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# PROPERTIES AND UTILISATION OF GOLDEN REDFISH (SEBASTES MARINUS) HEAD

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# ABSTRACT

This study characterises the chemical composition of golden redfish head with respect to size and explores the possibilities of new product development using a simple oil extraction process and other utilisations (minerals, collagen etc) of the red fish head. Respective yields of different head and carcass parts and their chemical composition are determined. Oil was extracted from the brain and eyes using the wet reduction method and composition evaluated. The gill had the highest (SH - 8.55 % and BH - 9.63 %) yield of all parts with the carcass constituting a larger (SH- 73.10 % and BH - 77.76 %) portion after other parts were separated. The brain had the highest lipid content (SH - 45.78 % and BH - 35.02) while the cheek had a high protein content (SH – 20.25 and BH – 18.25). The SH and BH brain had a lower free fatty acid while the SH eye and the BH eye had lower phospholipid. Profiles of fatty acid showed that the SH and BH gills are rich sources of polyunsaturated fatty acids eicosapentaenoic acid, docosahexaenoic acid n-3 fatty acids (about 26-32% of total fatty acids), and various minerals (Na, Ph, Ca, K, and Mg) with calcium being the most abundant micro element followed by phosphorus. Yield of SH brain oil extracted oil showed no significant difference with and without heat. BH samples showed a significant difference in oil extracted with and without heat. The SH oil samples had significant high free fatty acid content while no phospholipid was recorded in SH and BH oil samples. The fatty acid composition did not show significant difference between the mode of extraction. Results from this study show that redfish head contains large amounts of nutritional components and can be utilised to produce value-added products.

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#### 1 INTRODUCTION

During recent years, there has been an exponential increase in by-products discharged by fisheries. According to FAO, (2018), 88% of fisheries resources are used for actual human consumption while the remaining 12 % are used for non-food purposes. Therefore, a more efficient and economical way of utilising all the non-food parts known as by-products that might be generated for other purposes is of paramount importance.

The net increase in fish production, processing and changing consumer trends towards readyto-use products as observed in the African catfish (*Clarias gariepinus*), are likely to lead to an increase in the number of by-products that might be generated in Nigeria. Evaluation of the composition of some of the by-products that might be generated from fish processing and finding a possible use for them is needed.

The African catfish (*Clarias gariepinus*), has been in high demand as a protein source in Nigeria and other parts of the world (Musa *et al.*, 2012), and is currently the dominant fish culture in Nigeria, responsible for the major aquaculture output of the country (FAO, 2017). According to FAO reports, the contribution of African catfish aquaculture to total production in Nigeria jumped from 7.8% to 53.2% in 2001 and 2013, respectively (Figure 1).



Figure 1: *Clarias gariepinus* production in Nigeria, in comparison with total aquaculture production from 1995 to 2015. Data obtained from Food and Agricultural Organisation (FAO) and Federal Department of Fisheries (FDF), Nigeria, (FAO, 2017).

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The 2015 data by FAO put total African catfish production in Nigeria at 160,295 tonnes out of 316,727, which constitutes 50.61%. Adewumi *et al.* (2010) reported that *C. gariepinus* gave Nigeria a niche in the global aquaculture production, and it is currently the second highest producer of aquaculture products in Africa and the highest producer of African catfish in Africa as well as the world (FAO, 2017).

The success of catfish as an aquaculture fish species has increased the awareness of the possibility of producing more value-added products from the species asides from the popular smoked catfish. With this development, many stakeholders are coming into the catfish business and looking into the possibility of canning. The canning process tends to generate a lot of by-products (predominantly the head, viscera and cut offs). Although data on the amount of by-product that might be generated is not available but is of utmost importance to investigate ways of effectively valorising the by-products that would be generated in the process.

Research has revealed that the lipid content of African catfish ranges between 2.02% to 6.87%, and in-turn is referred to as a lean fish. This means African catfish compares favorably with the Golden redfish with a lipid content of about 3 % as reported by Sa and Pe (2005). For this study, Golden redfish (*Sebastes marinus*) will be used because of its similar fat content to the African catfish. Although in terms of physical appearance, the African catfish head is different from the golden redfish. On examination of the African catfish, the gills, liver, viscera, constitute about 35 % of the total body weight, and the muscle and bones from the head can be easily separated to work on. This implies that although the possibilities of utilisation and product development from parts of catfish may differ, the evaluation process of raw material would be similar to that of the redfish.

Golden redfish (Sebastes marinus) is a commercially important fish species in Icelandic waters. The catch of golden redfish increased from 39,000 tonnes in 2011 to 57,900 tonnes in 2015 (Statistics Iceland, 2016) and the economic importance of the fish has therefore increased. The main markets are Germany and Belgium, but a significant portion is also exported to other Western European countries and Eastern Asia where Japan and China are the largest buyers (Statistics Iceland, 2011).

Redfish processing generates a vast quantity of by-products using the head, frame, and viscera, which are converted to low value by-products such as fish meal. Many studies have shown that by-products from fish processing contain both valuable lipid and protein fractions as well as other interesting and valuable compounds. In addition, the frame and head of the fish has been

seen to contain high values of calcium and phosphorus (Bechtel *et al.* 2010 and Gencbay and Turhan, 2016).

Research has shown that by-products from fishery industries have been processed into high and low value products (fish oil, protein powders, supplements and fish feeds). During the last decade, interest in the nutritional importance of n-3 polyunsaturated fatty acid (PUFA) has increased markedly. This has been seen particularly in the important role n-3 PUFA, especially docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), play in retinal and brain function (Connor et al., 1992). Reports also exist which describe fish lipids and the fatty acid composition in different tissues of fish. Sahari et al. (2013) investigated the distribution of the fatty acid compositions of different parts of five commercially important fish species from the Persian Gulf (Scomberomorus commersoni, Thunnus tonggol, Euthynnus affinis, omberomorus guttatus and Dussumieria acuta). Hong, et al. (2013) did a comparative study on the lipid content and fatty acid profile of muscle, brain and eyes of seven freshwater fish. He et al. (2011) showed that the contents of protein and fat in processing wastes of Atlantic salmon (Salmo salar) and yellowtail kingfish (Seriola lalandi) were 10 - 20 and 20 - 30%, respectively. Gencbay and Turhan, 2016, also studied the nutritional profile of the black sea anchovy (Engraulis encrasicholus) by-products. Profiles of amino acids, fatty acids, and minerals of anchovy by-products showed they are rich sources of lysine, leucine, and several essential amino acids.

In addition, in fish meal production factories, the head of fish which is about 25-30 % of the total fish weigh has been found to be a major part of fish meal production. However, the fish meal in turn has been found to be of low value from the economic standpoint. Therefore, the complete utilisation of every part of the fish head to develop high value-added products when possible is becoming an important economic issue for every stakeholder in the fisheries sector. Also, based on the fact that DHA is a major component of the brain, eye retina and heart muscle of humans, information about the fatty acid profile of the brain, eyes, gills and tongue of redfish and the identification of which of the parts are an excellent source of PUFA, and which could be used to produce good quality fish oil for human consumption, is therefore of great interest.

A detailed characterisation of the fish head needs to be completed to assess the composition. This would further open for the opportunity to produce new bioactive products, oils rich in omega 3 and 6 fatty acids, or other products to maximise the value from the redfish head and therefore get an overall higher value from the redfish that is caught around Iceland. Also, a study like this will be of value due to the fact that Nigeria has large fishery resources available and a strong, rapidly growing aquaculture sector. This study could open a new area where money can be made in the fisheries sector and also carve a niche for Nigeria in Africa and the rest of the world to be a leader in the area of the complete utilisation of every part of a fish. The redfish will be evaluated in this study to understand the process techniques involved in characterising a fish head.

The main goal of this study is to characterise the chemical composition of golden redfish head with respect to size. To use the results to take a step into looking at the possibility of new product development using a simple oil extraction process, and to further reflect on further utilisation (such as minerals, collagen etc) of the red fish head. This will give insight into the possibility of using the same principle that is used on redfish in this study on fish heads generated from fish species both in the wild and in the aquaculture sector (e.g. Catfish) in Nigeria.

- determine the yield and chemical composition of various parts of the fish head
- evaluate the possibility of developing a new product from the brain and eyes and assess some quality parameters of oil extracted from the Golden redfish heads in processing
- to generate knowledge and experience on methods used to extract oil out of the brain of fish

#### 2 LITERATURE REVIEW

Golden redfish (Sebastes marinus) in Iceland (Figure 2), are caught all year round but the quality of fish is highest in late winter. Bottom trawl is the most popular method for redfish fishing. The total annual catches of golden redfish in Icelandic waters were around 150,000 tonnes from about 1955 to 1988, but in recent years, the catches of golden redfish have declined to about 40,000 tonnes a year (FAO, 2013; Nghi and Sigurdsson, 2002). The waters west and south-west of Iceland are the richest in this fish, where the ocean temperature is at 3 - 8 °C. Golden redfish are present mostly at a depth of 100 - 400 m, but have been found at depths of 1000 m. The species migrate vertically, depending on the amount of light, and tends to stay deeper during daytime than during night. Redfish is a slow growing fish that reaches maturity at the age of 12-15 years of age. Unlike most other fish species that spawn unfertilised eggs, redfish has an internal fertilisation. Life offspring, 37-350 thousand at a time, are born in April and May. Redfish feed mainly on plankton, crustaceans, and fish fries. Older redfish feed off herring, capelin, shrimp and some codfish as well (Magnússon, 2000). The size of the mature male is 31 - 34 cm, whereas the size of the mature female is 35 - 37 cm. A typical landing weight of the redfish is 0.5 to 1.5 kg. Redfish is one of the most important commercial species in Iceland, sold largely as fresh or frozen fillets, or whole, frozen at sea or ashore. In 2012, the export values of golden redfish reached 66% for products frozen at sea, 24% for fresh and chilled and 10% land-frozen products. Initially, Icelanders regarded the redfish as a side product of cod fishing and often discarded it or used it for fishmeal and oil. In the 1950s to the year 1978, foreign fleets, mainly West German, were coming to Icelandic waters for redfish fishing and catching more redfish than the local fleet. As foreign fleets were expelled from Icelandic waters, markets opened in Germany, and Icelandic boats increased their catch.

