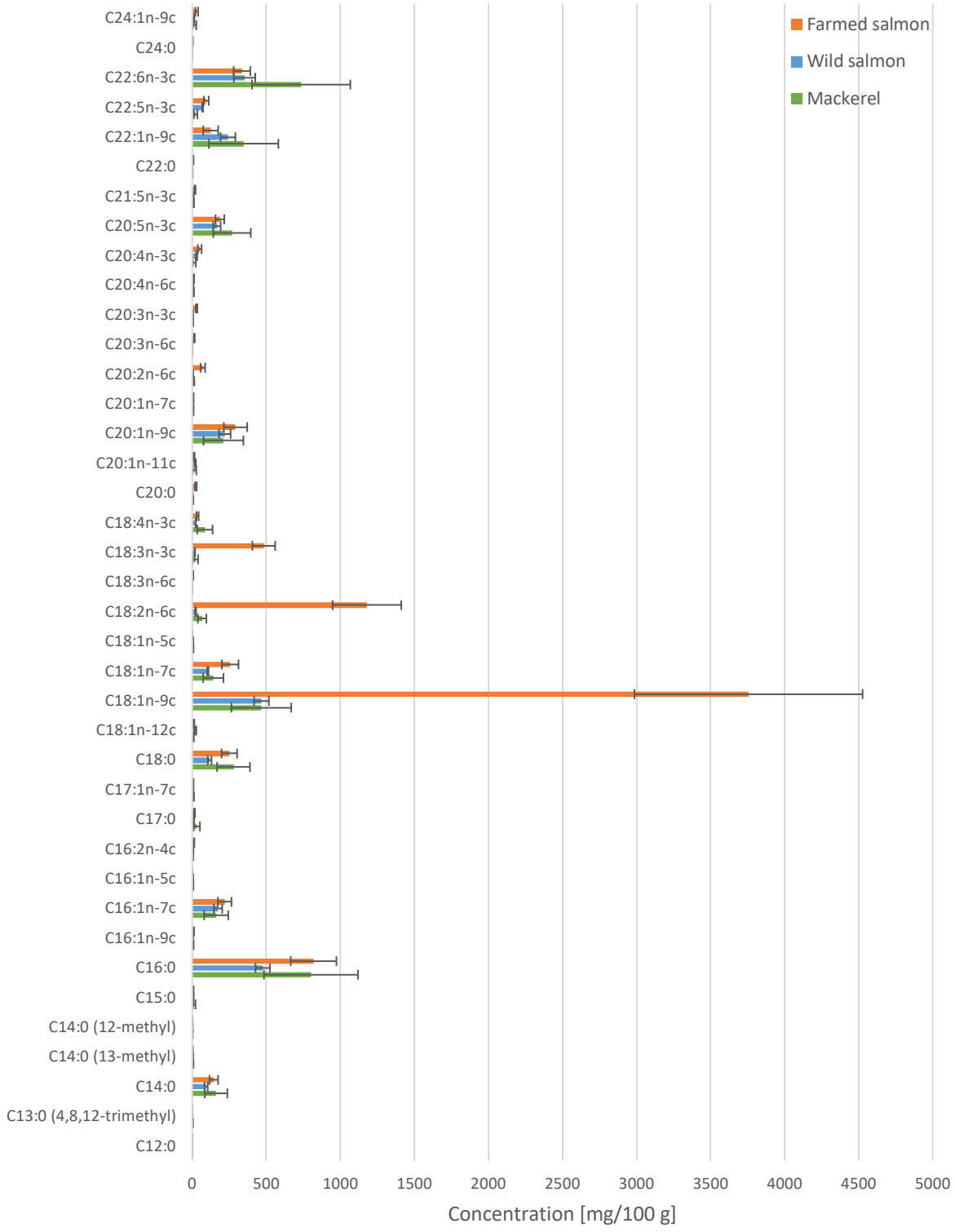


Figure 5.1: *Comparison of the total FA profile of Atlantic mackerel (n = 3), wild Atlantic salmon (n = 3) and farmed Atlantic salmon (n = 3). Concentrations are given in mg FA/100 g fish muscle with their associated standard deviations.*

In particular, four FAs stand out in the FA profile of farmed salmon; C16:0, C18:1n-9c, C18:2n-6c, and C18:3n-3c. These FAs are present in relatively high concentrations (482 – 3,756 mg/100 g) and together accounted for 73% of the FAs in farmed salmon. In the wild species, these four FAs exist at significantly lower concentrations and constitute only 36% and 34% of the FAs in wild salmon and mackerel, respectively. The majority of the FAs are found at low concentrations and are therefore presented in an additional plot, Figure 5.2, where FAs with concentrations higher than 100 mg/100 g have been excluded.

C18:1n-9c (OA) is the dominant peak found in farmed salmon. With a concentration of 3,756 mg/100 g, it represents as much as 44% of the fatty acid content in the fish. This is in good agreement with recent studies that also report high contents of OA in farmed salmon (Friesen et al. 2015; Sprague et al. 2016). This is an unsaturated n-9 fatty acid found naturally in various plant and animal sources and is among other things the major constituent of rapeseed oil (Mørkøre et al., 2014). As previously explored in section 3.1.5, the intake of OA has been associated with potential beneficial effects in patients suffering from type II diabetes (Vassiliou et al., 2009).

The second most dominant FA in farmed salmon is the n-6 fatty acid C18:2n-6c (LA). LA, an n-6 EFA, displayed a concentration of 1,179 mg/100 g and accounted for 14% of the FA content of farmed salmon. Furthermore, the farmed salmon also showed a high concentration of the n-3 EFA C18:3n-3c (ALA). As previously stated in section 3.1.5, ALA is the precursor to both EPA and DHA, and, along with LA, needs to be incorporated in the diet (Dewick, 2009). In total, these two EFAs make up 19.5% of the fatty acids in farmed salmon, only 1.5% in wild salmon and 2.2% in mackerel. OA, LA and ALA are found in greater concentrations in farmed salmon compared to the wild species. This is likely due to the feeding regime and will be discussed in section 5.4.

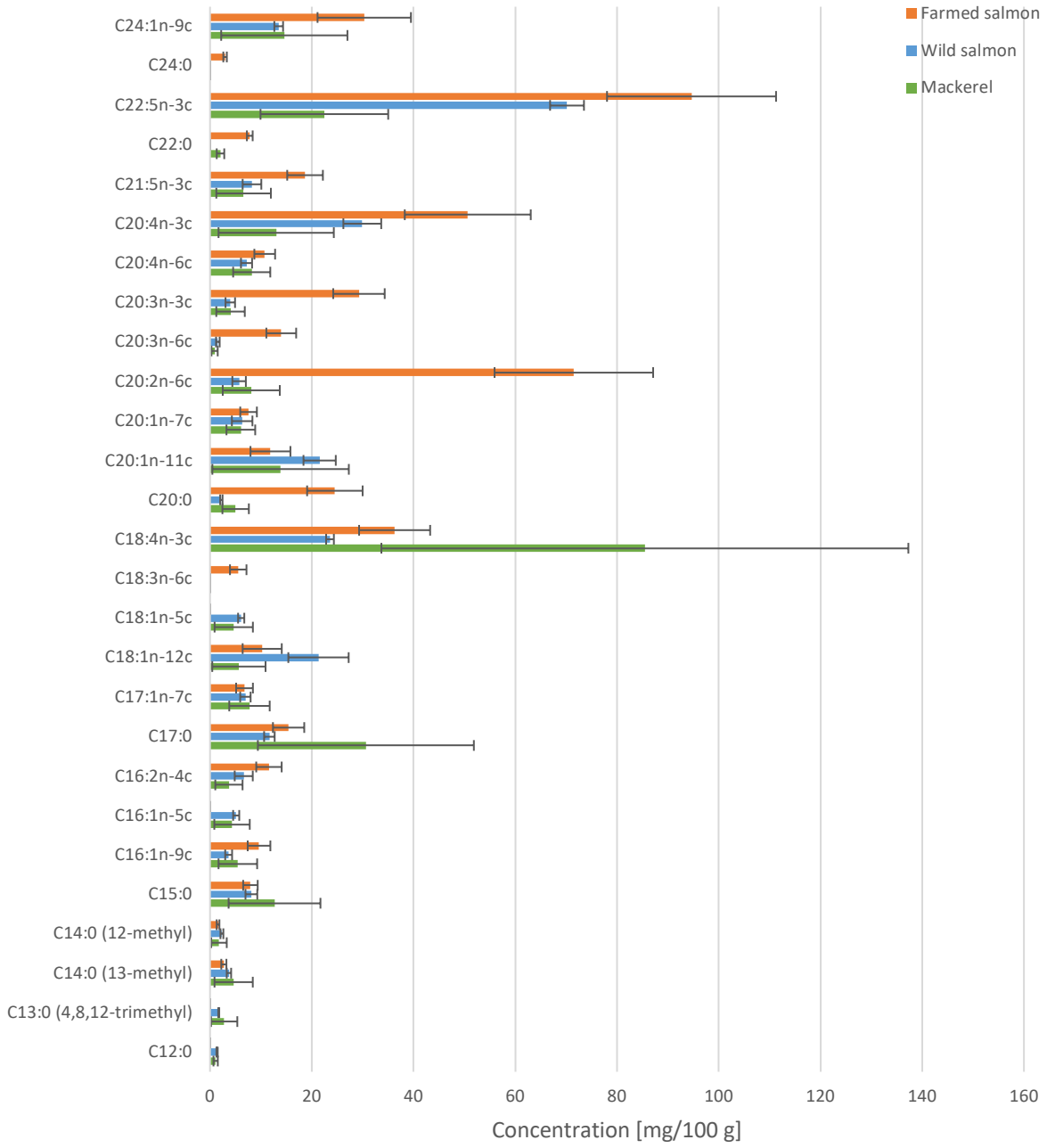


Figure 5.2: *Comparison of the total FA profile of Atlantic mackerel (n = 3), wild Atlantic salmon (n = 3) and farmed Atlantic salmon (n = 3). To highlight the FAs with low concentrations, the FAs with concentrations higher than 100 mg/100 g have been excluded from this Figure. Concentrations are given in mg FA/100 g fish muscle with their associated standard deviations*

By examining both Figure 5.1 and 5.2, it is apparent that the different fishes mostly share the same FAs. However, C18:3n-6c and C24:0 are unique to the FA profile of the farmed salmon. The farmed salmon contains more total fat than both the wild salmon and the mackerel and was therefore expected to display higher concentrations of most FAs. While that generally is the case, Figure 5.2 reveals FAs in both wild salmon and mackerel of higher concentrations than what are found in farmed salmon, for example, C17:0, C18:1n-5c, and C20:1n-11c.

The main peaks of wild salmon were found to be C16:0, C18:1n-9c, C20:1n-9c, C22:1n-9c, and DHA, which accounted for 64.5% of the total lipid content. These results are in good agreement with a study by Olsen et al. (2013) who also reported that these five FAs accounted for 65% of the FA content in wild salmon. The main peaks of mackerel were found to be C16:0, C18:0, C18:1n-9c, C22:1n-9c, EPA, and DHA, which accounted for 73.6% of the total lipid content of the mackerel. A study by Standal et al., (2018) showed that these six FAs represented 61% of the total lipid content. The results from this study are significantly higher. However, the mackerel FAs generally displayed a significantly higher standard deviations, due to the variations found in the lipid content of the fish, as previously stated in section 5.1.

Even though the farmed salmon contained more fat than the wild salmon, they both displayed relatively similar concentrations of both EPA and DHA. Compared to both types of salmon, the mackerel displayed two times the concentrations of EPA and DHA. This is believed to originate from the differences in the lifecycles of the mackerel and the salmon. The wild salmon travels up rivers to spawn. Thus, spending a significant period of their lifecycle in freshwater, making the marine sources of these n-3 FAs unavailable. Mackerels, however, lives exclusively in the sea and thus have receive these FAs regularly throughout their life. In literature, mackerel have also been shown to be twice as rich in EPA and DHA as salmon (Lundebye et al., 2017; Standal et al., 2018).

Another FA of interest were erucic acid (C22:1n-9c) which was found in higher concentrations in both the wild species compared to the farmed salmon (347 ± 235, 241.5 ± 49.9, and 124.8 ± 50.5 mg/100 g, respectively). The European Food Safety Authority, EFSA, issued a report in 2016 recommending a dietary limit of 7 mg/kg body weight per day, due to the potential negative health effects on children under the age of 10 (Knutsen et al., 2016). This means that a child of 25 kg has a recommended limit of 175 mg erucic acid per day. A consumption of 100 g of the fish fillets subjected to testing would yield 125, 242, and 347 mg from farmed salmon, wild salmon, and mackerel, respectively. Thus, consuming either wild salmon or mackerel would significantly exceed the recommended daily limit.

5.4. Comparison of the complete fatty acid profile of farmed salmon and feed

In order to evaluate the effect of the feed on the farmed salmon's FA composition, this study also included the total FA profile in salmon feed. The comparison between the various FAs in farmed salmon and salmon feed is presented in Figure 5.3 as percentages of the total lipid content. An overview of all FAs found in fish feed with associated retention time, match factor and concentration with standard deviation can be found in **appendix VIII**.

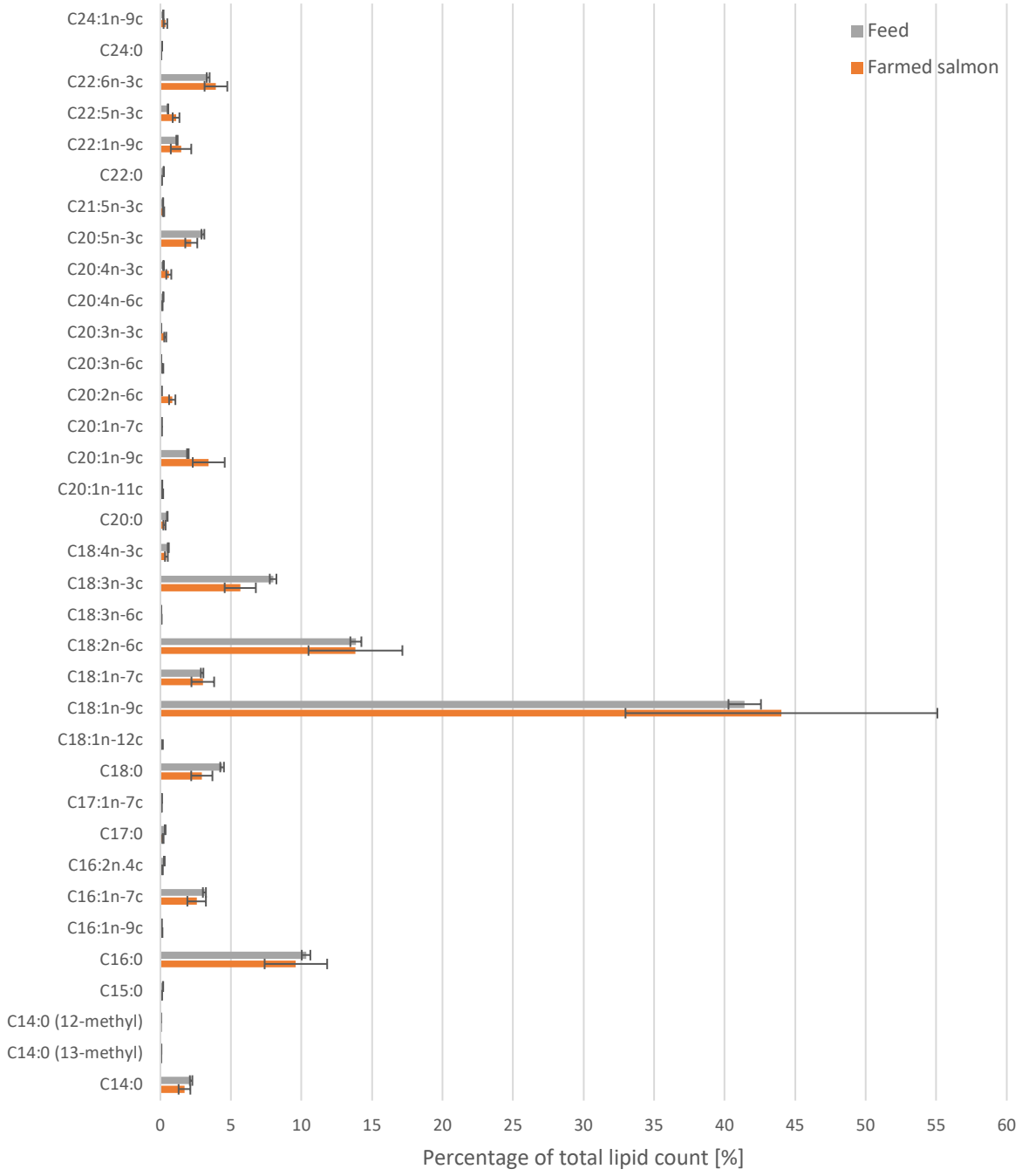


Figure 5.3: *Comparison of the FA profile of farmed salmon (n = 3) and the feed displayed as percentages relative to the total lipid amount with standard deviations.*

The majority of the FAs constitutes but a small fraction of the lipid content and are therefore presented in an additional plot, Figure 5.4, where FAs that constitute more than 1.5% of the lipid count have been excluded.

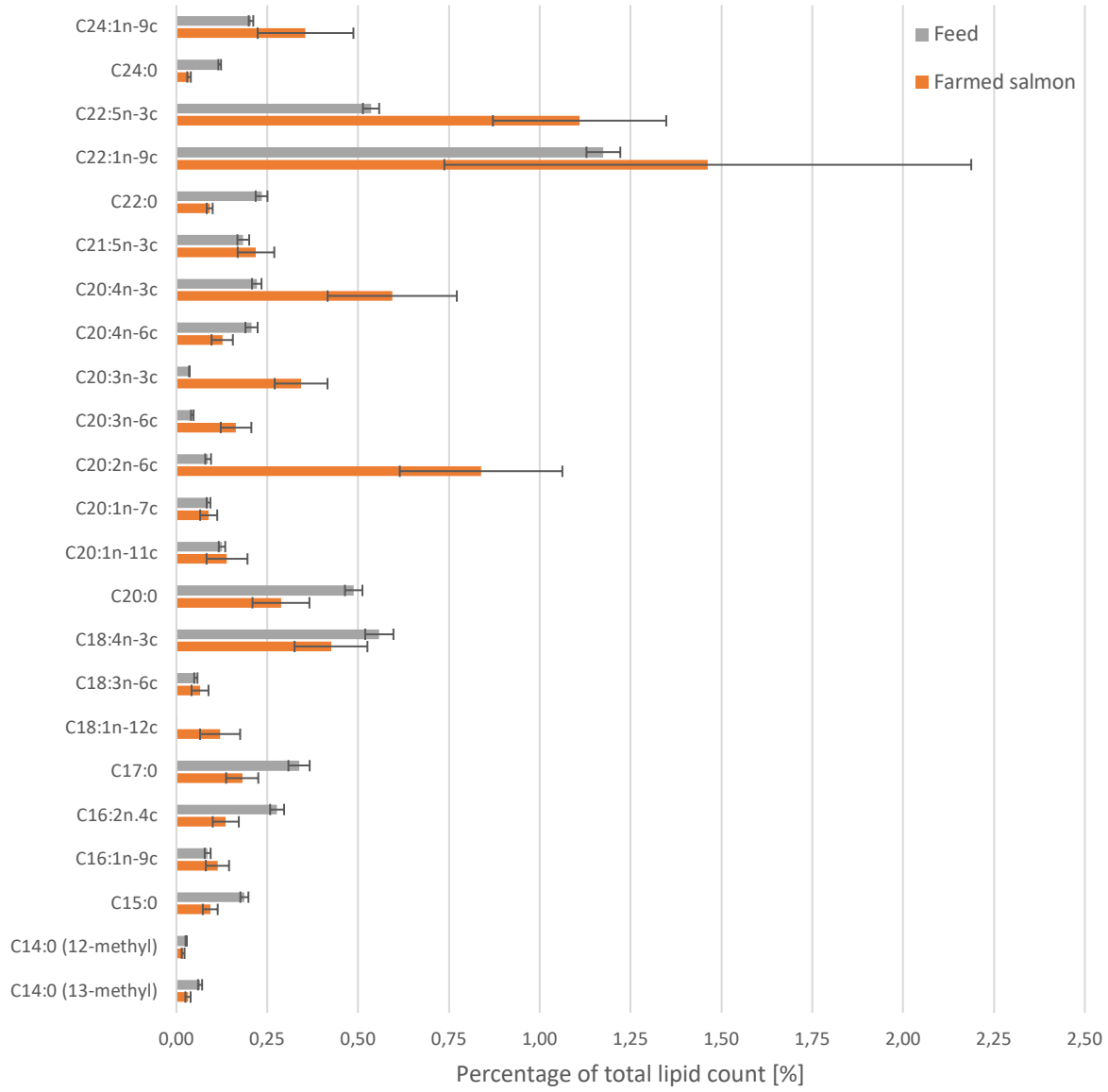


Figure 5.4: *Comparison of the FA profile of farmed salmon (n = 3) and the feed displayed as percentages relative to the total lipid count with standard deviations. FAs that constitute more than 1.5% of the lipid count have been excluded.*

As illustrated in Figure 5.3 and 5.4, the farmed salmon and the salmon feed share the same FAs at relatively similar proportions. However, the FA C18:1n-12c was only found in the farmed salmon. The proportions of the individual FAs are also relatively similar. OA (C18:1n-9c), which accounted for 44% of the FAs in farmed salmon, is also the most abundant FA in the feed. These results are in agreement with a study by Sprague et al. (2016) which showed the

amount of OA has increased in farmed salmon in accordance with the increased amount of plant-based ingredients in the feed. Furthermore, LA (C18:2n-6c) and, to a lesser extent, C16:0, and ALA (C18:3n-3c) are present in large quantities in both farmed salmon and the feed. OA, LA, and ALA are most commonly found in plant sources, and together with C16:0 they are the main constituents in rapeseed oil (Sahrafi et al., 2015). Rapeseed oil is one of the main ingredients in salmon feed in Norway today (Aas et al., 2019). Additionally, the feed contained greater proportions of EPA compared to farmed salmon (3.0 and 2.2 % respectively) and lower proportions of DHA (3.4 and 3.9 %, respectively).

