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(54) Title: DAIRY PRODUCT AND PROCESS

(57) Abstract: The present invention provides a method for the preparation of an alpha$_S$-casein fraction comprising: (a) providing a liquid sample of a dairy feedstock comprising casein as at least 40% by weight of the milk protein; (b) contacting the dairy feedstock with a cation exchanger loaded with sodium or potassium or both to exchange calcium for sodium or potassium or both; (c) adding calcium to the resulting calcium-depleted milk or milk protein concentrate; and (d) recovering the precipitated fraction. The invention also provides for a process for preparing a beta casein fraction having steps (a)-(c) as above and (d) removing the precipitated fraction and recovering the unprecipitated fraction. Among other uses the alpha$_S$-casein fraction may be used for controlling the texture of a cheese product.

DAIRY PRODUCT AND PROCESS

TECHNICAL FIELD

The invention relates to casein fractions and processes for their preparation. The invention also relates to dairy products enriched or depleted in particular casein fractions and processes for their preparation. The products are useful as agents for altering the properties of simulated cheese products and other dairy compositions. The fractions may also be used as a source of bioactive peptides or as a source of phosphopeptides for delivery of high levels of calcium.

BACKGROUND ART

Whole casein and caseinate consist of four primary proteins - alpha$_{s1}$-casein, alpha$_{s2}$-casein, beta-casein and kappa-casein. Each of these components, has a unique composition and structure.

The approximate content of the four proteins in casein/caseinate products (% by weight) is 36-40, 10-12, 33-40 and 10-12 for alpha$_{s1}$-, alpha$_{s2}$-, beta- and kappa-casein respectively.

Alpha$_{s1}$-casein and alpha$_{s2}$-casein are the products of two different genes. In this specification the term alpha$_s$-casein refers to a mixture of the two proteins.

Warner (J Am Chem Soc. 66, 1725-31, 1944) was the first to attempt the isolation of the alpha$_s$-casein by exploiting the solubility differences between the alpha$_s$- and beta-casein fractions. Caseinate solutions at 2°C and pH 6.5 were acidified to pH 4.9 prior to centrifuging to enable the precipitation of an alpha$_s$-casein enriched fraction. Since the precipitation neither allowed much yield nor significant purification, repeated extractions were necessary to improve product purity.

Hipp *et al* (J Dairy Res 35, 272-281, 1952) refined the method of Warner (1944) and performed the fractionation of whole casein by one of two different methods. In the first method a crude alpha$_s$-casein precipitate was obtained by acidifying (to pH 6.5) an ethanolic solution of whole casein (50% ethanol/0.4 M ammonium acetate). The precipitate was redissolved in weak ammonia, diluted with water to 6% protein and the solution made 50% in ethanol. Homogeneous purity alpha$_s$-casein was claimed to be recovered by acidifying the solution to pH 7.2 with ammonium acetate. In the second method whole casein was dissolved in 6.6 M urea

and purification was achieved on the basis of the different solubility of the fractions in urea. In such studies the alpha$_s$-casein fraction was typically recovered as a precipitate when the urea concentration was reduced to 4.6 M.

Adjustment of the pH of whole casein dispersions in urea have also been used to precipitate the alpha$_s$-casein. This has been achieved either by adjusting a 6.6 M urea solution of casein to pH 1.3-1.5 (Zittle & Custer, J Dairy Sci. 46, 1183-1188, 1963) or by adjusting a 3.3 M urea system to pH 4.5 (Fox & Guiney, J Dairy Sci. 39, 49-53, 1972).

The alpha$_s$-casein fraction is readily precipitated from solution when calcium chloride is added. This observation was used to advantage in a wide range of methods described for the isolation of the alpha$_s$-casein fraction.

Fox (J Dairy Sci. 41, 715, 1958) attempted to use equipment which was normally only found in the dairy laboratory. The method for the isolation of the 'calcium soluble' fraction of whole casein required a cold (5°C) sodium caseinate solution (2%) at pH 11.0 which was treated with 0.15 M $CaCl_2$. The pH of the mixture was then lowered to 8.3 with HCl and the precipitate was removed by a clarifier separation. The clarified solution was adjusted to pH 4.7 and the 'calcium soluble' casein recovered as a curd. No analyses were provided on the composition, purity and yield of the fractions obtained in the study. The focus was on the preparation of beta casein.

In a number of methods published for the fractionation of the caseins during the 1960s (and the subsequent period) the preparation of a alpha$_s$-casein fraction – via one of the abovementioned methods – has been the preferred step for the subsequent recovery of a highly purified alpha$_s$-casein fraction. Thus Gehrke *et al* (Separation Science 1, 431-442, 1966) combined urea fractionation with calcium precipitation of alpha$_s$-casein which was subsequently purified by DEAE-cellulose chromatography. In another study the urea fractionation method of Hipp *et al* (1952) was combined with DEAE-cellulose (see Cheesman & Jeffcoat, J Dairy Res. 37, 245-257, 1970). In methods of this type the aim has generally been either to prepare highly purified samples of alpha$_s$-casein (especially free of kappa-casein) or to prepare samples of highly purified kappa-casein (free of alpha$_s$-casein and beta-casein).

Kudo (NZ J Dairy Sci Tech 15, 245-254 1980) developed a solvent based precipitation in which n-propanol was used to remove the alpha$_{s2}$-casein fraction from a dispersion of whole casein at

pH 6.5; the alpha$_{s1}$-casein fraction, present in the supernatant (which contained 40% w/v n-propanol), was subsequently recovered by the addition of calcium. The further purification of the products was achieved by (both) repeated extractions and chromatography on DEAE-cellulose exchangers (see Kudo, 1980 for details). In another study polycations have been used to complex the alpha$_s$-casein fraction at high pH. This complex is precipitated by acidifying the alkaline caseinate mixture to pH 5.8 (or 8.0) and the alpha$_s$-casein product is subsequently recovered by alkaline extraction of the precipitate. DEAE-dextran has been shown to be effective for this purpose (see Gurov *et al*, J Dairy Sci 64, 380-383, 1981 for details).

JP 54-95768 relates to a process which comprises cooling an alkaline metal salt solution of casein having a process concentration of 0.5 to 6% and a pH of 7 to 10 to 0 to 15°C and adding a divalent cation followed by the removal of the resulting alpha$_s$-casein precipitate from the remaining solution.

