

NUTRITIONAL MINERAL FORTIFICATION OF MILK

Technical Field

The present invention relates to a process for producing calcium
5 or other nutritional mineral fortified milk, toned milk, or milk powders.

Background to the invention

Calcium is a mineral essential in human nutrition and comprises
approximately 1.5 to 2 percent of total adult body weight. Besides providing the
10 skeletal structure for bones and teeth, calcium plays a key role in many other
day-to-day functions of the body. Calcium is important for the normal clotting of
blood, the conduction of nerve impulses, the contraction and relaxation of
muscles as well as the regulation of body fluids, hormone secretion and cell
division.

15 Calcium has received increased attention in dietary regimes in
recent times because of its possible role in the prevention of diseases such as
osteoporosis. The recommended daily allowance (RDA) for calcium is 1200mg
for women and 800mg for men. Dairy products are considered an excellent
source of calcium and the RDA is essentially met through the intake of dairy
20 products.

Milk is a dairy product and as such a source of calcium. Milk
generally contains 1200mg per litre of calcium. However, as the demand for
calcium has increased, it has become necessary to produce calcium fortified
milk with a larger amount of calcium in a single serve.

25 A calcium fortified milk provides to people who do not choose to
consume large amounts of dairy products an alternative to calcium mineral
supplements.

Some Calcium fortified milk products use relatively insoluble forms of calcium
such as tricalcium phosphate or calcium carbonate that have the disadvantage
30 of having a gritty taste and sedimentation of the calcium salt. These forms of
calcium require the use of suspension agents to maintain the calcium in
suspension.

It has been observed that the addition of calcium salts to milk causes a drop in

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pH. This drop in pH is thought to be one reason for lack of heat stability in such calcium fortified milks where sedimentation occurs after pasteurisation. The raised calcium activity of milks with added calcium also contributes to heat instability. This has led some producers to add calcium after pasteurisation.

5 The specification of US Patent No. 5,449,523 discloses a process for the preparation of a calcium fortified yoghurt. The process includes the steps of mixing a fermentable dairy product with an alkaline agent, a chelating agent [preferably alkali metal citrates] and a source of soluble calcium. The alkaline agent and chelating agent are added to the mixture in amounts
10 effective to maintain the pH of the yoghurt base mix above about 6.7. The yoghurt base mix is then pasteurised, cooled and inoculated with a yoghurt starter. The specification states that the invention is based on the unexpected discovery that the heat stability of yoghurt can be dramatically improved if the pH is not allowed to drop below about 6.7 prior to pasteurisation. Furthermore,
15 a source of additional calcium can be added to the milk whilst maintaining the pH above 6.7 by adding a chelating agent and/or an alkaline agent at approximately the same time as the source of calcium in amounts effective to prevent the pH from dropping below 6.7. Experience has shown that milk stabilised with citrates and other chelating agents suffer from translucency
20 apparently caused by migration of calcium from the protein micelles and release of casein into the serum.

Increasingly other nutritional minerals such as magnesium iron, zinc are being added to milk and milk products.

It is an object of this invention to provide heat stable calcium and other
25 nutritional mineral fortified milks that do not suffer any of the defects discussed above.

A further object of this invention is to produce calcium and other nutritional mineral fortified milk powders that have good heat stability when reprocessed after reconstitution.

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Brief description of the invention

This invention is predicated on the discovery that calcium and/or nutritional mineral fortified milk can be stabilised by the addition of an effective

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amount of a phosphate which enables calcium and/or nutritional minerals to migrate into the protein micelles and maintains the pH so that it lies within a range from 6.5 to 7.5 preferably between pH 6.8 -7.0. This is in contrast to the effect of chelating agents such as citrates and polyphosphates which at high concentrations disintegrate the casein micelle causing migration of calcium from the micelles and releasing casein into the serum. In contrast the phosphate added according to the present invention preserves the integrity of the casein micelles.

Throughout this specification the term milk or fortified milk means a milk based product including fresh milk, reconstituted milk, modified milk or enriched milk that is either intended as a beverage or as an ingredient to be used for further processing. The ingredient may be milk, concentrated milk or milk powder and further processing includes processing to make other dairy products such as cheese, yoghurt, powdered milk starting from milk, concentrated milk or milk powder.

Nutritional minerals other than calcium are iron, magnesium, zinc or manganese.

Accordingly, the present invention provides a process for producing a calcium or nutritional mineral fortified milk, the process including the steps of:

- adding a soluble calcium and/or a nutritional mineral compound to a milk,
- adding either before or after the calcium and/or nutritional mineral addition an effective amount of a phosphate which enables the calcium and/or nutritional mineral to migrate into the protein micelles and maintains the pH so that it lies within a range from 6.5 to 7.5

The preferred phosphate is a pyrophosphate or orthophosphate and is preferably one or more of monosodium dihydrogen orthophosphate, disodium hydrogen orthophosphate, tri sodium orthophosphate, monopotassium dihydrogen orthophosphate, dipotassium hydrogen orthophosphate and tri potassium orthophosphate

In a further aspect, the present invention provides a calcium or

nutritional mineral fortified milk which includes milk, a soluble calcium or nutritional compound and an amount of a phosphate which enables calcium or the nutritional mineral to migrate into the protein micelles and maintains the pH so that it lies within a range from 6.5 to 7.5, effective in stabilising the milk during heat treatment.

The added calcium or nutritional mineral according to the present invention does not require the addition of suspension agents. Preferably the calcium and/or nutritional mineral is added as a soluble salt. It is thought that the minerals added to the milk migrates, in the presence of ortho phosphate, into the micelle phase of the milk where it is held in colloidal suspension in the micelles. Other forms of soluble calcium or nutritional mineral when added with chelating agents such as citrates have the opposite effect of encouraging calcium migration out of the micelles which results in the translucent appearance of the milk. Because the orthophosphates encourage migration into the micelles the upper limit to calcium fortified content in heat stable milks is also increased.

