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Final Project 2005

STABILITY AND QUALITY OF FISH OIL DURING TYPICAL DOMESTIC APPLICATION

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ABSTRACT

The objective of this project was to investigate the oxidation degree of fish oil and to improve its stability by addition of tocopherol during simulated domestic application. Fresh oil obtained from fish species containing high amounts of n-3 poly unsaturated fatty acids (EPA, 18.2%; DHA, 12.2%) was used for the study. Typical domestic application was simulated by storing the fish oil at 10°C and taking daily portions of fish oil while exposed to room temperature for 30 min. The stability and quality of fish oil were evaluated by measuring the peroxide value and the anisidine value. Minor changes were observed in the peroxide values of the fish oil during the initial 30 days, but for safe consumption of fish oil without antioxidant, a maximum of 36 days shelf life is suggested under storage conditions for domestic consumption. Addition of 0.01% tocopherol had no significance for retarding autoxidation of the fish oil and the anti-oxidative effect of tocopherol did not increase with concentrations of tocopherol higher than 0.05%. In case of fish oil with tocopherol 0.05%, the acceptable limit (PV = 8meq/kg) in peroxide value for human consumption was reached in 41 days, corresponding to a 14% improvement in stability, in comparison with fish oil without tocopherol. The longest storage time to PV = 8 meq/kg was found in the sample with Ronaxan 0.05% at 44 days, which means about a 22% improvement in oxidative stability of the fish oil. There was very little secondary oxidation of the fish oil and no significant effects of antioxidants on changes in anisidine values throughout the storage time.

Keywords: fish oil, EPA, DHA, stability, tocopherol, anti-oxidation.

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1 INTRODUCTION

Fish oils such as cod liver oil and Alaska Pollack liver oil are rich sources of DHA (docosahexaenoic acid) and EPA (eicosapentaenoic acid) called highly or poly unsaturated fatty acids (HUFA or PUFA). During the last several decades, interest in the dietary effects of n-3 PUFA has increased because of their ability to lower serum triacylglycerols and cholesterol and in their conversion to eicosanoids, which are known to reduce thrombosis. In addition, these fatty acids play an important role in the prevention and possible treatment of coronary heart disease, hypertension, arthritis, and other inflammatory and autoimmune disorders, and DHA is particularly important for brain development.

The emphasis on the importance of omega-3 long-chain PUFA has led to the commercial availability of purified fish oil supplements that are available in health food stores. Therefore, fish oil has become popular because of its useful effects on human health and nutrition, and quality and stability of it has gained more importance. However, despite their health benefits, fish oils are highly sensitive to oxidative deterioration, which entails practical problems. During the autoxidation of fish oils, undesirable flavours and odours develop at very low peroxide values, even during the induction period. Oxidation of lipids not only produces rancid odours and flavours, but can also decrease nutritional quality and safety by the formation of secondary products. In order to solve the problem, research for safer and effective natural antioxidants are underway and several natural sources are being examined.

The typical consumption of fish oil in Iceland is in the form of a daily spoon of cod liver oil or omega-3 fish oil at breakfast. A large bottle of fish oil (500 ml) may last for 1 to 2 months before it is empty. During that time the bottle may be taken daily from the refrigerator and left for standing for up to 30 minutes at the breakfast table. Similar consumption has been found for Alaska Pollack liver oil in North Korea. However, the correct method of antioxidation during consumption of fish oil has not been found and in some cases, because of oxidation of oil, undesirable flavours and odours occur, and oxidative changes of EPA and DHA are expected. This practice decreases consumers' trust in the safety and value of oil products. Therefore, it is important to improve stability of fish oil for safe consumption.

The objective of this project is to improve stability of fish oil during usual domestic application by the addition of antioxidants.

The goals of this project are:

- To investigate the oxidation degree during usual household application of fish oil without antioxidants.
- To evaluate the stability of fish oil with antioxidants.
- To decide the concentration of antioxidants for safe consumption of fish oil.

2 LITERATURE REVIEW

2.1 Importance of fish oil for human health

Marine oils are rich in polyunsaturated fatty acids (PUFA), especially those of the $\omega 3$ family such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Wanasundara and Shahidi 1998, Heinzelmann and Franke 1999). The n-3 polyunsaturated fatty (n-3 PUFA) have been recognised for their important role in health (Uauy and Valenzuela 2000, Paez *et al*. 2002, Liu *et al*. 2005).

2.1.1 What are n-3 (omega-3) PUFAs?

Originally, n-3 fatty acids were named omega-3 fatty acids, utilising the Greek word 'Omega' ('last' in English) and the first double bond being numbered from the last carbon (i.e. the methyl end of the fatty acid chain)(Curtis *et al*. 2004). Thus, for omega-3 fatty acids, the first unsaturated carbon bond occurs at the third carbon from the methyl end. Likewise, for omega – 6 and -9 fatty acids, the first unsaturated carbon bond occurs at the sixth and ninth carbon, respectively, from the methyl end. The designations omega-3, -6 and -9 were subsequently changed to n-3, n-6 and n-9, respectively.

Sources of n-3 PUFAs include some plant oils, such as linseed oil, and green leaves, which contain α-linolenic acid, which in mammals can be converted via desaturation and elongation to EPA and DHA (Curtis *et al*. 2004, Arkhipenko and Sazontova 1995). However EPA and DHA biosynthesis in animal and human organisms is rather a slow process which is further decelerated with ageing (Arkhipenko and Sazontova 1995), and because we cannot synthesise them, EPA and DHA are essential nutrients in our diet for optimal health (Leaf *et al*. 2003). EPA and DHA can be ingested directly as they are present in high concentrations in oily fish (e.g. mackerel, herring, sardines and salmon) and in fish oil extracts such as cod liver oil (Curtis *et al*. 2004).