JP 59-91849 relates to a method characterized by the addition of a divalent salt to a casein fraction containing alpha$_s$- and beta-casein as major ingredients at a temperature of 0-10°C and fractionating this into separate fractions, one of which has alpha$_s$-casein as a major ingredient and the other having beta-casein as a major ingredient.

US Patent 5,068,118 describes a process for separating whole casein into its components and includes the steps of dissolving whole casein in an aqueous urea solution having a urea concentration sufficient to provide the solution, reducing the urea concentration of the solution to about 4.6 molar by addition of water, removing precipitated alpha$_s$-casein, reducing the urea concentration of the remaining solution to about 1.7 molar by addition of water, removing precipitated beta-casein and then precipitating gamma-casein present in the remaining solution.

US Patent 5,068,118 indicates that alpha$_{s1}$-casein provided a simulated process cheese with melt properties close to that of sodium caseinate cheese but with somewhat firmer texture. This patent also indicates that beta-casein provided a simulated process cheese which was soft and had restricted melt properties.

The existing methods are useful for producing casein fractions. However there remains a need for a method for producing a alpha$_s$-casein rich fraction or a beta-casein rich fraction that allows production on an industrial scale and does not require use of agents (such as urea, chaotropic

agents, detergents and solvents) that are undesirable in a food preparation process. Therefore it is an object of the present invention to provide such a process and/or to provide the public with a useful choice.

DISCLOSURE OF THE INVENTION

In one aspect the invention provides a method for the preparation of an alpha$_s$-casein fraction comprising:

(a) providing a liquid sample of a dairy feedstock comprising casein as at least 40% by weight of the milk protein;

(b) contacting the dairy feedstock with a cation exchanger loaded with sodium or potassium or both to exchange calcium for sodium or potassium or both;

(c) adding calcium to the resulting calcium-depleted material.

(d) recovering the precipitated fraction.

Preferably the dairy feedstock is selected from the group comprising UF concentrated cheese milks, total milk proteins, milks or milk protein solutions previously diafiltered or dialysed or electrodialysed or ion exchanged for the removal of minerals, micellar casein preparations, UF concentrates of acidified cheese milks, skim milk, milk and other milk products having at least 40% of the milk protein as casein. The feedstock may be a liquid dairy stream or it may be reconstituted from a dried product.

Preferably the feedstock comprises at least 60% casein, more preferably at least 80%

Preferably the cation exchanger is loaded with sodium and exchanges calcium in the feedstock for sodium.

Preferably the feedstock is whey-depleted.

Preferably, the feedstock is selected from a milk (including skim milk) and a milk protein concentrate, preferably whey-depleted.

The term "alpha$_s$-casein fraction" refers to a fraction comprising casein in which the ratio of alpha$_s$-casein to beta-casein is at least 30% higher, preferably at least 60% higher, most preferably 100% higher than the material before fractionation

The term "beta-casein fraction" refers to a fraction comprising casein in which the ratio of beta-casein to alpha$_s$-casein is at least 30% higher, preferably at least 60% higher, most preferably 100% higher than the material before fractionation

The term "whey-depleted milk" includes "whey depleted whole milk" and "whey-depleted skim milk". The term "whey-depleted" means that relative to the corresponding product when not "whey-depleted" the level of whey proteins is at least 10%, preferably at least 20%, more preferably at least 50%, lower. A whey-depleted milk is obtainable by microfiltration of a milk that is not whey-depleted.

The term "milk protein concentrate" (MPC) refers to a milk protein product in which greater than 55% and preferably greater than 70%, more preferably 75% of the dry matter is milk protein. An MPC may be prepared fresh or may be a dried product for reconstitution with water, milk or another suitable aqueous liquid.

The term "milk protein isolate" (MPI) refers to a milk protein product in which at least 85% of the dry matter is milk protein. An MPI may be prepared fresh or may be a dried product for reconstitution with water, milk or another suitable aqueous liquid.

The term "total milk protein" refers to a milk protein product that is an MPC or MPI in which the whey protein is denatured.

The term "comprising" as used in this specification means "consisting at least in part of", that is to say when interpreting statements in this specification which include that term, the features, prefaced by that term in each statement, all need to be present but other features can also be present.

Preferably the starting material is whey-depleted skim milk retentate. In a preferred embodiment, milk is microfiltered at low pH (pH range 4.9-7.5, preferably 5.5-6.3, most preferably pH 5.8-6.1) in order to enhance calcium reduction while whey proteins are removed into the permeate. Membranes (e.g. of MWCO) having pore size between 0.1-0.8 microns are

preferred. The preferred temperature for this step is in the range 4-60°C, more preferably 10-50°C, most preferably 40-50°C).

An alternative starting material is an MPC or MPI without whey-depletion, preferably with at least 70%, more preferably 75%, more preferably at least 80%, most preferably at least 85% of the total solids as protein. Preferably, the MPC is prepared by ultrafiltration at a pH in the range of 4.9-7.5 preferably 5.5-6.5, most preferably 5.8-6.1.

Following ultrafiltration or microfiltration and prior to contacting the MPC with the cation exchanger, it is preferred to adjust the pH to 4.9-7.5, preferably 5.5-6.3, most preferably 5.8-6.1.

The preferred ion exchanger is a cation exchanger bearing strongly acidic groups in the sodium form or potassium form. Among preferred acidic groups are sulphonic acid groups. One preferred ion exchange resin is IMAC HP 111E manufactured by Rohm & Haas. This resin has a divinylbenzene copolymer matrix. The functional groups are sulphonic acid groups that can be obtained in the Na+ form. Another preferred ion exchanger is Amberlite SR1L.

The cation exchange step allows better precipitation in step (c).

Following the cation exchange step, it is preferred to use an anion exchange step. The anion exchange may be in the Cl or OH form. Preferably, the anion exchanger bears weak basic groups e.g. primary, secondary or tertiary amines. A preferred ion exchanger is Purelite A1035.

In another preferred alternative the cation-exchange and anion-exchange steps are carried out at the same time using a mixed bed of the two ion-exchange resins.

Use of the anion exchange step is preferred because it allows cleaner separation of alpha$_s$-casein from the beta-casein and kappa casein fraction. This is currently believed to be due to removal of phosphate ions. Use of this step is particularly advantageous when the starting material is an MPC.

