

## Comparison of different starter systems for water-buffalo Mozzarella cheese manufacture

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**Summary** — Natural whey cultures, a thermophilic multiple strain starter (*Lactobacillus helveticus* and *Streptococcus thermophilus*) and a more complex multiple strain starter, including both thermophilic and mesophilic bacteria and a yeast (*L. delbrueckii* subsp *lactis*, *S. thermophilus*, *Lactococcus lactis* subsp *lactis*, *Lactococcus lactis* subsp *diacetylactis*, *Enterococcus faecalis*, *Leuconostoc mesenteroides* subsp *dextranicum* and *Kluyveromyces marxianus*) and artificial acidification (addition of citric acid) were used for the manufacture of water-buffalo Mozzarella cheese. Whey acidity, fermentation end-products and microbial populations were monitored during cheese manufacture. A scorecard for sensory evaluation of water-buffalo Mozzarella cheese was developed and used to compare the cheeses obtained with the different procedures. The traditional technology (raw milk and natural whey cultures) allowed shorter manufacturing times due to faster acid production during ripening. Cheeses produced with the thermophilic multiple strain starter and citric acid addition obtained the lowest scores in sensory evaluation. When the complex multiple strain starter was used scores were slightly higher and more constant than those obtained using traditional technology.

### **Mozzarella cheese / water-buffalo milk / starter cultures**

**Résumé** — Comparaison entre différents levains pour la production du fromage Mozzarella avec du lait de bufflonne. Les auteurs utilisent, pour la production de fromage Mozzarella à partir du lait de bufflonne, différents levains et l'acidification artificielle avec de l'acide citrique. Les levains utilisés étaient les suivants : des levains mixtes (cultures naturelles dans le lactosérum), un levain thermophile à 2 souches (*Lactobacillus helveticus* et *Streptococcus thermophilus*), et un levain plus complexe à souches multiples contenant des souches mésophiles et des souches thermophiles (*L. delbrueckii* subsp *lactis*, *S. thermophilus*, *Lactococcus lactis* subsp *lactis*, *Lactococcus lactis* subsp *diacetylactis*, *Enterococcus faecalis*, *Leuconostoc mesenteroides* subsp *dextranicum* et *Kluyveromyces marxianus*). L'acidité du lactosérum, les produits finaux de la fermentation et les populations microbiennes ont été suivis au cours du processus de production du fromage. Une fiche d'évaluation sensorielle a été mise au point et utilisée pour comparer les fromages obtenus avec les différents processus. La technologie traditionnelle, qui utilise du lait cru et des cultures naturelles dans du lactosérum, permet des temps de production très courts à cause d'une plus rapide production d'acide. Les fromages produits avec le levain thermophile à 2 souches et ceux obtenus avec addition d'acide citrique ont obtenu les plus mauvais résultats dans l'évaluation sensorielle. Avec le levain complexe à souches multiples les résultats étaient, par contre, meilleurs et plus constants par rapport à ceux des fromages produits par la technologie traditionnelle.

### **fromage Mozzarella / lait de bufflonne / levains**

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## INTRODUCTION

It is well recognized that starter cultures fulfil several important functions in cheese-making: acid production from lactose, inhibition of spoilage and pathogenic microorganisms, improvement of cheese keeping quality, direct and indirect contributions to flavour and aroma (Sandine, 1979).

In the case of the manufacture of unripened "pasta filata" cheese, the main function of starter cultures is to ensure a rapid acidification of the curd which promotes the transformation of dicalcium paracasein into monocalcium paracasein during stretching in hot water (Chapman and Sharpe, 1981).

Starter cultures containing mixtures of thermophilic rods (*Lactobacillus delbrueckii* subsp *bulgaricus* and/or *L. helveticus*) and cocci (*Streptococcus thermophilus*) have been suggested (Kosikowski, 1977; Law, 1982; Vedamuthu and Washam, 1983) and used in the manufacture of Mozzarella cheese. A cocci:rods ratio of 2:1 to 3:1 is considered to be optimal (Thunell and Sandine, 1985). The role of secondary or accessory microflora has always been disregarded.

In Southern Italy water-buffalo Mozzarella cheese has been traditionally manufactured from raw milk employing natural whey cultures as starters. This cheese is attaining an increasing success; as for other "typical" cheeses its manufacture and composition is regulated by law (Repubblica Italiana, 1980). Although the pasteurization of milk is required by health authorities for the manufacture of unripened cheeses and the use of starter cultures composed of *L. delbrueckii* subsp *bulgaricus* or *L. helveticus*, and *S. thermophilus* was specifically suggested for pasta filata cheeses (Ministero della Sanita della Repubblica Italiana, 1978), we previously re-

ported (Coppola *et al*, 1988) that traditional technology is still widely employed. In fact, according to the cheesemakers, the use of the commercially available starters (mixtures of thermophilic rods and cocci) causes and impoverishment of the organoleptic characteristics of the products.

In a previous paper (Parente *et al*, 1989) we reported the development of a multiple strain starter whose composition "mimicked" that of natural whey cultures. In this paper the performance of different starter systems for water-buffalo Mozzarella cheese and the organoleptic characteristics of the cheeses made with them are compared. A scorecard for the evaluation of water-buffalo Mozzarella cheese was also tentatively developed and used.