The average compositions of redfish include 78% of water, 19% of protein, 3% of fat, 1.4% of minerals and 52% muscle of edible portion (Sa and Pe, 2005). Additionally, another result was reported that the proximate composition of the fresh redfish is 80% moisture, 1,9% minerals, 2.2% fat, 15.95% protein (Ayinsa and Maalekuu, 2013). The difference in proximate composition of fish muscle depends on species, season, sex, spawn cycle and environment (FAO, 2002). Other reports about chemical composition of redfish have been negligible to date.



Figure 2: Golden redfish (Source: Jón Baldur Hlíðberg)

# 2.1 By-products

There is no single definition of marine by-products. Usually, it refers to viscera, heads, cut-offs, bone, skin and fish that is damaged or unsuitable for human consumption (or further processing), and bycatch. In the regulatory papers, there is a division between by-products that can be used for human consumption and waste/discards/viscera (Rustad, 2003). In Norway, 'by-products' are defined as products that are not regarded as ordinary saleable products (fillet, round, eviscerated or beheaded fish), but which can be recycled after treatment. 'Waste' includes products that cannot be used for feed or value-added products, but which have to be composted, burned or destroyed (Bekkevold and Olafsen, 2007). The EC regulations on animal by-products (EC Nr 1774/2002, 2002), adopted on 3 October 2002, defines animal by-products as whole carcasses or parts of animals or products not intended for human consumption. The definition we will use to describe by-product is all parts left after all the edible portions have been removed.

The fish industry produces a wide range of by-products and solids including fish head, frame and viscera (Benhabiles *et al.*, 2012; Routray *et al.*, 2017a; Yao *et al.*, 2014), and liquids, which are generally wastewaters produced during processing. Solid wastes can be sources of bioactive proteins, peptides, amino-acids, enzymes, oils and fatty acids, which can be widely applied for nutraceutical extraction and/or preparation purposes (Gildberg, 2004; Routray *et al.*, 2017a). These wastes can also be sources of chitosan, chitin and carotenoids (Benhabiles *et al.*, 2013; Duan *et al.*, 2012; Dziril *et al.*, 2015; Hooshmand *et al.*, 2017). Apart from biomolecules and biochemicals, these wastes can be sources of various elements (calcium) and minerals (Yao *et* 

*al.*, 2014). Processing wastewaters can be potential sources of various biochemicals and biomolecules, which are dissolved or dispersed in it (Chowdhury *et al.*, 2010; Lin and Chiang, 1993). This can be converted to bioactive components with the application of appropriate and optimum unit-operations and extraction techniques. By-products and waste valorisation can lead to a reduction in pollution, generate an extra income which will support these industries during fluctuations and contribute towards increased economic stability.

#### 2.2 Fish head and its importance

The fish head has been an important part of fish by-products and has been used for various purposes such as in production of fish meal, pet foods, fish silage, in addition to being often dried. With the advancement in technology and for improved economic performance, a more efficient way of valorising this essential by-product is important.

In recent years, researchers have suggested various processing techniques that will ensure a better utilisation of the fish head. Karoud et al. (2017), produced antioxidative and angiotensin converting enzyme (ACE) inhibiting protein hydrolysates from hake (Merluccius. merluccius) heads. He et al. (2011) characterised the processing waste of Atlantic salmon (Salmo salar) and Yellowtail Kingfish (Seriola lalandi). The head components were found to be rich in omega-3 fatty acids while essential amino acids were also found to be abundant in the head components. There was a high presence of EPA and DHA in the brain oil while in the gill tissue, there was a high content of PUFA followed by MUFA and SFA. The EPA and DHA contents were also high as well as their ratio. In the report of Stoknes et al. (2004) about the fatty acid composition in the eyes and brain from teleosts and elasmobranchs, it was found that for the teleosts cod, saithe, redfish, salmon, and trout, results revealed that the eyes of the fatty species, salmon and trout, and the low fat redfish contained a higher percentage of the fatty acids 14:0, 16:1, 20:1, 22:1, 18:2n6, 18:4n3, and 20:4n3 when compared to the lean species, cod and saithe. However, it is to be noted here that although the PUFA in all the lean fishes seems high, if compared with the total lipid content, their resultant PUFA will be low. This gives the redfish an edge over the lean fishes because of the ratio of PUFA, MUFA to the total lipid content. Cod and saithe brain contained the highest levels of DHA while the DHA/EPA ratio was highest for the lean species, and particularly high for the cartilaginous species due to low levels of EPA. The level of DHA exceeded the level of EPA almost fivefold in brains from the cartilaginous species examined.

The global population is becoming increasingly reliant upon aquaculture to supply edible omega-3 long chain polyunsaturated fatty acids (n-3 LC PUFA). A series of stepwise reduction

in the dietary provision in commercial aquafeed formulations has resulted in findings that there is a direct relationship of dietary feed from commercial aquafeed formulations on the fatty acid profile of the fish. Hong *et al.* (2013), in a comparative study of the lipid content and fatty acid profile of muscle, brain and eyes of seven freshwater fishes, found that although n-3 PUFA were present in the various parts, most of the freshwater fish demonstrated much lower n-3 PUFA than n-6 PUFA in all tissues. The presence of EPA and DHA in fish head components indicates they can be utilised to produce fish oil. Sahari *et al.* (2013) in a study of n- fatty acid distribution of commercial fish species components reported that the richest sources of n-3 were found in the head, tail, fins, skin and liver.

In recent times, fish oil has been seen has one of the popular bioactive supplements, whose market value and demand can further increase with increasing consciousness about a healthy lifestyle. Polyunsaturated fatty acids and especially omega-3 fatty acids are the major components of fish oil, which attribute to its food value. This has led many research groups and markets to concentrate more on omega-3 fatty acid enriched food products or omega-3 fatty acids available in consumer-friendly forms (encapsulated and/or other concentrates), (Dave and Routray, 2018).

#### 2.3 Fish Oil

Better utilisation of marine fish processing by-products could be achieved by converting these materials into fish oil (Kim and Mendis, 2006). In general, the fat content of fish is varies from 2– 30% and it basically depends on the type of species, dietary, geographic, environmental, reproductive, and seasonal variations. However, with depleting marine fisheries resources it is not encouraged to fish for their oil. Therefore, a large amount of by-product generated from processing, would be a potential source to produce good quality fish oil for human consumption. Composition of fish oil is different from that of other oils and mainly composed of two types of fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These are polyunsaturated fatty acids classified as omega-3 fatty acids and predominantly found in many marine animals including cold-water fish species with a higher unsaturated fat content. Compared to saturated fats, polyunsaturated fatty acids in fish oil are readily digested for energy production and have been reported to have various bioactivities (Kim and Mendis, 2006).

#### 2.3.1 Omega-3 fatty acids

Health professionals and nutraceutical industry have been increasingly encouraging the consumption of poly-unsaturated fatty acids mainly omega-3 and omega-6 fatty acids, as these

fatty acids have been reported essential and beneficial for the proper maintenance and growth of humans. The health benefits of the omega-3 fatty acids include: prevention of atherosclerosis, heart attack, depression, stroke, diabetes, obesity, premature aging, hyper tension, cancer and improve the vision power and memory (Chin and Dart, 1995; Connor, 2000).

#### 2.3.2 Fish oil extraction

Fish oil can be produced by several methods which included hydraulic pressing, vacuum distillation, urea crystallization, supercritical fluid extraction, which all require high temperatures or high pressure in processing or reduction of moisture content in the sample prior to extraction (Mbatia *et al.* 2010). The most common method used for fish oil production is wet reduction, which involves three basic steps: cooking at high temperatures (85-95°C), pressing and centrifuging (FAO 2006). This process permits obtaining high volumes of crude fish oil, although subsequent refining steps are required in order to make the crude fish oil suitable for edible purposes. Enzymatic tissue disruption may be a valid alternative technique for releasing natural lipids from fish, which using commercial, low cost food grade neutral proteases provides an attractive alternative as reactions could be carried out under mild conditions for short periods of time.

Solvent based extraction can be accounted as the most common and traditional method of extraction, and some of the solvent extraction methods, considered as standard methods of total lipid extraction include soxhlet extraction, Bligh and Dyer, (Bligh and Dyer, 1959; Manirakiza *et al.*, 2001; Smedes and Askland, 1999). Some of the regularly used solvents include hexane, methanol, acetone, propanol, cyclohexane, petroleum ether, chloroform and a combination of these solvents (Manirakiza *et al.*, 2001). Based on extensive research in this field, some of the major challenges can be summarised as (a) development of eco-friendlier and food grade extraction methods (reduction of the amount of solvent), (b) reducing time and temperature of extraction (to avoid fatty acid destruction by oxidation). New methods which seem to be better than solvent extraction methods, have been increasingly applied in the extraction and purification of the marine oils and can be further explored for extraction of marine oils from the waste sources, for preparation of nutraceuticals. Those methods are mainly supercritical fluid and enzymatic extraction.

#### 2.4 Minerals

Minerals play an important role in maintaining body functions because they maintain acidbase balance and help blood formation (hemoglobin formation) (Njinkoue *et al.*, 2016). They also control the water balance in the body, help bone formation and teeth structure, and catalyse many metabolic reactions. The importance of minerals as food and feed ingredients is not only their nutritional and physiological roles, but they also contribute to food flavour and activate or inhibit enzyme-catalysed and other metabolic reactions, and they affect the texture of food (Njinkoue *et al.*, 2016). However, by-products from fish processing have also been found to be sources of essential minerals, (heads and viscera). Bechtel *et al.* (2010); Gencbay and Turhan (2016); and Bechtel and Johnson (2004) reported high values of calcium and phosphorus in the frame and heads of Pacific Ocean perch (*Sebastes alutus*), Black Sea Anchovy (*Engraulis encrasicholus*) and pink salmon heads. Bones have also been seen to constitute a significant part of the fish; and approximately 10–15% of total fish biomass are bones from the head. In a study by Toppe *et al.* (2006), on the mineral composition from bones of various fish species, it was recorded that the levels of macro minerals correlated to the level of ash in the bones, and that the bones of the fish species had a high level of macro minerals (calcium and phosphorus).

