Norwegian University of Life Sciences
Department of Animal and Aquacultural Sciences

Master Thesis 2016
30 credits

Effect of dietary enrichment with antioxidants on the sensory quality of raw and cooked Atlantic salmon (Salmo salar L.)

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Effect of dietary enrichment with antioxidants on the sensory quality of raw and cooked Atlantic salmon (*Salmo salar* L.)

Master thesis in aquaculture

(30 credits)

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March 2016

Acknowledgements

This master thesis has been written and submitted to Department of Animal and Aquacultural sciences at Norwegian University of Life Sciences (NMBU). The work presented in this thesis was carried out at Nofima, Ås.

First, I would like to express my sincere gratitude to my main supervisor, Dr. Turid Mørkøre, who is the consistent source of information with detailed guidance in doing experiments; statistical analyses, valuable comments, constructive suggestions and enabling me to better understand the subject that made the accomplishment of this manuscript. I am grateful and deeply indebted to my co-supervisor Professor Dr. Kjell-Arne Rørvik for the overview of my thesis, valuable comments and supports while writing my thesis. I would like to express my genuine gratitude to Thomas Larsson for the help while writing thesis and assistance during laboratory work.

Thanks Målfrid, Atik, Sarif, Meng and Wang for your company during sampling and lab work. Thanks Raul for the company while writing thesis. Thanks to all my friends who participated in sensory assessment of cooked salmon fillet.

Last, but not least, I feel proud to say thank to my family members for their continuous support and inspiration, my wife Nirmala Adhikari for her encouragement and my little boy Alvin for his patience while writing thesis.

Pradip Adhikari
Ås, 2016

Abstract

The main sensory quality parameters of Atlantic salmon are color, texture, fillet integrity, taste and odor. The aim of the present study was to investigate the effect of diet on sensory quality of raw salmon fillets, and sensory quality and consumer acceptability of cooked salmon fillets. Diets investigated were a standard commercial feed and the same feed added antioxidants (vitamin C, vitamin E and selenium). The experiment was carried out from 7th January to 13th March 2015. Salmon with an average body weight of 4123 ± 106 g and 4203 ± 108 g of control and antioxidant diet groups respectively were killed by percussive stunning. Fillet color, gaping and texture were analyzed in the raw fillets after 7 days of ice storage. Untrained assessors evaluated color, odor, tastiness, juiciness, firmness and acceptability of cooked fillets after 7 months of storage at -40°C. The result showed significant higher fillet weight, lower gaping and higher juiciness of salmon fed the antioxidant diet group compared to the control diet. Also a tendency to improved tastiness and firmness were observed for salmon fed the antioxidant diet. Females rated the cooked salmon as tastier, firmer and juicier than males. Color, odor, tastiness and juiciness, but not firmness, correlated significantly to the acceptability. It is concluded from the present experiment that dietary supplementation of antioxidants improved muscle growth, integrity of raw fillets and sensory quality of cooked fillets.

Key words: Sensory quality, Atlantic salmon, antioxidant diet, fillet weight, gaping and juiciness

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List of Abbreviations

HPLC	High performance liquid chromatography
VIS/NIS	Visible and near infrared
QIM	Quality Index Method
ROS	Reactive Oxygen Species
NQC	Norwegian Quality Cut
ANOVA	Analyses of variance
SEM	Standard error of means

1. Introduction

Global aquaculture has grown steadily by an average annual growth rate of 8.8 % from 1980 to 2012. World per capita fish consumption increased from an average of 9.9 kg in the 1960s to 19.2 kg in 2012. World´s population is expected to reach 9.6 billions in 2050, therefore the demand for fish production is projected to increase in the coming years (FAO 2014).

Fish accounted for 16.7 % of the global intake of animal protein and 6.5 % of all protein consumed in 2010 (FAO 2014). Fish is an important dietary source of iodine, selenium, and vitamins such as A, D and E (Tacon & Metian 2013). Seafood, and especially fatty fish species such as salmon, are rich sources of omega-3 (n–3) long-chain polyunsaturated fatty acid (Tur et al. 2012). Therefore, consumption of salmon is highly encouraged.

The farming of Atlantic salmon (*Salmo salar* L) has become an important enterprise especially in Norway. Norway has a coastline of 83,000 km, which is suitable for aquaculture activities of salmonids. Salmon farming started in Norway in the late 1960s on an experimental level and became commercial in the 1980s (Stickney 1991). Within a few decades, Norway has become the world´s largest producer of salmon with a production of 1,3 million tons of slaughtered salmon with a trade value of around 41,8 billions NOK in 2014 (Fiskeridirektoratet 2015).

Fish quality is a complex term, and specific parameters that are important in one part of the world may be less important in other parts of the world. Quality differs between marketing chains. For farmers, growth and feeding ratio are of great importance whereas positive perception is of main concern for consumers and processors (Rasmussen 2001). Flesh quality of salmon is influenced by several factors, such as breeding (Gjedrem 1997), feed composition (Bell et al. 2002; Thomassen & Røsjø 1989; Torstensen et al. 2008), metabolism (Mørkøre et al. 2008), stress (Mørkøre et al. 2008), season (Mørkøre

& Rørvik 2001) and starvation period prior to harvesting (Einen & Thomassen 1998; Mørkøre et al. 2008). Salmon is a perishable product (Korneliussen & Grønhaug 2003), so the producers are paying special attention to maintain the fresh quality during storage.

Sensory evaluation is a scientific discipline used to analyze and interpret characteristics of food as perceived by the sense of sight, smell, texture and taste (Olafsdottir et al. 1997). The main sensory quality factors in salmon are color, texture, fillet integrity, taste and odor. Moreover, freshness is significantly important for all food items (Olafsdottir et al. 2004).

The main aim of the present study was to investigate the effect of feed on the sensory quality of raw fillets and sensory quality and consumer acceptability of cooked fillets. Diets investigated were a standard commercial feed, and the same feed added vitamin C, vitamin E and selenium.

2. Theoretical background

This chapter gives general information about sensory quality, flesh quality characteristics and methods for the evaluation of sensory quality respectively. Additionally, information is given about antioxidants and muscle structure and composition.

Feed represents the highest single cost factor in the Norwegian salmon farming (Aas et al. 2015). Consequently, high growth performance and utilization of feed are important for the profitability. Dietary antioxidant supplementation is an effective strategy to introduce antioxidant into fish muscle in aquaculture. Chaiyapechara et al. (2003) reported that high dietary vitamin level during the grow-out period and a few weeks before harvest in a finishing diet safeguard product. Vitamins are a costly component of the fish diet and it is crucial to establish minimum feeding period required before slaughter to obtain maximum antioxidant effect (Ruff et al. 2003).

2.1. Sensory quality

The concept of sensory quality changes with time. Kramer defined sensory quality in 1959 as "The composite of those characteristics that differentiate among individual units of a product and have significance in determining the degree of acceptability of that unit by the user". Some authors' center their attention on the first part of the definition that the sensory quality is product oriented while other emphasis the second part of the definition that is consumer oriented.

Sensory evaluation is defined by Olafsdottir et al. (1997) as "A scientific discipline used to evoke, measure, analyze and interpret human reactions to products based on human senses".

According to Lawless and Heymann (2010), the principles and practices of sensory evaluation involve each of the four activities mentioned in the above definition and explained as:

1) ´To evoke´- sensory evaluation gives guidance for the preparation and serving of samples under controlled conditions for reducing the biasing factors;

2) `To measure`- sensory evaluation is a quantitative science in which numerical data are collected to establish relationships between product characteristics and human perception;

3) `Analysis´- proper data analysis is a critical part of sensory test. Data generation from human observers is highly variable that cannot be completely controlled in sensory test;

4) `Interpretation of results´ - sensory evaluation practices is an experiments. Data and statistical information are only useful when interpreted in the context of hypothesis, background knowledge, and implication for decisions and actions to be taken.

Traditionally, sensory evaluation was used as a subjective assessment of quality. However, it is turned to be an objective quality tool (Hyldig & Green-Petersen 2004). Sensory quality perceived by consumers has major effect in acceptance and market value of products. Sensory evaluation plays a key role in the food industry to reduce risk and uncertainty regarding ingredients modification, launches of new product in the market and shelf life stability. Instrumental methods are used for the evaluation of sensory characteristics, which gives objective results. However, for the description of the edible profile like odor and taste, a sensory panel consisting of trained or untrained persons is often employed (Rasmussen 2001).

Instrumental assessments of eating quality components can only be the approximation to the true measure of particular attributes. Instruments cannot measure the range of interacting characteristics that contribute to eating quality. Humans can assess several

attributes simultaneously, for example juiciness, flavor, odor, and mouth feel. On the other hand, a given instrumental determination is an objective evaluation of specific sensory property.

Sensory evaluation of salmon at different stage of marketing chain is shown in figure 2.1.