5.5. Comparison of SFA, MUFA and PUFA in fish

As emphasised in section 3.1.5, SFAs, MUFAs, and PUFAs are associated with different effects on human health. The quantitative differences found in SFAs, MUFAs, and PUFAs for Atlantic mackerel, wild and farmed Atlantic salmon are highlighted in Figure 5.5 as a percentage bar chart.

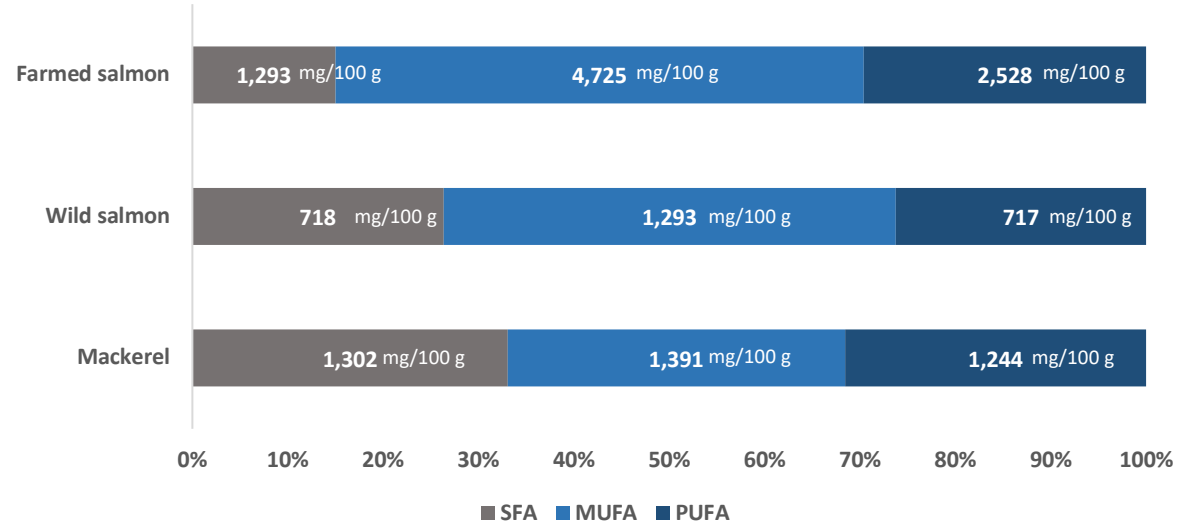


Figure 5.5: *Proportions of SFA, MUFA and PUFA relative to total lipid content given as percentages in farmed Atlantic salmon (n = 3), wild Atlantic salmon (n = 3) and Atlantic mackerel (n = 3). The categories' respective concentrations are given in mg FA/100 g fish muscle. Farmed Atlantic salmon consists of 15.0% SFA, 55.4% MUFA, and 29.6% PUFA. Wild Atlantic salmon consists of 26.3% SFA, 47.4% MUFA and 26.3% PUFA. Atlantic mackerel consists of 33.1% SFA, 35.3% MUFA, and 31.6% PUFA.*

The Atlantic mackerel was found to be the richest in SFAs. The SFAs constitute 33.1% of the total lipid content found in mackerel, while 26.3% for wild salmon and only 15.0% for farmed salmon. However, the mackerel and farmed salmon contain similar concentrations of SFAs (1,302 and 1,278 mg/100 g fish muscle, respectively), which is significantly higher than the

concentration found in wild salmon (718 mg/100 g fish muscle). The MUFAs compose the largest proportions in all three fishes. Whereas the mackerel displayed a proportion of 35.3% MUFAs, the salmons showed significantly higher proportions of 47.4 and 55.4% for wild and farmed salmon, respectively. Furthermore, the mackerel and wild salmon contain relatively similar concentrations of MUFAs (1,391 and 1,293 mg/100 g fish muscle, respectively). As anticipated, due to the higher lipid content of the farmed salmon, it displayed a much higher concentration of MUFAs (4,725 mg/100 g fish muscle). Relatively similar proportions of PUFAs was observed in mackerel and farmed salmon (31.6 and 29.6%, respectively). The wild salmon showed a somewhat lower proportion, however, not significantly lower (26.3%). The mackerel displayed almost equal proportions of all three categories. Furthermore, the FAs C16:0 and C18:0 constituted the majority of the total SFA content for all three fishes, while the FAs OA, C20:1n-9c, and C22:1n-9c were present in major quantities of the total MUFA content. The n-3 FAs EPA and DHA constituted the majority of the total PUFA content in both wild salmon and mackerel. However, LA and ALA were the major constituents of the total PUFA content in the farmed salmon.

5.6. Comparison of n-3 and n-6 FAs in fish

A percentage bar chart of the n-3 and n-6 FAs for Atlantic mackerel, wild and farmed Atlantic is presented in Figure 5.6. The remaining FAs are also included to show the proportions of the n-3 and n-6 FAs.

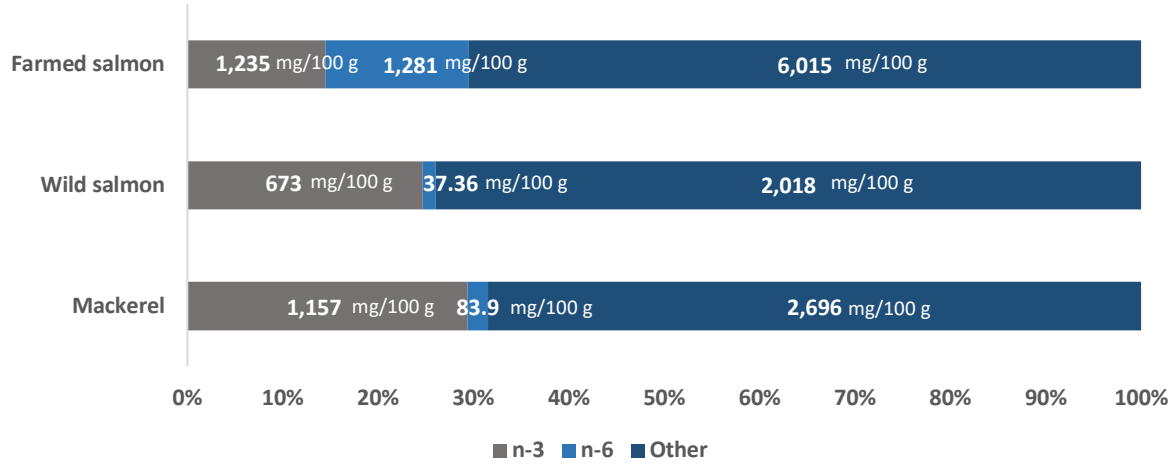


Figure 5.6: *The proportions of n-3, n-6 and the remaining FAs in farmed (n = 3) and wild Atlantic salmon (n = 3) and Atlantic mackerel (n = 3) given as percentages. The concentrations of the various categories are given in mg FA/100 g fish muscle. Farmed Atlantic salmon consists of 14.5% n-3 FAs, 15.0% n-6 FAs, and 70.5% other FAs. Wild Atlantic salmon consists of 24.7% n-3 FAs, 1.3% n-6 FAs, and 74.0% other FAs. Atlantic mackerel consists of 29.4% n-3 FAs, 2.1% n-6 FAs, and 68.5% other FAs.*

As a result of the higher lipid content, the farmed salmon comprised of higher concentrations of both n-3 and n-6 FAs compared to wild salmon and mackerel. The n-3 and n-6 FAs represent approximately 30% of the total lipid content in Atlantic mackerel and farmed Atlantic salmon, whereas 26% in the wild Atlantic salmon. Whereas the n-3 content was higher than the n-6 content in both wild salmon (24.7 and 1.4%, respectively) and mackerel (29.4 and 2.1%, respectively), the opposite was found in farmed salmon where more n-6 than n-3 was observed. The proportion of n-6 FAs were ten times higher in farmed salmon compared to the wild salmon, while seven times higher compared to the mackerel.

Judging by the results, consuming 200 g of fish fillets would provide 1,860 mg of n-3 and 1,920 mg of n-6 FAs from farmed salmon, 1,005 mg n-3 and 60 mg n-6 from wild salmon and 1,740 mg n-3 and 120 mg n-6 from mackerel. Due to their benefits to human health the marine n-3 FAs EPA and DHA are of particular interests and in 2012, EFSA set a dietary recommendation of these marine n-3 FAs of 250 mg/day, or 1.75 g/week (EFSA Panel on Dietetic Products & Allergies 2012). By eating a dinner portion (200 g) of fish fillets would provide 2,008, 1,040, and 1,045 mg EPA and DHA from mackerel, wild and farmed salmon, respectively. Thus, only 48 g of wild and farmed salmon, and 25 g of mackerel would be necessary to satisfy the recommended daily amount of EPA and DHA and eating salmon twice a week or mackerel just once a week would satisfy the recommended weekly intake. Thus, mackerel comprise of twice the amount of DHA and EPA as both wild and farmed salmon.

5.7. The fish lipid fractions

In addition to the elucidation of the complete FA profile, the extracted lipids from the fishes were to be separated into three fractions; NLs, FFAs, and PLs. The fractioning by off-line SPE yielded a total FA content of 20 – 50% lower than the value reported by following the method for elucidation of the complete FA profile. This might be explained by a poor mixing of the thawed ISs, especially the TAG IS, resulting in wrong concentrations. Nevertheless, the proportions (%) of the three fractions were calculated for the fishes and are presented in Table 5.4. The results of the lipid fractions in each fish is given as percentages of the total area (area %) and are presented in Table 5.5. The proportions of SFA, MUFA, PUFA, and n-3 and n-6 FAs found in the different fractions of the fishes are given in Table 5.6. An overview of all NLs, FFAs, and PLs found in the fishes can be found in **appendix IX**, **X**, and **XI**, respectively.

The appendices present the FAs with the corresponding retention time, match factor, probability and area % with standard deviations.

Table 5.4. The percentage proportions of NL, FFA, and PL in farmed (n = 3) and wild Atlantic salmon (n = 3) and Atlantic mackerel (n = 3).

	Proportions [%]		
	NL	FFA	PL
Atlantic mackerel	73.1	13.5	13.4
Wild Atlantic salmon	74.4	20.1	5.5
Farmed Atlantic salmon	86.9	6.1	7.0

Table 5.5. Comparison of the FA composition (area %) of the lipid fractions (NLs, FFAs, and PLs) in Atlantic mackerel (n = 3), wild Atlantic salmon (n = 3) and farmed Atlantic salmon (n = 3) given as percentages of the total peak area.

	Atlantic mackerel			Wild Atlantic salmon			Farmed Atlantic salmon		
	Mean ± SD [%]			Mean ± SD [%]			Mean ± SD [%]		
FA	NL	FFA	PL	NL	FFA	PL	NL	FFA	PL
C12:0	0.10 ± 0.04	n.d.[b)]	n.d.	0.071 ± 0.001	0.09 ± 0.01	n.d.	n.d.	n.d.	n.d.
C13:0 (4,8,12-trimethyl) [a)]	0.17 ± 0.05	n.d.	n.d.	0.09 ± 0.02	n.d.	n.d.	n.d.	n.d.	n.d.
C14:0	5.71 ± 0.38	2.97 ± 0.34	0.89 ± 0.41	4.28 ± 0.15	3.70 ± 0.31	0.97 ± 0.12	2.05 ± 0.03	2.88 ± 0.06	0.62 ± 0.05
C14:0 (13-methyl)	0.30 ± 0.02	n.d.	n.d.	0.19 ± 0.01	0.15 ± 0.02	n.d.	0.05 ± 0.00	n.d.	n.d.
C14:0 (12-methyl)	0.13 ± 0.01	n.d.	n.d.	0.13 ± 0.01	0.10 ± 0.01	n.d.	0.03 ± 0.01	n.d.	n.d.
C15:0	0.94 ± 0.09	0.69 ± 0.12	0.43 ± 0.08	0.42 ± 0.02	0.34 ± 0.02	0.23 ± 0.03	0.140 ± 0.003	0.26 ± 0.01	0.13 ± 0.01
C16:0	21.05 ± 3.16	25.58 ± 1.86	27.73 ± 3.13	18.08 ± 1.00	22.96 ± 0.83	22.86 ± 1.82	9.49 ± 0.09	19.14 ± 0.53	20.62 ± 0.89
C16:1n-9c	0.31 ± 0.03	0.24 ± 0.08	0.14 ± 0.03	0.15 ± 0.02	0.13 ± 0.02	n.d.	0.137 ± 0.004	0.13 ± 0.01	0.12 ± 0.01
C16:1n-7c	3.85 ± 0.73	3.90 ± 1.38	1.06 ± 0.39	6.22 ± 0.23	4.43 ± 0.47	1.05 ± 0.16	2.32 ± 0.04	2.06 ± 0.03	0.46 ± 0.03
C16:1n-5c	0.22 ± 0.01	0.46 ± 0.05	0.22 ± 0.02	0.27 ± 0.01	0.30 ± 0.02	n.d.	n.d.	n.d.	n.d.
C16:2n-4c	0.19 ± 0.05	0.21 ± 0.04	n.d.	0.27 ± 0.05	0.18 ± 0.02	n.d.	0.17 ± 0.01	0.20 ± 0.03	n.d.
C17:0	1.78 ± 0.15	1.19 ± 0.22	1.16 ± 0.11	0.57 ± 0.05	0.36 ± 0.05	0.53 ± 0.08	0.21 ± 0.01	0.32 ± 0.01	0.29 ± 0.01
C17:1n-7c	0.50 ± 0.14	0.57 ± 0.19	0.38 ± 0.06	0.24 ± 0.01	0.22 ± 0.02	n.d.	0.098 ± 0.004	n.d.	n.d.
C18:0	5.66 ± 1.18	9.51 ± 0.84	6.34 ± 0.39	3.67 ± 0.18	5.43 ± 0.23	4.01 ± 0.40	2.59 ± 0.07	7.55 ± 0.23	1.57 ± 0.06
C18:1n-12c	0.24 ± 0.03	0.23 ± 0.04	0.23 ± 0.05	0.86 ± 0.29	0.92 ± 0.14	0.64 ± 0.16	0.17 ± 0.03	n.d.	n.d.
C18:1n-9c	12.22 ± 0.84	10.20 ± 1.49	6.08 ± 1.01	17.76 ± 1.27	11.52 ± 0.47	7.42 ± 1.04	44.65 ± 0.45	28.34 ± 0.42	11.04 ± 0.79
C18:1n-7c	3.45 ± 0.64	3.37 ± 0.97	1.90 ± 0.48	4.14 ± 0.97	3.44 ± 0.50	1.98 ± 0.40	3.04 ± 0.05	2.68 ± 0.03	1.78 ± 0.08
C18:1n-5c	0.26 ± 0.04	n.d.	n.d.	0.28 ± 0.03	0.27 ± 0.03	n.d.	n.d.	n.d.	n.d.
C18:2n-6c	1.62 ± 0.38	1.76 ± 0.10	0.77 ± 0.16	1.08 ± 0.04	0.77 ± 0.03	0.34 ± 0.03	14.40 ± 0.12	13.88 ± 0.26	2.84 ± 0.16
C18:3n-6c	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.08 ± 0.01	n.d.	n.d.
C18:3n-3c	1.09 ± 0.36	1.34 ± 0.02	0.24 ± 0.03	0.88 ± 0.06	0.67 ± 0.02	0.20 ± 0.02	6.23 ± 0.31	7.13 ± 0.41	2.45 ± 0.18
C18:4n-3c	2.24 ± 1.31	2.26 ± 0.40	0.21 ± 0.07	1.23 ± 0.18	0.79 ± 0.14	0.21 ± 0.01	0.49 ± 0.04	0.43 ± 0.03	0.13 ± 0.02

a) The FA is not confirmed by a standard, only by NIST library search. b) n.d. – not detected.

Table 5.5 continued. Comparison of the FA composition (area %) of the lipid fractions (NLs, FFAs, and PLs) in Atlantic mackerel (n = 3), wild Atlantic salmon (n = 3) and farmed Atlantic salmon (n = 3) given as percentages of the total peak area.

	Atlantic mackerel			Wild Atlantic salmon			Farmed Atlantic salmon		
	Mean ± SD [%]			Mean ± SD [%]			Mean ± SD [%]		
FA	NL	FFA	PL	NL	FFA	PL	NL	FFA	PL
C20:0	0.40 ± 0.12	0.17 ± 0.04	0.12 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	n.d.[b)]	0.283 ± 0.004	0.220 ± 0.005	0.08 ± 0.01
C20:1n-11c	0.68 ± 0.15	0.25 ± 0.03	0.16 ± 0.04	0.89 ± 0.10	0.48 ± 0.06	0.23 ± 0.03	0.19 ± 0.03	n.d.	n.d.
C20:1n-9c	4.96 ± 2.00	2.35 ± 0.81	0.94 ± 0.60	7.54 ± 0.72	3.85 ± 0.20	1.24 ± 0.20	3.24 ± 0.25	1.67 ± 0.10	0.31 ± 0.03
C20:1n-7c [a)]	0.47 ± 0.17	0.21 ± 0.10	n.d.	0.26 ± 0.06	0.15 ± 0.03	n.d.	0.11 ± 0.01	n.d.	n.d.
C20:2n-6c	0.47 ± 0.01	0.28 ± 0.06	0.19 ± 0.01	0.24 ± 0.03	0.15 ± 0.02	n.d.	0.774 ± 0.003	0.61 ± 0.01	0.30 ± 0.03
C20:3n-6c	0.05 ± 0.01	n.d.	n.d.	0.06 ± 0.01	0.05 ± 0.01	n.d.	0.18 ± 0.02	0.15 ± 0.02	0.20 ± 0.02
C20:3n-3c	0.26 ± 0.02	0.18 ± 0.04	n.d.	0.19 ± 0.02	0.14 ± 0.02	n.d.	0.34 ± 0.01	0.31 ± 0.01	0.18 ± 0.03
C20:4n-6c	0.48 ± 0.01	0.55 ± 0.06	1.10 ± 0.07	0.29 ± 0.04	0.55 ± 0.03	0.60 ± 0.08	0.13 ± 0.01	0.16 ± 0.00	0.56 ± 0.03
C20:4n-3c	0.63 ± 0.24	0.59 ± 0.01	0.24 ± 0.03	1.33 ± 0.15	1.05 ± 0.06	0.44 ± 0.03	0.56 ± 0.05	0.55 ± 0.04	0.49 ± 0.06
C20:5n-3c	5.61 ± 0.55	9.44 ± 1.01	8.56 ± 1.00	6.32 ± 0.23	10.72 ± 0.66	8.15 ± 0.58	2.08 ± 0.06	4.30 ± 0.12	8.90 ± 0.24
C21:5n-3c	0.39 ± 0.02	0.22 ± 0.02	0.13 ± 0.04	0.38 ± 0.03	0.24 ± 0.02	0.31 ± 0.04	0.04 ± 0.02	0.116 ± 0.004	0.15 ± 0.02
C22:0	0.16 ± 0.11	n.d.	n.d.	n.d.	n.d.	n.d.	0.08 ± 0.01	n.d.	n.d.
C22:1n-9c	8.31 ± 3.68	2.58 ± 0.98	0.73 ± 0.55	8.07 ± 1.20	3.27 ± 0.20	0.30 ± 0.01	1.26 ± 0.29	0.46 ± 0.08	0.11 ± 0.01
C22:5n-3c	1.30 ± 0.14	0.88 ± 0.05	1.42 ± 0.09	2.67 ± 0.30	2.39 ± 0.31	2.84 ± 0.56	1.05 ± 0.04	0.66 ± 0.05	2.50 ± 0.25
C22:6n-3c	12.70 ± 3.59	17.55 ± 5.63	36.90 ± 4.86	10.08 ± 0.95	19.81 ± 1.70	44.44 ± 1.78	2.61 ± 0.22	3.88 ± 0.24	39.46 ± 0.81
C24:0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.07 ± 0.01	n.d.	n.d.
C24:1n-9c	0.85 ± 0.09	0.26 ± 0.03	0.22 ± 0.06	0.60 ± 0.10	0.29 ± 0.07	n.d.	0.07 ± 0.01	0.26 ± 0.01	0.28 ± 0.01

a) The FA is not confirmed by a standard, only by NIST library search. b) n.d. – not detected.

Table 5.6. Comparison of some FA classes within the lipid fractions (NLs, FFAs, and PLs) in Atlantic mackerel (n = 3), wild Atlantic salmon (n = 3) and farmed Atlantic salmon (n = 3) given as percentages of the total peak area.

	Atlantic mackerel [%] ± SD			Wild Atlantic salmon [%] ± SD			Farmed Atlantic salmon [%] ± SD		
	NL	FFA	PL	NL	FFA	PL	NL	FFA	PL
∑ SFA	36.39 ± 5.30	40.11 ± 3.40	36.67 ± 4.12	27.62 ± 1.45	33.21 ± 1.49	28.60 ± 2.46	15.00 ± 0.22	30.37 ± 0.85	23.30 ± 1.02
∑ MUFA	36.29 ± 8.56	24.61 ± 6.14	12.05 ± 3.29	47.29 ± 4.99	29.27 ± 2.21	12.86 ± 1.99	55.27 ± 1.15	35.60 ± 0.69	14.09 ± 0.97
∑ PUFA	27.01 ± 6.70	35.26 ± 7.43	49.75 ± 6.35	25.00 ± 2.19	37.49 ± 3.05	57.53 ± 3.13	29.15 ± 0.92	32.38 ± 1.23	58.16 ± 1.85
∑ n-3	24.21 ± 6.23	32.46 ± 7.17	47.69 ± 6.11	23.06 ± 2.02	35.79 ± 2.93	56.60 ± 3.02	13.40 ± 0.75	17.37 ± 0.91	54.26 ± 1.61
∑ n-6	2.62 ± 0.41	2.60 ± 0.21	2.05 ± 0.24	1.67 ± 0.11	1.52 ± 0.09	0.94 ± 0.11	15.57 ± 0.16	14.81 ± 0.29	3.90 ± 0.24

As expected, the storage lipids comprising the NL fractions were by far the most abundant in all fishes. The mackerel and wild salmon showed a similar proportion of NLs with respectively 73.1% and 74.4%. The farmed salmon, however, showed a significantly higher proportion of 86.9%. The PLs constituted the lowest proportion of the lipids in wild salmon (5.5%), whereas the second lowest in farmed salmon (7.0%). The mackerel showed approximately equal proportions of PLs and FFAs (13.4 and 13.5%, respectively). The FFAs constituted a total of 20.1 and 6.1% of the lipids in wild and farmed salmon, respectively.

