

Oxidation in Omega-3 Oils: An Overview



*A White Paper Prepared by
the Global Organization for EPA and DHA Omega-3s
and
the Council for Responsible Nutrition*

GOED OMEGA-3

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Executive Summary

Oxidation of omega-3 oils is a complicated topic, but it is important to understand. A significant number of consumers cite fishy burp and/or taste, thought to be the result of oxidation, as one of the main reasons they do not consume EPA and DHA oils. In addition, some papers have discussed the potential for adverse effects associated with consumption of oxidized oils.

Measuring oxidation in omega-3 oils is complex

due to the differences in chemical and physical characteristics of many commercially available products, which means not all methods to determine quality are appropriate for all types of oils. A number of consumer groups, organizations that issue product quality seals, and academic groups have published data on levels of oxidation in omega-3 oils. Overall, this data shows that commercially available omega-3 supplements are low in oxidation.

Key Points

Oxidation is a normal process that happens with all fats and oils that contain polyunsaturated fatty acids.

- Omega-3 products usually contain antioxidants and have specialized manufacturing that helps manage the oxidation process.

- There is some concern that oxidative products from lipids could be harmful, but this is being debated in the scientific literature. Current evidence suggests the potential for harm is related to dose, local concentration and type of oxidation product, as well as the activity of our own antioxidant defenses.

- The human clinical trials conducted so far with oxidized EPA and DHA oils have found no negative effects, and many of the human clinical trials with commercially available EPA and DHA oils have found beneficial impacts on the oxidative status of tissues in our body.

- Measuring oxidation in EPA and DHA oils is complex due to the wide variety of products available, but the two main tests currently used measure peroxide value and p-anisidine values.

- The p-anisidine value is NOT a valid test for flavored oils, or for oils with natural colors like krill or virgin salmon oils.

- The omega-3 industry has voluntarily established lower limits for oxidation than exist for other edible oils.

- The more than 2,000 test results available from scientific literature, third-party testing labs, and GOED's industry testing program show that more than 94% of products meet the stricter GOED limits for peroxide value and nearly 98% meet the limit for p-anisidine value.

What is Oxidation?

Oxidation is simply what happens to the unsaturated fatty acids found in fats and oils when they are exposed to oxygen. Upon reaction with oxygen from the air that surrounds us, the chemical bonds in the fatty acid molecules break down to form new molecules. All lipids containing unsaturated fatty acids oxidize over time, regardless of whether they come in the form of cooking oils or fish oil capsules, and this can ultimately lead to the oil becoming rancid. Therefore, consumer intake of oxidized oil is not limited to omega-3 products. In EPA and DHA omega-3 oils, this degradation is most often linked to a fishy taste or odor, which further limits consumption of these oils and is the reason why omega-3 companies take steps to reduce the oxidation process.

When fatty acids oxidize, they form a variety of oxidative products like fatty acid peroxides, alcohols and aldehydes. Some specific oxidation products resulting from the lipid peroxidation of highly unsaturated fatty acids include 4-hydroxy-2-hexenal (4-HHE), 4-hydroxy-2-nonenal (4-HNE), and a wide variety of isoprostanes, the presence of which are often measured as signs of oxidative stress in clinical trials.

Multiple factors contribute to the rate at which lipids oxidize, including: exposure to oxygen, light, heat and the degree of unsaturation of the fatty acids (Shahidi & Zhong, 2005). Highly unsaturated lipids, like EPA and DHA omega-3s, are more prone to oxidation from these factors and generally require special handling to

avoid off-flavors from developing. Some of these measures include the use of antioxidants to slow the rate of oxidation, limiting exposure to ambient air during manufacturing, refining oils in a vacuum, and blanketing storage containers with inert gases like nitrogen that displace oxygen. These strategies appear to be effective and are widely applied in the manufacturing of omega-3 products.

The same chemical oxidation of unsaturated fatty acids occurs inside the human body, but this process is tightly controlled by internal antioxidant defenses, effectively allowing humans to maintain a healthy life in an oxygen-rich atmosphere. This same strategy, defending against the oxidation process with antioxidants, is used in omega-3 products to keep oils from going rancid. For example, one experiment tested 19 commercially available brands of fish oils using antioxidants and noted that oxidation was stable in products stored at room temperature for 22 days with no noticeable changes in oxidation (Kolanowski, 2010). Consumers should expect the products they purchase to be below oxidation limits through the end of the products' shelf lives when these strategies are effectively utilized by manufacturers.

