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(54) EXTRACTION PROCESS

(71) We, RHONE-POULENC INDUSTRIES, a French body corporate, of 22, avenue Montaigne, 75 Paris (8), France, do hereby declare the invention for which we pray that a patent may be granted to us and the method by which it is to be performed to be particularly described in and by the following statement:—

The invention relates to the extraction of proteins from milk. The treatment of skimmed milk generally consists of firstly extracting casein by acid or enzymatic coagulation, then extracting the proteins from the milk serum by thermal coagulation, ultrafiltration or ion exchange, and finally separating the lactose, which may be hydrolysed.

However, this treatment has disadvantages. The separated casein is in the form of a precipitate, is partly degraded and may contain other entrained proteins. The other proteins extracted by thermal coagulation lose some of their biological properties and this also applies in the case of ultrafiltration because the length of the operation generally requires pasteurisation of the milk serum. It is difficult to perform ion-exchange separation industrially because the known ion exchangers based on cellulose or dextran have poor mechanical properties. Ion exchanges for the separation of proteins from milk serum that have better mechanical properties are described in the specification of our copending Application No. 35774/76. Serial No. 1513195 However, the casein is still in the precipitated form, is partly degraded and may contain entrained proteins.

The present invention is based on the discovery of an extractive distillation process for milk, which alleviates these disadvantages and makes it possible to obtain on an industrial scale pure proteins in the natural state and having all their biological properties.

The process of the present invention comprises extracting all the proteins from the skimmed milk leaving a solution of mineral salts and lactose by extracting the proteins other than casein by contacting the skimmed milk successively with at least one anion-exchange resin and then silica, or successively with silica and then at least one anion-exchange resin, fixation of the proteins and elution, the casein that has remained in solution then being separated from the mineral salts and the lactose, in which the or each anion-exchange resin is made up of an alumina or silica support coated with less than 20 mg/m² of a cross-linked polymer film containing or carrying functional groups, and the silica and any support of an anion-exchange resin have a grain size between 4 μm and 5 mm, a specific surface of approximately 5 to 150 m²/g, a pore volume of 0.4 to 2 ml/g and a pore diameter between 250 and 2500 Å.

When reference is made to proteins other than casein or proteins of milk serum, this is understood to mean lactalbumins, serum albumin, lactoglobulins and immunoglobulins.

The anion-exchange resins preferably have an exchange capacity below 2 meq./g. The functional groups are usually tertiary amine or quaternary ammonium salts of general formula

$$—CH_2—N(R)—CH_2—$$

or

$$—CH_2—N^{(\pm)}(R)_3X^{(-)}$$

in which each R, which is the same as or different from the others, represents an alkyl or hydroxyalkyl radical having 1 to 4 carbon atoms and X is an inorganic or organic anion, for example, chloride, sulphate, nitrate, phosphate or citrate.

The cross-linked polymers coating the surface of the supports are in themselves known products obtained from monomers such as (a) epoxy compounds, which cross-

link with polyamines as catalysts; (b) formaldehyde, which cross-links by polycondensation with urea, melamine, polyamines or phenols; or (c) vinyl monomers such as vinylpyridine, styrene and their derivatives, which cross-link with polyfunctional monomers such as diacrylates or dimethacrylates of mono- or poly-alkylene glycols, divinylbenzene, vinyltrialkoxysilanes, vinyltrihalosilanes and bis-methylene acrylamide, in the presence of an initiator or ultraviolet light.

The mineral support is coated with the cross-linked polymer by impregnating the support with a solution of the monomer or monomers and optionally the initiator in a solvent, which is then evaporated, and cross-linking the monomers by known processes. The solvent can be any product that dissolves the monomers and the initiator and whose boiling point is preferably as low as possible in order to aid its subsequent evaporation. Examples of suitable solvents are methylene chloride, diethyl ether, benzene, acetone and ethyl acetate.