Shortly after the ion exchange step the calcium-depleted whey-depleted material may be subjected to concentration, preferably ultrafiltration or ultrafiltration with diafiltration. Membranes of NMCO of cutoff 1-100 K Daltons, more preferably 5-30 K Daltons most

preferably 10-30 K Daltons. Alternatively, concentration could be carried out using rennet treatment or acidification. In this step lactose and ash is (further) reduced.

Before the precipitation step, the (heat-treated) calcium-depleted material is preferably diluted to give a casein concentration of 2-10%, preferably 4-7%.

The preferred temperature for the calcium precipitation step is 0-25°C, preferably 1-15°C, more preferably 4-10°C, most preferably 4-8°C. Where the concentrated calcium-depleted material is at a higher temperature, it may be cooled before or during calcium precipitation. The cooling time used is typically 5-200 minutes, preferably 30-200 minutes, more preferably 30-60 minutes.

Calcium is preferably added as a soluble calcium salt or as the hydroxide, more preferably as a calcium salt. Preferred salts include the chloride, acetate, ascorbate, citrate, lactate and tartrate salts. Calcium chloride is currently the most preferred. The calcium salt may be added as either a powder or as a solution.

Salts of other nutrient metal ions may be added before or with the calcium addition step. These may be selected from potassium, magnesium, manganese, zinc, chromium, copper and molybdenum salts. Potassium, magnesium, manganese and zinc are preferred for this purpose. Inclusion of such salts will result in binding of these salts to the casein molecules so that the eventual product will act as a source of these nutrients.

In one embodiment a soluble calcium salt is added with or following the addition of a soluble phosphate salt (for example a sodium phosphate salt). This allows formation of a calcium-alpha$_s$-casein-phosphate complex.

Following the calcium addition, alpha$_s$-casein flocculates. The alpha$_s$-deplete-casein stream remaining in solution is rich in beta-casein and kappa-casein.

The flocculated alpha$_s$-casein may be collected for example by sedimentation or centrifugation at (e.g. at 2,000-15,000 x g for 10 min). The alpha$_s$-casein fraction is preferably separated from the supernatant fraction by cold centrifugation. The precipitate may be minced in a colloid mill and have its pH adjusted to pH 4.2-5.2, preferably 4.6-4.9. The calcium content may be adjusted by washing the precipitate with acidified water until a desired level of calcium has been reached.

The precipitated and washed alpha$_s$-casein fraction may be converted to a sodium or potassium alpha$_s$-caseinate. Typically, the pH of the precipitate is adjusted to 6.0-7.0, preferably 6.5-7.0 at 50-60°C and blended with another dairy stream such as MPC or a cream. The precipitate may also be re-dissolved in water at pH 6.0-7.0 at 5°C-85°C, preferably 16°C-60°C more preferably 16°C -30°C.

The alpha-casein product or blended product may then be concentrated (for example by evaporation) and dried (for example by spray drying).

Preferably the pH of the protein material undergoing ion exchange treatment is in the range of pH 5 to pH 9.5, more preferably 5.5-6.5, most preferably 5.7-6.0. The preferred pH for carrying out the calcium precipitation of alpha$_s$-casein is pH 6.5 to pH 10.5, preferably 8.5-10.5, most preferably 9.5-10.3. Especially preferred is pH 9.8-10.2. These pH values are measured at the temperature of the process step.

The preferred concentration of calcium for use in precipitating the alpha$_s$-casein from the calcium-depleted whey-depleted MPC is 0.4-8% (w/w), preferably 1-8% (w/w), more preferably 2-8% (w/w), most preferably 4-6% w(Ca)/w(protein). The preferred concentrations are 5-200 mM, preferably 30-80 mM when the level of protein is between 2% and 4%. Mild agitation may be usefully employed during this step.

Alpha$_s$-casein produced by the above method is particularly useful as a process cheese ingredient. It can be blended into other dairy product streams to make fresh alpha enriched products or cheese milks, or converted into dry ingredients (eg caseinates, MPC or total milk protein products) for use in functional or nutraceutical foods. Caseinates can be prepared using ion exchange on a cation exchanger charged with potassium or sodium or by addition of KOH or NaOH.

The operation and methodology of the invention is simpler to carry out than other stated prior art methods.

The alpha$_s$-casein ingredient after separation from the fraction containing high beta-casein and kappa-casein may be adjusted to have high, medium or low calcium, 2500-6500 mg/100g, 1000-2500 mg/100 g or 0.5-1000 mg calcium per 100 g product respectively. The freshly precipitated

alpha$_s$-casein is high in calcium but where a low or medium calcium product is required, calcium can be removed, for example by dialysis.

The fraction with higher beta-casein and high kappa-casein may be recovered by the usual method used for casein recovery, e.g. by renneting or by acid precipitation, or alternatively by dialysis or ultrafiltration followed by e.g. spray drying. The calcium content of that fraction may be adjusted to provide low (0.5-100 mg calcium/100 g product), medium (100-1500 mg calcium/100 g product) or high calcium (greater than 1500 mg calcium per 100g product) as described for alpha$_s$-casein. The precipitated and washed fraction with higher beta-casein, high kappa-casein may be used to enrich another dairy product stream or may be converted to a sodium or potassium beta/kappa-caseinate.

The high beta-casein, high kappa-casein fraction may be used for a variety of purposes including as a source of glycomacropeptide for enhanced nutrition, and a source of bioactive peptides, and as a source of protein for infant formula.

In a further aspect of the invention, there is provided an alpha$_s$-casein fraction prepared by the method of the invention.

In yet a further aspect of the invention there is provided a method for controlling the texture and/or firmness of a cheese product comprising:

(a) preparing an alpha$_s$ casein fraction by a method of the invention.

(b) including the fraction in a milk or concentrated milk starting material

(c) treating the starting material with acid and/or a hydrolytic enzyme to produce curds.

(d) processing the curds to prepare cheese or a processed cheese.

Preferred embodiments are depicted in Figures 1, 2 and 3.

In a further aspect the invention provides a method for the preparation of a beta-casein fraction comprising:

(a) providing a liquid sample of a dairy feedstock comprising casein as at least 40% of the milk protein;

(b) contacting the dairy feedstock with a cation exchanger loaded with sodium to exchange calcium for sodium;

(c) adding calcium to the resulting calcium-depleted material.

(d) removing the precipitated fraction

(e) recovering the unprecipitated fraction

Steps (a)-(d) are preferably carried out as described above in relation to the preferred embodiments for preparation of the alpha$_s$-casein.