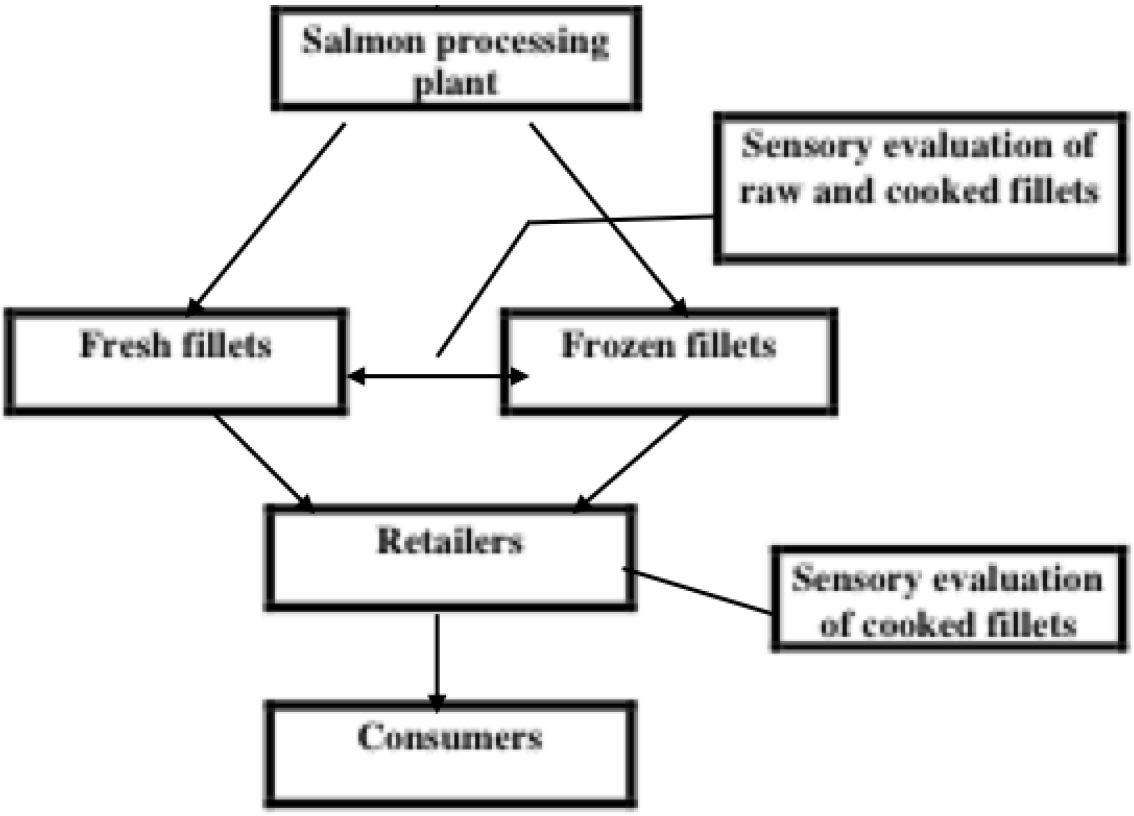


Figure 2.1. Sensory evaluation of Atlantic salmon (*Salmo salar* L.) during marketing chains. Adopted from Cheng et al. (2015).

2.2. Flesh quality characteristics

2.2.1. Color

Color is the visual display of the fillet. It is an important quality parameter and decision maker for the consumers when purchasing the salmon fillet. The increasing production of Atlantic salmon around the world leads to higher demands for quality. The characteristic pink color of salmon flesh is a major determinant for consumers preferred choice of product (Alfnes et al. 2006).

The red color of salmon is a result of carotenoids such as astaxanthin and canthaxanthin deposition in the flesh from the dietary sources because salmon cannot synthesis coloring pigments *de novo*. Atlantic salmon poorly utilizes dietary carotenoids and the retention of astaxanthin in the muscle is less than 12 % when the dietary inclusion level is around 50 mg kg^{-1} (Bjerkeng et al. 1999a; Bjerkeng et al. 1999b; Wathne et al. 1998) but may reach 15-20 % at lower dietary inclusion levels. In commercial farming practices dietary color pigments comprises less that 3 % of the feed cost (Rørvik et al. 2010).

Color of salmon fillets is affected by pre-mortem factors including dietary carotenoid concentration (Bjerkeng & Berge 2000; Hatlen et al. 1998), lipid level (Bjerkeng et al. 1997; Mørkøre et al. 2001; Nickell & Bromage 1998), pigment type (Buttle et al. 2001; Skrede & Storebakken 1986; Storebakken et al. 1987), seasonal variation (Mørkøre & Rørvik 2001), starvation and stress prior to slaughtering (Einen & Thomassen 1998; Erikson & Misimi 2008; Mørkøre et al. 2008) and oil sources (Regost et al. 2004).

The time of filleting and storage conditions significantly affect the fillet color. The fillet color is also affected by the time of filleting post-mortem. Filleting of the salmon before the unset of rigor mortis (pre-rigor filleting) gives better color than filleting the salmon after rigor resolution (post-rigor filleting) (Einen et al. 2002). Moreover, the concentration of astaxanthin decreases during frozen (Regost et al. 2004) and chilling storage (Gobantes et al. 1998).

Measurements of color

Traditional measurements of carotenoid pigments in salmon fillet by high-performance liquid-chromatography (HPLC) is described by Bjerkeng et al. (1997). Such methods are reliable, but slow, costly and destructive. Other objective method used for the analyses of visual color in the industry is by using the DSM Salmo Fan^{TM} Lineal (DSM, Switzerland)). SalmoFan^{TM} card ranges over 14 red colors with varying intensity. Each color is associated with a value ranging from 20 (very pale red) to 34 (very intense red) (Forsberg & Guttormsen 2006).

The fillet color can be determined using image analyses, for example as described by (Folkestad et al. 2008) (Figure2.2). Image analysis provides information about a product from a single image. Visible and near infrared (VIS/NIS) evaluates fat content and pigment in the salmon fillet based on VIS/NIR spectroscopy of salmon fillets (Folkestad et al. 2008).

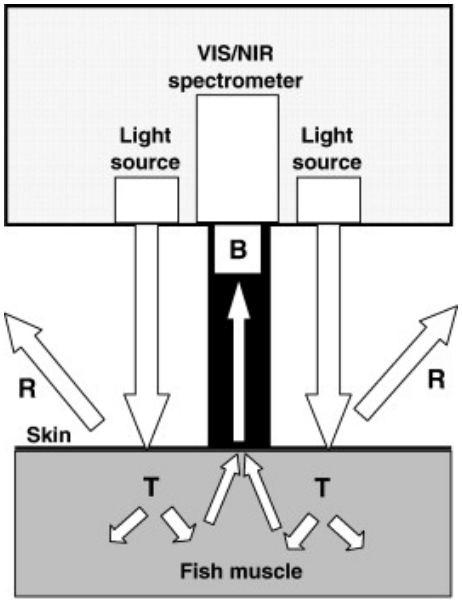


Figure 2.2. VIS/NIR spectrometer (Folkestad et al. 2008).

2.2.2. Gaping

Gaping is one of the most important quality issue facing the industry, which decreases the salmon value up to 38 % during secondary production (Michie 2001). Gaping is the tearing of the connective tissue between the muscle layers thus resulting in holes and slits in the fish fillet (Pittman et al. 2013). This leads to downgrading of the fillets and hence economic loss because of the rejection by the consumers due to its unattractive appearance (Skjervold et al. 2001b). The problem can become more severe when the skin is removed and the fillets are cut into small portions.

Gaping is caused by rupture of the connective tissue due to the interaction between forces pulling the muscle apart and the strength of the tissue thus producing flaking of the fillet (Kiessling et al. 2004). Pre mortem factors affecting gaping are the level of stress during prior to and at slaughter (Bahuaud et al. 2010b; Roth et al. 2006) which is linked to for example decrease in pH (Lavety et al. 1988; Skjervold et al. 2001a). Acidic condition also causes an increased activity of cathepsin L in the muscle tissue that can accelerate degradation of collagen, hence softening of the fillet (Bahuaud et al. 2010a). Gaping varies with season and higher growth may promote flesh softening in salmon (Mørkøre & Rørvik 2001). There is a general positive correlation between soft texture and the occurrence of gaping in salmonids, with firmer fillets having less gaping (Einen & Thomassen 1998).

Post mortem factors influencing gaping are leaving blood or other remains in the abdominal cavity after slaughtering and gutting (Jacobsen et al. 2015). Also the fiber density and the relative amount and the distribution of connective tissue affect gaping (Johnston 1999), with low fiber cross-sectional area (<12.5 μm^2 on average) giving significantly firmer texture compared with fillets comprised of large fibers (Mørkøre et al. 2009). This suggests that the muscle fiber characteristics can affect the degree of post-harvest gaping. According to Skjervold et al. (2001b), pre-rigor filleting of salmon have lower degree of gaping compared to post-rigor. Degree of gaping may also increase with

post-mortem handling and storage. Atlantic salmon with varying gaping is shown in figure 2.3.

Figure 2.3. Atlantic salmon fillet with gaping (Pittman et al. 2013).

Measurements of gaping

There are several methods to analyze gaping in salmon. The first commonly used method is on a scale from 1 to 4 according to Kiessling et al. (2004); where 1 represents no gaping, 2 minor gaping (1-5 cm longitudinally), 3 moderate gaping (6-10 cm) and 4 much gaping (>10 cm).

The other commonly used method is according to Andersen et al. (1994) on a scale from 0 to 5; where 0 = no slits, 1 = less than 5 small slits (< 2 cm), 2 = less than 10 small slits, 3 = more than 10 slits or some large (> 2 cm), 4 = many large slits and 5 = extreme gaping.

2.2.3. Texture

Fillet texture is a sensory attribute that is determined by touching the product or by assessment in the mouth. Fillet texture is one of the most important quality parameters for producers, processors and consumers. Many fish do not have a distinct flavor and therefore texture becomes more important for consumers acceptability (Hyldig & Nielsen 2001). The collagen and its properties contribute to the textural properties of fish. Fish muscles is generally softer compared to land animals due to their low content of collagen and fewer cross-links (Liu et al. 2013; Sato et al. 1986) that results in more tender product after cooking. Consumers commonly want salmon with firm texture and soft texture is associated with downgrading and economic loss (Merkin et al. 2014). On the other hand, consumers prefer juicy rather than dry fillet. Juiciness of salmon flesh is associated with the amount of moisture and the amount of intra-muscular fat (Ofstad et al. 1996). Liquid holding capacity of muscle is highly influenced by fibril swelling, contraction and the distribution of fluid between intra and extracellular locations (Offer & Trinick 1983).

Flesh texture is influenced by several factors. Ante-mortem factors affecting fillet quality are starvation before slaughtering (Einen & Thomassen 1998; Mørkøre et al. 2008), feed and feeding regimes (Mørkøre & Rørvik 2001), genetic background (Bahuaud et al. 2010a), fish species, harvesting season and photoperiod regimes (Espe et al. 2004; Hagen et al. 2007; Johnston et al. 2004). According to Einen and Thomassen (1998), starvation before slaughtering coincide with the increased pH and sensory evaluation shows increased hardness of fillet implying that the decreased pH is associated with flesh softening.

