

Process for the preparation of a nutritional composition

This invention relates to a process for the preparation of a nutritional composition. Further, the invention relates to a nutritional composition, in particular the nutritional composition as obtained in the process of the invention. The invention also relates to an infant formula comprising such a nutritional composition.

Technical Background

Mother's milk is recommended for all infants. However, in some cases breast feeding is inadequate or unsuccessful for medical reasons or the mother chooses not to breast feed. Infant formulas have been developed for these situations.

However, there is a continuing need to still further improve the protective effects of infant formulas and the like compositions by combining specific ingredients, probiotics, and prebiotics with particularly beneficial effects. Additionally and/or alternatively, the protective effect of such compositions may be improved by other production processes maintaining higher levels of protein nativity, for example by production processes requiring lower heat loads or less processing steps.

Infant formula products are subject to strict hygiene regulations and absence or very low levels of viable microbes is desired. In order to achieve these required low levels of viable microbes, conventional infant formula products are pasteurized or sterilized for example by using direct steam injection (DSI) heating the product to 95°C or higher for a number of seconds followed by rapid cooling.

A disadvantage of DSI is that it has a relatively poor energy efficiency. In addition, although such a heat treatment is reducing the number of viable microbes, the heat treatment also causes denaturation of beneficial proteins like immunoglobulins (Ig) and lactoferrin which is undesirable in view of their beneficial biological activity.

It is hence desirable to develop an improved process for the production of a nutritional composition, such as infant formula, which requires less heat treatment, has equally low counts of viable microbes (e.g. at least maintaining the microbial quality of conventional infant formula production processes) and has the same or higher concentration of one or more of native lactoferrin, native immunoglobulin, vitamin D3, and/or vitamin B2. Preferably, such a process is more sustainable as compared to DSI due to a better energy efficiency. Further, the sustainability of an infant formula production process may be improved by using an alternative for DSI that requires less energy, thereby providing an alternative production process which has higher flexibility in the ingredients that may be used while not compromising on ingredient quality.

The process of the invention solves one or more of these problems.

The inventors of the process of the invention surprisingly found that UV-C may be used to remove thermoresistant bacteria from an liquid nutritional composition such as a liquid infant formula or infant formula base. More precisely, the inventors surprisingly found that UV-C takes away the necessity to heat ingredients like milk, whey or the liquid nutritional composition to temperatures of higher than 90°C.

Thus, this invention provides a process and products of the kind mentioned in the first paragraph, which process and products are characterized in the claims.

Summary of the Invention

In a first aspect the invention relates to a process for the preparation of a liquid nutritional composition comprising protein, carbohydrate, and fat, comprising the steps of

- a. obtaining ingredients milk, and one or more ingredient milk fraction

selected from the group consisting of skimmed milk, whey, and milk fat;
and

b. mixing the ingredients of a. to obtain a liquid nutritional
composition;

5 wherein the milk and the liquid nutritional composition are each
individually not heated to a temperature of higher than 90°C;
characterised in that the liquid nutritional composition and/or the milk is
UV-C germicidal treated.

10 Further, the invention relates to nutritional composition as obtained in the
process of the invention.

In another aspect, the invention relates to an infant formula comprising
the nutritional composition of the invention.

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Description of the Figures

Figure 1: Example of a prior art process for the preparation of a nutritional
composition comprising whole milk. The heat treatment at high heat ($\geq$
95 °C) at the end of the process results in no bio-actives in the nutritional
20 composition. The high heat treatment causes denaturation of the bio-
active proteins.

Figure 2 and 3 disclose an example of a process according to the
invention.

Figure 4: Decimal reductions of three groups of microorganisms (total
plate count, low thermoresistant plate count, and high thermoresistant
25 plate count) are shown for Liquid nutritional composition prepared using
whole milk when treated with UV-C treatment in combination with low
pasteurization (73.5°C, 18s), exclusively low pasteurization, or direct
steam injection (100°C, 2s). ¹⁰Log reductions between sample point 1 and
30 2 were calculated from two sets of triplicate measurements and error bars
represent the cumulative standard deviation. A higher Log-reduction

means that a larger number of microbes has been inactivated between sample point 1 and 2.

Figure 5: Decimal reductions of three groups of microorganisms (total plate count, low thermoresistant plate count, and high thermoresistant plate count) are shown for Liquid nutritional composition prepared using skim milk when treated with UV-C treatment in combination with low pasteurization (73.5°C, 18s), exclusively low pasteurization, or direct steam injection (100°C, 2s). Log reductions between sampling points 1 and 2 were calculated from two sets of triplicate measurements and error bars represent the cumulative standard deviation.

Figure 6: The concentration of native whey proteins in the whole milk based product after pasteurization compared to the concentration in the liquid nutritional composition, expressed as a percentage.

Figure 7: The concentration of native whey proteins in the skim milk based product after pasteurization compared to the concentration in the liquid nutritional composition, expressed as a percentage.

Figure 8: Concentration of vitamin B2 and vitamin D3 before and after treatment with UV-C in whole milk-based liquid nutritional composition.

Figure 9: Concentration of vitamin B2 and vitamin D3 before and after treatment with UV-C in skim milk-based liquid nutritional composition.

Figure 10: Schematic representation of the process used in Example 1 showing the different ingredients and the location of the UV-treatment. In the process of the invention, the UC-treatment always takes place at one or more of positions 3 and 4.

Details of the Invention

The terms "infant formula" or "infant nutritional product" as used herein are used interchangeably to refer to synthetic nutritional compositions that have the proper balance of macronutrients, micro-nutrients, and calories to provide sole or supplemental nourishment for and generally

maintain or improve the health of infants, toddlers, or both. Infant formulas preferably comprise nutrients in accordance with the relevant infant formula guidelines for the targeted consumer or user population, an example of which would be the Infant Formula Act, 21 U.S.C. Section 5 350(a). Another example with guidelines for nutrients of an infant formula, in particular for a person of 0-12 months of age and for children up to 36 months old, may be found in the CODEX Alimentarius (CODEX STAN 72-1981), further referred to as the CODEX). Infant formula products may be typically suited for infants aged 0-6 months (stage 1), 10 infants 6-12 months (stage 2), and infants 1-3 years old (stage 3). Alternatively, the infant formula may be suited for older children, e.g. 3-5 years old.

