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(74) Agent: VOSSIUS & PARTNER; Siebertstr. 4, 81675
Munich (DE).

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(71) Applicant (for all designated States except US): TECH-
NISCHE UNIVERSITÄT MÜNCHEN [DE/DE]; Arcis-
strasse 21, 80333 München (DE).

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(72) Inventors; and

(75) Inventors/Applicants (for US only): BÖNISCH, Mar-
tin [DE/DE]; Schwannseestr. 11, 81539 München (DE).
KULOZIK, U. [DE/DE]; Ländestr. 14, 86354 Freising
(DE). HEIDEBACH, Thomas [DE/DE]; Josef-Ritz-Weg
25, 81673 München (DE). HUSS, Manfred [DE/DE]; Blu-
menstrasse 6, 85414 Kirchdorf a. d. Amper (DE).

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(54) Title: PREPARATION OF A FOOD STUFF FROM A PROTEIN ENRICHED SUBSTRATE UNDER THE SIMULTANE-
OUS USE OF TRANSGLUTAMINASE AND PROTEASE

(57) Abstract: The present invention relates to a method for the preparation of visco-plastic food stuff, comprising the step of
adding to a liquid, protein-enriched concentrate comprising at least 5% proteins and (a) reactive protein(s) for transglutaminase, a
transglutaminase and a rennet or a rennet replacement which comprises a proteolytic enzyme, whereby said transglutaminase and
said rennet or said rennet replacement are added simultaneously to said protein-enriched concentrate comprising a reactive protein
for transglutaminase. Also provided are means and kits for carrying out the inventive method.



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Preparation of a food stuff from a protein enriched substrate under the simultaneous use of transglutaminase and protease

The present invention relates to a method for the preparation of visco-plastic food stuff, comprising the step of adding to a liquid, protein-enriched concentrate comprising at least 5% proteins and (a) reactive protein(s) for transglutaminase, a transglutaminase and a rennet or a rennet replacement which comprises a proteolytic enzyme, whereby said transglutaminase and said rennet or said rennet replacement are added simultaneously to said protein-enriched concentrate comprising a reactive protein for transglutaminase. Also provided are means and kits for carrying out the inventive method.

A classical protein enrichment of foods, for example matured cheeses with rennet (soft, semi hard and hard cheese) is based on the fortification of the milk constituents, mainly of casein, by the drainage of whey after a clotting process and under the use of rennet. Also the preparation of Tofu is based on a shrinking process of a protein matrix by syneresis to enrich the protein content and to achieve a certain texture.

In the 1980ties, intensive research on the amelioration of yield of cheese from milk was carried out. Trials were done to produce cheese from an ultrafiltration (UF) concentrate, in which the protein is retained completely in the retentate, without drainage of whey. This effect is achieved when an UF-membrane with a cut off of 25,000 Dalton is employed. Separation of a water phase by syneresis is called "drainage of whey" and the water phase is "whey". A separation of a water phase at an upstream process, like membrane process, is called filtrate, permeate or serum. When the ingredients of a substrate are not traditional, often the product which results from the syneresis is also called serum, e.g. at a prevention of a drainage, the term is serum binding. The disadvantage of the ultrafiltration is that in mild heat-treated milk whey proteins are present in a native state. By ultrafiltration the whey proteins are concentrated in the same ration as casein so the natural ratio of casein to whey proteins remains at approx. 80/20 and the yield may be improved. However, native whey proteins are not significantly participating in the structure formation of the resulting cheese (Schreiber, 2000. Fortschritt-

Berichte VDI Reihe 3 Verfahrenstechnik Nr. 644). The cheese from ultrafiltered milk often shows a wet surface, which easily leads to an undesired and counter-productive growth of yeasts and other disadvantages, like losing the "skin". Due to the wet surface of the cheese this process became popular only for a few kinds of white cheeses, when this cheese is stored in brine.

With the microfiltration in the 1990ties, a membrane process was introduced to the dairy industry, which had a wider pore size (approx. 0,1 micron) than UF-membranes have and which makes it possible to separate casein and whey proteins. In said process, casein is concentrated to a greater extent than whey proteins. Whey proteins permeate into the filtrate and can be treated separately from the casein by the so-called microparticulation (SPIEGEL, 2000, Deutsche Milchwirtschaft 51 (7), S. 309-311, 2000). Microparticulation is a process wherein the whey proteins denature and aggregate due to heat and under shear conditions. Microparticulated whey proteins can be incorporated into the microfiltered concentrate. With this procedure it is assured that whey proteins are mainly present in cheese in an aggregated form. Denatured whey proteins are more relevant for the structure formation of cheese and they prevent from wet surfaces due to their serum binding capacity. Besides that the dry matter of cheese can be reduced due to the behavior of aggregated whey proteins. Furthermore, the incorporation of aggregated whey proteins leads to a more creamy structure and therefore fat reduced cheese with a pleasant consistency can be produced. The production of cheese from a micro-filtered milk concentrate is, inter alia, described in Bachmann, 2003, Agrar Forschung 10 (10), 406-410.

Generally there are several methods for milk clotting/gelation, in particular acidification and renneting. Besides that also an enzymatic, covalent cross-linking can induce a gelation of proteins (Ikura, 1992, Comments Agric & Food Chem, Vol. 2, No. 6, 389-407). An overview of possible applications of transglutaminase in milk products is given in Jaros, D., Partschfeld, C., Henle, T., Rohm, H. "Transglutaminase in dairy products: chemistry, physics, application", 2006. However, an incubation of transglutaminase in milk or milk concentrates even at high enzyme concentrations (e.g. 2 U/g protein) does not result in a gelation due to the electrostatic repulsion between the casein micelles at neutral pH-values. Therefore, transglutaminase alone is not a sufficient reagent for the preparation of a relatively firm cheese. Only by a reduction of this electrostatic repulsion through a pH-drop or through the effect of rennet at relevant protein

concentrations and relevant transglutaminase concentrations a gelation may be achieved. Yet, the knowledge of the use of transglutaminase in order to influence the behavior of a rennet gel is very limited; see, e.g. Lorenzen, 2000, *Milchw* 55: 433-437. The influence of the microbial transglutaminase, MTGase, (in combination with a thermal treatment of the used milk) on the gelling time at the preparation of rennet gels was here assessed. Untreated skim milk samples were treated with different temperature-time combinations (37-90 °C; 5-300 s) and were then incubated with MTGase (14.7 U /g milk protein, based on a protein content of 3.5 % in the milk) at 35 °C. After an incubation time of 0-120 minutes and the addition of 0.015 % rennet the gelling times were estimated. It was evident that with increasing pre-incubation time as well as with increasing thermal intensity of the milk either the gelling time increased or no visible gelling occurred. At a pre-heating treatment at 90 °C for more than 5 minutes and following pre-incubation of more than 5 minutes no gelling was visible. At a thermal treatment of 75 °C for a few seconds and a following pre-incubation with MTGase in the range of 0-120 minutes the gelling time increased from 13 to 35 minutes. Yet, a protein solution with 3.5 % protein and with a ratio of 80 % casein to 20 % whey proteins resulted, after a pre-incubation time of 120 min, in a non-visible flocculation. When rennet and TG was added simultaneously to a pasteurized milk (75 °C for a few seconds), a slightly extended gelling time compared to the control was detected. O'Sullivan, 2002, *J Dairy Res* 69: 433-442 described that the extended gelling time or the absence of gelling after an incubation with MTGase is due to a negative influence of the enzymatic phase during the renneting.

EP 1 197 152 describes an improved activity of microbial transglutaminase MTGase in pasteurized milk by the addition of glutathione rich yeast extract. It is described that an increase in yield at the preparation of Cheddar cheese through a modification of the renneting process by TG and a reducing agent such as a thiol compound is possible. However, in EP- 1 197 152, non-concentrated milk was used and the whey drainage of the curd was almost comparable to a conventionally produced cheese. The taught, modified cheese making process does not offer an advantage to the cheese making process.

The prior art has employed transglutaminase in milk products in combination with the microfiltration only in cross-linking of caseino makro peptide (CMP) in whey.

However, there is a general problem in the use of transglutaminase in particular TGs in the preparation of a rennet cheese. Such negative influence of TGs on the renneting process is also described in Lorenzen (2000), Habilitationsschrift Bundesforschungsanstalt für Milchwissenschaft Kiel and O'Sullivan (2002), J Dairy Res 69: 433-442.

EP-A2 1 048 218 describes the incorporation of whey proteins in cheese, using non-rennet protease and TG. Transglutaminase and the non-rennet protease is used in a heat-treated dairy liquid. EP-A2 1 048 218 teaches that a cheese product containing a substantial portion of the whey proteins can be produced by sequentially contacting a heat treated dairy liquid with transglutaminase and non-rennet protease. Here, "dairy liquid" is defined as milk, milk products obtained by fractioning raw milk to provide a liquid fraction. Similarly, EP-B1 1 057 411 teaches that a cheese curd containing a substantial proportion of whey protein products can be produced by sequentially (1) contacting a dairy liquid fortified with whey protein with transglutaminase, (2) blending the modified dairy liquid with a second dairy liquid containing casein; and (3) contacting the resulting mixture with a rennet.

EP-A1 1 249 176 describes a process for a production of a whey protein stabilized fatty emulsion which is acidified and heated. After adding it to food, e.g. processed cheese, an incubation step with transglutaminase follows. In particular, EP-A1 1 249 176 teaches that whey proteins can be incorporated into foodstuffs by modifying the whey proteins so that they form whey protein-stabilized fatty emulsions. It is also described that modified whey proteins display a "modified behaviour towards the action of transglutaminase".

WO 97/01961 describes a pre-incubation of transglutaminase (10 min to 2 hours) of cheese milk with 1 to 6% protein. As potential concentrating options, evaporation, spray drying or ultrafiltration are offered. These are methods, which lead to a complete retention of all proteins, and molecules, which are larger than 20,000 Dalton.

EP 1 442 663 reports about disadvantages concerning concentrated milk when an increase of the cheese yield is intended. It is reported that a pre-incubation inhibits the rennet effect and that even a simultaneous administration inhibits the rennet effect. In EP 1 442 663, rennet is added to cold milk. After adding transglutaminase, the milk is warmed. It is concluded that the current cheese preparation technique comprising a step of concentrating the raw material milk cannot satisfy the quality of the final product as required by consumers.