In the case where the polymer cross-linked on the surface of the support does not have functional groups in its chain, it must be modified. This is particularly the case with cross-linked polymers based on styrene and its derivatives and with polymers of formaldehyde with urea, melamine, polyamines or phenols.

In the case of polymers of styrene or phenol-formaldehyde this modification consists of fixing to the polymer chloromethyl groups and reacting them with a secondary or tertiary amine in known manner. In order to fix the chloro-methyl groups to the polymer, it is advantageous in the case of styrene polymers to disperse the polymer-coated mineral support in chloromethyl methyl ether in the hot state in the presence of a Lewis acid. However, in the case of a phenol-formaldehyde resin, it is possible to disperse the polymer-coated mineral support in epichlorohydrin and then react it hot.

This modification in the case of polymers of formaldehyde with polyamines, urea and melamine comprises transforming the primary amines present in the chain into tertiary amines or quaternary ammonium salts according to any known process, for example reaction with an alkyl sulphate or halide.

Contacting of the skimmed milk with the anion-exchange resin or resins and the silica takes place without any change in the pH of the milk at temperatures of 0 to 50°C, preferably 0 to 15°C.

The quantity of anion-exchange resins is preferably 5 to 15 g. per gram of all the proteins to be extracted and the quantity of silica is preferably 2 to 7 g. per gram of all the proteins to be extracted.

The proteins retained by the anion-exchange resin or resins are β-lactoglobulins, α-lactalbumins, serum-albumin and a small quantity of immunoglobulins, since silica fixes most of the immunoglobulins.

The separation of the proteins from the resin or resins and the silica is obtained by elution, either with a solution of high ionic strength, e.g. a molar solution of sodium chloride or ammonium carbonate, or with a solution of acid pH for the anion-exchange resin(s) and basic pH for the silica. The solution of acid pH is an organic or mineral acid solution, for example, hydrochloric, acetic, nitric, sulphuric or lactic acid and the solution of basic pH is a solution of an alkali metal hydroxide such as sodium hydroxide or potassium hydroxide, or ammonium hydroxide.

In order to obtain a more selective separation, it is possible to treat the milk successively with several like or different anion-exchange resins either before or after the silica treatment. Thus, in the case of two anion-exchange resins the eluted solution of the first resin is very rich in β-lactoglobulins while the eluted solution of the second resin contains α-lactalbumins and serum-albumin and very small quantities of β-lactoglobulins and immunoglobulins.

Extraction of the proteins from the skimmed milk can be performed with identical results discontinuously, semi-continuously in columns or continuously with groups or columns. Continuous operations are particularly suitable for industrial purposes, the resins permitting of an easy filling of the column, a high rate of flow and easy elution.

The solutions of proteins obtained contain only traces of lactose and mineral salts. They may be used as they are or the proteins can be separated by any known process and more particularly by atomisation.

After extraction by the anion-exchange resin or resins and the silica, the remaining solution contains casein, lactose and mineral salts but no longer any other proteins. The casein may be extracted by exclusion chromatography or more particularly by ultra-filtration using any suitable known process. Extraction can be performed continuously or discontinuously.

A solution of natural casein in water whose concentration is a function of the extraction process is thus obtained, together with a solution of lactose and mineral salts.

The solution of uncoagulated natural casein contains only traces of lactose and can be used as it is, or the casein can be

separated from the solution, particularly by atomisation.

According to a modification of the process according to the invention, the lactose and mineral salts are separated from the skimmed milk first, e.g. by exclusion chromatography or ultrafiltration, and the proteins other than casein are then extracted by contacting the milk successively with at least one anion-exchange resin and then silica, or successively with silica and then at least one anion-exchange resin, fixation of the proteins and then elution. The casein remains in solution.

The extraction of the proteins by the anion-exchange resin or resins and the silica is performed in the manner described hereinbefore.

After extracting the proteins other than casein, a solution of uncoagulated natural casein is obtained.

According to another modification, the process of the invention may be applied to milk serum, i.e. skimmed milk from which the casein has been removed. In this case the milk serum is successively contacted with at least one anion-exchange resin and then silica, or successively with silica and then at least one anion-exchange resin, followed by fixation of the proteins and elution.

