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(54) **Title**: STABILIZED MICELLAR CASEIN AND COMPOSITIONS

(57) **Abstract**: The invention pertains to a heat-treated liquid composition comprising protein, wherein the protein concentration is between 8 and 25 g per 100 ml, said composition comprising cross-linked micellar casein (transglutaminase treated), wherein the composition has been heat- treated by heat sterilisation or pasteurisation.

Stabilized micellar casein and compositions

FIELD OF THE INVENTION

The present invention relates in general to a liquid enteral composition for providing nutrition, either as a supplement, or as a complete nutrition, with high protein concentrations and high energy content.

BACKGROUND OF THE INVENTION

An elderly person's ability to consume products may diminish. They may have difficulty consuming a product due to the too large volume of product they need to consume to meet the daily intake of nutrients. Hence, compliance is not optimal, and often, the intake is suboptimal, leading to suboptimal nourishment. Therefore, elderly persons and ill patients need more concentrated nutritional liquid compositions having increased energy as well as increased protein per unit dosage.

Certain disease states or conditions may require restrictions on the diet a patient consumes. For example, renal patients may have fluid restrictive diets. Also, a number of patients need nutrition in the smallest liquid volume possible. Such patients may suffer from cachexia related to cancer or cancer treatment, or suffer from severe pulmonary diseases like COPD (Chronic Obstructive Pulmonary Disease), tuberculosis and other infection diseases or persons that experience severe surgery or trauma like burns. Furthermore, persons suffering from disorders in the throat or mouth such as oesophageal cancer or stomatitis and persons having problems with swallowing like dysphagic persons, require special liquid, low-volume nutrition. Additionally, many patients suffer from reduced appetite and/or loss of taste due to their illness and will also benefit from an energy and protein dense low-volume liquid nutritional composition.

However, when increasing calories and/or proteins in a nutritional liquid composition this increases the overall product viscosity and stability, and this has a disadvantageous effect on the palatability of the composition. An increased viscosity can make the liquid nutritional composition difficult to consume or administer, and can also diminish the taste of the composition. Furthermore, the stability of such protein and energy dense

liquid product may become a problem; salt crystal formation during shelf life can become an issue, while it is desired that such a medical nutritional product has a shelf life of at least 1 year.

WO 02/098242 A1 discloses a calorically dense liquid oral supplement (2.25 kcal/ml) based on a (60:40) soy protein isolate/caseinate mixture with a protein level of 9 g/100 ml (16 En%), 12,25 g/100 ml of fat (49 En%), and 19.7 g/100 ml of digestible carbohydrates (35 En%). According to WO 02/098242, because caseinate may increase the viscosity of the supplement, a blend of caseinate and soy protein isolate is desired. The effects of thermal stability are not addressed.

US 5,683,984 and the corresponding EP0686396B1 teach to replace all of the caseinate in a medium energy nutritional formulation (1 kcal/ml) by native micellar casein to obtain a formulation essentially containing native micellar casein with a low viscosity, and a thermal stability to withstand sterilization. It discloses a composition containing a maximum of 7 vol% of native micellar casein. The issues of high energy and the effects of further ingredients and minerals are left unattended, nor is it disclosed how to stabilise micellar casein in a high-energy high-protein nutritional formulation. It neither hints at the problems that would arise, nor does it teach the poor shelf and heat stability, let alone does it disclose measures to overcome said problems with the formulations.

WO2009/072885 [NV Nutricia] discloses a sterilized liquid enteral nutritional composition comprising 6 to 14 g of protein per 100 ml of the composition, said protein including micellar casein and caseinate, the composition having an energy density of at least 2.0 kcal/ml. A shelf-stable high-protein low viscous product is achieved. The contents of WO2009/072885 are herewith incorporated by reference. By using micellar casein and caseinate, this prior art document shows that it is possible to produce nutritional compositions, based on a small specific volume of micellar casein. These highly-densed micelles render it possible to add more protein to a liquid composition without adverse consequences of increased viscosity or particle formation (deposits).

However, a disadvantage of the micellar casein in the compositions as disclosed in the prior art mentioned above, is that this protein is relatively unstable under different

processing conditions. In addition, additional ingredients such as carbohydrates, fat and micronutrients put further restrictions on the use of micellar casein, particularly in nutritionally complete compositions which comply with recommended daily mineral intakes. All these ingredients, and in particular the monovalent and divalent ions that are present (as 'side-products') in these ingredients (and in micellar casein), have a pronounced destabilising effect on the micellar structure of casein. The casein micelle structures are unfortunately sensitive to physico-chemical changes, susceptible to changes attributed by processing steps and changes in product environment (e.g. pH), and sensitive to mineral content (ionic strength) and mineral sources of the final product. Sodium and/or potassium present in the composition exchanges with divalent cations in the casein micelle over time. This replacement of divalent cations will destabilise the micelles, which increase in size and become less compact. Similarly, chelators like citrate and phosphate that are present in the compositions, e.g. as pH buffers, similarly have destabilising effects on the micelles, by binding to divalent cations and depleting them from the micelle structure. All of the above will have a detrimental effect on the heat stability, viscosity and shelf stability of the product.

The object of the present invention is therefore to provide a more shelf-stable, heat-stable, attractive, palatable liquid enteral composition with low viscosity that is less sensitive to physiological or chemical changes, suitable for providing nutrition, either as a supplement, or as a complete nutrition, with high energy content of preferably at least 1.0 kcal/ml, more preferably at least 1.5 kcal/ml and a protein content of at least 8 g/100 ml, to a person, in particular to an elderly person or an ill patient.

SUMMARY OF THE INVENTION

To that end, the present inventors found new nutritionally applicable solutions suitable for making protein and energy dense nutritional compositions. The inventors came to the solution of using transglutaminase enzyme activity for cross-linking micellar casein. It was found that cross-linking the casein micelles renders these more stable and less sensitive to subsequent processing or ionic conditions. These stabilization effects are evidenced in the accompanying experiments and figures. The present inventors applied the transglutaminase [TG] treatment to prepare nutritional products with a high

protein density of at least 8 g/100 ml and high caloric content of preferably at least 1.5 kcal/ml, with improved stability.

It has been known for over ten years that casein micelles in milk can be cross-linked using TG treatment in order to improve the heat stability of milk, yet it was believed that these stabilizing effects were reserved for lower protein concentrations. Reference is made to Smiddy et al. "*Stability of casein micelles cross-linked by transglutaminase*" J. Dairy Sci. 89:1906-1914, disclosing the use of transglutaminase for stabilising casein micelles in milk (with a relatively low protein content of about 3.2%). It was expected that an increase in protein concentrations would stimulate intermicellar cross-links over intramicellar cross-links (due to the increased proximity of the proteins), and that such intermicellar cross-links would lead to particle formation, protein deposits and therefore poorer product stability.

The inventors however found ways to have intramicellar cross-linking prevail over intermicellar cross-linking, rendering cross-linked micellar caseins that have a higher temperature and acid stability compared to their non-cross-linked counterparts, even with a protein content of more than 8 g protein per 100 ml and an energy density of preferably more than 1.0 kcal per 100 ml. Additionally, it was surprisingly found that stable liquid products could be produced with a very high protein content of more than 14 gram, up to 25 g per 100 ml, denser than present commercially available products. These findings could be applied to reduce intake volumes, as the inventors also found that compliance, particularly in the above-identified target subjects, improves when products have a smaller volume (less than 150 ml) compared to the 200 ml doses (and more) typically applied in the art.

