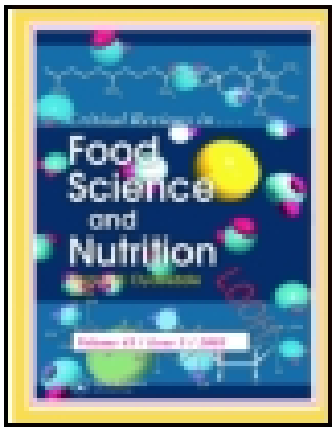

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ABSTRACT

Fish products contain various nutritionally beneficial components, namely, ω3 polyunsaturated fatty acids (ω3-PUFA), minerals, and vitamins. Particularly, tocopherols (α-, β-, γ-, and δ-tocopherol) can be provided by seafood and aquaculture products. Hence, this review shows the various aspects of tocopherols in seafood and aquaculture products. For tocopherol determination in these products, HPLC methods coupled with diode array detection in the UV area of the spectrum or fluorescence detection have been shown as sensitive and accurate. These newest methods have helped in understanding tocopherols fate upon ingestion by seafood organisms. Tocopherols pass through the intestinal mucosa mainly by the same passive diffusion mechanism as fats. After absorption, the transport mechanism is thought to consist of two loops. The first loop is dietary, including chylomicrons and fatty acids bound to carrier protein, transporting lipids mainly to the liver. The other is the transport from the liver to tissues and storage sites**.** Moreover, tocopherol levels in fish organisms correlate with diet levels, being adjusted in fish body depending on diet concentration. For farmed fish species, insufficient levels of tocopherols in the diet can lead to poor growth performance or to nutritional disease. The tocopherol quantity needed as a feed supplement depends on various factors, such as the vitamer mixture, the lipid level and source, the method of diet preparation, and the feed storage conditions. Other ingredients in diet may be of great importance, it has been proposed that α-tocopherol may behave as a prooxidant synergist at higher concentrations when prooxidants such as transition metals are present. However, the antioxidant action of tocopherols outweighs this prooxidant effect, provided that adequate conditions are used. In fact, muscle-based foods containing higher levels of tocopherol show, for instance, higher lipid stability. Besides, tocopherols are important not only from the nutritional point of view but also from the physiological one, since they are involved in many metabolic processes in the human organism. Moreover, synergistic interactions with selenium and ascorbic acid have been reported. It deserves attention that there is evidence tocopherols taken with food can prevent heart disease, while no such evidence was found for α-tocopherol as supplement. From this perspective, eating fish is advisable, since, for instance, a 100 g serving of salmon may provide nearly 14 % of recommended dietary allowance.

Keywords: tocopherols, antioxidants, analytical methodology, food processing, storage stability, health benefits

Introduction

Seafood and aquaculture products present a great importance in the human diet. Moreover, they offer a wide diversity of species, a broad range of products —from whole fresh fish to caviar— and large amounts of different nutrients, invaluable to human health. The consumption of fish and fish products has considerably increased over recent decades (SOFIA, 2009). In fact, the health benefits of a diet rich in fish have been extensively recognized in the last decade. These products contain various nutritionally beneficial components, namely, readily digested proteins, ω3 polyunsaturated fatty acids (ω3-PUFA), such as, eicosapentaenoic acid (EPA, 20:5ω3) and docosahexaenoic acid (DHA, 22:6ω3) —which are associated with decreased morbidity and mortality from cardiovascular and other diseases as well as with foetal development (Simopoulos, 2002; EFSA, 2005)—, minerals, and vitamins. Regarding latter nutrients, fatty fish are a rich source of liposoluble vitamins (A, D, E, and K). Particularly, the vitamin E, which comprises in nature four tocopherols (α-, β-, γ-, and δ-tocopherol) and four tocotrienols (α-, β-, γ-, and δ-tocotrienol), can be provided by seafood and aquaculture products. The four tocopherol vitamers —particularly α-tocopherol— are the most important (Yoshida et al., 2003). Among these vitamers, RRR-α-tocopherol (this molecule is chiral with three stereocenters, hence RRR) is considered to have the highest biological activity (Niki, 1996) (Figure 1) and is the only form of α-tocopherol that occurs naturally in foods. There are other stereoisomeric forms of α-tocopherol, such as *2R*- (*RRR*-, *RSR*-, *RRS*-, and *RSS*-α-tocopherol) and *2S*-stereoisomeric forms that occur in fortified foods and supplements (IOM, 2000).

Analysis of Tocopherols in Seafood and Aquaculture Products

In general, tocopherols are analysed through HPLC techniques (André et al., 2010; Rupérez et al., 2001). Effectively, HPLC techniques have widely replaced direct spectrophotometric and fluorometric methods (Gregory, 2000). Associated with HPLC separation systems, different detectors have been used, from flame ionization, diode array detection, fluorescence, mass spectrometry to atmosphere pressure chemical ionization and others (André et al., 2010). Particularly for seafood and aquaculture products, HPLC methods coupled with diode array detection in the UV area of the spectrum or fluorescence detection have been described (Huo et al., 1996; López-Cervantes et al., 2006). Since tocopherols are components of the unsaponifiable lipid fraction of seafood, this fraction must be previously extracted. There are two routes to perform this extraction, extracting the total lipid component, saponifying it, and removing the unsaponifiable fraction, or saponifying the lipid component within the sample and only extracting the unsaponifiable fraction. The latter route involves two main steps instead of three and, as such, limits processing and the oxidation process associated to extraction (Cayuela et al., 2003; Ryynänen et al., 2004). However, there are some drawbacks, namely, complexity (many factors to be optimized) and labour intensity, making usual techniques less attractive. But, recently, a methodology comprising microscale saponification and extraction with n-hexane for analysis of α-tocopherol has been developed and successfully applied to macroalgae (Sánchez-Machado et al., 2002). Furthermore, HPLC separation coupled with UV or fluorescence detection was used. For instance, a method widely used in the determination of tocopherols has been normal phase HPLC with a fluorescence detector with excitation wavelength of 292 nm and emission 324 nm and elution mixture of hexane:isopropanol (99.8:0.2) (Piironen et al., 1984).