The pH range which ensures heat stable fortified milk is wider when using the phosphates of this invention than is the case with chelating agents such as citrates. The pH of the calcium fortified milk may be controlled by the addition of an appropriate phosphate or orthophosphate or mix of phosphates or orthophosphates. For example, trisodium orthophosphate is highly alkaline, disodium orthophosphate is slightly alkaline and monosodium orthophosphate is mildly acidic. It is also within the scope of this invention to adjust the pH within the range of 6.5 to 7.5 using an appropriate food grade alkali or acid. The quantity of alkaline agent added to the calcium fortified milk is sufficient to cause the pH to fall within the specified range. Optimally this is close to pH 7, but adequate heat stability can be achieved if the pH lies in a range between 6.5 and 7.5.

The amount of orthophosphate added to the calcium fortified milk is largely dependent upon the amount of additional calcium added to the milk and the heat treatment to be applied. Normally, sufficient orthophosphate is added to the milk so that the ratio of added calcium to added orthophosphate is 1:1 on a molar basis. Higher or lower ratios may be utilised depending on

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the inherent heat stability of the milk and the level of calcium fortification.

However, the ratio of added calcium to added orthophosphate would normally lie in a range from 2:1 to 1:2 on a molar basis when a high heat treatment (for example 90°C for 10 minutes) is required. Lower levels of phosphate may be suitable to provide adequate heat stability under pasteurisation conditions (eg 72°C for 30 seconds).

In a preferred form of the invention, 8g of calcium is added per kilogram of finished calcium enriched skim milk solids. Thus, the actual level of calcium addition is dependent on the level of milk solids in the milk to be fortified. According to the standard text: P. Walstra and R. Jenness, "Dairy Chemistry and Physics", John Wiley & Sons, New York (1984), the calcium content of skimmed milk (containing 9 to 9.5% solids) is in the range 1200 to 1300 mg/litre. Therefore, if for example, a kilogram of standard skimmed milk contains 92.5g of solids and 1250 mg/litre of calcium, then the required level of calcium addition would be 785mg of calcium per kilogram of milk. The preferred level addition for the other nutritional minerals is intended to deliver the maximum recommended daily allowance per 100g of fortified milk solids. For iron zinc and manganese these are very small amounts compared to the daily requirements for calcium and magnesium.

In some cases when the correct form of orthophosphate is used, the alkaline agent may not be required depending on the condition of the milk and the season of the year. When other forms of orthophosphate are used, the quantity of alkaline agent required will be higher, but again it will also depend on inherent milk properties.

The order of addition of calcium compound orthophosphate and pH adjustment agent to the milk is not critical. Although it is obviously easier to correctly adjust the pH, if needed, after the calcium compound and the orthophosphate have been added.

The calcium and/or nutritional mineral fortified milk of the present invention can be pasteurised (eg 72°C for 30 seconds) or heat treated at a range of other conditions (eg: 90°C for 10 minutes). The low or high heat treatment of milk prior to concentration and drying to produce milk powder imparts specific attributes to milk powders. For example, a high heat treatment

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improves the water binding capacity, viscosity and gelation characteristics of the milk when reconstituted and used in specific applications. Low heat treatment and high heat treatment will produce milk powders that conform to either a low or high heat specification based on the whey protein nitrogen index (WPNI), the specification used in the international trade for skimmed milk powders. A multitude of other heating conditions is in use in the industry, which will achieve the same WPNI specifications.

The milk may be homogenised before or after addition of the calcium source.

The addition of an appropriate mix of pyrophosphates or orthophosphates to the milk or toned milk maintains the pH range similar to that of conventional milk. A resultant improvement in heat stability allows one to prepare a calcium-fortified milk wherein the calcium source can be added to the milk prior to heat treatment and/or pasteurisation without the undesirable formation of a milk protein precipitate.

The milk or toned milk may be homogenised. Any commonly employed homogenisation conditions can be satisfactorily employed in the present invention.

The heating step is carried out using techniques well known in the field, preferably at specified times and temperatures. At high levels of calcium fortification ultra-high temperatures are generally not employed for heating.

The milk or toned milk thus obtained can be enriched with calcium to a level 60% above that of the untreated milk.

In a third aspect of the invention, there is provided a process for producing a calcium fortified milk or toned milk powder, the process including the steps of:

- adding a soluble calcium and or/ a nutritional mineral compound to a milk,
- adding either before or after the calcium and/or nutritional mineral addition an effective amount of a phosphate which enables calcium and/or nutritional mineral to migrate into the protein micelles and maintains the pH so that it lies within a range

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from 6.5 to 7.5

- pasteurising or heating the calcium fortified milk, and
- concentrating and dehydrating the milk to form a calcium and/or nutritional mineral fortified dry milk or toned milk powder.

5 The calcium compound employed in the present invention is any food grade calcium source preferably a water-soluble calcium salt such as calcium gluconate, calcium chloride and calcium hydroxide. The nutritional mineral salts are also food grade water soluble chlorides or salts usually used in foods. The orthophosphate preferably employed in the present invention includes
10 monosodium dihydrogen orthophosphate, disodium hydrogen orthophosphate, trisodium orthophosphate, monopotassium dihydrogen orthophosphate, dipotassium hydrogen orthophosphate and tri potassium orthophosphate.

It is preferred that the sequence of addition of the above-mentioned substances to the milk is orthophosphate, followed by calcium
15 and/or nutritional mineral source and alkaline or acidifying agent if necessary.

The alkaline agent utilised in the process is preferably any food grade alkaline agent that has a minimal effect on the taste, or smell of the milk or toned milk. Preferred alkaline agents include alkali metal hydroxides. Preferred alkali metal hydroxides include potassium hydroxide, sodium
20 hydroxide or mixtures thereof.

