MASTER´S THESIS

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Chemical analysis of pigmented salmon (*Salmo salar*) fed diets containing 30% microalgae (*Tetraselmis chuii* and *Phaeodactylum tricornutum*)

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Abstract

In a feeding experiment using 30% induced microalgae (*Tetraselmis chuii* or *Phaeodactylum tricornutum*) in the feed for Atlantic salmon (*Salmo salar*), some fish turned yellow on the ventral side of the skin. When opening the fish, color differences were observed between salmon fed *Phaeodactylum* and control feed, and a question was raised to what could be the cause of this coloration. Thus, the work in this thesis has been mainly about identifying and quantifying the pigments present in the different groups. Lipids were also analyzed to see whether lipid profile was affected by the microalgae. In addition, retention of pigments and lipids in the muscle were compared between broken and intact cell walls of the microalgae, as cell walls may be an obstacle when salmon is absorbing nutrients, but little differences were observed, in both pigment content and lipid profile. Huge differences were found in total carotenoid content between *Phaeodactylum* fed salmon, and the other groups. Fucoxanthin and its derivative fucoxanthinol were the contributors to the pigmentation. The percentage of omega-3 was significantly lower in salmon fed *Phaeodactylum* than salmon fed control diet or *Tetraselmis*.

Keywords: Pigments, microalgae, *Salmo salar*, *Tetraselmi chuii*, *Phaeodactylum tricornutum*

Abstrakt

I et fôringsforsøk ble 30% mikroalger (*Tetraselmis chuii* eller *Phaeodactylum tricornutum*) tilsatt i for Atlantisk laks (*Salmo salar*), og fisk ble gul i skinnet på buken. Når fisken ble åpnet, ble det observert fargeforskjeller mellom fisk fôret *Phaeodactylum* og kontrollfôr, og det ble stilt spørsmål ved hva som var årsaken til denne fargen. Derfor har denne masteroppgaven hovedsakelig satt søkelys på å identifisere og kvantifisere pigmenter til stede i de ulike gruppene. Fettsyrer ble også analysert for å se om fettsyreprofilen ble påvirket av mikroalgen. I tillegg ble retensjon av pigment og fettsyrer sammenlignet for mikroalgene med hele og knuste cellevegger, fordi cellevegger kan være til hindring for opptak av næringsstoffer for laksen, men lite forskjell ble observert. Store forskjeller ble derimot funnet i totalt karotenoidinnhold mellom *Phaeodactylum* fôret laks og de andre gruppene. Fukoxantin og dets derivative fukoxantinol bidro til denne fargen. Prosenten av omega-3 var signifikant lavere i laks fôret *Phaeodactylum* enn laks fôret kontroll fôr eller *Tetraselmis*.

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Bodø, May 2021

Renate Wilhelmsen Hammerø

Contents

1 Introduction

The world's population is continuing to increase, and it is necessary to have enough food to sustain the increased population. By the 2050, the world population is expected to reach 9 billion people (Diana et al., 2013), and more food needs to be provided, and the ocean needs to be utilized to a larger extent. To maintain the oceans fish stocks, it is important to limit the harvest of species from the ocean to less than the oceans is able to produce. If species are more harvested than produced in the ocean, it will not be sustainable, and aquaculture and especially fish farming has been a good solution to produce more fish and crustaceans. However, the aquaculture industry still requires marine sources, like fish meal (FM) and fish oil (FO), for use in their feed. In 1990, 90% of ingredients fed to Norwegian salmon were of marine origin, mainly FM and FO, whereas in 2013, only 30% were marine ingredients (Ytrestøyl et al., 2015). FM and FO are extracted from fish waste, bycatch and waste of other marine species, but also fish are caught only to make feed for other animals, for instance fish or crustaceans in aquaculture or for poultry.

1.1 Norwegian seafood export

Even though 2020 was a different and in many ways a hard year, the salmon industry has not suffered. Both in 2019 and 2020, the export of salmon passed 100 billon NOK (Norwegian kroner) in export value. On the webpage fish.no, five reasons why the export has been so good have been stated; First of all, the NOK has been weak. In addition, the seafood industry has been good at adaption, and there has been a strong growth on single species like mackerel and herring for the industry. Norwegian seafood is sought globally and the export value for salmon is the second highest it has ever been. (FiskMediaAS, 2021)

In 2020, Norway exported 1.2 million ton fish from aquaculture, an increasing volume of 3% from 2019. Of these, 1.1 million ton was salmon, with a value of 70.1 billon NOK. Of all exported seafood, 45% is from aquaculture, and 55% is wild captured, the economic values are different, with 70% of the income from aquaculture and 30% from fisheries. (FiskMediaAS, 2021)

1.2 Salmonid quality

Quality of salmon is evaluated by flesh color, fat content, texture, and general appearance, in addition to country of origin, branding and ecolabels. Customers expect to see pink-colored salmon, as this is how the wild salmon appear. Coloration of food cause physiological and psychological expectations which are based on experience, tradition, and anticipated quality. Color of salmonid flesh is seen as an important quality parameter, and is caused by the pigment astaxanthin. Consumers have a preference of red-colored salmonid products, and an expectation that redder salmon is fresher, and has a better quality and flavor, and costumers are willing to pay significantly more for this. Einen and Skrede (1998) found a significant correlation between astaxanthin concentration of raw fillets and smoke odor of smoked fish. In 2001 a new study was performed to investigate the astaxanthin concentration (0.5, 5, 10, 11 and 27 mg astaxanthin/kg feed), and thus redness, had an impact on the flavor intensity of rainbow trout (Osterlie et al., 2001). The experiment was performed under white light to mask color differences of the samples. They found no significant difference between amount of astaxanthin and flavor intensity between the smallest and largest amount. In another study from 2007 it is stated that dietary carotenoids have a significant impact on taste and flavor of rainbow trout fillets, and that control diets gave the lowest score of taste and flavor, indicating that carotenoid supplementation may impact salmonids in a good way (Yanar et al., 2007). Other carotenoids than astaxanthin were not reported.

1.3 Feed production and sustainability

Aquaculture production is increasing every year, and to sustain this trend further, the industry is dependent on sustainable high quality feed ingredient. As mentioned earlier, FM and FO are no longer considered sustainable, as this requires more fish than the ocean can produce yearly, which also pushes the prices upwards. Due to this, the European fish feed industry has reduced the content of FM and FO, and replaced it with plant ingredients (Shepherd et al., 2017). The high levels of plant oils have however changed the lipid profile in the muscle of farmed Atlantic salmon. Since 2006, due to the transfer from marine ingredients to plant ingredients, the amount of 18:2n-6 (linoleic acid, LA), 18:3n-6 (γ-linolenic acid, GLA) and 18:1n-9 (oleic acid, OA) has increased, while C20:5n-3 (eicosapentaenoic acid, EPA) and C22:6n-3 (docosahexanoic acid, DHA) has been reduced. Reduction of EPA and DHA is concerning due to the nutritional benefits for Atlantic salmon (Sprague et al., 2016). Since microalgae are the primary producers of EPA and DHA, the interest of using them as supplement in feeds has increased (Gong et al., 2019).

Some attempts to solve the sustainability problem has been to increase the use of microalgae in the feed. Microalgae come from the marine environment and contains many of the same essential fatty acids as the wild salmon has in its natural feed. In aquaculture sustainability is measured in “fish in/fish out”, and less fish must be used in feed than what becomes produced. Now meals are mainly produced from vegetative ingredients, like soybean meal, corn meal, wheat meal etc. but also these ingredients have ethical and sustainability issues, in regard to deforestation and limited arable land. Oils are also produced from vegetative ingredients. In this study the two microalgae *Phaeodactylum tricornutum* and *Tetraselmis chuii* has been added in fish feed, fed to salmon, and the salmon was analyzed for pigments and fatty acids.

1.4 Lipid and pigment uptake in salmon

The digestive tract of the salmon consists of mouth, stomach, intestines and anus. Lipid digestion requires emulsifiers, mainly from proteins and phospholipids, but also endogenous bile acid and phospholipid secretion from the proximal part of the digestive tract. Most dietary lipids are absorbed as free fatty acids, either by diffusion or facilitated transport, in the proximal regions of the intestine and pyloric caeca. Short and medium chained fatty acids are absorbed rapidly in the proximal part of the intestine. Saturated long chain fatty acids (LC-FA) may not be as easily absorbed due to their high degree of hydrophobicity and lower micellar solubility. Free fatty acids (FA) are absorbed more easily than triglycerides. (Torrissen, 1985)

Pigments are absorbed in the digestive tract, then transported by the blood to the muscle where it is retained and metabolized (Buttle et al., 2001), but does not occur efficiently. Increased dietary lipid level did show higher deposition of astaxanthin in salmon muscle, probably due to increased mixed micellar incorporation and eased transfer to the enterocytes for absorption (Olsen & Baker, 2006). It is believed that free astaxanthin give better pigmentation than astaxanthin dipalmitate (Foss et al., 1987; Storebakken et al., 1987), but the astaxanthin deposition in the flesh is dependent on multiple factors, like genetical differences, in *Salmo Gairneri* (Torrissen & Naevdal, 1984), dietary pigment and dietary pigment level, stage of sexual maturation and feed intake, which is also dependent on size (Buttle et al., 2001). Johnson et al. (1980) also found that rupturing of cell walls of the yeast *Phaffia rhodozyma* increased pigment availability.

In a study from 1990, fish was fed with the dark orange flower *Adonis aestivalis* and the fish obtained more pigments than the control fish. The major pigment in the skin appeared to be astaxanthin diester, while free astaxanthin was dominant in the flesh. It is likely that the astaxanthin was incorporated as a free form in the flesh, and further transferred to the skin where it was esterified and stored (Simpson, 1990). The absorbed astaxanthin could also have been diesters that were hydrolyzed and transported as free astaxanthin. After transport to the skin, the free astaxanthin was re-esterified and stored. The study proposed a theory that carotenoid esters are absorbed from the intestine and hydrolyzed to free astaxanthin and fatty acids, then transported into the skin where they are re-esterified and stored. (Foss et al., 1987; Simpson, 1990; Storebakken et al., 1987)

1.5 Microalgae

Microalgae are in general seen as good ingredients in fish feed, as they are marine, and most of them contain the most important fatty acids, EPA and DHA, and also have good protein profiles. The biggest challenge in regards to using microalgae in fish feed, is the requirement of a large scale production, which has been hard due to technical and economic reasons. Usage of microalgae in fish feed was suggested already in the 1950s (Becker, 2007), and through the last decades, the increasing interest and requirement for these protein sources have led to mass production at a reasonable cost (Huntley et al., 2015). In 2000, the worldwide commercial production of algal biomass was about 5000 metric tons per year, about 1% of the amount of FM and FO used in 2000 (Strøm, 2002), and the estimated average price was 300 US$ per kg. In 2015, the cost had decreased to less than 5$ per kg (Das et al., 2015). Only a few microalgae are selected for mass production, due to their convenient amino acid profile and lipid profile, as well as their fast growth, robustness, and lack of toxin production.

Another problem with microalgae is their cell wall which act as a barrier for the salmon to access the nutrients inside the cell. The algae cell wall can normally not be digested by salmon, and it is evidence that disrupted cell walls increase the uptake of nutrients in the fish (Rumsey et al., 1990; Teuling et al., 2019), and thus the microalgae should be broken down before mixed into the fish feed. Cell walls are often made up of dietary fibre, which by its own definition, is resistant do digestion in the small intestine. Without cell wall disruption, digestive enzymes can only access the intracellular nutrition through natural pores in the cell wall (Capuano & Pellegrini, 2019). In a study performed on the microalgae *Nannochloropsis gaditana*, cell wall disruption lead to increased digestibility of protein to 62% to 78% and fat from 50% to 82% in Nile tilapia (*Oreochromis niloticus*) (Teuling et al., 2019). The present study focused on Atlantic salmon, with two other microalgae incorporated into the feed, *Tetraselmis chuii* and *Phaeodactylum tricornutum*. The experiment was performed with both intact cell walls, and disrupted cell walls, to see how this could have an impact on the uptake of pigment and fatty acid in the muscle.

1.5.1 Tetraselmis chuii

Tetraselmis chuii (Butcher, 1959) is a green, unicellular microalga in the marine environment, and belongs to the class Prasinophyceae. It was first isolated off the coast of Great Britain in the 1950s but found in most marine waters around the world. *T. chuii* does not produce toxins and the species is harmless toward marine species. (Mantecón et al., 2019)

T. chuii is easy to culture and has a high nutritional value, with a high protein content (Nunes et al., 2020), and sufficient amounts of lipid, carbohydrate and fatty acids. It is flagellated, have a rapid growth rate, and can grow in a broad range of temperatures and pH values. The species is a rich source of multiple bioactive compounds, such as vitamin E, carotenoids and phenolic compound, which shows antioxidant, anticancer and antimicrobial properties (Rahman et al., 2017). However, when carotenoids are extracted from the species, it degrades fast and is not a useful source of coloration. *Tetraselmis chuii* has a thin cell wall (Cardinaletti et al., 2018), which might make nutrients more available for the salmon than other microalgae with thicker cell walls would.

The nutritional profile of *T. chuii* contain proteins, carbohydrates and fats, and all the essential amino acids for salmon (arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine) (Halver et al., 1957) are present The species is rich in long-chain polyunsaturated fatty acids (LC PUFA), especially the omega-3 fatty acids EPA and DHA, as well as fat-soluble carotenoids. It contains about 35-40% protein, 5-10% fat, 30-35% carbohydrate and 15% ash. (Mantecón et al., 2019) *T. chuii* include chlorophyll *a* and *b*, β,β-carotene, lutein, zeaxanthin, antheraxanthin, violaxanthin and 9'-cis-neoxanthin (Brown & Jeffrey, 1992). However, it does not mean that all these carotenoids would be present in the salmon flesh, because the salmon may not be able to take them up, and some may be degraded while processed as feed. This is a problem also for astaxanthin which is relatively stable.

1.5.2 Phaeodactylum tricornutum

Phaeodactylum tricornutum is a diatom known to accumulate EPA, fucoxanthin, neutral lipids, and crysolaminarin (Gao et al., 2017). It is a commercially viable species for large-scale cultivation and can grow to high cell densities (Rebolloso-Fuentes et al., 2001). The microalgae are greatly adaptive, and have three different morphotypes; oval, fusiform and triradiate, where the fusiform (see figure 1, image E) is the most stable. The morphological types may change depending on external conditions where the microalgae are present, and the mechanisms are complex (Heimann & Katsaros, 2012). The different appearances also give different characteristics to each of them. The fusiform (figure 1, image E) and triradiate (figure 1, image D) shapes are more buoyant than the oval (figure 1, image A-C) shape, adapting them to a more planktonic lifestyle (Heimann & Katsaros, 2012). It may have been that the species was originally oval and lived in the benthic community, but during evolution realized it was convenient to become planktonic (Sabir et al., 2018).

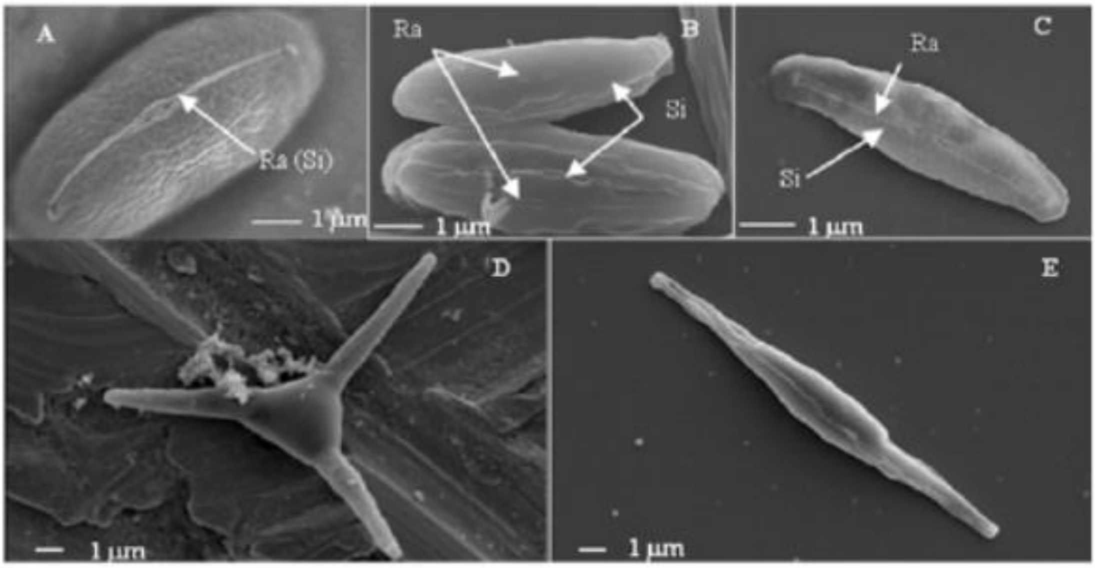

Figure 1:Electron micrographs of the morophotypes of Phaeodactylum tricornutum. A-C shows oval shapes with different degrees of silicification, from only raphe (A) to whole valve (B) to an intermediate (C), D: triradiate cell, E: fusiform cell. Picture borrowed from: https://ebookcentral-proquest-com.ezproxy.nord.no/lib/nord/reader.action?docID=893825&ppg=53

The species was first described in 1897 and is now widely cultivated as feed for larval mollusks and fish in aquaculture (Bowler & Falciatore, 2019). The optimum growth temperature is between 18 and 22 °C, making temperate climates their favorable habitat. (Rebolloso-Fuentes et al., 2001)

P. tricornutum is characterized by high levels of n-3 polyunsaturated fatty acids (PUFAs), where EPA is the dominant one, and high concentrations of the orange-colored carotenoid fucoxanthin. *P. tricornutum* always contains the chlorophylls *a* and *c,* as well as the xanthophylls fucoxanthin, diatoxanthin and diadinoxanthin, (Feijão et al., 2018), but also violaxanthin and β,β-carotene and β,ε-carotene has been reported (Rebolloso-Fuentes et al., 2001). Overall, the species contains about 35-40% protein, 15-20% lipids, 25-30% carbohydrates and 15% ash. (Rebolloso-Fuentes et al., 2001)

1.6 Pigments

Wild salmon obtain their naturally pink color from the accumulation of astaxanthin, which they obtain from consuming zooplankton and crustaceans rich in astaxanthin. In aquaculture however, this color is usually achieved by industrially synthesized astaxanthin in the feeds (Chitchumroonchokchai & Failla, 2017). The amount of astaxanthin incorporated in the diet, affect the redness of the muscular tissue of the salmon.

Astaxanthin is the main pigment expected to be found in the salmon in this experiment. The fish given experimental diets, may absorb some other pigments in addition, if pigments present in the microalgae used in the feed, *Tetraselmis* and *Phaeodactylum*, are absorbed by the salmon. Thus, for fish given *Tetraselmis*, lutein, zeaxanthin, violaxanthin, neoxanthin, β,β-carotene and antheraxanthin may be found in the flesh, while in the fish fed *Phaoedactylum*, fucoxanthin, diadinoxanthin and diatoxanthin may appear in the flesh. See figure 2 below for colors of the different carotenoids.

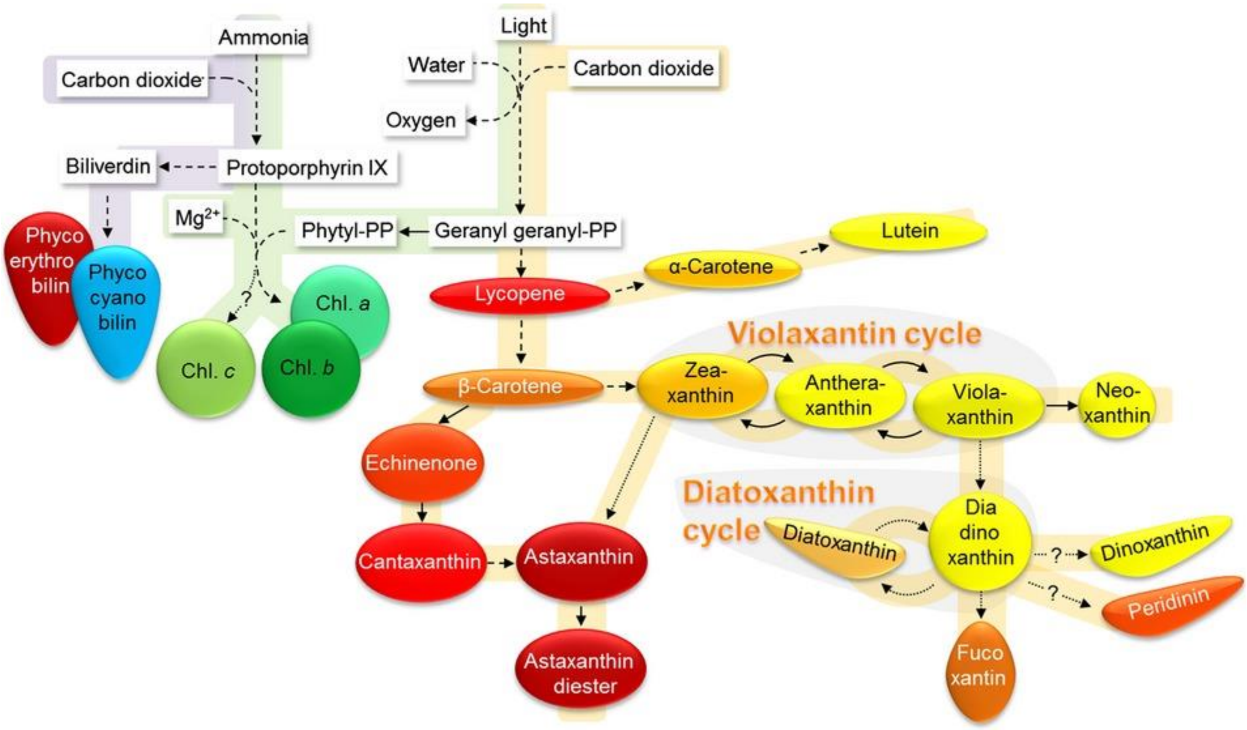

Figure 2: An overview of pigment biosynthesis pathways in microalgae, including the coloration of different pigments. (Mulders et al., 2014)

1.6.1 Carotenoids

Carotenoids are hydrophobic. The polyene chain is the main part of any carotenoid and causes its observed color. The color facilitates isolation and makes it easy to monitor purification steps and any chemical reactions. Changes in color, or total loss of color, notifies that there has been a decomposition or structural modification of the carotenoid. The intensity of the color provides the basis for a quantitative determination of carotenoids. Carotenoids are unstable, largely due to their polyene chain, which can react in oxygen in air or peroxides, addition of electrophiles may happen, and *trans/cis*-isomerization can be caused by heat, light, and/or chemicals. Carotenoids are also unstable in acids. Chemical reactions may also happen at the cyclic end of the carotenoids, causing unwanted modifications. 5,6-epoxides like neoxanthin and violaxanthin may undergo rearrangement to 5,8-furanoxide in reaction with acid. Most carotenoids are alkali stable. (Britton et al., 2004)

Carotenoids are poorly utilized by fish (Britton et al., 2008) and retention in muscle of Atlantic salmon is normally less than 12%. This is mainly because of poor absorption in the gut. 67% of astaxanthin absorbed by salmon was metabolically transformed or excreted through gills (Britton et al., 2008).

General precautions when it comes to carotenoids include avoiding oxygen, heat, light, and acid. This reduces the risk of unwanted reactions or destruction of the carotenoid. (Britton et al., 1995a) Nitrogen (>99.9% pure) should be used to replace the atmospheric air, by blowing nitrogen to the surrounding air of the sample before capping. Antioxidants may be used to protect carotenoids during saponification. *Trans/cis*-isomerization reactions are promoted at higher temperatures, and thus when operating with carotenoids the temperature should be as low as possible. Extraction of carotenoids are possible from room temperature and below and extracted solutions should be stored at -20˚C. Sunlight must be avoided and artificial light should be low, so lab procedures should be carried out in darkness.

1.6.1.1 Astaxanthin and idoxanthin

Wild salmon gets its red color from eating copepods that produce astaxanthin, like *Calanus*. Astaxanthin is an antioxidant, and mixture of *trans/cis* astaxanthin works better than (all-*trans*)-astaxanthin in vitro. *Trans/Cis* isomerization occurs during processing of food and feed. Light, heat treatment, cooking and cell breakage have impacts on the isomerization. Atlantic salmon accumulate (all-*cis*)-astaxanthin in muscle, when diet was supplemented with a mixture of *trans/cis* (all-*trans*:9*cis*:13*cis* ratio 75:3:22) in a study by B. Bjerkeng and G. M. Berge (2000). (Britton et al., 2008)

Astaxanthin is stored in muscle and skin in different forms. Free astaxanthin is deposited in the flesh of salmonids (Foss et al., 1987) while the skin usually contains astaxanthin esters, which are re-esterified from the free astaxanthin in the muscle. The inclusion of astaxanthin in the feed is usually in free astaxanthin form that is produced for aquaculture feeds. When fed natural forms of astaxanthin, like shrimp waste, krill or microalgae, the astaxanthin is incorporated as esterified forms (Bowen et al., 2002). The forms of astaxanthin may have an impact on different degradation of astaxanthin in the skin and muscle. Other reasons for observing degraded carotenoid in the skin and not in the muscle could be due to carotenoid binding receptors, which is salmon is known to be in the muscle. (Zoric, 2017) During sexual maturation astaxanthin is redistributed from muscle to skin, meaning that hormones may play a part in the distribution of astaxanthin. (Rajasingh et al., 2006)

Idoxanthin is a breakdown product from astaxanthin in salmonids, and levels of idoxanthin increase where levels of astaxanthin decreases. This means that coloration is weaker whenever the idoxanthin level is high. Stressed salmon is reported to have more idoxanthin, but the mechanisms of how stress affects quality are not properly understood. (Ytrestøyl & Krasnov, 2016) The structures of astaxanthin, *cis*-astaxanthin and idoxanthin can be seen in figure 1, 2 and 3 in appendix A.

1.6.1.2 Lutein

Lutein (see figure 4 in appendix A) is a yellow carotenoid naturally present in many fruits and vegetables, like parsley, spinach, and kale (Kim & Park, 2016), and it gives color to the poultry feathers and the yellow egg yolk (Becerra et al., 2020). The all-*trans* form is the most common, but the four *cis*-isomers 9-*cis*, 9'-*cis*, 13-*cis* and 13'-*cis* may also be present where *trans*-lutein is found (figure 5 in appendix A). Lutein is unstable and the bioaccessibility and bioavailability in food sources are low, so it is generally not used as a supplement in food. However, microalgae are a good source of lutein, and can potentially become a source for food coloration, as humans already get naturally in some vegetables. (Becerra et al., 2020). Some species, like the Chlorophyte *Chlamydomonas reinhardtii* can convert lutein to loroxanthin.

Lutein enriched feed given to goldfish led to higher survival rate in juvenile goldfish. (Besen et al., 2019) The natural red astaxanthin is more expensive than other carotenoids like lutein. Even though lutein appears yellow in most organisms, it was suggested that it could be used in goldfish to achieve a redder color. In the study, groups of goldfish were fed diets containing only one pigment for each group, and showed that lutein gave similar skin coloration as astaxanthin and canthaxanthin.

1.6.1.3 Fucoxanthin and fucoxanthinol

Fucoxanthin (see figure 6, top, in appendix A) is a naturally occurring, orange-colored carotenoid present in the chloroplasts of brown seaweed and many microalgae. It plays an important role in light harvesting and photoprotection for effective light use, and upregulation of photosynthesis. Fucoxanthin is absorbed in the intestine of salmon through the same path as the dietary fats. When fucoxanthin is ingested, it is metabolized mainly to fucoxanthinol (figure 6, bottom, in appendix A) in the gastrointestinal tract (Peng et al., 2011).

1.7 Fatty acids

Lipids include fats, oils, waxes, carotenoids, phospholipids, and steroids. Common for these compounds is that they are hydrophobic, non-polar and do not mix well with water. Lipids have many important roles and functions, as for instance storage of energy, making up cell membranes and providing building blocks for hormones.

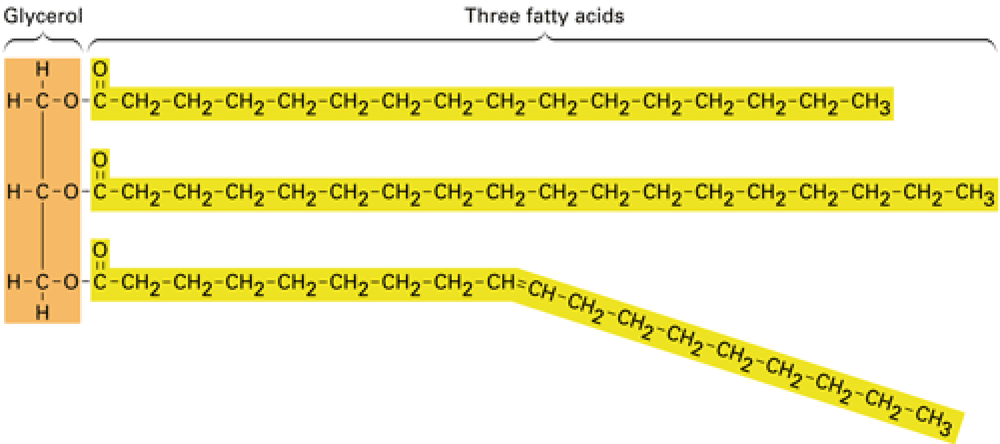

Figure 3: A fat molecule with a glycerol backbone and three fatty acid tails. Retrieved from: https://bodell.mtchs.org/OnlineBio/BIOCD/text/chapter5/concept5.3.html

A fat molecule consists of one glycerol backbone and three fatty acid tails (see figure 3 above). A fatty acid is made up of a long hydrocarbon chain, mainly 12-18 carbons. Not all fatty acids are found in fats, some are also free inside the body, and some are a part of phospholipids. Fatty acids can be saturated and unsaturated depending on double bond conjugations present between the carbons. Fatty acids are named by their number of carbons, followed by the number of double bonds, and the position of the first double bond, for instance C18:1n-9 has 18 carbon atoms, 1 double bond and the first double bond is on carbon 9, counted from the CH3 end, see figure 4 below. *Cis* and *trans* configurations are possible, depending on the position of the long chains and hydrogens on both sides of a double bond (see figure 4). (OpenStax, 2013) In order to separate and analyze fatty acids, they must be transformed to fatty acid methyl esters (FAMEs) by using methanol. The fatty acids must split from the glycerol and convert into derivatives with lower boiling points. Esterification is done prior to GC analysis. (Eder, 1995)

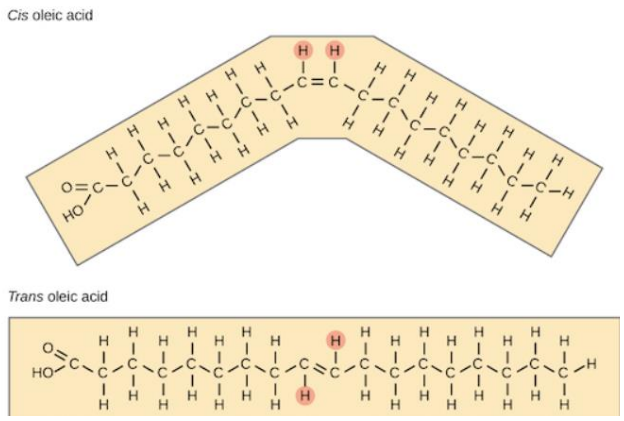

Figure 4: The upper image shows a cis-configuration and the bottom one a trans-configuration of the same fatty acid, the oleic acid, C18-9n-1. Figure from: https://bio.libretexts.org/Bookshelves/Introductory_and_General_Biology/Book%3A_General_Biology_(Boundless)/3%3A_Biological_Macromolecules/3.2%3A_Lipid_Molecules/3.2A%3A_Lipid_Molecules

EPA (eicosapentaenoic acid, C20:5n-3) and DHA (docosahexaenoic acid, C22:6n-3) are important for nutritional value of farmed salmon. Atlantic salmon require ALA (α-linolenic acid, C18:3n-3), (1% of diet) and EPA+DHA (0.5-1.0% of diet) (Emery et al., 2016). In a study on n-3 LC-PUFA in Atlantic salmon, lack of EPA and DHA did not have an impact on mortality, but the growth was significantly slower in fish fed less than 0.5% EPA+DHA. EPA and DHA are most important for the heart and brain, as that is where most EPA and DHA are conserved. Most of ALA and EPA was converted into DHA, when DHA was excluded from diets. (Bou et al., 2017)

Fish are poikilothermic and have a low body temperature, which means they require more PUFAs as this is the most liquid fats to maintain movement in cold water. When poikilothermic organisms are subjected to thermal change, the cell membrane lipids and fluidity is modified in order to maintain membrane properties and function. The response performed by the membrane in relation to temperature change is known as homeoviscous adaptation (HVA). (Malekar et al., 2018) More double bonds mean more fluidity, giving fish a greater chance to adapt to different water temperatures.

1.8 UV/Vis spectroscopy

Spectrophotometers are common basic equipment in most biochemistry laboratories. A spectrophotometer is used to measure absorbed light intensity as a function of wavelength. There are three main components of a spectrophotometer, the light source, a means of dispersing light into its component wavelength and a detector. The light source can either work in the UV region (180-400 nm) as a deuterium arc lamp, or in the visible region (above 400 nm) as for instance a tungsten-filament. A prism can be used as the means. The essentially monochromatic light is received by a detector, usually is some type of electron photomultiplier tube. The variation in intensity of the absorbed light, as a function of wavelength, generates the absorption spectrum. (Britton et al., 1995b)

The UV/Vis spectrum gives information about the conjugated double bonds, but not about any other functional groups. Because of the long conjugated double bond system, carotenoids show strong absorption in the visible or UV-region. Quantitative analysis of carotenoids can be achieved through spectrophotometric methods. This is done by measuring the light absorption of the sample relative to pure solvent (Britton et al., 1995b)

Carotenoids can be analyzed in spectrophotometers to find an estimated total carotenoid concentration in a mixture of carotenoids, or to quantify the concentration of a pure carotenoid. This is possible because carotenoids obey Beer-Lambert law that states that absorbance is linearly proportional to the concentration. The majority of carotenoids absorb light in the visible region of the spectrum, between 400 and 500 nm. (Scott, 2001)

The spectrum of a carotenoid is determined by multiple factors. The main band, specified by λ_{max} is the wavelength of the maximum absorption and provides information about the number of conjugated double bonds. The intensity of the absorption (A) is related to the structure and concentration of the sample. Most carotenoids show not a single band, but three more-or-less distinct peaks. As the overall shape of the specter give information about the conjugated double bonds, it is useful to compare spectra with respect to their shape and position of maximum absorption. (Britton et al., 1995b)

Astaxanthin, β,β-carotene and Ψ, Ψ- carotene all contain the same amount of conjugated double bonds (see figure 5 below), but the specters look different due to the ends. Both astaxanthin and β,β-carotene have ring structures, which shift the specter to the right and the peaks are less sharp. The ketones in astaxanthin make up the broad peak.

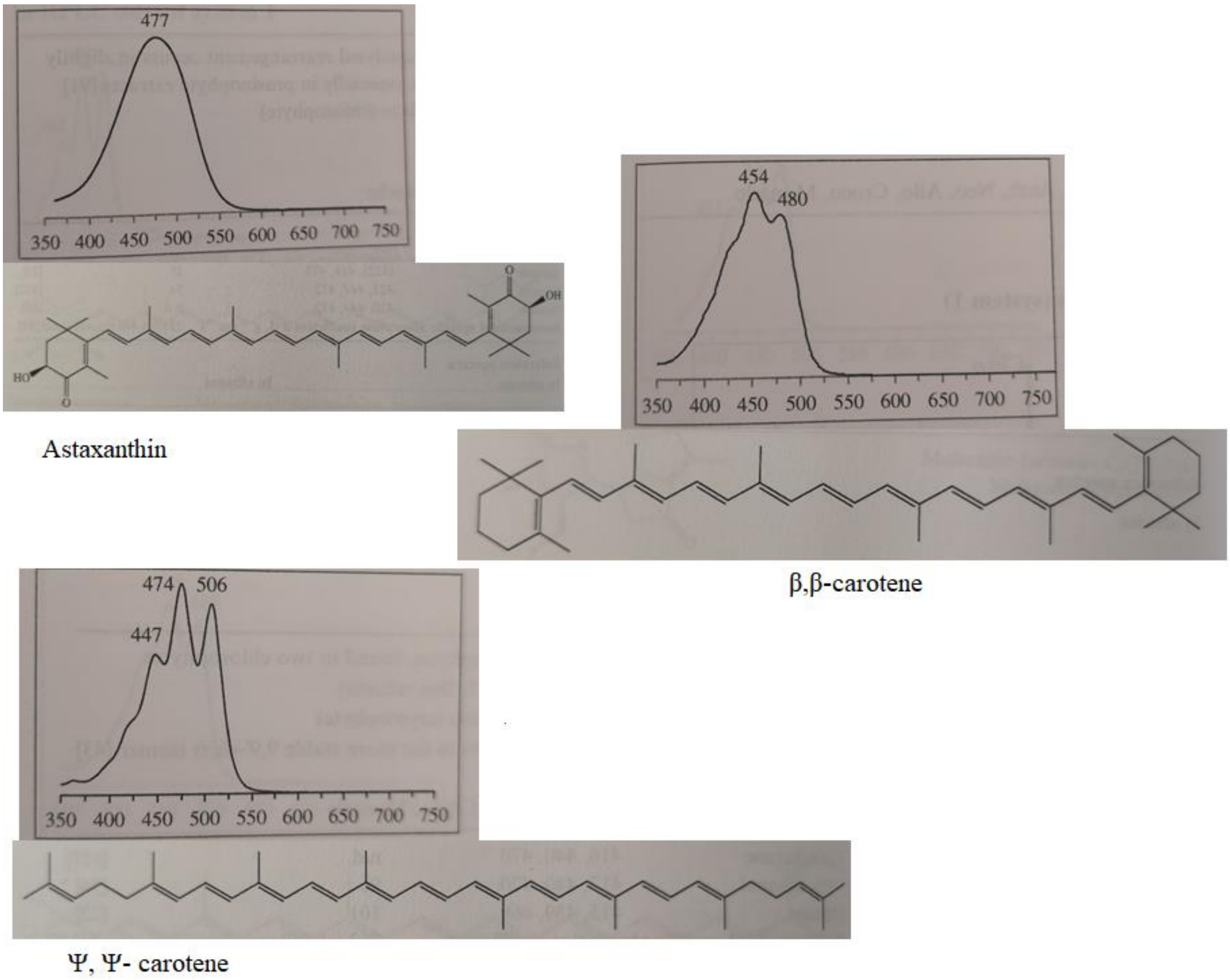

Figure 5: Astaxanthin, β,β-carotene and Ψ, Ψ- carotene with structural shape and specter from HPLC. Pictures are from the book Phytoplanktonic pigments (Roy et al., 2011).

1.9 Chromatography

Chromatography is any separation method that uses one stationary and one mobile phase to separate components in a mixture. The mobile phase passes through the stationary phase, and the mixture that is carried by the mobile phase, interacts with the stationary phase at different times and places in the column. (Britton et al., 1995a)

1.9.1 High-performance liquid chromatography (HPLC)

High-performance liquid chromatography, HPLC, is a good method for analyzing carotenoids and amino acids. The HPLC system consists of solvent pump(s), sample injector, analytical column, detector and a data recording device, usually a computer (see figure 6). Temperature controllers on injection autosampler and column compartments are advantageous. (Bidigare et al., 2005) The HPLC uses a column that contain a stationary phase and a liquid mobile phase to separate compounds of a mixture based on different affinities to the stationary phase and the mobile phase. The liquid sample is injected into the HPLC and flushed to the column by the mobile phase, and the sample gets separated due to different affinity between the sample compounds and the stationary and mobile phase on a non-polar column. The most polar and smallest compound will leave earlier than larger and less polar molecules. The non-polar molecules will have a higher affinity for the stationary phase in the column and will be retained and pass through slower. The compounds absorbance is measured as they leave the column, which is used to quantify the concentration in the sample injected. The compounds are recognized based on retention time and wavelength of maximum absorption. (Roy et al., 2011)

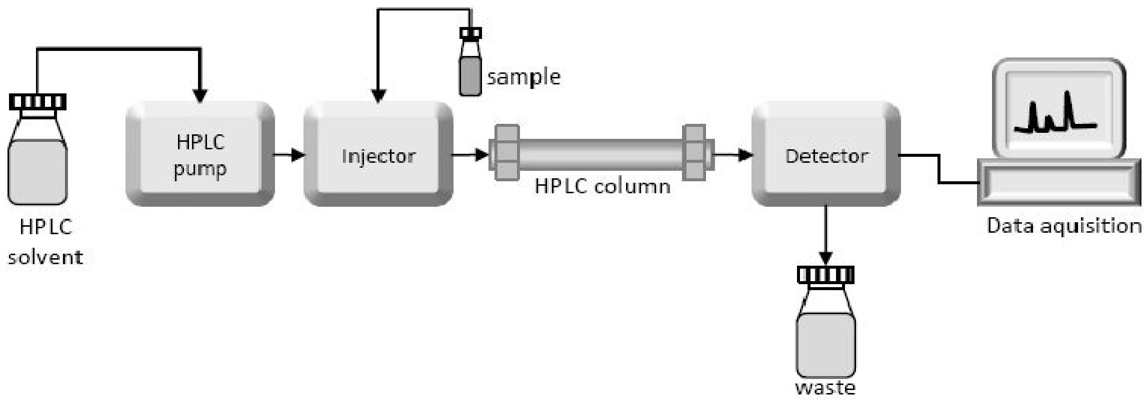

Figure 6: An overview of compartments and set-up for HPLC-analysis. Figure from: https://laboratoryinfo.com/hplc/

1.9.2 Gas chromatography (GC)

The modern gas chromatography (GC) was invented by Martin and James in 1952 and is a well-known method for analyzing lipids. The method is used to separate mixtures, by using a gas as a mobile phase and liquid as a stationary phase held in a column, as the mixture is injected into the machine by an autosampler injector. The mixture separates in the column, and a detector monitors the separated peaks and give the resulting chromatogram (see figure7 below for details). The most important part of the machine is the column. There are different types of columns, which can have an impact on the quality of the separation. The oldest ones were packed columns, but it has been replaced with capillary columns.

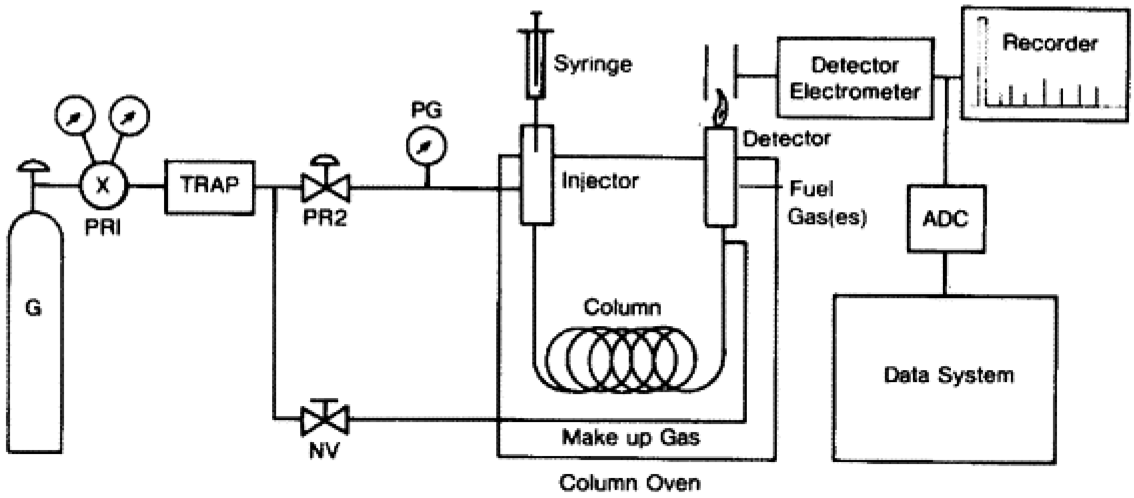

Figure 7: An overview of the compartments and set-up used for a GC separation. Figure from: https://ars.els-cdn.com/content/image/1-s2.0-S0165993602008063-gr2.gif

1.9.3 Standards

Standards are used in most chemical analysis to quantify results. An internal standard is a chemical substance added in a constant amount to samples, blank samples and to calibration standards. The internal standard must be a compound that shows similar, but not identical behavior as any analyte compound. This substance is used for calibration by plotting the ratio of the signal from the analyte to signal of the internal standard as function of the analyte standard concentration. The ratios are used to obtain analyte concentrations from a calibration curve. (Oliveira et al., 2010)

An external standard is similar to the internal, except that the substrate is not added to the samples. Samples are run alone, usually at different concentrations, to generate a standard curve. The area of the peaks are related to the known amounts of external standard run. The main difference is that internal standards correct for losses during preparation of samples, while external standards do not. (Oliveira et al., 2010) An advantage of external standard is that there is no risk of accidental overlaps between the standard and any of the sample compounds.

1.10 Purpose of the study

The main object of this experiment performed was to investigate the apparent digestibility coefficients (ADCs) of dry matter, protein, ash, and energy in whole or broken microalgae-derived feed for Atlantic salmon. During the experiment skin and flesh color of the salmon was observed visually to change, which lead to another interesting field of research, namely the pigments. Thus, this thesis is about chemical analysis of the salmon that was fed microalgae-derived feed, with the main focus on pigment identification and quantification, as well as lipid content. Lipid content will be the measure to see whether the different feed has an impact on the lipid content, and thus the quality of the fish. The study will also investigate if crushed cell walls in the microalgae result in better uptake of pigments and lipids in the salmon.

2 Materials and methods

The salmon feeding experiment was performed in Mørkvedbukta research station at the end of 2018, and a research paper is waiting to be published. Part 2.1 is mainly the same as in that paper and is written by Mette Sørensen.

2.1 Materials and experimental design

The microalgae used in the feeds were *Phaeodactylum tricornutum* B58 and *Tetraselmis chuii* UTEX LB232, both produced at the National Algae pilot Mongstad (NAM) (Mongstad, Norway) using four 800L photobioreactor systems (GemTube MK2-750 from LGem b.v), where the microalgae were harvested twice per week, concentrated to a paste by centrifugation (Evodos 50, Evodos b.v), vacuum packed and directly frozen at -20°C before further downstream processing. The microalgae paste, containing 22.9 % and 18.7 % dry matter (DM), for *P. tricornutim* and *T. chuii*, respectively, was processed by a single pass through a Dyno-Mill Multi Lab bead mill (WAB, Muttenz, Switzerland) using a 0.6 l chamber, small glass beads (0.25-0.4 mm) at 80% chamber filling, 12 m/sec agitator tip speed and 6-9 kg/h biomass flow rate. Following, whole and cell wall disrupted biomasses were spray dried for fine powders before used in feed production. (Mette Sørensen et al. in preparation)

The diets for this study were produced at the Nofima feed laboratory, Bergen, Norway.
The five feed mixes were prepared and homogenized (30 min) using a horizontal ribbon mixer. The feed mixes were conditioned with steam and water in an atmospheric double differential preconditioner prior to extrusion on a TX-52 co-rotating, fully intermeshing twin-screw extruder (Wenger Manufacturing Inc., Sabetha, KS, USA). The temperature of the feed mash entering the extruder was 84-87 °C. Temperature in the extruder outlet ranged from 109 °C in “Tetra Intact” (TI), “Tetra Broken” (TB) and “Phaeo Intact” (PI) to 113 and 117 °C in the “Referense diet” (RD) and “Phaeo Broken” (PB), respectively. The extruder outlet was restricted by 24 circular 2.5 mm die wholes. The wet extrudates were cut at the die surface with a rotating knife. Sampling of pellet was conducted after achievement of steady state conditions in the preconditioner and extruder. Extrudate was dried in a in a hot air dual layer carousel dryer (Paul Klockner, Nistertal, Germany) at constant air temperature (80 °C) to approximately 7-8% moist. Then each of the diets were coated in an experimental vacuum coater (Pegasus PG-10VC LAB, Dinnissen B.V., The Nederlands) with fish oil. (Mette Sørensen et al. in preparation)

A reference diet, containing 475.5 g/kg protein, 219 g/kg lipid and 0.1 g/kg yttrium oxide, was prepared. The main sources of protein and lipid was fishmeal and fish oil, respectively. The microalgae test diets were made by mixing 70% reference diet with 30% microalgae. No astaxanthin was added in the feed, besides the natural content in fish meal and fish oil.