Although α-tocopherol contents as determined in samples by UV and fluorescence systems were identical (Sánchez-Machado et al., 2002), fluorescence detection had a lower detection limit (10.4 *vs* 104 ng/ml), a better precision (relative standard deviation, 1.81 *vs* 2.12 %) and an acceptable recovery (94.3 %). The method was validated and proven sensitive, selective, precise, fast, simple, cheap, and convenient for these matrices. This same extraction procedure has been applied with some slight modifications to other seafood and aquaculture products (López-Cervantes et al., 2006). Concerning the chromatographic conditions, a SS Exil ODS column with 5 μm-particle size was used by these authors as well as a mobile phase 68:28:4 (v/v/v) methanol:acetonitrile:water at a flow rate of 1.4 ml/min. Detection was done at 208 nm. Given these conditions, the overall recovery was 100.8 % and the relative standard deviation (method precision) was 2.3 % (López-Cervantes et al., 2006). The quantification of the various vitamin E homologues can be carried out with a reverse-phase HPLC separation system coupled with an electrochemical detector (ECD) (Gotoh et al., 2011). A mobile phase consisting of a mixture of methanol and distilled water (50/1, v/v) containing 50 mM sodium perchlorate was utilized at a flow rate of 1.0 ml/min and room temperature. In addition, no major interference problems regarding the other components present in seafood and aquaculture products are nowadays reported in the scientific literature.

Tocopherols in Aquatic Food Products

Absorption, Transport, Storage, and Elimination in Fish

Vitamin E, like other fat soluble vitamins, passes through the intestinal mucosa mainly by the same passive diffusion mechanism as for fats (McDonald et al., 2002). After absorption, as a lipid soluble compound, α-tocopherol probably follows the transport mechanism of lipids between organs in fish (Lie et al., 1994). This mechanism is thought to consist of two loops, as is in mammals (Sheridan, 1988). The first loop is dietary (exogenous), including chylomicrons and fatty acids bound to carrier protein, transporting lipids mainly to the liver. The other is an endogenous loop which transports lipids from the liver to tissues and storage sites other than liver —for instance, perivisceral adipose tissue (Wang et al., 2006)— in the form of low density lipoproteins (LDL). In spite this subject warranting further studies, it has been reported that α-tocopherol is preferentially combined with LDL in rainbow trout *(Oncorhynchus mykiss)* (Hung et al., 1982). Moreover, the metabolism of LDL in rainbow trout seems to be similar to that of mammals (Gjoen and Berg 1992).

Nevertheless, concerning vitamin E isomers, there is very limited information available of their utilization and metabolism by fish and shrimp (NRC, 2011). Fish α-tocopherol is preferentially retained in the body when compared with the other tocopherols (Hamre, 2011). This fact is probably due to the presence of α-tocopherol transfer protein (α-TTP) in the liver which binds the tocopherols with different affinities and returns them to the plasma. The other

vitamin E forms (e.g., γ-tocopherol) bind weakly to α-TTP and are removed from the circulation (Traber, 2007) and to a greater extent excreted in the bile (Hamre, 2011).

Although hepatic α-TTP has not been identified in fish, there is evidence that this protein is present in fish (Hamre et al., 1998; Wang et al., 2006). Hepatic α-TTP is required to maintain plasma and tissue α-tocopherol levels (Traber, 2007). The liver regulates the body's vitamin E concentrations and it seems to be the major site of vitamin E metabolism and excretion. Tocopherols are metabolized in a way similar to xenobiotics. Firstly, they are ω-oxidized by cytochrome P450 enzymes, then are subjected to several steps of β-oxidation, and are conjugated and excreted. Liver α-tocopherol and other tocopherol concentrations are closely regulated; thus, any potential adverse vitamin E effects are limited (Traber, 2007). No conversion of α-tocopherol into other naturally occurring tocopherols (β-, γ-, δ- tocopherol) has been found in some cultured fish species, such as turbot (*Scophthalmus maximus*) (Schulz, 1986).

Hence, E vitamer levels in fish organisms correlate with diet levels. Fish adjust the tocopherol levels in their body depending on diet concentrations (Akhtar et al., 1999, Hamre, 2011). In general, within the studied dose ranges, there is a linear correlation between the increased level of tocopherol supplied in the feed and that present in the whole body fish (Hamre, 2011). Particularly, in liver, though some studies point to a linear relationship, most experimental works have observed that α-tocopherol increases exponentially in response to increased dietary concentration (Hamre, 2011). On the other hand, it has been observed that after a period of feeding with α-tocopherol supplementation, the fish body concentration remains constant, and the amount of α-tocopherol metabolized and excreted balances the amount absorbed (Akhtar et

al., 1999). It has also been stated that α-tocopherol appears to be retained in the tissues according to a "distribution key", independent of supplementation and body level.

Taking into account the previously mentioned issues, a bioavailability analysis is also of paramount importance. Bioavailability also depends on the specific E vitamer and the associated chemical anion. Indeed, several authors reported that α-tocopherol acetate is a source with higher bioavailability than α-tocopherol succinate for red drum (*Sciaenops ocellatus*) (NRC, 2011). In Atlantic salmon (*Salmo salar*), the relative uptake of α-, γ-, and δ-tocopherol seemed to be different in various organs, suggesting that each organ has specific mechanisms which ensure the take up of lipids and tocopherols from plasma (Hamre and Lie, 1997). The same authors observed that mixture of α-tocopherol with other vitamers in the diet reduces its retention in some organs, except liver, muscle and gonads. Liver is to be regarded as the main storage organ for either α-tocopherol or other vitamers (Schulz, 1986). However, in Atlantic salmon (*Salmo salar*), it was observed that γ and δ- tocopherol supplementation in diet can be accumulated preferentially in adipose and muscle tissue (Hamre and Lie, 1997).

Marine Products

In general, fish and other fish products contain low to moderate levels of tocopherols (Lall and Parazo, 1995). Nevertheless, fish contain higher concentrations than meat or poultry (Ackman and Cormier, 1967). This fact can be ascribed to biochemical adaptation of marine organisms to cold-water environments, since there are many seafood species living in cold waters (Fujisawa et al., 2010). It must be remarked that cell membrane homeostasis at low temperature is attained by production of high levels of unsaturated fatty acids (Crockett, 2008),

which are prone to oxidation, thereby imperilling normal cell function (Slater, 1984). Thus, these organisms elaborate high levels of biochemical defenses, including the production and/or sequestration of small-molecule antioxidants, such as tocopherols (Fujisawa et al., 2010), in order to stave off fat oxidation. According to Carballo-Cárdenas et al. (2003), tocopherols also have a role in electron transport reactions and cell membrane stabilization related to membrane permeability and fluidity, as is illustrated in Figure 2.

