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Quality of raw and cooked fillets of Atlantic salmon farmed in the Arctic: effect of dietary oil and thawing temperature.

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By

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ABSTRACT

The fatty acid content in fillets of Atlantic salmon cultured in the Arctic and fed diets containing varying levels of EPA and DHA (low, medium or high) was assessed. Also, dietary effects on fillet color and liquid holding capacity were investigated and analyses were performed in different areas of the salmon fillet in both raw and cooked products. The fillet fatty acid composition, texture, and color of thawed (4ºC and 20ºC) and cooked fillet of the various dietary groups were determined. Gutted weight (kg), length (cm), condition factor and fillet yield (%) were higher in the S1s (smolts transferred to seawater in spring) than S0s (underyearlings transferred in autumn). C18:1n9c (oleic acid) had the highest concentration with TestS showing higher concentration than TestP for the S0 salmon. Increasing dietary inclusion of EPA & DHA increases the fillet EPA and DHA composition in both S0 and S1 groups. TestS had a higher color score than TestP with SalmoFan scores for ventral fillet region (thawed at 4ºC and cooked). Thawing at 20ºC increased driploss in TestS than TestP. Temperature, time of transfer to sea water, sampling time and point accounted for most of the differences seen in the diets of the S0s or S1 salmon.

1 INTRODUCTION

Fish plays an important role in global nutrition and health by providing critical nutrients including proteins (FAO, 2020; Finegold, 2009; Tacon & Metian, 2013) and essential fatty acids, particularly n-3 long chain polyunsaturated fatty acids such as eicosapentaenoic (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) (Ghasemi Fard et al., 2019). Aside from its essence in human diets, fish oil has been a major ingredient in fish feeds for carnivorous fishes like salmonids owing to its richness in the polyunsaturated fatty acids (Nasopoulou & Zabetakis, 2012). Dwindling fish stocks resulting from the proliferation of unsustainable fishing over the last four decades (Kituyi, 2020) and exacerbated by changing climates (Rhodes & Tupper, 2007; Mohanty et al., 2010) as well as the high demand for fish for human consumption (Delgado et al., 2003, Merino et al., 2012) is, however, expected to have a consequentially negative impact on the availability of healthy oils from fish for human consumption and in fish feeds. Thus, the need for alternative oil sources that can replace fish oil in fish feeds has become a topical issue (Bell et al., 2004a; Morais et al., 2012; Wan et al., 2019).

Because of the limited availability and high prices, the level of fish oil in salmon diets has decreased gradually during the last two decades, to a current level of 10% in Norwegian salmon feeds, while the inclusion of rapeseed oil has increased (Aas et al., 2019). The reduced marine fish oil inclusion has resulted in decreased levels of the healthy EPA and DHA, while the content of omega-6 and monounsaturated fatty acids such as C18:1n-9 have increased in salmon feeds. Because the fatty acid composition of salmon fillets reflects the fatty acid composition of the feed, the edible salmon tissue has changed significantly. Studies on the effects of fish oil substitution by plant vegetable oils like the rapeseed, soybean, linseed oil (Bell et al., 2001a) have predominately focused on smaller fish and response parameters such as growth and health, while effects on fillet quality in slaughter sized fish are limited. Moreover, available studies on fish oil substitution by plant oils have used diets with higher inclusions of fish meal compared with current levels, and no studies have reported fish oil substitution by plant oils in salmon farmed in Arctic areas.

a. Important fillet quality parameters in salmon include nutritional composition and sensory quality, where the fillet color has a profound effect on consumer preferences (Alfines et al., 2006; Anderson, 2001; Steine et al., 2005). Moreover, the liquid holding capacity is important both for the economy, the appearance and the juiciness of the product. It is well

documented that nutritional composition and color varies among different parts of the salmon fillet. The fat content is highest in the belly area while the color intensity is highest in the tail part (Hikima et al., 2017; Munoz et al., 2007). Knowledge on how dietary fatty acid composition affects the sensory and technical quality of different fillet areas is warranted. Therefore, the aim of this thesis was to assess the fatty acid content in the fillets of Atlantic salmon farmed in Artic and fed diets containing varying levels of EPA and DHA. Dietary effects on fillet color and liquid holding capacity were focused and analyses were performed in different areas of the salmon fillet in both raw and cooked products. Moreover, effects of frozen storage were analyzed in fillets thawed as different temperatures ($4^{o}C$, $20^{o}C$).

2 THEORITICAL BACKGROUND

2.1 Aquaculture

The term aquaculture entails water (aqua) and culture, which is rearing of plants or animal species that thrive in water (Schnick, 2001; Stickney, 2000). Worldwide, there are more than 220 species of finfish and shellfish**,** which includes oysters, clams, shrimps, finfishes like tilapia or salmon, etc (Naylor et al., 2000).

Aquaculture has gained popularity in recent times due to the major contributions it makes towards food protein especially when capture from the wild has not been consistent and thus, creating a gap between high consumer demand and limited supply of marine protein.

Recent reports from FAO (2020) suggest that fish consumption globally has grown at an average yearly rate of 3.1% between 1961 to 2017, a figure that is almost double of the world population growth per year (1.6%) for the same period, and higher than that of all other protein nourishment from animals i.e., meat and dairy.

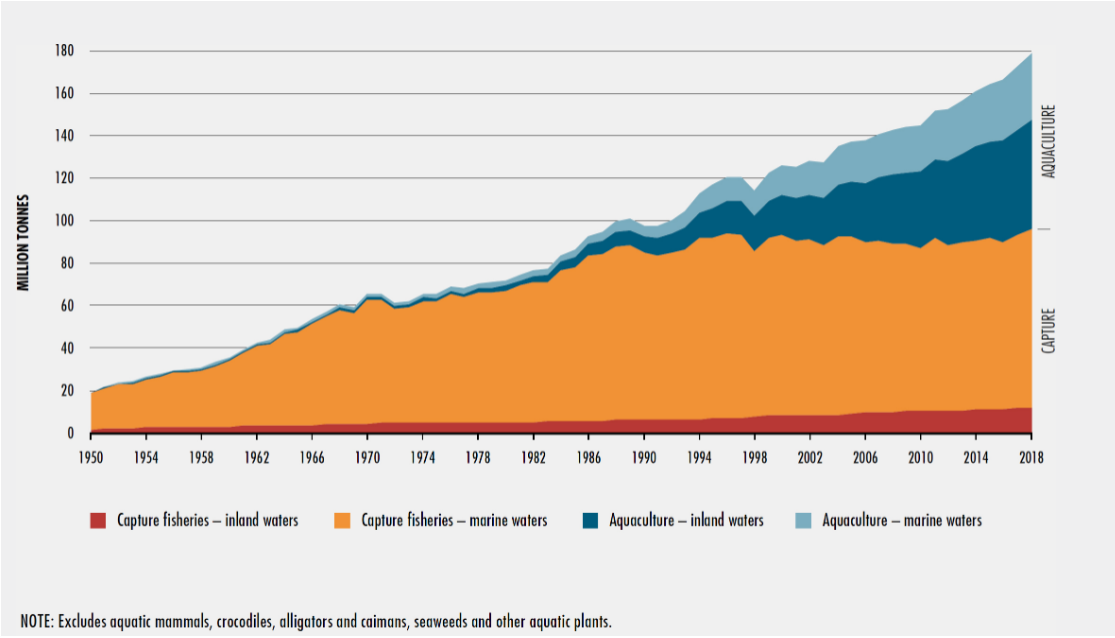

Figure 2.1. Capture fisheries versus aquaculture production worldwide (Adapted from FAO, 2020).

Based on these figures, one can arguably say that aquaculture has become the fastest growing food production sector worldwide. This shows the pace at which it contributes to world fish supplies over the years and it is still rising. World aquaculture contributes to approximately 60 million tons of seafood, a value worth more than US$70 billion yearly (Turchini et al., 2009). This value is expected to increase because of dwindling stocks from capture fisheries and ever-increasing total and per capita marine food consumption.

Globally, the fast expansion of aquaculture industry results in an increase intensification of culture systems and use of manufactured aquaculture feeds. Estimates suggest that 67% of all fish oils produced in 2012 were used solely for fish feed formulation as a lipid source (Ytrestøyl et al., 2015)

In salmonids feed, scientific research in 1990 showed that 90% of the ingredients for feed was from capture fisheries**,** however**,** this dropped to 30% in 2013. The total amount of marine ingredients incorporated in salmon feed production was reduced from 544,000 to 466,000 tons. This is mainly due to alternate source of planted-based oils to substitute fish oil for sustainability of the marine environment.

2.2 Salmon life cycle

The salmon is a carnivorous anadromous fish species which migrate from sea to freshwater to spawn (Schtickzelle & Quinn, 2007) and thus, complete their life cycle. Naturally, the salmon takes between 3 to 4 years to complete its life cycle, however**,** due to genetics and breeding programs, the life cycle in salmon farming has been reduced to about 1 and half years. It thrives in cold regions; with optimal growth in water temperatures below 14ºC (Ytterborg & Falconer, 2020).

In Norway, the most cultured salmon species is the Atlantic salmon (*Salmo salar*) (Heldal et al., 2019). Other cultured species include Pacific and Coho salmons. Salmon culture in Norway normally involves about 3 to 4 stages, which are broodstock/hatchery, smolt and ongrowing farm and lastly slaughtering.

After the alevin stage (Figure 2.2), the fish are transferred to bigger tanks for start feeding to commence. Smoltification is a complex series of physiological changes where young salmonid

fish adapt from living in fresh water to living in seawater. Smoltification has three primary triggers: a large increase in day length, an increase in temperature, and an increase in nutrient availability from increased feeding. Of these, an increase in day length is the most important (Towers, 2016). In aquaculture, it is possible to manipulate the photoperiod and temperature to control the time of smoltification. Salmon that are transferred to seawater during spring are termed S1 smolts, while underyearlings that are transferred to seawater during Autumn are termed S0 smolts**,** meaning they use less than a year for the smoltification. Prior to the sea transfer, the fish are vaccinated and undergo a sea test (Blackburn & Clarke, 1989 as cited in Noble et al., (2018) just for the farmer to be sure they are ready for sea (Lekang, 2007).

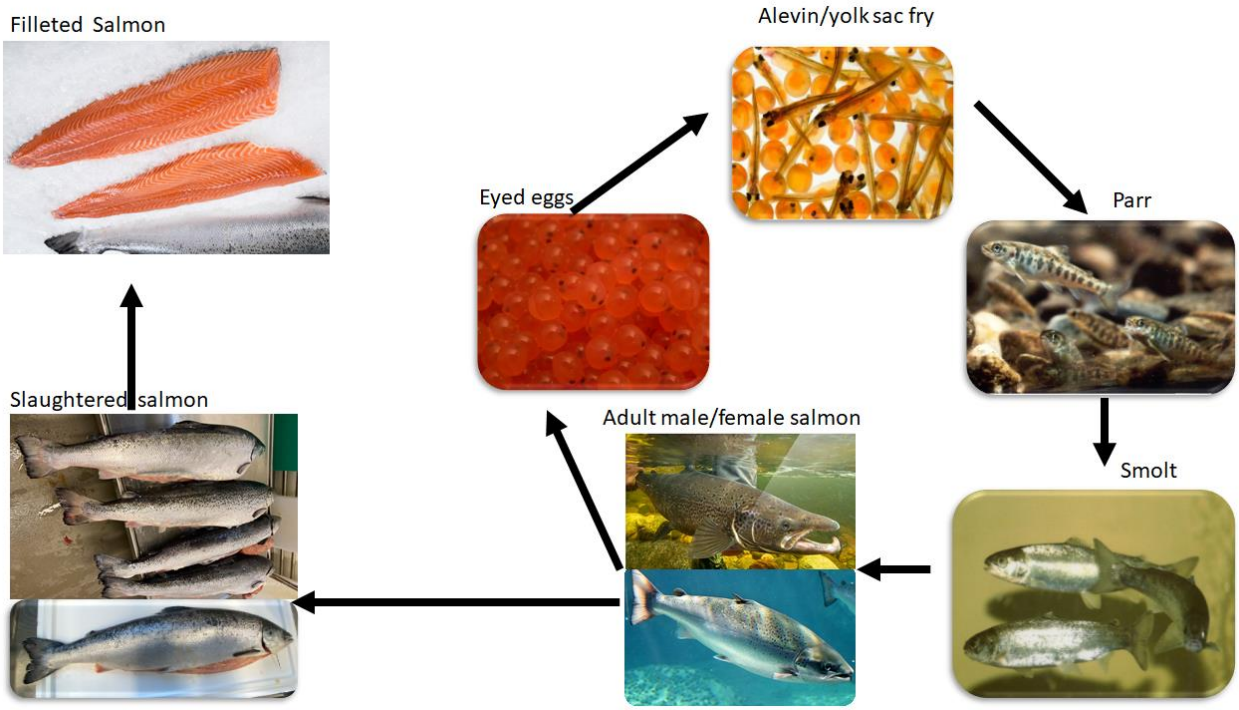

Figure 2.2. Typical life cycle of Salmon, from the egg stage to matured adults.

2.3 Post harvest processing

2.3.1 Evisceration and Filleting

This is part of primary processing which is defined as the first steps used to convert fish into attractive form for consumers. Evisceration involves removal of the intestines, visceral parts to enhance the shelf life of the product and make it hygienic. Normally farmed fish are starved for a few days prior to slaughter (Ginés et al., 2002), to reduce the activity of digestive enzymes which

can cause spoilage. Filleting involves the removal of bones from the flesh of fish. This can be done by machines or manually by hand.

2.3.2 Gaping

Gaping is a big issue with regards to fillet quality and refers to slits seen in the flesh of the filleted fish. Gaping can occur when there is poor handling, or severe stress during harvesting. Fillets with gaping are unattractive to consumers. Gaping severity is often scored visually according to a scale ranging from 0 to 5 (Andersen et al., 1994), where score 0 is no gaping and score 5 is severe gaping.

Figure 2.3. Salmon fillet with severe gaping (Adapted from Xelect, 2019).

2.3.4 Texture

Fish texture forms one of the important intrinsic traits when it comes to fish quality, and is influenced by species, age and size of fishes within same species and diet (Dunajski, 1980). Also, postmortem process such as rigor mortis development, glycolysis, storage and cooking temperature may affect textures of fish.

According to Johnston (2001) architecture and structure of fish muscle determine its textural characteristic specifically the connective tissue matrix and the muscle fibers (raw fish). Muscle fibers are subject to continuous changes as a result of growth and are divided into two main types: red/slow and white/fast (Luna et al., 2015). The former has its name red because there is presence of well-developed blood supply with high amount of myoglobin (Greek-Walker & Pull, 2006). The white muscles, on the other hand, do not have well developed blood supply, hence is powered by anaerobic metabolism.

Numbers of slow/red muscles are known to increase with length while the white reaches a limit and can grow only through exercise and continuous use of muscles (Johnston et al, 2006). Meaning that if continuous growth occurs via feeding, genetics and environmental conditions**,** it influences the texture of the raw salmon fillet.

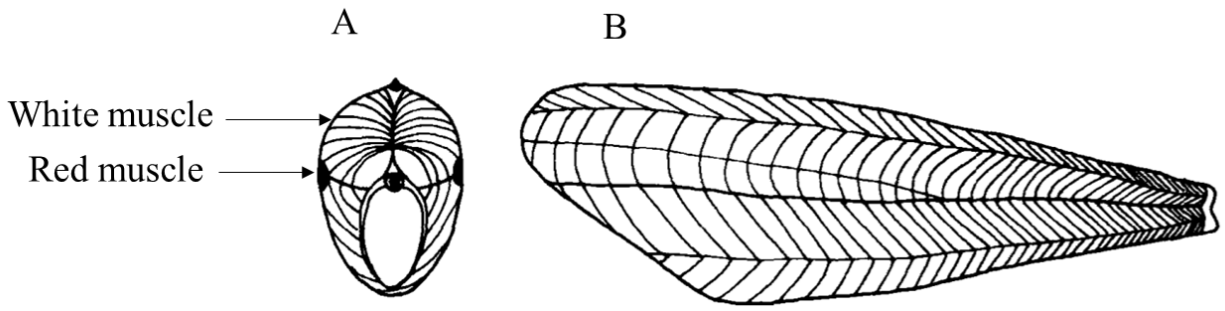

Figure 2.4. The metameric structure of fish muscles: The cross section of the fish muscle (A) and longitudinal (B) sections represent arrangement of layers of connective tissue in the muscles (Adapted from Dunajski, 1980).

2.3.5 Fillet color

The flesh of many fish species ranges from white to yellowish and orange in others. Salmon and the rainbow trout have orange or reddish flesh color which is highly sought after by consumers. (Hutchings, 1999) described it as an organoleptic property on which food products are accepted.

The quantity or degree of the orange colour of salmon is predominately dependent on the astaxanthin and canthaxanthin contents in the flesh (Baker et al., 2002). This in turn is influenced by the amount in feed, and the amount utilized by the fish for pigment deposition in eggs or skin during maturation in females and males**,** respectively.

In the salmon industry, several ways to measure the fillet colour exist although the most widely used is color charts. They are used to visually assign numbers to fillets of salmon, and it is the SalmoFan™ color measurement scale by DSM that is recognized as the industry standard across the world (Figure 2.5). Each colour of the SalmoFan™ has a number beginning from 20 (very pale red) to 34 (very intense red) The area used mostly to assess the fillet colour of salmon is dorsal region on the cutlet between the posterior end of the dorsal fin and the gut. This area is termed The Norwegian Quality Cut (NQC). Some factors like light intensity amongst others affect the reading of colors and should therefore be standardized.

Figure 2.5. The SalmoFan™ color measurement scale by DSM with the varying degree of colours assigned numbers (Adapted from Burros, 2003).