Atlantic salmon post-smolts (Aquagen strain, Aquagen AS, Sluppen, Trondheim, Norway) with an average weight of approximately 315 g were stocked into 1100 l tanks (55 fish per tank). These tanks were part of a flow-through system with seawater drawn from a depth of 250 m from the Saltenfjorden. The water was filtered and aerated before use. The average water temperature in the tanks was 7.7 °C and the oxygen was above 85% of saturation throughout the experimental period. The fish were reared under 24 h of continuous light condition. Fish were randomly assigned to 3 replicate tanks of the different dietary treatments, respectively. Fish were fed by automatic feeders - approximately 1.4% of the biomass - for 35 days, i.e., until the first feces collection. Feed was withdrawn for two days after the feces collection. The feeding continued for another 9 days and a second stripping was performed. (Mette Sørensen et al. in preparation)

2.2 Extraction of pigments from fish muscle

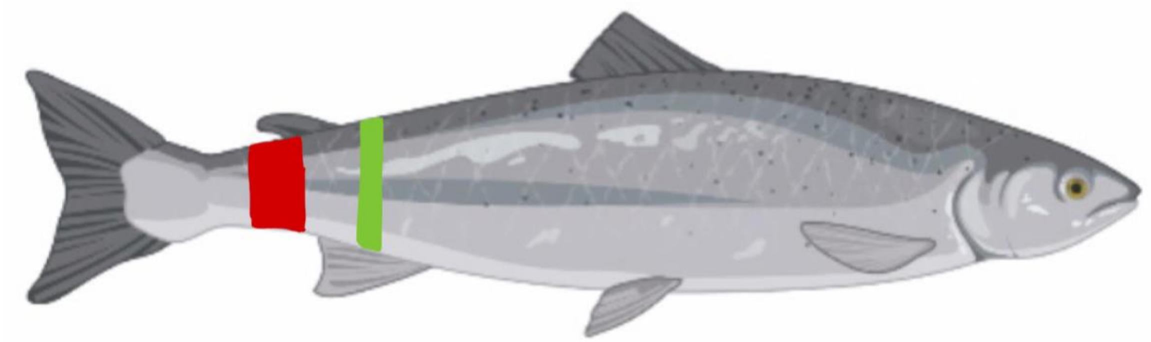

Figure 8: Atlantic salmon with an overview of where samples were taken. The green line shows pigment samples, while the red area was homogenized and used for lipid analysis.

Single fish were analyzed, 18 from each of the five groups, RD, PI, PB, TI, TB. On the first day, a vertical stripe of the posterior part of one filet side of the salmon muscle (see green line in figure 8) was cut into 8-10 mm sized cubes and weighted. 25 ml cold (-30°C) acetone was added to the fish cubes, making sure all cubes were covered with solvent. The mixture was flushed with nitrogen gas and kept at -30°C until the next day (for at least 22 hours).

On the second day, the liquid was decanted off the fish cubes, and filtered through a glass-sintered filter (porosity 3-5), and evaporated with approx. 50 ml benzene and 25 ml absolute ethanol under reduced pressure at max 30°C until dryness, then flushed with nitrogen gas, added a little acetone (approximately 4 ml) and flushed with nitrogen gas again before storage in freezer. This was Extract 1. The fish cubes were added another 25 ml acetone, flushed with nitrogen gas and kept at -30°C for approximately 20 hours.

The third day, the liquid was removed from the fish cubes as the previous day, the extract was placed in a new bottle, flushed with nitrogen gas, and kept at -30°C for at least 22 hours, this was Extract 2. The fish cubes were added 25 ml acetone, flushed with nitrogen gas, and kept at -30°C for at least 22 hours. The procedure for the third day was repeated on day four, and the extracts were combined in the same bottle, Extract 2.

The combined extracts from the third, fourth and fifth day (in the bottle labeled Extract 2) was added approximately 250 ml benzene and 75 ml absolute ethanol and evaporated under reduced pressure at max 30°C until dryness, then flushed with nitrogen gas, added a little acetone (approximately 5 ml), poured into the bottle labelled extract 1, flushed with nitrogen gas and stored in the freezer. Fish cubes were cut in two to ensure all color was extracted.

2.3 Extraction of pigments from fish skin

Three samples of ventral fish skin from PB, of the (previously) yellow-colored part and two samples from TB and three samples from TI, was analyzed. Thin stripes (approximately 5 x 50 mm) of the skin were cut out and placed in a bottle, and mass was determined. No shells were intentionally removed from the skin, but about half were naturally lost due to handling. 25 ml cold (-30°C) acetone was added to the stripes, making sure they were coved with solvent. The mixture was flushed with nitrogen gas and kept at -30°C until the next day (at least 22 hours).

The second day, the liquid was decanted of the fish stripes, and filtered through a glass-sintered filter (porosity 3-5), and evaporated with approx. 50 ml benzene and 25 ml absolute ethanol under reduced pressure at max 30°C until dryness, then flushed with nitrogen gas, added a little acetone (approximately 4 ml) and flushed with nitrogen gas again before storage in the freezer. This was Extract. The skin stripes were added another 25 ml acetone, flushed with nitrogen gas and kept at -30°C for about 20 hours.

The third day, the liquid was removed from the skin stripes, and the extract was placed in the bottle labeled Extract, flushed with nitrogen gas and kept at -30°C for at least 22 hours. The skin stripes were added 25 ml acetone, flushed with nitrogen gas and kept at -30°C for at least 22 hours.

The combined extracts from the second, third and fourth day (in the bottle labeled Extract) was added approximately 150 ml benzene and 75 ml absolute ethanol and evaporated under reduced pressure at max 30°C until dryness, then flushed with nitrogen, added a little acetone (approximately 4 ml), flushed with nitrogen gas and stored in the freezer. The colorless skin left in the bottle was thrown in the trash.

2.4 Preparation for spectrophotometer

The extracts from each sample were added in a 10 mL volumetric flask and acetone was added to the line, capped and shaken. A pipette was used to take out a small part of the sample and placed it in a quarts cuvette (micro cell type 115-QS, light path 10.0 mm, Hellma ®). The sample was placed in a baselined and zeroed spectrophotometer (Varian Cary 100, Agilent). When the spectrum was recorded, results were obtained from the screen, and the samples were prepared for HPLC analysis.

2.5 Preparation for HPLC

The combined extracts from the spectrophotometer analysis were evaporated under reduced pressure as described above. A small amount of acetone was used to dilute the sample slightly, and the sample was added to a 5 ml volumetric flask, and acetone was added to reach the 5ml line in the flask. The extract was filtered through a syringe filter (GHP membrane, 13 mm diameter, 0.2 µm pore size, Acrodisc®), flushed with a slow rate of nitrogen, capped and placed in the HPLC (1100 series, Agilent).

2.6 Settings (HPLC analysis)

The method used is described in the NASA report, as the UN method (Egeland, 2012). Two identical C_{18} columns were used (ACE 5 C_{18} part no. ACE-121-2546, 4.6x350 mm each, with 5 µm packing) with separate guard column (ACE). The solvents used as the mobile phase had a flow rate of 0.5 ml/min in the following order (minutes, % solvent A, % solvent B, % solvent C, % solvent D); (0, 19.9, 80.0, 0.0, 0.1), (60, 0.0, 69.9, 30.0, 0.1), (100, 0.0, 30.0, 50.0, 20.0), (110, 0.0, 0.0, 40.0, 60.0), (120, 0.0, 99.9, 0.0, 0.1), (130, 19.9, 80.0, 0.0, 0.1). Solvent A was 1 M ammonium acetate, solvent B was methanol, solvent C was acetone in water and solvent D was hexane. (Egeland, 2012) 10% propan-2-ol and was used for cleansing the pump heads.

2.7 Calibration

Calibration was performed after all samples had been analyzed by the HPLC. Peaks from each samples were identified with help from books (Roy et al., 2011) and (Britton et al., 2004) and my supervisor was consulted if in doubt.

Formula 1 was used for calculation:

$$pigment\ (mg) = \frac{volume\ (ml)\ x\ absorbance}{absorption\ coefficient\ d\ (l\ g^{-1}cm^{-2})}$$

The absorption coefficient d differs between the different pigments and in variable solvents, and values for standards used in calibration can be seen in table 1 below. One sample of each carotenoid with known concentration was injected into the HPLC at different volumes. 1 µl, 5 µl, 10µl, 25 µl and 50 µl.

2.8 Pigment identification

Table 1: An overview of all standards tested, included retention time and wavelength. D-value and solvent is added for standards used for calibration.

RT	*Pigment*	*Wavelength (nm)*	*d-value (l g^{-1})*	*Solvent*
35	Fucoxanthinol	450	141	Acetone
44	Fucoxanthin	450	166	Acetone
48	Neoxanthin	450		
50	Violaxanthin	450		
52	Idoxanthin	480	199	Acetone
54	Diadinoxanthin	450		
54	Anteraxanthin	450		
55	Astaxanthin	480	206	Methanol
58	Adinoxanthin	480		
59	Diatoxanthin	450	272	Acetone
60	Lutein	450	255	Ethanol
60	Zeaxanthin	450		
73	Chlorophyll *b*	450		
80	Chlorophyll *a*	420		
83	Astaxanthin monopalmitat	480		
93	β,ε-Carotene	450		
94	β,β-Carotene	450		
97	Astaxanthin dipalminat	480		

In order to identify the pigments present in the fish, several known standards were tested and compared to results of the samples tested. The sample must match the standard in both specter and retention time to be correctly identified. All relevant standards available were tested for can be seen in table 1 above. In addition to these standards, it is believed that loroxanthin and diadinochrome was also present, but no standards for these pigments were available.

2.9 Extraction and methylation of fatty acids

Single samples of the posterior part of the salmon flesh (see red area in figure 8 under **2.2**) were analyzed in duplication, 18 (36 in duplicate) samples from each of the five groups RD, TB, TI, PB and PI. About 50 mg homogenized, freeze dried sample was weight and added into a labeled Greiner centrifuge tube (Falcon, 15 mL, 17x120 mm, conical (V) bottom, w/ graduation, I.D. field, T1818-500EA). Lipid extraction of freeze-dried samples were carried out according to the chloroform and methanol gravimetric determination described by Bligh and Dyer (1959). 0.8 mL distilled water, 2.0 mL methanol and 1.0 mL trichloromethane (stabilized with 1% ethanol) were added to the sample and homogenized for 1 min. 1.0 mL chloroform was added, followed by 20 seconds of homogenization. 1.0 mL distilled water placed in the tube before the tubes were placed in the centrifuge (ThermoFisher Scientific Sorvall Legend X1R Centrifuge, Germany) for 4000 rpm, at 4 degrees Celsius for 10 min. 0.5 mL of the bottom chloroform layer was transferred into a labeled Kimax tube (DWK Life Sciences Kimble™ KIMAX™ Reusable Tubes with Rubber-Lined Screw Cap) which was evaporated by heat (up to 45 degrees) and nitrogen flow.

Fatty acid methyl esters (FAMEs) of samples were obtained by transesterification and methylation according to the method described previously by Metacalfe et al. (1966). (Metcalfe et al., 1966) To the dry sample, 1.0 mL 0.5 M NaOH-methanol was added, and placed on a heater at 100 degrees for 15 min, and then cooled on ice for a few minutes. 2.0 mL 12% BF3-methanol was added, and the tube was placed on the same heater for 5 min, and then cooled down on ice for a few minutes. 1.0 mL hexane was added to the tube, which was heated for 1 min and then cooled down for a few minutes. 3.0 mL saturated NaCl in distilled water was added to the tube, the tube was shaken and placed on ice for a few minutes. 0.6 mL of the upper hexane layer was placed in a small 2 mL GC-vial. The concentrated samples were placed in the freezer until all sampled were extracted and methylated. Samples were diluted to a concentration at about 0.2 mg/ml, and then placed in the GC.

To find the concentration of the FAMES in the samples, 0.5 mL of the chloroform layer after extraction was pipetted into a small pre-weight vial, evaporated on a heater at about 35 degrees and weighted on a microscale (UMX2 Mettler Toledo, Mettler-Toledo A/S, Switzerland).

Formula 2 was used to calculate the lipid concentration going into GC:

$$Lipid\ concentration\ into\ GC = \frac{amount\ of\ fat\ in\ the\ beaker\ (mg)}{0.5\ mL}$$

2.10 GC

FAMEs analyses were performed in a gas chromatograph (SCION 436-GC, Scion Instruments, Goes, Netherlands) fitted with a flame ionization detector at 250 °C in duplicate. The separation was achieved using a wax embedded column of 25m length, 0.25mm internal diameter and 0.2 μm film thickness (Agilent Technologies, Middelburg, Netherlands). Individual FAME was identified and quantified by comparison to known standard mixtures of common fatty acids (FAME MIX 2/GLC-473, Nu-Chek Prep, Elysian, MN, USA) and results are expressed as relative area percentage on the total fatty acid using a software Compass CDS, Bruker Co-operation.

2.11 Analysis

The analysis of the data proceeded as follows. At the start all the individual data series (measurements of pigments and fatty acids per test group) were tested for normality using the Shapiro-Wilk test (Shapiro & Wilk, 1965). The samples were found to be consistent with being normally distributed in most cases. The dominant exception are measurements series with many null results. This is to be expected as a consequence of limited measurement precision a low values, and should have limited effect on the later statistical tests, although the null values will create some bias towards lower means for the affected series.

For each data series the mean, standard deviation and the error on the mean (SEM) is calculated. For the error on the mean it is used a 95% confidence limit instead of the more usual 68%.

For individual pigments and fatty acids (and combination categories) ANOVA tests were performed (McDonald, 2014) across feeds. This revealed statistically significant differences for most measurements. To find the cause of these differences individual t-tests were performed between the feeds. The specific test used was Welch's unequal variances t-test (Welch, 1947) which is more robust to the underlying assumptions of normality and equal variance in the original t-test.

For all these tests the null hypothesis H0 states that there are no significant differences between the means of the samples analyzed. For each test, a p-value is calculated. If the p-value from the test is low, less than 0.05, we reject the H0 hypothesis. A higher p-value indicates that with the current data there is no significant differences between the samples studied. The statistical interpretation of this p-value is that if a set of measurements identical to ours had been repeated many times, and assuming that H0 is true, only in 5% of the measurement sets would H0 be rejected despite being true.

Plots were also made to show the differences between feeds for the different pigments and fatty acids. Some of these plots can be seen in appendix D. Here we print error bars showing the mean and the 95% confidence interval for the mean. For astaxanthin and idoxanthin, a correlation plot was made.

The numerical analysis was performed using either RStudio or python. For the python part we used the scipy statistical package (Virtanen et al., 2020), and RStudio without packages (Team, 2020).

3 Results

3.1 Total carotenoids

Observed color of the salmon flesh was light orange when samples were analyzed almost 2 years post slaughter of the fish. The skin of the salmon appeared to have normal salmon coloration with silver abdominal skin and going darker towards the back in all samples. Salmon analyzed shortly after slaughter was observed to be yellow in both flesh and skin in fish fed PB and PI diets (see figure 9 below). The pictures below are taken only moments after slaughter in January 2019.

Figure 9: Left: Muscle color in control fish (RD). Right: Muscle color of salmon fed Phaeodactylum tricornutum. Photo: Chris André Johnsen.

Figure 9 show muscle coloration of the the control fish to the left, and salmon fed *Phaeodactylum* to the right. The coloration was visibly different between the samples, and salmon fed *Phaeodactylum* showed a darker, more pigmented flesh, and the ventral part of the head and operculum also showed a more yellow color than the control salmon. The control fish appears pale and poorly pigmentmented.

Figure 10: Skin coloration of salmon fed Phaeodactylum (left) and Tetraselmis (right). Photo: Anjana.Mahesh Palihawadana

The *Phaeodactylum* fed salmon (left side in figure 10) was visibly yellow on the ventral skin, on the lower side of the operculum, and on the caudal fin. The *Tetraselmis* fed salmon (right side in figure 10) did not show any discoloration, and appears visually unaffected by the carotenoids in the microalgae *Tetraselmis* in the feed.

Figure 11: Left: PI fed salmon muscle. Right: Control salmon muscle. Photo: Renate Hammerø

Figure 11 show coloration of one PI sample and one control sample. The right side, RD, show a paler, but at the same time more red color than the left PI fillet, which looks strongly pigmented, but the color is more yellow-orange than red-orange. The photos were photographed in summer 2020.

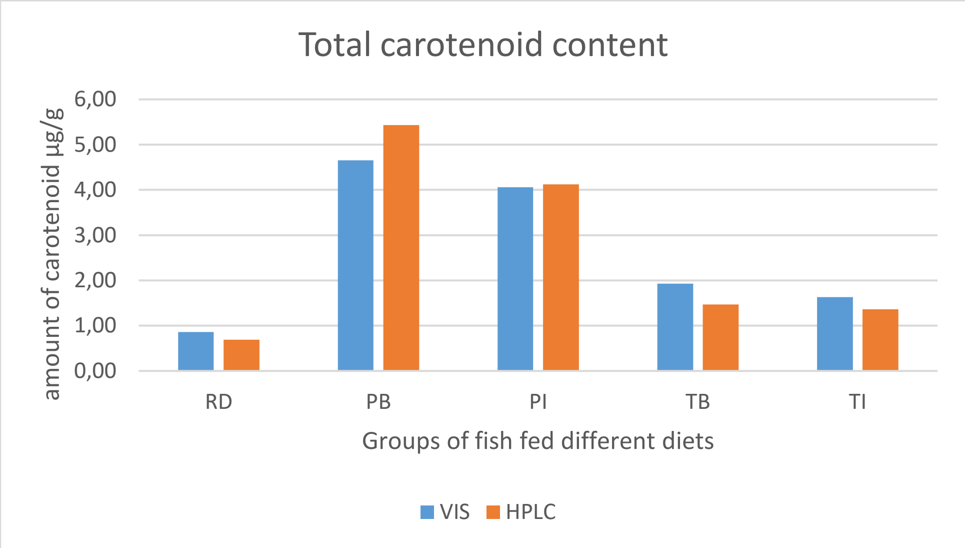

Figure 12: Total carotenoid content, calculated from both Vis and HPLC

As seen in figure 12 above, PI and PB show much higher amounts of carotenoids than the other groups. There is a difference in the amount between the calculations from UV/Vis spectrophotometer (shortened to Vis) and HPLC, and Vis show a higher amount in RD, TB and TI, while HPLC show the larger amount in PB. PI show close to identical values in both methods. The control group (RD) show the lowest content of carotenoids (0.86 µg/g in Vis and 0.69 µg/g in HPLC). Broken cell walls gave a higher carotenoid content than intact cell walls, that was significantly different between PB and PI, with a p-value = 0.0023, and amounts of 5.4 µg/g and 4.1 µg/g from HPLC in PB and PI, respectively and 4.7 µg/g and 4.1 µg/g from Vis. The differences between TB and TI were not significant, p-value = 0.49. TB and TI show similar amounts to each other with 1.5 µg/g and 1.4 µg/g in HPLC, and 1.9 µg/g and 1.6 µg/g in Vis respectively. From now, when referred to total carotenoid content, it means from HPLC, unless stated otherwise.

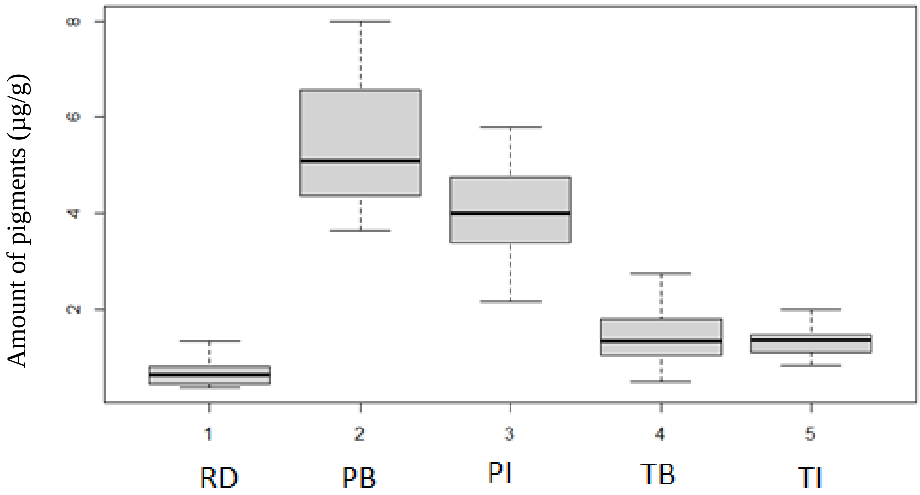

Figure 13: A boxplot of the total carotenoid content from HPLC.

PB and PI show larger varieties in their total carotenoid content (see figure 13). The difference between the means of TB and TI is insignificant, but TB shows slightly higher variations between values. PB has more than seven times as much carotenoid in total as RD.

3.2 Muscle pigments

Table 2 presents all pigments, and their amounts present in each of the fish groups. Diadinochrome appeared in PI, but was not detected in PB. In PB, the specter of lutein was pure and did not contain the astaxanthin *cis*-isomer all other groups had present. Loroxanthin was only present in fish fed *Tetraselmis*, and the unknown astaxanthinlike was only found in *Phaeodactylum* fed fish. Fucoxanthin and fucoxanthinol was present in all sample groups, and all samples of PB and PI, but only in a few samples of RD, TB and TI.

Table 2: An overview over pigments present in each sample given in µg pigment of g fish, standard deviation and SEM for 95% confidence interval. Lutein is in a mixture with cis-astaxanthin in all samples, and astaxanthin is in a mixture with diadinochrome in PI samples.

	RD			**PB**			**PI**			**TB**			**TI**		
Pigments	**mean**	**std**	**SEM**	**mean**	**std**	**SEM**	**mean**	**std**	**SEM**	**mean**	**std**	**SEM**	**mean**	**std**	**SEM**
Fucoxanthinol	0.01	0.04	0.02	1.40	0.51	0.25	1.04	0.30	0.15	0.01	0.04	0.02	0.02	0.03	0.01
Fucoxanthin	0.02	0.05	0.03	2.84	0.90	0.45	2.04	0.79	0.39	0.02	0.07	0.03	0.03	0.03	0.02
Loroxanthin										0.04	0.02	0.01	0.04	0.07	0.01
Idoxanthin	0.03	0.05	0.03	0.05	0.06	0.03	0.04	0.06	0.03	0.04	0.07	0.04	0.04	0.07	0.04
Astaxanthin	0.50	0.23	0.11	0.65	0.21	0.11	0.59	0.22	0.11	0.62	0.30	0.15	0.52	0.15	0.07
Diatoxanthin	0.01	0.01	0.01	0.25	0.08	0.04	0.16	0.05	0.02						
Lutein	0.11	0.05	0.02	0.14	0.03	0.01	0.17	0.05	0.03	0.66	0.33	0.17	0.63	0.20	0.10
Cis-lutein	0.01	0.01	0.01	0.05	0.03	0.01	0.04	0.05	0.02	0.08	0.08	0.04	0.09	0.07	0.03
Total	**0.69**	**0.28**	**0.15**	**5.43**	**1.32**	**0.66**	**4.12**	**1.04**	**0.52**	**1.47**	**0.60**	**0.30**	**1.36**	**0.30**	**0.15**

The mean and SEM presented is calculated by dividing on total samples, 18, even though some pigments were only present in some of the samples. Fucoxanthinol and fucoxanthin in

RD was only present in four and three samples respectively. In TB, fucoxanthinol and fucoxanthin appeared in eight and nine samples respectively and both appeared in twelve samples of TI. Idoxanthin was found in seven, ten, five, five and seven samples of the fish groups RD, PB, PI, TB and TI. Diatoxanthin was found in eight samples of control feed, but all samples of PB and PI.

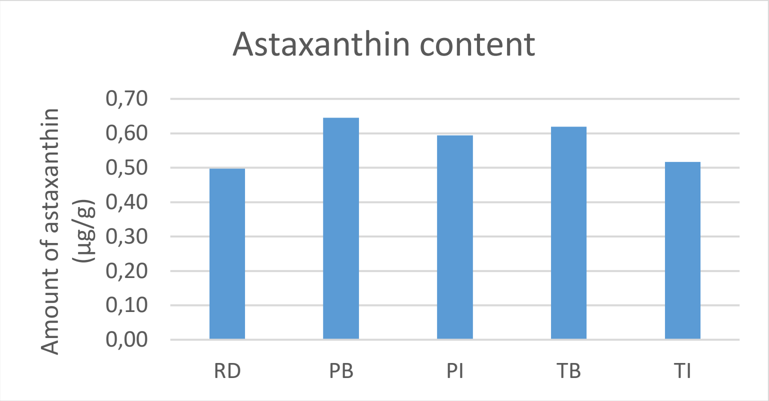

Figure 14: Astaxanthin content in the different groups

No significant differences in astaxanthin content were detected between the test groups (see figure 14 above), PB p-value=0.052, PI p-value=0.20, TB p-value=0.18 and TI p-value=0.77, and all tested versus the control group. While there are differences between the groups, they are not significant below p-value<0.05. A t-test was used for calculating p-values in R.

Figure 14 shows that the astaxanthin level in the different fish did not vary much, but some differences are seen. The fish fed feed with broken cell walls, seem to have a higher content of astaxanthin, than those with intact cell walls, but insignificant in both PI vs PB, p-value = 0.47 and TI vs. TB, p-value = 0.20. The control feed shows the lowest content of astaxanthin, but not significantly different from any of the other.

As idoxanthin is expected to be a breakdown product from astaxanthin, it should be possible to find a correlation between idoxanthin and astaxanthin. The analysis was done in Python to find a trend. The regression line is y=0.39+1.70x, with a correlation coefficient r=0.47. The scatterplot can be seen in figure 15 below. Idoxanthin did not appear in all samples and 0 values are ignored in the plot. All samples of all groups were combined for the plot.

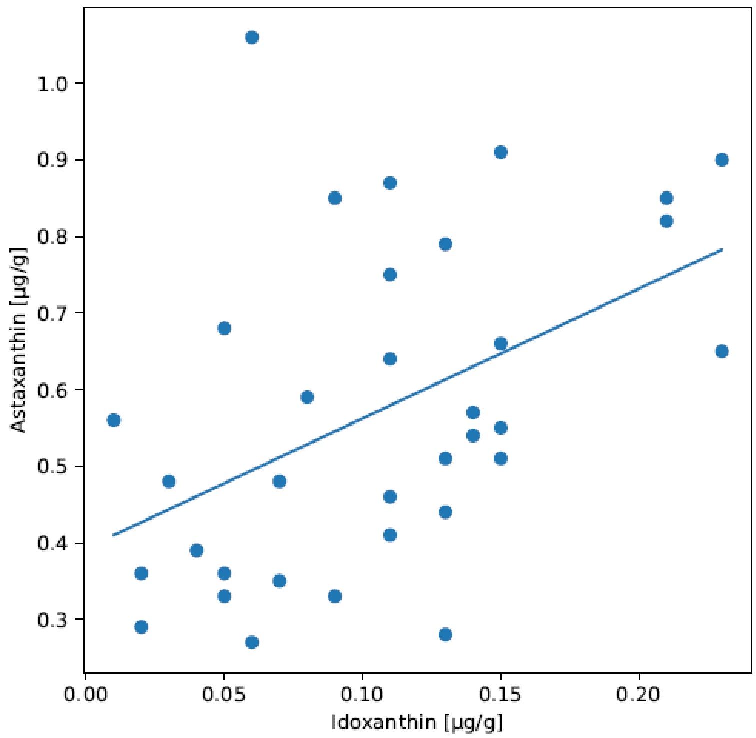

Figure 15: A scatter plot of astaxanthin vs. idoxanthin to find trend.

3.2.1 Reference diet

The control fish (RD) contained mainly astaxanthin and lutein (see figure 16), in addition to *cis*-isomers of both pigments. As the astaxanthin *cis*-isomer was found together in the same peak as lutein, it was impossible to separate them with the used HPLC method to get values for each of them. Thus, the area under the peak gave the value of lutein and astaxanthin *cis* combined. It is believed however, based on the specter, that the lutein content is the major contributor to the peak. Idoxanthin was present in about half the samples, and diatoxanthin was present in a few samples. Small amounts of fucoxanthinol and fucoxanthin appeared in three samples, and fucoxanthin in one additional sample.

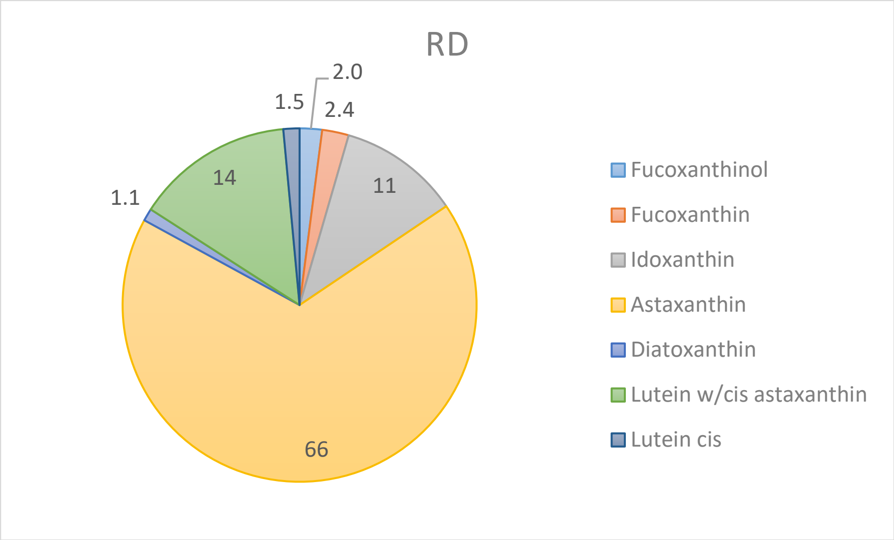

Figure 16: Overview of the carotenoid content in RD fed salmon muscle. Values are the percentage of single carotenoids in total carotenoid content.

3.2.2 Phaeodactylum fed salmon

Common for the fish fed *Phaeodactylum*, intact and broken, is that they contained large amounts of the fucoxanthinol and fucoxanthin which gave the yellowish color to the fish. Astaxanthin, lutein and diatoxanthin were also found in all samples, as wells as the *cis*-isomers of astaxanthin and lutein. Idoxanthin was found in about half of the samples. In the end of the chromatogram, several minor peaks appeared between R_t 77 and 88. These were not identified as they had a wavelength of 450, but the absorption spectra were shaped like astaxanthin that has a wavelength of 480. The multiple peaks were combined and is referred to as "unknown astaxanthinlike" in tables and figures. Small amounts of chlorophylls were detected in some samples, around R_t 47-48, but these were not identified either. It may be pheophorbide *a*.

Even though the percentage of each carotenoid seem to be similar between PI and PB (see figure 17 and 18 below), the total amount is clearly different (p-value=0.002) when a significance level is sat to 0.05 between the two groups. PB has more carotenoid retained in the muscle than PI. The values of astaxanthin and lutein were not different in the two groups, but fucoxanthinol and fucoxanthin were different.

The amount of both fucoxanthinol and fucoxanthin was significantly different between PB and PI, p-value<0.05, with fucoxanthinol, p-value=0.015, and fucoxanthin, p-value=0.0080, and PI had the highest content of both. The amount of fucoxanthin and fucoxanthinol in PB and PI was significantly larger than the amount of astaxanthin.

3.2.2.1 *Phaeodactylum Broken*

In the flesh of PB fish, the major peaks were identified as fucoxanthinol, fucoxanthin, astaxanthin and lutein (see figure 17). In addition, diatoxanthin was found in all samples, and idoxanthin in about half of them. No overlap of peaks or pigments were found. In the end of the chromatogram, between 77 and 88 R_t, several (<5) small peaks (combined, <1% of total carotenoid content) appeared, but were not identified, and is referred to as "unknown astaxanthinlike" in tables and figures. Some type of chlorophyll was found at R_t=47-48, but was not identified or quantified. It is believed to be pheophorbide *a*. The unknown astaxanthinlike, the *cis*-isomers of lutein and idoxanthin all showed 1% or less of the total carotenoid content in PB. Fucoxanthinol and fucoxanthin counted for 78% of the total carotenoid content.

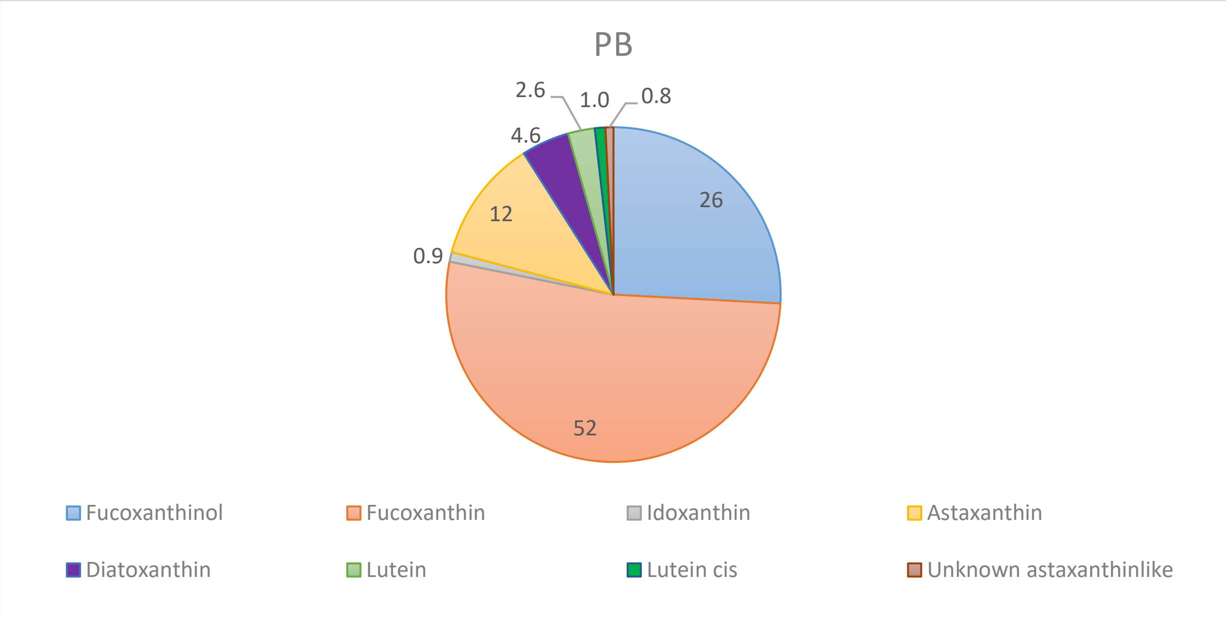

Figure 17: Overview of the carotenoid content in PB fed salmon muscle. Values are the percentage of single carotenoids in total carotenoid content.

3.2.2.2 *Phaeodactylum Intact*

As well as in PB, fucoxanthinol, fucoxanthin, astaxanthin and lutein were the main pigments in PI (see figure 18). However, some of the peaks indicated a mixture of pigments. When analyzing the peak of astaxanthin, another specter appeared as well, at 450, most likely diadinochrome. Also, in the peak of lutein, a smaller amount of an astaxanthin *cis*-isomer was found and identified based on the specter, but the exact amount was impossible to determine. Diatoxanthin was found in all samples, idoxanthin was found in about half of the samples, and as well as in PB, the same unknown astaxanthinlike structure and chlorophyll structure was found. Fucoxanthinol and fucoxanthin counted for about 75% of the total carotenoid content combined. Astaxanthin was only 14% of the total carotenoid content. The remaining carotenoid amounts can be seen in figure 18 below.

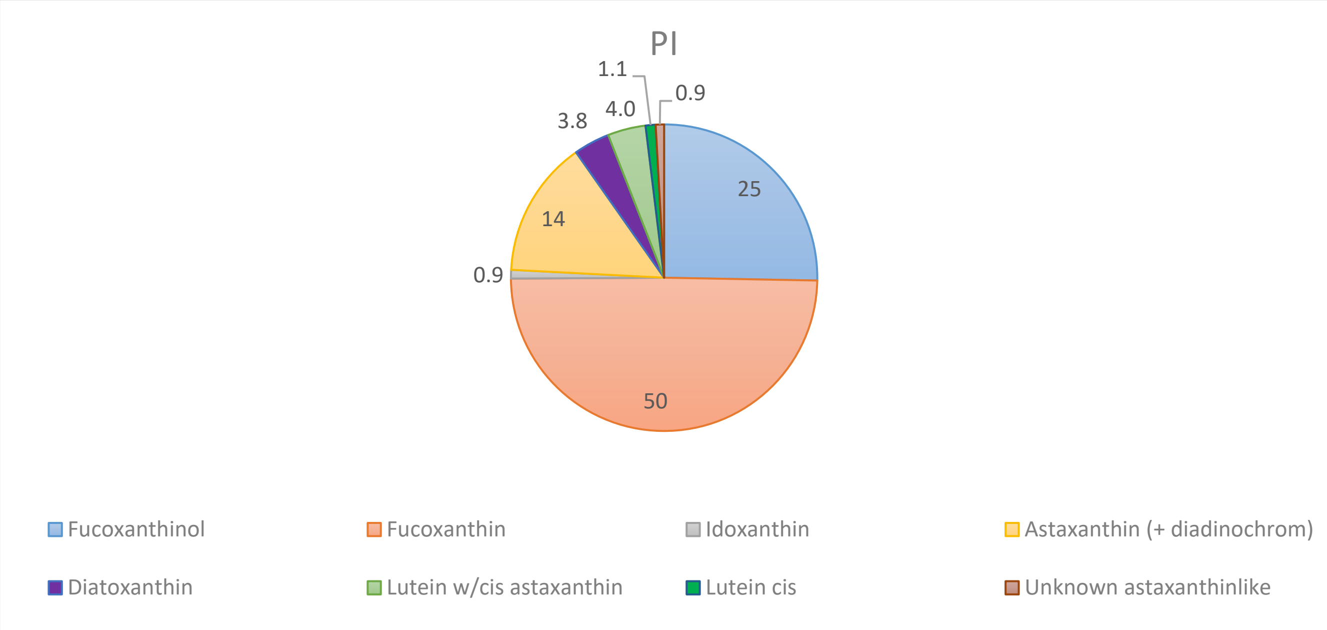

Figure 18: Overview of the carotenoid content in PI fed salmon muscle. Values are the percentage of single carotenoids in total carotenoid content.

3.2.3 Tetraselmis fed salmon

The main carotenoids in fish fed *Tetraselmis* diets were astaxanthin, lutein and their *cis*-isomers. As well as in *Pheaodactylum* fed fish, the major pigment in *Tetraselmis* fed fish was not astaxanthin. In *Tetraselmis*, the major carotenoid content was lutein, although not separated by HPLC, together with some amounts of *cis* astaxanthin. All samples contained astaxanthin and lutein as well as relatively low amounts of their *cis*-isomers. One pigment appeared in all samples and is believed to be loroxanthin, based on retention time, specter and knowledge about the presence of loroxanthin in *Tetraselmis* algae (Garrido et al., 2009). Up to two different chlorophyll derivatives were found in samples of TB and TI. One appeared between R_t 73-81 and one between 87-91. The last specter was suspected to be pyropheophytin *a*, or pheophytin *a* (see appendix B, figure 32, 33 and 34), and the first peak was not identified. Fucoxanthin and fucoxanthinol was found in small amounts in a few samples.

Between these two test groups there was no significant difference, p-value=0.49, in total carotenoid content. Even when testing all the different carotenoids against each other, no differences were found when the significant level was set to p-value=0.05. TB did show a higher variation between the values than TI.

3.2.3.1 Tetraselmis Broken

The peak of lutein also contained some amounts of a *cis*-isomer of astaxanthin, so the amount given is these two compounds combined. After the mixture of lutein and *cis* astaxanthin, one to three peaks of lutein *cis*-isomers appeared in the chromatogram, in small amounts. Another type of chlorophyll was found in some samples of TB at R_t 89-91 believed to be pyropheophytin *a*. The main pigments, astaxanthin and lutein, including their *cis*-isomers made up more than 90% of the total carotenoid content (see figure 19). Fucoxanthinol and fucoxanthin combined accounted for less than 3%. Idoxanthin was found in some samples, and loroxanthin was found in all samples.

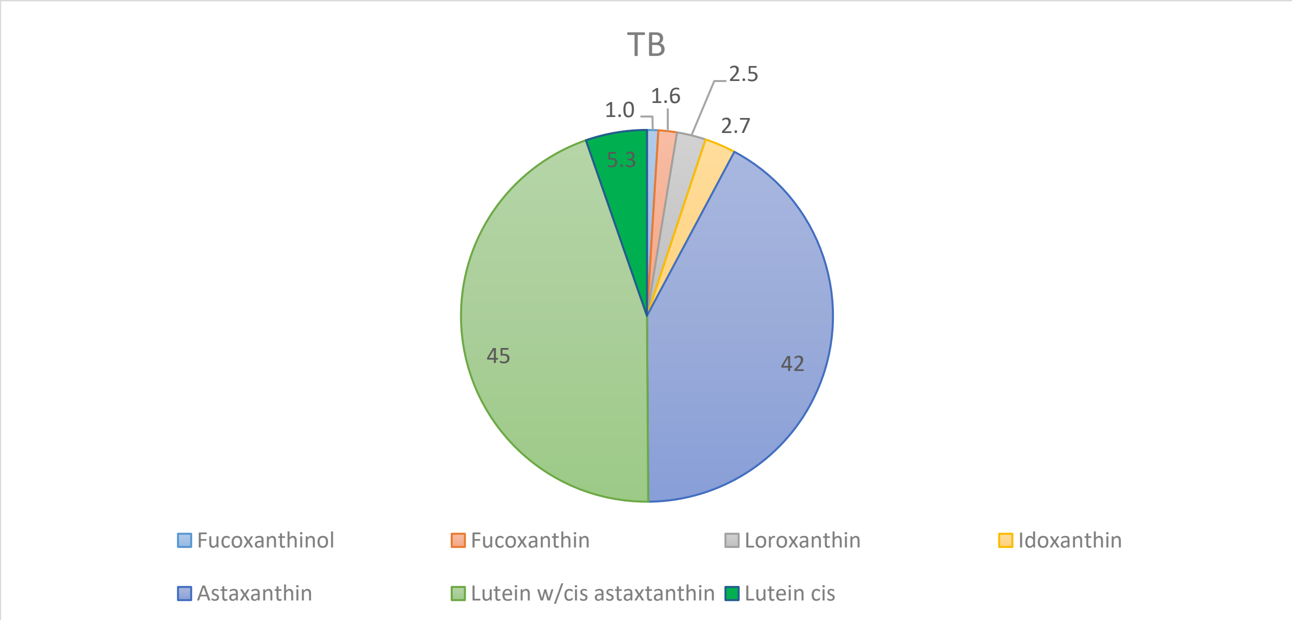

Figure 19: Overview of the carotenoid content in TB fed salmon muscle. Values are the percentage of single carotenoids in total carotenoid content.

3.2.3.2 Tetraselmis Intact

In TI the major pigments were lutein and astaxanthin and their *cis*-isomers, and accounted for more than 90% of the total carotenoid content (see figure 20 below). Small amounts of fucoxanthinol and fucoxanthin was found in a few samples. Loroxanthin was present in all samples and idoxanthin was present in some. The same chlorophyll as in TB, believed to be pyropheophytin *a*, was also found in TI. The other chlorophyll, found between R_t 73-81, was not identified.

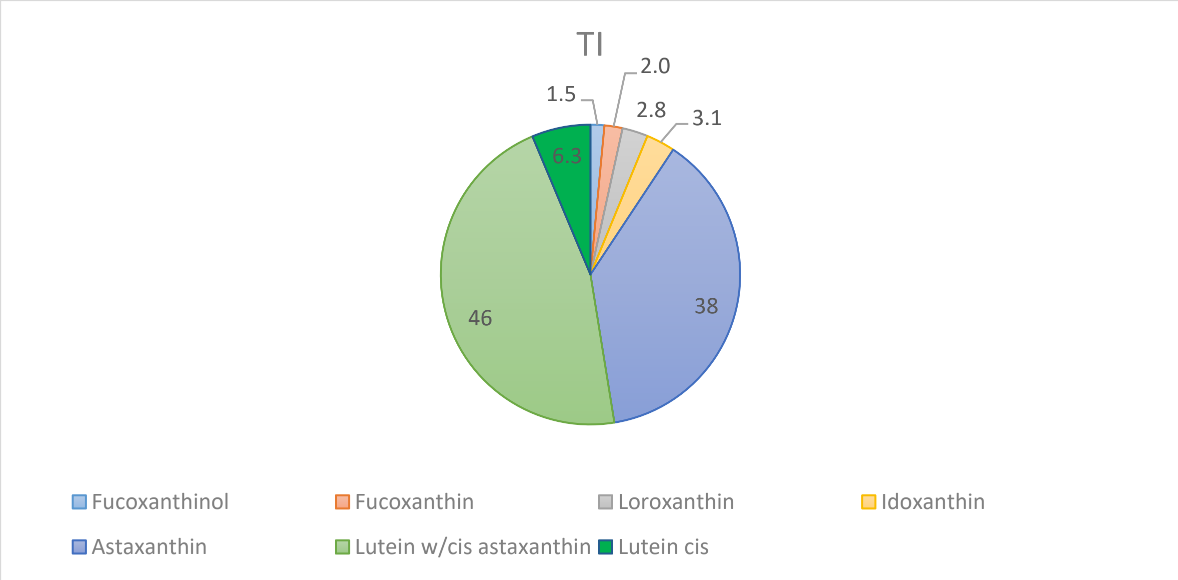

Figure 20: Overview of the carotenoid content in TI fed salmon muscle. Values are the percentage of single carotenoids in total carotenoid content.

3.3 Skin pigments

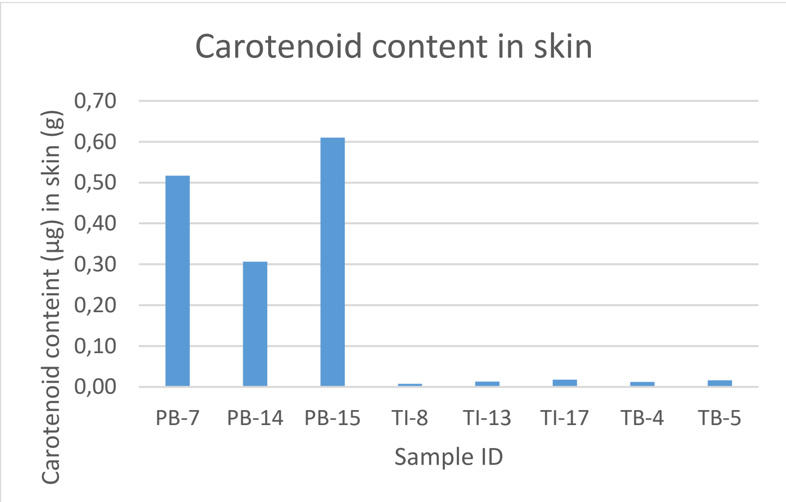

Figure 21: Carotenoid content in skin samples.

Of the few samples analyzed on the skin, close to no pigment was found (see figure 21). In the skin of fish fed TB feed, one peak appeared in both samples, at R_t 60.4-60.9 and indicated lutein. The amount found was 0.016 µg/g in TB-5 and 0.012 µg/g in TB-4. TI-8, TI-13 and TI-17 also showed minor amounts of pigments. TI-8 had 0.008 µg/g, TI-13 had 0.013 µg/g and TI-17 had 0.018 µg/g lutein.

The samples from PB showed two other pigments than did not appear in TB or TI, namely fucoxanthinol and fucoxanthin. In addition, the amounts of total carotenoid were significantly higher in PB than TB and TI. PB-7 contained 0.24 µg/g fucoxanthinol and 0.28 µg/g fucoxanthin, PB-14 contained 0.09 µg/g fucoxanthinol and 0.22 µg/g of fucoxanthin, while

PB-15 had 0.28 µg/g fucoxanthinol and 0.33 µg/g fucoxanthin. In addition, an unidentified chlorophyll derivate was found in all skin samples of PB, likely pheophorbide *a*. The amount of the chlorophyll was not quantified.

