Title: PROCESS FOR THE PREPARATION OF A FERMENTED MILK PRODUCT

Abstract: The present invention relates to a process for preparing a fermented milk product which comprises 4 to 15 wt/wt % of protein, comprising a contacting milk with an enzyme having carboxypeptidase activity, b. contacting milk with a composition comprising lactic acid bacteria. The invention further relates to the use of an enzyme having carboxypeptidase activity to increase fluidity of a fermented milk product.
PROCESS FOR THE PREPARATION OF A FERMENTED MILK PRODUCT

The present invention relates to a process for the preparation of a fermented milk product.

Background

Fermented milk products such as yoghurt are produced by inoculating milk with a so-called yoghurt culture consisting of lactic acid bacteria. Fermentation of lactose by these bacteria produces lactic acid, which acts on milk protein to give yogurt its texture and characteristic tang. The texture of yoghurt is a.o. influenced by the type of lactic acid bacteria used, eg. *Streptococcus thermophilus*. *Lactobacillus* sp. or *Bifidobacterium* sp., and the protein content in the milk. Usually, a higher protein content in the milk results in a higher viscosity of the resulting yoghurt. An example of a high protein yoghurt is Greek yoghurt, which is a firm yoghurt. Apart from the traditional yoghurts with relatively high viscosity, there is a growing need for healthy and easy drinking yoghurts high in protein content.

The aim of the present invention is a fermented milk product with improved properties.

Summary

The present disclosure relates to method for preparing a fermented milk product with an improved fluidity.

Disclosed herein is a process for preparing a fermented milk product which comprises 4 to 15 wt/wt % of protein, comprising

a. contacting milk with an enzyme having carboxypeptidase activity;

b. contacting the milk with a composition comprising lactic acid bacteria;

and preparing the fermented milk product.

In another aspect, the disclosure relates to the use of an enzyme having carboxypeptidase activity to increase fluidity of a fermented milk product.

Definitions
Sequence identity. It is defined herein that in order to determine the percentage of sequence identity of two amino acid sequences, the sequences are aligned for optimal comparison purposes. In order to optimize the alignment between the two sequences gaps may be introduced in any of the two sequences that are compared. Such alignment can be carried out over the full length of the sequences being compared. Alternatively, the alignment may be carried out over a shorter length, for example over about 20, about 50, about 100 or more amino acids. The sequence identity is the percentage of identical matches between the two sequences over the reported aligned region. The percent sequence identity between two amino acid sequences may be determined using the Needleman and Wunsch algorithm for the alignment of two sequences. (Needleman, S. B. and Wunsch, C. D. (1970) J. Mol. Biol. 48, 443-453). Both amino acid sequences and nucleotide sequences can be aligned by the algorithm. The Needleman-Wunsch algorithm has been implemented in the computer program NEEDLE. For the purpose of this invention the NEEDLE program from the EMBOS package was used (version 2.8.0 or higher, EMBOS: The European Molecular Biology Open Software Suite (2000) Rice, P. Longden, I. and Bleasby, A. Trends in Genetics 16, (6) pp276—277, http://emboss.bioinformatics.nl/). For protein sequences EBLASTSUM62 is used for the substitution matrix. The optional parameters used are a gap-open penalty of 10 and a gap extension penalty of 0.5. The skilled person will appreciate that all these different parameters will yield slightly different results but that the overall percentage identity of two sequences is not significantly altered when using different algorithms.

A “mature polypeptide” is defined herein as a polypeptide in its final form and is obtained after translation of a mRNA into polypeptide and post-translational modifications of said polypeptide. Post –translational modifications include N-terminal processing, C-terminal truncation, glycosylation, phosphorylation and removal of leader sequences such as signal peptides, propeptides and/or prepropeptides by cleavage.

