A literature review submitted in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in food science
Department of Food Science
University of Iceland

Endogenous antioxidants in fish

Margrét Bragadóttir
2001

Supervisors: Heiða Pálmadóttir M.S. and Kristberg Kristbergsson Ph.D.

LIST OF TABLES

LIST OF FIGURES

TABLE OF CONTENTS

ABSTRACT

Several types of antioxidative compounds are found in all fish species to protect their lipids against damage caused by reactive oxygen species. These compounds belong to various chemical groups and make use of their antioxidative effects via different modes of action. These include antioxidant enzymes, amino acids, peptides, ascorbic acid, carotenoids and phenolic compounds such as tocopherols and ubiquinones. The occurrence of endogenous antioxidants in fish is reviewed, including their concentration, chemical nature and antioxidative activity.

1 INTRODUCTION

Fish with its high content of unsaturated fatty acids (Ackman 1980, 1989a, 1989b; Morris and Culkin 1989) is highly susceptible towards lipid oxidation (Ackman 1988, Hardy 1980, Khayat and Schwall 1983). The resulting development of rancidity in fish leads to undesirable changes in flavor (Durnford and Shahidi 1998, Karahadian and Lindsay 1989), texture (Castell 1971, Sikorski *et al.* 1976), color (Haard 1992, Jensen *et al*. 1998, Smith and Hole 1991) and nutritional value (Opstvedt, 1971, 1975, Watanabe 1982). In fresh fish the balance between the prooxidative and antioxidative factors which control oxidative reactions is maintained by numerous systems (Hultin 1992, 1994; Undeland 1997). With processing and prolonged storage time the control of oxidation is lost and the onset of lipid oxidation can no longer be prevented (Flick *et al*. 1992). How long this will take is highly dependent on the type of handling the fish is subjected to and the level of antioxidants present in the fish tissue (Decker 1998, Petillo *et al.* 1998).

In this review, the role and mechanism of endogenous antioxidants in fish and seafoods are reviewed, including their presence, concentration and antioxidative mechanisms.

1.1 Lipid oxidation and antioxidants

Lipid oxidation has been well documented (Chan 1987, Frankel 1980, Labuza 1971, Pokorný 1987, Frankel 1991). Autoxidation is the direct reaction of molecular oxygen with organic compounds.

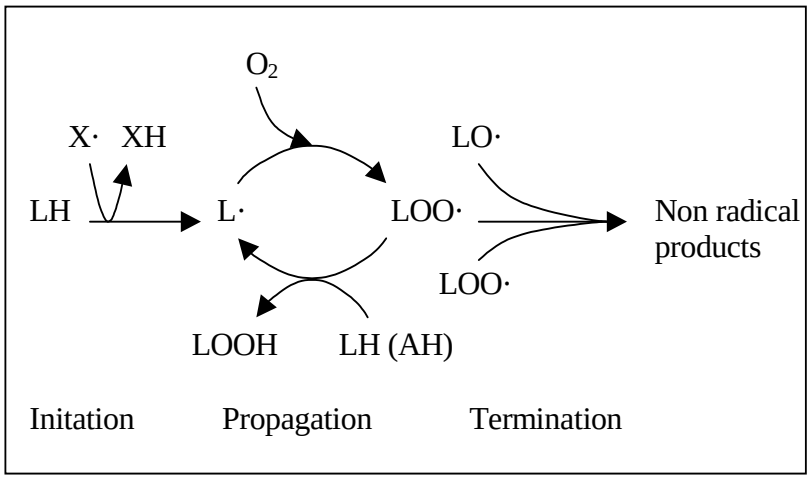


Figure 1.1. *Schematic illustration of the lipid oxidation process.*
LH = unsaturated lipid, X• = initiator, L• = alkyl radical, LO• = alkoxyl radical, LOO• = peroxy radical, LOOH = hydroperoxide, AH = antioxidant.

Autoxidation of lipids proceeds through a free radical chain mechanism involving initiation, propagation and termination steps (Figure 1.1). Lipid oxidation in fish is influenced by several catalytic systems for oxygen activation. To overcome the spin restriction between ground state oxygen and lipids, the reaction requires initiation (or initiator: X•) which may be the activation of ground state oxygen ($^3O_2$) into singlet oxygen ($^1O_2$), superoxide ($O_2^{\bullet -}$), hydroxyl radical (HOO•), or peroxides (LOOH), or transformation of unsaturated lipids into lipid radicals (L•). Under most circumstances, autoxidation starts in the presence of initiators with an extraction of H-atom from a fatty acid to produce the free radical. Subsequently the reaction proceeds through propagation reactions, which produce further free radicals. In the terminating reaction two free radicals combine to produce non-radical products.

Lipid hydroperoxides are formed in the propagation phase when lipid radicals react with oxygen. They are relatively stable, but transition metal ions (Me), such as iron and copper, as well as haem compounds catalyze their decomposition both by oxidation and by reduction;

$$LOOH + Me^{n+} \longrightarrow LO^{\bullet} + OH^- + Me^{n+1}$$

$$LOOH + Me^{n+1} \longrightarrow LOO^{\bullet} + H^+ + Me^{n+}$$

Transition metal ions are therefore important prooxidants for the initiation of lipid oxidation.

In biological tissues such as fish muscle, other components such as proteins, amino acids and ascorbate can interact with these free radicals to terminate the reaction. When components other than lipids terminate the reaction, they are often referred to as antioxidants.

The definition of an antioxidant as any substance that, when present at low concentrations compared to those of an oxidizable substrate (Halliwell and Gutterridge 1989) indicates that almost everything found in foods and in living tissues including proteins, lipids, carbohydrates and DNA is able to act as an antioxidant (Halliwell *et al*. 1995).

The antioxidant systems in living organisms may be divided in to two types. One is represented by enzymes, such as superoxide dismutase, catalase and the peroxidases, which remove reactive oxygen species. The other group of antioxidative compounds scavenges free radicals, they are generally of low molecular weight and may be water- or lipid soluble. These antioxidants reduce free radicals and are themselves oxidized. After that they may be reduced to their active forms by other reducing systems.

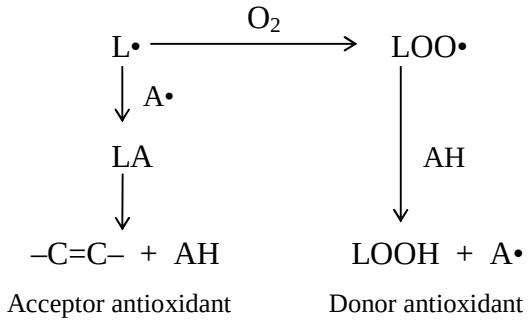
In biological systems the initiation of oxidation is balanced by the presence of natural antioxidants. Lipid-soluble antioxidants donate a hydrogen atom (H) to a fatty acid based free radical more readily than does an unoxidised fatty acid. Water-soluble antioxidants interacting between the aqueous and lipid phases can then reduce the lipid soluble antioxidant so they can continue to participate in these antioxidative reactions. The ability of an antioxidant to reduce another antioxidant or a lipid derived radical is determined by their reduction potentials (Buettner and Jurkiewicz 1996). In post mortem muscle tissue the ability to keep the antioxidants in the reduced state diminishes with time because of the loss of reducing compounds, the ability to stabilize lipid free radical is lost, and the lipids will eventually oxidize.

Antioxidants can act at different stages in the oxidation process and some may have more than one mechanism of action. Their mode of action can be classified into the following categories (Symons Gutteridge 1998):

- Removing oxygen or decreasing local O_2 concentrations.
- Removing catalytic metal ions.
- Removing reactive oxygen species such as $O_2\bullet^-$ and H_2O_2.
- Scavenging initiating radicals such as •OH, LO• and LOO•.

- Breaking the chain of an initiated sequence.
- Quenching or scavenging singlet oxygen.

Thus antioxidants may directly or indirectly inhibit the initiation and propagation steps of lipid oxidation. They sometimes have multiple effects and their mechanisms of action are therefore often difficult to interpret. According to their mode of action, antioxidants can be categorized into preventive inhibitors and true antioxidants. Preventive inhibitors are those which interfere with the lipid oxidation initiation step, for example with various prooxidants. Preventive inhibitors are divided into primary and secondary inhibitors. Primary inhibitors remove active reduction products of oxygen or convert transition metals to inactive forms. Secondary initiation inhibitors can affect the production of the primary catalysts of lipid oxidation. True antioxidants (AH), also called chain breaking antioxidants interfere with the propagation step of lipid oxidation by reacting with the lipid derived radicals. Chain breaking antioxidants can be further sub-divided into hydrogen or electron donors to peroxyl or hydroxyl radicals and hydrogen or electron acceptors from carbon-centered radicals (Scott 1997). The first class comprises phenol antioxidants and the second the stable phenoxyl radicals and quinonoid compounds (Scott 1997):



The most common food antioxidants are hydrogen donors, but inhibition by electron acceptors can become important in biological tissues since the oxygen pressure is much lower in healthy tissues than in the atmosphere (Gordon 1990).

The key reactions of chain-breaking antioxidants can be listed as:

$$LOO\bullet + AH\bullet \rightarrow LOOH + A\bullet$$

$$LO\bullet + AH \leftrightarrow LOH + A\bullet$$

$$A\bullet + LOO\bullet \rightarrow \text{Non-radical products}$$

$$A\bullet + A\bullet \rightarrow \text{Non-radical products}$$

A molecule will be able to act as a primary antioxidant if it is able to donate a hydrogen atom rapidly to a lipid radical and if the radical derived from the antioxidant is more stable than the lipid radical, or is converted to other stable products. The antioxidant radical (A•) formed from phenolic compounds is stabilised by delocalization of the unpaired electron around its aromatic ring and further increased by substituents at the *ortho* and *para* positions (Frankel 1998a).

Primary antioxidants can become less active at high concentration because of their tendency to act as chain-carriers and become prooxidants (Gordon 1990):

$$AH + O_2 \rightarrow A\bullet + HOO\bullet$$
$$AH + LOOH \rightarrow A\bullet + LO\bullet + H_2O_2$$

In multi-components systems, antioxidant compounds can reinforce each other by cooperative effects known as synergism (Frankel 1998b). Significant synergism is generally observed when chain-breaking antioxidants are used together with preventive antioxidants or peroxide destroyers because they suppress both initiation and propagation reactions of free radicals.

1.2 Fish as source of antioxidants

There are several natural compounds that participate in the antioxidative defense mechanism of fish (Hultin 1992, 1994; Undeland 1997). These include enzymes (catalase, peroxidase, glutathione and superoxide dismutase), carotenoids, peptides, amino acids and phenolic compounds (tocopherols, ubiquinones). These compounds are found in the cell plasma, mitochondria of cell membranes.

Table 1.1. Types of lipid oxidation inhibitors in fish.

Type of inhibitor	Mode of action	Chemical compound
Antioxidants	Propagation inhibitors, react with free radicals	Tocopherols, ubiquinone, carotenoids, ascorbic acid
Synergists	Reinforce activity of antioxidants	Tocopherol, amino acids, peptides, ascorbic acid, phospholipids
Retarders	Reduce hydroperoxides	Catalase, peroxidases, amines
Metal scavengers	Inhibit metals to catalyze production of free radicals	Amino acids, peptides, phospholipids, ascorbic acid
Singlet oxygen quenchers	Inactivates singlet oxygen	Superoxide dismutase, ascorbic acid, carotenoids

Types of lipid oxidation inhibitors found in fish is summarized in Table 1.1, including their modes of activity and chemical nature.

2 ENZYMES

Living cells possess very active enzyme defense mechanisms against reactive oxygen species, such as $O_2\bullet^-$ and H_2O_2. This includes catalase, glutathione peroxidase and superoxide dismutase. The level of enzyme activity varies with species and muscle type (Decker and Xu 1998).

2.1 Catalase

Catalase, which is a metal-containing enzyme, is the most efficient enzyme that promotes redox reaction:

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

Hydrogen peroxide itself is not particularly reactive with most biologically important molecules, however, it may acts as a precursor for more reactive oxidants such as •OH.

2.2 Superoxide Dismutase

Superoxide Dismutase (SOD) is a metalloenzyme acting as primary preventive inhibitor by catalyzing the conversion of superoxide anion ($O_2\bullet^-$) to H_2O_2 and O_2 (Symons and Gutteridge 1998). The content of antioxidant enzymes (superoxide dismutase and catalase) in elasmobranchs (shark and ray) is lower than in teleosts and seems to follow the overall metabolic oxygen consumption and oxidative activity of each major fish taxonomic group (Haard 2000).

2.3 Glutathion peroxidase

Glutathion peroxidase (GSH-px) is another propagation inhibitor in the aqueous phase of fish muscle that is located in the mitochondria and cytosol of skeletal muscle cells. GSH-px is a selenium-containing enzyme (Symons and Gutteridge 1998) that

catalyses the reduction of hydrogen of lipid peroxides (LOOH) with reduced glutathione (GSH).

$$2GSH + H_2O_2 \rightarrow 2H_2O + GSSG$$

GSSG is then reduced back to GSH by glutathione reductase at the expense of NADPH.

$$GSSG + NADPH + H^+ \rightarrow 2GSH + NADP^+$$

Glutathione peroxidase differs form catalase in that it is capable of reacting with both lipid and hydrogen peroxides (Halliwell and Gutterridge 1989). The specific activity of GSH-px in fish ranges form 7.7 to 65 nmol/min/mg protein as reported by Nakano and coworkers (1997). Selenium, which is required for glutathione peroxidase activity, is obtained from the diet and glutathione peroxidase activity can be enhanced in chicken muscle by increasing the level of selenium in the diet (DeVore *et al*. 1983). Same authors found conversely relationship between glutathione peroxidase activity and lipid oxidation in post mortem chicken muscle. GSH-px is the only antioxidant enzyme in muscle foods, which has been reported to be influenced by animal diet (Decker and Xu 1998). Antioxidative enzymes have little significance in food application and they are currently not of commercial significance in the food industry (Meyer and Isaksen 1995).

3 ASCORBIC ACID

3.1 Chemistry of ascorbic acid

Vitamin C or L-ascorbic acid is chemically simple, although its ene-diol structure provides it with a highly complex chemistry, which has been reviewed by Buettner and Jurkiewicz (1996).

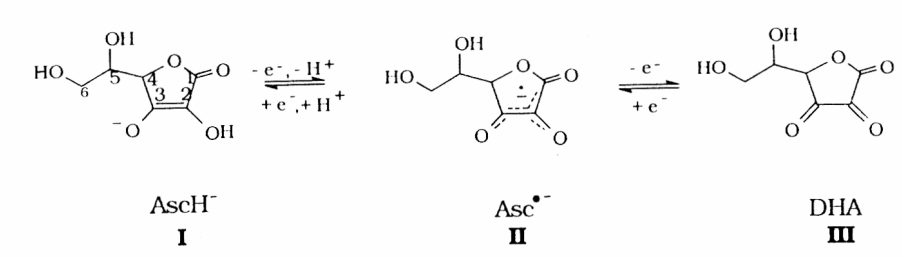


Figure 3.1. Structure and redox reactions of ascorbate.

Vitamin C is water-soluble and is present in its deprotonated state as ascorbate under most physiologic conditions (Sies and Stahl 1995). It has a redox chemistry with radical intermediates and it is modified by the acidic properties of the molecule. The chemical structure of ascorbate is α-keto lactone with a five-membered ring (Figure 3.1). Ascorbate (I) is an excellent reducing agent. The loss of an electron by ascorbate gives the stable semidehydroascorbate radical (II) due to the delocalized unpaired electron, which can be further oxidized to give dehydroascorbate (III). Ascorbic acid is a diacid. The dissociation constants are $pK_1 = 4.17$ (at the 3-OH) and $pK_2 = 11.57$ (at the 2-OH).

3.2 Function of ascorbic acid

The history of vitamin C is associated with the prevention of scurvy and it was named ascorbic acid due to its antiscorbutic properties. Scurvy is caused by deficiency of ascorbate from the human diet. Ascorbic acid is required as a cofactor for proline hydroxylase and lysine hydroxylase, involved in the biosynthesis of collagen. Collagen synthesized in the absence of ascorbic acid is insufficiently hydroxylated and does not form fibers properly, giving rise to the scurvy symptoms, which are poor wound-healing and fragility of blood vessels (Halliwell and Gutterridge 1989). Nearly all species of animals synthesize vitamin C and do not require it in their diets. However, humans, other primates, some birds and fish such as Coho salmon, rainbow trout, and carp cannot synthesize the vitamin because of the loss of a liver enzyme, L-glucono-γ-lactone oxidase, during the course of evolution (Deshpande *et al*. 1996). Ascorbate is required as a cofactor for several enzymes and for the action of the copper enzyme dopamine-β-hydroxylase which converts dopamine into noradrenalin (Halliwell and Gutterridge 1989). Ascorbic acid is an essential factor in several hydroxylation reactions, primarily because of its ability to act as a redox couple, but most physiological functions of ascorbic acid are related to its ability to act as an electron donor (Deshpande *et al*. 1996).