Tocopherols content varies between species and tissues. Dark muscle contains higher amounts of this essential component than light muscle due to the higher rate of metabolic activity associated to the dark muscle tissues (Ackman and Cormier, 1967). Moreover, muscle tissue of fish species considered fat and liver of lean species present higher concentrations of this vitamin (Lall and Parazo, 1995; Huss, 1995).

In fish and fish products, α-tocopherol is the main tocopherol (Syväoja and Salminen, 1985). Besides α-tocopherol, substantial albeit minor amounts of the β-, γ-, and δ-analogues occur in some algae species, for instance, in brown algae (Nakamura et al., 1994). Twelve years ago, a new vitamer was discovered in salmon roe (Yamamoto et al., 1999). This vitamin E form was termed “marine derived tocopherol” (MDT) (Gotoh et al., 2011). The structure of MDT is similar to that of α-tocopherol with the exception of a double bond at the end of the phytyl side chain (Figure 1). MDT is distributed in a wide range of marine products, from phytoplankton to fish (Yamamoto et al., 2001; Dunlap et al., 2002; Fujisawa et al., 2010). Moreover, it was found that the relative biological activity of vitamin E for MDT was higher than that for β-tocopherol, which is considered to show the highest relative vitamin E activity next to α-tocopherol (Gotoh et al., 2009). It is noteworthy that the tocopherol:fat ratio was lower in fatty fish than in lean fish

(Syväoja and Salminen, 1985). On the other hand, during processing and frozen storage of seafood and aquaculture products, tocopherol concentrations decrease as lipid peroxidation progresses (Lall and Parazo, 1995). Regarding this subject, experiments have shown that fish oils, which contain high levels of polyunsaturated fatty acids, are much more prone to autooxidation phenomena the lower their tocopherol content (Cruickshank, 1962). Likewise, rancid and other unpleasant flavours rapidly lead to sensory rejection of fishery products whenever tissue tocopherol levels are low. Hence, the quality of seafood and aquaculture products, not only from the nutritional point of view, but also for preservation purposes, is strongly connected with tocopherols (Lall and Parazo, 1995).

Tocopherols content in fish species depends on the various parameters affecting their biology. Effectively, the values range between 0.1 mg/100 g in some wild fish species to 3-4 mg/100 g in aquaculture fish species (Table 1). Diet is extremely important, since fish are unable to synthesize tocopherols (Ackman and Cormier, 1967). Season, age, and size also affect tocopherols levels. A study has observed that fish caught in the spawning season of numerous species presented a higher tocopherols level and a lower fat content than those caught in the other autumn (Syväoja and Salminen, 1985). In general, lean fish species have a small level of tocopherols in muscle with values around 0.3 mg/100 g for cod and 0.5 mg/100 g for hake. Nevertheless, Afonso et al. (2008) reported higher levels of tocopherols for a lean species such as black scabbard fish (*Aphanopus carbo*). Furthermore, α-tocopherol level of 2.4 mg/100 g was reported in a fatty fish species as eel (Dias et al., 2003).

Aquaculture and Other Products

Aquaculture production has a long tradition. In fact, in some world regions, such as China, fish have been confined and fed for several thousand years (Halver, 2002). However, recently, there has been a remarkable worldwide expansion of the aquaculture production, since wild fish resources are largely overexploited.

The nutrient levels, including vitamins, of farmed fish species reflect their diet (Hamre et al., 1997; Hasan, 2001; Hamre, 2011). Tocopherols are not biosynthesized by the animal organisms, so animals are thoroughly dependent on their diet to attain this vitamin (Nogala-Kałucka, 2003). Moreover, contrary to other fat-soluble vitamins, tocopherols are not stored in large amounts in the animal body, thereby entailing a regular dietary intake (McDonald *et al*., 2002). While wild fish rarely present symptoms of vitamin deficiency, farmed fish may display such symptoms whenever their diet is not adequately formulated (Hasan, 2001; Hamre, 2011). In fish, studies of the interactions between selenium (Se) and tocopherol have shown that Se deficiency may lead to reduced levels of tissue α-tocopherol (Hamre, 2011). Of course, tocopherols requirement varies with endogenous and exogenous factors such as fish species, size, growth rate, metabolic functions, feeding patterns, and environment, culture system included (Lovell, 1987, Hasan, 2001; Hamre, 2011). Accordingly, tocopherol requirement of aquaculture species can vary considerably with the species itself, the type of tocopherol source in the diet (supplementary and/or natural feed) (De Silva and Anderson, 1995; Hasan, 2001), as well as the amount of polyunsaturated fatty acids and other interacting nutrients in diet (McDonald et al., 2002; Halver, 2002; Hamre, 2011). Indeed, high levels of polyunsaturated fatty acids and low levels of vitamin

C, selenium, and astaxanthin increase the requirement of vitamin E (Hamre, 2011). This factor can be very important, leading to huge variations of the vitamin E requirement. For instance, whereas rainbow trout fed a diet with 10 g/kg linolenic acid as only polyunsaturated fatty acid source for 16 weeks did not display any pathologies with 5 mg/kg α-tocopherol (Cowey et al., 1981), same fish species fed a diet with 150 g/kg fish oil for 12 weeks presented vitamin E deficiency with 50 mg/kg α-tocopherol (Watanabe et al., 1981). Moreover, the variation range between species is wide, for instance, from 25 mg/kg α-tocopherol in blue tilapia (Roem et al., 1990) to 119 mg/kg α-tocopherol in yellowtail (Shimeno, 1991).

Hence, whenever data from a different species are used to guide the tocopherol requirements of a new farmed species, inappropriate tocopherols levels may be an undesirable outcome (De Silva and Anderson, 1995). Insufficient levels of tocopherols in the diet, as compared to the species requirements, can lead to poor growth performance or to a nutritional disease (Lovell, 1987; Hamre, 2011; NRC, 2011), frequently characterized by a fatty liver, muscular dystrophy, and other physiological disorders (Lovell, 1987; Halver, 2002; McDonald et al., 2002; Hamre, 2011; NRC, 2011). Deficiency of this vitamin also affects reproductive performance, causing immature gonads and lower hatching rate and survival of offspring (Izquierdo et al., 2001). It has been noticed that Se combined with tocopherol (Watanabe et al., 1997; NRC, 2011) and ascorbic acid (Halver, 2002) is essential for the prevention of any nutritional muscular dystrophy symptoms (Halver, 2002; Hamre, 2011).