Surprisingly, it was also found that the structure of the cross-linked micellar casein was not affected when calcium and/or phosphor was removed from the casein, e.g. by ultrafiltration. This renders it possible to still benefit from the compact structure of the casein micelle without having relatively high levels of calcium and/or phosphor present in the composition. The calcium concentration in micellar casein is about 10-fold higher than in sodium caseinate. In transglutaminase-treated micellar casein the concentration of calcium could be lowered to levels as low as when using the

equivalent amount of sodium caseinate as protein source, and even lower, thereby making it possible to have a low-viscous nutritional product with between 8 and 25 g / 100 ml protein and low levels of calcium and/or phosphor. The concentration of calcium is preferably below 500 mg/ 100 g protein and the relative amount of phosphor is preferably below 300 mg/100g protein. In a preferred embodiment, the composition comprises between 8 and 25 g protein per 100 ml and has a calcium concentration below 5 mg per g protein, preferably below 4.5 mg per g protein and even more preferred between 0.2 and 4.0 mg calcium per gram protein. A preferred composition according to the invention would comprise at least 8 g protein/100 ml and have energy content of at least 100 kcal/100 ml, wherein the calcium concentration is below 175 mg, preferably below 80 mg per 100 kcal.

LIST OF PREFERRED EMBODIMENTS

1. A heat-treated liquid composition comprising protein, wherein the protein concentration is between 8 and 25 g per 100 ml, said composition comprising cross-linked micellar casein, wherein the composition has been heat-treated by heat sterilisation or pasteurisation.

2. A heat-treated liquid composition comprising protein, wherein the protein concentration is between 8 and 25 g per 100 ml, said composition comprising cross-linked micellar casein, wherein the composition has been heat-treated by heat sterilisation or pasteurisation, and wherein the protein comprises at least 30 wt.%, preferably at least 50 wt.%, more preferably at least 70 wt.% micellar casein, based on total protein, wherein at least part of the micellar casein is cross-linked.

3. The liquid composition according to embodiment 1 or 2, further comprising fat and carbohydrates, and wherein the protein comprises at least 30 wt.% cross-linked micellar casein, based on total protein.

4. The liquid composition according to any of the preceding embodiments, wherein the protein comprises between 35 and 100 wt.% cross-linked micellar casein.

5. The liquid composition according to any of the preceding embodiments, wherein the composition has an energy density of at least 1.0 kcal/ml, preferably at least 1.5 kcal/ml and even more preferably between 2.0 and 3.0 kcal.

6. The liquid composition according to any of the preceding embodiments, wherein the calcium concentration is below 0.4 wt.%, based on the total protein content.

7. The liquid composition according to any of the preceding embodiments wherein the composition has a viscosity between 5 mPa.s and 200 mPa.s measured at 20 °C and with a shear rate of 100 s^{-1}.

8. The liquid composition according to any of the preceding embodiments, wherein the composition further comprises sodium or potassium caseinate, or both.

9. The liquid composition according to any of the preceding embodiments, comprising less than 0.25 wt.% phosphor based on the total weight of the protein in the composition.

10. The liquid composition according to any of the preceding embodiments, comprising at least 12 g/100 ml protein.

11. A process for preparing a composition comprising cross-linked micellar casein, comprising: (a) providing a liquid composition comprising between 7 and 15 wt.%, preferably between 8 and 10 wt.% micellar casein, based on total weight of the composition, at a pH between 6 and 8; (b) subjecting said composition to transglutaminase treatment, preferably at a temperature between 37 and 50 °C, preferably at about 45 °C, to obtain a composition comprising cross-linked micellar casein; and (c) optionally adjusting pH to above 6.6 and/or subjecting the composition to enzyme inactivation.

12. The process according to embodiment 11, wherein the composition comprising cross-linked micellar casein obtained in (b) is subjected to filtration.

13. The process according to embodiment 11 or 12, wherein the transglutaminase treatment involves contacting the composition at said temperature with at least 0.05 wt.%, preferably 0.1 – 0.35 wt.% transglutaminase for a period of preferably at least one hour, preferably between 1 and 5 hrs, more preferably between 2 and 4 hours .

14. The process according to any one of embodiments 11-13, wherein the cross-linked micellar casein composition is dried to form a powder, preferably by spray drying.

15. A composition comprising cross-linked micellar casein obtainable by the process according to any of embodiments 11 - 14.

16. A powder composition comprising at least 30 wt.% cross-linked micellar casein based on the total weight of the powder.

17. The powder according to embodiment 16, comprising between 90-100 wt.% cross-linked micellar casein based on the total weight of the powder.

18. The powder according to embodiment 16 or 17, comprising less than 0.8 wt.% calcium based on total protein content.

19. The powder according to embodiment 16 - 18, comprising less than 0.4 wt.% phosphor.

20. The powder according to embodiment 16 - 19, obtainable by the process according to any of embodiments 11 – 14.

21. The process according to any of embodiments 11 - 14, further providing the composition with protein, fat and carbohydrates to arrive at a liquid nutritional composition having a protein concentration between 8 and 25 g per 100 ml, said composition comprising at least 30 wt.% cross-linked micellar casein based on total protein.

22. Use of the composition according to embodiment 15 - 20 for improving the heat stability of a liquid nutritional composition.

23. A liquid enteral nutritional composition according to embodiment 1 or 2, comprising: a) between 9 - 20 g of protein per 100 ml, wherein the protein fraction comprising cross-linked micellar casein and at least one other protein, said protein fraction providing at least 16 % of the total energy content of the composition and comprising least 30 wt.% cross-linked micellar casein; b) fat providing at least 30 % of the total energy content of the composition; c) carbohydrate providing at least 30 % of the total energy content of the composition, said composition having an energy density of at least 10 kJ/ml [2.4 kcal/ml].

24. The liquid enteral nutritional composition according to embodiment 23, comprising less than 15 wt.% whey based on total protein.

LIST OF FIGURES

Figure 1 shows the particle size distribution of cross-linked micellar casein with and without addition of 200 mEq trisodium citrate (TSC). Also shown as control is the particle size distribution of non-cross-linked micellar casein with and without added trisodium citrate.

Figure 2 shows the viscosity as a function of shear rate of solutions of non-cross-linked (control) and cross-linked micellar casein in the presence of 0, 35 and 70 mEq/L trisodium citrate (TSC).

DETAILED DESCRIPTION OF THE INVENTION

A preferred embodiment according to the present invention is a nutritional composition with an energy density of at least 1.0 kcal per ml, more preferably at least 1.5 kcal per ml, most preferably at least 2.0 kcal/ml, and a protein content of at least 8 g/100 ml, more preferably between 8 and 25 g protein per 100 ml, more preferably between 9 and 25 g protein per 100 ml, more preferably between 10 and 25 g protein per 100 ml, even more preferably between 12 and 25 g protein per 100 ml and most preferably between 15 and 25 g protein per 100 ml, wherein the protein comprises at least 30 wt.% cross-linked micellar casein based on the total protein content, preferably between 30-95 wt.% cross-linked micellar casein, more preferably between 35-90 wt.% cross-linked micellar casein, even more preferably between 40 and 85 wt.%, most preferably between 45 and 80 wt.% cross-linked micellar casein, based on total protein content.

In one embodiment, the invention relates to a nutritional composition with an energy density of at least 1.0 kcal per ml, more preferably at least 1.5 kcal per ml, most preferably at least 2.0 kcal/ml, and a protein content of at least 8 g/100 ml, more preferably between 8 and 25 g protein per 100 ml, more preferably between 9 and 25 g protein per 100 ml, more preferably between 10 and 25 g protein per 100 ml, even more preferably between 12 and 25 g protein per 100 ml and most preferably between 15 and 25 g protein per 100 ml, wherein the protein comprises at least 30 wt.% micellar casein based on the total protein content, preferably between 30-95 wt.% micellar casein, more preferably between 35-90 wt.% micellar casein, even more preferably between 40 and 85 wt.%, most preferably between 45 and 80 wt.% micellar casein, based on total protein content, wherein at least part of the micellar casein is cross-linked. Preferably, at least 20 wt.%, more preferably at least 40 wt.%, even more preferably at least 60 wt.%, yet even more preferably at least 80 wt.%, most preferably at least 90 wt.% of the micellar casein is cross-linked.