The reported proportion of NLs in the farmed salmon are comparable to the value reported by Tsoupras et al. (2018) of 85%. Furthermore, the results for both wild and farmed salmon correspond to a study by Bell et al. (1998) which reported that wild and farmed salmon contained respectively 72% and 89% NLs. Halvorsen (2019) reported that the NL fractions constituted 83% and 97% of the lipids found in wild and farmed salmon, respectively, which are significantly higher than the results in the present study. However, unlike the present study, the subcutaneous fat was sampled.

It has been reported that FFAs constitute only 1 % of the lipids found in farmed salmon (Halvorsen, 2019; Ruiz-Lopez et al., 2015), while 8 % in wild salmon (Halvorsen, 2019). However, the proportions of FFAs found were significantly higher, especially in wild salmon (20.1 and 6.1 % for wild and farmed salmon, respectively). The reason for this might be that the wild salmon was not frozen quick enough after capture to prevent the lipases in the muscles to initiate decomposition. Thus, some of the FAs from NLs might have cleaved from the glycerol backbone turning into FFAs by lipid hydrolysis (Shewfelt, 1981). It is also worth mentioning that in literature the salmon might have been sampled at different periods of its life cycle, which would influence the results. The mackerel lipids have been reported to comprise of approximately 1.5% FFAs (Romotowska et al., 2016). The amount of FFAs have been shown to increase significantly in Atlantic mackerel during long-term frozen storage. Temperature fluctuations may increase enzyme activity causing accelerated lipid hydrolysis (Romotowska et al., 2017).

The proportions of PLs found in Atlantic salmon varies highly in literature from 2 – 16% (Halvorsen, 2019; Tsoupras et al., 2018; Tsoupras et al., 2019). The mackerel, however, showed a proportion of 13.4% PLs, which was significantly higher than the values reported by Romotowska et al. (2016) of 0.7 – 4%. Additionally, Romotowska et al. (2016) reported a

significant increase in phospholipids in Atlantic mackerel during prolonged storage in the freezer, which may be a result from the protein denaturation occurring during extended frozen storage (Saoussem, 2000). Considering that the mackerel was sampled in August 2019 and last lipid extraction were performed in late February 2020, it has been stored a considerable amount of time in the freezer, which may have increased the proportion of PLs.

It is also worth mentioning that if it, indeed, is the ISs that are the cause of the low recovery rate of the lipids, then these "missing lipids" might not be equally distributed between the three fractions. This would increase the uncertainty of the calculated proportions and may also explain the rather large differences between the calculated proportions and the ones found in literature.

The NLs found is presented in Figure 5.7 as percentages of the total peak area. However, the majority of the FAs constitutes but a small fraction of the NLs and are therefore presented in Figure 5.8, where FAs that contribute more than 5% to the total area have been excluded.

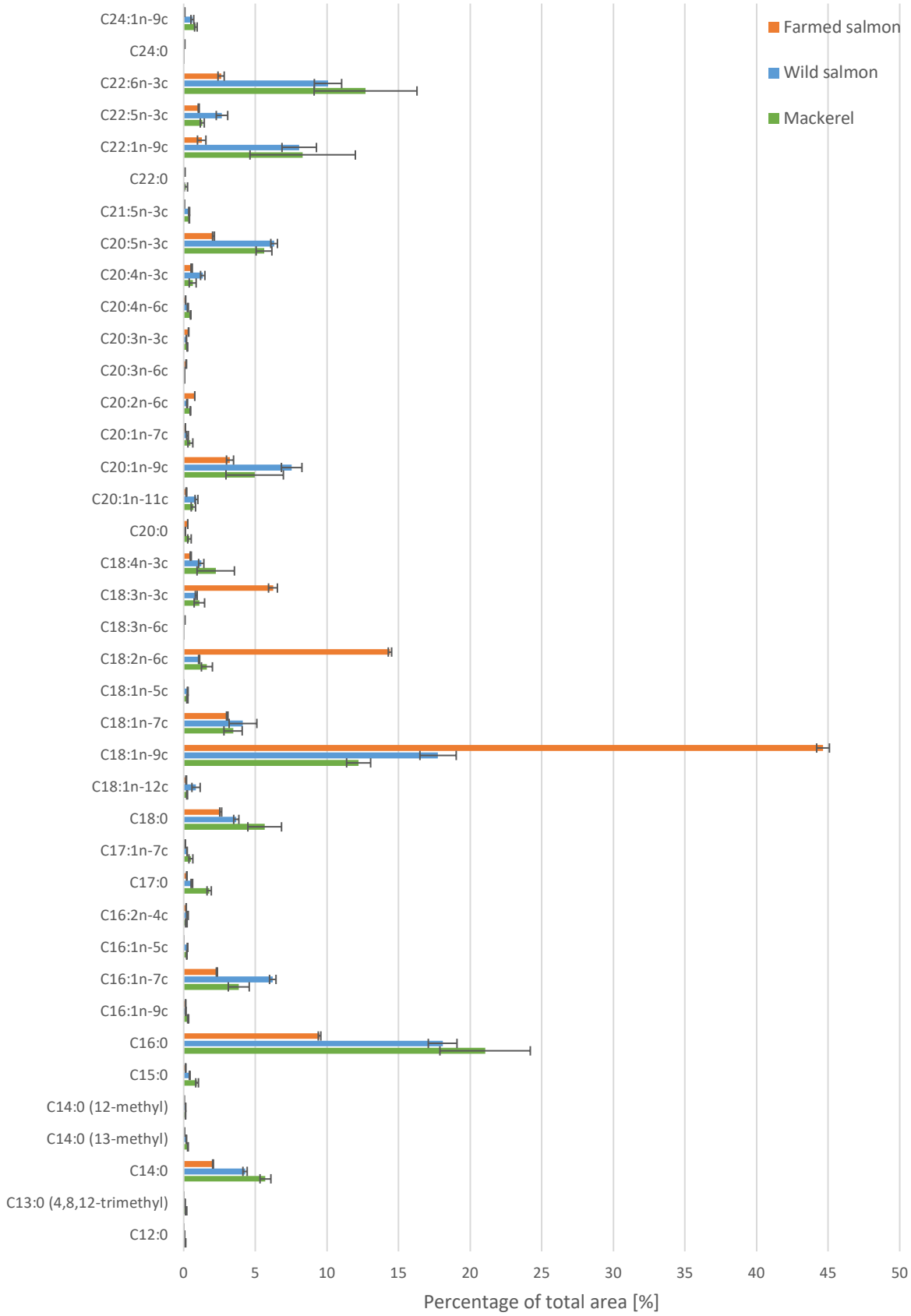


Figure 5.7: *Comparison of the NLs found in Atlantic mackerel (n = 3), wild Atlantic salmon (n = 3) and farmed Atlantic salmon (n = 3) displayed as percentages of the total area with standard deviations.*

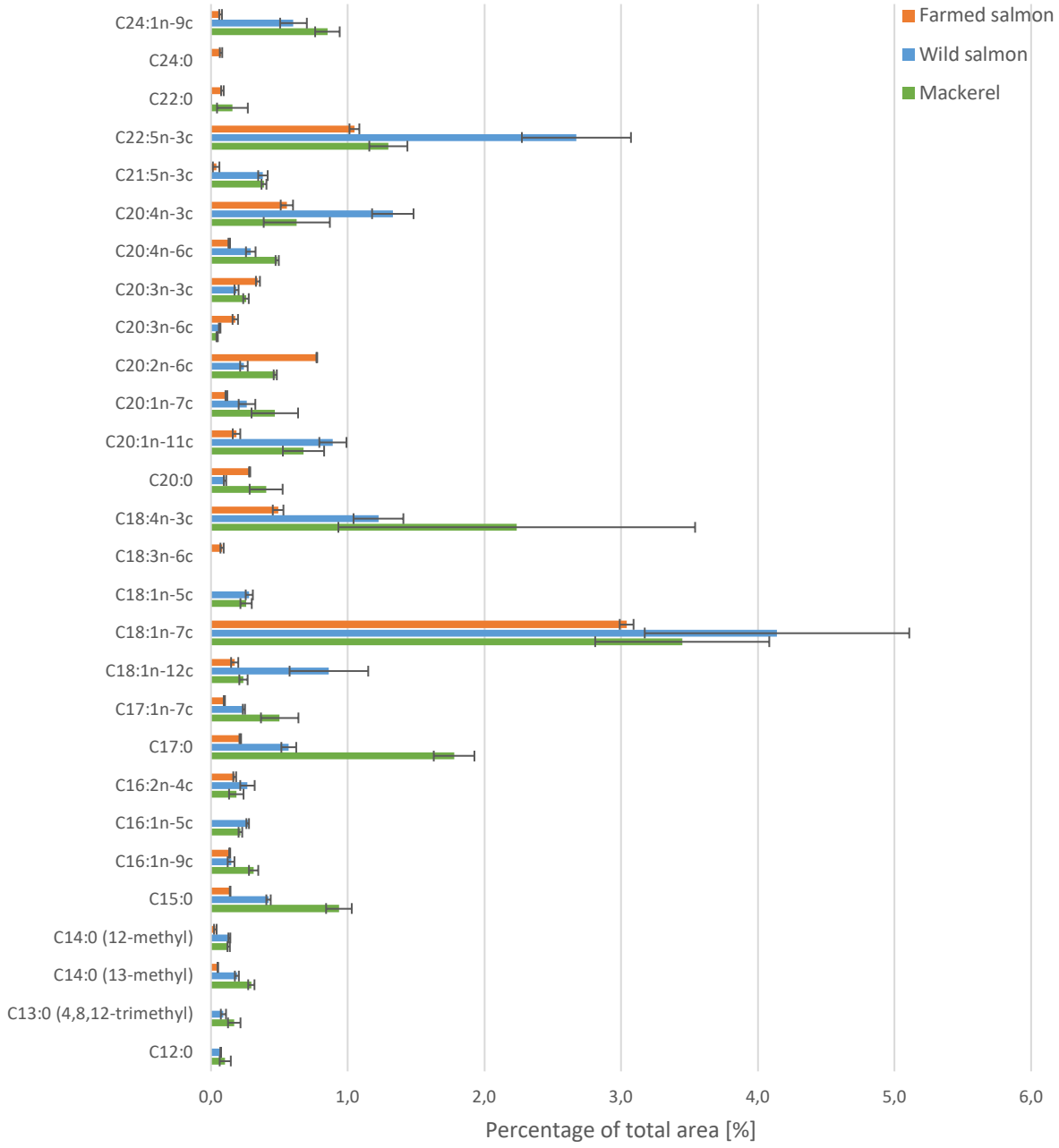


Figure 5.8: *Comparison of the NLs found in Atlantic mackerel (n = 3), wild Atlantic salmon (n = 3) and farmed Atlantic salmon (n = 3) displayed as percentages of the total area with standard deviations. NLs that constitute more than 5% of the total area have been excluded.*

Analogous to the complete FA profile, C16:0, C18:1n-9c, LA, and ALA were the dominant FAs in the NL fraction of the farmed salmon and constitutes 75% of the NLs found in farmed salmon. The dominant peaks of the wild salmon were C16:0, C18:1n-9c, C20:1n-9c, C22:1n-9c, and DHA, which accounted for 62% of the total NL peak area, and the dominant peaks of the mackerel were C14:0 C16:0, C18:0, C18:1n-9c, C22:1n-9c, EPA, and DHA, which accounted for 71% of the total NL peak area of the mackerel.

The composition of the FFA fraction is given in Figure 5.9 and 5.10. The majority of the FFAs constitutes but a small fraction of the total peak area and are presented in Figure 5.8 where FAs that contribute more than 5% to the total area have been excluded.

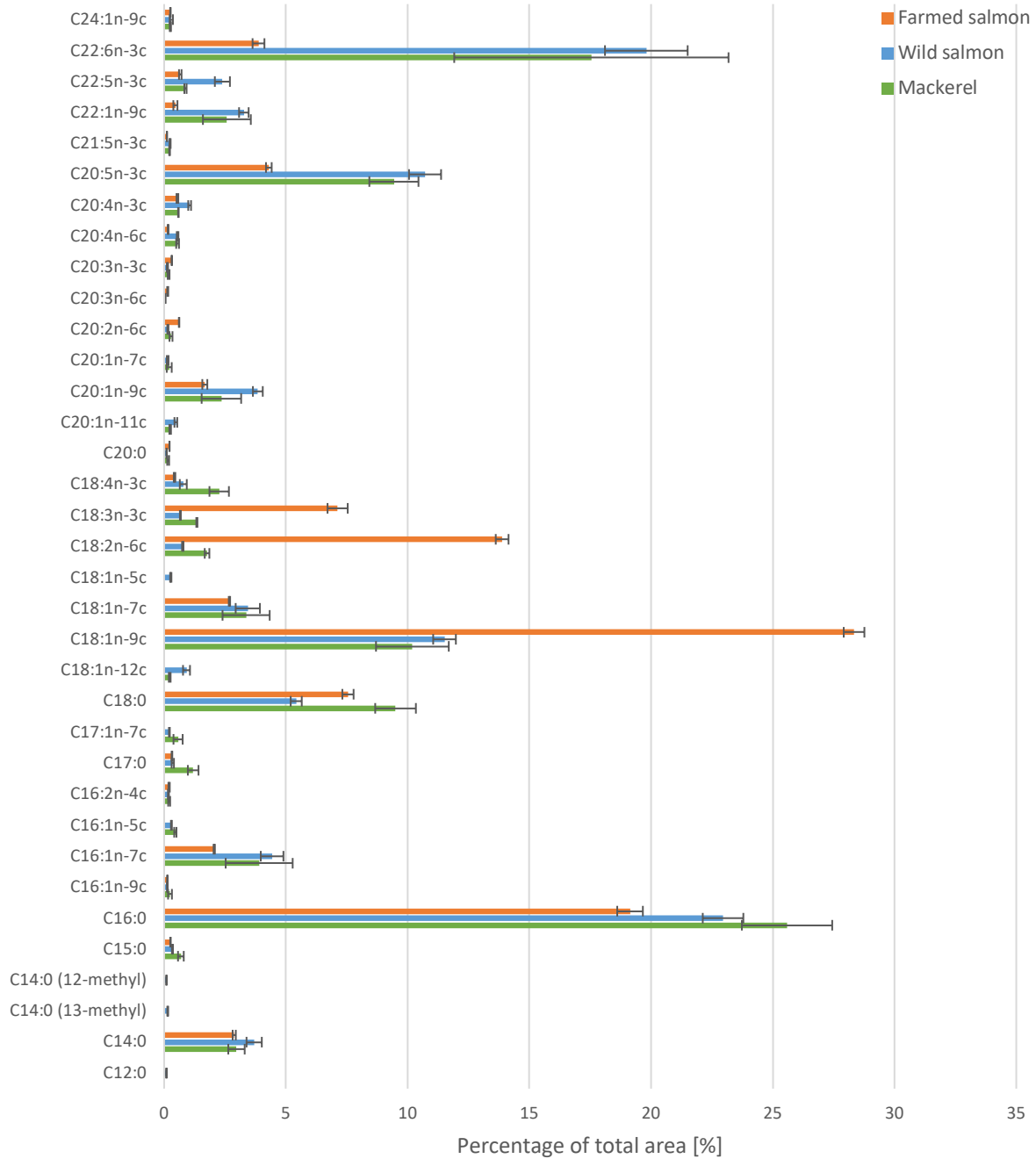


Figure 5.9: *Comparison of the FFAs found in Atlantic mackerel (n = 3), wild Atlantic salmon (n = 3) and farmed Atlantic salmon (n = 3) displayed as percentages of the total area with standard deviations.*

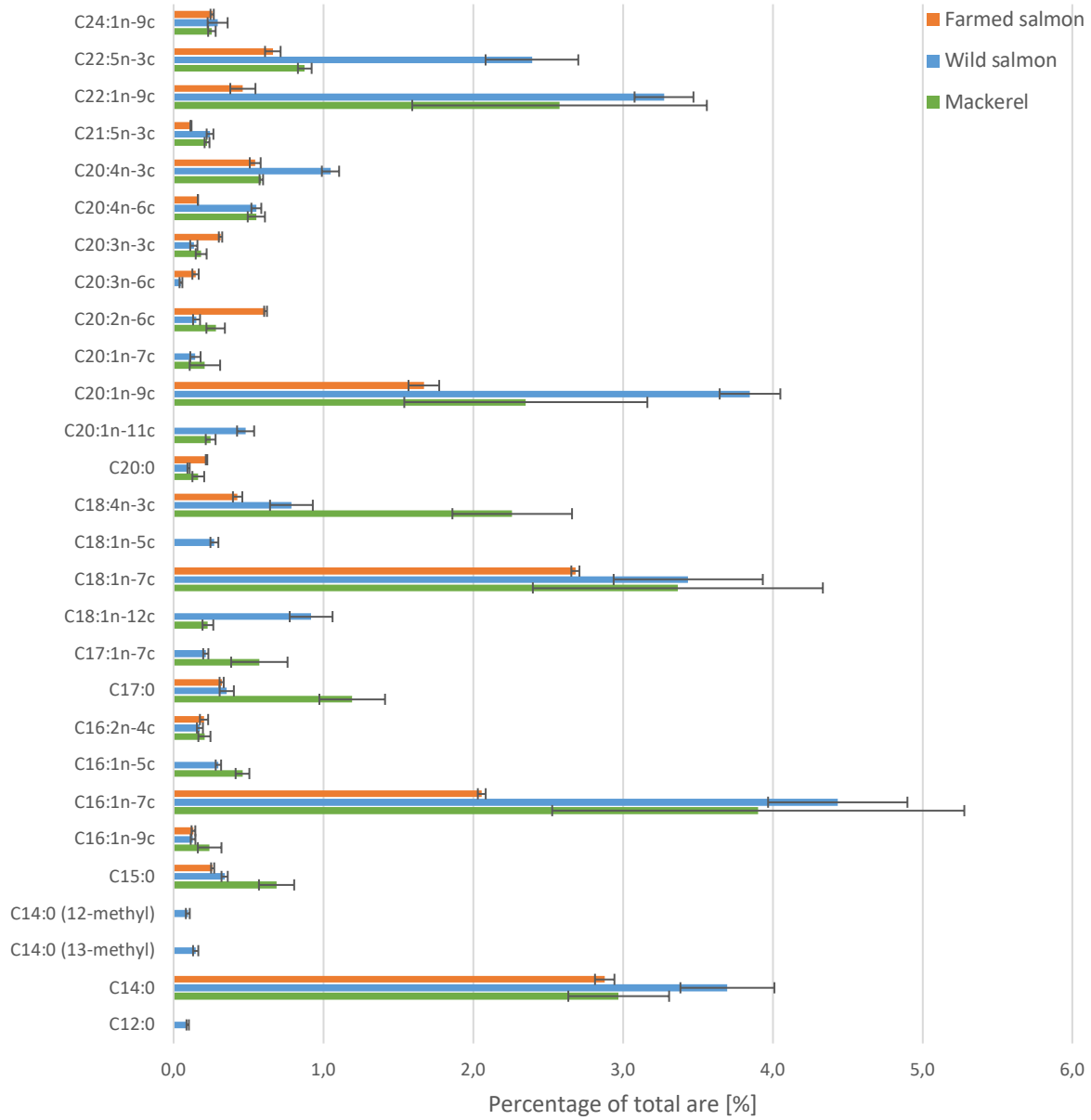


Figure 5.10: *Comparison of the FFAs found in Atlantic mackerel (n = 3), wild Atlantic salmon (n = 3) and farmed Atlantic salmon (n = 3) displayed as percentages of the total area with standard deviations. FFAs that constitute more than 5% of the total area have been excluded.*

Similar to the NLs, the four most dominant peaks found in the FFA fraction of farmed salmon were C16:0, C18:1n-9c, LA, and ALA which makes up 68% of the total peak area. The main peaks of the wild salmon were C16:0, C18:1n-9c, C22:1n-9c, and DHA, and accounted for 65% of the total peak area. The most dominant peaks of mackerel were C16:0, C18:0, C18:1n-9c, EPA, and DHA, which accounted for 72% of the total peak area.

The composition of the PL fraction is given in Figure 5.11 and 5.12. The majority of the PLs constitutes but a small fraction of the total peak area and are presented in Figure 5.10 where FAs that contribute more than 5% to the total area have been excluded.

