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QUALITY AND STABILITY OF CUBAN SHARK LIVER OIL: COMPARISON WITH ICELANDIC COD LIVER OIL

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ABSTRACT

The quality of shark liver oil obtained from a pool of livers of three shark species (*Ginglimostoma cirratum, Carcharhinus longimanus,* and *Carcharhinus falciformis)* caught in the Cuban coastal waters was studied by measuring some chemical characteristics such as squalene, vitamins (A, E, D) and free fatty acids. The quantity of some metals (copper and iron) was used as an indicator of the possible oxidation of the oil. As oxidative stability is one of the most important factors in keeping the quality of fish oils, volatile compounds, fatty acids in neutral fraction, peroxide values and induction period were evaluated as stability parameters in shark liver oil, in order to detect oxidative changes during storage. The oil was kept at two different temperatures (0°C and 30°C) for three weeks. Sensory analysis was done to evaluate the rancidity levels of the oil during the storage time. Dioxins and dioxin-like PCBs (polychlorinated biphenyls) are very persistent chemicals responsible for contamination in fish oils. Its content in shark liver oil was also evaluated in this study. Cod liver oil from *Gadus morhua* and other species of Gadus from South-West Iceland was also evaluated in order to establish a comparison between both oils in terms of quality and stability.

Key words: shark liver oil, quality, oxidative stability, cod liver oil.

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1 INTRODUCTION

Sharks are among the most important commercial fish stocks in Cuban waters nowadays and there are 23 commercial species which have been caught for the past 100 years (Garcia 2005). Levels of shark catches in Cuban waters have varied during the years in terms of fishing effort. Catch levels are already around 1500 or 2000 tonnes per year.

As demonstrated by Espinosa (1997), eight species of shark inhabit coastal areas, eight are pelagic, and three species migrate on to and from the continental shelf, and can be caught in coastal waters, depending on the type of fishing gear used. Four species are caught in deep waters. The common size of the coastal sharks landed in Cuba is between 60 and 255 cm, the pelagic shark size is between 149 and 348 cm and the deep water shark size is between 83 and 408 cm.

The Cuban continental shelf is not considered a targeted area for shark fishery. Sharks are commonly caught as by-catch in other types of fisheries.

Shark liver oil (also known as shark oil) is extracted from the livers of various species of sharks. Oil is yellow to brown, it has a strong flavour, and it is insoluble in water. It is used as a source of Vitamin A and Omega-3, and has a high level of squalene, which is used in biochemical research.

Studies have been conducted on oil extracted from shark livers from Cuban waters since the 1940s. Some exports of the product were carried out and its physical, chemical and microbiological properties were studied. It is known (Lopez 2007, personal communication reference) that comparative studies were made between shark oil and Atlantic cod liver oil, showing the similarity between them in terms of their properties. Unfortunately, there are no references published on the matter. However, after 1959 investigations concerning Cuban sharks were reopened and there are some studies on their population (Espinosa 1983, 1987, 1994, 1997), taxonomic characterisation, status of exploitation of the species, representative species and general characteristics of each one (Guitart 1975).

Although liver is a good source of oil in sharks, it is currently considered a waste in Cuba. Therefore, there is currently interest in Cuba to investigate the possibility of using shark liver oil as a health product, as well as developing new technological processes for extracting quality liver oil and to develop new food products for human consumption (Garcia 2005). Converting shark by-products into shark oil is an opportunity of adding value to by-products.

The weight of the liver of some shark species constitutes almost 20% of the shark's weight (Vannucini 1999), and it can be 5% of the body weight in other species (Navarro *et al.* 2000). Based on these figures, it would be possible to process between 75-100 and 300-400 tonnes of liver oil per year in Cuba.

Since only artisanal production of shark liver oil exists in Cuba at this moment, advanced processing is not possible. For this reason, to evaluate the quality and stability of the oil is a very important aspect to guarantee its safety as a nutritional

supplement for human consumption. Only one plant (supported by WWF) is actually working on developing methods to process shark oil as a by-product. Once it begins shark liver oil production, it will be possible to refine it with the aim of using it as a nutritional supplement for the Cuban population (Garcia 2007, personal communication).

The focus of this research is to study the quality and the stability of Cuban shark liver oil obtained from a pool of livers from sharks captured in Cuban waters, as well as to provide a comparison between Cuban shark liver oil and Icelandic cod liver oil.

Three species of sharks (caught off the Cuban coast) were used to extract the oil: *Ginglimostoma cirratum, Carcharhinus longimanus*, and *Carcharhinus falciformis.*

2 LITERATURE REVIEW

2.1 Fish oils and health effects

The good health effect of fish oils has been known now for many years and the American Heart Association recommend to patients with coronary heart disease, the consumption of 1 g of fish oil per day, preferably by eating fish (American Heart Association 2007).

Investigations of fish oils have not only shown their importance as a dietary source of vitamins A and D, but also that they are very rich in fatty acids of long chained omega-3 (Bragadóttir *et al.* 2005). Vitamin A accumulates in body fat, and can reach harmful levels sufficient to cause hypervitaminosis A. Overdose of vitamin A and D is not desirable.

As fish oil has high levels of omega-3 fatty acids, it has been demonstrated that it can help to regulate cholesterol in the body through the effects of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Fish oil also has anti-inflammatory properties and positive effects on body composition.

Hypertriglyceridemia, secondary cardiovascular disease prevention and high blood pressure are the main conditions for which fish oil and other omega-3 sources are most highly recommended (NIH Medline Plus 2006).

Fish oils may also help protect the brain from cognitive problems associated with Alzheimer's disease, as demonstrated in a study from Louisiana State University in September 2005 (Lukiw 2005).

Incorporating fish oil in foods, is not only a very effective way to increase the value of fish oils, but is also an important way to increase the consumption of fatty acids contained in these oils (Medina *et al.* 2003).

2.2 Polyunsaturated fatty acids

Fatty acids are an important source of energy in mammals and they are the major energy source for the heart. They can be saturated, monounsaturated or polyunsaturated. Saturated fatty acids contain no double bond, monounsaturated one double bond and polyunsaturated contains two to six double bonds. The polyunsaturated fatty acids of four to six double bonds are a characteristic for fish oil, resulting in the unique health property often referred as the Omega-3 fatty acids. They can also be classified by the length of the carbon chain (long chain, n=20 to 22; intermediate chain, n=18). Long chain and intermediate chain fatty acids must be consumed as part of the diet because they cannot be synthesised by humans (DeFilippis and Sperling 2006, Lands 1992).

Long chain polyunsaturated fatty acids (Nettleton 1995, Stone 1996, De Caterina 2003, Calder 2003) are an important source for health and human nutrition, as part of the basic and cellular structures involved in the metabolic processes in mammals (Pawlosky *et al.* 2003).

Omega-3 fatty acids, which are mainly present in marine products, are a family of polyunsaturated fatty acids which have in common a carbon-carbon double bond in the ω−3 position. Eicosapentaenoic acid (EPA) (20:5), docosahexaenoic acid (DHA) (22:6) and α-linolenic acid (ALA), are nutritionally essential omega−3 fatty acids. EPA and DHA, which are most often found in fish oils, are especially beneficial to human health (Aidos 2002, Market Biosciences Corporation 2007).

Numerous investigations have shown that consumption of fish or dietary supplementation of fish oils rich in long-chain omega-3 polyunsaturated fatty acids, not only lowers the risk of cardiovascular and coronary heart disease (Bigger 2001, Lee *et al.* 2003), but can also inhibit the development of cancers, stimulates immune functions (Side *et al.* 1998) and helps the development of the brain (Haag 2003).

An alternative to fresh fish could be omega-3 supplements, most of which are subject to contamination tests prior to sale. Omega-3 supplementation of products has been a major growth area in the neutraceutical market. Mintel's Global New Products Database (GNPD) showed 208 omega- 3-containing product launches across Europe in 2005 (International Fish Oil Standards 2006a).

The high level of unsaturated fatty acids in fish oil makes it highly susceptible to oxidative degradation, which leads to rancidity. That led to the limitation of incorporating omega-3 fatty acids and fish oils into functional food. The deterioration of fish oils is due to the oxidation of its lipids and it produces a rancid smell and flavour in the oil, by reducing its nutritional quality, causing the formation of other undesirable substances (Frankel 1996).

2.3 Sharks as a valuable resource

Sharks appeared around 450 million years ago during the Devonian era. Shark meat has been consumed since the fourth century. Persians and Cretans caught and sold sharks in the Persian Gulf and the Mediterranean some 5000 years ago.

Vannucini (1999) reported that sharks are a very valuable resource in many countries. Sharks also have been a cheap source of protein for coastal communities dependent on subsistence fisheries for many years. For centuries humans have used sharks for many purposes including its meat, fins, liver-oil, skin, teeth, cartilage and other internal organs.

