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(54) Title: FLAVOURING MIXTURE FOR PRODUCING MOZZARELLA

(57) Abstract: The present invention relates to a flavouring mixture comprising liquid, partially fermented milk, obtained through the development of an association of lactic ferments and yeasts until a pH comprised between 5.7 and 6.5 is reached, and its use in the industrial production of mozzarella. This flavouring mixture is able to give mozzarella a stronger taste, typical of artisanally produced mozzarella.



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Title: "Flavouring mixture for producing mozzarella."

DESCRIPTION

FIELD OF THE INVENTION

5 The present invention relates to a flavouring mixture for the production of mozzarella, the process of production of said mozzarella using such flavouring mixture.

STATE OF THE ART

10 Currently, the range of dairy products offers a wide variety of uses in the culinary field. Among the most used products, an important slice of the market is represented by mozzarella that can be consumed as such, or in hot dishes (for example pizza, pasta) or even in
15 take-away food products such as, for example, sandwiches, small pizzas, piadinas, etc. As known, mozzarella is prepared through an industrial process comprising the following steps.

20 A starter culture (starter), that is lactic ferments with acidifying activity, developed in milk or whey, or in the form of freeze-dried or frozen concentrated ferments, is added to pasteurized milk. Calf or microbial rennet having a titre of 1:10,000 is added to this mixture in an amount of about 25-30 ml/hl of total

pasteurized milk, at a temperature between 32 and 36°C so that coagulation is achieved in 20-30 min.

The obtained curd then undergoes a series of mechanical operations so as to promote separation of the whey, that is, a first cut into cubes of considerable size, in the order of several cm³, followed by about 10-15 min rest, then a second cut to reach a markedly smaller size (of a walnut). The next manufacturing steps, instead, include extraction of a part of the whey and subsequent ripening of the curd under whey, according to the traditional method, for a time comprised between 3 and 6 hours. Alternatively, fermentation can also occur in the absence of whey, as long as the maintenance of suitable temperature conditions is guaranteed.

The latter step can be substituted by transfer of the curd, which at first has a pH around 6, on drainage tables, followed by cutting and revolving the curd blocks, an operation that is carried out at room temperature for 2-6 hours, until a final pH around 5.2-5.4 is obtained.

The following phases include stretching with hot water, optionally supplemented with table salt, until an even mass is obtained, at a temperature between 58 and

82°C, and shaping the mass into different shapes and sizes (globes, provolas, braids, etc), cooling in cold water, followed by optional salting in brine containing a salt concentration of 12-14% by weight, and eventually by
5 packaging in storage liquid.

Such an industrial process guarantees a product suitable to be consumed as a fresh cheese or ready to be cooked, having good and consistent quality, then able to satisfy most consumers, while guaranteeing the highest
10 hygienic and sanitary level. However, the average consumer would be even more satisfied and would be more incline to fidelize to the brand of the producer if this product displayed peculiar sensorial qualities able to make it as close as possible to an artisanal type
15 product, which is generally characterized by typical and sometimes unmistakable taste and flavour. As the average consumer is generally more attracted by a mozzarella with a more prominent, natural and authentic taste, he/she will consequently tend to buy the product that satisfies
20 him/her the most again.

In the dairy field, yeasts are generally recognized as undesirable contaminants and, as such, they fall in the category of the so-called anti-dairy microorganisms.

Exception is made for some dairy processings, wherein the employment of yeasts is intended and pursued. In this case, yeasts are generally allowed to develop on the rind, being their proteolytic and deacidifying action
5 desired there. In very particular cases, and specifically in the production of blue-veined cheeses, such as gorgonzola cheese, the action of yeasts is desired inside the cheese. The production of gas due to alcoholic fermentation of sugars present in the cheese, allows
10 cavities to form where, following piercing with needles, typical moulds (*Penicillium roqueforti*) able to give the final product the characteristic colour, taste and flavour can develop.

For similar purposes, Eliskases-Lechner et al in:
15 "Tilsiter ohne Schmiere" DMZ. Lebensmittelindustrie und Milkwissenschaft Volks" vol. 115, no. 4, February 17, 1994, p. 174-177 disclose a process wherein yeast is used to deacidify the rind of Tilsiter and then to promote the development of *Corynebacteria*.

20 Aponte M. et al in "Short Communication: identification and technological buffalo Mozzarella Cheese" Journal of Dairy Science, American Dairy Science Association vol. 93, no. 6, June 1, 2010, p. 2358-2361

and Romano in "Yeasts from Water Buffalo Mozzarella Cheese a traditional cheese of the Mediterranean area" International Journal of Food Microbiology, September 19, 2001 p. 45-51 report the results of microbiological
5 investigations carried out on buffalo mozzarella, which emphasize the massive presence of yeasts in the final product as contaminants, and their respective characterization.

In fact, it is known that yeasts represent normal
10 contaminants of the natural whey culture not only of mozzarella, but also of other cheeses such as grana, provolone etc. and, more generally, in the dairy environment. In the case of buffalo mozzarella, brines are likely to be the origin of the massive presence of
15 yeasts in the final product. The authors of said scientific publications believe that the amount and type of yeasts may have an impact on the organoleptic characteristics of the final product.

As demonstrated in example 9, included in the present
20 description, what said authors supposed is not confirmed. In fact, this example emphasizes that flavouring additives based on milk, partially fermented only with yeasts, do not allow to obtain the intended results in

terms both of sapidity and acidity, even if the yeasts contained therein are present at concentrations four-fold higher than standard concentrations.