3.3 Occurrence of ascorbic acid

Vitamin C is found through out the plant and animal kingdom and its occurrence in all living tissues, both animal and vegetable gives an indication of its vital biological role

(Kläui and Pongracz 1981). Fish muscle is generally low in Vitamin C content and the levels may be considered as negligible (Nettleton and Exler 1992). Levels of ascorbic acid in the edible portion of fish and fish products have been reported by Gordon and Martin (1982). The ascorbic acid found in white fish muscle averaged 0.33 ± 0.19 mg/100 g with slightly more in oysters and 1.7 mg/100 g in salmon. Sidwell and coworkers (1978) reported somewhat higher values; 7.1 ± 1.9 in salmon, 1.1 ± 1.1 in trout, 2.6 ± 1.2 in tuna, 1.5 ± 0.6 in shrimp and prawns, and 9.0 ± 3.8 mg/100 g in herring. Nettleton and Exler (1992) measured ascorbic acid in edible portion of wild and cultivated channel catfish, coho salmon, rainbow trout, red swamp crayfish and White River crayfish. The vitamin C content of these fish was generally below 1 mg/100 g, except for rainbow trout with 1.8 mg/100 g in wild and 2.9 mg/100 g in cultivated fish, and eastern oysters with 3.1 in wild and 3.8 mg/100 g in cultivated oysters. Cultivated fish did not in general contain higher amounts of vitamin C.

Nettleton and Exler (1992) did also determine the vitamin C content in the relevant fish species after cooking. Vitamin C values for all cooked samples were greater than those in raw samples. The authors proposed that these findings were due to moisture loss during cooking and that vitamin C was well retained after cooking. Other processing of fish like hot smoking have also been found to have little effect on vitamin C in fish. Thus Bhuiyan and coworkers (1993) found 4.7 mg/100 g of ascorbic acid in fresh and 4.5 mg/100 g in hot-smoked fillets of Atlantic fall mackerel.

Storage time will probably affect the content of vitamin C in fish. Brannan and Erickson (1996) measured the effect of frozen storage on the stability of ascorbic acid in fillets and mince of channel catfish (*Ictalurus punctatus*). The initial ascorbic acid concentration in fillets and mince was around 1.47 mg/100 g and decreased by a first order regression equation for both fillets and mince during frozen storage at -6°C. The loss of vitamin C occurred at a faster rate in the mince, especially through the first four months. The ascorbic acid values for the fillets and mince at the end of the storage experiment (6 months) were 1.19 and 0.63 mg/100 g, respectively. Large losses of ascorbic acid in the initial phases of accelerated frozen storage (temperature fluctuations) have also been reported in minced muscle of cultivated channel catfish (*Ictalurus punctatus*) (Erickson 1993a) and striped bass (*Morone saxatilis*) (Erickson 1993b).

3.4 Antioxidant activity ascorbic acid

Ascorbate is readily oxidized, but the rate is dependent upon pH and the presence of catalytic metals. Ascorbic acid solutions are quite stable at low pH, but as the pH is raised above pK_1, $AscH^-$ becomes dominant and the stability of the solution decreases, usually as the result of the presence of catalytic transition metal ions (Buttner and Jurkiewicz 1996). However, even in absence of metals, the rate of oxidation increases. Consequently ascorbate undergoes true autoxidation:

$$Asc^{2-} + O_2 \rightarrow Asc^{\bullet -} + O_2^{\bullet -}$$

Ascorbate functions as metal chelator and oxygen scavenger. Approximately 3.5 mg of ascorbic acid will scavenge the oxygen contained in a 1 cm^3 headspace (Kitts 1997). Furthermore ascorbate is effective in trapping both singlet oxygen and superoxide (Kitts 1997).

Unlike the oxidation-reduction reactions in which ascorbate donates two electrons, the antioxidant reactions use its ability to donate a single electron to free-radical species (Deshpande 1996). According to Buettner and Jurkiewicz (1996) the low reduction potential of ascorbate ($AscH^-$) enables it to repair oxidizing free radicals with greater reduction potential (Table 3.1), including •OH, RO•, ROO•, GS•, urate and tocopheroxyl radical (TO•):

$$AscH^- + X\bullet \rightarrow Asc\bullet^- + XH$$

Table 3.1. Standard reduction potentials for selected compounds.

Reaction	E^θ (V)
$\bullet OH + e^- + H^+ \rightarrow H_2O$	2.31
$RO\bullet + e^- + H^+ \rightarrow ROH$ (aliphatic alkoxyl radical)	1.60
$ROO\bullet + e^- + H^+ \rightarrow ROOH$ (alkyl peroxyl radical)	1.00
$GS\bullet + e^- \rightarrow GS^-$ (glutathione)	0.92
$O_2^{\bullet -}\ e^- + 2H^+ \rightarrow H_2O_2$	0.94
$PUFA\bullet + e^- + H^+ \rightarrow$ PUFA-H (bis-allylic-H)	0.60
$HU^{\bullet -} + e^- + H^+ \rightarrow UH_2^-$ (urate)	0.59
$TO\bullet + e^- + H^+ \rightarrow TOH$ (tocopherol)	0.48
$H_2O_2 + e^- + H^+ \rightarrow H_2O + HO\bullet$	0.32
$Asc^{\bullet -} + e^- + H^+ \rightarrow Asc^-$ (ascorbate)	0.28
$Q\bullet + e^- + H^+ \rightarrow QH$	0.24
$O_2 + e^- \rightarrow O_2^{\bullet -}$	-0.33
$O_2 + e^- + H^+ \rightarrow HOO\bullet$	-0.46

Source: Adapted from Buettner (1993), Buettner and Jukiewicz (1996) and Kagan (1996).

Where X• is any of these oxidizing free radicals. Asc•- does not react by an addition reaction with O_2, but can be recycled by reduction back to ascorbate by enzyme systems (Buettner and Jurkiewicz 1996).

3.5 Recycling of other antioxidants by ascorbic acid

According to Buettner and Jurkiewicz (1996) ascorbate is capable of repairing tocopheroxyl radical due to the lower reduction potential of ascorbate. The fact that ascorbate is water soluble, while tocopherol is lipid soluble would suggest that in a biological system there would be little interaction between these species. It has, however, been well established in liposomes and experimental membrane preparations that the antioxidant active phenol group of tocopherol appears to be located at the water/membrane interface (Buettner and Jurkiewicz 1996). This physical arrangement allows the reaction between the tocopheroxyl free radical, TO• and $AscH^-$:

$$AscH^- + TO\bullet \rightarrow Asc^{\bullet-} + TOH$$

Thus the reaction of TO• with ascorbate allows the export of oxidative free radicals from the membrane in such way that TOH donates hydrogen to lipid peroxyl radicals, while ascorbate recycles tocopherol.

3.6 Prooxidant activity of ascorbic acid

Although ascorbate is considered to be the terminal small-molecule antioxidant in biological systems, it may act in a prooxidant manner due to its excellent reducing ability (Buettner and Jurkiewicz 1996). Ascorbate is able to reduce catalytic metals such as Fe^{3+} and Cu^{2+} to their more catalytically active valence state Fe^{2+} and Cu^{+}. In general low concentrations of ascorbate are required for prooxidant conditions, while high concentration is needed for antioxidant conditions. In the presence of ascorbate, catalytic metals will initiate radical chain oxidations, but when ascorbate concentration is high these radical processes will be less significant (Buettner and Jurkiewicz 1996).

3.7 Lipid oxidation and ascorbic acid

Ascorbic acid is noted for its complex multi-functional effects. Depending on condition ascorbic acid can act as an antioxidant, a pro-oxidant, a metal chelator, a reducing agent or as an oxygen scavenger (Frankel 1996).

Studies on the stability of ascorbic acid in fish and fish products compared to oxidative stability have been done. Erickson (1993a) studied ascorbic acid degradation in two strains (AQUA and LSU) of minced frozen Channel catfish under accelerated conditions (temperature fluctuations). Both strains contained similar amounts of α-tocopherol, but the LSU strain contained nearly 1.4 times more ascorbic acid than the AQUA strain. By nine months of storage, neither strain contained detectable amounts of ascorbic acid. Lipid oxidation measured as TBA values increased evenly until after 6 months storage, when a sharp rise was observed. Lipid oxidation was more pronounced in the AQUA strain with the lower initial ascorbic acid level.

Undeland (1998a) studied the lipid oxidation in herring fillets by measuring peroxide value and decline in ascorbate during 15 days storage in ice. The decrease in ascorbate was most rapid during the first 9 days of storage, where 61% of the ascorbic acid disappeared. At the end of storage a total decline of 75% was observed. High correlation between peroxide value and ascorbic acid results ($r^2 = 0.87$) indicated that ascorbic acid is a rather good indicator of lipid oxidation in herring fillets during ice storage.

Petillo and coworkers (1998) measured the kinetics of antioxidant loss of mackerel during 11 days storage in ice. The loss of ascorbate in light muscle was more rapid than the losses of the lipid soluble antioxidants (α-tocopherol and ubiquinone-10), but similar to glutathione loss. Ascorbate and glutathione gave the best correlation with sensory scores for light mackerel muscle and also along with ubiquinol in dark mackerel muscle.

Simulating effects of lipid oxidation by ascorbate in mackerel muscle have also been reported (Decker and Hultin 1990). It was suggested that ascorbate is capable of reducing ferric ions which results in a catalysis of lipid oxidation.

The influence of ascorbic acid addition was determined in a study with fish oil/lecithin/water system (Han *et al*. 1997). The individual addition of ascorbic acid

(0.04%) and δ-tocopherol (0.2%) increased the length of the induction period of fish oil from 4.4 h to 11.3 and 8.5 hours, respectively. When these antioxidants were combined they acted synergistically as the induction period increased to 40 h.

4 AMINES, AMINO ACIDS AND PEPTIDES

4.1 Chemistry of amino acids, amines and peptides

Nitrogenous constituents not classified as proteins comprise between 0.5 and 1% of total weight of the fish muscle (Spinelli 1982). Over 95% of the non-protein muscle constituents of fish and shellfish consist of amino acids, imidazole dipeptides, guanidine compounds, trimethylamine oxide (TMAO), urea, betaines, nucleotides and compounds related to nucleotides (Ikeda 1980).

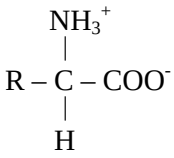


Figure 4.1. General structure of amino acids.

Amino acids contain basic amino groups, acidic carboxyl groups and a side chain (R) (Figure 4.1). The side chains influence the chemical properties of amino acids and proteins. The types of side chains may be classified into four groups; polar-uncharged are generally soluble in aqueous solutions, nonpolar are less soluble in aqueous solvents, positively charged and finally, negatively charged dicarboxylic amino acids (Anglemier and Montgomery 1976). Glycine, serine, threonine, cysteine, cystine, tyrosine, aspargine and glutamine are amino acids with polar-uncharged side chains. Alanine, valine, leucine, isoleucine, phenylalanine, tryptophan and methionine have nonpolar side chain. Aspartic acid and glutamic acid have negatively charged side chains, whereas lysine, arginine and histidine have positively charged side chains (Anglemier and Montgomery 1976).

Many of these compounds as well as their degradation products have been related to the flavor of fish. TMAO (Figure 4.2) can be degraded to trimethylamine (TMA), dimethylamine (DMA) and formaldehyde (FA) and the contents of these compounds have been used as spoilage indices in fish (Regenstein *et al.* 1982).

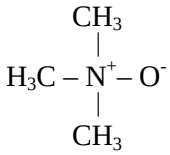


Figure 4.2. Trimethylamine oxide.

In frozen fish where bacterial activity is reduced, DMA and FA are formed both enzymatically and nonenzymatically by the breakdown of TMAO (Hultin 1992a).

Carnosine, anserine and ophidine are one of a limited number of peptides that have been identified in the extracts of fish. They are histidine containing dipeptides of similar structure (Figure 4.3).

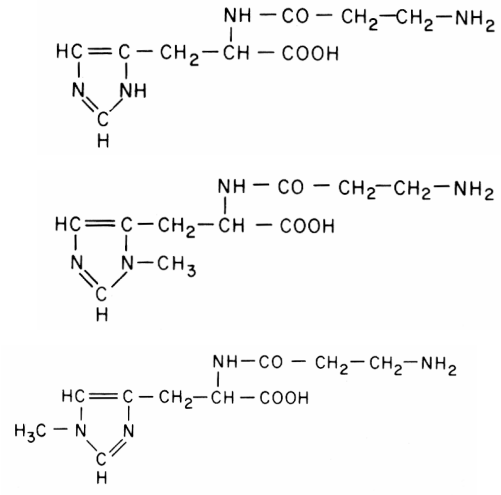


Figure 4.3. Carnosine (β-alanyl-L-histidine), anserine (β-alanyl-L-1-methylhistidine) and ophidine (β-alanyl-L-3-methylhistidine).

Other amino compounds considered with antioxidative properties in fish are glutathione, which is a tripeptide (γ-Glu-Cys-Gly), the polyamines, putrescine, spermidine and spermine (Figure 4.4) and uric acid (Figure 4.5) (Decker and Xu 1998).

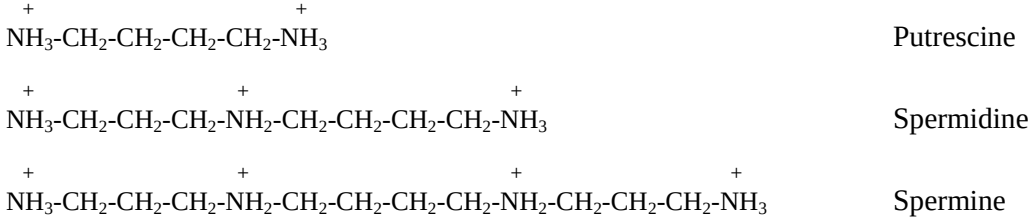


Figure 4.4. Polyamines.

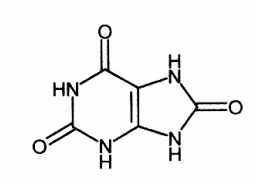


Figure 4.5. Uric acid.

4.2 Function of amino acids, amines and peptides

Free amino acids in fish muscle have been suggested to function in the regulation of osmotic pressure, as their concentrations were higher in marine species than in their freshwater counterparts and highest in crustacean muscle (Spurvey *et al*. 1998). Some amino acids, such as *β*-alanine are abundant in fishes living in waters of low temperature (Konosu and Yamaguchi 1992).

Amines are formed during normal metabolic processes in all living organisms, and certain amines fulfill a number of biological roles (Bardócz 1995). TMAO is also reported to be present in high levels in fish living in cold water (Shibamoto and Horiuchi 1997). Apparently TMAO is a part of the body's normal buffer system and also a part of the system used for osmoregulation of marine fish (Regenstein 1982).

The role of carnosine and anserine is also unceratain, but they have been suggested to act as buffers. Because pK_a of the imidazole ring of carnosine and anserine are 6.83 and 7.04, respectively, they exhibit excellent buffering capacity at physiological pH values (Chan and Decker 1994).

The role of polyamines is in cellular metabolism and in the synthesis of protein, RNA and DNA (Bardócz 1995). All organs of the body require polyamines for growth, renewal and metabolism (Anon 1995).

Glutathione is a hydrogen donor for the enzyme glutathione peroxidase, which catalyses the reduction of hydrogen peroxide and organic hydroperoxides. Glutathione is also a cofactor for several enzymes in widely different metabolic pathways (Halliwell and Gutterridge 1989).

Uric acid is a by-product of purine metabolism and has usually been thought to be a waste product with no biological function (Deshpande *et al*. 1996). More recently uric acid has become increasingly recognized as an important biological antioxidant (Halliwell 1996).

4.3 Occurrence of amino acids, amines and peptides

Fish and shellfish are high in nitrogen compounds compared to other foods, ranging from 10% to 40% (Venugopal and Shahidi 1996). The concentration and composition of the non-protein nitrogen fraction are dependent on the species, season, spawning migration, environmental conditions, diets, freshness, tissue parts etc. (Finne 1992). Free amino acid content in muscle of some fish and seafood is listed in Table 4.1. Free amino acids in relatively high amounts in fish are glycine, taurine, alanine and lysine. Shellfish contain larger amounts of glutamic acid, glycine and alanine than finfish. Migratory fishes such as mackerel and tuna contain high amounts of histamine and white-fleshed fishes are high in taurine content (Konosu and Yamaguchi 1982). The free amino acid content of fish is relatively low when compared to shellfish.

Carnosine, anserine and ophidine, also known as balerine are abundant peptides in fish and shellfish. Dark-fleshed fish, including eel, tuna and skipjack, contain considerable amounts of carnosine, while other species of fish may have very small amounts (Ikeda 1980). Anserine is abundant in tuna, skipjack and some species of shark (Konosu and Yamaguchi 1982) and dark-fleshed fish (Ikeda 1980). Carnosine and anserine contents of some fishes are listed in Table 4.2.

Table 4.1. Content of free amino acids in muscle of some fish and seafoods (mg/100 g)

Amino acid	Flounder	Mackerel	Yellowfin tuna	Shark	Abalone	Crab	Prawn	Krill
Asp	-	-	1	7	9	15	-	52
Thr	4	11	3	7	82	30	13	54
Ser	3	6	2	10	95	158	133	43
Glu	6	18	3	12	109	62	34	35
Pro	1	26	2	7	83	239	203	217
Gly	5	7	3	21	174	190	1222	116
Ala	13	26	7	19	98	173	43	106
Val	1	16	7	7	37	20	17	63
Met	1	2	3	6	13	9	12	34
Ile	1	7	3	5	18	17	9	48
Leu	1	14	7	8	24	33	13	86
Tyr	1	7	2	5	57	20	20	48
Phe	1	4	2	4	26	13	7	53
Try	-	-	-	-	20	-	-	-
Lys	17	93	35	3	76	18	52	145
His	1	676	1220	8	23	16	16	17
Arg	3	11	1	6	299	397	902	266
Tau	171	84	26	44	946	50	150	206

Adapted from Ikeda (1982).

Table 4.2. Carnosine and anserine content of some fish and seafood.

