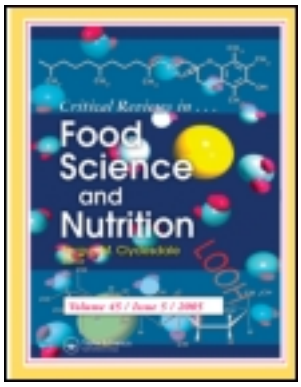

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Fortification of Foods with Omega-3 Polyunsaturated Fatty Acids

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Fortification of Foods with Omega-3 Polyunsaturated Fatty Acids

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A $600 million nutritional supplements market growing at 30% every year attests to consumer awareness of, and interests in, health benefits attributed to these supplements. For over 80 years the importance of polyunsaturated fatty acid (PUFA) consumption for human health has been established. The FDA recently approved the use of ω-3 PUFAs in supplements. Additionally, the market for ω-3 PUFA ingredients grew by 24.3% last year, which affirms their popularity and public awareness of their benefits. PUFAs are essential for normal human growth; however, only minor quantities of the beneficial ω-3 PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are synthesized by human metabolism. Rather PUFAs are obtained via dietary or nutritional supplementation and modified into other beneficial metabolites. A vast literature base is available on the health benefits and biological roles of ω-3 PUFAs and their metabolism; however, information on their dietary sources and palatability of foods incorporated with ω-3 PUFAs is limited. DHA and EPA are added to many foods that are commercially available, such as infant and pet formulae, and they are also supplemented in animal feed to incorporate them in consumer dairy, meat, and poultry products. The chief sources of EPA and DHA are fish oils or purified preparations from microalgae, which when added to foods, impart a fishy flavor that is considered unacceptable. This fishy flavor is completely eliminated by extensively purifying preparations of n-3 PUFA sources. While n-3 PUFA lipid autoxidation is considered the main cause of fishy flavor, the individual oxidation products identified thus far, such as unsaturated carbonyls, do not appear to contribute to fishy flavor or odor. Alternatively, various compound classes such as free fatty acids and volatile sulfur compounds are known to impart fishy flavor to foods. Identification of the causative compounds to reduce and eventually eliminate fishy flavor is important for consumer acceptance of PUFA-fortified foods.

[Supplementary materials are available for this article. Go to the publisher's online edition of Critical Reviews in Food Science and Nutrition for the following free supplemental files: Additional text, tables, and figures.]

Keywords PUFA, ω-3 fatty acid, DHA, EPA, fishy flavor

INTRODUCTION

Dietary fatty acids are a primary energy source for higher mammals. However, beyond providing calories, fatty acids from foods are also biomolecule precursors and become components of biological structures such as cell membranes (Hulbert et al., 2005). As progenitors of a wide variety of biological lipids and participants of myriad signaling pathways, fatty acids play a significant role in survival and well being of mammalian life (Hwang and Rhee, 1999). Fatty acids are usually derived from hydrolysis of triacylglycerols consumed as part of our diet and whether from plant or animal source, consist of straight chain, saturated molecules with single C–C bonds only (Gunstone, 1996), unsaturated fatty acids with one or more double bonds (C=C), or even branched chain fatty acids (Christie, 1995).

Fatty acids with multiple unsaturated double bonds are collectively termed polyunsaturated fatty acids (PUFAs) and have been shown to provide additional benefits for human health beyond energy (Takahata et al., 1998). α-Linolenic acid (ALA) is the most commonly available ω-3 PUFA through dietary oils from both plant and animal sources, whereas eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are available from marine food sources such as fish and fish oils, and algae. The molecular functions of PUFAs and their associated health benefits have been extensively characterized (Tapiero et al., 2002; Ruxton et al., 2004; Fewtrell, 2006). Since humans cannot make ALA, and both EPA and DHA are synthesized from the precursor ALA by metabolism of dietary lipids, all three PUFAs are necessarily acquired from various dietary sources. Considerable efforts have been undertaken to incorporate PUFAs into foods

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Table 1 Types of unsaturated fatty acids

Fatty acid (IUPAC name)	Fatty acid (common name)	Chain length (number of carbons)	Type of fatty acid (based on position of first double bond)	Degree of saturation (number of double bonds)
cis-9-Octadecenoic acid	Oleic acid	18	ω-9	1
cis, cis-9,12-Octadecadienoic acid	Linoleic acid	18	ω-6	2
all-cis-6,9,12-Octadecatrienoic acid	γ-Linolenic acid	18	ω-6	3
cis,cis,cis-9,12,15-Octadecatrienoic acid	α-Linolenic acid	18	ω-3	3
(6Z,9Z,12Z,15Z)-6,9,12,15-Octadecatetraenoic acid	Stearidonic acid	18	ω-3	4
all-cis-5,8,11,14-Eicosatetraenoic acid	Arachidonic acid	20	ω-6	3
all-cis 8,11,14,17-Eicosatetraenoic acid	Eicosatetraenoic acid	20	ω-3	4
(5Z,8Z,11Z,14Z,17Z)-Eicosa-5,8,11,14,17-pentenoic acid	Eicosapentaenoic acid	20	ω-3	5
(Z)-Docos-13-enoic acid	Erucic acid	22	ω-9	1
5Z,8Z,11Z)-Eicosa-5,8,11-trienoic acid	Mead acid	20	ω-9	3
(4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenoic acid	Docosahexaenoic acid	22	ω-3	6

for regular consumption. Such efforts are often deterred by the off-flavors that accompany the sources of lipids containing PUFAs. We highlight here the various health benefits of PUFAs and focus on their inclusion in foods and the associated challenges for their delivery.

Dietary Fatty Acids

Food consumption primarily allows organisms to derive energy, and facilitates metabolic precursor and energetic needs of a vast network of biosynthetic processes that support life (Prentice, 2005; Anderson et al., 2006). Irrespective of hierarchy in the biological realm, some form of carbon is usually ingested in order to derive energy, which for mammalian life, includes three main sources, carbohydrates, amino acids, and lipids (Prentice, 2005). Notably these molecules also possess other functions such as formation of structural components such as cell membranes (Hulbert et al., 2005) or providing building blocks for other functional proteins and genetic material (Saris et al., 1998).

Lipids are the richest sources of energy in mammalian diets (Tataranni and Ravussin, 1997) and are hydrolyzed by digestive lipases at their ester linkages, releasing the component fatty acids and the glycerol backbone (Masoro, 1977). The fatty acids of chain lengths up to 20 carbons are then degraded sequentially to the 2-carbon precursor acetyl-CoA, which is then utilized via the Krebs cycle and oxidized to carbon dioxide. This process yields energy in the form of ATP, with up to 10 ATP generated per acetyl-CoA entering the Krebs cycle. Thus, the sequential degradation of a 6-carbon fatty acid molecule such as caproic acid will yield ~30 ATP molecules. Additionally, acetyl-CoA acts as the precursor for in vivo biosynthesis of lipids, amino acids, and other biomolecules (Harwood, 1988).

Fatty acids present in triacylglycerols are either saturated, i.e., all carbons in the fatty acids are joined by single (C–C) bonds, or unsaturated, i.e., contain double bonds (C=C) between carbons in varying proportions (Gunstone, 1996). One or more double bonds are present in unsaturated fatty acids (mono- or poly-unsaturated, respectively) and the fatty acid chains are usually longer than 10 carbons (Table 1). The position of the first double bond in the alkyl side chain of the fatty acid determines the nomenclature of the PUFAs. For example, *cis, cis*, *cis* -9,12,15-octadecatrienoic acid (trivial name α-linolenic acid) has its first double bond at the third carbon from the alkyl terminal, and is classified as a ω-3 fatty acid. Similarly, *cis,cis* -9,12-octadecadienoic acid (trivial name linoleic acid) has its first double bond at the sixth carbon and is a ω-6 fatty acid. Unsaturated fatty acids that play important roles in human health usually belong to either of the ω-3 or ω-6 groups, while apart from oleic acid, the ω-9 fatty acids are considered detrimental to human health (Table 1).

PUFA Metabolism and Physiological Roles

The fatty acids necessary for an organism are either acquired from its diet or synthesized from acetyl-CoA produced by carbohydrate and amino acid metabolism. The proportions of saturated and unsaturated fatty acids vary by the source of lipids (Table 2). Marine sources such as fish and fish oils are uniquely rich in the ω-3 PUFAs EPA and DHA in varying amounts, while terrestrial sources such as plants, animals, or poultry vary in the types and quantity of saturated/unsaturated fatty acids. The major unsaturated fatty acids in terrestrial sources are oleic and linoleic acid, which account for 50–70% of total unsaturated fatty acids; while ALA content of terrestrial sources ranges between 1 and 100 mg/g of total unsaturated fatty acids (Sheppard et al., 1978; Christie, 1995), EPA and DHA are not normally produced. Dietary lipids are metabolized further to derive the various fatty acids for energy and cellular function.