An "Infant formula base" as used herein refers to nutritional compositions 15 that have the proper balance of macronutrients like (protein, carbohydrate and fat) and calories for an infant formula. It comprises a majority, preferably all the mandatory nutrients of an infant formula as defined by international legislations like Codex Alimentarius Standards for Infant Formula and Formulas for Special Medical Purposes Intended for Infants. 20 Codex Stan 72 – 1981, revision 2007. An infant formula may be prepared from an infant formula base by adding missing mandatory ingredients and by adding optional nutrients like vitamins, minerals, probiotics, prebiotics, and/or human milk oligosaccharides.

25 As used herein, "UV-C germicidal treatment" is referring to an UV-C treatment that destroys or inactivates pathogens (such as bacteria, viruses, and fungi). Such treatment systems are known in the art e.g. from Lyras as described in WO2023052418.

30 The term "synthetic composition" as used herein refers to a composition which is artificially prepared and means a composition comprising at least one compound that is produced *ex vivo* chemically and/or biologically, e.g.

by means of chemical reaction, enzymatic reaction or recombinantly, or purified by humans. The synthetic composition of the invention is not identical with a naturally occurring composition, e.g. human breast milk. The synthetic composition of the invention typically comprises nutrients
5 like protein, fat (i.e. lipid), and carbohydrates, but may further include other ingredients like minerals, vitamins, or Human Milk Oligosaccharides (HMOs).

In a first aspect the invention relates to a process for the preparation of a
10 liquid nutritional composition comprising protein, carbohydrate, and fat, comprising the steps of

- a. obtaining ingredients milk, and one or more ingredient milk fraction selected from the group consisting of skimmed milk, whey, and milk fat; and
- 15 b. mixing the ingredients of a. to obtain a liquid nutritional composition;

wherein the milk and the liquid nutritional composition are each individually not heated to a temperature of higher than 90°C; characterised in that the liquid nutritional composition and/or the milk is
20 UV-C germicidal treated.

Examples of such a process of the invention are shown in Figure 2 and Figure 3. These processes differ from the process of the prior art (an example of which is shown in Figure 1) in the UV-C treatment step and the low heat treatment of the composition after the mixing step. In other
25 words, in the process of the invention is no high heat treatment at product temperatures of higher than 90°C after the mixing step (i.e. the temperature of the wet product / wet bulb temperature). For the avoidance of doubt, a spray drying step is not a high heat treatment step as it does not involve the heating the wet product to a temperature of
30 higher than 90°C.

In the process of the invention as shown in Figure 2, the UV-C treatment is positioned between the mixing step and the low-heat treatment at the

end of the process. Alternatively, in another embodiment of the invention, only the whole milk (also referred to as raw milk or fresh milk) is UV-C treated. In yet another embodiment of the invention, the nutritional composition is comprising whole milk, and as an ingredient milk fraction whey and cream. In this embodiment both the whole milk and whey are UV-C germicidal treated prior to the mixing step, as shown in Figure 3. As indicated elsewhere herein, the UV-C germicidal treatment as used in the process of the invention, may be positioned before or after mixing step b. Preferably, it is positioned before the mixing step as that causes a lower volume to be UV-C treated. So, in a preferred embodiment, the process of the invention comprises a step of UV-germicidal treatment of the milk ingredient prior to mixing the milk with other ingredients. Optionally, other ingredient milk fractions whey and skimmed milk, if present, are also individually UV-C germicidal treated prior to mixing step b.

Ingredient milk fraction milk-fat is normally subjected to a heat treatment e.g. of 95°C or more, to kill pathogens and reduce the count of bacteria. It is important that a nutritional composition according to the invention especially an infant formula base or an infant formula, have an absence of pathogens and low counts of thermoresistant bacteria in accordance with regulatory requirements. Normally this is accomplished by heating the ingredients to temperatures of 95°C or higher. Such high temperatures kill / destroy thermoresistant bacteria and pathogens present in the product, but also cause denaturation of biologically active proteins. This is undesirable as denaturation leads to a reduction of the biological activity of such proteins. The higher the heat load the ingredient is exposed to, the greater the loss of biological activity. So, in a preferred embodiment the milk and the liquid nutritional composition being produced in the process of the invention are each individually not heated to a temperature of higher than 85°C, more preferably not heated to a temperature of higher than 75°C or higher, particularly preferably not heated to a temperature of higher than 70°C, most preferably not heated to a

temperature of higher than 68°C. In one embodiment the milk is not heated to a temperature of higher than 85°C, more preferably not heated to a temperature of higher than 75°C or higher, particularly preferably not heated to a temperature of higher than 70°C, most preferably not heated
5 to a temperature of higher than 68°C. In another embodiment, when whey is used as an ingredient milk fraction, the whey is not heated to a temperature of higher than 85°C, more preferably not heated to a temperature of higher than 75°C or higher, particularly preferably not heated to a temperature of higher than 70°C, most preferably not heated
10 to a temperature of higher than 68°C. In yet another embodiment the liquid nutritional composition as obtained in the process of the invention is not heated to a temperature of higher than 85°C, more preferably not heated to a temperature of higher than 75°C or higher, particularly preferably not heated to a temperature of higher than 70°C, most
15 preferably not heated to a temperature of higher than 68°C.

The milk fat as optionally used in the process of the invention can in principle be any available milk fat source, such as cream, anhydrous milk fat (AMF) or milk fat fractions resulting from dry fractionation, critical CO₂
20 extraction or other fractionation methods known in the art. Milk fat may be added in addition to the fat present in milk. The milk fat is preferably obtained from mammalian milk, more preferably from ruminants e.g. milk of sheep, cattle, or goat. Particularly preferably, the mammalian milk fat is milk fat obtained from cattle, most preferably cow's milk fat. It was
25 found particularly suitable to use cream as the milk fat source, more preferably to use bovine cream as the milk fat source. In one embodiment, the milk fat source is cream from bovine whole milk.