Sharks accumulate a considerable amount of oil in the liver and because of that they are a very interesting resource at the international level (Kjerstad *et al.* 2003). It was mentioned by Kreuzer and Ahmed (1999) and Vannucini (1999) that the height and weight of shark livers vary depending on the species and time of year. In some sharks, the liver can be up to 20% of the total animal weight.

Shark livers were in demand for their rich stores of Vitamin A before and during the Second World War. Some species of sharks contain higher yields of oil in their livers than others. This is the case of deep sea sharks such as gulper shark, small fin gulper shark, basking shark and tope shark.

In some countries such as the Solomon Islands and China, shark livers have been traditionally used as food. In the Solomon Islands, for instance, the liver is sliced, salted and eaten, and it can also be eaten fresh after harvesting and cooking or preserved by salting and, much later, cooked before eating. Numerous investigations show that shark liver oil has been extensively used for many years and for different purposes. In fact, the traditional uses of this important resource not only cover health, but also the food industries, cosmetics, textiles, pharmaceutical products, lubricants and fuels (Vannucini 1999).

Recent research has focused on certain components of shark liver oil (alkylglycerols, squalamine, and squalene). Alkylglycerols are thought to be helpful in several ways. It has been suggested that they fight cancer by killing tumour cells indirectly and reduce the side effects of chemotherapy and radiation treatment. Early lab studies suggest that they may have anti-tumour effects in animal models and this activity is said to be due to the ability of alkylglycerols to protect cell membranes. However, their effects in humans are not yet known. Clinical trials are currently under way (Akutsu *et al.* 2006). Alkylglycerols are also claimed to help against cold, flu, chronic infections, asthma, psoriasis, arthritis, and AIDS.

Shark liver oil is believed to strengthen the immune system in humans by stimulating macrophages (immune system cells that consume invading germs and damaged cells) and inhibiting protein kinase C (a key regulator of cell growth). It is well known that it can help fight the common cold, infections and heal wounds, ease symptoms of allergies, sinusitis, and asthma, raise white blood and T cell count, lower blood pressure, reduce blood sugar levels, decrease pain, boost energy, help people to sleep better and more (Solomon *et al.* 1997). It is one of the ingredients used in haemorrhoid creams such as Preparation H.

Other compounds in shark liver oil, such as squalamine and squalene, have also been promoted as having anti-cancer effects. Because some early studies have shown that squalamine can slow the growth of tumour blood vessels, proponents claim it may help to treat cancer, either alone or when combined with chemotherapy. It is also being studied for use against macular degeneration, an eye condition that results in loss of vision. Squalene has been promoted as having cell protective effects, which may reduce the side effects of chemotherapy. These claims are currently being studied (Akutsu *et al.* 2006).

Shark liver oil has been used as a folk remedy by people on the coasts of Norway and Sweden for hundreds of years. It was mainly used to promote wound healing and as a general remedy for conditions of the respiratory tract and the digestive system.

In the 1950s, a young Swedish doctor suggested that extracts of bone marrow helped boost the recovery of white blood cells in children getting radiation and chemotherapy for leukaemia. The active ingredient in the bone marrow extract was identified as alkylglycerols. Shark liver oil was found to be one of the richest sources of alkylglycerols. The first commercially purified shark liver oil with a "standard dose" of alkylglycerols was marketed around 1986. It is still widely used in many northern European countries (Akutsu *et al.* 2006).

2.4 Quality and stability in fish oils

Fish oil has important industrial applications in food and as a nutritional supplement. The unsaturation of the fatty acids makes the fish oil more vulnerable for spoilage than other oils. Fish oil spoils in two major ways, like oils from animal and vegetable sources: oxidative spoilage and hydrolytic spoilage (Cmolik *et al.* 2000). The positive nutritional value of omega-3 fatty acids in lipids of fish oils can become a negative factor if adequate care is not taken in the storage of the oil.

Oxidation is the most important cause for fish oil quality deterioration because of its high concentration of triglycerides (Pak 2005). Fish oil quality control is a complex concept involving a whole range of factors.

The simplest quality test is to determine flavour and odour of the oil. Sensory evaluation methods are very useful to determine quality of fish and fish oils (Ramos 2004) and in combination with chemical and microbiological methods to establish levels of tolerance through chemical indicators of deterioration. Sensory methods are an important tool for measuring freshness and quality in the fish sector and in fish inspection services (Luten and Martinsdóttir 1997).

The free fatty acid (FFA) concentration is usually measured because it is considered to be an important quality parameter for oils and give an idea of overall quality. It reflects adequate deodourisation. Indeed, high levels of FFA can be a presage for lipid oxidation development (Aidos 2002).

Quality specifications for crude fish oils state that the FFA content should vary usually between 2-5% but 4.0% has also been suggested as a maximum acceptance value (Bimbo 1998).

Oxidative stability is one of the most important quality indicators in fish oils. Peroxide value (PV) is one of the commonly used methods for evaluation of fish oil stability and monitoring of deterioration during storage. Regulatory agencies have established the limits for quality and acceptability of oils for human consumption, and 8 meq O_2/kg of oil is the limit of acceptability of PV (Huss 1988).

Aidos (2002) has pointed out that the products of lipid oxidation may be toxic. They may react with other nutrients such as proteins, vitamins, etc., reducing their available dietary levels.

In fish oils, the induction period (IP) is the time before the highest increase of lipids oxidation. It is widely useful to study the oxidative stability of edible oils (Coppin and Pike 2001).

The induction period can be determined by using the OXIPRES apparatus and it is expressed in hours. By showing the oxygen pressure changes, it can determine the induction period as the time after which the pressure begins to decrease abruptly. This is measured from the cross-section point of tangents of the first part and the subsequent part of the curve recording the pressure changes. For edible oils analysis, the optimum conditions are: 5.0 g samples; initial pressure 0.5 MPa; temperature 100°C (Trojáková 2001).

Fish oil is highly susceptible to oxidative spoilage (Huss 1988) because of its high content of polyunsaturated fatty acids. The rate of fish oil oxidation is significantly different from that of other oils. The break in the induction curve is not as strong for fish oils as for other oils and undesirable flavours and odours develop at very low values of peroxide at an early stage of oxidation, even during the induction period (Liston *et al.* 1963, Stansby 1967, Aidos 2002).

For fatty acids, the Food and Drug Administration (FDA) recommends that total dietary intake from fish should not exceed 3 g per day, of which no more than 2 g per day are from nutritional supplements (Food and Drug Administration 2004). Fatty acids profile is useful to follow the stability of fish oil by knowing its components.

Ólafsdottir (2005) suggests that the composition of volatile compounds is a very important quality and stability indicator in fish oils, since they contribute to odour changes in fish and fish oils. It was stated before that by measuring these components, it is possible to evaluate fish oils' freshness and spoilage (Ólafsdóttir and Fleurence 1997a).

However, it is not easy to identify the volatile components responsible for off-flavours in fish oil, because of their low levels. Therefore, several methods have been used in this sense. By using a gas chromatography-olfactometry (GC-O) technique, it is possible to detect those compounds that have very low odour thresholds (Jónsdóttir 2005).

In terms of volatile components, 2,4,7-decatrienals, 1-penten-3-one, 4-*cis*-heptenal, 2,4-(*trans, trans*)- heptadienal, and 2,6-(*trans, cis*)-nonadienal are the most common found in fish oils as a consequence of its oxidation. They contribute to the rancidity and undesirable odours and flavours in fish oils.

Several organic and inorganic substances, such as metals, can be also responsible by initiating and promoting oxidation in fish oils. Iron (Fe) and copper (Cu) may play an important role in metal peroxidation. The Fe^{2+} ion used to be responsible by producing the highly reactive hydroxyl radical (OH) which is the most important reactive oxygen species (ROS) to abstract hydrogen and to initiate the chain reaction of lipid peroxidation (Benedet and Shibamoto 2007). Therefore, high content of iron can result in a higher oxidation rate in fish oils.

2.5 Chemical properties of shark liver oil

Shark liver oil is offered only as a dietary supplement and its main ingredients are: squalamine, alkylglycerols (AKGs)- 20%, squalene, omega-3, free fatty acid, vitamin E (Natural), iron (Fe), zinc (Zn) and copper (Cu). It is sold, for instance, as "Ultramarine Shark Liver Oil" (570 mg/120 gel capsules), "Squalamax - Natural Squalamine Extract" (650 mg/100 capsules), and "Shark Liver Oil" (400 mg/120 Softgels) (Solomon *et al.* 1997).

