Influence of the Milk Bactofugation and Natural Whey Culture on the Microbiological and Physico-Chemical Characteristics of Mozzarella Cheese

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Abstract

In this work the effect of the milk bactofugation and natural whey culture on the microbiological and physico-chemical quality of Mozzarella was studied. To this aim, the microbiological, sensory and physico-chemical parameters of the Mozzarella cheese were monitored during the storage at 8°C. The bactofugation treatment did not significantly affect the growth of typical dairy microorganisms, while a significant decrease of Enterobacteriaceae in milk was found. The Mozzarella manufactured with natural whey culture and bactofugated milk showed a slower increase in the Pseudomonas spp. cell load during storage. Moreover, the absence of natural whey culture in the Mozzarella cheese manufacture caused a faster sensorial quality loss during storage (~4.5 days) respect to the product with natural starter (~6 days). However, the factor limiting the shelf life of Mozzarella cheese was the growth of Pseudomonas spp. In particular, a shelf life value of about 4.0 days was obtained for Mozzarella produced with bactofugated milk and natural whey culture versus 3.5 days for the control, with citric acidification, and samples manufactured with natural whey culture and no bactofugated milk. The compositional characteristics of the cheeses were influenced by the use of the natural starter, especially at the end of the storage period. In fact, the control cheese tended to lose soluble compounds (WSN and NaCl) faster and, increase in moisture content.

Keywords: Mozzarella cheese; Bactofugation; Natural whey culture; Shelf life

Introduction

The pasta filata process of cooking and stretching of cheese curd is typical during manufacture of many Italian cheeses such as the Mozzarella [1]. Two types of Mozzarella are produced, based on moisture content [2]. In particular, “low moisture” (LM) Mozzarella cheese (moisture 45-52%) is usually used for pizza toppings or as an ingredient in other recipes, whereas “high moisture” (HM) Mozzarella cheese (moisture 52-60%), making up most of the Italian Mozzarella export, is usually consumed fresh as a table cheese [3-5]. Traditional HM Mozzarella is packaged in a dilute salt solution (NaCl and/or CaCl₂) (called conditioning brine) and has a short shelf life (3-4 days). The short shelf life of traditional Mozzarella cheese has been attributed to microbiological spoilage. This spoilage is often caused by the growth of coliforms, Pseudomonas spp. and/or by psychrotrophic bacteria that grow on the cheese surface, mostly coming from water used in the manufacture [6-8].

Under industrial conditions, selected thermophilic lactic acid bacteria cultures are used as starters in Mozzarella cheese making [9], whereas for traditional processing, naturally fermented whey is used as the inoculum. The selection of starter cultures has a profound effect on the final characteristics of the cheese. Yun et al. [10] reported that an appropriate rod-to-coccus ratio influences the proteolysis, springiness and viscosity of Mozzarella cheese. In traditional cheese making procedures the natural whey cultures are natural microbial cultures occurring in the whey drained after curd ripening. Part of this whey is stored and employed as starter the following day. However, little information is available on the complex bacterial community of Mozzarella cheese from raw cows’ milk processed by adding the naturally fermented whey from the previous day as a starter culture [11,12].

Bactofugation is a process in which a specially designed hermetic centrifuge, the Bactofuge, is used to separate bacteria, and especially the spores formed by specific bacteria strains, from milk. Bactofugation has proved to be an efficient way of reducing the number of spores in milk, since their density is higher than that of milk. Bactofugation normally separates the milk into a fraction that is more or less free from bacteria, and a concentrate (bactofugate), which contains both spores and bacteria in general and amounts to up to 3% of the feed to the Bactofuge. It has principally been used in the cheese industry where its high-cleaning capabilities have been used to remove spores from cheese milk that could cause latent fermentation in semi-hard cheeses. It has now been adapted for processing consumption milk, where it holds out the promise of prolonging the life of fresh, pasteurised milk by 3-5 days [13].