Fish	Carnosine (mg/100 g tissue)	Anserine (mg/100 g tissue)	Reference
Yellow fin tuna	55	234	Ikeda 1980
Skipjack	252	559	-
Horse mackerel	0	2.6	-
Swordfish	130	370	-
Sea perch	0	-	-
Flat fish	0	-	-
Eel	542	-	-
Chum salmon	4-8	-	Sikorski *et al*. 1990

Balerine has been detected only in small quantities in a limited number of species of tuna and sharks, but it makes up the major part of the extractive components of baleen whales, amounting to more than 1500 mg (Konosu and Yamaguchi 1982).

Trimethylamine oxide (TMAO) exists in a large number of fish and shellfish, but is negligible in freshwater fish (Hebard *et al*. 1982). The highest values were reported in elasmobranch tissues (500-1500 mg/100 g), followed by squid and gadoid muscle (Ikeda 1980). In freshly caught gadoids such as cod and haddock, TMAO amount to 350 mg/100 g (Venugopal and Shahidi 1996). Some seaweeds have been reported to contain high levels of TMAO and there is a possibility of direct incorporation of TMAO by aquatic animals feeding on seaweed (Ikeda 1980). White fleshed fish generally contain larger quantities of TMAO than dark-fleshed (pelagic) fish (Hebard 1982). TMAO level in fish varies with the season, size and age of fish, as

well as environmental conditions to which the animal is subjected (Hebard *et al*. 1982). In addition white-fleshed fishes have higher TMAO in ordinary muscle than in the dark muscle, but the opposite is true for dark-fleshed fish such as sardine, mackerel, tuna and skipjack (Tokunaga 1970).

Polyamines are commonly found in foods (Bardócz 1995) and spermine and putrescine occur naturally in salmon spermary tissue (Sasaki *et al*. 1996). Fish has more putrescine than either spermidine or spermine (Bardócz 1995). All types of food contain putrescine and spermidine, which are the most common compounds, whereas spermine, agmatine and cadaverine may also occur naturally, and are not necessarily the result of bacterial contamination (Bardócz 1995). Dietary polyamines at levels normally present in foods are non-toxic, while biogenic amines, particularly histamine, are toxic at high intake levels (Bardócz 1995). Tuna and mackerel are

known for their risk of histamine production during spoilage (Ritchie and Mackie 1980).

Reported contents of glutathione in fish are 4 mg in carp and 13-20 mg in salmon (Konosu and Yamaguchi 1982). Petillo and coworkers (1998) found higher glutathione content in light muscle of mackerel compared to dark muscle.

4.4 Antioxidant activity of amino acids, amines and peptides

Amines, peptides and amino acids are known to have significant antioxidant properties. In general, they function as synergists or primary antioxidants. Amino acids, peptides and nucleotides are believed to be important metal chelators present in fish (Hultin 1992b).

Most of the amino acids have antioxidant properties depending on the pH of the medium and their concentration. Marcuse (1962) found that most amino acids had a significant antioxidative potential even in the absence of primary antioxidants in linoleic acid model system. Amino acids are also known to exert synergistic effect with antioxidants. Various amino acids have been shown to make covalent attachment to Trolox-C, a synthetic derivative of α-tocopherol, to produce Troloxyl-amino acids with higher antioxidant activities than Trolox-C alone (Taylor *et al*. 1981).

Amino acids are also suggested to have antioxidant properties as reaction products with carbonyls from oxidizing lipids. Various studies have shown results that suggests that reactions between oxidized lipids and amino acids produce many nonenzymic browning reaction compounds, which exert antioxidative properties (Alaiz *et al*. 1995, Alaiz *et al*. 1996, Ishtiaque *et al*. 1996). The reaction of sugars and carbonyl compounds with amino acids or proteins are known as Maillard reaction or non-enzymatic browning. Their antioxidant properties are attributed to the formation of reductone (enaminone) structures that have both reducing and metal complexing properties (Frankel 1998b). Furthermore the basic amino acids (histidine, lysine and arginine) are known to produce the most effective antioxidant products with sugars (Frankel 1998b).

A range of protein hydrolyzates of fish have been found to exhibit antioxidant activities. A polar fraction of krill extract, which was identified as a mixture of 13-20 free amino acids, was reported as having strong antioxidant activity (Seher and

Löschner 1985). Capelin protein hydrolyzates exhibited a significant antioxidant effect in β-carotene-linoleate emulsion, while seal protein hydrolyzate displayed a significant prooxidative effect at high concentration and antioxidative effect at low concentration (Shahidi and Amarowicz 1996).

Carnosine is capable of chelating copper but ineffective at inhibiting iron catalyzed oxidation of ascorbate (Chan and Decker 1997). Both carnosine and anserine have been found to scavenge hydroxyl radicals and quench singlet oxygen (Chan and Decker 1997). Carnosine has also been shown to quench unsaturated aldehydic oxidation products and is therefore suggested to decrease the toxicity of lipid oxidation products in muscle foods (Zhou and Decker 1999). The antioxidant activity of carnosine and anserine is proposed to involve both the amino acid composition and the peptide linkage between *β*-Ala and His, whereas neither imidazole group methylation nor free carboxyl-group amidation seems to interfere greatly with the antioxidant activity (Can and Decker 1997).

Trimethylamine oxide is best known for its synergistic effect with tocopherol in fats and oils. Ishikawa and coworkers (1978) postulated a synergistic mechanism between tocopherol dimer and TMAO. The reducing tocopherol dimer is regenerated by TMAO-H, which is an active intermediate from the reaction of TMAO with peroxide. Yuki and coworkers (1973) found that antioxidative activity of natural tocopherols and butylated hydroxyanisole was promoted remarkably by addition of TMAO, using lard and methyl esters of linseed oil under various oxidative conditions. TMAO alone had no antioxidative activity. Little is known about the antioxidant role of TMAO fish muscle. The formation of formaldehyde and DMA due to breakdown of TMAO is believed to be one of the mechanisms operative in protein denaturation in frozen fish tissue (Hultin 1992a). TMAO may be degraded by alternative unknown pathways, as it has been shown to degrade faster than the production of DMA and/or TMA in frozen gadoids at temperatures below -12°C (Sotelo *et al*. 1995) and in the presence of oxidized lipids (Joly *et al*. 1997).

Glutathione (GSH) functions as an antioxidant in tissue by inactivating free radicals and by providing a source of electrons which allows glutathione peroxidase to enzymically decompose hydrogen and lipid peroxides (Decker and Xu 1998).

$$2H_2O_2 + 2GSH \xrightarrow{\text{glutathione peroxidase}} GSSG + H_2O$$

Two molecules of GSH join together as the -SH groups of cysteine are oxidized to form a disulfide bridge, -S-S-. Oxidized glutathione, GSSG is reduced back to GSH by glutathione reductase to maintain a ratio of GSH/GSSG greater than 10 in normal cells (1998a). In fish muscle glutathione seems to protect against quality loss in the early stages of storage and it has been found to decrease faster during storage than other endogenous antioxidants in fish (Brannan and Erickson 1996, Petillo *et al*. 1998).

The polyamines, putrescine, spermidine, and spermine inhibit lipid oxidation by free-radical inactivation and inhibition of iron catalyzed reactions (Decker and Xu 1998). Antioxidant activity of the polyamines increases with increasing number of amine groups; spermine > spermidine > putrescine (Løvaas 1991).

Uric acid inhibits oxidative reaction by quenching and/or scavenging singlet oxygen, iron chelation and free radical scavenging (Halliwell 1996). Little is known about the antioxidant role of polyamines and uric acid in skeletal muscle (Decker and Xu 1998).

4.5 Prooxidant activity of amino acids, amines and peptides

Amino acids may serve both as catalysts and antioxidants, depending on their concentration, surrounding pH and other factors. Marcuse (1962) found that most amino acids had a significant antioxidative potential in linoleic acid and methyl esters of linoleic acid. However, cysteine was a notable exception and was found to be normally a prooxidant. Marcuse suggested that there might be a tendency towards a prooxidative inversion with relatively high amino acid concentrations, or at low pH.

Uric acid and glutathione are not always perfect antioxidants. Both uric acid and glutathione may form radicals through reactions with •OH, which might cause some biological problems. GSH reaction with superoxide radical can also lead to formation of singlet oxygen (Halliwell and Gutterridge 1989).

5 TOCOPHEROLS

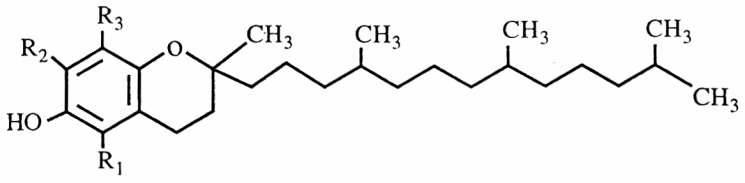
5.1 Chemistry of tocopherols

The chemistry of tocopherols and tocotrienols has been reviewed extensively (Schuler 1990, Madhavi *et al.* 1996, Niki 1996). The term vitamin E is used to describe all

tocol and tocotrienol derivatives that exhibit the biological activity of α-tocopherol. α-Tocopherol is one of the eight forms, or homologes of vitamin E. All of these have 6-chromanol ring structure with different number of methyl groups and a phytol side chain (Figure 5.1) (Madhavi *et al*. 1996). Based on the structure of the side chain, these compounds can be further classified as tocopherols (or tocols) and tocotrienols, containing an unsaturated side chain with double bonds at the 3´-, 7´- and 11´-positions.

5.2 Function of tocopherols

Vitamin E has long been known to be an essential nutritional factor for animals. A lack of vitamin E causes wide variety of symptoms including sterility in animals (Deshpande *et al*. 1996). In humans, vitamin E-deficient diets do not lead to a characteristic disease or disorder, however, recent research suggests that vitamin E deficiency is associated with an elevated risk of atherosclerosis and other degenerative diseases (Bramley *et al.* 2000). Vitamin deficiency in fish have also been reported (Watanabe 1982).



R_1	R_2	R_3	
CH_3	CH_3	CH_3	α-tocopherol
CH_3	H	CH_3	β-tocopherol
H	CH_3	CH_3	γ-tocopherol
H	H	CH_3	δ-tocopherol

Figure 5.1. Tocopherols.

Low content of tissue tocopherol was found in carp with "Sekoke" disease, histologically muscular dystrophy. This disease was induced when the carp was fed diets containing oxidized lipids. This disease of carp was effectively prevented by the addition of DL-α-tocopherol acetate (50 mg/100g diet) but not by antioxidants such as BHA, ethyl gallate and ethoxyquin. The author concluded that it was most likely that the oxidized lipids destroyed the α-tocopherol originally present. According to Watanabe and Takeuchi (1989) the tocopherol requirement of rainbow trout, which is

markedly lower than that of carp is also affected by dietary lipid content. Rainbow trout fed tocopherol-deficient diet containing 15% methyl esters of pollock liver oil (0.9 g in body weight) resulted in poor growth, low feed conversion, and a high mortality along with convulsion. Addition of 5 mg of α-tocopherol to the diet effectively prevented these symptoms.

Many early observations about the damaging effects of deficiency of vitamin E in animal diets could be partially or completely improved by synthetic antioxidants led to the conclusion that vitamin E functions in vivo as a protector against lipid peroxidation (Deshpande *et al*. 1996). The major function of vitamin E is namely as an antioxidant (Niki 1996) and it is the principal component of the secondary defense mechanisms against free-radical mediated cellular injuries (Deshpande *et al*. 1996). The phenolic hydroxyl group is critical for the antioxidant activity of vitamin E and the presence of at least one methyl group on the aromatic ring is also critical (Bramley *et al*. 2000). Vitamin E is lipophilic and located in the lipophilic compartment of membranes and lipoproteins, where it acts as an antioxidant primarily by scavenging active free radicals (Niki 1996).

Of all of the isomers and analogs, RRR-α-tocopherol, formerly known as d-α-tocopherol, has the highest biological activity (Niki 1996). The biopotencies of tocopherols, defined as the absorption in the gut, is in the order α-tocopherol > β-tocopherol > γ-tocopherol > δ-tocopherol, and α-tocotrienol, which has approximately 25% of the biopotency of α-tocopherol (Deshpande *et al*. 1996).

The stabilization of singlet oxygen may also be an important function of vitamin E (Niki 1996). According to Halliwell and Gutterridge (1989 vitamin E influences the metabolism of arachidonic acid in platelets and leukocytes and it affects the metabolism of vitamin A. Vitamin E possesses numerous beneficial effects that may arise from its function as a radical scavenging antioxidant but cannot be fully explained by its antioxidant function alone, since, for example other antioxidants having similar functions do not produce similar actions (Niki 1996).

5.3 Occurrence of tocopherols

In marine animals α-tocopherol has been found to be the principal tocopherol (Syväoja *et al*. 1985). The tocopherol content of foods is influenced by a large number

of factors e.g. seasonal differences and significant losses may occur during processing and storage of foods (Deshpande *et al*. 1996). Other factors may also be involved.

Each fish species has characteristic tocopherol levels in its tissue, which, since fish are unable to synthesize the vitamin, are related to diet (Ackman and Cormier 1967). The size or the age of the fish may also influence the tocopherol concentration. Thus, López and others (1995) found highest values for α-tocopherol in the youngest rainbow trout, but no significant difference was found between sexes in adult samples.

Considerable differences in α-tocopherol concentration have been reported between light and dark fish muscle. Ackman and Cormier (1967) found α-tocopherol values for the light and dark cod muscle 0.24 and 1.16 mg/100 g, respectively, corresponding to 0.30 and 0.63 mg α-tocopherol/g lipid. Petillo and others (1998) found 4.4 fold difference between the light and dark muscle concentration of tocopherol in mackerel muscle. When expressed per gram of lipid, there was a decrease in differences, where the concentration of α-tocopherol in the light muscle was 0.20 mg/g lipid compared to 0.64 mg/g lipid in the dark muscle.

The effect of processing on tocopherol content in fish has been studied. Erickson (1992) measured the effect of cooking on minced channel catfish. About 60% of the α-tocopherol remained after 5 minutes of heating at 177°C, and over 80% of the γ-tocopherol. Other cooking methods like frying in vegetable oil may even increase the tocopherol concentration in fish. Storozhok (1985) determined the tocopherol content of peled whitefish (*Coregonus peled*). The α-tocopherol content was 11 mg/100 g tissue in freshly caught fish and 37.1 mg/100 g in whitefish fried in vegetable oil. Same author reported that the α-tocopherol level was only 4.3 mg/100 g in dry cured whitefish. Lighter processing like freezing also affects the tocopherol content of fish. Syväoja and Salminen (1985) measured α-tocopherol in blast-frozen herring fillets. The tocopherol content fell from 420 mg/kg lipid to 270 mg/kg during six months frozen storage, that is over 60% of the α-tocopherol remained. Still, processes like hot-smoking of fall Atlantic mackerel (*Scomber scombrus*) left the vitamin E virtually unchanged (Bhuiyan *et al*. 1993).

Seasonal variations in tocopherol content of fish species may be considerable. Syväoja and Salminen (1985) measured tocopherols and tocotrienols in Finnish fish and fish products and found that fish caught in the spring, the spawning season of

most species, had a higher tocopherol content and lower fat content, than those caught in the autumn. Others have reported the same situation. Hardy and Mackie (1969) found a decrease in fat content, followed by an increase in tocopherol content in the period from October to March in sprats. Ackman (1974) found a corresponding inverse relationship between tocopherol content and fat content in commercial sole fillets. The tocopherol content was as high as 500-600 mg/kg lipid in the lean period of the year, and as low as 100 to 200 mg/kg lipid when lipid was at its highest in September and October. The results of Syväoja and Salminen (1985) showed that the tocopherol content of for example Baltic herring was 360 mg/kg lipid in the spring, but the values for the autumn herring was 200 mg/kg lipid, or about 55% that of the spring herring. The reason for this seasonal variation in tocopherol content has been suggested to be a consequence of the sexual maturation of the fish, reaching their peak during the spawning season (Syväoja and Salminen 1985; Hardy and Mackie 1969).

Table 5.1. α-Tocopherol content of some fish and fish products.

Fish[a]	α-Tocopherol (mg/100 g tissue)	α-Tocopherol/lipid (mg/g lipid)	Reference
Cod liver oil		0.26-0.32	O´Keefe & Ackman 1986
Mussel	0.5		Sidwell *et al*. 1978
Halibut	0.9		Schuler 1990
Shrimp	0.9		-
Cod	0.2		-
Flounder	0.36	0.25	Ackman & Cormier 1967
Sablefish	0.44	0.27	-
Lobster	1.5	1.51	-
Mackerel (autumn)	1.3	0.06	Bhuiyan *et al*. 1993
Baltic herring (spring)	2.45	0.36	Syväoja *et al*. 1985
Baltic herring (autumn)	1.48	0.20	-
Cod (spring)	1.05	1.24	-
Cod (autumn)	0.95	0.62	-
Rainbow trout[b]-spring	1.89	0.19	-
Rainbow trout[b]-autumn	1.43	0.09	-
Salmon-spring	2.02	0.16	-
Salmon-autumn	2.43	0.19	-
Salmon[b]	3.58		Refsgaard *et al*. 1998
Salmon (smoked)	0.38		Weber *et al.* 1997
Rainbow trout (steamed)	1.30		-
Herring (marinated)	0.43		-

[a] Cod (*Gadus morhua* spp.), mussel (*Mytilidae* spp.), halibut (*Hippoglossus hippoglossus*), shrimp (*Pandalus* spp.), flounder (*Pseudopleuronectes americanus*), sablefish (*Anoplopoma fimbria*), lobster (*Homarus americanus*), mackerel (*Scomber scombrus*), Baltic herring (*Clupea harengus membras*), salmon (*Salmo salar*), rainbow trout (*Salmo gairdneri*).
[b] Cultivated.