## MATERIALS AND METHODS

Whole water-buffalo milk provided by Cooperativa Produttori Latte Bufalino, Cancellone Arnone (Caserta) was used to produce, at the pilot plant of the Istituto di Microbiologia Agraria of the University of Naples, lots of Mozzarella cheese for each of the following experimental conditions:

A: Raw milk with natural whey culture (obtained from Cooperativa Produttori Latte Bufalino) as starter, according to the traditional technology (Addeo and Coppola, 1983);

B: Pasteurized milk (70°C, 1 min, Sordi SpA pasteurizer) inoculated with 10% (vol/vol) of a starter prepared according to the suggestions of Ministero della Sanita (1978): cultures of *L. helveticus* 6 and *S. thermophilus* 317 were grown in whole water-buffalo milk (treated at 120°C for 5 min) at 37°C for 18 h and mixed in a 1:3 ratio (vol/vol). Both the strains had been isolated from natural whey cultures (Coppola *et al*, 1988) and selected for their high acid production ability in water-buffalo milk.

C: Pasteurized milk (as above) inoculated with 10% (vol/vol) of the multiple strain starter described in Parente *et al* (1989). The bulk starters were grown in pasteurized (121°C, 5 min) whole water-buffalo milk using the following procedure: the mesophilic strains (*Lactococcus lac-*

*tis* subsp *lactis* 136, *Lactoc lactis* subsp *diacetylactis* RD, *Leuconostoc mesenteroides* subsp *dextranicum* L1, *Enterococcus faecalis* 221, *Kluyveromyces marxianus* 25Y) were grown together at 22 °C for 18 h while the thermophilic strains (*L. delbrueckii* subsp *lactis* 22 and 35, *S. thermophilus* 317) were grown together at 37 °C for 18 h; just before inoculation of the vat the mesophilic and thermophilic cultures were mixed in a 3:2 (vol/vol) ratio.

D: pasteurized milk plus citric acid; citric acid addition was adjusted to bring the pH of the curd to values suitable for stretching (pH 4.9 to 5.0) in 75 min. This treatment was included as a control with no starter added for sensory evaluation.

Three lots were produced for treatments A, C and D and 2 lots for B. All the other technological parameters (temperature, amount of rennet, etc) were the same (Addeo and Coppola, 1983) for all treatments. 90 l of water-buffalo milk were used for each trial. For treatments A, B and C curd was aseptically sampled hourly during ripening; 100 g of curd were then weighed and pressed (in a home-made press at  $\approx 2$  kg/cm<sup>2</sup> for 3 min) to obtain whey for microbiological and chemical analyses.

The following procedures were used: pH was measured with a Beckman PHI43 pH-meter. Enzymatic assays (Boehringer-Mannheim) were used to measure L(+)-lactic acid, D(-)-lactic acid (kit 139.084 and D-lactate dehydrogenase), ethanol (kit 176.290) and acetic acid (kit 148.261). The samples were deproteinized using the procedure suggested by the manufacturer; samples were filtered through Gelman Akrodisk (pore size 0.45  $\mu$ m) to obtain clear deproteinized whey for enzymatic assays.

Microbiological analyses were carried out for a single lot of treatment A (natural whey starter) and for all the lots of treatments B (thermophilic multiple strain starter) and C (complex multiple strain starter). In a previous study (Addeo and Coppola, 1983) it was shown that the differences between bacterial numbers in the curd and in the whey were not significant. However, yeasts were selectively retained in the curd; pressing the curd allowed a better recovery (unpublished data). In this study microbial counts were only carried out on the whey obtained by pressing the curd with the procedure described above.

After decimal dilutions in sterile quarter strength Ringer's solution enumerations were

carried out using the procedures described below: three-tube MPN procedures were used for the enumeration of mesophilic and thermophilic lactic acid bacteria, coliforms and enterococci. Lactic acid bacteria were counted in skim milk (Oxoid, UK) after incubation for 48 h at 22 °C (mesophilic lactic acid bacteria) or at 45 °C (thermophilic lactic acid bacteria). Coliforms were counted in brilliant green lactose bile broth (Oxoid, UK), incubated at 30 °C for 48 h. For enterococci azide dextrose broth (Oxoid, UK) tubes were inoculated and incubated at 37 °C for 48 h; ethyl violet azide broth (Oxoid, UK) was inoculated from presumptively positive tubes; after incubation at 37 °C for 24 h, positive tubes were confirmed by streaking plates of thallos acetate tetrazolium glucose agar (TITGA, Barnes, 1956). Yeasts were counted by inoculating sample dilutions onto yeast dextrose chloramphenicol agar (Anonymous, 1985) and incubating for 5 d at 30 °C.

The following procedures were used for the differential enumeration of lactic acid bacteria and yeasts in treatments B (thermophilic multiple strain starter) and C (complex multiple strain starter). Yeasts were counted as described for the natural whey culture. *L. helveticus* and *S. thermophilus* were differentially counted on tryptose proteose peptone yeast eriochrome black agar (Bracquart, 1981), incubated at 37 °C for 48 h in Gaspak jars supplied with CO<sub>2</sub>-generating kit (BBL, USA). *E. faecalis* was counted on surface-inoculated TATGA (Barnes, 1956) incubated at 45 °C for 24 h. *Lactoc lactis* subsp *lactis* and *Lactoc lactis* subsp *diacetylactis* were counted on Reddy's medium (Reddy *et al.*, 1972) incubated at 30 °C for 2–5 d in Gaspak jars supplied with CO<sub>2</sub>-generating kit (BBL, USA); since *E. faecalis* produced clear haloes on Reddy's medium *Lactoc lactis* subsp *diacetylactis* counts were obtained as a difference between the citrate-utilizing colonies on Reddy's medium and *E. faecalis* counts on TATGA. *Leuc. mesenteroides* subsp *dextranicum* were counted as slime-forming colonies on Elliker lactic agar (Elliker *et al.*, 1956) incubated for 48 h at 30 °C in a CO<sub>2</sub>-enriched atmosphere generated as described above.