Preferably the micellar casein is cross-linked enzymatically using the enzyme transglutaminase (TG), but other cross-linking enzymes can be suitably applied as well provided intramicellar cross-linking is achieved and inter-micellar cross-linking is controlled. The preferred cross-linked micellar casein is transglutaminase treated micellar casein, throughout the description abbreviated as 'TGMC'. Cross-linking of the micellar casein in the product can be assessed through acidification: The particle size distribution of the cross-linked protein micelles is acid-independent, and the micelle size will thus not decrease upon adding acid such as citrate. Functional tests are provided here below and in the examples.

Micellar casein (MC), also named native micellar casein, is a high quality milk protein naturally occurring in cow's milk in a concentration of about 2.6 g/100 ml. It is concentrated by a process that does not, or does not substantially denature the casein proteins and is marketed as Micellar Casein Isolate (MCI). The micellar structure in milk is stabilized by calcium phosphate and characterized by a high molecular weight. The casein micelle molecular size corresponds to about 10,000 casein monomers per micelle. It has an intrinsic low viscosity and a liquid composition comprising micellar casein is therefore easy to drink.

In contrast, non-micellar casein or caseinate, as it is used in the context of this invention, refers to the curd form of casein, which has lost its native micellar structure and is marketed as e.g. sodium caseinate and/or potassium caseinate. Optionally, the composition comprises 0 - 50 wt.%, preferably 1 to 20 wt.%, more preferably 5 to 10 wt.% of non-micellar casein or caseinate, or both, based on total weight of the protein. The caseinates are preferably in intact form. In one embodiment of the present invention, the casein or casein is Na-caseinate, Mg-caseinate, K-caseinate, Ca-caseinate or any mixture thereof. Mixtures such as Na/K-caseinate and Na/Mg caseinate can also be used as the source of caseinate. In a preferred embodiment, the composition comprises sodium or potassium caseinate, or both, preferably in a total amount of 0 - 50 wt.%, more preferably 1 to 40 wt.%, even more preferably 5 to 30 wt.%, most preferably 10 to 20 wt.% based on total weight of the protein. Preferably, Ca-caseinate, or a caseinate comprising Ca is not used, as sufficient amounts of calcium are already provided by the micellar casein and unnecessary calcium additions should be avoided.

As mentioned previously, the composition of the present invention should not contain large amounts of proteins other than micellar casein and caseinate. However, in a further embodiment of the present invention, the composition may comprise up to about 30 wt.% of whey, preferably 1 – 20 wt.% of whey, more preferably less than 15 wt.% of whey, most preferably less than 7.5 wt.% of whey of the total protein present in the liquid nutritional composition.

In the context of this application, the nutritional composition according to the invention is heat-treated in order to prolong its shelf-life and make the composition suitable for commercial use. Accordingly, the nutritional composition according to the invention is subjected to a heat-treatment such as pasteurisation or sterilisation such that the microbacterial load is reduced. Typical pasteurisation times are 30 sec at 85 °C. Typical sterilisation times are 4 minutes at 124 °C. The final heat treatment (preferably sterilisation) decreases the viscosity of the treated composition to such an extent that a microbial stable product is obtained with a low viscosity. Without wishing to be bound to any explanation, it is believed that during the extensive final heat treatment (sterilisation), restructuring of the micellar casein into a more compact structure results in a lowering of the viscosity. The product viscosity does not change substantially during storage over longer periods of time, which provides the product with a desirable long shelf life.

The composition preferably has an energy density of at least 1.8 kcal/ml, more preferably at least 2.0 kcal/ml, even more preferably at least 2.2 kcal/ml, more preferably at least 2.3 kcal/ml, even more preferably at least 2.4 kcal/ml, and less than 4 kcal/ml, preferably less than 3.5 kcal/ml. Although the composition has a high energy density, it also has a sufficiently low viscosity to allow it to be consumed by persons that may have difficulty swallowing products or those that are tube fed. Hence, in one embodiment, the nutritional composition is a liquid, heat-sterilized product having a viscosity of less than 500 mPa.s, more preferably less than 400 mPa.s, more preferably less than 300 mPa.s, even more preferably less than 200 mPa.s, preferably between 15 and 100 $mPa.s^{-1}$, as measured at 20 °C and at a shear rate of 100 /s. The viscosity may

be determined using a rotational viscosity meter using a cone/plate geometry.

Transglutaminase (TG) (EC 2.3.2.13) can be obtained from both animal and microbiological sources. In application to food system, it is more common to use bacterial TG such as e.g. the one obtained from *Streptoverticillium* (*Streptoverticillium* S-8112, *Streptoverticillium mobarense*, *Streptoverticillium sp.*, *Streptoverticillium ladakanum*, *Streptoverticillium lydicus*), since its better availability. Preferably Activa YG (Ajinomoto Food Europe, France) is used. This enzyme has an activity of approximately 1000 Units per g.

Presently, outside the field of the invention, TG in foodstuffs is used for improving the texture during meat and ham production where TG works like glue. In the protein solutions according to the invention TG can form two types of cross-linking upon reaction with casein micelles: *intra-* and *inter*micellar cross-links. While *intra*micellar cross-linking is believed to fixate the casein micelles internally, the *inter*micellar cross-links would increase the viscosity of the protein solution through gelling. Increased protein concentrations are expected to stimulate intermicellar cross-linking due to the closer proximity of the proteins. The inventors found however a way to stimulate intramicellar over intermicellar cross-linking, even at relatively high concentration of proteins and caseins. The results surprisingly show that at a pH ranging between 6 and 8, preferably between 6 and 7.5, more preferably between 6.1 and 6.9, even more preferably between 6.2 and 7.3, most preferably between 6.2 and 6.5, TG favors intramicellar cross-linking over intermicellar cross-linking of the casein micelles, even in solutions with a high protein concentration. Consequently, the size of the caseins is fixed and protein concentrations could be increased without affecting the viscosity disadvantageously. More details are given in the examples.

The inventors also found a way to control (reduce) issues associated with side-products that are generated when using transglutaminase. There are three reactions catalyzed by TG in various proteins, the most important reaction being cross-linking. However, amine incorporation and deamidation can also occur in a TG-catalyzed reaction. All three reactions disadvantageously result in the production of the by-product ammonia (NH_3). For the cross-linking reaction, TG catalyzes an acyl-transfer reaction of which

γ-carboxamide groups of protein-bound glutaminyl residues are acting as the acyl donors. At the carboxamide groups of glutamine residues, the enzymes exchange a wide variety of primary amines and ammonia.

As shown in Example 1, it is possible to determine the amount of ammonia released during transglutaminase treatment. The amount of ammonia released is directly related to the number of cross-links introduced in the protein and can therefore be used to monitor the transglutaminase reaction.

Also, when protein-bound lysine residues are acting as the acyl acceptor, cross-links between protein-bound glutamine and lysine residues, and ε-(γ-glutaminyl) lysine isopeptide bonds are formed. The degree of cross-linking reaction depends on the availability of glutamine and lysine involved in the reaction, of which the accessibility is in turn affected by the configuration, location and concentration of the proteins. If no measures are taken, cross-linking of proteins undesirably results in the formation of dimers, trimers, and larger protein polymers. This unwanted effect is expected to increase with protein concentration, yielding a viscous and instable product.