Both saturated and unsaturated fatty acids released from dietary lipid hydrolysis, undergo multiple metabolic fates in mammals (Figure 1) including β-oxidation to acetyl-CoA, esterification to form other lipids, or desaturation and conversion

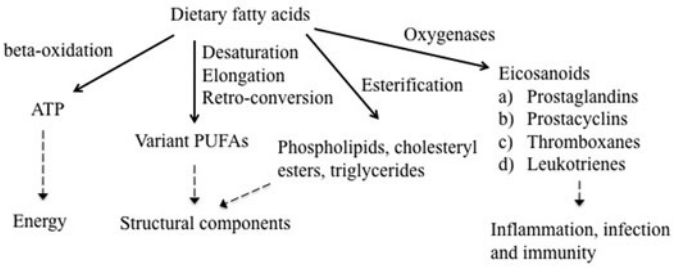

Figure 1 Metabolic fates of dietary fatty acids.

to other fatty acids (Masoro, 1977; Figure 2). Among PUFAs, ALA is mainly degraded by β-oxidation and small amounts are deposited in skin, adipose tissue, and carcass (Sinclair et al., 2002). Arachidonic acid present in tissue is primarily obtained from metabolic conversion of linoleic acid rather than directly from dietary sources and localizes to non-adipose tissue phospholipids (Zhou and Nilsson, 2001). PUFAs also circulate as free fatty acids in blood plasma; however, ALA is found only in limited quantities as it is mostly catabolized via β-oxidation (Arterburn et al., 2006). EPA and DHA, however, increase in plasma levels when supplemented in the diet. As free fatty acids are prone to β-oxidation in various tissues they may just act as substrates for energy.

In contrast to saturated fatty acids, PUFAs are directly incorporated into cell membrane phospholipids, and only deposited in limited amounts into adipose tissue. Membrane PUFAs fluctuate in response to diet while saturated or mono-unsaturated fatty acids remain constant (Hulbert et al., 2005), suggesting

Table 2 Saturated and unsaturated fatty acid content of dietary lipids[1]

	Total fatty acid (%, w/w)	
Lipid source	Saturated	Unsaturated
Coconut oil	86.3	7.9
Palm kernel oil	81.4	12.9
Cow milk fat	67.9	26.1
Butter	62.3	32.6
Palm oil	47.9	47.7
Cottonseed oil	26.1	69.6
Cod liver oil	17.3	77.1
Peanut oil	17.3	77.8
Sesame oil	15.2	80.5
Soybean oil	15.1	80.7
Olive oil	14.2	81.4
Corn oil	12.7	82.8
Safflower oil	9.5	86.3
Rapeseed oil	4.7	87.7
Safflower oil (high oleic)	6.4	89.2

[1]Compiled from Sheppard et al. (1978) and Christie (1995).

that membrane PUFA levels depend on dietary PUFA intake and not biosynthesis. A specific class of enzymes called desaturases participate in interconversion of PUFAs by introducing C=C double bonds. Mammals lack the Δ-12 desaturase enzyme required for PUFA synthesis from oleic acid (Figure 2) and are subsequently incapable of de novo ω-3 and ω-6 PUFA biosynthesis (Passorn et al., 1999). Hence, they depend on dietary sources to obtain the precursors linoleic acid and ALA (Arterburn et al., 2006) to further synthesize other ω-3 and ω-6 PUFAs (Figure 2) or at a last resort synthesize ω-9 PUFAs.

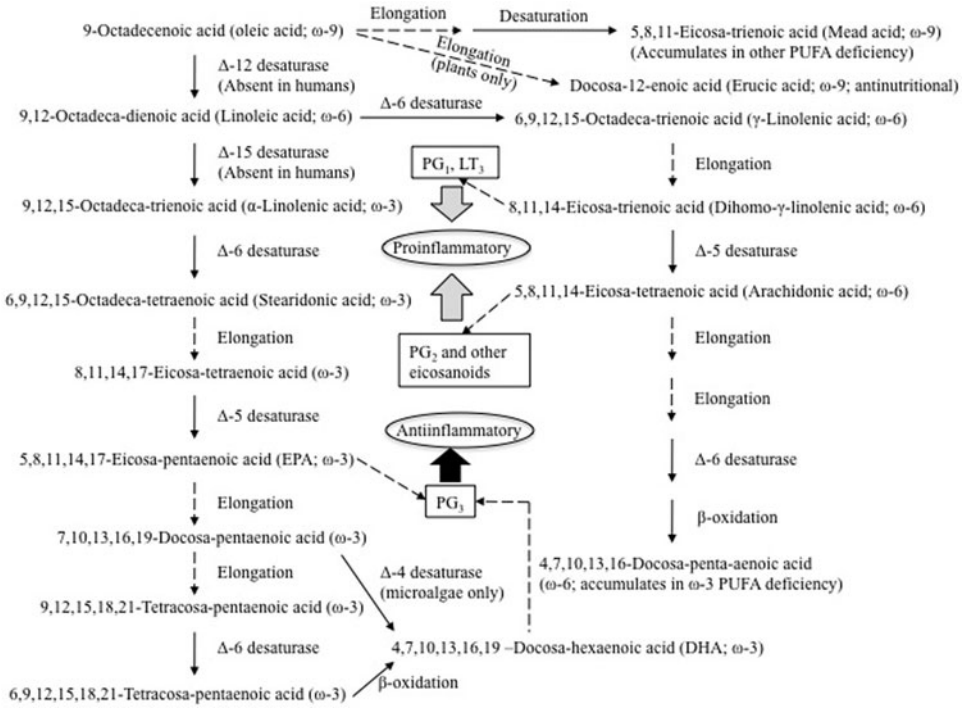

Figure 2 Metabolic pathways of PUFA biosynthesis from dietary unsaturated fatty acids. Solid arrows indicate single enzyme reactions, dashed arrows indicate multi-enzyme processes, and filled arrows point at the biological action of the respective compounds. PG, prostaglandins, LT, leukotrienes.

However, only 4% of ALA consumed by males and 10% in females are converted to EPA or DHA, and the rest is degraded by β-oxidation (Tapiero et al., 2002; Burdge, 2006; Childs et al., 2008).

PUFAs perform several biological roles, mainly being the sole precursors of eicosanoid hormones (Khanapure et al., 2007) that possess different roles targeted toward and against tissue inflammation. Normally, the eicosanoid hormones are generated from arachidonic acid, an ω-6 fatty acid, and consist of the prostaglandin, thromboxane, leukotriene, and lipoxin subgroups (Funk, 2001), but analogues of eicosanoids are also derived from other ω-6 and ω-3 PUFAs (Khanapure et al., 2007). The analogues from ω-3 PUFAs are also known as alternative eicosanoids and named differently, such as resolvins (products from EPA) and docosatrienes, protectins, and neuroprotectins (products from DHA) (Serhan, 2005). As a common set of enzymes is involved in the biosynthesis of various eicosanoids, we will continue to refer to ω-3 PUFA analogues as eicosanoid analogues in this review.

The biosynthesis of eicosanoids proceeds via a series of enzymes that release, cyclize, and oxidize PUFAs. Membrane PUFAs are cleaved from glycerophospholipids by the enzyme phospholipase A2 (Bingham and Austen, 1999) and converted by cycloxygenase into the intermediate prostaglandins G2 and H2. These are eventually converted into the various prostaglandins responsible for inflammatory response by the further action of cycloxygenases and by thromboxane synthase into thromboxane. Alternatively, the cleaved PUFAs are converted by 5-lipoxygenase to an epoxide intermediate, leukotriene A4, which is further converted to three types of leukotrienes depending on whether it is further hydrolyzed, conjugated with glutathione, or transformed into other eicosanoids via the action of lipoxygenases. Lipoxins are generated by the action of 15-lipoxygnase on arachidonic acid.

Apart from energy, hormonal production, and signaling, unsaturated fatty acids are part of the cell membrane structure and aid its fluidity. Cell membranes are composed of two layers of lipids, the inner and outer monolayers, which are asymmetrically distributed and form the lipid bilayer (Brenner, 1984). While the bilayer itself is structurally stable, the membrane lipids constantly move and rearrange themselves within the membrane depending on temperature. The rigidity or flexibility of cell membranes partly depends on the degree of unsaturation of fatty acids forming membrane phospholipids (Brenner, 1984; Quinn et al., 1989). Saturated fatty acids in phospholipids allow them to form a packed, paracrystalline structure with limited motion; while the double bonds in unsaturated fatty acids introduce kinks in the packing, and produce a more flexible cell membrane. The flexibility of the cell membrane hence increases with temperature and with higher levels of PUFAs. The physical interactions of PUFAs with cholesterol in the cell membrane are also associated with membrane fluidity (Wassall and Stillwell, 2009).