On the other hand, the acid alcoholic fermentation
5 performed by lactic bacteria in association with yeasts is well known, as it is also reported in the following documents for preparing diet supplements or food based on completely fermented milk.

WO00/60950 discloses diet supplements obtained from
10 completely dried kefir, that is completely fermented milk, in association with yeasts and lactic ferments, also supplemented with mineral salts.

WO2007055604 discloses a dairy product consisting of completely fermented longer duration milk obtained by
15 adding non-fermenting yeasts or non-galactose or non-lactose fermenting yeasts, added simultaneously with lactic acid bacteria.

Eventually, EP346884 discloses completely fermented kefir-like milk produced from a starting material
20 containing lactose and at least one sugar, submitting the lactose to fermentation by lactic bacteria and the sugar to alcoholic fermentation by a non-galactose or non-

lactose fermenting yeast, until complete removal of both sugars.

SUMMARY OF THE INVENTION

The Applicant has now found that it is possible to
5 give an industrially produced mozzarella a stronger and
more "authentic" taste, which then gets closer to that of
an artisanal-type mozzarella, by adding to pasteurized
milk, before the enzymatic coagulation, a flavouring
mixture, previously obtained through the development of
10 an association of lactic ferments and yeasts in milk.

An object of the present invention is represented by
what is claimed in the claims attached to the present
application.

In particular, the present invention relates to the
15 preparation of partially fermented milk in a liquid form,
obtained by adding an association of lactic ferments and
yeasts thereto, and allowing them to develop for a time
comprised between 6 and 18 hours, until pH values
comprised between 5.70 and 6.50 are reached.

20 A further object of the present invention relates to
the use of said partially fermented milk in a liquid
form, as a flavouring mixture in the process of
industrial production of mozzarella.

In particular, a further object of the present invention is the use of such a flavouring mixture in an industrial process for preparing mozzarella, comprising adding said flavouring mixture to pasteurized milk
5 destined to the production of mozzarella, at a total concentration comprised between 1 and 3%, as calculated on the total volume of said pasteurized milk.

In fact, the Applicant has surprisingly found that, by properly selecting the type of both lactic ferments
10 and yeasts, and properly dosing the respective amounts of these microorganisms, it is possible to obtain flavouring mixtures able to give mozzarella different tastes, that can be associated to those of artisanal-type mozzarella originating from different regions. For example, it is
15 possible to obtain mozzarella with a more pronounced taste, which can be associated with the taste of mozzarella produced in the Agro Pontino, or it is possible to prepare mozzarella with a milder and milkier taste, more similar to that of mozzarella from Apulia.

20 DETAILED DESCRIPTION OF THE INVENTION

For the purposes of the present invention, partially fermented milk means pasteurized milk that has undergone partial acidification until a pH comprised between 5.70

and 6.50, preferably comprised between 5.80 and 6.10, even more preferably between 6.00 and 6.10 was reached, and that consequently retains its own liquid state.

Hence, this type of milk is different from completely
5 fermented milk that is in an essentially solid or semi-liquid form displaying pH values lower than 5.0.

Lactic ferments present in the partially fermented milk object of the present invention, for use as a flavouring mixture in the preparation of mozzarella
10 according to the present invention, are preferably lactococci, and even more preferably are selected from *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *diacetylactis*, *Lactococcus lactis* subsp. *cremoris* and *Leuconostoc* spp. and combinations thereof.

15 Another class of lactic ferments preferably present in the flavouring mixture according to the present invention is formed by mesophilic lactobacilli and more preferably are selected from *Lactobacillus plantarum*, *Lactobacillus casei*, *Lactobacillus rhamnosus*,
20 *Lactobacillus pentosus*, *Lactobacillus paracasei* and combinations thereof.

Yeasts present in the mixture, instead, are generally selected based on their characteristics related to

aminopeptidasic activity, alcoholic fermentation of sugars and ability to neutralize curd, and are preferably selected in the class formed by: *Kluyveromyces marxianus* subsp. *marxianus*, *Debaryomyces hansenii*, *Candida*
5 *colliculosa*, *Kluyveromyces lactis*, *Saccharomyces cerevisiae* and combinations thereof.

The amount of lactic ferments to be used for preparing the flavouring mixture according to the present invention considerably ranges depending on the lactic
10 ferments employed, and is generally comprised between 5 billion and 100 billion cfu/50 l of pasteurized milk. The amount of yeasts also considerably ranges, depending on the types of yeast employed and is generally comprised between 20 millions and 3.2 billion cfu/50 l of milk.

15 Preferably, an association of two or three species of lactobacilli or lactococci is employed.

Even more preferably, in this association, when an association of two lactobacilli or lactococci is employed, the ratio of the cell population of one species
20 of lactobacilli or lactococci to the second species of lactobacilli or lactococci is comprised between 1:10 and 10:1. Even more preferably, it is comprised between 1:3 and 3:1. When an association of 3 lactococci is employed,

the ratio of the cell populations of the respective species is preferably comprised between 1:2:3 and 3:2:1.

Preferably, yeasts are also employed in preparing the flavouring mixture according to the present invention. In
5 this case, when only two species of yeasts are employed, the ratio between their cell populations is preferably comprised between 10:1 and 1:10, preferably between 3:1 and 1:3. When an association of three yeasts is present, the ratio between the respective cell populations is
10 preferably comprised between: 1:2:3 and 3:2:1.