In one embodiment, the process of the invention comprises a further step
30 c. of adding lactose as an additional ingredient during mixing step b. or to the liquid nutritional composition as obtained in step b. Adding lactose as an additional ingredient allows for a better control of the relative amounts

of protein, carbohydrate and fat in the nutritional composition which is important in view of the strict legal and/or regulatory requirements that may apply to infant nutrition.

In one embodiment, the nutritional composition as obtained in the process of the invention is an infant formula. In another embodiment it is a young child formula for a child aged 1-3 years old. In yet another embodiment it is an adult nutrition product. Preferably, it is an infant formula for an infant aged 0-12 months. An infant formula means a breast-milk substitute specially manufactured to satisfy, by itself, the nutritional requirements of infants during the first months of life up to the introduction of appropriate complementary feeding.

In another embodiment of the process of the invention the milk is selected from one or more of the group consisting of whole milk, and skimmed milk; preferably whole milk. In yet another embodiment the milk is microfiltrated milk i.e. it has been subjected to a microfiltration step, preferably, ceramic microfiltration. In still another embodiment, the milk is selected from one or more of the group consisting of heat treated whole milk, and heat treated skimmed milk; preferably heat-treated whole milk. In yet another embodiment, the milk is selected from one or more selected from the group consisting of unheated whole milk and unheated skimmed milk, preferably unheated whole milk. Heat-treated milk is heated to a temperature above room temperature such as between 25°C and 80°C, preferably between 25°C and 68°C, more preferably between 25°C and 60°C, most preferably between 25°C and 50°C. Unheated milk is referring to milk that is not heated i.e. not heated to a temperature above 30°C.

The whey as optionally used as an ingredient milk fraction in the process of the invention can be any kind of whey-protein fraction from milk. Preferably, the whey is selected from one or more selected from the group consisting of cheese whey, acid whey, and native whey. Preferably, the

whey is desalted whey, e.g. by desalting using ion exchange or electrodialysis, or desalted using ultrafiltration. Especially when the nutritional composition is an infant formula, it is desired to use desalted whey because the salt level in infant formula may not be too high.

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Whey is the product, which is obtained after precipitation and separation of casein-containing milk proteins from milk. When the precipitation is effected by the action of enzymes, the product after the separation of the casein is called cheese whey. When the milk is acidified e.g. by

10 fermentation or by adding acid to the milk, the product obtained after separation of casein is called acid whey. In the latter case the undissolved calcium salts present in the milk are dissolved due to the reduction of the pH. Native whey is obtained by subjecting milk to membrane filtration wherein casein is separated from whey protein material.

15 The milk being preferably mammalian milk, more preferably milk from ruminants e.g. milk of sheep, cattle, or goat. Particularly preferably, the mammalian milk is bovine milk, most preferably cow's milk.

The whey protein fraction may be obtained using milk processes known in the art. In one embodiment whey is an ingredient milk fraction and the

20 whey is desalted whey, preferably selected from one or more selected from the group consisting of desalted cheese whey, desalted acid whey, and desalted native whey. Alternatively, the whey comprises one or more of whey protein concentrate (WPC) and serum protein concentrate (SPC). Preferably, it is selected from one or more selected from the group

25 consisting of bovine WPC, bovine SPC, and bovine native whey. In another embodiment whey is an ingredient milk fraction and the whey is desalted whey not heated to a temperature of higher than 90°C and the whey is also UV-C germicidal treated; preferably the whey is selected from one or more selected from the group consisting of cheese whey, acid whey, and
30 native whey. Or alternatively, it is comprising as an ingredient milk fraction whey protein concentrate (WPC), serum protein concentrate (SPC). More preferably, the ingredient fraction is selected from one or

more selected from the group consisting of bovine WPC, bovine SPC, and bovine native whey.

Whey protein concentrate (WPC) is a product obtained by ultrafiltration optionally in combination with reverse osmosis or optionally

5 demineralization of acid whey (obtained by acidification of milk, i.e. caseinate production) or cheese whey (obtained by renneting, i.e. cheese making). By ultrafiltration, a large part of the water, lactose and ash are removed from the product, thereby concentrating the whey proteins.

Reverse osmosis can be used to remove water and to further concentrate
10 the WPC.

The preparation of SPC, on the other hand, involves microfiltration of (skimmed) milk. Said microfiltration results in a concentrated (micellar) casein retentate and a serum fraction containing most of the whey proteins as the permeate. The serum protein fraction is also called native
15 whey. Conventionally, this permeate fraction is then subjected to ultrafiltration, optionally in combination with reverse osmosis in order to remove lactose, ash, and water.

In one embodiment of the process of the invention, the milk is heat treated prior to step b. by heating the milk to 60-70°C, preferably heating
20 the milk to 60-70°C for 5-60 seconds, more preferably heating the milk to 65-70°C for 5-30 seconds, even more preferably heating the milk to 66-69°C for 10 -25 seconds.

In another embodiment of the process of the invention, the nutritional composition comprises whey as an ingredient milk fraction and the whey
25 is heat treated by heating the whey to 60-70°C, preferably heating the whey to 60-70°C for 5-60 seconds, more preferably heating the whey to 65-70°C for 5-30 seconds, even more preferably heating the whey to 66-69°C for 10 -25 seconds.

In still another embodiment of the process of the invention, the nutritional
30 composition comprises milk fat as an ingredient milk fraction and the milk fat is heat treated by heating the milk fat to 70-100 °C, preferably heating the milk fat to 70-100°C for 5-60 seconds, more preferably heating the

milk fat to 75-95°C for 5-30 seconds, even more preferably heating the milk fat to 80-90°C for 10 -25 seconds.