PUFAs present in membrane phospholipids also participate in signaling pathways. Longer chain PUFAs, such as arachidonic acid and linoleic acid, participate in signaling pathways, aid the release of calcium, and eventually affect signaling pathways targeting the cell nucleus (Jump and Clarke, 1999). Fatty acids also participate in body temperature regulation via interactions with glycerolipids (Prentki and Madiraju, 2008). Alterations in membrane lipids also change the cell's ability for carrier-mediated transport, alter membrane-bound enzymes, prostaglandin production, and cell growth (Spector and Yorek, 1985), and eventually affect the organism's health.

Health Benefits of ω-3 PUFAs

The ω-3 PUFAs provide a wide range of benefits (Table 3) from general improvements in health to protection against inflammation and disease. DHA and EPA have been used in a number of small clinical trials to understand their efficacy

Table 3 Health benefits of ω-3 PUFAs

Benefit	Fatty acid studied	Subject or model organism	Reference
Lowers insulin resistance	ALA	Human	(Vuksan et al., 2007)
Reduces atherosclerosis	DHA, EPA	Human	(Dyerberg et al., 2004)
Aids neural and brain development	ALA, DHA	Human, rodents, other primates	(Lauritzen et al., 2000; McCann and Ames, 2005)
Anti-tumor	DHA	Human, rat	(Conklin, 2002; Holian and Nelson, 1992)
Prevents apoptosis	DHA, EPA	Rat	(Calviello et al., 1999; German et al., 2006)
Prevents inflammation	ALA	Mouse, rat	(Ren et al., 2007)
Improves bone density	DHA	Human	(Hogstrom et al., 2007)
Alleviates inflammation in cystic fibrosis	DHA, EPA	Human	(De Vizia et al., 2003)
Combats oxidative stress	DHA	Cat, dog, human	(Brown, 2008; Yavin et al., 2002)
Anti-thrombosis	EPA	Human	(Tamura et al., 1992)
Anti-arrhythmia	DHA, EPA	Human	(Lombardi and Terranova, 2007; Nodari et al., 2009)
Immuno-modulation	DHA, EPA	Human	(Yaqoob and Calder, 2007)
Augments neural, vision, and brain functions	DHA, EPA	Human	(Chen et al., 2008; German et al., 2006; Lauritzen et al., 2000; Valentine and Valentine, 2004)
Mitigates fatality from cardiovascular disease	DHA, EPA	Human	(GISSI, 1999)

Note: ALA, alpha-linolenic acid, EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

(Table 3) and shown to possess immunomodulatory properties depending on their localization in different cell types (Yaqoob and Calder, 2007). They also reduce inflammation in many conditions, especially in cystic fibrosis (De Vizia et al., 2003). The ω-3 PUFAs possess anti-thrombotic properties, that in combination with their anti-inflammatory effect, is likely to positively aid cardiovascular disease treatment (Tamura et al., 1992). DHA and EPA also appear to possess anti-cancer and anti-apoptotic effects (Holian and Nelson, 1992; Calviello et al., 1999; Conklin, 2002; German et al., 2006). Additionally, these PUFAs suppress gene expression of lipogenic genes in the liver and trigger adipose fatty acid oxidation, suggesting a potential role against obesity (Nakamura et al., 2004). The ω-3 PUFAs are also shown to be beneficial for vision and cognition abilities as well as brain and neural development (Lauritzen et al., 2000; Valentine and Valentine, 2004; German et al., 2006; Chen et al., 2008).

Similar to ω-3 PUFAs, *trans*-PUFAs found in bovine milk such as conjugated linoleic acid (*cis-trans* isomers of 9:11-octadecadienoic acid) and its mono-unsaturated precursor 9-octadecenoic acid or vaccenic acid also possess anti-carcinogenic effects, inhibit atherosclerosis, increase lean body mass, and prevent airway inflammation (Kanwar et al., 2008). However, *trans*-octadecenoic acids have adverse effects, such as increasing LDL-cholesterol levels (Khosla and Hayes, 1996), which contrasts the benefits of ω-3 PUFAs. These health benefits of ω-3 PUFAs have recently been comprehensively summarized (NCBI, 2009).

The health benefits of ω-3 PUFAs and their anti-inflammatory properties are directly attributable to the eicosanoid hormones that control pro- and anti-inflammatory responses (Calder and Grimble, 2002; Khanapure et al., 2007). As described earlier these hormones are produced from arachidonic acid and other ω-6 PUFAs and transferred into extra cellular space to reach neighboring target cells. Funk (2001) has extensively reviewed eicosanoid synthesis and function. Briefly, inflammatory stimuli such as mechanical trauma, presence of cytokines, or various growth factors, activate phospholipases to release arachidonic acid from cell membrane phospholipids for prostaglandin production (Funk, 2001). Prostaglandin ω-3 analogues synthesized from EPA and DHA are anti-inflammatory and serve to balance the action of arachidinoyl and other ω-6 prostaglandins. Antithrombotic effects of EPA and DHA are linked to their ability to form the corresponding analogues of thromboxane that are either less effective or negate its effects (Whelan, 1996).

The ω-6 PUFAs also possess attributes important for human health; however, the resulting eicosanoid hormones synthesized using ω-6 PUFAs have a pro-inflammatory action. Apart from the monounsaturated oleic acid, other ω-9 fatty acids such as the PUFA mead acid (eicosa-5,8,11-trienoic acid) and monounsaturated erucic acid, do not possess beneficial attributes for mammalian health. Mead acid is synthesized only under abnormal conditions of PUFA metabolism such as when no ω-3 or ω-3 fatty acids are available to the cells for anabolism into needed prostaglandins, while mammals do not synthesize erucic acid (Figure 2).

Notably for human health, the eicosanoids from ω-3 and ω-6 PUFAs possess opposing modes of action, which requires an appropriate ratio of ω-6:ω-3 PUFAs to be maintained for avoiding toxicity and adverse reactions (Calder and Grimble, 2002). For example, contradictory to ω-3 PUFAs, ω-6 PUFAs promote the development of adipose tissue (Ailhaud et al., 2006). Since ω-6 PUFAs are present at higher levels than ω-3 PUFAs in most dietary lipids (Calder and Grimble, 2002), dietary ω-3 PUFAs are necessary to attain a proper balance for long-term health. This ω-6:ω-3 PUFA balance is altered during chronic diseases such as cardiovascular disease, cancer, diabetes, and many other disorders suggesting that alterations in this balance correlate with higher chronic disease risk (Zamaria, 2004). As PUFAs are deemed essential for normal growth and healthy life (Zamaria, 2004; Agostoni, 2008) their inclusion in foods consumed at various stages of life allows a steady supply of PUFAs from infancy to adulthood.

Re-emerging bacterial pathogens and rapid antibiotic resistance development are some of the bigger challenges in controlling infectious disease dissemination. As most bacteria do not produce EPA and DHA these PUFAs may possess antibacterial potential that we can harness for effective infectious disease control. However, studies intended to identify this potential suggest contrasting results. For example gut inflammation is mitigated in the presence of probiotic bacteria for which these bacteria need to adhere to intestinal mucosa. But the ability of probiotics to attach to intestinal cells is limited in the presence of ω-3 PUFAs at concentrations of 10–40 μg/mL (Kankaanpaa et al., 2001); whereas some bacteria adhere better at 5 μg/mL PUFA concentration. The ω-3 PUFAs DHA and EPA increase the minimum inhibitory concentrations of bactericides against *Escherichia coli* by at least 4–6-fold at concentrations of 50–100 μg/mL (Giamarellos-Bourboulis et al., 1994) and also reduce host resistance to *Listeria monocytogenes* (Fritsche et al., 2005). The ω-3 PUFA linolenic acid does possess antibacterial effects against multidrug-resistant *Staphylococcus aureus* at 10 μg/mL (Ohta et al., 1994; Lee et al., 2002). In contrast, Shin et al. (2007) suggest that 30–500-fold higher concentrations of DHA and EPA were required to inhibit 11 food borne pathogens. They also observed that Gram-positive bacteria are susceptible at lower concentrations (MIC 350–500 μg/mL) than Gram-negative bacteria (MIC 1,650–5,000 μg/mL). Potentially these contradictory results are because of physiological differences in susceptibility of bacteria used for testing, in combination with different concentrations and types of fatty acids tested. Further research is needed to critically understand the mechanistic effects of unsaturated fatty acids in bacterial survival and pathogenicity. However, the additional health attributes of ω-3 PUFAs suffice to direct our future efforts toward large-scale clinical studies on ω-3 PUFAs, understanding their mechanisms of action, and identifying mechanisms of their dietary incorporation.