#### 2.5 Collagen

Collagen is the foremost constituent of the extracellular matrix which is abundant fibrous structural protein in all higher entities (Sweeney *et al.*, 2008). It is mostly found in fibrous tissues such as skin, ligament and tendon in the form of elongated fibrils and is also abundant in cornea, blood vessels, bone, cartilage, intervertebral disc and the gut. (Pati *et al.*, 2010). The characteristic feature of a typical collagen molecule, tropo collagen, is its long, stiff, triple-stranded helix, in which three collagen polypeptide chains are wound around one another in the form of a rope-like super helix (Shanmugam *et al.*, 2012).

Nearly 28 types of collagen have been identified so far which are composed of 46 distinct polypeptide chains. All of them have a characteristic triple helix but the length of the helix and the size and nature of the non-helical portion varies from one to another type (Miller, 1984).

Collagen has been isolated from the skins of land-based animals, such as cow and pig, and has been widely used in food, cosmetic, biomedical and pharmaceutical industries (Ogawa *et al.*, 2004). However, the outbreak of bovine spongiform encephalopathy (BSE) and the foot and-mouth disease (FMD) crisis have resulted in anxiety among users of collagen and collagenderived products from land-based animals in recent years (Jongjareonrak *et al.*, 2005). Additionally, collagen obtained from pig cannot be used as a component of some foods for religious reasons (Sadowska *et al.*, 2003). Therefore, there is a strong need to develop alternative collagen sources. By-products from fish processing have been seen as an alternative source and have received attention as collagen sources. Fish processing by-products consist of skin, scale and bone, which are very rich in collagen (Kittiphattanabawon *et al.*, 2005)

Collagen from several fish species has been isolated and characterised (Jongjareonrak *et al.*, 2005; Morimura *et al.*, 2002). Wang et al. (2008) isolated collagen from the skin, scale and bones of deep-sea red fish (*Sebastes mentella*) and observed that isolated collagen was type I and manintaned its triple helical structures. Also, Shanmugam *et al.* (2011 and 2012) extracted acid soluble collagen from the outer skin of two species of cuttlefish; *Sepiella pharaonic* and *Sepiella inermis*.

The result of the study reveals the existence of helical arrangements of collagen and it could provide alternatives to mammalian collagen in food, cosmetics and biomedical materials.

#### 3 METHODOLOGY

#### 3.1 Experimental design

Redfish (*Sebastes marinus*) caught in December 2018 by bottom fish trawl were used. Frozen redfish heads of large and small sizes were provided by HB Grandi and divided into two groups (the big heads were collected fresh and frozen at the Matis laboratories while the smaller heads were collected in frozen blocks from HB Grandi). The study was undertaken in two phases: evaluation of the composition of different parts of the head and the crude oil extraction from components of head. The experiments were carried out at the Matis laboratories in Reykjavik, Iceland.

#### 3.1.1 Sample Preparation

Twenty-one small heads and fifteen big heads were separated into two different samples (A and B). The fish heads were weighed, and average mass of 352.2 g and 1406.8 g were recorded for both small and big heads, respectively. The eyes, brain, tongue, gill and cheek were removed while the fish was frozen (Appendix A).

For removal of eyes, this was done by piercing the fold of skin surrounding the eyes and a small sharp knife inserted into the back of the eye to cut the optic nerve inside the eye socket. Then using a small spoon and finger, the eyeball and surrounding tissue were popped out.

The brain was removed by first cutting through the top of the head above the location of the eye with a sharp knife as shown in Appendix A; then with the aid of a small spoon and spatula, the brain was removed and stored in a clean container. The fish head was then left for 5 hours to thaw, after which the gills and tongue were removed. The cheek was cut out with a sharp knife and all the sample parts were kept in small plastic containers and stored at -80° C. Prior to analysis, the samples (eyes, tongue, gills, cheek and brain) were removed from the -80° C freezers, thawed for few hours and were homogenized separately in a Waring blender.

It should be noted here that getting a homogenous mixture for the brain was quite difficult and success depended on obtaining a representative portion from the brain mixture for the evaluation and extraction process.

Finally, the gills and eye lens were removed separately and analysed for collagen and minerals.

# 3.1.2 Evaluation of composition and oil extraction

Figure 3 shows the setup for the evaluation of the composition of both small and big heads in all the sample parts (cheek, gill, tongue, eye and brain). The results obtained from the lipid analysis were used to ascertain the part used for the oil extraction.



Figure 3: Experimental setup for evaluation of composition

Figure 4 (below) shows the part of the head used for the oil extraction process. The oil extraction method used was based on the wet reduction method (with and without heat) according to the method of Taati *et al.* (2018) with slight modifications performed at the Matis laboratory.

#### 3.1.3 Wet reduction method

With heat: The small and large head brains (n = 2) were blended, and deionized water was added at a ratio of 10ml to 2 g of the sample and cooked in a water bath at 95 °C for 15 minutes. After cooking, the slurry was centrifuged (5.000rpm, 20 °C, 20 min) and the sample separated into four parts (the residue, water layer, emulsion layer and oil layer). The two topmost layers were collected into another tube (emulsion layer and oil layer) and 10 ml of deionized water was added and centrifuged (5.000rpm, 20 °C, 10 min). This was repeated twice in order to fully extract the oil from the emulsion phase. After this, the oil layer was recovered and weighed. For the eyes, water was not added as with the brain, because of their high-water content (80 %).

**Without heat:** The same method was used without heating the sample first. There was only centrifugation. The oil samples extracted were kept at -80° C prior to analysis.

#### 3.2 Chemical and physical analysis

#### 3.2.1 Yield analysis

The head was weighed after beheading and then every single part from the same head (eyes, brain, gills, cheeks and tongue) were also weighed to assess the yield of each part of the head and were expressed as a percentage. The oil yield was also expressed as a percentage of the crude oil extracted from the by-product

$$Yield (\%) = \frac{g \text{ individual part}}{g \text{ whole head}} \ge 100$$
$$Yield (\%) = \frac{wt \text{ of crude oil} \ge 100}{wt \text{ of by-product}}$$

### 3.2.2 Water analysis

The water content of the various parts of the head was analysed using ISO 6494 (1999). The results were expressed as a percentage.



Figure 4: Experimental setup for product development

# 3.2.3 Protein analysis

Protein content was analysed using the method described in ISO 5983-2:2005 using a Tecator. The results were expressed in percentage.

# 3.2.4 Ash analysis

Ash content was analysed using the method described in ISO 5984-2002 (E).

# 3.2.5 Lipid analysis

Lipids were extracted based on the Bligh and Dyer (1959) method with adaptations (the 25g of sample were replaced by the quantity of sample and water needed to have 80% water in it). The determination of lipid content is expressed as g lipid/100 g sample.

# 3.2.6 Phospholipid Content (PL)

Colorimetric method based on the formation of a complex between phospholipids and ammonium ferrothiocyanate was used (Stewart, 1980). The results expressed as g of PL/100g of lipids.

# 3.2.7 Free fatty acid analysis (FFA)

The free fatty acid (FFAs) content was determined by the method of Lowry and Tinsley (1979), with modifications as described by Bernárdez *et al.* (2005), from the lipid extractions provided by the Bligh and Dyer (1959) method, as described earlier. The results were expressed as g FFA/100 g lipids.

# 3.2.8 Fatty acid composition (FAC)

The fatty acid composition (FAC) was determined based on AOCS official method Ce 1b-89. The results expressed as percentage of total lipid.

# 3.2.9 Collagen

Twenty lenses were pooled from the SH eye and seven lenses were pooled from the BH eye, ground using a ceramic mortar. The collagen content of the eye lenses was determined using the standardised NMLK, 2002, 2<sup>nd</sup> edition method. The results were expressed as a percentage.

# 3.2.10 Minerals

The mineral content of the gills was determined using the standardised NMLK 186 (2007) method. The results were expressed as g of mineral/kg of wet weigh.

# 3.2.11 Statistical analysis

Data was analyzed using Microsoft Excel 2016 (Microsoft Inc. Redmond, Wash, USA) and SPSS. One-way analysis of variance (ANOVA), was performed on means of the variables and p values less than 0.05 were considered as a significant difference for all analyses.

#### 4 RESULTS AND DISCUSSION

#### 4.1 Yield

The yield proportion of the eyes, gills, cheek, tongue, brain and carcass (what was left of the head after removing the parts) are shown in Figure 5 (Table 1, Appendix C). Overall, the highest yield was obtained in the gills which constituted 8.5 % and 9.6 % for the small and big heads. The cheek and eyes of the SH and BH made up 3.11 %, 6.0 % and 3.94 %, 4.26 % of the total weight of the head respectively. The fishes with small heads gave a significantly higher brain yield (2.0 %) than fishes with the big heads (1.9 %). The lowest yield proportions were seen in the tongue (0.70 and 1.14 %) in the small and big heads. The other materials in the small and big heads which were left after all the parts had been removed, accounted for a larger proportion was the carcass (73.1 % and 77.76 %). Also, the cutting ability will determine the actual yield as this might vary between individuals. It should be noted that between 7 % and 2 % of the total weight in both the small and big heads were lost during the cutting process. This could be as a result of thawing the heads during the separation and some blood loss during the process. The increase observed in the gill yield as seen in the large heads agrees with the study of Hughes (1966), which stated that the bigger the gills get, the more oxygen uptake occurs, and the gills get bigger as the fish gets larger and more active.



Figure 5: Yield proportion (%) of the different parts of the Redfish (*Sebastes marinus*) head. SH-small head; BH- big head

#### 4.2 Water content

The water content of the eyes, gills, brain, tongue, and cheeks of the SH and BH are shown in Figure 6 (Table 1, Appendix C). The water content in the eye of both the SH and BH was higher and significantly different (p<0.05) than all other sample parts. However, no significant difference (p>0.05) was observed in the eye water content of both the SH and BH samples.

A significantly (p<0.05) low water content was observed in the SH brain (26.9 %) when compared to the BH brain (44.6 %) and other sample parts. The tongue also had a water content of 53.2 % and 46.5 % for both SH and BH samples respectively, but with no significant difference (p>0.05). Furthermore, a higher water content was recorded in the cheek (74.1 and 76.6 %) and gills (67.6 and 70.0 %) of both the SH and BH. However, no significant difference (p>0.05) was observed as a result of the different sizes of the fish. The eye water content reported in this study was comparable to the water content of redfish eye reported by Stokens *et al.* (2004) and lower than the water content of other similar fishes (cod, saithe, Portuguese dogfish, black dogfish and leaf scale gulper shark) but also higher than the water content in the eye of salmon and trout.