Furthermore, tocopherols associated to Se-containing glutathione peroxidase, other enzymes containing trace elements, and other vitamins protect fish cells against oxidative damage inflicted by free radicals (McDonald et al., 2002). This is an important issue due to the

importance of curbing oxidation of ω3-PUFA, which function as constituents of subcellular membranes and precursors of prostaglandins and reach very high levels in fish tissues. As a general rule, the higher the ω3-PUFA contents in the fish, also the higher the tocopherol requirements. Moreover, given the synergism between tocopherol and Se, tocopherol requirements are also greater in Se-depleted fish (Lovell, 1987; De Silva and Anderson, 1995; Hamre, 2011). In this way, a higher oxidative stability of the raw and processed products as well as an improved nutritional value may be achievable (Eitenmiller and Lee, 2004).

In the diet of farmed fish, tocopherols are required for an optimal fish growth (Sau et al., 2004; Abowei and Ekubo, 2011). Data obtained from studies on salmonids (rainbow trout; 30 – 50 mg/kg dry diet), common carp (80 – 300 mg/kg dry diet) or channel catfish (50 – 100 mg/kg dry diet) are usually applied to other species whenever the formulation of a complete diet for intensive culture is intended (Hasan, 2001). Besides fish productivity, tocopherols incorporation in the diet is warranted by the need of extending the shelf life of the fish carcasses (McDonald et al., 2002). Additionally, the tocopherol quantity needed as a feed supplement also depends on the vitamer mixture, the lipid level and source, the method of diet preparation, and the storage conditions under which the feeds are held before feeding (Cowey et al., 1981; Halver, 2002; Hamre, 2011).

Moreover, α-tocopherol may be added as a feed supplement, being produced synthetic α-tocopherol for this purpose. Concerning this, commercial preparations in the form of esterified acetate or phosphate are available and are commonly used as diet supplements (Lovell, 1987; Halver, 2002; Hamre, 2011). Besides, these esters are much more stable than the free form, which is rapidly decomposed by air oxidation or in the presence of labile compounds such as ω3-

PUFA from fish oils (Halver, 2002). In fish, the acetate ester is hydrolysed in the gut prior to absorption of α-tocopherol (Hung et al. 1982).

Recently, microalgae have also been produced with the purpose of obtaining significant amounts of natural tocopherols. In microalgae and higher plants, the tocopherols activity is mostly due to the α-tocopherol, which nevertheless varies from about 90 % of the total tocopherols in *Tetraselmis* microalgae (Huo et al., 1997) to less than 50 % in rapeseed oil (Schwartz et al., 2008). In microalgae, α-tocopherols are located in chloroplasts, whereas γ-and δ-tocopherols are found in extrachloroplastic fractions of the cell. Moreover, tocopherols have a widespread natural occurrence, being present in both photosynthetic (e.g. leaves) and non-photosynthetic (e.g. seedlings) tissues of higher plants and algae. In green microalgae, *Isochrysis galbana* and *Diacronema vlkianum*, an increase in α-tocopherol levels during the growing phases was reported by Bandarra et al. (2003), Donato et al. (2003) and Durmaz et al. (2008a,b). The levels of α-tocopherols were higher in these freeze dried microalgae when compared with conventional foods known to be rich in tocopherols. Brown algae, such as *Ishige okamurae*, present a high tocopherol content, encompassing not only α-tocopherol (13.4-21.4 mg/100 g dry weight), but also significant quantities of δ-tocopherol (12.9-17.5 mg/100 g dry weight) (Nakamura et al., 1994).

Although many microorganisms have been screened for the existence of tocopherols, they were mainly found in unicellular algae. Among them, according to several authors cited by Kusmic et al. (1999), *Dunaliella, Chlorella, Chlamydomonas* (all Chlorphyceae), *Ochromonas* (Chrysophyceae), and *Euglena* (Euglenophyceae) have attracted the attention of many scientists.

The Biochemical Role of Tocopherol

The physiological and/or pathological mechanisms occurring in living organisms generate intracellular reactive oxygen species that damage the structure of the cell membrane or even within the nucleus itself. Among these reactive oxygen species, the superoxide, hydroxyl, and hydrogen peroxide radicals have a very important role. The action of hydroxyl radical upon the phospholipid chains within the cell membranes initiates a chain of reactions commonly referred to as the phenomenon of lipid peroxidation, which, in turn, yield degradation products such as the peroxyl radical (ROO •) (Halliwell & Gutteridge, 2007). In addition, there are endogenous antioxidant systems such as the enzymes superoxide dismutase, glutathione peroxidase and catalase, which are the first line of defense against any formed radicals (McDonald et al., 2002, Halliwell & Gutteridge, 2007).

The peroxyl radical reacts with tocopherols (TOC-OH) through their hydroxyl group to form the corresponding organic hydroperoxide and tocopheroxyl radical (TOC-O$^{\bullet}$). Hence, in the presence of tocopherols occurs the following reaction:

$$ROO^{\bullet} + TOC\text{-}OH \rightarrow ROOH + TOC\text{-}O^{\bullet}$$ (IOM, 2000)

In fact, tocopherols act mainly as a primary antioxidant, interrupting the propagation of the autooxidation process by donation of hydrogens (and respective electrons) to lipid free radicals (Kamal-Eldin and Appelqvist, 1996). In particular, tocopherols block the reactivity of the peroxyl radical (Nogala-Kałucka, 2003; Mourente et al., 2007), contributing to the diminution of what is commonly called oxidative stress. Concerning this antioxidant action, the different

tocopherol homologues may present different activity levels, being α-tocopherol theoretically a more potent chain-breaking antioxidant than the β/γ/δ-tocopherols (Saldeen and Saldeen, 2005). This variability between homologues can be related to the different relative reactivities of substituted phenols (Figure 1), which result from the presence of electron-releasing substituents in positions ortho- and/or para- to the hydroxyl function and stereoelectronic effects concerning the substituents' orientation to the aromatic plane (Burton et al., 1985). The electron-releasing substituents in ortho- and/or para- positions to the hydroxyl function favour the rupture of the O-H bond, thereby enhancing the free radical reactivity (Pokorny, 1987), as is the case in α-tocopherol, but not in the other homologues, which lack one or two methyl ortho- groups. Of course, this straightforward reasoning fails to predict the relative effects of tocopherols *in vivo*, since the utilization of tocopherols in biological tissues is not only affected by their chemical reactivities, but also by the biokinetics of their transport and distribution and their bioavailability (Massey, 1984).