2.4 Lipids

These are a heterogenous group of compounds that are soluble in organic solvents and not water, hence termed hydrophobic. They include triacylglycerols, wax esters, sterols, phospholipids and are constituents of dietary fat. Lipids are required by fish because they are readily energy sources, bio membrane structural components, carriers of vitamins that are fat-soluble, precursors to eicosanoids, hormones and vitamin D, and as enzyme co-factors (Higgs & Dong, 2000). In fish, dietary lipids are an important source of essential fatty acids for regular growth, health, reproduction and bodily functions.

The roles of lipids in fish nutrition have become more important in recent years given the production and implementation of high-lipid, energy-dense diets. Improvements in growth, feed utilization efficiency and nutrient retention in fish fed such energy-dense diets (Bell et al., 2002a) benefit not only the fish farmer by giving a shorter grow-out period, but also the environment. Lipid levels as high as 40% are currently used in commercial salmon feeds.

Triglycerides form nearly 99 percent of the lipids that are reserved in the body and those found in foods. They are immediate sources of energy forms when needed by the body. Phospholipids on the other hand are different form triglycerides by the chemical makeup, in the sense that they consist of three fatty acids and a glycerol, but the former replaces a phosphate molecule for one of

the 3 fatty acids. Phospholipids form the membrane that make up the outer layer of the cells. They play a key role in determining what enters and exits every cell.

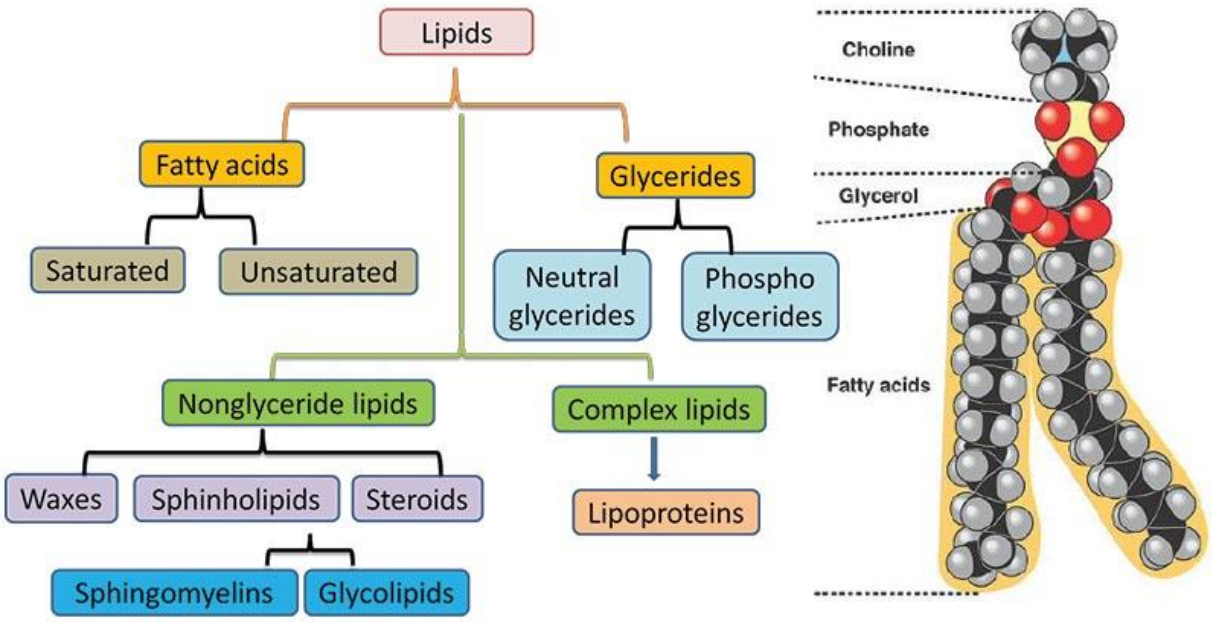

Figure 2.66. Constituents of lipids with further sub-categories (Adapted from Aryal, 2018).

2.5 Essential fatty acids

Essential fatty acids (EFAs) are FA that living organisms must ingest because the body requires them for good health but cannot synthesize them (Goodhart et al. 1980). The essential fatty acids are polyunsaturated fatty acids (PUFAs), which means that they have two or more double bonds in their chemical composition.

All vertebrate species, including fish, have an absolute dietary requirement for both n-6 and n-3 PUFAs. The biologically active forms of essential fatty acids are generally the C20 and C22 metabolites of linoleic acid (LA; 18:2 n-6) and a-linolenic acid (ALA; 18:3 n-3). Humans together with mammals do not have the ability to produce these and thus they are included in the diet of fish so in turn it will be of great benefit for humans. In the body, they are metabolized by adding carbon atoms and removing hydrogen- a term known as desaturation. b-oxidation of FA occur in either mitochondria or peroxisomes (Sargent et al., 1995; Rustan & Drevon, 2001).

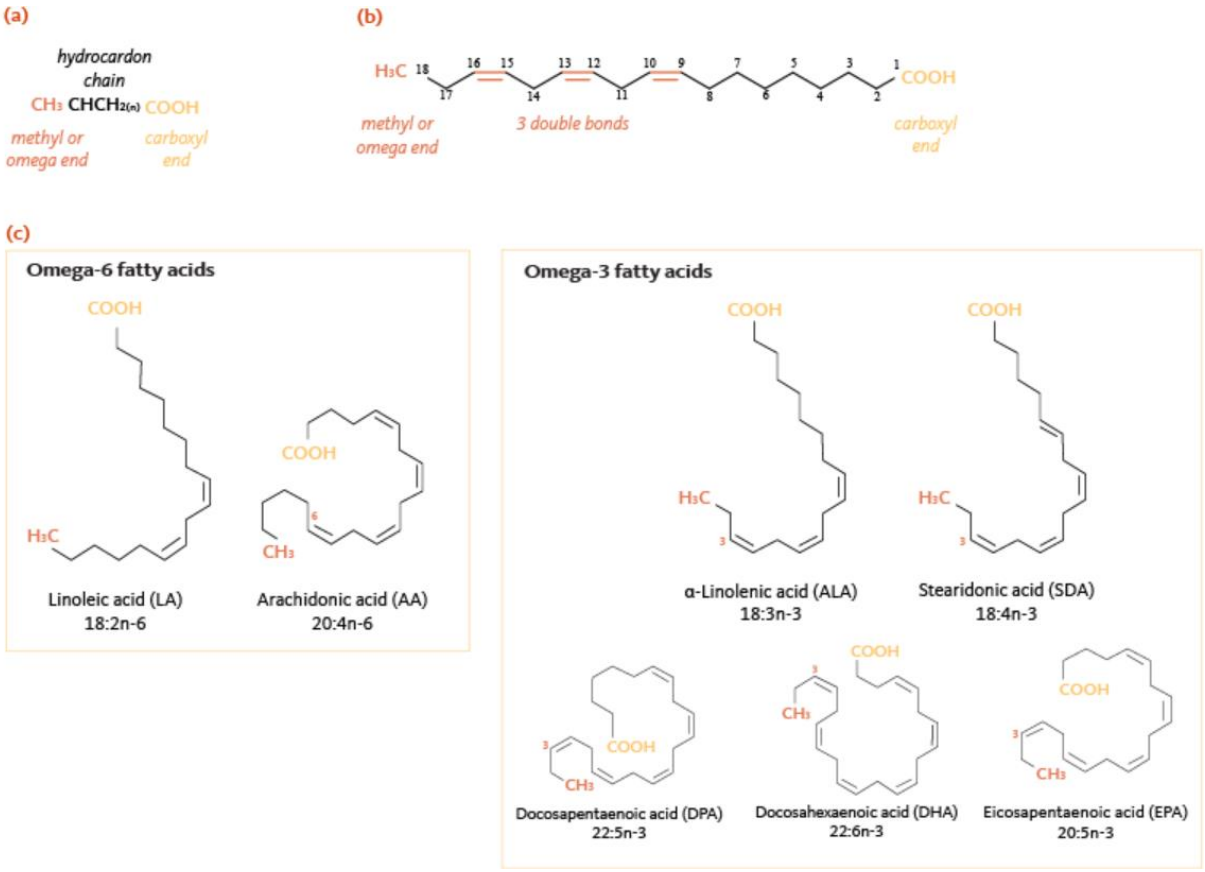

Figure 2.7. Chemical structure of the different constituent that make up essential fatty acids. (a) The structure of a fatty acid. (b) The chemical structure of alpha-linolenic acid (ALA), 18:3n-3. ALA has 18 carbon atoms (C) and 3 double bonds, the first of which is located on the third carbon atom from the terminal methyl group (omega end). (c) The molecular structures of dietary omega-6 and omega-3 fatty acids. The presence of a double bond in the hydrocarbon chain of polyunsaturated fatty acids (PUFA) introduces a 'kink' in the molecule, creating different secondary structures that influence physical properties (Adapted from Halver & Hardy, 2003).

2.5.1 Structure of Essential Fatty Acids

Fatty acids are carbon chains with a methyl group at one end of its chain and a carboxyl at its other end. Two types exist, saturated and unsaturated-the former is filled with hydrogen and the latter is not. Under unsaturated fatty acids, there are monosaturated which contain only one carbon -carbon double bond that may occur at different position in its chain. The other, polyunsaturated fatty acid has two or more double bonds with the first double bond found between the third and fourth carbon atom from the ω-carbon. These are termed as omega-3 fatty acids. Again, if the first double bond is found between the sixth and seventh carbon, it is called omega-6 fatty acid. Usually, double bonds in the PUFAs are separated by a methylene group.

Classes of Essential Fatty Acids (EFAs)

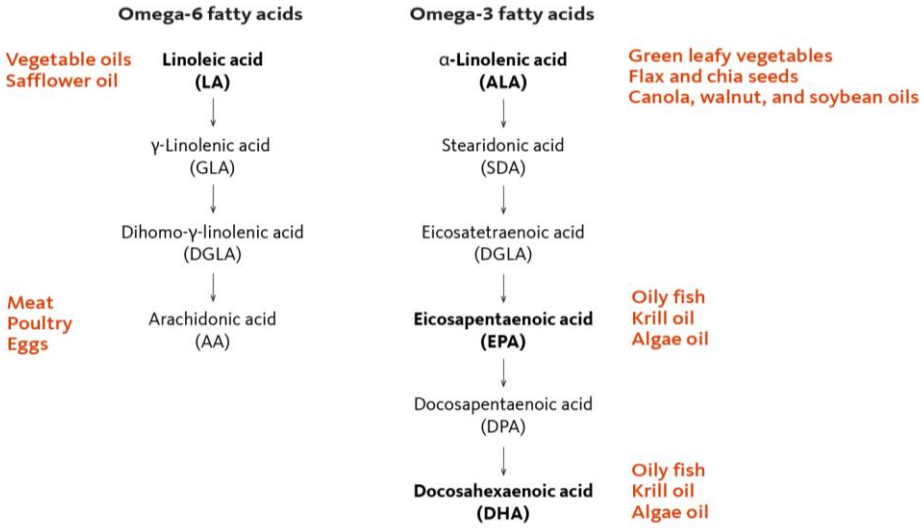

Figure 2.8. Various classes of essential fatty acids and its likely food sources. Omega-6 (n-6) and omega-3 (n-3) fatty acids comprise the two classes of essential fatty acids (EFA). The parent compounds of each class, linoleic acid (LA) and alpha-linolenic acid (ALA) (bold font), give rise to longer chain derivatives inside the body. Due to low efficiency of conversion of ALA to the long-chain omega-3 PUFA, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), it is recommended to obtain EPA and DHA from additional sources. Dietary sources of the different long chain PUFA are listed in the red font (Adapted from Higdon, 2003).

2.5.2 Synthesis and Metabolism of EFAs

Through the addition of double bonds between two carbon atoms, a term known as desaturation**,** and addition of two carbon atoms a term also referred to as elongation-biosynthesis of Omega-3 and 6 can be performed from the mother forms linoleic and linolenic acid by all animals including fish (Sargent et al, 1993). This is also similar in humans.

Some organisms have high ability for these processes than others, where an efficient example is the plankton group and is the major producers of these EFAs. Basically, the saturated fatty acids with 18 and 20 PUFA from omega-3, 6 and 9 are synthesized mainly by fatty acyl desaturates (fads) (which is normally denoted by ‘’ in many pathway synthesis) and elongases which are produced in the body. The *fads* enable the activity of the desaturases 5 and 6 which have been found to be responsible for all the desaturation of C_{18} into important $C_{20\text{-}22}$ PUFA: these include the ARA, EPA and DHA (Castro et al, 2016). On the other hand, the presence of elongases Elovl5 and Elovl2 in the Atlantic salmon enable the elongation aspect during the synthesis. During the elongation process, the Elovl5 has its preferred substrate as C_{18} while that of Elovl2 is for C_{22} PUFA substrate. In addition, both share similar substrates, however, the Elovl2 has the specific ability to elongate C_{22} to C_{24} and thus very essential for DHA synthesis. Both desaturation and

elongation synthesis reactions are known to occur in the liver except for one. The affinity of these desaturases and elongases are said (Halver & Hardy, 2003) to have higher affinity for the omega-3 than 6 and 9 series. Again, the affinity is higher for 6 when in comparison with 9 and thus, the synthesis of 6 and 9 occur only in the absence of the omega-3.

The desaturation of the 4th last carbon in 22:6n-3 (Figure 2.9) has challenges because the reaction is not direct. The 22:5n-3 is elongated to 24:5n-3 after which is changes by the 6 desaturases to 24:6n-3 and later converted to 22:6n-3. In the reaction, the 22:6n-3 is the final product for the synthesis of 18:3n-3 while 20:4n-6 (ARA) is the end product for 18:2n-6. Studies have shown (Halver & Hardy, 2003) that the 20:4n-6 can be changed to 22:5n-6 in the same manner as 22:6n-3 (DHA) is obtained. The product of 18:1n-9 (20-3n-9) cannot be elongated and desaturated further like the other two.

There are uncertainties in research concerning the ability of the fatty acid 6-desaturase to catalyze individual reaction steps or different forms of the desaturases involved for C18 and C24 PUFA. The vertical arrows pointing downwards (Figure 2.9) represent elongation and the horizontal ones for desaturation.

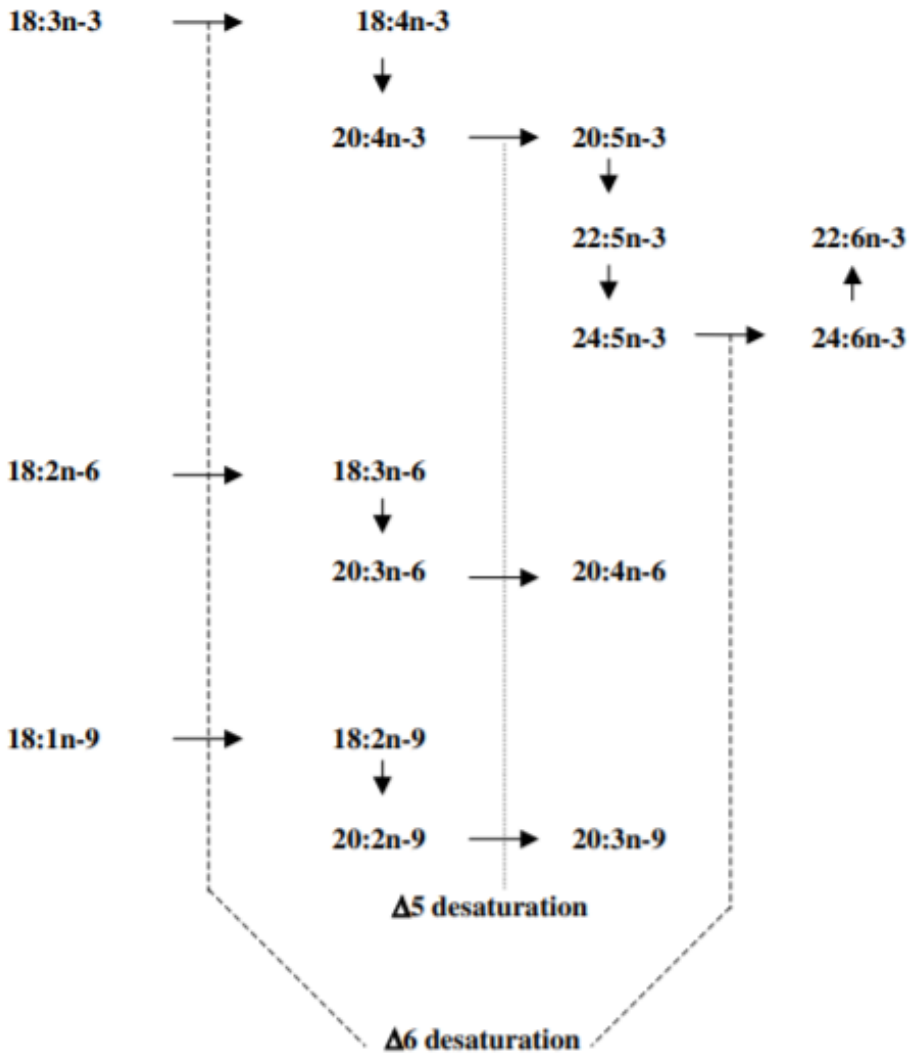

Figure 2.9. Desaturation and elongation pattern of omega-3, 6 and 9 in the tissues of living organisms (Adapted from Halver & Hardy, 2003).

2.6 Sources of oils for fish feed

2.6.1 Vegetable versus Animal sources

Rapeseed (also referred to as canola), soybean and sunflower oil have gained much attention as alternate sources to fish oil in feed production for both livestock and fish. Annual production of these vegetable oil has increased considerably more than fish oil. Also, the price of these plant-based oil sources is much cheaper and sustainable for the environment.