Availability and Fortification of Food and Pharmaceutical Products with ω-3 PUFAs

To obtain the health benefits of ω-3 PUFAs they need to be included in daily diets at appropriate amounts suitable to balance the effects of ω-6 fatty acids. Prior to 2002, only an acceptable intake for ω-3 PUFA ALA was suggested (1.6 g/d ALA for men and 1.1 g/d ALA for women) (Trumbo et al., 2002), while recent developments in ω-3 PUFA research have led to recommendations for dietary intakes of ω-3 PUFAs EPA and DHA (0.25–0.5 g/d of EPA and DHA; Harris et al., 2009). Current population surveys of PUFA consumption suggest that the average intake of EPA and DHA in North America is only 0.1 g/d (Ervin et al., 2004; Wang et al., 2004). Based on dietary recommendations, North American EPA and DHA intake levels need to be increased 5–15-fold, for which food sources rich in these PUFAs must be regularly consumed along with augmenting the production of EPA- and DHA-enriched foods.

The earliest known dietary sources of ω-3 PUFAs are fish and fish oil (Willie and Gonus, 1988). Marine microalgae consumed by fish and marine bacteria produce either EPA or DHA as part of their cell membrane lipids (Table 3) that eventually accumulate in fish lipids (El Abed et al., 2008). Other PUFAs are found in a wide range of fungal, plant, and animal sources (Table 3). This includes common dietary plant oils (linseed, rape seed, soybean, flaxseed, mustard) that contain linoleic and ALA as the major PUFAs, but not EPA and DHA. ALA is metabolized into EPA via desaturation and elongation (Figure 3). Since humans convert ALA into EPA and DHA with low efficiency (Carlier et al., 1991), dietary supplementation of EPA and DHA is necessary to acquire their health benefits. Also, vegans and vegetarians need supplemented foods in absence of botanical sources of EPA and DHA.

The availability of novel dietary sources of ω-3 PUFAs is extensively documented (Whelan and Rust, 2006), but whether these sources provide sufficient amounts of ω-3 PUFAs has not been affirmed. Natural sources of PUFAs rather than fortified foods have been recommended (Kris-Etherton and Hill, 2008) for optimal health benefits. However these recommendations can be universally achieved only by widespread availability of foods inherently rich in EPA and DHA and due to other regional, dietary, and socio-cultural preferences, supplementation of these PUFAs via commonly consumed foods is necessary.

Challenges in PUFA Fortification

In order to provide adequate PUFAs, the level of their fortification into foods needs extensive consideration. At least 0.5 g/d of ω-3 PUFAs EPA and DHA are recommended for daily consumption (Harris et al., 2009), while preferred intake levels are 2–3-fold higher (Trumbo et al., 2002). Even at 0.5 g/d, considering that dietary guidelines recommend fat only providing 20–35% of calories consumed (HHS and USDA, 2005), for an average adult male consuming 2,500 cal/d, 0.5–1% of total fat must comprise of EPA or DHA or both. When lesser amounts of lipids are consumed due to caloric restrictions, the percentage of lipid ω-3 PUFAs required for supplementation increases, as also with less effective ω-3 PUFAs such as ALA and stearidonic acid, and varies by the types of other non-ω-3 fatty acids attached to the glycerol backbone. For example, consumers that follow a reduced- or low-fat diet may require that up to 3–10% of dietary lipids consist of ω-3 PUFAs alone. The sources of ω-3 PUFAs are also critical, as fish oils are not prevalently used as dietary lipids, and a broader range of fat-containing foods must be fortified to achieve the 0.5 g/d target. In the following sections we will address current availability and challenges in ω-3 PUFA inclusion for different dietary sources.

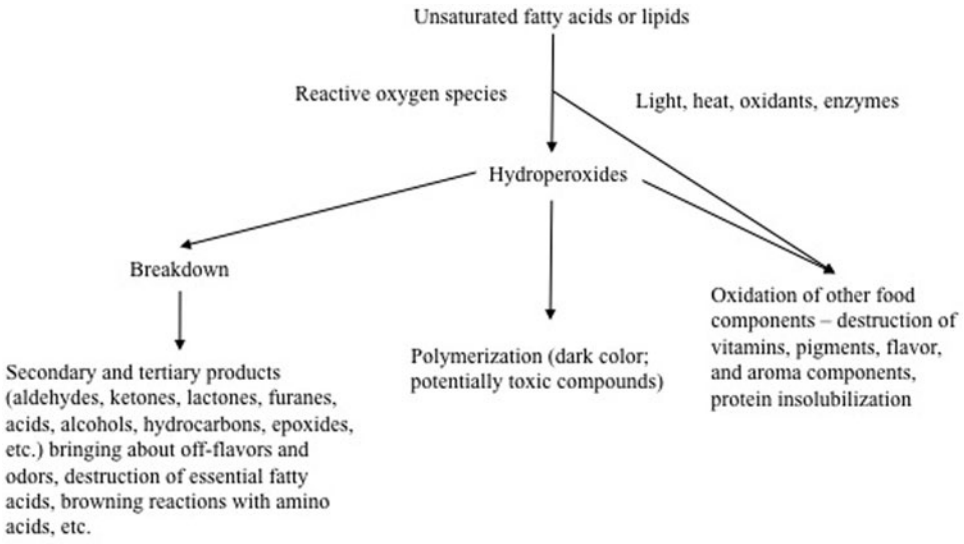

Figure 3 General mechanisms of lipid oxidation in foods (sourced from Kocchar, 1995).

Table 4 Biological sources of dietary lipids containing PUFAs

Source	Quantity (% w/w of total fat)			
	Linoleic	ALA	EPA	DHA
Plant sources				
Castor oil	4	–	–	–
Coconut oil	1.4	–	–	–
Corn oil	52	1	–	–
Cottonseed oil	50.5	–	–	–
Linseed oil	14.2	59.8	–	–
Olive oil	18.9	0.8	–	–
Palm oil	11	0.4	–	–
Peanut oil	41.1	0.3	–	–
Rapeseed oil (high erucic)	14	10	–	–
Rapeseed oil (low erucic)	26	10	–	–
Safflower oil (high oleic)	15.8	–	–	–
Safflower oil (high linoleic)	75.3	–	–	–
Sesame oil	45	0.6	–	–
Soybean oil	53	7.5	–	–
Sunflower oil	68.5	0.1	–	–
Terrestrial animal sources				
Human milk fat	7	1	0.1	0.2
Cow milk fat	2	1	–	–
Lard	11.4	1	–	–
Beef	3.7	0.18	–	–
Sheep	1.6	0.2	–	–
Lamb	8.1	1.6	–	–
Chicken breast	18.7	1.1	–	–
Chicken leg	23.5	1	–	–
Turkey	21.3	1.2	–	–
Pork	9.7	0.7	–	–
Chicken eggs	11.1	0.3	–	–
Marine animal sources				
Cod liver oil	22.6	–	7	7
Sardine oil	1	–	17	13
Cod	1	–	11	3.5
Sardine	1.3	0.9	16.9	12.9
Herring	2.9	1.1	8.8	10.8
Anchovy	1	–	18	11
Sand Eel	< 2	< 2	10.6	8.2
Krill	3.3	1.1	17..4	12.4
Mussels	–	–	10.2	13.4
Oyster	2	3.3	11.2	9.7
Shrimp	1.6	0.8	14.9	12.8
Scallop	0.1	1.2	26	24.1
King crab	3.2	3.3	21.5	10.2
Clam	–	–	10	15
Squid	0.7	0.1	14.6	30.4
Mullet	1.9	0.8	16.5	5.8
Hake	2.2	0.5	6.8	31
Sepia	1.2	0.3	20	20.9
Perch	5.5	0.2	5.6	8.1
Tropical halibut	–	–	2.5	32.3
Shark liver oil	4.1	–	4.3	30
Seal oil	10.3	–	4.7	5.1
Algal sources[2]				
Chlorella officinale		2.5	32	
Chlorella minutissima	–	–	44	–
Phaeodactylum tricornutum	–	–	26	11
Gonyaulox caterella	–	1.3	11.2	34
Crypthecodinium cohnii	–	–	–	P
Schizochytrium	–	–	P	–
Phaeophyceae	–	–	P	P
Phytophthora cinnamomi	–	–	P	–
Bacterial sources[2]				
Shewanella oneidensis	–		–	P
Moritella marina	–	–	P	–
Photobacterium profundum	–	–	–	P
Flexibacter polymorphus	–	–	–	P
Fungal sources				
Pythium spp.	–	–	P	–
Morierella	–	–	P	–
Pichia pastoris	–	–	P	–

[1]Compiled from Gunstone (1996).
[2]P, can produce PUFAs but not quantified.