Huss (1995) reported that differences in water content could be as result of variation between species, sexual maturation and feeding cycles. The changes in water content of the sample parts between the SH and BH might be influenced by one of the above-mentioned reasons.



Figure 6: Water contents (%) of the different parts of the Redfish (*Sebastes marinus*) head. SH-small head; BH- big head.

#### 4.3 Protein content

The protein content from the different parts of the small head and big head ranged from 2.7 % - 20.2 % as shown in Figure 7 (Table 2, Appendix D). The cheek had the highest protein content in both the SH (20.2 %) and BH (18.2 %) sample part with no significant difference (p>0.05). The lowest protein content was observed in the brain in both the SH (2.7 %) and BH (2.8 %).

However, high protein content was observed in the gills of the SH and BH (13.8 % and 16.2 %) as well as in the muscle tissue of the tongue (12.3 %) of the BH sample. It should be noted here that for the SH, no value was recorded because the tongue sample left for the protein content analysis was very small. A significantly (p<0.05) higher protein content was also observed in the SH eye (11.7 %) compared to the BH eye (8.0 %).

A correlation seems to exist with the protein and water content, and this can be seen in all the sample parts when the water content was high, the protein content was low and vice versa. However, an exception was observed with the brain where the protein and water content were low in both SH and BH. This is as a result of the high lipid content observed in both SH and BH. Comparing the protein content in the various parts with respect to the size of the fish, there seems to be a little variation. The variation observed in the protein content of the sample parts might be linked to the size of the fish as well as the different fishing grounds where the feeding cycle of the fish play an important role. With the high protein content observed in the cheeks and gills for the SH and BH as well as the tongue for the BH sample, a further study on these parts will be needed.



Figure 7: Protein content (%) of the different parts of the Redfish (*Sebastes marinus*) head SH-small head; BH- big head.

#### 4.4 Ash content

The ash content of the eyes, gills, brain, tongue and cheeks are shown in Figure 8 (Table 2, Appendix D). The gills in the SH and BH (6.6 %, 5.1 %) were significantly different (p<0.05) from each other and also from the BH tongue but the BH tongue (8.2 %) showed the highest ash content, whereas the other four parts (eyes, brain, cheeks and tongue) in both the SH and the BH contained similar low ash content, in the range from 0.5 % - 3.3 %. It should be noted here that for the SH, no value was recorded because there was no tongue sample left for the protein content analysis. The ash content in the brain were not significantly different (p>0.05) in both SH and BH but a significant difference (p<0.05) was observed in the cheek and eye ash contents.



Figure 8: Ash content (%) of the different parts of the Redfish (Sebastes marinus) head. SH-small head; BH- big head.

The presence of bones or cartilage, as well as fibrous filaments in the gills, which contain a higher amount of minerals than the softer tissue of the fish, such as the muscle, brain, or eyes might be responsible for the high ash content in the gill. According to Murray and Burt (2001), the ash content of the gills is also affected by the environment, because fishes use their gills in absorbing minerals from the external environment. External objects such as sand, stones present in the sea can also affect the ash content in the gills.

#### 4.5 Lipid content

The lipid content result is shown in Figure 9 (Table 3, Appendix E) and varied in different parts from 2.6 - 45.7 % within the SH and BH. The highest lipid content (45.7 %) was observed in

the brain of the SH samples, and was significantly higher (p<0.05) than the lipid content in the brain of the BH (35.0 %) and all other sample parts within the SH and BH. An increase was seen in the lipid content of the eye with increasing fish size and a significant difference (p<0.05) was recorded for SH eye (2.6 %) when compared to BH eye (8.2 %). This result shows that the brain and eye will be worth looking into. No significant difference (p>0.05) was observed between the lipid content in the tongue of both the SH and BH. However, there was a difference (p<0.05) between lipid content in the cheeks and gills in the SH and BH respectively.



Figure 9: Lipid content (%) of the different parts of the Redfish (*Sebastes marinus*) head. SH-small head; BH- big head.

The result obtained in this study compares favourably well with the report of Stoknes *et al.* (2004) on the lipid content in the eye and brain of redfish (*Sebastes marinus*) captured off the West coast of Norway. However, for the lipid content in the eye, the result obtained in this study were found to be lower than the lipid contents in the eye of salmon and trout which were from species farmed on the West coast of Norway. Also, the result obtained in this study for the brain and eye lipid content were higher than the brain and eye lipid content reported for salmon, trout, cod, saithe, Portuguese dogfish, black dogfish and leafscale gulper shark captured at Hatton Bank in the North Atlantic. The variation observed in lipid content may be due to size, age and different spawning feeding periods of the redfish and seasonal fluctuation (Nazemroaya *et al.*, 2011).

#### 4.6 Free fatty acid content (FFA)

The FFA content of the different parts of the redfish head (SH and BH) are shown in Figure 10 (Table 3, Appendix E). The FFA content in all the SH and BH parts varied from 0.09 g of FFA/100 g lipids (SH check) – 2.74 g of FFA/100 g lipids (BH Brain). A significant difference (p<0.05) was observed in the gills, eye and tongue between the SH and BH sample parts while no significant difference (p>0.05) was seen in the cheek in the SH and BH (2.74 g of FFA/100 g lipids and 1.97 g of FFA/100 g lipids). A lower value was recorded for the FFA in the brain of both SH and BH (0.13 g of FFA/100 g lipids and 0.09 g of FFA/100 g lipids), however, no significant difference was seen between them. It was observed that the FFA content of the SH sample parts were higher than that of the BH.



Figure 10: Free fatty acid content (g FFA/100 g lipid) of the different parts of the Redfish (*Sebastes marinus*) head. SH-small head; BH- big head.

The FFA of the eye for the redfish SH and BH in this study were found to be lower than what was reported by Stoknes *et al.* (2004) for the FFA of redfish eye, saithe and cod eyes. The difference in the FFA in the eyes of the redfish might be as a result of difference in size, age and different spawning and feeding periods or handling of the redfish.

#### 4.7 Phospholipid content (PL)

The PL content as shown in Figure 11 (Table 3, Appendix E) of all the parts are in the range of 0.3 g PL/ 100g - 8.7 g PL/ 100g with the SH eye having the lowest PL and BH cheek having the highest PL. A significant difference (p<0.05) was observed in the PL content of all the sample parts of the SH and BH. The PL content of the BH gill, cheek, eye and brain were found

to be significantly (p<0.05) higher than those reported for the SH. However, the PL content of the SH tongue was found to be significantly higher (p<0.05) than the BH tongue. The PL reported for eyes in this study were lower than the values reported for the eyes of cod, saithe, Portuguese dogfish, black dogfish and leafscale gulper shark (Stokness *et al.*, 2004).



Figure 11: Phospholipid content (g/100 g) of the different parts of the Redfish (*Sebastes marinus*) head. SH-small head; BH- big head.

#### 4.8 Mineral content

The mineral composition of the gills of both the SH and BH are shown in Figure 12 (Table Appendix C). There were significant differences (p<0.05) in the phosphorus (P) and calcium (Ca) contents between the SH and BH gills with the SH having a higher value of both minerals. While no significant difference (p>0.05) was observed in the sodium (Na), potassium (K) and magnesium (Mg) contents. Calcium was the most abundant micromineral detected followed by phosphorus in the SH and BH gills. It was observed that the values of phosphorus and calcium (10.12 g/kg, 7.93 g/kg and 22.25 g/kg, 16.16 g/kg) were higher in both the SH and BH gills compared to other elements while the magnesium value was quite low (0.5 g/kg and 0.38 g/kg) in both SH and BH.



Figure 12: Mineral composition of Redfish gills. SH-small head; BH- big head.

The results of this study compare with the findings of Gencbay and Turhan (2016); Wu *et al.* (2011); He *et al.* (2011); Bechtel *et al.* (2008) where the phosphorus and calcium contents of the fish heads, were found to be higher than other mineral component with calcium being the most abundant followed by phosphorus.

Due to the physiological importance of calcium in the soft tissues of the human body in providing strength to teeth and bones, calcium supplements from redfish gills could be an alternative.

# 4.9 Collagen content

The eye lens in both SH and BH samples were distinct and on touching, it has a jelly-like feeling. It was based on this that the collagen content of the eye lens was measured. However, the result reveals that the collagen content in the lens were very low in both the SH (0.016 %) and BH (0.019 %) eye lenses. Research claims that there are proteins present in the eyes, hence further research in categorising this protein and the feasibility of using them is needed.

# 4.10 Fatty acid profile

The fatty acid composition of the different parts from the redfish SH and BH are shown in Figure 13 and 14 (Table 4 and 5, Appendix F). The fatty acid profile of all parts of the head reveals a significant difference (p<0.05) between SH and BH sample parts. The samples were characterised by high amounts of monounsaturated fatty acids (MUFA) followed by polyunsaturated fatty acid (PUFA) and saturated fatty acid (SFA). The SFA, MUFA and PUFA

values of the SH sample parts were in the range of 20.44 % - 21.51 %, 46.46 % - 54.01 % and 20.32 % - 28 % while the BH sample parts are in the range of 17.72 - 19.38, 53.68 - 57.66 and 20.66 % - 23.49 % respectively.

In the SH samples, the cheek (8.71 %) and tongue (8.42 %) contained higher amounts of EPA while the eye (15.33 %) and cheek (13.34 %) contained high amounts of DHA. The values of EPA and DHA in the BH samples were higher in the cheek (7.71 %, 10.63 %) and eye (7.59 %, 9.35). EPA/DHA ratio a shown in Figure 15 was however higher in the brain in both the SH and BH (1.15 % and 0.96 %) samples.



Figure 13: Fatty acid composition (g/100 g of total lipids) of different parts of Redfish (*Sebastes marinus*) small head SFA- saturated fatty acid, MUFA- monounsaturated fatty acid, PUFA-polyunsaturated fatty acid.



Figure 14: Fatty acid composition (g/100 g of total lipids) of different parts of Redfish (*Sebastes marinus*) big head SFA- saturated fatty acid, MUFA- monounsaturated fatty acid, PUFA-polyunsaturated fatty acid.