In view thereof, the inventors found that it is preferred to minimize cross-linking to the extent needed to stabilize the micellar casein solution, thus advantageously maintaining side-product formation at low levels. To optimize glutamine and lysine availability, it is preferred that the concentration of micellar casein subjected to cross-linking is maintained between 7 and 15 wt.%, preferably between 8 and 10 wt.% micellar casein, based on total weight of the aqueous composition subjected to cross-linking. As described above, the pH of the composition with limited amount of micellar casein to be subjected to the transglutaminase enzyme treatment is preferably adjusted to between 6 and 8, or any of the preferred sub-ranges.

The micellar casein is preferably cross-linked to the extent that size of the casein micelles does not change (i.e., is not reduced) significantly if 70 mEq/L citrate is added to the solution comprising the cross-linked ('TG-treated') micellar casein. Changes in micellar size - as for instance determined using standardized Zetasizer technology - of less than 10 %, even more preferably less than 5 % are still regarded acceptable in the

context of the invention. Thus, in a composition according to the invention, comprising cross-linked micellar casein and optionally further comprising non-cross-linked micellar casein, the skilled person would find that upon addition of citrate at least part (if non-cross-linked micellar casein is present), and preferably a major part, of the particle (micelle) size distribution of the casein micelles would not shift to smaller micelle sizes, thus providing evidence that (at least part of) the micellar casein present in the product is cross-linked.

Preferably, the enzyme is subsequently inactivated, for instance by heating to at least 65 °C for at least 5 minutes, preferably between 65 and 110 °C for between 10 seconds and 20 minutes. The actual time depends on the temperature, but enzyme activation is considered to fall within the skilled person's knowledge. Higher inactivation temperatures typically require lower inactivation times. Preferably the inactivation is carried out between 65 and 75 °C for a period ranging between 20 seconds and 6 minutes. Enzyme activation is preferably carried out before the cross-linked micellar casein is mixed with any of the other ingredients, particularly any optional further proteinaceous material.

Optionally the composition comprising cross-linked micellar casein is dried, preferably by spray drying, in order to obtain a cross-linked micellar casein, e.g. a cross-linked MCI (micellar casein isolate) powder. The powder can be packaged and sold as such or mixed with other nutritional ingredients for preparing a nutritional composition with high protein density. Therefore, an embodiment according to the present invention relates to a powder comprising transglutaminase-treated cross-linked micellar casein (TGMC) wherein the cross-linked micellar casein is present in at least 30 wt.%, preferably at least 40 wt.%, more preferably at least 50 wt.%, even more preferably at between 40-100 wt.%, 50-95 wt.%, most preferably between 60 and 95 wt.%, based on the dry weight of the powder. In one embodiment, the powder does not comprise any non-cross-linked micellar casein. In one embodiment, the powder comprises between 90-100 wt.% cross-linked micellar casein based on the total weight of the powder.

Once cross-linked at the above preferred conditions, the cross-linked micellar casein is ready for use in a liquid composition according to the invention. In order to arrive at a

liquid low-viscous high-protein and high-caloric composition according to the invention, fat and carbohydrates and optionally other protein and minerals can be provided to the cross-linked micellar casein, preferably to an extent that the liquid nutritional composition has a protein concentration between 8 and 25 g per 100 ml, or any of the preferred sub-ranges, said composition comprising at least 30 wt.% micellar casein based on total protein, preferably at least 50 wt.% micellar casein, more preferably at least 70 wt.% micellar casein, most preferably at least 90 wt.% micellar casein based on total protein, wherein at least part of the micellar casein is cross-linked. In a preferred embodiment, fat and carbohydrates and optionally other protein and minerals are provided to the cross-linked micellar casein to an extent that the liquid nutritional composition has a protein concentration between 8 and 25 g per 100 ml, or any of the preferred sub-ranges, said composition comprising at least 30 wt.% cross-linked micellar casein based on total protein. Additional proteins may involve one ore more of the group consisting of casein and caseinates, whey protein.

Preferably the relative amount of cross-linked micellar casein related to the total protein content in the composition is at least 30 %, more preferably at least 35, 40, 45, 50, 55 or 60 % and even more preferable at least 75%, preferably up to 100 %, even more preferably up to 95 %. If more than one protein source is used in the nutritional composition, preferably these protein sources are mixed after the micellar casein protein source has been subjected to cross-linking. In one embodiment the protein in the composition comprises, in addition to TGMC, native micellar casein (not TG-treated) or caseinate, or both.

In one embodiment of the present invention, the combined amount of TGMC and micellar casein and caseinate in the liquid nutritional composition according to the invention is at least 70 wt.% more preferably at least 85 wt.%, more preferably at least 90 wt.%, more preferably at least 95 wt.% of the total protein present in the liquid nutritional composition. Additionally or alternatively, the weight ratio of TGMC to the sum of micellar casein and caseinate ranges from 90:10 to 35:65, more preferably 85:15 to 50:50, most preferably between 80:20 and 55:45. Most preferably, the weight ratio of TGMC to the sum of micellar casein and caseinate ranges from 80:20 to 60:40.

Protein sources that comprise micellar casein that can be used forg the TG cross-linking reaction include micellar casein isolate (MCI), e.g. obtainable from Ingredia, milk protein isolate (MPI) comprising about 80% micellar casein of the total protein content, milk protein concentrate (MPC) which is usually prepared by ultra filtration (ultra-filtered milk, UF milk) that also contains about 80 % micellar casein based on the total protein content, skimmed milk (defatted milk), and fresh or pasteurized milk. In principle any milk source can be used such as cow's milk, goat milk, sheep milk etc. Preferably cow's milk is used since this source is in general better available at industrial scale. These micellar casein sources can be used in combination with any of the micellar casein protein sources or with any of the sources selected from the group consisting of plant protein sources such as soy, pea and potato protein or hydrolysates of all mentioned plant protein sources; or milk protein sources such as caseinate, whey protein, or hydrolysates thereof.

Micronutrients

The micronutrients of untreated micellar casein are rather restricted due to the instability of the micellar casein. The calcium ions in micellar casein can be exchanged due to changes in pH or ion strength of the total composition. Normal micellar casein contains about 800-2700 mg calcium per 100g powder (i.e. 0.8 – 2.7 wt.% calcium based on protein content). It was found that the calcium content of the composition comprising the cross-linked micellar casein could be reduced to very low levels without destabilizing the micellar casein, or otherwise negatively affect the low viscosity of the composition. The calcium content, based on total protein content, is preferably less than 0.7 wt.%, more preferably less than 0.6 wt.%, even more preferably less than 0.5 wt.%, most preferably less than 0.4 wt.%, especially less than 0.3 wt.%, particularly less than 0.2 wt.% calcium.

An embodiment according to the present invention pertains to a powder comprising at least 30 wt.% transglutaminase treated micellar casein (TGMC) based on total weight of the composition and less than 0.8 wt.% calcium based on total protein content, preferably less than 0.7, 0.6, 0.5, 0.4, 0.3 or 0.2 wt.% calcium based on total protein content.

Low calcium levels are particularly relevant when high protein concentrations (more than 10 g/100 ml) are required; in such case, calcium levels could easily increase to above recommended levels: 175 mg/100 kcal (Foods for Special Medical Purposes [FSMP] EC directive) and 80 mg/100 kcal (NIMS). In a preferred embodiment of the present invention the composition therefore comprises calcium levels of less than 0.4 wt.% calcium based on total weight of protein, preferably below 0.2 wt.% and even more preferably below 0.1 wt.% calcium based on total protein content of the composition. In terms of caloric content, it is preferred that the composition comprises calcium concentrations which are in accordance with the above recommendations, i.e. preferably below 175 mg/100 kcal, more preferably below 80 mg/100 kcal. With the cross-linking technology it is possible to produce products with more than 9 g protein per 100 ml, more preferably more than 10 g/100 ml protein, most preferably more than 12 g/100 ml protein, and less than 90 mg of calcium per 100 ml, without compromising the liquid character of the product. This would be impossible when using intact protein.

