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(54) Title: BETA-SERUM DAIRY PRODUCTS, NEUTRAL LIPID-DEPLETED AND/OR POLAR LIPID-ENRICHED DAIRY PRODUCTS, AND PROCESSES FOR THEIR PRODUCTION

(57) Abstract: Processes for producing dairy products having lower levels of neutral lipids, and/or higher levels of polar lipids, by extraction using near critical carbon dioxide or dimethyl ether. These products may be used as ingredients in infant formulas. Infant formulas containing beta-serum are also claimed. "Beta-serum" means an aqueous dairy ingredient separated from dairy streams containing greater than 60% fat which have been through phase inversion from an oil-in-water to a water-in-oil emulsion, such as the serum produced during the production of butter oil.

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Infant formula comprising beta-serum or low-lactose beta-serum**FIELD OF THE INVENTION**

The present invention relates to infant formula comprising beta-serum or low-lactose beta-serum.

BACKGROUND

Commercially available infant formulas are typically produced using non-human milk. However the nutritional composition of human milk differs in some respects to that of non-human milk (such as cow, sheep, buffalo or goat).

Non-human whole milk such as cow, goat or sheep milk, contains a higher proportion of saturated fatty acids than human milk and is deficient in linoleic acid and alpha-linolenic acid, polyunsaturated fatty acids that are essential for normal infant growth and development. Also, butyric acid which is found in milk fat may cause putrid vomit in infants.

Therefore standard infant formulas are typically produced using low-fat dairy products such as skim milk. Using a reduced-fat dairy product means undesirable components in milk fat are not included in the infant formula, but it also means that phospholipid and (glyco)sphingolipid levels are significantly lower than those in human milk.

Research over the last 5-10 years has shown that increasing phospholipid and (glyco)sphingolipid levels in infant formulations to levels found in human milk (particularly ganglioside GM₃, ganglioside GD₃, ceramides and sphingomyelin) may lead to:

- enhanced gut maturation, thereby reducing the risk of infection;

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- prevention of infections by modifying gut intestinal flora and competitively binding antigens;
- prevention of the development of allergies; and
- optimal neural development.

It is therefore desirable to produce an infant formula containing sufficient levels of desirable lipids while minimising or eliminating undesirable ingredients.

One means currently used to achieve this is to add lipid-containing extracts and other individual ingredients to a base formulation thereby producing an infant formula with the desired nutritional profile. The lipid extracts may be produced using conventional extraction solvents (for example WO 94/18289 describes a method for extracting sphingomyelin from a phospholipid-containing fat concentrate using solvent extraction techniques). Such lipid extracts are expensive to produce. Furthermore, extensive toxicity and safety studies are required before regulatory approval in some jurisdictions can be obtained for their use.

Another means used to achieve this goal is to include buttermilk in infant formulas. Buttermilk is the aqueous by-product stream produced during one of three processes:

- (1) Traditional butter manufacture using either the Fritz buttermaking or batch buttermaking process;
- (2) Traditional production of butter-oil (also known as anhydrous milk fat or AMF) from cream as shown in Figure 1;
- (3) Production of butter-oil from cream using a two-sera process as shown in Figure 2, where the buttermilk is produced by blending the secondary skim and beta-serum streams together.

Infant formulas containing buttermilk contain lesser amounts of undesirable components of milk fat than non-human milk, but higher levels of phospholipids and (glyco)sphingolipids than reduced-fat dairy products. However, the levels of these desirable lipids are not high enough for buttermilk to be used in a whey-dominant infant

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formula in order to achieve phospholipid and (glyco)sphingolipid levels similar to those in human milk.

Supercritical extraction using carbon dioxide as the solvent is known to extract neutral lipids from buttermilk powders. Astaire J. C., Ward R., German J. B., and Jimenez-Flores R. (2003) *Concentration of Polar MFGM Lipids from Buttermilk by Microfiltration and Supercritical Fluid Extraction* J. Dairy Sci. 86, 2297-2307 describes the supercritical extraction of buttermilk using carbon dioxide as the solvent to produce a product rich in protein, and enhanced in levels of polar lipids. However, the buttermilk powder so produced still has low levels of polar lipids, at a maximum of 2% of the dry powder mass, and is thus unsuitable for infant formula.

One possible way to provide a suitable product would be to separate the protein components from the lipid components in a dairy product.

Dimethyl ether (DME) has previously been used in the extraction of lipids from raw egg yolk (Yano *et al* US 4,157,404) and dried egg powder (Yano *et al* US 4,234,619). The process causes the fractionation of the lipid and protein components into separate streams. In US 4,157,404, Yano states that while lipids can be extracted from raw egg yolks (75% moisture content), the proteins are denatured. In US 4,234,619, Yano states that proteins are not denatured if the egg yolk is dry, but the phospholipids can only be partially extracted.

WO 2004/066744 describes the extraction of lipids from an aqueous dairy stream using near critical extraction where dimethyl ether is the solvent. WO 2004/066744 also discloses that neither supercritical CO₂ or subcritical dimethyl ether can extract lipids in useful yields from whey protein concentrate (WPC) dairy powders. However, this document does not disclose the extraction of lipids from powders rich in milk fat globule membrane material.

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Attempts to extract lipids from dairy powder streams with high lactose contents (where high is at least 30% by mass of the total powder) by extraction using liquefied dimethyl ether have been unsuccessful.

It is therefore an object of the present invention to provide improved or alternative dairy products that can be used in infant formulations, and/or to at least provide the public with a useful choice.

DISCLOSURE OF THE INVENTION

The present invention relates to an infant formula comprising isolated beta-serum or low-lactose beta-serum.