According to a preferred embodiment, in the phase of preparation of the partially fermented milk or flavouring mixture object of the present invention, lactic ferments and yeasts are added to pasteurized milk in the form of
15 ready-to-use formulations, even more preferably in the form of a single formulation or single-dose bag.

The flavouring mixture according to the present invention is preferably developed at a temperature between 22 and 30°C, even more preferably between 24 and
20 26°C.

The time of development is preferably comprised between 16 and 18 hours, when lactic ferments are mesophilic lactobacilli.

For the purposes of the present invention, an "industrial process for the production of mozzarella" means a process carried out in the dairy industry, aimed at producing considerable amounts of such product, and
5 able to guarantee the strictest and most severe hygienic and sanitary standards.

The industrial process for preparing mozzarella with a flavour similar to that of artisanal one, employing the flavouring mixture according to the present invention,
10 can include that the formation of curd is carried out by adding acidifying lactic ferments or starter such as, for example, *Streptococcus thermophilus*, that is, forming the so-called lactic curd, or by adding citric acid and rennet and giving place to the formation of the so-called
15 chemical-enzymatic curd.

Even if the employment of the flavouring mixture is preferred in the process of preparation by lactic fermentation, the employment of such flavouring mixture in the process that includes the formation of chemical-
20 enzymatic curd, rather than obtaining mozzarella with a taste more similar to the artisanal type, advantageously gives it a taste similar to that of industrial mozzarella obtained with lactic curd.

In particular, the industrial process for the production of mozzarella including the formation of lactic curd and employing the flavouring mixture according to the present invention comprises the following steps:

a) adding the flavouring mixture to pasteurized milk destined to the production of mozzarella, said milk being optionally standardized for its own fat/protein ratio according to the good dairy practice well known to the experts in the field, and having an acidifying lactic ferment or starter previously or simultaneously added thereto;

b) enzymatic coagulation of the milk by adding calf or microbial rennet;

c) cutting the thus obtained curd, stirring, partial or total drainage of whey and separation of the curd;

d) ripening of the curd by fermentative acidification up to the desired pH, generally comprised between 5.2 and 5.4;

e) cutting the mature curd up;

f) stretching it in hot water or also under steam;

g) shaping the thus obtained stretched curd into mozzarellas of specific shape;

h) cooling the mozzarellas;
i) optionally salting them by dipping into brine;
j) packaging, generally but not necessarily, in storage liquid.

5 Preferably, in step (a) of said process, the flavouring mixture is added in amounts preferably comprised between 1.2 and 1.6% by weight on the total volume of pasteurized milk destined to the production of mozzarella.

10 Concerning the following steps (c)-(h) of said process, they are carried out following well-established and conventional-type operating conditions, such as for example those previously described in the "State of the art" of the present description.

15 In case the industrial process for the production of mozzarella included the step of formation of the curd taking place in a chemical-enzymatic way, it particularly comprises the following steps:

a') adding the flavouring mixture to pasteurized milk
20 destined to the production of mozzarella, said milk being optionally standardized for its own fat/protein ratio according to the good dairy practice well known to the experts in the field, and having citric acid and calf or

microbial rennet previously or simultaneously added thereto;

b') enzymatic coagulation of the milk by adding calf or microbial rennet;

5 c') cutting the thus obtained curd, stirring, total drainage of whey and separation of the curd;

d') cutting the curd up and stretching it in hot water or also under steam;

e') shaping the thus obtained stretched curd into
10 mozzarellas of specific shape;

f') cooling the mozzarellas;

g') optionally salting them by dipping in brine;

h') packaging, generally but not necessarily, in storage liquid.

15 Microbiological analyses performed on freshly prepared mozzarella (time $t=0$) employing the flavouring mixture of the invention, and on its expiry date (end of shelf life) have highlighted a presence of yeasts below 100 cfu/g of mozzarella.

20 These values are fully within the limits dictated by the regulation in force for the marketing of such products and are well far from the concentrations of yeasts present in artisanally produced buffalo

mozzarella, and analyzed in the above cited papers by Aponte and Romano, from which it emerges that buffalo mozzarella can be contaminated by such yeasts, not completely removed in the production phase, and that
5 therefore can remain in the finished marketed product.

The following examples of preparation of mozzarella and respective evaluation tests for flavour and taste are reported for illustrative but not limiting purposes.

Example 1 - Mozzarella 1:

10 1.1 Flavouring mixture preparation

Powder formulation A formed by:

Mesophilic lactococci: *Lactococcus lactis* subsp. *lactis* (GLL23; GLL17), *Lactococcus lactis* subsp. *cremoris* (GLC16), total concentration 20×10^9 cfu/50 l of milk.

15 Yeasts: *Kluyveromyces lactis* (GKL04) 2×10^6 cfu/50 litres of milk, *Debaryomyces hansenii* (GDH 02) 4×10^6 cfu/50 l of milk.

The formulation is dissolved in 50 l of pasteurized whole milk and allowed to develop for 4 h at 30°C until
20 pH=6.00-6.10 is reached.

1.2 Process for the preparation of mozzarella and evaluation of its properties

In step (a) of the process of the invention the flavouring mixture obtained as described above was added in an amount of 1.6% by weight on the total volume of pasteurized milk to be processed.