Post-mortem factors influencing quality include handling methods after filleting (Roth et al. 2002; Sigholt et al. 1997), slaughtering procedure (Merkin et al. 2014), processing technique (Veiseth-Kent et al. 2010) and storage temperature (Sigholt et al. 1997). Pre-rigor filleting of salmon gives firmer texture compared to post-rigor filleting (Skjervold et al. 2001b). Post-mortem pH falls rapidly due to anaerobic breakdown of glycogen to lactic acid. The muscle fibres become weakened as the pH falls and the muscles becomes soft (Kiessling et al. 2007). Other factors include tissue softening of salmon during frozen (Einen et al. 2002) and ice chilled storage (Hultmann 2003). Moreover, texture of the fish fillet is influenced by the diameter of the muscle fibers. The strength of raw fillet is higher when the diameter of fiber is smaller (Hatae et al. 1990). Mørkøre et al. (2009) observed that raw fillets with low fiber cross-sectional area (<12.5 μm^2 on average) had significantly firmer texture compared with fillets comprised of large fibers.

Measurements of texture

Various instrumental methods are in use for measurement of the texture quality of salmon fillets. The most common are automatic penetrometer using different types of devices such as flat-ended cylinders knifes, Kramer shear cell (multiple blades) or Warner - Bratzler blade (v-shaped blade).

2.2.4. Flavors

Flavor is very important when evaluating fish quality and freshness. The flavor plays an important role for food recognition, selection, acceptance and nutrition, as it is partly responsible for aiding the digestion of food in humans (Ensor 1989). There are two components of flavor: taste and odor. Taste is caused by relatively few non-volatile, water-soluble components and detected on the tongue. There is five taste perceptions: sweet, sour, salt, bitter and umami (Table 2.1).

Table 2.1. Taste sensations (Deisingh et al. 2004).

Sensation	**Elicited by these compounds**
Sweet	Sugar, amino acids, alcohols
Sour	Acids, e.g. acetic, citric
Salty	Table salt
Bitter	Quinine, caffeine, aspirin, nicotine
Umami	Monosodium glutamate (MSG), disodium inosinate in fish

Odor is produced by volatile substances that are detected by olfactory receptors in the passage at the back of the nose. The volatile substances responsible for odor of fish are mainly small molecules- aldehydes, ketones, alcohols and esters (Boscaino et al. 2014). Between 10-100 millions receptors for olfaction lie in the nasal epithelium in an area of about 5 cm^2 (Deisingh et al. 2004). The information from tongue and nose is integrated and interpreted by the brain.

The volatile compounds contributing to fish odor is divided into three groups based on their origin and shown in figure 2.4.

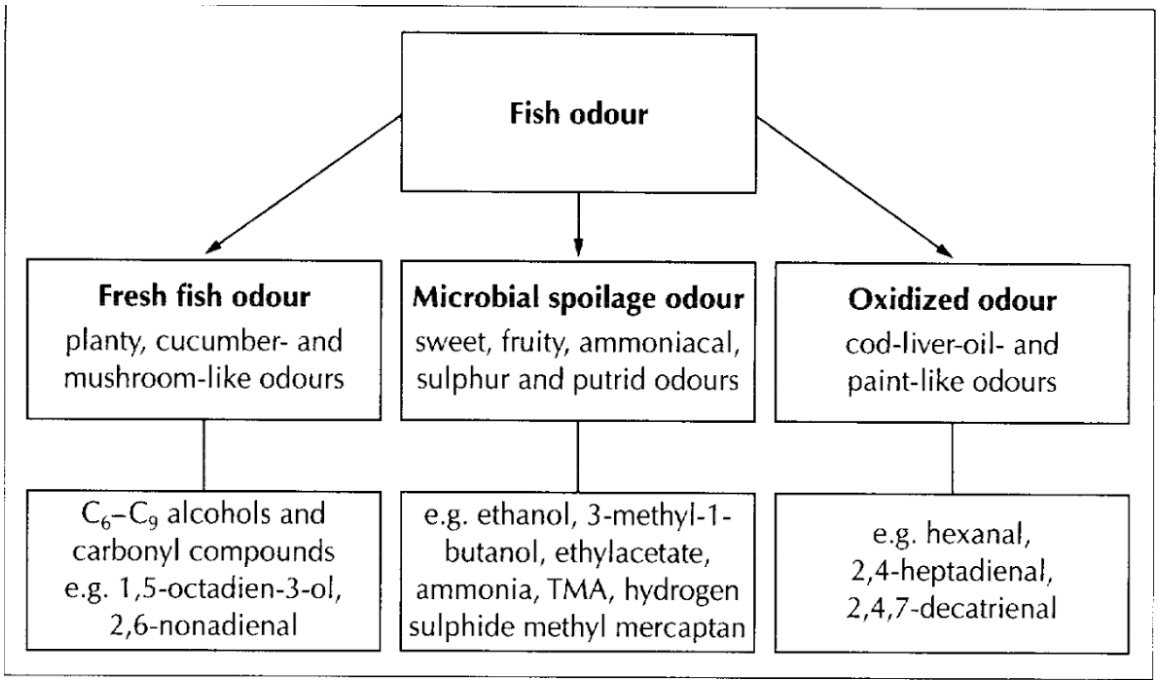


Figure 2.4. Categorization of fish odors and volatile compounds that contribute to the characteristic odor of fresh spoiled and oxidized fish. TMA, tri-methylamine (Olafsdottir et al. 1997).

Off-flavor in fish products is caused by bacterial reduction of tri-methylamine oxide to tri-methyl amine, which creates fishy odor (Connell 1990). Decreased flavor is also related to oxidation of unsaturated fatty acids that appears to be related to the antioxidant status as lipid oxidation during chilled storage of tropical farmed barramundi (Jones & Carton 2015) was reduced by dietary vitamin E enrichment. Post-mortem conditions are important for the fish flavor as correct handling and storage of fish influence flavor. During storage and packing, highly unsaturated fatty acid oxidizes in the presence of atmospheric oxygen causing rancidity. Odor is influenced by the treatment of salmon fillet by different sodium salts before storage (Sallam 2007).

Measurements of flavor

Electronic noses and electronic tongues are typically array of sensors used to characterize complex samples. Array of gas sensors are termed as electronic noses while array of gas sensors are termed as electronic tongues (Stetter & Penrose 2002). Electronic nose are used in quality control and process operations in the food industry while the electronic tongue are widely used in taste studies.

2.3. Methods for the evaluation of sensory quality

There are several methods for the evaluation of sensory quality. Selected methods are described below:

2.3.1. Quality Index Method

QIM (Quality Index Method) is used for the evaluation of freshness and quality of seafood. QIM is composed of precise description of quality parameters for a particular species and allocating scores to each attribute depending on the state of freshness or quality of the selected item. The scores are assigned in whole numbers ranging from 0, for fresh, to 3 for deterioration. The most commonly used attributes for salmon are the appearance of eyes, gills and skin together with texture and odor (Sveinsdottir et al. 2003). The sum of all the scores is used to predict the remaining shelf life of fish.

The QIM scheme developed for farmed salmon is presented in the table 2.2.

Table 2.2. The QIM scheme developed for farmed salmon (Hyldig & Green-Petersen 2004).

Quality parameters		**Description**	**Score**
Skin:	Color/appearance	Pearl-shiny all over the skin	0
		The skin is less pearl-shiny	1
		The fish is yellowish, mainly near the abdomen	2
	Mucus	Clear, not clotted	0
		Milky, clotted	1
		Yellow and clotted	2
	Odor	Fresh sea weedy, neutral	0
		Cucumber, metal, hey	1
		Sour, dish cloth	2
		Rotten	3
	Texture	In Rigor	0
		Finger mark disappears rapidly	1
		Finger leaves mark over 3 seconds	2
Eyes:	Pupils	Clear and black, metal shiny	0
		Dark grey	1
		Mat, grey	2
	Form	Convex	0
		Flat	1
		Sunken	2
Gills:	Color/appearance	Red/dark brown	0
		Light red, pink/hazel	1
		Grey-brown, brown, grey, green	2
	Mucus	Transparent	0
		Milky, clotted	1
		Brown, clotted	2
	Odor	Fresh, seaweed	0
		Metal, cucumber	1
		Sour, moldy	2
		Rotten	3
Abdomen:	Blood in abdomen	Blood red/not present	0
		Blood more brown, yellowish	1
	Odor	Neutral	0
		Cucumber, melon	1
		Sour, reminds of fermentation	2
		Rotten/rotten kale	3
Quality Index (0-24)			

2.3.2. Trained panels

A trained panel analyzes products with the purpose to find and express sensory details of the product without putting personal value to the result (Rødbotten 2009). Normally a panel consists of eight to ten individuals, inspected and trained for their sensory alertness (Warriss 2010). The training provides validity and reliability to the sensory assessment.

It is wise to schedule the evaluation of certain product types at the time of day when the product is normally used or consumed. Product testing just after meals or coffee breaks introduce bias and should be avoided (Meilgaard et al. 2006).

2.3.3. Consumer panels

Consumers panels evaluate products to give information about their impression and emotional value of the product (Rødbotten 2009). Assessments by consumer panels are done in less controlled conditions and require a large number of individuals.

2.4. Antioxidant

Free radicals or Reactive oxygen species (ROS) contain one or more unpaired electrons. Free radicals react quickly with other compounds and gain electrons and become stable. When the molecules loose electron they become free radicals themselves and start a chain reaction. The result is the initiation of lipid peroxidation that results in destabilization and disintegration of cell membranes and oxidation of the cellular components like proteins, DNA and finally resulting in the disruption of cells (Halliwell et al. 1995). Antioxidants neutralize free radicals by donating one of their own electrons or receiving an electron from the free radicals. Antioxidants are stable in either forms, hence the contribution of electron to free radical will not lead antioxidant to become free radicals (Kaur & Kapoor 2001).

Vitamin E is a lipid soluble vitamin that comprises four tocopherols and four tocotrienols in nature. Among them, α-tocopherol has the highest vitamin E activity (NRC 1993). Alpha-tocopherol has a protective role against lipid peroxidation. This is due to the ability to scavenge free radicals involved in the initiation and propagation of lipid peroxidation, thus preventing the formation of fatty acid hydro-peroxides (Machlin 1991), which can result in off-odors in the flesh. Increasing vitamin E deposition as α-tocopherol improves the quality and storage shelf life of fish flesh (Baker 2001). It was reported that the inclusion of vitamin E in the diet prior to harvest improved the robustness to stress during slaughter, improved gut health and muscle texture of salmon (Mørkøre 2012).

