UNIVERSITY OF TROMSØ UIT

NORWEGIAN COLLEGE OF FISHERIES SCIENCE

Fat, fatty acids and fat soluble nutrients in fillet of farmed and wild Atlantic salmon (*Salmo salar* L.)

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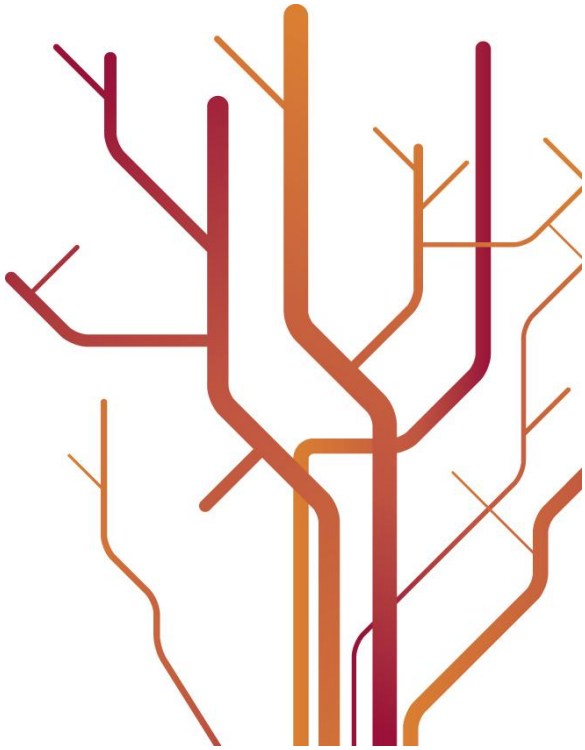
Master's Degree Thesis in Fisheries and Aquaculture
Field of study: Seafood Science (60 credits)

May 2011



Acknowledgements

The work behind this master thesis was carried out in at the Norwegian College of Fishery Science, University of Tromsø, marking the end of five fabulous and exciting years.
It is with great gratitude and respect I would like to thank my supervisor Ragnar L. Olsen. Your knowledge, advice, interest and good humor has been a driving force and foundation both professionally and life in general.

Special thanks go to Hanne K. Mæhre, Ida-Johanne Jensen, Birthe Vang, Alice Marie Pedersen, Sigmund Sperstad and Svein Kristian Stormo, Thank you for all your help and motivation through my work in the lab and in the writing process. The HPLC analysis for determination of vitamin D_3 in salmon muscle was carried out by Svein Kristian Stormo with the author as a "by-sitter".

My friends at school and in private, thank you for always make me laugh and making my life through the writing process and otherwise perfect.

And at last but not least, I thank my mother and my stepfather for always given me all the love and encouragement in the world, it means everything to me.

Siri Tømmerås
Tromsø, Mai 2011

Sammendrag

En av hovedgrunnene til at det er et høyt fokus på å spise fisk er det naturlige innholdet av de lang kjedede flerumettede omega-3 fettsyrene, eicosapentaen syre (EPA; 20:5n-3) og docosahexaen syre (DHA; 22:6n-3). Det anbefalte daglige inntaket av disse er fra 0,2 til 0,5g/dag eller to porsjoner fisk i uken, og da helst fet fisk. Oppdrettsnæringen produserer i dag nesten halvparten av all fisk og fiskeprodukter konsumert på verdensbasis. Tradisjonelt har fiskemel og fiskeolje vært hovedingrediensen i formulert fôr brukt ved intensiv oppdrett av marine og diadrome arter. På grunn av den økende produksjonen innen oppdrett har etterspørselen etter disse råvarene overgått tilgang, og ført til at industrien har måtte finne andre kilder for protein og fett. Det er nå vanlig å bytte ut noe av fiskemelet og fiskeoljen med vegetabilsk mel og olje. Atlantisk laks (*Salmo salar* L.) er den dominerende arten innen den diadrome gruppen med Norge som ledende produsent. I 2010 ble omtrent 1 million tonn produsert i Norge, noe som utgjorde 68% av total produksjon av oppdrettet Atlantisk laks på verdensbasis. Hovedmålet med denne oppgaven var å undersøke ernæringsverdien av oppdrettet Atlantisk laks produsert i Norge med fokus på fettsyresammensetning. I tillegg skulle Astaxanthin og vitamin D_3 innhold bestemmes. Dette ble sammenlignet med vill Atlantisk laks fra havet.

Resultatene viste at oppdrettet Atlantisk laks hadde et totalt fettinnhold på 11,4%, noe som var nesten dobbelt så mye som i vill Atlantisk laks. Videre var innholdet av EPA og DHA 0,42g og 0,61g/100g filet. Vill Atlantisk laks hadde et innhold på 0,19g og 0,36g/100g filet av EPA og DHA. n-6/n-3 ratioen var 0,37 og 0,07 i oppdrettet og vill Atlantisk laks. Ved målinger av astaxanthin innholdet fant vi ingen forskjell mellom de to gruppene. Oppdrettet Atlantisk laks hadde et gjennomsnittlig astaxanthin innhold på 5,2mg/g prøve, mens vill Atlantisk laks hadde et gjennomsnittlig innhold på 4,8mg/g prøve. Ved måling av vitamin D var resultatene svært tvetydig, noe som førte til at vi valgte å overse disse.

Konklusjonen i denne oppgaven er at oppdrettet Atlantisk laks er en utmerket kilde til EPA og DHA, og at en liten porsjon (50 gram) dekker det daglige anbefalte inntaket på 0,5g EPA og DHA.

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Summary

One of the main reasons for the advise to consume more seafood is to obtain adequate dietary levels of long chain polyunsaturated fatty acids, eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3). The recommended dietary intake of these fatty acids is between 0.2-0.5g/day, or consumption of at least two servings of fish, preferably fatty fish per week. The aquaculture industry today provides almost half of all fish and fish products consumed around the world. Traditionally, fish meal and fish oil have been the main ingredients in formulated feed used in intensive farming of marine and diadromous fish. Due to the rapid growth in aquaculture production, the demands for fish meal and fish oil has exceeded the supply, leading the industry to find other sources of protein and oils to satisfy the nutritional quality of the fish. It is now common to substitute some of the marine ingredients with vegetable meal and oil. Atlantic salmon (*Salmo salar* L.) is the major species in the diadromous group with Norway as the leading producer. In 2010, about one million tons were produced in Norway, accounting for approximately 68% of the world's total production of farmed Atlantic salmon. The overall aim of this thesis was to evaluate human nutritional value of farmed Atlantic salmon produced in Norway with regard to the composition and content of fatty acids, astaxanthin and vitamin D_3, and to compare with wild Atlantic salmon.

The results showed that farmed Atlantic salmon had total lipid content of 11.4% which is twice the amount in wild Atlantic salmon. Furthermore, the content of EPA and DHA was 0.42g and 0.61g/100g fillet, respectively in farmed Atlantic salmon. In wild Atlantic salmon the total content of EPA and DHA were 0.19g and 0.36g/100 g fillet. The n-6/n-3 ratio was 0.37 and 0.07 in farmed and wild Atlantic salmon, respectively. The content of astaxanthin in farmed and wild Atlantic salmon was similar. Farmed Atlantic salmon had an average astaxanthin content of 5.2mg/g sample, while wild had an average content of 4.8mg/g sample. In determining the vitamin D_3 content the results were very ambiguous, leading us to dismiss all data.

However, the high fat content makes the farmed salmon an excellent source of these health promoting fatty acids. Dependent on the suggested daily requirement of EPA and DHA, 20-50g of farmed Atlantic fillet is sufficient to satisfy the daily needs. In addition the ratio between n-6 and n-3 is very low and well below the value often recommended by nutritional experts.

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1. Introduction and aims

Since the 1960s worldwide farming of fish and shellfish has grown tremendously and today the amount of food produced from aquaculture is about the same as obtained from harvesting of wild species (FAO, 2010). The average annual growth rate has been almost 8% worldwide since 1970 to 2008, making it the fastest growing food producing sector (FAO, 2010). The major species groups in aquaculture are freshwater fishes, mollusks, crustaceans, diadromous fishes and marine fishes. The leading production countries are China (produced 32.5 million tons in 2008), India, Vietnam, Thailand, Chile, Japan, Norway and the Philippines. In 2008 the total aquaculture production of fish, crustaceans, mollusks and other aquatic organisms reached a total of 52.5 million tons worldwide with a value of almost US$100 billion, when excluding aquatic plants. The major species groups in aquaculture such as freshwater fishes, mollusks, crustaceans, diadromous fishes and marine fishes had a production volume of 28.8, 13.1, 9.5, 6.3 and 1.8 million tons respectively in 2008 (FAO, 2010).

Atlantic salmon (*Salmo salar* L.) is the dominant cultured species in the diadromous group with a production volume of 1.5 million tons in 2008 (FAO, 2010). Including rainbow-trout (*Oncorhynchus mykiss*, Walbaum.) it accounts for more than 80% of the total European aquaculture production, with Norway as the leading contributor on a worldwide basis (FAO, 2009). About 50% of all Atlantic salmon consumed worldwide is from fish farming (Norwegian Seafood Export Council). This has been driven by increased demands and establishment of new markets. The main producers of farmed Atlantic salmon were Norway and Chile with a production on 36% and 28%, respectively in 2008, but Chilean aquaculture sector experienced several outbreak of infectious Salmon Anemia (ISA) the same year, causing the industry to lose half its production volume (FAO, 2010). Norway produced approximately 68% of the farmed Atlantic salmon consumed worldwide in 2010 (Norwegian Seafood Export Council).