5 Table 1 reports organoleptic characteristics of the product (look, structure, taste) the day after manufacturing (D+1), as evaluated by attributes by a panel of 10 judges according to a 6-score scale ranging from 0 to 5 where:

10 0=unacceptable-very bad; 1=unacceptable-poor;
2=acceptable-mediocre; 3=acceptable-good; 4=very good;
5=extremely good.

TABLE 1 - PROPERTIES OF MOZZARELLA 1

Time of tasting	Look	Structure	Taste	Overall evaluation
D + 1	4	4	3	3

Example 2 - Mozzarella 2:

15 2.1. Flavouring mixture preparation

Powder formulation B formed by:

Mesophilic lactobacilli: *Lactobacillus paracasei*
subsp. *paracasei* (GP02) and *Lactobacillus plantarum*
(GP01), present in a 1:1 ratio for a total 100×10^9
20 cfu/50 l of milk.

Yeasts: *Kluyveromyces lactis* (GKL04) 2×10^6 cfu/50 l of milk, *Debaryomyces hansenii* (GDH02) 2×10^6 cfu/50 l of milk.

Formulation B is added to 50 l of whole milk and
5 allowed to develop for 16-18 h at 24-26°C until pH=5.9-6.10 is reached.

2.2 Process for preparing mozzarella and evaluation of the properties of mozzarella

In step (a) of the process of the invention the
10 flavouring mixture was added in an amount of 1% by weight on the total weight of pasteurized milk to be processed.

In the following table 2 organoleptic characteristics of the product (look, structure, taste) are reported, as evaluated by the same panel of judges of the previous
15 example, the day after its manufacturing and scored according to the attribute scale, which interpretation is reported in example 1.

Table 2 - PROPERTIES OF MOZZARELLA 2

Time of tasting	Look	Structure	Taste	Overall evaluation
D + 1	4	4	4	4

In this case, a mozzarella, which characteristic
20 flavour is judged as having very good intensity is obtained.

Example 3 - Mozzarella 3:**3.1. Flavouring mixture preparation**

Powder formulation C formed by:

Mesophilic lactococci: *Lactococcus lactis* subsp.

5 *lactis* (GLL23; GLL17); *Lactococcus lactis* subsp. *cremoris*
(GLC16); *Lactococcus lactis* subsp. *diacetylactis* (GLD13)
20 x 10⁹ cfu/50 l of milk.

Yeasts: *Candida colliculosa* (GCC05) 0.4 x 10⁸ cfu/50
1 of milk, *Kluyveromyces marxianus* subsp. *marxianus*
10 (GKM03) 1.4 x 10⁹ cfu/50 l of milk, *Debaryomyces hansenii*
(GDH01) 1.6 x 10⁹ cfu/50 l of milk.

Formulation C is added to 50 l of whole milk and
allowed to develop for 6 h at 24-25°C until pH=6.00-6.10
is reached.

15 **3.2 Process for the preparation of mozzarella and
evaluation of its properties**

In step (a) of the process of the invention the
flavouring mixture was added in an amount of 1% by weight
on the total weight of pasteurized milk.

20 In the following table 3 the organoleptic
characteristics of the product (look, structure, taste)
are reported, as evaluated by the same panel of judges of
the previous example, the day after its manufacturing and

scored according to the attribute scale, which interpretation is reported in example 1.

TABLE 3 - PROPERTIES OF MOZZARELLA 3

Time of tasting	Look	Structure	Taste	Overall evaluation
D + 1	4	4	5	5

Example 4 - Mozzarella 4:

5 4.1 Flavouring mixture preparation

Powder formulation D formed by:

10 Mesophilic lactobacilli in 1:1 ratio *Lactobacillus*
paracasei subsp. *paracasei* (GP02) and *Lactobacillus*
plantarum (GP01), total concentration 100×10^9 cfu/50 l
of milk.

Yeasts: *Candida colliculosa* (GCC05) 0.4×10^8 cfu/50
l of milk, *Kluyveromyces marxianus* subsp.
marxianus (GKM03) 1.4×10^9 cfu/50 l of milk, *Debaryomyces*
hansenii (GDH01) 1.6×10^9 cfu/50 l of milk.

15 Formulation D is added to 50 l of pasteurized milk
and allowed to develop for 16-18 h at 24-25°C until
pH=6.00-6.10 is reached.

4.2. Preparation and evaluation of mozzarella

20 In step (a) of the process of the invention the
flavouring mixture was added in an amount of 1% by weight
on the total weight of pasteurized milk.

In the following table 4, characteristics of the product (look, structure, taste) are reported, as evaluated by the same panel of judges of the previous example the day after its manufacturing and scored according to the attribute scale which interpretation is reported in the previous examples.

TABLE 4 - PROPERTIES OF MOZZARELLA 4

Time of tasting	Look	Structure	Taste	Overall evaluation
D + 1	4	3	4	4

Example 5 - Mozzarella 4A:

Mozzarella is prepared according to the methods used for preparing mozzarella 4, with the only difference of adding the flavouring mixture prepared as described in 4.1 in an amount equal to 1.2% by weight on the total weight of pasteurized milk.

In the following table 4A, properties of mozzarella, as evaluated by the same panel of judges of the previous example are reported the day after its manufacturing and scored according to the attribute scale which interpretation is reported in example 1.

TABLE 4A - PROPERTIES OF MOZZARELLA 4A

Time of tasting	Look	Structure	Taste	Overall evaluation
D + 1	5	4	5	5

Flavour is present and prominent.

