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Review

Beneficial effects and oxidative stability of omega-3 long-chain polyunsaturated fatty acids

Elmira Arab-Tehrany*, Muriel Jacquot, Claire Gaiani, Muhammad Imran, Stephane Desobry and Michel Linder

LiBio, Nancy Université, Laboratoire d'Ingénierie des Biomolécules, 2 avenue de la Forêt de Haye, B.P. 172, 54505 Vandoeuvre Les Nancy Cedex, France (Tel.: +33 0383 59 58 77; fax: +33 0383 59 58 04; e-mail: elmira.arab-tehrany@ensaia.inpl-nancy.fr)

Omega-3 Polyunsaturated fatty acids (n-3 PUFAs), especially long-chain eicosapentaenoic (EPA) and docosahexaenoic (DHA) fatty acids, exert a strong positive influence on human health. At present, fish oil is the major source of omega-3.

EPA and DHA are highly susceptible to lipid oxidation Lipid oxidation of fish oil and other PUFA-rich foods is a serious problem that often leads to loss of shelf-life, consumer acceptability, functionality, nutritional value, and safety. In this review, some beneficial effects of omega-3 fatty acids are presented. In addition, some approaches used to protect PUFAs such as antioxidants, microencapsulation and modified atmosphere packaging are reviewed.

Introduction

Interest in utilizing oils in sea food products is mainly due to their high content of long-chain polyunsaturated fatty acids (PUFAs). Fish are a source of omega-3 fatty acids, especially long-chain docosahexaenoic acid (DHA, C22:6n-3) and eicosapentaenoic acid (EPA, C20:5n-3) (Kolakowska, Zienkowicz, Domiszewski, & Bienkiewicz, 2006). Three characteristic features of fish oil make it unique compared to other commercially available oils. These are (i) the high degree of unsaturation and number of carbon atoms of the constituent fatty acids; (ii) the high content of the long-chain omega-3 type PUFAs; and (iii) the great number and variety of fatty acids present in the triacylglycerols (Ackman & Lamothe, 1989).

Depending on age, season, fish species and area of living, fish oils may contain 100–300 g kg^{-1} of omega-3 polyunsaturated fatty acids. Low-temperature acclimatization and transfer to seawater result in an increase in n-3 PUFAs (Kolakowska *et al*., 2006).

In Table 1 the values of the total SFAs (Saturated fatty acids), MUFAs (Monounsaturated fatty acids), PUFAs (Polyunsaturated fatty acids), n-6 PUFAs (omega-6 Polyunsaturated fatty acids), n-3 PUFAs (omega-3 Polyunsaturated fatty acids), and the fatty acid composition of 5 fish species are shown. The fish containing the highest amount of total lipids are salmon and halibut, with respective average levels of 13.5 and 11.7 g of lipids for 100 g of fish. Marckerel and sardine also contain important amounts of lipids (7.1 and 5.7 g/100 g) but unlike the fish mentioned previously, the levels are not homogeneous between the different zones. These differences may be explained by the difference of size observed during the sampling, a seasonal effect, different provisioning origins or a reproduction period dependent on the region. However, the successful production of food products enriched with n-3 PUFAs, both DHA and EPA, is impeded by their high susceptibility toward oxidative deterioration (Romeu-Nadal, Castellote, & López-Sabater, 2004). Oxidation has negative, both nutritional and organoleptic, consequences; namely, changes in nutritional value of products such as the destruction of essential fatty acids and the lipid-soluble vitamins A, D, E, and K; decrease in caloric content; rancidity which produces off-flavors and pronounced odors; color changes such as darkening of fats and oils and lightening of pigments, as well as flavor loss.

Oxidation of PUFAs produces a complex mixture of volatile secondary oxidation products, and these cause particularly objectionable off-flavors (Let, Jacobsen, & Meyer, 2005).

The main objectives of this review are to better understand some health aspects, stabilization mechanisms of PUFAs in controlling lipid oxidation.

* Corresponding author.