The term "milk" is intended to encompass milks from mammals and plant sources or mixtures thereof. Preferably, the milk is from a mammal source. Mammal sources of milk include, but are not limited to cow, sheep, goat, buffalo, camel, llama, mare and deer. In an embodiment, the milk is from a mammal selected from the group consisting of cow, sheep, goat, buffalo, camel, llama, mare and deer, and combinations thereof. Plant sources of milk include, but are not limited to, milk extracted from soy bean, pea, peanut, barley, rice, oat, quinoa, almond, cashew, coconut, hazelnut, hemp,
sesame seed and sunflower seed. In addition, the term "milk" refers to not only whole milk, but also skim milk or any liquid component derived thereof.

As used in the present specification, the term "fermented milk product" refers to a product that has been fermented with lactic acid bacteria such as *Streptococcus thermophilus* and/or *Lactobacillus delbrueckii* subsp. *Bulgarius*. Other microorganisms such as *Lactobacillus delbrueckii* subsp. *lactis*, *Bifidobacterium animalis* subsp. *lactis*, *Lactococcus lactis*, *Lactobacillus acidophilus* and *Lactobacillus casei*, may also be used. Lactic acid strains other than *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, are intended to give the finished product various properties, such as the property of promoting the equilibrium of the flora. Fermentation of lactose in the milk by lactic acid bacteria produces lactic acid which acts on the milk protein to give the fermented milk product texture. The fermentation process further increases the shelf-life of the product while enhancing and improving the digestibility of milk. Many different types of fermented milk products can be found in the world today. Examples are soured milk (e.g. buttermilk), soured cream and yogurt.

As used herein, the term "yogurt or yoghurt" is a fermented milk product produced by fermentation of milk by lactic acid bacteria, also known as "yoghurt cultures". Yogurt may be made from cow milk, the protein of which mainly comprises casein, which is most commonly used to make yoghurt, but milk from other mammal sources, or milk from plant sources and combinations thereof may be used as well. The term "yoghurt" furthermore encompasses, but is not limited to, yogurt as defined according to French and European regulations, e.g. coagulated dairy products obtained by lactic acid fermentation by means of specific thermophilic lactic acid bacteria only (i.e. *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*) which are cultured simultaneously and are found to be living in the final product in an amount of at least 10 million CFU (colony-forming unit) per gram of the yogurt. Preferably, the yoghurt is not heat-treated after fermentation. Yogurts may contain added dairy raw materials (e.g. cream and/or protein) or other ingredients such as sugar or sweetening agents, one or more flavouring(s), cereals or nutritional substances, especially vitamins, minerals and fibers. Such yoghurt advantageously meets the specifications for fermented milks and yoghurts of the AFNOR NF 04-600 standard and/or the codex StanA-Ila-1975 standard. Yoghurt encompasses set yogurt, stirred yoghurt, drinking yoghurt, Petit Suisse, heat treated yogurt and yogurt-like products.
**Detailed description**

The present invention relates to a process for preparing a fermented milk product, which comprises 4 to 15 wt/wt % protein, comprising

a. contacting milk with an enzyme having carboxypeptidase activity;

b. contacting milk with a composition comprising lactic acid bacteria; and

preparing the fermented milk product.

Surprisingly, it was found that a fermented milk product comprising 4 to 15 wt/wt% protein with improved properties was prepared by a process as disclosed herein, as compared to a fermented milk product comprising 4 to 15 wt/wt% protein that was prepared by a process in the absence of carboxypeptidase. An improved property as used herein may be improved fluidity or pourability, texture, flavor or taste of the fermented milk product, such as improved fluidity.

A process for preparing a fermented milk product may be a process for preparing a fermented milk product with an improved fluidity, such as compared to the fluidity of a fermented milk product that has been prepared in a process without contacting milk with an enzyme having carboxypeptidase activity.

Accordingly, in one aspect the present disclosure relates to a method for improving fluidity of a fermented milk product comprising

a. contacting a milk with an enzyme having carboxypeptidase activity; and

b. contacting the milk with a composition comprising lactic acid bacteria.

In a process for improving the fluidity of the fermented milk product, the fluidity is improved compared to a fermented milk product that was prepared in the absence of an enzyme having carboxypeptidase activity.