However, unlike fish oil- vegetable lipids have maximum 18 carbon and 3 double bonds and thus lacking the n-3 fatty acids EPA and DHA. Studies show that the replacement of vegetable oils

such as rapeseed in diverse fractions or even mixture of two or more do not have adverse effects in overall composition or lipid content in fillets and whole body of numerous fish species (Bell et al., 2003a). In general, dietary composition of fish diet reflects in their flesh.

Animal lipid sources such as lard (pigs) or poultry fat are estimated to be cheaper than vegetable oils for the aquatic feed industry. However, the n-3 Highly Unsaturated Fatty Acid (HUFA) content of animal fat is found only at trace level, and hence these lipid sources can be considered lacking in n-3 HUFA. Extensively, investigations have shown that 50% incorporation of animal fats into dietary lipid level of fish have no negative effects on growth performance if the EFA requirements are met (Mugrditchian et al., 1981; Bureau & Gibson, 2004 as cited in Sonu, 2018; Turchini et al., 2003)

2.6.2 Rapeseed Oil

Rapeseed is bright yellow flowering plant of the family *Brassica*, and several species exist in this genus such as *B.napus, campestris, rapa and B.juncea*. It has a variety of common names such as mustard and rapa. Species of this family can be of winter or spring variety. It is the second most produced oilseed crop after soybean (Burel & Kaushik, 2008). They are used for edible oils for human consumption, animal feed production and some biofuels.

Originally, the oil extracted from the seed of the natural and unselected rapeseed had high levels of erucic acid likewise the meal which had high amounts of glucosinolates (Alexander et al., 2008). Glucosinolates refer to a class of organic compounds that contain sulphur and nitrogen. They are characterized by a very strong bitterness when the plant is chewed or cut, acting as natural defense mechanisms against pests (Alexander et al., 2008). They are known to be toxic if ingested in high amounts. As a result, utilization of the rapeseed for livestock feed materials were reduced. Also, oils extracted from the rapeseed had erucic acid accounting for 50% of total fatty acids (Turchini & Mailer, 2010) and thus becoming harmful when too much intake occurs in humans and animals (Charlton et al., 1975)

The current rapeseed varieties that are used for replacement in fish feeds and livestock feeds are improved versions of selected cultivars in early 1970's (Turchini & Mailer, 2010) that contained reduced amounts of erucic acid and glucosinates that had been developed through traditional selective-breeding procedures. Popular amongst new varieties is the Canola, which is derived from

the name CANadian Oil is a generic (Turchini & Mailer, 2010) and common name used for varieties of rapeseed oil containing low amounts of erucic acid.

Figure 2.10. Rapeseed plant with its seeds and derived oil. (Adapted from http://www.oilmillmachinery.net/rapeseed-oil-production.html.http://rencompany.org/agricultural-commodities/rapeseed-2/)

2.6.3 Chemical Composition and Nutritional Value of Rapeseed Oil

The rapeseed contains about 40% oil and 38% protein, the rest are hulls and moisture content (Bell, 1984b). Of the 40% oil, 95-99% of the fatty acids that it may contain are triglycerides and their molecular structures determine the nature that is physical, chemical and nutritional aspects of the oil. Widely, the most common fatty acids are found in position 1 or 3 of rapeseed oil (canola), in an ascending manner (Ratnayake & Daun, 2004), 18:0 (2.8%), alpha-linolenic acid (ALA) (5.9%), 16:0 (6.6%), linoleic acid (LA) (16.4%), and oleic acid (OA) (60.9%). While those commonly found in position 2 are 18:0 (0.7%), 16:0 (1.4%), ALA (13.7%), LA (29.5%) and OA (52.7%), according to Ratnayake & Daun (2004).

Even though OA is still more abundant, the presence of LA molecules in large numbers with respect to position 2, gives an interesting observation from a nutritional point (Turchini & Mailer, 2010).

Table 2.1. Chemical composition of de-oiled rapeseed, protein isolates from rapeseed and de-oiled soybean. (Adapted from Yoshie-Stark et al., 2008)

	Dry matter (%)	Protein (%, DM)	Ash (%, DM)	Fat (%, DM)	Fibre (%, DM)
Rapeseed (raw)	92.99	19.0	3.60	54.2	23.2
Rapeseed (hexane de-oiled)	92.20	48.2	7.90	6.37	37.5
Precipitated protein isolate	91.01	70.8	10.8	8.23	10.2
Ultrafiltered protein isolate	92.25	98.7	3.05	1.18	-
Hexane de-oiled soybean	90.00[a]	61.0[a]	6.00[a]	2.00[a]	6.00

2.7 Fish oil and Fish meal in Feed Production

In the past, fish oil (FO) and fish meal (FM) were the basic ingredients for production of fish feed for aquaculture due to good practical, scientific and economic reasons (Bell et al., 2003a). Also, they are the most palatable and digestible ingredient in fish feeds. They are palatable for the fish, have good amino acid profile and its lipid residue contain more EPA and DHA (Gonçalves et al., 2012; Miles & Chapman, 2006). They are mainly constituents from pelagic seawater fishery.

Anchovies, sardines and herrings are the most popular species used (FAO, 2018). However, the abundance and availability of these small pelagics are dependent on El Niño phenomena (Kumar et al., 2014; Pincinato et al., 2020; Velarde et al., 2004), coupled with pollution and climate change which in turn causes inconsistencies in the marine food web (Degerman, 2015; Durant et al., 2019; Ullah et al., 2018). In 1994, the production of fishmeal increased to 30 million tonnes equivalence in live weight (FAO; 2018). Since then, these values have been dwindling. Fish oil and meal have become very limited and production now is around 7 million tonnes per year. In 2016, fisheries landings apportioned for fishmeal production were minimized to values below 15 million tonnes (live weight equivalent); mainly as a result of reduced catches of anchoveta (FAO, 2018). Fluctuations in availability and variations in quality makes marine feed sources vulnerable.

Fish meal is generally around 5% in a grower's diet and about 10-12% in the later stages of cultured salmon. On average the percentage in salmon feed is about 16%. Studies conducted by (Rainuzzo et al., 1997) states that lipids in broodstock nutrition are marked as important criteria for the quality of the larvae. Lipids have effects on the spawning and the egg quality of numerous fish species and a deficiency in omega-3 HUFA in broodstock negatively influence fecundity, fertilization and hatching rate of the species studied (Yanes-Roca et al., 2009; Yıldız et al., 2020); Navas et al.,

1997). They are increasingly used selectively, for example for specific stages of production, particularly for hatchery, broodstock and finishing diets. Their incorporation in grower diets has decreased over time.

2.8 The effects of fish oil replacement on fish performance and feed efficiency on salmonids

No negative effects were reported in the following investigations on partial, complete or mixture of vegetable oils replacement of fish oil by linseed oil (Menoyo et al., 2005), rapeseed oil (Bell et al., 2001a, 2003; Ng et al., 2004), soybean oil (Ruyter et al, 2006) in different stages in the salmon life cycle.

However, the fatty acid profile in tissue is highly influenced by these replacement (Caballero et al., 2002) as fatty acid composition in fish's feed is reflected in its muscles, organs and lipid stores (Mourente et al., 2005; Tocher et al., 2002; Richard et al., 2006). Although, this means that dietary oleic, linoleic and alpha-linolenic will increase in the fish flesh and organs, while n-3 fatty acids specifically EPA and DHA will decrease.

An example being the organs of seawater fishes have increased sensitivity to dietary lipid changes because of their poor ability to convert saturated compounds to unsaturated forms as previously mentioned (Ganga et al., 2005; Izquierdo et al., 2005; Mourente et al., 2005). Finally, depending on the components of the fatty acid profile of a particular vegetable oil, the fish tissue composition maybe modified differently by a lower or higher extent depending on certain priorities that benefit the fish, like those with fast renovation such as gills, gut epithelia, blood cells or with a higher ratio of phospholipids such as neural tissues.

Also, quantity of dietary saturated fatty acids affects lipid digestion at low temperatures (Ng et al., 2004; Torstensen et al., 2000). Particularly in Atlantic salmon and rainbow trout the substitution of fish oil with rapeseed oil showed that saturated fatty acid (SFA) decreased while monounsaturated fatty acid (MUFA) increased, indicating that lipid digestibility was altered at low water temperatures (Caballero et al., 2002; Karalazos et al., 2007). In addition, reduction in protein inclusion in feed due to high lipid inclusion results in elevated protein use in Atlantic salmon at low water temperatures (Karalazos et al., 2007). In short, low water temperature affects dietary lipid sources and protein levels in both Atlantic salmon and rainbow trout (Norambuena et al., 2015, 2016).

Solutions to address the issue of low n-3 long chain polyunsaturated fatty acid (LC-PUFA) in the fish fillet as a result of replacement by vegetable oils has been considered. For example, feeding adult salmon a finishing fish oil diet which is rich in n-3 LC-PUFA prior to harvesting. By this approach, there will be increment of marine n-3 PUFA in the fillets at harvesting, so that vegetable based fatty acids will be reduced to mimic same levels as fishes fed fish oil diets.

Length of time needed to achieve this effect in salmonids have been shown to be the onset of periods when the fish are doubling their weight (Bell et al., 2003a; Torstensen et al., 2005). This depends also on the fish species from which these oils are produced since some species have higher or diverse levels of n-3 LC PUFA than others.

Also, the levels of these essential fatty acids determine the time required to restore the fatty acids in the flesh. For example, the levels of both EPA and DHA in fillets of gilthead sea bream fed vegetable oil-based feed (even at an 80% substitution level) were recovered after 60 days of FO feeding. This was the same and even higher than values in wild fish (Izquierdo et al., 2005).

3 Materials & Methods

3.1 Fish

The fish used were Atlantic salmon (*Salmo salar* L) farmed by Cermaq in sea in Alta area Finnmark, Norway's northernmost county (70° N 23° E). S0 and S1 smolts were transferred to sea in Autumn and Spring, 2018 respectively. The fish were distributed randomly into 12 commercial sized net pens and fed diets with varying level of omega-3 (EPA and DHA) in triplicates (Table 3.1). Sampling of S0 salmon was carried out in October and the S1 salmon were received for analyses in November 2019 and January 2020; eight fish from each. The S0 salmon were fed a diet with low omega-3 content (TestS) or medium omega-3 content (TestP) while the S1 salmon were fed a diet with medium content of omega-3 (TestN) or high omega-3 (TestO).

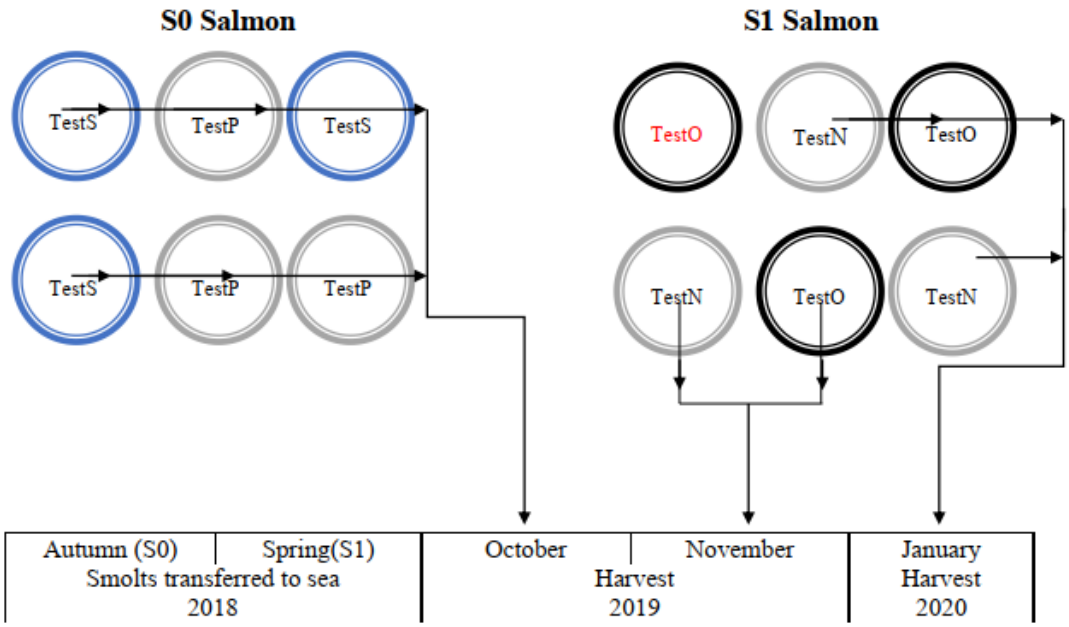

Figure 3.1. Schematic overview of the experimental design with smolt groups (S0, S1) assigned varying levels of n-3 diets (TestS, TestP, TestN and TestO). One sample was missing during analysis, from the S1 group (red font). Compositions of the diets are available below (Table 3.1).

3.2 Experimental diet

Table 3.1. Fatty acid compositions (%) of the experimental diets with varying levels of n-3.

Fish group	**S0 salmon**		**S1 salmon**	
Diet	TestS	TestP	TestN	TestO
Level of n-3	Low	Medium	Medium	High
Total fat content, %	29.1	29.9	30.9	29.5
C14:0 (Myristic)	2.1	3	2.4	2.9
C16:0 (Palmitic)	7.7	9.3	8.1	8.8
C18:0 (Stearic)	3.6	3.9	3.8	3.8
C22:0 (Behenic)	0.3	0.2	0.3	0.3
C24:0 (Lignoceric)	0.2	0.1	0.2	0.2
Total saturated [1]	*14.3*	*17.2*	*15.3*	*16.5*
C16:1n7 (Palmitoleic)	1.9	2.6	2.2	2.6
C18:1n9c (Oleic)	41.8	36.4	38.8	35.9
C20:1n9 (Eicosenoic)	2.8	3.1	3.1	3.4
C22:1n9 (Erucic)	0.9	0.7	0.9	0.8
Total monoenes [2]	*47.6*	*43.2*	*45.3*	*43.1*
C18:2n6c (Linoleic)	15.6	14.2	14.7	13.6
C18:3n6 (g-Linolenic)	0.1	0.1	0.1	0.1
C20:2n6 (Eicosadienoic)	1	1.3	1.2	1.4
C20:3n6 (Eicosatrienoic)	0.1	0.1	0.1	0.1
C20:4n6 (Arachidonic)	0.2	0.2	0.2	0.2
Total n-6 PUFA	*16.9*	*15.9*	*16.3*	*15.4*
C18:3n3 (Linolenic)	7.1	6.5	6.7	6.3
C20:3n3 (Eicosatrienoic)	0.1	0.1	0.1	0.1
C20:5n3 (Eicosapentaenoic, EPA)	3.1	4.2	3.9	4.6
C22:5n3 (Docosapentaenoic, DPA)	0.4	0.5	0.5	0.6
C22:6n3 (Docosahexaenoic; DHA)	2.8	3.6	3.5	4.1
Total n-3 PUFA [3]	*12.5*	*13.5*	*13.5*	*14.4*

[1]includes C12:0, C15:0, C17:0, C19:0, C21:0, C23:0

[2]includes C14:1 & C24:1

[3]includes C22:2

3.3 Slaughter and Filleting

The fish were slaughtered according to standard procedures, eviscerated, packed in Styrofoam boxes containing ice and stored for one week prior to filleting by hand.

Figure 3.2. Gutted salmon taken out from Styrofoam boxes prior to measurement of body weight and filleting.

3.4 Quality analyses for fresh fish

Body weights and total length of the gutted salmon were measured and recorded. Filleting was done, and the first fillet was weighed. Fillets were then subjected to visual assessment of gaping and colour and instrumental determination of fillet texture. The cutlet between the posterior end of the dorsal fin and the gut (Norwegian Quality Cut) was then cut out and stored at -40ºC until analyses of fat and fatty acids (6 weeks storage).

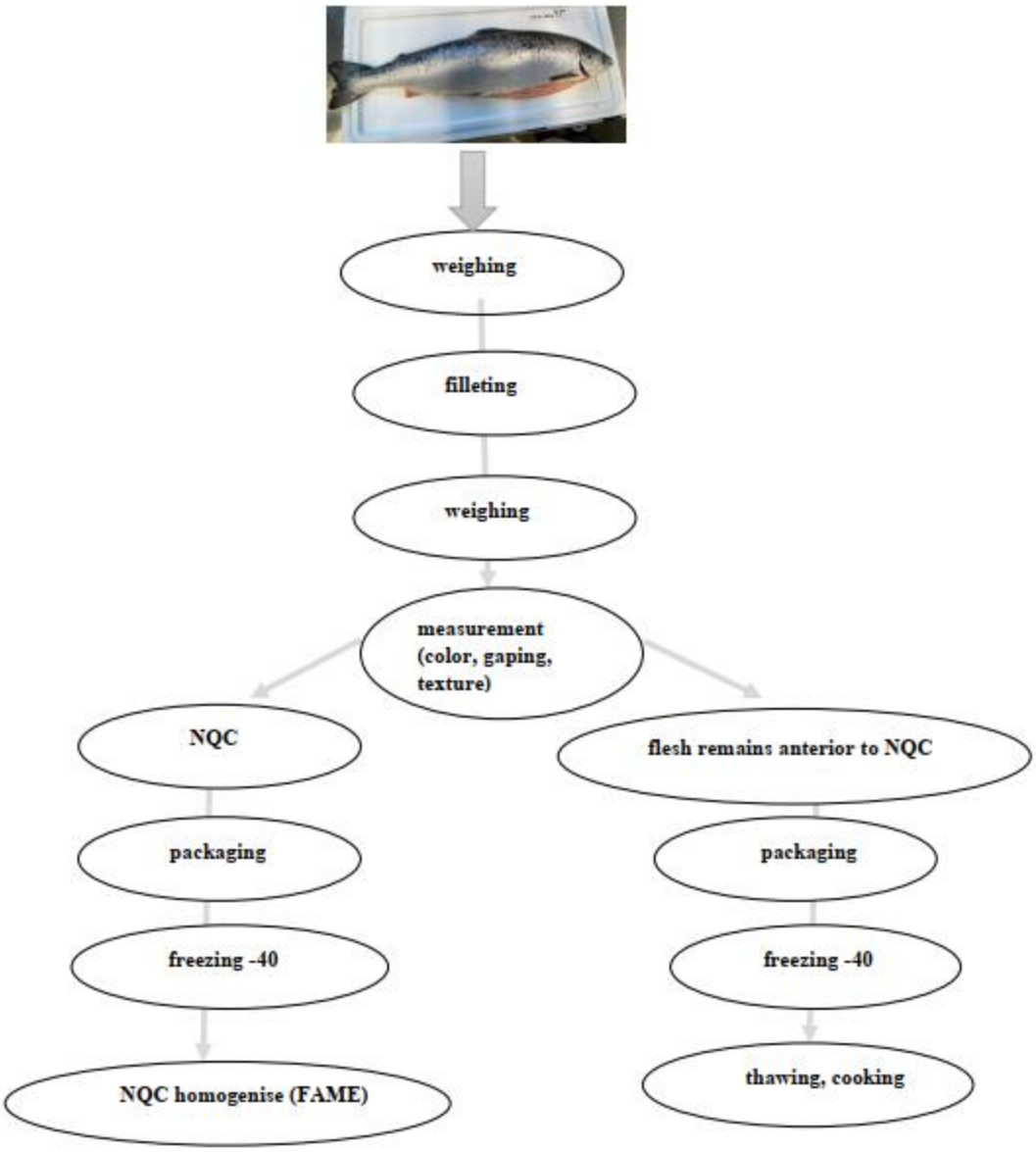

Figure 3.3. Flow diagram for preparation of Atlantic salmon muscles for Fatty Acid Methyl Ester (FAME) and thawing/cooking procedures.