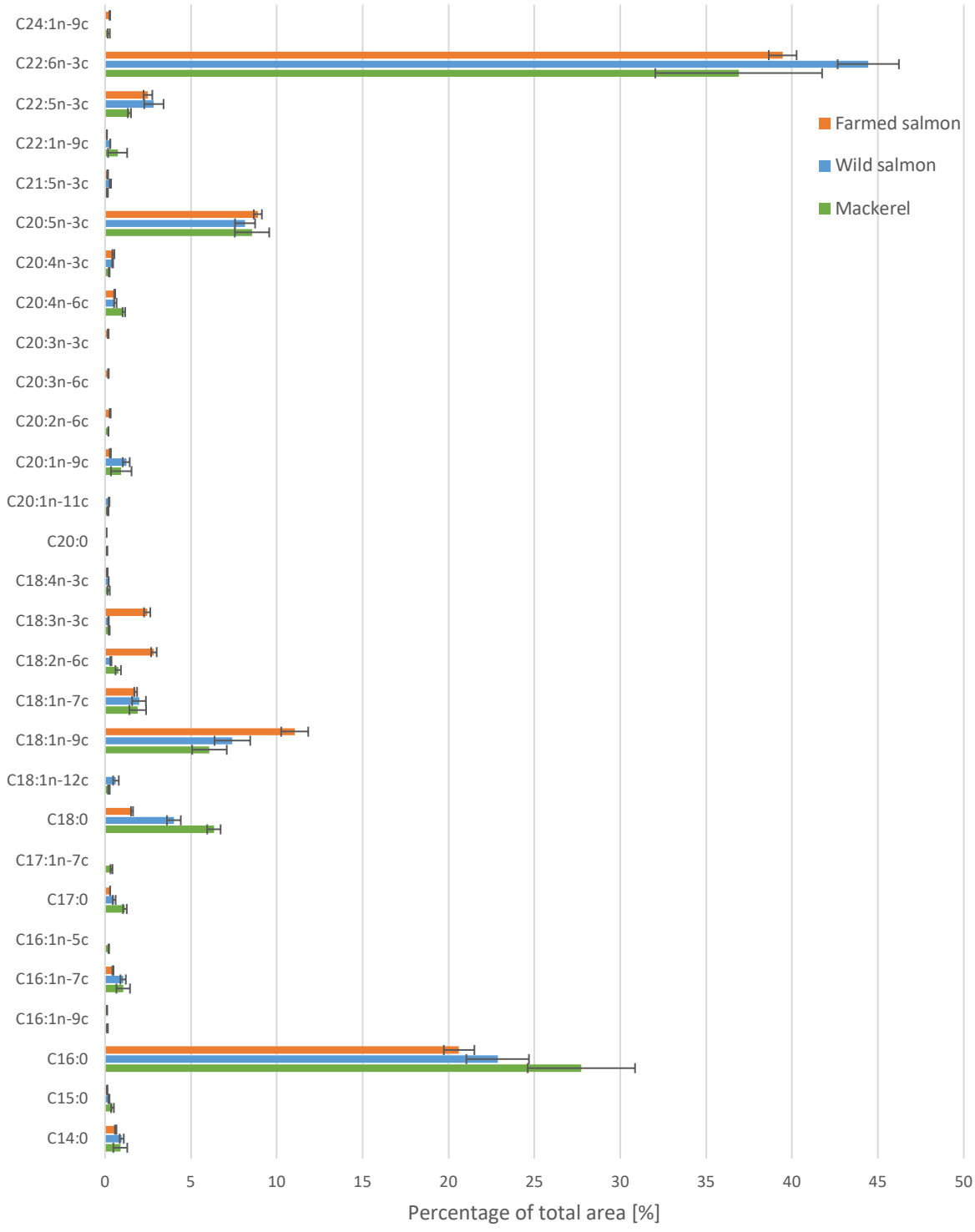


Figure 5.11: *Comparison of the PLs found in Atlantic mackerel (n = 3), wild Atlantic salmon (n = 3) and farmed Atlantic salmon (n = 3) displayed as percentages of the total area with standard deviations*.

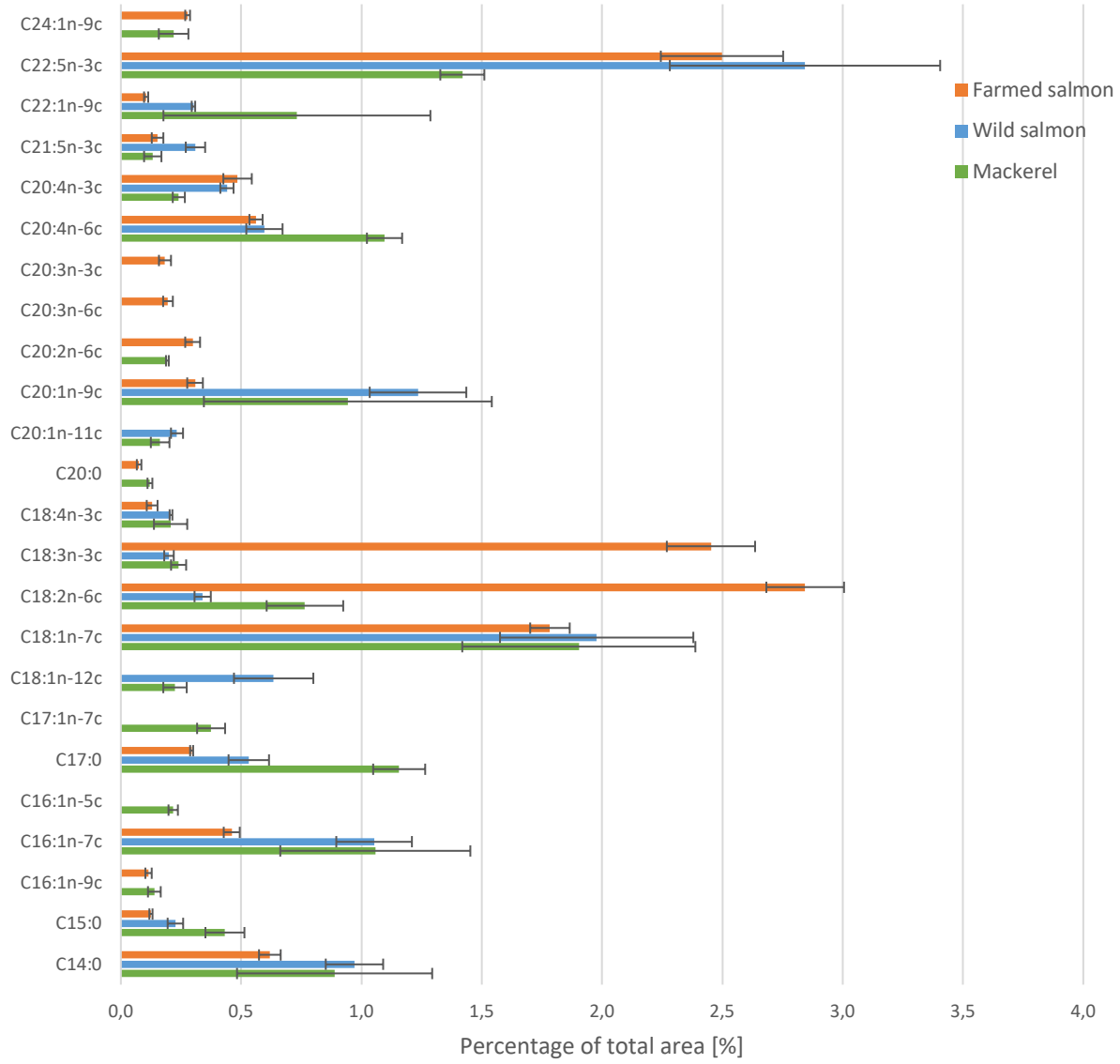


Figure 5.12: *Comparison of the PLs found in Atlantic mackerel (n = 3), wild Atlantic salmon (n = 3) and farmed Atlantic salmon (n = 3) displayed as percentages of the total area with standard deviations. PLs that constitute more than 5% of the total area have been excluded.*

DHA was the dominant peak in all three fishes, which alone constitute 37, 44, and 39% of the PLs in mackerel, wild and farmed salmon, respectively. C16:0, C18:1n-9c, C18:2n-6c, and EPA are the other dominant FAs amongst the PLs in farmed salmon, and together with DHA, make up 95% of the PLs in farmed salmon. C16:0, C18:0, C18:1n-9c, and EPA, together with DHA, make up as much as 85 and 87% of the PLs in mackerel and wild salmon, respectively.

As revealed in Table 5.5 and 5.6, the NL fractions closely resembled the FA profile and the proportions of SFAs, MUFAs, PUFAs, n-3- and n-6 FAs compared to the complete FA profiles found in their respective fish. This was due to the NLs displaying the largest proportions of the lipids. The FFA fractions were the richest in SFAs, whereas the PL fractions in PUFAs, and the NL fractions in MUFAs. Analogous to the complete FA profile, the FAs C16:0 and C18:0 constituted the majority of the total proportion of SFAs within each respective fraction for all three fishes, while the FAs OA, C20:1n-9c, and C22:1n-9c constituted the majority of the total proportions of MUFAs. The n-3 FAs EPA and DHA were the major constituents of the proportions of PUFAs within each fraction of both the wild salmon and mackerel. This was also the case for the PL fraction of the farmed salmon. However, the PUFAs LA and ALA constituted the major proportions within the NL and FFA fraction. The PL fraction was the richest in n-3 FAs (47, 57, and 54 % of the total peak area in mackerel, wild and farmed salmon, respectively). Our results show higher proportions of n-6 FAs in the NL and FFA fractions of the farmed salmon, where the n-6 FAs constituted 16 and 15 % of the NL and FFA fraction, respectively, while only 4 % in the PL fraction. This might be due to the lipid fraction of the feed primarily consisting of rapeseed oil, which is rich in n-6 FAs, and has been reported to comprise of 92 % NLs (Zaderimowski & Sosulski, 1978). In contrast, the n-6 FAs constituted approximately 2 % of the NL and FFA fraction, while 1 % in the PL fraction in the wild salmon. These results correspond with the findings of Halvorsen (2019). In mackerel, the n-6 FAs constituted approximately 3 % of the NL and FFA fraction, while 2 % in the PL fraction.

5.8. Nutritional quality indices of the lipids in fish

The n-6/n-3 ratio, AI and TI were calculated have been listed in Table 5.3. The farmed salmon and the feed displayed relatively similar n-6/n-3 ratios, with calculated ratios of 1.04/1 and 0.89/1 respectively. Thus, the feed displayed a slightly more beneficial composition of n-6 and n-3 FAs. This was considerably higher than that of wild salmon and mackerel, however, which was found to be 0.06/1 and 0.07/1, respectively. An n-6/n-3 ratio below 5/1 is considered beneficial for human health (Simopoulos, 2008; Yang et al., 2016). Given that the Western diets are rich in n-6 FAs and lack in n-3 FAs (Simopoulos, 2008), mackerel and wild salmon therefore displayed a more favourable n-6/n-3 ratio compared to farmed salmon.

The calculated AI value for farmed salmon was 0.19, which was significantly lower than that of wild salmon and mackerel (0.43 and 0.55, respectively). Relatively similar TI values were

observed amongst the salmons. The farmed salmon had a TI value of 0.18, whereas the wild salmon had a value of 0.22. The mackerel showed a higher TI value than the salmons of 0.29. High AI and TI values (> 1.0) have been reported to be detrimental to human health (Ouraji et al., 2009; Stancheva et al., 2014). The values in the present study were all lower than 1.0, which indicates that muscle tissue of the fishes in the present study is beneficial from a health perspective.

6. Conclusion and further work

The use of GC-MS for the analysis of derivatised FAs was found to yield satisfactory results for lipids extracted from the fishes. The LOD and LOQ, in full scan mode, was determined to be in the ng/mL and µg/mL range, respectively. A total of 37, 36, 35, and 34 FAs were found in respectively mackerel, wild salmon, farmed salmon, and salmon feed adding up to 39 unique FAs. Three IS, in conjunction with the determined RRF-values, allowed for the quantitation of all FAs present in mackerel, wild and farmed salmon, and salmon feed. The lipid content in fish muscle was found to be 3.1 ± 1.5%, 2.14 ± 0.32, and 8.97 ± 0.63% for mackerel, wild and farmed salmon, respectively. All three fishes were rich in both MUFAs and PUFAs, including the n-3 PUFAs EPA and DHA. The mackerel was especially rich in the latter. Both mackerel and farmed salmon contained roughly equal amounts of PUFAs relative to the total FA content, 31.6 and 29.6%, respectively, which was slightly higher than the amount found in wild salmon (26.3%). MUFAs constituted 35.3, 47.4, and 55.4% of the FA content in mackerel, wild and farmed salmon, respectively. The mackerel was richer in SFAs compared to both wild and farmed salmon (33.1, 26.3, and 15.0%, respectively). The FA profile of the salmon feed was strongly reflected in the farmed salmon. C18:1n-9c was the most dominant FA in the farmed salmon, accounting for as much as 44% of the total FAs content. The farmed salmon consisted of similar amounts of n-6 FAs and n-3 FAs. The proportions of the three fractions were respectively 73.0, 13.5, and 13.4% of total peak area in mackerel, while respectively 74.4, 20.1, and 5.5 % in wild salmon, and respectively 86.9, 6.1, and 7.0 % in farmed salmon. The nutritional quality of the three fishes were assessed by the contents of n-3 FAs in conjunction with the nutritional quality indices; AI, TI and the n-6/n-3 ratio. Purely based on the FA composition, all three types of fish displayed nutritionally beneficial profiles. However, the high contents of MUFAs and n-3 PUFAs relative to SFAs, in conjunction with the AI, TI, n-6/n-3 ratio, suggested that substituting farmed salmon with either wild salmon or mackerel might prove more nutritionally favourable.

It is also worth mentioning that an increased number of parallels of a larger sample size of fish can be analysed to obtain a more representative result. Several types of fish can also be analysed, such as Atlantic halibut (*Hippoglossus hippoglossus*) and Atlantic cod (*Gadus morhua*). It is possible to compare wild and farmed halibut, as well as wild and farmed cod with their feed to determine how reflective the FA composition is of the feed and compare it to the wild and farmed salmon. It could also prove interesting to look at the total fat content and

difference in the n-6/n-3 ratio across different species of fish. Additionally, the LOD and LOQ of the GC-MS could also be determined for all FAMEs in the Supelco 37 FAME mix in TIC, reconstructed ion chromatogram, and single ion monitoring acquisition modes. Other analytical parameters like linearity, sensitivity, accuracy, and repeatability could also be a subject for further testing.

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Paper I

Identification and quantification of lipids in wild Atlantic salmon, farmed Atlantic salmon (*Salmo salar*), and salmon feed by GC-MS

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Abstract:

The fatty acid (FA) profiles of wild and farmed salmon (*Salmo salar*), and salmon feed was elucidated and quantitated. Due to the increasing proportion of vegetable oils in salmon feed, it was of interest to evaluate the effects on the farmed salmon FA profile. There were found 36, 35, and 34 FAs in respectively wild salmon, farmed salmon, and salmon feed adding up to 39 unique FAs. There was a significant difference in the muscle lipid content of the muscles in farmed and wild salmon. The farmed salmon (8.97 ± 0.63 %) was clearly richer in lipid content than the wild salmon (2.14 ± 0.32 %). The contents of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs) in farmed Atlantic salmon were respectively 15.0, 55.4, and 29.6 %, respectively. In wild salmon the contents of SFAs, MUFAs, and PUFAs were respectively 26.3, 47.4, and 26.3 %. The fish lipids were fractioned into neutral lipids, free fatty acids, and polar lipids by off-line solid-phase extraction. Both wild salmon and farmed salmon contained approximately the same amount of the two major marine n-3 FAs eicosapentaenoic acid and docosahexaenoic acid with 520 and 523 mg/100 g fish muscle, respectively. The salmons were evaluated from a health perspective by discussing the contents of n-3 and n-6 FAs, SFAs, MUFAs, and PUFAs in both types together with nutritional quality indices. In conjunction with a significantly lower fat intake by consumption, the wild Atlantic salmon displayed the most nutritionally beneficial profile.

Keywords: Atlantic salmon, fatty acid composition, GC-MS, lipid content, n-3

Abbreviations: FA, fatty acid; **FFA**, free fatty acid; **FAME**, fatty acid methyl ester; **IS**, internal standard; **MUFA**, monounsaturated fatty acid**; NL**, neutral lipid; **PL**, polar lipid; **PUFA**, polyunsaturated fatty acid; **SFA**, saturated fatty acid

1. Introduction

The Atlantic salmon (*Salmo salar*) is a fish rich in lipids, in particular both eicosapentaenoic acid (EPA; C20:5n-3c) and docosahexaenoic acid (DHA; C22:6n-3c), and is one on the most important species in aquaculture in Europe, where Norway is the world's largest producer (Asche et al., 2020). However, there has been reported a decreased concentration of n-3 fatty acids (FAs) in farmed salmon compared to the level in previous years (Aas et al., 2019). Due to the scarcity and increasing price of marine oils, the feed that previously consisted of 90 % fish meal and fish oils have been reduced to 25 %, while the rest has been substituted with plant-based ingredients (Aas et al., 2019; Sprague et al., 2016). This substitution enabled a growth of 5.8 % per annum in aquaculture production without a considerable increase in fish meal and fish oil consumption (Hamilton et al., 2020). In recent years in Norway, the proportion of plant-based ingredients like plant oil and plant protein in the feed have increased. Recently, up to 2/3 of the lipid fraction in salmon feed is of rapeseed oil origin. In Norway today, the feed consists of 70 % plant-based ingredients as opposed to 60 % in 2012 (Aas et al., 2019; Mørkøre et al., 2014). In contrast, the diet of wild salmon is based on small fish and crustaceans. Hence the feed provided to farmed salmon differs from the natural diet (Renkawitz & Sheehan, 2011). This has ultimately altered the FA profile of farmed salmon and resulted in an approximate 50 % reduction in the proportion of n-3, and an increase in proportion of n-6 FAs (FAO, 2018; Sissener, 2018; Sprague et al., 2016). The FA composition in salmon fillets have been shown to reflect that of the feed, possibly due to their limited ability to elongate and desaturate FAs (Sissener, 2018; Torstensen et al., 2005). This decrease in n-3 FAs in fish feed can potentially have negative effects on both the fish health and the consumer (Rosenlund et al., 2016).

Throughout the years, many studies have been conducted to establish the importance of fatty acids (FAs) on human health. By far the most extensively studied are the n-3 long-chained polyunsaturated fatty acids (PUFAs), which play a key role in human growth and development (Simopoulos, 1991). EPA and DHA are known to exhibit key roles in membrane functions, immunology and inflammation, as well prostaglandin metabolism (Simopoulos, 1991). Several diseases and disorders have been linked to deficiencies of DHA and n-3 PUFAs. Namely, cardiovascular disease (CVD), attention deficit hyperactivity disorder, unipolar depression and cystic fibrosis, among others (Horrocks & Yeo, 1999). Although both EPA and DHA can be produced by humans, the rate of biosynthesis is low and insufficient, and they are recommended to be supplemented in the diet (Dewick, 2009).

The dietary intake ratio of n-6 to n-3 FAs has also been reported to be of significance in overall health (Liu et al., 2013; Riediger et al., 2008; Russo, 2009; Yang et al., 2016). Apart from the n-6/n-3 ratio, two other nutritional quality indices, the atherogenicity (AI) and thrombogenicity index (TI), are commonly employed to estimate of the nutritional value of PUFAs in human metabolism (Simopoulos, 2002; Ulbricht & Southgate, 1991). These indices are strongly associated with disease prevention and are claimed to promote health (Cherifi et al., 2018; Rhee et al., 2017; Simopoulos, 2002).

The main objective of this study was to determine and quantitate the FA levels in wild and farmed Atlantic salmon, with a focus on the saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), PUFAs, n-3 and n-6 FAs, as well as the nutritional quality indices; AI, TI, and the n-6/n-3 ratio. This is evaluated in the context of nutritional differences by consumption of these two products. Additionally, the FA profile of salmon feed was also of interest to compare the similarities between the FA composition of farmed salmon and its feed.

2. Materials and methods

2.1 Chemicals and standards

The chloroform used for internal standard (IS) and lipid extraction from the fish muscle samples, was supplied by VWR Chemicals and was of Chromanorm quality (France). The methanol, used in conjunction with chloroform for the extraction procedure and to make the sodium methoxide solution, was supplied by Sigma Aldrich and was of Chromasolv quality (Poland). The methylation of the lipids into FAMEs was performed by using a 14 % boron-trifluoride-methanol solution supplied by Sigma-Aldrich (Switzerland). Heptane ≥99 % n-heptane basis (GC) was supplied by Acros Organics (Belgium). The solution used to elute FFAs by off-line solid-phase extraction (SPE) contained acetic acid and diethyl ether. The acetic acid 99.9 % puriss. p.a was supplied by VWR Chemicals (France) and the diethyl ether puriss. p.a. ≥99.8 % was supplied by Sigma Aldrich (Poland).

A total of three different IS; nonadecanoic acid (C19:0 FFA), trinonadecanoin (C19:0 TAG) and 1,2-Dinonadecanoyl-sn-Glycero-3-phosphatidylcholine (C19:0 PL), all supplied by Larodan AB (Malmö, Sweden), were chosen for quantitation of the FAMEs. The TAG IS stock solution was prepared by dissolving 200 mg of standard with 20 mL of chloroform to a final concentration of 10 mg/mL. Both the FFA and PL IS were prepared for two concentrations, 10 and 1 mg/mL. This was done by separately dissolving 20 mg of standard with 2 and 20 mL of chloroform, respectively. All IS stock solutions were transferred to GC vials, sealed, and stored in darkness at -20 °C until use.

A fatty acid methyl ester (FAME) mix containing 37 different components was used for the identification of FAMEs resulting from the derivatisation of FAs from the Atlantic salmon. The 37 Component FAME Mix was supplied by Supelco (Schnelldorf, Germany) and had a

total concentration of 10 mg/mL. For further identification, 12-methyl-tetradecanoate, 13-methyl-tetradecanoate, *cis*-7-hexadecenoic acid methyl ester, *cis*-11-hexadecenoic acid methyl ester, all-*cis*-9,12-hexadecadienoic acid methyl ester, *cis*-6-octadecenoic acid methyl ester, *cis*-11-octadecenoic acid methyl ester, *cis*-13-octadecenoic acid methyl ester, all-*cis*-6,9,12,15-octadecatetraenoic acid methyl ester, *cis*-9-eicosenoic acid methyl ester, all-*cis*-8,11,14,17-eicosatetraenoic acid methyl ester, all-*cis*-6,9,12,15,18-Heneicosapentaenoic acid methyl ester, and all-*cis*-7,10,13,16,19-docosapentaenoic acid methyl ester were all purchased from Larodan AB (Malmö, Sweden).

2.2 Samples and sample preparation

The farmed Atlantic salmons (n = 3) purchased fresh from "Son brygge og fiskebutikk", in Son, Norway. Both the farmed salmon and the feed came from Vikenco AS located in Aukra (62°50'45"N, 6°46'34"E), Norway. The feed was of the type "Rapid HF 1000 HQ 50A" and was produced on November 17th by EWOS AS, Scotland. The wild salmons (n = 3) were acquired from Finnmarkfisk AS and were caught with salmon traps in Namsenfjorden (64°27'22"N, 11°30'09"E), outside of Namsen, Norway. The wild salmons were frozen fresh at -20 ℃ since June 2019.