EP-A1 1 232 692 discloses that a whey-less cream cheese can be produced by simultaneously treating a dairy liquid containing dairy proteins with transglutaminase and a lactic acid producing culture.

EP-A1 1 254 601 describes that a cheese product can be made by simultaneously adding a acidifying agent and transglutaminase to a dairy liquid, sufficient to crosslink at least a portion of the dairy proteins and to form a curd and a liquid whey.

US 6,767,575 teaches that a concentrate of denatured whey protein with a specific aggregate size can be produced by thermal denaturation under specific conditions.

Cozzolino (2003), *Biotechnology-and-Applied-Biochemistry* 38, 289-295 reports an increased incorporation of whey proteins into curd by transglutaminase. The yield increased proportional with the added amount of whey proteins. Yet, transglutaminase was added to cheese milk simultaneously or 30 min before the rennet or after cutting the curd. The disadvantage of the applied technology is a retardation of the gelling time in the case of the pre-incubation, the problem of a homogeneous mixture of TG and the curd. Furthermore, only very sensitive curd can be obtained by the technology as disclosed in Cozzolino (2003). This requires an undesired additional curd treatment in a cheese vat.

US 6,749,873 teaches that cheese yield can be enhanced by elaborately utilizing a milk whey protein or milk whey protein and transglutaminase. Therefore, a partial hydrolysate of the milk whey protein is mixed with the material milk, subjecting the resulting mixture to the milk coagulating treatment. Alternatively, transglutaminase is allowed to act on the mixture prior to subjecting the mixture to the milk coagulating treatment. Furthermore, it is taught that the partial hydrolysis of the milk whey proteins allows the transglutaminase to act on the whey proteins.

US 2006/0083822 discloses a method for making cheese, which involves combining a slurry with a cheese precursor that is subsequently processed to form the final product. It is taught that enzymes, inter alia transglutaminase, may be used to create flavors, texture, melt and/or

other functional characteristics in the final cheese product, and/or in the slurry that can be transferred to the final cheese product once the slurry and cheese have been mixed together.

Therefore, the prior art described a pre-treatment of milk or whey proteins with transglutaminase before the actual cheese making processes extensively. However, a real improvement in final yield has never been reported.

Heidebach, 2004, "Einfluss der enzymatischen Quervernetzung auf die Labgelbildung", Diploma-thesis at the Technische Universität München, has documented that the pre-incubation with transglutaminase generally leads to negative gelling effects. Even a simultaneous addition of transglutaminase together with rennet to non-concentrated cheese milk resulted in a drastically retarded gelling time. Furthermore, the simultaneous addition of TG as taught by Heidebach (2004), loc. cit. resulted in a cheese with a grainy structure and with an unpleasant water binding capacity. In addition, the yield of the final cheese product (in this case Mozzarella) was only (slightly) increased when whey proteins were added. Without whey proteins the mass of cheese was even lower than the control cheese when transglutaminase was added, i.e. even a loss compared to the standard was documented by Heidebach (2004) loc. cit.

Considering the prior art, the technical problem to be solved in accordance with the present invention is the provision of means and methods, which allow for the production of food stuff in high yield, in a particular of cheese and cheese curd.

This technical problem has been solved by the embodiments provided herein and as characterized in the appended claims.

Accordingly, the present invention relates to a method for the preparation of visco-plastic food stuff, said method comprising the step of adding to a liquid, protein-enriched concentrate comprising at least 5% proteins and (a) reactive protein(s) for transglutaminase, a transglutaminase and a rennet or a rennet replacement which comprises a proteolytic enzyme, whereby said transglutaminase and said rennet or said rennet replacement are added simultaneously to said protein-enriched concentrate comprising a reactive protein for transglutaminase.

In accordance with the present invention it was surprisingly found (as illustrated in the appended examples, that the simultaneous addition of rennet (or rennet replacements, like fruit juices comprising proteases, i.e. melon juices or papaya juices etc) and transglutaminase to a liquid, protein enriched or protein rich basic concentrate with a protein content of at least 5% (like microfiltered milk), leads to a final product of increased weight and additionally to a product which comprises gel like product wherein serum drainage was almost completely stopped. Therefore, the method of the present invention leads to a surprising elevation of the amount of resulting food product (like cheese) and said food product comprises a lower dry matter content and a higher water (or serum) content. A further, unexpected advantage of the present invention is a high stability of the curd against further treatments, like mechanical treatments (e.g. pressing, kneading, stretching or netting) or like thermal treatments. Furthermore, the efficiency of the cheese vats/vessels/machinery for the gelling process can substantially be increased, due to the higher starting protein content and the shorter processing time.

It is of note that, in accordance with this invention, a curd with a low protein content (as, inter alia provided in EP1197152), cannot represent a physically stabile food structure even if cross linking occurs, unless considerable amounts of whey are removed from the curd. The advantage of the present invention is a resulting physically stabile food structure, which forms spontaneously after the gelling process. This is, inter alia, in stark contrast to EP-1197152 wherein a regular curd treatment is carried out and the resulting whey drainage of the curd was almost comparable to a conventionally produced cheese. In contrast thereto, with the teaching of the present invention, a conventional curd treatment is not necessary and the curd can directly be filled in corresponding final forms, like perforated forms. This can, in accordance with this invention be done within (few) minutes after the gel/curd is cut. In those cases when the protein content is already adjusted to the final protein content of the said final visco-elastic foodstuff by, e.g. microfiltration, the product does not even necessarily need to be filled in perforated forms and the gel does not need to necessarily undergo a curd treatment.

Most importantly, and shown in the appended examples, with the teachings of the present invention it is now possible to influence/control the syneresis and therefore to set a limit to the serum drainage. Furthermore, a desired firmness of a final product (cheese) at a remarkable higher serum content can be obtained. In one embodiment and as a gist of the present

invention, the inventive method leads to a substantial higher yield in particular of cheese/cheese products or cheese curd. It is one important teaching of the present invention that as starting material a high-protein concentrate is used which comprises a high content of proteins that are susceptible to the enzymatic activity of transglutaminase. One important example of these proteins is casein. Accordingly, and in contrast to the prior art, whey proteins are not necessary in the present inventive method and can, if desired, optionally be added to the "protein-enriched concentrate". Therefore, in one embodiment, a desired "protein-enriched concentrate" to be employed in the inventive method is casein-rich, micro-filtered milk.

Accordingly, the method of the present invention leads to a substantial higher yield of final food product (like cheese/cheese curd) even when no whey proteins are added when the transglutaminase is used in an (acidified) MF-concentrate. The concentration of the reactive protein (susceptible to the action of transglutaminase, like casein) is in accordance with the present invention as high so that the enzyme-substrate-reaction causes an extremely improved firmness of the curd, which is not possible, when milk is first treated with transglutaminase and then concentrated.

Yet, as described herein below, it is also part of this invention that e.g. whey proteins are added to the "protein-enriched concentrate" to be employed as starting material in the method of the present invention. However, the incorporation/addition of whey proteins serves primarily the regulation of the product structure and is not relevant for a higher yield of final product. In many cases the desired product structure is achieved without the addition of (denatured) whey proteins. In relation to "cheese" as a final product of the present inventive method, the structure of said cheese/cheese product will change dependent on the amount of (denatured) whey proteins additionally added to the protein-enriched concentrate, e.g. the MF-milk concentrate. Without whey proteins the resulting cheese has a very long structure (Mozzarella-like) and with addition of (denatured) whey proteins it results in a short structure (Provolone-like). In case of additional addition of whey proteins, it is envisaged that these whey proteins are only added to a final concentration of protein reactive with transglutaminase (e.g. casein) versus whey proteins of 80% versus 20% or 70% versus 30% the most. Accordingly, if whey proteins are to be added to the protein-enriched concentrate to be employed as starting material of the inventive method, these whey proteins, in particular denatured whey proteins, are to be added up to not more than the natural casein/whey protein ratio of 80/20 or 70/30. One of the

advantages of the present invention is that an increase in final yield is not achieved by an increased incorporation of whey proteins through the use of transglutaminase, but an extremely improved effect of the cross-linking of protein susceptible to the enzymatic activity of transglutaminase (in particular casein) due to a higher density of said susceptible protein in the herein defined "protein-enriched concentrate", e.g. MF-milk concentrate. This results in a non-expected, improved incorporation of whey/serum respectively serum binding capacity. It is of note that even if the exemplified final product /exemplified visco-plastic food stuff is cheese, the present invention is not limited to the production of cheese or cheese curd.

The term "visco-plastic" means a elastic texture of the food-stuff to be obtained by the method of this invention. Visco-plastic food stuff comprises, but is not limited to cheeses, like quarg, Feta, Mozzarella, soft-cheeses, semi-hard cheeses or hard-cheeses (like Cheddar). Also "visco-plastic food stuff" are food stuffs like TOFU products or like SURIMI. However, in context of this invention also the production of other food-stuff, like thick drinks, e.g. protein drinks, are envisaged and part of this invention. Accordingly, the method of this invention is not limited to the production of cheeses and cheese-like products but also encompasses the production of other protein-enriched food-stuff and drinks.

The term "visco-plastic food stuff" also comprises curds, like cheddar curds.

The term "liquid, protein-enriched concentrate" as used herein relates to a protein solution which comprises a total protein content of at least 5%, at least 6%, at least 7%, at least 8%, at least 9%, at least 10%, at least 11%, at least 12% or at least 13% protein. All protein contents herein and in particular also in the appended examples are expressed as w/w (weight per weight). Said protein-enriched concentrate is also characterized in comprising in said at least 5% protein content, (a) protein(s) which is(are) reactive for transglutaminase in vitro. Said protein(s) reactive for transglutaminase are in particular casein, casein macro peptide, α -casein fraction, β -casein fraction, caseinate and the like. Accordingly, also other proteins susceptible to the enzymatic action of transglutaminase are envisaged. These proteins comprise but are not limited to proteins from soybean (like the non-limiting examples: globuline fraction(s), vicilin(s), legumin(s)/glycinin(s) or albumin(s)), proteins from leguminosae like peas or seed of lupins (like the non-limiting examples: globuline fraction(s), vicilin(s), legumin(s)/glycinin(s) albumin(s) or glutelin(s)). As mentioned above and in a particular embodiment, said (a)

protein(s) which is(are) reactive for transglutaminase is already comprised in said at least 5% total protein content of the liquid, protein-enriched concentrate. However, it is also envisaged that further and/or additional "protein(s) which is(are) reactive for transglutaminase" is/are added to said liquid, protein-enriched concentrate before, in accordance with this invention, the transglutaminase and the rennet/rennet replacement are added simultaneously.