A descriptive test was used for sensory evaluation of the cheeses produced for each lot. A panel of 25 judges was selected among untrained consumers of water-buffalo Mozzarella cheese. A scorecard based on that proposed by

Duthie *et al* (1980) was developed (table I) and the judges were briefed on the use of the scorecard before the test. Statements for "ideal" appearance, body/texture and flavour, description of defects and their intensity scores are based on the typical expectations of local consumers for water-buffalo Mozzarella cheese. For each lot a letter-coded 200 g sample for each type of cheese (see A, B, C and D above) was submitted to each judge according to a completely random design. Total scores for appearance, body/texture and flavour were obtained subtracting from the maximum ("excellent") score the intensity scores of the defects.

## RESULTS

### Acid production and evolution of fermentation end-products

The time course of acidification for experimental conditions A, B and C is reported in figures 1 (pH) and 2 (isomers of lactic acid). Acid production was faster when natural whey cultures were used: pH 4.9–5.1 (optimal pH value for stretching) was reached in about 4 h while 4.5 and 5 h were needed for the complex multiple strain starter (C) and the thermophilic starter (B) respectively.

Figure 2 shows the time course of the production of L(+) and D(-) lactic acid for the 3 starter systems. Although at the end of curd acidification (between 4 and 5 h) the 2 isomers were present in about the same amount, their evolution in time varied among the starters. For both the natural whey culture and the complex multiple strain starter (C) the L(+) isomer was produced at a high rate during the first 3 h while the D(-) isomer was produced mainly during the last h. For the complex multiple strain starter an early production of the L(+) isomer was backed up by a higher initial population of streptococci; the D(-) isomer evolution closely followed that of *L*

*delbrueckii* subsp *lactis*. Regarding the natural whey culture, enterococci were probably responsible for the production of L(+) lactic acid during the first hours. Since individual species of lactic acid bacteria were not counted, the role of thermophilic lactobacilli in the production of the D(-) isomer can only be hypothesized. The 2 isomers were produced at approximately the same rate for the thermophilic starter (B). This is consistent with the evolution of the populations of microorganisms produc-

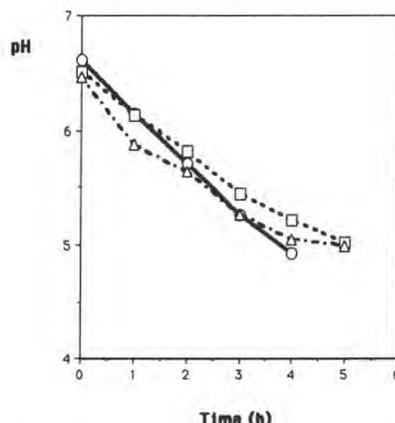


Fig 1. pH evolution during acidification of the curd in the manufacture of water-buffalo Mozzarella cheese with different starter systems. O: natural whey culture; □: thermophilic multiple strain starter (*L. helveticus* and *S. thermophilus*); Δ: complex multiple strain starter (*L. delbrueckii* subsp *lactis*, *S. thermophilus*, *Lactoc. lactis* subsp *lactis*, *Lactoc. lactis* subsp *diacetylactis*, *Leuc. mesenteroides* subsp *dextranicum*, *E. faecalis*, *K. marxianus*).

Evolution du pH pendant l'acidification du caillé au cours de la fabrication du fromage Mozzarella à partir du lait de bufflonne utilisant différents levains. O: levain mixte (culture naturelle dans du lactosérum); □: levain thermophile à 2 souches (*L. helveticus* et *S. thermophilus*); Δ: levain complexe à souches multiples (*L. delbrueckii* subsp *lactis*, *S. thermophilus*, *Lactoc. lactis* subsp *lactis*, *Lactoc. lactis* subsp *diacetylactis*, *Leuc. mesenteroides* subsp *dextranicum*, *E. faecalis*, *K. marxianus*).

**Table I.** Scorecard for water-buffalo Mozzarella cheese.  
*Fiche pour l'évaluation sensorielle du fromage Mozzarella.*

*Appearance**Excellent = 3*

Ideal: Globular shape, with well-sealed and typical "whiskers" caused by the cutting of the paste.  
 Crust: very thin, smooth, shiny, bright white; sheen, attractive surface.

<i>Defects</i>	<i>Intensity scores</i>		
	<i>Slight</i>	<i>Definite</i>	<i>Pronounced</i>
Misshapen	0	-1	-2
Unsealed or badly sealed	-1	-2	-3
Unnatural colour	-3	-3	-3
Opaque surface	-1	-2	-3
Rough surface	-1	-2	-3
Peelable in thin layers by touching	-1	-3	-3
Peelable in thick layers by touching	-3	-3	-3

*Body /Texture**Excellent = 5*

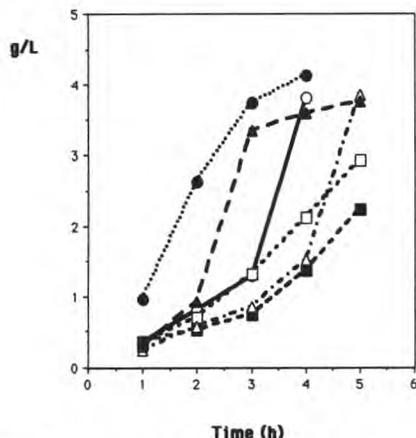
Ideal: Body: flexible and elastic. Texture: soft and smooth; thinly sheeted structure with progressive tendency to homogeneity in the inner part. Milky droplets are apparent upon cutting; small eyes are acceptable.

