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(54) Title of the invention: METHOD FOR EXTRACTING KRILL PHOSPHOLIPIDS, FUNCTIONAL FOOD PRODUCT HAVING BRAIN FUNCTION ENHANCING EFFECT, AND BRAIN FUNCTION ENHANCING AGENT

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SPECIFICATION

1. Title of the Invention

Method for Extracting Krill Phospholipids, Functional Food Product Having Brain Function Enhancing Effect, and Brain Function Enhancing Agent

2. Scope of Patent Claims

(1) A method for extracting krill phospholipids wherein raw krill is dewatered using a vacuum freezing drying method, all the lipids therein are extracted using ethanol, an elution of the total lipids thus obtained is made with an ethanol solvent, an acetone solvent, or a hexane solvent, the phosphatidyl choline and, using adsorption column chromatography with a silica gel as a filler, phosphatidyl ethanol amine are fractionated and then isolated using a faction collector.

(2) A functional food product having a brain function enhancing effect, wherein at least one or more of phosphatidyl choline or phosphatidyl ethanol amine isolated from krill or a derivative of these is mixed into a base food ingredient as an active ingredient.

(3) A brain function enhancing agent, wherein at least one or more of phosphatidyl choline or phosphatidyl ethanol amine isolated from krill or a derivative of these is contained as an active ingredient.

3. Detailed Description of the Invention

[Industrial Field of Use]

The present invention is a method for separating and extracting phospholipids from krill and is more particularly a method for isolating phosphatidyl choline and phosphatidyl ethanol amine which show important bioactivity in living bodies, relating to a technology characterized in that phosphatidyl choline and phosphatidyl ethanol amine thus extracted can be used as a food product or pharmaceutical product.

[Prior Art]

We have begun entering an era of the aging society in recent years, and as this has happened senile dementia has become a major societal issue.

Senile dementia can broadly be divided into Alzheimer's-type dementia which is caused by dysfunction of the nervous system and cerebrovascular dementia which is caused by dysfunction of the cerebral blood vessels. With Alzheimer's-type dementia, it is known that production of acetyl choline, a neurotransmitter, drops significantly as a neurochemical change in the brain. To prevent and treat this ailment, an attempt is made to restore biological function by supplementing metabolism of the reduced choline. Examples include "Method and Composition for Treating an Illness by Administering Lecithin" in PCT Application S56-500374 A, "Brain Hyperfunction Agent Composition" in JP S59-167514 A, and "Therapeutic Composition and Treatment Method for Neurological Damage and Chemotaxis" in S60-214734 A.

In other words, by ingesting phosphatidyl choline, which is a choline-containing phospholipid, acetyl choline is supplied to the brain, which is expected to prevent and treat Alzheimer's-type dementia and other neurological disorders.

Phosphatidyl ethanol amine, which is another type of phospholipid, is converted into phosphatidyl choline through a methyl group transition reaction from S-adenosylmethionine. Consequently, phosphatidyl ethanol amine is also expected to be usable in treatment of Alzheimer's -type dementia and other neurological disorders and as a therapeutic agent therefor.

The inventors focused particularly on the phospholipids phosphatidyl choline and phosphatidyl ethanol amine, which are glycerophospholipids in researching and developing a method for industrially extracting these in a form usable as a raw ingredient in food and pharmaceutical products.

Conventionally, soy beans have typically been used as a raw material when refining phospholipids from a natural substance for industrial use. Soy bean phospholipids have mainly been commercialized as health food products and the like. Conventional soy bean phospholipid refining processes involve extracting the total phospholipids from raw soy beans with a chloroform ethanol solvent. The total phospholipids are then fractionated with acetone, thereby separating them into a soluble section and an insoluble section. In the acetone-soluble section, neutral lipids, cholesterol, freed lipids, and so on are fractionated. In the acetone-insoluble section, phospholipids are fractionated. Next, the acetone-insoluble section is treated with a 90% ethanol solution, thereby obtaining phosphatidyl choline, which is soluble in alcohol, and phosphatidyl ethanol amine, which is not soluble.

[Problem to be Solved by the Invention]

However, when refining phospholipids from soy beans, the phosphatidyl choline and phosphatidyl ethanol amine which are obtained have a purity of around 70% to 80% and it is very difficult to obtain a refined product with a purity of 90% or more. The method using chloroform ethanol described above also entails the fear that harmful substances might remain, no matter how the soy beans are refined and fractionated, making it difficult to use this in food products, which is a problem.

The inventors noticed that while krill has gotten attention as a rich source of protein, it nevertheless rots easily and has a high water content, making it expensive to store and transport, which is why no effective method of use has been established. The inventors also noticed that while krill has a high phospholipid content, there has been no technological development which would allow use of phospholipids, which the inventors focused on and which are an active ingredient, in high-value-added and economically valuable functional food or pharmaceutical products.

The inventors therefore realized that if useful phospholipids could be obtained from krill, which is an untapped marine resource, at high purities, this would be extremely profitable as an effective method for utilizing krill. The inventors therefore proceeded with R&D of such a refinement method, and arrived at the present invention.