A protein-enriched concentrate to be processed in accordance with this invention is most preferably microfiltered milk, but also protein-enriched other dairy products/liquids are envisaged as long as they comprise more than 5%, more than 6%, more than 7%, more than 8%, more than 9%, more than 10%, more than 11%, more than 12%, or more than 13% total protein content or at the highest a protein content of 15%, or 16%, or 17%, or 18% or 19% or, preferably a highest protein content of 20%. As will be detailed below, protein enriched concentration can also be obtained by adding endogenous or foreign proteins to a corresponding liquid, like, e.g. casein (and the like as defined above) to food liquids, like vegetable juices, fruit juices etc.

In a further embodiment, it is also envisaged that the method of this invention comprises the addition of fat to the liquid, protein-enriched concentrate, as e.g. shown in example 3 below, said fat can, inter alia, be added in the form of natural milk fat globules, like cream, homogenised cream (reduced fat globule size) or reemulsified butterfat/oil or emulsified fat from other sources, like, e.g. palm oil or any other plant or animal fat/oil. The person skilled in the art is readily in a position to emulsify different fats or oils or fractions of fats or oils from different sources with milk proteins or many other proteins due to their behaviour to act as emulsifiers, see e.g. Dickinson, E.; Properties of emulsions stabilized with milk proteins: overview of some recent developments; J. Dairy Sci; 80:2607-2619. Due to the fact that the addition of fat reduces the total protein content the addition of fat is limited to maximal 70 % fat in dry matter. Preferably, the addition of fat is limited to 60%, more preferably 50%, more preferably 40%, more preferably 30%, more preferably 20% and most preferably 10% fat in the original liquid, protein-enriched concentrate. Again, in context of this invention, a fat content of maximal 70% fat in the final dry matter is advantageous. The addition of fat to the liquid protein concentrate leads to a reduction of the protein concentration in the resulting mixture. Therefore, in context of the invention, the maximal addition of fat to the liquid, protein-enriched concentrate as employed in the inventive method is an utmost amount that the resulting liquid, protein-enriched concentrate still comprises at least 5% proteins. The fat is

added so that the ratio of fat content to total protein content in the liquid, protein-enriched concentrate is 2.5(fat):1(protein) at the most, i.e. e.g. 2:1, 1:1 or 0:1 (no fat added). An example of a corresponding "protein-enriched concentrate" also comprising fat is given in the appended, illustrative example; i.e. in Example 3 where a fat content of (about) 8% was employed.

In this example, the addition of 20kg of cream with a fat content of 40% to 80kg of liquid, protein-enriched concentrate resulted in a total fat content of 8%. All fat contents herein and in particular also in the appended examples are expressed as w/w (weight per weight).

However, the addition of any additional ingredient results in a liquid, protein-enriched concentrate still comprising at least 5% proteins.

The term "total protein content" is known in the art (in the present case, the total protein content comprised in the protein-enriched concentrate to be employed in the inventive method is at least 5%). The total protein content of the samples can, inter alia, be assessed by the nitrogen content using a Leco FP 528 system (Leco Instrumente GmbH, Moenchengladbach, Germany) based on the Dumas method; see Wiles, 1998, Journal of AOAC International, 81, 620-632. "Total protein content" can then be calculated by multiplying the total nitrogen by 6.38. As mentioned above, the protein contents are expressed as w/w (weight per weight).

The "protein-enriched concentrate" to be employed as basic food liquid in the method of the present invention is a liquid with high protein content, i.e. comprising more than 5%, more than 6%, more than 7%, more than 8%, more than 9%, more than 10%, more than 11%, more than 12%, more than 13%, up to approximately 20%. A significant higher protein content than 20% can only be obtained by a diafiltration e.g. with drinking water, additional to the microfiltration or by addition of protein powders to a liquid protein concentrate. Accordingly, it is also envisaged that the "protein-enriched concentrate" may, exceptionally, comprise more than 20% protein content. In particular when dairy liquids (like microfiltered milk) are used, the proteins comprised in said liquids are in particular casein or casein-like proteins. Particular preferred "protein-enriched concentrates" to be used in context of the present invention are micro-filtered milk or other protein-enriched dairy liquids. Also envisaged are protein-enriched vegetable or fruit juices, or coco milk, milk permeates from ultrafiltration, microfiltration or/and nanofiltration in which added proteins or a microfiltered protein concentrate are mixed/solved. In context of this invention, it is also envisaged that the food liquids to be employed as "protein-enriched concentrates" are obtained by the addition of exogenous proteins (i.e. proteins not originally comprised in said food liquids). These proteins may in

particular comprise casein and casein like- proteins or any other protein(s) which are reactive with transglutaminase/reactive for transglutaminase. In accordance with this invention, the term “reactive with transglutaminase/reactive for transglutaminase” means that the corresponding proteins are susceptible (in particular in vitro and in corresponding food processing processes, like cheese-making) to the enzymatic activity of transglutaminase (TG) as defined herein.

As discussed above, it also envisaged that whey proteins be added to the starting “protein-enriched concentrate” before, in accordance with this invention the rennet/rennet replacement and said TG is added. Said whey proteins may, inter alia, be obtained by microparticulation, this being a process wherein the whey proteins are denatured and aggregated by heat and under shear conditions. Microparticulated whey proteins can be incorporated into, e.g. the microfiltered milk-concentrate to be employed, in one embodiment as starting “protein-enriched concentrate”.

The particularly preferred “protein-enriched concentrate” comprising at least 5% protein is micro-filtered milk. The process of obtaining micro-filtered milk is well known in the art, see e.g. Bachmann, 2003 (loc. cit) or Kersten, (2000), Fortschritt-Berichte VDI Reihe 3 Verfahrenstechnik, Nr. 709, VDI Verlag and also illustrated in the appended examples. For example, it is possible to obtain said micro-filtered milk by passing normal milk (up to 3.5% protein content) through a 0.05 – 0.2 μm membrane. Preferable and employed in the appended examples is a membrane of 0.1 μm cut-off.

In one embodiment of the present invention, said at least 5% total protein content in said protein-enriched dairy liquid comprises is at least 70%, preferable about 80% casein, casein-like protein or the protein which is susceptible for transglutaminase (“reactive for transglutaminase”). As illustrated in the appended examples, an advantageous ratio between casein/casein-like proteins/proteins, which are susceptible for transglutaminase, and the remaining proteins (like whey proteins) is 80/20, i.e. 80% of casein/casein-like proteins versus rest or other proteins, which are susceptible for transglutaminase.

Therefore, the present invention is particularly useful when said protein-enriched concentrate comprises a casein/rest protein(s) ratio of at least 70/30, preferably of 80/20. In one

embodiment of the invention, said "rest protein(s)" is/are whey proteins and the main protein susceptible for TG(s) is casein.

As mentioned above, it is also envisaged in context of this invention that a "protein-enriched concentrate" is prepared prior to the inventive simultaneous addition of TG(s) and the rennet or rennet replacement as defined herein. In a preferred embodiment, the exogenous or additional protein to be added in order to prepare a "high protein concentrate" (i.e. more than 5% total protein content and up to, preferably, 20% total protein content) is a protein susceptible to the enzymatic action of transglutaminase as defined herein, e.g. casein(s). It is envisaged that said casein(s) is/are added before said transglutaminase and said rennet/rennet replacement is/are added simultaneously to said protein-enriched concentrate. However, also the addition of further proteins, like and non-limiting whey proteins (also denatured whey proteins), egg white proteins or egg yolk proteins (also denatured egg proteins), soybean protein or proteins from other leguminosae to the protein concentrate before the TG and the rennet/rennet replacement is added, is within the scope of the present invention. However, whey proteins in their native structure are less prone to the cross-linking reaction (by TG(s)) mainly due to their globular conformation stabilised by disulphide bonds. However, the cross-linking of whey proteins can be improved by a foregoing denaturation by heat treatment (Han, 1996, J. Agricultural Food Chemistry 44, 1211-1217; Sharma, 2001, International Dairy Journal, 11, 785-793). In a mixed protein system, such as milk, the caseins are preferentially cross-linked. This is in contrast to any cross-linking between whey proteins, which is less preferred (Han, 1996, loc. cit).

In accordance with the invention, the "casein", which is the most important protein reactive for/with transglutaminase, comprises, but is not limited to casein/rennin itself, casein submicellae, caseinate preparation (like, e.g. rennet caseinate, acid caseinate, Na⁺-caseinate, K⁺-caseinate, Ca⁺⁺-caseinate, CMP (caseino macro peptide) or glycosylated versions thereof, like GMP (glycol-caseino macro peptide). As used herein, "casein" relates to any, or all, of the phosphoproteins in milk. An important characteristic of casein is that it forms micelles in naturally occurring milk and in the dairy liquids employed in the present invention, and that clotting a dairy liquid containing casein by any suitable means provides a coagulated curd phase and a liquid whey phase that are separable from one another. Many casein components have been identified, including, but not limited to, alpha-casein (including alpha s1-casein and alpha s2-casein), beta-casein, kappa-casein, their genetic variants, and mixtures thereof.

The term "transglutaminase" is known in the art and relates, also in context of this invention, to microbial as well as non-microbial transglutaminase (TG). In context of this invention, several TGs are useful and in the appended examples the microbial TG (MTG or MTGase) from *Streptovercillium* (mobaraense) is employed.