Figure 15: EPA/DHA ratio (g/100 g of total lipids) of different parts of Redfish (*Sebastes marinus*) small head SFA- saturated fatty acid, MUFA- monounsaturated fatty acid, PUFA-polyunsaturated fatty acid.

The results revealed that the dominating fatty acid group in all the head parts were MUFAs, followed by PUFAs and finally SFAs, which is in agreement with the findings of Stoknes *et al.* (2004) where the fatty acids composition in redfish eye and brain were characterised by high amount of MUFAs followed by PUFAs and SFAs. The result however does not agree with the

findings of Duan *et al.* (2010) in cod muscle and Stoknes et al. (2004) for fatty acid in the brain and eyes of cod, saithe, salmon and trout, because the fish species are different.

The n-3/n-6 ratio was also determined for all the parts. The results showed that the parts generally contained high n-3/n-6 ratios. The highest n-3/n-6 ratio in the SH was in the eyes (9.43) while it was found in the cheek for BH. The n-3/n-6 ratio in the brain was higher in the BH (7.48) than in the SH (4.99). The ratio was generally quite high in the different head parts and therefore, they may possibly be used in further development of health products.

The results obtained show that the different parts of the head are good sources of fatty acids, including omega-3 and omega-6 fatty acids, which are essential fatty acids that need to be included in the diet, since the human body cannot synthetize them from other food source fatty acids. Also, looking at the high lipid content of the brain and the high EPA/DHA ratio of the eye and the brain, they are worth looking into for the development of a value-added oil products.

### 4.11 Oil extraction

#### 4.11.1 Brain

#### 4.11.1.1 Yield

The amount of oil obtained by the wet reduction method (with heat and without heat) process is shown in Figure 16. There was no significant difference (p>0.05) in the yield of the small head with respect to the extraction method (with or without heat). However, significant difference was observed in the yield of the SH with application of Bligh and Dyer. Therefore, it can be said that the other two extraction methods (with heat and without heat) are not as efficient as Bligh and Dyer in extracting the oil. For the BH samples, significant difference (p<0.05) was seen in the oil extracted as a higher amount of oil was extracted with heat. But no significant difference (p>0.05) was found compared with the B&D method.



Figure 16: Yield proportion (%) of the Redfish (Sebastes marinus) brain.

SH-small head; BH- big head; BWH- brain without heat; BH- brain heated.

A similar result was recorded by Taati *et al.* (2018) in the extraction of fish oil from tuna byproducts where a high oil content was extracted as a result of heating the by-product.

The higher percentage of oil yield from the BH when heated can be linked to the heating which causes denaturation of the protein matrixes of the tissue that the oil is strongly bound to and causes the opening of oil globules and fat cells, resulting in the release and fluidity of the oil, which resulted in an increase the yield (Chantachum *et al.*, 2000). The lower oil yield from the SH when heated might be as a result of the strong bonds between oil and protein phase. This agrees with the report of Chantachum *et al.* (2000), where an observation was made as to how the tightly packed protein structure becomes when denatured leading to the prevention of oil release. Ahren and Klibanov (1985) also reported that when proteins are heated at higher temperatures (90 – 100 C), they undergo irreversible denaturation, hence preventing the release of oil.

#### 4.11.1.2 Free fatty acid content (FFA)

The FFA content of oil extracted from the redfish brain with heat and without heat (SH and BH) are shown in Figure 17. A significant difference was seen between the FFA of oil from SH and BH brain samples under the two-extraction method. A low FFA was recorded for the BH brain samples at the two extraction conditions.



Figure 17: Free fatty acid content (g FFA/100 g lipid) of the different parts of the Redfish (*Sebastes marinus*) head SH-small head; BH- big head; B&D- Bligh and Dyer.

For the BH brain samples, no significant difference (p>0.05) was found between the three extraction methods, but with the heat extraction for the SH, an increase was seen in the FFA. This can be explained by the fact that when oil is heated, the triglycerides bonds will be broken, hence creating more free fatty acids. It should be noted that the high FFA in the extracted oil and as seen in the B&D extract as well is as a result of more enzymatic activities in the SH samples as compared with BH samples. However, the handling of the raw material (storing at different temperatures) could also be a reason for the high and low FFA in both the SH and BH samples.

#### 4.11.1.3 Phospholipid content

The phospholipid content of the SH and BH oil samples under the two extraction conditions reveals that there was no phospholipid in the SH and BH brain oil samples. Phospholipids are primarily found in the cell membrane and to extract them, they need to be broken. It should be mentioned here that for the raw material (SH and BH), PL was extracted using B&D method (0.25 and 0.46). But for the oil extracted with heat and without heat, none of the process could break the cell membrane, hence no PL was extracted.

#### 4.11.1.4 Fatty acid profile

The fatty acid profile of the SH and BH brain oil extracted by the wet reduction method and that of the raw material is shown in Figure 18. The result reveals no significant difference (p<0.05) was observed in the saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) within SH and BH brain samples. They were all

characterised by high amounts of (MUFA) followed by polyunsaturated fatty acid (PUFA) and saturated fatty acid (SFA). The SFA, MUFA and PUFA values of the SH and BH with heat and without heat were in the range of 17.45 % - 18.72 %; 55.89 % - 60.02 %; 17.34 % - 19.98 % and 16.99 % - 18.74 %; 55.98 % - 60.06 %; 17.66 % - 19.94 % respectively. In the SH brain, the EPA (6.42 % and 6.54 %) was found to be higher than the DHA (5.93 % and 6.10 %) in the two extraction methods while the DHA (7.57 % and 7.54 %) was found to be higher than the EPA (7.27 % and 7.25 %) for the BH brain. This was the same trend for the raw material where in the SH, the EPA (6.23) was higher than the DHA (6.06) and for the BH, the DHA (7.83) was higher than the EPA (7.17).



Figure 18: Fatty acid composition (g/100 g of total lipids) of Redfish (*Sebastes marinus*) small head and big head brain. SFA- saturated fatty acid, MUFA- monounsaturated fatty acid, PUFA-polyunsaturated fatty acid.

The n-3/n-6 ratio results showed that the SH (7.46 and 7.61) and BH (8.81 and 8.75) brains under the two extraction conditions had high n-3/n-6 ratios. The highest n-3/n-6 ratio was found in the BH brain and was significantly higher that of the SH brain. The same trend was also seen in the n-3/n-6 ratio in the raw material. The ratio was generally quite high in both extraction methods and therefore, they may possibly be used in further development of health products.

The results obtained show that the extraction methods did not have any major significant effect on the SFA, MUFA and PUFA within the SH and BH samples.

### 4.12 Eyes

### 4.12.1 Yield

The oil extraction process (with heat and without heat) for the eye was not successful for the SH. However, for the BH, the extraction with heat gave no yield, but for the extraction without heat, a low yield of 0.53 % was recorded. For the raw material, the Bligh and dyer extraction for the SH and BH samples were successful and gave a yield of 6.29 for the SH and 8.45 for the BH.

The result obtained in this study with the extraction with heat for the SH eye and BH eye does not agree with the literature for extraction of fish oil with heat. A better oil release is obtained when samples are heated because the heating coagulates the protein which enhances better oil release. However, it should be noted that for the eye in both the SH and BH samples, the lipid content was low and such a simple method (extraction with heat and without heat) will not be able to separate the oil from the eye matrix.

### 4.12.2 Free fatty acid (FFA)

The free fatty acid for the BH eye oil extracted without heat was low (0.22 g FFA/100 g lipid) while no result was recorded for the BH extracted with heat and SH extracted with heat and without heat. For the raw material, the FFA for the BH eye was also low (0.62 g FFA/100 g lipid) while a high FFA was recorded for the SH eye (8.57 g FFA/100 g lipid). This result agrees with that recorded for the FFA of the SH and BH for the brain samples.

# 4.12.3 Phospholipid (PL)

Result of analysis of PL showed that no PL was present in the extracted oil.

# 4.12.4 Fatty acid profile

The result of the fatty acid content for the BH eye oil extracted without heat shows it was characterised by high amount of MUFA (56.45 %) followed by PUFA (21.56 %) and SFA (18.03 %). The DHA (8.52 %) was also seen to be higher than the EPA (7.57 %) and the n3/n6 ratio was also high (8.14). However, for the raw material, the same trend was observed for the BH.

#### **5. CONCLUSION**

In the present study, the aim of separating and characterising the different parts of the golden redfish head as well as evaluation of possibilities of product development from parts of redfish head, such as the oil extraction was achieved. There were challenges in the separation of the different parts from the head, especially in getting out all the brain fluid and so to get the maximum yield. Cutting out the tongue was also challenging. The strong bond between the oil and the protein phase for the brain samples made the oil extraction method under laboratory conditions difficult leading to a variation seen in the oil quantity, especially with the SH brain. (It was difficult to homogenize the sample for the B&D to have a representative portion of the brain).

It was revealed that the brain had high lipid content in both the SH and BH samples, although there was a significant difference in the lipid content between the SH and the BH. The cheek was also seen to have a high protein content. All the parts of the redfish head in the SH and BH were found to be rich sources of PUFA, n-3 fatty acids, EPA + DHA, and can be considered for future product development. The result also reveals that the redfish gills are a good source of essential micro elements. For the eye's lens, the collagen content was low, but with the size of the lens, other nutritional properties could be investigated (e.g. protein). The yield of extracted oil from the SH and BH brain using the wet reduction method (this is used to have edible oil for human consumption devoid of organic solvent), revealed that the heat extraction method for the big head brain is more efficient than the extraction without heat. For the small head, no significant difference was found. Also, for the oil quality between the samples, it was characterised by high EPA, DHA and n-3/n-6 ratio. However, comparing the individual fatty acid of the oil extracted with the two methods with the Bligh and dyer extract, there were significant difference was observed in there SFA, MUFA and PUFA.

The gills however contain a high quantity of calcium and phosphorus which could be exploited for further use. Also, looking at the carcass (left over after all the parts have been removed), it could also be separated into muscle and bones, which has been shown to contain high deposits of important micro elements (calcium and phosphorus).

The results from this study revealed that different parts of the redfish head contained large amounts of nutritional components, and they can be utilised to produce value-added products. The brain and cheek could be used to produce edible fish oil for human consumption, protein powder and protein hydrolysates, while mineral supplements, collagenous proteins could be extracted from the gills and carcass.