<i>Defects</i>	<i>Intensity scores</i>		
	<i>Slight</i>	<i>Definite</i>	<i>Pronounced</i>
Coarse	-1	-3	-5
Gassy	-2	-3	-5
Lacks flexibility or elasticity	-2	-3	-5
Pasty	-2	-3	-5
Open or empty	-1	-2	-3
Gummy	-0	-1	-2
Dry	-1	-2	-4
Hard	-2	-3	-4

*Flavour**Excellent = 10*

Ideal: bland and nutty, fresh and odorous, slightly and pleasantly acid.

<i>Defects</i>	<i>Intensity scores</i>		
	<i>Slight</i>	<i>Definite</i>	<i>Pronounced</i>
Flat	-1	-3	-5
Coarse	-3	-5	-7
Acid (too much acid)	-3	-5	-8
Bitter	-5	-7	-10
Foreign	-3	-6	-10
Fruity	-2	-4	-6
Musty	-3	-5	-7
Lipolyzed	-4	-6	-10
Salty	-1	-2	-3
Whey taint	-2	-3	-5
Yeasty	-4	-6	-10
Greasy	-1	-2	-3

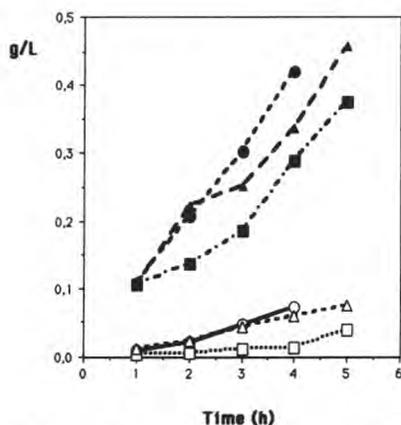


**Fig 2.** Production of D(-) (empty symbols) and L(+)-lactic acid (closed symbols) during acidification of the curd in the manufacture of water-buffalo Mozzarella cheese with different starter systems. ○ : natural whey culture; □ : thermophilic multiple strain starter (*L. helveticus* and *S. thermophilus*); △ : complex multiple strain starter (*L. delbrueckii* subsp. *lactis*, *S. thermophilus*, *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *diacetylactis*, *Leucon mesenteroides* subsp. *dextranicum*, *E. faecalis*, *K. marxianus*).

Production d'acide lactique D(-) (symboles vides) et L(+) (symboles pleins) pendant l'acidification du caillé au cours de la fabrication du fromage Mozzarella à partir du lait de bufflonne utilisant différents levains. ○ : levain mixte (culture naturelle dans du lactosérum); □ : levain thermophile à 2 souches (*L. helveticus* et *S. thermophilus*); △ : levain complexe à souches multiples (*L. delbrueckii* subsp. *lactis*, *S. thermophilus*, *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *diacetylactis*, *Leucon mesenteroides* subsp. *dextranicum*, *E. faecalis*, *K. marxianus*).

ing the 2 different isomers shown in figure 5 (*L. helveticus* can produce both D(-)- and L(+)-isomers).

The production of acetic acid and ethanol during curd ripening is shown in figure 3. The presence of these compounds re-



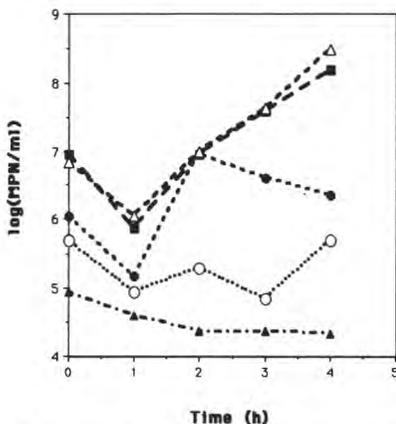
**Fig 3.** Production of acetic acid (closed symbols) and ethanol (empty symbols) during acidification of the curd in the manufacture of water-buffalo Mozzarella cheese with different starter systems. ○ : natural whey culture; □ : thermophilic multiple strain starter (*L. helveticus* and *S. thermophilus*); △ : complex multiple strain starter (*L. delbrueckii* subsp. *lactis*, *S. thermophilus*, *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *diacetylactis*, *Leucon mesenteroides* subsp. *dextranicum*, *E. faecalis*, *K. marxianus*).

Productions d'acide acétique (symboles pleins) et éthanol (symboles vides) pendant l'acidification du caillé au cours de la fabrication du fromage Mozzarella à partir du lait de bufflonne utilisant différents levains. ○ : levain mixte (culture naturelle dans du lactosérum); □ : levain thermophile à 2 souches (*L. helveticus* et *S. thermophilus*); △ : levain complexe à souches multiples (*L. delbrueckii* subsp. *lactis*, *S. thermophilus*, *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *diacetylactis*, *Leucon mesenteroides* subsp. *dextranicum*, *E. faecalis*, *K. marxianus*).

flects the microbiological complexity (*ie*, the presence of heterofermentative microorganisms, like coliforms and leuconostocs) of the starter system used: their concentration was lower when the thermophilic starter was used.