Squalene is a natural organic compound (a poly-unsaturated hydrocarbon) present in some shark liver oils, mainly of the family Squalidae, and in cod liver oil, olive oil, wheat germ oil, rice bran oil and other vegetable oils. It is a highly unsaturated aliphatic hydrocarbon. Its occurrence was first reported by Tsujimoto (Tsujimoto 1906) who first assigned the correct empirical formula C30H50 to squalene, an unsaturated hydrocarbon he discovered in 1906, and it was isolated in 1926 by Heilborn *et al*. (Vannucini 1999). Although shark liver oil is a natural source for this hydrocarbon, all higher organisms produce squalene, including humans (Vannucini 1999).

Some sharks have as much as 90% squalene in the liver and, because of its low specific gravity; they can maintain their buoyancy in water. Squalene is extensively used for many purposes nowadays such as in cosmetics, health food, and as high-grade machine oil (Vannucini 1999).

The most abundant source of squalene is the livers of deep sea sharks to be found at depths of as much as 1,500 m. It occurs in shark liver oils as the major component, comprising up to 85% of the oil. Squalene is not found in sharks living in shallow areas.

Vitamin A is an essential human nutrient. It does not exist as a single compound, but in several forms. In foods of animal origin, the major form of vitamin A is an alcohol (retinol), but it can also exist as an aldehyde (retinal), or as an acid (retinoic acid). Precursors to the vitamin (a provitamin) are present in foods of plant origin as some of the members of the carotenoid family of compounds (Berdanier 1997)

Vitamin A is absorbed from dietary animal fats (especially liver, fish, egg yolks, and milk fat) and from dietary supplements. It is stored in the liver until needed by the body, so it does not need to be consumed every day.

The recommended daily allowance (RDA) of vitamin A is 2,310 International Units (0.7 mg) per day for women (slightly more for women who are pregnant or

breastfeeding) and 3,000 IU (0.9 mg) per day for men (Office of Dietary Supplements 2007).
In foods, there are many sources of vitamin E. Fish and fish oils are one of the best known sources of vitamin E (Bauernfeind 1980).

Vitamin E (fat-soluble vitamin) is actually a family of eight antioxidant compounds. These consist of four tocopherols (alpha, beta, gamma and delta) and four tocotrienols (also, alpha, through, delta). Each form has its own biological activity, which is the measure of potency or functional use in the body (Traber and Packer 1995). Alpha-tocopherol (α-tocopherol) is the most active form of vitamin E in humans. It is also a powerful biological antioxidant (Traber 1999, Farrell and Roberts 1994).

Antioxidants such as vitamin E act to protect the human cells against the effects of free radicals, which are potentially damaging by-products of energy metabolism. Free radicals can damage cells and may contribute to the development of cardiovascular disease and cancer. Studies are underway to determine whether vitamin E, through its ability to limit production of free radicals, might help prevent or delay the development of those chronic diseases. Vitamin E has also been shown to play a role in immune function, in DNA repair, and other metabolic processes (Traber 1999, Farrell and Roberts 1994).

Vitamin D plays an important role in the maintenance of organ systems. Five micrograms/day (200 IU/day) of vitamin D for infants, children and men and women aged 19–50 is recommended by the U.S. Dietary Reference Intake.

Since vitamin D is fat soluble and accumulates in lipid stores, fish liver oil is a rich source of it. The content varies a lot in liver oil depending on the species and season.

As very few foods naturally contain significant amounts of vitamin D, fortified foods represent the major dietary sources of this vitamin.

Fish liver oils are natural sources of vitamin D. Cod liver oil, for instance, provides 1,360 IU f this vitamin in 1 Tbs. (15 mL) (Dietary Supplement Fact Sheet 2007).

2.6 Pollutants of shark liver oil

The issue of potential toxicity in shark liver oil may be due to its content of polychlorinated biphenyls (PCBs) and dioxins.

Dioxins and dioxin-like PCBs (polychlorinated biphenyls) are very persistent chemicals that are widely present in the environment and in foods at very low levels. They are found in all foods, but the highest levels are found in edible fish and other products with a high animal fat content.

Because of the high levels of toxic contaminants such as mercury, dioxin, PCBs and chlordane in fatty fish, the FDA recommends limiting its consumption. Due to this limitation, many people have turned to fish oil supplements to get adequate omega-3 fatty acids (EPA 2007).

Shark liver oil supplements can be contaminated with polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs). This can have harmful effects in humans, and may increase the risk of various types of cancer.
Dioxins can build up in the human body if too high levels are consumed. High levels have been linked to a number of health problems, such as cancer, immune and nervous system disorders, liver damage and sterility. The tolerable daily intake of dioxins (that is the amount that may be consumed every day over a lifetime without causing harm) is 2 pg (picograms) per kg of bodyweight. The EU limits for dioxins in fish oil intended for human consumption were set in July 2002 at 2 pg/g. In fact, the permitted levels for fish oils are much lower than for other foodstuffs.

It is clear that fish oils, especially from fish livers, can be good for human health. At the same time, if used improperly, they can cause problems. Shark liver oil has many good substances. According to current advice, in terms of contaminants, fish oils pose no risk if consumed only at small levels. Therefore, it is a global trend to reduce dioxin levels in fish oils, in order to ensure the reliability of suppliers to consumers (International Fish Oil Standards 2006b).

3 MATERIALS AND METHODS

The quality of Cuban shark liver oil was studied and it was compared with Icelandic cod liver oil in terms of its main properties and stability.

3.1 Raw material

Cuban shark liver oil and Icelandic cod liver oil were the raw materials used in this report.

The shark liver oil was obtained from a pool of livers from sharks *(Ginglimostoma cirratum, Carcharhinus longimanus,* and *Carcharhinus falciformis)* captured in Cuban waters under artisanal conditions of production. The oil was sent from Cuba (through Spain) to Iceland and the shipping took nine days.

The cod liver oil was obtained by rendering fresh liver of cod (*Gadus morhua*) and other species of Gadus from South-West Iceland. No refining of the oil from shark and cod had taken place.

By keeping both oils (poured into brown bottles) at two different temperatures (0°C and 30°C), during three weeks, the storage test was done. The reference sample was kept at -80°C. Kept samples were used to measure volatiles, peroxides and for sensory analysis.

3.2 Chemical properties of shark liver oil

Squalene content in Cuban shark liver oil was determined by Gas Chromatography (GC) in-house method developed at Lysi Ltd.

Vitamins A, E, D were measured by HPLC in-house method developed at Lysi Ltd.

Free fatty acid (FFA) measurements were done with titration of sodium hydroxide (0.1M) calculated as oleic acid (AOAC 1990).
Fatty acids were analysed in the lipid. Saponification, methylation and gas chromatography were performed according to standard methods (AOAC 1990). The fatty acid methyl esters were separated and quantified by gas chromatography (Varian 3900) with an FID detector and Varian Factor Four Capillary Column (VF-WAXms 30mx0.32mm, inner dia 0.25µm).

Iron and copper concentrations in shark liver oil and cod liver oil were determined by an inductively coupled plasma mass spectrometry (ICP-MS) (Agilent 7500ce, Waldbronn, Germany) in the full quantitative mode after mineralisation, and complete destruction of the organic matrix of samples with closed vessel acid digestion. Dogfish Muscle Certified Reference Material for Trace Metals (DORM-2) measurement control was used.

Prior to analysis, portions (up to 200 mg) of the samples together with 3 mL HNO_3 were transferred to 50 mL Par digestion bombs. Samples were digested for ~ 18 hours at 180^{o}C. The digested sample solutions were quantitatively transferred to 50 mL polypropylene tubes (Sarstedt, Germany) and diluted appropriately with ultra-pure laboratory Milli-Q water (18.2 MΩ.cm quality; Millipore SAS, France). The digests were analysed for total iron and copper.

The ICP-MS instrument was tuned daily by 1 µg L^{-1} tuning solution. Operation conditions of ICP-MS were as follows: RF power 1500 W; argon plasma gas 15 $L.min^{-1}$; nebulizer gas 0.9 $L.min^{-1}$; make up gas 0.16 $L.min^{-1}$; hydrogen gas 3.6 $mL.min^{-1}$; helium gas 3.9 $mL.min^{-1}$; skimmer cone Ni; spray chamber temperature 2^{o}C; integration time 0.1 sec; replicates 4; nebulizer type concentric; sample delivery ~ 0.5 $mL.min^{-1}$.

Fish oil samples were collected in previously acid (nitric acid 65% suprapur; Merck, Germany) washed 50 mL sample tubes (Sarstedt, Germany).

3.3 Stability indicators in shark liver oil and cod liver oil

3.3.1 Volatile compounds

Volatile compounds were detected by Gas Chromatography analysis (GC-O and GC-FID) and Gas Chromatography- Mass-Spectrometry (GC-MS). The samples were analysed after three weeks of storage at 30°C. Samples of shark liver oil and cod liver oil were weighted (5 g) into a 25-mL sample vial and the headspace was collected.

