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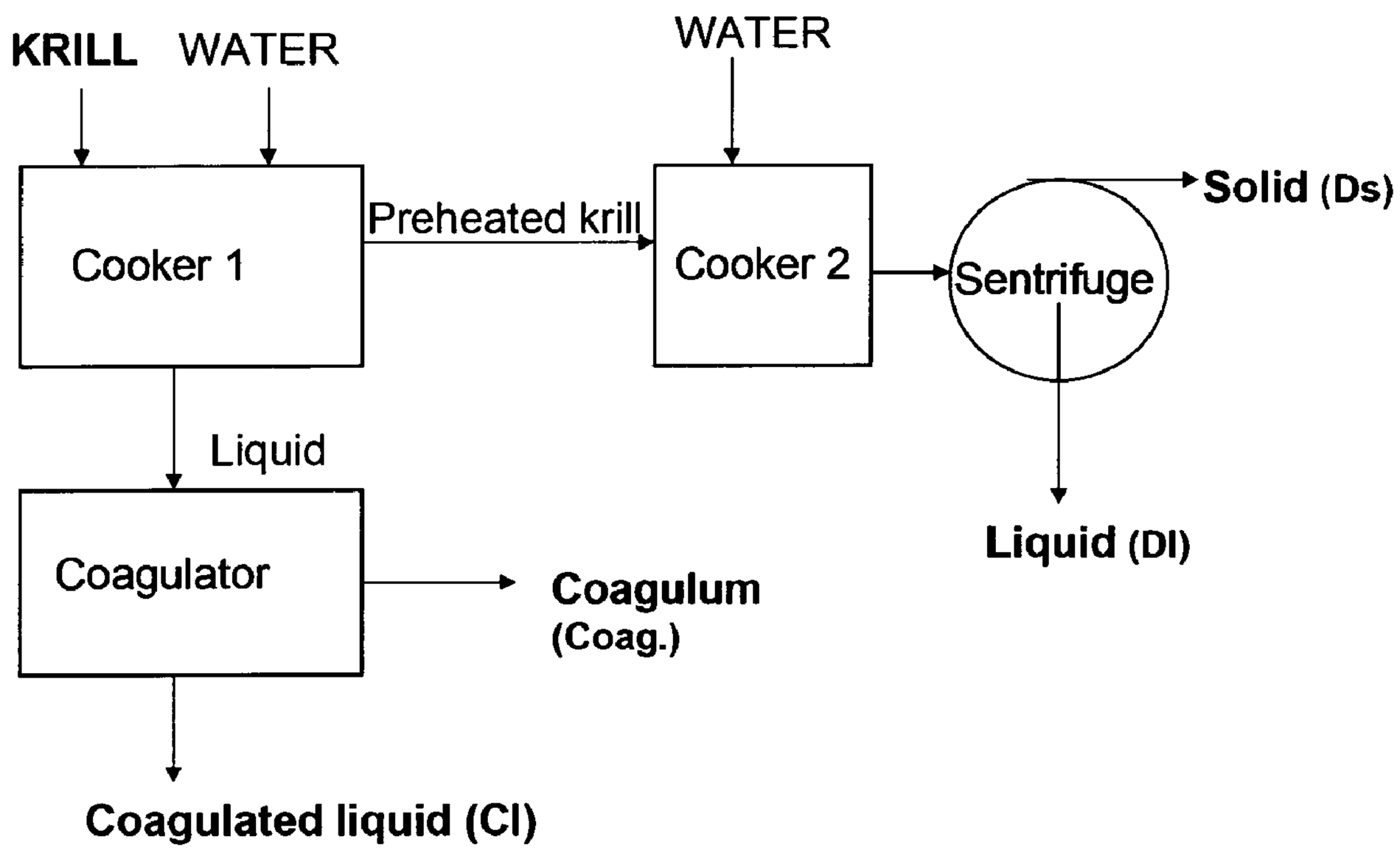

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A new method for krill meal production has been developed using a two step cooking process. In the first step the proteins and phospholipids are removed from the krill and precipitated as a coagulum. In the second stage the krill without phos-pholipids are cooked. Following this, residual fat and astaxanthin are removed from the krill using mechanical separation methods. A novel krill meal product with superior nutritional and technical properties is prepared.

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(54) Title: A NEW METHOD FOR MAKING KRILL MEAL

(57) Abstract: A new method for krill meal production has been developed using a two step cooking process. In the first step the proteins and phospholipids are removed from the krill and precipitated as a coagulum. In the second stage the krill without phospholipids are cooked. Following this, residual fat and astaxanthin are removed from the krill using mechanical separation methods. A novel krill meal product with superior nutritional and technical properties is prepared.

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A new method for making krill meal

FIELD OF THE INVENTION

The invention relates to processing crustaceans such as krill to provide oil and meal products, and in particular to the production of oils containing astaxanthin and phospholipids comprising omega-3 fatty acid moieties and meal rich in astaxanthin.

BACKGROUND OF THE INVENTION

Krill is a small crustacean which lives in all the major oceans world-wide. For example, it can be found in the Pacific Ocean (*Euphausia pacifica*), in the Northern Atlantic (*Meganyctiphanes norvegica*) and in the Southern Ocean off the coast of Antarctica (*Euphausia superba*). Krill is a key species in the ocean as it is the food source for many animals such as fish, birds, sharks and whales. Krill can be found in large quantities in the ocean and the total biomass of Antarctic krill (*E. superba*) is estimated to be in the range of 300-500 million metric tons. Antarctic krill feeds on phytoplankton during the short Antarctic summer. During winter, however, its food supply is limited to ice algae, bacteria, marine detritus as well as depleting body protein for energy. Virtue et al., Mar. Biol. 126, 521-527. For this reason, the nutritional values of krill vary during the season and to some extent annually. Phleger et al., Comp. Biochem. Physiol. 131B (2002) 733. In order to accommodate variations in food supply, krill has developed an efficient enzymatic digestive apparatus resulting in a rapid breakdown of the proteins into amino acids. Ellingsen et al., Biochem. J. (1987) 246, 295-305. This autoproteolysis is highly efficient also post mortem, making it a challenge to catch and store the krill in a way that preserves the nutritional quality of the krill. Therefore, in order to prevent the degradation of krill the enzymatic activity is either reduced by storing the krill at low temperatures or the krill is made into a krill meal.

During the krill meal process the krill is cooked so that all the active enzymes are denatured in order to eliminate all enzymatic activity. Krill is rich in phospholipids which act as emulsifiers. Thus it is more difficult to separate water, fat and proteins using mechanical separation methods than it is in a regular fish meal production line. In addition, krill becomes solid, gains weight and loose liquid more easily when mixed with hot water. Eventually this may lead to a gradual build up of coagulated krill proteins in the cooker and a non-continuous operation due to severe clogging problems. In order to alleviate this, hot steam must be added directly into the cooker. This operation is energy demanding and may also result in a degradation of unstable bioactive components in the krill such as omega-3 fatty acids,

phospholipids and astaxanthin. The presence of these compounds, make krill oil an attractive source as a food supplement, a functional food products and a pharmaceutical for the animal and human applications.

Omega-3 fatty acids have recently been shown to have potential effect of preventing cardiovascular disease, cognitive disorders, joint disease and inflammation related diseases such as rheumatoid arthritis. Astaxanthin is a strong antioxidant and may therefore assist in promoting optimal health. Hence, there is a need for a method of processing krill into a krill meal at more gentle conditions which prevents the degradation of these valuable bioactive compounds.

SUMMARY OF THE INVENTION

The invention relates to processing crustaceans such as krill to provide oil and meal products, and in particular to the production of oils and other lipid extracts containing astaxanthin and phospholipids comprising omega-3 fatty acid moieties and meal rich in astaxanthin.

In some embodiments, the present invention provides compositions comprising less than about 150, 100, 10, 5, 2 or 1 mg/kg astaxanthin or from about 0.1 to about 1, 2, 5, 10 or 200 mg/kg astaxanthin, preferably endogenous, naturally occurring astaxanthin, from about 20% to about 50%, 15% to 45%, or 25% to 35% phospholipids on a w/w basis, and about 15% to 60%, about 20% to 50%, or about 25% to 40% protein on a w/w basis, wherein said phospholipids comprise omega-3 fatty acid residues. In some embodiments, the composition comprises a lipid fraction having an omega-3 fatty acid content of from about 5% to about 30%, from 10% to about 30%, or from about 12% to about 18% on a w/w basis. In some embodiments, the phospholipids comprise greater than about 60%, 65%, 80%, 85% or 90% phosphatidylcholine on a w/w basis. In some embodiments, the phospholipids comprise less than about 15%, 10%, 8% or 5% ethanolamine on a w/w basis. In some embodiments, the compositions comprise from about 1% to 10%, preferably 2% to 8%, and most preferably about 2% to 6% alkylacylphosphatidylcholine. In some embodiments, the compositions comprise from about 40% to about 70% triacylglycerol on a w/w basis. In further embodiments, the compositions comprise less than about 1% cholesterol. In some embodiments, the protein comprises from about 8% to about 14% leucine on a w/w basis and from about 5% to 11% isoleucine on a w/w basis.

In some embodiments, the present invention comprises an aqueous phase and a solid phase, said solid phase comprising from about 20% to about 40% phospholipids on a w/w

basis, and about 20% to 50% protein on a w/w basis, wherein said phospholipids comprise from about 10% to about 20% omega-3 fatty acid residues.

In other embodiments, the present invention provides krill compositions comprising astaxanthin, a protein fraction, and a lipid fraction, wherein said lipid fraction comprises less than about 10%, 5% or 3% phospholipids on a w/w basis. In some embodiments, the phospholipids comprise less than about 15%, 10% or 5% phosphatidylcholine on a w/w basis.

In some embodiments, the present invention provides a krill meal comprising astaxanthin and from about 8 % to about 31 % lipids, preferably from about 8% to about 10 or 18 % lipids, wherein said lipids comprises greater than about 80% neutral lipids on a w/w basis. In some embodiments, the krill meal comprises less than about 15%, 10%, 5%, 3% or 1% phospholipids. In some embodiments, the phospholipids comprise less than about 15%, 10% or 5% phosphatidylcholine on a w/w basis.

In some embodiments, the present invention provides methods of preparing a phospholipid composition from biological material or biomass comprising: mixing said biological material or biomass with water at a suitable temperature to form a solid phase and an aqueous phase comprising phospholipids and proteins; separating said solid phase from said aqueous phase; heating said aqueous phase at a temperature sufficient to form a phospholipid-protein precipitate; and separating said phospholipid-protein precipitate from said aqueous phase. In some embodiments, the present invention provides a phospholipid-protein precipitate obtained by using the foregoing method. In some embodiments, the biological material or biomass is krill. In other embodiments, the biological material or biomass is selected from crabs, shrimp, calanus, plankton, crayfish, eggs or other phospholipid containing biological materials or biomass. In some embodiments, the methods further comprise the step of forming a meal from said solid phase. In some embodiments, the step of forming a meal comprises: heating the solid phase in the presence of water; separating fat and protein in said solid phase; and drying said protein to form a meal. In some embodiments, the processes further comprise the steps of pressing and drying the coagulum to form a coagulum meal. In some embodiments, the drying is by hot air or steam. In some embodiments, the present invention provides a phospholipid-protein precipitate obtained by using the foregoing method. In some embodiments, the present invention provides a composition comprising a krill solid phase according to the foregoing methods. In some embodiments, the present invention provides a krill meal obtained by the foregoing methods.

In some embodiments, the present invention provides processes comprising: extracting a first lipid fraction from a krill biomass; extracting a second lipid fraction from a krill

biomass; and blending said first lipid fraction and said second lipid fraction to provide a krill lipid composition having a desired composition. In some embodiments, the one or more of the extracting steps are performed in the absence of substantial amounts of organic solvents. In some embodiments, the first lipid fraction is extracted by: mixing krill with water at a suitable temperature to form a solid phase and an aqueous phase comprising phospholipids and protein; separating said solid phase from said aqueous phase; heating said aqueous phase at a temperature sufficient to form a phospholipid-protein precipitate; separating said phospholipid-protein precipitate from said aqueous phase; and separating said phospholipids from said protein. In some embodiments, the second lipid fraction is extracted by: heating the solid phase in the presence of water; and separating fat and protein in said solid phase. In some embodiments, the first lipid fraction comprises a phospholipid fraction comprising greater than about 90% phosphatidylcholine on a w/w basis. In some embodiments, the second lipid fraction comprises greater than about 80% neutral lipids on a w/w basis.

In some embodiments, the present invention provides processes of producing a phospholipid composition from biological material or biomass comprising: mixing said biological material or biomass with water to increase the temperature of said biological material to about 25 to 80 °C , preferably to about 50 to 75 °C, and most preferably to about 60 to 75 °C to form a first solid phase and a first aqueous phase comprising phospholipids and proteins; separating said first solid phase from said first aqueous phase; and separating a protein and phospholipid fraction from said first aqueous phase. In some embodiments, the biomass is heated to the first temperature for at least 3 minutes, preferably from about 3 minutes to 60 minutes, more preferably from about 3 minutes to 20 minutes, and most preferably from about 3 minutes to 10 minutes. The present invention is not limited to the use of any particular biological materials or biomass. In some embodiments, the biological material is a marine biomass. In some preferred embodiments, the biological material or biomass comprises krill crabs, shrimp, calanus, plankton, crayfish, eggs or other phospholipid containing biological materials or biomass. The present invention is not limited to the use of any particular type of krill. In some embodiments, the krill is fresh, while in other embodiments, the krill is frozen. In some embodiments, the krill is of the species *Euphausia superba*. In some embodiments, the step of separating a protein and phospholipid fraction from said first aqueous phase comprises heating said first aqueous phase at a temperature sufficient to form a phospholipid-protein coagulate and separating said phospholipid-protein coagulate from said aqueous phase. In some embodiments, the processes utilize a second heating step. In some embodiments, the first aqueous phase is heated to over 80 °C, preferably

to about 80 to 120 °C, and most preferably to about 90 to 100 °C. In some embodiments, the krill milk is held at these temperatures for from about 1 minute to about 60 minutes, preferably about 1 minute to about 10 minutes, and most preferably for about 2 minutes to 8 minutes. In some embodiments, the heating is at atmospheric pressure, while in other embodiments, the pressure is greater than atmospheric pressure. In some embodiments, the processes further comprise the step of pressing said phospholipid-protein coagulate to form a coagulate liquid phase and a coagulate press cake. In some embodiments, the processes further comprise drying said coagulate press cake to form a coagulate meal. In some embodiments, the processes further comprise extracting a coagulate oil from said coagulate meal. In some embodiments, the processes further comprise the steps of pressing and drying the coagulum to form a coagulum meal. In some embodiments, the drying is by hot air or steam.

In some embodiments, the step of separating a protein and phospholipid fraction from said first aqueous phase comprises filtration of said aqueous phase to provide a phospholipid-protein retentate comprising proteins and phospholipids. In some embodiments, filtration is via membrane filtration. In some embodiments, the filtration comprises filtering said aqueous phase through a microfilter with a pore size of from about 50 to 500 nm. In some embodiments, the processes further comprise the step of dewatering said phospholipid-protein retentate to form a retentate liquid phase and a retentate concentrate. In some embodiments, the processes further comprise the step of removing water from said retentate concentrate so that said retentate concentrate is microbially stable. In some embodiments, the processes further comprise the step of extracting a retentate oil from said retentate concentrate. In some embodiments, the processes further comprise the step of heating said first solid phase and then pressing said first solid phase to form a first press cake and a second liquid phase. In some embodiments, the processes further comprise the step of drying said first press cake to provide a first krill meal. In some embodiments, the processes further comprise the steps of heating said second liquid phase and then separating said second liquid phase to provide a first krill oil and stickwater. In some embodiments, the stickwater is evaporated and added to said first press cake, and a meal is formed from said evaporated stickwater and said first press cake to provide a second krill meal. In some embodiments, the second liquid phase is heated to over 80 °C, preferably to about 80 to 120 °C, and most preferably to about 90 to 100 °C prior to said separation. In some embodiments, the processes further comprise the step of combining the previously described coagulate oil or the retentate oil and the first krill oil to provide a blended oil. In other embodiments, the coagulate oil, retentate oil, or oil pressed from the first

solid phase are combined with the coagulate meal or retentate. In further embodiments, the processes of the present invention comprise the further step of supplementing the meals or oils produced as described above with additional proteins, phospholipids, triglycerides, fatty acids, and/or astaxanthin to produce an oil or meal with a desired defined composition. As such, a person of skill in the art will readily recognize that the processes described above serve as a starting point for producing compositions that are further supplemented in subsequent process steps to produce a desired composition, such a composition containing elevated levels of proteins, lipids or astaxanthin. In some embodiments, the present invention provides the lipid-protein composition produced by the foregoing processes. In some embodiments, the present invention provides the coagulate meal produced by the foregoing processes. In some embodiments, the present invention provides the coagulate oil produced by the foregoing processes. In some embodiments, the present invention provides the retentate meal produced by the foregoing processes. In some embodiments, the present invention provides the retentate oil produced by the foregoing processes. In some embodiments, the present invention provides the krill meal produced by the foregoing processes. In some embodiments, the present invention provides a krill oil produced by the foregoing processes. In some embodiments, the present invention provides a blended oil produced by the foregoing processes. In some embodiments, the compositions of the present invention are supplemented with additional proteins, phospholipids, triglycerides, fatty acids, and/or astaxanthin to produce an oil or meal with a desired defined composition. As such, a person of skill in the art will readily recognize that the compositions described above serve as a starting point for producing compositions that are further supplemented in subsequent process steps to produce a desired composition, such a composition containing elevated levels of proteins, lipids or astaxanthin.

In some embodiments, the present invention provides processes comprising: heating a krill biomass to about 25 to 80 °C, preferably to about 50 to 75 °C, and most preferably to about 60 to 75 °C; separating said krill biomass into solid and liquid phases; extracting a first lipid fraction from said solid phase; extracting a second lipid fraction from said liquid phases; and blending said first lipid fraction and said second lipid fraction to provide a krill lipid composition having a desired composition. In some embodiments, the extracting steps are performed in the absence of substantial amounts of organic solvents. In some embodiments, the first lipid fraction comprises a phospholipid fraction comprising greater than about 90% phosphatidylcholine on a w/w basis. In some embodiments, the second lipid fraction comprises greater than about 80% neutral lipids on a w/w basis.

In some embodiments, the present invention provides krill compositions comprising from about 0.01 to about 200 mg/kg astaxanthin, from about 45% to about 65% fat w/w, and about 20% to 50% protein w/w, wherein said fat comprises omega-3 fatty acid residues. In some embodiments, the fat has an omega-3 fatty acid content of from about 10% to 30 %, preferably 15% to about 25% on a w/w basis. In some embodiments, the fat comprises from about 20% to about 50% phospholipids w/w, wherein said phospholipids comprise greater than about 65% phosphatidylcholine w/w and from about 1% to about 10% alkylacylphosphatidylcholine. In some embodiments, the phospholipids comprise less than about 10% ethanolamine on a w/w basis. In some embodiments, the fat comprises from about 40% to about 70% triacylglycerol w/w. In some embodiments, the compositions further comprise less than about 1% cholesterol. In some embodiments, the protein comprises from about 8% to about 14% leucine on a w/w basis and from about 5% to 11% isoleucine on a w/w basis.