The farmed salmons were filleted, deboned and deskinned. The subcutaneous fat was removed so only the fish muscle remained. Figure 1 shows a diagram of the muscles in both a salmon fillet (a) and cutlet (b). From the farmed salmon, both red and white muscles were sampled from all over the fillet as indicated by the blue rectangles in Figure 1a. The flesh was cut into smaller pieces and homogenised using a stave mixer. This was done separately for each fish. The resulting muscle mass was stored in blue-capped tubes in darkness at -20 ℃. The wild salmons came in the form as cutlets, but the same procedure for acquiring the muscle mass was

used, however, one half of every cutlet in their respective packs were sampled as indicated in Figure 1b. The feed was delivered as pellets. The pellets were grinded into a homogenous mixture using a mortar. To keep the feed as fresh as possible, the pellets were grinded prior to the lipid extraction.

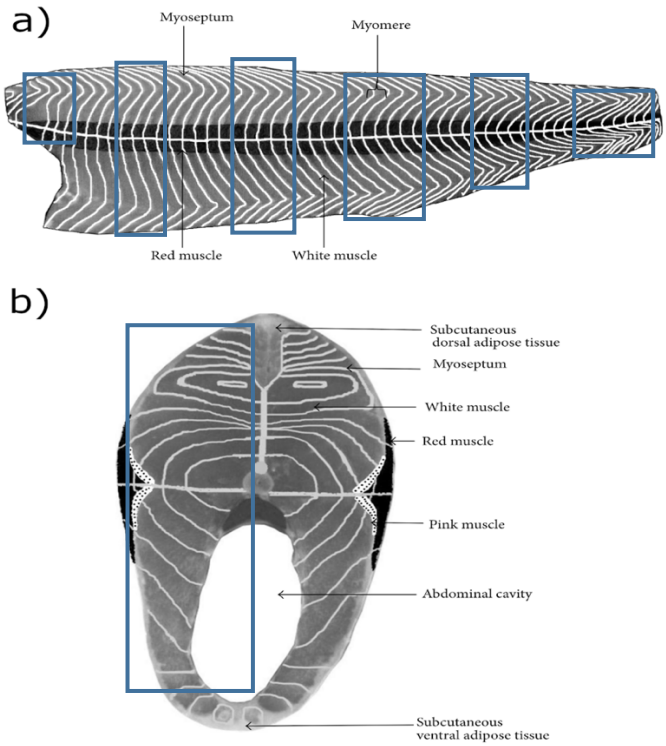

Figure 1: *A diagram of: (a) salmon fillet in longitudinal section presenting the W-shape of myomere and the two muscle types, and (b) the cross section of a salmon cutlet. The blue rectangles indicate where the samples were sampled. Adapted from Listrat et al. (2016).*

2.3 Lipid extraction procedure for determining lipid content

The lipids were extracted following Folch's method (1957). In brief, three grams of homogenous muscle mass were transferred to 100 mL Erlenmeyer flasks, and added 60 mL of a 2:1 chloroform:methanol (v/v) solution. Lids were placed on top of the beakers, with subsequent shaking on an orbital shaker (Biosan PSU-10i, Riga, Latvia) at 390 rpm for 30 minutes. The contents of the Erlenmeyer flasks were transferred to separatory funnels and

added 12 mL of a 0.9 % NaCl in Milli-Q water solution. Chloroform was used to wash the flasks for any lipid residues. The separatory funnels were shaken vigorously until satisfactory separation of the two phases was achieved, and the lower organic phase was transferred to 120 mL Büchi reagent tubes. Two additional liquid-liquid extractions were carried out with 10 mL chloroform and collected in the same reagent tubes. The collected organic phase was dried using a vacuum evaporator system (Büchi, Syncore® Polyvap equipped with a V-700 vacuum pump and a V-855 vacuum controller) at 40 ℃, 100 rpm, and an air pressure at 207 mbar. When most of the solvent had evaporated, the content was transferred to pre-weighed culture tubes (DURAN®, GL14). The complete removal of solvent was carried out by inserting the tubes in heating blocks at 40 ℃ under pure nitrogen. The dry residues were weighed to calculate the total lipid content of the fish.

2.4 Lipid extraction and methylation

Different volumes of C19:0 TAG IS were added in two series to allow quantitation of the compounds in the chromatogram. The added volumes for the 1st series were 200 µL and 100 µL for farmed and wild salmon, respectively, while in the 2nd series were 50 µL and 10 µL for farmed and wild salmon, respectively. The salmon feed shared the same added volumes as the farmed salmon.

To a 50 mL screw cap tube (Greiner Bio-One, Cellstar® Tubes), 0.5 g muscle mass was added in two series. IS and 10 mL of a 2:1 chloroform:methanol (v/v) solution was added and shaken at 390 rpm for 20 minutes using an orbital shaker. Then, 2 mL of a 0.9 % NaCl in Milli-Q water solution was added and shaken using a vortex mixer (IKA®-Werke, Yellowstone TTS-2). The two phases were then separated by centrifugation (Beckman CoulterTM, AvantiTM J-25 equipped with a JA-12 fixed-angle rotor), 5 minutes at 2000 rpm. The upper aqueous phases

were discarded, and the organic phases were transferred to test tubes. The samples were heated to 40 ℃ under N_2-gas flow until dryness. The complete removal of solvent was carried out by inserting the tubes in heating blocks at 40 ℃ under pure nitrogen. The dry residues were resolved in 1 mL of n-heptane and transferred to culture tubes. A sodium methoxide solution was prepared by dissolving metallic sodium, supplied by Merck (Darmstadt, Germany), in methanol to a final concentration of 5 mg/mL. To each of the culture tubes, 1 mL of the sodium methoxide solution were added, followed by horizontal shaking using an orbital shaker at 390 rpm for 30 minutes. 1 mL of 14 % boron-trifluoride-methanol solution was added to each of the culture tubes and heated in a water bath at 80 ℃ for 20 minutes. The tubes were cooled to room temperature and the two phases were separated by centrifugation (Hettich®, EBA 20) for 5 minutes at 2000 rpm. The upper heptane phase was transferred to GC vials and diluted with n-heptane. The wild salmon samples were diluted 1:10, the farmed salmon samples were diluted 1:50 and the salmon feed samples were diluted 1:100. The samples were stored in darkness at -20 ℃ until analysis with GC-MS.

2.5 Solid-Phase Extraction and methylation

The lipids were extracted in two series, following the same procedure as section 2.4, however, three IS were added. The added volumes for the 1st series were 200 and 100 μL of C19:0 TAG, 15 and 10 μL of C19:0 FFA (10 mg/mL) and 50 and 25 μL of C19:0 PL (10 mg/mL), for farmed and wild salmon, respectively. The added volumes for the 2nd series were 20 and 10 μL of C19:0 TAG, 15 and 10 μL of C19:0 FFA (1 mg/mL) and 50 and 25 μL of C19:0 PL (1 mg/mL), for farmed and wild salmon, respectively. Furthermore, the dry extracted lipids were resolved in 1 mL of chloroform and transferred to GC vials. Blank samples of pure chloroform were also prepared. The samples were stored in darkness at -20 ℃ until fractioning.

The method using SPE for lipid fractionation was based on the previous works of Pinkart et al. (1998) and Ruiz et al. (2004), and was carried out using a GX-274 ASPEC™ (Gilson, Middleton, WI, USA), and the accompanying software TRILUTION® LH Software v.3.0 (Gilson, Middleton, WI, USA). Two different columns were used as the stationary phase for the different series. Discovery DSC-NH_2 500 mg and 3 mL columns (Sigma Aldrich, USA) were used in the 1st series, while Bond-Elut NH_2 500 mg and 3 mL columns (Agilent Technologies, USA) were used in the 2nd series. The columns were conditioned using 7.5 mL heptane and a flow rate of 1.0 mL/min, prior to the application of the samples (500 µL). The neutral lipids (NLs) were eluted into glass vials using 5.0 mL chloroform, the free fatty acids (FFAs) using 5.0 mL of a 98:2 diethyl ether:acetic acid (v/v) solution, and the polar lipids (PLs) using 5.0 mL of methanol. The contents of the glass vials were transferred to culture tubes. Chloroform was used to wash the glass vials for any lipid residues.

Blanks samples were prepared and analysed for both column types. In both columns, the FFA fraction showed a contribution of C14:0, C16:0 and C18:0. To account for this, the mean areas of the contributions were subtracted from their respective counterparts in the FFA samples.

The complete removal of solvent was carried out by inserting the tubes containing the three fractions and blanks in heating blocks at 40 °C under pure nitrogen until dryness. The methylation procedure followed the same procedure as section 2.4, with some modifications. The dry residues of the NL and PL fractions were resolved in 2 mL n-heptane, added 1.5 mL of sodium methoxide (5.0 mg/mL), and horizontally shaken at 390 rpm for 30 minutes using an orbital shaker. To separate the two phases the tubes were left in vertical position for 30 minutes. The dry residues of the FFA fraction were added 1 mL of 14 % boron-trifluoride-methanol solution and heated for 5 minutes at 80 °C in a water bath. The tubes were cooled to

237 room temperature, added 2 mL n-heptane and shaken by a vortex mixer. The tubes were left in
238 vertical position for 15 minutes. The upper heptane phases of all fractions were transferred to
239 GC vials, and stored in darkness at -20 ℃ until analysis with GC-MS. The NL fractions of
240 farmed salmon were diluted 1:10 with n-heptane.
241
242 **2.6 Analysis of FAMEs by GC-MS**
243 An ISQ^{TM} QD GC-MS (Thermo Fisher Scientific, Waltham, MA, USA) was used to identify
244 the FAMEs in the samples. The MS was a single quadrupole. Electron ionisation was used as
245 the ionisation method (70 eV electrons), and a mass range of m/z 50 – 600 was chosen. Both
246 the ion source and transfer line were kept at a temperature of 250 °C. Full-scan acquisition
247 mode was utilised.
248
249 The GC used in combination with the MS was a TRACE^{TM} 1310 (Thermo Fisher Scientific,
250 Waltham, MA, USA), equipped with a 60 m Rtx®-2330 column with an inner diameter of 0.25
251 mm and a 0.2 µm film thickness of fused silica biscyanopropyl cyanopropylphenyl
252 polysiloxane stationary phase (Restek, Bellefonte, PA, USA). To inject the sample, an AI/AS
253 1310 Series Autosampler was utilised (Thermo Fisher Scientific, Waltham, MA, USA),
254 injecting 1.0 µL at a split ratio of 1:10 into an injection chamber set to 250 °C, using helium as
255 a carrier gas (99.9 %, AGA, Norway) at a constant flow of 1.0 mL/min. The total run time was
256 set to 110 minutes, with the initial GC oven temperature set to 50 °C for 5 minutes, before
257 increasing, at a rate of 100 °C/min, to 140 °C and held for 30 minutes. The temperature was
258 increased to 145 °C, at a rate of 10 °C/min and held for 30 minutes, before increased further,
259 at a rate of 3 °C/min, to 175 °C and held for an additional 20 minutes. Finally, at a rate of
260 50 °C/min, the temperature was held at 260 °C for 10 minutes.
261

For the identification and quantitation of the complete FA profiles, a single injection of each diluted quadruplicates was subjected to analysis by GC-MS. A single injection of n-heptane was carried out in-between samples replicates of different fish. For the samples prepared using off-line SPE, undiluted quadruplicates were made for each of the following fractions: NLs, FFAs, and PLs for both kinds of salmons, with the exception of the NL fractions of the farmed salmon which was diluted. Undiluted quadruplicates were also prepared for the three fractions of the blank samples. A single injection was carried out for each sample replicate, with one injection of heptane in-between samples replicates of different fish. The software used for the GC-MS analysis was Chromeleon v7.2.8 (Thermo Fisher Scientific, Waltham, MA, USA). To aid in the identification of FAMEs, NIST 08 Mass Spectral Library (Gaithersburg, MD, USA) was used in conjunction with the retention times of the independent standards as well as the standards present in Supelco 37 Component FAME Mix.

2.7 Nutritional quality indices of the lipids

To estimate the nutritional quality of the lipids, two separate indices were to be calculated in addition to the n-6/n-3 ratio. The AI and TI were calculated by using the empirical equations, equation 1 and 2, respectively, according to Ulbricht and Southgate (1991).

(Eq. 1) $$AI = \frac{[C12{:}0 + (4{*}C14{:}0) + C16{:}0]}{(\sum MUFAs + \sum n{-}6 + \sum n{-}3)}$$

(Eq. 2) $$TI = \frac{[C14{:}0 + C16{:}0 + C18{:}0]}{[(0.5{*}\sum MUFAs){+}(0.5{*}\sum n{-}6 {+}(3{*}\sum n{-}3) {+}\left(\frac{\sum n{-}3}{\sum n{-}6}\right))]}$$

3. Results and discussion

3.1 Lipid content

The average lipid content of the farmed salmon muscle (wet weight) was four times that of the wild salmon (8.97 ± 0.63 % and 2.14 ± 0.32 %, respectively). These results confirm the observations of Jensen et al. (2012) and Lundbye et al. (2017) that the lipid content is significantly higher in farmed salmon. However, both Jensen et al. (2012) and Lundebye et al. (2017), reported average lipid content in the range of 6 – 8 % and 12 – 14 % for wild and farmed salmon, respectively, thus significantly higher values than our results. Apart from the biological factors and individual differences, this is believed to originate from differences in sampling methods. Both Jensen et al., (2012) and Lundebye et al., (2017) sampled the salmon following the Norwegian Quality Cut, where only the flesh cut between the dorsal and adipose fin, and down to the gut is sampled. Furthermore, the subcutaneous fat is not removed. Our study focused on determining the lipid content in fish muscle and deemed it appropriate to remove the subcutaneous fat and sample cuts from all of the fish to get a representative muscle sample. Additionally, the wild salmon had been frozen since June 2019 and albeit being frozen fresh and stored in the freezer, some of the FAs may have been oxidised, or otherwise decomposed (Dawson et al., 2018). Furthermore, the fat cells might break due to freezing resulting in loss of some acylglycerides from the muscles. The results could thus have been better comparable if both farmed salmon and wild salmon had been fresh. However, most commercially available fish products have been frozen at some point, so these results might offer the most relevant picture for the nutritional values. Based on the present study, and assuming that a dinner portion of fish fillet is 200 g, one would receive 4.3 g of fat from wild salmon and 17.9 g of fat from farmed salmon. Thus, consuming farmed salmon results in a significantly higher fat intake.

3.2 FA profile of wild and farmed Atlantic salmon and salmon feed

The FA composition of the muscles of wild and farmed salmon together with the composite values for the feed given to the farmed salmon are provided in Table 1. A total of 36, 35, and 34 FAs were found in respectively wild salmon, farmed salmon, and salmon feed adding up to 39 unique FAs, where C12:0 being the shortest while C24:1n-9c was the longest FA. All the unsaturated FAs found exhibited a cis configuration. The FA composition is mainly reflected by the FA composition of the feed (Jensen et al., 2012). As the feeding regime is widely different for the farmed and wild salmon, it was expected to be reflected in the FA profiles. Compared to the wild salmon, four FAs in particular stand out in the FA profile of the farmed salmon. C16:0, oleic acid (OA; C18:1n-9c), linoleic acid (LA; C18:2n-6c), and alpha-linolenic acid (ALA; C18:3n-3c), are present in relatively high concentrations (482 – 3,756 mg/100g fish muscle) and together accounted for 73 % of the FAs in farmed salmon. In the wild counterpart, these four FAs exist at significantly lower concentrations and constitute only 36 % of the total FA content. ALA is the precursor to both EPA and DHA, and, along with LA, make up the essential fatty acids (EFAs) which needs to be incorporated in the diet (Dewick, 2009). In total, these two EFAs make up 19.5 % of the FA content in farmed salmon, whereas only 1.5 % in wild salmon. OA, LA and ALA are found in greater concentrations in farmed salmon compared to the wild counterpart.

A monounsaturated n-9 FA, OA, was the dominant peak found in farmed salmon and its feed and represents as much as respectively 44 and 41 % of the FA content, respectively. This corresponds well with previously published literature, which also report elevated contents of OA in farmed salmon in accordance with the increased amount of plant-based ingredients in the feed (Friesen et al. 2015; Sprague et al. 2016). The intake of OA has been associated with potential beneficial effects in patients suffering from type II diabetes (Vassiliou et al., 2009).

335 Furthermore, LA and, to a lesser extent, C16:0, and ALA are present in large quantities in both
336 farmed salmon and the feed. OA, LA, and ALA are most commonly found in plant sources,
337 and together with C16:0 they are the main constituents in rapeseed oil (Sahrafi et al., 2015).
338 Rapeseed oil is one of the main ingredients in salmon feed in Norway today (Aas et al., 2019).
339 Additionally, the feed contained greater proportions of EPA compared to farmed salmon (3.0
340 and 2.2 % respectively) and lower proportions of DHA (3.4 and 3.9 %, respectively).
341
342 As a direct consequence of the higher lipid content of the farmed salmon, it displayed higher
343 concentrations of most FAs. However, similar concentrations of both EPA (167 and 188
344 mg/100g fish muscle, respectively) and DHA (353 and 335 mg/100g fish muscle, respectively)
345 were found in wild and farmed salmon. Albeit similar concentrations, the proportions of these
346 n-3 FAs were three times higher in wild salmon (6 and 13 % of the FA content, respectively)
347 compared to the farmed salmon (2 and 4 % of the FA content, respectively). The main peaks
348 of wild salmon were C16:0, C18:1n-9c, C20:1n-9c, C22:1n-9c, and DHA, which accounted for
349 64.5 % of the total lipid content. These results correspond with a study by Olsen et al. (2013)
350 who reported that these five FAs accounted for 65 % of the FA content in wild salmon.
351
352 Erucic acid (C22:1n-9c), which has been associated with a health risk to children under the age
353 of 10, was found at roughly twice the concentration in the wild salmon compared to the farmed
354 salmon (241.5 and 124.8 mg/100g fish muscle, respectively). The European Food Safety
355 Authority (EFSA) issued a report in 2016 recommending a dietary limit of 7 mg/kg body
356 weight per day (Knutsen et al., 2016). This means that a child of 25 kg has a recommended
357 limit of 175 mg erucic acid per day. By consuming 100 g of the fish subjected to testing one
358 would receive 242 mg and 125 mg from wild and farmed salmon, respectively. Thus,
359 consuming wild Atlantic salmon would significantly exceed than the recommended daily limit.

3.3 Comparison SFAs, MUFAs, and PUFAs in Atlantic salmon

The SFAs, MUFAs, and PUFAs are associated with different effects on human health. Contrary to SFAs, MUFAs and especially PUFAs are believed to have positive effects on human health, and recommendations for substituting SFAs with MUFAs and PUFAs are well established. An overwhelming amount of studies have been conducted linking the substitution of SFAs with MUFAs and PUFAs to a decreased risk of CVD (Hooper et al., 2015; Kris-Etherton & Krauss, 2020; Siri-Tarino et al., 2015). Even this is a debated topic, and newer research indicated no significant association between intake of SFAs and CVDs (Krauss & Kris-Etherton, 2020; Zhu et al., 2019).

The wild salmon was found to be the richest in SFAs. The SFAs constitute 26.3 % of the total lipid content found wild salmon, while only 15.0 % for farmed salmon. However, due to the higher total lipid content of the farmed salmon, it displayed a much higher concentration of SFAs (1,278 mg/100g fish muscle), compared to wild salmon (718 mg/100g fish muscle). The MUFAs compose the largest proportions in both wild and farmed salmon (47.4 and 55.4 %, respectively). As expected, due to the higher lipid content of the farmed salmon, it displayed a much higher concentration of MUFAs (4,725 mg/100g fish muscle), compared to wild salmon (1,293 mg/100g fish muscle). Relatively similar proportions of PUFAs was observed in both wild and farmed salmon (26.3 and 29.6 %, respectively). Furthermore, the FAs C16:0 and C18:0 constituted the majority of the total SFA content for both fish, while the FAs OA, C20:1n-9c, and C22:1n-9c were present in major quantities of the total MUFA content. The n-3 FAs EPA and DHA constituted the majority of the total PUFA content in wild salmon, however, LA and ALA were the major constituents of the total PUFA content in the farmed salmon.

3.4 Comparison of n-6 and n-3 FAs in Atlantic salmon

The n-3 and n-6 FAs exhibits different biological effects. The n-6 FAs have a tendency of being pro-inflammatory, whereas the n-3 FAs, like EPA and DHA, inhibits inflammation (Simopoulos, 2008). As a result of the higher lipid content, the farmed salmon comprised of higher concentrations of both n-3 and n-6 FAs compared to wild salmon. However, the wild and farmed salmon displayed similar proportions of n-3 and n-6 FAs (26.1 and 29.5 %, respectively of the lipid content). Whereas the wild salmon comprised of more n-3 than n-6 FAs (24.7 and 1.4 %, respectively), the opposite was found in farmed salmon where slightly more n-6 than n-3 FAs (15.0 and 14.5 %, respectively) was observed. The proportion of n-6 FAs were ten times higher in farmed salmon compared to the wild salmon and are believed to be a result of the feed composition.

Judging by our results, consuming 200 g of fish fillets would provide 2,470 mg of n-3 and 2,562 mg of n-6 FAs from farmed salmon, and 1,346 mg n-3 and 75 mg n-6 from wild salmon. Due to their benefits to human health the marine n-3 FAs EPA and DHA are of particular interest and in 2012, EFSA set a dietary recommendation of these marine n-3 FAs of 250 mg/day, or 1.75 g/week (EFSA Panel on Dietetic Products & Allergies 2012). By eating a dinner portion (200 g) of fish fillets would provide 1,040 mg, and 1,045 mg EPA and DHA from wild and farmed salmon, respectively. Thus, only 48 g of wild and farmed salmon would be necessary to satisfy the recommended daily intake of EPA and DHA and eating salmon twice a week would satisfy the recommended weekly intake. Furthermore, the consumption of wild salmon would yield approximately equal amounts of EPA and DHA compared to the farmed salmon, however, at a lower energy intake due to the lower lipid content.

3.5 The fish lipid fractions

The lipids were fractioned into NLs, FFAs, and PLs and the identified FAs from each fraction is presented as percentages of the total area (area %) in Table 2. The proportions of SFA, MUFA, PUFA, and n-3 and n-6 FAs found in the different fractions of the fish are also provided. The NLs, comprising the triacylglycerides, were by far the most abundant in both wild and farmed salmon composing a total of respectively 74.4 and 86.9 % of the lipids. The PLs constituted the lowest proportions of the lipids in wild salmon (5.5 %), whereas the second lowest in farmed salmon (7.0 %). As the phospholipids play a key role in cell membranes, the PLs were anticipated to constitute a small fraction of the lipids. The FFAs, however, constituted a total of 20.1 and 6.1 % of the lipids in wild and farmed salmon, respectively.

