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(74) Mandataire: BREESE-MAJEROWICZ; 3, avenue de
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(71) Déposant (pour tous les États désignés sauf US): LABO-
RATOIRES LAVIPHARM S.A. [FR/FR]; 23, avenue de
Neuilly, F-75116 Paris (FR).

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(72) Inventeurs; et

(75) Inventeurs/Déposants (pour US seulement): DE-
SCHAMPS, Frantz [FR/FR]; 13 rue Gandon, Escalier
3 - Etage 2, F-75013 Paris (FR). MAJEWSKI, Wies-
law [FR/FR]; 4 Immeuble Méridien, Terrasse Vosges,
F-54520 Laxou (FR). PERRUT, Michel [FR/FR]; 25 rue
Santifontaine, F-54000 Nancy (FR). LAIMAY, François
[FR/FR]; 39bis Bd. Joffre, F-77300 Fontainebleau (FR).

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(54) Title: METHOD FOR FRACTIONATING A RAW MATERIAL CONSISTING OF SEVERAL CONSTITUENTS USING A
SUPERCRITICAL PRESSURE SOLVENT

(54) Titre: PROCEDE DE FRACTIONNEMENT D'UNE MATIERE COMPOSEE DE PLUSIEURS CONSTITUANTS A L'AIDE
D'UN SOLVANT A PRESSION SUPERCRITIQUE

(57) Abstract: The invention concerns a method for fractionating a synthetic or natural material consisting of several constituents
and containing at least an amphiphilic constituent. The invention is characterised in that said fractionating is carried out by means of
a supercritical pressure solvent by dispersion of said material inside an insoluble or hardly soluble liquid in the supercritical pressure
solvent. The inventive method is advantageously carried out for a mixture of lipids such as, for example an oil extracted from a
cereal.

(57) Abrégé: La présente invention concerne un procédé de fractionnement d'une matière naturelle ou synthétique composée de
plusieurs constituants et contenant au moins un composant à caractère amphiphile, caractérisé en ce que ledit fractionnement est
réalisé à l'aide d'un solvant à pression supercritique à partir d'une dispersion de ladite matière au sein d'un liquide insoluble ou très
faiblement soluble dans le solvant à pression supercritique. Avantagusement le procédé de l'invention est mis en oeuvre sur un
mélange de lipides comme par exemple une huile extraite de céréale.

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METHOD FOR FRACTIONATING A RAW MATERIAL CONSISTING OF
SEVERAL CONSTITUENTS USING A SUPERCRITICAL PRESSURE
SOLVENT

The invention concerns a method for fractionating a raw material made up of several constituents using a solvent under supercritical pressure. A solvent under supercritical pressure in the meaning of the invention
5 denotes a solvent in supercritical state or a subcritical liquid. More particularly, the invention relates to a method for fractionating a mixture of lipids in order to obtain fractions rich in some polar lipids belonging to the sphingolipids including ceramides, and to the
10 glycolipids and phospholipids.

A solvent in supercritical state is characterized either by a pressure and temperature respectively greater than the critical pressure and temperature for a pure body, or by a representative point (pressure, temperature)
15 located beyond the envelope of critical points shown on a diagram (pressure, temperature) for a mixture. It is known that a solvent in supercritical state, for numerous substances, has high solvent power out of all comparison with the power observed in the same solvent in the
20 compressed gas state. The same applies to so-called "subcritical" liquids. Subcritical liquids are in a state characterized either by a pressure greater than the critical pressure and by a temperature lower than critical temperature for a pure body, or by a pressure greater than
25 the critical pressures and a temperature lower than the critical temperatures of the components of a mixture (Michel PERRUT, Les Techniques de l'Ingénieur, Extraction

par fluid supercritique, J 2, 770, 1 to 12, 1999). The substantial, adjustable variations of the solvent power of these fluids are moreover used in numerous extraction processes (solid/fluid), fractionating processes (liquid/fluid), analytical or preparative chromatography, in the treatment of materials (ceramics, polymers, etc..). Chemical or biochemical reactions are also produced in such solvents. It is to be noted that the physico-chemical properties of carbon dioxide and its critical coordinates (critical pressure: 7.4 MPa and critical temperature: 31°C) make this the preferred solvent for numerous applications, especially as it has no toxicity and is available at very low cost and in large quantities. When brought to supercritical pressure carbon dioxide, which is a non-polar solvent, is sometimes used with a co-solvent consisting of a polar organic solvent which modifies the solvent power in considerable manner especially in respect of molecules having some extent of polarity, ethanol being frequently used for this purpose.

It is to be recalled that bodies are generally known in three states: solid, liquid and gas. The changeover from one state to another is achieved by causing the temperature and/or pressure to vary. There exists a point, however, beyond which it is possible to change from the liquid state to the gas or vapour state without boiling, or conversely without condensation, but in continuous manner, this point being called the critical point.

One of the chief advantages of methods using solvents under supercritical pressure lies in the ease of separation between the solvent (the fluid) and the extracts and solutes, as described in numerous

publications, such as French patent published under n° 2 584 618 for example. Moreover, the interesting properties of these fluids have long been used for solid-fluid extraction and liquid-fluid fractionation as
5 described in the above-cited article.

The method of the invention is particularly adapted to fractionating natural or synthetic raw materials made up of several constituents and containing at least one component of amphiphilic type, from which it is desired to
10 extract one or more constituents in purified form. This purpose is achieved by means of a method for fractionating a natural or synthetic raw material made up of several constituents and containing at least one component of amphiphilic type, characterized in that said fractionation
15 is conducted using a solvent under supercritical pressure on a dispersion of said raw material in a liquid that is immiscible or nearly immiscible in the solvent under supercritical pressure.