From the beginning of the Norwegian aquaculture adventure in the 1970s, the industry has grown with a great success in terms of production and profitability. Production of Atlantic salmon has been the main contributor to this and has grown from 46 tons in 1972 to 944 600 tons in 2010 (Knapp *et al.*, 2007; Norwegian Seafood Export Council). The success story is amongst other things due to the country's ideal long coast line, deep fjords and optimal water conditions. Other species are also being cultivated such as rainbow-trout, cod (*Gadus morhua*

L.), halibut (*Hippoglossus hippoglossus* L.), wolffish (*Anarhichas spp*.), char (*Salvelinus alpines* L.) and turbot (*Psetta maxima* L.) (Norwegian Seafood Export Council).

Aquaculture production is playing an increasing role in satisfying the demands for fish and fish products for human consumption, and it has been an increased focus on the nutritional quality and safety associated with fish consumption. As a nutritional source, fish has been proven to be a good source of proteins, and compared to animal meat it is low in saturated fat and cholesterol. The main focus however, has been on the natural content of the healthy highly unsaturated n-3 fatty acids (FAs), eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) (Wall *et al.*, 2010). These FAs are essential for normal development and growth, and are associated with reduced risks of cardiovascular diseases, hypertension, diabetes mellitus, cancer, arthritis and a wide range of other inflammatory or autoimmune disorders (Simopoulos, 2000; Morris *et al.*, 2003; Hibbeln *et al.*, 2006; Lunn and Theobald, 2006; Wall *et al.*, 2010).

The dietary recommendations of EPA and DHA are set to 0.2-0.5g/day or consumption of at least two servings of fish, preferably fatty fish per week (Calder and Yaqoob, 2009; Kris-Etherton *et al.*, 2009). A dietary intake of n-3 FAs also contribute to lower the n-6/n-3 ratio in the modern Western society were the diet is dominated by processed food and vegetable oils high in n-6 FAs (Kris-Etherton *et al.*, 2009). The n-6/n-3 recommended optimal ratio is 4:1, but in Western societies, due to the high levels of n-6 FAs in the diet, the average ratio is 15-16:1 (Wall *et al.*, 2010). It is worthwhile to note that if one meal of fish substitutes one meal of sausages the adverse effects associated with saturated fat and cholesterol such as promotion of inflammation, atherosclerosis and coronary heart disease (CHD) is decreased (Mozaffarian *et al.*, 2004; Lopez-Garcia *et al.*, 2005) .

As the aquaculture industry represent the fastest growing food industry, the demand for fish meal (FM) and fish oil (FO) for inclusion in formulated fish feeds has exceeded the supply, leading the industry to find other sources of high quality protein and essential FAs to satisfy the nutritional requirements of the fish. Of particular importance has been to produce salmon with a high content of long chain polyunsaturated fatty acids (Lc-PUFA), EPA and DHA. Production of farmed Atlantic salmon has been depending on formulated feeds dominated by marine raw materials (FM and FO) commonly obtained from small pelagic fish such as anchoveta (*Engraulis ringens,* Jenyns.), blue witting (*Micromesistius poutassou*, Risso.), capelin (*Mallotus villosus,* Müller.), herring (*Clupea harengus* L./*Clupea harungus*), sandeel (*Ammodytes* spp.) and sprat (*Sprattus sprattus*, Girgensohn.) (EWOS, 2010; Helland,

2010; Skretting, 2010). As the global pelagic capture fisheries have reached maximum capacity, and the prices of FM and FO have increased, the industry has been substituting FM and FO with vegetable ingredients in the feed (Naylor *et al.*, 2009). The most common substitute for FM is soybean meal, mainly due to the high nutritional value, cost and already established marked availability (Tacon, 1994), but also rapeseed, corn gluten, wheat gluten, barley, pea and lupin meal may be suitable substitutes (Tacon *et al.*, 2006). Sunflower, maize, canola/rapeseed and soybean are suitable substitutes for FOs (Naylor *et al.*, 2009). Studies have shown that vegetable oil (VO) can replace up to 75% of the FOs without compromising growth, performance and fish health as long as the requirements of the essential long chain n-3 FAs are met (Bell *et al.*, 2001; Torstensen *et al.*, 2005; Naylor *et al.*, 2009; Turchini *et al.*, 2009).

The FA profile of the salmon fillets reflects the FA composition of feed. Vegetable oils have generally a high content of n-6 FAs, particular linolenic acid (LA; 18:2n-6), and some medium chain n-3 FA; α- linolenic acid (ALA; 18:3n-3). Plant oils are however devoid of EPA and DHA. FO has very little LA and ALA, but is rich in EPA and DHA. Replacing FO with VO increases the n-6/n-3 ratio in the flesh of the fish and lowers the content of EPA and DHA. This may compromise the health benefits of consuming farmed Atlantic salmon in societies were the n-6/n-3 ratio in the diet is already too high (Torstensen *et al.*, 2005).

In addition to EPA and DHA, Atlantic salmon may contain high amounts of fat soluble nutrients like astaxanthin and vitamin D. Astaxanthin is a carotenoid that gives the salmon its desirable pink-red color, which is one of the most important quality traits to the consumers, and is known for its antioxidative capacity. The salmon assimilate astaxanthin through consumption of a prey organisms like zooplankton or astaxanthin manufactured by chemical synthesis added in the feed.

Atlantic salmon is one of the few natural sources of vitamin D, and based on dietary tables it is suggested that wild raw salmon consist of 8µg vitamin D per 100g (Matportalen). Reasons why fatty fish like salmon has a high level of vitamin D in the tissue is debated, but it is most probably due to the food web containing amongst other photosynthesizing phytoplankton (Holick, 2003), and their great ability to store fat soluble nutrients like vitamin D in the adipose tissue. Other dietary sources of vitamin D are cod-liver oil, mushroom and fortified foods like margarine and milk.

The overall aim of this thesis was to evaluate human nutritional value of farmed Atlantic salmon produced in Norway with regard to the composition and content of fatty acids, astaxanthin and vitamin D_3, and to compare with wild Atlantic salmon.

2. Background

2.1 Fatty acids

Fatty acids (FAs) are hydrocarbon chains with a methyl group at one end (methyl end) and a carboxyl group in the opposite end (carboxyl end). Common FAs in foods have between 12 to 22 carbons atoms. The FAs from plants and animal fat normally have 16 to 18 carbons, while marine organisms including fish, have chains from 14 to 22 carbons. Milk and butter fat also have a high content of short chained FAs with only 4 to 12 carbons. FAs are divided in two main groups; saturated FAs (SFA) with no carbon-carbon (C-C) double bonds and unsaturated FAs with 1 to 6 C-C double bonds. The unsaturated FAs are also divided in two groups; mono- (MUFAs) and polyunsaturated fatty acids (PUFAs). MUFAs have only one C-C double bond, while PUFAs have 2 to 6 C-C double bonds. The PUFAs with $\geq$ 20 carbon atoms and 4 to 6 C-C double bonds are also called highly unsaturated fatty acids (HUFAs) or long chain PUFAs (Lc-PUFA). Normally, the main focus with regards of PUFAs is not on the numbers of C-C double bonds, but where the double bonds are placed from the methyl end. This is what creates the two main families of PUFAs, classified as omega-3 FAs (n-3 FAs) and omega-6 FAs (n-6 FAs). In n-3 FAs the first double bond is on the third carbon position and in the n-6 FAs the first double bond is on the sixth carbon position from the methyl end.
FAs are also differentiated into essential and non-essential FAs. For humans and most animals the essential FAs (EFAs) are linoleic acid (LA; 18:2n-6) and α-linoleic acid (ALA; 18:3n-3). These are desaturated and elongated further to arachidonic acid (ARA; 20:4n-6), EPA and DHA by $\Delta^{12}$and Δ^{15} desaturase, and elongase (Kris-Etherton *et al.*, 2009). Marine fish however, have to a large extent lost the ability to synthesize these HUFAs and they are thus classified as essential FAs in fish. It shall also be mentioned that humans have a limited capacity to synthesize EPA and DHA from the precursor ALA, and hence may need to have these FAs supplemented through their diet to meet dietary requirements (Kris-Etherton *et al.*, 2009).

2.1.2 FAs in food

Fish and shellfish are in general known for the high natural content of the HUFAs EPA and DHA (Farrell *et al.*, 2010). However, the absolute amounts present are dependent on the fat content of the edible parts. The high levels of HUFAs come mainly from phytoplankton in the food-web. Phytoplankton are very good producers of EPA and DHA in

contrast to terrestrial plants which cannot elongate and desaturate LA and ALA. Furthermore, fish have a high ability to store these FAs in their adipose tissue which in fatty fish is in the fillet. In meat from pork and beef, the majority of FAs are the SFAs palmitic acid (16:0), stearic acid (18:0) and the MUFA oleic acid (18:1n-9), and with no or just a very low content of long chain n-3 PUFAs such as EPA and DHA. Soybean, corn, sunflower, safflower and cotton seed oil is rich in n-6 PUFAs, especially LA, and there is just a few of the VOs that has a reasonable content of n-3 PUFAs (linseed and canola/rapeseed oil) (Wall *et al.*, 2010).