3.4.1 Color measurements

Color was evaluated by comparing salmon fillets against SalmoFan™ color scale (DSM) under standard illumination Salmon colour box, where scores ranged from 20 (very pale red) to 34 (very intense red; (Forsberg & Guttormsen, 2006). Colour was assessedabove and below the vertebral column.

3.4.2 Fillet gaping and Texture Analysis

Fillet gaping was assessed immediately after filleting. Instrumental texture analyses were performed using Texture Analyzer TA-XT2 (SMS Ltd., Surrey, England) equipped with a 5 kg load cell. The probe was a flat-ended cylinder (12.5 mm diameter; type P/0.5). Firmness was determined as the force required to penetrate the cylinder through the fillet surface (i.e. the breaking force).

3.5 Analysis of frozen fish

Fillet portions anterior to the NQC (Figure 3.4) were trimmed, labelled, packed in sealed plastic bags, placed in Styrofoam boxes and immediately transferred to a -40ºC freezer.

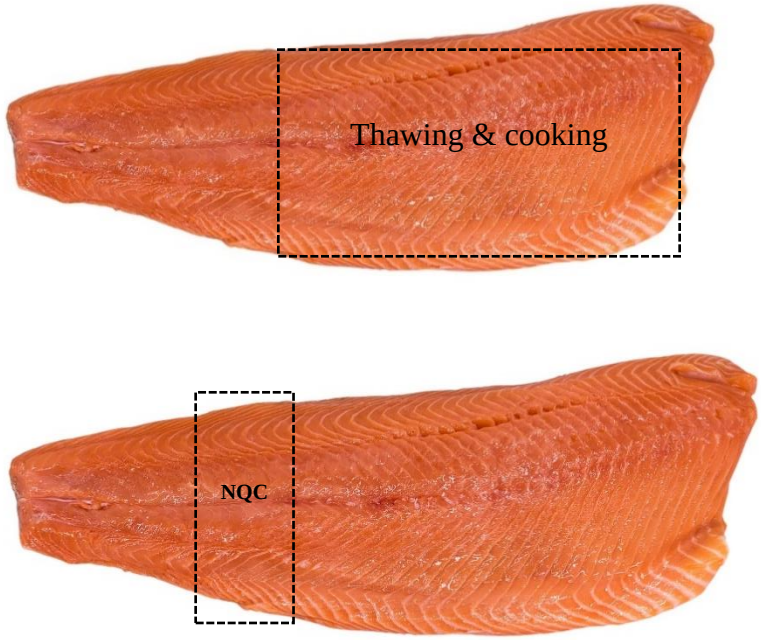

Figure 3.4. Fillet portions used for analysis.

3.6 Thawing and cooking

Salmon fillets from each net pen were taken from -20ºC after 5 weeks and placed in controlled rooms at 4ºC and 20ºC; eight pieces per thawing cabinet (2 per net pen). Temperature recording probes were inserted to monitor the development in thawing temperature (Figure 3.6). Thawing at 4ºC took about 10hours while thawing at 20ºC took about 5 hours. For fillets thawed at 4ºC, weight and color were measured when the core temperature reached approximately 4ºC. Thereafter, the

fillets were put back and left in the thawing chamber until the core temperature stabilized at 20ºC. Stabilizing from 4ºC to 20ºC took approximately 4-5 hours approximately depending on the fillet size.

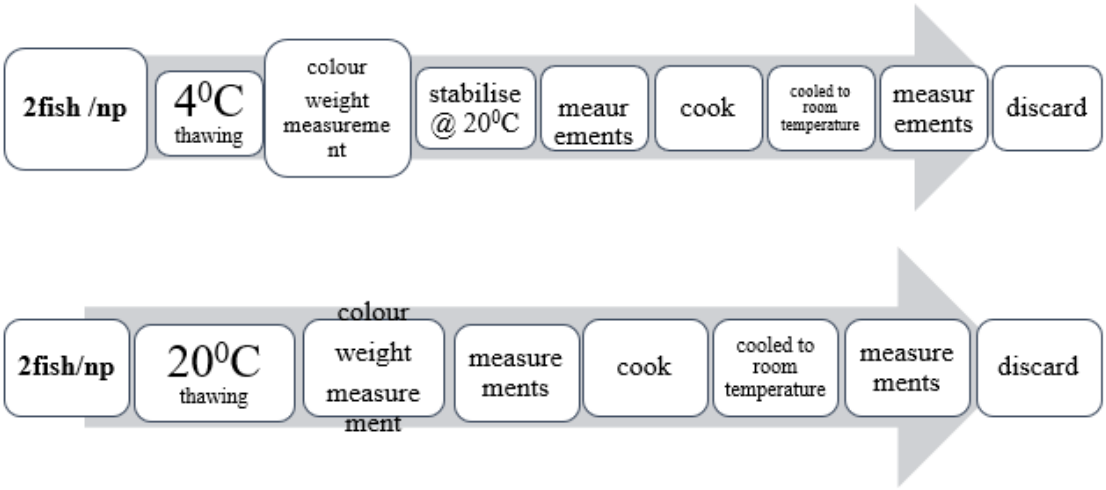

Figure 3.5. Flow diagram for thawing and cooking procedure.

Figure 3.6. Temperature recording probes (A), controlled climate rooms (B).

Thawed fillets with core temperature of 20ºC were placed in a microwave (Samsung MC28M6065) after colorimetric and weight measurements for cooking. The fillets were cooked at 180ºC till the core temperature reached 70ºC (Figure 3.7). After cooking, they were cooled to room temperature before measurements were taken.

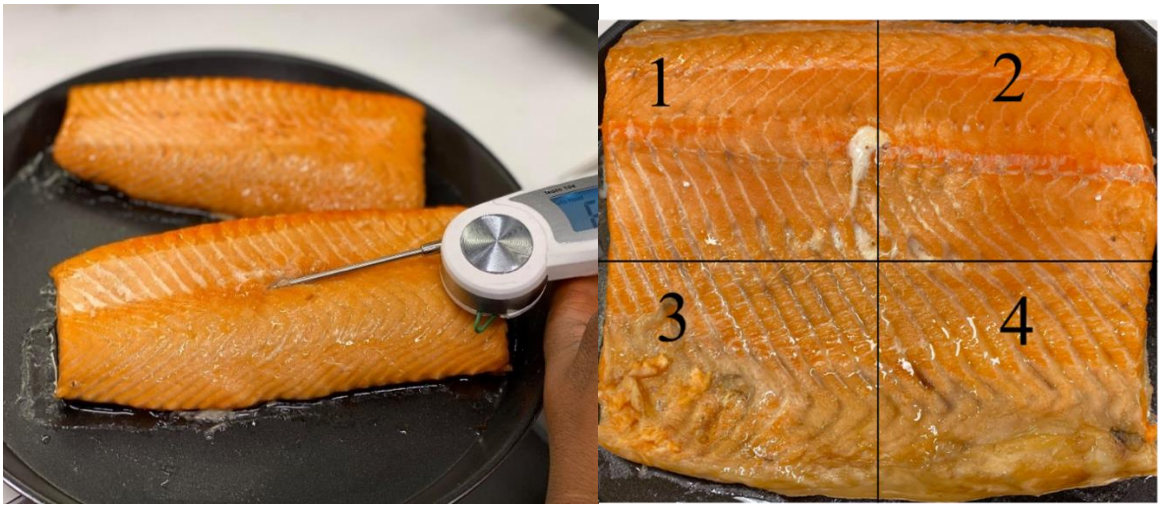

Figure 3.7. Measurement of core temperature during cooking in the microwave (left) and locations on Atlantic salmon fillet where color measurements were taken for thawed and cooked fish (right).

3.6.3 Quality analyses for cooked fish

Colour measurements were performed using both colorimetric analyses (Minolta CIE L*a*b*) and SalmoFan™ scoring. The measurements were taken in four regions to have an average picture of the dorsal and belly regions since the latter is known to have paler color than the former (Figure 3.7).

3.7 Lipid Analysis

Half frozen NQCs pooled from each net pen were deskinned and homogenized using a blender with sharp knives at room temperature. This was done within a short time frame to reduce fat smearing on walls of the grinding container. Then 0.8g of the frozen sample was measured and placed in 12mL screw-cap glass tube to which 0.8 mL of the C13:0 internal standard (0.5 mg of C13:0/mL of MeOH), 0.56mL of 10 *N* KOH, in water, and 4.2 mL of MeOH were added.

The tube was incubated at 55°C in a water bath for 1.5 h with vigorous shaking using a multitube vortex for 5s every 20 min. This allows for proper permeation, dissolution, and hydrolyzation of the sample. After, the samples were cooled below room temperature in a cold tap water bath, 0.46 mL of 24 *N* H_2SO_4 in water was then added (Figure 3.8).

The tube was mixed by inversion and with precipitated K_2SO_4 present was incubated again at 55°C in a water bath for 1.5 h with multitube vortex for 5 s very 20 min. After FAME synthesis, the tube was cooled in a cold tap water bath. 2.4 milliliters of heptane (for safety reasons) were added, and the tube was vortex-mixed for 5 min on a multitube vortex.

The tube was centrifuged for 5 min in a Heraeus Multicentrifuge X1R from Thermo Scientific under 3000rpm with a temperature of 20^0C, after the hexane layer, containing the FAME, was siphoned (Figure 3.8) into a GC vial using a calibrated micropipette. The vial was capped, labelled and placed in GC (Hewlett Packard GC 6890).

Figure 3.8. Addition of H_2SO_4 after cooling in cold water bath (left) and hexane layer siphoned into labelled GC vials for gas chromatography (right).

Fatty acid composition was analyzed using Trace Gas Chromatography System Ultra with auto-injector (Thermo Scientific) with software Chromeleon 7.2 (Thermo Scientific). The GC was equipped with capillary column: Rt - 2560, 100 m, 0,25 mm internal diameter, 0,20 µm dt (Restek, Cat# 13198), injector with a temperature of 250 °C, flame ionization detector, and split injector with 1:40 split ratio, injection volume of 1µL.

Both the injector and the flame ionization detector were set at 250°C. Initial oven temperature was 140°C, held for 5 min, subsequently increased to 240°C at a rate of 4°C min-1, and then held for 20 min. Helium was used as the carrier gas at a flow rate of 0.5 mLmin-1, and the constant pressure was 2.70 bar.

Analysis took 50 min per sample. Fatty acids were determined by measurement of the area beneath peaks corresponding to the retention times of the fatty acid methyl standards. In total forty-seven lipids were identified.

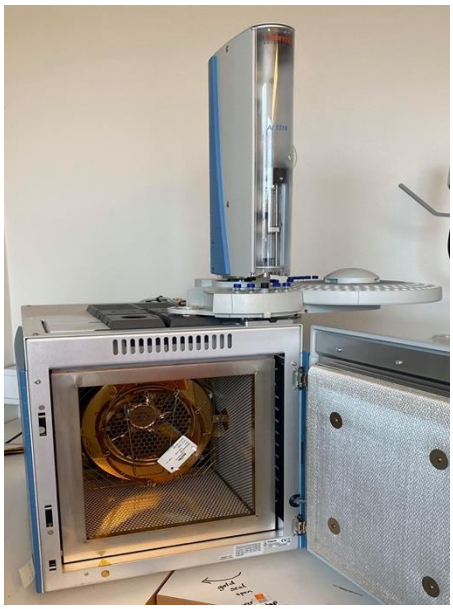

Figure 3.9. Gas chromatograph used for FAME analysis.

3.8 Calculations and Statistical analysis

Filet yield was calculated as weight of both fillets/gutted weight*100%, while slaughter yield was calculated as gutted weight/whole body weight*100%. Condition factor =100*gutted weight*(length, cm)$^{-3}$

One-way analyses of variance (ANOVA) were carried out separately with dietary treatment as explanatory variable. Net-pens were used as the units of observation. Also, two-way ANOVA were carried out with dietary treatment and temperature (explanatory variables) for thawing or cooking data sets. Significant differences among means of dietary treatment, fatty acid compositions and temperature were ranked by the pdiff and Duncans multiple range test. Proportion of the total variation explained by the model is expressed by R^2 and the level of significance was set at 5% ($p<0.05$). All statistical analyses were carried out using the SAS software package (SAS Institute, Cary, NC, USA).

4 RESULTS

4.0 Body weight and slaughter parameters

The average gutted weight was 4.4kg for the S1 salmon and 1.5kg for the S0 salmon on average (Table 4.1). The condition factor was significantly higher of the S1 salmon, but dietary treatment did not affect the condition factor significantly. The fillet yield was 3% units higher of the S1 salmon (77.5 on average) compared with the S0 salmon (74.6% on average), but no significant difference was observed due to dietary treatment (Table 4.1).

Table 4.1. Gutted body weight, length, condition factor and fillets yield of Atlantic salmon fed diets with varying levels of n-3.

Fish group	S0 salmon		S1 salmon	
Diet	TestS	TestP	TestN	TestO
Level of n-3	Low	Medium	Medium	High
Gutted weight, kg	1.6 ± 0.7^{b}	1.4 ± 0.6^{b}	4.3 ± 0.1^{a}	4.5 ± 0.1^{a}
Length, cm	52.0 ± 0.7^{b}	50.5 ± 0.6^{b}	71.3 ± 0.9^{a}	72.2 ± 0.7^{a}
Condition factor	1.1 ± 0.0^{b}	1.1 ± 0.0^{b}	1.2 ± 0.0^{a}	1.2 ± 0.0^{a}
Fillet yield, %	74.6 ± 0.5^{b}	74.6 ± 0.6^{b}	77.3 ± 0.6^{a}	77.9 ± 0.5^{a}

Results are presented as least square means ± standard error.

Values in the same row assigned a different superscript letter differ significantly, $P < 0.05$.

$$\textit{Condition factor} = \frac{\textit{Gutted weight (kg)} * 100}{\textit{(Fish length, (cm))}^3}$$

4.1 Lipid content and composition of skeletal muscle

Fillet fat content ranged from 11.8-15.4g per 100g tissue (Table 4.2). There was no significant difference between dietary treatment within the S0 or S1 salmon group. However, fillet fat content was higher in the TestN (medium, S1 salmon) than in the TestP (low, S0 salmon) (Table 4.2).

Total saturated fatty acids (SFA) ranged between 13.9-14.7% and monounsaturated fatty acids (monoenes) (MUFA) between 44.5-45.6% showed no significant differences amongst the dietary groups. The FA with the highest concentration was C18:1n9c (oleic acid), ranging from 37.4 to 39.2%. The 18:1n9c was higher in S0 salmon fed TestS diet (39.2±0.3) than TestP (37.4±0.5) (P=0.05) in S0 salmon, while the content of C16:1n7 and C20:1n11 were higher in TestP. No FA of the SFA or MUFA differed significantly between the S1 dietary groups (Table 4.2).

Total n-6 polyunsaturated fatty acids (PUFA) ranged from 15.2-16.0%. The level was higher in the TestS (16.0±0.1) compared with the TestP (15.2±0.1) (P=0.01) of the S0 group. No significant difference was seen between TestN and TestO of the S1 group (Table 4.2).

The n-6 PUFA with the highest level was C18:2n6c (linoleic acid), ranging from 13.4-14.1%. The C18:2n6c was higher in S0 salmon fed TestS diet (14.1±0.2) than TestP (13.4±0.2) (P=0.01). The C18:2n6c did not differ significantly amongst the S1salmon dietary groups (Table 4.2).

Total amount of n-3 PUFA ranged from 14.9% – 16.5%. For the S0 salmon group, TestP (15.9±0.0) had a content higher than TestS (14.9±0.0) (P=0.03). No significant differences were seen between the dietary groups of the S1 salmon group (P = 0.16) (Table 4.2).

EPA was higher in TestP (3.3±0.0) than TestS (2.9±0.1) (P=0.001) for the S0 salmon group, while TestO (3.1±0.0) was higher than TestN (2.9±0.0) (P=0.03) for the S1 salmon group (Table 4.2).

