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(71) Applicant *(for all designated States except US)*: **PRONOVA BIOPHARMA NORGE AS** [NO/NO]; Lysaker Torg 8, N-1327 Lysaker (NO).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: **BREIVIK, Harald** [NO/NO]; Uranusveien 22, N-3942 Porsgrunn (NO).

(74) Agent: **LILLEGRAVEN, Rita**; Zacco Norway AS, P.O. Box 2003, N-0125 Oslo (NO).

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(54) Title: PROCESS FOR PRODUCTION OF OMEGA-3 RICH MARINE PHOSPHOLIPIDS FROM KRILL

(57) Abstract: The present invention relates to a process for preparing a substantially total lipid fraction from fresh krill, a process for separating phospholipids from the other lipids, and a process for producing krill meal.

PROCESS FOR PRODUCTION OF OMEGA-3 RICH MARINE PHOSPHOLIPIDS FROM KRILL

Field of the invention

The present invention relates to a process for preparing a substantially total lipid fraction from fresh krill, and a process for separating phospholipids from the other lipids. The invention also relates to a process for production of high quality krill meal.

Background of the invention

Marine phospholipids are useful in medical products, health food and human nutrition, as well as in fish feed and means for increasing the rate of survival of fish larval and fry of marine species like cod, halibut and turbot.

Phospholipids from marine organisms comprise omega-3 fatty acids. Omega-3 fatty acids bound to marine phospholipids are assumed to have particularly useful properties.

Products such as fish milt and roe are traditional raw materials for marine phospholipids. However, these raw materials are available in limited volumes and the price of said raw materials is high.

Krill are small, shrimp-like animals, containing relatively high concentrations of phospholipids. In the group *Euphasiids,* there is more than 80 species, of which the Antarctic krill is one of these. The current greatest potential for commercial utilisation is the Antarctic *Euphausia superba. E. superba* has a length of 2-6 cm. Another Antarctic krill species is *E. crystallorphias. Meganyctiphanes norvegica, Thysanoessa inermis* and *T. raschii* are examples of northern krill.

Fresh krill contains up to around 10 % of lipids, of that approximately 50 of % phospholipids in Euphausia superba. Phospholipids from krill comprise a very high level of omega-3 fatty acids, whereof the content of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) is above 40 %. The approximate composition of lipids from the two main species of Antarctic krill is given in Table 1.

Table 1: *Composition of krill lipids. Lipid classes, (approximate sum EPA + DHA)*

	Wax esters	**Glycerides**	**Phospholipids**	**Ratio EPA/DHA**
Euphausia superba	1	50 (7)	50 (40-45)	1.4-1.5
Euphausia crystallorphias	40	20 (4)	40 (30-33)	1.3

Furthermore, Antarctic krill has lower level of environmental pollutants than traditional fish oils.

The krill has a digestive system with enzymes, including lipases that are very active around 0 °C. The lipases stay active after the krill is dead, hydrolysing part of the krill lipids. An unwanted effect of this is that krill oil normally contains several percents of free fatty acids. If the krill has to be cut into smaller fragments before being processed, the person skilled in the art will immediately realise that this will increase the degree of hydrolysis. Thus, it is a desire to find a process that can utilise whole, fresh krill, or whole body parts from krill, as such a process will provide a product with improved quality and low degree of hydrolysis of lipids. This improved quality will affect all groups of krill lipids, including phospholipids, triglycerides and astaxanthin esters.

Krill lipids are to a large extent located in the animals' head. A process that can utilise fresh krill is therefore also well suited for immediate processing of the by-products from krill wherefrom the head is peeled off, a product that can be produced onboard the fishing vessel.

From US Patent No. 6,800,299 of Beaudion et al. it is disclosed a method for extracting total lipid fractions from krill by successive extraction at low temperatures using organic solvents like acetone and ethanol. This process involves extraction with large amounts of organic solvents which is unfavourable.

