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(54) Title: FAST ACIDIFYING AND PHAGE-RESISTANT LACTIC ACID BACTERIA FOR PASTA FILATA

(57) Abstract: The present disclosure is in the field of dairy technology. It relates to methods for producing pasta filata type cheese products and products produced therefrom. The disclosure describes new strains and starter cultures comprising said strains suitable for preparing pasta filata type cheese products.

WO 2025/040656 A1

FAST ACIDIFYING AND PHAGE-RESISTANT LACTIC ACID BACTERIA FOR PASTA FILATA**FIELD**

The present disclosure generally relates to the field of fermented milk. In particular to compounds,
5 compositions and methods for the production of cheese.

BACKGROUND

Streptococcus thermophilus (ST) is a thermophilic lactic acid bacterium (LAB), widely used as a
starter to produce fermented dairy products. For this application, the fast-growing capacity of these
10 bacteria in milk is crucial to enable intense and rapid acidification of milk. ST strains with the ability
to acidify milk quickly are essential for several applications amongst other the production of cheese
such as e.g. Pasta filata.

Phage attacks is a challenge in the manufacturing of fermented milk products. Often a set of several
cultures having the same performance are generated for the Dairies to apply a rotation of cultures
15 having different phage-resistance profiles. Thereby, when a phage attack takes place the phage-hit
(phage-sensitive) culture can be replaced/rotated with another culture which are phage-resistant.

WO2019/137983 describes an optimized method for increasing the yield of Low-Moisture
Mozzarella Cheese (LMMC).

Thus there is a continuous need for new strains to replace phage-hit strains.

20

SUMMARY

One aspect of the present disclosure relates to a method for producing a pasta filata type cheese
product comprising the steps of:

- a) Providing a milk base;
- 25 b) Adding one or more strains of a lactic acid bacteria of the species *Streptococcus thermophilus* to the milk base, wherein the strains are selected from DSM 34679, DSM 34680, and DSM 34684;
- c) Adding a coagulant;
- d) Scalding;
- 30 e) Draining of whey;
- f) Cheddaring;
- g) Stretching; and

h) Moulding.

A second aspect of the present disclosure relates to a composition or kit-of-parts comprising one or more strains of a lactic acid bacteria of the species *Streptococcus thermophilus* selected from
5 DSM 34679; DSM 34680; and DSM 34684.

A third aspect of the present disclosure relates to a lactic acid bacteria strain of the species *Streptococcus thermophilus* selected from DSM 34679; DSM 34680; and DSM 34684.

A fourth aspect of the present disclosure relates to use of the strain, or the composition or kit-of-parts for producing a pasta filata type cheese product.

10 A fifth aspect of the present disclosure relates to a pasta filata type cheese product made by the method or by the use.

DETAILED DESCRIPTION OF THE DISCLOSURE

Pasta filata is a technique in the manufacture of pasta filata cheese also known as stretched-
15 curd, pulled-curd, and plastic-curd cheese. The technique includes a plasticizing and kneading treatment of the fresh curd in hot water, which gives the cheeses its fibrous structure. Examples of cheeses made with this technique include but are not limited to Mozzarella, Burrata, Straciatella, Scamorza, Caciocavallo, Ragusano, Kashkaval, Provolone and Pizza cheese.

The technique is typically comprising the following steps:

- 20 a) Providing a milk that may be standardized regarding casein and/or fat
- b) Pasteurizing the milk
- c) Pre-fermentation / Hot maturation by addition of a Starter culture
- d) Renneting by addition of a Coagulant
- e) Cutting
- 25 f) Scalding / Cooking by raising the temperature typically to between 37°C to 42°C
- g) Draining of whey
- h) Cheddaring of curd
- i) Milling of curd into chips which
- j) Stretching in warm water
- 30 k) Molding into shape
- l) Brining / Salting
- m) Package and storage.

The method of producing pasta filata type cheese is a combination of milk acidification, heat, addition of coagulant/enzymes, and mechanical actions. Most productions starts from a standardized and pasteurized milk. The heat treatment prevents the presence of critical amounts of unwanted bacteria, spores and bacteriophages.