3.4 Kimberly's analysis

Eleven samples of salmon flesh and belonging skin from fish fed PI diets were analyzed by Kimberly Sara, a bachelor student in the spring of 2019. Four similar samples, but from control diets were analyzed as well. The salmons were a part of the same experiment as the rest of the salmon in this thesis, but analysis was performed almost one and a half year earlier than the other samples. Only samples of PI and RD was analyzed in 2019.

3.4.1 Kimberly's muscle analysis of fish fed PI diets

In the control fed fish, only astaxanthin and lutein were present in all four samples, while idoxanthin was present in three and diatoxanthin only in one sample. See table 3 below. PI contained almost 10 times as much pigments as the RD.

Table 3: Overview of muscle pigments from analysis performed in spring 2019 by Kimberly. Values presented in (µg/g)±sd

Pigments	RD	PI
Fucoxanthinol		0.79±0.39
Fucoxanthin		1.85±0.56
Idoxanthin	0.04±0.01	0.09±0.02
Astaxanthin	0.28±0.13	
Astaxanthin and diadinochrome		0.55±0.17
Diatoxanthin	0.02±0.00	0.18±0.06
Lutein	0.06±0.03	0.16±0.03
Total	**0.37±0.18**	**3.55±0.94**

Between 2019 and 2020, the total carotenoid content in the *Phaeodactylum* fed salmon did not show any significant difference, with p-value=0.15. The difference between RD salmon from 2019 and 2020 was significant, p-value = 0.019, and the highest content was found in the samples from 2020.

In 2020, the amounts of the major pigments astaxanthin (+diadinochrome), lutein (+ astaxanthin *cis*-isomer), fucoxanthin and fucoxanthinol were insignificantly different from the same group of fish muscle analyzed one and a half year earlier, p-value=0.53, p-value=0.49, p-value=0.47 and p-value=0.089, respectively. All pigments were present in all samples of 2019, except for idoxanthin which was only found in three of the eleven samples and diatoxanthin that was found in one.

3.4.2 Kimberly's skin analysis of fish fed PI diets

The eleven skin samples of PI analyzed by Kimberly in 2019 showed only the two carotenoids fucoxanthinol and fucoxanthin, and the amount was 0.80±0.35 µg/g and 1.82±0.83 µg/g respectively. The carotenoids are present in almost the exact same concentration in the skin as in the muscle. Five samples of RD skin were also analyzed, but only one sample showed any amounts of pigments. The pigments in that sample were identified as astaxanthin and lutein in the following respective amounts; 0.48 µg/g and 0.07 µg/g. A small comparison between skin samples from 2019 and 2020, gave a significant difference with p-value = 0.00014, where the amount was much higher in 2019 as in 2020.

The comparative amounts are presented in figure 22 below, with Kimberly's eleven PI samples to the left, and the three PB samples of this study to the right.

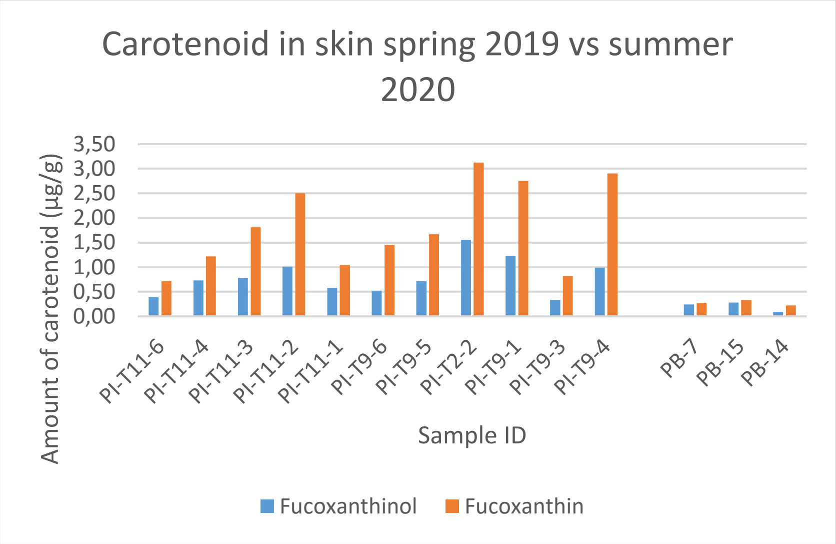

Figure 22: Kimberly's samples (PI) to the left, the present study with the three samples (PB) to the right.

In PI samples analyzed more than a year earlier, in spring 2019, the skin samples contained a lot more of the two carotenoids fucoxanthinol and fucoxanthin. The amount of fucoxanthinol and fucoxanthin in those samples were in average 0.80 µg/g and 1.82 µg/g, respectively. Compared to the skin samples analyzed autumn 2020 which contained an average of 0.20 µg/g fucoxanthinol and 0.27 µg/g fucoxanthin, the difference was more than four times as high for fucoxanthinol and more than six times as high for fucoxanthin in 2019 than the amount in 2020.

3.5 Fatty acid content

For total fatty acid content, the measures are given without a unit, due to lack of standard. 18 duplicate samples of each feed were analyzed. PB shows the highest content with a total area of 19573 in average and PI comes next with 18314. TB has an area of 18186 and TI has 16078. RD has the second lowest content of all samples with 18021 (see figure 23 below). The results show that broken cell walls give higher fatty acid content than when cell walls are intact, but the results are not significant, p-value = 0.39 for *Phaeodactylum* and 0.23 for *Tetraselmis*. None of the test diet fed salmon were significantly different from the control fish. The difference was slightly bigger between intact and broken cell walls in *Tetraselmis* fed salmon than in *Phaeodactylum* fed salmon.

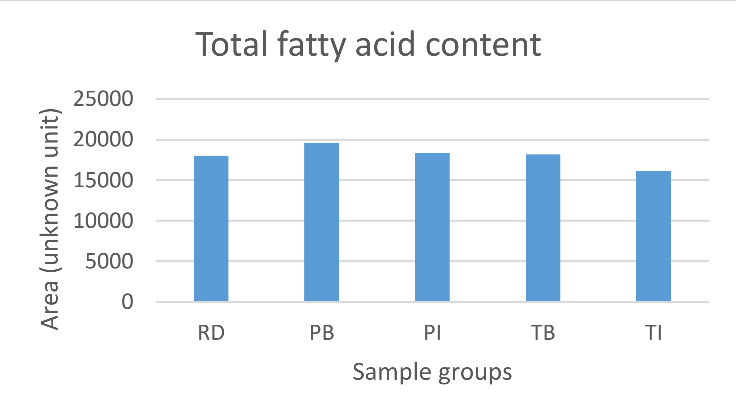

Figure 23: Total fatty acid content based on area from the CG

Table 4: Mean % of all fatty acids present in the different test groups, with std and SEM values in addition, given with a 95% confidence level.

Sample	***RD***			***PB***			***PI***			***TB***			***TI***		
	Mean	STD	SEM	Mean	STD	SEM	Mean	STD	SEM	Mean	STD	SEM	Mean	STD	SEM
C14:0	3.04	0.19	0.07	3.03	0.19	0.07	2.94	0.14	0.05	2.97	0.35	0.11	2.81	0.25	0.08
C16:0	13.44	0.45	0.15	13.53	0.58	0.20	13.43	0.54	0.18	13.93	1.29	0.42	13.74	0.64	0.22
C18:0	3.00	0.12	0.04	2.99	0.15	0.05	3.04	0.15	0.05	3.16	0.30	0.10	3.20	0.23	0.08
C22:0	4.85	0.37	0.13	4.15	0.33	0.11	4.12	0.32	0.11	4.13	0.39	0.13	3.95	0.41	0.14
SUM SAF	**24.33**	**0.80**	**0.27**	**23.69**	**0.81**	**0.28**	**23.52**	**0.80**	**0.27**	**24.17**	**2.04**	**0.67**	**23.70**	**0.93**	**0.32**
C16:1n-7	4.07	0.17	0.06	5.44	0.41	0.14	4.92	0.36	0.12	3.96	0.32	0.11	3.85	0.20	0.07
C18:1n-9	25.55	1.26	0.43	27.20	1.44	0.49	27.0	1.67	0.56	28.01	1.52	0.50	26.71	2.06	0.71
C18:1n-7	3.02	0.05	0.02	3.25	0.06	0.02	3.24	0.05	0.02	3.27	0.21	0.07	3.15	0.07	0.03
C20:1n-9	4.57	0.27	0.09	4.05	0.50	0.17	4.31	0.25	0.09	4.21	0.46	0.15	4.19	0.33	0.11
SUM MUFA	**37.21**	**1.36**	**0.46**	**39.93**	**1.41**	**0.48**	**39.42**	**1.66**	**0.56**	**37.98**	**6.21**	**2.04**	**37.14**	**4.93**	**1.69**
C18:2n-6	7.42	0.50	0.17	8.37	0.48	0.16	8.36	0.63	0.21	8.62	0.78	0.26	8.00	0.74	0.25
C18:3n-3	2.90	0.19	0.06	3.07	0.12	0.04	3.09	0.20	0.07	3.71	0.28	0.09	3.61	0.15	0.05
C18:4n-3	1.29	0.34	0.11	1.11	0.10	0.03	1.03	0.09	0.03	1.48	0.18	0.06	1.42	0.16	0.06
C20:4n-3	1.21	0.06	0.02	1.12	0.06	0.02	1.12	0.07	0.02	1.26	0.12	0.04	1.27	0.13	0.04
C20:5n-3	4.97	0.37	0.13	5.06	0.52	0.18	4.80	0.52	0.18	4.38	0.64	0.21	4.80	0.67	0.23
C22:5n-3	1.81	0.11	0.04	1.86	0.12	0.04	1.89	0.12	0.04	1.71	0.16	0.05	1.74	0.15	0.05
C22:6n-3	14.60	1.41	0.48	12.64	2.09	0.71	13.68	1.71	0.58	13.93	2.53	0.83	14.17	2.57	0.88
SUM PUFA	**34.19**	**1.29**	**0.44**	**33.16**	**2.14**	**0.73**	**33.97**	**1.67**	**0.56**	**34.20**	**4.44**	**1.46**	**35.02**	**2.62**	**0.90**
SUM n-3	26.77	1.49	0.50	24.79	2.32	0.78	25.61	2.05	0.69	26.04	3.56	1.17	27.02	3.10	1.07
EPA + DHA	**19.57**	**1.47**	**0.50**	**17.69**	**2.41**	**0.81**	**18.48**	**2.02**	**0.68**	**18.31**	**2.96**	**0.97**	**18.98**	**3.04**	**1.04**

Table 4 above present percentage values of the fatty acids present in each sample, in addition to std (standard deviation) and SEM (standard error mean) value given with a 95% confidence interval. Statistical analysis of the lipids show that there are significant differences only between some fatty acids in some samples. For instance that the control fish show the lowest percentage of n-6 fatty acids, and that all test groups show a significant difference in C20:1n-9 from the control fish, where all values are lower than the control fish.

The table shows that the control feed contained the highest percentage of SFA, significantly similar only to TB, p-value = 0.66. RD and TI show similar percentage of MUFA, around 37%, while TB is 1% higher, and PB and PI show almost 40%. The percentage of PUFA is highest in TB, 34%, and lowest in PB, 33%, but neither are significantly different from the control group. There were a few fatty acids that all groups showed a significant difference from the control group, RD. RD showed a significantly higher percentage of C20:1n-9 and C22:0, while RD had significantly lower percentages than all test groups in C18:1n-7, C18:2n-6 and C18:3n-3 compared to all test diets. TB and TI contained a higher percentage, 3.71 and 3.61, of C18:3n-3 than any other the other groups.

The control fish showed the highest percentage of EPA+DHA, but PB is the only test group that is significantly lower, p-value = 0.0002. Only PB showed higher percentage of EPA than the control, but the difference is insignificant. TB had a significantly lower percentage of EPA than RD, p-value = 8.0×10^{-6}. For DHA, the control feed was still the highest, significantly different from PB and PI, p-value = 1.7×10^{-5} and 0.016, respectively.

The dominating fatty acid in all sample groups was oleic acid (C18:1n-9). However, all test groups showed a significant higher percentage of C18:1n-9, when compared to RD, P-value = 0.0064 for TI, p-value = 2.0×10^{-10} in TB, p-value = 2.1×10^{-6} PB and p-value = 1.4×10^{-4} in PI. For cetoleic acid (C22:1n-11), it was the opposite, and the fatty acid appeared in a much higher percentage in RD than in the four test diets.

In total, the fish fed intact cell walls show a higher percentage of omega-3 fatty acids, but the difference does not seem to be significant, p-value = 0.211 for *Tetraselmis* and p-value = 0.115 for *Phaeodactylum*. There are some differences when comparing intact cell walls with broken cell walls, for instance for EPA, which is significantly higher in TI than in TB, p-value = 0.007, and higher in PB than in PI, p-value = 0.040. Only PB and PI showed a significant difference between DHA, with PI having the highest percentage, and a p-value = 0.023.

4 Discussion

4.1 Pigments

Figure 12 in result part showed differences in amounts of carotenoids between Vis and HPLC. Vis is likely to be less accurate, as only one d-value is used, 206 for astaxanthin in RD, TB and TI, and 166 for fucoxanthin in PB and PI, which gives an incorrect amount as the sample is a mixture of all carotenoids present. PI showed a higher amount of total carotenoid in HPLC than in Vis, which may be because fucoxanthin and fucoxanthinol are the major pigments, and fucoxanthinol has an even lower d-value (144), which would give a higher amount of total content.

Multiple standards have been checked to find out which pigments existed in the fish in this experiment. Some standards were confirmed, while others could not be present in the salmon flesh. The correct standards were found based on their retention time, specter and probability of finding the pigment in either salmon, the algae or in the normal feed. In Appendix B, different chromatograms of samples and spectra of carotenoids can be seen, and these were used to identify the carotenoids.

Loroxanthin was in 2009 claimed to be reported for the first time in six different *Tetraslemis* species, including *Tetraselmis chuii.* (Garrido et al., 2009). However, as the standard of loroxanthin was not available, it could not be tested, and thus it cannot be proven that the pigment was present in the salmon flesh. Loroxanthin is a carotenoid similar to lutein, but has three OH-groups attached, one at each end and one attached to the central polyene chain. The retention time seem to be appropriate in what could be expected by loroxanthin, based on its characteristics. Loroxanthin has been reported in several green algae with same pigmentation as *Tetraselmis*.

Even though neoxanthin and violaxanthin are common carotenoids in green algae, also in *Tetraselmis*, none of these were discovered in any of the samples. Maybe it is due to the uptake regulation of the salmon, that the salmon does not want these carotenoids, but it is no evidence on this. The epoxy carotenoids are also highly unstable and may have been degraded in the diets, or in the intestine of the salmon. *Tetraselmis* contains acids which degrade epoxide carotenoids and must be analyzed immediately after extraction. (Mulders et al., 2014)

Lutein and zeaxanthin are pigments naturally present in corn gluten. In the trend of giving vegetable feed to fish, white fleshed fish is turning slightly yellow, and the acceptance on the marked is reduced. Bleaching of corn gluten meal with soy flour remove carotenoids from corn gluten. (Francis et al., 2001) The bleaching, with the use of soy flour as a lipoxygenase source, removed more than half of the carotenoid content in corn gluten meal (Park et al., 1997). Maybe this could be an option for fish fed fucoxanthin and fucoxanthinol, in order to reduce the amounts of the yellow carotenoids. It might be possible to remove the yellow carotenoids from the feed, and then add astaxanthin as normal, to get the dark orange coloration that customers are used to.

The supplementation of lutein did not influence the astaxanthin pigmentation in the flesh of the Atlantic salmon according to Olsen and Baker (2006). In the experiment three feeds were formulated and contained 51.9, 54.9 and 54.5 mg astaxanthin per kg feed, and 0.7, 11.8 and 23.3 mg lutein per kg feed respectively. The content of retained astaxanthin after 58 and 138 days showed significantly no difference. Based on this experiment, it can be reasonable that TB and TI did not show any big color differences during visual inspection, even though lutein was present in a relatively high amount, 50.1% and 52.5%, respectively, of total carotenoid content.

Chlorophylls were found in several samples, which was not expected as the fish did not show any visible signs of green coloration. The amounts were relatively low, <1% of total area under peaks. Tests of a few standards of chlorophyll were performed, but as none of them were identical, it was believed to be other chlorophylls or breakdown products of chlorophyll. In *Tetraselmis* fed salmon the peaks appeared late in the specter, around R_t 73-81 and is likely to be chlorophyll *b* degraded products.

In most samples of *Phaedactylum* fed fish and some samples *Tetraelmis* fed fish, one chlorophyll derivative compound was found around R_t 47-48, between fucoxanthin and idoxanthin in the chromatograms. As it is known that both microalgae contain chlorophyll *a*, it is likely that the peak present in the chromatogram is a derivative from the chlorophyll *a*. Chlorophyll *a* is non-polar and comes late in the chromatogram, but as chlorophyll *a* loose its “tail” (see figure 24 and 25 below), and add an OH group instead, it becomes smaller and more polar and appears earlier in the chromatogram. Based on this information and the specter of the chlorophyll it is likely that the peak is pheophorbide *a*. (Roy et al., 2011)

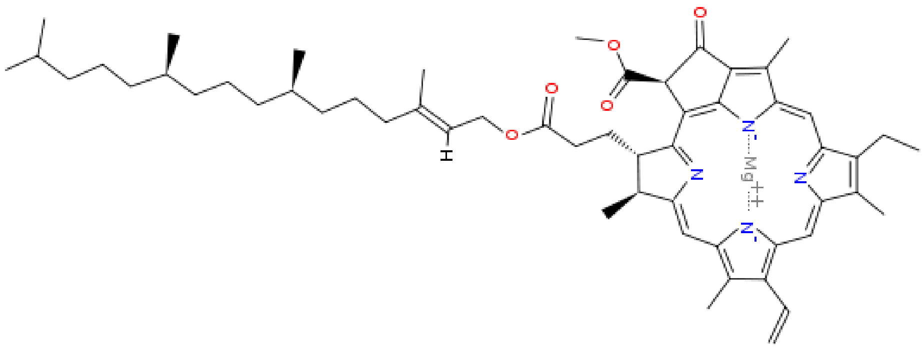

Figure 24: Chlorophyll a. Figure from: https://en.wikipedia.org/wiki/Chlorophyll_a

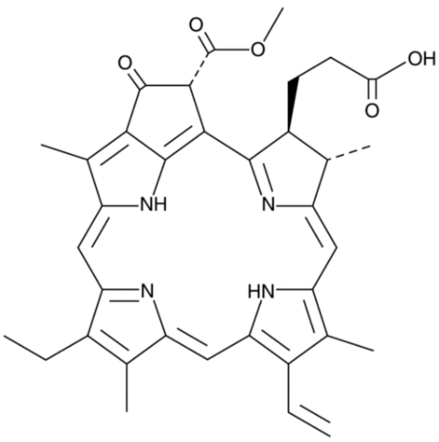

Figure 25: Pheophorbide a. Figure from: https://www.caymanchem.com/product/16072/pheophorbide-a

Even though Rebolloso-Fuentes et al. (2001) stated that total pigment in *Phaeodactylum* was mainly chlorophyll, this was not observed in the salmon. It may be that the chlorophylls are not as easily absorbed in the salmon, and also that the chlorophylls degrade faster, and thus were not found in nearly as high amounts as the carotenoids. Chlorophyll is not common to find in fish, but degraded chlorophylls have also been found in stickleback gonads. (Nordeide et al., 2006) In deep sea dragon fish (*Malaxosteus niger*) chlorophyll is used for vision and chlorophyll a derivatives were found in the retina suspensions from the dragon fish. (Douglas et al., 1998)

Lutein was found in all samples in all groups, mainly together with *cis* astaxanthin, most likely. Lutein *cis*-isomers were also found, and were easily identified as the standard of lutein that was tested also contained three minor peaks after the main lutein peak. These three were named lutein cis1, lutein cis2 and lutein cis3, but were later grouped together in *cis* lutein. Not all three lutein isomers were present in all samples, but usually at least one of the *cis*-isomers were present. In specter, *cis*-isomers are similar to the *trans*, but with a slight elevation to the left in the specter (see Appendix B).

Astaxanthin was easily detected because of its characteristic broad, one-peak specter. The broad specter comes from the ketone structure, and astaxanthin has one ketone in each end of its structure (see figure 5, in **1.8**). Idoxanthin, one of astaxanthins derivatives show a similar specter, but one of the ketone groups are converted to OH, giving a less broad specter and increasing the polarity, so that idoxanthin appear before astaxanthin in the chromatogram.

Nofima had an experiment earlier and reported that omega-3 content in the feed determines the quantity of the color uptake in salmon (Ytrestøyl & Krasnov, 2016). They also reported that stress play a role in interaction between color and diet. They assumed salmon fed low levels of marine omega-3 (0% and 1%, compared to 2.2% in commercial diets) while exposed to stress metabolize larger amounts of astaxanthin, giving the muscle a less intense color. Growth was not significantly impacted, but survival rate was lower in fish fed low levels of omega 3. No stress was measured in this experiment, but based on the results in this thesis it is still believed that when more lipids were absorbed, more carotenoids were also absorbed.

Analysis of correlation between the amount of astaxanthin and idoxanthin in all samples. Zero values were removed, thus the result is only valid in cases where idoxanthin was present in samples. In the scatterplot of astaxanthin and idoxanthin (see figure 15 in **3.2**), it appears that more astaxanthin is correlated with more idoxanthin. This makes sense because if there is more astaxanthin present in samples, more of it is able to transform into idoxanthin, as one of the ketone groups are being reduced to alcohol. The calculated regression line $y=0.39+1.70x$, where x is the amount of idoxanthin and y is the corresponding amount of astaxanthin, indicate that when the amount of idoxanthin increases by one unit, the amount of astaxanthin increases by 1.70. The H0 hypothesis of no correlation is rejected with a p-value 0.005, and the corresponding correlation coefficient, $r=0.47$ indicate a relatively strong correlation. The casual relationship between the two pigments cannot be investigated in this experiment because stress was not a part of the experiment, thus impossible to see if astaxanthin is converted to idoxanthin when salmon was exposed to stress or other unideal situations. The idoxanthin content is however higher in small fish, indicating that the metabolic capacity, or need, to transform absorbed astaxanthin is decreased with the age of salmon fish. (Zoric, 2017)

4.2 Tetraselmis

A study was performed with incorporating *Tetraselmis* in feed for juvenile sea bass (*Dicentrarchus labrax*), and the skin pigmentation changed, and showed a significant increase of greenness on the skin. As the study was only analyzing lipid content, it was not discovered the source of the coloration, weather it was from chlorophyll or epoxy carotenoids ions which appear blue, and combined with yellow pigments, like lutein or other carotenoids, it may appear green. Even though the fish turned greener, the growth and other major quality factors, like EPA content and essential amino acids was not compressed with up to 20% *Tetraselmis* in the feed, and it was concluded that the microalgae could be used in fish feed, and replacing up to 20% of the protein. (Tulli et al., 2012) *Tetraselmis chuii* has also been added as an ingredient in bread, and color change was observed very much, even with only 1% *Tetraselmis*. At only 4% incorporation, the bread was as green as the algae itself. (Nunes et al., 2020) In the present study, the *Tetraselmis* fed salmon did not show a visible color change, and essential fatty acids were not negatively affected based on results from the GC.

In other studies, the use of *Tetraselmis* (*T. lutea* and *T. suecica*), has shown to affect the coloration of European sea bass skin to appear greenish when fed inclusions of 10, 20 and 30% microalgae in the diet. Growth of the fish was not affected in the trial and neither was the feed conversion ratio. However, a slight decrease in digestibily to dry matter and protein was observed, but this was compensated by increased feeding intake. The study concluded that microalgae are a good and sustainable source for the future, when availability becomes higher due to large-scale production and the price goes down. (Cardinaletti et al., 2018)

The appearance of fucoxanthinol and fucoxanthin in *Tetraselmis* fed salmon was not expected, and is likely to be due to contamination from muscle of *Phaeodactylum* fed salmon. As samples were prepared and analyzed randomly, instead of one group at a time, it may have led to some contamination during preparation of samples. The same goes for the control salmon, which also showed small amounts in a few samples. Diatoxanthin appeared in some samples of the control group, which was most likely a contamination from *Phaeodactylum* fed salmon. Another option is that some fish were fed wrong pellets, or that samples were labeled with the wrong group. However, as the values were so different from *Tetraselmis* and control fed salmon to *Phaeocatylum* fed salmon to, only about 1-2%, it is most likely because of contamination.

The fact that there was almost no difference between pigment amounts in TB and TI, may be due to the thin cell wall of the *Tetraselmis*, indicating that salmon’s digestive enzymes are able to get through to the cell wall more easily than for other microalgae with thicker cell walls, like *Phaeodactylum* for instance.

4.3 Phaeodactylum

The orange carotenoid fucoxanthin has been shown to change the coloration, in relation to the control feed, of the seabream (*Sparus aurata*) flesh when *P. tricornutum* was incorporated into the diet, even at only 2.5%. The visual coloration difference was observed as lighter and more yellow in the operculum and the ventral skin of the seabream. The experiment concluded that *Phaeodactylum* could be used as a functional ingredient which improves the pigmentation. (Ribeiro et al., 2017)

Another study was performed in regards to the health effects of fucoxanthin for humans. The study shows that fucoxanthin and fucoxanthinol may have anti-cancer and anti-diabetic effects, as well as its antioxidant properties. However, the study was worked out on mice, instead of humans, but due to similar gene material, it is likely that fucoxanthin and its derivative fucoxanthinol can be used for human health later, but more research is still needed in the field. (Mikami & Hosokawa, 2013)

During the analysis of the data, the specter of astaxanthin appeared different in PI than in any of the other groups. It was checked in the computer belonging to the HPLC and it was discovered that another pigment was present in the same peak as astaxanthin. Diatoxanthin and diadinoxanthin was tested, but none of them fit. Diadinochrome, similar to the two other pigments was suspected to be in the mixture, but as the standard was not available for testing, the identification could not be proved.

The epoxidic diadinoxanthin is rapidly rearranged to the furanoxide diadinochrome under acidic conditions (see figure 7, (V) and (VI) respectively, in Appendix A) (Repeta & Gagosian, 1982). Diadinoxanthin was not found in any samples of *Phaeodactylum* fed salmon, but diadinochrome was found in PI. It is possible that diadinochrome appeared in PI only because PI was not processed as much as PB. It can also be that the carotenoid was more degraded in PB and was thus not found. Diatoxanthin was found in all samples of PI and PB.

Even though all test groups had more total carotenoid than the control group, there was no difference in astaxanthin content, indicating that the test groups absorbed the same amount of astaxanthin, but also absorbed other carotenoids in addition. Astaxanthin was not added in the feed, and thus it is likely that the astaxanthin content of the salmon came from the feed fed prior to the experiment. Another possibility is that the fish meal contained some natural astaxanthin obtained from the sources of fish meal. This indicate however, that the amount of astaxanthin was very low compared to salmon. Maybe the coloration of fucoxanthin and fucoxanthinol would be camouflaged, if astaxanthin was added in the feed in commercial amounts.

4.4 Comparison with Kimberly Sara's analysis

Kimberly Sara performed pigment analysis using HPLC in the spring 2019, on fish from the same experiment as in this thesis. As the lab work for this thesis was performed in the summer/autumn of 2020, the samples had been in the freezer for over a year after the experiment was done. When these samples were analyzed in the summer of 2020, none of the fish appeared yellow, neither in the skin nor in the muscle. Thus, it was interesting to compare the results of the lab work done for this thesis with the lab work that another student did back in spring 2019, when the coloration of the salmon was clearly affected. The peaks in the chromatogram are slightly shifted to the left in the samples analyzed summer 2020. This is most likely due to the use of different columns in the HPLC. The carotenoids were still identified as the same in 2019 and 2020 due to the positioning of the carotenoid in relation to the other carotenoids.

Calibration of the HPLC was not performed by Kimberly in 2019, but area under peaks were given for all peaks, and weight of fish analyzed was noted. This allowed for calculating amounts of carotenoid from 2019 and 2020 based on the calibration of standards performed in autumn 2020.

4.4.1 Kimberly's muscle analysis

When looking at the fish fed diets containing *Phaeodactylum* and comparing them to Kimberly's result, it seemed that the main carotenoids are the same, in almost the same amounts in the muscle, after more than a year in the freezer. In most samples; fucoxanthinol, fucoxanthin, a little idoxanthin, astaxanthin in a mixture with diadinochrome, diatoxantin and lutein was found. The three *cis*-isomers for lutein also seemed to be present in samples from both 2019 and 2020. In the present study, there were also a lot of astaxanthinlike degraded peaks around R_t 80-85, but their wavelength is 450. These were not observed in the analysis from 2019. The total carotenoid content was slightly higher in 2020 than in 2019, not significantly with p-value = 0.15, but this may be due to different people handling the analysis, or the fact the salmon handled were different individuals.

4.5 Skin analysis

The skin samples in this trial contained little carotenoids, as low as 0.014 µg/g in TB and 0.013 µg/g in TI. Due to these low values, it was considered reasonable to not analyze more samples after the first run. In PB, the amount was higher, 0.48 µg/g in PB, but still less than 15 % of the corresponding muscle sample, leading to the same conclusion to not analyze more samples. Skin for analysis was provided only for TI, TB and PB samples, thus it was not possible to compare with the control skin. Even though no visual color differences were observed between the sample groups, PB showed completely different pigments in the skin during HPLC analysis compared to TI and TB. Fucoxanthinol and fucoxanthin were the only pigments found in the skin of PB, the same as in the muscle, but the muscle contained other pigments in addition to the fucoxanthinol and fucoxanthin.

Comparing the skin carotenoid content from 2019 with the data achieved in this study, showed a high decrease in carotenoid levels over time. From 0.80 µg/g and 1.82 µg/g of fucoxanthinol and fucoxanthin in PI, to 0.20 µg/g and 0.27 µg/g in PB, respectively, the p-value was 0.0001 and 0.0005, indicating a clear decrease in carotenoid amounts of fish fed *Pheaodactylum* diets. It must be remembered that the fish were not fed the same diets, but different versions of *Phaeodactylum* diets, broken and intact cell walls. This decrease of pigmentation was not found in the muscle of the respective salmon, PI, where the p-value was 0.15 when checking PI in muscle for both 2019 and 2020. Salmon analyzed in 2020 showed slightly higher values than what was observed in 2019.

Kimberly analyzed skin coloration in RD and PI in 2019. Comparing the PB skin samples from this study, with Kimberly's PI skin analysis from 2019, there are some clear differences in the amount of pigments of the skin. Keep in mind that these are different test groups, and the *Phaeodactylum* algae is processed differently in the feed, but with such a big difference in amount, the broken cell walls is not likely to be the cause of the degradation, and it is likely that the same result would be seen when using the same test group both times. In addition, the number of samples are not the same, and sample numbers are low.

4.6 Pigment degradation

Even though some studies indicate pigment degradations in salmonid muscle during storage in freezer, it was not observed in the present study. Only pigments present in the salmon skin was shown to decrease in amount from spring 2019 to autumn 2020. The muscle analyzed in 2019 and 2020, appeared to have similar amounts of carotenoid, while the skin coloration was visibly and chemically less colored after one and a half year longer storage time in the freezer.

One interesting part about the samples analyzed in 2019, is that the carotenoid concentration of the muscle and the skin was the same. The total carotenoid content of the PI muscle analyzed in 2019 appeared to have 0.79 (±0.39) µg/g fucoxanthinol and 1.85 (±0.56) µg/g fucoxanthin, almost identical values as for the pigmentation in the PI skin from the same samples, containing 0.80 (±0.35) µg/g fucoxanthinol and 1.82 (±0.83) µg/g fucoxanthin.

Carotenoids can affect the coloration of the salmon flesh, and the color fades faster in freezer (-20°C) than in fridge (1-2°C), with 47% and 90% retained carotenoids after 14 days. Disruption of muscle tissue before freezing may also lead to carotenoid oxidation. In ground crayfish, more than 65% of the astaxanthin was lost in three months at -20°C, while whole crayfish lost only 30%, indicating that both chemical and enzymatic pathways for lipid oxidation may contribute to oxidation of carotenoids. Lipogynases or myeloperoxidases may be factors contributing to carotenoid decoloration. (Erickson, 1997)

4.7 HPLC calibration

When deciding how to calibrate for HPLC, two methods were considered. Either solutions with different concentrations had to be premade in the lab, or one solution with known concentration was inserted into the HPLC at different volumes, 1 µl, 5 µl, 10 µl, 25 µl and 50 µl. There were different advantages of each, and the conclusion of choosing to make one solution and insert different amounts was based on human errors vs computer errors. When the machine is set to insert a specific amount, it is expected to be accurate. Five solutions prepared by a student is more likely to be inaccurate.

When analyzing samples on the HPLC, external standard was used. This is done by making a known calibration curve for each standard wanted to use. The samples contain unknown data, but when it is combined with the known data from the calibration curve it is possible to make a quantitative report. The external method was chosen because it was simple, and gave results suitable for its purpose.

4.8 Fatty acid discussion

The reason for not reaching 100% fatty acids in each group is because there were some small peaks in the chromatogram that could not be explained or identified. These peaks were removed, but the values of fatty acids were not changed according to this, as it is not possible to know what the peaks were, and if they were a part of the sample or just residues. The identification of fatty acids was done automatically by the GC computer, but modifications were done when peaks were wrongly identified due to very similar retention times. Some identification may still be incorrect, but this is discussed further down.

All test groups were compared to the control group. Differences between broken and intact cells were also compared for the most important fatty acids. There was found no differences between broken and intact cell walls, in both *Phaeodactylum* and *Tetraselmis* diets when comparing the groups saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). However, there were differences between intact and broken cell walls in some important fatty acids, for instance for EPA which was higher in PI than in PB and higher in TI than TB. This result is opposite as of what is expected because broken cell walls should lead to higher absorptions of nutrients. The data achieved is however just in percentage, and it is therefore impossible to find out if the values are higher or lower in total lipid content. When the cell walls are broken, all nutrients should be absorbed in a higher amount, not only the most essential nutrients.

More than 2.7% of the fatty acid profile should be EPA and DHA. (Rosenlund et al., 2016) The content of EPA+DHA in the different groups tested were between 17 and 20%, which is much more than required. The reason for this is could be that the control feed used in this experiment, was mainly based on FM and FO as the main sources of protein and lipids, while commercial production uses more vegetable sources. This may also be the reason for having a low percentage of n-6 fatty acids, as these mainly come from vegetable sources.

Gadoleic acid (C20:1n-11), together with cetolei acid (C22:1n-11), is the major long-chain polyunsaturated fatty acid (LCMUFA) in marine sources, but none of these were identified in the samples. It is possible that the peak of C22:0 is wrongly identified and should be C22:1n-11 instead. This makes sense based on literature from for instance (Yang et al., 2016) and (Thomassen & Røsjø, 1989) and the fact that C22:1n-11 is actually a major marine fatty acid. The amount of the fatty acid, named C22:0 is much higher than what would be expected (Thomassen & Røsjø, 1989), while it would be a decent value for C22:1n-11 (Thomassen & Røsjø, 1989). See figures and tables in Appendix B. The amount of this fatty acid was significantly higher in RD than in all other groups, indicating that the source of the fatty acid is the marine fish oil, and does not come from the microalgae.

Both PI and PB show significantly higher percentages of MUFAs than the three other groups, p-value = 4.2×10^{-8} for PI and p-value = 4.1×10^{-12} for PB. C18:1n-9, oleic acid was the fatty acid that was present in the highest amount in all groups studied. The FA is naturally occurring in both animal and vegetable fats and oils. As the peak of C18:1n-9 is highest also in the control group, it seems that C18:1n-9 comes mainly from the marine fish meal and fish oil, but also from the microalgae as the amount in the test groups were higher there than in the control group. Another possibility, as the values are only given in percentage of total fatty acid content in the salmon, is that the microalgae contained less of other fatty acids, and thus the percentage would raise, even though the amount is the same. As total fatty acid is 100%, when one fatty acid increases, another has to decrease.

The amount of lipid could not be calculated as a standard was not used, regrettably. The total amount of fatty acid presented in figure 23 in the result part is therefore not proper results, but an indication that broken cell walls actually allows higher absorption of nutrients. The values were collected from the GC, that gave total volume of the areas in a sample. This includes also residues, which was not removed from the calculation. It may affect the results, but the residues seemed to appear in equal amounts throughout the sample groups. This is not the optimal way to get correct measures of the fatty acids, but when a standard was not used, this seemed to be one of the better solutions. The result can only be used to compare total fatty acids between each other, and nothing else. No unit was given, other than that it presents the area of the total peaks in a sample.

4.9 Improvements

If the experiment were to be performed another time, there are several things that should be done differently, which is likely to give better results. Mainly, samples should be extracted and analyzed earlier, to make sure there is less color or lipid degradation during storage time in the freezer. It would also be preferable to do one sample group at a time, to avoid any possible contamination, when analyzing pigments. The HPLC system should also have been changed, so that lutein and *cis*-astaxanthin also could be separated. For fatty acid analysis, an internal standard should have been used, so that amounts of fatty acids could be achieved, and not just percentages in relation to total fatty acid content of samples. It would also be more valuable to add astaxanthin in the feed, and see how astaxanthin and fucoxanthin combined would have an impact on the coloration.

4.10 Suggestions for future studies

For further studies, it could be interesting to look at salmon fed the *Phaeodactylum* feed, but with added astaxanthin in addition, to see if it would mask the color from fucoxanthin and fucoxanthinol. Another thing that could be interesting to test, would be to add soy flour in the feed, and see if that reduces the coloration of fucoxanthin in *Phaeodactylum* in the same way it removed lutein from corn flour (Francis et al., 2001). If astaxanthin is added after this bleaching, the coloration of the salmon may appear as normal salmon color. In addition, a stress experiment could be used to check if salmon becomes paler, and if this is due to the transformation of astaxanthin to idoxanthin.

5.0 Conclusion

Based on information from previous studies and the results obtained in this study, it seems that it is possible to use these microalgae in salmon feed to some extent, without compromising the fatty acid content of the salmon. The pigmentation in *Tetraselmis* fed salmon was not visually affected by the content of carotenoids in the microalgae, and the most important fatty acids were still present in sufficient percentage, not deviating from the control. *Tetraselmis Chuii* seem to be a good ingredient in salmon feed in regards to maintaining a good fatty acid profile, and not providing visual or nutritional disadvantages.

Salmon was visibly impacted by the fucoxanthinol and fucoxanthin present in the microalgae *Phaeodactylum* and appeared yellow on the ventral skin and in the muscle. The color of the muscle was much stronger in fish fed *Phaeodactylum* than in the control. The fatty acid content was however affected by the microalgae, and total omega-3 content was significantly lower than in the control fish. Based on this study and the analysis performed, it may seem like 30% inclusion of the microalgae *Phaeodactylum* in salmon feed is too much.

Cell wall disruption gave higher amount of total carotenoid, fucoxanthinol and fucoxanthin in salmon fed *Phaeodactylum*, but astaxanthin and lutein was not observed to be different between the two groups. In *Tetraselmis*, no significant differences in pigmentation were found between intact or broken cell walls of the microalgae in the salmon, which could be due to the thin cell wall of the microalgae. TI contained slightly more percentage of omega-3 than TB. Skin carotenoids were seen to degrade in freezer over time.

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Appendix A – Pigment structures

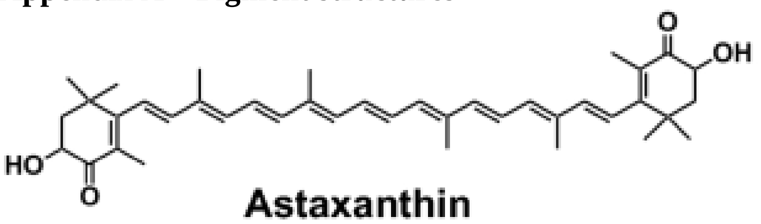


Figure 1: Astaxanthin. Picture from: https://link.springer.com/article/10.1007/s11418-019-01364-x

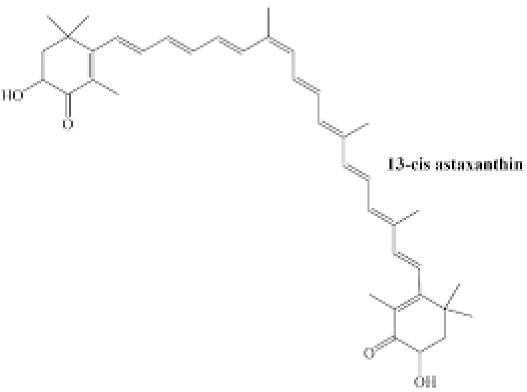


Figure 2: Cis-astaxanthin. Picture from: https://www.researchgate.net/publication/343629365_Oxidative_Stress_and_Marine_Carotenoids_Application_by_Using_Nanoformulations/figures?lo=1&utm_source=google&utm_medium=organic

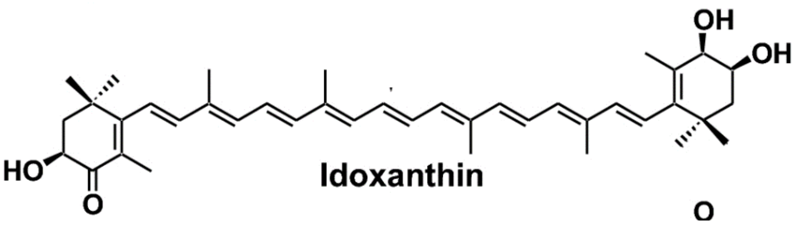


Figure 3: Idoxanthin. Retrieved from: https://link.springer.com/article/10.1007/s11418-019-01364-x

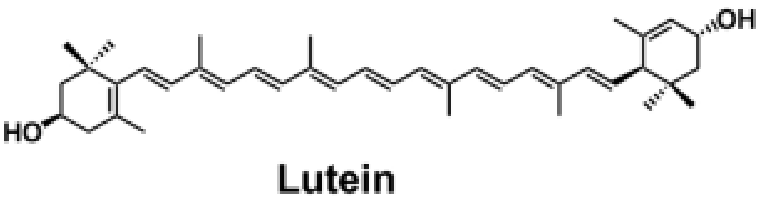


Figure 4: Lutein. Picture from: https://link.springer.com/article/10.1007/s11418-019-01364-x

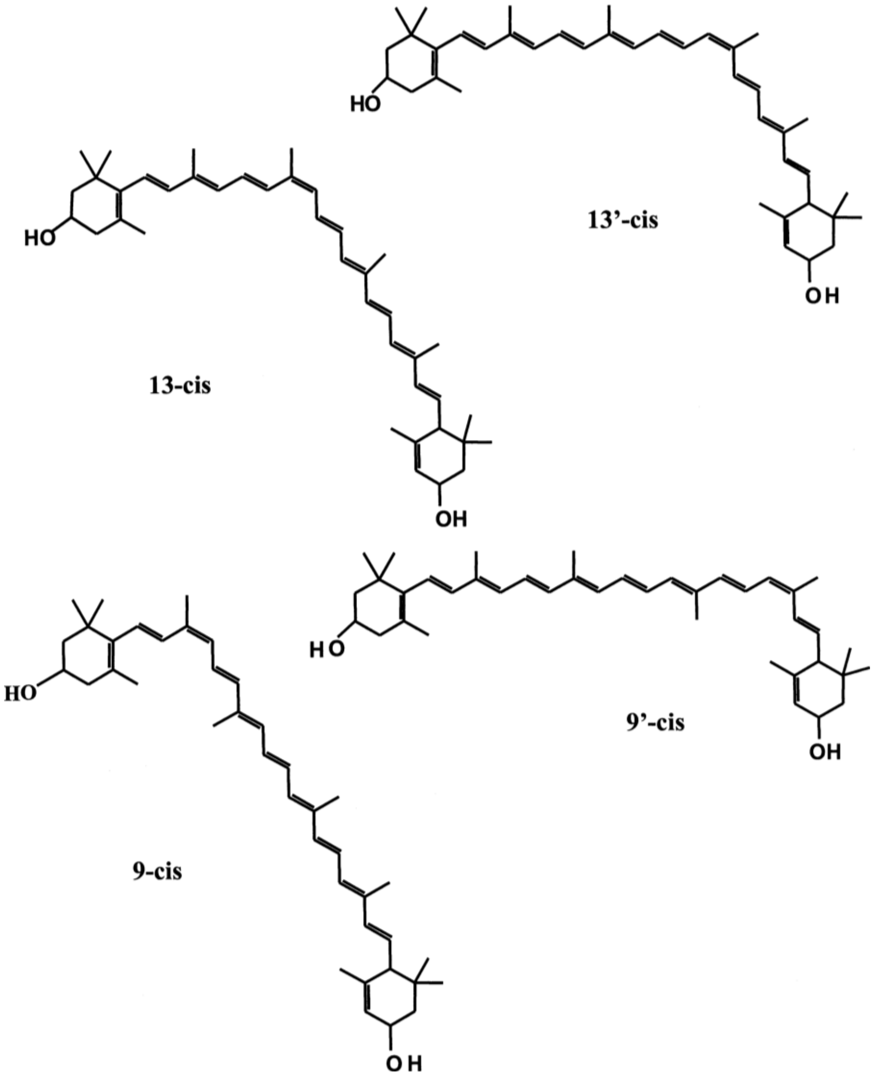


Figure 5: An overview of trans-lutein and the four main cis-isomers. Picture from: https://www.tandfonline.com/doi/full/10.1080/10408690590957034?casa_token=mD2TyVnqPMYAAAAA%3A5-V9UdEh2V9F0mDbXKEk6RRvQHMOMEwZJLZnM0c-78QSPViRMSrJ9kk_yjjQojAR2eNtXC8Bicam

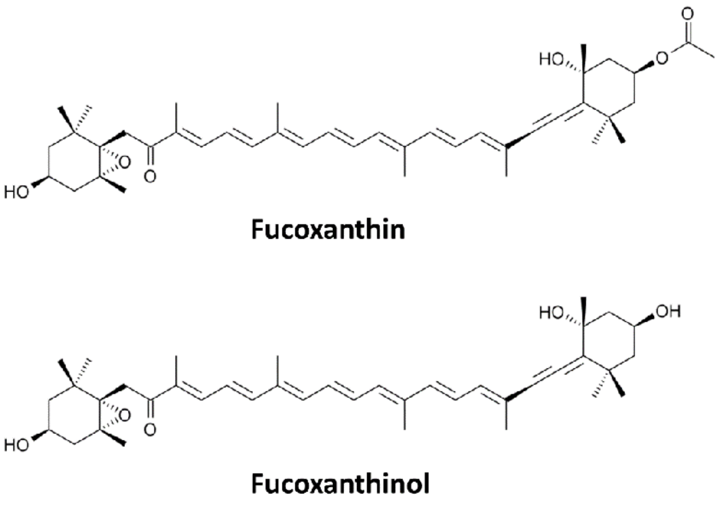
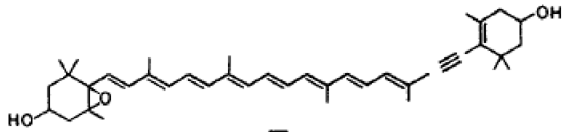
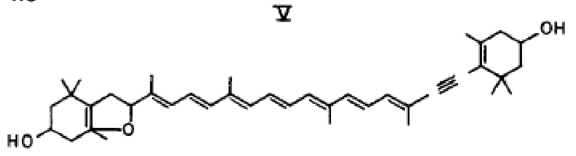


Figure 6: Fucoxanthin (top) and fucoxanthinol (bottom) from (Martin, 2015)



V



VI

Figure 7: Diadinoxanthin (V) and diadinochrome (VI) (Repeta & Gagosian, 1982)

Appendix B – Chromatograms and spectra

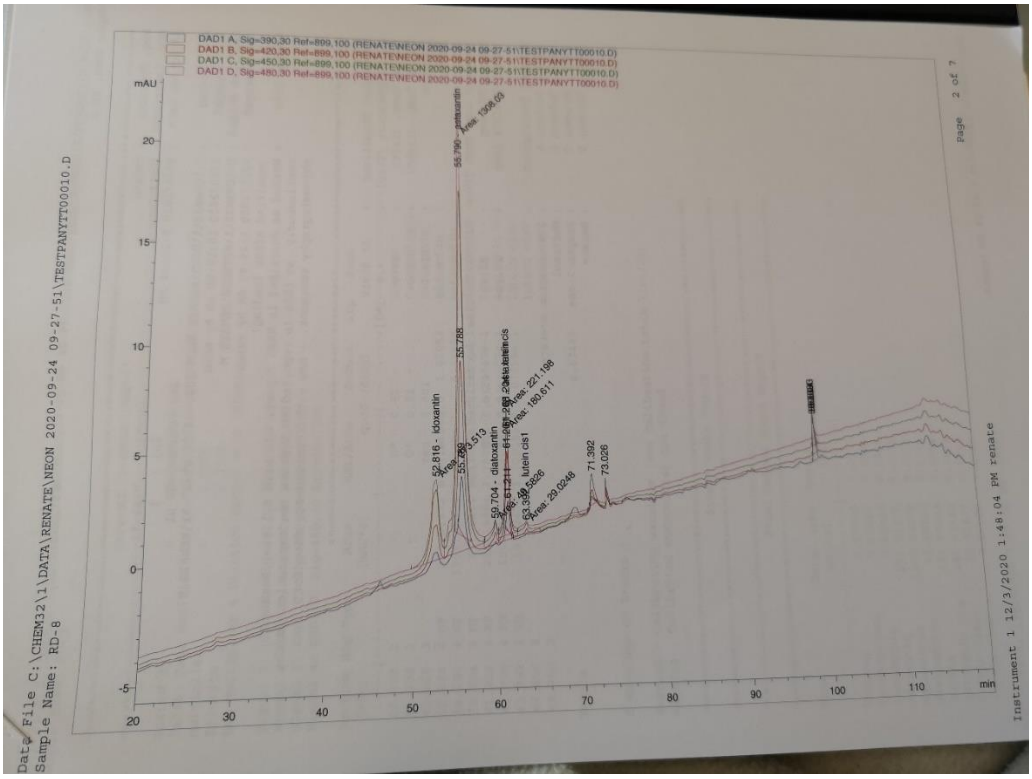


Figure 1: Chromatogram of RD-8. A control sample of muscle analyzed in 2020.