Consumer Acceptance of Oxidized Omega-3 Supplements

The Global Organization for EPA and DHA Omega-3s (GOED), an industry association, has conducted

Percent of Consumers Citing Fishy Taste as a Primary Reason for Not Taking O-3 Supplements

	Australia	Brazil	China	France	UK	USA	Russia	Germany	Japan
% of Omega-3 Non-Users	23.2%	14.1%	15.5%	12.9%	18.8%	11.5%	14.7%	20.3%	6.5%
% of Total Population	10.6%	4.4%	6.0%	6.6%	8.5%	1.8%	3.4%	7.5%	4.6%

surveys in nine countries about the main reasons consumers do not try to get more EPA and DHA in their diets (see table on previous page). Between 7% and 23% of non-users, depending on the country, say the fishy taste is a barrier to trying an omega-3 product. In addition, data from the US shows that 18% of people who stop taking omega-3 supplements cite a fishy burp as the reason. GOED has always been concerned that the development of a fishy burp is one of the primary reasons consumers avoid omega-3 products, effectively limiting their intakes of a vital nutrient.

Health Effects of Oxidized EPA and DHA Oils

Research on the health effects of oxidized EPA and DHA omega-3 oils is still emerging, but there is no evidence that normal usage of omega-3 oils results in adverse health effects due to oxidation. The theory with regards to adverse health effects is that consumption of oxidized products will lead to inflammation and oxidation of tissues in the human body.

It is currently poorly understood if oxidized lipid products that may be present in our food effectively enter our bodies, and if they play any significant role in comparison to the established roles of oxygenated lipids that are used in our body as hormones and autacoids. Our bodies are well-equipped to handle oxygen, and we also consume a certain level of oxidized lipids in our normal diets. In fact, a range of oxygenated and oxidized lipid products produced within our bodies are known to be beneficial in human health, for example in both initiating and resolving inflammation. Oxidized lipid products are formed in inflamed tissues within the body, but it does not mean that ingesting the same substances from rancid oil has the same biological effect. The concern that oxidized lipids present in food is bad for human health is also overrated because the assumption is that anything oxidized is bad. Like most unwanted molecules

we ingest, these substances are typically neutralized quickly after ingestion by multiple detoxification and antioxidant systems in the body, as well as "filtered out" by the liver or excreted into urine. In addition, we have an innate aversion for consuming rancid foods, which effectively self-limits our ingestion of food items that are oxidized.

In 2012, GOED commissioned a thorough safety assessment on EPA and DHA oils that concluded:

"Studies in both healthy and unhealthy populations looked at effects on specific lipid oxidation or oxidative stress parameters, which are difficult to interpret. End-points such as TBARS, lymphocyte phagocytic activity, in vitro or ex vivo determination of lag time and oxidation rate of LDL, and in vitro rate of formation of conjugated dienes do not appear to have a strong evidence base to support their validated in vivo relevance as biomarkers for a disease or compromised health state" (Spherix Consulting, 2012).

Two studies assessing the health impacts of oxidized fish oils have been conducted to date in humans. Both studies were gold-standard randomized, double-blind, placebo-controlled trials and both compared the impact of a highly oxidized fish oil, a regular fish oil, and a placebo on a wide variety of established markers of oxidation and antioxidative systems.

In the first study, after seven weeks of supplementation with 8g of oil per day, the authors found no signs of oxidative stress in any of the groups after looking at nine different measures of oxidative stress in the blood and urine (4-HHE, 4-HNE, 8-iso-PGF₂α, alpha-tocopherol, total GSH, GR, GPx, CAT, and C-Reactive Protein). This study demonstrated that high dosages of highly oxidized fish oil do not induce oxidative stress in our bodies (Ottestad, et al., 2012).

The second study was conducted by the same group and used the same study design, but looked at four additional markers of oxidative stress related to vascular inflammation (sICAM-2, sVCAM-1, IL-6, and oxidized LDL cholesterol). Again, no impact on these markers

was found with consumption of abnormally oxidized fish oil (Ottestad, et al., 2013).