Selenium is an essential trace element for fish metabolism and important micronutrients in the human diet (Ames 1998). Selenium is a vital component of glutathione peroxidase, which protects cells from oxidative damage (Watanabe et al. 1997).

Vitamin C is an antioxidant that together with α-tocopherol helps in the prevention of lipid oxidation in Atlantic salmon (Hamre et al. 1997). Ascorbic acid function as a cofactor for proline hydroxylase and lysine hydroxylase that are involved in the biosynthesis of collagen. Collagen synthesized in the absence of ascorbic acid is insufficiently hydroxylated and does not form fibers properly giving rise to scurvy (Halliwell & Gutteridge 1989).

2.5. Muscle structure and composition

The chemical composition of fish fillets varies from species to species and even among the same species of fish depending on the age, sex, season and environment. Generally, fish muscle contains 66-81% water, 16-21 % protein, 0.2-25% lipids, <0.5% carbohydrates and 1.2 – 1.5% ash (Murray & Burt 2001).

The skeletal muscle is the major edible portion of fish. There are three structural factors that contribute to the tenderness in fish: collagen, amino acid content and muscle

cellularity. Fish muscle has lower amounts of collagen and contains less of the amino acid hydroxylproline in its connective tissue. Fish muscles, unlike other meats, are arranged in layers of short fibers (myotomes), which are separated by connective tissue called myocommata (Brown 2013). These sheets run parallel adjacent to each other by making a complex W shaped folded structure along the fillet (Figure 2.5).

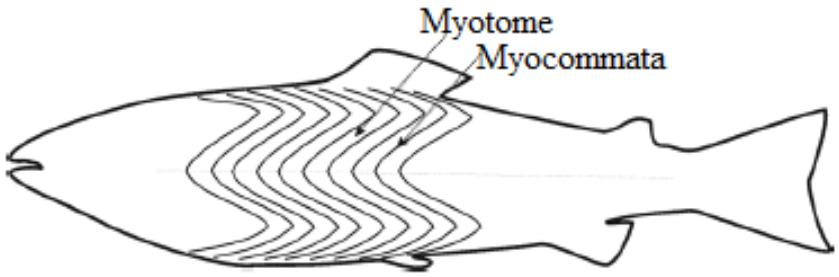


Figure 2.5. Schematic illustration of arrangement of myotomes and myocommata (Kiessling et al. 2006).

Muscle proteins, based on their solubility, are divided into three groups: sarcoplasmic, myofibrillar and insoluble protein (connective tissue protein). Sarcoplasmic proteins are water-soluble. Myofibrillar proteins are salt soluble. These proteins are primarily bound to the contractile network; hence they are called contractile proteins. Collagen is the main component of the insoluble proteins (Dunajski 1980).

The strength of the tissue depends on the amount of collagen and the stability of collagen in the connective tissue (Moreno et al. 2012). Glycine is the amino acid that facilitates the triple helical conformation of collagen (Alberts et al. 2002). Proline has a complex structure that stabilizes the collagen helix. Hydroxyproline is synthesized from hydroxylation of proline by the action of the enzyme prolyl hydroxylase. Hydroxyproline helps in the stabilization of the triple-stranded of helix by hydrogen bonding (Johnston et al. 2006; Ramachandran 1988). Lysine is present in the helical and non-helical region of the collagen. Lysine gives rise to cross-link and giving stability to collagen (Asghar &

Henrickson 1982; Li et al. 2005). Sulfur containing amino acids; methionine and cysteine tied two different helical or loop within a same helical by covalent sulfur – sulfur bonds. Higher cross links will give stability to collagen (Alberts et al. 2002).

3. Materials and Methods

3.1. Fish materials and treatments

The study was carried out in seawater at Marine Harvest research station on the Norwegian west coast (Averøy). Atlantic salmon (*Salmo salar* L) 0^{+} smolts were transferred to sea in the size of $343m^3$ (7m x 7m x 7m) net pen in Autumn 2013 and fed a standard commercial feed until January 2015 by automatic feeders (Sterner Maxi, Sterner, Leksand, Sweden). In December 2014, the fish were randomly distributed into 125 m^3 net-pens (5m x 5m x 5m), on average 170 fish per net pen. From 7^{th} January to 13^{th} March (around 10 weeks), the salmon were fed either a commercial diet or the same diet added antioxidants in triplicates. The average seawater temperature recorded at 3m depths was 5.5°C from January 2015 to March 2015.

The commercial diet used was a standard extruded feed (Optiline V 2500 40A, 9 mm, Skretting, Averøy, Norway) containing 37.9 % protein, 33.5 % fat, 7.1 % water, 4.6 % ash and 50 mg kg^{-1} astaxanthin. The level of vitamin C and vitamin E were 50 mg kg^{-1} and 201 mg kg^{-1} respectively. The antioxidant diet was prepared by coating 25 kg feed with 600 ml water, supplemented with 350 mg kg^{-1} vitamin C (ROVIMIX® STAY-C® 35, DSM Nutritional Products Ltd, Basel, Switzerland), 500 mg kg^{-1} vitamin E (dl-α-tocopherol acetate-DSM Nutritional products Ltd, Basel, Switzerland) and 0.2 mg kg^{-1} selenium (Organic selenium Sel-Plex®, Alltech) in a blender. Diet was spread on a tray for 3 days at approximately 15°C for drying and finally coated with 250 ml rapeseed oil to prevent leaching of nutrients and taste effect.

3.2. Sampling

The fish were anaesthetized and harvested 17^{th} to 19^{th} March 2015 by percussive stunning, two net pens each day. Both gill arches were cut and the salmon were bled in circulated seawater at ambient temperature. Immediately, salmon were gutted, cleaned

and the fillets were removed manually. Sixty salmon from each group were used for recording body weight and fillet weight. The right fillet of 28 salmon from each group were ice-packed in standard Styrofoam boxes and stored in a cooling room (4°C) for a week and 16 raw fillets from each groups were used for determination of sensory properties like color, gaping and texture in a post rigor stage in Nofima; and taking samples for the analyses of chemical composition.

For the consumers' sensory test analyses, 12 fillets from each of three control and antioxidant net pens from the dorsal side of right fillet, just above the pin bone, were individually vacuum packed in coded plastic bags and stored at -40°C. There were a total of 72 (12 x 6) fillets. Sensory assessment was done after 7 months of storage at -40^{o} C.

3.3. Color measurements

Visual color was evaluated by comparing the fillets against the DSM SalmoFan^{TM} card which has a scale ranging from 20-34; where 20 is very pale red and 34 is very intense red (Figure 3.1). The color analyses were performed under light conditions within a controlled environment ("Salmon Color Box", Skretting, Stavanger, Norway). The color card readings were performed on the dorsal fillet part, between the posterior end of the dorsal fin and the gut (NQC) and the under the anterior end of the dorsal fin.

Figure 3.1. Illustration of visual color measurement of salmon fillet in lab (Salmon Color Box not shown).

3.4. Gaping

The gaping was assessed visually (Figure 3.2) according to Andersen et al. (1994) using a scale ranging from 0 to 5, depending on the amount and size of the slits; where 0 = no slits, 1 = less than 5 small slits (<2 cm), 2 = less than 10 small slits, 3 = more than 10 slits or some large (> 2 cm), 4 = many large slits and 5 = extreme gaping.

Figure 3.2. Assessment of gaping in Atlantic salmon in lab.

3.5. Texture Analyses

Instrumental determination of firmness was performed by using a TA-XT2; (stable Micro Systems Ltd., Surrey, England) equipped with a 30 kg load by pressing a flat-ended cylinder (12.5 mm diameter, type P/0.5) into the surface of the fillet just above the spine below the dorsal fin (Figure 3.3). The compression analyses are performed perpendicular to the muscle fiber at 1mm/sec (Mørkøre & Einen 2003). Force-time graphs were recorded and fillet firmness was determined as the total area (N*s) under the force-time

graphs. This parameter has previously shown a good correlation with sensory assessment of firmness in raw and smoked salmon fillets (Mørkøre & Einen 2003). In the result chapter, the determination of total area (N*s) from the mechanical analyses is termed "firmness".

Figure 3.3. Texture analyses performed in lab.

3.6. Thawing loss

The weight of fillet pieces cut just below the spine under the dorsal fin were recorded before and after frozen storage at -25°C (thawing overnight at 4°C). Thawing loss is presented as: (Initial weight-Final weight)/Initial weight * 100 %.

3.7. Sensory assessment of cooked fillet

The fillet pieces were thawed overnight at 4°C and cut into 2cm x 2cm cubes that were kept in a cooling room at 4°C for 24 hours. Thereafter the muscle cubes were heated in an oven (Termaks, Bergen, Norway) adjusted to 75°C for 9 minutes before they were served to the assessors. Samples were drawn from the same anatomical region from each fillet as shown in figure 3.4.

A triangle test was performed to evaluate whether the assessors were able to differentiate between salmon fed the control or antioxidant diet. Nineteen untrained assessors participated in the sensory assessment. The assessors consisted of 7 females and 12 males, aged between 20-40 years. The assessors reported the number of fish meals per week (0.5-1 times or > 2times a week).

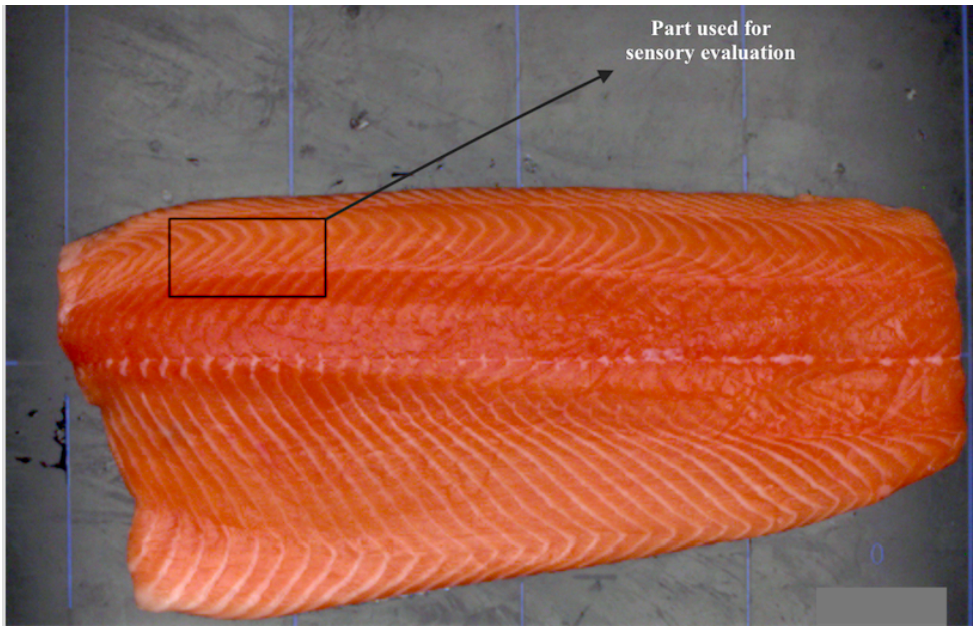


Figure 3.4. Sampling location for evaluation of sensory quality.