According to statistics in Iceland, about 57,900 tonnes of redfish were caught in 2015. Working with this and assuming a catch of 10,000 tonnes, 50.221 kg of oil could be extracted as well as 6473.05 kg of calcium and 3041.87 kg of phosphorus. Below is a flow diagram from the redfish (10,000 tonnes) showing the breakdown of what could be extracted from the heads.



Figure 19: Flow diagram from the redfish (10,000 tonnes) showing the breakdown of extraction possibilities.

#### 6. RECOMMENDATIONS

Further characterisation of the oil from the brain is needed to fully understand the oil properties and refining process to enable its usage for human consumption. The eye and the lens also need to be researched further to understand what other components it has to put it into proper use. For the gills, identifying the type of calcium present is needed and for the carcass (bones from the head) which constitute a large proportion of the head, further research needs to be conducted to determine what it could be used for to completely valorise the head.

Also, in relation to catfish, identifying which part of the head would be easy to separate, other possible by-products and what simple process techniques could be put in place in extracting the valuable components from the parts to completely put then into direct human use.

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#### 8. REFERENCES

- Adam, F., Abert-Vian, M., Peltier, G.and Chemat, F. (2012). Solvent-free" ultrasound assisted extraction of lipids from fresh microalgae cells: a green, clean and scalable process. *Bioresour. Technol.* 114, 457-465
- Adewolu, M. A. and Adeoti, A. J. (2010). Effect of mixed feeding schedules with varying dietary crude protein levels on the growth and feed utilization of *Clarias gariepinus* fingerlings. *Journal of Fisheries and Aquatic Science*, 5, 304–310. https://doi.org/10. 3923/jfas.2010.304.310
- Adewumi, A. A. Olaleye, V. F. (2010) Catfish culture in Nigeria: Progress, prospects and problems. *African Journal of Agricultural Research* 6: 1281-1285.
- Ahren, T. J. and Klibanov, A. M. (1985). The mechanism of irreversible enzyme inactivation at 100 C. Science, 288, 1280 1284.
- Antoine, F. R., Wei, C. I., Littell, R. C. and Marshall, M. R. (1999). HPLC method for analysis of free amino acids in fish using o-phthaldialdehyde precolumn derivatization. *Journal of Agriculture and Food Chemistry*, 47: 5100–5107.
- Arab-Tehrany, E., Jacquot, M., Gaiani, C., Imran, M., Desobry, S. and Linder, M. (2012). Beneficial effects and oxidativestabilityofomega-3 long-chain polyunsaturated fatty acids. *Trends in Food Science and Technology*, 25, 24e33.
- Arterburn, L. M., Hall, E. B. and Oken, H. (2006). Distribution, interconversion, and dose response of n-3 fatty acids in humans. *American Journal of Clinical Nutrition* 83:S1467–S1476
- Ayinsa, K. and Maalekuu, B. K. (2013). Effect of traditional fish processing methods on the proximate composition of red fish stored under ambient room conditions Department of Horticulture Faculty of Agriculture Kwame Nkrumah University of Science and Technology Kumasi. *American Journal of Food and Nutrition*, 15–24. http://doi.org/10.5251/ajfn.2013.3.2.73.82
- Bechtel, P. J., Morey, A., Oliveira, A. C. M., Wu, T. H., Plante, S. and Bower, C. K. 2010. Chemical and nutritional properties of Pacific Ocean perch (Sebastes alutus) whole fish and by-products. *Journal of Food Processing and Preservation*. 34: 55–72.

Bekkevold, S. and Olafsen, T. (2007). Råvarer Med Muligheter. Trondheim: RUBIN AS

- Bligh, E. G. and Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Can J Bichem Physiol*, *37*(8), 911-917.
- Bruinsma, K. A. and Taren, D. L. (2000). Dieting, essential fattyacid intake, and depression. *Nutr. Rev.* 58 (4), 98-108
- Carr, H. Y. and Purcell, E. M. (1954). Effects of diffusion on free precession in nuclear magnetic reasonance experiments. *Physical Reviwe*, 94(3), 630-638.
- Charles, E. O. (2009). Virtual Chembook. Elmhurst College
- Chantachum, S., Benjakul S. and Sriwirat N. (2000). Separation and quality of fish oil from precooked and non-precooked tuna heads. Food Chemistry 69:289-294
- Chin, J. P., Dart, A. M. (1995). How do fish oils affect vascular function? *Clin. Exp. Pharmacol. Physiol.* 22 (2), 71-81.
- Cho, S.-Y., Miyashita, K., Miyazawa, T., Fujimoto, K. and Kaneda, T. (1987). Autoxidation of ethyl eicosapentaenoate and docosahexaenoate. J. *Am. Oil Chem. Soc.* 64 (6), 876-879.
- Connor, W. E., Neuringer, M. and Reisbick, S. (1992). Essential fatty acids: the importance of n-3 fatty acids in the retina and brain. *Nutr Rev* 50:21–29
- Connor, W.E. (2000). Importance of n3 fattyacids in health and disease. *Am. J. Clin. Nutr.* 71 (1), 1715-1755
- Covington, M.B. (2004b). Omega-3 Fatty Acids. Atlantic, 1(2.0).
- Cunnane, S. C., Hamadeh, M. J., Liede, A. C., Thompson, L. U., Wolever, T. and Jenkins, D. (1995). Nutritional attributes of traditional flaxseed in healthy young adults. *Am. J. Clin. Nutr.* 61 (1), 62-68
- Da Porto, C., Decorti, D. and Tubaro, F. (2012). Fatty acid composition and oxidation stability of hemp (Cannabis sativa L.) seed oil extracted by supercritical carbon dioxide. *Ind. Crop. Prod.* 36 (1), 401-404
- Deepika, D., Vegneshwaran V. R., Julia P., Sukhinder K. C., Sheila T., Heather M. and Wade M. (2014). Investigation on oil extraction methods and its influence on omega-3 content from cultured salmon. *Journal of Food Processing and Technology* 5(12):113

- Dunford, N. T., Temmeli, F. and LeBlanc, E. (1997). Supercritical CO2 extraction of oil and residual proteins from Atlantic mackerel (*Scomber scombrus*) as affected by moisture content. *Journal of Food Science*, 62, 289–294.
- Din, J., Newby, D. E. and Flapan, A. D. (2004). Omega 3 fatty acids and the cardiovascular disease-fishing for a natural treatment. *British Medical Journal*, 328, 30e35.
- EC Nr 1774/2002 (2002). The EC regulation on animal by-products. URL document Available at: http://ec.europa.eu/food/food/biosafety/animalbyproducts/legisl\_en.htm
- FAO. (2002). Chemical Composition. Quality quality changes in fresh fish. http://doi.org/10.1002/0471743984.vse1661
- FAO. (2006). The production of fish meal and oil. FAO Fisheries Technical Paper, No. 142, Rome, Italy, 63 pp
- FAO. (2013). Icelandic Golden Redfish Commercial Fishery.
- FAO, (2017). Fishery and Aquaculture Statistics. Global aquaculture production 1950-2015 (FishstatJ). In: FAO Fisheries and Aquaculture Department. Rome.
- FAO, (2018). Fishery and Aquaculture Statistics. The State of World fisheries and Aquaculture (FishstatJ). In: FAO Fisheries and Aquaculture Department. Rome.
- Fiori, L., Solana, M., Tosi, P., Manfrini, M., Strim, C. and Guella, G. (2012). Lipid profiles of
- oil from trout (*Oncorhynchus mykiss*) heads, spines and viscera: trout by-products as a possible source of omega-3 lipids? *Food Chem.* 134 (2), 1088-1095
- Gencbay, G. and Turhan, S. (2016). Proximate Composition and Nutritional Profile of the Black Sea Anchovy (*Engraulis encrasicholus*) Whole Fish, Fillets, and By-Products, *Journal* of Aquatic Food Product Technology, 25:6, 864-874, DOI: 10.1080/10498850.2014.945199
- Gill, I. and Valivety, R. (1997). Polyunsaturated fatty acids, part 1: occurrence, biological activities and applications. *Trends Biotechnol*. 15 (10), 401-409.
- He, S., Franco, C. and Zhang, W. (2011). Characterisation of processing wastes of Atlantic Salmon (*Salmo salar*) and Yellowtail Kingfish (*Seriola lalandi*) harvested in Australia. *International Journal of Food Science and Technology*, 46, 1898–1904

- Herrero, M., Mendiola, J. A., Cifuentes, A. and Ibanez, E. (2010). Supercritical fluid extraction: recent advances and applications. *J. Chromatogr. A* 1217 (16), 2495-2511.
- Hong, H., Zhou, Y. and Wu, H. (2013). Lipid Content and Fatty Acid Profile of Muscle, Brain and Eyes of Seven Freshwater Fish: A Comparative Study. *Journal of the American Oil Chemists' Society*, 91: 795. <u>https://doi.org/10.1007/s11746-014-2414-5</u>
- Joensen, H and Grahl-Nielsen, O. (2001). The redfish species *Sebastes viviparus, Sebastes* marinus and *Sebastes mentella* have different composition of their tissue fatty acids. *Comparative Biochemistry and Physiology*, 129, 73-85
- Karoud, W., Sila, A., Krichen, F., Martinez-Alvarez, O. and Bougatef, A. (2017). Characterization, Surface Properties and Biological Activities of Protein Hydrolysates Obtained from Hake (Merluccius merluccius) Heads. Waste Biomass Valorization, DOI 10.1007/s12649-017-0069-9
- Khoomrung, S., Chumnanpuen, P., Jansa-Ard, S., Stahlman, M., Nookaew, I., Boren, J. and Nielsen, J. (2013). Rapid quantification of yeast lipid using microwave-assisted total lipid extraction and HPLC-CAD. *Anal. Chem.* 85 (10), 4912-4919.
- Kim, S.-K. and Mendis, E. (2006). Bioactive compounds from marine processing by-products – a review. *Food Research International*, **39**, 383–393
- Larsson, S. C., Kumlin, M., Ingelman-Sundberg, M. and Wolk, A. (2004). Dietary lingchain n-3 fatty acids for the prevention of cancer: A review of potential mechanism. *American Journal of Clinical Nutrition*, 79, 935e945.
- Linder, M., Fanni, J. and Parmentier, M. (2005). Proteolytic extraction of salmon oil and PUFA concentration by lipases. *Mar. Biotechnol.* 7 (1), 70-76.
- Linder, M., Matouba, E., Fanni, J. and Parmentier, M. (2002). Enrichment of salmon oil with n-3 PUFA by lipolysis, filtration and enzymatic re-esterification. *Eur. J. Lipid Sci. Technol.* 104 (8), 455-462
- Lowry, R. R. and Tinsley, I. J. (1979). Rapid colorimetric determination of free fatty acids. *Journal of the American Oil Chemists Society* 53(7), 470-472. doi:http://doi.org/10.1007/BF02636814
- Manirakiza, P., Covaci, A. and Schepens, P. (2001). Comparative study on total lipid determination using soxhlet, roese-gottlieb, bligh & dyer, and modified bligh & dyer extraction methods. *J. Food Compos. Anal.* 14 (1), 93-100