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Table 1. Mean fatty acid composition of fresh fish (mg/100 g).[a]

Fatty acid	Halibut	Mackerel	Salmon	Sardine	Tuna	Anglerfish	Cod
n[b] Lipids (g/100 g)	11.7	7.07	13.5	5.72	0.73	0.21	0.30
C12:0	–	–	–	–	–	–	–
C14:0	366	179	640	179	6	8	1
C14:1 (n-5)	–	–	7	–	–	–	–
C15:0	–	10	8	11	1	–	–
C16:0	1832	1198	2472	1200	109	32	45
C16:1 (n-7)	808	145	576	197	10	6	2
C18:0	295	327	360	276	53	12	10
C18:1 (trans)	109	15	248	–	–	1	2
C18:1 (cis, n-9)	1544	1258	2204	808	94	18	19
C18:1 (cis, n-7)	329	–	74	3	1	2	2
C18:2 (n-6, LA)	75	145	577	37	12	1	1
C18:3 (n-6)	–	–	13	4	1	–	–
C18:3 (n-3, ALA)	9	58	174	40	3	–	–
C18:4 (n-3)	1296	343	672	146	4	1	5
C20:0	143	120	105	42	3	–	–
C20:2 (n-6)	–	–	–	–	–	–	–
C20:4 (n-6, AA)	116	114	81	89	18	6	7
C20:5 (n-3, EPA)	969	662	1112	638	35	26	28
C22:5 (n-3, DPA)	285	118	349	178	7	2	4
C22:6 (n-3, DHA)	1400	1404	2164	1269	131	37	75
SFA[c]	**3041**	**1867**	**4006**	**1779**	**187**	**52**	**57**
MUFA[c]	**3069**	**1436**	**3237**	**1081**	**113**	**33**	**24**
PUFA[c]	**4186**	**2845**	**5146**	**2407**	**211**	**71**	**121**
LC-PUFA (n-3)[c]	**3960**	**2585**	**4472**	**2270**	**179**	**66**	**112**
LC-PUFA (n-6)[c]	**191**	**259**	**671**	**130**	**31**	**7**	**8**

Data from Sirot, Oseredczuk, Bemrah-Aouachria, Volatier, and Leblanc (2008).
[a] A dash means the value is under the LOQ (0.1 g/100 g of total lipids).
[b] The totals for SFA, MUFA, PUFA, and LC-PUFA include all the analyzed fatty acids (all the 48 analyzed fatty acids do not appear in the table).
[c] Carbon.

Health effects of PUFAs

The beneficial effects of omega-3 fatty acids are mainly seen with fatal cardiovascular disease. More specifically, the most pronounced effects of omeg-3 fatty acids are shown on sudden death (Brouwer, 2008).

Intake of EPA and DHA may prevent cardiovascular diseases (CVD) (Kris-Etherton, Harris, & Appel, 2002) and reduce the symptoms in rheumatoid arthritis. Furthermore, (n-3) PUFAs plays a role in preventing the promotion and progression stages of some types of cancer. For example, the ethyl ester of eicosapentaenoic acid has been used in the treatment of arteriosclerosis and hyperlipidemia since 1990 in Japan. Docosahexaenoic acid plays a role in the prevention of a number of diseases in humans, including cardiovascular disease (Schram *et al.*, 2007), inflammation (Mickleborough, 2009), and cancer (Schram *et al.*, 2007). Far more studies have investigated the effect of n-3 PUFAs on blood pressure, in an attempt to identify how these fatty acids influence CVD risk in light of their neutral effect on blood cholesterol. Such studies suggest a blood pressure-lowering effect of 2–3 g/day of long-chain n-3 PUFAs in individuals with hypertension. Guillot *et al.* (2009) showed that low consumption of DHA could be an effective and nonpharmacological way to protect healthy men from platelet-related cardiovascular events.

In addition, recent research has suggested that (n-3) PUFA may have a positive effect in the treatment of depression and schizophrenia (Schram *et al.*, 2007). Additionally DHA is essential for the development of brain, mammalian nervous system, eye development in fetus and infants (Kolanowski, Jaworska, Weiβbrodt, & Kunz, 2007). DHA is the predominant type of long-chain polyunsaturated fatty acids in the brain and represents around 15% of total fatty acids in that tissue. DHA is contained in the phospholipids that build the structure of neuronal membranes. Most of its accumulation occurs during late prenatal and early postnatal development, coinciding with the formation of synapses (Green, Glozman, Kamensky, & Yavin, 1999). Adequate dietary availability of DHA during this period is essential for optimal central nervous system development and functioning. More recently, epidemiological studies (Larrieu, Letenneur, Helmer, Dartigues, & Barberger-Gateau, 2004) have suggested that high fish consumption is inversely associated with cognitive impairment, cognitive decline, and/or development of dementia or Alzheimer's disease (AD).