Similarly, the phosphate concentrations in micellar casein are responsible for a steep increase in phosphor levels in the protein composition. In a preferred embodiment the phosphor levels in the protein are below 0.25 wt.% based on the total weight of the protein, more preferably below 0.2 wt.% and even more preferably below 0.1 wt.% based on total protein weight.

A preferred composition according to the invention is a liquid product with micellar casein wherein the calcium level is below 0.4 wt.% and the phosphate level is below 0.25 wt.% based on the total protein content of the composition. A method for decreasing phosphate and calcium from micellar casein would comprise a filtration step to be performed after cross-linking the micellar casein. Through filtrations, the phosphate and calcium levels in the protein fraction comprising the TG-treated micellar casein could be reduced to a desirably low level. Any suitable filtration process could be used e.g. ultrafiltration, microfiltration, nanofiltration, diafiltration, dialysis, osmosis etc. Preferably ultrafiltration is used.

A preferred nutritional composition according to the invention comprises at least 150 kcal per 100 ml, at least 9 g protein per 100 ml and less than 80 mg calcium/100 kcal, more preferably at least 150 kcal/100 ml, at least 10 g protein per 100 ml and less than 80 mg calcium/100 kcal final product, and most preferably at least 200 kcal/100 ml, at least 12 g protein and less than 80 mg calcium/100 kcal nutritional composition. The nutritional composition preferably comprises cross-linked micellar casein in an amount of at least 30 wt.%, more preferably at least 50 wt.% based on the total protein content, wherein the other protein is selected from the group consisting of casein and caseinate (sodium and/or potassium), whey, soy and pea protein.

Digestible carbohydrates

In one embodiment of the present invention, the liquid nutritional composition according to the invention further comprises digestible carbohydrate, said digestible carbohydrate providing between 30 to 60 % of the total energy content of the composition. For a 2.0 kcal/ml composition, this amounts to 80 to 120 kcal per 100 ml. Preferably, the digestible carbohydrate provides at least 40 % of the total energy content of the composition according to the invention. The digestible carbohydrate may comprise either simple or complex carbohydrates, or any mixture thereof. Suitable for use in the present invention are glucose, fructose, sucrose, lactose, trehalose, palatinose, corn syrup, malt, maltose, isomaltose, partially hydrolysed corn starch, maltodextrins, glucose oligo- and polysaccharides.

For the present invention concerning products with high caloric density, the carbohydrate concentration is relatively high and should preferably be selected from the group of polysaccharides, because these carbohydrates contribute the least to the osmolarity of the liquid nutritional composition. In one embodiment of the present invention, the digestible carbohydrate includes maltodextrose with a high DE (dextrose equivalent). In one embodiment the digestible carbohydrate includes maltodextrose with a DE of > 20, preferably > 30 or even > 40, such as a DE of about 47.

The use of maltodextrose leads to few or no Maillard reaction products upon heating. Without being bound to any explanation, it is thought that this effect might be attributed to the fact that the compact micellar structure of the micellar casein offers

few lysine reaction sites for a Maillard reaction. In one embodiment of the present invention, the digestible carbohydrate includes maltodextrose with a high DE in an amount of at least 35 wt.%, preferably at least 50 wt.%, preferably at least 65 wt.%, preferably at least 90 wt.% of the total weight of digestible carbohydrate. In one embodiment of the present invention, the digestible carbohydrate includes maltodextrose with a low DE of 2 to 20. In one embodiment of the present invention, the digestible carbohydrate includes maltodextrose with a low DE of 2 to 10, preferably with a low DE of about 2. In one embodiment of the present invention, the digestible carbohydrate includes maltodextrose with a low DE in an amount of less than 35 wt.%, preferably less than 20 wt.%, preferably less than 10 wt.% of the digestible carbohydrate. Maltodextrose with a low DE may also be referred to as maltodextrine. In another embodiment of the present invention, the digestible carbohydrate includes maltodextrose with a high DE, preferably a DE of > 20, preferably > 30 or even > 40, most preferably a DE of about 47 in combination with maltodextrose with a low DE, preferably a low DE of 2 to 20, more preferably a low DE of 2 to 10, most preferably with a low DE of about 2. As is known, maltodextrose with a low DE, such as of about 2, gives rise to a high viscosity. Maltodextrose with a high DE, such as of about 47 gives rise to a low viscosity, but is very sweet. The combination of both maltodextroses optimizes the balance between sweetness and viscosity. In one embodiment of the present invention, the digestible carbohydrate includes at least 65 wt.%, preferably at least 90 wt.%, based on total weight of digestible carbohydrate of maltodextrose with a DE >40, preferably with a DE of about 47 and 0 to 10 wt.% of maltodextrose with a DE 2 to 10, preferably with a DE of about 2.

In another embodiment of the present invention, the digestible carbohydrate includes trehalose. As was indicated, it is one of the main objects of the invention to provide a nutritional composition with a low viscosity. Sucrose is very well suited for such purpose, but gives rise to very sweet compositions, which are in general disliked by the consumer. Maltodextrose with a low DE, such as of about 2, does not suffer from the latter drawback, but gives rise to a high viscosity. Maltodextrose with a high DE, such as of about 47 gives rise to a low viscosity, but is again very sweet, and gives further rise to the undesired Maillard reactions. Trehalose is a preferred choice of digestible carbohydrate, as it gives rise to a low viscosity, no undesired Maillard reactions and it

has a sweetness about half of that of sucrose. In one embodiment of the present invention, the digestible carbohydrate includes trehalose in an amount of 20 wt.% to 60 wt.%.

Process for making transglutaminase intramicellar cross-linked micellar casein (TGMC)

In one aspect of the invention, the process for making the nutritional product according to the invention comprises the step of preparing a micellar casein solution with a concentration of between 7 and 15 g micellar casein per 100 ml, preferably between 8 and 12 g per 100 ml and even more preferably between 9 and 11 g per 100 ml. The pH is preferably adjusted and maintained at a level ranging between 6 and 8, preferably between 6.0 and 7.5, more preferably between 6.1 and 6.9, even more preferably between 6.2 and 7.3. The smallest micellar sizes were obtained at a pH between 6.2 and 6.8. With pH, the protein concentration in the above range is one of the parameters to control cross-linking, such that intramicellar cross-links are formed predominantly over or instead of intermicellar cross-links. The micellar casein solution is preferably prepared by filtration of milk protein in order to remove the whey fraction, according to standard procedures known in the art. This has the advantage that the casein already in solution can directly be used for the cross-linking reaction with transglutaminase without the need of any preceding processing steps.

The protein solution is subsequently incubated using standard incubation technology, with TG enzyme preferably at a final concentration between 5-100 Units/g protein, preferably between 10 and 75 Units/g protein and even more preferably between 15 and 25 Units/g protein, and preferably at a protein concentration of between 7 and 15 g per 100 ml, preferably between 8 and 12 g per 100 ml and even more preferably between 9 and 11 g per 100 ml. This concentration is important in the management of the formation of intramicellar cross-links over intermicellar cross-links. The incubation preferably takes place at a temperature between 30 and 55 °C, more preferably between 35 and 50 °C and even more preferably between 40 and 50 °C. The optimal temperature is between 44 and 46 °C. The incubation time and temperature are related to one another. At lower temperature, longer incubation times are required to achieve cross-linking to satisfactory levels. At an incubation temperature of between 30 and 55

°C an incubation time of between 6 and 1 hrs is preferred. At a temperature between 40 and 50 °C the incubation time is preferably between 2.5 and 3.5 hrs. Incubation time and temperature are however parameters within the ambit of the skilled person's knowledge.