The effect of various regimes of milk bactofugation on the removal of bacteria was studied by Dilanyan et al. [14]. Bactofugation of either cold milk or of milk pre-heated to 43-45°C eliminated about 67-85% of the bacteria present; when double treatment was carried out the bacterial removal increased to about 95%. Bactofugation can be an interesting alternative to pasteurization in cheese-making: it allows to use raw milk, avoiding the negative effects of heating to the coagulation properties [15]. This aspect is particularly interesting for cheeses that

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require curd with good firmness and structure, such as hard, semi-hard and pasta filata cheeses. For this latter type of cheese, adequate curd structure and low presence of whey proteins are very important to guarantee good stretching properties [16]. For Italian Mozzarella the structural characteristics are particularly important for one more reason, that is the storage of the cheese in water or diluted brine, which tends to impair firmness of the product. Indeed, bactofugation is not comparable to pasteurization as to safety, even though it is very effective against coliforms [17]: nevertheless, the presence of a thermic step (the stretching process) during the manufacture, makes the bactofugation process compatible with the safety of the final product. For all these reasons some dairies are re-considering bactofugation as an alternative to pasteurization; unfortunately, information regarding the application of this technique for the manufacture of Mozzarella is lacking.

Taking into account all the above considerations, the objective of the present study was to evaluate the effect of the milk bactofugation and natural whey culture on the microbiological, sensory and physico-chemical quality of HM Mozzarella cheese.

Materials and Methods

Bactofugation process

The bactofugation process was carried out in a industrial dairy, using the one-phase Westfalia bactofuge CNE 300 at the following operating conditions: temperature 55°C; flow rate 30.000 L/h; feeding pressure 1 bar; pressure at discharge 5 bar.

Mozzarella cheese manufacture

Samples of Mozzarella were produced from raw (NWC
10
) or bactofuged milk (NWC
off
), both inoculated with 5% natural whey culture obtained by spontaneous fermentation, at 38°C, of whey derived from the cheese production of the previous day. Bovine rennet addition (strength 1:10.000 at the dose 0.25 mLL
−1
of milk) was performed at 35°C and coagulation took place in 20 min. After removal of about 80% whey the curd was kept warm for fermentation, which was completed within 3 hours. Samples of Mozzarella were also produced from the same raw milk by direct acidification with citric culture obtained by spontaneous fermentation, at 38°C, of bactofuged milk (NWC
off
). The curd stretching was performed mechanically, using hot water at 85°C. During the stretching, the temperature of the curd increased to 60°C. After shaping, the cheeses were placed in cold water (10°C) and stored at 4°C until sampling.

Microbiological analyses

The preparation of the test samples, initial suspension and decimal dilutions for microbiological examination was performed according to the International Standard ISO 8261:2001. Plate Count Agar (PCA) incubated at 30°C for 24-48 h was used as media for total viable count; Pseudomonas Agar Base (PAB), added with CFC selective supplement, incubated at 25°C for 48 h for Pseudomonas spp. count. For Enterobacteriaceae, Violet Red Bile Glucose Agar (VRBGA, Oxoid) were used and plates were incubated at 37°C for 18-24 h. Lactic acid bacilli (LAB) were plated on de Man Rogosa Sharpe agar (MRS, Oxoid) and incubated anaerobically in the jars HP 11 (Oxoid, Milan, Italy) at 30°C for 2-4 days. M17 agar (Oxoid), incubated at 37°C for 48 h for coccus-shaped lactic acid bacteria. For the enumeration of enterococci, Slanetz-bartley medium incubated at 37°C for 48-72 h, was used. Sabouraud Dextrose Agar incubated at 25°C for 48 h, was used as media for yeast count. The microbiological analyses were carried out twice on two different batches.

In order to evaluate the effect of the bactofugation on the microbiological quality of milk, "Microbial Growth Decrease" index was calculated as:

\[
MGD = \frac{\log CFU_{ml}^{-1} - \log CFU_{ml}^{-1}}{\log CFU_{ml}^{-1}} \times 100
\]

where \( \log CFU_{ml}^{-1} \) represents the cell load of control milk and \( \log CFU_{ml}^{-1} \) the cell load of bactofugated milk.