Eicosapentaenoic acid (EPA) may play a more important role in cardiovascular and immunological health (Ryan *et al.*, 2010). Most recently, in the prospective, randomized open-label, Japan EPA Lipid Intervention Study (JELIS), patients who were randomly assigned 1800 mg of EPA daily (with statin) had a 19% relative reduction in major coronary events compared with those who received the statin only. Although only EPA was used in this study, it appears that it has benefits in preventing major coronary events and especially non-fatal coronary events. This dose, however, is much greater than would typically be obtained by consuming fish (Kris-Etherton, Grieger, & Etherton, 2009). Khan *et al*. (2005, pp. 172–173) showed that eicosapentaenoic acid (EPA) has inhibitory effects against the inflammatory symptoms of edema, erythema, and blood flow. Additionally, intervention studies have revealed that the administration of EPA for 6 months to Alzheimer-type and cerebrovascular dementia patients improves cognitive function (Otsuka & Ueki, 2001).

DHA in infant nutrition

This is becoming an increasing challenge with the introduction of many nutraceuticals and functional foods, a prime example being the debate surrounding the introduction of long-chain polyunsaturated fatty acids (LC-PUFAs) into infant formulas (Gibson & Makrides, 2000).

Whether long-chain polyunsaturated fatty acids should be added to infant formula is one of the most debated issues in infant nutrition. The debate concerns two families of non-interconvertible fatty acids of the n-3 and n-6 series (Innis, 1992). Lucas *et al*. (1999) showed that addition of n-3 and n-6 LC-PUFAs to infant formula milk during the first 6 months promotes long-term cognitive and motor development, without adverse consequences.

The role of DHA in infant nutrition is important to visual and neural development in term breast-fed infants (Innis, 2007a; Uauy, Hoffman, Peirano, Brich, & Brich, 2001). A 14-month observation thus may have been a transient effect of DHA supplementation on early vocabulary development (Auestad *et al*., 2003). The findings suggest that early LC-PUFA status may be important in terms of optimizing brain development and would imply that the average DHA status of pregnant women has specific relevance for brain development of their infants (Krabbendam, Bakker, Hornstra, & van Os, 2007). Accretion of DHA in the infant brain is dependent on DHA status, intake, and metabolism (Jørgensen, Kjær Nielsen, Fleischer Michaelsen, Lund, & Lauritzen, 2006). High levels of docosahexaenoic acid are found in the gray matter of the cerebral cortex and in the retina, and it seems as if the availability of long-chain PUFAs may be limiting cerebral development. Infant visual acuity at 2 months of age is significantly and positively associated with maternal DHA status at 36 weeks of gestation (Lacroix, 2007). The proportion of DHA in erythrocyte total lipids of infants breast-fed by vegans was 1.9% compared with 3.7% in infants fed a milk formula containing butterfat as the sole source of fat and 6.2% in infants breast-fed by omnivores at 14 weeks postpartum (Sanders & Reddy, 1992). The DHA + EPA dose would be based on doses previously found to be safe for the mother and fetus and to significantly reduce recurrence risk of preterm delivery (about 2–3 g/day of DHA + EPA from fish oil). Maternal DHA + EPA supplementation during pregnancy may also offer additional advantages for schizophrenic mothers and their offspring (McNamara & Carlson, 2006). The dietary fat intake in pregnancy and lactation (energy%) should also be recommended for the general population; pregnant and lactating women should aim to achieve an average daily dietary intake of at least 200 mg DHA; or intakes of up to 1 g/d DHA or 2.7 g/d n-3 long-chain PUFA (Koletzko, Cetin, & Brenna, 2007).

Approximately 4 g DHA could be obtained *via* mobilization of 4 kg adipose tissue accumulated during pregnancy. Based on these values, estimates of DHA requirements for pregnancy and lactation are therefore 23 g over a 15-month period. The results show a gap between requirement and provision of approximately 17.5 g DHA for each single pregnancy (Williams & Burdge, 2006).

Consumption of n-3 fatty acids and recommendations

The intake of total omega-3 fatty acids in the United States is approximately 1.6 g/d and only 0.1–0.2 g/d comes from EPA and DHA (Kris-Etherton *et al*., 2000). Typical recommendations from the World Health and North Atlantic Treaty Organizations are 0.3–0.5 g/d of EPA + DHA (Kris-Etherton *et al*., 2002). In the United States, DHA per capita disappearance from fish is 0.25 g/d, which is similar to the worldwide average of 0.23 g/d; that of EPA is 0.16 g/d, compared with a worldwide average of 0.15 g/d (Kris-Etherton *et al*., 2000). In Japan, n-3 PUFA intake is about 1–2 g/d (Sugano & Hirahara, 2000). Canada recommends a total n-3 fatty acid intake of 1.2–1.6 g/d (Scientific Review Committee, 1990).