In some embodiments, the present invention provides krill compositions comprising from about 10% to about 20% protein w/w, about 15% to about 30% fat w/w, and from about 0.01 to about 200 mg/kg astaxanthin. In some embodiments, the fat has an omega-3 fatty acid content of from about 10% to about 30% on a w/w basis. In some embodiments, the fat comprises from about 30% to about 50% phospholipids w/w. In some embodiments, the phospholipids comprise greater than about 65% phosphatidylcholine w/w. In some embodiments, the phospholipids comprise less than about 10% ethanolamine on a w/w basis. In some embodiments, the fat comprises from about 40% to about 70% triacylglycerol w/w. In some embodiments, the compositions comprise less than about 1% cholesterol. In some embodiments, the protein comprises from about 7% to about 13% leucine on a w/w basis and from about 4% to 10% isoleucine on a w/w basis.

In some embodiments, the present invention provides krill meal press cakes comprising from about 65% to about 75% protein w/w (dry matter) , from about 10% to about 25% fat w/w (dry matter), and from about 1 to about 200 mg/kg astaxanthin (wet base). In some embodiments, the fat comprises greater than about 30% neutral lipids and greater than about 30% phospholipids on a w/w basis. In some embodiments, the fat comprises from about 50 to about 60% neutral lipids w/w and from about 40% to about 55% polar lipids w/w. In some embodiments, the protein comprises from about 5% to about 11% leucine w/w and from about 3% to about 7% isoleucine w/w.

In some embodiments, the present invention provides krill meals comprising from about 65% to about 75% protein w/w (dry matter) , from about 10% to about 25% fat w/w

(dry matter), and from about 1 to about 200 mg/kg astaxanthin (wet base). In some embodiments, the fat comprises greater than about 30% neutral lipids and greater than about 30% phospholipids on a w/w basis. In some embodiments, the fat comprises from about 50 to about 60% neutral lipids w/w and from about 40% to about 55% polar lipids w/w. In some embodiments, the polar lipids comprise greater than about 90% phosphatidyl choline w/w. In some embodiments, the polar lipids comprise less than about 10% phosphatidyl ethanolamine w/w. In some embodiments, the protein comprises from about 5% to about 11% leucine w/w and from about 3% to about 7% isoleucine w/w.

In some embodiments, the present invention provides krill oil compositions comprising greater than about 1500 mg/kg total esterified astaxanthin, wherein said esterified astaxanthin comprises from about 25 to 35% astaxanthin monoester on a w/w basis and from about 50 to 70% astaxanthin diester on a w/w basis, and greater than about 20 mg/kg free astaxanthin.

In some embodiments, the present invention provides krill compositions comprising from about 3% to about 10% protein w/w, about 8% to about 20% dry matter w/w, and about 4% to about 10% fat w/w. In some embodiments, the fat comprises from about 50% to about 70% triacylglycerol w/w. In some embodiments, the fat comprises from about 30% to about 50% phospholipids w/w. In some embodiments, the phospholipids comprise greater than about 90% phosphatidyl choline w/w. In some embodiments, the fat comprises from about 10% to about 25% n-3 fatty acids. In some embodiments, the fat comprises from about 10% to about 20% EPA and DHA.

In some embodiments, the krill compositions of the present invention are supplemented with additional proteins, phospholipids, triglycerides, fatty acids, and/or astaxanthin to produce an oil or meal with a desired defined composition. As such, a person of skill in the art will readily recognize that the krill compositions described above serve as a starting point for producing compositions that are further supplemented in subsequent process steps to produce a desired composition, such a composition containing elevated levels of proteins, lipids or astaxanthin.

The meal and oil compositions of the present invention described above are characterized in containing low levels, or being substantially free of many volatile compounds that are commonly found in products derived from marine biomass. In some embodiments, the meals and oils of the present invention are characterized as being substantially free of one or more of the following volatile compounds: acetone, acetic acid, methyl vinyl ketone, 1-penten-3-one, n-heptane, 2-ethyl furan, ethyl propionate, 2-methyl-2-pentenal, pyridine,

acetamide, toluene, N,N-dimethyl formamide, ethyl butyrate, butyl acetate, 3-methyl-1,4-heptadiene, isovaleric acid, methyl pyrazine, ethyl isovalerate, N,N-dimethyl acetamide, 2-heptanone, 2-ethyl pyridine, butyrolactone, 2,5-dimethyl pyrazine, ethyl pyrazine, N,N-dimethyl propanamide, benzaldehyde, 2-octanone, β-myrcene, dimethyl trisulfide, trimethyl pyrazine, 1-methyl-2-pyrrolidone. In other embodiments, the meals and oils of the present invention are characterized in containing less than 1000, 100, 10, 1 or 0.1 ppm (alternatively less than 10 mg/100g, preferably less than 1 mg/100 g and most preferably less than 0.1 mg/100 g) of one or more of the following volatile compounds: acetone, acetic acid, methyl vinyl ketone, 1-penten-3-one, n-heptane, 2-ethyl furan, ethyl propionate, 2-methyl-2-pentenal, pyridine, acetamide, toluene, N,N-dimethyl formamide, ethyl butyrate, butyl acetate, 3-methyl-1,4-heptadiene, isovaleric acid, methyl pyrazine, ethyl isovalerate, N,N-dimethyl acetamide, 2-heptanone, 2-ethyl pyridine, butyrolactone, 2,5-dimethyl pyrazine, ethyl pyrazine, N,N-dimethyl propanamide, benzaldehyde, 2-octanone, β-myrcene, dimethyl trisulfide, trimethyl pyrazine, 1-methyl-2-pyrrolidone. In further embodiments, the compositions of the present invention are characterized in comprising less than 10 mg/100g, and preferably less than 1mg/100 g (dry weight) of trimethylamine (TMA), trimethylamine oxide (TMAO) and/or lysophosphatidylcholine.

In some embodiments, the present invention provides systems for processing of marine biomass comprising: a mixer for mixing marine biomass and water to form a mixture having a defined temperature, wherein said mixture has a first solid phase and a first liquid phase. In some embodiments, the water is heated and said defined temperature of said mixture is from about 25 to 80 °C, preferably to about 50 to 75 °C, and most preferably to about 60 to 75 °C. In some embodiments, the systems further comprise a separator in fluid communication with said mixer for separating said first solid phase and said first liquid phase. In some embodiments, the first separator is a filter. In some embodiments, the systems further comprise a first heater unit in fluid communication with said first separator, wherein said first heater unit heats said first liquid phase to a defined temperature. In some embodiments, the defined temperature is about 80°C to about 100°C, preferably 90°C to about 100°C, most preferably 95°C to about 100°C. In some embodiments, the systems further comprise a microfilter in fluid communication with said mixer, wherein said liquid phase is separated into a retentate phase and a permeate phase by said microfilter. In some embodiments, the systems further comprise a prefilter in line with said microfilter. In some embodiments, the prefilter is a sieve In some embodiments, the water is heated and said defined temperature of said mixture is from about 25 to 80 °C, preferably to about 50 to 75 °C, and most preferably to

about 60 to 75 °C. In some embodiments, the systems further comprise a first separator in fluid communication with said mixer for separating said first solid phase and said first liquid phase. In some embodiments, the first separator is a filter.

In some embodiments, the present invention provides krill compositions comprising from about 10% to about 20% protein w/w, about 15% to about 30% fat w/w, from about 0.01% to about 200 mg/kg astaxanthin, and less than about 1 mg/100g trimethyl amine, trimethyl amine, volatile nitrogen, or 1g/100g lysophosphatidylcholine or combinations thereof. In some embodiments, the fat has an omega-3 fatty acid content of from about 10% to about 25% on a w/w basis. In some embodiments, the fat comprises from about 35% to about 50% phospholipids w/w. In some embodiments, the phospholipids comprise greater than about 90% phosphatidylcholine w/w. In some embodiments, the phospholipids comprise less than about 10% ethanolamine on a w/w basis. In some embodiments, the fat comprises from about 40% to about 60% triacylglycerol w/w. In some embodiments, the compositions further comprise less than about 1% cholesterol. In some embodiments, the protein comprises from about 7% to about 13% leucine on a w/w basis and from about 4% to 10% isoleucine on a w/w basis.

In some embodiments, the present invention provides processes for processing of marine biomass comprising: providing a marine biomass and a mixer for mixing marine biomass and water to form a mixture having a defined temperature, wherein said mixture comprises a first solid phase and a first liquid phase. In some embodiments, the defined temperature of said mixture is from about 25 to 80 °C, preferably to about 50 to 75 °C, and most preferably to about 60 to 75 °C. In some embodiments, the processes further comprise the steps of separating said liquid phase from said solid phase, and heating said liquid phase to about 80°C to about 100°C, preferably 90°C to about 100°C, most preferably 95°C to about 100°C, to produce a coagulate. In some embodiments, the coagulate comprises proteins and lipids. In some embodiments, the coagulate is separated from residual liquid by filtering.

In some embodiments, the present invention provides systems for processing of marine biomass comprising: a ship; a trawl net towable from said ship, said trawl net configured to catch the marine biomass; and a mixer for mixing said marine biomass and water to form a mixture having a defined temperature, wherein said mixture has a first solid phase and a first liquid phase. In some embodiments, the marine biomass is krill. In some embodiments, the krill is fresh krill and the trawl and ship are configured to deliver the fresh krill to the mixer. In some embodiments, system comprises a pump to transfer the biomass from the krill to the ship. In some embodiments, the system comprises a microfilter in fluid

communication with said mixer, wherein said microfilter separates said first solid phase and said first liquid phase. In some embodiments, the marine biomass is krill. In some embodiments, the krill is fresh krill.

In some embodiments, the present invention provides a pharmaceutical composition comprising one or more of the compositions described above in combination with a pharmaceutically acceptable carrier. In some embodiments, the present invention provides a food product comprising one or of the foregoing compositions. In some embodiments, the present invention provides a dietary supplement comprising one or more of the foregoing compositions. In some embodiments, the present invention provides an animal feed comprising one or more of the foregoing compositions.

DESCRIPTION OF THE FIGURES

Figure 1 shows an overview of the process of making krill meal with a two stage cooking process.

Figure 2 is a graph of the Permeate flux as function of dry matter of the retentate (%) (°Brix).

Figure 3 is a graph of Average Flux as function of dry matter in retentate.

Figure 4 is a GC of the neutral fraction extracted from krill coagulate.

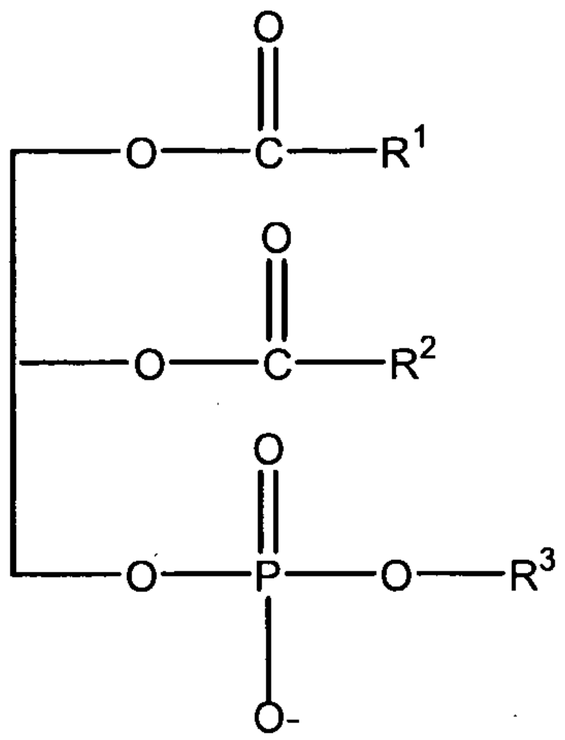
Figure 5 is a GC analysis of the neutral fraction extracted from krill coagulate.

Figure 6 is a GC of the polar fraction extracted from krill coagulate.

Figure 7 is a GC analysis of the polar fraction extracted from krill coagulate.

DEFINITIONS

As used herein, "phospholipid" refers to an organic compound having the following general structure:

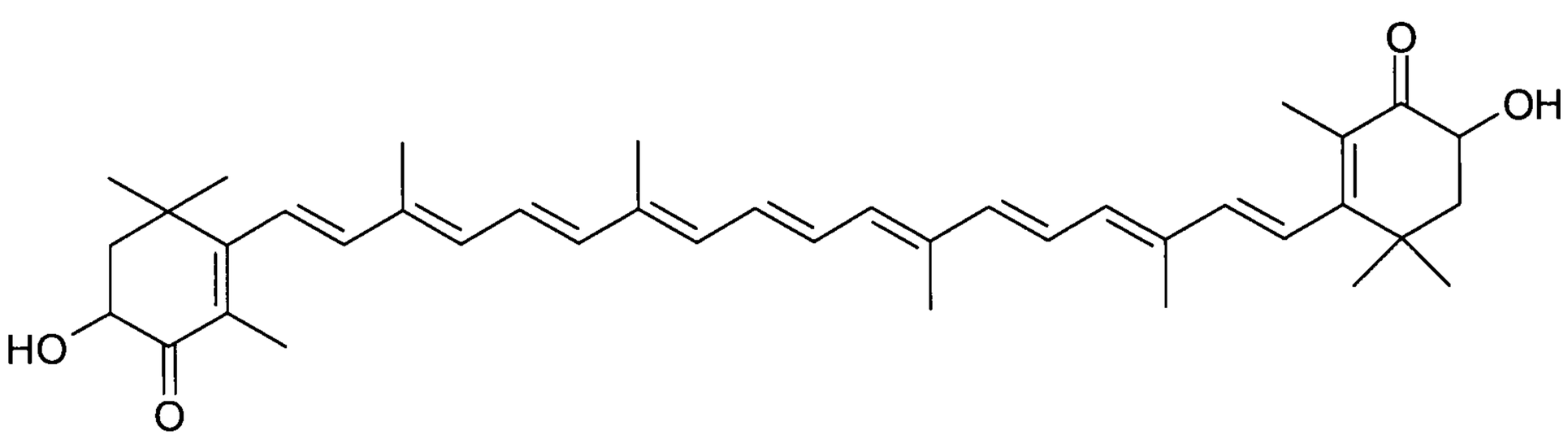


wherein R1 is a fatty acid residue, R2 is a fatty acid residue or –OH, and R3 is a –H or nitrogen containing compound choline ($HOCH_2CH_2N^+(CH_3)_3OH^-$), ethanolamine ($HOCH_2CH_2NH_2$), inositol or serine. R1 and R2 cannot simultaneously be OH. When R3 is an –OH, the compound is a diacylglycerophosphate, while when R3 is a nitrogen-containing compound, the compound is a phosphatide such as lecithin, cephalin, phosphatidyl serine or plasmalogen.

An "ether phospholipid" as used herein refers to a phospholipid having an ether bond at position 1 the glycerol backbone. Examples of ether phospholipids include, but are not limited to, alkylacylphosphatidylcholine (AAPC), lyso-alkylacylphosphatidylcholine (LAAPC), and alkylacylphosphatidylethanolamine (AAPE). A "non-ether phospholipid" is a phospholipid that does not have an ether bond at position 1 of the glycerol backbone.

As used herein, the term omega-3 fatty acid refers to polyunsaturated fatty acids that have the final double bond in the hydrocarbon chain between the third and fourth carbon atoms from the methyl end of the molecule. Non-limiting examples of omega-3 fatty acids include, 5,8,11,14,17-eicosapentaenoic acid (EPA), 4,7,10,13,16,19-docosahexanoic acid (DHA) and 7,10,13,16,19-docosapentanoic acid (DPA).

As used herein, astaxanthin refers to the following chemical structure:



As used herein, astaxanthin esters refer to the fatty acids esterified to OH group in the astaxanthin molecule.

As used herein, the term w/w (weight/weight) refers to the amount of a given substance in a composition on weight basis. For example, a composition comprising 50% w/w phospholipids means that the mass of the phospholipids is 50% of the total mass of the composition (i.e., 50 grams of phospholipids in 100 grams of the composition, such as an oil).

As used herein, the term "fresh krill" refers to krill that is has been harvested less than about 12, 6, 4, 2 or preferably 1 hour prior to processing. "Fresh krill" is characterized in that products made from the fresh krill such as coagulum comprise less than 1 mg/100g TMA, volatile nitrogen or Trimetylamine oxide-N, alone or in combination, and less than 1g/100 g lysophosphatidylcholine.

DETAILED DESCRIPTION OF THE INVENTION

The invention relates to processing crustaceans such as krill to provide oil and meal products, and in particular to the production of oils containing astaxanthin and phospholipids comprising omega-3 fatty acid moieties and meal rich in astaxanthin. In some embodiments, the present invention provides systems and methods for the continuous processing of fresh or frozen krill into useful products, including krill oil, krill meal, and a krill protein/phospholipid coagulum.

Previous processes for treating marine biomasses such as krill have utilized a single high temperature treatment to provide a proteinaceous product. Pat No. SU220741; "Removing fats from the protein paste "Okean". Gulyaev and Bugrova, Konservnaya i Ovoshchesushil'naya Promyshlennost (1976), (4), 37-8; Amino acid composition of protein-coagulate in krill. Nikolaeva, VNIRO (1967), 63 161-4. However, these methods result in a product with a relatively low lipid content. The present invention describes a process in which the marine biomass such as krill is first heated at moderate temperatures to provide an aqueous phase which is subsequently heated at a higher temperature. This process provides a novel protein-lipid composition that has a higher lipid content than previously described compositions produced from marine biomasses. The compositions of the present invention are further distinguished from other krill oil supplements marketed for human use in that the described compositions are, in some embodiments, provided as solids or powders comprising a combination of krill lipids, including krill phospholipids and krill triglycerides, and krill-derived protein. These solids/powders may preferably be provided in capsules, gel capsules, or as tablets or caplets.

In some embodiments, the present invention provides solvent-free methods to produce a phospholipid-containing composition from a biomass such as krill, crabs, Calanus, plankton, eggs, crayfish, shrimp and the like without using organic solvents. In some embodiments, the biomass (preferably krill, freshly harvested or frozen) is heated to a temperature in the range of 25 to 80°C, preferably 40 to 75°C, and most preferably 60 to 75°C in order to dissolve/disperse lipids and proteins from the krill into the water phase, which is called krill milk. In some embodiments, the biomass is heated to and held at this first temperature for at least 3 minutes, preferably from about 3 minutes to 60 minutes, more preferably from about 3 minutes to 20 minutes, and most preferably from about 3 minutes to 10 minutes. In some embodiments, the processes then utilize a second heating step. The proteins and phospholipids are precipitated out of the water phase produced from the first heating step by heating the krill milk (after removal of the krill solids) to a temperature of greater than about 80°C, preferably 80 to 120°C, most preferably 95 to 100°C. In some embodiments, the krill milk is held at these temperatures for from about 1 minute to about 60 minutes, preferably about 1 minute to about 10 minutes, and most preferably for about 2 minutes to 8 minutes. The water phase may be heated at atmospheric pressure, or the water phase may be heated in a closed system at an elevated pressure so that the temperature can be increased above 100°C. Accordingly, in some embodiments, the heating is at atmospheric pressure, while in other embodiments, the pressure is greater than atmospheric pressure. The precipitate formed (hereafter called a coagulum) can be isolated and characterized. In some embodiments, the processes further comprise the steps of pressing and drying the coagulum to form a coagulum meal. In some embodiments, the drying is by hot air or steam.

The solid phase (e.g., krill solids) is preferably used to make a krill meal which also has a novel composition. In other embodiments, the krill milk is microfiltrated. The solid phase produced by microfiltration (called the retentate) is similar to that of the coagulum. Data show that the coagulum and retentate are low in cholesterol. In some embodiments, the retentate and coagulum are substantially free of cholesterol. In some embodiments, the retentate and coagulum comprise less than 1% cholesterol, preferably less than 0.1% cholesterol. This is a novel method to remove at least a portion of the lipids, such as phospholipids, from the krill. Removal of lipids from krill has previously required solvent extraction using liquids such as ethanol or other polar solvents. Solvent extraction is time-consuming and may also result in loss of material and is therefore not wanted. The krill used to separate out the coagulum had been stored frozen for 10 months prior to the experimentation. It is believed that due to the release of proteolytic enzyme activity during a

freezing/thawing process, more protein can be expected to be solubilized based on the processing of frozen krill than from fresh krill.