Seafood and Fish Oil

Seafood is a readily available source of PUFAs from organisms thriving in marine environments (Bourre and Paquotte, 2008; Kris-Etherton and Hill, 2008). Fish oil, one of the most common sources of ω-3 PUFAs, is mainly produced from marine fish like cod, sardine, herring, anchovy, sand eel, krill, and similar oil-rich fish and crustacean varieties. Fish oil obtained from these species is rich in both essential ω-3 PUFAs EPA and DHA that range from 0.2–15% of the total fatty acids (Table 4). EPA and DHA are also found in groundwater and estuarial fish and crustaceans such as mussels, oysters, shrimp, scallop, crabs, and clams (0.1–2.5%; Ackman, 2000). The concentrations of ω-3 PUFAs also vary by region and fish varieties. For example, Mediterranean marine fish such as squid, mullet, hake, and sepia contain comparably higher amounts of EPA and DHA (5–30%; Ackman, 2000). Similarly, Australian and tropical fish such as perch and tropical halibut contain 20–30% fatty acids as EPA and DHA (Table 4). Consequently the consumption of 5–100 g of fish, depending on the variety, provides sufficient amounts of ω-3 PUFAs to deliver health benefits. Fish is also considered a good protein source (18–25% protein) and most varieties are low in cholesterol (15–25 mg/100 g; Ackman, 2000) and hence highly suitable as a ω-3 PUFA source.

However, fish and fish oil pose many challenges as prime sources of ω-3 PUFAs because of their non-nutritional contents. The increasing mercury content of aquatic environments also raises concerns about the safety of fish and fish oils (Diez, 2009). Inorganic and organic mercury, particularly alkyl mercury forms, are extremely toxic to humans (Diez, 2009). The bioaccumulation of mercury in lower aquatic life and subsequent consumption by higher life forms leads to "biomagnification" in fish, wherein mercury consumed is retained and passed on to human consumers. Knowledge of risks associated with methyl mercury consumption via fish dates back to our knowledge of ω-3 PUFA benefits (Anonymous, 2007). Currently, aquatic foods are recognized as the primary source of methyl mercury exposure. Similarly, pesticides and other marine environmental pollutants are also enriched in fish and fish oil reducing their safety for consumption (Borga et al., 2004; Nfon et al., 2008).

Mercury accumulation in aquatic life and subsequent toxicity risk varies in different regions and parts of the world and does not require termination of fish consumption, but the risk is widely acknowledged. Alternate and safer sources of EPA and DHA other than fish need to be identified for safer long-term dietary fortification.

Fish oil forms <1% of dietary oil consumed worldwide and hence is not a widespread source of dietary PUFAs (USDA, 2005). Additionally a fishy off flavor is often associated with fish oils and fish oil-incorporated foods (Ramaswamy et al., 2001; Perez-Mateos et al., 2004; Venkateshwarlu et al., 2004a) that make it unacceptable to most consumers.

Infant Formulae and Natal Diet

Prior to conception, maternal lipids are transferred to the placenta either as intact triacylglycerols via low-density lipoprotein, or as free PUFAs by a membrane fatty acid binding protein (Herrera et al., 2006). Post-conception infants can absorb and synthesize PUFAs from parental essential fatty acids (Herrera, 2002). Infant and neonatal cognitive and visual development is strongly correlated to inclusion of ω-3 PUFAs in their diet (Innis et al., 1999; Fewtrell, 2006; Agostoni, 2008). Human milk contains similar amounts of fat to bovine milk and its phospholipids have similar total amounts of PUFAs, but nearly 7-fold higher amounts of DHA. At least 12 different ω-6 PUFAs and seven different ω-3 PUFAs have been found in human milk (Jensen et al., 1992). Human milk is, however, low in EPA, which along with DHA, is required for hair and dermal growth (Gunstone, 1996). In order to fortify human milk, dietary increases in PUFA-rich oils, accompanied with adjustments for caloric intake of lipids, are recommended for lactating mothers (Agostoni, 2008). This also aids in taste conditioning of foods containing PUFAs for natal dietary supplementation (Ackroff et al., 2005).

Various pre- and post-natal supplements and food products serve the ω-3 PUFA needs of mothers (Table S1; supplementary data available online). The concentration of total PUFAs vary between 13% and 26% (w/w) in commercial formulae (Jensen et al., 1992), which initially only contained ALA as the ω-3 PUFA (Jensen et al., 1992), while infant formulae that contain EPA and DHA have only become recently available (Douaud, 2006; Starling, 2008). With low efficiency of incorporation into membrane phospholipids and less benefit from plasma ω-3 PUFAs, it is unclear if human milk fortification delivers sufficient ω-3 PUFAs to infants. The need, however, can be fulfilled via fortified infant formulae, many brands of which are commercially available (Table S1; supplementary data available online).

Dairy Products

Milk and dairy products are widely consumed by persons of all ages throughout their life span and are prominent sources of fatty acids for humans. Bovine milk usually contains 3–4% fat (MacGibbon and Taylor, 2006) with higher levels in other ruminants. While more than 400 minor fatty acids are present in milk, the major *cis*-PUFA is ALA (~10 mg/g fat) with EPA (~0.9 mg/g fat) and DHA (~1 mg/g fat) being present at much lower levels (MacGibbon and Taylor, 2006). Bovine milk also contains seven other unique *cis*-PUFAs at lower levels (<1 mg/g fat) that are polyunsaturated isomers of 20 or 22 carbon-length. However, none of these alternate PUFAs are associated with any known health benefits. Fatty acids are also attached to the 0.5–1% phospholipids in bovine milk fat, with glycerophospholipids containing *cis*-PUFAs in 22–25% (w/w) of their total fatty acids, while sphingolipids have <1% (w/w) *cis*-PUFAs (MacGibbon and Taylor, 2006). Ceramides and gangliosides are at low levels in milk (2–4 μg/g), contain only ALA, and are not significant contributors of PUFAs. The concentrations of *cis*-PUFAs decrease over lactation. Bovine milk also contains small amounts (~15 mg/g fat) of *trans*-PUFAs that arise from incomplete ruminal biohydrogenation of unsaturated lipids. The total ω-3 PUFAs available from milk is only 0.33 mg/100 mL, so in order to achieve 0.5 g/d ω-3 PUFA intake of at least 150 L of milk or 15 kg cheese or 5 kg butter is required, suggesting that unfortified milk and dairy products supply inadequate amounts of dietary PUFAs.

Commercial dairy products such as liquid milk and yoghurt are currently fortified with ω-3 PUFAs obtained from flaxseed, fish oil, or marine microalgae (Table S1; supplementary data available online). Milk is fortified either by direct addition of oil or ω-3 PUFA carrier, or indirectly via animal feeding of ω-3 PUFA sources to increase levels in the subsequent milk produced in the mammary glands. However, ruminant feed incorporation only mildly increases (<4%) DHA and EPA content of milk fat due to losses in biohydrogenation by ruminal bacteria (Lock and Bauman, 2004). Calcium salts of PUFAs may be fed to livestock to prevent ruminal digestion and modification (Theurer et al., 2009), but they are either excreted due to poor water solubility (Graham and Sackman, 1983) or form soaps and may lead to poor absorption in both livestock and humans. Bovine milk containing high levels of ALA provides cheese of acceptable flavor (Hauswirth et al., 2004), and adding ω-3 PUFAs to animal feed to enhance conjugated linoleic acid, EPA, and DHA in milk, did not alter milk flavor (Nelson and Martini, 2009).

Martini et al. (2009) made Cheddar cheese from milk fortified with EPA and DHA and noted that the PUFAs were stable over 3 mo of aging and the fortified cheeses had comparable flavor to cheese without PUFA addition. Addition of ω-3 PUFAs directly into dairy foods during their manufacturing process has more likelihood of impacting dietary intakes of ω-3 PUFAs. This necessitates better comprehension of metabolic and biochemical changes to lipids after addition to dairy foods in order to prevent off flavor development and changes to ω-3 PUFAs.

Meat

Dietary lipids are also obtained from meat and meat products, which form a larger component of adult diets than dairy products. As terrestrial animals do not produce ALA, EPA, or DHA,

beef, pork, and poultry meats do not naturally contain these fatty acids. Other PUFAs are found in higher amounts in membrane lipids than adipose tissue, mainly the ω-6 PUFAs linoleic acid, the dominant PUFA in both adipose and muscle tissues of beef ($\sim$40–80 mg/g fat), pork ($\sim$70–149 mg/g fat) or poultry meat ($\sim$180–220 mg/g fat), followed by traces of arachidonic acid (Rhee, 2000). Hence, dietary meats are broadly poor sources of ω-3 PUFAs. Thermal processing of dietary meats (temperatures $> 60^{\circ}$C) also leads to loss of free PUFAs except for PUFAs bound to membrane phospholipids that are generally stable to heating (Rhee, 2000).

