

METHOD FOR PRODUCING A DEMINERALISED MILK PROTEIN COMPOSITION, SUITABLE
IN PARTICULAR FOR THE ORGANIC SECTOR, AND DEMINERALISED MILK PROTEIN
COMPOSITION

5 This invention essentially relates to a method for demineralising a milk protein composition.

 The invention is also based on a demineralised milk protein composition likely to be obtained by such a method.

 Among known milk protein compositions, whey is the liquid part that comes
10 from the curdling of the milk during the production of cheese.

 Whey is essentially formed of water, lactose, proteins and minerals. One of the ways of enhancing whey relates to the production of whey powder, serving as an ingredient for producing baby milk. To do this, it is particularly necessary to proceed with reducing the mineral content to obtain a product rich in proteins and lactose.

15 In a manner known per se, the method of demineralising whey comprises, in particular, a creaming step enabling the whey fat content to be reduced, followed by an electrodialysis step, by which a dialysed whey containing 50% to 60% in weight of less minerals is obtained. The creamed and dialysed whey then goes into a cation column, from where it emerges that it is highly cation-depleted, then in an anion column,
20 whereupon exit the whey is known as demineralised.

 But, the regeneration of ion-exchange resins leads to the use of strong bases and acids such as soda, potash or hydrochloric acid which can damage the quality of the demineralised product obtained. Through their functioning, ion-exchange resins induce the addition of exogenous mineral species and are, in this regard, considered as
25 technological auxiliaries in the sense of the European standard, EC 889/2008. Their use in the organic sector is forbidden because of this.

 In this context, the present invention aims for a method for demineralising a milk protein composition, free of any operation implementing ion-exchange resins and enabling to obtain a level of demineralisation that is at least equal to 90%, while
30 respecting a mineral profile that is compatible with the development of a product such as baby milk.

 To this end, the method for producing the demineralised milk protein composition is essentially characterised in that it comprises at least the steps of:

- preparing or providing a milk protein composition,
- ultrafiltration of said milk protein composition,
- nanofiltration of the ultrafiltration retentate obtained in the preceding step,

and

5 - electrodialysis of the nanofiltration retentate obtained in the preceding step,
said method being free of any step that involves passing over ion-exchange ions.

Thus, the method of the invention is exclusively a membrane method involving, in a specific order, the steps of ultrafiltration, nanofiltration and electrodialysis. The method of the invention enables to obtain a demineralised milk protein composition up to 90% wherein the mineral content is controlled by enabling, in particular, the maintenance of the ratio between calcium and phosphorus as close as possible to milk, which is not the case for a conventional demineralisation method, passing over ion-exchange ions, which has a lower calcium to phosphorus ratio. The milk protein composition further has a protein denaturation rate that is lower than that of the compositions obtained by the demineralisation method in the prior art, involving ion-exchange resins.

The method of the invention can also comprise the following optional characteristics considered by themselves or according to all the possible technical combinations:

20 - the ultrafiltration retentate has a total nitrogenous substance content at least equal to 14% of dry extract.

- according to a first variant, the milk protein composition is cheese whey.

- the cheese whey comes from organic farming.

25 - according to a second variant, the milk protein composition comes from a method comprising at least the steps of:

- provision of raw milk,

- creaming, heat treatment and bacterial purification of said raw milk,

- microfiltration on milk membrane obtained in the preceding step, and

30 -- recovery of the permeate from microfiltration forming the milk protein composition.

- the microfiltration membrane has a porosity of between 0.1 and 0.2 micrometres.

- the raw milk comes from organic farming.

The invention is also based on a demineralised milk protein composition, likely to be obtained by the method such as defined above.

The milk protein composition of the invention can also comprise the following optional characteristics, by themselves or according to all possible technical combinations:

- in the milk protein composition likely to be obtained by the method such as defined above, the ratio between calcium and phosphorus contents is more than 0.65.

- in this case, the demineralised milk protein composition can comprise:

- calcium at a content less than 11 milligrams per gram of total nitrogenous substance,

- sodium at a content less than 5 milligrams per gram of total nitrogenous substance,

- magnesium at a content less than 3 milligrams per gram of total nitrogenous substance,

- potassium at a content less than 5.5 milligrams per gram of total nitrogenous substance,

- chloride at a content less than 1.7 milligrams per gram of total nitrogenous substance, and

- phosphorus at a content less than 8.5 milligrams per gram of total nitrogenous substance.