The removal of the precipitated fraction may be achieved for example by sedimentation or by centrifugation (e.g. at 2,000-15,000 x g for 10 min).

The protein component of the unprecipitated fraction recovered may be obtained by the usual method used for casein recovery, e.g. by renneting or by acid precipitation, or alternatively by dialysis or ultrafiltration followed by e.g. spray drying.

For this method the preferred methods of carrying out steps (a) – (c) are as for the preparation of the alpha$_s$-casein fraction. In a preferred method both the precipitated fraction and the unprecipitated fraction are recovered to provide both an alpha$_s$-casein fraction and a beta-casein fraction. The beta-casein fraction will generally also contain kappa-casein.

In a further embodiment there is provided a beta-casein fraction prepared by the method of the invention.

The invention also provides a method of making a sodium or sodium/calcium caseinate product comprising the steps of:

(a) microfiltering milk or skim milk at low pH (pH range 4.9-7.5, preferably 5.5-6.3, most preferably 5.8-6.1);

(b) contacting the retentate with a cation exchange loaded with sodium to exchange calcium for sodium; and

(c) recovering the sodium or sodium/calcium caseinate fraction.

This process may be manipulated to allow production of caseinates with desired mineral profiles. For example, requirements can be met to provide particular Ca/Mg mineral mixtures. The

process can be used to provide sodium potassium mixtures for food functional applications, new MPCs, total milk proteins and milk compositions for cheese manufacture.

Preferred cation exchangers are as described for the methods for preparing alpha$_s$-casein and beta-casein.

Preferred microfiltration methods are as described above.

The resulting caseinate is useful as an intermediate for producing alpha$_s$-casein or beta-casein. It may be used directly or spray dried for future use.

BRIEF DESCRIPTION OF THE DRAWING

Figure 1 is a schematic drawing of a preferred embodiment of the process for preparing casein fractions.

Figure 2 is a schematic drawing of a preferred embodiment of the process for preparing casein fractions.

Figure 3 is a schematic drawing of a preferred embodiment of the process for preparing casein fractions.

The following Example further illustrates practice of the invention.

EXAMPLE 1

Skim milk (25 L, casein to whey ratio of 3.2) was fractionated by batch microfiltration to a VCF of 2.1 at 50°C (using the MFS1 unit from Alpha Laval fitted with 0.1μm membrane). The retentate was diafiltered twice (13.6 L water addition each time) to further remove whey proteins, lactose and minerals. A 10 L sample of the diafiltered retentate (casein: whey ratio of 9.1, 5.57% protein) was passed twice through a cation exchange resin (Rohm & Haas Amberlite SR1L Na form (bed volume 4L) to fully substitute sodium for calcium as the counterion

associated with the protein in solution. Prior to the second pass through the cation exchange resin, the breakthrough was adjusted from pH 8.2 to pH 6.1 with HCl. The breakthrough (calcium depleted, sodium form of the proteinate) was recovered (4% w/v protein), was cooled to 7°C, and adjusted to a pH of between 10.0-10.43 using 10% NaOH. Calcium chloride (74.04 g) was added to 10 L of the breakthrough, the mixture stirred and left at 5°C overnight. The flocculated protein was recovered by centrifugation (30 min, 10000 rpm with rotor J14 at 8°C).

The sedimented protein (885 g) was slurried in 200 mL of water and acidulated to pH 4.93 with HCl. This acidulated mixture was left in the cold room held at 4°C overnight and the protein recovered by centrifugation and freeze-dried.

The alpha$_s$-casein to beta-casein ratio in skim milk and standard lactic casein is 1:0.94. The preparation above had an alpha$_s$-casein to beta-casein ratio of 1.81:1.

Beta casein can be recovered from the liquid fraction by the standard methods used for the recovery of casein, namely adjusting the pH of the liquid to pH 4.6 using a mineral acid (typically 2 M HCl) and heating at 45°C-47°C followed by recovery of the precipitate by centrifugation. Alternatively, the liquid stream may be concentrated by ultrafiltering with a 10 K Dalton membrane, and the concentrated protein is then spray dried.

EXAMPLE 2

MPI (milk protein isolate, 85% milk protein, Fonterra) 200 g was mixed with 1800 mL of water at 50°C, then chilled to 4°C and adjusted to pH 5.95 with 10% HCl.

The pH adjusted solution was passed twice through a one litre bed of cation exchange resin (Amberlite SR1L Na form, Rohm & Haas) to fully substitute sodium for calcium as the counterion associated with the protein in solution. The breakthrough (calcium depleted, sodium form of the proteinate) was recovered, was cooled to below 10°C, diluted to 2-3% casein and adjusted to a pH of 10.5 using 10% NaOH. Calcium chloride was added to 100 mL aliquots of the breakthrough (1.25, 1.50, 1.75 and 2.00 mL of 43% $CaCl_2.2H_2O$ per 100 mL aliquot). The mixtures were stirred and held at 4°C overnight. The flocculated protein was recovered by centrifugation (10 min, 8000 rpm with rotor J14 at 8°C).

The chilled precipitates were diluted 1/20 using alkali urea buffer and subjected to alkaline urea polyacrylaminde gel electrophoresis to separate the caseins prior to staining with Amido Black and analysing using densitometry.

The $alpha_s$-casein to beta-casein ratio in skim milk and standard lactic casein is 1:0.94. Each preparation above had an $alpha_s$-casein to beta-casein ratio in the range 1.47-1.59.1

EXAMPLE 3

MPC (milk protein concentrate, 70% milk protein, Fonterra) 200 g was mixed with 1800mL of water at 50°C, chilled to 4°C and adjusted to pH 5.95 with 10% HCl.

The pH adjusted solution was passed twice through a one litre bed of cation exchange resin (Amberlite SR1L Na form, Rohm & Haas) to fully substitute sodium for calcium as the counterion associated with the protein in solution. The breakthrough (calcium depleted, sodium form of the proteinate) was recovered and cooled to below 10°C. 2 L of the calcium-depleted solution was passed through 2 L of anion exchange resin (A103S, Purelite 1035, chloride form). The breakthrough was diluted to 2-3% casein and adjusted to a pH of 10.5 using 10% NaOH. Calcium chloride was added to 100 mL aliquots of the breakthrough (1.25, 1.50, 1.75 and 2.00 mL of 43% $CaCl_2.2H_2O$ -per 100 mL aliquot). The mixtures were stirred for 3 minutes. The precipitated protein was recovered by centrifugation (10min, 8000 rpm with rotor J14 at 8°C).