Three representative muscle samples of either two controls or an antioxidant or vice versa (figure 3.5) were served individually in small porcelain cups to each assessors. Each consumer got 3 samples that were coded with A, B and C (n=9 in total) and they were not informed about the treatment of the fish. The assessors were served water and crackers to rinse the mouth after eating each sample. The evaluation included color, odor, tastiness, juiciness, firmness and overall acceptability according to a categorical scale from 1- 5 (Table 3.1).

Figure 3.5. A serving for sensory assessment to the assessors.

Table 3.1. Scoring system used by the assessors to assess the sensory quality of cooked salmon fillets.

Parameters	**Scores**				
	1	2	3	4	5
Color	Poor	Acceptable	Medium	Good	Excellent
Odor	Poor	Acceptable	Medium	Good	Excellent
Tastiness	Poor	Acceptable	Medium	Good	Excellent
Juiciness	Dry	Acceptable	Medium	Good	Juicy
Firmness	Soft	Acceptable	Medium	Good	Firm
Overall acceptability	Poor	Acceptable	Medium	Good	Excellent

3.8. Analyses of protein and amino acids

Connective tissue from NQC was isolated at Instituto de Ciencia y Tecnología de Alimentos y Nutrición (ICTAN-CSIC; Madrid) as described by Moreno et al. (2012). The isolated connective tissue was dried and stabilized at -80^{o} C until analyses were carried out. Three samples were analyzed in triplicate for each group. A small freeze dried section from the connective tissue was used for the analyses of hydroxyproline and amino acids by HPLC as described (Moreno et al. 2012).

Protein was analyzed in white skeletal muscle using the Kjeldahl total nitrogen method at ICTAN-CSIC.

3.9. Data Analyses

Data were analyzed by ANOVA using the SAS program (Version 9.4; SAS Institute Inc., Cary, USA). All results are expressed as means ± SEM (standard error of means). Differences were considered significant if $P < 0.05$, and if $0.05 < P < 0.1$, this is reported as a trend. Pearson´s correlations between different variables were also calculated.

4. Results

The result chapter is divided into four main sections. The first section deals with body weight. The second and third sections deal with sensory properties of raw and sensory assessment of cooked fillets respectively. The fourth section deals with the composition of fillets.

4.1. Body weight

4.1.1. Body weight

The average body weight of the control and antioxidant groups were 4123 g and 4203 g respectively (Table 4.1). The body weight showed no significant difference between the dietary groups (Table 4.1).

4.1.2. Fillet weight

Body weight, fillet weight, fillet dorsal thickness of the anterior and posterior part and thawing loss of the control and antioxidant groups are shown in Table 4.1. The antioxidant diet group has significantly higher fillet weight that the control group. The fillet thickness showed no significant difference between the dietary groups, but the antioxidant diet group tended to have higher dorsal thickness than the control group (Table 4.1).

Table 4.1. Body weight (g), fillet weight (g), anterior fillet thickness (mm), posterior fillet thickness (mm) and thawing loss (%) of fillets of Atlantic salmon between control and antioxidant groups.

Parameters	Control	Antioxidant	P-value
Body weight, g	4123 ± 106	4203 ± 107	0.5961
Fillet weight, g	2401 ± 138a	2818 ± 132b	0.0359
Anterior fillet thickness, mm	36.6 ± 0.7	38.4 ± 1.0	0.0982
Posterior fillet thickness, mm	30.2 ± 0.7	31.5 ± 0.8	0.1623
Thawing loss, %	3.8 ± 0.1	3.9 ± 0.2	0.6510

Results are means ± SEM. The level of significant is $P<0.05$.

4.1.3. Thawing loss

The fillet thawing loss showed no significant difference between control and antioxidant diet groups (Table 4.1).

4.2. Sensory properties of raw fillets

4.2.1. Fillet color

The salmon color card readings ranged from 25-26 for both dietary groups (Figure 4.1). The fillet color showed no significant difference between dietary groups (Figure 4.1).

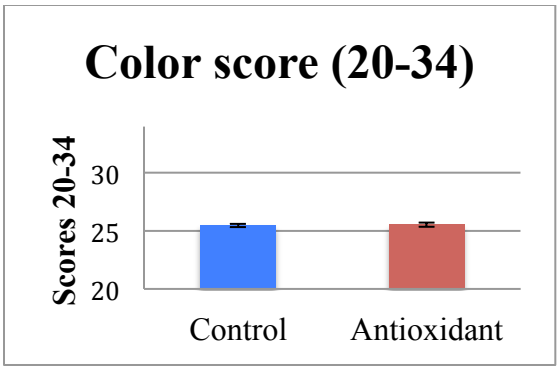


Figure 4.1. Fillet color score of Atlantic salmon (*Salmo salar* L.) fed a standard diet (Control) or the same diet added vitamin C, vitamin E and selenium (Antioxidant). Results are means ± SEM (standard error of means). No significant difference was observed at $P<0.05$.

4.2.2. Gaping

There was a significant difference in gaping between the dietary groups (Figure 4.2). Gaping was significantly lower in the antioxidant (score 0.6) compared with the control group (score 0.9).

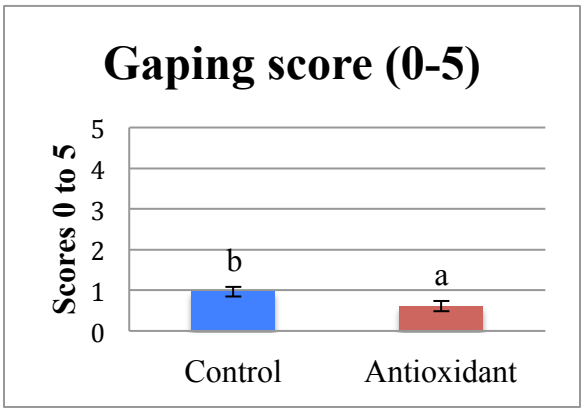


Figure 4.2. Gaping score of Atlantic salmon (*Salmo salar* L.) fed a standard diet (Control) or the same diet added vitamin C, vitamin E and selenium (Antioxidant). Results are means ± SEM. Different letters in superscripts indicate significant differences between dietary groups at $P<0.05$.

4.2.3. Firmness

The dorsal firmness (N*s) and NQC firmness (N*s) data showed no significant difference between the dietary groups (Table 4.2).

Table 4.2. Dorsal and NQC firmness (total area, N*s) of fillets of Atlantic salmon between control and antioxidant diet groups.

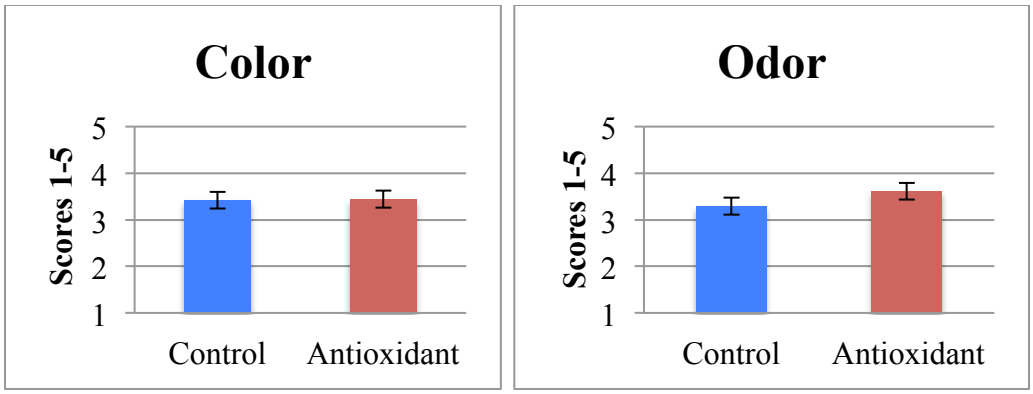
Parameters	Control	Antioxidant	P-value
Dorsal firmness, N*s	257.2 ± 10.4	269.10 ± 11.0	0.4683
NQC firmness, N*s	232.3 ± 7.1	234.90 ± 7.0	0.7924

Results are means ± SEM. The level of significant is $P<0.05$.

4.3. Sensory assessment of cooked fillet

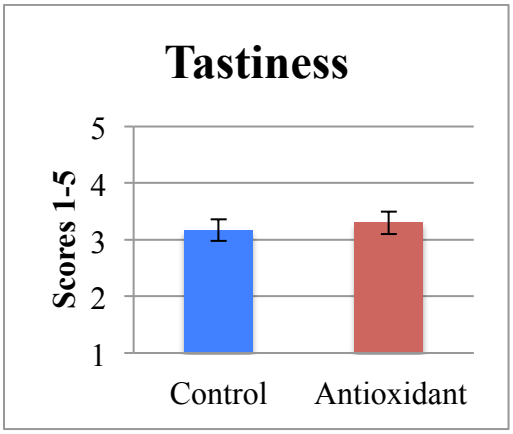
4.3.1. Effect of feed on the assessors

The dietary effects on color, odor, tastiness, juiciness, firmness and acceptability are shown in figure 4.3. There was no significant difference in color, odor, tastiness and firmness between control and antioxidant diet groups. Juiciness was significantly higher of salmon fed the antioxidant diet compared to the control feed. The acceptability of antioxidant feed tended to be higher compared with the control feed (P = 0833).