In some embodiments, the present invention provides systems and processes for processing a marine biomass. In preferred embodiments, the marine biomass is krill, preferably the Antarctic krill *Euphausia superba*. Other krill species may also be processed using the systems and processes of the present invention. In some embodiments, the krill is processed in a fresh state as defined herein. In some embodiments, the krill is processed on board a ship as described below within 12, 10, 8, 6, 4, or preferably 2 hours of catching the krill. In some embodiments, the krill is processed on board a ship within 1 or preferably 0.5 hours of catching the krill. In some embodiments, the ship tows a trawl that is configured to catch krill. The krill is then transferred from the trawl to the ship and processed. In some embodiments, the trawl comprises a pump system to pump the freshly caught krill from the trawl to the ship so that the krill can be processed in a fresh state. In preferred embodiments, the pump system comprises a tube that extends below the water the trawl and a pumping action is provided by injecting air into the tube below the waterline so that the krill is continuously drawn or pumped from the trawl, through the tube and on board the ship. Preferred trawling systems with pumps are described in PCT Applications WO 07/108702 and WO 05/004593, incorporated herein by reference.

Some embodiments of the systems and processes of the present invention are shown in Figure 1. As shown in Figure 1, fresh or frozen is krill is mixed in mixer with a sufficient amount of hot water from water heater to increase the temperature of the krill mass to approximately 40 to 75°C, preferably 50 to 75 °C, more preferably 60 to 75 °C, and most preferably about 60 to 70 °C. Many different types of water heaters are useful in the present invention. In some embodiments, the water heater is a steam heated kettle, while in other embodiments, the water heater is a scraped surface heat exchanger. The heated mass is then separated into liquid (krill milk) and krill solid fractions in a filter. In some embodiments, the separation is performed by sieving through a metal sieve. After separation, the krill milk is heated to approximately 90°C to 100°C, preferably to about 95°C to 100°C in a heater. Any type of suitable water or liquid heater may be used. In preferred embodiments, the heater is a scraped surface heat exchanger. This heating step produced a solid fraction (the coagulum described above) and a liquid fraction. In some preferred embodiments, the separator utilizes a filter as previously described. The present invention is not limited to the use of any particular type of filter. In some embodiments, the filter is a woven filter. In some embodiments, the filter comprises polymeric fibers. The coagulum is introduced into a

dewaterer. In some embodiments, the dewaterer is a press such as screw press. Pressing produces a liquid fraction and a press cake. The press cake is dried in a drier to produce coagulum meal.

The solid krill fraction is introduced into a dewaterer for dewatering. In some embodiments, the dewaterer is a press such as screw press. Pressing produces a press cake and a liquid fraction. The press cake is dried in a drier, such as an air drier or steam drier, to provide krill meal. The liquid fraction is centrifuged to produce a neutral krill oil containing high levels of astaxanthin and stickwater. In preferred embodiments, the stick water is added back into the krill press cake to make a full meal, including the various components of the stick water such as soluble proteins, amino acids, etc.

In alternative embodiments, the krill milk can be treated by microfiltration instead of by heating to form a coagulum. The krill milk is introduced into a microfilter. Microfiltration produces a fraction called a retentate and a liquid permeate. The retentate is concentrated by evaporation under vacuum to stability, water activity <0.5 Aw. Membrane filtration of cooking liquid is preferably performed at about 70 °C with a filter having a pore size of about 10 nm to about 1000nm, more preferably about 50 to about 500 nm, and most preferably about 100 nm. An exemplary filter is the P19-40 100 nm ZrO_2 membrane. In some embodiments, the liquid fraction is prefiltered prior to microfiltration. In preferred embodiments, the prefilter is a roto-fluid sieve (air opening 100 μm).

In yet another embodiment of the invention is a novel and more efficient method of preparing krill meal. By removing the coagulum, the krill meal process is less susceptible to clogging problems and the use of hot steam in the cooker can be avoided. The data disclosed show the coagulum contains a high percentage of phospholipids, hence the separation of the fat in the new krill meal process can be obtained using mechanical methods as in standard fish meal processes. In fact, the separation of fat from the meal is important. Ideally, the krill meal should have a low fat value in order to have satisfactory technical properties. Mechanically separating the fat from the meal will result in a neutral oil rich in astaxanthin. If the neutral oil rich in astaxanthin stays in the meal, the astaxanthin may be degraded during the drying.

In some embodiments, the present invention provides a krill coagulate and retentate compositions. The compositions are characterized in containing a combination of protein and lipids, especially phospholipids. In preferred embodiments, the compositions are solids or powders and are provided as a meal. In some embodiments, the compositions comprise from about 20% to about 50% protein w/w, preferably about 30% to 40% protein w/w, and about 40% to 70% lipids w/w, preferably about 50% to 65% lipids w/w, so that the total amount of

proteins and lipids in the compositions of from 90 to 100%. In some embodiments, the lipid fraction contains from about 10 g to 30 g omega-3 fatty acid residues per 100 g of lipid, preferably about 15 g to 25 g omega-3 fatty acids residues per 100 g lipids (i.e., from 10 to 30% or preferably from 15 to 25% omega-3 residues expressed w/w as a percentage of total lipids in the composition). In some embodiments, the lipid fraction of the composition comprises from about 25 to 50 g polar lipids per 100 g lipids (25 to 50% w/w expressed as percentage of total lipids), preferably about 30 to 45 g polar lipids per 100 g total lipids (30 to 45% w/w expressed as percentage of total lipids), and about 50 to 70 g nonpolar lipids per 100 g lipids (50 to 70% w/w expressed as percentage of total lipids), so that the total amount of polar and nonpolar lipids is 90 to 100% of the lipid fraction. In some embodiments, the phospholipids comprise greater than about 60% phosphatidylcholine on a w/w basis. In some embodiments, the phospholipids comprise less than about 10% ethanolamine on a w/w basis. In some embodiments, the compositions comprise from about 20% to about 50% triacylglycerol on a w/w basis. In some embodiments, the compositions comprise less than about 1% cholesterol. In some embodiments, the protein fraction comprises from about 8% to about 14% leucine on a w/w basis and from about 5% to 11% isoleucine on a w/w basis. In some embodiments, the compositions comprise less than about 200, 10, 5 or 1 mg/kg naturally occurring or endogenous astaxanthin. In some embodiments, the compositions comprise from about 0.01 to about 200 mg/kg naturally-occurring astaxanthin. It will be recognized that the astaxanthin content of the composition can be increased by adding in astaxanthin from other (exogenous) sources, both natural and non-natural. Likewise, the compositions can be supplemented with exogenous proteins, triglycerides, phospholipids and fatty acids such as omega-3 fatty acids to produce a desired composition.

In yet another embodiment of the invention is a pre-heated krill composition. Non-limiting examples of the pre-heated krill composition is a krill composition comprising lipids with less than 10% or 5% phospholipids, and in particular phosphatidylcholine.

In yet another embodiment of the invention is a novel krill meal product produced from the solid phase left after the first heating step (i.e., the heating step at below 80 C). The krill meal has good nutritional and technical qualities such as a high protein content, low fat content and has a high flow number. Unexpectedly, the ratios of polar lipids to neutral lipids and EPA to DHA is substantially enhanced as compared to normal krill meal. In some embodiments, the krill meals comprise from about 60% to about 80% protein on a w/w basis, preferably from about 70% to 80% protein on a w/w basis, from about 5% to about 20% fat on a w/w basis, and from about 1 to about 200 mg/kg astaxanthin, preferably from about 50 to

about 200 mg/kg astaxanthin. In some embodiments, the fat comprises from about 20 to 40% total neutral lipids and from about 50 to 70% total polar lipids on a w/w basis (total lipids). In some embodiments, the ratio of polar to neutral lipids in the meal is from about 1.5:1 to 3:1, preferably about 1.8:1 to 2.5:1, and most preferably from about 1.8:1 to 2.2:1. In some embodiments, the fat comprises from about 20% to 40% omega-3 fatty acids, preferably about 20% to 30% omega-3 fatty acids. In some embodiments, the ratio of EPA:DHA is from about 1.8:1 to 1:0.9, preferably from about 1.4:1 to 1:1.

In still other embodiments, the present invention provides oil produced by the processes described above. In some embodiments, the oils comprise greater than about 1800 mg/kg total esterified astaxanthin, wherein said esterified astaxanthin comprises from about 25 to 35% astaxanthin monoester on a w/w basis and from about 50 to 70% astaxanthin diester on a w/w basis, and less than about 40 mg/kg free astaxanthin.

The compositions of the present invention are highly palatable humans and other animals. In particular the oil and meal compositions of the present invention are characterized as containing low levels of undesirable volatile compounds or being substantially free of many volatile compounds that are commonly found in products derived from marine biomass. In some embodiments, the meals and oils of the present invention are characterized as being substantially free of one or more of the following volatile compounds: acetone, acetic acid, methyl vinyl ketone, 1-penten-3-one, n-heptane, 2-ethyl furan, ethyl propionate, 2-methyl-2-pentenal, pyridine, acetamide, toluene, N,N-dimethyl formamide, ethyl butyrate, butyl acetate, 3-methyl-1,4-heptadiene, isovaleric acid, methyl pyrazine, ethyl isovalerate, N,N-dimethyl acetamide, 2-heptanone, 2-ethyl pyridine, butyrolactone, 2,5-dimethyl pyrazine, ethyl pyrazine, N,N-dimethyl propanamide, benzaldehyde, 2-octanone, β-myrcene, dimethyl trisulfide, trimethyl pyrazine, 1-methyl-2-pyrrolidone. In other embodiments, the meals and oils of the present invention are characterized in containing less than 1000, 100, 10, 1 or 0.1 ppm (alternatively less than 10 mg/100g, preferably less than 1 mg/100 g and most preferably less than 0.1 mg/100 g) of one or more of the following volatile compounds: acetone, acetic acid, methyl vinyl ketone, 1-penten-3-one, n-heptane, 2-ethyl furan, ethyl propionate, 2-methyl-2-pentenal, pyridine, acetamide, toluene, N,N-dimethyl formamide, ethyl butyrate, butyl acetate, 3-methyl-1,4-heptadiene, isovaleric acid, methyl pyrazine, ethyl isovalerate, N,N-dimethyl acetamide, 2-heptanone, 2-ethyl pyridine, butyrolactone, 2,5-dimethyl pyrazine, ethyl pyrazine, N,N-dimethyl propanamide, benzaldehyde, 2-octanone, β-myrcene, dimethyl trisulfide, trimethyl pyrazine, 1-methyl-2-pyrrolidone. In further embodiments, the compositions of the present invention are characterized in comprising less than 10 mg/100g,

and preferably less than 1mg/100 g (dry weight) of trimethylamine (TMA), trimethylamine oxide (TMAO) and/or lysophosphatidylcholine.

In some embodiments, the compositions of this invention (such as those described in the preceding sections) are contained in acceptable excipients and/or carriers for oral consumption. In some embodiments, the present invention provides a pharmaceutical compositions one or more of the foregoing compositions in combination with a pharmaceutically acceptable carrier. The actual form of the carrier, and thus, the composition itself, is not critical. The carrier may be a liquid, gel, gelcap, capsule, powder, solid tablet (coated caplet or non-coated), tea, or the like. The composition is preferably in the form of a tablet or capsule and most preferably in the form of a soft gel capsule. Suitable excipient and/or carriers include maltodextrin, calcium carbonate, dicalcium phosphate, tricalcium phosphate, microcrystalline cellulose, dextrose, rice flour, magnesium stearate, stearic acid, croscarmellose sodium, sodium starch glycolate, crospovidone, sucrose, vegetable gums, lactose, methylcellulose, povidone, carboxymethylcellulose, corn starch, and the like (including mixtures thereof). Preferred carriers include calcium carbonate, magnesium stearate, maltodextrin, and mixtures thereof. The various ingredients and the excipient and/or carrier are mixed and formed into the desired form using conventional techniques. The tablet or capsule of the present invention may be coated with an enteric coating that dissolves at a pH of about 6.0 to 7.0. A suitable enteric coating that dissolves in the small intestine but not in the stomach is cellulose acetate phthalate. Further details on techniques for formulation for and administration may be found in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing Co., Easton, PA).

The dietary supplement may comprise one or more inert ingredients, especially if it is desirable to limit the number of calories added to the diet by the dietary supplement. For example, the dietary supplement of the present invention may also contain optional ingredients including, for example, herbs, vitamins, minerals, enhancers, colorants, sweeteners, flavorants, inert ingredients, and the like. For example, the dietary supplement of the present invention may contain one or more of the following: ascorbates (ascorbic acid, mineral ascorbate salts, rose hips, acerola, and the like), dehydroepiandosterone (DHEA), Fo-Ti or Ho Shu Wu (herb common to traditional Asian treatments), Cat's Claw (ancient herbal ingredient), green tea (polyphenols), inositol, kelp, dulse, bioflavinoids, maltodextrin, nettles, niacin, niacinamide, rosemary, selenium, silica (silicon dioxide, silica gel, horsetail, shavegrass, and the like), spirulina, zinc, and the like. Such optional ingredients may be either naturally occurring or concentrated forms.

In some embodiments, the dietary supplements further comprise vitamins and minerals including, but not limited to, calcium phosphate or acetate, tribasic; potassium phosphate, dibasic; magnesium sulfate or oxide; salt (sodium chloride); potassium chloride or acetate; ascorbic acid; ferric orthophosphate; niacinamide; zinc sulfate or oxide; calcium pantothenate; copper gluconate; riboflavin; beta-carotene; pyridoxine hydrochloride; thiamin mononitrate; folic acid; biotin; chromium chloride or picolonate; potassium iodide; sodium selenate; sodium molybdate; phylloquinone; vitamin D3; cyanocobalamin; sodium selenite; copper sulfate; vitamin A; vitamin C; inositol; potassium iodide. Suitable dosages for vitamins and minerals may be obtained, for example, by consulting the U.S. RDA guidelines.

In further embodiments, the compositions comprise at least one food flavoring such as acetaldehyde (ethanal), acetoin (acetyl methylcarbinol), anethole (parapropenyl anisole), benzaldehyde (benzoic aldehyde), N butyric acid (butanoic acid), d or l carvone (carvol), cinnamaldehyde (cinnamic aldehyde), citral (2,6 dimethyloctadien 2,6 al 8, gera nial, neral), decanal (N decylaldehyde, capraldehyde, capric aldehyde, caprinaldehyde, aldehyde C 10), ethyl acetate, ethyl butyrate, 3 methyl 3 phenyl glycidic acid ethyl ester (ethyl methyl phenyl glycidate, strawberry aldehyde, C 16 aldehyde), ethyl vanillin, geraniol (3,7 dimethyl 2,6 and 3,6 octadien 1 ol), geranyl acetate (geraniol acetate), limonene (d , l , and dl), linalool (linalol, 3,7 dimethyl 1,6 octadien 3 ol), linalyl acetate (bergamol), methyl anthranilate (methyl 2 aminobenzoate), piperonal (3,4 methylenedioxy benzaldehyde, heliotropin), vanillin, alfalfa (Medicago sativa L.), allspice (Pimenta officinalis), ambrette seed (Hibiscus abelmoschus), angelic (Angelica archangelica), Angostura (Galipea officinalis), anise (Pimpinella anisum), star anise (Illicium verum), balm (Melissa officinalis), basil (Ocimum basilicum), bay (Laurus nobilis), calendula (Calendula officinalis), (Anthemis nobilis), capsicum (Capsicum frutescens), caraway (Carum carvi), cardamom (Elettaria cardamomum), cassia, (Cinnamomum cassia), cayenne pepper (Capsicum frutescens), Celery seed (Apium graveolens), chervil (Anthriscus cerefolium), chives (Allium schoenoprasum), coriander (Coriandrum sativum), cumin (Cuminum cyminum), elder flowers (Sambucus canadensis), fennel (Foeniculum vulgare), fenugreek (Trigonella foenum graecum), ginger (Zingiber officinale), horehound (Marrubium vulgare), horseradish (Armoracia lapathifolia), hyssop (Hyssopus officinalis), lavender (Lavandula officinalis), mace (Myristica fragrans), marjoram (Majorana hortensis), mustard (Brassica nigra, Brassica juncea, Brassica hirta), nutmeg (Myristica fragrans), paprika (Capsicum annuum), black pepper (Piper nigrum), peppermint (Mentha piperita), poppy seed (Papayer somniferum), rosemary (Rosmarinus officinalis), saffron (Crocus sativus), sage (Salvia officinalis), savory (Satureia hortensis, Satureia

montana), sesame (Sesamum indicum), spearmint (Mentha spicata), tarragon (Artemisia dracunculus), thyme (Thymus vulgaris, Thymus serpyllum), turmeric (Curcuma longa), vanilla (Vanilla planifolia), zedoary (Curcuma zedoaria), sucrose, glucose, saccharin, sorbitol, mannitol, aspartame. Other suitable flavoring are disclosed in such references as Remington's Pharmaceutical Sciences, 18th Edition, Mack Publishing, p. 1288-1300 (1990), and Furia and Pellanca, Fenaroli's Handbook of Flavor Ingredients, The Chemical Rubber Company, Cleveland, Ohio, (1971), known to those skilled in the art.

In other embodiments, the compositions comprise at least one synthetic or natural food coloring (e.g., annatto extract, astaxanthin, beet powder, ultramarine blue, canthaxanthin, caramel, carotenal, beta carotene, carmine, toasted cottonseed flour, ferrous gluconate, ferrous lactate, grape color extract, grape skin extract, iron oxide, fruit juice, vegetable juice, dried algae meal, tagetes meal, carrot oil, corn endosperm oil, paprika, paprika oleoresin, riboflavin, saffron, tumeric, tumeric and oleoresin).

In still further embodiments, the compositions comprise at least one phytonutrient (e.g., soy isoflavonoids, oligomeric proanthcyanidins, indol 3 carbinol, sulforaphone, fibrous ligands, plant phytosterols, ferulic acid, anthocyanocides, triterpenes, omega 3/6 fatty acids, conjugated fatty acids such as conjugated linoleic acid and conjugated linolenic acid, polyacetylene, quinones, terpenes, cathechins, gallates, and quercitin). Sources of plant phytonutrients include, but are not limited to, soy lecithin, soy isoflavones, brown rice germ, royal jelly, bee propolis, acerola berry juice powder, Japanese green tea, grape seed extract, grape skin extract, carrot juice, bilberry, flaxseed meal, bee pollen, ginkgo biloba, primrose (evening primrose oil), red clover, burdock root, dandelion, parsley, rose hips, milk thistle, ginger, Siberian ginseng, rosemary, curcumin, garlic, lycopene, grapefruit seed extract, spinach, and broccoli.

In still other embodiments, the compositions comprise at least one vitamin (e.g., vitamin A, thiamin (B1), riboflavin (B2), pyridoxine (B6), cyanocobalamin (B12), biotin, ascorbic acid (vitamin C), retinoic acid (vitamin D), vitamin E, folic acid and other folates, vitamin K, niacin, and pantothenic acid). In some embodiments, the particles comprise at least one mineral (e.g., sodium, potassium, magnesium, calcium, phosphorus, chlorine, iron, zinc, manganese, flourine, copper, molybdenum, chromium, selenium, and iodine). In some particularly preferred embodiments, a dosage of a plurality of particles includes vitamins or minerals in the range of the recommended daily allowance (RDA) as specified by the United States Department of Agriculture. In still other embodiments, the particles comprise an amino

acid supplement formula in which at least one amino acid is included (e.g., l-carnitine or tryptophan).