Syväoja and Salminen (1985) did observe that in high-fat fish the tocopherol:fat ratio was lower than in low-fat fish. α-Tocopherol content of low-fat fish was on the average 1100 mg/kg fat, with 300 mg/kg in the medium-fat and 160 mg/kg in the high-fat species.

In the research of Syväoja and Salminen (1985), α-tocopherol was found to be the principal tocopherol in both marine and lake fish, although the α-tocopherol content of marine fish was higher than that of freshwater fish. Ackman and Cormier (1967) examined α-tocopherol content in five species of fish (cod, dogfish, mackerel, flounder and sablefish). The values in both lean and fat major muscle tissues fell in the range of 210-330 mg/kg lipid (Table 5.1).

5.4 Antioxidant activity of tocopherols

Vitamin E is stable to heat and alkali in the absence of oxygen and is unaffected by acids at temperatures up to 100°C. It is, however, slowly oxidized by oxygen to tocopheroxide, tocopherylquinone, and tocopheryl hydroquinone as well as to dimers an trimers (Deshpande *et al*. 1996). The oxidation rate is greatly enhanced by iron and copper. The esters of vitamin E, e.g. tocopheryl acetate, are stable to oxidation and cannot function as antioxidants. The active hydroxyl group is protected. However, under certain conditions, e.g. acidic aqueous systems (as in the stomach), a slow hydrolysis of the tocopheryl acetate can be observed. The released tocopherol then acts as an antioxidant (Schuler 1990).

The dynamics and mechanism of inhibition of oxidation by α-tocopherol have been studied extensively and are now fairly well understood (Halliwell and Gutterridge 1995, Niki 1996). α-Tocopherol (TOH) acts as an antioxidant by scavenging lipid peroxyl oxygen radical (LOO•):

$$LOO\bullet + TOH \rightarrow LOOH + TO\bullet$$

According to Niki (1996) α-tocopherol scavenges the peroxyl radical about 10 times faster than the lipid reacts with the radical. However, it is unlikely that vitamin E scavenges hydroxyl and alkoxyl radical efficiently in vivo (Niki 1996). The total antioxidant potency is however not only determined by rate of the reaction but also by the fate of the radical derived.

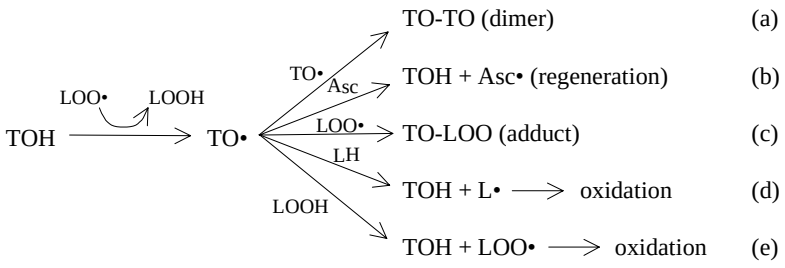


Figure 5.2. Proposed reaction pathways for tocopherol.

The tocopheroxyl radical (TO•) is relatively stable because the unpaired electron of the oxygen atom can be delocalized into the aromatic ring structure of the molecule. The resulting α-tocopheroxyl radical may react further in a proxidative or antioxidative nature, depending on concentration and mobility of the antioxidant, the site of formation and activity of attacking radical, and the interaction with other antioxidants (Figure 5.2) (Niki 1996). The α-tocopheroxyl radical may react with another vitamin E radical to give a dimer (reaction a). It may be converted back to tocopherol by ascorbate (Asc), ubiquinone, or enzymes (reaction b). It may scavenge another peroxyl radical to give an adduct (reaction c). Finally the vitamin E radical may act as a prooxidant by abstracting hydrogen atom from lipids (LH) or hydroperoxides (LOOH) to give lipid radical or peroxyl radical, respectively (reactions d and e).

The pro-oxidant action of α-tocopherol is mainly observed in foods at high concentrations of the antioxidant and according to Schuler (1990) this has to be considered mainly with α- and β-tocopherol, whereas γ- and δ-tocopherol have less prooxidant effect.

The relative antioxidant activity of different tocopherol homologs has been studied with much attention. According to Frankel (1996) there are inconsistencies in these results, which can be attributed to the wide differences in substrates tested, the level of oxidation used in the tests and the method used to analyze oxidation. On basis of oxidation of methyl linoleate, lard, cottonseed, and linseed esters, γ- and δ-tocopherols have been reported to be relatively more effective than β- and α-tocopherols (Lea 1960, Lea and Ward 1959). However, Burton and Ingold (1981) found that the antioxidative effectiveness of tocopherol isomers during initiated oxidation of styrene in chlorobenzene solution increased in the same order as their vitamin E activities; α > β > γ > δ.

According to Niki (1996) ascorbic acid acts as a potent antioxidant when α-tocopherol is present in the membrane and spares α-tocopherol by reduction of the tocopheroxyl radical. Apparently the two vitamins interact at the surface of the membrane. Whether this means that ascorbic acid acts as a synergist with α-tocopherol in vivo is not evident. To be synergistic, the antioxidant effect of the combination must be more extensive than the sum of the effects caused by either vitamin alone.

5.5 Tocopherol and lipid oxidation in fish

A number of studies on the stability of tocopherol in fish and fish products compared to their oxidative stability have appeared. Petillo and co-workers (1998) measured the α-tocopherol loss of both light and dark muscle of mackerel during 11 days storage in ice. At the completion of storage, the α-tocopherol content of light muscle had dropped to 40% of the initial level and about three-quarters of the α-tocopherol was lost from the dark muscle. Rates of sensory quality loss and increase in TBA values in the light and dark muscles were similar except that initial loss of quality in the dark muscle was greater than that in the light muscle.

Bandarra and others (1997) measured the α-tocopherol loss of sardine (10% fat) during 6 days ice storage. Fast reduction of the level of α-tocopherol was registered, and not being detected at the 4th day. High correlations were obtained for α-tocopherol, peroxide value and conjugated trienes.

Other research have shown remarkably good stability of tocopherols in fish. Erickson (1992) found no decrease in either α- or γ-tocopherols (0.2 mg/100 g tissue each) in minced channel catfish during seven days of refrigerated storage. No significant increase was observed in the oxidative response of the same samples.

In a study with commercial sole fillets, the natural content of tocopherol showed an inverse relationship to lipid content (Ackman 1974). Rancidity during frozen storage was reported to develop more rapidly in fish caught in the summer months, during the post-spawning feeding period with high lipid accumulation.

Notevarp and Chahine (1972) determined the tocopherol content of crude capelin (*Mallotus villosus*) oils from five different processing plants. The mean tocopherol content for the four oils possessing the best oxidative stability (24 days) was 0.33 mg/g, compared to 0.10 mg/g for the two with the lowest oxidative stability (11 days).

According to Parazo (1998) cultured Atlantic salmon are usually supplemented with tocopherol, solely in the α-tocopherol form. α-Tocopherol is expected to function as antioxidant in vivo, but membrane bound α-tocopherol also reduces postmortem lipid oxidation in fish products (O´Keefe and Noble 1978, Sigurgisladottir *et al*. 1994, Waagbo *et al*. 1993). Moreover, Parazo and others (1998) found that both α-tocopherol and γ-tocopherol were effective stabilizers of salmon muscle lipids during frozen storage.

In an experiment by Frigg and others (1990) rainbow trout (150 g) was fed 0, 50, 100 and 200 IU of vitamin E/kg feed for 85 days. At the end of the feeding period, the oxidative stability of the trout fillets was measured as TBA values under forced oxidative conditions. The oxidative stability was relative to the amount of vitamin E supplementation and the relationship of TBA values to the levels of α-tocopherol in fillets tended to be hyperbolic.

Jensen and others (1998) measured lipid oxidation in rainbow trout fed different levels of astaxanthin and α-tocopheryl acetate. Frozen storage (-28°C) of whole, gutted fish for 18 months significantly reduced astaxanthin and α-tocopherol levels, while lipid oxidation measured as TBA was limited. Lipid oxidation was however, pronounced in smoked trout, but a high level of α-tocopherol in the fillet significantly reduced lipid oxidation during chill storage of the smoked product. The authors concluded that α-tocopherol seems to be more important as an antioxidant at more advanced stages of lipid oxidation.

Erickson (1993a) studied tocopherol degradation in minced frozen Channel catfish under accelerated conditions (temperature fluctuations). The tocopherol was lost slowly during the first 6 months of storage, whereas after 9 months, less than 10% of the initial tocopherol was detected. The author suggested that a critical nonprotecting concentration of tocopherol had been reached after 6 months. Very good correlation was obtained between tocopherol levels and oxidative response. According to Erickson (1993b), monitoring the degradation of tocopherol is a sensitive indicator of oxidative stability at early stages prior to formation of oxidation products.

According to Chan and Decker (1994), the most efficient method for using α-tocopherol as an antioxidant is the incorporation of α-tocopherol into skeletal muscle

by dietary means. In that way the α-tocopherol is incorporated into membrane lipids where initial oxidation of skeletal muscle takes place.

6 CAROTENOIDS

6.1 Chemistry of carotenoids

Carotenoids are isoprenoid polyenes of eight C_5 -isoprene units. There are two main classes of carotenoids. The carotenes which are highly unsaturated hydrocarbons corresponding to the empirical formula $C_{40}H_{56}$ and the xanthophylls that are oxygenated carotenes. Carotenoids are either dissolved in fats or combined with proteins in the aqueous phase, and they occur in free form as well as in the forms of esters, glycosides, sulfates and caroteoproteins (Matsuno and Hirao 1989). Carotenoids contribute to the yellow, orange and red colors found in the skin, shell or exoskeleton of several important fish and shellfish. A series of conjugated double bonds constitutes a chromatophore of variable length, resulting in the characteristic yellow to red colors (Britton 1985). Some important carotenoids are shown in Figure 6.1.

6.2 Occurrence of carotenoids

Carotenoids are the most widespread pigments found in nature, as they occur in bacteria, yeast, mold and green plants, and many animals. About 180 carotenoids have so far been reported from marine life (Matsuno and Hirao 1989). Carotenoids in fish are found in the skin, flesh, eggs, gonads, milt, liver and eyes. Most of the skin and muscle pigments of fish and shellfish are xanthophylls (Haard 1992). In the skin the xanthophylls occur in esterified forms, while in other organs they are in free forms (Matsuno and Hirao 1989).

Crustaceans like lobster, crab and shrimp owe their diverse shades of red, brown, blue, green to astaxanthin and other carotenoids which are conjugated with protein to form carotenoprotein (Shahidi *et al*. 1998).

Astaxanthin is probably the most important marine pigment and is found in a very diverse group of sea animals, such as lobster, crab, shrimp, salmon and others.

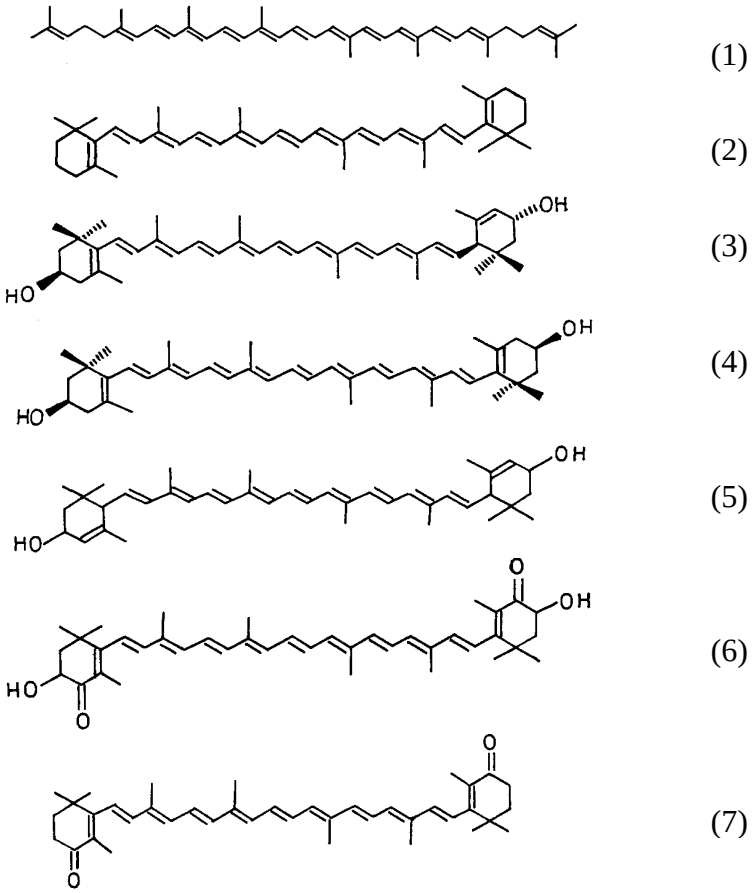


Figure 6.1. Some important carotenoids: (1) lycopene, (2) β-carotene, (3) lutein, (4) zeaxanthin, (5) tunaxanthin, (6) astaxanthin and (7) canthaxanthin (Matsuno and Hirao 1989).

Tunaxanthin has been shown to be widely distributed in various marine species, whereas lutein is a dominant pigment in freshwater fish and is generally of minor significance in marine fish (Simpson 1982). Carotenoids are less widely distributed in muscle than in integument (Haard 1992). Economically important fish species that typically exhibit flesh pigmentation include salmon and trout. The color of flesh is normally due to astaxanthin although the more stable canthaxanthin is used as feed additive in aquaculture (Mortensen and Skibsted 2000). Canthaxanthin colors both salmon skin and flesh but seems to be deposited rather than converted to astaxanthin (Simpson *et al.* 1981).

According to Shahidi and others (1998), 88% of the carotenoids in the flesh of chum salmon, 99.8% in sockeye salmon, 95.7% in soho salmon, 82.1% in pink salmon and 79.2% in masu salmon were composed of astaxanthin. In gonads of salmonids astaxanthin was the main carotenoid present, whereas in the skin salmoxanthin and zeaxanthin were mainly present (Matsuno and Hirao 1989).

The amount of carotenoids deposited in the flesh is very variable and related to various factors, including the content of carotenoids in the diet and the size and sex of the fish (Hatlen *et al*. 1995). Sexual maturation may also influence the carotenoid concentration in the flesh, because salmonids undergoing sexual maturation mobilize carotenoids from the flesh and selectively transfer them to the skin and gonads (Crozier 1970). Both astaxanthin and canthaxanthin have been found to increase with increasing dietary lipid levels, thus resulting in higher content of carotenoids in the flesh (Jensen *et al*. 1998, Storebakken and No 1992). Carotenoid concentration in rainbow trout weighing between 100 and 500 g may reach 6 to 7 mg/kg in the flesh, whereas larger trouts may contain as much as 25 mg/kg (Storebakken and No 1992). The astaxanthin concentration of wild salmon (*Salmo salar*) was reported by Schiedt and others (1981) to lie between 3.1 and 8.1 mg/kg. The astaxanthin concentration of farmed salmon *(S. salar*) was determined in six different fillet sections of 145 Atlantic salmon weighing from 4 to 4.5 kg, given feed containing 90 mg/kg astaxanthin (Refsgaard *et al*. 1998). The average astaxanthin concentration was 5.5 mg/kg muscle with a biological standard deviation of 1.1 mg/kg. They found significant variations of the astaxanthin content through out the salmon, with high levels in the tail and dorsal regions and a low level near the head. Matsuno and Hirao (1989) reported values for total carotenoids in salmon and trout as high as 7.5 mg/kg flesh of salmon (*Salmo salar*), 3.9 for chum salmon (*Oncorhynchus keta*), 37.5 for sockeye salmon (*Oncorhynchus nerka*) and 23.4 for coho salmon (*Oncorhynchus kisutch*).

6.3 Function of carotenoids

One of the most important physiological functions of carotenoids is due to their action as vitamin A precursors in animals. Although close to 600 carotenoids have been investigated and characterized in nature, only about 50 possess the biological activity of vitamin A (Olson and Krinsky 1995). In order to be a vitamin A precursor, a carotenoid must contain at least one terminal β-ione ring (Figure 6.2) (Shahidi *et al*. 1998). Vitamin A, as such, is not available from plants. Xanthophylls that are not vitamin A precursors in mammals were found to be active in lower animals because these animals are able to metabolize xanthophylls to β-carotene, which is finally converted to vitamin A (Shahidi *et al*. 1998).

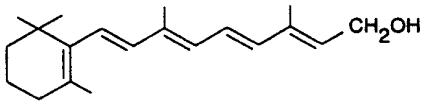


Figure 6.2. Structure of vitamin A.

The biological role of carotenoids in animals is not quite clear, except for the role of carotenes as vitamin A precursors (Tacon. 1981). So far as is known, only green plants, fungi and bacteria can perform a *de novo* synthesis of the carotenoids. Animals obtain carotenoids from their diet, phytoplankton being the primary source in the oceanic food chain. According to Haard (1992) the fate of dietary carotenoids varies with the types of carotenoid and the animal.

Carotenoids ingested by an animal may be: (1) passed form the animal via feces in chemically unchanged condition, (2) assimilated and stored in an unmodified form, (3), assimilated and converted to another storage form, and (4) assimilated and completely catabolized.

Simpson (1982) describes the division of fish into three subgroups according to their biosynthetic capabilities: (1) "Red carp type" can convert lutein, zeaxanthin, or intermediates to astaxanthin, but β-carotene is not an efficient precursor of astaxanthin. Astaxanthin can be stored directly, (2) "Prawn type" can convert β-carotene or intermediates to astaxanthin and (3) "Sea bream type" which can not convert β-carotene, lutein or zeaxanthin to astaxanthin. Can transfer pigments form the diet to tissue pigments as free or esterified form. Trout and salmon would best be listed under the "sea bream type".

