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

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(57) **ABSTRACT**

The present disclosure 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.

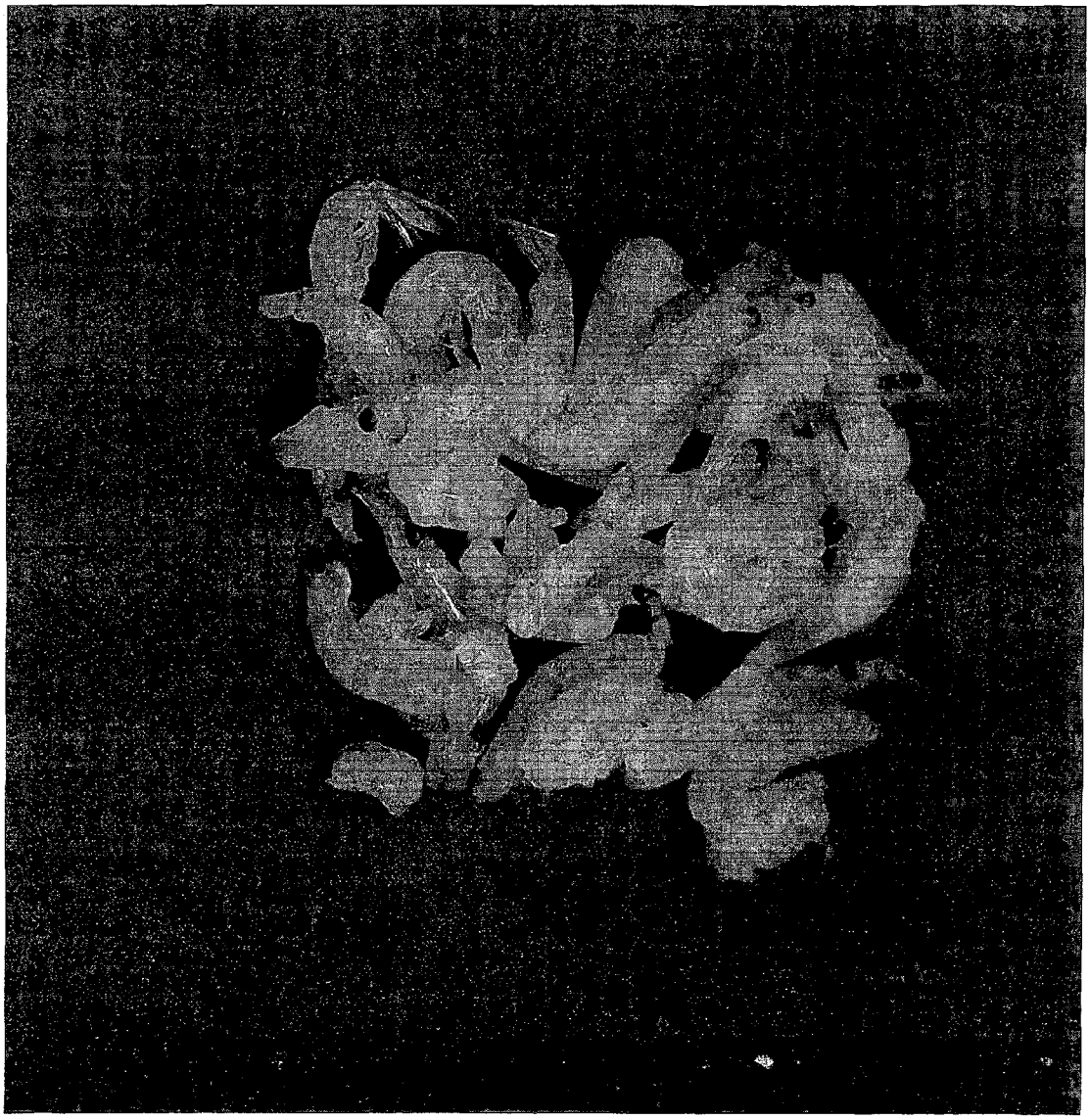

Fig. 1

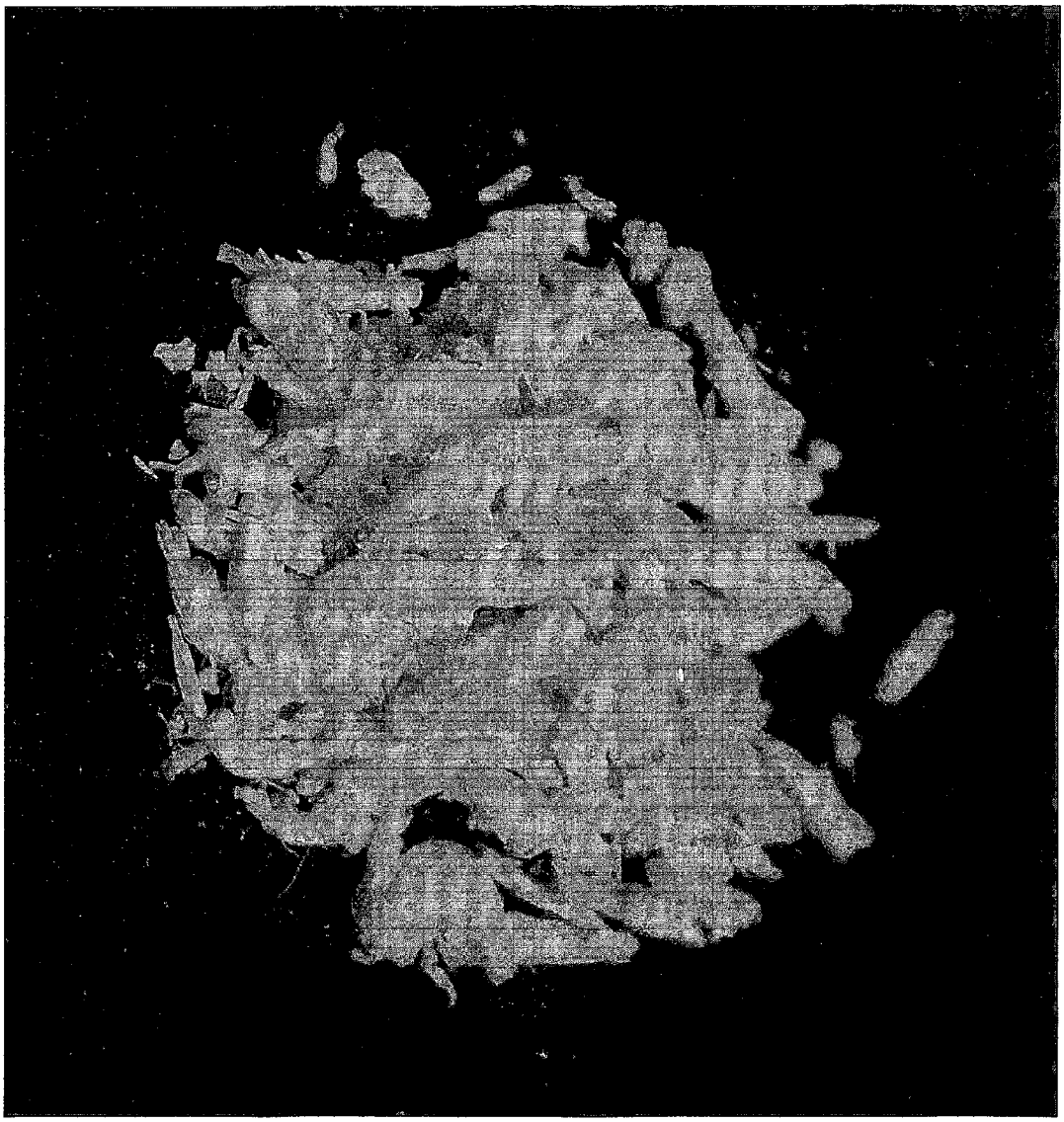

Fig. 2

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

FIELD OF THE INVENTION

[0001] The present invention relates to a process for preparing a substantially total lipid fraction from fresh hill, 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

[0002] 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.

[0003] Phospholipids from marine organisms comprise omega-3 fatty acids. Omega-3 fatty acids bound to marine phospholipids are assumed to have particularly useful properties.

[0004] 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.

[0005] 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.

[0006] Fresh hill 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

[0007] Furthermore, Antarctic krill has lower level of environmental pollutants than traditional fish oils.

[0008] 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 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.

[0009] 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.

[0010] From U.S. Pat. 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.

[0011] 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.

[0012] 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.

[0013] 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

[0014] 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.