2.1.2 The health benefits of n-3 PUFAs

2.1.2.1 Cardiovascular benefits

The benefits of high consumption of n-3 polyunsaturated fatty acids (n-3 PUFAs) on cardiovascular disease mortality was first noted over two decades ago (Abeywardena and Head 2001). The strongest evidence for an effect of n-3 fatty acids and disease is the inverse relation between the amount of n-3 fatty acids in the diet, in blood, and in tissues, and the occurrence of coronary heart disease (Uauy and Valenzuela 2000). Effects of n-3 fatty acids on coronary heart disease have been shown in hundreds of experiments in animals and in humans, tissue culture studies, and clinical trials (Leaf *et al*. 2003, Uauy and Valenzuela 2000, Arkhipenko and Sazontova 1995). Dietary n-3 fatty acids act to prevent heart disease through a variety of actions: by preventing arrhythmias, generating prostanoids and leukotrienes with anti-inflammatory actions, and by inhibiting synthesis of cytokines and mitogens that augment the inflammation and promote plaque formation (Uauy and Valenzuela 2000). In addition, these compounds stimulate endothelial-derived

nitric oxide, which relaxes vascular smooth muscle, promoting endothelial repair, and lower plasma lipids, mainly triacylglycerols and VLDL (very low density lipoprotein).

2.1.2.2 Benefits for the brain and eyes

The brain is one of the organs where the omega-3 PUFAs are essential (Garcia *et al*. 2004, Meza *et al*. 2003). The tissue of this organ is particularly rich in DHA, showing a close correlation between the consumption of this acid and its deposition in the cellular membrane. DHA is particularly abundant in the membranes of retinal photoreceptors and in neural tissue, especially in the grey matter of the brain, comprising 30-50% of the lipids in these tissues (Arterburn *et al*. 2000). DHA take part in the brain development and retina formation of a child during pregnancy (Garcia *et al*. 2004) and the most rapid rate of retinal development and brain growth occurs in third trimester of gestation, and DHA accretion rates are highest during this period (Arterburn *et al*. 2000). Therefore, it is recommended that future mothers incorporate fish into her diet as a source of omega-3 PUFAs (Garcia *et al*. 2004). Brain development and DHA accretion continue at a rapid pace throughout the first 2 years of life (Arterburn *et al*. 2000). Long-chain polyunsaturated fatty acid (LC-PUFA) composition of neural membranes is a key factor for brain development (Högyes *et al*. 2003) and a normal adult brain contains more than 20 g of DHA (Paez *et al*. 2002). DHA is required for maintenance of normal brain functions in adults (Horrocks and Yeo 1999). The inclusion of plentiful DHA in the diet improves learning ability, whereas deficiencies of DHA are associated with deficits in learning. Maintaining concentrations of PUFA is likely to favour enhanced cognitive, learning and memory functions (Youdim *et al*. 2000).

2.1.2.3 Effects on cancer

There are many reports on the effects of PUFAs on cancer development (Jiang *et al*. 1998, Moyad 2005, Horrocks and Yeo 1999, Nano *et al*. 2003, Horia *et al*. 2005, Smyth *et al*. 2005, Mahéo *et al*. 2005, Barascu *et al*. 2006). It is clear that some properties of PUFAs make them attractive options in the treatment of cancer (Jiang *et al*. 1998).

PUFAs:

- modify cell membrane phospholipids,
- modify cellular functions which may reduce tumour motile/invasive potential,
- are directly toxic to tumour cells,
- modify the sensitivity of tumour cells to chemotherapeutic agents and to radiation,
- exert a protective role towards normal tissues (in radiation) and
- are low in cytoxicity to normal cells.

Many studies have shown that fish oil has important roles in prevention of some types of cancer, including colon (Nano *et al*. 2003, Moyad 2005, Jiang *et al*. 1998), breast (Horia and Watkins 2005, Mahéo *et al*. 2005, Barascu *et al*. 2006, Jiang *et al*. 1998), renal (Smyth and McGlynn 2005, Moyad 2005), prostate, pancreatic cell and liver (Jiang *et al.* 1998, Moyad 2005).

2.1.2.4 n-3 PUFAs and human immune and inflammatory responses

During the past years, many studies have investigated the effects of n-3 PUFA on human immune and inflammatory responses (Kelley 2001, Calder 2001, Uauy and Valenzuela 2000, Horrocks and Yeo 1999, Curtis *et al*. 2004, Teitelbaum and Walker 2001). The effects of LCPUFA supplementation on different physiologic functions may be explained by production of various eicosanoids (Uauy and Valenzuela 2000). Eicosanoids are a second group of chemical messengers which act within the immune system (Calder 2001). These compounds provide a link between PUFAs, inflammation and immune functions. Eicisanoids are synthesised from PUFAs and include prostaglandins (PG), thromboxanes, leucotrienes (LT), lipoxins, hydroperoxyeicosatetraenoic acids (HPETE) and hydroxyeicosatetraenoic acids (HETE). Prostaglandins, prostacyclins, throboxanes, and leucotrienes derived from LCPUFA play a key role in modulating inflammation, cytokine release, immune response, platelet aggregation, vascular reactivity, thrombosis, and allergic phenomenon (Uauy and Valenzuela 2000). The balance between AA (arachidonic acid, n-6) and EPA (n-3) in biological membranes is regulated based on dietary supply. The n-6/n-3 ratio in phospholipids modulates the balance between prostanoids of the 2 and 3 series derived from AA and EPA, respectively. Series 3 prostanoids are weak agonists or in some cases antagonise the activity of series 2 prostanoids. Eicosanoids of the 2 series promote inflammation and platelet aggregation, and activate the immune response. On the other hand, series 3 prostanoids tend to ameliorate these effects.

2.1.2.5 Effects on plasma lipids

Marine oils have a demonstrable effect on lowering triglycerides in normal or hyperlipemic subjects (Uauy and Valenzuela 2000). This is quite different from the effect of n-6-predominant vegetable oils, which lower LDL (low density lipoprotein) cholesterol. Studies in both humans and animals have shown that fish oil concentrate inhibits hepatic triacylglycerol synthesis and the secretion of VLDL from the liver. Because n-3 fatty acid treatment has failed consistently to stimulate postheparin lipoprotein lipase or hepatic lipase activity, it has been assumed that the primary mechanism by which n-3 fatty acids lower triglycerides is by lowering production, not by enhanced clearance. Although n-3 fatty acids do not affect postheparin enzyme activities, it is possible that n-3 fatty acids increase susceptibility of VLDL to lipoprotein lipase and/or hepatic lipase-mediated lipolysis and thereby increase the production of LDL from VLDL.