The term "beta-serum" as used herein means an aqueous dairy ingredient separated from dairy streams containing greater than 60% fat which have been through phase inversion from an oil-in-water to a water-in-oil emulsion. Cream is the preferred starting material for the production of beta-serum. For example beta-serum is produced during the production of butter-oil (also known as anhydrous milk fat or AMF) from cream as shown in Figure 2. The lactose content of beta-serum may be reduced by ultrafiltration. Beta-serum and low-lactose beta-serum may be powders.

Preferably, the infant formula comprises:

- (a) 30 – 60 % lactose
- (b) 15 – 35% vegetable oils
- (c) 0 – 40% skim milk powder
- (d) 0 – 40% whey protein concentrate
- (e) 1 – 50% isolated beta-serum or low-lactose beta-serum powder.

More preferably, the infant formula comprises:

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- (a) 40 – 60 % lactose
- (b) 20 – 30% vegetable oils
- (c) 10 – 15% skim milk powder
- (d) 6 – 8% of an 80% protein whey protein concentrate (WPC80)
- (e) 1 – 10% isolated beta-serum or low-lactose beta-serum powder.

Most preferably, the infant formula comprises:

- (a) 40 – 60 % lactose
- (b) 20 – 30% vegetable oils
- (c) 10 – 15% skim milk powder
- (d) 6 – 8% of an 80% protein whey protein concentrate (WPC80)
- (e) 2 – 5% isolated beta-serum or low-lactose beta-serum powder.

The infant formula may also comprise 2 – 4% of at least one of the following:

- (a) vitamin premix
- (b) mineral premix
- (c) lecithin
- (d) antioxidant
- (e) stabiliser
- (f) nucleotides.

In a preferred embodiment, these infant formulas may be formulated to provide between 2700 and 3000 kJ/L.

The infant formulas of the present invention may be administered to provide health benefits.

For example, the following health benefits are contemplated:

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- enhancing gut maturation
- reducing the risk of infection
- modifying gut intestinal flora and competitively binding antigens
- preventing infections
- preventing the development of allergies
- optimizing neural development
- treating a dermatological condition
- optimizing immune system development
- maintaining optimal immune function
- preventing or treating colon cancer.

The inventors have discovered that the levels of phospholipids and gangliosides in beta-serum make it suitable to be used in the fortification of infant formulas.

In the description in this specification reference may be made to subject matter which is not within the scope of the claims of the current application. That subject matter should be readily identifiable by a person skilled in the art and may assist in putting into practice the invention as defined in the claims of this application.

Described are processes for producing dairy products that have lower levels of neutral lipids, or higher levels of polar lipids, or both. These products may be used as ingredients in infant formulas.

Described is a process for producing a neutral lipid-depleted beta-serum dairy product comprising the following steps:

- (1) providing dried beta-serum; and
- (2) subjecting the beta-serum to a near critical carbon dioxide extraction process.

Preferably the dried beta-serum is a powder.

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Preferably the near critical carbon dioxide pressure is at least 73.2 bar and the temperature is in the range 304.2 to 373 K (supercritical region); or the carbon dioxide pressure is greater than or equal to the vapour pressure, and the temperature is in the range 273 to 304.1 K (subcritical region). More preferably, the carbon dioxide pressure is at least 250 bar, and the temperature in the range 313 to 353 K.

Preferably the lactose content of beta-serum is reduced before the extraction process. Preferably the lactose content is reduced by ultrafiltration. Preferably the lactose content is further reduced by diafiltration during ultrafiltration.

Preferably, the lactose content is reduced to less than or equal to 30% (on a dry weight basis) before the near critical carbon dioxide step. More preferably, the lactose content is reduced to less than or equal to 25% (on a dry weight basis) before the near critical carbon dioxide step. More preferably, the lactose content is reduced to less than or equal to 20% (on a dry weight basis) before the near critical carbon dioxide step. Most preferably, the lactose content is reduced to less than or equal to 10% (on a dry weight basis) before the near critical carbon dioxide step.

Preferably the neutral lipid-depleted beta-serum dairy product is enriched in phospholipids, sphingolipids and glycolipids. Preferably the dairy product comprises approximately 50-70% protein (TN x 6.38), preferably at least 60% protein (TN x 6.38); 5-25% phospholipids and glycolipid, preferably 15-25% phospholipids and glycolipid; and 0-10% neutral lipid, preferably approximately 5% neutral lipid. Most preferably the dairy product comprises less than about 5% neutral lipids.

Also described is a process for producing a neutral lipid-depleted dairy product comprising the following steps:

- (1) providing a dried high-fat low-lactose dairy starting material;
- (2) subjecting the starting material to a near critical carbon dioxide extraction process, and

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subsequently fractionating the dairy product to produce a lipid enriched fraction and a lipid depleted fraction comprising the step of:

- (3) subjecting this lipid-depleted dairy product to a liquid dimethyl ether extraction process.

Preferably the near critical carbon dioxide pressure is at least 73.2 bar and the temperature is in the range 304.2 to 373 K (supercritical region); or the carbon dioxide pressure is greater than or equal to the vapour pressure, and the temperature is in the range 273 to 304.1 K (subcritical region). More preferably, the carbon dioxide pressure is at least 250 bar, and the temperature in the range 313 to 353 K.

Preferably, the dimethyl ether is liquefied and pressurised to a pressure at least equal to the vapour pressure at the temperature of the extraction, and more preferably is at least 10 bar greater than the vapour pressure. Preferably the temperature is in the range 273 – 373 K, and more preferably in the range 293 – 353 K.

The term "high fat" means containing greater than 10% fat w/w of solids, preferably greater than 15% fat w/w of solids and most preferably greater than 20% fat w/w of solids.

The term "low-lactose" means that the lactose content is less than or equal to 30% (on a dry weight basis). More preferably, the lactose content is less than or equal to 25% (on a dry weight basis). More preferably, the lactose content is less than or equal to 20% (on a dry weight basis). Most preferably, the lactose content is less than or equal to 10% (on a dry weight basis).

