



- (72) BEAUDOIN, Adrien, CA
- (72) MARTIN, Geneviève, CA
- (71) UNIVERSITÉ DE SHERBROOKE, CA
- (51) Int.Cl.⁶ C11B 1/10, A23J 1/04, A23D 9/02
- (54) **PROCEDE D'EXTRACTION DES LIPIDES DE TISSUS
D'ANIMAUX AQUATIQUES PRODUISANT UN RESIDU
DESHYDRATE**
- (54) **PROCESS FOR LIPID EXTRACTION OF AQUATIC ANIMAL
TISSUES PRODUCING A DEHYDRATED RESIDUE**

(57) The procedure includes the suspension of freshly collected material in an equal volume of acetone under inert gas atmosphere. Lipids are extracted by successive acetone and ethanol treatments. The procedure produces two lipid fractions and a dry residue enriched in protein and other material insoluble in organic solvents. Recovery of total lipids is comparable or superior to the Folch et al. (1957) procedure. It has been tested with krill, Calanus and fish tissues.

TITLE OF THE INVENTION

**PROCESS FOR LIPID EXTRACTION OF AQUATIC ANIMAL TISSUES
PRODUCING A DEHYDRATED RESIDUE**

FIELD OF THE INVENTION

The present invention relates to a method for lipid extraction of animal tissues and to the lipid and dry residue fractions obtained therefrom. More particularly, the present invention relates to a lipid extraction method using krill, *Calanus* and fish tissues as starting material.

SUMMARY OF THE INVENTION

Extraction process

Fresh (or frozen) material (*Euphausia pacifica* and other species) is suspended in cold acetone for a given period of time at low temperature (5°C or lower). A ratio of krill-acetone 1:6 (w/v) and an incubation time of 2 h in acetone were found to be optimal. Alternatively the material can be kept in an equal volume of acetone at low temperature for long periods of time (months) under inert atmosphere. The size of the material is an important factor for the penetration of acetone. Indeed, it is preferable to grind material with dimensions superior to 5 mm before getting it in contact with acetone. The suspension is swirled for a short period of time (about 20 min) after acetone addition. After filtration on an organic solvent resistant filter (metal, glass or paper) the residue is washed with two volumes of pure acetone. The combined filtrates are evaporated under reduced pressure. The water residue obtained after evaporation is allowed to separate from the oil phase (fraction I) at low temperature. The solid residue collected on the filter is suspended and extracted with two volumes (original volume of frozen material) of 100% ethanol. The ethanol filtrate is evaporated leaving a second fraction of lipids (identified as fraction II).

Variations of the process

Variable volumes of acetone relative to the levels of sample can be used. It is also applicable to the volume of acetone used to wash and to the volume of ethanol used to extract. Incubation times in solvents may vary. Particle size affect the recovery of lipids and the material could be ground in various sizes of particles, depending on the grinder used. Temperature of the organic solvents and temperature of the sample are not critical parameters, but it is preferable to be as cold as possible.

Methods

To compare the efficiency of the extraction process, a classical technique (Folch et al. 1957) implying chloroform and methanol was applied to krill. This is the standard of reference for the efficiency of the extraction process. Lipid recovery was estimated by suspending lipid fractions in small volumes of their original solvents and measuring by gravimetry small aliquots after evaporation.

To analyze lipid composition, small aliquots of the various extracts were loaded on silica-gel plates and fractionated by thin layer chromatography, TLC (Bowyer et al. 1962) with the following solvents. Neutral lipids: hexane, ethyl ether, acetic acid (90:10:1 v/v) and phospholipids: chloroform, methanol, water (80:25:2 v/v). Fatty acid composition of *E. pacifica* was analyzed by gas liquid chromatography, GLC (Bowyer et al. 1962) including some modifications to the original technique: 1h at 65°C instead of 2h at 80°C, three washes with hexane instead of two and no wash with water.

The dry residue is wetted with ethanol to facilitate a progressive rehydration of the proteins.

To get rid of traces of organic solvents, lipid fraction I and II are warmed (60°C for fraction I and 70°C for fraction II) for 5 min under inert atmosphere.

Applications

The different fractions (oil, proteins, and others) of aquatic animal biomass extracted by the current procedure could be used in many fields:

1-Aquaculture

As mentioned in results, fatty acids 20:5 (eicosapentaenoic acid) and 22:6 (docosahexaenoic acid) are found in high concentrations in krill, *Calanus*, and fish. Farming fish on high quality marine oils rich in docosahexaenoic and eicosapentaenoic (EPA) acids is an efficient means of delivering these essential nutrients in human diets and also efficiently exploiting a strictly limited marine bioresource (Sargent 1997). Krill may be used as food supplement for fish and shrimp (Sargent 1997) because of its capacity to improve growth and survival capacity against diseases (Runge 1994), as pigmentation enhancer for ornamental fish species and as starter diet for marine and fresh water species (Prawn Hatchery Food 1997).

2-Nutraceuticals

Considering the beneficial effects of omega-3 fatty acids, the marine oils from krill, *Calanus* and fish could be used as dietary supplements to human diet. 22:6 *n*-3 fatty acid is essential for proper development of the brain and the eye (Sargent 1997). The beneficial effects of *n*-3 polyunsaturated fatty acids in reducing the incidence of cardiovascular disease by lowering plasma triacylglycerol level and altering platelet function towards a more anti-atherogenic state has been reviewed (Christensen 1994). Also, dietary krill oil, like fish oil, can suppress the development of autoimmune murine lupus: EPA substitutes for arachidonic acid, a substrate for cyclooxygenase thereby reducing the production of prostaglandins (Chandrasekar 1996). The effects of dietary supplementation with *w*-3 lipid-rich krill oil includes decreased expression of TGF β in kidneys and of the oncogene-*c-ras* in splenocytes (Chandrasekar 1996). Krill oil has beneficial effects on life span and amelioration of renal disease similar to those previously described in studies with fish oil (Chandrasekar 1996).

3-Animal food

Feeding the animals with omega-3 fatty acids may increase the level of unsaturated fatty acids and decrease cholesterol levels of meat. This property is exploited in the poultry industry to improve the quality of eggs. *Calanus*, in particular, is a full of promise ingredient of domestic animal's food (Runge 1994).

4-Cosmetic industry

Calanus is used for the production of moisturizing creams (Runge 1994).

5-Medical applications

Krill may be used as a source of enzymes for medical application like the debridement of ulcers and wounds (Hellgren 1991) or to facilitate food digestion.

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