An enzyme having carboxypeptidase activity may be contacted with milk during any suitable step in a process for the preparation of a fermented milk product or a method for improving fluidity of a fermented milk product. The enzyme having carboxypeptidase activity may be added to milk prior, during or after contacting the milk with a composition comprising lactic acid bacteria. Preferably, an enzyme having carboxypeptidase activity is added to milk prior to contacting the milk with a composition comprising lactic acid bacteria. Alternatively, an enzyme having carboxypeptidase activity may be added to the milk during contacting milk with lactic acid bacteria. Contacting milk with an enzyme having carboxypeptidase activity may comprise incubating the milk with an enzyme having carboxypeptidase activity at a
suitable temperature and time. For instance incubating milk with an enzyme having carboxypeptidase activity may be performed at a temperature of 1 to 10 degrees Celsius, such as 2 to 8 degrees Celsius, for instance during 6 to 24 hrs or during 8 to 18 hrs, or 10 to 16 hrs.

Alternatively, milk may be incubated with an enzyme having carboxypeptidase activity at a temperature of between 25 and 50 °C, or between 30 and 45 °C for instance during contacting milk with lactic acid bacteria.

Incubating milk with an enzyme having carboxypeptidase activity may be performed at any suitable pH value, for instance at the pH of milk, or for instance a pH from 3 to 9, or a pH from 4 to 8.

An enzyme having carboxypeptidase activity may be a carboxypeptidase, for instance a serine carboxypeptidase which has enzyme classification number EC 3.4.16. A carboxypeptidase may be derived from any suitable microorganism, for instance Aspergillus sp. such as Aspergillus niger, or Aspergillus oryzae. A carboxypeptidase may be carboxypeptidase I from Aspergillus niger as disclosed in Del Degan (1992) Applied Environmental Microbiology, 58, 2144-2152. A carboxypeptidase is a protease enzyme that hydrolyses a peptide bond at the carboxy- (C-) terminal end of a protein or peptide. The wording “derived” or “derivable” from with respect to the origin of a polypeptide as disclosed herein, means that when carrying out a BLAST search with a polypeptide as disclosed herein, the polypeptide may be derivable from a natural source, such as a microbial cell, of which an endogenous polypeptide shows the highest percentage homology or identity with the polypeptide as disclosed herein.

In one embodiment a carboxypeptidase is a polypeptide having carboxypeptidase activity which has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the mature polypeptide sequence of SEQ ID NO: 1. The mature polypeptide sequence may comprise amino acids 53 to 520 of SEQ ID NO:1, wherein methionine at position 1 in SEQ ID NO: 1 is counted as number 1.

A carboxypeptidase as disclosed herein may be produced in any suitable host organism, for instance in fungi Aspergilli, eg Aspergillus niger or Aspergillus oryzae, Trichoderma, or the yeasts Saccharomyces, and Kluyveromyces or the bacteria of the genus Streptomyces, Escherichia, such as Escherichia coli or Bacilli, such as Bacillus subtilis by known methods in the art, for instance using standard molecular techniques

In one embodiment, an enzyme having carboxypeptidase activity is a purified or a pure enzyme. A pure of purified enzyme is an enzyme that may be at least 50% pure, e.g., at least 60% pure, at least 70% pure, at least 75% pure, at least 80% pure, at least 85% pure, at least 90% pure, at least 95% pure, 96%, 97%, 98%, 99%, 99.5%, 99.9% pure for instance as determined by SDS-PAGE or any other analytical method suitable for this purpose and known to the person skilled in the art.

A process for preparing a fermented milk product or a method for improving fluidity of a fermented milk product as disclosed herein may comprise pasteurizing or sterilizing the milk. For instance, the milk may be pasteurized or sterilized after contacting the milk with an enzyme having carboxypeptidase activity. For instance the milk is pasteurized or sterilized after adding an enzyme having carboxypeptidase activity to the milk and prior to contacting the milk with a composition comprising lactic acid bacteria. Advantageously, an enzyme having carboxypeptidase activity is inactivated after pasteurization or sterilization of milk.