Example 6 - Mozzarellas 5-10 and 13

The following mozzarellas were prepared employing
flavouring mixtures prepared in the following way: each
5 of the formulations (D5, D6, D8, D9, D13, D13*) reported
below are added to 50 l of pasteurized whole milk and the
mixture is allowed to develop at 24°C for 16-18 h until
pH=6.00-6.10 is reached

Formulation D5 (mozzarella 5)

10 Mesophilic lactobacilli in 1:1 ratio, *Lactobacillus*
paracasei subsp. *paracasei* (GP02) and *Lactobacillus*
plantarum (GP01) with a total concentration of 100×10^9
cfu/50 l of milk.

Yeasts: *Candida colliculosa* (GCC05) 0.4×10^7 cfu/50
15 l of milk, *Kluyveromyces marxianus* subsp. *marxianus*
(GKM03) 1.4×10^9 cfu/50 l of milk, *Debaryomyces hansenii*
(GDH01) 1.6×10^9 cfu/50 l of milk.

Formulation D6 (mozzarella 6)

Mesophilic lactobacilli in 1:1 ratio, *Lactobacillus*
20 *paracasei* subsp. *paracasei* (GP02) and *Lactobacillus*
plantarum (GP01) with a total concentration of 100×10^9
cfu/50 l of milk.

Yeasts: *Candida colliculosa* (GCC05) 0.8×10^8 cfu/50 l of milk, *Kluyveromyces marxianus* subsp. *marxianus* (GKM03) 1.4×10^9 cfu/50 l of milk, *Debaryomyces hansenii* (GDH01) 1.6×10^9 cfu/50 l of milk.

5 **Formulation D8 (mozzarella 7)**

Mesophilic lactobacilli in 1:1 ratio, *Lactobacillus paracasei* subsp. *paracasei* (GP02) and *Lactobacillus plantarum* (GP01) with a total concentration of 100×10^9 cfu/50 l of milk.

10 Yeasts: *Candida colliculosa* (GCC05) 0.8×10^8 cfu/50 l of milk, *Kluyveromyces marxianus* subsp. *marxianus* (GKM03) 2.8×10^9 cfu/50 l of milk, *Debaryomyces hansenii* (GDH01) 1.6×10^9 cfu/50 l of milk.

Formulation D9 (mozzarella 8)

15 Mesophilic lactobacilli in 1:1 ratio, *Lactobacillus paracasei* subsp. *paracasei* (GP02) and *Lactobacillus plantarum* (GP01) with a total concentration 100×10^9 cfu/50 l of milk.

20 Yeasts: *Candida colliculosa* (GCC05) 0.4×10^8 , *Kluyveromyces marxianus* subsp. *marxianus* (GKM03) 2.8×10^9 cfu/50 l of milk, *Debaryomyces hansenii* (GDH01) 3.2×10^9 cfu/50 l of milk.

Formulation D13 (mozzarella 9)

Mesophilic lactobacilli in 1:1 ratio, *Lactobacillus paracasei* subsp. *paracasei* (GP02) and *Lactobacillus plantarum* (GP01) with a total concentration of 100×10^9 cfu/50 l of milk.

5 Yeasts: *Kluyveromyces marxianus* subsp. *marxianus* (GKM03) 5.6×10^9 cfu/50 l of milk, *Debaryomyces hansenii* (GDH01) 4.8×10^9 cfu/50 l of milk.

Formulation D13* (Mozzarella 10)

10 Mesophilic lactobacilli in 1:1 ratio, *Lactobacillus paracasei* subsp. *paracasei* (GP02) and *Lactobacillus plantarum* (GP01) with a total concentration of 100×10^9 cfu/50 l of milk.

Yeasts: *Kluyveromyces marxianus* subsp. *marxianus* (GKM03) 5.6×10^9 cfu/50 l of milk.

15 **Example 7 - Preparation of mozzarellas 11 and 12**

Mozzarellas 11 and 12 are prepared employing the flavouring mixtures prepared according to the following experimental conditions, respectively. Formulations A2 and C2 reported below are added to 50 l of pasteurized
20 whole milk, the resulting mixture is allowed to develop at 24°C for 6 h, until pH= 6.00-6.10 is reached.

Formulation C2 (mozzarella 11)

Mesophilic lactococci in 1:1:1 ratio, *Lactococcus lactis* subsp. *lactis* (GLL23; GLL17); *Lactococcus lactis* subsp. *cremoris* (GLC16), *Lactococcus lactis* subsp. *diacetylactis* (GLD13) 5×10^9 cfu/50 l of milk.

5 Yeasts: *Kluyveromyces marxianus* subsp. *marxianus* (GKM03) 5.6×10^9 cfu/50 l of milk.

Formulation A2 (mozzarella 12)

Mesophilic lactococci in 1:1:1 ratio, *Lactococcus lactis* subsp. *lactis* (GLL23; GLL17); *Lactococcus lactis* subsp. *cremoris* (GLC16), *Lactococcus lactis* subsp. *diacetylactis* (GLD13): 5×10^9 cfu/50 l of milk.

Yeasts: *Kluyveromyces lactis* (GKL04) 1.6×10^9 cfu/50 l of milk, *Debaryomyces hansenii* (GDH02) 0.4×10^8 cfu/50 l of milk.