The chilled precipitates were diluted 1/20 using alkali urea buffer and subjected to alkaline urea polyacrylamide gel electrophoresis to separate the caseins prior to staining with Amido Black and analysing using densitometry.

The $alpha_s$-casein to beta-casein ratio in skim milk and standard lactic casein is 1:0.94. The precipitate had $alpha_s$-casein to beta-casein ratios of 3.7, 13.9, 7.88 and 6.9 for addition of 1.25, 1.50, 1.75 and 2.00 mL of the calcium chloride solution respectively.

EXAMPLE 4

Skim milk (34 L, casein to whey ratio of 3.2) was adjusted in pH to 6.0 by addition of 5% NaOH. It was then fractionated by batch microfiltration to a VCF of 3 at 10°C (using the MFS1

unit from Alpha Laval fitted with 0.1μm membrane). The retentate was diafiltered twice (16 L water addition each time) to further remove whey proteins, lactose and minerals. A 2 L sample of the diafiltered retentate (8-10% total solids) was passed twice through a cation exchange resin (Rohm & Haas Amberlite SR1L Na form (bed volume 1 litre)) to fully substitute sodium for calcium as the counterion associated with the protein in solution. The breakthrough (calcium depleted, sodium form of the proteinate) was recovered (**5.7** w/w protein of which 5.5% was casein), diluted to 2-3% casein, cooled to 7°C, and adjusted to a pH of 10.5 using 10% NaOH. To six 600 mL aliquots of calcium chloride was added (1.25 mL, 1.35 mL, 1.5 mL, 1.7 mL, 2.0 mL and 2.5 mL of 43% $CaCl_2.2H_2O$). The mixtures were stirred for 3 minutes with a glass rod. The precipitated protein was recovered by centrifugation (30 min, 8000 rpm with rotor J14 at 8°C).

The precipitated protein was diluted 1/20 into alkali urea buffer and subjected to alkali urea polyacrylamide gel electrophoresis to separate the caseins prior to staining with Amido Black and analysing using densitometry. The $alpha_s$-casein to beta-casein ratio was 3.4, 3.2, 3.2, 3.2, 3.1 and 2.8

EXAMPLE 5

MPC70 powder (Fonterra, milk protein concentrate 70% protein, 20kg) was reconstituted to 5% total solids with water at 50°C and adjusted to pH 6.00 with 5% HCl.

The pH adjusted solution was processed in a UFS4 with continuous diafiltration until the protein level was 85% of total solids. Ultrafiltration retentate (2.5 L) was diluted to 10% total solids. The pH was adjusted to pH 5.9. The retentate was then passed through a cation exchange resin (Amberlite SR1L, Na form, Rohm and Haas bed volume 1L) to remove calcium. The breakthrough (calcium depleted, sodium form of the proteinate) was recovered and divided into 3 portions. One portion was diluted to 2% casein. The second was diluted to 3% casein and the third to 4% casein. Each portion was cooled to below 10°C and then the portions were split as shown in Table 1. Each portion was adjusted to a preselected pH using 10% NaOH (see Table 1). Calcium chloride was added as $CaCl_2.2H_2O$ in the amounts shown in Table 1. The mixtures were stirred and left at 4°C for 15 minutes. The flocculated protein was recovered by centrifugation (10min, 8000rpm with rotor J14 at 8°C).

The chilled precipitates were diluted 1/20 into alkali urea buffer and subjected to alkali urea gel electrophoresis to separate the caseins prior to staining with Amido Black and analysing using densitometry.

The alpha$_s$-casein to beta-casein ratio in skim milk and standard lactic casein as measured by densitometry is 1:0.94. Table 1 shows the alpha$_s$-casein to beta-casein ratios obtained.

TABLE 1

DS number	Casein (TN-NCN) *6.38	Calcium (g/100 mL)	pH	Alpha$_s$:beta ratio	wet weight (g)
23	6.3	1.1	9.98	3.29	33
24	6.3	1.1	10.51	6.45	13
25	6.3	0.8	10.25	1.73	17
26	6.3	1.4	10.24	2.56	31
27	3.4	0.55	10.01	none	none
28	3.4	0.55	10.52	none	none
29	3.4	0.4	10.25	none	none
30	3.4	0.7	10.26	?	0.8
31	3.4	0.6	10	3.47	No data
32	4.2	1.05	10	3.15	25
33	4.2	0.6	10.5	4.6	2.2
34	4.2	1.05	10.5	6.7	9
35	4.2	0.825	10.25	4.1	7
36	4.2	0.825	10.25	4.0	13
37	4.2	0.825	10.25	4.6	8

EXAMPLE 6

MPC 70 (milk protein concentrate, 70% milk protein, Fonterra) 250 g was mixed with water at 50°C to form a reconstituted MPC 70 with 5% total solids, chilled to 4°C and adjusted to pH 6.0 with 5% HCl.

The pH adjusted material was subjected to diafiltration in a 4 loop ultrafitration system with 5-10 K Dalton membranes in continuous mode until the permeate was less than 0.5° Brix and less than 0.8mS/cm conductivity. The pH was adjusted to pH 5.9 with 5% HCl.

A portion of the pH adjusted solution was passed twice through a 125 litre bed of cation exchange resin (Amberlite SR1L Na form, Rohm & Haas) (first cycle 900L, recharged with sodium, second cycle 1068L, 200L not used) to fully substitute sodium for calcium as the counterion associated with the protein in solution. The breakthrough (calcium depleted, sodium form of the proteinate) was recovered, cooled to below 10°C, diluted to 4% casein and adjusted

to a pH of 10.0 using 10% NaOH. The remaining portion of the diafiltered material was not subjected to ion exchange and was diluted to 4% casein. The extent of calcium depletion required for precipitation of the alpha$_s$-casein fraction was tested using blends of the ion-exchanged material and the material without ion exchange. Six test samples were prepared. These contained 80%, 70%, 60%, 50%, 40% and 30% of the ion-exchanged material. The pH of each was adjusted to pH 10 with 10% NaOH. No precipitation was observed.

The above examples illustrate the practice of the invention. It will be appreciated by those skilled in the art that the invention can be carried out with numerous modifications and variations. For example, the material subjected to calcium depletion can show variations in protein concentration and pH, the method of calcium depletion can be varied, the percentage calcium depletion and percentage whey depletion can also be varied.