The Microbial Acceptability Limit (MAL) (i.e., the storage time at which the viable cell concentration reached the threshold), was calculated as reported by Del Nobile et al. [18]. The limit of the CFU g
−1
was fixed at 10
6
CFU/g for Pseudomonas spp. [19].

Chemical analyses

Mozzarella cheese samples were analysed for moisture [20], protein by macro-Kjeldahl method [21], fat by Soxhlet method, pH [22], Water-Soluble Nitrogen (WSN) by the method of Kuchroo and Fox [23]. All compositional analyses were performed in triplicate. The profile of WSN was studied by High Performance Liquid Chromatography using a Waters 990E pump, equipped with a VyDAC18 column and a Diode Array Detector 996; the separation was carried out with a gradient of acetonitrile in water, both containing 0.1% trifluoracetic acid, from 5 to 70% in 45 min.

Sensory analysis

Mozzarella samples were subjected to sensory analysis by a panel consisting of seven trained evaluators. Panelists from the food packaging laboratory of the University of Foggia were selected on the basis of international standards ISO 8586-1:1993 (Sensory analysis-General guidance for the selection, training and monitoring of assessors-Part 1: Selected assessors) and ISO 8586-2:1994 (Sensory analysis- General guidance for the selection, training and monitoring of assessors-Part 2: Experts). Mozzarella samples were presented to panelists without brine and they were asked to describe differences between samples by using a scale from 1 to 7 [24]. Panelists were asked to base their decision on the sample "overall quality" only taking into account color, odor, and firmness. Therefore, the samples "overall quality" has to be considered as an average of the above-mentioned sensory attributes (i.e., color, odor, and firmness) as weighted by the panelists [4]. A score of 4 was the minimum threshold value for cheese acceptability. The Sensory Acceptability Limit (SAL) (i.e., the storage time at which the sensory attribute reaches the threshold) was calculated as reported by Del Nobile et al. [4].

Statistical analysis

Experimental data of all tested samples were compared by one-way Anova analysis. A Duncan’s multiple range test, with the option of homogenous groups (P<0.05), was used to determine significance among treatments. To this aim, Statistica 7.1 for Windows (StatSoft Inc, Tulsa, OK, USA) was used.

Results and Discussion

Effect of bactofugation on milk microbial quality

The effect of the bactofugation on the microbiological quality of milk is shown in figure 1. It has been found that, on the average, milk treated in a bactofuge contains 90% fewer microorganisms than the untreated milk [25]. In our conditions, among the anaerobe-facultative microorganisms the highest cell load decrease was found for
Enterobacteriaceae (about 72%), in agreement with the results reported by Kosikowsky and Fox [17]. The bactofugation did not significantly affect the growth of typical dairy microorganisms. In particular, a low MGD index for enterococci (7%), coccus-shaped LAB (26%) and lactic acid bacilli (LAB) (33%) was obtained. Moreover, the microbial growth decrease was about 10% for total viable count and 55% and 21% for yeast and Pseudomonas spp., respectively. These results are interesting for the Mozzarella cheese-making process, since they demonstrate that the conditions used for the bactofugation process allow to obtain a good selection of microorganisms without using high temperature. Moreover, it is well known that the effectiveness of bactofugation increases with the temperature of the milk.

The figure 2 shows the microbial cell load of the Natural Whey Cultures (NWC) used in the cheese-making process. The natural whey cultures consisted of a large number of microorganisms and a great variety of microbial groups. The results showed a total viable count of about 6.0 log CFU/ml with a predominance of lactic acid bacteria (6.0 log CFU/ml) and enterococci (5.0 log CFU/ml). Their ability to produce flavor compounds and to synthetize large amounts of extracellular polysaccharides could be important in Mozzarella manufacture. Yeasts and coccus-shaped LAB reached counts in the range of 4 log CFU/ml and they may be responsible for some biochemical activity in the cheese-making process. Enterobacteriaceae in the natural whey cultures were below the detection limit while a cell load of about 4.5 log CFU/ml for Pseudomonas spp. was recorded.