The separation of proteins from milk serum by using anion-exchange resins and cation-exchange resins was described in the specification of our copending Application No. 35774/76. Serial No. 1513195. However, the use of silica in place of a cation-exchange resin permits of an easier elution and gives more concentrated protein solutions. Therefore during the drying of the proteins the quantity of water to be eliminated is smaller, resulting in a shorter treatment, which is less harmful to the proteins. In addition, silica is a simpler product than cation-exchange resins and is recognized for use in the foodstuffs industry.

The proteins are extracted from the milk serum by the anion-exchange resin or resins and silica in the manner described for milk except that the contacting of the milk serum with the anion-exchange resin or resins and the silica is performed at a pH above 4 and preferably between 5.5 and 7.5 at temperatures between 0 and 50°C and preferably between 0 and 30°C. After extracting the proteins, the remaining solution contains lactose and mineral salts but no proteins.

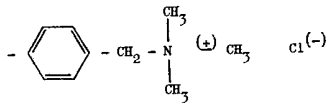
No matter what its origin, the lactose in solution may be chemically or enzymatically hydrolysed by any known process to obtain a solution of glucose and galactose.

The process of the invention can be used in the dairy industry for the preparation of proteins, including casein, and is particularly suitable for use in the foodstuffs, dietetic, pharmaceutical and veterinary industries.

The following examples illustrate the invention.

EXAMPLE 1

Into a first column with a diameter of 2.5 cm (herein called "column 1") is placed 20 g of an anion-exchange resin constituted by a silica having a grain size of 100 to 200 μm, a specific surface of 24 m²/g, an average pore diameter of 1400 Å and a pore volume of 1 ml/g, coated with 3.3 mg/m² of a styrene-vinyltriethoxysilane copolymer carrying functional groups of formula



This resin has the following characteristics:

Carbon content	4.8%
Chlorine content	2%
Nitrogen content	0.9%
Exchange capacity	0.6 meq./g

Into a second column with a diameter of 2.5 cm (herein called "column 2") are placed 10 g of silica spheres having a grain size of 100 to 200 μm, a specific surface of 25 m²/g, a pore diameter of 1400 Å and a pore volume of 1.1 ml/g.

After placing the two columns in series, the resin and silica are washed by passing 500 ml of water through them. 250 ml of skimmed milk containing 7 g of casein, 1.6 g of other proteins and 12 g of lactose is successively percolated through column 1 and then column 2 at a rate of 100 ml/h. The resin and silica from the two columns are then washed by passing 200 ml of water through them.

By passing an *N*/100 hydrochloric acid solution through column 1 the fixed proteins are eluted. 33 ml of solution containing 1.3 g of proteins is obtained. These proteins are α-lactalbumins, β-lactoglobulins, serum-albumin and a small amount of immunoglobulins.

By passing an N-ammonium carbonate solution through column 2 the fixed proteins are eluted and 12 ml of solution containing 0.3 g of immunoglobulins is obtained.

The two solutions of proteins contain less than 1% by weight of fatty substances and lactose. The electrophoretic migration of the proteins is the same as that for milk, so that they are not denatured.

The solutions from column 2 (milk and wash waters) contain no proteins other than

casein and are ultrafiltered. The material held back contains about 7 g of natural casein at a concentration of 20% by weight. The ultrafiltrate contains almost all the lactose at a concentration of about 42 g/l and the mineral salts.

EXAMPLE 2

Example 1 is repeated but the treatment of the skimmed milk with anion-exchange resin and silica is performed at 50°C.

The results are the same as those in Example 1.

EXAMPLE 3

Example 1 is repeated by placing in front of column 1 a column A with a diameter of 1 cm and containing 3 g of the same anion-exchange resin as used in column 1. By elution of column A with an N/100 HCl solution, 15 ml of a solution containing 0.3 g of proteins formed almost exclusively of β-lactoglobulins is obtained.