### Microbial counts

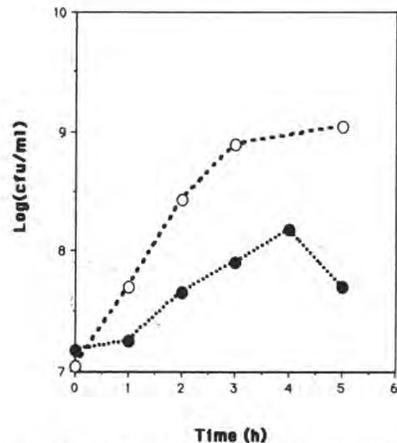
Figures 4, 5 and 6 and 7 show the results of the microbial counts for the natural whey culture, the thermophilic and the complex multiple strain starters, respectively. More than  $10^7$  lactic acid bacteria were present for each starter system at the beginning of the fermentation. The evolution of microbial populations during the manufacture of Mozzarella cheese using natural whey cultures (fig 4) confirmed the results obtained in previous experiments (Coppola *et al.*, 1985). Although in a previous study (Addeo and Coppola, 1983) no significant differences were noted between bacterial numbers in the whey and in the curd, en-



**Fig 4.** Microbial counts in whey during acidification of the curd in the manufacture of water-buffalo Mozzarella cheese with a natural whey starter. Log (MPN/ml). ■ : thermophilic lactic acid bacteria; ○ : mesophilic lactic acid bacteria; △ : enterococci; ● : coliforms; ▲ : yeasts (Log (cfu/ml)).

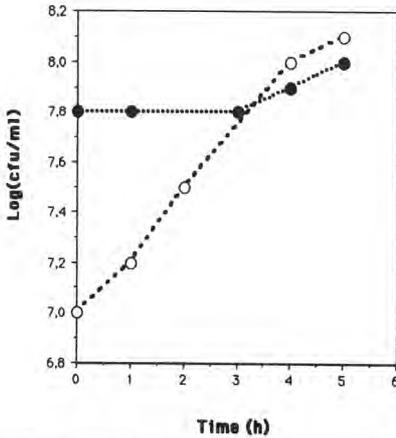
*Dénombrements microbiens dans le lactosérum durant l'acidification du caillé au cours de la fabrication du fromage Mozzarella à partir du lait de bufflonne utilisant une culture naturelle en lactosérum.* Log (NPP/ml) ○ : bactéries lactiques mésophiles; ■ : bactéries lactiques thermophiles; △ : entérocoques; ● : coliformes; ▲ : levures (log (ufc/ml)).

trapment of bacteria in the curd seems to be the only possible explanation for reduction in numbers between 0 and 1 h. However, this was not observed when multiple strain starters were used. A significant growth was observed only for thermophilic lactic acid bacteria and enterococci. Due to the counting methods used, the results for these 2 groups largely overlap. The increasing acidity of the curd may explain inhibition of coliforms after 2 h. When pasteurized milk and starters with a defined composition were used (B and C) an attempt was made to estimate the numbers of the single components of the starters. This was easily accomplished for starter B (fig 5), due to its simple composition and to the good differential characteristics of the medium used for counting (TPPYA). Both the strains multiplied appreciably during the entire manufacturing period, but *L hel-*



**Fig 5.** Microbial counts in whey during acidification of the curd in the manufacture of water-buffalo Mozzarella cheese with a simple thermophilic starter. ○ : *L. helveticus*; ● : *S. thermophilus*.

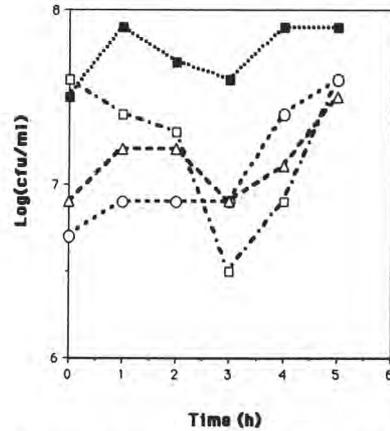
*Dénombrements microbiens dans le lactosérum pendant l'acidification du caillé au cours de la fabrication du fromage Mozzarella à partir du lait de bufflonne utilisant un levain thermophile à 2 souches.* ○ : *L. helveticus*; ● : *S. thermophilus*.



**Fig 6.** Microbial counts in whey during the manufacture of water-buffalo Mozzarella cheese with a complex multiple strain starter : thermophilic starter. ○ : *L. delbrueckii* subsp *lactis*; ● : *S. thermophilus*.

Dénombrements microbiens dans le lactosérum pendant l'acidification du caillé au cours de la fabrication du fromage Mozzarella à partir du lait de bufflonne utilisant un levain complexe à souches multiples. Souches thermophiles. ○ : *L. delbrueckii* subsp *lactis*; ● : *S. thermophilus*.