The lack of ω-3 PUFAs in dietary meats suggests that fortification is necessary to achieve 0.5 g/d ω-3 PUFA intake. Currently commercial meat products supplemented with ω-3 PUFAs via animal feed are available (Table S1; supplementary data available online) but only one is a DHA-containing product (beef from Dalco Foods) and the others contain ALA. As only 4–10% of ALA is converted to DHA and EPA, at least 10 times the amount of meat ($\sim$1 kg) needs to be consumed daily to provide sufficient DHA/EPA, suggesting that the current supplementation level of ALA ($\sim$4–5 mg/g fat) is insufficient to meet dietary needs. Such consumption would also increase dietary ω-6 PUFAs and total dietary fat consumption, both of which increase the risk for cardiovascular and other lipidemic diseases. Direct incorporation of DHA and EPA surmounts these risks and may provide the required ω-3 PUFA content needed for healthy life.

Other considerations related to PUFA incorporation via animal feed are losses due to digestive metabolism such as ruminant biohydrogenation and suppression by EPA and DHA of the proliferation of cellulolytic bacteria essential for ruminant digestion (Maia et al., 2007). The effect of ruminal metabolism is evident on comparing feed incorporation of ω-3 PUFAs in animals with different digestive systems, where poultry meat and pork (from single stomach animals) showed a 7–10-fold and 6-fold increase, respectively, while only a 2-fold increase was observed for beef (Bourre, 2005). Addition of encapsulated ω-3 PUFA powder directly to processed meat avoids issues related to biohydrogenation in ruminant animals.

PUFAs of varying levels of unsaturation tend to localize into different tissue types, which alters the amount incorporated and the tissue to be consumed for health benefits. In poultry, ALA from feed is predominantly incorporated into adipose tissue (Rymer and Givens, 2005), as opposed to arachidonic acid, EPA, and DHA that are absorbed into membrane phospholipids, and hence are richer in lean meats. Although some researchers (Rymer and Givens, 2006) found that ALA-inclusion in feed did not increase EPA and DHA levels in poultry, suggesting that either the birds cannot convert ALA into longer ω-3 PUFAs or the converted PUFAs are not deposited into edible tissue. Additionally, as animal weight (and age) at time of harvest increases, the PUFA content of meat decreases (Komprda et al., 2005), and the animal's health needs to be optimized prior to harvest to deliver meat with a consistent amount of dietary PUFAs.

Eggs

Eggs are rich in both protein and lipid content and hence highly suitable as a macronutrient source. The eggs of a large number of animals are edible, including amphibians, reptiles, and terrestrial and aquatic birds, and even fish eggs, like the expensive delicacy caviar. However, commercial dietary eggs are mainly derived from chicken and contain 10.6% fat and 12.6% protein. Eggs are considered highly nutritious as egg protein contains all 20 common amino acids. But the high fat content and high levels of cholesterol (5% of total lipids) reduces the widespread acceptability of their healthiness.

Without providing diets formulated to be rich in egg ω-3 PUFAs, chicken eggs are poor sources of PUFAs (10–15 mg/g fat) and the main PUFA is linoleic acid (>95% of PUFAs), while ω-3 PUFAs are below detectable levels (Cantor et al., 2000). Dietary ω-3 PUFAs can be obtained only from eggs of chickens fed high ω-3 PUFAs in their diet by including sources such as fish meal, linseed oil, or salmon oil. Multiple commercials brand of eggs containing DHA are currently available that deliver 0.125 g/egg, which increases egg ω-3 PUFAs by 10% (Table S1; supplementary data available online). Thus, the consumption of four eggs will provide 0.5 g/day required for health benefits of ω-3 PUFAs. However, it is unclear whether ω-3 PUFA incorporation correspondingly reduces the level of ω-6 PUFAs in eggs. This also increases the amount of dietary fat consumed from eggs by 4-fold, requiring dietary adjustments for fat consumption. The crucial challenge for eggs as sources of PUFAs is their high cholesterol content (Cantor et al., 2000), a risk factor for cardiovascular disease. It is also unclear if cholesterol negates the beneficial effect of ω-3 PUFAs.

Bakery Products

Wheat is the staple grain of the Western diet and the third most widely produced grain after corn and rice. Wheat is usually ground into flour to make bread with or without dough fermentation. Different types of fat are added during dough-making depending on the type of bread, and hence, wheat can be easily fortified by addition of ω-3 PUFA-rich fats during bread making. Bakery products such as cakes, cookies, and pastries are manufactured from multiple ingredients wherein ω-3 PUFA-enriched ingredients such as eggs and dairy products can be specifically used in combination to provide enhanced health benefits. However, only bread is commercially produced with added ω-3 PUFAs (Table S1; supplementary data available online).

Bakery products do not usually include products from fish or fish oil. Inclusion of oils rich in ω-3 PUFAs, while increasing plasma ω-3 PUFA levels upon consumption (Yep et al., 2002), also causes deterioration in the quality and acceptance of bakery products upon storage (Serna-Saldivar et al., 2006), which is likely due to heat-generated susceptibility to oxidative lipid deterioration in cereal products (Fellers and Bean, 1977; Frankel et al., 2002). Apart from heat, physical forces also appear to

play a role in lipid instability. For example, (Laignelet and Dumas, 1984) showed that ∼45% of flour lipids were lost due to lipid oxidation even within 10 min of dough mixing. Additional studies are needed to improve the storage stability and consumer acceptance of bakery products fortified with ω-3 PUFAs.

Vegetarian and Vegan Diets

Plant oils used for cooking are the most common source of ω-3 PUFAs for vegan and vegetarian consumers. Plant cooking oils are rich in linoleic acid and ALA but are still deficient in EPA and DHA (Table 4). With rising levels of obesity worldwide, especially in developing countries, the increased consumption of plant oils for PUFA combined with caloric monitoring and restriction of dietary lipid intake for proper health has been recommended (Ackroff et al., 2005). Plant oils can be supplemented with EPA and DHA from vegetable sources such as marine algae or fungi and a limited number of such products are commercially available. Since the major portion of dietary fat for vegetarians is obtained from cooking oil, the high amount of oil used provides sufficient ω-3 PUFAs upon fortification.

A major challenge with ω-3 PUFA addition to plant oils is loss of heat stability (Goburdhun and Jhurree, 1995; Choe and Min, 2007; Ayadi et al., 2009; Hack et al., 2009). Oils containing higher amounts of PUFAs are less stable at high temperatures and form free radicals, free fatty acids, and other hydrolysis and oxidation products (Eunok and David, 2006) that are detrimental to cardiovascular health (Patrignani, 2001; Waddington et al., 2003). Increase in ω-3 PUFA content may cause the oil to be more heat labile and negate the cardiovascular benefits of ω-3 PUFA inclusion. Novel formulations of cooking oils that include ω-3 PUFAs and desirable amounts of antioxidants would need to be developed to provide health benefits of ω-3 PUFAs.

Other Food Products

Breakfast products such as cereals and bars are convenient modes for PUFA fortification (Table S1; supplementary data available online). Juices consumed during breakfast can also be fortified, as also spreadable products such as jams, jellies, peanut butter, and dairy spreads. Unique food products such as fortified cheese sauce and chocolate are also commercially available. In all these products, challenges exist for incorporation of ω-3 PUFAs (that have already been highlighted in previous sections) for stable maintenance and delivery in sufficient quantities to meet RDA recommendations. As these products do not form part of staple diets worldwide, and are not consumed in high quantities, fortification may provide the intended health benefits only to specific populations and in limited amounts.

Pharmaceutical Supplements

A large number of nutritional supplements that include ω-3 PUFAs as one of the active ingredients are commercially available (Table 4). Over 15 brands sell products that directly provide ω-3 PUFAs, mainly in the forms of fish liver oil or cod liver oil that are encapsulated in soft gels. A variety of benefit claims such as joint relief, reduction in triglycerides, heart health, and immunity are made by manufacturers as part of the marketing strategy for these supplements. However, the FDA has recognized the role of ω-3 PUFAs only in reducing risk of coronary heart disease and has permitted ω-3 PUFA use with a qualified health claim (FDA, 2004). Any additional benefits touted by manufacturers are not FDA-recognized and subject to verification.

Commercial ω-3 PUFA Supply

The increasing demand for ω-3 PUFA supplements necessitates their production at a larger scale than their availability via fish oils. Over 33 suppliers of ω-3 PUFAs are found worldwide (ICIS, 2009) of which 26 are based in the US. Currently, PUFA-rich oil is extracted directly from fermentations by microalgae isolated from marine environments (Certik and Shimizu, 1999; Spolaore et al., 2006) in place of oil extraction from fish. Biotechnological production of ω-3 PUFAs from a non-animal source is suitable for vegetarians and avoids environmental toxicity and poor infantile and neonate absorption issues associated with fish oils (Nutting et al., 2002). While ω-3 PUFAs are added to foods and supplements, consistent delivery requires their presence in such products over time, bringing into question the stability of PUFAs in food products.