- more specifically, the demineralised milk protein composition can comprise:

- calcium at a content less than 7.7 milligrams per gram of total nitrogenous substance,

- sodium at a content less than 3.65 milligrams per gram of total nitrogenous substance,

- magnesium at a content less than 2.15 milligrams per gram of total nitrogenous substance,

- potassium at a content less than 3.70 milligrams per gram of total nitrogenous substance,

- chloride at a content less than 1.5 milligrams per gram of total nitrogenous substance, and

- phosphorus at a content less than 7.7 milligrams per gram of total nitrogenous substance.

- the demineralised milk protein composition likely to be obtained by the method such as defined above has a percentage of native proteins in relation to the total proteins which is more than 85.

5 - the demineralised milk protein composition has a percentage of native proteins in relation to the total proteins which is more than 90.

- the clustering rate of A-lactalbumin protein in said composition is less than 5%.

- the average diameter of type D4.3 of particles of the demineralised milk protein composition likely to be obtained by the method defined above is less than 0.3 micrometres.

10 - the average diameter of type D4.3 of particles of the demineralised milk protein composition is less than 0.2 micrometres and in that at least 40% of particles in the volume have a size less than 0.15 micrometres.

The invention is finally based on the use of the demineralised protein composition such as defined above, wherein the use of this composition is used as an ingredient for developing baby milk.

15 Advantageously, baby milk comes from organic farming.

Other characteristics and advantages of the invention will clearly emerge from the description which is given below, for information purposes and not at all exhaustive, and in reference to the following figures

20 - figure 1 is a synoptic diagram of the method for producing a demineralised milk protein composition according to the invention and according to two different ways of providing or producing the milk protein composition intended to undergo steps of demineralisation,

25 - figure 2 illustrates the mineral contents respectively obtained from the demineralised composition of the invention and from the composition of the prior art, as well as the maximum regulatory levels authorised for these minerals in a baby milk,

- figure 3 represents the fluorescence spectrum of a pure alpha lactalbumin solution subject to the pH level fluctuations of the method of the invention,

30 - figure 4 represents the fluorescence spectrum of a pure alpha lactalbumin solution subject to the pH level fluctuations of the method of the prior art, and

- figure 5 illustrates the distribution of the size of the particles in the composition of the invention and in the composition of the prior art.

The method of the invention essentially resides in the succession, in order, of the steps of ultrafiltration, nanofiltration and electrodialysis of a milk protein composition. This milk protein composition can, as an example, be cheese whey or creamed raw milk, heat-treated, bactofugated and microfiltered.

5 In the following description, the use of the expression “prior art” refers to the method and to the associated demineralised composition obtained when ion-exchange resins are used.

In reference to figure 1, a first place of developing the milk protein composition 1 from raw milk 2 is defined. The raw milk 2 preferably comes from the organic sector.

10 The raw milk 2 undergoes, in the first place, a creaming 3 under standard conditions, with a creaming temperature of around 50°C, then the creamed milk 4 undergoes a heat treatment 5 at a temperature of around 65°C-68°C for less than one minute. This heat treatment is voluntarily limited to this step, to avoid the clustering of serum proteins on the casein micelles, and to obtain the best protein yield in the later
15 step of microfiltration.

The creamed and heat-treated milk 6 then undergoes a mechanical bactofugation 7 to ensure the bacterial purification thereof, and to perfect the creaming thereof. Alternatively, another bacterial purification operation can be provided such as, for example, microfiltration.

20 The creamed, heat-treated and bactofugated milk then undergoes a holding step (not represented), at a temperature of around 50°C-52°C in view of the preparation thereof for the microfiltration step 10.

The composition of creamed, heat-treated and bactofugated milk is given in Table 1 below. The total nitrogenous substance mainly surrounds proteins, peptides and
25 non-protein nitrogen, for example, urea. In the results shown below, the total nitrogenous substance is quantified by the dosage of total nitrogen per distillation according to the Kjeldahl method.

pH level at 20°C	6.6-6.7
Dry extract (g/L)	90-96
Total nitrogenous substance (as a % of dry extract)	36-39
Non-protein nitrogenous fraction (g/L)	1.8-2.2

Residual fatty substance (g/L)	0.4-1
Ash (g/L)	<9
Lactose	46-50

Table 1: Composition of the creamed, heat-treated and bactofugated milk

The microfiltration 10 is a tangential microfiltration, of which the ceramic membrane has a porosity of 0.1 micrometres. The functioning temperature thereof is between 49°C and 53°C, preferably 52°C. The permeate 11 undergoes a cooling 12 and is returned to a temperature of between 10°C and 15°C, preferably 12°C. The cooled permeate 11 then forms a milk protein composition 1a that comes from the milk and is intended to undergo later successive steps of concentration and demineralisation, which will be defined later.