K. Yamaguchi *et al.* (*J. Agric. Food Chem.* 1986 34, 904-907) showed that supercritical fluid extraction with carbon dioxide, which is the most common solvent for supercritical fluid extraction, of freeze dried Antarctic krill resulted in a product mainly consisting of unpolar lipids (mostly triglycerides), and no phospholipids. Yamaguchi *et al.* reported that oil in krill meal was deteriorated by oxidation or polymerisation to such an extent that only limited extraction occurred with supercritical CO_2.

Y. Tanaka and T. Ohkubo (*J. Oleo. Sci. (2003),* 52, 295-301) quotes the work of Yamaguci *et al.* in relation to their own work on extraction of lipids from salmon roe. In a more recent publication (Y. Tanaka *et al. (2004), J. Oleo. Sci.*, 53, 417-424) the same authors try to solve this problem by using a mixture of ethanol and CO_2 for extracting the phospholipids. By using CO_2 with 5 % ethanol no phospholipids were removed from freeze dried salmon roe, while by adding 10 % ethanol, 30 % of the phospholipids were removed, and by adding as much as 30 % ethanol, more than 80 % of the phospholipids were removed. Freeze drying is a costly and energy consuming process, and not suited for treatment of the very large volumes of raw materials that will become available by commercial krill fisheries.

Tanaka *et al.* tried to optimise the process by varying the temperature of the extraction, and found that low temperatures gave the best results. 33°C, a temperature just above the critical temperature for CO_2, was chosen as giving best results.

Contrary to these findings, we have surprisingly found a process for extraction of a substantially total lipid fraction from fresh krill, without the need for complicated and costly pre-treatment like freeze drying of large volumes. The lipid fraction contained triglycerides, astaxanthin and phospholipids. We did not have to dry or deoil the raw material before processing. Contrary to Tanaka et al. we have found that a short heating of the marine raw material was positive for the extraction yield. It was also shown that pre-treatment like a short-time heating to moderate temperatures, or contact with a solid drying agent like molecular sieve, of the krill can make ethanol wash alone efficient in removing phospholipids from fresh krill.

Summary of the invention

It is a main object of the present invention to provide a process for preparing a substantially total lipid fraction from fresh krill without using organic solvents like acetone.

The exposure to the fluid under supercritical pressure will prevent oxidation from taking place, and the combined carbon dioxide/ethanol is expected to deactivate any enzymatic hydrolysis of the krill lipids. As the process according to the invention requires a minimum of handling of the raw materials, and is well suited to be used on fresh krill, for example onboard the fishing vessel, the product according to the invention is

expected to contain substantially less hydrolysed and/or oxidised lipids than lipid produced by conventional processes. This also means that there is expected to be less deterioration of the krill lipid antioxidants than from conventional processing. The optional pre-treatment involving short-time heating of the fresh krill will also give an inactivation of enzymatic decomposition of the lipids, thus ensuring a product with very low levels of free fatty acids.

Another object of the present invention is to provide a process for preparing a substantially total lipid fraction from other marine raw materials like fish gonads, *Calanus* species, or high quality krill meal.

Another object of the present invention is to provide a substantially total lipid fraction high in long chain polyunsaturated omega-3 fatty acids.

These and other objects are obtained by the process and lipid fraction as defined in the accompanying claims.

According to the invention it is provided a process for extracting a substantially total lipid fraction from fresh krill, comprising the steps of:
a) reducing the water content of krill raw material; and
b) isolating the lipid fraction.

Optionally, the above-mentioned process comprising a further step of:
a-1) extracting the water reduced krill material from step a) with CO_2 at supercritical pressure containing ethanol, methanol, propanol or iso-propanol. This step, a-1), is performed directly after step a).

In a preferred embodiment of the invention it is provided a process for extracting a substantially total lipid fraction from fresh krill, comprising the steps of:
a) reducing the water content of krill raw material;
a-1) extracting the water reduced krill material from step a) with CO_2 containing ethanol, the extraction taking place at supercritical pressure; and
b) isolating the lipid fraction from the ethanol.

In a preferred embodiment of the invention, step a) comprises washing of the krill raw material with ethanol, methanol, propanol and/or iso-propanol in a weight ratio 1:0.5 to 1:5. Preferably, the krill raw material is heated to 60-100°C, more preferred to

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