For GC-FID and GC-O analysis the headspace was collected using Solid Phase Microextraction (HS-SPME) and the volatile compounds were separated and identified on a DB-5ms column (30mx0.25-nm inner dia x 0.25µm, J&W Scientific, Folsom, Calif., USA). Measurements were performed on a GC (HP 5890, Hewlett-Packard, Palo Alto, Calif. USA).

For GC-MS analysis the samples were prepared in the same way except that the volatile compounds were collected on 250 mg Tenax 60/80 (Alltech, I1, USA) in

stainless steel tubes (Perkin-Elmer, Buchinghamshire, UK) for the combined ATD 400 and GC-MS measurements.

Volatile compounds were thermally desorbed (ATD 400, Perkin-Elmer, Buchinghamshire, UK) from the Tenax tubes, separated with the same type of column as for the GC-O and detected by GC-MS (HP G1800C GCD, Hewlett-Packard, Palo Alto, CA) (Jónsdóttir *et al.* 2005). The identification and quantification of the volatile compounds was done as described by Jónsdóttir *et al.* (2005).

3.3.2 Oxidative stability

The oxidative stability of shark liver oil and cod oil was measured electronically, under oxygen pressure (5bar) in an ML OXIPRES apparatus. In this equipment, consumption of oxygen results in a pressure drop which is measured by means of pressure transducers and the samples are heated to accelerate the process and shorten the analysis time.

Samples (5 g) were weighed into reaction flasks (125 mL), and the pressure signal was recorded at 60°C. Two samples were running at the same time in each unit and the pressure change signals were sent to a PC in order to be processed by using the PARALOG software. The induction period was determined graphically as a cross-section of the line during the induction period and the second line along the pressure decline. Each sample was measured in triplicate.

3.3.3 Peroxide value

The peroxide value in shark and cod liver oil was measured in terms of milliequivalents of peroxide per 1000 g of sample; which oxidise potassium iodine (KI) (AOAC 1990).

3.3.4 Sensory analysis

Cuban shark liver oil and Icelandic cod liver oil were subjected to sensory analysis by trained panellists once a week during three weeks in order to evaluate its rancidity levels.

Samples were evaluated by using a 6-point category scale with a description of intensity of rancid odour at each score (ISO 1985, 1987): 0 = no rancidity, 0.5 = thresholds or just detectable, 1 = very slight, 2 = slight, 3 = moderate, 4 = very rancid.

The sensory panellists were trained in odour analysis to assess shark and cod liver oils in three sessions. Some of the panellists have several years of experience in evaluating rancidity of fish, fish oils, and vegetable oils and have been trained according to international standards including detection and recognition of odours.

The order of samples presentation to the panellists was balanced to minimise possible carryover effects between samples. All observations were conducted under standardised conditions, with as little interruption as possible, at room temperature, and under white fluorescent light.

3.4 Pollutants of shark liver oil

In order to determine its content of dioxins, Cuban shark liver oil was analysed by partner laboratory Eurofins GfA, Hamburg in Germany, by using Method: EN 1948 modified HRMS for Polychlorinated dibenzodioxins and -furans (PCDDs/PCDFs) and HRMS for Dioxin-like PCBs (DL-PCBs).

4 RESULTS

4.1 Chemical properties of shark liver oil

The quality analysis of shark liver oil was based on squalene, vitamins A, E and D, free fatty acids (FFA), fatty acids composition in neutral fraction and content of metals (iron and copper).

4.1.1 Content of squalene, vitamins and FFA in shark liver oil

The results for squalene, vitamins (A, E, D) and FFA are shown in Table 1.

Table 1: Quality properties (squalene, vitamins A, E, D and free fatty acids) of shark liver oil.

Property	**Value**
Squalene (%)	0.03
Vitamin A (mgg^{-1})	439.5
Vitamin E (mgg^{-1})	0.76
Vitamin D (mgg^{-1})	Not detected
FFA (%)	0.428

The FFA concentration was also measured in cod liver oil in order to compare it with shark liver oil content. The result, in this case, was 3.297%.

4.1.2 Fatty acids profile in neutral fraction

The fatty acid composition of shark liver oil and cod liver oil is reported in Table 2. In cod liver oil 94.9% of the total fatty acids were identified and 5.1% are unknown fatty acids and others. For shark liver oil 91.5% was identified leaving 8.5% unknown.

Table 2: Fatty acid composition (%) of cod liver oil and shark liver oil in neutral fraction.

Sample name	Cod liver oil (%)	Shark liver oil (%)
C14:0	4.1	4.0
C16:0	12.9	32.3
C16:1n-7	6.3	6.6
C18:0	2.8	8.5
C18:1n-9	16.7	16.4
C18:1n-7	4.4	5.3
C18:1n-5	0.4	0.0
C18:2n-6	1.2	1.8
C18:3n-3	0.8	0.0
C18:4n-3	2.4	0.0
C20:1n-9+7	9.3	2.0
C20:1	0.5	0.0
C20:4n-6	0.7	6.1
C20:5n-3	10.1	1.0
C22:1n-11+9	7.5	0.0
C21:5n-3	0.6	0.0
C22:5n-3	1.2	2.4
C22:6n-3	12.5	5.1
C24:1	0.4	0.0
total identified	94.9	91.5
others + unidentified	5.1	8.5
SFA	19.9	44.8
MUFA	45.5	30.3
PUFA	29.5	16.4
EPA+DPA+DHA	23.8%	8.5%

4.1.3 Iron and copper content in shark liver oil

Iron (Fe) and copper (Cu) were evaluated in shark liver oil. Content of the same metals was also measured in cod liver oil to establish comparison between both oils content (Table 2). The results are presented as mean±s.d. in mg per kg of product.

Table 3: Iron ($mgkg^{-1}$) and copper ($mgkg^{-1}$) content in shark and cod liver oil (values presented as mean±s.d).

Samples	Fe ($mgkg^{-1}$)	Cu ($mgkg^{-1}$)
Cod liver oil	0.754 ± 0.176	0.030 ± 0.005
Shark liver oil	1.02 ± 0.17	0.052 ± 0.021

4.2 Stability indicators in shark liver oil and cod liver oil

4.2.1 Volatile compounds

Same volatile components, but in different concentrations, were identified in the shark and cod liver oils (Table 4) during the storage test as can be seen in Appendix 1 and Appendix 2.

The odour description and odour intensity of the compounds are shown in Table 4, as well as the possible origin of some of them and the percentage of each one in cod and shark liver oils.

Table 4: Volatile compounds in cod and shark liver oils stored at 30°C during three weeks, odour evaluation by GC-O and quantification by GC-MS expressed as mean area (%).

	RI DB-5ms[a]	ID	Odour description	CGC-O intensity		GC-MS (%)		
Compound		means[b]	(GC-O)	Cod oil	Shark oil	Cod oil	Shark oil	Possible origin
Ethanol	<165	MS	n.d.[c]			2,5	3,3	
Acetic acid	188	MS	n.d.[c]				2,7	
2-Butanone	190	MS,1	Rancid	4		25,9	5,9	
2-Butenal	214	MS	Flower	3		1,1		
Unkown	220	2	Malty	4				
1-Penten-3-ol	271	MS,1	n.d.[c]			13,1	5,9	n-3 fatty acids
3-Methyl-butanal	282	MS, 1, 2	Rancid, sweet	5	2	26,2	8,5	
3-Methyl-1-butanol	316	MS,1	n.d.[c]			0,6	1,3	
Dimethyl disulfide	320	MS,1	n.d.[c]				1,4	
2-Pentenal	330	MS	Flower	2	3	2,1	0,8	
Hexanal	403	MS, 1, 2	Grass	5	3	2,0	28,3	n-6 fatty acids
2-Hexenal	430	MS	n.d.[c]			0,3	0,3	
cis -4-Heptenal	499	1, 2	Rancid	5	5			n-3/ n-6 fatty acids
Heptenal	505	1, 2	Boiled potato	2				
1-Octen-3-ol	579	MS,1, 2	Mushroom	5	4	0,0	0,5	n-6 fatty acids
6-Methyl-5-hepten-2-one	582	MS	n.d.[c]			0,3	0,0	
Decane	599	MS	n.d.[c]			0,4	0,5	
(E,E)-2,4-Heptadienal	624	MS,1, 2	Sweet, fatty	4	3	0,2	0,2	n-3 fatty acids
1-Nonanol	647	MS	n.d.[c]				0,2	
Nonanal	705	MS	n.d.[c]		3	0,6		
2-Nonenal	759	1, 2	Cucumber	4	3			
Decanal	800	MS	n.d.[c]	3		0,2	0,4	

[a]Calculated ethyl ester retention index on DB-5ms capillary column

[b]Identification means: MS=mass spectra; 1=authentic standards; 2=odour identification

[c]n.d.=odour not detected by GC-O

4.2.2 Oxidative stability

Figure 1 shows the behaviour of the pressure as a function of time at a predetermined temperature of 60°C for samples of shark and cod liver oil. The induction period (hours) was determined graphically by drawing tangents at every curve. For shark liver oil, the induction period had a value of 26.5 hours and for the cod liver oil, 23.8 hours.