WHAT WE CLAIM IS:

1. A method for the preparation of an alpha$_s$-casein fraction comprising:
   (a) providing a liquid sample of a dairy feedstock comprising casein as at least 40% by weight of the milk protein;
   (b) contacting the dairy feedstock with a cation exchanger loaded with sodium or potassium or both to exchange calcium for sodium or potassium or both;
   (c) adding calcium to the resulting calcium-depleted material;
   (d) recovering the precipitated fraction.

2. A method as claimed in claim 1 wherein the dairy feedstock is a milk or milk protein concentrate that is whey-depleted.

3. A method as claimed in claim 2 wherein the whey-depletion is due to microfiltration at pH 4.9-7.5.

4. A method as claimed in claim 1, wherein the liquid sample is an MPC with at least 70% of the total solids as protein.

5. A method as claimed in claim 4 wherein the MPC is prepared by ultrafiltration at a pH in the range 4.9-7.5.

6. A method as claimed in any one of claims 1-5 wherein the cation exchanger bears strongly acidic groups in the sodium or potassium form.

7. A method as claimed in any one of claims 1-6 wherein following the cation exchange step, the calcium-depleted material is contacted with an anion exchanger in the Cl or OH form.

8. A method as claimed in claim 7 wherein the anion exchanger bears weak basic groups.

9. A method as claimed in any one of claims 6-8 wherein in step (b) the dairy feedstock is contacted with a cation exchanger in a mixed bed with an anion exchanger.

10. A method as claimed in any one of claims 1-9 wherein before or after ion exchange a heat treatment step is carried out under alkaline conditions to denature whey proteins.

11. A method as claimed in any one of claims 1-10 wherein the calcium precipitation step is carried out at 4-10°C.

12. A method as claimed in claim 11 wherein the cooling time for the precipitation step is in the range 5-200 minutes.

13. The method as claimed in any one of claims 1-12 wherein the calcium is added as a soluble calcium salt or as calcium hydroxide.

14. A method as claimed in claim 13 wherein calcium is added as calcium chloride.

15. A method as claimed in any one of claims 1-14 wherein the alpha-casein is collected following sedimentation or centrifugation.

16. A method as claimed in any one of claims 1-15 wherein the pH is in the range 9.8-10.2 during the calcium precipitation step.

17. A method as claimed in any one of claims 1-16 wherein the concentration of calcium during precipitation of the $alpha_s$-casein is 5-200 mM.

18. A method for controlling the texture and/or firmness of a cheese product comprising:
    (a) preparing an $alpha_s$-casein fraction by a method claimed in any one of claims 1-17.
    (b) including the fraction in a milk or concentrated milk starting material
    (c) treating the starting material with acid and/or a hydrolytic enzyme to produce curds.
    (d) processing the curds to prepare cheese or a processed cheese.

19. A method for the preparation of a beta-casein fraction comprising:
    (a) providing a liquid sample of a dairy feedstock;

(b) contacting the dairy feedstock with a cation exchanger loaded with sodium to exchange calcium for sodium;

(c) adding calcium to the resulting calcium-depleted material;

(d) removing the precipitated fraction;

(e) recovering the unprecipitated fraction.

20. A method as claimed in claim 19 wherein the dairy feedstock is milk or milk protein concentrate is whey-depleted.

21. A method as claimed in claim 20 wherein the whey-depletion is due to microfiltration at pH 4.9-7.5.

22. A method as claimed in claim 19 wherein the liquid sample is an MPC with at least 70% of the total solids as protein.

23. A method as claimed in claim 22 wherein the MPC is prepared by ultrafiltration at a pH in the range 4.9-7.5.

24. A method as claimed in any one of claims 19-23 wherein the cation exchanger bears strongly acidic groups in the sodium or potassium form.

25. A method as claimed in any one of claims 19-24 wherein following the cation exchange step the calcium depleted material is contacted with an anion exchanger in the Cl or OH form.

26. A method as claimed in claim 25 wherein the anion exchanger bears weak basic groups.

27. A method as claimed in any one of claims 19-25 wherein in step (b) the dairy feedstock is contacted with a cation exchanger in a mixed bed with an anion exchanger.

28. A method as claimed in any one of claims 19-27 wherein before or after ion exchange a heat treatment step is carried out under alkaline conditions to denature whey proteins.

29. A method as claimed in any one of claims 19-28 wherein the calcium precipitation step is carried out at 4-10°C.

30. A method as claimed in claim 29 wherein the cooling time for the precipitation step is in the range 5-200 minutes.

31. The method as claimed in any one of claims 19-30 wherein the calcium is added as a soluble calcium salt or as calcium hydroxide.

32. A method as claimed in claim 31 wherein calcium is added as calcium chloride.

33. A method as claimed in any one of claims 19-32 wherein the alpha-casein is collected following sedimentation or centrifugation.

34. A method as claimed in any one of claims 19-33 wherein the pH is in the range 9.8-10.2 during the calcium precipitation step.

35. A method as claimed in any one of claims 19-34 wherein the concentration of calcium during precipitation of the $alpha_s$-casein is 5-200mM.