15 In the following tables 5 and 6, properties of mozzarellas 11 and 12 are reported, as evaluated by the same panel of judges of the previous example the day after their manufacturing, and scored according to the attribute scale which interpretation is reported in
20 example 1.

TABLE 5 - PROPERTIES OF MOZZARELLA 11

Time of tasting	Look	Structure	Taste	Overall evaluation
D + 1	4	3	4	4

TABLE 6 - PROPERTIES OF MOZZARELLA 12

Time of tasting	Look	Structure	Taste	Overall evaluation
D + 1	4	3	3	3

Example 8 - Subjective and objective sensorial analyses

A thorough study was carried out using both a sample
 5 of average consumers and a panel of judges trained to
 evaluate dairy industry products as well, in order to
 verify if mozzarellas according to the present invention
 confirmed the results in terms of look, structure and
 taste of the preliminary studies reported in the previous
 10 examples.

8.1 Samples examined

In this study, 3 types of 125 g-mozzarella were
 analysed that will be defined as follows:

- Mozzarella from the market
- 15 - Prototype 1 obtained as described in example 1 with
 type A formulation
- Prototype 2 obtained as described in example 6
 (mozzarella 10 obtained with formulation D13*)

8.2 SUBJECTIVE SENSORIAL ANALYSIS

20 8.2.1 Structure of the test and recording design

A blind test was carried out in specific centres with a sample of consumers so composed:

- 100 women consumers between 25 and 55 years of age;
- users of "traditional" cow mozzarella sold in supermarkets, at least twice in the last 3 months.

Interviewed women had gone to the centres where the test was taking place and had tasted the 3 samples in sequential monadic, according to a balanced rotation design.

10 8.2.2 Evaluation of samples

For each sample, women consumers expressed an overall liking score (scale 1-9) for the following properties: look, taste, softness, juiciness in the mouth.

Interviewed women were also asked to express an opinion on the suitability of the intensity of the following properties:

colour, taste, milky taste, compactness, juiciness, amount of milk, acidity and sapidity/degree of salt (scale 1-9). The propensity to buy was also recorded (scale 1-5).

8.3 OBJECTIVE SENSORIAL ANALYSIS

Tested products were subjected to evaluation by a panel of 8 trained judges that specialize in analysis of dairy products.

For each product, intensity of the following typical
5 descriptive qualities and of those concerning more general properties was measured on a 0-10 scale in 3 measurement sessions, that is: look, smell and flavour, taste, compactness, previously defined during appropriate preliminary sessions.

10 In fact, descriptive qualities concerning the look are inside colour, outside colour, surface brightness, surface smoothness and visual fibrousness.

Descriptive qualities defining smell and flavour are overall smell, butter flavour, cream flavour, milky
15 flavour and whey flavour.

Descriptive qualities regarding taste are overall taste, and in particular salty, bitter, acid tastes.

Eventually, qualities defining compactness are hardness at cutting, cohesivity, flexibility, flaking,
20 juiciness.

8.4 Results

8.4.1 Subjective sensorial analysis

In the following table 5 results are reported in terms of overall liking, and of results concerning look, taste, softness, juiciness in the mouth of each of the samples tested, following the subjective sensorial analysis described above.

TABLE 5

	Prototype 1	Mozzarella from the market	Prototype 2	*LSD
Overall liking	6.2	5.4	6.6	0.5
Look	6.8	5.4	6.7	0.5
Taste	5.6	5.4	6.4	0.6
Softness	6.5	3.9	6.6	0.6
Juiciness in the mouth	5.6	4.2	6.3	0.6

LSD 95% = least significant difference (P=95%)

In table 6 mean scores of suitability scores are reported, classified according to scales ranging from 1 to 9 for each property and so that:

Colour: 1 = too light, 9= too dark; Taste/milky taste 1 = very weak, 9 = too intense; Compactness 1 = too soft,

9 = too hard; Juiciness in the mouth: 1 = very little juice, 9 = too juicy; Amount of milk seeping: 1 = too little, 9 = too much; Acidity: 1 = very little acidity, 9 = too acid.

5 Table 6 - Suitability scores

	Prototype 1	Mozzarella from the market	Prototype 2	*LSD
Colour	5.2	5.3	5.3	0.4
Amount of milk	3.8	2.2	3.9	0.5
Taste	4.1	4.0	4.8	0.5
Milky taste	4.0	3.3	4.3	0.5
Acidity	5.0	4.6	5.2	0.4
Juiciness	4.3	3.1	4.2	0.4

LSD 95% = least significant difference (P=95%)

Values in bold are significantly lower or higher than the suitability value.

10 In table 7 data regarding propensity to buy are reported.

	Mean scores	I certainly/probably	I would certainly
--	----------------	-------------------------	-------------------------

		buy it	buy it
Prototype 1	3.3	47%	21%
Mozzarella from the market	2.8	32%	5%
Prototype 2	3.6	59%	24%
LSD 95%	0.3		

* LSD 95% = least significant difference (P=95%)

8.4.2 Objective sensorial analysis

In the following table 8, results of the evaluation are reported, as expressed by the panel of external
5 judges on a scale ranging from 1 to 9, wherein 1 represents the lowest value for the test properties while 9 represents the highest value for the same properties.