Preferably the lactose content of beta-serum is reduced before the extraction process. Preferably the lactose content is reduced by ultrafiltration. Preferably the lactose content is further reduced by diafiltration during ultrafiltration.

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Preferably the high-fat low-lactose dairy starting material is lactose-reduced beta-serum. Alternatively the high-fat low-lactose dairy starting material is lactose-reduced buttermilk.

Preferably the high-fat low-lactose dairy starting material is spray dried before the near critical carbon dioxide step. Alternatively high-fat low-lactose dairy starting material is freeze-dried or vacuum dried before the near critical carbon dioxide step.

The lipid enriched fraction preferably contains at least 50% phospholipids and glycolipids and is substantially reduced in neutral lipids. More preferably, the lipid enriched fraction contains at least 80% phospholipids and glycolipids, and is substantially free of neutral lipids.

In a preferred embodiment the lipid depleted fraction is enriched in milk fat globule membrane proteins and contains, on a powder basis 65-79% (TN x 6.38) protein, 8-12% lactose, 5-11% fat including phospholipids (4-9% of fraction), more preferably about 72% protein (TN x 6.38), 9% lactose, 8% fat including phospholipids (6% of fraction), 5% ash and 4% moisture.

Also described is a process for producing a lipid enriched fraction and a lipid depleted fraction comprising the following steps:

- (1) providing a high-fat low-lactose dairy starting material; and
- (2) subjecting the starting material to a liquid dimethyl ether extraction process.

The lipid depleted fraction contains all of the milk fat globule membrane proteins and therefore could be used in infant formulas to deliver health benefits that are more strongly associated with milk fat globule membrane protein components.

Preferably, the dimethyl ether is liquefied and pressurised to a pressure at least equal to the vapour pressure at the temperature of the extraction, and more preferably is at least 10

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bar greater than the vapour pressure. Preferably the temperature is in the range 273 – 373 K, and more preferably in the range 293 – 353 K.

The term “high fat” means containing greater than 10% fat w/w of solids, preferably greater than 15% fat w/w of solids and most preferably greater than 20% fat w/w of solids.

The term “low-lactose” means that the lactose content is less than or equal to 30% (on a dry weight basis). More preferably, the lactose content is less than or equal to 25% (on a dry weight basis). More preferably, the lactose content is less than or equal to 20% (on a dry weight basis). Most preferably, the lactose content is less than or equal to 10% (on a dry weight basis).

Preferably the lactose content of beta-serum is reduced before the extraction process. Preferably the lactose content is reduced in beta-serum or the starting material by ultrafiltration. Preferably the lactose content is further reduced by diafiltration during ultrafiltration.

Preferably the high-fat low-lactose dairy starting material is lactose-reduced beta-serum. Alternatively the high-fat low-lactose dairy starting material is lactose-reduced buttermilk.

Preferably the high-fat low-lactose dairy starting material is spray dried before the liquid dimethyl ether extraction step. Alternatively high-fat low-lactose dairy starting material is freeze-dried or vacuum dried before the liquid dimethyl ether extraction step.

In one embodiment, the lipid enriched fraction contains at least 40% phospholipids and glycolipids. This process does not take out the neutral lipids first with CO₂, and because DME extracts both polar and neutral lipids, the DME lipid extract will contain about the same ratio of neutral lipid-to-phospholipid as in the feed. For example, for a feed containing a neutral lipid to phospholipid ratio of about 1.2, the composition of the DME

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lipid extract on a 3% moisture basis is 80-90% fat including phospholipids (35-45% of fraction), 5-9% ash, 2-5% lactose and 2-3% moisture.

The lipid depleted fraction is enriched in milk fat globule membrane proteins and contains, on a powder basis, about 60-80% protein (TN x 6.38), 6-12% lactose, 5-11% fat including phospholipids (5-9% of fraction), preferably 73% protein (TN x 6.38), 9% lactose, 8% fat including phospholipids (7% of fraction), 5% ash and 3% moisture.

The level of protein in the products described in this specification is calculated from the total nitrogen (TN) in a sample multiplied by a factor of 6.38.

Also described are products produced by the described processes, and compositions and infant formulas containing such products.

The term "infant formula" as used herein includes formulas designed for infants 0-12 months old, formulas designed for infants 6-12 months old (follow-on-formula) and formulas designed for toddlers and young children (1-7 years, growing-up milks / milk powders).

A preferred infant formula comprises:

- (a) 30 – 60 % lactose
- (b) 15 – 35% vegetable oils
- (c) 0 – 40% skim milk powder
- (d) 0 – 40% whey protein concentrate
- (e) 1 – 50% a product produced by a process described herein.

A more preferred infant formula comprises:

- (a) 40 – 60 % lactose
- (b) 20 – 30% vegetable oils

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- (c) 10 – 15% skim milk powder
- (d) 6 – 8% 80% protein whey protein concentrate (WPC80)
- (e) 1 – 5% a product produced by a process of the present invention.

The infant formula may also comprise 2 – 4% of at least one of the following:

- (a) vitamin premix
- (b) mineral premix
- (c) lecithin
- (d) antioxidant
- (e) stabiliser
- (f) nucleotides.

In a preferred embodiment, these infant formulas may be formulated to provide between 2700 and 3000 kJ/L.

Also described is an infant formula comprising as an ingredient a fraction obtained from beta-serum enriched in polar lipids or depleted in neutral lipids or both.

Preferably the infant formula comprises:

- (a) 30 – 60 % lactose
- (b) 15 – 35% vegetable oils
- (c) 0 – 40% skim milk powder
- (d) 0 – 40% whey protein concentrate
- (e) 1 – 50% a fraction obtained from beta-serum enriched in polar lipids or depleted in neutral lipids or both.