On the other hand, tocopherols may also act as prooxidants (Kamal-Eldin and Appelqvist, 1996). The reason for this lies in the radicals generated by tocopherols. These radicals may be reactive toward stable molecules or to participate in reactions other than donation of hydrogens to radicals or radical-radical coupling. The importance of these phenomena is determined by different factors, such as concentration and temperature (Lea, 1960). In particular, the prooxidant action of α-tocopherol was linked to its tocopheroxyl radicals (Pokorny, 1987) and enhanced by high concentrations (Cillard et al., 1980; Koskas et al., 1984; Terao and Matsushita, 1986). Regarding temperature, it has been reported that the higher the temperature, the less the prooxidant action of α-tocopherol, even at high concentration (Marinova and Yanishlieva, 1992).

This may be related to the lower oxygen solubility in oils at higher temperatures. However, a study conducted with fish oil showed that a lower temperature appeared to reduce the prooxidant effect of α-tocopherol (Zuta et al., 2007). Hence, further research on this subject would be important. Another variable that influences the relative prooxidant/antioxidant potency is the medium nature (Cort, 1974). It was observed a higher antioxidant potency of tocopherols in animal fats than in vegetable oils (Cort, 1974) and that their stability was higher in ω3-PUFA rich oils (Yuki and Ishikawa, 1976), such as fish oils. In addition, the presence of synergists (for instance, ascorbic acid, carotenes, amines, and phospholipids) in the medium may increase the antioxidant potency by regeneration or metal chelation (Kamal-Eldin and Appelqvist, 1996). It was also recorded a significant effect between α-tocopherol and phospholipids Bandarra et al., 1999). On the other hand, the presence of prooxidant substances may lead the tocopherols to behave as prooxidant synergists. Namely, it has been suggested that α-tocopherol may not be a prooxidant *per se*, but may be a prooxidant synergist at higher concentrations when prooxidants such as transition metals are present (Fuster et al., 1998).

In seafood and aquaculture products, different studies have corroborated some of the previous observations. Namely, it has been observed a prooxidant effect of tocopherol in a study on the oxidative stability of sardine skin lipids as measured by oxygen uptake determination (Vicetti et al., 2005). This occurred for higher α-tocopherol contents (equal to or above 1 %, w/w, in fish oil). It has been suggested that after the reaction between α-tocopherol and a free radical, the functional part of this antioxidant becomes a radical compound, which can take a hydrogen atom (plus its electron) from an active methyl group of a non-oxidized α-tocopherol molecule (Terao and Matsushita, 1986). This hypothesis has been confirmed by other authors (Parker, 1989).

Precisely to prevent this prooxidant action, it has been proposed the utilization of small amounts of ascorbic acid as a reductor agent (Niki, 1987). Phospholipids, such as lecithin, are another possible solution (Bandarra et al. 1999; Vicetti et al., 2005). They are able to decompose hydroperoxides with the concomitant formation of carbonyls and volatile compounds (Lee et al., 1984). In fact, positive results in delaying fish lipid oxidation were attained with lecithin coupled with α-tocopherol and ascorbic acid (Vicetti et al., 2005). According to other authors (Saldeen and Saldeen, 2005) that reviewed experimental evidence, the counteraction of the prooxidant effect of α-tocopherol may require the presence of γ- and δ-tocopherol. Furthermore, a mixture of γ-, δ-, and α-tocopherols (proportions, 5:2:1) presented a better antioxidant action than α-tocopherol alone (Saldeen and Saldeen, 2005). Similar results were reported by other authors (Liu et al., 2002). Hence, instead of incorporating α-tocopherol alone into seafood and aquaculture products whether by post-mortem addition or diet supplementation, mixtures of tocopherols would be preferable, particularly those mimicking the mixture ratio commonly found in nature (Saldeen and Saldeen, 2005).

The Role and Stability of Tocopherols during Seafood Storage and Processing

The group of seafood and aquaculture products is much more easily spoilable than other food groups due to their own characteristics and to their natural habitats. Namely, the high moisture level, the presence of many nitrogen-containing compounds with a low molecular weight and, as such, very volatile, the protein profile, the low importance of the connective tissues, the high levels of ω3-PUFA in fatty fish, and the importance of psychrophilic bacterial flora promote various alterations that rapidly lead to significant quality losses and to product rejection. These

changes occur even at low refrigeration temperatures (0-2 ºC) in unprocessed whole fish. In particular, ω3-PUFA have a greater tendency to oxidize due to their very high unsaturation levels, thereby leading to the formation of off-flavours and substantial reduction of the products' shelf life (Pérez-Mateos et al., 2004). Hydrolytic and oxidative reactions during processing and storage are among the basic processes causing the production of hydroperoxides (Osman et al., 2001), free fatty acids (Chaijan et al., 2006), and rancidity (Donelli and Robinson, 1995). Hydroperoxides are unstable and decompose, yielding a complex mixture of secondary oxidation products, mainly aldehydes and ketones, which are responsible for the changes in aroma and flavour (Frankel, 1991). The basic mechanism of free radical induced lipid oxidation may be described by three different steps: initiation, propagation, and termination. This phenomenon is affected by both intrinsic and extrinsic factors, such as fatty acid composition, concentration of pro-oxidants, endogenous ferrous iron, myoglobin, enzymes, pH, temperature, ionic strength, and oxygen consumption (Andreo et al., 2003).

Hence, the decrease of tocopherol content in the products may jeopardize the quality of the products, particularly, those containing higher fat levels. As a consequence, it is of great importance to ensure the stability of endogenous tocopherols during storage and processing for the sake of overall product quality as well as for their own intrinsic nutritional value. Conversely, tocopherol —mainly α-tocopherol— has been added to seafood and aquaculture products, particularly fish oils, as a means to curb fish lipid oxidation (Selmi et al., 2010; Zuta et al., 2007). Tocopherols intercept radicals before they react with unsaturated fats (Gatellier et al., 2000). In fact, muscle-based foods containing higher levels of tocopherol (at maximum antioxidant effect threshold concentrations) show higher lipid stability (Faustman et al., 1999).