After incubation, the enzyme may optionally be inactivated and the composition with cross-linked micellar casein may conveniently be dried for further use as described here above, i.e. for providing heat stability to a liquid composition comprising high amounts of protein according to the invention.

A nutritional composition according to the invention preferably comprises protein, fat and carbohydrate wherein the protein content is between 8 and 25 wt.% and comprises between 30-95 wt.% TGMC based on the total protein content of the composition, the fat content is between 6-25 wt.% based on total weight of the composition, and digestible carbohydrate between 1-60 wt.% based on the total weight of the composition, and wherein the energy density is between 1.5 and 4.0 kcal per ml.

Example 1: Effect of TG treatment on micellar casein size, viscosity and stability

Sample preparation

A micellar casein isolate (MCI) protein powder having a protein content of 82% (w/w%), of which approximately 5% (w/w%) is whey protein (Ingredia, France), was dissolved in warm water (± 70°C) to reach a protein concentration of about 10 % (w/w) and stirred with a laboratory stirrer (RW 20.n, IKA Labortechnik, Staufen, Germany) at 600 rpm for 15 minutes. Afterwards, the protein solution was subjected to homogenization (NS2006L, GEA Niro Soari S.P.A., Parma, Italy) at a pressure of 350/50 bar. The particle size of the protein solution was determined using a Mastersizer 2000 apparatus containing a hydro 2000G water bath (Malvern Instruments, Worcestershire, England), ensuring that single casein micelles, having a diameter D[4,3] of ~0.15 µm, were obtained. After homogenization, the pH of the MCI solution was ~6.6-6.7 and the temperature was ~40 °C.

Transglutaminase (Activa YG, Ajinomoto Food Europe, France) was dissolved in demineralized water to achieve a concentration of 5% (w/w). The solution was stirred for 2 hours to ensure complete dissolution of the enzyme. A volume of 10 ml transglutaminase solution was added to 180 ml of the MCI solution and was subsequently incubated in a water bath at 45 °C for 3 hours, while constant stirring was applied. The enzyme inactivation was conducted at 70 °C for 10 min in a water bath, followed by cooling to room temperature by submerging the solution in a cold water bath. The non-cross-linked (control) MCI solution was prepared by adding 10 ml demineralized water instead of Activa YG solution to the 180 ml MCI solution.

The transglutaminase-treated (cross-linked) MCI solution and control (non-cross-linked) MCI solution were subsequently divided in volumes of 95 ml. To determine the extent of cross-linking of the casein micelles in the MCI solution, 5 mL of trisodium citrate (TSC) (4000 mEq.L-1) was added to one of the solutions to obtain a final concentration of 200 mEq.L-1 TSC in the MCI solutions, while 5 mL of demineralized water (demiwater) was added to the other MCI solutions as reference. Also a TSC concentration range (0, 35 and 70 mEq.L-1 TSC) was added to the cross-linked MCI solution and the control MCI solution. At this point, the protein concentration of each solution was 9% (w/w) and the pH was adjusted to 6.6 ± 0.05. The protein solutions were gently stirred at ambient temperature for ~17 h to obtain equilibrium in the solutions. Final pH adjustments were made in case deviations had occurred during storage. Deviations in pH were always small and samples did not show any visible spoilage.

Particle size distribution

The particle size distribution of the protein solutions was measured by using a Zetasizer Nano ZS (Malvern Instruments, Worcestershire, England) to determine the effect of transglutaminase cross-linking on the integrity of the casein micelles after trisodium citrate (TSC) addition. Protein solutions were diluted 100 times in demineralized water, filtered with 0.8 μm syringe filter and measured at 25 °C in disposable sizing cuvettes (type DTS0012). Protein solutions were equilibrated for 120 s and measured at 90 °C with backscattering. Refractive index used for the casein micelles was 1.57 and for the continuous phase 1.33 (water). Each measurement was performed in triplicate. Z-

average as well as volume percentage of the particle size distribution were obtained. The results are shown in Figure 1.

Viscosity

The viscosity of the protein solutions was determined at 20 °C with an MCR 300 rheometer (Anton Paar Physica, Graz, Austria) using a cup and bob geometry (CC27 cylinder). The viscosity was measured at shear rates of 1 – 1000 s^{-1}. Each measurement was performed in triplicate and the viscosities determined were expressed in mPa.s. The results are shown in Figure 2.

Ammonia concentration

The ammonia concentration was determined by using the Ammonia Assay Kit (Catalog Number AA0100, Sigma-Aldrich Co. LLC, USA). The samples were diluted 10 times by using demineralized water and a sample volume of 100 µl was mixed with 1.0 ml ammonia assay reagent in cuvettes. The blank was made by using 100 µl demineralized water, and the standard was made by using 50 µl ammonia standard solution (with ammonia concentration ~10 $\mu g.ml^{-1}$) in 1.0 ml ammonia assay reagent. All mixtures were incubated for ~30 min at ambient temperature and the absorbance of each mixture was measured at 340 nm (AInitial). Subsequently, 10 µl of L-glutamate dehydrogenase solution was added to each cuvette and the mixtures were incubated for 5 min. Then, the absorbance of each mixture was measured at 340 nm (AFinal). The ammonia concentration (mg NH_3 per ml of original sample volume) was calculated using the following formulas.

- $\Delta A340 = AInitial - AFinal$
- $\Delta(\Delta A340)$ sample or standard = $\Delta A340$(test or Standard) - $\Delta A340$(blank)

- $mg\ NH_3/ml = [A \times TV \times MW_{NH3} \times F]/[\varepsilon \times d \times SV \times 1000]$
  $= [A \times TV \times F]/[SV \times 0.00273]$

with

A = $\Delta(\Delta 340)$, absorbance sample or standard

TV = total assay volume (ml)

SV = sample volume (ml)

MW of ammonia = 17 $g.mole^{-1}$

F = dilution factor from sample preparation

ε = extinction coefficient of NADPH at 340 nm = 6.22 mM^{-1} cm^{-1}

d = cuvette light path (cm) = 1 cm

Results

Figure 1 shows that addition of trisodium citrate (TSC) to non-cross-linked MCI results in a reduction of particle size, most likely due to disintegration of the casein micelles. After treatment with transglutaminase according to the method of the invention, the size distribution of the resulting cross-linked casein micelles is not affected by TSC addition.

Figure 2 shows that following addition of trisodium citrate (TSC) to non-cross-linked casein micelles, the disintegrated micelles bind much more water than the intact, cross-linked micelles, resulting in increased viscosity of the composition.

It was also found that more time was needed to heat-coagulate a liquid emulsion comprising cross-linked casein micelles (25 minutes) compared to an analogous liquid emulsion comprising non-cross-linked casein micelles (control) (about 22 minutes). These changes are regarded significant, particularly when realizing that batch sterilization in the art is typically carried out at 120 °C for about 20 minutes. Thus, cross-linking improves the heat-stability of micellar casein solutions according to example 1.

Example 2: Product examples

Table 1 gives a representation of sterilized high-protein compositions (1) – (7) with various amounts of TG-treated micellar casein and their viscosities.