Stability of PUFAs in Foods

Dietary lipids are susceptible to oxidation that generates unsaturated carbonyls and other reaction products during food manufacturing processes (Eunok and David, 2006). Heat or ultraviolet light exposure, or the presence of metal catalysts, initiates the conversion of fatty acids or acyl glycerols to the lipid alkyl radical form by removing a hydrogen atom. The lipid alkyl radicals thus formed from lipids react with atmospheric triplet oxygen to generate allylic hydroperoxides. These lipid peroxyls then abstract hydrogens from other lipid molecules, thus forming new lipid peroxyl radicals that are highly reactive. Transition metals present in oils, such as iron and copper, reduce the activation energy for initiation and catalytically cycle the hydroperoxides to increase autoxidation. Additionally, triplet oxygen is also converted to singlet oxygen in the presence of light and a type II photosensitizer. Singlet oxygen thus generated can also then react directly with the lipids to form peroxyl radicals. Notably, the hydroperoxides formed from the initial reactions retain the double bonds found in the fatty acid moieties but the nature of the double bond, such as its position or configuration, are likely to be altered. The autoxidation process is eventually terminated when the free radicals and/or hydroperoxides react to form stable end products. The presence of free fatty acids greatly enhances the initiation of lipid oxidation.

Dietary lipids from different sources contain varying proportions of saturated and unsaturated fatty acids (Table 2). Lipids that contain more saturated fatty acids are relatively more stable to oxidative reactions, whereas lipids rich in unsaturated fatty acids are less stable and are readily oxidized. Thus, PUFAs are more reactive to chemical modifications like hydrogenation and metal-catalyzed autoxidation than saturated fatty acids as they possess multiple C=C bonds that are susceptible to electrophilic attack (Gunstone, 1996). With more unsaturated fatty acids attached to lipids, the susceptibility to oxidation by free radicals increases and further reactive processes such as hydroxylation and oxidation are facilitated (Eunok and David, 2006), and the products of such reactions are known to be harmful to the cardiovascular system (Patrignani, 2001; Waddington et al., 2003) and are carcinogenic.

Oxidative loss of PUFAs in foods and supplements is limited by adding antioxidants to foods. Antioxidants inhibit oxidative deterioration by delaying the induction of autoxidation or reducing the rate of oxidation by either scavenging free radicals and lipid peroxides or controlling transition metals (Choe and Min, 2007). These processes prolong shelf life and promote palatability of the products. Emulsification of fish oil also enhances oxidative stability for specific foods by limiting access of lipids to oxidants (Let et al., 2007). For example, emulsification by lactoglobulins electrostatically inhibits iron-hydroperoxide interactions and stabilizes lipid hydroperoxides, eventually reducing hydroperoxide concentration, and enhancing lipid stability (Kellerby et al., 2006).

Fish oil and algal oil lipids are rich in ω-3 PUFAs and are consequently highly susceptible to autoxidation. Therefore, processes for controlling oxidative loss of ω-3 PUFAs have already been developed for fish oil. For example, the addition of alpha-tocopherol delays the onset of oxidation and maintains fish oil in a stabilized form in microencapsulated powder (Hogan et al., 2003). Similarly, the removal of tocopherol and other antioxidants from fish oil led to loss of DHA stability and needed addition of EDTA for restoring oil stability (Frankel et al., 2002). Microencapsulated multilayer emulsions made from lecithin and chitosan that contain corn syrup solids and EDTA, also effectively prevent fish oil oxidation by limiting hydroperoxide formation (Shaw et al., 2007) as do lactoglobulin emulsions (Katsuda et al., 2008). However, lengthy storage of supplements containing microencapsulated fish oil can lead to oxidation of fatty acids and a fishy off flavor due to air exposure (Kolanowski and Weißbrodt, 2008). Hence, the prevalence of fishy off flavor from ω-3 PUFA sources, even in stabilized fish oil products, needs to be addressed in order to extend shelf life and provide products of acceptable flavor. Multiple methods are essential to limit autoxidation of PUFA-rich foods (Wang and Wang, 2008). The oxidative breakdown of PUFAs results in unsaturated aldehydes and carbonyls, which are potentially detrimental to human health (Figure 2; Guiotto et al., 2005; Aldini et al., 2007; Hill et al., 2008). Additionally, off-flavors are also generated and consequently the product becomes unacceptable for consumption (Brewer, 2009).

Sources of Fishy Flavor in Foods

The characteristic fishy flavor of fresh fish is a desirable attribute for its palatability (Ganeko et al., 2008) but fishy flavor is not a desirable attribute in other foods (Im and Kurata, 2003) and severely limits our ability to include PUFA-fortified foods as a regular part of our diets. The unwanted fishy flavor in foods refers to an oxidative flavor that appears to be the results of products formed from lipid autoxidation and is variably described as rancid, train oil-like, or metallic (Venkateshwarlu et al., 2004a; Venkateshwarlu et al., 2004b; Gudipati et al., 2010).

Lipid autoxidation products have been strongly attributed to cause fishy off flavor (Venkateshwarlu et al., 2004a; Venkateshwarlu et al., 2004b). High purity preparations of fish and algal oils are low in autoxidation products and also possess less intense fishy flavor (Miller et al., 2007), suggesting that lipid oxidation may be the primary mechanism by which fishy flavor precursors are generated. The potential involvement of lipid oxidation in fishy flavor defects has been widely addressed by developing solutions to address lipid oxidative stability. The physical properties of lipid solutions have been extensively studied to understand their stability (Chaiyasit et al., 2007; Katsuda et al., 2008), including the application of surfactants (Chaiyasit et al., 2008), as well as proteins and peptides (Kellerby et al., 2006), milk whey (Tong et al., 2000), organic acids (Ke et al., 2008), SDS-fish gelatin membranes (Surh et al., 2005), alginate (Gudipati et al., 2010), and other multi-layered membranes (Gudipati et al., 2010). Other applications to improve PUFA stability in foods include the use of spice extracts and commercial antioxidants (Galobart et al., 2001), micro-encapsulation of spray-dried fish oils (Hogan et al., 2003), fish oil pre-emulsification (Let et al., 2007), and oil interesterification (Aguedo et al., 2008).

In order to decipher the role of lipid oxidation end products in fishy flavor, many researchers have added DHA and EPA to foods and shown that fishy flavor increases, or have created large libraries of compounds considered as lipid oxidation products (Im and Kurata, 2003; Allred et al., 2006; Let et al., 2007; Brewer, 2009). For example, surimi seafood gels (used as crab analogues) fortified with fish oils, developed fishy flavor within 30 d (Perez-Mateos et al., 2004). Autoxidized soybean, linseed, and fish oils that possessed a whale-like or fishy flavor was shown to contain an unsaturated aldehyde, 2-trans,4-cis,7-cis-decatrienal to which this flavor was attributed (Meijboom and Stronk, 1972). The isolated or selected inclusion of these compounds in foods has also been shown to increase fishy flavor such as by addition to milk (Venkateshwarlu et al., 2004a) or salmon (Refsgaard et al., 2000). Cumulatively, from all the above studies, over 100 different unsaturated carbonyls have been found to be present and supposedly associated with fishy flavor development in ω-3 PUFA-fortified foods.

Another approach to understand the components of fishy flavor in PUFA-fortified foods added antioxidants to ameliorate the lipid autoxidation product formation and further tested their sensory attributes (Jacobsen, C., Adler-Nissen et al.,

1999; Jacobsen, C., Hartvigsen et al., 1999; Jacobsen et al., 2000; Jacobsen et al., 2001). Using this approach, Jacobsen's group showed that tocopherol reduced rancid flavor formation, but tocopherol and propyl gallate unexpectedly increased fishy flavor in mayonnaise, suggesting that these antioxidants may be incapable of improving peroxide decomposition from metal ion catalysis. They further performed GC-MS analysis to identify unsaturated carbonyls and alcohols (trans-2-heptenal, 4-octen-3-one, 1-octen-3-ol, trans, cis-2,4-heptadienal, trans, trans-2,4-heptadienal, trans-2-octenal, nonanal and trans, cis-2,6-nonadienal in tocopherol-added fish oil-fortified mayonnaise, and 3-furaldehyde, 2,4-heptadienal, 2,4-decadienal and ethyl benzene in propyl gallate-added fish oil-fortified mayonnaise) were correlated to fishy odor and flavor perception increases. The presence of 2,4-heptadienal in both tocopherol- and propyl gallate-added fish oil-containing mayonnaise adds credence to the hypothesis that unsaturated carbonyls arising from lipid autoxidation are the cause of fishy flavor. Further studies by this group reveal that different matrices respond variably to altering the antioxidants used for improving fishy flavor, such as EDTA being effective in salad dressings as opposed to ascorbyl palmitate being effective in milk (Jacobsen, C. et al., 2008). For further discussions about the potential mechanisms of fish oil containing foods, the reader is referred to an extensive literature base from Jacobsen's group.