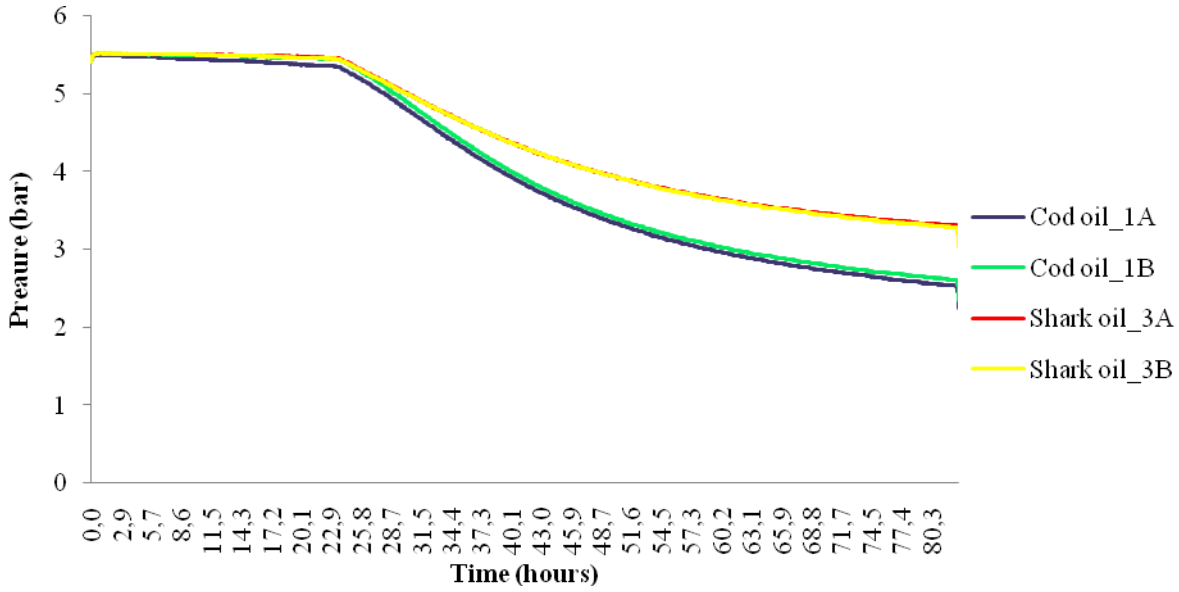

Figure 1: Induction period results from cod and shark liver oils.

Four samples of cod and shark liver oils, identified as: Cod oil_1A, Cod oil_1B, Shark oil_3A and Shark oil_3B, were kept at the same temperature (60°C) in four bombs respectively to measure their oxygen pressure during the selected time and the respective induction period.

4.2.3 Peroxide value

Peroxide values for shark and cod liver oils at two temperatures (0°C and 30°C) are shown in Table 5 and Table 6 respectively. The results are expressed in mg equivalent O_2 per kg of oil ($meqkg^{-1}$).

Table 5: Peroxide values (PV) in shark liver oil at two different temperatures (0°C and 30°C) during three weeks of storage.

Time	PV at 0°C ($meqkg^{-1}$)	PV at 30°C ($meqkg^{-1}$)
Zero point	1.8457	1.8457
One week	0.466	1.2773
Two weeks	0.4557	0.6992

Table 6: Peroxide values (PV) in cod liver oil at two different temperatures (0°C and 30°C) during three weeks of storage.

Time	PV at 0°C ($meqkg^{-1}$)	PV at 30°C ($meqkg^{-1}$)
Zero point	1.6947	1.6947
One week	0.963	1.8562
Two weeks	0.6894	2.1601

The following diagram (Figure 2) clearly shows the behaviour of peroxides measured in cod and shark liver oil at 0°C and 30°C during three weeks of storage.
Error! Objects cannot be created from editing field codes.
Figure 2: Peroxide values for shark and cod liver oils at 0°C and 30°C during three weeks of storage.

4.2.4 Sensory analysis

Intensity of rancid odour, according to the 6-point category scale (referring to shark and cod liver oils) is shown in Appendix 5. Values are presented as mean±s.d.

Figure 3 shows the results for rancidity levels of shark and cod liver oil during the storage time at two different temperatures (0°C and 30°C).

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Figure 3: Rancidity levels for shark liver oil at two different temperatures: 0°C and 30°C (standard at -80°C) during three weeks of storage.

Error! Objects cannot be created from editing field codes.
Figure 4: Rancidity levels for cod liver oil at two different temperatures: 0°C and 30°C (standard at -80°C) during three weeks of storage.

4.3 Pollutants in shark liver oil

Contaminants (dioxins) were evaluated in shark liver oil. The results are presented in nanograms per kg of product (Table 7).

Table 7: Content of dioxins ($ngkg^{-1}$) in shark liver oil.

Dioxins	value ($ngkg^{-1}$)
Dioxins PCDD/F incl. LOQ	3.69
Dioxin-like PCBs incl. LOQ	14.3

5 DISCUSSION

5.1 Chemical properties of shark liver oil

As part of the objectives of this paper, squalene, vitamins (A, D and E), free fatty acids and fatty acids composition, were measured in order to study the quality of Cuban shark liver oil. These parameters are compared with standard values, carried out in previous investigations.

5.1.1 Content of squalene, vitamins and FFA in shark liver oil

It is known that shark liver oil is recommended as a nutritional supplement because of its content of hydrocarbon squalene. Cuban shark liver oil was analysed and its squalene content is reported at 0.03% in this study (Table 1), which is considered low. The most abundant source of squalene is from the livers of deep sea sharks (found at depths of up to 1500 m). Therefore it is understandable that the shark species studied (caught off the Cuban coast) do not have a high content of squalene. Due to its low squalene content the shark liver oil must be minimally processed to ensure that the squalene content and all of its natural trace elements are maintained.

Vitamin D was not detected in the shark liver oil analysed (Table 1). However, the oil was found to have a high amount of vitamin A (439.5 mgg^{-1}). Vitamin A (as a fat soluble vitamin) is considered good for human health and provides shark liver oil with some essential properties to help human health, as it is necessary for the maintenance of healthy epithelial tissue which is found in the skin, eyes, respiratory system, GI and urinary tracts. Its detection in the shark oil is considered a good quality parameter. As the recommended daily allowance for men and women is 900-700 microgram/day, one spoon of the shark liver oil studied gives the 50% of the RDI.

Vitamin E was also found (0.76 mgg^{-1}) in the shark liver oil (Table 1) and it is known that this vitamin plays an important role as a natural antioxidant.

It has been reported by Bimbo (1998) that the free fatty acids (FFA) content for crude oils, should vary usually between 2-5%. A low FFA content was found in the shark liver oil (0.428%); it has less than the permitted limit. Cod liver oil has higher levels of FFA (3.297%) than shark liver oil at the conditions of the study, which is normal in crude oils, as long as during the refining process the excess of FFA can be removed. The results are reported in Table 1.

5.1.2 Fatty acids profile in neutral fraction

Some fatty acids (21 of them) were identified in the neutral fraction of shark and cod liver oil in this study. However, the ratios of saturated, monounsaturated, and polyunsaturated fatty acids were different among (Table 3).

Saturated fatty acids (SFA) were more abundant in shark liver oil (44.8%) than in cod liver oil (19.9%). Palmitic (C16:0) was the prevalent of the three saturated fatty acids identified in the neutral fraction of shark liver oil (32.3%) and stearic acid (C18:0) was the second most abundant (8.5%) (Table 3). This is in accordance with previous

results (Garcia *et al.* 2005), where palmitic and stearic were reported as the predominant saturated fatty acids (24.2%) and (6.1%), respectively, in the same species of shark studied in this research *(Ginglimostoma cirratun*, *Carcharhinus longimanus*, and *Carcharhinus falciformes).*

Oleic acid (C18:1n-9) was the predominant monounsaturated fatty acid (MUFA) in both shark and cod liver oil (16.4% in shark liver oil and 16.7% in cod liver oil). Garcia *et al.* (2005) reported similar amounts of oleic acid (15.6%) and a 32.6% of total MUFA for shark liver oil, corroborating the amount obtained here (30.3%). Cod liver oil presented higher content of MUFA (45.5%) than shark liver oil.