A resultant improvement in heat stability allows one to prepare a calcium-fortified milk wherein the calcium source can be added to the milk prior to heat treatment and/or pasteurisation without the undesirable formation of a milk protein precipitate.

25 The dehydration step can be carried out using a variety of conventional techniques including spray drying, roller drying and freeze drying, however, spray drying is a preferred means for dehydration. Spray drying of the calcium fortified and concentrated milk or toned milk can be conducted under conventional conditions.

30 The milk or toned milk powder thus obtained is enriched with calcium some 60% above that of the untreated milk or toned milk.

In another aspect of this invention there is provided a method of testing to determine the heat sensitivity of the milk and consequently the type

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and quantity of phosphate needed to stabilise the fortified milk.

The first method is simply to add a predetermined amount of calcium salt and orthophosphate and measure the pH. If the pH is within the range of 6.5 to 7.5 no further action is needed. If the pH lies outside that range the pH may be
 5 adjusted or the mix of ortho phosphates changed to bring the pH within the desired range of 6.5 to 7.5.

Detailed Description of the Invention

In order to demonstrate the present invention, a number of
 10 experiments were conducted by the present inventors. The experiments investigated a process of producing calcium fortified milk or toned milk and further to this the production of calcium fortified milk and toned milk dry powder.

15 Example1

The use of a range of phosphates (orthophosphate, pyrophosphate, tripolyphosphate, polyphosphate) for stabilising Ca fortified milks was examined. Each of these has a differing effect on milk and could be used to stabilise Ca fortified milks (See Table 1). Without additives, skim milks (10%
 20 milk solids) with 20mM added calcium form a precipitate on heating at 90 °C for 10 min. The effect of added citrate and longer chain phosphates (polyphosphate), which is commonly used to stabilise Ca fortified milks, are included for comparison.

It was observed that citrate and the polyphosphate have a tendency to cause
 25 the milk to lose its whiteness (before heating) and it may take on a undesirable greenish - yellowish hue.

Table 1: Use of different additives for stabilising reconstituted skim milks (10% skim milk solids) with added ~20mM added Ca under selected conditions.

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Additive	Before heat		After heat (90°C/10 min)		
	pH	Free Ca activity (mM)	Viscosity (cPoise)	Total Ca (mg/litre)	Supernatant Ca (mg/litre)

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Control skim milk (10% milk solids) without added Ca at the natural pH

None*	6.63	1.78	1.7	1470	1450
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Skim milk(10% milk solids) with added Ca at pH~7

20mM orthophosphate* (K ₃ PO ₄ /K ₂ HPO ₄ mix)*	6.91	1.29	2.3	2290	2280
4mM pyrophosphate (Na ₄ P ₂ O ₇)	7.12	2.5	2.3	nd	nd
4mM tripolyphosphate (Na ₃ P ₃ O ₁₀)	7.00	2.54	2.4	2270	2220
8mM tripolyphosphate (Na ₃ P ₃ O ₁₀)	7.00	1.1	2.7	2240	2220
12mM tripolyphosphate (Na ₃ P ₃ O ₁₀)	7.01	0.64	3.2	2260	2260
0.15% Polyphosphate (Calgon T)	7.01	2.45	2.4	2580	2470
10mM Na ₃ citrate	7.00	2.65	2.0	2250	2230
20mM Na ₃ citrate	7.00	1.86	2.0	2090	2120

- 5 * In these systems, NaOH was not used. In all other systems, NaOH was used to adjust the pH of the milks to pH 7
nd - not determined

- 10 Supernatant Ca is that remaining in the supernatant of heated milk after low speed centrifugation (182 g for 10 min). It is an indication of calcium that is not precipitated by the heating process; n = data not recorded, na – not applicable

Example 2

- 15 Table 2 shows the effects of added orthophosphates on heat stability of Ca fortified milks with 20 mM added calcium. When no orthophosphate is added, skim milks (10% milk solids) with 20mM added calcium form a precipitate on heating at 90 °C for 10 min. Milks with a 1 to 1 molar ratio of added Ca to added phosphate are stable from ~pH 6.4 to pH 7.8. By altering the ratios of
20 added tri - di - mono- potassium orthophosphate the pH may be adjusted within the range 7.1 to 5.8 without the use of added NaOH.

Table 2 : Heat stability- pH profile for Ca fortified skim milks (10 % milk solids containing 20 mM added calcium) stabilised with added orthophosphates

Type of added phosphate			Before heat		After heat (90 °C/10min)	
Added K_3PO_4	Added K_2HPO_4	Added KH_2PO_4	pH	Free Calcium (mM)	Viscosity ¹ (cPoise)	Sediment ² (ml/50ml milk)
-	-	-	6.19	13.1	precipitated	nd
20 mM	-	-	7.67	0.55	2.8	0.1
20 mM	-	-	7.61	0.77	3.2	0.3
20 mM	-	-	7.56	0.64	2.6	0.2
20 mM	-	-	7.43	0.76	2.6	0.1
20 mM	-	-	7.33	0.88	2.5	0.2
20 mM	-	-	7.23	0.95	2.6	0.1
20 mM	-	-	7.19	1.02	2.7	0.1
20 mM	-	-	7.04	1.38	2.5	<0.1
16 mM	4 mM	-	6.89	1.62	2.3	0.1
12 mM	8 mM	-	6.75	1.98	2.4	0.2
8 mM	12 mM	-	6.63	2.22	2.5	<0.1
4 mM	16 mM	-	6.52	2.72	2.5	0.2
-	20 mM	-	6.41	3.36	4.2	0.1
-	16 mM	4 mM	6.28	4.16	7.0	0.5
-	12 mM	8 mM	6.14	5.33	precipitated	nd
-	8 mM	12 mM	6.01	6.71	precipitated	nd
-	4 mM	16 mM	5.86	9.49	precipitated	nd
-	-	20 mM	5.79	9.42	precipitated	nd