In a preferred embodiment, the nutritional composition comprises whey and milk fat as an ingredient milk fraction and each of the milk, whey, and milk fat are heat treated prior to step b).

In another embodiment, the nutritional composition comprises whey and milk fat as an ingredient milk fraction and the milk, whey and milk fat as used in the process of the invention are heat treated prior to step b) wherein the heat treatment of each ingredient is defined as:

- i. the milk is heat treated by heating the milk to 60-70°C;
- ii. The whey is heat treatment by heating the whey to 60-70°C,;
- iii. The milk fat is heat treated by heating the milk fat to 70-100 °C, preferably heating the milk fat to 70-100°C for 5-60 seconds, more preferably heating the milk fat to 75-95°C for 5-30 seconds, even more preferably heating the milk to 80-90°C for 10 -25 seconds.

In yet another embodiment, the nutritional composition comprises whey and milk fat as an ingredient milk fraction and the milk, whey and milk fat as used in the process of the invention are heat treated prior to step b) wherein the heat treatment of each ingredient is defined as:

- i. the milk is heat treated by heating the milk to 60-70°C;
- ii. The whey is heat treatment by heating the whey to 60-70°C, preferably heating the whey to 60-70°C for 5-60 seconds, more preferably heating the whey to 65-70°C for 5-30 seconds, even more preferably heating the whey to 66-69°C for 10 -25 seconds;
- iii. The milk fat is heat treated by heating the milk fat to 70-100 °C.

In still another embodiment, the nutritional composition comprises whey and milk fat as an ingredient milk fraction and the milk, whey and milk fat as used in the process of the invention are heat treated prior to step b)

wherein the heat treatment of each ingredient is defined as:

- i. the milk is heat treated by heating the milk to 60-70°C, preferably heating the milk to 60-70°C for 5-60 seconds, more preferably heating

the milk to 65-70°C for 5-30 seconds, even more preferably heating the milk to 66-69°C for 10 -25 seconds;

- ii. The whey is heat treatment by heating the whey to 60-70°C;
- iii. The milk fat is heat treated by heating the milk fat to 70-100 °C.

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In an embodiment of the invention, the liquid nutritional composition is dried into a powder. The drying may be carried out by any method generally used in the field, such as spray drying. The powder can optionally be dry blended with other ingredients and may be recombined

10 with water to provide a ready to drink nutritional composition in liquid form. Optionally, a vegetable fat blend is added to the liquid nutritional as obtained in the process of the invention prior to the drying step.

In one embodiment, the liquid nutritional composition as obtained in the process of the invention is an infant formula comprising a total protein
15 content of about 1.0 to about 2.5wt%, a carbohydrate content of about 6.0 to about 8.5wt% and a fat content of about 3.0 to about 5.0wt%. Vegetable oils and/or fats may be added to the composition as obtained in the process of the invention to obtain sufficient amounts of essential fatty acids.

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The ratio of whey protein to casein in the nutritional composition preferably is between 20:80 and 100:0, more preferably between 25:75 and 70:30, even more preferably between 30:70 and 60:40.

25 In one embodiment the nutritional composition as referred to in the process of the invention has one or more selected from the group consisting of:

- a. a dry matter content of between 4 and 30 wt%;
- b. a fat content as determined on dry mass of between 5.0 and 30
30 wt%, preferably between 7.0 and 28 wt%, more preferably between 9.0 and 25 wt%;

c. a protein content as determined on dry mass of at least 5.0 wt%, ;
and

d. a carbohydrate content as determined on dry mass of between 35
and 75 wt%, preferably between 40 and 70 wt%, particularly preferably
5 between 45 and 67 wt%.

In one embodiment the nutritional composition as referred to in the
process of the invention has a dry matter content of between 8 and 30
wt%, preferably between 10 and 28 wt%, more preferably between 12
and 25 wt%.

10 In one embodiment the nutritional composition as referred to in the
process of the invention has a fat content as determined on dry mass of at
least 4.0 wt% preferably between 5.0 and 30 wt%, more preferably
between 7.0 and 28 wt%, most preferably between 9.0 and 25 wt%.

In one embodiment the nutritional composition as referred to in the
15 process of the invention has a protein content as determined on dry mass
of at least 5.0 wt%, preferably between 5.0 and 35 wt%, more preferably
between 10 and 30 wt%, particularly preferably between 12 and 26 wt%;

In one embodiment the nutritional composition as referred to in the
process of the invention has a carbohydrate content as determined on dry
20 mass of between 35 and 75 wt%, preferably between 40 and 70 wt%,
more preferably between 45 and 67 wt%. In another embodiment at least
80 wt% of the carbohydrate in the composition of the invention is lactose
and the carbohydrate content as determined on dry mass of between 35
and 75 wt%, preferably between 40 and 70 wt%, more preferably
25 between 45 and 67 wt%.

The ultraviolet spectra may be broken down into several smaller areas
such as ultraviolet A (UV-A), 315-400 nm; ultraviolet B (UV-B), 280-315
nm; ultraviolet C (UV-C), 100-300 nm; near ultraviolet (N-UV), 300-400
30 nm; middle ultraviolet (M-UV), 200-300 nm; far ultraviolet (F-UV), 122-
200 nm.

In one or more embodiments, the UV germicidal treatment system further comprises one or more filters positioned between the one or more light sources and the one or more spiral-shaped tubes, wherein the one or more filters prevent light above a wavelength of 300 nm from reaching the one or more spiral-shaped tubes. By preventing light above a wavelength of 300 nm from reaching the one or more spiral-shaped tubes is meant that light above 300 nm, but below 500 nm (300-500nm), is attenuated by a substantial amount, e.g. at least a factor of 100, or a factor of 1,000 or more. In one or more embodiments, the one or more filters prevent light above a wavelength of 280 nm from reaching the one or more spiral-shaped tubes. One of the advantages using one or more filters is that photo oxidation from higher wavelengths may be avoided. E.g. avoiding photo oxidation of riboflavin (around a wavelength of 446 nm) is preferred, but also avoiding photo oxidation of other components in the liquid food product, which enhances a bitter and bad flavour/taste in said product, is preferred. Additionally, the filters may avoid hot air from contacting the one or more spiral-shaped coils, hereby avoiding heating of the liquid product.