Notably, the putatively identified compounds from PUFA-fortified foods show fishy flavor or odor only when tested in foods and not individually in aqueous solutions (Venkateshwarlu et al., 2004a). Additionally, the requirement of different compounds in different matrices further confounds fishy flavor amelioration; for example, hexanal found among volatiles of fishy flavor mayonnaise was not detected in liquid milk. Considering that the identification of a compound that possesses fishy flavor by itself has proven elusive, a role for the compounds identified thus far for fishy flavor is unclear. Other possible alternative mechanisms include their participation in a cohort to cause fishy flavor (the combination of which is yet to be determined) or that they enhance fishy flavor sensations contributed by compounds originating from additional mechanisms such as microbial metabolism.

While it is commonly believed that EPA and DHA contribute to fishy flavor, the original flavor of these fatty acids or their glycerides has not been examined. Since esterified fatty acids cannot be selectively removed from lipids, alternate processes that aid removal of fishy flavor are essential for an acceptable carrier of EPA and DHA. Interestingly, very few compounds are conclusively linked to fishy flavor even though the flavor defect occurs in a wide variety of foods. Industrial processes such as deodorization of fish oils reduce their fishy flavor (Willie and Gonus, 1988); however, they also reduce ω-3 content of fish oils. Besides, the flavor profile of glycerides, or esters, of long chain PUFAs is not known; hence, a direct link between PUFAs and fishy flavor is not evident. Fatty acids of comparable chain lengths to DHA and EPA (18–22 carbons) are typically associated with a waxy or oily flavor and possess low volatility (Brennand et al., 1989) that likely precludes their role in fishy flavor and suggest roles for other compound classes.

Table 5 Examples of commercial brands selling products that contain ω-3 PUFAs as a supplement

Advocare	Sundown	Nutri-Supreme
Mega Smarts	Eniva	Vitamin Shoppe
Pure Encapsulations	Nature's Sunshine	Integrative Therapeutics
Aristo	Swanson	OmegaBrite
Minami	GNC	Vitamin World
Puritan's Pride	New Chapter	Jarrow
Berkley and Jensen (BJ's)	Tropicana	Ω-Gel
Mommy's Bliss	Great American Products	Wegmans
Shaklee	Nordic Naturals	Kirkland (Costco)
Carlson	Twinlab	Origin (Target)
Natural Factors	Health from the Sea	Weil
Silk (White-Wave)	Now	Life Extension Foundation
Coromega	USANA	PharmAssure
Nature Made	Healthy Hide	Yoplait
Spring Valley (Wal-Mart)	Nutramax	Lipiderm
CVS	VitalOils1000	Pharmanex
Nature's Bounty	Iceland Health	

Apart from the fatty acids themselves, the end products of PUFA metabolism, such as short chain fatty acids, alcohols, and carbonyls and other compounds present in foods arising from reactions with these products, may be responsible for fishy off flavor. A broad variety of compounds that includes unsaturated aldehydes, ketones, volatile sulfur compounds, and medium chain fatty acids have been implicated in fishy flavor of food products (Table 5). Of these groups, unsaturated medium chain aldehydes, like the hexenal isomers derived from the catabolism of long chain PUFAs, are directly involved in fishy flavor (Ramaswamy et al., 2001). Similarly 2-trans,4-cis,7-cis-decatrienal is a product of ALA autoxidation (Meijboom and Stronk, 1972). These compounds are typically produced by lipid autoxidation, and processes for fishy flavor reduction have been developed to further oxidize these and other aldehydes in foods to flavorless compounds (Garter et al., 2008). But such processes may also alter other compounds responsible for beneficial flavor and oxidize the PUFAs, thus reducing the nutritional value of PUFA addition and changing the acceptability of the product.

Ketones and volatile sulfur compounds also cause fishy flavor defects in foods (Table 6). Dimethyl trisulfide alone causes fishy off flavor in meat (Brewer, 2009), while 1,5-octadien-ω-3-one and methional in a 1:100 ratio are responsible for fishy odor in dried spinach (Masanetz et al., 1998). Ketones are derived from both autoxidation and microbial degradation of fatty acids and aldehydes, while volatile sulfur compounds result only from microbial amino acid metabolism (Gao et al., 1998). Notably, the addition of amino acids and whey proteins is reported to reduce fishy off flavors in breakfast cereals by binding the responsible aldehydes (Garter et al., 2008); but not all amino acids are correlated to reduction in fishy aroma. Subsequent loss or resurgence of fishy flavor over storage was also not tested; hence, the stability of flavor amelioration is unclear. The addition of

Table 6 Compounds linked with fishy flavors in foods

Compound class	Compound	Food product	Off flavor	Potential mechanism	Reference
Aldehydes	n-hexanal	butter	fishy	lipid autoxidation	(Ramaswamy et al., 2001)
	n-hex-2-enal	butter	fishy	lipid autoxidation	(Ramaswamy et al., 2001)
	n-heptanal	butter	fishy	lipid autoxidation	(Ramaswamy et al., 2001)
	2,4-Heptadienal	Porcine liver	fishy	lipid autoxidation	(Im and Kurata, 2003)
	2-trans,4-cis,7-cis-decatrienal	Porcine liver	fishy	lipid autoxidation	(Meijboom and Stronk, 1972)
Ketones	1,5-octadien-ω-3-one	dried spinach	fishy	microbial metabolism	(Masanetz et al., 1998)
Volatile sulfur compounds	Methional	dried spinach	fishy	microbial metabolism	(Masanetz et al., 1998)
	Dimethyl trisulfide	Meat	fishy	Autoxidation during irradiation	(Brewer, 2009)

alpha-tocopheryl acetate to chicken diet also prevents PUFA oxidation in enriched eggs (Galobart et al., 2001). Given the variety of compounds likely to contribute to fishy flavor, our ability to attribute aldehydes as causative to fishy flavor and amino acids as direct modulators of aldehydes' oxidation states is limited at this point. Also, amino acid metabolism may eventually add to fishy off flavor via amine generation (Lunden et al., 2002; Honkatukia et al., 2005). Considering that oxidation of the fishy flavor impact-compounds reduces the flavor perception, simultaneous oxidation of these compounds may aid flavor improvement.

The role of antioxidants in particular in ameliorating fishy flavor from fish oil-fortified foods has been chiefly used to understand the mechanism of unsaturated aldehyde generation by linking to lipid autoxidation (Jacobsen et al., 2008). However, the role of antioxidants in reducing other compound classes has not been discussed in these studies. Antioxidants are capable of oxidizing a larger variety of compounds than unsaturated carbonyls alone; for example, the pro-oxidant ascorbate can oxidize methanethiol to dimethyl sulfide and dimethyl disulfide (Chin and Lindsay, 1994). Similarly, reductions in levels of volatile sulfurs and carbonyls were also observed when antioxidants such as tocopherol, gallate, and sesamol were added to pork patties (Nam and Ahn, 2003). Thus, while the role of antioxidants in improving fishy flavor defects in foods is clear, the role of a particular group of compounds is yet to be fully established. Considering that carbonyls, medium chain fatty acids, and volatile sulfurs are all lipid-soluble (Christensen et al., 1981; Christensen and Reineccius, 1992) and become less volatile when they are mixed into lipids than in water (Brennand et al., 1989) and are all subject to antioxidant action (Chin and Lindsay, 1994; Nam and Ahn, 2003), the causant role of these compounds classes deserves further investigation.

In summary, a wide range of compounds appears to be responsible for fishy flavor in foods. However, the role of such compounds in foods fortified with ω-3 PUFAs is unclear except for unsaturated aldehydes proposed to arise from PUFA degradation and autoxidation (Ramaswamy et al., 2001). As some of the putative impact compounds are products of amino acid degradation, it appears unlikely that any group of compounds and their metabolism or autoxidation, singlehandedly contribute to fishy off flavor. Further characterization of fishy flavor associated with ω-3 PUFAs is a necessary step toward improving the flavor profile of foods fortified with ω-3 PUFAs.

Future Needs

The benefits of ω-3 PUFAs to human health are widely acknowledged. Dietary consumption of ω-3 PUFAs via incorporation into foods is ultimately the most effective mechanism of providing them to the average consumer. However, the fishy odor and flavor that pervades products naturally rich in ω-3 PUFAs, like fish oil, deters direct consumption. The ω-3 PUFAs themselves do not apparently contribute to this fishy flavor. In order to make ω-3 PUFA-containing foods palatable, the sources of fishy flavor need to be identified and mechanisms for their elimination need to be discovered. This would then allow fortification of more foods with ω-3 PUFAs, that would then increase the likelihood that Western diets would have the overall and consistent increase in ω-3 PUFAs, needed to bring about long term health improvements.

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