- 5 One aspect of the present disclosure relates to a method for producing a pasta filata type cheese product comprising the steps of:
- a) Providing a milk base;
 - b) Adding one or more strains of a lactic acid bacteria of the species *Streptococcus thermophilus* to the milk base, wherein the strains are selected from DSM 34679,
10 DSM 34680, and DSM 34684;
 - c) Adding a coagulant;
 - d) Scalding;
 - e) Draining of whey;
 - f) Cheddaring;
 - 15 g) Stretching; and
 - h) Moulding.

In one embodiment the disclosure relates to a method, wherein the one or more strains are added comprised in a composition or in a kit-of-parts.

- The terms "Milk" or "Milk base" as used herein means a composition comprising lacteal secretion
20 obtained from any mammal, such as an animal of a species belonging to the subfamily Bovinae (which includes the domestic cow (*Bos taurus*) and buffalo); an animal of a species belonging to the subfamily Caprinae (which includes goat and sheep); or an animal of the species Camelidae (which includes camels). Optionally the milk or milk base is acidified, e.g. by addition of an acid (such as citric, acetic or lactic acid) or by addition of an acid producing microorganism. The milk or milk base
25 may be raw or processed, e.g. by filtering, sterilizing, pasteurizing, homogenizing, fractionating (e.g. reducing the fat content of the milk) etc., or it may be reconstituted dried milk. An important example of milk or milk base according to the present disclosure is pasteurized cow's milk. It is understood that the milk may be acidified, mixed or processed before, during and/or after the adding of bacterial cultures. The milk or milk base may further comprise protein, calcium or other
30 additives added.

Starter cultures comprising one or more lactic acid bacteria (LAB) are added to acidify the milk. The acidification (decreased pH) is a result of lactic acid generated during hydrolysis/fermentation by the LAB strains of the disaccharide lactose comprised in milk into the monosaccharides glucose and galactose. The starter culture is typically added to the milk having a temperature from 32°C to 37°C,

from 33°C to 36°C, or from 34°C to 35°C. The composition of the starter culture influences the functional characteristics of the curd, such as e.g. melting and stretching, by the amount of lactic acid produced and the moisture level obtained.

The term “Starter” or “Starter culture” as used herein means a culture or composition comprising one or more lactic acid bacteria strains able to acidify a milk base according to general practice in the cheese making industry. One embodiment of the present disclosure relates to a starter culture comprising one or more LAB strains of the species *Streptococcus thermophilus* (ST). one embodiment of the present disclosure relates to a strain of the species *Streptococcus thermophilus* (ST). One embodiment of the present disclosure relates to a protease positive ST strain. One embodiment of the present disclosure relates to the starter further comprising one or more strains of the genus *Lactobacillus*. One embodiment of the present disclosure relates to a starter culture, wherein the one or more strains of the genus *Lactobacillus* is selected from *L. delbrueckii* ssp. *bulgaricus*, *L. delbrueckii* ssp. *lactis*, *L. fermentum* and *L. helveticus*.

The starter comprises a concentration of viable cells in the range of 10^4 to 10^{14} , 10^6 to 10^{13} , 10^8 to 10^{12} , or 10^{10} to 10^{11} cfu per gram of the composition.

In one embodiment the disclosure relates to a method, wherein the composition or the kit-of-parts further comprise one or more strains of the genus *Lactobacillus*, preferably *L. delbrueckii* ssp. *bulgaricus*, *L. delbrueckii* ssp. *lactis*, *L. fermentum* and/or *L. helveticus*.

Phage attacks is a continuous challenge during production of fermented milk products, in particular where the fermented milk product is cheese due to the long production time. Accordingly, there is a continuous need for phage-resistant LAB strains for producing suitable starter cultures to replace the phage-hit LAB strains and/or starter cultures.

The terms "bacteriophage" or “phage” as used herein have the conventional meaning as understood in the art, i.e., a virus that selectively infects one or more bacteria. Many bacteriophages are specific to a particular genus or species or strain of bacteria. Bacteriophages may include, but are not limited to, bacteriophages that belong to any of the following virus families: Corticoviridae, Cystoviridae, Inoviridae, Leviviridae, Microviridae, Myoviridae, Podoviridae, Siphoviridae, or Tectiviridae. The bacteriophage may be a lytic bacteriophage or a lysogenic bacteriophage. A lytic bacteriophage is one that follows the lytic pathway through completion of the lytic cycle, rather than entering the lysogenic pathway. A lytic bacteriophage undergoes viral replication leading to lysis of the cell membrane, destruction of the cell, and release of progeny bacteriophage particles capable of infecting other cells. A lysogenic bacteriophage is one capable of entering the lysogenic pathway, in which the bacteriophage becomes a dormant, passive part of the cell’s genome through prior to completion of its lytic cycle.