The preferred raw materials for which the method of
20 the invention is particularly well suited are those containing at least one constituent which, in the purified state, is a semi-solid, paste or even a solid, and whose viscous nature prevents their treatment in a column.

The amphiphilic component may be added to the
25 starting raw material to be fractionated, or it may be one of its constituents as is the case for a raw material made up of a mixture of lipids.

By dispersion is meant both an emulsion and a suspension of the material in a liquid that is immiscible
30 or nearly immiscible in the solvent under supercritical pressure.

The method of the invention is therefore particularly advantageous for fractionating, extracting or isolating one or more constituents of the initial raw material. More particularly it consists of conducting the following steps:

a) a dispersion containing said raw material is prepared in a liquid that is immiscible or nearly immiscible in the solvent under supercritical pressure.

b) said dispersion is subjected to extraction using a solvent under supercritical pressure,

c) after said extraction, two fractions are collected of which one is enriched in at least one of the constituents of the raw material,

d) optionally, the extraction is repeated on at least one of the fractions collected during the previous extraction, a sufficient number of times to obtain one of the constituents of the raw material substantially purified in a fraction.

After the first extraction of the method of the invention a raffinate and an extract are collected and, in the case of a raw material made up of a mixture of lipids, preference is given to using the raffinate obtained after the preceding extraction to conduct further extractions.

The method of the invention applies more particularly to the fractionation of lipids of natural origin for the purpose of obtaining different polar or neutral components in the purified state.

Polar lipids are known for having physicochemical properties that are particularly suitable for preparing dispersions - in water, in an organic solvent or in a mixture of water and organic phase(s) - which enter into

the composition of cosmetic, dermatological or pharmaceutical products. These polar lipids are indeed remarkable surfactants for stabilizing water-oil interfaces, and they are involved in the constitution of living cell membranes. At the present time, it is extremely difficult to obtain most polar lipids in the pure state on a scale permitting industrial applications. Therefore, with a few exceptions, these products are only available as costly, laboratory reagents. On the other hand, mixtures rich in polar lipids are available on a large scale, such as lecithins widely used in the food industry and in health foods. It therefore appears of interest to fractionate these mixtures so as to obtain some of their components in the purified state for their subsequent use in applications with high added value, since their exceptional primary properties such as their viral safety on account of their plant origin are difficult to find in other classes of products, even synthetic products.

Neutral lipids are also useful in the area of cosmetics but in addition, like polar lipids, in the area of food-processing. Therefore the method of the invention is of especial interest for stripping food oils to remove the polar lipids from the oil. Polar lipids are likely to limit the organoleptic and technical properties of these oils.

Therefore, the method of the invention more particularly concerns a raw material chiefly containing a mixture of lipids, such as an oil extracted from cereals, from corn for example, corn gluten, barley, oats, millet, rice etc..

More particularly, the method of the invention consists of fractionating the mixture of lipids in relation to the polarity of its constituents. Therefore, with the method of the invention it is possible, after one
5 or more extractions, to obtain one or more fractions highly rich in some polar lipids belonging to the following different classes: the sphingolipids including ceramides and cerebrosides, the glycolipids including monogalactosyldiglycerides and digalactosyldiglycerides,
10 the phospholipids and among the latter phosphatidylcholines, phosphatidyl-ethanolamines, acid phospholipids in particular and their derivatives.

Advantageously, dispersion is conducted in water or in an aqueous solution of water-soluble organic solvents,
15 such as alcohols and preferably ethanol, ketones and preferably acetone, esters and preferably ethyl acetate.

According to one preferred embodiment of the method of the invention, as solvent under supercritical pressure for at least one of the extractions, carbon dioxide is
20 used either pure or in a mixture with different co-solvents chosen from among the light hydrocarbons having 2 to 8 carbon atoms, alcohols and preferably ethanol, esters preferably ethyl acetate, halogenated hydrocarbons and preferably
fluorine-containing hydrocarbons.
25 Advantageously, at last one of the extractions is therefore conducted with carbon dioxide either pure or in a mixture with one or more of the above-mentioned co-solvents, at a pressure lying between 7.4 and 50 MPa, preferably between 10 and 40 MPa, and at a temperature of
30 between 0 and 80°C.

According to another preferred embodiment of the method of the invention, as solvent under supercritical pressure for at least one of the extractions, a hydrocarbon is used with 2 to 5 carbon atoms, preferably with 3 or 4 carbon atoms. For fractionating a mixture of lipids this second type of solvent proves to perform particularly well during the first separation phase of the neutral lipids from the polar lipids. Consequently, the first extraction in step (b) is advantageously conducted with a hydrocarbon having between 2 and 5 carbon atoms, favourably 3 or 4 carbon atoms, at a pressure lying between 4.2 and 20 MPa favourably between 5 and 15 MPa, and at a temperature of between 0 and 80°C.

The method of the invention may be implemented using a discontinuous fractionating system using successive samples, or a continuous system on a column and in this latter case advantageously on a column in countercurrent operation. A fractionating method on a column with countercurrent operation is also called "fluid-liquid or liquid-liquid countercurrent fractionation" in the prior art. The uses and applications of countercurrent fractionation are widely described in the prior art (G. BRUNNER "Gas Extraction", chapter 8, published in 1994, Ed. Springer, ISBN 0-387-91477-3), in particular for lipids (Supercritical Fluid Technology in Oil and Lipid Chemistry, 1996, published by J.W. King and G.R. LIST, ISBN 0-935315-71-3). It is helpful to recall that lipids, soluble in organic solvents and insoluble in aqueous solvents, have varying solubility in solvents under supercritical pressure depending upon the polarity of the molecules concerned and the type of solvents used.