DHA showed the same pattern, with significantly higher content in TestP (4.8±0.0) than Test S (4.2±0.1) and TestN (3.7±0.1) than Test O (4.1±0.0) (Table 4.2).

The level of DPA was higher in TestP (1.4±0.0) than TestS (1.2±0.0) (p=0.002) of the S0 group, but no significant difference was observed amongst the S1 dietary groups (P=0.11) (Table 4.2).

Table 4.2. Fatty acid composition (%) in fillets of Atlantic salmon fed diets with varying levels of n-3.

Fish group	**S0 salmon**		**S1 salmon**	
Diet	TestS	TestP	TestN	TestO
Level of n-3	Low	Medium	Medium	High
Total fat content, %	12.6±0.4^{ab}	11.8±0.3^{b}	15.4±1.0^{a}	14.6±2.7^{ab}
C14:0 (Myristic)	2.2±0.1	2.4±0.1	2.4±0.2	2.2±0.0
C16:0 (Palmitic)	8.8±0.0	9.2±0.1	9.0±0.5	8.8±0.3
C18:0 (Stearic)	2.4±0.1	2.4±0.0	2.3±0.0	2.4±0.1
Total saturated [1]	*13.9±0.0*	*14.7±0.2*	*14.4±0.8*	*14.0±0.5*
C16:1n7 (Palmitoleic)	2.3±0.0^{b}	2.6±0.0^{a}	2.6±0.1^{a}	2.5±0.1^{a}
C18:1n9c (Oleic)	39.2±0.3^{a}	37.4±0.5^{b}	38.5±0.6^{ab}	38.8±0.9^{ab}
C20:1n11 (Gondoic)	3.4±0.1^{b}	3.7±0.0^{a}	3.3±0.1^{b}	3.4±0.1^{ab}
C22:1n11 (Cetoleic)	0.5±0.0^{ac}	0.5±0.0^{a}	0.4±0.0^{b}	0.4±0.0^{bc}
Total monoenes [2]	*45.6±0.4*	*44.5±0.6*	*44.9±0.5*	*45.4±0.9*
C18:2n6c (Linoleic)	14.1±0.2^{a}	13.4±0.2^{b}	13.8±0.1^{ab}	14.0±0.1^{a}
C20:2n6 (Eicosadienoic)	1.1±0.0	1.1±0.0	1.1±0.0	1.1±0.0
C20:3n6 (Eicosatrienoic)	0.3±0.0^{a}	0.3±0.0^{ab}	0.3±0.0^{b}	0.3±0.0^{ab}
C20:4n6 (Arachidonic)	0.3±0.0^{a}	0.3±0.0^{bc}	0.3±0.0^{a}	0.3±0.0^{b}
Total n-6 PUFA [3]	*16.0±0.1^{a}*	*15.2±0.1^{b}*	*15.6±0.1^{ab}*	*15.8±0.1^{a}*
C18:3n3 (Linolenic)	6.1±0.2^{b}	5.9±0.1^{b}	7.2±0.1^{a}	7.2±0.2^{a}
C20:3n3 (Eicosatrienoic)	0.5±0.0^{b}	0.5±0.0^{b}	0.7±0.0^{a}	0.6±0.0^{a}
C20:5n3 (Eicosapentaenoic, EPA)	2.9±0.1^{bc}	3.3±0.0^{a}	2.9±0.0^{c}	3.1±0.0^{b}
C22:5n3 (Docosapentaenoic, DPA)	1.2±0.0^{b}	1.4±0.0^{a}	1.4±0.1^{a}	1.5±0.1^{a}
C22:6n3 (Docosahexaenoic, DHA)	4.2±0.1^{b}	4.8±0.0^{a}	3.7±0.1^{c}	4.1±0.0^{b}
Total n-3 PUFA	*14.9±0.4^{b}*	*15.9±0.0^{a}*	*15.8±0.3^{a}*	*16.5±0.3^{a}*
Sum FA	91.4±0.1	91.4±0.9	91.8±0.2	92.8±1.2

Mean fatty acids of muscle above 0.2 are presented. Values are means ± se, n=3. Values in the same row assigned a different superscript letter differ significantly, $P < 0.05$.
[1]Includes, 12:0, 15:0, 17:0, 21:0, 22:0 and 23:0.
[2]Includes 14:1.
[3]Includes C18:3n6

The sum of EPA&DHA ranged from 6.6%-8.1%. Significant difference was seen between the different dietary treatments. The amount of EPA&DHA was higher in TestP (8.1±0.0) than TestS

(7.1±0.2) (P=0.001) for S0 salmon group. TestO (7.2±0.0) was higher than TestN (6.6±0.1) (P=0.03) for S1 salmon group (Figure 4.1).

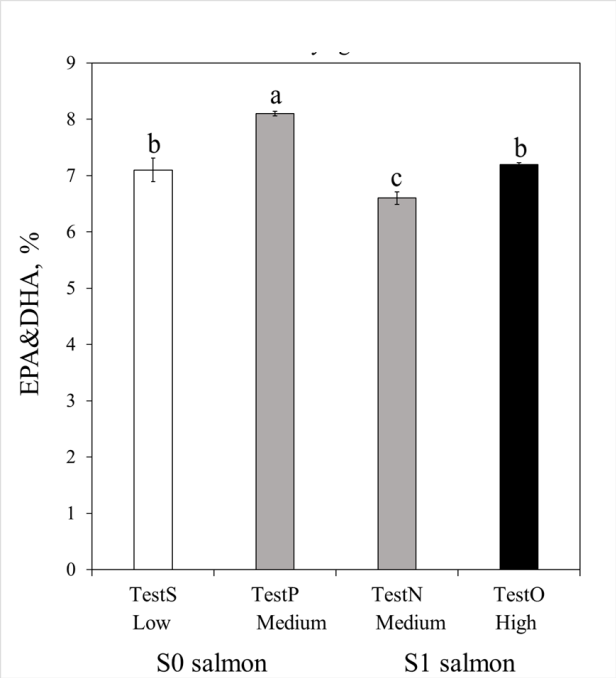

Figure 4.1. Sum of EPA&DHA, % in fillets of S0 and S1 Atlantic salmon fed diets with varying levels of n-3. Different superscripts indicate significant differences ($p<0.05$). variations are standard error bars.

4.5 Quality of fresh fillets

The fillets had a gaping score of 0.4 and 0.6 for the S0 and S1 salmon group respectively. No significant differences were observed in neither between dietary treatment nor within the S0 (P = 0.40) or S1 (P =0.19) salmon groups (Table 4.3).

Color scores measured with SalmoFan™ ranged from 24-25 on the dorsal fillet region and from 24-26 for the Norwegian Quality Cut (NQC). There were no significant differences observed for the salmon groups (Table 4.3).

Firmness measured instrumentally ranged between 6.9-9.4N, with numerically higher values for the S1 salmon compared with the S0 salmon. Dietary treatment had no significant effect on firmness (Table 4.3).

Fillet thickness ranged from 24.5-36mm, with higher values for the S1 salmon compared with the S0 salmon. Thickness was higher in TestS (25.4±0.5) than TestP (24.5±0.5) (P=0.03) for the S0 salmon group. No differences were observed for the S1 salmon group (Table 4.3).

Table 4.3. Evaluation of sensory parameters and texture of fillets of Atlantic salmon fed diets with varying levels of n-3.

Fish group	S0 salmon			S1 salmon		
Diet	TestS	TestP		TestN	TestO	
Level n-3	Low	Medium	P-value	Medium	High	P-value
Gaping, score (0-5)	0.4±0.1	0.3±0.1	0.40	0.7±0.1	0.5±0.2	0.19
Colour Dorsal, score	24±0.1	24±0.1	0.15	25±0.2	25±0.1	0.36
Colour NQC, score	24±0.1	24±0.2	0.07	26±0.2	26±0.1	0.59
Colour average, score	24±0.1	24±0.1	0.10	26±0.2	26±01	0.45
Firmness, N	7.1±0.3	6.9±0.2	0.62	8.8±0.2	9.4±0.4	0.16
Thickness, mm	25.4±0.5	24.5±0.5	0.03	35.6±0.7	36±0.6	0.64

Both fish groups were fed either low/medium or medium/high n-3 diets.
Values are means ± se.

*4.6 Colorimetric analyses (CIE L*a*b*) of thawed and cooked salmon fillets*

4.6.1 Fillets thawed at 4°C

4.6.1.1 Dorsal fillet region

Lightness (L* value) ranged from 40-42 for both S0 and S1. No differences in lightness were observed between diets of the S0 or S1 salmon group (Figure 4.2).

Redness ranged from 11.7-15.1 for both S0 and S1. No differences in redness were observed between diets of the S0 or S1 salmon group (Figure 4.2).

Yellowness ranged from 12.3-15.3. No differences in yellowness were observed between diets of the S0 or S1 salmon groups (Figure 4.2).

4.6.1.2 Ventral fillet region

Lightness ranged from 43-45.8. For S0 salmon group (Figure 4.2g). TestS (43.11±0.7) was lower in lightness than TestP (44.94±0.79) (P=0.03). No differences in lightness were observed between diets of the S1 salmon group (Figure 4.2).

Redness ranged from 12.3-16.6 for both groups. No differences in redness were observed between diets of the S0 or S1 salmon group (Figure 4.2).

Yellowness ranged from 13.8-18.05. There were no differences in yellowness between diets of the S0 or S1 salmon groups (Figure 4.2).

4.6.1.3 Dorsal and ventral fillet region (SalmoFan™)

For the S0 salmon group, SalmoFan™ scores ranged from 23.8-25 for the dorsal fillet region (Figure 4.3a, 4.3c). No significant color differences were observed between diets of the S0 or S1 salmon on the dorsal fillet region.

The color score of the ventral fillet region ranged from 23.4-24.6. No color differences were observed between diets of the S0 or S1 salmon group on the ventral fillet region (Figure 4.3)

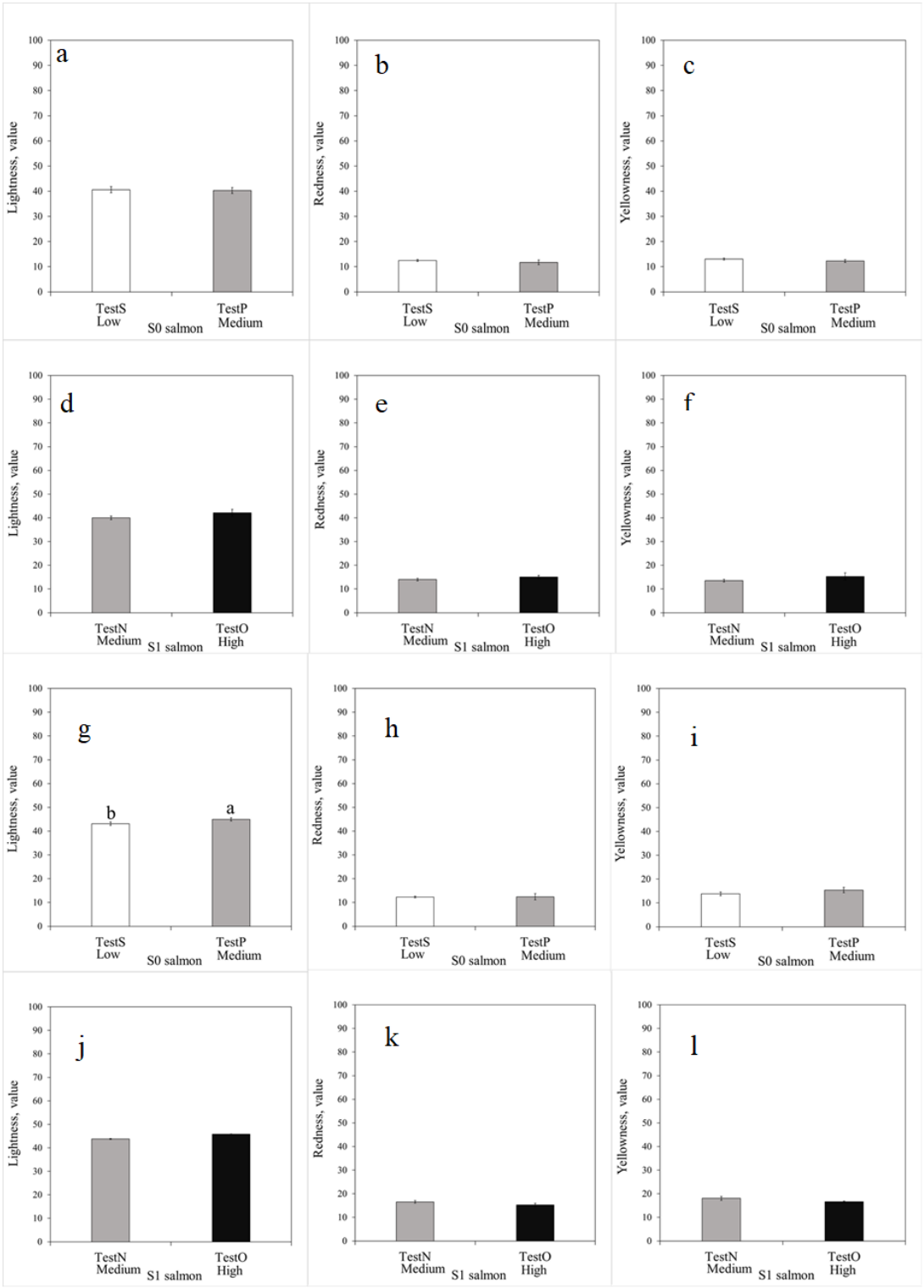

Figure 4.2. Comparison of colorimetric lightness (L* value), redness (a* value) and yellowness (b*value) of S0 (a-c, g-i) and S1 (d-f, j-l) Atlantic salmon fillets thawed at 4°C. The salmon were fed diets with low (TestS), medium (TestP, TestN) or high (TestO) levels of n-3. Color measurements were done on the dorsal (a-f) and ventral (g-l) fillet regions.

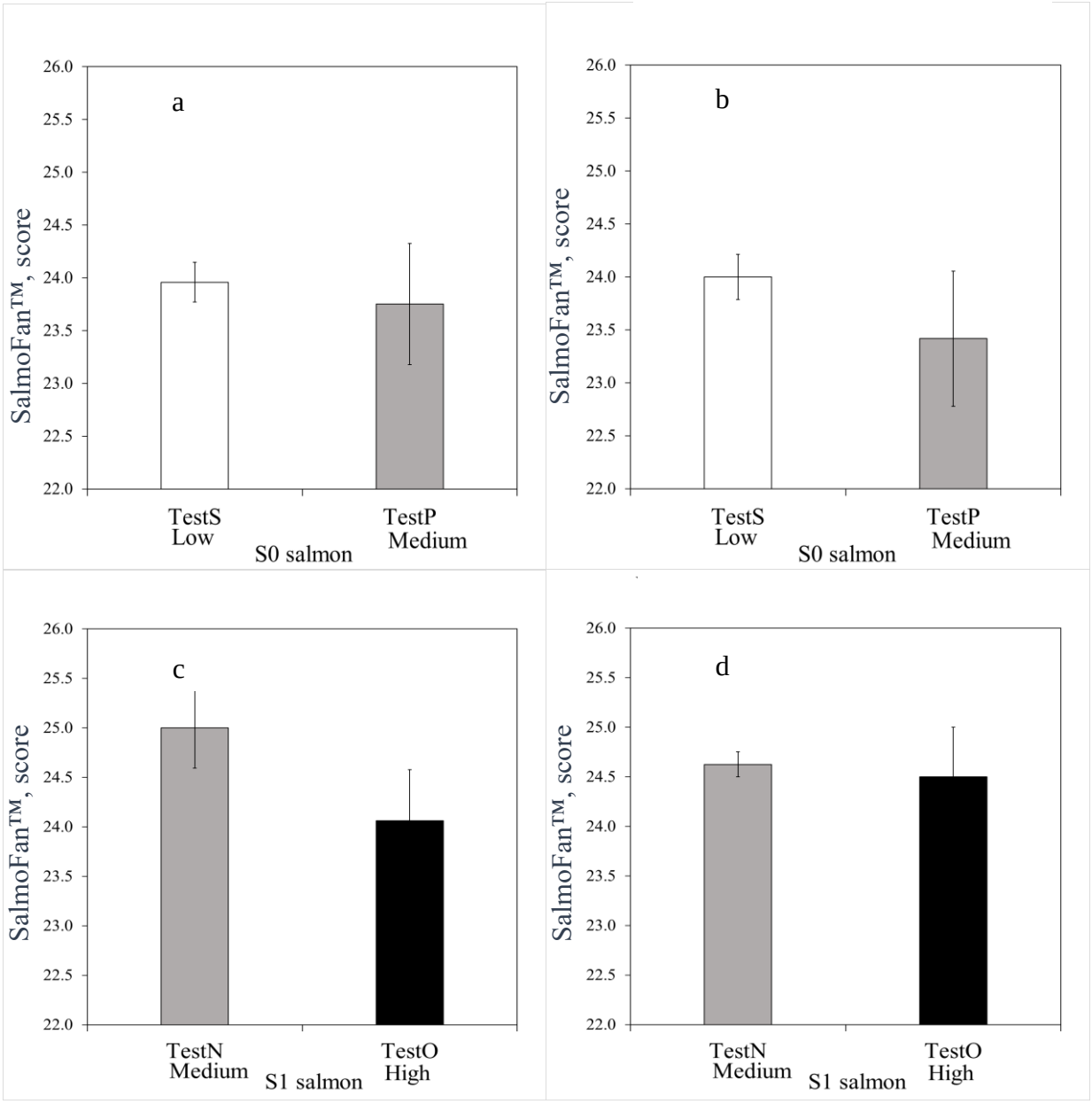

Figure 4.3. Visual color (SalmoFan™ scores) of S0 and S1 Atlantic salmon thawed at 4°C. The salmon were fed diets with low (TestS), medium (TestP, TestN) or high (TestO) levels of n-3. Color measurements were done on the dorsal (a, c) and ventral (b, d) fillet regions.