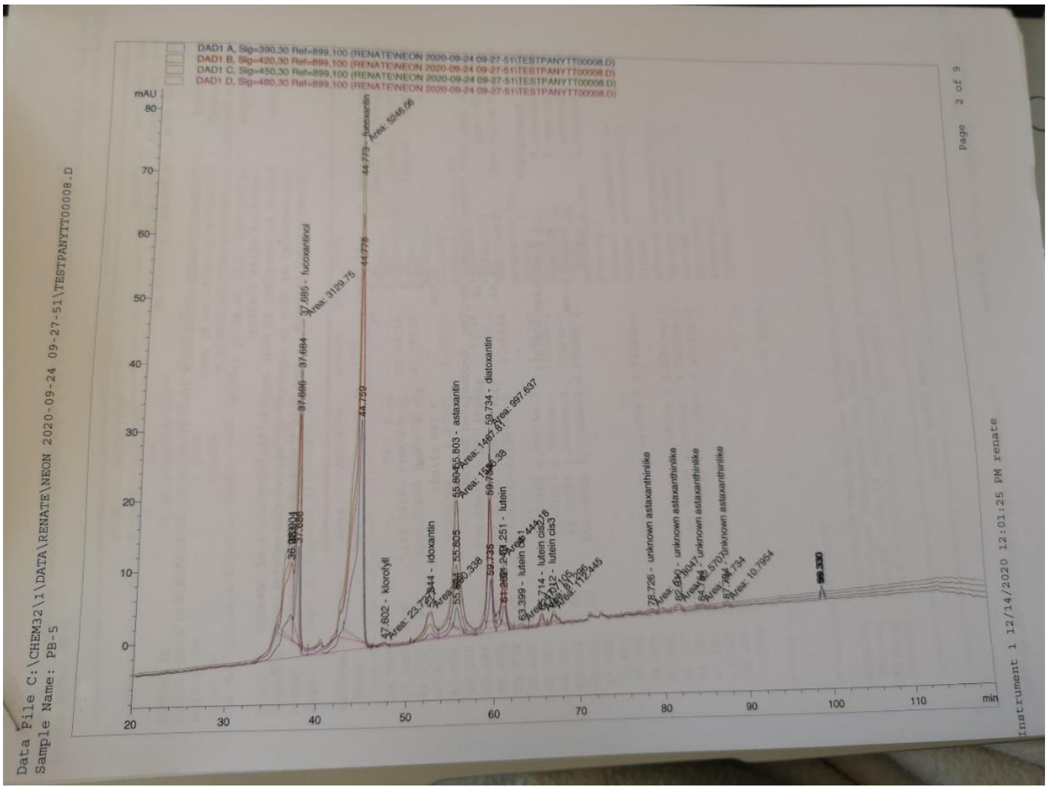


Figure 2: Chromatogram of PB-5. A muscle sample of salmon fed Phaeodactylum broken feed. Analyzed in 2020.

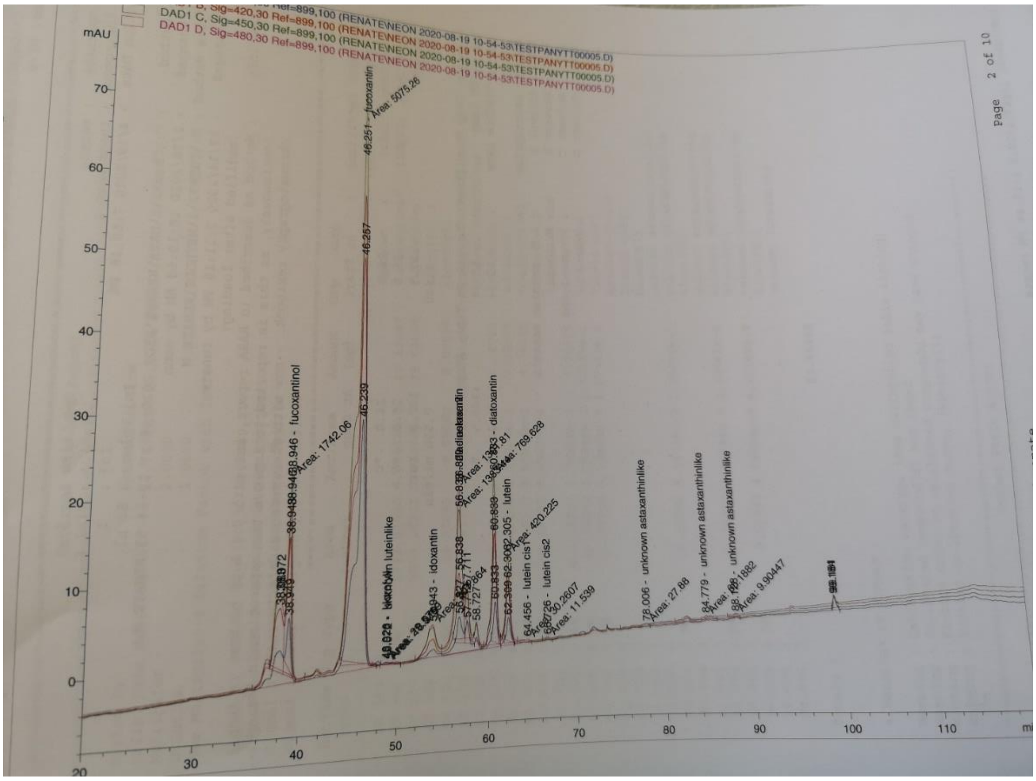


Figure 3: Chromatogram of PI-9. A muscle sample of salmon fed Phaeodactylum with intact cell walls. Analyzed in 2020.

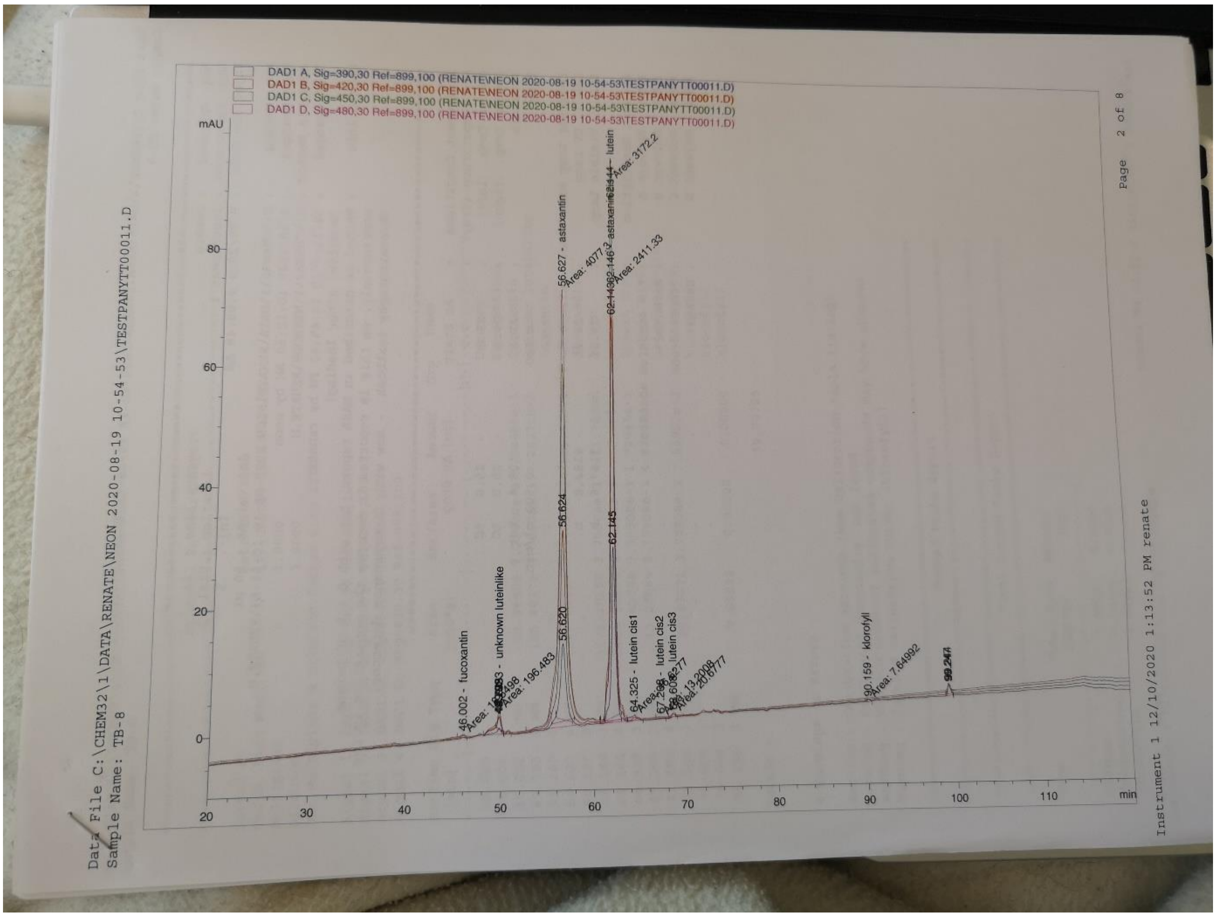


Figure 4: Chromatogram of TB-8. A muscle sample of salmon fed Tetraselmis with broken cell walls. Analyzed in 2020.

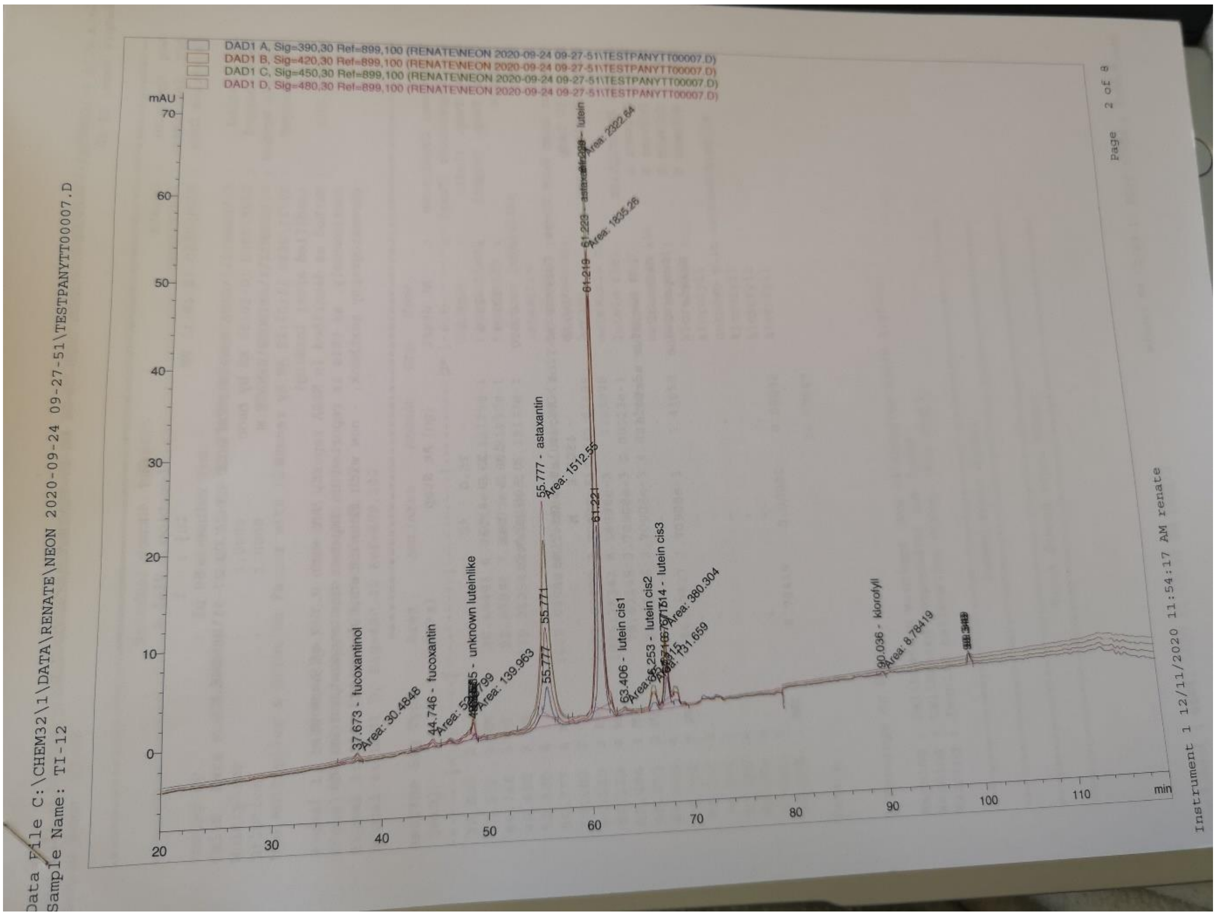


Figure 5: Chromatogram of TI-12. A muscle sample of salmon fed Tetraselmis with intact call walls. Analyzed in 2020.

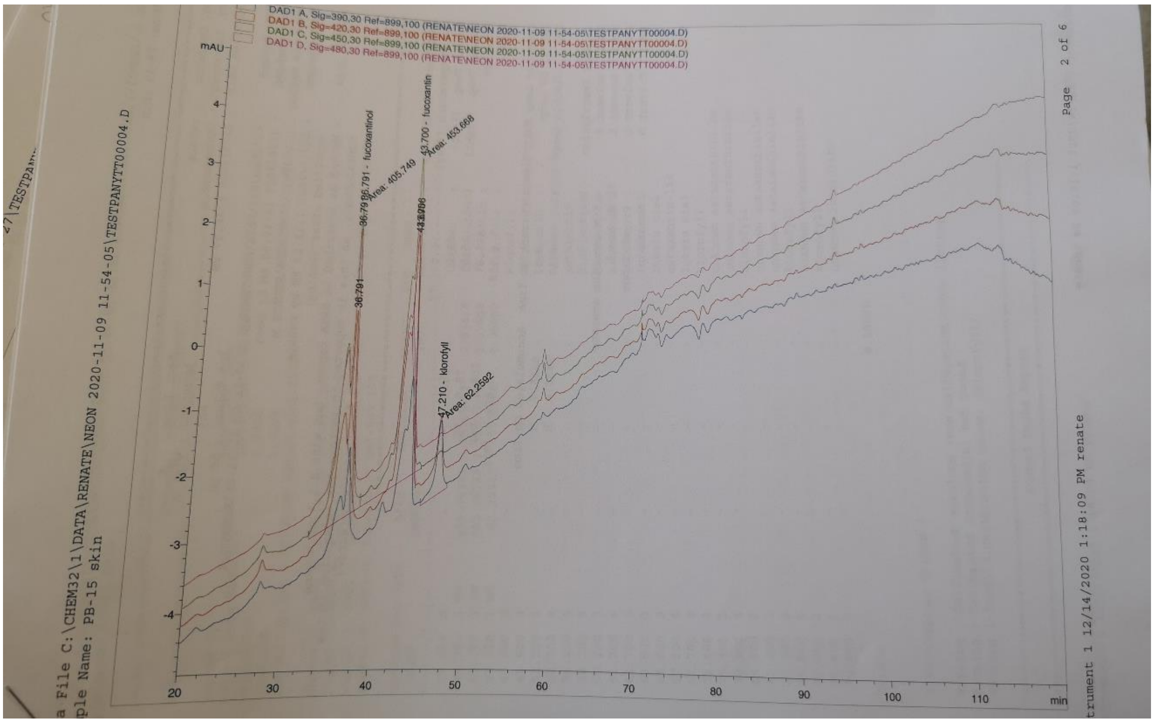


Figure 6: Chromatogram of skin sample from salmon fed Phaeodactylum with broken cell walls. Analyzed in 2020.

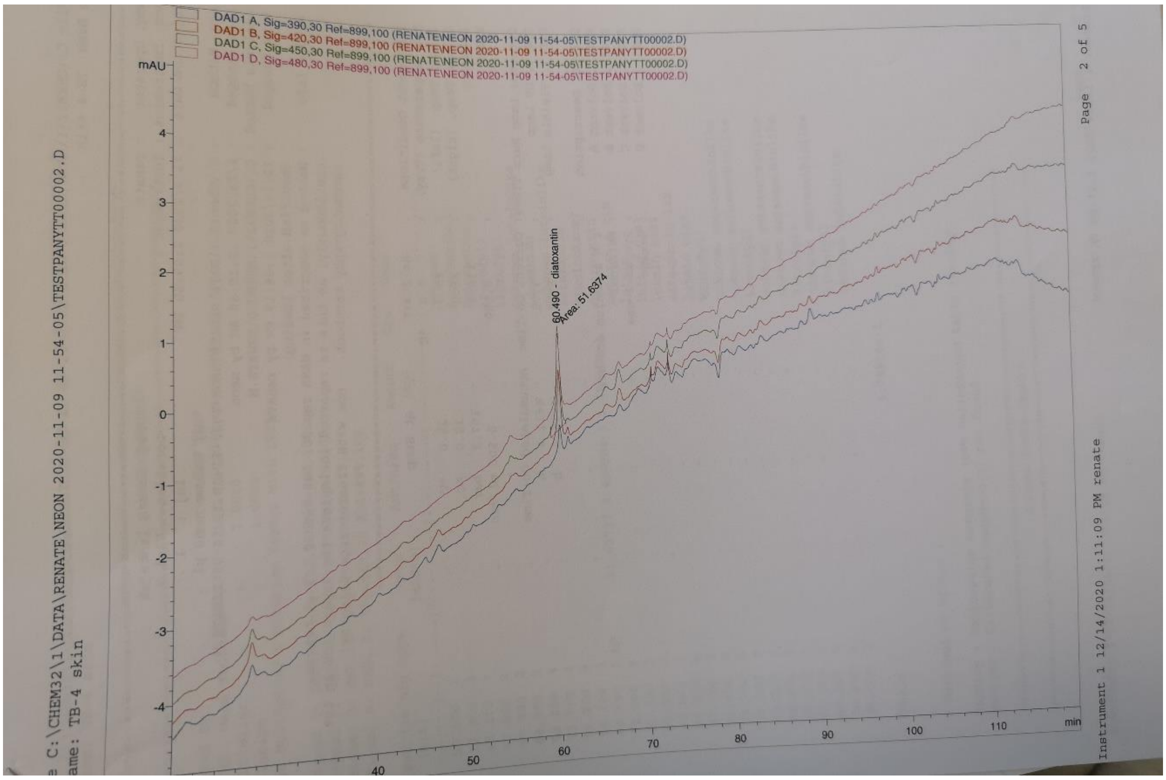


Figure 7: Chromatogram of skin sample from salmon fed Tetraselmis with broken cell walls. Analyzed in 2020. OBS! Wrong identification on the peak, should be lutein.

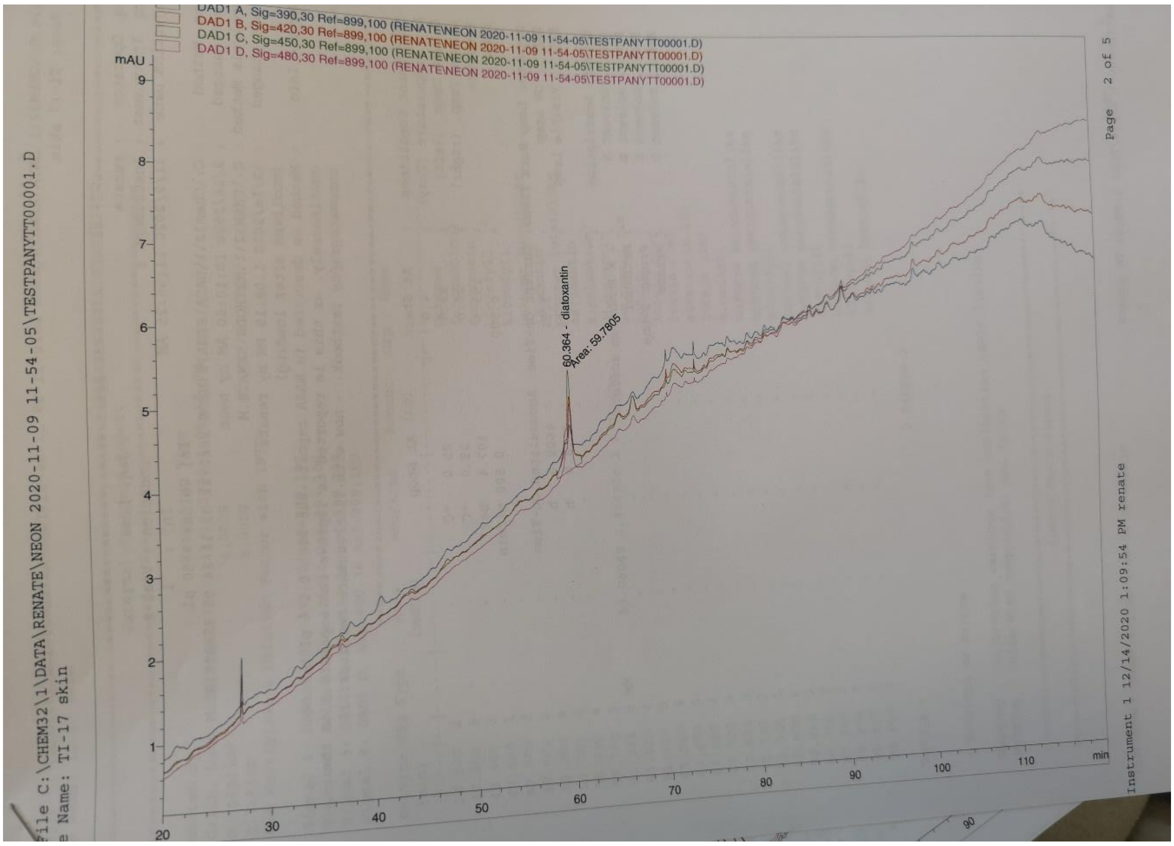


Figure 8: Chromatogram of skin sample from salmon fed Tetraselmis with intact cell walls. Analyzed in 2020. OBS! Wrong identification on the peak, should be lutein.

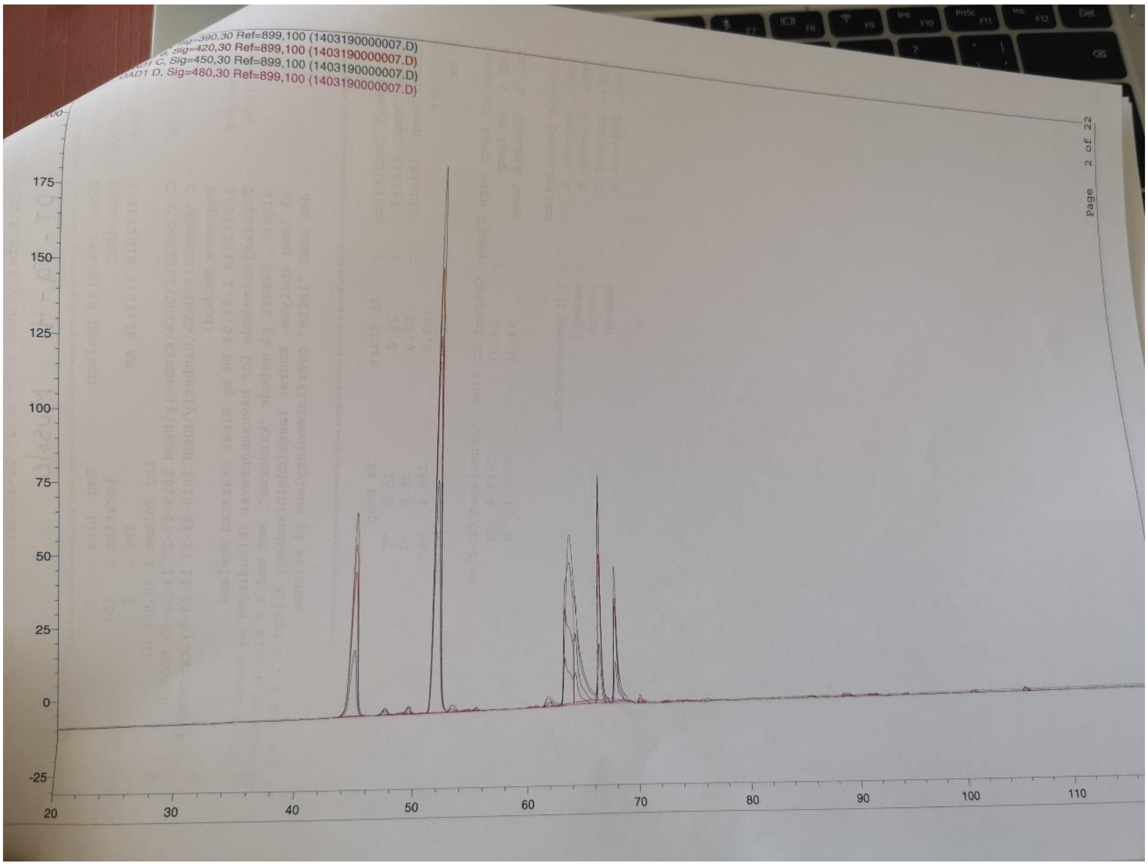


Figure 9: Chromatogram of Kimberly's analysis of muscle in salmon fed Phaeodactylum with intact cell walls. Analyzed in 2019.

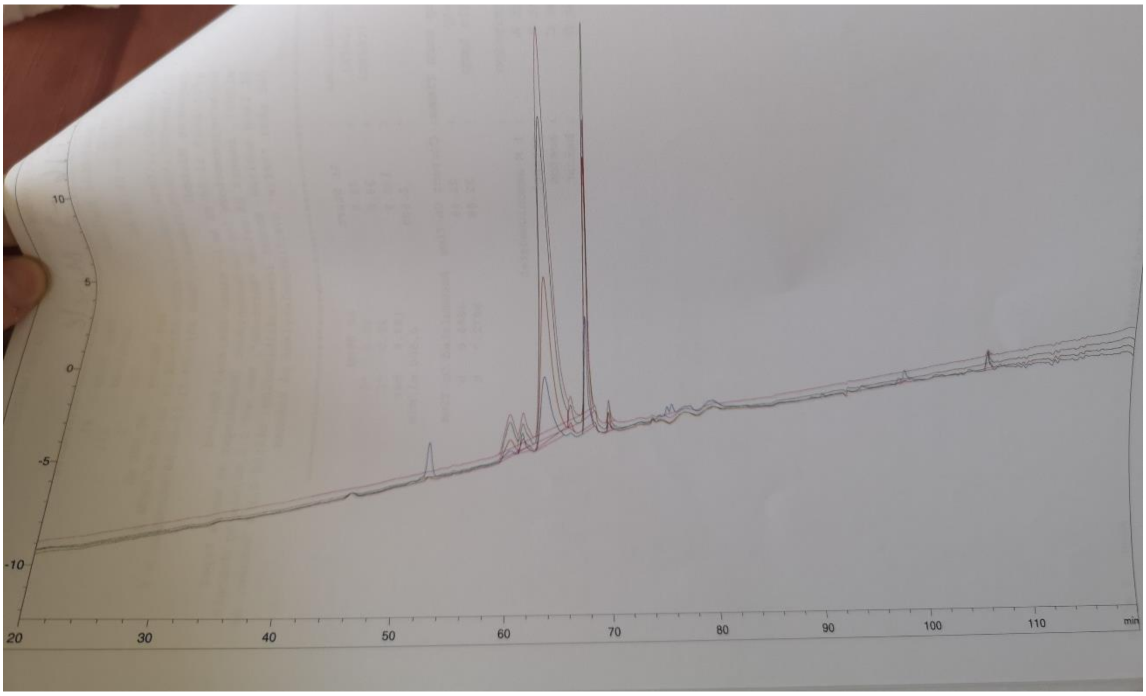


Figure 10: Chromatogram of Kimberly's analysis of muscle from salmon fed control diet. Analyzed in 2019.

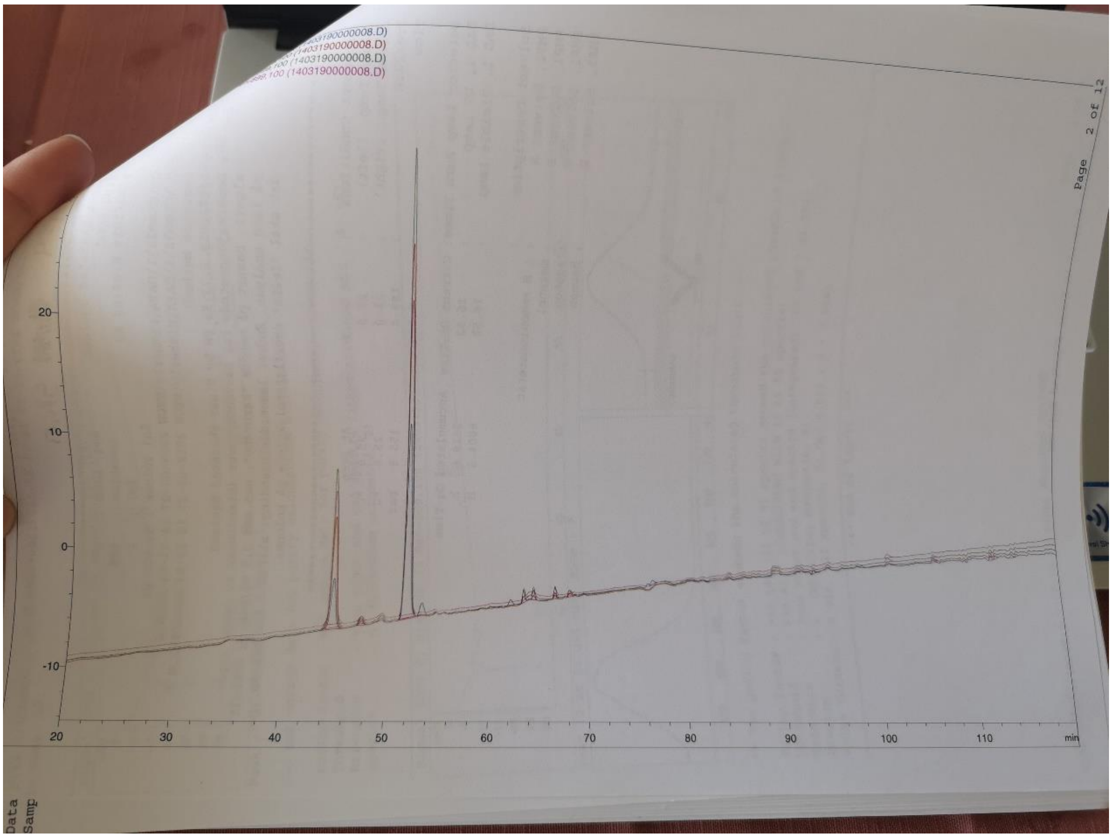


Figure 11: Chromatogram of Kimberlys analysis of skin from salmon fed Phaeodactylum with intact cell walls. Analyzed in 2019.

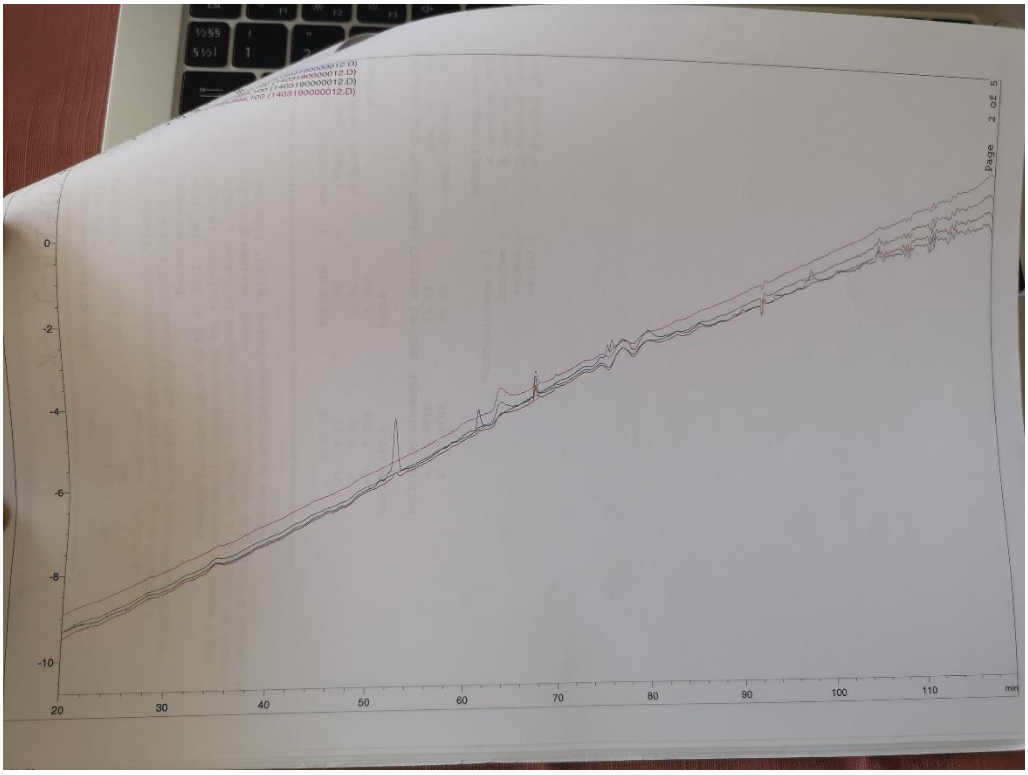


Figure 12: Chromatogram of Kimberly's analysis of skin from salmon fed control feed. Analyzed in 2019.

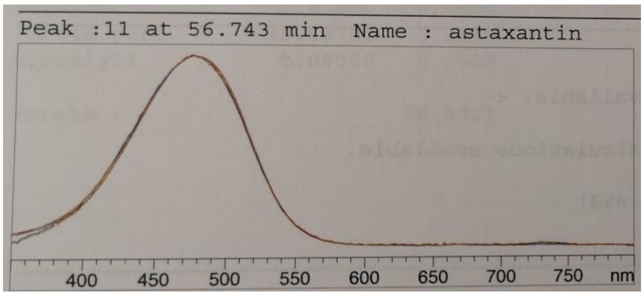


Figure 13: Specter of astaxanthin in the muscle sample TB-3

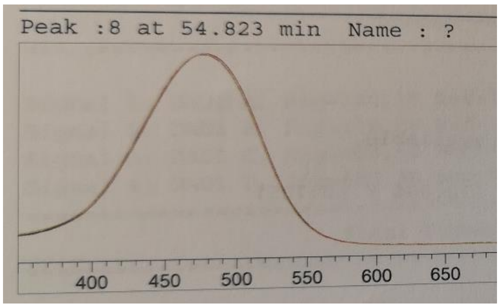


Figure 14: Specter of astaxanthin in standard

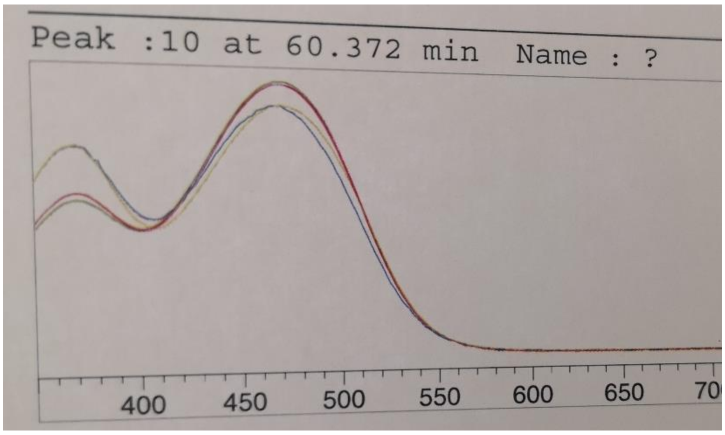


Figure 15: Specter of cis-astaxanthin in standard

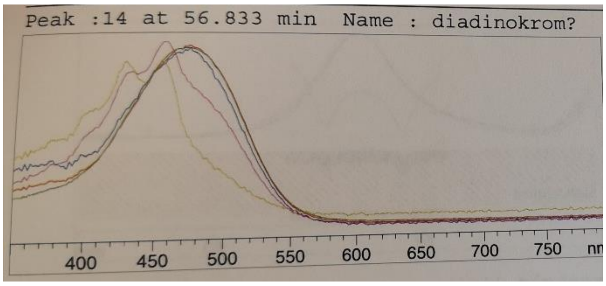


Figure 16: Specter of astaxanthin and diadinochrome in mixture in the muscle sample PI-9

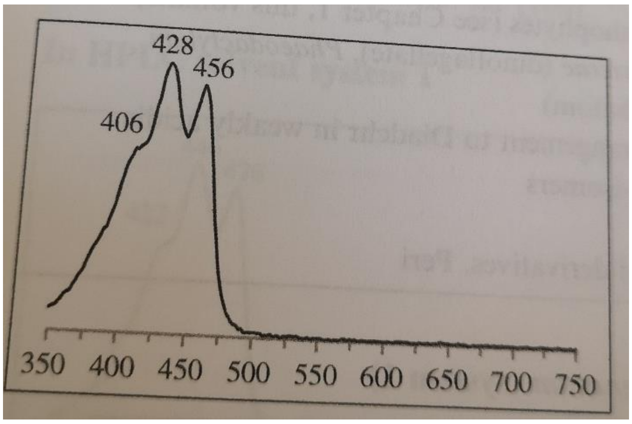


Figure 17: Specter of diadinochrome. From: (Roy et al., 2011)

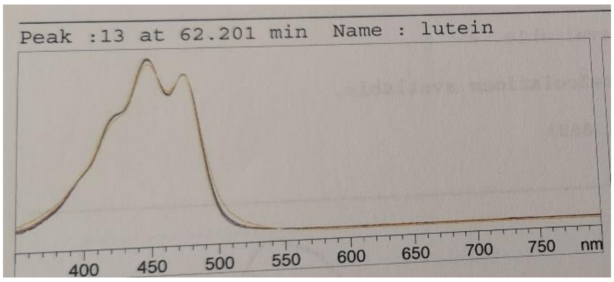


Figure 18: Specter of lutein in the muscle sample TB-3

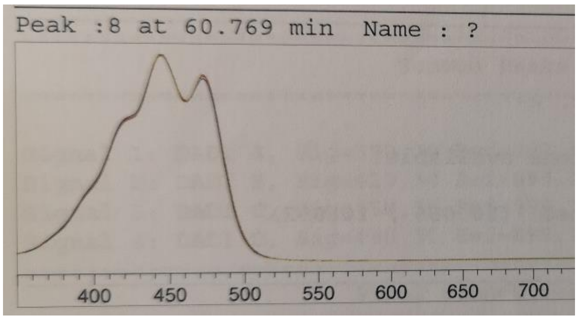


Figure 19: Specter of lutein in standard

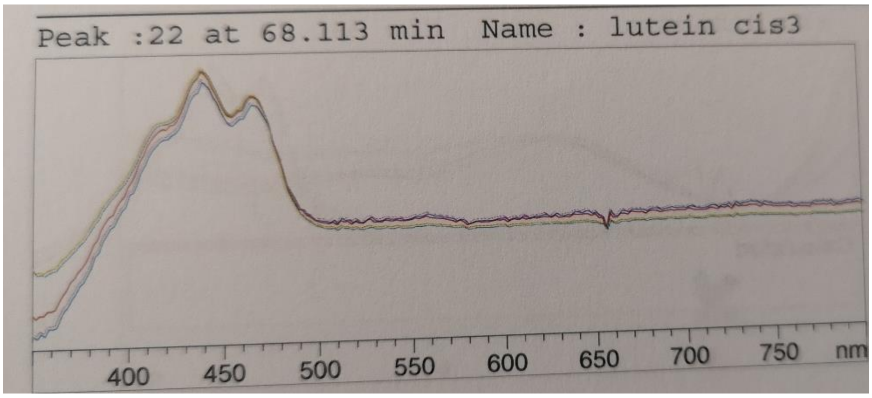


Figure 20: Specter of cis-lutein in the muscle sample TI-11

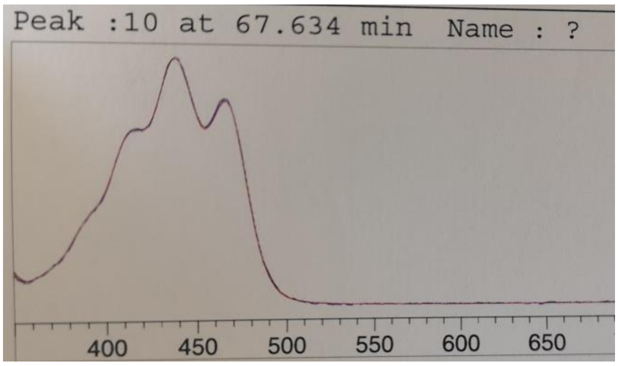


Figure 21: Specter of cis-lutein from standard

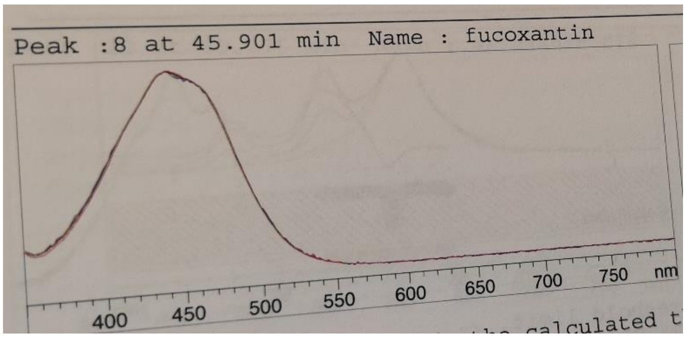


Figure 22: Specter of fucoxanthin in in the muscle sample PI-9

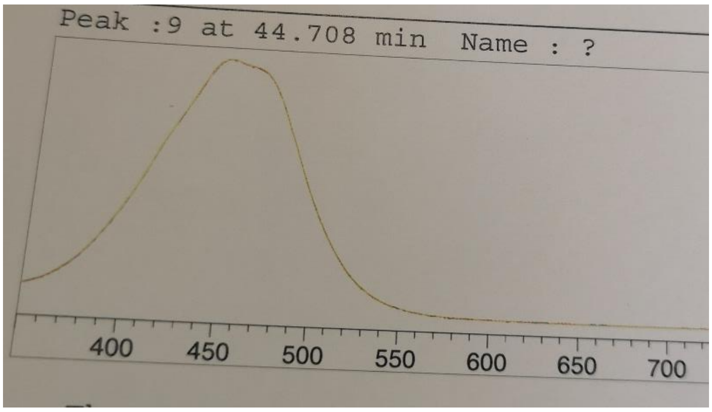


Figure 23: Specter of fucoxanthin from standard.

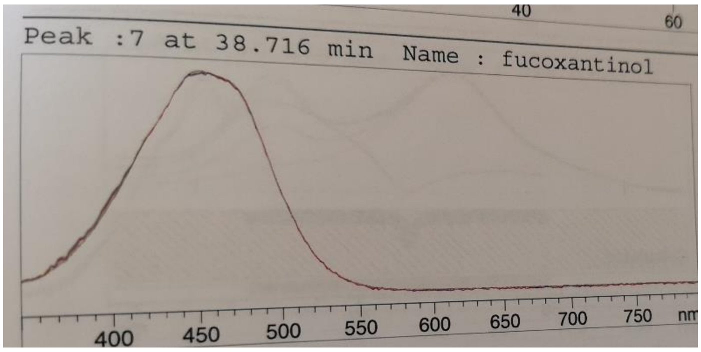


Figure 24: Specter of fucoxanthinol in the muscle sample PI-9

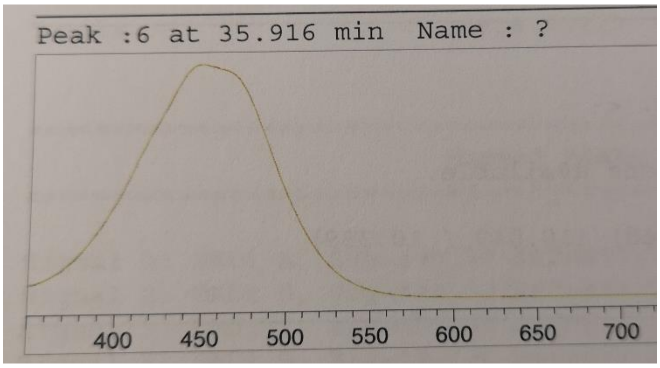


Figure 25: Specter of fucoxanthinol in standard

Specter of idoxanthin was not detected in the HPLC, as the peak was too low. However, when analyzing on the computer, a specter identical to figure 26 was observed. No picture was taken of this, unfortunately.

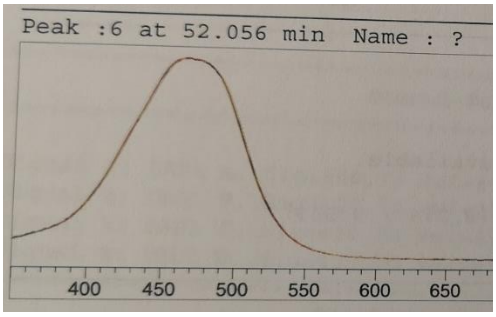


Figure 26: Specter of idoxanthin from standard

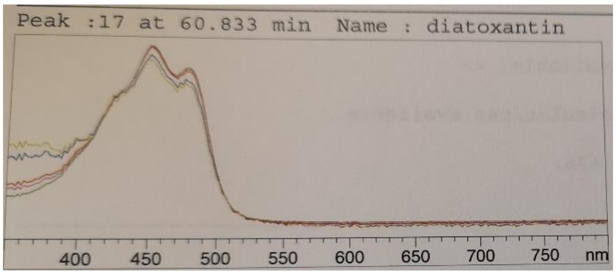


Figure 27: Specter of diatoxanthin in the muscle sample PI-9

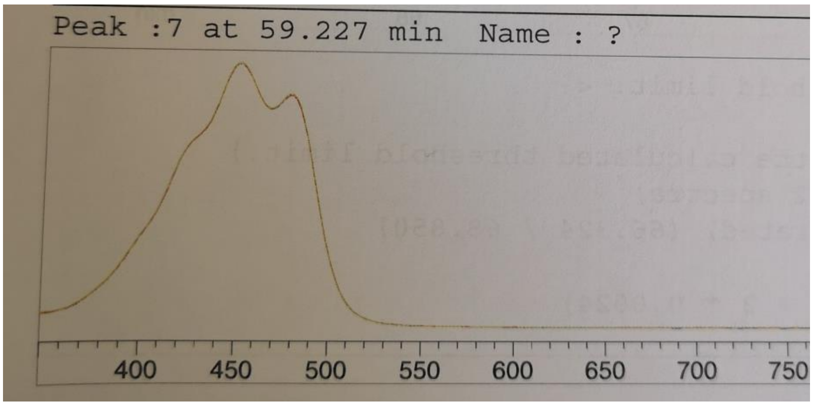


Figure 28: Specter of diatoxanthin from standard.