Tocopherols are deemed more “consumer friendly” and regarded as “natural” antioxidants in opposition to synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene, or tertiary-butyl hydroquinone (Zuta et al., 2007). Therefore, tocopherols may be important for quality preservation in seafood products during cold and frozen storage (Calder, 2003). In particular, a study on the effects of antioxidants on lipid oxidation of common carp fillets stored at 5 ºC for 16 days revealed that tocopherols were effective in reducing thiobarbituric acid reactive substances (TBARS) values, an indicator of the formation of secondary oxidation products, with respect to control fish samples (Khalil and Mansour, 1998). Another study investigated the influence of blending sorbitol with tocopherol (mixture of the various homologues) in the antioxidant protection of cooked frozen crab (Calder, 2003). It was found out that 1 g sorbitol + 2.5 g of tocopherol per crab had a positive effect on the sensory ratings of whole cooked, cryogenically frozen crab, thereby being beneficial for the product’s shelf-life. Moreover, addition of α-tocopherol to tilapia hamburgers (10 mg per 100 g) had a significantly positive effect on storage stability of the products as measured by TBARS values (Fogaça and Sant’Ana, 2007). Effectively, α-tocopherol has been the most commonly used form of vitamin E incorporation, given its higher vitamin E activity compared to the other homologues (National Research Council, 1993). The same authors also found out that tocopherol supplementation in diets protected the hamburgers more effectively from lipid oxidation than post-mortem addition (Fogaça and Sant’Ana, 2007). With respect to this issue, it must be taken into account that fish (e.g. Atlantic salmon) adjust their body levels to the dietary α-tocopherol concentrations after approximately 3 months (Hamre and Lie 1995; Hamre et al., 1997). Hence, appropriate tocopherol retention in farmed fish tissues is of paramount importance. Indeed, it has been shown

that α-tocopherol is more efficient in protecting catfish fillets against oxidation than synthetic antioxidants, possibly owing to a better retention in the fillet (Gatlin et al., 1992). Moreover, the combination of low tocopherol and a high level of ω3-PUFA in diets enhanced rancid flavour and colouration alteration in farmed fish fillet stored at different conditions (Waagbø et al., 1993; Hamre, 2011).

Other researchers have focused on the combination of tocopherols with secondary antioxidants (Vicetti et al., 2005), such as citric acid, lecithin, ascorbic acid, and tartaric acid (Reische et al., 1998). These compounds act differently from primary antioxidants in that they slow down autooxidation by chelating prooxidant metals, decomposing hydroperoxides formed during the propagation step, and providing hydrogens to primary antioxidants. Hence, secondary antioxidants may act synergistically with primary antioxidants to increase the latter's antioxidant activity (Reische et al., 1998). It has been observed that the most effective solution for restraining oxidation of sardine skin lipids was a combination of α-tocopherol, lecithin, and ascorbic acid (Vicetti et al., 2005). Whereas this treatment prolonged during 14 days the oxidation initiation step, α-tocopherol *per se* at identical concentration only delayed oxidation for four days. Nevertheless, α-tocopherol alone can also be very effective, namely in fish oils (Selmi et al., 2010; Zuta et al., 2007). It was reported that treatment of mackerel oil with 5 or 10 mg/100 g α-tocopherol yielded the lowest oxidation level as measured by peroxide value (PV), conjugated diene (CD) value and TBARS over a 66-day period at -40 ºC (Zuta et al., 2007). The absence of significant differences between the effects of 5 and 10 mg/100 g of α-tocopherol after 66 days led the authors to consider 5 mg/100 g of α-tocopherol as the preferred concentration for controlling oxidation on the basis of both cost and efficacy. Other authors (Selmi et al., 2010),

which carried out a study on the oxidative stability of sardine oil, concluded that storage at 4 ºC coupled with addition of 10 mg/100 g α-tocopherol had a favourable influence upon sardine oil stability. On the other hand, γ/δ-tocopherol has also been successfully tested in a binary system with lecithin, being reported a strongly synergistic effect in delaying peroxidation of fish (menhaden or Chilean anchovy) oil as measured by PV (Hamilton et al., 1998). However, the ternary blends of α/γ/δ-tocopherols, lecithin, and ascorbyl palmitate delivered the greatest protection against autooxidation. Namely, oil containing 2 % δ-tocopherol, 0.1 % ascorbyl palmitate, and 0.5 % lecithin presented no significant oxidation at 20 ºC during a six month period, when assessed by chemical parameters. But, the antioxidant mixture did not avoid oxidation flavour deterioration as evaluated by sensory analysis.

Concerning oils, emulsions have received growing attention from researchers, given their various applications in food products (Jacobsen et al., 2008). Although this review showed that available data did not support an efficient antioxidant effect of tocopherols on real food emulsions enriched with ω3-PUFA, in a model emulsion of water and menhaden oil, both α- and δ-tocopherol efficiently prevented oxidation (Chaiyasit et al., 2005). However, it is possible that these different outcomes are related to the fact that oxidation in tested model emulsions was catalysed by heat and not by the addition of metal ions. Under these circumstances, the ability of tocopherol to scavenge free radicals may suffice to prevent lipid oxidation, while this is not the case in the more complex food emulsions characterized by the occurrence of oxidation reactions along multifarious pathways (Jacobsen et al., 2008). Furthermore, it was shown that the relative efficiency of δ-tocopherol was higher than that of α-tocopherol, precisely the opposite of the observed in bulk menhaden oil (Chaiyasit et al., 2005). This result is much more remarkable,

since, according to the so called “antioxidant polar paradox”, the α-tocopherol should be more efficient in an emulsion system. The antioxidant polar paradox is based on numerous studies, which have found out that polar antioxidants are more effective in bulk oil than in oil-in-water emulsions and, conversely, nonpolar antioxidants are more effective in emulsions (Frankel et al., 1996). The paradox has been ascribed to retention of nonpolar antioxidants in the lipid phase of oil-in-water emulsions or the affinity of polar antioxidants for the oil/air or oil/water interfaces in bulk oils. But, in fact, α-tocopherol (three methyl groups in benzene ring) has less polarity than δ-tocopherol (containing only one methyl group). This suggests that δ-tocopherol may outperform α-tocopherol as an antioxidant stabilizer. However, this and other questions concerning synergies with other antioxidants still warrant further research (Jacobsen et al., 2008).