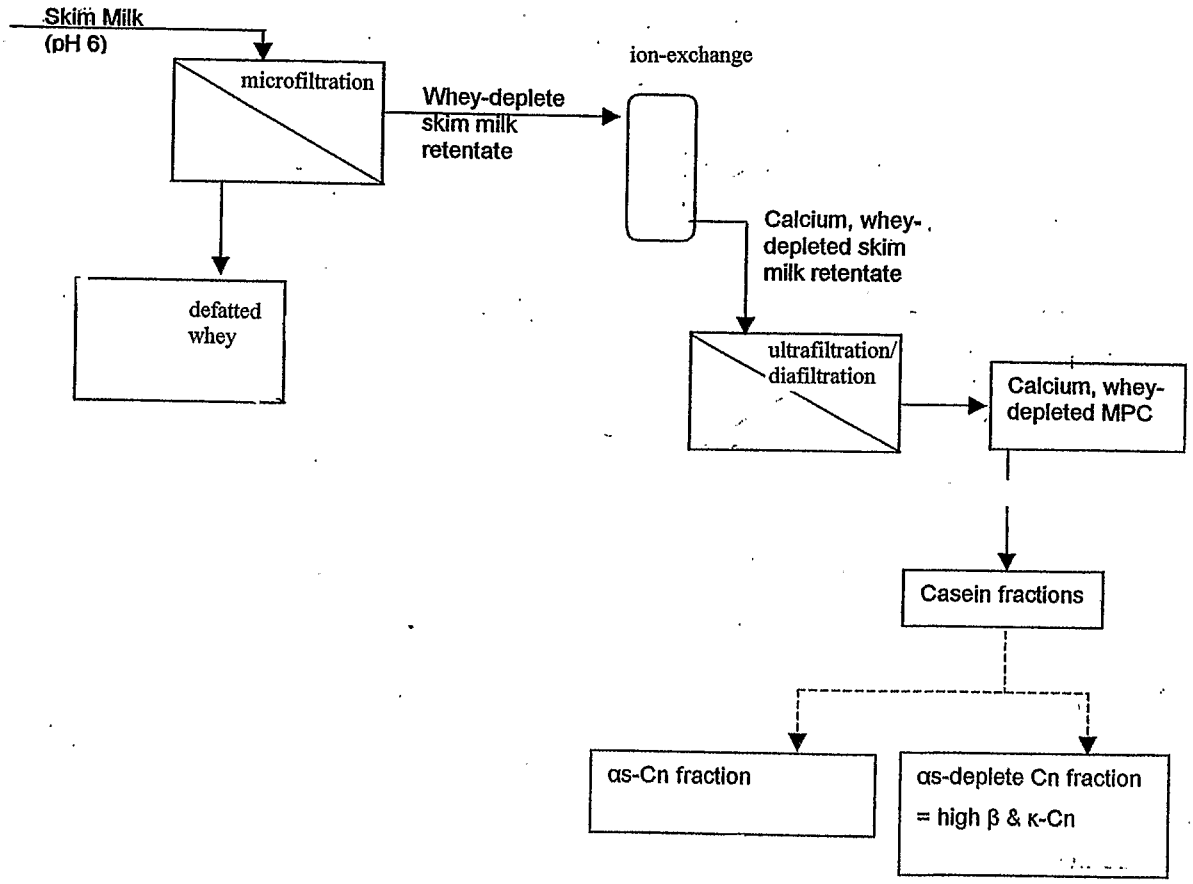
36. A method as claimed in any one of claims 19-35 wherein following removal of the precipitated faction the pH is adjusted to pH 4.0-5.5 to precipitate the beta-casein fraction.

37. A method as claimed in any one of claims 1-17 wherein the product is converted to a caseinate by addition of KOH or NaOH or by ion exchange on a cation exchanger charged with sodium or potassium or both.

38. A method as claimed in any one of claims 19-36 wherein the product is converted to a caseinate by addition of KOH or NaOH or by ion exchange on a cation exchanger charged with sodium or potassium or both.

FIGURE 1

PREFERRED EMBODIMENT – PROCESS FLOW DIAGRAM



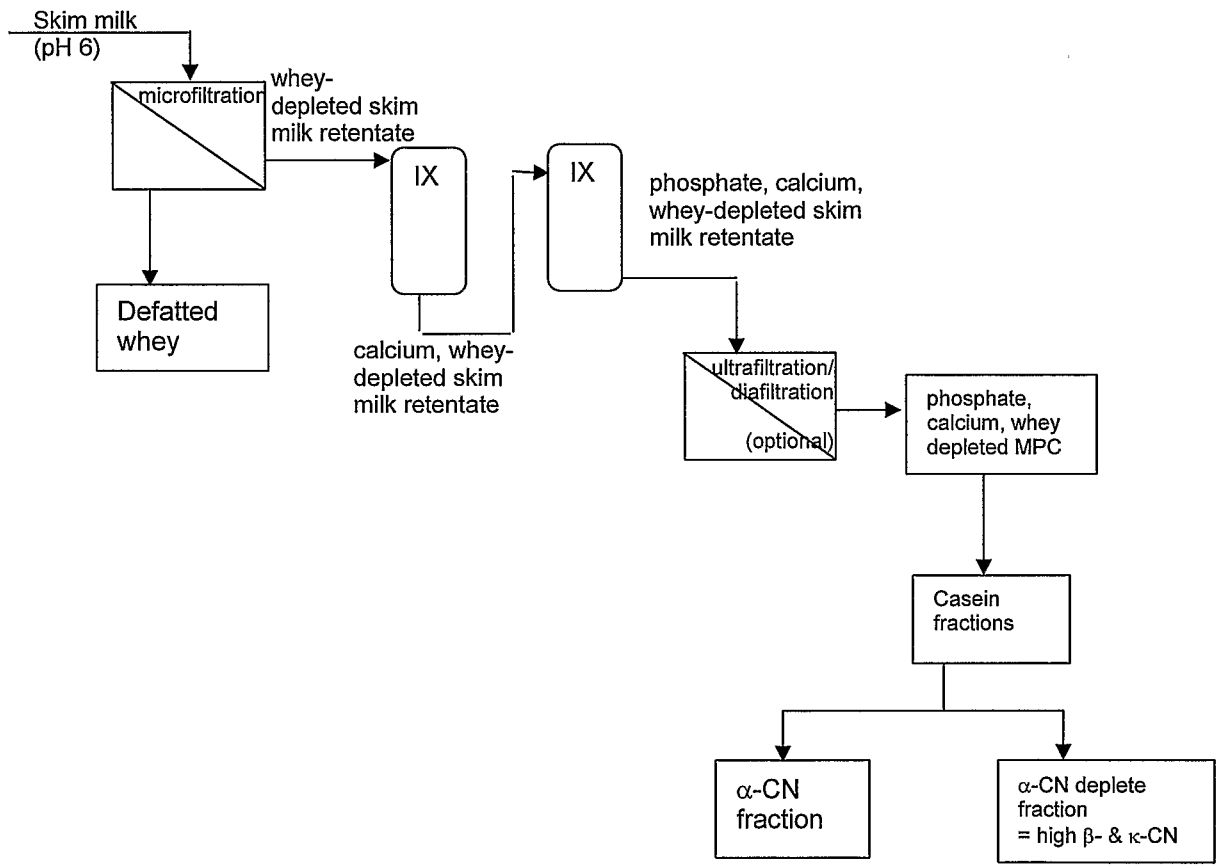

Skim milk
(pH 6)
microfiltration
whey-depleted skim milk retentate
IX
IX
phosphate, calcium, whey-depleted skim milk retentate
Defatted whey
calcium, whey-depleted skim milk retentate
ultrafiltration/ diafiltration
(optional)
phosphate, calcium, whey depleted MPC
Casein fractions
α-CN fraction
α-CN deplete fraction = high β- & κ-CN

