Oxidation and Anti-Oxidation
Natural *vs* Synthetic Antioxidants (AOx)

What, How and When to Use Natural AOxs

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Abstract

Oxidation either on dried meals, oils or frozen krill is a complicated topic. In krill meal, it degrades the quality of the fat content, gives a rancidity odor, creates the loss of typical orange coloration due to astaxanthin damage and poses a risk of spontaneous fire, which results in the loss of clients and lowering prices. In marine oils, consumers cite fishy burp and rancidity taste as their primary concern on what is seen as a poor-quality product. Prices drop and brands are badly affected. Some research discusses the potential of adverse effects associated with the consumption of oxidized oils.

Tharos' laboratory running at sea, onboard factory trawlers, and on-land, measuring oxidation in omega-3 oils, confirms that it is a complex matter due to the differences in chemical and physical characteristics of many commercially available marine meals and oils. This means that not all methods that determine quality are appropriate for all types of meals and oils.

Krill meal and oil have their own particulars. For example, it is not possible to get a high certainty of confidence analyzing p-anisidine as its astaxanthin colorimetric properties interfere with this analysis.

For marine-origin dried meals, krill meal included, analytical methods that measure oxidation are well known, as peroxides value, acid value, EPA & DHA level, organoleptic assessment, applicable for almost all such meals.

A good krill meal oxidation control and protection is a combination of antioxidants (AOx)[1], packaging systems[2] and low storage temperature[3].

Regarding AOxs, the decision is between potentially harmful synthetic AOx such as ethoxyquin (EQ) and natural AOxs such as NATUROX. Several studies have indicated undesirable health-effects in animal-fed diets containing EQ.

[1] Prevent fats to ignite fire spontaneously, for example.
[2] To remove oxygen, nitrogen barred and packaging material with oxygen barrier .
[3] Preferable less than 5ºC, ideally at -18ºC.

Europe is the main market banning EQ[4]. On the contrary, synthetic AOxs, either single-added or blended as BHT (*butyl hydroxytoluene*), BHA (*butyl hydroxyanisole*), propyl gallate or TBHQ (*tertiary butylhydroquinone*), have been re-authorized. Authorized natural single-added or blended AOxs include Vitamin E, lecithin, alpha-tocopherol, tocopherol and rosemary rich extracts mainly ascorbic acid, sodium + calcium ascorbate and ascorbyl palmitate as well. BHA, BHT, TBHQ and propyl gallate have already been approved for use as food additives.

EQ has proven to be a very efficient AOx. It remains accepted in several countries as a safe way to protect feed-grade marine-origin meals and oils in China, Japan, and the USA. An IFFO study (see Annex) showed that 300 ppm EQ, as well as a low EQ dosage of 50 ppm, is effective protecting oxidation of anchovy fishmeal, more so than any of the alternative AOxs evaluated in the same trial. IFFO trials showed that after 18 months of storage, residual antioxidants remained at >35% in 50kg bags and >45% in one-ton bags, providing protection for at least another six months.

These results supported the amendments to the *Model Regulations for the Shipping of Dangerous Goods* of the United Nations' Transport for Dangerous Good (and IMO 9) where the reduced residual EQ level minimum at shipment time (50 ppm instead of current 100 ppm) were accepted into the Model Regulations.

It also accepted the inclusion of alternative antioxidants as BHT with a 100 ppm residual level, and natural tocopherols with 250 ppm residual level at shipment date. See below:

CHINA:
https://gain.fas.usda.gov/Recent%20GAIN%20Publications/China%20Regulatory%20System%20on%20Imports%20of%20Feed_Beijing_China%20-%20Peoples%20Republic%20of_12-7-2015.pdf

USA
https://www.fda.gov/animal-veterinary/ingredients-additives/labeling-and-use-ethoxyquin-animal-feed

[4] https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32017R0962&from=EN

JAPAN

Accepts EQ, BHT and BHA.

EQ remains the preferred AOx for feed-grade marine meals. For food-grade krill meals, e.g. oil extraction, EQ must be replaced by natural AOxs, mainly natural tocopherols mixed with rosemary extract and lecithin. Alternatively, food-grade synthetic AOx can be replaced by active components such as BHA, BHT and lecithin.

In addition to oxidation matters, June 2020's South Korean health authorities recalled several krill oil brands due to solvent-contamination, banned solvents plus other nasty chemicals. Solvent contamination is a very delicate human health concern. Rancidity and oxidized marine oils are not such a dangerous health problem, but surely a matter to take care of.

Recommendation

For **feed-grade** krill meal, Tharos recommends maintaining **EQ** as primary AOx, when approved by the client. Tharos' minimal dosage recommendation is 300 ppm (or 0.3 kilos per ton of meal). It protects krill meal quality and meets IMO minimum level of 50 ppm at the date of shipment (considering EQ normal auto-consumption, or reduction curve during krill meal storage). The estimated cost impact per ton of krill meal is $3-4 USD/ton. In addition, we recommend store feed-grade krill meal at a temperature <5ºC.

For **food-grade** krill meals using **Natural** AOx, Tharos recommends the use of KEMIN's NATUROX, alternatively OXY´BLOCK (Ref. BAB20014) from NATUREX, at a dosage of 1,500 ppm (1.5 kg/ton meal). In addition, we recommend store food-grade krill meal at a temperature <5ºC preferably using bags with oxygen barrier and valves to evacuate oxygen, and nitrogen barred. Prices are in the range of $70-80 USD/ton of krill meal.

Regarding natural-AOx OXY´BLOCK, it has more tocopherols as an active component, 38-41% versus NATUROX's 25%.