As also illustrated in EP-A2 1 048 218 transglutaminases are enzymes which catalyze the transfer of the gamma-carboxamide group of a glutaminy residue in a protein or peptide to the epsilon-amino of a lysyl residue of the same or a different protein or peptide, thereby forming a gamma-carboxyl-epsilon-amino crosslink. Transglutaminases have a broad occurrence in living systems, and may be obtained, for example, from microorganisms such as those belonging to the genus *Streptovercillium*, *Bacillus subtilis*, various *Actinomycetes* and *Myxomycetes*, or from plants, fish species, and mammalian sources including pig liver and the blood clotting protein activated Factor XIII. In general, transglutaminases from animal sources require calcium ions for activity. Recombinant forms of transglutaminase enzymes may be obtained by genetic engineering methods as heterologous proteins produced in bacterial, yeast, and insect or mammalian cell culture systems. The principal requirement of any transglutaminase employed in the instant invention is that it has the cross linking activity. Any enzyme having transglutaminase activity may be employed in the methods of the present invention. In a preferred embodiment, the transglutaminase is obtained from the genus *Streptovercillium*. Most preferred is Activa YG[®], as also employed in the appended examples. However, it is also envisaged that enzymes or enzymatic fragments are employed which show transglutaminase activity. Transglutaminase activity may be determined using known procedures. One such technique comprises colorimetric procedures, like the use of benzyloxycarbonyl-L-glutaminy-glycine and hydroxylamine to form a gamma-carboxyl-hydroxamic acid if transglutaminase is present. An iron complex of the hydroxamic acid can be formed in the presence of ferric chloride and trichloroacetic acid. Using the absorbance at 525 nm with appropriate standards, the activity of enzyme present may be determined; see, US 5,681,598.

As illustrated in the appended examples, microbial transglutaminases (TGs) can successfully be employed in context of this invention. Examples of such transglutaminases are TGs from *Streptovercillium* (like *Streptovercillium mobaraense*) or *Bacillus subtilis*. Yet, also other TGs, as mentioned above may be useful in context of this invention and comprise, e.g. non-

microbial transglutaminases, like TGs from fungi, from plants or from animals. Particularly, the TGs from fungi may be from actinomycetes or myxomycetes.

In context of this invention, the usage of TGs in terms of corresponding concentrations to be employed is within the skill of the artisan. However, as shown in the appended examples transglutaminase may be added to said protein-enriched concentrate in concentration of about 0.1 U/g protein to about 3 U/g protein, preferably from 1 U/g protein to 2 U/g protein of transglutaminase is employed. However, it is also within the skill of the artisan that higher amount, e.g. up to 10 U/g are employed in context of this invention. Per definition in the art, "U" describes "international units". International units are defined as the amount of enzyme which catalyses the formation of 0.5 μ mol of hydroxamate per min from N α -CBZ-L-glutaminyglycine and hydroxylamine at pH 6.0 and 37°C; see Folk, (1966). The Journal of Biological Chemistry, 241, 5518-5525. As shown in the examples, impressive results can be obtained when 1 or 2 U/g protein is used.

The term "rennet or rennet replacement" relates in accordance with this invention to a preparation with an enzymatic activity which comprises proteolytic enzymatic activity, in particular "proteases"/protease activity. Such proteases are most preferably chymosin or pepsin, in particular chymosin. For example, when milk or dairy products (with protein contents of at least 5%) are employed as "protein-enriched concentrate", the rennet to be employed is preferably natural "rennet", like the rennet obtainable from several animal species or recombinant versions thereof. Such "rennet" is, e.g., natural rennet from bovine (calf), goat, buffalo or sheep. Recombinant "rennet" is, e.g. recombinant chymosin or recombinant rennet of cattle (calf) origin produced in different microbial hosts.

Also comprised under the term "rennet or rennet replacement" are enzyme preparations comprising rennin/chymosin (EC 3.4.23.4) or even prechymosin(s). Also comprised are other enzyme preparations, which have protease activity and/or co-agulating activity. Non-limiting examples include other aspartyl proteases such as various pepsins, and a large number of proteases from nonmammalian sources, including plants, microorganisms, and marine fishes. As used herein, a "rennet protease" relates to any such protease having milk-clotting activity. Also genetically engineered mutant proteins derived from such natural proteases and having the corresponding protease activity, are available as recombinant protein products, obtained upon introducing genes encoding these proteins as heterologous genes into suitable host

organisms to produce the protein products. As used herein, all such recombinant proteases having milk-clotting activity are included in the term "rennet or rennet replacement".

Also envisaged as "rennet or rennet replacement with proteolytic enzyme activity" as to be employed in context of this invention are vegetable rennets (like rennets from fig tree bark, nettles, thistles, melon, papaya). Preferred "rennets" in this context are, e.g., thistle press juice or juice from melons or papaya.

Other "rennets or rennet replacements" are, in context of this invention, rennets of fungal origin, also known as "genetic rennet", like genetic rennet produced from *Aspergillus niger*, *Rhizopus oryzae* (Sushil-Kumar et. al *Journal-of-Food-Science-and-Technology*. 2004; 41(3): 279-283; 28., *Fusarium subglutinans* (Ghareib,-M et. al *Acta-Microbiologica-Polonica*. 2001; 50(2): 139-147; 22), *Mucor miehei* (El-Tanboly,-E et. al *Milchwissenschaft*. 2000; 55(11): 624-628; 24.), *Mucor pusillus* var. *lindt* (Wahba,-A et. al *Alexandria-Journal-of-Agricultural-Research*. 1986, recd. 1988; 31(3): 139-150; 11 ref.), *Fungus Irpex lacteus* (Kobayashi,-H *Agricultural-and-Biological-Chemistry*. 1985; 49(6): 1605-1609; 13 ref.), *Rhizopus oligosporus* (Krishnaswamy,-M-A et. al *Journal-of-Food-Science-and-Technology,-India*. 1976; 13(4): 187-191; 18 ref.), or *Basidiomycetes* (Kawai,-M; Mukai,-N *Agricultural-and-Biological-Chemistry*. 1970; 34(2): 159-63; 25

In context of this invention, it is also envisaged that chymosin/rennin is used as "rennet or rennet replacement" in the methods and means provided herein.

Accordingly, in context of this invention, the rennet or rennet replacement to be employed in the inventive method comprises a proteolytic enzyme and may be selected from the group consisting of bovine/calf rennet, buffalo rennet, sheep rennet, goat rennet, porcine rennet, microbial rennet like from *Bacillus subtilis* (Masek,-J *Conference-Proceedings XIX International Dairy Congress*. 1974; 1E, 692.), *Streptomyces* strains (Abdel-Fattah,-A-F *Acta-Microbiologica-Polonica,-B* 1974; 6(1): 27-32; 14), *Streptococcus liquefaciens*-108 (Srinivasan,-R-A et. al *Indian-Journal-of-Dairy-Science*. 1968; 21(3): 149-54; 11.), *Bacillus polymyxa* (Denkov,-Ts; Vasileva,-S *Nauchni-Trudove,-Institut-po-Mlechna-Promishlenost*. 1979; 9, 158-165; 8.) genetic rennet, recombinant rennet, chymosin preparations. Also envisaged are fruit or vegetable juices and/or pulps and the like, which have "renneting activity". Such fruit/vegetable or plant pulps or juices comprise, e.g. melon juice, papaya juice,

proteinases from *Opuntia ficus-indica* fruits (Garcia,-M-T; et. al *Biotechnology-Progress*. 2006; 22(3): 847-852; 31), extracts from berries of the plant *Solanum dobium* (Yousif,-B-H *International-Dairy-Journal*. 1996; 6(6): 637-644; 10), enzymatic complex from pineapple pulp Cattaneo,-T-M-P *Milchwissenschaft*. 2005; 60(4): 399-402; 14), vegetable juice, like from Ash gourd (*Benincasa cerifera*) (Gupta,-C-B; Eskin-N-A-M *Food-Technology* 1977; 31(5): 62-64, 66; 10 ref.), plant derived coagulating enzymes from Cardoon extract e.g. strain *Cynara cardunculus* (ALVARES, S. et. al *Alimentacion-Equipos-y-Tecnologia*. 2005; 24(203): 63-66; 11 ref.), Ficin and Papain (Yit-Hwei-Low et. al *International-Dairy-Journal*. 2006; 16(4): 335-343; 19), extract from the flowers of wild globe artichokes (Tsouli,-J *Comptes-Rendus-Hebdomadaires-des-Seances-de-l'Academie-des-Sciences,-Serie-D-Science-Naturelles*. 1970; 270(2): 396-99; 6 ref.), protease isolated from *Dieffenbachia maculata*, a stem of *Dieffenbachia maculata* (a plant of the Araceae family widely cultivated in India) (Sriram-Padmanabhan et. al *Nahrung*. 1993; 37(1): 99-101; 11). As is evident from the above, in accordance with this invention, the term "juice form plants, fruits, vegetables, is not limited to the fluids pressed from said plants, fruits or vegetables, but also comprises corresponding preparations, like pulp, extracts, isolated enzymes and the like. Further rennets or rennet replacements are also rennet from stomach of seal (US 4,526,868) or from stomach lining of Atlantic cod (Brewer,-P *Canadian-Institute-of-Food-Science-and-Technology-Journal*. 1984; 17(1): 38-43; 19).

As shown in the appended examples, in particular natural rennet, like calf rennet, can successfully be employed in context of this invention.

The concentration of the rennet/rennet replacement added simultaneously with TG(s) to protein concentrates with high protein content as defined herein can easily be established by the person skilled in the art. It is shown in the appended examples, that said rennet or rennet replacement may be added to said protein-enriched concentrate in concentration of 0.01 to 1.0 IMCU/g protein (International Milk Clotting Units) preferably 0.1 to 0.5 IMCU/g protein. International Milk Clotting Units are described from SAUERER, V. in *dmz Deutsche Molkereizeitung* 1/2001. Before the IMCU, the REMCAT method was employed. The international method "REMCAT" is the relative milk clotting activity test for which any rennet sample is compared to two standard samples. One standard rennet sample from a calf origin control powder consists of at least 98% Chymosin and to a maximum 2% bovine pepsine. The second standard rennet sample is a cattle origin control powder consisting of at least 99% bovine pepsine and to a maximum 1% chymosin. The standard is determined in reconstituted skim milk. For the

actual determination of the activity a calf and a cattle origin control powder is mixed in a ratio of the enzymes the assay consists of. The time between the addition of the rennet and the visible flocculation in the reconstituted skim milk at a pH-value of 6.5 and a temperature of 32°C is taken. Parallel, under the same conditions, the time period for the standard rennet is determined. The actual activity is calculated by comparison of the renneting substance to be assayed with standard rennet and is specified as International Milk Clotting Units (IMCU).

