Introduction to MSO™
Multi Stage Oil Extraction Process
Introduction

Krill Oil is one of the fastest growing, most valuable Omega-3 ingredients in recent years. Clinical and pre-clinical studies that compare Krill Oil and Fish Oil suggest that Krill Oil may be more effective than Fish Oil in terms of bioavailability for some indications. Although Krill Oil and Fish Oil possess a similar name, the term "oil" can be misleading not just because it implies a similar physical structure but also may suggest the two products are being produced and purified using similar techniques. Indeed, both of these perceptions are incorrect and misleading. First and foremost, Krill Oil composition is fundamentally different than Fish Oil. Unlike Fish Oil, Krill Oil contains additional elements within its matrix, which seems to enhance its biological activity beyond what is known in fish oils.

Those elements are:
- Omega-3 bound Phospholipids - Human clinical trials and pre-clinical studies suggest that phospholipids are better Fatty acids (FA) carriers when compared to triglycerides and therefore omega-3 FAs attached to Phospholipids are considered to have high bioavailability.
- Astaxanthin - Recognized as one of the most potent natural antioxidants known to man, astaxanthin is one of the most desirable and important components in Krill Oil. Clinical studies conducted on Astaxanthin demonstrate the beneficial effect of this antioxidant on different health problems, such as: cardiovascular health, immune-related issues, inflammatory and neurodegenerative diseases.

**Introduction to MSO™ Multi Stage Oil Extraction Process**

**Krill Oil**
- Omega-3 Triglycerides
- Omega-3 Phospholipids
- Astaxanthin

**Fish Oil**
- Omega-3 Triglycerides

**Figure 1a. Triglyceride**

**Figure 1b. Phosphatidylcholine**

**Fig 1: Schematic composition of Krill Oil vs. Fish Oil**
Due to its unique and sensitive components, Krill Oil requires specialized knowledge of chemistry and process engineering in order to maintain appropriate purity, stability, and quality under mild processing conditions. This procedure is fundamentally different than that of Fish Oil Triglycerides (TG) purification, normally performed under the conditions of high temperature and aggressive chemicals.

The purpose of this paper is to explain in simple terms the differences in production / purification processes of Krill and Fish Oils and to examine the big impact those processes have on quality and efficacy of the end product.

Marine biomass processing, Fish vs. Krill

Immediately after death, marine animals begin to deteriorate; once autolysis begins, bacteria present in the gut and skin or in water can easily enter tissues causing further deterioration and spoilage. Unfortunately, Krill is more susceptible to spoilage and deterioration than other marine sources, therefore Krill biomass must be processed immediately after harvesting on board the vessels, while fish processing can be done in land-based factories. Therefore, the first stage for having quality Krill Oil is the controlled stage of Krill biomass preparation. If not properly controlled no quality Krill Oil could be processed, regardless of technology used. Part of the MSO™ quality pledge, which will be described further, is the proper quality assurance program which ensures the selection of the right raw materials and suppliers.

Purification of Fish Oil vs. Krill Oil

Normally, oil processing for food applications (e.g. corn oil, olive oil, palm oil, etc.) involves several stages, among which are crushing the seed, filtration, deodorization and secondary purification processes. To obtain high-quality Fish Oil with virtually no contaminants (pesticides, polycyclic aromatic hydrocarbons, dioxins and polychlorinated biphenyls), the purification process must include refining and in some cases deodorization and distillation stages. The refining stage involves thermal and chemical treatment. It is therefore considered a very aggressive purification process, significantly affecting the quality of the oil and requiring quality parameters mainly to control its oxidation level PV, TOTOX value, and P-Anisidine - which are therefore carefully monitored. Oxidized oil is both not tasty and hazardous for your health. In some cases even considered carcinogenic.

In all matters concerning oil quality, high levels of the above parameters would specify it as adulterated.

It is clear therefore that such a process, though efficient, is primarily dependent on exposing the biomass to high temperatures and aggressive chemicals, especially when involving oils. The physical character of this cleaning process of oils in general cannot be applied in Krill Oil as Omega-3 Phospholipids and Astaxanthin are extremely sensitive. Furthermore, a different set of quality parameters should be implemented as TOTOX, PV, etc. are inapplicable. This type of process is called Solvent Extraction, when for example the solid biomass is placed in an agitated vessel that contains a solvent such as alcohol. While mixing the solid particles and the solvent, certain elements from the solid particles are dissolving in the solvent (for example: dirt in soap), while others do not dissolve. The end result is a solvent “loaded” with certain elements and solid elements and solid particles all agitated in a single vessel.
The solid particles and the loaded solvent can be easily separated through the forces of gravity and filtration. Then a second stage occurs. Under certain conditions (for example: temperature, acidity or presence of yet another liquid), the dissolved load could be separated from its carrier and form a separate phase, sometimes forming again solids, which in their turn, must again be separated through forces of gravity and filtration. This process, when conducted under controlled conditions, can ensure low temperature, zero oxidation and high yields. This is generally the method utilized by all suppliers of Krill Oil. Fig 2 provides a general overview of Fish Oil and Krill Oil processing and purification stages.