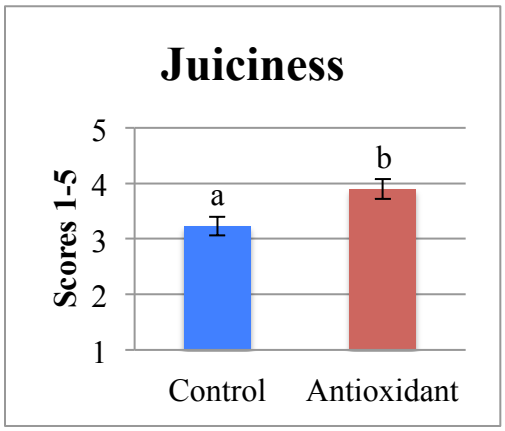


(a)

(b)



(c)



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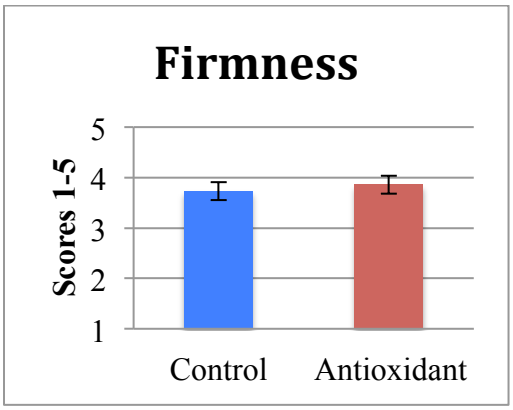
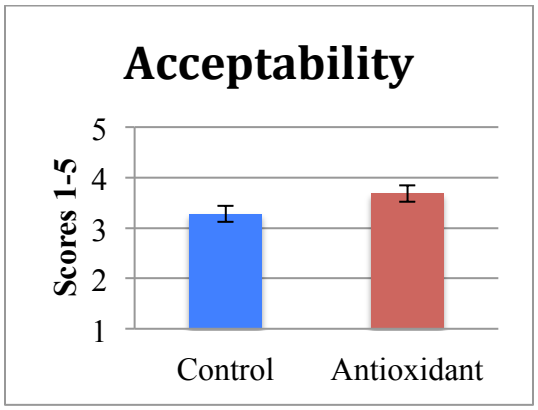


(e)



(f)

Figure 4.3. Sensory assessment of (a) color, (b) odor, (c) tastiness (d) juiciness (e) firmness and (f) acceptability of cooked Atlantic salmon (*Salmo salar* L.) fillet fed a standard diet (Control) or the same diet added with vitamin C, vitamin E and selenium (Antioxidant). Results are presented as means ± SEM. Different letters in superscripts indicate significant differences between dietary groups at $P<0.05$.

4.3.2. Differences between female and male assessors

The effect of gender on the assessment of color, odor, tastiness, juiciness, firmness and acceptability is shown in figure 4.4. There was no significant difference in color, odor or acceptability between female and male assessors. The scores for tastiness, juiciness and firmness were significantly higher for female assessors compared to male assessors.

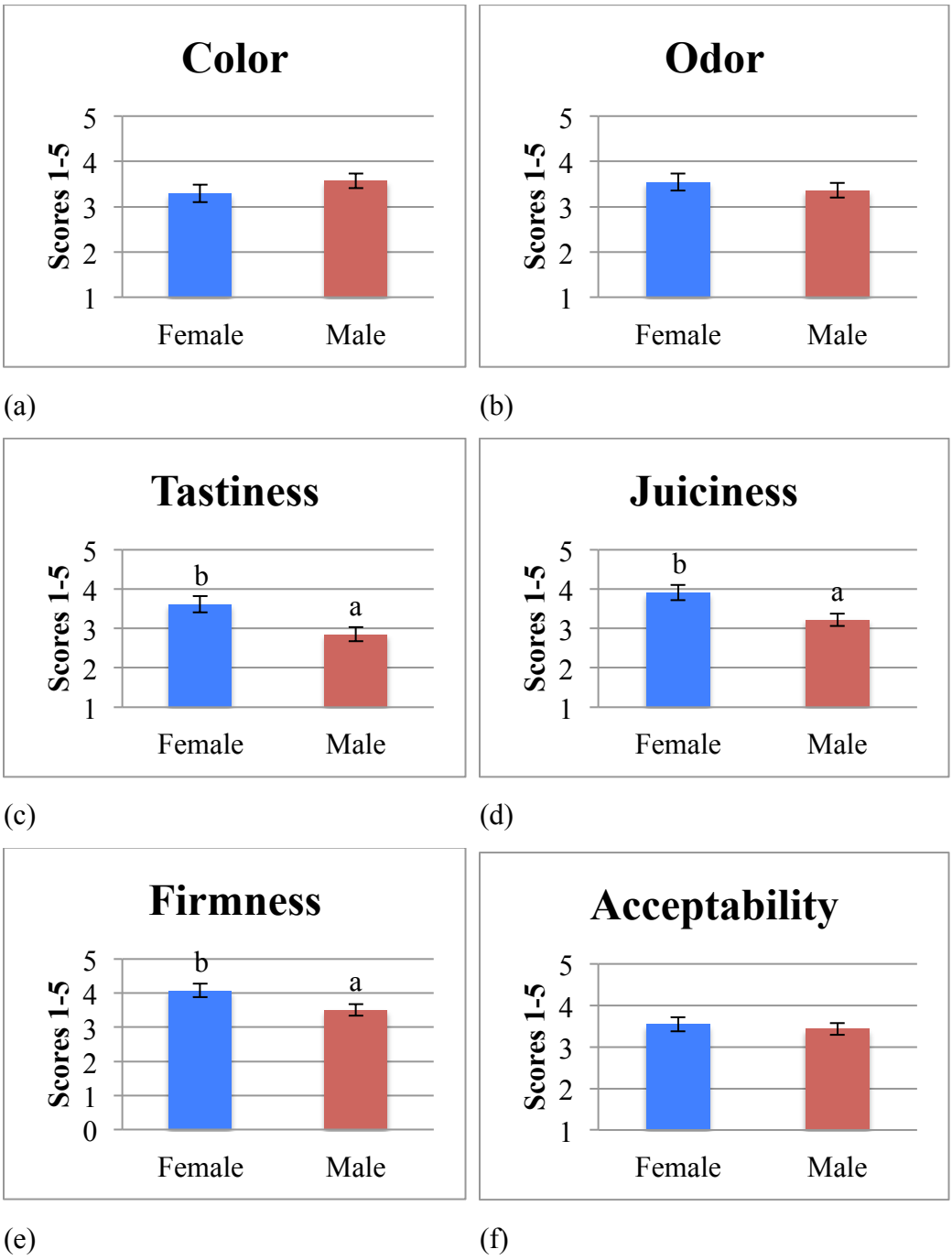


Figure 4.4. Sensory assessment of (a) color (b) odor (c) tastiness (d) juiciness (e) firmness and (f) acceptability of cooked Atlantic salmon (*Salmo salar* L.) fillets. Results are presented as means ± SEM for female and male assessors. Different letters in superscripts indicate significant differences between genders at $P<0.05$.

4.3.3. Effect of age of the assessors

The effect of age on the assessment of color, odor, tastiness, juiciness, firmness and acceptability is shown in figure 4.5. There was no significant difference in color, odor, tastiness, firmness and acceptability between 20-30 and 30-40 years age groups. The assessment of juiciness tended to be higher for the 30-40 years age group compared with the 20-30 age groups (P=0.0689).

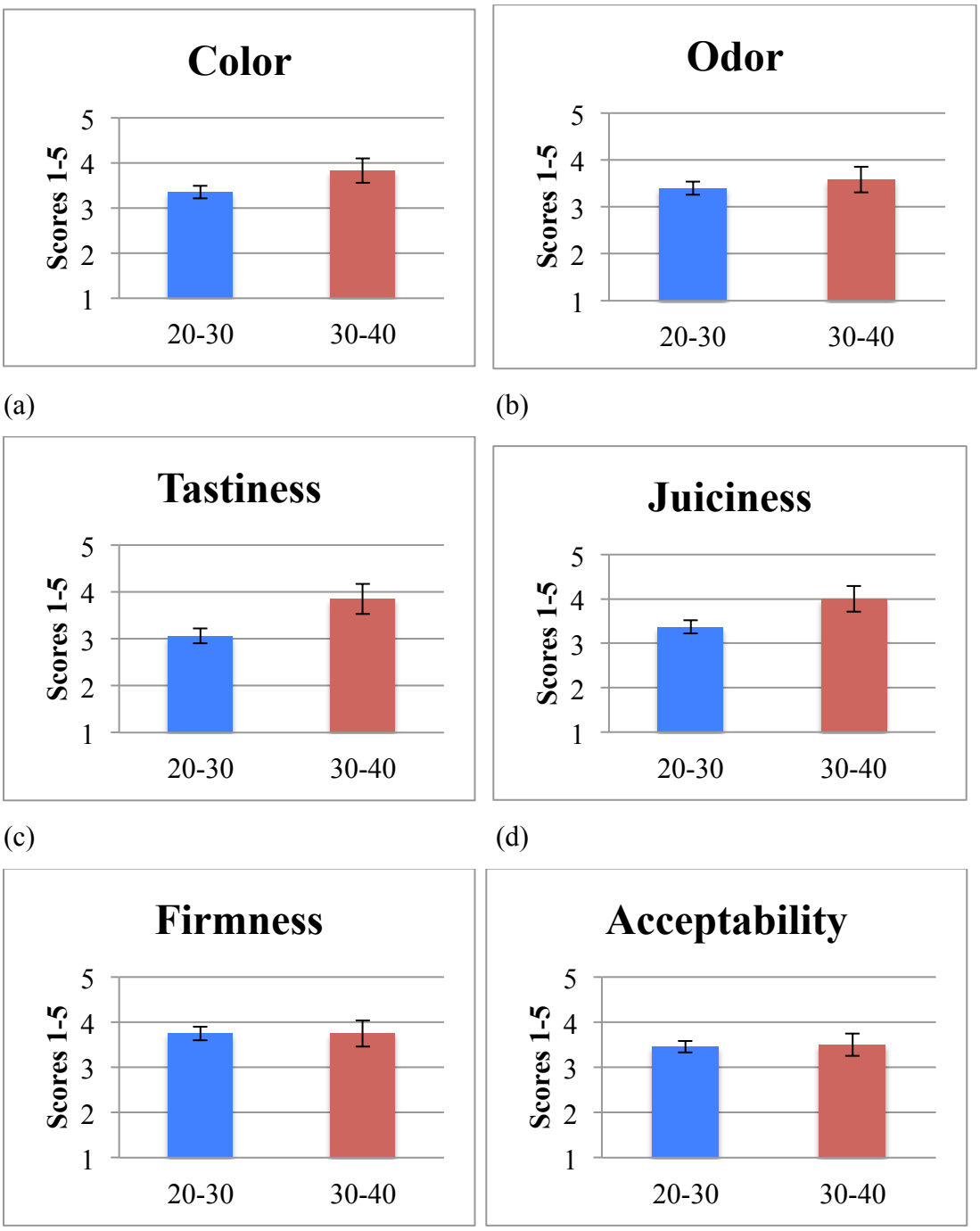


Figure 4.5. Sensory assessment of (a) color (b) odor (c) tastiness (d) juiciness (e) firmness and (f) acceptability of cooked Atlantic salmon (*Salmo salar* L.) Results are presented as means ± SEM for 20-30 (n=15) and 30-40 (n=4) years' age assessors. No significant difference were observed between age groups at $P<0.05$.