Therefore, pure carbon dioxide is used as solvent for triglycerides and is used to extract oils from different natural sources such as oleaginous grains (British patents n° 1 356 749 and n° 1 356 750; US patent 3 939 281). It is also known that this solvent does not extract polar lipids such as lecithins present in these same grains. This selectivity is, moreover, used for the extraction of lipids from egg yolk from which it is preferable not to extract the phospholipids whose surfactant properties contribute favourably to the organoleptic quality of foods containing this de-lipidated egg yolk, in the cake industry in particular. It is also known that it is possible to obtain a lecithin totally rid of neutral lipids from oils of plant origin extracted using the conventional hexane method. Nevertheless, lipid fractionating operations using solvents under supercritical pressure often come up against serious implementation difficulties, since the initial or resulting phases are often paste-like, making contact with the solvent fluid difficult even impossible to conduct. Some systems have been described to treat this problem, such as the jet extractor system put forward by Eggers E. and Wagner H., ("Proceedings of the Third International Symposium on Supercritical Fluids", ISBN 2-905267-2368), 1994, Volume 2, p. 125-130) to de-oil a soy lecithin. However, it appears that this system corresponds to a theoretical level and can only validly be used to conduct separations for which the separation factor is high. On the other hand, this system cannot be used to conduct the fractionating of closely similar components requiring a high number of theoretical plates generally used on multi-

stage countercurrent columns with perforated plates or packing, the use of this equipment not being possible with products having very high viscosity, like some lipid mixtures, or likely to crystallize or solidify, leading to clogging of the columns.

For the fractionation of a mixture of lipids, particular preference is therefore given to using a solvent under supercritical pressure on a column with countercurrent operation and extract reflux, so that the fraction obtained at the head of the column after separation of the solvent, generally called extract, is considerably enriched in the least polar lipids relative to the starting raw material. The fraction collected at the foot of the column, generally called raffinate, is in the form of a dispersion in a liquid and is considerably enriched in the most polar lipids. The cut-off threshold of this enrichment is determined by operating conditions. For example, a first treatment of a mixture of neutral and polar lipids according to this embodiment makes it possible to obtain an extract considerably enriched in neutral lipids at the head of the column and a raffinate considerably enriched in polar lipids. This first step is frequently called de-oiling. For the refining of raw lecithins, it provides a raffinate in the form of a dispersion of polar lipids. This first raffinate may be treated directly using the same method, under operating conditions defining a higher fractionation threshold in terms of polarity. Therefore the treatment of this first raffinate according to steps (b) and (c) of the method of the present invention makes it possible to obtain an extract enriched in the least polar lipids and a new

raffinate enriched in the most polar lipids. This second raffinate is in the form of a dispersion of polar lipids and may in turn be treated according to steps (b) and (c) of the method of the invention under new operating
5 conditions defining a higher cut-off threshold than the previous threshold in terms of polarity.

This sequence in treatment consisting of repeating steps (b) and (c) may be conducted directly on each new raffinate so as to achieve fine fractionating of the raw
10 material.

An example of said method according to the invention consists of conducting steps (b) and (c) on a column with countercurrent operation and extract reflux, using a solvent under supercritical pressure made up of carbon
15 dioxide mixed with ethanol at a concentration of between 1 and 5 % by weight, brought to a pressure of between 7.4 and 50 MPa, preferably between 15 and 30 MPa, at a temperature of between 32 and 80°C, so that the fractionation of a sample no longer containing any neutral
20 lipids leads to obtaining a fraction at the head of the column, after separation of the solvent, which is highly enriched in sphingolipids, including ceramides and cerebroside, and in monogalactosyldiglycerides relative to the sample. Steps (b) and (c) are repeated on a
25 countercurrent column with extract reflux, using a solvent under supercritical pressure made up of carbon dioxide mixed with ethanol at a concentration of between 3 and 8 % by weight, at a pressure of between 7.4 and 50 MPa, preferably between 12 and 30 MPa, at a temperature of
30 between 32 and 80°C, so that the fractionation of a sample formed by the previously obtained raffinate leads to

obtaining a fraction at the head of the column after separation of the solvent, that is highly enriched in digalactosyldiglycerides relative to the sample.

Another method for fractionating lipids has recently
5 been described in American patent US 5 759 549, consisting
of adsorbing the mixture to be fractionated on a porous
solid from which the different components are successively
extracted by a solvent under supercritical pressure whose
solvent power and polarity are successively increased.
10 This method, whose concept has been used for several years
by several authors, is known under the name "extrography"
designating the combination of extraction and
chromatography. This method may be conducted with high
selectivity, by combining the selectivity of the adsorbing
15 solid and of the solvent under supercritical pressure, but
requires complex means and can only be implemented in
discontinuous mode with successive samples, leading to
very high operating costs.

Another embodiment of use of the method of the
20 invention on a mixture of lipids therefore consists of
conducting the fractionation in discontinuous manner in a
contactor formed of a container under pressure, optionally
provided with packing intended to improve contact quality
between the two phases of the mixture to be fractionated
25 initially placed in emulsion in step (a), using a solvent
under supercritical pressure whose solvent power is to be
modified over several successive steps so as successively
to extract the different families of lipids in increasing
order of polarity. This operation is made possible by the
30 fact that the lipids not yet extracted do not precipitate
in paste form as would be the case if the initial mixture

was treated as such, but remain in dispersion in a liquid phase in which the solvent fluid can be caused to percolate under good conditions for the transfer of material between phases. In addition, at the end of the operation, contactor emptying is problem-free since the raffinate is in the form of a liquid phase having low viscosity.