One of the main roles of carotenoids in living tissues seems to relate to the light absorption functions. In plants carotenoids function as light harvesting or accessory antennal pigments, light or energy conductors and finally as singlet oxygen and triplet molecule quenchers, or as protector of photooxidation (Shahidi *et al*. 1998). Carotenoid concentrations are occasionally high in the reproductive tissues of fungi, algae, and animals, which may suggest they participate in the reproduction (Matsuno and Hirao 1989, Shahidi *et al*. 1998). In terms of free-radical pathology, the most important biological functions of carotenoids appear to be their antioxidant nature, their ability to quench singlet oxygen, and possible roles in enhancing the immune responses and the inhibition of mutagenisis (Deshpande *et al*. 1996, Shahidi *et al*. 1998).

In muscle food carotenoids may play two roles closely related to the consumers acceptance of the food: Providing color to the food as in the flesh of salmon and trout and as an antioxidant to prevent lipid oxidation (Mortensen and Skibsted 2000).

6.4 Antioxidant activity of carotenoids

Carotenoids are known to scavenge and deactivate free radicals both in vitro and in vivo (Deshpande *et al*. 1996). The antioxidative behavior of carotenes is closely related to their own oxidation. The polyene chain of the molecule is highly reactive, electron-rich system that is susceptible to attack by peroxyl radicals and other electrophilic reagents. It is responsible for the instability of carotenoids toward oxidation and at the same time an important property of the molecule concerning free-radical chemistry (Britton 1995). Carotenoids are therefore sensitive to oxygen, light, heat, acid and alkali, particularly combinations of these factors (Britton 1985).

Although majority of the work on antioxidative activity of carotenoids has been done on β-carotene (Yanishlieva *et al*. 1998), theoretically, all carotenoids with similar conjugated double bond system should have antioxidant properties (Britton 1995, Shahidi *et al*. 1998). Carotenoids are able to quench free radical species such as singlet oxygen. In addition to quenching reactive species formed by photochemical reaction, carotenoids may also act as chain-breaking antioxidants, although they do not have the characteristic structural features associated with this class of antioxidants (Deshpande *et al*. 1996). The antioxidant potency of carotenes depends on several factors such as oxygen pressure, intrinsic chemical reactivity of the molecule toward radicals, site of generation and reactivity of the radicals, concentration and mobility in the microenvironment, stability and fate of carotene-derived radicals, and interaction with other antioxidants (Palozza and Krinsky 1994, Yanishlieva *et al*. 1998).

In some food and model systems, carotenoids work as prooxidants under some conditions and as antioxidants under other condition depending on their concentration. The balance between prooxidant and antioxidant behavior is very delicate, and the antioxidant behavior is most pronounced at low oxygen partial pressure (Jørgensen and Skibsted 1993).

6.5 Carotenoids as a free radical scavenger

Carotenoids are suggested to react with peroxyl radicals to form a resonance-stabilized radical (Burton and Ingold 1984) and perhaps by electron transfer to form the alkyl peroxide anion and carotenoid radical cation (Britton 1995). Alkylperoxyl radicals do not abstract hydrogen from carotenoids, so the antioxidant mechanism of carotenoids is different from that of phenolic compounds. β-Carotene acts as an antioxidant by a mechanism, in which the chain-propagating radical ROO• is trapped by addition to the conjugated polyene system of β-carotene (Figure 6.3).

ROO• + β-carotene → ROO-β-carotene•

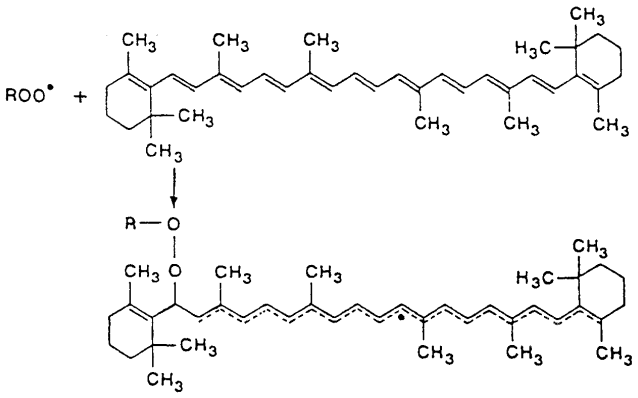


Figure 6.3. A proposed interaction between β-carotene and a peroxyl radical (ROO•) to produce a resonance-stabilized carbon centered radical (Yanishlieva et al. 1998).

The carbon-centered radical is resonance-stabilized because of the delocalization of the unpaired electron in the conjugated polyene system, leading to chain termination. The resulting β-carotene radical (LOO-βCar•) may react further in a proxidative or antioxidative nature, depending on the oxygen pressure, interactions with other antioxidants and properties of the prooxidant agent (Figure 6.4). It may react with another radical (X•) to give a stable product (reaction a). It may react with oxygen to yield a β-carotene peroxyl radical (LOO-βCarOO•), which may react with another radical to give a stable product (reaction b), attack another β-carotene molecule to induce the autoxidation of β-carotene (βC) (reaction c), or attack lipid substrate (LH) to generate a lipid radical (L•) and induce chain oxidation (reaction d). α-Tocopherol (α-Toc) may possibly scavenge the β-carotene peroxyl radical (reaction e). Finally the β-carotene radical may undergo β-scission to give an epoxide and alkoxyl radical, which may continue oxidation (reaction f). Reactions a, b and e lead to antioxidation, whereas reactions d and f do not (Yanishlieva *et al*. 1998).

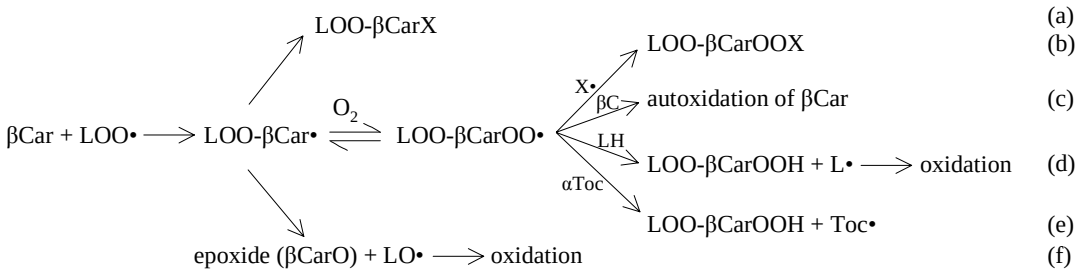


Figure 6.4. Proposed reaction pathways for β-carotene during lipid autoxidation (adapted from Yanishlieva et al. 1998).

Thus under conditions of low oxygen pressure the concentration of the reactive β-carotene peroxyl radical (LOO-β-caroteneOO•) is greatly reduced and leads to antioxidation. However, β-carotene does not behave as an antioxidant at normal oxygen concentration (Britton 1995). The antioxidant function of β-carotene therefore complements the action of other antioxidant molecules such as catalase, peroxidase, vitamin C and vitamin E, which are very effective at normal oxygen concentrations (Deshpande *et al*. 1996).

6.6 Carotenoids in photosensitized oxidation

Photosensitized oxidation in foods has been thoroughly reviewed by Bradley and Min (1992). The effect of light on the flavor stability of food can be explained by both photolytic autoxidation and photosensitized oxidation. Photolytic autoxidation is the production of free radicals primarily form lipids during exposure to light. Direct interaction of UV light and the lipid portion of food products is minimal, and is thus not a primary concern. Photosensitized oxidation, however, occurs in the presence of sensitizer (Sen) and visible light (hν). Natural pigments present in food, such as chlorophyll, hematoporphyrins, and riboflavin, are known to be efficient photosensitizers due to their conjugated double-bond system that easily absorbs visible light energy. When the sensitizer absorbs light energy, it is boosted to a higher energy level to a singlet-excited state (^{1}Sen*). The light energy may then be removed by inter-system crossing (ISC) to a triplet-excited state (^{3}Sen*). Finally the triplet-excited sensitizer transfers energy to atmospheric triplet oxygen ($^3O_2$) to produce the reactive singlet state oxygen ($^1O_2$).

$$\text{Sen} \xrightarrow{h\nu} {}^1\text{Sen}^* \xrightarrow{\text{ISC}} {}^3\text{Sen}^* \xrightarrow{{}^3O_2} {}^1O_2 + \text{Sen}$$

Plants containing chlorophyll are well protected by carotenoids against atmospheric damage of photosensitized oxidation (Olson and Krinsky 1995). Carotenoids protect unsaturated lipids against photosensitized oxidation by interfering with the activation of triplet oxygen into singlet oxygen. Carotenoids like β-carotene have sufficiently low singlet energy state to accept energy from singlet oxygen and effectively quench $^1O_2$ (Bradley and Min 1992).

$$^1\text{Carotene} + {}^1O_2 \longrightarrow {}^3\text{Carotene} + {}^3O_2 \longrightarrow {}^1\text{Carotene}$$

Carotenoids with nine or more conjugated double bonds can absorb the energy from the singlet oxygen, which is then distributed over all the single and double bonds in the molecule (Bradley and Min 1992). The triplet state carotene releases the energy in the form of heat, so that a regenerating reaction is not required (Yanishlieva *et al*. 1998). Carotenoids are therefor not destroyed during the process and one molecule of β-carotene is estimated to be able to quench up to 1000 molecules of singlet oxygen (Bradley and Min 1992).

6.7 Comparison of antioxidant activity of carotenoids

Several studies have been done to compare the relative antioxidant activity of carotenoids. Astaxanthin is perhaps the most important marine pigment and is expected to function in an antioxidative manner similar to that of most carotenoids (Britton 1995, Shahidi *et al*. 1998). On the other hand, several investigators have demonstrated that carbonyl carotenoids such as canthaxanthin (4,4´-diketo-β-carotene) and astaxanthin (3,3´-dihydroxy-4,4´diketo-β-carotne) are better free-radical trapping compounds than β-carotene (Miki 1991, Palozza and Krinsky 1994).

Terao (1989) observed that astaxanthin and canthaxanthin retard hydroperoxide formation in solution and that their effects are more pronounced than those of β-carotene and zeaxanthin.

Miki (1991) found approximately 10 times stronger scavenger activities of oxygen species by astaxanthin than by zeaxanthin, lutein, tunaxanthin, canthaxanthin and β-carotene and 100 times greater than those of α-tocopherol.

Jørgensen and Skibsted (1993) found that the antioxidative effectiveness of carotenoids during initiated oxidation of methyl linoleate in chloroform decreased in the following order; astaxanthin > canthaxanthin > β-carotene. However, during initiated oxidation of a heterogeneous lipid/water system, no differences in the antioxidative behavior of the carotenoids were observed (Jørgensen and Skibsted 1993).

Naguib (2000) determined the antioxidant activities of astaxanthin, lutein, lycopene and α- and β-carotenes, and α-tocopherol by employing novel fluorometric assays based on BODIOY class of dyes as indicators, a lipophilic peroxyl radical generator AMVN, and lipophilic media. Astaxanthin was found to exhibit the highest antioxidant activity of the selected carotenoids.

It is generally accepted that alkylperoxyl radicals do not abstract hydrogen from carotenoids, so the antioxidant mechanism of carotenoids is different from that of phenolic compounds. Carotenoids are suggested to react with peroxyl radicals to form a resonance-stabilized radical and perhaps by electron transfer to form the alkyl peroxide anion and carotenoid radical cation (Britton 1995, Palozza and Krinsky 1994, Yanishlieva *et al*. 1998). Naguib (2000), however proposes to explain the high antioxidant activity of the α-hydroketocarotenoid astaxanthin as compared to other carotenoids, that in solution astaxanthin exists in an equilibrium with the enol form of the ketone, thus the resulting ortho-dihydroxyl-conjugated polyene system possess a hydrogen atom capable of acting as an chain breaking antioxidant in free radical reaction in a way similar to the hydroxyl group of α-tocopherol.

Astaxanthin has two hydroxy groups in the 3 and 3´position, which makes it more hydrophilic than β-carotene (Yanishlieva *et al*. 1998). In a complex system like flesh, it was postulated that higher hydrophilicity of astaxanthin provides a better contact to hydroperoxides and thus a more effective protection against lipid oxidation (Andersen *et al*. 1990).

6.8 Cooperative action of carotenoids with other antioxidants

Carotenoids are, like other antioxidants, degraded by radicals when functioning as antioxidants and the presence of other antioxidants is thus important for the preservation of color. Carotenoids may be protected by other antioxidants in two

ways: (1) the other antioxidant scavenges the radicals before they react with the carotenoids or (2) the oxidized carotenoid is recycled by another antioxidant (Mortensen and Skipstedt 2000).

Synergistic interaction between carotenoids and tocopherols in preventing oxidation in foods has been reviewed by Mortensen and Skibsted (2000) and Yanishlieva and others (1998). Whether carotenoids are recycled by tocopherols or the reverse reaction was dominant may not be evident, but tocopherols and carotenes in combination are found to provide a higher antioxidative capacity than tocopherols or carotenes alone (Palozza and Krinsky 1992).

6.9 Lipid oxidation and carotenoids

The degradation of carotenoids is of extreme importance to seafood. In the case of aquaculturally raised fish, the quantity and quality of the carotenoids in the diet are of importance, as is the maintenance of color in the product. The changes that can occur in carotenoids can range from slight shifts in color, in the case of cis/trans isomerization, to loss of color in the presence of strong light, lipoxygenase, or drying conditions (Simpson 1982).

Jensen and others (1998) investigated the effect of feeding astaxanthin on lipid oxidation during frozen storage of rainbow trout. Lipid oxidation as measured by TBARS was less significant after 21 months frozen storage for rainbow trouts that had been fed 100 ppm astaxanthin compared to 40 ppm. The level of α-tocopherol in the feed (100 or 600 ppm), was however, not important. It was concluded that astaxanthin and α-tocopherol operated via different mechanisms and at different stages of the oxidative deterioration of lipids in salmonid products.

The carotenoids have long been known to be a substrate for lipoxygenase type enzymes. Tsukunda and Amano (1968) observed that discoloration of red fish occurred at refrigeration temperatures in the dark, and that homogenates from the skin muscle and liver were able to degrade astaxanthin, tunaxanthin, and β-carotene to colorless compounds. These authors isolated and partially purified a heat labile lipoxygenase-like enzyme from the skin of red fish, *Sebastes thompsoni* and *Chelidonichthys kumu*. The enzyme discolored tunaxanthin in the presence of linoleic and linolenic acids.

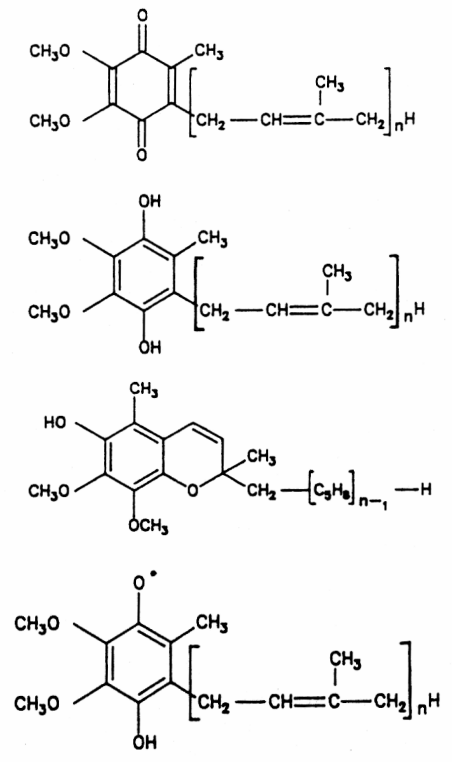
7 UBIQUINONE

7.1 Chemistry of ubiquinone

Coenzyme Q_{10} is a group of homologous quinones (Figure 7.1). Coenzyme Q_{10} (CoQ_{10}) or ubiquinone is a benzoquine with an isoprenoid side chain (Kagan *et al*. 1996). The isoprenoid tail is found in various lengths, from 6-10 isoprenoid units in plants and microorganism, whereas rodents have CoQ_9 and man CoQ_{10} as the dominating form (Weber *et al*. 1997). CoQ is found it two redox forms and the redox cycle involves a reversible reduction to the respective ubiquinols (Lang *et al*. 1986).

7.2 Function of ubiquinone

Ubiquinone has various bioactivities concerning energy metabolism, immunological competence and antioxidation. Ubiquinone is an electron carrier of the electron transport chain of the inner membrane of the mitochondria, which are known as the energy factories, where it aids in generating ATP (Kagan *et al*. 1996). In its role as an electron transport agent this compound undergoes continuos addition and loss of an electron (Martino *et al*. 1995).



Coenzyme Q (n=1-12)

Ubiquinol

Ubichromenol

Semiquinone

Figure 7.1. Structure of ubiquinone and related compounds.

In addition to its multiple roles in energy metabolism, coenzyme Q as well as its reduced and isomerized forms, ubiquinol and ubichromenols (Figure 7.1) may play an important role in controlling lipid oxidation (Lambelet *et al*. 1992, Kagan *et al*. 1996). This may be of particular importance in fish dark muscle where mitochondria can make up to 45% of the total volume of the cell (Petillo *et al*. 1998). Other functions of coenzyme Q_{10} may be related to growth control, calcium transport and secretion-related membrane flow (Kagan *et al*. 1996).

Ubiquinol is the only known lipid-soluble antioxidant that can be synthesized "de novo" in animal cells and for which there exists an enzymic mechanism-mitochondrial and microsomal electron-transport systems- that can regenerate the antioxidant from its oxidized form resulting from its inhibitory effect on lipid peroxidation (Kagan *et al*. 1996).