The method as provided herein may also comprise an additional acidification step as routinely used and employed in the food processing and food preparation methods, like cheese making. Said additional acidification of the protein-enriched concentrate may take place before or during the simultaneous addition of transglutaminase and rennet or rennet replacement. In one embodiment, said acidification of the protein-enriched concentrate comprises the adjustment of the pH a value between 5.2 and 6.8, preferably between 6.0 to 6.8. The acidification can be carried out by methods known in the art and may be obtained by the addition of a starter culture or the addition of an organic acid. Such starter culture may be from selected milk bacteria or may be from mesophilic bacteria e.g. *Lactobacillus lactis* subsp. *lactis*, *Streptococcus lactis* subsp. *cremoris*, *Lactobacillus lactis* subsp. *lactis* biovar *diacetylactis*, *Leuconostoc mesenteroides* subsp. *cremoris*, *Leuconostoc mesenteroides* subsp. *dextranicum*, and/or thermophilic bacteria e.g. *Streptococcus thermophilus*, *Lactobacillus desbruekii* subsp. *bulgaricus* (*Lactobacillus bulgaricus*), *Lactobacillus acidophilus*, *Lactobacillus helveticus*, *Lactobacillus casei* susp. *casei*.

Also useful, food-compatible organic acids are known and comprise, but are not limited to, citric acid, lactic acid, acetic acid, malic acid, formic acid or glucono δ lactone.

In a further embodiment of this invention, it is envisaged and may be useful that the method as provided herein comprises a further step, wherein an inhibitor of inhibitors of transglutaminase is added to said protein concentrate before said transglutaminase and said rennet or rennet replacement is added simultaneously to said protein-enriched concentrate. For example, glutathione may be added as such an inhibitor. Glutathione is well known in the art and is, inter alia, described in EP 1 197 152. In context of this invention, glutathione means the tri-peptide epsilon-L-glutamyl-L-cysteinyl-glycin, which is reversibly oxidizable due to its free thiol-groupe. Glutathione is, e.g., present in different yeast extracts in relatively high concentrations. Glutathione is a reducing agent.

The inventive method as provided herein requires that the transglutaminase (or an enzyme with transglutaminase activity or a fragment of such an enzyme with said activity) is simultaneously added to the protein-enriched concentrate with rennet/rennet replacement. Said "simultaneous addition" of said transglutaminase and said rennet/rennet replacement to said protein-enriched concentrate means that the addition of transglutaminase and rennet or rennet replacement is preferably carried out at least within 10 minutes or less, preferably within 9 minutes or less, more preferably within 8 minutes, more preferably within 7 minutes or less, more preferably within 6 minutes or less, more preferably within 5 minutes or less, more preferably within 4 minutes or less, more preferably within 3 minutes or less, more preferably within 2 minutes or less, more preferably within 1 minute or less or most preferably at the same time.

If not added at the same time, the addition of TG before the rennet/rennet replacement is preferred. However, a person skilled in the art is aware of the, less preferred, possibility of adding TG after the rennet/rennet replacement, which is also comprised as an embodiment of this invention.

A particular example of the present invention is provided in the appended experimental part. Here, the production of cheese or cheese curd is described, wherein said protein-enriched concentrate employed is micro-filtered milk with a protein content of more than 5%, and wherein said transglutaminase employed is from *Streptovercillium* and wherein said rennet is calf rennet.

The obtained visco-plastic food stuff as provided by the inventive method is characterized as being a physically stabile product with high gel formability, a high water binding capacity and/or having a relatively dry surface, which is suitable for a growth of different maturing microorganisms e.g. mould like *Penicillium candidum*, yeast like *Geotrichum candidum* or mixed cheese flora like red smear culture. The product is also suitable to be packed in foil e.g. shrink-wrap foil or wax without further maturing. The food stuff is also suitable to generate a rind. At high protein content, the curd as obtainable by the inventive method is stable for example to be mixed with table salt and offered as unripened food stuff without further forming the curd (like cheddar curd).

Said visco-plastic food stuff, like curd, obtainable by the inventive method is particularly useful in the preparation of cheese or cheese products, like quarg, rennet cheese, soft-cheese, semi-hard cheese and hard-cheese. Corresponding examples for the texture are Mozzarella,

Cashcaval, Feta, Neufchatel, Petit Suisse, Queso Blanco, Camembert, Edam, Gouda, Limburger, Muenster, Provolone, Roquefort, Cheddar, Cheddar curd or Emmental.

Also provided in this invention is a kit for carrying out the inventive method for preparation of visco-plastic food stuff. Said kit comprises (a) transglutaminase(s) as characterized above and comprises (a) rennet(s) or rennet replacement(s) as characterized herein, whereby said (a) transglutaminase(s) and said rennet(s) or rennet replacement(s) are to be administered to protein-enriched concentrate as defined herein simultaneously.

The Figures show:

Figure 1: Resistance force of MF-Gels dependent on protein content and dependent on the clotting process

This figure shows that an increasing protein content leads to an increased gel firmness on different levels dependent on the method for gelling. The use of 1 U transglutaminase per g protein plus fermentation (acidification) leads to a resistance of 100 g at a protein content of 13.7%. The use of 2 U transglutaminase under ambient conditions leads to almost the same resistance like the use of rennet plus acidification (resistance 250 g at a protein content of 13.7%). A combination of rennet, transglutaminase and acidification resulted in doubling the firmness (approx. 500 g at a protein content of 13.7%). This synergistic effect was surprising. As rennet a natural rennet was used which had an activity of 140 IMCU/ml (international milk clotting units). Per g protein 0.3 IMCU rennet was used.

Figure 2: Amount of serum of MF-Gels dependent on the protein content and dependent on the clotting process

The following examples illustrate but do not limit the invention:

Example 1: Use of TG and rennet in non-concentrated 3.3 to 3.5% milk or curd.

Pasteurized whole milk (fat content 3.5%) from the local dairy with its natural protein content between 3.3 and 3.5% was obtained. Three cheese vessels were filled with 8 kg whole milk each and at a temperature of 10°C citric acid with a concentration of 9% was added until a pH-value of 5.9 was reached. The citric acid was prepared from 90 g crystalline technical citric acid and 910 g distilled water. After that the milk was warmed up by warm water in the double jacket wall to a temperature of 33 °C. At that temperature in all cases 0.02% natural rennet preparation (Naturen™, Chr. Hansen, Denmark) which was diluted with 50 parts of water was used. One trial/vessel was performed as a standard, one trial/vessel was performed with 0.59 IU transglutaminase per g protein and with glutathion (Activa YG) (simultaneous with rennet) and to the third vessel transglutaminase preparation (Activa YG) was added when the curd was already prepared, i.e. after rennet was previously added.

The gelling time was estimated manual by the common method to consider the intersection of a cut in the curd with a knife or a spatula. When the curd brakes smooth the time to cut the complete curd is defined. The gelling time was 25 min for the standard and the third vessel and 28 min for the trial with simultaneous addition of transglutaminase and rennet. The curd was cut in walnut size curd cubes and was not stirred for 3 to 5 minutes. In the third vessel 0.59 Units Activa YG per g protein was added to the curd. In one case 3 Units/g protein was added. The curd was stirred carefully for 40 to 60 minutes. After that the curd was transferred to perforated forms for the drainage of whey and for an adhesion. After spending 60 to 80 min in the forms at room temperature, the weight of the adhered curd was taken (mass of the curd). The formed curd from each vessel was cut in 6 equal pieces each and then manually formed in hot water (never below 80°C). The hot water was prepared in amounts of 8 L with 1% table salt. The netting process (=stretching and kneading) of the mozzarella took about 5 min per bowl. After forming the mozzarella bowls the cheese were placed in iced water with a table salt concentration of 0.5% for approx. 30 min. Now the weight of the cheese was measured.

In further experiments denatured whey proteins was added to cheese milk. As denatured whey proteins a liquid WPC (whey protein concentrate) with a protein content of 23.6% was used. The whey was origin for a mozzarella production and was ultrafiltered and diafiltered to this protein concentration with an ultrafiltration plant with a cut off of 25.000 Dalton at a low temperature (10 °C). The WPC was heated by means of a scraped surface heat exchanger at a shear rate of 630 s⁻¹ and at a temperature of 85 °C for 3 minutes. The mean diameter of the denatured whey protein aggregates was approx. 2.0 micron while the particle measurement of

the concentrate (native) before the heating and shearing process showed a medium particle size of approx. 0.2 micron. The medium particle size is defined as d50.3, the volume based diameter, which means that 50% of the volume of the particles is covered by particles smaller than the mentioned size and 50% of the volume is covered by particles larger than the mentioned size. The particles were measured by a laser scattering spectroscopy from the company Coulter (calculation model: protein model). At the described heating conditions the denaturation of the whey proteins is very high (> 90% denaturation of β -lactoglobulin).

These experiments were carried out as described above, yet 0.2% whey proteins (0.4%, respectively) were added to the whole milk at the begin of the procedure. Accordingly, the protein concentrate was increased to 3.7% (or 3.9%)

The yield of the curd (raw curd) and the cheese (mozzarella) was defined as Yield = kg curd or cheese/100 kg milk.

The result of this equation was defined as 100% yield for the standard and the result for the trials was calculated as relative yield.

The following results were achieved:

Table 1: Yield of curd and mozzarella cheese

	Rel. Yield of the curd	Rel. Yield of the mozzarella
Standard (without TG)	100%	100%
simultaneously with rennet 0.59 U TG/g protein	118%	91%
mixed with the curd 0.59 U TG/g	102%	95%
mixed with the curd 3 U TG/g	103%	98%
Standard (without TG and without whey proteins)	100%	100%
Standard (without TG and + 0.2% whey proteins)	116%	105%
simultaneously with rennet 0.59	>120%	101%

U TG/g protein + 0.2% whey proteins		
mixed with the curd 0.59 U TG/g + 0.2% whey proteins	117%	108%
Standard (without TG and + 0.4% whey proteins)	118%	110%
simultaneously with rennet 0.59 U TG/g protein + 0.4% whey proteins	130%	105% (grainy product; leakage of whey)
mixed with the curd 0.59 U TG/g + 0.4% whey proteins	121%	104%

The results provided above document that in low protein milk (3.3 to 3.9%) an intermediate increase of an intermediate product (and) could be obtained when in particular whey proteins were added in combination with TG. However, in contrast to the examples below, further processing of said card let to rather disappointing yield of finalized products. The above results document that rennet/TG administration to low protein milk (up to 3.9%) let to a final yield of 105% at the most.

In addition, it could be shown that the final product obtained in the combination of rennet/TG and a protein content in the milk of 3.9% was not a stabilized cheese product, was short-structured (grainy) and an undesired leakage of whey was observed. However, the curd obtained by the method as provided in this Example 1 was sensitive against further mechanical and thermal treatments.