5 ¹ Determined at 30 rpm with a Brookfield LVDT-II viscometer fitted with a UL adaptor (equivalent to 36.7 s⁻¹)

² Determined as volume of sediment obtained following centrifugation of a 50 mL sample (Beckman J6-HC centrifuge, JA4.2 rotor, 800 rpm, 10 minutes - equivalent to ~182xg 10 minutes)

10 * The pH of these samples was adjusted by the addition of NaOH
nd- not determined

Example 3

15 Table 3 shows the effects of added tripolyphosphate on heat stability of Ca fortified milks with 20 mM added Ca. These examples demonstrate that tripolyphosphate is effective in stabilising Ca fortified milks over a wide range of pH when the correct ratio of added Ca : added tripolyphosphate is used. Visual examination of the milks with added tripolyphosphate indicated that
20 these milks developed an undesirable translucency

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Table 3 : Heat stability- pH profile for Ca fortified skim milks (10 % milk solids containing 20 mM added calcium) containing tripolyphosphate at three concentrations

Added Sodium Tripolyphosphate ($\text{Na}_3\text{P}_3\text{O}_{10}$)	Before heat		After heat (90 °C/10min)	
	pH	Free Calcium (mM)	Viscosity ¹ (cPoise)	Sediment ² (mg/50ml)
0	6.19	13.61	precipitated	nd
4 mM	6.14	6.38	>20	nd
4 mM	6.39*	4.96	7.4	14
4 mM	6.65*	3.64	2.6	0.7
4 mM	6.69*	3.23	2.5	1
4 mM	6.84*	2.93	2.6	0.2
4 mM	7.02*	2.41	2.3	0.1
4 mM	7.22*	1.89	2.5	0.2
4 mM	7.51*	1.50	2.7	0.2
8 mM	6.29	2.47	2.6	0.9
8 mM	6.56*	1.76	2.6	0.5
8 mM	6.69*	1.53	2.7	0.2
8 mM	6.84*	1.28	2.8	0.2
8 mM	7.08*	0.93	2.7	0.1
8 mM	7.17*	0.85	2.8	0.2
8 mM	7.36*	0.71	2.9	0.1
8 mM	7.82*	0.48	4.7	0.4
12 mM	6.51	1.27	2.4	0.1
12 mM	6.75*	1	2.6	0.2
12 mM	6.88*	0.8	2.8	0.1
12 mM	7.03*	0.71	3.8	0.2
12 mM	7.14*	0.62	14.4	0.3
12 mM	7.34*	0.53	>20	0.2
12 mM	7.54*	0.44	>20	nd
12 mM	7.79*	0.34	>20	nd

¹ Determined at 30 rpm with a Brookfield LVDT-II viscometer fitted with a UL adaptor (equivalent to 36.7 s^{-1})

² Determined as volume of sediment obtained following centrifugation of a 50 mL sample (Beckman J6-HC centrifuge, JA4.2 rotor, 800 rpm, 10 minutes - equivalent to $\sim 182 \times g$ 10 minutes)

* The pH of these samples was adjusted by the addition of NaOH;

n.d – not determined

Example 4

Table 4 shows the effects of added pyrophosphate on heat stability of Ca fortified milks with 20 mM added Ca. Pyrophosphates are less effective in

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providing heat stability and they also cause an increase in the viscosity of the calcium fortified milks, both before and after heating. At lower pH values, gels form in Ca fortified milks with added pyrophosphate before heating. Other studies have previously shown that pyrophosphates increase the viscosity of milk. Vujicic I., deMan J.M., and Woodrow I.L. "Interaction of Polyphosphates and Citrates with Skim milk Proteins.", *Can. Inst. Food Technol. J.* **1** 17 (1968)

Table 4 : Heat stability- pH profile for Ca fortified skim milks (10 % milk solids containing 20 mM added calcium) containing pyrophosphate at two concentrations

milk solids non-fat	Added Sodium Pyrophosphate (Na ₄ P ₂ O ₇)	Before heat			After heat (90 °C/10min)	
		pH	Free Calcium (mM)	Viscosity (cPoise)	Viscosity ¹ (cPoise)	Sediment ² (mL/50ml)
10%	0	6.19	13.61	nd	precipitated	nd
10%	4 mM	6.19	7.37	nd	nd	nd
10%	4 mM	6.44	5.31	nd	<20	nd
10%	4 mM	6.57	4.57	nd	2.8	1.6
10%	4 mM	6.68	4.03	nd	2.3	1.2
10%	4 mM	6.81	3.24	nd	2.2	0.6
10%	4 mM	6.94	3.35	2.18	2.5	0.6
10%	4 mM	7.12	2.5	nd	2.3	0.5
10%	4 mM	7.28	2.17	nd	2.3	0.1
10%	4 mM	7.56	1.66	nd	2.4	0.4
10%	8 mM	6.30	nd	gel	nd	nd
10%	8 mM	6.53*	nd	gel	nd	5.0
10%	8 mM	6.75*	nd	gel	nd	3.5
10%	8 mM	6.85*	nd	5.7	8.3	5.0
10%	8 mM	7.02*	nd	4.9	4.5	1.3
10%	8 mM	7.21*	nd	4.1	4.5	1.0
10%	8 mM	7.49*	nd	3.7	4.0	0.1
10%	8 mM	8.09*	nd	3.2	3.8	0.3

¹ Determined at 30 rpm with a Brookfield LVDT-II viscometer fitted with a UL adaptor (equivalent to 36.7 s⁻¹)

² Determined as volume of sediment obtained following centrifugation of a 50 mL sample (Beckman J6-HC centrifuge, JA4.2 rotor, 800 rpm, 10 minutes - equivalent to ~182xg 10 minutes)

* The pH of these samples was adjusted by the addition of NaOH
nd- not determined

The method for production of selected Ca fortified powders are given in Examples 5-10 below. The characteristics of the powders and the heat stability of milks reconstituted from these powders are given in Table 5.