2.1.3 Health related aspects

The correlation between n-3 HUFAs and health was postulated in the 1970s when Dyerberg and Bang (1979) found a positive correlation between low rates of coronary heart disease (CHD) amongst Greenland Eskimos and their high consumption of these FAs through their diet. The ratio of death by CHD was 1:10 compared to the Scandinavian population (Kromann and Green, 1980). Since then it has been shown that n-3 HUFAs play a major role in many physiological processes, and have positive effect on a wide range of illnesses with an inflammatory element, such as inflammatory bowel disease, atherosclerosis and rheumatism. It may also reduce the incidents of certain types of cancer, diabetes mellitus and mental diseases like dementia and depression (Bougnoux *et al.*, 1994; Simopoulos, 2000; Das, 2001; Morris *et al.*, 2003; Hibbeln *et al.*, 2006; Lunn and Theobald, 2006; Calder, 2008; Wall *et al.*, 2010). The n-3 HUFAs, especially DHA are also essential for fetal health and normal neurological and visual development in humans and vertebrates (Uauy *et al.*, 1992; Lauritzen *et al.*, 2001; Das, 2002; Nugent, 2004). In addition there has been shown that n-3 HUFAs have a role in cognitive development and performance, and have been associated with childhood and attention-deficit/hyperactivity disorder (ADHD) (Bell *et al.*, 2000b; Richardson, 2006).

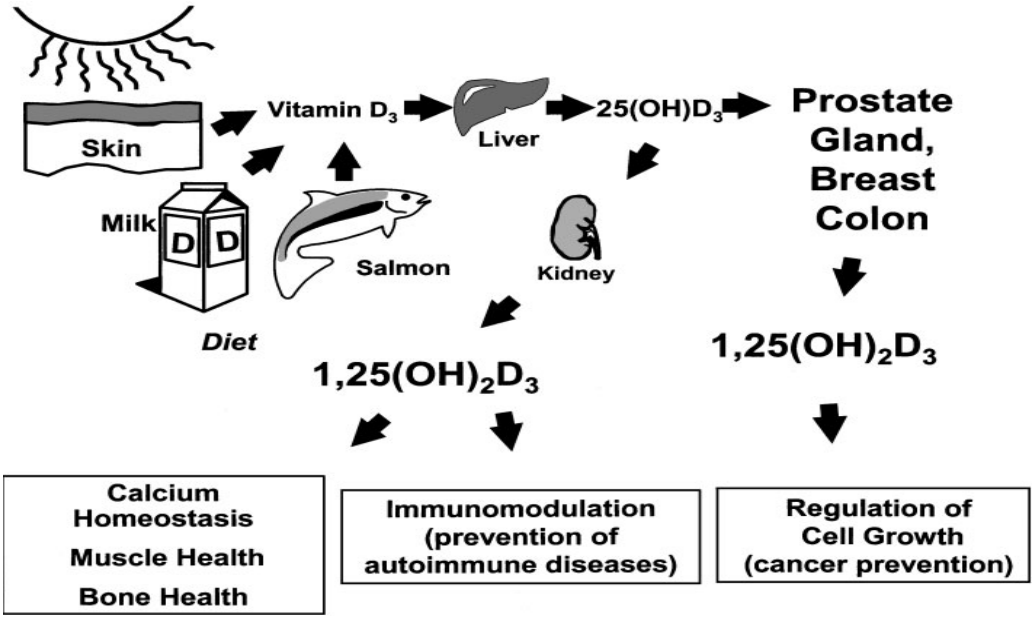
Reasons why they have such positive effects on human health have been widely debated, but one reason might be the production of anti-inflammatory eicosanoids (FA hormones) derived from EPA that modulate immune reactivity, and amongst other might inhibit the synthesis of pro-inflammatory cytokines (Kelley and Rudolph, 2000). This is in contrast to the n-6 eicosanoids derived from ARA that have shown to be pro–inflammatory, thrombotic and immune active (Babcock *et al.*, 2000; Funk, 2001).

2.2 Vitamin D

The positive effect of consuming vitamin D has been known since the industrial revolution (in the mid-1700), when a correlation between vitamin D deficiency and rickets was found (Holick, 1994). About 150 years ago it was discovered that taking cod-liver oil prevented this disease in people (children) who did not get enough sun. In the beginning of the 1990s the research on vitamin D attracted greater attention, and it was shown that a large proportion of the population had inadequate levels of blood serum 25(OH) vitamin D (Byrdwell, 2009). It was revealed the vitamin had a much wider range of function through the entire body than first known (Tangpricha *et al.*, 2010).

The most established role of vitamin D is controlling the calcium absorption and calsiumphosphate homeostasis to obtain healthy mineralization of bones and teeth (Alappat *et al.*, 2010), preventing diseases like osteomalacia and osteoporosis in adults and rickets in children (Holick, 2003). Receptors for vitamin D have also however been discovered in the brain, heart, stomach, pancreas, skin and gonads etc. suggesting a connection between vitamin D deficiency and a wide range of illnesses, such as increased risk of hypertension, diabetes mellitus (Holick, 2003), and psychosomatic disorders like autism, multiple sclerosis and schizophrenia (Ganji *et al.*, 2010). Correlation between vitamin D deficiencies and some types of cancer like prostate, colon, breast and ovary have also been suggested. This may be due to the vitamin involvement in cell growth, differentiation and proliferation (Garland *et al.*, 1989; Garland *et al.*, 1990; Hanchette and Schwartz, 1992; Holick, 2003; Ostermeyer and Schmidt, 2006)

The most efficient way to obtain an adequate level of serum vitamin D is through endogenous synthesis in the skin from 7-dehydrocholesterol (provitamin D_3) to previtamin D_3 with exposure of ultraviolet B (UVB) radiation (Holick *et al.*, 1981; MacLaughlin *et al.*, 1982; Holick, 2002). The pre-vitamin D_3 is then transformed in the liver to 25(OH) vitamin D_3 (cholecalciferol), which is the principal form of circulating vitamin D. (Figure 1). Furthermore, 25(OH) vitamin D_3 is hydroxylated to $1.25(OH)_2$ vitamin D_3 (calciferol) in the kidneys which is the biologically most active metabolite of vitamin D (Garland *et al.*, 2006), also called vitamin D hormone. In seasons with insufficient sunlight we have to obtain the levels of serum vitamin D through the diet. Natural dietary sources of vitamin D are fatty fish such as Atlantic salmon, mackerel and herring, cod-liver oil and some types of mushroom. In addition, we also have a range of fortified food like milk, margarine and butter that are enriched with vitamin D during processing (Byrdwell, 2009). Vitamin D from endogenous



Figur 1: Production, sources, metabolism and biologic function of vitamin D_3 (Holick, 2003).

synthesis and from the diet is stored in adipose tissue and in the liver (Ostermeyer and Schmidt, 2006).

The required dietary intake of vitamin D varies through the year because of exposure to UVB radiation on the skin. The Norwegian Council of Nutrition estimates a recommended intake of 7.5µg for both men and women between 2 and 60 years old, with a maximum dietary intake of 50µg. People over 60 years should have a daily intake of 10µg. Furthermore, they recommend that infants from four weeks up to six months of age are given 2.5-5ml cod-liver oil or vitamin D drops. In Norway, the incidence of rickets is exclusively linked to children from developing countries; and the government has therefore started a program giving out vitamin D drops for immigrant children from developing countries the first six months (National council for nutrition, 2011).

Studies have shown that deficiency often strike people with high skin pigmentation (colored may have 25(OH) vitamin D_3 levels that are only half those of Caucasians) (Holick, 2003). Elderly also have a tendency to have low amounts of serum 25(OH) vitamin D_3. This comes mainly from immobility and that the skin with age loses its capability to synthesize pre-vitamin D_3 (MacLaughlin and Holick, 1985). However, studies have shown that the skin has such a high capability to synthesize pre-vitamin D_3 that as long as it is exposed to sunlight the body will obtain adequate levels of the vitamin (Reid *et al.*, 1986; Holick, 1994; Chel *et al.*, 1998). One study has shown that diabetes mellitus also may cause a low vitamin D status, but in this case there is no definitive conclusion (Mitri and Pittas, 2010).

2.3 Astaxanthin

Astaxanthin is a non-polar lipid classified as a carotenoid and gives Atlantic salmon its characteristic and desirable pink-red muscle color which is one of the most important quality traits for the consumers (Sigurgisladottir *et al.*, 1997). Astaxanthin is one of at least 700 different types of carotenoids that are produced by photosynthesizing organisms. The majorities are biosynthesized by algae. Apart from serving as a colorant, the carotenoid may have a variety of metabolic functions in humans and vertebrates e.g. as an antioxidant that have the ability to quench and inactivate reactive oxygen species, especially singlet oxygen ($^1O_2$), neutralize "sensitizing"-compounds (that may cause oxidation or form singlet oxygen) and capture free radicals. It has also been suggested that carotenoids such as astaxanthin operate synergistically with vitamin E (Bell *et al.*, 2000a), and may play a major role in protecting the lipid tissue from peroxidation in cold water fish like Atlantic salmon that have a high level of PUFAs in their membranes (Bell *et al.*, 2000a)

Furthermore, carotenoids have shown to serve as a UV protector on fish eggs (Craik, 1985; Pavlov *et al.*, 2007), in addition to be essential for growth and survival in the start feeding process of farmed fish (Christiansen *et al.*, 1995). It is also important for normal embryonic development and could affect hatching rate and larval survival (Torrissen, 1984; Craik, 1985; George *et al.*, 2001). But the most important function for some carotenoids is the ability to function as precursors of vitamin A in diet of humans and animals.

Wild species of Atlantic salmon assimilate astaxanthin and its esters by consuming a number of pray organisms like zooplankton and crustaceans. (Storebakken and No, 1992) Zooplankton produce astaxanthin from β-carotene by consuming phytoplankton (Matsuno, 2001; Anderson *et al.*, 2003), which is the most common carotenoid in plant tissue (Damodaran *et al.*, 2008). In krill (*Euphausiacea,* Dana.) and the copepod (*Calanus finmarchicus* ,Gunnerus.*)* almost 85-90% of the pigment is astaxanthin (Funk and Hobson, 1991; Lotocka and Styczynska-Jurewicz, 2001). Astaxanthin which are either manufactured by chemical synthesis, or obtained from biosynthesizing organisms are included in the feed for farmed Atlantic salmon (Bjerkeng *et al.*, 2000).