2.1.3 Recommended intake of DHA and EPA

The emphasis on the importance of omega-3 long-chain PUFA has led to the commercial availability of purified fish oil supplements that are available in health food stores (Horrocks and Yeo 1999). Some consumers do not pay sufficient attention to the recommended dosage and abuse these encapsulated omega-3 supplements. An excessive intake of DHA can disturb membrane permeability and some enzymic activities and also without adequate antioxidants can cause the accumulation of lipid peroxides.

Recommendations for daily intakes of ω-3 PUFAs have been published by several international scientific authorities (Kroes *et al*. 2003). Nutrition recommendations published by Health and Welfare Canada provide a recommended daily intake of 1.0-1.8 g ω-3 PUFAs/day, although differentiation between the individual ω-3 PUFAs was not identified. The International Society for the study of Fatty Acids and Lipids (ISSFAL) recommended Adequate Intakes (AIs) of a minimum of 0.22 g/day for DHA and EPA combined, while the British Nutrition Foundation (BNF) has recommended a desirable population intake of 1.1 g (females) and 1.4 g (males) of DHA and EPA/day. In the United States, the Institute of Medicine (IOM) published a recommended AI of 0.5g ω-3 PUFAs (including DHA)/day for infants. The United States Food and Drug Administration (US FDA) concluded that consumption of up to 3 g/day of combined DHA and EPA in menhaden oil is safe.

2.2 Deterioration in quality of fish oil during storage

2.2.1 Oxidative deterioration of fish oil

As in other common oils that contain a high concentration of triglycerides, the most important cause of deterioration in the quality of fish oil, from a flavour and odour standpoint, is oxidation by atmospheric oxygen. The sites of attack by oxygen are the unsaturated portions of the fatty acid moieties of triglycerides (Stansby 1967). Due to its high content of polyunsaturated fatty acids, including EPA and DHA, fish oils are highly susceptible to oxidative spoilage and the rate of fish oil oxidation is significantly different from that of other oils (Boran *et al*. 2006). Normally with polyunsaturated oils, initiation of rancidity begins slowly, with the polyenoic ester (LH) giving the free-radical (Reaction 1) (Beddows *et al*. 2001, Frankel 1996). This reacts with oxygen (Reaction 2) to give a peroxy species immediately, which in turn, reacts with a fresh alkyl-proton to give a new free-radical (Reaction 3).

$$LH \rightarrow L\cdot \qquad (1)$$
$$L\cdot + O2 \rightarrow LOO\cdot \qquad (2)$$
$$LOO\cdot + LH \rightarrow LOOH + L\cdot \qquad (3)$$

Various factors govern the oxidative reactions that occur at centres of unsaturation (Stansby 1967). In addition to being affected by temperature and the degree of unsaturation, oxidation may be accelerated or retarded by various catalytic agents. Certain metals, visible light and light of shorter wavelengths, some oxidative enzymes, and other biological substances, such as hemoglobin, markedly accelerate this type of oxidative deterioration. During the autoxidation of fish oils, undesirable flavours and odours develop at very low peroxide values at an early stage of oxidation, even during the induction period (Boran *et al*. 2006). The hydro peroxides do not themselves contribute appreciably to the deteriorated flavours and odours of oxidised oils (Stanby 1967). In most cases, the organoleptically detectable materials appear to have low molecular weights and are formed by the decomposition of peroxides and by further oxidation of the peroxides and their breakdown products. A large number of saturated and unsaturated

aldehydes, ketones, acids, and other products have been isolated from oxidised oils, and have been shown to contribute to the undesirable flavours and odours.

2.2.2 Health hazard of oxidised lipids

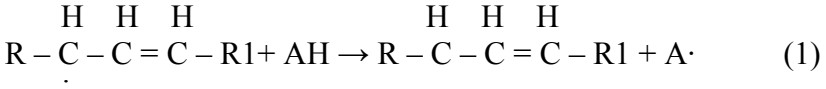
Oxidation of lipids not only produces rancid odours and flavours, but also can decrease nutritional quality and safety by the formation of secondary products (Frankel 1996). The products of lipid oxidation are known to be health hazards since they are associated with aging, membrane damage, heart disease and cancer (Suja *et al*. 2004). The consumption of such oxidised fats has been reported to cause diarrhoea, liver enlargement, growth depression and histological changes in tissues of experimental animals (Nwanguma *et al*. 1999). The production of biologically active carbonyl compounds including acrolein, malonaldehyde (MA) and 4-hydroxyl-2-nonenal (4-HN) from lipids during oxidation has been reported by many researchers (Miyake and Shibamoto 1996). These chemicals have been associated with human diseases such as atherosclerosis, cataracts and ageing. For example, acrolein reportedly caused several cytopathic effects that relate to multistage carcinogenesis in the human bronchial epithelium. MA has been implicated in ageing, mutagenesis, and carcinogenesis. The toxicity of these aldehydes is due to their ability to crosslink to proteins and bind covalently to nucleic acids. Almost all amino acids react with primary and secondary products of oxidised lipids, thereby decreasing the digestive utilisation of protein, amino acids and fats, which may affect weight gain (Varela *et al*. 1995).

As an example of acute poisoning, food poisoning caused by deteriorated fat and oil in instant noodles was reported in Japan approximately 40 years ago (Gotoh *et al*. 2005). No one died from these incidents; however, many people who ate degraded instant noodles developed acute symptoms such as diarrhoea, nausea, emesis, abdominal pain, fatigue and headache. The degree of oxidation of the lipids in the instant noodles that induced food poisoning was at least 100 meq/kg in peroxide value (PV).