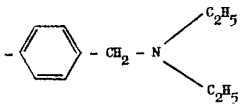
Elution with an *N*/100 HCl solution from column 1 gives 33 ml of a solution containing 1 g of proteins formed mainly of α-lactalbumins and serum albumin as well as a small proportion of β-lactoglobulins and immunoglobulins.

Elution of column 2 gives the same results as in Example 1.

EXAMPLE 4

In a column 1 with a diameter of 2.5 cm are placed 10 g of silica balls with a grain size of 100 to 200 μm, a specific surface of 25 m^2/g, a pore diameter of 1400 Å and a pore volume of 1.1 ml/g.

In a column 2 with a diameter of 2.5 cm is placed 15 g of an anion-exchange resin comprising a silica with a grain size of 100 to 200 μm, a specific surface of 24 m^2/g, an average pore diameter of 1400 Å and a pore volume of 1 ml/g, coated with 6 mg/m^2 of a styrene-vinyltriethoxysilane co-polymer carrying functional groups of formula



and having the following characteristics:

Carbon content	7.5%
Nitrogen content	1.5%
Exchange capacity	1.07 meq/g.

After placing the two columns in series, the silica and resin are washed by passing 500 ml of water through them.

250 ml of skimmed milk containing 7 g of casein, 1.6 g of other proteins and 12 g of lactose is percolated through at a rate of 100 ml/h successively into column 1 and then column 2. The silica and resin from the two columns are then washed by passing 200 ml of water through them.

By passing an *N*/100 ammonia solution through column 1, the fixed proteins undergo elution. 12 ml of solution containing 0.4 g of immunoglobulins, i.e. most of the latter, is obtained.

By passing an *N*/10 hydrochloric acid solution through column 2 the fixed proteins undergo elution. The proteins are α-lactalbumins, β-lactoglobulins, serum-albumin and traces of immunoglobulins. 23 ml of solution containing 1.2 g of these proteins is obtained.

The two solutions of the proteins contain less than 1% by weight of lactose. The electrophoretic migration of the proteins is the same as that with milk. Therefore they are not denatured.

The solution from column 2, i.e. approximately 260 ml, contains no proteins other than casein, and is percolated into a third column to separate the casein by exclusion chromatography. This column, which has a diameter of 3 cm, contains 450 g of silica with a grain size 100—200 μm, a specific surface of 400 m^2/g, an average pore diameter of 80 Å and a pore volume of 1 ml/g. It is eluted with water.

330 ml of a solution is obtained which contain almost all the natural casein, together with a solution containing the lactose and mineral salts.

EXAMPLE 5

Into a column 1 with a diameter of 2.5 cm is placed 15 g of the same anion-exchange resin as used in column 2 of Example 4.

Into a column 2 with a diameter of 2.5 cm are placed 10 g of silica balls having a grain size of 100 to 200 μm, a specific surface of 50 m^2/g, a pore diameter of 800 Å and a pore volume of 1.1 ml/g.

After placing the two columns in series the resin and silica are washed by passing 500 ml of water through them.

300 ml of milk serum with a pH of 6.5 and containing 1.5 g of proteins and 11 g of lactose is percolated through at ambient temperature successively into column 1 and then column 2, at a rate of 400 ml/h.

The resin and silica from the two columns are washed by passing 200 ml of water through them.

By passing a solution of *N*/100 hydrochloric acid through column 1, the fixed proteins are eluted and 33 ml of solution containing 1.25 g of proteins is obtained. These proteins are α-lactalbumins, β-lactoglobulins, serum-albumin and a small amount of immunoglobulins.

By passing an *N*/100 ammonia solution through column 2 the fixed proteins are eluted and 10 ml of solution containing 0.25

g of proteins constituted almost exclusively of immunoglobulins is obtained.

The two solutions of the proteins contain less than 1% by weight of fatty substances and lactose. The electrophoretic migration of the proteins is identical to that with the milk serum. They are therefore not denatured.