Our reported proportion of NLs in farmed salmon was comparable to a study by Tsoupras et al. (2018) that reported a proportion of NLs of 85 %. Additionally, our results correspond with a study by Bell et al. (1998) reported that wild and farmed salmon contained respectively 72 and 89 % NLs. Halvorsen (2019) reported that the NL fractions constituted 83 and 97 % of the lipids found in wild and farmed salmon, respectively, which are significantly higher than the results in the present study. However, unlike the present study, the subcutaneous fat was sampled. The proportions of PLs found in Atlantic salmon varies highly in literature from 2 – 16 % (Halvorsen, 2019; Tsoupras et al., 2018; Tsoupras et al., 2019). It has been reported that FFAs constitute only 1 % of the lipids found in farmed salmon (Halvorsen, 2019; Ruiz-Lopez et al., 2015), while 8 % in wild salmon (Halvorsen, 2019). However, the proportions of FFAs found were significantly higher, especially in wild salmon (20.1 and 6.1 % for wild and farmed salmon, respectively). The reason for this might be that the wild salmon was not frozen quick enough after capture to prevent the lipases in the muscles to initiate decomposition. Thus, some of the FAs from NLs might have cleaved from the glycerol backbone turning into FFAs by

lipid hydrolysis (Shewfelt, 1981). It is also worth mentioning that in literature the salmon might have been sampled at different periods of its life cycle, which would influence the results.

As revealed in Table 2, the NL fractions closely resembled the FA profile and the proportions of SFAs, MUFAs, PUFAs, n-3- and n-6 FAs compared to the complete FA profiles found in their respective fish. This was due to the NLs displaying the largest proportions of the lipids. The FFA fractions were the richest in SFAs, whereas the PL fractions in PUFAs, and the NL fractions in MUFAs. Analogous to the complete FA profile, the FAs C16:0 and C18:0 constituted the majority of the total proportion of SFAs within each respective fraction for both wild and farmed salmon, while the FAs OA, C20:1n-9c, and C22:1n-9c constituted the majority of the total proportions of MUFAs. The n-3 FAs EPA and DHA were the major constituents of the proportions of PUFAs within each fraction of the wild salmon. This was also the case for the PL fraction of the farmed salmon. However, the PUFAs LA and ALA constituted the major proportions within the NL and FFA fraction. DHA alone constituted 44 and 39 % of the total area of the PL fractions of wild and farmed salmon, respectively. The PL fraction was the richest in n-3 FAs (57 and 54 % of the total peak area in wild and farmed salmon, respectively). Our results show higher proportions of n-6 FAs in the NL and FFA fractions of the farmed salmon, where the n-6 FAs constituted 16 and 15 % of the NL and FFA fraction, respectively, while only 4 % in the PL fraction. This might be due to the lipid fraction of the feed primarily consisting of rapeseed oil, which is rich in n-6 FAs, and has been reported to comprise of 92 % triacylglycerides (Zaderimowski & Sosulski, 1978). In contrast, the n-6 FAs constituted approximately 2 % of the NL and FFA fraction, and 1 % of the PL fraction in the wild salmon. These results correspond with the findings of Halvorsen (2019).

3.6 Nutritional quality indices of the lipids

The n-6/n-3 ratio, AI, and TI were calculated and are listed in Table 1. The n-6/n-3 ratio of the modern Western diets have been estimated to be 15 – 17/1 (Simopoulos, 2008). A high imbalance in the n-6/n-3 ratio has been linked to many chronic diseases, including coronary heart disease and CVD (Simopoulos, 2008). However, the importance of this ratio is debated, and the FAO does not give any specific recommendations (FAO, 2010). For years, nutritionists have emphasised adding fish rich in n-3 FAs to the Western diets, with the purpose of obtaining a more optimal n-6/n-3 ratio (Simopoulos, 2002). The n-6/n-3 ratio of the farmed salmon was calculated to be 1.04/1, which corresponds well with the findings of Aas et al. (2019) that reported values of approximately 1/1. However, this was considerably higher than that of wild salmon (0.06/1). The higher ratio of the farmed salmon reflects the increased use of vegetable oils in salmon feed, which had a ratio of 0.89/1. An n-6/n-3 ratio below 5/1 is considered beneficial for human health (Simopoulos, 2002; Yang et al., 2016). Thus, consumption of both farmed and wild salmon could contribute to reduce the n-6/n-3 ratio. Assuming that the Western diets are rich in n-6 FAs, wild salmon therefore displayed a more beneficial n-6/n-3 ratio.

The calculated AI value for farmed salmon 0.19, which was significantly lower than that of wild salmon (0.43). Relatively similar TI values were observed amongst the salmons. The farmed salmon had a TI value of 0.18, whereas the wild salmon had a value of 0.22. High AI and TI values (> 1.0) have been reported to be detrimental to human health (Ouraji et al., 2009; Stancheva et al., 2014). The values in the present study were all lower than 1, which indicates that muscle tissue of both wild and farmed salmon is beneficial from a health perspective.

4. Conclusions

The results presented in this study highlighted the quantitative diversity of FAs for wild and farmed Atlantic salmon. Significant differences between the lipid contents of wild and farmed salmon were observed (2.14 and 8.97 % of fish muscle, respectively). As a result of the feeding regime, farmed salmon were richer in MUFAs (55.4 %) and PUFAs (29.6 %) than the wild counterpart (47.4 and 26.3 % for MUFAs and PUFAs, respectively) and contained significantly higher amounts of the EFAs C18:2n-6c (13.8 %) and C18:3n-3c (5.6 %) as well as the MUFA C18:1n-9c (44.0 %). Furthermore, farmed salmon were far richer in n-6 FAs (15.0 %). In contrast, wild salmon was richer in SFAs (26.3%) and n-3 FAs (24.7 %). Additionally, the content of the marine n-3 FAs EPA and DHA were almost identical in the wild and farmed salmon (520 and 523 mg/100g fish muscle, respectively). The proportions of the three fractions were respectively 74.4, 20.1, and 5.5 % of total peak area in wild salmon, while respectively 86.9, 6.1, and 7.0 % in farmed salmon. The high contents of MUFAs and n-3 PUFAs relative to SFAs, along with favourable n-6/n-3 ratios, and AI and TI values suggest that both the wild and farmed Atlantic salmon display nutritionally beneficial profiles. However, wild salmon displayed the most beneficial of the two. Furthermore, consuming wild Atlantic salmon would yield a significantly lower total fat intake, thus suggesting a substitution from farmed to wild Atlantic salmon may prove nutritionally favourable.

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509 **Table 1.** Fatty acid composition (% of total FAs) and amount of FA (mg per 100g of muscle (wet weight)) in farmed (n = 3) and wild (n = 3) Atlantic salmon and salmon feed.

Fatty acids	Wild salmon		Farmed salmon		Feed	
	Composition [%]	Amount [mg/100 g]	Composition [%]	Amount [mg/100 g]	Composition [%]	Amount [mg/100 g]
C12:0	0.05 ± 0.01	1.38 ± 0.15	n.d.[b)]	n.d.	n.d.	n.d.
C13:0 (4,8,12-trimethyl) [a)]	0.063 ± 0.004	1.71 ± 0.12	n.d.	n.d.	n.d.	n.d.
C14:0	3.43 ± 0.49	93.7 ± 13.2	1.71 ± 0.42	145.5 ± 35.0	2.18 ± 0.09	653.1 ± 26.2
C14:0 (13-methyl)	0.14 ± 0.02	3.71 ± 0.53	0.03 ± 0.01	2.71 ± 0.62	0.07 ± 0.01	19.53 ± 1.69
C14:0 (12-methyl)	0.09 ± 0.01	2.33 ± 0.35	0.018 ± 0.004	1.57 ± 0.33	0.027 ± 0.002	8.04 ± 0.50
C15:0	0.30 ± 0.05	8.13 ± 1.42	0.09 ± 0.02	7.94 ± 1.75	0.19 ± 0.01	56.11 ± 3.32
C16:0	17.43 ± 2.18	475.7 ± 59.5	9.61 ± 2.22	819 ±189	10.32 ± 0.30	3,097.4 ± 91.5
C17:0	0.43 ± 0.05	11.67 ± 1.27	0.18 ± 0.04	15.44 ± 3.77	0.34 ± 0.03	101.22 ± 8.77
C18:0	4.31 ± 0.56	117.7 ± 15.4	2.94 ± 0.75	250.4 ± 64.1	4.37 ± 0.13	1,312 ± 38.9
C20:0	0.08 ± 0.01	2.22 ± 0.29	0.29 ± 0.08	24.54 ± 6.68	0.49 ± 0.02	146.34 ± 7.20
C22:0	n.d.	n.d.	0.09 ± 0.01	7.81 ± 0.69	0.23 ± 0.02	70.27 ± 4.88
C24:0	n.d.	n.d.	0.035 ± 0.005	2.95 ± 0.42	0.119 ± 0.004	35.71 ± 1.10
∑ SFAs	**26.32 ± 3.38**	**718.2 ± 92.3**	**14.98 ± 3.55**	**1,278 ± 303**	**18.33 ± 0.61**	**5,500 ± 184**
C16:1n-9c	0.13 ± 0.03	3.66 ± 0.83	0.11 ± 0.03	9.63 ± 2.73	0.09 ± 0.01	25.77 ± 2.45
C16:1n-7c	6.39 ± 1.26	174.5 ± 34.5	2.57 ± 0.66	219.1 ± 56.1	3.12 ± 0.11	935.8 ± 32.2
C16:1n-5c	0.19 ± 0.03	5.14 ± 0.73	n.d.	n.d.	n.d.	n.d.
C17:1n-7c	0.26 ± 0.05	6.95 ± 1.25	0.08 ± 0.02	6.78 ± 2.02	0.09 ± 0.01	27.50 ± 2.23
C18:1n-12c	0.78 ± 0.27	21.32 ± 7.26	0.12 ± 0.06	10.25 ± 4.71	n.d.	n.d.
C18:1n-9c	17.14 ± 2.24	467.7 ± 61.0	44.0 ± 11.1	3,756 ± 943	41.42 ± 1.15	12,427 ± 345
C18:1n-7c	3.86 ± 0.19	105.46 ± 5.12	3.00 ± 0.80	256.3 ± 68.6	2.96 ± 0.09	887.6 ± 26.8
C18:1n-5c	0.22 ± 0.03	6.12 ± 0.75	n.d.	n.d.	n.d.	n.d.
C20:1n-11c	0.79 ± 0.14	21.54 ± 3.89	0.14 ± 0.06	11.88 ± 4.81	0.13 ± 0.01	37.60 ± 2.67
C20:1n-9c	8.05 ± 1.76	219 ± 48.1	3.43 ± 1.13	292.3 ± 93.6	1.95 ± 0.06	583.9 ± 17.3
C20:1n-7c [a)]	0.23 ± 0.09	6.31 ± 2.28	0.09 ± 0.02	7.47 ± 2.00	0.09 ± 0.01	26.48 ± 1.54
C22:1n-9c	8.85 ± 2.24	241.5 ± 61.1	1.46 ± 0.73	124.8 ± 61.9	1.17 ± 0.05	352.5 ± 14.0
C24:1n-9c	0.50 ± 0.04	13.50 ± 1.05	0.36 ± 0.13	30.3 ± 11.2	0.21 ± 0.01	61.57 ± 1.87
∑ MUFAs	**47.40 ± 8.36**	**1,293 ± 228**	**55.4 ± 14.7**	**4,725 ± 1,254**	**51.22 ± 1.49**	**15,366 ± 446**

C16:2n-4c	0.24 ± 0.08	6.62 ± 2.17	0.14 ± 0.04	11.58 ± 3.06	0.28 ± 0.02	83.11 ± 5.77
C18:2n-6c (LA)	0.84 ± 0.14	22.94 ± 3.90	13.83 ± 3.33	1179 ± 284	13.86 ± 0.39	4157 ± 117
C18:3n-6c	n.d.	n.d.	0.06 ± 0.02	5.54 ± 1.99	0.053 ± 0.004	16.04 ± 1.32
C18:3n-3c (ALA)	0.64 ± 0.09	17.51 ± 2.34	5.66 ± 1.10	482.9 ± 94.2	7.99 ± 0.23	2,396.5 ± 70.5
C18:4n-3c	0.86 ± 0.03	23.58 ± 0.93	0.43 ± 0.10	36.29 ± 8.55	0.56 ± 0.04	167.5 ± 11.7
C20:2n-6c	0.21 ± 0.06	5.71 ± 1.63	0.84 ± 0.22	71.5 ± 19.1	0.09 ± 0.01	26.17 ± 2.38
C20:3n-6c	0.06 ± 0.02	1.54 ± 0.43	0.16 ± 0.04	14.00 ± 3.59	0.044 ± 0.004	62.00 ± 1.06
C20:3n-3c	0.15 ± 0.04	3.98 ± 1.15	0.34 ± 0.07	29.27 ± 6.20	0.036 ± 0.001	10.67 ± 0.29
C20:4n-6c	0.26 ± 0.05	7.17 ± 1.34	0.13 ± 0.03	10.75 ± 2.50	0.21 ± 0.02	10.01 ± 5.05
C20:4n-3c	1.10 ± 0.17	29.93 ± 4.56	0.59 ± 0.18	50.7 ± 15.2	0.22 ± 0.01	66.29 ± 3.92
C20:5n-3c (EPA)	6.11 ± 1.13	166.8 ± 30.8	2.19 ± 0.43	186.7 ± 36.3	3.01 ± 0.10	903.8 ± 30.2
C21:5n-3c	0.30 ± 0.08	8.24 ± 2.25	0.22 ± 0.05	18.68 ± 4.29	0.18 ± 0.02	55.20 ± 4.85
C22:5n-3c	2.57 ± 0.15	70.18 ± 4.08	1.11 ± 0.24	94.7 ± 20.3	0.54 ± 0.02	160 ± 6.71
C22:6n-3c (DHA)	12.94 ± 3.26	353.2 ± 88.9	3.94 ± 0.81	335.8 ± 68.7	3.39 ± 0.10	1,016.1 ± 30.5
∑ **PUFAs**	**26.29 ± 5.30**	**717 ± 145**	**29.63 ± 6.66**	**2,524 ± 568**	**30.45 ± 0.97**	**9,134 ± 292**
Total		**2,729**		**8,531**		**30,000**
∑ n-6	1.37 ± 0.27	37.36 ± 7.30	15.02 ± 3.65	1,281 ± 311	14.25 ± 0.42	4,275 ± 127
∑ n-3	24.68 ± 4.95	673 ± 135	14.48 ± 2.98	1,234 ± 254	15.92 ± 0.53	4,777 ± 159
n-6/n-3	0.06		1.04		0.89	
AI	0.43		0.19		0.23	
TI	0.22		0.18		0.21	

Values are expressed as mean ± standard deviation. a) The FA is not confirmed by a standard, only by NIST library search. b) n.d. – not detected.

516 **Table 2.** Comparison of the FA composition (area %) of the lipid fractions (NLs, FFAs, and PLs) in wild (n = 3) and farmed (n = 3) Atlantic salmon given as percentages of the total peak area.

	Wild salmon Composition [%]			Farmed salmon Composition [%]		
Fatty acid	NL	FFA	PL	NL	FFA	PL
C12:0	0.071 ± 0.001	0.09 ± 0.01	n.d. [b)]	n.d.	n.d.	n.d.
C13:0 (4,8,12-trimethyl) [a)]	0.09 ± 0.02	n.d.	n.d.	n.d.	n.d.	n.d.
C14:0	4.28 ± 0.15	3.70 ± 0.31	0.97 ± 0.12	2.05 ± 0.03	2.88 ± 0.06	0.62 ± 0.05
C14:0 (13-methyl)	0.19 ± 0.01	0.15 ± 0.02	n.d.	0.05 ± 0.00	n.d.	n.d.
C14:0 (12-methyl)	0.13 ± 0.01	0.10 ± 0.01	n.d.	0.03 ± 0.01	n.d.	n.d.
C15:0	0.42 ± 0.02	0.34 ± 0.02	0.23 ± 0.03	0.140 ± 0.003	0.26 ± 0.01	0.13 ± 0.01
C16:0	18.08 ± 1.00	22.96 ± 0.83	22.86 ± 1.82	9.49 ± 0.09	19.14 ± 0.53	20.62 ± 0.89
C17:0	0.57 ± 0.05	0.36 ± 0.05	0.53 ± 0.08	0.21 ± 0.01	0.32 ± 0.01	0.29 ± 0.01
C18:0	3.67 ± 0.18	5.43 ± 0.23	4.01 ± 0.40	2.59 ± 0.07	7.55 ± 0.23	1.57 ± 0.06
C20:0	0.10 ± 0.01	0.10 ± 0.01	n.d.	0.283 ± 0.004	0.220 ± 0.005	0.08 ± 0.01
C22:0	n.d.	n.d.	n.d.	0.08 ± 0.01	n.d.	n.d.
C24:0	n.d.	n.d.	n.d.	0.07 ± 0.01	n.d.	n.d.
∑ SFAs	**27.62 ± 1.45**	**33.21 ± 1.49**	**28.60 ± 2.46**	**15.00 ± 0.22**	**30.37 ± 0.85**	**23.30 ± 1.02**
C16:1n-9c	0.15 ± 0.02	0.13 ± 0.02	n.d.	0.137 ± 0.004	0.13 ± 0.01	0.12 ± 0.01
C16:1n-7c	6.22 ± 0.23	4.43 ± 0.47	1.05 ± 0.16	2.32 ± 0.04	2.06 ± 0.03	0.46 ± 0.03
C16:1n-5c	0.27 ± 0.01	0.30 ± 0.02	n.d.	n.d.	n.d.	n.d.
C17:1n-7c	0.24 ± 0.01	0.22 ± 0.02	n.d.	0.098 ± 0.004	n.d.	n.d.
C18:1n-12c	0.86 ± 0.29	0.92 ± 0.14	0.64 ± 0.16	0.17 ± 0.03	n.d.	n.d.
C18:1n-9c	17.76 ± 1.27	11.52 ± 0.47	7.42 ± 1.04	44.65 ± 0.45	28.34 ± 0.42	11.04 ± 0.79
C18:1n-7c	4.14 ± 0.97	3.44 ± 0.50	1.98 ± 0.40	3.04 ± 0.05	2.68 ± 0.03	1.78 ± 0.08
C18:1n-5c	0.28 ± 0.03	0.27 ± 0.03	n.d.	n.d.	n.d.	n.d.
C20:1n-11c	0.89 ± 0.10	0.48 ± 0.06	0.23 ± 0.03	0.19 ± 0.03	n.d.	n.d.
C20:1n-9c	7.54 ± 0.72	3.85 ± 0.20	1.24 ± 0.20	3.24 ± 0.25	1.67 ± 0.10	0.31 ± 0.03
C20:1n-7c [a)]	0.26 ± 0.06	0.15 ± 0.03	n.d.	0.11 ± 0.01	n.d.	n.d.
C22:1n-9c	8.07 ± 1.20	3.27 ± 0.20	0.30 ± 0.01	1.26 ± 0.29	0.46 ± 0.08	0.11 ± 0.01
C24:1n-9c	0.60 ± 0.10	0.29 ± 0.07	n.d.	0.07 ± 0.01	0.26 ± 0.01	0.28 ± 0.01
∑ MUFAs	**47.29 ± 4.99**	**29.27 ± 2.21**	**12.86 ± 1.99**	**55.27 ± 1.15**	**35.60 ± 0.69**	**14.09 ± 0.97**
C16:2n-4c	0.27 ± 0.05	0.18 ± 0.02	n.d.	0.17 ± 0.01	0.20 ± 0.03	n.d.

C18:2n-6c (LA)	1.08 ± 0.04	0.77 ± 0.03	0.34 ± 0.03	14.40 ± 0.12	13.88 ± 0.26	2.84 ± 0.16
C18:3n-6c	n.d.	n.d.	n.d.	0.08 ± 0.01	n.d.	n.d.
C18:3n-3c (ALA)	0.88 ± 0.06	0.67 ± 0.02	0.20 ± 0.02	6.23 ± 0.31	7.13 ± 0.41	2.45 ± 0.18
C18:4n-3c	1.23 ± 0.18	0.79 ± 0.14	0.21 ± 0.01	0.49 ± 0.04	0.43 ± 0.03	0.13 ± 0.02
C20:2n-6c	0.24 ± 0.03	0.15 ± 0.02	n.d.	0.774 ± 0.003	0.61 ± 0.01	0.30 ± 0.03
C20:3n-6c	0.06 ± 0.01	0.05 ± 0.01	n.d.	0.18 ± 0.02	0.15 ± 0.02	0.20 ± 0.02
C20:3n-3c	0.19 ± 0.02	0.14 ± 0.02	n.d.	0.34 ± 0.01	0.31 ± 0.01	0.18 ± 0.03
C20:4n-6c	0.29 ± 0.04	0.55 ± 0.03	0.60 ± 0.08	0.13 ± 0.01	0.16 ± 0.00	0.56 ± 0.03
C20:4n-3c	1.33 ± 0.15	1.05 ± 0.06	0.44 ± 0.03	0.56 ± 0.05	0.55 ± 0.04	0.49 ± 0.06
C20:5n-3c (EPA)	6.32 ± 0.23	10.72 ± 0.66	8.15 ± 0.58	2.08 ± 0.06	4.30 ± 0.12	8.90 ± 0.24
C21:5n-3c	0.38 ± 0.03	0.24 ± 0.02	0.31 ± 0.04	0.04 ± 0.02	0.116 ± 0.004	0.15 ± 0.02
C22:5n-3c	2.67 ± 0.30	2.39 ± 0.31	2.84 ± 0.56	1.05 ± 0.04	0.66 ± 0.05	2.50 ± 0.25
C22:6n-3c (DHA)	10.08 ± 0.95	19.81 ± 1.70	44.44 ± 1.78	2.61 ± 0.22	3.88 ± 0.24	39.46 ± 0.81
∑ PUFAs	**25.00 ± 2.19**	**37.49 ± 3.05**	**57.53 ± 3.13**	**29.15 ± 0.92**	**32.38 ± 1.23**	**58.16 ± 1.85**
∑ n-3	23.06 ± 2.02	35.79 ± 2.93	56.60 ± 3.02	13.40 ± 0.75	17.37 ± 0.91	54.26 ± 1.61
∑ n-6	1.67 ± 0.11	1.52 ± 0.09	0.94 ± 0.11	15.57 ± 0.16	14.81 ± 0.29	3.90 ± 0.24

Values are expressed as mean ± standard deviation. a) The FA is not confirmed by a standard, only by NIST library search. b) n.d. – not detected.