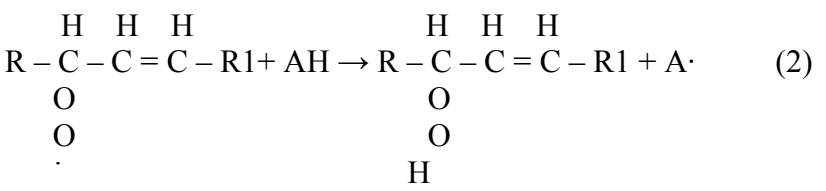
2.3 Utilisation of antioxidants

2.3.1 Mechanism of antioxidant action

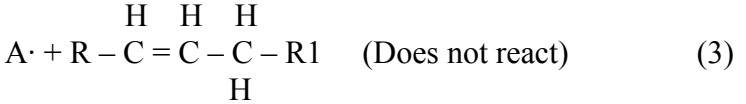
An antioxidant may be defined as a substance which, in relatively low concentration, markedly inhibits the rate of the reaction with oxygen (Stansby 1967, Markley 1961). Information about this phenomenon is particularly important with fish oil, the fatty acids of which are generally highly unsaturated and hence unusually susceptible to attack by the oxygen of air (Stansby 1967). Antioxidants are those substances that interfere either with the initiation step or with the early stages of the propagation steps. An antioxidant reacts with either the original free radical or with one formed in the early stages to give an intermediate, which is not capable of continuing the chain. The following are simplified versions of these reactions (AH represents the antioxidant):



(1)



(2)



(3)

(4)

Substances capable of acting as antioxidants have readily removable protons and the remaining free radicals are not capable of abstracting a proton from another molecule of an unsaturated substrate. Hence the reaction chain is stopped.

2.3.2 Utilisation of natural antioxidants

2.3.2.1 Restriction reasons of synthetic antioxidants

In order to overcome the stability problems of oil and fats, synthetic antioxidants, such as butylated hydroyanisole (BHA), butylated hydroxytoluene (BHT), ter-butyl hydroquinone (TBHQ) have been used as food additives (Iqbal and Bhanger 2005, Suja *et al*. 2004, Krings *et al*. 2000), because they are effective and less expensive than natural antioxidants (Suja *et al*. 2004). But recent reports reveal that these compounds may be implicated in many health risks, including cancer and carcinogenesis (Iqbal and Bhanger 2005). Therefore, the most powerful synthetic antioxidant (TBHQ) is not allowed for food application in Japan, Canada and Europe. Similarly, BHA and BHT have also been removed from the generally recognised as safe list of compounds (Iqbal and Bhanger 2005, Suja *et al*. 2004). Due to these safety concerns, there is an increasing trend among food scientists to replace these synthetic antioxidants with natural ones, which, in general, are supposed to be safer.

2.3.2.2 Major natural antioxidants

The interest in natural antioxidants continues to grow because they are presumed to be safe since they occur in foods and have been used for centuries, and the question of safety of synthetic compounds can thus be avoided (Frankel 1996). Research for a safer and effective natural antioxidant is underway and several natural sources are being examined (Suja *et al.* 2004, Wanasundara and Shahidi 1998, Lee and Shibamoto 2001, Yu *et al*. 2005, Peschel *et al.* 2005, Balasundram *et al*. 2005).

The technologically most important natural antioxidants today are: tocopherols and spice extracts, ascorbic and citric acid and their salts (Hraš *et al*. 2000, Tsimidou *et al*. 1995). The majority of published work in the area of natural antioxidants has focused on tocopherols (Kalucka *et al*. 2005). The α -, β -, γ - and δ - tocopherols occur as mixtures in vegetable oils and are the main natural antioxidants in fats (Hraš *et al*. 2000). The most abundant and the most biologically active tocopherol in food is α - tocopherol. α - Tocopherol inhibits free radical oxidation by reacting with peroxyl radicals to stop chain propagation, and with the alkoxyl radical to inhibit the decomposition of the hydroperoxides and decrease the formation of aldehydes (Frankel 1996).

Depending on the conditions, ascorbic acid can act as an antioxidant, a pro-oxidant, a metal chelator, a reducing agent or as an oxygen scavenger. Ascorbyl palmitate (AP), a lipid-soluble ester of vitamin C, is an approved synthetic antioxidant that delays the onset of rancidity (Beddows *et al*. 2001).

Likewise, it has also been reported that the phospholipids show a synergic interaction with alpha-tocopherol. Phosphatidylethanolamine is more effective than phosphatidylcholine. However, the latter is more available commercially (Vicetti *et al.* 2005). Phospholipids decompose hydroperoxides with concomitant formation of carbonyls and volatile products. Therefore, when phospholipids get in contact with triglyceride, PV values do not increase during incubation.

Citric acid is found in almost all plant and animal species (Hraš *et al.* 2000). It can chelate metal ions by forming bonds between the metal and the carboxyl or hydroxyl groups of the citric acid molecule. Citric acid is very effective in retarding the oxidative deterioration of lipids in foods and is commonly added to vegetable oils after deodorisation.

The use of spices and herbs as antioxidants in processed foods is a promising alternative to the use of synthetic antioxidants (Madsen *et al*. 1998). Numerous reports of anti-oxidative activity of spices have appeared, strongly inspired by an increasing consumer interest in natural food additives. Many spices or their extracts have been assessed for antioxidant activity in a variety of food products and lipid systems (Tsimidou *et al*. 1995). Most studies have so far concentrated on the anti-oxidative effects of rosemary and rosemary extract (Hraš *et al*. 2000, Madsen *et al*. 1998). The compounds responsible for anti-oxidative activity of rosemary are mainly phenolic diterpenes such as carnosic acid, carnosol, rosmanol, epirosmanol, isorosmanol, methyl carnosate and other phenolic acids, such as rosmarinic acid (Hraš *et al*. 2000, Kalucka *et al*. 2005). Many rosemary extracts, for use in food systems, are today available on the market. However, the strong and characteristic flavour of rosemary might limit the use of this spice despite the well established and very high anti-oxidative capacity (Madsen *et al.* 1998). The antioxidant activity of clove bud extract and its major aroma components, eugenol and eugenyl acetate, were comparable to that of the natural antioxidant, α-tocopherol (vitamin E) (Lee and Shibamoto 2001). Clove bud extract inhibited malonaldehyde formation in cod liver oil.