More preferably, the infant formula comprises:

- (a) 40 – 60 % lactose

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- (b) 20 – 30% vegetable oils
- (c) 10 – 15% skim milk powder
- (d) 6 – 8% 80% protein whey protein concentrate (WPC80)
- (e) 1 – 5% a fraction obtained from beta-serum enriched in polar lipids or depleted in neutral lipids or both.

The infant formula may also comprise 2 – 4% of at least one of the following:

- (a) vitamin premix
- (b) mineral premix
- (c) lecithin
- (d) antioxidant
- (e) stabiliser
- (f) nucleotides.

In a preferred embodiment, these infant formulas may be formulated to provide between 2700 and 3000 kJ/L.

The inventors have also discovered that dairy products which are high in fat but low in lactose (including low-lactose beta-serum) may be processed to reduce the levels of neutral lipids, or increase the levels of polar lipids, or both, thus creating products which are even more suitable in the fortification of infant formulas.

The processes described herein utilise processing and extraction techniques which do not leave toxic residues, therefore further processing of the final dairy product is not required.

Additionally, the use of ultrafiltration and near critical extraction with carbon dioxide as the solvent means it should be easier to obtain regulatory approval for the use of this product as there is minimal or no solvent residue in the product compared with the use of conventional solvents such as acetone and ethanol. Additionally conventional solvents

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extensively denature proteins, making the use of these solvents unsuitable for producing dairy products for infant formula applications.

The term "dairy" as used herein means of, containing, or concerning milk and its products. It includes milk produced by humans, cows, buffalo and goats but is not limited to these animals.

Every substance has its own "critical" point at which the liquid and vapour state of the substance become identical. Above but close to the critical point of a substance, the substance is in a fluid state that has properties of both liquids and gases. The fluid has a density similar to a liquid, and viscosity and diffusivity similar to a gas. The term "supercritical" as used herein refers to the pressure-temperature region above the critical point of a substance. The term "subcritical" as used herein refers to the pressure-temperature region equal to or above the vapour pressure for the liquid, but below the critical temperature. The term "near critical" as used herein encompasses both "supercritical" and "subcritical" regions, and refers to pressures and temperatures near the critical point.

BRIEF DESCRIPTION OF THE DRAWING

Figure 1 shows a schematic drawing of the traditional process for producing butter-oil from cream and the production of buttermilk during this process.

Figure 2 shows a schematic drawing of the process for producing butter-oil from cream and the production of beta-serum during this process.

EXAMPLES

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The following Examples further illustrate the products and processes described herein. Examples 6, 7 and 8 illustrate the invention.

Example 1: Extraction of whey protein concentrate powder

This example shows that the extraction of lipids from powder with high concentrations of whey proteins results in very low yields of lipid. Whey protein concentrate powders containing 80.26 % by mass protein, 6.83 % by mass lipid, and 3.57% moisture were extracted with the near critical solvents carbon dioxide, propane, and dimethyl ether (DME). The solvent, pressure, temperature, mass of solids used, mass of solvent used, and extract solids and lipid yields are given in table 1.

Table 1: Lipid yields for the extraction of WPC solids with various solvents

Solvent	Pressure bar	Temperature K	Mass of solids g	Mass of solvent used, kg	Mass of Extract, g	Yield, % solids	Yield, % lipids
CO ₂	300	317.1	3600.0	18.9	2.69	0.07	1.05
Propane	32	314.1	3600.0	11.8	3.97	0.11	1.56
DME	32	314.1	3600.0	10.9	4.58	0.13	1.80
DME	55	323.9	129.2	0.41	0.34	0.26	3.60
DME	55	333.1	129.1	0.42	0.56	0.43	5.95

The lipid yields are very low and an increase in extraction temperature does not increase the extraction yield to desired levels.

Example 2: Extraction of standard lactose beta serum powders with supercritical CO₂

This example shows that extraction of neutral lipids is possible from beta serum powder with standard lactose content, but that the yield is significantly less than with powders where the lactose content has been reduced. The protein and total phospholipid content of the final powder are low. Beta serum powders with the following compositions were

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extracted with supercritical CO₂ at 300 bar and 313 K: batch 1 total protein 29.4 %, lactose 42.5 %, total fat 19.7 %, moisture 3.1 % and ash 6 %; batch 2 total protein 31.7 %, lactose 44.6 %, total fat 20.6 %, moisture 2.3 % and ash 6.1 %. The total fat is made up of neutral lipids, phospholipids, gangliosides, ceramides and cerebroside, such as lactosylceramide. The fat extraction results, and mass of phospholipids in the extract are shown in table 2. Only neutral lipids are extracted by supercritical CO₂ as the other types of fat, and especially phospholipids, are not soluble in this solvent.

Table 2: Lipid yields for the extraction of standard lactose beta serum powders with CO₂

Batch	Mass of solids	CO ₂ used	Mass of extract	Mass of phospholipid	% Yield, total lipids	% Yield, neutral lipids
	g	kg	g	g		
1	11108.3	73.305	944.1	0	43.14	72.3
2	9618.4	69.894	714.3	0	36.05	62.6

The powder compositions after extraction were: batch 1 total protein 32.0 %, lactose 47.9 %, total fat 13.6 %, moisture 3.8 %, and ash 3 %; batch 2 total protein 34.2 %, lactose 44.2 %, total fat 11.3 %, moisture 3.5 %, and ash 6.3 %.

The powder from batch 2 was tested for whey protein denaturation. It was assumed that the casein proteins were not denatured. A representative sample of powder was taken, and mixed with water to give approximately 3 % whey proteins in solution. The caseins were precipitated at pH 4.6 with hydrochloric acid, and removed from solution by centrifuging. The composition of the remaining soluble whey proteins was determined by reverse phase chromatography. The soluble whey proteins decreased from 13.43 g/100 g of protein in the feed to 8.39 g/100 g of protein in the extracted powder. There was a very large decrease in native (undenatured) beta-lactoglobulin. Denaturation of the protein

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makes the powder less suitable for infant formula than those products described in Example 3.