The terms "phage-resistant strain" and "phage robust strain" as used herein refer to a bacterial strain which has a defense mechanism against phages. One embodiment of the present disclosure relates to a lactic acid bacterium strain, wherein the strain is resistant to one or more bacteriophage or one or more sets of bacteriophages. One embodiment of the present disclosure relates to a LAB
5 strain with phage-resistant properties. In particular, the phage-resistant LAB strain is resistant towards the phages that are attacking the phage-hit strains, i.e. towards which the phage-hit strains are sensitive. One embodiment of the present disclosure relates to a phage-resistant strain. One embodiment of the present disclosure relates to a phage-resistant strain of the species *Streptococcus thermophilus*. One aspect of the present disclosure relates to a lactic acid bacteria
10 strain of the species *Streptococcus thermophilus* selected from DSM 34679; DSM 34680; and DSM 34684.

The strains may be comprised in a composition or a kit-of-parts. One aspect of the present disclosure relates to a composition or a kit-of-parts comprising one or more strains of a lactic acid bacteria of the species *Streptococcus thermophilus* selected from DSM 34679; DSM 34680; and
15 DSM 34684. The composition or kit-of parts may comprise additional strains. In particular strains of the genus *Lactobacillus*. Preferably the one or more strains may be selected from *Lactobacillus delbrueckii* ssp. *bulgaricus*, *Lactobacillus delbrueckii* ssp. *lactis*, *Lactobacillus fermentum* and/or *Lactobacillus helveticus*. One embodiment of the present disclosure relates to a composition or kit-of-parts, further comprising one or more strains of the genus *Lactobacillus*, preferably *L. delbrueckii*
20 ssp. *bulgaricus*, *L. delbrueckii* ssp. *lactis*, *L. fermentum* and/or *L. helveticus*.

Rennet is typically added at a temperature from 34°C to 38°C in order to coagulate milk proteins. It may be of animal or vegetable origin and can be liquid, tablet, paste or in powder format. A proteolytic enzyme (protease) comprised in rennet coagulates the milk, causing it to separate into solids and whey. The main enzymatic action comes from chymosin but also pepsin and lipase affect
25 the final result in terms of texture and flavor. Vegetable rennet may originate from the thistle family of plants or from a mold (*Mucor Miehei*).

The term "Coagulant" as used herein means any agent or compound which when added to a milk base results in curd formation by coagulation of casein. The coagulant may be a natural coagulant such as animal rennet such as e.g. calves rennet. It may be of bovine or porcine origin. It may also
30 be a microbial coagulant derived from species such as e.g. *Rhizomucor miehei*. The active enzyme is called chymosin. The chymosin may be of Camelidae or bovine origin. Examples of commercially available coagulants are e.g. Naturen®, Microlant®, Hannilase® and CHY-MAX® (all from Chr. Hansen A/S). One embodiment of the present disclosure relates to a method, wherein the coagulant is a rennet or a microbial chymosin, preferably a camel chymosin.

After coagulation the curd is cut to promote whey separation. In one embodiment of the present disclosure the curd is cut into cubes about 1cm^3 . Curd particles begin shrinking and separating from whey in response to stirring, scalding and acidification. Scalding is a heating step where the temperature is gradually increased to a temperature from 37°C to 43°C . In one embodiment of the present disclosure the scalding temperature is gradually increased to a temperature in the range from 39°C to 43°C , from 41°C to 42°C , or about 41.5°C . After scalding the cut curds may be kept at a temperature in the range from 30°C to 38°C for up to 8 hours for further acidification and release of whey.