In further embodiments, the present invention provide animal feeds comprising one or more the compositions described in detail above. The animal feeds preferably form a ration for the desired animal and is balanced to meet the animals nutritional needs. The compositions may be used in the formulation of feed or as feed for animals such as fish, including fish fry, poultry, cattle, pigs, sheep, shrimp and the like.

EXAMPLE 1

Four portions of krill were analysed for dry matter, fat, and protein. Most of the variation in the composition can be expected to be due to variation in the sampling. To include the effect of variation in storage time after thawing, raw material samples were also taken at different times during the working day. The observed variation in raw material input is inherent in all calculations of fat, dry matter and protein distributions based on the reported examples.

Table 1. Composition of krill (g/100 g)

	Dry matter	Fat	Fat free dry matter	Protein
Krill 1	21,40	7,80	13,60	11,80
Krill 2	22,13	7,47	14,66	12,96
Krill 3	23,78	7,44	16,34	14,60
Krill 4	23,07	7,55	15,52	13,83
Mean	**22,60**	**7,57**	**15,03**	**13,30**
SD	1,04	0,16	1,17	1,20
RSD	4,6 %	2,2 %	7,8 %	9,0 %

EXAMPLE 2

In this example a novel method for preparing krill meal was investigated. 800 g of preheated water (95-100 °C) and 200g of frozen krill (0 °C) were mixed in a cooker (cooker 1) at a temperature of 75 °C for 6 minutes. Next, the heated krill and the hot water were

separated by filtration. The preheated krill was further cooked (cooker 2) by mixing with 300 g hot water (95 °C) in a kitchen pan and kept at 90 °C for 2 minutes before separation over a sieve (1,0 × 1,5 mm opening). The heated krill was separated from the liquid and transferred to a food mixer and cut for 10 seconds. The disintegrated hot krill was added back to the hot water and centrifuged at 8600 × g (RCF average) for 10 minutes. The supernatant corresponding to a decanter liquid (Dl) was decanted off. The liquid from cooking step 1 was heated to 95-100 °C to coagulate the extracted protein. The coagulum was separated over a sieve (1.0 × 1.5 mm opening) and a weight of 40 g was found. Figure 1 shows an overview of the process of making krill meal with a two stage cooking process.

EXAMPLE 3

The total volatile nitrogen (TVN), trimethylamine (TMA) and trimethylamine oxide (TMAO) content were determined in the four products from the cooking test in example 2 (Table 2). The krill was fresh when frozen, so no TMA was detected in the products. The results show that TMAO is evenly distributed in the water phase during cooking of krill.

Table 2. Distribution of total volatile nitrogen (TVN), trimethylamine (TMA) and trimethylamine oxide (TMAO) in the products from the cooking procedure.

Products from test no.	10	Krill	Coagulum from cooker	Coagulated cooker liquid	Decanter solids	Decanter liquid	SUM
Weight (wb)	g	200	97,6	711,1	90,3	294,7	
Dry matter	g/100 g	21,4	14,2	1,0	22,2	0,9	
Analytical values							
Total volatile nitrogen	mg N/100 g	8	1,3	1,2	2,3	1	
Trimetylamine-N	mg N/100 g	<1	<1	<1	<1	<1	
Trimetylamine oxid-N	mg N/100 g	107	19,2	13,5	10,4	13,1	
Quantities							
Total volatile nitrogen	mg N	15,0	1,3	8,5	2,1	2,9	14,8
Trimetylamine-N	mg N	-	-	-	-	-	-

Trimetylamine oxid-N	mg N	214	18,7	96,0	9,4	38,6	163
Distribution							
Total volatile nitrogen	% of input	100 %	8 %	57 %	14 %	20 %	99 %
Trimetylamine-N	% of input						
Trimetylamine oxid-N	% of input	100 %	9 %	45 %	4 %	18 %	76 %

In addition, fat, dry matter and astaxanthin were determined in the products (Table 3). It was observed that the major part of the astaxanthin in the krill was found in the press cake (*Table* 3). Only a minor part is found in the coagulum which contains more than 60 % of the lipid in the krill raw material. The cooking procedure with leaching of a protein-lipid emulsion increases the concentration of astaxanthin in the remaining fat. The results also show that the water free coagulum contains approximately 40% dry matter and 60% fat. The dry matter consist of mostly protein.

Table 3. Distribution of astaxanthin in the products from the cooking procedure.

Products from test no.	10		Coagulum from cooker	Coagulated cooker liquid	Decanter solids	Decanter liquid	
		Krill					SUM
Weight (wb)	g	200	97,6	711,1	90,3	294,7	
Fat	g/100 g	7,8	10,3	0,1	5,3	0,2	
Fat free dry matter	g/100 g	13,6	3,9	0,9	16,9	0,8	
Analytical values							
Fri Astaxanthin	mg/kg	3	<1	<1	4,5	<1	
Astaxanthin esters	mg/kg	33	1,2	<0,02	59	0,18	
Conc. in lipid							
Fri Astaxanthin	mg/kg lipid	38	-	-	85	-	
Astaxanthin esters	mg/kg lipid	423	12	-	1111	113	
Quantities							
Free Astaxanthin	mg	0,6	-	-	0,4	-	0,4
Astaxanthin esters	mg	6,6	0,1	-	5,3	0,1	6,2
Distribution							
Free Astaxanthin	% of	100 %	-	-	68 %	-	68 %

Astaxanthin esters	input % of input	100 %	2 %	-	81 %	1 %	83 %

The coagulum from the cooking experiment in Example 2 were analysed for lipid classes. The coagulum lipid was dominated by triacylglycerol and phosphatidyl choline with a small quantity of phosphatidyl ethanolamine (Table 4).

Table 4. Distribution of lipid classes in the coagulum from cooking experiments.

Experiment		Krill	Coagulum F5	Coagulum F6
Fat (Bligh & Dyer)	g/100 g sample	7,8	11,8	9,9
Triacylglycerol	g/100 g fat	47	40	50
Diacylglycerol	g/100 g fat	<0,5	1	0,7
Monocylglycerol	g/100 g fat	<1	<1	<1
Free fatty acids	g/100 g fat	12	0,2	0,4
Cholesterol	g/100 g fat	0,3	<0,3	<0,3
Cholesterol esters	g/100 g fat	0,8	<0,3	<0,3
Phosphatidyl ethanolamine	g/100 g fat	5,3	2,3	2,2
Phosphatidyl inositol	g/100 g fat	<1	<1	<1
Phosphatidyl serine	g/100 g fat	<1	<1	<1
Phosphatidyl choline	g/100 g fat	33	43,1	42,3
Lyso-Phosphatidyl choline	g/100 g fat	2,4	<1	<1
Total polar lipids	g/100 g fat	41,3	45,5	44,5
Total neutral lipids	g/100 g fat	61,0	41,3	51,2
Sum lipids	g/100 g fat	102,3	86,8	95,7

The proportion of phosphatidyl choline increased from 33 % in krill to 42 – 46 % in the coagulum. The other phospholipids quantified, phosphatidyl ethanolamine and lyso-phosphatidyl choline, had lower concentrations in the coagulum than in krill. The free fatty acids were almost absent in the coagulum.

The cooking time in test F5 was 6.75 min, in test F6 it was 4.00 min. The results in *Table* 4 show no dependence of the distribution of the lipid classes with the cooking time.

The amino acid composition of the coagulum is not much different the amino acid composition in krill. There seems to be a slight increase in the apolar amino acids in the coagulum compared to krill (Table 5). For a protein to have good emulsion properties it is the distribution of amino acids within the protein that is of importance more than the amino acid composition.

Table 5. Amino acids in coagulum from cooking Example 2.

		Coagulum F 10-2 mar/apr 2007	Coagulum 70-100°C 24.06.2006	Krill 24.06.2006
Aspartic acid	g/100 g protein	8,8	10,8	7,8
Glutamic acid	g/100 g protein	10,1	11,6	10,7
Hydroxiproline	g/100 g protein	<0,10	<0,10	<0,10
Serine	g/100 g protein	4,3	4,6	3,0
Glycine	g/100 g protein	3,7	3,4	4,1
Histidine	g/100 g protein	1,7	1,6	1,6
Arginine	g/100 g protein	4,4	4,4	5,7
Threonine	g/100 g protein	5,2	5,6	3,4
Alanine	g/100 g protein	4,7	4,6	4,7
Proline	g/100 g protein	4,2	4,3	3,9
Tyrosine	g/100 g protein	4,3	4,7	2,7
Valine	g/100 g protein	6,4	6,6	4,2
Methionine	g/100 g protein	2,1	2,1	2,4
Isoleucine	g/100 g protein	8,0	8,5	4,5
Leucine	g/100 g protein	10,8	11,6	6,7

Phenylalanine	g/100 g protein	4,3	4,3	3,6
Lysine	g/100 g protein	7,5	8,2	6,2
Cysteine/Cystine	g/100 g protein	0,75		
Tryptophan	g/100 g protein	0,63		
Sum amino acids		91,9	96,9	75,2
Polar amino		47 %	48 %	51 %
Apolar amino acids		53 %	52 %	49 %

The fatty acid profile of the coagulum is presented in Table 6. The content of EPA (20:5) is about 12.4 g/100 g extracted fat and the content of DHA (22:6) is about 5.0 g/100 g extracted fat.

Table 6. Fatty acid content of coagulum

Fatty acid	**Unit**	**Amount**
14:0	g/100 extracted fat	11,5
16:0	g/100 extracted fat	19,4
18:0	g/100 extracted fat	1,1
20:0	g/100 extracted fat	<0,1
22:0	g/100 extracted fat	<0,1
16:1 n-7	g/100 extracted fat	7,0
18:1 (n-9) + (n-7) + (n-5)	g/100 extracted fat	18,4
20:1 (n-9) + (n-7)	g/100 extracted fat	1,3
22:1 (n-11) + (n-9) + (n-7)	g/100 extracted fat	0,8
24:1 n-9	g/100 extracted fat	0,1
16:2 n-4	g/100 extracted fat	0,6
16:3 n-4	g/100 extracted fat	0,2
16:4 n-4	g/100 extracted fat	<0,1
18:2 n-6	g/100 extracted fat	1,2
18:3 n-6	g/100 extracted fat	0,1
20:2 n-6	g/100 extracted fat	<0,1
20:3 n-6	g/100 extracted fat	<0,1
20:4 n-6	g/100 extracted fat	0,2
22:4 n-6	g/100 extracted fat	<0,1
18:3 n-3	g/100 extracted fat	0,8
18:4 n-3	g/100 extracted fat	2,5
20:3 n-3	g/100 extracted fat	<0,1
20:4 n-3	g/100 extracted fat	0,4
20:5 n-3	g/100 extracted fat	12,4
21:5 n-3	g/100 extracted fat	0,4

22:5 n-3	g/100 extracted fat	0,3
22:6 n-3	g/100 extracted fat	5,0

EXAMPLE 4

To evaluate the two stage cooking process described above, a laboratory scale test was performed. The tests are described below.

Materials and methods

Raw material. Frozen krill were obtained by Aker Biomarine and 10 tons were stored at Norway Pelagic, Bergen, and retrieved as required. The krill was packed in plastic bags in cardboard boxes with 2×12.5 kg krill. The boxes with krill were placed in a single layer on the floor of the process plant the day before processing. By the time of processing the krill varied from + 3 °C to -3 °C.

Analytical methods.

Protein, Kjeldahl's method: Nitrogen in the sample is transformed to ammonium by dissolution in concentrated sulfuric acid with cupper as catalyst. The ammonia is liberated in a basic distillation and determined by titration, (ISO 5983:1997(E), Method A 01). Uncertainty: 1 %.

Protein, Combustion: Liberation of nitrogen by burning the sample at high temperature in pure oxygen. Detection by thermal conductivity. Percent protein in the sample is calculated by a multiplication of analysed percent nitrogen and a given protein factor, (AOAC Official Method 990.03, 16th ed. 1996, Method A 25).

Moisture: Determination of the loss in mass on drying at 103 °C during four hours (ISO 6496 (1999). Method A 04). Uncertainty: 4 %.

Ash: Combustion of organic matter at 550 °C. The residue remaining after combustion is defined as the ash content of the sample. (ISO 5984:2002. Method A 02). Uncertainty: 3 %.

Fat, Ethyl acetate extraction: Absorption of moisture in wet sample by sodium sulphate, followed by extraction of fat by ethyl acetate (NS 9402, 1994 (modified calculation). Method A 29).

Fat, Soxhlet: Extraction of fat by petroleum ether. Mainly the content of triglycerides is determined, (AOCS Official Method Ba 3-38 Reapproved 1993. Method A 03).

Fat, Bligh and Dyer: Extraction of fat by a mixture of chloroform, methanol, and water in the proportion 1:2:0.8 which build a single phase system. Addition of chloroform and water gives a chloroform phase with the lipids and a water/methanol phase. The lipids are determined in an aliquot of the chloroform phase after evaporation and weighing. The extraction includes both triglycerides and phospholipids. (E.G. Bligh & W.J. Dyer: A rapid method of total lipid extraction and purification. Can.J.Biochem.Physiol. Vol 37 (1959). Metode A 56).

Astaxanthin: Extraction with ethanol and di-chloromethane. Polar products are removed by open column chromatography on silica gel. Isomers are separated on normal phase HPLC on Si 60 column and detection at 470 nm. (Schierle J. & Härdi W. 1994. Determination of stabilized astaxanthin in Carophyll® Pink, premixes and fish feeds. Edition 3. Revised Supplement to: Hoffman P, Keller HE, Schierle J., Schuep W. Analytical methods for vitamins and carotenoids in feed. Basel: Department of Vitamin Research and Development, Roche. Method A 23)

Moisture in oil: Determination of actual water content of fats and oils by titration with Karl Fischer reagent, which reacts quantitatively with water, (AOCS Official Method CA 2e-84. Reapproved 1993. Method A 13).

Dry matter in stick water during processing is correlated to refract meter which gives ° Brix. Amino acids were determined as urea derivatives by reversed phase HPLC with fluorescence detection. (Cohen S. A. and Michaud D. P., Synthesis of a Fluorescent Derivatizing Reagent, 6-Aminoquinolyl-N-Hydroxysuccinimidyl Carbamate, and Its Application for the Analysis of Hydrolysate Amino Acids via High-Performance Liquid Chromatography. Analytical Biochemistry **211**, 279-287, 1993. Method A42). TVB-N, TMA-N and TMAO-N were determined in a 6% trichloro-acetic acid extract by micro diffusion and titration. (Conway, E. I., and A. Byrne. An absorption apparatus for the micro determination of certain volatile substances. Biochem. J. 27:419-429, 1933, and Larsen, T, SSF rapport nr. A-152, 1991). Fatty acids were determined by esterifying the fatty acids to methyl esters, separate the esters by GLC, and quantify by use of C23:0 fatty acid methyl ester as internal standard.(AOCS Official Method Ce 1b-89, Method A 68). Lipids were separated by HPLC and detected with a Charged Aerosol Detector. Vitamins A, D and E were analysed at AnalyCen, Kambo.

Results and discussion

Raw material of krill. Table 7 gives the results of analysis of the raw material of the krill that was used in the pilot trials. Besides the first trial, the same shipment of krill was used for all trials. The dry matter was about 21-22 %, fat 6 %, protein 13-14 %, salt 1 % pH, total volatile nitrogen (TVN) 18 mgN/100g, trimethylamine (TMA) 4 mg N/100g and trimethylamineoxide (TMAO) 135 mg N/100g. Compared to fish pH, TMAO and salt (Cl -) is high for krill.

Table 7. Analysis of raw krill on wet base (wb)

Sample:	**Raw material of krill**									
Analysis:	Dry matter	Fat, B&D	Protein	Ash	Salt	pH	TVN	TMA	TMAO	
Date:	g/100 g	g/100 g	g/100 g	g/100 g	g/100 g		mg N/100 g	mg N/100 g	mg N/100 g	Marks
07.08.2007	22,8	7,1	13,5	2,5						Saga Sea 04.07.06 Lot. L1
18.09.2007	21,3	6,0								
04.10.2007	21,6	6,3	13,5							Krillråstoff CO5S
04.10.2007	20,5	5,9	12,8							Krillråstoff AO6S
25.10.2007	22,1	6,0	13,9	2,9	1,1	7,4	20,8	5,8	128,3	Krillråstoff CO5S
25.10.2007	21,3	6,0	13,2	2,7	1,1	7,4	15,0	2,3	140,6	Krillråstoff AO6S
22.11.2007	21,9	5,9				7,8	17,9	3,5	123,7	
Average	**21,6**	**6,2**	**13,5**	**2,7**	**1,1**	**7,4**	**17,9**	**4,0**	**134,5**	

Table 8 gives the analysis of raw krill on dry base. If these figures are multiplied with 0.93 it will give the figures on meal base with 7 % water.

Table 8 Analysis of raw krill on dry base (db)

Sample:	**Raw material of krill**							
Analysis:	Dry matter	Fat, B&D	Protein	Ash	Salt	TVN	TMA	TMAO
Date:	g/100 g	g/100 g	g/100 g	g/100 g	g/100 g	mg N/100 g	mg N/100 g	mg N/100 g
07.08.2007	100	31,1	59,2	11,0				
18.09.2007	100	28,2		0,0				
04.10.2007	100	29,2	62,5	0,0				
04.10.2007	100	28,8	62,4	0,0				
25.10.2007	100	27,1	62,9	13,1	5,0	94,1	26,1	580,5
25.10.2007	100	28,2	62,0	12,7	5,2	70,6	10,9	660,2
22.11.2007	100	26,9				81,7	16,0	564,8
Average	**100**	**28,5**	**62,5**	**12,3**	**5,1**	**82,4**	**18,5**	**620,4**

Separation of coagulum and pressing for krill oil. 99 kg krill was processed by adding batches of 20 kg krill to 80 l of water at 95 °C in a steam heated kettle (200 l). The steam on the kettle was closed, and the krill and water were gently mixed manually for 3 minutes, and the mixed temperature became 75 °C (heating step no. 1). The heated krill was separated from the water by sieving. Sieved preheated krill (75°C) was added 20 kg hot water and heated to 85 °C within a minute, (heating step 2). The krill was sieved again and feed into the press. The liquid from step1 (krill milk) was coagulated at 95 °C. All the krill was cooked and the press liquid was separated for oil. From 99 kg krill about 0.5 kg of unpolished krill oil

was separated from the press liquid. Tables 9 and 10 provide an analysis of cooked krill after first cooking step on wet base and dry base.

Table 9 Analysis of cooked krill on wet base (wb)

Sample:	**Cooked krill**							
Analysis:	Dry matter	Fat, B&D	Protein	Ash	pH	TVN	TMA	TMAO
Date:	g/100 g	g/100 g	g/100 g	g/100 g		mg N/100 g	mg N/100 g	mg N/100 g
07.08.2007	20,2	4,7	13,5	2,2				
18.09.2007	19,8	4,6						
25.10.2007	15,2	3,2	10,3	2,0	8,2	10,5	3,5	75,4

Table 10 Analysis of cooked krill on dry base (db)

Sample:	**Cooked krill**						
Analysis:	Dry matter	Fat, B&D	Protein	Ash	TVN	TMA	TMAO
Date:	g/100 g	g/100 g	g/100 g	g/100 g	mg N/100 g	mg N/100 g	mg N/100 g
07.08.2007	100,0	23,3	66,8	10,9			
18.09.2007	100	23,2					
25.10.2007	100	21,1	67,8	13,2	69,3	23,1	496,3

Compared to raw krill (Table 8) there is a reduction in dry matter for cooked krill. The fat content in dry matter is reduced because of the fat in the krill milk which is separated from the cooked krill. The content of protein is increased on dry base, but the ash seems to be at the same level. TMAO in the krill is reduced and is found in the cooking liquid.