Navarro *et al.* (2000), on the other hand, reported 19.46% of MUFA for two species of shark *(Galeocerdo cuvier* and *Carcharhinus falciformis)* from the Cuban Caribbean and the Gulf of California, Mexico. The diet composition and water temperature might explain the difference in the MUFA content between the same species *(Carcharhinus falciformis)* but living in different habitat.

The present results confirm that the content of polyunsaturated fatty acids (PUFA) is higher in cod liver oil (29.5%) than in shark liver oil (16.4%) with the predominant docosahexaenoic acid (C22:6n-3) in the first one (12.5%), while the shark liver oil showed higher percentages (6.1%) of arachidonic acid (C20:4n-6). This is in accordance with other studies (Bakes and Nichols 1995, Deprez *et al.* 1990, Kayama *et al.* 1971, Malins *et al.* 1965).

Eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids content in shark liver oil were 1.0% and 5.1% respectively, and they are similar to those reported by Garcia *et al.* (2005), (EPA=1.7% and DHA=5.3% respectively). However cod liver oil showed higher percentages in those cases (EPA=10.1% and DHA=12.5%). The differences may have been influenced by many factors, such as species, location, fish age, gender, diet, water temperature, nutritional habitats and season.

Diet composition and water temperature may be two important factors responsible for the low content of EPA and DHA in liver oil of Cuban sharks, as well as the fact that planktonic crustaceans are an important source of food for them, and its EPA and DHA concentration is affected by the environmental temperature in this sense: an increased in water temperature could cause a decrease in the EPA and DHA levels in shark liver oil (Malins *et al*. 1965).

Docosapentaenoic acid (DPA), an intermediary between eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3) was in higher concentration in shark liver oil compared to cod liver oil (2.4% and 1.2% respectively). Seal oil is a rich source of DPA (Omega 3 Seal Oil Studies and Research 2006).

Two unknown compounds were detected, possibly C22:4 n-6 (4.6%) and C22:5n-6 (1.4%).

Results from the present study suggest that lipid content and composition in shark liver oil could be affected by different known factors mentioned above, such as fishing season, species, location and availability of food.

5.1.3 Iron and copper content in shark liver oil

Content of iron (Fe) and copper (Cu) in shark liver oil were 1.02 $mgkg^{-1}$ and 0.052 $mgkg^{-1}$ respectively (Table 2). Cod liver oil had, on the other hand, lower values of Fe (0.754 $mgkg^{-1}$) and Cu (0.030 $mgkg^{-1}$) in this study. The iron (as reported by Benedet and Shibamoto 2007) is an important catalyst in the formation of the OH radical, which is the most relevant initiator of the lipid peroxidation in fish oils. Iron is, however, an essential nutrient and it is not a negative property in the oil studied. If high values of iron were found, it can be removed by alkali refining and bleaching during the purification step of the oil production. Copper, in this amount, can be considered an important mineral in protecting against free radical production.

5.2 Stability indicators in shark liver oil

5.2.1 Volatile compounds

The same key volatile compounds (but in different concentrations) were detected in shark and cod liver oil, they are: 2-butanone, 3-methyl-butanal, hexanal, *cis*-4-heptenal, 1-octen-3-ol, and 2.4-heptadienal (Table 4).

Aldehydes and ketones were the most abundant group of volatiles. All of them have been associated with lipid oxidation as well as being part of the secondary lipid oxidation in fish oils. They have low flavour thresholds in oil and thus have a high impact on flavour, although they are not present in high concentrations (Karahadian and Lindsay 1990). Most of the volatiles detected in the cod liver oil and shark liver oil have previously been reported in fish oils and fish oil-enriched mayonnaise, as reviewed in Jónsdóttir *et al.* (2005).

Hexanal and 2,4-heptadienal have been shown to cause oxidised, rancid and painty flavours in fish oils and *cis*-4-heptenal has been associated with fishy off-flavours in oxidised fish oil (Karahadian and Lindsay 1989). Hexanal (which is much higher in shark than in cod liver oil) can be a quality marker for oxidation in shark liver oil.

Appendix 1 and Appendix 2 show the chromatograms for volatile compounds identified in cod and shark liver oil respectively, storage during three weeks at 30°C by using Headspace Solid Phase Microextraction (HS-SPME) / Gas Chromatography analysis (GC-O and GC-FID).

5.2.2 Oxidative stability

The oxidative stability of shark and cod liver oil, evaluated by using Oxipres test at 60°C under changes of oxygen pressure, showed that both oils behaved similarly. However, the oxygen pressure decreased a bit more slowly in the cod liver oil than in the shark liver oil. The induction period (Figure 1) was similar in both (23.8 hours in cod liver oil and 26.5 hours in shark liver oil), showing that there is no significant difference in the stability of the oils analysed.

5.2.3 Peroxide value

The peroxide values obtained in shark liver oil stored at 0°C (Table 5), are similar to those reported by Garcia (2005) for Cuban shark liver oil and Aidos (2002) for crude herring oils.

According to Rossel (1989), it can be confirmed that the Cuban shark liver oil is a good quality oil obtained from fresh raw material, because it has less than 1 meqkg^{-1} as peroxide value after one and two weeks of storage.

At zero point the peroxide value was a little high, but this may be due to the manipulation of oil during its transportation from Cuba to Iceland, which took some days and this may have affected the initial point of the experiment. However, during the storage at the temperatures defined for the study, it was found that the value of peroxide behaved as reported in the literature for this type of oil in similar conditions.

There was a slight increase in the shark liver oil stored at 30°C peroxide value (Table 5), which is normal in these cases, because the peroxide value tends to increase at the beginning until the end of the first oxidation phase and then drop. Because the period studied was very short (three weeks), it was not possible to observe the end value of this parameter, but is supposed to follow the trend known for fish oils under similar conditions.

The levels of peroxide in the cod liver oil, both 0°C and 30°C (Table 6) are higher compared with those obtained for shark liver oil. At 30° C the peroxide levels also increased much more than at 0°C confirming that the level of peroxides increases with temperature until the end of the first phase of oxidation in fish oils, when the first products of lipid oxidation are produced. It is important to maintain proper control of the product at this stage because it will define the quality of the final product.

Figure 2 shows that according to the interval of time studied and the temperatures established for the study, shark liver oil is more stable than cod liver oil. However, there was very little time to get an oxidation indicative result through the peroxide levels.

5.2.4 Sensory analysis

Rancidity levels in shark and cod liver oil kept at 0°C and 30°C during three weeks were analysed by a sensory panel. Since the time of the storage was quite short, the evaluation was very difficult to carry out. Shark and cod liver oils have a very characteristic smell and it was complicated for the panellists to recognise the rancidity level in the samples. However, the results (Figure 3 and Figure 4) show a small increase of rancidity in cod liver oil in comparison with the shark liver oil. Significant differences were not found in the oil samples analysed (P_{shark}=0.464 and P_{cod}=0,671), as can be seen in Appendix 5.

5.3 Pollutants in shark liver oil

Even though dioxins and PCBs are usually high in fish oils, especially in fish liver oils, the present study indicates that Cuban shark liver oil does not have such high content of dioxins. Its value (3.69 $ngkg^{-1}$) is lower that the limit permissible for crude oils (4.99 $ngkg^{-1}$ for pure cod liver oil) (Food Standard Agency 2002). Dioxins, like PCBs (14.3 $ngkg^{-1}$) found in the oil, can be removed during the production by purification processes.

6 CONCLUSIONS AND RECOMMENDATIONS

Even though Cuban shark liver oil had a low content of squalene (which is well documented for shallow water sharks), its amount of vitamin E and vitamin A as well as its low free fatty acids content indicate that it can potentially be used as a nutritional supplement, contributing to a complete use of the Cuban sharks' livers. Due to their low levels, iron and copper are not considerate negative quality factors in shark liver oil, but they seem to be a good mineral source in this product.

Shark liver oil contains a higher amount of saturated fatty acids (SFA) compared to cod liver oil. However, it constitutes a natural source of polyunsaturated fatty acids (PUFA), despite the higher amount found in cod liver oil (which was expected and well documented in previous research).

The same key volatile compounds (but in different concentrations) were detected in shark and cod liver oils. Most of them have previously been reported in fish oils and fish oil-enriched mayonnaise, as reviewed in Jónsdóttir *et al.* (2005). Hexanal can be a quality marker for oxidation in shark liver oil.

Shark and cod liver oils presented similar induction periods (23.8 h and 23 h respectively) showing that there are not significant differences in their stability.