The following table summarizes the differences between quality assays used for Fish and Krill Oil.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Fish Oil</th>
<th>Krill Oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay of actives</td>
<td>GC</td>
<td>P-NMR, GC, HPLC</td>
</tr>
<tr>
<td>Oxidation</td>
<td>PV, P-Anisidine, TOTOX</td>
<td>PV, Astaxanthin levels over time</td>
</tr>
<tr>
<td>Other qualifying tests</td>
<td>NA</td>
<td>TVN, TMA</td>
</tr>
<tr>
<td>Qualitative spec</td>
<td>Smell</td>
<td>Smell, Color, Flowability</td>
</tr>
</tbody>
</table>

**Table 1: Summary of quality essays**

As mentioned before, in theory a controlled extraction process would ensure a high-quality product. But in reality, different producers and production plants employ various methods with different levels of expertise, creating an output without standardized results. Therefore a careful examination of the output of each and one of the Krill Oils currently available shows dramatic differences in both qualitative aspects of the oils such as: odor, flowability, color and quantitative aspects such as levels of oxidation or volatile amine-derivatives.

**Fig 2: Schematic processing of Fish Oil vs. Krill Oil.**
**Introduction to MSO™ Multi Stage Oil Extraction Process**

**MSO™ vs. Standard Extraction**

MSO™ by Enzymotec is a multi-stage solvent extraction process exclusively developed and designed by Enzymotec Engineers specifically for Krill Oil extraction and purification. It involves careful and controlled steps to assure the highest quality, stability and efficacy of Krill Oil.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>MSO™ (Krill)</th>
<th>Standard Extraction (Krill)</th>
<th>Refining (Fish)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>Low</td>
<td>Low</td>
<td>Very High</td>
</tr>
<tr>
<td>Stability</td>
<td>High</td>
<td>Poor</td>
<td>Good / Standard</td>
</tr>
<tr>
<td>Smell</td>
<td>Good / Standard</td>
<td>Poor</td>
<td>Good / Standard</td>
</tr>
</tbody>
</table>

**The Impact of MSO™ on efficacy and quality of Krill Oil**

Generally speaking, there are three known parameters used to measure Krill Oil efficacy and quality; levels of EPA/DHA, Total Phospholipids (PL) and Astaxanthin. Krill Oil is comprised of all three of these active elements.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Typical Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>% w/w Phospholipids</td>
<td>40-50%</td>
</tr>
<tr>
<td>% w/w EPA+DHA</td>
<td>16-18%</td>
</tr>
<tr>
<td>Astaxanthin (ppm)</td>
<td>200-400</td>
</tr>
</tbody>
</table>

**Table 3: Standard quality parameters for Krill Oil**

In reality, the term “quality” is broadly used within a wide range of definitions and applications.

In the fishing industry the term “quality fish” is synonymous with aesthetic appearance and freshness and refers to the degree of spoilage the fish has undergone. A European Union directive on fish hygiene specifies that if the organoleptic examination reveals any doubt regarding the freshness of the fish, inspectors must use total volatile basic nitrogen (TVN) as a chemical test.

TVN is a comprehensive spoilage index that includes trimethylamine (TMA), ammonia and other basic nitrogenous compounds.
Additional quality features and criteria significantly affect Krill Oil’s ability to have a positive biological effect on the body. As will be further demonstrated, MSO™ by Enzymotec, does ensure low TVN levels over time. Furthermore, careful analysis of certain actives over time may also provide an important insight into the bio-efficacy of Krill Oil.

**Standard list of TVN values for different degrees of freshness of fish is as follows:**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>TVN [mgN/100gr]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh Fish</td>
<td>12</td>
</tr>
<tr>
<td>Slight decomposition (edible)</td>
<td>12-20</td>
</tr>
<tr>
<td>Badly decomposed/non edible</td>
<td>&gt; 25</td>
</tr>
</tbody>
</table>

**Table 4: Levels of TVN and the freshness degree of fish**

As previously shown, higher TVN levels represents a degraded marine bio-mass. Uncontrolled extraction may cause Krill Oil to further deteriorate after processing and encapsulation.

**MSO™ impact on Bio-activity**

Astaxanthin is a powerful yet sensitive antioxidant. The presence of oxidizing elements within Krill Oil quickly reacts with the Astaxanthin and degrades it. The figure below shows how quickly this can happen when utilizing utilizing improper extraction methods.

**Conclusion**

While public awareness, of Omega-3 in general and Krill Oil in particular, is constantly increasing, so is the corresponding need to understand the physical and biological differences between the various sources of these omega-3 oils, in particular Fish and Krill Oils.

The growing body of knowledge simply emphasizes the differences between the types of oils and their chemical structure, while the significant differences between the various purification techniques and their impact on quality and efficacy of the end product is ignored.

Enzymotec defines a new quality level through its exclusive MSO™ technology, with improved physical properties as confirmed by third party assays. These physical properties include decreased odor, increased flow-ability and better biological activity (levels of oxidation and amine derivative).

Enzymotec’s MSO™ technology helps overcome application challenges and guarantees the highest quality standards available in market.