7.3 Occurrence of ubiquinone

Ubiquinone is widely distributed in animals, plants and microorganisms, and almost every cell of a living organism contains CoQ (Lambelet *et al*. 1992). The CoQ content varies in different organs, being highest in those that produce large amounts of energy as in dark muscle of fish.

Weber and coworkers (1997) determined the coenzyme Q_{10} content in selected food items. The richest sources of CoQ_{10} ranged from 0.8-20.3 mg/100 g in meat and poultry as well as 4-27 mg/100 g in fish, which is in accordance with the presence of a high content of mitochondria in muscle tissue. Kamei and coworkers (1986) determined the content of ubiquinone in fish and shellfish as well as other foods. All seafoods examined were found to contain Q_{10} mainly. The ubiquinone content of some fish and shellfish is listed in Table 7.1.

Martino and coworkers (1995) determined the coenzyme Q_{10} content of meat, fish, eggs, milk and cheese. They found positive correlation between CoQ_{10} contents and cholesterol, protein and ash contents, but no significant correlations with lipid, fatty acid and mineral levels.

Petillo and coworkers (1998) found considerable differences in both forms of coenzyme Q (ubiquinone and ubiquinol) concentration between light and dark muscle of mackerel (*Scomber scombrus*).

Table 7.1. Ubiquinone content of some fish and fish products.

Fish[a]	Ubiquinone $_{10}$ (mg/100 g tissue)	Reference
Mackerel	4.33	Kamei *et al*. 1986
Sardine	6.43	-
Horse mackerel	2.07	-
Yellow tail	2.07	-
Cattle fish	2.38	-
Flat fish	0.55	-
Eel[b]	1.11	-
Trout	1.28	Martino *et al*. 1995
Herring (marinated)	0.27	Weber *et al*. 1997
Rainbow trout (steamed)	0.11	-
Salmon (smoked)	0.43	-

[a] Mackerel (*Scomber scombrus*), s*ardine (Sardina pilchardus*), horse mackerel (*Trachurus trachurus*), eel (*Anguilla anguilla*), herring (*Clupea harengus*), salmon (*Salmo salar*), rainbow trout (*Salmo gairdneri*).
[b] Cultivated.

The dark muscle concentrations on a wet weight basis were more than an order of magnitude higher than they were in the light muscle. Weber and coworkers (1997) evaluated the effect of cooking on coenzyme Q_{10} in selected foods. In the case of frying of meat, the loss of CoQ_{10} was in the range of 14-32%, but in the case of boiling of vegetables and eggs, the values tended to be higher for the cooked samples.

7.4 Antioxidant activity of ubiquinone

The mechanisms proposed for antioxidant activity of ubiquinones are still conjectural (Kagan *et al*. 1996). Ubiquinol (QH) may act as a chain -breaking antioxidant by hydrogen donation to reduce peroxyl radicals (ROO•):

$$QH + ROO\bullet \rightarrow Q\bullet + ROOH$$

7.5 Recycling of other antioxidants by ubiquinone

Another mechanism proposed for antioxidant activity of ubiquinones is the recycling of the phenoxyl radical (TO•) of vitamin E (Kagan *et al*. 1996):

$$QH + TO\bullet \rightarrow Q\bullet + TOH.$$

7.6 Prooxidant activity of ubiquinone

Like many antioxidants, ubiquinone may also function as a prooxidant. Ubisemiquinone ($SO^{-}\bullet$), an intermediate of ubiquinone redox cycling by electron transport, can undergo autoxidation (Kagan *et al*. 1996):

$$SQ^{-}\bullet + O_2 + H^{+} \leftrightarrow Q + HOO\bullet$$

This reaction requires protons and since protons have no access to intact phospholipid membranes where naturally occurring ubiquinones are located, autoxidation is therefore not to be expected (Kagan *et al*. 1996).

7.7 Lipid oxidation and ubiquinone

Very little is known about the antioxidant role of ubiquinones in skeletal muscle but their presence in mitochondria suggests that they could be important in the oxidative stability of red muscle (Decker and Xu 1998).

Petillo and coworkers (1998) evaluated the loss of antioxidants in mackerel during 11 days storage in ice. Ubiquinone-10 was the most stable antioxidant in light muscle and almost three-quarters was still present at the completion of storage. Ubiquinol gave the best correlation with sensory scores for dark mackerel muscle along with ascorbate and glutathione, which showed the best relationship with sensory scores for light mackerel muscle. Although the great majority of the coenzyme Q in mackerel muscle was in the oxidized form, the authors found it unlikely to function directly as an antioxidant. The authors proposed antioxidant participation where ubiquinone would be reduced to semi-ubiquinone by ascorbate radical, while being oxidized to dehydroascorbate. The semiubiquinone radical could then either reduce a tocopheroxyl radical for regenerating tocopherol or dismutate to one oxidized and one reduced molecule of the coenzyme ($2QH\bullet \rightarrow Q + QH_2$).

Lambelet and coworkers (1992) measured the antioxidant properties of coenzyme Q_{10} and its reduced form, ubiquinol 10 ($CoQ_{10}H_2$) in fish oil by means of ethane formation and oxygen consumption in headspace. CoQ_{10} only enhanced the stability marginally but $CoQ_{10}H_2$ protected the oil slightly better. Investigation of the radical reactions of these compounds in autoxidizing chicken fat revealed that CoQ_{10} did not react with the peroxidizing lipid, but $CoQ_{10}H_2$ formed the corresponding semiquinone radical that was rapidly transformed into the corresponding inactive quinone in air.

The authors concluded that CoQ_{10} is not a food antioxidant but in conjunction with a reducing agent such as vitamin C it will be active as a food antioxidant.

8 PHOSPHOLIPIDS

8.1 Chemistry of phospholipids

Phospholipids (PL), also known as phosphatides are phosphorus-containing lipids. Those based on glycerol are phosphoglycerides, whereas sphingolipids are based on sphingosine. Phosphoglycerides are based on phosphatitic acids which are 1,2-diacyl esters of 3- glycerol phosphoric acid, linked to organic bases or other moieties. The individual components of fish phospholipids consist principally of phosphatidyl choline (PC or lecitin), phosphatidyl ethanolamine (PE or cephalin), phosphatidyl serine (PS), and phosphatidyl inositol (PI) (Figure 8.1), with minor contents of sphingomyelin, lysophosphatidylcholine, and cardiolipin (diphosphatidyl glycerol) (Morris and Culkin 1989).

Phospholipids are more polar than the other lipid classes. The fatty acids occupying the *sn*-1 and *sn*-2 positions vary in chain length and in unsaturation. The *sn*-1 position in PC of animal origin is largely esterified by saturated fatty acids while the *sn*-2 position contains polyunsaturated fatty acids (Dugan 1976). Marine phospholipids are usually richer in the polyunsaturated fatty acids than are the neutral lipids (Vaskovsky 1989). PE is usually the most unsaturated phospholipid, but more than 40 different fatty acids may be identified in PL of marine animals. The essential fatty acids in PL of the majority of marine animals are 16:0, 16:1, 18:0, 18:1, 20:4ω6, 20:5ω3, and 22:6ω3 (Vaskovsky 1989). Enzymatic hydrolysis of phospholipids occurs selectively at different ester sites by several phospholipases (Frankel 1998c).

8.2 Occurrence of phospholipids

Phospholipids are important for function and structure of biomembranes. They are the major structural lipids in fish tissues, and they also occur in high concentration in the brain tissues (Morris and Culkin 1989). According to Ackman (1980), lean white fish muscle contains a minimum of about 0.7% of basic cellular lipid, of which 85-95% is polar lipids, mostly PE and PC.

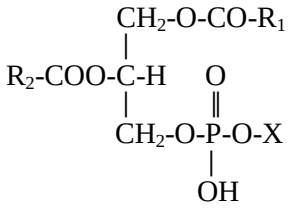


Figure 8.1. General formula of phosphoglycerides.

R_1 and R_2 are alkyl portions of fatty acids and X can be:
X = H: Phosphatidic acid (PA)
X = $CH_2CH_2N^+Me_3$ or choline: Phosphatidyl choline (PC)
X = $CH_2CH_2N^+H_3$ or ethanolamine: Phosphatidyl ethanolamine (PE)
X = $CH_2CH(NH_3)^+COO^-$ or serine: Phosphatidyl serine (PS)
X = $C_6H_{11}O_5$ or inositol: Phosphatidyl inositol (PI)

Posphatidyl choline is the main phospholipid in the majority of marine invertebrates and vertebrates (Vaskovsky 1989). PE is the second essential PL of marine organisms and usually it amounts to 20-25% of the total PL in marine animals. PC is usually twice the PE (Ackman 1980). PS is a common component of marine animals. PI is an acidic phospholipid as is PS. PI is less abundant than PS in marine animals. Average concentration of PI are 2-5% of the total PL. Phosphatidylglycerol is a minor PL in marine animals but has been detected in several marine animals such as krill and scallop (Varcovsky 1989).

The content and composition of phospholipids as well as the fatty acid composition undergo seasonal variations, which are influenced by temperature, nutrition and reproduction (Vaskovsky 1989). Starvation decreases the level of neutral lipids more than phospholipids and therefore the relative concentration of phospholipids increases.

8.3 Antioxidant activity of phospholipids

The reported antioxidant effect of various phospholipids is contradictory. Physical and structural differences among and within each PL class may in part contribute to the differences observed in their antioxidant properties (Nwosu *et al.* 1997). They are believed to function as synergists and metal chelators and may also bring about decomposition of hydroperoxides (Pokorný 1987). Lee and coworkers (1984) found methyl linoleate hydroperoxides-decomposing activity in all krill phospholipids, composed of PC, PE and lysoPC. Hydrogenation of PC inhibited its browning as well

as the peroxide composing activity, suggesting that oxidative degradation is necessary for phospholipids to show peroxide-decomposing activity. This was in agreement with the results of Ohshima and coworkers (1993) who found less oxygen uptake in the neutral lipid fraction of sardine with added 10% sardine PE compared to 10% hydrogenated sardine PE. According to Pokorný (1987), phospholipids catalyze the destruction of hydroperoxides probably by forming labile complexes with dimeric hydroperoxides, which are decomposed without formation of free radicals.

Phospholipases are responsible for phospholipid hydrolysis in biological systems like fish muscle. While triglyceride hydrolysis leads to increased oxidation, phospholipid hydrolysis produces the opposite effect (Shwefelt 1981). Addition of phospholipase A has been shown to decrease both enzymic and nonenzymic lipid oxidation in flounder muscle microsomes, while phospholipase C lowered enzymic oxidation but did not affect non-enzymic oxidation (Shewfelt *et al.* 1981). Phospholipase A has also been shown to inhibit myoglobin oxidation in ground beef (Govindarajan *et al.* 1977). Phospholipase A has been classified as a secondary preventive inhibitor of lipid oxidation (Hultin 1994, Undeland 1998b). The specific cause of this effect is not clear, but hydrolysis of phospholipids may cause membrane rearrangements, formation or activation of antioxidants and inhibition of electron transfer enzymes.

8.4 Synergism of phospholipids

Secondary antioxidant effects of phospholipids arise from the synergistic activity in mixtures with natural tocopherols and synthetic antioxidants. PC and PE have received the most attention for this purpose (Kikugawa *et al.* 1990). Dipalmitoyl phosphatidyl ethanolamine acted as a potent synergist for a wide range of antioxidants such as probyl gallate, α-tocopherol, BHA, BHT and TBHQ at elevated temperatures (above 80°C). At 120°C the synergistic effects increases progressively as the concentration of synergist increases from 0.025% to 0.25% (Dziedzic and Hudson 1984).

The mechanism responsible for the observed synergy of tocopherols and phospholipids against oxidation is not very well understood, but seems to be related to the involvement of the amino group of phospholipids in the regeneration of tocopherol, by hydrogen transfer or electron donation (Hildebrand *et al.* 1984).

Ohshima and coworkers (1993) studied the effect of phosphoryl bases in the presence of α-tocopherol (30 μg/g lipid) on the oxidation rate of nonpolar lipids of sardine and trilinolein. The synergistic ability of *O*-posphoethanolamine, the base moiety of PE, was higher than that of *O*-phosphoserine. *O*-Phosphocholine was only slightly effective.

Segawa and coworkers (1994) studied the synergistic effect of phospholipids and tocopherols on the oxidation of fish oil at 30°C. Tocopherols were consumed in the order α-, γ-, δ-isomers, with or without phospholipid. During oxidation, tocopherols remained 1.5 and 3 times longer in PC- and PE-containing oils, respectively.

8.5 Antioxidant effect of browning reaction products from phospholipids

Phospholipids with free amino groups participate in aldehyde-amine reactions typical of nonenzymic browning (Dugan 1976). Several studies have shown most antioxidant activity of phospholipids at elevated temperatures or where browning of phospholipids was considerable (Dziedzic and Hudson 1984, Husain *et al.* 1986, Lee *et al.* 1984, Ohshima *et al.* 1993). It was postulated by Bandarra and coworkers (1999), that the high synergy between PE and tocopherol is due to the occurrence of a simultaneous antioxidant mechanism involving Maillard compounds. King and coworkers (1992) observed an inverse relationship between oxidation index (C22:6/C16:0) and color intensity for phospholipids in a refined salmon oil model system following 2 hours of heating at 180°C. The authors suggested that Maillard-type reaction products may have contributed to the oxidative stability of PL-supplemented fish oils.

8.6 Prooxidant activity of phospholipids

Unsaturated fatty acids in phospholipids are susceptible to lipid oxidation and the phosphoglycerides in fish oxidize prior to substantial oxidation of the triglycerides (Dugan 1976). Few examples of prooxidant activity of phospholipids are, however, found in the literature. Phospholipids are believed to facilitate lipid hydroperoxide formation in buttermilk by binding metal ions to a metal-phospholipid complex at the oil-water interface (Allen 1983). Prooxidant effect of PG was also observed by Segawa and coworkers (1994) in fish oil.

8.7 Lipid oxidation and phospholipids

Nwosu and coworkers (1997) compared the antioxidant properties of sphingomyelin, PC, and PE from salmon and menhaden by Rancimat induction time at 100°C. The induction time was influenced by the headgroup and fatty acid composition of the phospholipid in the salmon oil but not in the menhaden oil. Phospholipids that contained a choline headgroup possessed improved antioxidant activity compared to ethanolamine and phosphatide-containing phospholipids. Phospholipids containing more saturated fatty acids and phospholipids with longer fatty acid chains (C18 and above) had longer oxidation induction times. The authors suggested that fatty acid profiles of individual oils may influence the antioxidant properties of each phospholipid and the degree of saturation is a critical factor in the antioxidant activity of phospholipids. Differences in chainlength of the phospholipid fatty acids may also be a contributing factor to the variations detected in their antioxidant properties.

Bandarra and coworkers (1999) investigated the antioxidant activity of α-tocopherol at 0.04% and several phospholipids at 0.5% in refined sardine oil. Lipid oxidation was monitored by measuring peroxide value at elevated temperature (40°C). The most effective individual antioxidant was PC, followed by cardiolipin, and PE was the least effective phospholipid. The highest synergistic effect was obtained with a mixture of α-tocopherol and PE, and the second and third best by mixtures made with PC and cardiolipin CL, respectively.

Segawa and coworkers (1994) studied the antioxidative effects of phospholipids in fish oils kept at 30°C in the dark. PC was found to have a strong antioxidative effect, PE a strong synergistic effect, PG a prooxidative effect and PI had no effect on autoxidation of fish oil.

King and coworkers (1992) established the antioxidant effectiveness of individual phospholipids in salmon oil (heated for 3 hours at 180°C) in the order of sphingomyelin = LPC = PC = PE > PS > PI > PG.

9 OTHER ANTIOXIDATIVE COMPOUNDS IN FISH

Fish and seafoods may contain several other antioxidative compounds than hitherto mentioned. Seaweeds are known to contain antioxidative compounds (Ramarathnam *et al*. 1995) and some of the active compounds have been identified as compounds related to chlorophyll a and bromophenols (Sakata *et al*. 1994). Fish and marine animals feeding directly or indirectly on algae may also contain these antioxidants. The edible part of short necked clam was thus found to contain several antioxidative compounds related to chlorophyll a, pyropheophorbide a, and purpurin (Sakata *et al*. 1994). Scallop, oyster and abalone have also been shown to contain compounds related to chlorophyll a (Sakata *et al*. 1994). Antioxidative compounds have also been found in shrimp. Pasquel and Babitt (1991) isolated and characterized an antioxidative compound in shrimp and suggested that it was a polyhydroxylated derivative of an aromatic amino acid. Seymour and coworkers (1996) isolated and characterized a natural antioxidant from shrimp shell waste, which results indicated a phenolic compound with a primary amino group. The antioxidant was proposed to be 1,2-diamino-1-(*o*-hydroxyphenyl)propene.

10 CONCLUSIONS

Fish contains various antioxidants for protection of their unsaturated lipids and other nutrients from reactive oxygen species. Oxidative protection of fish includes enzymic, various water-soluble and lipid soluble antioxidant systems. The function of these antioxidants is to control prooxidants, scavenge free radicals and inactivate reactive oxygen species. The chemistry and function of well established food antioxidants such as ascorbic acid and tocopherol that also occur naturally is fairly well understood. The antioxidative mechanism and of other more recently discovered antioxidants such as Q_{10} is much less identified. Recently research on antioxidants has gained much interest due to increased evidence of the importance of antioxidants in human health. This interest has brought about considerable research on defining and finding components with antioxidative activity in humans and in biological systems. Relatively few studies on the various antioxidants in fish are currently available. Studies on the interaction between the various endogenous antioxidants in fish are also very few and may be difficult to rationalize because of the multiplicity of the

antioxidant system in fish. However, the occurrence of the most abundant endogenous antioxidants found in fish such as tocopherols and carotenoids is fairly well established. The relative concentration of antioxidants varies with the fish species as well as the type of fish muscle tissue. The quantity of these antioxidants may also fluctuate with storage time and handling post mortem. Control of lipid oxidation in fish involves utilizing processing and storage techniques that do not greatly decrease the activity of endogenous antioxidants.