4.6.2 Fillets thawed at 20°C

4.6.2.1 Dorsal fillet region

Lightness ranged from 41-43.8. No differences in lightness were observed between diets of the S0 or S1 salmon group (Figure 4.4).

Redness ranged from 11.5-15.1. No differences in redness were observed between diets of the S0 or S1 salmon group (Figure 4.4).

Yellowness ranged from 12.8-15.9. There were no differences in yellowness between diets of the S0 or S1 salmon groups (Figure 4.4).

4.6.2.2 Ventral fillet region

Lightness ranged from 43.0-46.0. No differences in lightness were observed between diets of the S0 or S1 salmon group (Figure 4.4).

Redness ranged from 12.4-13.8. No differences in redness were observed between diets of the S0 or S1 salmon group (Figure 4.4).

Yellowness ranged from 14.1-15.5. There were no differences in yellowness between diets of the S0 or S1 salmon groups (Figure 4.4).

4.6.2.3 Dorsal and ventral fillet region (SalmoFan™)

SalmoFan™ scores for dorsal fillet region (Figure 4.5a, 4,5c) ranged from 23-24.4 for both S0 and S1 group.

No significant color differences were observed between diets of the S0 or S1 salmon group on the dorsal fillet region (Figure 4.5).

The ventral fillet region had a color score from 22.9-24.8 (Figure 4.5b, 4,5d) No color differences were observed between diets of the S0 or S1 salmon group on the ventral fillet region.

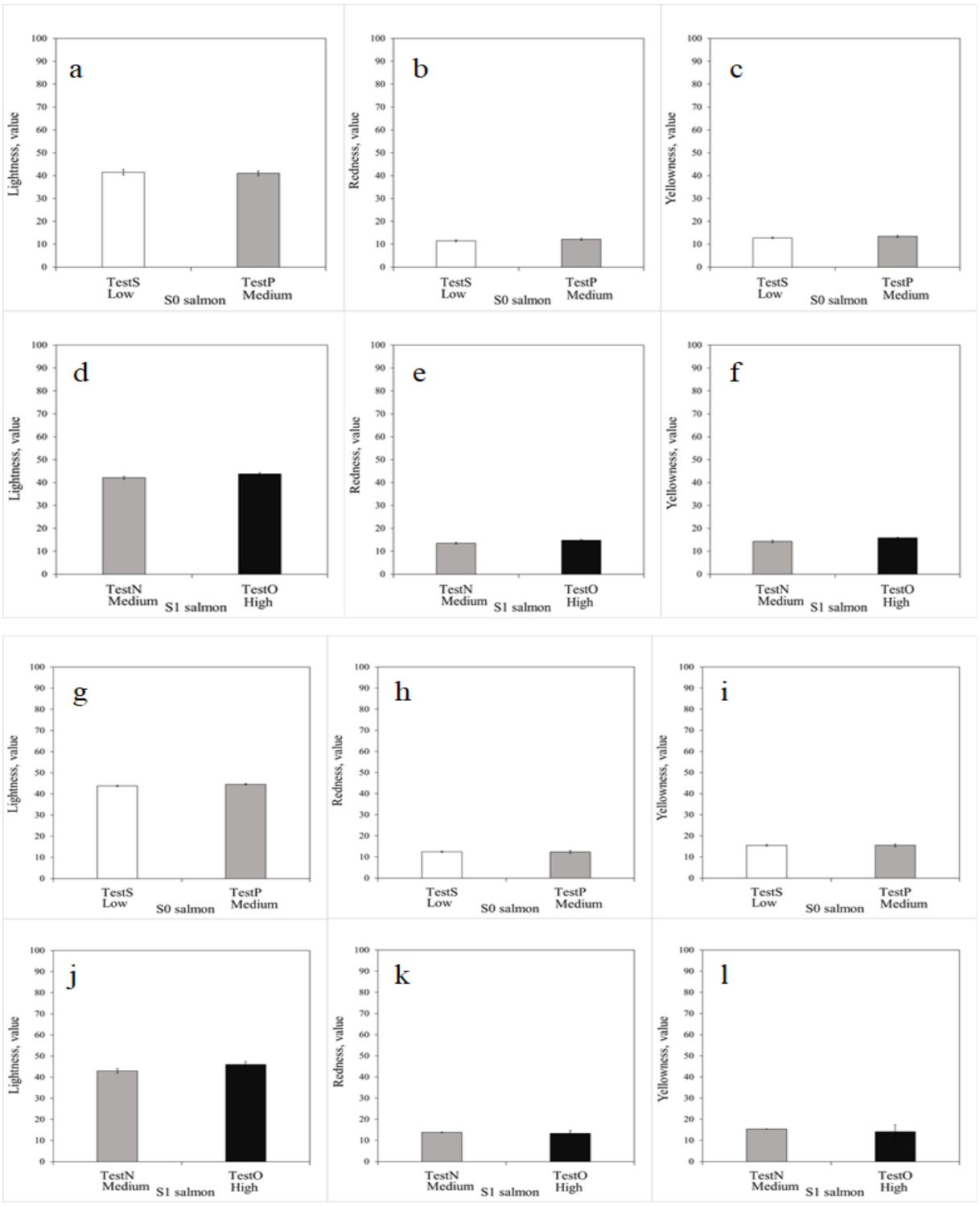

Figure 4.4. Comparison of colorimetric lightness (L* value), redness (a* value) and yellowness (b* value) of S0 (a-c, g-i) and S1 (d-f, j-l) Atlantic salmon fillets thawed at 20^0C. The salmon were fed diets with low (TestS), medium (TestP, TestN) or high (TestO) levels of n-3. Color measurements were done on the dorsal (a-f) and ventral (g-l) fillet region.

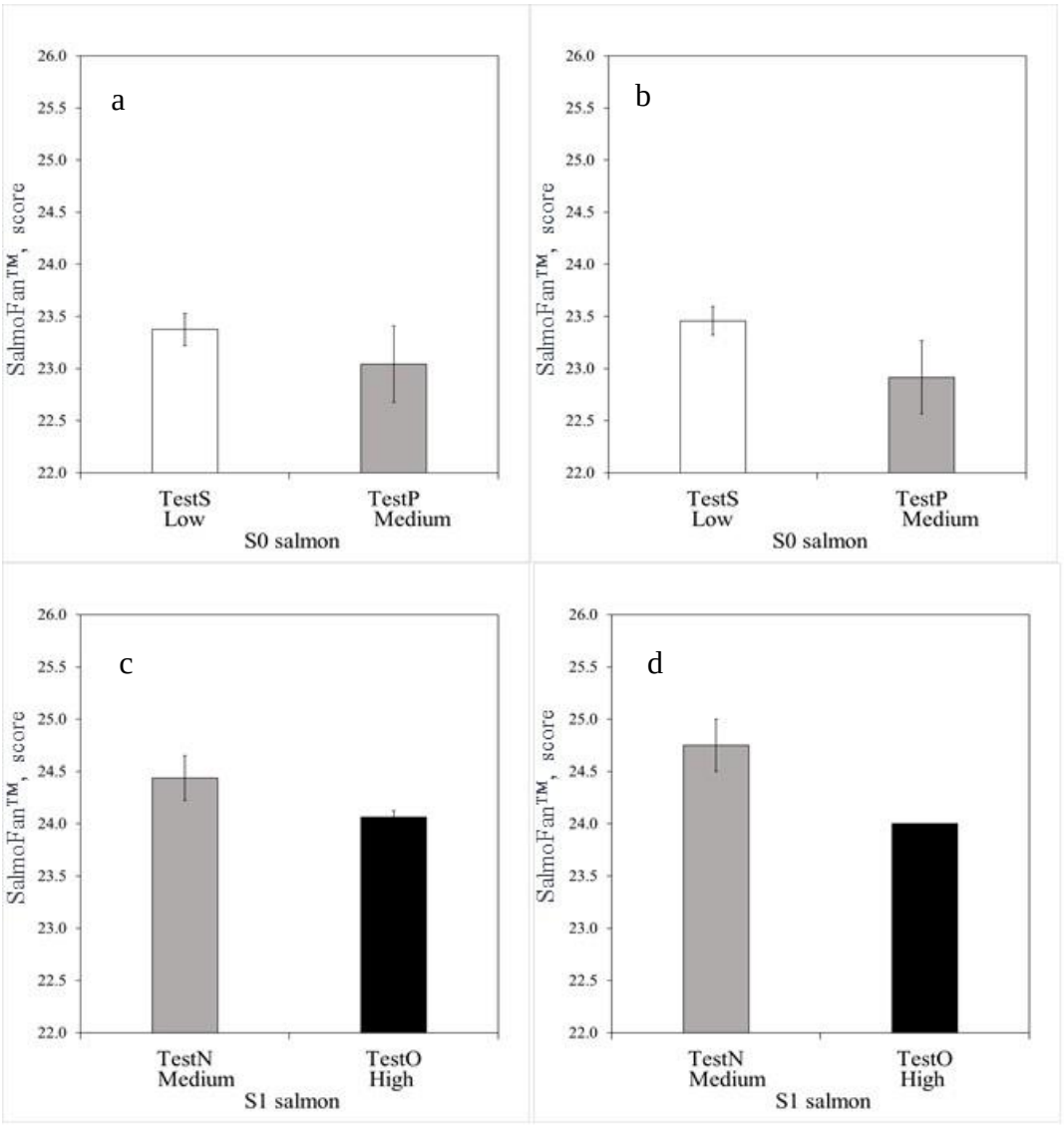

Figure 4.5. Visual color (SalmoFan™ scores) of S0 and S1 Atlantic salmon thawed at 20°C. The salmon were fed diets with low (TestS), medium (TestP, TestN) or high (TestO) levels of n-3. Color measurements were done on the dorsal (a, c) and ventral (b, d) fillet regions.

4.6.3 Fillets cooked after 4°C thawing

4.6.3.1 Dorsal fillet region

Lightness ranged from 65.6-68.3. No differences in lightness were observed between diets of the S0 or S1 salmon group (Figure 4.6).

Redness ranged from 16.3-18.9. No differences in redness were observed between diets of the S0 or S1 salmon group (Figure 4.6).

Yellowness ranged from 26.2-27.7. There were no differences in yellowness observed between diets of the S0 or S1 salmon groups (Figure 4.6).

4.6.3.2 Ventral fillet region

Lightness ranged from 65.1-67.5. No differences in lightness were observed between diets of the S0 or S1 salmon group (Figure 4.6).

Redness ranged from 14.6-17.9. No differences in redness were observed between diets of the S0 or S1 salmon group (Figure 4.6).

Yellowness ranged from 23.8-27.4. There were no differences in yellowness between diets of the S0 or S1 salmon groups (Figure 4.6).

4.6.3.3 Dorsal and ventral fillet region (SalmoFan™)

SalmoFan™ scores for the dorsal fillet region (Figure 4.7a, 4.7c) ranged from 20.5-21.5 for the S0 and S1 salmon groups. No significant differences were observed between diets of the S0 or S1 salmon group.

For ventral fillet region (Figure 4.7b, 4.7d) the range was from 20.4-21.2. TestS (21.2±0.3) had a higher color score on the ventral fillet region than TestP (20.4±0.2) (P=0.04) in the S0 group (Figure 4.7b). No significant differences were observed for the S1 salmon group.

4.6.4 Fillets cooked after 20^0C thawing

4.6.4.1 Dorsal fillet region

Lightness (L* value) ranged from 68.4-69.2. No differences in lightness were observed between diets of the S0 or S1 salmon group (Figure 4.8).

Redness (a* value) ranged from 13.3-17.7. No differences in redness were observed between diets of the S0 or S1 salmon group (Figure 4.8).

Yellowness (b* value) ranged from 21.9-25.1. There were no differences in yellowness between diets of the S0 or S1 salmon groups (Figure 4.8).

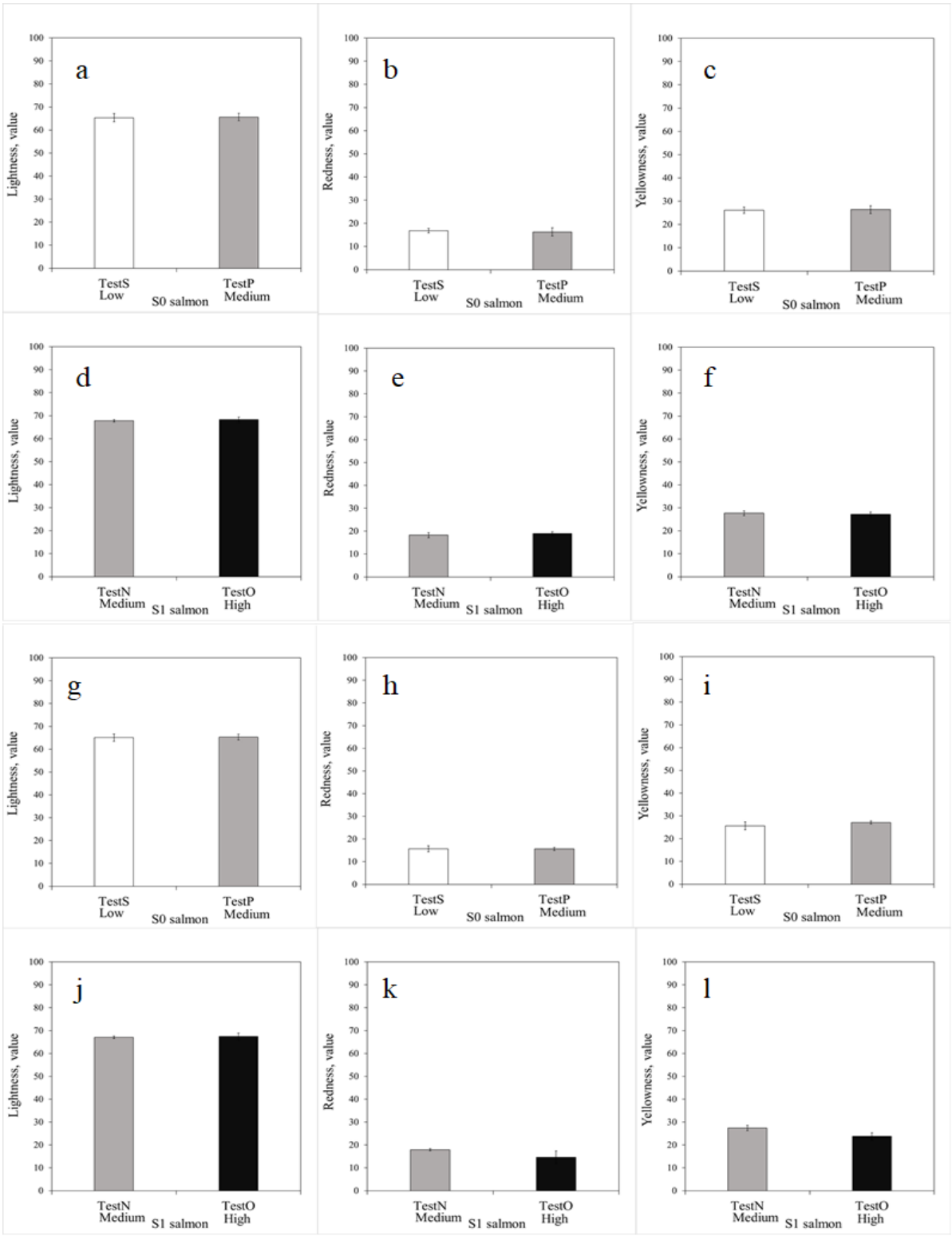

Figure 4.6. Comparison of colorimetric lightness (L* value), redness (a* value) and yellowness (b* value) of S0 (a-c, g-i) and S1 (d-f, j-l) Atlantic salmon fillets thawed at 4ºC and cooked. The salmon were fed diets with low (TestS), medium (TestP, TestN) or high (TestO) levels of n-3. Color measurements were done on the dorsal (a-f) and ventral (g-l) fillet regions.