Microbiological quality of Mozzarella cheese

The evolution of Pseudomonas spp. cell load plotted as a function of storage time for Mozzarella samples is shown in figure 3a. In the figure the horizontal solid line represents the viable cell concentration threshold value (10^6 CFU g). It is worth noting that microbial cell load was monitored until the packed food reached either its microbial or sensory threshold value. As can be seen the NWCamples showed the lowest initial cell load of Pseudomonas spp. (below 2 log CFU/g). Regards the CNTR and the NWCamples a cell load of about 4 and 3 log CFU/g, respectively, was observed. However, during the storage time, Pseudomonas spp. cell load of the latter two samples exceeded the threshold value at earlier storage time if compared to sample manufactured with NWC and bactofugated milk (NWCamples). In fact, the MALPseudomonas values listed in table 1 highlight that the addition of NWC and the use of bactofugated milk (NWCamples) significantly affected the microbial quality of Mozzarella cheese (P<0.05). On the other hand, the NWCamples samples showed not substantial differences compared the Mozzarella without NWC (CNTR). For these samples a MALPseudomonas values of about 3.5 days was recorded; whereas, for the NWCamples a value of about 4.5 days was obtained.

Figure 3b shows the evolution of total viable count for Mozzarella samples during the storage. As can be seen from data shown in the figure, a faster increase of the total viable count was observed for CNTR sample. In particular, the initial cell load was about 5.0 log CFU/g and reached a value higher than 8.0 log CFU/g at 6 days of storage. It is worth noting that for the NWCamples samples a slower growth respect to the NWCamples was observed. Moreover, a lower cell load of about 0.65 log cycle for NWCamples at 7 days of storage was obtained.

Lactic acid bacilli for NWCamples showed a lower increase at 5 days of storage respect the other samples (Figure 3c). However, at the end of storage the same cell load for all samples was observed.

It is worth noting that for NWCamples samples the coccus-shaped lactic acid bacteria showed a lowest increase respect to that from no bactofugated milk (NWCamples) and CNTR (Figure 3d). In particular, a final cell load of about 7.5 log CFU/g for CNTR and NWCamples was recorded. A difference of about 1.0 log cycle for NWCamples samples was observed.

The enterobacteria cell load in the NWCamples was below 4 log CFU/g until day 5 and reached a cell load of about 6 log CFU/g at the end of storage (Figure 3e). For Mozzarella samples NWCamples a faster increase of the Enterobacteriaceae cell load was observed. The same evolution for the CNTR samples was also obtained; moreover, in this case, a higher initial (3.6 log CFU/g) and final cell load (7.3 log CFU/g) was recorded.

Chemical analyses

The gross composition of the three types of Mozzarella at 1st day were not statistically different as to moisture, fat, protein and NaCl, whereas pH and WSN showed some differences between the two samples made with natural starter and the control (Table 1). As expected, pH was higher in the control cheese due to the fact that it was produced by direct acidification. The WSN content was very low in all samples indicating very poor proteolysis, as previously reported [26], but it was particularly low in the control. The values of WSN decreased with time: the data at day 7, beside confirming scarce casein degradation, suggests that a progressive loss of low molecular weight compounds (probably migrating into the storage solution) takes place. This hypothesis is confirmed by the evolution of the concentration of sodium chloride: for all samples a decrease was found, but it was more...
Table 1: Chemical composition of Mozzarella samples during storage (g kg⁻¹).