4.3.4. Effect of frequency of fish consumption

The effect of high (>2 times/week) or low (0.5-1 time/week) weekly fish consumption on the assessment of color, odor, tastiness, juiciness, firmness and acceptability is shown in figure 4.6. There was no significant difference for any of the sensory properties.

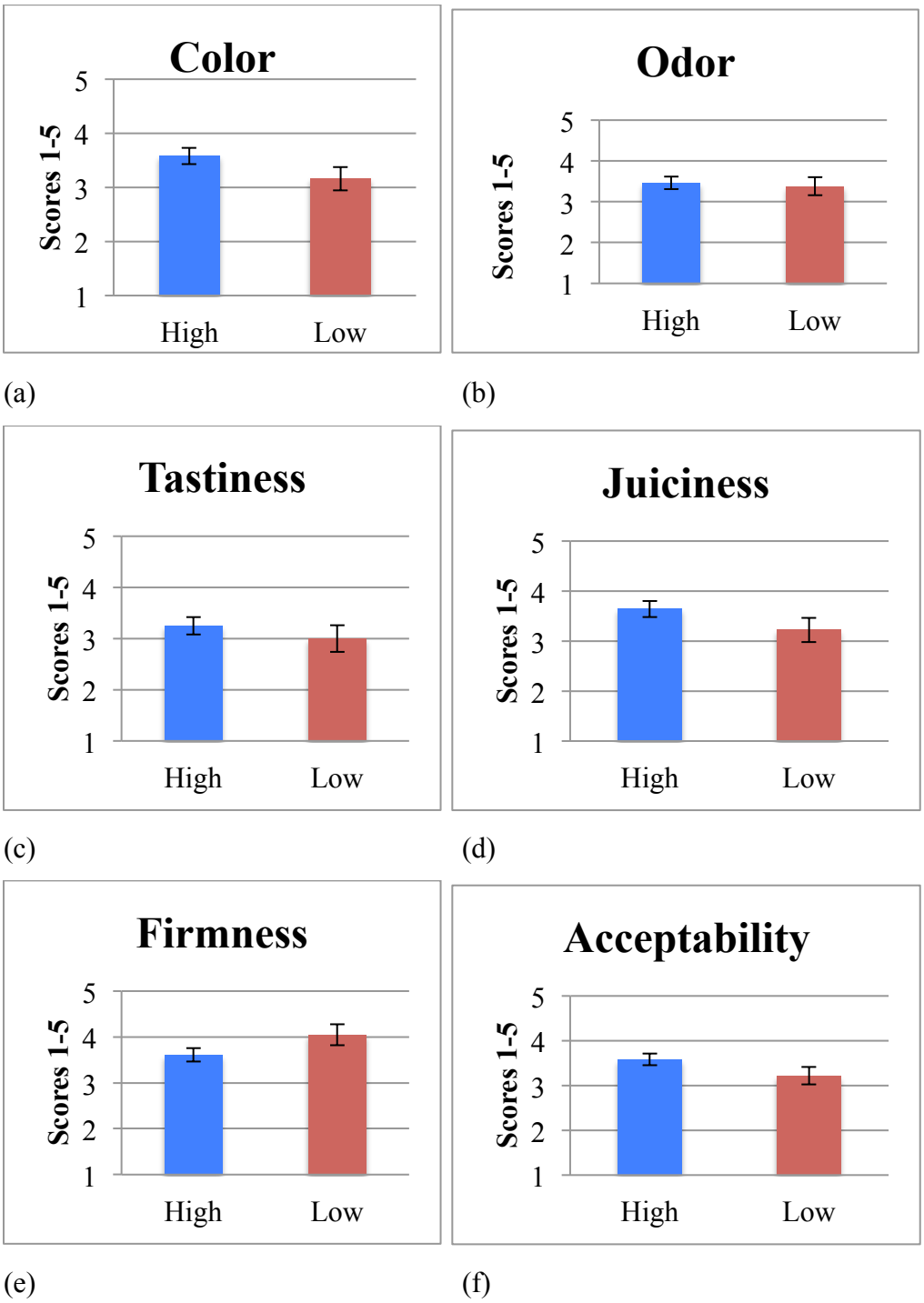


Figure 4.6. Sensory assessment of (a) color (b) odor (c) tastiness (d) juiciness (e) firmness and (f) acceptability of cooked Atlantic salmon (*Salmo salar* L.) Results are shown as means ± SEM for assessors with high (>2 times/week; n=13) or low (0.5-1 times/week; n=6) amounts of fish meals per week. No significant difference were observed at $P<0.05$.

4.4. Composition of fillets

The fillet protein and hydroxyproline (estimation for total collagen) content showed no significant difference between the dietary groups (Table 4.3). Glycine was the dominant amino acid in both the control and antioxidant group. The proline content tended to be higher of the antioxidant diet group while the methionine content in the connective tissue tended to be higher of control group. There was no significant difference in cysteine or glycine between the dietary groups (Table 4.3.).

Table 4.3. Protein content and collagenous content of hydroxyproline, glycine, proline, methionine and cysteine of Atlantic salmon (*Salmo salar* L.) that was fed a standard diet or the same diet added antioxidants (vitamin C, vitamin E and selenium).

Parameters	**Control**	**Antioxidant**	**P-value**
Protein %	20.50 ± 0.50	21.10 ± 0.40	0.4141
Amino acids in connected tissue (residues/1000)			
Hydroxyproline	52.20 ± 2.84	52.30 ± 1.83	0.9929
Glycine	317.30 ± 7.36	305.90 ± 4.50	0.1486
Proline	85.20 ± 1.78	89.00 ± 1.97	0.0600
Methionine	19.70 ± 0.39	18.90 ± 0.38	0.0954
Cysteine	3.54 ± 0.19	3.65 ± 0.14	0.6436

Values are means ± SEM. The level of significant is $P<0.05$.

4.5. Correlation of overall acceptability

Pearson correlation coefficient among color, odor, tastiness, juiciness, firmness and acceptability is presented in Table 4.4. Color, odor, tastiness and juiciness correlated significantly to the acceptability whereas firmness was not correlating significantly to the acceptability or any other quality traits.

Table 4.4. Pearson correlation coefficient among color, odor, tastiness, juiciness, firmness and acceptability.

	Color	Odor	Tastiness	Juiciness	Firmness	Acceptability
Color		0.54***	0.33*	0.36*	-0.10 ns	0.50***
Odor			0.30*	0.35*	-0.01ns	0.51***
Tastiness				0.63***	0.05ns	0.51***
Juiciness					0.05ns	0.53***
Firmness						0.15ns

Ns = non significant difference at P> 0.05; *P<0.05; **P<0.001 and ***P<0.0001.

5. Discussion

The discussion chapter is divided into three main sections. The first section discusses body weight; the second discusses the sensory properties of raw fillet, whereas the third section discusses the sensory assessment of cooked fillet.

5.1. Body weight

The result showed that the fillet weight was significantly higher of the antioxidant diet group and the fillets also tended to be thicker compared with the control group. This is a good indication from an economic point of view to the salmon farming industry as the fillets are the most valuable products of salmon (Gjedrem 2008). Hence, higher fillet weight means higher return from salmon farming. No negative effects of diet supplemented with antioxidants were observed in this experiment.

Antioxidants supplementation had no effect on salmon growth in this trial, which is in agreement with earlier studies done by Rafiq (2015) and Hang (2012) with a diet supplemented with vitamin E and selenium, and vitamin E in Atlantic salmon respectively. Jones and Carton (2015) did not find any significant difference in weight in farmed barramundi when the standard diet was enriched with α-tocopherol acetate. Hamre et al. (1997) recorded higher growth in Atlantic salmon when the diet was supplemented with both vitamin C and E as compared to the diet deficient in both vitamin C and E. Tocher et al. (2002) also reported that the growth was increased in sea bream when the diet was supplemented with vitamin E. The fillet weight of salmon is higher in this experiment as compared to the result of Ruff et al. (2003) who reported lower fillet weight for turbot when diets supplemented with vitamin C and vitamin E.

5.2. Sensory properties of raw fillet

The fillet gaping score was significantly lower of the antioxidant group compared to the control group. Gaping is caused by rupture of the connective tissue due to the interaction between forces pulling the muscle apart and the strength of the tissue thus producing flaking of the fillet (Kiessling et al. 2004). Collagen is the most important constituent of connective tissues in the muscle (Andersen et al. 1994; Sikorski et al. 1990). The strength of the tissue depends on the amount of collagen and the stability of collagen in the connective tissue (Moreno et al. 2012). Low amount or weak connective tissue can lead to gaping. The total amount of collagen in the fillet is not reported because analytical errors occurred, but indications of the collagens strength could be determined from the amino/imino acid profile.

