# Omega-3 oxidative stability: an overview of lipid oxidation and its relevance to product quality, patient compliance and clinical outcomes

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The importance of omega-3 fatty acids in naturopathic practice is well established. While the clinical applications of omega-3 fatty acids are well understood, the qualitative aspects (product quality and in particular lipid peroxidation) and its impact on patient care is not.

Oil chemistry is a very complex subject and presents a significant technical challenge to manufacturers of omega-3 fish oils. At the root of this complex subject is lipid oxidation. This article attempts to review the basics of lipid oxidation and its impact on product quality and patient compliance. The focus will be placed on 3 primary factors that influence overall quality and sensory impact 1) capsule vs. liquid format, 2) ethyl ester vs. triglyceride form, and 3) the importance and efficacy of additive lipid antioxidants.

## What is oxidation?

Oxidation is a chemical reaction that occurs between oxygen and an unsaturated fatty acid, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA),1 resulting in fishy and/or rancid flavors that are often found in fish oil supplements. Though oxidation is a problem in all oils and oil-containing foods, the high concentration of polyunsaturated fatty acids (PUFA) and the high number of double bonds in EPA and DHA (PUFA oils have at least one double bond. EPA and DHA have 5 and 6 respectively) make fish oil very susceptible to oxidation.[2] There are a number of factors that can initiate or accelerate the rate of oxidation.

**Table 1 – Catalysts and factors that accelerate lipid oxidation**

| |
|---|
| Ultraviolet light |
| Elevated temperatures |
| Oxygen |
| Metals |
| Free radicals |
| Ethyl esters |
| Free fatty acids |

## How does oxidation occur?

There are many different types of oxidation, the mechanism that occurs in fish oil and other edible oils is known as "free radical oxidation" or autoxidation.[1] Free radicals formed from any one of the sources mentioned in Table 1 attack the double bonds of unsaturated fatty acids,[3] generating more free radicals, as seen in Figure 1. This is referred to as the *initiation* phase of oxidation[1].

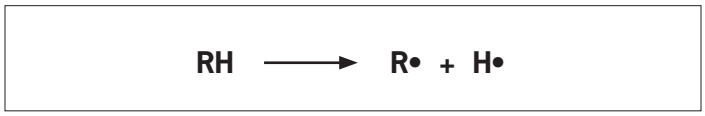


Figure 1 - Initiation phase: Oxidation at a double bond (RH) gives a free radical (R•) and a hydrogen atom (H•)

Once enough free radicals have built up in the oil, oxidation proceeds to the next phase, propagation1. This oxidation phase results in the formation of more free radicals as well as hydroperoxides and hydroperoxide radicals (Figure 2). Hydroperoxides are relatively stable at room temperature, but will readily breakdown at elevated temperatures, or in the presence of metals[4]. The propagation phase is comprised of chain reactions; the more oxidation that occurs, the more free radicals are formed, causing more oxidation. As a result, hydroperoxide formation is rapidly occurring. Eventually, all of the fatty acid substrate will be oxidized and no more hydroperoxides will form. This is known as the *termination* phase, and it is at this point that rapid breakdown of hydroperoxides begins.

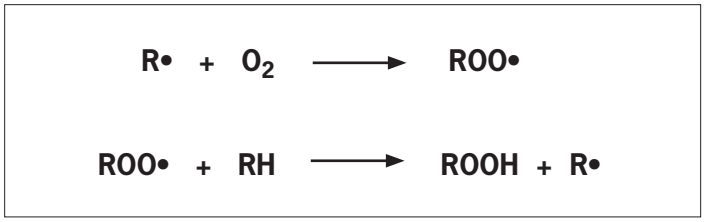


Figure 2 - Propagation phase: A free radical (R•) reacts with oxygen to from a lipid hydroperoxide radical (ROO•). The hydroperoxide radical then reacts with another double bond (RH) to form a hydroperoxide (ROOH) and another free radical (R•)

It should be noted that peroxides on their own do not cause negative flavours in fish oil, but they break down into other volatile compounds, such as aldehydes, ketones and acids, that have very strong tastes and odours[4,5] (Figure 3). These volatile compounds are known as secondary oxidation products and are formed rapidly during the termination phase. Though the most rapid breakdown occurs during termination, hydroperoxides are degraded throughout the oxidation process, sometimes quite slowly.[5] This constant breakdown can result in the formation of off-flavours

even if the peroxide value remains very low. For this reason it is commonly misunderstood that peroxide value (PV) does not relate to fish oil taste and smell.