EXAMPLE 6

Example 5 is repeated but the treatment of the milk serum with anion-exchange resin and silica is performed at 50°C.

The results are identical to those of Example 5.

EXAMPLE 7

Example 5 is repeated by a column A with a diameter of 1 cm containing 3 g of the same anion-exchange resin as in column 1 is placed in front of the latter. By elution of column A with an *N*/100 HCl solution, 15 ml of a solution is obtained containing 0.3 g of proteins constituted almost exclusively of β-lactoglobulins.

Elution with an *N*/100 HCl solution of column 1 gives 33 ml of a solution containing 0.95 g of proteins constituted largely by α-lactalbumins and serum-albumin and a very small amount of β-lactoglobulins and immunoglobulins.

Elution of column 2 gives the same results as in Example 5.

EXAMPLE 8

Into a column 1 with a diameter of 2.5 cm are placed 10 g of silica balls with a grain size 100 to 200 μm, a specific surface of 25 m^2/g, a pore diameter of 1400 Å and a pore volume of 1.1 ml/g.

Into a column 2 with a diameter of 2.5 cm is placed 20 g of the same anion-exchange resin as used in column 1 of Example 1.

After placing the two columns in series the silica and resin are washed by passing 500 ml of water through them.

300 ml of milk serum of pH 6.5 containing 1.5 g of proteins and 11 g of lactose is percolated through at ambient temperature at a rate of 600 ml/h successively through column 1 and then column 2. The silica and resin from the two columns are then washed by passing 200 ml of water through them.

The fixed proteins are eluted by passing an *N*/100 ammonia solution through column 1. 12 ml of solution containing 0.4 g of immunoglobulins, i.e. most of the latter, is obtained.

By passing an *N*/10 sulphuric acid solution through column 2, the fixed proteins are eluted. These proteins consist of α-lactalbumins, β-lactoglobulins, serum-albumin and traces of immunoglobulins. 20 ml of solution containing 1.1 g of these proteins is obtained.

The two solutions of proteins contain less than 1% by weight of lactose. Electrophoretic migration of the proteins is the same as that with milk serum. They are therefore not denatured.

EXAMPLE 9

Into column 1 with a diameter of 2.5 cm is placed 20 g of the same anion-exchange resin as used in column 1 of Example 1.

Into a column 2 with a diameter of 2.5 cm are placed 10 g of silica balls with a grain size 100 to 200 μm, a specific surface of 25 m^2/g, a pore diameter of 1400 Å and a pore volume of 1.1 ml/g.

After placing the two columns in series the resin and silica are washed by passing 500 ml of water through them.

500 ml of milk serum adjusted to pH 7.5 by adding 0.1 *N* sodium hydroxide, filtered to eliminate insoluble substances and containing 2.5 g of proteins and 18 g of lactose, is percolated successively into column 1 and then column 2 at a rate of 300 ml/h at ambient temperature. The resin and silica from the two columns are washed by passing 100 ml of water through them.

By passing an *N*/100 hydrochloric acid solution through column 1 the fixed proteins are eluted. 35 ml of solution containing 2.05 g of proteins is obtained. These proteins are α-lactalbumins, β-lactoglobulins, serum-albumin and a small amount of immunoglobulins.

By passing a molar ammonium carbonate solution through column 2 the fixed proteins are eluted. 12 ml of solution containing 0.45 g of proteins constituted almost exclusively of immunoglobulins is obtained.

For comparison purposes, this example is repeated but the 10 g of silica in column 2 is replaced by 10 g of a cation exchange resin comprising a silica with a grain size 100 to 200 μm, a specific surface of 25 m^2/g, an average pore diameter of 1400 Å and a pore volume of 1.1 ml/g coated with 7.2 mg/m^2 of an acrylic acid -diethyleneglycol -dimethacrylate copolymer carrying —COOH functional groups.

This resin has the following characteristics:

Carbon content	10.55%
Exchange capacity	1.05 meq./g.