So, in one embodiment the UV-C germicidal treatment as used in the process of the invention, involves exposing the milk and/or nutritional composition to a light source emitting light in a wavelength range between 180-300 nm, preferably between 250 nm and 258 nm, more preferably between 252 nm and 256 nm, particularly preferably between 253 nm and 255 nm, most preferably wherein the UV-C has a wavelength of 254 nm.

In one preferred embodiment, the UV-C germicidal treatment lasts shorter than 60 seconds to allow for a large scale production process. The UV-C germicidal treatment duration may be altered by increasing the UV-C light intensity and/or increasing the turbulence of the flow (lower laminar flow behaviour).

In another embodiment, the process of any of the preceding claims, wherein the duration of the UV-C treatment is less than 40 seconds, preferably less than 20 seconds. More preferably, wherein the UV-C

intensity is below 10mW/cm² and the duration of the UV-C treatment is less than 40 seconds, preferably less than 20 seconds.

Applying the UV-C germicidal treatment to the milk and/or the liquid
5 nutritional composition as in the process of the invention allows a lower heat load to be applied to the product while still adhering to the strict hygiene regulations that apply to infant formulae and obtaining an infant formula base with no or very low levels of viable microbes. Hence, in one embodiment, whey and milk fat are an ingredient milk fraction of the
10 liquid nutritional composition and the liquid nutrition composition as obtained in the process of the invention and its ingredients the milk, the whey, and the milk fat are not exposed to temperatures of higher than 86 °C. The benefit of a lower heat load is less denaturation of biologically active proteins present in the nutritional composition. Preferably, the milk,
15 and the ingredient milk fractions the whey, and the milk fat are not heated to a temperature higher than 85°C, more preferably not heated to a temperature of higher than 75°C or higher, particularly preferably not heated to a temperature of higher than 70°C, most preferably not heated to a temperature of higher than 68°C.

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In one embodiment the invention relates to a process for the preparation of a powdered nutritional composition comprising the step of drying the liquid nutritional composition as obtained in the process of the invention. Preferably, the nutritional composition is an infant formula. The liquid
25 nutritional composition may be dried using methods known in the art such as freeze drying, or spray drying; spray drying being more preferred. In another embodiment, the process for the preparation of a powdered nutritional composition is comprising a further step of blending additional ingredients into the dried powdered nutritional composition, preferably
30 wherein the additional ingredients are selected from one or more selected from the group consisting of minerals, vitamins, prebiotics, probiotics, and human milk oligosaccharides.

In one embodiment, one or more of minerals, vitamins and lactose may be added to the nutritional composition prior to mixing step b. of the process of the invention.

5 In another aspect the invention relates to a nutritional composition as obtained in the process of the invention. In one embodiment the milk as used in the preparation process of the invention is whole milk, more preferably unheated whole milk. As used herein, unheated whole milk may also be referred to as fresh milk. Alternatively, in yet another embodiment
10 the whole milk is heat-treated to a temperature above room temperature such as between 25°C and 80°C, preferably between 25°C and 68°C, more preferably between 25°C and 60°C, most preferably between 25°C and 50°C.

In yet another embodiment, the nutritional composition as obtained in the
15 process of the invention comprises whole milk and the aerobic plate count of the nutritional composition is less than 500 cfu/g of sample, preferably less than 100 cfu/g of sample, more preferably less than 50 cfu/g of sample. The aerobic plate count may be determined using methods known in the art such as the method of Sani et al (N. A. Sani, S. Hartantyo, S.J.
20 Forsythe, Journal of Dairy Science, Volume 96, Issue 1, 2013, Pages 1-8, <https://doi.org/10.3168/jds.2012-5409>).

In still another aspect, the invention relates to an infant formula comprising the nutritional composition of the invention. In one
25 embodiment it is an infant formula for infants from 0-6 months, in another embodiment for infants 6-12 months. In still another embodiment for infants of 1 year to 3 years old.

In one embodiment the nutritional composition of the invention, or the infant formula according to the invention are comprising immunoglobulin
30 G (IgG) and at least 80% of the immunoglobulin is in its native form, preferably at least 85% of the IgG is in its native form. Nativity of IgG is preferably determined using HPLC-UV as defined in the Examples section.

In another embodiment the infant formula base of the invention, or the infant formula according to the invention are comprising beta-lactoglobulin and at least 80 % of the beta-lactoglobulin is in its native form; alpha lactalbumin and at least 80 % of the alpha lactalbumin is in its native
5 form; and immunoglobulin G and at least 80% of the immunoglobulin is in its native form. In still another embodiment the infant formula base of the invention, or the infant formula according to the invention are comprising beta-lactoglobulin and at least 85 % of the beta-lactoglobulin is in its native form; alpha lactalbumin and at least 85 % of the alpha lactalbumin
10 is in its native form; and immunoglobulin G and at least 85% of the immunoglobulin is in its native form.

Preferably, in still another embodiment, the infant formula is comprising lactoferrin and at least 80% of the lactoferrin is its native form.

15 It must be noted that, as used in the specification and the appended claims, the singular form "a", "an", and "the" comprise plural referents unless the context clearly indicates otherwise. For example, reference to a component in the singular is intended to comprise a plurality of components.

20 It will be understood that within this disclosure, any reference to a weight, weight ratio, and the like pertains to the dry matter, in particular the dry matter of the composition, unless defined otherwise.

Unless defined otherwise, all technical and scientific terms used herein generally have the same meaning as commonly understood by one of
25 ordinary skill in the art to which this invention belongs.

To the extent that the term "includes" or "including" is used in the description or the claims, it is intended to be inclusive of additional elements or steps, in a manner similar to the term "comprising" as that term is interpreted when employed as a transitional word in a claim.