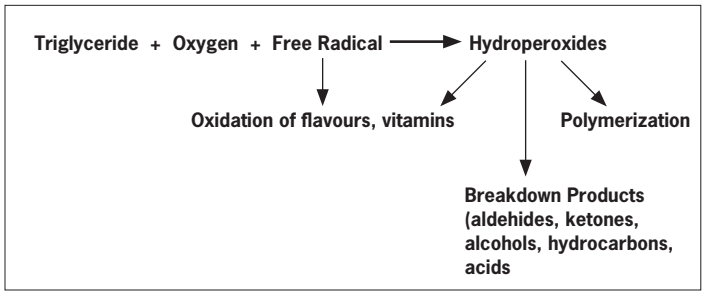


Figure 3. Termination phase: Triglycerides react with oxygen and free radicals to form hydroperoxides. These hydroperoxides break down into secondary oxidation products. Free radicals can also oxidize flavours and vitamins that are present in the oils. Modified from Labuza[1] (1971).

## Oxidation in fish oil supplements

Now that we've detailed the process of oxidation, the next step is to examine how this process is influenced, positively and negatively, by the various forms and attributes of fish oil supplements – capsules vs. liquids and ethyl esters vs. triglycerides. The result of preventing oxidation is a fish oil with pleasant sensory properties as well as preserving levels of actives (EPA & DHA) and endogenous antioxidants.

### *Capsules vs. Liquids*

Fish oil supplements are generally sold as soft gelatin capsules and free flowing liquids.

Although the liquid format is more convenient for swallowing it is less preferred due to the negative sensory attributes of most fish oils. To overcome the fishy taste and smell, many fish oil liquids incorporate very strong citrus based flavours which in themselves may reduce patient compliance. The capsule format on the other hand bypasses the initial sensory impact but can still result in what is referred to as fish burp back, which occurs after the capsule contents are released high in the GI tract. Some manufacturers have attempted to minimise fish burp back by using an enteric coating on the capsule which results is the capsule releasing its contents further down the digestive track.

Because oxygen is the primary catalyst for oxidation, one strategy to prevent lipid peroxidation is to minimize oxygen contact with the oil. Oxygen, and other catalysts, come into play at three time points: 1) processing/refining, 2) bulk and finished storage/shelf life, and 3) breakdown in the intestinal milieu. The impact of the processing of the fish oil applies equally to both liquids and capsules. Oxidation as it relates to finished product storage, however, slightly favours capsules over liquids. The gelatin based capsule provides a barrier against oxygen and thus slows the rate of oxidation of the oil inside. Liquids are bottled in such a way that there is space left in the top of the bottle known as headspace. Careful purging of the headspace with nitrogen gas removes the atmospheric oxygen and slows the rate of oxidation.

Once the bottle is opened, capsules offer further protection against oxidation because the oil is still protected by the gelatin capsule. Liquids on the other hand, once opened, introduce atmospheric oxygen into the headspace which can accelerate the rate of oxidation.

Once the oil is orally consumed, in either liquid or capsule form, the oil is exposed to the harsh environment of the gastrointestinal tract where the rate of oxidation is again accelerated.

While the capsule format inherently offers greater protection to the fish oil, there are two other variables that predominate as determinants for overall oxidative stability and sensory impact 1) ethyl esters vs. triglycerides, and 2) antioxidants.