Under one particularly advantageous embodiment, the fractionating method is conducted in continuous manner. The method of the invention in continuous mode applied to a mixture of lipids comprises the following steps:

- an emulsion containing the mixture of lipids to be fractionated is prepared in water;
- the emulsion is treated on a column with countercurrent operation and extract reflux, using carbon dioxide either pure or with an added co-solvent under supercritical pressure, under conditions of pressure and temperature such that the fraction obtained at the head of the column after separation of the solvent, generally called the extract, is considerably enriched in neutral lipids, such as triglycerides and sterols, and low in polar components which are practically all collected at the foot of the column in the fraction called the raffinate in the form of a dispersion in water, or a water-cosolvent mixture.

- the raffinate is fractionated on a column with countercurrent operation and extract reflux, using carbon dioxide with an added polar co-solvent under supercritical pressure as solvent under conditions of pressure and temperature such that the fraction obtained at the head of the column after separation of the solvent is considerably

enriched in the least polar components, such as the sphingolipids, including ceramides and monogalactosyldiglycerides (MGDG), and low in the most polar components such as the glycosphingolipids and the digalactosyldiglycerides (DGDG) and phospholipids which are all collected at the foot of the column in the form of a dispersion in an aqueous solution of the polar co-solvent used,

- the raffinate obtained during the previous step is fractionated on a column with countercurrent operation and extract reflux, using carbon dioxide as solvent under supercritical pressure to which a polar co-solvent is added advantageously chosen to be identical to the one used in the previous step, under conditions of pressure and temperature such that the fraction obtained at the head of the column after separation of the solvent is considerably enriched in components of average polarity such as digalactosyldiglycerides (DGDG) and low in the most polar components such as phospholipids which are all collected at the foot of the column in the form of a dispersion in an aqueous solution of the polar co-solvent used,

- the fractionation of the raffinate obtained during the previous step is repeated several times on the same type of equipment, but increasing the polarity of the solvent fluid each time by varying its pressure, temperature and co-solvent content, so as to successively separate each of the families of phospholipids and their derivatives, among which may be cited: phosphatidyl-ethanolamine, phosphatidyl-choline, acid phospholipids.

The invention therefore also relates to a purification method for polar lipids starting from a natural or synthetic material chiefly made up of a mixture of lipids, characterized in that said material is
 5 fractionated by an above-described method.

Other advantages and characteristics of the invention will become apparent from the following examples given by way of illustration which refer to the appended drawings in which:

- 10 - figure 1 shows a contactor for implementing the fractionation method of the invention,
- figure 2 shows a column for implementing the fractionating method of the invention.

15 Example 1: Materials

1) Oils

The lipid mixtures used are oils extracted from corn gluten by conventional methods, whose weight compositions
 20 obtained by liquid phase chromatography are the following:

OIL 1

Tryglycerides: 38 %

Sterols: 4 %

Sphingolipids 8.5 %

25 Monogalactosyldiglycerides (MGDG): 10.2 %

Digalactosyldiglycerides (DGDG): 25.5 %

Phospholipids: 13.8 %

Including: Phosphatidyl-ethanolamine: 5.1 %

 Phosphatidyl-choline: 5.4 %

30 OIL 2

Neutral lipids: 49 %

Sphingolipids: 9 %

MGDG: 12 %

DGDG: 30 %

The oil is placed in emulsion in a mixture of water
5 and ethanol under mechanical stirring, with a weight
composition of: 84% water, 10 % oil and 6 % ethanol.

2) Equipment

Two items of equipment were used: a contactor
10 operating in discontinuous mode and a column operating in
continuous mode. The equipment shown in figure 1 is
chiefly formed of a cylindrical contactor 1, having a
volume of 0.5 litre filled with packing 2 of Intalox type
having a nominal size of 10mm, through which a solvent
15 under supercritical pressure is able to flow over a
temperature range of 20 to 80°C and up to a pressure of 30
MPa. The solvent is then depressurised in a valve 3 and
the extract is separated in separators 4, 5, 6 such as
those described in French patent n° 2 584 618.

20 The equipment shown in figure 2 is chiefly formed of
a fractionating column 1 having a diameter of 58 mm and
height of 4 m filled with 10 mm Intalox-type packing in
stainless steel, fitted with a double casing divided into
four superimposed sections allowing the circulation of
25 coolants at different temperatures to obtain a temperature
gradient that will induce an internal extract reflux as is
known in the prior art. The solvent fluid is made up of
carbon dioxide, compressed to the liquid state by a
membrane volumetric pump 2 having an adjustable flow rate
30 of between 10 and 60 kg/h, to which a liquid co-solvent
may be added, butane for example supplied by a

commercially available reservoir and added by means of another pump 3 of similar type whose flow rate is adjustable between 2 and 10 kg/h. The solvent thus compressed to the desired pressure is heated in a heat exchanger 4 formed of a double tube whose outer tube contains a flow of hot water at suitable temperature. The sample to be treated, previously brought to sufficient temperature to enable its pumping, is injected between two stages of the column via a volumetric membrane pump 5 with an adjustable flow rate of between 2 and 10 kg/h. It is also possible to directly inject the liquid co-solvent in the column, favourably between the first and second stages. The raffinate leaves via the foot of the column and is decompressed to atmospheric pressure via an airlock system formed of two successive containers 6, 7 in which depressurisation takes place to pressures lower than the pressure prevailing inside column 1, enabling degassing of the liquid and partial recycling of the solvent vaporized from the container 6. The solvent containing the extract leaves via the head of the column and is decompressed via a discharge-type valve 8, the drop in pressure leading to de-mixing of the mixture which enters a group of separators 9, 10, 11 which according to the system described in French patent FR 2 584 618, cited above, is formed of cyclone chambers assuring total separation of the liquid phase and the gas phase with heat contributed via the walls of the separators whose double-casing contains a flow of hot water providing the required enthalpy to ensure vaporization of the solvent; the liquid phase is drawn off at atmospheric pressure via an airlock system 12, 13, 14 which operates according to the system