Example 3: Extraction of low lactose beta serum powders with supercritical CO₂

This example shows that extraction of neutral lipids with greater than 90 % yield is possible from low lactose beta serum powders. The reduction in lactose content of the beta serum was carried out by ultrafiltration to a volume concentration factor of 8. The protein and total phospholipid contents of the final powder are high. Low lactose beta serum powders with the following compositions were extracted with supercritical CO₂ at 300 bar and 313 K: batch 3 low lactose total protein 48.3 %, lactose 14.4 %, total fat 30.1 %, moisture 3.0 % and ash 4.8 %; batch 4 total protein 52.0 %, lactose 7.8 %, total fat 31.9 %, moisture 2.7 % and ash 4.8 %. The fat extraction results, and mass of phospholipids in the extract are shown in table 3. Only neutral lipids are extracted by supercritical CO₂ as the other types of fat, and especially phospholipids, are not soluble in this solvent.

Table 3 Lipid yields for the extraction of low lactose beta serum powders with CO₂

Batch	Mass of solids	CO ₂ used	Mass of extract	Mass of phospholipid in extract	% yield, total lipids	% yield, neutral lipids
	g	kg	g	g		
3	6981.2	80.399	1085.7	0	51.6	90.2
4	6375.2	79.601	1085.4	0	53.4	94.0

The powder compositions after extraction were: batch 3 total protein 57.3 %, lactose 15.1 %, total fat 18.7 %, total phospholipids 14.4 %, moisture 4.1 %, and ash 5.7 %; batch 4 total protein 61.6 %, lactose 10.1 %, total fat 21.9 %, total phospholipids 16.8 %, moisture 4.5 %, and ash 5.6 %. Batches 3 and 4 extracted with supercritical CO₂ also had

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enhanced levels of gangliosides at ~ 0.7 % by mass. The remaining difference between the total fat in the residual powder, and the phospholipid and ganglioside content, is made up of mostly ceramides and cerebroside, especially lactosylceramide.

The powder from batches 3 (low lactose) and 4 (very low lactose) were tested for protein denaturation to ensure that it was suitable for use in infant formula as per example 2. The soluble whey proteins *increased* from 12.20 g/100 g of protein in the feed to 13.57 g/100 g of protein in the extracted powder for batch 3; and from 12.44 g/100 g of protein in the feed to 12.94 g/100 g of protein in the extracted powder for batch 4. The lack of denaturation of the protein, and the high protein and phospholipid contents of the extracted low lactose powders make them very suitable for infant formula.

Example 4: Extraction of standard and low lactose beta serum powders with supercritical CO₂ followed by near critical dimethyl ether

This example shows that the extraction of phospholipids in high yield from beta serum powder that has been pre-extracted with supercritical CO₂ is only possible when the lactose content of the powder has been reduced; and that dimethyl ether extraction temperature influences the extraction yield. The example also shows that it is possible to control the final phospholipids content in the powder after extraction by controlling the extraction temperature. Partially defatted powder batches 2 (standard lactose content, feed mass 4318.7 g), 3 (low lactose, feed mass 2952.6 g) and 4 (very low lactose, feed mass 2668.2 g) produced in examples 2 and 3 were re-extracted with dimethyl ether at 40 bar and 293 K using 12.236, 13.828 and 5.117 kg respectively; and then re-extracted with dimethyl ether at 40 bar and 323 K using 13.037, 10.962 and 6.965 kg respectively. The extraction yield results are shown in table 4

Table 4 Phospholipid and total lipid yields from standard and low lactose powders using dimethyl ether after supercritical CO₂ extraction

Batch	Lactose	DME extraction	DME extraction	Overall lipid yields, %
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	content	yields at 293 K		yields at 323 K		Phospholi pid	Total lipid
		Total lipid, g	% Phospholi pid	Total lipid, g	% Phospholi pid		
2	47.9	56.1	64.3	14.5	63.7	14.2	61.0
3	15.1	338.6	76.8	38.9	75.5	69.1	82.6
4	10.1	318.8	77.8	32.2	77.3	60.3	82.1

The total lipid extract also contained significant levels of ganglioside, at 2.5 % by mass for batch 4 at 293 K; and 1 % by mass for batch 4 at 323 K. The protein contents of all powders increased relative to the feed after dimethyl ether extraction. The powder compositions after CO₂ and dimethyl ether extractions were: batch 2 total protein 34.6 %, lactose 47.1 %, total fat 8.9 %, total phospholipids 6.3 %, moisture 2.7 %, and ash 6.7 %; batch 3 total protein 64.4 %, lactose 17.9 %, total fat 8.4 %, total phospholipids 5.7 %, moisture 3.6 %, and ash 5.4 %; batch 4 total protein 73.2 %, lactose 8.7 %, total fat 7.6 %, moisture 4.3 %, and ash 5.1 %. Both powders had significant levels of gangliosides, at approximately 0.4 % by mass. The remaining difference between the total fat in the residual powder, and the phospholipid and ganglioside content, is made up of mostly ceramides and cerebroside, especially lactosylceramide.

The powder from batches 2 (standard lactose content), 3 (low lactose) and 4 (very low lactose) after supercritical CO₂ and dimethyl ether extraction were tested for protein denaturation as per example 2. The soluble whey proteins decreased from 13.43 g/100 g of protein for batch 2 to 8.00 g/100 g of protein in the DME extracted powder. The soluble whey proteins *increased* from 12.20 g/100 g of protein in the feed to 15.23 g/100 g of protein in the extracted powder for batch 3; and from 12.44 g/100 g of protein in the feed to 16.98 g/100 g of protein in the extracted powder for batch 4. The lack of protein denaturation, and the high protein and phospholipid contents of the extracted low lactose powders make them very suitable for infant formula. Extraction with dimethyl ether has had the unexpected effect of increasing the apparent whey protein solubility, which is initially diminished by the removal of lactose from the feed.