- Magnússon, J. (2000). Lífríki sjávar Gullkarfi. Námsgagnastofnunin-Hafrannsóknarstofnunin, Reykjavík, Ísland
- Mbatia, B., Adlercreutz, D., Adlercreutz, P., Mahadhy, A., Mulaa, F. and Mattiasson, B. (2010).
   Enzymatic oil extraction and positional analysis of u-3 fatty acids in Nile perch and salmon heads. *Process Biochem.* 45 (5), 815-819
- McKeigue, P. (1994). Diets for secondary prevention of coronary heart disease: can linolenic acid substitute for oily fish? *Lancet* 343 (8911), 1445
- Mercer, P. and Armenta, R. E. (2011). Developments in oil extraction from microalgae. *Eur. J. Lipid Sci. Technol.* 113 (5), 539-547
- Ministry of Fisheries and Agriculture. (2014). Main species, Redfish, Golden Redfish. Retrieved Jan. 20<sup>th</sup>, 2014 from http://www.fisheries.is/main-species/ redfishes/ golden-redfish/
- Murray, J. and Burt, J. R. (2001). The Composition of Fish. Ministry of Technology, Torry Research Station. (NO. 38). Torry advisory note
- Musa, S. M., Aura, C. M., Ngugi, C. C. and Kundu, R. (2012). The effect of three different feed types on growth performance and survival of African catfish fry (Clarias gariepinus) reared in a hatchery. ISRN Zoology, 861364,1–6. https://doi.org/10.5402/2012/861364
- Nazemroaya, S., Sahari, M. A. and Rezaei, M. (2011). Identification of fatty acid in mackerel (*Scomberomorus commersoni*) and shark (*Carcharhinus dussumieri*) fillets and their changes during six month of frozen storage. *Journal of Agricultural Science and Technology*, 13:553–566
- Nghi, N. Q. and Sigurdsson, T. (2002). Redfish (Sebastes marinus) a marine protected area west of Iceland.
- Oomah, B. D., Busson, M., Godfrey, D. V. and Drover, J. C. G. (2002). Characteristics of hemp (Cannabis sativa L.) seed oil. *Food Chem.* 76 (1), 33-43
- Pena, T., Pensado, L., Casais, C., Mejuto, C., Phan-Tan-Luu, R. and Cela, R. (2006). Optimization of a microwave-assisted extraction method for the analysis of polycyclic aromatic hydrocarbons from fish samples. *J. Chromatogr.* A 1121 (2), 163-169.

- Qi-yuan L., Jun-qing Q. and Xiao-ge W. (2016). Optimization of enzymatic fish oil extraction from mackerel viscera by response surface methodology. *International Food Research Journal* 23(3):992-997
- Ramakrishnan V. V., Ghaly A. E., Brooks M. S. and Budge S. M. (2013). Extraction of oil from mackerel fish processing waste using alcalase enzyme. *Enzyme Engineering* 2(2): 1-10
- Rubio-Rodríguez, N., de Diego, S. M., Beltran, S., Jaime, I., Sanz, M. T. and Rovira, J. (2012). Supercritical fluid extraction of fish oil from fish by-products: a comparison with other extraction methods. *J. Food Eng.* 109 (2), 238-248.
- Rustad, T. (2003). Utilisation of marine by-products. *Electronic Journal of Environmental, Agricultural and Food Chemistry*, **2**, 458–463.
- Sa, E. and Pe, M. S. (2005). *Food Chemistry*. Vitis (Vol. 92). http://doi.org/10.1016/j.foodchem.2004.08.025
- Sahari, M., Farahani, F., Soleimanian, Y. and Mokhlesi, A. (2013)). n-3 Fatty acid distribution
- of commercial fish species components. Journal of the American Oil Chemists Society 90:1167-1178
- Sahena, F., Zaidul, I., Jinap, S., Saari, N., Jahurul, H., Abbas, K. and Norulaini, N. (2009a). PUFAs in fish: extraction, fractionation, importance in health. *Compr. Rev. Food Sci. Food Saf.* 8 (2), 59-74.
- Sahena, F., Zaidul, I. S. M., Jinap, S., Karim, A. A., Abbas, K. A., Norulaini, N. A. N. and Omar, A. K. M. (2009b). Application of supercritical CO2 in lipid extraction: a review. *J. Food Eng.* 95 (2), 240-253.
- Shahidi, F. and Wanasundara, U. N. (1998). Omega-3 fatty acid concentrates: nutritional aspects and production technologies. Trends Food Sci. Technol. 9 (6), 230e240.
- Statisitics Iceland (2011). Export of marine products. [February 2013] <]http://www.statice.is
- Statistics Iceland (2016). Fisheries. In Iceland in figures 2016. Vol. 21. Reykjavik, Iceland: Statistics Iceland
- Stewart, L. C. M. (1980). "Colorimetrc determination of phospholipids with ammonium ferrothiocyanate." *Analytical Biochemistry* 104(1): 10-14.

- Stoknes, I. S., Okland, H. M. W., Falch, E., and Synnes, M. (2004). Fatty acid and lipid class composition in eyes and brain from teleosts and elasmobranchs. *Comparative Biochemistry and Physiology*, 138, 183-191
- Stroescu, M., Stoica-Guzun, A., Ghergu, S., Chira, N. and Jipa, I. (2013). Optimization of fatty acids extraction from Portulaca oleracea seed using response surface methodology. *Ind. Crop. Prod.* 43 (0), 405-411.
- Temelli, F. (2009). Perspectives on supercritical fluid processing of fats and oils. J. Supercrit. Fluids 47 (3), 583-590
- Iceland Seafood International, Kollunarklettsvegur 2, Reykjavik, I. Golden redfish in Iceland (2014).
- Valenzuela, B. R., Tapia, O. G., Gonzalez, E. M. and Valenzuela, B. A. (2011). Acidos Grasos omega-3 (EPA y DHA) y su aplicaci on en diversas situaciones clínicas. *Revista Chilena de Nutricion*, 38, 356-367
- Weichbrodt, M., Vetter, W. and Luckas, B. (2000). Microwave-assisted extraction and accelerated solvent extraction with ethyl acetateecyclohexane before determination of organochlorines in fish tissue by gas chromatography with electron-capture detection. *J. AOAC Int.* 83 (6), 1334e1343
- Xiaobo, Z., Jiewen, Z., Povey, M. J. W., Holmes, M., and Hanpin, M. (2010). "Variables selection methods in near-infrared spectroscopy." *Analytica Chimica Acta*, 667(1–2), 14–32
- Yamaguchi, K., Murakami, M., Nakano, H., Konosu, S., Kokura, T., Yamamoto, H., Kosaka, M. and Hata, K. (1986). Supercritical carbon dioxide extraction of oils from Antarctic krill. J. Agric. Food Chem. 34 (5), 904-907.
- Yashodhara, B. M., Umakanth, S., Pappachan, J. M., Bhat, S. K., Kamath, R. and Choo, B. H. (2009). Omega-3 fatty acids: A comprehensive review of theirrolein health and disease. *Postgraduate Medical Journal*, 85, 84e90.

# Appendix A

This appendix shows the different parts of the head; eye, brain, cheek, gills, and tongue.



# Appendix B

This appendix contains table with values of the yield, water content protein and ash content from different parts from the head. (Figure 5 and 6).

	Yield (%)		Water content	
			(%)	
Raw material	Small head	Big head	Small head	Big head
Eye	6.09	4.26		80.3
Gill	8.55	9.63	70.04	67.68
Cheek	3.11	3.94	76.69	74.12
Tongue	0.70	1.14	46.57	53.24
Brain	2.05	1.91	26.98	44.65
Carcass	73.10	77.76		

Table 1: Yield and water content (%) between sizes

Table 2: Protein and ash content (%) between sizes

	Protein (%)		Ash (%)	
Raw material	SH	BH	SH	BH
Gill	13.85	16.2	6.65	5.15
Cheek	20.25	18.25	3.3	2.15
Eye	11.75	8.05	1.1	1.2
Brain	2.7	2.8	0.5	0.55
Tongue		12.25		8.2

# Appendix C

This appendix contains table with values of the lipid content, free fatty acid content (FFA), Phospholipid content (PL) from different parts from the head and mineral content from the gill.

	Lipid (%)		FFA (g FFA/100g	5	PL (g PL/100g	
			lipid)		lipid)	
Raw	Small	Big head	Small head	Big head	Small head	Big head
material	head					
Gill	9.14	10.66	1.95	1.49	1.77	5.11
Cheek	3.34	5.26	2.74	1.98	1.88	8.72
Eye	2.66	8.26	2.23	0.45	0.32	4.49
Brain	45.78	35.02	0.14	0.09	0.58	1.85
Tongue	24.42	19.07	0.70	0.25	6.58	3.13

Table 3: Lipid, free fatty acid and phospholipid content between sizes

Table 4: Mineral content of the gills

Element	Small head	Big head
Na	2.73	2.82
К	2.14	2.01
Ph	10.12	7.93
Ca	22.25	16.16
Mg	0.5	0.38

# Appendix D

This appendix contains tables with values of the fatty acid, saturated fatty acid content, monounsaturated fatty acid content and polyunsaturated fatty acid, EPA + DHA, EPA/DHA ratio and n3/n6 ratio of different parts from the heads.