Specifically, the present invention is an extraction method, wherein krill is used as a raw ingredient, total phospholipids are fractionated, and high-purity phosphatidyl choline and phosphatidyl ethanol amine are refined and isolated from the total phospholipids thus obtained, being a technology whereby the bioactive substances thus obtained are used as a functional food product having a brain function enhancing effect and a brain function enhancing agent.

[Means for Solving the Problem]

The present invention uses the following means to solve this problem.

The present invention is a method for extracting krill phospholipids wherein raw krill is dewatered using a vacuum freezing drying method, all the lipids therein are extracted using ethanol, an elution of the total lipids thus obtained is made with an ethanol solvent, an acetone solvent, or a hexane solvent, the phosphatidyl choline and, using adsorption column chromatography with a silica gel as a filler, phosphatidyl ethanol amine are fractionated and then isolated using a faction collector.

First step: Blocks of raw krill quick-frozen at sea have a water content of 90% or more, so drying is a problem. With the present invention, a vacuum

freezing and drying machine is used as a pre-process to extraction using adsorption column chromatography to achieve dewatered, dried krill. It is desirable for dewatering and drying to reduce the water content to 6% or less. This minimizes admixture of water-soluble proteins in the ethanol extract, allowing a greater purity of separated components.

Second step: Total lipids are extracted by homogenizing the dried krill obtained in the first step with ethanol.

Third step: As much of the ethanol as possible is removed from the total lipids next, and an elution is obtained with an acetone solvent or a hexane solvent, fractionated into a soluble section and an insoluble section. For example, in the case of an acetone solvent, most of the phospholipids are in the insoluble section, so if the solvent is washed off, crude phospholipids are obtained.

Fourth step: An elution is formed of the crude phospholipids with an ethanol solvent, an acetone solvent, or a hexane solvent, which is then fractionated into phosphatidyl choline and phosphatidyl ethanol amine using adsorption column chromatography, from which the phospholipids are isolated using a faction collector with a high purity of 90% or more and around 95%.

The present invention is a method for extracting krill phospholipids such as phosphatidyl choline and phosphatidyl ethanol amine at high purities using the method above.

Next, focusing on the fact that the high-purity phosphatidyl choline or phosphatidyl ethanol amine isolated from the krill using the above method is a biofunction activating substance that enhances brain functions, the present invention is a functional food product having a brain function enhancing effect, wherein at least one or more of phosphatidyl choline or phosphatidyl ethanol amine isolated from krill or a derivative of these is mixed into a base food ingredient as an active ingredient.

The present invention is also a brain function enhancing agent, wherein at least one or more of phosphatidyl choline or phosphatidyl ethanol amine isolated from krill or a derivative of these is contained as an active ingredient. In this case the brain function enhancing agent can be made into a pharmaceutical product in tablet, capsule, granular, or liquid form.

[Operation]

With Alzheimer's-type dementia, it is known that production of acetyl choline, a neurotransmitter, drops significantly as a neurochemical change in the brain. To prevent and treat this ailment, an attempt is made to restore biological function by supplementing metabolism of the reduced choline.

In particular, in the case of humans, when choline or lecithin, a naturally-produced compound which dissociates into choline, is administered orally, it is known that the resulting biofunction is an increase in the blood choline sufficient to promote synthesis and release of acetyl choline in the brain and an increase in the amount of choline in the cerebrospinal fluid.

Accordingly, an object of the present invention is to expect prevention and treatment of Alzheimer's-type dementia and other neurological disorders by supplying acetyl choline to the brain by extracting phosphatidyl choline, which is a phospholipid, from krill in as safe and efficient a manner as possible and ingesting this as a food or pharmaceutical product.

[Examples]

The present invention is described in detail below, with reference to examples.

<Example 1.>

20 kg of raw krill that was quick-frozen at sea was dried until a water content of around 4% was achieved using a vacuum drying machine, obtaining 2.2 kg of dried krill. Table 1 gives the results of an iatroscan analysis of the lipid composition of the dried krill which was the raw material.

Next, 2 kg of the dried krill thus obtained was homogenized using 40 kg of ethanol and the total lipids were extracted. Extraction was repeated, this time with 20 kg of ethanol.

The total lipids, which were the extract, were condensed and as much of the ethanol as possible was removed. The total lipids were dissolved in acetone and fractionated into a soluble section and an insoluble section. The majority of the phospholipids were fractionated into the insoluble section. Accordingly, the substances fractionated into the insoluble section were repeatedly washed with acetone, obtaining 408 g of crude phospholipids.