Micro filtration. The krill milk (70 °C) from step 1 was coagulated at > 95 °C and separated from the liquid through microfiltration (Soby Miljøfilter). Coagulum was then pressed in a press and dried. Tables 11 and 12 gives analyses of coagulum on wet base and dry base. The dry matter of the coagulum was between 12.8 and 16.7 %. On dry base the fat content about 60 % and TMAO 340 mg N/100 g. The dry matter of the coagulum increased to 34-38 % by pressing. The fat content also increased on dry base (Table 13), but the TMAO was reduced to 145 mg N/100 g. After washing the press cake with 1 part water to 1 part press cake of coagulum and then press again, the TMAO was reduced to 45 mg N/100g on dry base (Table 18).

Table 11 Analysis of coagulum on wet base (wb)

Sample:	**Coagulum**						
Analysis:	Dry matter	Fat, B&D	Protein	Ash	TVN	TMA	TMAO
Date:	g/100 g	g/100 g	g/100 g	g/100 g	mg N/100 g	mg N/100 g	mg N/100 g
10.10.2007	12,8	7,9					
25.10.2007	14,3	8,3	5,4	1,0	5,9	2,3	48,6
31.10.2007	16,7	9,3	6,2				
Average	**14,6**	**8,5**	**5,8**				

Table 12 Analysis of coagulum on dry base (db)

Sample:	**Coagulum**						
Analysis:	Dry matter	Fat, B&D	Protein	Ash	TVN	TMA	TMAO
Date:	g/100 g	g/100 g	g/100 g	g/100 g	mg N/100 g	mg N/100 g	mg N/100 g
10.10.2007	100	61,7					
25.10.2007	100	58,0	37,8	7,0	41,0	16,4	340,1
31.10.2007	100	55,7	37,1				
Average	**100**	**58,5**	**37,4**				

Table 13 Analysis of press cake from coagulum on wet base

Sample:	**Press cake of coagulum**					Raw krill	Coagulum	**Coagulum PK**
Analysis:	Dry matter	Fat, B&D	TVN	TMA	TMAO	worked up	perss cake	**per kg raw krill**
Date:	g/100 g	g/100 g	mg N/100 g	mg N/100 g	mg N/100 g	kg	kg	**kg/kg**
22.11.2007	38,8	23,6	7,9	4,5	56,1	1000	54,2	**0,0542**
11.12.2007	33,8	22,5	3,4	0	45,3	500	21,92	**0,0438**
11.12.2007*	33,6	21,3	0	0	15,3	500	15	**0,0300**

*) After 1 wash (Press cake : water = 1:1)

Membrane filtration. Another way to collect the lipids from the krill milk is to separate by membrane filtration. For this to be possible the milk must not coagulate, but be brought to the membrane filter from the sieve (heating step no. 1).

Before the krill milk could enter the membrane filter the milk is pre-filtrated, which was done by the sieve (100 μm). The opening of the micro-filter was 100 nm. 80 kg krill was processed by starting by 80 kg water (95 °C) and 20 kg krill into the kettle as described. For the first 2 batches of krill clean water was used (160 kg), but for the last 2 batches permeate from the membrane filter was used instead of water. The membrane filtration was followed with a refract meter calibrated for sugar solution (°Brix). The Brix-value is near the dry matter concentration in the process liquids. The flux value for the filter at about 60 °C was 350 l/m2/h for retentate with 7.8 °Brix (refract meter) and reduced to 290 l/m2/h when the Brix value increased to 9.9 °. The Brix value for the permeate was only 1 ° due to high

dilution when the amount to be filtered is small. See Figures 2 and 3. The permeate was golden and transparent.

All permeate was evaporated in a kettle to > 65 ° Brix. Retentate, 2 liter, was evaporated in a laboratory evaporator at 70 °C and 12 mm Hg. At 27.5 °Brix the retentate was still flowing well. As the concentration continued the retentate became more and more viscous, first as a paste and finely to a dry mass. The concentrated retentate (27 °Brix), permeate (> 65 °Brix) and dry retentate were analyzed and the results are given in Table 14 on sample base (% wb) and Table 15 on dry matter base (% db) (sample no 1, 2 and 3). A sample of coagulum was dried as for the retentate (sample no 4).

Table 14 Analysis of concentrate from retentate, permeate and coagulum on wet base (wb)

	Dry matter	Fat (polar+apolar)	Crude Protein	Ash	TVN	TMA	TMAO	Water activity
		Bligh & Dyer						25 °C
Sample	% wb	% wb	% wb	% wb	mg N/100g wb	mg N/100g wb	mg N/100g wb	aw
No. 1 Concentrate of retentat	26,0	16,3	9,5	1,6	5,7	<1	99	0,978
No. 2 Consentrate of permeat	72,7	1,0	51,1	24,7	138	110	1 157	0,385
No. 3 Vakuum dried retentate	64,9	39,3	24	4,1	12,8	29,4	196	0,875
No. 4 Vakuum died coagulum	60,3	37,1	20,9	4,4	52,9	28,1	216	0,912

Table 15 Analysis of concentrate from retentate, permeate and coagulum on dry matter base (db)

	Dry matter	Fat (polar+apolar)	Crude Protein	Ash	TVN	TMA	TMAO
		Bligh & Dyer					
Sample	% db	% db	% db	% db	mg N/100g db	mg N/100g db	mg N/100g db
No. 1 Concentrate of retentat	100,0	62,7	36,5	6,2	21,9	<1	382
No. 2 Consentrate of permeat	100,0	1,4	70,3	34,0	190	152	1 592
No. 3 Vakuum dried retentate	100,0	60,6	37,0	6,3	19,7	45,3	302
No. 4 Vakuum died coagulum	100,0	61,5	34,7	7,3	87,7	46,6	358

These results indicate that micro filtration of krill milk was promising and is an alternative to coagulate the krill milk. The protein portion was high in taurine. The content of fat, protein, ash and TMAO were almost similar between retentate and coagulum. Permeate can be concentrated to 70 % dry matter and will have a water activity below 0.4 at 25 °C which means that it can be stored at ambient temperature.

Press cake and press liquid. Tables 16 and 17 provide an analysis of press cake on wet and dry base from the different trials. The average amount of press cake per kg raw krill was found to be 0.23 kg. The dry matter of the press cake was between 44 and 48 %. The fat content in dry matter was reduced from 21 % before to 15-20 % after pressing. This will give a press cake meal from 14 to 18.5 % fat, about 67 % protein and 7 % moisture. TMAO was

reduced from about 500 mg N/100g dry matter in cooked krill to 95mg N/100g dry matter in the press cake.

Table 16 Analysis on wet base (wb) of press cake and calculations

Sample:	**Press cake**						Raw krill	Press cake	Kg press cake
Analysis:	Dry matter	Fat, B&D	Protein	TVN	TMA	TMAO	worked up		per kg raw krill
Date:	g/100 g	g/100 g	g/100 g	mg N/100 g	mg N/100 g	mg N/100 g	kg	kg	kg/kg
18.09.2007	48,1	8,0					327	90	0,28
04.10.2007	47,9	7,0	34,8						
10.10.2007	44,8	9,3					250	55	0,22
31.10.2007	47,4	7,2	33,8				709	143	0,20
22.11.2007	44,4	8,1		8,4	2,1	42,2	1000	226	0,23
11.12.2007	43,8	7,3		5,6	2,2	46,7	500	117	0,23
Average:	**46,1**	**7,8**	**34,3**	**7**	**2,2**	**44,5**			**0,23**

Table 17 Analysis on dry base (db) of press cake

Press cake					
Dry matter	Fat, B&D	Protein	TVN	TMA	TMAO
g/100 g	g/100 g	g/100 g	mg N/100 g	mg N/100 g	mg N/100 g
100	16,6				
100	14,6	72,7			
100	20,8				
100	15,2	71,3			
100	18,2		18,9	4,7	95,0
100	16,7		12,8	5,0	106,6
100	**17,0**	**72,0**	**15,9**	**4,9**	**100,8**

Oil was produced from the krill solids by centrifugation. Table 18. The oil was almost free for water and the content of astaxanthin was quite high (1.8 g/kg).

Table 18 Analysis of krill oil

		Date:	Date:
Tricanter oil (krill oil)		31.10.2007	22.11.2007
Astaxanthin, Free	mg/kg	22	29
Trans	mg/kg	12	14
9-cis	mg/kg	2,3	3,2
13-cis	mg/kg	5,4	7,8
Astaxanthin, Esters	mg/kg	1802	1785
Diester	mg/kg	1142	1116
Monoester	mg/kg	660	669
Astaxanthin - total	**mg/kg**	**1824**	**1814**
Water, Karl F.	g/100 g	0,17	0,04
FFA	g/100 g		0,9
Vitamin A	IE/kg		602730
Vitamin D3	IE/kg		<1000
Vitamin E (alfa-tokoferol)	mg/kg		630

Table 19 Analysis of press cake from coagulum on dry base

Sample:	**Press cake of coagulum**				
Analysis:	Dry matter	Fat, B&D	TVN	TMA	TMAO
Date:	g/100 g	g/100 g	mg N/100 g	mg N/100 g	mg N/100 g
22.11.2007	100	60,8	20,4	11,6	144,6
11.12.2007	100	66,6	10,1	0,0	134,0
11.12.2007*	100	63,4	0,0	0,0	45,5
*) After 1 wash (Press cake : water = 1:1)					

The yield of coagulum press cake was about 5 % of raw krill. The compositions of coagulum and retentate from micro filtration is compared in Table 20. There was hardly any difference between the products from the two process alternatives. Press cake of coagulum was dried, and Table 21 gives the analysis of the coagulum and final coagulum meal. The proximate composition based on dry matter did not change during drying, and the amino acid composition and fatty acid composition is near identical. There was some loss of phospholipids during drying. This is most probable caused by oxidation of fatty acids, but other chemical modification of the phospholipids may also be of consequence.

Table 20 Analysis of Retentate from micro filtration and Coagulum

		Retentat 25.10.07	Coagulum 25.10.07
Protein	g/100 g	5,8	5,4
Dry matter	g/100 g	13,5	14,3
Ash	g/100 g	1,1	1,0
Fat (B&D)	g/100 g	7,3	8,3
pH		8,5	
TFN	mg N/100 g	5,9	5,9
TMA	mg N/100 g	2,3	2,3
TMAO	mg N/100 g	61,0	48,6
Lipd classes:			
Triacylglycerol	g/100 g extracted fat	59,0	51
Diacylglycerol	g/100 g extracted fat	1,3	1
Monocylglycerol	g/100 g extracted fat	<1	<1
Free fatty acids	g/100 g extracted fat	3,8	3,2
Cholesterol	g/100 g extracted fat	<0,5	<0,5
Cholesterol esters	g/100 g extracted fat	1,0	0,8
Phosphatidyl ethanolamine	g/100 g extracted fat	1,8	3
Phosphatidyl inositol	g/100 g extracted fat	<1	<1
Phosphatidyl serine	g/100 g extracted fat	<1	<1
Phosphatidyl choline	g/100 g extracted fat	35,0	40
Lyso-Phosphatidyl choline	g/100 g extracted fat	0,8	1,2
Total polar lipids	g/100 g extracted fat	37,6	44,2
Total neutral lipids	g/100 g extracted fat	67,1	56,0
Sum lipids	g/100 g extracted fat	103,4	100,2
Fatty acid composition:			
14:0	g/100 g extracted fat	10,6	10,4
16:0	g/100 g extracted fat	16,4	16,2
18:0	g/100 g extracted fat	1,1	1,2
20:0	g/100 g extracted fat	0,1	0,1
22:0	g/100 g extracted fat	<0,1	<0,1
16:1 n-7	g/100 g extracted fat	6,3	6,4
18:1 (n-9)+(n-7)+(n-5)	g/100 g extracted fat	15,5	15,4
20:1 (n-9)+(n-7)	g/100 g extracted fat	1,1	1,1
22:1 (n-11)+(n-9)+(n-7)	g/100 g extracted fat	0,6	0,5
24:1 n-9	g/100 g extracted fat	0,1	0,1
16:2 n-4	g/100 g extracted fat	0,5	0,5
16:3 n-4	g/100 g extracted fat	0,2	0,2
18:2 n-6	g/100 g extracted fat	1,4	1,4
18:3 n-6	g/100 g extracted fat	0,2	0,2
20:2 n-6	g/100 g extracted fat	0,1	0,1
20:3 n-6	g/100 g extracted fat	0,1	0,1
20:4 n-6	g/100 g extracted fat	0,3	0,3
22:4 n-6	g/100 g extracted fat	<0,1	<0,1
18:3 n-3	g/100 g extracted fat	0,7	0,7
18:4 n-3	g/100 g extracted fat	1,7	1,7
20:3 n-3	g/100 g extracted fat	<0,1	<0,1
20:4 n-3	g/100 g extracted fat	0,3	0,3
20:5 n-3 (EPA)	g/100 g extracted fat	10,5	10,3
21:5 n-3	g/100 g extracted fat	0,3	0,3
22:5 n-3	g/100 g extracted fat	0,5	0,4
22:6 n-3 (DHA)	g/100 g extracted fat	5,1	5,0
Sum saturated fat acides	g/100 g extracted fat	28,2	27,9
Sum monoene fat acides	g/100 g extracted fat	23,6	23,4
Sum PUFA (n-6) fat acides	g/100 g extracted fat	2,1	2
Sum PUFA (n-3) feat acides	g/100 g extracted fat	19,1	18,7
Sum PUFA fat acides total	g/100 g extracted fat	21,9	21,4
Sum fat acides total	g/100 g extracted fat	73,7	72,7
EPA/DHA		2,1	2,1

Table 21 Analysis of Coagulum press cake and meal dried in a Rotadisc dryer on wet and dry base

		Coagulum	Coagulum	Coagulum	Coagulum
		press cake	meal	press cake	meal
		22.11.2007	22.11.2007	22.11.2007	22.11.2007
Analysis:		**wb**	**wb**	**db**	**db**
Protein	g/100 g	14,6	35,3	37,6	37,4
Moisture	g/100 g	61,2	5,7	0,0	0,0
Fat B&D	g/100 g	23,6	55,1	60,8	58,4
Ash	g/100 g		5,9		6,3
TMA	mg N/100 g	4,5	7	11,6	7
TMAO	mg N/100 g	56,1	140	144,6	148
Fatty acid composition:					
14:0	g/100 g extracted fat	10,4	10,4		
16:0	g/100 g extracted fat	17	17		
18:0	g/100 g extracted fat	1,2	1,2		
20:0	g/100 g extracted fat	0,1	0,1		
22:0	g/100 g extracted fat	0,1	0,1		
16:1 n-7	g/100 g extracted fat	6,4	6,4		
18:1 (n-9)+(n-7)+(n-5)	g/100 g extracted fat	15,2	15,3		
20:1 (n-9)+(n-7)	g/100 g extracted fat	1,1	1,1		
22:1 (n-11)+(n-9)+(n-7)	g/100 g extracted fat	0,5	0,6		
24:1 n-9	g/100 g extracted fat	0,1	0,1		
16:2 n-4	g/100 g extracted fat	0,5	0,5		
16:3 n-4	g/100 g extracted fat	0,2	0,2		
18:2 n-6	g/100 g extracted fat	1,5	1,4		
18:3 n-6	g/100 g extracted fat	0,2	0,2		
20:2 n-6	g/100 g extracted fat	0,1	0,1		
20:3 n-6	g/100 g extracted fat	<0,1	<0,1		
20:4 n-6	g/100 g extracted fat	0,3	0,3		
22:4 n-6	g/100 g extracted fat	<0,1	<0,1		
18:3 n-3	g/100 g extracted fat	0,7	0,7		
18:4 n-3	g/100 g extracted fat	1,7	1,7		
20:3 n-3	g/100 g extracted fat	<0,1	<0,1		
20:4 n-3	g/100 g extracted fat	0,4	0,4		
20:5 n-3 (EPA)	g/100 g extracted fat	10,9	10,5		
21:5 n-3	g/100 g extracted fat	0,3	0,3		
22:5 n-3	g/100 g extracted fat	0,3	0,3		
22:6 n-3 (DHA)	g/100 g extracted fat	5,3	5,1		
Sum saturated fat acides	g/100 g extracted fat	28,7	28,7		
Sum monoene fat acides	g/100 g extracted fat	23,3	23,3		
Sum PUFA (n-6) fat acides	g/100 g extracted fat	2	2		
Sum PUFA (n-3) feat acides	g/100 g extracted fat	19,7	19		
Sum PUFA fat acides total	g/100 g extracted fat	22,4	21,7		
Sum fat acides total	g/100 g extracted fat	74,4	73,8		
Amino acids:					
Aspartic acid	g/100 g protein	10,5	10,5		
Glutamic acid	g/100 g protein	11,2	11,6		
Hydroxiproline	g/100 g protein	<0,10	<0,10		
Serine	g/100 g protein	4,3	4,2		
Glycine	g/100 g protein	4	4		
Histidine	g/100 g protein	2	1,9		
Arginine	g/100 g protein	4,8	4,7		
Threonine	g/100 g protein	4,9	4,9		
Alanine	g/100 g protein	4,8	4,9		
Proline	g/100 g protein	4,2	4,1		
Tyrosine	g/100 g protein	3,7	3,5		
Valine	g/100 g protein	6	5,9		
Methionine	g/100 g protein	2,4	2,4		
Isoleucine	g/100 g protein	6,9	6,7		
Leucine	g/100 g protein	9,6	9,4		
Phenylalanine	g/100 g protein	4,5	4,4		
Lysine	g/100 g protein	7,7	7,6		
Sum AA	g/100 g protein	91,5	90,7		
Lipid classes:					
Triacylglycerol	g/100 g extracted fat	48	63		
Diacylglycerol	g/100 g extracted fat	1,2	1,3		
Monocylglycerol	g/100 g extracted fat	<1	<1		
Free fatty acids	g/100 g extracted fat	3,2	3,1		
Cholesterol	g/100 g extracted fat	1,2	<0,5		
Cholesterol esters	g/100 g extracted fat	0,5	0,9		
Phosphatidyl ethanolamine	g/100 g extracted fat	3,1	1,1		
Phosphatidyl inositol	g/100 g extracted fat	<1	<1		
Phosphatidyl serine	g/100 g extracted fat	<1	<1		
Phosphatidyl choline	g/100 g extracted fat	38	34		
Lyso-Phosphatidyl choline	g/100 g extracted fat	1,2	<1		
Total polar lipids	g/100 g extracted fat	42	34,8		
Total neutral lipids	g/100 g extracted fat	54,6	67,9		
Sum lipids	g/100 g extracted fat	96,7	103,6		

Krill meal. Final krill meal was produced. Press cake and press cake with stick water concentrate were dried in a hot air dryer or steam drier. Table 22.