described in above-cited French patent FR 2 584 618. Thus
rid of the extract and part of the co-solvent, the solvent
is liquefied in a dual-tube condenser 15 whose outer tube
contains a flow of a water-ethylene glycol mixture cooled
5 to around 0°C and stored in the liquid state at around 5°C
in a reservoir 16, whose level is maintained stable
through the additional supply of carbon dioxide from an
outside tank.

10 3) HPLC analysis of polar lipids

- Stationary phase, non-grafted silica Nucleosil
100-5, 2 cartridges 125x2 mm coupled, preceded by a
precolumn filled with the same medium (8x3mm) (Macherey-
Nagel, Hoechst, Düren, Germany),
- 15 - Column oven, temperature 30°C
- P10000XR quaternary gradient pump, with membrane
degasser under partial pressure (Thermo Separation
Products, San Jose, California, USA),
- Kontron 360 autosampler fitted with a 5 µl
20 injection loop (Kontron Instruments, Milan, Italy),
- Cunow DDL II evaporator detector with light
diffusion (Eurosep, Cergy, France) temperature of
evaporator tube 35°C and nitrogen pressure of 1 bar.

After evaporation of the solvents and dilution in a
25 1/1 v/v chloroform/methanol mixture, the extracts are
analysed with the following gradient at a flow rate of 0.4
ml/min as indicated in table 1 below.

Table 1

Time (min)	Chloroform	Chloroform/ Methanol 60/40	Chloroform/ Methanol/Water 60/34/6
0	100	0	0
1	100	0	0
11	00	100	0
27	0	0	100
31	0	0	100
32	0	100	0
35	0	100	0
36	100	0	0
50	100	0	0

Example 2: Oil treatment on a contactor in discontinuous mode

5

1) Extraction of the neutral lipids from oil 1

Oil 1 is dispersed in emulsion form in a water/ethanol mixture to water/oil/ethanol proportions of 84/10/6.

10 50 g of this emulsion are added to the contactor.

Extraction is conducted with a solvent made up of carbon dioxide with added 10 % heptane, at a pressure of 250 bar and a temperature of 60°C.

15 The separators are maintained at a pressure of 50 bar and a temperature of 50°C.

The solvent flow rate is 2 kg/h. After an extraction time of two hours, an extract in heptane is collected. After evaporation of the solvent, 2 g of neutral lipids are recovered.

The contactor then contains a dispersed system of polar lipids containing 2.9 g polar lipids having the following lipid composition:

- Sphingolipids: 14.5 % including 2 % ceramides and
5 12.5 % glycosphingolipids,
- MGDG: 18.5 %
- DGDG: 45 %
- Phospholipids: 22 %

This stable suspension can be perfectly well drawn
10 off from the contactor in this state. Successive extractions may be conducted directly on this emulsion of polar lipids without re-sampling.

2) Extraction of neutral lipids from oil 2

15 Oil 2 is dispersed in emulsion form in a water/ethanol mixture to water/oil/ethanol proportions of 84/10/6.

50 g of this emulsion are added to the contactor.

Extraction is conducted with a solvent made up of
20 carbon dioxide with added 10 % heptane, at a pressure of 250 bar and a temperature of 60°C.

The separators are maintained at a pressure of 50 bar and a temperature of 50°C.

The solvent flow rate is 2 kg/h. After an extraction
25 time of two hours, an extract in heptane is collected. After evaporation of the solvent, 2.3 g of neutral lipids are recovered.

The contactor then contains a dispersed system of polar lipids in a water/ethanol mixture containing 2.5 g
30 polar lipids having the following composition:

- Sphingolipids: 17.5 % including 2.5 % ceramides and 15 % glycosphingolipids,

- MGDG: 23.5 %

- DGDG: 59 %

5 This stable suspension can be perfectly well drawn off in this state from the contactor. Successive extractions can be made directly on this emulsion of polar lipids without re-sampling.

10 3) Obtaining a fraction rich in sphingolipids and MGDG.

Consecutive to the operation described above under (1) the de-oiled dispersion is subjected to extraction conducted with carbon dioxide with added 5 % ethanol, at a pressure of 250 bar and a temperature of 60°C.

15 The separators are maintained at a pressure of 50 bar and a temperature of 50°C.

The solvent flow rate is 2 kg/h. After an extraction time of three hours, an extract in ethanol is collected. After evaporation of the solvent, 0.6 g of polar lipids are recovered having the following composition:

- Sphingolipids (ceramides): 12 %

- Monogalactosyldiglycerides: 88 %

25 The contactor then contains a stable suspension in a water/ethanol mixture containing 2.2 g polar lipids having the following composition:

Sphingolipids (glycosphingolipids): 16 %

DGDG: 57 %

Phospholipids: 27 %

30 4) Obtaining a fraction rich in glycolipids

The raffinate dispersion obtained above under (3) is subjected to extraction conducted with carbon dioxide with added 12 % ethanol, at a pressure of 250 bar and a temperature of 60°C.