The milk protein composition obtained 1a has a dry extract content of around 57g/kg to 62g/kg, a total nitrogenous substance of around 9% to 12% of dry extract, and a fatty substance content of 0.5% of dry extract.

The milk protein composition 1 can alternatively come from cheese whey of an organic origin 13, which undergoes a cooling 14 to be recovered to a temperature of between 10°C and 15°C, preferably 12°C, and form a milk protein composition 1b.

The milk protein composition 1 to be demineralised can thus either be a milk protein composition 1a that comes from an organic origin, or a milk protein composition 1b that comes from cheese whey of an organic origin. The milk protein composition can, of course, not be from an organic origin.

The milk protein composition 1 then undergoes a succession of membrane operations. The example below relates to the demineralisation of the milk protein composition 1a that comes from milk of an organic origin 2.

First, the milk protein composition 1a undergoes an ultrafiltration operation 15, from which the retentate 16 is recovered. This ultrafiltration operation is likened to a pre-concentration and a first demineralisation of the milk protein composition 1. This ultrafiltration operation 15 is led so that the retentate 16 has a dry extract content of more than 60g/kg, a fatty substance content of less than 0.5% of dry extract, but in particular, a total nitrogenous substance content at least equal to 15% of dry extract. The nitrate and nitrate content is zero or almost zero. Moreover, this operation enables

an increase in the total nitrogenous substance content, and the partial elimination of the soluble cations, the result of which being the decrease in the ratio between divalent cations and the total nitrogenous substance content.

The ultrafiltration retentate 16 then undergoes a nanofiltration operation 17, which enables to concentrate the ultrafiltration retentate 16 to more than 19% dry extract and preferably more than 21% dry extract. This operation enables to optimise the functioning of the later electrodialysis step, by further ensuring a pre-demineralisation up to 30% to 35% in mineral weight. The nanofiltration retentate 18 is recovered, and has, other than a dry extract content of more than 21%, a total nitrogenous substance content of more than 13.6% of dry extract, and still a fatty substance content of less than 0.5% of dry extract.

The nanofiltration retentate 18 then undergoes an electrodialysis operation 19, led under conditions suitable for obtaining a demineralised milk protein composition 20. During the electrodialysis, the nanofiltration retentate 18 circulates in a diluate chamber 21. Under the effect of an electric field, the nanofiltration retentate 18 demineralises and the ions pass into a concentration chamber 22, selectively crossing the anion and cation membranes 23. The circulation of the nanofiltration retentate 18 is done until the level of demineralisation is reached for a conductivity of between 0.3mS/cm and 0.5mS/cm, preferably between 0.3mS/cm and 0.4mS/cm to obtain better still demineralisation performances.

The demineralised whey 20 has a dry extract content of around 192 to 194 grams per kilogram, in any case, more than 190 grams per kilogram, a fatty substance content less than 0.5% of dry extract, a nitrogenous substance content of around 15% to 16% of dry extract, in any case at least equal to 14% of dry extract, a lactose content of less than 83% of dry extract, and an ash content of less than 1% of dry extract, preferably less than 0.7% of dry extract. The nitrate and nitrite content is zero or almost zero.

Table 2 below highlights the synergy between the nanofiltration and electrodialysis operations. From a milk protein composition 1 (column 1) obtained from raw milk of an organic origin that has undergone the treatment defined before, and experiencing percentages respective of demineralisation for nanofiltration (column 2) and for electrodialysis (column 4), the theoretical contents have been calculated of a demineralised whey (column 5). These theoretical contents corresponding to the sum of

the percentages of demineralisation of nanofiltration and of electrodialysis. In column 6, the mineral contents actually obtained after going through nanofiltration and electrodialysis have been reported, in the framework of the method of the invention.