Pasteurizing milk may be performed by any suitable process known in the art. For instance, pasteurizing milk comprises bringing the milk to a temperature of between 60 and 65 degrees Celsius during a period of between 5 to 30 min, such as for instance between 10 and 20 min, or bringing the milk to a temperature to a temperature of between 70 and 75 degrees Celsius for 5 to 30 seconds.

Sterilizing milk may be performed by any suitable process known in the art. Sterilizing milk may comprise bringing the milk to a temperature of between 80 and 100 degrees Celsius during 5 to 40 min. Sterilization may also be performed ultra-high temperature (UHT) sterilization, which comprises bringing the milk to a temperature of between 110 and 140 degrees Celsius during 1 to 5 seconds.

Contacting milk with a composition comprising lactic acid bacteria, may comprise adding a composition comprising lactic acid bacteria to milk. Contacting milk with a composition comprising lactic acid bacteria may further comprise fermenting or incubating the milk with lactic acid bacteria, for instance at a temperature of between 25 to 50 degrees Celsius, such as between 30 and 45 degrees Celsius during 2 to 10 hrs, for instance during 3 to 8 hrs or during 4 to 6 hrs, or during 10 to 16 hrs. Suitable lactic acid bacteria in a process for the preparation of a fermented milk product as
disclosed herein are for instance strains of *Streptococcus thermophiles*, and / or *Lactobacillus delbrueckii* subsp. *bulgaricus*.

In one embodiment, a process or method as disclosed herein may further comprise bringing the fermented milk product to a temperature of 1 to 10 degrees Celsius, such as a temperature of between 2 and 8 degrees Celsius, such as a temperature of between 4 and 6 degrees Celsius.

In another embodiment a process for preparing a fermented milk product and a method for improving fluidity of a fermented milk product as disclosed herein may further comprise smoothening the fermented milk product. Smoothening usually comprises breaking the texture of the fermented milk product and / or homogenizing the fermented milk product Smoothening the fermented milk product may be performed by any suitable method known in the art. For instance smoothening may be performed by stirring the fermented milk product and / or pumping the fermented milk product, for instance through a tube. Smoothening the fermented milk product may be performed prior to or after bringing the fermented milk product to a temperature of 1 to 10 degrees Celsius. It was found that smoothening the fermented milk product increased the fluidity or pourability of the fermented milk product. Surprisingly, it was found that a fermented milk product comprising 2 to 15 wt/wt % or 4 to 15 wt/wt% of protein which was prepared by a process as disclosed herein was more fluid, or exhibited an increased pourability as compared to a fermented milk product that was prepared in the absence of an enzyme having carboxypeptidase activity.

In one embodiment a fermented milk product comprises 2 to 15 wt/wt% of protein, or 4 to 15 wt/wt% of protein, or 3 to 12 wt/wt% of protein, or 3 to 14 wt/wt% of protein, or 4 to 12 wt/wt% of protein, or 4 to 11 wt/wt% of protein, or 5 to 10 wt/wt% of protein, or 6 to 9 wt/wt% of protein. Wt/wt% of protein as used herein is defined as dry weight protein per wet weight of milk, or fermented milk product. Protein may be mammalian milk protein such as casein or whey protein, or plant protein such as soy protein. Whey protein may comprise beta-lactoglobuline or alpha-lactalbumin.

A process and method as disclosed herein may comprise enriching milk with protein, for instance adding protein to milk prior or during contacting milk with an enzyme having carboxypeptidase activity. Protein that is added to milk may be protein as defined herein above. Milk in a process as disclosed herein may comprise 2 to 15 wet wt/wt % of protein, or 4 to 15 wt/wt% of protein, or 3 to 12 wt/wt% of protein, or 3 to
14 wt/wt % of protein, or 4 to 13 wt/wt% of protein, or 4 to 11 wt/wt % of protein, or 5 to 12 wt/wt% of protein, or 5 to 10 wt/wt% of protein or 6 to 9 wt/wt % of protein.

In one embodiment, a fermented milk product as disclosed herein is a fluid fermented milk product. A fluid fermented milk product as used herein is a fermented milk product that is pourable fermented milk product.