The UK Committee on Medical Aspect of Food and Nutrition Policy recommends that the intake of EPA and DHA be 0.2 g/d or 1.5 g/wk (Kris-Etherton *et al*., 2002). The International Society for the Study of Fatty Acids and Lipids has proposed an adequate intake of EPA plus DHA to be 0.65 mg per day and even more in the case of pregnant and lactating women (Kolanowski, Jaworska, Weißbrodt, & Kunz, 2007).

Women following vegetarian diets have $<0.1\%$ DHA in their milk fat, while women consuming about 80, 130, or 200 mg/d DHA had 0.17%, 0.32% and 0.5% DHA (Innis, 2007b).

An increasing amount of evidence complied over the past 30 years supports the nutritional benefits of long-chain polyunsaturated fatty acids in human milk and as additives to infant formulas. LC-PUFAs are essential for energy and growth, organ differentiation, and immune function, and cellular metabolism of preterm and term infants (Let, Jacobsen, & Meyer, 2007b).

Oxidation of omega-3 LC-PUFA

Lipid oxidation of fish oil and other PUFA-rich foods is a serious problem that leads to loss of shelf-life, consumer acceptability, functionality, nutritional value, and safety.

Polyunsaturated fatty acids oxidation affects the quality and nutritional value of foods (Dacaranhe & Terao, 2001). Consequently, the presence of fatty acid oxidation products in human foods, especially the aldehydes have been implicated in aging, mutagenesis, and carcinogenesis (Kähkönen *et al*., 1999; Kampa, Niffi, Notas, & Castanas, 2007). The toxicity of these aldehydes such as malondialdehyde (MDA) and 4-hydroxy-2-nonenal (4-HNE) is due to their ability to crosslink to proteins and bind covalently to nucleic acids (Nair, Cooper, Vietti, & Turner, 1986).

Access to oxygen and light, surface area, heating, and irradiation accelerate lipid oxidation, decreasing stability and shelf-life of products containing fish oil. However, the low oxidative stability of polyunsaturated marine n-3 fatty acids calls for effective antioxidant protection to avoid oxidative deterioration and off-flavor development of such oil-enriched foods (Jacobsen *et al*., 2001).

Lipid oxidation causes 3 main problems: it gives rise to the formation of objectionable off-flavors, it reduces the nutritional value of lipid-containing food products, and free radicals formed during oxidation may participate in development of atherosclerosis (Jacobsen *et al*., 1999).

When fish oils oxidize, they produce unstable intermediary compounds, such as free radicals and hydroperoxides, which are susceptible to further decomposition into products such as aldehydes and ketones (Valero, Villamiel, Miralles, Sanz, & Martínez-Castro, 2001). During peroxidation of unsaturated fatty acids, a complex mixture of secondary lipid oxidation products (alkanes, alkenes, aldehydes, ketones, and so on) is generated (Vichi, Pizzale, Conte, Buxaderas, & López-Tamames, 2003). Current assays to assess food oxidative rancidity involve the measurement of hydroperoxides for the determination of primary oxidation products and low-molecular-weight aldehydes for secondary products (Romeu-Nadal, Chávez-Servín, Castellote, Rivero, & López-Sabater, 2007). Pro-oxidants, such as lipoxygenases, singlet oxygen, and transition metals accelerate lipid oxidation. Transition metals, and in particular iron, are naturally present at levels high enough to promote lipid oxidation in many foods (Nuchi, McClements, & Decker, 2001).

Oxidation of fish oil, as other PUFA-containing lipids, involves the reaction of unsaturated fatty acids with oxygen and occurs in 3 phases: an initiation or induction phase, a propagation phase, and a termination phase.

The reactive products of the initiation phase react with additional lipid molecule creating other reactive chemical products. This chain reaction, the continuation of further oxidation by propagation phase products, gives rise to the term 'autoxidation' (Kolanowski, Jaworska, & Weißbrodt, 2007). The autoxidation (peroxidation) of lipids (lipid hydroperoxide: LH) in homogeneous solution is a free radical chain reaction (Lagarde, 2010). This is a main mechanism for lipid oxidation, an autocatalytic process initiated by formation of radicals in unsaturated lipids followed by oxygen attack (Frankel, 2005). Hydroperoxides are the primary oxidation products formed and further oxidation, decomposition and polymerization reactions lead to formation of a complex mixture of intermediate and secondary oxidation products which include a multitude of compounds. Non-volatile and volatile compounds, of different molecular weight and polarity, and bearing different oxygenated functions, such as hydroperoxy, hydroxy, aldehyde, epoxy and ketone functions, are formed. Hence great difficulties are normally encountered to evaluate the degree of oxidation (Dobarganes & Márquez-Ruz, 2003).