	Milk protein composition (1a)	% of demineralisation by nanofiltration	% of demineralisation by electrodialysis	Theoretical demineralised whey	Demineralised whey according to the method of the invention
calcium (mg/kg of dry extract)	5010	2.5	37.3	3066	651
magnesium (mg/kg of dry extract)	1095	3.2	24.7	798	208
potassium (mg/kg of dry extract)	24585	35.6	71.0	4593	313
sodium (mg/kg of dry extract)	5920	36.8	54.5	1703	339
phosphorus (mg/kg of dry extract)	5615	-7.5	45.0	3321	990

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Table 2: Highlighting of the synergy between the steps of nanofiltration and electrodialysis

Prior art 1: Organic demineralised powder whey obtained by a first method comprising passing over ion-exchange resins

Prior art 2: Demineralised powder whey obtained by a second method comprising passing over ion-exchange resins

Invention 1: Demineralised liquid whey obtained from a milk protein composition that comes from milk of an organic origin – conductivity at the end of electrodialysis: 0.5mS/cm – without pH level adjustment – results expressed in milligrams per gram of Total Nitrogenous Substance

Invention 2: Demineralised liquid whey obtained from a milk protein composition that comes from milk of an organic origin – conductivity at the end of electrodialysis: 0.4mS/cm – without pH level adjustment – results expressed in milligrams per gram of Total Nitrogenous Substance

	Prior art 1 (mg/100mg)	Prior art 2 (mg/100mg)	Inventio n 1 (mg/g MAT)	Inventio n 2 (mg/g MAT)	Inventio n 3 (mg/g MAT)	Inventio n 4 (mg/g MAT)
Sodium	7	80	3.63	2.48	2.35	5
Potassium	3.5	185	3.30	2.59	1.80	5.5
Calcium	15	30	7.66	5.78	4.71	11
Magnesium	8	16.5	2.14	1.64	1.38	3
Phosphorus	85	100	7.62	7.15	6.86	8.5
Chlorides	30.5	15	1.25	1.45	1.14	1.7
Ash	0.4% of dry extract	0.9% of dry extract	0.6% of dry extract	0.4% of dry extract	0.4% of dry extract	-
Ca/P ratio	0.49	0.3	1.01	0.81	0.69	1.29

Invention 3: Demineralised liquid whey obtained from a milk protein composition that comes from milk of an organic origin – conductivity at the end of electrodialysis: 0.35mS/cm – without pH level adjustment – results expressed in milligrams per gram of Total Nitrogenous Substance

Invention 4: Extrapolation of the results of the Inventions 1, 2 and 3 for a conductivity at the end of electrodialysis of 0.7mS/cm – results expressed in milligrams per gram of Total Nitrogenous Substance

Table 3: Mineral compositions of demineralised milk protein compositions of the prior art and of the invention – comparison of ratios of calcium and phosphorus contents

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It can be observed in Tables 2 and 3, that the method of the invention enables to obtain a demineralised milk protein composition up to 90%, by a succession of membrane methods and this, without using cation-exchange resins or anion-exchange resins.

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Moreover, it can be observed, relating to Table 2 above, that the actual demineralisation rates obtained by the method of the invention are at least four times more than those theoretically calculated.

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Moreover, it can be observed, that the demineralised whey of the invention, advantageously has a low phosphorus content and a calcium content that is sufficiently controlled, so that the ratio of the calcium and phosphorus contents are between 0.65 and 1.29. This ratio comes close to that of milk, which is around 1.25, and this contrary to the demineralised whey of the two methods of the prior art, which have respective calcium to phosphorus ratios of 0.49 and 0.3.

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Obtaining a higher ratio of calcium and phosphorus contents advantageously enables to limit the quantity of minerals to add during the later development of baby milk.

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Figure 2 illustrates the content of certain minerals (sodium, potassium, calcium, phosphorus, magnesium and chlorides), expressed in weight per 100kcal in a mixture made from a demineralised milk protein composition and of milk, before any addition of minerals, in view of developing a first-age baby milk. The results referenced 30 relate to the case where the demineralised milk protein composition is that of the invention, and

the results referenced 31 relate to the case where the demineralised milk protein composition is that of the prior art. In this figure, maximum regulatory levels authorised for these minerals (reference 32) in a first-age baby milk are also represented. It is observed that the demineralised milk protein composition of the invention, when mixed with milk, in view of producing a baby milk, for example, a first-age milk, has a mineral profile less than the maximum regulatory levels authorised. These results also illustrate the high latency possible to add certain minerals according to the type of baby milk in question, and this, without exceeding the maximum levels authorised.