Table 1. Low-viscous sterilized high-protein compositions with various amounts of TG-treated micellar casein

	(1)	(2)	(3)	(4)	(5)	(6)	(7)
g per 100 ml	**Amount per 100 ml of product**	**Amount per 100 ml of product**	**Amount per 100 ml of product**	**Amount per 100 ml of product**	**Amount per 100 ml of product**	**Amount per 100 ml of product**	**Amount per 100 ml of product**
Energy (kCal)	400	320	275	250	240	240	239.8
Protein (En%)	16%	20%	23%	32%	42%	16%	24%
protein (g)	16	16	16	20	25	9.6	14.4
TG-treated micellar casein (g)[wt.%]	6 [38]	15 [94]	8 [50]	16 [80]	18 [72]	8 [83]	12 [83]
Carbohydrates (En%)	50%	38%	51%	32%	36%	50%	41%
Carbohydrates (g)	50	30	35	20	21.5	29.7	24.4
Fat (En%)	34%	42%	33%	36%	23%	35%	35%
Fat (g)	15	15	10	10	6	9.3	9.4
Dietary fibre (g)	2	-	1.5	-	-	-	-
Vitamins	16% of RDA	16% of RDA	16% of RDA	16% of RDA	16% of RDA	16% of RDA	16% of RDA
Calcium (mg)	160 -700	110 - 450	115 -400	95 -185	88 - 350	<200	<250
Viscosity (mPa.s at 20°C at 100 s^{-1})	<200	<200	<200	<200	<200	<100	<150

CLAIMS

1. A heat-treated liquid composition comprising protein, wherein the protein concentration is between 8 and 25 g per 100 ml, said composition comprising cross-linked micellar casein, wherein the composition has been heat-treated by heat sterilisation or pasteurisation.

2. The liquid composition according to claim 1, further comprising fat and carbohydrates, and wherein the protein comprises at least 30 wt.% cross-linked micellar casein, based on total protein.

3. The liquid composition according to any of the preceding claims, wherein the protein comprises between 35 and 100 wt.% cross-linked micellar casein.

4. The liquid composition according to any of the preceding claims, wherein the composition has an energy density of at least 1.0 kcal/ml, preferably at least 1.5 kcal/ml and even more preferably between 2.0 and 3.0 kcal.

5. The liquid composition according to any of the preceding claims, wherein the calcium concentration is below 0.4 wt.%, based on the total protein content.

6. The liquid composition according to any of the preceding claims wherein the composition has a viscosity between 5 mPa.s and 200 mPa.s measured at 20 °C and with a shear rate of 100 s^{-1}.

7. The liquid composition according to any of the preceding claims, wherein the composition further comprises sodium or potassium caseinate, or both.

8. The liquid composition according to any of the preceding claims, comprising less than 0.25 wt.% phosphor based on the total weight of the protein in the composition.

9. The liquid composition according to any of the preceding claims, comprising at least 12 g/100 ml protein.

10. A process for preparing a composition comprising cross-linked micellar casein, comprising: (a) providing a liquid composition comprising between 7 and 15 wt.%, preferably between 8 and 10 wt.% micellar casein, based on total weight of the composition, at a pH between 6 and 8; (b) subjecting said composition to transglutaminase treatment, preferably at a temperature between 37 and 50 °C, preferably at about 45 °C, to obtain a composition comprising cross-linked micellar casein; and (c) optionally adjusting pH to above 6.6 and/or subjecting the composition to enzyme inactivation.

11. The process according to claim 10, wherein the composition comprising cross-linked micellar casein obtained in (b) is subjected to filtration.

12. The process according to claim 10 or 11, wherein the transglutaminase treatment involves contacting the composition at said temperature with at least 0.05 wt.%, preferably 0.1 – 0.35 wt.% transglutaminase for a period of preferably at least one hour, preferably between 1 and 5 hrs, more preferably between 2 and 4 hours .

13. The process according to any one of claims 10-12, wherein the cross-linked micellar casein composition is dried to form a powder, preferably by spray drying.

14. A composition comprising cross-linked micellar casein obtainable by the process according to any of claims 10 - 13.

15. A powder composition comprising at least 30 wt.% cross-linked micellar casein based on the total weight of the powder.

16. The powder according to claim 15, comprising between 90-100 wt.% cross-linked micellar casein based on the total weight of the powder.

17. The powder according to claim 15 or 16, comprising less than 0.8 wt.% calcium based on total protein content.

18. The powder according to claim 15 - 17, comprising less than 0.4 wt.% phosphor.

19. The powder according to claim 15 - 18, obtainable by the process according to any of claims 10 – 13.

20. The process according to any of claims 10 - 13, further providing the composition with protein, fat and carbohydrates to arrive at a liquid nutritional composition having a protein concentration between 8 and 25 g per 100 ml, said composition comprising at least 30 wt.% cross-linked micellar casein based on total protein.

21. Use of the composition according to claim 14 - 19 for improving the heat stability of a liquid nutritional composition.

22. A liquid enteral nutritional composition according to claim 1, comprising: a) between 9 - 20 g of protein per 100 ml, wherein the protein fraction comprising cross-linked micellar casein and at least one other protein, said protein fraction providing at least 16 % of the total energy content of the composition and comprising least 30 wt.% cross-linked micellar casein; b) fat providing at least 30 % of the total energy content of the composition; c) carbohydrate providing at least 30 % of the total energy content of the composition, said composition having an energy density of at least 10 kJ/ml [2.4 kcal/ml].

23. The liquid enteral nutritional composition according to claim 22, comprising less than 15 wt.% whey based on total protein.