Considering active component proportion and prices, Tharos' preliminary selection is NATUREX's Brand **OXY'Block** BAB20014.

Remarks:

a) *Tharos' preference is preliminary, as we need to know current AOx set-up, operators' preferences, and final stability tests.*
b) *NATUREX has not provided full technical information missing, for example, how OXY'-Block was developed, if it was tested on high-fat krill meals, or simply on high-fat marine meals.*

Some AOx suppliers are manufacturers themselves, KEMIN on the contrary buys its ingredients from third parties, blending in-house following their patented models.

What is a Stability Trial?

A stability trial helps to understand how the AOx application performs in time, the real capacity for krill meal protection, if it prevents cargo spoilage, and if a reduction or an increase of the recommended AOx dosage is needed.

For the natural AOx, Tharos recommends six bags at a dosage of 1,000 ppm and six bags at a dosage of 2,000 ppm with bags with oxygen barrier material and nitrogen barred, stored at <5ºC for food-grade krill meal. Periodically check every two months, for a total of 12 months, the meal quality evaluation (sensory, PV, EPA, DHA, Astaxanthin) and determine which AOx level is best, specifically for JU conditions. It provides crucial information to decide the best conditions to avoid oxidation, complying IMO regulations at the lowest cost.

An alternative option for the **Synthetic food-grade AOx**, is the use of PET-OX from Kemin, at a dosage of 2,000 ppm (2 kg/ton of meal), with an estimated cost per ton of krill meal of $30 USD.

The operator will finally decide if it prefers Natural or Synthetic AOx for its food-grade krill meal, according to their import/export regulations, meal cost and the quality message it plans to give its end-buyers.

What is Oxidation?

Oxidation happens to the unsaturated fatty acids found in fats and oils when they are exposed to oxygen, either the product is oil or the fat is embedded in the dried meal. Upon reaction with the surrounding oxygen, the chemical bonds

in the fatty acid molecules break down to form new molecules. All products containing unsaturated fatty acids oxidize over time, regardless of whether they come in the form of cooking oils, dried meals or fish oil capsules. This can ultimately lead to the product becoming rancid.

Raw krill is known for its high content of valuable unsaturated fats that make oxidation a matter of high concern. Therefore, consumer intake of oxidized oil is not limited to omega-3 products. In the EPA and DHA omega-3 oils, this degradation is most often linked to a fishy taste or odor, which limits consumption and is the reason 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. Multiple factors contribute to the rate at which lipids oxidize, including the exposure to oxygen, light, heat and the degree of unsaturation of the fatty acids. Highly unsaturated lipids, like EPA and DHA omega-3s, are more prone to oxidation from these factors and generally require special handling. The same is for dried meal.

Some of these measures include the use of AOxs to slow the rate of oxidation, limiting exposure to ambient air during manufacturing, refining oils in vacuum and blanketing storage containers with inert gases like nitrogen that displace oxygen.

Banning Ethoxyquin – Undesirable Health Effects in Marine Aquaculture

The merit for prohibiting the use of EQ is sustained on several studies that have indicated the undesirable health-effect it has in animals fed on diets containing EQ. Yet, to date, limited information is available on possible effects and effect levels of EQ present in fish feed:

a) Yellow croaker (*Pseudosciaena crocea*). Ten weeks of dietary exposure to 1,350 mg EQ/kg feed decreased specific growth rate, condition factor and hepatosomatic index. Indications of perturbation of the lipid metabolism were seen at the whole fish level
b) Turbot (*Scophthalmus maximus L.*). Disturbed ion-regulatory mechanisms were suggested in fish exposed for 16 days to 200 and 400

mg EQ/kg feed, affecting plasma concentrations of sodium, chloride and calcium.

c) Tilapia (*Oreochromis niloticus*). 30 days of dietary exposure to 150 mg EQ/kg feed was found to have immunosuppressive effects and cause histopathological changes in the liver.
d) Atlantic salmon (*Salmo salar L.*). EQ was shown to affect gene and protein expression patterns associated with the hepatic biotransformation of EQ after 90 days of exposure to doses of up to 1,800 mg/kg feed. However, no differences were seen in growth rate, feed conversion rate, hepato-somatic index or mortality.

Europe - Main Market Banning EQ

- Within the re-authorization process: BHT, BHA, propyl gallate, rosemary extract, citric acid.
- Authorized: Vitamin E, lecithin, alpha-tocopherol and tocopherol rich extracts, ascorbic acid, sodium + calcium ascorbate, ascorbyl palmitate.
- BHA, BHT, TBHQ and propyl gallate have already been approved for use as **food** additive (different legislation).

  - EUROPEAN COMMISSION IMPLEMENTING REGULATION (EU) 2017/962 of 7 June 2017 suspending the authorization of ethoxyquin as a feed additive for all animal species and categories.
  - EU Feed Additives Regulation: Regulation (EC) 1831/2003. International Maritime Organization: IMDG and IMSBC Codes.

Current Status of Ethoxyquin in the EU

- EQ was legal for use as a feed additive in fishmeal until September 30, 2019.
- Existing feeds stocks with EQ may continue on the market until March 31, 2020.
- Deadline provided time for a further EFSA Opinion on safety.

- EFSA[5] Opinion will be followed by a European Commission decision (likely mid-2019). Remark*: There is no published decision as of February 2020.*
- The market is moving away from EQ (especially in salmon).

The Global Salmon Initiative (GSI) applied a voluntary maximum limit of 400 ppm in 2017 for fishmeal received at feed plants. Media interest has been slow, but it can be expected a lot more to come when the EFSA opinion is published and the EC decision is taken.

Remark: There is no published decision as of February 2020.

[5] European Food Safety Authority - http://www.efsa.europa.eu/