<table>
<thead>
<tr>
<th></th>
<th>NWC_bacto 1d</th>
<th>NWC_bacto 7d</th>
<th>NWC_no_bacto 1d</th>
<th>NWC_no_bacto 7d</th>
<th>CNTR 1d</th>
<th>CNTR 7d</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>5.35 ± 0.01(a)</td>
<td>5.41 ± 0.03(a)</td>
<td>5.31 ± 0.04(a)</td>
<td>5.43 ± 0.02(a)</td>
<td>5.86 ± 0.03(a)</td>
<td>5.96 ± 0.03(a)</td>
</tr>
<tr>
<td>Moisture</td>
<td>620 ± 6.0(a)</td>
<td>626 ± 5.5(a)</td>
<td>615 ± 4.0(a)</td>
<td>621 ± 5.5(a)</td>
<td>616 ± 5.5(a)</td>
<td>639 ± 5.0(a)</td>
</tr>
<tr>
<td>Fat</td>
<td>155 ± 4.5(a,b)</td>
<td>153 ± 3.5(a,b)</td>
<td>156 ± 1.0(a)</td>
<td>154 ± 5.0(a)</td>
<td>158 ± 3.0(a)</td>
<td>151 ± 3.0(a)</td>
</tr>
<tr>
<td>Protein</td>
<td>148 ± 2.0(a,b)</td>
<td>149 ± 4.0(a,b)</td>
<td>149 ± 2.5(a,b)</td>
<td>148 ± 2.0(a)</td>
<td>151 ± 2.0(a)</td>
<td>143 ± 3.5(a)</td>
</tr>
<tr>
<td>NaCl</td>
<td>7.4 ± 0.3(a)</td>
<td>3.9 ± 0.4(a)</td>
<td>7.6 ± 0.2(a)</td>
<td>3.8 ± 0.2(a)</td>
<td>8.0 ± 0.5(a)</td>
<td>2.5 ± 0.2(a)</td>
</tr>
<tr>
<td>WSN</td>
<td>0.12 ± 0.01(a)</td>
<td>0.10 ± 0.003(a)</td>
<td>0.14 ± 0.05(a)</td>
<td>0.11 ± 0.02(a)</td>
<td>0.07 ± 0.01(a)</td>
<td>0.04 ± 0.01(a)</td>
</tr>
</tbody>
</table>

Values with different superscripts are different at P<0.01 (One-way ANOVA and Duncan’s test)

Figure 3: Evolution of *Pseudomonas* spp. (a), total viable count (b), lactic acid bacilli (c), coccus-shaped lactic acid bacteria (d), Enterobacteriaceae (e) for Mozzarella cheese during the storage.
Figure 4: HPLC profiles of WSN of the Mozzarella samples.
marked for the control (from 8 to 2.5 g.kg$^{-1}$). For this sample, a slight increase of the moisture content was also detected.

The HPLC profiles of WSN at day 7 are reported in figure 4: they evidenced some differences. The profiles of the samples made natural starter were very similar under a qualitative point of view, but that of the No Bacto sample had slightly higher peaks. The profile of the control was instead totally different: the peaks were less high and were mainly shifted towards the final part of the chromatogram, indicating a more marked hydrophobicity.

**Sensory quality decay**

Figure 5a shows the evolution during storage of the odor in Mozzarella cheese. The score for odor decreased faster for the CNTR samples, reaching first the odor threshold value. The panelists judged the samples manufactured with NWC with highest odor score; moreover, the bactofugation treatment did not affect this parameter. Color attribute showed a high score in the first 4 days of storage and then decreased however no difference between the samples was observed (Figure 5b). It is worth noting that the texture was affected by both the addition of NWC in the production process and the bactofugation treatment. In particular, the texture score for the CNTR sample decreased when compared to the other formulations, reaching the threshold value at about 5 days, followed by the NWC$_{no\_bacto}$ samples at 6 days. The bactofugation process positively affected the texture of the product that reached the threshold value at 7 days (Figure 5c).

The overall quality assessment of Mozzarella samples is shown in figure 5d. As can be seen, the overall quality trend coincides with score of the odor, thus proving that this attribute represented the factor limiting cheese storability. In particular, the overall quality of the CNTR samples, after the first three days, rapidly decreased reaching first the "overall quality" threshold value (score 4). The addition of NWC during the manufacture process affected positively the sensorial quality of Mozzarella. In fact, the overall quality for this sample remained above the threshold value up to the 6$^{th}$ day. After this period the quality decay occurred. It should be noted also that the bactofugation did not affect the sensorial quality of produced Mozzarella.