Example 2: Protein combination modifications in simultaneous addition of TG and rennet to a protein enriched dairy liquid

These following trials were carried out with pasteurized skim milk from a local dairy.

The pasteurized skim milk was microfiltered (MF) by means of a pilot plant with microfiltration moduls from the company APV Invensys, Denmark with ceramic tubular filter

elements with a declared pore size of 0.1 micron and a membrane surface of 1.68 m². With this process, microfiltered milk (MF-milk) with a protein content of at least 5% can be obtained. As shown below, even protein combinations of more than 13% can be achieved.

Even if the current experiments have been carried out in a pilot plant, microfiltration of milk is commonly used and for example described in Kersten, 2000 (Fortschritt-Berichte VDI Reihe 3 Verfahrenstechnik, Nr. 709, VDI Verlag)

In the here used pilot plant, the permeate and the retentate was circulated by centrifugal pumps in a batch process in order to adjust an equal pressure difference of 0.4 bar over the whole length of the moduls. Accordingly, uniform transmembrane pressure (UTP-principle by APV) was employed . The milk was warmed up by a plate heat exchanger to a temperature of 52 °C ± 2 °C and kept at that temperature over the whole process by cooling with water. The result was a good permeation of the whey proteins to the permeate while the casein remained in the retentate. 120 kg starting material pasteurized skim milk resulted in 25 kg retentate and 95 kg permeate. The permeate was visually absolute clear over the whole process of microfiltration. The permeation rate (flux) was approx. 50 l/m² h at the begin and approx. 25 l/m² h at the end of the filtration process.

The pasteurized skim milk and the retentate as well as the permeate was determined for the total protein content by the Dumas method (LECO FP 528). The protein content was calculated by multiplying total nitrogen by 6.38. The following protein contents were achieved: retentate: 13.7%; permeate: 0.6%

A part of the microfiltered retentate was then diluted with a permeate from the ultrafiltration (pasteurized skim milk from the local dairy; process temperature 50°C; cut off 25.000 Dalton, pilot plant from the Technical University of Munich) to different protein contents. The dilution was done with the UF-permeate to keep the milieu (minerals, lactose) constant but to keep the casein/whey protein ratio in all cheese milk preparations on the highest level of 93:7 (calculated from the protein content of the MF-permeate and the total protein content of the skim milk, under the presumption that the natural ratio of casein to whey protein in skim milk is 80:20). In the MF-permeate only whey proteins are found and in UF-permeate only peptides but no casein and no major whey proteins are present.

The samples with different protein contents were warmed up to 35 °C and 200 g of it were filled in cups. As a starter culture 2 ml of a pre-culture per kg cheese milk preparation was used. The acidified pre-culture was prepared by solving a deep frozen thermophilic, probiotic starter culture (ABT 21; CHR HANSEN) in high heated and again cooled skim milk in a ratio of 20 g starter culture to 180 g skim milk. From this amount of 200 g pre-culture 0.4 ml were added to each cup with 200 g cheese milk preparation. This procedure was done for a better dosing of the starter culture. After the starter culture was added, an amount of 0.3 IMCU of rennet per g protein (NaturenTM Premium 145; CHR HANSEN) was added. The rennet preparation has an IMCU (International Milk Clotting Unity) activity of 140 IMCU/ml (REMCAT strength), see e.g. Sauerer, dmz Deutsche Molkereizeitung (1/2001).

In accordance with the present invention, the transglutaminase was added simultaneously with the rennet. Due to the small amounts of samples the transglutaminase preparation Activa YG[®] from the company Ajinomoto was not solved in water before but added as a powder to the milk preparation. Activa YG[®] is a mixture of MTGase, Lactose, Maltodextrin, GSH, added as yeast extract and safflower oil. In other documented experiments, 1 Unit transglutaminase per g protein or 2 Units per g protein were used.

The assay samples were incubated in variation as shown in diagram 1 and 2 at a temperature of 35 °C in a pre-heated stove for approx. 9 hours. After cooling down to a temperature below 10 °C, the assay samples were measured by the texture analyser (Stevens Texure Analyser) with a cylindric probe with a diameter of 4 mm with a speed of 0.5 mm/s and a depth of 15 mm.

In the resulting samples, pH measurements showed certain variations depending on protein content in the starting material.

The following, surprising results have been obtained. Starting from high (more than 4.5%) protein content, it was surprisingly found that a final product (gel/curd) could be obtained with high firmness under the condition that the TG and the rennet was added simultaneously; see Figure 1. Furthermore, as documented in Figure 2, much less serum drainage/whey drainage could be observed when high protein content milk was employed in simultaneous addition of TG and rennet. Accordingly, a high and surprising serum/whey binding capacity could be observed. This was and is in clear contrast to the examples as provided herein above under Example 1. Accordingly, the results obtained with high protein milk (protein enriched milk

concentrate comprising more than 5% protein) are surprising, since the final product has the following unexpected advantages:

- A physically stabile product against mechanical treatments of pressing, kneading, stretching, netting;
- A high stability against thermal treatments with high gel formability;
- A high water binding capacity and a relatively dry surface which is suitable for a growth of different maturing microorganisms e.g. mould like *Penicillium candidum*, yeast like *Geotrichum candidum* or mixed cheese flora like red smear culture;
- Ability for being packed in foil e.g. shrink wrap foil or wax without further maturing;
- Ability to generate a rind under maturing conditions;
- At high protein contents the curd is stable for example to be mixed with table salt;
- Can be offered as fresh food stuff without further forming of the curd (behavior like cheddar curd);
- The curd can be filled in perforated forms only a few minutes after the gel/curd is cut;
- In those cases when the protein content is already adjusted to the final protein content of the final visco-elastic foodstuff e.g. by means of microfiltration (instead of achieving the final protein content by whey drainage), the product does not even necessarily need to be filled in perforated forms and the gel does not need to necessarily undergo a curd treatment;
- Substantially increased efficiency of the cheese vats/vessels/machinery due to the higher starting protein content and the shorter processing time;
- Low whey drainage.

This was unexpected, since the experiments documented in Example 1 led to a completely different result. In Example 1 an undesired sensitive curd was obtained and the whey drainage was basically not reduced. In accordance with the results presented in Example 1, the complete cheese making process shown in said example was not more efficient than a standard cheese making process.

Several assay samples from example 2 were further treated by a Thermomix (company Vorwerk) mixer and it resulted in granulous curd products.

Therefore, when transglutaminase was used (also in common combination with an acidification) at 1 U/g (as well as at 2 U/g), serum drainage was almost completely stopped especially at high protein contents. In contrast, rennet in combination with acidification let to

very intensive serum drainage. Simultaneous use of rennet and transglutaminase leads to a minimal serum drainage. One advantage of the present invention is that it is now possible to influence/control the syneresis and therefore to set a limit to the serum drainage. Furthermore, a desired firmness of a final product (cheese) at a remarkable higher serum content can be obtained.

When a milk gel shows a low serum drainage and still reaches a higher firmness, an increase in yield without a loss in texture firmness can be achieved.

Example 3: Large scale production of FETA-like cheese from concentrated milk (in contrast to example 1)

Pasteurized skim milk and pasteurized cream was obtained from the local dairy. The pasteurized skim milk was microfiltered (MF, as detailed also in example 2) and then the retentate was mixed with the cream to adjust the fat content. In particular, microfiltration was done in a pilot plant with microfiltration modules from the company APV with ceramic tubular filter elements with a declared pore size of 0.1 micron and a membrane surface of 1.68 m². At this pilot plant the permeate was circulated as well as the retentate by centrifugal pumps in a batch process to adjust an equal pressure difference of 0.4 bar over the whole length of the modules, i.e. it followed the principle of an uniform transmembrane pressure (UTP-principle; patented by APV). The milk was warmed up by a plate heat exchanger to a temperature of 52 °C ± 2 °C and kept at that temperature over the whole process by cooling with water. The result was a good permeation of the whey proteins to the permeate while the casein remained in the retentate. 300 kg starting material pasteurized skim milk resulted in 95 kg retentate and 205 kg Permeate. The permeate was visually absolute clear over the whole process of microfiltration. The permeation rate (flux) was approx. 50 l/m² h at the beginning and approx. 30 l/m² h at the end of the filtration process.

The pasteurized skim milk and the retentate as well as the permeate was determined for the total protein content by the Dumas method (LECO FP 528). The protein content was calculated by multiplying total nitrogen by 6.38. The following protein contents were achieved:

Product	protein content (%)
Pasteurized skim milk	3.5%

<u>MF-retentate</u>	<u>11.5%</u>
MF-permeate	0.55%

In the assumption that milk has a natural casein/whey protein-ratio of 80:20 and the fact that in the permeate only whey proteins are present a casein/whey protein ratio of the retentate can be calculated by a mass balance.

$$300 \text{ kg skim milk} = 95 \text{ kg Retentate} + 205 \text{ kg Permeate}$$

Protein mass in skim milk:

$$300 \text{ kg skim milk} \times 0.7\% \text{ whey proteins} = 2.1 \text{ kg whey protein}$$

$$300 \text{ kg skim milk} \times 2.8\% \text{ casein} = 8.4 \text{ kg casein}$$

$$300 \text{ kg skim milk} \times 3.5\% \text{ total protein} = 10.5 \text{ kg total protein}$$

Protein mass in the permeate:

$$205 \text{ kg permeate} \times 0.55\% \text{ whey proteins} = 1.12 \text{ kg whey protein}$$

Protein mass in the retentate:

$$95 \text{ kg retentate contains } 8.4 \text{ kg casein} + 2.1 \text{ kg whey protein} - 1.12 \text{ kg whey protein}$$

$$95 \text{ kg retentate contains}$$

$$8.4 \text{ kg casein and } 0.98 \text{ kg whey protein represents a casein/whey protein ratio of approx. } 90:10.$$

The slight difference in calculation versus measured protein content in the retentate was 11.6% (calculated to 11.5%).

The total protein concentration in the liquid, protein-enriched concentrate was 11.5% as analyzed and 9.88% as calculated referring to the total protein content:

The MF retentate (11.5% analyzed protein content) was cooled down by the plate heat exchanger to a temperature of 4 °C and stored over night at that temperature.