Whey viscosity is impacting the whey off i.e. the whey draining step. If the whey is too viscous it is difficult to whey off. Therefore, it is desirable to include strains providing low or no viscosity. The viscosity of fermented milk made with lactic acid bacteria may be measured by TADM (as described in the examples) which provides an indication of the whey viscosity as viscous/texturizing samples would typically have a viscous whey. Strains providing a fermented milk with a TADM value of 1×10^6 or more is considered to be viscous/texturizing. One embodiment of the present disclosure relates to strains providing a fermented milk with a TADM value below 1×10^6 .

After the whey off cheddaring of curd takes place for further acidifying the curd and draining of more whey. It is conducted by cutting the curd into blocks, some of which are optionally piled on top of each other, which are turned several times. Once a target pH has been reached the curd is milled. The target pH of the curd is from pH 5.0 to pH 5.4. In one embodiment of the present disclosure the target pH is pH 5.0, pH 5.1, pH 5.2, pH 5.3, or pH 5.4.

Curds acidified to the target pH are milled and cut into small pieces which are optionally salted before stirring with hot water at a temperature in the range from 55°C to 70°C . An embodiment of the present disclosure the temperature of the water is in the range from 60°C to 70°C , from 65°C to 70°C , or about 66°C , 67°C , 68°C , or 69°C . In one embodiment of the present invention the milled curd is salted in 2% NaCl. The salting may be dry salting. The melted and fluid curd is stretched, shaped, and optionally plunged into cool water. Stretching also called kneading is a result of rearranged casein molecules which depends on the loss of calcium phosphate from casein. The soft curd mass is kneaded into balls or blocks, cooled and placed in brine for a short time. In the case of mozzarella or fior di latte, the process is now essentially complete. In some cases further processing steps such as drying, ageing, longer brining, and/or smoking may follow.

One aspect of the present disclosure relates to use of the strain, or the composition or kit-of-parts for producing a pasta filata type cheese product.

One aspect of the present disclosure relates to a pasta filata type cheese product made by the method or by the use. The cheese product may be fresh cheese such as Mozzarella, Halloumi,

Burrata, Straciatelle, Scamorza, and Pizza cheese; low moisture cheese such as low-moisture mozzarella; aged cheese such as Provolone, Ragusano and Caciocavallo, and/or smoked cheese such as Provolone or Scamorza affumicata. There are many different types of pasta filata cheeses each characterized by the specific method used in the region of origin. One embodiment of the present disclosure relates to a product, wherein the cheese is Mozzarella, Burrata, Straciatella, Scamorza, Caciocavallo, Ragusano, Kashkaval, Provolone or Pizza cheese.

One embodiment of the present disclosure relates to a product, wherein the product is a low-moisture cheese. Low-moisture is defined as a moisture content measured in w/w% below 52%, below 51%, below 50%, or in the range of 43-51%, 44-50%, 45-49%, 46-48%, or about 45%, about 46%, about 47%, about 48%, or about 49%.

DEPOSIT AND EXPERT SOLUTION

The applicant requests that a sample of the deposited microorganisms stated below may only be made available to an expert, subject to available provisions governed by Industrial Property Offices of States Party to the Budapest Treaty, until the date on which the patent is granted.

Table 1: The applicant has made the following deposits at a Depository institution having acquired the status of international depository authority under the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure: *Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures* Inhoffenstr. 7B, 38124 Braunschweig, Germany.

Strain	Accession No.	Deposit date
<i>Streptococcus thermophilus</i>	DSM 34679	2023.06.28
<i>Streptococcus thermophilus</i>	DSM 34680	2023.06.28
<i>Streptococcus thermophilus</i>	DSM 34684	2023.06.28

EXAMPLES

EXAMPLE 1 – ACIDIFICATION PROFILE OF SINGLE STRAINS AND COMPOSITIONS

Single strains were prepared as follows. Working ampoules were made from frozen glycerol stocks by inoculating each single strain in M17 + 0,5% or 2% lactose and incubating overnight (o/n) at 37°C. The cell material was spun down and resuspended in a sterile milk substrate (reconstituting low fat skim milk powder to a dry matter level of 9.5%, autoclaved at 114°C for 15 min.) with 20% glycerol v/v and stored at -80°C.