3. Materials and methods

3.1 Sampling and preparing of materials

The farmed salmons (n=20) were obtained from Leroey Aurora AS Skjervøy, Norway, in July 2010. The fish had been fed Opal feed (EWOS AS, Norway). The wild Atlantic salmon (n=20) were caught with pound net at "Loppa" off the coast of Finnmark, Norway, in June 2010, and bought from a local fishing shops, Joker and Eide handel, Tromsø. The average gutted weight for farmed and wild Atlantic salmon was 3.5 ± 0.3kg and 3.3 ± 0.5kg, respectively. The gutted fish were manually filleted and skinned within 24 hours after landing and slaughtering. Visible fat was removed from the belly flaps and dorsal fin areas prior to mincing. The minced fillets were stored in plastic bags at -50°C for a total time of 6 months before being analyzed.

3.2 Water and ash content

Water content was determined with a modified version of AOAC 925.08 (Horwitz, 2004) by weighing out approximately 1g homogenized tissue (3 parallels), and then drying the samples at 105°C. Ash content was determined after heating the dried samples in an oven (incinerator) at 500°C for 16 hours (AOAC 983.08) (Horwitz, 2004).

3.3 Lipid content

The extraction of the total lipid was based on the method described by Folch *et al*. (1957), replacing chloroform with dichloromethane for safety reasons. Approximately 1g tissue was mixed with 20ml dichloromethane/methanol (2:1, v/v). The sampling was done in triplicates from each fillet. Heptadecanoic acid (Sigma Chemicals Co, St. Louis, MO, USA) was used as internal standard (IS) and mixed with dichloromethane/methanol (2:1, v/v). The IS was added (200µl) to every sample before 20 minutes of vortexing. The samples were then filtrated and 4ml of 0.9% NaCl added before centrifuging at 2000rpm at 4°C for 10 minutes. The upper phase was then removed before the final filtrate was evaporated using an evaporator (Heidolph Laborata 4000, Büchi Vacuum Controller B-721, Flawil, Switzerland) at 90mbar and 40ºC. Finally the lipid content was determined gravimetrically.

3.4 Lipid composition

The extracted lipids were dissolved to a concentration of approximately 10mg/ml in dichloromethane/methanol (2:1, v/v). These solutions were then methylated as described by

Stoffel *et al.* (1959) with minor adjustments. The solvent (100µl) was mixed with 900µl dichloromethane/methanol (2:1, v/v) and 2ml 2% H_2SO_4 in methanol, and then boiled at 100ºC for one hour. Thereafter, 3.5ml of heptane and 3.5ml 5% NaCl was added, and the tubes were thoroughly shaken before top phase was transferred and evaporated under a stream of nitrogen with an N-EVAP (Organomation Assoc. Inc., Berlin, Germany). The samples were then resuspended in 100µl heptane. A FA profile from all of the samples was obtained by a capillary gas chromatograph, Agilent 6890N equipped with a 7638B auto injector and a flame ionization detector (FID) (Agilent Technologies, Santa Clara, CA, USA). It was collected 1µl from each sample. Helium was used as the carrier gas. The column used was a Varian CP7419 capillary column (50m x 250µm nominal), (Varian Inc., Middelburg, Holland). Injector and detector temperature programme was used to ensure the best possible separation of the FAs (50ºC for two minutes, then 10ºC per min to 150ºC followed by 2ºC per min to 205ºC).

FA composition in farmed and wild Atlantic salmon samples were determined by comparison of chromatographic peak areas and retention time with known FA standards PUFA no 1, PUFA no 2 and PUFA no 3 from Sigma (Sigma Chemicals Co, St. Louis, MO, USA). It was analyzed triplets of each sample. The FA composition of the feed given to the farmed Atlantic salmon was also determined.

3.5 Muscle pH measurement

Muscle pH was measured in a homogenized fish tissue mixed with 0.15M KCl (1:1, w/v), using a pH-meter. The analysis was performed as described by Wang *et al*. (2011).

3.6 Astaxanthine content

The analysis was done after Olsen & Jakobsen (1995). Approximately 2g of homogenized tissue was added 10ml acetone before carefully vortexed for 15 minutes. The samples were then filtrated and absorption was measured by a spectrophotometer (Hitachi U-2001, Mannheim, Germany) at 470nm. Acetone was used as blank sample.

To estimate the amount of astaxanthin in each sample the following formula was used.

$$\text{ppm astaxanthin (mg/kg)} = \frac{OD \text{ x10}\left(\frac{\text{mg}}{\text{ml}}\right)\text{x 30(ml)x 1000}\left(\frac{\text{g}}{\text{kg}}\right)}{E_{1\text{cm}}^{1\%} \text{ x m(g)}}$$

OD = absorption at 470nm

m = sample weight (g)

$E_{1cm}^{1\%}$ = 1900cm^{-1} (extinction coefficient of 1% astaxanthin in acetone) (Foss *et al*., 1984)

The measurement was done in triplicate for all samples.

3.7 Vitamin D_3 analysis

Approximately 2g of homogenized tissue was added to 10ml 5% ascorbic acid in ethanol, 100µl D_3 IS (10ug/ml) and 2ml of 50% potassium hydroxide (KOH). The solution was mixed thoroughly and boiled for 20 minutes or until complete dissolution of the tissue. After cooling to room temperature the solution was transferred to a separating funnel, and 15ml of toluene was added. The solution was then washed with 10ml 1.0M KOH to separate the inorganic phase from the organic phase. The washing process was continued with 5ml 0.5M KOH followed by 3 rounds of 5ml distilled water. Organic phase was transferred and added approximately 2g of anhydrous sodium sulphate for removal of water. An amount of 4ml was evaporated under nitrogen in an N-EVAP (Organomation Assoc. Inc., Berlin, Germany) before dissolved in 0.5ml methanol. This solution was filtered through a 0.45µm PTFE syringe filter prior to HPLC-MS analysis. LC-MS analyses were performed on a Shimadzu separations module equipped with a 2010EV mass spectrometer in *atmospheric-pressure chemical ionization (APCI) mode*, using an Ascentis Express reverse phase C-18 column (Supelco, 30×2.1 mm i.d., 2.7 µm). The compounds of interest were eluted isocratically at ambient temperature using a mobile phase comprising 95% acetonitrile (ACN) with 0.1% trifluoroacetic acid (TFA). Flow-rate was maintained at 0.4 ml/min. Internal standard (deuterized vitamin D_3) and the natural vitamin D_3 were delivered from Sigma (Sigma Chemicals Co, St. Louis, MO, USA).

4. Results

4.1 Composition and pH of fillets

The average total lipid content in minced fillets of farmed Atlantic salmon was almost twice that of wild Atlantic salmon, 11.4% and 6.1% respectively (Table 1). The fillets of farmed Atlantic salmon had a water and ash content of 65.6% and 1.1%, respectively, while values of wild salmon were 71.0% and 1.1% respectively. The muscle pH was 6.18 ± 0.03 and 6.31 ± 0.1 in farmed and wild Atlantic salmon, respectively. The farmed fish feed had a lipid content of 23.8%, and a water and ash content of 6.9% and 5.2%.

Table 1: Proximate composition, % wet weight, and post-rigor pH in minced fillets of farmed and wild Atlantic salmon. The lipid, water and ash content in the feed given to the farmed Atlantic salmon is also included (values are expressed as mean ± SD).

	Wild salmon (n=20)	Farmed salmon (n=20)	Farmed fish feed (n=1)
Water content	70.99 ± 3.62	65.63 ± 2.66	6.96
Lipid content	6.05 ± 2.15	11.43 ± 2.63	23.8
Ash content	1.17 ± 0. 05	1.14 ± 0. 04	5.20
Muscle pH	6.31 ± 0. 1	6.18 ± 0.03*	

*Denotes a significant difference between farmed and wild at $p \leq 0.05$.

In Table 2, the lipid content of each of the individuals from both groups are presented. The content in the fillets of the farmed Atlantic salmon varied from 7.0% to 17.2% fat. In the wild Atlantic salmon the fat content were from 1.2% to 9.7%.

Table 2: The lipid content (% wet weight) of individual farmed and wild Atlantic salmon fillets (each of the individual fillets from both groups are given names from 1 to 20).

Fish fillet sample:	Farmed salmon (%)	Wild salmon (%)
1	12.98	5.15
2	7.96	6.88
3	11.27	6.62
4	10.18	7.08
5	10.97	6.54
6	7.00	3.18
7	13.08	2.80
8	10.01	3.81
9	10.47	9.21
10	13.88	4.78
11	13.07	7.31
12	13.69	4.59
13	8.51	6.89
14	12.64	1.19
15	11.24	9.71
16	11.06	5.57
17	12.36	7.29
18	15.33	8.04
19	17.17	6.93
20	16.46	7.38

The individual pH values are presented in Figure 2. The pH values in farmed Atlantic salmon varied from 6.12 to 6.24. The individual wild Atlantic salmon fillet had a larger variation in muscle pH with a range from 6.17 to 6.52. Wild Atlantic salmon number 15 and 17 had the lowest pH of the fillets, with values of 6.17 and 6.19, respectively.