Fatty acid	Brain	Tongue	Cheek	Eye	Gill
C14:0	5.34±0.06	5.02±0.04	3.88±0.02	3.99±0.24	4.56±0.04
C16:0	12.97±0.07	13.29±0.06	13.77±0.08	12.73±0.20	14.39±0.12
C16:1n7	7.09±0.03	6.92±0.03	5.91±0.03	5.89±0.15	6.45±0.05
C16:2n4	0.44±0.00	0.45±0.20	$0.25 \pm 0.01$	$0.22 \pm 0.02$	0.64±0.13
C18:0	2.34±0.05	2.44±0.01	$2.78 \pm 0.02$	$3.07 \pm 0.07$	2.55±0.04
C18:1n9	12.91±0.06	13.44±0.10	$14.18 \pm 0.08$	14.83±0.15	14.95±0.02
C18:1n7	3.94±0.03	4.17±0.01	4.05±0.03	$3.87 \pm 0.04$	4.52±0.02
C18:1n5	1.21±0.01	1.22±0.01	1.23±0.01	$1.15 \pm 0.00$	$1.17 \pm 0.01$
C18:2n6	0.64±0.01	0.62±0.01	$0.51 \pm 0.01$	$0.52 \pm 0.02$	$0.59 \pm 0.01$
C18:3n6	2.26±0.01	1.87±0.03	$1.29\pm0.00$	$1.35 \pm 0.02$	$1.69 \pm 0.01$
C20:1n11	12.67±0.06	$11.80\pm0.01$	10.18±0.03	$9.66 \pm 0.09$	9.98±0.10
C20:1n9	$0.60\pm0.00$	0.55±0.01	$0.46 \pm 0.01$	$0.42 \pm 0.01$	$0.45 \pm 0.01$
C20:2	$0.20\pm0.00$	0.21±0.00	$0.19 \pm 0.00$	$0.18 \pm 0.00$	$0.19 \pm 0.00$
C20:4n6	0.39±0.00	$0.45 \pm 0.00$	$0.73 \pm 0.01$	$0.74{\pm}0.01$	$0.60 \pm 0.01$
C20:4n3	0.47±0.00	0.49±0.01	$0.52 \pm 0.01$	0.51±0.01	0.53±0.00
C20:5n3 (EPA)	8.08±0.04	8.42±0.09	8.71±0.07	7.39±0.07	8.38±0.12
C22:1n11	13.32±0.12	12.51±0.09	$10.09 \pm 0.02$	9.93±0.12	9.97±0.13
C22:1n9	1.64±0.03	$1.48\pm0.02$	$1.24\pm0.01$	$1.29 \pm 0.03$	$1.18 \pm 0.01$
C22:5n3	0.77±0.01	$0.88 \pm 0.00$	$1.28\pm0.02$	1.31±0.01	1.31±0.02
C22:6n3 (DHA)	7.06±0.02	7.82±0.04	13.34±0.17	15.33±0.33	10.23±0.16
C24:1n9	0.62±0.01	0.66±0.03	$0.72 \pm 0.04$	0.93±0.13	0.72±0.03
SFA	20.66±0.08	20.76±0.11	$20.44 \pm 0.67$	20.70±1.30	21.50±0.71
MUFA	54.01±0.12	52.75±0.34	48.06±2.55	46.46±2.14	49.39±1.36
PUFA	20.31±0.21	21.20±0.44	26.82±1.83	28.00±0.64	24.16±1.13
EPA+DHA	15.14±0.15	16.24±0.48	22.05±1.59	22.73±0.02	18.61±0.80
EPA/DHA					
ratio	1.15±0.03	1.08±0.09	$0.66 \pm 0.05$	0.62±0.19	0.82±0.09
n3/n6	4.99	5.98	9.41	9.43	7.11

Table 5: Fatty acid composition of the small head sample parts

Fatty acid	BRAIN	TONGUE	CHEEK	EYE	GILL
C14:0	4.05±0.08	3.57±0.03	3.35±0.04	3.83±0.20	3.61±0.00
C16:0	12.01±0.09	11.44±0.05	12.26±0.07	12.66±0.13	11.76±0.08
C16:1n7	7.08±0.02	6.76±0.01	6.52±0.01	7.13±0.11	6.70±0.01
C18:0	2.57±0.03	2.71±0.02	3.06±0.02	2.89±0.08	3.00±0.04
C18:1n9	15.90±0.13	16.68±0.01	$15.88 \pm 0.00$	16.71±0.12	16.61±0.06
C18:1n7	4.36±0.03	4.27±0.01	4.21±0.02	4.42±0.01	4.44±0.04
C18:1n5	0.52±0.00	$0.52 \pm 0.00$	$0.52 \pm 0.00$	0.55±0.01	$0.50 \pm 0.00$
C18:2n6	1.23±0.00	1.24±0.01	1.23±0.00	1.30±0.01	1.25±0.00
C18:3n6	0.56±0.00	0.52±0.02	$0.49 \pm 0.00$	0.56±0.01	0.50±0.01
C18:3n4	2.11±0.00	1.52±0.02	$1.44\pm0.01$	$1.86 \pm 0.00$	$1.48\pm0.01$
C20:1n11	13.46±0.03	13.64±0.05	12.52±0.03	11.90±0.02	13.10±0.02
C20:1n9	0.69±0.02	$0.66 \pm 0.00$	$0.64 \pm 0.00$	$0.60 \pm 0.01$	0.64±0.01
C20:2	0.22±0.00	0.22±0.00	$0.22\pm0.00$	0.21±0.00	0.22±0.00
C20:3n6	0.38±0.00	$0.47 \pm 0.00$	$0.58 \pm 0.00$	$0.47 \pm 0.00$	0.56±0.01
C20:4n3	$0.46 \pm 0.00$	0.46±0.00	$0.44 \pm 0.00$	$0.44 \pm 0.01$	0.45±0.00
C20:5n3					
(EPA)	7.36±0.04	7.34±0.04	7.71±0.02	$7.59 \pm 0.04$	7.03±0.02
C22:1n11	$12.35 \pm 0.01$	12.47±0.07	$10.96 \pm 0.02$	$10.39 \pm 0.04$	11.67±0.06
C22:1n9	$2.01 \pm 0.03$	$1.96 \pm 0.02$	$1.78 \pm 0.01$	$1.68 \pm 0.01$	$1.90\pm0.01$
C22:5n3	$0.66 \pm 0.01$	$0.72 \pm 0.00$	$0.74 \pm 0.01$	$0.70 \pm 0.00$	$0.77 \pm 0.00$
C22:6n3					
(DHA)	7.70±0.02	8.38±0.02	$10.63 \pm 0.10$	9.35±0.04	9.03±0.03
C24:1n9	$0.57 \pm 0.01$	0.68±0.01	$0.64 \pm 0.00$	$0.65 \pm 0.00$	0.71±0.01
SFA	18.62±0.02	17.72±0.26	18.68±0.56	19.38±0.51	18.37±0.22
MUFA	56.95±0.32	57.66±0.22	53.67±0.92	54.01±0.45	56.28±1.80
PUFA	20.66±0.06	20.88±0.26	23.48±0.30	22.47±0.27	21.28±1.47
EPA+DHA	15.06±0.04	15.72±0.23	18.34±0.16	16.93±0.22	16.06±1.40
EPA/DHA					
ratio	0.96±0.00	0.87±0.00	0.73±0.02	0.82±0.01	0.780±0.05
n3/n6	7.48	7.58	8.47	7.77	7.50

Table 6: Fatty acid composition of the big head sample parts

# Appendix E

This appendix contains tables with values of the fatty acid, saturated fatty acid content, monounsaturated fatty acid content and polyunsaturated fatty acid, EPA + DHA, EPA/DHA ratio and n3/n6 ratio of small head and big head brains extracted oil.

Name	SHB-H	SHB-WH	BHB-H	BHB-WH
C14:0	4.79±0.17	4.57±0.05	4.16±0.20	4.20±0.15
C16:0	10.32±0.11	10.05±0.00	11.83±0.29	11.85±0.26
C16:1n7	7.65±0.08	7.47±0.07	7.48±0.13	7.47±0.11
C18:0	2.34±0.04	2.37±0.02	2.73±0.02	2.69±0.03
C18:1n9	12.55±0.02	12.45±0.10	15.38±0.09	15.37±0.02
C18:1n7	3.31±0.02	3.28±0.02	4.21±0.02	4.17±0.02
C18:1n5	0.57±0.00	0.57±0.00	0.60±0.00	$0.60 \pm 0.00$
C18:2n6	1.22±0.00	1.22±0.01	1.22±0.00	1.22±0.01
C18:3n6	0.56±0.00	0.57±0.00	0.60±0.00	$0.60 \pm 0.00$
C18:3n4	2.22±0.00	2.23±0.00	2.18±0.00	2.18±0.01
C20:1n11	15.64±0.02	15.77±0.11	13.37±0.18	13.43±0.20
C20:1n9	0.85±0.01	0.85±0.01	0.71±0.00	0.71±0.01
C20:4n3	0.45±0.00	0.46±0.00	0.47±0.01	$0.47{\pm}0.01$
C20:5n3				
(EPA)	6.42±0.02	6.54±0.05	7.27±0.06	7.25±0.06
C22:1n11	16.33±0.03	16.52±0.12	11.73±0.19	11.79±0.27
C22:1n9	2.49±0.04	2.50±0.05	$1.88 \pm 0.04$	1.89±0.05
C22:5n3	0.53±0.01	0.54±0.01	0.69±0.00	$0.68 \pm 0.00$
C22:6n3				
(DHA)	5.93±0.04	6.10±0.12	7.57±0.21	7.54±0.15
C24:1n9	0.64±0.01	0.65±0.02	0.54±0.00	0.54±0.00
SFA	17.45±0.32	16.99±0.27	18.72±0.01	18.74±0.03
MUFA	60.02±0.35	60.06±0.20	55.89±0.04	55.98±0.08
PUFA	17.34±0.35	17.66±0.16	19.98±0.09	19.94±0.16
EPA+DHA	12.35±0.31	12.64±0.34	14.84±0.09	14.79±0.16
EPA/DHA				
ratio	1.08±0.02	1.07±0.52	0.96±0.01	0.96±0.01
n3/n6	7.46	7.61	8.81	8.75

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