In one embodiment a fluid fermented milk product is a yoghurt, preferably a drinking yoghurt or drinkable yoghurt.

In one embodiment milk is from a mammalian source, for instance milk is a cow’s, ewe’s, or goat’s milk.

In one aspect the present disclosure relates to the use of an enzyme having carboxypeptidase activity to increase fluidity or pourability of a fermented milk product. The use of an enzyme having carboxypeptidase activity to increase fluidity or pourability of a fermented milk product, comprises preparing a fermented milk product as disclosed herein above.

In another aspect the present invention relates to a fermented milk product obtainable by a process as disclosed herein.

EXAMPLES

MATERIALS AND METHODS

Carboxypeptidase and culture
Yoghurt starter culture TS-80J (batch 9802, UW 43) in Example 1, starter culture YS-131 (batch 8618) in Example 2 and carboxypeptidase Maxipro CPP (batch 6131344701; 954 CPGU/g) were obtained from DSM Food Specialties, Delft, the Netherlands.

Carboxypeptidase activity
Carboxypeptidase activity is expressed in CPGU = Carboxy Peptidase G Unit.

One CPGU is the amount of enzyme needed to decrease the optical density at 340 nm by one absorbency unit per minute (1 AU/min) applying the conditions of the test (hydrolysis of 1.5 mM FA-Phe-Ala, pH 4.5 and 37°C).

Acidification activity test
Acidification activity was tested according to the Method of Analysis Carboxyfluorescein measurement in yoghurt in MTP format (AMI 15.1).
Fluorescent was measured by Tecan Infinitie pro2000 with the following parameters: Excitation: 491nm; Emission: 519nm, Gain: 56.
For bottom reading no Z-position is needed.
No multiplate reads per plate
Shaking: before measurement, duration 3 sec, mode: linear, intensity: low, settle time: 3 sec
Kinetic: measured every 5 minutes, for 6 hours.
RFU is put out in a graph against the real pH of the calibration buffers in exponential.
An equation comes out and to calculate the real pH of the samples.

EXAMPLE 1. Preparation of high protein fermented milk product
Preparation of fermented milk product with carboxypeptidase

Milk with 5 wt/wt % proteins was prepared by adding 2 wt % extra proteins of skimmed milk powder with 35% proteins to full fat commercial pasteurized milk (CPM-ff) with. The milk was stirred at room temperature for 30 minutes and subsequently 20 ml divided in Greiner tubes.
Maxipro CPP was diluted 100 x by 0.1 ml in 9.9ml milli-Q mixed wells and kept at room temperature. 0.1%, 1% and 5% Maxipro CPP / milk protein (wt wt/ wt %) was added to milk (final concentrations ~50 CPGU/L milk, ~500 CPGU/L milk and ~2500 CPGU/L milk respectively) and stored in a cold room overnight.

After incubation, the activity of Maxipro CPP was stopped by putting the Greiner tubes comprising 20 ml milk in a water bath of 85 °C for 5 minutes. After that the milk samples were cooled to room temperature.

Starter culture was a direct vat culture (DVC) frozen pellets. Inoculation level was 86 g / 1000L of thawn culture for the DSM cultures.
For each culture a tube with 10 ml was brought to 20 degrees Celsius. Dilutions were made by diluting 1 gram in 9 ml of saline. Tubes of milk were inoculated with the culture and then 1800 µl was pipetted in 6 separate 24-wells plates with flat bottom and 200ul of 1mmol carboxyfluorescein was mixed together. Incubation was performed at 42 degrees Celsius for 6 hours. One plate was smoothened directly by pipetting three times up and down. Subsequently all plates were stored in a cold room at 4 to 8 °C.
Viscosity measurement

Plates were brought to room temperature before viscosity measurement. The back pressure (viscosity) was measured by the Hamilton Microlab STARlet. The Hamilton monitors aspiration and dispense steps in real time during sample transfers using a software TADM (Total Aspirate and Dispense Monitoring). TADM_viscosity 24 wells_tipcount_21NOV14 tipcut 4, Z: 176, asp 100 µl disp 200 µl. Measurements were performed according to the suppliers instructions.