Example 5: Production of low heat skim milk powder containing approximately 8g added calcium per kg final powder (1:1 molar ratio of calcium chloride to tripotassium orthophosphate)

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Solutions of calcium chloride (9.515 kg of 0.2 mole / kg CaCl_2 solution) and tripotassium orthophosphate (9.515 kg of 0.2 mole / kg K_3PO_4 solution) were mixed with skim milk (100 kg milk containing 8.9 % milk solids non-fat). To this mixture was added 0.644 kg hydrochloric acid solution (1 mole / kg). The pH of the final mixture was 7.1. The Ca fortified milk mixture was pasteurised at 72 °C for 30 sec, concentrated to ~45 % total solids using a double effect falling film evaporator and dried to ~4 % moisture in a Niro Production Minor.

15 **Example 6:** Production of high heat skim milk powder containing approximately 8g added calcium per kg final powder (1:1 molar ratio of calcium chloride to tripotassium orthophosphate)

Solutions of calcium chloride (9.515 kg of 0.2 mole / kg CaCl_2 solution) and tripotassium orthophosphate (9.515 kg of 0.2 mole / kg K_3PO_4 solution) were mixed with skim milk (100 kg milk containing 8.9 % milk solids non-fat). To this mixture was added 0.644 kg hydrochloric acid solution (1 mole / kg). The pH of the final mixture was 7.1. The Ca fortified milk mixture was heated at 90 °C for 10 min, concentrated to ~45 % total solids using a double effect falling film evaporator and dried to ~4 % moisture in a Niro Production Minor.

25 **Example 7:** Production of low heat skim milk powder containing approximately 8g added calcium per kg final powder (1:1 molar ratio of calcium chloride to monosodium dihydrogen orthophosphate)

Solutions of calcium chloride (9.510 kg of 0.2 mole / kg CaCl_2 solution) and tripotassium orthophosphate (9.510 kg of a solution containing 0.2 mole NaH_2PO_4 and 0.32 mole KOH / kg) were mixed with skim milk (100 kg milk containing 8.9 % milk solids non-fat). To this mixture was added 0.6 kg potassium hydroxide solution (1.0 mole / kg). The pH of the final mixture was

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7.0. The Ca fortified milk mixture was pasteurised at 72 °C for 30 sec, concentrated to ~45 % total solids using a double effect falling film evaporator and dried to ~4 % moisture in a Niro Production Minor.

- 5 **Example 8:** Production of high heat skim milk powder containing approximately 8g added calcium per kg final powder (1:1 molar ratio of calcium chloride to monosodium dihydrogen orthophosphate)

Solutions of calcium chloride (9.510 kg of 0.2 mole / kg CaCl_2 solution) and tripotassium orthophosphate (9.510 kg of a solution containing 0.2 mole

- 10 NaH_2PO_4 and 0.32 mole KOH / kg) were mixed with skim milk (100 kg milk containing 8.9 % milk solids non-fat). To this mixture was added 0.6 kg potassium hydroxide solution (1.0 mole / kg). The pH of the final mixture was 7.0. The Ca fortified milk mixture was heated at 90 °C for 10 min, concentrated to ~45 % total solids using a double effect falling film evaporator and dried to ~4 % moisture in a Niro Production Minor.
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Example 9: Production of low heat full-cream milk powder containing approximately 8g added calcium per kg final powder (1:1 molar ratio of calcium chloride to monosodium dihydrogen orthophosphate)

- 20 Solutions of calcium chloride (13.89 kg of 0.2 mole / kg CaCl_2 solution) and monosodium dihydrogen orthophosphate (13.89 kg of a solution containing 0.2 mole NaH_2PO_4 and 0.32 mol KOH / kg) were mixed with full-cream milk (100 kg milk containing 8.9 % milk solids non-fat and 4 % fat). The pH of the final mixture was 6.9. The Ca fortified milk mixture was pasteurised at 72 °C for 30
- 25 sec, concentrated to ~45 % total solids using a double effect falling film evaporator and dried to ~4 % moisture in a Niro Production Minor.

Example 10: Production of high heat full-cream milk powder containing approximately 8g added calcium per kg final powder (1:1 molar ratio of calcium chloride to monosodium dihydrogen orthophosphate)

- 30 Solutions of calcium chloride (13.89 kg of 0.2 mole / kg CaCl_2 solution) and monosodium dihydrogen orthophosphate (13.89 of a solution containing 0.2 mole NaH_2PO_4 and 0.32 mol KOH / kg) were mixed with full-cream milk (100

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kg milk containing 8.9 % milk solids non-fat and 4 % fat). The pH of the final mixture was 6.9. The Ca fortified milk mixture was heated at 90 °C for 10 min, concentrated to ~45 % total solids using a double effect falling film evaporator and dried to ~4 % moisture in a Niro Production Minor.