TABLE 8. Sensorial profiles of the products

	Prototype 1	Mozzarella from the market	Prototype 2	*LSD
Outside colour	3.9	5.5	1.9	2.5
Surface brightness	7.3	3.8	7.4	2.0
Surface smoothness	6.1	3.3	8.0	2.3

Compactness	5.3	6.8	5.3	0.4
Visual flexibility	5.1	5.4	5.0	1.7
Hardness at cutting	2.8	7.1	4.9	2.4
Inside colour	4.7	8.5	2.1	2.0
Visual fibrousness	5.3	1.9	4.5	2.5
Overall smell	2.8	4.9	4.6	2.6
Overall taste	3.1	2.2	6.4	2.0
Sweet	6.5	7.8	3.3	2.4
Salty	1.8	1.2	5.4	1.5
Bitter	4.5	2.2	3.9	2.5
Acid	4.5	1.6	7.5	2.2
Butter flavour	3.6	6.5	3.3	2.6
Milky flavour	5.3	4.5	6.5	2.6
Cream flavour	4.9	6.4	4.3	2.5
Whey flavour	5.5	2.9	6.3	2.5
Sapidity/salty taste	4.3	4.2	4.9	0.4
Cohesivity	2.7	8.9	4.0	2.1
Flaking	4.6	4.6	4.4	2.6
Juiciness	6.5	1.4	6.8	2.2

As it can be inferred from both subjective and objective sensorial analyses, the prototypes according to the invention show significantly improved flavour and taste in comparison with the marketed product that was not prepared using the flavouring mixture according to the present invention, in addition, prototype 2 is also the one giving better results non only in terms of taste, but also of colour, smoothness and cohesivity.

Example 9

Data obtained from 50 l of pasteurized milk fermented in the presence of the following yeasts only are reported hereinafter:

Candida colliculosa, *Kluyveromyces marxianus* subsp. *marxianus*, *Debaryomyces hansenii*.

Example 9 -A-

Flavouring yeasts for mozzarella

Activities performed in high quality, pasteurized, marketed whole milk

pH/acidity of the milk = 6.79/14°D

Standard concentration	Four-fold concentration
yeasts	yeasts
<i>Candida colliculosa</i>	<i>Candida colliculosa</i>
T°C=24°C	T°C=24°C

Time (h)	pH	Time (h)	pH
0	6.79	0	6.79
1	6.69	1	6.67
2	6.68	2	6.65
3	6.67	3	6.64
4	6.67	4	6.64
5	6.66	5	6.63
6	6.66	6	6.63
7	6.66	7	6.62
8	6.66	8	6.62
9	6.65	9	6.62
10	6.65	10	6.62
11	6.65	11	6.62
12	6.65	12	6.61
13	6.65	13	6.61
14	6.65	14	6.61
15	6.65	15	6.61
16	6.65	16	6.61
17	6.64	17	6.60
18	6.64	18	6.60
19	6.64	19	6.59
20	6.64	20	6.59

As it can be observed, not even at a four-fold concentration and after 20 h, the pH of such mixture reaches the pH required to give the required acidity.

Mozzarella produced using milk wherein said yeast was
 5 allowed to develop as a flavouring mixture was also analyzed by the same panel of 10 judges that examined mozzarellas obtained with the flavouring mixtures of examples 1-7, only concerning the taste the day after manufacturing. The evaluation of such product was 0,
 10 since the mozzarella shows a taste that is almost identical to that of those obtained in the absence of flavouring mixture.

Example 9 -B- *Debaryomyces hansenii*

Activities performed in pasteurized, marketed whole
 15 milk

pH/acidity of the milk = 6.72/16°D

Standard concentration		four-fold concentration	
yeasts		yeasts	
<i>Debaryomyces hansenii</i>		<i>Debaryomyces hansenii</i>	
T°C=24°C		T°C=24°C	
Time (h)	pH	Time (h)	pH
0	6.72	0	6.72
1	6.68	1	6.64
2	6.67	2	6.64

3	6.67	3	6.64
4	6.67	4	6.64
5	6.66	5	6.64
6	6.66	6	6.64
7	6.66	7	6.63
8	6.66	8	6.63
9	6.66	9	6.63
10	6.66	10	6.63
11	6.66	11	6.63
12	6.65	12	6.63
13	6.65	13	6.62
14	6.65	14	6.62
15	6.64	15	6.62
16	6.63	16	6.61
17	6.63	17	6.60
18	6.62	18	6.59
19	6.60	19	6.56
20	6.57	20	6.51

Also in this case the flavouring mixture does not reach the desired values of acidity even employing a four-fold concentration of yeast and after 20 hours.

Mozzarella produced with the flavouring mixture
5 containing said yeast only, the day after its production was evaluated by the same panel of 10 judges of examples

1-7 only concerning the taste. Also in this case, the evaluation of such product was 0, since the mozzarella shows a taste almost identical to that of those obtained in the absence of such flavouring mixture.

5 Example 9 -C- *Kluyveromyces marxianus* subsp. *marxianus*

Standard concentration	four-fold concentration
------------------------	-------------------------

yeasts

Kluyveromyces marxianus
subsp. *marx.*

T°C=24°C

yeasts

Kluyveromyces marxianus
subsp. *marx.*

T°C=24°C

Time (h)	pH	Time (h)	pH
0	6.72	0	6.72
1	6.65	1	6.67
2	6.64	2	6.66
3	6.64	3	6.66
4	6.63	4	6.65
5	6.63	5	6.65
6	6.63	6	6.64
7	6.63	7	6.64
8	6.62	8	6.64
9	6.62	9	6.63
10	6.62	10	6.63
11	6.62	11	6.63

12	6.62	12	6.62
13	6.61	13	6.62
14	6.61	14	6.61
15	6.60	15	6.60
16	6.60	16	6.58
17	6.59	17	6.56
18	6.58	18	6.54
19	6.56	19	6.51
20	6.54	20	6.48

As it results from the above data, only after 20 h and with a concentration four-fold higher than the standard, a flavouring mixture with a pH slightly lower than the maximum acceptable value (6.50) is obtained.