Dozens of human studies have measured the effects of consuming regular EPA and DHA supplements on oxidative stress in humans as well. Overall, the evidence suggests that intake of commercially available products does not increase markers of oxidative stress in humans, but rather to the contrary, beneficially reduces some of these markers. The current understanding of these observations points to the role of EPA and DHA as sensors for oxidative stress in the body that activate our antioxidative defenses to help us protect our bodies from oxidation. Part of the disagreement regarding the effects of oxidized EPA and DHA on human health is around the fact that some studies suggest that oxidation byproducts from EPA and DHA have the potential to be beneficial to human health. For example, Nakagawa et al observed that a 4-hydroxy hexenal (4-HHE) increase after fish oil consumption protects vascular function (Nakagawa, et al., 2014).

Most toxicology studies with respect to oxidized oils have been conducted in animals utilizing dosages or levels of oxidation that are unrealistic in the human diet. The animal studies conducted on consumption of oxidized oils have primarily used vegetable oils and have identified isoprostanes, malonaldehyde (MDA), and 4-hydroxynonenal (4-HNE) as potentially atherogenic and genotoxic compounds. However, in the past 15 years:

- 35 human clinical studies have measured the effects of EPA and DHA oils on isoprostane levels, and nearly all have either found no effect, or reductions in the levels of these markers. The couple of studies that observed increases in isoprostane levels were at very high dosage levels or in conditions where the body was put under stress.
- 20 human clinical studies have measured the effects of EPA and DHA oils on MDA levels, and none have observed an increase in MDA levels.
- One human clinical study has measured the effect of EPA and DHA oils on 4-HNE and found no effect.

Measuring Oxidization in EPA and DHA Oils

There are three primary analytical measures used to measure oxidation in omega-3 oils, the Peroxide Value (PV), the para-Anisidine Value (pAV) and TOTOX, but since the primary concern is whether or not a product tastes fishy, simply smelling or trying the product is often the easiest method to determine rancidity for a consumer. GOED has always recommended that if consumers have a poor sensory experience with their omega-3 product, they should try another product as an alternative.

The Peroxide Value is a measure of how much peroxide is present in oil. When polyunsaturated fatty acids oxidize, the first compounds created are peroxides, so this is a measure of primary oxidation. The method is fairly robust and is used in a wide variety of oils, not just omega-3 oils. However, while the PV initially increases as oil oxidizes, it can actually decrease as the peroxides are consumed during further oxidative reactions. So a low PV is not necessarily an indicator of high quality oils by itself, which means that measures of secondary oxidation are also often used to determine the true “freshness” of an oil. Secondary oxidation products are the products formed from the initially formed peroxides during further steps in the oxidative process, and include chain-shortened aldehydes and alcohols.

The p-Anisidine Value is a colorimetric method where the absorbance of a specific wavelength of light, 350nm, by a solution of oil and acetic acid is measured after para-anisidine is added. It primarily measures the presence of 2-alkenals and 2,4-alkadienals, secondary oxidation products that react with para-anisidine in acidic conditions to turn yellow, absorbing that specific wavelength of light (Fennema, 1996) (Kiokias, Varzakas, Arvanitoyannis, & Labropoulos, 2009). There is significant variance in pAV results between various types of oils (Guillen & Cabo, 2002), and GOED only recommends the test for EPA and DHA oils in triglyceride or ethyl ester form, that do not

contain natural pigments or added ingredients, other than antioxidants.

The p-Anisidine test is not appropriate for measuring secondary oxidation in omega-3 oils that have a strong color or contain added flavorings. Oils like krill oil or virgin salmon oils naturally contain levels of carotenoids, such as astaxanthin, which interfere with the p-Anisidine assay and yield invalid results.

In addition, phospholipid sources of omega-3s are polar by nature and it is possible that they absorb light differently in the organic solvent used for the pAV measurement. In a paper by Lu et al, it was observed that pAV developed erratically, with results increasing and decreasing randomly over time within the same samples (Lu, Bruheim, Haugsgjerd, & Jacobsen, 2014). This work suggests that pAV does not yield an accurate read-out for secondary oxidation in krill oils. A Norwegian report noted that these same issues are observed in salmon oils due to the similarly high astaxanthin content (Rubin, 2009).

Flavorings consist of a variety of compounds. In many fruit-derived flavors the desirable odors, taste and colors are carried by compounds containing aldehydes. Since pAV measures the presence of aldehydes, these flavorings can often interfere with pAV results when added to oils. An experiment by Thea Norveel Semb demonstrated this when a variety of compounds were added to the same sample of cod liver oil. Most of the antioxidants had no significant effect on the pAV result of the oil compared to the control, but a 2% inclusion of lemon flavoring increased pAV more than 12-fold. She concluded that “[pAV] measurement on oil with added lemon extract give highly unreliable results” (Norveel Semb, 2012).