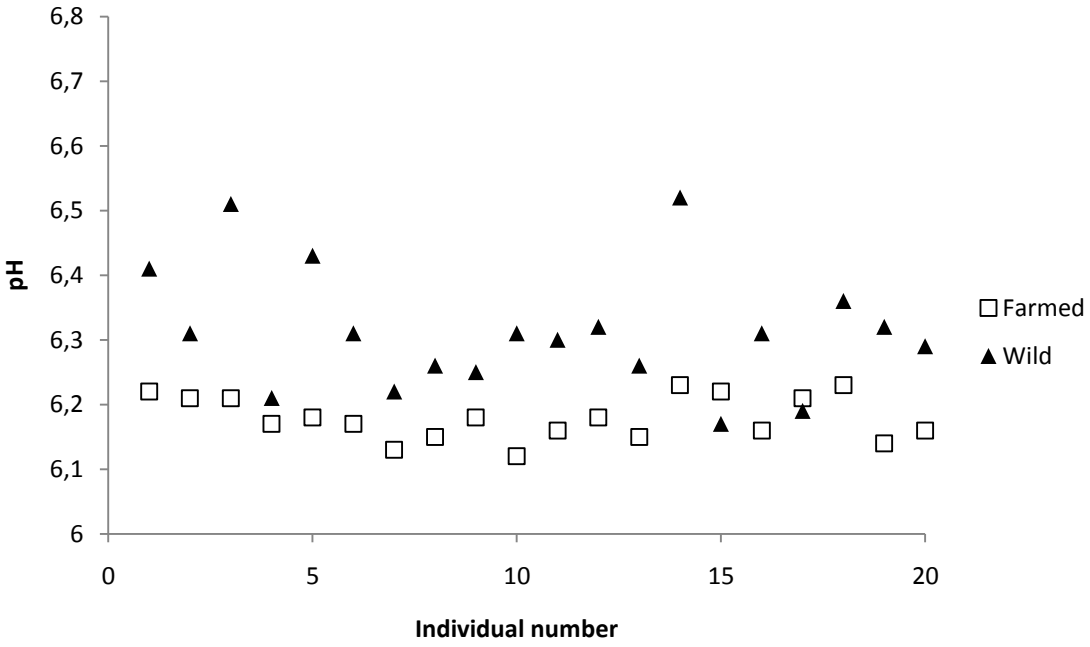


Figure 2: Post rigor muscle pH of the individual farmed (n=20) and wild Atlantic salmon (n=20).

To see if there was any notable correlation between the variations in lipid content and the post rigor pH in wild Atlantic salmon; the results were plotted against each other (Figure 3). There are no specific patterns between the lipid content and pH of the fillets.

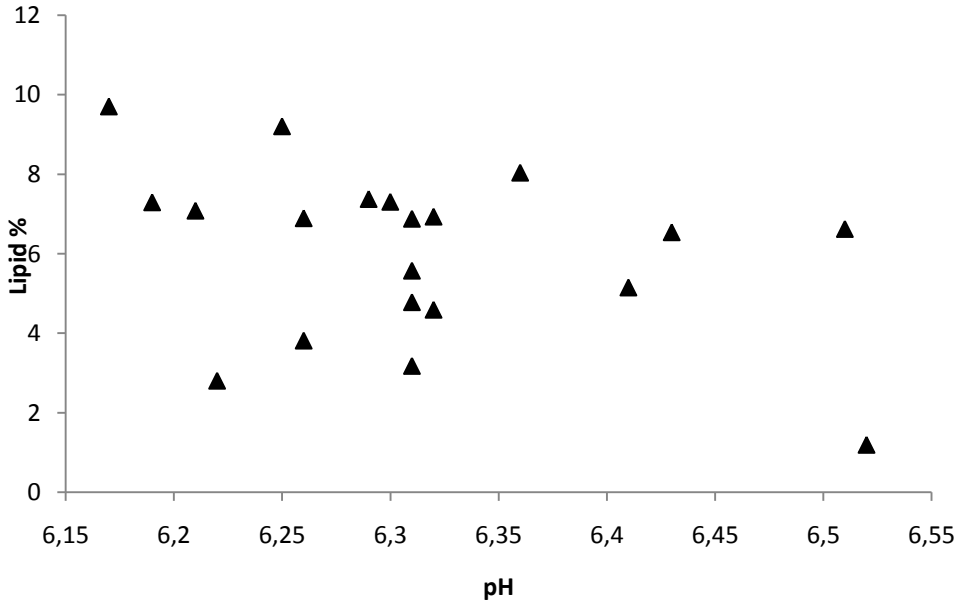


Figure 3: Lipid content of the individual wild Atlantic salmon (n=20) fillets compared with the post rigor pH of the fillet.

4.2 Fatty acid composition and content in farmed and wild Atlantic salmon

The fatty acid compositions (% of total FA) and the total amount of the FAs in g per 100g fillets of farmed and wild Atlantic salmon and of the feed are presented in Table 3. The lipids in farmed Atlantic salmon had a content of oleic acid (18:1n-9), linoleic acid (LA; 18:2n-6) and α-linoleic acid (ALA; 18:3n-3) of 27.6 ± 2.0%, 8.7 ± 0.9% and 3.2 ± 0.4%, respectively. The content of eicosapentaenoic acid (EPA; 20:5n-3), docosapentaenoic acid (DPA; 22:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) were 5.2 ± 0.7%, 2.7 ± 0.9% and 7.6 ± 1.0%, respectively.

Table 3: Fatty acid composition (% of total fatty acids) and total amount of fatty acids (g/100g muscle) in fillet from wild and farmed Atlantic salmon (values are expressed as mean ± SD) and farmed fish feed

Fatty acids	Farmed salmon (n=20)		Wild salmon (n=19)		Farmed fish feed (n=1)	
	Composition	Total amount	Composition	Total amount	Composition	Total amount
14:0	4.38 ± 2.79	0.35 ± 0.16	4.90 ± 1.01	0.17 ± 0.08	4.07	0.46
16:0	12.88 ± 1.35	1.01 ± 0.23	17.49 ± 2.67	0.60 ± 0.25	12.0	1.47
16:1 n7	4.36 ± 0.53	0.34 ± 0.23	5.03 ± 1.46	0.18 ± 0.10	2.66	0.30
18:0	2.99 ± 0.41	0.23 ± 0.06	3.55 ± 0.79	0.12 ± 0.05	2.94	0.34
18:1 n9	27.61 ± 1.98	2.07 ± 0.65	15.86 ± 3.20	0.56 ± 0.28	30.8	3.52
18:1 n7	3.03 ± 0.23	0.24 ± 0.05	3.60 ± 0.78	0.13 ± 0.06	2.26	0.26
18:2 n6	8.72 ± 0.93	0.70 ± 0.17	1.38 ± 0.26	0.05 ± 0.02	9.56	1.09
18:3 n3	3.19 ± 0.44	0.25 ± 0.06	0.85 ± 0.14	0.02 ± 0.01	4.03	0.46
18:4 n3	0.82 ± 0.89	0.11 ± 0.02	1.13 ± 0.38	0.04 ± 0.01	5.83	0.67
20:1 n9	0.55 ± 0.03	0.04 ± 0.02	9.63 ± 2.09	0.34 ± 0.15	0.00	0.00
22:1 n9	1.95 ± 0.31	0.14 ± 0.02	1.88 ± 0.50	0.06 ± 0.02	0.62	0.07
20:5 n3	5.23 ± 0.67	0.42 ± 0.10	5.54 ± 1.09	0.19 ± 0.08	3.90	0.45
22:5 n3	2.66 ± 0.88	0.21 ± 0.23	2.26 ± 0.61	0.07 ± 0.03	1.25	0.14
22:6 n3	7.64 ± 0.97	0.61 ± 0.15	11.34 ± 4.23	0.36 ± 0.13	3.42	0.39
SFA	20.26 ± 3.02	1.60 ± 0.49	24.99 ± 5.31	0.85 ± 0.41	20.21	2.27
MUFA	41.96 ± 3.12	3.32 ± 0.93	43.03 ± 7.51	1.52 ± 0.76	43.29	4.87
PUFA	32.66 ± 4.56	2.60 ± 0.84	21.87 ± 5.91	0.71 ± 0.31	28.40	3.20
n3	23.87 ± 3.95	1.90 ± 0.64	20.56 ± 5.69	0.66 ± 0.28	18.27	2.06
Lc-n3	14.33 ± 2.84	1.23 ± 0.29	19.05 ± 5.62	0.62 ± 0.22	8.63	0.10
n6/n3	0.37 ± 0.10		0.07 ± 0.01		0.53	

The lipids of wild Atlantic salmon contained values of oleic acid, linoleic acid and α-linoleic acid of 15.9 ± 3.2%, 1.4 ± 0.3% and 0.9 ± 0.1%, respectively. Wild Atlantic salmon number 15 which had 5.0% 18:2n-6 (Figure 4) was not included in the table showing the FA

composition. The content of the EPA, DPA and DHA were 5.5 ± 1.1%, 2.3 ± 0.6% and 11.3 ± 4.2%, respectively.

The total amount of long chain n-3 FAs EPA and DHA in g per 100g fillets of farmed Atlantic salmon were 0.42 ± 0.10 and 0.61 ± 0.15, respectively. In wild Atlantic salmon the total amounts of EPA and DHA in g per 100g were 0.19 ± 0.08 and 0.36 ± 0.13, respectively. When including DPA, the total amount of long chain n-3 FAs was 1.23 ± 0.29g/100 g fillet in farmed and 0.62 ± 0.22g/100g fillet in wild Atlantic salmon. The lipids in farmed Atlantic salmon contained 20.3% SFA, 41.9% MUFA and 32.6% PUFA while the values in wild Atlantic salmon were 24.9%, 43.0% and 21.9%, respectively. The n-6/n-3 ratio was 0.37 in farmed Atlantic salmon and 0.07 in wild Atlantic salmon. The lipids in the farmed fish feed had a content of oleic acid, linoleic acid and α-linoleic acid of 30.8%, 9.6% and 4.0%. The amount of EPA, DPA and DHA were 3.9%, 1.6% and 3.4%, respectively. The feed lipids contained 20.2%, 43.3% and 28.4% of SFA, MUFA and PUFA, and had an n-6/n-3 ratio of 0.53.

