

Why is it Better to Manufacture Phospholipid's Enriched Krill Oils At-Sea?

Tharos' (at sea) krill oil extraction process opens a new category in the Omega-3 industry.

Tharos keeps encouraging its customers to be informed of what type of krill oil they consume, who's the manufacturer, if the label gives clean and accurate information, how it was processed and if the richness of the raw krill was well preserved.

<u>All current krill oils</u> are manufactured on-land, very far from where the raw fresh krill was firstly captured and processed.

At present, all traded krill oils are not processed from raw fresh krill. Quite on the contrary. Krill oils come from materials previously processed at-sea, from where krill oil is finally extracted on land using solvents;

- From dried krill meal = Aker's Superba™ + Enzymotec's K-Real® + some Chinese brands
- From pelletized protein/lipids complex = Olympic's Rimfrost Sublime
- From frozen krill = Neptune's NKO® + some Chinese brands

There is at present no high-quality, phospholipids enriched krill oils that are high in EPA and DHA plus high natural antioxidants as astaxanthin that are manufactured entirely on-board (at-sea) from fully and traceable freshly captured raw krill, with a process that it is 100% chemical and solvent-free.

Lets address the technical explanation why it is better to process at-sea

To preserve krill's unique health and nutritional properties, krill oil extraction should be very fast after capture has taken place at sea. Tharos' process takes less than 2 hours after raw fresh krill has been fished from the clean South Antarctic Ocean, until the final product is obtained, preserving freshness and avoiding oxidation.

This unique concept sources the health and food industries with a highly competitively priced krill oil that preserves krill's original nutritional and medical properties;

- No lipids oxidation
- No aftertaste neither burping problems
- No residual material/solvents

The process extracts the entire phospholipids' profile that raw fresh krill contains, preserving natural Omega-3 content and a high content of pure and natural antioxidant as astaxanthin.

Tharos' process avoids normal temperature and/or oxidation impact when manufacturing dried meal, lipid/protein complex and frozen krill on-board.

Tharos' process works perfectly well on krill's normal low or high-fat seasons, sourcing in parallel triglycerides-enriched krill oils and food and feed-grade dried proteins.

As Tharos' krill oil is entirely obtained onboard with high processing yields, financial margin for the fishing vessel is very attractive. And as there is no need of extra processing investments on land, all favors sourcing a competitively priced, fresh and high quality krill oil for the consumer.

Tharos identified three stages that dead krill follows after capture, when stored at app 0°C, that reinforces why it is better to get phospholipids' enriched krill oils manufactured at-sea.

Stage 1

Before rigor mortis, which takes $1.0 \sim 2.0$ h after capture, krill is transparent and its carapace is shiny, coloring from brick to pale pink and green to yellow spots (thanks to phytoplankton). Once stored, krill gradually becomes opaque opal pink, jelly-shaped. Recently captured krill pH is 7.3 or higher.

Stage 2

Krill becomes stiff in $1.0 \sim 2.0$ h, ending after $2.5 \sim 3.0$ h. The neck becomes firm and acquires an arched shape. PH (below 7.2), and solubility of salt-soluble proteins come to a minimum.

Stage 3

Severe autolysis for krill stored over 4.0 hours. As a result of the intense hydrolysis, meat protein softens, valuable lipids are lost, pH and protein solubility at krill's neck rises and there is a sharp increase in the content of non-protein substances plus harmful volatile nitrogen bases. For krill stored for more than 5 hours, there is autolysis and microbial spoilage of viscera and muscles.

At this stage, the amount of nitrogen volatile bases range $30 \sim 50$ mg/100 g while recently captured raw krill is $8.0 \sim 13.7$ mg/100 g. Within the first six hours of storage, krill color fades and texture softens. By the 10^{th} hour there is blackening of the cephalothorax.

Other damaging effects:

(a) Acceleration of autolysis and degradation of krill is also impacted by capture volume of each trawl (tonnage per haul). For large tows (> 10 tons) there is a long-term damage resulting in a significant loss of proteins and acceleration of hydrolysis.

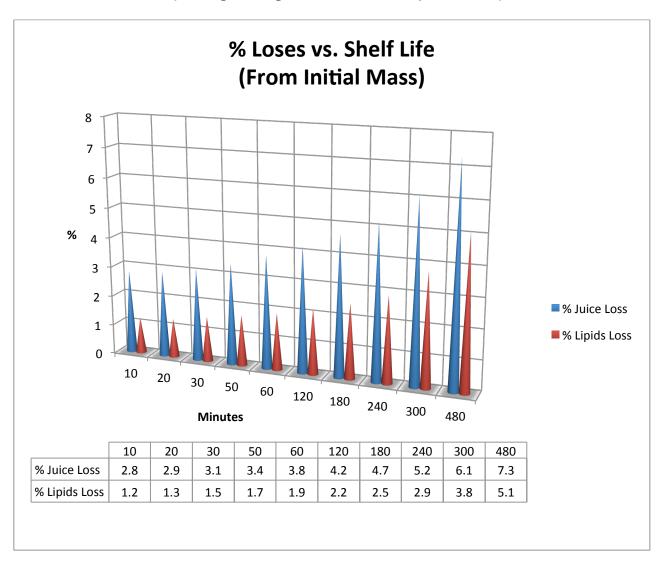
When raw krill is captured by continuous pumping system instead of the regular net-gear system, squezzing problems diminish. Raw krill arrives to the processing line almost alive. This is a good fishing method to prevent problems described herein.