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Example 5: Extraction of standard and low lactose powders with dimethyl ether

This example shows that the extraction of both neutral lipids and phospholipids in high yield from beta serum powder is only possible when the lactose content of the powder has been reduced when using dimethyl ether as the solvent without previously extracting the powder with supercritical CO₂; and that dimethyl ether extraction temperature influences the extraction yield. The example also shows that it is possible to control the final phospholipids content in the powder after extraction by controlling the extraction temperature. Batch 2 (standard lactose content, feed mass 4245.6 g) with composition as given in example 2; and batches 3 (low lactose, feed mass 3407.5 g) and 4 (very low lactose, feed mass 3204.4 g) with compositions as given in example 3 were extracted with dimethyl ether at 40 bar and 273.293 K using 13.426, 12.666 and 13.938 kg respectively; and then re-extracted with dimethyl ether at 40 bar and 323 K using 15.727, 11.673 and 11.123 kg respectively. The extraction yield results are shown in table 5

Table 5 Phospholipid and total lipid yields from standard and low lactose powders using dimethyl ether at 293 K and 323 K

Batch	Lactose content	DME extraction yields at 293 K		DME extraction yields at 323 K,		Overall lipid yields, %	
		Total lipid, g	% Phospholipid	Total lipid, g	% Phospholipid	Phospholipid, %	Total
2	44.6	189.4	21.8	50.7	27.3	14.9	26.9
3	14.4	752.9	27.5	101.9	66.8	62.9	82.3
4	7.8	869.1	31.6	72.7	69.9	67.4	85.3

The protein contents of all powders increased relative to the feed after dimethyl ether extraction. The powder compositions after dimethyl ether extractions were: batch 2 total protein 34.8 %, lactose 44.2 %, total fat 16.3 %, phospholipids 8.3 %, moisture 2.3 %,

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and ash 6.2 %; batch 3 total protein 65.1 %, lactose 15.3 %, total fat 8.3 %, phospholipids 6.7 %, moisture 2.2 %, and ash 5.3 %; batch 4 total protein 73.3 %, lactose 8.8 %, total fat 8.3 %, total phospholipids 6.8 %, moisture 2.6 %, and ash 5.2 %. For batches 3 and 4, the difference between the total fat and phospholipids content is made up of gangliosides, ceramides and cerebroside.

The powder from batches 2 (standard lactose content), 3 (low lactose) and 4 (very low lactose) after dimethyl ether extraction were tested for protein denaturation as per example 2. The soluble whey proteins increased from 13.43 g/100 g of protein to 14.38 g/100 g for batch 1; from 12.20 g/100 g of protein in the feed to 15.47 g/100 g of protein in the extracted powder for batch 3; and from 12.44 g/100 g of protein in the feed to 15.55 g/100 g of protein in the extracted powder for batch 4. The lack of protein denaturation and the high protein content of the DME extracted low lactose powders make them suitable for a wide range of food applications, especially sports nutrition. Extraction with dimethyl ether has had the unexpected effect of increasing the apparent whey protein solubility, which is initially diminished by the removal of lactose from the feed. The extraction yield of total lipids and phospholipids is very low for powder with high lactose contents (batch 2) when using dimethyl ether alone as the extraction solvent. The high content of neutral lipids makes this powder less suitable for infant formula.

Example 6: Levels of phospholipids and gangliosides in selected dairy products

The phospholipid content of human milk typically ranges from 200-400 mg/l. (Jensen RG (1989) *Textbook of Gastroenterology and Nutrition in Infancy*, 2nd Edition, E. Lebenthal (Ed), Raven Press Ltd, New York, 157-208).

According to Harzer G, Haug M, Dieterich I & Gentner PR (1983) Changing patterns of human milk lipids in the course of the lactation and during the day. *American Journal of Clinical Nutrition*, 37, 612-621, the phospholipid composition of human milk 36 days postpartum is phosphatidylcholine (PC) 24.9%, phosphatidylethanolamine (PE) 27.7%,

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phosphatidylserine (PS) 9.3%, phosphatidylinositol (PI) 5.4% and sphingomyelin (SM) 32.4%.

Table 6 shows the phospholipid contents of:

- beta-serum powder (product A),
- low lactose beta-serum powder (product B),
- neutral lipid-depleted beta serum powder (product C), and
- the lipid extract (product D) and residual powder (product E) of product C after DME extraction.

These products are derived from bovine milk.

Product A (beta-serum powder) was produced using the method illustrated in Figure 2. Product B (low lactose beta-serum powder) was produced by ultrafiltration of product A. Product C was produced using the process as described in Example 3. Products D and E were produced using the process as described for batch 4 in Example 4, except that a single stage DME extraction was carried out at 55°C.

The total lipid content was measured by a modified Röse-Gottlieb method where the lipid extracts were vacuum evaporated and freeze-dried cf. oven drying (low temperature drying minimises the phospholipid hydrolysis that occurs during oven drying due to the presence of ammonia in the lipid extracts). The total phospholipid content was calculated by multiplying the phosphorus content of the modified Röse-Gottlieb fat extract by 25.5 (refer McDowell AKR (1958) Phospholipids in New Zealand dairy products. *Journal of Dairy Research*, 25, 192-202.)

Individual phospholipids were measured by ^{31}P NMR.