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Appendices

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Appendix I: Internal standards

Table A.1: The internal standards utilised for the quantitation of the complete fatty acid profiles of Atlantic mackerel, wild and farmed Atlantic salmon and fish feed. The triacylglyceride of C19:0 were used.

Internal standard		Molecular weight [g/mole]	Concentration [mg/mL]	IS used [mL]	Amount IS [mg]	Moles IS	Moles FAs
C19:0 TAG	Mackerel	933.60	10	0.10	1.0	$1.07 * 10^{-6}$	$3.21 * 10^{-6}$
C19:0 TAG		933.60	10	0.02	0.2	$2.14 * 10^{-7}$	$6.43 * 10^{-7}$
C19:0 TAG	Farmed	933.60	10	0.20	2.0	$2.14 * 10^{-6}$	$6.43 * 10^{-6}$
C19:0 TAG	salmon	933.60	10	0.05	0.5	$5.35 * 10^{-7}$	$1.61 * 10^{-6}$
C19:0 TAG	Wild salmon	933.60	10	0.10	1.0	$1.07 * 10^{-6}$	$3.21 * 10^{-6}$
C19:0 TAG		933.60	10	0.02	0.2	$2.14 * 10^{-7}$	$6.43 * 10^{-7}$
C19:0 TAG	Fish feed	933.60	10	0.20	2.0	$2.14 * 10^{-6}$	$6.43 * 10^{-6}$
C19:0 TAG		933.60	10	0.05	0.5	$5.35 * 10^{-7}$	$1.61 * 10^{-6}$

Table A.2: The internal standards utilised for the quantitation of the NLs, FFAs, and PLs of wild Atlantic salmon and Atlantic mackerel.

Internal standard	Molecular weight [g/mole]	Concentration [mg/mL]	IS used [mL]	Amount IS [mg]	Moles IS	Moles FAs
C19:0 NL	933.60	10	0.100	1.00	$1.07 * 10^{-6}$	$3.21 * 10^{-6}$
C19:0 NL	933.60	10	0.010	0.100	$1.07 * 10^{-7}$	$3.21 * 10^{-7}$
C19:0 FFA	298.52	10	0.010	0.10	$3.35 * 10^{-7}$	$3.35 * 10^{-7}$
C19:0 FFA	298.52	1	0.010	0.010	$3.35 * 10^{-8}$	$3.35 * 10^{-8}$
C19:0 PL	818.20	10	0.025	0.25	$3.06 * 10^{-7}$	$6.11 * 10^{-7}$
C19:0 PL	818.20	1	0.025	0.025	$3.06 * 10^{-8}$	$6.11 * 10^{-8}$

Table A.3: The internal standards utilised for the quantitation of the NLs, FFAs, and PLs of farmed Atlantic salmon.

Internal standard	Molecular weight [g/mole]	Concentration [mg/mL]	IS used [mL]	Amount IS [mg]	Moles IS	Moles FAs
C19:0 NL	933.60	10	0.200	2.00	$2.14 * 10^{-6}$	$6.43 * 10^{-6}$
C19:0 NL	933.60	10	0.020	0.200	$2.14 * 10^{-7}$	$6.43 * 10^{-7}$
C19:0 FFA	298.52	10	0.015	0.15	$5.02 * 10^{-7}$	$5.02 * 10^{-7}$
C19:0 FFA	298.52	1	0.015	0.015	$5.02 * 10^{-8}$	$5.02 * 10^{-8}$
C19:0 PL	818.20	10	0.050	0.50	$6.11 * 10^{-7}$	$1.22 * 10^{-6}$
C19:0 PL	818.20	1	0.050	0.050	$6.11 * 10^{-8}$	$1.22 * 10^{-7}$

Appendix II: Reference standards

Table A.4: The FAME components of the Supelco 37 Component FAME mix used as reference standards for FAMEs from the mackerel, farmed and wild salmon, and the feed. Weight% of each component in the FAME mix is also given.

FAME	Name	Weight% [c)]
C4:0	Butanoic acid, ME [d)]	4
C6:0	Hexanoic acid, ME	4
C8:0	Octanoic acid, ME	4
C10:0	Decanoic acid, ME	4
C11:0	Undecanoic acid, ME	2
C12:0	Dodecanoic acid, ME	4
C13:0	Tridecanoic acid, ME	2
C14:0	Tetradecanoic acid, ME	4
C14:1n-5c	*cis*-9-Tetradecenoic acid, ME	2
C15:0	Pentadecanoic acid, ME	2
C15:1n-5c	*cis*-10-Pentadecenoic acid, ME	2
C16:0	Hexadecanoic acid, ME	6
C16:1n-7c	*cis*-9-Hexadecenoic acid, ME	2
C17:0	Heptadecanoic acid, ME	2
C17:1n-7c	*cis*-10-Heptadecenoic acid, ME	2
C18:0	Octadecanoic acid, ME	4
C18:1n-9tr	*trans*-9-Octadecenoic acid, ME	2
C18:1n-9c	*cis*-9-Octadecenoic acid, ME	4
C18:2n-6tr	all-*trans*-9,12-Octadecadienoic acid, ME	2
C18:2n-6c	all-*cis*-9,12-Octadecadienoic acid, ME	2
C18:3n-6c	all-*cis*-6,9,12-Octadecatrienoic acid, ME	4
C18:3n-3c	all-*cis*-9,12,15-Octadecatrienoic acid, ME	2
C20:0	Eicosanoic acid, ME	2
C20:1n-9c	*cis*-11-Eicosenoic acid, ME	2
C20:2n-6c	all-*cis*-11,14-Eicosadienoic acid, ME	2
C20:3n-6c	all-*cis*-8,11,14-Eicosatrienoic acid, ME	4
C20:3n-3c	all-*cis*-11,14,17-Eicosatrienoic acid, ME	2
C20:4n-6c	all-*cis*-5,8,11,14-Eicosatetraenoic acid, ME	2
C20:5n-3c	all-*cis*-5,8,11,14,17-Eicosapentaenoic acid, ME	2
C21:0	Heneicosanoic acid, ME	2
C22:0	Docosanoic acid, ME	2
C22:1n-9c	*cis*-13-Docosenoic acid, ME	2
C22:2n-6c	*cis*-13,16-Docasadienoic acid, ME	4
C22:6n-3c	all-*cis*-4,7,10,13,16,19-Docosahexaenoic acid, ME	2
C23:0	Tricosanoic acid, ME	2
C24:0	Tetracosanoic acid, ME	2
C24:1n-9c	*cis*-15-Tetracosenoic acid, ME	2

c) Weight% of the individual FAMEs in the Supelco 37 Component FAME mix. d) ME – methyl ester

Table A.5: FAMEs used as reference standards for the FAMEs found in Atlantic mackerel, farmed and wild Atlantic salmon, and salmon feed, not present in the Supelco 37 Component FAME mix

FAME	Name
C7:0	Heptanoic acid, ME
C9:0	Nonanoic acid, ME
C14:0 (12-methyl)	12-Methyltetradecanoic acid, ME
C14:0 (13-methyl)	13-Methyltetradecanoic acid, ME
C16:1n-9c	*cis*-7-Hexadecenoic acid, ME
C16:1n-5c	*cis*-11-Hexadecenoic acid, ME
C16:2n-4c	all-*cis*-9,12-Hexadecadienoic acid, ME
C18:1n-12c	*cis*-6-Octadecenoic acid, ME
C18:1n-7c	*cis*-11-Octadecenoic acid, ME
C18:1n-5c	*cis*-13-Octadecenoic acid, ME
C18:4n-3c	all-*cis*-6,9,12,15-Octadecatetraenoic acid, ME
C19:0	Nonadecanoic acid, ME
C20:1n-11c	*cis*-9-Eicosenoic acid, ME
C20:4n-3c	all-*cis*-8,11,14,17-Eicosatetraenoic acid, ME
C21:5n-3c	all-*cis*-6,9,12,15,18-Heneicosapentaenoic acid, ME
C22:5n-3c	all-*cis*-7,10,13,16,19-Docosapentaenoic acid, ME

Appendix III: Limit of detection and limit of quantitation

Table A.21: LOD and LOQ-values for the four selected FAMEs using full scan mode.

FAME	Full scan	
	LOD [ng/mL]	LOQ [µg/mL]
C10:0	37.1	0.14
C18:0	495.2	1.33
C18:1n-9c	578.5	0.99
C20:0	866.5	1.95

Appendix IV: RRF-values

Table A.6: RRF-values with associated molecular weight for the respective FAMEs in the Supelco 37 Component FAME mix with individually added C7:0, C9:0, and C19:0 FAMEs. The RRF-values obtained by the different set of personnel are given as S1 and S2.

FAME	Molecular weight [g/mol]	RRF-value S1	RRF-value S2	Mean RRF-value
C4:0	102.13	0.69	0.33	0.51
C6:0	130.18	0.66	0.56	0.61
C7:0 e)	144.21	0.72	0.58	0.65
C8:0	158.24	0.94	0.79	0.87
C9:0 e)	172.26	0.97	0.78	0.88
C10:0	186.29	1.06	0.94	1.00
C11:0	200.32	1.00	1.00	1.00
C12:0	214.34	1.16	1.07	1.11
C13:0	228.37	1.07	1.11	1.09
C14:0	242.40	1.19	1.16	1.17
C14:1n-5c	240.38	1.04	1.10	1.07
C15:0	256.42	1.07	1.16	1.12
C15:1n-5c	254.40	0.96	1.10	1.03
C16:0	270.50	1.10	1.07	1.09
C16:1n-7c	268.48	0.86	0.92	0.89
C17:0	284.53	0.92	1.00	0.96
C17:1n-7c	282.51	0.88	0.94	0.91
C18:0	298.55	1.00	1.02	1.01
C18:1n-9tr	296.53	0.88	0.91	0.90
C18:1n-9c	296.53	1.04	1.03	1.04
C18:2n-6tr	294.52	0.87	0.87	0.87
C18:2n-6c	294.52	1.15	0.99	1.07
C18:3n-6c	292.50	0.99	0.93	0.96
C18:3n-3c	292.50	1.15	1.02	1.09
C19:0 e)	312.58	1.00	1.00	1.00
C20:0	326.60	1.15	1.06	1.11
C20:1n-9c	324.58	1.00	1.00	1.00
C20:2n-6c	322.57	1.02	0.99	1.00
C20:3n-6c	320.55	0.96	0.91	0.94
C20:3n-3c	320.55	1.15	0.97	1.06
C20:4n-6c	318.53	1.26	1.01	1.13
C20:5n-3c	316.52	1.29	1.08	1.19
C21:0	340.63	0.97	0.96	0.97
C22:0	354.66	1.04	0.97	1.01
C22:1n-9c	352.64	1.03	0.94	0.98
C22:2n-6c	350.63	1.37	1.09	1.23
C22:6n-3c	342.56	1.24	0.99	1.11
C23:0	368.68	1.07	0.92	1.00
C24:0	382.71	1.26	1.02	1.14
C24:1n-9c	380.69	1.24	1.05	1.15

e) Individually added.

Table A.7: The RRF-values and molecular weight for the FAMEs found in the mackerel, wild and farmed salmon, and feed.

FAME	Molecular weight [g/mol]	RRF-value
C12:0	214.34	1.11
C13:0 (4,8,12-trimethyl) [f)]	270.50	1.17
C14:0	242.40	1.09
C14:0 (13-methyl) [f)]	256.42	1.12
C14:0 (12-methyl) [f)]	256.42	1.12
C15:0	256.42	1.12
C16:0	270.50	1.09
C16:1n-9c [f)]	268.48	0.89
C16:1n-7c	268.48	0.89
C16:1n-5c [f)]	268.48	0.89
C16:2n-4c [f)]	266.47	0.89
C17:0	284.53	0.96
C17:1n-7c	282.51	0.91
C18:0	298.55	1.01
C18:1n-12c [f)]	296.53	1.04
C18:1n-9c	296.53	1.04
C18:1n-7c [f)]	296.53	1.04
C18:1n-5c [f)]	296.53	1.04
C18:2n-6c	294.52	1.07
C18:3n-6c	292.50	0.96
C18:3n-3c	293.50	1.09
C18:4n-3c [f)]	290.48	1.09
C20:0	326.60	1.11
C20:1n-11c [f)]	324.58	1.00
C20:1n-9c	324.58	1.00
C20:1n-7c [f)]	324.58	1.00
C20:2n-6c	322.57	1.00
C20:3n-6c	320.55	0.94
C20:3n-3c	320.55	1.06
C20:4n-6c	318.53	1.13
C20:4n-3c [f)]	318.53	1.08
C20:5n-3c	316.52	1.15
C21:5n-3c [f)]	330.55	1.15
C22:0	354.66	1.01
C22:1n-9c	352.64	0.98
C22:5n-3c [f)]	344.58	1.11
C22:6n-3c	342.56	1.11
C24:0	382.71	1.14
C24:1n-9c	380.69	1.15

f) Manually assigned RRF-value

Appendix V: Complete FA profile of Atlantic mackerel

Table A.8: Overview of the FAs found in Atlantic mackerel (n = 3). The FAs are listed with their associated retention times, match factors and concentrations with standard deviations. The match factor measures how well the spectrum match with the one found in the library (max value: 999). The FAs in the table were confirmed with reference standards. The concentration is given in mg FA/100 g fish muscle.

Fatty acid	Retention time [min]	Match factor	Concentration Mean ± SD [mg/100g]
C12:0	14.65	905	1.10 ± 0.43
C13:0 (4,8,12-trimethyl) [a)]	20.72	887	2.78 ± 2.58
C14:0	20.48	950	161.0 ± 76.1
C14:0 (13-methyl)	22.78	906	4.64 ± 3.76
C14:0 (12-methyl)	23.65	817	1.76 ± 1.53
C15:0	25.38	946	12.7 ± 9.03
C16:0	32.56	954	801 ± 317
C16:1n-9c	36.39	899	5.46 ± 3.79
C16:1n-7c	37.16	950	161.7 ± 81.3
C16:1n-5c	38.27	877	4.32 ± 3.48
C16:2n-4c	45.43	813	3.71 ± 2.68
C17:0	41.28	750	30.6 ± 21.2
C17:1n-7c	46.22	859	7.75 ± 3.97
C18:0	52.74	961	279 ± 111
C18:1n-12c	57.78	840	5.56 ± 5.24
C18:1n-9c	58.74	947	467 ± 202
C18:1n-7c	60.02	946	142.4 ± 68.6
C18:1n-5c	62.35	848	4.66+ 3.76
C18:2n-6c	70.22	938	66.7 ± 28.7
C18:3n-3c	77.42	931	20.9 ± 18.6
C18:4n-3c	81.15	949	85.5 ± 51.8
C20:0	77.06	925	5.03 ± 2.60
C20:1n-11c	79.34	897	13.9 ± 13.4
C20:1n-9c	79.73	948	211 ± 135
C20:1n-7c [a)]	80.47	886	6.05 ± 2.83
C20:2n-6c	85.32	895	8.10 ± 5.61
C20:3n-6c	89.56	692	0.89 ± 0.61
C20:3n-3c	93.26	849	4.03 ± 2.80
C20:4n-6c	92.94	908	8.19 ± 3.65
C20:4n-3c	97.39	937	13.0 ± 11.3
C20:5n-3c	98.12	952	269 ± 127
C21:5n-3c	99.60	926	6.60 ± 5.36
C22:0	92.47	796	2.05 ± 0.74
C22:1n-9c	95.78	950	347 ± 235
C22:5n-3c	100.65	946	22.5 ±12.6
C22:6n-3c	101.05	958	735 ± 332
C24:1n-9c	99.78	952	14.6 ± 12.4

a) Not confirmed by a standard, only by NIST library search.

Appendix VI: Complete FA profile of wild Atlantic salmon

Table A.9: Overview of the FAs found in wild Atlantic salmon (n = 3). The FAs are listed with their associated retention times, match factors and concentrations with standard deviations. The match factor measures how well the spectrum match with the one found in the library (max value: 999). The FAs in the table were confirmed with reference standards. The concentration is given in mg FA/100 g fish muscle.

Fatty acid	Retention time [min]	Match factor	Concentration Mean ± SD [mg/100g]
C12:0	14.68	890	1.38 ± 0.15
C13:0 (4,8,12-trimethyl) [a)]	20.79	811	1.71 ± 0.12
C14:0	20.55	947	93.7 ± 13.2
C14:0 (13-methyl)	22.86	877	3.71 ± 0.53
C14:0 (12-methyl)	23.74	761	2.33 ± 0.35
C15:0	25.48	933	8.13 ± 1.42
C16:0	32.78	946	475.7 ± 59.5
C16:1n-9c	36.55	879	3.66 ± 0.83
C16:1n-7c	37.34	952	174.5 ± 34.5
C16:1n-5c	38.41	898	5.14 ± 0.73
C16:2n-4c	45.63	839	6.62 ± 2.17
C17:0	41.43	722	11.67 ± 1.27
C17:1n-7c	46.40	841	6.95 ± 1.25
C18:0	53.05	963	117.7 ± 15.4
C18:1n-12c	58.14	917	21.32 ± 7.26
C18:1n-9c	59.23	942	467.7 ± 61.0
C18:1n-7c	60.39	949	105.46 ± 5.12
C18:1n-5c	62.69	865	6.12 ± 0.75
C18:2n-6c	70.48	919	22.94 ± 3.90
C18:3n-3c	77.58	919	17.51 ± 2.34
C18:4n-3c	81.33	928	23.58 ± 0.93
C20:0	77.23	880	2.22 ± 0.29
C20:1n-11c	79.54	901	21.54 ± 3.89
C20:1n-9c	79.99	948	219 ± 48.1
C20:1n-7c [a)]	80.67	885	6.31 ± 2.28
C20:2n-6c	85.55	853	5.71 ± 1.63
C20:3n-6c	89.80	684	1.54 ± 0.43
C20:3n-3c	93.53	805	3.98 ± 1.15
C20:4n-6c	93.21	892	7.17 ± 1.34
C20:4n-3c	97.46	944	29.93 ± 4.56
C20:5n-3c	98.17	958	166.8 ± 30.8
C21:5n-3c	99.65	909	8.24 ± 2.25
C22:1n-9c	96.24	941	241.5 ± 61.1
C22:5n-3c	100.69	941	70.18 ± 4.08
C22:6n-3c	101.10	958	353.2 ± 88.9
C24:1n-9c	99.82	939	13.50 ± 1.05

a) Not confirmed by a standard, only NIST library search.

Appendix VII: Complete FA profile of farmed Atlantic salmon

Table A.10: Overview of the FAs found in farmed Atlantic salmon (n = 3). The FAs are listed with their associated retention times, match factors and concentrations with standard deviations. The match factor measures how well the spectrum match with the one found in the library (max value: 999). The FAs in the table were confirmed with reference standards. The concentration is given in mg FA/100 g fish muscle.

Fatty acid	Retention time [min]	Match factor	Concentration Mean ± SD [mg/100g]
C14:0	20.61	949	145.5 ± 35.0
C14:0 (13-methyl)	22.94	674	2.71 ± 0.62
C14:0 (12-methyl)	23.83	530	1.57 ± 0.33
C15:0	25.59	851	7.94 ± 1.75
C16:0	32.75	951	819 ±189
C16:1n-9c	36.69	795	9.63 ± 2.73
C16:1n-7c	37.43	942	219.1 ± 56.1
C16:2n-4c	45.83	780	11.58 ± 3.06
C17:0	41.61	730	15.44 ± 3.77
C17:1n-7c	46.62	653	6.78 ± 2.02
C18:0	53.17	943	250.4 ± 64.1
C18:1n-12c	58.39	659	10.25 ± 4.71
C18:1n-9c	59.54	950	3,756 ± 943
C18:1n-7c	60.65	928	256.3 ± 68.6
C18:2n-6c	70.76	946	1179 ± 284
C18:3n-6c	75.21	729	5.54 ± 1.99
C18:3n-3c	77.75	937	482.9 ± 94.2
C18:4n-3c	81.50	900	36.29 ± 8.55
C20:0	77.31	891	24.54 ± 6.68
C20:1n-11c	79.64	804	11.88 ± 4.81
C20:1n-9c	80.03	944	292.3 ± 93.6
C20:1n-7c [a)]	80.79	784	7.47 ± 2.00
C20:2n-6c	85.72	893	71.5 ± 19.1
C20:3n-6c	90.04	776	14.00 ± 3.59
C20:3n-3c	93.78	839	29.27 ± 6.20
C20:4n-6c	93.47	784	10.75 ± 2.50
C20:4n-3c	97.52	929	50.7 ± 15.2
C20:5n-3c	98.20	959	186.7 ± 36.3
C21:5n-3c	99.69	851	18.68 ± 4.29
C22:0	92.82	708	7.81 ± 0.69
C22:1n-9c	96.18	908	124.8 ± 61.9
C22:5n-3c	100.74	928	94.7 ± 20.3
C22:6n-3c	101.13	957	335.8 ± 68.7
C24:0	99.35	803	2.95 ± 0.42
C24:1n-9c	99.84	897	30.3 ± 11.2

a) Not confirmed by a standard, only NIST library search.

Appendix VIII: Complete FA profile of salmon feed

Table A.11: Overview of the FAs found in salmon feed. The FAs are listed with their associated retention times, match factors and concentrations with standard deviations. The match factor measures how well the spectrum match with the one found in the library (max value: 999). The FAs in the table were confirmed with reference standards. The concentration is given in mg FA/100 g feed.