Fig. 1

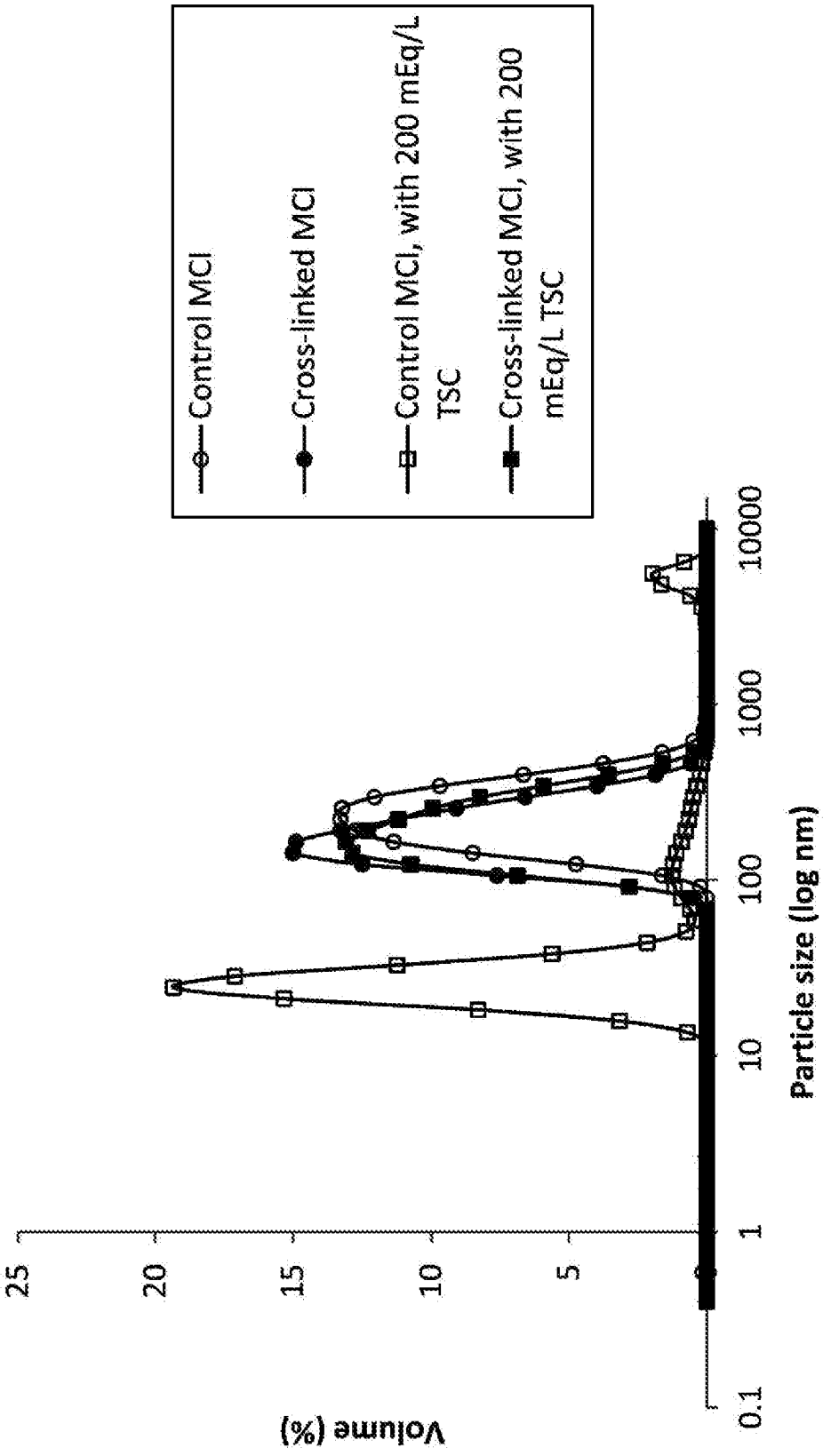
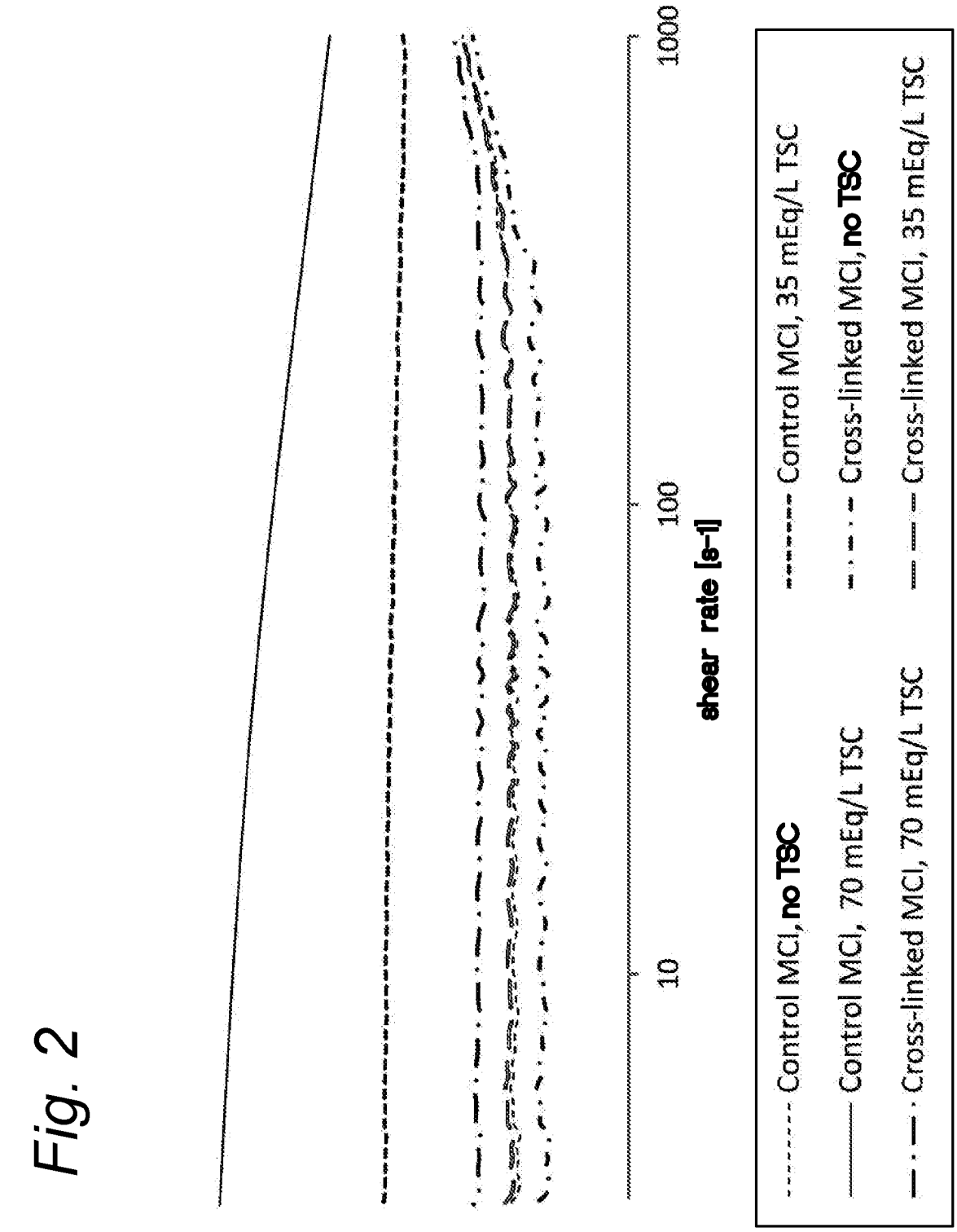

Control MCI
Cross-linked MCI
Control MCI, with 200 mEq/L TSC
Cross-linked MCI, with 200 mEq/L TSC
Volume (%)
25
20
15
10
5
0.1
1
10
100
1000
10000
Particle size (log nm)

Fig. 2


Viscosity [log10 mPa.s]
3.5
3
2.5
2
1.5
1
0.5
0
10
100
1000
shear rate [s-1]
Control MCl, no TSC
Control MCl, 70 mEq/L TSC
Cross-linked MCl, 70 mEq/L TSC
Control MCl, 35 mEq/L TSC
Cross-linked MCl, no TSC
Cross-linked MCl, 35 mEq/L TSC

INTERNATIONAL SEARCH REPORT

International application No
PCT/NL2014/050229

A. CLASSIFICATION OF SUBJECT MATTER
INV. A23L1/305 A23L1/29 A23C9/142 A23C9/152 A23J3/10
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A23L A23C A23J

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CA 2 310 659 A1 (NESTLE SA [CH]) 17 June 1999 (1999-06-17) page 10, line 18 - line 27 page 11, line 32 - page 1, line 14; examples 1,3-9; tables 1-7 ----- -/--	1-23

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 November 2014	04/12/2014

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Graham, Judith

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/NL2014/050229

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	M.P. BÖNISCH ET AL: "Effect of Ultra-high Temperature Treatment on the Enzymatic Cross-linking of Micellar Casein and Sodium Caseinate by Transglutaminase", JOURNAL OF FOOD SCIENCE, vol. 69, no. 8, 31 October 2004 (2004-10-31), pages E398-E404, XP055079416, ISSN: 0022-1147, DOI: 10.1111/j.1365-2621.2004.tb09902.x table 1 Materials and Methods; page E399	1-23
A	BONISCH ET AL: "Improvement of enzymatic cross-linking of casein micelles with transglutaminase by glutathione addition", INTERNATIONAL DAIRY JOURNAL, ELSEVIER APPLIED SCIENCE, BARKING, GB, vol. 17, no. 1, 9 November 2006 (2006-11-09), pages 3-11, XP005755145, ISSN: 0958-6946, DOI: 10.1016/J.IDAIRYJ.2006.01.007 paragraph [2.2.5] paragraph [02.3]	1-23
A	WO 2009/072885 A1 (NUTRICIA NV [NL]; SLIWINSKI EDWARD LUCIAN [NL]; LE FUR AUDREY [NL]; KI) 11 June 2009 (2009-06-11) page 4, line 27 - line 14 page 7, line 16 - page 9, line 16 table 1	1-23
A	US 5 683 984 A (JOST ROLF [CH]) 4 November 1997 (1997-11-04) examples 1,2	1-23

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/NL2014/050229

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		CA 2310659 A1	17-06-1999
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