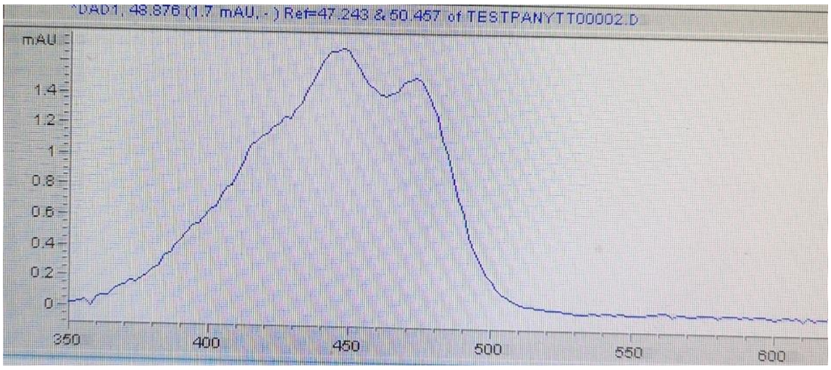


Figure 29: Loroxanthin in a muscle sample of TB-14. Peak was too small to be calculated by the HPLC.

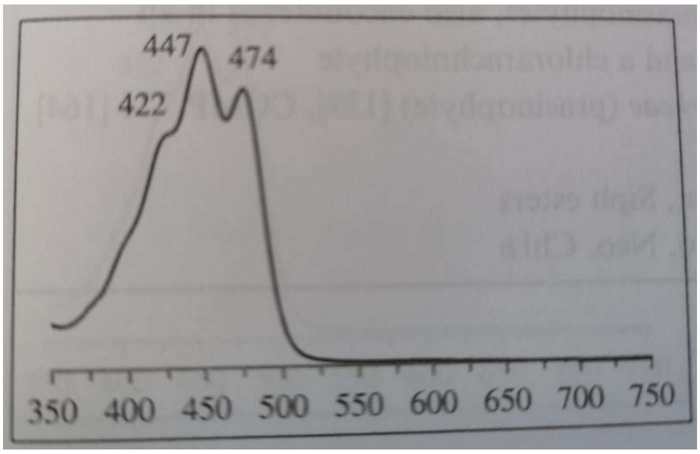


Figure 30: Specter of loroxanthin. From (Roy et al., 2011)

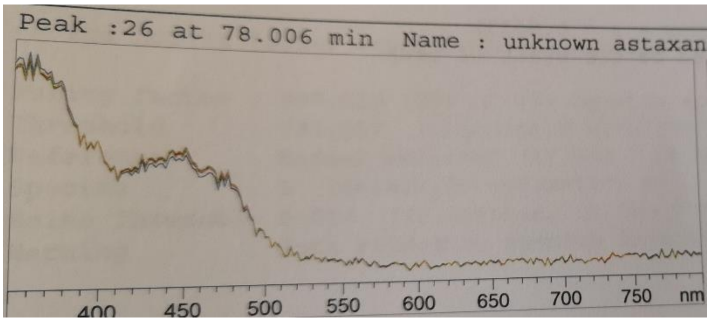


Figure 31: Specter of unknown astaxanthinlike in muscle sample PI-9.

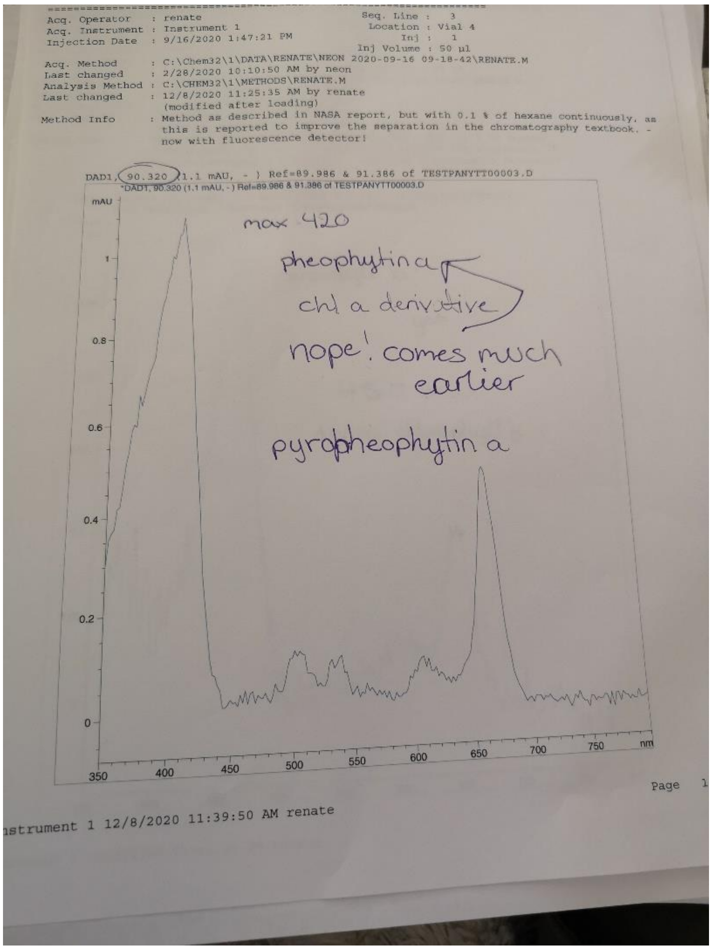


Figure 32: Specter of pyropheophytin a (most likely) from a muscle sample of TB-11. Analyzed in 2020.

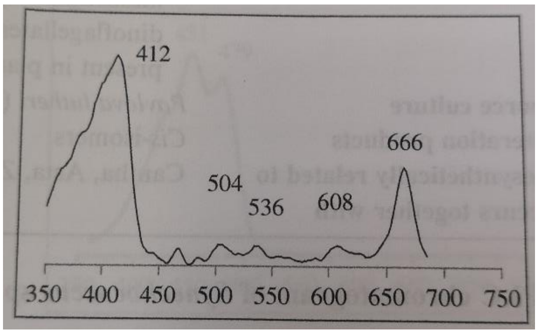


Figure 33: Specter of pyropheophytin a. From: (Roy et al., 2011)

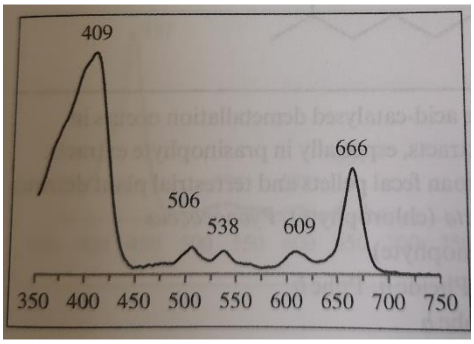


Figure 34: Specter of pheophytin a. From: (Roy et al., 2011)

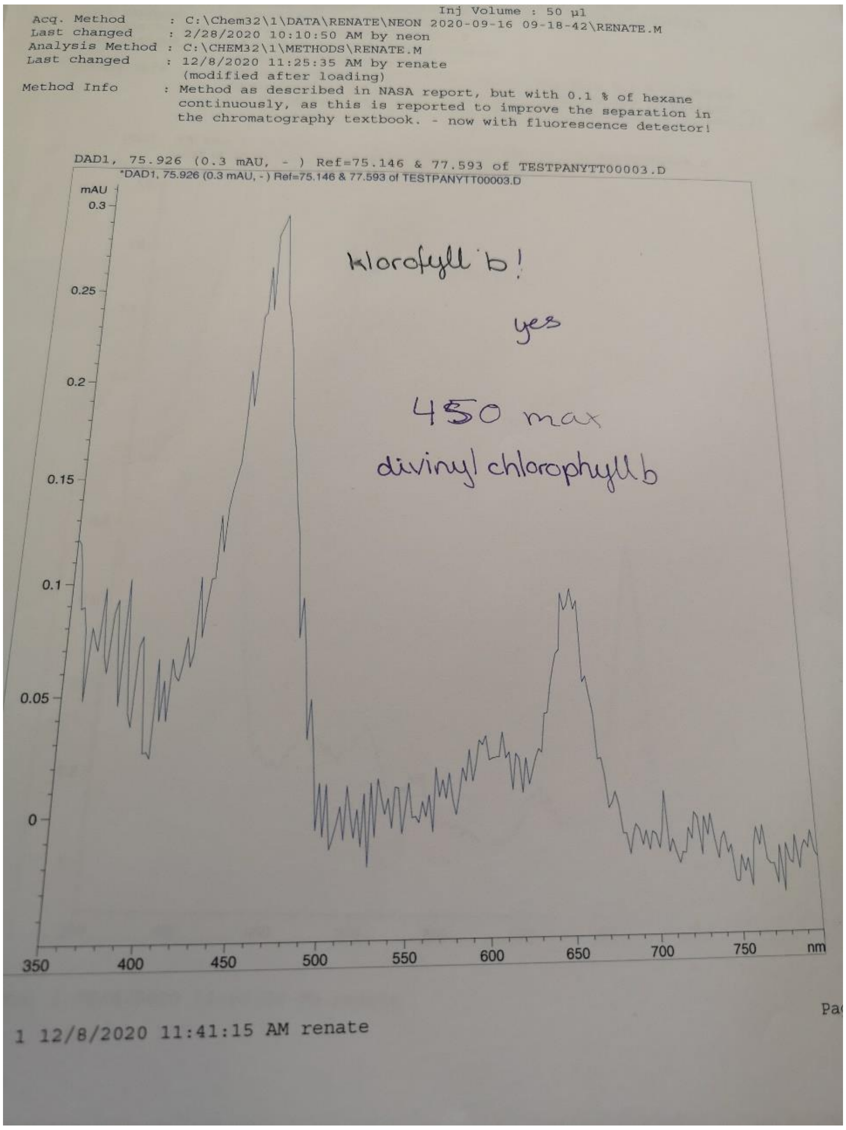


Figure 35: A degraded chlorophyll b in muscle sample TB-11. NOT divinyl chlorophyll b as it says in the photo.

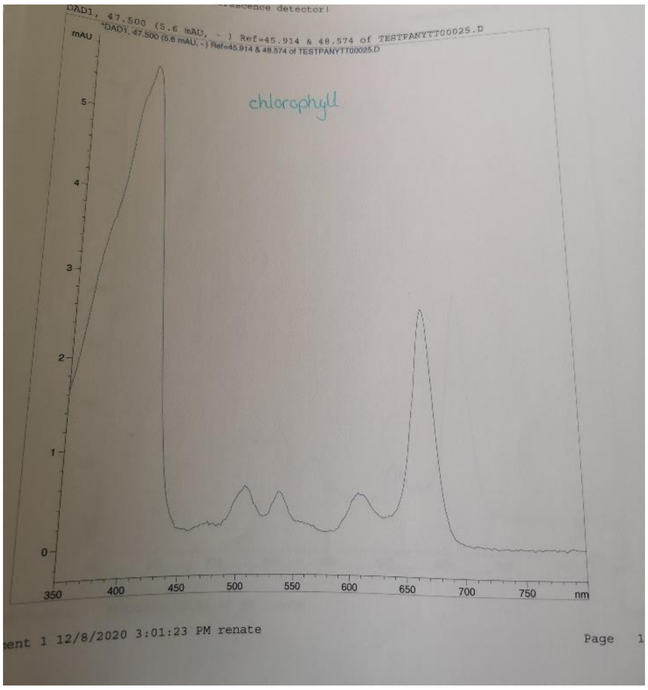


Figure 36: Specter of pheophorbide a (most likely), found in a muscle sample of PB-7. Analyzed in 2020.

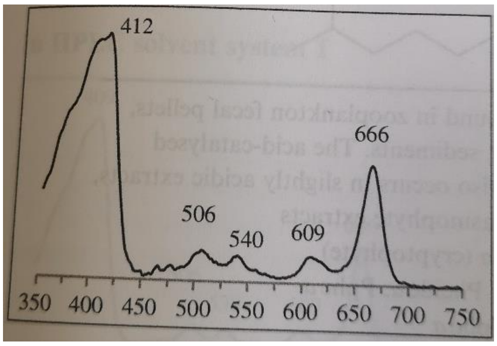


Figure 37: Specter of pheophorbide a. From:(Roy et al., 2011)

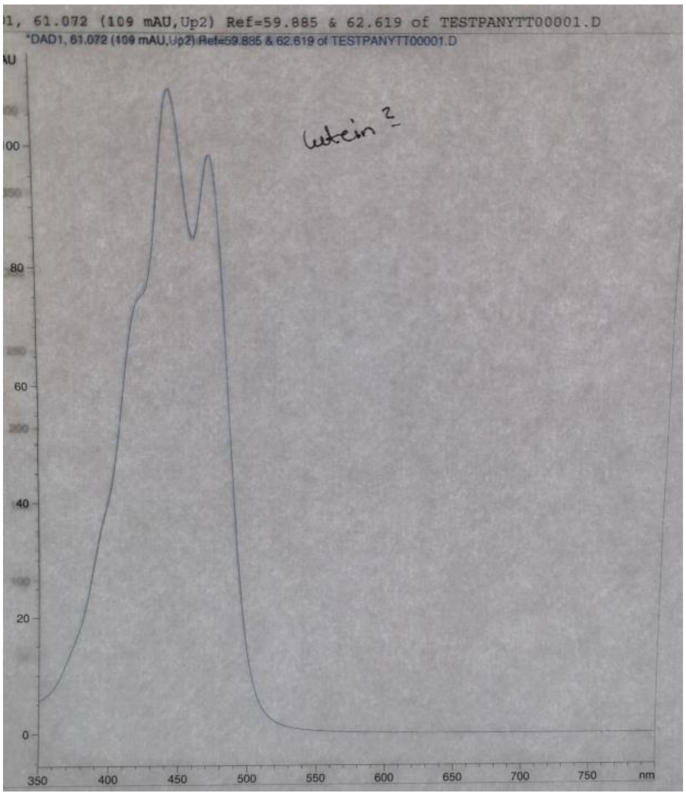


Figure 38: Specter from a peak in TB-13 in front of a specter from lutein, in front of a window. Specter overlap, and peak in TB-13 is thus lutein.

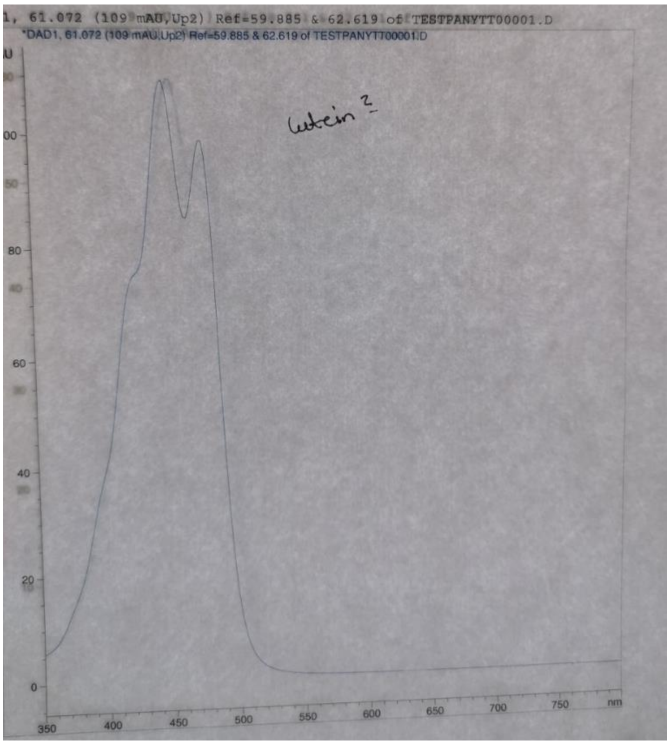


Figure 39: Specter of a peak in TB-13 in front of a specter of diatoxanthin in front of a window. TB-13 and diatoxanthin spectra are different, thus the peak in TB-13 is not diatoxanthin.

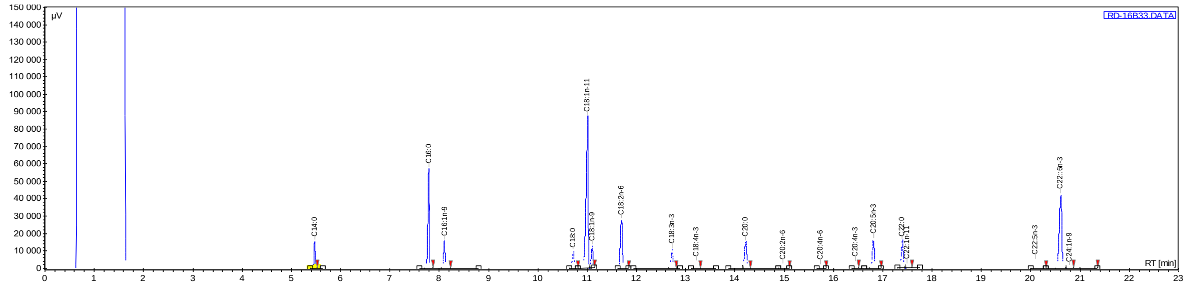


Figure 40: Chromatogram from GC, of RD-16. With identifications of FAMEs.

Table 1: Raw data from GC. Sample RD-16

#	Name	Time [Min]	Area [µV.Min]	Area % [%]
1	C14:0	5.47	474.3	2.85
2	C16:0	7.79	2160.9	12.986
3	C16:1n-9	8.1	645.2	3.877
4	C18:0	10.71	497.3	2.988
5	C18:1n-11	11.01	4258.6	25.592
6	C18:1n-9	11.1	507	3.047
7	C18:2n-6	11.7	1243	7.47
8	C18:3n-3	12.72	492.1	2.957
9	C18:4n-3	13.21	192	1.154
10	C20:0	14.22	757.1	4.55
11	C20:2n-6	14.97	124.7	0.75
12	C20:4n-6	15.74	101.4	0.609
13	C20:4n-3	16.45	195.4	1.174
14	C20:5n-3	16.81	791.5	4.756
15	C22:0	17.4	797.5	4.793
16	C22:1n-11	17.49	108.7	0.653
17	C22:5n-3	20.09	294.8	1.772
18	C22::6n-3	20.61	2603.7	15.647
19	C24:1n-9	20.78	132.8	0.798
20	UNKNOWN	21.2	262.7	1.579
Total			16640.4	100

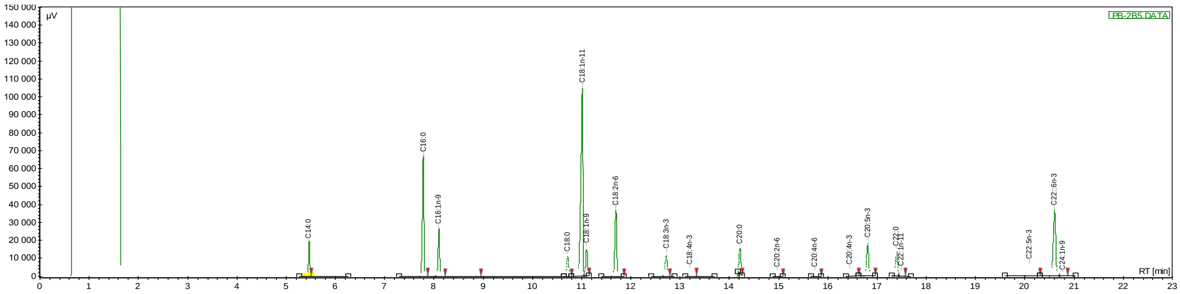


Figure 41: Chromatogram from GC, of PB-2. With identifications of FAMEs.

Table 2: Raw data from GC. Sample PB-2.

#		Name	Time [Min]	Area [µV.Min]	Area % [%]
	1	C14:0	5.47	575.9	3.102
	2	C16:0	7.79	2437.6	13.13
	3	C16:1n-9	8.1	993.7	5.353
	4	UNKNOWN	8.91	103.4	0.557
	5	C18:0	10.72	542.8	2.924
	6	C18:1n-11	11.02	5284.4	28.464
	7	C18:1n-9	11.1	615.5	3.315
	8	C18:2n-6	11.7	1612.3	8.684
	9	C18:3n-3	12.72	591.7	3.187
	10	C18:4n-3	13.2	212.8	1.146
	11	C20:0	14.22	651	3.507
	12	C20:2n-6	14.97	160.5	0.865
	13	C20:4n-6	15.74	138.6	0.746
	14	C20:4n-3	16.45	211.4	1.139
	15	C20:5n-3	16.81	925.4	4.985
	16	C22:0	17.4	738	3.975
	17	C22:1n-11	17.49	110.8	0.597
	18	C22:5n-3	20.09	355.8	1.916
	19	C22::6n-3	20.6	2166.9	11.672
	20	C24:1n-9	20.77	136.6	0.736
Total				18565.2	100

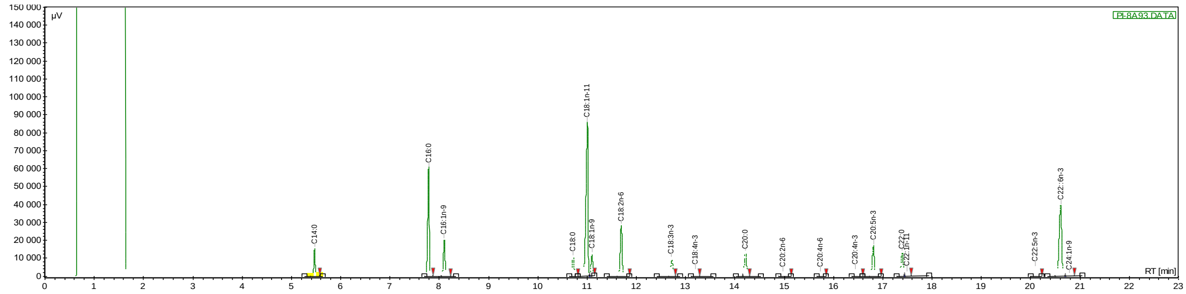


Figure 42: Chromatogram from GC, of PI-8. With identifications of FAMEs.

Table 3: Raw data from GC. Sample PI-8.

#	Name	Time [Min]	Area [µV.Min]	Area % [%]
1	C14:0	5.47	480.8	2.967
2	C16:0	7.79	2289.4	14.126
3	C16:1n-9	8.1	803	4.955
4	C18:0	10.71	509.8	3.145
5	C18:1n-11	11.01	4109.5	25.357
6	C18:1n-9	11.1	515.7	3.182
7	C18:2n-6	11.69	1248.2	7.702
8	C18:3n-3	12.72	471.8	2.911
9	C18:4n-3	13.21	167.9	1.036
10	C20:0	14.21	684.3	4.223
11	C20:2n-6	14.97	119.3	0.736
12	C20:4n-6	15.74	127.2	0.785
13	C20:4n-3	16.45	184.8	1.14
14	C20:5n-3	16.81	840.4	5.186
15	C22:0	17.4	682.1	4.209
16	C22:1n-11	17.48	110	0.679
17	C22:5n-3	20.09	311.6	1.922
18	C22::6n-3	20.61	2418.3	14.922
19	C24:1n-9	20.78	132.5	0.817
Total			16206.4	100

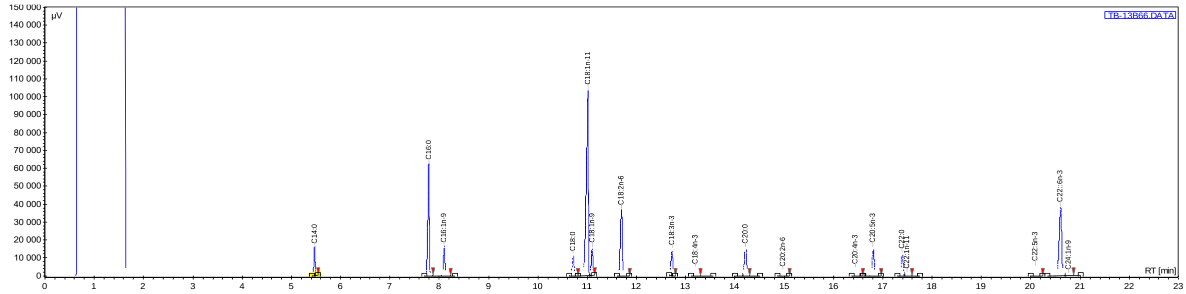


Figure 43: Chromatogram from GC, of TB-13. With identifications of FAMEs.

Table 4: Raw data from GC. Sample TB-13.

#	Name	Time [Min]	Area [µV.Min]	Area % [%]
1	C14:0	5.47	489.7	2.781
2	C16:0	7.79	2379.8	13.516
3	C16:1n-9	8.1	672.2	3.818
4	C18:0	10.72	566.1	3.215
5	C18:1n-11	11.02	5113.3	29.04
6	C18:1n-9	11.1	576.7	3.275
7	C18:2n-6	11.7	1573	8.934
8	C18:3n-3	12.72	635.3	3.608
9	C18:4n-3	13.21	227.7	1.293
10	C20:0	14.22	761.9	4.327
11	C20:2n-6	14.97	150.5	0.855
12	C20:4n-3	16.45	222.3	1.263
13	C20:5n-3	16.81	746.6	4.24
14	C22:0	17.39	669.9	3.805
15	C22:1n-11	17.49	104.8	0.595
16	C22:5n-3	20.09	301	1.709
17	C22::6n-3	20.61	2293.5	13.026
18	C24:1n-9	20.77	123.2	0.7
Total			17607.7	100

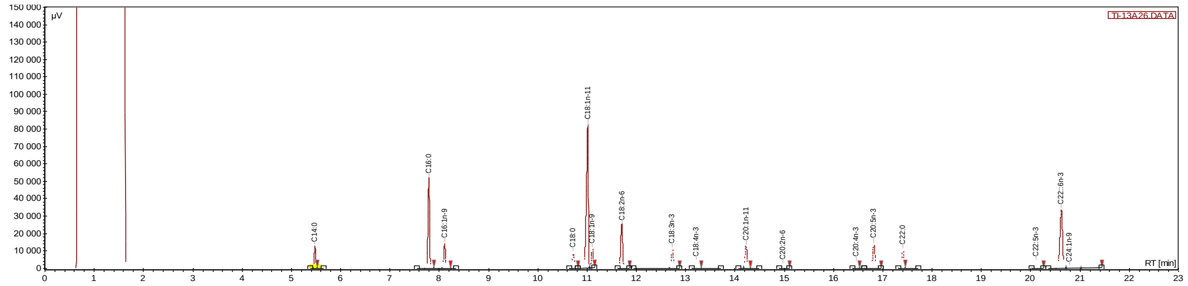


Figure 44: Chromatogram from GC, of TI-13. With identifications of FAMEs

Table 5: Raw data from GC. Sample TI-13.

#	Name	Time [Min]	Area [µV.Min]	Area % [%]
1	C14:0	5.48	393.8	2.7
2	C16:0	7.79	1946.3	13.343
3	C16:1n-9	8.11	555.2	3.806
4	C18:0	10.72	436	2.989
5	C18:1n-11	11.01	3891	26.676
6	C18:1n-9	11.11	461.8	3.166
7	C18:2n-6	11.7	1182.4	8.106
8	C18:3n-3	12.73	525.4	3.602
9	C18:4n-3	13.22	206.5	1.416
10	C20:1n-11	14.22	621.6	4.261
11	C20:2n-6	14.98	118.4	0.812
12	C20:4n-3	16.46	198.9	1.364
13	C20:5n-3	16.82	742.5	5.091
14	C22:0	17.41	557.9	3.825
15	C22:5n-3	20.11	270.5	1.855
16	C22::6n-3	20.62	2099.2	14.392
17	C24:1n-9	20.79	117.4	0.805
18	UNKNOWN	21.23	261.4	1.792
Total			14586.1	100

Appendix C – Raw data

Table 1: Raw data from HPLC, RD muscle samples.

Sample	Pigment	Area	Amount (ng/50microliter	Amount (ng) in 5 ml whole sampl	Weight of sample	Amount of pigment in whole sample ng/	Amount of total sample (%)
RD-1	Idoxanthin	301,92905	1,55708	155,708	10,43	14,93	11,0
	Astaxanthin	2293,30103	9,4965	949,65	10,43	91,05	67,0
	Diatoxanthir	45,39287	0,156922	15,6922	10,43	1,50	1,1
	Lutein	389,41916	1,44439	144,439	10,43	13,85	10,2
	Astaxanthin	335,50159	1,3893	138,93	10,43	13,32	9,8
	Lutein cis	35,11812	0,1302565	13,02565	10,43	1,25	0,9
	Total	3400,66182	14,1744485	1417,44485		135,9007526	
RD-2							
	Idoxanthin	25,68938	0,132483	13,2483	10,28	1,29	1,9
	Astaxanthin	1386,71008	5,74233	574,233	10,28	55,86	82,4
	Lutein	168,625	0,625444	62,5444	10,28	6,08	9,0
	Astaxanthin	137,21727	0,568213	56,8213	10,28	5,53	8,2
	Lutein cis	9,19623	0,0341096	3,41096	10,28	0,33	0,5
	Total	1701,74858	6,9700966	710,25796		67,80249611	
RD-3							
	Unknown	22,9053	0	0	9,34	0,00	0,0
	Idoxanthin	-	-	-			
	Astaxanthin	769,6839	3,18724	318,724	9,34	34,12	79,2
	Lutein	111,32515	0,412914	41,2914	9,34	4,42	10,3
	Astaxanthin	96,75191	0,400647	40,0647	9,34	4,29	10,0
	Lutein cis	6,79069	0,0251872	2,51872	9,34	0,27	0,6
	Total	1007,45695	4,0259882	402,59882		43,10479872	
RD-4							
	Astaxanthin	1111,28	4,60	460,18	10,62	43,33	63,01
	Lutein	328,67	1,22	121,91	10,62	11,48	16,69
	Astaxanthin	314,16	1,30	130,09	10,62	12,25	17,81
	Lutein cis	35,78	0,13	13,27	10,62	1,25	1,82
	????	13,10	0,05	4,87	10,62	0,46	0,67
	Total	1802,99652	8,6032143	730,32143		68,76849623	
RD-5							
	Idoxanthin	262,62	1,35	135,43	10,71	12,65	19,72
	Astaxanthin	735,97	3,05	304,77	10,71	28,46	44,37
	Lutein	316,87	1,18	117,53	10,71	10,97	17,11
	Astaxanthin	280,56	1,16	116,18	10,71	10,85	16,91
	Lutein cis	35,03	0,13	12,99	10,71	1,21	1,89
	Total	1631,05	6,87	686,90		64,14	
RD-6							
	Fucoxanthin	6,03088	0,0	4,1979	12,35	0,34	0,45
	Idoxanthin						
	Astaxanthin	1520,62439	6,3	629,687	12,35	50,99	67,07
	Lutein	390,84506	1,4	144,968	12,35	11,74	15,44
	Astaxanthin	346,66446	1,4	143,553	12,35	11,62	15,29
	Lutein cis	44,32101	0,2	16,43903	12,35	1,33	1,75
	Total	2308,5	9,4	938,8		76,0	
RD-7							
	Fucoxanthin	5,27	0,04	3,67	9,41	0,39	0,40
	Fucoxanthin	21,11	0,15	15,24	9,41	1,62	1,66
	Idoxanthin	-	-				
	Astaxanthin	1287,22	5,33	533,04	9,41	56,65	58,00
	Diatoxanthir	50,86	0,18	17,58	9,41	1,87	1,91
	Lutein	455,63	1,69	169,00	9,41	17,96	18,39
	Astaxanthin	375,72	1,56	155,59	9,41	16,53	16,93
	Lutein cis	67,25	0,25	24,94	9,41	2,65	2,71
	Total	2263,05	9,19	919,05		97,67	
RD-8							
	Idoxanthin	273,51273	1,41053	141,053	10,66	13,23	16,26068652
	Astaxanthin	1308,03479	5,41654	541,654	10,66	50,81	62,44224438
	Diatoxanthir	49,5826	0,171406	17,1406	10,66	1,61	1,98
	Lutein	221,19804	0,820442	82,0442	10,66	7,70	9,45811161
	Astaxanthin	180,61122	0,747907	74,7907	10,66	7,02	8,621923159
	Lutein cis1	29,02477	0,107655	10,7655	10,66	1,01	1,241054219
	Total	2061,96415	8,67448	867,448		81,37410882	
RD-9							
	Unknown	49,34103	0	0	10,48	0,00	
	Idoxanthin	-	-				
	Astaxanthin	824,45	3,41	341,40	10,48	32,58	76,34
	Diatoxanthir	27,12	0,09	9,37	10,48	0,89	2,10
	Lutein	128,46	0,48	47,65	10,48	4,55	10,65
	Astaxanthin	110,36	0,46	45,70	10,48	4,36	10,22
	Lutein cis1	8,28	0,03	3,07	10,48	0,29	0,69
	Total	1148,01	4,47	447,19		42,67	

Table 2: Raw data from HPLC, RD muscle samples continued.

Sample	Pigment	Area	Amount (ng/50microliter)	Amount (ng) in 5 ml whole sample	Weight of sample	Amount of pigment in whole sample ng/g	Amount of total sample (%)
RD-10							
	Unknown	25.32	0.00		12.09	0.00	
	Idoxanthin	149.58	0.77	77.14	12.09	6.38	10.86
	Astaxanthin	788.83	3.27	326.65	12.09	27.02	45.97
	Diatoxanthin	82.35	0.28	28.47	12.09	2.35	4.01
	Lutein	344.90	1.28	127.93	12.09	10.58	18.00
	Astaxanthin ci	314.53	1.30	130.25	12.09	10.77	18.33
	Lutein cis	54.23	0.20	20.11	12.09	1.66	2.83
	Total	1759.74	7.11	710.55		58.77	100.00
RD-11							
	Astaxanthin	842.13	3.49	348.72	10.46	33.34	65.62
	Lutein	249.83	0.93	92.67	10.46	8.86	17.44
	Astaxanthin ci	195.92	0.81	81.13	10.46	7.76	15.27
	Lutein cis	24.05	0.09	8.92	10.46	0.85	1.68
	Total	1311.93	5.31	531.44		50.81	100.00
RD-12							
	Idoxanthin	126.76	0.65	65.37	10.44	6.26	4.04
	Astaxanthin	2677.22	11.09	1108.63	10.44	106.19	68.43
	Lutein	567.29	2.10	210.41	10.44	20.15	12.99
	Astaxanthin ci	529.64	2.19	219.32	10.44	21.01	13.54
	Lutein cis	43.81	0.16	16.25	10.44	1.56	1.00
	Total	3944.72	16.20	1619.99		155.17	100.00
RD-13							
	Astaxanthin	2259.6958	9.4	935.734	11.71	79.91	68.06
	Lutein	588.89343	2.2	218.425	11.71	18.65	15.89
	Astaxanthin ci	484.4917	2.0	200.627	11.71	17.13	14.59
	Lutein cis	54.02555	0.2	20.03849	11.71	1.71	1.46
	Total	3387.10648	13.7482449	1374.82449		117.4060196	100
RD-14							
	Astaxanthin	975.41302	4.0	403.916	10.32	39.14	77.78
	Lutein	156.00264	0.578627	57.8627	10.32	5.61	11.14
	Astaxanthin ci	131.55615	0.5	54.4771	10.32	5.28	10.49
	Lutein cis	8.14037	0.0301933	3.01933	10.32	0.29	0.58
	Total	1271.11218	5.1927513	519.27513		50.31735756	100
RD-15							
	Unknown pigr	41.62254	0	0	10.21	0.00	?
	Idoxanthin	-	-				
	Astaxanthin	1577.92749	6.53416	653.416	10.21	64.00	79.03918478
	Diatoxanthin	25.60999	0.0885333	8.85333	10.21	0.87	1.070925698
	Lutein	221.5134	0.821612	82.1612	10.21	8.05	9.938468401
	Astaxanthin ci	187.48454	0.776369	77.6369	10.21	7.60	9.391195325
	Lutein cis	12.48659	0.0463138	4.63138	10.21	0.45	0.560225797
	Total	2066.64455	8.2669881	826.69881		80.96952106	
RD-16							
	Fucoxanthino	147.05186	1.02358	102.358	10	10.24	12.26855328
	Fucoxanthin	157.39577	1.13651	113.651	10	11.37	13.62212381
	Astaxanthin	936.87354	3.87957	387.957	10	38.80	46.5002357
	Diatoxanthin	81.04556	0.280173	28.0173	10	2.80	3.358132612
	Lutein	261.37622	0.969466	96.9466	10	9.69	11.61994693
	Astaxanthin ic	243.13611	1.00682	100.682	10	10.07	12.06766918
	Lutein cis	12.7	0.047	4.7	10	0.47	0.563338483
	Total	1839.57906	8.343119	834.3119		83.43119	100
RD-17							
	Fucoxanthino	289.29791	2.01371	201.371	12.43	16.20	17.25529355
	Fucoxanthin	333.38501	2.40727	240.727	12.43	19.37	20.62767256
	Idoxanthin	-	-				
	Astaxanthin	1029.92651	4.2649	426.49	12.43	34.31	36.55
	Diatoxanthin	112.66798	0.389491	38.9491	12.43	3.13	3.34
	Lutein	316.535	1.17405	117.405	12.43	9.45	10.06
	Astaxanthin ci	285.25165	1.18122	118.122	12.43	9.50	10.12
	Lutein cis	47.78725	0.177247	17.7247	12.43	1.43	1.52
	Unknown asta	15.02351	0.062211924	6.221192402	12.43	0.50	0.53
	Totalt	2429.87482	11.67009992	1167.009992		93.88656415	100
RD-18							
	Idoxanthin	74.22234	0.382772	38.2772	15.47	2.47	4.269608275
	Astaxanthin	1088.98547	4.50946	450.946	15.47	29.15	50.30051239
	Lutein	495.86679	1.83921	183.921	15.47	11.89	20.51536224
	Astaxanthin ci	482.92279	1.99977	199.977	15.47	12.93	22.30631953
	Lutein cis	63.04149	0.2338259	23.38259	15.47	1.51	2.608197563
	Total	2205.03888	8.9650379	896.50379		57.95111765	100

Table 3: Raw data from HPLC, PB muscle samples.

PB-14	Area	Amount (ng/50microliter)	Amount (ng) in 5 ml whole sample	Weight of sample	Amount of pigment in whole sample ng/g	In %
Fucoxanthino	691.15674	4.81092	481.092	10.45	46.03751196	14.5409096
Fucoxanthin	2891.52686	20.87885	2087.885	10.45	199.7976077	63.1059072
Clorophyll	-	-				
Idoxanthin	127.06396	0.655282	65.5282	10.45	6.270641148	1.98057676
Astaxanthin	851.66888	3.52674	352.674	10.45	33.74870813	10.6595012
Diatoxanthin	575.58551	1.98979	198.979	10.45	19.04105263	6.01410054
Lutein	257.01199	0.953279	95.3279	10.45	9.122287081	2.88126674
Astaxanthin ci	-	-		10.45		0
Lutein cis	38.76003	0.1437642	14.37642	10.45	1.375733971	0.43452442
unknown asta	30.61785	0.12678778	12.678778	10.45	1.213280191	0.38321353
Totalt	5463.39182	33.08541298	3308.541298	94.05	316.6068228	100
PB-13						
Fucoxanthino	2572.54614	17.9066	1790.66	11.25	159.1697778	19.8948093
Fucoxanthin	7428.78174	53.641	5364.1	11.25	476.8088889	59.5968784
Clorophyll	-	-		11.25		
Idoxanthin	243.21603	1.25429	125.429	11.25	11.14924444	1.39355658
Astaxanthin	2375.6936	9.83769	983.769	11.25	87.44613333	10.9299904
Diatoxanthin	1361.44751	4.7065	470.65	11.25	41.83555556	5.22907307
Lutein	430.47479	1.59667	159.667	11.25	14.19262222	1.77395179
Astaxanthin ci	-	-		11.25		0
Lutein cis	98.78291	0.3663933	36.63933	11.25	3.256829333	0.40707476
unknown asta	156.81381	0.64936221	64.936221	11.25	5.772108533	0.72146233
unknown lute	12.91056	0.04788644	4.788644	11.25	0.425657244	0.05320338
Totalt	14680.6671	90.00639195	9000.639195		800.0568173	100
PB-3						
Fucoxanthino	2046.5	14.2450242	1424.50242	10.63	134.0077535	20.4016149
Fucoxanthin	5822.87402	42.04522	4204.522	10.63	395.5335842	60.2168429
Clorophyll	27.52025	-		10.63	0	0
Idoxanthin	-	-				
Astaxanthin	2089.10156	8.65092	865.092	10.63	81.38212606	12.3897815
Diatoxanthin	873.12335	3.01837	301.837	10.63	28.39482596	4.32288646
Lutein	327.62936	1.2152	121.52	10.63	11.4317968	1.74040016
Astaxanthin ci	-	-				
Lutein cis	78.52367	0.2912512	29.12512	10.63	2.739898401	0.41712774
unknown asta	86.2206	0.35703743	35.703743	10.63	3.358771684	0.51134628
unknown lute	-	-				
Totalt	11351.4928	69.82302283	6982.302283		656.8487566	100
PB-2						
Fucoxanthino	2383.08594	16.58789	1658.789	10.71	154.8822596	20.991629
Fucoxanthin	6423.73975	46.3839	4638.39	10.71	433.0896359	58.6978587
Clorophyll	69.6572	0	0	10.71	0	0
Idoxanthin	196.85661	1.01521	101.521	10.71	9.479084967	1.28472709
Astaxanthin	2200.50806	9.11225	911.225	10.71	85.08169935	11.5313624
Diatoxanthin	957.96472	3.31167	331.167	10.71	30.92128852	4.19084936
Lutein	478.21222	1.77373	177.373	10.71	16.56143791	2.24461834
Astaxanthin ci	-	-		10.71		0
Lutein cis	94.13138	0.3491414	34.91414	10.71	3.259957049	0.44183116
unknown asta	117.76461	0.48766025	48.766025	10.71	4.553316993	0.61712388
unknown lute	-	-		10.71		0
Totalt	12921.9205	79.02145165	7902.145165		737.8286802	100
PB-15						
Fucoxanthino	1303.73071	9.07485	907.485	11.2	81.02544643	18.5712204
Fucoxanthin	3778.65381	27.28452	2728.452	11.2	243.6117857	55.8363867
Clorophyll	37.58186	0	0	11.2	0	0
Idoxanthin	172.41144	0.889144	88.9144	11.2	7.938785714	1.81958811
Astaxanthin	1597.92163	6.61695	661.695	11.2	59.07991071	13.5412527
Diatoxanthin	879.43207	3.04018	304.018	11.2	27.14446429	6.22157422
Lutein	354.11249	1.31343	131.343	11.2	11.72705357	2.6878679
Astaxanthin ci	-	-				
Lutein cis	93.31138	0.3460996	34.60996	11.2	3.090175	0.70827528
unknown asta	72.43479	0.2999511	29.99511	11.2	2.678134821	0.61383472
unknown lute	-	-				
Totalt	8289.59018	48.8651247	4886.51247		436.2957563	100
PB-9						
Fucoxanthino	1263.33508	8.79367	879.367	12.66	69.46026856	14.7461382
Fucoxanthin	4107.81787	29.66132	2966.132	12.66	234.2916272	49.7391786
Clorophyll	33.9684	0	0	12.66	0	0
Idoxanthin	-	-				
Astaxanthin	3491.6853	14.45898	1445.898	12.66	114.2099526	24.2463177
Diatoxanthin	1188.6853	4.10917	410.917	12.66	32.45789889	6.89068256
Lutein	435.07227	1.61372	161.372	12.66	12.74660348	2.70605311
Astaxanthin ci	-	-				
Lutein cis	118.99296	0.441355	44.1355	12.66	3.48621643	0.74010985
unknown asta	134.14722	0.5555	55.55	12.66	4.387835703	0.93152003
unknown lute	-	-				
Totalt	10773.7044	59.633715	5963.3715		471.0404028	100
PB-7						
Unknown	19.03444	finn ut		9.79	0	0
Fucoxanthino	1472.66235	10.25073	1025.073	9.79	104.7061287	20.2197505
Fucoxanthin	3810.64331	27.51551	2751.551	9.79	281.0573034	54.2748416
Clorophyll	266.18188	0	0	9.79	0	0
Idoxanthin	-	-				
Astaxanthin	1927.3573	7.98114	798.114	9.79	81.52339122	15.7429431
Diatoxanthin	774.39154	2.67706	267.706	9.79	27.34484168	5.28054932
Lutein	401.38452	1.48877	148.877	9.79	15.20704801	2.93662578
Astaxanthin ci	-	-				
Lutein cis	104.63291	0.3880921	38.80921	9.79	3.964168539	0.76551869
unknown asta	95.46493	0.3953175	39.53175	9.79	4.037972421	0.77977093
unknown lute	-	-				
Totalt	8871.75318	50.6966196	5069.66196		517.8408539	100

Table 4: Raw data from HPLC, PB muscle samples continued.