*veticus* 6 rapidly outnumbered *S. thermophilus* 317, whose growth stopped after 4 h, reaching a population 100-fold higher at the end of curd ripening. The decline in coccal population after 4 h could be explained by growth of lytic phages. Such phages can be isolated from both water-buffalo milk and natural whey cultures, but rarely reach high levels (Tramma, 1988). The interpretation of the results of the counts for the multiple strain starter (fig 6, thermophiles and fig 7, mesophiles) was more complicated. The media and methods used for enumeration lack good selectivity and differential capacity (Parente *et al*, 1989): the numbers of *Lactoc lactis* subsp *diacetylactis* RD and *Lactoc lactis* subsp *lactis* 136 were estimated using the counts from different media; *Leuc mesen-*



**Fig 7.** Microbial counts in whey during the manufacture of water-buffalo Mozzarella cheese with a complex multiple strain starter : mesophilic strains. ■ : *Lactoc lactis* subsp *lactis*; □ : *Lactoc lactis* subsp *diacetylactis*; ○ : *Leuc mesenteroides* subsp *dextranicum*; Δ : *E. faecalis*.

Dénombrements microbiens dans le lactosérum pendant l'acidification du caillé au cours de la fabrication du fromage Mozzarella à partir du lait de bufflonne utilisant un levain complexe à souches multiples. Souches mésophiles. ■ : *Lactoc lactis* subsp *lactis*; □ : *Lactoc lactis* subsp *diacetylactis*; ○ : *Leuc mesenteroides* subsp *dextranicum*; Δ : *E. faecalis*.

*teroides* subsp *dextranicum* was counted on Elliker lactic agar; although its colonies were clearly distinguishable, they had to be counted on plates which were crowded with colonies of *mesophilic streptococci*. An increase in numbers in whey during curd acidification was evident only for *L. delbrueckii* subsp *lactis* 22 and 35 and *Leuc mesenteroides* subsp *dextranicum* L1, although all the strains in the starter (except the yeast) were present in fairly high numbers throughout ripening. Yeast number were always below  $10^3$  cfu/ml (data not shown). However, their real amount in the curd might have been underestimated.

### Sensory evaluation

The results of sensory evaluation of Mozzarella cheese obtained using different starter systems or citric acid are shown in table II. Total scores as well as partial scores for appearance, body/texture and flavour are reported as mean with standard deviation in parentheses. Attempts to use principal component analysis to isolate the individual variables which gave the largest contribution to the scores were unsuccessful because of the high variability in the results of sensory evaluation within individual lots of cheese.

Mozzarella cheese produced with a simple thermophilic starter (B) obtained consistently lower scores for appearance, body/texture and flavour. The defects which were most frequently observed when the cheese was manufactured with this starter were: peelability in thin (70 and 50% of the samples for lots 1 and 2) and/or thick layers (54 and 50%); coarseness (46 and 86%), pastiness (38 and 21%) and lack of flexibility (61 and 86%) in body and texture; flatness (100% and 93%) and/or slightly excessive acidity (61 and 29%) in flavour. According to 46% of the judges, both lots also had a foreign flavour.

**Table II.** Sensory evaluation scores for water-buffalo Mozzarella cheese manufactured with different starter systems. Mean and standard deviation. A: Natural whey culture. B: thermophilic multiple strain starter (*L. helveticus* and *S. thermophilus*). C: complex multiple strain starter (*L. delbrueckii* subsp *lactis*, *S. thermophilus*, *Lactoc lactis* subsp *lactis*, *Lactoc lactis* subsp *diacetylactis*, *Leuc mesenteroides* subsp *dextranicum*, *E. faecalis*, *K. marxianus*). D: artificial addition of citric acid.

*Évaluation sensorielle du fromage Mozzarella produit à partir du lait de bufflonne en utilisant différents levains. Moyenne et écart type. A: culture naturelle en lactosérum. B: levain thermophile à 2 souches (L. helveticus et S. thermophilus). C: levain à souches multiples (L. delbrueckii subsp lactis, S. thermophilus, Lactoc lactis subsp lactis, Lactoc lactis subsp diacetylactis, Leuc mesenteroides subsp dextranicum, E. faecalis, K. marxianus). D: Addition d'acide citrique.*

Starter system	Lot	Appearance	Body/Texture	Flavour	Total
A	1	1.05(0.10)	1.55(1.14)	7.80(2.61)	10.8(2.61)
	2	2.67(0.62)	4.80(0.56)	8.30(1.88)	15.7(2.43)
	3	2.64(0.61)	3.47(1.73)	7.76(2.79)	12.2(4.42)
	Mean	2.03(0.64)	3.27(1.70)	7.94(2.45)	12.6(3.81)
B	1	0.31(0.75)	1.23(1.74)	1.08(1.19)	3.3(3.40)
	2	0	0	3.50(3.65)	3.5(3.65)
	Mean	0.15(0.36)	0.59(1.34)	2.33(2.97)	3.4(3.47)
C	1	2.50(0.96)	4.59(0.85)	9.72(0.70)	16.7(1.55)
	2	2.81(0.40)	4.75(0.77)	9.62(0.72)	17.2(1.11)
	3	2.75(0.73)	4.50(1.21)	9.75(0.45)	17.1(1.50)
	Mean	2.67(0.73)	4.61(0.94)	9.70(0.63)	17.0(1.40)
D	1	0.93(1.03)	4.07(1.03)	5.13(0.52)	10.1(1.60)
	2	0.26(0.70)	3.27(0.59)	5.40(0.83)	9.0(1.22)
	3	0.75(1.0)	4.56(3.14)	5.37(0.81)	9.4(1.15)
	Mean	0.65(0.94)	3.97(2.00)	5.30(0.72)	9.5(1.15)

Mozzarella cheese produced by acidification with citric acid (D) was included as a non-starter reference for sensory evaluation and obtained higher scores (see table II). The main defects noted in this type of cheese were peelability in thin or thick layers in 100% of the samples, gumminess (100% of samples) and flatness (100% of samples).