This phenomenon can be influenced by both intrinsic and extrinsic factors, such as the fatty acid composition, the concentration of pro-oxidants, endogenous ferrous iron, myoglobin, enzymes, pH, temperature, ionic strength, and oxygen consumption (Muggli, 2007).

Stabilization of omega-3 LC-PUFA-containing foods with antioxidants

Lipid oxidation is a highly deteriorative process in foods, as it leads to unacceptable properties for customers and a loss in nutritional value. In addition, oxidation may lead to health disorders such as atherosclerosis and carcinogenesis. Hence, the presence of antioxidants in foods is essential for their quality retention and safety (Koleva *et al*., 2003). Toxicological effects of synthetic antioxidants and consumer preference for natural products have resulted in increased interest in the application of natural antioxidants (Arabshahi, Vishalakshi, & Urooj, 2007). Recently, many attempts have been made to prevent the oxidative deterioration of lipids by using natural antioxidants (Frankel, 1998). Some components in natural products such as vitamin E, carotenoids, flavonoids, anthocyanins, and phenolic compounds are known to function as scavengers in both 1 and 2 oxidation processes (Ahn, Kim, Seo, Choi, & Kim, 2008).

The main antioxidative mechanisms necessary to protect food systems from oxidation are radical scavenging, metal chelation, and oxygen scavenging. Some antioxidants are able to contribute more than one possible mode of action. Furthermore, the polarity and solubility of an antioxidant determine the actual location of the antioxidant in a given food matrix, which again influences the antioxidative efficacy of the antioxidant (Frankel, 2005). Overall, many factors complicate the prediction of antioxidant efficacy in real food systems (Let, Jacobsen, & Meyer, 2007a).

Tocopherol

Vitamin E has been known as an essential nutrient for reproduction since 1922 when Evans and Bishop observed fetal resorption in rats fed with rancid fat (Bolle, Evandri, & Saso, 2002) when 2 compounds with vitamin E activity were isolated and characterized from wheat germ oil in 1936, the name tocopherol "tokos" (childbirth) + "phorein" (bring forth) was assigned (Azzi & Stocker, 2000).

Since the 1940s, it is known that vitamin E is a major lipophilic chain-breaking antioxidant which protects tissue polyunsaturated fatty acids against peroxidation (Buettner, 1993).

Furthermore, a calculation of the vitamin E requirement, using recent nutritional intake data, shows that a reduction in total fat intake with a concomitant increase in PUFA consumption, including EPA and DHA, will result in an increased amount of vitamin E required (Valk & Hornstra, 2000). The American Food and Nutrition Board in 1968 officially recognized the essential nature of vitamin E (Azzi & Stocker, 2000).

Vitamin E includes 8 naturally occurring compounds in 2 classes designated as tocopherols and tocotrienols with different biological activities (Meydani, 1995).

Tocopherols are fat-soluble antioxidants that function as scavengers of lipid peroxyl radicals. Kushi, Folsom, Prineas, Wu, and Bostick (1996) demonstrated that the tocopherol content in food is inversely associated with mortality from cardiovascular disease. In addition, tocopherols, due to their capacity to quench free radical damage, play a putative role in the prevention of Alzheimer's disease and cancer (Ryan, Galvin, O'Connor, & Maguire, 2007). However, several trials of high-dosage vitamin E supplementation have reported non-statistically significant increases in total mortality. There is even evidence that vitamin E supplements could be harmful, although the report has been challenged (Miller *et al.*, 2005). They show that high-dosage (>or = 400 IU/d) vitamin E supplements may increase all-cause mortality and should be avoided.

Vegetables and seed oils including soybean, safflower and corn, sunflower seeds, nuts, whole grains, and wheat germ are the main sources of tocopherols, whereas animal products are generally poor sources of this vitamin. Absorption of vitamin E is dependent upon digestion and absorption of fat (Meydani, 1995).

∝-Tocopherol has the highest biological activity. It is the most effective chain-breaking lipid-soluble antioxidant. The ability of ∝-tocopherol to have an antioxidant, neutral, or pro-oxidant effect in foods depends on temperature, lipid composition, physical state (bulk phase or emulsion) and its concentration.

Although γ-tocopherol makes a significant contribution to the vitamin E content in foods, it is less effective in animal and human tissues (Valk & Hornstra, 2000).

Serfert, Drusch, and Schwarz (2009) used α-tocopherol as a chemical stabilizer of oils rich in long-chain polyunsaturated fatty acids during homogenization, microencapsulation and storage. Horn, Nielsen, and Jacobsen (2009) were studied the effects of caffeic acid, ascorbyl palmitate and γ-tocopherol on the protection of fish oil-enriched energy bars against lipid oxidation during storage for 10 weeks at room temperature.