TOTOX is a third way to measure oxidation and is just a calculation combining PV and pAV. It was conceived as a way to give a complete picture of oxidation by including primary and secondary oxidation measurements. However, it too has its limitations. As Shahidi and Zhong noted in the seminal text, *Bailey's Industrial Oil and Fat Products*, TOTOX has no scientific basis for its use, but instead is a convenient measure of ox-

idation (Shahidi & Zhong, 2005). Additionally, since one component of the TOTOX calculation is pAV, it also is not valid for any oils containing other ingredients or that have strong colors, including flavored oils, krill oils and virgin salmon oils.

Analytical Tests on EPA and DHA Oils

In 2002, industry representatives established the Council for Responsible Nutrition (CRN) Voluntary Monograph, now known as the GOED Voluntary Monograph. The limits for EPA and DHA oils within the scope of the Monograph were voluntarily set at levels lower than other edible oils, and GOED has advocated for regulatory authorities to codify these limits, seen in the table below, into regulation.

Parameter	Limit
Peroxide Value	5 meq/kg
p-Anisidine Value	20
TOTOX	26

The fact is that vegetable oils have much higher oxidation limits that also allow for post-purchase, high-temperature frying and consumption in quantities much greater than EPA and DHA oils. In fact, the peroxide limit for refined vegetable oils in most countries is set at 10meq/kg and for extra virgin olive oil at 20meq/kg. In addition, the British Pharmacopoeia and European Pharmacopoeia, as well as Australian regulatory authorities, set limits for refined omega-3 oils at 10meq/kg. These authorities take the position that levels of oxidation similar to vegetable oils are acceptable, and some authorities have established even higher acceptable limits. The table on the next page

Standard	Peroxide Value	p-Anisidine Value
GOED Voluntary Monograph	5	20
Australia Natural Fish Oil	10	30
Australia DHA-rich oil derived from microalgae <i>Schizochytrium</i> sp.	5	—
Australia DHA/EPA rich <i>Schizochytrium</i> algal oil	5	20
Australia Squid Oil	5	15
Canada-NHP-Cod Liver Oil	5	20
Canada-NHP-Fish Oil	5	20
Canada-NHP-Krill Oil	5	20
Canada-NHP-Seal Oil	5	20
Canada Quality of NHP Guide	5	20
China-SCT 3502-2000 (Grade A Refined)	5	—
China-SCT 3502-2000 (Grade B Refined)	6	—
NSF/ANSI Standard 173 - 2013	10	20
FCC DHA Algal Oil, Crypthecodinium	5	20
FCC DHA Algal Oil, Schizochytrium	5	20
FCC DHA Algal Oil, Ulkenia	5	—
FCC Menhaden Oil, Refined	5	—
PhEur Fish Oil, Rich in Omega-3 Acids, Type I	10	30
PhEur Fish Oil, Rich in Omega-3 Acids, Type II	5	15
PhEur Cod Liver Oil, Types A	10	30
PhEur Cod Liver Oil, Type B	10	—
PhEur Cod Liver Oil, Farmed	5	10
PhEur Salmon Oil, Farmed	5	10
British Pharm Omega-3-Marine Triglycerides	10	30
British Pharm Fish Oil, Rich in Omega-3-Acids Type I	10	30
British Pharm Fish Oil, Rich in Omega-3-Acids Type II	5	15
Korean Pharm Cod Liver Oil	—	30
USP Cod Liver Oil	—	30
USP <i>Crypthecodinium cohnii</i> Oil	5	20
USP Fish Oil containing Omega-3 Acids	5	20
USP Omega-3 Acid Triglycerides	10	30
USP Schizochytrium Oil	5	20
USP Krill Oil	5	—
PhEur Omega-3 Acid Triglycerides	10	30
PhEur Omega-3-Acid EEs 60%	10	20
PhEur Omega-3-Acid EEs 90%	10	20
PhEur Omega-3-Acid EEs 90% DRAFT	10	20
USP Omega-3 Acid Ethyl Esters	10	15

lays out the limits established by various organizations and governments.

A handful of scientific studies and third-party testing organizations have tested oxidation in commercially available omega-3 supplements. In addition, GOED has conducted a number of tests in its efforts to monitor global product quality of omega-3 oils. We have compiled these into a single dataset and analyzed overall compliance with various regulatory, monograph and pharmacopoeial limits.