5 The separators are maintained at a pressure of 50 bar and a temperature of 50°C.

 The solvent flow rate is 2 kg/h. After an extraction time of three hours, an extract in ethanol is collected. After evaporation of the solvent, 1.6 g of polar lipids
10 are recovered having the following composition:

- Sphingolipids (glycosphingolipids): 22 %
- DGDG: 78 %

 The contactor then contains a stable dispersed system of polar lipids in a water/ethanol mixture containing 0.6 g
15 polar lipids with a phospholipid content of more than 98 %.

5) Obtaining a fraction rich in DGDG

 Consecutive to the operation described under (2)
20 above, the de-oiled raffinate dispersion is subjected to extraction conducted with carbon dioxide with added 8 % ethanol, at a pressure of 250 bar and a temperature 60°C.

 The separators are maintained at a pressure of 50 bar and a temperature of 50°C.

25 The solvent flow rate is 2 kg/h. After an extraction time of three hours, an extract in ethanol is collected. After evaporation of the solvent, 1 g of polar lipids is recovered having the following composition:

- Sphingolipids: 43 %
- 30 - Monogalactosyldiglycerides: 51 %

The contactor then contains a dispersion of polar lipids in a water/ethanol mixture containing 1.5 g polar lipids with a DGDG content greater than 95 %.

5 Example 3 : Oil treatment in successive steps on a single column in continuous mode

 The oil, previously placed in emulsion as indicated above, is treated over several steps under conditions in which the polarity of the solvent fluid under
10 supercritical pressure is successively increased, as described above.

1) Extraction of neutral lipids

 Oil 1 is placed in emulsion in a water/ethanol
15 mixture to water/oil/ethanol proportions of 84/10/6.

 This emulsion is injected into the column between the third stage and the stage above at a flow rate of 1.2 kg/h. Sprinkling is conducted at the head of the column using water at a flow rate of 1.5 kg/h.

20 Extraction is conducted with a solvent made up of carbon dioxide with added 9.7 % ethanol, at 200 bar and 60°C, injected at the foot of the column at a flow rate of 28.4 kg/h.

 An equilibrium period of two hours is observed.

25 At the head of the column a lipid extract is collected whose neutral lipid content is more than 99 %.

 The raffinate is collected at the foot of the column in the form of a dispersion of polar lipids in a water/ethanol mixture, having the following lipid
30 composition:

- No neutral lipids (triglycerides and sterols),

- Sphingolipids: 14.5 % including 2 % ceramides,
12.5 % glycosphingolipids,
- MGDG: 18.5 %
- DGDG: 45 %
- 5 - Phospholipids: 22 %

2) Extraction of neutral lipids

Oil 1 is placed in emulsion in a water/ethanol mixture to water/oil/ethanol proportions of 84/10/6.

10 This emulsion is injected at the head of the column at a flow rate of 2 kg/h.

Extraction is conducted at 200 bar and 60°C with a solvent made up of carbon dioxide injected at the foot of the column at a flow rate of 23 kg/h and ethanol injected
15 between the first and second stages of the column at a flow rate of 2.4 kg/h.

An equilibrium period of two hours is observed.

A lipid extract is collected at the head of the column whose neutral lipid content is greater than 99 %
20 after evaporation of the solvent.

The raffinate is collected at the foot of the column in the form of a dispersed system, having the following lipid composition:

- No neutral lipids (triglycerides and sterols)
- 25 - Sphingolipids: 14.5 % including 2 % ceramides and
12.5 % glycosphingolipids
- MGDG: 1.5 %
- DGDG: 45 %
- Phospholipids: 22 %

30

3) Extraction of neutral lipids

Oil 2 is placed in emulsion in a water/ethanol mixture to water/oil/ethanol proportions of 84/10/6.

This emulsion is injected at the head of the column at a flow rate of 2 kg/h.

5 Extraction is conducted at 200 bar and 60°C with a solvent made up of carbon dioxide injected at the foot of the column at a flow rate of 25 kg/h and ethanol injected between the first and second stages of the column at a flow rate of 2.1 kg/h.

10 An equilibrium period of two hours is observed.

A lipid extract is collected at the head of the column whose neutral lipid content is greater than 99 %.

The raffinate is collected at the foot of the column in the form of a dispersed system of polar lipids in a water/ethanol mixture, having the following lipid composition:

- No neutral lipids (triglycerides and sterols),
- Sphingolipids: 17.5 % including 2.5 % ceramides, 15 % glycosphingolipids,
- 20 - MGDG: 23.5 %
- DGDG: 59 %

4) Obtaining a fraction rich in sphingolipids and MGDG

25 The raffinate obtained above under (2) is injected at the head of the column at a flow rate of 1 kg/h.

Extraction is conducted at 250 bar and 60°C with a solvent made up of carbon dioxide injected at the foot of the column at a flow rate of 20 kg/h and ethanol injected between the first and second stages of the column at a flow rate of 3 kg/h.

30

After an equilibrium time of two hours, the extract collected at the head of the column is enriched in the least polar lipids of the sphingolipid and MGDG class having the following lipid composition:

- 5 - Sphingolipids (ceramides): 8 %
- MGDG: 82 %
- Glycosphingolipids: 6 %
- DGDG: 4 %

The raffinate is collected at the foot of the column in the form of a stable dispersion of polar lipids considerably enriched in the most polar lipids of the DGDG and phospholipid class in a water/ethanol mixture, having the following lipid composition:

- Glycosphingolipids: 13 %
- 15 - DGDG: 59 %
- Phospholipids: 28 %

5) Obtaining a fraction rich in glycolipids (DGDG).

The raffinate dispersion under (3) above is injected at the head of the column at a flow rate of 1 kg/h.

Extraction is conducted at 250 bar and 60°C with a solvent made up of carbon dioxide injected at the foot of the column at a flow rate of 20 kg/h and ethanol injected between the first and second stages of the column at a flow rate of 3 kg/h.