Table 22 Analysis of krill meal from

		Forberg	Forberg	Rota disc.
		Air dried	**Air dried**	**Steam dried**
		Press cake	Krill meal	Krill meal
Date: 22.11.2007		meal of krill	with stickwater	with stickwater
Wet base:				
Protein	g/100 g	66,4	63,6	66,3
Moisture	g/100 g	5,9	7,1	3,7
Fat Soxhlet	g/100 g	8,7	10,4	
Fat B&D	g/100 g	15,9	15,6	15,2
Ash	g/100 g	9,8	13,0	13,4
Salt	g/100 g	1,3	4,3	4,4
Water sol. protein	g/100 g prot.	11,1	28,0	27,1
pH		8,6	8,3	
TVN	mg N/100 g	18,8	39,9	38,6
TMA	mg N/100 g	11,1	22,2	29,8
TMAO	mg N/100 g	109,7	442,1	399,5
Dry matter base:				
Protein	g/100 g db	70,6	68,5	
Fat Soxhlet	g/100 g db	9,2	11,2	
Fat B&D	g/100 g db	16,9	16,8	15,8
Ash	g/100 g db	10,4	14,0	
Salt	g/100 g db	1,4	4,6	
TVN	mg N/100 g db	20,0	42,9	40,1
TMA	mg N/100 g db	11,8	23,9	30,9
TMAO	mg N/100 g db	116,6	475,9	414,9
Astaxanthin on wet base:				
Astaxanthin, Free	mg/kg	4,6	3,6	<1
Trans	mg/kg	2,5	1,9	<1
9-cis	mg/kg	0,4	0,4	<1
13-cis	mg/kg	1,3	0,9	<1
Astaxanthin, Esters	mg/kg	112,0	100	58,0
Diester	mg/kg	80,0	72,0	50,0
Monoester	mg/kg	32,0	27,0	8,1
Astaxanthin - total	mg/kg	116,6	103,6	58,0
Astaxanthin on fat base:				
Astaxanthin, Fritt	mg/kg fat	28,9	23,1	<7
Trans	mg/kg fat	15,7	12,2	<7
9-cis	mg/kg fat	2,5	2,6	<7
13-cis	mg/kg fat	8,2	5,8	<7
Astaxanthin, Estere	mg/kg fat	704,4	641,0	381,6
Diester	mg/kg fat	503,1	461,5	328,9
Monoester	mg/kg fat	201,3	173,1	53,3
Astaxanthin - totalt	mg/kg fat	733,3	664,1	381,6
Amino acids:				
Aspartic acid	g/100 g protein	10,6	9,2	9,2
Glutamic acid	g/100 g protein	14,1	12,4	12,3
Hydroxiproline	g/100 g protein	<0,5	<0,5	0,1
Serine	g/100 g protein	4,2	3,7	3,8
Glycine	g/100 g protein	4,4	4,4	4,5
Histidine	g/100 g protein	2,3	1,9	1,9
Arginine	g/100 g protein	6,6	6,0	6,1
Threonine	g/100 g protein	4,3	3,7	4,1
Alanine	g/100 g protein	5,4	4,9	5,3
Proline	g/100 g protein	3,7	4,1	4
Tyrosine	g/100 g protein	4,4	3,1	4,7
Valine	g/100 g protein	5,1	4,4	4,5
Methionine	g/100 g protein	3,2	2,7	2,7
Isoleucine	g/100 g protein	5,3	4,5	4,5
Leucine	g/100 g protein	8,0	6,9	6,9
Phenylalanine	g/100 g protein	4,6	3,9	4
Lysine	g/100 g protein	8,2	7,0	6,6
Sum AA	g/100 g protein	94,4	82,8	85,2
Lipide classes:				
Triacylglycerol	g/100 g extracted fat		41,0	63
Diacylglycerol	g/100 g extracted fat		1,7	1,3
Monocylglycerol	g/100 g extracted fat		<1	<1
Free fatty acids	g/100 g extracted fat		8,8	3,1
Cholesterol	g/100 g extracted fat		2,4	<0,5
Cholesterol esters	g/100 g extracted fat		<0,5	0,9
Phosphatidyl ethanolamine	g/100 g extracted fat		3,6	1,1
Phosphatidyl inositol	g/100 g extracted fat		<1	<1
Phosphatidyl serine	g/100 g extracted fat		<1	<1
Phosphatidyl choline	g/100 g extracted fat		43,0	34
Lyso-Phosphatidyl choline	g/100 g extracted fat		1,1	<1
Total polar lipids	g/100 g extracted fat		47,2	34,8
Total neutral lipids	g/100 g extracted fat		54,2	67,9
Sum lipids	g/100 g extracted fat		101,4	103,6

EXAMPLE 5

Coagulum meal produced as described in Example 4 was extracted using lab scale SFE. 4,885g of coagulum (freeze dried over night) via a two step extraction: 1) SFE: CO_2, 500 Bar, 60°C, 70min at a medium flow rate of 1,8ml/min of CO_2; 2) SFE: CO_2+15%EtOH, 500 Bar, 60°C, 70min at a medium flow rate of 2,5ml/min of CO_2+EtOH. The first step extracted 1,576g of extracted neutral fraction (NF). As shown in Figures 4 and 5, the analysis at HPLC show lower than the detectable limit content on PL in the NF. It was extracted about 32.25% of the total material. Table 29 provides the peak areas of the components of the neutral fraction as determined by GC.

Table 29.

Rel.Area	Peakname	Ret.Time	Area	Height	Rel.Area
%		min	mV*min	mV	%
0,29	n.a.	17,455	0,2864	2,271	0,29
19,49	**C14:0**	**24,073**	**19,0301**	**105,696**	**19,49**
21,16	**C16:0**	**32,992**	**20,6601**	**88,859**	**21,16**
11,99	**C16:1**	**36,197**	**11,7032**	**48,125**	**11,99**
3,5	n.a.	37,28	3,4166	14,344	3,5
1,57	n.a.	43,331	1,5375	6,141	1,57
15,6	**n.a.**	**46,425**	**15,2285**	**58,605**	**15,6**
8,81	n.a.	46,873	8,5983	30,65	8,81
0,93	n.a.	50,499	0,9055	3,164	0,93
1,56	n.a.	51,292	1,5216	5,746	1,56
1,67	n.a.	57,312	1,6281	4,78	1,67
2,03	n.a.	60,985	1,98	6,963	2,03
0,02	n.a.	67,761	0,0189	0,116	0,02
0,11	n.a.	68,833	0,1066	0,423	0,11
0,11	n.a.	71,705	0,1028	0,497	0,11
0,08	n.a.	74,053	0,0806	0,398	0,08
3,92	**C20:5 EPA**	**74,489**	**3,826**	**12,07**	**3,92**
0,11	n.a.	80,519	0,1095	0,48	0,11
0,08	C22:5 DPA	85,369	0,0785	0,41	0,08
1,3	**C22:6 DHA**	**87,787**	**1,2719**	**4,253**	**1,3**

The second step extracted a polar fraction of 1,023g corresponding to 20,95% of the total material. The polar fraction consisted mostly of PL and just less than 1% TG. See Figures 6 and 7. Table 30 provides the peak areas of the components of the polar fraction as determined by GC.

Table 30.

Rel.Area %	**Peakname**	**Ret.Time min**	**Area mV*min**	**Height mV**	Rel.Area %
2,87	C14:0	24,025	4,8099	28,243	2,87
28,5	**C16:0**	**33,084**	**47,7079**	**182,756**	**28,5**
1,82	C16:1	36,155	3,0402	13,166	1,82
1,13	n.a.	43,304	1,8848	8,208	1,13
3,89	n.a.	46,336	6,5129	27,429	3,89
5,46	n.a.	46,852	9,1467	35,825	5,46
2,15	n.a.	51,265	3,6015	14,095	2,15
1,6	n.a.	57,121	2,6735	7,213	1,6
1,72	n.a.	60,944	2,8832	10,686	1,72
2,03	n.a.	68,259	3,3913	8,025	2,03
30,09	**C20:5 EPA**	**74,599**	**50,3768**	**163,312**	**30,09**
12,11	**C22:6 DHA**	**87,832**	**20,2774**	**68,714**	**12,11**

The coagulate was dried over night with a weight loss of about 5,53% w/w. The total extracted was about 53,2% of the starting weight of the dried material.

EXAMPLE 6

Freshly harvested krill were processed into coagulum on board the ship either 10 minutes or six hours post harvest. The coagulum produced from both the 10 minute post harvest krill and the 6 hour post harvest krill contained less than 1mg/100g volatile nitrogen, less than 1 mg/100 g trimethylamine (TMA), and less than 1g/100g lysophosphatidylcholine. This can be compared to the coagulum produced from frozen krill in Example 4 above, which contained higher levels of volatile nitrogen, and lysophosphatidylcholine. The methods of the invention which utilize freshly harvested krill provide krill products that are characterized in being essentially free of TMA, volatile nitrogen, and lysophosphatidylcholine.

EXAMPLE 7

Coagulum meal, 250 g, and krill oil were mixed in a kitchen mixer. The aim was to add 300 – 500 mg astaxanthin/kg coagulum meal. If the oil contains 1500 mg astaxanthin/kg krill oil, at least 200 g oil should be added to one kg of coagulum meal. The flow of the meal was markedly reduced by addition of 10 % oil, and the oil came off on the packaging when the addition of oil was increased to 14 and 20 %. 3.5 kg coagulum from was thawed and milled on a Retsch ZM1 with a 2 mm sieve. The quantity of milled powder was 2.96 kg. The 2.96 kg dried coagulum was added 300 g krill oil in three portions. The knives in the mixer (Stephan UM12) were to far from the bottom to give a good mixing, so the mixture was mixed by hand and mixer intermittently. The astaxanthin content in the final mixture was 40 % lower than calculated. New analyses of astaxanthin were performed on the oil and on the fortified meal. The krill oil had been stored in a cold room at 3 °C for 4 months, and the astaxanthin content in the oil did not change during this storage . A new sample were drawn from the fortified meal after 4 weeks frozen storage, and the astaxanthin content was the same in both samples (Table 31).

Table 31. Composition of steam dried coagulum fortified with 10 % krill oil.

		Analysed Meal with oil	Calculated Meal with oil	New analysis Krill oil	New analysis Meal with oil
Dry matter	g/100 g	98.0	99.2		
Protein	g/100 g		33.6		
Fat (B&D)	g/100 g	58.9	60.7		
Ash	g/100 g		5.9		
Water soluble protein	g/100 g protein		15.8		
TFN	mg N/100 g		10		
TMA	mg N/100 g		10		
TMAO	mg N/100 g		113		
Astaxanthin, Free	mg/kg	2.5	4.9	27	2.8
Trans	mg/kg	1.4	2.5	14	1.5
9-cis	mg/kg	0.35	0.6	3.1	0.4
13-cis	mg/kg	0.57	1.2	6.2	0.7
Astaxanthin, Esters	mg/kg	193	338	1805	197
Diester	mg/kg	126	216	1128	127
Monoester	mg/kg	67	122	677	70
Astaxanthin - total	mg/kg	196	343	1832	200
Astaxanthin, Free	mg/kg lipid	4.2	8.1		
Trans	mg/kg lipid	2.4	4.2		
9-cis	mg/kg lipid	0.6	1.0		

13-cis	mg/kg lipid	1.0	2.0
Astaxanthin, Esters	mg/kg lipid	328	556
Diester	mg/kg lipid	214	356
Monoester	mg/kg lipid	114	200
Astaxanthin - total	mg/kg lipid	332	564
Ffa	g/100 g extracted fat		4.4
Total polar lipids	g/100 g extracted fat		39.7
Total neutral lipids	g/100 g extracted fat		60.1

The astaxanthin content in fortified coagulum meal is 58 % of the amount in the ingredients. This reduction in astaxanthin takes place during mixing of dried coagulum and krill oil, and indicate that dried coagulum is easily oxidized.

Example 8

The dried coagulum meal was extracted by supercritical fluid extraction. The extracted oil was analyzed as presented in Tables 32-34.

Table 32. Lipid composition

Phosphatidylcholine	34 g/100 g lipid
Phosphatidylethanolamine	1,3 g/100 g lipid
Triglycerides	48 g/100 g lipid
Cholesterol	n.d.
Free fatty acids	1,0 g/100 g lipid

Table 33. Fatty acid profile

Total saturated fatty acids	26,3 g/100 g lipid
Total omega-3 fatty acids	18,1 g/100 g lipid
Total fatty acids	67,3 g/100 g lipid

Table 34. Miscellaneous properties

Astaxanthin	130 mg/kg
TMAO	87 mg N/100 g

TMA	<1 mg N/100 g
Viscosity at 25°C	61 mPa s

Example 9

Coagulum meal prepared as described above was administered to two human subjects and absorption of the product was determined by measuring omega-3 fatty acids in total lipids and in phospholipids in plasma. Subject 1 consumed 8g of coagulum in combination with yoghurt, whereas subject 2 consumed 8g of krill oil without yoghurt. The data is presented in Tables 35 (Subject 1) and 36 (Subject 2).

Table 35

Time (h)	C20:5 W3 (EPA)	C22:5 W3 (DPA)	C22:6 W3(DHA)
0	0.117	0.062	0.267
0.5	0.118	0.063	0.270
1	0.113	0.061	0.260
1.5	0.117	0.064	0.272
2	0.116	0.063	0.271
2.5	0.119	0.063	0.271
3	0.123	0.065	0.281
3.5	0.122	0.063	0.275
4	0.123	0.063	0.275
5	0.141	0.065	0.294
6	0.153	0.064	0.286
7	0.154	0.062	0.277
8	0.165	0.063	0.292
10	0.167	0.063	0.291
12	0.163	0.061	0.275
16	0.169	0.062	0.301
24	0.173	0.074	0.323

Table 36

Time (h)	C20:5 W3 (EPA)	C22:5 W3 (DPA)	C22:6 W3(DHA)
0	0.146	0.052	0.260
0.5	0.142	0.052	0.260
1	0.146	0.054	0.268
1.5	0.142	0.053	0.263
2	0.145	0.054	0.267
2.5	0.140	0.053	0.258

3	0.143	0.054	0.264
3.5	0.155	0.056	0.278
4	0.155	0.055	0.277
5	0.179	0.057	0.295
6	0.217	0.057	0.316
7	0.204	0.057	0.304
8	0.211	0.060	0.320
10	0.187	0.057	0.293
12	0.171	0.054	0.272
16	0.166	0.052	0.272
24	0.169	0.061	0.290

These data show that absorption patterns of the coagulum and krill oil are different for the two subjects. The EPA pattern in subject 1 (coagulum) shows that a high EPA level is maintained over a long time despite the fact that coagulum contains less lipid than the krill oil. The coagulum has also enriched the circulating PL pool which could be an indication of absorption/incorporation of krill oil fatty acids in PL form. We have previously observed that krill oil is more efficient in enriching tissue lipid fatty acid profiles than fish oil. These data indicate that coagulum is even more bioeffective than krill oil.

Example 10.

The phospholipid content of the retentate was further analyzed by NMR. Table 37 provides the results.

Table 37.

Phospholipid	**% (w/w)**
Phosphatidylcholine	16,5
Alkylacylphosphatidylcholine	1,7
Lyso-alkylacylphosphatidylcholine	0,28
2-lysophosphatidylcholine	0,52
Phosphatidylethanolamine	0,59
N-acylphosphatidylethanolamine	3,6
Total phospholipid	23,23

Example 11

This example provides an analysis of the volatile compounds in oil extracted from krill meal and oil extracted from coagulum meal. Table 38. Briefly, oil was extracted by SFE from regular krill meal or meal prepared from coagulum as described above. The oil prepared from coagulum meal had substantially reduced amounts of volatile compounds as compared to the oil prepared from regular krill meal. In particular, 1-penten-3-one was detected in oil prepared from regular krill meal and was absent in oil prepared from coagulum meal. 1-pentene-3-one have previously been identified has a key marker of fishy and metallic off-flavor in fish oil and fish oil enriched food products (Jacobsen et al., J. Agric Food Chem, 2004, 52, 1635-1641).

Table 38.

Compound	TIC peak area (Krill oil extracted from krill meal using SFE)	Description	TIC peak area (Krill oil extracted from coagulum using SFE)	Description
dimethyl amine	180403283		22848535	
trimethyl amine	255213688	old fish, strong bad	49040416	old fish
Ethanol	394615326	fresh	1426886614	vodka, ethanol
Acetone	875959		0	
acetic acid	36136270	weak smell	0	
methyl vinyl ketone	515892		0	
2-butanone	2807131	sweet	23124362	
ethyl acetate	6231705		404501	
1-[dimethylamino]-2-propanone	23316404		15380603	
1-penten-3-one	5627101	rubbery	0	weak dishcloth

n-heptane	291386		0	
2-ethyl furan	1640866	weak sweet	0	
ethyl propionate	909959		0	
2-methyl-2-pentenal	6996219		0	
Pyridine	2085743		0	
Acetamide	6169014	pleasant	0	
Toluene	4359806		0	
N,N-dimethyl formamide	177968590	garden hose, mint	0	garden hose
ethyl butyrate	1122805		0	
2-ethyl-5-methyl furan	1550476	good, flower	427805	
butyl acetate	306001		856292	
3-methyl-1,4-heptadiene	1617339		0	weak smell, rubber
Isovaleric acid	1528541	foot sweat, weak	0	
methyl pyrazine	1335979	peculiar	0	
ethyl isovalerate	1043918	fruity	0	fruity
N,N-dimethyl acetamide	9895351		0	smell, solvent
2-heptanone	7397187	blue cheese	0	
2-ethyl pyridine	317424		0	
Butyrolactone	652076	butter, pleasant	0	
2,5-dimethyl pyrazine	2414087		0	
ethyl pyrazine	1909284	metallic	0	soft
N,N-dimethyl propanamide	1160830	unpleasant	0	
Benzaldehyde	3134653		0	
2-octanone	2068169	disgusting	0	
β-myrcene	2618870		0	

dimethyl trisulfide	3279406	sewer	0	
n-decane	1851488		331629	
trimethyl pyrazine	4186679	unpleasant	0	
1-methyl-2-pyrrolidone	9577873		0	
Eucalyptol	0	peppermint	868411	
Asetofenoni	1146348	smell, pleasant	350688	

Example 12

Krill meal produced by the traditional process (Tables 39-42) was compared with krill meal produced from the solid fraction remaining after removal of krill milk (Tables 43-46).