Antioxidants also have been detected in a number of food and agricultural products, including cereal grains, vegetables, fruits, and oil seeds (Yu *et al.* 2005, Madsen *et al.* 1998). A few byproduct derived antioxidants have been developed successfully from the vast quantities of plant residues produced by the food processing industry in Europe, primarily grape seed and olive waste extracts (Peschel *et al.* 2006). Recently, cold-pressed seed oils, including black caraway, carrot, hemp, and cranberry seed oils, have become commercially available (Yu *et al.* 2005). In a study of the total antioxidant activities of 12 fruits and five commercial fruit juices it has been recorded that strawberry has the highest antioxidant activity followed by plum, orange, red grape, kiwi fruit, pink grapefruit, white grape, banana, apple, tomato, pear and honeydew melon (Mokbel and Hashinaga 2006). Anti-oxidative efficacy of sesame cake extracts have also been investigated (Suja *et al.* 2004). The anti-oxidative protection offered by methanolic extract of sesame cake is comparable with, or in some cases better than, that of the widely used synthetic antioxidant BHT.

Many works have been carried out on antioxidant actions of tea (Wanasundara and Shahidi 1998, Zandi and Gordon 1999, Krings *et al*. 2000). Green tea leaves contain up to 36% polyphenols, on a dry weight basis and tea extracts are powerful antioxidants owing mainly to the presence of the flavanols epigallocatechin gallate, epicatechin gallate, epigallocatechin and epicatechin. Tea catechins retard oxidation of marine oils with a similar effectiveness as synthetic antioxidants and green tea extracts have the potential for large-scale application as natural antioxidants. Tea catechins are effective scavengers of free radicals and also act as metal chelators. Chlorophyll present in organic extracts from green tea also affects the antioxidant activity of the extracts.

3 MATERIALS AND METHOD

3.1 Materials

3.1.1 Fish oil

The fish oil, which was supplied from Lysi Ltd. in Iceland, was processed from fish species containing high amounts of n-3 polyunsaturated fatty acids, with a pale yellow colour and almost without door and taste. The fish oil was refined, bleached, dried, cold filtered and deodorised. It was free from genetically modified materials and components. Physicochemical characteristics of the fish oil are shown in Table 1.

Table 1: Physicochemical characteristics of the fish oil sample.

Characteristic index	Values
Free fatty acids, %	0.5
Saponification value	180
Unsaponifiable matter, %	2.0
Water and mucilage, %	0.15
Weight, g/ml at 20°C	1.481-1.485
Cold test, hour (0°C)	3
Colour; Gardner units	8
Peroxide value, meq. O2/kg	0.6
Dioxin, ng TE/kg	2
PCB congeners (28;52;101;138;153;180) mg/kg	0.5
Anicidine value	19.8
Iodine value	202
Eicosapentaenoic acid (EPA), %	18.2
Docosahexaenoic acid (DHA), %	12.2

3.1.2 Antioxidants

The antioxidants used for the experiment were Coviox T-70 (Cognis) containing 70% tocopherol mixture and Ronoxan (DMS) containing 5% dl-alpha tocopherol, 25% ascorbyl palmitate and 70% lecithin. Concentrations of antioxidants added in samples were prepared on the basis of content of active antioxidants. The antioxidants were provided by Lysi Ltd. in Iceland.

3.2 Methods

3.2.1 Sample preparation

The antioxidants were added to fish oil in the following quantities:
Sample 1 - 0.02 wt % of tocopherol,
Sample 2 - 0.05 wt % of tocopherol,
Sample 3 - 0.1 wt % of tocopherol
Sample 4 – 0.05wt % of Ronoxan
For the control, the sample without antioxidants was used (control). After careful mixing, the samples were transferred into 500 ml brown bottles (2 bottles for each fish oil sample).

3.2.2 Assessment of oxidative stability

The fish oil samples with or without antioxidants that were put into 500 ml brown bottles were stored at 10°C in a refrigerator. For sampling, the bottles were taken daily (5 times per week) from the refrigerator and stored for 30 minutes at room temperature (20-24°C), and portions (15 ml) were taken for measurements. Oxidative stability was determined by measuring the peroxide value every other day (3 times per week) for 6-7 weeks and the anisidine value weekly.

3.2.3 Peroxide value

Primary oxidation products – hydro peroxides- are determined by peroxide value measurement. Peroxide values of oils were measured by titration of liberated iodine with standardised sodium thiosulphate solution according to the AOAC official method 965.33 (AOAC 1990).

3.2.4 Anisidine value

Formation of secondary oxidation products was measured by p-anisidine value. Anisidine value of oils was determined by the reaction of aldehydic compounds in oil and the p-anisidine, and absorbance measured at 350 nm, according to standard methods (IUPAC 1987).

3.2.5 Statistical analysis

Statistical analysis was done on the data by analysis of variance (ANOVA) on Number Cruncher Statistical Software (NCSS 2000 and Pass Trial, Kaysville, Utah). A Tukey comparison test was used to determine differences between the samples *(P*<0.05*).*

4 RESULTS

4.1 Changes in the peroxide value of fish oil during domestic consumption

4.1.1 Changes in the peroxide value of fish oil without antioxidants

Primary oxidation of oils was determined by measuring peroxide value. Changes in peroxide values of the fish oil without antioxidants during domestic consumption are shown in Figure 1.