After an equilibrium time of two hours, the extract collected at the head of the column is enriched in the least polar lipids belonging to the sphingolipid and DGDG class, having the following lipid composition:

- 30 - Sphingolipids: 40 %
- MGDG: 54 %

- DGDG: 6 %

At the foot of the column a dispersion of polar lipids is collected in a water/ethanol mixture, having the following lipid composition:

5 - Glycosphingolipids: 4 %

- DGDG: 96 %

CLAIMS

1. Method for fractionating a natural or synthetic raw material made up of several constituents and containing at least one component of amphiphilic type, characterized in that said fractionation is conducted with a solvent under
5 supercritical pressure using a dispersion of said raw material in a liquid that is immiscible or nearly immiscible in the solvent under supercritical pressure.
2. Method according to claim 1, characterized in that:
10 a) a dispersion is prepared containing said raw material in a liquid that is immiscible or nearly immiscible in the solvent under supercritical pressure,
 b) said dispersion is subjected to extraction using a solvent under supercritical pressure,
15 c) after said extraction two fractions are collected, of which one is enriched in at least one of the constituents of the raw material,
 d) optionally, the extraction is repeated on at least one of the fractions collected after the previous
20 extraction, a sufficient number of times to obtain one of the constituents of the raw material substantially purified in a fraction.
3. Method according to claim 2, characterized in that
25 after each extraction a raffinate and an extract are collected, and in that the raffinate is subjected to a further extraction.

4. Method according to any of the preceding claims, characterized in that the natural or synthetic raw material made up of several constituents and containing at least one component of amphiphilic type, is chiefly formed
5 of a mixture of lipids such as an oil extracted from cereal, for example from corn, corn gluten, barley, oats, millet, rice, etc..
5. Method according to claim 4, characterized in that
10 fractionation is conducted in relation to the polarity of the constituents.
6. Method according to either claim 4 or 5, characterized in that after one or more extractions a
15 raffinate is collected enriched in polar lipids belonging to at least one of the following classes: the sphingolipids including ceramides and cerebrosides, the glycolipids including monogalactosyldiglycerides and digalactosyldiglycerides, the phospholipids and among
20 these in particular the phosphatidyl-cholines, phosphatidyl-ethanolamines, acid phospholipids and their derivatives.
7. Method according to any of the preceding claims,
25 characterized in that dispersion is conducted in water or in an aqueous solution of water-soluble organic solvents, such as alcohols and preferably ethanol, ketones and preferably acetone, esters and preferably ethyl acetate.
- 30 8. Method according to any of the preceding claims, characterized in that for at least one of the extractions,

as solvent under supercritical pressure, carbon dioxide is used either pure or in a mixture with different co-solvents chosen from among the light hydrocarbons containing between 2 and 8 carbon atoms, alcohols and preferably ethanol, ketones and preferably acetone, esters and preferably ethyl acetate, halogenated hydrocarbons and preferably fluorine-containing hydrocarbons.

9. Method according to any of claims 2 to 8, characterized in that for at least one of the extractions, as solvent under critical pressure, carbon dioxide is used either pure or in a mixture with one or more of the co-solvents, at a pressure of between 7.4 and 50 MPa, preferably between 10 and 40 MPa, and at a temperature of between 0 and 80°C.

10. Method according to any of claims 2 to 9, characterized in that for at least one of the extractions, as solvent under supercritical pressure, a hydrocarbon is used having between 2 and 5 carbon atoms, preferably 3 or 4 carbon atoms.

11. Method according to any of claims 2 to 10, characterized in that for at least one of the extractions, as solvent under supercritical pressure, a hydrocarbon is used having between 2 and 5 carbon atoms, preferably 3 or 4 carbon atoms, at a pressure of between 4.2 and 20 MPa, more favourably between 5 and 15 MPa, and at a temperature of between 0 and 80°C.

12. Method according to any of the preceding claims, characterized in that it is applied using a discontinuous fractionating system in successive samples or a continuous system on a column, and in the latter case advantageously
5 on a column with countercurrent operation.

13. Method according to any of claims 4 to 12, characterized in that several successive extractions are conducted on a column in continuous mode with
10 countercurrent operation and extract reflux, such that the fraction obtained at the head of the column after separation of the solvent is considerably enriched in the least polar lipids relative to the starting raw material, and in that the fraction collected at the foot of the
15 column is in the form of a dispersion in a liquid which is considerably enriched in the most polar lipids.

14. Method according to claim 13, characterized in that several successive extractions are made on a column with
20 countercurrent operation in continuous mode and extract reflux, using a solvent under supercritical pressure made up of carbon dioxide in a mixture with ethanol at a concentration of between 1 and 5 % by weight, brought to a pressure of between 7.4 and 50 MPa, preferably between 15
25 and 30 MPa, at a temperature of between 32 and 80°C such that the fractionating of a sample no longer containing any neutral lipids leads to obtaining a fraction at the head of the column after separation of the solvent which is highly enriched in sphingolipids, including ceramides
30 and cerebrosides, relative to the sample.

15. Method according to claim 14, characterized in that several successive extractions are conducted on a column operating continuously with countercurrent and extract reflux, using a solvent under supercritical pressure
5 formed of carbon dioxide in a mixture with ethanol at a concentration of between 3 and 8 % by weight, at a pressure of between 7.4 and 50 MPa, preferably between 12 and 30 MPa, at a temperature of between 32 and 80°C, such that fractionation leads to obtaining a fraction at the
10 head of the column after separation of the solvent which is highly enriched in digalactosyldiglycerides relative to the sample.