Polyphenols

Bioactive compounds, particularly polyphenols found in fruits, vegetables, herbs, and other plants, have been shown to have possible health benefits with antioxidative, anticarcinogenic (Kalogeropoulos, Mylona, Chiou, Ioannou, & Andrikopoulos, 2007; Simopoulos, 2001), anti-inflammatory (Tapiero, Tew, Nguyen, & Mathe, 2002), and angiogenesis inhibitory activities (Yoo, Lee, Lee, Moon, & Lee, 2008). They show protective effects on brain degenerative processes (Conte, Pellegrini, & Tagliazucchi, 2003) and cardioprotective (Simopoulos, 2001; Tapiero *et al.*, 2002) effects and inhibition of platelet-activating factor activity (Verzelloni, Tagliazucchi, & Conte, 2007).

Polyphenols are multifunctional antioxidants by acting as reducing agents, hydrogen donating antioxidants and singlet oxygen quenchers (Rice-Evans, Miller, & Paganga, 1996). The dietary intake of these polyphenols has been estimated to be from 20 mg to 1 g per day.

Flavonoids have been shown to be highly effective scavengers of most types of oxidizing molecules, including singlet oxygen and various free radicals that are probably involved in several diseases. The mechanism of antioxidant action can include suppressing reactive oxygen species formation, either by inhibition of enzymes or by chelating trace elements involved in free radical production, scavenging reactive species, and upregulating or protecting antioxidant defenses (Montero, Giménez, Pérez-Mateos, & Gómez-Guillén, 2005).

Flavonols are a sub-group of flavonoids, found ubiquitously in fruits, vegetables and many medicinal and aromatic plants (Rupasinghe, 2008). Quercetin, a common flavonol, has been shown as an effective antioxidant in several *in vitro* systems such as the oxygen radical absorbance capacity (ORAC) (Ou, Hampsch-Woodill, & Prior, 2001). Quercetin has been shown to inhibit lipid oxidation in cereal grains and marine oils rich in PUFA (Montero *et al.*, 2005).

Huber, Rupasinghe, and Shahidi (2009) studied inhibition of oxidation of omega-3 polyunsaturated fatty acids and fish oil by quercetin glycosides.

Rupasinghe, Erkan, and Yasmin (2010) were presented the antioxidant protection of eicosapentaenoic acid and fish oil oxidation by polyphenolic-enriched apple skin extract.

Encapsulation

However, due to the high degree of unsaturation, the n-3 PUFAs are very susceptible to oxidation, resulting in the formation of toxic hydroperoxides, off-flavors, and shorter shelf-life of the products (Kolanowski, Swiderski, & Berger, 1999). This means that fish oil which is incorporated into food components has to be protected against oxidation (Heinzelmann & Franke, 1999). Another approach to protect lipids from oxidation is to microencapsulate the lipid to be protected.

Micoencapsulation can protect polyunsaturated fatty acids from light and heat damage, and suppress or retard their oxidation (Kagami *et al.*, 2003; Minemoto, Fang *et al.*, 2002; Minemoto, Hakamata, Adachi, & Matsuno, 2002).

Encapsulation efficiency and storage stability of omega-3 oil microcapsules are important considerations in the development of appropriate encapsulation systems. This depends on the selection of the encapsulant, the formulation, and processing conditions used for production of microcapsules (Augustin & Hemar, 2009; Gouin, 2004).

Microencapsulation can provide more prolonged shelf-life by protecting oils with encapsulating agent such as milk protein or dextrin, modified cellulose, gelatine, plants gums, modified starch, and others (Ahn *et al*., 2008). However, the selection of an adequate coating material for PUFA depends on its capacity to stabilize and protect the oil from degradation during processing and storage conditions, and it also has to be approved for food use (Quispe-Condori, Saldaña, & Temelli, 2011).

There are a number of techniques used for fish oil microencapsulation, the method with the lowest costs being spray-drying; other techniques are freeze-drying, spray-nozzle and double-coating (Kolanowski, Jaworska, Weißbrodt, & Kunz, 2007; Legako & Dunford, 2010). One limitation of the spray-drying technique is the limited number of wall materials available (Desai & Park, 2005).