Pre-cultures were prepared by inoculating 100mL pre-warmed pasteurized low-fat milk (past-LFM) to 1% v/v with thawed ampoule material and incubated at 38°C for 3,5 hours. The pre-cultures were used to inoculate past-LFM in 200mL bottles at 1% v/v. The bottles were incubated at 38°C o/n and pH was monitored every 4 minutes using the Cinac system (Scientific Solutions).

5 CFU (colony forming units) counts were conducted on the pre-cultures using the automated RAPID CFU method, and the counts were used to normalize the results.

The lag phase (Ta) was determined as time in minutes from initial pH at the start of fermentation until a pH drop of 0.08 unit was achieved.

10 All the new strains were faster at reaching target pH 5.15 as compared to the *Streptococcus thermophilus* reference strain ST-6 comprised in a variant of Blend R. The fast acidification time was obtained by both a shorter lag phase as well as a shorter acidification time.

Table 2 – Acidification profile of single strains.

STRAIN	Ta (min)	Time to pH 5.50 (min)	Time to pH 5.15 (min)
ST-6	152	272	308
DSM 34679	128	236	272
ST-6	152	272	304
DSM 34680	136	256	292
ST-6	152	260	296
DSM 34684	104	236	284

15 Blends comprising the single strains were prepared from single strains grown at the Chr. Hansen A/S pilot plant, harvested and formulated as frozen culture material. The content of strains in the two experimental blends Blend A and Blend B is shown in the table below. A commercially available starter culture for pasta Filata, STI-06 (Chr. Hansen A/S), was used as reference (Blend R). The reference comprises none of the strains comprised in the experimental blends.

Table 3 – Content of strains in the Blends

STRAIN	Blend A	Blend B
ST-A	+	-
DSM 34679	+	+
DSM 34680	+	+
DSM 34684	+	+
ST-B	-	+

The blends were inoculated at 0,01% w/w in 200mL pre-warmed past-LFM at 34°C. From t=100min to t=140min, the milk was gradually heated up to 41°C was measured every 4 minutes in a Cinac, the experiments ran o/n.

Table 4 – Acidification profile of blends.

BLEND	Ta (min)	Time to pH 5.50 (min)	Time to pH 5.15 (min)
TEST 1			
Blend R	87	189	216
Blend A	79	194	209
TEST 2			
Blend R	96	194	228
Blend A	84	191	219
TEST 3			
Blend R	87	192	224
Blend B	85	196	224

5

EXAMPLE 2 – PHAGE PROFILE OF STRAINS

The phage profiles of the new strains DSM 34679; DSM 34680; and DSM 34684 were investigated using Chr. Hansen’s *Streptococcus thermophilus* phage collection composed out of phages with different host ranges. The spot test for phage profiling was conducted as follows: 0.1mL of the log phase of the culture was mixed with 3mL M17 molten soft agar (0.5% w/v) and plated as a thin top layer on M17-Ca/Mg (10mM CaCl₂, 10mM MgCl₂) agar (1% w/v) plates. After solidification aliquots of 10µL of 10-fold dilutions of phage lysate were spotted on the plates. After incubation at the conditions of growth, the presence of single plaques in a certain dilution was recorded and the original phage titer calculated based on this.

10

The new strains were phage negative (Neg) i.e. phage resistant whereas the reference strains were phage positive (Pos) i.e. phage sensitive as can be seen in the table below. The new strains were also phage negative when tested against 45 different additional phages (data not shown). The reference strains ST-1 to ST-5 were all comprised in Blend R and ST-3 to ST-6 were comprised in a variant of Blend R.

15

Table 5 – Phage profile of new strains (DSM) and reference strains (ST).

Phage	DSM 34679	DSM 34680	DSM 34684	ST-1	ST-2	ST-3	ST-4	ST-5	ST-6
Ph-1	Neg	Neg	Neg	Pos	Neg	Neg	Neg	Neg	Neg
Ph-2	Neg	Neg	Neg	Neg	Neg	Neg	Pos	Neg	Neg
Ph-3	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Pos

Ph-4	Neg	Neg	Neg	Neg	Neg	Pos	Neg	Neg	Neg
Ph-5	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Pos	Neg
Ph-6	Neg	Neg	Neg	Neg	Pos	Neg	Neg	Neg	Neg