30 As used herein, the term "comprising", which is synonymous with "including" or "containing", is open-ended, and does not exclude additional, unrecited element(s), ingredient(s) or method step(s), whereas

the term "consisting" of is a closed term, which excludes any additional element, step, or ingredient which is not explicitly recited.

Furthermore, to the extent that the term "or" is employed (e.g., A or B), it is intended to mean "A or B or both." When the "only A or B but not both" is intended, then the term "only A or B but not both" is employed. Thus, use of the term "or" herein is the inclusive, and not the exclusive use.

When the term "and" as well as "or" are used together, as in "A and/or B" this indicates A or B as well as A and B.

Throughout this application, where publications are referenced, the

disclosures of these publications in their entireties are hereby incorporated by reference into this application to more fully describe the state of the art to which this invention pertains.

Except in the examples, or where otherwise expressly indicated, all numerical quantities in this description indicating amounts of material or conditions of reaction and/or use are to be understood as modified by the word "about" in describing the broadest scope of the invention. Practice within the numerical limits stated is generally preferred. Also, unless expressly stated to the contrary: percent, "parts of," and ratio values are by weight; the description of a group or class of materials as suitable or preferred for a given purpose in connection with the invention implies that mixtures of any two or more of the members of the group or class are equally suitable or preferred; description of constituents in chemical terms refers to the constituents at the time of addition to any combination specified in the description, and does not necessarily preclude chemical interactions among the constituents of a mixture once mixed; the first definition of an acronym or other abbreviation applies to all subsequent uses herein of the same abbreviation and applies, mutatis mutandis, to normal grammatical variations of the initially defined abbreviation; and, unless expressly stated to the contrary, measurement of a property is determined by the same technique as previously or later referenced for the same property.

It is also to be understood that this invention is not limited to the specific embodiments and methods described herein, as specific components and/or conditions may, of course, vary. Furthermore, the terminology used herein is used only for the purpose of describing particular
5 embodiments of the present invention and is not intended to be limiting in any way.

The invention is hereinafter illustrated with reference to the following, non-limiting, examples.

10 **Examples**

Microbial analyses

For the aerobic mesophilic count, also known as the *total plate count* (TPC), 1 ml of product was poured in plate count milk agar and plates were incubated for 72 hours aerobically at 30°C. Plates were subsequently
15 counted and the number of colony forming units (CFU) per ml of product was reported for a dilution where the number of observed colonies on the plate was between 10 and 300. This method is equivalent to NEN-EN-ISO 4833-1.

For the *low thermoresistant plate count*, the sample was treated at 63.5°C
20 for 30 minutes prior to plating. This method is equivalent to NEN 6807.

For the *high thermoresistant plate count*, the sample was treated at 80°C for 5 minutes prior to plating.

Three independent samples were used for each plate count determination.

25 Chemical analyses

Determination of native Immunoglobulin G (IgG) by HPLC-UV analysis was performed after the sample was dissolved in a sodium chloride buffer to precipitate interferent proteins. A protein G affinity cartridge was used to separate the protein and external IgG standards are used. This method is
30 equivalent to Abernethy et al. (2010) (Abernethy, G., Otter, D., & Collaborators: (2010). Determination of Immunoglobulin G in Bovine Colostrum and Milk Powders, and in Dietary Supplements of Bovine Origin

by Protein G Affinity Liquid Chromatography: Collaborative Study. Journal of AOAC INTERNATIONAL, 93(2), 622–627. <https://doi.org/10.1093/jaoac/93.2.622>).

Determination of native alpha-lactalbumin and beta-lactoglobulin was performed by precipitating denatured whey proteins using 1% acetic acid and analysing the supernatant using a C18 reversed-phase LC-UV quantification using external references.

Determination of Vitamin B2. The sample was dissolved in water and internal standard was added. An enzymatic digest was performed at 37°C overnight to convert vitamin B phosphate complexes into the active form. Active vitamin B was extracted using a 20% solution of trichloroacetic acid and subsequently centrifuged and diluted with acetonitrile before LC-MS/MS quantification. This method was accredited under ISO 17025 and ISO 21470 and is equivalent to McClure (2020). (McClure, S. (2020).

Simultaneous Determination of Total Vitamins B1, B2, B3, and B6 in Infant Formula and Related Nutritionals by Enzymatic Digestion and LC-MS/MS—A Multi-Laboratory Testing Study Final Action: AOAC Method 2015.14. In Journal of AOAC INTERNATIONAL (Vol. 103, Issue 4, pp. 1060–1072)).

Determination of Vitamin D3 using LC-MS/MS. The sample was dissolved in water and isotope labelled vitamin D3 internal standard was added. Samples were saponified and fat-soluble compounds were separated using isooctane extraction. Vitamin D was derivatized using 4-phenyl-1,2,4-triazoline-3,5-dione and the resulting adduct was extracted using acetonitrile before C18 reversed-phase LC-MS/MS quantification. This method was accredited under ISO 17025 and is equivalent to NEN-ISO 20636.

Example 1 Suitability of UV-C

In order to test the suitability of UV-C as a germicidal treatment in the production of an infant formula a number of infant formulas was prepared using raw milk (i.e. fresh whole milk, also referred to as RWM), skimmed

milk, cream, and desalted whey. Some physical data of these ingredients is given in Table 1.