Legako and Dunford (2010) studied the effect of spray-nozzle design on fish oil encapsulation efficiency and microcapsule properties. Spray-nozzles utilize multiple fluid channels allowing for mixing of wall and core materials at the point of atomization. Sonic energy has also been employed as a means of atomization. With regard to uniformity of size and shape, microcapsules produced by the 2-channel ultrasonic nozzle were observed to be more uniform in size and shape, determined by particle size distribution and image comparisons. Disadvantages were also observed for ultrasonic nozzle microcapsules having lower oil encapsulating efficiency compared to freeze dried microcapsules at the same core to wall ratio.

Drusch (2007) microencapsulated a fish oil rich in long-chain polyunsaturated fatty acids in a matrix of sugar beet pectin and glucose syrup.

Microencapsulation of fish oil by complex coacervation with gum arabic and gelatine has also been reported. The results showed that it is not easy to dry them by spray-drying since there is too much water in coacervates (Lamprecht, Schäfer, & Lehr, 2001).

Wu, Chai, and Chen (2005) encapsulated fish oil by simple coacervation of hydroxypropyl methylcellulose with spray-drying. Use of microencapsulated fish oil makes possible the production of enriched bread and infant formula (Kolanowski & Laufenberg, 2006).

One disadvantage of this technology is the elevated temperature which is necessary for drying. High temperatures and the presence of oxygen may lead to an increased oxidation of PUFAs so that a drying process at low temperatures (freeze-drying) is expected to be an alternative for the microencapsulation of fish oil (Heinzelmann & Franke, 1999; Heinzelmann, Franke, Jensen, & Haahr, 2000). Studies on microencapsulation of oils rich in LC-PUFAs, like fish oil, with sodium caseinate, dextrins and highly branched cyclic dextrins or modified cellulose are available (Drusch & Schwarz, 2006; Kagami *et al*., 2003).

As shown in Table 2 spray-drying has been largely used for fish oil microencapsulation. It is the most common and cheapest technique to produce microencapsulated food materials. Compared to freeze-drying, the cost of spray-drying method is 30–50 times cheaper (Gharsallaoui, Roudaut, Chambin, Voilley, & Saurel, 2007).

Stabilization of omega-3 LC-PUFA-containing foods with modified atmosphere packaging

The modified atmosphere packaging (MAP) is a technique, which is widely used to extend the shelf-life and to improve the quality of perishable foods including meat, meat products, fish, fruits and fresh-vegetables.

Table 2. Comparison of different ingredients and methods of fish oil microencapsulation.

Authors	Ingredients	Method
Minemoto, Adachi, and Matsuno (1997)	Gum arabic	Spray and freeze-drying
Heinzelmann and Franke (1999)	Lactose and maltodextrin	Spray and freeze-drying
Heinzelmann et al. (2000)	Sodium caseinate, carbohydrate	Freeze-drying
Keogh et al. (2001)	Casein and lactose	Spray-drying
Hogan, O'Riordan, and O'Sullivan (2003)	Sodium caseinate and dextrose equivalence	Spray-drying
Kagami et al. (2003)	Highly branched cyclic dextrin and sodium caseinate	Spray-drying
Kolanowski, Laufenberg, and Kunz (2004)	Methylcellulose Hydroxypropyl methylcellulose	Spray-drying
Fang, Shima, and Adachi (2005)	Gum arabic	Spray-drying
Wu et al. (2005)	Hydroxypropyl methylcellulose	Spray-drying
Augustin, Sanguansri, and Bode (2006)	Sodium caseinate, glucose, glucose syrup	Spray-drying
Drusch, Serfert, and Schwarz (2006)	n-octenylsuccinate Derivatized starch/glucose syrup or terhalose	Spray-drying
Drusch et al. (2006)	n-octenylsuccinate Derivatized starch/glucose syrup	Spray-drying
Kolanowski,Ziolkowski, Weiβbrodt, Kunz, & Laufenberg(2006)	Methylcellulose Hydroxypropyl methylcellulose	Spray-drying
Drusch (2007)	Sugar beet pectin and glucose syrup	Spray-drying
Shaw, McClements, and Decker (2007)	Corn syrup solids	Spray-drying

Oxidation is especially a problem in game meat as it is rich in myoglobin (and thus iron (Fe)) and polyunsaturated fatty acids, and relatively high in protein. These are all pro-oxidants that increase the meat's susceptibility to oxidation (Chaijan, 2008).

Goulas, Chouliara, Nessi, Kontominas, and Savvaidis (2005) studied the microbiological, biochemical and sensory changes of mussels during storage under aerobic, vacuum packaging (VP) and modified atmosphere packaging (MAP) conditions at 4 °C, and to determine shelf-life of mussels under the same packaging conditions using the above assessment parameters.