[0015] The exposure to the fluid under supercritical pressure will prevent oxidation from taking place, and the com-

according to the invention requires a minimum of handling of the raw materials, and is well suited to be used on fresh hill, 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.

[0016] 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.

[0017] Another object of the present invention is to provide a substantially total lipid fraction high in long chain polyunsaturated omega-3 fatty acids.

[0018] These and other objects are obtained by the process and lipid fraction as defined in the accompanying claims.

[0019] 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.

[0020] 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).

[0021] 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.

[0022] 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 70-100° C., and most preferred to 80-95° C., before washing. Furthermore, the krill raw material is preferably heated for about 1 to 40 minutes, more preferred about 1 to 15 minutes, and most preferred for about 1 to 5 minutes, before washing.

[0023] In another preferred embodiment of the invention, step a) comprises bringing the krill raw material in contact with molecular sieve or another form of membrane, such as a water absorbing membrane, for removal of water.

[0024] Preferably, the amount of ethanol, methanol, propanol and/or iso-propanol in step a-1) is 5-20% by weight, more preferably 10-15% by weight.

[0025] In addition to producing a product containing the total lipids of krill, the invention also can be used for separating phospholipids from the other lipids. To separate the total lipids obtained by extraction at supercritical pressure, according to the present invention into the different lipid [illegible] rich phospholipids. Extraction of the total lipids with carbon dioxide containing less than 5% ethanol or methanol is another option.

[0026] As the phospholipids are much richer in the valuable omega-3 fatty acids than the other lipid classes, this makes the invention useful for producing high concentrates of omega-3 fatty acids. While commercially available fish oils contain 11-33% total omega-3 fatty acids (Hjaltason, B and Haraldsson, G G (2006) Fish oils and lipids from marine sources, In: *Modifying Lipids for Use in Food* (FD Gunstone, ed), Woodhead Publishing Ltd, Cambridge, pp. 56-79), the phospholipids of krill contain much higher levels (Ellingsen, T E (1982) Biokjemiske studier over antarktisk krill, PhD thesis, Norges tekniske høyskole, Trondheim. English summary in Publication no. 52 of the Norwegian Antarctic Research Expeditions (1976/77 and 1978/79)), see also Table 1. The omega-3 rich phospholipids can be used as they are, giving the various positive biological effects that are attributed to omega-3 containing phospholipids. Alternatively, the phospholipids can be transesterified or hydrolysed in order to give esters (typically ethyl esters) or free fatty acids or other derivatives that are suitable for further concentration of the omega-3 fatty acids. As examples, the ethyl esters of krill phospholipids will be valuable as an intermediate product for producing concentrates that comply with the European Pharmacopoeia monographs no. 1250 (Omega-3-acid ethyl ester 90), 2062 (Omega-3-acid ethyl esters 60) and 1352 (Omega-3-acid triglycerides). At the same time, the remaining lipids (astaxanthin, antioxidants, triglycerides, wax esters) can be used as they are for various applications, including feed in aquaculture, or the lipid classes can be further separated.

[0027] Thus, still another object of the present invention is to provide a process for separating phospholipids from the other lipids as described above.

[0028] Another object of the invention is to produce a high quality krill meal. As the lipids are removed at an initial step of the process, the meal will be substantially free of oxidised and polymerised lipids. This will make the meal very well suited for applications where it is important to avoid oxidative stress, i.e. for use in aquaculture feed, especially starting feed for marine fish species. The krill meal of the present invention is thus well suited for feeding fish larvae and fry, as well as fish and crustaceans. Furthermore, the krill meal of the invention may be used as a source for production of high quality chitosan.

DETAILED DESCRIPTION OF THE INVENTION

[0029] The process can be performed with a wide variety of processing conditions, some of which are exemplified below.

[0030] In the following "fresh" krill is defined as krill that is treated immediately after harvesting, or sufficiently short time after harvesting to avoid quality deterioration like hydrolysis or oxidation of lipids, or krill that is frozen immediately after harvesting. Fresh krill can be the whole krill, or by-products from fresh krill (i.e. after peeling). Fresh krill can also be hill, or by-products from krill, that have been frozen shortly after harvesting.

[0031] Moreover "krill" also includes krill meal.

BRIEF DESCRIPTION OF THE FIGURES

DOCKET ALARM

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