Fatty acid	Retention time [min]	Match factor	Concentration Mean ± SD [mg/100g]
C14:0	20.61	957	653.1 ± 26.2
C14:0 (13-methyl)	22.92	807	19.53 ± 1.69
C14:0 (12-methyl)	23.82	667	8.04 ± 0.50
C15:0	25.56	929	56.11 ± 3.32
C16:0	32.86	947	3,097.4 ± 91.5
C16:1c7	36.65	790	25.77 ± 2.45
C16:1c9	37.45	953	935.8 ± 32.2
C16:2c9,12	45.80	839	83.11 ± 5.77
C17:0	41.58	791	101.22 ± 8.77
C17:1c10	46.57	740	27.50 ± 2.23
C18:0	53.49	964	1,312 ± 38.9
C18:1n-9c	60.00	953	12,427 ± 345
C18:1n-7c	60.89	927	887.6 ± 26.8
C18:2n-6c	70.91	950	4,157 ± 117
C18:3n-6c	75.23	699	16.04 ± 1.32
C18:3n-3c	77.84	946	2,396.5 ± 70.5
C18:4n-3c	81.53	916	167.5 ± 11.7
C20:0	77.40	939	146.34 ± 7.20
C20:1n-11c	79.70	809	37.60 ± 2.67
C20:1n-9c	80.09	947	583.9 ± 17.3
C20:1n-7c [a)]	80.83	809	26.48 ± 1.54
C20:2n-6c	85.77	774	26.17 ± 2.38
C20:3n-6c	90.08	638	62.00 ± 1.06
C20:3n-3c	93.86	605	10.67 ± 0.29
C20:4n-6c	93.55	864	10.01 ± 5.05
C20:4n-3c	97.54	911	66.29 ± 3.92
C20:5n-3c	98.22	958	903.8 ± 30.2
C21:5n-3c	99.70	878	55.20 ± 4.85
C22:0	92.89	851	70.27 ± 4.88
C22:1n-9c	96.28	939	352.5 ± 14.0
C22:5n-3c	100.75	928	160 ± 6.71
C22:6n-3c	101.15	959	1,016.1 ± 30.5
C24:0	99.36	866	35.71 ± 1.10
C24:1n-9c	99.85	902	61.57 ± 1.87

a) Not confirmed by a standard, only NIST library search.

Appendix IX: Neutral lipid fraction

Table A.12: Overview of the NLs found in Atlantic mackerel (n = 3). The FAs are listed with their associated retention times, match factors and percentage of total area with standard deviations. The match factor measures how well the spectrum match with the one found in the library (max value: 999). The FAs in the table were confirmed with reference standards.

Fatty acid	Retention time [min]	Match factor	Area % Mean ± SD [%]
C12:0	14.40	933	0.10 ± 0.04
C13:0 (4,8,12-trimethyl) [a)]	20.16	879	0.17 ± 0.05
C14:0	19.97	949	5.71 ± 0.38
C14:0 (13-methyl)	22.10	881	0.30 ± 0.02
C14:0 (12-methyl)	22.94	839	0.13 ± 0.01
C15:0	24.57	942	0.94 ± 0.09
C16:0	31.58	950	21.05 ± 3.16
C16:1n-9c	35.05	945	0.31 ± 0.03
C16:1n-7c	36.00	931	3.85 ± 0.73
C16:1n-5c	37.04	927	0.22 ± 0.01
C16:2n-4c	43.82	903	0.19 ± 0.05
C17:0	39.89	771	1.78 ± 0.15
C17:1n-7c	44.51	935	0.50 ± 0.14
C18:0	50.93	953	5.66 ± 1.18
C18:1n-12c	55.55	859	0.24 ± 0.03
C18:1n-9c	56.62	955	12.22 ± 0.84
C18:1n-7c	57.75	925	3.45 ± 0.64
C18:1n-5c	59.82	930	0.26 ± 0.04
C18:2n-6c	68.13	951	1.62 ± 0.38
C18:3n-3c	76.28	946	1.09 ± 0.36
C18:4n-3c	79.83	955	2.24 ± 1.31
C20:0	75.94	939	0.40 ± 0.12
C20:1n-11c	78.07	908	0.68 ± 0.15
C20:1n-9c	78.53	949	4.96 ± 2.00
C20:1n-7c [a)]	79.17	932	0.47 ± 0.17
C20:2n-6c	83.72	939	0.47 ± 0.01
C20:3n-6c	87.70	847	0.05 ± 0.01
C20:3n-3c	91.15	912	0.26 ± 0.02
C20:4n-6c	90.89	900	0.48 ± 0.01
C20:4n-3c	96.27	941	0.63 ± 0.24
C20:5n-3c	97.74	956	5.61 ± 0.55
C21:5n-3c	99.24	938	0.39 ± 0.02
C22:0	90.50	905	0.16 ± 0.11
C22:1n-9c	93.73	949	8.31 ± 3.68
C22:5n-3c	100.29	941	1.30 ± 0.14
C22:6n-3c	100.70	961	12.70 ± 3.59
C24:1n-9c	99.44	953	0.85 ± 0.09

a) Not confirmed by a standard, only NIST library search.

Table A.13: Overview of the NLs found in wild Atlantic salmon (n = 3). The FAs are listed with their associated retention times, match factors and percentage of total area with standard deviations. The match factor measures how well the spectrum match with the one found in the library (max value: 999). The FAs in the table were confirmed with reference standards.

Fatty acid	Retention time [min]	Match factor	Area % Mean ± SD [%]
C12:0	14.33	926	0.071 ± 0.001
C13:0 (4,8,12-trimethyl) [a)]	20.01	875	0.09 ± 0.02
C14:0	19.81	953	4.28 ± 0.15
C14:0 (13-methyl)	21.92	886	0.19 ± 0.01
C14:0 (12-methyl)	22.75	772	0.13 ± 0.01
C15:0	24.35	942	0.42 ± 0.02
C16:0	31.28	949	18.08 ± 1.00
C16:1n-9c	34.66	911	0.15 ± 0.02
C16:1n-7c	35.65	949	6.22 ± 0.23
C16:1n-5c	36.70	921	0.27 ± 0.01
C16:2n-4c	43.37	881	0.27 ± 0.05
C17:0	39.46	727	0.57 ± 0.05
C17:1n-7c	44.02	907	0.24 ± 0.01
C18:0	50.36	950	3.67 ± 0.18
C18:1n-12c	54.97	925	0.86 ± 0.29
C18:1n-9c	56.14	943	17.76 ± 1.27
C18:1n-7c	57.11	930	4.14 ± 0.97
C18:1n-5c	59.12	893	0.28 ± 0.03
C18:2n-6c	67.40	926	1.08 ± 0.04
C18:3n-3c	75.92	934	0.88 ± 0.06
C18:4n-3c	79.45	948	1.23 ± 0.18
C20:0	75.60	908	0.10 ± 0.01
C20:1n-11c	77.74	911	0.89 ± 0.10
C20:1n-9c	78.27	949	7.54 ± 0.72
C20:1n-7c [a)]	78.80	894	0.26 ± 0.06
C20:2n-6c	83.27	895	0.24 ± 0.03
C20:3n-6c	87.18	803	0.06 ± 0.01
C20:3n-3c	90.55	872	0.19 ± 0.02
C20:4n-6c	90.29	907	0.29 ± 0.04
C20:4n-3c	95.62	934	1.33 ± 0.15
C20:5n-3c	97.61	963	6.32 ± 0.23
C21:5n-3c	99.14	926	0.38 ± 0.03
C22:1n-9c	93.23	945	8.07 ± 1.20
C22:5n-3c	100.20	946	2.67 ± 0.30
C22:6n-3c	100.62	962	10.08 ± 0.95
C24:1n-9c	99.35	946	0.60 ± 0.10

a) Not confirmed by a standard, only NIST library search.

Table A.14: Overview of the NLs found in farmed Atlantic salmon (n = 3). The FAs are listed with their associated retention times, match factors and percentage of total area with standard deviations. The match factor measures how well the spectrum match with the one found in the library (max value: 999). The FAs in the table were confirmed with reference standards.

Fatty acid	Retention time [min]	Match factor	Area % Mean ± SD [%]
C14:0	19.85	954	2.05 ± 0.03
C14:0 (13-methyl)	22.01	739	0.05 ± 0.00
C14:0 (12-methyl)	22.84	567	0.03 ± 0.01
C15:0	24.44	818	0.140 ± 0.003
C16:0	31.07	946	9.49 ± 0.09
C16:1n-9c	34.79	800	0.137 ± 0.004
C16:1n-7c	35.65	932	2.32 ± 0.04
C16:2n-4c	43.57	778	0.17 ± 0.01
C17:0	39.59	697	0.21 ± 0.01
C17:1n-7c	44.19	726	0.098 ± 0.004
C18:0	50.26	959	2.59 ± 0.07
C18:1n-12c	55.04	702	0.17 ± 0.03
C18:1n-9c	56.15	952	44.65 ± 0.45
C18:1n-7c	57.07	945	3.04 ± 0.05
C18:2n-6c	67.68	941	14.40 ± 0.12
C18:3n-6c	73.22	709	0.08 ± 0.01
C18:3n-3c	76.03	943	6.23 ± 0.31
C18:4n-3c	79.53	891	0.49 ± 0.04
C20:0	75.66	901	0.283 ± 0.004
C20:1n-11c	77.78	842	0.19 ± 0.03
C20:1n-9c	78.16	939	3.24 ± 0.25
C20:1n-7c [a)]	78.86	756	0.11 ± 0.01
C20:2n-6c	83.37	901	0.774 ± 0.003
C20:3n-6c	87.30	811	0.18 ± 0.02
C20:3n-3c	90.71	845	0.34 ± 0.01
C20:4n-6c	90.46	778	0.13 ± 0.01
C20:4n-3c	95.72	870	0.56 ± 0.05
C20:5n-3c	97.61	954	2.08 ± 0.06
C21:5n-3c	99.17	822	0.04 ± 0.02
C22:0	90.02	673	0.08 ± 0.01
C22:1n-9c	92.92	904	1.26 ± 0.29
C22:5n-3c	100.22	927	1.05 ± 0.04
C22:6n-3c	100.61	955	2.61 ± 0.22
C24:0	98.94	745	0.07 ± 0.01
C24:1n-9c	99.40	862	0.07 ± 0.01

a) Not confirmed by a standard, only NIST library search.

Appendix X: Free fatty acid fraction

Table A.15: Overview of the FFAs found in Atlantic mackerel (n = 3). The FAs are listed with their associated retention times, match factors and percentage of total area with standard deviations. The match factor measures how well the spectrum match with the one found in the library (max value: 999). The FAs in the table were confirmed with reference standards.

Fatty acid	Retention time [min]	Match factor	Area % Mean ± SD [%]
C14:0	19.89	949	2.97 ± 0.34
C15:0	24.51	883	0.69 ± 0.12
C16:0	31.24	951	25.58 ± 1.86
C16:1n-9c	34.93	698	0.24 ± 0.08
C16:1n-7c	35.80	912	3.90 ± 1.38
C16:1n-5c	36.93	820	0.46 ± 0.05
C16:2n-4c	43.71	645	0.21 ± 0.04
C17:0	39.73	806	1.19 ± 0.22
C17:1n-7c	44.38	838	0.57 ± 0.19
C18:0	50.45	962	9.51 ± 0.84
C18:1n-12c	55.17	741	0.23 ± 0.04
C18:1n-9c	56.06	942	10.20 ± 1.49
C18:1n-7c	57.28	887	3.37 ± 0.97
C18:2n-6c	67.80	895	1.76 ± 0.10
C18:3n-3c	76.15	919	1.34 ± 0.02
C18:4n-3c	79.69	931	2.26 ± 0.40
C20:0	75.77	739	0.17 ± 0.04
C20:1n-11c	77.91	806	0.25 ± 0.03
C20:1n-9c	78.28	936	2.35 ± 0.81
C20:1n-7c [a)]	79.00	632	0.21 ± 0.10
C20:2n-6c	83.56	751	0.28 ± 0.06
C20:3n-3c	90.96	624	0.18 ± 0.04
C20:4n-6c	90.68	831	0.55 ± 0.06
C20:4n-3c	96.01	835	0.59 ± 0.01
C20:5n-3c	97.68	960	9.44 ± 1.01
C21:5n-3c	99.21	852	0.22 ± 0.02
C22:1n-9c	93.21	915	2.58 ± 0.98
C22:5n-3c	100.26	903	0.88 ± 0.05
C22:6n-3c	100.65	959	17.55 ± 5.63
C24:1n-9c	99.41	839	0.26 ± 0.03

a) Not confirmed by a standard, only NIST library search.

Table A.16: Overview of the FFAs found in wild Atlantic salmon (n = 3). The FAs are listed with their associated retention times, match factors and percentage of total area with standard deviations. The match factor measures how well the spectrum match with the one found in the library (max value: 999). The FAs in the table were confirmed with reference standards.

Fatty acid	Retention time [min]	Match factor	Area % Mean ± SD [%]
C12:0	14.35	853	0.09 ± 0.01
C14:0	19.73	955	3.70 ± 0.31
C14:0 (13-methyl)	21.86	791	0.15 ± 0.02
C14:0 (12-methyl)	22.71	705	0.10 ± 0.01
C15:0	24.26	915	0.34 ± 0.02
C16:0	30.86	950	22.96 ± 0.83
C16:1n-9c	34.49	736	0.13 ± 0.02
C16:1n-7c	35.35	938	4.43 ± 0.47
C16:1n-5c	36.53	838	0.30 ± 0.02
C16:2n-4c	43.21	781	0.18 ± 0.02
C17:0	39.28	729	0.36 ± 0.05
C17:1n-7c	43.86	762	0.22 ± 0.02
C18:0	49.76	954	5.43 ± 0.23
C18:1n-12c	54.43	887	0.92 ± 0.14
C18:1n-9c	55.27	939	11.52 ± 0.47
C18:1n-7c	56.44	915	3.44 ± 0.50
C18:1n-5c	58.60	759	0.27 ± 0.03
C18:2n-6c	66.90	855	0.77 ± 0.03
C18:3n-3c	75.73	904	0.67 ± 0.02
C18:4n-3c	79.24	885	0.79 ± 0.14
C20:0	75.47	675	0.10 ± 0.01
C20:1n-11c	77.50	869	0.48 ± 0.06
C20:1n-9c	77.87	940	3.85 ± 0.20
C20:1n-7c [a)]	78.57	757	0.15 ± 0.03
C20:2n-6c	83.03	740	0.15 ± 0.02
C20:3n-6c	86.94	571	0.05 ± 0.01
C20:3n-3c	90.26	686	0.14 ± 0.02
C20:4n-6c	90.00	868	0.55 ± 0.03
C20:4n-3c	95.21	887	1.05 ± 0.06
C20:5n-3c	97.51	958	10.72 ± 0.66
C21:5n-3c	99.11	888	0.24 ± 0.02
C22:1n-9c	92.47	921	3.27 ± 0.20
C22:5n-3c	100.16	942	2.39 ± 0.31
C22:6n-3c	100.56	964	19.81 ± 1.70
C24:1n-9c	99.34	824	0.29 ± 0.07

a) Not confirmed by a standard, only NIST library search.

Table A.17: Overview of the FFAs found in farmed Atlantic salmon (n = 3). The FAs are listed with their associated retention times, match factors and percentage of total area with standard deviations. The match factor measures how well the spectrum match with the one found in the library (max value: 999). All FAs in the table were confirmed with reference standards.

Fatty acid	Retention time [min]	Match factor	Area % Mean ± SD [%]
C14:0	20.06	906	2.88 ± 0.06
C15:0	24.78	834	0.26 ± 0.01
C16:0	31.59	950	19.14 ± 0.53
C16:1n-9c	35.40	637	0.13 ± 0.01
C16:1n-7c	36.24	920	2.06 ± 0.03
C16:2n-4c	44.26	671	0.20 ± 0.03
C17:0	40.22	768	0.32 ± 0.01
C18:0	51.11	953	7.55 ± 0.23
C18:1n-9c	56.94	950	28.34 ± 0.42
C18:1n-7c	58.11	889	2.68 ± 0.03
C18:2n-6c	68.70	929	13.88 ± 0.26
C18:3n-3c	76.58	933	7.13 ± 0.41
C18:4n-3c	80.18	825	0.43 ± 0.03
C20:0	76.19	784	0.220 ± 0.005
C20:1n-9c	78.73	890	1.67 ± 0.10
C20:2n-6c	84.13	817	0.61 ± 0.01
C20:3n-6c	88.22	643	0.15 ± 0.02
C20:3n-3c	91.73	751	0.31 ± 0.01
C20:4n-6c	91.47	667	0.16 ± 0.00
C20:4n-3c	96.78	856	0.55 ± 0.04
C20:5n-3c	97.84	956	4.30 ± 0.12
C21:5n-3c	99.35	731	0.116 ± 0.004
C22:1n-9c	93.98	781	0.46 ± 0.08
C22:5n-3c	100.39	882	0.66 ± 0.05
C22:6n-3c	100.78	942	3.88 ± 0.24
C24:1n-9c	99.52	793	0.26 ± 0.01

Appendix XI: Polar lipid fraction

Table A.18: Overview of the PLs found in Atlantic mackerel (n = 3). The FAs are listed with their associated retention times, match factors and percentage of total area with standard deviations. The match factor measures how well the spectrum match with the one found in the library (max value: 999). All FAs in the table were confirmed with reference standards.

Fatty acid	Retention time [min]	Match factor	Area % Mean ± SD [%]
C14:0	19.86	927	0.89 ± 0.41
C15:0	24.44	845	0.43 ± 0.08
C16:0	31.11	946	27.73 ± 3.13
C16:1n-9c	34.83	550	0.14 ± 0.03
C16:1n-7c	35.67	843	1.06 ± 0.39
C16:1n-5c	36.86	682	0.22 ± 0.02
C17:0	39.63	734	1.16 ± 0.11
C17:1n-7c	44.31	639	0.38 ± 0.06
C18:0	50.21	937	6.34 ± 0.39
C18:1n-12c	55.02	649	0.23 ± 0.05
C18:1n-9c	55.82	928	6.08 ± 1.01
C18:1n-7c	57.04	859	1.90 ± 0.48
C18:2n-6c	67.58	768	0.77 ± 0.16
C18:3n-3c	76.06	779	0.24 ± 0.03
C18:4n-3c	79.59	735	0.21 ± 0.07
C20:0	75.69	577	0.12 ± 0.01
C20:1n-11c	77.82	703	0.16 ± 0.04
C20:1n-9c	78.17	890	0.94 ± 0.60
C20:2n-6c	83.43	661	0.19 ± 0.01
C20:4n-6c	90.50	851	1.10 ± 0.07
C20:4n-3c	95.82	659	0.24 ± 0.03
C20:5n-3c	97.63	954	8.56 ± 1.00
C21:5n-3c	99.18	731	0.13 ± 0.04
C22:1n-9c	92.99	861	0.73 ± 0.55
C22:5n-3c	100.23	894	1.42 ± 0.09
C22:6n-3c	100.63	958	36.90 ± 4.86
C24:1n-9c	99.38	820	0.22 ± 0.06

Table A.19: Overview of the PLs found in wild Atlantic salmon (n = 3). The FAs are listed with their associated retention times, match factors and percentage of total area with standard deviations. The match factor measures how well the spectrum match with the one found in the library (max value: 999). All FAs in the table were confirmed with reference standards.

Fatty acid	Retention time [min]	Match factor	Area % Mean ± SD [%]
C14:0	19.71	920	0.97 ± 0.12
C15:0	24.21	625	0.23 ± 0.03
C16:0	30.66	950	22.86 ± 1.82
C16:1n-7c	35.18	820	1.05 ± 0.16
C17:0	39.17	611	0.53 ± 0.08
C18:0	49.47	910	4.01 ± 0.40
C18:1n-12c	54.17	746	0.64 ± 0.16
C18:1n-9c	54.92	932	7.42 ± 1.04
C18:1n-7c	56.18	844	1.98 ± 0.40
C18:2n-6c	66.60	487	0.34 ± 0.03
C18:3n-3c	75.60	561	0.20 ± 0.02
C18:4n-3c	79.10	584	0.21 ± 0.01
C20:1n-11c	77.33	604	0.23 ± 0.03
C20:1n-9c	77.68	826	1.24 ± 0.20
C20:4n-6c	89.75	787	0.60 ± 0.08
C20:4n-3c	94.89	771	0.44 ± 0.03
C20:5n-3c	97.43	943	8.15 ± 0.58
C21:5n-3c	99.06	720	0.31 ± 0.04
C22:1n-9c	92.17	535	0.30 ± 0.01
C22:5n-3c	100.12	855	2.84 ± 0.56
C22:6n-3c	100.52	958	44.44 ± 1.78

Table A.20: Overview of the PLs found in farmed Atlantic salmon (n = 3). The FAs are listed with their associated retention times, match factors and percentage of total area with standard deviations. The match factor measures how well the spectrum match with the one found in the library (max value: 999). All FAs in the table were confirmed with reference standards.

Fatty acid	Retention time [min]	Match factor	Area % Mean ± SD [%]
C14:0	20.07	898	0.62 ± 0.05
C15:0	24.78	614	0.13 ± 0.01
C16:0	31.54	948	20.62 ± 0.89
C16:1n-9c	35.37	742	0.12 ± 0.01
C16:1n-7c	36.23	789	0.46 ± 0.03
C17:0	40.16	596	0.29 ± 0.01
C18:0	50.97	908	1.57 ± 0.06
C18:1n-9c	56.73	935	11.04 ± 0.79
C18:1n-7c	58.00	850	1.78 ± 0.08
C18:2n-6c	68.56	901	2.84 ± 0.16
C18:3n-3c	76.51	917	2.45 ± 0.18
C18:4n-3c	80.12	647	0.13 ± 0.02
C20:0	76.12	524	0.08 ± 0.01
C20:1n-9c	78.67	724	0.31 ± 0.03
C20:2n-6c	84.06	711	0.30 ± 0.03
C20:3n-6c	88.13	621	0.20 ± 0.02
C20:3n-3c	91.61	630	0.18 ± 0.03
C20:4n-6c	91.33	817	0.56 ± 0.03
C20:4n-3c	96.70	825	0.49 ± 0.06
C20:5n-3c	97.82	958	8.90 ± 0.24
C21:5n-3c	99.32	703	0.15 ± 0.02
C22:1n-9c	93.92	509	0.11 ± 0.01
C22:5n-3c	100.36	922	2.50 ± 0.25
C22:6n.3c	100.77	958	39.46 ± 0.81
C24:1n-3c	99.50	814	0.28 ± 0.01

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