5 **Table 5** : Characteristics of milks obtained on reconstitution of Ca fortified powders at (skim milks:10 %, full cream milks 12.5% total solids,)

Examples	Reconstituted Milks			
	pH	Total Ca mg/litre	Viscosity before heating (cPoise)	Viscosity after heating (90°C/10min) (cPoise)
Low heat skim milk powder containing approximately 8g added calcium per kg final powder (1:1 molar ratio of calcium chloride to tripotassium orthophosphate)	7.0	2170	2.2	2.1
High heat skim milk powder containing approximately 8g added calcium per kg final powder (1:1 molar ratio of calcium chloride to tripotassium orthophosphate)	7.0	2125	2.3	2.1
Low heat skim milk powder containing approximately 8g added calcium per kg final powder (1:1 molar ratio of calcium chloride to monosodium dihydrogen orthophosphate)	6.8	2110	2.2	2.0
High heat skim milk powder containing approximately 8g added calcium per kg final powder (1:1 molar ratio of calcium chloride to monosodium dihydrogen orthophosphate)	6.8	2095	2.4	2.0
Low heat full-cream milk powder containing approximately 8g added calcium per kg final powder (1:1 molar ratio of calcium chloride to monosodium dihydrogen orthophosphate)	6.6	2210	3.5	4.4
High heat full-cream milk powder containing approximately 8g added calcium per kg final powder (1:1 molar ratio of calcium chloride to monosodium dihydrogen orthophosphate) powder	6.5	2205	3.5	2.5

All milks made by reconstituting the milk powders produced in examples 5 to 10 were stable to heat processing at 90°C for 10 minutes.

10 **Example 11**

The addition of magnesium to milk is illustrated in table 6. 20mM magnesium was added with orthophosphates. When no orthophosphate is added, skim milks (10% milk solids) with 20mM added magnesium form a precipitate on heating at 90 °C for 10 min. The non fat milk solids in all of the compositions was 10%.

Table 6 Heat stability- pH profile for Ca fortified skim milks (10 % milk solids containing 20 mM added magnesium) stabilised with added orthophosphates

Added K_3PO_4	Added K_2HPO_4	Added KH_2PO_4	Before heat		After heat (90 °C/ 10 min)	
			pH	Free Calcium	Viscosity ¹	Sediment ²
0	0	0	6.37	4.31	precipitated	nd
20 mM			8.42*	0.09	3.6	<0.1
20 mM			8.14*	0.14	3.9	0.1
20 mM			8.00*	0.18	3.2	<0.1
20 mM			7.89*	0.20	2.8	0.1
20 mM			7.77*	0.24	2.4	<0.1
20 mM			7.71*	0.24	2.3	<0.1
20 mM			7.56	0.3	2.7	<0.1
16 mM	4 mM		7.29	0.36	2.1	0.1
12 mM	8 mM		7.10	0.54	2.0	0.1
8 mM	12 mM		6.91	0.71	2.0	<0.1
4 mM	16 mM		6.76	0.94	2.0	0.1
	20 mM		6.60	1.30	2.1	0.1
	16 mM	4 mM	6.50	1.71	4.2	<0.1
	12 mM	8 mM	6.37	2.17	precipitated	nd
	8 mM	12 mM	6.26	2.75	precipitated	nd
	4 mM	16 mM	6.15	3.38	precipitated	nd
		20 mM	6.03	4.18	precipitated	nd

¹ Determined at 30 rpm with a Brookfield LVDT-II viscometer fitted with a UL adaptor (equivalent to 36.7 s^{-1})

² Determined as volume of sediment obtained following centrifugation (Beckman J6-HC centrifuge, JA4.2 rotor, 800 rpm, 10 minutes - equivalent to $\sim 182 \times g$ 10 minutes)

* The pH of these samples was adjusted by the addition of NaOH

Example 12

The profile of milk additions of magnesium and calcium with orthophosphates is shown in table 7

Table 7

Added $MgCl_2$	Added $CaCl_2$	Added K_3PO_4	Added K_2HPO_4	Before heat		After heat (90 °C/ 10 min)	
				pH	Free Calcium	Viscosity ¹	Sediment ²
20 mM	-	-	-	6.37	4.31	precipitated	nd
20 mM	0	12 mM	8 mM	7.10	0.52	2.05	0.1

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16 mM	4 mM	12 mM	8 mM	7.05	0.65	2.05	0.2
12 mM	8 mM	14 mM	6 mM	7.09	0.66	2.1	<0.1
8 mM	12 mM	14 mM	6 mM	7.06	0.76	2.16	<0.1
4 mM	16 mM	16 mM	4 mM	7.16	0.74	2.3	0.1
2 mM	18 mM	16 mM	4 mM	7.13	0.83	2.34	0.3
0	20 mM	16 mM	4 mM	7.12	0.85	2.37	0.4

Example 13

The relative performance of orthophosphates and other complexing agents on the distribution of calcium and protein in the serum phase of skim milk or mineral adjusted skim milk was examined.

Skim milk and mineral adjusted skim milks (20mM added calcium) were prepared and the milk serum fractions were obtained by centrifugation (70,000×g 90 minutes). The calcium and protein concentrations of each of the milks and their serum fractions were analysed. The types of protein present in the serum fractions of the skim milk and mineral adjusted skim milk were determined by capillary electrophoresis. The results of experiments are shown in table 8.

Distribution of Calcium

The control (unadjusted) skim milk demonstrates that the calcium concentration of the supernatant (serum) phase of the milk was of the order of one quarter of the concentration in the un-fractionated milk. The addition of calcium alone to the milk, caused little change in the proportion of the calcium in the serum phase. The addition of orthophosphate (PO_4^{3-}) in association with calcium caused a considerable reduction in the proportion of calcium in the serum phase. When longer chain forms of phosphate; pyrophosphate (P_2O_5), tripolyphosphate (P_3O_{10}) or Calgon T ($\text{P}_n\text{O}_{3n+1}$ $n=10\sim12$) were added in combination with calcium chloride there was also a reduction in the proportion of the calcium in the serum phase but to a lesser extent than that occurring with orthophosphate. The addition of citrate caused a major increase in the proportion of calcium in the serum fraction increasing it to more than twice that seen in either unadjusted milk or milk with 20mM added calcium.