The method of the invention defined in this example of an embodiment applies to the demineralisation of any milk protein composition.

The method of the invention enables to produce a new production line of a demineralised milk protein composition up to 90% with a specific mineral profile, compatible with the baby milk formulation, and this, only by filtration membrane and demineralisation methods. The demineralised milk protein composition obtained thus respects specific ionic specifications, in particular to serve as an ingredient for producing baby milk. The method of the invention does not require any technological auxiliary addition which changes the intrinsic composition of the product. The absence of ion-exchange resins enables the demineralised milk protein composition obtained to satisfy the conditions defined in European regulation CE 889/2008 governing the production of transformed products of an organic origin, on the single condition that the raw milk or cheese whey used to produce the milk protein composition comes from organic farming.

The Applicant was surprised to observe that the method of the invention led to advantageous consequences regarding the demineralised milk protein composition obtained, going beyond the main objective of producing a demineralised milk composition without using ion-exchange resins.

It has indeed been observed, in the first place, that the rate of native proteins in the demineralised milk protein composition of the invention is clearly higher than the rate of native proteins presents in the demineralised composition of the prior art. Or, in other words, the rate of protein denaturation in the demineralised milk protein composition of the invention is lower than the protein denaturation rate in the demineralised composition of the prior art.

This low denaturation rate is directly connected to the method of the invention which involves no significant variation in pH level, nor substantial heat treatment as is demonstrate above.

Figure 3 and 4 present, respectively for the demineralised protein composition of the invention and for the demineralised composition of the prior art, fluorescence spectrums intrinsic to a pure alpha lactalbumin solution, to which pH level fluctuations of the method of the invention and of the method of the prior art have been applied. These figures reflect the fluorescence emitted during the excitation of "tryptophan" amino acids present within alpha lactalbumin.

In the method of the invention, wherein no pH level adjustment is made, the pH level fluctuates from around 6.7 to 5.3.

In reference to figure 3, the curve referenced 33 represents the fluorescence spectrum of the alpha lactalbumin solution at a pH level of 6.7. This curve has a maximum lambda of 329 nanometres, characteristic of the native state of this protein. The curve referenced 34 represents the fluorescence spectrum of the alpha lactalbumin solution at a pH level of 5.3. It is observed, that the look of the spectrum changes by expanding and by having a maximum decreased fluorescence intensity. This modification of the spectrum illustrates a change in the structure of protein structures, characteristic of a protein clustering phenomenon.

The curve reference 35 represents the fluorescence spectrum of the alpha lactalbumin solution when the pH level of 5.3 is raised to 6.7, in order to check that the modifications of the protein structures can be reversed. It has been observed, that while the look of the curve comes back to be the same as and superimposable on the initial curve 33. In particular, the maximum fluorescence intensity is again 329 nanometres.

These results illustrate that the acidification of a minimum pH level of 5.3 leads to a protein clustering phenomenon, this clustering is totally reversible. Thus, the structure of the proteins in the demineralised composition of the invention undergoing a low acidification of a pH level of 5.3, is totally protected.

In the method of the prior art, wherein ion-exchange resins are used, the pH level fluctuates from around 6.5 down to a maximum pH level of between 2 and 2.5 before being adjusted to 6.5. The method of the prior art also involves the implementation of a heat treatment higher than 90°C, as will be detailed later.

In reference to figure 4, the curve 36, like the curve 33 in figure 3, represents the fluorescence spectrum of the alpha lactalbumin solution at a pH level of 6.7 and has a maximum emitting wavelength of 329 nanometres. The curve 37 illustrates the spectrum obtained when the alpha lactalbumin solution is brought to a pH level of 2.4, and the curve 38 illustrates the spectrum obtained when the alpha lactalbumin solution at a pH level of 2.4 undergoes a heat treatment of 95°C.

It is observed that if the curves 37 and 38 superimpose each other, they present a difference in relation to the curve 36 that has a maximum emitting wavelength of 334 nanometres. This difference towards larger maximum emitting wavelengths, illustrates the partial unfolding of the protein unit structure.

The curve 39 represents the fluorescence spectrum of the alpha lactalbumin solution when the pH level of 2.4 with heat treatment (curve 38) is raised to 6.5.

It is observed that the curve 39 cannot be superimposed with the initial curve 36, the fluorescence intensity being lower, and the maximum wavelength being decreased to around 324 nanometres.