Pan XL & Izumi T (2000) Variation of the ganglioside compositions of human milk, cow's milk and infant formulas. *Early Human Development*, 57, 25-31 show that ganglioside GD3 and ganglioside GM3 account for about 60% of the total gangliosides in

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human milk and that the total ganglioside content of infant formula, as measured by the lipid-bound sialic acid (LBSA) content, is significantly lower than that of human milk. The content of ganglioside GD3 plus ganglioside GM3 in human milk ranges from 10-16 mg/l, depending on the stage of lactation (Nakano *et al.*, 2001 Sialic acid in human milk: Composition and functions. *Acta Paediatrica Taiwanica*, 42, 11-17). Table 6 shows the ganglioside GD3 and ganglioside GM3 contents of product A, product B, product C, product D and product E. The ganglioside GD3 and ganglioside GM3 levels were calculated as follows: samples were dissolved in chloroform/methanol/water 6:3:0.45 and filtered. The gangliosides were then separated into GM3 and GD3 fractions by strong anion exchange solid phase extraction and sialic acid quantified by the Resorcinol method of Svennerholm (Svennerholm, L. 1957. Quantitative estimation of sialic acids. II. A colorimetric resorcinol-hydrochloric acid method. *Biochim. Biophys. Acta*. 24:604-611). The sialic acid levels were then used to calculate the concentrations of GM3 and GD3.

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Table 6 - Polar lipid compositions of products A-E

Component (% w/w)	A	B	C	D	E
Total lipid	20.6	33.9	20.9	86.1	6.3
Total phospholipid	9.7	15.1	17.5	66.6	5.2
Phosphatidylcholine	2.6 ¹	4.1	4.9	13.3	1.7
Phosphatidylethanolamine	2.7 ¹	4.2	4.8	22.0	1.0
Phosphatidylserine	0.8 ¹	1.3	1.7	8.2	0.36
Phosphatidylinositol	0.6 ¹	1.0	1.2	6.1	0.35
Sphingomyelin	2.7 ¹	4.2	4.4	15.1	1.8
Ganglioside GD3	0.36	0.58 ²	0.66 ³	2.09	0.28
Ganglioside GM3	0.04	0.06 ²	0.05 ³	0.34	0.0

Example 7: Infant formulas containing selected dairy product

Table 7 shows the percentage of each product (A, B, C, D and E) that needs to be added to infant formula on a powder basis in order to increase the "total" ganglioside (ganglioside GD3 plus ganglioside GM3) content of ready-to-feed (RTF) infant formula (IF) by 16 mg/L.

The assumptions are that the baseline levels of these components in standard infant formula are 0%, that the infant formula powder is reconstituted to 13% total solids, and that the density of the RTF IF is 1.0 kg/L.

The beta serum product addition rates shown in Table 7 also increase the individual phospholipid contents of the infant formula to levels greater than those found in human milk, the only exception being product E, where the added PE and PS levels are slightly lower than those found in human milk (base levels of these components in standard infant formula would probably compensate for these shortfalls). Note that product D could be

¹ Estimated from measured values for LLBSP.

² Estimated from mean values for standard beta serum powder.

³ Estimated. These are the results of another BPC6C sample.

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used instead of soy lecithin, which is commonly used to instantise infant formulas, thereby making them easier to reconstitute.

Table 7 Added levels of polar lipid components in RTF IF at 13% total solids

Added Component (mg/L)	Human Milk	A	B	C	D	E
Addition rate (% of powdered infant formula)	NA	3.08	1.92	1.73	0.51	4.40
Total phospholipid	300 ⁴	388	377	394	442	297
Phosphatidylcholine	75	105	102	110	88	97
Phosphatidylethanolamine	83	109	105	108	146	57
Phosphatidylserine	28	34	32	38	54	21
Phosphatidylinositol	16	26	25	27	40	20
Sphingomyelin	97	108	105	99	100	103
Ganglioside GD3+GM3	16	16	16	16	16	16
Added neutral lipid ⁵	NA	436	469	76	129	63

Example 8: Infant formulas

Ready-to-feed total solids = 13.0%

Whey-to-casein ratio – 60:40 (whey protein at least 60.0% of total protein)

Protein target⁶ = 14 g/L

Fat target^{6,7} = 35.4-37.6 g/L

⁴ Mean of range reported by Jensen (1989). Individual phospholipids are calculated based on this mean value and the percentages reported by Harzer *et al.* (1983).

⁵ Neutral lipid is estimated as the difference between the total lipid and phospholipid. This is, however, an over-estimate as it includes the glycolipids (e.g. ceramides, gangliosides). To put the added amount of neutral lipid into perspective, the ANZFA range for fat in RTF IF is 21600-45000 mg/kg i.e. the impact of added neutral lipid on the balanced fatty acid profile of IF is small, particularly with products C, D and E.

⁶ Based on Similac Advance with Iron and Enfamil LIPIL with Iron levels, noting that the Enfamil LIPIL with Iron levels were reported as g per 100 cal (cf g/100 g) so that a RTF energy content of 2800 kJ/L for this product has been assumed.

⁷ Estimated levels for Similac and Enfamil products respectively.

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Carbohydrate target⁶ = 72.1-72.9 g/L

These target levels meet the ANZFA energy requirements of infant formula (2700-3000 kJ/L). The difference between the sum of the protein, carbohydrate and fat levels and the 13% total solids target was assumed to be the vitamin and mineral premixes, antioxidants, lecithin (used to instantise the final infant formula), and possibly nucleotides/nucleosides. These components typically amounted to about 3% of the powdered infant formula.

The oil mix used in preparing infant formulas typically comprises a blend of vegetable oils in order to achieve a fatty acid profile close to that of human milk. Vegetable oils that are commonly used in infant formula are high oleic palm olein, high oleic sunflower oil, high oleic safflower oil, coconut oil and soy oil. Furthermore, many of the premium brands also contain fish/microalgal and fungal oils as sources of docosahexaenoic acid and arachidonic acid respectively.