Concerning tocopherols themselves, there is stability to heat under anaerobic conditions, but they are slowly oxidized by oxygen to tocopheroxide, tocopherylquinone, and tocopheryl hydroquinone as well as to dimers and trimers (Deshpande et al., 1996). However, the oxidation reaction is catalysed and accelerated by iron and copper. Though seafood and aquaculture products, in general, are not particularly rich in these metals, studies on the subject are obviously of paramount importance. Some experimental works on the tocopherol stability in seafood and aquaculture products are found in the literature. Namely, the decrease of α-tocopherol in the light and dark muscles of mackerel over iced storage time was studied (Petillo et al., 1998). It was found out that whereas in the light muscle α-tocopherol decreased by 60 %, in the dark muscle a larger 70 % decline was measured. This difference can be ascribed to the high heme pigment (containing iron) in fish dark muscle (Kijowski, 2001). Moreover, a strong reduction in

α-tocopherol content was also reported for sardine (Bandarra et al., 1997). However, other studies have shown stability of tocopherols in seafood products. A study on the tocopherol levels in minced channel catfish over chilled storage time did not measure any decline in either α- or γ-tocopherol after seven days (Erickson, 1992). The same author also studied channel catfish subjected to frozen storage for up to nine months under fluctuating temperatures in the range of -6 to -18 ºC (Erickson, 1993a). Tocopherol was slowly lost during the first six storage months. Accelerated degradation of tocopherol after six months coincided with increased generation of lipid oxidation products, thereby pointing to an insufficient tocopherol level to protect membrane lipids and prevent propagation (Erickson, 1993a). In addition, tocopherol levels and oxidative response were strongly correlated. Furthermore, it has been uphold that the evolution of tocopherol level may be a sensitive indicator of oxidative stability at the first oxidation steps (Erickson, 1993b). Another interesting study has shown that in a system of mixed palm triolein and fish oil the concentration of α-tocopherol decreased during storage (Yi et al., 2011). It was also reported that ascorbyl palmitate reduced the losses of α-tocopherol. Besides storage time, food processing operations may affect tocopherol concentrations. For instance, the effect of cooking on the tocopherol content of minced channel catfish was assessed (Erickson, 1992). It was reported a greater loss of α-tocopherol than of γ-tocopherol (40 *vs* 20 %) after five minutes at 177 ºC. Interestingly, according to some authors (Storozhok, 1985), frying in vegetable oil (rich in tocopherols) may lead to a higher tocopherol content in the processed seafood.

Health Benefits and Dietary Recommendations

Tocopherols deserve attention not only from the nutritional point of view but also from the physiological one (Belitz et al., 2004), because they are involved in many metabolic processes in the human organism. The promotion of health by tocopherols is linked to their antioxidant function (IOM, 2000; McDonald et al., 2002; Nogala-Kałucka, 2003; Belitz et al., 2004; Halliwell and Gutteridge, 2007). Such function has been related to the inhibition of oxidative stress reactions (Pryor, 2000). It is also known that tocopherols display an important antioxidant action regarding such reactions caused by the decomposition of mercury organic forms (Parazo et al., 1998; Rao and Sharma, 2001).

On the other hand, tocopherols may present a synergistic interaction with Se, whose content in seafood products make these foods an important Se source (Watanabe et al., 1997). Tocopherol behaves as a membrane-associated antioxidant and/or scavenger of free radicals, thereby inhibiting peroxide formation (McDonald et al., 2002). These two nutrients complement each other and protect biological membranes against lipid oxidation. Besides, the dietary level of tocopherol affects the minimum Se requirement (Watanabe et al., 1997).

Ascorbic acid also displays synergistic interaction with tocopherols in the maintenance of the levels of intracellular antioxidants and free radical traps. Moreover, it acts synergistically with tocopherols and Se in order to guarantee acceptable activities of glutathione peroxidase and superoxide dismutase (Halver, 2002).

Therefore, the antioxidant function has contributed to the importance of tocopherols in all pathophysiological situations based upon the production of reactive oxygen species. This is the

case of cardiovascular diseases (IOM, 2000; Visioli and Hagen, 2007), neurodegenerative diseases, cancer (IOM, 2000), and light-induced pathologies of skin and eyes, which have been the subject of several studies (Clark 1996; Eitenmiller 1997; Gómez-Coronado et al. 2004). However, several studies mentioned by Saldeen and Saldeen (2005) have reported that α-tocopherol alone has no effect on cardiovascular events and death. Besides, there is a report carried out by the Swedish Council on Technology Assessment in Health Care —performed in Sweden on the basis of all scientific studies on tocopherols published from 1989 to 1996— that concluded that tocopherols, when taken with food, can prevent heart disease, but no such evidence was found for α-tocopherol as supplement (Saldeen and Saldeen, 2005).

Hence, consumption of seafood and aquaculture products containing significant levels of tocopherols, other vitamins, and Se may have a positive effect on the consumers' health and may contribute for the reduced morbidity and mortality (IOM, 2000).

The formulation of general dietary recommendations on seafood consumption regarding its tocopherols levels is a difficult matter, given the wide range of nutrients and other components found in the edible portion of these foods. Fish flesh presents low to moderate tocopherols concentrations, since, for instance, a 100 g serving of salmon may provide nearly 14 % of recommended dietary allowance for this vitamin (Lall and Parazo, 1995). Tocopherol concentration in the edible flesh is listed in Table 1. Data showing the vitamin content of raw fish and shellfish provide useful information. However, taking into account those factors that may alter vitamin composition before food consumption, any estimate of the vitamin intake based on those data is seldom accurate. Moreover, it must be noticed that dietary tocopherol

intake values are usually expressed in α-tocopherol equivalents to account for the biopotencies of both α- and non- α-tocopherols (Lall and Parazo, 1995).

Intake recommendations for α-tocopherol have been set by the Food and Nutrition Board (FNB) at the Institute of Medicine (IOM) of The National Academies (IOM, 2000) and are provided as Dietary Reference Intakes (DRIs). The DRIs are reference values that are used to plan and measure nutrient intakes for apparently healthy people. These values vary by life stage and gender group and include four different reference values: recommended dietary allowances (RDA – dietary intake level sufficient to meet the nutrient requirements of nearly all (97 %–98 %) healthy people); adequate intake (AI – used when the RDA can not be established and set at a level to ensure the nutritional adequacy); estimated average requirement (EAR - estimated nutrient needs of half of the individuals in a life stage and group); and tolerable upper intake level (UL - highest intake improbable to cause adverse health effects) (IOM, 2000). For the assessment of tocopherol requirements and recommending daily intake, the panel on dietary antioxidants and related compounds of FNB of IOM (2000) used the α-tocopherol level alone (IOM, 2000). The complete information about α-tocopherol DRIs is shown in Table 2. The RDA, EAR, and UL for individuals aged over 19 years have been set respectively at 15, 12 and 1000 mg (IOM, 2000). The RDA value of 15 mg must be increased when the diet is rich in unsaturated fatty acids (Weber et al., 1997).