Aqua-cultured mussels (*Mytilus galloprovincialis*) were obtained from a local culture farm, packaged aerobically under VP and MAP (50%/50% CO_2/N_2: M1, 80%/20% CO_2/N_2: M2, 40%/30%/30% $CO_2/N_2/O_2$: M3), and stored at 4 °C. Quality evaluation was carried out using microbiological, chemical and sensory analyses. Microbiological results revealed that the M2 and VP delayed microbial growth compared with that of air-packaged samples.

Based on odor and taste evaluation, they showed that the M1 and M3 samples remained acceptable until 11–12 days, the M2 samples remained acceptable until 14–15 days while the VP and air-packaged mussel samples remained acceptable until 10–11 and 8–9 days of storage respectively. Based primarily on sensory, but also on biochemical and microbiological parameters determined, M2 gas mixture was the most effective for mussel preservation achieving a shelf-life of 14–15 days.

Gonçalves, López-Caballero, and Nunes (2003) studied the storage of deepwater pink shrimp (*Parapenaeus longirostris*) in modified atmosphere. Two gas mixtures were tested (40% CO_2/30% O_2/30% N_2 and 45% CO_2/5% O_2/50% N_2), combined with sulfites-based pretreatment, in comparison with air storage. The quality of shrimp was evaluated by sensory (black spot presence) and chemical analyses (pH value and nucleotides breakdown products). Generally, both atmospheres preserved the shrimp quality up to 9 days compared with 4–7 days of ice storage (only with pretreatment), although it seems that atmosphere containing 45% CO_2/5% O_2/50% N_2 was the most effective. The mixtures containing 40%–45% CO_2, combined with O_2 and pretreatment with sulfites, were very efficient in the preservation of shrimp quality, contributing to the extension of shelf-life, at least in 2 days, compared with the ice storage (4–7 days), although it seems that the mixture with low O_2 concentration was slightly more effective.

It has been suggested that packaging in modified atmospheres depleted of oxygen may improve color stability and prevent lipid oxidation in chill-stored shrimps (Sivertsvik, 1995).

Bak, Andersen, Andersen, and Bertelsen (1999) presented the effect of modified atmosphere packaging on oxidative changes in frozen stored cold-water shrimp. The purpose of the present study was to investigate the effect of packaging atmosphere, temperature fluctuations and light exposure on frost formation, lipid oxidation, discoloration and meat toughness of shell-on cold-water shrimps during 12 months of frozen storage. The results show that packaging in modified atmosphere resulted in overall better quality in relation to color fading, development of rancid flavor and toughening of the meat. Light exposure influenced both color fading and lipid oxidation negatively. Temperature fluctuations resulted in very pronounced formation of frost in the packages.

Further, MAP has been found to increase the shelf-life of seer fish (*Scomberomorus commerson*) (Yesudhason, Gopal, Ravishankar, Lalitha, & Kumar, 2009, 2010), and Mediterranean swordfish (Kykkidou, Giatrakou, Papavergou, & Kontominas, Savvaidis, 2009).

Summary

There has been considerable interest during the past 3 decades in the highly polyunsaturated marine n-3 fatty acids EPA and DHA due to their reported positive effects on cardiovascular health and visual function. Consumption of the n-3 polyunsaturated fatty acids and fish is believed to be associated with reduced risk of Alzheimer's disease. The oxidative stability of any long-chain polyunsaturated fatty acid and docosahexaenoic acid containing fish varies widely according to their fatty acid composition, the physical and colloidal states of the lipid and the contents of tocopherols and other antioxidants.

Antioxidants, encapsulation or modified atmosphere packaging can be used to delay or avoid the phenomenon of oxidation. Antioxidants, especially the natural ones are of major interest for their benefits on food quality, like the protection against oxidation, and on health in general. The antioxidant effect of flavonoids and tocopherols has already been demonstrated. Quercetin is most abundant of the flavonol-type flavonoid that is found in fruits and vegetables; it exhibits a high antioxidant activity although not being liposoluble.

The use of microencapsulation technology allows the incorporation of sensitive ingredients, such as polyunsaturated omega-3 fatty acids, into foods without affecting the taste, aroma or texture of the food.

One disadvantage of spray-drying technology is the elevated temperature which is necessary for drying. High temperatures lead to an increased oxidation of PUFAs so that a drying process at low temperatures (freeze-drying) is expected to be an alternative for the microencapsulation of fish oil. Spray-drying is rather inexpensive and straightforward.

It can be summarized that the production of dried microencapsulated fish oil by freezing and subsequent freeze-drying offers an opportunity to achieve a product with good oxidation stability. Finally, the modified atmosphere packaging stabilizes both the color and oxidation of products.

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