FIGURE 2

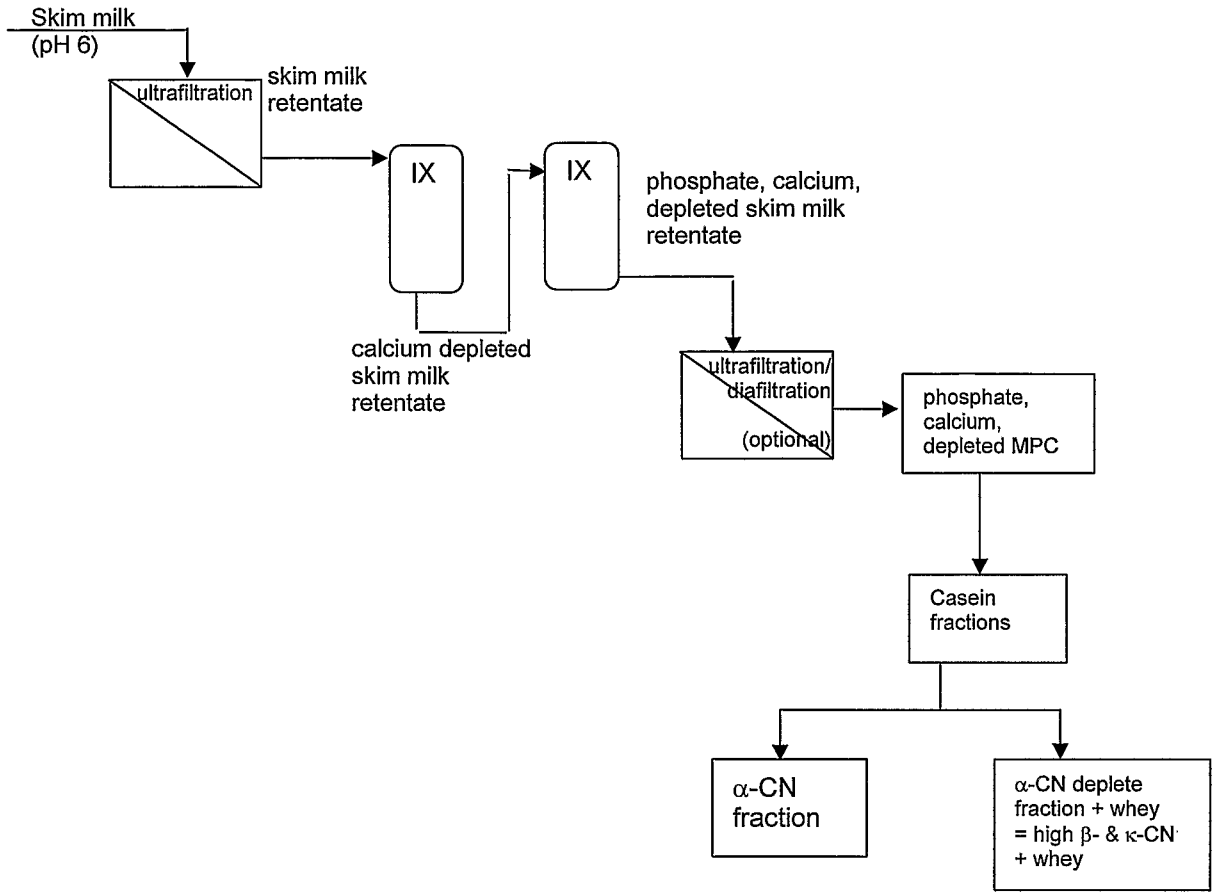

Skim milk
(pH 6)
ultrafiltration
skim milk
retentate
IX
IX
phosphate, calcium,
depleted skim milk
retentate
calcium depleted
skim milk
retentate
ultrafiltration/
diafiltration
(optional)
phosphate,
calcium,
depleted MPC
Casein
fractions
α-CN
fraction
α-CN deplete
fraction + whey
= high β- & κ-CN
+ whey

FIGURE 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NZ2006/000168

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

A23C 21/00 (2006.01) *A23J 1/20* (2006.01) *A23J 3/10* (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
STN files: Medline, CA, FSTA, WIPDS. Key words: alpha s1 or alpha s2 (w) casein, cation exchange?, potassium or sodium, calcium or Ca, precipit?, Prep?

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2003/003847 (HANNAH RESEARCH INSTITUTE) 16 January 2003 see whole document, especially page 14, line 24-page 15, line 4, page 32, line 25-page 33, line 17 and Fig 7, page 16, lines 10-16, page 20, lines 8-9, page 1, line 21-page 2, line 6	
A	Fox, K.K. "Separation of a calcium-soluble fraction of casein from isoelectric casein". Journal of Dairy Science (1958), 41:715 (cited in specification) see whole document	
A	Zittle C.A. "Influence of Heat on κ-casein: Effect of α_s-Casein and Concentration of Calcium Chloride and Sodium Chloride". Journal of Dairy Science (1969), Vol 52(9): 1356-1358 Abstract and Fig 3	

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 September 2006	21 SEP 2006
Name and mailing address of the ISA/AU	Authorized officer
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	**DAVID GRIFFITHS** Telephone No : (02) 6283 2628

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Haza A.I. et al., "Immunoreactivity of Goat's Milk Casein Fractionated by Ion-Exchange Chromatography". J. Agric. Food Chem. (1995), 43: 2025-2029 Abstract, Material and Methods on page 2025 and 2026	
A	Law A.J.R. et al., "Quantitative fractionation of ovine casein by cation-exchange FPLC" Milchwissenschaft (1992), 47(5): 279-281. Introduction and Materials and Methods	
A	Hollar C.M. et al. "Separation of Major Casein fractions Using Cation-Exchange Fast Protein Liquid Chromatography." Journal of Dairy science (1991), Vol 74 (8): 2403-2409 Abstract, page 2404, left hand column: materials and methods	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/NZ2006/000168

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report	Patent Family Member
WO 03003847	EP 1406507 US 2004234666

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

END OF ANNEX