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, Lactoc 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.

Mozzarela 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, Lactoc 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 cheesemaking: 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 reported (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, Cancello Amone (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 diacetyl-
lactis RD, Leuconostoc mesenteroides subsp
dextranicum L1, Enterococcus faecalis 221,
Kluyveromyces marxianus 252Y) were grown to-
gether 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 con-
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 technologi-
ical 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 rip-
ening; 100 g of curd were then weighed and
pressed (in a home-made press at = 2 kg/cm²
for 3 min) to obtain whey for microbiological and
chemical analyses.

The following procedures were used: pH was
measured with a Beckman PH143 pH-meter. En-
zymatic assays (Boeringher–Mannheim) were
used to measure L(+)-lactic acid, D(-)-lactic acid
(kit 139.084 and D-lactate dehydrogenase), eth-
anol (kit 176.290) and acetic acid (kit 148.261).
The samples were deproteinized using the pro-
cedure suggested by the manufacturer; samples
were filtered through Gelman Akrodisk (pore
size 0.45 µm) 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 differenc-
es 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 be-
low: 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 (ther-
mophilic lactic acid bacteria). Coliforms were
counted in brilliant green lactose bile broth (Ox-
oid, UK), incubated at 30°C for 48 h. For entero-
cocci azide dextrose broth (Oxoid, UK) tubes
were inoculated and incubated at 37°C for 48 h;
ethyl violet azide broth (Oxoid, UK) was inoculat-
ed from presumptively positive tubes; after incu-
bation at 37°C for 24 h, positive tubes were con-
firmed by streaking plates of thallous acetate
tetrazolium glucose agar (TITGA, Barnes,
1956). Yeasts were counted by inoculating sam-
ple dilutions onto yeast dextrose chlorampheni-
col 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 multi-
ple 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 (Braquart, 1981), incubated at 37°C
for 48 h in Gaspak jars supplied with CO₂-
generating kit (BBL, USA). E faecalis was count-
ed 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 sup-
plied with CO₂-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 cit-
rate-utilizing colonies on Reddy’s medium and
E faecalis counts on TATGA. Leuc mesente-
roides subsp dextranicum were counted as
slime-forming colonies on Elliker lactic agar (Ell-
liker et al, 1956) incubated for 48 h at 30°C in a
CO₂-enriched atmosphere generated as de-
scribed above.

A descriptive test was used for sensory eval-
uation of the cheeses produced for each lot. A
panel of 25 judges was selected among un-
trained consumers of water-buffalo Mozzarella
cheese. A scorecard based on that proposed by
Duthie et al (1980) was developed (table 1) 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 baked 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-
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.

<table>
<thead>
<tr>
<th>Defects</th>
<th>Slight</th>
<th>Definite</th>
<th>Pronounced</th>
</tr>
</thead>
<tbody>
<tr>
<td>Misshapen</td>
<td>0</td>
<td>-1</td>
<td>-2</td>
</tr>
<tr>
<td>Unsealed or badly sealed</td>
<td>-1</td>
<td>-2</td>
<td>-3</td>
</tr>
<tr>
<td>Unnatural colour</td>
<td>-3</td>
<td>-3</td>
<td>-3</td>
</tr>
<tr>
<td>Opaque surface</td>
<td>-1</td>
<td>-2</td>
<td>-3</td>
</tr>
<tr>
<td>Rough surface</td>
<td>-1</td>
<td>-2</td>
<td>-3</td>
</tr>
<tr>
<td>Peelable in thin layers by touching</td>
<td>-1</td>
<td>-3</td>
<td>-3</td>
</tr>
<tr>
<td>Peelable in thick layers by touching</td>
<td>-3</td>
<td>-3</td>
<td>-3</td>
</tr>
</tbody>
</table>

### Intensity scores

- Slight: 1, 2, 3
- Definite: 2, 3, 4
- Pronounced: 3, 4, 5

### 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.

<table>
<thead>
<tr>
<th>Defects</th>
<th>Slight</th>
<th>Definite</th>
<th>Pronounced</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coarse</td>
<td>-1</td>
<td>-3</td>
<td>-5</td>
</tr>
<tr>
<td>Gassy</td>
<td>-2</td>
<td>-3</td>
<td>-5</td>
</tr>
<tr>
<td>Lacks flexibility or elasticity</td>
<td>-2</td>
<td>-3</td>
<td>-5</td>
</tr>
<tr>
<td>Pasty</td>
<td>-2</td>
<td>-3</td>
<td>-5</td>
</tr>
<tr>
<td>Open or empty</td>
<td>-1</td>
<td>-2</td>
<td>-3</td>
</tr>
<tr>
<td>Gummy</td>
<td>-0</td>
<td>-1</td>
<td>-2</td>
</tr>
<tr>
<td>Dry</td>
<td>-1</td>
<td>-2</td>
<td>-4</td>
</tr>
<tr>
<td>Hard</td>
<td>-2</td>
<td>-3</td>
<td>-4</td>
</tr>
</tbody>
</table>

### Flavour

**Excellent** = 10

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

<table>
<thead>
<tr>
<th>Defects</th>
<th>Slight</th>
<th>Definite</th>
<th>Pronounced</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flat</td>
<td>-1</td>
<td>-3</td>
<td>-5</td>
</tr>
<tr>
<td>Coarse</td>
<td>-3</td>
<td>-5</td>
<td>-7</td>
</tr>
<tr>
<td>Acid (too much acid)</td>
<td>-3</td>
<td>-5</td>
<td>-8</td>
</tr>
<tr>
<td>Bitter</td>
<td>-5</td>
<td>-7</td>
<td>-10</td>
</tr>
<tr>
<td>Foreign</td>
<td>-3</td>
<td>-6</td>
<td>-10</td>
</tr>
<tr>
<td>Fruity</td>
<td>-2</td>
<td>-4</td>
<td>-6</td>
</tr>
<tr>
<td>Musty</td>
<td>-3</td>
<td>-5</td>
<td>-7</td>
</tr>
<tr>
<td>Lipolyzed</td>
<td>-4</td>
<td>-6</td>
<td>-10</td>
</tr>
<tr>
<td>Salty</td>
<td>-1</td>
<td>-2</td>
<td>-3</td>
</tr>
<tr>
<td>Whey taint</td>
<td>-2</td>
<td>-3</td>
<td>-5</td>
</tr>
<tr>
<td>Yeasty</td>
<td>-4</td>
<td>-6</td>
<td>-10</td>
</tr>
<tr>
<td>Greasy</td>
<td>-1</td>
<td>-2</td>
<td>-3</td>
</tr>
</tbody>
</table>
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); 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).

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); 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).

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); 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).

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); 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).

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 reflects the microbiological complexity (i.e., 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, entrapment 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 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)).

**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.
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 lactic subsp diacetylactis* RD and *Lactoc lactic subsp lactis* 136 were estimated using the counts from different media; *Leuc mesenteroides 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.

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

Yeast populations were constantly low (<1 x \( 10^5\) cfu/ml for treatment A, natural whey culture and 1 x \( 10^2\) 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 x \( 10^7\) 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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