80 kg of the MF-retentate were then mixed with 20 kg pasteurized cream with a fat content of 40% and a protein content of 2 %. The mixture considered as cheese milk preparation had a fat

content of 8% and a protein content of 9.2 kg coming from the retentate and 0.4 kg origin from the cream. The total protein content in the cheese milk preparation therefore was 9.6 %. The fat content was determined by the Gerber method.

The cheese milk preparation was warmed up in two cheese vessels with a volume of 50 kg each by warm water in a double jacketed wall to 35 °C. As a starter culture 2 ml of a pre-culture per kg cheese milk preparation was used. The acidifying pre-culture was prepared by solving a deep frozen mesophilic, homofermentative starter culture (R-604; CHR HANSEN) in high heated and again cooled skim milk in a ratio of 200 g starter culture to 1800 g skim milk. From this amount of 2000 g pre-culture 100 ml were added to each cheese vessel. This procedure was chosen in order to facilitate dosing of the starter culture. After the starter culture was added in the case of the control production only an amount of 0.2 ml rennet per liter cheese milk preparation (NaturenTM Premium 145; CHR HANSEN) was added. The rennet preparation has a labeled IMCU (International Milk Clotting Unit) activity of 140 IMCU/ml (REMCAT strength). Based on the 100 kg cheese milk preparation it means per g protein 0.29 IMCU rennet were used (20 ml rennet to 100 kg cheese milk preparation with 9,600 g; 20 ml x 140 IMCU/ml = 2,800 IMCU; 2,800 IMCU/9,600 g protein = 0.29 IMCU/g protein). Here, transglutaminase was added simultaneously with rennet. In case of the use of 1 U TG/g protein an amount of 96 g of the transglutaminase preparation Activa YG[®] from the company Ajinomoto was dissolved in 1000 g distilled water before adding to the cheese milk (50% of the solution in each cheese vessel) together with the same amount of rennet as in the control cheese production). The preparation Activa YG[®] is a mixture of MTGase, Lactose, Maltodextrin, GSH, added as yeast extract and safflower oil. In the case of the use of 2 U TG/g protein, an amount of 192 g of the transglutaminase preparation Activa YG[®] was solved in 1000 g distilled water before adding to the cheese milk. Again, the amount of rennet (0.2 ml/l) was kept the same.

The trials were done in three following days.

The visual control of the gelling showed that the curd was ready to be cut after 26 min for the control cheese milk preparation (gelling time 26 min) whereas the cheese milk preparation with 1 U TG/g protein showed a delay in gelling of 4 min (gelling time 30 min). When the curd was cut with a spatula and the curd was lifted up with the spatula then and the cut surface showed the typical porcelain-like structure, the curd was cut with a wired frame to a size of cubes with

a length 2 cm x 2 cm x 2 cm. The cheese milk preparation with 2 U TG/g protein had a further delay in gelling time and it was possible to cut the curd 38 min after adding the enzymes. Compared to the control, the process was prolonged for 12 min. Compared to conventional cheese making with non-concentrated milk this is still an advantageous gelling time. The gelling time at conventional processes often is 40 min.

After cutting the curd the complete mass of curd and whey was circulated in the vat and was transferred to forms in between a time of some minutes and then pressed at a pressure of 0.035 kg/cm². By pressing, a smooth surface was achieved. Due to the high serum binding capacity of the curd as obtained by the method of the present invention, the pressing procedure is not related to a high loss of whey and the cheese is well formable. The weight for pressing remained over night on the surface of the cheese and the curd remained in the forms over night at room temperature so the acidification could continue. On the next day a pH of 5.0 ± 0.5 was measured in the cheese mass. The weight remained over night but after the cheese received a smooth surface the weight could have been removed earlier.

On the following day the cheese was spread with table salt (but could also be immersed in brine which is common for many kind of cheese). Due to the rather firm curd, a technology similar to the technology for the production of cheddar could have also been used. Cheddar production is, inter alia, described in Kosikowski (1997), "Cheese and Fermented Milk Foods"; Kosikowski, Westport, USA. For cheddar a curd which passes the so called cheddaring process and is milled and mixed with dry salt and then filled up in forms. If the weight of the cheese is about 200 kg the cheese does not need to be pressed in the form due to high weight of the cheese itself. It is sufficient when the form is turned 180 ° then two or more times. If the pieces of cheese are lower in weight it is pressed like rennet cheese is usually pressed. For the production of a cheese with the presented technology using a microfiltered milk and transglutaminase a similar technique like some steps for producing a cheddar can be used. One advantage of the present invention is the finding that high protein concentrate prepared by simultaneous addition of TG and rennet/rennet replacements leads to a firm food stuff even without "cheddaring process". Accordingly, the present invention avoids one additional step in (e.g.) cheese making, namely the "cheddaring process", i.e. a maturing step of curd after whey drainage. Further advantages of the method of the present invention have been described herein above.

In particular, it was surprisingly found that in accordance with the present invention, it was possible to produce 48 kg cheese from 80 kg acidified MF-concentrate (total protein content 11.5%; fat 0,2 %) + 20 kg cream (fat 40 %; total protein content 2%) = 100 kg milk preparation under the use of 1 U TG/g protein. This represents an increase in yield of approx. +12% (the control, i.e. only rennet, was only 43 kg). A doubling of the concentration of the transglutaminase to 2 U/g protein increases the yield to 53 kg cheese representing a further increase of +10% (total increase of + 23%).

Surprisingly, even if a higher weight increase of final product was obtained, the firmness of the final product remained on a similar high level. Firmness was measured by a penetration test (Stevens Texture Analyser) and is documented also in the following table.

The example also shows that fat can be added as a part of the protein enriched dairy liquid. Fat was added in the form of natural milk fat globules (cream) but the source could have also been a homogenised cream (reduced fat globule size) or reemulsified butterfat/oil or emulsified fat from other sources like palm oil or any other plant or animal fat/oil. It is state of art to emulsify different fats or oils or fractions of fats or oils from different sources with milk proteins or many other proteins due to their behaviour to act as emulsifiers. Due to the fact that the addition of fat reduces the total protein content the addition of fat is limited to 70 % fat in dry matter. It is still important that protein is a dominant ingredient of the substrate.

Procedure	Amount of cheese from 100 kg milk preparation	Penetration value (g) *) depth 10 mm/5 mm	<u>Weight</u> <u>yield</u>
Only rennet (control)	43 kg	695/250	100%
+ 1 U TG/g protein	48 kg	670/233	111%
+ 2 U TG/g protein	53 kg	655/220	123%

*) Penetration probe cylinder Ø 13 mm, penetration speed 0.2 mm/s

The variation of the penetrometer values was $\pm 10\%$ of the total value. The relatively high deviation is due to the fact that the pieces of cheese cut for this test show a certain variation in size and structure. The values shown in this table were calculated as the mean value from at least 4 measurements.

The following table illustrates the dry matter balance in this experiment

Dry matter balance

Product	dry matter	amount	total amount of dry matter
Retentate	18.1%	80 kg	14.5 kg
Cream	45.0%	20 kg	9 kg
Cheese	44.2%	43 kg	19 kg
Whey	8.0%	57 kg	4.5 kg
Cheese	40.4%	48 kg	19.4 kg
Whey	8.0%	52 kg	4.1 kg
Cheese	37.2%	53 kg	19.7 kg
Whey	8.0%	47 kg	3.8 kg

Accordingly, the surprising elevation of the amount of resulting cheese corresponds to lower dry matter content/higher water (or serum) content.

Example 4: Simultaneous addition of TG and rennet as compared to longer incubation times

The same product- and process-parameters according to example 2 were used in the following example, where the incubation time of TG was varied before rennet was added (preincubation of TG). In all cases 2 U TG/g protein and a protein concentration of 14 % was used. After the MF-concentrate was warmed up to 35 °C the starter culture and the TG was added at the same time. The starter culture was added to have comparable conditions with example 2 but due to the relatively short incubation times the starter culture does only play a minor role in this example. Rennet was added in one case 30 min after incubation of TG and starter culture, in one case 10 min after incubation of TG and starter culture, in one case 5 min after incubation of TG and starter culture, in one case 3 min after incubation of TG and starter culture and in

one case simultaneous with TG and rennet (no preincubation time). The following results were obtained:

Sample no.	incubation time of TG and starter culture before rennet addition	gelling time after addition of rennet
1	0 min	37 ± 1 min
2	3 min	40 ± 1 min
3	5 min	$40,5 \pm 1$ min
4	10 min	46 ± 1 min
5	30 min	54 ± 1 min

The gelling time was visually controlled. The gelling time is the time until the curd is ready to be cut after rennet addition, as described in Example 3. The gel strength was measured by the texture analyzer additionally, as described in Example 2. The resistance force was measured immediately after the gelling time was visually proofed. All samples showed a resistance force of $19 \text{ g} \pm 5\%$ when the gelling point was defined visually. This means in all cases the gel has reached a comparable resistance. The results of this example document, that a simultaneous addition, i.e. addition at the same time, of TG and rennet is most preferable, since a pre-incubation of TG before rennet addition leads to remarkable and undesirable elongation of the gelling time. Therefore the rennet should be added within 10 min or less after incubation with TG.

Claims

1. Method for the preparation of visco-plastic food stuff, comprising the step of adding to a liquid, protein-enriched concentrate comprising at least 5% proteins and (a) reactive protein(s) for transglutaminase, a transglutaminase and a rennet or a rennet replacement which comprises a proteolytic enzyme, whereby said transglutaminase and said rennet or said rennet replacement are added simultaneously to said protein-enriched concentrate comprising a reactive protein for transglutaminase.
2. The method of claim 1, wherein said protein-enriched concentrate is selected from the group consisting of micro-filtered milk, a protein-enriched dairy liquid, protein-enriched vegetable or fruit juices.
3. The method of claim 2, wherein said micro-filtered milk is obtainable by passing milk through a 0.05 – 0.2 μm membrane.
4. The method of claim 2, wherein said protein-enriched dairy liquid comprises at least 70% casein in said at least 5% total protein content.
5. The method of any one of claims 1 to 4, wherein said transglutaminase is a microbial transglutaminase (TG) from *Streptoverticillium* or *Bacillus subtilis*.
6. The method of claim 5, wherein said microbial transglutaminase is transglutaminase from *Streptoverticillium mobaraense*.
7. The method of any one of claims 1 to 6, wherein said transglutaminase is a non microbial transglutaminase and selected from the group consisting of fungi, plants or animals.
8. The method of claim 7, wherein said non-microbial transglutaminase from fungi is selected from the group consisting of actinomycetes and myxomycetes.