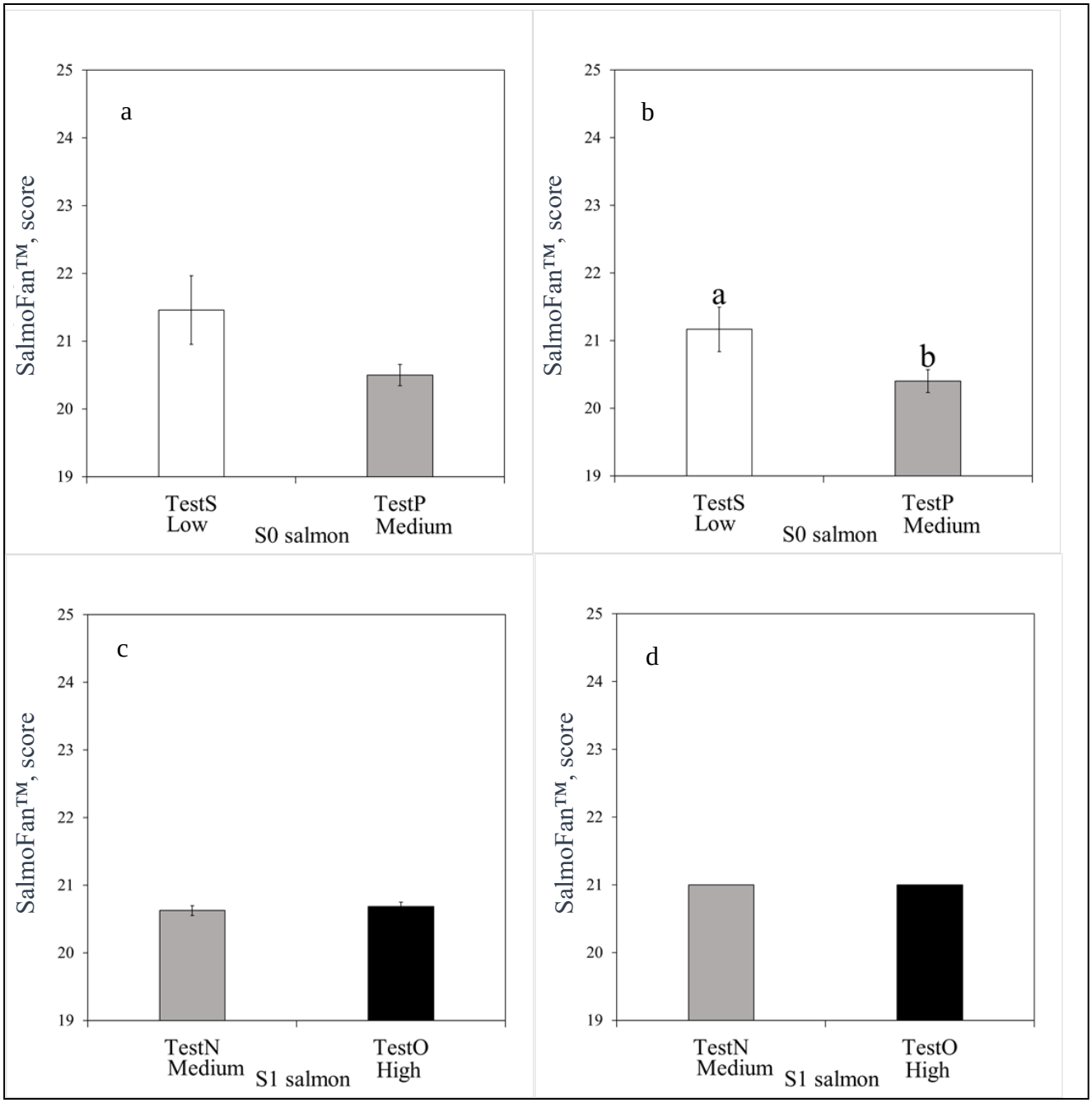

Figure 4.7. Comparison of SalmoFan™ color scores of S0 and S1 Atlantic salmon fillets thawed at 4°C and cooked. The salmon were fed diets with low (TestS), medium (TestP, TestN) or high (TestO) levels of n-3. Color measurements were done on the dorsal (a, c) and ventral (b, d) fillet regions.

4.6.4.2 Ventral fillet region

Lightness ranged from 64.9-68.2. No differences in lightness were observed between diets of the S0 or S1 salmon group (Figure 4.8).

Redness ranged from 13.5-15.4. No differences in redness were observed between diets of the S0 or S1 salmon group (Figure 4.8).

Yellowness ranged from 21.1-23.8. There were no differences in yellowness between diets of the S0 or S1 salmon groups (Figure 4.8).

4.6.4.3 Dorsal and ventral fillet region (SalmoFan™)

SalmoFan™ scores for the dorsal fillet region (Figure 4.9a, 4.9c) ranged from 20.4-21.3 for both the S0 and S1 salmon groups. No significant differences were observed between diets of the S0 or S1 salmon group on the dorsal fillet region.

For ventral fillet region (Figure 4.9b, 4.9d), the range was from 20.7-21.3. No significant differences were observed for the S0 or S1 salmon group on the ventral fillet region.

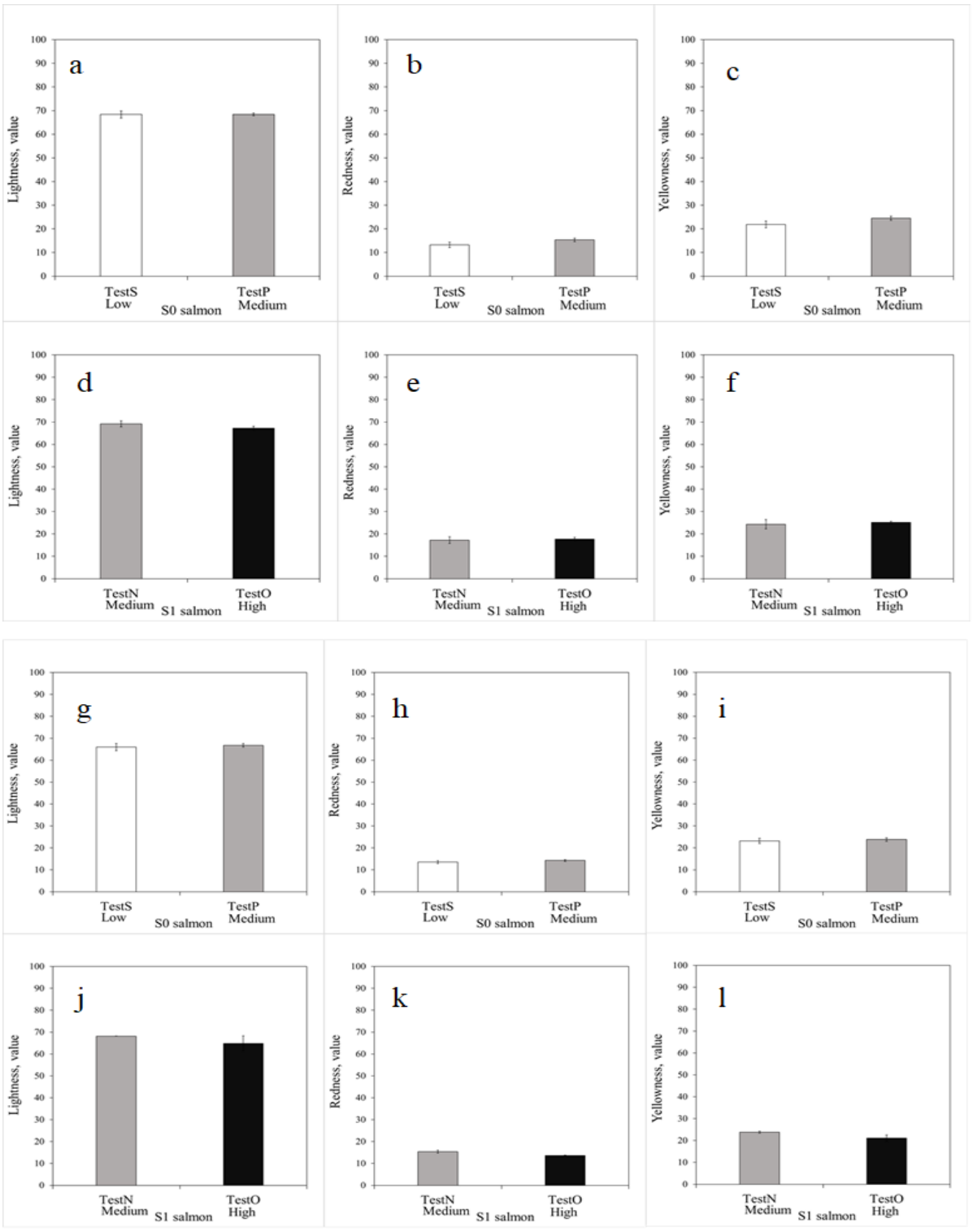

Figure 4.8. Comparison of colorimetric lightness (L* value), redness (a*value) and yellowness (b* value) of S0 and S1 Atlantic salmon fillets thawed at 20°C and cooked. The salmon were fed diets with low (TestS), medium (TestP, TestN) or high (TestO) levels of n-3. Color measurements were done on the dorsal (a-f) and ventral (g-l) regions.

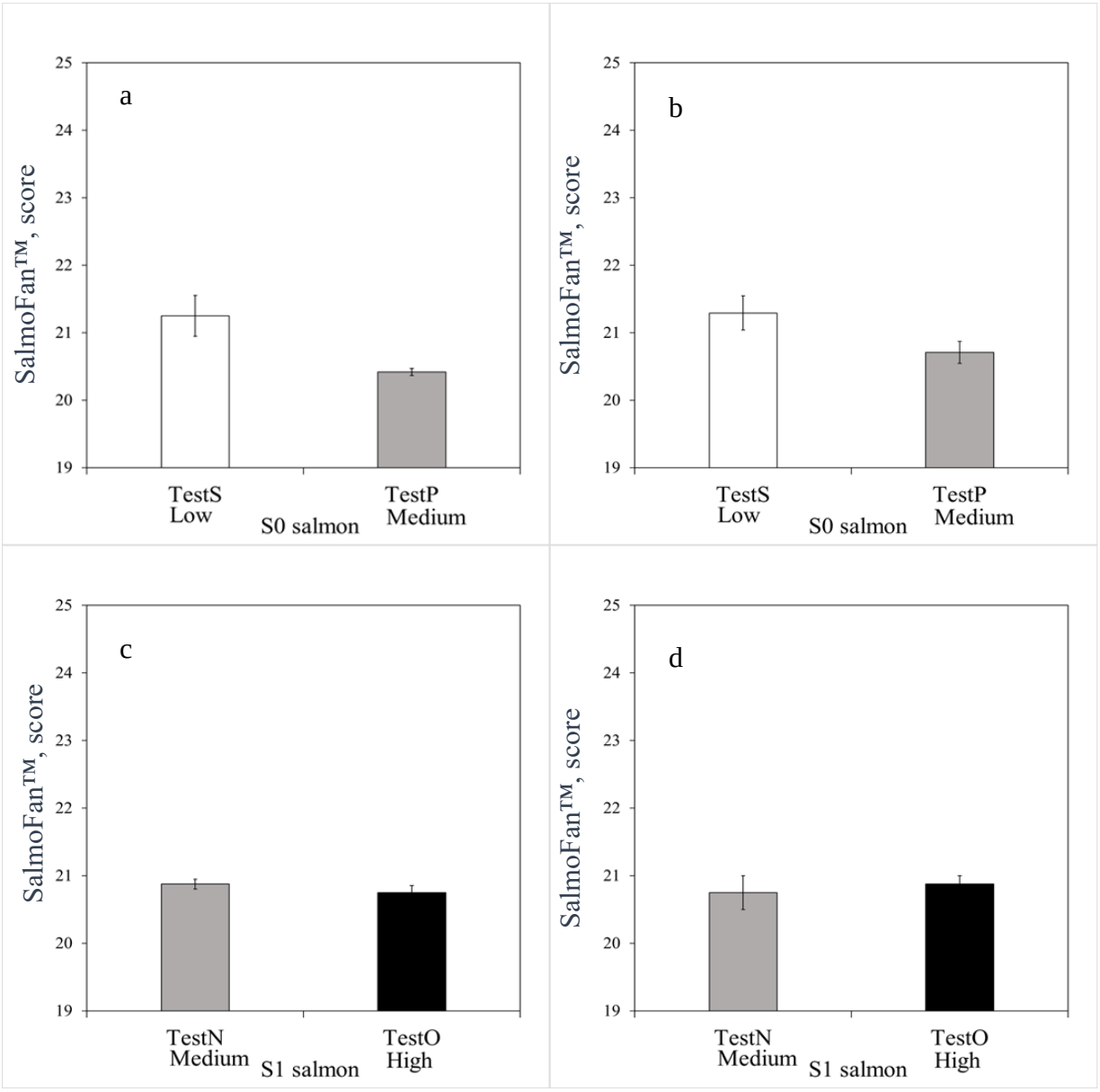

Figure 4.9. Comparison of SalmoFan™ color scores of S0 and S1 Atlantic salmon fillets thawed at 20°C and cooked. The salmon were fed diets with low (TestS), medium (TestP, TestN) or high (TestO) levels of n-3. Color measurements were done on the dorsal (a, c) and ventral (b, d) fillet regions.

4.6.5 Drip loss, cook loss and total liquid loss during thawing and cooking of Atlantic salmon fillets

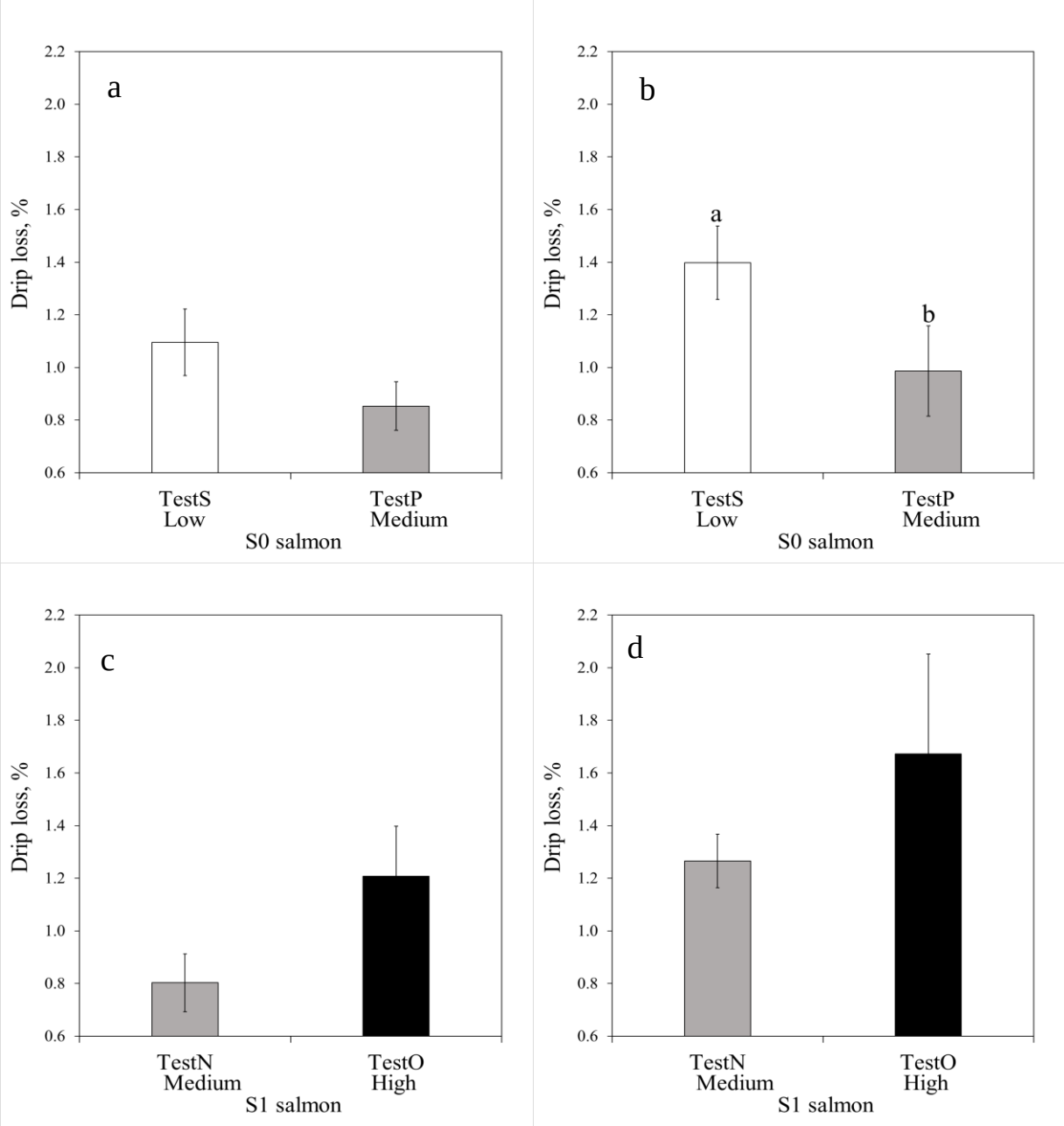

Figure 4.10. Comparison of drip loss of S0 and S1 Atlantic salmon fillets thawed at 4°C (a, c) or 20°C (b, d). The salmon were fed diets with low (TestS), medium (TestP, TestN) or high (TestO) levels of n-3.

4.6.5.1 Drip loss for 4°C and 20°C thawing temperature

Range for drip loss during 4°C (Figure 4.10a, 4.10c) thawing was between 0.8-1.2% for both S0 and S1 salmon. There were no significant differences for the S0 or S1 salmon group.

Range for drip loss during 20ºC thawing (Figure 4.10b, 4.10d) was between 1.0-1.7%.