Table 39

14:0	g/100g total fat	8,3		
16:0	g/100g total fat	15,4		
18:0	g/100g total fat	1,0		
20:0	g/100g total fat	<0,1		
22:0	g/100g total fat	<0,1		
16:1 n-7	g/100g total fat	4,7		
18:1 (n-9)+(n-7)+(n-5)	g/100g total fat	13,5		
20:1 (n-9)+(n-7)	g/100g total fat	0,9		
22:1 (n-11)+(n-9)+(n-7)	g/100g total fat	0,6		
24:1 n-9	g/100g total fat	0,1		
16:2 n-4	g/100g total fat	0,6		
16:3 n-4	g/100g total fat	0,3		
18:2 n-6	g/100g total fat	1,1		
18:3 n-6	g/100g total fat	0,1		
20:2 n-6	g/100g total fat	<0,1		
20:3 n-6	g/100g total fat	<0,1		
20:4 n-6	g/100g total fat	0,3		
22:4 n-6	g/100g total fat	<0,1		
18:3 n-3	g/100g total fat	0,8		
18:4 n-3	g/100g total fat	1,8		
20:3 n-3	g/100g total fat	<0,1		
20:4 n-3	g/100g total fat	0,4		
20:5 n-3	g/100g total fat	11,3		
21:5 n-3	g/100g total fat	0,4		
22:5 n-3	g/100g total fat	0,3		
22:6 n-3	g/100g total fat	6,5		

Table 40

* Fat Bligh & Dyer	%	22,8		
Sum saturated fatty acids	g/100g total fat	24,7		
Sum monounsaturated fatty acids	g/100g total fat	19,8		
Sum PUFA (n-6)	g/100g total fat	1,6		
Sum PUFA (n-3)	g/100g total fat	21,5		
Sum PUFA	g/100g total fat	24,0		
Sum fatty acids total	g/100g total fat	68,5		

Table 41

Triacylglycerol	g/100g total fat	46		
Diacylgyycerol	g/100g total fat	1,0		
Monoacylglycerol	g/100g total fat	<1		
Free fatty acids	g/100g total fat	4,4		
Cholesterol	g/100g total fat	1,6		
Cholesterol ester	g/100g total fat	0,8		
Phosphatidylethanolamine	g/100g total fat	4,6		
Phosphatidylinositol	g/100g total fat	<1		
Phosphatidylserine	g/100g total fat	<1		
Phosphatidylcholine	g/100g total fat	37		
Lyso-Phosphatidylcholine	g/100g total fat	2,0		
Total polar lipids	g/100g total fat	36,2		
Totale neutral lipids	g/100g total fat	54,0		
Total sum lipids	g/100g total fat	96,2		

Table 42

Protein Kjeldahl (N*6,25)	%	60,9		
Total	%	92,7		
Salt (NaCI)	%	2,9		
Trimetylamine-N	Mg N/100 gram	4		
Trimethylaminoxide-N	Mg N/100 gram	149		
Free Astaxanthin	Mg/kg	<1		
Astaxanthin ester	Mg/kg	122		

Table 43

14:0	g/100g total fat	5,0		
16:0	g/100g total fat	13,9		
18:0	g/100g total fat	0,8		
20:0	g/100g total fat	<0,1		
22:0	g/100g total fat	<0,1		
16:1 n-7	g/100g total fat	3,0		
18:1 (n-9)+(n-7)+(n-5)	g/100g total fat	11,4		
20:1 (n-9)+(n-7)	g/100g total fat	0,5		
22:1 (n-11)+(n-9)+(n-7)	g/100g total fat	0,4		
24:1 n-9	g/100g total fat	0,1		
16:2 n-4	g/100g total fat	0,4		
16:3 n-4	g/100g total fat	0,2		
18:2 n-6	g/100g total fat	1,2		
18:3 n-6	g/100g total fat	0,1		
20:2 n-6	g/100g total fat	0,1		
20:3 n-6	g/100g total fat	0,1		
20:4 n-6	g/100g total fat	0,4		
22:4 n-6	g/100g total fat	<0,1		
18:3 n-3	g/100g total fat	0,7		
18:4 n-3	g/100g total fat	1,2		
20:3 n-3	g/100g total fat	0,1		
20:4 n-3	g/100g total fat	0,3		
20:5 n-3	g/100g total fat	13,1		
21:5 n-3	g/100g total fat	0,3		
22:5 n-3	g/100g total fat	0,3		
22:6 n-3	g/100g total fat	10,0		

Table 44

* Fat Bligh & Dyer	%	10,2		
Sum saturated fatty acids	g/100g total fat	19,7		
Sum monounsaturated fatty acids	g/100g total fat	15,3		
Sum PUFA (n-6)	g/100g total fat	1,8		
Sum PUFA (n-3)	g/100g total fat	26,1		
Sum PUFA	g/100g total fat	28,5		
Sum fatty acids	g/100g total fat	63,5		

Table 45

Triacylglycerol	g/100g total fat	25		
Diacylgyycerol	g/100g total fat	0,7		
Monoacylglycerol	g/100g total fat	<1		
Free fatty acids	g/100g total fat	0,9		
Cholesterol	g/100g total fat	3,1		
Cholesterol ester	g/100g total fat	<0,5		
Phosphatidylethanolamine	g/100g total fat	12,8		
Phosphatidylinositol	g/100g total fat	<1		
Phosphatidylserine	g/100g total fat	<1		
Phosphatidylcholine	g/100g total fat	49		
Lyso-Phosphatidylcholine	g/100g total fat	1,3		
Total polar lipid	g/100g total fat	63,2		
Total neutral lipid	g/100g total fat	29,7		
Total sum lipid	g/100g total fat	92,9		

Table 46

Protein Kjeldahl (N*6,25)	%	73,9		
Total	%	90,2		
Salt (NaCI)	%	1,9		
Trimetylamine-N	Mg N/100 gram	7		
Trimethylaminoxide-N	Mg N/100 gram	224		
Free Astaxanthin	Mg/kg	2,8		
Astaxanthin ester	Mg/kg	89		

CLAIMS

1. A process for preparing phospholipid compositions from krill comprising phospholipids and proteins comprising:

mixing said krill with water to increase the temperature of said krill to about 65 to 80 °C to form a first solid phase and a first aqueous phase comprising said phospholipids and proteins;

separating said first solid phase from said first aqueous phase; and

separating a phospholipid-protein coagulate fraction from said first aqueous phase by heating said first aqueous phase at a temperature sufficient to form said phospholipid-protein coagulate fraction and separating said phospholipid-protein coagulate fraction from said aqueous phase, wherein said phospholipid-protein coagulate fraction comprises about 20% to 50% protein w/w and about 50% to 65% lipids w/w on a dry base and wherein said lipids further comprise 25% to 50% w/w phospholipids expressed as percentage of total lipids.

2. The process of claims 1, wherein said krill is freshly harvested.

3. The process of claim 1, wherein said krill is frozen.

4. The process of claim 1, 2, or 3, wherein said first aqueous phase is heated to greater than 80 °C to provide said phospholipid-protein coagulate.

5. The process of any one of claims 1 to 4, further comprising the step of washing said phospholipid-protein coagulate.

6. The process of any one of claims 1 to 5, further comprising the step of pressing said phospholipid-protein coagulate to form a coagulate liquid phase and a coagulate press cake.

7. The process of claim 6, further comprising drying said coagulate press cake to form a coagulate meal.

8. The process of claim 7, further comprising extracting a coagulate oil from said coagulate meal.

9. The process of claim 1, 2, or 3, wherein said separating a protein and phospholipid fraction from said first aqueous phase comprises filtration of said aqueous phase to provide a phospholipid-protein retentate comprising proteins and phospholipids.

10. The process of claim 9, wherein said filtration is via membrane filtration.

11. The process of claim 9 or claim 10, further comprising the step of dewatering said phospholipid-protein retentate to form a retentate liquid phase and a retentate concentrate.

12. The process of claim 11, further comprising extracting a retentate oil from said retentate concentrate.

13. The process of any one of claims 1 to 12, further comprising the step of supplementing the protein and phospholipid fraction with additional proteins, lipids, astaxanthin and combinations thereof.

14. An aqueous phase composition obtained by the process of claim 1, 2, or 3.

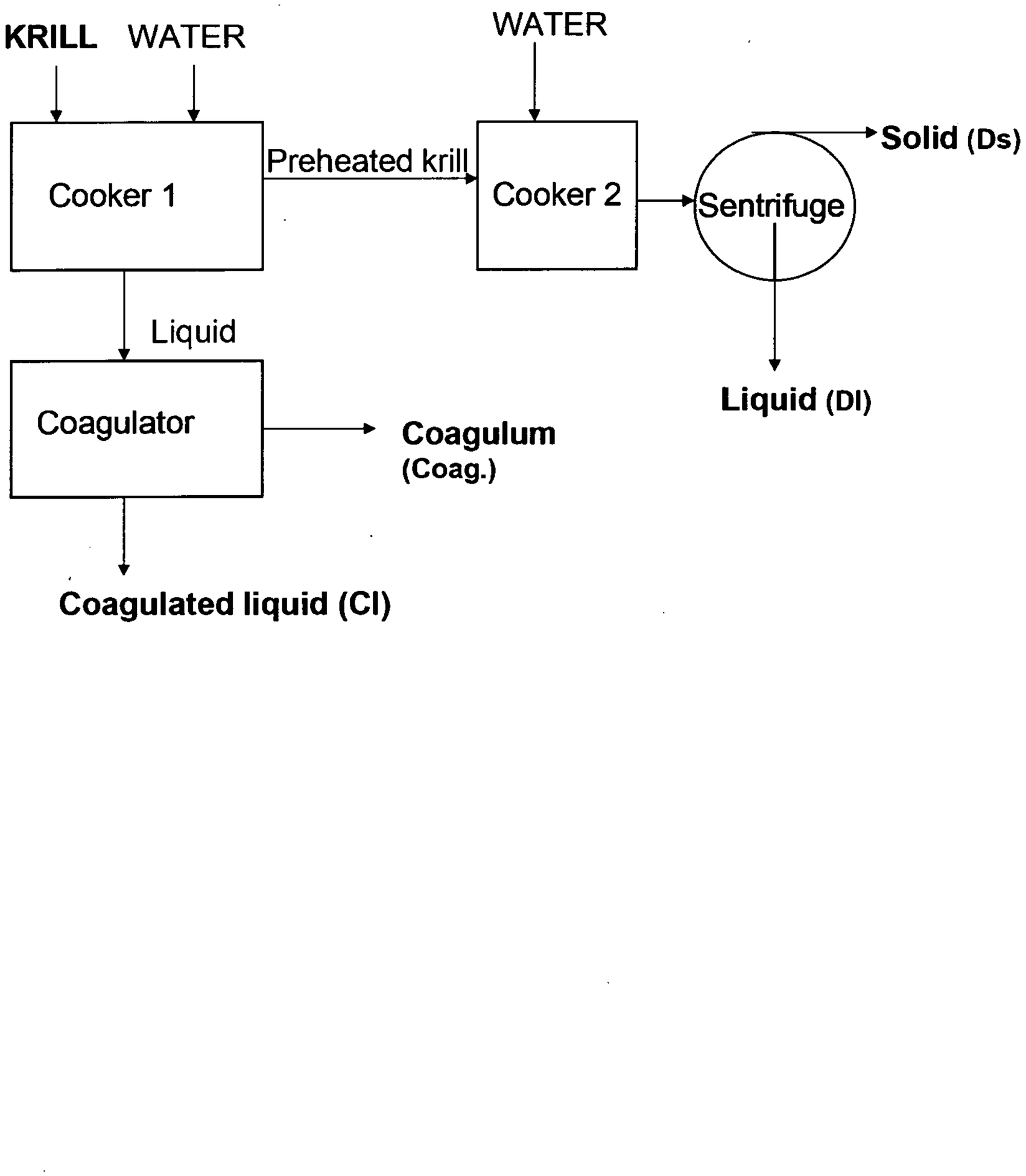
15. A coagulate meal obtained by the process of claim 7.

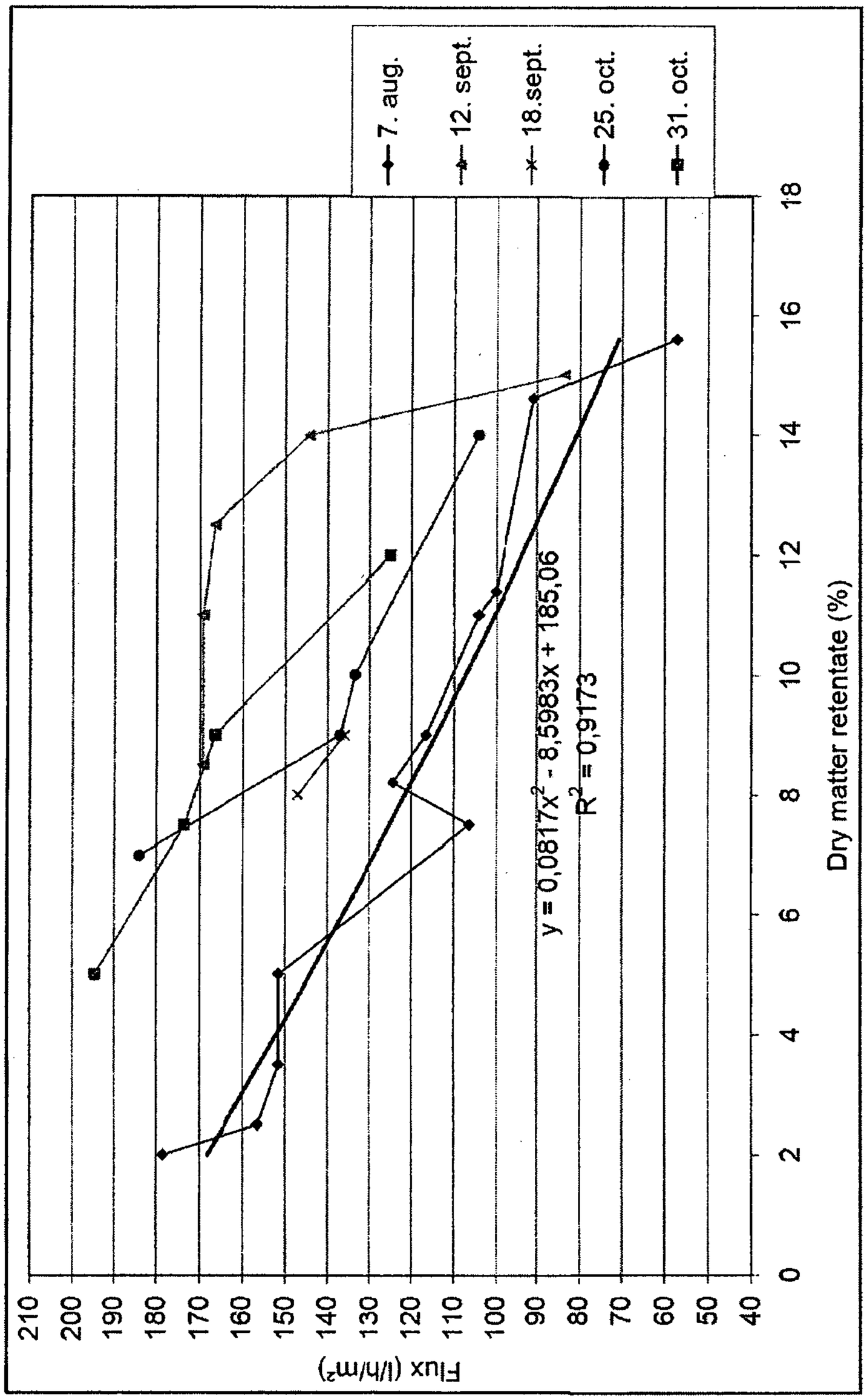
16. A coagulate oil obtained by the process of claim 8.

17. A retentate concentrate obtained by the process of claim 11.

18. A retentate oil obtained by the process of claim 12.

FIGURE 1


KRILL
WATER
WATER
Cooker 1
Preheated krill
Cooker 2
Sentrifuge
Solid (Ds)
Liquid
Liquid (Dl)
Coagulator
Coagulum
(Coag.)
Coagulated liquid (Cl)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12. sept.
18.sept.
25. oct.
31. oct.
y = 0,0817x² - 8,5983x + 185,06
R² = 0,9173
Dry matter retentate (%)
Flux (l/h/m²)

FIGURE 2

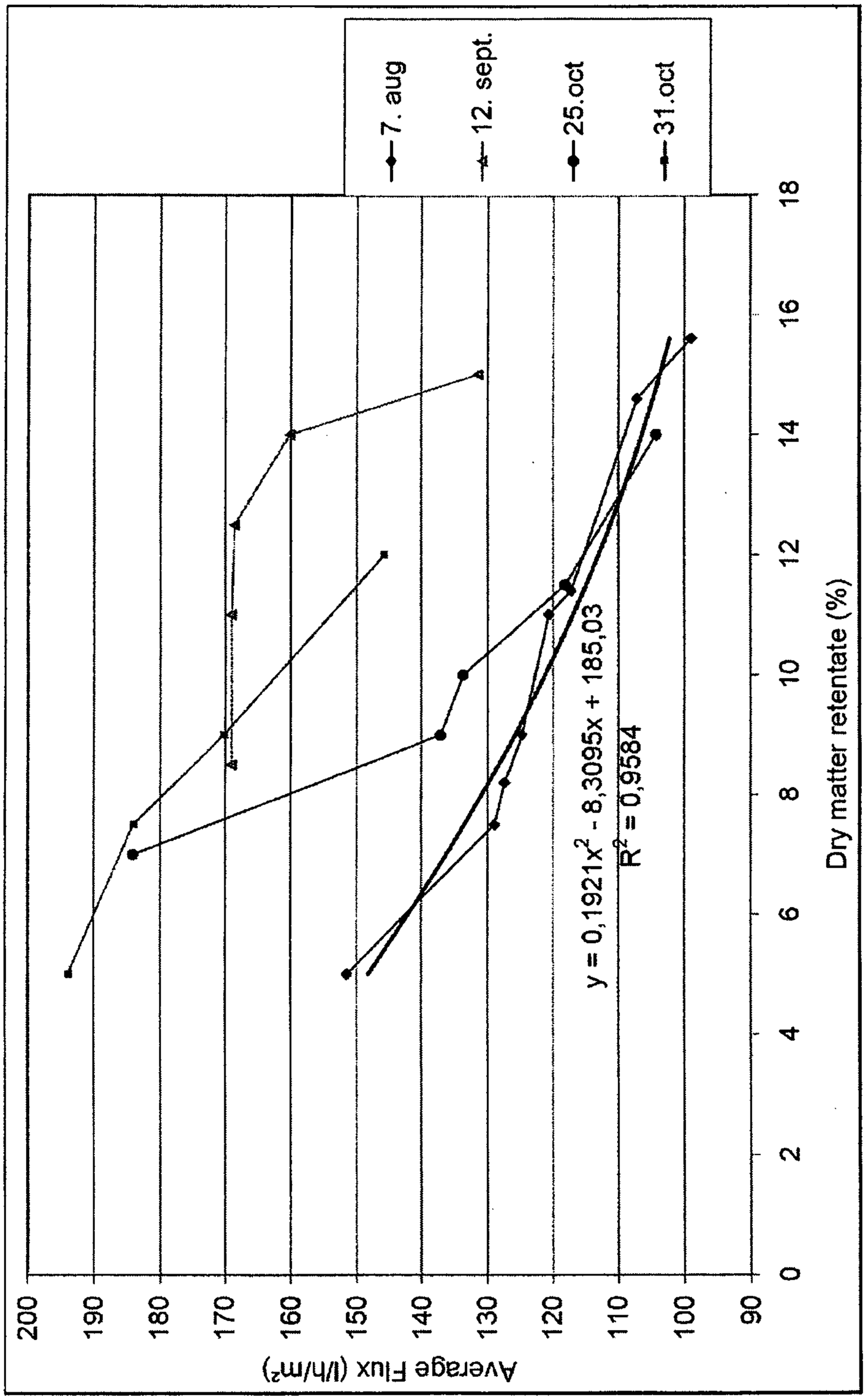
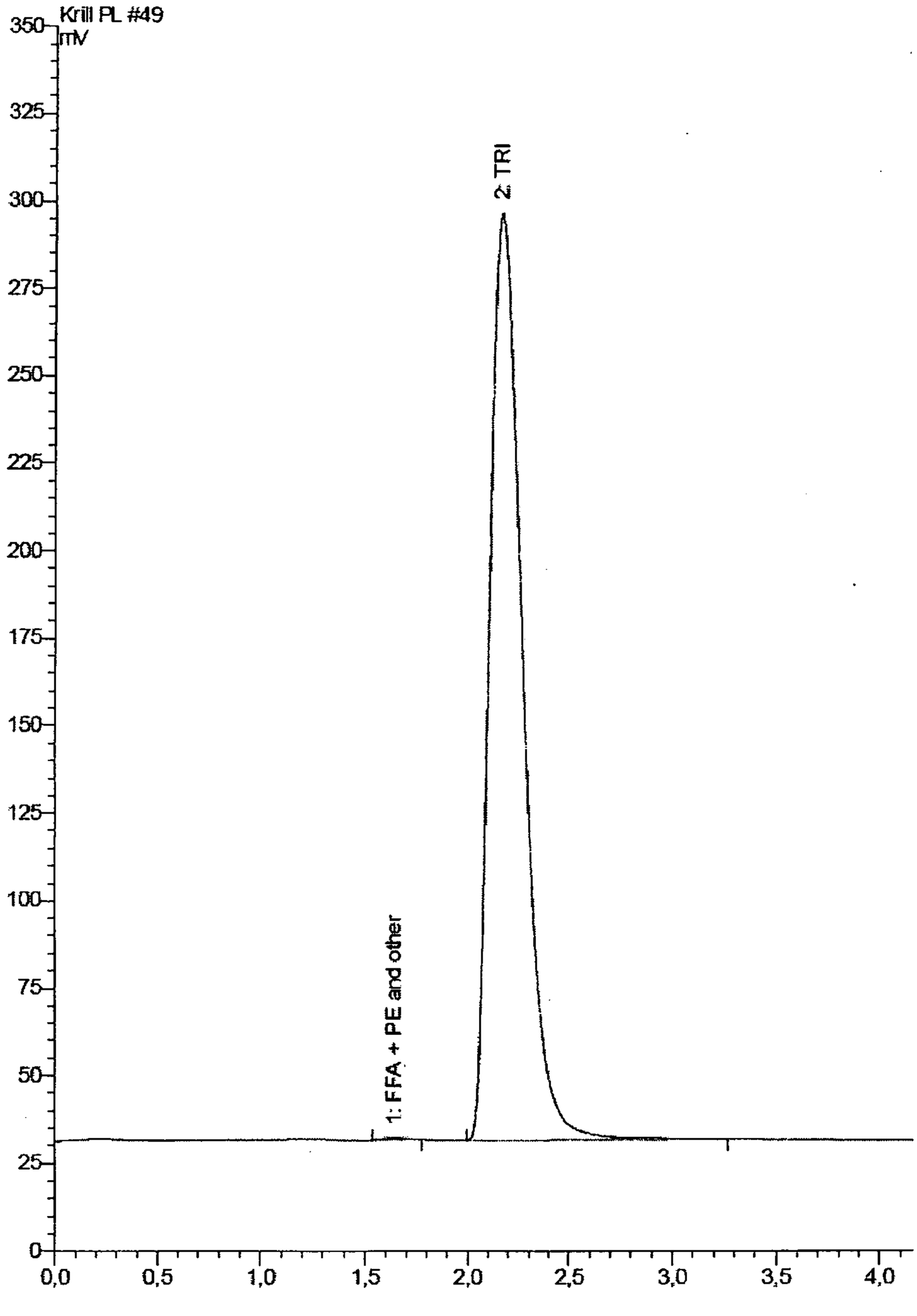