### *Ethyl esters vs. triglyceride form*

Omega-3 fatty acids in the form of ethyl esters (EEs) are much less stable than those in the natural triglyceride (TG) form, and readily oxidize. The oxidation kinetics of DHA as an EE or as a TG was assessed by measuring the concentration of oxygen found in the head space of a reaction vessel.[6] The EE form of DHA was more reactive, and quickly oxidized, demonstrating that EEs are far less stable and can more readily produce negative sensory by-products and free radicals. In another study the stability of a phospholipid, triglyceride and EE containing DHA was assessed. After a ten-week oxidation period, the EE DHA oil decayed 33% more rapidly.[7] Due to the oxidative instability of the EE form, the goal of minimising oxidation would necessitate that only the TG form be used.

### *Antioxidants*

Antioxidants are molecules that inhibit or neutralize free radicals that occur during oxidation reactions.[8] In the case of fish oil, antioxidants can be added to the product to inhibit or neutralize oxidation reactions that lead to the development of fishy or rancid notes. Without the use of antioxidants, refined fish oils would be completely unpalatable and potentially harmful to consume due to the oxidative stress impact on the body. The efficacy of antioxidants in fish oil varies considerably. Common antioxidants such as vitamin E are minimally effective and do not prevent oxidation, leading to fishy tasting oils.

### *Optimal stability, sensory, and patient compliance*

In addition to improving compliance, the oxidative stability of the product confers further benefit once consumed. Because the stability of the fish oil has a negative correlation to the amount of free radicals generated during the digestive process, it follows that less stable fish oils increase the oxidative stress burden on the body.

While fish oil chemistry is a complex process as it relates to oxidation, the human sensory organs for taste and smell are highly effective in detecting oxidation by-products. Our senses easily tell us when a piece of fish is not fit to eat, it equally can tell us when a fish oil has undergone even low levels of oxidation. For patients and practitioners, tasting a teaspoon of liquid or biting down on a capsule will tell us if the product is fit to be consumed.

In addition to fishy taste and odour, rancid fish oil may also taste metallic, like mushrooms or grass. Many of the compounds that are responsible for these off-notes can be detected at parts per million or parts per million levels. Table 2 shows some of these compounds, their taste and their detection limit when tasted.

**Table 2 - Some common oxidation products found in fish oils**

| Compound | Odour/Flavour | Sensory Threshold (ppm) |
|---|---|---|
| 1-octen-3-ol[9 10] | Mushroom[9] | 0.01[9] |
| trans-2-heptenal[9,10] | Fatty, bitter almond[11] | 0.20[9] |
| trans,trans-2,4-heptadienal[9] | Painty, fatty[9] | 0.055[9] |
| trans,cis-2,6-nonadienal[9] | Cucumber[11] | 0.022[9] |
| 2-nonenal[9,10,11] | Tallow, cucumber[11] | 0.1[9,10] |

An unopened bottle of liquid TG fish oil stored in an amber bottle, out of direct sunlight will maintain its quality for approximately two years, sometimes longer depending on the product. An opened bottle of liquid TG fish oil stored in the refrigerator will maintain its quality for approximately 100 days. Encapsulated fish oil does not need to be stored in the refrigerator after opening as the capsule material protects the oil from oxygen. Fish oil capsules stored in an amber bottle at room temperature out of direct sunlight can maintain their quality for over two years. These estimations are approximate and may vary from product to product. Every fish oil product has an expiry date for unopened product that is clearly noted on the label. In order to sell fish oil in Canada, the shelf life of the product must be verified by the manufacturer. For liquid fish oils, a time period is also stated for opened products stored in the fridge. It is recommended that patients follow the guidelines on the package.

## About the Author

**Marc St-Onge** is the founder and CEO of Ascenta. He is a renowned expert in the field of omega-3 and formulator of the Ascenta product line. Marc passionately pursues the goal of establishing Ascenta as a world leader in health, environmental stewardship and social responsibility. Marc has a Bachelor of Science degree from Dalhousie University in Halifax, Nova Scotia, Canada. He is a contributing author of "Walking with the Wise for Health & Vitality" and was a 2005 Ernst & Young Entrepreneur of the Year finalist and the 2005 BDC Young Entrepreneur of the Year.
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