Three samples (plates) were measured:

One plate contained set yoghurt that was cooled directly after incubation, one plate was smoothened before cooling by pipetting three times and one plate was smoothened after cooling by pipetting three times.

Table 1. Back pressure (viscosity) and fluorescence of fermented milk products with and without carboxypeptidase treatment

<table>
<thead>
<tr>
<th>Protease (%)</th>
<th>back pressure</th>
<th>Acidification (fluorescence)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>smooth before cooling</td>
<td>smooth after cooling</td>
</tr>
<tr>
<td>0.1 CPP</td>
<td>-1333</td>
<td>-1440</td>
</tr>
<tr>
<td>1 CPP</td>
<td>-649</td>
<td>-</td>
</tr>
<tr>
<td>5 CPP</td>
<td>-332</td>
<td>-761</td>
</tr>
<tr>
<td>Reference</td>
<td>-1132</td>
<td>-1850</td>
</tr>
<tr>
<td>Reference</td>
<td>-1289</td>
<td>-1728</td>
</tr>
<tr>
<td>Reference</td>
<td>-1293</td>
<td>-1884</td>
</tr>
</tbody>
</table>

The results in Table 1 show that addition of carboxypeptidase Maxipro CPP leads to a decrease in viscosity, while acidification is comparable to the reference fermented milk product. All samples had undergone a proper acidification which is indicated by an RFU of below 550.

When the fermented milk product was smoothened before or after cooling, the back pressure (viscosity) of the fermented milk treated with carboxypeptidase (CPP) is reduced as compared to the reference fermented milk product. The back pressure (viscosity) of the set fermented milk product is higher than the back pressure (viscosity) of smoothened fermented milk product, also in the fermented milk product treated with carboxypeptidase, as can be seen by the higher ‘back pressure’.
EXAMPLE 2: Preparation of fermented milk product with enzyme added during fermentation

10% skimmed milk powder (SMP) solution containing 3.5 wt/wt % protein was prepared in 1 L bottles by dissolving SMP in water and stirring at room temperature for 30 minutes. The milk was heated at 85 °C for 30 minutes.

Starter culture was a direct vat culture (DVC) frozen pellets. Inoculation level was 4U / 1000L of thawed culture YS-131 for the DSM culture. Maxipro CPP was added at a dose of 2.5% (v/v) to the milk. An amount of 200ml from this milk was used for fermentation at 42°C with continuous monitoring of the pH. When the pH reached a value of 4.6, the fermented milk was texturized by passing through a peristaltic pump (1330 ml / min), followed by cooling of the fermented milk to 4 °C. In the same way, fermented milk was prepared from SMP without enzyme addition. The texturized fermented milks were split in 4 X 125 ml cups and kept at this temperature for 5 days prior to the viscosity measurement.

Viscosity measurement

The viscosity of the fermented milks (4 yoghurt cups per sample) was measured by Brookfield. The Brookfield viscometer determines the viscosity by measuring the force required to turn the spindle into the product at a given rate. The Helipath system with a T -spindle is used as it is designed for non-flowing thixotropic materials. The viscometer measures constantly the viscosity in fresh material and is considered the standard measurement for stirred and set yoghurt viscosity. The changes in the viscosity (the average of four sample measurements) for the Maxipro CPP containing fermented milk and the control were shown below in Table 2.

The results in Table 2 show that the viscosity of yoghurt comprising a protein content of 3.5 wet wt/wt % was reduced when Maxipro CPP was added during fermentation of the milk.

<table>
<thead>
<tr>
<th>Fermented milk</th>
<th>Viscosity, mPa</th>
</tr>
</thead>
<tbody>
<tr>
<td>No enzyme</td>
<td>7220.20</td>
</tr>
<tr>
<td>Maxipro CPP</td>
<td>4583.84</td>
</tr>
</tbody>
</table>
CLAIMS

1. Process for preparing a fermented milk product which comprises 4 to 15 wt/wt % of protein, comprising
   a. contacting milk with an enzyme having carboxypeptidase activity;
   b. contacting milk with a composition comprising lactic acid bacteria.