7. aug
12. sept.
25.oct
31.oct
y = 0,1921x² - 8,3095x + 185,03
R² = 0,9584
Dry matter retentate (%)
Average Flux (l/h/m²)
0
2
4
6
8
10
12
14
16
18
90
100
110
120
130
140
150
160
170
180
190
200

FIGURE 3

FIGURE 4


Krill PL #49
mV
350
325
300
275
250
225
200
175
150
125
100
75
50
25
0
2: TRI
1: FFA + PE and other
0,0
0,5
1,0
1,5
2,0
2,5
3,0
3,5
4,0

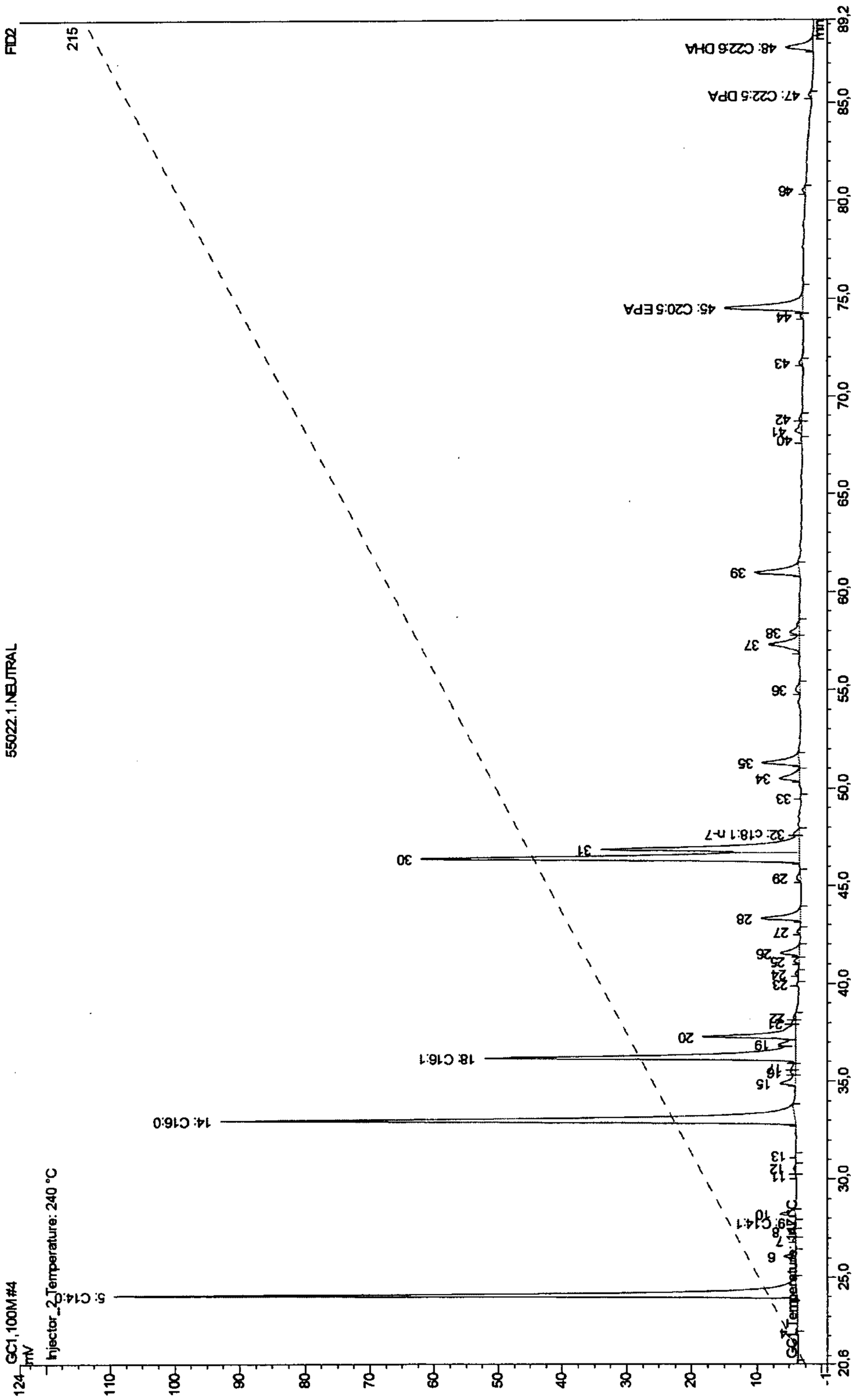
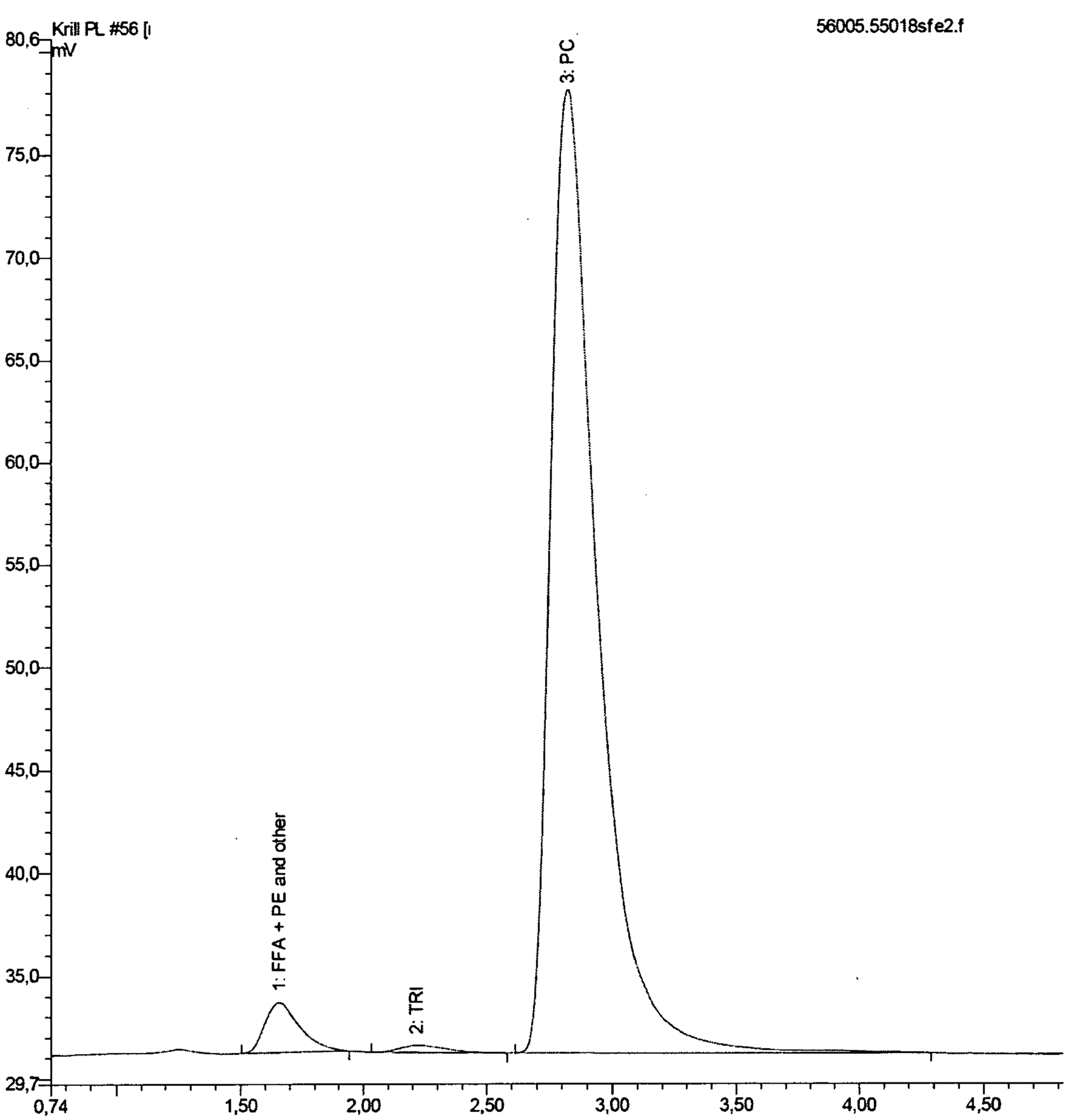

FID2
55022.1.NEUTRAL
GC1,100M#4
mV
Injector_2 Temperature: 240 °C
GC1 Temperature: 140 °C
48: C22:6 DHA
47: C22:5 DPA
45: C20:5 EPA
32: C18:1 n-7
18: C16:1
14: C16:0
5: C14:0
min
89,2
85,0
80,0
75,0
70,0
65,0
60,0
55,0
50,0
45,0
40,0
35,0
30,0
25,0
20,6
124
110
100
90
80
70
60
50
40
30
20
10
-1

FIGURE 5

FIGURE 6


Krill PL #56 [
mV
80,6
75,0
70,0
65,0
60,0
55,0
50,0
45,0
40,0
35,0
29,7
3: PC
1: FFA + PE and other
2: TRI
0,74
1,50
2,00
2,50
3,00
3,50
4,00
4,50
56005.55018sfe2.f

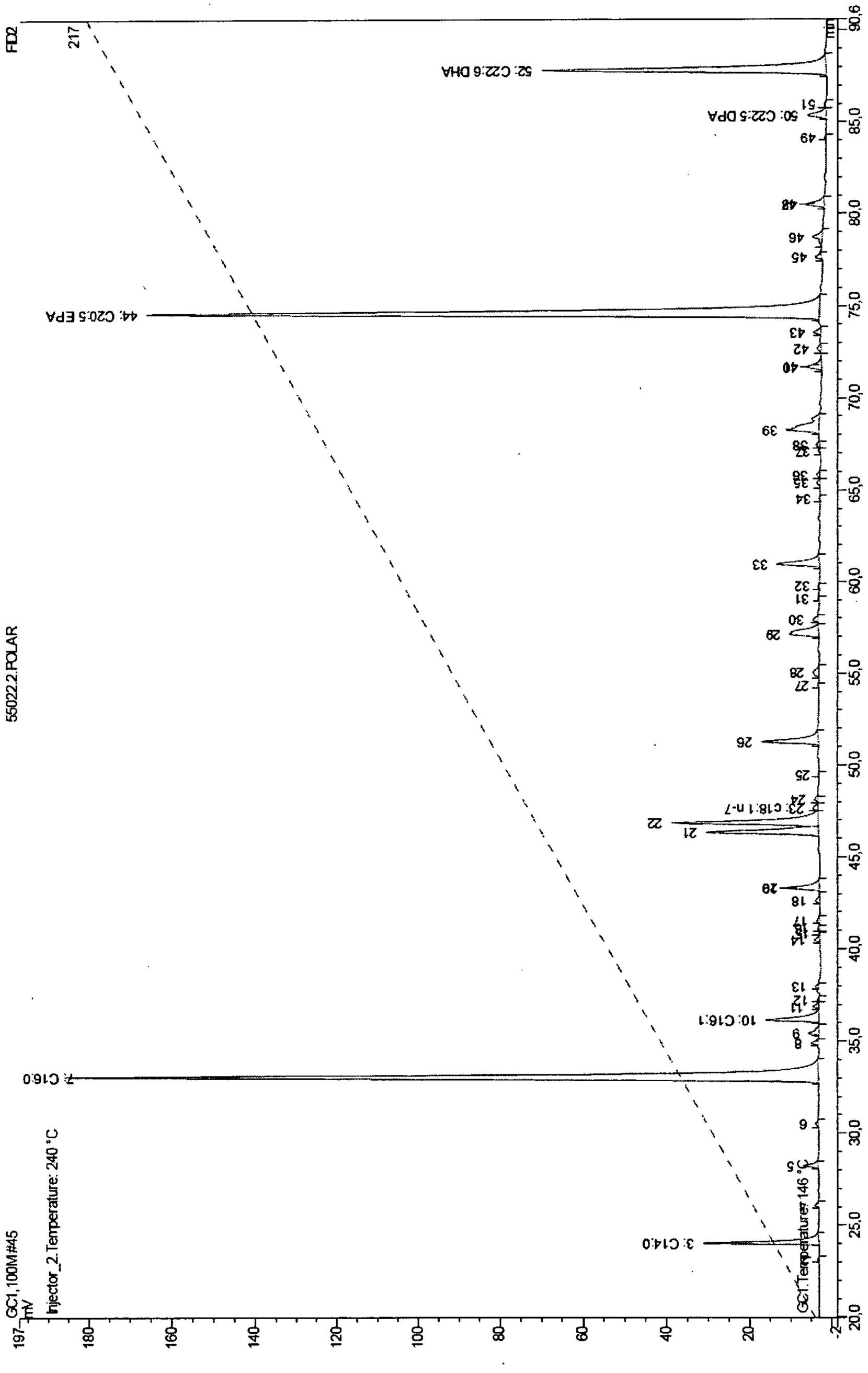
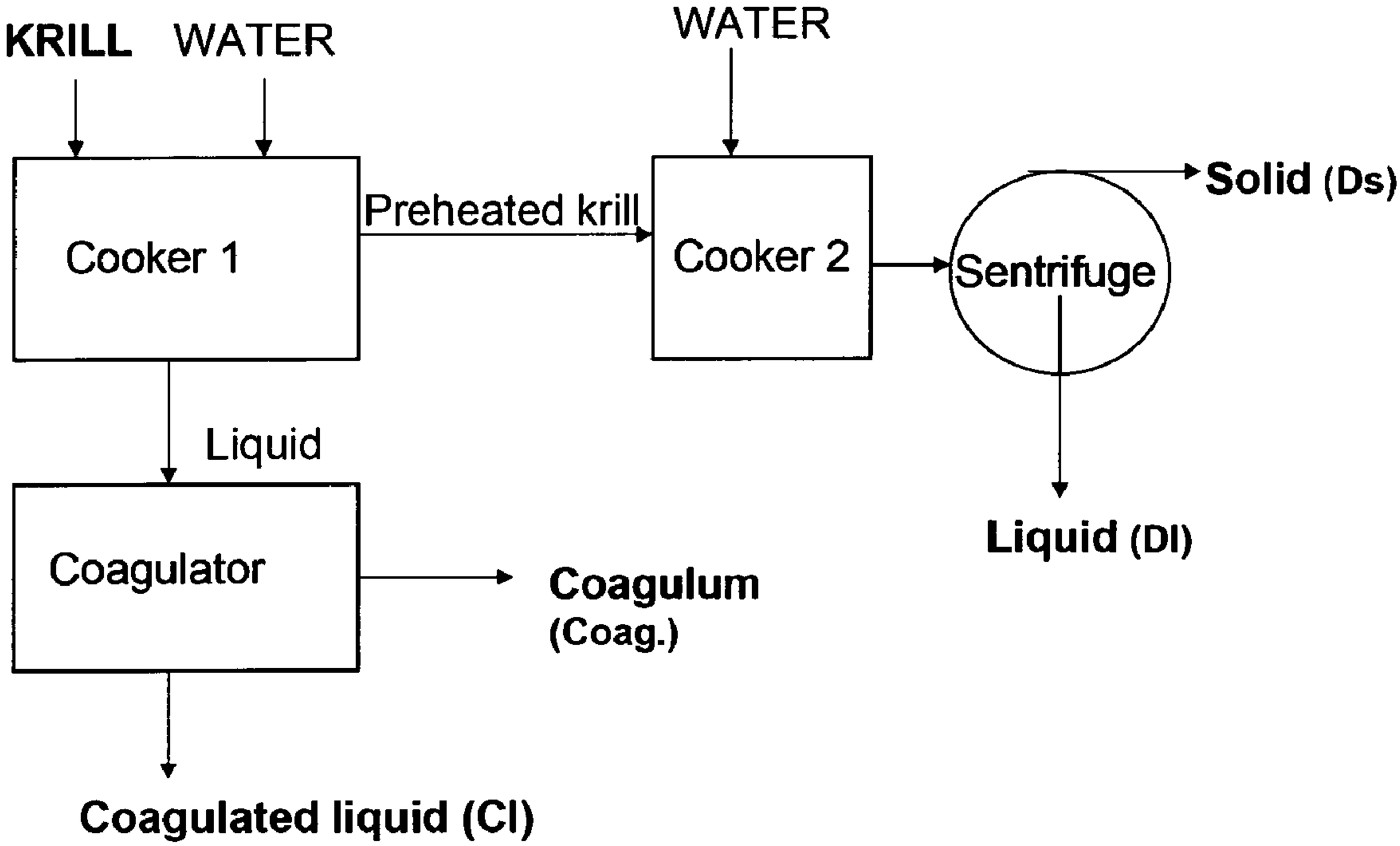

FID2
217
55022.2.POLAR
GC1,100M#45
mV
Injector_2.Temperature: 240 °C
GC1.Temperature: 146 °C
197
180
160
140
120
100
80
60
40
20
-2
90,0
85,0
80,0
75,0
70,0
65,0
60,0
55,0
50,0
45,0
40,0
35,0
30,0
25,0
20,0
52: C22:6 DHA
50: C22:5 DPA
44: C20:5 EPA
22
7: C16:0
10: C16:1
3: C14:0

FIGURE 7


KRILL
WATER
WATER
Cooker 1
Preheated krill
Cooker 2
Sentrifuge
Solid (Ds)
Liquid
Coagulator
Coagulum
(Coag.)
Liquid (Dl)
Coagulated liquid (Cl)