Distribution of protein

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The control (unadjusted) milk demonstrates that the normal protein concentration of the milk serum was about one fifth of the protein concentration of the unfractionated milk. The majority of the serum proteins are the "whey" proteins but a small proportion are serum caseins which occur in equilibrium with the micellar caseins. The addition of calcium alone, or calcium and ortho-phosphate to the milk caused a reduction in the relative concentration of protein in the milk serum, implying that the calcium, ortho-phosphate, and protein migrate together into the micellar phase. Addition of pyro-phosphate has a similar effect to the addition of ortho-phosphate, while tripoly-phosphate and citrate cause an increase in the proportion of protein found in the serum. Calgon T has an effect intermediate between ortho-phosphate and citrate.

Distribution of protein types

Analysis of the control (unadjusted) milk and the serum phases of the control and adjusted milks demonstrated little change in the levels of the two major whey proteins β lactoglobulin and α -lactalbumin with any of the treatments. This indicates that these proteins, which form the majority of the serum proteins, are not involved in the mineral induced re-distribution between serum and micellar phases. Considerable changes were observed in the distribution of caseins. In general the patterns shown follow the changes observed in the analysis of protein nitrogen. Adding calcium causes a reduction in the supernatant casein concentration compared to the supernatant from unmodified skim milk. This is also observed when orthophosphate or pyrophosphate are added together with the calcium. The addition of tripolyphosphate, or citrate with calcium cause an increase in the levels of the caseins in the serum phase to varying degrees. Calgon T has an effect intermediate between ortho-phosphate and citrate.

The patterns shown here demonstrate that there are clearly identifiable differences in the effect that a number of different calcium complexing agents have on the distributions of protein and calcium between the serum and micellar phases of skim milk. The preferred ingredient for our process,

orthophosphate can easily be differentiated from other typically used agents such as citrate or poly phosphate through an evaluation of the protein contents of the supernatant phases. Citrate also causes a markedly different distribution of calcium. Of the two other forms of phosphate tested,

- 5 tripolyphosphate shows considerable differences in the protein distribution, while although pyrophosphate is not easily distinguished by these methods other tests have shown that it is less effective in providing stabilisation.

Table 8 Levels of calcium and protein in complete and serum fractions of
10 control milk (unadjusted) or mineral adjusted skim milks:

	trial	Calcium			Protein		
		total solution mg/litre	supernatant mg/litre	relative concentration	total solution %	supernatant %	relative concentration
Control milk	1	1240	307	24.8%	3.16	0.68	21.6%
	2	1230	326	26.5%	3.11	0.66	21.2%
Milk+ 20mM CaCl ₂	1	2085	496	23.8%	3.11	0.52	16.8%
	2	2205	602	27.3%	3.55	0.57	16.0%
Milk+ 20mM CaCl ₂ +20mM orthophosphate	1	2123	264	12.4%	3.22	0.57	17.8%
	2	2063	310	15.0%	3.11	0.53	17.1%
Milk+20mM CaCl ₂ +4mM pyrophosphate	1	2058	365	17.7%	3.09	0.58	18.8%
	2	2068	484	23.4%	2.87	0.52	18.2%
Milk+20mM CaCl ₂ +8mM tripolyphosphate	1	2068	352	17.0%	3.09	1.01	32.9%
	2	2088	389	18.6%	3.20	0.80	24.9%
Milk+20mM CaCl ₂ +0.15% Calgon T	1	2223	367	16.5%	3.36	0.69	20.5%
	2	2078	471	22.6%	3.19	0.54	17.0%
Milk+20mM CaCl ₂ +20mM Citrate	1	2098	1325	63.2%	3.16	0.82	25.9%
	2	2020	1151	57.0%	2.94	0.74	25.2%

Relative concentrations are calculated as $100 \times \text{concentration in supernatant} / \text{concentration in total solution}$.

- 15 **Example 14** Use of fortified milk powders and milk concentrates in pilot scale production of Ice Cream

Ice creams were manufactured at a pilot scale to investigate the suitability of using calcium fortified milk powder with 8g added calcium /kg fortified solids or calcium fortified milk concentrates manufactured as described in Example 9 as
20 ingredients. Control ice creams were produced using a non-fortified milk powder or concentrate manufactured at the same time as a control. Milk solids

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were incorporated into the formulation by either using skim milk powder or skim milk concentrates..

Formulation

- 5 The compositions of the ice cream pre-mixes used were: Milk-solids-not-fat 11.00%, Milk fat 11.00%, Sucrose 14.00%, Guar Gum 0.1% Carboxymethyl cellulose (CMC) 0.1%, and Glycerol monostearate (GMS) 0.2%, Vanilla flavour 0.35%. The remainder of the formulation comprised of water

10 Production of ice cream

The ice cream mixes were prepared by combining the milk concentrates (obtained either directly from the plant or by reconstituting milk powder) with a blend of sugar, Guar Gum, and CMC in water then adding an emulsifier-cream mix of GMS in cream. The complete mix was then homogenised (40°C, 15 2500psi first stage 500 psi second stage) and pasteurised (80°C, 30 seconds) using a tubular heat exchanger. The appropriate amount of vanilla flavouring was then added. This mix was then "aged" at 4°C for 24 hours. The aged ice cream mix was frozen using a GELMAK 160 continuous ice cream freezer. The machine was operated to give a temperature at the outlet of -5°C.

- 20 Following freezing samples were stored at -30°C for hardening (24 hours) then transferred to -20°C for storage.

Analysis

Analysis of the ice cream took place seven days after freezing.

25 Meltdown

- Meltdown tests samples were performed on samples filled directly from the ice-cream freezer into specially prepared two-piece cylindrical plastic moulds. The samples were hardened as described above then equilibrated at -20°C before testing. Samples were removed from the moulds and placed on a stainless-30 steel mesh screen (3mm mesh) and the mass of the melted portion passing through the screen was recorded at 15 minute intervals for up to two hours. Ice creams made with either the fortified or non-fortified milk powders melted at about the same rate and melted completely within 105 minutes. Where concentrates were used, the melting rates were different with the calcium

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fortified ice cream melting within 75 minutes while the control ice cream was still melting after 2 hours.