Despite it being a very short storage time, the peroxide value at 0°C behaved as reported by Garcia 2005 for this shark species. Adequate care must be taken during the liver oil production from the beginning, because it will define the quality of the final product.

Sensory analysis showed a slightly high rancidity level in cod liver oil in comparison with the shark liver oil. Significant differences were not found in the oil samples analysed.

Cuban shark liver oil had a fairly low content of dioxins (3.69 $ngkg^{-1}$), lower that the limit permissible for crude oils (4.99 $ngkg^{-1}$) (Food Standard Agency 2002). Dioxins like PCBs (14.3 $ngkg^{-1}$) found in the oil, can be removed during the production by purification processes.

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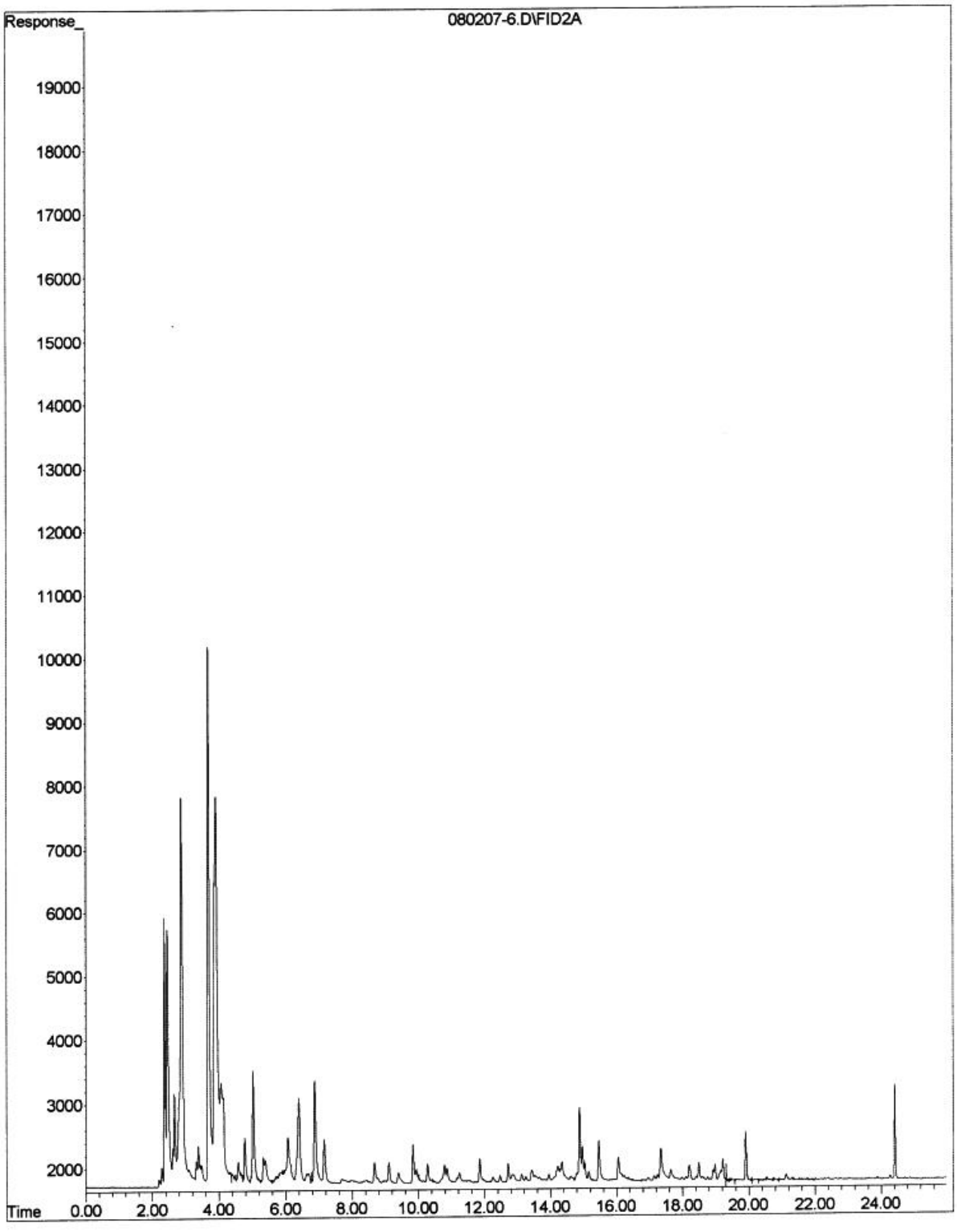
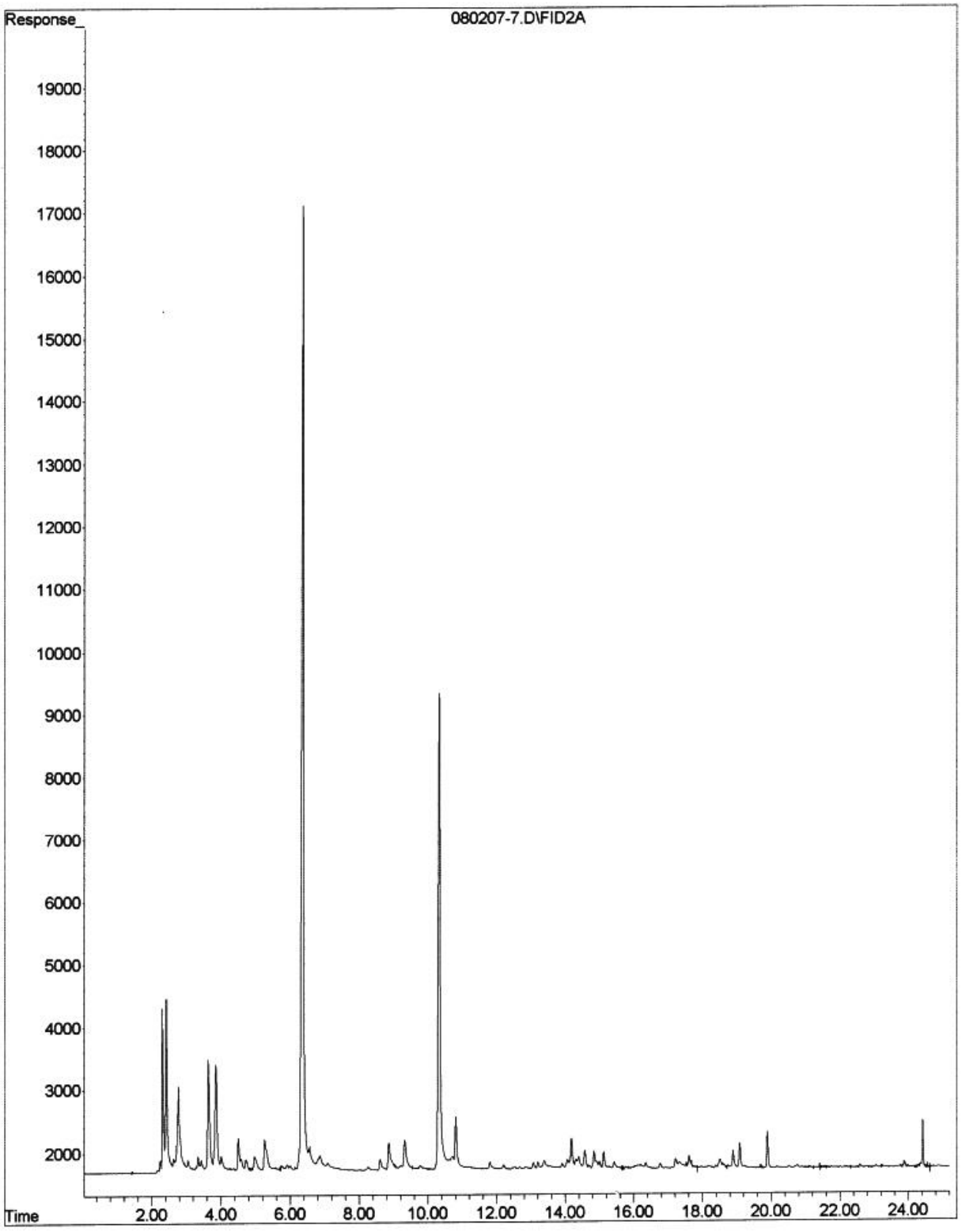
APPENDICES