Composition of the amino acids contributing to the stability of the collagen was measured. According to Nalinanon et al. (2010), glycine is a dominant amino acid in all fractions. Accordingly, Glycine was most abundant in both control and antioxidant groups in the present experiment. Glycine facilitates the triple helical conformation of collagen (Alberts et al. 2002). Proline stabilizes the collagen helix. Higher hydroxyproline in the connective tissue stabilizes the triple-stranded of helix by hydrogen bonding (Johnston et al. 2006; Ramachandran 1988). Sulfur containing amino acids: methionine and cysteine tied two different helical or loop stability to collagen (Alberts et al. 2002). Moreno et al. (2012) observed that amino acids contributed significantly to the collagen stability, which is the main constituent of connective tissue. No significant variations in amino acids were observed in the present experiment, but there was a trend to higher proline content to the antioxidant group and lower methionine content. No significant difference was observed among amino acids cysteine and glycine or hydroxyproline that are contributing to the structure of the helix between the dietary groups. In order to explain gaping differences between the dietary treatments, further knowledge is needed about composition of the extracellular matrix. However, higher protein content of the antioxidant group may have contributed to lower gaping frequency

as Andersen et al. (1994) observed significant higher protein content in fillets without gaping compared to fillet with gaping.

In the previous experiment done by Hang (2012), firmer texture but no significant difference in gaping was observed when the diet was supplemented with 1200 mg kg^{-1} vitamin E in Atlantic salmon. In the present experiment, diet was supplemented with, 350 mg kg^{-1} vitamin C, 500 mg kg^{-1} vitamin E and 0.2 mg kg^{-1} selenium. It is therefore possible that gaping can be reduced by dietary inclusion of different antioxidants instead of increasing a single antioxidant in higher proportion.

5.3. Sensory assessment of cooked fillet

5.3.1. Effect of feed

The sensory assessment showed significantly higher scores for juiciness of the antioxidant group compared with the control group. Positive and significant correlations were observed between juiciness and color, odor as well as tastiness (Table 4.4). In the present experiment, fillets were stored at -40^{o}C for 7 months and then thawed at 4^{o}C before they were cooked and served for sensory assessment to the assessors. Therefore, water loss from salmon fillet was expected during these three stages: storage, thawing and heating.

Water holding capacity refers to the ability of the protein to absorb water and retain it against gravitational forces within a protein matrix (Damodarn 1996). Tissue water is often characterized as bound water, entrapped water and free water (Huff-Lonergan & Lonergan 2005; Pearce et al. 2011). Bound water is closely associated with proteins by interaction with amino acids. This water has lower mobility and is not considerable affected by freezing or heating. Entrapped water is either attracted to the bound water, or held my space effect or tension forces. Free water is held by weak forces and easily lost from the muscles. Entrapped water together with free water comprises approximately 90

% of the muscle water. The entrapped and free water may be lost from the tissue during processing and storage (Belton 2011; Erikson et al. 2012). In the muscle of raw fillet, the majority of water is present in the spaces between the thick (myosin) and a thin (actin) filament of the myofibrils. The water is bound together due to the cohesive force between the water molecules. The larger pores have lower attraction force and lose more water from cell and vice versa (Offer & Trinick 1983). It is expected to have different pore space inside the salmon fillet; hence the antioxidant group could have smaller pore space with higher retention of water inside the cells. That water could have contributed to the higher juiciness in the antioxidant group. On the other hand, antioxidants may have protected lipids from hydrolysis during storage and that lipid could have contributed to the juiciness in antioxidant diet group since benefits of dietary antioxidants on the oxidation stability of muscle lipids have been reported in Atlantic salmon (Onibi et al. 1996; Scaife et al. 2000), trout (Frigg et al. 1990; Kamireddy et al. 2011) and turbot (Stéphan et al. 1995). No significant difference in loss of water was found during the thawing process between the dietary groups.

Lipid and water make up about 80 % of fish muscle (Ofstad et al. 1996). The fillets lose water during cooking that contains soluble proteins and fat (Bertola et al. 1994; Leander et al. 1980). The decrease in juiciness is likely to be a consequence of a reduction in water holding capacity. Thus, the antioxidant diet could have protected proteins from denaturation, enabling the proteins to retained more water.

In order to give more through explanation about the difference between the dietary groups, further knowledge is needed on the pore size distribution within fish fillets and the relative importance of various pore sizes present for the water-holding properties. Additionally, more detailed knowledge about dietary antioxidants and their effect on lipid peroxidation during storage.

The results are in agreement with Khan et al. (2011) and Kennedy et al. (2005) who reported higher juiciness in *Labeo rohita* when the diet was added 35 % protein and in poultry meat when the inclusion of 250 mg kg^{-1} vitamin E in the diets respectively.

5.3.2. Effect of gender and age

The sensory assessment of cooked fillets showed that female assessors gave significantly higher scores for tastiness than male assessors. The testing capacity depends on the number of fungiform papillae or the taste buds that are present in the tongue. Earlier studies have shown that the number of fungiform papillae or the taste buds are higher in females than in males (Bartoshuk et al. 1996). This could be the reason for the taste difference between male and female. It is expected that taste is related with other factors like firmness, mouth feel and the juiciness. The scores for firmness and juiciness were assessed as significantly higher by females; therefore firmness and juiciness could have contributed to the higher scores for tastiness of the cooked salmon fillet by female assessors.

It is perceived that the human senses of juiciness and tenderness appeared to be interrelated. Juicy flesh may be perceived as more tender than a similar sample, which has inherently the same texture. That could be the reason for the sensory difference in the firmness between genders while no significant difference in the mechanical texture between the dietary groups were observed.

The sensory assessment of the salmon fillet showed no significant difference in tastiness between age groups. In this study, the difference was not observed probably due to relatively low numbers of assessors. In earlier studies significant difference was observed for taste between the age groups (Sveinsdóttir et al. 2009). The explanation was that taste differs due to personal background, belief and attitude towards fish.

6. Conclusion

The antioxidant diet added extra vitamin C; vitamin E and selenium demonstrated significant effect on fillet weight, sensory properties of raw fillet and sensory assessment of cooked fillet compared to control diet. The results can be summarized as follows:

The antioxidant diet significantly increased the fillet weight compared to the control diet.

The antioxidant diet significantly reduced gaping compared to control diet.

The antioxidant diet significantly improved the juiciness of cooked salmon. Also a tendency to improved tastiness and firmness were observed for the antioxidant diet compared with the control diet.

No negative effect of diet supplemented with antioxidant was found on the present study. The overall acceptability of cooked salmon was affected by a combination of color, odor, tastiness and juiciness, but not firmness.

Females rated the cooked salmon as tastier, firmer and juicier than males.

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8. Appendix

Appendix 1. Color and gaping score of Atlantic salmon (*Salmo salar* L.) fed a standard diet (Control) or the same diet added vitamin C, vitamin E and selenium (Antioxidant).

Parameters	Control	Antioxidant	P-value
Color	25.47 ± 0.16	25.55 ± 0.19	0.7592
Gaping	0.96 ± 0.12	0.61 ± 0.13	0.0500

Results are means ± SEM. The level of significant is $P<0.05$.

Appendix 2. Sensory assessment of (a) color (b) odor (c) tastiness (d) juiciness (e) firmness and (f) acceptability of cooked Atlantic salmon (*Salmo salar* L.) fillet between control and antioxidant diet groups.

Parameters	Control	Antioxidant	P-value
Color	3.42 ± 0.18	3.44 ± 0.18	0.9377
Odor	3.29 ± 0.18	3.61 ± 0.18	0.2031
Tastiness	3.17 ± 0.19	3.30 ± 0.20	0.6350
Juiciness	3.23 ± 0.17	3.90 ± 0.18	0.0099
Firmness	3.73 ± 0.18	3.86 ± 0.18	0.6365
Acceptability	3.28 ± 0.16	3.68 ± 0.16	0.0833

Results are means ± SEM. The level of significant is $P<0.05$.

Appendix 3. Sensory assessment of (a) color (b) odor (c) tastiness (d) juiciness (e) firmness and (f) acceptability of cooked Atlantic salmon (*Salmo salar* L.) fillet between female (n=7) and male (n=12) assessors.

Parameters	Female	Male	P-value
Color	3.29 ± 0.19	3.57 ± 0.16	0.2803
Odor	3.54 ± 0.19	3.36 ± 0.16	0.5058
Tastiness	3.62 ± 0.21	2.85 ± 0.18	0.0092
Juiciness	3.91 ± 0.19	3.22 ± 0.16	0.0088
Firmness	4.08 ± 0.20	3.51 ± 0.17	0.0357
Acceptability	3.54 ± 0.17	3.43 ± 0.14	0.6305

Results are means ± SEM. The level of significant is $P<0.05$.

Appendix 4. Sensory assessment of (a) color (b) odor (c) tastiness (d) juiciness (e) firmness and (f) acceptability of cooked Atlantic salmon (*Salmo salar* L.) fillet between consumers of 20-30 (n=15) and 30-40 (n=4) years age groups.

Parameters	20-30	30-40	P-value
Color	3.35 ± 0.14	3.83 ± 0.27	0.1286
Odor	3.40 ± 0.14	3.58 ± 0.27	0.5630
Tastiness	3.06 ± 0.16	3.85 ± 0.32	0.1575
Juiciness	3.37 ± 0.15	4.00 ± 0.29	0.0689
Firmness	3.75 ± 0.15	3.75 ± 0.29	0.9866
Acceptability	3.46 ± 0.13	3.50 ± 0.25	0.9072

Results are means ± SEM. The level of significant is $P<0.05$.

Appendix 5. Sensory assessment of (a) color (b) odor (c) tastiness (d) juiciness (e) firmness and (f) acceptability of cooked Atlantic salmon (*Salmo salar* L.) fillet between consumers eating high (>2 times/week; n=13) or low (0.5-1 times/week; n=6) salmon.

Parameters	High	Low	P-value
Color	3.58 ± 0.15	3.16 ± 0.22	0.1249
Odor	3.46 ± 0.15	3.38 ± 0.22	0.7941
Tastiness	3.25 ± 0.17	3.00 ± 0.26	0.4267
Juiciness	3.64 ± 0.16	3.22 ± 0.24	0.1653
Firmness	3.61 ± 0.15	4.05 ± 0.23	0.1253
Acceptability	3.58 ± 0.13	3.22 ± 0.20	0.1388

Results are means ± SEM. The level of significant is $P<0.05$.