Table 1 – Physical data of ingredients

	Dry matter (wt%)	Protein (wt%)	Casein (wt%)	Whey protein (wt%)	Fat (wt%)	Lactose (wt%)
Raw whole milk (RWM)	12.6	3.4	2.7	0.7	4.0	4.4
Skimmed milk (SM)	9.2	3.6	2.7	0.7	0.1	4.6
Cream (CR)	45.5	2.1	1.6	0.5	41.7	2.6
Desalted whey (DW)	10.2	1.4	0	1.4	0.1	8.7

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From these ingredients, six (6) batches of nutritional composition (being an infant formula in this example) were prepared. Batch numbers I, II, and III were consisting of Raw Whole Milk (RWM), Cream (CR), and Desalted Whey (DW) (i.e. demineralized whey) and were prepared using the process as shown in Figure 10. Batch numbers IV, V, and VI were consisting of Skimmed Milk (SM), CR, and DW. These were prepared in the same way, except that skimmed milk (SM) was used instead of RWM. All of these nutritional compositions had a fat content of 3.2 wt%, a protein content of 2.1 wt%, a lactose content of 6.5 wt%, and a dry matter (DM) of 12.3 wt%. As shown in Figure 10, one or more of the ingredients, or the blend of ingredients was subjected to a UV-C treatment. In other words, UV-C was either used for single raw materials or for the blends of dairy ingredients (the nutritional compositions) recipe – indicated with the combination of ingredients between brackets followed by UV-C. Table 2 (below) summarises the results of the pasteurised blends (i.e. batch nr) which were the end products in this example. TPC, LTR, and HTR were determined for each batch nr. It can be seen that no UV-C treatment (nr. I and IV) leads to log 2 to log 3 bacterial counts for

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each indicator. Meaning that there are log 2 to log 3 of high thermoresistant bacteria left after pasteurisation. If UV-C is applied on the blend (nr. III and VI) the lowest bacterial counts (log 1 or less) are achieved. UV-C treatment of individual raw materials is also possible and results in lower counts compared to no UV-C treatment.

Table 2: Microbial data of different blends treated with UV-C
(RWM: raw whole milk; TSM: thermized skimmed milk; DW: desalted whey; CR: cream)

Batch nr	Description **	TPC log (CFU/ml)	LTR log(CFU/ml)	HTR log(CFU/ml)
I	RWM+DW+CR	3.1	3.1	2.4
II	RWM(UV-C) +DW(UV-C)+CR	2.0	1.8	0.0
III	(RWM+DW+CR) UV-C	1.0	1.0	0.0
IV	TSM+DW+CR	3.0	2.6	2.3
V	TSM (UV-C) +DW(UV-C) +CR	1.0	1.0	0.8
VI	(TSM+DW+CR) UV-C	1.0	1.0	0.0

**. Raw Whole Milk (RWM), Cream (CR), Desalted Whey (DW), Thermized Skimmed Milk (TSM) (68°C, 20s).

This example shows that UV-C may be used in addition to pasteurisation to reduce the TPC, LTR, and HTR of an infant formula.

Example 2 Liquid infant formula

A liquid nutritional composition (in this example being an infant formula) was made by mixing thermized desalted whey (68°C, 20s), thermized whole- or skim milk (68°C, 20s), pasteurized cream (85°C, 20s) and
5 lactose. The ratios of these ingredients was determined based on their composition and to obtain a liquid nutritional composition with a composition of approximately 13% dry matter, 2.5% fat, 2.0% protein and 7% lactose.

Before processing, samples were taken from the liquid nutritional
10 composition for microbial and chemical analysis (sample point 1). Half of the liquid nutritional composition was treated with UV-C radiation using a Lyras Polaris (Lyras A/S, Aalborg, Denmark) Raslysation unit using the following settings: A 7 mm coil was installed using a two-pass system, allowing for double the exposure time, a narrow-band UV-C filter was
15 placed between the product and the light-source, light intensity was set at 80% and a flow of 400 L/h was applied. Samples for microbial and chemical analysis were taken directly after UV-C treatment (sample point 2). This UV-C treated product was subsequently heated rapidly using a tubular heat exchanger and pasteurized using an FSH pasteurizer at
20 73.5°C with a holding time of 18 seconds (Low Pasteurisation) before cooling to 63°C in a flash vessel.

The other half of the liquid nutritional composition bypassed the UV-C treatment and only underwent conventional pasteurization. For both products, further samples were taken for microbial and chemical analysis
25 after the flash vessel.

In another – comparative process – another liquid nutritional composition was prepared using Direct Steam Injection (No UV-C treatment) - a Liquid nutritional composition was produced as described above, the milk was
30 pasteurized (73.5°C, 18s) before blending, pre-heated to 80°C and exposed to Direct Steam Injection pasteurization (100°C, 2s) before flash-cooling to 63°C. For this process, samples were also taken from the

untreated Liquid nutritional product and the pasteurized product after the flash cooling.

As shown in Figures 4 and 5, treatment of Liquid nutritional composition with UV and low pasteurization (73.5°C, 18 seconds) results in higher decimal reductions compared to treatment using only low pasteurization or direct steam injection. Negative log reductions were observed for some tests when only applying low pasteurization, suggesting that organisms were able to increase in numbers throughout the process.

As shown in Figures 6 and 7, the UV-C treatment in combination with low pasteurization results in a higher remaining concentration of immunoglobulin G compared to the direct steam injection process. It is hence shown that the process of the invention provides a nutritional composition wherein at least 80% of Immunoglobulin G is in its native form, preferably at least 85%.

As can be seen in Figures 8, and 9, UV-C treatment only resulted in a minor decrease (<10% in whole milk, <3% on average) in the concentration of the light sensitive vitamin B2, but a large increase in the concentration of vitamin D3.

All UV-C treated nutritional compositions as prepared in this example met regulatory hygiene requirements (TPC, HTR, LTR) for infant formulae.

Example 3 Infant formula and Growing up milk base powders comprising whole milk / raw milk.

An example of the composition of an infant formula and growing up milk base powders comprising whole milk / raw milk that may be prepared using the process of the invention is given below in Table 3. Oils and the vegetable fat blend may be mixed with the product of the invention after the flash-cooling step as referred to in Example 1.

Table 3: Example of Infant formula and Growing up milk base powders comprising whole milk / raw milk according to the invention.

Ingredient		Base with low milk fat	Base with high milk fat
Whole Milk	kg solids/100 kg	16-20	18-45
Skimmed milk	kg solids/100 kg	4-24	0-16
Demineralized whey concentrate	kg/100 kg	20-45	20-45
Cream	kg solids/100 kg		0-12
Lactose	kg/100 kg	5-15	5-15
Vegetable fat blend	kg/100 kg	20-30	10-15
Minerals & vitamins	kg/100 kg	1.5-3.5	1.5-3.5

Example 4 Infant formula and Growing up milk comprising

5 skimmed milk, WPC, and SPC.