9. The method of any one of claims 1 to 8, wherein said transglutaminase is added to said protein-enriched concentrate in concentration of at least 0.1 U/g protein to 10 U/g protein transglutaminase/ g protein.
10. The method of any one of claims 1 to 9, wherein said rennet or rennet replacement comprising a proteolytic enzyme is selected from the group consisting of calf rennet, buffalo rennet, sheep rennet, goat rennet, microbial rennet, genetic rennet, recombinant rennet, chymosin preparations and fruit, vegetable or plant juice with renneting activity.
11. The method of claim 10, wherein said rennet is calf rennet.
12. The method of claim 10, wherein said fruit, vegetable or plant juice with renneting activity is selected from the group consisting of melon juice, papaya juice, proteinases from *Opuntia ficus-indica* fruits, extracts from berries of the plant *Solanum dobium*, pineapple pulp extracts, juices from Ash gourd, Cardoon extracts and extract from the flowers of wild globe artichokes.
13. The method of any one of claims 1 to 12, wherein said rennet or rennet replacement is added to said protein-enriched concentrate in concentration of 0.1 to 1 IMCU.
14. The method of any one of claims 1 to 13, wherein said method comprises an additional acidification of the protein-enriched concentrate before or during the simultaneous addition of transglutaminase and rennet or rennet replacement.
15. The method of claim 14, wherein said acidification of the protein-enriched concentrate comprises the adjustment of the pH to a value between 5.2 and 6.8.
16. The method of claim 14 or 15, wherein said acidification is obtained by the addition of starter culture or the addition of an organic acid.
17. The method of claim 16, wherein said starter culture is from or are mesophilic and/or thermophilic bacteria.

18. The method of claim 16, wherein said organic acid is citric acid, lactic acid, acetic acid, malic acid, formic acid or glucono δ lactone.
19. The method of any one of claims 1 to 18, wherein said protein-enriched concentrate comprises a casein/rest protein(s) ratio of at least 70/30.
20. The method of claim 19, wherein said rest protein(s) is/are whey proteins.
21. The method of claim 19 or 20, wherein said casein is added before said transglutaminase and said rennet or rennet replacement is added simultaneously to said protein-enriched concentrate.
22. The method of any one of claims 1 to 21, further comprising the addition of whey proteins and/or casein to the protein-enriched concentrate.
23. The method of claim 22, wherein said whey proteins are denatured.
24. The method of any one of claims 1 to 23, further comprising the addition of fat.
25. The method of claim 24, wherein the fat is added so that the ratio of fat content to total protein content in the liquid, protein-enriched concentrate is 2.5(fat):1(protein) at the most.
26. The method of any one of claims 1 to 25 further comprising a step of adding glutathione before said transglutaminase and said rennet or rennet replacement is added simultaneously to said protein-enriched concentrate.
27. The method of any one of claims 1 to 26, wherein said simultaneous addition of said transglutaminase and said rennet to said protein-enriched concentrate comprises the addition of transglutaminase and rennet within 10 minutes or less.

28. The method of any one of claims 1 to 27, wherein said simultaneous addition of said transglutaminase and said rennet to said protein-enriched concentrate comprises the addition of said transglutaminase and said rennet at the same time.
29. The method of any one of claims 1 to 28 for the production of cheese or cheese curd, wherein said protein-enriched concentrate is microfiltered milk, wherein said transglutaminase is from Streptococcus and wherein said rennet is calf rennet.
30. The method of any of the claims 1 to 29, wherein said (a) reactive protein(s) for transglutaminase is comprised in said at least 5% proteins of the liquid, protein-enriched concentrate.
31. Use of the visco-plastic food stuff as obtained by the method of any one of claims 1 to 30 for the preparation of cheese or cheese products.
32. The use of claim 18, wherein said cheese or cheese product is selected from the group consisting of quarg, rennet cheese, soft-cheese, semi-hard cheese and hard-cheese.
33. A kit for carrying out the method of any one of claims 1 to 32, said kit comprising transglutaminase as characterized in any one of claims 5 to 8 and comprising said rennet or rennet replacement as characterized in any one of claims 10 to 12.

Fig. 1

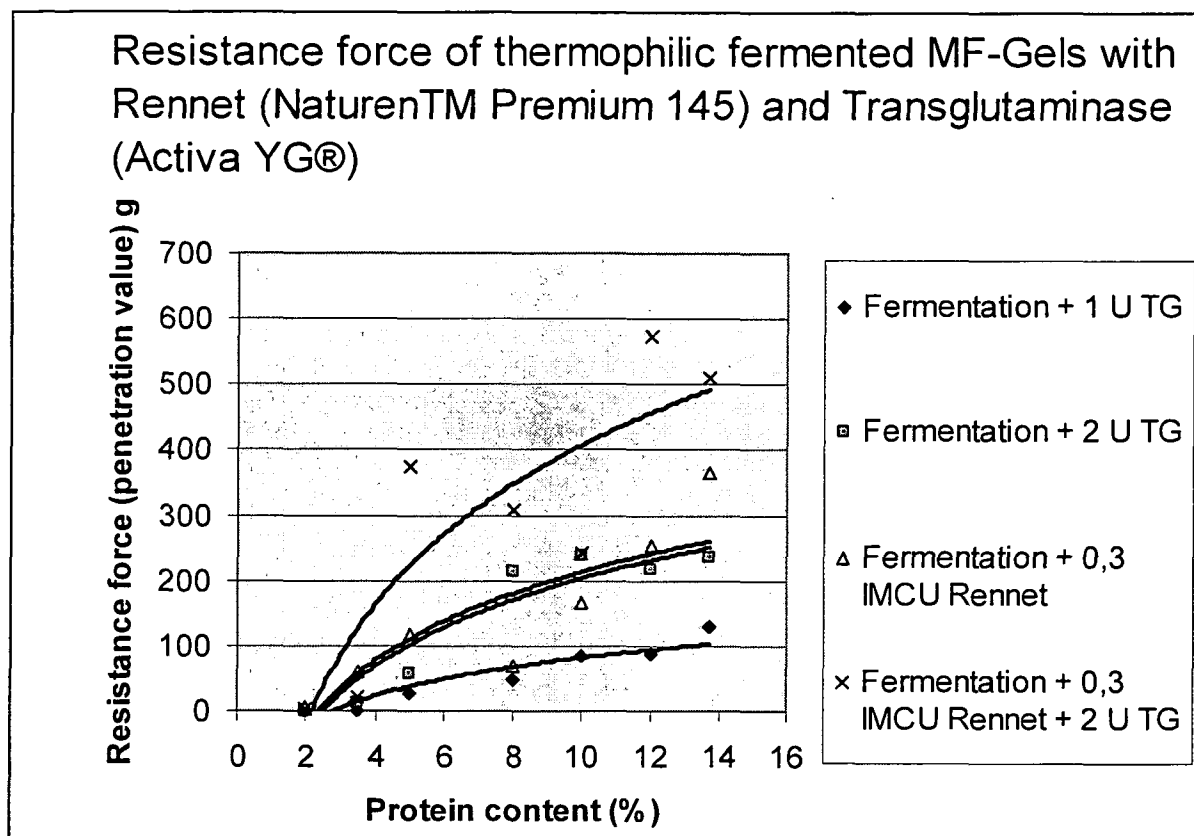
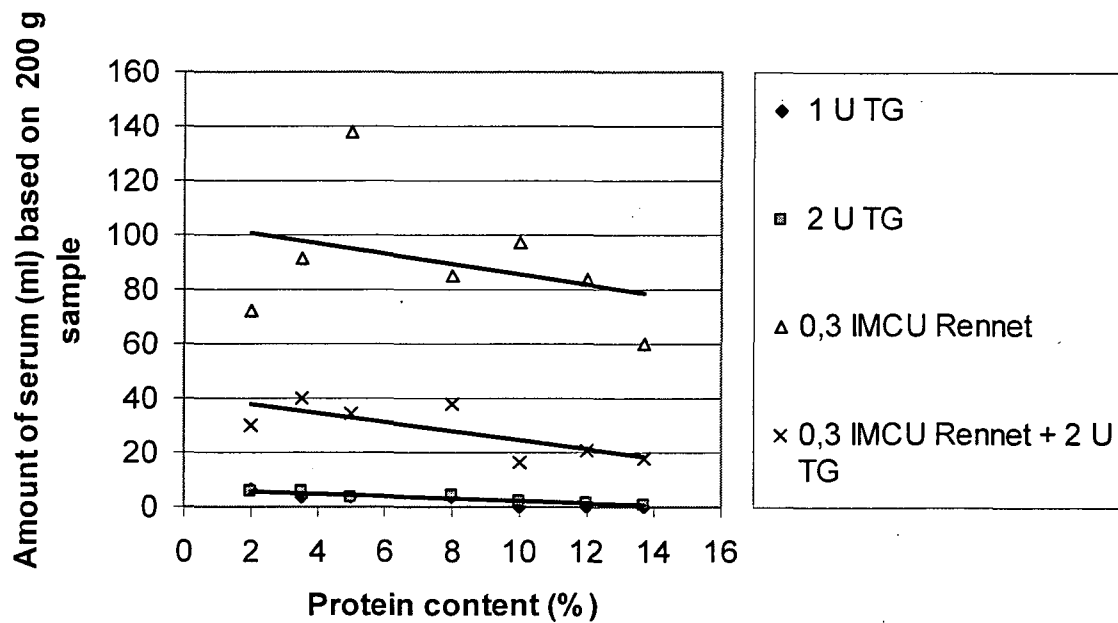


Fig. 2

Serum drainage of thermophilic fermented MF-Gels with
Rennet (Naturen™ Premium 145) and
Transglutaminase (Activa YG®)



INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2007/007100

A. CLASSIFICATION OF SUBJECT MATTER

INV. A23C19/032 A23C19/05 A23C19/068

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)

A23C

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 practical, search terms used)

EPO-Internal, WPI Data, FSTA, BIOSIS, COMPENDEX

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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A	paragraph [0027]; example 1	1-32
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X	page 7, paragraph 4; example 1	33
	-/--	

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

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Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Koch, Jürgen

INTERNATIONAL SEARCH REPORT

International application No

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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