11 REFERENCES

Ackman, R.G. 1974. Marine Lipids and Fatty Acids in Human Nutrition. In: *Fishery Products*, R. Kreuzer (Ed.), Fishing News (Books) Ltd., Surrey, pp 112-131.

Ackman R.G. 1980 Fish lipids. Part 1. In: *Advances in Fish Science and Technology*, J.J. Connell (Ed.), Fishing News Books Ltd. Farnham, Surrey, pp 86-103.

Ackman, R.G. 1988. Concerns for Utilization of Marine Lipids and Oils. *Food Technol. 42* (5): 151-155.

Ackman, R.G. 1989a. Fatty acids. In: *Marine Biogenic Lipids, Fats, and Oils*. Vol. I., R. G. Ackman (Ed.), pp. 103-137.

Ackman, R.G. 1989b. Nutritional composition of fats in seafoods. In: *Progress in Food and Nutrition Science*, Vol. 13, Pergamon Press, pp. 161-241.

Ackman, R.G. and Cormier, M.G. 1967. α-Tocopherol in some Atlantic fish and shellfish with particular reference to live-holding without food. *J. Fish. Res. Bd. Can. 24* (2): 357-373.

Alaiz, M., Zamora, R. and Hidalgo, J. 1995. Natural antioxidants produced in oxidized lipid/amino acid browning reactions. *JAOCS 72*: 1571-1575.

Alaiz, M., Zamora, R. and Hidalgo, F.J. 1996. Contribution of the formation of oxidized lipid/amino acid reaction products to the protective role of amino acids in oils and fats. *J. Agric. Food Chem. 44*: 1890-1895.

Allen, J.C. 1983. Rancidity in dairy products. In: *Rancidity in Foods*, J.C. Allen and R.J. Hamilton (Eds.), Applied Science Publishers, London, pp 169-178.

Anglemier, A.F. and Montgomery, M.W. 1976. Amino acids, peptides and proteins. In*: Food Chemistry*, O.R. Fennema (Ed.), Marcel Dekker, New York, pp 205-284.

Anon. 1995. Polyamines from food - we need them. *Fair Flow Rep. F-FE 160*/95, 1 p.

Bandarra, N.M. Campos, R.M., Batista, I., Nunes, M.L. and Empis, J.M. 1999. Antioxidant synergy of α-tocopherol and phospholipids. *JAOCS 76*: 905-913.

Bandarra, N.M., Undeland, I., Nunes, M.L., Batista, I. and Empis, J.M. 1997. Lipid oxidation indices to evaluate sardine freshness. In*: Methods to Determine the Freshness of Fish in Research and Industry*, G. Olafsdóttir, J. Luten, P. Dalgaard, M. Careche, V. Verrez-Bagnis, E. Martinsdóttir, K. Heia (Eds.), International Institute of Refrigeration, Paris, pp 263-265.

Bardócz, S. 1995. Polyamines in food an their consequences for food quality and human health. *Trends Food Sci. Technol. 6* (10): 341-346.

Bhuiyan, A.K.M., Ratnayake, W.M.N. and Ackman, R.G. 1993. Nutritional composition of raw and smoked Atlantic mackerel (*Scomber scombrus*): Oil- and water-soluble vitamins. *J. Food Composit. Anal. 6*: 172-184.

Bradley, D.G. and Min. D.B. 1992. Singlet oxygen oxidation of foods. *Crit. Rev. Food Sci. Nutr. 31*: 211-236.

Bramley, P.M., Elmadfa, I., Kafatos, A., Kelly, F.J., Manios, Y, Roxborough, H.E., Schuch, W., Sheehy, P.J.A. and Wagner, K.H. 2000. Vitamin E. *J. Sci. Food Agric. 80*: 913-938.

Brannan, R.G. and Erickson, M.C 1996. Quantification of antioxidants in channel catfish during frozen storage. *J. Agric. Food Chem. 44*: 1361-1366.

Britton, G. 1985. General carotenoid methods. *Method. Enzymol. 111*: 113-149.

Britton, G. 1995. Structure and properties of carotenoids in relation to function. *FASEB J. 9*: 1551-1558.

Buettner, G.R. 1993. The pecking order of free radicals and antioxidants: lipid peroxidation, α-tocopherol, and ascorbate. *Arch. Biochem. Biophys. 300*: 535-543.

Buettner, G.R. and Jurkiewicz, B.A. 1996. Chemistry and biochemistry of ascorbic acid. In: *Handbook of Antioxidants* (Vol 3), E. Cadenas and L. Packer (Eds.), Marcel Dekker, New York, pp 91-116.

Burton, G.W. and Ingold, K.U. 1981. Autoxidaton of biological molecules. 1. The antioxidant activity of vitamin E and related chain-breaking phenolic antioxidants in vitro. *J. Am. Chem. Soc. 103*: 6472-6477.

Burton, G.W. and Ingold, K.U. 1984. β-Carotene: an unusual type of lipid antioxidant. *Science 224*: 569-573.

Castell, C.H. 1971. Metal-catalysed lipid oxidation and changes of proteins in fish. *JAOCS 48*: 645-649.

Chan, H.W.-S. 1987. The mechanism of autoxidation. In: *Autoxidation of unsaturated lipids*, H. W. -S. Chan (Ed.), Academic Press, London, pp 1-16.

Chan, K.M. and Decker, E.A. 1994. Endogenous skeletal muscle antioxidants. *Crit. Rev. Food Sci. Nutr. 34*: 403-426.

Crozier, G.F. 1970. Tissue carotenoids in prespawning spawning sockeye salmon (*O. nerka). J. Fish. Res. Bd. Can. 27*: 973-975.

Decker, E.A. 1998. Strategies for manipulating the prooxidative/antioxidative balance of foods to maximize oxidative stability. *Trends Food Sci. Technol. 9*: 241:248.

Decker, E. A. and Xu, Z. 1998. Minimizing rancidity in muscle foods. *Food Technol. 52* (10): 54-59.

Decker, E.A. and Hultin, H.O. 1990. Factors influencing catalysis of lipid oxidation by the soluble fraction of mackerel muscle. *J. Food Sci. 55*: 947-950, 953.

Deshpande, S.S., Deshpande, U.S. and Salunkhe, D.K. 1996. Nutritional and health aspects of food antioxidants. In: *Food Antioxidants. Technological, Toxicological, and Health Perspectives*, D.L. Madhavi, S.S. Deshpande and D.K. Salunkhe (Eds.), Marcel Dekker, New York, pp 361-470.

DeVore, V.R., Colnago, G.L., Jensen, L.S. and Greene, B.E. 1983. Thiobarbituric acid values and glutathione peroxidase activity in meat from chickens fed a selenium-supplemented diet. *J. Food Sci. 48*: 300-301.

Dugan, L. 1976. Lipids. In: *Food Chemistry*, O.R. Fennema (Ed.), Marcel Dekker, New York, pp 139-203.

Durnford, E. and Shahidi, F. 1998. Flavour of fish meat. In*: Flavor of Meat, Meat Products and Seafoods*, 2nd ed., F.Shahidi (Ed.). Blackie Academic & Professional, London, pp. 131-158.

Dziedzic, S.Z. and Hudson, B.J.F. 1990. Phosphatidyl ethanolamine as a synergist for primary antioxidants in edible oils. *JAOCS 61*: 1042-1045.

Erickson, M.C. 1992. Changes in lipid oxidation during cooking of refrigerated minced channel catfish muscle. In: *Lipid Oxidation in Food*, A.J. St. Angelo (Ed.), American Chemical Society, Washington, DC, pp 344-350.

Erickson, M.C. 1993a. Compositional parameters and their relationship to oxidative stability of channel catfish *J. Agric. Food Chem. 41*: 1213-1218.

Erickson, M.C. 1993b. Ability of chemical measurements to differentiate oxidative stabilities of frozen minced muscle tissue from farm-raised striped bass and hybrid striped bass. *Food Chem. 48:* 381-385.

Finne, G. 1992. Non-protein nitrogen compounds in fish and shellfish. In*: Advances in Seafood Biochemistry. Composition and Quality*, G.J. Flick and R.E. Martin (Eds.), Technomic Publishing, Lancaster, Pennsylvania, pp 393-401.

Flick, G.J., Hong, G. and Knobl, G.M. 1992. Lipid oxidation of seafood during storage. In: *Lipid Oxidation in Food*, A.J. St. Angelo (Ed.), American Chemical Society, Washington, DC, pp 181-207.

Frankel, E.N. 1980. Lipid oxidation. *Prog. Lipid Res*. *19:* 1-22.

Frankel, E.N. 1991. Review. Recent advances in lipid oxidation. *J. Sci. Food Agric 54*: 495-511.

Frankel, E.N 1996. Antioxidants in lipid foods and their impact on food quality. *Food Chem. 57:* 51-55.

Frankel, E.N. 1998a. Antioxidants. In: *Lipid oxidation*, E.N. Frankel (Ed.), The Oily Press, Dundee, pp 129-160.

Frankel, E.N. 1998b. Foods. In: *Lipid oxidation*, E.N. Frankel (Ed.), The Oily Press, Dundee, pp 187-225.

Frankel, E.N. 1998c. Introduction. In: *Lipid oxidation*, E.N. Frankel (Ed.), The Oily Press, Dundee, pp 1-12.

Frigg, M., Prabucki, A.L. and Ruhdel, E.U. 1990. Effect of dietary vitamin E levels on oxidative stability of trout fillets. *Aquaculture 84*: 145-158.

Gordon, D.T. 1990. The mechanism of antioxidant action *in vitro*. In: *Food Antioxidants*, B.J.F. Hudson (Ed.), Elsevier Applied Science, London, pp 1-18.

Gordon, D.T. and Martin, R.E. 1982. Vitamins and minerals in seafoods of the Pacific Northwest. In: *Chemistry & Biochemistry of Marine Food Products*, R.E. Martin, G.J. Flick, C.E. Hebard and D.R. Ward (Eds.), AVI Publishing Company, Westport, Connecticut, pp 429-445.

Govindarajan, S., Hultin, H.O. and Kotula, A.W. 1977. Myoglobin oxidation in ground beef: mechanistic studies. *J. Food Sci. 42*: 571-577, 582.

Haard, N.F. 1992. Biochemistry and chemistry of color and color change in seafoods. In: *Advances in Seafood Biochemistry. Composition and Quality*. G.J. Flick and R.E. Martin (Eds.), Technomic Publishing, Lancaster, Pennsylvania, pp 305-360.

Haard, N.F. 2000. Seafood enzymes: The role of adaptation and other intraspecific factors. In: *Seafood Enzymes. Utilization and Influence on Postharvest Seafood Quality*, N.F. Haard and B.K. Simpson (Eds.), Marcel Dekker, New York, pp 1-36.

Halliwell, B. 1996. Uric acid: An example of antioxidant evaluation. In: *Handbook of Antioxidants* (Vol 3), E. Cadenas and L. Packer (Eds.), Marcel Dekker, New York, pp 243-256.

Halliwell, B. and Gutterridge, J.M. 1989. Lipid peroxidation: a radical chain reaction. In: *Free Radicals in Biology and Medicine*, 2nd ed., Clarendon Press, Oxford, pp 188-276.

Halliwell, B., Aeschbach, R., Loelinger, H. and Aruoma, O.E. 1995. The characterization of antioxidants. *Food Chem. Toxic. 33*: 601-617.

Han, D., Shin, H. and Yoon S.H. 1997. Separation of ω3 polyunsaturated fatty acids from fish oil and stabilization of the oil against autoxidation. In: *Flavor of Lipid Chemistry of Seafoods*, F. Shahidi and K.R. Cadwallader (Eds.), American Chemical Society, Washington, DC, pp 255-263.

Hardy, R. 1980. Fish lipids. Part 2. In: *Advances in Fish Science and Technology*, J.J. Connell (Ed.), Fishing News Books Ltd. Farnham, Surrey, England, pp 103-111.

Hardy, R. and Mackie, P. 1969. Seasonal variation in some of the lipid components of sprats (*Sprattus sprattus*). *J. Sci. Food Agric. 20*: 193-198.

Hatlen, B., Aao, G.H., Jørgensen, E.H. Storebakken, T. and Goswami, U.C. 1995. Pigmentation of 1-, 2-, and 3-year-old Arctic charr (*Salvelinus alpinus*) fed on different dietary astaxanthin concentrations. *Aquaculture 238*: 303-312.

Hildebrand, D.H., Terao, J. and Kito, M. 1984. Phospholipids plus tocopherols increase soybean oil stability. *JAOCS 61*: 552-555.

Hultin, H.O. 1992a. Trimethylamine-N-oxide (TMAO) demethylation and protein denaturation in fish muscle. In: *Advances in Seafood Biochemistry. Composition and Quality*, G.J. Flick and R.E. Martin (Eds.), Technomic Publishing, Lancaster, Pennsylvania, pp 25-42.

Hultin, H.O. 1992b. Lipid oxidation in fish muscle. In: *Advances in Seafood Biochemistry. Composition and Quality*, G.J. Flick and R.E. Martin (Eds.), Technomic Publishing Company, Lancaster, Pennsylvania, pp 99-122.

Hultin, H.O. 1994. Oxidation of lipids in seafoods In: *Seafoods: Chemistry, processing technology and quality*, F. Shahidi and J.F. Botta (Eds.), Blackie Academic and Professional. Glasgow, pp 49-74.

Husain, S.R., Terao, J. and Matsushita, S. 1986. Effect of browning reaction products of phospholipids on autoxidation of methyl linoleate. *JAOCS 63*: 1457-1460.

Ikeda, S. 1980. Other organic components and inorganic components. In*: Advances in Fish Science and Technology*, J.J. Connell (Ed.), Fishing News Books Ltd., Farnham, Surrey, pp 111-124.

Ishikawa, Y., Yuki, E., Kato, H. and Fujimaki, M. 1978. The mechanism of synergism between tocopherols and trimethylamine oxide in the inhibition of the autoxidation of methyl linoleate. *Agric. Biol. Chem. 42*: 711-716.

Ishtiaque, A., Alaiz, M., Zamora, R. and Hidalgo, F.J. 1996. Antioxidative activity of lysine/13-hydroperoxy-9(Z),11(E)-octadecadienoic acid reaction products. *J. Agric. Food Chem. 44*: 3646-3949.

Jensen, C., Birk, E., Jokumsen, A., Skibsted, L.H. and Bertelsen, G. 1998. Effect of dietary levels of fat, α-tocopherol and astaxanthin on colour and lipid oxidation during storage of frozen rainbow trout (*Oncorhynchus mykiss*) and during chill storage of smoked trout. *Z. Lebensm. Unters. Forshch. A. 207*: 189-196.

Joly, A., Huidobro, A. and Tejada, M. 1997. Influence of lipids on dimethylamine formation in model systems of hake (*Merluccius merluccius*) kidney during frozen storage. *Z. Lebensm. Unters. Forsh. A 205*: 14-18.

Jørgensen, K. and Skibsted, L.H. 1993. Carotenoid scavenging of radicals. Effect of carotenoid structure and oxygen partial pressure on antioxidative activity. *Z. Lebensm. Unters. Forsh. 196*: 423-429.

Kagan, V.E., Nohl, H. and Quinn, P.J. 1996. Coenzyme Q: Its role in scavenging and generation of radicals in membranes. In: *Handbook of Antioxidants*, E. Cadenas and L. Packer (Eds.), Marcel Dekker, New York, pp 157-201.

Kamei, M, Fujita, T, Kanabe, T, Sasaki, K, Oshiba, K, Otani, S., Matsui-Yuasa, I. and Morisawa, S. 1986. Distribution and content of ubiquinone in foods. *Internat. J. Vit. Nutr. Res. 56*: 57-63.

Karahadian, C. and Lindsay, R.C. 1989. Role of oxidative processes in the formation of and stability of fish flavors. In: *Flavor Chemistry Trends and Developments*, R. Teranishi, R. G. Buttery and F. Shahidi (Eds.), American Chemical Society, Washington, DC, pp 60-65.

Khayat, A. and Schwall, D. 1983. Lipid oxidation in seafood. *Food Technol. 37* (7): 130-140.

Kikugawa, K., Kunugi, A. and Kurechi T. 1990. Natural antioxidants exploited commercially. In: *Food Antioxidants*, B.J.F. Hudson (Ed.), Elsevier Applied Science, London, pp 65-98.

King, M.F., Boyd, L.S. and Sheldon, B.W. 1992. Antioxidant properties of individual phospholipids in salmon oil model system. *JAOCS 69*: 545-551.

Kitts, David D. 1997. An evaluation of the multiple effects of the antioxidant vitamins. *Trends Food Sci. Technol. 8*: 198-203.

Kläui, H. and Pongracz, G. 1981. Ascorbic acid and derivatives as antioxidants in oils and fats. In: *Vitamin C*, J.N. Counsell and D.H. Horing (Eds.), Applied Science Publishers, London, pp 139-166.

Konosu, S. and Yamaguchi, K. 1982. The flavor components in fish and shellfish. In*: Chemistry & Biochemistry of Marine Food Products*, R.E. Martin, G.J. Flick, C.E. Hebard and D.R. Ward (Eds.), AVI Publishing Company, Westport, Connecticut, pp 367-404.