An example of the composition of an infant formula and growing up milk comprising skimmed milk, WPC, and SPC that may be prepared using the process of the invention is given below in Table 4. Oils and the vegetable fat blend may be mixed with the product of the invention after the flash-cooling step as referred to in Example 1.

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Table 4: Example of Infant formula and Growing up milk comprising skimmed milk, WPC, and SPC according to the invention

Ingredient		Infant Formula	Growing-Up milk
skimmed milk (liquid)	kg solids /100 kg	3-4	14-18
milk serum protein concentrate (SPC)	kg solids /100 kg	14-16	17-21
Whey Protein Concentrate (WPC)	kg /100 kg	1.5-5	2-4
cream	kg solids /100 kg	14-16	9-12
Lactose	kg/100 kg	40-44	33-35
vegetable fat blend	kg /100 kg	12-15	8-12
oil / minerals / vitamins	kg/100 kg	2-4	1-3
Galacto-oligosacharides	kg/100 kg	4-5	4-6
nucleotides, trace elements	kg/100 kg	1.1-1.9	0.5-2
Lactoferrin	kg/100 kg	0.1-0.5	0.1-0.5
HMO	kg/100 kg	0.1-0.5	0.1-0.6

Claims

1. Process for the preparation of a liquid nutritional composition comprising protein, carbohydrate, and fat,
5 comprising the steps of
 - a. obtaining ingredients milk and one or more ingredient milk fraction selected from the group consisting of skimmed milk, whey, and milk fat;
 - b. mixing the ingredients of a. to obtain a liquid nutritional
10 composition; and
 - c. optionally add lactose as an additional ingredient during mixing step b. or to the liquid nutritional composition as obtained in step b.;wherein the milk and the liquid nutritional composition are each
15 individually not heated to a temperature of higher than 90°C;
characterised in that the liquid nutritional composition and/or the milk is UV-C germicidal treated.
2. The process of claim 1, wherein the milk is selected from one or
20 more selected of the group consisting of whole milk, and skimmed milk; preferably whole milk.
3. The process of any of the preceding claims, wherein the one or more
25 ingredient milk fraction comprises whey and the whey is desalted whey, preferably the desalted whey is selected from one or more selected from the group consisting of desalted cheese whey, desalted acid whey, and desalted native whey.
4. The process of any of the preceding claims, wherein
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 - i. the milk is heat treated prior to step b) by heating the milk to 60-70°C, preferably heating the milk to 60-70°C for 5-60 seconds, more preferably heating the milk to 65-70°C for 5-

30 seconds, even more preferably heating the milk to 66-69°C for 10 -25 seconds; and/or

ii. the nutritional composition comprises whey as an ingredient milk fraction and the whey is heat treatment prior to step b) by heating the whey to 60-70°C, preferably heating the whey to 60-70°C for 5-60 seconds, more preferably heating the whey to 65-70°C for 5-30 seconds, even more preferably heating the whey to 66-69°C for 10 -25 seconds; and/or

iii. the nutritional composition comprises milk fat as an ingredient milk fraction and the milk fat is heat treated prior to step b) by heating the milk fat to 70-100 °C, preferably heating the milk fat to 70-100°C for 5-60 seconds, more preferably heating the milk fat to 75-95°C for 5-30 seconds, even more preferably heating the milk to 80-90°C for 10 -25 seconds;

preferably wherein the milk is heat treated as defined under i. more preferably wherein whey and milk fat are an ingredient milk fraction of the nutritional composition and each of the milk, whey, and milk fat are heat treated prior to step b) as defined under i., ii., and iii.

5. The process of any of the preceding claims, wherein the liquid nutritional composition has one or more selected from the group consisting of:
- a. a dry matter content of between 4 and 30 wt%;
 - b. a fat content as determined on dry mass of between 5.0 and 30wt%;
 - c. a protein content as determined on dry mass of at least 5.0 wt%; and
 - d. a carbohydrate content as determined on dry mass of between 35 and 75 wt%,.

6. The process of any of the preceding claims, wherein the UV-C germicidal treatment involves exposing the milk and/or liquid nutritional composition to a light source emitting light in a wavelength range between 180-300 nm, preferably between 250 nm and 258 nm, more preferably between 252 nm and 256 nm, particularly preferably between 253 nm and 255 nm, most preferably wherein the UV-C has a wavelength of 254 nm.
7. The process of any of the preceding claims wherein whey and milk fat are an ingredient milk fraction of the nutritional composition and wherein the liquid nutritional composition and its ingredients the milk, the whey, and the milk fat are not exposed to temperatures of higher than 86 °C.
8. The process of any of the preceding claims, wherein the duration of the UV-C treatment is less than 40 seconds.
9. Process for the preparation of a powdered nutritional composition comprising the step of drying the liquid nutritional composition of any of the preceding claims; preferably wherein the drying is using spray drying or freeze drying, spray drying being more preferred.
10. The process for the preparation of a powdered nutritional composition according to claim 9, comprising a further step of blending additional ingredients into the dried powdered nutritional composition, preferably wherein the additional ingredients are selected from one or more selected from the group consisting of minerals, vitamins, prebiotics, probiotics, and human milk oligosaccharides.
11. A nutritional composition as obtained in any one of claims 1 – 10.

12. The nutritional composition of claim 11, wherein the milk is whole milk, preferably comprising unheated whole milk.
13. The nutritional composition of claim 11 or 12 wherein the total aerobic plate count is less than 100 cfu/g of sample.
14. An infant formula comprising the nutritional composition of anyone of claim 11, 12, and/or 13.
15. The nutritional composition according to anyone of claim 11, 12 or 13, or the infant formula according to claim 14, comprising immunoglobulin G (IgG) and at least 80% of the immunoglobulin is in its native form.