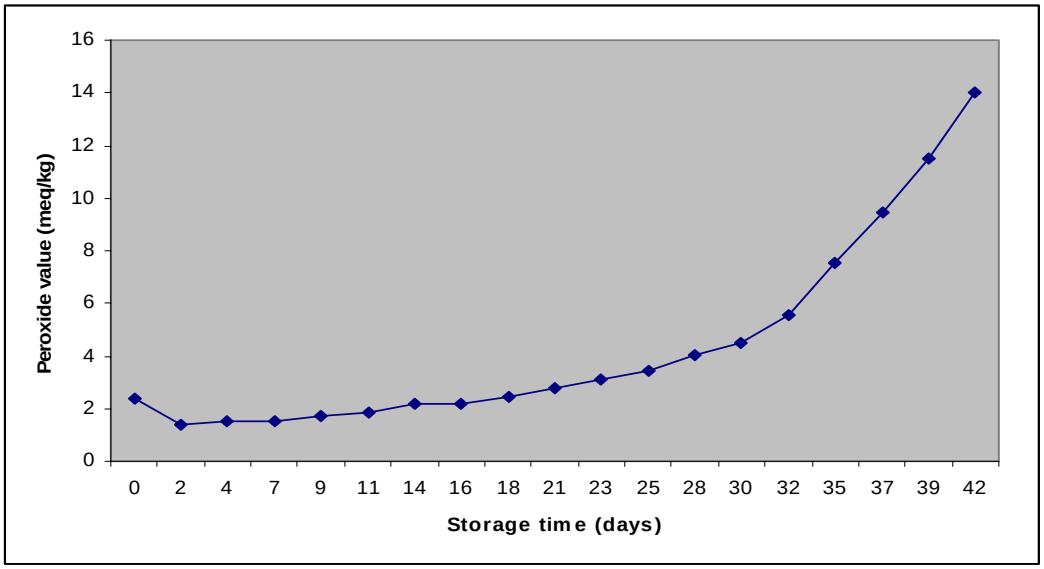


Figure 1: Changes in the peroxide values of fish oil without antioxidants during domestic consumption.

Initially, there were little changes in the peroxide values of the fish oil. Namely, the peroxide value increased from approximately 2 to 4.5 (meq/kg) during storage of up to 30 days. But after that, a rapid increase in peroxide value was observed and at end of the experiment (42nd day of storage) that value had increased to 14 (meq/kg).

4.1.2 Changes in the peroxide value of fish oil with antioxidants

Changes in the peroxide value of fish oil with different concentrations of tocopherol during domestic consumption are shown in Figure 2.

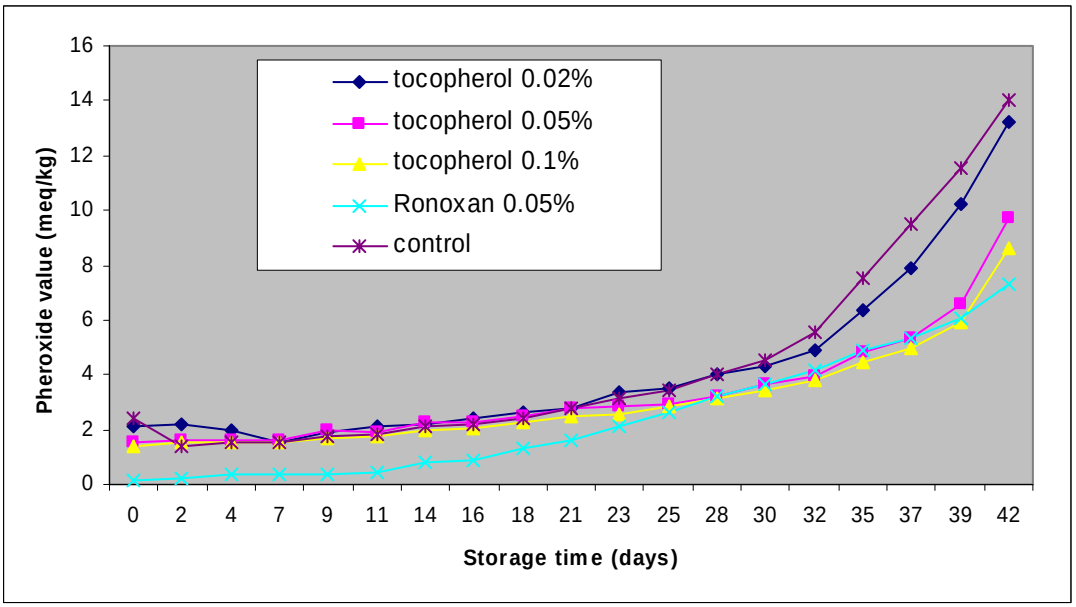


Figure 2: Changes in the peroxide values of fish oil with antioxidants during simulated domestic consumption.

There were very little changes and no differences in the peroxide values of samples with and without tocopherol from the beginning to the 3rd week of the experiment. Small variations were observed between samples until the 23rd day. Thereafter apparent differences were observed with increasing storage time. In the case of Ronoxan addition, the peroxide value was lower in comparison with other samples' value from the beginning to the 23rd day, and a similar tendency was found with tocopherol 0.05% and tocopherol 0.1%. At the end of the experiment (42nd day of storage), the peroxide values of the control sample had the highest value of 14 meq/kg, followed by tocopherol 0.02% (PV = 13. meq/kg), tocopherol 0.05% (PV = 9.7 meq/kg), tocopherol 0.1% (PV = 8.6 meq/kg) and Ronoxan 0.05% (PV = 7.3 meq/kg).

The results of statistical analysis of the samples with tocopherol showed no differences ($P>0.05$) between the control and the sample with tocopherol 0.02%, and between samples of tocopherol 0.05% and 0.1%. A significant difference ($P<0.05$) was observed between the control and tocopherol 0.05% (tocopherol 0.1%). In the case of the Ronoxan sample, the peroxide value was found to be lower than in one of the other samples from the beginning of the experiment ($P<0.05$), and showed no significant difference ($P>0.05$) with samples of tocopherol 0.05% and 0.1% at the end of storage.

Peroxide values in all of the samples did not rise to 20 meq/kg, which is generally considered necessary for oils to become rancid (Hraš *et al.* 2000).

4.1.3 Storage times to PV = 8

A suggested limit of peroxide value for quality and acceptability of oils for human consumption is 8 meq/kg (Boran *et al.* 2006). Storage times to PV = 8 of fish oils with and without antioxidants are shown in Figure 3.