EXAMPLE 3 – TEXTURIZING CAPACITY OF STRAINS

Heat-treated skimmed milk was inoculated (1% v/v inoculum) with strains pre-grown in M17-K medium with 2% w/v lactose at 37°C overnight (o/n). The inoculated milk plates were incubated o/n at 40°C and cooled down to 4°C before analysis. The texture assessment of the fermented milk samples was performed using Total Aspiration Dispense Monitoring (TADM) in 2mL microtiter plates as described in Poulsen et al., *FEMS Microbiology Letters*, Volume 369, Issue 1, 2022, fnac117 (<https://doi.org/10.1093/femsle/fnac117>). Strains resulting in fermented milk with TADM values under 1 million Pa × ms are considered non-texturing strains. Two high texturing benchmark strains ST-7 and ST-8 providing broth viscosity were included as references.

The table shows a TADM value below 1×10^6 of the new strains and are thus not considered to be viscous/texturizing.

Table 6 – Texturizing capacity of single strains

Strain	TADM area, Pa * ms
ST-7	1647913
ST-8	1384513
DSM 34679	935962
DSM 34680	816435
DSM 34684	976150

EXAMPLE 4 – PRODUCTION OF PASTA FILATA

Pasta filata cheese was produced in 150L Cheese Vats according to the method outlined in the table below. Milk contained 3.8% protein adjusted with UF retentate powder, and 2.8% fat. Two blends comprising the new strains Blend A and Blend B were tested as starter cultures for fermentation in two separate experiments each including F-DVS® STI-06 (Catalogue no. 704889 Chr. Hansen A/S) as reference (Blend R). In Experiment 1 Blend A and Blend R were inoculation in an amount of 0.013%, and in Experiment 2 Blend B and Blend R were inoculated in an amount of 0.008%.

Chy-Max M (Catalogue no. 127405, Chr. Hansen A/S) was used as coagulant for renneting according to providers recommendation.

Table 7 – Method for making cheese.

Step	Process	Minutes
1	Pre-fermentation of milk with a starter culture at 34.5°C	60

2	Renneting with a coagulant	28
3	Cutting in to squares of 1 cm ³ .	5
4	Pre-stirring	10
5	Scalding by gradually increasing the temperature to 41.5°C.	30
6	Final stirring	20
7	Whey off	20
8	Cutting the curd into blocks and 1 st . turn of curd	10
9	2 nd turn	10
10	3 rd turn	10
11	Milling of curd into chips at pH 5.10	5
12	Dry salting of chips by addition of 2% NaCl	5
13	Stretching of curd in 68°C warm water	18
14	Cooling in 10°C water	20
15	Salt brining 22°Bé (Baumé), pH 5.2	90
16	Drying at 10°C	30
17	Packaging	10

Acidification time was determined by measuring the time in minutes from start of fermentation to target pH 5.10.

Cheese yield was determined as the amount in kg of cheese produced from 100 kg milk.

- 5 Cheese moisture content was measured in % moisture of total mass by a FoodScan unit (Foss Analytics).

Shredability was evaluated after 1 week by shredding a cold (5°C) block of cheese. In a mechanical sieve, the obtained shreds are divided into fractions depending on the size. Typically, fractions comprise shreds of different lengths, the smallest fraction being “fines”, which have the least desirable size. Passing through a sieve having a mesh size of 2,36 mm. The final result of the Shredability test is expressed as the amount of “fines” as a percentage of the total weight of shredded cheese tested, the lower the result, the better.

10

Browning was measured after 3 or 5 weeks (Blend A and Blend B respectively) by baking a standard industrial pizza on a crust with tomato sauce in a conveyor oven. The melted cheese on the pizza crust is then visually inspected and analyzed by image analysis based on a greyscale conversion of photos of the pizzas. The median value of the image analysis data will give an indication of the overall browning of the cheese on the pizza. A high score indicates low browning.

15

Meltability was measured after 3 or 5 weeks (Blend A and Blend B respectively) according to the Schreiber test as described in Kosikowski, F. 1977. Pages 337–340 in Cheese and Fermented Milk

Foods. F. V. Kosikowski & Assoc., Brooktondale, NY. The value for each blend in the table below is a mean of 8 measurements of 6 cheeses. The higher the meltability the higher the index value.

Table 8 – Results of production and storage of pasta filata.