SAL values determined for Mozzarella cheese "overall quality" (SAL$_{\text{Overall Quality}}$) were listed in table 2. As stated beforehand, any substantial difference between Mozzarella manufactured with NWC and milk subjected or not to the bactofugation treatment was observed (P>0.05). On the other hand, the absence of NWC in the Mozzarella cheese manufacture caused a faster sensorial quality loss during
Mean values in the same column followed by different superscript are significantly different at P<0.05 (One-way ANOVA and Duncan’s test).

Table 2: Shelf life of Mozzarella samples evaluated on the basis of microbial acceptability limit (MAL) of *Pseudomonas* spp. (MAL	extsubscript{Pseudomonas}) and sensorial acceptability limits (SAL	extsubscript{Overall Quality}).

<table>
<thead>
<tr>
<th>Samples</th>
<th>MAL	extsubscript{Pseudomonas}</th>
<th>SAL	extsubscript{Overall Quality}</th>
<th>Shelf Life</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNTR</td>
<td>3.57 ± 0.16*</td>
<td>4.48 ± 0.23*</td>
<td>3.57 ± 0.16*</td>
</tr>
<tr>
<td>NWC	extsubscript{CNTR}</td>
<td>4.33 ± 0.38*</td>
<td>6.33 ± 0.18*</td>
<td>4.33 ± 0.38*</td>
</tr>
<tr>
<td>NWC	extsubscript{bacto}</td>
<td>3.40 ± 0.20*</td>
<td>5.95 ± 0.17*</td>
<td>3.40 ± 0.20*</td>
</tr>
</tbody>
</table>

Means in the same column followed by different superscript are significantly different at P<0.05 (One-way ANOVA and Duncan’s test).

Storage. In particular, a SAL	extsubscript{Overall Quality} value of about 6 and 4.5 days for Mozzarella with (NWC	extsubscript{bacto}, NWC	extsubscript{no bacto}) and without NWC (CNTR) was recorded.

**Shelf life evaluation**

Mozzarella shelf life was calculated as the lowest value between MAL	extsubscript{Pseudomonas} and SAL	extsubscript{Overall Quality} (Table 1). It is worth noting that the shelf life of Mozzarella cheese seemed to be strongly dependent on the microbial quality. In particular, *Pseudomonas* spp. growth was the limiting factor the product acceptability, whereas the sensorial quality did not limit the Mozzarella shelf life. As can be inferred from data, for CNTR and NWC	extsubscript{CNTR} samples a shelf life value of about 3.5 days and 4.5 for the NWC	extsubscript{bacto} samples was obtained.

**Conclusion**

Results of this study suggested that bactofugation can be successfully used to remove the bacteria of milk prior to processing. Therefore, the bactofugation process can be an interesting alternative to pasteurization in cheese-making as it avoid the negative effects of heating to the coagulation properties. Moreover, this technique is able to improve the shelf life of Mozzarella obtained with raw milk with the addition of natural whey culture. In fact, for these samples a lower increase of the *Pseudomonas* spp. cell load was obtained. Moreover, the absence of natural whey culture in the Mozzarella cheese manufacture caused a faster sensorial quality loss during storage respect to the product with natural whey culture. However, the factor limiting the shelf life of Mozzarella cheese was the growth of *Pseudomonas* spp., whereas the sensorial quality did not limit the product acceptability. In particular, a shelf life value of about 4.5 days for Mozzarella with natural whey culture and bactofugated milk and 3.5 days for the control and sample with natural whey culture and no bactofugated milk was obtained. The compositional characteristics of the cheeses were influenced by the use of the natural starter, in particular at the end of the storage period, when the control cheese tended to loss faster the soluble compounds (WSN and NaCl) and to increase the moisture content. These results suggest a more marked “permeability” of the casein matrix made by direct acidification: the reasons of this different behavior among the cheeses need more investigation.

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**References**

20. IDF (1982) Cheese and processed cheese: Determination of the total solids content. IDF References method 4A.