	Area	Amount (ng/50microliter)	Amount (ng) in 5 ml whole sample	Weight of sample	Amount of pigment in whole sample ng/g	In %
PB-1						
Unknown	14.30223	finn ut		12	0	0
Fucoxanthino	1037.44104	7.22129	722.129	12	60.17741667	14.1697407
Fucoxanthin	4047.66919	29.227	2922.7	12	243.5583333	57.3497271
Clorophyll	97.12606	0	0	12	0	0
Idoxanthin	338.14175	1.74383	174.383	12	14.53191667	3.42177352
Astaxanthin	1906.5199	7.89485	789.485	12	65.79041667	15.4914118
Diatoxanthin	745.98151	2.57885	257.885	12	21.49041667	5.06026427
Lutein	356.52716	1.32239	132.239	12	11.01991667	2.59481663
Astaxanthin ci	-	-				
Lutein cis	127.45663	0.472747	47.2747	12	3.939558333	0.92763238
unknown asta	121.17827	0.5017964	50.17964	12	4.181636667	0.98463361
unknown lute	-	-				
Totalt	8792.34374	50.9627534	5096.27534		424.6896117	100
PB-10						
Unknown	10.71464	finn ut		11.09		0
Fucoxanthino	2138.31885	14.88415	1488.415	11.09	134.2123535	27.0542632
Fucoxanthin	3957.6814	28.57723	2857.723	11.09	257.6846709	51.943571
Clorophyll	16.75731	0	0	11.09	0	0
Idoxanthin	64.80528	0.334208	33.4208	11.09	3.013597836	0.60747515
Astaxanthin	1272.81921	5.27071	527.071	11.09	47.52669071	9.58033719
Diatoxanthin	889.33497	3.07442	307.442	11.09	27.72245266	5.58823769
Lutein	371.83148	1.37915	137.915	11.09	12.43597836	2.50682015
Astaxanthin ci	-	-				
Lutein cis	239.04728	0.886646	88.6646	11.09	7.995004509	1.61161734
unknown asta	147.1631	0.6093993	60.93993	11.09	5.495034265	1.10767824
unknown lute	-	-				
Totalt	9108.47352	55.0159133	5501.59133		496.0857827	100
PB-8						
Unknown	13.98563	finn ut		12.54	0	0
Fucoxanthino	2879.70117	20.04467	2004.467	12.54	159.8458533	23.7973843
Fucoxanthin	6750.60742	48.74411	4874.411	12.54	388.7090112	57.8698635
Clorophyll	76.39868	0	0	12.54	0	0
Idoxanthin	130.41913	0.672585	67.2585	12.54	5.363516746	0.79850472
Astaxanthin	2063.50366	8.54492	854.492	12.54	68.14130781	10.1446791
Diatoxanthin	1100.32617	3.80381	380.381	12.54	30.33341308	4.51595004
Lutein	393.44345	1.45931	145.931	12.54	11.63724083	1.73251846
Astaxanthin ci	-	-				
Lutein cis	116.24362	0.431156	43.1156	12.54	3.438245614	0.51187598
unknown asta	127.98871	0.5299989	52.99989	12.54	4.226466507	0.629224
unknown lute	-	-				
Totalt	13652.6176	84.2305599	8423.05599		671.695055	100
PB-16						
Unknown	-	-				
Fucoxanthino	4362.60791	30.36671	3036.671	11.76	258.2203231	39.5103445
Fucoxanthin	4745.79199	34.26794	3426.794	11.76	291.3940476	44.5862629
Clorophyll	36.57347		0	11.76	0	0
Idoxanthin	-	-		11.76		0
unknown lute	22.17982	0.0822668	8.22668	11.76	0.699547619	0.10703792
Astaxanthin	1703.42212	7.05383	705.383	11.76	59.98154762	9.17778889
Diatoxanthin	559.672	1.93478	193.478	11.76	16.45221088	2.51735616
Lutein	486.39429	1.80408	180.408	11.76	15.34081633	2.34730145
Astaxanthin ci	-	-		11.76		0
Lutein cis	201.02349	0.753647	75.3647	11.76	6.408562925	0.98057553
unknown asta	143.53272	0.594365	59.4365	11.76	5.05412415	0.77333257
unknown lute	-	-		11.76		0
Totalt	12261.1978	76.8576188	7685.76188		653.5511803	100
PB-6						
Fucoxanthino	1597.23193	11.11782	1111.782	10.61	104.7862394	23.8189624
Fucoxanthin	2927.98267	21.14208	2114.208	10.61	199.2655985	45.2950676
Clorophyll	72.15926	0	0	10.61	0	0
Idoxanthin	-	-				
unknown lute	45.86537	0.170118	17.0118	10.61	1.603374175	0.36446302
Astaxanthin	1945.27136	8.05532	805.532	10.61	75.92196041	17.2578225
Diatoxanthin	837.86292	2.89648	289.648	10.61	27.29952875	6.20545649
Lutein	542.43658	2.01194	201.194	10.61	18.96267672	4.31040647
Astaxanthin ci	-	-				
Lutein cis	237.64479	0.881444	88.1444	10.61	8.307672008	1.88841711
unknown asta	96.87055	0.4011385	40.11385	10.61	3.780758718	0.85940435
unknown lute	-	-				
Totalt	8303.32543	46.6763405	4667.63405		439.9278087	100
PB-12						
Fucoxanthino	1764.31384	12.28082	1228.082	10.27	119.5795521	23.8131924
Fucoxanthin	2354.21313	26.99907	2699.907	10.27	262.8925998	52.3526969
Clorophyll	18.75561	0	0	10.27	0	0
Idoxanthin	267.84485	1.3813	138.13	10.27	13.44985394	2.67841745
unknown lute	-	-				
Astaxanthin	1089.81299	4.51289	451.289	10.27	43.94245375	8.75074447
Diatoxanthin	880.2384	3.04297	304.297	10.27	29.62969815	5.90048791
Lutein	489.94708	1.81725	181.725	10.27	17.69474197	3.52374873
Astaxanthin ci	-	-				
Lutein cis	317.43746	1.177402	117.7402	10.27	11.46447907	2.2830479
unknown asta	86.88664	0.3597959	35.97959	10.27	3.503368062	0.69766424
unknown lute	-	-				
Totalt	7269.45	51.5714979	5157.14979		502.1567468	100
PB-5						
Fucoxanthino	3129.74683	21.78516	2178.516	10.66	204.3636023	29.4878921
Fucoxanthin	5246.05908	37.88022	3788.022	10.66	355.3491557	51.2737956
Clorophyll	23.72766	0	0	10.66	0	0
Idoxanthin	290.33801	1.4973	149.73	10.66	14.04596623	2.02671089
unknown lute	-	-				
Astaxanthin	1467.80969	6.07816	607.816	10.66	57.01838649	8.22725775
Diatoxanthin	997.63733	3.44882	344.882	10.66	32.35290807	4.66824353
Lutein	444.17963	1.6475	164.75	10.66	15.45497186	2.23001816
Astaxanthin ci	-	-				
Lutein cis	241.45155	0.895564	89.5564	10.66	8.401163227	1.21221486
unknown asta	155.90476	0.6455983	64.55983	10.66	6.056269231	0.87386703
unknown lute	-	-				
Totalt	11996.8545	73.8783223	7387.83223		693.0424231	100

Table 5: Raw data from HPLC, PB muscle samples continued.

	Area	Amount (ng/50microliter)	Amount (ng) in 5 ml whole sample	Weight of sample	Amount of pigment in whole sample ng/g	In %
PB-17						
Fucoxanthino	1694.22485	11.79295	1179.295	9.91	119.0005045	31.6155681
Fucoxanthin	2660.90039	19.21356	1921.356	9.91	193.8805247	51.5093861
Clorophyll	22.86655	0	0	9.91	0	0
Idoxanthin	-	-				
unknown lute	-	-				
Astaxanthin	866.70905	3.58902	358.902	9.91	36.21614531	9.6217576
Diatoxanthin	251.35713	0.868938	86.8938	9.91	8.768294652	2.32952472
Lutein	314.93323	1.16811	116.811	9.91	11.78718466	3.13157109
Astaxanthin ci	-	-				
Lutein cis	97.33299	0.3610166	36.10166	9.91	3.642952573	0.96784476
unknown asta	74.25552	0.3074906	30.74906	9.91	3.102831483	0.8243476
unknown lute	-	-				
Totalt	5982.57971	37.3010852	3730.10852		376.3984379	100
PB-18						
Fucoxanthino	3008.21997	20.93925	2093.925	12.92	162.0684985	40.3781498
Fucoxanthin	3055.42017	22.06227	2206.227	12.92	170.7606037	42.5437226
Clorophyll	64.52967	0	0	12.92	0	0
Idoxanthin	182.25551	0.939911	93.9911	12.92	7.274852941	1.812475
unknown lute	-	-				
Astaxanthin	1093.95459	4.53004	453.004	12.92	35.0622291	8.73549118
Diatoxanthin	342.34174	1.18347	118.347	12.92	9.15998452	2.28214138
Lutein	344.10291	1.27631	127.631	12.92	9.878560372	2.46116916
Astaxanthin ci	-	-				
Lutein cis	148.6135	0.55122	55.122	12.92	4.266408669	1.06294369
unknown asta	90.65554	0.3754029	37.54029	12.92	2.905595201	0.72390723
unknown lute	-	-				
Totalt	8330.0936	51.8578739	5185.78739		401.376733	100
PB-14n						
Fucoxanthino	1821.99231	12.6823	1268.23	11.23	112.9323241	30.9900755
Fucoxanthin	2695.11401	19.46061	1946.061	11.23	173.2912734	47.5533439
Clorophyll	-	-				
Idoxanthin	110.26912	0.568669	56.8669	11.23	5.063837934	1.38958196
unknown lute	-	-				
Astaxanthin	886.94971	3.67284	367.284	11.23	32.70560997	8.97483807
Diatoxanthin	623.70392	2.15613	215.613	11.23	19.19973286	5.26865249
Lutein	362.46338	1.34441	134.441	11.23	11.97159394	3.28515864
Astaxanthin ci	-	-				
Lutein cis	205.23966	0.761251	76.1251	11.23	6.778726625	1.86016937
unknown asta	67.02207	0.2775367	27.75367	11.23	2.471386465	0.67818008
unknown lute	-	-				
Totalt	6772.75418	40.9237467	4092.37467		364.4144853	100
PB-11						
Fucoxanthino	2604.71924	18.13061	1813.061	10.02	180.9442116	33.1527036
Fucoxanthin	3378.25864	24.39411	2439.411	10.02	243.4541916	44.6058185
Clorophyll	21.70895	0	0	10.02	0	0
Idoxanthin	-	-				
unknown lute	-	-				
Astaxanthin	1727.6438	7.15413	715.413	10.02	71.39850299	13.0816752
Diatoxanthin	624.70105	2.15958	215.958	10.02	21.55269461	3.94889723
Lutein	456.16776	1.69196	169.196	10.02	16.88582834	3.09383128
Astaxanthin ci	-	-				
Lutein cis	208.35458	0.773176	77.3176	10.02	7.716327345	1.41378998
unknown asta	92.87994	0.3846133	38.46133	10.02	3.838456088	0.70328416
unknown lute	-	-				
Totalt	9114.43396	54.6881793	5468.81793		545.7902126	100
PB-4						
Fucoxanthino	3447.25635	23.99524	2399.524	11.68	205.4386986	35.2179133
Fucoxanthin	4300.99951	31.05623	3105.623	11.68	265.8923801	45.5813576
Clorophyll	23.80588	0	0	11.68	0	0
Idoxanthin	-	-				
unknown lute	39.90218	0.148	14.8	11.68	1.267123288	0.21722021
Astaxanthin	1658.74243	6.86881	686.881	11.68	58.80830479	10.0813809
Diatoxanthin	700.32568	2.42101	242.101	11.68	20.72782534	3.55332642
Lutein	572.86218	2.12479	212.479	11.68	18.19169521	3.1185631
Astaxanthin ci	-	-				
Lutein cis	249.71358	0.926209	92.6209	11.68	7.929871575	1.35940079
unknown asta	143.28345	0.5933332	59.33332	11.68	5.079907534	0.8708376
Totalt	11136.8912	68.1336222	6813.36222		583.3358065	100

Table 6: Raw data from HPLC, PI muscle samples.

PI-10	Area	Amount (ng/50microliter)	Amount (ng) in 5 ml whole sample	Weight of sample	Amount of pigment in whole sample ng/g	In %
Fucoxanthino	1587.48157	11.04995	1104.995	11.74	94.12223169	25.0209785
Fucoxanthin	2217.7019	16.01336	1601.336	11.74	136.4	36.2598868
Clorophyll	8.75263	0	0	11.74	0	0
Astaxanthin	1776.45239	7.35625	735.625	11.74	62.65971039	16.6571408
Diadinochrom	913.14502	3.15673	315.673	11.74	26.88867121	7.14794849
Diatoxanthin	478.63263	1.654622429	165.4622429	11.74	14.09388781	3.74664792
Lutein	538.03802	1.99563	199.563	11.74	16.99855196	4.51880916
Astaxanthin ci	465.98245	1.92192	192.192	11.74	16.37069847	4.35190376
Lutein cis	166.35483	0.6170242	61.70242	11.74	5.25574276	1.3971601
unknown asta	95.93257	0.3972547	39.72547	11.74	3.383770869	0.89952455
Totalt	8248.47401	44.16274133	4416.274133	117.4	376.1732652	100
PI-6						
Fucoxanthino	1198.73437	8.344	834.4	11.12	75.03597122	27.8185923
Fucoxanthin	1933.69165	13.96261	1396.261	11.12	125.5630396	46.5508336
Clorophyll	-	-		11.12	0	0
Astaxanthin	573.5826	2.37519	237.519	11.12	21.3596223	7.91879702
Diadinochrom	399.06528	1.37956	137.956	11.12	12.40611511	4.59940284
Diatoxanthin	336.76724	1.1642	116.42	11.12	10.46942446	3.88140043
Lutein	294.90515	1.09383	109.383	11.12	9.836600719	3.64678941
Astaxanthin ci	221.84084	0.918637	91.8637	11.12	8.261124101	3.06270233
Lutein cis	96.36423	0.3574236	35.74236	11.12	3.214241007	1.19163728
unknown asta	96.32478	0.398878	39.8878	11.12	3.587032374	1.32984474
Totalt	5151.27614	29.9943286	2999.43286		269.7331709	100
PI-16						
Fucoxanthino	1425.09021	9.91959	991.959	10.06	98.60427435	25.4058494
Fucoxanthin	1614.04675	11.65455	1165.455	10.06	115.8503976	29.8493932
Clorophyll	-	-				0
Idoxanthin	283.37491	1.46139	146.139	10.06	14.52673956	3.74288194
Astaxanthin	1330.59558	5.50996	550.996	10.06	54.77097416	14.111996
Diadinochrom	700.30579	2.42095	242.095	10.06	24.06510934	6.20048723
Diatoxanthin	508.58893	1.75818	175.818	10.06	17.47693837	4.50301437
Lutein	719.81616	2.66986	266.986	10.06	26.53936382	6.83799039
Astaxanthin ci	587.97516	2.43479	243.479	10.06	24.2026839	6.23593395
Lutein cis	241.12844	0.894365	89.4365	10.06	8.890308151	2.2906292
unknown asta	77.48831	0.3208773	32.08773	10.06	3.189635189	0.82182433
Totalt	7488.41024	39.0445123	3904.45123		388.1164245	100
PI-11						
Fucoxanthino	1134.74707	7.89861	789.861	12.43	63.54473049	25.947359
Fucoxanthin	1589.51831	11.47743	1147.743	12.43	92.33652454	37.7039754
Clorophyll	30.01157	0	0	12.43	0	0
Idoxanthin	-	-		12.43	0	0
Astaxanthin	1182.21191	4.89551	489.551	12.43	39.38463395	16.0820139
Diadinochrom	674.41302	2.33143	233.143	12.43	18.75647627	7.65887305
Diatoxanthin	207.50963	0.717357	71.7357	12.43	5.771174578	2.35655636
Lutein	355.47617	1.31849	131.849	12.43	10.607321	4.33131062
Astaxanthin ci	297.52512	1.23204	123.204	12.43	9.911826227	4.04731772
Lutein cis	73.72248	0.2734428	27.34428	12.43	2.199861625	0.89827432
unknown asta	71.62356	0.2965917	29.65917	12.43	2.386095736	0.9743197
Totalt	5616.75884	30.4409015	3044.09015		244.8986444	100
PI-5						
Fucoxanthino	1689.44348	11.75967	1175.967	10.76	109.2906134	29.954654
Fucoxanthin	2505.57007	18.09197	1809.197	10.76	168.1409851	46.0845162
Clorophyll	-	-		10.76	0	0
Idoxanthin	-	-		10.76	0	0
Astaxanthin	904.56836	3.7458	374.58	10.76	34.81226766	9.54143637
Diadinochrom	514.42609	1.77836	177.836	10.76	16.52750929	4.5299025
Diatoxanthin	290.60297	1.00461	100.461	10.76	9.336524164	2.55897869
Lutein	283.58911	1.05186	105.186	10.76	9.775650558	2.67933559
Astaxanthin ci	241.23413	0.998945	99.8945	10.76	9.283875465	2.5445486
Lutein cis	113.4883	0.420937	42.0937	10.76	3.912053903	1.07222585
unknown asta	98.06576	0.4060881	40.60881	10.76	3.774052974	1.0344022
Totalt	6640.98827	39.2582401	3925.82401		364.8535325	100
PI-18						
Fucoxanthino	2552.11401	17.76444	1776.444	14	126.8888571	30.1456991
Fucoxanthin	2482.33618	17.9242	1792.42	14	128.03	30.4168068
Clorophyll	25.23825	0	0	14	0	0
Idoxanthin	-	-		14	0	0
Astaxanthin	2502.58252	10.36313	1036.313	14	74.02235714	17.5859075
Diadinochrom	1298.73462	4.4897	448.97	14	32.06928571	7.61888048
Diatoxanthin	470.15396	1.62531	162.531	14	11.60935714	2.75810024
Lutein	779.85852	2.89256	289.256	14	20.66114286	4.90858385
Astaxanthin ci	661.30029	2.73843	273.843	14	19.56021429	4.64703006
Lutein cis	186.80562	0.692916	69.2916	14	4.9494	1.17585678
unknown asta	105.752813	0.4379192	43.79192	14	3.127994286	0.74313519
Totalt	11064.8768	58.9286052	5892.86052		420.9186086	100
PI-17						
Fucoxanthino	2131.6	14.8373786	1483.73786	10.53	140.9057797	27.1194248
Fucoxanthin	3005.12305	21.69909	2169.909	10.53	206.0692308	39.6611056
Clorophyll	-		0	10.53	0	0
Idoxanthin	-		0	10.53	0	0
Astaxanthin	1598.83142	6.62072	662.072	10.53	62.87483381	12.1012022
Diadinochrom	948.28784	3.27822	327.822	10.53	31.13219373	5.99185632
Diatoxanthin	633.80609	2.19106	219.106	10.53	20.80778727	4.00476988
Lutein	667.27124	2.47496	247.496	10.53	23.50389364	4.52367587
Astaxanthin ci	535.05511	2.21565	221.565	10.53	21.04131054	4.04971492
Lutein cis	257.52469	0.955181	95.5181	10.53	9.071044634	1.74585821
unknown asta	106.01342	0.4389989	43.89989	10.53	4.169030389	0.80239225
Totalt	9883.51286	54.7112585	5471.12585		519.5751045	100

Table 7: Raw data from HPLC, PI muscle samples continued.

	Area	Amount (ng/50microliter)	Amount (ng) in 5 ml whole sample	Weight of sample	Amount of pigment in whole sample ng/g	In %
PI-12						
Fucoxanthino	2607.39575	18.14924	1814.924	10.73	169.1448276	34.1756553
Fucoxanthin	2531.95117	18.28968	1828.968	10.73	170.4536813	34.4401088
Clorophyll	9.61183	-		10.73	0	0
Idoxanthin	-	-				0
Astaxanthin	1200.55664	4.97148	497.148	10.73	46.33252563	9.36147117
Diadinochrom	813.12811	2.81097	281.097	10.73	26.1972973	5.29315508
Diatoxanthin	627.08527	2.16782	216.782	10.73	20.20335508	4.08208108
Lutein	645.58929	2.39454	239.454	10.73	22.31630941	4.50900279
Astaxanthin ci	560.80927	2.3223	232.23	10.73	21.64305685	4.37297233
Lutein cis	459.53395	1.704449	170.4449	10.73	15.88489282	3.20953724
unknown asta	71.40705	0.2952766	29.52766	10.73	2.751878844	0.55601619
Totatl	9527.06833	53.1057556	5310.57556		494.9278248	100
PI-4						
Fucoxanthino	1945.44543	13.54162	1354.162	10.15	133.4149754	32.7027139
Fucoxanthin	1994.18298	14.3994	1439.94	10.15	141.8660099	34.7742337
Clorophyll	-	-				0
Idoxanthin	-	-				0
Astaxanthin	899.97192	3.72676	372.676	10.15	36.71684729	9.00004328
Diadinochrom	553.53131	1.91355	191.355	10.15	18.85270936	4.62118109
Diatoxanthin	458.09366	1.58362	158.362	10.15	15.60216749	3.82440741
Lutein	630.91309	2.34011	234.011	10.15	23.05527094	5.65131409
Astaxanthin ci	500.98383	2.07456	207.456	10.15	20.43901478	5.01001669
Lutein cis	386.5138	1.4336117	143.36117	10.15	14.1242532	3.46214067
unknown asta	95.39132	0.3950136	39.50136	10.15	3.891759606	0.95394914
Totalt	7465.02734	41.4082453	4140.82453		407.9630079	100
PI-14v						
Fucoxanthino	1014.28345	7.0601	706.01	11.28	62.58953901	12.5836992
Fucoxanthin	3388.06689	24.46421	2446.421	11.28	216.8812943	43.6042352
Clorophyll	-	-		11.28	0	0
Idoxanthin	-	-		11.28	0	0
Astaxanthin	2950.05957	12.21612	1221.612	11.28	108.2989362	21.7736264
Diadinochrom	1548.99	5.35483	535.483	11.28	47.47189716	9.54427986
Diatoxanthin	629.9668	2.17779	217.779	11.28	19.30664894	3.88162411
Lutein	585.06012	2.17004	217.004	11.28	19.23794326	3.86781075
Astaxanthin ci	482.91452	1.99974	199.974	11.28	17.72819149	3.56427341
Lutein cis	55.68491	0.2065399	20.65399	11.28	1.831027482	0.36813019
unknown asta	110.05954	0.4557536	45.57536	11.28	4.040368794	0.81232082
Totalt	10765.0858	56.1051235	5610.51235		497.3858466	100
PI-9						
Fucoxanthino	1742.0553	12.12589	1212.589	10.62	114.1797552	18.6051603
Fucoxanthin	5075.26221	36.64694	3664.694	10.62	345.0747646	56.2286308
Clorophyll	29.577692	-		10.62	0	0
Idoxanthin	307.86429	1.58769	158.769	10.62	14.95	2.43604609
Astaxanthin	1301.81104	5.39077	539.077	10.62	50.76054614	8.27123945
Diadinochrom	940.62848	3.25174	325.174	10.62	30.61902072	4.98925389
Diatoxanthin	769.62811	2.66059	266.059	10.62	25.05263653	4.08223259
Lutein	420.22507	1.55865	155.865	10.62	14.67655367	2.39148904
Astaxanthin ci	331.11932	1.37116	137.116	10.62	12.91111111	2.10381684
Lutein cis	41.7997	0.1550381	15.50381	10.62	1.459869115	0.23788016
unknown asta	102.97268	0.4264072	42.64072	10.62	4.01513371	0.65425089
Totalt	11062.9439	65.1748753	6517.48753		613.6993908	100
PI-15v						
Fucoxanthino	908.49628	6.32375	632.375	11.21	56.41168599	13.9794811
Fucoxanthin	3244.40723	23.42689	2342.689	11.21	208.9820696	51.7882215
Clorophyll	-	-		11.21	0	0
Idoxanthin	298.83765	1.54114	154.114	11.21	13.74790366	3.40689266
Astaxanthin	1468.8512	6.08248	608.248	11.21	54.25941124	13.446122
Diadinochrom	876.2771	3.02928	302.928	11.21	27.02301517	6.69662186
Diatoxanthin	537.05878	1.8566	185.66	11.21	16.56199822	4.10425848
Lutein	365.80099	1.35679	135.679	11.21	12.10338983	2.99936274
Astaxanthin ci	289.64594	1.19942	119.942	11.21	10.69955397	2.65147566
Lutein cis	26.10071	0.0968097	9.68097	11.21	0.863601249	0.21401057
unknown asta	77.94841	0.3227826	32.27826	11.21	2.879416592	0.71355339
Totalt	8093.42429	45.2359423	4523.59423		403.5320455	100
PI-14						
Fucoxanthino	1017.30615	7.08114	708.114	10.68	66.30280899	12.7323896
Fucoxanthin	3453.58594	24.93731	2493.731	10.68	233.495412	44.8390437
Clorophyll	-	-		10.68	0	0
Idoxanthin	-	-		10.68	0	0
Astaxanthin	2891.59082	11.97401	1197.401	10.68	112.1161985	21.5301152
Diadinochrom	1443.5332	4.99027	499.027	10.68	46.72537453	8.97285771
Diatoxanthin	695.29523	2.40362	240.362	10.68	22.50580524	4.32187843
Lutein	535.54083	1.98637	198.637	10.68	18.59897004	3.57163347
Astaxanthin ci	425.38211	1.7615	176.15	10.68	16.49344569	3.16730134
Lutein cis	55.76487	0.2068362	20.68362	10.68	1.936668539	0.37190609
unknown asta	63.78035	0.274113	27.4113	10.68	2.566601124	0.49287452
Totalt	10581.7795	55.6151692	5561.51692	117.48	520.7412846	100
PI-3						
Fucoxanthino	2207.3728	15.36481	1536.481	13.99	109.8270908	21.8588969
Fucoxanthin	4156.97021	30.01623	3001.623	13.99	214.5548964	42.7028825
Clorophyll	-	-		13.99	0	0
Idoxanthin	-	-		13.99	0	0
Astaxanthin	3181.57935	13.17484	1317.484	13.99	94.17326662	18.7433147
Diadinochrom	1502.11609	5.19279	519.279	13.99	37.11786991	7.38757336
Diatoxanthin	529.71002	1.8312	183.12	13.99	13.08934954	2.60517455
Lutein	626.73236	2.3246	232.46	13.99	16.6161544	3.30711487
Astaxanthin ci	493.48492	2.04351	204.351	13.99	14.60693352	2.90721944
Lutein cis	39.38235	0.146072	14.6072	13.99	1.044117227	0.20781076
unknown asta	34.28476	0.1419813	14.19813	13.99	1.014877055	0.20199108
unknown lute	13.24382	0.0548423	5.48423	13.99	0.392010722	0.07802193
Totalt	12784.8767	70.2908756	7029.08756		502.4365661	100

Table 8: Raw data from HPLC, PI muscle samples continued.

	Area	Amount (ng/50microliter)	Amount (ng) in 5 ml whole sample	Weight of sample	Amount of pigment in whole sample ng/g	In %
PI-8						
Fucoxanthino	1820.94775	12.67503	1267.503	11.15	113.6773991	18.0864308
Fucoxanthin	5532.31104	39.94716	3994.716	11.15	358.2704933	57.0019592
Clorophyll	18.39879	-		11.15	0	0
Idoxanthin	-	-		11.15	0	0
Astaxanthin	1923.84314	7.96659	796.659	11.15	71.44923767	11.3677978
Diadinochrom	1162.9187	4.02019	402.019	11.15	36.0555157	5.73654564
Diatoxanthin	585.97656	2.02571	202.571	11.15	18.16780269	2.89055439
Lutein	363.16031	1.34699	134.699	11.15	12.0806278	1.92206578
Astaxanthin ci	309.22256	1.28048	128.048	11.15	11.48412556	1.8271604
Lutein cis	-	-		11.15	0	0
unknown asta	197.58105	0.818178	81.8178	11.15	7.337919283	1.16748597
Totalt	11914.3599	70.080328	7008.0328		628.5231211	100
PI-1						
Fucoxanthino	1620.63159	11.28069	1128.069	10.99	102.6450409	17.2396416
Fucoxanthin	4582.38477	33.08802	3308.802	10.99	301.0738854	50.5665527
Clorophyll	67.7118	0	0	10.99	0	0
Idoxanthin	282.00073	1.45431	145.431	10.99	13.23303003	2.22253986
Astaxanthin	2094.54639	8.67347	867.347	10.99	78.92147407	13.2551745
Diadinochrom	1211.02197	4.18648	418.648	10.99	38.09353958	6.39796101
Diatoxanthin	641.81079	2.21873	221.873	10.99	20.18862602	3.39075978
Lutein	520.63342	1.93107	193.107	10.99	17.5711556	2.95114525
Astaxanthin ci	399.73727	1.6553	165.53	10.99	15.06187443	2.52970153
Lutein cis	46.16756	0.171239	17.1239	10.99	1.558134668	0.2616949
unknown asta	187.22338	0.775288	77.5288	10.99	7.054485896	1.18482888
Totalt	11653.8697	65.434597	6543.4597		595.4012466	100
PI-15						
Fucoxanthino	1516.39746	10.55515	1055.515	13.44	78.53534226	13.7543191
Fucoxanthin	5529.88184	39.92962	3992.962	13.44	297.0953869	52.0319214
Clorophyll	27.60083	0	0	13.44	0	0
Idoxanthin	489.80969	2.526	252.6	13.44	18.79464286	3.29160742
Astaxanthin	2478.04272	10.26151	1026.151	13.44	76.35052083	13.3716795
Diadinochrom	1452.96326	5.02287	502.287	13.44	37.37254464	6.5452558
Diatoxanthin	915.36963	3.16442	316.442	13.44	23.54479167	4.12352666
Lutein	623.08429	2.31107	231.107	13.44	17.19546131	3.01153411
Astaxanthin ci	489.61075	2.02747	202.747	13.44	15.08534226	2.64197755
Lutein cis	68.22286	0.2530442	25.30442	13.44	1.882769345	0.32973957
unknown asta	166.49881	0.6894676	68.94676	13.44	5.129967262	0.8984389
Totalt	13757.4821	76.7406218	7674.06218		570.9867693	100
PI-7						
Fucoxanthino	1779.66016	12.38764	1238.764	10.23	121.0913001	21.7857319
Fucoxanthin	4133.39062	29.84597	2984.597	10.23	291.7494624	52.4891181
Clorophyll	45.24955	0	0	10.23	0	0
Idoxanthin	-	-		10.23	0	0
Astaxanthin	1676.57336	6.94265	694.265	10.23	67.8655914	12.2098084
Diadinochrom	887.72272	3.06884	306.884	10.23	29.99843597	5.39706719
Diatoxanthin	534.97736	1.84941	184.941	10.23	18.07829912	3.25249606
Lutein	331.37271	1.22909	122.909	10.23	12.014565	2.16155984
Astaxanthin ci	251.76654	1.04256	104.256	10.23	10.19120235	1.83351571
Lutein cis	29.22584	0.108401	10.8401	10.23	1.059638319	0.19064125
unknown asta	93.3817	0.3866915	38.66915	10.23	3.779975562	0.68006152
Totalt	9763.32056	56.8612525	5686.12525		555.8284702	100
PI-13						
Fucoxanthino	981.91931	6.83482	683.482	9.85	69.38903553	18.4184163
Fucoxanthin	2727.94092	19.69764	1969.764	9.85	199.9760406	53.0810372
Clorophyll	41.96231	0	0	9.85	0	0
Idoxanthin	0	0	0	9.85	0	0
Astaxanthin	1105.76147	4.57893	457.893	9.85	46.48659898	12.3392626
Diadinochrom	570.90558	1.97361	197.361	9.85	20.03664975	5.31846788
Diatoxanthin	397.25012	1.37329	137.329	9.85	13.94203046	3.70073052
Lutein	345.8067	1.28263	128.263	9.85	13.02162437	3.4564207
Astaxanthin ci	254.37285	1.05335	105.335	9.85	10.69390863	2.83855886
Lutein cis	37.30559	0.1383695	13.83695	9.85	1.404766497	0.37287698
unknown asta	42.4971	0.1759798	17.59798	9.85	1.786596954	0.47422891
Totalt	6505.72195	37.1086193	3710.86193		376.7372518	100
PI-2						
Fucoxanthino	1867.55469	12.99945	1299.945	11.49	113.1370757	21.9037656
Fucoxanthin	4050.06396	29.2443	2924.43	11.49	254.5195822	49.2759535
Clorophyll	35.43066	0	0	11.49	0	0
Idoxanthin	244.60947	1.26148	126.148	11.49	10.97893821	2.12556395
Astaxanthin	1767.71936	7.32008	732.008	11.49	63.70826806	12.3341616
Diadinochrom	874.13159	3.02186	302.186	11.49	26.29991297	5.09176259
Diatoxanthin	402.1207	1.39012	139.012	11.49	12.09852045	2.3423193
Lutein	514.242	1.90737	190.737	11.49	16.6002611	3.21387331
Astaxanthin ci	399.79819	1.65555	165.555	11.49	14.40861619	2.78956257
Lutein cis	52.08503	0.1931873	19.31873	11.49	1.68135161	0.32551603
unknown asta	85.63635	0.3546172	35.46172	11.49	3.086311575	0.59752159
Totalt	10293.392	59.3480145	5934.80145		516.5188381	100

Table 9: Raw data from HPLC, TB muscle samples.

TB-5	Area	Amount (ng/50microliter)	Amount (ng) in 5 ml whole sample	Weight of sample (g)	Amount of pigment in whole sample ng/g	In %
Unknown	9.70498	0	0	11.13	0	0
Fucoxanthino	248.04129	1.72654	172.654	11.13	15.51248877	9.16180568
Fucoxanthin	445.83243	3.21922	321.922	11.13	28.92380952	17.082644
Unknown lute	29.09097	0.107901	10.7901	11.13	0.969460916	0.57257173
Chlorophyll	-		0	11.13	0	0
Idoxanthin	-		0	11.13	0	0
Astaxanthin	1349.8877	5.58985	558.985	11.13	50.22327044	29.6622838
Diatoxanthin	119.2691	0.412311	41.2311	11.13	3.704501348	2.1879095
Lutein	933.87622	3.46382	346.382	11.13	31.1214735	18.3806027
Astaxanthin ci	876.39569	3.62913	362.913	11.13	32.60673854	19.2578127
Lutein cis	187.70218	0.6962031	69.62031	11.13	6.25519407	3.69436996
Chlorophyll						
Chlorophyll						
Chlorophyll						
Totalt	4199.80056	18.8449751	1884.49751		169.3169371	100
TB-6						
Unknown	-					
Fucoxanthino	-					
Fucoxanthin	-					
Unknown lute	60.13533	0.223047	22.3047	10.68	2.088455056	1.78130044
Chlorophyll	-					
Idoxanthin	-					
Astaxanthin	1342.18066	5.55794	555.794	10.68	52.0406367	44.3868823
Diatoxanthin	-					
Lutein	857.52399	3.18063	318.063	10.68	29.78117978	25.4011827
Astaxanthin ci	707.03821	2.92783	292.783	10.68	27.41413858	23.3822685
Lutein cis	170.42912	0.6321353	63.21353	10.68	5.91886985	5.04836597
Chlorophyll						
Chlorophyll						
Chlorophyll						
Totalt	3137.30731	12.5215823	1252.15823		117.24328	100
TB-12						
Unknown	-					
Fucoxanthino	-					
Fucoxanthin	-					
Unknown lute	72.04163	0.267208	26.7208	10.07	2.653505462	1.55851114
Chlorophyll	-					
Idoxanthin	-					
Astaxanthin	1447.20093	5.99282	599.282	10.07	59.51161867	34.9535819
Diatoxanthin	-					
Lutein	1421.08069	5.27091	527.091	10.07	52.34270109	30.7429865
Astaxanthin ci	1142.65369	4.7317	473.17	10.07	46.98808342	27.5980029
Lutein cis	237.91404	0.8824432	88.24432	10.07	8.763090367	5.14691759
Chlorophyll	6.32229		0	10.07	0	
Totalt	4327.21327	17.1450812	1714.50812		170.258999	100
TB-2v						
Unknown						
Fucoxanthino	5.84383	0.040677	4.0677	12.99	0.313140878	0.16362275
Fucoxanthin	29.15054	0.210487	21.0487	12.99	1.620377213	0.84668145
Unknown lute	130.67593	0.484688	48.4688	12.99	3.731239415	1.94965171
Chlorophyll	-					
Idoxanthin	-					
Astaxanthin	1825.51111	7.5594	755.94	12.99	58.19399538	30.4075965
Diatoxanthin	-					
Lutein	2324.76855	8.62276	862.276	12.99	66.3799846	34.6849494
Astaxanthin ci	1822.87634	7.54849	754.849	12.99	58.1100077	30.3637111
Lutein cis	106.15366	0.3937332	39.37332	12.99	3.031048499	1.58378711
Chlorophyll	7.09189		0	12.99	0	0
Totalt	6252.07185	24.8602352	2486.02352	103.92	191.3797937	100
TB-17						
Unknown	-					
Fucoxanthino	6.61297	0.0460307	4.60307	13.12	0.35084375	0.24586347
Fucoxanthin	8.79482	0.0635048	6.35048	13.12	0.484030488	0.33919776
Unknown lute	58.62139	0.217432	21.7432	13.12	1.657256098	1.16136808
Chlorophyll	-		0	13.12	0	0
Idoxanthin	-		0	13.12	0	0
Astaxanthin	1219.7854	5.0511	505.11	13.12	38.4992378	26.9794065
Diatoxanthin	-		0	13.12	0	0
Lutein	1679.01575	6.22761	622.761	13.12	47.46653963	33.2634915
Astaxanthin ci	1281.5094	5.3067	530.67	13.12	40.44740854	28.3446411
Lutein cis	487.90503	1.80968	180.968	13.12	13.79329268	9.66603163
Chlorophyll	-		0	13.12	0	0
Totalt	4742.24476	18.7220575	1872.20575	131.2	142.698609	100

Table 10: Raw data from HPLC, TB muscle samples continued.

	Area	Amount (ng/50microliter)	Amount (ng) in 5 ml whole sample	Weight of sample (g)	Amount of pigment in whole sample ng/g	In %
TB-4						
Unknown	-					
Fucoxanthino	63.20988	0.439984	43.9984	10.49	4.194318398	1.98940729
Fucoxanthin	-					
Unknown lute	87.7149	0.325342	32.5342	10.49	3.101448999	1.47104837
Chlorophyll	-					
Idoxanthin	-					
Astaxanthin	1438.53198	5.95693	595.693	10.49	56.78674929	26.9345248
Diatoxanthin	-					
Lutein	1959.81848	7.26913	726.913	10.49	69.29580553	32.8676956
Astaxanthin ci	1567.53479	6.49112	649.112	10.49	61.87912297	29.3498887
Lutein cis	440.49563	1.63383	163.383	10.49	15.57511916	7.38743524
Chlorophyll						
Totalt	5557.30566	22.116336	2211.6336		210.8325643	100
TB-9v						
Unknown	-					
Fucoxanthino	23.25135	0.161845	16.1845	9.17	1.764940022	1.02815759
Fucoxanthin	60.59494	0.437538	43.7538	9.17	4.771406761	2.77956078
Unknown lute	112.80363	0.418398	41.8398	9.17	4.562682661	2.65796953
Chlorophyll	-					
Idoxanthin	-					
Astaxanthin	1741.78186	7.21268	721.268	9.17	78.65517993	45.8202086
Diatoxanthin	-					
Lutein	1057.11816	3.92094	392.094	9.17	42.75834242	24.9086732
Astaxanthin ci	814.38959	3.37237	337.237	9.17	36.77611778	21.4237561
Lutein cis	58.63786	0.217493	21.7493	9.17	2.371788441	1.38167431
Chlorophyll	12.71245		0	9.17	0	0
Totalt	3881.28984	15.741264	1574.1264		171.660458	100
TB-18						
Unknown	-					
Fucoxanthino	-					
Fucoxanthin	-					
Unknown lute	68.16195	0.252818	25.2818	9.71	2.603686921	1.5635393
Chlorophyll	-					
Idoxanthin	212.12666	1.09396	109.396	9.71	11.26632338	6.76553666
Astaxanthin	951.75739	3.9412	394.12	9.71	40.58908342	24.374139
Diatoxanthin	-					
Lutein	1391.76208	5.16216	516.216	9.71	53.16333677	31.9251003
Astaxanthin ci	1117.24927	4.6265	462.65	9.71	47.64675592	28.6123399
Lutein cis	294.67087	1.0929588	109.29588	9.71	11.25601236	6.7593448
Chlorophyll	13.40372		0	9.71	0	0
Totalt	4049.13194	16.1695968	1616.95968	67.97	166.5251988	100
TB-10						
Unknown	-					
Fucoxanthino	8.33433	0.0590126	5.90126	12.71	0.464300551	0.27884265
Fucoxanthin	-					
Unknown lute	75.91174	0.281563	28.1563	12.71	2.215287175	1.33042387
Chlorophyll	-					
Idoxanthin	212.7401	1.09712	109.712	12.71	8.631943352	5.18404278
Astaxanthin	998.62109	4.13527	413.527	12.71	32.53556255	19.5397191
Diatoxanthin	-					
Lutein	1961.47485	7.27527	727.527	12.71	57.24051928	34.3766506
Astaxanthin ci	1537.44714	6.36653	636.653	12.71	50.09071597	30.0827292
Lutein cis	525.36895	1.94864	194.864	12.71	15.33154996	9.20759181
Chlorophyll	10.74341		0	12.71	0	0
Chlorophyll	8.84598		0	12.71	0	0
Chlorophyll	32.78532		0	12.71	0	0
Totalt	5372.27291	21.1634056	2116.34056		166.5098788	100
TB-16						
Unknown	-					
Fucoxanthino	-					
Fucoxanthin	-					
Unknown lute	21.75408	0.0806877	8.06877	11.51	0.701022589	1.03563738
Chlorophyll	-					
Idoxanthin	-					
Astaxanthin	604.80188	2.50447	250.447	11.51	21.75907906	32.145206
Diatoxanthin	-					
Lutein	628.34558	2.33059	233.059	11.51	20.2483927	29.9134331
Astaxanthin ci	515.96381	2.13659	213.659	11.51	18.56290182	27.4234172
Lutein cis	199.18057	0.7387774	73.87774	11.51	6.418569939	9.48230633
Chlorophyll	5.52583		0	11.51	0	0
Totalt	1975.57175	7.7911151	779.11151		67.68996612	100
TB-11						
Unknown	-					
Fucoxanthino	34.41434	0.239547	23.9547	10.96	2.18564781	0.87048843
Fucoxanthin	8.55217	0.0617526	6.17526	10.96	0.563436131	0.22440241
Unknown lute	154.74689	0.573969	57.3969	10.96	5.236943431	2.08574256
Chlorophyll	-					
Idoxanthin	-					
Astaxanthin	1283.06738	5.31315	531.315	10.96	48.47764599	19.3074244
Diatoxanthin	-					
Lutein	2667.15186	9.89269	989.269	10.96	90.26177007	35.9489878
Astaxanthin ci	1897.01978	7.85551	785.551	10.96	71.67436131	28.5460914
Lutein cis	965.75724	3.58207	358.207	10.96	32.68312044	13.016863
Chlorophyll	10.03041		0	10.96	0	0
Chlorophyll	25.26008		0	10.96	0	0
Totalt	7046.00015	27.5186886	2751.86886		251.0829252	100

Table 11: Raw data from HPLC, TB muscle samples continued.

	Area	Amount (ng/50microliter)	Amount (ng) in 5 ml whole sample	Weight of sample (g)	Amount of pigment in whole sample ng/g	In %
TB-8						
Unknown	-					
Fucoxanthino	-					
Fucoxanthin	18.64984	0.134665	13.4665	11.13	1.209928122	0.33837773
Unknown lute	196.48279	0.728771	72.8771	11.13	6.547807727	1.83120989
Chlorophyll	-					
Idoxanthin	-					
Astaxanthin	4077.30127	16.884	1688.4	11.13	151.6981132	42.4250523
Diatoxanthin	-					
Lutein	3172.2019	11.76596	1176.596	11.13	105.7139263	29.5647636
Astaxanthin ci	2411.32666	9.98524	998.524	11.13	89.7146451	25.0902825
Lutein cis	80.50629	0.2986043	29.86043	11.13	2.682877808	0.75031409
Chlorophyll	7.64992		0	11.13	0	0
Totalt	9964.11867	39.7972403	3979.72403		357.5672983	100
TB-2						
Unknown	-					
Fucoxanthino	-					
Fucoxanthin	30.42824	0.219713	21.9713	11.87	1.850994103	0.83479907
Unknown lute	165.52936	0.613962	61.3962	11.87	5.172384162	2.33274729
Chlorophyll	-					
Idoxanthin	-					
Astaxanthin	1926.34668	7.97695	797.695	11.87	67.20261163	30.3084043
Diatoxanthin	-					
Lutein	2511.99268	9.31719	931.719	11.87	78.4935973	35.4006433
Astaxanthin ci	1904.2854	7.8856	788.56	11.87	66.43302443	29.9613202
Lutein cis	82.46047	0.3058525	30.58525	11.87	2.57668492	1.16208591
Chlorophyll	10.13376		0	11.87	0	0
Totalt	6631.17659	26.3192675	2631.92675		221.7292965	100
TB-15						
Unknown	-					
Fucoxanthino	-					
Fucoxanthin	34.11559	0.246338	24.6338	12.77	1.929036805	0.63157431
Unknown lute	210.54576	0.780932	78.0932	12.77	6.115364135	2.00219451
Chlorophyll	-					
Idoxanthin	-					
Astaxanthin	2845.87939	11.78472	1178.472	12.77	92.2844166	30.2142846
Diatoxanthin	-					
Lutein	3746.72778	13.89693	1389.693	12.77	108.8248238	35.6296796
Astaxanthin ci	2904.84473	12.02889	1202.889	12.77	94.19647612	30.8403004
Lutein cis	71.71378	0.2659929	26.59929	12.77	2.082951449	0.68196658
Chlorophyll	37.05774		0	12.77	0	0
Totalt	9850.88477	39.0038029	3900.38029		305.4330689	100
TB-3						
Unknown	-					
Fucoxanthino	18.09945	0.125984	12.5984	10.76	1.170855019	0.43604951
Fucoxanthin	29.52682	0.213204	21.3204	10.76	1.981449814	0.737931
Unknown lute	158.22755	0.586879	58.6879	10.76	5.454265799	2.03127618
Chlorophyll	-					
Idoxanthin	440.59402	2.27219	227.219	10.76	21.11700743	7.86439015
Astaxanthin	2216.00488	9.17642	917.642	10.76	85.28271375	31.760965
Diatoxanthin	-					
Lutein	2352.38794	8.7252	872.52	10.76	81.08921933	30.199225
Astaxanthin ci	1810.52856	7.49735	749.735	10.76	69.67797398	25.9494521
Lutein cis	79.50889	0.2949052	29.49052	10.76	2.740754647	1.0207111
Chlorophyll						
Totalt	7104.87811	28.8921322	2889.21322		268.5142398	100
TB-9						
Unknown	-					
Fucoxanthino	139.58698	0.97162	97.162	11.81	8.227095682	3.82189309
Fucoxanthin	451.60388	3.2609	326.09	11.81	27.61134632	12.8268368
Unknown lute	186.00189	0.689897	68.9897	11.81	5.841634208	2.71372819
Chlorophyll	-					
Idoxanthin	-					
Astaxanthin	2399.07642	9.93452	993.452	11.81	84.1195597	39.0776985
Diatoxanthin	132.91103	0.459471	45.9471	11.81	3.890524979	1.80734139
Lutein	1415.84033	5.25147	525.147	11.81	44.46629975	20.6567968
Astaxanthin ci	1111.48218	4.60262	460.262	11.81	38.97222693	18.1045281
Lutein cis	67.93651	0.2519818	25.19818	11.81	2.133630821	0.99117711
Chlorophyll	28.40012		0	11.81	0	0
Totalt	5932.83934	25.4224798	2542.24798		215.2623184	100
TB-1						
Unknown	-					
Fucoxanthino	-					
Fucoxanthin	14.21537	0.102645	10.2645	10	1.02645	0.42451232
Unknown lute	148.47699	0.550714	55.0714	10	5.50714	2.27760609
Chlorophyll	-					
Idoxanthin	-					
Astaxanthin	1451.02	6.00863587	600.863587	10	60.0863587	24.8501139
Diatoxanthin	-					
Lutein	2507	9.2986728	929.86728	10	92.986728	38.4568284
Astaxanthin ci	1912.49915	7.91961	791.961	10	79.1961	32.7533928
Lutein cis	80.67564	0.2992327	29.92327	10	2.992327	1.23754656
Chlorophyll	14.6896		0	10	0	0
Chlorophyll	14.39439		0	10	0	0
Chlorophyll	49.01331		0	10	0	0
Totalt	6191.98445	24.17951037	2417.951037		241.7951037	100

Table 12: Raw data from HPLC, TB muscle samples continued.

	Area	Amount (ng/50microliter)	Amount (ng) in 5 ml whole sample	Weight of sample (g)	Amount of pigment in whole sample ng/g	In %
TB-7						
Unknown	-					
Fucoxanthino	-					
Fucoxanthin	-					
Unknown lute	80.5205	0.298657	29.8657	11.86	2.518187184	1.88716811
Chlorophyll	-					
Idoxanthin	-					
Astaxanthin	1398.5	5.79115192	579.115192	11.86	48.8292742	36.5934073
Diatoxanthin	-					
Lutein	1384.38647	5.1348	513.48	11.86	43.29510961	32.4460194
Astaxanthin ci	1068.67651	4.42536	442.536	11.86	37.31332209	27.9631761
Lutein cis	47.37052	0.1757012	17.57012	11.86	1.481460371	1.11022913
Chlorophyll	-					
Chlorophyll	-					
Chlorophyll	-					
Totalt	3979.454	15.82567012	1582.567012		133.4373535	100
TB-14						
Unknown	-					
Fucoxanthino	-					
Fucoxanthin	-					
Unknown lute	97.88731	0.363072	36.3072	10.33	3.514733785	2.49196966
Chlorophyll	-					
Idoxanthin	147.1483	0.758859	75.8859	10.33	7.346166505	5.20848097
Astaxanthin	1194.69482	4.9472	494.72	10.33	47.89157793	33.9554477
Diatoxanthin	-					
Lutein	1198.00024	4.44348	444.348	10.33	43.01529526	30.4981308
Astaxanthin ci	935.31934	3.87313	387.313	10.33	37.49399806	26.5834943
Lutein cis	49.59145	0.1839388	18.39388	10.33	1.780627299	1.26247661
Chlorophyll	-					
Chlorophyll	-					
Chlorophyll	-					
Totalt	3622.64146	14.5696798	1456.96798		141.0423988	100
TB-13	?????					
Unknown	-					
Fucoxanthino	-					
Fucoxanthin	-					
Unknown lute	208.73714	0.774223	77.4223	10.45	7.408832536	1.83724187
Chlorophyll	-					
Idoxanthin	458.7623	2.36589	236.589	10.45	22.64009569	5.61428962
Astaxanthin	2277.62988	9.43161	943.161	10.45	90.25464115	22.3813407
Diatoxanthin	-					
Lutein	4210.24072	15.61613	1561.613	10.45	149.4366507	37.057292
Astaxanthin ci	3226.97949	13.36284	1336.284	10.45	127.874067	31.7102038
Lutein cis	159.01836	0.589812	58.9812	10.45	5.644133971	1.39963202
Chlorophyll	-					
Chlorophyll	-					
Chlorophyll	-					
Totalt	10541.3679	42.140505	4214.0505	62.7	403.2584211	100

Table 13: Raw data from HPLC, TI muscle samples.