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TABLES CAPTIONS

Table 1 – Tocopherols contents in seafood and aquaculture products (weight of tocopherol (mg)/sample weight (100 g)).[a]

Table 2 – Dietary reference intake (DRIs) values for the α-tocopherol (mg α-tocopherol/day).

Table 1 – Tocopherols contents in seafood and aquaculture products (weight of tocopherol (mg)/sample weight (100 g)).[a]

Seafood product	n	α-tocopherol	γ-tocopherol	δ-tocopherol	MDT
Wild fish					
Angler[b]	10	0.11 ± 0.14	n.d.	n.d.	n.d.
Blackbelly rosefish[b]	10	0.44 ± 0.50	n.d.	n.d.	n.d.
Black scabbardfish[b]	10	1.59 ± 2.03	n.d.	n.d.	n.d.
Megrim[b]	8	0.66 ± 0.86	n.d.	n.d.	n.d.
Pike-perch[c]	9	2.62 ± 0.292	n.d.	n.d.	n.d.
Trout[c]	8	1.80 ± 0.234	n.d.	n.d.	n.d.
Carp[c]	7	0.48 ± 0.051	n.d.	n.d.	n.d.
Sprat[c]	7	0.35 ± 0.053	n.d.	n.d.	n.d.
Mackerel[c]	8	1.59 ± 0.201	n.d.	n.d.	n.d.
Eel[d]	4	2.4	n.d.	n.d.	n.d.
Salmon[e]	4	1.69	0.00	0.00	0.22
Bogue (March)[f]	3	0.54 ± 0.11	n.d.	0.04 ± 0.03	n.d.
Bogue (September)[f]	3	0.70 ± 0.23	n.d.	0.08 ± 0.04	n.d.
Horse mackerel (March)[f]	3	0.64 ± 0.24	n.d.	0.07 ± 0.02	n.d.
Horse mackerel (September)[f]	3	0.52 ± 0.32	n.d.	0.09 ± 0.07	n.d.
Horse mackerel (December)[f]	2	0.45 ± 0.00	n.d.	n.d.	n.d.
Blue-tailed cod[e]	1	0.18	0.00	0.00	0.00
Processed fish products					
Sturgeon eggs[e]	1	0.61	0.01	0.00	0.20

Hering fillets[g]	3	0.15 ± 0.01	n.d.	n.d.	n.d.
Farmed fish					
Farmed salmon[e]	4	2.60	0.06	0.02	0.01
Farmed trout oil[h]	6	35.9 ± 2.9	n.d.	n.d.	n.d.
Molluscs					
Clam[i]	2	0.57 ± 0.13	n.d.	n.d.	n.d.
Crustaceans					
Shrimp[j]	1	17.5	42.4	7.1	n.d.
Plankton					
Phytoplankton sample[e]	---	0.03	0.00	0.00	0.01
Zooplankton sample[e]	---	0.20	0.00	0.00	0.02

[a]Data are expressed as mean±standard deviation with exception of data from Yamamoto et al., 2001.

Data from: [b]Afonso et al., 2008; [c]Ribarova et al., 2003; [d]Dias et al., 2003; [e]Yamamoto et al., 2001; [f]Orban et al., 2011; [g]Undeland et al., 1999; and [h]López et al., 1995. [i]Kuhnlein et al., 2006. [j]Monge-Rojas and Campos, 2011.

n.d. – not detected.

Table 2 – Dietary reference intake (DRIs) values for the α-tocopherol (mg α-tocopherol/day).

	DRIs			
	RDA[a]*	AI[b]*	EAR[c]*	UL[d]
Life Stage				
0 -6 mo		4		ND
6-12 mo		5		ND
1-3 y	6		5	200
4-8 y	7		6	300
9-13 y	11		9	600
14-18y	15		12	800
19- >70 y	15		12	1000
Pregnancy				
14-18 y	15		12	800
19-50 y	15		12	1000
Lactation				
14-18 y	19		16	800
19-50 y	19		16	1000

[a]RDA, recommended dietary allowance: the intake that meets the nutrient needs of almost all (97%–98%) of individuals in a group.

[b]AI, adequate intake: the observed average or experimentally determined intake by a defined population or subgroup that appears to sustain a defined nutritional status, such as a growth rate, normal circulating nutrient values, or other functional indicators of health. The AI is used if sufficient scientific evidence is not available to derive an EAR. The AI is not equivalent to an RDA.

[c]EAR, estimated average requirement: the intake that meets the estimated nutrient needs of half of the individuals in a group.

[d]UL, tolerable upper intake level.

*α-Tocopherol comprises natural *RRR*-α-tocopherol and the *2R*-stereoisomeric forms of α-tocopherol used in supplements and fortified foods (*RRR*-, *RSR*-, *RRS*-, and *RSS*-α-tocopherol) (source: IOM, 2000).

ND, not determinable.

FIGURES CAPTIONS

Figure 1 – Structures of vitamin E homologues and novel tocomonoenol (from Gotoh et al., 2011).

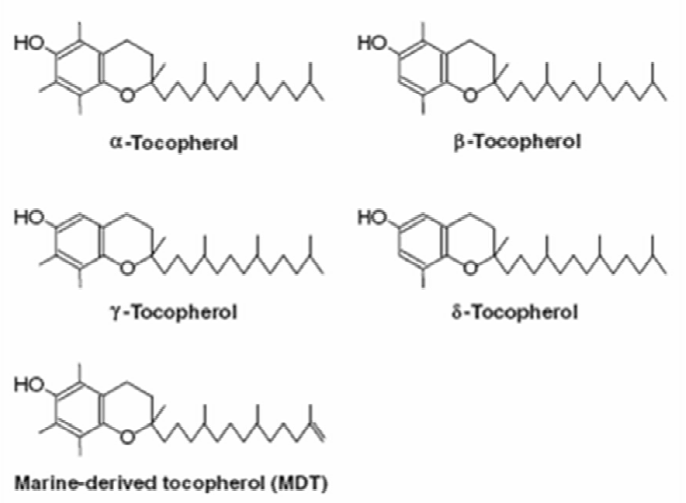

Figure 2 – Tocopherols importance in membrane permeability and fluidity (from Sen et al., 2007).