	EXPERIMENT 1		EXPERIMENT 2	
	Blend R	Blend A	Blend R	Blend B
Time to pH 5.10 [min]	198	195	201	201
Cheese Yield [kg]	11.59	11.45	11.56	11.63
Cheese moisture [%]	46.70	46.36	47.14	47.04
Shredability [%fines]	-	-	2.95	4.17
Browning color index	143	139	139	136
Meltability index	4.83	4.55	4.05	4.04

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The present invention has been described with reference to various embodiments, aspects, examples, or the like. It is not intended that these elements be read in isolation from one another. Thus, the present disclosure provides for the combination of two or more of the embodiments, aspects, examples, or the like.

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All embodiments described herein are intended to be within the scope of the invention disclosed. These and other embodiments of the present invention will become readily apparent to those skilled in the art from the following detailed description of the preferred embodiments having reference to the whole description, the invention not being limited to any particular preferred embodiment(s) disclosed.

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CLAIMS

1. A method for producing a pasta filata type cheese product comprising the steps of:
 - a) Providing a milk base;
 - b) Adding one or more strains of a lactic acid bacteria of the species *Streptococcus thermophilus* to the milk base, wherein the strains are selected from DSM 34679,
5 DSM 34680, and DSM 34684;
 - c) Adding a coagulant;
 - d) Scalding;
 - e) Draining of whey;
 - 10 f) Cheddaring;
 - g) Stretching; and
 - h) Moulding.
2. The method according to claim 1, wherein the one or more strains are added comprised in a composition or as a kit-of-parts.
- 15 3. The method according to claim 2, wherein the composition or the kit-of-parts further comprise one or more strains of the genus *Lactobacillus*, preferably *L. delbrueckii* ssp. *bulgaricus*, *L. delbrueckii* ssp. *lactis*, *L. fermentum* and/or *L. helveticus*.
4. The method according to any one of claims 1-3, wherein the coagulant is a rennet or a microbial chymosin, preferably a camel chymosin.
- 20 5. A composition or kit-of-parts comprising one or more strains of a lactic acid bacteria of the species *Streptococcus thermophilus* selected from DSM 34679; DSM 34680; and DSM 34684.
6. The composition or kit-of-parts according to claim 5, further comprising one or more strains of the genus *Lactobacillus*, preferably *L. delbrueckii* ssp. *bulgaricus*, *L. delbrueckii* ssp. *lactis*,
25 *L. fermentum* and/or *L. helveticus*.
7. A lactic acid bacteria strain of the species *Streptococcus thermophilus* selected from DSM 34679; DSM 34680; and DSM 34684.
8. Use of the strain of claim 7 or the composition or kit-of-parts of claims 5-6 for producing a pasta filata type cheese product.
- 30 9. A pasta filata type cheese product made by the method according to any one of claims 1-4 or by the use according to claim 8.
10. The product according to claim 9, wherein the cheese is Mozzarella, Burrata, Straciatella, Scamorza, Caciocavallo, Ragusano, Kashkaval, Provolone or Pizza cheese.

11. The product according to any one of claims 9-10, wherein the product is a low-moisture cheese.

INTERNATIONAL SEARCH REPORT

International application No PCT/EP2024/073295

A. CLASSIFICATION OF SUBJECT MATTER
 INV. A23C19/032 A23C19/068
 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 data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2021/076696 A1 (ROUSTEL SEBASTIEN [DK] ET AL) 18 March 2021 (2021-03-18) claims 1,2,5,6 -----	1 - 11
X	US 2017/135360 A1 (GARRIGUES CHRISTEL [DK] ET AL) 18 May 2017 (2017-05-18) paragraphs [0284] - [0296], [0300] -----	1 - 3, 5 - 9
X	US 2015/201634 A1 (FREMAUX CHRISTOPHE [FR] ET AL) 23 July 2015 (2015-07-23) paragraph [0261]; claims 1,43 -----	7
	- / - -	

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

* Special categories of cited documents :

<p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier application or patent but published on or after the international filing date</p> <p>"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)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p>	<p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&" document member of the same patent family</p>
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Date of the actual completion of the international search	Date of mailing of the international search report
6 November 2024	21/11/2024

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

International application No

PCT/EP2024/073295

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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