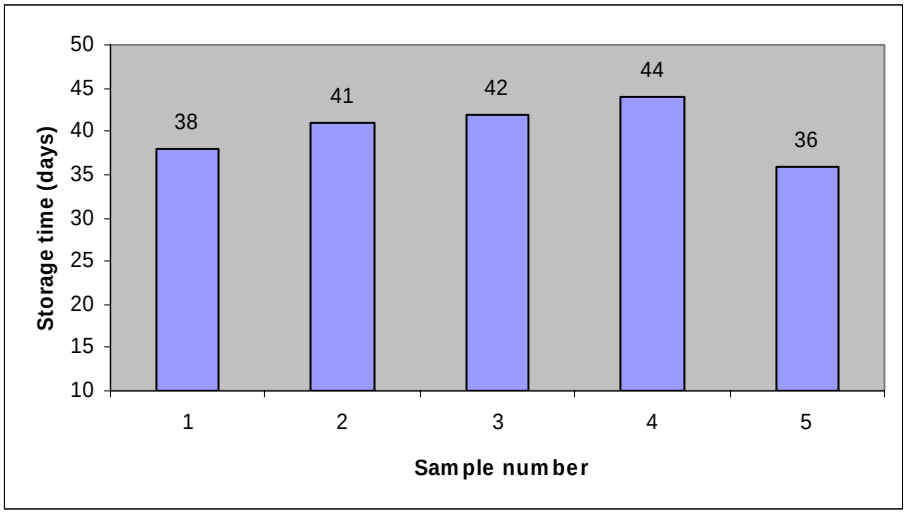


Figure 3: Storage times to PV = 8 of fish oils with and without antioxidants (Sample 1: tocopherol 0.02%, Sample 2: tocopherol 0.05%, Sample 3: tocopherol 0.1%, Sample 4: Ronaxan 0.05% and Sample 5: control).

Storage time to PV = 8, during simulated domestic consumption was 36 days for the control sample and increased with increase in tocopherol concentration to 38 days (tocopherol 0.02%), 41 days (tocopherol 0.05%) and 42 days (tocopherol 0.1%), and was the longest at approximately 44 days for the Ronoxan sample. This was estimated graphically from Figure 2, because the Ronoxan sample did not reach PV = 8 before the end of the experiment. The results showed no difference ($P>0.05$) between the control and sample with tocopherol 0.02%, and between samples of tocopherol 0.05%, 0.1% and Ronoxan 0.05%. A significant difference ($P<0.05$) was observed between the control and tocopherol 0.05% (tocopherol 0.1% and Ronoxan 0.05%).

4.2 Changes in the anisidine values of fish oil during domestic consumption

Formation of secondary oxidation products was measured by p-anisidine values. Changes in anisidine values of the fish oil with and without antioxidants during simulated domestic consumption are shown in Figure 4.

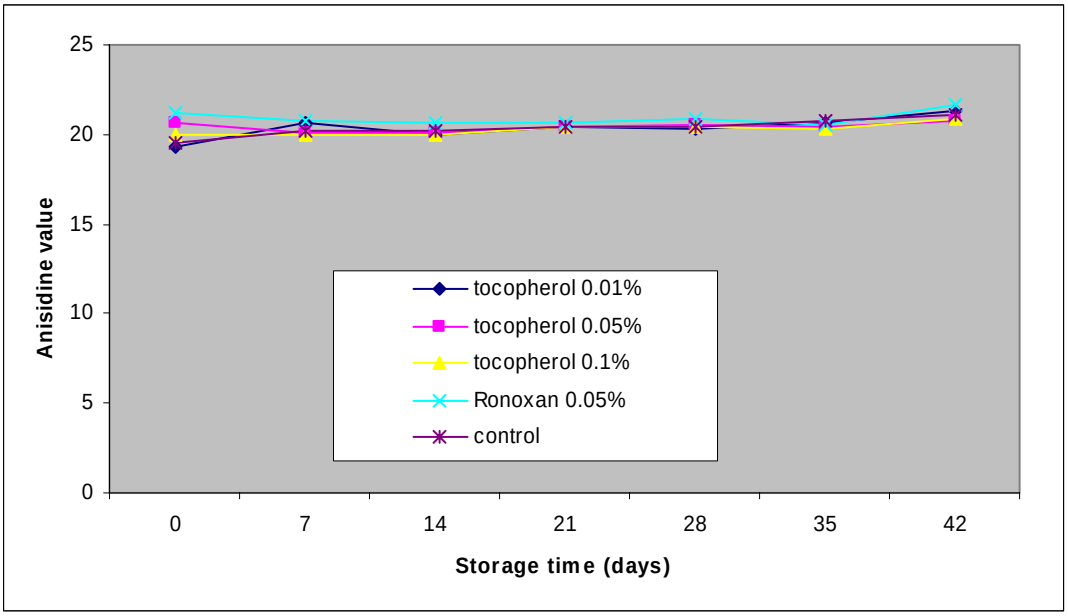


Figure 4: Changes in the anisidine values of fish oil with and without antioxidants during domestic consumption.

As shown in Figure 4, there were almost no changes and differences in anisidine value of the fish oil with and without antioxidants from the beginning to the end of the experiment. The anisidine value of samples that was 19.8 at beginning, increased slightly to 21 (control), 21.3 (tocopherol 0.02%), 20.8 (tocopherol 0.05 %), 20.8 (tocopherol 0.1%) and 21.6 (Ronoxan 0.05%) at the end of the experiment. No significant differences were found (P>0.05) in anisidine values between fish oils with and without antioxidants.

5 DISCUSSION

5.1 Peroxide increase in fish oil during domestic consumption

Fish oil, containing high levels of polyunsaturated fatty acids, is very susceptible to oxidative deterioration at varying velocities, strongly depending on the storage conditions and fatty acid profile. In the present condition of simulated domestic consumption, very little change was found in the peroxide values of the fish oil for the initial 30 days and at that time, the peroxide value was approximately 4.5 meq/kg. After that, rapid changes in peroxide value were observed, rising to 14 meq/kg on the 42nd day of the experiment. A peroxide value of 8 meq/kg, an acceptability limit of oil for human consumption, was reached in 36 days.