It results in the modifications of the protein structure induced by the acidification at a pH level of 2.4, with or without heat treatment, not being reversible for the demineralised composition of the prior art, which conveys the protein losing the native state thereof.

Further to the variations in pH level that are less significant in the method of the invention than in the method of the prior art, the variations in temperatures to which the proteins are subjected in the method of the invention, are also less significant than in the method of the prior art, which guarantees keeping the native structure of the proteins.

In the method of the invention, a temperature of around 65°C-68°C is applied during the heat treatment of the creamed milk, then the temperature is brought down to 10°C-12°C during demineralisation. Optionally, a step of pasteurisation at 72°C for a few seconds can be applied to the demineralised composition. In the method of the prior art, the use as a raw material of a cheese whey requires a first pasteurisation operation, carried out at a temperature higher than 72°C, then, after demineralisation, a second pasteurisation step at a temperature higher than 90°C is carried out.

If it is considered that the temperatures of thermal denaturation, irreversible from three major protein species present in the soluble phase of the milk, namely

bovine serum albumin, alpha lactalbumin and beta lactoglobulin, respectively of 63°C, 69°C and 72°C, it is observed that the temperatures of the method of the invention are less than the temperatures of denaturation of alpha lactalbumin and beta lactalbumin. It results in the state of these proteins not being changed by the method of the invention.

5 On the contrary, the temperatures of the method of the prior art lead to a change in the structure of these three protein species.

Table 4 below is referred to, which presents the analytical protein dosage results, enabling to characterise the protein contents in their native state and their clustering rate.

10 The protein composition of the demineralised milk protein compositions has been determined by means of a chemical method (dosage of nitrogen by the Kjeldahl technique) and by means of liquid chromatography in the reverse phase led, using analyses under different operational conditions.

15 The composition in soluble proteins has been determined by chromatography in the liquid phase, following the elimination of denatured proteins and residual caseins by precipitation at a pH level of 4.6. This analysis enables to determine the protein contents present in their native state.

20 The analysis of the total proteins has also been determined after separating clusters. This technique enables, in particular, to measure the overall protein denaturation of the sample (clustering rate), but also that of individual proteins.

The results in Table 4 are also presented for the most representative proteins, namely beta lactoglobulin and the Kappa casein, the 2 heat-sensitive proteins which co-cluster under the effect of heat treatments, as well as for alpha lactalbumin, which is the nutritional protein of interest in baby nutrition.

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	Demineralised composition of the invention	Demineralised composition of the prior art
Nitrogenous fraction, soluble at a pH level = 4.6 (in relation to the total nitrogenous fraction)	96%	69%
% of native proteins (in	93%-97%	<85%

relation to the % of total proteins)		
Clustering rate of beta lactoglobulin (in relation to the total beta lactoglobulin)	<8%	15%
Clustering rate of the Kappa casein (in relation to the total Kappa casein)	≤20%	55%
Clustering rate of alpha lactalbumin (in relation to the total alpha lactalbumin)	<5%	Not determined

Table 4: Analytical results of protein dosage in the composition of the invention and in the composition of the prior art.

5 It is confirmed by the results presented in Table 4, that the percentage of native proteins in the demineralised composition of the invention is clearly higher than that of the composition of the prior art. Moreover, it is observed that the clustering rates of beta lactoglobulin and of the Kappa casein are lower in the composition of the invention. Finally, the clustering rate of alpha lactalbumin is also low.

10 Figure 5 is referred to, which illustrates the size of the particles present in the composition of the invention (curve 41) and in the composition of the prior art (curve 42). The size of the particles is measured by the grain size technique. In the composition of the invention, it is observed that a size distribution of quite consistent particles, with a highly predominant population of small-sized particles and very low peak
15 corresponding to the particles (or particle clusters), of a slightly larger size. The average diameter of type D4.3 is 0.19 micrometres and 50% of the particles (in volume) have a smaller size of 0.14 micrometres.

 On the contrary, the size distribution is bimodal for the composition of the prior art, with a small-sized population and a population corresponding to large clusters. This
20 data is found in characteristic sizes, with an average diameter of type D4.3 of 5.17

micrometres and respectively 50% and 90% of the particles (in volume) having a larger size of 0.75 micrometres and 11.8 micrometres.

These results fall into the continuity of keeping the protein structure resulting from the method of the invention, since it is thus observed that the main part of the particles of the composition of the invention are not clustered, while on the contrary, in the composition of the prior art, further to undergoing denaturation, the proteins are, for a large part of them, in the form of clusters.