Table 1. Lipid Composition of Dried Krill

Lipid Composition	wt. %
Phosphatidyl choline	31.1
phosphatidyl ethanol amine	7.5
Triglycerides	43.2
Free fatty acids	6.5
Other	5.7

Next, 400 g of the crud phospholipids was dissolved in 2000 ml of ethanol and 20 ml per batch was automatically injected into an extraction column (column length x width: 50 cm x 50 mm, cross-sectional area: 19.6 cm^3) mounted on a fully-automated extraction-type high-performance liquid chromatography [sic] filled with spherical silica gel

(adsorption agent) having a particle diameter of 10 μm. The elution was introduced with 100% ethanol at a flowrate of 30 ml/min. The column constant temperature tank was at 40°C. Peak detection was monitored using a UV absorption detector (205 μm). The chromatograph shown in FIG. 1 was obtained. The fraction section of the first peak is A and the fraction section of the second large peak is B. These were extracted using a fraction collector. The purity of the phosphatidyl choline in the fraction section B was 98% or higher according to the iatroscan analysis. The cycle time per batch was 30 min. The base solution was automatically filled every 30 min, repeated over 100 cycles for a total time of around 50 hr. As a result, approximately 239 g of high-purity phosphatidyl choline was obtained from 2 kg of dried krill.

Approximately 45 g of high-purity phosphatidyl ethanol amine with a purity of 95% or higher was similarly obtained from the fraction section A.

<Example 2.>

Wexler memory and intelligent quotient tests were performed. A patient with memory loss, having a memory quotient of 123, was orally administered 10 g of the high-purity phosphatidyl choline (98% purity) obtained from the krill in the first example by mixture into food at every meal, three times a day for six weeks.

Blood samples were taken from the patient before the test treatment and after six weeks of administration of the high-purity phosphatidyl choline. The blood plasma was separated and frozen and analyzed using an ordinary radiation oxygen method to determine the choline content. As a result, the blood plasma choline content in the blood taken before the test treatment was 13.4±2.5 nanomol/ml. After four weeks of high-purity phosphatidyl choline administration, the blood plasma choline content had increased to 31.3±2.5 nanomol/ml ($P<0.01$).

Moreover, the patient's memory quotient had improved to 142 after six weeks of high-purity phosphatidyl choline administration.

[Effects]

The invention which is submitted for protection according to claim 1 is an extraction method wherein krill, which is an untapped marine resource, is used as a raw material to refine and isolate useful phosphatidyl choline and phosphatidyl ethanol amine therein to a high purity of 90% or higher. This extraction method not only achieves a high purity in the refined and isolated components thereof, but also uses no solvents having toxicity in the refinement process. Therefore, this method is characterized in being able to be used safely for food and pharmaceutical products.

Furthermore, the inventions which are submitted for protection according to claims 2 and 3 are of a functional food product having a brain function enhancing effect and a brain function enhancing agent, because the phosphatidyl choline and phosphatidyl ethanol amine, which are the krill phospholipids thus obtained, have biological functions for enhancing brain functions which can be expected to be used in preventing and treating Alzheimer's disease.

4. BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a fraction table of components obtained from a chromatogram according to the present invention.

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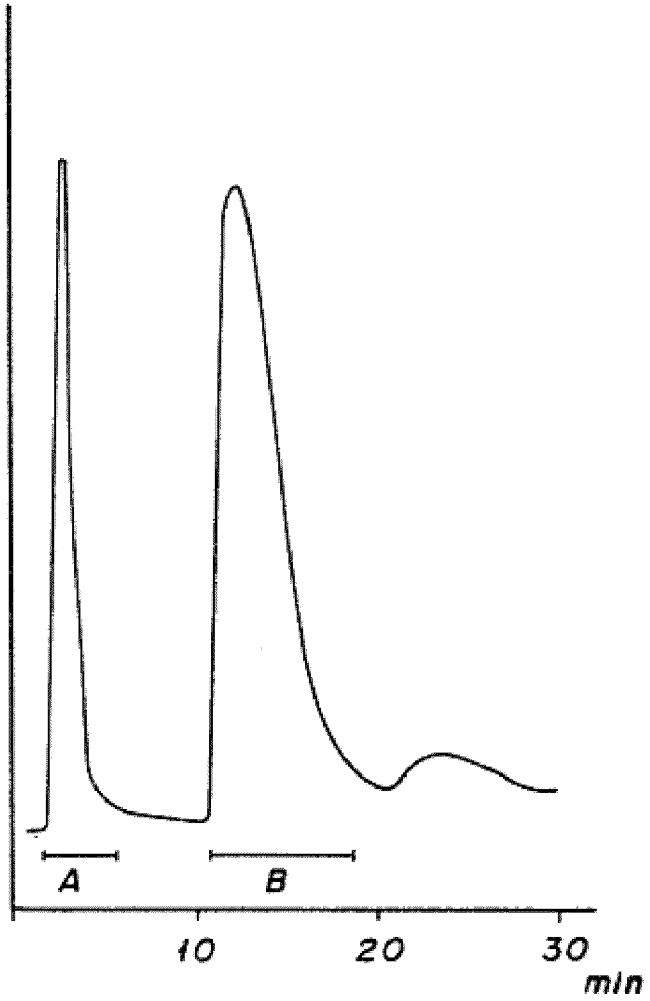
[SEAL: illegible]

FIG. 1



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