Labuza, T.P. 1971. Kinetics of lipid oxidation in foods. *CRC Food Technol. 2*: 355-405.

Lambelet, P., Löliger, J. Saucy, F. and Bracco, U. 1992 Antioxidant properties of coenzyme Q_{10} in food systems. *J. Agric. Food Chem. 40*: 581-584.

Lang, J. K., Gohil, K. and Packer, L. 1986. Simultaneous determination of tocopherols, ubiquinols, and ubiquinones in blood, plasma, tissue homogenates, and subcellular fractions. *Anal. Biochem. 157*: 106-116.

Lea, C.H. 1960. On the antioxidant activities of the tocopherols. II. Influence of the substrate, temperature and level of oxidation. *J. Sci. Food Agric. 11*: 212-218.

Lea, C.H. and Ward, R.J. 1959. Relative antioxidant activities of the seven tocopherols. *J. Sci. Food Agric. 10*: 537-548.

Lee, J., Fujimoto, K. and Kaneda, T. 1984. Importance of oxidative degradation on peroxide-decomposing activities of Antarctic krill phospholipids. *Bull. Japan. Soc. Sci. Fish. 50*: 1863-1868.

López, M.C., Satué, M.T., González, M.L. and Agromont, A. 1995. α-Tocopherol content in trout oil. *Food Chem. 53*: 67-70.

Løvaas, E. 1991. Antioxidative effects of polyamines. *JAOCS 68*: 353-358.

Madhavi, D.L., Singhal, R.S., and Kulkarni, P.R. 1996. Technological aspects of food antioxidants. In: *Food Antioxidants. Technological, Toxicological, and Health Perspectives*, D.L. Madhavi, S.S. Deshpande and D.K. Salunkhe (Eds.), Marcel Dekker, New York, pp 159-266.

Marcuse, R. 1962. The effect of some amino acids on the oxidation of linoleic acid and its methyl ester. *JAOCS 39*: 97-107.

Martino, G., Haouet, C., Reali, C., Olivieri, O. and Valfre, F. 1995. Coenzyme Q_{10} and its relationship to other nutritional components of animal products. *Ital. J. Food Sci. 7*: 19-26.

Matsuno, T. and Hirao, S. 1989. Marine carotenoids. In*: Marine Biogenic Lipids, Fats, and Oils*. Vol. I, R. G. Ackman (Ed.), CRC Press, Boca Raton, Florida, pp 251-388.

Meyer, A. S., Isaksen, A. 1995. Application of enzymes as food antioxidants. *Trends Food Sci. Technol. 8*: 198-203.

Miki, W. 1991. Biological functions and activities of animal carotenoids. *Pure & Appl. Chem. 63*: 141-146.

Morris, R.J. and Culkin, F. 1989. Fish. In: *Marine Biogenic Lipids, Fats, and Oils*. Vol. II, R.G. Ackman (Ed.), CRC Press, Boca Raton, Florida, pp 145-178.

Mortensen, A. and Skibsted, L.H. 2000. Antioxidant activity of carotenoids in muscle foods. In: *Antioxidants in Muscle Foods*, E.A. Decker, C. Faustman and C.J. Lopez-Bote (Eds.), Wiley, John & Sons, New York, pp 61-83.

Naguib, Y.M.A. 2000. Antioxidant activities of astaxanthin and related carotenoids. *J. Agric. Food Chem. 48*: 1150-1154.

Nakano, T., Sato, M. and Takeuchi, M. 1992. Glutathione peroxidase of fish. *J. Food Sci. 5*: 1116-1119.

Nettleton, J.A. and Exler, J. 1992. Nutrients in wild and farmed fish and shellfish. *J. Food Sci. 57*: 257-260.

Niki, E. 1996. α-Tocopherol. In: *Handbook of Antioxidants*, E. Cadenas and L. Packer (Eds.), Marcel Dekker, New York, pp 3-25.

Notevarp, O. and Chahine, M.H. 1972. Trace metal contents, chemical properties and oxidative stability of capelin and herring oils produced in Norwegian plants. *JAOCS 49*: 274-277.

Nwosu, C.V., Boyd, L.C. and Sheldon, B. 1997. Effect of fatty acid composition of phospholipids on their antioxidant properties and activity index. *JAOCS 74*: 293-297.

Opstvedt, J. 1971. Fish taints in eggs and poultry meat. In*: University of Nottingham Nutrition Conference for Feed Manuf.* No. 5, H. Swan and D. Lewis (Eds.), Churchill Livingstone, London, pp 70-99.

Opstvedt, J. 1975. Influence of residual lipids on the nutritive value of fish meal. VII. Effect of lipid oxidation on protein quality of fish meal. *Acta Agric. Scand. 25*: 53-71.

O´Keefe, S.F. and Ackman, R.G. 1986. Vitamins A, D_3 and E in Nova Scotian cod liver oils. *Proc. N.S. Inst. Sci. 37*: 1-7.

O´Keefe, S.F. and Noble, R.L. 1978. Storage stability of channel catfish (*Ictalurus punctatus*) in relation to dietary level of α-tocopherol. *J. Fish. Res. Board Can. 35*: 457-460.

Ohshima, T., Fujita, Y. and Koizumi, C. 1993. Oxidative stability of sardine and mackerel lipids with reference to synergism between phospholipids and α-tocopherol. JAOCS 70: 269-279.

Olson, J.A. and Krinsky, N.I. 1995. Introduction: The colorful, fascinating world of the carotenoids: important physiologic modulators. *FASEB J. 9*: 1549-1550.

Palozza, P. and Krinsky, N.I. 1992. β-Carotene and a-tocopherol are synergistic antioxidants. *Arch. Biochem. Biophys. 297*: 184-187.

Palozza, P. and Krinsky, N.I. 1994. Antioxidant properties of carotenoids. In: *Retinoids: From Basic Science to Clinical Applications*, M.A. Livera and G. Vialdi (Eds.), Birkhäuser Verlag Basel, pp 35-41.

Parazo, M.P.M., Lall, S.P., Castell, J.D. and Ackman, R.G. 1998. Distribution of α- and γ-tocopherols in Atlantic salmon (*Salmo Salar*) tissues. *Lipids 33*: 697-704.

Pasquel, L.J. and Babbitt, J.K. 1991. Isolation and partial characterization of a natural antioxidant from shrimp (*Pandalus jordani*). *J. Food Sci. 56*: 143-145.

Petillo, D., Hultin, H.O., Krzynowek, J. and Autio, W.R. 1998. Kinetics of antioxidant loss in mackerel light and dark muscle. *J. Agric. Food Chem. 46*: 4128-4137.

Pokorný, J. 1987. Major factors affecting the autoxidation of lipids. In*: Autoxidation of unsaturated lipids*, H.W.-S. Chan (Ed.), Academic Press, London, pp 141-206.

Ramarathnam, N., Osawa, T., Ochi, H. and Kawakishi, S. 1995. The contribution of plant food antioxidants to human health. *Trends Food Sci. Technol. 6*: 75-82.

Refsgaard, H.H.F., Brockhoff, P.B. and Jensen, B. 1998. Biological variation of lipid constituents and distribution of tocopherols and astaxanthin in farmed Atlantic salmon (*Salmo salar*). *J. Agric. Food Chem. 46*: 808-812.

Regenstein, J.M., Schlosser, M.A., Samson, A. and Fey, F. 1982. Chemical changes of trimethylamine oxide during fresh and frozen storage of fish. In: *Chemistry & Biochemistry of Marine Food Product*s, R.E. Martin, G.J. Flick, C.E. Hebard and D.R. Ward (Eds.), AVI Publishing Company, Westport, Connecticut, pp 137-148.

Ritchie, A.H. and Mackie, I.M. 1980. The formation of diamines an polyamines during storage of mackerel (*Scomber scombrus*). In: *Advances in Fish Science and Technology*, J.J. Connell (Ed.), Fishing News Books Ltd., Farnham, Surrey, pp 489-494.

Sakata, K., Yamamoto, K., and Watanabe, N. 1994. Antioxidative compounds from marine organisms. In: *Food Phytochemicals for Cancer Prevention II. Teas, Spices and Herbs*, C.T. Ho, T. Osawa, M.T. Huang and R.T. Rosen (Eds.), American Chemical Society, Washington DC, pp 164-182.

Sasaki, S., Ohta, T. and Decker, E.A. 1996. Antioxidant activity of water-soluble fractions of salmon spermary tissue. *J. Agric. Food Chem. 44*: 1682-1686.

Schiedt, K., Leuenberger, F.J. and Vecchi, M. 1981. Natural occurrence of enantiomeric and meso-astaxanthin. 5. Ex wild salmon (*Salmo salar* and *Oncorhynchus*). *Helv. Chim. Acta. 64*: 449-457.

Schuler, P. 1990. Natural antioxidants exploited commercially. In: *Food Antioxidants*, B.J.F. Hudson (Ed.), Elsevier Applied Science, London, pp 99-170.

Scott, G. 1997. Chain-breaking antioxidants. In: *Antioxidants in Science, Technology, Medicine and Nutrition*, G.Scott (Ed.), Albion Publishing, Chichester, pp 80-92.

Segawa, T. Hara, S. and Totanai, Y. 1994. Antioxidative behavior of phospholipids for polyunsaturated fatty acids of fish oil. II. Synergistic effect of phospholipids for tocopherol. *J. Jpn. Oil Chem. Soc. 46*: 515-519.

Seher, V.A. and Löschner, D. 1985. Natural antioxidants V: Antioxidants and synergists of Antarctic krill. *Fette Seifen Anstrichm. 87* (11): 454-457.

Seymour, T.A., Li, S.J. and Morrissey, M. 1996. Characterization of natural antioxidant from shrimp shell waste. *J. Agric. Food Chem. 44*: 682-685.

Shahidi, F. and Amarowicz, R. 1996. Antioxidant activity of protein hydrolyzates from aquatic species. *JAOCS 73*: 1197-1199.

Shahidi, F., Metusalach and Brown, J.A. 1998. Carotenoid pigments in seafood and aquaculture. *Crit. Rev. Food Sci. 38*: 1-67.

Shewfelt, R.L. 1981. Fish muscle lipolysis - a review. *J. Food Biochem. 5* (2): 79-100.

Shewfelt, R.L., McDonald, R.E. and Hultin, H.O. 1981. Effect of phospholipid hydrolysis on lipid oxidation in flounder muscle microsomes. *J. Food Sci. 46*: 1297-1301.

Shibamoto, T. and Horiuchi, M. 1997. Role of aldehydes in cooked fish flavors. In*: Flavor of Lipid Chemistry of Seafoods*, F. Shahidi and K.R. Cadwallader (Eds.), American Chemical Society, Washington DC, pp 20-30.

Sidwell, V.D., Loomis, A.L., Foncannon, P.R. and Buzzell, D.H. 1978. Composition of the edible portion of raw (fresh or frozen) crustaceans, finfish, and mollusks. IV. Vitamins. *Mar. Fish. Rev. 40*: 1-16.

Sies, H. and Stahl W. 1995. Vitamins E and C, β-carotene, and other carotenoids as antioxidants. *Am. J. Clin. Nutr. 62*(suppl): 1315S-1321S.

Sigurgisladottir, A., Parrish, C.C., Lall, S.P. and Ackman, R.G. 1994. Effects of feeding natural tocopherols and astaxanthin on Atlantic salmon (*Salmo salar*) fillet quality. *Food Res. Int. 27*: 23-32.

Sikorski, Z.E., Kolakowska, A. and Pan, B.S. 1990. The nutritive composition of the major groups of marine food organisms. In: *Seafood: Resources, Nutritional Composition and Preservation*, Z.E. Sikorski (Ed.), CRC Press, Boca Raton, Fl., pp 29-54.

Sikorski, Z.E., Kostuch, S., Olley, J. 1976. Protein changes in frozen fish. *CRC Critic. Rev. Food Sci. Nutr. 8*: 97-129.

Simpson, K.L. 1982. Carotenoid Pigments in Seafood. In: *Chemistry & Biochemistry of Marine Food Products*, R.E. Martin, G.J. Flick, C.E. Hebard and D.R. Ward (Eds.), AVI Publishing Company, Westport, Connecticut, pp 115-136.

Simpson, K.L., Katayama, T. and Chichester, C.O. 1981. Carotenoids in fish feeds. In: *Carotenoids as Colorants and Vitamin A Precursors*, J.C. Bauernfeind (Ed.), Academic Press, New York, pp 463-538.

Smith, G. and Hole, M. 1991. Browning of salted sun-dried fish. *J. Sci. Food Agric*. *55*: 291-301.

Sotelo, C.G., Gallardo, J.M., Pineiro, C. and Pérez-Martin, R. 1995. Trimethylamine oxide and derived compounds´ changes during frozen storage of hake (*Merluccius merluccius*). *Food Chem. 53*: 61-65.

Spinelli, J. and Dassow, J.A. 1982. Fish proteins: Their modification and potential uses in the food industry. In: *Chemistry & Biochemistry of Marine Food Products*, R.E. Martin, G.J. Flick, C.E. Hebard and D.R. Ward (Eds.), AVI Publishing Company, Westport, Connecticut, pp 13-37.

Spurvey, S., Pan, B.S. and Shahidi, F. 1998. Flavour of shellfish. In: *Flavor of Meat, Meat Products and Seafoods* 2.ed., F. Shahidi (Ed.), Blackie Academic & Professional, London, pp. 158-196.

Storebakken, T. and No, H.K. 1992. Pigmentation of rainbow trout. *Aquaculture 100*: 209-229.

Storozhok, N.M. 1985. Tocopherol content of whitefish and its products. *Voprosy Pitaniya 1*: 73-74.

Symons, M.C.R. and Gutteridge, J.M.C. 1998. Superoxide, peroxides, and iron in biological systems In: Free Radicals and Iron: *Chemistry, Biology, and Medicine*, M.C.R. Symons and J.M.C. Gutteridge (Eds.), University Press, Oxford, pp 113-137.

Syvaojaa, E. L. and Salminen, K. 1985. Tocopherols and tocotrienols in Finnish foods: Fish and fish products. *JAOCS 62*: 1245-1248.

Tacon, A.G.J. 1981. Speculative review of possible carotenoid function in fish. *Prog. Fish-Cult. 43*: 205-207.

Taylor, M.J., Richardson, T. and Jasensky, R.D. 1981. Antioxidant activity of amino acids bound to Trolox-C. *JAOCS 58*: 622-626.

Terao, J. 1989. Antioxidant activity of β-carotene-related carotenoids in solution. *Lipids 24*:659-661.

Tokunaga, T. 1970. Trimethylamine oxide and its decomposition in the dark muscle of fish. I. TMAO, TMA and DMA contents in ordinary and dark muscles. *Bull. Jpn. Soc. Sci. Fish. 36*: 502-509.

Tsukunda, N. and Amano, K. 1968. Studies on the discoloration of red fishes - V. Enzyme involved in the discoloration of carotenoid pigments in fish skin tissues. *Bull. Jpn. Soc. Sci. Fish. 34*: 633-639.

Undeland, I. 1997. Lipid oxidation in fish - causes, changes and measurements. In: *Methods to Determine the Freshness of Fish in Research and Industry*, G. Olafsdóttir, J. Luten, P. Dalgaard, M. Careche, V. Verrez-Bagnis, E. Martinsdóttir, K. Heia (Eds.), International Institute of Refrigeration. Paris, France, pp 241-257.

Undeland, I. 1998a. Investigation of antioxidant stability in herring fillets during ice storage. In: *Final report of the concerted action "Evaluation of fish freshness" AIR3 CT94 2283, December 1997- April 1998*, G. Ólafsdóttir and E. Martinsdóttir (Eds.), Icelandic Fisheries Laboratories, Reykjavík, Report IFL 12, Appendix 4, pp 1-13.

Undeland, I. 1998b. Lipid oxidation in fillets of herring (*Clupea harengus*) during processing and storage. (Ph. thesis), Department of Food Science, Chalmers University of Technology and SIK- The Swedish Institute for Food and Biotechnology, Göteborg.

Vaskovsky, V.E. 1989. Phospholipids. In: *Marine Biogenic Lipids, Fats, and Oils*. Vol. I, R.G. Ackman (Ed.), CRC Press, Boca Raton, Florida, pp 199-242.

Venugopal, R. and Shahidi, F. 1996. Structure and composition of fish muscle. *Food Rev. Int. 12* (2): 175-197.

Waagbø, R.K., Sandnes, K., Torrissen, O., Sandvin, A., and Lie, O. 1993. Chemical and sensory evaluation of fillets from Atlantic salmon (*Salmo salar*) fed tree levels of n-3 polyunsaturated fatty acids at two levels of vitamin E. *Food Chem. 46*: 361-366.

Watanabe, T. 1982. Lipid nutrition in fish. *Comp. Biochem. Physiol. 73B*: 3-15.

Watanabe, T. and Takeuchi, M. 1989. Implications of marine oils and lipids in aquaculture. In: *Marine Biogenic Lipids, Fats and Oils*, Vol I, R.G. Ackman (Ed.), CRC Press, Boca Raton, Florida, pp 457-479.

Weber, C., Bysted, A. and Hølmer, G. 1997. The coenzyme Q_{10} content of the average Danish diet. *Int. J. Vit. Nutr. Res. 67*: 123-129.

Yanishlieva, N.V., Aitzetmüller, K. and Raneva, V.G. 1998. β-Carotene and lipid oxidation. *Fett/Lipid 100*: 444-462.

Zhou, S., Decker, E.A. 1999. Ability of carnosine and other skeletal muscle components to quench unsaturated aldehydic lipid oxidation products. *J. Agric. Food Chem. 47*: 21-55.