The multiple strain starter gave significantly ( $P < 0.05$ ) better results than the natural whey culture: higher scores (an overall average total score of 17.0 and 12.6 respectively) and a significantly ( $P < 0.01$ ) lower variance was obtained. The most frequent defect in appearance for both starter systems was a slight peelability in thin layers (in 15, 7 and 12% of samples for lot 1, 2 and 3 for Mozzarella cheese produced with natural starter culture and 19, 19 and 4.5% of samples for lots 1, 2 and 3 respectively for the product obtained with the multiple strain starter). Flatness (35, 20 and 11.8 for the 3 lots of the natural whey culture and 19, 25 and 18 for the multiple strain starter) was the most frequent flavour defect. A whey taint was also noted in 15, 20 and 41% of samples for lots 1, 2 and 3 respectively, for the cheese produced with the natural whey culture.

## DISCUSSION

The starter systems used varied in their acid producing ability. Thermophilic starters composed of *L. delbrueckii* subsp. *bulgaricus* and/or *L. helveticus* and *S. thermophilus* have been suggested (Kosikowski, 1977; Ministero della Sanita della Repubblica Italiana, 1978; Law, 1982; Vedamuthu and Washam, 1983) and are currently employed for the manufacture of Mozzarella cheese. Although 2 of the best acid producers in our culture collection were

chosen for this type of starter: it showed the lowest acid producing ability. The acid production of the complex multiple strain starter (C) was intermediate between that of the thermophilic starter (B) and that of the natural whey culture (A). Similar results were obtained by Parente *et al* (1989). The time needed to reach a pH suitable for stretching of the curd (4.9–5.1) is an important technological parameter; more study is needed in the selection and improvement of strains to be used in the multiple strain starter.

The evolution of lactose fermentation end-products, as expected, closely followed that of the producing microorganisms. The time course of the production of the optical isomers of lactic acid was strikingly similar in the natural whey culture and in the multiple strain starter. L(+)-lactic acid was produced at a high rate during the first 3 h while the D(-)-isomer was produced at an increasing rate as of the 2nd h of curd ripening. This can be easily explained in the multiple strain starter system where D(-)-lactic acid production clearly correlated with the population of *L. delbrueckii* subsp. *lactis* 22 and 35 which outnumbered the L(+)-lactic acid producers (*mesophilic* and *thermophilic streptococci*) only after 3 h. The complexity of the microflora involved in traditional manufacture of Mozzarella cheese discouraged individual counting of lactic acid bacteria species. In a previous study (Coppola *et al*, 1988) we showed that the species most frequently isolated for natural whey cultures were *L. delbrueckii* subsp. *lactis* and *L. helveticus* among the lactobacilli, *S. thermophilus*, *E. faecium* and *Lactoc. lactis* subsp. *lactis* among the streptococci. The early accumulation of L(+)-lactic acid may reflect the growth of enterococci while the accumulation of the D(-)- isomer could be explained by the growth of thermophilic lactobacilli. Acetic acid and ethanol reached higher

levels when heterofermentative organisms were present (natural whey culture, A, and multiple strain starter, C).

It is impossible to compare the results of the counts for the natural whey culture and the defined starter systems (B and C) since different methods were used (MPN vs plate counts). The evolution of microbial populations during the manufacture of Mozzarella cheese using natural whey cultures described by Coppola *et al* (1985) was confirmed in this study. The microflora of whey during curd acidification is dominated by enterococci and other thermophilic lactic acid bacteria. Although their growth was somewhat inhibited, coliforms reached dangerously high levels at the end of curd ripening. The use of raw milk and natural whey cultures represents a health risk for consumers: stretching of the curd in hot water (which brings the curd to 80–90 °C) may destroy indicator and pathogenic organisms, but it cannot provide a substitute for pasteurization of milk.

When the complex multiple strain starter was used, a significant increase in numbers was observed only for *L. delbrueckii* subsp. *lactis* and *Leuc. mesenteroides* subsp. *dextranicum*; it is interesting to note that 35–36 °C (the temperature of the curd from milk clotting to the end of acidification in water-buffalo Mozzarella cheese manufacture) is very close to the maximum temperature for the growth of the latter strain (Garvie, 1986). Although some of them (*S. thermophilus* and *Lactoc. lactis* subsp. *lactis*) were present in high numbers (around  $5 \times 10^7$  cfu/ml) in the whey throughout the acidification of the curd, no particular trend was evident in the populations of the mesophilic and thermophilic streptococci. The difficulty in obtaining a correct estimate of populations of the mesophilic streptococci (especially *Lactoc. lactis* subsp. *diacetylactis*) can be attributed to the lack of selectiv-

ity and good differential ability of the media used for enumeration.

Yeast populations were constantly low ( $<1 \times 10^5$  cfu/ml for treatment A, natural whey culture and  $1 \times 10^2$  cfu/ml for treatment C, complex multiple strain starter) during curd acidification. In a previous study (Addeo and Coppola, 1983) it was shown that they were the only microbial group which was selectively retained in the curd, where they reached  $1 \times 10^7$  cfu/ml during the traditional manufacture of water-buffalo Mozzarella cheese. Their number in the whey obtained by the sampling procedure used in this study may not reflect their number in the curd.