It has thus been demonstrated previously, that serum proteins of the demineralised composition of the invention were predominantly kept in their non-clustered, native state. On the contrary, the serum proteins of the composition of the prior art are in a partially denatured and clustered state, with a mixture of individual proteins and larger-sized clusters.

It results in a different in performance of serum proteins of the composition of the invention and of the composition of the prior art, and more specifically, a difference in interacting with the casein micelles provided by the milk ingredient of the baby formula.

This difference in the structural state of serum proteins will have an impact on their capacity to interact with the micelle structure during the heat treatment step prior to dehydrating the baby milk.

Indeed, native serum proteins have a larger capacity to bond with the surface of the micelle structure than denatured, clustered serum proteins. The consistency and the thickness of the serum cortex to the micelle surface will thus be more significant in the case of the composition of the invention, which could have an impact on the accessibility of casein micelles, slow down their degradation by digestive enzymes in the gastrointestinal tract and have an impact on the level of allergenicity of the caseins by reducing de facto the allergenicity of the composition of the invention.

Table 5 below presents the contents of the essential amino acids of major nutritional interest (histidine, tryptophan, tyrosine and leucine), in essential and semi-essential amino acids and in amino acids connected to the demineralised composition of the invention and to the demineralised composition of the prior art. The essential amino acid content is a particularly significant parameter in baby milk, the infant not having the capacities to synthesise them; these must therefore be provided by means of food, more specifically before the weaning phase.

	Composition of the invention	Composition of the prior art	Difference
Histidine	2.30 ± 0.05	1.89 ± 0.05	+22%
Tryptophan	2.20 ± 0.14	1.85 ± 0.25	+19%
Tyrosine	3.23 ± 0.15	2.68 ± 0.14	+21%
Leucine	12.10 ± 0.23	10.84 ± 0.12	+12%
Essential and semi-essential amino acids	56.5 ± 0.5	54.4 ± 0.4	+4%
Chained amino acids	22.4	21.8	+3%

Table 5: Contents of the essential amino acids of major nutritional interest, in essential and semi-essential amino acids, and in chained amino acids in the composition of the invention and to the composition of the prior art

It is observed that the composition of the invention has a high essential amino acid content, particularly for those of major nutritional interest.

This result is particularly significant for tryptophan. Indeed, regulations forbid the addition of tryptophan in all the forms thereof in organic baby milk. Yet, tryptophan is present in a large quantity in breastmilk and is an active component in the infant's proper development. Thus, the composition of the invention naturally comes closer to the composition of breastmilk, also from the viewpoint of tryptophan content.

Furthermore, tryptophan is defined as a precursor of serotonin and melatonin. Tryptophan would thus intervene positively in regulating sleep and stress, likewise for concentration.

Finally, grain size analyses have been carried out on the fatty substance forming supernatant, after centrifugation, of a reconstituted baby milk, this being developed from the composition of the invention or from the composition of the prior art.

The size of the particles is measured by the grain size technique, which enables to measure the individual fat droplet size when the measurement is taken in the presence of SDS, and the size of fat droplet clusters, in the case where there is

flocculation (measurement only in water). In this case, the results are expressed in D4.3 (in micrometres), which increases with the clustering rate of the droplets.

It has been determined that the average diameter of type D4.3 for the composition of the invention is 0.75 ± 0.03 micrometres, whereas the average diameter of type D4.3 for the composition of the prior art is 4.59 ± 0.05 micrometres. It is thus observed, that the flocculation phenomenon is less significant in a reconstituted baby milk, developed from the composition of the invention, the majority of fatty globules being in an individual state. On the contrary, in a reconstituted milk developed from the composition of the prior art, a significant proportion of fatty globule clusters is observed.

It has also been determined that the average size of individual fatty globules for the composition of the invention is 0.51 ± 0.02 micrometres, whereas the average size of individual fatty globules for the composition of the prior art is 0.59 ± 0.02 micrometres.

It results in a more significant stability in a reconstituted baby milk from the composition of the invention. This more favourable organisation of the fatty substance is, without doubt, linked to the reduced protein denaturation during the method of the invention and to the favourable interaction of proteins of the composition with micelle structures, such as stated above.

It has thus been demonstrated above, that further to the beneficial effects on the quality of the proteins, the method of the invention and the resulting demineralised composition, also contribute to a better functionality of the end product.