The nature of post-mortem changes is similar to that of other crustaceans, but much faster on krill.

The increase of volatile nitrogen occurs through the action of krill enzymes adenosine deaminase and adenosine-mono-phosphatase. There is also growth of microorganisms alongside the decrease of enzyme activity.

(b) Other significant effect on recently captured krill is storage temperature, with a predominance of microbial spoilage and the formation of nitrogen-containing volatiles.

Amount of Liquids Released Post-Raw Krill Catch (During Storage - Chemical Composition %)



Source: Tharos' Unpublished data

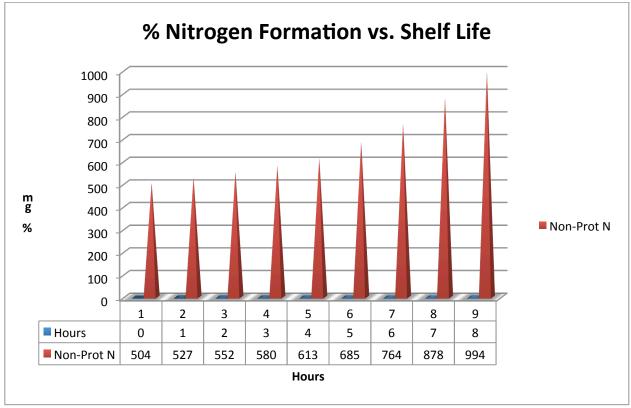
Remarks: (a) Krill stored at app 0°C (b) Stored max 4 hrs onboard vessel holds

Characteristics of Fresh and Frozen Krill (Stored up to 4 months-Content %)

Sample	рН	Total	No Protein	Sarcoplasmic proteins		Myofibrillar proteins	
		Nitrogen	Nitrogen	Total	% total	Total	% total
Fresh krill	7.3	2.5	0.6	1.1	43.4	0.8	31.2
Storage 1 mont	h 7.6	2.5	1.1	1.2	48.6	0.7	25.3
Storage 4 mont	h 7.7	2.5	1.6	1.9	75.0	0.6	21.8

Source: Tharos' Unpublished data

Krill's post-mortem changes have a strong autolytic and microbial effect that is accentuated after 4 hours after catch that negatively influences shelf life of raw krill components such as lipids and proteins.



Source: Tharos' Unpublished data

Tharos New Krill Oil - September. 2015

Consequently, generating valuable PL-enriched krill oil, preserving all natural rich krill compounds, krill oil extraction should be done at-sea from fresh raw krill, showing no detrimental impact of such forces.

Great difficulties in the processing of crustaceans, including krill, are caused by their rapid post-mortem deterioration caused by the high enzymatic activity of the proteolytic system of the raw material, after stored for 4 hr at 0°C, subject that the raw material (krill in this case) is properly stored.

As a result of the autolytic process, it modifies salt-soluble proteins of krill. There is also the worsening of organoleptic characteristics of whole krill when it is later frozen; color, taste, smell, texture which are intensified after 4hrs.

Why dried meal, pellets or frozen krill is not the best option for krill oil extraction;

- 1. Oxidation and rancidity increases (masked by soft-gels, capsules).
- 2. Krill oil odor worsens.
- 3. Increased FFA content.
- 4. Phospholipids' deterioration.
- 5. Increased lysophosphatidylcholine.

On a 1986 research conducted with frozen krill (*Euphausia superba* Dana), lipid classes where compared between frozen krill and fresh krill after various periods of storage at 251 K (-22°C). Fresh krill lipid composition differed from that determined in frozen samples, depending on storage duration, season of harvest, and developmental stage.

Phospholipids proved very susceptible to changes, as opposed to triglycerides, which were most resistant; diglycerides and cholesterol esters were also destroyed. The freezing process per se affected the lipid composition only slightly; however, after 30 days of storage the amount of free fatty acids almost doubled. After 6 months with storage temperature of 251 K (-22°C), 70% of phospholipids were decomposed and the amount of free fatty acids increased by a factor of 6 to 20. Monoglycerides, absent from fresh krill, appeared after several months of frozen storage.