16. Method according to any of claims 4 to 12,
15 characterized in that several successive extractions are conducted discontinuously in a contactor formed of a container under pressure optionally fitted with packing intend to improve contact quality between the two phases of the mixture to be fractionated initially placed in
20 dispersion, using a solvent under supercritical pressure whose solvent power is to be modified in several successive steps such as successively to extract the different families of lipids in increasing order of polarity.

25

17. Method according to any of claims 4 to 12, characterized in that it comprises the following steps:

- a dispersion is prepared in water containing the mixture of lipids to be fractionated,
- 30 - the emulsion is treated on a column with countercurrent operation and extract reflux, using carbon

dioxide either pure or with an added non-polar co-solvent, under supercritical pressure and under conditions of pressure and temperature such that the fraction obtained at the head of the column after separation of the solvent, generally called the extract, is considerably enriched in neutral lipids, such as triglycerides and sterols, and low in polar compounds which are practically all recovered at the foot of the column in the fraction called the raffinate in the form of an emulsion in water;

10 - the raffinate is fractionated on a column with countercurrent operation and extract reflux, using carbon dioxide as solvent under supercritical pressure, to which a polar co-solvent is added, under conditions of pressure and temperature such that the fraction obtained at the head of the column after separation of the solvent is considerably enriched in the least polar components, such as the sphingolipids, including ceramides and monogalactosyldiglycerides (MGDG), and low in the most polar components, such as the glycosphingolipids and digalactosyldiglycerides (DGDG) and phospholipids, which are all recovered at the foot of the column in the form of an emulsion in an aqueous solution of the polar co-solvent used;

25 - the raffinate, obtained during the previous step, is fractionated on a column with countercurrent operation and extract reflux, using carbon dioxide as solvent under supercritical pressure, to which a polar co-solvent is added advantageously chosen to be identical to the one used in the preceding step, under conditions of pressure and temperature such that the fraction obtained at the head of the column after separation of the solvent is

considerably enriched in components of average polarity, such as the digalactosyldiglycerides (DGDG) and low in the most polar components such as the phospholipids, which are all recovered at the foot of the column in the form of an emulsion in an aqueous solution of the polar co-solvent used,

- the fractionation of the raffinate obtained in the previous step is repeated several times on the same type of equipment but increasing the polarity of the solvent fluid each time by varying its pressure, temperature and co-solvent content, such as successively to separate each of the families of phospholipids and their derivatives, among which the following may be cited: phosphatidyl-ethanolamine, phosphatidyl-choline, acid phospholipids.

18) Method for purifying polar lipids from a natural or synthetic material chiefly made up of a mixture of lipids, characterized in that said material is fractionated using a method according to any of claims 4 to 17.

ABSTRACT

The invention concerns a method for fractionating a synthetic or natural material consisting of several constituents and containing at least an amphiphilic constituent. The invention is characterised in that said fractionating is carried out by means of a supercritical pressure solvent by dispersion of said material inside an insoluble or hardly soluble liquid in the supercritical pressure solvent. The inventive method is advantageously carried out for a mixture of lipids such as, for example an oil extracted from a cereal.

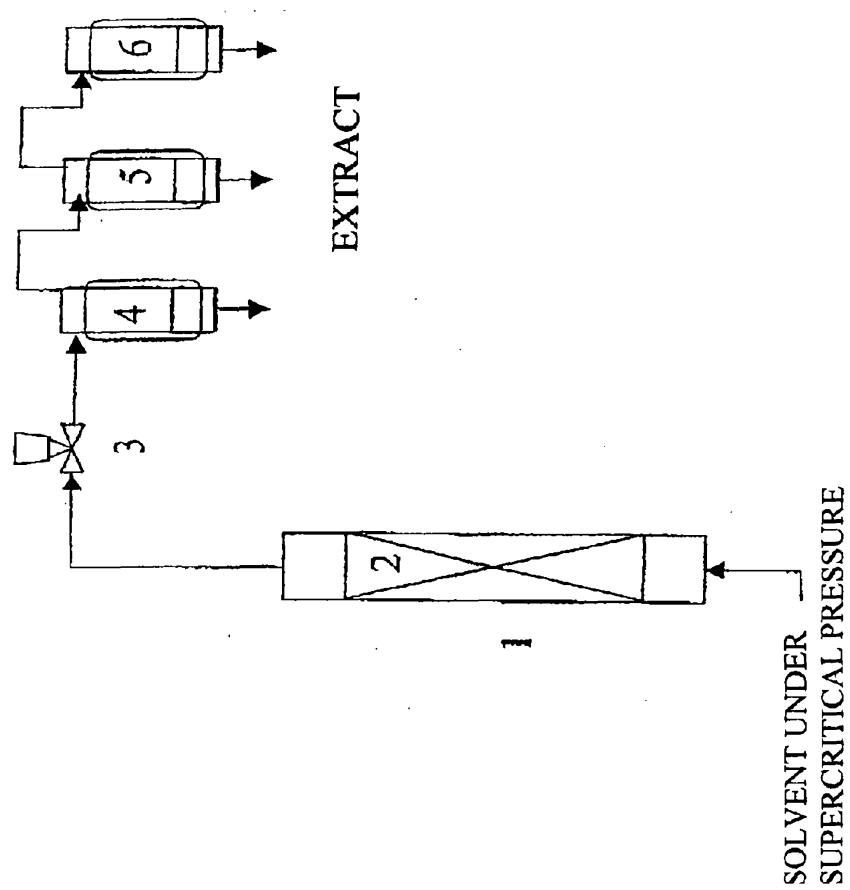


FIG. 1

2/2