2. Method for improving fluidity of a fermented milk product comprising
   a. contacting milk with an enzyme having carboxypeptidase activity; and
   b. contacting milk with a composition comprising lactic acid bacteria.

3. Process according to claim 1 or method according to claim 2, wherein the enzyme having carboxypeptidase activity is contacted with milk prior, during or after contacting the milk with a composition comprising lactic acid bacteria.

4. Process according to any one of the claims 1 or 3 or a method according to claim 2 or 3, wherein the enzyme having carboxypeptidase activity has at least 80% identity to the mature polypeptide sequence of SEQ ID NO: 1.

5. Process according to any one of the claims 1, 3, or 4 or a method according to any one of the claims 2 to 4, further comprising bringing the fermented milk product to a temperature of 1 to 10 degrees Celsius.

6. Process according to any one of the claims 1, 3 to 5 or a method according to any one of the claims 2 to 5, further comprising smoothening the fermented milk product.

7. Process or method according to claim 6, wherein smoothening the fermented milk product is performed prior or after bringing the fermented milk product to a temperature of 1 to 10 degrees Celsius.

8. Process according to any one of the claims 1, 3 to 7, or a method according to any one of the claims 2 to 7, wherein the process further comprises adding protein to the milk.
9. Process according to any one of the claims 1, 3 to 8, or a method according to any one of the claims 2 to 8, wherein the fermented milk product is a fluid fermented milk product.

10. Process according to any one of the claims 1, 3 to 9, or a method according to any one of the claims 2 to 9, wherein the fermented milk product is a yoghurt, preferably a drinking yoghurt.

11. Process according to any one of the claims 1, 3 to 10, or a method according to any one of the claims 2 to 10, wherein the milk is a cow’s, ewe’s, or goat’s milk.

12. Use of an enzyme having carboxypeptidase activity to increase fluidity of a fermented milk product.

13. Use according to claim 12, or process according to claim 2, wherein the fermented milk product comprises 2 to 15 wt/wt % of protein.

14. A fermented milk product obtainable by a process according to any one of the claims 1, 3 to 11.
**INTERNATIONAL SEARCH REPORT**

**A. CLASSIFICATION OF SUBJECT MATTER**

INV. A23C9/12 A23C9/127

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)

A23C

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

Electronic database consulted during the international search (name of database and, where practical, search terms used)

EPO-Internal, WPI Data, COMPENDEX, FSTA, Sequence Search

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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<tr>
<td>X</td>
<td>WO 2012/022112 A1 (DSM IP ASSETS BV [NL]; WANG JIANMING [CN]; ZHAO SHAOHUA [CN]) 23 February 2012 (2012-02-23) claims 1,2,8-10; examples 1,2</td>
<td>1,3,5,7, 9,11,14</td>
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<tr>
<td>Y</td>
<td>JP H07 147898 A (OGAWA KORYO KK) 13 June 1995 (1995-06-13) example 1</td>
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**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
  
  "E" earlier application or patent but published on or after the international filing date
  
  "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

**Date of the actual completion of the international search**

4 August 2016

**Date of mailing of the international search report**

18/08/2016

**Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV RIJWIK Tel: (+31-70) 340-3040, Fax: (+31-70) 340-3016**

Götz, Michael
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<td>Y</td>
<td>WO 02/068623 A2 (DSM NV [NL]; EDENS LUPPO [NL]; DIJK VAN ALBERTUS ALARD [NL]; KRUBASIK) 6 September 2002 (2002-09-06)</td>
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<td>A</td>
<td>SEQ ID n° 1 of claim 3 has 100.00 % identity (100.00 % similarity) over 520 positions in a common overlap (range (q:s): 1-520:1-520) with subject GSP:ABR38863 (length: 520) from this document.</td>
<td>2,12,13</td>
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<td>Patent document cited in search report</td>
<td>Publication date</td>
<td>Patent family member(s)</td>
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