By eluting the first column the same results are obtained as in Example 5.

By eluting the second column, 17 ml of solution containing 0.45 g of proteins constituted almost exclusively of immunoglobulins is obtained.

It is obvious that the use of silica, a material that is much simpler than the cation-exchange resin, leads to a more

concentrated solution of the immunoglobulins:

With exchange resin 2.65 g/100 ml,

With silica 3.75 g/100 ml, i.e. 41.5% more proteins.

WHAT WE CLAIM IS:

1. A method of extracting proteins from milk, comprising extracting the proteins other than casein by contacting the skimmed milk successively with at least one anion-exchange resin and then silica, or successively with silica and then at least one anion-exchange resin, fixation of the proteins and elution, the casein that has remained in solution then being separated from the mineral salts and the lactose, in which the or each anion-exchange resin is made up of an alumina or silica support coated with less than 20 mg/m² of a cross-linked polymer film containing or carrying functional groups, and the silica and any support of an anion-exchange resin have a grain size between 4 μm and 5 mm, a specific surface of approximately 5 to 150 m²/g, a pore volume of 0.4 to 2 ml/g and a pore diameter between 250 and 2500 Å.

2. A method as claimed in Claim 1 in which the or each anion-exchange resin has an exchange capacity below 2 meq./g.

3. A method as claimed in Claim 2 in which the functional groups are tertiary amines or quaternary ammonium salts of general formula

$$-CH_2-\underset{\displaystyle R}{\overset{\displaystyle |}{N}}-CH_2-$$

or

$$-CH_2-N^{(+)}(R)_3X^{(-)},$$

in which each R, which is the same as or different from the others, represents an alkyl or hydroxyalkyl radical having 1 to 4 carbon atoms and X is a mineral or organic anion.

4. A method as claimed in Claim 2 or 3 in which the cross-linked polymer is obtained from one or more epoxy compounds cross-linked with polyamines as the catalyst; formaldehyde cross-linked by polycondensation with urea, melamine, polyamines or phenols; and one or more vinyl monomers linked with one or more polyfunctional monomers.

5. A method as claimed in Claim 4 in which the vinyl monomer(s) is/are vinylpyridine, styrene and/or a substituted styrene and the poly-functional monomer(s) is/are one or more diacrylates or dimethacrylates of mono- or poly-alkalene glycols, divinylbenzene, vinyl-trialkoxysilane, vinyltrihalogenosilane and/or bis methylene-acrylamide.

6. A method as claimed in any preceding claim in which contacting of the skimmed milk with the anion-exchange resins and the silica is performed at a temperature between 0 and 50°C.

7. A method as claimed in any preceding claim in which the quantity of anion-exchange resin or resins is 5 to 15 g per gram of proteins to be extracted and the quantity of silica is 2 to 7 g. per gram of proteins to be extracted.

8. A method as claimed in any one of Claims 1 to 7 in which the proteins held back by the resins and silica are eluted with a solution of high ionic strength.

9. A method as claimed in any one of Claims 1 to 7 in which the proteins held back by the resins and silica are eluted with a solution of acid pH for the anion-exchange resins and a solution of basic pH for the silica.

10. A method as claimed in any preceding claim in which the proteins are extracted discontinuously, semi-continuously or continuously.

11. A method as claimed in any preceding claim in which after extracting the proteins the natural casein is extracted by exclusion chromatography or ultrafiltration.

12. A modification of a method as claimed in any preceding claim in which the lactose and the mineral salts are separated from the skimmed milk by ultrafiltration or exclusion chromatography before the milk is treated with the anion-exchange resin(s) and the silica.

13. A modification of a method as claimed in any preceding claim in which milk serum is used instead of milk.

14. A method of extracting proteins from milk or milk serum, substantially as hereinbefore described in any one of the Examples.

15. Natural proteins, viz. casein, β-lactoglobulins, α-lactalbumins, serum-albumin and immunoglobulins and mixtures of them, obtained by a method as claimed in any one of Claims 1 to 14.

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