4.3 Content of linoleic acid (18:2 n-6) in wild and farmed salmon

The individual contents of linoleic acid in farmed and wild salmon are shown in Figure 4. The content of 18:2n-6 in farmed Atlantic salmon varied from 7.9% to 10.1%. The content of 18:2n-6 in 19 of the wild Atlantic salmon fillets varied from 1.0% to 1.8%. One of the wild Atlantic salmon (fillet number 15) had as much as 5.0% 18:2n-6 of total FA.

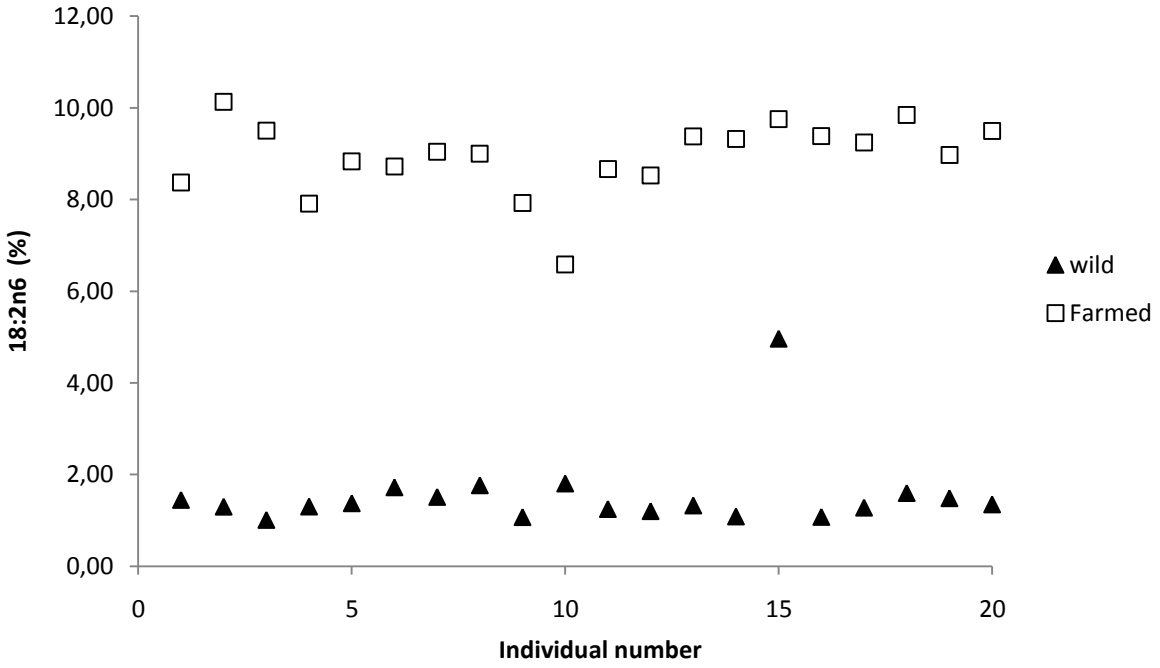


Figure 4: Linoleic acid (18:2n-6) (% of total fat) in fillets of the individual farmed (n=20) and wild Atlantic salmon (n=20).

4.5 Astaxanthin

The astaxanthin content of frozen farmed and wild Atlantic salmon was quite similar with a content of 5.2mg/g and 4.8mg/g sample, respectively (Table 4). Fresh fillet of salmon had almost double the content with 9.45mg/g sample.

Table 4: Astaxanthin content in the fillets of frozen (n=20) and fresh (n=1) farmed Atlantic salmon and frozen wild Atlantic salmon (n=20).

Fish sample	mg/g sample
Wild salmon	4,75 ± 1,63
Frozen farmed salmon	5,15 ± 1,32
Fresh farmed salmon	9,45 ± 0,36

Visible spectra of the extracted astaxanthin were also determined (Figure 5). The spectra from the 2 groups of farmed Atlantic salmon were exactly similar with the major peak at 470nm. Additional peaks were at approximately 965nm, 870nm and 340nm. The spectra from the fillet of wild Atlantic salmon was similar except that the peak near the UV area was at approximately 360nm.

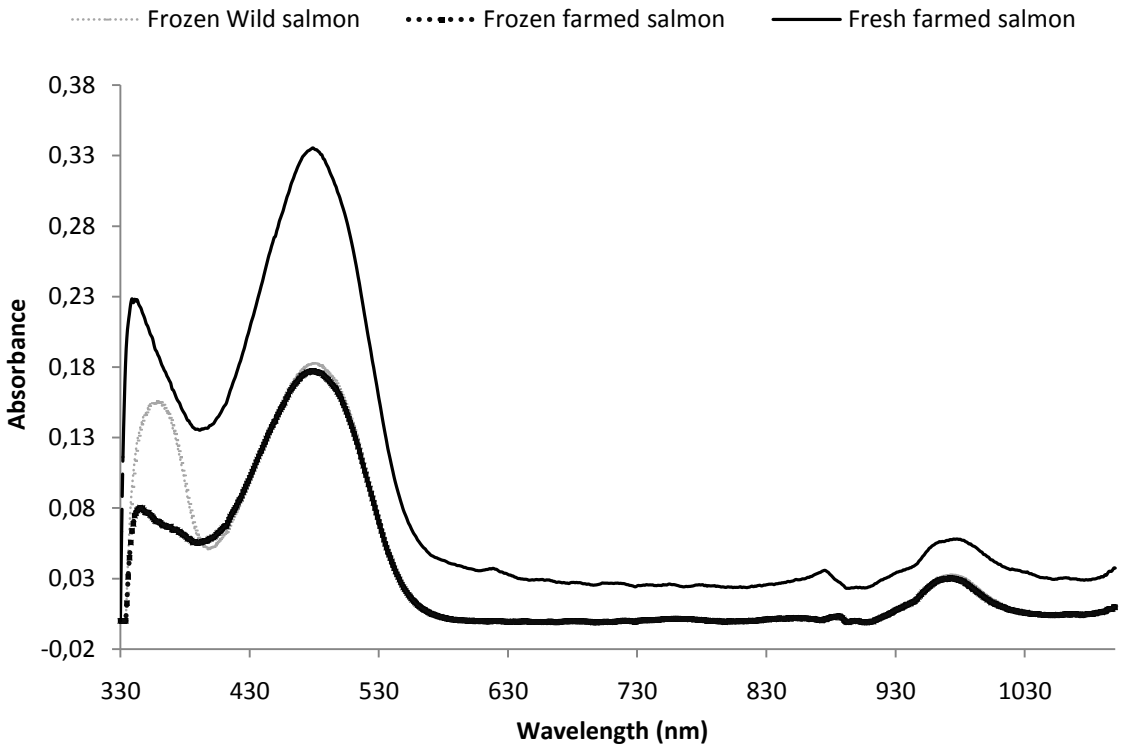


Figure 5: Visible spectra of the astaxanthin extracted with acetone from fresh Atlantic salmon fillets and of thawed fillets of farmed and wild Atlantic salmon after freezing.

The astaxanthin content (mg/g fillet) and the total lipid content (%) of 12 of the individual fillets of farmed and wild Atlantic salmon were plotted against each other in Figure 6. The results were different for farmed and wild salmon. In farmed salmon it may appear that the astaxanthin content which varied from 3.0mg/g fillet to 7.0mg/g fillet had some correlation with the lipid content in the tissue. The astaxanthin content in wild salmon varied from 2.1mg/g fillet to 7.0mg/g fillet and there were no correlation with the lipid content.

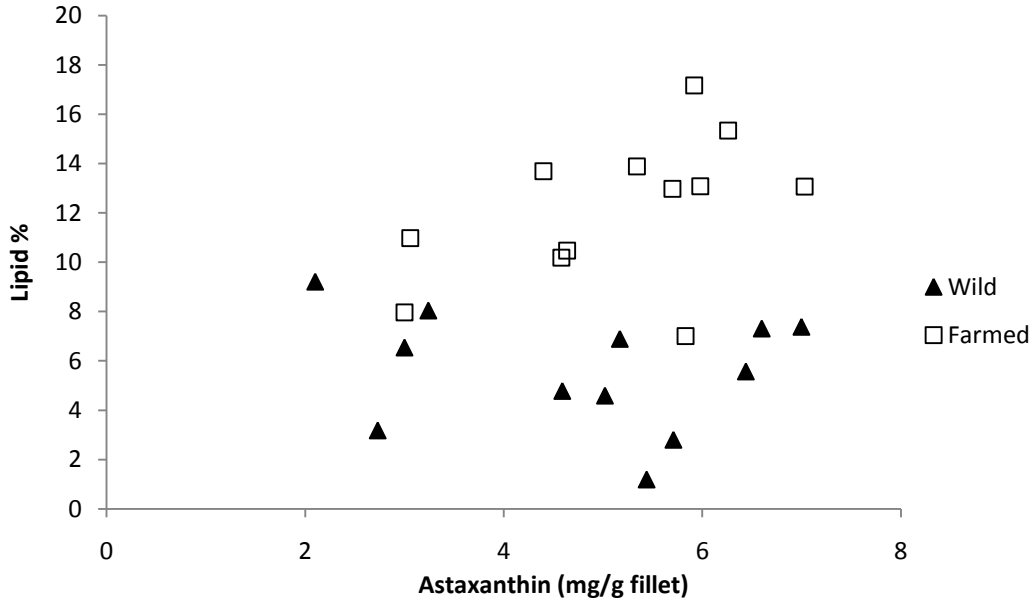


Figure 6: The lipid content plotted against the OD values at 470nm for each of the fillets of farmed (n=12) and wild Atlantic salmon (n=12).