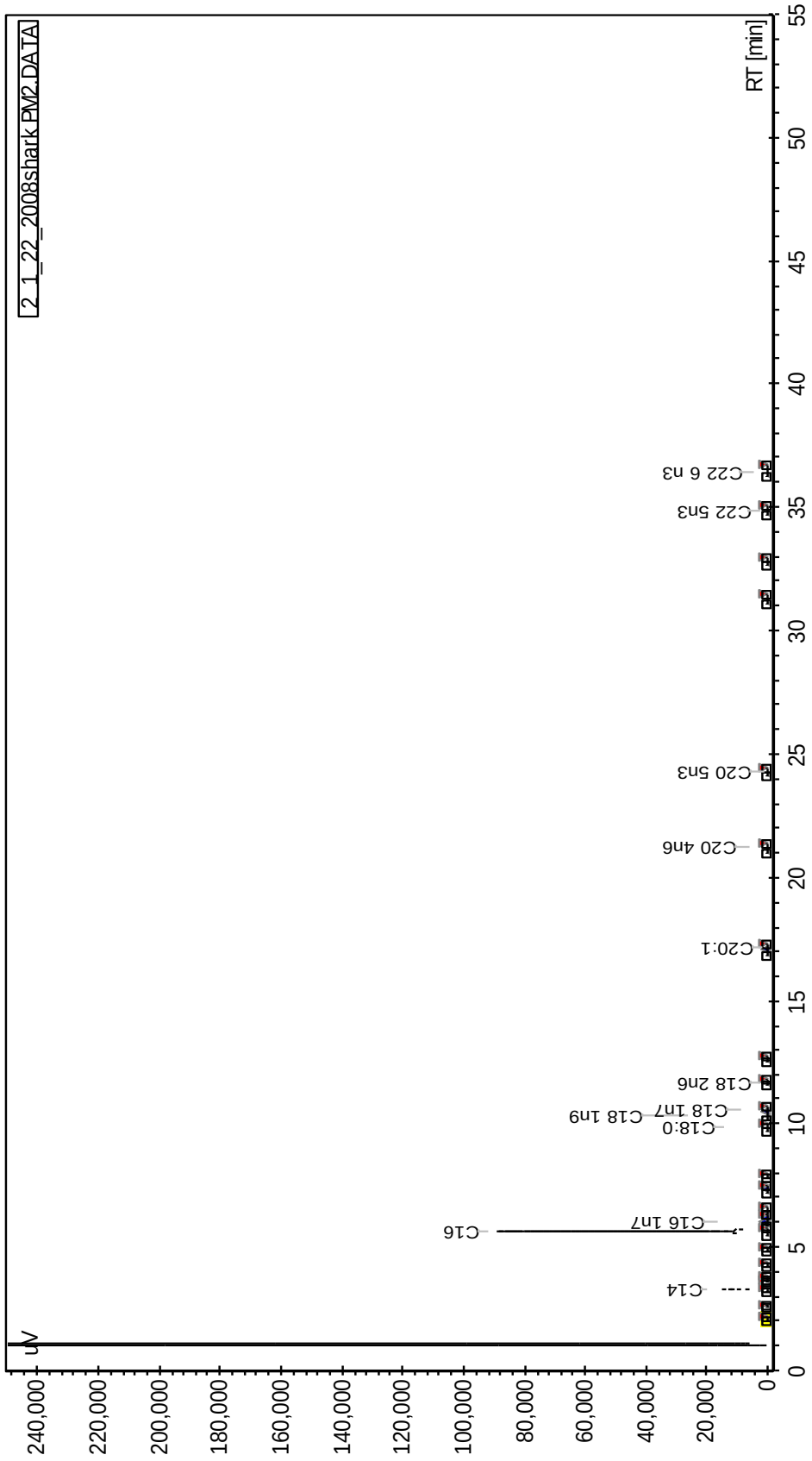
Appendix 1. Volatile compounds identified in cod liver oil storage during three weeks at 30°C of temperature by using Headspace Solid Phase Microextraction (HS-SPME) / Gas Chromatography analysis (GC-O and GC-FID).



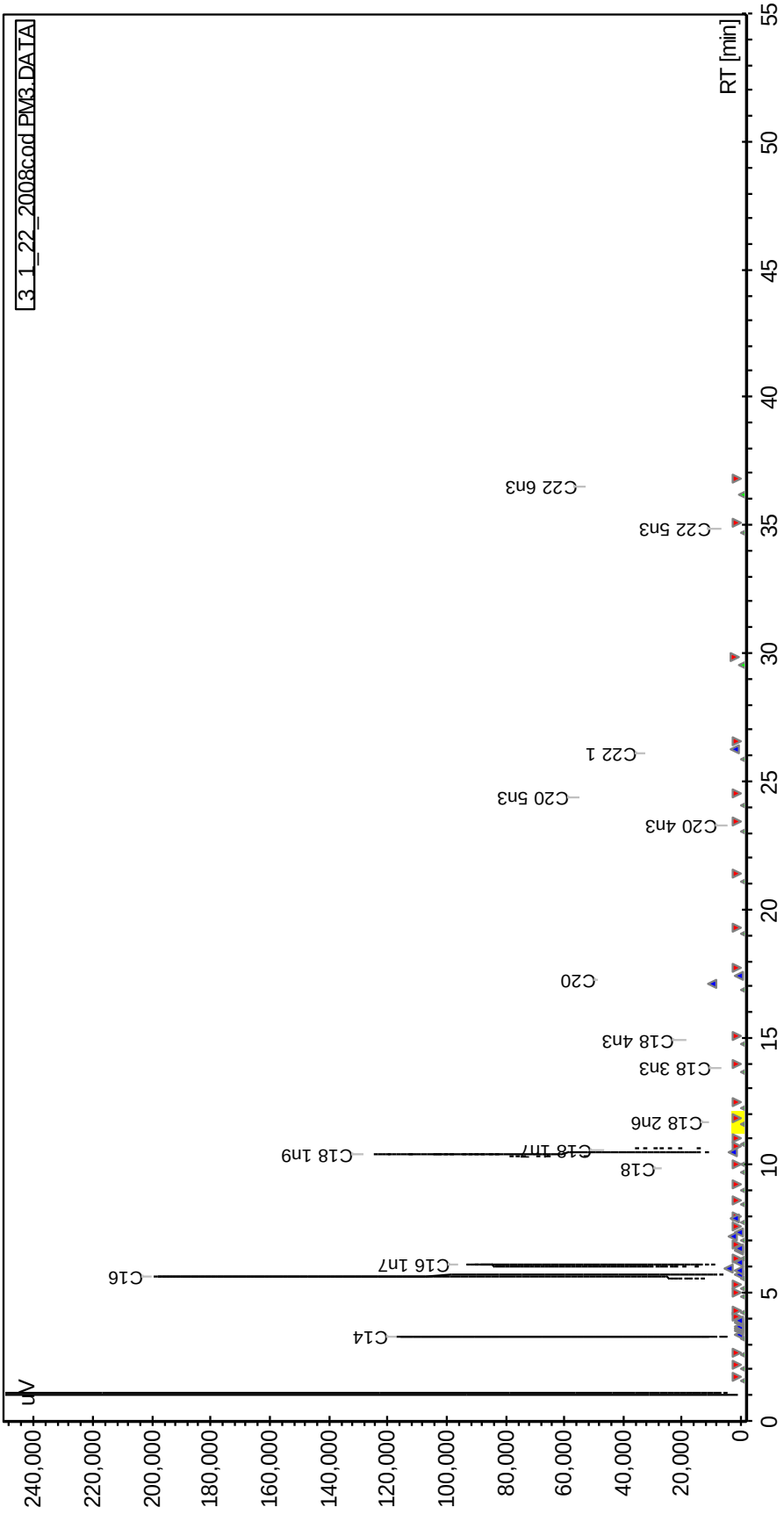
Appendix 2. Volatile compounds identified in shark liver oil storage during three weeks at 30°C of temperature by using Headspace Solid Phase Microextraction (HS-SPME) / Gas Chromatography analysis (GC-O and GC-FID).



Appendix 3. Fatty acids profile of shark liver oil carried out on GLC equipment, based on AOCS Official Method Ce 1b-89 with minor adjustments.



Appendix 4. Fatty acids profile of cod liver oil carried out on GLC equipment, based on AOCS Official Method Ce 1b-89 with minor adjustments.



Appendix 5. Rancidity levels in cod and shark liver oils at two different temperatures: 0°C and 30°C during three weeks of storage. Values expressed as mean ± s.d.

Days of storage	0°C	30°C	-80°C (standard)
Shark			
0	0.65 (0.80)	0.55 (0.72)	0.40 (0.45)
5	0.50 (0.59)	0.39 (0.63)	0.21 (0.38)
15	0.58 (0.80)	0.67 (0.82)	0.08 (0.20)
Cod			
0	0.10 (0.35)	0.20 (0.32)	0.20 (0.35)
5	0.50 (0.23)	0.64 (0.52)	0.14 (0.23)
15	0.85 (0.66)	1.10 (1.18)	0.60 (0.66)