TI-3	Area	Amount (ng/50microliter)	Amount (ng) in 5 ml whole sample	Weight of sample	Amount of pigment in whole sample n	In %
Unknown	41.52628	0	0	9.32	0	0
Unknown lute	184.76477	0.685308	68.5308	9.32	7.353090129	2.53168681
Chlorophyll	33.28526	0	0	9.32	0	0
Idoxanthin	192.73029	0.99393	99.393	9.32	10.66448498	3.67180811
Astaxanthin	1690.51001	7.00036	700.036	9.32	75.1111588	25.8609546
Diatoxanthin	47.17151	0.163071	16.3071	9.32	1.749688841	0.60242212
Lutein	2526.36475	9.3705	937.05	9.32	100.5418455	34.6168019
Astaxanthin ci	1980.5387	8.20136	820.136	9.32	87.99742489	30.2977274
Lutein cis	176.51172	0.654696	65.4696	9.32	7.024635193	2.41859898
Chlorophyll	63.38132	?		9.32	0	0
Chlorophyll	12.0593	?		9.32	0	0
Chlorophyll	51.07677	?		9.32	0	0
Totalt	6999.92068	27.069225	2706.9225		290.4423283	100
TI-7						
Unknown	32.17622	0	0	10.54	0	0
Fucoxanthino	10.58206	0.0736583	7.36583	10.54	0.698845351	0.49612427
Fucoxanthin	56.31337	0.406622	40.6622	10.54	3.857893738	2.73879579
Unknown lute	80.60223	0.29896	29.896	10.54	2.836432638	2.01364016
Chlorophyll	-	-				
Idoxanthin	-	-				
Astaxanthin	975.51135	4.03957	403.957	10.54	38.32609108	27.2084573
Diatoxanthin	-	-				
Lutein	1797.24353	6.66613	666.613	10.54	63.24601518	44.899609
Astaxanthin ci	765.92596	3.17168	317.168	10.54	30.09184061	21.3627985
Lutein cis	51.25896	0.1901237	19.01237	10.54	1.803830171	1.28057505
Chlorophyll	14.96746		0	10.54	0	0
Chlorophyll	32.26964		0	10.54	0	0
Totalt	3816.85078	14.846744	1484.6744		140.8609488	100
TI-1						
Unknown	39.1062	0	0	12.02	0	0
Fucoxanthino	8.13435	0.0566206	5.66206	12.02	0.471053245	0.25247591
Fucoxanthin	13.45931	0.0971856	9.71856	12.02	0.808532446	0.43335859
Unknown lute	193.88716	0.719144	71.9144	12.02	5.982895175	3.20672227
Chlorophyll	132.86673	0	0	12.02	0	0
Idoxanthin	-	-		12.02	0	0
Astaxanthin	1539.59167	6.37541	637.541	12.02	53.04001664	28.4284778
Diatoxanthin	-	-		12.02	0	0
Lutein	2130.28882	7.90142	790.142	12.02	65.73560732	35.2330819
Astaxanthin ci	1624.62573	6.72754	672.754	12.02	55.96955075	29.9986544
Lutein cis	147.96603	0.548819	54.8819	12.02	4.565881864	2.44722908
Totalt	5829.926	22.4261392	2242.61392		186.5735374	100
TI-15						
Unknown	33.57552	0	0	9.77	0	0
Fucoxanthino	-					
Fucoxanthin	-					
Unknown lute	114.29685	0.423937	42.3937	9.77	4.339170931	2.0450487
Chlorophyll	20.15618	0	0	9.77	0	0
Idoxanthin	-	-				
Astaxanthin	1086.84045	4.50058	450.058	9.77	46.06530194	21.7105496
Diatoxanthin	-	-				
Lutein	2230.92969	8.2747	827.47	9.77	84.69498465	39.9166963
Astaxanthin ci	1717.34375	7.11148	711.148	9.77	72.78894575	34.3053872
Lutein cis	113.02669	0.419225	41.9225	9.77	4.290941658	2.02231827
Chlorophyll	62.16527		0	9.77	0	0
Chlorophyll	10.87039		0	9.77	0	0
Chlorophyll	20.71394		0	9.77	0	0
Chlorophyll	47.1376		0	9.77	0	0
Totalt	5457.05633	20.729922	2072.9922		212.1793449	100
TI-11						
Unknown	26.76645	0	0	10.31	0	0
Fucoxanthino	99.10077	0.689809	68.9809	10.31	6.690678952	3.29687509
Fucoxanthin	157.4559	1.13694	113.694	10.31	11.02754607	5.43389426
Unknown lute	153.17249	0.56813	56.813	10.31	5.510475267	2.71532213
Chlorophyll	151.13129	0	0	10.31	0	0
Idoxanthin	46.56541	0.240143	24.0143	10.31	2.329224054	1.14774014
Astaxanthin	898.73694	3.72165	372.165	10.31	36.09747818	17.7872646
Diatoxanthin	-	-				0
Lutein	1849.58313	6.86026	686.026	10.31	66.53986421	32.7879461
Astaxanthin ci	1458.39417	6.03917	603.917	10.31	58.57584869	28.8636262
Lutein cis	449.44097	1.667014	166.7014	10.31	16.16890398	7.96733144
Chlorophyll	186.3091		0	10.31	0	0
Chlorophyll	64.80898		0	10.31	0	0
Chlorophyll	61.35386		0	10.31	0	0
Chlorophyll	128.48743		0	10.31	0	0
Totalt	5731.30689	20.923116	2092.3116	144.34	202.9400194	100
TI-13						
Unknown	38.87924	0	0	10.17	0	0
Fucoxanthino	107.49944	0.748269	74.8269	10.17	7.357610619	5.44230459
Fucoxanthin	111.06078	0.801937	80.1937	10.17	7.885319567	5.8326423
Unknown lute	51.3856	0.190593	19.0593	10.17	1.874070796	1.38621961
Chlorophyll	-	-				
Idoxanthin	69.14035	0.356564	35.6564	10.17	3.506037365	2.59335866
Astaxanthin	957.48669	3.96493	396.493	10.17	38.98652901	28.8376997
Diatoxanthin	-	-				
Lutein	1079.50244	4.00396	400.396	10.17	39.37030482	29.1215724
Astaxanthin ci	841.65515	3.48527	348.527	10.17	34.27010816	25.3490402
Lutein cis	53.27398	0.1975972	19.75972	10.17	1.942941986	1.4371625
Chlorophyll	8.38481		0	10.17	0	0
Chlorophyll	19.40376		0	10.17	0	0
Totalt	3337.67224	13.7491202	1374.91202		135.1929223	100

Table 14: Raw data from HPLC, TI muscle samples continued.

	Area	Amount (ng/50microliter)	Amount (ng) in 5 ml whole sample	Weight of sample	Amount of pigment in whole sample n	In %
TI-17						
Unknown	27.51276	0	0	11.5	0	0
Fucoxanthino	-					
Fucoxanthin	-					
Unknown lute	126.403	0.468839	46.8839	11.5	4.07686087	1.79279129
Chlorophyll	-					
Idoxanthin	-					
Astaxanthin	1419.21765	5.87695	587.695	11.5	51.10391304	22.472842
Diatoxanthin	-					
Lutein	2607.10083	9.66995	966.995	11.5	84.08652174	36.9768772
Astaxanthin ci	2029.19897	8.40286	840.286	11.5	73.06834783	32.1316576
Lutein cis	467.1635	1.732744	173.2744	11.5	15.06733913	6.6258318
Totalt	6676.59671	26.151343	2615.1343		227.4029826	100
			0			
			0			
			0			
TI-18			0			
Unknown						
Fucoxanthino	26.00607	0.18102	18.102	10.19	1.776447498	0.65157291
Fucoxanthin	14.99638	0.108284	10.8284	10.19	1.062649657	0.38976313
Unknown lute	105.41989	0.391011	39.1011	10.19	3.83720314	1.40742557
Chlorophyll	-					
Idoxanthin	-					
Astaxanthin	1818.57996	7.53069	753.069	10.19	73.90274779	27.1063619
Diatoxanthin	-					
Lutein	2499.73511	9.27173	927.173	10.19	90.98851816	33.3731529
Astaxanthin ci	1978.39587	8.19249	819.249	10.19	80.39735034	29.4884796
Lutein cis	568.00488	2.106777	210.6777	10.19	20.67494603	7.583244
Totalt	7011.13816	27.782002	2778.2002		272.6398626	100
TI-12						
Unknown						
Fucoxanthino	30.48481	0.212195	21.2195	11.08	1.915117329	0.82339746
Fucoxanthin	52.87994	0.38183	38.183	11.08	3.446119134	1.48164589
Unknown lute	139.96254	0.519133	51.9133	11.08	4.685315884	2.01443385
Chlorophyll	-					
Idoxanthin	-					
Astaxanthin	1512.54968	6.26343	626.343	11.08	56.52915162	24.3044951
Diatoxanthin	-	-				
Lutein	2322.63892	8.61486	861.486	11.08	77.75144404	33.4289395
Astaxanthin ci	1835.26343	7.59978	759.978	11.08	68.5900722	29.4900423
Lutein cis	587.59471	2.179437	217.9437	11.08	19.67000903	8.45704603
Chlorophyll	8.78419		0	11.08	0	0
Totalt	6490.15822	25.770665	2577.0665		232.5872292	100
TI-6						
Unknown						
Fucoxanthinol						
Fucoxanthin						
Unknown lute	56.06812	0.207961	20.7961	10.41	1.997704131	1.05264628
Chlorophyll	-					
Idoxanthin	470.741	2.42766	242.766	10.41	23.3204611	12.2882044
Astaxanthin	1623.28247	6.72197	672.197	10.41	64.57223823	34.0249217
Diatoxanthin	-					
Lutein	1340.73523	4.9729	497.29	10.41	47.77041306	25.1715692
Astaxanthin ci	1099.96216	4.55492	455.492	10.41	43.75523535	23.0558596
Lutein cis	234.72326	0.870608	87.0608	10.41	8.363189241	4.40679876
Chlorophyll	17.62793		0	10.41	0	0
Chlorophyll	29.46696		0	10.41	0	0
Totalt	4872.60713	19.756019	1975.6019		189.7792411	100
TI-4						
Unknown	-					
Fucoxanthino	46.42904	0.323178	32.3178	10.72	3.014720149	1.44769836
Fucoxanthin	47.4314	0.342488	34.2488	10.72	3.194850746	1.53419885
Unknown lute	91.65342	0.33995	33.995	10.72	3.171175373	1.5228297
Chlorophyll	-					
Idoxanthin	219.98131	1.13447	113.447	10.72	10.58274254	5.08193738
Astaxanthin	1198.08569	4.96124	496.124	10.72	46.28022388	22.2242201
Diatoxanthin	-					
Lutein	1988.06665	7.37391	737.391	10.72	68.78647388	33.0319434
Astaxanthin ci	1548.61279	6.41277	641.277	10.72	59.82061567	28.7264498
Lutein cis	387.04075	1.435567	143.5567	10.72	13.39148321	6.43072236
Unknown asta	218.04671		0	10.72	0	0
Chlorophyll	7.07851		0	10.72	0	0
Chlorophyll	21.50833		0	10.72	0	0
Chlorophyll	40.47		0	10.72	0	0
Totalt	5814.4046	22.323573	2232.3573		208.2422854	100
TI-5						
Unknown	-					
Fucoxanthino	22.43524	0.156165	15.6165	10.66	1.464962477	0.90496773
Fucoxanthin	7.918	0.0571735	5.71735	10.66	0.536336773	0.33131734
Unknown lute	98.17397	0.364135	36.4135	10.66	3.415900563	2.11014262
Chlorophyll	-					
Idoxanthin	-					
Astaxanthin	1052.80396	4.35964	435.964	10.66	40.89718574	25.2638779
Diatoxanthin	-					
Lutein	1636.14905	6.06861	606.861	10.66	56.92879925	35.1672666
Astaxanthin ci	1113.0271	4.606861	460.6861	10.66	43.21633208	26.6965103
Lutein cis	443.19035	1.643832	164.3832	10.66	15.42056285	9.5259175
Chlorophyll	5.33331		0	10.66	0	0
Chlorophyll	21.91235		0	10.66	0	0
Chlorophyll	31.62561		0	10.66	0	0
Totalt	4432.56894	17.2564165	1725.64165		161.8800797	100

Table 15: Raw data from HPLC, TI muscle samples continued.

	Area	Amount (ng/50microliter)	Amount (ng) in 5 ml whole sample	Weight of sample	Amount of pigment in whole sample n	In %
TI-10						
Unknown	-					
Fucoxanthino	24.79956	0.172622	17.2622	9.99	1.727947948	1.10678782
Fucoxanthin	19.76199	0.142695	14.2695	9.99	1.428378378	0.91490707
Unknown lute	79.1152	0.293445	29.3445	9.99	2.937387387	1.88145979
Chlorophyll	-					
Idoxanthin	-					
Astaxanthin	1135.5105	4.70212	470.212	9.99	47.06826827	30.1482381
Diatoxanthin	-					
Lutein	1319.6947	4.89486	489.486	9.99	48.9975976	31.384015
Astaxanthin ci	1070.06897	4.43113	443.113	9.99	44.35565566	28.4107514
Lutein cis	258.77018	0.959794	95.9794	9.99	9.607547548	6.15384083
Chlorophyll	14.30517		0	9.99	0	0
Chlorophyll	6.80482		0	9.99	0	0
Totalt	3928.83109	15.596666	1559.6666		156.1227828	100
TI-2						
Unknown	-					
Fucoxanthino	30.4103	0.211676	21.1676	11.55	1.832692641	0.98951884
Fucoxanthin	71.5032	0.516303	51.6303	11.55	4.470155844	2.41355442
Unknown lute	176.707	0.655421	65.5421	11.55	5.674640693	3.06388739
Chlorophyll	-					
Idoxanthin	-					
Astaxanthin	1770.34167	7.33094	733.094	11.55	63.47134199	34.2698428
Diatoxanthin	-					
Lutein	1764.65308	6.54525	654.525	11.55	56.66883117	30.5969887
Astaxanthin ci	1376.48291	5.69998	569.998	11.55	49.35047619	26.6456169
Lutein cis	116.53579	0.432241	43.2241	11.55	3.74234632	2.02059096
Unknown asta	73.3603		0	11.55	0	0
Chlorophyll	7.84565		0	11.55	0	0
Chlorophyll	6.28573		0	11.55	0	0
Chlorophyll	23.64407		0	11.55	0	0
Chlorophyll	48.32499		0	11.55	0	0
Totalt	5466.09469	21.391811	2139.1811		185.2104848	100
TI-8						
Unknown	-					
Fucoxanthino	-					
Fucoxanthin	-					
Unknown lute	39.23896	0.145541	14.5541	11.07	1.314733514	0.79448196
Chlorophyll	-					
Idoxanthin	446.38141	2.30204	230.204	11.07	20.79530262	12.5664195
Astaxanthin	2204.35181	9.12817	912.817	11.07	82.45862692	49.8290271
Diatoxanthin	-					
Lutein	938.65808	3.48156	348.156	11.07	31.4504065	19.0052056
Astaxanthin ci	745.88013	3.08867	308.867	11.07	27.90126468	16.8604902
Lutein cis	46.64225	0.173	17.3	11.07	1.562782294	0.94437567
Unknown asta	-					
Chlorophyll	8.10858		0	11.07	0	0
Totalt	4429.26122	18.318981	1831.8981		165.4831165	100
TI-9						
Unknown	-					
Fucoxanthino	-					
Fucoxanthin	-					
Unknown lute	80.91472	0.300119	30.0119	9.56	3.139320084	2.01588397
Chlorophyll	-					
Idoxanthin	84.12421	0.433837	43.3837	9.56	4.538043933	2.91406094
Astaxanthin	837.09143	3.46638	346.638	9.56	36.25920502	23.2834972
Diatoxanthin	-					
Lutein	1444.30505	5.35705	535.705	9.56	56.03608787	35.9830308
Astaxanthin ci	1111.52258	4.60279	460.279	9.56	48.14633891	30.916705
Lutein cis	78.98544	0.727536	72.7536	9.56	7.610209205	4.8868221
Unknown astaxanthinlike						
Chlorophyll	12.93778			9.56	0	0
Totalt	3649.88121	14.887712	1488.7712		155.729205	100
TI-14						
Unknown	-					
Fucoxanthino	16.1802	0.112625	11.2625	11.01	1.022933697	0.62278716
Fucoxanthin	40.4602	0.292151	29.2151	11.01	2.653505904	1.61551957
Unknown lute	128.27939	0.475799	47.5799	11.01	4.321516803	2.63104557
Chlorophyll	-					
Idoxanthin	-					
Astaxanthin	1233.07312	5.10613	510.613	11.01	46.37720254	28.23558
Diatoxanthin	-					
Lutein	1682.69421	6.24125	624.125	11.01	56.68710263	34.5125004
Astaxanthin ci	1314.84888	5.44476	544.476	11.01	49.45286104	30.1081165
Lutein cis	110.89316	0.4113123	41.13123	11.01	3.73580654	2.27445078
Unknown asta	157.98315	0.65420391		11.01	0	0
Chlorophyll	11.75953			11.01	0	0
Totalt	4696.17184	18.73823121	1808.40273		164.2509292	100
TI-16						
Unknown	-					
Fucoxanthino	151.55867	1.05495	105.495	12.31	8.569861901	6.9565279
Fucoxanthin	125.2087	0.904094	90.4094	12.31	7.344386677	5.96175661
Unknown lute	36.05717	0.133739	13.3739	12.31	1.08642567	0.88189875
Chlorophyll	-					
Idoxanthin	-					
Astaxanthin	1017.93958	4.21526	421.526	12.31	34.24256702	27.796174
Diatoxanthin	-					
Lutein	1064.49377	3.9483	394.83	12.31	32.07392364	26.0357923
Astaxanthin ci	897.09418	3.71484	371.484	12.31	30.17741673	24.4963156
Lutein cis	321.83393	1.19370982	119.370982	12.31	9.697074086	7.87153483
Unknown asta	-					
Chlorophyll	7.737		0	12.31	0	0
Totalt	3621.923	15.16489282	1516.489282		123.1916557	100

Table 16: Raw data from VIS of RD muscle samples. Analyzed in 2020.

	Wavelength	Abs	ng in 100 ml sample		ng/g
RD-1	476.41	0.285	0.13834951	10.43	0.01326457
RD-2	475.57	0.175	0.08495146	10.28	0.00826376
RD-3	477.24	0.161	0.07815534	9.34	0.00836781
RD-4	475.57	0.167	0.08106796	10.62	0.00763352
	449.05	0.151	0.07330097		
RD-5	473.91	0.128	0.06213592	10.71	0.00580167
RD-6	475.57	0.23	0.11165049	12.35	0.00904053
RD-7	473.91	0.165	0.08009709	9.41	0.00851191
RD-8	474.32	0.179	0.0868932	10.66	0.00815133
RD-9	474.93	0.097	0.04708738	10.48	0.00449307
RD-10	467.36	0.154	0.07475728	12.09	0.0061834
RD-11	475.57	0.234	0.11359223	10.46	0.01085968
RD-12	476.41	0.329	0.15970874	10.44	0.01529777
RD-13	477.24	0.304	0.14757282	11.71	0.01260229
RD-14	472.09	0.212	0.10291262	10.32	0.00997215
RD-15	475.16	0.178	0.08640777	10.21	0.00846305
RD-16	473.91	0.12	0.05825243	10	0.00582524
RD-17	473.91	0.156	0.07572816	12.43	0.00609237
RD-18	474.74	0.195	0.09466019	15.47	0.00611895

Table 17: Raw data from VIS of PB muscle samples. Analyzed in 2020.

	Wavelength	Abs	ng	Weight of fish (g)	
PB-1	448.05	0.789	0.4753012	12	0.03960843
	474.93	0.687	0.41385542		
			0		
PB-2	447.22	1.139	0.68614458	10.71	0.06406579
	471.1	1.031	0.62108434		
	423.47	0.92	0.55421687		
			0		
PB-3	446.8	1.019	0.61385542	10.63	0.05774745
mg/g	471.42	0.92	0.55421687		
			0		
PB-4	448.05	0.989	0.59578313	11.68	0.05100883
	473.67	0.865	0.52108434		
			0		
PB-5	447.64	1.057	0.63674699	10.66	0.05973236
	474.3	0.898	0.54096386		
			0		
PB-6	450.97	0.733	0.44156627	10.61	0.04161793
	476.51	0.644	0.38795181		
			0		
PB-7	448.47	0.707	0.42590361	9.79	0.04350394
	475.56	0.619	0.37289157		
			0		
PB-8	447.64	1.318	0.7939759	12.54	0.06331546
	471.78	1.174	0.70722892		
			0		
PB-9	452.22	0.936	0.56385542	12.66	0.04453834
	475.26	0.872	0.5253012		
			0		
PB-10	448.05	0.897	0.54036145	11.09	0.04872511
	473.67	0.777	0.46807229		
			0		
PB-11	448.05	0.783	0.47168675	10.02	0.04707453
	475.25	0.682	0.41084337		
			0		
PB-12	450.14	0.864	0.52048193	10.27	0.05067984
	476.2	0.747	0.45		
			0		
PB-13	447.22	1.297	0.7813253	11.25	0.06945114
	472.06	1.163	0.70060241		
	422.2	1.031	0.62108434		
			0		
PB-14	448.05	0.631	0.38012048	11.23	0.03384866
	476.2	0.522	0.31445783		
			0		
PB-15	448.05	0.81	0.48795181	11.2	0.04356713
	475.26	0.705	0.4246988		
			0		
PB-16	446.8	1.091	0.65722892	111.76	0.00588072
	473.35	0.963	0.58012048		
	419.39	0.815	0.49096386		
			0		
PB-17	445.55	0.552	0.33253012	9.91	0.03355501
	472.09	0.48	0.28915663		
			0		
PB-18	447.64	0.839	0.50542169	12.92	0.03911933
	474.3	0.74	0.44578313		

Table 18: Raw data from VIS on PI muscle samples. Analyzed in 2020.

	Wavelength	Abs		Weight of fish (g)	(…/g) in total
PI-1	448.89	0.921	0.55481928	10.99	0.05048401
	474.62	0.742	0.44698795		
			0		
PI-2	447.22	0.859	0.51746988	11.49	0.04503654
			0		
PI-3	448.89	1.023	0.61626506	13.99	0.0440504
	474.62	0.924	0.55662651		
			0		
PI-4	449.72	0.592	0.35662651	10.15	0.03513562
	476.85	0.484	0.29156627		
			0		
PI-5	446.38	0.554	0.33373494	10.76	0.03101626
	475.88	0.442	0.26626506		
			0		
PI-6	446.38	0.469	0.28253012	11.12	0.02540738
	475.25	0.371	0.22349398		
			0		
PI-7	448.05	0.788	0.4746988	10.23	0.04640262
	472.38	0.706	0.4253012		
			0		
PI-8	446.38	0.93	0.56024096	11.15	0.05024583
	470.78	0.833	0.50180723		
			0		
PI-9	447.22	0.942	0.56746988	10.62	0.05343408
			0		
PI-10	449.72	0.562	0.33855422	11.74	0.02883767
	480.3	0.474	0.28554217		
			0		
PI-11	448.89	0.422	0.25421687	12.43	0.02045188
	474.62	0.367	0.22108434		
			0		
PI-12	449.3	0.877	0.52831325	10.73	0.04923702
	475.26	0.747	0.45		
			0		
PI-13	448.47	0.605	0.36445783	9.85	0.0370008
			0		
PI-14	452.22	0.753	0.45361446	10.68	0.04247326
			0		
PI-14v	453.06	0.823	0.49578313	11.28	0.04395241
	476.53	0.721	0.43433735		
			0		
PI-15	449.72	1.023	0.61626506	13.44	0.04585306
	474.62	0.897	0.54036145		
PI-15v	448.89	0.839	0.50542169	11.21	0.04508668
			0		
PI-16	450.97	0.569	0.34277108	10.06	0.03407267
	480.3	0.474	0.28554217		
			0		
PI-17	448.89	0.846	0.50963855	10.53	0.04839872
	475.89	0.707	0.42590361		
			0		
PI-18	450.97	0.816	0.49156627	14	0.03511188
	475.56	0.739	0.44518072		

Table 19: Raw data from VIS on TB muscle samples. Analyzed in 2020.

	Wavelength	Abs	mg?	fisk i gram	mg/g?
TB-1	449.72	0.499	0.24223301	10	0.0242233
	476.53	0.487	0.23640777		
	421.56	0.374	0.1815534		
			0		
TB-2	450.14	0.451	0.21893204	11.87	0.01844415
	476.85	0.44	0.21359223		
	424.11	0.321	0.15582524		
	396.63	0.181	0.08786408		
			0		
TB-2v	449.72	0.445	0.21601942	12.99	0.01662967
	476.51	0.435	0.21116505		
	420.97	0.3	0.14563107		
	399.19	0.221	0.10728155		
			0		
TB-3	476.41	0.596	0.28932039	10.76	0.02688851
	450.64	0.595	0.28883495		
	424.43	0.454	0.22038835		
	397.58	0.335	0.16262136		
			0		
TB-4	449.3	0.345	0.16747573	10.49	0.01596527
	475.26	0.335	0.16262136		
	421.88	0.251	0.12184466		
	398.54	0.174	0.08446602		
			0		
TB-5	475.16	0.327	0.15873786	11.13	0.01426216
	450.63	0.313	0.15194175		
	420.97	0.222	0.10776699		
			0		
TB-6	473.49	0.248	0.12038835	10.68	0.01127232
	451.26	0.235	0.11407767		
	424.12	0.166	0.08058252		
			0		
TB-7	450.55	0.303	0.14708738	11.86	0.01240197
	476.21	0.296	0.14368932		
	422.2	0.218	0.10582524		
			0		
TB-8	477.24	0.643	0.31213592	11.13	0.02804456
	450	0.595	0.28883495		
	424.75	0.405	0.19660194		
	398.22	0.23	0.11165049		
			0		
TB-9	475.57	0.41	0.19902913	11.81	0.01685259
	450.96	0.389	0.18883495		
	424.11	0.293	0.14223301		
	396.31	0.209	0.10145631		
			0		
TB-9v	475.57	0.302	0.14660194	9.17	0.01598713
	450.64	0.296	0.14368932		
	423.47	0.213	0.10339806		
	398.54	0.151	0.07330097		
			0		
TB-10	448.89	0.406	0.19708738	12.71	0.01550648
	474.3	0.373	0.18106796		
	422.84	0.303	0.14708738		
	398.54	0.213	0.10339806		
			0		
TB-11	449.3	0.543	0.26359223	10.96	0.02405039
	475.57	0.518	0.25145631		
	423.16	0.393	0.1907767		
	396.31	0.241	0.11699029		
			0		
TB-12	475.16	0.379	0.18398058	10.07	0.01827017
	450.32	0.383	0.18592233		
	423.49	0.292	0.14174757		
			0		
TB-13	476.41	0.672	0.32621359	10.45	0.03121661
	450.14	0.679	0.32961165		
	423.16	0.473	0.22961165		
			0		
TB-14	475.57	0.278	0.13495146	10.33	0.01306403
	450.96	0.275	0.13349515		
	422.2	0.183	0.08883495		
			0		
TB-15	476.41	0.643	0.31213592	12.77	0.02444291
	450.55	0.642	0.31165049		
	422.84	0.44	0.21359223		
	396.95	0.272	0.13203883		
			0		
TB-16	449.3	0.19	0.09223301	11.51	0.00801329
	474.62	0.179	0.0868932		
	421.24	0.144	0.06990291		
			0		
TB-17	449.3	0.507	0.2461165	13.12	0.01875888
	475.25	0.492	0.23883495		
	419.39	0.345	0.16747573		
	397.3	0.221	0.10728155		
			0		
TB-18	449.3	0.643	0.31213592	9.71	0.03214582
	476.21	0.622			
	421.56	0.56			
	399.18	0.518			

Table 20: Raw data from VIS of TI muscle samples. Analyzed in 2020.

	Wavelength	Abs			
TI-1	450.12	0.393	0.1907767	12.02	0.01587161
	476.83	0.369	0.17912621		
	422.23	0.279	0.13543689		
	399.5	0.188	0.09126214		
			0		
TI-2	450.97	0.36	0.17475728	11.55	0.0151305
	476.53	0.352	0.17087379		
	422.52	0.262	0.12718447		
	395.99	0.193	0.09368932		
			0		
TI-3	450.14	0.516	0.25048544	9.32	0.02687612
	476.2	0.494	0.23980583		
	422.86	0.381	0.18495146		
	399.82	0.267	0.12961165		
			0		
TI-4	449.3	0.372	0.18058252	10.72	0.01684538
	475.25	0.362	0.17572816		
	420.97	0.247	0.11990291		
	396.03	0.167	0.08106796		
			0		
TI-5	450.14	0.348	0.16893204	10.66	0.01584728
	476.21	0.331	0.16067961		
	421.24	0.248	0.12038835		
	397.9	0.172	0.08349515		
			0		
TI-6	474.74	0.34	0.16504854	10.41	0.01585481
	451.89	0.322	0.15631068		
	419.7	0.21	0.10194175		
			0		
TI-7	450.55	0.215	0.10436893	10.54	0.00990218
	474.62	0.211	0.10242718		
	423.81	0.154	0.07475728		
			0		
TI-8	476.41	0.385	0.1868932	11.07	0.01688285
	449.68	0.345	0.16747573		
	420.92	0.227	0.11019417		
	399.5	0.151	0.07330097		
			0		
TI-9	449.72	0.278	0.13495146	9.56	0.01411626
	476.85	0.262	0.12718447		
	423.16	0.206	0.1		
	400.78	0.13	0.0631068		
			0		
TI-10	449.3	0.31	0.15048544	9.99	0.01506361
	475.89	0.29	0.1407767		
	424.75	0.241	0.11699029		
	399.82	0.193	0.09368932		
			0		
TI-11	448.47	0.394	0.19126214	10.31	0.01855113
	475.25	0.347	0.1684466		
	418.13	0.29	0.1407767		
			0		
TI-12	449.72	0.412	0.2	11.08	0.01805054
	475.25	0.401	0.19466019		
	420.97	0.277	0.13446602		
	401.08	0.176	0.08543689		
			0		
TI-13	450.14	0.25	0.12135922	10.17	0.01193306
	475.56	0.245	0.11893204		
	417.81	0.154	0.07475728		
			0		
TI-14	449.72	0.346	0.16796117	11.01	0.01525533
	476.53	0.331	0.16067961		
	423.17	0.248	0.12038835		
	397.9	0.186	0.09029126		
			0		
TI-15	449.72	0.35	0.16990291	9.77	0.01739027
	476.51	0.335	0.16262136		
	419.39	0.233	0.1131068		
			0		
TI-16	450.14	0.257	0.12475728	12.31	0.01013463
	475.26	0.248	0.12038835		
	422.52	0.193	0.09368932		
			0		
TI-17	449.3	0.431	0.2092233	11.5	0.01819333
	475.25	0.417	0.20242718		
	420.97	0.28	0.13592233		
	397.93	0.178	0.08640777		
			0		
TI-18	449.3	0.47	0.22815534	10.19	0.02239012
	475.56	0.451	0.21893204		
	420.02	0.314	0.15242718		
	396.66	0.212	0.10291262		

Table 21: Skin samples analyzed in 2020. Wrong identification of peaks in TB and TI. It should be lutein, not diatoxanthin.

PB-7	Area	Amount (ng) in the injected sample.	In total sample	Weight of sample (g)		ng/g
Fucoxanthino	250.4631	1.74339	17.4339	0.72		24.21375
Fucoxanthin	274.05807	1.97889	19.7889	0.72		27.48458333
Klorofyll	136.61169	0		0.72		
PB-15						
Fucoxanthino	405.74921	2.82429	28.2429	1		28.2429
Fucoxanthin	453.66766	3.2758	32.758	1		32.758
Klorofyll	62.2592	0		1		
PB-14						
Fucoxanthino	94.20996	0.655765	6.55765	0.75		8.743533333
Fucoxanthin	227.3438	1.64158	16.4158	0.75		21.88773333
Klorofyll	8.86849	0		0.75		
TI-13		I 50 microliter	*10=5ml			
Diatoxanthin	49.86621	0.172387	1.72387	1.31		1.315931298
Klorofyll	26.73468	0		1.31		
	33.27968	0.115047				
TB-5						
Diatoxanthin	33.27968	0.115047	1.15047	0.72		1.597875
TI-8						
Diatoxanthin	17.73825	0.0613209	0.613209	0.81		0.757048148
TB-4						
Diatoxanthin	51.63737	0.17851	1.7851	1.45		1.231103448
TI-17						
Diatoxanthin	59.78049	0.20666	2.0666	1.17		1.766324786

Table 22: Table of Kimberly's pigment analysis on muscle from HPLC. Analyzed in 2019

	RT	value from calib	Area	PI-T2-2	ng in whole sample (5ml)	Weight of sample	ng/g pigment in sample
450	44.45	24.82656599	3566.58447	Fucoxanthino	2482.656599	14.19	174.9581817
450	51.65	38.02570359	5266.17969	Fucoxanthin	3802.570359	14.19	267.97536
480	63.77	6.155698166	1486.53955	Astaxanthin a	615.5698166	14.19	43.38053676
450	63.77	2.034433194	588.50049	Diadinochrom	203.4433194	14.19	14.33709087
450	66.4	2.032682338	587.99402	Diatoxanthin	203.2682338	14.19	14.32475221
450	67.8	1.990200438	536.57794	Lutein?	199.0200438	14.19	14.02537306
	RT		Area	PI-T9-1			
450	44.34	15.88867221	2282.56665	Fucoxanthino	1588.867221	16.25	97.77644438
450	51.48	41.4522928	5740.72803	Fucoxanthino	4145.22928	16.25	255.0910326
480	63.42	9.182395917	2217.45679	Astaxanthin	918.2395917	16.25	56.5070518
450	63.21	7.314229301	2115.78711	Diadinochrom	731.4229301	16.25	45.01064185
450	66.23	3.161515816	914.53168	Diatoxanthin	316.1515816	16.25	19.45548194
450	67.7	2.53162672	682.55188	Lutein?	253.162672	16.25	15.57924135
	RT		Area	PI-T9-3			
450	44.25	7.748926772	1113.21082	Fucoxanthino	774.8926772	15.05	51.48788553
450	51.39	25.99475536	3600.01367	Fucoxanthino	2599.475536	15.05	172.722627
480	60.4	1.867734774	362.17245	Idoxanthin	186.7734774	15.05	12.41019783
480	63.74	6.817313802	1646.31311	Astaxanthin	681.7313802	15.05	45.29776613
450	64.07	2.991179901	865.25861	Diadinochrom	299.1179901	15.05	19.87494951
450	66.34	2.73848249	792.16083	Diatoxanthin	273.848249	15.05	18.19589695
450	67.8	2.158712585	582.0105	Lutein?	215.8712585	15.05	14.34360521
				PI-T9-4			
450	44.6	18.04756801	2592.71362	Fucoxanthino	1804.756801	19.08	94.58893086
450	51.75	45.81839945	6345.39014	Fucoxanthino	4581.839945	19.08	240.1383619
480	63.74	17.36872152	4194.37256	Astaxanthin	1736.872152	19.08	91.03103522
450	63.71	9.015656584	2607.95898	Diadinochrom	901.5656584	19.08	47.25186889
450	66.46	5.419334877	1567.651	Diatoxanthin	541.9334877	19.08	28.40322263
450	67.92	4.266845295	1150.38416	Lutein?	426.6845295	19.08	22.36292083
				PI-T9-5			
450	44.45	6.2994306	904.9762	Fucoxanthino	629.94306	15.60	40.38096538
450	51.54	16.23029208	2247.73315	Fucoxanthino	1623.029208	15.60	104.0403338
480	60.2	1.395600278	270.62085	Idoxanthin	139.5600278	15.60	8.946155631
480	63.5	8.378784132	2023.39258	Astaxanthin	837.8784132	15.60	53.71015469
450	63.45	6.622742559	1915.76074	Diadinochrom	662.2742559	15.60	42.45347794
450	66.33	2.017686763	583.65625	Diatoxanthin	201.7686763	15.60	12.93388951
450	67.79	2.659142688	716.93146	Lutein?	265.9142688	15.60	17.04578646
				PI-T9-6			
450	44.42	7.378055965	1059.93152	Fucoxanthino	737.8055965	15.79	46.72613024
450	51.57	21.89534891	3032.28687	Fucoxanthino	2189.534891	15.79	138.6659209
480	63.67	9.592592405	2316.51514	Astaxanthin	959.2592405	15.79	60.7510602
450	63.67	7.391103156	2138.02441	Diadinochrom	739.1103156	15.79	46.8087597
450	66.4	2.227821827	644.44202	Diatoxanthin	222.7821827	15.79	14.10906794
450	67.87	2.174422425	586.24603	Lutein?	217.4422425	15.79	13.770883
				PI-T11-1			
450	44.04	6.713291104	964.4314	Fucoxanthino	671.3291104	19.46	34.49789879
450	51.64	18.40046761	2548.28076	Fucoxanthino	1840.046761	19.46	94.55533204
480	63.37	14.55748503	3515.48706	Astaxanthin	1455.748503	19.46	74.80722009
450	63.37	10.61104601	3069.45728	Diadinochrom	1061.104601	19.46	54.5274718
450	66.34	2.389604971	691.24103	Diatoxanthin	238.9604971	19.46	12.27957334
450	67.797	3.494270947	942.09039	Lutein?	349.4270947	19.46	17.95617136
				PI-T11-2			
450	44.5	11.55674642	1660.24219	Fucoxanthino	1155.674642	19.70	58.66368739
450	51.59	39.06736198	5410.43896	Fucoxanthino	3906.736198	19.70	198.3114821
480	63.37	10.47880293	2530.52612	Astaxanthin	1047.880293	19.70	53.19189305
450	63.19	5.939532409	1718.12854	Diadinochrom	593.9532409	19.70	30.14991071
450	66.3	3.722103882	1076.69299	Diatoxanthin	372.2103882	19.70	18.89392834
450	67.76	3.087532695	832.42969	Lutein?	308.7532695	19.70	15.6727548
				PI-T11-3			
450	44.41	10.83228721	1556.16638	Fucoxanthino	1083.228721	15.36	70.52270316
450	51.51	27.88228204	3861.41724	Fucoxanthino	2788.228204	15.36	181.5252737
480	60.11	1.049165489	203.44368	Idoxanthin	104.9165489	15.36	6.830504487
480	63.43	5.561985755	1343.16394	Astaxanthin	556.1985755	15.36	36.21084476
450	63.15	3.36706271	973.99023	Diadinochrom	336.706271	15.36	21.92098118
450	66.28	3.182727763	920.66766	Diatoxanthin	318.2727763	15.36	20.72088387
450	67.74	2.163284856	583.24323	Lutein?	216.3284856	15.36	14.08388578
				PI-T11-4			
450	43.82	15.57163267	2237.02075	Fucoxanthino	1557.163267	14.73	105.7137317
450	50.92	33.21038804	4599.30664	Fucoxanthino	3321.038804	14.73	225.4608828
480	63.03	9.001145058	2173.68652	Astaxanthin	900.1145058	14.73	61.10756998
450	63	3.307900162	956.87628	Diadinochrom	330.7900162	14.73	22.4568918
450	65.78	4.222051613	1221.31287	Diatoxanthin	422.2051613	14.73	28.66294374
450	67.25	2.601368569	701.35498	Lutein?	260.1368569	14.73	17.66034331
				PI-T11-6			
450	44.21	15.81113045	2271.427	Fucoxanthino	1581.113045	17.91	88.28101869
450	51.31	28.83231677	3992.98755	Fucoxanthino	2883.231677	17.91	160.9844599
480	63.72	4.497426767	1086.08359	Astaxanthin	449.7426767	17.91	25.11126056
450	64.02	1.64212853	475.01852	Diadinochrom	164.212853	17.91	9.168780181
450	66.27	2.208023093	638.71484	Diatoxanthin	220.8023093	17.91	12.32843714
450	67.74	1.517775416	409.20743	Lutein?	151.7775416	17.91	8.474457936

Table 23: Table of Kimberly's analysis on skin samples from HPLC. Analyzed in 2019.

Wavelength	RT	PI-T2-2	Area		in 5 ml sample	Weight of sample	ng/g pigment in sample
450	44.62	5.93512516	852.64008	Fucoxanthinol	593.5125157	3.82	155.3697685
450	51.71	11.9351906	1652.90454	Fucoxanthin	1193.519056	3.82	312.4395433
							PI-T9-1
450	44.4	4.319989	620.60962	Fucoxanthinol	431.9989002	3.53	122.3792918
450	51.51	9.72465933	1346.76807	Fucoxanthin	972.4659326	3.53	275.4860999
							PI-T9-3
450	44.29	0.70146429	100.77236	Fucoxanthinol	70.14642907	2.12	33.08793824
450	51.38	1.73313192	240.02144	Fucoxanthin	173.3131923	2.12	81.7515058
							PI-T9-4
450	44.67	3.24124126	465.63672	Fucoxanthinol	324.1241264	3.27	99.12052795
450	51.77	9.49099632	1314.40808	Fucoxanthin	949.0996317	3.27	290.2445357
							PI-T9-5
450	44.5	2.34808249	337.32553	Fucoxanthinol	234.8082486	3.29	71.37028834
450	51.56	5.48697285	759.89087	Fucoxanthin	548.697285	3.29	166.7772903
							PI-T9-6
450	44.49	1.37625811	197.71324	Fucoxanthinol	137.6258109	2.64	52.13098899
450	51.58	3.83961636	531.74847	Fucoxanthin	383.9616362	2.64	145.4400137
							PI-T11-1
450	44.52	1.59241334	228.7661	Fucoxanthinol	159.2413337	2.74	58.11727507
450	51.55	2.84873514	394.52133	Fucoxanthin	284.8735143	2.74	103.9684359
							PI-T11-2
450	44.45	2.47975101	356.24103	Fucoxanthinol	247.9751009	2.46	100.8028866
450	51.54	6.15455925	852.34491	Fucoxanthin	615.4559246	2.46	250.1853352
							PI-T11-3
450	44.44	2.94855464	423.58936	Fucoxanthinol	294.8554643	3.77	78.21099849
450	51.54	6.8243162	945.09955	Fucoxanthin	682.4316196	3.77	181.0163447
							PI-T11-4
450	44.55	2.23964778	321.7478	Fucoxanthinol	223.9647779	3.06	73.1911039
450	51.66	3.72086555	515.30267	Fucoxanthin	372.086555	3.06	121.5969134
							PI-T11-6
450	44.24	1.47192621	211.45692	Fucoxanthinol	147.1926215	3.74	39.3563159
450	51.38	2.69116904	372.7	Fucoxanthin	269.1169037	3.74	71.95639138

Table 24: Raw data from GC. RD muscle samples.

RD	Area %/C14:0	Area %/C16:0	Area %/C16:1n-7	Area %/C18:0	Area %/C18:1n-9	Area %/C18:1n-7	Area %/C18:2n-6	Area %/C18:3n-3	Area %/C18:4n-3	Area %/C20:1n-9	Area %/C20:4n-3	Area %/C20:5n-3	Area %/C22:0	Area %/C22:5n-3	Area %/C22::6n-3
Anjana\2021\02\11\RD-18B37.DATA	3.417	13.639	4.319	2.951	26.296	3.121	7.808	3.112	1.22	4.872	1.224	5.125	5.001	1.937	13.074
Anjana\2021\02\11\RD-18A36.DATA	3.302	13.287	4.191	2.862	25.901	3.054	7.778	3.022	1.234	4.765	1.231	4.984	5.125	1.889	12.514
Anjana\2021\02\11\RD-17B35.DATA	3.069	13.099	3.99	2.913	25.269	3.046	7.454	2.962	1.204	3.759	1.294	5.217	5.13	1.977	15.128
Anjana\2021\02\11\RD-17A34.DATA	3.069	13.113	3.957	3.009	25.011	3.012	7.356	2.897	1.247	4.592	1.269	5.074	5.047	1.917	14.76
Anjana\2021\02\11\RD-16B33.DATA	2.85	12.986	3.877	2.988	25.592	3.047	7.47	2.957	1.154	4.55	1.174	4.756	4.793	1.772	15.647
Anjana\2021\02\11\RD-16A32.DATA	3.047	13.223	3.912	3.01	25.42	3.025	7.391	2.935	1.161	4.5	1.188	4.81	4.625	1.772	15.429
Anjana\2021\02\11\RD-15B31.DATA	3.389	14.134	4.152	2.99	24.466	3.027	7.005	2.766	1.255	4.78	1.353	5.26	4.78	1.96	14.196
Anjana\2021\02\11\RD-15A30.DATA	3.275	13.89	4.077	2.989	24.44	3.01	7.053	2.781	1.203	4.69	1.335	5.238	4.717	1.96	14.496
Anjana\2021\02\11\RD-14B29.DATA	2.92	13.635	3.848	3.182	26.602	3.037	7.951	3.135	1.123	4.192	1.142	4.949	3.959	1.728	16.006
Anjana\2021\02\11\RD-14A28.DATA	2.754	13.403	3.831	3.082	26.462	2.993	7.92	3.093	1.128	4.217	1.162	4.991	4.046	1.742	15.723
Anjana\2021\02\11\RD-13B27.DATA	3.089	13.614	4.091	3.06	25.062	2.988	7.356	2.771	1.278	4.78	1.232	5.329	5.025	1.825	14.171
Anjana\2021\02\11\RD-13A26.DATA	3.182	13.298	4.08	2.968	25.058	2.963	7.409	2.789	1.294	4.68	1.224	5.324	4.899	1.808	14.218
Anjana\2021\02\11\RD-12B25.DATA	2.916	12.388	4.119	2.702	27.067	3.032	8.161	3.235	1.274	4.68	1.177	4.976	4.814	1.812	13.277
Anjana\2021\02\11\RD-12A24.DATA	2.921	12.795	4.06	2.823	26.97	3.042	8.021	3.192	1.202	4.623	1.159	4.987	4.648	1.794	13.341
Anjana\2021\02\11\RD-11B23.DATA	3.023	13.604	4.135	3.163	26.169	3.102	7.432	2.988	1.196	4.708	1.287	5.437	4.902	1.957	14.333
Anjana\2021\02\11\RD-11A22.DATA	3.023	13.935	4.143	3.22	25.552	3.059	7.198	2.89	1.243	4.651	1.268	5.396	4.998	1.97	14.531
Anjana\2021\02\11\RD-10B21.DATA	3.275	13.595	4.367	2.953	25.064	3.002	6.815	2.731	1.274	4.892	1.218	5.138	5.344	1.895	13.58
Anjana\2021\02\11\RD-10A20.DATA	3.335	13.799	4.397	3.031	25.33	3.032	6.895	2.729	1.3	4.944	1.215	5.086	5.325	1.878	13.462
Anjana\2021\02\11\RD-9B19.DATA	3.069	13.083	4.053	3.043	25.661	2.991	7.027	2.597	1.217	4.63	1.103	4.251	5.224	1.55	15.286
Anjana\2021\02\11\RD-9A18.DATA	2.846	13.303	4.172	3.066	26.18	3.056	7.228	2.717	1.269	4.694	1.112	4.314	5.248	1.561	15.322
Anjana\2021\02\11\RD-8B17.DATA	3.035	13.431	3.907	2.836	24.609	3.026	7.186	2.815	1.135	4.542	1.169	4.637	4.822	1.756	16.657
Anjana\2021\02\11\RD-8A16.DATA	2.751	13.902	3.898	2.893	24.446	3.018	7.084	2.755	1.195	3.673	1.195	4.841	4.735	1.797	17.525
Anjana\2021\02\11\RD-7B15.DATA	3.122	14.071	4.413	2.815	23.818	2.971	6.694	2.638	1.411	4.852	1.303	5.148	5.584	1.853	14.469
Anjana\2021\02\11\RD-7A14.DATA	3.135	13.959	4.465	2.774	23.922	2.987	6.736	2.635	1.521	4.904	1.311	5.128	5.666	1.854	14.308
Anjana\2021\02\11\RD-6B13.DATA	2.912	13.899	4.11	3.095	25.108	2.963	7.007	2.704	1.224	4.533	1.164	4.488	5.006	1.717	14.933
Anjana\2021\02\11\RD-6A12.DATA	2.86	14.142	4.095	3.202	25.044	2.97	7.01	2.715	1.289	4.515	1.186	4.728	4.908	1.757	15.881
Anjana\2021\02\11\RD-5B11.DATA	2.821	12.908	3.96	3.065	27.71	3.073	8.15	3.067	1.167	4.515	1.156	4.129	4.275	1.649	13.859
Anjana\2021\02\11\RD-5A10.DATA	2.787	12.606	4.011	2.931	27.502	3.074	8.167	3.134	1.202	4.619	1.178	4.158	4.605	1.693	13.588
Anjana\2021\02\11\RD-4B9.DATA	2.746	12.93	4.008	2.947	27.969	3.092	8.405	3.207	1.219	4.584	1.151	4.644	4.51	1.774	11.995
Anjana\2021\02\11\RD-4A8.DATA	3.175	12.886	4.045	2.974	28.359	3.113	8.555	3.233	1.184	4.589	1.167	4.615	4.326	1.748	11.738
Anjana\2021\02\11\RD-3B7.DATA	3.056	13.623	3.917	3.21	26.316	3.065	7.833	3.026	1.182	4.641	1.141	5.019	4.773	1.863	14.943
Anjana\2021\02\11\RD-3A6.DATA	2.884	13.054	3.771	3.103	24.826	2.976	7.361	2.936	1.175	4.404	1.123	5.187	4.618	1.848	15.857
Anjana\2021\02\11\RD-2B5.DATA	3.385	13.971	4.222	3.098	25.33	2.961	7.239	2.909	1.314	4.622	1.239	5.383	5.002	1.796	13.157
Anjana\2021\02\11\RD-2A4.DATA	2.942	13.755	4.173	3.026	25.086	2.933	7.267	2.958	1.334	4.541	1.242	5.537	4.888	1.797	13.614
Anjana\2021\02\11\RD-1B3.DATA	3.061	13.593	3.954	2.988	23.367	2.94	6.897	2.711	1.298	4.343	1.168	5.491	4.651	1.753	17.738
Anjana\2021\02\11\RD-1A2.DATA	3.023	13.401	3.845	2.933	22.842	2.866	6.817	2.682	3.211	4.334	1.13	5.156	4.673	1.702	16.703

Table 25: Raw data from GC. PB muscle samples.