4.6 Vitamin D_3

In Figure 7, a chromatogram where the mass of the internal standard (deuterized vitamin D_3 mass 270Da; pink line) and the content of natural D_3 (mass 267Da; black line) is shown. The peak with retention time (rt) of 2.694 (pink line) shows the signal of the internal standard (deuterized vitamin D_3), and rt 2.781 (black line) is suggested to be natural vitamin D_3.

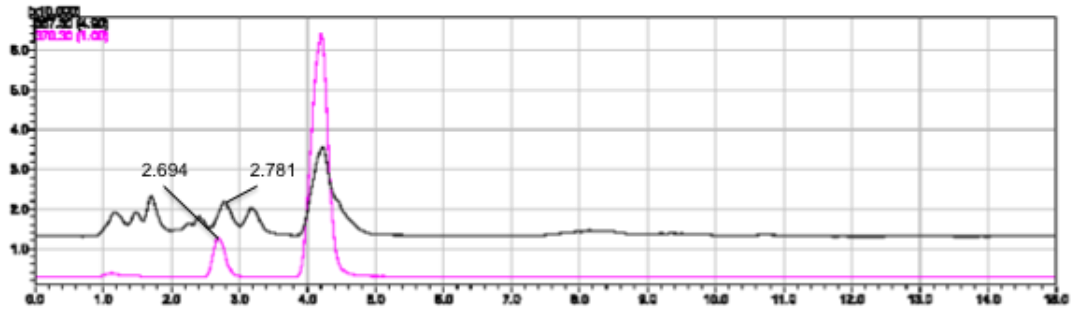


Figure 7: Pink line shows the mass that correspond to the deuterized D_3 (internal standard (IS) 10ng/ml), and black line shows the endogenous (natural) D_3. (X-axis shows minute and Y-axis shows response).

To validate the results the muscle was spiked with 2 levels of natural vitamin D_3. In Figure 8 the sample was spiked with 0.1ng of natural D_3 (along with internal standard (10ng/ml) before

extraction. The rt was not influenced by the spiking of the sample with natural 0.1ng vitamin D_3. The internal standard had an rt of 2.713 while the natural vitamin D_3 had 2.779.

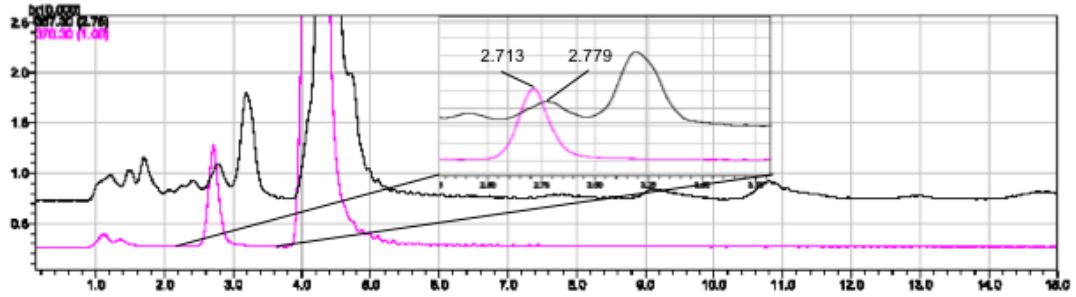


Figure 8: Spiking the sample with 0.1ng (natural) D_3 before extraction (along with 10ng/ml IS). (X-axis shows minute and Y-axis shows response).

However, using 1ng natural vitamin D_3 gave a different result. (Figure 9). The internal standard had a rt as in the previous runs; 2.705, but the natural standard had a lower rt of 2.734.

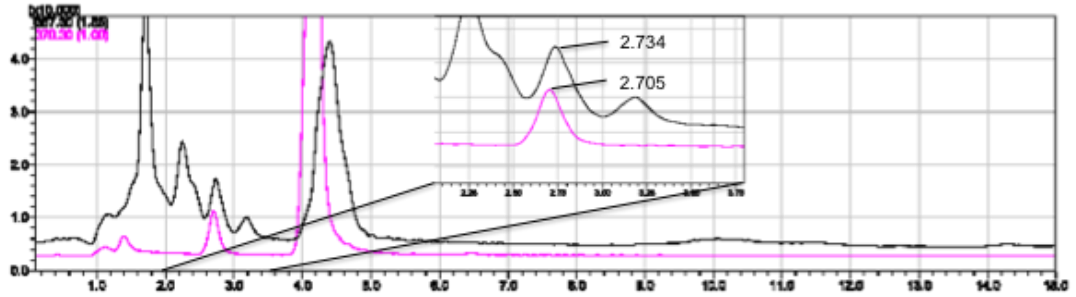


Figure 9: Spiked sample with 1ng of natural D_3 and internal standard (10ng/ml). (X-axis shows minute and Y-axis shows response).

5. Discussion

5.1 General discussion

Academic and public interest concerning the nutritional quality of farmed fish compared to wild fish of the same species has increased as the aquaculture industry now contributes to almost half of the fish and fish products consumed worldwide. The increased attention on the subject has spurred many publications and the nutritional quality concerning fatty acids, protein and other essential nutrients have been measured and reported from several cultivated species and their wild counterparts. Amongst other, Atlantic halibut (Olsson *et al.*, 2003), sea bass (Periago *et al.*, 2005; Fuentes *et al.*, 2010), Atlantic salmon (Hamilton *et al.*, 2005; Johnston *et al.*, 2006; Farrell *et al.*, 2010), turbot (Sérot *et al.*, 1998) and rainbow trout (Blanchet *et al.*, 2005).

One of the aims of this study was to determine the total lipid content in farmed and wild Atlantic salmon. As shown in the results (Table 1), the fillets of farmed Atlantic salmon had an average of total lipid content of 11.4% which is much higher than the fillets of wild Atlantic salmon which only had an average lipid content of 6.0%. The high lipid content found in the tissue of farmed Atlantic salmon compared to wild specimens reflects the high lipid content of the formulated fish feed and perhaps most important, *ad libitum* access to feed for the farmed fish. Reasons why the lipid content in the tissue of farmed fish is lower than presented in the feed, is because some lipids in the feed are used as an energy source through β-oxidation. This is explained more thoroughly in the FA composition section. Previous studies have also concluded with higher lipid content in the tissue of farmed fish compared to wild. Olsson *et al*. (2003) found that farmed and wild Atlantic halibut had a fat content of 3.4-7.4% and < 0.2%, respectively. In previous studies of farmed and wild Atlantic salmon, Bell *et al.* (1998) reports a fat content of 11.3% in farmed and 5.4% in wild Atlantic salmon, while Hamilton *et al.* (2005) found a fat content of 16.6% and 6.4% in farmed and wild Atlantic salmon. Furthermore, Bendiksen *et al.* (2011) found a fat content of 17.2% (Norwegian Quality Cut) in farmed Atlantic salmon. However, Blanchet *et al.* (2005) found no difference in fat content of farmed and wild Atlantic salmon. The opposite does also occur; in sea bass Periago *et al.* (2005) found a fat content of approximately 6.7% in farmed and 9.2% in wild specimens. There were quite large variations in the individual lipid content of the fillets of both farmed and wild Atlantic. The lipid content of the farmed Atlantic salmon ranged from 7.0% to 17.2%, while the total lipid content of wild Atlantic salmon had a range from 1.2% to

9.7%. These individual differences may be explained by the nutritional status of each individual. However, the individual differences in farmed Atlantic salmon fillets are very surprising since they are all given the same access to feed. Explanations could be hierarchy or differences in appetite. The variation in the wild salmons fillets can may be due to individual feed excess, or perhaps appetite.

The higher water content in fillets of farmed Atlantic salmon is due to the difference in total lipid content. Water and fat content constitutes approximately 80% of total body weight in most fish species (Love, 1988), and if the lipid content goes up the water content goes down and vice versa.

The protein content was not determined; however it is well known that farmed fish has higher protein content than wild fish because of high availability of feed.

Post rigor pH was measured in both groups. The farmed Atlantic salmon had a marginally significant lower muscle pH then the wild Atlantic salmon, with values of 6.18 and 6.31, respectively. A difference between farmed and wild fish has previously been observed in other fish species (Orban *et al.*, 2002; Periago *et al.*, 2005; Olsson *et al.*, 2007; Fuentes *et al.*, 2010). The difference in post rigor pH may be due to the difference in the access of feed in the two groups. In intensive farming the fish has access to unlimited amounts of feed, which leads to a high muscle glycogen level, and subsequently a low ultimate muscle pH post rigor due to anaerobic degradation of glycogen after slaughtering (Ofstad *et al.*, 1996). Whereas the muscle glycogen levels in the wild specimens depends on the availability of food in the marine environment, leading to a lower glycogen level. It has also been reported seasonal variations in muscle pH in farmed salmon (Lavèty *et al.*, 1988). In studies of farmed and wild Atlantic cod the difference in post-rigor pH was much higher (Kristoffersen *et al.*, 2006) than observed in this present study, which may be due to species differences or that the wild Atlantic salmon also was in almost the same good biological conditions as the farmed Atlantic salmon. The relatively high fat content may indicate this.

