

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Beaudoin et al.

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Examiner: Witz, Jean C.

Title: METHOD OF EXTRACTING LIPIDS FROM MARINE AND AQUATIC ANIMAL TISSUES

AMENDMENT

CERTIFICATE UNDER 37 CFR 1.8(a)
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PETITIONS OFFICE

Dear Sirs:

This amendment is in response to the Office Action dated February 25, 2003.

Amendments to the claims begin on page 2 of this paper.

Remarks/arguments begin on page 8 of this paper.

{WP146887:1}

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AMENDMENTS TO THE CLAIMS

1-51. (Cancelled)

52. (New) A method for extracting total lipid fractions from marine and aquatic animal material, said method comprising the steps of:

- (a) placing marine and aquatic animal material in a ketone solvent to achieve extraction of the soluble lipid fraction from said marine and aquatic animal material;
- (b) separating the liquid and solid contents;
- (c) recovering a first total lipid rich fraction from the liquid contents of b) by evaporation of the solvent present in the liquid contents;
- (d) placing said solid contents in an organic solvent selected from the group of solvents consisting of alcohol and esters of acetic acid to achieve extraction of the remaining soluble total lipid fraction from said marine and aquatic animal material;
- (e) separating the liquid and solid contents;
- (f) recovering a second total lipid rich fraction by evaporation of the solvent from the liquid contents of e); and
- (g) recovering the solid contents.

53. (New) A method as in claim 52, wherein steps (b) and (d) are conducted under inert gas atmosphere.

54. (New) A method as in claim 52, wherein steps (b) and (e) are effected by techniques selected from the group consisting of filtration, centrifugation and sedimentation.

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55. (New) A method as in claim 52, wherein steps (c) and (f) are effected by techniques selected from the group consisting of vacuum evaporation, flash evaporation and spray drying.

56. (New) A method as in claim 52, wherein after step (b) and before step (c), the method additionally comprises the intervening step of washing the solid contents with the solvent and adding the resulting washing solution to the liquid contents of step (b).

57. (New) A method as in claim 52, wherein after step (e) and before step (f), the method additionally comprises the intervening step of washing the solid contents with the organic solvent selected in step (d).

58. (New) A method as in claim 52, wherein prior to step (a) the marine and aquatic animal material is finely divided.

59. (New) A method as in claim 52, wherein steps (a) and (b) are conducted at solvent temperatures of not more than about 5°C.

60. (New) A method as in claim 52, wherein said marine and aquatic animal is zooplankton.

61. (New) A method as in claim 60, wherein said zooplankton is selected from krill and *Calanus*.

62. (New) A method as in claim 52, wherein said marine and aquatic animal is fish.

63. (New) A method for extracting an astaxanthin-and-canthaxantin-containing total lipid fraction from a marine and aquatic animal material selected from zooplankton and fish, said method comprising the steps of:

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- (a) placing said animal material in a ketone solvent to achieve an extraction of the soluble lipid fraction from said marine and aquatic animal material;
 - (b) separating the liquid and solid contents; and
 - (c) recovering a lipid rich fraction from the liquid contents by evaporation of the solvent present in the liquid contents,
- whereby an astaxanthin-and-canthaxantin-containing total lipid fraction is obtained.

64. (New) A method for extracting a total lipid fraction from a marine and aquatic animal material selected from zooplankton and fish, said method comprising the steps of:

- (a) placing said animal material in a solvent mixture comprising acetone and ethanol to achieve an extraction of the soluble lipid fraction from said marine and aquatic animal material;
 - (b) separating the liquid and solid contents; and
 - (c) recovering a lipid rich fraction from the liquid contents by evaporation of the solvents present in the liquid contents,
- whereby a total lipid fraction is obtained.

65. (New) A method as in claim 63, wherein the animal material is selected from krill and *Calanus*.

66. (New) A method as in any one of claim 63, wherein during step (a), the animal material is homogenized.

67. (New) A method as in claim 63, wherein steps (b) and (d) are conducted under inert gas atmosphere.

68. (New) A method as in claim 63, wherein step (b) is effected by a technique selected from the group consisting of filtration, centrifugation and sedimentation.

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69. (New) A method as in claim 63, wherein step (c) is effected by a technique selected from the group consisting of vacuum evaporation, flash evaporation and spray drying.

70. (New) A method as in claim 63, wherein after step (b) and before step (c), the method additionally comprises a step of washing said solid contents with solvent and adding the resulting washing solution to the liquid contents of step (b).

71. (New) A method as in claim 63, wherein prior to step (a) the marine and aquatic animal material is finely divided.

72. (New) A method as in claim 63, wherein steps (a) and (b) are conducted at solvent temperatures of about 5°C or less.

73. (New) A method of lipid extraction as in claim 52, wherein the solid contents of step b) is recovered and consists of a dehydrated residue containing active enzymes.

74. (New) A method of lipid extraction as in claim 63, wherein the solid contents of step b) is recovered and consists of a dehydrated residue containing active enzymes.

75. (New) A method for extracting total lipid fractions from marine and aquatic animal material, said method comprising the steps of:

- (a) placing marine and aquatic animal material in a ketone solvent to achieve extraction of the soluble total lipid fraction from said marine and aquatic animal material;
- (b) separating the liquid and solid contents;
- (c) recovering a first total lipid-rich fraction from the liquid contents of b) by evaporation of the solvent present in the liquid contents;
- (d) placing said solid contents in an organic solvent selected from the group of solvents consisting of alcohol and esters of acetic acid to achieve extraction

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