The qualities stated above of the composition of the invention are mainly linked to the fact that the method of the invention leads to no excessive acidification or heat treatment that alter proteins and other components of the raw material.

Remaining in the framework of the invention, the demineralised composition defined above could be obtained by another method than that defined above, on the condition that this method respects the low fluctuations in pH level and temperature.

CLAIMS

1. Method for producing a demineralised milk protein composition, characterised in that it comprises at least the steps of:
 - 5 - preparing (2, 3, 5, 7, 9, 10, 12) or providing a milk protein composition (1, 1a, 1b),
 - ultrafiltration (15) of said milk protein composition (1),
 - nanofiltration (17) of the ultrafiltration retentate (16) obtained in the preceding step, and
 - 10 - electrodialysis (19) of the nanofiltration retentate (18) obtained in the preceding step,
 - said method being free of any step that involves passing over ion-exchange ions.
2. Method according to claim 1, characterised in that the ultrafiltration retentate

15 (16) has a total nitrogenous substance content at least equal to 14% of dry extract.
3. Method according to any one of claims 1 and 2, characterised in that the milk protein composition (1) is cheese whey (13).
- 20 4. Method according to claim 3, characterised in that the cheese whey (13) comes from organic farming.
5. Method according to any one of claims 1 and 2, characterised in that the milk protein composition (1) comes from a method comprising at least the steps of:
 - 25 - provision of raw milk (2),
 - creaming (3), heat treatment (5) and bacterial purification (7) of said raw milk (2),
 - microfiltration on milk membrane (10) obtained in the preceding step, and
 - recovery of the microfiltration permeate (11) forming the milk protein

30 composition (1a).
6. Method according to claim 5, characterised in that the microfiltration membrane has a porosity of between 0.1 and 0.2 micrometres.

7. Method according to any one of claims 5 and 6, characterised in that the raw milk (2) comes from organic farming.

5 8. Demineralised milk protein composition, characterised in that it is likely to be obtained by the method according to any one of the claims 1 to 7.

9. Demineralised milk protein composition according to claim 8, characterised in that the ratio between calcium and phosphorus contents is more than 0.65.

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10. Demineralised milk protein composition according to claim 9, characterised in that it comprises:

- calcium at a content less than 11 milligrams per gram of total nitrogenous substance,

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- sodium at a content less than 5 milligrams per gram of total nitrogenous substance,

- magnesium at a content less than 3 milligrams per gram of total nitrogenous substance,

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- potassium at a content less than 5.5 milligrams per gram of total nitrogenous substance,

- chloride at a content less than 1.7 milligrams per gram of total nitrogenous substance, and

- phosphorus at a content less than 8.5 milligrams per gram of total nitrogenous substance.

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11. Demineralised milk protein composition according to any one of the claims 9 and 10, characterised in that it comprises:

- calcium at a content less than 7.7 milligrams per gram of total nitrogenous substance,

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- sodium at a content less than 3.65 milligrams per gram of total nitrogenous substance,

- magnesium at a content less than 2.15 milligrams per gram of total nitrogenous substance,

- potassium at a content less than 3.70 milligrams per gram of total nitrogenous substance,

- chloride at a content less than 1.5 milligrams per gram of total nitrogenous substance, and

5 - phosphorus at a content less than 7.7 milligrams per gram of total nitrogenous substance.

12. Demineralised milk protein composition according to claim 8, characterised in that it has a percentage of native proteins in relation to the total proteins which is more
10 than 85.

13. Demineralised milk protein composition according to claim 12, characterised in that it has a percentage of native proteins in relation to the total proteins which is more
15 than 90.

14. Demineralised milk protein composition according to any one of the claims 12 and 13, characterised in that the clustering rate of A-lactalbumin protein in said composition is less than 5%.

20 15. Demineralised milk protein composition according to claim 8, characterised in that the average diameter of type D4.3 of particles of said composition is less than 0.3 micrometres.

25 16. Demineralised milk protein composition according to claim 15, characterised in that the average diameter of type D4.3 of particles of said composition is less than 0.2 micrometres and in that at least 40% of particles in the volume have a size less than 0.15 micrometres.

30 17. Use of a demineralised milk protein composition according to any one of the claims 8 to 16, wherein said demineralised milk protein composition is used as an ingredient for developing a baby milk.

18. Use according to claim 17, characterised in that the baby milk comes from organic farming.