Similar results were found in a study by Boran *et al.* (2006). They reported that during storage at 4°C, peroxide values of several fish oils reached the acceptable limit of 8 meq/kg after 60 or 90 days and after at least 150 days at –18°C. This fact showed that the storage temperature had important effects on the storage stability of the fish oil. The short storage period to the acceptable limit observed in our experiment, compared with the study of Boran, can be explained by the effects of storage temperature as well as the daily removal of the oil from the bottles, introducing fresh oxygen into the bottles. Another reason may be found in the difference in iodine values of the fish oils used. The iodine value of the fish oil used in our experiment was 202, which was higher than the iodine values (177 to 197) of fish oil used in Boran's study. It is a well-known fact that the higher the degree of unsaturation, the higher the rate of autoxidation of oils (Stansby 1967).

The results indicate that for safe consumption of this fish oil without antioxidants, a maximum of 36 days shelf life is suggested under storage conditions of domestic consumption.

5.2 Inhibition of peroxide formation by antioxidants during domestic consumption of fish oil

The anti-oxidative effects of tocopherol have been shown in many studies and it is known as a common natural antioxidant. Ascorbyl palmitate and lecithin are also known as synergists. In our experiment the anti-oxidative effects of tocopherol and Ronoxan was also obvious (Figure 2). There was no difference ($P>0.05$) between the control and the sample with tocopherol 0.02%, and between samples of tocopherol 0.05%, 0.1% and Ronoxan. A significant difference ($P<0.05$) was observed between the control and tocopherol 0.05% (tocopherol 0.1% and Ronoxan 0.05%). Addition of 0.02% tocopherol had in fact no significance for the retardation of autoxidation of fish oil and the anti-oxidative effects of tocopherol did not increase with a concentration of tocopherol at higher concentrations than 0.05%. A similar phenomenon with anti-oxidative activity of tocopherol being optimal at concentrations between 400 and 600 μg/g, was found in Frankel's report (1996). As a result, in the case of addition of only tocopherol, it was found that 0.05% of tocopherol was optimal for improving the stability of fish oil during

simulated domestic consumption. In this case, an acceptable limit in peroxide value for human consumption was reached in 41 days, which means about 14% improvement in oxidative stability of fish oil, in comparison with fish oil without antioxidant. The fish oil with Ronoxan showed some difference from the other samples, which was apparent in lower peroxide values from the start of the experiment. Ronoxan contains lecithin and it is a known fact that phospholipids like lecithin are able to decompose hydroperoxides (Vicetti *et al.* 2005). But sufficient clear data was not found in the literature, which might suggest a need for further investigation. In this study, the longest storage time to PV = 8 meq/kg was found in the sample with Ronoxan 0.05% at 44 days. That result means signifies 22% improvement in oxidative stability of the fish oil and suggests an important action of lecithin and ascorbyl palmitate in combination with tocopherol.

For further improvement in oxidative stability, it has to be considered to use synergists such as lecithin and ascorbyl palmitate with tocopherol, but not to increase the concentration of tocopherol addition. Regarding oxidative stability of fish oil, it may also be considered to use a smaller bottle than 500 ml for consumption in a short time, for example, 250 ml or 300ml bottles.

5.3 Secondary oxidation of fish oil during domestic consumption

Formation of secondary oxidation products was expressed by an increase in anisidine values. Almost no changes were observed from the beginning to the end of the experiment and differences in anisidine values of fish oil with and without antioxidants were insignificant ($P>0.05$) (Figure 4). The initial anisidine value of 19.8 was rather high compared to other oils including safflower oil (anisidine value 1.1), rapeseed (anisidine value 3.5) and olive oil (anisidine value 5.1) (Guillén and Cabo 2002). High anisidine value in initial samples may however be a property of fish oil rather than proceeding of oil oxidation. High anisidine value in fresh oil has been reported, where the anisidine value of sunflower was 18.8 and of fish oil was 19.8 (Suja *et al.* 2004, United States Patent 6623774). Some authors have indicated that anisidine values are comparable only within each oil type because the initial value varies among oil sources (Guillén and Cabo 2002).

Experimental results in this study showed that there was very little secondary oxidation in the fish oil and no significant effects of antioxidants on the change of anisidine values during simulated domestic consumption.

6 CONCLUSIONS

Under conditions of domestic consumption of fish oil, little changes were observed in the peroxide values of the fish oil without antioxidants for the first 30 days and for safe consumption of fish oil without antioxidants, a maximum of 36 days shelf life is suggested.

Anti-oxidative effects of tocopherol and Ronoxan were observed in the fish oil. Anti-oxidative effects of tocopherol did not increase with concentration of tocopherol higher than 0.05% and addition of 0.01% tocopherol had no significance for retarding auto-oxidation. In a case with 0.05% of tocopherol, the acceptable limit in peroxide value for human consumption was reached in 41 days, which means about 14% improvement in oxidative stability, in comparison with fish oil without antioxidants. The longest storage time to PV = 8 meq/kg was found in the sample with Ronoxan 0.05% as 44 days. That means about 22% improvement in oxidation stability of fish oil and suggests important action of lecithin and ascorbyl palmitate.

There was very little secondary oxidation of fish oil and no significant effects of tocopherol on changes of anisidine values during the storage period.

For further improvement in oxidation stability, it may be considered to use synergist such as lecithin, citric and ascorbic acid with tocopherol, rather than increase the concentration of tocopherol. Regarding the oxidative stability of fish oil, it may also be considered to use smaller bottles than 500ml, for consumption in a short time, for example, 250 ml or 300ml bottles.

ACKNOWLEDGEMENTS

I would like to thank my supervisor Margrét Bragadóttir for her active guidance, valuable advice and statistical analysis. I am grateful to Erla M. Karelsdóttir, Ingibjörg Jónsdóttir, Ingibjörg Þorvaldsdóttir, Eyrún Þorsteinsdóttir and Greta Garðarsdóttir for their warm assistance.

I would like to thank Dr. Tumi Tómasson, Director and Mr. Þór Heiðar Ásgeirsson, Deputy Director of the Fisheries Training Programme of the United Nations University for making it possible for me to attend this programme and their help, and Sigríður Kr. Ingvarsdóttir for her careful help.

I would also like to thank all the people of the Marine Research Institute, Iceland Fisheries Laboratories and fellows for their contribution to my learning and life in Iceland.

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