5 *Firmness*

Firmness was measured using an Instron Universal Testing Machine (model 5564) equipped with a 500N load cell. The force required to penetrate the sample with a 5mm cylindrical probe at a speed of 50mm/min to a depth of 50mm was recorded. Where fortified or non-fortified milk powders were used
10 no significant differences could be identified between the control and calcium fortified samples. With concentrates control (unfortified) ice cream was slightly firmer.

Sensory testing

15 Informal sensory testing was conducted by giving coded samples to individuals and eliciting spontaneous impressions. Little difference in flavour could be found between the two products.

This work demonstrates that the milk powders or concentrates with enhanced
20 levels of calcium, produced by the process as claimed in claim 1, may be used to produce calcium fortified ice-cream which has generally similar physical characteristics to non-fortified ice cream. The fortified ice cream has minimal flavour differences compared to the non-fortified ice-cream.

25 **Example 15** Use of fortified milk powders (8g added calcium / kg fortified solids) in UHT applications.

Milk based beverages were prepared using calcium fortified milk powders with 8g added calcium /kg fortified solids produced as described in Example 9 as the source of milk solids. The pH of the samples was further adjusted through
30 the use of Sodium hydroxide. They were processed in model UHT equipment giving a heat treatment of 140°C for three seconds. The viscosity of the samples was determined after heating. This demonstrates the potential for

these powders to be utilised in sweetened dairy based beverages, provided the appropriate pH is selected.

Table 9 Properties of milk based beverages (8.5% milk solids non-fat, 3.5% milk fat, and 6% sugar) produced with calcium fortified milk solids before and after UHT treatment.

Sample	Before UHT treatment	After UHT treatment (140°C / 3 seconds)	
		Viscosity (cPoise) ¹	Sediment (ml / 50 ml)
Calcium fortified low heat milk with fat and sugar	6.72	off scale	>20
Calcium fortified low heat milk with fat and sugar. pH adjusted to ~7.0	7.07	30.7 (12rpm)	4
Calcium fortified low heat milk with fat and sugar. pH adjusted to ~7.2	7.24	16.3	2
Calcium fortified low heat milk with fat and sugar. pH adjusted to ~7.4	7.44	8.12	1.4

¹ Determined at 30 rpm with a Brookfield LVDT-II viscometer fitted with a UL adaptor (equivalent to 36.7 s⁻¹)

Conclusions

The mechanism of action of orthophosphate in milk is unique. This is because of its effects on the dynamic equilibria between Ca, phosphate and the casein micelle. In milk, addition of orthophosphates reduces Ca activity as well as impacts on the composition of the casein micelle. Whilst orthophosphate and other agents such as polyphosphate and citrate can be used to improve the heat stability of other proteins such as soy proteins that are sensitive to heat, the action in systems other than milk is mainly due to the lowering of the Ca activity. This reduces the sensitivity of the proteins to heat aggregation.

Heat stability of Ca fortified milk with up to 25 mM added Ca has been successfully achieved with judicious addition of orthophosphate and pH control. A 1:1 molar ratio of added Ca:added orthophosphate has generally been used to achieve heat stability in Ca fortified milks. Lower ratios may be used but this decreased the range of pH over which the Ca fortified milks are heat stable.

By selecting suitable combinations of added orthophosphates, the viscosity of the heated solution should be low, and preferably less than about 3 cPoise and

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the sediment obtained on centrifugation of heated solutions should be < 0.5 ml/50 mL milk.

CLAIMS

1. A process for producing a calcium and/or nutritional metal fortified milk, the process including the steps of:

- adding a soluble calcium and/or nutritional metal compound to a milk,
- 5 - adding either before or after the calcium and/or nutritional metal addition an effective amount of a phosphate which enables calcium and/or nutritional metal to migrate into the protein micelles and maintains the pH so that it lies within a range from 6.5 to 7.5 wherein the phosphate is selected from one or more of monosodium dihydrogen orthophosphate, disodium hydrogen orthophosphate,
- 10 trisodium orthophosphate, monopotassium dihydrogen orthophosphate, dipotassium hydrogen orthophosphate and tri potassium orthophosphate.

2. A calcium and/or nutritional metal fortified milk which includes milk, a calcium and/or nutritional metal compound and an amount of a phosphate which enables calcium and/or

15 nutritional metal to migrate into the protein micelles and maintains the pH so that it lies within a range from 6.5 to 7.5, effective in stabilising the milk during heat treatment said phosphate being selected from one or more of monosodium dihydrogen orthophosphate, disodium hydrogen orthophosphate, trisodium orthophosphate, monopotassium dihydrogen orthophosphate, dipotassium hydrogen orthophosphate and tri potassium

20 orthophosphate.

3. A process for producing a calcium and/or nutritional metal fortified milk or toned milk powder, the process including the steps of:

- adding a soluble calcium and/or nutritional metal compound to a milk,
- 25 - adding either before or after the calcium and/or nutritional metal addition an effective amount of a phosphate selected from one or more of monosodium

dihydrogen orthophosphate, disodium hydrogen orthophosphate, trisodium orthophosphate, monopotassium dihydrogen orthophosphate, dipotassium hydrogen orthophosphate and tri potassium orthophosphate which enables calcium and/or nutritional metal to migrate into the protein micelles and maintains the pH so that it lies within a range from 6.5 to 7.5,

- pasteurising the calcium and/or nutritional metal fortified milk, and
- concentrating and dehydrating the milk to form a calcium and/or nutritional metal fortified dry milk or toned milk powder.

10 4. A fortified milk as claimed in claim 2 in which the nutritional metal is selected from one or more water soluble compounds of magnesium, iron, zinc and manganese.

5. A fortified milk powder produced by the process of claim 3.