At 20ºC thawing, TestS (1.4±0.1) was higher than TestP (1.0±02) (P=0.04) for the S0 salmon group. There were no differences between diets in the S1 salmon group (Figure 4.10)

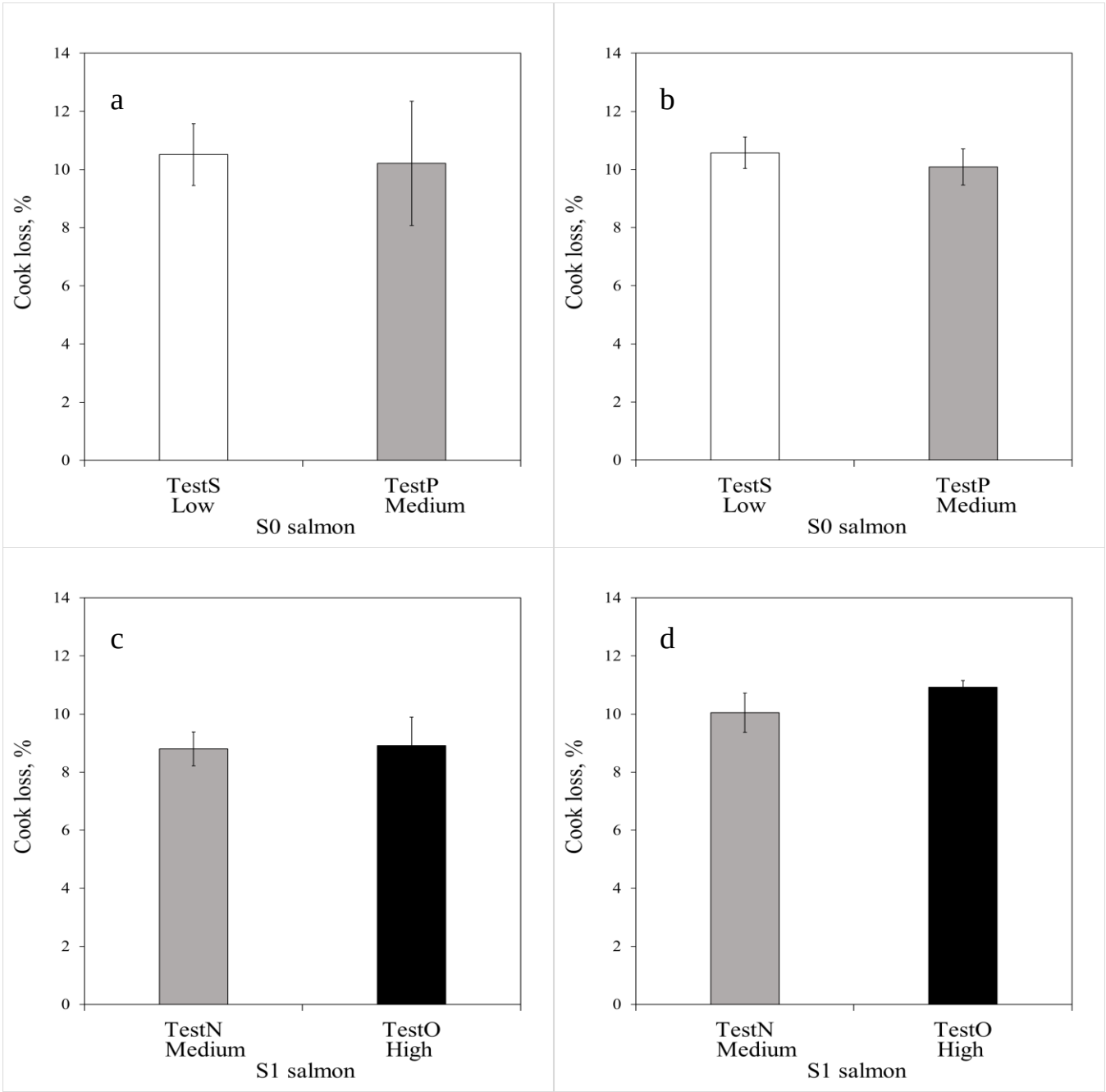

Figure 4.11. Comparison of cook loss of S0 and S1 Atlantic salmon fillets thawed at 4ºC (a, c) or 20ºC (b, d) prior to cooking. The salmon were fed diets with low (TestS), medium (TestP, TestN) or high (TestO) levels of n-3.

4.6.5.2 Cook loss for fillets thawed at 4ºC or 20ºC

Range for cook loss was 8.8-10.5% for fillets thawed at 4ºC (Figure 4.11a, 4.11c) There were no significant differences for the S0 or S1 salmon group thawed at 4ºC.

Range for cook loss was 10.1-10.6% for fillets thawed at 20ºC (Figure 4.11b, 4.11d) There were no differences between diets of the S0 or S1 salmon group.

4.6.5.3 Total liquid loss for fillets thawed at 4ºC or 20ºC

Range for total liquid loss was between 11.6-13.6.5% for 4ºC thawing (Figure 4.12a, 4.12c). There were no significant differences between diets of the S0 or S1 salmon group thawed at 4ºC.
Range for liquid loss during cooking was between 11.0-12.4% for 20ºC thawing (Figure 4.12b, 4.12d). There were no differences between diets of the S0 or S1 salmon group.

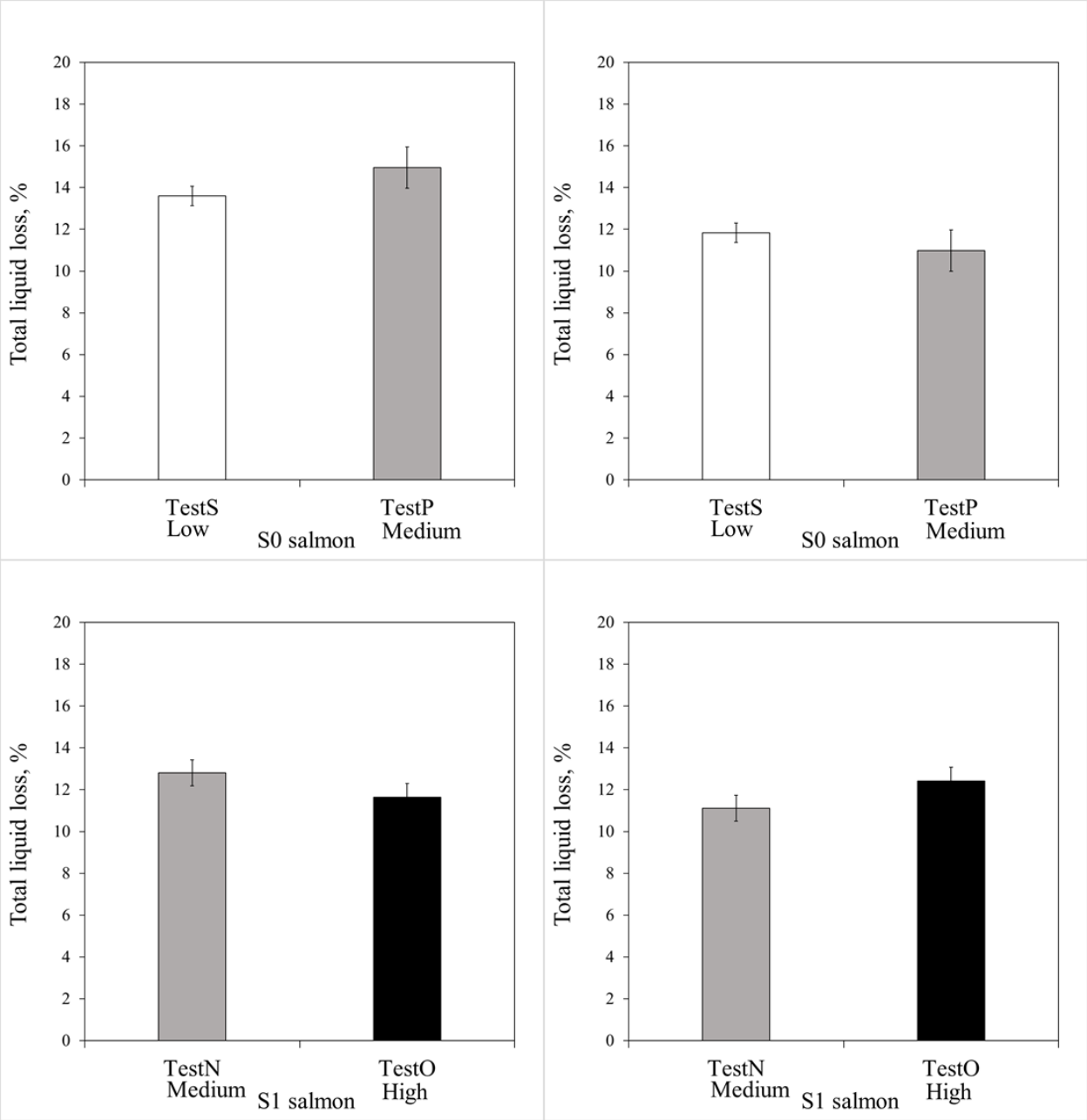

Figure 4.12. Comparison of total liquid loss of S0 and S1 Atlantic salmon fillets thawed at 4°C (a, c) or 20°C (b, d) prior to cooking. The salmon were fed diets with low (TestS), medium (TestP, TestN) or high (TestO) levels of n-3.

5 DISCUSSION

This study assessed the fatty acid profile in the fillets of Atlantic salmon farmed in Arctic and fed diets containing varying levels of EPA and DHA. In addition, effects of frozen storage at different thawing temperatures (4ºC, 20ºC) and cooking on flesh quality were investigated.

Body length, condition factor and fillet yield showed a similar pattern among the dietary groups, although the gutted weight differed significantly (1.5kg for the S0 salmon and 4.4kg for the S1 salmon). The reason being that weight and length have somewhat isometric associations (Froese and Pauly, 2009) and which in turn affects the condition factor and fillet yield.

The fish sampled for analyses represented the average weight of the fish in the net pens. The lower weight of the S0 salmon was according to expectations since the fish were transferred to seawater approximately half a year later than the S1 salmon. Nevertheless, the body weight of the S0 salmon is considered to be low after one year in sea. The lower growth of the S0 salmon is however consistent with investigations by Thrush et al. (1994) and Duncan et al. (1998,1999) who reported that underyearlings (S0) autumn-transferred smolts get low and reduced daylength after transfer to sea with variable growth performance than normal spring-transferred smolts. Moreover, Wade (2019) reported that Atlantic salmon has reduced growth in the summer and undergoes compensatory growth in the autumn, which is a plausible reason that the S0s were smaller in this current study. However, since harvest was before autumn the compensation was not achieved as opposed to S1s which were harvested during the winter. Again, the different sampling schedules may explain the higher fat content in the S1 salmon. In a contrasting study by Mørkøre & Rørvik (2001), the authors observed no effect of seasonal variations on growth even though the opposite occurs in production efficiency and quality of fish. An example of the latter is fat deposition. Fillet fat was not higher than 16% and thus acceptable since values above 18% have an adverse effect on texture and other quality parameters (Gjedrem, 1997).

Fishes are what they eat (Caballero et al., 2002; Rosenlund et al, 2001; Torstensen et al., 2000), therefore the muscle composition reflects the nutritional composition. This is not always true since the rate of usage maybe not be balanced as some nutrients are utilized at a faster rate than others (Bell, 2003, Torstensen et al., 2004a).

Moreover, lipid content in fish muscles increase with body weight (Hemre & Sandnes 1999; Powell, et al. 2008; Torstensen et al., 2005). It is therefore expected that more fat is accumulated

in fish with larger weight, as was observed in the present study (12% in S0 and 15% in S1 salmon). The sample size presented for the FAME analysis was short of one net pen (TestO) from the S1 salmon group and this may explain why there was no significant difference in fat percentages between the S1s (n=2 rather than n=3).

High concentrations of C18:1n9c (oleic acid) and C18:2n6c (linoleic acid) has been associated with fish fed diets with rapeseed oil (Bell et al., 2002; Ng et al., 2004; Sissener, 2018). Accordingly, C18:1n9c was the highest FA followed by C18:2n6c. The C18:1n9c concentration in the S0 salmon was higher in TestS than TestP, corresponding with the high amount of rapeseed present in the feed.

Research has shown that C20:1n-11 act as long chain erucic acid during metabolism in fish and may be the reason why they were present in the flesh as gondoic acid and not erucic acid. Dietary forms of C22:1n-11 are known to be high energy sources for salmon and trout and in addition, enhance the synthesis of EPA and increase the ability of retaining EPA and DHA by the whole body (Østbye, 2019).

The higher content of C16:1n7 and C20:1n11 in the TestP may be because during the feeding period, TestP had a constant EPA&DHA concentrations in the feed while TestS had a variable EPA&DHA and thus the larger build up in the former diet. This was also the case for the TestN and TestO, where the former had a constant EPA&DHA concentrations whiles the latter had a constant level, thus resulting in inconsistent and no patterns in the various diet groups that corresponds with the literature.

The higher levels of n-6 PUFA observed in the TestS compared with the TestP reflected the higher dietary level. This further allowed for higher concentration n-6 than n-3 PUFAs. In addition to dietary differences, research has shown that n-3 PUFA compete with n-6 PUFAs for similar enzymes used in elongation and desaturation of their parent forms (Strobel, 2012). Therefore, the concentration of one can be an antithesis of the other due to this competition for the enzymes for the purported modifications. The ratio of n-3 to n-6 PUFA is essential in human, where a ratio of 1:1 is deemed appropriate and health beneficial (Simopoulos, 2002). This was not exactly the case for this study. Linolenic acid is known for maintenance and function of membranes in cells of organism (Bell et al, 1991) and confirms why it was the predominant PUFA. Study by Norambuena et al. (2016) indicate that their converted forms ARA are critical during temperature fluctuations.

EPA increase enzymes activities for mitochondrial β-oxidation whiles DHA is peroxisomal in rats (Madsen et al. 1999; Willumsen et al. 1993, 1996). TestO was higher than TestN for the S1 salmon group in terms of EPA and DHA concentrations. Apart from a mistakenly overdose of EPA and DHA in one of the TestP feed batches, as reported by one of the technicians, it may be that excess amount of the EPA&DHA with respect to EPA led to conversions to DPA, in consistent with literature (Hatlen et al 2012; Kjær et al., 2008; Østbye et al., 2011; Todorcevic et al., 2009). These authors found that Atlantic salmon possess a high capacity for elongation of EPA to DPA, particularly in a high EPA diet. Generally, S1 salmon showed a better growth than S0 in terms of both body weight and length. According to (Madsen et al. 1999; Willumsen et al. 1993, 1996), when dietary levels of certain fatty acids are increased, they could induce an increase in β-oxidation capacity generating energy for metabolism, in which sense spare protein for muscle growth in fish. This phenomenon is prominent in cold conditions where the relative capacity for oxidizing lipids tends to increase. Considering that conditions were colder in the S1 group than the S0, the growth disparity observed between the S1 and the S0 salmon groups is probably due to temperature difference. In addition, there is known effect of hepatic desaturation and elongation of alpha linolenic acid from the diets which cause some levels of reduction in EPA&DHA and therefore maybe the reason for reduced levels of EPA&DHA in TestO compared with TestP.

Studies have shown that the quality of fish fillets are influenced by season, largely because it affects growth rate, fat content and maturation (Lavety et al.,1988). Thickness of fillets of Atlantic salmon varies from the head to tail region, with normal values around 23mm (Weihe, 2019) which was similar in the present study. With regards to the diet, thickness was higher in TestS than TestP for the S0 salmon group. This may be because they were larger in size than TestP, since their larger weight means more deposition of flesh/and or fat than smaller ones.

Texture Profile Analysis mimic the biting by humans/consumers, and thus hardness, chewiness, resilience and gumminess may vary based on the thickness of the fillet. Hardness increases with increasing thickness (Veland, 1999) although this contrasts the findings of Gregory, (2014) who did not confirm a positive correlation between thickness and hardness. Preservation and cooking methods such as salting/brining are known to be influenced by fillet thickness and as a result the thicker the fish, the more time it takes to be well cooked (Mørkøre, 2009). In the salmon industry, thickness around the dorsal fin region is a good predictor of yield of individual fishes during

slaughtering and processing from the farm, thus meeting consumer demand to prevent loss of edible flesh and increasing profit for fillet companies.

Studies by Dawson (2018) showed that increase in lightness value occur with high freezing rate and vice versa in reduced lightness. Furthermore, higher concentration of astaxanthin in TestS diets maybe another possible reason for the higher color score for it than TestP when the Salmonfan was used after cooking and the differences in lightness between TestS and TestP. Besides, size is known to have effect on pigmentation, but this should have been noticed for TestN and TestO fed to the S1 groups which had larger body sizes. However, this was not observed. Thus, supporting the fact that its due to high levels of astaxanthin in TestS (Wathne, 1988).

In Atlantic salmon, sex plays an important role in pigmentation. Males tend to be more pigmented than females since a significant proportion of pigments used in their feed is channeled to their eggs during vitellogenesis, but also in immature fish, the color may differ. Therefore, if the sex ratio between the test groups is skewed, it might lead to observed disparity in fillet coloration. In the present study, gender was included in the statistical model.

Even though cooked fillets were paler than the fresh ones, it was seen that some levels of coloration was maintained, and thawing at lower temperatures appeared to preserve the color of salmon fillets better than elevated temperatures. Additionally, lower thawing temperatures may be beneficial as Haugland (2002) suggested that high surface temperatures of fillets during thawing may promote the growth of microbes. Thawing at lower temperatures takes more time to obtain a completely thawed fish and may not be ideal in cases when time is a constraint. Since cooking procedures were the same for both thawing groups, the results suggest that thawing at lower temperature (4^{o} C) helps to retain the fillet color than thawing at higher temperature (20^{o}C) before cooking.

The higher concentration of oleic acid in TestS diet (from the rapeseed/canola oil in feed) may explain the higher drip loss in TestS diet group than TestP for the S0 salmon at 20^{o}C. Rapeseed oils have a melting point between 13^{o}C to 16^{o}C (Lutton, 1946; Abrahamsson, 1962). Therefore, thawing at temperatures above this threshold could convert these oils in their liquid form and leave the fillet with the water during thawing. This will result in the oils easily escaping from the fillet in such high amounts during thawing. However, there seem to be factors besides from the fatty acid composition that affect the drip loss, as TestO tended to have a higher drip loss compared with TestN.

CONCLUSION

This study revealed that time at which smolts were transferred to sea and sampling time affected body size, length and fillet fat content in Atlantic salmon farmed in the Arctic. The dietary fatty acid profile significantly affected the fatty acid profile in the fillets in both S0 and S1 salmon groups, with a significant increased percentage of EPA&DHA in fillets of salmon fed diets with increased levels. However, when expressed as g/kg, no dietary effect was observed for the S1 group. Fillet quality of the S1 group showed no differences between dietary groups. For the S0 group, thicker fillets, more intense color of cooked fillets thawed at 4 °C and higher drip loss was observed for salmon fed low omega-3 levels. Thawing fillets at 4°C compared with 20°C resulted in less drip loss during thawing and cooking and improved color of the cooked fillets

Recommendation

Future studies are recommended to repeat this study with increase in sample size to reveal the full effects. In addition, effect of different packaging methods on the thawing and cooking loss and rate in salmon fillets should be considered since observations were made in the early stages of this study that vacuum packaging tended to have a slower thawing rate than air packaging.

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