- 5 In any case, also mozzarellas produced with said flavouring mixture were given a taste value = 0, since they showed a taste almost identical to that of those obtained in the absence of flavouring mixture.

CLAIMS

1. Partially fermented pasteurized milk in a liquid form obtained by addition of an association of lactic ferments and yeasts and their development for a time
5 comprised between 6 and 18 hours, until pH values comprised between 5.70 and 6.50 are reached.

2. Partially fermented pasteurized milk in a liquid form according to claim 1, wherein said development is carried out until pH values comprised between 5.80 and
10 6.10 are reached.

3. Partially fermented pasteurized milk in a liquid form according to claim 2, wherein said pH is comprised between 6.00 and 6.10.

4. Partially fermented pasteurized milk in a liquid
15 form according to any one of claims 1-3, wherein the lactic ferments are selected from mesophilic lactococci and/or lactobacilli.

5. Partially fermented pasteurized milk in a liquid form according to claim 4, wherein the mesophilic
20 lactobacilli are selected in the group consisting of:
Lactobacillus plantarum, *Lactobacillus casei*;
Lactobacillus rhamnosus; *Lactobacillus pentosus*;
Lactobacillus paracasei and combinations thereof.

6. Partially fermented pasteurized milk in a liquid form according to claim 4 wherein the mesophilic lactococci are selected in the group consisting of: *Lactococcus lactis* subsp. *lactis*; *Lactococcus lactis* subsp. *diacetylactis*; *Lactococcus lactis* subsp. *cremoris*; and combinations thereof.

7. Partially fermented pasteurized milk in a liquid form according to any one of claims 1-6, wherein the yeasts are selected in the group consisting of: *Kluyveromyces marxianus* subsp. *marxianus*, *Debaryomyces hansenii*, *Candida Colliculosa*, *Kluyveromyces lactis*; *Saccharomyces cerevisiae*.

8. Use of the partially fermented milk according to any one of claims 1-7, as a flavouring mixture in the industrial process of production of mozzarella.

9. Use according to claim 8 wherein said process for preparing mozzarella comprises adding said flavouring mixture to pasteurized milk destined to the production of mozzarella, in amounts between 1 and 3% by weight on the total volume of said pasteurized milk, with said flavouring mixture being added during said process before forming the curd.

10. Use according to claim 9, wherein in said process the curd is produced by adding at least one acidifying lactic ferment or starter simultaneously or before adding the flavouring mixture.

5 11. Use according to claim 10 wherein the process of preparation of mozzarella comprises the following steps:

a) adding the flavouring mixture to pasteurized milk destined to the production of mozzarella, said milk being optionally standardized for its own fat/protein ratio
10 according to the good dairy practice well known to the experts in the field, and having an acidifying lactic ferment or starter previously or simultaneously added thereto;

b) forming curd by adding calf or microbial rennet;

15 c) cutting the thus obtained curd, stirring, partial or total drainage of whey and separation of the curd;

d) ripening of the curd, by fermentative acidification up to the desired pH generally comprised between 5.2 and 5.4;

20 e) cutting the curd up in hot water or also under steam;

f) stretching it in hot water or also under steam;

g) shaping the thus obtained stretched curd into mozzarellas of specific shape;

h) cooling the mozzarellas;

i) optionally salting them by dipping in brine;

5 j) packaging, generally but not necessarily in storage liquid.

12. Use according to claim 11, wherein in step (a) the flavouring mixture is added at concentrations between 1.2 and 1.6% by weight/total volume of pasteurized milk
10 destined to the production of mozzarella.

13. Use according to claim 9 wherein, in the industrial process for the production of mozzarella, the formation of the curd is achieved by adding citric acid and microbial or calf rennet.

15 14. Use according to claim 13 wherein the process of production of mozzarella comprises the following steps:

a') adding the flavouring mixture to pasteurized milk, said milk being optionally standardized for its own fat/protein ratio according to the good dairy practice
20 well known to the experts in the field and having citric acid previously or simultaneously added thereto;

b') forming curd by adding calf or microbial rennet;

c') cutting the thus obtained curd, stirring, total drainage of whey and separation of the curd;

d') cutting the curd up and stretching it in hot water or also under steam;

5 e') shaping the thus obtained stretched curd into mozzarellas of specific shape;

f') cooling the mozzarellas;

g') optionally salting them by dipping in brine;

h') packaging, generally but not necessarily, in
10 storage liquid.

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2013/061282

A. CLASSIFICATION OF SUBJECT MATTER

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

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

C. 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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X	----- WO 2007/055604 A1 (FONTERRA CO OPERATIVE GROUP [NZ]; TSAO MARLENE [NZ]; LIU SHAO QUAN [NZ]) 18 May 2007 (2007-05-18) cited in the application examples 3, 5, 7-17	1-7
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☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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Date of the actual completion of the international search

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INTERNATIONAL SEARCH REPORT

International application No

PCT/IB2013/061282

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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Information on patent family members

International application No

PCT/IB2013/061282

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