The cheese produced using the different starter systems were graded by a sensory evaluation panel using the scorecard proposed in this study. The panel was composed of local consumers of water-buffalo Mozzarella cheese; since the quality of the cheese produced by the traditional technology is extremely variable and many of the characteristics used in the evaluation (*ie*, peelability, gumminess, flatness, acidity) lacked any objective reference the variability in the results of the sensory evaluation within a single lot of cheese was not surprising. However, Mozzarella cheese produced with water-buffalo milk using a simple thermophilic starter obtained consistently low scores for appearance, body/texture and flavour (0.15, 0.59 and 2.33; mean of 2 lots). This confirms the claim that using this starter for the manufacture of Mozzarella cheese from water-buffalo milk causes a severe impoverishment in organoleptic characteristics. It is interesting to note that artificial acidification with citric acid gave slightly better results (0.65, 3.97 and 5.30 respectively for appearance, body texture and flavour; mean of 3 lots). In both cases, flatness of flavour and a severe defect in appearance,

peelability in thin or thick layers, was recognized by almost all the judges. The latter defect is generally attributed to improper acidification during the ripening of the curd before stretching.

Scores obtained by Mozzarella cheese produced with the natural whey culture were much higher (2.03, 3.27, 7.94; mean of 3 lots) but rather variable (lot 1 had definitely lower scores). The highest and more constant scores were obtained when the multiple strain starter was used (2.67, 4.61, 9.70; mean of 3 lots for appearance, body/texture and flavour respectively).

It can be concluded that although the multiple starter culture we developed for water-buffalo Mozzarella cheese has still to be improved as regards acid producing ability to reduce the manufacturing time to a minimum; it is definitely superior to simpler starters (*L. helveticus* and/or *L. delbrueckii* subsp. *bulgaricus* and *S. thermophilus*) and natural whey cultures. Its use would make possible water-buffalo Mozzarella cheese manufacture, eliminating the health risks connected with the use of raw milk and natural whey cultures while preserving the organoleptic characteristic of the product obtained by the traditional technology.

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## REFERENCES

- Addeo F, Coppola S (1983) Aspetti tecnologici e microbiologici della trasformazione del latte

di bufala in Mozzarella. *Latte* 8, 706-723

- Anonymous (1985) General guidance for enumeration of yeasts and molds. Colony count technique at 25 °C. ISO/DIS 7954
- Barnes EM (1956) Methods for the isolation of faecal streptococci (Lancefield Group D) from bacon factories. *J Appl Bacteriol* 19, 193-203
- Bracquart P (1981) An agar medium for the differential enumeration of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in yogurt. *J Appl Bacteriol* 51, 303-305
- Chapman HR, Sharpe ME (1981) Microbiology of cheese. In: *Dairy Microbiology* (Robinson RK, ed) Applied Science Publ, London, vol 2, 157-243
- Coppola S, Parente E, Dumontet S, Coppola R (1985) La fabbricazione della mozzarella di bufala: attività fermentative durante la maturazione della cagliata. *Ann Microbiol* 35, 211-220
- Coppola S, Parente E, Dumontet S, La Peccearella A (1988) The microflora of natural whey cultures utilized as starters in the manufacture of Mozzarella cheese from water-buffalo milk. *Lait* 68, 295-310
- Duthie AH, Lemaire OT, Nilson KM, Partridge JA, Atherton HV (1980) Scorecard for Mozzarella cheese. *Cult Dairy Prod J* 15, 5-7
- Elliker PR, Anderson AW, Hannesson G (1956) An agar culture medium for lactic streptococci and lactobacilli. *J Dairy Sci* 39, 1611-1612
- Garvie EI (1986) Genus *Leuconostoc*. In: *Bergey's Manual of Systematic Bacteriology* (Sneath PHA, Mair NS, Sharpe ME, Holt JG, eds) Williams and Wilkins, Baltimore, vol II, 1071-1075
- Kosikowski F (1977) *Cheese and Fermented Milk Foods*. Edward Brothers, Ann Harbour MI, 2nd edn, 158
- Law BA (1982) Cheese. In: *Fermented Foods* (Rose AH, ed) Academic Press, London, 148-198
- Ministero della Sanita della Repubblica Italiana (1978) Circolare, 88, Dec 15
- Parente E, Villani F, Coppola R, Coppola S (1989) A multiple strain starter for water-buffalo Mozzarella cheese manufacture. *Lait* 69, 271-279
- Reddy MS, Vedamuthu ER, Washam CJ, Reinbold GW (1972) Agar medium for differential

- enumeration of lactic streptococci. *Appl Microbiol* 24, 947-952
- Repubblica Italiana (1980) *Gazzetta Ufficiale*, 40, Feb 11
- Sandine WE (1979) Lactic starter culture technology. In: *Pfizer Cheese Monographs*. Pfizer Co, vol 6, NY
- Thunell RK, Sandine WE (1985) Types of starter cultures. In: *Bacterial Starter Cultures for Foods* (Gilliland SE, ed) CRC Press, Boca Raton, F, 127-144
- Tramma L (1988) Isolamento di batteriofagi di streptococchi di interesse caseario e studio delle interazioni ospite-fago. Thesis, University of Naples
- Vedamuthu ER, Washam C (1983) Cheese. In: *Biotechnology* (Rehm HJ, Reed G, eds) Verlag Chemie, Weinheim, vol 5, 213-313