Infant formula 1 (Using beta serum powder – product A):

A nutritional formula comprising:

- a. About 46.54% lactose
- b. About 26.92% oil mix (comprising 45% high oleic palm olein, 20% soy oil, 20% coconut oil and 15% of either high oleic safflower oil or high oleic sunflower oil)
- c. About 13.85% skim milk powder (SMP)
- d. About 6.54% ALACEN 392 (80% protein whey protein concentrate)
- e. About 3.15% beta serum powder (product A)
- f. About 3.00% vitamin and mineral premixes, lecithin, antioxidants/stabilisers, optional components e.g. nucleotides

Infant formula 2 (Using low lactose beta serum powder – product B):

A nutritional formula comprising:

- a. About 47.69% lactose
- b. About 26.92% oil mix

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- c. About 13.85% SMP
- d. About 6.54% ALACEN 392
- e. About 1.92% low lactose beta serum powder (product B)
- f. About 3.08% vitamin and mineral premixes, lecithin, antioxidants/stabilisers, optional components e.g. nucleotides

Infant formula 3 (Using -- product C):

A nutritional formula comprising:

- a. About 47.69% lactose
- b. About 26.92% oil mix
- c. About 13.85% SMP
- d. About 6.54% ALACEN 392
- e. About 1.73% product C
- f. About 3.27% vitamin and mineral premixes, lecithin, antioxidants/stabilisers, optional components e.g. nucleotides

Infant formula 4 (Using product D):

A nutritional formula comprising:

- a. About 46.54% lactose
- b. About 26.92% oil mix
- c. About 16.15% SMP
- d. About 6.77% ALACEN 392
- e. About 0.51% product D
- f. About 3.11% vitamin and mineral premixes, lecithin, antioxidants/stabilisers, optional components e.g. nucleotides

Infant formula 5 (Using product E):

A nutritional formula comprising:

- a. About 50.77% lactose
- b. About 26.92% oil mix
- c. About 8.23% SMP

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- d. About 6.00% ALACEN 392
- c. About 4.40% product F
- f. About 3.68% vitamin and mineral premixes, lecithin, antioxidants/stabilisers, optional components e.g. nucleotides

The above examples are illustrations of practice of the invention. It will be appreciated by those skilled in the art that the invention may be carried out with numerous variations and modifications. For example temperatures and pressures for the extractions may be varied as can the protein and lactose contents of the starting materials.

Also, it will be appreciated that the dairy products of the present invention may also be used in products for dermatological or general nutritional benefit in the consumer, including sports nutrition and food for the elderly.

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.

In this specification where reference has been made to patent specifications, other external documents, or other sources of information, this is generally for the purpose of providing a context for discussing the features of the invention. Unless specifically stated otherwise, reference to such external documents is not to be construed as an admission that such documents, or such sources of information, in any jurisdiction, are prior art, or form part of the common general knowledge in the art.

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WHAT WE CLAIM IS:

1. An infant formula comprising isolated beta-serum or low-lactose beta-serum.
2. The infant formula of claim 1 comprising:
 - (a) 30 – 60 % lactose
 - (b) 15 – 35% vegetable oils
 - (c) 0 – 40% skim milk powder
 - (d) 0 – 40% whey protein concentrate
 - (e) 1 – 50% isolated beta-serum or low-lactose beta-serum powder.
3. The infant formula of claim 2 comprising:
 - (a) 40 – 60 % lactose
 - (b) 20 – 30% vegetable oils
 - (c) 10 – 15% skim milk powder
 - (d) 6 – 8% of an 80% protein whey protein concentrate (WPC80)
 - (e) 1 – 10% isolated beta-serum or low-lactose beta-serum powder.
4. The infant formula of claim 3 comprising:
 - (a) 40 – 60 % lactose
 - (b) 20 – 30% vegetable oils
 - (c) 10 – 15% skim milk powder
 - (d) 6 – 8% of an 80% protein whey protein concentrate (WPC80)
 - (e) 2 – 5% isolated beta-serum or low-lactose beta-serum powder.
5. The infant formula of any one of claims 1 to 4 further comprising 2 – 4% of at least one of the following:

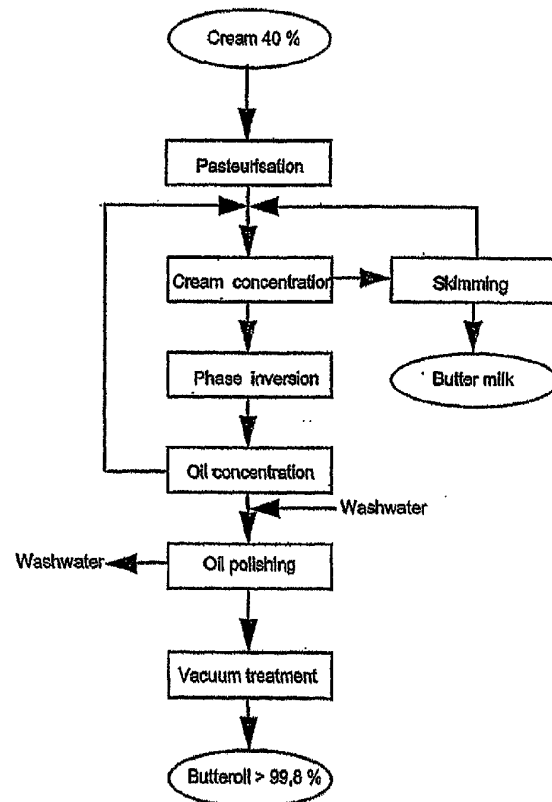
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- (a) vitamin premix
 - (b) mineral premix
 - (c) lecithin
 - (d) antioxidant
 - (e) stabiliser
 - (f) nucleotides.
6. The infant formula of any one of claims 1 to 5 wherein the infant formula provides between 2700 and 3000 kJ/L.
7. An infant formula comprising isolated beta-serum or low-lactose beta-serum substantially as herein described with reference to any example thereof.

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Figure 1



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Figure 2