PB	Area %/C14:0	Area %/C16:0	Area %/C16:1n-7	Area %/C18:0	Area %/C18:1n-9	Area %/C18:1n-7	Area %/C18:2n-6	Area %/C18:3n-3	Area %/C18:4n-3	Area %/C20:1n-9	Area %/C20:4n-3	Area %/C20:5n-3	Area %/C22:0	Area %/C22:5n-3	Area %/C22::6n-3
Anjana\2021\02\15\PB-18B37.DATA	3.027	13.957	4.408	3.191	29.922	3.241	9.374	3.339	0.895	3.445	0.988	3.924	3.437	1.632	12.975
Anjana\2021\02\15\PB-18A36.DATA	2.827	13.937	4.39	3.206	27.997	3.243	8.868	3.222	0.925	3.253	1.055	4.553	3.415	1.799	16.216
Anjana\2021\02\15\PB-17B35.DATA	2.867	13.577	5.595	3.063	25.513	3.288	7.781	2.96	1.072	4.337	1.176	5.367	4.484	1.955	14.518
Anjana\2021\02\15\PB-17A34.DATA	2.875	14.167	5.682	3.209	25.528	3.34	7.74	2.945	1.108	4.343	1.226	5.748	4.341	2.054	15.693
Anjana\2021\02\15\PB-16B33.DATA	2.855	14.252	5.672	3.014	25.909	3.263	8.252	3.118	1.141	4.207	1.137	5.922	4.073	2.03	15.154
Anjana\2021\02\15\PB-16A32.DATA	3.016	14.362	5.395	3.07	25.298	3.213	7.948	3.028	1.087	3.236	1.126	6.067	3.969	2.033	16.205
Anjana\2021\02\15\PB-15B31.DATA	2.974	13.621	5.026	3.063	27.697	3.128	8.623	3.156	1.029	4.247	1.109	5.286	3.83	1.731	11.676
Anjana\2021\02\15\PB-15A30.DATA	3.064	13.423	5.12	2.991	29.018	3.197	9.103	3.23	1.013	4.407	1.091	4.872	3.956	1.679	10.055
Anjana\2021\02\15\PB-14B29.DATA	2.947	12.611	5.245	2.848	28.967	3.316	8.922	3.158	1.084	4.44	1.162	4.672	3.919	1.742	11.005
Anjana\2021\02\15\PB-14A28.DATA	2.978	12.3	5.444	2.722	29.37	3.32	9.139	3.095	1.104	4.512	1.176	4.545	4.028	1.751	10.223
Anjana\2021\02\15\PB-13B27.DATA	3.078	13.382	5.797	2.761	25.312	3.144	8.107	3.053	1.115	4.219	1.089	5.123	4.24	1.957	13.418
Anjana\2021\02\15\PB-13A26.DATA	2.846	13.45	5.851	2.817	25.411	3.198	8.134	3.077	1.131	3.446	1.123	5.329	4.272	2.033	14.281
Anjana\2021\02\15\PB-12B25.DATA	3.183	14.189	5.821	2.974	25.72	3.251	7.825	2.919	1.091	4.707	1.269	5.56	4.559	1.917	11.332
Anjana\2021\02\15\PB-12A24.DATA	3.397	13.923	5.878	2.908	25.24	3.243	7.76	2.889	1.117	4.608	1.286	5.689	4.507	1.973	11.901
Anjana\2021\02\15\PB-11B23.DATA	3.081	13.29	5.335	3.051	26.435	3.138	7.996	2.958	1.055	3.453	1.118	4.986	4.087	1.926	13.449
Anjana\2021\02\15\PB-11A22.DATA	3.157	13.561	5.497	3.051	27.037	3.226	8.123	3.011	1.166	3.551	1.144	5.154	4.194	1.969	13.823
Anjana\2021\02\15\PB-10B 21.DATA	3.141	12.741	6.036	2.782	27.778	3.264	8.448	3.052	1.256	3.693	1.152	4.911	4.434	1.815	10.657
Anjana\2021\02\15\PB-10A20.DATA	3.106	13.525	5.845	2.968	27.478	3.291	8.072	2.933	1.224	3.611	1.157	5.169	4.255	1.858	11.97
Anjana\2021\02\15\PB-9B19.DATA	2.855	13.151	5.091	3.103	28.721	3.269	8.745	3.153	1.071	3.553	1.055	4.56	4.032	1.774	12.97
Anjana\2021\02\15\PB-9A18.DATA	2.91	12.922	5.112	2.99	28.39	3.208	8.612	3.102	1.266	4.313	1.02	4.305	3.986	1.698	11.845
Anjana\2021\02\15\PB-8B17.DATA	3.004	12.561	5.299	3.004	29.902	3.288	9.323	3.301	1.14	3.656	1.049	4.326	3.825	1.744	10.25
Anjana\2021\02\15\PB-8A16.DATA	2.989	12.717	5.238	3.042	29.486	3.271	9.09	3.204	1.081	4.344	1.053	4.351	3.797	1.732	10.785
Anjana\2021\02\15\PB-7B15.DATA	2.879	13.215	5.06	3.002	27.491	3.213	8.766	3.183	1.006	4.177	1.087	4.952	3.803	1.811	13.499
Anjana\2021\02\15\PB-7A14.DATA	3.053	13.362	5.03	3.029	27.461	3.208	8.685	3.181	1.037	3.449	1.08	4.966	3.771	1.793	13.517
Anjana\2021\02\15\PB-6B13.DATA	3.196	13.476	5.591	2.846	27.221	3.267	8.205	2.959	1.139	4.8	1.073	4.599	4.639	1.789	9.752
Anjana\2021\02\15\PB-6A12.DATA	3.234	13.506	5.627	2.853	27.304	3.262	8.256	2.992	1.167	4.77	1.069	4.675	4.6	1.812	9.973
Anjana\2021\02\15\PB-5B11.DATA	3.271	13.791	6.165	2.826	26.399	3.221	7.74	2.889	1.323	4.58	1.123	4.861	4.544	1.801	10.597
Anjana\2021\02\15\PB-5A10.DATA	3.319	13.204	6.182	2.725	27.048	3.25	8.153	3.043	1.334	4.649	1.149	4.91	4.632	1.846	10.466
Anjana\2021\02\15\PB-4B9.DATA	3.177	13.767	5.575	2.95	25.603	3.185	7.764	2.945	1.034	4.565	1.188	5.619	4.414	1.923	12.09
Anjana\2021\02\15\PB-4A8.DATA	3.321	13.812	5.764	2.89	27.103	3.242	8.278	3.1	1.143	4.005	1.175	5.222	4.606	1.849	10.104
Anjana\2021\02\15\PB-3B7.DATA	2.59	13.795	5.733	2.982	26.931	3.356	8.494	3.158	1.073	4.381	1.074	5.307	4.454	1.987	13.003
Anjana\2021\02\15\PB-3A6.DATA	3.283	15.115	5.42	3.394	25.568	3.353	7.841	2.975		3.274		6.248	4.101	2.038	17.39
Anjana\2021\02\15\PB-2B5.DATA	3.102	13.13	5.353	2.924	28.464	3.315	8.684	3.187	1.146	3.507	1.139	4.985	3.975	1.916	11.672
Anjana\2021\02\15\PB-2A4.DATA	3.03	13.141	5.126	2.962	28.152	3.259	8.452	3.112	1.046	4.305	1.145	5.005	3.868	1.872	12.041
Anjana\2021\02\15\PB-1B3.DATA	2.511	14.388	5.474	3.257	26.683	3.326	8.24	2.976	1.223	3.472	1.132	5.347	4.244	2.001	15.727
Anjana\2021\02\15\PB-1A2.DATA	2.929	13.653	5.027	3.109	25.512	3.14	7.853	2.842	1.005	4.126	1.069	4.89	3.953	1.862	14.615

Table 26: Raw data from GC. PI muscle samples.

PI	Area %/C14:0	Area %/C16:0	Area %/C16:1n-7	Area %/C18:0	Area %/C18:1n-9	Area %/C18:1n-7	Area %/C18:2n-6	Area %/C18:3n-3	Area %/C18:4n-3	Area %/C20:1n-9	Area %/C20:4n-3	Area %/C20:5n-3	Area %/C22:0	Area %/C22:5n-3
Anjana\2021\02\15\PI-18B112.DATA	2.906	12.9	4.487	2.992	30.039	3.295	9.463	3.432	0.959	4.319	1.02	4.002	3.639	1.659
Anjana\2021\02\15\PI-18A111.DATA	2.794	12.873	4.528	3.01	29.042	3.255	9.207	3.384	1.062	4.232	1.049	4.224	3.649	1.736
Anjana\2021\02\15\PI-17B110.DATA	3.134	13.719	5.566	2.912	25.178	3.187	7.794	2.887	1.13	4.549	1.189	5.249	4.738	2.013
Anjana\2021\02\15\PI-17A109.DATA	3.232	13.994	5.361	3.124	25.012	3.23	7.792	2.964	1.121	4.439	1.207	5.765	4.515	2.07
Anjana\2021\02\15\PI-16B108.DATA	2.92	12.907	5.149	2.833	27.1	3.273	8.4	3.135	1.114	4.557	1.051	4.237	4.699	1.787
Anjana\2021\02\15\PI-16A107.DATA	2.888	12.601	4.88	2.823	25.333	3.16	8.105	2.963	1.036	4.295	1.043	4.391	4.397	1.803
Anjana\2021\02\15\PI-15B106.DATA	2.962	12.99	5.36	2.96	27.309	3.235	8.423	3.119	1.095	4.468	1.184	5.306	4.377	1.975
Anjana\2021\02\15\PI-15A105.DATA	2.993	13.108	5.279	2.911	27.613	3.227	8.519	3.015	1.081	4.425	1.173	5.377	4.21	1.956
Anjana\2021\02\15\PI-14B104.DATA	2.879	13.286	4.914	3.025	27.271	3.225	8.49	2.903	1.213	4.406	1.02	4.477	4.252	1.768
Anjana\2021\02\15\PI-14A103.DATA	2.919	12.933	5.05	2.789	27.353	3.202	8.5	3.051	1.166	4.415	1.002	4.369	4.326	1.777
Anjana\2021\02\15\PI-13B102.DATA	2.788	13.1	4.829	3.093	27.409	3.225	8.297	2.872	1.084	4.428	1.153	4.666	4.032	1.958
Anjana\2021\02\15\PI-13A101.DATA	2.848	12.599	4.996	2.87	27.259	3.184	8.496	3.092	1.024	4.448	1.127	4.466	4.243	1.923
Anjana\2021\02\15\PI-12B100.DATA	2.968	14.635	5.146	3.414	23.372	3.196	6.861	2.719	1.022	4.094	1.27	5.959	4.375	2.176
Anjana\2021\02\15\PI-12A99.DATA	3.005	14.213	5.214	3.103	24.287	3.172	7.166	2.779	1.022	4.206	1.241	5.534	4.455	2.061
Anjana\2021\02\15\PI-11B98.DATA	3.147	13.062	4.573	2.968	30.146	3.298	9.489	3.504	0.954	4.534	1.021	4.06	3.803	1.682
Anjana\2021\02\15\PI-11A97.DATA	3.034	12.668	4.46	2.869	29.396	3.222	9.328	3.438	0.876	4.388	1.001	3.985	3.727	1.659
Anjana\2021\02\15\PI-10B96.DATA	3.042	13.264	5.332	2.786	27.208	3.25	8.579	3.098	1.115	4.588	1.117	4.734	4.541	1.828
Anjana\2021\02\15\PI-10A96.DATA	2.962	13.259	5.25	2.842	26.623	3.229	8.29	3.19	1.103	4.507	1.163	4.922	4.431	1.881
Anjana\2021\02\15\PI-9B95.DATA	3.113	13.795	5.456	3.044	26.823	3.263	8.172	3.058	1.097	4.424	1.139	4.795	4.267	1.787
Anjana\2021\02\15\PI-9A95.DATA	3.373	14.098	5.595	2.931	26.401	3.28	8.122	3.103	1.17	4.356	1.19	5.195	4.207	1.885
Anjana\2021\02\15\PI-8B94.DATA	2.937	13.519	5.113	2.974	25.759	3.182	7.946	3.019	1.1	4.318	1.143	5.099	4.391	1.931
Anjana\2021\02\15\PI-8A93.DATA	2.967	14.126	4.955	3.145	25.357	3.182	7.702	2.911	1.036	4.223	1.14	5.186	4.209	1.922
Anjana\2021\02\15\PI-7B92.DATA	2.862	13.719	4.874	3.243	26.971	3.238	8.335	3.229	1.006	4.295	1.157	5.352	3.991	2.076
Anjana\2021\02\15\PI-7A91.DATA	3.046	13.33	4.757	3.059	28.519	3.25	8.895	3.268	0.98	4.457	1.121	4.691	3.94	1.874
Anjana\2021\02\15\PI-6B90.DATA	2.909	14.21	5.005	3.176	25.412	3.2	7.638	2.877	1.054	4.33	1.077	5.14	4.19	1.98
Anjana\2021\02\15\PI-6A89.DATA	2.781	13.573	4.887	3.124	25.135	3.146	7.624	2.883	1.015	3.429	1.064	4.976	4.242	1.935
Anjana\2021\02\15\PI-5B88.DATA	2.737	13.553	4.664	3.154	28.338	3.307	8.463	3.08	0.924	4.387	1.082	4.12	3.915	1.804
Anjana\2021\02\15\PI-5A87.DATA	3.008	13.618	4.494	3.206	29.354	3.277	8.776	3.168	0.9	4.378	1.072	3.918	3.629	1.735
Anjana\2021\02\15\PI-4B86.DATA	2.932	13.59	5.032	2.976	25.989	3.23	7.918	2.876	1.017	4.353	1.154	4.816	4.128	1.894
Anjana\2021\02\15\PI-4A85.DATA	2.786	13.733	4.886	3.082	25.167	3.22	7.687	2.839	0.984	4.182	1.18	5.153	3.994	1.961
Anjana\2021\02\15\PI-3B84.DATA	2.802	12.603	4.397	3.07	28.904	3.335	9.338	3.329	0.896	4.285	1.108	4.471	3.725	1.859
Anjana\2021\02\15\PI-3A83.DATA	2.804	13.328	4.21	3.282	28.351	3.322	9.043	3.386	0.899	4.196	1.121	4.698	3.549	1.879
Anjana\2021\02\15\PI-2B82.DATA	2.849	13.076	4.709	3.024	28.647	3.33	9.136	3.139	0.976	4.452	1.148	4.465	3.907	1.896
Anjana\2021\02\15\PI-2A81.DATA	2.929	13.959	4.409	3.266	27.036	3.244	8.573	3.257	1.007	4.132	1.189	5.098	3.555	1.983
Anjana\2021\02\15\PI-1B80.DATA	2.963	14.269	4.621	3.264	26.297	3.293	8.281	3.117	0.974	3.402	1.152	5.182	3.959	2.07
Anjana\2021\02\15\PI-1A79.DATA	2.757	13.074	4.497	2.983	25.201	3.136	8.039	2.989	0.901	4.171	1.093	4.727	3.986	1.967

Table 27: Raw data from GC. TB muscle samples.

TB	Area %/C14:0	Area %/C16:0	Area %/C16:1n-7	Area %/C18:0	Area %/C18:1n-9	Area %/C18:1n-7	Area %/C18:2n-6	Area %/C18:3n-3	Area %/C18:4n-3	Area %/C20:1n-9	Area %/C20:4n-3	Area %/C20:5n-3	Area %/C22:0	Area %/C22:5n-3	Area %/C22::6n-3
Anjana\2021\02\15\TB-18B77.DATA	2.863	12.357	4.077	2.634		3.049	8.496	3.846	1.806	4.558	1.218	3.903	4.535	1.625	11.458
Anjana\2021\02\15\TB-18A76.DATA	2.7	13.047	3.832	2.966	26.748	3.108	8.095	3.48	1.556	4.403	1.229	4.421	4.295	1.687	14.688
Anjana\2021\02\15\TB-17C75.DATA	3.9	15.672	4.298	3.586	29.638	3.62	9.152	4.094		4.844		4.611	4.559		12.024
Anjana\2021\02\15\TB-17B74.DATA	2.892	13.484	3.837	3.014	27.009	3.246	8.422	3.779	1.442	3.538	1.323	4.503	4.528	1.867	15.494
Anjana\2021\02\15\TB-17A73.DATA	2.624	14.211	3.637	3.24	25.82	3.144	7.89	3.724	1.311	4.055	1.312	4.783	3.867	1.861	17.007
Anjana\2021\02\15\TB-16B72.DATA	2.788	13.312	3.987	3.171	29.802	3.291	8.933	3.736	1.402	3.509	1.222	4.3	3.907	1.728	12.188
Anjana\2021\02\15\TB-16A71.DATA	2.811	12.877	3.917	3.136	29.78	3.209	9.011	3.717	1.337	4.173	1.163	4.018	3.696	1.623	10.601
Anjana\2021\02\15\TB-15B70.DATA	2.893	13.971	4.011	3.138	27.672	3.218	8.278	3.687	1.497	4.483	1.291	4.964	4.284	1.795	11.57
Anjana\2021\02\15\TB-15A69.DATA	3.04	15.096	3.956	3.456	26.865	3.276	8.04	3.644	1.676	3.347	1.357	5.628	4.094	1.944	14.581
Anjana\2021\02\15\TB-14B68.DATA	3.015	13.217	4.109	3	29.711	3.25	8.918	3.645	1.476	4.689	1.23	3.738	4.262	1.591	9.909
Anjana\2021\02\15\TB-14A67.DATA	3.019	13.303	4.111	3.016	29.731	3.267	8.924	3.635	1.407	4.649	1.218	3.823	4.19	1.607	10.203
Anjana\2021\02\15\TB-13B66.DATA	2.781	13.516	3.818	3.215	29.04	3.275	8.934	3.608	1.293	4.327	1.263	4.24	3.805	1.709	13.026
Anjana\2021\02\15\TB-13A65.DATA	2.923	13.186	3.791	2.983	28.684	3.243	8.899	3.649	1.292	4.27	1.291	4.323	3.728	1.716	13.364
Anjana\2021\02\15\TB-12C64.DATA	3.786	15.473	4.487	3.414	30.361	3.604	9.41	4.045		4.669		4.215	4.223		12.314
Anjana\2021\02\15\TB-12B63.DATA	2.866	14.77	3.733	3.377	25.732	3.154	7.796	3.485	1.46	3.99	1.245	4.727	3.598	1.73	18.337
Anjana\2021\02\15\TB-12A62.DATA	2.898	14.632	3.709	3.396	26.622	3.176	8.029	3.586	1.399	4.094	1.23	4.6	3.608	1.683	17.337
Anjana\2021\02\15\TB-11B61.DATA	2.865	14.588	3.625	3.148	26.067	3.202	8.167	3.724	1.377	4.063	1.269	5.06	3.697	1.799	17.35
Anjana\2021\02\15\TB-11A60.DATA	3.024	13.518	3.857	2.86	28.245	3.237	8.889	3.669	1.379	3.469	1.215	4.17	3.999	1.626	12.5
Anjana\2021\02\15\TB-10B59.DATA	3.09	13.583	4.21	3.01	29.429	3.263	8.479	3.49	1.632	4.67	1.19	3.524	4.238	1.447	10.627
Anjana\2021\02\15\TB-10A58.DATA	3.031	13.501	4.181	3.041	29.308	3.258	8.464	3.647	1.548	4.672	1.193	3.562	4.259	1.476	10.895
Anjana\2021\02\15\TB-9B57.DATA	3.042	12.956	3.779	2.973	30.286	3.278	9.283	3.478	1.411	4.391	1.144	3.668	3.759	1.551	13.449
Anjana\2021\02\15\TB-9A56.DATA	2.899	12.473	3.888	2.813	29.958	3.297	9.343	3.665	1.476	3.68	1.162	3.625	4.225	1.608	13.077
Anjana\2021\02\15\TB-8B55.DATA	2.763	14.257	3.832	3.183	27.134	3.203			1.441	3.591	1.228	4.807	4.201	1.768	15.005
Anjana\2021\02\15\TB-8A54.DATA	3.032	14.817	3.699	3.403	26.842	3.196	8.204	3.563	1.372	4.317	1.208	4.966	3.978	1.755	15.649
Anjana\2021\02\15\TB-7B53.DATA	2.634	13.869	3.904	3.011	26.003	3.225	8.155	3.684	1.54	4.378	1.328	4.811	4.479	1.904	14.98
Anjana\2021\02\15\TB-7A52.DATA	2.508	14.118	3.882	3.096	26.418	3.249	8.229	3.693	1.627	3.515	1.34	4.909	4.419	1.91	15.526
Anjana\2021\02\15\TB-6B51.DATA	2.594	12.312	3.735	2.937	29.148	3.242	9.139	3.518	1.357	4.323	1.086	3.421	3.838	1.526	14.633
Anjana\2021\02\15\TB-6A50.DATA	2.704	12.294	3.621	2.954	28.673	3.217	8.964	3.57	1.287	4.223	1.054	3.4	3.707	1.483	14.714
Anjana\2021\02\15\TB-5B49.DATA	2.758	13.651	3.575	3.296	28.129	3.216	8.716	3.68	1.262	3.297	1.187	4.144	3.492	1.663	15.105
Anjana\2021\02\15\TB-5A48.DATA	2.881	13.616	3.822	3.232	29.508	3.352	9.306	3.758	1.327	3.462	1.223	4.142	3.855	1.67	13.97
Anjana\2021\02\15\TB-4B47.DATA	3.091	14.097	3.832	3.157	28.023	3.269	8.531	3.613	1.415	4.39	1.233	4.42	3.802	1.548	13.913
Anjana\2021\02\15\TB-4A46.DATA	4.249	19.834	5.352	4.515		4.33	11.901	5.128	2.076	4.809	1.786	6.667	5.24	2.275	21.837
Anjana\2021\02\15\TB-3B45.DATA	2.56	13.137	3.717	3.21	28.09	3.223	8.422	3.598	1.296	4.32	1.16	3.85	3.827	1.671	16.334
Anjana\2021\02\15\TB-3A44.DATA	2.83	13.469	3.845	3.246	28.528	3.28			1.376	4.4	1.177	3.958	3.973	1.67	16.016
Anjana\2021\02\15\TB-2B43.DATA	2.933	14.044	4.139	3.082	27.17	3.244	8.117	3.748	1.597	4.576	1.259	4.489	4.639	1.735	13.003
Anjana\2021\02\15\TB-2A42.DATA	3.059	14.455	4.145	3.18	26.883	3.245	7.902	3.749	1.779	4.567	1.311	4.786	4.528	1.781	13.726
Anjana\2021\02\15\TB-1B41.DATA	3.185	14.254	4.32	2.945	25.944	3.156	7.515	3.546	1.818	4.732	1.418	4.553	4.786	1.728	11.42
Anjana\2021\02\15\TB-1A40.DATA	3.161	14.185	4.223	2.94	25.31	3.101	7.327	3.637	1.695	4.594	1.442	4.563	4.64	1.712	11.602

Table 28: Raw data from CG. TI muscle samples.

TI	Area %/C14:0	Area %/C16:0	Area %/C16:1n-7	Area %/C18:0	Area %/C18:1n-9	Area %/C18:1n-7	Area %/C18:2n-6	Area %/C18:3n-3	Area %/C18:4n-3	Area %/C20:1n-9	Area %/C20:4n-3	Area %/C20:5n-3	Area %/C22:0	Area %/C22:5n-3	Area %/C22::6n-3
Anjana\2021\02\10\TI-18B37.DATA	2.822	13.959	3.702	3.345	26.765	3.23	7.883	3.689	1.288	3.217	1.287	5.006	3.733	1.832	16.678
Anjana\2021\02\10\TI-18A36.DATA	2.232	14.26	3.83	3.392	27.45	3.303	8.069	3.667	1.32	3.324	1.321	5.161	3.802	1.876	16.993
Anjana\2021\02\10\TI-17B35.DATA															
Anjana\2021\02\10\TI-17A34.DATA	2.843	13.864	3.893	3.308	25.79	3.138	7.532	3.698	1.524	4.254	1.362	5.137	4.213	1.866	14.797
Anjana\2021\02\10\TI-16B33.DATA	2.999	14.061	4.047	3.456	28.772	3.263	8.306	3.72	1.306	4.295	1.257	4.305	4.236	1.758	14.219
Anjana\2021\02\10\TI-16A32.DATA	2.938	14.68	3.842	3.587	27.37	3.084	7.655	3.375	1.238	3.971	1.226	4.396	3.544	1.715	14.708
Anjana\2021\02\10\TI-15B31.DATA	2.136	14.149	3.612	3.33	24.118	3.092	6.961	3.341	1.276	4.071	1.283	4.997	3.959	1.778	18.385
Anjana\2021\02\10\TI-15A30.DATA	2.784	14.416	3.56	3.387	24.193	3.118	7.083	3.275	1.354	3.982	1.286	5.147	3.77	1.812	18.623
Anjana\2021\02\10\TI-14B29.DATA	2.936	13.519	4.214	2.812	26.628	3.167	7.814	3.687	1.702	4.632	1.479	4.716	4.495	1.75	9.955
Anjana\2021\02\10\TI-14A28.DATA	3.131	13.508	4.258	2.821	26.884	3.182	7.884	3.743	1.717	4.714	1.466	4.816	4.582	1.783	10.554
Anjana\2021\02\10\TI-13B27.DATA	2.771	13.206	3.913	2.987	27.421	3.184	8.433	3.675	1.485	4.418	1.393	4.87	3.904	1.859	13.284
Anjana\2021\02\10\TI-13A26.DATA	2.7	13.343	3.806	2.989	26.676	3.166	8.106	3.602	1.416	4.261	1.364	5.091	3.825	1.855	14.392
Anjana\2021\02\10\TI-12B25.DATA	2.99	14.144	3.857	3.047	24.318	3.111	7.491	3.737	1.653	4.252	1.393	6.111	4.388	1.981	15.165
Anjana\2021\02\10\TI-12A24.DATA	2.833	13.806	3.878	2.937	24.397	3.07	7.535	3.692	1.635	4.23	1.379	5.878	4.409	1.934	14.441
Anjana\2021\02\10\TI-11B23.DATA	2.277	15.109	3.597	3.429	22.038	2.921	6.54	3.402	1.498	3.781	1.428	6.274	4.01	1.857	19.637
Anjana\2021\02\10\TI-11A22.DATA	2.22	15.225	3.646	3.463	22.901	2.991	6.713	3.456	1.527	3.876	1.414	6.237	3.917	1.863	19.165
Anjana\2021\02\10\TI-10B21.DATA	3.032	13.397	4.079	2.935		3.15	7.982	3.6	1.529	4.664	1.294	4.028	4.47	1.589	10.897
Anjana\2021\02\10\TI-10A20.DATA	3.119	14.289	4.109	3.134	26.799	3.151	7.568	3.468	1.509	4.56	1.327	4.322	4.249	1.607	11.751
Anjana\2021\02\10\TI-9B19.DATA	2.832	13.614	3.952	3.444	26.715	3.168	7.691	3.551	1.408	4.144	1.335	5.485	3.948	2.031	14.284
Anjana\2021\02\10\TI-9A18.DATA	2.878	13.699	3.97	3.782	26.811	3.142	7.726	3.516	1.353	4.076	1.277	5.2	3.68	1.93	13.501
Anjana\2021\02\10\TI-8B17.DATA	2.788	12.263	3.815	2.957	31.915	3.235	9.923	3.85	1.173	4.323	0.996	3.393	3.203	1.393	10.473
Anjana\2021\02\10\TI-8A16.DATA	2.889	12.461	3.809	2.994	31.451	3.228	9.746	3.775	1.18	4.3	1.026	3.518	3.201	1.411	11.094
Anjana\2021\02\10\TI-7B15.DATA	2.819	13.256	3.945	2.907	27.147	3.18	8.357	3.804	1.52	4.205	1.335	4.82	3.957	1.669	13.177
Anjana\2021\02\10\TI-7A14.DATA	3.004	13.544	4.034	2.978	27.109	3.19	8.364	3.638	1.568	4.173	1.352	4.94	3.955	1.689	13.575
Anjana\2021\02\10\TI-6B13.DATA	2.761	13.17	3.5	3.262	28.496	3.148	9.125	3.639	1.113	3.917	1.02	4.276	3.136	1.577	16.022
Anjana\2021\02\10\TI-6A12.DATA	2.721	13.197	3.471	3.218	28.341	3.121	8.945	3.341	1.09	3.951	1.012	4.097	3.178	1.548	15.151
Anjana\2021\02\10\TI-5B11.DATA	2.868	13.853	3.663	3.255	26.337	3.152	8.023	3.677	1.268	4.144	1.298	4.804	3.689	1.689	15.174
Anjana\2021\02\10\TI-5A10.DATA	2.756	13.597	3.567	3.451	25.586	3.08	7.82	3.536	1.259	3.995	1.278	4.796	3.623	1.688	15.744
Anjana\2021\02\10\TI-4B9.DATA	2.882	13.372	3.696	3.063	26.382	3.132	8.079	3.617	1.541	4.239	1.177	4.512	3.991	1.669	15.25
Anjana\2021\02\10\TI-4A8.DATA	2.846	13.413	3.658	3.065	26.316	3.124	7.983	3.557	1.466	4.202	1.155	4.422	3.94	1.645	14.81
Anjana\2021\02\10\TI-3B7.DATA	2.819	13.564	3.901	2.984	25.708	3.071	7.642	3.595	1.518	4.515	1.302	4.708	4.728	1.757	13.419
Anjana\2021\02\10\TI-3A6.DATA	2.842	14.664	3.777	3.354	25.084	3.056	7.318	3.906	1.543	4.217	1.268	5.152	4.011	1.765	15.286
Anjana\2021\02\10\TI-2B5.DATA	2.926	13.186	3.979	3.178	29.436	3.205	8.961	3.697	1.351	4.344	1.124	4.131	3.81	1.646	10.917
Anjana\2021\02\10\TI-2A4.DATA	2.852	13.059	3.916	3.152	28.564	3.176	8.746	3.823	1.432	4.304	1.14	4.269	3.925	1.69	11.404
Anjana\2021\02\10\TI-1B3.DATA	3.018	13.994	4.065	3.233	27.299	3.197	7.95	3.511	1.468	4.629	1.262	4.545	4.467	1.672	11.689
Anjana\2021\02\10\TI-1A2.DATA	3.17	14.033	4.008	3.242	27.07	3.196	7.899	3.642	1.435	4.604	1.257	4.536	4.409	1.638	11.462

Appendix D – Plots

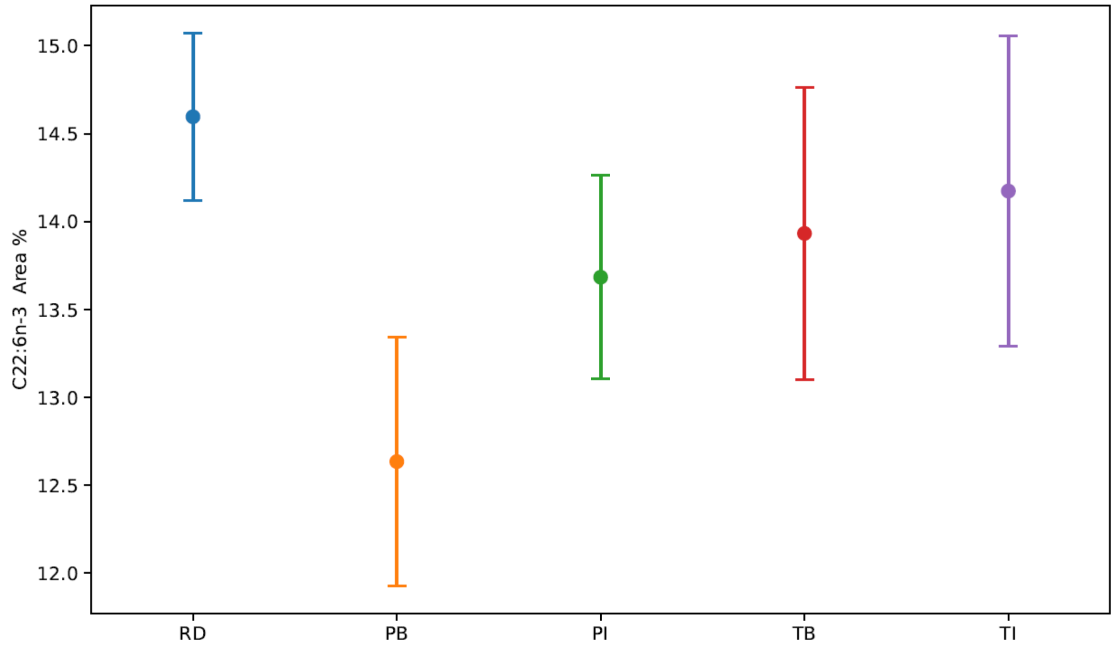


Figure 1: Plot of DHA in all muscle sample groups analyzed in 2020.

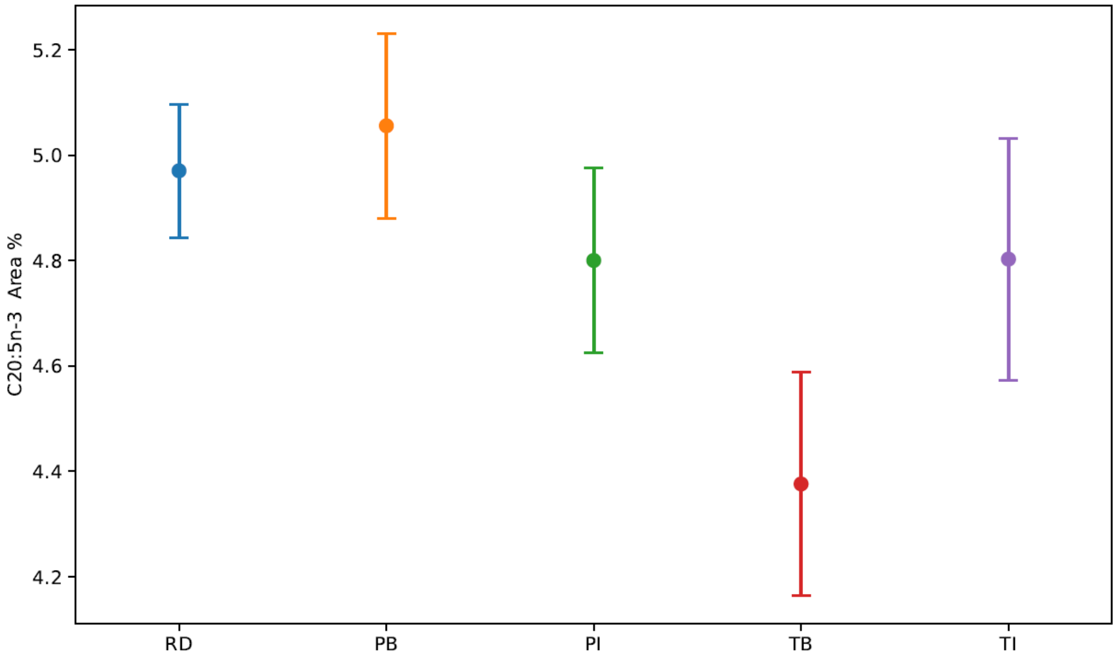


Figure 2: Plot of EPA in all muscle sample groups analyzed in 2020.

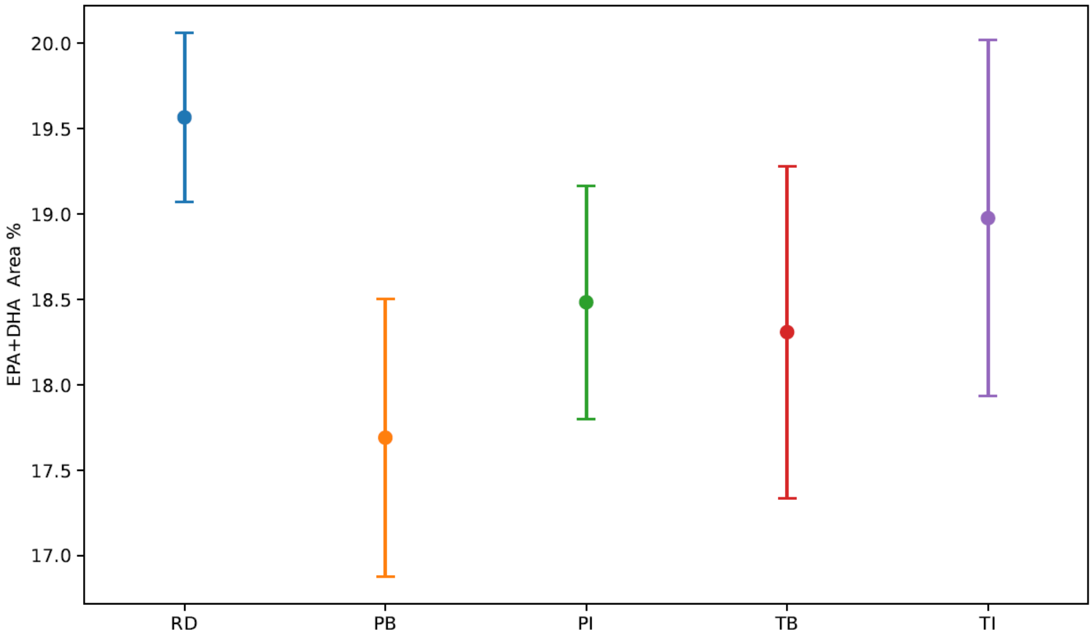


Figure 3: Plot of EPA+DHA in all muscle sample groups analyzed in 2020.

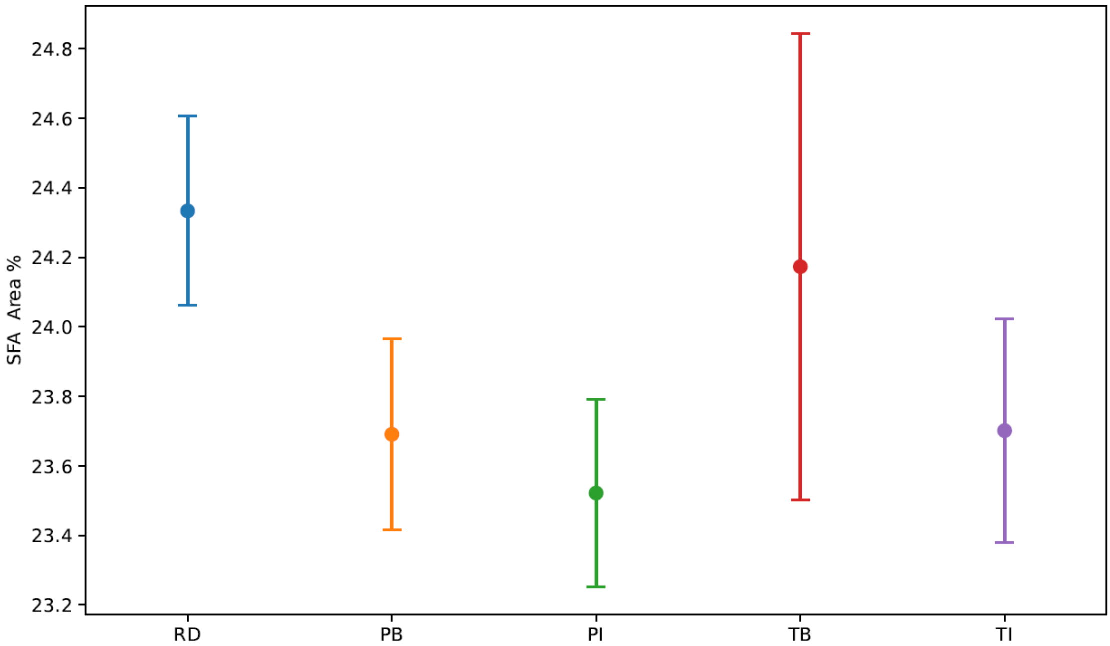


Figure 4: Plot of SFA in all muscle sample groups analyzed in 2020.

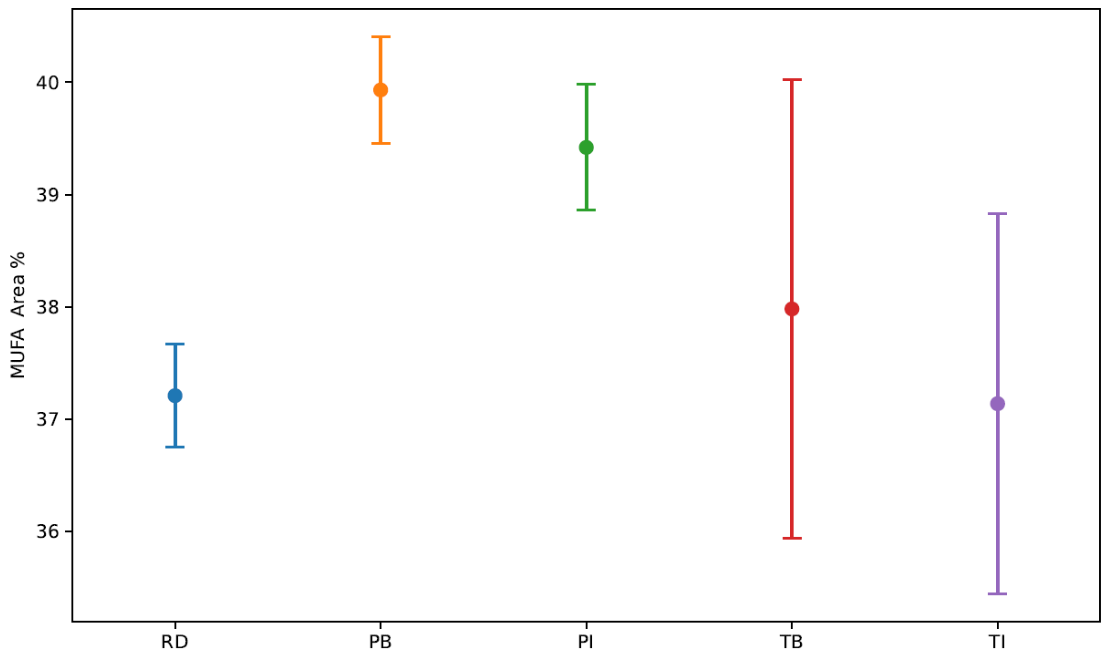


Figure 5: Plot of MUFA in all muscle sample groups analyzed in 2020.

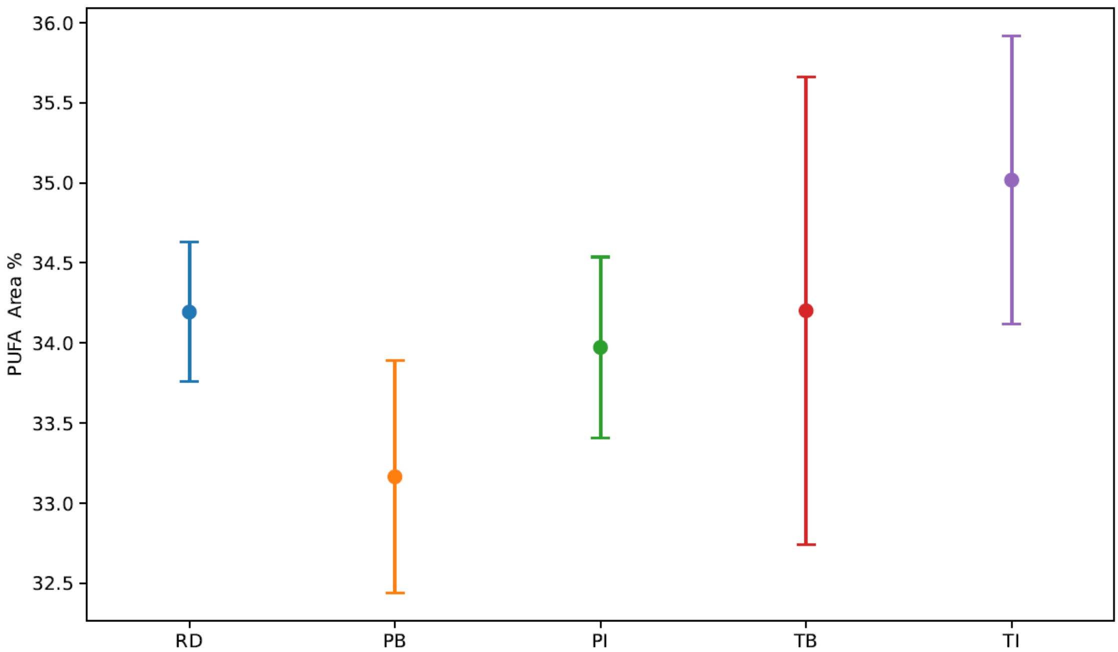


Figure 6: Plot of PUFA in all muscle sample groups analyzed in 2020.