To see if there was any correlation between the total fat content in the tissue and the large variation of pH in wild Atlantic salmon the values were plotted against each other in Figure 3. From this figure we could not see any specific patterns that could explain the variations in pH values depending on the lipid content. A possible explanation for the large variation in pH might be difference in nutritional status when captured and slaughtered in addition to basic individual differences.

The strong relationship between dietary FA and tissue FA composition is well documented (Bell *et al.*, 2001; Blanchet *et al.*, 2005), and therefore it is expected that the FA composition of the feed is assimilated into the tissue of the fish in proportion to dietary content, minus any amount used for energy through β-oxidation. In tissue of adult Atlantic salmon mitochondrial β-oxidation dominates, while peroxisomal β-oxidation controls in the liver (Henderson, 1996). In fish, mitochondrial β-oxidation prefers SFA 16:0 and MUFAs such as 18:1n-9, 20:1n-9 and 22:1n-11 instead of PUFA and EPA which is in general oxidized to a lesser extent. DHA is selectively concentrated in the tissue rather than being a substrate for oxidation (Sargent *et al.*, 1989; Tocher, 2003).

The FA composition of both groups were measured and determined by comparison with known fatty acid standards delivered from Sigma and reported in Table 3. In addition the FA composition of the formulated fish feed given to the farmed fish was analyzed. The SFA and MUFA composition (%) were slightly higher in wild Atlantic salmon compared to farmed Atlantic salmon, whereas farmed specimens showed a higher content of PUFA. Palmitic acid (16:0) was the primary saturated fatty acid in all samples from both groups, followed by myristic acid (14:0), these contents being higher in wild fish. Oleic acid (18:1n-9) was identified as the major MUFA in both farmed and wild Atlantic salmon, the content being higher in farmed specimens. The high amount of oleic acid (27.6%) in farmed samples could be due to its dominance in the formulated feed, which had oleic acid content.

With regards to PUFA, Atlantic salmon is considered as a good source of Lc-n-3 FAs, particularly EPA and DHA. The percentage of EPA and DHA in the two groups was quite similar, with a slightly higher percentage of DHA in the wild samples, but the concentration of this HUFA is higher in the farmed fish on an absolute basis (g/100g of tissue) because of the higher total lipid content. As a result of this one serving (200g) of farmed salmon contain approximately 2.40g of Lc-n-3 PUFA, which constitute to almost five times the recommended daily intake of these FAs. This result agrees with several other studies (Bell *et al.*, 1998; Blanchet *et al.*, 2005; Hamilton *et al.*, 2005). Bell *et al*. (1998) found that the absolute concentration of Lc-n-3 PUFA in farmed salmon were twice the amount of wild, while Blancet *et al.* (2005) reports the same Lc-n-3 PUFA content in both farmed and wild Atlantic salmon. Furthermore, Hamilton *et al.* (2005) found an Lc-n-3 PUFA content in farmed Pacific salmon that was twice and sometimes three times the values of wild Pacific salmon.

The reason why DHA tends to be conserved is, as mentioned earlier, primarily due to it being a relatively poor substrate for β-oxidation (Sargent *et al.*, 1989). Furthermore, the

farmed samples show comparatively high levels of ALA (3.2%) in the fillet. This is just slightly lower than the content of ALA in the formulated fish feed that had ALA values of 4%. This FA is accumulated largely unchained in the lipids of marine fish due to their reduced capability to chain elongation and desaturation.

In general, the total amounts of LA found in FOs are less than 2%, but in the formulated feed given to the farmed Atlantic salmon, LA constituted with 9.6% of the total FA. This indicates that there is an inclusion of plant ingredients (VOs) in the formulated feed. LA is a major constituent of corn, rapeseed and soybean oils typically used as component in the formulated feed. Plant meal also contains fat, approximately 10% of that in fish meal. The different levels of LA in dietary FOs and VOs reflects the amount of LA found in the tissue of farmed and wild Atlantic salmon which were 8.7% and 1.4%, respectively. A previous study also found the percentage of LA to be higher in farmed then in wild fish, but with only half the total amount of lipids (Blanchet *et al.*, 2005). Reasons why this n-6 FA tends to be stored in the tissue of marine fishes is because of reduced or lack of capability to elongate and desaturate LA into ALA. This is confirmed by the fact that arachidonic acid (20:4n-6) which is formed from 18:2n-6, is not detected in the farmed Atlantic salmon.

With an inclusion of plant materials in the formulated fish feed the FA composition in the diet tends to have a lower amount of Lc-n3 PUFAs, and a higher amount of n-6 PUFA. This results in a higher n-6/n-3 ratio in the tissue of the fish. The n-6/n-3 ratio was higher in farmed than in wild Atlantic salmon, with a value of 0.37 and 0.07 respectively. The higher n-6/n-3 ratio of farmed fish leads to a reduction of the nutritional quality in the lipid components of farmed Atlantic salmon. This may be due to, as mentioned in the introduction, the harmful effects of a high dietary n-6/n-3 ratio. The same results in FA composition are found in other farmed and wild fish species such as Atlantic halibut (Olsson *et al.*, 2003). For health reasons for the consumer a lowest possible dietary n-6/n-3 ratio is preferred (Simopoulos, 2008), and in a healthy diet the n-6/n-3 ratio should be lower than 5 (Kris-Etherton *et al.*, 2009). Based on this it is possible to conclude that the farmed Atlantic salmon constitute to lower the dietary n-6/n-3 ratio.

The higher percentage of LA in the tissue of the fish is a brilliant marker for distinguishing farmed and wild salmon, based on present feeding practices (Megdal *et al.*, 2009). Through the FA composition determination it was detected a much higher LA content in the tissue in one of the wild Atlantic salmon fillets (number 15), with a LA value of 4.97% (Figure 4), whereas the average content of LA in farmed and wild Atlantic salmon were as

mentioned, 8.72% and 1.38%, respectively. Earlier studies have shown that it takes months for the fatty acid profile to change, and that escaped farmed salmon may have a FA profile with a ratio between what's typical for farmed and wild salmon (Megdal *et al.*, 2009). Thus, it is presumed that this particular fish is an escaped farmed fish. The pH profile of this exact sample somewhat ambiguous as well. The total lipid content of fillet number 15 was quite high with a total content of 9.7%. This is higher than the average total lipid content of the wild Atlantic salmon on 6%. But there were also other samples from the wild Atlantic salmon group that had higher lipid content than average.

The content of Astaxanthin in the flesh of farmed and wild Atlantic salmon was also determined. The results showed no difference in the two groups. This may indicate that the wild Atlantic salmon had a good access to feed. In addition the high levels of astaxanthin in the tissue implies that the fish was not sexually mature. Other studies done on the subject did find a higher carotenoid pigment concentration in farmed Atlantic salmon (Johnston *et al.*, 2006). Because this study compared fish from only two different sources the results cannot represent all farmed and wild Atlantic salmon since results observed might be due to location. (Farmer *et al.*, 2000)

The levels of astaxanthin in the fresh fillets were almost twice that of frozen which gives the fillet a much brighter pink-red color compared to the frozen fillets. The color differences may be explained by the ability of astaxanthin to function as an antioxidant when the muscle is exposed to oxidation which often happens through prolonged storage of fillets, particular if it is minced.

As seen in Figure 5 the peak near the UV area is of the wild Atlantic salmon around 360nm, while this peak is around 340nm in frozen and fresh farmed Atlantic salmon. These differences may be caused by the amount and type of pigmentation source in the diet. As mentioned in the introduction, wild Atlantic salmon obtain astaxanthin by consuming prey organisms such as zooplankton and crustaceans, while farmed Atlantic salmon get the favorable pink-red color through synthetic astaxanthin and/or cantaxanthin incorporated in the formulated feed. The exact type of carotenoid presented in the flesh of the fish is not possible to determine using this method based on Wang *et al.* (2011). Some studies have also shown that coloration of the flesh in affected by rearing temperature (Fuentes *et al.*, 2010)

The astaxanthin levels and the lipid content of each of the individual fillets from both groups were plotted against each other in Figure 6. The plot shows that many of the individuals of farmed Atlantic salmon with high lipid content also had the highest amount of

flesh carotenoid. This may indicate a correlation between astaxanthin level in the tissue and fat content. But it is not possible to come to any conclusion without further examination.

When determining the vitamin D_3 content in the farmed and wild Atlantic salmon we met some obstacles. The results are very ambiguous and we can therefore not make any conclusion. Spiking of muscle with a high amount of internal D_3 did not confirm the rt of compound believed to be vitamin D_3 in the non-spiked muscle. This suggests that the initial peak believed to be vitamin D_3 may be a different compound or that vitamin D_3 was co-eluting with another compound. Due to time constrains this could not be investigated further.

5.2 Conclusion

The fat content of farmed at salmon demonstrate that it is produced under intensive conditions with free access of feed. The substitution of marine ingredients with plant meal and oils clearly affects the fatty acid composition of the farmed salmon. Linoleic acid (18: 2n-6) which is a signature fatty acid for plant oils, constitute almost 9% of fatty acids in the farmed salmon. The concentration of long chain n-3 PUFA are reduced compared with wild Atlantic salmon.

However, the high fat content makes the farmed salmon an excellent source of these health promoting fatty acids. Dependent on the suggested daily requirement of EPA and DHA, 20-50g of farmed Atlantic fillet is sufficient to satisfy the daily needs. In addition the ratio between n-6 and n-3 is very low and well below the value often recommended by nutritional experts.

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