In total, of the 2,187 individual PV test results that have been reported, only 82, or 3.7%, have exceeded the limit established in the GOED Voluntary Monograph. Since the GOED Voluntary Monograph is a stricter standard than most others, it is also important to look at compliance with international regulatory and pharmacopoeial limits. The Australian government, British Pharmacopoeia, and European Pharmacopoeia have set the PV limit at 10meq/kg for fish oils, the same limit applicable to vegetable oils. Only 16 of the test results, or 0.7% of the products tested, exceeded this standard.

There are 2,117 individual pAV value test results reported from these sources, and only 44, or 2.1%, have exceeded the limit established in the GOED Voluntary Monograph. Similarly, the Australian government, British Pharmacopoeia, and European Pharmacopoeia have established higher pAV value limits than in the GOED Voluntary Monograph, in this case a limit of 30 for fish oils. Only 25 of these test results, or 1.2% of the products tested, exceeded these limits. Keep in mind that flavored oils cause the pAV test to report artificially high results, and this dataset mixes flavored and non-flavored oils, so the true compliance rate is likely even higher.

If you have any further questions or require additional information, please contact GOED at info@goedomega3.com.

References

- Fennema, O. (1996). *Food Chemistry, Third Edition*. CRC Press.
- Guillen, M.D., & Cabo, N. (2002). Fourier transform infrared spectra data versus peroxide and anisidine values to determine oxidative stability of edible oils. *Food Chem*, 77: 503–510.
- Higdon, J., Liu, J., Du, S., Morrow, J., Ames, B., & Wander, R. (2000). Supplementation of postmenopausal women with fish oil rich in eicosapentaenoic acid and docosahexaenoic acid is not associated with greater *in vivo* lipid peroxidation compared with oils rich in oleate and linoleate as assessed by plasma malondialdehyde and F(2)-isoprostanes. *Am J Clin Nutr*, 72:714-22.
- Kiokias, S., Varzakas, T., Arvanitoyannis, I., & Labropoulos, A. (2009). Lipid Oxidation and Control of Oxidation. In F. Yildiz, *Advances in Food Biochemistry* (pp. 383-408). CRC Press.
- Kolanowski, W. (2010). Omega-3 LC PUFA Contents and Oxidative Stability of Encapsulated Fish Oil Dietary Supplements. *Int J Food Prop*, 13:498-511.
- Lu, F., Bruheim, I., Haugsgjerd, B., & Jacobsen, C. (2014). Effect of temperature towards lipid oxidation and non-enzymatic browning reactions in krill oil upon storage. *Food Chem* 157: 398-407.
- Nakagawa, F., Morino, K., Ugi, S., Ishikado, A., Kondo, K., Sato, D., et al. (2014). 4-Hydroxy hexenal derived from dietary n-3 polyunsaturated fatty acids induces anti-oxidative enzyme heme oxygenase-1 in multiple organs. *Biochem Biophys Res Commun*, 443:991-6.
- Norveel Semb, T. (2012). *Analytical Methods for Determination of the Oxidative Status in Oils*. Trondheim: Norwegian University of Science and Technology.
- Ottestad, I., Retterstøl, K., Myhrstad, M., Andersen, L., Vogt, G., Nilsson, A., et al. (2013). Intake of oxidised fish oil does not affect circulating levels of oxidised LDL or inflammatory markers in healthy subjects. *Nutr Metab Cardiovasc Dis*, 23:e3-4.
- Ottestad, I., Vogt, G., Retterstøl, K., Myhrstad, M.C., Haugen, J., Nilsson, A., et al. (2012). Oxidised fish oil does not influence established markers of oxidative stress in healthy human subjects: a randomised controlled trial. *Br J Nutr*, 108:315-26.
- Rubin. (2009). *Omega-3 oljer fra ferskt marint råstoff*. Trondheim.
- Shahidi, F., & Zhong, Y. (2005). Lipid Oxidation: Measurement Methods. In F. Shahidi, *Bailey's Industrial Oil and Fat Products* (pp. 357-385). Newfoundland: John Wiley & Sons.
- Spherix Consulting. (2012). *Hazard Characterization of the Long-Chain Polyunsaturated n-3 Fatty Acids, DHA, EPA and DPA*. Bethesda.